Università di Pisa

Scuola di Dottorato di Ricerca in Biologia

XXIX Ciclo

Diversity and evolution in the family Paraonidae (Annelida, Polychaeta): a morphological and molecular perspective

Candidate:

Joachim Langeneck

Tutors:

Prof. Alberto Castelli

Dr. Ferruccio Maltagliati

To Nicola Tomassone

and Enrico Pappalettere

who first awakened and cultivated my curiosity

In loving memory of Klaus Langeneck

Son teneri, rosei ed inermi
i vermi di Forte dei Marmi
che in coro mi cantano: "Dormi!"
Cullato dal canto dei vermi
se dormo non posso sognarmi
che un mare di vermi che mormori.

(Toti Scialoja)

Harmless, rosy, and tender to touch
are the worms of Burnham-on-Crouch
whose choir now sings me to sleep.
And cradled by their song, so warm,
I dream of a murmuring swarm
of worms that in mind I must keep.

Table of contents

1. Introduction

1.1 The study of polychaete diversity and taxonomy

1.1.1 Historical perspective: the change of taxonomic paradigms and schemes

One of the most fruitful field of investigation in zoology has historically been the study of vermiform invertebrates, which were reunited by Linnaeus (1758) in the class of Vermes. This class was subsequently recognized as artificial and split in several groups, or phyla, namely Cnidaria, Nematoda, Platyhelminthes, Priapulida, Echiura, Sipuncula, Annelida, Mollusca. A traditional taxonomic scheme recognized three classes within Annelida, namely Polychaeta, Oligochaeta, and Hirudinea. Following this view, Echiura and Sipuncula were considered distinct phyla closely related to Annelida (Meglitsch, 1972). Recently, two deep-water related groups of tubeworms, Pogonophora and Vestimentifera, have been considered additional distinct phyla closely related to Annelida (Jones, 1985). A more recent perspective, based on the use of molecular tools, unravelled a more complex taxonomic scheme. The group of Clitellata, including Oligochaeta and Hirudinea, is a monophyletic clade within Annelida and is related to groups traditionally referred to Polychaeta. As a consequence, Polychaeta is paraphyletic with regard to Clitellata (Struck et al., 2007; 2011). Moreover, molecular and morphological investigations showed that Vestimentifera and Pogonophora are actually a clade derived from typical Annelida (Boore & Brown, 2000; Rouse, 2001) and, more recently, also Echiura and Sipuncula were reunited to Annelida on the basis of compelling molecular evidence (Struck et al., 2007; 2011). The position of the small group of Myzostomida, small non-segmented worms with parapodia and parasitic behaviour, has been historically questioned (Riedl, 1983). Molecular data are somewhat ambiguous and place this group at the basis of the Trochozoa radiation (Eeckhaut et al., 2000; Zrzavy et al., 2001) or within the Annelida radiation (Bleidorn et al., 2007). This last view seems the most supported by the recent phylogenetic reconstructions (Bleidorn et al., 2009). Actually, the traditional view considering Annelida as composed only by segmented organisms proved to be wrong, and a more comprehensive view includes several non-segmented groups that are not directly related (Halanych et al., 2002) (Fig. 1).

An updated taxonomy of Annelida should therefore start from the splitting of the paraphyletic group of polychaetes, and the recognizing of actual monophyletic lineages. An old view recognized two large groups within polychaetes, namely Errantia and Sedentaria (see Fauvel, 1923); this classification was provisionally retained mainly for practical issues, even though it was dismissed in the 1970s as unrepresentative of the actual phylogenetic relationships among polychaete groups (Day, 1967).

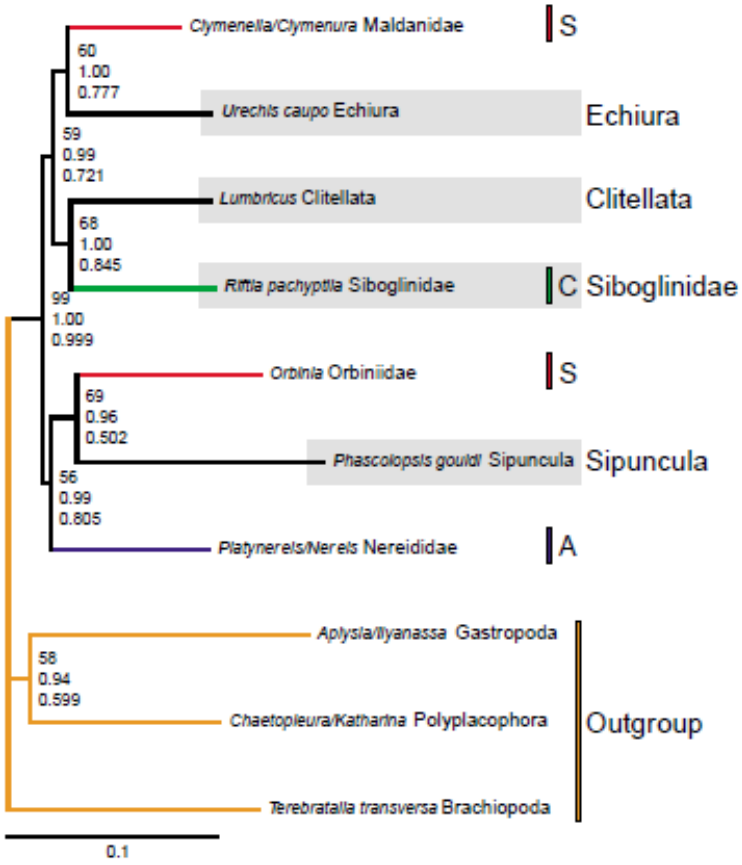

Figure 1: Evolutionary relationships within Annelida according to the latest molecular reconstructions (from Struck et al., 2007)

A later view identified within a monophyletic class of Polychaeta two main lineages, namely Scolecida and Palpata, with the latter further divided in two clades, Canalipalpata and Aciculata (this latter including a large part of the former Errantia) (Rouse & Fauchald, 1997). Struck et al. (2007) demonstrated that neither Scolecida, nor Canalipalpata are monophyletic, and Aciculata represents a monophyletic group with the addition of Orbiniidae and removal of Amphinomidae. A later work confirms that Scolecida and Canalipalpata are artificial groups and identifies a large clade including the former Sedentaria (with the exception of Orbiniidae and Chaetopteridae), Echiura and Clitellata, and another one comprising the former Errantia (included Amphinomidae) and Orbiniidae (Struck et al., 2011). Sipuncula, Myzostomida and the family of Chaetopteridae ramify basally to the two clades. Struck et al. (2011) identified therefore two polychaete groups coarsely corresponding to Errantia and Sedentaria; within these groups, several traditionally recognized orders (Terebelliformia, Cirratuliformia, Sabellida, Spionida, Phyllodocida, Eunicida) have been confirmed by molecular studies. The morphology of polychaetes, and of annelids in a more general way, seems therefore ineffective as indicator of evolutionary processes and phylogenetic relationships, at least at the higher taxonomic levels, whereas already at the order level, groups identified on the basis

of similar structures proved generally to be monophyletic. Struck et al. (2011) suggested that in several cases, similar morphologies in Annelida are the result of similar adaptive strategies and processes, and do not reflect common ancestries, as is the case of Canalipalpata and Scolecida.

The reconstruction of phylogenies is a valuable tool for improving traditional taxonomy at lower taxonomic levels, and to infer on the main ecological processes that determined the current diversity of polychaete families. Phylogenies based on morphological features have been reconstructed for a number of families, or sub-familiar groups, but as Struck et al. (2011) demonstrated, morphological characters are not always reliable, since similar features can arise in unrelated groups as consequence of similar evolutionary histories (Struck et al., 2015) and strong morphological differences can be due to different adaptive strategies within the same group, or among closely related groups (Bleidorn, 2005). Moreover, fossil records of polychaetes are very poor, except for few groups with sclerotized parts, such as Eunicida and Glyceridae. Therefore, the use of molecular tools is of paramount importance in order *i*) to assess the correctness of phylogenies based on morphological features, *ii*) to infer about the evolutionary history of polychaete groups, and *iii*) to obtain a taxonomy as much as possible accurate with regard to the actual phylogenetic relationships within the group. Although the use of molecular tools in phylogenetic reconstruction is widespread and useful, this approach is still poorly adopted in polychaete research, mainly because the majority of the material available in polychaete collections has been traditionally treated with formaldehyde, which preserves at best the soft parts of polychaete anatomy, but makes samples useless for molecular studies. Every molecular study on polychaetes, therefore, must start from the gathering of new genotypable material, extending the times and costs needed for research. Until now, molecular phylogenies, even partial, have been obtained for Orbiniidae (Bleidorn, 2005), Arenicolidae (Bleidorn et al., 2005), Aphroditiformia (Wiklund et al., 2005; Norlinder et al., 2012), Serpulidae (Kupriyanova et al., 2006; Lehrke et al., 2007; Kupriyanova et al., 2008), Phyllodocidae (Eklöf et al., 2007), Syllidae (Aguado et al., 2007; 2012), Amphinomida (Wiklund et al., 2008; Borda et al., 2015), Sabellidae (Capa et al., 2011), Opheliidae (Law et al., 2012) and Eunicidae (Zanol et al., 2014). Moreover, comprehensive molecular studies have been carried out on some groups belonging to Clitellata (Apakupakul et al., 1999; Erséus et al., 2000; Siddall et al., 2001; Bely & Wray, 2004; Borda & Siddall, 2004; Erséus & Källersjö, 2004; Erséus et al., 2010), on Echiura (Goto et al., 2013), and at a lesser extent, on Sipuncula (Maxmen et al., 2003; Schulze et al., 2005). Several families that display high species diversity, such as Nereididae, Glyceridae, Maldanidae, and of course Paraonidae, are

still poorly known from the molecular point of view. Molecular phylogenetic inference for these groups could be useful in order to give a deeper insight into the evolutionary pathways within Annelida.

1.1.2 Cryptic speciation in the marine environments

With the progress of scientific knowledge and molecular techniques, the evidence of cryptic speciation in marine invertebrates has become overwhelming (Knowlton, 2000). Although the occurrence of cryptic species is known from the first half of the XX century (Sonneborn, 1939; Mayr, 1942) and has been the subject of important terminological and theoretical debates (Dobzhansky, 1972; Steyskal, 1972), this phenomenon revealed itself more widespread and frequent than previously thought, especially in marine invertebrates (Knowlton, 2000), where species boundaries can be concealed by phenotypic plasticity, lack of morphological differentiation between reproductively isolated lineages, or high degree of morphological variation (Meyer et al., 2008; Petraccioli et al., 2010; Couceiro et al., 2012; Sanna et al., 2012). Cryptic speciation was thought to occur mainly in species with low dispersal capability, such as sessile or sedentary invertebrates, especially those with direct development or short planktonic lifespan of the larvae (Valentine & Jablonski, 1983; Palumbi, 1994). However, recent evidence showed that the occurrence of cryptic species is frequent in both low dispersal (Sponer & Roy, 2002; Iannotta et al., 2009a; Pérez-Portela et al., 2013; Amor et al., 2014) and high dispersal species (Goetze, 2003; Nikulina et al., 2007; Ladner & Palumbi, 2012; Cabezas et al., 2013). Cryptic speciation, therefore, appears a complex phenomenon, caused by a number of interacting factors, rather than exclusively related to life cycle and dispersal features. Barriers to gene flow should be considered fundamental factors that can determine population genetic divergence and, ultimately, cryptic speciation in marine invertebrates. In fact, the presence of extrinsic barriers to gene flow, represented by stretches of unsuitable habitat (Casu et al., 2006), or by fronts, unfavourable currents, and land barriers (Pannacciulli et al., 1997; Pérez-Losada et al., 2002; Sá-Pinto et al., 2012; Cabezas et al., 2013), may prevent the passive dispersal of larval stages (Palumbi, 1994). Also strong, sudden ecological breaks in marine environments could affect the connectivity between populations, since the occurrence of a strong divergent selection driven by environmental factors may lead to reproductive isolation even in absence of intrinsic barriers to gene flow, according to Rice & Hostert's (1993) divergence-with-gene-flow speciation model. This model has been proposed as a suitable explanation for speciation patterns observed in a number of marine organisms (Ferguson & Taggart, 1991; Johannesson et al., 1995;

Tatarenkov & Johannesson, 1999; Beheregaray & Sunnucks, 2001; Johanesson, 2003; Maltagliati et al., 2004; 2005). In this perspective, partially isolated environments, such as brackish-water environments (Cognetti & Maltagliati, 2000; Bilton et al., 2002; Iannotta et al., 2009a), hydrothermal vents (Peek et al., 1997; Howe, 2008), cold seeps (Peek et al., 1997; Sibuet & Olu, 1998; Kiel & Little, 2006; Calosi et al., 2013), and seamounts (de Forges et al., 2000; Shank, 2010) have been often considered evolutionary hotspots, where differentiation processes occur at a significantly higher rate if compared with other well connected marine environments.

Among invertebrate taxa, polychaete worms are interesting models for the understanding of microevolutionary processes and cryptic speciation, because of the high number of lifecycles and strategies that occur in this group, and of the absence of human impact on the genetic structure of the vast majority of species. A number of polychaete species have been reported worldwide, but, since actually cosmopolitan species are rare in macrofaunal taxa (Wilson & Hessler, 1987; Wilkinson, 2001), we should suppose that the majority of these cosmopolitan species are actually characterised by cryptic diversity and should be considered as species complexes (Bleidorn et al., 2006; Barroso et al., 2010). The occurrence of cryptic species in polychaetes has been verified with molecular techniques for 86 nominal species, belonging to 30 families (Nygren, 2014). In several cases, a fine morphological characterisation, or the re-evaluation of overlooked morphological features, revealed that genetically divergent lineages are morphologically diagnosable as well (Wu et al., 1991; Maltagliati et al., 2004; Luttikhuizen & Dekker, 2010; Nygren et al., 2010; Nygren & Pleijel, 2011). Interestingly, such kind of study has never been carried out on Paraonidae species, even though there is a general agreement about the probable state of species complex of several nominal taxa (Hartley, 1984; Gaston & McLelland, 1996; A. S. Y. Mackie, *pers. comm.*, M. E. Çinar, *pers. comm.*).

The identification of cryptic and pseudocryptic species is not only useful to understand evolutionary processes, or to update taxonomical views, but has relevant consequences for management and conservation purposes. In fact, the overlooking of cryptic species, along with taxonomic uncertainties, may lead to overestimate similarity between different geographical areas and different environments (Giangrande, 2003). A consequence of this apparently high similarity is that different environments are erroneously treated as a coherent whole, and management politics based on this conclusion turn out to be ineffective for conservation of biodiversity and natural resources (Hutchings & Ponder, 2003).

1.2 The family Paraonidae

1.2.1 Historical review and taxonomy

The family Paraonidae Cerruti 1909 is one of the most diverse polychaete families, and includes currently 134 species, belonging to seven genera: *Aricidea* (75 species), *Cirrophorus* (7), *Levinsenia* (20), *Paradoneis* (20), *Paraonella* (7), *Paraonis* (4), and *Sabidius* (1) (Blake, 2016).The monotypic genus *Aparaonis* Hartman, 1965 according to Reuscher (2013) has been described on the basis of a juvenile Opheliidae, and even if Blake (2016) still lists this species within Paraonidae, his extremely detailed redescription is consistent with Reuscher's (2013) view (Fig. 2A-B). Moreover, the enigmatic *Xandaros acanthodes* Maciolek, 1981, the only species of the genus *Xandaros* collected from hydrothermal vents in the Pacific Ocean (Maciolek, 1981) (Fig. 2C-D) probably does not belong to Spionidae, and has been considered close to Paraonidae, even if its true affinities are still uncertain (Blake & Arnofsky, 1999). The first species of this family were erroneously assigned to similar families such as Orbiniidae (Webster, 1879), Spionidae (Grube, 1872; McIntosh, 1878; Tauber, 1879; Levinsen, 1883) and Cirratulidae (Ehlers, 1908). Later, Mesnil and Caullery (1898) were the first to identify all the species described at time as belonging to a different family, to which they gave the name of "Levinseniidae". Cerruti (1909) reviewed the information available on the family, also with interesting morphological and anatomical investigations on new material from the Mediterranean Sea, and identified *Paraonis tenera* Grube, 1872 as representative of this family, stating that the correct name should be Paraonidae. Although *Paraonis tenera* is clearly a *nomen dubium*, the widespread use of this genus led finally to an application to ICZN and to the opinion 1139 (Melville, 1979), that stabilized the use of *Paraonis* Cerruti, 1909 (*non* Grube, 1872), *Levinsenia* Mesnil, 1897, and Paraonidae Cerruti, 1909 as the correct name of the family.

The only complete revision of Paraonidae was carried out by Strelzov (1973), who identified seven genera within this family (among them *Aparaonis*) and put *Paradoneis* Hartman, 1965 and *Paraonides* Cerruti, 1909 in synonymy with *Cirrophorus* Ehlers, 1908. Strelzov's (1973) synonymy between *Cirrophorus* and *Paradoneis* was not widely accepted and *Paradoneis* remained in use for the species with notopodial modified chetae and without median antenna. Also the synonymy between *Paraonides* and *Cirrophorus* and the subsequent replacement of *Paraonides sensu* Hartman, 1965 with *Paraonella* Strelzov, 1973 for species without median antenna and without modified chetae has not been widely accepted (Katzmann & Laubier, 1975). The main problem with *Paraonides* is the correct identification of the type species, namely *Paraonides neapolitana* Cerruti, 1909. In my opinion, Strelzov (1973) reasonably

demonstrated that the diagnostic character of this species, namely the presence of notopodial leaf-shaped chaetae, is a fixation artefact. Since the type specimen is currently lost (Castelli, 1987; A. Travaglini, *pers. comm.*), a positive identification of the species described by Cerruti (1909) would need topotypic material from the Gulf of Naples. Therefore, the identification of *Cirrophorus neapolitanus* given by Strelzov (1973), based on Atlantic specimens and probably referred to *Paradoneis ilvana* Castelli, 1985, cannot be confirmed (Katzmann & Laubier, 1975). However, the use of *Paraonides* stated by Hartman (1965) cannot be supported, since the type species cannot be positively identified (despite the high amount of records, mainly in environmental monitoring campaigns). In my opinion, for species without median antenna and without modified chaetae the use of *Paraonides* should be avoided and the use of *Paraonella*, whose type species is clear (*Paraonella nordica* (Strelzov, 1968)) should be preferred.

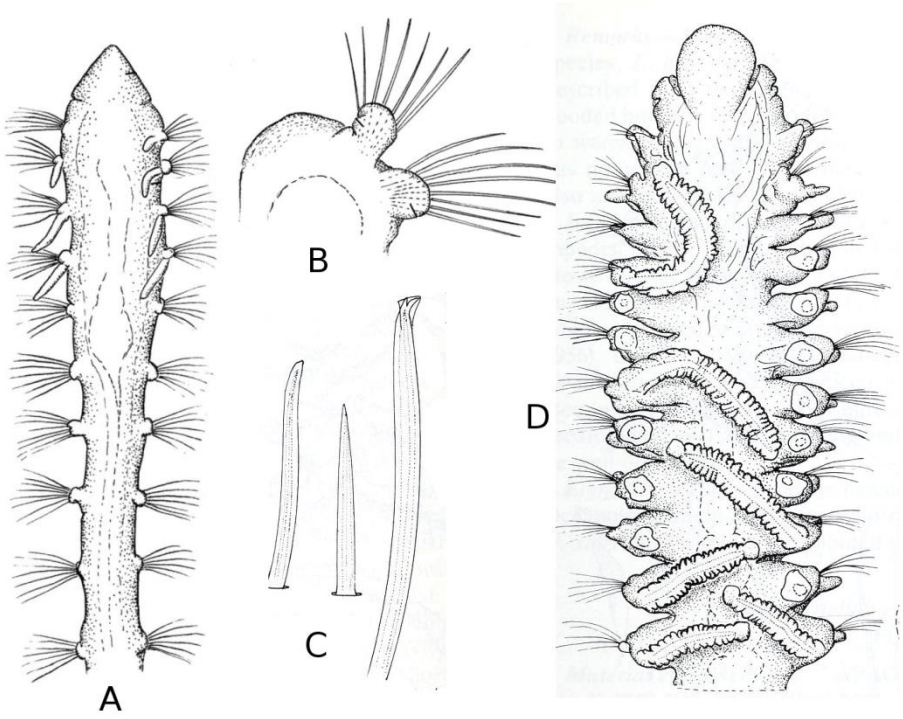

Figure 2: Problematic taxa considered close to Paraonidae. *Aparaonis abyssalis* Hartman, 1957: A) Dorsal view of the holotype; B) mid-body parapodium. *Xandaros acanthodes* Maciolek, 1981: C) Mid-body and posterior neurochaetae; D) Dorsal view of the holotype (A and B after Blake, 2016; C and D after Maciolek, 1981).

The replacement of *Levinsenia* Mesnil, 1897 with the new genus *Tauberia* Strelzov, 1973 has been lastly rejected, with relation to the ICZN opinion 1139 (Melville, 1979), which stabilized *Aonides gracilis* Tauber, 1879 as type species of *Levinsenia*.

Strelzov (1973) also split the extremely species-rich genus *Aricidea* Webster, 1879 in four subgenera based on the presence and shape of modified neuropodial chetae, namely *Aricidea*,

Acesta Strelzov, 1973, *Allia* Strelzov, 1973, *Aedicira* Hartman, 1957. Later *Acesta* was found to be homonymous of *Acesta* H. Adams & A. Adams, 1858 (Bivalvia) and replaced by *Acmira* (Hartley, 1981), and *Allia* was recognized as homonymous of *Allia* Walker, 1867 (Lepidoptera) and replaced by *Strelzovia* (Aguirrezabalaga, 2012).The current opinion of the majority of scholars is that the four subgenera are largely artificial groups and do not represent monophyletic lineages; it must be noted that Strelzov himself appeared not confident in the monophyly of the four subgenera, and often stressed the high similarity between species belonging to different subgenera. Moreover, the actual boundaries of *Aedicira* are debatable; even though several species are occasionally assigned to this subgenus, the type species *Aricidea (Aedicira) pacifica* Hartman, 1944, shows very unusual features, and, on the other hand, modified neuropodial chaetae are often poorly characterised and/or occur in the posterior part of the body in several species of the subgenus *Strelzovia*. According to Hartman (1957) and Laubier & Ramos (1974), *Aedicira* s.s. could actually represent a valid genus within Paraonidae; however, the majority of the species currently assigned to this genus are not closely related to *A. pacifica*.

Until few years before Strelzov's (1973) revision, Paraonidae were considered a relatively poor family in term of species number. The first to highlight a high diversity within a restricted area was Laubier (1967). Apart from the more complete work by Strelzov (1973), dealing also with anatomical and evolutionary aspects, all subsequent works focused on taxonomical issues and on the description of new species. The most important works on this topic were those by Imajima (1973), Laubier & Ramos (1974), Katzmann & Laubier (1975), Hartley (1981; 1984), Blake (1996), de Leon Gonzalez et al. (2006), Zhou & Li (2007), Aguirrezabalaga & Gil (2009), and Çinar et al. (2011). Giere et al. (2007) did not concentrate on Paraonidae, but attributed the two enigmatic known species of the genus *Periquesta* Brito & Nuñez, 2002, to *Levinsenia*. The correctness of such synonymy, as well as of the attribution of these species to Paraonidae, is questionable and will be discussed further (see also Reuscher, 2013). Revisions, even partial, of the family were not published after Strelzov's (1973) comprehensive work.

The first attempt to infer on Paraonidae phylogeny was made by Reuscher (2013), who set up a cladistic analysis based on morphological data only, given the difficulty of collecting genotypable specimens of species belonging to this family. Reuscher's (2013) analyses highlighted the monophyly of the family, with a dorsal anus and complete fusion of prostomium and peristomium as symplesiomorphic characters. He found the monophyly of *Aricidea* s.l. and highlighted that the two species of *Periquesta* do not belong to *Levinsenia*

due to the absence of the terminal organ on the prostomium and the presence of three instead of two anal cirri. In addition, they do not belong neither to Paraonidae, because prostomium and peristomium are not fused and parapodia lack post-chaetal notopodial lobes. He subsequently re-established *Periquesta* as a valid genus and removed it from Paraonidae. Working on type material he subsequently concentrated on the possible synonymy of *Paradoneis* and *Cirrophorus*, and found that *Cirrophorus* does consist of two different lineages arising within the larger group of *Paradoneis*; thus, *Cirrophorus* is a polyphyletic group and *Paradoneis* a paraphyletic one. The whole *Paradoneis*-*Cirrophorus* clade was found to be the sister group of the *Paraonides* (= *Paraonella*) clade, and the species of *Paradoneis* with notopodial spines instead of harpoon-like or lyrate chetae represented the sister clade of the group including the remaining species of *Paradoneis* and *Cirrophorus*. *Paradoneis* was therefore synonymised with *Cirrophorus*, and *Paradoneis* spp. with notopodial spines belong to a genus yet to be described (M. Reuscher *pers. comm.*). The relationships among the remaining genera of Paraonidae remain still unclear; Reuscher (2013) remarked that the really simple anatomy and the low number of useful characters in Paraonidae make morphology-based phylogenetic inference poorly reliable and demanded further analyses to ultrastructural and molecular characters. In addition, several features traditionally considered highly informative could actually be misleading: Reuscher (2013) pointed out that the median antenna, traditionally considered a relevant character in higher rank taxonomy of Paraonidae, evolved independently at least twice within the genus *Cirrophorus sensu* Reuscher (2013). The same problem could also occur for other morphological characters that have been considered highly informative, and should actually be tested by means of molecular markers.

1.2.2 Morphological features and possible phylogenetic relationships

Paraonidae are medium size polychaetes, with length varying from 2-3 mm up to 30-40 (100) mm and width between 0.1 and 2 mm. The cephalic region is composed by a prostomium and a buccal segment or peristomium, that in this family is always reduced, fused in a certain way to the prostomium and does not form a peristomial, achaetous ring. The presence of a peristomial ring in the drawings referred to some Paraonidae (Webster & Benedict, 1887; Monro, 1930; Castelli, 1985) is probably a fixation artefact, since the fusion of prostomium and peristomium is regarded as a symplesiomorphic feature for this family (Reuscher, 2013). The prostomium is very simple, devoid of palps and triangle- to trapezoid- shaped. Eyes, if present, are simple, ventrally placed eyespots (Fig. 3) and their presence can depend on the

growth stage of the animal. In some species of the genera *Paradoneis* and *Cirrophorus* eyes are frequent in juveniles and lack in adults, whereas in other species they are present also in adults, or lack in any growth stage. On the posterior part of the prostomium, two paired, often very conspicuous nuchal organs are noticeable. The majority of species shows an apical, unpaired and retractile sensorial button. The antenna, when present (in *Aricidea* s.l. and *Cirrophorus*), is inserted in the central area of the prostomium (Fig. 3); its shape and length are highly variable and in *Cirrophorus* it can be absent in juveniles. In several species the presence of a ring of ciliate cells has been observed on the prostomium (Cerruti, 1909; Jones, 1968; Strelzov, 1973). This structure has probably a sensorial function and is very difficult or impossible to observe in preserved material. The mouth opening is ventral and surrounded by four lips, not always simple to notice. The pharynx is simple, muscular, poorly developed and devoid of hard structures.

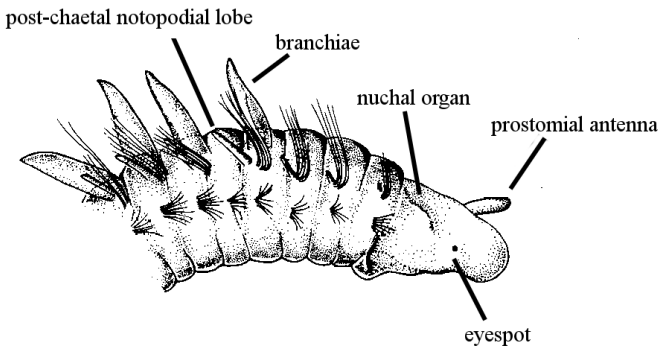

Figure 3: Anterior part of a Paraonidae, with the main morphological features highlighted (after Laubier, 1967)

The body is elongate, composed by numerous segments and can be divided in pre-branchial, branchial and post-branchial regions. Although the segments show certain characters of heteronomy, such as the presence/absence of modified chaetae and branchiae, or the presence of notopodial post-chaetal lobes (Fig. 3) with different lengths, in this family the identification of well-defined thoracic and abdominal regions is impossible. From this point of view, among the families traditionally referred to Sedentaria (Fauvel, 1923), Paraonidae show similarities with Orbiniidae, Spionidae, Apistobranchidae and Poecilochaetidae (Strelzov, 1973). Branchiae (Fig. 3) are present in the majority of described species, lacking only in few abyssal *Aricidea, Paradoneis*, *Paraonella* and *Levinsenia*; their position, number, and shape are considered taxonomically relevant characters. Branchiae are typically simple, unbranched processes more or less elongate with acute tip (sometimes tapering). Typically, branchiae in Paraonidae are ciliated and often reddish in the live animal due to the presence of

respiratory pigments. The number of branchiae typically varies with the size of the animal, whereas the number of pre-branchial chaetigers is constant in the majority of species, changing with the animal size only in a few species (Strelzov, 1968).

Parapodia are always biramous, typically the notopodium has longer and slender capillary chaetae, whereas the neuropodium has shorter and thicker chaetae. Notopodial post-chaetal lobes are typically well developed, and can be short, oval, or long and slender; in some species their length dramatically changes from the branchial to the post-branchial region, whereas in other species they remain approximately of the same length. In the post-branchial region, the number of chaetae rapidly decreases and segments become more elongate. In the genera *Cirrophorus* and *Paradoneis* modified chaetae are dorsal, and are typically lyrate, or derived from a modification of lyrate chaetae. In *Paradoneis spinifera* (Hobson, 1972) and *Paradoneis drachi* Laubier & Ramos, 1974, dorsal modified chaetae are thick spines, without the trace of a ramification. According to Reuscher (2013; *pers. comm.*) these species should be assigned to a new genus. Dorsal modified chaetae typically start from the middle of the branchial region, or even from the pre-branchial region. In some species (e.g. *Cirrophorus branchiatus*, *Paradoneis ilvana*, *Paradoneis armata*) the shape of modified chaetae changes along the body, with posterior chaetae thicker than the anterior ones, whereas in other species (e.g. *Paradoneis lyra*, *Cirrophorus furcatus*) modified chaetae remain approximately of the same size and shape for the whole body length. Ventral modified chaetae are, however, the most common in Paraonidae and are present in the genera *Aricidea* (subgenera *Acmira*, *Aricidea*, and *Strelzovia*), *Levinsenia*, and *Paraonis*; moreover, in some *Paradoneis* and *Cirrophorus* species posterior segments have neuropodial spines. In *Acmira*, *Levinsenia* and *Paraonis* ventral modified chaetae are hook-shaped, short and thick, with (*Acmira partim*, *Paraonis*) or without (*Acmira partim*, *Levinsenia*) a significantly thinner terminal arista that can be easily damaged and go unnoticed (Castelli, 1985). In *Aricidea* s.s. modified chaetae are typically pseudo-articulate, with a subterminal arista inserted ventrally or dorsally, but leaving a conspicuous subdistal notch on the main ramus of the chaeta. Lastly, in *Strelzovia* modified chaetae are shorter and thicker capillaries, often with a terminal slender extension that can be similar to an arista, but thicker in relation to the rest of the chaeta (Fig. 4).

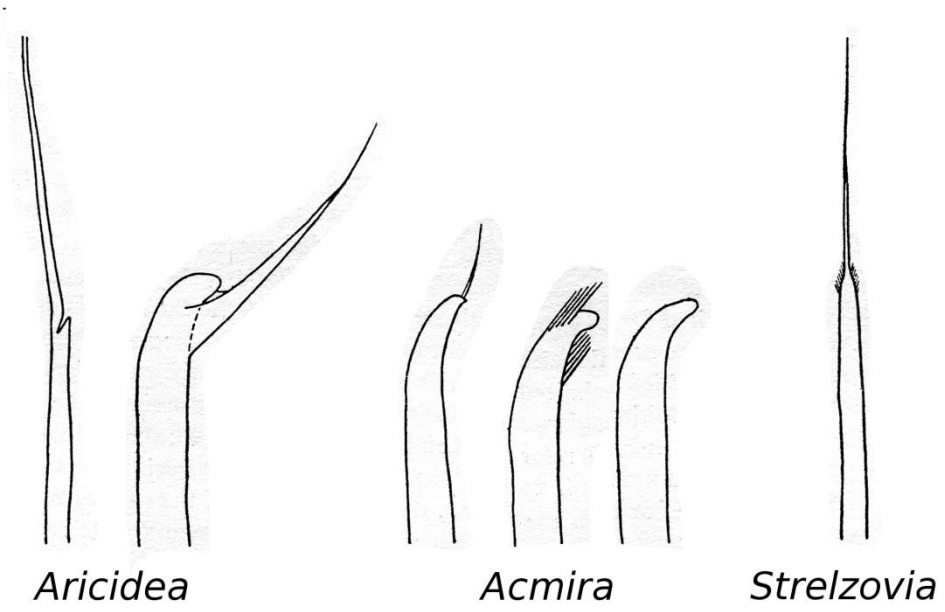

Figure 4: Modified chaetae occurring in the three subgenera of *Aricidea* Webster, 1879 (after Strelzov, 1973)

Paraonidae without modified chaetae are attributed to *Paraonella* (species without median antenna) and *Aedicira* (species with median antenna). The representatives of *Paraonella* are considered very similar to *Paradoneis* (Reuscher, 2013) and the possibility that the lacking of modified chaetae is not taxonomically informative cannot be excluded. Since "the absence of evidence is not evidence of the absence" (Altman & Bland, 1995), the secondary loss of modified dorsal chaetae could be a hypothesis for features that are peculiar to *Paraonella* spp. as reasonable as its identification as a basal lineage in the *Cirrophorus/Paradoneis* group (Reuscher, 2013). In a general way, the secondary loss of structures is a process that can lead to serious mistakes in the reconstruction of phylogenetic relationships. Great care must be taken, therefore, when considering organisms lacking modified structures as basal lineages (Jenner, 2004). The systematic position of the subgenus *Aedicira* seems even more unclear. The genus *Aedicira* was erected for *Aricidea pacifica*, a species that shows peculiar features in the shape of the prostomium and was used for several species, the majority of which has been lastly attributed to *Strelzovia*. Also in this case, the absence of modified ventral chaetae is a character whose informativeness is questionable.

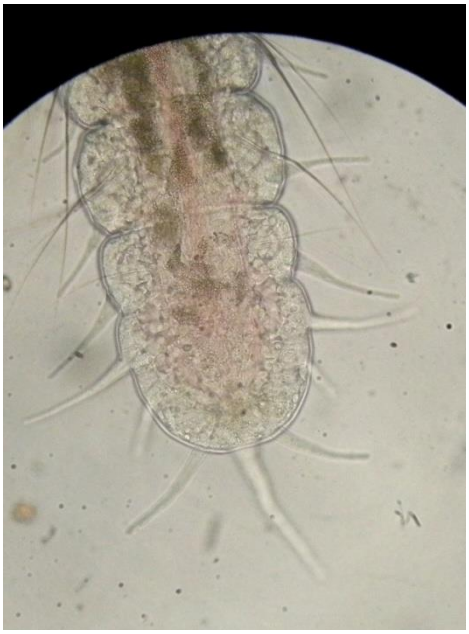

Figure 5: Pygidium of juvenile *Paradoneis* cf. *ilvana* showing four additional anal cirri, actually corresponding to the notopodial lobes of the last two chaetigers, still developing.

The anal region is not known in all species, but it does not seem to vary in a relevant way. It is characterised by an anal lobe obliquely slanted dorsally, with two (*Levinsenia*) or three (other genera) anal cirri. In juvenile specimens it is easy to confuse the notopodial post-chaetal lobes of the last, developing chaetigers with anal cirri (Fig. 5). Some species, as *Paraonis pygoenigmatica* Jones, 1968, show several additional anal cirri that may represent the trace of a fusion between the anal segment and the last two-three chaetigers. The anal opening is always located dorsally (Strelzov, 1973; Reuscher, 2013). Reports of a ventral opening of the anus (e.g. Katzmann & Laubier, 1975) are typically due to mistakes (Reuscher, 2013).

Based on their morphological features, Paraonidae have been considered similar to some families belonging to a well characterised group of sedentary polychaetes, the Scolecida (Rouse & Fauchald, 1997). The monophyly of Scolecida, however, has not been supported by subsequent molecular studies (Struck et al., 2007). Among Scolecida, candidate sister families to Paraonidae are Orbiniidae, Cossuridae, and Opheliidae. Orbiniidae have been often considered the group closest to Paraonidae; however, this group is characterised by the presence of aciculae (absent in all other Scolecida) and, although the first molecular phylogeny of this family confirmed their belonging to Scolecida (Bleidorn, 2005), further molecular analyses suggested that actually, Orbiniidae could be closer to Errantia (Struck et al., 2007). Other morphological differences between Paraonidae and Orbiniidae refer to the peristomium (fused to the prostomium in Paraonidae, separated and forming peristomial ring(s) in Orbiniidae), the presence of rows of thoracic hooks in Orbiniidae, the beginning of the branchiae in the posterior region (branchiae are limited to some anterior segments in Paraonidae). Cossuridae do not have modified chaetae and have a distinct peristomial ring, and the branchial structure is single, unpaired and filamentous, quite different from those of Paraonidae and probably not homologous. Lastly, Opheliidae typically have a short body, composed by a stable number of segments, thin branchiae typically located along the central and posterior part of the body, and lack modified chaetae. An older, less widespread view considered Paraonidae as close to some group referred today to Spionida, such as Apistobranchidae, Spionidae, and Magelonidae (Hartman, 1957; Strelzov, 1973). All these families are characterised by the presence of paired palps (which are completely absent in Paraonidae), and multidentate, hooded ventral hooks that most likely are not homologous with the ventral modified chaetae of Paraonidae. Nonetheless, this hypothesis is provisionally retained by some scholars (V. Radashevsky *pers. comm.*).

Molecular data about the phylogenetic relationships of Paraonidae are still scarce. The position of *Cirrophorus lyra* in the first complete molecular phylogeny of Annelida by Rousset et al. (2006) is ambiguous, whereas Bleidorn (2005) confirmed that Paraonidae are closely related to Opheliidae, Scalibregmatidae and Cossuridae, further suggesting that the morphologically divergent Sternaspidae may belong to Paraonidae.

1.2.3 Ecological features

Paraonidae are strictly related to soft bottoms, even though sometimes stray specimens are reported from hard bottoms with a high degree of sedimentation (Martín, 1987) and some species show a strict association with seagrass and *Caulerpa* beds (Brito et al., 2005; Box et al., 2010; Çinar & Dağli, 2013). The species belonging to this family are typically marine and stenohaline, and only few have been reported from estuary environments and coastal lagoons. Brackish-water Paraonidae, on the other hand, show low tolerance towards wide variations of salinity and in most cases they can be found in marine environments with organic pollution impact, as well as in enclosed environments with narrow salinity variations (Arriaga-Hernandez et al., 2013; see also Chapter 1 below). Brackish-water Paraonidae, therefore, can be considered marine species with high tolerance towards eutrophic conditions.

Approximately the half of the currently known Paraonidae species is related to deep environments (up to 6000 m depth); according to Strelzov (1973), with the increase of depth, the number of species remains virtually constant, while the number of genera and subgenera decreases. *Paraonis*, *Aricidea s.s.* and *Aedicira* seem related to shallow environments, whereas *Acmira*, *Strelzovia*, *Cirrophorus*, *Paradoneis* and *Levinsenia* show a high diversity also in deep environments. In shallow environments Paraonidae are present in gravel, coarse sand, fine sand and silt; typically shallow environments show a low Paraonidae diversity, with few syntopic species, of which one is often dominant (Castelli, 1985; *pers. obs.*). Deeper environments are characterised by the prevalence of silt and clay in the sediments and show a higher species diversity, even though the majority of them is composed by "rare species" (*pers. obs.*). Several species have been considered highly eurybathic; for instance, *Cirrophorus branchiatus* Ehlers, 1908 has been reported from 8 to 2700 m; *Aricidea (Acmira) simonae* Laubier & Ramos, 1974 from 8 to 1000 m; *Paradoneis armata* Glémarec, 1966 from 1 to 1200. Other species are more strictly related to the bathyal and abyssal stages (Aguirrezabalaga & Gil, 2009). Extremely wide bathymetric ranges are not uncommon in polychaetes, but this feature could actually be an artefact due to incorrect identification, or absence of reliable morphological features, because the probability of the adaptation to such a

wide range of environmental conditions is very low (Brown & Thatje, 2014). As already observed in several groups of polychaetes (Nygren, 2014), the occurrence of the same species in very different habitats could suggest the existence of different cryptic species, more than an extreme adaptability of a single species. The same hypothesis can account also for the high number of species with assumed cosmopolitanism, or very wide geographical distribution.

Paraonidae live on the surface of soft bottoms, or in their uppermost layer. Many species build temporary mucous tubes, covered by soil particles, that are difficult to observe due to their brittleness. Traces (ichnofossils) of burrows that are very similar to those described by Röder (1971) for *Paraonis fulgens* have been observed in Cretaceous sediments (Hänztschel, 1975), however there is scarce evidence that such burrows actually belong to Paraonidae. The living animals have a characteristic "corkscrew" position (Fig. 3), with the middle and posterior parts of the body folded forming several spiral circles and only the anterior, branchial region of the body more or less straight. *Levinsenia* spp. rarely show a regular "corkscrew" folding, but the posterior part of the body is irregularly tangled, often around vegetal fibres that are common in circalittoral silts and offer protection to the animal (Fig. 3). Coarse bottom species, such as *Aricidea (Acmira) cerrutii* Laubier, 1966 and *Paradoneis ilvana*, are less sedentary and seem not to build tubes. This group includes the few known pedomorphic species, in which even mature animals show features typical of juveniles, such as a pygidium with supernumerary anal cirri and well developed cilia on the prostomium. These features can be interpreted as adaptations to interstitial environment (McLelland & Gaston, 1994). Paraonidae typically feed on microbenthos and microbial films on the surface layer of the soil without moving from their tube. The few intestine content analyses available show that diatoms and foraminiferans are among the most common food items (Strelzov, 1973). In most cases, an active selection of food items has been observed, rather than the indiscriminate ingestion of sediment (Röder, 1971; Gaston et al., 1992). Although the majority of works about the identification of feeding guilds in macrobenthic communities list Paraonidae as sub-surface deposit feeders (Jumars et al., 2015) and the occurrence of some species in deeper sediment layers supports this view, at least in some cases (Castelli, *unp.*), the few specific studies carried out on feeding habits in this group clearly identify them as surface deposit feeders, and probably this category includes the majority of species.

Figure 6: Live Paraonidae from the Mediterranean Sea. A) *Aricidea catherinae* Laubier, 1967, Cinquale, Tyrrhenian Sea, 19 m. B) *Levinsenia kosswigi* Çinar, Açik & Dağli, 2011, Tuscan Archipelago, Tyrrhenian Sea, 110 m. C) *Paradoneis armata* Glémarec, 1966, Cinquale, Tyrrhenian Sea, 9.5 m. D) *Cirrophorus branchiatus* Ehlers, 1908, Tuscan Archipelago, Tyrrhenian Sea, 110 m. E) *Levinsenia demiri* Çinar, Açik & Dağli, 2011, Tuscan Archipelago, Tyrrhenian Sea, 110 m. F) *Cirrophorus* sp. A, Porto Pozzo, Tyrrhenian Sea, 0.8 m.

The reproduction of Paraonidae is poorly known. Fewkes (1883) identified planktonic polychaete larvae similar to Spionidae but without trace of palps as *Aricidea* sp.; however, this identification was later questioned (Thorson, 1946). Successively Bhaud (1983) described larval stages of polychaetes he assigned to Paraonidae. However, the large size and high number of segments of Bhaud's (1983) specimens do not seem very compatible to the known juvenile stages of Paraonidae (typically, with few number of segments and really small size) (Cerruti, 1909). Also the observed chaetae did not match typical Paraonidae chaetae. The absence of reliable reports of planktonic larvae, as well as the large size of the eggs, that implies a high amount of food reserves, and the presence of epitoke forms in some species (Strelzov, 1973; López-Jamar et al., 1987), support the hypothesis of direct development (Hartman, 1957; Strelzov, 1973; Giangrande, 1997) or really short planktonic lifespan. However, according to Rouse & Pleijel (2001), currently available data do not allow to clarify this issue. The occurrence of epitoke forms is known for a few species, namely *Paraonis fulgens* (Levinsen, 1884), *Paradoneis lyra* (Southern, 1914), *Aricidea (Strelzovia) claudiae* Laubier, 1967 (Strelzov, 1973), and *Paradoneis armata* Glémarec, 1966 (López-Jamar et al., 1987). There are no data about the occurrence of epitoky in other species of this family and, unlike several other polychaete families, such as Syllidae and Nereididae, epitoke forms are quite difficult to observe. Strelzov (1973) suggested that only few species undergo morphological reproductive modifications. However, the majority of Paraonidae are poorly known and there is the possibility that epitoke stages are present in the majority of species, even though they have not been observed. For instance, some features of the recently described *Aricidea (Strelzovia) longisetosa* and *Paradoneis hirsuta* (Sardá et al., 2009), as well as the elongate chaetae and branchial tips of *Aricidea* sp. A (see the "Species analysed" section) could refer to epitoke specimens, rather than to the atoke, vegetative form.

1.2.4 Distribution and biogeography

Paraonidae are known from all the oceans of the globe and also for inner seas with low salinity such as the Baltic Sea and the Black Sea. Members of this family are known from both the Arctic and Antarctic, where they show a relatively high diversity (Strelzov, 1973). As suggested by Strelzov himself, the absence of this family in several biogeographical areas, such as the Central Eastern Atlantic, the Central Pacific and the Indian Ocean, is due to the lack of relevant sampling campaigns, than to an actual scarcity of Paraonidae in these areas. Since more than half of currently known Paraonidae have been described after Strelzov's revision, his considerations about Paraonidae biogeography and distribution cannot be

considered valid. Several species belonging to this family have been reported from extremely wide geographical ranges, also with a really wide bathymetric and ecological occurrence. Some species have an alleged cosmopolitan distribution, whereas other species show in Strelzov's opinion a bipolar distribution, that however could be an artefact due to the scarce knowledge about tropical and equatorial Paraonidae diversity. Other species seem restricted to particular geographical areas: *Aricidea trilobata sensu* Laubier & Ramos, 1974 and *Aricidea (Strelzovia) pseudannae* Katzmann & Laubier, 1975 are known until now only for the Mediterranean Sea, and the majority of Atlantic-Mediterranean Paraonidae are restricted to the Mediterranean Sea and the North-Eastern Atlantic Ocean (the so-called Lusitanian biogeographical province). Since the Lusitanian province is one of the best known in terms of species diversity, a preliminary biogeographical analysis of the Paraonidae reported from the area could be useful. Currently, 49 species of Paraonidae have been reported for the Lusitanian province (Laubier & Ramos, 1974; Katzmann & Laubier, 1975; Çinar et al., 2011; Aguirrezabalaga, 2012; Çinar & Dağli, 2013). Among them, 13 species (26.5%) have an alleged "cosmopolitan" distribution; namely, they have been reported at least from both the Atlantic and the Pacific Oceans; 13 (26.5%) have been reported from both the Atlantic Ocean and the Mediterranean Sea; 9 (18.4%) are known only for the Atlantic Ocean; and 14 (28.6%) are known only for the Mediterranean Sea. Even if several of the recently described Mediterranean species are likely to be reported also from the eastern Atlantic Ocean, these data show that only a minor part of Paraonidae reported from the Lusitanian region has a wide cosmopolitan distribution. The majority of them, instead, has a narrower distribution, that is consistent with current biogeographical theories (Oliver & Irwin, 2008; Almada et al., 2013; Watling et al., 2013). Moreover, some of the species with alleged cosmopolitan distribution, such as *Aricidea (Acmira) catherinae*, *Aricidea (Acmira) cerrutii*, and *Levinsenia gracilis*, are commonly considered probable species complex (Aguirrezabalaga, 2012; A. S. Y. Mackie, *in litt.*; M. E. Çinar, *in litt.*) and the actual species' distributions are probably significantly narrower than currently thought. Reasonably, a more accurate analysis of Paraonidae diversity in other biogeographical provinces will show a similar trend, with few true cosmopolitan species, and a number of species with a narrower geographical distribution. The simple anatomy of Paraonidae and the uncertain taxonomy of this group make this kind of analysis difficult and ultimately unreliable. Molecular data will represent a precious tool to understand which characters can be used for taxonomical purposes, and which are the actual biogeographical boundaries that impinge on species' distribution.

1.3 Aims of the work

Within polychaete worms, Paraonidae are among the less known families; actually, we have really few data about their ecology, reproduction, biogeography, and evolutionary history. As regards the taxonomy of this group, even if there is a well-established taxonomic scheme, based on some morphological features, the reliability of such a scheme is far to be proved. After Strelzov's (1973) revision, the majority of the aspects regarding the biology and ecology of Paraonidae have been only fragmentarily addressed; as a consequence, in the last forty years the state of the knowledge regarding this family did not show significant improvements. Molecular markers will provide an important contribution in order to disentangle many ecological and biological problems.

As previously highlighted, about the half of known Paraonidae species is strictly deep-water related, whereas the remaining half occurs also in shallow waters. It would be interesting to investigate whether Paraonidae are primarily deep-water polychaetes that secondarily adapted to shallow environments, or instead the colonisation of deep environments happened only secondarily. The higher occurrence of deep-water forms in respect to other polychaete families seems to support the first view, but a molecular phylogeny, based on sequence data, could provide an important contribution to clarify this issue, to understand which is the ancestral ecological state and which is the later adaptation to different environments (Valdés, 2004; Hundt et al., 2014).

Molecular data would also help to infer on reproductive features in Paraonidae. As Strelzov (1973) pointed out, there are really few data about Paraonidae reproduction; the occurrence of pelagic larvae is sporadic, but there is no clear evidence of direct development in members of this family. Also the occurrence of epitoke spawning forms is known only for some species, and by anecdotic evidence (Strelzov, 1973; López-Jamar et al., 1987). Phylogeographical studies on this family could help to estimate the dispersal range by estimating gene flow in relation to geographical distance and thus support different hypotheses about reproductive dispersal phases in this family. As previously outlined, the effects of biogeographical boundaries and barriers on Paraonidae dispersal and evolution is still poorly understood. The majority of the species traditionally considered cosmopolitan probably consists of species complexes. Molecular data could help to clarify the status of several groups within Paraonidae and to infer on the relevance of geographical features on their diversification.

Lastly, molecular data may help to better understand the evolutionary history and the actual diversity of Paraonidae. Currently Paraonidae include more than 130 species belonging to six genera; moreover, the genus *Aricidea* is composed by four subgenera. Within Paraonidae

Reuscher (2013) identified five well characterised and probably monophyletic lineages, namely *Paraonis*, *Levinsenia*, *Aricidea s.l.*, *Sabidius* and *Paradoneis – Cirrophorus – Paraonella*. Molecular data may help to understand whether these genera, as well as the four subgenera of *Aricidea*, are monophyletic and, in a more general way, the evolutionary relationships within Paraonidae.

On the other hand, the comparison of DNA sequences of different morphospecies can help to understand which morphological characters are actually useful for Paraonidae taxonomy, and which should be discarded. The understanding of the degree of reliability of morphological characters is particularly relevant, because Paraonidae have a really simple external anatomy and only few morphological features can be taken into account. Moreover, several of them refer to soft parts, that can be damaged by sampling techniques, or strongly altered by the fixation, which led to severe mistakes, in particular in historical descriptions (Strelzov, 1973; Reuscher, 2013). Therefore, I expect that molecular data would give also relevant cues about which morphological characters are useful for Paraonidae taxonomy and which are not.

2. Species analysed

2.1 Collection of material

Paraonidae were collected by diving, with a Van Veen grab, or with a box-corer from different environments in many localities covering a large part of the Mediterranean Sea. When possible, specimens were examined alive, subsequently fixed in 96% ethanol and preserved at 4 °C until DNA extraction. Additional material came from ARPAT (Regional Agency for Environmental Protection of Tuscany) environmental monitoring campaigns, and several samples were obtained thank to the kind collaboration of colleagues. In particular, deep-water Paraonidae from the Pacific Ocean were loaned by the Scripps Institution of Oceanography, where they were returned after morphological characterisation. In some instances a small part of the individuals was excised and used for molecular analyses. A part of the material from the north-eastern Atlantic Ocean came from the collection of the National Museum of Wales.

Despite the recent flowering of molecular studies, the vast majority of polychaete samples are still treated with formalin, and the collection of genotypable material is challenging, in particular for small, soft-bottom species. Therefore, this work would not have been possible without the support of a number of colleagues that helped me in obtaining samples from all over the world (see Acknowledgements section).

2.2 Taxonomic and ecological issues of examined species

The present work allowed the examination of a high number of Paraonidae (approximately 1150 individuals belonging to 44 nominal species, 39 of which are available for molecular studies). Since the taxonomy of Paraonidae is still uncertain and quite complex, and only three species will be the subject of specific chapters (see Chapters 1, 2, 3), I considered worthwhile to include a section with nomenclatural and ecological notes on the examined species. This section will eventually represent the basis of a more comprehensive critical revision of the family Paraonidae in the Mediterranean Sea. Each sub-section will be dedicated to a single species, and will be structured in the following way.

Material: Examined material, divided in material potentially useful for both morphology and genetics (*Genetics and morphology*) and material fixed with formalin, that can be used to support results obtained with molecular techniques (*Only morphology*). Not all the material listed in the *Genetics and morphology* section has been used in subsequent studies, often because not all specimens gave technically satisfying results.

Description: Either a short description, with drawings, or the main references where the species is described.

Taxonomic notes: Nomenclatural issues or uncertainties regarding the species.

Ecological features: Ecological features based on available literature.

Distribution: Distribution reconstructed on the basis of available literature.

Aricidea (Acmira) assimilis Tebble, 1959

= *Aricidea (Acmira) mutabilis* Laubier & Ramos, 1974

= ?*Aricidea (Acmira) fauveli* Hartman, 1957 *sensu* Bellan (1965)

= *Aricidea (Aricidea) fragilis* Webster 1879 *sensu* Amoureux (1970)

= *Aricidea (Acmira) lopezi* Berkeley & Berkeley, 1956 *sensu* Strelzov (1973) *partim*

Material

Genetics and morphology: Bari, Adriatic Sea, 75 m (03/2015); Cala di Forno, Tyrrhenian Sea, 7 m (06/2014); Cinquale, Tyrrhenian Sea, 19 m (05/2015); Lavagna, Ligurian Sea, 20 m (06/2014); Marina di Ravenna, Adriatic Sea, 8,5 m (09/2014); Ombrone River Mouth, Tyrrhenian Sea, 7 m (06/2014); Rosignano, Tyrrhenian Sea, 7 m (06/2014); Sea of Marmara, 200 m (04/2014); Strait of Otranto, Adriatic Sea, 120 m (03/2015); Tuscan Archipelago, Tyrrhenian Sea, 110 m (12/2015).

Only morphology: Arno River Mouth, Tyrrhenian Sea, 8-20 m (06/1985); Castiglione della Pescaia, Tyrrhenian Sea, 10 m (07/1984); Cyprus, Levant Sea, 10 m (07/2014); Litorale Ravennate, Adriatic Sea, 15 m (07/1987); Porto Pozzo, Tyrrhenian Sea, 1 m (07/1987); Taranto Gulf, Ionian Sea, 20 m (1983-84); Trieste, Adriatic Sea, 15 m (10/1995); Tuscan Archipelago, Tyrrhenian Sea, 20-110 m (08/1985; 03/2015).

Description: See Katzmann & Laubier (1975) and Aguirrezabalaga (2012).

Taxonomic notes: This species occurs in two different forms: a long-antenna form, corresponding to the original description by Tebble (1959) and a short-antenna form. The latter form was reported by Bellan (1965) as *Aricidea fauveli* Hartman, 1957 (now considered a junior synonym of *Aricidea lopezi* Berkeley & Berkeley, 1956), and by Strelzov (1973) as *A. lopezi*. Later, Laubier & Ramos (1974) stressed the high similarity between the two forms, considering them within the same species, that was redescribed as *Aricidea mutabilis*. After the examination of the type material of *Aricidea assimilis*, Katzmann & Laubier (1975) stated the synonymy between *Aricidea assimilis*, *A. mutabilis*, *A. fauveli sensu* Bellan (1965), and *A. lopezi sensu* Strelzov (1973).

Ecological features: On muddy or mixed bottoms, common at 10-60 m, rarely from 1 to 150 m depth. Often abundant, sometimes syntopic with *Acmira catherinae* (e.g. Porto Pozzo, Rosignano).

Distribution: Mediterranean Sea (Laubier & Ramos, 1974; Katzmann & Laubier, 1975; Castelli, 1987; Çinar, 2005); Atlantic Ocean (Strelzov, 1973); the Pacific Ocean records (Strelzov, 1973; Hobson, 1976; Lovell, 2002) should probably be referred to different species (Blake, 1996).

Aricidea (Acmira) catherinae Laubier, 1967

= ?*Aricidea (Acmira) elongata* Imajima, 1973

= ?*Aricidea (Acmira) eximia* Imajima, 1973

Material

Genetics and morphology: Albegna River Mouth, Tyrrhenian Sea, 10 m (06/2014); Beals, Maine, Western Atlantic Ocean, 10 m (05/2016); Belfast Lough, Irish Sea, 24 m (09/2008); Cala di Forno, Tyrrhenian Sea, 7 m (06/2014); Capraia Island, Tyrrhenian Sea, 7 m (06/2014); Cinquale, Tyrrhenian Sea, 19 m (07/2015); Galicia, Eastern Atlantic Ocean, 30 m (04/2016); Rosignano, Tyrrhenian Sea, 7 m (06/2014); Sea of Marmara, 25 m (04/2014).

Only morphology: Asinara Gulf, Sea of Sardinia, 15 m (02/2000); Calich Pond, Sea of Sardinia, 1 m (07/1987); Casaraccio Pond, Sea of Sardinia, 1 m (03/1994; 12/1994); Cyprus, Levant Sea, 10 m (07/2014); Gulf of Follonica, Tyrrhenian Sea, 8 m (12/1987); Litorale Ravennate, Adriatic Sea, 15 m (07/1987); Porto Pozzo, Tyrrhenian Sea, 1-14 m (07/1987; 10/1987); Sa Mesa Longa, Sea of Sardinia, 3 m (03/2011); Strait of Messina, 25-99 m (07/1992); Trieste, Adriatic Sea, 15 m (10/1995).

Description: See Laubier (1967).

Taxonomic notes: Type species of *Acmira* Hartley, 1981 (replacement for *Acesta* Strelzov, 1973). The synonymy between this species, *A. elongata* and *A. eximia* suggested by Lovell (2002) appears questionable on both morphological and biogeographical basis.

Ecological features: A species with wide ecological requirements; it lives on both gravel/coarse sand (frequent but not very common) and fine sand, up to mud or mixed bottoms. Typically from 5 to 20 m depth; in enclosed and brackish-water environments, also in shallower (1-3 m), more rarely deeper (up to 100 m) bottoms. Reports from the bathyal stage (Carpine, 1970) probably refer to different species. On mixed bottoms it is sometimes syntopic with *Aricidea assimilis*.

Distribution: Allegedly cosmopolitan; reported from the Mediterranean Sea (Laubier, 1967; Laubier & Ramos, 1974; Katzmann & Laubier, 1975; Castelli, 1985; Castelli, 1987; Çinar, 2005), Atlantic Ocean (Hartley, 1981; Aguirrezabalaga, 2012; Ravara & Moreira, 2013), Pacific Ocean (Blake, 1996; Lovell, 2002); it could actually represent a species complex (A. S. Y. Mackie, *in litt.*).

Aricidea (Acmira) cerrutii Laubier, 1966

= *Aricidea jeffreysii* (McIntosh, 1879) *sensu* Auctt.

= ?*Paraonis paucibranchiata* Cerruti, 1909

Material

Genetics and morphology: Anglesey, Irish Sea, 105 m (09/2008); Capraia Island, Tyrrhenian Sea, 14 m (05/2014); Elba Island, Tyrrhenian Sea, 10 m (06/2014); L'Estartit, Balearic Sea, 6 m (07/2014); Palmaiola Island, Tyrrhenian Sea, 10 m (08/2014); Pianosa Island, Tyrrhenian Sea, 0.8 m (04/2015); Porto Pozzo, Tyrrhenian Sea, 0.5 m (07/2015).

Only morphology: Calich Pond, Sea of Sardinia, 1 m (03/1988; 03/1995); Casaraccio Pond, Sea of Sardinia, 0.5-1 m (03/1994; 10/1994; 12/1994); Elba Island, Tyrrhenian Sea, 5-10 m (10/1982; 10/1983; 05/1990); Giglio Island, Tyrrhenian Sea, 8 m (08/2012); Golfo Aranci, Tyrrhenian Sea, 5 m (12/1997); Magra River Mouth, Ligurian Sea, 0 m (1988); Porto Pozzo, Tyrrhenian Sea, 1-4 m (06/1987; 07/1987; 10/1987); Rosignano Solvay, Tyrrhenian Sea, 8 m (07/1984); Sa Mesa Longa, Sea of Sardinia, 3 m (03/2011); Strait of Messina, 15-99 m (07/1992).

Description: See Cerruti (1909 – as *A. jeffreysii*) and Laubier (1967).

Taxonomic notes: This species has been reported extensively in the Mediterranean Sea as *A. jeffreysii* (currently considered a *nomen dubium* – see Strelzov, 1973) until Laubier's (1966a) re-description with a new name. *Paraonis paucibranchiata* Cerruti, 1909 is clearly a juvenile form, which probably should be referred to this species.

Deep Atlantic records have been referred to *Aricidea cerrutii pacifica* Imajima, 1973 (Aguirrezabalaga, 2012). However, the use of subspecific rank in marine invertebrate taxonomy, though commonly used in Paraonidae (Imajima, 1973; Laubier & Ramos, 1974), should be avoided (Winston, 1999). The two forms appear different enough in morphology and ecology to be treated as different species. Moreover, the identification of Atlantic specimens with *A. cerrutii pacifica* is questionable.

Ecological features: Common, shallow species, typically occurs on coarse bottoms from 1 to 15 m depth (rarely deeper in peculiar environments, such as the Strait of Messina). Circalittoral records in the Atlantic Ocean probably refer to a different species.

Distribution: Mediterranean Sea (Cerruti, 1909; Laubier, 1966a; 1967; Laubier & Ramos, 1974; Katzmann & Laubier, 1975; Castelli, 1985; 1987; Çinar, 2005); Atlantic Ocean (Hartley, 1981; Ravara & Moreira, 2013).

Aricidea (Acmira) elongata Imajima, 1973 (Fig. 7A-C)

= ?*Aricidea (Acmira) eximia* Imajima, 1973

Material

Genetics and morphology: Jinhae Bay, Korea, Pacific Ocean, tide level (05/2016)

Description: See Imajima (1973). The available individual corresponds to the original description by Imajima (1973), even though it is smaller than the type material. For this reason it has fewer branchiae and modified chaetae start from the 15th chaetiger. Moreover, the posterior-most branchiae are not very elongated. The peculiar shape of the branchiae in the type series, however, could be due to epitoke modifications. The presence of eyes has not been reported by Imajima; however, in Paraonidae eyespots are often present in juveniles, and then fade in adults.

Taxonomic notes: Listed by Lovell (2002) as synonym of *A. catherinae*, it shows different features with respect to the original description by Laubier (1967). *A. eximia* is a very close species that differs only in fine characters of modified chaetae, and might be synonymous with *A. elongata*.

Ecological features: On muddy bottoms from the surface to 130 m depth (Imajima, 1973).

Distribution: Japan Sea (Imajima, 1973; present data).

Aricidea (Acmira) cf. ***laubieri*** Hartley, 1981 (Fig. 7D-E)

= ?*Aricidea* sp. *sensu* Laubier, 1966b

Material

Genetics and morphology: San Teodoro Pond, Tyrrhenian Sea, 0.5 m (07/2015).

Morphology only: Calich Pond, Sea of Sardinia, 1 m (07/1987); Cyprus, Levant Sea, 10 m (07/2014); Elba Island, Tyrrhenian Sea, 12 m (10/1983); Gulf of Follonica, Tyrrhenian Sea, 4 m (04/1981).

Description: The examined individuals are medium sized *Aricidea* (0.2-0.5 mm maximum width). Prostomium triangular, with ventral eyespots and a cirriform median antenna with constant width and blunt tip that usually does not exceed the 2/3 of the prostomial length. Three pre-branchial chaetigers; first two chaetigers with tubercular notopodial lobes, third one with elongated lobe. From 10 to 26 leaf-shaped, relatively elongated branchiae (number is size-depending); the size of branchiae decreases in the posterior part of the branchial region, and tips are never thin and tapering as in *A. assimilis* (Fig. 7D). Notopodial lobes remain slender and elongated throughout the whole branchial and post-branchial regions. Parapodia biramous, composed by two bundles of thick capillaries. Modified neuropodial chaetae begin shortly after the end of the branchial region and are thick, relatively straight hooks with abruptly crooked tip; the apical arista is inserted near to the dorsal edge, and additional hairs may be present (Fig. 7E). Live colour bright yellow, with pale pink thoracic inclusions.

Taxonomic notes: This species has never been officially reported from the Mediterranean Sea; it shows strong similarities with *A. assimilis*, but it has a shorter antenna, with different shape and it exhibits differences in the shape of parapodia. J. Gil (*in litt.*) tentatively attributed to this species an individual reported by Laubier (1966b) from the coast of Lebanon, later assigned by Laubier & Ramos (1974) to the short-antenna form of *A. assimilis*. M. Rousou (*in litt.*) reported this species from Cyprus. The examined individuals closely match the description given by Hartley (1981). It is likely that the species actually occurs in the Mediterranean Sea, but it has been overlooked due to its strong similarity with *A. assimilis*.

Ecological features: *A. laubieri* is poorly known from an ecological point of view; it occurs on sandy and mixed bottoms between 10 and 100 m depth. In the Mediterranean Sea, it is a shallow species, with most records between 4 and 12 m depth; in brackish-water environments it is even shallower (0.5-2 m depth).

Distribution: Eastern Atlantic Ocean (Hartley, 1981; O'Connor et al., 1984; Aguirrezabalaga & Gil, 2008; Ravara & Moreira, 2013); Mediterranean Sea: Levant Sea (Rousou, *in litt.*), Tyrrhenian Sea (present data).

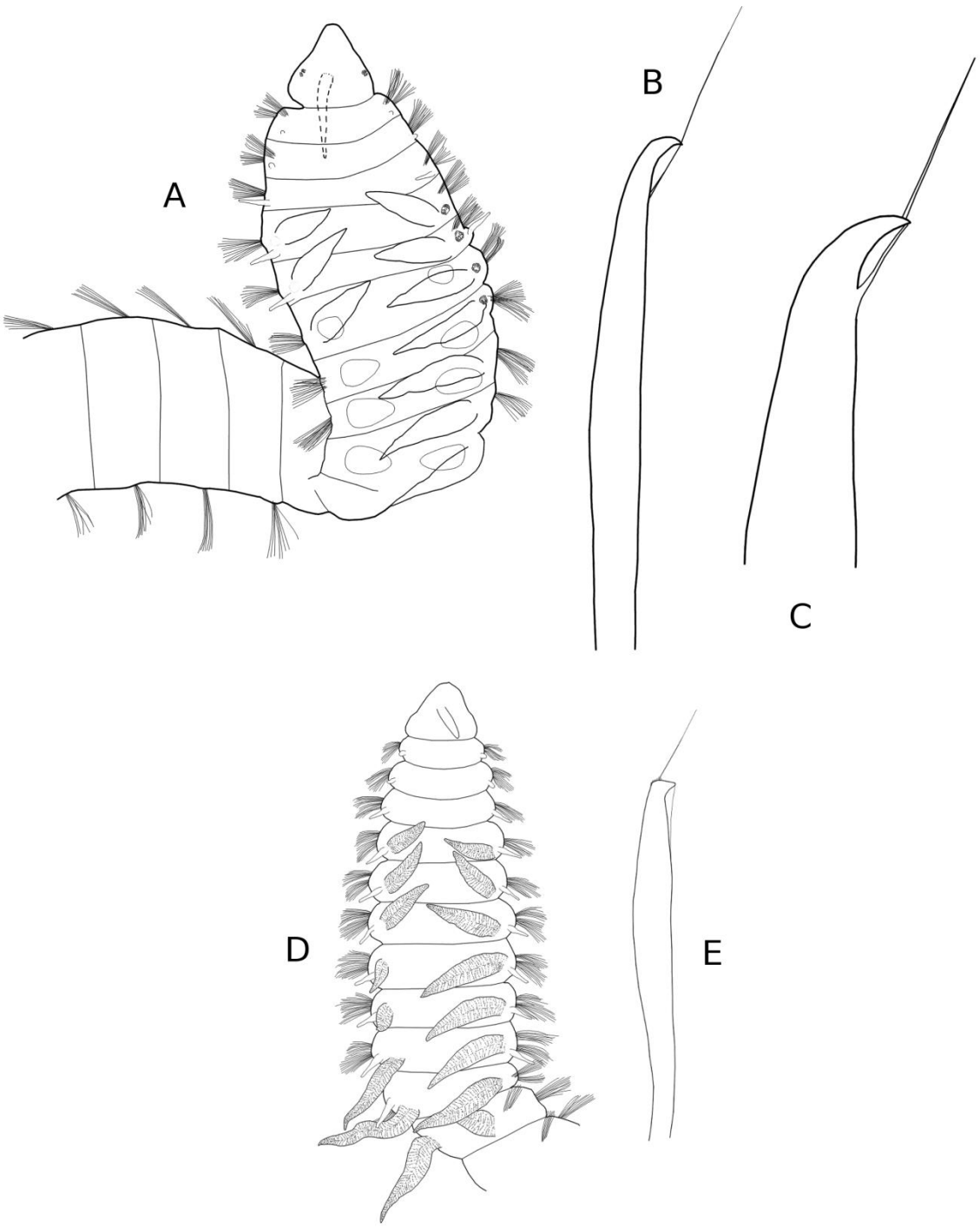

Figure 7: *Aricidea (Acmira) elongata* Imajima, 1973: A) Anterior part of the body in dorsal view, antenna reconstructed on the basis of Imajima (1973); B) Modified neurochaeta; C) Tip of modified neurochaeta. *Aricidea (Acmira)* cf. *laubieri* Hartley, 1981: D) Anterior part of the body in dorsal view; E) modified neurochaeta. A-C: Jinhae Bay, South Korea, Pacific Ocean; D-E: Calich Pond, Mediterranean Sea.

Aricidea (Acmira) mirifica Strelzov, 1973

Material

Genetics and morphology: Cold seep near Costa Rica, Pacific Ocean, depth unknown (02/2009)

Description: See Strelzov (1973). The only difference between the examined individual and the original description is the number of modified chaetae (2-6 in Strelzov's specimens, 1 in the examined specimen). This could be due to the really small size of the Costa Rica individual – since number of chaetae is generally size-dependent.

Taxonomic notes: Despite its attribution to *Acmira* Hartley, 1981, this species closely resembles *Aricidea (Strelzovia) quadrilobata* Webster & Benedict, 1887, an eurybathic species reported from a really wide geographic range. The main similarities regard the shape of the prostomium and of the modified chaetae; however, in *A. mirifica* the modified chaetae abruptly begin in the post-branchial region, whereas in *A. quadrilobata* they are already present in the neuropodia of the branchial region.

Ecological features: On muddy and mixed bottoms between 80 and 2900 m depth; the examined individuals come from undefined depths, but probably between 1000 and 2000 m depth.

Distribution: Pacific Ocean (Strelzov, 1973; present data); Antarctic Ocean (Strelzov, 1973).

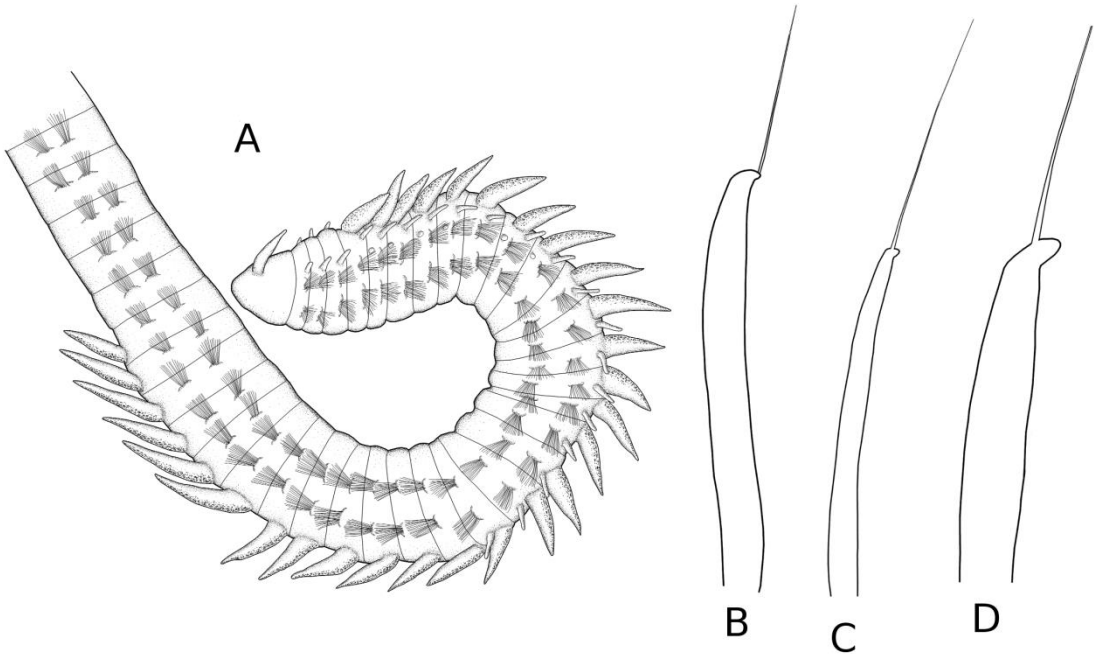

Figure 8: *Aricidea (Acmira) rubra* Hartman, 1963 from cold seeps in Costa Rica (Pacific Ocean): A) Anterior part of the body; B) Modified neurochaeta from the anterior part of the body; C) Modified neurochaeta from the posterior part of the body; D) Detail of C showing the insertion of the arista.

Aricidea (Acmira) rubra Hartman, 1963 (Fig. 8)

= *Aricidea lopezi rubra* Hartman, 1963

= *Aricidea (Acesta) finitima* Strelzov, 1973 *partim*

Material

Genetics and morphology: Costa Rica Mound 2, Pacific Ocean, 1001 m (03/2009); Jaco Scarp, Costa Rica, Pacific Ocean, 1800 m (01/2010).

Morphology only: Cold seep near Costa Rica, Pacific Ocean, depth unknown (02/2009).

Description: Relatively large species (complete individual approx. 20 mm for 122 chaetigers, and 0.5 mm maximum width). Prostomium triangular, eyeless, with antenna spindle-shaped, relatively thick, with pointed tip, shorter than the prostomium, not reaching the first chaetiger behind. Three pre-branchial chaetigers, with post-chaetal notopodial lobes spindle-shaped, gradually increasing, not tubercular on the first chaetiger. Up to 28-30 pairs of elongate, pointed branchiae, with length increasing in the posterior part of the branchial region; branchiae contain reddish pigment inclusions, especially along the anterior edge. Notopodial lobes increasing in the branchial region, very long and threadlike in the post-branchial region (Fig. 8A). Parapodia biramous, with thick bundles of long capillaries, notopodial chaetae distinctly longer than the neuropodial ones, especially in the post-branchial region; neuropodial modified chaetae begin in the post-branchial region and are thick, short hooks, with changing shape along the body length. In the anterior part of the post-branchial region modified chaetae are similar to those of *A. catherinae*, straight, with slightly curved tip and terminal, thin and not very long (easily damaged) arista (Fig. 8B); whereas more posterior modified chaetae are thinner, with blunt, angled tip, and sub-terminal, thicker and longer arista, with insertion approximately at the half of the tip (neither terminal, as in *A. catherinae*, nor dorsal, as in *A. assimilis*) (Fig. 8C-D). Both types of chaetae were described for *A. finitima* by Strelzov (1973). Live colour grey-yellowish, with reddish branchiae, and red inclusions in the branchial region. See as well Blake (1996).

Taxonomic notes: The synonymy between *Aricidea rubra* and *A. finitima* is due to Blake (1996). According to Strelzov (1973: under synonymy) *A. rubra* shows a remarkably high intraspecific variability, in particular in the shape and length of the antenna, and a wide distribution, and is probably a species complex. The examined individuals differ from the original description of *A. finitima* in particular in the length of the pre-branchial notopodial lobes, that are already spindle-shaped and relatively long in the first two chaetigers, whereas in *A. finitima* they are short and conical (Strelzov, 1973). The individual from Hartman's

collection (Fig. 40 B-F in Strelzov, 1973), from epibathyal bottoms along California coast (400-500 m), corresponding to *A. rubra*, shows longer post-chaetal lobes and distinctly shorter antenna, and could belong to the examined species. There is a possibility that actually *A. rubra* and *A. finitima* represent closely related, yet different species.

Ecological features: *A. rubra* is known from muddy to sandy bottoms between the infralittoral (30 m) and the bathyal (3800 m) regions. Present material comes from bathyal muddy bottoms near to cold seeps.

Distribution: Pacific Ocean (Hartman, 1963; Strelzov, 1973; Blake, 1996); Southern Atlantic Ocean (*doubtful* - Strelzov, 1973).

Aricidea (Acmira) simonae Laubier & Ramos, 1974

= *Aricidea punctata* Katzmann, 1972

= *?Aricidea* cf. *neosuecica* Hartman, 1965 *sensu* Laubier & Ramos, 1974

= *?Aricidea neosuecica* Hartman, 1965 *sensu* Çinar, 2005

= *?Acmira simplex* (Day, 1963) *sensu* Zaâbi et al., 2012

Material

Genetics and morphology: Elba Island, Tyrrhenian Sea, 10 m (06/2014)

Only morphology: Elba Island, Tyrrhenian Sea, 10-12 m (10/1983); Montecristo Island, Tyrrhenian Sea, 8 m (06/2012); Strait of Messina, 25 m (07/1992); Tuscan Archipelago, Tyrrhenian Sea, 50-275 m (08/1985; 12/1986).

Description: See Aguirrezabalaga (2012).

Taxonomic notes: This species was firstly described by Katzmann (1973) as *Aricidea punctata*; however, this name was preoccupied by *Aricidea (Aedicira) punctata* Hartmann-Schröder, 1962, which Strelzov (1973) considered a doubtful synonym of *Aricidea (Acmira) lopezi* Berkeley & Berkeley, 1956. The species was also described by Laubier & Ramos (1974) with the current name and the synonymy was stated by Katzmann & Laubier (1975).

A. simonae is a very conspicuous species, and the combination of morphological features (branchiae beginning at the 3rd chaetiger; very short antenna; modified chaetae short and thick, pointed, knife-shaped; reddish inclusions throughout the body) makes it unmistakable among Mediterranean paraonids.

The report of *Aricidea (Acmira) simplex* Day, 1963 in a checklist of Tunisian polychaetes (Zaâbi et al., 2012) should probably be referred to this species, which is absent from the

checklist. Also the records of *Aricidea* cf. *neosuecica* for the Mediterranean Sea (Katzmann & Laubier, 1975; Çinar, 2005) probably refer to this species (see Hartley, 1981).

Ecological features: Rare, but regular, from 8 to 300 m depth, mainly on sandy or mixed bottoms.

Distribution: Mediterranean Sea (Katzmann, 1973; Laubier & Ramos, 1974; Katzmann & Laubier, 1975; Çinar, 2005); Eastern Atlantic Ocean (Hartley, 1981; Aguirrezabalaga & Gil, 2009; Ravara & Moreira, 2013).

Aricidea (Acmira) simplex Day, 1963

= *Aricidea suecica simplex* Day, 1963

= *Aricidea neosuecica* Hartman, 1965

= *Aricidea neosuecica nipponica* Imajima, 1973

Material

Genetics and morphology: Coronado Bank, Pacific Ocean, 1100 m (7/2012).

Description: See Aguirrezabalaga (2012).

Taxonomic notes: *A. simplex* is a poorly characterised species, that shows some similarities with *A. simonae* in the shape of modified chaetae and the short antenna, but it shows differences in the overall shape of prostomium and body. Both *A. neosuecica* and *A. neosuecica nipponica* have been synonymised with this species, but the scarcity of morphological diagnostic features in this species makes this synonymy poorly reliable. As a consequence, both ecology and distribution of this species are quite confused.

The few Mediterranean records, and more generally, shallow Atlantic records probably refer to *A. simonae* rather than to this species.

Ecological features: Mainly bathyal, on muddy bottoms between 200 and 3000 m depth.

Distribution: Eastern Atlantic Ocean (Day, 1963; Amoureux, 1973); Western Atlantic Ocean (Strelzov, 1973); Antarctic Ocean (Strelzov, 1973); Pacific Ocean (Hobson, 1972; Strelzov, 1973; Imajima, 1973; Blake, 1996). The few records for the Mediterranean Sea (Laubier & Ramos, 1974 – as *Aricidea* cf. *neosuecica*; Çinar, 2005 – as *Aricidea neosuecica*; Zaâbi et al., 2012) should probably be referred to *A. simonae*.

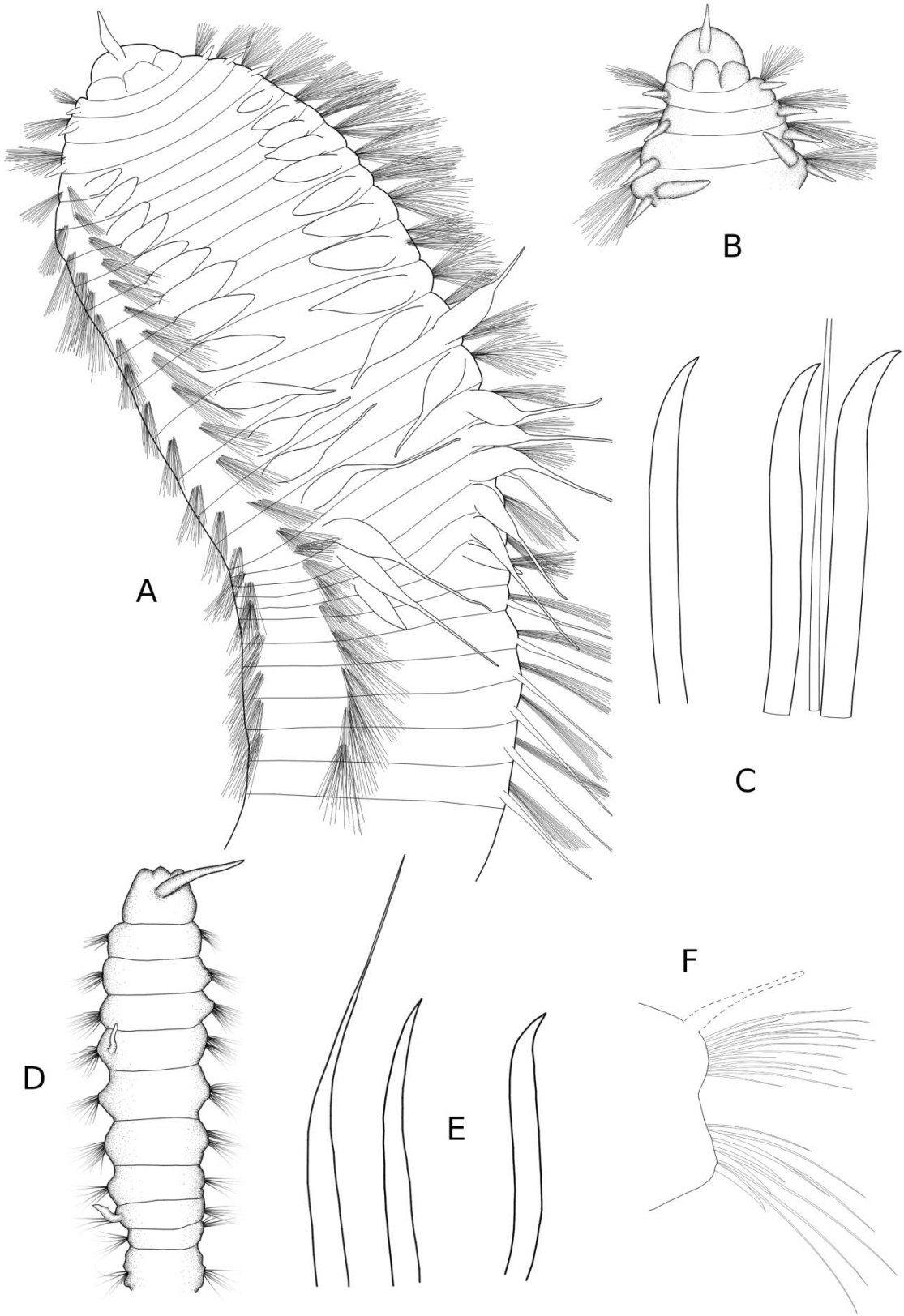

Figure 9: *Aricidea (Acmira)* sp. A: A) Anterior part of the body in dorsal view; B) Prostomium and first chaetigers; C) Modified chaetae from posterior neuropodia. *Aricidea (Acmira)* sp. B: D) Anterior part of the body in dorsal view; E) Modified chaetae from posterior neuropodia; F) Schematic drawing of a posterior parapodium (notopodial lobe reconstructed after Laubier & Ramos, 1974). A-C: Costa Rica, Pacific Ocean; D-F: Malta Escarpment, Mediterranean Sea.

***Aricidea (Acmira)* sp. A** (Fig. 9A-C)

= *Aedicira longocirrata* Fauchald, 1972

Material

Genetics and morphology: Jaco Sharp, Costa Rica, Pacific Ocean, 1802 m (01/2010).

Description: The only examined individual of this species is an incomplete, anterior fragment (DNA extracted from some posterior chaetigers). Relatively large species, 12 mm long for approximately 50 chaetigers; maximum width approximately 2 mm. Prostomium wide, rounded, with conspicuous paired nuchal organs, a short, spindle-shaped and thin central antenna, with pointed tip, which length is approximately ¾ of the prostomium length (Fig 9B). Three pre-branchial chaetigers, 17 pairs of branchiae, wide and rounded in the anterior part, with pointed tip progressively elongated; in the last pairs, the elongated tip is up to 2-3 times the length of the branchia. Post-chaetal notopodial lobes spindle-shaped in the pre-branchial and early branchial region, progressively elongated; already in the posterior branchial region they are very long and thin, more than ½ of the body width (Fig 9A). Chaetae are thick and very long capillaries, occurring in thick bundles;. In the posterior region they are even longer, and accompanied by several modified chaetae, thicker and shorter, but still longer than in the majority of Paraonidae. Modified chaetae are straight, with slightly curved tip, sharply pointes, without any trace of hood, hairs or arista (Fig. 9C). Live colouration pale pink, with red inclusions in the branchial region; branchiae greenish-yellowish.

Taxonomic notes: This poorly-known species has been described by Fauchald (1972) as *Aedicira longicirrata.* Considering the genus *Aedicira* as a subgenus of *Aricidea*, this name is preoccupied by *Aricidea (Aricidea) longicirrata* Hartmann-Schröder, 1965. Moreover, the examination of modified chaetae, that are lacking in Fauchald's description, yet present in the examined individual, allows to consider this individual close to *A. simplex*. This species should therefore be assigned to the subgenus *Acmira* according to the current definition of subgenera, and since the name given by Fauchald is preoccupied, it should be replaced by a new name. Among other Paraonidae, this species resembles at most *A. simplex* and *Aricidea (Strelzovia) pulchra* Strelzov, 1973, two large, deep-water species, in size, number of branchiae, shape of the prostomium, length of the antenna and shape of modified chaetae. It differs from both species in the shape of the antenna (pointed in *Aricidea* sp. A, blunt in *A. simplex* and *A. pulchra*); moreover, *A. pulchra* has narrow branchiae (whereas in this species branchiae are wide) and *A. simplex* has distinctly thicker modified chaetae and shorter

notopodial lobes in the pre-branchial region. Even though some peculiar features of the examined specimen (such as really long capillary chaetae and elongated tip of branchiae) are probably due to the maturative stage (i.e. probably the individual is an epitoke form), the differences observed with the most similar species suggest that it is a separated species.

Ecological features: On muddy bottoms, sometimes near cold seeps, at 1200-2900 m depth (Fauchald, 1972).

Distribution: Eastern Pacific Ocean, from California and western Mexico (Fauchald, 1972) to Costa Rica (present data).

***Aricidea (Acmira)* sp. B** (Fig. 9D-F)

= *Aricidea trilobata* Laubier & Ramos, 1974

Material

Morphology only: Malta Escarpment, Ionian Sea, 1800-2100 m (05/2009)

Description: Very small species, 25 chaetigers for 2.5 mm, with a maximum width of approximately 0.2 mm. Prostomium sub-trapezoidal, with anterior edge clearly divided in three triangular lobes. Antenna well-developed, with central insertion, elongated, slightly tapering, measuring approximately 1½ of the prostomium length. Body relatively slender, with well-developed parapodia (Fig. 9D). Appendages (notopodial lobes and branchiae) very brittle, easily broken. Notopodial lobes are elongated, approximately as long as the branchiae, and bottle-shaped, whereas branchiae are relatively short and wide, somewhat corrugated (Fig. 9F). Semi-circular dorsal lobes are present at certain chaetigers in the branchial region.

Parapodia bear mostly strong capillaries in the pre-branchial and branchial region. Capillaries are strongly curved in the first parapodia, becoming straighter and thinner in the posterior part of the branchial region, and are somewhat thicker in the neuropodium. Modified chaetae occur from chaetiger 20 and are represented by up to five thick, slightly curved and strongly pointed hooks. At chaetigers 20-21 transition chaetae, thick but with elongated tip, are noticeable. At chaetigers 24-25 modified chaetae are fewer and have a strongly curved tip (Fig. 9E). See as well Laubier & Ramos (1974).

Taxonomic notes: This species has been originally described by Laubier & Ramos (1974) for bathyal bottoms of the western Mediterranean Sea. However, this name is preoccupied by *Aricidea (Acmira) trilobata* Imajima, 1973, a poorly known Pacific species that occurs on circalittoral bottoms. *Aricidea (Acmira) trilobata sensu* Laubier & Ramos, 1974 has been redescribed with a new name in a paper that is currently under revision (Langeneck et al., *in*

press). The two species are very similar as regards the main morphological features, but differ in size, presence/absence of dorsal lobes and number of branchiae. Moreover, geographical distribution and ecological requirements are clearly different (Langeneck et al., *in press*). Laubier & Ramos' (1974) individuals were short anterior fragments, broken before the beginning of modified chaetae; thus, on the basis of this description it was impossible to assign it to any of the subgenera of *Aricidea*. On the basis of more complete material, I hereby confirm that this species shows strong, slightly curved hooks as modified chaetae and, based on the current definition of subgenera, should be assigned to *Acmira* Hartley, 1981.

Ecological features: On deep, muddy bottoms between 600 and 2800 m depth (Laubier & Ramos, 1974; Çinar, 2005).

Distribution: Mediterranean Sea: Western Mediterranean Sea (Laubier & Ramos, 1974), Levant Sea (Çinar, 2005), Ionian Sea (Langeneck et al., *in press*).

Aricidea (Aricidea) bansei Laubier & Ramos, 1974

= *Aricidea (Aricidea) capensis bansei* Laubier & Ramos, 1974

Material

Genetics and morphology: Albegna River Mouth, Tyrrhenian Sea, 10 m (06/2014); Elba Island, Tyrrhenian Sea, 5 m (06/2014).

Only morphology: Cyprus, Levant Sea, 10 m (07/2014); Elba Island, Tyrrhenian Sea, 12 m (10/1983); Gulf of Follonica, Tyrrhenian Sea, 6-8 m (04/1981; 07/1987; 02/1988; 05/1988); Strait of Messina, 25 m (07/1992).

Description: See Aguirrezabalaga (2012) (as *Aricidea capensis bansei*).

Taxonomic notes: This species has been originally described as *Aricidea capensis bansei*. According to Winston (1999), however, the use of subspecific rank in marine invertebrates should be avoided, especially when their biology and biogeography are poorly known. Although *Aricidea capensis* Day, 1961 appears similar to this species, the differences observed between the two taxa (postchaetal lobes long *vs* short in the pre-branchial chaetigers; postchaetal lobes shorter than the notopodial chaetae *vs* longer in the post-branchial region; modified chaetae with only one *vs* one-three accessory tooth) justify the raising of this taxon to specific rank, as *A. bansei*.

Ecological features: Frequent, but usually not abundant, in shallow environments (5-15 m, rarely deeper), on clean fine sand, sometimes on coarser sediments or mixed bottoms.

Distribution: Mediterranean Sea (Laubier & Ramos, 1974; Castelli, 1985; Çinar, 2005; Zaâbi et al., 2012); Eastern Atlantic Ocean (Hartley, 1981; O'Connor et al., 1984; Gil & Sardá, 1999).

Aricidea (Aricidea) fragilis Webster, 1879 (Fig. 10)

= *Aricidea (Aricidea) fragilis mediterranea* Laubier & Ramos, 1974 *partim*

Material

Genetics and morphology: Candelaro River Mouth, Adriatic Sea, 8 m (09/2014); Viareggio, Tyrrhenian Sea, 10 m (06/2014).

Only morphology: Candelaro River Mouth, Adriatic Sea, 3.5 m (04/2016); Cattolica, Adriatic Sea, 11 m (11/2010); Cavallino-Treporti, Adriatic Sea, 13 m (10/2014); Gulf of Trieste, 10 m (03/2016); Po River Mouth, Adriatic Sea, 14 m (10/2014); Porto Pozzo, Tyrrhenian Sea, 1 m (07/1987); River Jadro Estuary, Adriatic Sea, depth unknown (08/2014).

Description: Mediterranean individuals show large size if compared to other *Aricidea* s.l. species; maximum width from 0.6 to 1.4 mm; maximum length unknown, since all specimens are incomplete, but probably around 40-50 mm in the larger individuals (anterior fragments up to 32 mm for 108 chaetigers). Prostomium roughly triangular, with median antenna cirriform, pointed, approximately 1-1.5 times the prostomium length, with slightly enlarged basis; eyespots are generally present. Three pre-branchial chaetigers; 25-37 pairs of well developed, fragile branchiae. The number of branchiae depends on the individual size: small individuals (0.6-0.8 mm width) show 25-30 pairs of branchiae, whereas larger ones (1.2-1.4 mm width) have 34-37. Branchiae are pointed and leaf-shaped, not excessively elongate; some (4-6) posterior pairs are narrower and show elongate tips, and the last 2-3 branchiae are distinctly smaller, without elongate tips (Fig. 10A). Postchaetal notopodial lobes are elongate throughout the whole body length; in pre-branchial and branchial chaetigers they are thicker and can have enlarged basis, whereas in the post-branchial region they remain long, but are distinctly thinner. Parapodia biramous, poorly developed, with capillary chaetae throughout the body. Modified neuropodial chaetae are pseudo-articulate, with a shallow notch approximately at the half of their length, in proximity of which clearly noticeable hairs are present; the distal part of the chaeta is often deflected with respect to the proximal part (Fig. 10C). Modified chaetae occur in thick bundles in the posterior part of the body, together with simple capillaries and transition chaetae.

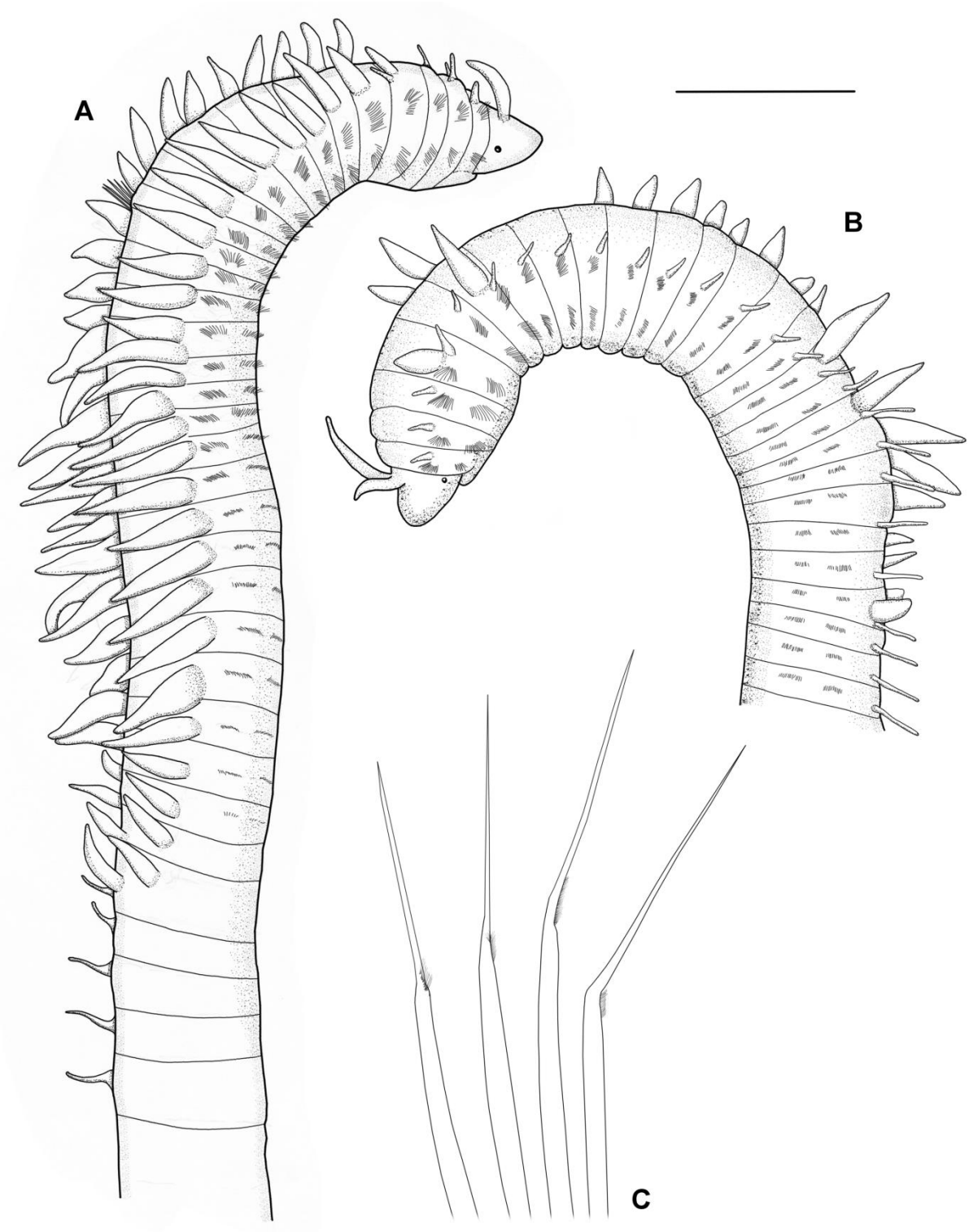

Figure 10: *Aricidea (Aricidea) fragilis* Webster, 1879. A) Typical individual in dorsal view; B) Individual with bifurcate antenna; C) Modified neurochaetae. A: Cattolica, Adriatic Sea; B-C: Jadro River Estuary, Adriatic Sea.

Two individuals from River Jadro Estuary (Croatia) and one from the Gulf of Trieste are completely indistinguishable from the remaining material, but they have a double antenna, formed by two rami originating from the same basis (Fig. 10B). Live colour unknown; preserved individuals whitish or yellowish (if not stained). See also Strelzov (1973).

Taxonomic notes: Type species of *Aricidea* Webster, 1879. Despite the re-descriptions given by Pettibone (1965) and Strelzov (1973), the actual identity of this species remained puzzling until recently; material assigned to *A. fragilis* probably belongs to several species, many of them undescribed (Gaston & McLelland, 1996). Some individuals described as *Aricidea fragilis mediterranea* by Laubier & Ramos (1974) clearly correspond to this species, but the type material of *A. fragilis mediterranea* refers to a different species (*Aricidea pseudoarticulata* Hobson, 1972).

Mediterranean material matches well topotypic individuals as regards size, number and features of branchiae, and modified chaetae, although these latter are often not easy to notice, and less characterised than in Strelzov (1973) and Laubier & Ramos (1974), whilst closely resembling the drawings by Pettibone (1965), with the occurrence of transitional chaetae between the simple capillary and the pseudo-articulate types. This species has probably been misidentified as *Aricidea (Strelzovia) meridionalis* Laubier & Ramos, 1974.

Ecological features: In shallow environments, typically on muddy bottoms. Mediterranean records between 1 and 15 m depth. In the English Channel and in the Mediterranean Sea this species shows a patchy and relatively narrow distribution, where however it reaches remarkably high densities (Quiroz-Martinez et al., 2012; T. Scirocco, *pers. comm.*) and is probably an alien species, even though the way of introduction is still unknown.

Distribution: Western Atlantic Ocean (Webster, 1879; Pettibone, 1965; Strelzov, 1973; Gaston & McLelland, 1996); English Channel (Dauvin & Gentil, 1980; Dauvin et al., 2003; Quiroz-Martinez et al., 2012); Mediterranean Sea (Laubier & Ramos, 1974; present data). Records for the Pacific Ocean (Strelzov, 1973) are doubtful.

Aricidea (Aricidea) minuta Southward, 1956

Material

Genetics and morphology: Beals, Maine, Western Atlantic Ocean, 10 m (05/2016); St. Mary's Road, North Sea, 15 m (06/2009).

Only morphology: Gulf of Trieste, Adriatic Sea, 15 m (10/1995).

Description: See Aguirrezabalaga (2012).

Taxonomic notes: -

Ecological notes: *A. minuta* is common in the Eastern Atlantic Ocean, where it could reach remarkably high densities (Gibbs, 1965), mainly on sandy bottoms. In the Mediterranean Sea it is a rare species, occurring mainly on muddy bottoms between 15 and 50 m depth, but

Mediterranean records are very scarce. The individual from the Adriatic Sea represents the first occurrence of the species in Italian waters and shows a good correspondence with Atlantic material.

Distribution: Eastern Atlantic Ocean (Southward, 1956; Eliason, 1962; Gibbs, 1965); Western Atlantic Ocean (present data); Western Mediterranean Sea (Laubier & Ramos, 1974; Sardá, 1984; present data); Pacific Ocean (de León-González et al., 2006 - *doubtful*).

Aricidea (Aricidea) pseudoarticulata Hobson, 1972

= *Aricidea (Aricidea) fragilis mediterranea* Laubier & Ramos, 1974 *partim*

Material

Genetics and morphology: Cala di Forno, Tyrrhenian Sea, 10 m (06/2014); Elba Island, Tyrrhenian Sea, 5 m (06/2014); Galicia, Eastern Atlantic Ocean, 30 m (04/2016).

Only morphology: Castiglione della Pescaia, Tyrrhenian Sea, 20 m (07/1984); Cyprus, Levant Sea, 10 m (07/2014); Gulf of Follonica, Tyrrhenian Sea, 8 m (12/1987); Jadro River Estuary, Adriatic Sea (11/2014); Porto Pozzo, Tyrrhenian Sea, 1 m (07/1987); Tuscan Archipelago, Tyrrhenian Sea, 13 m (12/1986).

Description: See Aguirrezabalaga (2012).

Taxonomic notes: Aguirrezabalaga & Gil (2008) stated the synonymy between *A. pseudoarticulata* and *A. fragilis mediterranea*; even though the type locality of *A. pseudoarticulata* is in the Northern Pacific Ocean, the two taxa are identical as regards morphological features. Thus, I retain the synonymy, precautionarily stating that, if the two taxa turn out to be distinct, my material refers to *A. fragilis mediterranea*.

The description of this taxon as subspecies of *A. fragilis* may have contributed to the overlooking of the true *A. fragilis* in the Mediterranean Sea – even though the two taxa strongly differ in a number of features and could actually turn out to be only distantly related. This species, on the other hand, shows striking similarities with *A. minuta*, but they differ in number and shape of branchiae and in the presence of two types of modified chaetae, one of which, similar to the modified chaetae of *A. catherinae*, is lacking in *A. minuta*.

Ecological features: Uncommon, but regular, on clean fine sand between 5 and 20 m depth, rarely shallower (1 m) in environments affected by brackish water inflow.

Distribution: Pacific Ocean (Hobson, 1972; Blake, 1996); Western Atlantic Ocean (Hobson, 1972); Eastern Atlantic Ocean (Aguirrezabalaga, 2012; Ravara & Moreira, 2013); Mediterranean Sea (Laubier & Ramos, 1974; Capaccioni, 1987; Castelli, 1987).

Aricidea (Aricidea) wassi Pettibone, 1965

Material

Genetics and morphology: Capraia Island, Tyrrhenian Sea, 100 m (08/2016).

Description: see Katzmann & Laubier (1975).

Taxonomic notes: -

Ecological features: This species occurs mainly on muddy bottoms between 10 and 125 m depth. In the Mediterranean Sea it is a circalittoral and usually uncommon species, and all recorded specimens have been sampled between 60 and 125 m depth, whereas in the Atlantic Ocean this species appears shallower (10-50 m).

Distribution: Western Atlantic Ocean (Pettibone, 1965; Strelzov, 1973); Eastern Atlantic Ocean (Hartley, 1981; O'Connor, 1984; Aguirrezabalaga & Gil, 2008; Ravara & Moreira, 2013); Pacific Ocean (Blake, 1996; Aguado & López, 2003), In the Mediterranean Sea this species has been reported from the Adriatic Sea (Katzmann & Laubier, 1975) and from the Aegean Sea (Simboura & Zenetos, 2005). The hereby reported individual represents the first record of the species for the Tyrrhenian Sea.

Aricidea (Strelzovia) abyssalis Laubier & Ramos, 1974

Material

Morphology only: Sardinian Slope, Sea of Sardinia, 1200 m (10/2009).

Description: see Laubier & Ramos (1974).

Taxonomic notes: this species is relatively easy to identify because of the absence of branchiae and the presence of dark red sub-epidermic inclusions that may recall those of *A. simonae*. However, modified chaetae are clearly different between the two species.

Ecological features: on bathyal bottoms between 1100 and 2800 m depth (Laubier & Ramos, 1974; Aguirrezabalaga & Gil, 2009).

Distribution: western Mediterranean Sea (Laubier & Ramos, 1974; present data); eastern Atlantic Ocean (Aguirrezabalaga & Gil, 2009).

Aricidea (Strelzovia) balearica Castelli, 1987

= *Aedicira mediterranea* Laubier & Ramos, 1974

= *Aricidea (Allia) mediterranea* (Laubier & Ramos, 1974) (Aguirrezabalaga & Gil, 2009)

= *Aricidea (Strelzovia) mediterranea* (Laubier & Ramos, 1974) (Aguirrezabalaga, 2012)

= ?*Aricidea (Strelzovia) sardai* Aguirrezabalaga & Gil, 2009

Material

Morphology only: Maltese Escarpment, Ionian Sea, 1500 m (05/2009).

Description: see Laubier & Ramos (1974) as *Aedicira mediterranea*.

Taxonomic notes: in the original description, this species is assigned to *Aedicira* Hartman, 1957, there considered as a separate genus from *Aricidea* Webster, 1879 (Laubier & Ramos, 1974). These Authors were however dubious about the correct assignment of this species to *Aedicira*. Considering *Aedicira* as a subgenus of *Aricidea*, Castelli (1987) remarked a homonymy problem with *Aricidea fragilis mediterranea*, and created *Aricidea (Aedicira) balearica* as new name for this entity. This taxonomic change, however, was not received by the scientific community. Later, Aguirrezabalaga & Gil (2009) assigned *Aedicira mediterranea* to the genus *Aricidea* and subgenus *Allia*, pointing out that modified chaetae in this species are present. The homonymy problem was not addressed by these Authors, but it still remains, and in my opinion the correct name would be *Aricidea (Strelzovia) balearica*.

Aricidea (Strelzovia) sardai Aguirrezabalaga & Gil, 2009 is a very similar species, and differs from *A. balearica* only in the shape of the antenna, which is bifurcate in *A. balearica*, simple in *A. sardai* (Aguirrezabalaga & Gil, 2009). Since bifurcate antennae were observed in other species (Cerruti, 1909; Dauvin et al., 1980), *A. sardai* could fall in the morphological variation range of *A. balearica*.

Ecological features: bathyal, between 600 and 2850 m depth (Laubier & Ramos, 1974; Çinar, 2005).

Distribution: Mediterranean Sea (Laubier & Ramos, 1974; Çinar, 2005), possibly Atlantic Ocean (Aguirrezabalaga & Gil, 2009).

Aricidea (Strelzovia) claudiae Laubier, 1967

Material

Genetics and morphology: Cattolica, Adriatic Sea, 10 m (05/2016); Tuscan Archipelago, Tyrrhenian Sea, 110 m (11/2014; 08/2015; 11/2015; 02/2016)

Only morphology: Arno River Mouth, Tyrrhenian Sea, 10-15 m (06/1985); Tuscan Archipelago, Tyrrhenian Sea, 110 m (08/2012; 03/2015); Litorale Ravennate, Adriatic Sea, 15 m (1987); Porto Pozzo, Tyrrhenian Sea, 6 m (06/1987); Trieste, Adriatic Sea, 10-15 m (10/1995; 2014).

Description: see Laubier (1967) and Aguirrezabalaga (2012).

Taxonomic notes: this species occurs in two forms, one with bottle-shaped antenna, apparently articulated, with long narrowed tip, the other with thicker antenna, without narrowed tip, or just with a distal button. The two forms are otherwise indistinguishable; this species is mainly characterised by the presence of a large mid-dorsal tubercle on the 4th chaetiger – sometimes not easy to notice.

Ecological features: on muddy or mixed bottoms, from shallow waters (10-15 m) up to the circalittoral zone (100-150 m). Sometimes deeper (Aguirrezabalaga & Gil, 2009). In the Black Sea this species can reach remarkably high densities (Strelzov, 1973). Often syntopic with *A. assimilis*.

Distribution: Mediterranean Sea (Laubier, 1967; Strelzov, 1973; Laubier & Ramos, 1974; Katzmann & Laubier, 1975; Çinar, 2005); Black Sea (Strelzov, 1973); eastern Atlantic Ocean (Gil & Sardá, 1999; Aguirrezabalaga & Gil, 2008; 2009).

Aricidea (Strelzovia) hartleyi Blake *in* Blake, Hilbig & Scott, 1996

= ?*Aricidea jeffreysii* (McIntosh, 1879) *sensu* Imajima, 1973

= *Aricidea* cf. *nolani* Webster & Benedict *sensu* Lissner et al., 1986 *fide* Blake, 1996

Material

Genetics and morphology: Point Loma, Pacific Ocean, 240 m (07/2012).

Description: see Blake (1996).

Taxonomic notes: see Blake (1996).

Ecological features: on circalittoral, muddy bottoms. Poorly known.

Distribution: eastern Pacific Ocean (Blake, 1996; present data); western Pacific Ocean (Imajima, 1973, as *A. jeffreysii*; Lovell, 2002).

Aricidea (Strelzovia) mariannae Katzmann & Laubier, 1975 (Fig. 11)

Material

Genetics and morphology: Tuscan Archipelago, Tyrrhenian Sea, 110 m (12/2015).

Morphology only: Sardinian Slope, Sea of Sardinia, 600 m (10/2009).

Description: medium-sized *Aricidea*, with moderately slender and slightly flattened body; maximum width approximately 0.5 mm. Prostomium conical, with short, thick and distally pointed median antenna that does not reach the first chaetiger. Nuchal organs conspicuous, clearly noticeable as short grooves on the posterior part of the prostomium, with a glandular, subtriangular yellowish part. Eyes absent. Bright red inclusions clearly noticeable from the 4th to the 16th chaetiger; branchiae relatively numerous, at least 14 pairs, from the 4th to the 17th chaetiger, slender, pointed and not very long, easily broken. In the pre-branchial chaetigers the post-chaetal notopodial lobes show a clear increase in length, then from the 4th chaetiger they remain approximately of the same length through the whole branchial region. The examined individuals are anterior fragments; modified chaetae are scarcely modified capillaries, similar to those of *A. roberti* and *A. claudiae*. Live colouration greenish or yellowish. See as well Katzmann & Laubier (1975).

Taxonomic remarks: this species shows striking similarities with *Aricidea (Strelzovia) mirunekoa* Aguirrezabalaga & Gil, 2009, from which it differs from the shape of the antenna and the notopodial lobes of the pre-branchial region (Aguirrezabalaga & Gil, 2009).

Ecological features: on circalittoral muddy bottoms, between 100 and 300 m depth.

Distribution: Mediterranean Sea: Adriatic Sea (Katzmann & Laubier, 1975); Tyrrhenian Sea (Castelli et al., 2008; present data); Sea of Sardinia (present data). Atlantic Ocean: Bay of Biscay (Aguirrezabalaga & Gil, 2009).

Aricidea (Strelzovia) meridionalis Laubier & Ramos, 1974

= *Aricidea suecica meridionalis* Laubier & Ramos, 1974

Material

Morphology only: Gulf of Cagliari, Sea of Sardinia, 20 m (1982); Gulf of Trieste, Adriatic Sea, 15 m (10/1995); Livorno, Tyrrhenian Sea, 14 m (02/1992).

Description: see Laubier & Ramos (1974).

Taxonomic notes: this species has been described as the Mediterranean subspecies of *Aricidea suecica* Eliason, 1920. The main differences with *A. suecica suecica* are represented by the shape of the notopodial lobes in the pre-branchial region (first two lobes tubercular, third elongate in *A. suecica suecica vs* all lobes elongate in *A. suecica meridionalis*), the shape of the anterior part of the body (width of segments gradually increasing in *A. suecica suecicavs* segments briskly wider in the pre-branchial region in *A. suecica meridionalis*) and the length of the antenna (less than the prostomial length in *A. suecica suecica vs* more than the

prostomial length in *A. suecica meridionalis*). These differences would actually justify the elevation of *A. suecica meridionalis* at the species rank, as *Aricidea meridionalis* Laubier & Ramos, 1974. The redescription by Aguirrezabalaga (2012) is somewhat ambiguous; in particular, Figure 91 B-C-D and Figure 92 probably refer to *Aricidea (Aricidea) fragilis* Webster, 1879.

Ecological features: on mixed bottoms, between 15 and 80 m depth. This is a large and relatively shallow species, that however rarely occurs in benthic samples.

Distribution: Mediterranean Sea (Laubier & Ramos, 1974; Castelli, 1987; Çinar et al., 2014); eastern Atlantic Ocean (Aguirrezabalaga, 2012).

Aricidea (Strelzovia) monicae Laubier, 1967

= ?*Aricidea (Strelzovia) bifurcata* Aguirrezabalaga & Gil, 2009

Material

Genetics and morphology: Otranto Strait, Adriatic Sea, 120 m (03/2015); Tuscan Archipelago, Tyrrhenian Sea, 110 m (02/2016).

Only morphology: Sardinian Slope, Sea of Sardinia, 900 m (10/2009); Tuscan Archipelago, Tyrrhenian Sea, 30-88 m (05/1985).

Description: see Aguirrezabalaga (2012).

Taxonomic notes: within the subgenus *Strelzovia*, this species resembles at most *Aricidea bifurcata* Aguirrezabalaga & Gil, 2009, which differs mainly in the shape of antenna (bifurcate in *A. bifurcata*, unbranched in *A. monicae*). Since the number of branches in the antenna in some species shows some variation (see for instance *A. fragilis*), probably the statement by Laubier & Ramos (1974) that double-antenna and single-antenna forms represent only a part of the variation of the species should be held as correct.

A. monicae is chiefly characterised by sub-trapezoidal prostomium, a really short prostomial antenna, few pairs of branchiae and the presence of 1-6 short, finger-like pre-chaetal lobes in the branchial region.

Ecological features: relatively sporadic species, generally reported between 200 and 1500 m depth, occasionally deeper or shallower. Always rare.

Distribution: Mediterranean Sea (Laubier, 1967; Katzmann & Laubier, 1975; Çinar, 2005); eastern Atlantic Ocean (Aguirrezabalaga & Gil, 2009); eastern Pacific Ocean (Strelzov, 1973; Blake, 1996 - doubtful).

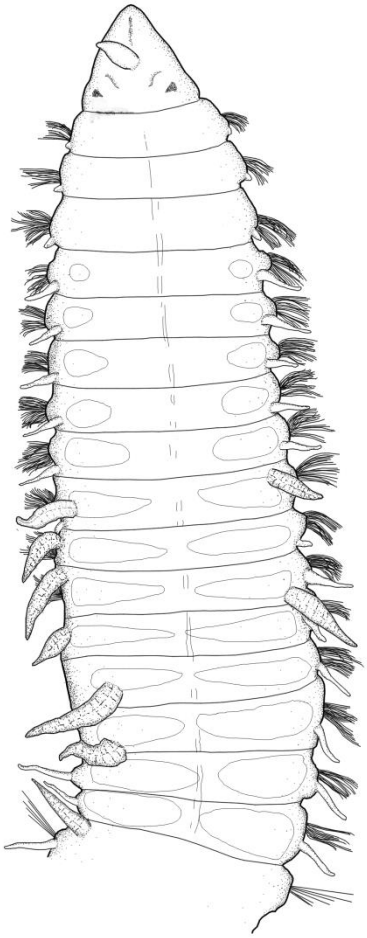

Figure 11: *Aricidea (Strelzovia) mariannae* Katzmann & Laubier, 1975 from the Tuscan Archipelago, Mediterranean Sea.

Aricidea (Strelzovia) quadrilobata Webster & Benedict, 1887

= *Aricidea annae* Laubier, 1967

= ?*Aricidea antennata* Annenkova, 1934

= ?*Aricidea uschakowi* Annenkova, 1937

= ?*Aricidea longicornuta* Berkeley & Berkeley, 1950

= *Aricidea suecica* Eliason, 1920 *sensu* Annenkova (1938) *fide* Strelzov (1973)

Material

Morphology only: Malta Escarpment, Ionian Sea, 1200-1500 m (05/2009).

Description: see Aguirrezabalaga (2012).

Taxonomic notes: according to Strelzov (1973) this species has a number of synonyms. The examination of Mediterranean individuals, referred by Laubier (1967) to *A. annae*, showed no differences towards western Atlantic topotypic individuals. On the other hand, the Pacific material is often referred to *Aricidea (Strelzovia) antennata* Annenkova, 1934, that is considered by some Authors as a different species (Aguirrezabalaga, 2012). As for other species with very wide distribution, molecular data are needed to clarify whether all individuals identified as *A. quadrilobata* actually belong to the same species.
Ecological notes: on mud, between 5 and 5600 m depth. Such a wide depth range suggests that not all records refer to the same species.
Distribution: allegedly cosmopolitan. Originally described for the western Atlantic Ocean (Webster & Benedict, 1887), it has subsequently been reported for the Arctic (Annenkova, 1934; Strelzov, 1973) and for the Pacific Ocean (Strelzov, 1973; Zhou & Li, 2007). In the Mediterranean Sea this is an uncommon species, mainly reported for circalittoral to bathyal environments (Laubier, 1967; Langeneck et al., *in press*),

Aricidea (Strelzovia) ramosa Annenkova, 1934

Material
Genetics and morphology: Coronado Bank, Pacific Ocean, 1100 m (07/2012).
Description: see Strelzov (1973) and Blake (1996).
Taxonomic notes: despite being one of the few *Aricidea* s.l. with branched antenna, *A. ramosa* is a poorly known species, and could actually represent a species complex, especially considering the really wide depth range where it occurs. Since type material is lost, Strelzov (1973) re-described the species on the basis of several Pacific individuals. Later, Eastern Pacific individuals have been considered as belonging to an undescribed species (Parker, 1996), characterised by a lower number of branches in the antenna. Since the number of branches in the antenna is a variable feature and the overall features of the examined individual match those of *A. ramosa*, I consider it as belonging to this taxon, even though a re-description based on Arctic material is needed to clarify the identity of this species.
Ecological features: an eurybathic species, mainly occurring in bathyal environments between 600 and 2000 m depth; locally shallower (10-80 m) (Strelzov, 1973).
Distribution: Arctic (Annenkova, 1934; Strelzov, 1973); Pacific Ocean (Strelzov, 1973; Blake, 1996).

Aricidea (Strelzovia) roberti Hartley, 1984

Material

Genetics and morphology: Galicia, Atlantic Ocean, 35 m (04/2015; 04/2016)

Description: see Aguirrezabalaga (2012).

Taxonomic notes: this species has been described in the frame of a revision of the material identified as *Aricidea suecica* Eliason, 1920 (Hartley, 1984) and belongs to a small group of *Strelzovia* with short prostomial antenna, relatively slender body and poorly modified neuropodial chaetae, comprising also *Aricidea bulbosa* Hartley, 1984, *A. hartleyi*, *Aricidea hartmani* Strelzov, 1968, *A. mariannae*, and *A. mirunekoa*. Among this group of poorly known species, *A. roberti* is characterised by slender body, and posterior branchiae with elongate tips.

Ecological features: this poorly known species appears related mainly to muddy environments between 25 and 200 m depth. Probably confused with other species. Locally common.

Distribution: eastern Atlantic Ocean (Hartley, 1984; Aguirrezabalaga & Gil, 2008; Ravara & Moreira, 2013).

Aricidea (Strelzovia) cf. ***suecica*** Eliason, 1920

= *Aricidea (Allia) nolani* Webster & Benedict, 1887 *sensu* Strelzov, 1973

= ?*Aricidea uschakovi* Zachs, 1925

= ?*Aricidea heteroseta* Hartman, 1948

Material

Genetics and morphology: Beals, Maine, Western Atlantic Ocean, 10 m (05/2016); Tuscan Archipelago, 110 m (08/2015)

Description: see Hartley (1984).

Taxonomic notes: the identity, and the validity, of *A. suecica* has been object of debate until Hartley's (1984) redescription. Strelzov (1973) considered *Aricidea nolani* Webster & Benedict, 1887 as the correct name, but Hartley (1984) demonstrated that the type material of *A. nolani* refers to two different species and thus this taxon should be considered as a *nomen dubium.*

Mediterranean records have been referred to *Aricidea (Strelzovia) suecica meridionalis* Laubier & Ramos, 1974. However, the individual sampled in the Tuscan Archipelago corresponds morphologically to the nominal subspecies, and is on the other hand quite different from *A. suecica meridionalis*. On the other hand, the Mediterranean and the Atlantic

individual are quite different with regard to the shape of the prostomium, and since the type locality of *A. suecica* is in the North Sea, it is possible that both represent undescribed species.

Ecological features: on muddy bottoms, from shallow waters (3-12 m) to bathyal depths (600-900 m) (Strelzov, 1973). Probably not all records actually refer to the same species.

Distribution: eastern Atlantic Ocean (Eliason, 1920; Strelzov, 1973; Hartley, 1984); western Atlantic Ocean (Strelzov, 1973); Mediterranean Sea (present data): northern Pacific Ocean (Strelzov, 1973).

Cirrophorus branchiatus Ehlers, 1908

= *Cirrophorus lyriformis* Annenkova, 1934

= ?*Cirrophorus aciculatus* (Hartman, 1957)

Material

Genetics and morphology: Loch Creran, Atlantic Ocean, 22 m (09/2003); Strait of Otranto, Adriatic Sea, 120 m (03/2015); Tuscan Archipelago, Tyrrhenian Sea, 110 m (11/2014).

Morphology only: Castiglione della Pescaia, Tyrrhenian Sea, 20 m (07/1984); Croatia, Adriatic Sea, depth unknown (11/2014); Cyprus, Levant Sea, 58 m (07/2014); Tuscan Archipelago, Tyrrhenian Sea, 76-120 m (08/1985).

Description: see Aguirrezabalaga (2012).

Taxonomic notes: although Strelzov (1973) showed that notopodial modified chaetae of the anterior-most segments are lyrate (whereas they are acicular in the post-branchial region) and thus *Cirrophorus lyriformis* is a junior synonym of this species, some authors still list the latter taxon as a valid species (see, for instance, Çinar et al., 2014). Strelzov (1973) listed as well *Cirrophorus aciculatus* among synonyms of *C. branchiatus*, however Blake (2016) rejected this claim, considering *C. aciculatus* as a valid species.

Ecological features: uncommon, but relatively regular, typically occurring on muddy bottoms between 20 and 150 m depth; sporadically deeper or shallower. Locally more abundant in enriched environments (e.g. under fish-farming cages).

Distribution: allegedly cosmopolitan; Strelzov (1973) reported it as a species with bipolar distribution, but this is probably an artefact due to sampling scarcity in the tropical and equatorial areas. The type locality is in the Southern Atlantic Ocean (Ehlers, 1908); reported from the Northern Atlantic Ocean (Glémarec, 1966; Hartley, 1981); Mediterranean Sea

(Laubier & Ramos, 1974; Katzmann & Laubier, 1975; Çinar, 2005; Çinar et al., 2014); Pacific Ocean (Hartman, 1957; Strelzov, 1973; Imajima, 1973).

Cirrophorus furcatus (Hartman, 1957)

Material

Genetics and morphology: Santa Monica Bay, Pacific Ocean (07/2002).

Description: see Blake (1996).

Taxonomic notes: although this species has been reported from the Mediterranean Sea and the Atlantic Ocean, Atlantic-Mediterranean records should be probably referred to undescribed species (herein reported as *Cirrophorus* sp. A, currently under description, and *Cirrophorus* sp. B) and/or to *Cirrophorus americanus* Strelzov, 1973. These three species are closely related and are difficult to identify, especially juvenile specimens.

Ecological features: on muddy bottoms, from the infralittoral to the bathyal zone (Blake, 1996).

Distribution: Pacific Ocean (Hartman, 1957; Blake, 1996).

***Cirrophorus* sp. A** (Fig. 12A-E)

= *Cirrophorus furcatus* (Hartman, 1957) *sensu* Auctt.

= *Paradoneis lyra* (Southern, 1914) *sensu* Auctt.

Material

Genetics and morphology: Livorno, Tyrrhenian Sea, 3 m (04/2016); Porto Pozzo, Tyrrhenian Sea, 0.5 m (07/2015); Tortolì Pond, Tyrrhenian Sea, 0,8 m (05/2016); Varano Lake, Adriatic Sea, 3 m (11/2014); Venice Lagoon, Adriatic Sea, 1 m (03/2015)

Morphology only: Calich Pond, Sea of Sardinia, 1 m (03/1988; 03/1995); Golfo Aranci, Tyrrhenian Sea, 5 m (01/1997; 12/1997); Gulf of Cagliari, Sea of Sardinia, 20 m (1982); Gulf of Follonica, Tyrrhenian Sea, 8 m (12/1987); Porto Pozzo, Tyrrhenian Sea, 4 m (07/1987); Portoferraio, Tyrrhenian Sea, 8 m (11/1982; 05/1990); Varano Lake, Adriatic Sea, 3 m (10/2015); Venice Lagoon, Adriatic Sea, 1 m (2002-2003).

Description: a medium-sized *Cirrophorus*, with maximum width between 0.2 and 0.45 mm, total length up to 15 mm. Complete individuals with 70-100 chaetigers, approximately. Prostomium roughly triangular, with a short, oval median antenna with central insertion; the antenna length is usually approximately 1/6 of the prostomium length and may be difficult to notice or it lacks in juvenile individuals. Three pre-branchial chaetigers, with short post-

chaetal notopodial lobes, gradually increasing towards the branchial region. This species shows a high number of branchiae (45-66); smaller individuals often show a lower number of branchiae (24-36) (Fig. 12A). The first 10-15 branchiae pairs are long, tapering and pointed; then branchiae become distinctly shorter and blunt, and in the last branchial chaetigers are noticeable as knobs (Fig. 12B). Notopodial lobes in the branchial region are conical, vaguely tapering, and remain approximately of the same length throughout the whole body length; in the last, pre-anal chaetigers they may be longer (even though not as long as in *C. americanus*). Pygidium wide, rounded, with three terminal cirri approximately of the same length. Parapodia biramous, with thick capillaries in both rami; notopodial modified chaetae from chaetiger 2-3 (occasionally 1 or 4) – usually from chaetiger 3; neuropodial modified chaetae in the posterior region, more evident in the last chaetigers (Fig. 12C). Notopodial modified chaetae lyriform, with the two rami approximately of the same width, one clearly longer than the other; internal edge of the rami distinctly serrated (Fig. 12D). In the first chaetigers are present only 1-2 lyrate chaetae, up to 3-4 around the 10^{th}-15^{th} chaetiger, then again 2 until the end of the body. Neuropodial modified chaetae are thickened capillaries, appearing in the post-branchial region, or in the last branchial chaetigers; initially there is only one, distinctly shorter and knife-shaped chaeta, then the number rises to 3-4; in the pre-anal chaetigers of well preserved individuals the neuropodium shows a row of 5-8 capillaries and a parallel row of 4-7 knife-shaped thickened capillaries. Modified neuropodial capillaries may be simply knife-shaped, or with a terminal thin arista; the arista can be contiguous to the blade or clearly deviated, sometimes with a subtle fringe between proximal and distal parts (Fig. 12E). Live colour bright orange, with golden-green posterior part; preserved individuals dark red.

Taxonomic notes: this species has been reported from the Mediterranean Sea, and possibly from the Atlantic Ocean, as *C. furcatus*. It is actually a new species, under description (Langeneck et al., *subm.*). Reports of *P. lyra* in Mediterranean brackish environments (e.g. Cognetti et al., 1978; Rossi & Lardicci, 1995; Maggiore & Keppel, 2007; Simboura et al., 2007) refer to this species as well.

Ecological features: in brackish, eutrophic environments, usually at shallow depths (0-3 m), often in proximity to seagrass (*Cymodocea*, *Zostera*, *Nanozostera*) meadows. Also in marine environments affected by strong organic pollution, such as ports and fish farming cages, where it may live deeper (5-20 m).

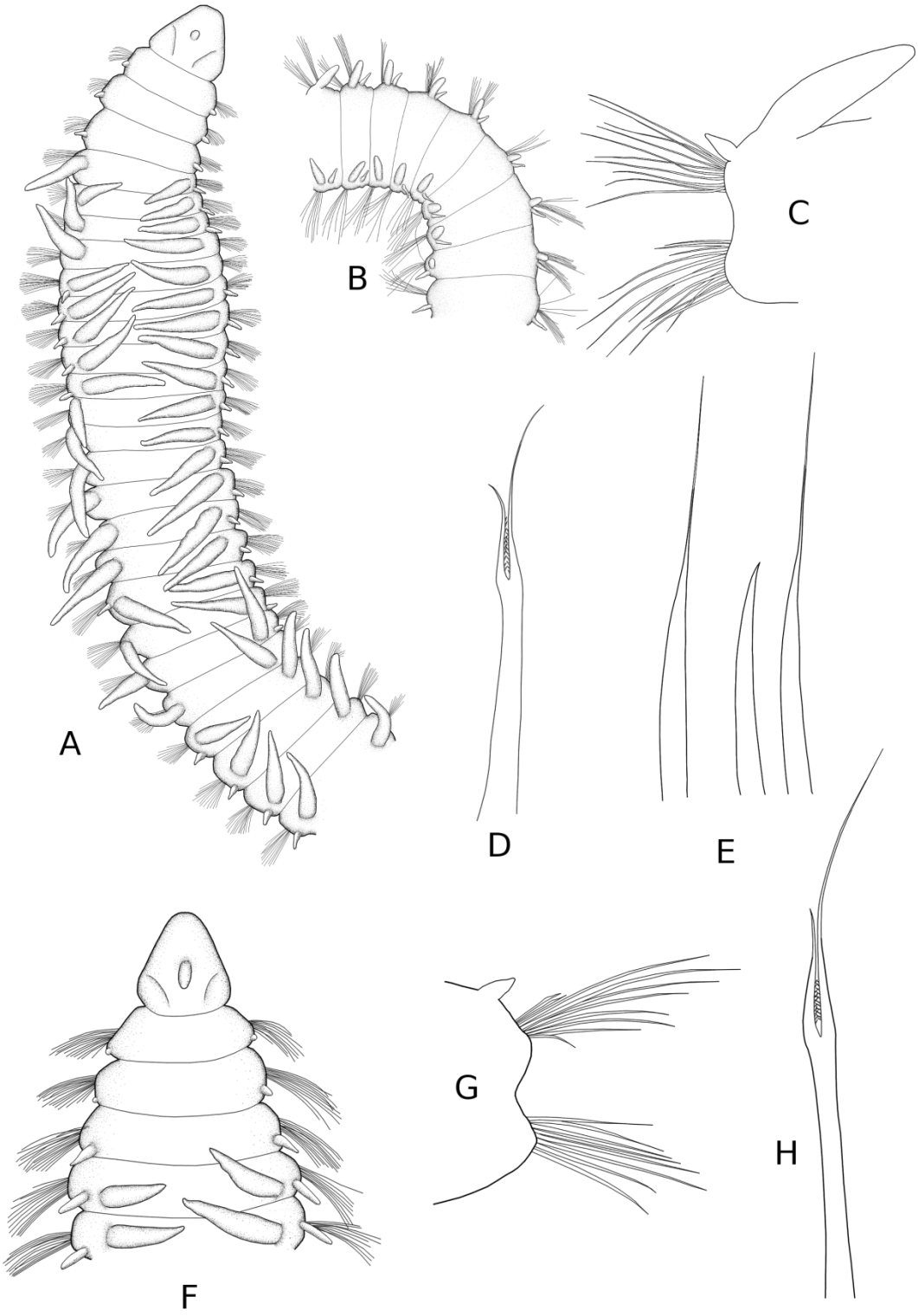

Figure 12: *Cirrophorus* sp. A: A) Anterior part of the body in dorsal view; B) End of the branchial region; C) Schematic view of a parapodium in the branchial region; D) Modified notochaeta from the posterior part of the body; E) Modified neurochaetae from the posterior part of the body. *Cirrophorus* sp. B: F) Anterior part of the body in dorsal view; G) Schematic view of a parapodium in the post-branchial region; H) Modified notochaeta from the posterior part of the body. A-E: Calich Pond, Mediterranean Sea; F-H: Bay of Limassol, Cyprus, Mediterranean Sea.

Distribution: Mediterranean Sea: Balearic Sea, Sea of Sardinia, Tyrrhenian, Adriatic and Aegean Sea. Possibly Atlantic Ocean.

***Cirrophorus* sp. B** (Fig. 12F-H)

= *Cirrophorus* cf. *lyriformis* Annenkova, 1934 *sensu* Laubier, 1966c

= *Cirrophorus furcatus* (Hartman, 1957) *sensu* Katzmann & Laubier, 1975

Genetics and morphology: Livorno, Tyrrhenian Sea, 3 m (04/2016).

Morphology only: Cyprus, Levant Sea, 6 m (07/2014).

Description: medium sized species (0.2-0.45 mm maximum width). Prostomium roughly triangular, with short, blister-like or cirriform median antenna. Eyes absent. Three pre-branchial chaetigers, with notopodial lobes gradually increasing in length. 16-22 pairs of elongated, pointed branchiae; the branchial region ends abruptly, without a strong and gradual reduction in branchiae length (12F). Parapodia biramous, composed by two bundles of thick capillaries; notopodial lobes strongly decreasing in length in the post-branchial region (Fig. 12G). Notopodial modified chaetae are lyrate and start from chaetiger 2-3 (4), usually from chaetiger 2. In the posterior part of the body one ramus of the lyrate chaetae is thicker and shorter than the other one (Fig. 12H). Live colour yellowish-orange, similar to *Cirrophorus* sp. A.

Taxonomic notes: among the described species, *Cirrophorus* sp. B resembles at most *Cirrophorus americanus* Strelzov, 1973 in the beginning of modified chaetae at the 2nd chaetiger. However, in this species the median antenna is longer, the number of branchiae is higher and the notopodial lobes do not show a gradual increase towards the pre-branchial region. It is probably an undescribed Mediterranean species.

Ecological features: in enriched environments, at shallow depths, often with high density. This species can occur in syntopy with *Cirrophorus* sp. A (e.g. Livorno), but seems to be less tolerant towards salinity variations, and occurs mainly in strictly marine environments.

Distribution: Mediterranean Sea: Levant Sea; Adriatic Sea (Katzmann & Laubier, 1975, as *Cirrophorus furcatus*); Tyrrhenian Sea; Balearic Sea (Laubier, 1966c; Laubier & Ramos, 1974, as *Cirrophorus* cf. *lyriformis*).

Levinsenia demiri Çinar, Açik & Dağli, 2011

Material

Genetics and morphology: Southern Adriatic Sea, 75-120 m (03/2015); Tuscan Archipelago, Tyrrhenian Sea, 110 m (11/2014; 02/2016).

Morphology only: Tuscan Archipelago, Tyrrhenian Sea, 110 m (02/2016).

Description: see Çinar et al. (2011).

Taxonomic notes: similarly to the majority of the Mediterranean *Levinsenia* spp., *L. demiri* has been historically confused with *Levinsenia gracilis* (Tauber, 1879), a species with allegedly cosmopolitan distribution, but probably less widespread than commonly thought. *L. demiri* shows strong similarities with *Levinsenia kantauriensis* Aguirrezabalaga & Gil, 2009, but it differs in the shape of notopodial lobes and in the main ecological traits.

Ecological features: on muddy bottoms, between 20 and 120 m depth, generally with high densities. This species occurs often in bottoms with vegetal debris and is the most common *Levinsenia* in Mediterranean environments.

Distribution: eastern Mediterranean Sea (Çinar et al., 2011); western Mediterranean Sea and Adriatic Sea (present data).

Levinsenia gracilis (Tauber, 1879)

Material

Genetics and morphology: Loch Creran, Atlantic Ocean, 19 m (09/2003); Tuscan Archipelago, Tyrrhenian Sea, 110 m (11/2014).

Description: see Aguirrezabalaga (2012).

Taxonomic notes: this taxon is considered cosmopolitan and it has been used as a dumping ground for a number of small Atlantic and Mediterranean *Levinsenia* spp. After its description, *L. gracilis* has not been reviewed nor re-described, as a consequence, there is the possibility this name has been used for several different organisms.

Ecological features: on muddy bottoms, between 10 and 120 m depth. Deeper records probably refer to different species.

Distribution: allegedly cosmopolitan (Strelzov, 1973; Hartley, 1981; Blake, 1996; Aguirrezabalaga, 2012). Its presence in the Mediterranean Sea has been considered controversial, but morphologically close individuals have been reported from both the western and eastern Mediterranean Sea (present data; M. Rousou, *pers. comm.*).

Levinsenia kantauriensis Aguirrezabalaga & Gil, 2009

Material

Genetics and morphology: Southern Adriatic Sea, 598-970 m (03/2015).

Description: see Aguirrezabalaga (2012).

Taxonomic notes: this species shows remarkable similarities with *L. demiri*, from which it differs mainly for the shape of the notopodial lobes; the two species show different ecological features as well.

Ecological features: on bathyal mud, between 500 and 1100 m depth.

Distribution: eastern Atlantic Ocean (Aguirrezabalaga & Gil, 2009); Mediterranean Sea (present data).

Levinsenia kosswigi Çinar, Açik & Dağli, 2011

Material

Genetics and morphology: Tuscan Archipelago, Tyrrhenian Sea, 110 m (11/2014).

Morphology only: Tuscan Archipelago, Tyrrhenian Sea, 110 m (09/2012)

Description: see Çinar et al. (2011).

Taxonomic notes: along with *L. materi*, this species has been historically confused with *Levinsenia oculata* (Hartman, 1957), a Pacific species (Castelli et al., 1995). Previous records of *L. oculata* should be carefully re-evaluated in order to assess the actual distribution of this species.

Ecological features: uncommon, but regular, on circalittoral muddy bottoms between 60 and 110 m depth.

Distribution: eastern Mediterranean Sea (Çinar et al., 2011); western Mediterranean Sea (present data).

Levinsenia materi Çinar & Dağli, 2013

Material

Genetics and morphology: Gulf of Palermo, Tyrrhenian Sea, 10 m (11/2014); Porto S. Stefano, Tyrrhenian Sea, 8 m (06/2013)

Morphology only: Cyprus, Levant Sea, 20 m (07/2014)

Description: see Çinar & Dağli (2013).

Taxonomic notes: probably this species, similarly to *L. kosswigi*, has been confused with *L. oculata.*

Ecological features: uncommon, originally described from *Posidonia oceanica* matte in the Levant Sea (Çinar & Dağli, 2013) between 20 and 40 m depth, rare on sandy or mixed bottoms. In Italian waters it has been rarely reported from mixed bottoms between 8 and 10 m depth.

Distribution: eastern Mediterranean Sea (Çinar & Dağli, 2013); western Mediterranean Sea (present data).

***Levinsenia* sp. A** (Fig. 13)

= *Levinsenia* sp. 1 *sensu* Langeneck et al. (*in press*)

Material

Genetics and morphology: Strait of Otranto, Adriatic Sea, 120 m (03/2015).

Morphology only: Maltese Slope, Strait of Sicily, 1200-2100 m (05/2009); Sardinian Slope, Sea of Sardinia, 600 m (10/2009).

Description: a small-sized species (0.18 mm maximum width) of *Levinsenia* with pointed prostomium, clearly observable apical organ. 7-8 pre-branchial chaetigers, 10 (8-12) pairs of short, leaf-shaped and pointed branchiae (Fig. 13A-B). Notopodial lobes short, poorly noticeable. Modified chaetae almost straight, with only slightly curved tip, without hood, longitudinally striated (Fig. 13C). Live colour unknown, preserved individual whitish-yellowish.

Taxonomic notes: within the genus *Levinsenia* this species shows strong similarities with *L. oculata*, *L. materi*, *Levinsenia kirbyae* Lovell, 2002, *Levinsenia reducta* (Hartman, 1965) and *Levinsenia acutibranchiata* (Strelzov, 1973). *L. oculata* is the only species of the genus without a noticeable hood on the dorsal edge of the hooks and it has been described as a highly variable species. The shape of the prostomium and absence of large, conspicuous nuchal organs allow to distinguish this species from *L. oculata*. *L. materi*, *L. kirbyae, L. reducta* and *L. acutibranchiata* are characterised by the presence of a dorsal hood on the hooks. Moreover, *L. materi* shows a higher number of longer branchiae, *L. kirbyae* has neuropodial hooks in double row, and *L. reducta* is a bathyal species with an almost rounded prostomium and strongly crooked hooks. This species resembles at most *L. acutibranchiata*, but probably is an undescribed species, belonging to the same group of *L. kosswigi* and *L. materi*, but related to deeper environments.

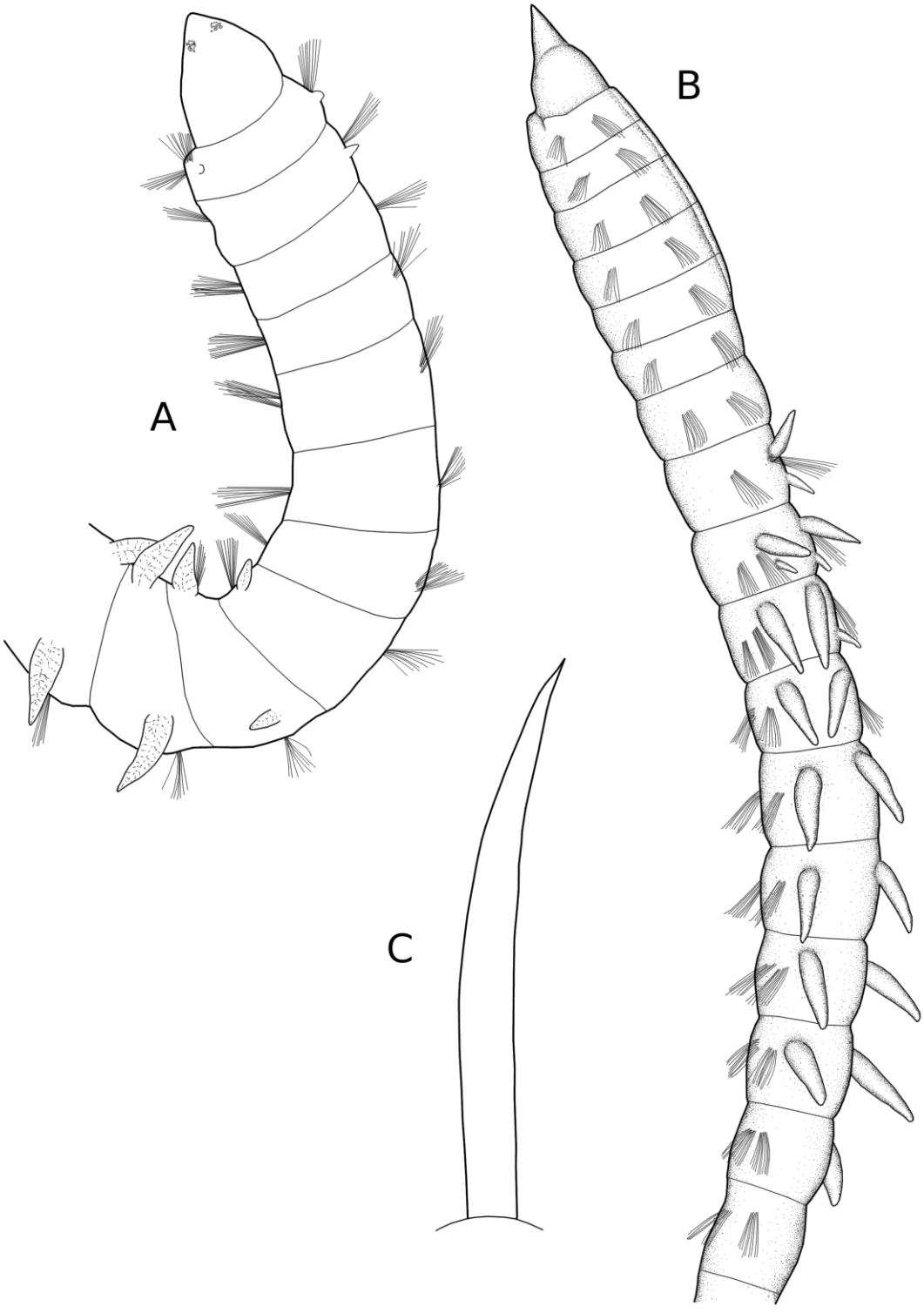

Figure 13: *Levinsenia* sp. A: A) Anterior part of the body in dorsal view; B) Anterior part of the body in lateral view; C) Modified neurochaeta from the posterior part of the body. A: Southern Adriatic Sea, Mediterranean Sea; B-C: Malta Escarpment, Mediterranean Sea.

Ecological features: on compact mud from the lower circalittoral to bathyal environments (120-2100 m depth).

Distribution: until now known only for the western and central Mediterranean Sea.

Paradoneis armata Glémarec, 1966

= ?*Paradoneis harpagonea* Storch, 1967

Material

Genetics and morphology: Albegna River Mouth, Tyrrhenian Sea, 7 m (06/2014); Ansedonia, Tyrrhenian Sea, 10 m (06/2014); Bay of Rosas, Balearic Sea, 9.5 m (07/2014); Cala di Forno, Tyrrhenian Sea, 10 m (04/2013; 06/2014); Cinquale, Ligurian Sea, 9 m (03/2014; 07/2014); Cyprus, Levant Sea, 8 m (07/2015); Elba Island, Tyrrhenian Sea, 10 m (06/2014); Galicia, Atlantic Ocean, 30-35 m (04/2015; 04/2016); Gulf of Palermo, Tyrrhenian Sea, 10 m (11/2014); Marina di Carrara, Tyrrhenian Sea, 10 m (06/2015); Ombrone River Mouth, Tyrrhenian Sea, 10 m (06/2014); Salivoli, Tyrrhenian Sea, 10 m (04/2014).

Morphology only: Arno River Mouth, Tyrrhenian Sea, 2-20 m (06/1985); Calich Pond, Sea of Sardinia, 1 m (11/1987; 03/1995); Castiglione della Pescaia, Tyrrhenian Sea, 10 m (07/1984); Cyprus, Levant Sea, 10 m (07/2014); Giglio Island, Tyrrhenian Sea, 8 m (08/2012); Golfo Aranci, Tyrrhenian Sea, 5 m (12/1997); Gulf of Follonica, Tyrrhenian Sea, 6-8 m (12/1987; 05/1988); Porto Pozzo, Tyrrhenian Sea, 1 m (07/1987).

Description: see Aguirrezabalaga (2012).

Taxonomic notes: even though López-Jamar et al. (1987) assessed the synonymy between *P. armata* and *P. harpagonea*, stating that the differences observed by Strelzov (1973) depended on the stage of growth of the individual and on the preservation state of chaetae, there is still some uncertainty on the correctness of such synonymy. Reuscher (2013) considers *P. harpagonea* as a valid species and biogeographic features support this consideration. In fact, *P. armata* has been described in the Atlantic Ocean, whereas the type locality of *P. harpagonea* is in the Red Sea. On the other hand, since type material of *P. harpagonea* went lost and Strelzov's re-description is based on Black Sea individuals, this re-description probably refers to *P. armata*. Topotypic material is needed in order to clarify the taxonomic status of these two taxa.

Ecological features: very common on fine sandy bottoms (SFBC biocoenoses *sensu* Péres & Picard, 1964) at 7-10 m depth (although it has been also reported from deeper environments) (Castelli, 1985). It becomes uncommon on mixed bottoms at the same depths (e.g. in the

proximity of river mouths, where the bottom is affected by silt inclusions). Shallower in enclosed, brackish-water environments (where it is uncommon). Deeper in the Atlantic Ocean. The report by Aguirrezabalaga & Gil (2009) from 1000 m depth is puzzling, and could refer to an undescribed deep-water species.

Distribution: eastern Atlantic Ocean (Glémarec, 1966; Aguirrezabalaga & Gil, 2008; 2009); Mediterranean Sea (Laubier, 1971; Laubier & Ramos, 1974; Castelli, 1985; Çinar, 2005; Zaâbi et al., 2012); Black Sea (Strelzov, 1973); Red Sea? (Storch, 1967); Pacific Ocean? (Lovell, 2002).

Paradoneis ilvana Castelli, 1985

= ?*Paraonis (Paraonides) neapolitana* Cerruti, 1909

= ?*Paradoneis lyra capensis* (Day, 1955)

= ?*Paradoneis capensis* (Day, 1955)

= *Cirrophorus neapolitanus* (Cerruti, 1909) *sensu* Strelzov, 1973

Material

Genetics and morphology: Capraia Island, Tyrrhenian Sea, 14 m (05/2014); Pianosa Island, Tyrrhenian Sea, 0,8 m (07/2014; 04/2015); Porto Pozzo, Tyrrhenian Sea, 1 m (07/2015).

Morphology only: Calich Pond, Sea of Sardinia, 1 m (07/1987; 11/1987; 03/1995); Elba Island, Tyrrhenian Sea, 8-10 m (10/1982; 11/1982 [holotype]); Giglio Island, Tyrrhenian Sea, 8 m (08/2012); Gulf of Follonica, Tyrrhenian Sea, 8-10 m (12/1987); Porto Pozzo, Tyrrhenian Sea, 1 m (07/1987); Strait of Messina, 25-99 m (07/1992); Trieste, Adriatic Sea, 15 m (10/1995).

Description: see Aguirrezabalaga (2012). In the original description (Castelli, 1985) the species is described with an achaetous peristomial ring (absent in Paraonidae). A close examination of the holotype, currently preserved in the polychaete collection of the University of Pisa showed that this is a fixation artefact.

Taxonomic notes: this recently described species has a complex taxonomic history. The type species of *Paraonides*, namely *P. neapolitana*, probably has been described on an individual that, according to Strelzov (1973), has morphological features matching *P. ilvana*. However, as correctly observed Katzmann & Laubier (1975), since type material went lost and Strelzov did not examine Mediterranean material, the identification and re-description of *Cirrophorus neapolitanus* given by Strelzov cannot be assumed as correct. The Mediterranean species was later described by Castelli (1985) with the currently used name.

Strelzov synonymised also *Paradoneis lyra capensis* (Day, 1955) with *P. neapolitana*. Reuscher (2013) examined type material of *P. capensis* and *P. ilvana*, raising the first taxon at species level and concluding that these species are very similar and included in a well-characterised clade, but they are probably distinct. However, with the current state of knowledge, a synonymy between *P. capensis* and *P. ilvana* cannot be excluded – in this case, the correct name would be *Paradoneis capensis* (Day, 1955).

Ecological features: shallow species (typically 1-15 m depth), on fine to coarse sandy bottoms; locally (Elba Island, Giglio Island) syntopic with *P. armata*. It is one of the shallower species in the Mediterranean Sea, and locally can be found in brackish-water environments, sometimes together with *Cirrophorus* sp. A and *A. cerrutii*.

Distribution: Mediterranean Sea (Castelli, 1985; Rossi & Lardicci, 1995; Çinar, 2005; Çinar et al., 2014); eastern Atlantic Ocean (Strelzov, 1973; Aguirrezabalaga & Gil, 2008); Black Sea (Strelzov, 1973).

Paradoneis lyra (Southern, 1914)

= ?*Paradoneis mikeli* Aguirrezabalaga & Gil, 2009

= ?*Paradoneis hirsuta* Sardá, Gil, Taboada & Gili, 2009

Material

Genetics and morphology: Bari, Adriatic Sea, 75 m (03/2015); Loch Creran, Irish Sea, 25 m (09/2003); Southern Adriatic Sea, 217 m (03/2015); Tuscan Archipelago, Tyrrhenian Sea, 110 m (02/2016).

Morphology only: Asinara Gulf, Sea of Sardinia, 15 m (02/2000); Golfo Aranci, Tyrrhenian Sea, 5 m (12/1997); Gulf of Trieste, Adriatic Sea, 15 m (10/1995); Ravenna, Adriatic Sea, 15 m (07/1987); Tuscan Archipelago, Tyrrhenian Sea, 50-380 m (08/1985).

Description: see Aguirrezabalaga (2012) and Mackie (1991).

Taxonomic notes: despite being one of the first Paraonidae to be described (Southern, 1914), *P. lyra* is still a poorly known species. The two subspecies *P. lyra capensis* (Day, 1955) and *P. lyra guadalupensis* (Amoureux, 1985) are currently considered valid species and *P. lyra* has been only recently separated from the similar *Paradoneis eliasoni* Mackie, 1991. Deep-water forms have been described as different species even more recently, as *P. mikeli* and *P. hirsuta*; the differences observed by Aguirrezabalaga & Gil (2009) between *P. lyra* and *P. mikeli* can be attributed to the different environments where the individuals occur and fall within the variability of a single species. On the other hand, *P. hirsuta* is known only from a

single, epitoke individual (Sardá et al., 2009) and could just be the reproductive form of *P. lyra*, which has been already described by Southern (1914).

Ecological features: on muddy bottoms, from 15-20 m depth to higher depths (300-400 m, maybe more). In the Mediterranean Sea it is generally a rare, deep-water species, commonly occurring in shallow environments only in the Adriatic Sea.

Distribution: eastern Atlantic Ocean (Southern, 1914; Strelzov, 1973; Aguirrezabalaga, 2012); Mediterranean Sea (Katzmann & Laubier, 1975; Aguirrezabalaga, 2012); Pacific Ocean (Imajima, 1973 – *dubious*).

Paradoneis mikeli Aguirrezabalaga & Gil, 2009

= ?*Paradoneis hirsuta* Sardá, Gil, Taboada & Gili, 2009

Material

Genetics and morphology: Southern Adriatic Sea, 598 m (03/2015).

Morphology only: Sardinian Slope, Sea of Sardinia, 600 m (10/2009).

Description: see Aguirrezabalaga (2012).

Taxonomic notes: this species has been described as a deep-water form of *P. lyra* (Aguirrezabalaga & Gil, 2009); nevertheless, the most striking difference observed between the two species, namely the position of the anus, is clearly a mistake, since the dorsal opening of the anus is a symplesiomorphic feature of this family (Reuscher, 2013). The other differences observed may be considered within the intraspecific variability of *P. lyra*. Also *P. hirsuta*, an epitoke form described on the basis of a single individual, could be included within the variability of *P. mikeli*. If the two taxa were found to be synonymous, for priority rules *P. hirsuta* would be the correct name of the species.

Ecological features: bathyal, on compact mud, between 500 and 1100 m depth.

Distribution: eastern Atlantic Ocean (Aguirrezabalaga & Gil, 2009); Mediterranean Sea (Amoureux, 1982?; present data).

Paradoneis spinifera (Hobson, 1972)

Material

Genetics and morphology: Point Loma, Pacific Ocean, 240 m (07/2012).

Description: see Blake (1996).

Taxonomic notes: according to Reuscher (*in litt.*) the *Paradoneis* spp. with simple acicular notopodial modified chaetae (*P. spinifera* and *Paradoneis drachi* Laubier & Ramos, 1974) is

included in a clade sister to the remaining *Cirrophorus*/*Paradoneis* spp. and should be assigned, therefore, to a genus yet to be described. The claim of possible synonymy with the Atlantic-Mediterranean *P. drachi* (Blake, 1996) is, in my opinion, unsubstantiated.

Ecological features: deep circalittoral and bathyal, on mud.

Distribution: eastern Pacific Ocean (Hobson, 1972; Blake, 1996).

Paraonis fulgens (Levinsen, 1884) (Fig. 14)

Material

Genetics and morphology: Albegna River Mouth, Tyrrhenian Sea, 7 m (06/2014); Cinquale, Tyrrhenian Sea, 7 m (03/2014); Mola, Tyrrhenian Sea, 10 m (06/2014); Newton-by-the-Sea, North Sea, tide level (08/2003).

Morphology only: Carbonifera, Tyrrhenian Sea, 7 m (06/2015); Elba Island, Tyrrhenian Sea, 5-12 m (10/1982; 10/1983); Gulf of Follonica, Tyrrhenian Sea, 6 m (05/1988); Rosignano Solvay, Tyrrhenian Sea, 5-8 m (02/1983; 02/1985); Tirrenia, Tyrrhenian Sea, 1.5 m (07/1985).

Description: see Aguirrezabalaga (2012) and Castelli (1985). In contrast to what Aguirrezabalaga (2012) writes, the pygidium of this species is actually well-known, showing three filiform cirri, similarly to the majority of Paraonidae. The last chaetiger may be reduced, devoid of modified neurochaetae (Fig. 14C), but is not fused to the pygidium as in *Paraonis pygoenigmatica* Jones, 1968.

Taxonomic notes: Mediterranean individuals are smaller than Atlantic ones and this led to differentiate them at least at subspecific level (A. Castelli, *pers. comm.*); this claim was later rejected and does not appear in Castelli (1985). However, further research is needed to clarify whether Mediterranean and Atlantic *P. fulgens* actually belong to the same species, given the peculiar environment where this species commonly lives.

Ecological features: common, but rarely abundant, on fine to coarse sandy bottoms between tide level and 10 m depth. Because of its really small size this species may often pass unnoticed, or confused with other species, in particular juveniles of *Paradoneis armata*, with which it lives often in sympatry. *P. fulgens* is bioluminescent, however the ecological and evolutionary meaning of this trait is still unknown.

Distribution: eastern Atlantic Ocean (Levinsen, 1884; Aguirrezabalaga, 2012); western Atlantic Ocean (Pettibone, 1963; Gaston et al., 1992); Mediterranean Sea (Laubier & Ramos, 1974; Castelli, 1985).

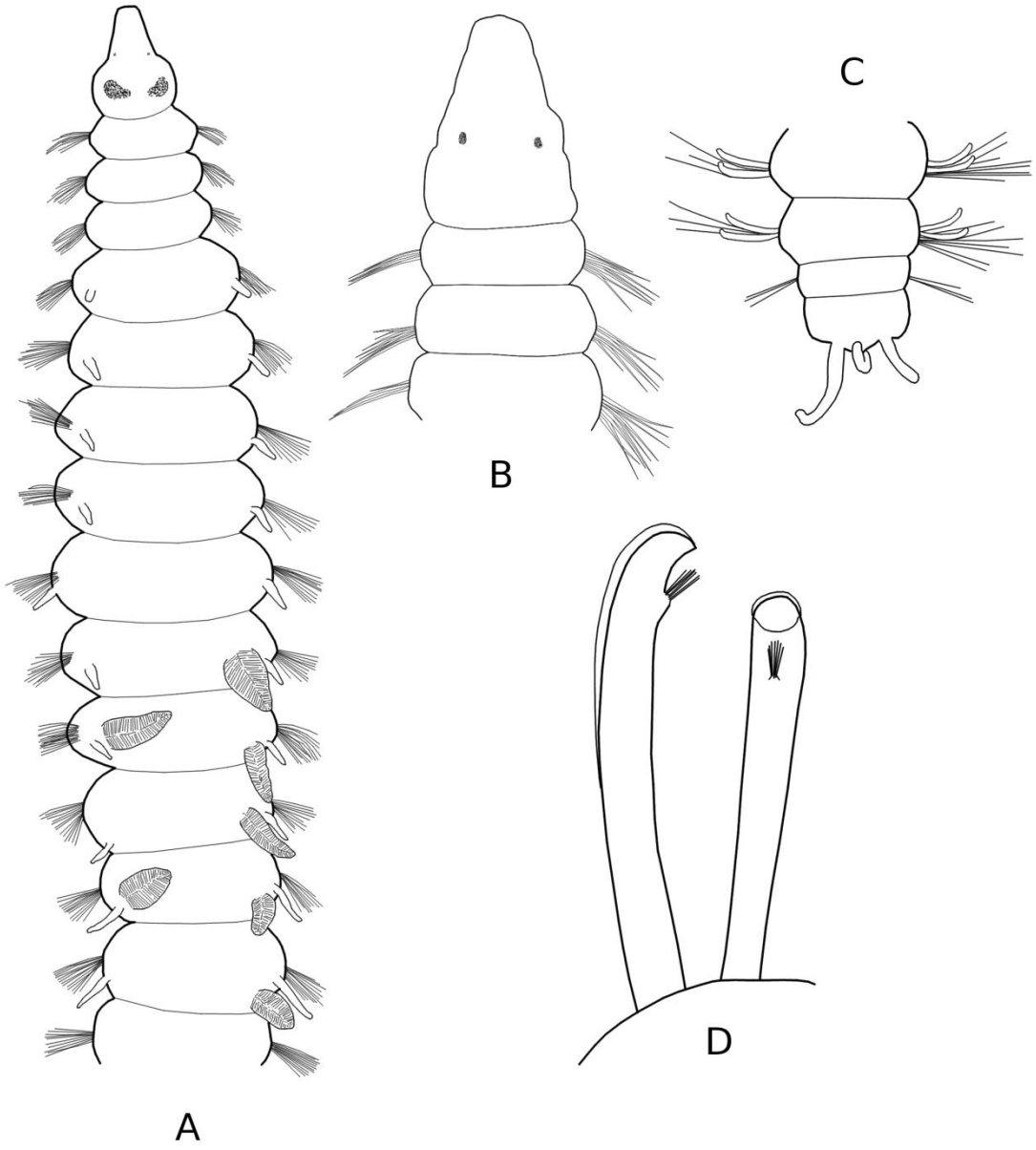

Figure 14: *Paraonis fulgens* (Levinsen, 1884) from the Mediterranean Sea: A) Anterior part of the body in dorsal view; specimen with partially swollen prostomium, clearly showing the nuchal organs; B) Prostomium and pre-branchial chaetigers; C) Pygidium and last chaetigers in ventral view; D) Modified neurochaetae from the posterior part of the body. A, C and D: Carbonifera, Tyrrhenian Sea; B: Cinquale, Tyrrhenian Sea.

3. Chapter 1: A new species of *Cirrophorus* (Annelida: Paraonidae) from the Mediterranean Sea, with taxonomic notes on the genera *Cirrophorus*, *Paradoneis* and *Paraonides*[1]

3.1 Abstract

Cirrophorus sp. A is described from brackish-water and organically enriched marine environments of the Mediterranean Sea. The new species is characterised by a very small prostomial antenna and a high number of branchiae pairs. A phylogenetic analysis carried out through the use of three molecular markers (16S rRNA, 18S rRNA and COI) supports the distinction between *Cirrophorus* sp. A and *C. furcatus*, a closely related species with which it has been misidentified. Preliminary results obtained show that the genera *Cirrophorus* and *Paradoneis* are not reciprocally monophyletic, with uncertain relationships with the remaining genera of Paraonidae. This outcome suggests that the evolutionary history of Paraonidae is less straightforward than previously supposed. Moreover, the uncertainty about the taxonomic status of *Paraonides neapolitana*, type species of the genus *Paraonides*, makes the revamping of the taxonomy of Paraonidae more challenging. Awaiting support from studies including more species, and based on morphological and genetic data as well, I suggest to provisionally maintain the current use of *Cirrophorus* and *Paradoneis*, and to assign to *Paraonella* the species traditionally assigned to *Paraonides*.

3.2 Introduction

The Paraonidae constitutes one of the most diverse and taxonomically complex family among polychaetes. This family has been investigated in the Mediterranean Sea by several authors (Cerruti, 1909; Laubier, 1967; Laubier & Ramos, 1974; Katzmann & Laubier, 1975; Castelli, 1985), but the knowledge of the Mediterranean Paraonidae is far from being complete, and new species were recently described, even from shallow environments where polychaete fauna is better known (Sardá et al., 2009; Çinar et al., 2011; Çinar & Dağli, 2013). Moreover, within Paraonidae, the taxonomy of several genera is unclear and the whole family needs a taxonomic revision based on the re-examination of existing type material and the study of new material, covering also poorly known areas, using both molecular and morphological data.

[1] *Manuscript submitted to Journal of the Marine Biological Association of the U.K.*

The genus *Cirrophorus* Ehlers, 1908 has been generally considered to be represented in the Mediterranean Sea by two species, namely *Cirrophorus branchiatus* Ehlers, 1908 and *Cirrophorus furcatus* (Hartman, 1957). Some authors list an additional species, *Cirrophorus lyriformis* Annenkova, 1934 (Çinar et al., 2014), a species synonymised with *C. branchiatus* by Strelzov (1973) even if most of its Mediterranean records were later attributed to *C. furcatus* (see Katzmann & Laubier, 1975, and references therein). On the other hand, the identification of the Mediterranean *C. furcatus* has been more problematic, and several different species, or at least morphotypes, seem to be involved. Mediterranean individuals of the shallow water *Cirrophorus* near *furcatus***,** commonly found in brackish-water habitats and, in a more general way, organically-enriched environments, was questioned previously (Castelli et al., 1995), as the Mediterranean individuals show morphological and ecological differences with respect to those from California, the type locality of the species (Hartman, 1957).

Previous works on polychaetes have widely demonstrated that the combined use of morphological and molecular data is a powerful approach to disentangle complex systematic problems and make attempts to clarify evolutionary processes (i.a. Cadman & Nelson-Smith, 1990; Wu et al., 1991; Maltagliati et al., 2001; 2004; 2005; Nygren & Pleijel, 2011). In the frame of a systematic revision of the Mediterranean Paraonidae, in this work I identify on the basis of morphological and molecular data two divergent morphotypes that were historically identified as *Cirrophorus furcatus*. One of these morphotypes is described as a new species, whereas the other one represents a putative new species, but is not described because of the scarce material available. Moreover, the synonymy between the genera *Cirrophorus* and *Paradoneis* Hartman, 1965, and the use of the generic names *Paraonides* Cerruti, 1909 and *Paraonella* Strelzov, 1973 are discussed with base on the results from molecular data and the critical analysis of the literature.

3.3 Materials and methods

Samples of *Cirrophorus* near *furcatus* for morphological study were obtained from the collection of the University of Pisa. Specimens were fixed with 4% neutralized formaldehyde in seawater and subsequently preserved in 70% ethanol. Measurements and counts were performed with a Primo Star Zeiss light microscope equipped with an ocular micrometer; drawings were made from pictures taken with a digital camera, and refined with GIMP 2.8.18 (software downloadable and documentation available at http://www.gimp.org), following the

guidelines in Montesanto (2015). Reference material was deposited in the polychaete collection stored at the Department of Biology, University of Pisa. Live Paraonidae for molecular analyses were collected in several localities of the Mediterranean Sea and Atlantic Ocean (Table 1), fixed directly in 96% or 70% ethanol and preserved at 4 °C until DNA extraction. Among the available material, I chose all species of *Cirrophorus* and *Paradoneis* and the type species of *Aricidea* Webster, 1879, *Levinsenia* Mesnil & Caullery, 1898, and *Paraonis* Cerruti, 1909; when possible, I used material from type localities.

Table 1: Paraonidae analysed from the molecular point of view in the study. Ad = Adriatic Sea; At = Atlantic Ocean; T = Tyrrhenian Sea.

Species	Locality	Depth	Date	N
Aricidea fragilis Webster, 1879	Gulf of Manfredonia (Ad)	8 m	09/2014	1
Cirrophorus branchiatus Ehlers, 1908	Tuscan Archipelago (T)	110 m	11/2014	1
Cirrophorus sp. A	Livorno port (T)	3 m	04/2016	1
Cirrophorus sp. A	Porto Pozzo (T)	0.5 m	07/2015	2
Cirrophorus sp. A	Venice Lagoon (Ad)	1 m	03/2015	4
Cirrophorus sp. B	Livorno port (T)	3 m	04/2016	1
Levinsenia gracilis (Tauber, 1879)	Loch Creran (At)	19 m	09/2003	1
Paradoneis armata Glémarec, 1966 I	Bay of Biscay (At)	30 m	04/2015	1
Paradoneis armata Glémarec, 1966 II	Elba Island (T)	7 m	06/2014	1
Paradoneis ilvana Castelli, 1985 I	Pianosa Island (T)	0.8 m	04/2015	1
Paradoneis ilvana Castelli, 1985 II	Capraia Island (T)	20 m	07/2015	1
Paradoneis lyra (Southern, 1914) I	Loch Creran (At)	25 m	09/2003	1
Paradoneis lyra (Southern, 1914) II	Tuscan Archipelago (T)	110 m	02/2016	1
Paraonis fulgens (Levinsen, 1884)	Newton-by-the-Sea (At)	0.2 m	08/2003	1

DNA extraction was carried out using the GenElute™ Mammalian Genomic DNA Miniprep Kit distributed by Sigma-Aldrich, following the manufacturer's instructions. For phylogenetic reconstruction I amplified the genes for 16S rRNA and COI (mitochondrial) and 18S rRNA (nuclear). 16S rDNA amplification was obtained using the primer pair 16SarL (5'-CGCCTGTTTAACAAAAACAT-3') and H3080 (5′-CCGGTCTGAACTCAGATCACGT-3′) (Palumbi et al., 1991), whereas for COI amplification I used the universal primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994) and the annelid-specific primers POLYLCO (5'-GAYTATWTTCAACAAATCATAAAGATATTGG-3') and POLYHCO (5'-TAMACTTCWGGGTGACCAAARAATCA-3') (Carr et al., 2011). 18S rDNA amplification was obtained using the primers F9 (5'-CTGGTTGATCCTGCCAG- 3') (Medlin et al., 1988) and R1513 (5'-TGATCCTTCYGCAGGTTC-3') (Petroni et al., 2002). Polymerase chain reaction (PCR) amplifications were carried out in 20 μL solutions using 1.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.1 μM of each primer, 1 U of DreamTaq DNA polymerase (Thermo Scientific), and ~2.5 ng of template DNA. For 16S rDNA and COI the PCR profile was set as follows: initial denaturing step at 94 °C for 3 min; 34 cycles of denaturing at 94 °C for 45 s, annealing at 54 °C for 1 min, and extending ay 72 °C for 1 min,

and a final extending step at 72 °C for 7 min. A negative control was included in each reaction. For 18S rDNA, PCRs were carried out in 45 μL using a protocol with low ramp speed, and annealing temperature set at 50 °C (Lorenz, 2012). PCR products were precipitated with sodium acetate and absolute ethanol and sent to Macrogen Europe for sequencing.

Sequences from each gene were aligned with ClustalX 2.1 (Larkin et al., 2007), and alignments were edited in BIOEDIT version 7.2.5 (Hall, 1999). The program jModelTest 2.1.6 (Guindon & Gascuel, 2003; Darriba et al., 2012), based on the hierarchical likelihood ratio test, was used to assess the best model of evolution for the sequences under the Akaike Information Criterion (AIC) (Akaike, 1974). For molecular comparison and phylogenetic reconstruction, I used additional sequences downloaded from GenBank for *Cirrophorus furcatus* (accession numbers AY532349.1 and AY532330.1); moreover, I used *Ophelina acuminata* Örsted, 1843 as outgroup (accession numbers AY340471.1, AY340439.1 and HQ024164.1). The choice of the outgroup was based on Bleidorn's (2005) remarks, who identified Opheliidae as a likely sister taxon of Paraonidae.

A Bayesian consensus phylogenetic tree based on the three concatenated markers was constructed using MrBayes 3.2 (Ronquist et al., 2011), which allowed phylogenetic inference by treating each gene with its own substitution model. Four replicate runs were carried out with a total of three Markov chains per run for 2 x 10^6 generations. The chain was sampled every 100 generations to obtain 20 000 sampled trees. The first 5000 sampled trees (25%) were discarded as burn-in phase, with the remaining 15 000 trees used to estimate the Bayesian posterior probability (*PP*) of tree nodes. The convergence of Bayesian analyses was checked through the standard deviation of split frequencies, that should reach a value < 0.01 at the end of the analysis (Ronquist et al., 2011).

3.4 Results

3.4.1 Systematics

Class ANNELIDA

SCOLECIDA Rouse & Fauchald, 1997

Family PARAONIDAE Cerruti, 1909

Genus *Cirrophorus* Ehlers, 1908

Cirrophorus sp. A (Figure 15A-F)

Cirrophorus furcatus [*non* (Hartman, 1957)]: Castelli, 1985; Castelli & Lardicci, 1985; Castelli, 1987; Rossi & Lardicci, 1995; Como et al., 2004; Chessa et al., 2007; Schirosi et al.,

2010. *Paradoneis lyra* [*non* (Southern, 1914)]: Bonvicini Pagliai & Cognetti, 1982; Maggiore & Keppel, 2007; Simboura et al., 2007.

EXAMINED MATERIAL

Reference specimen 1: Calich Pond (40° 35.8'N 8° 17.3'E), 1 m (March 1995) (P/3800)

Reference specimen 2: Calich Pond (40° 35.8'N 8° 17.3'E), 1 m (March 1995) (P/3801)

COMPARATIVE MATERIAL EXAMINED

Acquatina Pond (40° 26.7'N 18° 14.3'E), 2 m: 68 individuals (November 2014); Agiasma Lagoon (40° 52.9'N 24° 37.1'E), 2 m: 25 individuals (date unknown); Alfacs Bay (40° 36.8'N 0° 36.5'E), 4 m: 3 individuals (September 1992); Calich Pond (40° 35.8'N 8° 17.3'E), 1 m: 4 individuals (March 1988); 26 individuals (March 1995); Capraia Island (43° 2.4'N 9° 50.8'E), under fish cages, 33.5 m: 43 individuals (July 2003); Golfo Aranci, Gulf of Olbia (40° 59.9'N 9° 37.1'E), under fish cages, 5 m: 1 individual (January 1997); Gulf of Cagliari (39° 5.9'N 9° 2.6'E), 20 m: 1 individual (1982); Gulf of Follonica (42° 55.1'N 10° 44.3'E), 8 m: 8 individuals (December 1987); Livorno port (43° 34.4'N 10° 18.9'E), 3 m: 15 individuals (April 2016); Porto Pozzo (41° 11.5'N 9° 16.7'E), 0.8 m: 4 individuals (July 1987); 22 individuals (July 2015); Portoferraio Bay, Elba Island (42° 48.7'N 10° 18.5'E), 8 m: 2 individuals (November 1982); 5 individuals (May 1990); Tortolì Pond (39° 56.9'N 9° 41.2'E), 1 m: 5 individuals (May 2016); Varano Lagoon (41° 52.4'N 15° 42.3'E), 3 m: 4 individuals (November 2014); 7 individuals (October 2015); Venice Lagoon (45° 29.2'N 12° 29.4'E), 0.8 m: 66 individuals (March 2015).

Cirrophorus sp. B (see below in the remarks section): Livorno port (43° 34.4'N 10° 18.9'E), 3 m: 1 individual (April 2016)

Description: Reference specimen complete, approximately 12 mm long, 0.45 mm wide for 126 chaetigers (Fig. 15A). Prostomium roughly trapezoidal, slightly longer than wider, antenna short, blister-like, with median insertion, approximately 1/6 of the prostomium length; in juveniles the prostomial antenna extremely reduced and might be difficult to notice. Eyes absent, nuchal organs small, comma-shaped, difficult to examine in preserved individuals, more conspicuous in living specimens. Three pre-branchial chaetigers, with post-chaetal notopodial lobes gradually increasing in length towards the branchial region; notopodial lobes are short, onion-shaped in the anterior branchial region, then gradually increase in the posterior part, being distinctly longer, tapered in the post-branchial region. Seventy-five pairs of branchiae from chaetiger 4; branchiae are pointed, approximately as

long as the body width in the anterior part, approximately six to seven times the notopodial lobes, then become gradually shorter, and in the posterior part are almost tubercular, shorter than the notopodial lobes (Fig. 15B). In the additional material examined their number goes from 24 to 72, more frequently from 40 to 65; the number of branchiae is coarsely correlated with the size of the animal (Fig. 16). Pygidium rounded, with three elongated anal cirri.

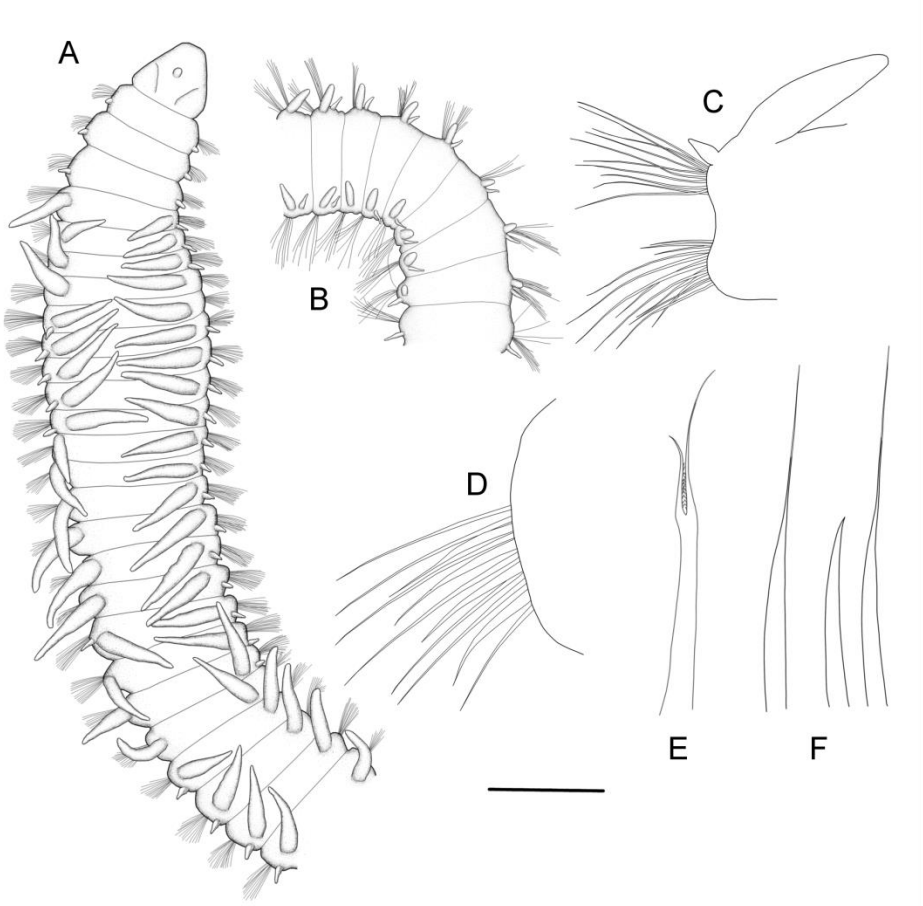

Figure 15: *Cirrophorus* sp. A, reference specimen 1 (P/3800): (A) dorsal view of the anterior region; (B) end of the branchial region (71th to 79th chaetiger); (C) left parapodium in the anterior region (6th chaetiger); (D) ventral part of a parapodium in the post-branchial region (110th chaetiger); (E) lyrate notopodial chaeta; (F) thickened neuropodial chaetae from the post-branchial region. Scale-bar: 0.3 mm (A, B), 0.1 mm (C), 50 μm (D), 12 μm (E), 30 μm (F)

Parapodia biramous, composed by several thick, slightly curved capillaries (Fig. 15C); 1-4 (usually 2-3) notopodial lyrate chaetae (Fig. 15E) from chaetiger 3, with branches sub-equal in thickness, the one approximately twice as long as the other. The internal edges of the two branches show the presence of several short, thin spines. Starting from chaetiger 96 modified neurochaetae are present, gradually increasing in number from 1-2 to 6-7, mixed to capillaries (Fig. 15D). Neuropodial modified chaetae are thickened capillaries, slightly curved, with or without tapered tip (Fig. 15E). In the additional material, some individuals show lyrate

chaetae from the 2nd chaetiger. The starting point of modified neurochaetae shows wide variation, ranging from the 44th to the 88th chaetiger, and it is not always easy to identify.

Preserved individuals are brownish to reddish; live colour bright orange, often with golden-green posterior part of the body.

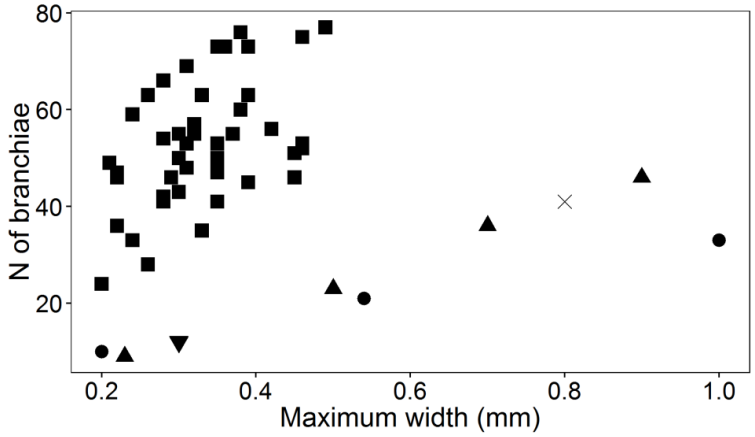

Figure 16: Ratio between maximum width and number of branchiae in five *Cirrophorus* species with lyrate notopodial chaetae. Legend: ■: *Cirrophorus* sp. A (present study); ▲: *Cirrophorus americanus* Strelzov, 1973 (from Strelzov, 1973); ●: *Cirrophorus furcatus* (Hartman, 1957) (from Strelzov, 1973); x: *Cirrophorus miyakoensis* Imajima, 1973 (from Imajima, 1973); ▼: *Cirrophorus* sp. B (present study).

Distribution: Mediterranean Sea. Collected in the Balearic Sea, Sea of Sardinia, Tyrrhenian, Adriatic and Aegean Sea (Fig. 17).

Ecology: Present in organically-enriched environments, between 0.5 and at least 30 m depth; shallower in brackish-water lagoons and coastal ponds, deeper in marine environments. In brackish-water environments it is often associated with *Zostera* and *Nanozostera* meadows, whereas in marine environments its presence is typically related to organic enrichment, such as sewer pollution and fish farms. *Cirrophorus* sp. A. shows remarkably high densities and a patchy distribution, typical of an opportunistic species.

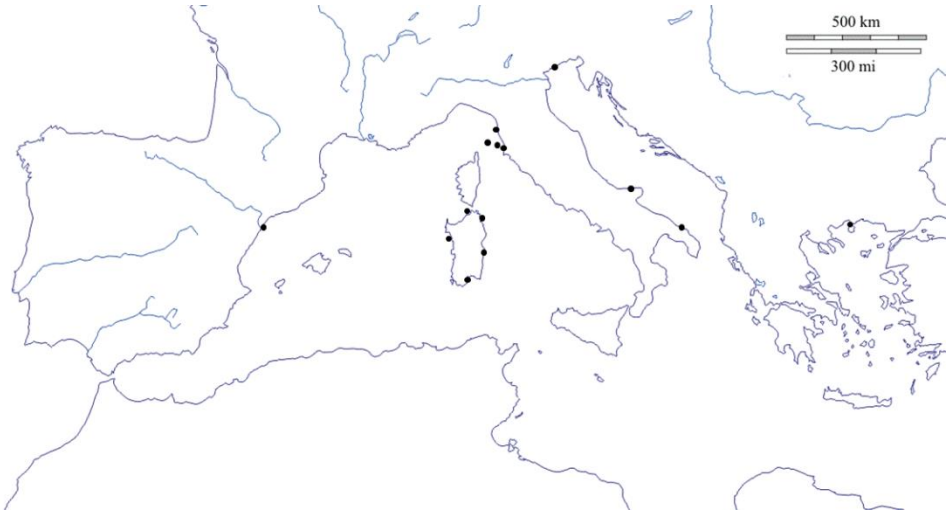

Figure 17: Known distribution of *Cirrophorus* sp. A.

Remarks: Currently, the genus *Cirrophorus* Ehlers, 1908 comprises seven valid species; the majority of them are poorly known and need to be re-described. Among them, *Cirrophorus branchiatus* Ehlers, 1908, which includes *Cirrophorus lyriformis* (Annenkova, 1934) as a synonym, according to Strelzov (1973), and *Cirrophorus aciculatus* (Hartman, 1957) can be easily distinguished due to the presence of thick notopodial acicular chaetae and the branchiae starting at chaetiger 5. The remaining species are characterised by the presence of a median antenna in adults (stated to be absent in juveniles or small specimens of *Cirrophorus americanus* Strelzov, 1973, and *Cirrophorus brevicirratus* Strelzov, 1973) and lyrate notopodial chaetae (Table 2). *Cirrophorus* sp. A clearly differs from *C. brevicirratus* Strelzov, 1973 in the shape of the prostomium (triangular and elongated in *C. brevicirratus*, trapezoidal in *Cirrophorus* sp. A), the segment of appearance of lyrate chaetae (chaetiger 6 in *C. brevicirratus*, chaetiger 2-3 in *Cirrophorus* sp. A) and the number of branchiae (up to 14-15 in *C. brevicirratus*, against an usual number of 40-65 in the new species). *Cirrophorus americanus* Strelzov, 1973, *Cirrophorus furcatus* (Hartman, 1957), *Cirrophorus longifurcatus* (Hartmann-Schröder, 1965) and *Cirrophorus miyakoensis* Imajima, 1973 are closer to *Cirrophorus* sp. A as regards the shape of the prostomium, but they show some differences, in particular in the shape and size of the antenna. The description of *C. americanus* is ambiguous and could refer to two different species, as the holotype shows the presence of thickened neuropodial chaetae in the posterior chaetigers and lacks the median antenna, whereas the remaining examined material has a well-developed median antenna and lacks modified neuropodial chaetae. Provisionally accepting that the holotype and the remaining material are conspecific, *Cirrophorus* sp. A differs from *C. americanus* in the size of the median antenna (short, blister-like in *Cirrophorus* sp. A, cirriform and approximately 1/3 of the prostomium length in *C. americanus*), in the lower number of branchiae (up to 46 pairs in *C. americanus*, against an usual number of 40-65 in the new species) even in larger individuals (up to 0.9 mm). Moreover, in *C. americanus* notopodial lobes in the pre-branchial region are of the same size, while in *Cirrophorus* sp. A they gradually increase in size. As the new species, *C. furcatus* has lyrate chaetae from the 3rd chaetiger and notopodial lobes of increasing size in the pre-branchial region and a short median antenna; nevertheless, this species shows a remarkably larger size (up to 1 mm), a distinctly lower number of branchiae (up to 33 pairs), a slender and longer cirriform antenna, and neuropodial thickened chaetae are absent. Moreover, in *C. furcatus* the size of the notopodial lobes decreases towards the pygidium (whereas it increases in *Cirrophorus* sp. A) (Blake, 1996). *C. longifurcatus*, a species known from Chile and rarely reported after the original description, shows a long prostomial

Table 2: Comparison among species of *Cirrophorus* Ehlers, 1908 with lyrate chaetae, highlighting the main differences in the most important taxonomic characters.

Species	Antenna (shape)	Antenna : prostomium (length)	Branchiae (pairs)	Notopodial lobes of prebranchial, branchial and postbranchial segments	Starting point of modified notochaetae (chaetiger)	Modified neurochaetae	Reference
Cirrophorus americanus Strelzov, 1973*	Absent or cirriform	1:3	9-46	Lobes gradually elongating in pre-branchial chaetigers; lobes long and slender in the branchial and postbranchial regions.	2-3	+/-	Strelzov (1973)
Cirrophorus brevicirratus Strelzov, 1973	Absent or blister-like	<1:10	9-15	Very short in the prebranchial region, gradually elongating towards the pygidium.	6	-	Strelzov (1973)
Cirrophorus furcatus (Hartman, 1957)	Cirriform	1:4	25-33	Relatively long and slender in the prebranchial region, decreasing in size in the branchial region, very short in the postbranchial region.	3	-	Hartman (1957); Blake (1996)
Cirrophorus longifurcatus (Hartmann-Schröder, 1965)	Cirriform, elongate	2:3	10-22	Very short in the prebranchial region, slender and increasing in length in the branchial region, still elongate but shorter in the postbranchial region	8	-	Hartmann-Schröder (1965)
Cirrophorus miyakoensis Imajima, 1973	Cirriform	1:3	39-42	Relatively long and slender in the prebranchial region, decreasing in size in the postbranchial region.	2	-	Imajima (1973)
Cirrophorus nikebianchii sp. nov.	Blister-like	1:6 or less	24-76 (usually 40-65)	Very short in the prebranchial region, gradually increasing in length in the branchial region, long and slender in the postbranchial region.	2-3	+	Present study
Cirrophorus sp. B**	Blister-like or cirriform	1:6 to 1:3	11-27	Short in the prebranchial region, longer and slender in the branchial region, decreasing again in the postbranchial region.	2-3	-	Laubier & Ramos (1974); Katzmann & Laubier (1975); present study

*based on the debatable assumption that all material described by Strelzov (1973) should be referred to the same species.
**corresponding to *Cirrophorus* cf. *lyriformis sensu* Laubier & Ramos (1974) and *Cirrophorus furcatus sensu* Katzmann & Laubier (1975)

antenna, almost reaching the 2nd chaetiger (whereas it is very short, blister-like in the new species), and a strong difference in length between the notopodial lobes in the pre-branchial and branchial region (of similar size in *Cirrophorus* sp. A) (Hartmann-Schröder, 1965). This species, therefore, is clearly different from *Cirrophorus* sp. A. Lastly, *C. miyakoensis* is similar to *Cirrophorus* sp. A, but the median antenna and notopodial lobes are distinctly slender, cirriform, and the size of the notopodial lobes decreases towards the pygidium (whereas it increases in *Cirrophorus* sp. A). Moreover, this species also shows a lower number of branchiae (up to 42 for 0.8 mm maximum width). Another Mediterranean morphotype differs from *Cirrophorus* sp. A mainly in the number of branchiae (usually 15-22 pairs) and in the pattern of notopodial lobes, that become very short in the posterior part of the body (whereas in *Cirrophorus* sp. A they remain long and slender). This morphotype has been collected until now only in a marine organically-enriched environment and morphologically corresponds to *Cirrophorus lyriformis sensu* Laubier & Ramos (1974) and to *Cirrophorus furcatus sensu* Katzmann & Laubier (1975). Since molecular data did not support its assignment to *Cirrophorus* sp. A, and given the scarce material available, I provisionally consider it as a putative new species, *Cirrophorus* sp. B.

Within the genus *Cirrophorus*, *Cirrophorus* sp. A can be easily identified based on the extremely small size of the median antenna, the relatively small body size (maximum width = 0.5 mm) and the high number of branchiae (up to more than 70 in large individuals, and an average number of 40-65). The ratio between the number of branchiae and maximum width of the animal is similar for *C. americanus*, *C. furcatus*, *C. miyakoensis* and *Cirrophorus* sp. B, but it is clearly different in *Cirrophorus* sp. A (Fig. 16). Finally, the occurrence in brackish-water environments at very shallow depths seems to be a peculiarity of this species.

3.4.2. Molecular phylogenetic analysis

I obtained sequences of 471 bp for 16S rDNA (GenBank accession numbers: KX901418 to KX901433), 670 bp for COI (GenBank accession numbers: KX901434 to KX901446), and 1790 bp for 18S rDNA (GenBank accession numbers: KX901405 to KX901417). The best fitting nucleotide substitution models were GTR+G for 16S rDNA, and GTR+I+G for COI and 18S rDNA. The tree showed the presence of a well supported clade (clade I: posterior probability, *PP* = 1) with two separated lineages that corresponded morphologically to *Paradoneis ilvana*, a third one relative to *Paradoneis armata* (*PP* = 1), and a fourth with *Cirrophorus* sp. B (Fig. 18). Another well supported clade (clade II: *PP* = 1) included *Paradoneis lyra*, *C. furcatus* and *Cirrophorus* sp. A (Fig. 18). *P. lyra* individuals, in their

turn, clustered in a strongly supported clade (*PP* = 1) as well as *C. furcatus* and *Cirrophorus* sp. A (*PP* = 1). Although *C. furcatus* and *Cirrophorus* sp. A are very close and represent a well supported clade, the divergence between the two lineages is comparable to that observed between different species, thus supporting the distinction at species level. In a third clade *Paraonis fulgens*, and *Aricidea fragilis* clustered with high statistical support (clade III: *PP* = 1). *Levinsenia gracilis* represents the sister group of all remaining Paraonidae with high statistical support (*PP* = 1). The position of *C. branchiatus*, and the relationships among the three clades, are however not resolved in this reconstruction (Fig. 18).

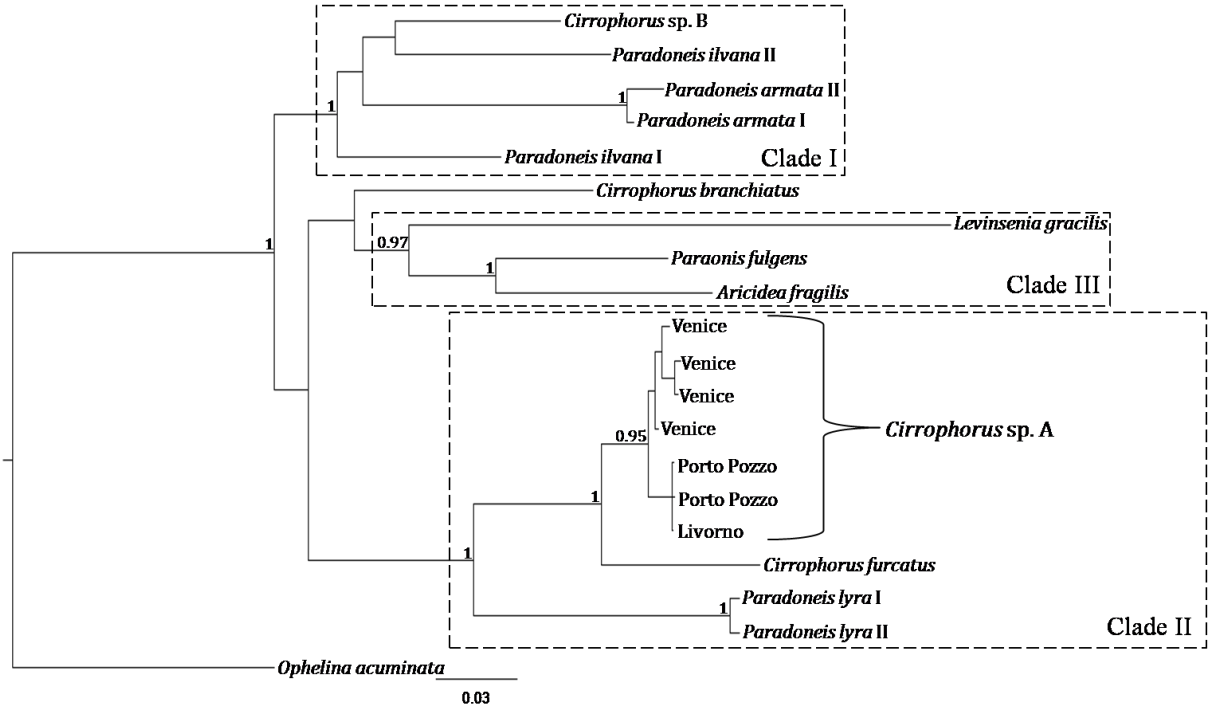

Figure 18: Bayesian tree obtained from the concatenated 16S rDNA, 18S rDNA, and COI sequences. Node values are Bayesian posterior probabilities; only statistically significant values are reported.

3.5 Discussion

According to present results, the doubts on the identity of the Mediterranean individuals identified as *Cirrophorus* near *furcatus* (Castelli et al., 1995) were founded, since both molecular and morphological data supported the distinction between *Cirrophorus* sp. A and *Cirrophorus furcatus*. It is noteworthy, however, that not all Mediterranean reports of *C. furcatus* can be referred to *Cirrophorus* sp. A; in particular, the descriptions of individuals referred to *C. furcatus* (or to *C. lyriformis*) in marine environments (Laubier, 1966c; Laubier & Ramos, 1974; Katzmann & Laubier, 1975) are morphologically different from *Cirrophorus* sp. A, mainly as regards the number of branchiae (11-27 *vs* 24-75, respectively) and the

length of the antenna. These individuals appear more similar to *Cirrophorus* sp. B. Unfortunately, the material available for this last putative species is scarce and not sufficient for a formal species description. On the other hand, the extremely small size of the median antenna led several authors to misidentify *Cirrophorus* sp. A as *Paradoneis lyra*. Shallow-water individuals, and in particular brackish-water records of the latter species in the Mediterranean Sea probably can all be referred to *Cirrophorus* sp. A. *P. lyra* is probably less widespread in the Mediterranean than commonly stated, and its distribution could be restricted to circalittoral and epibathyal bottoms.

The distinction between *Cirrophorus* Ehlers, 1908 and *Paradoneis* Hartman, 1965, based on the presence of a median antenna in the former genus and absence in the latter, was questioned by Strelzov (1968; 1973), who observed that in some species, such as *Cirrophorus americanus* and *Cirrophorus brevicirratus*, the antenna is present only in large adults, and absent in juveniles. The proposal of a synonymy between *Cirrophorus* and *Paradoneis*, however, was rejected by most of the subsequent authors (e.g. Laubier & Ramos, 1974; Katzmann & Laubier, 1975; Blake, 1996; 2016). Recently, Reuscher (2013) carried out a cladistic analysis of Paraonidae and identified a clade including all species of *Paradoneis* and *Cirrophorus*. This cladistic analysis highlighted that the median antenna characteristic of *Cirrophorus* was acquired and lost several times in the evolutionary history of the clade and, therefore, it cannot be considered a useful taxonomic trait. In Reuscher's (2013) morphology-based phylogenetic reconstruction, the species with notopodial spines, *Paradoneis spinifera* (Hobson, 1972) and *Paradoneis drachi* Laubier & Ramos, 1974, are the sister group of the remaining *Cirrophorus/Paradoneis* clade, and Reuscher (2013) assigned these two species to a new genus yet to be described. Species without notopodial modified chaetae and median antenna, assigned by Reuscher (2013) to the genus *Paraonides* Cerruti, 1909, are basal to the whole *Cirrophorus/Paradoneis* clade. Molecular data from this work do not support this reconstruction, and the tree obtained shows a very different topology, even if the low reliability of the median antenna as a useful taxonomic feature stated by Strelzov (1973) and Reuscher (2013) is confirmed. Species assigned to the genera *Cirrophorus* and *Paradoneis* are distributed in two different clades with high statistical support: the first clade includes *C. furcatus*, *Cirrophorus* sp. A and *P. lyra* and the second clade includes *Paradoneis armata* Glémarec, 1966, two strongly divergent lineages that were morphologically assigned to *Paradoneis ilvana* Castelli, 1985, and *Cirrophorus* sp. B, that probably represents an undescribed species. *Cirrophorus branchiatus* Ehlers, 1908 does not belong to any of the clades, with a doubtful position in the tree. Each clade comprises species with and without the

median antenna, but the relationship of genera *Aricidea* and *Paraonis* with the two clades are still uncertain, and the *Cirrophorus/Paradoneis* group resulted to be paraphyletic in this reconstruction. A possible explanation for this outcome may take into account an ancestral presence of notopodial modified chaetae, subsequently lost in several groups. The incorrect interpretation of this trait as derived character possibly led to an overestimation of its taxonomic value and, on the other hand, to underestimate other important features. Similarly, inconsistencies between molecular and morphological data have been already observed in several polychaete families (e.g. Bleidorn, 2005; Zanol et al., 2014).

Even though the results of the molecular phylogenetic reconstruction applied to the *Cirrophorus/Paradoneis* group are quite striking and statistically well-supported, I prefer to adopt a conservative approach to the Paraonidae taxonomy, awaiting for a more complete phylogenetic reconstruction, preferably based on both molecular and morphological data. However, some nomenclatural notes on the genera *Cirrophorus*, *Paradoneis* and *Paraonides* are in my opinion useful in anticipation of a necessary taxonomic revision of this family.

The genus *Cirrophorus* Ehlers, 1908 was created for *C. branchiatus*, and subsequently considered as a subgenus of *Aricidea* Webster, 1879 (Hartman, 1957). This taxonomic arrangement was questioned by Day (1963) and Laubier (1966c) and lastly rejected by Strelzov (1968), who stressed the similarity between *Cirrophorus* and *Paradoneis*. On the other hand, *Paradoneis* Hartman, 1965 was created for species lacking median antenna and with notopodial modified chaetae, with *Paraonis (Paraonides) lyra* Southern, 1914 as type species. The genus *Paraonides* Cerruti, 1909, created as subgenus of *Paraonis* Cerruti, 1909 with *Paraonis (Paraonides) neapolitana* Cerruti, 1909 as type species, was initially used for species with notopodial modified chaetae (Southern, 1914; Fauvel, 1927), but its diagnosis was subsequently emended (Hartman, 1965) and this genus was used to define species without modified chaetae. The question of the correct use of *Paraonides* has been long debated, and the main problem regarding this issue is the uncertain identity of the type species. In fact, *P. neapolitana* was described for the Gulf of Naples as a species without median antenna, with three pre-branchial chaetigers, nine pairs of branchiae and modified notopodial chaetae from the 12th chaetiger (Cerruti, 1909). The notopodial modified chaetae are apparently shorter than the capillaries, somewhat thicker and leaf-shaped, even though this kind of modified chaetae has never been reported in later descriptions of Paraonidae. Strelzov (1973) demonstrated that leaf-shaped chaetae could be an artefact of the fixation of lyrate chaetae in Canada balsam, since balsam and chaetae have a very similar refraction index that could prevent a clear distinction of the two branches of the lyrate chaeta. For this reason,

Strelzov (1973) considered *Paraonides* as a synonym of *Cirrophorus* and *Paradoneis*. Since type material is lost (Castelli, 1987), Strelzov (1973) re-described *Cirrophorus neapolitanus* on the basis of North and South Atlantic and Black Sea individuals; this conclusion was considered to be highly questionable without the examination of topotypic material (Katzmann & Laubier, 1975). The rejection of this re-description led to discard the identity of *Paraonides* and to maintain the diagnosis by Hartman & Fauchald (1971), even if in the possibility that Strelzov's (1973) remarks on the peculiar chaetae of *P. neapolitana* were correct. Topotypic material in good preservation conditions is necessary to clarify the identity of *P. neapolitana*; it is noteworthy, however, that this species has never been re-described in taxonomic works (Laubier & Ramos, 1974; Katzmann & Laubier, 1975; Castelli, 1985; Çinar et al., 2011; Çinar & Dağli, 2013) and in recent years this species has been cited only in species checklists of soft bottom ecology works (Gambi et al., 1998; Simonini et al., 2007; De Biasi & Pacciardi, 2008). Thus, it is likely that *P. neapolitana* is actually a species with lyrate chaetae, and that the genus *Paraonides* is not suitable for species lacking of modified notopodial chaetae. Because of the unclear identity of the type species of *Paraonides*, I suggest to precautionarily use *Paraonella* Strelzov, 1973 for species without notopodial modified chaetae and prostomial antenna, though considering that this group may turn out to be artificial, since the loss of modified chaetae could have happened several times in the evolutionary history of Paraonidae.

The paraphyletic condition of the *Cirrophorus/Paradoneis* group makes taxonomic revisions challenging. Actually, the type species of *Cirrophorus* is not included in any of the two highly supported clades and its relationships with the other groups within Paraonidae are still unclear. On the other hand, the genus *Paradoneis* could be used for the group comprising *P. lyra*, *C. furcatus* and *Cirrophorus* sp. A, but to do so, its diagnosis should be emended, while the morphological traits that can be considered diagnostic of this group are still unclear. Moreover, the uncertainty about the identity of *Paraonides neapolitana* does not allow to settle the question related to its possible synonymy with *Paradoneis*, or its possible use for the second monophyletic group identified by this analysis, which includes *P. armata*, the two cryptic species assigned to *P. ilvana* and *Cirrophorus* sp. B. These results highlight that, as observed in other polychaete families, morphological traits of the family Paraonidae may be misleading with regard to the actual evolutionary history of the group, and their evolutionary meaning should be, therefore, critically evaluated. An important contribution to Paraonidae systematics can be provided by molecular tools, in order to obtain a more sound classification of this taxonomically complex family.

3.6 Acknowledgements

I would like to thank F. Aguirrezabalaga, G. Benedettini, O. Bresciani, T. Darbyshire, A. Giangrande, A. S. Y. Mackie, D. Martin, A. M. Pastorelli, A. Pavia, and K. Vasileiadou for providing useful material, both for the morphological and the molecular works; M. Casu, A. M. Di Biasi, I. Guarneri, M. Oliva, L. Pacciardi, M. Pertusati, E. Pollonara, C. Pretti, F. Scarpa, M. Sigovini, D. Tagliapietra, and A. Vannucci for their invaluable help in sampling Mediterranean Paraonidae from several environments; G. Di Giuseppe, F. Erra, G. Montesanto and F. Verni for their technical support in microscope measurements and scientific drawing; T. Ravaglia and F. Squarcia for their help in molecular laboratory work; J. A. Blake and K. Meißner for providing literature; two anonymous referees for their valuable comments and corrections.

4. Chapter 2: Is cryptic speciation in *Aricidea assimilis* (Annelida, Paraonidae) driven by environmental features?

4.1 Abstract

The family Paraonidae is characterised by high diversity, even within restricted areas. It is, however, poorly known from several points of view, such as its reproductive features and the role played by environmental factors in shaping its diversity.

In this study I investigated the molecular diversity within *Aricidea assimilis* Tebble, 1959, a species that commonly occurs in different habitat types of the Mediterranean Sea. In order to test the effect of environmental factors and biogeographical barriers on molecular and morphological diversity, individuals of *A. assimilis* were collected in two Mediterranean areas (Tyrrhenian Sea - western Mediterranean - and Adriatic Sea - eastern Mediterranean), considering different habitats (marine and brackish) and depths [shallow (0.5 to 8 meters) and deep (75 to 120 meters)]. Phylogenetic reconstruction was obtained using two mitochondrial (16S rDNA and cytochrome oxidase c subunit I – COI) and one nuclear (18S rDNA) markers and the occurrence of cryptic species was tested using two species delimitation tests (Automatic Barcoding Gap Discovery and Poisson Tree Processes). The combined dataset highlighted the presence of two highly divergent clades, one including deep-water individuals, the other represented by shallow-water (brackish and marine) individuals. Moreover, a shallower divergence was detected between brackish-water and marine shallow-water individuals. In this case morphological and molecular diversity patterns were consistent. The occurrence of separate species could account for the divergence observed between the deep-water and shallow-water lineages. On the other hand, species delimitation tests applied to brackish-water and marine shallow sub-lineages gave ambiguous results. This outcome suggests possible incipient speciation between the two groups, which however needs further investigations.

Present result validated for the first time past speculations on the occurrence of cryptic species in Paraonidae. Environmental features such as depth and, at a lesser extent, brackish *vs*. marine habitats, were proved to be highly relevant in driving genetic divergence and ultimately cryptic speciation in *A. assimilis*. On the other hand, at this spatial scale geographical distance seemed to have a less pronounced effect on diversification of *A. assimilis*, suggesting that the species has wide-range dispersal phases, probably corresponding to planktonic larval stages, whose occurrence is still to be verified in Paraonidae.

4.2 Introduction

Among polychaetes, Paraonidae are a very diverse group, currently including more than 120 described species, occurring on soft bottoms from the tide level to the abyssal depths (Strelzov, 1973). In several marine environments Paraonidae represent the dominant taxon in terms of abundance and biomass and are therefore supposed to have a relevant role in sediment dynamics, trophic nets and several other ecological processes (Gibbs, 1965; Blake, 1996; Quiroz-Martinez et al., 2012). Despite their importance, many aspects of the biology of Paraonidae, such as reproductive features and population connectivity, are still scarcely known (Rouse & Pleijel, 2001). Moreover, a great number of the described species show a very wide distribution and some of them are considered cosmopolitan (Strelzov, 1973). This extremely wide distribution, together with the high intraspecific morphological variability, has been interpreted as a possible clue of cryptic speciation (Laubier & Ramos, 1974). However, until now experimental evidence of the occurrence of cryptic species in Paraonidae is not available. This is also true for the role played by environmental factors and biogeographical breaks and boundaries in the diversification of this family.

According to scientific literature, *Aricidea assimilis* Tebble, 1959 is a species that commonly occurs in sandy and muddy bottoms of the Mediterranean Sea from 2 down to 300 meters depth (Castelli, 1987). As for several Paraonidae, its actual distribution is somewhat unclear, and even though Strelzov (1973) reported the species from the Northern Pacific Ocean, the Red Sea and the Southern Atlantic Ocean, the majority of records are referred to the Mediterranean Sea and adjacent Atlantic waters (Tebble, 1959; Laubier & Ramos, 1974; Katzmann & Laubier, 1975; Castelli, 1985; Castelli, 1987; Çinar et al., 2014). Records from the Pacific Ocean by Strelzov (1973), Hobson (1976) and Lovell (2002) should probably be referred to different species (Blake, 1996). In addition, the high degree of morphological variability in *A. assimilis* accounts for its taxonomic uncertainty. In fact, the species has been misidentified as *Aricidea fauveli* Hartman, 1957 (= *A. lopezi*) (Bellan, 1965), *Aricidea fragilis* Webster, 1879 (Amoureux, 1970), and *Aricidea lopezi* Berkeley & Berkeley, 1956 (Strelzov, 1973). Moreover, *A. assimilis* has been redescribed as *Aricidea mutabilis* by Laubier & Ramos (1974), who highlighted the strong morphological variability of this species, regarding, in particular, the size and shape of prostomial antenna. In this species the prostomial antenna may vary from very long to relatively short. Even though Laubier & Ramos (1974) raised the doubt that short-antenna and long-antenna forms could actually represent separate species, they provisionally considered them conspecific. The short-antenna form of *A. assimilis* has been erroneously interpreted as conspecific with the Pacific *A. lopezi*

(Strelzov, 1973; Castelli, 1987) and occasionally it can be encountered in Mediterranean species lists (Çinar et al., 2014).

In this study I addressed the taxonomic problems regarding *A. assimilis*, in order to understand *i)* if cryptic species occur within the currently accepted *Aricidea assimilis*; *ii)* if long-antenna and short-antenna forms represent different species; *iii)* if lineages identified by molecular markers are morphologically distinguishable; *iv)* which are the drivers of genetic divergence and ultimately cryptic speciation in *A. assimilis*.

4.3 Materials and methods

4.3.1 Sampling

Individuals of *A. assimilis* were collected in seven localities from two Mediterranean biogeographical areas (Tyrrhenian Sea – western Mediterranean - and Adriatic Sea - eastern Mediterranean), considering different habitats (marine and brackish) and depths [shallow (0.5 to 8 meters) and deep (75 to 120 meters)] (Fig 19; Tab. 3). Sediment samples from suitable environments were collected with a Van Veen grab or where possible with a corer by skin-diving, and subsequently sieved with a 0.5 mm mesh. When possible, individuals of *A. assimilis* were sorted alive, otherwise after fixation of the whole sample in 96% ethanol. The main morphological feature, namely the length and shape of the prostomial antenna, was noted directly on alive individuals, when possible. All individuals were stored in 96% ethanol at 4 °C until DNA extraction.

Table 3: Sampling localities of the individuals of *Aricidea assimilis* employed in this study. Legend: Ad= Adriatic Sea; Ty= Tyrrhenian Sea; BW= Brackish-water; SM= Shallow marine; DM= Deep marine

Locality	Depth	Environment	N	Date
Cattolica (Ad)	5 m	SM	2	05/2016
Marina di Ravenna (Ad)	8 m	SM	3	09/2014
Rosignano (Ty)	7 m	SM	1	06/2014
Cala di Forno (Ty)	7 m	SM	1	06/2014
S. Teodoro Pond (Ty)	0.5 m	BW	2	07/2015
Strait of Otranto (Ad)	75-120 m	DW	2	03/2015
Tuscan Archipelago (Ty)	110 m	DW	1	11/2015

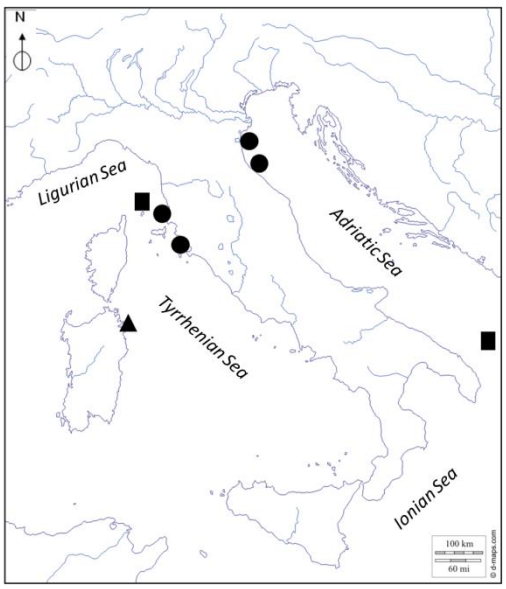

Figure 19: Sampling localities of *A. assimilis* along the Italian coasts: ▲: brackish-water ■: deep-water marine ●: shallow-water marine (for details see Table 3).

4.3.2 Genetic analyses

DNA extraction was carried out using the GenElute™ Mammalian Genomic DNA Miniprep Kit distributed by Sigma-Aldrich, following the manufacturer's instructions. The mitochondrial regions coding for 16S rRNA and cytochrome c oxidase subunit I (COI) and the nuclear region for 18S rRNA were amplified. 16S rDNA amplification was obtained using the primer pairs 16SarL (5'-CGCCTGTTTAACAAAAACAT-3') and H3080 (5′-CCGGTCTGAACTCAGATCACGT-3′) (Palumbi et al., 1991) and the annelid-specific primers 16S_ANNF (5'-GCGGTATCCTGACCGTRCWAAGGTA-3') and 16S_ANNR (5'-TCCTAAGCCAACATCGAGGTGCCAA-3') (Sjölin et al., 2005), whereas for COI amplification the annelid-specific primers POLYLCO (5'-GAYTATWTTCAACAAATCATAAAGATATTGG-3') and POLYHCO (5'-TAMACTTCWGGGTGACCAAARAATCA-3') (Carr et al., 2011) were employed. 18S rDNA amplification was carried out using the primers F9 (5'-CTGGTTGATCCTGCCAG-3') (Medlin et al., 1988) and R1513 (5'-TGATCCTTCYGCAGGTTC-3') (Petroni et al., 2002). Polymerase chain reaction (PCR) amplifications were carried out in 20 µL solutions using 1.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.1 µM of each primer, 1 U of DreamTaq DNA polymerase (Thermo Scientific), and ~2.5 ng of template DNA. For 16S rDNA and COI the PCR profile was set as follows: initial denaturing step at 94 °C for 3 min; 34 cycles of denaturing at 94 °C for 45 s, annealing at 54 °C for 1 min, and extending at 72 °C for 1 min, and a final extending step at 72 °C for 7 min. For 18S rDNA, PCRs were carried out in 45 µL using a protocol with low ramp speed, and annealing temperature set at 50 °C (Lorenz, 2012). A negative control was included in each reaction. PCR products were precipitated with sodium acetate and absolute ethanol and sent to Macrogen Europe for sequencing.

4.3.3 Data treatment

Sequences from each gene were aligned with ClustalX 2.1 (Larkin et al., 2007), and alignments were edited in BIOEDIT version 7.2.5 (Hall, 1999). The program jModelTest 2.1.6 (Guindon & Gascuel, 2003; Darriba et al., 2012), based on the hierarchical likelihood ratio test, was used to assess the best model of evolution for the sequences under the Akaike Information Criterion (AIC) (Akaike, 1974). An individual of *Aricidea (Aricidea) fragilis* Webster, 1879 from the Adriatic Sea was used as outgroup (GenBank accession numbers KX901405, KX901418, KX901445).

A Bayesian consensus phylogenetic tree based on the three concatenated markers was constructed using MrBayes 3.2 (Ronquist et al., 2011), which allowed phylogenetic inference by treating each gene with its own substitution model. Four replicate runs were carried out with three Markov chains per run for 2 x 10^6 generations. The chain was sampled every 100 generations to obtain 20 000 sampled trees. The first 5000 sampled trees (25%) were discarded as burn-in phase, with the remaining 15 000 trees used to estimate the Bayesian posterior probability (*PP*) of tree nodes. The convergence of Bayesian analyses was checked through the standard deviation of split frequencies, that should reach a value < 0.01 at the end of the analysis (Ronquist et al., 2011).

Pairwise K2P distances (Kimura, 1980) were calculated using the software MEGA 7.0.14 (Kumar et al., 2016). The separation at species level of the identified lineages was tested using two different single-locus species delimitation tests. The Automatic Barcoding Gap Discovery approach (ABGD) uses a range of prior intraspecific divergences to infer from sequence data a model-based one-sided confidence limit for intraspecific divergence. Thereafter, the algorithm detects the barcoding gap as the first significant gap beyond this limit and uses it to partition data, automatically sorting sequences into hypothetical species (Puillandre et al., 2012). The Poisson Tree Processes approach (PTP), on the other hand, uses phylogenetic trees, and in particular branch length (as proxy of number of substitutions), based on the principle that the number of substitution between species is significantly higher than the number of substitutions within species (Zhang et al., 2013).

4.4 Results

4.4.1 Morphological characterisation

Within the sampled material two clearly different morphotypes were identified (Fig. 20). The most widespread morphotype, which closely matches the description of *Aricidea assimilis* (Laubier & Ramos, 1974, under the name *Aricidea mutabilis*), was identified in both shallow and deep marine samples. It is characterised by tapering and pointed prostomial antenna, which can be either long or short, by branchiae with elongated and tapering tip in the posterior part of the branchial region and by brownish live colour, with bright red thoracic inclusions corresponding to enlarged commissural vessels of the branchial region (Strelzov, 1973). Brackish-water individuals exhibited a clearly different morphotype, characterised by short prostomial antenna with blunt tip, branchiae without elongated tip throughout the whole branchial region and bright yellow live colour, with pale orange thoracic inclusions. The second morphotype was tentatively referred to *Aricidea laubieri* Hartley, 1981, a species

known only for the Eastern Atlantic Ocean until now. The majority of morphological features were consistent with the available descriptions of *A. laubieri* (Hartley, 1981; Aguirrezabalaga & Gil, 2008), but chaetae were slightly different, being more crooked and thicker, with additional hairs less developed than in *A. laubieri*. The presence and development of additional hairs in the *Aricidea assimilis* group is a highly variable feature, varying from numerous, thick hairs (Strelzov, 1973; Laubier & Ramos, 1974) to almost smooth (*pers. obs.*). It is worth noting that this variability may be an artefact due to preservation of the individuals and observation conditions such as microscope lighting and optical properties. However, since the shape of chaetae recalls *A. assimilis* more than *A. laubieri*, I decided to consider the assignment to *Aricidea laubieri* only tentative. Both morphotypes share similar modified chaetae and small, ventral eyespots, whose absence in fixed material is due to pigment fading in ethanol.

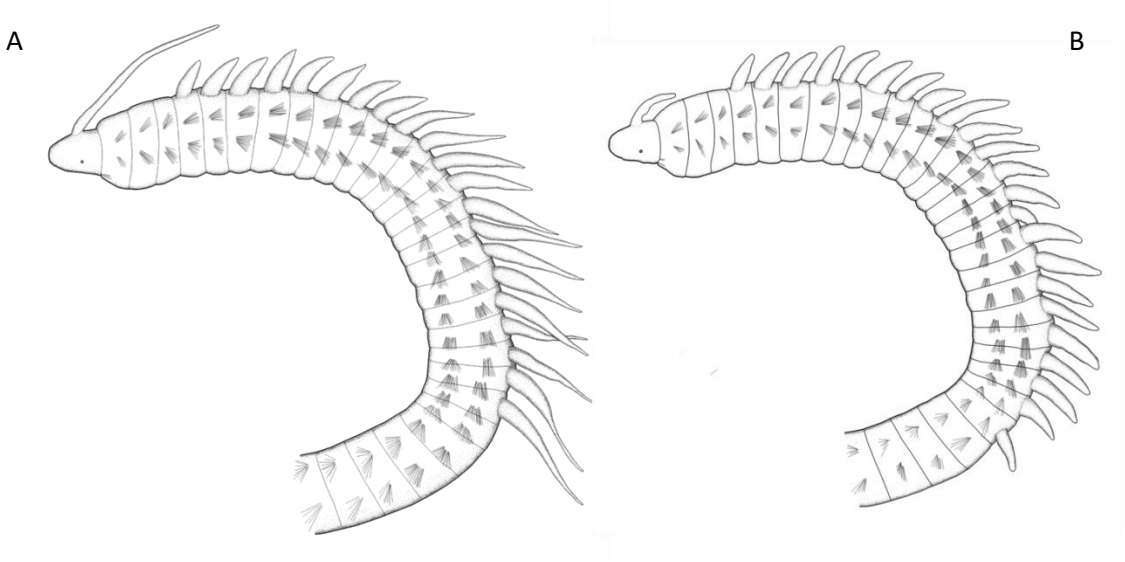

Figure 20: The two morphotypes of *Aricidea assimilis* identified in this study. A) *Aricidea assimilis* s.s. (deep and shallow marine); B) *Aricidea* cf. *laubieri* (brackish-water).

4.4.2 Phylogenetic reconstruction and species delimitation

I obtained sequences of 461 bp for 16S rDNA, 624 bp for COI, and 1786 bp for 18S rDNA. The best fitting nucleotide substitution models were GTR+G for 16S, GTR+I for COI, and HKY for 18S. The Bayesian tree (Fig. 21) showed a deep divergence between a group composed by all deep-water individuals and a group including both marine and brackish shallow-water individuals. A second, shallower divergence can be observed between a group including shallow-water marine individuals, from both the Adriatic and Tyrrhenian Sea, and a cluster composed by brackish-water individuals from S. Teodoro pond. The above described clusters are supported by high values of posterior probability ($PP > 0.95$) (Fig. 21).

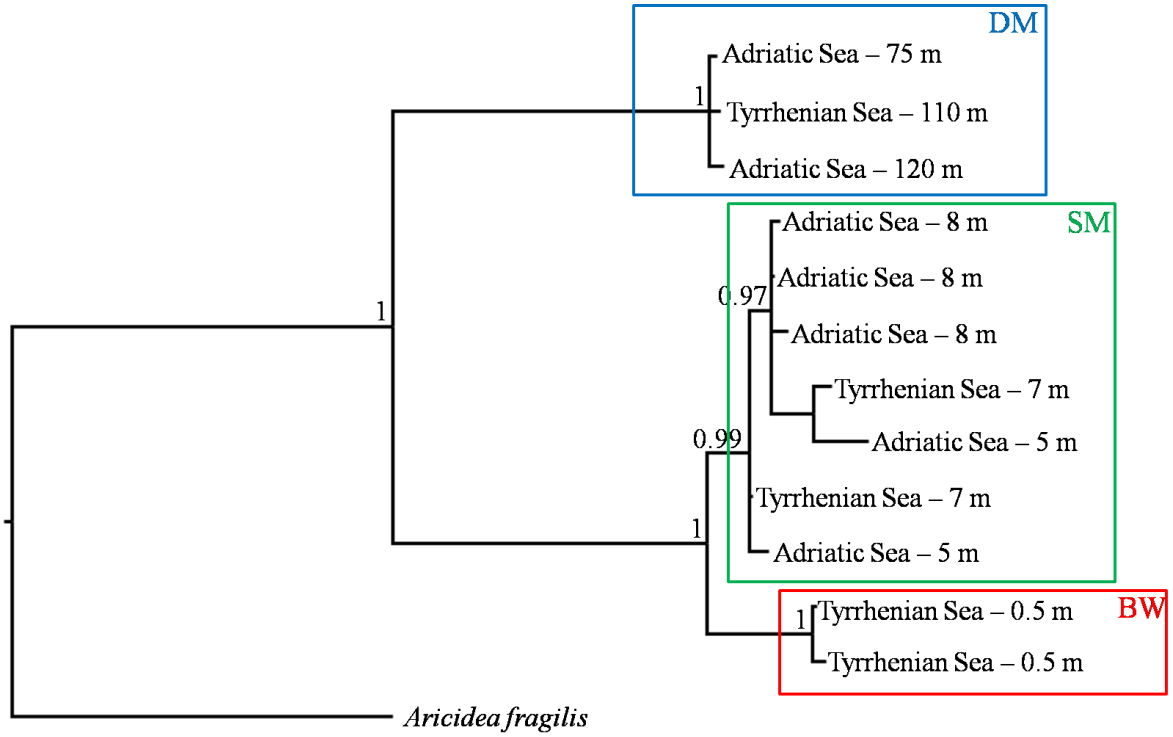

Figure 21: Bayesian tree of the combined gene dataset for *Aricidea assimilis* group from different areas and environments. Shown are node values with significant Bayesian posterior probabilities. DM: Deep marine; SM: shallow marine; BW: brackish-water.

K2P distances within groups ranged between 0 and 0.3% for 16S rDNA sequences. The distance between marine and brackish shallow-water individuals ranged from 2.0 to 2.3%; whereas the distance between deep-water and shallow-water individuals ranged from 14.8 to 15.8% (Fig. 22). K2P distances calculated on the COI sequence dataset, on the other hand, ranged between 0 and 0.2% within groups. The distance between marine and brackish shallow-water individuals ranged from 6.2 to 6.4%, and the distance between deep-water and shallow-water individuals ranged from 22.2 to 26.8% (Fig. 22).

Pairwise K2P distances calculated for 18S sequences were always under 1%. Distances within groups ranged from 0 to 0.1%, the distance between marine and brackish shallow-water individuals was 0.2%, and lastly, the distance between deep-water and shallow-water individuals ranged from 0.2 to 0.4% (Fig. 22). This result is coarsely consistent with the pattern identified with the mitochondrial genes, even if detected distances are distinctly lower.

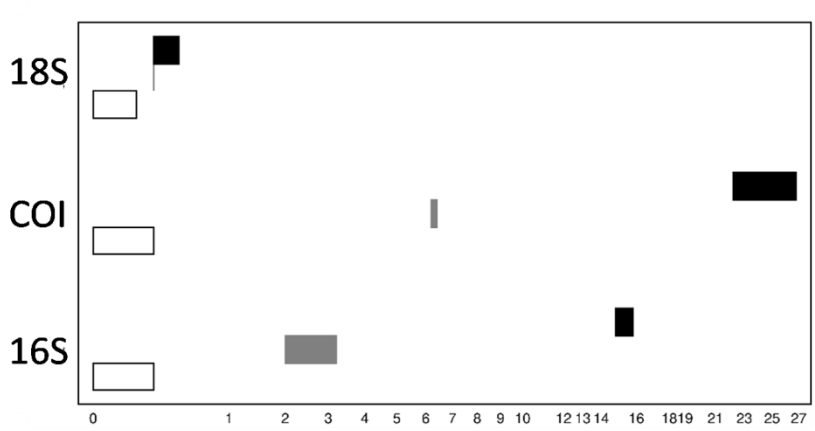

Figure 22: K2P distance ranges observed in the three markers. White: distances between individuals sampled in the same habitat type; Gray: distances between brackish-water and marine shallow individuals; Black: distances between deep- and shallow-water individuals.

Species delimitation tests strongly supported the distinction at species level between the shallow-water and the deep-water lineages, even though they provided ambiguous results regarding the distinction between brackish-water and marine shallow individuals (Tab. 4). The ABGD approach identified in the COI dataset two groups, corresponding respectively to the deep-water clade and to the shallow-water clade. The result dealing with the 16S dataset is more ambiguous, as the test identified two or three species as possible outcomes. A separation between the brackish-water and the shallow marine sub-lineages is partially supported, but the probability for their conspecificity is substantially higher. The results of the PTP approach were not consistent with those of the ABGD, because this test identified three separated lineages in COI sequences, and only two in 16S sequences. All tests performed on mitochondrial markers identified the deep-water lineage as a separate species, whereas the separation at species level between the shallow marine and the brackish-water sub-lineages is identified only by the PTP test on COI sequences, and with some probability by the ABGD test on 16S sequences. As already suggested by the low K2P pairwise distances, 18S rDNA sequences did not allow to distinguish any lineage within the *Aricidea assimilis* group (Tab. 4).

Table 4: Results of single-locus species delimitation tests for the three molecular markers. No. of species: number of species identified by the test; Deep/Shallow: separation at species level between deep- and shallow-water individuals; Brackish/Marine: separation at species level between brackish and marine shallow-water individuals.

	ABGD			PTP		
	No of species	Deep/Shallow	Brackish/Marine	No of species	Deep/Shallow	Brackish/Marine
16S	2/3	+	?	2	+	-
COI	2	+	-	3	+	+
18S	1	-	-	1	-	-

4.5 Discussion

The present study showed for the first time the occurrence of cryptic speciation within Paraonidae and confirmed the Laubier & Ramos' (1974) hypothesis about the actual status of species complex of *Aricidea assimilis*. Nevertheless, results did not support the Strelzov's (1973) and Katzmann & Laubier's (1975) hypothesis on taxonomic separation between short- and long-antenna individuals, suggesting that a correct interpretation of morphological characters is more complex than previously considered. Available data do not allow to univocally identify *A. assimilis s.s.*. The holotypes of *A. assimilis* and *Aricidea mutabilis*, which is considered synonymous (Katzmann & Laubier, 1975), were sampled on low infralittoral – high circalittoral bottoms *sensu* Péres & Picard (1964) (50 to 60 m depth) (Tebble, 1959; Laubier & Ramos, 1974), suggesting that this name could be applied to the deep-water lineage (75-120 m) analysed in this study. However, this statement needs further validation, as no topotypic material for both taxa was examined in the present study. Furthermore, the inconsistency between morphotypes and lineages suggests that morphological features may depend on factors other than phylogenetic relationships and should be taken with caution when using them to diagnose Paraonidae species, as already observed in other polychaete families such as Orbiniidae (Meyer et al., 2008) and Eunicidae (Iannotta et al., 2009b).

Results of the present study highlighted that *A. assimilis* is actually composed by three mitochondrial lineages. The deep-water lineage is clearly distinguished from the shallow-water counterpart, which in turn is composed by one marine and one brackish-water sub-lineages, as depicted in the phylogenetic tree (Fig. 21). Even if all nodes at the basis of these groups showed high statistical support, the divergence between the deep-water and the shallow-water groups is remarkably higher than that between brackish-water and marine lineages. Genetic distance values between deep-water and shallow-water individuals (Fig. 22) are clearly in the range of interspecific distances detected by other studies on polychaetes (Pleijel et al., 2009; Nygren & Pleijel, 2011; Neal et al., 2014). This outcome, along with the consistent result of the two species delimitation tests on both mitochondrial genes, allowed to consider the deep- and shallow-water lineages separated at species level. On the other hand, the distances observed between the brackish-water and the marine lineage are approximately four- to five-fold lower, even though the distance in COI sequences is remarkably higher than the 3% proposed as boundary by Hebert et al. (2003). It is worth noting, however, that recent studies stress that the identification of a barcoding gap is more important than the setting of a fixed threshold (Čandek & Kuntner, 2015; Kvist, 2016). Moreover, in several annelid taxa

intraspecific distances turned out to be higher than the commonly used 3%, and closer to the values of 6.2-6.4% identified in this study (Kvist, 2016; Lobo et al., 2016)[2]. The divergence observed between brackish-water and shallow marine individuals, therefore, is consistent with the hypothesis of conspecific individuals. The interpretation of the brackish-water and the shallow water marine sub-lineages as belonging to the same species is however poorly satisfying for two reasons. The first clue towards a different interpretation of these results is represented by the absence of geographical segregation between the two sub-lineages. In fact, Tyrrhenian marine individuals are genetically closer to Adriatic marine ones than to brackish-water individuals from the Tyrrhenian Sea. This suggests that, even if the separation between the brackish-water and the shallow marine sub-lineages is more recent than the separation between the deep and the shallow clades, it is nevertheless old enough to be detected over the geographical separation. A more formal clue was provided by the ambiguous results of species delimitation tests, that in some cases separated the two groups at species level (Tab. 4). The absence of geographical segregation, together with the ambiguous result of species delimitation tests, allowed to consider the two sub-lineages as two incipient species (Mallet, 2007). This ambiguity is consistent with Hausdorf's (2011) observation that randomly sampled molecular markers do not always allow to distinguish between incipient species.

This study identified depth as the most important factor contributing to shape genetic structure of *A. assimilis*, ultimately leading to a cryptic speciation process. The main environmental factor that changes within the considered depth range is represented by sediment granulometry; in fact, shallow-water samples have been collected on sand or silty sand, whereas deep-water samples have been obtained from silty clay. The relevant role of sediment features in Paraonidae diversification is confirmed by results on the *Aricidea catherinae* species complex reported below in Chapter 3. On the other hand, the effect of brackish-water environments seems to be less pronounced, probably because of the level of connectivity between brackish-water and marine environments, that allows a degree of gene flow (Cognetti & Maltagliati, 2000). The occurrence of speciation processes in brackish-water environments has been confirmed in recent years by molecular studies (Maltagliati et al., 2000; Beheregaray & Sunnucks, 2001; Maltagliati et al., 2001; Trabelsi et al., 2002; Iannotta et al., 2009a; Sanna et al., 2013; Taugbøl et al., 2014) and, at some extent, present results confirm that these environments may play an important role in lineage diversification. The

[2] The range of intraspecific distances in polychaetes reported by Lobo et al. (2016) appears however too wide, reaching a maximum value of 33%. Such intraspecific distance values are suspiciously high, suggesting that in some cases undetected cryptic species have been considered conspecific.

selective pressure of brackish-water environments is often considered a strong driver for morphological diversification; in fact, brackish-water environments are often characterised by clearly differentiated morphotypes (Cognetti, 1954; Maltagliati et al., 2001; Ferrito et al., 2007), even if these differences often do not match with genetic diversity patterns (Heras & Roldán, 2011; Jimoh et al., 2013) or appear to be distinctly wider than molecular data would suggest (Maltagliati et al., 2001). This seems to be the case of the brackish-water morphotype identified in the *Aricidea assimilis* complex: the strong morphological divergence observed towards all marine individuals led to identify them as *Aricidea* cf. *laubieri*, but turned out to be inconsistent with the molecular diversity pattern, and should be considered as the result of selective pressures and genetic drift (Cognetti & Maltagliati, 2000). The identity of the studied specimens with *A. laubieri*, a species known until now from the Atlantic Ocean, is uncertain, and the hypothesis that *A. laubieri* actually represents only a morphotype of the *Aricidea assimilis* complex should be tested using topotypic material.

On the other hand, geographical boundaries seem to have a distinctly lower effect on diversification within the *Aricidea assimilis* complex. On the basis of present data, Adriatic and Tyrrhenian individuals belonging to the same clade are not segregated, but appear to be mixed. Even though the size of the dataset does not allow to infer on haplotype diversity and distribution, this result allows to make some preliminary considerations on Paraonidae reproduction and dispersal. The reproduction of Paraonidae is still a puzzling issue. Despite the high ecological relevance of this family in marine environments (Strelzov, 1973; Blake, 1996), there are only few data about their reproduction, and mainly from indirect evidence. The claim by Fewkes (1883) to have found larvae of Paraonidae in planktonic samples was questioned by Thorson (1946) and the findings by Bhaud (1983) appeared questionable as well. Even accepting Fewkes' (1883) and Bhaud's (1983) identifications, Paraonidae larvae are remarkably sporadic in the water column. For this reason, some authors considered likely the possibility of direct development, or the occurrence of a short larval phase, in the majority of Paraonidae. This hypothesis is supported chiefly by the presence of epitoke forms and the large size of eggs in some species (Rasmussen, 1973; Strelzov, 1973; López-Jamar et al., 1987; Giangrande, 1997). Available data do not allow, at present, to support any of the above-mentioned hypotheses (Rouse & Pleijel, 2001). However, polychaete species with direct development usually revealed a clear geographical structuring between the Adriatic and the Tyrrhenian Sea (Abbiati & Maltagliati, 1996; Virgilio & Abbiati, 2004; Cossu et al., 2015), whereas for species with dispersal phases genetic divergence between the two basins is lower (Abbiati & Maltagliati, 1992; Iannotta et al., 2007). In the present study both the deep-water

and shallow-water lineages showed the absence of geographical structuring, suggesting that the development in *A. assimilis* complex is not direct; rather, relatively long-lived pelagic larval phases with high potential for dispersal are expected. The reason for the scarcity of Paraonidae larvae in planktonic samples is unclear, but could take into account sporadic reproductive events that make difficult their detection in the water column. Given the small size of the dataset, this is only a preliminary remark, but molecular surveys appear a promising approach to infer on potential for dispersal in Paraonidae. Further studies based on a higher number of individuals from a wide geographical area may give a contribution to understand the effect of geographical boundaries on Paraonidae genetic diversity.

4.6 Acknowledgements

I would like to thank S. Aliani, O. Bresciani, C. Mazziotti and A. Pavia for providing ethanol-fixed individuals of *Aricidea assimilis* for this work; M. Casu, F. Maltagliati, M. Oliva, L. Pacciardi, M. Pertusati, M. Ponti, C. Pretti and A. Vannucci for their invaluable help in field sampling; M. Barbieri, T. Ravaglia and F. Squarcia for their help in molecular laboratory work; F. Guatieri and L. Rindi for their technical support in drawing charts.

5. Chapter 3: The Name of the Worm: disentangling the *Aricidea catherinae* (Annelida, Paraonidae) species complex

5.1 Abstract

In recent years, the occurrence of cryptic and pseudocryptic species in polychaetes has been stressed as a possible explanation for the cosmopolitan distributions reported for several species. In this work I analysed from both the morphological and molecular point of views individuals identified as *Aricidea catherinae* from six localities in the Atlantic Ocean and Mediterranean Sea. Moreover, I included in the study individuals of the closely related species *Aricidea elongata* and *Aricidea rubra* from the Pacific Ocean. Molecular data based on COI (634 bp), 16S rDNA (472 bp) and 18S rDNA (1786 bp) sequences highlighted the presence of seven highly divergent lineages, which can be considered as different species on the basis of species delimitation tests. Three of them correspond to *A. catherinae*, *A. elongata* and *A. rubra*, whereas the others are referred to undescribed species (*Aricidea* sp. 1, sp. 2, sp. 3 and sp. 4). These lineages are included in three highly supported clades: clade I, with *A. elongata*, *A. rubra* and two undescribed species from the eastern Atlantic Ocean and the Mediterranean Sea (*Aricidea* sp. 2 and *Aricidea* sp. 3); clade II, with *A. catherinae* and one undescribed species from the western Atlantic Ocean (*Aricidea* sp. 1); clade III, with only one undescribed species from the Mediterranean Sea (*Aricidea* sp. 4). The seven identified lineages are morphologically diagnosable based on morphological characters that were overlooked by previous descriptions, consistently with the definition of pseudocryptic species. In addition, the three species occurring in the Mediterranean Sea are characterised by different ecological requirements, as they dwell on different substrates and often at different depths.

This study confirmed the importance of a combined approach in species identification, highlighting a previously unexpected diversity within individuals assigned to a single nominal species. Present data, along with other recent studies, allow to reject the historical view that considered cosmopolitan a large part of polychaete species. Instead, biogeographical barriers as well as ecological drivers play important roles in determining polychaete diversity and distribution. As a consequence, checklists containing a large part of cosmopolitan species need careful and critical re-examination.

5.2 Introduction

The study of polychaete diversity has been greatly improved by the use of molecular techniques, that may highlight the occurrence of cryptic lineages within a number of morphospecies (Nygren, 2014). The definition of cryptic species is somewhat ambiguous, as it should refer to species that are impossible to distinguish at morphological level, because of the absence of discerning features (Bastrop et al., 1998; Maltagliati et al., 2000; Barroso et al., 2010; Carr et al., 2011; Brasier et al., 2016) or the wide morphological variability and the possibility of phenotypic plasticity, that may conceal morphological diversity patterns (Maltagliati et al., 2001; Bleidorn et al., 2006; Maltagliati et al., 2006). In several cases, however, a fine morphological analysis that takes into account characters that have been overlooked by previous descriptions, such as live colour pattern, staining patterns and fine details of chaetae, may highlight morphological differences that are consistent with molecular diversity patterns (Wu et al., 1991; Maltagliati et al., 2004; Luttikhuizen & Dekker, 2010; Nygren et al., 2010; Nygren & Pleijel, 2011). Although in several works these cases were referred to 'cryptic species' issues (Nygren & Pleijel, 2011; Nygren, 2014), as stressed by Luttikhuizen & Dekker (2010), species identified at a molecular level and also morphologically characterised at a fine level, should be considered 'pseudocryptic' (Knowlton, 1993; 2000). The occurrence of pseudocryptic species is remarkably common in all taxa, especially in marine ones (Knowlton, 1993; Goetze, 2003; Saez et al., 2003) and their detection showed a quick and strong increase in the last two decades, mainly due to the advance of DNA sequencing methods (Knowlton, 2000; Bickford et al., 2007).

Since polychaetes are important components of marine benthic assemblages, and their diversity and distribution are commonly used in environmental monitoring campaigns, the missed detection of pseudocryptic species may represent an important issue. Actually, the lumping of different species under the same taxon may lead to underestimate differences between environmental condition, with the possibility to underrate the effect of environmental alterations on benthic assemblages (Giangrande, 2003), in particular if this problem deals with poorly-known ecosystems (Terlizzi et al., 2003). The study of cryptic species issues, therefore, represents more than an intellectual pastime for skilled taxonomists, assuming instead a paramount relevance in environmental monitoring and management.

The polychaete worm *Aricidea catherinae* Laubier, 1967 is a common and widely distributed Paraonidae that is often found in local species lists all over the world. It has been originally described from the western Mediterranean Sea, on muddy bottoms between 35 and 40 meters depths (Laubier, 1967). Afterwards, it has been reported from the Mediterranean Sea (Laubier

& Ramos, 1974; Katzmann & Laubier, 1975; Castelli, 1985; Castelli, 1987; Çinar, 2005; Zaâbi et al., 2012; Çinar et al., 2014); the eastern Atlantic Ocean (Hartley, 1981; Narayanaswamy et al., 2005; Aguirrezabalaga & Gil, 2008); the western Atlantic Ocean (Strelzov, 1973; Maurer & Leathem, 1980); the Pacific Ocean (Strelzov, 1973; Blake, 1996; Lovell, 2002). This species shows, therefore, an almost cosmopolitan distribution. Moreover, species' bathymetric range is extremely wide, ranging from the surface to approximately 2000 meters depth (Strelzov, 1973; Castelli, 1987). These data rise the expectation that *A. catherinae* may actually be a species complex (A.S.Y. Mackie, *in litt.*).

In this work I tried to disentangle the *Aricidea catherinae* species complex by comparing individuals identified as *A. catherinae* from different areas of the Mediterranean Sea and the Atlantic Ocean with a combined morphological and molecular approach. My aims were *i)* to clarify how many cryptic species occur within the material identified as *A. catherinae* and *ii)* to understand if these species can be distinguished on morphological bases.

5.3 Material and methods

5.3.1 Sampling

Individuals morphologically identified as *Aricidea catherinae* were collected by SCUBA diving with a corer or by a boat-driven Van Veen Grab from six localities in the Mediterranean Sea and in the Atlantic Ocean (Tab. 5). Depth of collection and sediment grain were recorded in order to obtain a rough ecological characterisation of the different sampling sites. Samples were sieved with a 0.5 mm mesh, and sorted alive when possible. Specimens of *A. catherinae* were isolated from the samples and fixed in 96% ethanol. In both morphological and molecular analyses I also included individuals of the closely related species *Aricidea elongata* Imajima, 1973, which is listed among the synonyms of *A. catherinae* by Lovell (2002), and *Aricidea rubra* Hartman, 1963 (Tab. 5). Individuals of the latter species were loaned from the Benthic Invertebrate Collection (BIC) of the Scripps Institution of Oceanography.

Table 5: Individuals of the *Aricidea catherinae* species complex that were genetically characterised in the present study. EAt: Eastern Atlantic Ocean; EPa: Eastern Pacific Ocean; WAt: Western Atlantic Ocean; WPa: Western Pacific Ocean; T: Tyrrhenian Sea.

Species	Locality	Depth	Sediment	Date	N
Aricidea catherinae	Bay of Biscay (EAt)	34 m	Sandy mud	04/2015	2
Aricidea catherinae	Beals, Maine (WAt)	Tide level	Mud	05/2016	2
Aricidea catherinae	Belfast Lough (EAt)	24 m	Sandy mud	09/2008	1
Aricidea catherinae	Capraia Island (T)	11 m	Gravel	06/2014	1
Aricidea catherinae	Maremma (T)	7 m	Fine sand	06/2014	3
Aricidea catherinae	Versilia (T)	19 m	Sandy mud	07/2015	2
Aricidea elongata	Jinhae Bay (WPa)	Tide level	Mud	05/2016	1
Aricidea rubra	Costa Rica (EPa)	1000 m	Clayish mud	03/2009	2

Thirty-two Mediterranean individuals of *A. catherinae* fixed with 4% buffered formaldehyde in seawater, preserved in 70% ethanol and deposited in the polychaete collection of the University of Pisa were used only for morphological characterisation.

5.3.2 Morphological characterisation

Early morphological studies on Paraonidae overlooked some details of morphological traits, considering them unsuitable and subject to intraspecific variability (Strelzov, 1973). However, more recent literature considered that some of those characters are taxonomically informative (Gaston & McLelland, 1996; Montiel & Hilbig, 2004; Aguirrezabalaga & Gil, 2009; Arriaga-Hernández et al., 2013; Zhou & Reuscher, 2013). Accordingly, I used the width at the 6th chaetiger to standardise size-dependent parameters and I recorded *i)* the number of branchiae; *ii)* the shape of the posterior-most branchiae; *iii)* the starting chaetiger of modified chaetae; *iv)* the presence and shape of a sub-distal spine on the ventral edge of modified chaetae; *v)* the presence of eyes; *vi)* the length of antenna.

Measurements and counts were performed with a Primo Star Zeiss light microscope equipped with an ocular micrometer; drawings were realised with a *camera lucida*, or starting from pictures taken by a digital camera, and refined with GIMP 2.8.18 (available at http://www.gimp.org) following Montesanto's (2015) guidelines. Specimens identified as type material were deposited in the institutional collection of the University of Pisa.

5.3.3 Molecular characterisation

DNA extraction was carried out using the GenElute™ Mammalian Genomic DNA Miniprep Kit distributed by Sigma-Aldrich, following the manufacturer's instructions. For phylogenetic reconstruction I amplified the genes for 16S rRNA and COI (mitochondrial) and 18S rRNA (nuclear). 16S rDNA amplification was obtained using the primer pair 16SarL (5'-CGCCTGTTTAACAAAAACAT-3') and H3080 (5′-CCGGTCTGAACTCAGATCACGT-3′) (Palumbi et al., 1991), whereas for COI amplification I used the annelid-specific primers POLYLCO (5'-GAYTATWTTCAACAAATCATAAAGATATTGG-3') and POLYHCO (5'-TAMACTTCWGGGTGACCAAARAATCA-3') (Carr et al., 2011). 18S rDNA amplification was obtained using the primers F9 (5'-CTGGTTGATCCTGCCAG- 3') (Medlin et al., 1988) and R1513 (5'-TGATCCTTCYGCAGGTTC-3') (Petroni et al., 2002). Polymerase chain reaction (PCR) amplifications were carried out in 20 µL solutions using 1.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.1 µM of each primer, 1 U of DreamTaq DNA polymerase (Thermo

Scientific), and ~2.5 ng of template DNA. For 16S rDNA and COI the PCR profile was set as follows: initial denaturing step at 94 °C for 3 min; 34 cycles of denaturing at 94 °C for 45 s, annealing at 54 °C for 1 min, and extending at 72 °C for 1 min, and a final extending step at 72 °C for 7 min. For 18S rDNA, PCRs were carried out in 45 µL using a protocol with low ramp speed, and annealing temperature of 50 °C (Lorenz, 2012). A negative control was included in each reaction. PCR products were precipitated with sodium acetate and absolute ethanol and sent to Macrogen Europe for sequencing.

5.3.4 Genetic data treatment

Sequences from each gene were aligned with ClustalX 2.1 (Larkin et al., 2007) and alignments were edited in BIOEDIT version 7.2.5 (Hall, 1999). The program jModelTest 2.1.6 (Guindon & Gascuel, 2003; Darriba et al., 2012), based on the hierarchical likelihood ratio test, was used to assess the best model of evolution for the sequences under the Akaike Information Criterion (AIC) (Akaike, 1974). I used sequences of *Cirrophorus branchiatus* as outgroup (accession numbers KX901406, KX901419 and KX901442) according to results of a preliminary phylogenetic reconstruction (see Chapter 1).

A Bayesian consensus phylogenetic tree based on the three concatenated markers was constructed using MrBayes 3.2 (Ronquist et al., 2011), which allowed phylogenetic inference by treating each gene with its own substitution model. Four replicate runs were carried out with a total of three Markov chains per run for 2 x 10^6 generations. The chain was sampled every 100 generations to obtain 20 000 sampled trees. The first 5000 sampled trees (25%) were discarded as burn-in phase, with the remaining 15 000 trees used to estimate the Bayesian posterior probability (*PP*) of tree nodes. The convergence of Bayesian analyses was checked through the standard deviation of split frequencies, that should reach a value < 0.01 at the end of the analysis (Ronquist et al., 2011).

Pairwise K2P distances (Kimura, 1980) were calculated using the software MEGA 7.0.14 (Kumar et al., 2016). The separation at species level of the identified lineages was tested using two different single-locus species delimitation tests. The Automatic Barcoding Gap Discovery approach (ABGD) uses a range of prior intraspecific divergence to infer from sequence data a model-based one-sided confidence limit for intraspecific divergence. Thereafter, the algorithm detects the barcode gap as the first significant gap beyond this limit and uses it to partition the data, by sorting automatically sequences into hypothetical species (Puillandre et al., 2012). The Poisson Tree Processes approach (PTP), on the other hand, uses phylogenetic trees, and in particular branch length (as proxy of number of substitutions),

assuming that the number of substitution between species is significantly higher than the number of substitutions within species (Zhang et al., 2013).

5.4 Results

5.4.1 Phylogenetic reconstruction and species delimitation

I obtained sequences of 472 bp for 16S rDNA, 634 bp for COI, and 1786 bp for 18S rDNA (length of concatenated sequences = 2892 bp). The best fitting nucleotide substitution models were GTR+G for 16S, GTR+I+G for COI, and GTR+I for 18S. The Bayesian tree highlighted the presence of seven highly supported and deeply divergent lineages that I hereby consider as putative species (Fig. 23).

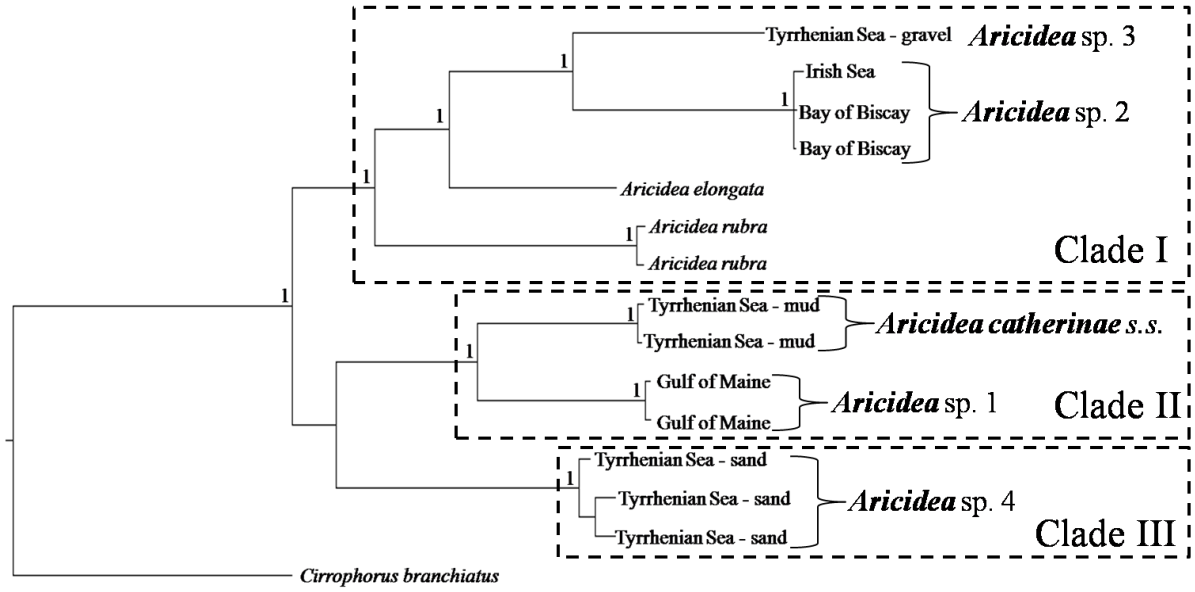

Figure 23: Bayesian tree of the combined gene dataset for the *Aricidea catherinae* species complex. Shown are node values with significant Bayesian posterior probabilities.

The identified lineages were included in three highly supported clades (Fig. 23). Clade I included *Aricidea rubra* and *A. elongata* from the Pacific Ocean, and two divergent lineages corresponding to individuals identified as *A. catherinae* from the Eastern Atlantic Ocean (*Aricidea* sp. 2) and from gravel bottoms from the Tyrrhenian Sea (*Aricidea* sp. 3). Clade II included two lineages with individuals identified as *A. catherinae*. Within clade II, a Mediterranean lineage corresponding to individuals sampled on muddy bottoms was identified as *Aricidea catherinae s.s.* based on morphological features that match those of the original description (see below in the systematic section), whereas the remaining lineage corresponds to individuals sampled in shallow environments of the Gulf of Maine (*Aricidea* sp. 1). Lastly, clade III showed unclear relationships with the other two clades, and included

only one lineage, corresponding to individuals identified as *A. catherinae* sampled on shallow sandy bottoms in the Tyrrhenian Sea (*Aricidea* sp. 4). All lineages showed high statistical support in the Bayesian tree (*PP*= 1).

Table 6: Pairwise K2P interspecific distances (%) ± standard deviation detected in the *Aricidea catherinae* species complex for 16S rDNA sequences (below diagonal) and for COI sequences (above diagonal). Intraspecific distances for 16S are reported on the diagonal, whereas those for COI are not shown due to the small amount of data. COI sequence data for *A. elongata* are not available (n.a.).

		1	2	3	4	5	6	7	8
1	*Aricidea catherinae*	0.0 ± 0.0	25.8	28.5 ± 0.0	n.a.	32.6 ± 0.1	27.4	32.0	37.4
2	*Aricidea* sp. 1	9.7 ± 0.0	0.0 ± 0.0	28.3 ± 0.0	n.a.	36.8 ± 0.0	28.3	38.4	34.6
3	*Aricidea rubra*	37.2 ± 0.5	41.3 ± 0.5	0.4 ± 0.0	n.a.	33.5 ± 0.8	28.6 ± 0.3	32.5 ± 0.3	32.6 ± 0.3
4	*Aricidea elongata*	39.0 ± 0.0	33.6 ± 0.0	32.3 ± 0.6	n.a.	n.a.	n.a.	n.a.	n.a.
5	*Aricidea* sp. 2	35.7 ± 0.4	35.8 ± 0.5	32.8 ± 0.5	28.8 ± 0.0	0.5 ± 0.4	28.1 ± 0.3	31.8 ± 0.9	39.6 ± 0.1
6	*Aricidea* sp. 3	38.3 ± 0.0	44.0 ± 0.0	40.9 ± 0.7	25.6	15.3 ± 0.3	n.a.	36.7 ± 0.0	36.5
7	*Aricidea* sp. 4	22.7 ± 1.1	27.5 ± 0.5	34.1 ± 0.4	37.1 ± 0.9	44.0 ± 1.6	40.0 ± 3.5	2.0 ± 0.3	36.4
8	*Cirrophorus branchiatus*	45.3 ± 0.0	50.1 ± 0.0	62.2 ± 1.0	54.2	50.2 ± 1.6	48.4	50.8 ± 2.0	n.a.

K2P distances calculated for 16S rDNA and COI sequence datasets (Tab. 6) were always remarkably high. For the 16S rDNA dataset, distances between lineages ranged from 9.7 to 44.0%, whereas for COI distances ranged from 25.8 to 38.4%. It is noteworthy that, considering the 16S rDNA database, the distances from to the outgroup *Cirrophorus branchiatus* were only slightly higher, ranging from 45.3 to 62.2%; whereas for COI, the distances from the outgroup were included in the distance interval of the species complex (32.6 to 39.6%). K2P distances detected within the same lineage with mitochondrial markers were very low, ranging from 0.0 to 0.5%, with the sole exception of *Aricidea* sp. 4, showing an average intraspecific distance of 2.0% relative to 16S rDNA data. As already observed (Chapter 2), 18S rDNA showed a much lower variability, and even if the trend of divergence observed is very similar to that obtained with mitochondrial markers, K2P distances between different lineages were distinctly lower, ranging from 0.1 to 0.6%. K2P distances from the outgroup were remarkably higher, ranging from 1.8 to 2.0%. 18S rDNA did not show intraspecific variation.

The two species delimitation tests gave identical results (Fig. 24). For mitochondrial markers both tests identified seven lineages, supported by high probability values in the PTP test (85 – 100% for 16S rDNA, 99 – 100% for COI), identified without ambiguity by the ABGD test. The results of the two tests, therefore, strongly support the preliminary conclusions about the status of phylogenetic species derived by tree topology and K2P distance matrix. The same tests applied to the 18S rDNA dataset, on the other hand, identify only two clusters, the former composed by *Aricidea* sp. 2 and *Aricidea* sp. 3 and the latter including all the remaining species. The statistical support for the two clusters was low (44.7 – 58.6% for

PTP), thus confirming the poor reliability of nuclear markers for species delimitation purpose (see Chapter 2).

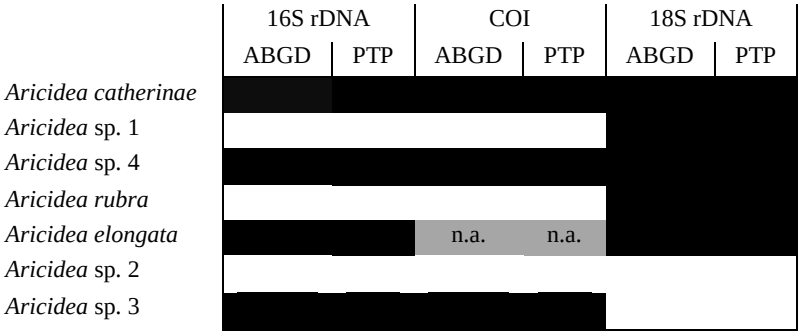

Figure 24: Graphical representation of the result of the two species delimitation tests on the three genes. Colour shifts indicate the recognition as separate species. COI data for *A. elongata* are not available (n.a.).

5.4.2 Systematics

Aricidea catherinae Laubier, 1967 (Fig. 25)

Examined material: Cinquale, Tyrrhenian Sea (43.9307° N, 10.0150° E), 19 m: 6 individuals (05/2015); Gulf of Follonica, Tyrrhenian Sea (42.9508° N, 10.7317° E), 8 m: 1 individual; Sea of Marmara (40.3767° N; 28.6614° E), 25 m: 3 individuals (06/2013).

Description: The examined individuals are all incomplete and relatively small, measuring approximately 0.24-0.37 mm of maximum width. Prostomium triangular, approximately 1.2 times longer than wide, without eyes. Prostomial antenna well developed, slightly shorter than the prostomium length, reaching backwards up to the end of the first chaetiger. The antenna is somewhat bottle-shaped, increasing in thickness in the middle part, and then tapering in the distal part. In particular, in living individuals some long, scattered cilia are noticeable on the distal part of the antenna. Two clearly noticeable nuchal slits on the posterior part of the prostomium (Fig. 25B). Three pre-branchial chaetigers; the first two show very short, tubercular notopodial post-chaetal lobes, whereas the third has a distinctly longer, cirriform notopodial lobe. Notopodial lobes are cirriform, slender in the branchial and post-branchial region. Branchiae are well developed, with very large basis and tapering, pointed tip; in the posterior part of the branchial region the elongated tip increases in length and can attain the length of the remaining part of the branchia. In the examined material, the number of branchiae ranges from 8 to 13 pairs (usually 10-12) (Fig. 25A).

Parapodia biramous, with relatively short and thick capillaries at both rami in the pre-branchial and branchial region. Capillary chaetae become thinner in the post-branchial region, where modified neuropodial chaetae occur. Modified chaetae begin at the 19^{th}-22^{nd} chaetiger and are 2-3 in the first chaetigers; afterwards their number increases, and in the posterior part

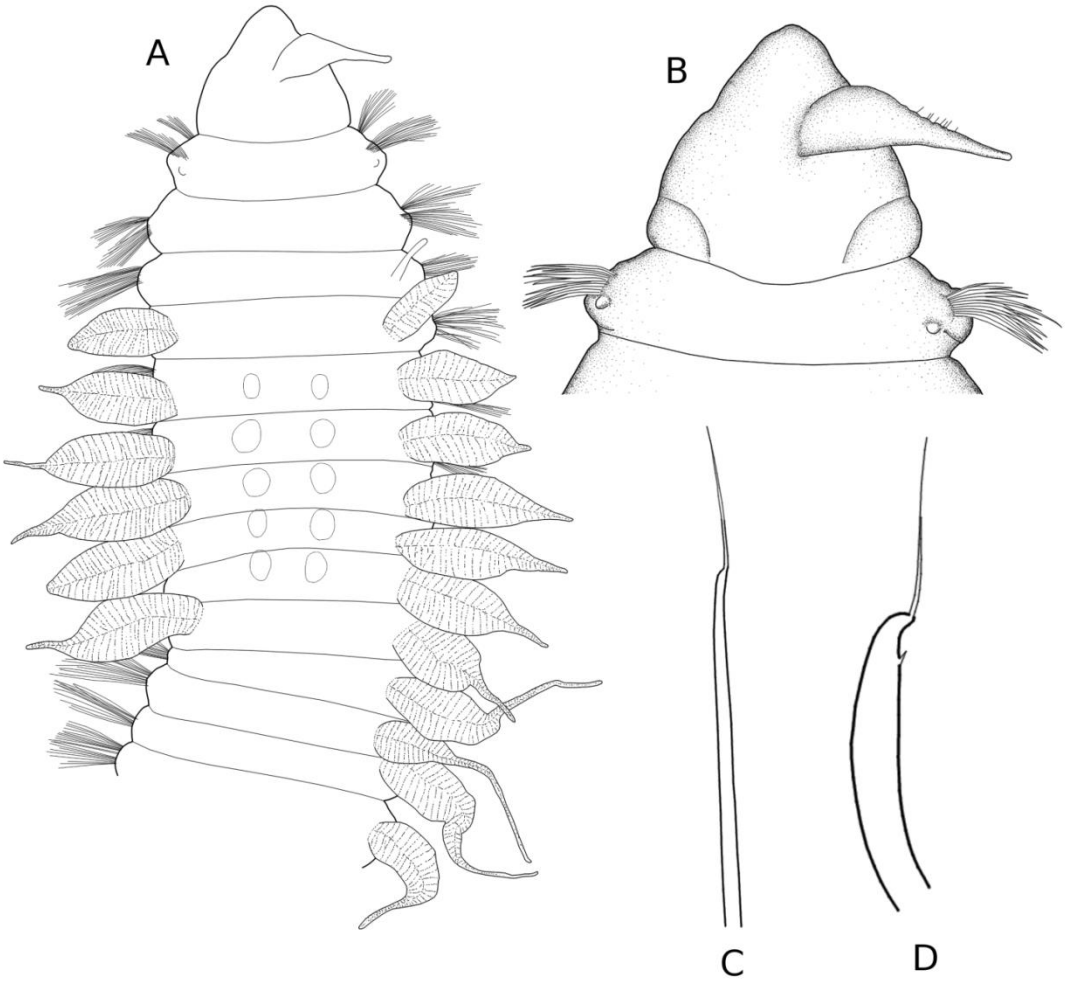

Figure 25: *Aricidea catherinae* Laubier, 1967: A) Individual from Cinquale (Tyrrhenian Sea); B) Prostomium of a live individual, showing nuchal slits and cilia on the antenna; C) Transitional chaeta from the upper part of the neuropodium (after Laubier, 1967); D) modified chaeta from the lower part of the neuropodium (after Laubier, 1967).

of the body their number ranges from 5 to 7. In the first post-branchial chaetigers, transition chaetae occur, that are relatively thin and elongate, with long, relatively thick and straight arista. True modified chaetae are however thick, almost straight hooks, with gently curved tip and a terminal, thin and straight arista that arises from their tip (Fig. 25D). Higher chaetae are thinner and straighter, with proportionally thicker arista (Fig. 25C). These structures might be interpreted as chaetae that are transitional between a typical capillary and a fully modified neuropodial hook. Ventral spine absent or very small, not reaching the tip of the chaeta.

Live colour greenish-brown, or yellowish-green, with bright pinkish-red thoracic inclusions and reddish branchiae.

Distribution: Gulf of Lion (Laubier, 1967); Tyrrhenian Sea (present data); Sea of Marmara (present data). Probably this species occurs in the whole Mediterranean Sea. The majority of

Mediterranean records, however, probably refer to different species, and this is true also for extra-Mediterranean records.

Ecology: On muddy bottoms, sometimes sand, between 8 and 40 m depth. More frequent between 20 and 40 m.

Remarks: The examined individuals match the original species' description (Laubier, 1967). *Aricidea catherinae s.s.* is a small species and does not show a wide variation range of the main morphological traits. Subsequent descriptions are often referred to larger individuals, with a wide variation range of both the number of branchiae and beginning of modified chaetae. Moreover, sometimes they showed features that are clearly different from *A. catherinae*, such as a sub-trapezoidal prostomium, a long prostomial antenna and eyes (Strelzov, 1973; Blake, 1996; Lovell, 2002). The vast majority of records referred to *A. catherinae* probably should be assigned to other species, some of which are described hereafter.

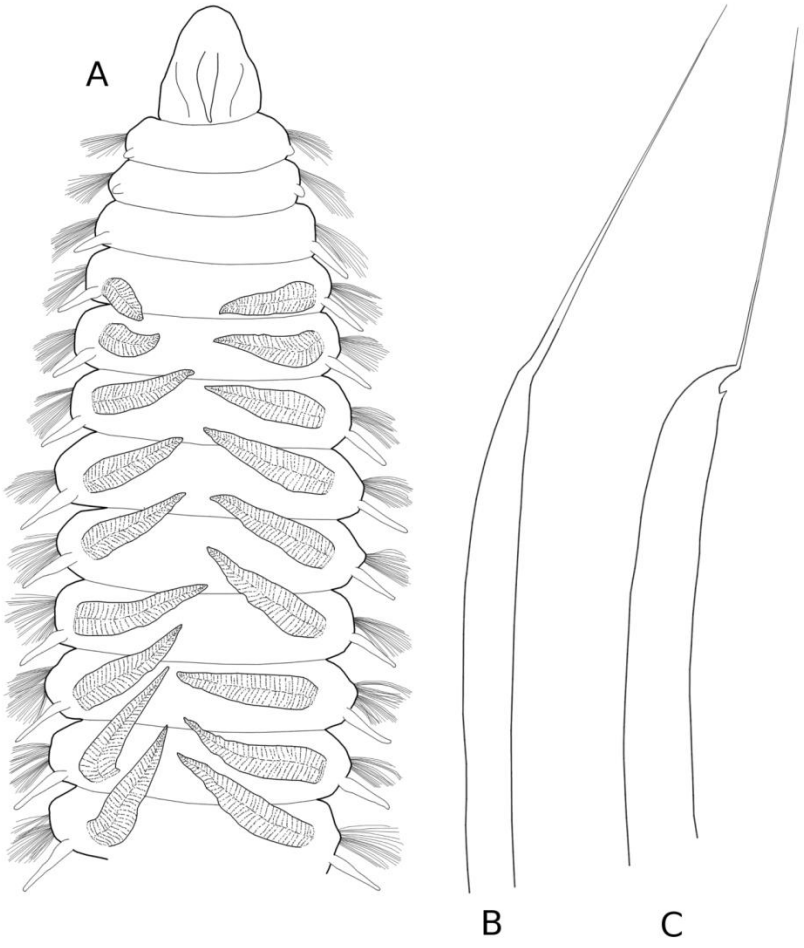

Figure 26: *Aricidea* sp. 1: A) Reference individual, anterior part in dorsal view; B) Transitional chaeta from the anterior part of the post-branchial region; C) Modified chaeta from the posterior part.

Aricidea sp. 1 (Fig. 26)

Examined material: **Reference individual**: Beals, Maine, Atlantic Ocean (44.5201° N; 67.6195° W), tide level. Additional material: Beals, Maine, Atlantic Ocean (44.5201° N; 67.6195° W), tide level: 30 individuals.

Description: Reference individual incomplete, approximately 12 mm for 76 chaetigers, 0.50 mm of maximum width. Body elongated, with branchial region somewhat flattened, quite wide. Prostomium sub-triangular, approximately 1.5 times longer than wide, with relatively wide, distinctly rounded anterior edge, without eyes. Two evident nuchal slits on the posterior part of the prostomium. Antenna relatively short, reaching backwards the end of the prostomium, slightly enlarged in the basal part, with tapered tip. Three pre-branchial chaetigers, the first two with short, tubercular notopodial post-chaetal lobe, the third with cirriform, distinctly longer notopodial lobe. Notopodial lobes are cirriform, long throughout the whole branchial and post-branchial regions. Pairs of branchiae are 18, well-developed, elongated, flattened in the anterior part of the branchial region, sub-cylindrical thereafter, without tapering tip in the posterior part of the branchial region (Fig. 26A).

Parapodia biramous, composed by two thick bundles of stocky, slightly curved capillaries. Modified chaetae begin at the 27th chaetiger and are 2-3 in the first chaetigers, increasing backwards to 5-7. In the first chaetigers, modified chaetae are accompanied by a transitional chaeta, thicker than the other capillaries and slightly curved, with deviated, elongated tip forming a long, robust arista (Fig. 26B). Modified chaetae are strong, relatively short hooks with slightly curved tip, becoming stockier towards the posterior part of the body. A very thin, straight and easily broken arista arises from the tip of the modified chaetae (Fig. 26C). Ventral spine absent, or barely noticeable as a small notch, in anterior modified chaetae, more developed but still very short in posterior modified chaetae.

In the remaining individuals (width interval = 0.44 to 0.55 mm), branchiae range from 14 to 17, the starting point of modified chaetae ranges from the 19th to the 28th chaetiger. In some individuals the median antenna is longer and reaches the middle of the first chaetiger. Posterior branchiae may have slightly tapering ends, but do not show the abrupt constriction that is evident in *A. catherinae*. Live colour unknown; preserved individuals brownish.

Distribution: Maine (present data). Probably elsewhere along the north-western Atlantic coast.

Ecology: Common on muddy bottoms at moderate depths, from tide level.

Remarks: *Aricidea* sp. 1 is close to *Aricidea catherinae* from both the morphological and molecular points of view. In particular, these two species share a very similar shape of

modified chaetae and a relatively short prostomial antenna. *Aricidea* sp. 1 is, however, a distinctly larger species, with a higher number of branchiae, a different prostomium shape and a clearly flattened body, with branchial region distinctly wider than the post-branchial part. I suggest that the majority of west-Atlantic records of *A. catherinae* can be actually referred to this species.

Aricidea rubra Hartman, 1963 (Fig. 27)

= *Aricidea lopezi rubra* Hartman, 1963

= *Aricidea finitima* Strelzov, 1973 *partim*

= *Acmira assimilis* (Tebble, 1959) *sensu* Lissner et al., 1986 (*partim*) *fide* Blake (1996)

= *Aedicira* sp. A *sensu* Lissner et al., 1986 *fide* Blake (1996)

= *Acmira* sp. B *sensu* Steinhauer & Imamura, 1990 *fide* Blake (1996)

= ?*Aricidea* near *suecica* Eliason, 1920 *sensu* Hartman, 1957 *fide* Blake (1996)

= ?*Aricidea longobranchiata* Day, 1961 *partim fide* Blake (1996)

= ?*Aricidea jeffreysii* McIntosh, 1879 *sensu* Hartman, 1955 *fide* Blake (1996)

Examined material: Costa Rica, Pacific Ocean (8.9317° N, 84.3167° W), depth unknown: 1 individual (02/2009) (BIC A1299); Costa Rica, Pacific Ocean (8.9305° N, 84.3123° W), 1000 m: 2 individuals (03/2009) (BIC A1444); Costa Rica, Pacific Ocean (8.9305° N; 84.3123° W), 1000 m: 1 individual (03/2009) (BIC A1616); Costa Rica, Pacific Ocean (9.1176° N, 84.8395° W), 1800 m: 1 individual (BIC A1952).

Description: The examined individuals show relatively large size; a complete individual measures approx. 20 mm of length for 122 chaetigers, and 0.70 mm maximum width. Prostomium triangular, eyeless, with antenna cirriform, with enlarged basis and pointed tip, shorter than the prostomium, not reaching the first chaetiger behind. Three pre-branchial chaetigers, with post-chaetal notopodial lobes spindle-shaped, gradually increasing, already cirriform on the first two chaetigers. Up to thirty pairs of elongate, pointed branchiae, with length increasing in the posterior part of the branchial region. Along the anterior edge of the branchiae, reddish pigment inclusions are clearly noticeable. Notopodial lobes increasing in the branchial region, very long and threadlike in the post-branchial region (Fig. 27A).

Parapodia biramous, with thick bundles of long capillaries, notopodial chaetae distinctly longer than the neuropodial ones, especially in the post-branchial region. The notopodium of some segments of the branchial region can be provided with a rounded additional papilla.

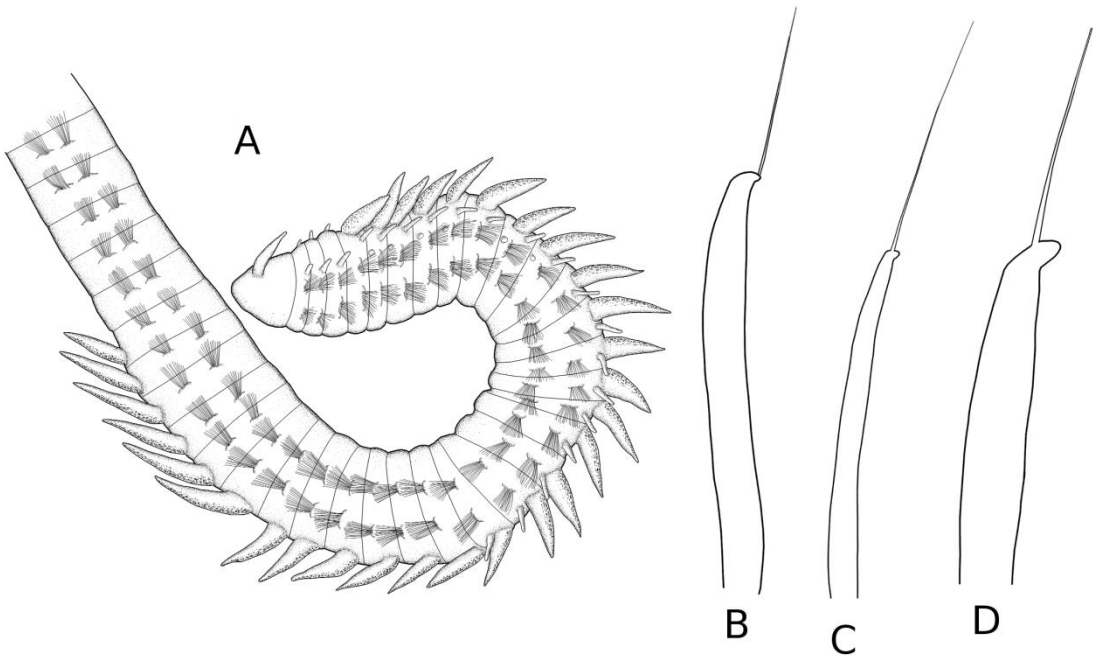

Figure 27: *Aricidea rubra* Hartman, 1963: A) Individual from Costa Rica in lateral view; B) Modified neurochaeta from the anterior part of the post-branchial region; C) Modified neurochaeta from the posterior part of the post-branchial region; D) Close-up of C showing the intermediate insertion of the arista.

Neuropodial modified chaetae, which occur in the post-branchial region, are thick, short hooks, with changing shape along the body length. In the anterior part of the post-branchial region modified chaetae are typical of the *Aricidea catherinae* complex, thick and straight, with slightly curved tip and terminal, thin and not very long (easily damaged) arista (Fig. 27B). Ventral spines are absent. Posterior modified chaetae are thinner, with blunt, angled tip (Fig. 27C), and sub-terminal, thicker and longer arista, with insertion approximately at the half of the tip (Fig. 27D).

Live colour yellowish-green, with bright red branchiae and red inclusions in the branchial region.

Distribution: Pacific Ocean: California (Hartman, 1963; Blake, 1996), Costa Rica (present data). Atlantic Ocean: Uruguay (Strelzov, 1973) (dubious); Antarctic Ocean: Scotia Sea (Strelzov, 1973) (dubious).

Ecology: On muddy bottoms, usually between 600 and 2000 m depth (Hartman, 1963; Strelzov, 1973; Blake, 1996). Records from infralittoral and circalittoral bottoms (Strelzov, 1973; Blake, 1996) might be referred to a different species.

Remarks: *A. rubra* has been described from bathyal environments off California by Hartman (1963) as a subspecies of *Aricidea lopezi* Berkeley & Berkeley, 1956. Later on, this taxon was forgotten, until Blake (1996) resurrected it as senior synonym of *Aricidea finitima* Strelzov,

1973 and erected it at species level. Present individuals are not completely consistent with Blake's (1996) redescription, since this Author did not highlight the presence of modified chaetae with terminal arista, and only described chaetae with sub-terminal arista. However, in his description of *A. finitima*, Strelzov (1973) clearly referred to chaetae with both terminal and subterminal arista. This pattern is consistent to that observed in the examined specimens, which show a gradual change from thicker chaetae with terminal arista, typical of the *Aricidea catherinae* complex, to thinner chaetae with crooked tip and subterminal arista, more similar to those of *A. lopezi*.

Based on Strelzov's (1973) original drawings and description, the synonymy between *A. finitima* and *A. rubra* stated by Blake (1996) appears somewhat questionable. In particular, Pacific individuals from Hartman's collection show elongated, cirriform post-chaetal notopodial lobes in the pre-branchial region, whereas the remaining material shows the first two lobes tubercular, and the third one distinctly slender. Moreover, the antenna is distinctly longer and slender in Atlantic material. Such differences suggest that *A. finitima*, as described by Strelzov (1973), might represent a species complex. The material examined in this work, however, closely corresponds to Pacific material and in my opinion it must be undoubtedly assigned to *A. rubra*.

Aricidea elongata Imajima, 1973 (Fig. 28)

= ?*Aricidea eximia* Imajima, 1973

= *Aricidea catherinae* Laubier, 1967 *sensu* Lovell, 2002 *partim*

Examined material: Jinhae Bay, South Korea, Pacific Ocean (34.9930° N; 128.6735° E), tide level: 1 individual (05/2016).

Description: A relatively large species, maximum width about 0.70 mm, 7.5 mm long for 65 chaetigers. Prostomium sub-triangular, posteriorly slightly wider, with rounded anterior margin, apparently without eyes. A pair of nuchal slits on the posterior part of the prostomium. Antenna slender, pointed, with median part not inflated, extending backwards to the 2^{nd}-4^{th} chaetiger. Three pre-branchial chaetigers, first two with very short, tubercular notopodial post-chaetal lobes, third one with distinctly longer, cirriform notopodial lobe. Notopodial lobes cirriform, slender in the branchial region, very thin, thread-like in the post-branchial region. Up to 20 sub-cylindrical branchiae with pointed, tapering tip; the length of branchial tips quickly increases in the posterior part of the branchial region, but the branchial tip becomes gradually thinner, without an abrupt constriction (see *A. catherinae*) (Fig. 28A).

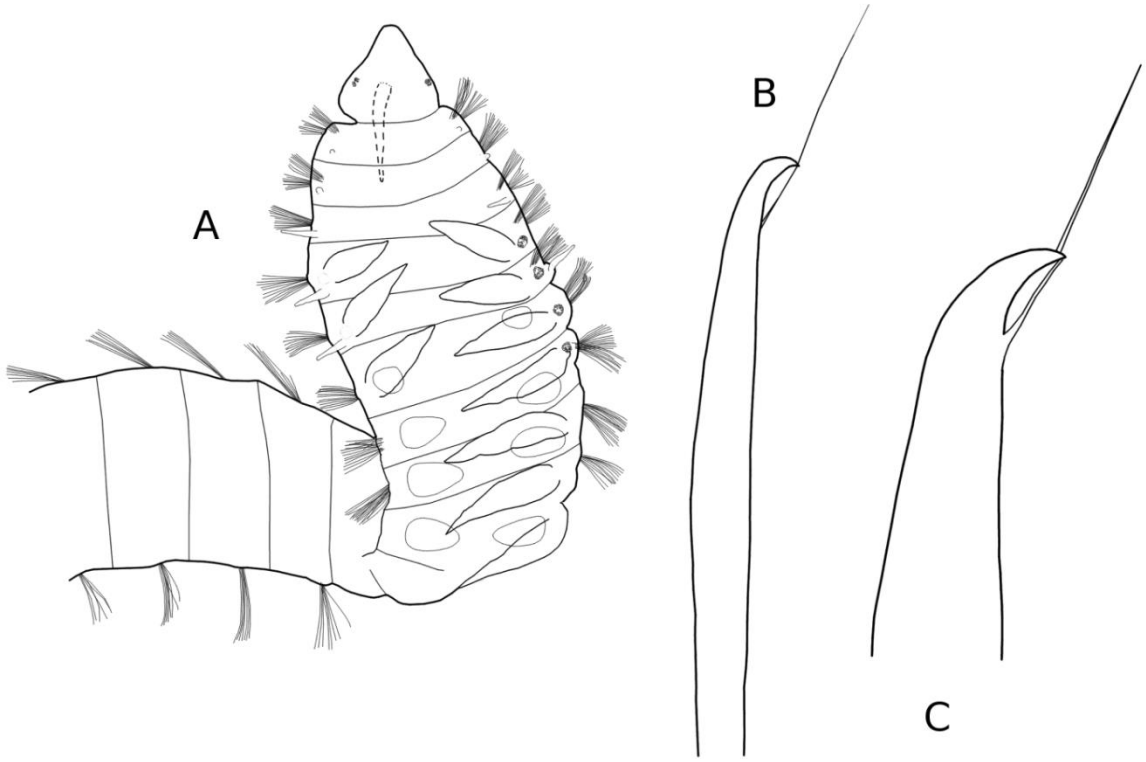

Figure 28: *Aricidea elongata* Imajima, 1973: A) Individual from Jinhae Bay, Sea of Japan, anterior part in dorsal view, antenna reconstructed on the basis of Imajima (1973); B) Neuropodial modified chaeta from chaetiger 15; C) Close-up of the tip of the modified chaeta.

Parapodia biramous, composed by two bundles of thick capillaries in the pre-branchial and branchial regions. Neuropodial modified chaetae begin after the branchial region and are relatively slender hooks, with curved tip. Ventral spine long, clearly noticeable, reaching the tip of the hook. Arista terminal, thin, easily broken (after Imajima, 1973) (Fig. 2B-C).

The examined individual matches the original description as regards the shape of neuropodial modified chaetae. It is however smaller (0.40 mm maximum width), with fewer branchiae (13). Modified hooks start at the 18th chaetiger and small, black eyespots are present.

Distribution: Sea of Japan (Imajima, 1973; present data).

Ecology: From the intertidal belt (present data) to 130 m depth (Imajima, 1973).

Remarks: Lovell (2002) listed *A. elongata* among the synonyms of *A. catherinae*. The present redescription shows that the two species clearly differ in the shape of antenna, modified chaetae (ventral spine present in *A. elongata*, absent in *A. catherinae*), and branchiae. Moreover, *A. elongata* is a distinctly larger species. *Aricidea eximia* Imajima, 1973 appears morphologically very close to *A. elongata* and Lovell (2002) considered this species synonymous with *A. catherinae* as well. The main difference between *A. elongata* and *A. eximia* is represented by modified chaetae, that are distinctly stockier in *A. eximia*, with

briskly crooked tip and apparently without arista. However, similar modified chaetae occur in posterior chaetigers of *Aricidea* sp. 2 and *Aricidea* sp. 3, whereas anterior chaetigers of the two species have slender hooks that are more similar to those of *A. elongata*. Both *A. eximia* and *A. elongata* are known from a very low number of individuals, and the possibility that the same change of chaetal shape observed in *Aricidea* sp. 2 and *Aricidea* sp. 3 occurs in *A. elongata* cannot be ruled out. If that would be the case, *A. eximia* and *A. elongata* should be synonymous, with *A. eximia* possessing nomenclatural priority.

Aricidea sp. 2 (Fig. 29)

= *Aricidea catherinae* Laubier, 1967 *sensu* Aguirrezabalaga, 2012

Examined material: **Reference individual**: Bay of Biscay (43.3039° N; 2.1479° E), 34 m (04/2016); Additional material: Belfast Lough, Irish Sea (54.7133° N; 5.6020° W), 24 m: 1 individual (09/2008); Bay of Biscay (43.3039° N; 2.1479° E), 34 m: 9 individuals (04/2016); Bay of Biscay (43.3060° N; 2.152° E), 35 m: 5 individuals (04/2016); Bay of Biscay (43.3041° N; 2.1504° E), 31 m: 5 individuals (04/2016).

Description: Reference individual incomplete, 4 mm for 35 chaetigers, 0.35 mm maximum width. Body elongated, sub-cylindrical. Prostomium sub-trapezoidal, approximately 1.2 times longer than wide, with squared anterior edge. Eyes present. Prostomial antenna relatively long and slender, reaching the middle of the 2nd chaetiger, slightly enlarged at the basis, gradually tapering towards the tip. Three pre-branchial chaetigers, the first two with very short, tubercular notopodial post-chaetal lobes, the third one with slender, cirriform notopodial lobe. Notopodial lobes are cirriform and slender in the branchial region, than tapered and thread-like in the post-branchial region. Branchiae are 13 pairs, sub-cylindrical, with somewhat pointed tip; their length gradually increases backwards, and in the posterior part of the branchial region tips are slightly tapering, acutely pointed. Last two pairs of branchiae are slightly smaller than the previous ones (Fig. 29A).

Parapodia biramous, composed by two bundles of thick capillaries in the pre-branchial and branchial regions. Neuropodial modified chaetae occur from the 27th chaetiger and are strong hooks with a long, thin terminal arista that easily breaks. In the first chaetigers of occurrence, modified chaetae are relatively slender, with gently curved tip, then become stockier, with briskly crooked distal end. Ventral spine present and well developed, reaching the tip of the hook (Fig. 29B).

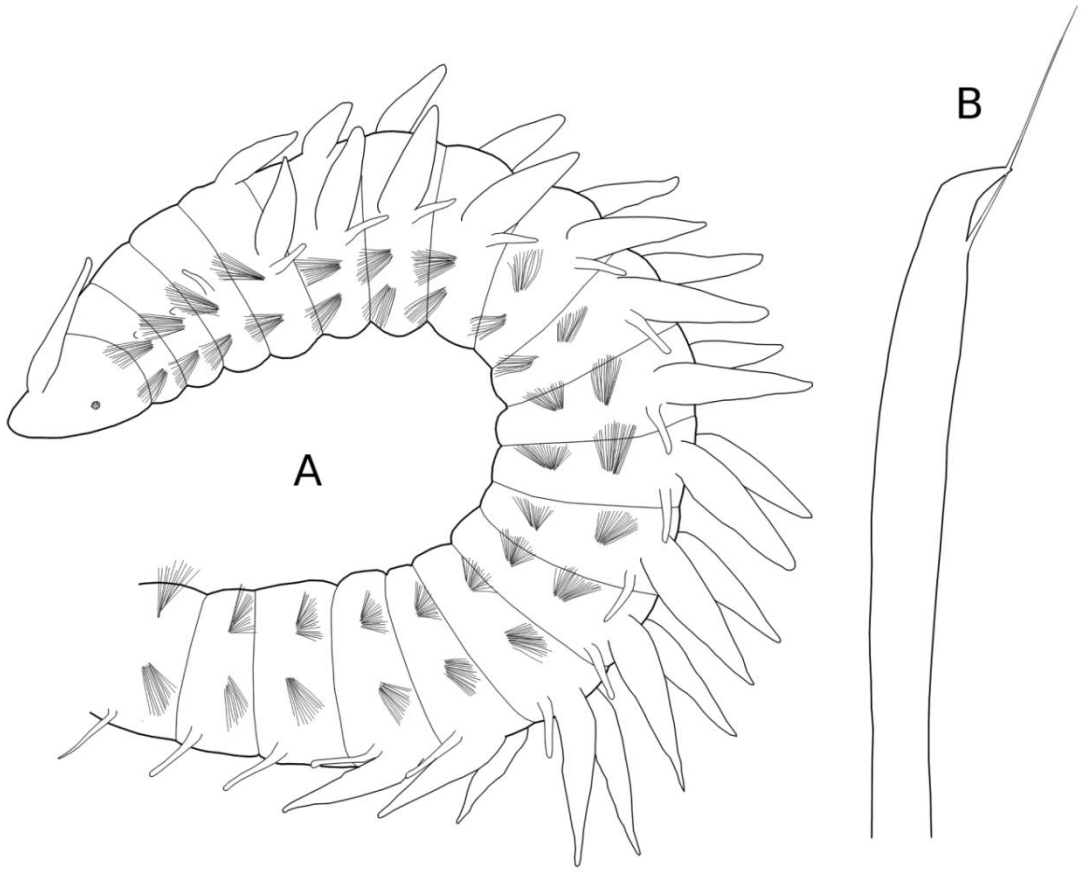

Figure 29: *Aricidea* sp. 2: A) Reference individual, anterior part in lateral view; B) Modified neuropodial chaeta.

The remaining individuals are very close to the described individual as regards meristic features. Branchiae are 13-14 pairs and maximum width ranges from 0.35 to 0.37 mm. Eyes may be absent – probably faded in preserved material. Live colour unknown, preserved animals greenish-brown.

Distribution: Eastern Atlantic Ocean, from the Irish Sea to the Bay of Biscay. The distribution of *Aricidea* sp. 2 might be wider.

Ecology: On muddy bottoms between 24 and 35 m depth.

Remarks: Among the *Aricidea catherinae* species complex *Aricidea* sp. 2 is closer to *Aricidea elongata* Imajima, 1973, and *Aricidea* sp. 3. These three species belong to a highly supported clade in the phylogenetic reconstruction of the present work and share some morphological features, such as in particular, the presence of eyes (easily faded in ethanol), a long prostomial antenna and a strong, well-developed ventral spine on modified chaetae. Nevertheless, *Aricidea* sp. 2 clearly differs from *A. elongata* in the shape of the prostomium (sub-trapezoidal in *Aricidea* sp. 2, sub-triangular in *A. elongata*), the absence of elongated tip in posterior branchiae, and the presence of stockier modified chaetae in the posterior part of the

body (although this last feature might be present in *A. elongata* as well). Moreover, *Aricidea* sp. 2 is a distinctly smaller species, with lower number of branchiae and modified chaetae starting posteriorly (from the 27th chaetiger in all the examined individuals, from the 18th chaetiger in an individual of *A. elongata* of similar size). *Aricidea* sp. 2 appears closer to *Aricidea* sp. 3, with which it shares the sub-trapezoidal prostomium and the presence of very thick hooks in the posterior part of the body. The two species differ in size, number of branchiae and beginning of modified chaetae, with *Aricidea* sp. 3 being a slightly larger species. Moreover, in *Aricidea* sp. 3 the ventral spine does not reach the tip of the hook. The drawing referred to *A. catherinae* in Aguirrezabalaga (2012) matches with *Aricidea* sp. 2 in number of branchiae, presence of eyes, shape of the prostomium and presence of a well-developed ventral spine on modified chaetae. I suggest that the majority of records referred to *Aricidea catherinae* from the north-eastern Atlantic Ocean actually refer to *Aricidea* sp. 2.

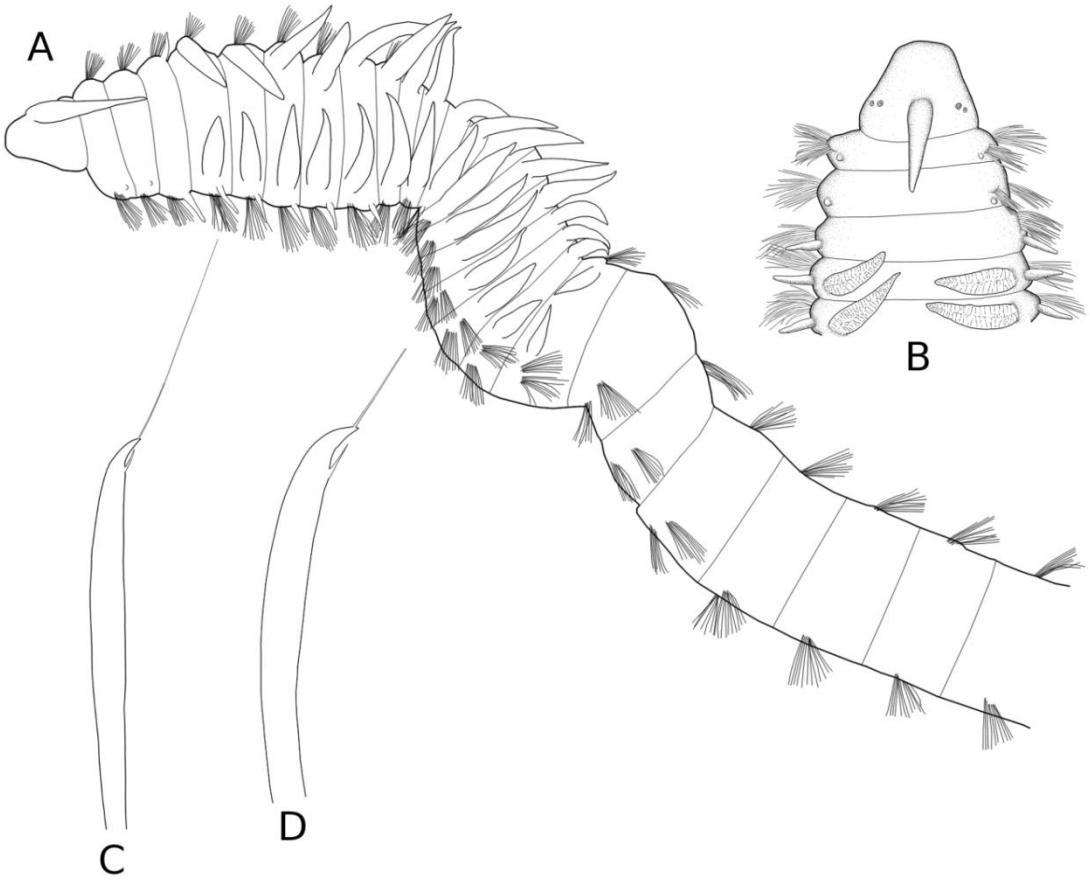

Figure 30: *Aricidea* sp. 3: A) Reference individual 1, anterior part in dorsal view; B) Reference individual 2, particular of the prostomium and first chaetigers; C) Modified chaeta from the anterior part of the post-branchial region; D) Modified chaeta from the posterior part of the post-branchial region.

Aricidea sp. 3 (Fig. 30)

Examined material: **Reference individual 1**: Sa Mesa Longa, Sea of Sardinia (40.0470° N; 8.3986° E), 3 m (03/2011); **Reference individual 2**: Strait of Messina (38.2084° N; 15.6291° E), 25 m (05/1992); Additional material: Capraia Island, Tyrrhenian Sea (43.0211° N; 9.835° E), 11 m: 1 individual (05/2014); Casaraccio Pond, Sea of Sardinia (40.9152° N; 8.2268° E), 1 m: 12 individuals (03/1994); Gulf of Trieste, Adriatic Sea (45.7468° N; 13.6075° E), 15 m: 1 individual (10/1995); Porto Pozzo, Tyrrhenian Sea (41.1928° N; 9.2781° E), 1-3 m: 2 individuals (07/1987); Strait of Messina (38.2084° N; 15.6291° E), 25-51 m: 16 individuals (05/1992).

Description: Reference individual 1 incomplete, approximately 10 mm for 63 chaetigers, 0.41 mm maximum width. Body elongated, slightly wider and flattened in the branchial region, chaetigers more elongate in the post-branchial region. Prostomium sub-trapezoidal, slightly longer than wide, with squared anterior edge. Eyes absent. Prostomial antenna long and slender, reaching the middle of the 2nd chaetiger, slightly enlarged at the basis, then tapering until the tip (Fig. 30B). Three pre-branchial chaetigers, the first two with very short, tubercular notopodial post-chaetal lobes, the third with long, cirriform post-chaetal lobe. Notopodial lobes are cirriform, elongated throughout the branchial region, then tapered and thread-like in the post-branchial region. Branchiae are 16 pairs, sub-cilindrical, with pointed tip, narrower and with slightly tapered, pointed end in the posterior part (Fig. 30A).

Parapodia biramous, composed by thick bundles of thick capillaries in the pre-branchial and branchial regions. Modified chaetae occur after the 36thchaetiger and are strong hooks with a terminal arista that is easily broken. In the anterior part of the post-branchial region modified chaetae are slender and with only slightly curved tip (Fig. 30C), whereas in the posterior part they are stockier, with briskly crooked tip (Fig. 30D). Ventral spine present, well developed but not reaching the tip of the hook.

The remaining individuals vary from 0.30 to 0.62 mm maximum width, and show 10 to 19 pairs of branchiae. In the majority of the examined individuals, eyes are present and may be simple or doubled, small eye-spots (Fig. 30B). Modified chaetae start after the 32nd – 45th chaetiger, depending on the size of the specimen. Live colour yellowish, with bright pink inclusions in the first branchial chaetigers. Preserved specimens whitish or yellowish.

Distribution: Mediterranean Sea: Adriatic Sea, Strait of Messina, Tyrrhenian Sea, Sea of Sardinia. Probably present in the remaining parts of the basin as well.

Ecology: On gravel, and mixed bottoms, usually between 1 and 15 m depth. Deeper records in the Strait of Messina (25-51 m) are due to the extremely peculiar environmental features of

the area. A part of the examined material was collected in a confined brackish-water basin (Casaraccio Pond). Among the *Aricidea catherinae* species complex, *Aricidea* sp. 3 is the only one that occurs in enclosed environments.

Remarks: *Aricidea* sp. 3 is closely related, and morphologically close, to *A. elongata* and *Aricidea* sp. 2. *Aricidea* sp. 3 differs from *A. elongata* in the shape of the prostomium (sub-trapezoidal in *Aricidea* sp. 3, sub-triangular in *A. elongata*), the absence of elongated tip in posterior branchiae and the presence of stockier modified chaetae in the posterior part of the body. It is also a slightly smaller species, with modified chaetae starting posteriorly (from the 32nd-45th chaetiger in the examined individuals, from the 18th chaetiger in an individual of *A. elongata* of similar size). Conversely, *Aricidea* sp. 3 appears almost identical to *Aricidea* sp. 2. However, in addition to the clear genetic divergence between *Aricidea* sp. 3 and *Aricidea* sp. 2, the two species appear differentiated at fine morphological level too. The prostomium is sub-trapezoidal in both species; however, in *Aricidea* sp. 3 it is wider than in *Aricidea* sp. 2. On average, *Aricidea* sp. 3 is slightly larger than *Aricidea* sp. 2 (0.40-0.60 mm *vs* 0.35-0.37 mm maximum width), with a higher number of branchiae (16-19 *vs* 13-14). Modified chaetae show the presence of a well-developed ventral spine, as in *A. elongata* and *Aricidea* sp. 2; however, in *Aricidea* sp. 3 the ventral spine does not reach the tip of the chaeta. Lastly, in *Aricidea* sp. 3 modified chaetae begin after the 32nd chaetiger (usually between the 35th and the 45th), whereas in *Aricidea* sp. 2 they start at the 27th chaetiger.

Aricidea sp. 4 (Fig. 31)

Examined material: **Reference individual**: Vasiliko Bay, Cyprus, Levant Sea (34.7161° N; 33.3200° E), 10 m (07/2014); Additional material: Albegna River Mouth, Tyrrhenian Sea (42.5020° N; 11.1878° E), 7 m: 1 individual (06/2014); Cala di Forno, Tyrrhenian Sea (42.6193° N; 11.0850° E), 7 m: 1 individual (06/2014); Gulf of Trieste, Adriatic Sea (45.7468° N; 13.6075° E), 15 m: 2 individuals (10/1995); Nettuno, Tyrrhenian Sea (41.4496° N; 12.6616° E), 7 m: 5 individuals (06/2015); Ombrone River Mouth, Tyrrhenian Sea (42.6591° N; 11.0088° E), 7 m: 1 individual; Porto Pozzo, Tyrrhenian Sea (41.1928° N; 9.2781° E), 14 m: 1 individual (10/1987); Rosignano, Tyrrhenian Sea (43.3755° N; 10.4259° E), 7 m: 1 individual (06/2014); Vasiliko Bay, Cyprus, Levant Sea (34.7161° N; 33.3200° E), 10 m: 3 individuals (07/2014).

Description: Reference individual incomplete, approximately 5 mm for 46 chaetigers, 0.28 mm maximum width. Body elongate, dorsally slightly flattened in the branchial region.

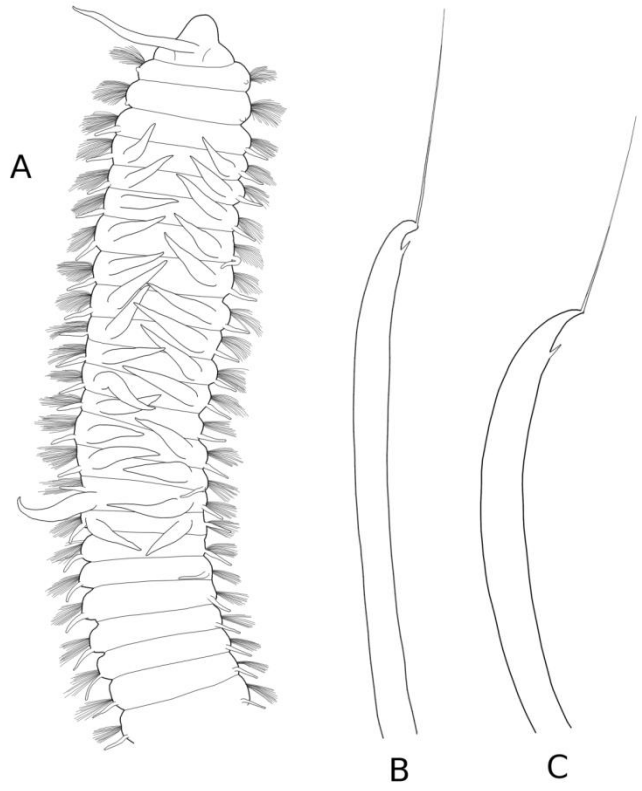

Figure 31: *Aricidea* sp. 4: A) Reference individual, anterior part in dorsal view; B) Dorsal-most modified chaeta at the 40th chaetiger; C) Ventral-most modified chaeta at the 40th chaetiger.

Prostomium triangular, with pointed tip, slightly enlarged posterior region with prominent nuchal organs. Eyes absent. Antenna very long and slender, posteriorly reaching the 4th chaetiger, tapering towards the tip, and devoid of noticeable basal enlargement. Three pre-branchial chaetigers, the first two with very short, tubercular notopodial post-chaetal lobes, the third one with long, cirriform notopodial lobe. Notopodial lobes are slender, cirriform, with slightly enlarged basis in the branchial region; in the post-branchial region they become thinner and thread-like, even if they remain of the same length. Branchiae are 14 pairs, relatively slender, slightly flattened and with pointed, not tapering tip (Fig. 31A).

Parapodia biramous, composed by two thick bundles of capillaries in the anterior region. Modified chaetae occur after the 29th chaetiger and are 1-4 strong hooks with terminal arista. Modified chaetae in the higher part of the neuropodium are long and relatively straight, with a very small ventral spine that is noticeable as a subdistal notch on the ventral edge (Fig. 31B); modified chaetae in the lower part of the neuropodium are strongly curved, shorter and thicker, with slightly more developed ventral spine, that however does not reach the tip of the

hook (Fig. 31C). The shape of modified chaetae remain similar throughout the whole body length, even if the number increases from 1 to 4 towards the pygidium. Other examined individuals have 13 to 22 branchiae for a maximum width of 0.25-0.30 mm. Modified chaetae start from the 26th-33rd chaetiger, depending on the size of the specimen, and eyes are sometimes noticeable. Preserved colour greenish, live colour unknown.

Distribution: Mediterranean Sea: Adriatic Sea, Tyrrhenian Sea, Levant Sea. Probably frequent also in the remaining parts of the basin.

Ecology: On fine sand, sometimes on muddy bottoms, between 7 and 15 m depth.

Remarks: *Aricidea* sp. 4 is morphologically very close to the other species of the complex, showing intermediate features between species belonging to clade I and species belonging to clade II. Similarly to species of clade I (*Aricidea elongata*, *A.* sp. 2 and *A.* sp. 3), it has a clearly noticeable subdistal spine on the ventral edge of the modified chaetae, but it is shorter than in the other species, and modified chaetae remain of the same shape throughout the body length. On the other hand, *Aricidea* sp. 4 shares with *Aricidea catherinae* and *A.* sp. 1 the triangular shape of the prostomium, but transitional chaetae are absent, and the ventral spine is more evident. Moreover, in this species the antenna is longer than in all other species, with the possible exception of large individuals of *A. elongata*. This combination of features, along with results of the molecular analysis, allows to consider *Aricidea* sp. 4 as a distinct species.

5.5 Discussion

Based on present data, individuals identified as *Aricidea catherinae* turned out to belong to five cryptic species, belonging to three clades, whose relationships are still unclear. Moreover, *Aricidea rubra* is closely related to one of the clades, including as well *Aricidea elongata*, a species that was considered a synonym of *A. catherinae* (Lovell, 2002), but that on the basis of both morphological and molecular data should be considered valid. In all cases, high values of interspecific distances were detected. The K2P distance range is between 9.7 and 44.0% for 16S rDNA, and between 25.8 and 38.4% for COI. These values are strikingly high, considering that a 3% divergence is commonly employed as a threshold to discriminate between different species (Hebert et al., 2003). Even if intraspecific distance values detected in annelids are often higher than this threshold value (Kvist, 2016), the detected values are higher than those identified within species complexes in other annelid families such as Hesionidae (Pleijel et al., 2009), Phyllodocidae (Nygren & Pleijel, 2011) and Polynoidae (Neal et al., 2014). A comparison with the results of Chapter 2 shows that distances between species within the same clade are comparable with those between the two highly divergent

Table 7: Prospect summarizing the major morphological characters of the species described within the *Aricidea catherinae* complex

Species	Antenna	Prostomium	Eyes	No. Branchiae	Starting chaetiger of modified chaetae	Ventral spine on modified chaetae
Aricidea catherinae	To the 1st chaetiger	Triangular	-	8-13	19-22	Very small
Aricidea sp. 1	To the 1st chaetiger	Subtriangular, anteriorly rounded	-	14-17	19-28	Very small
Aricidea rubra	To the end of prostomium	Subtriangular	-	25-30	22-32	Absent
Aricidea elongata	To the 2nd-4th chaetiger	Subtriangular	+/-	15-20	>18	Large
Aricidea sp. 2	To the 2nd chaetiger	Subtrapezoidal	+/-	13-14	27	Large
Aricidea sp. 3	To the 2nd chaetiger	Subtrapezoidal	+/-	10-19	32-45	Large, not reaching the tip
Aricidea sp. 4	To the 4th chaetiger	Triangular	+/-	13-22	26-33	Small

lineages identified within *Aricidea assimilis* Tebble, 1959 (9.7-25.6% *vs* 14.8-15.8% for 16S rDNA, 25.3-28.5% *vs* 22.2-26.8% for COI); whereas, considering both molecular markers, the distances between species belonging to different clades are distinctly higher. As a matter of fact, genetic distances between *Aricidea* species belonging to different clades are only slightly lower than distances towards *Cirrophorus branchiatus*, the non-congeneric species that I employed as outgroup. The comparison between the two mitochondrial markers employed shows that the distance pattern identified by 16S rDNA allows to infer on the actual phylogenetic relationships, whereas in COI sequences variable positions are probably saturated after a certain degree of divergence, making this marker poorly reliable in phylogenetic inference at the higher taxonomic levels (Xia et al., 2003). On the other hand, the genetic distance detected at the level of 18S rDNA towards *C. branchiatus* is at least three-fold the distance between any of the identified species, thus confirming that this marker, with its slower evolution rate, is far more suitable than mitochondrial markers for higher rank phylogenetic reconstruction (Hillis & Dixon, 1991). Species delimitation tests confirm this interpretation of molecular data, unambiguously identifying each lineage as a separate species for mitochondrial datasets. As previously observed (see Chapter 2), 18S rDNA is not suitable for species delimitation purposes, even if in this case it allows the identification of two divergent groups, that, however, do not correspond to clades identified in the Bayesian reconstruction based on the whole molecular dataset (Fig. 23).

According to this reconstruction, different lineages are also characterised by morphological differences (Tab. 7) that are recognizable at the clade level as well. Clade I is composed by two morphologically homogeneous sub-clades, the former corresponding to *Aricidea rubra*, a large, deep-water species bearing modified chaetae with both terminal and sub-terminal arista, and the latter including *Aricidea elongata*, *Aricidea* sp. 2, and *Aricidea* sp. 3. The latter group is characterised by the frequent occurrence of eyespots (that may fade in ethanol), a relatively long prostomial antenna, that reaches the 2nd-3rd chaetiger, and modified chaetae with a long, well-developed ventral spine. The other two clades are composed by species without a well-developed ventral spine. Clade II includes *Aricidea catherinae s.s.* and *Aricidea* sp. 1, a west-Atlantic shallow-water species. Both species are characterised by a very small, or absent, ventral spine, stocky modified chaetae and the presence of clearly noticeable transition chaetae, by absence of eyes, and by a short, thick prostomial antenna. Lastly, clade III includes only *Aricidea* sp. 4, a common shallow-water Mediterranean species, with a short ventral spine on modified chaetae and sub-triangular, pointed prostomium that may recall *A. catherinae*, but with a long, slender prostomial antenna. Different clades including individuals

identified as *Aricidea catherinae*, therefore, were primarily identified by molecular markers, but turned out to be morphologically distinct as well. The same can be stated for different species, even though in this case morphological differences are more subtle. For instance, *Aricidea* sp. 2 and *Aricidea* sp. 3 are clearly differentiated at molecular level, but morphological differences are less obvious, and mainly related to size, number of branchiae, beginning of modified chaetae and shape of the ventral spine. The current use of a combined molecular and morphological approach, thence, highlighted the presence of several pseudo-cryptic lineages that can be considered as different species following Cracraft's (1989) definition of phylogenetic species ("a phylogenetic species is an irreducible [...] cluster of organisms, diagnosably distinct from other such clusters, and within which there is a parental pattern of ancestry and descent").

It is worth noting that the strong morphological variability among individuals identified as *A. catherinae* has been already, although indirectly, remarked. Katzmann & Laubier (1975) stated that Strelzov (1973) "imperfectly represented the terminal hood of modified chaetae", and actually, chaetae drawn by Strelzov (1973) are rather different from those of *A. catherinae*, with a well-developed ventral spine. This suggests that Strelzov's (1973) material actually belonged to clade I, rather than to the true *A. catherinae*. In this case, both Laubier's (1967) and Strelzov's (1973) drawings were accurate, but there is the suspicion they did not refer to the same species. On the other hand, Lovell (2002) identified in Pacific material referred to *A. catherinae* additional inter-ramal papillae in the branchial region, that were never described for this species. Interestingly, discrepancies in drawings and descriptions referred to the same species have been always referred to the lack of technical skills or to intraspecific variability, whereas the possibility that they actually referred to different species was never stressed, at least in published literature. A possible explanation is that, based on present data, different species of the *Aricidea catherinae* species complex usually have relatively narrow, and scarcely overlapping, distributions. The vast majority of benthologist are used to work on specific geographical areas, where only one, or few, species are supposed to occur, and this may have led each research group to identify 'his' *Aricidea catherinae*, referring to morphological features observed in local material rather than to the original description. This process is evident for instance in Aguirrezabalaga (2012), whose extremely detailed and accurate drawing of *A. catherinae* strongly differs from the original drawings in Laubier (1967), but matches *Aricidea* sp. 2. The overlooking of morphological differences, along with the incorrect, yet widespread belief that "polychaetes generally are poor biogeographical indicators" (Ekman, 1953) are the main reasons for the alleged

cosmopolitanism of *A. catherinae*. Based on present data and in agreement with Fauchald (1984), species belonging to clades identified in this work showed a distribution that is strongly consistent with current biogeographical theories (Oliver & Irwin, 2008; Almada et al., 2013; Watling et al., 2013). Even though present results are instrumental in rejecting the hypothesis of *A. catherinae* cosmopolitanism, they are based on material coming from a relatively narrow geographical range. It is very likely that a further development of this work, taking in account material from other biogeographical areas and different environments, may highlight a number of undiscovered species that were until now concealed under an overused name. Misquoting Eco (1983) we can therefore state that "yesterday's *worm* stands only in name", as the majority of reports of *A. catherinae* should actually be referred to different species, and a thorough revision of worldwide material is necessary to correctly assess the true diversity hidden in the *Aricidea catherinae* species complex.

On the other hand, the distribution of different clades broadly overlaps: each clade includes one Mediterranean species, and in particular clade I includes Pacific, Atlantic and Mediterranean species. The three identified Mediterranean species are not only not directly related, i.e. do not belong to the same clade, but they differ in their ecological requirements as well. In fact, *Aricidea catherinae* is a muddy bottoms species that commonly occurs between 20 and 60 m depth, *Aricidea* sp. 3 chiefly occurs in shallow waters (1-15 m) on gravel or mixed bottoms, and *Aricidea* sp. 4 has been reported from clean sand between 7 and 15 m depth. The absence of a direct relationship among these species suggests that their differentiation should have occurred outside from the Mediterranean Sea, and that the three lineages should have colonised the Mediterranean basin afterwards, adapting to different ecological features. Based on present data, hypotheses taking into account human-mediated accidental translocations cannot be ruled out. However, invasive species belonging to Paraonidae have never been recorded, and, also on the basis of the ecological features of these organisms, I consider this possibility very unlikely. Moreover, the relationship between the Mediterranean *Aricidea* sp. 3 and the Atlantic *Aricidea* sp. 2 strongly suggests that *Aricidea* sp. 3 originated as a Mediterranean vicariant of the Atlantic species, probably as a consequence of glacial cycles (Bianchi & Morri, 2000). The relationship between different clades is unclear, and somewhat ambiguous: even though all clades share a feature that was traditionally considered as diagnostic for *A. catherinae*, that is, the terminal insertion of the arista on hook-shaped modified chaetae, they apparently are only distantly related, and do not form a monophyletic group (see as well Chapter 4). The insertion of the arista, therefore, does not represent a reliable taxonomic feature. This result might have different explanations. A

possibility is that the insertion of the arista on modified chaetae, and their shape are highly variable characters, and this variability makes inference based on this feature highly unreliable. Another suitable explanation, however, is that thick hooks with a thin, terminal or sub-terminal, arista represent the ancestral state for modified chaetae in the genus *Aricidea*, and therefore are independently retained by different groups within this genus. The latter view is supported by results of a more complete phylogenetic reconstruction (see Chapter 4) and may be an additional explanation for the extremely high diversity encountered within an allegedly single species.

Present data allow to reject the alleged cosmopolitanism of *A. catherinae*. At least in the Mediterranean Sea, different species may occur sympatrically, even though they are differentiated at the level of ecological requirements and thrive in different microhabitats (as for instance, sediment patches characterised by different granulometry). This result is consistent with recent studies on other allegedly cosmopolitan polychaete species (Bleidorn et al., 2006; Barroso et al., 2010; Carr et al., 2011; Álvarez-Campos et al., 2017), that strongly contradict previous views that suggest that a major part of polychaete species have very wide, and often cosmopolitan, distributions (Fauvel, 1923; 1927; Ekman, 1953). Interestingly, a large part of Paraonidae have been considered cosmopolitan (Strelzov, 1973; Imajima, 1973; Blake, 1996; Lovell, 2002), and only in recent years diversity patterns corresponding to biogeographical areas have been detected (Aguirrezabalaga & Gil, 2009; Çinar et al., 2011). The present study based on the *Aricidea catherinae* species complex suggests that actual species may have relatively narrow distributions. Species lists referring to European waters are largely based on species described in that area (Glémarec, 1966; Laubier, 1967; Laubier & Ramos, 1974; Katzmann & Laubier, 1975; Hartley, 1981; Castelli, 1985; Aguirrezabalaga & Gil, 2008; 2009), even if with some exceptions (Çinar et al., 2011; Langeneck et al., *subm.*). Conversely, in other geographical areas, the majority of taxa reported in species lists have been described for European waters (Strelzov, 1973; Blake, 1996; Lovell, 2002; Zhou & Li, 2007). Hence, I speculate that further revisions will highlight a strikingly high occurrence of undescribed species, as already suggested by some taxonomic keys, that consider provisional those species that are morphologically distinct, but that have not been officially described (Lissner et al., 1986; Lovell, 2002; Barwick, 2006). In this frame, the re-evaluation of synonymies and fine morphological features (Strelzov, 1973; Blake, 1996), as well as the combined use of morphological and molecular data may represent a very effective approach to reveal species complexes in Paraonidae, and to understand the actual biogeographical patterns in this family.

5.6 Acknowledgements

I would like to thank F. Aguirrezabalaga, B. Beals, O. Bresciani, H. Cha, M.E. Çinar, J.A. Commito, T. Darbyshire, D. Erdoğan, C. Jourdet, M. Lezzi, A.S.Y. Mackie, A. Pavia, V. Radashevsky, and G. Rouse for providing ethanol-fixed individuals of *Aricidea catherinae, A. elongata* and *A. rubra* for this work; C. Ravaglioli for her help in field sampling; T. Ravaglia and F. Squarcia for their help in molecular laboratory work.

6. Chapter 4: Molecular phylogeny of Paraonidae (Annelida)

6.1 Abstract

A molecular phylogeny of the family Paraonidae was reconstructed on the basis of 16S rDNA, COI and 18S rDNA sequences obtained from 60 individuals belonging to 34 nominal species and subspecies. Consistently with previous findings, Paraonidae represent a monophyletic group, and the closest polychaete family is found to be Sternaspidae. Neither the traditional view on Paraonidae evolution, nor a more recent cladistic analysis were consistent with the topology highlighted by the Bayesian analysis on the combined 2866 bp-sequence dataset. I found that Paraonidae can be divided in five well supported clades. The earliest branching clade (clade I) included *Cirrophorus* and *Paradoneis* with lyrate chaetae throughout the whole body length, whereas the remaining species of these genera, with lyrate chaetae changing in shape towards the pygidium, were included in a further clade (clade II), with the exception of *Cirrophorus branchiatus*, that cannot be assigned to any of the identified clades. The genus *Levinsenia* is monophyletic and represents the sister group of a highly supported clade that includes *Aricidea simplex*, *A. monicae*, *A. simonae* and an unnamed deep-water Pacific species; this clade represents a new genus, yet to be named. This clade is also morphologically characterised, including mainly deep-water species with rounded or squared prostomium, very small prostomial antenna and modified chaetae blade-shaped, without arista or additional hairs. All remaining species of *Aricidea* clustered in a highly supported clade that includes *Paraonis* as well. *Paraonis* is here interpreted as a pedomorphic form of *Aricidea*, and this would account for the strong morphological divergence between the two genera. For priority rules, *Paraonis* should be synonymised with *Aricidea*. None of the subgenera traditionally recognised within *Aricidea* turned out to be monophyletic, and the shallow molecular divergence identified among species, in particular at the level of 18S rDNA sequences, suggests that the adaptive radiation of the genus *Aricidea* is relatively recent.

The phylogenetic reconstruction shed light on the evolution of morphological features in Paraonidae. The median antenna seems to have evolved independently several times, even though it is very small in all genera except *Aricidea*, and the basal number of pre-branchial chaetigers is most likely three, even though probably arrangements with a higher number of chaetigers have been achieved at least twice independently. Notopodial modified chaetae appear to be a plesiomorphic feature of Paraonidae and have been lost subsequently; the absence of notopodial modified chaetae is a synapomorphy of a clade including *Aricidea*,

Levinsenia and the unnamed new genus. Neuropodial modified chaetae are present in all clades, even though in *Cirrophorus* and *Paradoneis* their presence is unstable. The ancestral state of this feature is most likely represented by short, thick and almost straight spines, that occur in some species of *Cirrophorus* and *Paradoneis*, and in the unnamed new genus; modified chaetae in *Levinsenia* are only slightly different from this basal type. In the genus *Aricidea*, instead, early branching groups bear hooks with terminal or subterminal arista, and other arrangements most likely originated from this ancestral modified chaeta.

The family Paraonidae show a strikingly high occurrence of cryptic and pseudocryptic species; on the basis of present data, 10-12 Mediterranean species are still undescribed, and in other biogeographical areas the number of undescribed taxa is expected to be far higher. Results of the present work suggest that environmental features play an important role in the diversification of Paraonidae, whereas the influence of geographical distance is less pronounced. Lastly, despite their importance in deep environments, Paraonidae appear to be a primarily shallow-water family, that radiated in the deep sea only secondarily.

6.2 Introduction

In recent years the development of molecular techniques allowed to better understand the evolution of annelids, and to update their systematics. Classifications based on the interpretation of morphological features and their evolution (Rouse & Fauchald, 1997) have been only recently tested by means of molecular markers (Rousset et al., 2006; Struck et al., 2007; 2011), often highlighting inconsistency between morphological and molecular data. More specifically, the traditional classification dividing annelids in three classes, and polychaetes in the groups of Errantia and Sedentaria (Fauvel, 1923), is not supported by molecular data. Similarly, the more updated view considering Clitellata and Hirudinea as derived from typical polychaete groups, and dividing polychaetes in Scolecida and Palpata (Rouse & Fauchald, 1997) were neither supported by molecular data. Recent phylogenetic reconstructions demonstrated that several non-segmented groups, i.e. Sipuncula, Echiura, Myzostomida, Vestimentifera and Pogonophora, are nested within the annelid evolutionary radiation (Halanych et al., 2002), and that the relationships among different polychaete groups are less linear than previously suggested (Struck et al., 2007; 2011). The discrepancy between morphology- and molecular-based phylogenetic reconstructions was also detected in phylogenies referred to single polychaete families (Bleidorn, 2005; Zanol et al., 2014). Possible explanations for this inconsistency might take in account *i*) convergent adaptive strategies, that may determine the appearance of similar morphologies in groups that are only

distantly related (Struck et al., 2015); *ii*) divergent adaptive strategies, that cause strong morphological differences in closely related organisms (Bleidorn, 2005); and *iii*) the role of pedomorphism and neoteny in the appearance of strongly divergent phenotypes that are not consistent with genetic divergences (Zanol et al., 2014). Complex intra-familiar relationships may be expected especially in groups that show a high species diversity (Aguado et al., 2012; Zanol et al., 2014), or very simple anatomy, with few morphological characters that are commonly employed in taxonomy (Bleidorn et al., 2005; Law et al., 2012). Molecular tools assumed a relevant role in unravelling annelid taxonomy and evolution since few decades (Grassle & Grassle, 1976), but the earlier studies concentrated chiefly on cryptic species issues and population genetics (Grassle & Grassle, 1976; Cadman & Nelson-Smith, 1990; Wu et al., 1991; Abbiati & Maltagliati, 1992; Manchenko & Radashevsky, 1993; Abbiati & Maltagliati, 1996; Röhner et al., 1996; 1997; Sato & Masuda, 1997). The use of DNA sequence data in polychaete phylogenetic inference is relatively recent, and early works focused on monophyly and relationships of Annelida with other phyla (McHugh, 2000; Bleidorn et al., 2003), whereas studies addressing the evolutionary history of single polychaete families are more recent (Bleidorn, 2005; Bleidorn et al., 2005). Until now, molecular phylogenies, even partial, are available for Orbiniidae (Bleidorn, 2005), Arenicolidae (Bleidorn et al., 2005), Aphroditiformia (Wiklund et al., 2005; Norlinder et al., 2012), Serpulidae (Kupriyanova et al., 2006; Lehrke et al., 2007; Kupriyanova et al., 2008), Phyllodocidae (Eklöf et al., 2007), Syllidae (Aguado et al., 2007; 2012), Amphinomida (Wiklund et al., 2008; Borda et al., 2015), Sabellidae (Capa et al., 2011), Opheliidae (Law et al., 2012) and Eunicidae (Zanol et al., 2014). The majority of polychaete families, most of which display high species diversity, are poorly known from the molecular point of view, even though ongoing research is being currently carried out on some families, such as Maldanidae (Kobayashi et al., 2016), Capitellidae (Tomioka & Kajihara, 2016) and Ampharetidae (Heggernes Eilertsen et al., 2016).

Within this framework, the family Paraonidae Cerruti, 1909 represents an interesting case study because of its high species diversity, its extremely wide bathymetric range of occurrence and the fact that its radiation occurred only on soft bottoms, where however this group was able to colonize several different environments (Strelzov, 1973). From a historical taxonomy point of view, the first Paraonidae species described were assigned to Spionidae (Grube, 1872; McIntosh, 1878; Tauber, 1879; Levinsen, 1884), Orbiniidae (Webster, 1879) and Cirratulidae (Ehlers, 1908). Mesnil & Caullery (1898) were the first to recognise Paraonidae as a coherent group, under the name Levinseniidae. Later on, Cerruti (1909)

identified *Paraonis tenera* Grube, 1872 as the first Paraonidae described ever, and stated the synonymy between *Levinsenia* and *Paraonis*, subsequently changing the family name in Paraonidae. Since those historical times, the knowledge about Paraonidae remained, however, fragmentary, with only few described species characterised by extremely wide putative distributions. Moreover, very often taxonomic uncertainties were associated to species, mainly regarding the correct use of genera and subgenera, and the relationships among them. For instance, the genus *Paraonis* was created by Grube (1872) for *Paraonis tenera*, which should be considered as a *nomen dubium* (Strelzov, 1973). The interpretation given by Cerruti (1909) for this taxon, which accounted for the use of Paraonidae instead of Levinseniidae, most probably did not correspond to its original meaning: Cerruti (1909) interpreted the caruncle described by Grube (1872) as a fixation artefact; whereas Strelzov (1973) pointed out that most likely it is the median antenna that is typical of the genus *Aricidea*. However, Cerruti's (1909) interpretation of *Paraonis* was commonly used in following literature, and this led the ICZN to stabilise the use of Paraonidae Cerruti, 1909, *Paraonis* Cerruti, 1909 (*non* Grube, 1872) and *Levinsenia* Mesnil, 1897 with the opinion 1139 (Melville, 1979).

Paraonidae were revised by Strelzov (1973), who highlighted a previously overlooked diversity within this family and tried to disentangle intra-generic relationships of the species-rich genus *Aricidea* Webster, 1879 by dividing it in four sub-genera, and synonymised *Paraonides* Cerruti, 1909 and *Paradoneis* Hartman, 1965 with *Cirrophorus* Ehlers, 1908. Moreover, Strelzov (1973) created the genera *Paraonella* Strelzov, 1973 to include species without antenna and modified chaetae (formerly assigned to *Paraonides*), *Sabidius* Strelzov, 1973 for the strange deep-water *Paraonis cornatus* Hartman, 1965, and *Tauberia* Strelzov, 1973 to include a part of the species traditionally assigned to *Paraonis* (currently assigned to *Levinsenia*). Following works dealt chiefly with taxonomy at species level (Imajima, 1973; Laubier & Ramos, 1974; Katzmann & Laubier, 1975; Hartley, 1981; 1984; Blake, 1996; de Leon Gonzalez et al., 2006; Aguirrezabalaga & Gil, 2009; Çinar et al., 2011), neglecting higher rank taxonomy and evolutionary relationships within Paraonidae. The first attempt to infer on Paraonidae phylogeny was made by Reuscher (2013), who focused on morphological features. His cladistic analysis confirmed the synonymy between *Cirrophorus* and *Paradoneis* (see Chapter 1), found the monophyly of *Aricidea* and allowed to exclude from Paraonidae the two known species of the genus *Periquesta* Brito & Núñez, 2002, which were assigned to *Levinsenia* by Giere et al. (2007), and *Aparaonis* Hartman, 1965, whose type specimen is a juvenile Opheliidae. Reuscher's (2013) phylogenetic analysis, however, did not allow to resolve the relationships among the other genera, mainly because of the simple external

anatomy of Paraonidae, with few morphological characters available. Despite the high level of species richness displayed by this family, its common occurrence from tide level to the abyssal depths (Strelzov, 1973), and the prominent role in sediment dynamics and trophic nets suggested by the high abundance of several species (Gibbs, 1965; Blake, 1996), Paraonidae are virtually unknown from the molecular point of view. In Rousset et al.'s (2006) phylogeny, this family exhibited an ambiguous placement and was not very close to any of the other considered groups; whereas in Bleidorn's (2005) reconstruction, Paraonidae appeared close to Opheliidae and Scalibregmatidae and the morphologically divergent family of Sternaspidae appears nested within Paraonidae. In all cases, only one or two species, and few molecular markers were employed, and therefore these reconstructions should be taken with considerable caution.

This work is aimed to reconstruct a molecular phylogeny of Paraonidae and, more specifically, to *i*) test the monophyly of the family and of the main groups (genera and subgenera); *ii*) assess the usefulness of currently employed morphological characters in Paraonidae taxonomy *iii*) identify morphological and ecological ancestral states in Paraonidae; *iv*) highlight possible clues on biogeography and reproduction of Paraonidae, that are still largely unknown; *v*) detect cases of cryptic or pseudocryptic speciation in this family.

6.3 Materials and methods

A total of 60 genotypable individuals belonging to 34 nominal species and subspecies of the family Paraonidae were obtained by direct sampling in suitable environments, from environmental monitoring programmes, from colleagues or from institutional collections (Table 8). It is noteworthy that some species have not been positively identified with any of the available taxa, and most likely represent undescribed taxa. When possible, Paraonidae were sorted and identified alive. All material was preserved in 96% or 70% ethanol at 4 °C until DNA extraction.

DNA extraction was carried out using the GenElute™ Mammalian Genomic DNA Miniprep Kit distributed by Sigma-Aldrich, following the manufacturer's instructions. For phylogenetic reconstruction I amplified the mitochondrial genes for 16S rRNA and COI and the nuclear gene for 18S rRNA. 16S rDNA amplification was obtained using the universal primer pair 16SarL (5'-CGCCTGTTTAACAAAAACAT-3') and H3080 (5′-CCGGTCTGAACTCAGATCACGT-3′) (Palumbi et al., 1991) and the annelid-specific primers 16S_ANNF (5'- GCGGTATCCTGACCGTRCWAAGGTA-3') and 16S_ANNR (5'-TCCTAAGCCAACATCGAGGTGCCAA-3') (Sjölin et al., 2005); whereas for COI

Table 8: Paraonidae employed in the analysis with sampling site, depth and genes available

Species	Locality	Depth	16S rDNA	COI	18S rDNA
Aricidea (Acmira) assimilis Tebble, 1959 1	Cala di Forno, Mediterranean Sea	7 m	x		x
Aricidea (Acmira) assimilis Tebble, 1959 2	Ravenna, Mediterranean Sea	8 m	x	x	x
Aricidea (Acmira) assimilis Tebble, 1959 3	Strait of Otranto, Mediterranean Sea	75 m	x	x	x
Aricidea (Acmira) assimilis Tebble, 1959 4	Tuscan Archipelago, Mediterranean Sea	110 m	x	x	x
Aricidea (Acmira) catherinae Laubier, 1967 1	Bay of Biscay, Atlantic Ocean	34 m	x	x	x
Aricidea (Acmira) catherinae Laubier, 1967 2	Beals, Maine, Atlantic Ocean	Tide level	x	x	x
Aricidea (Acmira) catherinae Laubier, 1967 3	Belfast Lough, Atlantic Ocean	24 m	x	x	x
Aricidea (Acmira) catherinae Laubier, 1967 4	Capraia Island, Mediterranean Sea	11 m	x	x	x
Aricidea (Acmira) catherinae Laubier, 1967 5	Maremma, Mediterranean Sea	7 m	x	x	x
Aricidea (Acmira) catherinae Laubier, 1967 6	Versilia, Mediterranean Sea	19 m	x		x
Aricidea (Acmira) cerrutii cerrutii Laubier, 1966 1	Bay of Rosas, Mediterranean Sea	6 m	x	x	x
Aricidea (Acmira) cerrutii cerrutii Laubier, 1966 2	Pianosa Island, Mediterranean Sea	0.5 m	x		x
Aricidea (Acmira) cerrutii cerrutii Laubier, 1966 3	Porto Pozzo Bay, Mediterranean Sea	0.8 m	x		x
Aricidea (Acmira) cf. *cerrutii pacifica* Imajima, 1973	Anglesey, Atlantic Ocean	110 m	x		x
Aricidea (Acmira) elongata Imajima, 1973	Jinhae Bay, S. Korea, Pacific Ocean	Tide level	x		x
Aricidea (Acmira) cf. *laubieri* Hartley, 1981	S. Teodoro Pond, Mediterranean Sea	0.5 m	x	x	x
Aricidea (Acmira) mirifica Strelzov, 1973	Jaco Scarp, Costa Rica, Pacific Ocean	d.u.	x	x	
Aricidea (Acmira) rubra Hartman, 1963	Jaco Scarp, Costa Rica, Pacific Ocean	1000 m	x	x	x
Aricidea (Acmira) simonae Laubier & Ramos, 1974	Elba Island, Mediterranean Sea	10 m	x		x
Aricidea (Acmira) simplex Day, 1963	Coronado Bank, California, Pacific Ocean	1100 m	x	x	x
Aricidea (Acmira) simplex Day, 1963	La Jolla, California, Pacific Ocean	60 m	x		
Aricidea (Acmira) sp. A*	Jaco Scarp, Costa Rica, Pacific Ocean	1800 m	x		x
Aricidea (Aricidea) capensis bansei Laubier & Ramos, 1974	Albegna River Mouth, Mediterranean Sea	10 m	x	x	x
Aricidea (Aricidea) fragilis Webster, 1879	Candelaro River Mouth, Mediterranean Sea	10 m	x	x	x
Aricidea (Aricidea) minuta Southward, 1956 1	Beals, Maine, Atlantic Ocean	Tide level	x		
Aricidea (Aricidea) minuta Southward, 1956 2	St.Mary's Road, Atlantic Ocean	15 m	x		x
Aricidea (Aricidea) pseudoarticulata Hobson, 1972 1	Bay of Biscay, Atlantic Ocean	35 m	x		x
Aricidea (Aricidea) pseudoarticulata Hobson, 1972 2	Cala di Forno, Mediterranean Sea	10 m	x		x
Aricidea (Strelzovia) claudiae Laubier, 1967 1	Cattolica, Mediterranean Sea	5 m	x		
Aricidea (Strelzovia) claudiae Laubier, 1967 2	Tuscan Archipelago, Mediterranean Sea	110 m	x	x	x
Aricidea (Strelzovia) mariannae Katzmann & Laubier, 1975	Tuscan Archipelago, Mediterranean Sea	110 m	x		x
Aricidea (Strelzovia) monicae Laubier, 1967	Tuscan Archipelago, Mediterranean Sea	110 m	x		x

Aricidea (Strelzovia) ramosa Annenkova, 1934	Coronado Bank, California, Pacific Ocean	1100 m	x		x
Aricidea (Strelzovia) roberti Hartley, 1984	Bay of Biscay, Atlantic Ocean	35 m	x		x
Aricidea (Strelzovia) cf. *suecica suecica* Eliason, 1920	Tuscan Archipelago, Mediterranean Sea	110 m	x		
Cirrophorus branchiatus Ehlers, 1908 1	Loch Creran, Irish Sea, Atlantic Ocean	22 m	x	x	x
Cirrophorus branchiatus Ehlers, 1908 2	Tuscan Archipelago, Mediterranean Sea	110 m	x		x
Cirrophorus furcatus (Hartman, 1957)	Santa Monica Bay, California, Pacific Ocean	d.u.	x		x
Cirrophorus sp. A 1	Livorno, Mediterranean Sea	3 m	x		x
Cirrophorus sp. A 2	Porto Pozzo Bay, Mediterranean Sea	0.8 m	x	x	x
Cirrophorus sp. A 3	Venezia, Mediterranean Sea	1 m	x	x	x
Cirrophorus sp. B	Livorno, Mediterranean Sea	3 m	x	x	x
Levinsenia demiri Çinar, Açik & Dağli, 2011 1	Strait of Otranto, Mediterranean Sea	75 m	x		x
Levinsenia demiri Çinar, Açik & Dağli, 2011 2	Tuscan Archipelago, Mediterranean Sea	110 m	x	x	x
Levinsenia gracilis (Tauber, 1879)	Loch Creran, Irish Sea, Atlantic Ocean	19 m	x	x	
Levinsenia kantauriensis Aguirrezabalaga & Gil, 2009 1	Southern Adriatic Sea, Mediterranean Sea	730 m	x		
Levinsenia kantauriensis Aguirrezabalaga & Gil, 2009 2	Southern Adriatic Sea, Mediterranean Sea	970 m	x		
Levinsenia kosswigi Çinar, Açik & Dağli, 2011	Tuscan Archipelago, Mediterranean Sea	110 m	x		x
Levinsenia materi Çinar & Dağli, 2013	Porto S. Stefano, Mediterranean Sea	8 m	x		x
Levinsenia sp. A 1	Southern Adriatic Sea, Mediterranean Sea	120 m	x		
Levinsenia sp. A 2	Southern Adriatic Sea, Mediterranean Sea	600 m	x		
Paradoneis armata Glémarec, 1966 1	Bay of Biscay, Atlantic Ocean	35 m	x		x
Paradoneis armata Glémarec, 1966 2	Elba Island, Mediterranean Sea	7 m	x	x	x
Paradoneis cf. *ilvana* Castelli, 1985	Capraia Island, Mediterranean Sea	14 m	x		
Paradoneis ilvana Castelli, 1985 1	Capraia Island, Mediterranean Sea	20 m	x	x	x
Paradoneis ilvana Castelli, 1985 2	Pianosa Island, Mediterranean Sea	0.5 m	x	x	x
Paradoneis lyra (Southern, 1914) 1	Loch Creran, Irish Sea, Atlantic Ocean	25 m	x	x	x
Paradoneis lyra (Southern, 1914) 2	Tuscan Archipelago, Mediterranean Sea	110 m	x	x	x
Paraonis fulgens (Levinsen, 1884) 1	Newton-on-Sea, Atlantic Ocean	Tide level	x	x	x
Paraonis fulgens (Levinsen, 1884) 2	Albegna River Mouth, Mediterranean Sea	7 m	x		x

**This species corresponds to* Aricidea *(*Aedicira) longicirrata *Fauchald, 1972, but the name is preoccupied by* Aricidea (Aricidea) longicirrata *Hartmann-Schröder, 1965, and the species is therefore unnamed. Moreover, the observed features of modified chaetae allow to assign this species to the subgenus* Acmira *Hartley, 1981.*

amplification I used the universal primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994) and the annelid-specific primers POLYLCO (5'-GAYTATWTTCAACAAATCATAAAGATATTGG-3') and POLYHCO (5'-TAMACTTCWGGGTGACCAAARAATCA-3') (Carr et al., 2011). 18S rDNA amplification was obtained using the primers F9 (5'-CTGGTTGATCCTGCCAG- 3') (Medlin et al., 1988) and R1513 (5'-TGATCCTTCYGCAGGTTC-3') (Petroni et al., 2002). Polymerase chain reaction (PCR) amplifications were carried out in 20 μL solutions using 1.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.1 μM of each primer, 1 U of DreamTaq DNA polymerase (Thermo Scientific), and ~2.5 ng of template DNA. For 16S rDNA and COI the PCR profile was set as follows: initial denaturing step at 94 °C for 3 min; 34 cycles of denaturing at 94 °C for 45 s, annealing at 54 °C for 1 min, and extending at 72 °C for 1 min, and a final extending step at 72 °C for 7 min. A negative control was included in each reaction. For 18S rDNA, PCRs were carried out in 45 μL using a protocol with low ramp speed, and annealing temperature set at 50 °C (Lorenz, 2012). PCR products were precipitated with sodium acetate and absolute ethanol and sent to Macrogen Europe for sequencing.

Table 9: Annelid taxa employed in the preliminary phylogenetic reconstruction, with GenBank accession numbers of sequences. If possible were employed sequences obtained from the same individual.

Species	**16S rDNA**	**COI**	**18S rDNA**
Bonellia viridis Rolando, 1821	KM187642.1	KM187650.1	AF123307.1
Chloeia viridis Schmarda, 1861	JN086555.1	JN086546.1	JN086537.1
Cossura candida Hartman, 1955	HM746710.1	-	AY532350.1
Eulalia viridis (Linnaeus, 1767)	AY340455.1	AY996122.1	AY340428.1
Eunice harassii Audouin & Milne-Edwards, 1833	GQ478140.1	GQ497535.1	GQ497486.1
Naineris dendritica (Kinberg, 1867)	FJ612462.1	FJ612504.1	AY532358.1
Ophelina acuminata Örsted, 1843	KF511811.1	HQ023899.1	KF511826.1
Phascolosoma granulatum Leuckart, 1828	GU230181.1	DQ300138.1	AF519252.2
Prionospio ehlersi Fauvel, 1928	EU340081.1	KT307690.1	EU340095.1
Sabella spallanzanii Gmelin, 1805	HQ015113.1	AY436349.1	AY436350.1
Sternaspis scutata Ranzani, 1817	AY532329.1	KJ466057.1	AY532353.1
Outgroup			
Chiton olivaceus Spengler, 1797	AY377605.1	AY377716.1	AY377651.1

Sequences from each gene were aligned with ClustalX 2.1 (Larkin et al., 2007), and alignments were edited in BIOEDIT version 7.2.5 (Hall, 1999). The program jModelTest 2.1.6 (Guindon & Gascuel, 2003; Darriba et al., 2012), based on the hierarchical likelihood ratio test, was used to assess the best model of evolution for the sequences under the Akaike Information Criterion (AIC) (Akaike, 1974). For molecular comparison and phylogenetic reconstruction, I used additional sequences downloaded from GenBank for *Cirrophorus*

furcatus (accession numbers AY532349.1 and AY532330.1). A preliminary reconstruction was carried out using the annelid taxa in Table 9, with *Chiton olivaceus* as outgroup, in order to *i*) test the monophyly of Paraonidae; *ii*) infer on evolutionary relationships and similarities with other annelid groups, that are still poorly understood and, according to available literature, quite ambiguous (Bleidorn, 2005; Rousset et al., 2006); and *iii*) identify a suitable outgroup for a more detailed phylogenetic reconstruction.

Bayesian consensus phylogenetic trees based on the single genes and on the three concatenated markers were constructed using MrBayes 3.2 (Ronquist et al., 2011). In the tree constructed with the concatenated markers each gene was treated according to its own substitution model. Four replicate runs were carried out with three Markov chains per run for 2×10^6 generations. The chain was sampled every 100 generations to obtain 20 000 sampled trees. The first 5000 sampled trees (25%) were discarded as burn-in phase, with the remaining 15 000 trees used to estimate the Bayesian posterior probability (*PP*) of tree nodes. The convergence of Bayesian analyses was checked through the standard deviation of split frequencies, that should reach a value < 0.01 at the end of the analysis (Ronquist et al., 2011).

6.4 Results

A preliminary phylogenetic reconstruction was based on sequences retrieved from GenBank, and comprising one species for each available genus within Paraonidae. I used 444 bp 16S rDNA, 563 bp COI, and 1859 bp 18S rDNA sequences. The substitution models were the generalised time reversible (GTR, Tavaré, 1986) +I+G for 16S and 18S, and GTR+G for COI. The Bayesian tree based on the concatenated molecular markers (Fig. 32) confirmed the monophyly of Paraonidae, as already stated by Reuscher (2013) on the basis of morphological features. However, none of the putative sister groups suggested by Strelzov (1973) (Opheliidae, Orbiniidae, Cossuridae) turned out to be close to Paraonidae. Instead, as suggested by Bleidorn (2005) on the basis of preliminary molecular data, Sternaspidae were strongly supported as sister group of Paraonidae. Accordingly I decided to use *Sternaspis scutata* sequences as outgroup in the following, more detailed phylogenetic reconstruction. The monophyly of Paraonidae was retrieved also in the two Bayesian trees based on single mitochondrial markers (Fig. 33-34), although not statistically supported in the COI tree (Fig. 34); whereas the phylogenetic reconstruction based on 18S rDNA sequences (Fig. 35) identified Sternaspidae as an in-group of Paraonidae, as already reported by Bleidorn (2005). In all trees the majority of nodes were weakly supported, and therefore this reconstruction did not allow to infer on higher-rank relationships between annelid taxa.

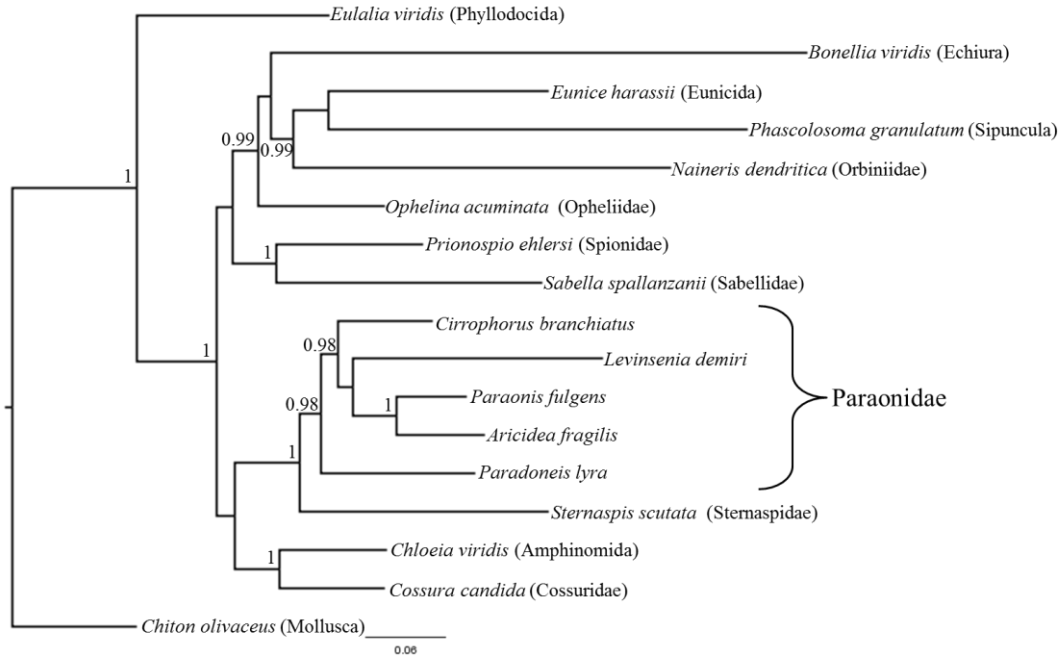

Figure 32: High-level Bayesian phylogenetic reconstruction based on the three concatenated molecular markers, showing the position of Paraonidae in the annelid evolutionary radiation. Shown are nodes with significant posterior probability values.

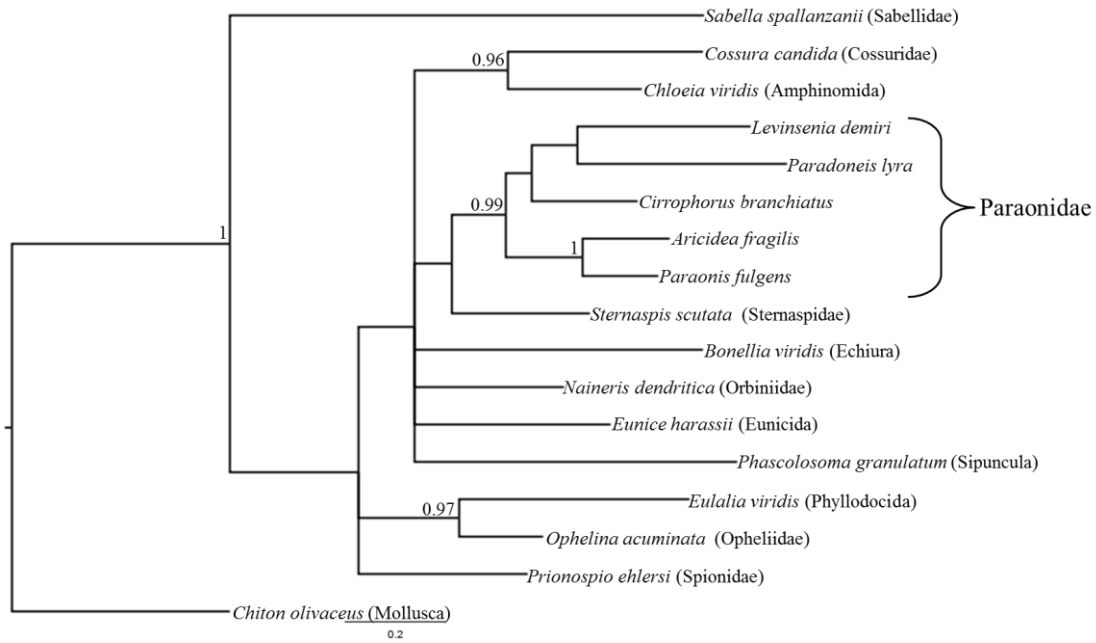

Figure 33: High-level Bayesian phylogenetic reconstruction based on 16S rDNA sequences

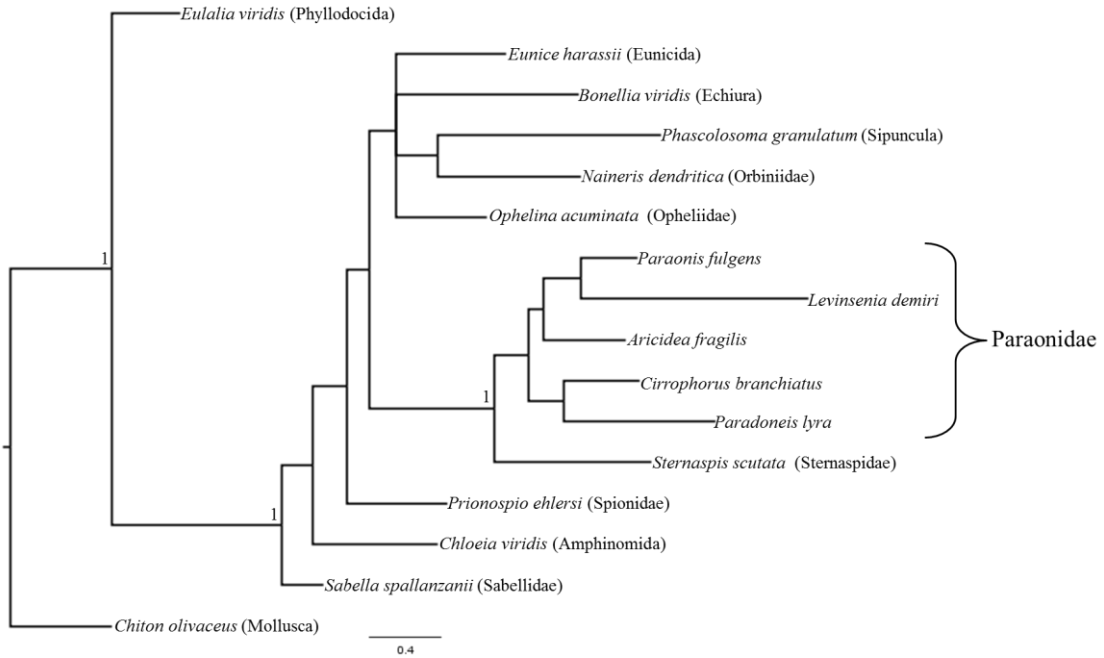

Figure 34: High-level Bayesian phylogenetic reconstruction based on COI sequences

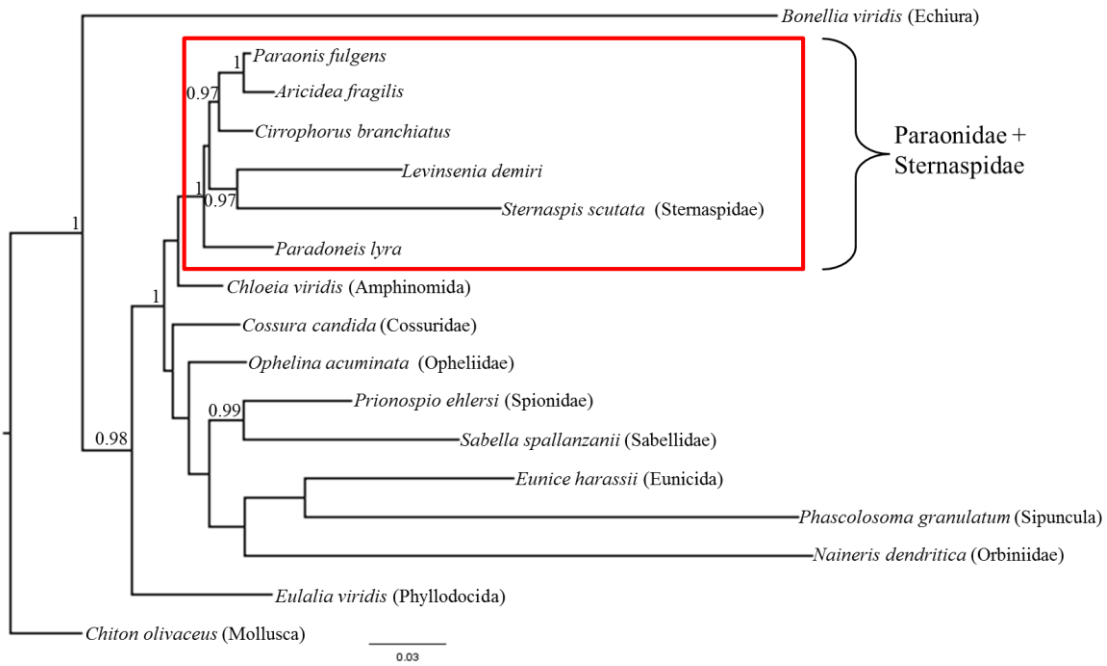

Figure 35: High-level Bayesian phylogenetic reconstruction based on 18S rDNA sequences. Unlike for mitochondrial markers, the monophyly of Paraonidae is not retrieved, and Sternaspidae are nested within Paraonidae.

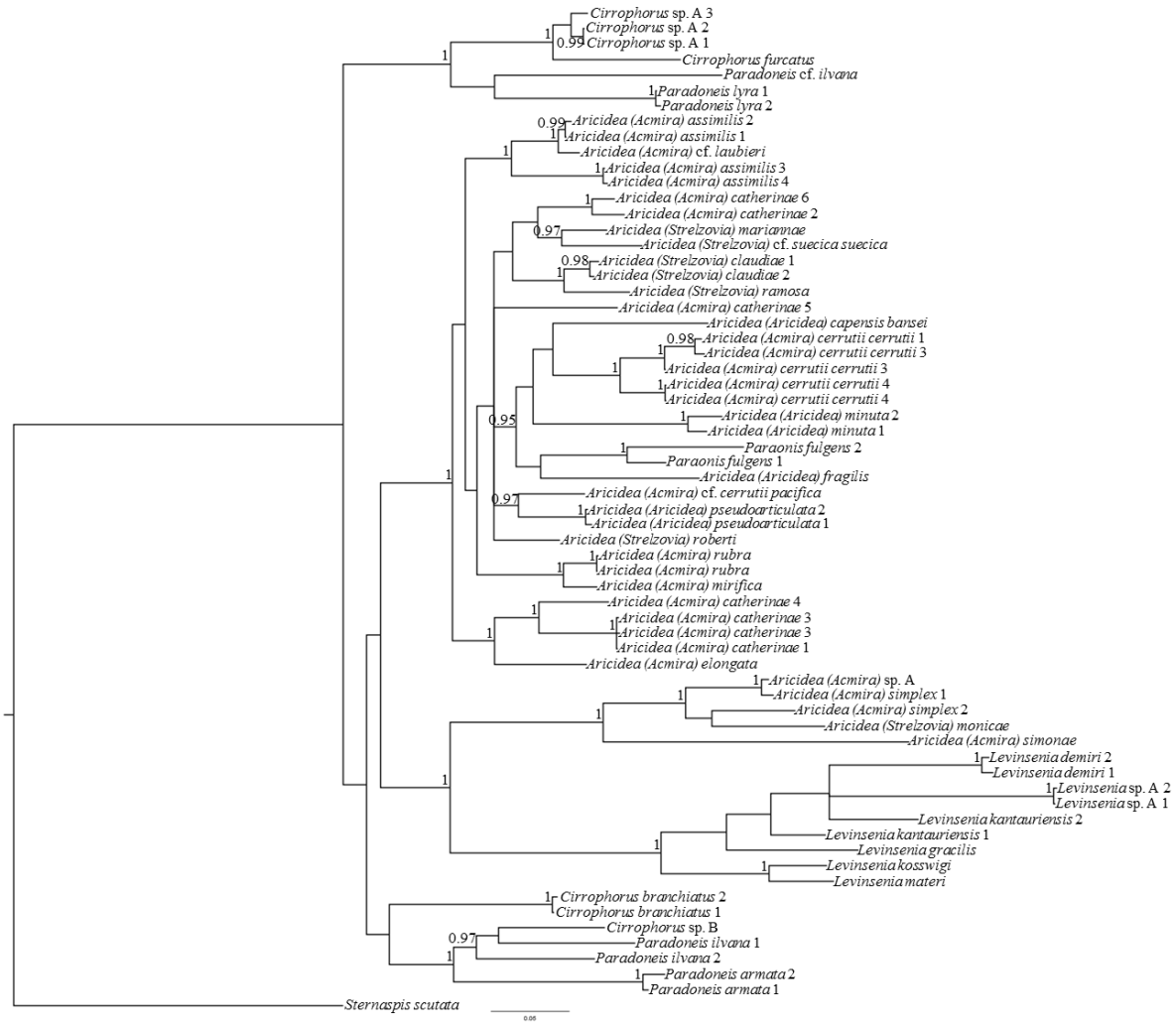

Figure 36: Bayesian phylogenetic tree of the family Paraonidae based on the three concatenated molecular markers. Only significant node values are shown.

A more detailed phylogeny, including 34 nominal Paraonidae species and subspecies, was constructed on the basis of 491 bp 16S, 638 bp COI, and 1834 bp 18S sequences; for all markers the most suitable substitution model was GTR+I+G. Different affinities of universal primers across the analysed species and the consequent use of specific primer pairs accounted for different sequence lengths (for details see Appendix 1). The Bayesian tree of the combined dataset (Fig. 36) showed a complex topology, with the majority of nodes that were weakly supported. However, in this reconstruction it was possible to identify five highly supported clades (*PP* = 1) within Paraonidae (Fig. 37). *Cirrophorus* sp. A, *Cirrophorus furcatus* and *Paradoneis lyra* were included in the earliest-diverging clade (Clade I). The remaining species of genera *Cirrophorus* and *Paradoneis* were included in a weakly supported clade that appears sister to all remaining Paraonidae. However, when *Cirrophorus branchiatus* was excluded from this group, the remaining species formed a highly supported clade (Clade II).

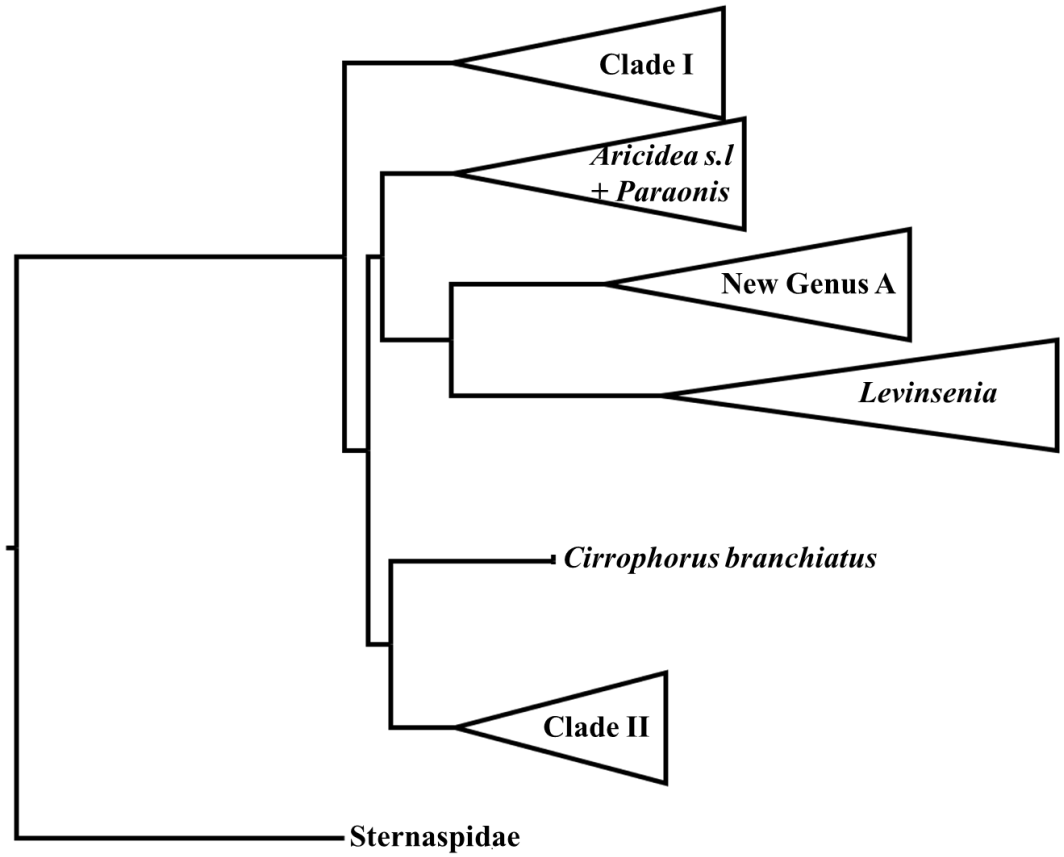

Figure 37: Collapsed tree showing the highly supported clades that can be identified within Paraonidae according to the phylogenetic reconstruction shown in Fig. 36.

All species of the genus *Aricidea* with the exceptions of *Aricidea (Acmira) simonae*, *Aricidea (Acmira) simplex*, *Aricidea (Acmira)* sp. A, and *Aricidea (Strelzovia) monicae* formed a monophyletic group that included *Paraonis* as well. This last genus was represented only by one nominal species, but the examined individuals showed high genetic divergence, even if they did form a clade. Lastly, the genus *Levinsenia* was monophyletic and represented the sister group of a clade composed by *Aricidea (Acmira) simonae*, *Aricidea (Acmira) simplex*, *Aricidea (Acmira)* sp. A, and *Aricidea (Strelzovia) monicae*, that showed divergence from the other clades that is consistent with the hypothesis of a new genus (New Genus A). None of the subgenera of *Aricidea* was monophyletic, and relationships among *Aricidea* species were often unclear. Within the genus *Aricidea* the following clades are highly supported: *1*) the so-called *Aricidea (Acmira) assimilis* complex (see Chapter 2); *2*) the *Aricidea (Acmira) elongata* and *Aricidea (Acmira) catherinae* complexes (see Chapter 3); and *3*) three smaller groups composed by *i*) *Aricidea (Aricidea) pseudoarticulata* and *Aricidea (Acmira)* cf. *cerrutii pacifica*, *ii*) *Aricidea (Strelzovia) claudiae* and *Aricidea (Strelzovia) ramosa*, and *iii*) *Aricidea (Strelzovia) mariannae* and *Aricidea (Strelzovia)* cf. *suecica suecica*. Nevertheless, a common pattern between morphological and molecular diversity is difficult to identify.

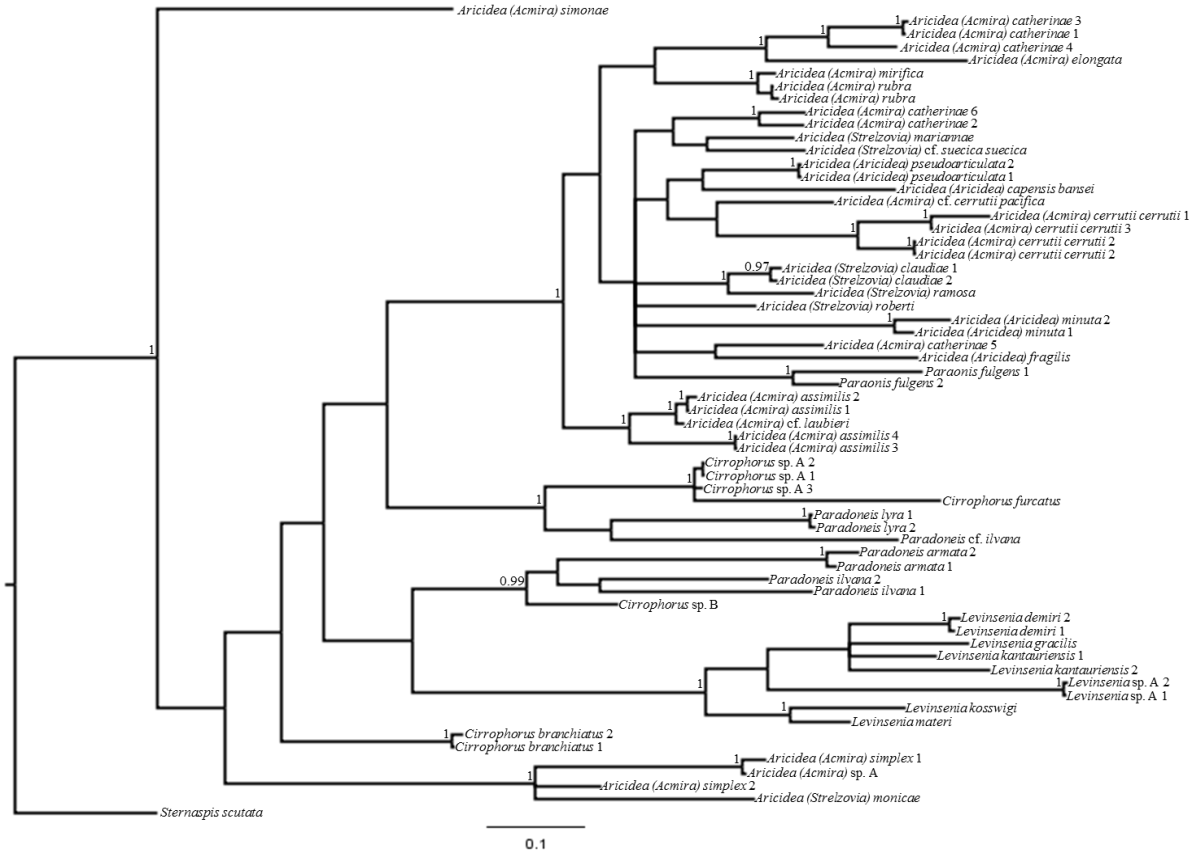

Figure 68: Bayesian phylogenetic tree of the family Paraonidae based on 16S rDNA sequences.

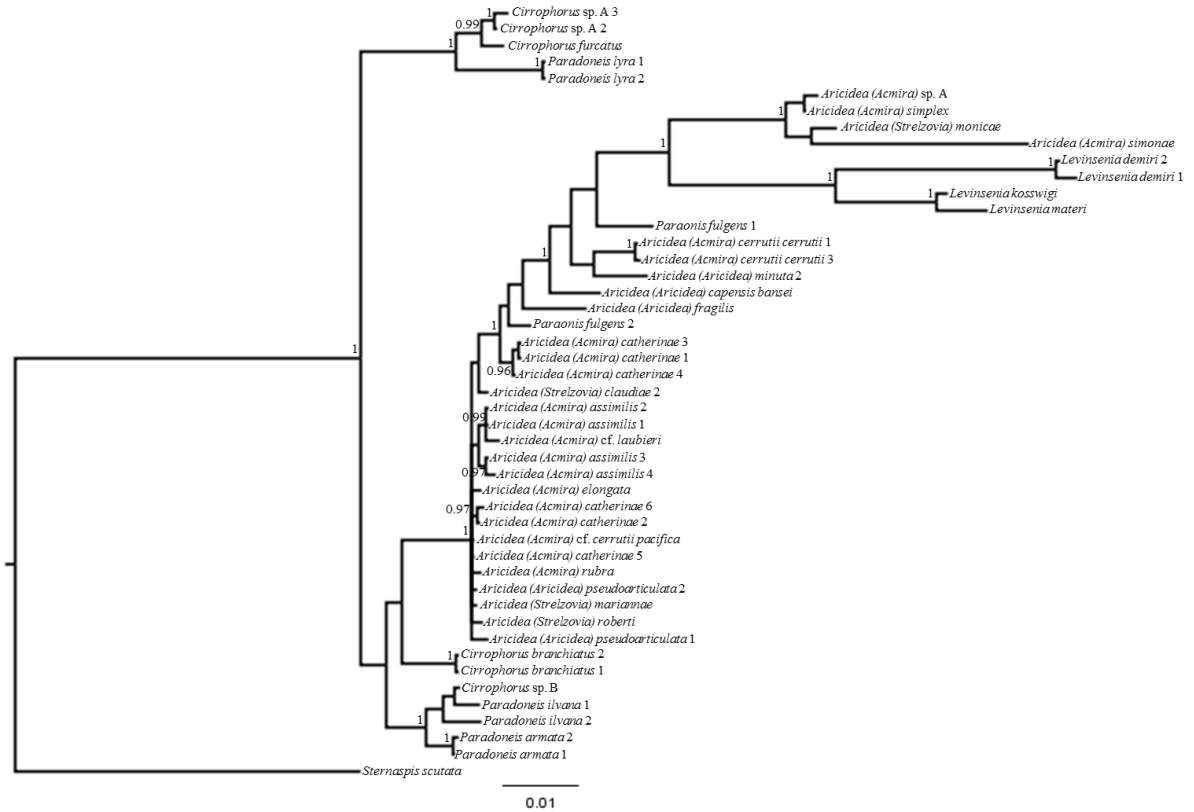

Figure 39: Bayesian phylogenetic tree of the family Paraonidae based on 18S rDNA sequences.

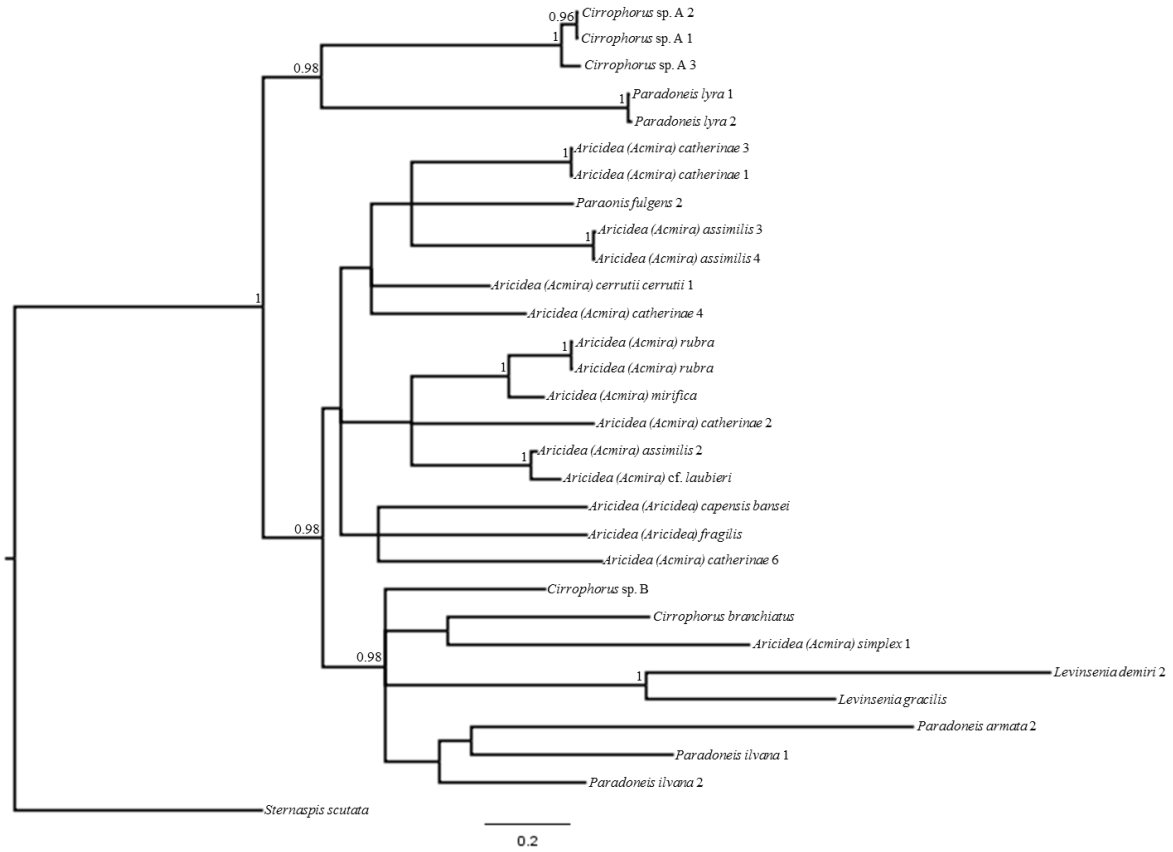

Figure 40: Bayesian phylogenetic tree of the family Paraonidae based on COI sequences.

Bayesian trees built using the single genes coarsely confirmed the five clades identified in the phylogenetic analysis with the concatenated markers. The tree based only on the 16S rDNA dataset (Fig. 38) highlighted *A. simonae* as sister group of all other Paraonidae, and New Genus A (in this case not including *A. simonae*) represented the earliest-diverging clade. Clade II was the sister group of *Levinsenia*, whereas clade I was the sister group of *Aricidea s.l.*; the position of *C. branchiatus* was ambiguous. The topology of this tree was not consistent with that of the tree based on the combined dataset; however, even if all clades were highly supported, the nodes explaining evolutionary relationships among them were only weakly supported. The tree based on the 18S rDNA dataset (Fig. 39) showed highly supported clade I, clade II, *Levinsenia* and New Genus A; moreover, the topology of these groups was very similar to that of the tree based on the combined dataset. However, *Levinsenia* and New Genus A were nested within the clade of *Aricidea s.l.*; the very long branches connecting these genera to the remaining part of the clade suggested that this is an artefact due to the phenomenon of long-branch attraction (Bergtsen, 2005; Kolaczkowski & Thornton, 2009). Lastly, in the COI tree (Fig. 40), clade I, *Aricidea s.l.* and *Levinsenia* were monophyletic, but the monophyly of *Aricidea s.l.* is weakly supported. On the other hand, the monophyly of clade II was not retrieved, and a highly supported clade including all species of

clade II included *C. branchiatus*, *A. simplex* (assigned to New Genus A) and *Levinsenia*. Moreover, New Genus A appeared the sister group of *C. branchiatus* rather than *Levinsenia*, although nodes were only weakly supported.

6.5 Discussion

6.5.1 Relationships between Paraonidae and other annelid taxa

Based on the higher-rank phylogenetic reconstruction (Fig. 32), all Strelzov's (1973) hypotheses about putative similarities of Paraonidae with other polychaete groups turned out to be inconsistent. The similarity between Orbiniidae and Paraonidae, historically stressed by some authors (e.g. Mesnil & Caullery, 1898) has been already rejected on both morphological and molecular bases (Bleidorn, 2005), and the affinity of body plans is most probably due to similar adaptive strategies, rather than to common descent (Struck et al., 2015). On the other hand, recent studies on annelid phylogeny considered Opheliidae, Scalibregmatidae, Cossuridae, Sternaspidae and Paraonidae as a probable monophyletic clade (Bleidorn, 2005; Struck et al., 2007), and therefore, results regarding the relationships between these families and Paraonidae are rather surprising. Neither Cossuridae, nor Opheliidae, despite their morphological similarity, were closely related to Paraonidae, and this is true for both the reconstruction based on concatenated markers and single-marker ones. On the other hand, the similarity of Paraonidae with Sternaspidae suggested by Bleidorn (2005) is confirmed in all phylogenetic reconstructions. In the phylogeny based on 18S rDNA sequences, *Sternaspis scutata* turned out to be nested within Paraonidae, as sister group of *Levinsenia demiri*. However, the branch connecting *Sternaspis scutata* to the remaining Paraonidae is unusually long, and in this reconstruction the position of Sternaspidae within Paraonidae is most likely an artefact due to the long-branch attraction phenomenon (Bergtsen, 2005; Kolaczkowski & Thornton, 2009). More generally, if these trees allow to infer on the monophyly of Paraonidae, the high distances among selected families make inference of their relationships unreliable, and probably for this reason, the majority of nodes are inconsistent with previous findings of more complete higher-rank annelid phylogenies (Rousset et al., 2006; Struck et al., 2007; Struck et al., 2011). Based on the preliminary reconstruction, I conclude that Paraonidae represent a monophyletic group of annelids, and that Sternaspidae represent their sister group, even though morphological features seem to be against this statement. The strong morphological divergence between Sternaspidae and Paraonidae most likely represents the consequence of different adaptive strategies, as already observed in other polychaete families (Bleidorn, 2005; Struck et al., 2015).

6.5.2 Phylogeny of Paraonidae and taxonomic implications

The phylogenetic reconstruction of evolutionary relationships within Paraonidae showed that the current taxonomic scheme is largely incorrect. Genera *Cirrophorus* and *Paradoneis* do not represent different clades, and this supports Strelzov's (1973) and Reuscher's (2013) views on the unreliability of the median antenna as diagnostic character between these two genera. On the other hand, *Cirrophorus* and *Paradoneis* species belong to two different, not directly related clades, and therefore would represent a polyphyletic group. Based on the present phylogeny, *Cirrophorus* and *Paradoneis* species characterised by modified notochaetae of the same shape throughout the whole body length are included in Clade I; whereas species with modified notochaetae of changing shape towards the pygidium are included in Clade II (Fig. 41).

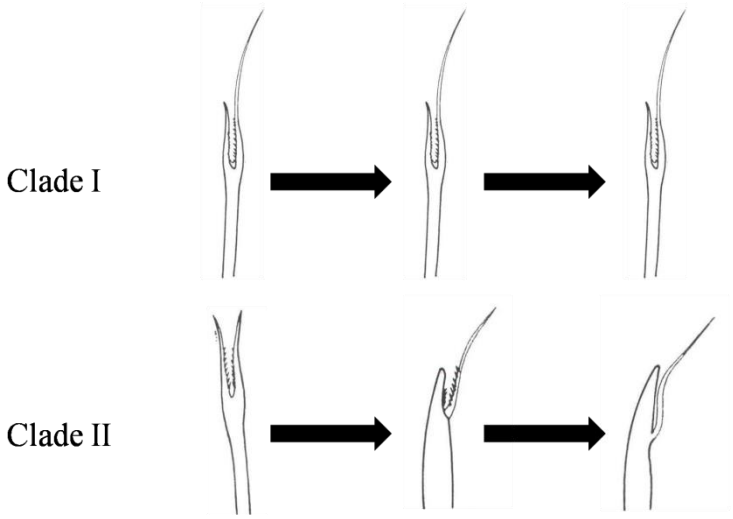

Figure 41: Shape of notochaetae throughout the body length in clade I and clade II (after Castelli, 1985)

This result suggests that changing *vs* not changing modified notochaetae represent a useful taxonomic character. However, this view needs to be validated on a higher number of species. Since clade I includes *Paradoneis lyra*, the type species of *Paradoneis* Hartman, 1965, species included in this clade could be assigned to *Paradoneis*; however, to do so, the diagnosis of *Paradoneis* should be emended, and, at present, data about morphological variability of clade I are not available, being all evidence based on four species only. The same observation is true for clade II, with the additional issues related to the uncertain clustering of *Cirrophorus branchiatus*, the type species of *Cirrophorus*, and the problematic identification of *Paraonides neapolitana* Cerruti, 1909, type species of *Paraonides*. Awaiting more complete material, I hereby avoid to anticipate possible taxonomic changes, highlighting however that, in contrast to Reuscher's (2013) reconstruction, the genera *Cirrophorus* and *Paradoneis* do not represent a monophyletic group, and a higher level taxonomic revision

based on combined molecular and morphological data is strongly advisable. The absence of *Paraonella* spp. and *Paradoneis* spp. with notopodial spines in this phylogenetic reconstruction does not allow to infer on their phylogenetic placement. *Paradoneis* spp. with notopodial spines were considered by Reuscher (2013) as the sister group of all remaining *Cirrophorus* and *Paradoneis*, and *Paraonella* Strelzov, 1973[3] represents the sister group of all Paraonidae with notopodial modified chaetae. However, the rejection of the hypothesis of a *Cirrophorus/Paradoneis* monophyletic group suggests that also these placements are incorrect. More specifically, *Paraonella* differs from *Paradoneis* only in the absence of modified notopodial chaetae, and since such chaetae appear to be an ancestral character, which has been secondarily lost in Paraonidae evolutionary history, the interpretation of *Paraonella* as a basal group is likely incorrect. More generally, the secondary loss of structures can lead to serious mistakes in phylogenetic inference, and the interpretation of organisms lacking specific characters as basal lineages is often misleading (Jenner, 2004).

The genus *Aricidea* is monophyletic if *Aricidea (Acmira) simonae*, *Aricidea (Acmira) simplex*, *Aricidea (Acmira)* sp. A, and *Aricidea (Strelzovia) monicae* are excluded, and *Paraonis fulgens* is included. Despite the striking morphological divergence, *Paraonis* is nested within *Aricidea*, of which it seems to be a derived group. A possible explanation is that *Paraonis* represents a pedomorphic form of *Aricidea* that retained features that are typical of juvenile individuals, such as the presence of a thick ciliation [interpreted also in other Paraonidae as clue of pedomorphism, as suggested by McLelland & Gaston (1994)] and the absence of the prostomial antenna. In other polychaete families, pedomorphism has been considered responsible for the appearance of strongly divergent morphologies that are apparently inconsistent with molecular patterns (Zanol et al., 2014); in my opinion, it represents the most likely explanation for the peculiar position of *Paraonis*. Since *Aricidea* Webster, 1879 has priority over *Paraonis* Cerruti, 1909, these two genera should be considered synonymous, and species of *Paraonis* should be moved to *Aricidea*. The partition of *Aricidea* spp. in four subgenera proposed by Strelzov (1973), and currently widely accepted (Aguirrezabalaga, 2012), was widely inconsistent with the phylogenetic reconstruction obtained in the present work. It is noteworthy that these subgenera were created mostly for practical purposes, that often Strelzov (1973) himself stressed similarities

[3] Reuscher (2013) referred species without a prostomial antenna and without modified chaetae at both parapodial rami to the genus *Paraonides* Cerruti, 1909, following the use of the genus stated by Hartman & Fauchald (1971). Given the uncertain identity of the type species of *Paraonides*, that however most likely bears modified notopodial chaetae (see Chapter 1), I here provisionally support the use of *Paraonella* Strelzov, 1973 for this group (see as well Blake, 2016).

between species assigned to different subgenera, and that this schematisation was often criticised as possibly artificial (Hartley, 1981). Present data allow to reject the monophyly of subgenera; in particular, the type species of *Aricidea* (*Aricidea fragilis*) is the sister taxon of *Paraonis fulgens s.l.*, and the relationship of the type species of *Acmira* Hartley, 1981 (*Aricidea catherinae*) with other *Aricidea* spp. is unclear. The type species of *Strelzovia* Aguirrezabalaga, 2012, namely *Aricidea albatrossae* Pettibone, 1957, was not examined in this study, but Pettibone (1957) herself highlighted the strong similarity between this species and *A. fragilis* (going as far as to suggest in a later work a synonymy between these two taxa – see Pettibone, 1965) and species of the subgenus *Strelzovia* analysed in this study do not form a monophyletic group. No species assigned to *Aedicira* Hartman, 1957 were examined in this work[4], but the vast majority of taxa referred to this subgenus have been later assigned to other subgenera (Aguirrezabalaga, 2012), and its morphological variability, as well as the actual number of included species, is still unclear. Despite the high number of species assigned to *Aricidea*, and the striking morphological variation displayed by this genus, genetic divergences among different species are relatively shallow, especially if compared to those observed in the other clades. Moreover, the vast majority of examined species showed extremely low genetic divergences at the level of 18S rDNA sequences. As a matter of fact, species and species complexes are clearly identified by mitochondrial markers, but apparently the nuclear marker employed has an excessively low mutation rate to allow correct phylogenetic inference at lowest taxonomic ranks. Conversely, 18S rDNA sequences were effective in resolving phylogenetic relationships at higher taxonomic levels. These observations suggest that the adaptive radiation within the genus *Aricidea* is relatively recent, and gave rise to an extremely wide variety of different species, that however are less differentiated from the molecular point of view than their morphology would suggest. The identification of morphologically coherent groups within *Aricidea* is a challenging task, and, even though the subgenus *Aricidea* (including *Aricidea (Acmira) cerrutii s.l.* and *Paraonis*) might represent a monophyletic group, the majority of clades identified in this phylogeny are morphologically heterogeneous. This might represent a consequence of the recent diversification of this group. Even if a subdivision in subgenera would be useful for taxonomic purposes, the topology identified in the genus *Aricidea*, and the relatively low

[4] With the exception of *Aricidea (Aedicira) longicirrata* Fauchald, 1972, a name pre-occupied by *Aricidea (Aricidea) longicirrata* Hartmann-Schröder, 1965, that corresponds to the individual reported as *Aricidea (Acmira)* sp. A. The examined individual clearly corresponds to Fauchald's (1972) description, but it has thick, blade-shaped modified chaetae that are very similar to those of *Aricidea (Acmira) simplex*, and probably went unnoticed because of the poor condition of Fauchald's (1972) type material. This species therefore should be assigned to *Acmira*, at least based on the current taxonomic schematisation.

genetic divergences among different species, compel to avoid further subdivisions that most likely would turn out to be artificial.

The genus *Levinsenia* is confirmed as a monophyletic group, even if the relationships within species belonging to the genus are largely unclear, probably also because of the few data available for several species. All species identified on the basis of morphological features were distinct also at molecular level, allowing to refuse the historically supported hypothesis of high intraspecific morphological variability (Hartman, 1957; Strelzov, 1973) and confirming the high diversity of the genus within restricted geographical areas stated by Çinar et al. (2011). Moreover, *Levinsenia* from deep environments of the Mediterranean Sea showed a remarkably high diversity and might represent undescribed species. However, the amplification of genes in the genus *Levinsenia* revealed itself challenging, and the incomplete dataset available makes unclear interspecific relationships. The same accounts for the last group, including *Aricidea (Acmira) simonae*, *Aricidea (Acmira) simplex*, *Aricidea (Acmira)* sp. A, and *Aricidea (Strelzovia) monicae*. These species have been traditionally assigned to *Aricidea* and are characterised by rounded or squared prostomium, approximately as long as wide, modified chaetae without additional hairs or arista, and two-three pre-branchial chaetigers; the prostomial antenna is always blister-like and very small. The species belonging to this clade represent therefore a morphologically coherent group, but interestingly they have never been recognised as such in previous works. I hereby propose to consider this group as a new genus, New Genus A. In addition to the mentioned species, taking in consideration morphological features, New Genus A should include *Aricidea (Strelzovia) aberrans* Laubier & Ramos, 1974, *Aricidea (Strelzovia) abyssalis* Laubier & Ramos, 1974, *Aricidea (Strelzovia) balearica* Castelli, 1987, *Aricidea (Strelzovia) bifurcata* Aguirrezabalaga & Gil, 2009, *Aricidea (Strelzovia) crassicapitis* Fauchald, 1972, *Aricidea (Strelzovia) pulchra* Strelzov, 1973, *Aricidea (Strelzovia) sardai* Aguirrezabalaga & Gil, 2009 (Fauchald, 1972; Strelzov, 1973; Laubier & Ramos, 1974; Aguirrezabalaga & Gil, 2009). *Aricidea jeffreysii* (McIntosh, 1878), currently considered as a *nomen dubium*, has probably been described on the basis of an individual belonging to this genus. Moreover, two species without prostomial antenna, namely *Aricidea (Strelzovia) belgicae* (Fauvel, 1936) and *Levinsenia duodecimbranchiata* Cantone, 1994 are most likely to be referred to this genus. *A. belgicae* is the only *Aricidea* species without prostomial antenna known to date (López, 2008). Remarks on the uncertain attribution of *L. duodecimbranchiata* to *Levinsenia* have been already raised by Aguirrezabalaga & Gil (2009) since this species has a rounded prostomium and only three pre-branchial chaetigers (Cantone, 1994). In fact, these features allow to place this species

near to New Genus A, rather than to *Levinsenia*, that is characterised by prostomium pointed and longer than wide, and five or more pre-branchial chaetigers. On the other hand, modified chaetae of New Genus A are quite similar to those of *Levinsenia*, being blade-shaped, stocky hooks without any trace of arista or additional hairs. Also the general external structure of New Genus A is more similar to *Levinsenia* than to *Aricidea*, and therefore it is not surprising that the two former genera are sister groups. A diagnosis of New Genus A is therefore: Paraonidae with rounded or squared prostomium, as wide as long; eyes absent, median antenna if present very small, blister-like; three (sometimes two) pre-branchial chaetigers, branchiae usually present; notopodial modified chaetae absent; neuropodial modified chaetae are slightly thicker and shorter capillaries, often with slightly deviated tip, or short, robust blade-shaped hooks, always without arista or additional hairs, always non articulated. I propose *Aricidea (Acmira) simplex* Day, 1963 as type species of New Genus sp. A, as this species has been described relatively early and therefore is not likely to be synonymised, and is known from the molecular point of view. Interestingly, the vast majority of the species referred to New Genus A comes from deep environments, with the exception of *A. simonae*, occuring in shallow bottoms.

Following the distinction of New Genus A from *Aricidea*, and the inclusion of *Paraonis* Cerruti, 1909, the diagnosis of *Aricidea* Webster, 1879 should be emended as follows: Paraonidae with triangular or sub-trapezoidal prostomium, usually longer than wide; eyes often present; the median antenna is usually present and well-developed, reaching more than the half of the prostomium length; it is sometimes articulated, rarely blister-like or absent; three pre-branchial chaetigers; notopodial modified chaetae absent; neuropodial modified chaetae present, with highly variable shape.

Some species show intermediate features between *Aricidea* and New Genus A. For instance, *Aricidea (Acmira) trilobata* Imajima, 1973 and *Aricidea (Acmira)* sp. B[5] show blade-shaped neuropodial hooks as in New Genus A, but the prostomium and antenna shapes are closer to *Aricidea* (Imajima, 1973; Laubier & Ramos, 1974). Genotypable material of these species is needed to clarify whether these species belong to *Aricidea*, to New Genus A, or even to a third genus yet to be described. The genus *Sabidius* Strelzov, 1973 includes only one deep-water species, and was not included in the analysis because of the absence of genotypable material. This genus shares with New Genus A the shape of modified chaetae and the presence of three pre-branchial chaetigers, but the shape of the prostomium is clearly

[5] Provisional name for *Aricidea (Acmira) trilobata* Laubier & Ramos, 1974, that is preoccupied by *Aricidea (Acmira) trilobata* Imajima, 1973.

different, with a hard cephalic cage characterised by trilobed anterior edge. Moreover, the body is very thin and elongate as in *Levinsenia*, and branchiae are digitiform, very small. According to Blake (2016), the pygidium of *Sabidius* is an expanded lobe, apparently devoid of anal cirri, and thence it is different from both that of New Genus A (bearing three anal cirri) and that of *Levinsenia* (bearing two anal cirri). *Sabidius* is most likely close to *Levinsenia* (Blake, 2016) and New Genus A, but a more precise placement is impossible based on present data.

6.5.3 Evolutionary insights on Paraonidae morphology

The present phylogenetic reconstruction allowed to infer on the evolution of morphological traits in Paraonidae, and thence on their taxonomic informativeness and usefulness. As suggested by Strelzov (1973) and Reuscher (2013), the prostomial antenna evolved independently several times in the evolutionary history of Paraonidae. The occurrence of the prostomial antenna is more widespread than previously thought, as only the genus *Levinsenia* includes only antenna-lacking species. In the present study, however, each clade identified by the phylogenetic analysis included one or a few species without prostomial antenna. This character appeared therefore to be variable across Paraonidae genera. As a consequence, it is advisable that its use in taxonomy is supported by other characters, such as chaetal shape. When present, the prostomial antenna is papillar or blister-like, very short in the majority of groups. Only *Aricidea* includes species with a long antenna showing the highest variability among paraonids. In fact, *Aricidea* antenna can be branched, articulated, cirriform and tapered or very short, with blunt tip. In New Genus A the antenna is always very small and it can be bifurcate; whereas in clade I and clade II, only species with simple, blister-like antenna are known. It is worth noting, however, that some poorly known species assigned to the genus *Cirrophorus* might have a prostomial antenna with length comparable to that of *Aricidea* (see description by Hartmann-Schröder, 1965). Based on present data it is impossible to understand whether the prostomial antenna represents a plesiomorphic character in Paraonidae. However, a well-developed antenna, which extends beyond the prostomium, occurs only in the genus *Aricidea* and probably represents a synapomorphy of this group.

The number of pre-branchial chaetigers shows a less obvious variation pattern. In the majority of species there are three pre-branchial chaetigers, regardless of the position in the phylogenetic tree. Among the analysed species, only *Cirrophorus branchiatus* and the genus *Levinsenia* show more than three pre-branchial chaetigers. According to Strelzov (1973), intraspecific variation in the number of pre-branchial chaetigers can be observed in both

genera and this is in partial agreement with observations on *Paradoneis* and *Levinsenia* spp. (Blake, 1996; Aguirrezabalaga & Gil, 2009; *pers. obs.*). However, the diversity of the genus *Levinsenia* has been recently re-assessed, showing that the intraspecific variability is far less wide than previously considered (Çinar et al., 2011), suggesting that variable pre-branchial chaetigers arrangements may represent clues of pseudo-cryptic speciation, rather than intra-specific variations. Less than three pre-branchial chaetigers have been observed only in large adults of *Aricidea (Acmira) simonae*, which should be assigned to New Genus A. According to phylogenetic tree, the most likely basal number of pre-branchial chaetigers is three, and arrangements different from that can be considered derived. Although arrangements with four-five pre-branchial chaetigers sporadically occur in the genera *Cirrophorus* and *Paradoneis*, only the genus *Levinsenia* shows a stable arrangement with five or more pre-branchial chaetigers. It may be argued that the stable arrangement with five pre-branchial chaetigers (the most common in *Levinsenia*) has been attained starting from the basal three-chaetigers arrangement in a similar way as it evolved in *Cirrophorus* and *Paradoneis*, whereas the seven- and eight-chaetigers arrangements evolved secondarily. However, the phylogenetic reconstruction does not support this scenario. Large species with seven-eight pre-branchial chaetigers (the clade including *Levinsenia materi* and *Levinsenia kosswigi*) appear to have diverged early in the evolutionary history of the genus *Levinsenia* (Fig. 36). The remaining part of the genus is composed by small species that usually have five pre-branchial chaetigers, with the exception of *Levinsenia* sp. A (a small species with seven-eight pre-branchial chaetigers).

Strelzov (1973) identified simple capillaries with round cross section as the primitive chaetal type. Since simple capillaries are widespread among polychaetes, and shared by a large part of polychaete families, this conclusion appears reasonable. Chaetae that differ from this type are considered specialised or modified by Strelzov (1973) and I here adopt his terminology, even though the evolutionary process suggested by Strelzov (1973) (Fig. 42) is inconsistent with the current view. Strelzov (1973) suggested that notopodial and neuropodial modified chaetae followed two different evolutionary pathways, with notopodial chaetae gradually thickening, and changing in shape from lyrate to acicular or harpoon-like. Neuropodial chaetae in Strelzov's (1973) opinion should have followed two different, gradual evolutionary processes. A first one would have led to acicular hooks with subdistal arista starting from pseudo-compound capillaries with a median notch; a second one would have led to thick hooks, with or without subterminal fringe, starting from chaetae with abruptly tapered tip (Fig. 42). If the suggestion by Strelzov (1973) about the evolutionary history of notopodial

chaetae appears partially substantiated by present findings, the evolution of modified neuropodial chaetae seems to have happened in a completely different direction.

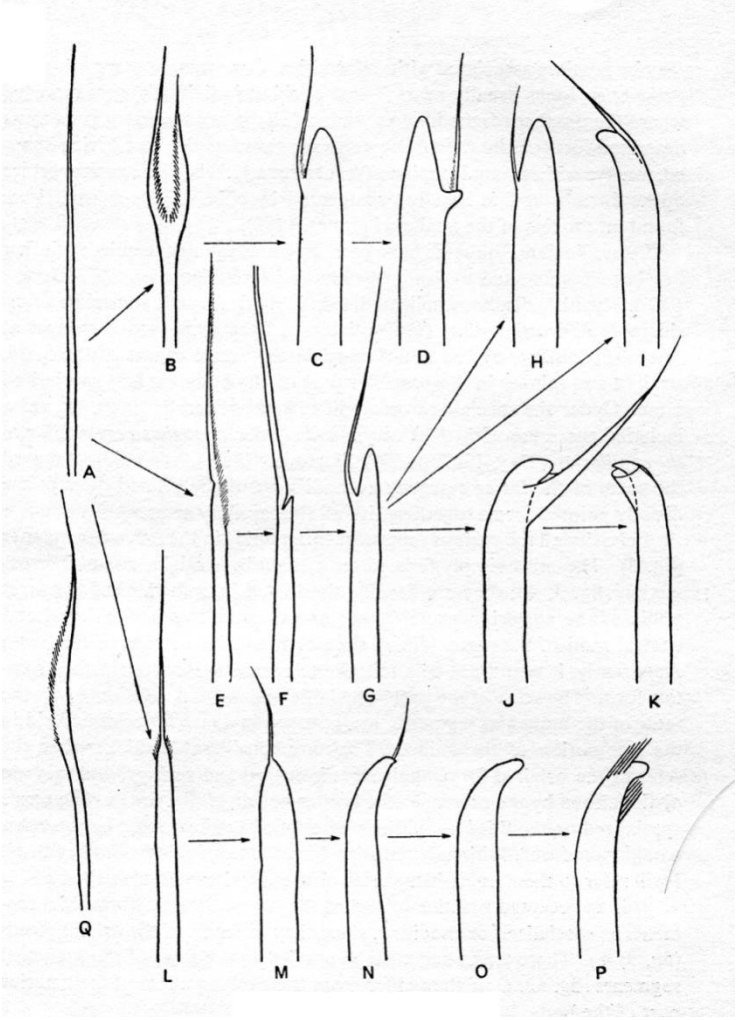

Figure 42: Strelzov's hypothesis on evolution of chaetal shape (from Strelzov, 1973).

According to Reuscher (2013), the presence of modified notopodial chaetae is a synapomorphy of genera *Cirrophorus* and *Paradoneis*. The present phylogenetic reconstruction did not support this view, as *Cirrophorus* and *Paradoneis* do not form a coherent group. Instead, they represent at least two ancient independently evolved clades. Moreover, present data suggested that the presence of modified notopodial can be an ancestral state in Paraonidae, even if they are lacking in the majority of species. The loss of modified notopodial chaetae seems to have happened only once in the evolutionary history of Paraonidae, as all lineages without modified notopodial chaetae are grouped in the same clade (Fig. 36-37). *Paraonella* differs from *Paradoneis* only in the absence of notopodial modified chaetae, and might be polyphyletic, since the species described until now are not very similar from the morphological point of view. However, it is likely that the loss of notopodial chaetae in this group occurred independently, and that *Paraonella* is not strictly related to *Aricidea*,

Levinsenia and New Genus A. The shape of modified notopodial chaetae is apparently a taxonomically sound character, as clade I includes only species with typical lyrate chaetae, whose shape does not change along the body, such as the cases of *Paradoneis lyra* and *Cirrophorus furcatus*, and clade II includes mainly species with lyrate chaetae gradually changing in shape to harpoon-like, as for instance in *Paradoneis armata* and *Paradoneis ilvana*. As suggested by Strelzov (1973), harpoon-like and acicular chaetae seem to have derived from typical lyrate chaetae, as molecular phylogeny suggested that clade II is more recent than clade I.

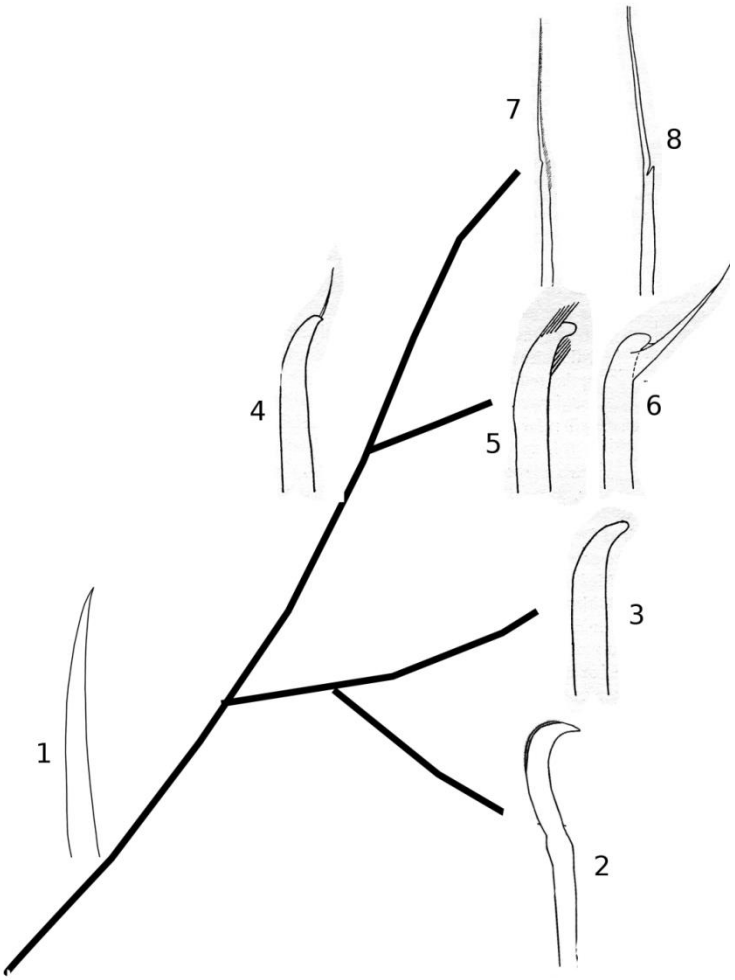

Figure 43: Evolution of neuropodial modified chaetae according to the results of the molecular phylogeny. 1. *Cirrophorus/Paradoneis*; 2. *Levinsenia*; 3. New Genus A; 4-5. *Acmira*-like; 6. *Aricidea*-like type 2; 6. *Strelzovia*-like; 7. *Aricidea*-like type 1.

Neuropodial modified chaetae are widespread in Paraonidae, and show an extremely wide variation in shape, size and distribution. These chaetae typically occur in the post-branchial region, but in some species they might be present only in the last chaetigers. Even if the genera *Cirrophorus* and *Paradoneis* are often described as genera lacking neuropodial

modified chaetae, in some species this feature is present, and may represent a relevant diagnostic character (Strelzov, 1973; Mackie, 1991; Blake, 1996). Modified neuropodial chaetae in *Cirrophorus* and *Paradoneis* are typically thickened capillaries, sometimes with strongly tapered tip, always longer, thinner and in lower number than the strong hooks that are typical of *Levinsenia* and New Genus A. Moreover, species with and without neuropodial modified chaetae might be closely related, suggesting that this character is highly variable in these groups. On the other hand, modified neuropodial chaetae are present in all species of *Aricidea s.l.*, *Levinsenia* and New Genus A. *Levinsenia* species bears several unmistakable neuropodial hooks that usually have a more or less developed dorsal hood. In the majority of the analysed species of New Genus A, modified chaetae are similar to those of *Levinsenia*, but they are straighter and always without dorsal hood; in *Aricidea (Strelzovia) monicae* modified chaetae are only slightly thicker than capillary chaetae, in other species that have been referred to this genus, modified chaetae are intermediate between thick hooks and capillaries. The genus *Aricidea s.l.* has the widest variety of different modified neuropodial chaetae. Usually modified neuropodial chaetae are clearly different from capillaries, even if in several species traditionally assigned to the subgenus *Strelzovia* modified chaetae are only slightly thicker and shorter, sometimes slightly curved capillaries. Species assigned to the subgenus *Aedicira* should lack modified neuropodial chaetae; however, the majority of species historically assigned to this subgenus turned out to actually bear modified chaetae and, on the other hand, it is often difficult to distinguish between slightly modified and non-modified capillaries. The distinction between *Strelzovia* and *Aedicira* is therefore uncertain and most likely artificial. Although representing a good starting point to review chaetal morphology within the genus *Aricidea*, the schematisation proposed by Strelzov (1973) about the evolution of modified chaetae (Fig. 42) appears inconsistent with the phylogenetic relationships identified in this study (Fig. 43). A widespread modified neuropodial chaeta is represented by strong hooks with a terminal or subterminal arista, and sometimes with additional hairs, that are considered typical for subgenus *Acmira*. The earliest branching clades are characterised by the presence of species with only this chaetal type, whereas the other clades include species with and without *Acmira*-like modified chaetae. *Acmira*-like modified chaetae probably represent the ancestral status for *Aricidea s.l.*, whereas other chaetal types, such as slightly thickened capillaries, pseudo-articulate chaetae and chaetae with ventrally inserted arista are derived (Fig. 43). Slightly thickened capillaries, that are typical of the subgenus *Strelzovia* (*Strelzovia*-like modified chaetae) occur in three unrelated clades, and this suggests that the loss of *Acmira*-like chaetae and their substitution with

Strelzovia-like chaetae occurred several times in the evolutionary history of *Aricidea*. Pseudo-articulate chaetae and chaetae with ventrally inserted arista have been considered diagnostic of the subgenus *Aricidea s.s.* and in Strelzov's (1973) opinion should represent variations on the same chaetal structure. However, pseudo-articulate chaetae (*Aricidea*-like type 1) are more likely to develop from a *Strelzovia*-like chaeta, whereas chaetae with ventrally inserted arista (*Aricidea*-like type 2) probably derive from typical *Acmira*-like chaetae (Fig. 43). Moreover, some species of *Aricidea s.s.*, such as *Aricidea (Aricidea) pseudoarticulata*, show the presence of both *Acmira*-like and *Aricidea*-like type 1 chaetae in posterior neuropodia. Interestingly, all examined *Aricidea s.s.* belong to two well-supported clades, also including *Aricidea (Acmira) cerrutii s.l.* and *Paraonis fulgens*. The relationship between the two clades is unresolved in the phylogenetic reconstruction, but morphology suggests that they might represent a coherent group. This group includes only shallow-water species, with antenna often articulated and *Aricidea*-like chaetae of both types. *Aricidea*-like type 1 chaetae are present in *Aricidea (Aricidea) fragilis*, *Aricidea (Aricidea) minuta* and *A. pseudoarticulata*, whereas *Aricidea (Aricidea) capensis bansei* shows *Aricidea*-like type 2 chaetae. On the other hand, chaetae of *Aricidea (Acmira) cerrutii s.l.* differ from *Aricidea*-like type 2 chaetae for only the absence of the arista. Lastly, modified chaetae of *Paraonis fulgens* may have a terminal arista (Castelli, 1985), but they also show a subdistal pubescence on the ventral edge that might recall the ventral arista typical of *Aricidea*-like type 2 chaetae. Interestingly, and despite their striking morphological similarity, *A. pseudoarticulata* and *Aricidea (Aricidea) minuta* are not closely related, and the same accounts for *Aricidea (Acmira) cerrutii cerrutii* and *Aricidea (Acmira)* cf. *cerrutii pacifica*. This outcome suggests that similar adaptations arose independently in these groups, leading to extremely similar morphologies.

6.5.4 Occurrence of cryptic species

As already suggested by the pervasive occurrence of cryptic speciation in polychaetes (Nygren, 2014), several nominal species of Paraonidae are expected to be complexes of cryptic or pseudocryptic species. In this phylogenetic reconstruction I identified cryptic species in all considered genera, following Cracraft's (1989) definition of phylogenetic species ("a phylogenetic species is an irreducible [...] cluster of organisms, diagnosably distinct from other such clusters, and within which there is a parental pattern of ancestry and descent"). The most striking case is that of *Aricidea (Acmira) catherinae*, that turned out to be composed by at least five species belonging to three different clades (see Chapter 3). However, species complexes are relatively frequent within the genus *Aricidea s.l.*. Aside from

the monophyletic *Aricidea (Acmira) assimilis* species complex, including *Aricidea (Acmira)* cf. *laubieri* as well (see Chapter 2), *Aricidea (Acmira) cerrutii cerrutii* is clearly separated from a morphologically divergent deep-water Atlantic form identified as *Aricidea (Acmira)* cf. *cerrutii pacifica*, and appears divided in two clearly separated shallow water Mediterranean lineages. Also Mediterranean and Atlantic *Paraonis fulgens* appear differentiated at species level, and the same accounts for west-Atlantic and east-Atlantic *Aricidea (Aricidea) minuta*.

In New Genus A, the bathyal form of *Aricidea (Acmira) simplex* and *Aricidea (Acmira)* sp. A are clearly conspecific, thus corroborating the hypothesis that the peculiar morphology of *Aricidea (Acmira)* sp. A is related to reproductive modifications, whereas the circalittoral *Aricidea (Acmira) simplex* is only distantly related. The occurrence of cryptic species in morphologically homogeneous species referred to this genus has been already remarked by Brasier et al. (2016), who identified at least two pseudocryptic species in Antarctic material originally referred to *A. simplex*. It is likely that the actual species diversity of New Genus A is greatly underestimated, mainly because of the extremely simple external anatomy, and of the few reliable morphological features.

The genus *Levinsenia* revealed a previously unexpected diversity in deep environments of the Mediterranean Sea. My results on deep Mediterranean *Levinsenia* are consistent with the occurrence of three species. Bathyal individuals with five pre-branchial chaetigers from 700-1000 m represent two divergent lineages, and their identity with *Levinsenia kantauriensis* is uncertain, whereas individuals with seven-eight pre-branchial chaetigers from 120-600 m probably belong to an undescribed species, here defined as *Levinsenia* sp. A. In addition, all species described by Çinar et al. (2011) and Çinar & Dağli (2013) included in the analysis proved to be valid, thus confirming the effectiveness of the number of pre-branchial and branchial chaetigers for taxonomic purposes in this genus.

As regards the genera *Cirrophorus* and *Paradoneis*, I identified two morphologically diagnosable provisional species identified as *Cirrophorus* sp. A and *Cirrophorus* sp. B (see Chapter 1). Moreover, individuals identified as *Paradoneis ilvana* from different environments in the same geographical area showed a deep molecular divergence. A single individual tentatively identified as a juvenile of *Paradoneis ilvana* and collected on gravel at moderate depth turned out to belong to clade I, and is more closely related to *Paradoneis lyra*. It is likely that this individual is not a juvenile, rather it could belong to an interstitial, pedomorphic species, close or identical to *Paradoneis perdidoensis* (McLelland & Gaston, 1994), never reported from the Mediterranean Sea until now.

The occurrence of cryptic species in Paraonidae suggested in previous works (Laubier & Ramos, 1974; Hartley, 1984; Brasier et al., 2016) is therefore confirmed by this study. In particular, according to molecular data, 10 to 12 Paraonidae species recorded in the Mediterranean Sea are still undescribed. A part of them is characterised also at morphological level (see Chapter 1 and Chapter 3); in some cases morphological differences are inconsistent with molecular diversity patterns (see Chapter 2); lastly, some species, such as *Cirrophorus* sp. B and *Paradoneis* cf. *perdidoensis*, are probably morphologically characterised, but the available material is too scarce for a complete description. Considering that the Mediterranean Sea is one of the best known marine basins in the world, it is likely that at global scale a large part of the extant species of Paraonidae are still undescribed (Lovell, 2002; Blake, 2016).

6.5.5 Patterns of biogeographical and bathymetric diversity in Paraonidae

The comparison among allegedly conspecific individuals coming from different geographical areas and/or environments allowed to make some preliminary observations about biogeographical and bathymetric diversity patterns in Paraonidae. A number of Paraonidae species have been reported from extremely wide geographical and bathymetric ranges (Strelzov, 1973), raising the suspect that they might actually represent species complexes (see Chapter 3). Among the examined species, the comparison between eastern Atlantic and Mediterranean individuals of *Aricidea (Aricidea) pseudoarticulata*, *Cirrophorus branchiatus*, *Paradoneis armata* and *Paradoneis lyra* highlighted scarce or no differentiation between the two basins, and the same high similarity has been observed between separated basins within the Mediterranean Sea for *Aricidea (Acmira) assimilis*, *Aricidea (Strelzovia) claudiae* and *Levinsenia demiri*. Usually high connectivity is taken into account to explain such results. Another possible explanation deals with recent divergence between spatially discrete groups; however, in the majority of cases mentioned above this interpretation is inconsistent with the age of biogeographical barriers (Bianchi & Morri, 2000). On the other hand, Adriatic and Tyrrhenian individuals of *Cirrophorus* sp. A appear differentiated, suggesting that populations of this species are actually geographically segregated, probably due to adaptation to brackish habitat, that is likely to reduce population connectivity (see Chapter 1). The connectivity between the two sides of the Atlantic Ocean seems distinctly lower, as both *Aricidea (Acmira) catherinae* and *Aricidea (Aricidea) minuta* from the two areas are differentiated at species level.

The primary driver of molecular differentiation in Paraonidae is represented by environmental features. The role of depth in the differentiation of lineages within the *A. assimilis* species

complex has been already investigated in Chapter 1; interestingly, *A. claudiae* shows a similar bathymetric repartition and it often occurs in the same environments in which *A. assimilis* is found, but individuals from shallow and deep environments are only weakly differentiated, suggesting that environmental features may play different roles in the evolution of different Paraonidae lineages. Strelzov (1973) remarked that, unlike other polychaete groups, Paraonidae show high diversity also in deep environments, comparable to that observed in shallow bottoms. This observation leads to suggest that Paraonidae represent primarily deep-water polychaetes that colonised shallower environments subsequently. However, this phylogenetic reconstruction does not support neither the origin of Paraonidae, nor the origin of single genera in the deep sea. Instead, early branching clades include mainly shallow-water species. This outcome might be due to the absence of deep-sea *Cirrophorus* and *Paradoneis* species in the available dataset. However, also in genera *Aricidea s.l.*, *Levinsenia* and New Genus A, the earliest branching species are shallow-water related, and deep-water species are nested within typically shallow-water groups, suggesting that deep environments have been colonised only secondarily, and ancestral Paraonidae were shallow-water polychaetes. On the other hand, New Genus A, and at a lesser extent *Levinsenia*, are characterised by relatively low diversity in shallow environments, and by a high number of deep-water species, and therefore, even if the ancestral state of these genera is shallow-water related, the adaptive radiation within the genus probably developed in deeper environments. The majority of Paraonidae, on the other hand, lives below tide level, in fine to very fine sediments. The adaptation of Paraonidae to coarse bottoms, often in very shallow environments, occurred independently several times along the family evolutionary history. The species from this kind of environment I examined were included in four unrelated groups, and at least *A. cerrutii cerrutii* and *P. fulgens* show strong molecular divergence also at lower spatial scale; whereas different cryptic species identified as *Paradoneis ilvana* are associated to different depths (0.5 *vs* 20 m depth). Coarse bottoms are often characterised by environmental patchiness, being scattered within wide stretches of sediment characterised by different grain. These features promote organisms diversification, and present data about Paraonidae confirm this paradigm.

6.6 Acknowledgements

A molecular phylogeny of an entire polychaete family is an ambitious task, and even though results achieved in this study are open to improvement, this work would not have been possible without the help of several colleagues. I am especially grateful to F. Aguirrezabalaga, S. Aliani, B. Beals, G. Benedettini, C. Bleidorn, O. Bresciani, H. Cha, T. Darbyshire, C.

Jourdet, L. Lovell, A.S.Y Mackie, C. Mazziotti, A. Pastorelli, A. Pavia, V. Radashevsky, and G. Rouse for providing ethanol-fixed Paraonidae that have been examined in this study; to S. Acunto, M. Barbieri, M. Casu, I. Guarneri, F. Maltagliati, M. Oliva, L. Pacciardi, M. Pertusati, L. Planella, E. Pollonara, M. Ponti, C. Pretti, C. Ravaglioli, M. Roldán, F. Scarpa, M. Sigovini, D. Tagliapietra, J. Tempesti, and A. Vannucci for their invaluable help in field sampling; to F. Aguirrezabalaga, J. A. Blake, M. E. Çinar, L. Lovell, A. S. Y. Mackie, V. Radashevsky, N. Ranauro, M. G. Reuscher and M. Rousou for the interesting discussions about Paraonidae taxonomy and phylogeny; to M. Barbieri, T. Ravaglia, and F. Squarcia for their support in laboratory work.

6.7 Appendix 1

Length of DNA sequences employed in the molecular phylogenetic study

Species	**16S rDNA (bp)**	**COI (bp)**	**18S rDNA (bp)**
Aricidea (Acmira) assimilis Tebble, 1959 1	471		1834
Aricidea (Acmira) assimilis Tebble, 1959 2	491	616	1834
Aricidea (Acmira) assimilis Tebble, 1959 3	491	598	1834
Aricidea (Acmira) assimilis Tebble, 1959 4	488	598	1834
Aricidea (Acmira) catherinae Laubier, 1967 1	491	628	1834
Aricidea (Acmira) catherinae Laubier, 1967 2	471	618	1834
Aricidea (Acmira) catherinae Laubier, 1967 3	491	638	1834
Aricidea (Acmira) catherinae Laubier, 1967 4	480	638	1834
Aricidea (Acmira) catherinae Laubier, 1967 5	475	595	1834
Aricidea (Acmira) catherinae Laubier, 1967 6	491		1834
Aricidea (Acmira) cerrutii cerrutii Laubier, 1966 1	446	590	1834
Aricidea (Acmira) cerrutii cerrutii Laubier, 1966 2	491		1834
Aricidea (Acmira) cerrutii cerrutii Laubier, 1966 3	484		1834
Aricidea (Acmira) cf. *cerrutii pacifica* Imajima, 1973	491		1834
Aricidea (Acmira) elongata Imajima, 1973	456		1834
Aricidea (Acmira) cf. *laubieri* Hartley, 1981	491	631	1834
Aricidea (Acmira) mirifica Strelzov, 1973	472	633	
Aricidea (Acmira) rubra Hartman, 1963	491	604	1834
Aricidea (Acmira) simonae Laubier & Ramos, 1974	349		1834
Aricidea (Acmira) simplex Day, 1963 1	476	598	1834
Aricidea (Acmira) simplex Day, 1963 2	485		
Aricidea (Acmira) sp. A*	491		1834
Aricidea (Aricidea) capensis bansei Laubier & Ramos, 1974	491	619	1834
Aricidea (Aricidea) fragilis Webster, 1879	488	629	1834
Aricidea (Aricidea) minuta Southward, 1956 1	491		
Aricidea (Aricidea) minuta Southward, 1956 2	473		1834
Aricidea (Aricidea) pseudoarticulata Hobson, 1972 1	491		1834
Aricidea (Aricidea) pseudoarticulata Hobson, 1972 2	491		1834
Aricidea (Strelzovia) claudiae Laubier, 1967 1	353		
Aricidea (Strelzovia) claudiae Laubier, 1967 2	491		1834
Aricidea (Strelzovia) mariannae Katzmann & Laubier, 1975	491		1834
Aricidea (Strelzovia) monicae Laubier, 1967	367		1834
Aricidea (Strelzovia) ramosa Annenkova, 1934	453		1834

Aricidea (Strelzovia) roberti Hartley, 1984	491		1834
Aricidea (Strelzovia) cf. *suecica suecica* Eliason, 1920	491		
Cirrophorus branchiatus Ehlers, 1908 1	491	593	1834
Cirrophorus branchiatus Ehlers, 1908 2	491		1834
Cirrophorus furcatus (Hartman, 1957)	471		1834
Cirrophorus sp. A 1	459	636	1834
Cirrophorus sp. A 2	471	632	1834
Cirrophorus sp. A 3	483	617	1834
Cirrophorus sp. B	488	638	1834
Levinsenia demiri Çinar, Açik & Dağli, 2011 1	491		1834
Levinsenia demiri Çinar, Açik & Dağli, 2011 2	206	620	1834
Levinsenia gracilis (Tauber, 1879)	491	628	
Levinsenia kantauriensis Aguirrezabalaga & Gil, 2009 1	476		
Levinsenia kantauriensis Aguirrezabalaga & Gil, 2009 2	359		
Levinsenia kosswigi Çinar, Açik & Dağli, 2011	491		1834
Levinsenia materi Çinar & Dağli, 2013	451		1834
Levinsenia sp. A 1	359		
Levinsenia sp. A 2	353		
Paradoneis armata Glémarec, 1966 1	491		1834
Paradoneis armata Glémarec, 1966 2	377	614	1834
Paradoneis cf. *ilvana* Castelli, 1985	365		
Paradoneis ilvana Castelli, 1985 1	491	607	1834
Paradoneis ilvana Castelli, 1985 2	491	638	1834
Paradoneis lyra (Southern, 1914) 1	485	638	1834
Paradoneis lyra (Southern, 1914) 2	464	614	1834
Paraonis fulgens (Levinsen, 1884) 1	491	614	1834
Paraonis fulgens (Levinsen, 1884) 2	491		1834

7. Conclusions

7.1 Synopsis and general remarks on results obtained

Results of the investigations carried out in my PhD work shed light on diversity and evolution of Paraonidae, a very diverse family of polychaetes that until recently was almost completely unknown from the molecular point of view. More specifically, this thesis addressed different research topics, provided answer to many of them, and opened new perspectives worthy of further investigation.

- The identity of the Mediterranean material traditionally referred to *Cirrophorus furcatus* (Hartman, 1957) was critically discussed on the basis of morphological and molecular data. I concluded that actually *C. furcatus* is absent from the Mediterranean Sea. The Mediterranean material historically referred to this taxon can be assigned to two undescribed species, *Cirrophorus* sp. A and *Cirrophorus* sp. B. The former species is characterised by high number of branchiae and commonly occurs in brackish-water and other organically enriched environments; whereas *Cirrophorus* sp. B is characterised by low number of branchiae and it is probably restricted to marine environments. Moreover, a preliminary phylogenetic analysis allowed to reject the synonymy between *Cirrophorus* and *Paradoneis* suggested by Strelzov (1973) and Reuscher (2013). In fact, I identified two highly supported clades, each including both *Cirrophorus* and *Paradoneis* species. This outcome was a first clue that evolutionary history of Paraonidae is less linear than previously suggested (Strelzov, 1973).
- I tested the effect of environmental breaks and biogeographical barriers on molecular diversity of *Aricidea assimilis* Tebble, 1959, a common Mediterranean paraonid. Molecular data showed a high degree of genetic divergence between deep-water and shallow-water lineages. A less pronounced, but statistically significant, divergence was detected within the shallow-water lineage, between brackish-water and marine sub-lineages. Moreover, the brackish-water sub-lineage is morphologically differentiated from the remaining individuals of *A. assimilis* examined. The levels of molecular divergence between the deep-water and the shallow-water lineages are consistent with the hypothesis of cryptic species, whereas the divergence identified between brackish-water and marine individuals can be considered representative of incipient species. Interestingly, geographical genetic structuring was not detected in any of the examined lineages, suggesting that Paraonidae have wide dispersal larval

phases, in contrast to the widespread belief on the predominance of direct development in this family (López-Jamar et al., 1987; Giangrande, 1997).

- A combined morphological and molecular approach was employed to test the alleged cosmopolitanism of *Aricidea catherinae* Laubier, 1967. The examination of individuals from the western Pacific Ocean, western and eastern Atlantic Ocean and the Mediterranean Sea identified as *A. catherinae* led to the conclusion that they belong to six different species, four of which are undescribed. Individuals identified as *A. catherinae* on the basis of chaetal shape belonged to three highly supported clades that were not directly related, suggesting that neuropodial modified chaetae, which were considered typical of *A. catherinae*, are actually widespread within the genus *Aricidea* and might represent a symplesiomorphic character for this genus. Conversely, several overlooked morphological features, such as fine details of the chaetae and shape of the antenna, have been re-evaluated as taxonomically informative. This study on *A. catherinae* highlighted the occurrence of several undescribed species within an allegedly cosmopolitan nominal taxon. Similar cases have been reported in a number of polychaete families (Bleidorn et al., 2006; Carr et al., 2011; Álvarez-Campos et al., 2017) and are expected to impinge on species checklists and ultimately on environmental assessment and monitoring (Hutchings & Ponder, 2003).
- A comprehensive phylogeny of all available Paraonidae genera, based on 60 individuals belonging to 34 nominal species, has been carried out. This phylogenetic works employed two mitochondrial (COI and 16S rDNA) and one nuclear (18S rDNA) markers and showed a more complex evolutionary history than suggested by previous works (Strelzov, 1973; Reuscher, 2013). Paraonidae analysed in the present work are composed by five highly supported clades. Species of the genera *Cirrophorus* and *Paradoneis* were included in two independent clades, as already described. The genus *Aricidea* is monophyletic with the inclusion of *Paraonis* and the exclusion of four morphologically homogeneous nominal species that represent an undescribed genus, here denominated New Genus A. New Genus A is morphologically close to *Levinsenia* as regards the shape of prostomium and that of modified neurochaetae, and molecular analyses confirmed this close relatedness. In addition, this phylogenetic reconstruction shed light on the evolution of some morphological features of Paraonidae. The evolutionary model I propose provides that

lyrate modified notochaetae represent the ancestral trait that later underwent modifications. In one lineage lyrate chaetae become thicker and harpoon-like, whereas in the other lineage, including *Aricidea*, *Levinsenia* and New Genus A, neuropodial modified chaetae are completely lost. On the other hand, modified neurochaetae were sporadically present in the earliest branching lineages, and became firmly stabilised only in *Aricidea*, *Levinsenia* and New Genus A, reaching the greatest variability in *Aricidea*. The adaptation to bathyal environments seems to have occurred several times along the evolutionary history of this family, always starting from shallow-water ancestors. Based on present data, therefore, the ancestral Paraonidae should have been a shallow-water polychaete with three pre-branchial chaetigers and lyrate notochaetae, with or without modified neurochaetae, with or without prostomial antenna.

- From a strict taxonomic perspective, the phylogenetic reconstruction highlighted the occurrence of a number of cryptic or pseudocryptic species within nominal taxa. Overall, according to results of the present work, I estimated that 10 to 12 Mediterranean Paraonidae are currently undescribed. This result is consistent with Blake's (2016) remarks, stating that a great number of Paraonidae is still undescribed at a global scale.

7.2 Implications for surveys on marine biodiversity

My thesis work represents a contribution to the study of marine biodiversity, and to ecological and evolutionary processes that determined its currently observed patterns. Accordingly to several other studies (Moura et al., 2008; Carr et al., 2011; Payo et al., 2013; Brasier et al., 2016), molecular tools often revealed previously unexpected hidden diversity in the marine biota and this outcome assumes a greater relevance in marine invertebrates. Even if an accurate estimate of the true number of marine species is virtually impossible, molecular studies highlighted that marine biodiversity is largely underestimated and a great number of marine species still awaits to be discovered (Appeltans et al., 2012) and Paraonidae do not make an exception to this general pattern. Results of my thesis suggest that ecological breaks and geographical boundaries have played a relevant role in shaping the current diversity pattern at both intra- and interspecific level. Adaptive radiations seem to be driven mainly by ecological factors, and in particular by the sudden appearance of empty ecological niches (Rainey & Travisano, 1998; Gavrilets & Losos, 2009). Such a process can be suggested as a general explanation for the diversification of Paraonidae, but, in particular, it can be an especially suitable explanation for the high diversity of the genus *Aricidea* Webster, 1879. In

fact, this genus shows a wide ecological and morphological variety, that does not correspond to the identified molecular pattern, in particular if nuclear markers showing low mutation rates are employed. An important ecological factor that is expected to have contributed to the high diversity observed in Paraonidae is represented by sediment granulometry; the occurrence of patches of different sediment across a restricted area of seafloor may have promoted diversification even at small spatial scale and, ultimately, the achievement of the high diversity reported for this family in a number of geographical areas (Katzmann & Laubier, 1975; Lovell, 2002; Aguirrezabalaga & Gil, 2009). Confined environments, on the other hand, are scarcely inhabited by Paraonidae, but their role as enhancer and catalyser of microevolutionary processes (Cognetti & Maltagliati, 2000) is confirmed by data about *A. assimilis* and *Cirrophorus* sp. A. Conversely, the role of geographical barriers in the diversification of lineages seems to be limited; whereas those barriers are expected to represent a key factor in lineage differentiation and, ultimately, cryptic speciation, in particular between Atlantic and Mediterranean, or west-Atlantic and east-Atlantic groups (Bianchi & Morri, 2000; Knowlton, 2000). The role of other evolutionary processes, such as pedomorphism, in fostering lineage diversification is not unknown in polychaete worms (Zanol et al., 2014). Present data suggest that pedomorphic Paraonidae might have been favoured in the colonisation of coarse sand and gravel bottoms, as already observed in other polychaete groups (Struck et al., 2015). More generally, the interpretation of organisms lacking specific structures as basal to a specific group and the hypothesis of a later development of such structures, were rejected in several phylogenetic studies (Litvaitis et al., 1996; Jenner, 2004; Puniamoorthy et al., 2008). The absence of specific structures is often due to loss that might have occurred several times in the evolutionary history of the group, representing a clue of convergent evolution (i.e. homoplasy) rather than of common descent (Wake et al., 2011). Present study on Paraonidae strongly supports this view, and past schematisations (Strelzov, 1973; Reuscher, 2013) turned out to be oversimplified and largely incorrect. Integrative taxonomy, employing both morphological and molecular data, allows therefore to give a deeper insight into organisms diversity and evolution, and to avoid a large part of the errors that inevitably would raise from the use of only one type of data (Schlick-Steiner et al., 2010).

7.3 The role of integrative taxonomy in environmental management

The evolution of marine organisms is a complex phenomenon, driven by ecological breaks, ontogenetic shifts and local diversification (Pianka, 1966; Palumbi, 1994; Knowlton, 2000). As a consequence of this complexity, and the interaction among different processes, diversity patterns recognisable in the marine biota are often unpredicted and non obvious. On the other hand, inference on the effect of climate change, global warming and anthropogenic activities on marine ecosystems is virtually impossible if we do not understand how this diversity was originated, and how different ecological processes contribute to change it (Parmesan, 2006; Hoffmann & Sgrò, 2011). Thence, a solid baseline on marine biodiversity patterns and evolution is not a pure science matter, but has important consequences on evaluation and management of marine systems, and ultimately on our quality of life. However, while the use of molecular tools is more and more widespread in the study of biological diversity (Karp et al., 1998; Singer & Hajibabaei, 2009), such a baseline would be impossible without the contribution of "traditional", morphology-based taxonomy, that is generally underrated and neglected (Boero, 2010; Tahseen, 2014; Boero, 2015).

In the last decades the missed turnover of taxonomists has raised concern in the scientific community, and in some fields this kind of scientific expertise is expected to undergo extinction in a few years (Giangrande et al., 2005; Lücking, 2008). This is allegedly due to the fact that the education of a taxonomist is a money- and time-consuming process, often taking several years before the first valuable contributions, whereas molecular techniques are highly mechanised and a student is expected to take only few months to master them (Boero, 2001; Giangrande et al., 2005; Boero, 2010). On a merely economic basis, therefore, molecular techniques seem to be the wisest choice. As a consequence, molecular data, such as DNA sequences, have already begun to be employed as proxy of biological diversity (Taberlet et al., 2012; Yu et al., 2012), even if, after a first bout of enthusiasm, technical and theoretical issues of such an approach have become evident (Coissac et al., 2012; Beng et al., 2016). The utility of the huge improvement that molecular techniques underwent in the last ten years is undeniable and metabarcoding techniques greatly contributed to understand diversity, ecology and evolution of microbial communities (Luna et al., 2009a; Luna et al., 2009b; Thomsen & Willerslev, 2015). Conversely, the study of macrofaunal assemblages with such techniques is prone to severe errors and flaws (Chariton et al., 2015), in particular because of the absence of a reliable reference library (Cowart et al., 2015; Leray & Knowlton, 2015). In this frame, integrative taxonomy represents the link between the identification and description of taxa (traditional taxonomy) and the quick molecular assessment of biological diversity

(metabarcoding). The starting point of the process, however, is represented by the training of traditional taxonomists (Boero, 2015). My studies on Paraonidae highlight not only that the diversity of this family is underestimated, but also that a significant contribution to a correct assessment of this diversity may come from traditional taxonomy. Moreover, only the use of morphological data allows to correctly understand the origin of molecular diversity patterns. It is strongly advisable, therefore, that more resources are invested in taxonomists formation, in order to close the virtuous cycle between traditional taxonomy, integrative taxonomy, and molecular assessment of biological diversity.

On the other hand, metabarcoding techniques are not a widespread tool in environmental assessment. In fact, the majority of environmental monitoring campaigns in marine environments are based on the morphological analysis of macrobenthic samples. The identification at species level of organisms is a time-consuming process, and can delay the obtaining of results; for these reasons, in the second half of the XX Century was created the concept of "taxonomic sufficiency", i.e. the hypothesis that in several cases an identification at a higher taxonomic level is sufficient to correctly assess the environmental status of a specific area (Ellis, 1985; Heip et al., 1988). This approach revealed itself promising chiefly in coastal stressed environments, that host a moderate diversity of individuals, and whose macrofauna is overall well-known (Ferraro & Cole, 1990; Tataranni et al., 2009). Even in this situation, however, the alternation of taxonomic sufficiency with fine taxonomic characterisation of the assemblages is advisable, in order to check the reliability of the obtained results (Musco et al., 2011). Conversely, taxonomic sufficiency is a disastrous approach in poorly known environments, such as the deep sea (Terlizzi et al., 2003), or in pristine environments, that often display high species diversity within the same family or the same genus (Giangrande, 2003). In these cases, the presence, or the absence, of taxonomic expertise on specific taxa might be the source of technical artefacts that lead to overestimate or underestimate ecological differences (Giangrande, 2003). This "taxonomic impediment", as defined by Giangrande (2003), represents a strong hurdle to a correct environmental management (Hutchings & Ponder, 2003). A possible solution to this impasse is represented by the building of taxonomic expertise networks. This has been already accomplished in some countries (SCAMIT, 2002), and the recent creation of the Italian network for marine organisms taxonomy (MOTax: Cirino et al., 2016) goes in this promising direction. However, this renewed attention to taxonomy is not sufficient: as previously stated, traditional taxonomy is time- and money-consuming, and scientists' interest and goodwill would not

make the change if not supported by the political decision to invest resources on this kind of expertise.

7.4 Concluding remarks

Even if it concentrated only on one polychaete family, thus covering a very small part of the marine biota, my work confirms the importance of integrative taxonomy as a synergistic approach to evaluate marine biodiversity patterns. As already highlighted, even if the importance of such studies is often underestimated, they are crucial in establishing a baseline on which rely the study of anthropogenic modifications and climate changes, the study of ecological processes in marine environments, and environmental monitoring aimed to assess health and quality status of coastal water bodies. Nonetheless, several points are still unclear and need to be more thoroughly encompassed. As for the research in itself, reproductive features of Paraonidae are still unclear, and this is a crucial step to understand the influence of ecological and geographical factors on Paraonidae diversity. A better understanding of this issue could be obtained by comparing phylogeographical patterns in related species showing different ecological requirements. Moreover, deep-sea Paraonidae are poorly explored, and the phylogenetic relationships of some enigmatic species is still unclear; in this case, the use of both molecular and morphological data in a more complete phylogeny, as well as the possible analysis of deep-sea species would help to better clarify how deep environments contributed to shape Paraonidae diversity. From a more general point of view, however, the reassessment of biological diversity patterns has been carried out with an integrative taxonomy approach on only few groups in marine invertebrates. Hopefully, thank to the improvement of molecular techniques combined to the reappraisal of the importance of taxonomic expertise, next years will see an increase in this kind of works, and as a consequence, in our knowledge and understanding of marine systems.

8. References

Abbiati M., Maltagliati F. (1992) Genetic population structure of *Neanthes succinea* (Polychaeta, Nereididae). *Journal of the Marine Biological Association of the U.K.* 72, 511-517.

Abbiati M., Maltagliati F. (1996) Allozyme evidence of genetic differentiation between populations of *Hediste diversicolor* (Polychaeta: Nereididae) from the western Mediterranean. *Journal of the Marine Biological Association of the U.K.* 76, 637-647.

Aguado M.T., López E. (2003) Paraonidae (Annelida: Polychaeta) del Parque Nacional de Coiba (Pacifico, Panamá), con la descripción de una nueva especie de *Aricidea* Webster, 1879. *Revista Chilena de Historia Natural* 76, 363-370.

Aguado M.T., Nygren A., Siddall M.E. (2007) Phylogeny of Syllidae (Polychaeta) based on combined molecular analysis of nuclear and mitochondrial genes. *Cladistics* 23, 552-564.

Aguado M.T., San Martín G., Siddall M.E. (2012) Systematics and evolution of syllids (Annelida, Syllidae). *Cladistics* 28, 234-250.

Aguirrezabalaga F. (2012) Familia Paraonidae Cerruti, 1909. In: *Annelida Polychaeta III*. J. Parapar, C. Alós, J. Núñez, J. Moreira, E. López, F. Aguirrezabalaga, C. Besteiro, A. Martínez (Eds.), pp. 160-272. Museo Nacional de Ciencias Naturales, CSIC, Madrid, Spain.

Aguirrezabalaga F., Gil J. (2008) Paraonidae (Annelida: Polychaeta) of the inlet of Zarautz (Basque Coast, Bay of Biscay), with new records from the Atlantic and the Iberian Coasts. *Cahiers de Biologie Marine* 49, 37-57.

Aguirrezabalaga F., Gil J. (2009) Paraonidae (Polychaeta) from the Capbreton Canyon (Bay of Biscay, NE Atlantic) with the description of eight new species. *Scientia Marina* 73, 631-666.

Akaike H. (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19, 716-723

Almada V.C., Falcón Toledo J., Brito A., Levy A., Floeter S.R., Robalo J.I., Martins J., Almada F. (2013) Complex origins of the Lusitania biogeographic province and northeastern Atlantic fishes. *Frontiers of Biogeography* 5, 20-28.

Altman D.G., Bland J.M. (1995) Absence of evidence is not evidence of absence. *British Medical Journal* 311, 485.

Álvarez-Campos P., Giribet G., Riesgo A. (2017) The *Syllis gracilis* species complex: A molecular approach to a difficult taxonomic problem (Annelida, Syllidae). *Molecular Phylogenetics and Evolution* 109, 138-250.

Amor M.D., Norman M.D., Cameron H.E., Strugnell J.M. (2014) Allopatric speciation within a cryptic species complex of Australasian octopuses. *PLoS One* 9, e98982.

Amoureux L. (1970) Annélides Polychètes du Golfe de Tarente. Resultats des nouvelles croisieres de l'Albatros (1968, 1969). *Annali del Museo Civico di Storia Naturale di Genova* 78, 1-11.

Amoureux L. (1973) Quelques annélides polychètes de l'Afrique occidentale et équatoriale. *Cahiers O.R.S.T.O.M.: Série Océanographique* 11, 41-65.

Amoureux L. (1982) Annélides Polychètes recuellies sur la pente continentale de la Bretagne à l'Irlande, Campagne 1973 de la "Thalassa" (suite et fin) avec la description de quatre espèces nouvelles pour la science. II Inventaire taxonomique annoté de toutes les Polychètes Sédentaires. *Cahiers de Biologie Marine* 22, 179-214.

Annenkova N.P. (1934) Paraoniden der Meeren des Fernen Osten der USSR. *Doklady Akademii Nauk SSSR* 3, 656-658.

Apakupakul K., Siddall M.E., Burreson E.M. (1999) Higher level relationships of leeches (Annelida: Clitellata: Euhirudinea) based on morphology and gene sequences. *Molecular Phylogenetics and Evolution* 12, 350-359.

Appeltans W., Ahyong S.T., Anderson G., Angel M.V., Artois T., Bailly N., Bamber R., Bartsch I., Berta A., Blażewicz-Paszkowycz M., Bock P., Boxshall G., Boyko C.B., Nunes Brandão S., Bray R.A., Bruce N.L. et al. (2012) The magnitude of global marine species diversity. *Current Biology* 22, 2189-2202.

Arriaga-Hernández S., Hernández-Alcántara P., Solís-Weiss V. (2013) Description and distribution of two new species of Paraonidae (Annelida: Polychaeta) from a lagoon-estuarine ecosystem in the Southern Gulf of Mexico. *Zootaxa* 3686, 51-64.

Barroso R., Klautau M., Solé-Cava A.M., Paiva P.C. (2010) *Eurythoe complanata* (Polychaeta: Amphinomidae), the "cosmopolitan" fireworm, consists of at least three cryptic species. *Marine Biology* 157, 69-80.

Barwick K. (2006) Key to the Paraonidae (Annelida: Polychaeta) reported from the City of San Diego Ocean Monitoring program with description of 7 provisional species. *SCAMIT, 2006. Available at http://www.scamit.org/tools/toolbox-new/ANNELIDA. Accessed on 07/12/2016.*

Bastrop R., Jürss K., Sturmbauer C. (1998) Cryptic species in a marine polychaete and their independent introduction from north America to Europe. *Molecular Biology and Evolution* 15, 97-103.

Beheregaray L.B., Sunnucks P. (2001) Fine-scale genetic structure, estuarine colonization and incipient speciation in the marine silverside fish *Odontesthes argentinensis. Molecular Ecology* 10, 2849-2866.

Bellan G. (1965) Contribution à l'étude des Polychètes profondes des parages de Monaco et des cotes de la Corse. *Bulletin de l'Institut Oceanographique de Monaco* 65, 1-24.

Bely A.E., Wray G.A. (2004) Molecular phylogeny of naidid worms (Annelida: Clitellata) based on cytochrome oxidase I. *Molecular Phylogenetics and Evolution* 30, 50-63.

Beng K.C., Tomlinson K.W., Shen X.H., Surget-Groba Y., Hughes A.C., Corlett R.T., Slik J.W.F. (2016) The utility of DNA metabarcoding for studying the response of arthropod diversity and composition to land-use change in the tropics. *Scientific Reports* 6, 24965.

Bergtsen J. (2005) A review of long-branch attraction. *Cladistics* 21, 163-193.

Bhaud M. (1983) Premières observations de la larve planctonique récoltée en haute mer d'un représentant des Paraonidae (Annélide Polychète). *Vie et Milieu* 33, 41-48.

Bianchi C.N., Morri C. (2000) Marine biodiversity of the Mediterranean Sea: situation, problems and prospects for future research. *Marine Pollution Bulletin* 40, 367-376.

Bickford D., Lohman D.J., Sodhi N.S., Ng P.L.K., Meier R., Winker K., Ingram K.K., Das I. (2007) Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* 22, 148-155.

Bilton D.T., Paula J., Bishop J.D.D. (2002) Dispersal, genetic differentiation and speciation in estuarine organisms. *Estuarine, Coastal and Shelf Science* 55, 937-952.

Blake J.A. (1996) Family Paraonidae Cerruti, 1909. In: *Taxonomic Atlas of the Benthic Fauna of the Santa Maria Basin and Western Santa Barbara Channel. 6 - The Annelida Part 3. Polychaeta: Orbiniidae to Cossuridae.* J.A. Blake, B. Hilbig, P.H. Scott (Eds), pp. 27-70. Santa Barbara Museum of Natural History, Santa Barbara, California, the U.S.A.

Blake J.A. (2016) Paraonidae Cerruti, 1909. In: *Handbook of Zoology Online, Annelida: Polychaetes Edition*. W. Westheide, G. Purschke (Eds), pp. 6-52. De Gruyter, Berlin, Germany.

Blake J.A., Arnofsky P.L. (1999) Reproduction and larval development of the spioniform Polychaeta with application to systematics and phylogeny. *Hydrobiologia* 402, 57-106.

Bleidorn C. (2005) Phylogenetic relationships and evolution of Orbiniidae (Annelida, Polychaeta) based on molecular data. *Zoological Journal of the Linnean Society* 144, 59-73.

Bleidorn C., Eeckhaut I., Podsiadlowski L., Schult N., McHugh D., Halanych K.M., Milinkovitch M.C., Tiedemann R. (2007) Mitochondrial genome and nuclear sequence data support myzostomida as part of the annelid radiation. *Molecular Biology and Evolution* 24, 1690-1701.

Bleidorn C., Kruse I., Albrecht S., Bartolomaeus T. (2006) Mitochondrial sequence data expose the putative cosmopolitan polychaete *Scoloplos armiger* (Annelida, Orbiniidae) as a species complex. *BMC Evolutionary Biology* 6, 47.

Bleidorn C., Podsiadlowski L., Zhong M., Eeckhaut I., Hartmann S., Halanych K.M., Tiedemann R. (2009) On the phylogenetic position of Myzostomida: can 77 genes get it wrong? *BMC Evolutionary Biology* 9, 150.

Bleidorn C., Vogt L., Bartolomaeus T. (2003) New insights into polychaete phylogeny (Annelida) inferred from 18S rDNA sequences. *Molecular Phylogenetics and Evolution* 29, 279-288.

Bleidorn C., Vogt L., Bartolomaeus T. (2005) Molecular phylogeny of lugworms (Annelida, Arenicolidae) inferred from three genes. *Molecular Phylogenetics and Evolution* 34, 673-679.

Boero F. (2001) Light after dark: the partnership for enhancing expertise in taxonomy. *Tree* 16, 206.

Boero F. (2010) The study of species in the era of biodiversity: a tale of stupidity. *Diversity* 2, 115-126.

Boero F. (2015) We need monographs, and revisions. *Italian Journal of Zoology* 82, 149-150.

Bonvicini Pagliai A.M., Cognetti G. (1982) Ecology of Polychaetes in the Orbetello lagoon (Tuscany). *Bollettino di Zoologia* 49, 51-60.

Boore J.L., Brown W.M. (2000) Mitochondrial genomes of *Galathealinum*, *Helobdella*, and *Platynereis*: sequence and gene arrangement comparisons indicate that Pogonophora is not a phylum and Annelida and Arthropoda are not sister taxa. *Molecular Biology and Evolution* 17, 87-106.

Borda E., Siddall M.E. (2004) Arhynchobdellida (Annelida: Oligochaeta: Hirudinida): phylogenetic relationships and evolution. *Molecular Phylogenetics and Evolution* 30, 213-225.

Borda E., Yáñez-Rivera B., Ochoa G.M., Kudenov J.D., Sanchez-Ortiz C., Schulze A., Rouse G.W. (2015) Revamping Amphinomidae (Annelida: Amphinomida), with the inclusion of *Notopygos*. *Zoologica Scripta* 44, 324-333.

Box A., Martín D., Deudero S. (2010) Changes in seagrass polychaete assemblages after invasion by *Caulerpa racemosa* var. *cylindracea* (Chlorophyta: Caulerpales): community structure, trophic guilds and taxonomic distinctness. *Scientia Marina* 74, 317-329.

Brasier M.J., Wiklund H., Neal L., Jeffreys R., Linse K., Ruhl H., Glover A.G. (2016) DNA barcoding uncovers cryptic diversity in 50% of deep-sea Antarctic polychaetes. *Royal Society open science* 3, 160432.

Brito M.C., Martín D., Nuñez J. (2005) Polychaetes associated to a *Cymodocea nodosa* meadow in the Canary Islands: Assemblge structure, temporal variability and vertical distribution compared to other Mediterranean seagrass meadows. *Marine Biology* 146, 467-481.

Brown A., Thatje S. (2014) Explaining bathymetric diversity patterns in marine benthic invertebrates and demersal fishes: physiological contributions to adaptation of life at depth. *Biological Reviews* 89, 406-426.

Cabezas M.P., Cabezas P., Machordom A., Guerra-García J.M. (2013) Hidden diversity and cryptic speciation refute cosmopolitan distribution in *Caprella penantis* (Crustacea: Amphipoda: Caprellidae). *Journal of Zoological Systematics and Evolutionary Research* 51, 85-99.

Cadman P.S., Nelson-Smith A. (1990) Genetic evidence for two species of lugworm (*Arenicola*) in South Wales. *Marine Ecology Progress Series* 64, 107-112.

Calosi P., Rastrick S.P.S., Lombardi C., de Guzman H.J., Davidson L., Jahnke M., Giangrande A., Hardege J.D., Schulze A., Spicer J.I., Gambi M.C. (2013) Adaptation and acclimatization to ocean acidification in marine ectotherms: an *in situ* transplant experiment with polychaetes at a shallow CO_2 vent system. *Philosophical Transactions of the Royal Society, Ser B.* 368, 20120444.

Čandek K., Kuntner M. (2015) DNA barcoding gap: reliable species identification over morphological and geographical scales. *Molecular Ecology Resources* 15, 268-277.

Cantone G. (1994) Polychaeta “Sedentaria” of Terra Nova Bay (Ross Sea, Antarctica): Orbiniidae to Oweniidae. *Animalia* 21, 35-47.

Capa M., Hutchings P., Aguado M.T., Bott N.J. (2011) Phylogeny of Sabellidae (Annelida) and relationships with other taxa inferred from morphology and multiple genes. *Cladistics* 27, 449-469.

Capaccioni R. (1987) *Anélidos Poliquetos de la Ensenada de los Alfaques (Delta del Ebro, Mediterráneo Occidental)*. PhD thesis, University of Valencia, Valencia, Spain, 533 pp.

Carpine C. (1970) Ecologie de l’étage bathyal dans la Méditerranée occidentale. *Mémoires de l’Institut Océanographique de Monaco* 2, 1-146.

Carr C.M., Hardy S.M., Brown T.M., Macdonald T.A., Hebert P.D.N. (2011) A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. *PLoS ONE* 6. e22232.

Castelli A. (1985) Paraonidae (Annelida, Polychaeta) des fonds meubles infralittoraux des cotes toscanes. *Cahiers de Biologie Marine* 26, 267-279.

Castelli A. (1987) Censimento dei policheti dei mari italiani: Paraonidae Cerruti, 1909. *Atti della Società Toscana di Scienze Naturali, Memorie, Serie B* 94, 319-340.

Castelli A., Abbiati M., Badalamenti F., Bianchi C.N., Cantone G., Gambi M.C., Giangrande A., Gravina M.F., Lanera P., Lardicci C., Somaschini A., Sordino P. (1995) Annelida Polychaeta, Pogonophora, Echiura, Sipuncula. In *Checklist delle specie della fauna italiana*. Minelli A., Ruffo S., La Posta S. (eds.). Calderini, Bologna, 19, 45 pp.

Castelli A., Bianchi C.N., Cantone G., Çinar M.E., Gambi M.C., Giangrande A., Iraci Sareri D., Lanera P., Licciano M., Musco L., Sanfilippo R., Simonini R. (2008) Annelida Polychaeta. *Biologia Marina Mediterranea* 15, 323-374.

Castelli A., Lardicci C. (1985) Note preliminari allo studio dei Policheti della Baia di Portoferraio (Isola d’Elba). *Oebalia* 11, 763-766.

Casu M., Casu D., Lai T., Cossu P., Curini-Galletti M. (2006) Inter-simple sequence repeat markers reveal strong genetic differentiation among populations of the endangered mollusc *Patella ferruginea* (Gastropoda: Patellidae) from two Sardinian marine protected areas. *Marine Biology* 149, 1163-1174.

Cerruti A. (1909) Contributo all’anatomia, biologia e sistematica delle Paraonidae (Levinsenidae), con particolare riguardo alle specie del golfo di Napoli. *Mitteilungen aus der zoologischen Station zu Neapel* 19, 459-512.

Chariton A.A., Stephenson S., Morgan M.J., Steven A.D., Colloff M.J., Court L.N., Hardy C.M. (2015) Metabarcoding of benthic eukaryote communities predicts the ecological condition of estuaries. *Environmental Pollution* 203, 165-174.

Chessa L.A., Scardi M., Serra S., Pais A., Lanera P., Plastina N., Valiante L.M., Vinci D. (2007) Small-scale perturbation on soft bottom macrozoobenthos after mechanical cleaning operations in a Central-Western Mediterranean lagoon. *Transitional Waters Bulletin* 2, 9-19.

Çinar M.E. (2005) Polychaetes from the coast of northern Cyprus (eastern Mediterranean Sea), with two new records for the Mediterranean Sea. *Cahiers de Biologie Marine* 46, 143-160.

Çinar M.E., Dağli E. (2013) Polychaetes (Annelida: Polychaeta) from the Aegean and Levantine coasts of Turkey, with descriptions of two new species. *Journal of Natural History* 47, 911-947.

Çinar M.E., Dağli E., Açik, S. (2011) Annelids (Polychaeta and Oligochaeta) from the Sea of Marmara, with descriptions of five new species. *Journal of Natural History* 45, 2105-2143.

Çinar M.E., Dağli E., Kurt-Şahin G. (2014) Checklist of the Annelida from the coasts of Turkey. *Turkish Journal of Zoology* 38, 734-764.

Cirino P., Di Capua I., Guglielmo R., Modica M.V. (2016) *MOTax Newsletter, Dicembre 2016, n. 0.* Stazione Zoologica di Napoli.

Cognetti G. (1954) Forme della *Mercierella enigmatica* nella nuova stazione del Lago di Patria. *Bollettino di Zoologia* 21, 41-45

Cognetti G. (1982) Adaptive strategies of brackish-water fauna in pure and polluted waters. *Marine Pollution Bulletin* 13, 247-250.

Cognetti G., De Angelis C.M., Orlando E., Bonvicini Pagliai A.M., Cognetti Varriale A.M., Crema R., Mari M., Mauri M., Tongiorgi P., Zunarelli Vandini R. (1978) Risanamento e protezione dell'ambiente idrobiologico di Orbetello. Situazione ecologica e ittiologica. *Ingegneria Ambientale* 7, 312-406.

Cognetti G., Maltagliati F. (2000) Biodiversity and adaptive mechanisms in brackish water fauna. *Marine Pollution Bulletin* 40, 7-14.

Coissac E., Riaz T., Puillandre N. (2012) Bioinformatic challenges for DNA metabarcoding of plants and animals. *Molecular Ecology* 21, 1834-1847.

Como S., Rossi F., Lardicci C. (2004) Response of deposit-feeders to exclusion of epibenthic predators in a Mediterranean intertidal flat. *Journal of Marine Biology and Ecology* 303, 157-171

Cossu P., Maltagliati F., Pannacciulli F.G., Simonini R., Massamba-N'Siala G., Casu M., Lardicci C., Prevedelli D., Castelli A. (2015) Phylogeography of *Ophryotrocha labronica* (Polychaeta, Dorvilleidae) along the Italian coasts. *Marine Ecology* 36, 1088-1097.

Couceiro L., López L., Sotka E.E., Ruiz J.M., Barreiro R. (2012) Molecular data delineate cryptic *Nassarius* species and characterize spatial genetic structure of *N. nitidus*. *Journal of the Marine Biological Association of the U.K.* 92, 1175-1182.

Cowart D.A., Pinheiro M., Mouchel O., Maguer M., Grall J., Miné J., Arnaud-Haond S. (2015) Metabarcoding is powerful yet still blind: a comparative analysis of morphological and molecular surveys of seagrass communities. *PLoS ONE* 10, e00117562.

Cracraft J. (1989) Speciation and its ontology: the empirical consequences of alternative species concepts for understanding patterns and processes of differentiation. In: *Speciation and its consequences*. D. Otte, J. Endler (Eds.), pp. 28-79. Sinauer Assoc., Sunderland, Massachusetts, the U.S.A.

Darriba D., Taboada G.L., Doallo R., Posada D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9, 772.

Dauvin J.-C., Dewarumez J.-M., Gentil F. (2003) Liste actualisée des espèces d'Annélides Polychètes présentes en Manche. *Cahiers de Biologie Marine* 44, 67-95.

Dauvin J.-C., Gentil F. (1980) Nouvelles espèces pour l'inventaire de la faune marine de la région de Roscoff: Annélides Polychètes et Crustacés Amphipodes. *Travaux de la Station Biologique de Roscoff* 26, 5-10.

Day J.H. (1963) Polychaete Fauna of South Africa: Part 7. Species from depths between 1,000 and 3,330 meters west of Cape Town. *Annals of the South African Museum* 46, 353-371.

Day J.H. (1967) *A monograph on the Polychaeta of Southern Africa*. Volume British Museum (Natural History) Publication. British Museum (Natural History). London. 878 pp.

De Biasi A.M., Pacciardi L. (2008) Macrobenthic communities in a fishery exclusion zone and in a trawled area of the middle Adriatic Sea (Italy). *Ciencias Marinas* 34, 433-444.

Dobzhansky T. (1972) Species of *Drosophila. Science* 177, 664-669.

Eco U. (1983) *The Name of the Rose.* Harcourt, San Diego, California, the U.S.A., 512 pp.

Eeckhaut I., McHugh D., Mardulyn P., Tiedemann R., Monteyne D., Jangoux M., Milinkovitch M.C. (2000) Myzostomida: a link between trochozoans and flatworms? *Proceedings of the Royal Society, Ser. B* 267, 1383-1392.

Ehlers E. (1908) Die bodensässigen Anneliden aus den Sammlungen der deutschen Tiefsee-Expedition. In: *Wissenschaftlichte Ergebnisse der deutschen Tiefsee-Expedition auf dem Dampfer 'Valdivia' 1898-1899, vol. 16.* C. Chun (Ed.), pp. 1-168.

Eklöf J., Pleijel F., Sundberg P. (2007) Phylogeny of benthic Phyllodocidae (Polychaeta) based on morphological and molecular data. *Molecular Phylogenetics and Evolution* 45, 261-271.

Ekman S. (1953) *Zoogeography of the Sea*. Sidgwick and Jackson, London, 417 pp.

Eliason A. (1920) Biologisch-faunistiche Untersuchungen aus dem Öresund. V. Polychaeta. *Lunds Universitets Årsskrift, new series, sec. 2* 16, 1-103.

Eliason A. (1962) Die Polychaeten der Skagerrak-Expedition 1933. *Zoologiska bidrag från Uppsala* 33, 207-293.

Ellis D. (1985) Taxonomic sufficiency in pollution assessment. *Marine Pollution Bulletin* 16, 459.

Erséus C., Källersjö M. (2004) 18S rDNA phylogeny of Clitellata (Annelida). *Zoologica Scripta* 33, 187-196.

Erséus C., Prestegaard T., Källersjö M. (2000) Phylogenetic analysis of Tubificidae (Annelida, Clitellata) based on 18S rDNA sequences. *Molecular Phylogenetics and Evolution* 15, 381-389.

Erséus C., Rota E., Matamoros L., De Wit P. (2010) Molecular phylogeny of Enchytraeidae (Annelida, Clitellata). *Molecular Phylogenetics and Evolution* 57, 849-858.

Fauchald K. (1972) Benthic polychaetous annelids from deep water off western Mexico and adjacent areas in the Eastern Pacific Ocean. *Allan Hancock Monographs in Marine Biology* 7, 1-525.

Fauchald K. (1984) Polychaete distribution patterns, or: can animals with Palaeozoic cousins show large-scale geographical patterns? In: *Proceedings of the First International Polychaete Conference.* P. Hutchings (Ed.), pp. 1-6. Published by The Linnean Society of New South Wales.

Fauvel P. (1923) *Polychètes errantes*. Faune de France, ed. Lechevalier, Paris, 488 pp.

Fauvel P. (1927) *Polychètes sédentaires.* Faune de France, ed. Lechevalier, Paris, 494 pp.

Ferguson A., Taggart J. (1991) Genetic differentiation among the sympatric brown trout (*Salmo trutta*) populations of Lough Melvin, Ireland. *Biological Journal of the Linnean Society* 43, 221-237.

Ferraro S.P., Cole F.A. (1990) Taxonomic level and sample size sufficient for assessing pollution impacts on the Southern California Bight macrobenthos. *Marine Ecology Progress Series* 67, 251-262.

Ferrito V., Mannino M.C., Pappalardo A.M., Tigano C. (2007) Morphological variation among populations of *Aphanius fasciatus* Nardo, 1827 (Teleostei, Cyprinodontidae) from the Mediterranean. *Journal of Fish Biology* 70, 1-20.

Fewkes J. W. (1883) On the development of certain worm larvae. *Bulletin of the Museum of Comparative Zoology at Harvard College* 11, 167-208.

Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3, 294-299.

Forges B.R. de, Koslow J.A., Poore G.C.B. (2000) Diversity and endemism of the benthic seamount fauna in the southwest Pacific. *Nature* 405, 944-947.

Gambi M.C., Conti G., Bremec C.S. (1998) Polychaete distribution, diversity and seasonality related to seagrass cover in shallow soft bottoms of the Tyrrhenian Sea (Italy). *Scientia Marina* 62, 1-17.

Gaston G.R., McLelland J.A. (1996) *Aricidea (Allia) bryani*, a new species of polychaete (Polychaeta: Paraonidae) from the northern Gulf of Mexico. *Gulf Research Reports* 9, 189-195.

Gaston G.R., McLelland J.A., Heard R.W. (1992) Feeding biology, distribution, and ecology of two species of Gulf of Mexico polychaetes: *Paraonis fulgens* and *P. pygoenigmatica* (Paraonidae, Annelida). *Gulf Research Reports* 8, 395-399.

Gavrilets S., Losos J.B. (2009) Adaptive radiation: contrasting theory with data. *Science* 323, 732-737.

Giangrande A. (1997) Polychaete reproductive patterns, life cycles and life histories: an overview. *Oceanography and Marine Biology: an Annual Review* 35, 323-386.

Giangrande A. (2003) Biodiversity, conservation, and the 'Taxonomic impediment'. *Aquatic Conservation* 13, 451-459.

Giangrande A., Rouse G.W., Pleijel F.J., Gambi M.C., Curini-Galletti M., Cavallo A., Licciano M., Musco L., Pierri C., Trianni L. (2005) La "rivoluzione cladistica" in

tassonomia, nuovi strumenti e implicazioni per gli ecologi. *Biologia Marina Mediterranea* 12, 23-30.

Gibbs P.A. (1965) Recent additions to the marine fauna of Whitstable, with a description of *Aricidea minuta* Southward (Annelida: Polychaeta). *Annals and Magazine of Natural History, series 13* 7, 33-36.

Giere O., Ebbe B., Erséus C. (2007) *Questa* (Annelida, Polychaeta, Orbiniidae) from Pacific regions – new species and reassessment of the genus *Periquesta*. *Organisms Diversity & Evolution* 7, 304-319.

Gil J., Sardá R. (1999) New records of Annelida Polychaeta for the Portuguese fauna (with comments on some already known species). *Arquivos do Museu Bocage, nova série* 3, 287-336.

Glémarec M. (1966) Paraonidae de Bretagne: description de *Paradoneis armata* nov. sp. *Vie et Milieu, Ser. A* 17, 1045-1052.

Goetze E. (2003) Cryptic speciation on the high seas: global phylogenetics of the copepod family Eucalanidae. *Proceedings of the Royal Society, Ser. B* 270, 2321-2331.

Goto R., Okamoto T., Ishikawa H., Hamamura Y., Kato M. (2013) Molecular phylogeny of echiuran worms (Phylum: Annelida) reveals evolutionary pattern of feeding mode and sexual dimorphism. *PLoS One* 8, e56809.

Grassle J., Grassle J.F. (1976) Sibling species in the marine pollution indicator *Capitella* (polychaeta). *Science* 192, 567-569.

Grube A.E. (1872) Über ein paar neue Anneliden aus der Familie der Spiodeen. *Jahres-Bericht der Schlesischen Gesellschaft für vaterländische Cultur* 50, 57-58.

Guindon S., Gascuel O. (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* 52, 696-704.

Halanych K.M., Dahlgren T.G., McHugh D. (2002) Unsegmented annelids? Possible origins of four lophotrochozoan worm taxa. *Integrative and Comparative Biology* 42, 678-684.

Hall T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acid Symposium Series* 41, 95-98.

Häntzschel W. (1975) Trace fossils and problematica. In: *Treatise on Invertebrate Paleontology, pt. W, Miscellanea, suppl. 1.* pp. 3-269. Geological Society of America and Kansas University Press, Kansas, the U.S.A.

Hartley J.P. (1981) The family Paraonidae (Polychaeta) in British Waters: a new species and new records with a key to species. *Journal of the Marine Biological Association of the U.K.* 61, 133-149.

Hartley J.P. (1984) Cosmopolitan polychaete species: the status of *Aricidea belgicae* (Fauvel, 1936) and notes on the identity of *A. suecica* Eliason, 1920 (Polychaeta: Paraonidae). In *Proceedings of the First International Polychaete Conference.* P. Hutchings (Ed.), pp. 7-20. The Linnean Society of New South Wales, Sydney, Australia.

Hartman O. (1955) Quantitative survey of the benthos of San Pedro Basin, southern California. Part I. Preliminary results. *Allan Hancock Pacific Expeditions* 19, 1-185.

Hartman O. (1957) Orbiniidae, Apistobranchidae, Paraonidae and Longosomidae. *Allan Hancock Pacific Expeditions* 15, 211-393.

Hartman O. (1963) Submarine canyons of southern California. Part II. Biology. *Allan Hancock Pacific Expeditions* 27, 1-424.

Hartman O. (1965) Deep-water benthic polychaetous annelids off New England to Bermuda and other North Atlantic areas. *Occasional Papers of the Allan Hancock Foundation* 28, 1-378.

Hartman O., Fauchald K. (1971) Deep-water benthic polychaetous annelids off New England to Bermuda and other North Atlantic Areas. Part II. *Allan Hancock Monographs in Marine Biology* 6, 1-327.

Hartmann-Schröder G. (1965) Die Polychaeten des Sublitorals. In: *Zur Kenntnis des Sublitorals der chilenischen Küste unter besonderer Berücksichtigung der Polychaeten und Ostracoden. (Mit bemerkungen über den Einfluss sauerstoffarmer Strömungen auf die Besiedlung von marinen Sedimenten.).* Hartmann-Schröder G., Hartmann G. (Eds.). *Mitteilungen aus dem Hamburgischen zoologischen Museum und Institut* 62, 59-305.

Hausdorf B. (2011) Progress towards a general species concept. *Evolution* 65, 923-931.

Hebert P.D.N., Cywinska A., Ball S.L., deWaard J.R. (2003) Biological identification through DNA barcode. *Proceedings of the Royal Society of London B* 270, 313-321.

Heggernes Eilertsen M., Alvestad T., Rapp H.T., Kongsrud J.A. (2016) Phylogeny of Ampharetidae. *Poster presented at the 12th International Polychaete Conference, 1-5 August 2016, Cardiff, Wales, the U.K.*

Heip C., Warwick R.M., Carr M.R., Herman P.M.J., Huys R., Smol N., van Holsbeke K. (1988) Analysis of community attributes of the benthic meiofauna of Frierfiord/Langesundfjord. *Marine Ecology Progress Series* 46, 171-180.

Heras S., Roldán M.I. (2011) Phylogenetic inference in *Odontesthes* and *Atherina* (Teleostei: Atheriniformes) with insights into ecological adaptation. *Comptes Rendues Biologie* 334, 273-281.

Hillis D.M., Dixon M.T. (1991) Ribosomal DNA: Molecular evolution and phylogenetic inference. *The Quarterly Review of Biology* 66, 411-453.

Hobson K.D. (1972) Two new species and two new records of the family Paraonidae (Annelida, Polychaeta) from the northeastern Pacific Ocean. *Proceedings of the Biological Society of Washington* 85, 549-556.

Hobson K.D. (1976) *Protoariciella oligobranchia* new species (Orbiniidae) and six new records of Orbiniidae, Questidae, and Paraonidae (Annelida, Polychaeta) from British Columbia. *Canadian Journal of Zoology* 54, 591-596.

Hoffmann A.A., Sgrò C.M. (2011) Climate change and evolutionary adaptation. *Nature* 470, 479-485.

Howe A. (2008) Deep-sea hydrothermal vent fauna: evolution, dispersal, succession and biogeography. *Macalester Reviews in Biogeography* 1, 6.

Hundt P.J., Iglésias S.P., Hoey A.S., Simons A.M. (2014) A multilocus molecular phylogeny of combtooth blennies (Percomorpha: Blennioidei: Blenniidae): multiple invasions of intertidal habitats. *Molecular Phylogenetics and Evolution* 70, 47-56.

Hutchings P., Ponder W. (2003) Marine invertebrates and their conservation. *Marine Pollution Bulletin* 46, 153-154.

Iannotta M.A., Gambi M.C., Patti F.P. (2009b) Molecular evidence of intraspecific variability in *Lysidice ninetta* (Polychaeta: Eunicidae) in the Mediterranean Sea. *Aquatic Biology* 6, 121-132.

Iannotta M.A., Patti F.P., Ambrosino M., Procaccini G., Gambi M.C. (2007) Phylogeography of two species of *Lysidice* (Polychaeta, Eunicidae) associated to the seagrass *Posidonia oceanica* in the Mediterranean Sea. *Marine Biology* 150, 1115-1126.

Iannotta M.A., Toscano F., Patti F.P. (2009a) *Nassarius corniculus* (*sic*) (Olivi, 1792) (Caenogastropoda: Nassariidae): a model of environmental complexity of Italian brackish and marine habitats. *Marine Ecology* 30, 106-115.

Imajima M. (1973) Paraonidae (Polychaeta) from Japan. *Bulletin of the National Scientific Museum of Tokio* 16, 253-292.

Jenner R.A. (2004) Towards a phylogeny of the Metazoa: evaluating alternative phylogenetic positions of Platyhelminthes, Nemertea, and Gnathostomulida, with a critical reappraisal of cladistics characters. *Contributions to Zoology* 73, 3-163.

Jimoh A.A., Anetekhai M.A., Cummings S., Abanikanda O.T.F., Turner G.F., van Oosterhout C., Hänfling B. (2013) Mismatch between molecular (mtDNA) and morphological classification of *Macrobrachium* prawns from Southern Nigeria: Cryptic freshwater species and brackish water morphotypes. *Aquaculture* 410-411, 25-31.

Johannesson K. (2003) Evolution in *Littorina*: ecology matters. *Journal of Sea Research* 49, 107-117.

Johannesson K., Rolán-Alvarez E., Ekendahl A. (1995) Incipient reproductive isolation between two sympatric morphs of the intertidal snail *Littorina saxatilis*. *Evolution* 49, 1180-1190.

Jones M.L. (1968) *Paraonis pygoenigmatica* new species, a new annelid from Massachusetts (Polychaeta: Paraonidae). *Proceedings of the Biological Society of Washington* 81, 323-334.

Jones M.L. (1985) On the Vestimentifera, new phylum: six new species, and other taxa, from hydrothermal vents and elsewhere. *Bulletin of the Biological Society of Washington* 6, 117-158.

Jumars P.A., Dorgan K.M., Lindsay S.M. (2015) Diet of worms emended: an update of polychaete feeding guilds. *Annual Review in Marine Science* 7, 497-520.

Karp A., Ingram D.S., Isaac P.G. (2012) *Molecular tools for screening biodiversity. Plants and animals.* Chapman & Hall, 498 pp.

Katzmann W. (1973) *Aricidea punctata* n. sp., ein neuer Paraonidae (Polychaeta) aus der Adria. *Annalen des Naturhistorischen Museums in Wien* 77, 287-288.

Katzmann W., Laubier L. (1975) Paraonidae (Polychètes Sédentaires) de l'Adriatique. *Annales der Naturhistorischen Museum in Wien* 79, 567-588.

Kiel S., Little C.T.S. (2006) Cold-seep mollusks are older than the general marine mollusk fauna. *Science* 313, 1429-1431.

Kimura M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16, 111-120.

Knowlton N. (1993) Sibling species in the sea. *Annual Review of Ecology and Systematics* 24, 189-216.

Knowlton N. (2000) Molecular genetic analyses of species boundaries in the sea. *Hydrobiologia* 420, 73-90.

Kobayashi G., Goto R., Kojima S. (2016) Molecular phylogeny of bamboo worms (Annelida: Maldanidae). *Oral communication at the 12th International Polychaete Conference, 1-5 August 2016, Cardiff, Wales, the U.K.*

Kolaczkowski B., Thornton J.W. (2009) Long-branch attraction bias and inconsistency in Bayesian phylogenetics. *PLoS One* 4, e7891.

Kumar S., Stecher G., Tamura K. (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* first published online March 22, 2016 doi:10.1093/molbev/msw054.

Kupriyanova E.K., Bastida-Zavala R., Halt M.N., Lee M.S.Y., Rouse G.W. (2008) Phylogeny of the *Serpula-Crucigera-Hydroides* clade (Serpulidae: Annelida) using molecular and morphological data: implications for operculum evolution. *Invertebrate Systematics* 22, 425-437.

Kupriyanova E.K., Macdonald T.A., Rouse G.W. (2006) Phylogenetic relationships within Serpulidae (Sabellida, Annelida) inferred from molecular and morphological data. *Zoologica Scripta* 35, 421-439.

Kvist S. (2016) Does a global barcoding gap exist in Annelida? *Mitochondrial DNA part A* 27, 2241-2252.

Ladner J.T., Palumbi S.R. (2012) Extensive sympatry, cryptic diversity and introgression throughout the geographic distribution of two coral species complexes. *Molecular Ecology* 21, 2224-2238.

Langeneck J., Barbieri M., Maltagliati F., Castelli A. (*subm.*) A new species of *Cirrophorus* (Annelida: Paraonidae) from the Mediterranean Sea, with taxonomic notes on the genera *Cirrophorus, Paradoneis* and *Paraonides*. *Journal of the Marine Biological Association of the U.K.*

Langeneck J., Busoni G., Aliani S., Castelli A. (*in press*). Deep-sea polychaetes (Annelida) from the Malta Escarpment (Western Ionian Sea). *European Zoological Journal*.

Larkin M.A., Blackshields G., Brown N.P., Chenna R., McGettigan P.A., McWilliam H., Valentin F., Wallace I.M., Wilm A., Lopez R., Thompson J.D., Gibson T.J. and Higgins D.G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23, 2947-2948.

Laubier L. (1966a) Le coralligène des Albères. Monographie biocénotique. *Annales de l'Institut Océanographique, Monaco, Nouvelle Série* 43, 137-316.

Laubier L. (1966b) Sur quelques annélides polychètes de la région de Beyrouth. *American University of Beirut – miscellaneous Paper on natural Sciences* 5, 9-22.

Laubier L. (1966c) Sur la présence du genre *Cirrophorus* (Polychètes Paraonidae) en Méditerranée. *Bulletin de la Sociètè zoologique de France* 90, 469-477.

Laubier L. (1967) Sur quelques *Aricidea* (Polychètes, Paraonidae) de Banyuls-sur-Mer. *Vie et Milieu, Ser. A.* 18, 99-132.

Laubier L., Ramos J. (1974) Paraonidae (Polychètes Sédentaires) de Méditerranée. *Bulletin du Muséum National d'Histoire Naturelle. Ser. 3*: 168, Zool. 113, 1097-1148.

Law C.J., Dorgan K.M., Rouse G.W. (2012) Kicking out the grubs: a new Opheliidae (Polychaeta: Annelida) phylogeny based on molecular data. *2012 Environmental Systems Symposium, University of California, San Diego, CA. Poster.*

Lehrke J., ten Hove H.A., Macdonald T.A., Bartolomaeus T., Bleidorn C. (2007) Phylogenetic relationships of Serpulidae (Annelida: Polychaeta) based on 18S rDNA sequence data, and implications for opercular evolution. *Organisms Diversity & Evolution* 7, 195-206.

León-González J.A. de, Hernández-Guevara N.A., Rodríguez-Valencia J.A. (2006) Paraonidae (Polychaeta) from western Mexico, with description of two new species. *Journal of the Marine Biological Association of the U.K.* 86, 253-252.

Leray M., Knowlton N. (2015) DNA barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity. *Proceedings of the National Academy of Sciences* 112, 2076-2081.

Levinsen G.M.R. (1884) Systematisk-geografisk Oversigt over de nordiske Annulata, Gephyrea, Chaetognathi og Balanoglossi. *Videnskabelige Meddelelser fra den naturhistoriske Forening i Kjöbenhavn* 45, 92-350, plates II-III.

Liessner A., Phillips C., Cadien D., Smith R., Bernstein B., Cimberg R., Kauwling T., Anikouchine W. (1986) *Assessment of long-term changes in biological communities in the Santa Maria Basin and Western Santa Barbara Channel. Phase I.* Final report submitted for the Minerals Management Service, U.S., Department of the Interior, Pacific OCS Office, Los Angeles, California.

Linnaeus C. (1758) *Systema Naturae, Tomus I, ed. 10.* Holmiae, Laurentii Salvii. 824 pp.

Litvaitis M.K., Curini-Galletti M.C., Martens P.M., Kocher T.D. (1996) A reappraisal of the systematics of the Monocelididae (Platyhelminthes, Proseriata): inferences from rDNA sequenze. *Molecular Phylogenetics and Evolution* 6, 150-156.

Lobo J., Teixeira M.A.L., Borges L.M.S., Ferreira M.S.G., Hollatz C., Gomes P.T., Sousa R., Ravara A., Costa M.H., Costa F.O. (2016) Starting a DNA barcode reference library

for shallow water polychaetes from the southern European Atlantic coast. *Molecular Ecology Resources* 16, 298-313.

López E. (2008) Redescription of *Aricidea belgicae* (Fauvel, 1936), an Antarctic Paraonidae (Annelida: Polychaeta), with reassessment of its taxonomic status based on new material. *Proceedings of the Biological Society of Washington* 121, 297-307.

López-Jamar E., O'Connor B., González G. (1987) Demography and gametogenic cycle of *Paradoneis armata* Glémarec (Polychaeta, Paraonidae) in Ría de la Coruña, North-West Spain. *Ophelia* 27, 127-136.

Lorenz T.C. (2012) Polymerase chain reaction: basic protocol plus troubleshooting and optimization strategies. *Journal of Visualized Experiments* 63, e3998.

Lovell L.L. (2002) Paraonidae (Annelida: Polychaeta) of the Andaman Sea, Thailand. *Phuket Marine Biological Center Special Publication* 24, 33-56.

Lücking R. (2008) Taxonomy: a discipline on the brink of extinction. *Archives de Sciences* 61, 75-88.

Luna G.M., Dell'Anno A., Corinaldesi C., Armeni M., Danovaro R. (2009a) Diversity and spatial distribution of metal reducing bacterial assemblages in groundwaters of different redox conditions. *International Microbiology* 12, 153-159.

Luna G.M., Stumm K., Pusceddu A., Danovaro R. (2009b) Archaeal diversity in deep-sea sediments estimated by means of different Termina-Restriction Fragment Length Polymorphism (T-RFLP) protocols. *Current Microbiology* 59, 356-361.

Luttikhuizen P.C., Dekker R. (2010) Pseudo-cryptic species *Arenicola defodiens* and *Arenicola marina* (Polychaeta: Arenicolidae) in Waddens Sea, North Sea and Skagerrak: morphological and molecular variation. *Journal of Sea Research* 63, 17-23.

Maciolek N.J. (1981) Spionidae (Annelida: Polychaeta) from the Galapagos Rift geothermal vents. *Proceedings of the Biological Society of Washington* 94, 826-837.

Mackie A.S.Y. (1991) *Paradoneis eliasoni* sp. nov. (Polychaeta: Paraonidae) from northern European waters, with a redescription of *Paradoneis lyra* (Southern, 1914). *Ophelia* S5, 147-155.

Maggiore F., Keppel E. (2007) Biodiversity and distribution of polychaetes and molluscs along the Dese estuary (Lagoon of Venice, Italy). *Hydrobiologia* 588, 189-203.

Mallet J. (2007) Species, concept of. In: *Encyclopedia of Biodiversity. Vol 5.* S. Levin et al. (Eds). p. 427-440. Academic Press.

Maltagliati F., Camilli L., Lardicci C., Castelli A. (2001) Evidence for morphological and genetic divergence in *Perinereis cultrifera* (Polychaeta: Nereididae) from two habitat

types at Elba Island. *Journal of the Marine Biological Association of the UK* 81, 411-414.

Maltagliati F., Casu M., Castelli A. (2004) Morphological and genetic evidence supports the existence of two species in the genus *Ophelia* (Annelida, Polychaeta) from the Western Mediterranean. *Biological Journal of the Linnean Society* 83, 101-113.

Maltagliati F., Casu M., Lai T., Iraci Sareri D., Casu D., Curini-Galletti M., Cantone G., Castelli A. (2005) Taxonomic distinction of *Ophelia barquii* and *O. bicornis* (Annelida, Polychaeta) in the Mediterranean as revealed by ISSR markers and the number of nephridiopores. *Journal of the Marine Biological Association of the U.K.* 85, 835-841.

Maltagliati F., Massaro L., Cossu P., Castelli A. (2006) Morphological differentiation in the ragworm, *Hediste diversicolor* (Polychaeta, Nereididae), as revealed by variation of paragnath number and distribution. *Italian Journal of Zoology* 73, 255-262.

Maltagliati F., Peru A.P., Casu M., Rossi F., Lardicci C., Curini-Galletti M., Castelli A. (2000) Is *Syllis gracilis* (Polychaeta: Syllidae) a species complex? An allozyme perspective. *Marine Biology* 136, 871-879.

Manchenko G.P., Radashevsky V.I. (1993) Genetic differences between two sibling species of the *Polydora ciliata* complex. *Biochemical Systematics and Ecology* 21, 543-548.

Martín D. (1987) La comunidad de Anélidos Poliquetos de las concreciones de algas calcáreas del litoral catalán. Caracterización de las especies. *Publicaciones del Departamiento de Zoologia de Barcelona* 13, 45-54.

Maurer D., Leathem W. (1980) Dominant species of polychaetous annelids of Georges Banks. *Marine Ecology Progress Series* 3, 135-144.

Maxmen A.B., King B.F., Cutler E.B., Giribet G. (2003) Evolutionary relationships within the protostome phylum Sipuncula: a molecular analysis of ribosomal genes and histone H3 sequence data. *Molecular Phylogenetics and Evolution* 27, 489-503.

Mayr E. (1942) *Systematics and the origin of species from the viewpoint of a zoologist*. Columbia University Press, 334 pp.

McHugh D. (2000) Molecular phylogeny of the Annelida. *Canadian Journal of Zoology* 78, 1873-1884.

McIntosh W.C. (1878) On the Annelida obtained during the Cruise of H.M.S. 'Valorous' to Davis Strait in 1875. *Transactions of the Linnean Society in London* 1, 499-511.

McLelland J.A., Gaston G.R. (1994) Two new species of *Cirrophorus* (Annelida, Polychaeta, Paraonidae) from the northern Gulf of Mexico. *Proceedings of the Biological Society of Washington* 107, 524-531.

Medlin L., Elwood H.J., Stickel S., Sogin M.L. (1988) The characterization of enzymatically amplified 16Slike rRNA-coding regions. *Gene* 71, 491-499

Meglitsch P.A. (1972) *Invertebrate Zoology*. Oxford University Press. 834 pp.

Melville R.V. (1979) Opinion 1139 *Paraonis* Grube, 1873 (Polychaeta, Paraonidae): Designation of a type species under the plenary power. *Bulletin of Zoological Nomenclature* 36, 114-118.

Mesnil F., Caullery M. (1898) Etudes de morphologie externe chez les annélides. IV. La famille nouvelle des Levinseniens. Révision des Ariciens. Affinités des deux familles. Les Apistobranchiens. *Bulletin scientifique de France et de Belgique* 31, 126-151.

Meyer A., Bleidorn C., Rouse G.W., Hausen H. (2008) Morphological and molecular data suggest a cosmopolitan distribution of the polychaete *Proscoloplos cygnochaetus* Day, 1954 (Annelida, Orbiniidae). *Marine Biology* 153, 879-889.

Monro C.C.A. (1930) Polychaete worms. *Discovery Reports, Cambridge* 2, 1-222.

Montesanto G. (2015) A fast GNU method to draw accurate scientific illustrations for taxonomy. *Zookeys* 515, 191-206.

Montiel A., Hilbig B. (2004) *Aricidea pisanoi* (Annelida: Polychaeta), a new species of Paraonidae from the southernmost waters of South America (Chile). *Journal of the Marine Biological Association of the U.K.* 84, 43-45.

Moura C.J., Harris D.J., Cunha M.R., Rogers A.D. (2008) DNA barcoding reveals cryptic diversity in marine hydroids (Cnidaria, Hydrozoa) from coastal and deep-sea environments. *Zoologica Scripta* 37, 93-108.

Musco L., Mikac B., Tataranni M., Giangrande A., Terlizzi A. (2011) The use of coarser taxonomy in the detection of long-term changes in polychaete assemblages. *Marine Environmental Research* 71, 131-138.

Narayanaswamy B.E., Bett B.J., Gage J.D. (2005) Ecology of bathyal polychaete fauna at an Arctic-Atlantic boundary (Faroe-Shetland Channel, North-east Atlantic). *Marine Biology Research* 1, 20-32.

Neal L., Wiklund H., Muir A.I., Linse K., Glover A.G. (2014) The identity of juvenile Polynoidae (Annelida) in the Southern Ocean revealed by DNA taxonomy, with notes on the status of *Herdmanella gracilis* Ehlers *sensu* Augener. *Memoirs of Museum Victoria* 71, 203-216.

Nikulina E.A., Hanel R., Schäfer P. (2007) Cryptic speciation and paraphyly in the cosmopolitan bryozoan *Electra pilosa* – impact of the Tethys closing on species evolution. *Molecular Phylogenetics and Evolution* 45, 765-776.

Norlinder E., Nygren A., Wiklund H., Pleijel F. (2012) Phylogeny of scale-worms (Aphroditiformia, Annelida) assessed from 18S rRNA, 28Ss rRNA, 16S rRNA, mitochondrial cytochrome c oxidase subunit I (COI), and morphology. *Molecular Phylogenetics and Evolution* 65, 490-500.

Nygren A. (2014) Cryptic polychaete diversity: a review. *Zoologica Scripta* 43, 172-183.

Nygren A., Eklöf J., Pleijel F. (2010) Cryptic species of *Notophyllum* (Polychaeta: Phyllodocidae) in Scandinavian waters. *Organisms Diversity & Evolution* 10, 193-204.

Nygren A., Pleijel F. (2011) From one to ten in a single stroke – resolving the European *Eumida sanguinea* (Phyllodocidae, Annelida) species complex. *Molecular Phylogenetics and Evolution* 58, 132-141.

O'Connor B.D.S., Dinneen P., Conneely M., Bowmer T. (1984) Notes on the Irish Paraonidae (Polychaeta) with records of two species new to Ireland. *Irish Naturalists' Journal* 21, 221-226.

Oliver M.J., Irwin A.J. (2008) Objective global ocean biogeographic provinces. *Geophysical Research Letters* 35, L15601.

Palumbi S.R. (1994) Genetic divergence, reproductive isolation, and marine speciation. *Annual Review in Ecology and Systematics* 25, 547-572.

Palumbi S.R., Martin A., Romano S., McMillan W .O., Stice L., Grabowski G. (1991) *The simple fool's guide to PCR, version 2.0.* Honolulu: Department of Zoology and Kewalo Marine Laboratory, University of Hawaii.

Pannacciulli F.G., Bishop J.D.D., Hawkins S.J. (1997) Genetic structure of populations of two species of *Chthamalus* (Crustacea: Cirripedia) in the north-east Atlantic and Mediterranean. *Marine Biology* 128, 73-82.

Parker T. (1996) Branching out for new species… or splitting hairs over split antennae. *SCAMIT Newsletter* 14 (10), 6-7.

Parmesan C. (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution and Systematics* 37, 637-669.

Payo D.A., Leliaert F., Verbruggen H., D'hondt S., Calumpong H.P., De Clerck O. (2013) Extensive cryptic species diversity and fine-scale endemism in the marine red alga

Portieria in the Philippines. *Proceedings of the Royal Society B: Biological Sciences* 280: 20122660.

Peek A.S., Gustafson R.G., Lutz R.A., Vrijenhoek R.C. (1997) Evolutionary relationships of deep-sea hydrothermal vent and cold-water seep clams (Bivalvia: Vesicomyidae): results from the mitochondrial cytochrome oxidase subunit I. *Marine Biology* 130, 151-161.

Pérès J.M., Picard J. (1964) Nouveau manuel de bionomie benthique. *Recueil des Travaux de la Station marine d'Endoume* 31, 5-137.

Pérez-Losada M., Guerra A., Carvalho G.R., Sanjuan A., Shaw P.W. (2002) Extensive population subdivision of the cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda) around the Iberian Peninsula indicated by microsatellite DNA variation. *Heredity* 89, 417-424.

Pérez-Portela R., Arranz V., Rius M., Turon X. (2013) Cryptic speciation or global spread? The case of a cosmopolitan marine invertebrate with limited dispersal capabilities. *Scientific Reports* 3, 3197.

Petroni G., Dini F., Verni F., Rosati G. (2002) A molecular approach to the tangled intrageneric relationships underlying phylogeny in *Euplotes* (Ciliophora, Spirotrichea). *Molecular Phylogenetics and Evolution* 22, 118-130.

Pettibone M.H. (1957) A new polychaetous annelid of the family Paraonidae from the North Atlantic. *Journal of the Washington Academy of Sciences* 47, 354-356.

Pettibone M.H. (1963) Marine polychaete worms of the New England region. I. Aphroditidae through Trochochaetidae. *Bulletin of the United States National Museum* 227, 1-356.

Pettibone M.H. (1965) Two new species of *Aricidea* (Polychaeta, Paraonidae) from Virginia and Florida, and redescription of *Aricidea fragilis* Webster. *Proceedings of the Biological Society of Washington* 78, 127-140.

Petraccioli A., Guarino F.M., Maio N., Odierna G. (2010) Molecular cytogenetic study of three common Mediterranean limpets, *Patella caerulea*, *P. rustica* and *P. ulyssiponensis* (Archaeogastropoda, Mollusca). *Genetica* 138, 219-225.

Pianka E.R. (1966) Latitudinal gradients in species diversity: a review of concepts. *The American Naturalist* 100, 33-46.

Pleijel F., Rouse G., Nygren A. (2009) Five colour morphs and three new species of *Gyptis* (Hesionidae, Annelida) under a jetty in Edithburg, South Australia. *Zoologica Scripta* 38, 89-99.

Puillandre N., Lambert A., Brouillet S., Achaz G. (2012) ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology* 21, 1864-1877.

Puniamoorthy N., Su K.F.-Y., Meier R. (2008) Bending for love: losses and gains of sexual dimorphisms are strictly correlated with changes in the mounting position of sepsid flies (Sepsidae: Diptera). *BMC Evolutionary Biology* 8, 155.

Quintas P., Cacabelos E., Troncoso J.S. (2013) Spatial distribution of soft-bottom polychaetes in seagrass beds of the Ensenada de O Grove (NW Spain). *Thalassas* 29, 45-58.

Quiroz-Martinez B., Schmitt F.G., Dauvin J.-C. (2012) Statistical analysis of polychaete population density: dynamics of dominant species and scaling properties in relative abundance fluctuations. *Nonlinear Processes in Geophysics* 19, 45-52.

Rainey P.B., Travisano M. (1998) Adaptive radiation in a heterogeneous environment. *Nature* 394, 69-72.

Rasmussen E. (1973) Systematics and ecology of the Isefjord marine fauna (Denmark). *Ophelia* 11, 1-507.

Ravara A., Moreira M.H. (2013) Polychaeta (Annelida) from the continental shelf off Aveiro (NW Portugal): Species composition and community structure. *Check List* 9, 533-539.

Reuscher M.G. (2013) Cladistic analysis of the family Paraonidae (Annelida: Polychaeta) and the genera *Cirrophorus* and *Paradoneis* based on morphological characters. In: Reuscher M.G. *Biodiversity, ecology, and natural history of polychaetous annelids from the Gulf of Mexico.* PhD thesis, Corpus Christi University, Corpus Christi, USA, pp. 192–237.

Rice W.R., Hostert E.E. (1993) Laboratory experiments on speciation: what have we learned in 40 years? *Evolution* 47, 1637-1653.

Riedl R. (1983) *Fauna und Flora des Mittelmeeres*. Parey Verlag, Berlin u. Hamburg, 836 pp.

Riera R., Núñez J., Brito M.C. (2010) Check-list of interstitial polychaetes from intertidal and shallow subtidal soft bottoms of Tenerife, Canary Islands. *Arquipelago. Life and Marine Science* 27, 21-39.

Röder H. (1971) Gangsysteme von *Paraonis fulgens* Levinsen 1883 (Polychaeta) in ökologischer, ethologischer und aktuopaläontologischer Sicht. *Senckenbergiana maritima* 3, 3-51.

Röhner M., Bastrop R., Jürss K. (1996) Colonization of Europe by two American genetic types or species of the genus *Marenzelleria* (Polychaeta: Spionidae). An electrophoretic analysis of allozymes. *Marine Biology* 127, 277-287.

Röhner M., Bastrop R., Jürss K. (1997) Genetic differentiation of *Hediste diversicolor* (Polychaeta: Nereididae) for the North Sea and the Baltic Sea. *Marine Biology* 130, 171-180.

Ronquist F., Teslenko M., van der Mark P., Ayres D., Darling A., Höhna S., Larget B., Liu L., Suchard M.A., Huelsenbeck J.P. (2011) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61, 539-542.

Rossi F., Lardicci C. (1995) Evoluzione delle comunità zoobentoniche di fondo molle della Laguna di Orbetello: variazioni nella composizione specifica. *Atti della Società Toscana di Scienze Naturali, Memorie, Ser. B* 102, 31-36.

Rouse G.W. (2001) A cladistics analysis of Siboglinidae Caullery, 1914 (Polychaeta, Annelida): formerly the phyla Pogonophora and Vestimentifera. *Zoological Journal of the Linnean Society* 132, 55-80.

Rouse G.W., Fauchald K. (1997) Cladistics and polychaetes. *Zoologica Scripta* 26, 139-204.

Rouse G.W., Pleijel F. (2001) *Polychaetes.* Oxford University Press, 384 pp.

Rousset V., Pleijel F., Rouse G.W., Erséus C., Siddall M.E. (2006) A molecular phylogeny of annelids. *Cladistics* 22, 1-23.

Saéz A.G., Probert I., Geisen M., Quinn P., Young J.R., Medlin L.K. (2003) Pseudo-cryptic speciation in coccolithophores. *Proceedings of the National Academy of Sciences* 100, 7163-7168.

Sanna D., Biagi F., Alaya H.B., Maltagliati F., Addis A., Romero A., De Juan J., Quignard J.-P., Castelli A., Franzoi P., Torricelli P., Casu M., Carcupino M., Francalacci P. (2013) Mitochondrial variability of the pipefish *Syngnathus abaster*. *Journal of Fish Biology* 82, 856-876.

Sanna D., Dedola G.L., Lai T., Curini-Galletti M., Casu M. (2011) PCR-RFLP: a practical method for the identification of specimens of *Patella ulyssiponensis s.l.* (Gastropoda: Patellidae). *Italian Journal of Zoology* 79, 50-59.

Sá-Pinto A., Branco M.S., Alexandrino P.B., Fontaine M.C., Baird S.J.E. (2012) Barriers to gene flow in the marine environment: insight from two common intertidal limpet species of the Atlantic and Mediterranean. *PLoS One* 7, e50330.

Sardá R. (1984) Nuevas citas de Annélidos Poliquetos para la peninsula Iberica. *Publicaciones del Departamento de Zoologia de Barcelona* 9, 13-21.

Sardá R., Gil J., Taboada S., Gili J.M. (2009) Polychaete species captured in sediment traps moored in northwestern Mediterranean submarine canyons. *Zoological Journal of the Linnean Society* 155, 1-21.

Sato M., Masuda Y. (1997) Genetic differentiation in two sibling species of the brackish-water polychaete *Hediste japonica* complex (Nereididae). *Marine Biology* 130, 163-170.

SCAMIT (2002) *SCAMIT Newsletter* 20, 11.

Schirosi R., Musco L., Giangrande A. (2010) Benthic assemblage of Acquatina Lake (South Adriatic Sea): present state and long-term faunistic changes. *Scientia Marina* 74, 235-246.

Schlick-Steiner B.C., Steiner F.M., Seifert B., Stauffer C., Christian E., Crozier R.H. (2010) Integrative taxonomy: a multisource approach to exploring biodiversity. *Annual Review of Entomology* 55, 421-438.

Schulze A., Cutler E.B., Giribet G. (2005) Reconstructing the phylogeny of the Sipuncula. *Hydrobiologia* 535/536, 277-296.

Shank T.S. (2010) Seamounts: deep-ocean laboratories of faunal connectivity, evolution, and endemism. *Oceanography* 23, 109-122.

Sibuet M., Olu K. (1998) Biogeography, biodiversity and fluid dependence of deep-esa cold-seep communities at active and passive margins. *Deep Sea Research Part II Topical Studies in Oceanography* 45, 517-557.

Siddall M.E., Apakupakul K., Burreson E.M., Coates K.A., Erséus C., Gelder S.R., Källersjö M., Trapido-Rosenthal H. (2001) Validating Livanow: molecular data agree that leeches, branchiobdellidans, and *Acanthobdella peledina* form a monophyletic group of oligochaetes. *Molecular Phylogenetics and Evolution* 21, 346-351.

Simboura N., Reizopoulou S., Arvanitidis C., Basset A. (2007) New information on the biogeographical and ecological distribution of *Pionosyllis anophthalma* Capaccioni & San Martín, 1989 (Polychaeta, Syllidae). *Transitional Waters Bulletin* 4, 53-58.

Simboura N., Zenetos A. (2005) Increasing polychaete diversity as a consequence of increasing research effort in Greek waters: new records and exotic species. *Mediterranean Marine Science* 6, 75-88.

Simonini R., Ansaloni I., Bonini P., Grandi V., Graziosi F., Iotti M., Massamba N'Siala G., Mauri M., Montanari G., Preti M., De Nigris N., Prevedelli D. (2007) Recolonization and recovery dynamics of the macrozoobenthos after sand extraction in relict sand bottoms of the Northern Adriatic Sea. *Marine Environmental Research* 64, 574-589.

Singer G.A., Hajibabaei M. (2009) iBarcode.org: web-based molecular biodiversity analysis. *BMC Bioinformatics* 10, S14

Sjölin E., Erséus C., Källersjö M. (2005) Phylogeny of Tubificidae (Annelida, Clitellata) based on mitochondrial and nuclear sequence data. *Molecular Phylogenetics and Evolution* 35, 431-441.

Sonneborn T.M. (1939) *Paramecium aurelia*: mating types and groups; lethal interactions; determination and inheritance. *The American Naturalist* 73, 390-413.

Southern R. (1914) Clare Island Survey. Archiannelida and Polychaeta. *Proceedings of the Royal Irish Academy* 31, 1-160.

Southward E.C. (1956) On some Polychaeta of the Isle of Man. *Annals and Magazine of Natural History* 9, 257-270.

Sponer R., Roy M.S. (2002) Phylogeographic analysis of the brooding brittle star *Amphipholis squamata* (Echinodermata) along the coast of New Zealand reveals high cryptic genetic variation and cryptic dispersal potential. *Evolution* 56, 1954-1967.

Steinhauer M., Imamura E. (1990) *California OCS Phase II Monitoring Program, Year-three Annual Report.* U.S. Department of the Interior, Minerals Management Service, Pacific OCS Office, Los Angeles, California.

Steyskal G.C. (1972) The meaning of the term "sibling species". *Systematics Zoology* 21, 446.

Storch V. (1967) Neue Polychaeten aus der Sandfauna des Roten Meeres. *Zoologischer Anzeiger* 178, 102-110.

Strelzov V.E. (1968) [Polychaete worms of the family Paraonidae (Polychaeta, Sedentaria) from the Barents Sea]. *Trudy Murmansk Marine Biological Institute, Akademia Nauk SSSR* 17, 74–95

Strelzov V.E. (1973) [*Polychaete worms of the family Paraonidae Cerruti, 1909 (Polychaeta, Sedentaria)*]. Akademia Nauk. Moscow, 170 pp.

Struck T.H., Golombek A., Weigert A., Franke F.A., Westheide W., Purschke G., Bleidorn C., Halanych K.M. (2015) The evolution of annelids reveals two adaptive routes to the interstitial realm. *Current Biology* 25, 1993-1999.

Struck T.H., Paul C., Hill N., Hartmann S., Hösel C., Kube M., Lieb B., Meyer A., Tiedemann R., Purschke G., Bleidorn C. (2011) Phylogenomic analyses unravel annelid evolution. *Nature* 471, 95-98.

Struck T.H., Schult N., Kusen T., Hickamn E., Bleidorn C., McHugh D. & Halanych K.M. (2007) Annelid phylogeny and the status of Sipuncula and Echiura. *BMC Evolutionary Biology* 7, 57.

Taberlet P., Coissac E., Pompanon F., Brochmann C., Willerslev E. (2012) Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology* 21, 2045-2050.

Tahseen Q. (2014) Taxonomy – the crucial yet misunderstood and disregarded tool for studying biodiversity. *Journal of Biodiversity & Endangered Species* 2, 128.

Tataranni M., Maltagliati F., Floris A., Castelli A., Lardicci C. (2009) Variance estimate and taxonomic resolution: an analysis of macrobenthic spatial patterns at different scales in a Western Mediterranean coastal lagoon. *Marine Environmental Research* 67, 219-229.

Tatarenkov A., Johannesson K. (1999) Micro- and macrogeographic allozyme variation in *Littorina fabalis*: do sheltered and exposed forms hybridize? *Biological Journal of the Linnean Society* 67, 199-212.

Tauber P. (1879) *Annulata Danica. En kritisk revision af de in Danmark fundne Annulata Chaetognatha, Gephyrea, Balanoglossi, Discophoreae, Oligochaeta, Gymnocopa og Polychaeta.* Reitzel, Kobenhavn, 143 pp.

Taugbøl A., Junge C., Quinn T.P., Herland A., Vøllestad L.A. (2014) Genetic and morphometric divergence in threespine stickleback in the Chignik catchment, Alaska. *Ecology and Evolution* 4, 144-156.

Tavaré S. (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* 17, 57-86.

Tebble N.(1959) On a collection of Polychaetes from the Mediterranean coast of Israel. *Bulletin of the Research Council of Israel* B8, 9-30.

Terlizzi A., Bevilacqua S., Fraschetti S., Boero F. (2003) Taxonomic sufficiency and the increasing insufficiency of taxonomic expertise. *Marine Pollution Bulletin* 46, 556-561.

Thomsen P.F., Willerslev E. (2015) Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation* 183, 4-18.

Thorson G. (1946) Reproduction and larval development of Danish marine bottom invertebrates with special reference to the planktonic larvae in the Sound (Øresund). *Medd. Komm. Danmarks Fisk. Havundersøgelser, Ser: Plankton* 4, 1-523.

Tomioka S., Kajihara H. (2016) Phylogenetic study of the family Capitellidae (Annelida) in Japan. *Oral communication at the 12th International Polychaete Conference, 1-5 August 2016, Cardiff, Wales, the U.K.*

Valdés A. (2004) Phylogeography and phyloecology of dorid nudibranchs (Mollusca, Gastropoda). *Biological Journal of the Linnean Society* 83, 551-559.

Valentine J.M., Jablonski D. (1983) Speciation in the shallow sea: general patterns and biogeographic control. In: *Evolution, time and space: the emergence of the biosphere.* R.W. Sims, J.H. Price, P.E.S. Whalley (Eds.), pp. 201-226. Academic Press, London and New York.

Virgilio M., Abbiati M. (2004) Habitat discontinuity and genetic structure in populations of the estuarine species *Hediste diversicolor* (Polychaeta: Nereididae). *Estuarine, Coastal and Shelf Science* 61, 361-367.

Wake D.B., Wake M.H., Specht C.D. (2011) Homoplasy: from detecting pattern to determining process and mechanism of evolution. *Science* 331, 1032-1035.

Watling L., Guinotte J., Clark M.R., Smith C.R. (2013) A proposed biogeography of the deep ocean floor. *Progress in Oceanography* 111, 91-112.

Webster H.E. (1879) The Annelida Chaetopoda of the Virginian Coast. *Transactions of the Albany Institute* 9, 202-269.

Webster H.E., Benedict J.E. (1887) The Annelida Chaetopoda from Eastport, Maine. *U.S. Commission of Fish & Fisheries. Report of the United States Commissioner of Fisheries. 1885 part 13, II. Appendix to report of commissioner* 22, 707-758.

Wiklund H., Nygren A., Pleijel F., Sundberg P. (2005) Phylogeny of Aphroditiformia (Polychaeta) based on molecular and morphological data. *Molecular Phylogenetics and Evoluton* 37, 494-502.

Wiklund H., Nygren A., Pleijel F., Sundberg P. (2008) The phylogenetic relationships between Amphinomidae, Archinomidae and Euphrosinidae (Amphinomida: Aciculata: Polychaeta), inferred from molecular data. *Journal of the Marine Biological Association of the U.K.* 88, 509-513.

Wilkinson D.M. (2001) What is the upper size limit for cosmopolitan distribution in free-living microorganisms? *Journal of Biogeography* 28, 285-291.

Wilson G.D.F., Hessler R.R. (1987) Speciation in the deep sea. *Annual Review of Ecology and Systematics* 18, 185-207.

Winston J. (1999) *Describing species: practical taxonomic procedures for biologists.* Columbia University Press, 517 pp.

Wu B., Qian P.-Y., Zhang S. (1991) Morphology, reproduction, ecology and allozyme electrophoresis of three *Capitella* sibling species in Qingdao (Polychaeta: Capitellidae). *Ophelia* S5, 391-400.

Xia X., Xie Z., Salemi M., Chen L., Wang Y. (2003) An index of substitution saturation and its application. *Molecular Phylogenetics and Evolution* 26, 1-7.

Yu D.W., Ji Y., Emerson B.C., Wang X., Ye C., Yang C., Ding Z. (2012) Biodiversity soup: metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring. *Methods in Ecology and Evolution* 3, 613-623.

Zaâbi S., Gillet P., Chambers S., Afli A., Boumaiza M. (2012) Inventory and new records of polychaete species from the Cap Bon Peninsula, north-east coast of Tunisia, Western Mediterranean Sea. *Mediterranean Marine Science* 13, 36-48.

Zanol J., Halanych K.M. & Fauchald K. (2014) Reconciling taxonomy and phylogeny in the bristleworm family Eunicidae (polychaete, Annelida). *Zoologica Scripta* 43, 79-100

Zhang J., Kapli P., Pavlidis P., Stamatakis A. (2013) A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* 29, 2869-2876.

Zhou J., Li X.Z. (2007) A report of the family Paraonidae (Annelida, Polychaeta) from China seas. *Acta Zootaxonomica Sinica* 32, 275-282.

Zhou J., Reuscher M.G. (2013) Description of a new species of *Aricidea* (Polychaeta: Paraonidae) from Chinese Seas. *Proceedings of the Biological Society of Washington* 126, 276-283.

Zrzavý J., Hypša V., Tietz D.F. (2001) Myzostomida are not annelids: molecular and morphological support for a clade of animals with anterior sperm flagella. *Cladistics* 17, 170-198.

9. Acknowledgements

A PhD project is such a hard and demanding task that enthusiasm, interest, technical and theoretical skills of a single person are not enough to comply it. This work would not have been possible without the precious contribution of a number of people, of which a large part was directly involved in the research, a minor part did not actively participate, but was essential in maintaining my mental health status within acceptable levels.

First of all I would like to express my gratitude to my tutor, Prof. Alberto Castelli, who invested on this PhD project and on my education as marine biologist, showing an infectious interest not only to this specific research project, but also on eventual side-quest about polychaete biology, and always encouraged me to investigate, question, compare literature and experimental data – in a single word, to research. An essential contribution came from Dr. Ferruccio Maltagliati, who first introduced me to the use of molecular tools in ecology, and then in these last three years carefully followed the development of the project. I am unspeakably grateful to Dr. Michele Barbieri, who helped me in handling molecular techniques, cheering me up in the most difficult situations through his witty humour and an overwhelming supply of spicy chili pepper. Since practical laboratory work has never been one of my best skills, I am particularly grateful to Tainara Ravaglia and Federica Squarcia, whose skilful help substantially contributed to the final results. Dr. Gipo Montesanto introduced me in the art of scientific drawing; without his kind help, the vast majority of the figures in this thesis would not be there.

A pleasant work environment is crucial for any kind of work, and people is the factor that mostly influences this aspect. I owe these three fruitful years of work also to Prof. Lisandro Benedetti Cecchi, Prof. Fabio Bulleri and Prof. Claudio Lardicci, as well as to Dr. Elena Balestri, Dr. Martina Dal Bello, Dr. Elena Maggi, Dr. Chiara Ravaglioli, Dr. Luca Rindi, Dr. Laura Tamburello, and Dr. Marc Uyá Yrayzoz, who were always available for a cup of coffee, a glass of wine or a short chat, that often developed in unexpected directions, leading to a substantial increase in my knowledge of ecology. I am also greatly indebted to the students I had the opportunity to follow in their bachelor or master thesis work: in addition to the abovementioned T. Ravaglia and F. Squarcia, I want to mention Costanza Bonomo, Jure dell'Omodarme, Marco Fattorini, Giada Fogli, Luciano Gintoli, Nicoletta Magrini, Angelo Satta, Martino Severini, Giulio Stefanelli, and Jonathan Tempesti. I hope that my contribution did not significantly damage their biological education.

The collection of samples of Paraonidae needed a substantial travel effort, and the logistic support of several colleagues and research units. I am greatly indebted to Nicola Bigongiari,

Anna Maria Di Biasi, Matteo Oliva, Lorenzo Pacciardi, Marco Pertusati, Carlo Pretti and Andrea Vannucci (CIBM, Livorno, Italy), Marco Casu, Piero Cossu, Marco Curini-Galletti, Tiziana Lai, Daria Sanna, Fabio Scarpa (University of Sassari, Sassari, Italy), Laia Planella and Marina Roldán (University of Girona, Girona, Spain), Lucrezia Cilenti, Raffaele D'Adamo, Sergio Pelosi, Angela Santucci and Tommaso Scirocco (CNR – ISMAR Lesina, Italy), Irene Guarneri, Marco Sigovini and Davide Tagliapietra (CNR – ISMAR Venezia, Italy), Demetris Kletou, Periklis Kleitou and Maria Rousou (MER Laboratory, Lemesos, Cyprus). A great amount of valuable material, without which this project would not have been possible, was obtained thank to Stefano Acunto, Fabio Bulleri, Enrica Pollonara and Chiara Ravaglioli (University of Pisa, Pisa, Italy), Gioia Benedettini, Ornella Bresciani, Marco Lezzi and Annamaria Pavia (ARPAT, Pisa, Italy), Teresa Darbyshire and Andy Mackie (National Museum of Wales, Cardiff, Wales, the U.K.), Florencio Aguirrezabalaga (University of the Basque Country, Bilbao, Spain), Harim Cha and Greg Rouse (Scripps Institution of Oceanography, La Jolla, California, the U.S.A.), Cristina Mazziotti (ARPAE, Cesenatico, Italy), Katerina Vasileiadou (IMBBC, HCMR, Thalassokosmos, Crete, Greece), Melih Ertan Çinar and Deniz Erdoğan (Ege University, Izmir, Turkey), Brian Beal and Cody Jourdet (University of Maine, Machias, Maine, the U.S.A.),Vassily Radashevsky and Vitaly Syomin (Russian Academy of Sciences, Moscow, Russian Federation), and Larry Lovell (Natural History Museum of Los Angeles, California, the U.S.A.).

An important help in understanding biology, taxonomy and ecology of Paraonidae came from several polychaete biologists. I am greatly indebted to Florencio Aguirrezabalaga, James A. Blake, Melih Ertan Çinar, Larry Lovell, Andy Mackie, Vassily Radashevsky, Natalia Ranauro, Michael Reuscher and Maria Rousou for their sharing of hypotheses, data and opinions about this interesting group of polychaete.

I thank my family for their continuous support, even in the most difficult moments. My mother Erika, my late father Klaus and my sister Sophie, my grandmother Laura, my aunt Alessandra, my uncle Peppino and my cousins Camilla and Matilde. I must mention also my adoptive families, family Andreozzi-Del Bianco (Enrico, Gloria, Teresa, Francesco and Giuliana), family Bonucci-Renzi (Angelo, Silvia, Tommaso and Matilde), and family Coatto-Bouchard (Daniele, Laura, Francesco, Marta and Giona), from whom I scrounged innumerable meals, with whom I passed a number of pleasant evenings and a number of important events, and who definitely made this three years in Pisa less lonesome and difficult than I would have thought.

Last but not least, I would like to thank my friends (in rigorous alphabetical order) Alberto, Aline, Annapaola, Antonio, Azzurra, Chiara, Elena, Eleonora, Erica, Esther, Fanny, Francesca, Giulio, Luca, Martina, Massimiliano, Maurizio, Niccolò, Pietro, Silvia, Stefania, and Stefano. Everyone of you knows why our relationship has been important, and since it does not regard science, I will keep it out from this thesis. I will just say that there is more in life than science, and thank to you these three years have been full of sense from every point of view.