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Lampriformes in the Mediterranean Sea: distribution and life-history traits with whole genome sequencing of *Zu cristatus* (Bonelli, 1819)

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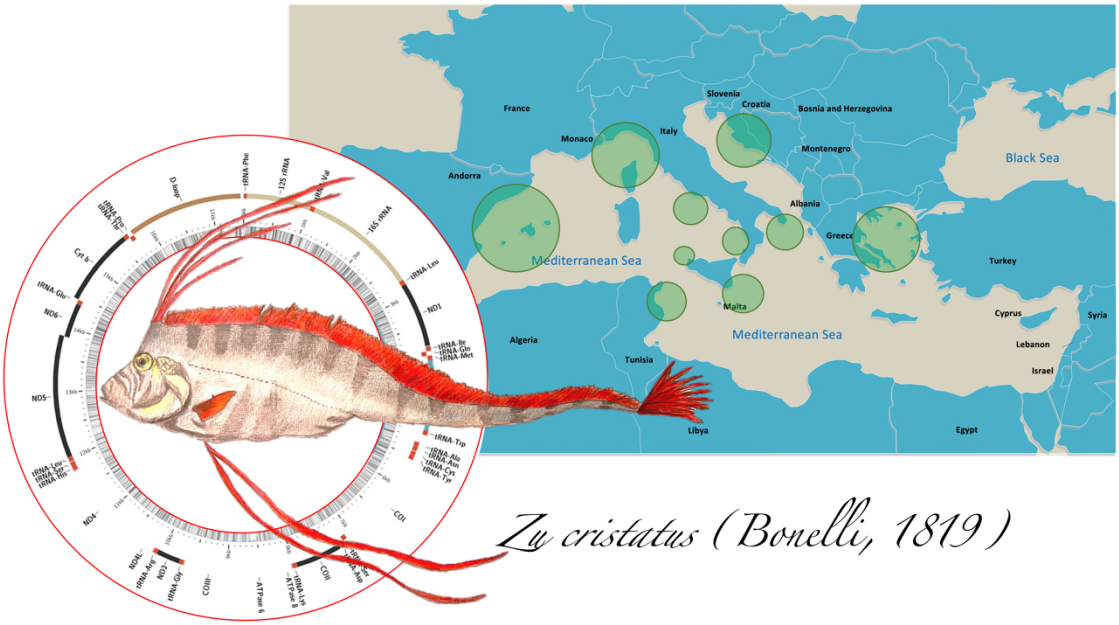
Graphical Abstract



Zu cristatus (Bonelli, 1819)

Illustration credit to Prof. Serena Savoca

Abstract

The biodiversity of Mediterranean fish has been continuously evolving in the last decades because of non-indigenous species' invasions and the influences of global climate change. The distribution of teleost fishes is of fundamental importance to comprehending how these species contribute to the ecological equilibria of marine areas, especially in a semi-closed basin such as the Mediterranean Sea. Moreover, due to their biological features, several rare fish species are challenging to detect and identify during commercial fishing activities, and both morphological and genetic data on their regard are poor. Molecular approaches have recently provided several new insights into phylogeny classifications and genetics, but a broader database is required to boost these research fields. The fishes of the order Lampriformes are circumglobally distributed and characterized by a peculiar morphology, but the information on this fish group is scarce to absent due to their rarity.

Here we report the occurrence in the Ionian Sea of *Zu cristatus* (Bonelli, 1819), a mesopelagic species from the order Lampriformes considered rare in the Mediterranean basin. The specimen was captured off the coast of Noto (Sicily, Italy) by deep-sea longline fisheries at a depth of 720 m. The sample was transported to the University of Messina laboratories for morphological identification and in-depth morphometric and meristic data analysis. The whole genome sequencing of the species was carried out on Illumina and Nanopore platforms in collaboration with the Nord University of Bodø, Norway. The final draft shows 82% completeness (BUSCO) compared to the actinopterygian genes database.

The first complete mitogenome for the species was isolated and annotated on the GenBank database (Acc. PRJNA845808), and an extensive analysis of its features reveals exciting insights. Phylogenetic reconstruction of the order Lampriformes base of the *mt-co1* sequences was performed; moreover, the phylogenetics relationships with other related orders were evaluated based on the entire mtDNA structure. An investigation of the opsin-like gene products found in the *Z. cristatus* genome draft was performed, with a reconstruction of the five sub-families. Identity comparisons and alignment analysis reveal

similarities and discordances with some teleost fish species from different orders, mainly related to different habitats. Considering the difficulty of collecting data on this rare taxon, this contribution can help improve the knowledge of this species' distribution, genetic structure, and features. Further analysis will lead to the complete annotation of the whole genome of the species, a milestone that allows future studies on morpho-physiological and genetic adaptation to the geographical and bathymetrical distributions of this species.

Nasciamo soli e unici,

moriamo soli come chiunque altro.

La vita è un lungo confronto con sé stessi e con il mondo per cercare di non perdere la propria unicità, e solo le figure il cui educativo riflesso alberga saldamente ancorato dentro di noi sono capaci di farci ritrovare la strada di casa, anche in mezzo al deserto.

Ad Anna e Sebastiano, sorgente di ogni mia capacità.

Summary

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At the end of my Ph.D. path, I want to reserve a reflection on my choice to leave a good permanent position for something temporary but much more rewarding on a personal level. Not being satisfied sometimes it can be trap, however, it must never be missing in the life of those who want to make scientific research their work. It was the right choice.

Next up...

INTRODUCTION

The order Lampriformes is a globally distributed taxon containing several families of strictly marine bony fishes with a peculiar morphology [1,2]. Despite their wide distribution, they are not commonly reported due to their rarity and the life cycle that causes their elusiveness [3,4]. The scarcity of reports in the literature results in little information regarding their presence and distribution. Indeed, even if biologically interesting species, they represent a fishing waste from a commercial point of view. Therefore, they are not retained and reported, leading to a huge gap in data collection [5]. Their morphological aspects led to characteristic common names of these species (ribbonfish, velifers, tube-eye, crestfish, oarfish, dealfish, tapertails, unicornfish, and inkfish). Due to the distinctive shape of most of them, jointly with their iridescent colors, these fishes were historically involved in several legends and popular myths [4]. Moreover, the larval stages of these species are often very different from the adult ones, both in morphology and lifestyle aspects [6]. All these factors contribute to promoting the interest of marine biology researchers and public curiosity.

The research interest in this order is linked to the ecological role of lampriform species involved in many bathypelagic food webs interactions. Moreover, during evolution, deep-sea species have developed highly specialized features to inhabit deep-sea environments, such as the maintenance of a vertical position in the water column during predation reported also for some Lampriformes species [7,8]. This feature might help these fish in the visualization of prey against downwelling light, and, at the same time, allow these elongated-shaped fish in minimize their appearance [9]. However, the study related to adaptations of Lampriformes species to deep-sea life are very scarce and, in considerations of their morphological features, needs further in-depth investigations, especially from a functional and genetics points of view [10,11]. Studying the adaptation to deep-sea life is essential both from an anatomical and physiological point of view in relation to their ecological features [12]. Moreover, this group is quite important from a phylogenetic point of view due to their ancient origins among teleost [13,14].

Lampriformes systematics is affected by the limitation in biometric, meristic, and molecular data and, for this reason, passed through several rearrangements during the past years. Some researchers have included other deep-sea fish families (e.g., Ateleopodidae, Megalomycteridae, Mirapinnidae, and Stylephoridae) based on the morphological identifications of the group. Still, the recent phylogenetic analysis, also based on molecular approaches, excluded these additional families that are currently considered unrelated [15,16]. However, this aspect is under continuous debate, and not many phylogenetic studies of species-level relationships were carried out on Lampriformes. Hence, to better assess the phylogenetic relationship of the group, more in-depth molecular data on families, genera, and species-level are required.

Environmental evolution in global marine ecosystems leads to the continuous rearrangement of faunal stands [17]. In recent years, it is known that global climate changes have contributed modifying several important environmental parameters of the aquatic ecosystems, such as salinity, temperature, and pH (for the strong influence on dissolved CO_2) [18,19]. Currently, the scientific community is working on understanding how these changes could affect aquatic organisms by trying to perform further predictive models [20,21]. The influences on marine faunal assemblages are various and not only attributable to climate change. It is known that reproductive behaviors, habitat preferences, feeding behaviors, genetic adaptation, and especially anthropic pressure (with its double impacts of fishing and pollution) [22,23], lead to complex biocenotics dynamics. These cannot be explained without intersecting all these factors on the species. Biological monitoring assumes a pivotal role with records of previously undiscovered species, new records of invasive alien or uncommon species, rarely reported ones [24–26].

Summarizing how pelagic organisms such as many teleost are distributed is essential to understanding how they face environmental modifications or biological interactions. Indeed, phenomena such as areal rearrangement can lead the species to different fates, depending mainly on their ecological plasticity [27]. Fishes have two different ways of dispersing in the marine environment, geographical and bathymetrical [28,29]. Especially the bathymetrical ones require specific adaptations by the organisms to be realized, which

mainly involve the sense organs and other structures. Indeed, moving from shallow to deep environments, fishes encounter high illumination differences that drive them to visual adaptations [9,30]. The increase in depth also influences structural hearing adaptations in fish that usually live under increased hydrostatic pressure [31]. Not secondary are the modifications that occur in several species due to altered pressure in the gills [32], and the muscles [33,34], or responding to the oxygen limitation [35,36].

The Mediterranean Sea covers about 2,000,000 km^2 on the European and the African continents [23]. It has a western communication with the Atlantic Ocean via the Strait of Gibraltar and an eastern one with the Black Sea via the Dardanelles and the Bosphorus [37]. The recorded maximum depth of the basin was 5,093 m [38], while the average depth is about 1,500 m, but due to its fragmented nature, significant differences exist [26,39]. Indeed, the Mediterranean Sea is divided into several subbasins that are variously connected and separated from them by different geological elements such as thresholds, peninsulas, or islands, of which the basin is rich [40,41].

The trophic relationships in marine ecosystems have been well investigated, studying their complex dynamics [42]. Considering the complexity that involves all the marine compartments, understanding the various ecological relations, which exist among these different habitats and the organisms is essential, especially the trophic ones [43]. In recent years, the increased sources of anthropogenic pressures on marine ecosystems, such as overfishing, pollution, and habitat degradation, lead to reassemblies in species composition with implications also on food webs at various levels [44]. Consequently, the ecological equilibria of the marine ecosystem have changed, following the different contributions of species to ecological dynamics [45]. Indeed, human activities in coastal and open sea areas have strongly increased in the last centuries, leading to the worst effect on the marine environment caused by various kinds of pollution, eutrophication, and excessive fishing pressure on natural stocks [46].

Moreover, the effects of global climate change on the Mediterranean Sea marine ecosystem, lead to changes in the complex dynamics of this semi-enclosed and fragmented basin, such as primary production, species distribution, and migrations, threatening to its biodiversity

[47,48]. Indeed, in recent years, these new dynamics have led to an increase in alien species in the whole basin, mainly from the Red Sea, and more in general an expansion of thermophilic species from the southern to the northern areas of the Mediterranean Sea basin [49,50].

Commonly, fishing activities focus on top predators, as these represent an excellent commercial resource in terms of size and quality (e.g., swordfish and tunas). These species are often overexploited or at risk of extinction, because characterized by low reproductive performance, slow growth, and long generation times [51,52]. Consequently, population declines for large pelagic fish are occurring worldwide [53]. In these regards, monitoring the status of key species is essential, also for establishing regulation services in different marine geographical areas [54]. Indeed, a reduction in top-predator activity negatively reflects on the dynamics of the entire marine ecosystems, influencing the equilibria of lower trophic levels of trophic webs [55]. Nonetheless, several fishing gears are very often non-selective systems affecting target and non-target species, thus having direct and indirect impacts on the ecological dynamics [56,57]. Particularly, the by-catch of bottom trawlers and longline fisheries affect many ecologically important meso- and bathypelagic species [58,59]; hence, also if they are not commercially important, these species populations suffer these indirect fishing impacts [5,60].

Despite their abundance, mesopelagic fishes remain among the less investigated groups of the marine ecosystem, with several aspects of their biology and adaptations still unknown. Trawling data estimates suggest that the global biomass of these fishes is about 1,000 million tons [61], but, due to the efficiency of deep waters fishing gear, it is highly probable that this data is underestimated [62,63]. Indeed, these species inhabit the water column from the surface to 1000m, also being part of one of the most characteristic and ecologically essential organisms' aggregations of marine ecosystems, the deep scattering layer [64,65].

For example, worldwide distributed orders such as Mictophiformes and Stomiiformes, which are highly represented in this assemblage, take part in vertical migration from deep-sea up to the epipelagic layers, especially during the night [66,67]. During this behavior, these species take interactions with many trophic levels, as prey of pelagic and demersal

fishes [68], cephalopods [67], and marine mammals [69], and as predators of zooplanktonic and small nektonic organisms; therefore, these fishes contribute to enhancing the complexity of the marine food web structure and their dynamics. Indeed, several mesopelagic fishes are selective predators [70], exerting an important top-down control on the zooplanktonic community structure [71]. In this manner, these species contribute, at an intermediate trophic level, to transfer the energy from primary production to top predators [72,73]. Understanding the functioning of deep-pelagic food webs is essential to comprise some related mechanisms, such as the population dynamics, and energy pathways, and influence the resilience of communities to perturbation [74].

The biodiversity of Mediterranean fishes has been continuously evolving in the last decades because of non-indigenous species invasions and the influence of described climate change effects [75–77]. Monitoring the occurrence and distribution of fish species is of fundamental importance, especially in a semi-closed basin like the Mediterranean Sea. The peculiar morphology of this basin makes it divided into several biologically different areas, which leads to enormous difficulties in carrying out research surveys that can cover it entirely [78]. Moreover, several fish species are difficult to detect during commercial fishing activities and to be correctly identified. Indeed, biologically interesting species often represent a fishing waste and therefore are not reported, leading to a huge gap in data collection [79,80]. Only *Lampris guttatus* (Brünnich, 1788) is appreciated and prized in some areas [81]. The rest of the lampriform species are considered inedible; hence, discarded in the sea immediately after their capture [2].

Deep-sea adaptation of teleost

The oceans represent the largest environment on the planet [82,83]. The shallower and more illuminated waters are those mainly inhabited by marine organisms. In contrast, a relative smaller number of organisms are able, through morphological and functional evolutive adaptations, to live in the more inhospitable deep-sea waters [84,85]. Indeed, deep seas are characterized by extremely harsh conditions, such as high hydrostatic pressure, darkness, low temperature, scarce food, and low oxygen availability [11,86]. Therefore, deep-sea

organisms have developed specific morphologic and genetic adaptations to survive in these extreme habitats [87,88]. Due to the environment, animals living in the deep-sea are run into unique challenges and require complex solutions to encounter and identify food, escape from predators, and meet their conspecific during reproductive behavior [89]. Studying these adaptations and responses to environmental stimulation is exciting and essential better to understand the relationships between these organisms and their habitat. However, as it happens for the organisms, the researchers have encountered many difficulties in exploring and sampling this habitat due to the high-depth conditions that require specific and highly costly instruments [90]. The classic approach historically used in the past century provided several sampling instruments such as extensive research vessels with a scientific crew, rosette samplers, box corer, remotely operated vehicles (ROVs), baited cages, and trawling nets. These methods were upgraded through new technologies with the support of advanced techniques and sensors that, in the last years, revealed new species in deep-sea habitats worldwide [91]. Moreover, the molecular support to these investigations permitted to minimize the efforts contemporary enhancing the efficacy of organisms detection, particularly for the rare or cryptic ones, as in the case of the environmental DNA (eDNA) approach [92,93].

Fishes represent the main components of the deep sea megafauna [94]. Evolutionary adaptations to deep-sea life appear to have occurred independently in at least 22 fish orders [95,96]. Several studies have investigated the genetic adaptations that allow vision in the dark of deep environments [97,98]. Indeed, the darkness of the deep-sea lead to the absence of reference marks to correctly evaluate the dimension or the distance from the other organisms or biotic obstacles using the common monocular vision [85,99]. Hence, for the predation result, binocular vision is essential, as in the case of *Stylephorus chordatus*, a species previously related to the Lampriformes order that possesses forward-facing tubular eyes advantageous during its predation activity [15]. Moreover, mesopelagic fishes commonly are provided by larger eyes compared to more shallow water living species, a valuable adaptation to capture more light radiation in a dark environment.

Other studies have focused on morphological and structural adaptation to high hydrostatic pressure [100,101]. Due to their biochemical structure, very high pressures typical of deep-sea environments denature proteins completely [102]. Consequently, to survive at a certain depth (>4000 m) and exploit all their normal functions correctly, fishes must adapt physiologically and morphologically, starting from their molecular architecture [32]. An increase in pressure, accompanied by a decrease in temperature typical of a deep environment, could also reduce the membrane fluidity. Consequently, the trans-membrane ion fluxes influence the membrane-based processes, which process extremely pressure-sensitive [103]. In deeper-living teleost, a natural selection occurs, responding to environmental pressure, favoring phospholipids with high inherent fluidity [101]. Cossins and MacDonald reported that mitochondrial membranes from the liver of deep-sea species contain a higher quantity of unsaturated fatty acids compared to shallow-living species [104]. However, apart from membrane lipid changes, pressure adaptation involves other structural adaptations on behalf of enzymes and structural proteins. Several authors demonstrate that actin extracted from deep-sea fishes' muscle fibers is characterized by their tertiary structure's structural properties, which consent it to manage pressure modification effects [33,101]. Moreover, this also provides high thermal stability at the same time [105].

Generally, a decrease in body size with depth is registered in deep-sea organisms. Thiel interpreted this phenomenon in benthic invertebrates as an adaptation concerning low food availability [106]. On the contrary, Heinke described an increase in average body size of plaice (*Pleuronectes platessa*) with increasing depth [107], the one known as Heinke's law, also described by other authors in many deep-sea fish species [108]. However, as confirmed by Heinke, it is conceivable that this phenomenon is related to most fish species' lifecycle patterns, whereby juveniles and larvae first appear in shallow water, where the fecundated eggs hatch. Subsequently, with the development of structures and functions of the body, the individuals migrate deeper to exploit their feeding and reproductive behaviors. This behavior is also reported and confirmed for Lampriformes species, whose life cycle perfect follows this style, so adult organisms generally live at higher depths [3,6,109]. Regarding the influence of depth on the body shape of fishes, some authors observed an elongation

trend in the body with depth in several deep-sea orders (e.g., Gadiformes, Argentiniformes). These findings also suggest the relationship between an elongated body and the low-speed swimming mode, with rare quick clicks during the predation mainly controlled by the fin used by deep-sea fishes [110]. This elongated shape may also be an advantage from a sense organ point of view, resulting in a longer lateral line that finely allows these species to detect sensing vibrations in the darkness [111]. However, there are numerous exceptions to this body shape trend, for example, the deep-sea species of genera *Lophius*, *Beryx*, and *Hoplostethus*, typically characterized by a short and round-oval shape.

Considering the low amount of food that characterizes the deep-sea environments, reducing the energy employed in swimming is essential for these species. For this reason, several of these demersal species, such as the flatfishes, are negatively buoyant to maintain regular contact with the seafloor, reducing the negative influence of bottom currents on their swimming activity [112]. Some other mesopelagic and bathypelagic species can regulate their neutral buoyancy with organs and fins to station in a vertical position during feeding activity to reduce the current effects and save energy [113]. Differently, the fast swimmer species like Scombriformes would not derive any advantage from the use of different buoyancy and are adapted to reduce as much as possible their volume and consequently the hydrodynamic resistance of their body, with the right shape and inserting their fins inside apposite splits during the active swimming [114]. A well-developed red muscle system also characterizes these species, whereas most sedentary bottom-living fish, slow-swimmer, possess reduced to absent red muscle fibers. Some authors reported that strictly demersal fishes generally have less red muscle fibers the more profound they live, and in the same manner, benthic fishes have less red muscle than the benthopelagic species [115]. From a functional point of view, these anatomical features imply that deep-sea fishes' swimming speed and endurance-swimming capabilities are less compared to shallow-water species.

The low light to darkness condition of the deep-sea environments leads to developing adaptations related to the coloration of deep-sea species about the necessity to be invisible to their prey/predators [116]. Many larval and juvenile stages of marine invertebrates and

teleost are transparent and with a flat form to be less visible as possible to predators in these defenseless stages of the life cycle [85]. During metamorphosis, this transparency is lost, and no adult fishes are transparent but develop the most suitable pigmentation for their needs. Many mesopelagic species have developed silvery reflectors in the skin giving a lighting appearance, such as many Mictophiformes, reflecting the light into the observer's eye at precisely the expected angle and intensity, but not if this comes vertically from below [67]. Dark blue or black pigmentation on the dorsal body surface gives less visibility against predators that come from lower depths [117]. Bioluminescence is another feature that characterizes deep-sea organisms that use it for multiple purposes. Indeed, bioluminescent light emissions can function as a defense in a teleost, for example, miming the down-welling light to escape from predators with counterillumination from the ventral surface of the body [9]. Photophores dispersed on the body or around the head can be used to illuminate potential prey or confuse them by giving the impression of a different shape.

Moreover, bioluminescence is also used for intraspecific communication. Confirming that, in some families like the Stomiidae and Myctophidae, photophores' location is species-specific and used in schooling, and also crucial in reproductive behavior as a sexually dimorphic characters [118]. This process occurs in fishes using intrinsic light organs through the oxidation of a specific substrate known as a luciferin that, in the presence of the enzyme luciferase, causes the excitation of this molecule that leads to the release of a photon [119]. However, teleost bioluminescence can also originate from symbiotic aquatic bacteria of the Vibrionaceae family, such as the genera *Vibrio* and *Photobacterium*. Despite these bacteria are not able to produce luminous radiations, they become able to emit a steady light when reaching the quorum sensing. This feeble light radiation is amplified or filtered by specific fish structures as reflectors and shutters [120].

However, despite several studies being carried out on some species, the details of these processes are to be deepened, considering a broad range of organisms and other adaptations remain largely unknown. The gaps in existing knowledge are also due to the difficulties associated with sampling efforts, finding uncommon specimens and the consequent studies, as well as the probability of collecting rare species [121–123]. In this context, the analysis of

fish genomes acquires fundamental importance, providing a precious resource for understanding the molecular mechanisms underlying environmental adaptation, especially in extreme environments such as the depth waters [124–126].

Among the deep-sea fish species, it is sporadic to find specimens of the family Trachipteridae (Swainson, 1839) of the order Lampriformes [3,127]. Actually, beyond Trachipteridae, other five families belong to this order: Lampridae, Veliferidae, Radiicephalidae, Lophotidae, and Regalecidae [2,4]. Although they are uncommon, are occurring in all the oceans. They generally present a naked body with some cycloid or modified ctenoid scales along the lateral line, often spiny and deciduous. Only some species of the families Lampridae and Veliferidae show the body partially covered by thin scales [81,128]. An elongated and compressed ribbon-like body characterizes the entire family. Two characteristics shared by all the species belonging to the Trachipteridae family are the total absence of an anal fin and a long caudal fin, with the presence of the upper lobe only, oriented perpendicularly to the body [2,129]. Also, the dorsal fin is often very long, with its origin well behind the tip of the snout, showing the typical crimson red color of trachipterids fins. Due to their life in deep-sea environments, all these species present large eyes, which are more suitable for capturing the lowest light radiation [2]. Moreover, like all Lampriformes, these fish possess the capacity to protrude and expand significantly their buccal cavity during their feeding activities [1]. In this way, they can hunt pelagic organisms with fewer difficulties in the darkness.

Biological and ecological features of Lampriformes

Morphologically, most Lampriformes are characterized by bright silvery colors and very colorful fins, mainly in red shades [2,4]. Sometimes the first ray of the dorsal fin is quite elongated and evident, characterizing the appearance as in the case of *Zu cristatus* (Bonelli, 1819) [130]. As members of Subphylum Vertebrata and Subclass Teleostei, lampriform fishes are characterized by a developed axial skeleton. The elongated form, like trachipterids, has a highly variable number of vertebrae comprising between 60 and 150 [1,131]. Instead, the most compressed body species possess fewer vertebrae (33 to 46), such as the case of

lamprids. Notably, this order has quite different patterns of small bones and ligaments associated with anterior vertebrae, probably developed during their evolution, based on their characteristic swimming behavior and the deep-sea life [1,132,133]. Other structural differences with the common teleost are mainly the head structure, with the lack of a ligamentous link between the cheekbones and the upper jaws (maxillae), supported by the nasal cartilage placed in the frontal region of the skull in a hollow [1]. These peculiar features are significant from a functional point of view for lampriform species because they enable the upper jaws to be stretched forward during feeding behavior.

This extreme jaw protrusion in some species permits the expansion of the mouth up to 40 times in dimension during predation allowing to capture even the most evasive of planktonic prey items in the darkness. Indeed, lampriforms feed mainly on small Polychaeta, medium/small-sized pelagic cephalopods, and crustaceans, Malacostraca included [134,135]. Also, they prey on small fishes not simple to capture in the dark deep-sea environment [134,135]. Borme and Voltolina also reported the occurrence in the stomach of *Trachipterus trachypterus* of some macroalgae such as *Ulva intestinalis* (Linnaeus, 1753) and *Cystoseira compressa* (Esper) Gerloff & Nizamuddin, 1975) showing a fascinating omnivorous, opportunistic nature of the species [134]. Regarding feeding interactions as preys, lampriform fishes have been sporadically found in the stomachs of cetaceans like the sperm whale (*Physeter macrocephalus,* Linnaeus, 1758), chondrichthyans as blue shark (*Prionace glauca*, Linnaeus, 1758) or big-sized bony fishes predators such as tunas [136,137]. Moreover, some typical enteric parasites of fishes, like *Ascaris capsularia* (Rudolphi, 1802), *Scolex polymorphus*, (Rudolphi, 1819), and *Anisakis physeteris* (Baylis, 1923), have been reported in some lampriformes species [138].

Information about the reproduction of Lampriformes is very scarce due to their elusive nature. Even though spawning has never been observed, lampriform fishes are considered broadcast spawners because eggs are planktonic, and several authors have used them to record the presence of some species [6,139]. Eggs have a diameter of about 2–6 mm and remind of the species' coloration by being bright with reddish hues. At hatching, larvae have fully developed mouths and digestive tracts and begin to feed immediately on minute

plankton [6]. The larval stages of lampriforms are beautiful and identifiable fishes characterized by evident, ornamented both dorsal and pelvic long fin rays. It is known that trachipterids undergo metamorphosis, passing from the larval to the juvenile form [113,140].

Moreover, from a habitat point of view, some species prefer shallow water during larval/juvenile stages while moving to the deep-sea environment during adult life. These transitions from shallow, nearshore habitats to the deep open ocean is one of the two significant events hypothesized to have characterized the evolution of the order Lampriformes [13]. The family of Veliferidae is a moderate-sized coastal fish group that inhabits for their entire life cycle in shallow waters. Some authors have reported it at a maximum depth of 110 m. It seems to be the most ancient group within the Lampriformes order [141]. In the adult stage, all other lampriforms are open-ocean, epipelagic, mesopelagic, or bathypelagic fishes. Some adult specimens of the Lampridae family were also detected in shallow waters near the surface, but, apart from occasional events, it is not considered a coastal water group [4]. An essential event in the evolution of the lampriform lineage is represented by the colonization of deep-sea environments made by the other families of the group because of the necessary functional adaptations [33,97]. The second significant evolutionary transition regards the body shape, passing from oval-shaped and deep-bodied veliferids and lamprids to elongate forms of oar-ribbon fishes (Regalecidae, Trachipteridae) [113].

As mentioned, knowledge about this taxon is scarce in relation to difficulties in finding and monitoring live specimens, events that occur rarely. This difficulty is also due to their loner nature, confirmed by the records present in literature and collected in this thesis, which mainly concern the occurrence of one specimen, very rarely two or three [5]. Whereas the generalist fishing methods through which they are caught, such as trawling nets or longline fisheries, the rare occurrence of one specimen highlights their loner nature and the already known rarity. Experimental studies are absent in the literature, and very little is known about the most suitable habitats of these species, their preferences or interaction in aquatic communities, and their role in deep-sea food webs. Occasional underwater surveys with

ROVs or diver direct observations and video recordings suggest that more elongated species like the oar-ribbon fishes usually orientate their body vertically [17]. This characteristic head-up position swims vertically using all their long fins through the water column, sometimes rapidly [129]. Indeed, despite the appearance of not robust fishes, histological analysis had also confirmed soft pale muscle tissues, especially when disturbed, these species can swim fast but briefly. On the contrary, veliferids, and lamprids usually swim horizontally in the typical teleost way, also confirming, in this case, a different evolutive history. These species are considered powerful swimmers, using their large pectoral fins for forwarding propulsion [81].

From the scarce data in the literature, even if active predators, Lampriformes seem not to be aggressive fishes, provided with extraordinary features to offend the prey or from predators. The above-mentioned head-maxillae structure provides them efficacy in predation. At the same time, from a defense point of view, fast swimming in escaping seems to be the primary mechanism, with some exceptions. Particularly curious is the case of the regalecid *Agrostichthys parkeri* (Benham, 1904), which has been reported to give a mild electric shock if handled [142]. Moreover, some species of the genus *Lophotus* and *Radiicephalus* seem to have specialized organs that, as defense mechanisms, expel a dark, squid-like ink through the cloaca if disturbed [113,143]. *T. trachypterus* (Gmelin, 1789) showed a Batesian mimicry strategy when observed alive, and its feeding behavior seems to be strictly influenced by up-welling currents and the moon phases [17]. Moreover, the areal distribution of this species seems to be related to the movements of different aquatic masses within the water column [134].

Currently, no lampriform species are listed by the IUCN, but of course, it depends on the rarity of these fishes and the scarcity of data about them. Considering the discussed role of Lampriformes in deep-sea food webs, fishermen and researchers should take more care in reporting the related data of their occurrence as by-catch [5,60], to deepen the stock assessment evaluation and consequently to understand better if conservation measures are required.

Essential systematics and phylogeny of the order

Taxonomy and systematics of Lampriformes passed through many insights in the last years due to the advent of new molecular approaches to phylogenetic relationships [16,144–146]. For this reason, clarifying the taxonomy of the group is essential to better evaluate and compare the literature in the field. Currently, the order Lampriformes comprises six families (Table 1): Lampridae (Gill, 1862), Lophotidae (Bonaparte, 1845), Radiicephalidae (Osorio, 1917), Regalecidae (Gill, 1884), Trachipteridae (Swainson, 1839) and Veliferidae (Bleeker, 1859) [147,148]. The family Lampridae consists of the genus *Lampris* (Retzius, 1799), which contains five species, three of that are very recent: *Lampris australensis* (Underkoffler, Luers, Hyde & Craig, 2018), *Lampris guttatus* (Brünnich, 1788), *Lampris immaculatus* (Gilchrist, 1904), *Lampris incognitus* (Underkoffler, Luers, Hyde & Craig, 2018), *Lampris megalopsis* (Underkoffler, Luers, Hyde & Craig, 2018) [81]. The family Lophotidae contains two genera, *Eumecichthys* (Regan, 1907), which includes a single species *Eumecichthys fiski* (Günther, 1890), and the genus *Lophotus* (Giorna, 1809), which is constituted by four species: *Lophotus capellei* (Temminck & Schlegel, 1845), *Lophotus guntheri* (Johnston, 1883), *Lophotus lacepede* (Giorna, 1809), and *Lophotus machadoi* (Miranda Ribeiro, 1927) [148,149]. The family Radiicephalidae is constituted by only one genus, *Radiicephalus* (Osório, 1917), which contains two species: *Radiicephalus elongatus* (Osório, 1917) and the recent annotated *Radiicephalus kessinger* (Koeda & Ho, 2018) [150]. The family Regalecidae passed through many rearrangements that, over time, added or deleted species and genera considered synonyms or sisters. Currently, the accepted classification attributes two genera to this family, *Agrostichthys* (Phillipps, 1924) with *Agrostichthys parkeri* (Benham, 1904) as unique species, and *Regalecus* (Ascanius, 1772), which contains the two species *Regalecus glesne* (Ascanius, 1772) and *Regalecus russellii* (Cuvier, 1816). These species have several synonyms (e.g., *Regalecus kinoi, Regalecus masterii, Regalecus woodjonesi, Gymnetrus hawkenii, Gymnetrus russellii*) [147,148]. Similarly, the family Trachipteridae consists of three genera, *Desmodema* (Walters & Fitch, 1960), which consist of two species *Desmodema lorum* (Rosenblatt & Butler, 1977) and *Desmodema polystictum* (Ogilby, 1898), the genus *Zu* (Walters & Fitch, 1960) with two species *Zu cristatus* (Bonelli, 1819) and *Zu elongatus* (Heemstra & Kannemeyer, 1984)

and the most prominent genus *Trachipterus* (Goüan, 1770) with its six species: *Trachipterus altivelis* (Kner, 1859), *Trachipterus arcticus* (Brünnich, 1788), *Trachipterus fukuzakii* (Fitch, 1964), *Trachipterus ishikawae* (Jordan & Snyder, 1901), *Trachipterus jacksonensis* (Ramsay, 1881), *Trachipterus trachypterus* (Gmelin, 1789), with several species belonging to this family that have more synonyms (e.g., *Trachipterus misakiensis, Trachypterus altivelis, Trachypterus nigrifrons*) [147,148]. Over 30 nominal species have been assigned to the family Trachipteridae, even if the valid species probably do not exceed ten [113]. However, an actual complete global synthesis of the family was missed due to the scarcity of data. The family Veliferidae is constituted by the genus *Metavelifer* (Walters, 1960) with the species *Metavelifer multiradiatus* (Regan, 1907) and the genus *Velifer* (Temminck & Schlegel, 1850), which contains *Velifer hypselopterus* (Bleeker, 1879) as its unique species [147].

The older classification also comprised the family Stylephoridae, which was recently moved based on molecular data into the new separate order of Stilephoriformes, which contains one monospecific genus, *Stylephorus* (Shaw, 1791) [15,151–153]. This thesis represents one of the first documents based on this new classification.

Table 1. Summary table of the currently accepted 6 families, 11 genera and 27 species of the Lampriformes order.

Family	Genus	Species
Lampridae (Gill 1862)	*Lampris* (Retzius, 1799)	*Lampris australensis* (Underkoffler, Luers, Hyde & Craig, 2018)
		Lampris guttatus (Brünnich, 1788)
		Lampris immaculatus (Gilchrist, 1904)
		Lampris incognitus (Underkoffler, Luers, Hyde & Craig, 2018)
		Lampris megalopsis (Underkoffler, Luers, Hyde & Craig, 2018)
Lophotidae (Bonaparte, 1845)	*Eumecichthys* (Regan, 1907)	*Eumecichthys fiski* (Günther, 1890)
	Lophotus (Giorna, 1809)	*Lophotus capellei* (Temminck & Schlegel, 1845)
		Lophotus guntheri (Johnston, 1883),
		Lophotus lacepede (Giorna, 1809),
		Lophotus machadoi (Miranda Ribeiro, 1927)
Radiicephalidae (Osorio, 1917)	*Radiicephalus* (Osório, 1917)	*Radiicephalus elongatus* (Osório, 1917)
		Radiicephalus kessinger (Koeda & Ho, 2018)
Regalecidae (Gill, 1884)	*Agrostichthys* (Phillipps, 1924)	*Agrostichthys parkeri* (Benham, 1904)
	Regalecus (Ascanius, 1772)	*Regalecus glesne* (Ascanius, 1772)
		Regalecus russellii (Cuvier, 1816)
Trachipteridae (Swainson, 1839)	*Desmodema* (Walters & Fitch, 1960)	*Desmodema lorum* (Rosenblatt & Butler, 1977)

		Desmodema polystictum (Ogilby, 1898)
	Trachipterus (Goüan, 1770)	*Trachipterus altivelis* (Kner, 1859)
		Trachipterus arcticus (Brünnich, 1788)
		Trachipterus fukuzakii (Fitch, 1964)
		Trachipterus ishikawae (Jordan & Snyder, 1901)
		Trachipterus jacksonensis (Ramsay, 1881)
		Trachipterus trachypterus (Gmelin, 1789)
	Zu (Walters & Fitch, 1960)	*Zu cristatus* (Bonelli, 1819)
		Zu elongatus (Heemstra & Kannemeyer, 1984)
Veliferidae (Bleeker, 1859)	*Metavelifer* (Walters, 1960)	*Metavelifer multiradiatus* (Regan, 1907)
	Velifer (Temminck & Schlegel, 1850)	*Velifer hypselopterus* (Bleeker, 1879)

Different phylogenetic surveys conducted with morphological and molecular approaches have placed the Lampriformes order within the Acanthomorpha clade [154,155]. Lampriform fishes are primitive compared to the Percomorpha, but their precise placement among basal Acanthomorpha remains undetermined [132]. The monophyly of Lampriformes is based on four apomorphias, three of which are correlated and involve evolutionary modifications of the unique feeding mechanism. In these species, the maxilla slides forward with the premaxilla during jaw protrusion [113]. The Veliferidae family is deemed the sister group of all other lampriforms, with the oarfish and related that evolved from a common velifer-like ancestor during the late Cretaceous or early Eocene [1]. Effectively, the order Lampriformes is considered one of the ancient sister taxa to approximately 60% of all known teleost species [113,132]. This pivotal systematic position makes the order essential to the evolution researchers, considering the difficulty of establishing this kind of phyletic relationship in classifying Acanthomorpha fishes [156].

In this regard, the inclusion of fossils in the analysis also assumes importance in phylogenetic studies to deepen the knowledge of morphological characters and their evolution. During archaeological research in the Mediterranean area, some skeletal evidence of about another ten extinct Acanthomorpha species have been found in fossil findings [14,157,158]. These fossil relics have been related to ancestral Lampriformes [14,154,158,159]. In 1999, Sorbini and Sorbini described the fossil of the oldest known lampriform fish, *Nardovelifer altipinnis*, found in the Cretaceous deposits of Nardò, Italy

[141]. Carnevale and Bannikov [160], and Papazzone et al. [161] described in Eocene deposits from Verona, Italy, two ancient species, *Bajaichthys elegans* and *Veronavelifer sorbinii*. Carnevale reports about the first fossil of the ribbonfish *Trachipterus mauritanicus* from a Miocene locality in northwestern Algeria [157]. Additional fossil taxa from other areas of the world reported some other Lampris-like species discovered in Miocene deposits in California (*Lampris zatima*) [162], two Oligocene lophotids, *Protomecicthys* and *Protolophotus* [158], and in Oligocene deposits from New Zealand (*Megalampris keyesi*) [163].

The role of molecular biology in the knowledge of rare species

In recent years, the improvement of marine genomic sequencing tools and technologies allowed taxonomists to better investigate on evolutionary relationships of lesser-known taxa. Correct identification of these tie results is also essential for improving the knowledge of some linked topics, such as evolutionary biology, ethology, anatomy, and functional morphology of these rare species, which often are characterized by fragmented and vague information [17,50]. Moreover, rare species are an active part of the marine trophic webs, having trophic relationships with key species, as confirmed by some authors for Lampriformes species [164–167]. Indeed, due to their predator habit, these species play an essential role in regulating ecosystem function and species diversity [168,169]. At the same time, these organisms interact with pathogens becoming a vector for the dissemination of sometimes unknown metazoan species [138].

A powerful tool in monitoring aquatic ecosystems is the environmental DNA method (eDNA), which allows the detection of living organisms' presence with reasonable accuracy in a non-invasive way [170,171]. For this to happen correctly, the species to be identified must be annotated in the reference sequence databases commonly used, such as GenBank [172]. It means that this tool loses its effectiveness without the adequate data previously collected by researchers during their studies. Therefore, it is essential to promote the research in this field to increase the pool of data available for these innovative and beneficial techniques compared to the methods traditionally used in environmental monitoring, which are much slower, expensive, and in some cases, inaccurate [93,173]. However, other

ecological details, such as sex and maturity stage, age, body size, morphological features, and behavioral information, are not obtained through the exclusive use of the eDNA metabarcoding approach. Some other complementary methods for detecting these features are required, such as direct observation by scuba divers or remotely operated vehicles (ROV), which, over the presence of the species, also provide insight into population size and dynamics, relative abundance, feeding, and reproductive behaviors, and other conspecific or heterospecific relationships [174,175].

These aspects are even more critical when considering rare or elusive species, sometimes tough to detect with just a method, and for this reason, still unknown for most of their features. In this sense, a good contribution in the last years has come from citizen science, which has been a considerable tool for increasing the knowledge of temporal and spatial distribution data of rare marine species [176,177]. This kind of contribution is often not controlled by the researcher and comes as cost-free contributions, although inaccurate in several cases. However, even if not reliable, in the study of rare species, this kind of contribution represents an essential resource, even because not commercially beneficial organisms often represent these species; for this reason, they are neglected by professional fisheries [5]. Hence, it is easy to understand how developing highly reliable and scientifically valid tools, such as molecular approaches, is fundamental in studying these organisms. Moreover, assessing the genetic structure of many organisms could lead to a better comprehension of their evolution and adaptation during their history of environmental variability and influence in the following years.

Phylogeny and teleost classification is rapidly changing under the boost of molecular phylogenetic approaches based on increased and more taxonomically valid datasets. Classical approaches based on textbook classification schemes were funded on older formulated syntheses that often come from largely independent studies [178]. Morphological cladistic studies of bony fish relationships have classically focused on lower taxonomic levels. Moreover, few attempts were made to investigate morphology at higher taxonomic levels based on this approach, obtaining relatively limited success [179]. The development of the first molecular markers as new evaluation criteria, firstly based on

sequences of mitochondrial DNA (mtDNA) genes or on the complete mitogenomes, enhanced the bony fish relationships topic, providing new essential information across broader taxonomic scales [16,156].

For this reason, recent studies based on mtDNA phylogenetics data consented to re-evaluate the relationship for several taxa at all levels, destabilizing older arrangements by proposing alternative views compared to previous classifications [180]. Despite the evident efficacy of mitogenomic hypotheses, this method is still not universally agreed upon by the scientific community, which criticizes the single locus nature of this information. For this reason, additional genomic regions are still analyzed to propose new nuclear DNA markers (e.g., rag1, rag2, 28S) that are already developed and applied in combination with the mtDNA ones (e.g., COI, COII, Cyt b) to well-assess teleost relationships. Recent approaches using 20 of these mixes of nuclear and mitochondrial markers demonstrated an improved resolution of phylogenetic relationships of bony fishes at all taxonomic levels [181]. Through this new information, it is possible to assess better the relationships of the early-branching lineages of early extant actinopterygians, which have been resolved using a mix of both morphological and mitochondrial sequences evidence [182]. In the same manner, recent molecular insights based on nuclear gene approaches consented to updating the relationships among major bony fish groups, such as Euteleostea and Elopomorpha [183]. On the contrary, more apical Acanthomorpha groups, such as percomorphs, are still not well assessed, and more studies are needed [155,184]. Similarly, basal branching events within Osteichthyes remain unsolved, such as the ancient relationships among lungfishes, coelacanths, and tetrapods [185].

More insights could also come from the whole genome sequencing approach. Some decades ago, sequencing living organisms' entire genomes was a complex and costly challenge for researchers in the biological and environmental sciences field. The first attention in this regard was dedicated to human whole genome sequencing, under the need of the medical sector to comprehend and try to solve numerous problems related to the genetic nature of various pathologies [186,187]. In recent years, the giant steps made by second and third-generation sequencing technologies, combined with a massive reduction in time, effort, and

cost of methodologies, have allowed a more straightforward and more widespread use of these techniques, resulting in a considerable increase in the effectiveness and quality of data [92]. These advantages have allowed researchers in the zoological and ecological fields to use these tools to deepen both phylogenetic classifications and morpho-functional studies on living organisms [10,188]. As in the case of human necessity, in marine genomics, the first relapses of this tool focused on the sequencing of commercially relevant species from an aquaculture or fisheries point of view [189,190]. Applications related to organisms used as study models or diseases related to farmed organisms are also not of secondary importance [191–193]. The diffusion of this approach has recently made it possible to address it to study rare and less commercially important but biologically relevant species [194,195]. In fact, through the sequencing of an increasing number of species, it is possible to thoroughly investigate the adaptive evolution of organisms according to the environment, both in response to climate change that occurred throughout history, concerning extreme environments (e.g., thermophilic organisms, organisms of the deep marine environment) [10,196].

Recently, due to their ecological importance, was observed an increased interest on mesopelagic and bathypelagic communities of the Mediterranean Sea [144,197–199]. Despite this, the information about Lampriformes distribution is very scarce and fragmented [200]. Currently, the species reported in this basin, with the documented capture of very few specimens, are: *L. guttatus*, *L. lacepede*, *T. arcticus*, *T. trachypterus*, *R. glesne* and *Z. cristatus* [4,129].

Due to the scarcity of data, the evolutionary relationships within Trachipteridae are poorly known [113,129,156]. Moreover, a bit of confusion about nomenclature and classification of trachipterids genera and species have been recorded over time [140]. Due to the known allometric growth that characterizes this family, drastic morphological changes occur during the ontogeny, with significant morphological and functional differences between juvenile and adult stages within the same species. This variability, combined with their rarity, has led to the description of different life stages as separate species rather than as part of the ontogenetic continuum of a single species, thus inflating the apparent diversity at

species level [133,156]. For these reasons, deepening the knowledge of this taxon based on new molecular information will be essential to assess the phylogenetic relationships within the order better and investigate the genetic structure of these fishes about their adaptative responses during their evolution which has determined their essential features.

AIMS AND OBJECTIVES

The poor status of current knowledge about the biology, distribution, and phylogenetic relationships of order Lampriformes led us to investigate the above-mentioned topics. Specifically, the lack of a document summarizing the state of the art of these fish in the Mediterranean Sea, and the scarce presence of molecular attributes of these ecologically important species on genetic databases, gave us the opportunity to investigate on these essential species for marine mesopelagic and bathypelagic trophic webs; as a consequence, deepening these aspects about one of the most representative Lampriformes species of the Mediterranean Sea, as *Z. cristatus*, allow to deepen the knowledge on their genetics features in relation to deep-sea adaptations.

Therefore, the present thesis aims to:

a) Provide the first comprehensive revision of all the Mediterranean Sea references related to the distribution and life-history traits of Lampriformes species.
b) Describe a new record of *Z. cristatus* in the Ionian Sea, with an in-depth analysis of the morphometric and meristic data compared with all the other similar Mediterranean Sea reports.
c) Produce the first whole genome assembly draft for this species, applying innovative methods and investigate on the use of hybrid technologies.
d) Isolate and annotate the first complete mitochondrial DNA genome of *Z. cristatus*, providing a detailed investigation on its structure, usefulness, and new phylogenetic insights.
e) Predict, isolate, and characterize the opsin-like gene products from the whole genome draft, providing new information on their gene family expansion in teleost in relation to deep-sea adaptations.

1. DISTRIBUTION AND LIFE-HISTORY TRAITS OF LAMPRIFORMES IN THE MEDITERRANEAN SEA

Specimens of Lampriformes order are rarely seen alive, especially in the adult stage. Very few cases were recorded and shared online regarding shabby animals in coastal areas or watered on the surface [4]. Some expert scuba diving photographers have caught juvenile stages of some lampriforms on camera, showing the incredible morphology that characterizes most of the group in this life stage [2]. Occasionally, relatively small specimens have been taken during scientific surveys using midwater or bottom trawls [24,77,198,199]. Among the Lampriformes, Lampridae and Veliferidae comprise species that inhabit shallow coastal waters, mainly distributed in the Indian and Pacific Oceans [113]. Sometimes, specimens of Trachipteridae and Regalecidae can be found in surface waters, but more by chance than by choice, driven by vertical currents [201]. Based on the literature, the remaining lampriform families consist of mostly mesopelagic but often epipelagic and bathypelagic species, reported beyond 2000 m [24]. This high depth range of distribution is common in eurybathic fishes, which, like Lampriformes, rarely show the typical decompression signs after the capture from depth bottoms [202]. The general distribution of the order covers all the Oceans worldwide [1,2]. Almost all these species are widely distributed in tropical and temperate zones, but they are considered extremely rare despite this typical distribution. From the record in literature, these species do not seem to inhabit extreme latitudes, apart for two species. One fish of the family Lampridae, the rare *L. immaculatus*, was reported in the subantarctic zone (Falkland–Patagonia) around 56° south latitude in 1985 [109]. On the other pole, the trachipterids, *T. articus,* can inhabit as far as the 60th parallel north [203].

As the main aim of this manuscript, an extensive literature review on the records of the Lampriformes families in the Mediterranean Sea is hereafter individually treated.

1.1 Geographical distribution

1.1.1 - Lampridae (Gill, 1862)

The family Lampridae, commonly named opahs, is currently represented by the single genus Lampris that is characterized by a compressed oval-shaped body, covered with very smooth and small scales, brightly colored with a dorsally bluish-sighted bottom that becomes paler reddish ventrally and very visible fins tending to bright red, the entire body exhibit scattered round silvery spots [1,81,204]. The dorsal and anal fins are elongated and retractable in a dedicated slot to facilitate swimming. Indeed, they are the most skilled group in swimming within the Lampriformes order [205,206]. The maximum size reported is around 190 cm in length and over 250 kg in weight, making it the heaviest representative of the order [207]; for shape and size, opah is often confused with the Atlantic sunfish *Mola mola*. The species of this family have coastal habits and seem to prefer the shallow and warm waters, but occasionally they have been found at higher depths (up to 500 meters) off the coasts [205,208]. Lamprids possess a small toothless mouth through which they prey on small cephalopods, fishes, and crustaceans [209,210]. However, it is an opportunistic family, and some authors also reported the occurrence in their stomachs of clams and crabs. Occasionally caught in the longline fisheries for large pelagic such as tunas and swordfishes, lamprids have the most appreciated flesh among the order Lampriformes, and it is marketed as by-catch [109,211]. Due to the coastal nature, the species *L. guttatus* is often caught by amateur fishermen with various fishing methods, therefore being one of the rare Lampriformes species occasionally fished in some areas, even from the shore.

During an interesting study, Runcie et al. [208] investigated on the capacity of *L. guttatus* to maintain a cranial endothermy. From their results, the authors concluded that this capacity was developed by opah through the proximal region of the paired lateral rectus extraocular muscle, which works in this species as a source of heat. The higher citrate synthase activity of this muscle, highlight the higher capacity for aerobic heat production. Moreover, in *L. guttatus* this muscle is insulated by a layer of fat and surrounded by a network of arteries and veins witch functions as a heat exchanger. This endothermal capacity of *L. guttatus* was also studied by Wegner et al. [212], which investigate on the whole body endothermy on

this species, relating it to a constant movement of pectoral fins and the particular structure of gills, provided by heat exchangers structures. Bo and colleagues [213] reported in this sense some recent insights on *L. megalopsis.* The investigation was conducted from a genetic point of view, following the whole genome sequencing of the species, which lead to the detection of positive selection on several genes, known to be involved in muscle differentiation and development. This feature could represent an important key for the opahs to expand their distribution habitat, both in a geographical and bathymetrical way, exploiting the thermal tolerance to move into deep and colder waters. Moreover, it represents a very rare feature among fish species, worthy of future in-depth analysis.

Worldwide distributed in tropical and temperate waters, mainly reported in the North Pacific Ocean [81], in the Mediterranean Sea the family Lampridae is represented only by the species *L. guttatus* anciently reported in the Ligurian Sea by Spinola in 1807 [214] and Risso in 1826 [215] (Table 2). The occurrence in the North-Wester area of the basin was confirmed by several other authors [216–220]. The presence of this species was successively detected in some other areas of the Mediterranean basin, with an increase of areal of distribution in the last 50 years. In the central part of the basin, the presence of *L. guttatus* was detected in the southern Tyrrhenian Sea [221,222], and both Italian and Albanian parts of the Adriatic basin [166,223,224]. Particularly for the Adriatic Sea, the presence of the species was reported as *Lampris luna* by Katurić in 1902 [225], and successfully by Cnrković in 1957 [226]. Moving to the Eastern Mediterranean Sea, the species was previously detected in the Aegean Greek Sea by Sinis [227]; successively Corsini-Foka [228], reported the occurrence of a specimen captured by longline fisheries near the surface off Pigadi in Karpathos Island, the central part of Aegean Sea. More recently, the species was detected in some new areas, such as in the Eastern Mediterranean in Turkey [229], and the central-eastern part of the basin in Tunis [230]. Francour et al. [231] reported in 2010 that at least 23 specimens in the Western Mediterranean along the French coasts were collected between 1997 and 2009. In the same study, it was reported the occurrence of the opah in Gouraya, from Northern Algeria. Considering the massive presence of *L. guttatus* in the area among the Gulf of Lion and the Ligurian Sea, is probable that the North-Western Mediterranean

represents the species preference area in the basin. The estimated distribution of records is resumed in Figure 1.

Table 2. Bibliographic references of the Lampridae family records in the Mediterranean Sea.

Family, Genus	Species	Year	Mediterranean area	Number of specimens	References
Lampridae, *Lampris*	*Lampris guttatus*	before 1807	Ligurian Sea (Italy)	1	Spinola (1807)
		before 1826	Nice (France)	1	Risso (1826)
		1829	Toulon (France)	1	Cuvier and Valenciennes (1835)
		1829	Marseille (France)	1	Cuvier and Valenciennes (1835)
		1898	Viareggio (Italy)	1	Ariola (1904)
		1901	Camogli (Italy)	1	Ariola (1904)
		1902	Novigrad Sea (Croatia)	2	Katurić (1902)
		1956	Bakar Bay (Croatia)	1	Crnković (1957)
		before 1970	Finale Ligure (Italy)	1	Tortonese (1970)
		1974	Camogli (Italy)	1	Cattaneo & Bava (2009)
		1979	Pizzo (Italy)	1	Andaloro & Di Natale (1979)
		1983	Unknown	1	Parin & Kukuyew (1983)
		1994	Neretva estuary (Croatia)	1	Bartulović (in Dulčić et al. 2005)
		1997	Toulon/Embiez (France)	1	Francour et al. (2010)
		1997	Bandol (France)	1	Francour et al. (2010)
		1998	Anzio (Italy)	3	Psomadakis et al. (2006)
		2000	Porquerolles Island (France)	1	Francour et al. (2010)
		2000	Sète (France)	1	Francour et al. (2010)
		2001	Anzio (Italy)	1	Psomadakis et al. (2006)
		2002	Nea Skioni (Greece)	1	Sinis (2004)
		2003	Embiez (France)	1	Francour et al. (2010)
		2003	Vir Island (Croatia)	1	Dulčić et al. (2005)
		2004	Bormes-les-Mimosas (France)	2	Francour et al. (2010)
		2007	Antibes (France)	2	Francour et al. (2010)
		2007	Embiez (France)	1	Francour et al. (2010)
		2007	Marseille (France)	1	Francour et al. (2010)
		2007	North Bastia (France)	1	Francour et al. (2010)
		2008	Antibes (France)	1	Francour et al. (2010)
		2008	Giens (France)	1	Francour et al. (2010)
		2008	off Le Levant Island (France)	1	Francour et al. (2010)
		2008	Karpathos Island (Greece)	1	Corsini-Foka (2009)
		2008	off Nice (France)	2	Francour et al. (2010)
		2008	Porquerolles Island (France)	1	Francour et al. (2010)
		2008	off Cagnes sur Mer (France)	1	Francour et al. (2010)
		2008	Saint-Raphaël (France)	2	Francour et al. (2010)
		2008	Gouraya (Algeria)	1	Francour et al. (2010)

2008	Camogli (Italy)	3	Francour et al. (2010)
2009	Camogli (Italy)	1	Francour et al. (2010)
2009	Cannes (France)	1	Francour et al. (2010)
2009	East Corsica (France)	1	Francour et al. (2010)
2009	Radhima, Vlora Bay (Albania)	1	Bego and Kashta (2012)
2012	Mali Ston Bay (Croatia)	1	Šprem et al. (2014)
2017	off Erdemli coast (Turkey)	1	Ergüden et al. (2019)
2021	Ghar El Melh (Tunisia)	1	Ennajar et al. (2020)

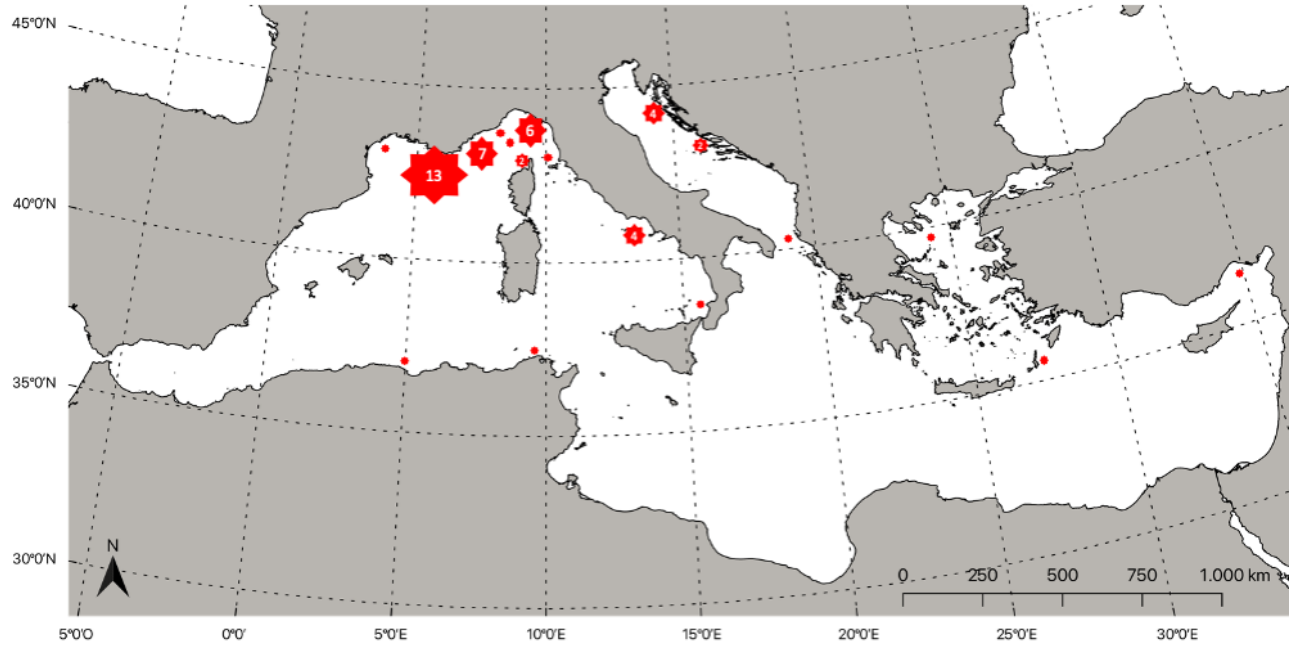


Figure 1. Distribution map of the Lampridae family occurrence in the Mediterranean Sea, in this case of the unique species reported in the basin *Lampris guttatus*. Red stars show the occurrence of a single specimen, the number inside the stars means the occurrence of the specified number of specimens in the area.

1.1.2 - Lophotidae (Bonaparte, 1845)

The family Lophotidae, commonly named crestfishes, consists of two mesopelagic fish genera, *Eumecichthys* and *Lophotus*, the first monospecific, while the second comprises four different species [135]. The elongated and compressed morphology of the body, which sometimes can reach 2 meters in length, is characterized by a large freshly crest or horn extending the jaw in the species of genus *Lophotus* and protruding far forward of the jaw genus *Eumecichthys* [1,131]. The scales are absent, except for the lateral line ones that show the characteristics of tubular morphology shared between some Lampriformes families. The color of the body is silver with numerous dark vertical bands in *Eumecichthys*; in contrast, in

Lophotus, the bands are absent, and the body color tends to be blue dorsally, grading to silver ventrally with multiple silver/white spots. As with most Lampriformes, the long evident dorsal fin is reddish, such as the pectoral, pelvic, and caudal, which in some species are absent (especially pelvic fins) or reduced (caudal fin). Upper jaw protrusible armed on jaws and vomer of small conical teeth used to prey on cephalopods and small fishes [232]. Crestfishes can use a tubular gland located in the back intestine that can emit through the anus an ink-like black liquid to deter their predators [143,233]. No fisheries information was reported on these fish, considered rare and free of commercial interest. Moreover, the group's taxonomy is uncertain, especially for the genus *Lophotus*, whose main species seems to be *L. lacepede*, while the others need further molecular investigation to be better clarified [156,224].

From the scarce data in the literature, their distribution seems to comprise all the oceans at warm latitudes, recorded from the surface to about 1000 meters of depth [234]. Two specimens of *L. guntheri* were recently reported for the first time in Taiwan by Koeda and Ho in 2017 [233]. Despite this, several other authors historically reported the presence of this family in Asian waters [235,236]. Moreover, Craig and colleagues reported in 2004 the presence of this family in all areas of the Pacific Ocean and part of the Atlantic [237].

Mediterranean distribution of the family Lophotidae is affected by a data deficiency due to the rarity of these fishes but probably to a lower presence in the basin if compared to other families of the order [234]. In the Mediterranean Sea, it was reported the presence of a single species of Lophotidae, *L. lacepede*, occasionally caught with longline or trawling fisheries (Table 3). The occurrence was originally reported in 1890 by Kolombatović, followed by other reports all from the Adriatic Sea before 1950 [238–240]. Some following other authors have reported the presence of the species in the central Mediterranean Sea [135,220,241,242]. Within the Adriatic sea, the presence of *L. lacepede* was confirmed at least six times by other authors [224,243,244].

Regarding the central part of the basin, Tortonese reported in 1970 the occurrence of *L. lacepede* in the Strait of Messina [220], without providing specific details. However, one year later, Magazzù and Zaccone reported the same occurrence in this important ecological area

[245]; another record confirmed the presence of this species in 1980 [246]. Several authors also reported the occurrence in southern Tyrrhenian waters [247–249]. Falsone et al. reported in 2017 on the occurrence off San Vito Lo Capo (northeastern Sicily, Italy) coast of a *L. lacepede* specimen caught with a longline drifting fishery, a typical system used in this area for common dolphinfish (*Coryphaena hippurus*, Linnaeus, 1758) capture [3]. A specimen was reported in 1979 from the western Mediterranean Sea by Portan and Del Cerro [250]. After one year, in 1980, Rey reported the capture of one specimen in the Strait of Gibraltar area [251]. The presence in the western part of the basin was confirmed in 2005 by Rodriguez et al., that reported the occurrence of the larval stage of *L. lacepede* in the Balearic region [252]. The eastern part of the Mediterranean Sea seems to be the preferred area for this species in the basin, which was also reported previously by some other authors. The first documented record in the southern part of this area was by Bilecenoglu et al. in 2001, from Gökova Bay [253]. An interesting report of an adult specimen of 650 mm in length occurred at 329 meters in 2017 in the northern part of the Turkish Aegean Sea [254]. Minos et al. e Minasidis & Kaminas reported respectively, in 2015 and 2021, the occurrence of *L. lacepede* from the northern part of the Aegean Sea in Greece. In 2019, Aga-Spyridopoulou et al. reported, through a citizen science project, the occurrence in the central and northern Aegean Sea of 5 specimens detected between 2016 and 2018 [255]. In the same year, Yapici reported the occurrence in the eastern part of the Mediterranean basin of a specimen photographed by a scuba diver and successively identified as *L. lacepede* in the Levantine Sea (Turkey) [256]. A very recent manuscript reported for the first time the species off the coast of Syria [257]. Figure 2 shows the estimated distribution of the mentioned records.

Table 3. Bibliographic references of the Lophotidae family records in the Mediterranean Sea. N.R. means not reported.

Family, Genus	Species	Year	Mediterranean area	Number of specimens	References
Lophotidae, *Lophotus*	*Lophotus lacepede*	before 1890	Adriatic Sea (Croatia)	1	Kolombatović (1890)
		before 1948	North Adriatic Sea	N.R.	Soljan (1948)
		before 1950	Adriatic Sea	1	Morović (1950)
		1970	Strait of Messina (Italy)	1	Tortonese (1970)
		before 1970	Genova (Italy)	1	Tortonese (1970)
		1971	Strait of Messina (Italy)	1	Magazzù & Zaccone (1971)

1979	Sitges (Spain)	1	Portas & Del Cerro (1979)
1980	Strait of Gibraltar	1	Ray (1983)
1980	Strait of Messina (Italy)	1	Giuffrè et al. (1980)
before 1986	Ionian Sea	N.R.	Palmer (1986)
before 1987	Central Mediterranean	N.R.	Bauchot (1987)
before 1988	Greek Sea	N.R.	Papacostantinou (1988)
1989	Sitia, Crete Island (Greece)	1	Minos et al. (2015)
1999	Ischia Island (Italy)	1	Bussotti et al. (1999)
2001	Gökova Bay (Turkey)	1	Bilecenoglu et al. (2001)
2002	Calabria (Italy)	1	Tripepi et al. (2004)
2003	Souda Bay, Crete Island (Greece)	1	Minos et al. (2015)
2005	Balearic region (Spain)	1	Rodriguez et al. (2013)
2007	North Adriatic Sea (Croatia)	2	Dulčic & Ahnelt (2007)
2008	North Adriatic Sea (Croatia)	1	Dulčic & Soldo (2008)
2011	Southern Adriatic (Croatia)	1	Sprem et al. (2014)
2011	cape Poseidi (Greece)	1	Minos et al. (2015)
2012	Punta Licosa (Italy)	1	Psomadakis et al. (2012)
2015	San Vito Lo Capo (Italy)	1	Falsone et al. (2017)
2016	Aegean Sea (Greece)	1	Aga-Spyridopoulou et al. (2019)
2017	northern Aegean Sea (Turkey)	1	Dalyan et al. (2021)
2017	Aegean Sea (Greece)	3	Aga-Spyridopoulou et al. (2019)
2018	Aegean Sea (Greece)	1	Aga-Spyridopoulou et al. (2019)
2018	Çanakkale (Turkey)	1	Tunçer & Kanat (2019)
2019	Levantine Sea (Turkey)	1	Yapici (2019)
2021	Ammouliani Island (Greece)	1	Minasidis & Kaminas (2021)
2021	off Banias (Syria)	1	Ali et al. (2021)

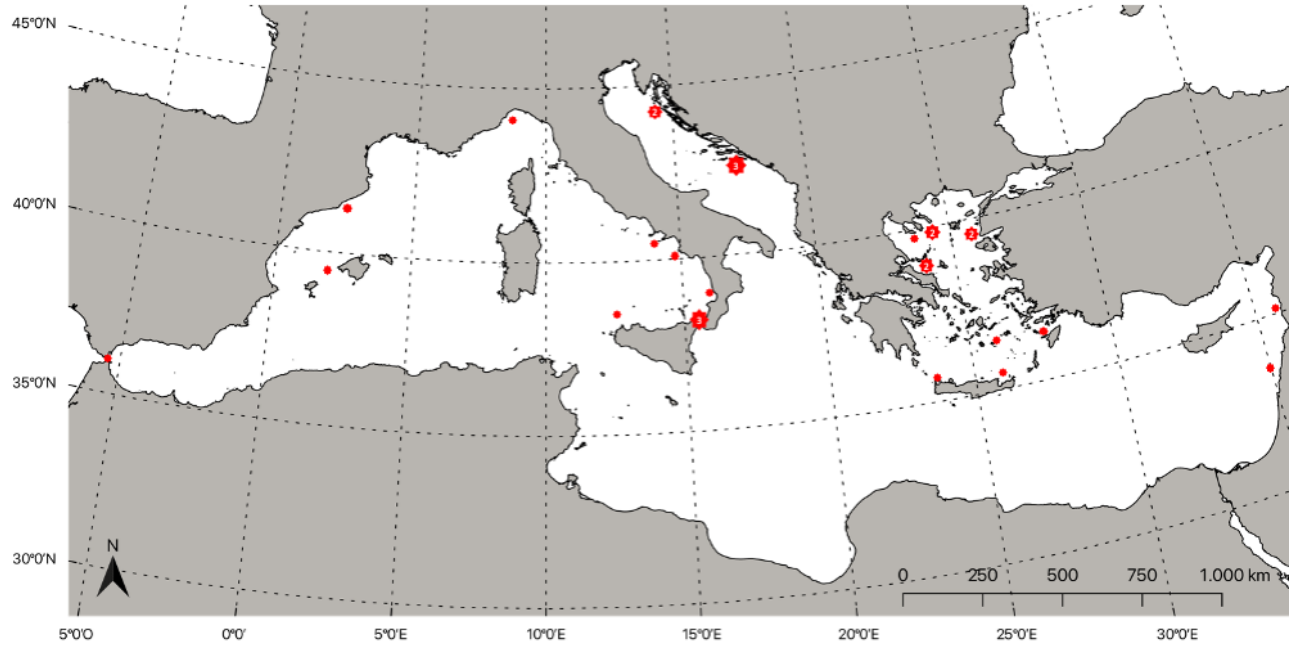


Figure 2. Distribution map of the Lophotidae family occurrence in the Mediterranean Sea, in this case of the unique species reported in the basin *Lophotus lacepede*. Red stars show the occurrence of a single specimen, the number inside the stars means the occurrence of the specified number of specimens in the area.

1.1.3 - Radiicephalidae (Osorio, 1917)

The family Radiicephalidae comprises the genus *Radiicephalus* with two species currently annotated; one of these, *R. kessinger*, was very recently described as a new holotype by Koeda & Ho [150], that based on a molecular approach, was separated from the historical one, *R. elongatus*, originally described by Osório in 1917 [258]. Commonly named tapertails, compared to the previously described families, these fishes are characterized by the smaller dimensions of the body that retain the typical morphological features of the order [1]. Indeed, they present an elongated and compressed naked body, silver-colored with evident red fins. The dorsal one inserts over the eye and develops along the entire body length [127]. The long caudal fin forms a posterior projection that, in live specimens, can reach the body length. Unfortunately, as for all the Lampriformes, it is tough to find undamaged specimens that can perfectly show these peculiar features [259]. The anal fin is reduced and distinguishes them from the members of the Trachipteridae family in which it is absent. Scales are absent, except for the lateral line ones that are tubular. A distinctive characteristic of this family is the fourth, fifth, and sixth preural centra with elongate haemal spines that

pierce the ventral margin of the body [1]. Like Lophotidae, they possess a defense mechanism based on a similar ink gland [131,143], used when threatened. As for the other members of the order, radiicephalids show the protrusibility of the upper jaw, which is toothless and is used to prey on small fishes, crustacean as euphausiid, and molluscs in meso- and bathypelagic environments. The species of this family are considered very rare, and few records occurred until now; hence the knowledge of this group is the poorest of the whole order and needs further records to be adequately enhanced and deepened.

The confirmed distribution of this family spans the Atlantic Ocean, from Spain to South Africa, and the Eastern Pacific [127,135,259]. The recent new annotation of *R. kessinger* in the Western Pacific Ocean (Taiwan) represents the first confirmed report of the Radiicephalidae family in this geographical area [150,233]. From the original description of Osòrio from the Atlantic coastal zone of Morocco [258], the presence of the species in this area was successfully confirmed by some other authors, also by juvenile specimens that are always important to establish the nursery areas [260]. Some other records occurred in the Eastern Atlantic area until its southern part of South Africa [127,200,261–263]. Similarly, the Eastern Pacific Ocean was confirmed as a suitable area for the species by some authors [264–266].

There is no record of *Radiicephalus* specimens in the whole Mediterranean Sea, suggesting the absence of these species in the basin. However, their occurrence in the Atlantic area outside Gibraltar Strait highlights the preference for the oceanic ecological and trophic conditions and the possibility that these fishes could be present, but very rare, in the nearby Mediterranean basin. Moreover, the morphology of this family reminds to little-experienced eyes the Trachipteridae family members that are most common in the Mediterranean Sea. For this reason, incorrect identifications of tapertails as trachipterids may have happened over time. It is also to consider that these lesser-known and much smaller Lampriformes are not interesting from a commercial point of view and rarely collected on scientific surveys. Specific studies in this regard are needed, but to date, based on the present literature, we confirm the absence of this family from the Mediterranean area.

1.1.4 - Regalecidae (Gill, 1884)

The family Regalecidae is one of the most common and, consequently, a well-known taxon of the order [267]. Currently, for this family, two genera are accepted, *Agrostichthys* and *Regalecus*. Commonly named oarfishes, to this group belongs iconic species as *R. glesne*, a fabled silvery fish significantly length (with its maximum recorded length of about 17 meters represent the longest of all bony fishes), bright crimson reddish fins with long rays in the dorsal one [1,2]. Some ancient sea legends and myths are historically linked to this species due to its morphology and dimension and the habit to stands at the surface or swimming out of the water standing on the beaches, especially after windstorms, leading to its association with sea "monsters" [268]. The morphology of the entire family is characterized by the very elongate and compressed shape of the naked (except for tubular lateral-line scales) body, with a long dorsal fin constituted by over 400 elements in some species, with the characteristics of first 8-10 rays extremely flexibles and elongate. Even the pelvic fins' soft rays are significantly developed in length, while the anal fin is often absent or constituted by some few elongated rays. In regalecids, the dorsal and pelvic-fin rays are provided with small spinules that project it laterally, such as the caudal-fin rays, if present. The anal fin is absent, a shared feature with the Trachipteridae family [1,2].

A particular feature of this group is represented by the self-amputation (autotomy) ability [268]. This process involves just the posterior part of the body over the anus and the caudal fin. It probably may occur more times during life because it does not involve any vital organ. The records on lengths over 1.5 meters often show clear signs attributable to autotomy, such as a healed-over stump or "terminus", and the lost part is never regenerated. Maybe this mechanism represents a defense strategy against predators, such as many other examples in both marine and terrestrial organisms [268]. From literature information, some insights on reproduction are known for this family. Sexually mature specimens meet and spawn, as broadcast spawners, between July and December in the North Atlantic Ocean, Mediterranean Sea (Straits of Messina), and the South Pacific (New Zealand-Australia) [241,268,269]. Recently, Oka and colleagues [270] have successfully performed artificial insemination, starting from the gametes of two dead sexually mature specimens of *R.*

russellii that washed up off the coast of Okinawa Island. This study obtained meaningful information on this species' hatching and the larval stage, such as the 18 days needed for the hatching and morphological and behavioral description of the larvae.

The head structure is the same for most Lampriformes, characterized by a protrusible jaw that preys on small fishes, cephalopods, and euphausiids [113]. Regalecids species have mainly mesopelagic habits, and are considered inedible; for this reason, even if sometimes could appear as by-catch in long-line or trawl fisheries pointed to pelagic species, they have no commercial value [60,131]. Despite this, due to its fascinating morphology is considered one of the most common Lampriformes groups (despite being a rare species), and especially with the increase in the importance of citizen science, even the personal records of living oarfish are essential to increase the knowledge on these species. Underwater footage highlighted their ability to swim in a vertical position, commonly called head-up swimming, without muscular effort but by exploiting the movements of the fins and their long appendages. Probably this habit is due to the feeding behavior of these species [268]. Recently, the complete mitochondrial genome of *R. glesne* was annotated by Yu et al. [271]; this represents an important step in deepening the knowledge of this species and its phylogenetic position.

The Regalecidae family is considered distributed in all the oceans, even at high latitudes, from the surface to about 1000 m depth. However, only *R. glesne* seems to have a confirmed worldwide distribution [4,241,272]. In 2002, Roberts reported about a specimen of *R. glesne* that some years before beached in a Naval war base in southern California [273], reporting exciting information on some myths linked to this fish. Other authors also confirmed the presence in the Atlantic Ocean in several areas [241,267,268]. However, the Pacific Ocean represents the geographical area with the most significant number of records in literature. Both species of genus *Regalecus* and the one of genus *Agrostichthys* occurred in their various zones [274–277]. While the genus *Regalecus* is widely distributed in all the Oceans, the genus *Agrostichthys* occurred only in the Southern Oceans and seemed to appreciate these latitudinal areas [2].

Regarding the distribution of the family Regalecidae in the Mediterranean Sea, only the genus Regalecus occurred in 1826 when Risso reported the occurrence of two regalecids in Nice under the name *Gymnetrius gladius* [215]. Many other authors also reported the occurrence of these species in subsequent years, with different specific names until the current annotation, made by Gill in 1884. The distribution of these ancient reports is strictly connected to the central part of the Mediterranean basin, comprehending the geographical zone between the Ligurian Sea and the Gulf of Leon area [218,278–281]. Some more recent authors confirmed the presence in this area [220,247,282–284]. Lozano-Cabo reported in 1969 the occurrence of an oarfish specimen from Mazzarrón, in the Spanish western Mediterranean waters, highlighting its very rare presence in this area of the basin [285]. Indeed, still today this remains the unique record from the Western Mediterranean.

Some ancient reports regard the central part of the Tyrrhenian Sea [217,281], an occurrence confirmed recently by Psomadakis et al. in 2012 with a record of *R. glesne* from Terracina [247]. The presence of this species was also confirmed in the southern Tyrrhenian sea by some authors [286,287], that reported its occurrence in the area near the Strait of Messina, that from the other Lampriformes occurrence data, seems to be an exciting area to study more in-depth the entire order. Another geographical area in which was recorded the presence of *R. glesne* is represented by the Adriatic Sea from both the Italian and Croatian coasts. The occurrence of this species was reported, as *Regalecus gladius,* in 1933 by Padovani in the northern part of this sub-basin [288]; more recently, two different authors confirmed this occurrence (with the currently accepted name) in the central Adriatic sea [8,289]. Dragičević recorded in Palagruža Island the occurrence of the early life stage of the species, an essential reference to deepen the study of breeding areas in the Mediterranean basin.

Just a few authors have recently reported the presence of *R. glesne* in Aegean Sea [290,291], which however was confirmed by Corsini-Foka in 2009 [228], that reported the occurrence of one specimen in 1993 off the coast of Gennadi (Rhodes). From the review of the reports currently available in the literature regarding the Regalecidae family, just the presence of *R. glesne* was confirmed in the Mediterranean Sea (Table 4). In detail, the northern part of the central Mediterranean Sea seems to be the area of preference for this species (Figure 3).

However, ancient records present some problems related to the different nomenclature of the species over the past centuries. The ancient morphological identification of these fishes is not entirely reliable and should be considered with caution. Moreover, it cannot be neglected that some of the records reported in the list were not caught fish but just observed alive, so it was not simple to discriminate between the two *Regalecus* species, in our opinion. Nevertheless, these fishes are considered rare in the Mediterranean basin.

Table 4. Bibliographic references of the Regalecidae family records in the Mediterranean Sea. N.R. means not reported.

Family, Genus	**Species**	**Year**	**Mediterranean area**	**Number of specimens**	**References**
Regalecidae,	*Regalecus*				
Regalecus	*glesne*	1826	Nice (France)	2	Risso (1826)
		1830	Nice (France)	3	Cuvier and Valenciennes (1835)
		1877	Nice (France)	1	Giglioli (1880)
		1891	Isola d'Elba (Italy)	1	Damiani (1918)
		1897	Beaulieu saint-Jean (Francia)	1	Vayssiére (1917)
		1903	Noli (Italy)	1	Ariola (1904)
		1906	Borghetto S. Spirito (Italy)	1	Vinciguerra (1918)
		1908	Arenzano (Italy)	1	Vinciguerra (1918)
		1910	Monaco (France)	1	Vayssiére (1917)
		1913	Castiglioncello (Italy)	1	Vinciguerra (1918)
		1915	Albissola (Italy)	1	Vinciguerra (1918)
		1917	S Margherita Ligure (Italy)	1	Vinciguerra (1918)
		1932	Rimini (Italy)	1	Padovani (1933)
		1950	Genova (Italy)	1	Guiglia (1950)
		1969	Mazzarrón (Spain)	1	Lozano-Cabo (1969)
		before 1970	Ligurian Sea	N.R.	Tortonese (1970)
		before 1971	Aegean Sea	N.R.	Ondrias (1971)
		1974	Olivieri (Italy)	3	Berdar et al. (1975)
		1980	Strait of Messina (Italy)	1	Cavallaro et al. (1980)
		before 1988	Aegean Sea	N.R.	Papacostantinou (1988)
		1993	Gennadi (Greece)	1	Corsini-Foka (2009)
		2002	Marseille (France)	2	Quero et al. (2003)
		2003	Arenzano (Italy)	1	Psomadakis et al. (2008)
		2009	Stobrec (Croatia)	1	Dulčic et al. (2009)
		2010	Palagruža Island (Croatia)	1	Dragičević et al. (2011)
		2012	Arenzano (Italy)	1	Psomadakis et al. (2012)
		2012	Terracina (Italy)	1	Psomadakis et al. (2012)

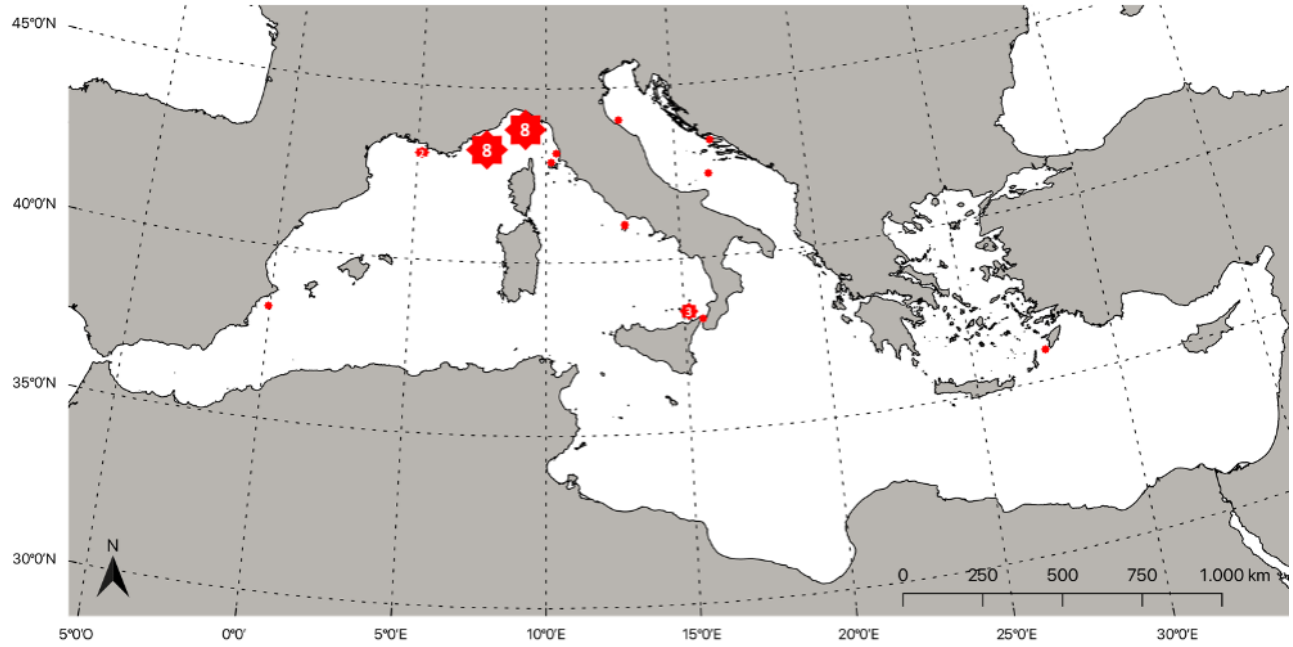


Figure 3. Distribution map of the Regalecidae family occurrence in the Mediterranean Sea, in this case of the unique species reported in the basin *Regalecus glesne*. Red stars show the occurrence of a single specimen, the number inside the stars means the occurrence of the specified number of specimens in the area.

1.1.5 - Trachipteridae (Swainson, 1839)

The family Trachipteridae comprises the common named dealfish and ribbonfish, and it is one of the widely distributed taxa of the Lampriformes order. Currently, three genera are accepted: *Desmodema*, *Trachipterus*, and *Zu*, including ten different species [113]. The largest one is Trachipterus which comprehends six species of dealfish. An elongate and compressed ribbon-like body characterizes the entire family. Notably, in genera *Desmodema* and *Zu*, the body depth decreased, reaching the caudal peduncle; this shape development resulted more accentuated in the species of genus *Zu* [1]. The bright silvery body sometimes shows dark spots (*Desmodema* and *Zu*) or vertical oblique bars (*Trachipterus*), adorned with crimson reddish fins characteristics of the entire order. The dorsal and pelvic fins are usually high elongated, more in larval stages than in adult specimens compared to the body length. Also, the caudal fin shows the upper lobe rays wide elongated as a palette, especially in *Zu* [2]. The anal fin is absent and helps the researchers discriminate between this family and the Radiicephalidae, in which is present even if reduced.

Unlike most Lampriformes, a few small and deciduous scales are present in the dealfish of the genus *Trachipterus* [292]. The lateral line scales are tubular and present sharp spines, sometimes used as identification characters [293]. Also, lateral spiny fins rays, which are founded in oarfish's dorsal, pelvic, and caudal fins, are shown by trachipterids but, in this case, are better developed. Regarding dimensions, the genus *Trachipterus* seems to reach 3 meters of body length, even if the detailed record in literature, with the collection of morphometric and meristic data, regards mainly specimens with maximum length of 1,6 m [220]. The maximum recorded length for *Zu* and *Desmodema* was about 1,4 and 1,1 meters, respectively, confirming the smaller size of these genera than *Trachipterus* [129].

As a distinctive feature of the entire order, in trachipterids, the absence of anterior palatomaxillary ligament and palatine prong leaves the axis maxilla-premaxilla free to extend during jaw protrusion [127]. Trachipterids use this buccal apparat to feed on pelagic cephalopods, crustaceans, and small fishes. The significant quantity of data for this family consent to better explore the feeding behavior of these fishes, which seem to be omnivorous, also comprehending terrestrial food as fragments of pollen cones of pine, petals of terrestrial plant, other fragments of terrestrial grass and beetles [294]. They also feed on aquatic plants and algae and different taxa of crustaceans such as Cladocera, Copepoda, Decapoda, and Isopoda [134]. They are meso- bathypelagic fishes with a wide distribution between surface (mainly for larval stages) and deep-sea environments (up to 2000 m) [24].

Very little is known about their reproductive biology, but eggs are free-floating, large, and red, such as for the other families of the order [6]. Interesting notes on larval/juvenile stage features come from a recent study in the Mediterranean Sea, in which some specimens were observed alive in shallow waters during the early nighttime [17]. These specimens show the typical head-up position during swimming, being carried away by the current avoidance just with dorsal fin movements to maintain their buoyancy. However, when alarmed, a fast-swimming movement used the whole body was shown. Also, feeding behavior characterized by extremely fast protrusions of jaws to prey planktonic organisms was confirmed. From the observed behavior, the authors have confirmed a moon's influence on

this species' occurrence within the planktotrophic web and hypothesized a Batesian mimicry strategy of the species to escape from predators.

The high fragility of these fishes results in unavoidable damage when captured in trawling nets, which is the most common way to capture these fishes as by-catch, along with long lines for big pelagic [5,60]. This feature leads to severe difficulties in morphological identification and discrimination between the vast number of species, and the revision of the literature needs care. For example, differentiating *T. trachypterus* and *T. arcticus* is only reliably through vertebral counts, which differ about ten (84–96 vs. 99–102 respectively) [295]. Molecular approaches could lead in the following years to a new rearrangement of the family taxonomy [16].

The general distribution of the family Trachipteridae comprehends all the Oceans circumglobally [296]. Indeed, their presence was historically reported in the entire Pacific Ocean [293,297,298], Atlantic Ocean [299–301], and Indian Ocean [302,303]. Considering that all the three Trachipterids genera passed through some taxonomical rearrangements, in literature is possible to find many reports with different scientific names for the species.

Within the Lampriformes order, the family Trachipteridae is the better represented in the Mediterranean Sea, which Bonelli reported for the first time in 1920, with the description of the species *Trachipterus cristatus*, today known by the accepted name *Z. cristatus*. Indeed, from the literature, two different genera are present in this basin, *Trachipterus* and *Zu*, with three species in total, *T. trachypterus, T. arcticus,* and *Z. cristatus*. The genus *Desmodema* was never recorded in the Mediterranean basin [304], with any one of the two species, originally described in 1898 by Ogilby, *D. polystictum*, and the "recently" described one by Rosenblatt and Butler in 1977 named *D. lorum* [305].

The genus *Trachipterus* is well distributed in the Mediterranean Sea, represented by the species *T. trachypterus* and *T. arcticus*. Regarding the last one, only a single record occurred in the Mediterranean Sea, from the coasts of Spain not too far from the Gibraltar Strait [267]. However, the presence of this species in the Atlantic Ocean could influenced this occurrence; moreover, the record was not well documented. About the Mediterranean dealfish *T. trachypterus*, apart from the documented record analyzed in this review, some

authors reported on the quite common occurrence of this fish (even with several personal communications). Costa, in 1991 reported in his atlas about the usual occurrence in the Strait of Messina area, of some specimens of Mediterranean dealfish [306].

Also, some authors recorded and confirmed the occurrence from the Aegean Sea [242,253], despite this, the occurrence of *T. trachypterus* in this area remains rare. Jardas, in 1980, reported about the occurrence, in the previous hundred years, of at least forty-six adult specimens from the Adriatic Sea [307]. The Adriatic Sea represents one of the most prevalent areas in the Mediterranean basin for these species, with several records of various life stages of *T. trachypterus,* starting from 1881 to the recent years [220,239,294,307–309]. Some of that comes from the Gulf of Trieste in the Northern Adriatic Sea. It is interesting to note how sometimes the specimens were not in good health conditions, as reported by Borme and Voltolina [134]. Another ecologically interesting feature linked to the occurrence of *T. trachypterus* in the Adriatic Sea is the inconsistency of its records [310]. Indeed, the presence of this species in the basin, especially in the northern part, seems to be related to oceanographical and climatological parameters, linked to the input of intermediate waters in the basin that influence the water characteristics [134]. These thermohaline anomalies coincide with rare species in the Adriatic Sea, trachipterids included, as reported by Jardas and Pallaoro [311]. The occurrence of *T. trachypterus* in the Ligurian Sea, historically reported by Tortonese [312], was confirmed by Garibaldi in 2015 [5], with an interesting analysis of the local longline swordfish fisheries by-catch. The manuscript is based on the percentage of observed non-commercial specimens on board, compared to the commercial ones, among which the species represent about 10% of the former. However, occurrence numbers were not reported.

The occurrence of the Mediterranean dealfish in the central Tyrrhenian Sea was firstly reported by Cau in 1980 [313] with three specimens from off the Sardinia coast, and successively by Psomadakis et al. (2006) [222] and Tiralongo et al. (2020) [50], that recorded one specimen from Anzio and Pianosa respectively. Recently, it was well assessed by Macali and colleagues in 2020, in a fascinating study from Ponza [17], that provided some significant findings on the species. During their study, the authors observed and recorded

eighteen specimens alive with scuba video equipment, of which four were collected and analyzed. This study provided essential information on this species' biological and ecological features, such as the influence of upwelling currents on their presence in shallow waters and the swimming style in vertical positions, with some good video references. The presence of the species in the Eastern Ionian Sea was recently recorded by Mytilineou and colleagues in 2010, with a record from Cephalonia Island [314]. This occurrence was confirmed by three more recent records from the western and central Ionian sea, reported by Tiralongo et al. [24].

Regarding the eastern part of the Mediterranean Basin, the presence of *T. trachypterus* was reported by Golani (1996) [315] from the Israelian Levantine Sea. More recently, Yapici reported about a juvenile specimen video recorded in 2016 from the Çeşme coastline, representing the first record from the Turkish Aegean Sea [256]. More recently, Gökoğlu & Özen (2021) recorded the occurrence of the species from the Gulf of Antalya, extending the information from the Turkish waters [316]. The occurrence in the Aegean Sea was recently confirmed by Kaminas et al. [177] with a conference poster based on citizen science contributions, which reported six different specimens observed in Aegean waters.

The genus *Zu* is constituted of two species, *Z. elongatus* and *Z. cristatus*, whose general distribution covers all the oceans, even if not in an equal way. Indeed, these fishes are more commonly recorded in some areas, like the Pacific Ocean [135,150,200,303,317,318]. Moreover, the distribution of the two species is not comparable, especially in particular areas such as the Mediterranean Sea, in which many authors assessed the presence of *Z. cristatus*, while *Z. elongatus* was never recorded and considered absent [2,129,148]. In the western Mediterranean basin, the scalloped ribbonfish was originally reported in the Balearic Sea as *Trachiptenes cristatus* by Oliver in 1955 [319]. Ibáñez and Gállego (1974) reported about the occurrence from Blanes (Spain) [320], and successively other two specimens were detailed reported by Roig and Demestre in 1982 from the same area on Cataluña [321]. More recently, García–Barcelona et al. (2014) have reported on the occurrence in swordfish longline fisheries of two *Z. cristatus* specimens off the coasts of the Balearic Islands.

Moving to the central part of the Mediterranean Sea, many records of *Z. cristatus* occurred in the Ligurian Sea, starting from the original description (with the original name of *T. cristatus*) of the species by Bonelli, that in 1920 reported on the capture in La Spezia of a specimen of 70 cm with crustacean and cephalopod remains in their stomach [322]. Several other authors have successfully reported this species' presence in this area [130,202,323]. Garibaldi (2015) has recently included in his analysis of the local longline swordfish fisheries by-catch, even this species, over than the already mentioned *T. trachypterus* [5]. In this case, the percentage of scalloped ribbonfish observed on board among the non-commercial specimens, compared to the Mediterranean dealfish one, more than doubled, representing about 25% of the species not saleable. Unfortunately, the raw data were not reported, but it is conceivable that over 50 specimens were observed during the study.

Some other reports regarding the occurrence of the species come from the central/south part of the Tyrrhenian Sea [222,313,324]. Falsone and colleagues have recently reported a specimen that occurred off the coast of San Vito lo Capo (Sicily), with some important information about age and sexual maturation [3]. Indeed, from the otolith and vertebrae analysis of the adult specimens of about 88 cm in total length, eleven accretion rings were counted. Moreover, the gonads of the male specimen caught during the Summer of 2015 developed at maturity stage 3. During the same year, Zenetos et al. [77] reported the occurrence of *Z. cristatus* unusually captured with a fishing rod in Vibo Valentia (south Tyrrhenian Sea). Two recent studies by Tiralongo and colleagues have collected some different occurrences in the central part of the basin involving all of the Tyrrhenian Sea in latitudinal extension (from Capraia to Vibo Valentia) and the Ionian Sea. [24,50]. Bradai and El Ouaer [325]studied more in-depth in 2012 a specimen captured in 1954 in Tunisian waters (Gulf of Tunis) previously described by Postel in 1955 [326] and conserved in the oceanographic museum of the Institut National des Sciences et Technologies de la Mer (Salammbô, Tunisia). Comparing that specimen with a new one that occurred in 2009 in Mahdia, the authors have confirmed the presence in the southern part of the central Mediterranean Sea [325,326].

Regarding the Adriatic Sea, in a similar way to *T. trachypterus*, Jardas reported in 1980 that at least 16 specimens of *Z. cristatus* occurred between 1946 and 1973 in the central and north Adriatic Sea [307]. In 2002, Dulčic reported the occurrence between planktonic samples of some eggs attributable to *Z. cristatus*, with some important information on their morphology and features [6]. Indeed, the author confirmed the autumnal occurrence of the eggs of this species in accordance with the previous hypothesized spawning period. The same author has reported, with some colleagues in 2014, the occurrence of three separate juvenile specimens from Croatian waters [327]. The occurrence in the Aegean Sea was recently confirmed by Kaminas et al. (2021) with a poster presentation based on citizen science contributions, which reported two different specimens observed in Dodecanese Islands and Levos Island, respectively [177].

Considering the above reported wide distribution in all areas of the Mediterranean basin (Table 5) and that this fish occurred in the same manner as all the other families (as by-catch in trawling nets or longline fisheries in mid and depth waters), it is reasonable to state that this family is more common, and better adapted to the biological and trophic conditions of the Mediterranean Sea. For this reason, more information about Trachipterids ecological interactions is available and their role in the trophic webs is more known and defined [134,328,329]. For this reason, they are even more interesting from a research point of view. Figure 4 shows the estimated distribution of the mentioned records.

Table 5. Bibliographic references of the Trachipteridae family records in the Mediterranean Sea. N.R. means not reported. *the number outside the brackets indicates the collected specimens; the number outside the brackets indicates the observed specimens.

Family, Genus	Species	Year	Mediterranean area	Number of specimens	References
Trachipteridae, *Trachipterus*	*Trachipterus arcticus*	before 1986	Spanish coasts	1	Robins and Ray (1986)
Trachipteridae, *Trachipterus*	*Trachipterus trachypterus*	1881	Adriatic Sea (Croatia)	N.R.	Kolombatović (1890)
		1888	Gulf of Trieste (Italy)	2	Marcuzzi (1972)
		before 1980	Grignano (Italy)	46	Jardas (1980)
		1980	off Sardinia coast (Italy)	3	Cau (1980)
		1992	Grignano (Italy)	1	Bussani (1992)
		1992	Ronek Cape (Slovenia)	1	Dulčic & Lipej (1997)

		1996	Eastern Levant Sea (Israel)	1	Golani (1996)
		1996	Stončica (Croatia)	1	Dulčic (1996)
		2000	Anzio (Italy)	1	Psomadakis et al. (2006)
		2006	Gulf of Trieste (Italy)	5	Borme & Voltolina (2006)
		2010	Cephalonia Island (Greece)	1	Mytilineou et al. (2013)
		2010-2013	Ligurian Sea (Italy)	N.R.	Garibaldi (2015)
		2016	Çeşme (Turkey)	1	Yapici (2019)
		2017	Scanzano Ionico (Italy)	1	Tirlongo et al. (2019)
		2018	Porto Cesareo (Italy)	1	Tirlongo et al. (2019)
		2018	Marzamemi (Italy)	1	Tirlongo et al. (2019)
		2018	Izola (Slovenia)	1	Lipej et al. (2018)
		2018	Ponza (Italy)	4 (18)*	Macali et al. (2020)
		2019	Pianosa (Italy)	1	Tiralongo et al. (2020)
		2020	Gulf of Antalya (Turkey)	1	Gökoğlu & Özen (2021)
		2021	Maliakos Gulf (Greece)	1	Kaminas et al. (2021)
		2021	Pagasitikos Gulf (Greece)	1	Kaminas et al. (2021)
		2021	Attica Peninsula (Greece)	2	Kaminas et al. (2021)
		2021	Kerkira Island (Greece)	2	Kaminas et al. (2021)
Trachipteridae, *Zu*	*Zu cristatus*	1918	La Spezia (Italy)	1	Bonelli (1920)
		1954	Gulf of Tunis (Tunis)	1	Postel (1955)
		1955	Palma de Mallorca (Spain)	1	Oliver (1955)
		1958	Gulf of Genova (Italy)	1	Tortonese (1958)
		1969	Blanes (Spain)	1	Ibáñez & Gállego (1974)
		1976	Gulf of Genova (Italy)	1	Gavagnin (1976)
		1979	off Sardinia coast (Italy)	1	Cau (1980)
		before 1980	central/northern Adriatic Sea	16	Jardas (1980)
		1980	Arenys de Mar (Spain)	1	Roig & Demestre (1982)
		1981	Malgrat de Mar (Spain)	1	Roig & Demestre (1982)
		1998	Gulf of Castellammare (Italy)	2	Bianco et al. (2006)
		1998-2000	Duće (Croatia)	eggs	Dulčic (2002)
		2000	Anzio (Italy)	1	Psomadakis et al. (2006)
		2003	Gulf of Genova (Italy)	2	Psomadakis et al. (2007)
		2004	Vis Island (Croatia)	1	Dulčic et al. (2014)
		2009	Zadar (Croatia)	1	Dulčic et al. (2014)
		2009	Mahdia (Tunis)	1	Bradai & El Ouaer (2012)
		2010-2013	Ligurian Sea (Italy)	N.R.	Garibaldi (2015)
		2013	Isla de Cabrera (Spain)	1	García–Barcelona et al. (2014)
		2013	Hvar Island (Croatia)	1	Dulčic et al. (2014)
		2014	Isla de Formentera (Spain)	1	García–Barcelona et al. (2014)
		2014	Vibo Valentia (Italy)	1	Zenetos et al. (2015)
		2015	San Vito lo Capo (Italy)	1	Fasone et al. (2017)
		2017	Civitavecchia (Italy)	1	Tiralongo et al. (2019)

	2018	Ponza (Italy)	1	Tiralongo et al. (2019)
	2018	Avola (Italy)	1	Tiralongo et al. (2019)
	2018	Porto Cesareo (Italy)	2	Tiralongo et al. (2019)
	2018	Briatico (Italy)	1	Tiralongo et al. (2020)
	2019	Pianosa (Italy)	1	Tiralongo et al. (2020)
	2019	Capraia (Italy)	1	Tiralongo et al. (2020)
	2019	Fiumicino (Italy)	1	Tiralongo et al. (2020)
	2021	Dodecanese Islands (Greece)	1	Kaminas et al. (2021)
	2021	Lesvos Island (Greece)	1	Kaminas et al. (2021)

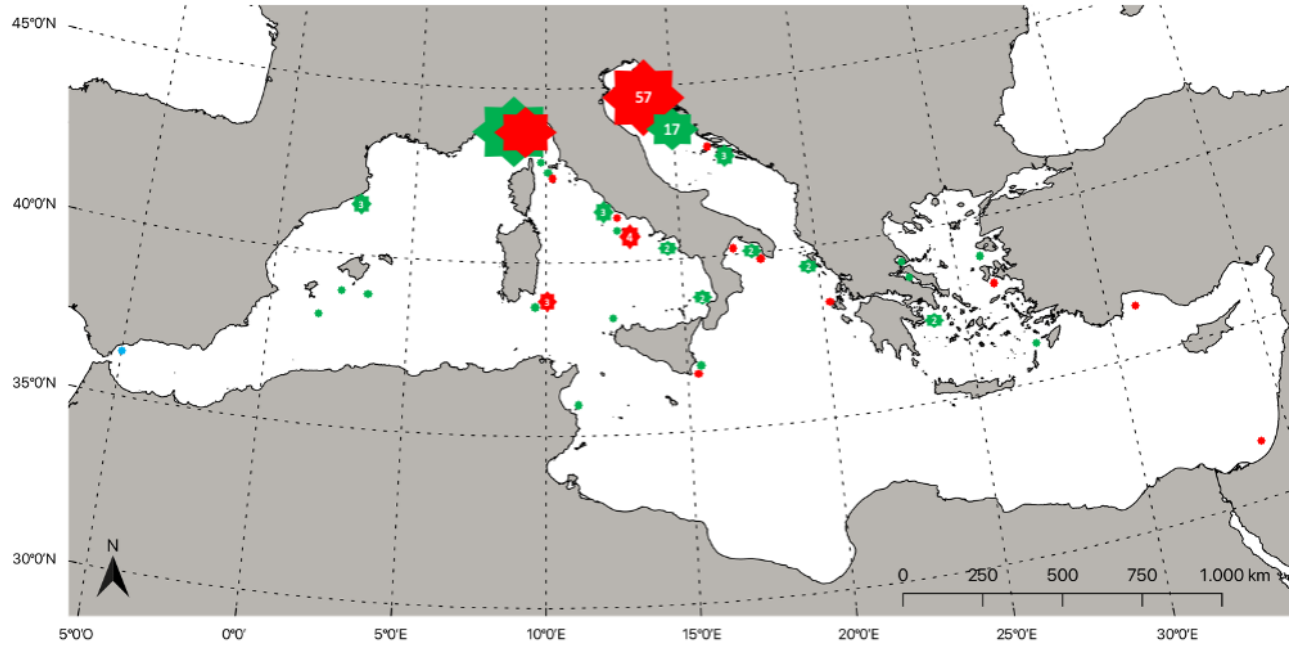


Figure 4. Distribution map of the Trachipteridae family occurrence in the Mediterranean Sea. The three species reported in the basin were identified by the different colors of the stars. Blue stars indicate the distribution of *Trachipterus arcticus*; red stars indicate the distribution of *Trachipterus trachypterus*; green stars indicate the distribution of *Zu cristatus*. Small stars show the occurrence of a single specimen, the number inside the stars means the occurrence of the specified number of specimens in the area; for the Ligurian Sea distribution of *T. trachypterus* and *Z. cristatus* the numbers were not indicated because were not shown in some studies, but the dimension of the stars approximately estimate the quantity.

1.1.6 - Veliferidae (Bleeker, 1859)

The Veliferidae, commonly named velifers, is a family that comprehends two monospecific genera, *Metavelifer* with the species *M. multiradiatus* and *Velifer* with the species *V. hypselopterus* [147]. This group is one of the rarest among Lampriformes families, and the information on their regard is very scarce. The two species of this family are of small dimension, especially if compared to the other families of the order [128]. Indeed, *M. multiradiatus* and *V. hypselopterus* can reach 28 cm and 40 cm maximum length, respectively.

The body of veliferids is compressed and disc-shaped, dorsal silvery and whitish in the ventral part, with some vertical dark bars in the sailfin velifer (*V. hypselopterus*) and brown spots in the spinyfin velifer (*M. multiradiatus*) [330]. The morphology of these fishes is characterized by a cranial crest constituted by the frontal bones and by the evident dorsal fins (which led to the family's common name) that covered the body entirely from the dorsal raising to the caudal fin. The dorsal fin is colored by evident yellow bands in *V. hypselopterus* and brown spots in *M. multiradiatus.* The first dorsal fin ray is equal in length to body depth in *V. hypselopterus* [331]. The anal fin presents the same features as the dorsal one but is reduced in dimension. The forked caudal fin shows the same coloration as the dorsal one. A thick, scaly sheath of skin lies at the base of portions of both dorsal and anal fins [128]. The external morphology is characteristic and not mistakable with other Lampriformes; however, a detective character of this group is the reduced number of vertebrae (33-46) compared to the more elongated ones that can reach over 150 vertebrae in some cases [1].

Veliferids are signaled as demersal epi- and mesopelagic species, particularly *M. multiradiatus,* which can reach higher depth (maximum reported 240), while *V. hypselopterus* prefer shallower water [113]. No information about the trophic behavior of this family was reported; considering the typical jaw's morphology and body dimension, they probably fed on tiny pelagic organisms, like the other Lampriformes at the juvenile stages. The Veliferidae family is considered the sister group of all other Lampriformes [113]. For this reason, despite being very rare, it remains one of the most studied families from a phylogenetic relationship point of view [158,159,332].

The distribution of these species seems to be very strictly reduced to the Indo-West Pacific area of the world. Indeed, based on the record present in the literature, the presence of veliferids was reported in the waters of Mozambique and Madagascar, the Australian continent, India, Japan, Indonesia, and Hawaii [333–336]. Recently, five specimens of *V. hypselopterus* occurred in trawling nets off the coast of Oman [337]. From the same area, Al-Mamry and Jawad reported in 2021 the occurrence of ten specimens (six females, four males) of sailfin velifer [331], with the essential collection of biometric and meristic data, very useful to deepen the knowledge of this rare species.

The occurrence in Mediterranean Sea waters was never recorded. As for the Radiicephalidae family, based on the current literature, we can state that this family is not present in the Mediterranean basin.

1.2 Considerations on the Mediterranean Lampriformes

Monitoring the distribution of living organisms is essential, especially in a climate global changing period that exerts environmental pressure on all organisms. In response to this, the living organisms modify their living range, both in geographical terms and, especially for aquatic organisms, including bathymetric movements. This topic is even more critical when it involves rare organisms due to their specific characteristics, as in the case of the members of the order Lampriformes. The literature review shows that these organisms are within marine trophic webs along most of the water column, inhabiting it from the surface down to the abyssal belt.

Despite this, very little is known about them due to their elusiveness and limited reports, partly due to their scant importance in fisheries. However, from a research point of view, these species represent an exciting group to deepen and better assess their ecological roles, morphological and molecular features, and phylogenetic relationships. Indeed, all these topics are little explored, and many issues are unclear or not well explored. For these reasons, this thesis aimed to clarify the Mediterranean Sea distribution of the entire Lampriformes order, provide researchers in this area with a comprehensive reference to support their studies, and enhance the importance of deepening it. The present document summarizes what is known on this topic, from the first species descriptions in the Mediterranean basin.

This topic results affected by fragmentation of reports, which very often have been treated superficially and in the margins of more extensive studies on species deemed commercially more important. Moreover, the phylogenetic positions and relationships within the order have changed many times in its history, making it harder to investigate them. Only recently, with the increased use of molecular approaches to support phylogenies, we have begun to understand these aspects more appropriately.

We show that four families of this rather vast order are represented in the Mediterranean Sea, which counts six worldwide distributed [296]. Indeed, Lampridae, Lophotidae, Regalecidae, and Trachipteridae, have been reported since early 1800 in several areas of the Mediterranean basin. Lampridae family is represented by the species *L. guttatus*, primarily present in the northern area of the Central Mediterranean Sea, from both French and Italian parts of the Ligurian Sea and the Adriatic Sea. The family Lophotidae is, in the same manner, represented by only one species, *L. lacepede*, historically present in Aegean waters and secondly in the Adriatic Sea, while recently occurring in some other areas of the basin. Indeed, this family show records widespread in the whole Mediterranean basin from the Gibraltar Strait to the Turkish waters. The Regalecidae family is represented by *R. glesne*, with an evident prevalence of records from the central part of the basin (Ligurian Sea) like Lampridae, even if supported by a less amount of data in this case. The Trachipteridae family is the most abundant and widely distributed taxon among Lampriformes fishes in the Mediterranean Sea. Indeed, it is represented by three species, *T. arcticus*, *T. trachypterus*, and *Z. cristatus*. Regarding the first one, it was recorded once a time in the basin, in the Spanish waters near the Gibraltar Strait, probably representing a specimen entering from the nearby Atlantic Ocean, where it is more common. On the contrary, *T. trachypterus* and *Z. cristatus* are the most represented species in the Mediterranean Sea, showing a preference for the central part of the basin, with a relatively high number of records from the Adriatic Sea, Ligurian Sea, and the whole Tyrrhenian Sea. The families Radiicephalidae and Veliferidae are not currently reported in the Mediterranean Sea.

Considering that, in the Mediterranean basin, the occurrence of these fishes is mainly linked to occasional capture by longline and midwater trawl fisheries as by-catch, a considerable amount of potential data gets lost every day, as often these specimens are thrown back into the sea without being considered. This reflects what happens in the rest of the world's seas, where these species are considered rare but not endangered, and all listed as Least Concern (LC) by IUCN Red List [338]. In fact, the limited knowledge on their regards does not allow to give an adequate evaluation of the conservation status of these species. Moreover, specimens caught with the trawling nets often show morphologically altered features and

are difficult to identify adequately. In this perspective, it is even more essential to use molecular approaches to identify fish that are phenotypically similar and distinguishing among them. Indeed, identification keys have changed many times such as the classification, for this reason, this literature review was not given particular attention to the older, not detailed identifications or personal communications. The current citizen science, supported by appropriate, photographic material, is instead a resource to be exploited more in the study of rare species such as Lampriformes. In any case, without the support of the research it will not be possible to obtain better knowledge, so it is required that the scientific community makes a more significant effort with dedicated projects to deepen the study of this interesting order.

2. *Zu cristatus* (BONELLI, 1820)

The scalloped ribbonfish *Z. cristatus* (Bonelli, 1820) is a meso-bathypelagic and cosmopolitan species which inhabits the Mediterranean Sea, the Azores and Madeira in the Atlantic, Pacific and Indian Oceans [3]. Data on the biology and ecology of this species are limited. Literature sources report that this is the only species of the genus inhabits the Mediterranean Sea [296]. It was reported in tropical and temperate waters of all the oceans [3,6,24,130,324,325]. In particular, in the Mediterranean basin, individuals of this family are often caught accidentally with professional fishing gear (mainly deep-sea longline and trawling nets), but rarely and in small numbers [3,5,17,24]. Its presence has been reported at various depths, from shallow water at juvenile stages to deep-sea environments, until 2000m, as recently documented by Tiralongo and colleagues, highlighting its meso-bathypelagic nature strictly related to its ontogenetic development [24].

The body shape of this species appears elongated and laterally compressed, with a typical taper posterior portion of the body until the tail. The ventral edge of the body shows the typical scalloped progress, which justifies the common name of "scalloped ribbonfish", just after the anus. As with other Trachipteridae, some dermal tubercles are present throughout the trunk. The body is almost naked, with a few cycloid and deciduous scales predominantly on the caudal peduncle and along lateral-line plates. These plates are armed with conical spines which point laterally (features that characterized the genus *Zu* from the rest of the Trachipteridae family members) [133]. The lateral line runs in the high portion of the trunk until the pelvic fins, then proceed ventrally until the anus, which assumes a zigzag pattern with a significant elongation of lateral-line plates. The maximum recorded dimension of the body was around 120-140cm in length and 4-5kg in weight.

The pigmentation that characterizes the species is predominantly silver with bronze reflections all over the body, with some (generally 6-7) brown or darker transversal bands more or less visible from the anterior to the posterior body portion, depending on the conservation and the life cycle phase of the specimens. However, some authors reported juveniles and adult specimens without the typical banding patterns caught in deep-water trawling fishing [133]. Hence, the coloration pattern can be influenced by the deep (with its

consequential light/darkness condition) and, not negligible, by the conservation status of the samples. Evident bright crimson reddish fins, especially the dorsal and caudal ones. The dorsal fin is elongated (over 120 rays), with the first six rays crested, originating from the posterior margin of the eye. Two parts form the caudal fin; the dorsal lobe consists of 9-12 rays, while the ventral one with up to 5 rays. The ventral lobe appears as a peduncle created by the partial fusion of these rays. Pelvic fins and anal fins appear absent in adults. Sometimes, a short bony base, like a peduncle, appears in pelvic fins insertions. The pectoral fin is composed of 10-13 rays, the first of which appears shorter and stouter. The dorsal, pectoral, and caudal fins are provided with reduced to absent spinules on their rays [129].

Despite its elongated shape *Z. cristatus* possess a total vertebrae number of 62-69, the lesser within the Trachipteridae family. The buccal cavity is armed with 14-21 caniniform solid teeth on the upper jaw and 10-12 on the lower one, while vomerine and palatine (if present) teeth result in 2 to 4. Generally, the total gill rakers are 11, eight of which are ceratobranchial and three epibranchial [113].

The juvenile stage of *Z. cristatus* shows some morphological differences compared to the adult stage. The body is much more elongated and laterally compressed than adults, with the same tapered shape in the posterior portion. The scalloped shape that characterizes the species is also present, and the dermal tubercles are present throughout the trunk, not as evident as in adults. A higher number of deciduous scales are more diffused on the body, while lateral-line plates already present in the caudal region result in stronger and more prominent ones in their anterior part compared to the adult stage [339]. Moreover, in the juvenile stage, the first six dorsal-fin rays are incredibly elongated, as pennant, as well as the pelvic and caudal fins, which successfully are reduced or lost becoming adults [127]. Despite some authors attributing the metamorphosis stage between larvae and juveniles to a size matter, it seems conceivable that this change occurs in *Z. cristatus* through some morpho-functional modification. Among these, the main ones are the elongation of the first six dorsal-fin rays; an anterior displacement of the pelvic fin with the contemporary elongation of its rays; the ventral constriction that occurs immediately after the anus, forming a thin elongate tail; the increasing of the depth in the anterior part of the body; the

formation of wavy progress of spined lateral-line scales in the caudal region; the formation of evident dark vertical bars along the body [140,304].

During the transition between larval and juvenile stages, in connection with the ontogenic changes, a modification in habitat preference is frequently recorded. Indeed, the small specimens have been generally collected from shallow waters relatively near the shore. Similarly, larval, and juvenile stages prefer to inhabit the photic zone. However, this more profound bathymetrical migration probably occurs gradually because some later juvenile stages were also found in deep waters [339]. This bathymetrical preference was apparent when considering that adult scalloped ribbonfish samples are sporadic in systematic collections. Indeed, due to the fisheries' pressure on the nearshore habitats, result more common to caught larval and juvenile stages compared to adults, which inhabit offshore epipelagic-to mesopelagic habitats, rarely approached because of expensive and often poor commercially exciting prey. Just rarely *Z. cristatus* is collected as a by-catch with particular fishing systems such as mid- and deep-water trawling nets and longlines fisheries [5,60].

From video-recorded images of live juvenile species, it is prominent how these long filaments are the main locomotory engine in this life cycle stage. This species is also known for its locomotory features, particularly its head-up swimming style [340]. Even more than adults with a more developed muscle mass, the ability to swim in the typical vertical position is possible in the juvenile stage thanks to the dorsal/caudal fins undulations, favorited by the scalloped shape of the body with the tapering of the caudal portion. Adult scalloped ribbonfish's diet mainly comprises small fish and cephalopods [341]. The few records of *Z. cristatus* in the Mediterranean basin show that this fish is present in the entire geographical area, with prevalence in the Central Mediterranean Sea [3,6,177]. From the scarce literature, the capture of scalloped ribbonfish seems to be related to the spawning season, which occurs in the late spring-first summer, when these fish are more mobile and defenseless [6,342]. Eggs are red, planktonic, and relatively big-sized (up to 2.3mm) [6]. In particular, the occurrence of *Z. cristatus* has been reported in the Adriatic Sea [6], Ionian Sea [24], Ligurian Sea [5,343,344], Tyrrhenian Sea [3,77,324], the western Mediterranean between

the coasts of Spain and Algeria [234,345], Catalunya [320,321] and in the Gulf of Tunis (North of Tunisia) [325], the Eastern Mediterranean in Greece [291] and Turkey [346].

Due to the scarcity of the adult stage collection and their limited data from literature, the knowledge in their regard is currently limited and marginally assessed. For this reason, most identification keys, and diagnostic characters available for this species were based mainly on juvenile characters. Considering that, as already mentioned, the larval, juvenile, and adult stages of *Z. cristatus* are somewhat markedly different, every new contribution adds essential information. Therefore, here below, we report the data related to the capture, identification, and in-depth analysis of all the morphometric and meristic features of an adult specimen of *Z. cristatus*, as one of the largest ever reported in the Mediterranean Sea.

2.1 Experimental procedures

2.1.1 - Sampling

An adult specimen of *Z. cristatus* was occasionally captured at about 720m of depth by longline swordfish fisheries off the coast of Noto, Syracuse, Italy (36°50'05" N 15°16'49" E) (Figure 5).

Figure 5. Capture site of *Z. cristatus* (36°50'05" N 15°16'49" E). Figure obtained with GIS software [347].

Historically, the fishery of this area is focused on capturing commercially important species such as *Lepidopus caudatus* (Euphrasen, 1788) and *Xiphias gladius* (Linnaeus, 1758). Lampridiformes are considered as by-catch species and often discarded as fishing waste [49,91]. The analyzed sample was retained upon our request to fisherman, partially identified on board, and stored at -20° for further analysis on the specimen (Figure 6).

Despite this species has been reported at various depths, from shallow water at juvenile stages to deep-sea environments, in the Ionian Sea this is the second record, following the recent one documented by Tiralongo and colleagues for the same area [24]. It is interesting to note how both these two specimens are among the deepest overall (720m and 2000m, respectively), thus denoting, based on the few available data, a tendency of this species to live deeper than normal in this area of the Mediterranean Sea.

Figure 6. *Z. cristatus* specimen of this study just hoisted on board.

2.1.2 - Morphological identification: results and morpho-meristic data discussion

The specimen identification and necroscopy procedures were performed at the Department of Chemical, Biological, Pharmacological and Environmental Sciences of the University of Messina (Messina, Italy). The sample was transported frozen and immediately processed after its arrived at the laboratory. All the measurements for identification were taken following identification keys proposed by Olney in 1999 for the Trachipteridae family [1]. Biometric data were compared to the Standard Length (SL), Total Length (TL) and Head Length (HL), as a percentage (Table 6). Epaxial musculature samples were taken for further

molecular identification and stored at -80°C. Length data were collected using a standard icthyometer of 100cm length and precision of 1mm, additionally other precision measuring sticks (0.1mm in accuracy) for the fins and detailed measures, while a precision scale was used for the total weight (UW8200S, Shimadzu Corporation, Kyoto, Japan).

The analyzed specimen was identified, through morphological identification, as *Z. cristatus*, a species characterized by the standard morphological features of the Trachipteridae family [1,127] (Figure 7). The analysis of diagnostic characters shows the presence of 62 vertebrae and 11 total gill rakes (3 epibranchial, 8 ceratobranchial); dorsal fin rays were 122, pectoral fin rays 9, while two lobes formed the caudal fin; spiny plates adorned the ventral portion of the tail; the lateral line resulted formed by 101 scales, of which the last 47 were spiny and point in an alternate direction, while the rest of the body resulted naked; the caudal portion of the body was evidently scalloped; the maximum body height resulted in 21.7% of SL (standard length), and eye diameter 34.3% of HL (head length).

Figure 7. Biometric and meristic data collection of the *Z. cristatus* specimen during the necroscopy. a, entire specimen; b, measurement check; c, posterior view of gill rakes; d, detail of the maxilla and the premaxilla extension during jaw protrusion.

Tables 6 and 7 reported all the biometric and meristic data obtained from the morphological examination of the specimen, also providing a comparison with literature data of other specimens previously reported in the Mediterranean area.

Table 6. Biometric data of the specimen of this study, compared with some *Z. cristatus* specimens reported with details in the Mediterranean area. [1] Falsone et al., 2017; [2] Psomadakis et al., 2007; [3] Tortonese, 1958; [4] Roig & Demestre, 1982; [5] Bianco et al., 2006; [6] Ibanez & Gallego, 1974; [7] Garcia-Barcelona et al., 2014.

% SL was referred to as a percentage of the standard length; % TL was referred to as a percentage of the total length; % HL was referred to as a percentage of the head length. * Identify the studies with two described specimens. The first column numbers were all referred to the specimens 1 reported in the study; the second column numbers were all referred to specimens 2 reported in the study.

Biometric data (mm)	**Present study (Ionian Sea)**		**[1]Southern Tyrrhenian Sea**		**[2]Ligurian Sea***		**[3]Ligurian Sea**		**[4]Iberian Sea***		**[5]Central Tyrrhenian Sea**		**[6]Iberian Sea**		**[7]Balearic Sea**	
Total length	1210		876		1031 - 1219		1105		1115 - 700				875		878	
Fork length	1090															
		% TL		**% TL**		**% TL**		**% TL**		**% TL**		**% TL**		**% TL**		**% TL**
Standard length	1060	87,6	733	83.7	926 - 1105	89.8 - 90.6	980	88.7	1000	89.7	180		785	89.7	803	91.5
		% SL		**% SL**		**% SL**		**% SL**		**% SL**		**% SL**		**% SL**		**% SL**
Head length	181	17,1	128	17.5	153 - 191	16.5 - 17.3	160	16.3	165 - 85		38	21	175	22.3	165	20.5
Pre-orbital length	50	4,7	39.6	5.4		5.6		5.6	5 - 3				55	7		
Post-orbital length	58	5,5							5.5 - 2.7							
Eye diameter	62	5,8	47.8	6.5		6.2		5.8					66	8.4	54	6.7
Opeculum height	198	18,7	146.2	19.9												
Upper jaw length	75	7,1	53.7	7.3											68	8.5
Lower jaw length	107	10,1	75.3	10.3		9.2									93	11.6

Pre-pectoral length	161	15,2												
Pectoral fin length	74	7,0	47.7	6.5		6.1 - 6.5		6.6		7 - 3.5			65	8.3	61	7.6
Width of pectoral fin base	13	1,2	10	1.4												
Maximum height of dorsal fin	67	6,3	91.3	12.5		6.6		7.1								
Dorsal fin length	950	89,6													840	104.6
Caudal fin length	128	12,1	130.2	17.3		10.3		12.8		11.5 - 11			90	11.5	66	8.2
Lateral line length	1000	94,3														
Spiny lateral line length	520	49,1														
Maximum height of the body	230	21,7				19.7 - 21.3		21.4		20.5 - 11			195	24.8	125	15.6
		% HL		**% HL**		**% HL**		**% HL**		**% HL**		**% HL**		**% HL**		**% HL**
Pre-orbital length	50	27,6	39.6	30.9		29.4 - 32.5		34.4					55	31.4		
Post-orbital length	58	32,0	46.5	36.3												
Eye diameter	62	34,3	47.8	37.3		34.6 - 35.6		35.6			2.1	5.6	66	37.7	54	32.7
Opeculum height	198	109,4	146.2	114,2												
Upper jaw length	75	41,4	53.7	41.9											68	41.2
Lower jaw length	107	59,1	75.3	58.8											93	56.4
Total weight (g)	4000		1301		4400		2800		2160 - 500							

Table 7. Meristic data of the specimen of this study, compared with some *Z. cristatus* specimens reported with details in the Mediterranean area. [1] Falsone et al., 2017; [2] Psomadakis et al., 2007; [3] Tortonese, 1958; [4] Roig & Demestre, 1982; [5] Bianco et al., 2006; [6] Ibanez & Gallego, 1974; [7] Garcia-Barcelona et al., 2014. * Identify the studies with two described specimens. The first column numbers were all referred to the specimens 1 reported in the study; the second column numbers were all referred to specimens 2 reported in the study.

Meristic data (counts)	**Present study (Ionian Sea)**	**[1]Southern Tyrrhenian Sea**	**[2]Ligurian Sea***	**[3]Ligurian Sea**	**[4]Iberian Sea***	**[5]Central Tyrrhenian Sea**	**[6]Iberian Sea**	**[7]Balearic Sea**
Lateral line scales	101		102 - 96			107		96
Last spine	47							38
Pectoral fin rays	10	11	10 - 11	10	11 -11	12	11	11
Dorsal fin spines	6							
Dorsal fin rays	122	126	125 - 130	125	117 (6-111) - 132 (6-	120	120	119
Caudal fin rays	9+3	9	9 - 9	9+1	126)		9+3	
Caudal fin spines	9				9+4 - 9+4			
Upper jaw teeth	18	18	14 - 21				16	8
Lower jaw teeth	12	12	10 - 10				10	12
Palatine teeth	4	4	4 - 4				2	4
Vomerine teeth	4	3	3 - 4				2	3
Gill rakes total	11	11	10 - 11	11	10 - 10	11		11
Gill rakes epibranchial	3	3	3 - 3	2	2 - 2	3		3
Gill rakes ceratobranchial	8	8	7 - 8	9	8 - 8	8		8
Vertebrae	62	62						

The specimen resulted in 1210mm in total length (TL) and 4000g in weight, and the SL resulted in 87.6% of the TL. It was a mature female with evidently developed gonads (Figure 8). The elongate and compressed, ribbon-like silver body was adorned by evident crimson reddish fins with some (apparently six) oblique dusky bars, more noticeable in the fresh specimen (Figure 6) but still presents after death (Figure 7). Particularly evident was the scalloped shape of the body proceeding towards the tail, resulting in higher pectoral fins insertion, and gradually decrease moving to the caudal portion of the body. The head appears round compared to the shape of rest of the body, with a length of 17.1% compared to the SL, with the upper jaw and lower jaw respectively 41.4% and 59.1% of the HL (respectively 7.1% and 10.1% of SL). The upper jaw was provided with 18 teeth and the lower one with 12, while four palatine and four vomerine teeth were present. The absence of the anterior palatomaxillary ligament and the palatine prong permitted the maxilla to extend freely with the premaxilla during jaw protrusion (Figure 7). The pre-orbital length resulted in 27.6% of the HL (4.7% of SL), the post-orbital length in 32% of HL (5.5 of SL), and the operculum height resulted in 109.4% of HL (18.7 of SL). The eye diameter resulted in 5.8% and 34.3% respectively of SL and HL.

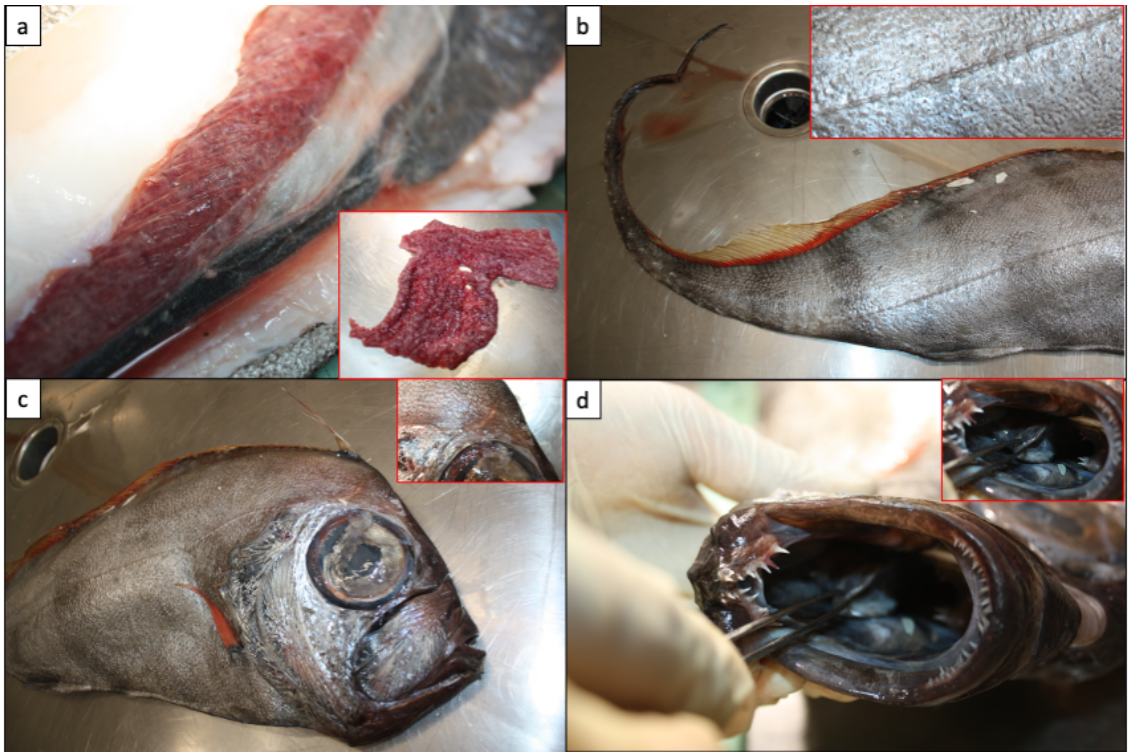


Figure 8. Details of the *Z. cristatus* specimen analyzed in the present study. a, mature female gonads, with details of eggs in the red box; b, posterior part of the *Z. cristatus* with evident ventral and tail spines, and tubular lateral line scales in the red box; c, anterior part of the specimens, with the large eye, oblique pectoral fin, and the first evidently elongated rays of the dorsal fin, insertion of the lateral line in the red box; d, mouth cavity armed with upper and lower jaws teeth, and some palatine ones in the red box.

The dorsal fin originated with six elongated rays above the orbit. It continued for the entire body length until the tail, resulting in 89.6% of SL in length, with a maximum height of 6.3% compared to SL. The dorsal fin showed six evident spines. Among the two lobes which constituted the caudal fin, the upper was upturned and formed by nine elongated rays, while the lower was constituted by three rays fused in a single spiny nubbin. Moreover, the caudal fin showed nine evident spines, resulting in 12.1% in length compared to SL. The anal and the pelvic fins were absent, with the pelvic one just sketched by some spiny nubbins. The pectoral fins were constituted by ten relatively short rays (7% of SL) with the base resulting in 1.2% of SL originating in the ventral half of the body, with an oblique, almost vertical orientation. The lateral line resulted in 94.3% of SL and was constituted by 101 tubular scales, 47 of which bore sharp last spines for a length of this spiny portion resulting in 49.1% of SL.

The Trachipteridae family represents the most diffused in the Mediterranean Sea within the Lampriformes order, with the confirmed presence of at least three species, *T. arcticus*, *T.*

trachypterus, and *Z. cristatus* [129]. Despite this, the knowledge base on these species is still scarce, especially from a molecular point of view. For these reasons, each contribution related to their geographical and bathymetrical occurrence is essential, particularly those accompanied by good morphometric data, description of biological and reproductive features, and genetic achievements. These aspects are essential, especially for species such as *Z. cristatus* that during their life cycle carry out a metamorphosis in the passage from juvenile to the adult stage. Heemstra and Kannemeyer in 1984 related this passage to the size of about 600-800mm, but Palmer in 1961 previously reported that the typical features of juvenile specimens could retain over this size range, relating it to a "definitive developmental stage" not strictly correlated with age or size [127,304]. Supporting this second theory, Ji et al. reported in 2009 the occurrence of a specimen of 528mm in SL that showed almost all the typical features of the adult stage, well below the range indicated by Heemstra and Kannemeyer [140].

The specimen of *Z. cristatus* captured in the late spring of 2020 and described in this study, was an adult female, with well-developed amber gonads extended for the entire abdominal cavity length, ready for eggs emission [348] (Figure 8). Some authors previously reported the late spring/early summer as the spawning season of this species in the Mediterranean Sea [3,130,342]. The eggs appeared reddish colored with a large diameter of over 2mm, as reported by Walters and Fitch in 1960 [339]. This information is also consistent with the founding of some embryonated scalloped ribbonfish eggs by Dulčic et al. in September of 1998 in the Adriatic Sea [6]. Our findings, according to these previous contributions, could suggest for the Ionian Sea the spawning season for this species between May and August. Considering that this is the first contribution of some reproductive features of *Z. cristatus* from the Ionian Sea, we can give no other interpretations of his early life stages in this geographical area.

At the macroscopical examination, the dorsoventrally compressed body, with a pronounced scalloped shape after the anus, is typical of the species *Z. cristatus* in comparison to *Z. elongatus* that usually shows a lighter decreasing of body depth proceeding until the tail [133]. This peculiar body shape could be involved in the typical swimming style of the

species, usually in a head-up position, with a major contribution of the fins compared to muscles. This is confirmed by the reduction in ventral scalloping observed in the adult specimens compared to the juvenile ones; indeed during the adult stage, the contribution of muscles in swimming behavior increases, while in the juveniles the very reduced muscular posterior part of the body is functionally replaced by the fins [304]. The silvery color of the body, with six dark oblique bars, is characteristics for the genus, such as the reddish crimson fins. Despite some authors describing these bars as present only in the juvenile stage, our specimen showed rather evidently this feature, which probably depends on the conservation status of the samples [127]. Despite this, in accordance with Palmer, a reduction in the evidence of dark body bars was observed, proceedings from the head to the caudal region of the body, typical of the adult stage of the species [304]. Occasionally could be present some dark spots on the body or fins, especially in juvenile individuals [133]. Our adult specimen did not show these features.

The specimens analyzed in the current study had a TL of 1210mm, resulting in the second-longest specimen caught among the reported Mediterranean ones, compared to the specimens with a TL of 1219mm one described by Psomadakis et al. in 2007 from the Ligurian Sea [130]. The SL was 1060mm, resulting in 87.6% of the TL. This value results average compared to all other Mediterranean records, which start from 83.7% for a specimen from the Southern Tyrrhenian Sea [3], up to 91.5% of a specimen from the Balearic waters [345]. This percentage is almost identical in all the specimens with a TL comparable to this study, confirming the order of this relation between SL and TL on about 88-89% for the adult stage. Also, the head length shows a similar trend resulting in 17.1% of SL, a common value compared to the other similar specimens analyzed that reached the 16.5% to 17.5% range for this relation. In comparison, the smaller ones show values over 20%, highlighting that in juvenile stages, the head length is greater proportionally to the TL [324].

The pre-orbital length, post-orbital length, and operculum height data did not show differences when compared with the SL among literature data on the studied species, in this case, between adult and juvenile stages. Differently, the evaluation of these measures with the HL showed a not linear relation among the compared specimens, highlighting that the

development of head structure is more variable in this species, depending on different factors, and further studies are needed to clarify the ontogenetic development of this species. Remarkably, the relation between pre-orbital length and HL varies from 27.6% detected in this study, to 35.3% reported for a specimen from the Iberian Sea [321], while the post-orbital length showed a less broad range (31.8%-36.3%). The value of the relationship between the operculum height and the HL was over 100%, 109.4% for this study and 114.2% for the specimen from the Southern Tyrrhenian Sea [3] respectively, due to the particular shape of this species.

Regarding the eye diameter, this species is known for its giant orbits. It is helpful to have a better view in the darkness of deep-sea environments, in which it usually moves during adult life. The specimen of this study showed an eye diameter length that resulted in 5.8% and 34.3%, respectively of SL and HL. These values are comparable with those of specimens of similar size (5.7%-6.2% of SL, 34.3%-35.8% of HL) [130,323] while resulting both lower when compared to smaller specimens (6.5%-8.6% of SL, 32.7%-37.3% of HL), which showed a more prominent eye size compared to the rest of the body [3,320,345]. It is interesting to note how the juvenile specimen reported by Bianco and colleagues [324] shows values of eye diameter length resulting in 5.6% of SL and 26.3% of HL. This shows how the development of the head structure in this species is a priority, if compared to the rest of the body. Indeed, in larval and juvenile stages, swimming is guaranteed by the movements of the long fins [129,131]; consequently lateral musculature is less used and developed compared to the adults..

The studied specimen's upper jaw and lower jaw lengths did not show values significantly different from the others used for different size comparisons with SL, resulting in a range of 7.1%-8.5% and 9.2%-11.6% for the upper jaw and the lower jaw, respectively. Similarly, the comparisons of these measures with the HL resulted in 41.2%-41.9% and 53.4%-59.1% in range for the upper and lower jaw, respectively. However, these represent high values compared to the average of the teleost due to the importance of the buccal apparatus in this species, which could be highly protruded during predation activity, and being essential in a dark environment such as the deep sea.

Regarding pre-pectoral length, lateral line length, and spiny lateral line length, resulted respectively in 15.2%, 94.3%, and 49.1% of SL; this document represents the first reporting these data for this species in the Mediterranean Sea, so no comparisons with literature data were possible. However, the pre-pectoral length value resulted similarly to the juvenile specimens reported by Angulo and Lopez-Sanchez in 2017, and originally caught in 1988 in the eastern Pacific Ocean (Gulf of Papagayo, Costa Rica), which showed a value of 14.2% of SL.

The pectoral fin length resulted in 7% of SL, which is considered normal as an upper limit, compared to the similar size specimens that report the same value (6.1%-7% of SL in range) [130,321,323], while the smaller specimens showed more variable values with a range of 5.9%-8.3% of SL [3,320,321,345]. Considering some higher values reported for smaller-sized specimens, it is probable that this feature reduces its length proportionally to the SL, during the transition from the juvenile to the adult stage. This transition is occurring because, during the juvenile phase, all the fins are mainly used for swimming. At the same time, in the adult stage, the role of the pectoral fins becomes more stabilizing, with more power provided by the muscular structure. For the same reasons, a similar trend is shown by the caudal fin length resulting in 12.1% of SL at the upper limit of the range for same-size specimens (10.3%-12.1% of SL) [130,321,323], while smaller-sized specimens show a more comprehensive range with higher values (8.2%-18.6% of SL) [3,320,321,345]. It is interesting to note how these fins' measurements (and generally all the recorded features) from the specimen reported by Garcia-Barcelona and colleagues, resulted in unusually low values compared to other specimens of similar size, altering ranges [345]. Considering that this latter is the unique record from the Balearic Sea, it would be exciting to have some new data from these waters to study more in-depth if exist is a relationship between this species' features and the source geographical area. The pectoral fin base width resulted in 1.2% of SL, a similar value with the one reported by Falsone and colleagues for their small-sized specimen (1.4% of SL), which is the only other value reported for this parameter from the Mediterranean Sea [3].

Regarding the dorsal fin length, our specimen data resulted in 89.6% of the SL, a relatively lower value compared to the only other available, the one reported by Garcia-Barcelona et al. in 2014 [345], which indeed resulted in 104.6% of the SL. However, it is conceivable that measurement error could have occurred during that study, as the authors probably have wrongly considered part of the dorsal fin as caudal, which is too short compared with all the other studies. The measurements of the fins in these fishes are conditioned by the state of conservation of the specimens. Particularly delicate, the fins can be partially damaged during the capture and storage operations, altering the measurements in those cases. This affects especially the fish caught with trawling nets due to the destructive nature of this fishing method. We believe that in this case, our value is accurate because the specimen analyzed in the present study was caught with longline fishing and was in good status. Considering some other related taxa references, other authors reported on extended dorsal fins in *Trachipterus* and *Desmodema* species, but never exceeding the whole length of the back and in any case the SL [7,134,305].

Differently, some authors collected the dorsal fin's maximum height. From our specimen, we reported a value of 6.3% of the SL, comparable with those from the other two big-sized specimens, which resulted in 6.6% and 7.1% of SL, recorded by Psomadakis et al. in 2007, and Tortonese [130,323]. Psomadakis and colleagues also report another small-sized individual with the highest value of this parameter (16.4% of the SL), similarly to what was documented by Falsone et al. in 2017, which reported in another specimen of similar size a maximum height of the dorsal fin of 12.5% of SL [3], confirming that, also for the dorsal fin, the young specimens show fins more developed in length compared to body size.

Regarding the body height (or body depth), the comparison between the Mediterranean specimens reveals some interesting question marks. Indeed, this is one of the most important features considered decisive in the identification of the *Zu* genus specimens. In detail, *Z. elongatus* should be characterized by a maximum body height of 12-16 of the SL, while the range of this parameter for identifying a specimen as *Z. cristatus* is 20-26% of the SL [1,113,127,131,259,267]. Our specimens reveal a maximum body height of 21.7% of the SL, a value within the species range and comparable to other reports by different authors

for six of the evaluated specimens [130,320,321,323,324]. Differently, two smaller-sized specimens reported respectively by Roig & Demestre in 1982 [321] and by Garcia-Barcelona et al. in 2014 [345], showed values of this parameter more in line with the range of *Z. elongatus*. Indeed, the body depth record for these two specimens was 11% and 15.6% of the SL. These values represent an interesting anomaly, although all other parameters measured confirmed the identification as *Z. cristatus*. Hence, it is probable that the validity of this parameter as an identifying character can in some way be put in question, especially among the juvenile specimens; greater deepening needs a wider basin of analyzed samples.

Regarding the total weight, the Mediterranean Sea literature provide data coming from only seven specimens. Hence, an evaluation approach to the length-weight relationship would not be significant. However, looking at the recorded data in relation to the SL, some interesting insights come out. Our specimen showed a total weight of 4000g with SL of 1060mm, values comparable with just another specimen recorded by Psomadakis et al. in 2007 from the Ligurian Sea, with a total weight of 4400g with SL of 1105mm [130]. Comparing the three small-sized specimens in a range of 926-1000mm of SL between them, the total weight resulted comprised of 2160g and 2800g [130,321,323]. According to literature, the two smallest recorded specimens showed a total weight of 500g and 1301g, respectively with 590mm and 733mm of SL [3,321]. From an evaluation of these data, it is evident that the development of *Z. cristatus* favors the length in the juvenile phase, increasing in weight going towards the adult stage. Particularly interesting is to note how, comparing the big-sized specimens with the medium-sized ones, a difference of a few mm in SL could lead to the highest difference in total weight. This is due to the peculiar shape of the species that, during the adult phase of the life cycle, becomes more dorsoventrally compressed and consequently weighted. Moreover, as reported by Palmer in 1961, passing from juvenile to adult stage *Z. cristatus* become less laterally compressed, and thus massive [304]. These developmental features were confirmed by the relationship between the maximum height of the body and the SL of the specimens compared in this manuscript. Indeed, the abnormal low values recorded for the smaller size specimens that resembling *Z. elongatus* for body depth values, are revealed also in the lowest weight compared to the SL.

Those apparently abnormal values probably reveal information on the development of the species, that appears like *Z. elongatus* in the juvenile stage, while takes on a certain shape and height of the body that characterizes the species becoming adult. More data on weight, in the various stages of growth, may lead in the future to establish a real length-weight relationship, which will certainly help to revise the identification keys in these regards.

Meristic count comparisons between the specimens herein reported, and the literature show some interesting points of discussion. Indeed, these features are historically involved in the process of morphological identification of the species, discriminating between the Lampriformes order members [1,113,127,131,259,267]. Vertebrae count is one of the main features to discriminate between *Z. cristatus* and *Z. elongatus*, with the last one usually revealing a higher number of elements (84-87). Our specimen reveals 62 elements, the same as the one reported by Falsone and colleagues [3], confirming the species identification and the validity of this discriminant character. Similarly, the lateral line scales are considered as a discriminant character between the two species of the genus *Zu*, with *Z. cristatus* that show a few total elements (between tubular and spiny scales) comprise in a range of 99-106, while *Z. elongatus* is identified by a higher number of total elements in a range of 126-130 [1,113,127,131]. Our specimen reveals 101 total lateral line elements, of which the last 47 were spiny. Comparing this data with the literature specimens, it is interesting to note how just a specimen reported by Psomadakis and colleagues [130] show a similar number of 102. All the other references reported values out of the above-mentioned range for the species identification, between 96 (in two different cases) to 107 [130,324,345]. Even if these values do not deviate sufficiently to question the identification of the species, is evident how a revision of morphological identification keys should be carried out based on these data. The lateral line is also important in the discrimination among the genera of the Trachipteridae family [113,127]. Particularly, the last lateral line portion of species belonging to the genus *Zu* usually slides along the ventral side of the tail, showing several sharp spines pointing in an alternate direction, both in the juvenile and the adult stages [133]. Differently, the species of the genus *Trachipterus* are characterized by a posterior portion of the lateral line that

proceeds well above the ventral edge of the tail, showing the lateral line spines that project laterally without pointing in alternate directions [1,267].

Pectoral fin rays were 10 in the analyzed specimen, and, comparing this count with those from literature (from 10 to 12), it is in line with the expected value for this species. The caudal fin of our sample consisted of two lobes, the upper of which was sharply upturned. This is an important feature within the Trachipteridae family, to distinguish the genera *Zu* and *Trachipterus*, from the genus *Desmodema* which does not show the evident upturned tail. Moreover, the ventral edge of the tails was provided by nine spiny plates, another feature that characterizes the members of the *Zu* and *Trachipterus* genera.

Regarding caudal-fin rays, our specimen has shown nine rays on the upper lobe and three on the lower one. These values are comparable with the others from the literature reporting uniformly nine rays on the upper lobe [3,130,320,321,323], while more variability was shown for the caudal lobe. Indeed, just Ibanez and Gallego described in 1974 a specimen with 3 rays on the lower caudal lobe, while Roig and Demestre documented in 1982 two specimens with 2 rays on the lower caudal lobe, and Tortonese in 1958 recorded a specimen with a lower caudal lobe constituted by just 1 single ray [320,321,323]. The rays reduction in the lower caudal lobe characterize this species during the shift to adult stage; this can explain the observed data variability, and probably led to this abnormal range of annotated data [304]. Moreover, despite some authors reported the lower lobe of the caudal fin as constituted by one or two long filaments [127,339], their fragility makes them difficult to record, as very often they are lost during capture phases. Apart from rare cases, only on living recorded specimens through underwater filming, has been possible to observe these features [133].

The pelvic and the anal fins were absent in our specimen. As confirmed by previous authors the anal fin is considered absent in this species, while the pelvic fins seem to be present only in the juvenile stage of the species, probably because in this phase all the fins are essential for swimming. Shifting to the adult stage, at an estimated size of 800mm, these fins disappeared, and a small nubbin is usually found at pelvic fins insertion [127,349]. However, Palmer in 1961 has untied this, like other characteristics of development, from

just a concern of size, as influenced by the development of the single specimen [304]. Considering the size of our specimen, we confirm these previous descriptions about the absence of the pelvic fins in the adult stage of *Z. cristatus*.

The dorsal fin is the most evident in *Z. cristatus*, due to its reddish crimson color and the first very elongated rays, for this reason, Walters and Fitch described in 1960 as an evident pennant [339]. Palmer reported in 1961 that the first six elongated rays are interested during the developing of the fish by a reduction in length, compared to the TL [304], so is conceivable that juvenile specimens shows very elongated rays compared to the adults. Moreover, the number of the dorsal fin rays is another important feature used for the discrimination between the genus *Desmodema*, which usually show a total number between 120 and 124, and the genera *Zu* and *Trachipterus*, which usually have more than 124 rays on their dorsal fin [1,127,259,267]. Even more specifically, the genus *Zu* usually has less than 150 elements, while the genus *Trachipterus* is the one with the more complex dorsal fin, showing usually more than 150 elements [1,127,259,267]. Our specimen showed a dorsal fin consisting of 122 rays with six evident spines in its posterior portion. This value is considered if it does not allow to distinguish between the genera *Zu* and *Desmodema*. The other references from Mediterranean literature show a range for this data between 117 and 132 total dorsal-fin elements, with half references that report values common to the *Desmodema* genus, denoting a high variability of this parameter [3,130,320,321,323,324,345]. However, curiously the two data that delimit the range, are reported by the same author for the two different specimens annotated during the same study, recording six elongated dorsal fin rays for both the specimens, followed by 111 and 126, respectively for the bigger and the smaller ones [321]. It is evident how, as already discussed, the fins cannot represent a fundamental character for the identification of Trachipteridae species, as they are often missed or damaged through the fishing methods and therefore lead, as in this case, too unreliable data because influenced by third factors. For example, Hayashi refers, in a general description of the species, no separation between the first six elongated rays and the rest of the dorsal fin rays [350]; on the contrary, in our specimen, the first rays were lightly separated from the rest of the dorsal fin, as described by Martin in 2015 [133]. Our

suggestion is to consider the counts and features of all the fin elements, and their length and height ratios with respect to the rest of the body (e.g., SL) of Trachipteridae species, as secondary factors alongside them by several other parameters during the morphological identification of the species.

Regarding the buccal apparatus, our specimen has shown 18 upper jaw teeth, 12 lower jaw teeth, four palatine teeth, and four vomerine teeth. The same numbers, apart from one less vomerine tooth, were reported by Falsone et al. 2017 for their specimen from the Southern Tyrrhenian Sea [3]. The other studied adult specimens showed a more variable number of teeth, particularly the upper jaw ones with a range from 8 to 21 [130,320,345]. It is interesting to note how the specimen reported by Ibanez and Gallego in 1974 from the Iberian Sea, had a low number of all teeth (16 on the upper jaw, 10 on the lower jaw, 2 palatines, 2 vomerine) [320]. Equally attractive, are the just eight upper jaw teeth shown by the specimen reported from the Balearic Sea by Garcia-Barcelona et al. in 2014 [345]. It is highly probable that *Z. cristatus* being an active predator of fishes, molluscs, and crustaceans may encounter the accidental loss of some tooth during its feeding behavior. Moreover, the geographical area of these recorded specimens with a lower teeth number is similar and could be interested in a different kind of scalloped ribbonfish' preys that led to a greater loss of teeth [351]. However, to support this hypothesis more information on the diet of these fishes would be required. Hence, another interesting literature data is the occurrence of a specimen with 21 upper jaw teeth recorded by Psomadakis et al. in 2007 from the Ligurian Sea [130]; a strange value which could be explained by an abnormal regeneration after the loss of some teeth or genetical-based alterations [352,353].

About the gill rakes, all the literature data showed a total number of 10-11, of which 2 or 3 epibranchial and 7 to 9 ceratobranchial. Our specimen has shown 3 epibranchial and 8 ceratobranchial gill rakes, the most shared values across the literature specimens, and considered as "normal" [3,130,321,323,324,345].

2.2 Considerations on our record of *Zu cristatus*

Our knowledge of the distribution of some marine teleost is affected by a scarcity of data, especially for those rare and difficult to sample. Moreover, the poor knowledge, combined with the low commercial value of these organisms, leads to a lack of interest in them. Therefore, more detailed information on these taxa is essential, and each new contribution can lead to interesting new insights.

Our report represents the second occurrence of the scalloped ribbonfish in the Ionian Sea, but the first with collected in-depth data [354]. Furthermore, this adult big-sized specimen represents one of the deepest occurrences overall for the species (720m). From the comparison between our morphometric and meristic data with the few references with comparable data, much information resulted fragmented or, in several cases, missed. It's evident how in this species the data of the juvenile specimens appear different from those of the adults, resulting partially incomparable.

Due to the scarcity of the adult stage data from literature, most identification keys and diagnoses available for *Z. cristatus* were based on juvenile characters, increasing the inaccuracy. New benchmarks should be established for the two main developmental stages separately, to correlate new data more appropriately. The increasing information about Lampriformes will certainly be useful in understanding their ecological roles and morphological adaptation to deep-sea life. Hence, our report, representing the most carefully recorded occurrence from the Mediterranean Sea, could be a new essential reference for future studies.

3. *DE NOVO* WHOLE GENOME SEQUENCING

In recent years, the introduction and advances of next-generation sequencing technology led to a rapid expansion of genomic resources and their application in ecology and zoology, even for non-model or commercially important species. Indeed, the researchers in this field were discouraged from undertaking these projects when the high costs and difficulties of whole genome sequencing were linked to the only available technology of Sanger sequencing [355]. For this reason, still few whole genomes of teleosts have been fully sequenced and well assembled, treating mainly model species or commercially important ones, comprised in recent years the even more interesting aquaculture species, such as zebrafish (*Danio rerio*), medaka (*Oryzias latipes*), Atlantic cod (*Gadus morhua*), Atlantic salmon (*Salmo salar*) [356–359]. Comparative genomic analysis is helpful in the absence of whole genome sequences for interesting species from a research point of view. Furthermore, comparing nuclear or mitochondrial genome traits results is helpful in conservation studies to evaluate syntenies, directional selection, and other features which facilitate understanding genome structure and, consequentially, the evolutive functional adaptation of the organisms [360]. These investigations are essential in the fish study because they provide physical evidence for orthology and genome duplication events, which is particularly important in exploring gene family expansion. Indeed, as it is known, teleost fishes passed through some whole genome duplication events during their evolutionary history, as confirmed by molecular evidence [361]. These events lead to an increased number of orthologous genes in fish species, compared to the rest of the vertebrates, which is very interesting to investigate from a functional point of view. However, the lack of well-assessed and annotated genomic sequences affects this research field, and the necessity for even more material started has begun to be partially satisfied thanks to the advent of new generation sequencing platforms such as Illumina (second generation) and even more performant Nanopore one (third generation) [362,363]. Nowadays, through developing and assessing these innovative technologies, it is possible to entire sequence genomes very quickly compared to some decades ago, most cheaply and more accurately.

Moreover, an increased number of annotated sequences in the databases could help in phylogenetics reconstructions of rare and scarcely known taxa, for which scarce reports and information on their morphology and distribution are present in the literature. This broad data basin could also help in using exciting tools such as eDNA, making them more effective and accurate in identifying a complete number of organisms and their relative abundances. Hence, it is predictable that in the following years, the research in marine ecology and zoology may undergo a significant change in the new light of these tools of investigation that are proving to be very useful in better understanding structures and functions, little or nothing known.

Second-generation sequencing technologies include the widely used sequencing by synthesis, ion semiconductor sequencing, sequencing by ligation platforms and pyrosequencing [364]. In the last decade, single-molecule third-generation sequencing platforms, such as the Helicos, Nanopore and Pacific Biosciences ones, have been developed. Some of these technologies have not had an excellent commercial response for inherent technological defects, while others are being rapidly replaced by their competitors. One of the most utilized ones was Illumina, which capitalized on an early edge with more rapid evolution tips in the sequencing process and simple bioinformatic tools. The main limitation of most of these platforms, such as Illumina and Ion Torrent, is that the sequencing output often generates a lot of short reads or significantly increased error rates, as in the case of the Pacific Biosciences.

Over the methodological defect of each of these technologies, the most shared problem was the investments required by the platforms, reagents, and pipelines of all these sequencing routes [365]. This problem led to the development of dedicated companies to exploit sequencing out of the competence of the researchers' laboratory.

Illumina's sequencing platforms have reached the best equilibrium between reads length, error rates and cost [366]. Nevertheless, the increased request in quality of the assembled genomes or gene sequences required longer reads output than this technology could provide. Indeed, several of the annotated sequences produced through short reads sequencing were characterized by misalignments and mis-mappings, leading to regions of

heterozygous, fragmented genes, altered structural variation, repetitive regions of the genome being inaccessible and impaired haplotypic structure [367].

Despite all these limitations, next-generation sequencing technologies have evolved rapidly, particularly nanopore sequencing, which promises to significantly increase the read length, considerably decreasing costs [364]. Furthermore, this third-generation single-molecule sequencing platform, by single-pore sequencing technologies with a simple library preparation process, promised to make nucleic acid sequencing affordable to a larger community and sustainable even by small laboratories [368].

Recently Oxford Nanopore released some portable instruments, the MinION sequencers, with incredible performance compared to previous technologies. This technology amazed researchers for its ease of use (despite the intrinsic complexity of the sequencing process); at the same time, the user can sequence his samples anywhere without excessively processing genomic DNA or amplifying it, using dedicated kits for the libraries' preparation [369]. In addition, the bioinformatics applications downstream of the process also seems improved and made more accessible even to not-too-experienced users. However, during the last years, the scientific community has interacted with Oxford Nanopore to provide revisions and evaluations of these new technologies and instruments to enhance the performance level and give the users all the appropriate support to get the best results in their applications [370,371].

Here we report the first *Zu cristatus* whole genome sequencing procedures and results using Illumina and Nanopore technologies, responding to the necessity of deepening the molecular database resources, enhancing the knowledge of rare fish species, and investigating the use of new generation sequencing technologies on their regards. The described procedures were carried out in the laboratories of the Department of Chemical, Biological, Pharmaceutical and Environmental Sciences of the University of Messina concerning the Illumina sequencing procedures. At the same time, the Nanopore approach was entirely exploited during the Erasmus period at the Faculty of Biosciences and Aquaculture of Nord University, Bodø, Norway. This chapter's project evolved with the additional technical objective of evaluating the technological approaches to fish whole

genome sequencing, describing the limitations and the advantages of both platforms and their coupled use.

3.1 DNA extraction and processing

3.1.1 - Phenol-chloroform protocol

The first approach of this research project focused on using an Illumina platform and short reads library approach, and a routine DNA extraction method was carried out. At Messina University laboratories, total genomic DNA was extracted from 0.25g of tissue using a mechanical glass-beads disruption method followed by a standard phenol-chloroform protocol (Figure 9) [372]. Epaxial muscle tissues of *Z. cristatus* were then dried on a filter paper, cut into small pieces, and placed in 900 µl of a lysis buffer (200 mM Tris-HCl pH 8.0; 100 mM EDTA; 250 mM NaCl) inside a 2 ml tube, and the tissues were incubated with Proteinase K (10 mg/ml) (Thermo Fisher Scientific, Waltham, MA, USA) at 42°C for at least 10 h. After this period, tissues were vortexed, and the top aqueous layer was moved to a new 2 ml tube. To isolate the DNA, 900 µl of phenol:chloroform:isoamyl alcohol (25:24:1) were added to the manually inverted tubes, briefly vortexed and centrifugated for 5 min at 15000 rpm. Seven hundred microliters of the top aqueous phase were moved to a new 2 ml tube. An equal quantity of 700 µl of chloroform:isoamyl alcohol (24:1) was added to the tubes that were manually inverted, briefly vortexed and centrifugated for 5 min at 15000 rpm. A quantity of 500 µl of the top aqueous phase was moved to new 2 ml tubes, and 1,25 ml of cooled absolute ethanol and 20µl of NaCl 5M were added to precipitate the DNA; hence the tubes were incubated overnight at -20°C. After this period, the DNA was recovered by 15.000 rpm centrifugation for 10 min. To eliminate the excess of absolute ethanol, the tubes were stored for 10 min at 65°C in a ThermoMixer C (Eppendorf Milano, Italy).

Further, the DNA pellet was washed briefly in 70 % ethanol, air dried and resuspended in 50 µl of TE buffer (10 mM Tris HCl pH 8.0; 1 mM EDTA pH 8.0) and incubated with 2 µl of RNase A (Thermo Fisher Scientific, Waltham MA, USA) for 45 min at 38°C. After it became

completely soluble, the DNA was stored at −20°C. DNA integrity and purity were evaluated spectrophotometrically and by 1.2% agarose gel electrophoresis. The best samples resulted from NanoPhotometer N60 (Implen, München, Germany) measurements in about 2500 ng/µl in concentration, with purity values of 1.9 and 2.2, respectively, for absorbance at 260/280 and 260/230 ratios. The representative workflow of these procedures is shown in Figure 9.

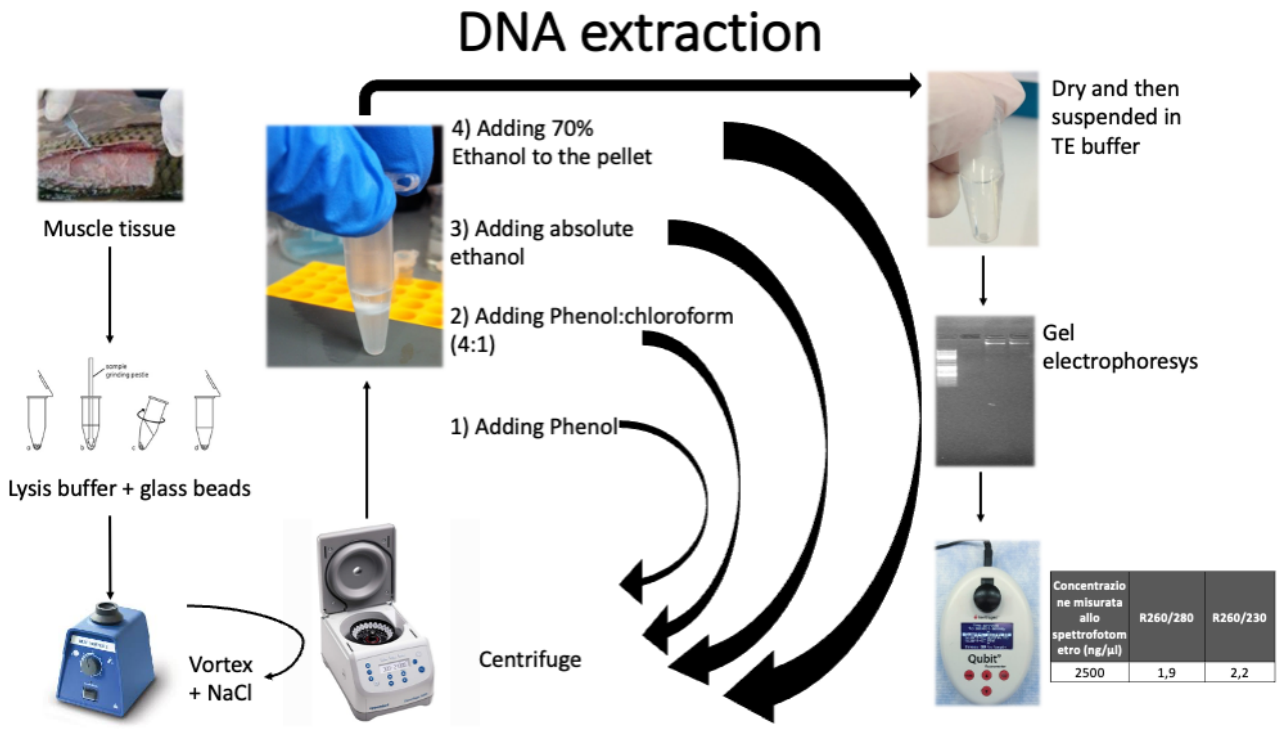


Figure 9. Extraction protocol scheme of the Illumina approach.

3.1.2 - Qiagen DNeasy blood and tissue kit approach

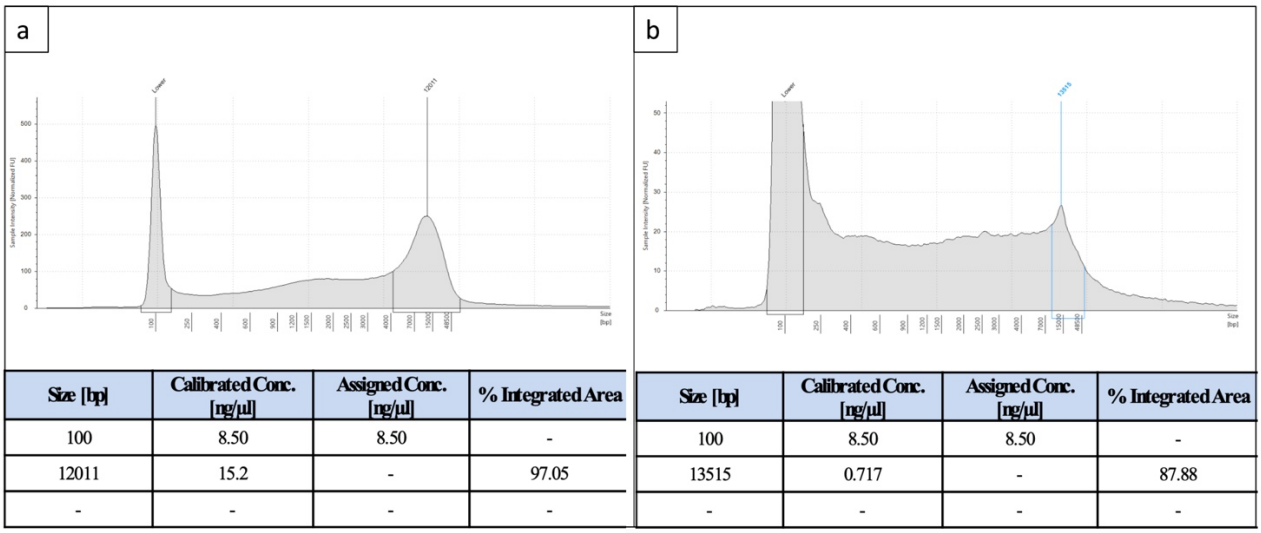
A second DNA extraction approach was used for the samples sequenced with Nanopore technology at Nord University of Bodø, Norway. In this case, prioritizing speed of execution due to the more samples required during the study, a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) was selected to carry out the total genomic DNA isolation from the *Zu cristatus* muscle tissue. As suggested by Piskata et al. in 2017 [373], we applied the standard protocol recommended by the manufacturer, with some modifications. Remarkably, due to the necessity to improve our sequencing input in terms of DNA integrity and length, we strongly reduced the vortexing during the entire protocol, starting with gentle lysis without the use of any mechanical fragmentation of the tissue, contemporary reducing the lysis

temperature to 37°C and increasing the time to 12h. For the same reasons, pipetting was carefully performed with the exclusive use of wide bore tips. The extracted genomic DNA was long-term stored at −20°C or 4°C when used within the next 24h. DNA purity was evaluated spectrophotometrically on NanoDrop One Microvolume UV-Vis (Thermo Fisher Scientific, Waltham, MA, United States), quantified on Qbit 3 Fluorometer (Thermo Fisher Scientific, Waltham, MA, United States), Integrity was assessed using the DNA broad-range tape on the TapeStation System 4150 (Agilent Technologies, Santa Clara, CA, United States). Representative results of these procedures are shown in Figure 11.

3.1.3 - Short Fragment Eliminator (EXP-SFE001) trials

Focusing on the necessity to increase the average length of our genomic DNA, we make some trials using the new Nanopore Short Fragment Eliminator, an expansion pack recommended by Oxford Nanopore for users who want to size-select high molecular weight (HMW) gDNA to deplete short fragments (<25000 bp). Using this buffer and some centrifugations, the manufacturer almost entirely guarantees the removal of fragments under 10 kb. However, based on our trials, the results were not satisfactory, probably due to the large amounts of short fragments in our samples, which, as suggested by the manufacturer, will lead to a low yield at the end of the process. Figure 10 shows how this kit was useless in our case to reach a better quality of genomic DNA. Indeed, despite an increase in high peak average length, of about a thousand base pairs, an additional fragmentation was recorded, as well as a considerable loss in sample total concentration of about 95%.

Considering that no references are available about the performances of this kit, it is understandable that this buffer still needs to be optimized and tested better, even through feedback from users like ours.



Size [bp]	Calibrated Conc. [ng/µl]	Assigned Conc. [ng/µl]	% Integrated Area
100	8.50	8.50	-
12011	15.2	-	97.05
-	-	-	-

Size [bp]	Calibrated Conc. [ng/µl]	Assigned Conc. [ng/µl]	% Integrated Area
100	8.50	8.50	-
13515	0.717	-	87.88
-	-	-	-

Figure 10. Representative graphics of the *Z. cristatus* genomic DNA isolated during this study and processed with the Nanopore Short Fragment Eliminator buffer. Images were obtained as TapeStation analysis output. **a)** Representative sample of freshly isolated genomic DNA before the processing; **b)** Output of the same sample after the processing protocol with SFE buffer.

3.1.4 - Zymoclean Large Fragment DNA Recovery Kit processing

Although the tips and care tried to obtain the best results in yield and high molecular weight DNA retention, the DNase Blood and Tissue Kit showed some limitations from these points of view. Indeed, despite the manufacturer's guarantees of a better yield, the average length of the extracted DNA fragments rarely exceeded 10000 bp due to the large percentage of short fragments in the samples. It was possible that long-term storage and multiple kinds of transport of the *Z. cristatus* muscle tissues have influenced these results, causing a higher degradation of the cellular material and consequent DNA, as previously reported by other authors [372,374].

After the failure of the trials with the Oxford Nanopore SFE buffer, our necessity to increase the quality of the DNA used for the preparation of the libraries to the MinION and not frustrate the ability of the instrument to produce long reads, led us to process several samples extracted with the kit Qiagen with a second one, the Zymoclean Large Fragment DNA Recovery Kit [375]. This gel DNA extraction kit provides a streamlined method for rapidly purifying and concentrating high-quality large-sized DNA (>25000 bp) from agarose gels. About 30 extracts (3 ml) of isolated DNA were processed with this method, merging the contents of about 75 columns from the kit and reaching at the end the best 1000

ng of high molecular weight DNA required for starting the library preparation for the second sequencing on MinION Mk1c, with an average length of over 25000 bp of the high peak (Figure 11). Analyzing the compared yield of these lengthy and articulate DNA extractions and purification processes, result evident how the size selection done with the Zymo kit resulted effective for our needs, allowing us to obtain an acceptable result, considering the starting material and the use of a non-specific kit for high molecular weight DNA isolation upstream, which probably would make the process faster.

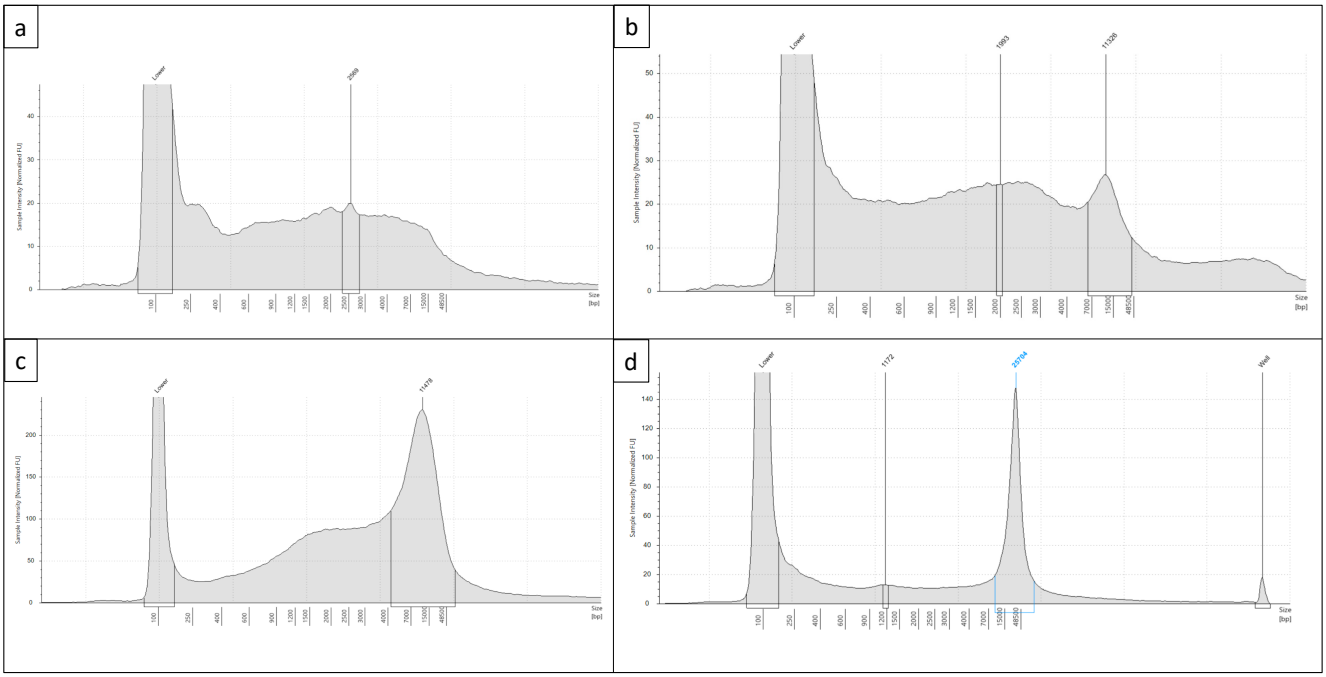


Figure 11. Representative graphics of the *Z. cristatus* DNA isolated during this study with different methods. Images were obtained as TapeStation analysis output. **a)** Example of a failed extraction with a very fragmented final product without a higher peak and very lower average length; **b)** Normal DNA isolated sample with the use of manufacturer suggested protocol with Qiagen DNeasy Blood and Tissue Kit. The final product shows high fragmentation and no evident higher peak; **c)** DNA isolated sample adopting the tips described in the main text to the protocol of Qiagen DNeasy Blood and Tissue Kit. The final product shows moderate fragmentation and a quite good average length of the higher peak; **d)** Final result of the processing of several samples as the previous, with the additional use of the Zymoclean Large Fragment DNA Recovery Kit. The final product shows absent or less fragmentation and a very good average length of the higher peak.

3.2 DNA sequencing

3.2.1 - Illumina Platform HiSeq 4000

Five thousand nanograms of purified total genomic DNA extracted with the phenol-chloroform protocol were sent to the Galseq company (www.galseq.com) for the preparation of two sequencing libraries containing different insert sizes (300 and 550 bp).

The libraries were sequenced in paired-end mode (2x150 bp) on an Illumina HiSeq 4000 platform. A total of 169,273,456 and 219,941,430 raw reads were obtained from the sequencing of the 300 bp and 550 bp insert-size libraries, respectively. After sequencing, Illumina raw reads were first inspected using the FASTQC program [376], and then cleaned with Trimmomatic v.0.39 software [377] by applying the following options: HEADCROP:10, ILLUMINACLIP:~/Trimmomatic-0.39/adapters/All_adapters.fa:2:25:10, LEADING:25, TRAILING:25, SLIDINGWINDOW:4:25, MINLEN:35. A resume of the cleaned data is shown in figure 12.

CLEANED DATA

	ZUC-300 Total cluster 84,636,728		ZUC-550 Total cluster 109,970,715	
Trimmomatic options	ILLUMINACLIP: 2:25:10 LEADING/TRAILING: 25 SLIDINGWINDOW: 4:25 MINLEN:35	HEADCROP: 10 ILLUMINACLIP: 2:25:10 LEADING/TRAILING: 25 SLIDINGWINDOW: 4:25 MINLEN:35	ILLUMINACLIP: 2:25:10 LEADING/TRAILING: 25 SLIDINGWINDOW: 4:25 MINLEN:35	HEADCROP: 10 ILLUMINACLIP: 2:25:10 LEADING/TRAILING: 25 SLIDINGWINDOW: 4:25 MINLEN:35
Both Surviving	68634431 (81.09%)	67673002 (79.96%)	74666321 (67.90%)	73012510 (66.39%)
R1 only	12018840 (14.20%)	12246375 (14.47%)	28417383 (25.84%)	28620450 (26.03%)
R2 only	1386418 (1.64%)	1576600 (1.86%)	1446968 (1.32%)	1680502 (1.53%)
Discarded	2597039 (3.07%)	3140751 (3.71%)	5440043 (4.95%)	6657253 (6.05%)

Figure 12. Illumina sequencing cleaned reads data from the two distinct libraries 300 bp and 550 bp.

3.2.2 - MinION Mk1c (Oxford Nanopore Technologies, Oxford Science Park, UK)

Two different *Z. cristatus* genomic DNA sequencing were carried out during the Erasmus period at the Nord University of Bodø. During these runs, no evident practical problems were encountered during library preparation and flow cell priming and loading, confirming the ease of execution proposed by the manufacturer. The utilized flow cell was the FLO-MIN106D (R9.4.1) for both runs, as suggested by Oxford Nanopore. Also, the setting of the options on the Mk1c touchscreen panel to start the sequencing was simple and intuitive, and customizable according to our needs. Our setting parameters were PCR-free protocol,

no multiplexing, 72-hour running at -180 mV, no automatic basecalling, filtering reads <1000 bp, output format FAST5, and no pores reserving.

For the library preparation, we used the Ligation Sequencing Kit (SQK-LSK110, Oxford Nanopore), which allows for performing several steps rapidly and independently, such as repairing the DNA, preparing the DNA ends for adapter attachment, and then proceeding to prime the flow cell, load the DNA library into the flow cell and start the sequencing [378]. This kit also required the use of NEBNext Companion Module for Oxford Nanopore Technologies Ligation Sequencing Kit, recommended for DNA repair, ends repair and adapter ligation steps [379].

The first trial was carried out on 1500 ng of genomic DNA previously extracted and purified with the Qiagen DNeasy Blood and Tissue Kit, without size selection processing. Before the library preparation, the samples had an average high peak length of about 12 kb.

The metrics of this first sequencing output are shown in Figure 13.

After the first sequencing, our evaluation of the possible insufficient relapses in merging those reads with the Illumina ones to reach a better assembly lead us to repeat this step with better genomic DNA as input. Indeed, the length of the reads obtained during the first run was not fully satisfactory, compared to the Illumina ones. The previously described procedures of DNA size selection will result essential to obtain the best quality reads to reach a better final hybrid assembly.

Hence, the second sequencing started with 1000 ng of genomic DNA, the minimum quantity suggested by the manufacturer to reach good results. Our choice was guided by the idea to not overload the flow cell with too much data, but to make the most of the pores to sequence the good starting material obtained from the previous steps. Before the library preparation, the samples had an average high peak length of about 25 kb. The metrics of this second sequencing output are shown in Table 8, in comparison with the first ones.

Table 8. *Z. cristatus* sequencing reports stats of the two MinION Mk1c runs in comparison. Data obtained from NanoPlot online [380].

Features	First run output	Second run output
Active channels	479.0	497.0
Mean read length	1922.4	6718.5

Mean read quality	10.2	12.2
Median read length	1079.0	5730.0
Median read quality	10.6	13.1
Number of reads	4,981,468.0	3,112,478.0
N50	3318.0	8576.0
STDEV read length	2589.5	4,160.0
Total bases	9,576,318,637.0	20,911,327,694.0
Number, percentage and megabases of reads above quality cutoff		
> Q5	4870325 (97.8%) 9407.8Mb	1508783 (97.0%) 10197.7Mb
> Q10	3145248 (63.1%) 6085.9Mb	1197524 (76.9%) 8036.5Mb
> Q15	3785 (0.1%) 3.3Mb	284074 (18.3%) 1821.1Mb
Top 3 highest mean basecall quality scores and their read lengths		
1	19.8 (204)	23.9 (169)
2	19.2 (617)	23.9 (5800)
3	19.2 (860)	22.4 (209)
Top 3 longest reads and their mean basecall quality scores		
1	242783 (3.9)	333512 (6.9)
1	108673 (8.2)	133504 (13.9)
3	106221 (4.8)	83706 (4.8)

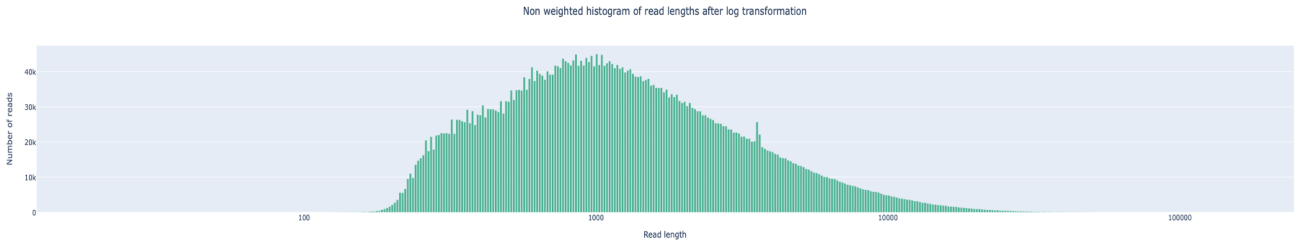


Figure 13. Detail of the *Z. cristatus* sequencing read length after the first MinION run. Graphic obtained with NanoPlot online [380].

3.3 Whole genome assembly

3.3.1 - Illumina reads

After quality filtering and trimming, over 93% (149,168,979 and 176,325,972 reads from the 300 bp and 550 bp library, respectively) of the total reads were used for the genome assembly. The Illumina reads' bioinformatics workflow is shown in Figure 14.

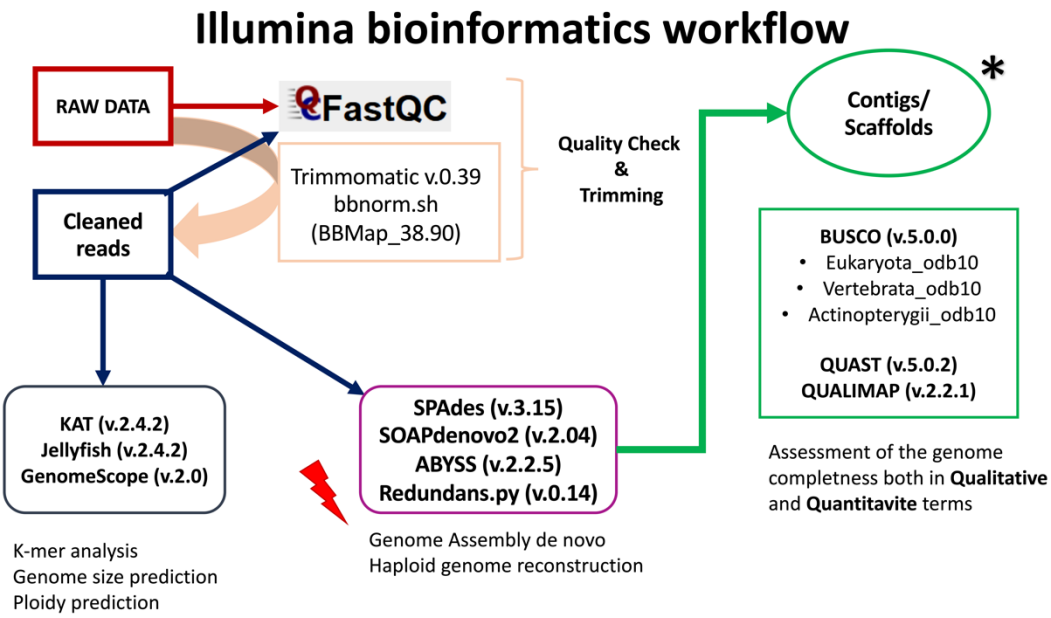


Figure 14. Illumina bioinformatics workflow resuming scheme.

After the inspection with the FASTQC program [376], and the cleaning with Trimmomatic v.0.39 software [377], some investigations were carried out being an assembly "*de novo*" without a reference or more information about the expected final output. Indeed, within the Lampriformes order, only two species of 27 (Table 1) have an annotated whole genome draft, *R. glesne* (OMLC00000000.1) and *L. guttatus* (OMLH00000000.1), both submitted in March 2018 by CEES, University of Oslo, Norway; the genomes size of these species was respectively 656,003,707 bp and 849,277,706 bp. The best model predicted a haploid genome size between 698,811,755 and 710,628,368 bp (for library ZUC-300) and between 682,270,576

and 693,566,216 bp (for library ZUC-550), with values in line with what is expected for a teleost and in comparison, with the few references of the order in the database (Figure 15). To estimate the level of heterozygosity and duplication of the genome draft a K-mer frequency analysis (k=27) was carried out employing KAT software (v.2.4.2) [381] and GenomeScope [382].

K-mers Data Analysis

Haploid Genome Model
p = **1**
k = 27

property	min	max
Homozygous (a)	100%	100%
Genome Haploid Length	674,865,664 bp	691,755,199 bp
Genome Repeat Length	262,793,655 bp	269,370,464 bp
Genome Unique Length	412,072,008 bp	422,384,734 bp
Model Fit	54.7983%	95.4206%
Read Error Rate	0.996299%	0.996299%

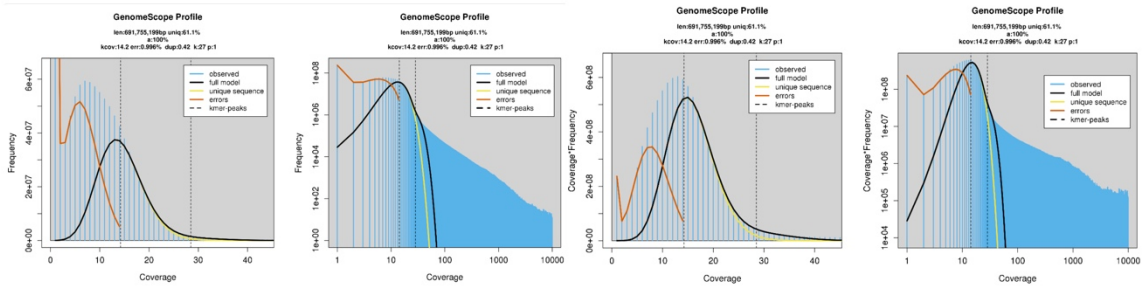


Figure 15. K-mers analysis of the best haploid genome model obtained from the Illumina data assembly.

Using the cleaned reads, some *de novo* genome assembly trials were performed using SPAdes (v.3.15.0) assembler [383], SOAPdenovo2 (v.2.04) [384], and ABySS (v.2.2.5) [385], and the resulting contigs were further processed with Redundans.py (v.0.14) software [386] to obtain scaffold sequences. The best result was obtained from Redundans on ABySS

(Figure 16). Hence, GapFiller (v.1-10) program [387] was used to close the gaps within pre-assembled scaffolds by reducing the number of undetermined bases in the genome draft.

Worst Median Best ☑ Show heatmap

***De novo* Genome Assembly**

Statistics without reference	SoapDeNovo2	Redundans_on_Soap	ABYSS	Redundans_on_ABYSS
# contigs	1 285 752	736 650	1 434 526	706 597
# contigs (>= 0 bp)	4 247 675	736 650	3 211 700	706 597
# contigs (>= 1000 bp)	320 057	263 285	184 432	179 698
# contigs (>= 5000 bp)	28 827	20 945	20 271	20 247
# contigs (>= 10000 bp)	4965	3554	3038	3041
# contigs (>= 25000 bp)	181	134	62	62
# contigs (>= 50000 bp)	8	8	0	0
Largest contig	78 295	74 528	42 359	42 360
Total length	1 219 869 242	880 757 489	983 668 090	751 753 356
Total length (>= 0 bp)	1 606 575 600	880 757 489	1 223 177 436	751 753 356
Total length (>= 1000 bp)	812 677 992	649 674 547	516 336 538	508 885 444
Total length (>= 5000 bp)	228 587 624	165 359 835	154 865 392	154 726 273
Total length (>= 10000 bp)	69 963 897	50 167 358	40 674 833	40 702 160
Total length (>= 25000 bp)	5 768 772	4 318 573	1 784 909	1 784 938
Total length (>= 50000 bp)	473 683	458 624	0	0
N50	1752	1987	1207	2095
N75	722	960	417	768
L50	165 550	113 367	162 120	91 171
L75	440 437	274 406	542 785	242 499
GC (%)	44.9	44.93	45.07	44.96
Reads mapping				
# mapped	146 829 585	138 260 770	136 736 114	138 711 087
Mapped (%)	98.71	98.6	99.46	99.06
# properly paired	87 336 910	103 365 740	93 471 966	98 170 682
Properly paired (%)	58.71	73.71	67.99	70.11
# singletons	983 775	1 034 534	491 905	879 734
Singletons (%)	0.66	0.74	0.36	0.63
# misjoint mates	43 632 144	28 061 216	40 383 248	34 630 964
Misjoint mates (%)	29.33	20.01	29.37	24.73
Avg. coverage depth	13	18	17	22
Coverage >= 1x (%)	79.74	97.53	99.18	99.83
Coverage >= 5x (%)	68.47	87.16	89.66	96.3
Coverage >= 10x (%)	47.37	69.05	69.1	83.62
Mismatches				
# N's	241 608 831	6 262 331	1 021 767	157 851
# N's per 100 kbp	19 806	711.02	103.87	21

Figure 16. Final statistics of the whole genome assemblies from Illumina data using Redundans on SoapDeNovo2 and Abyss pipelines.

The genome assembly draft quality was assessed using the QUAST (v.5.0.2) program [388], and QUALIMAP (v.2.2.1) [389]whereas their completeness was quantitatively evaluated with Benchmarking Universal Single-Copy Orthologs (BUSCO, v.5.0.0) [390] by searching for universal single-copy orthologs in some related lineage-specific datasets (Eukaryota_odb10; Vertebrata_odb10; Actinopterygii_odb10). Results of this analysis are shown in Figure 17.

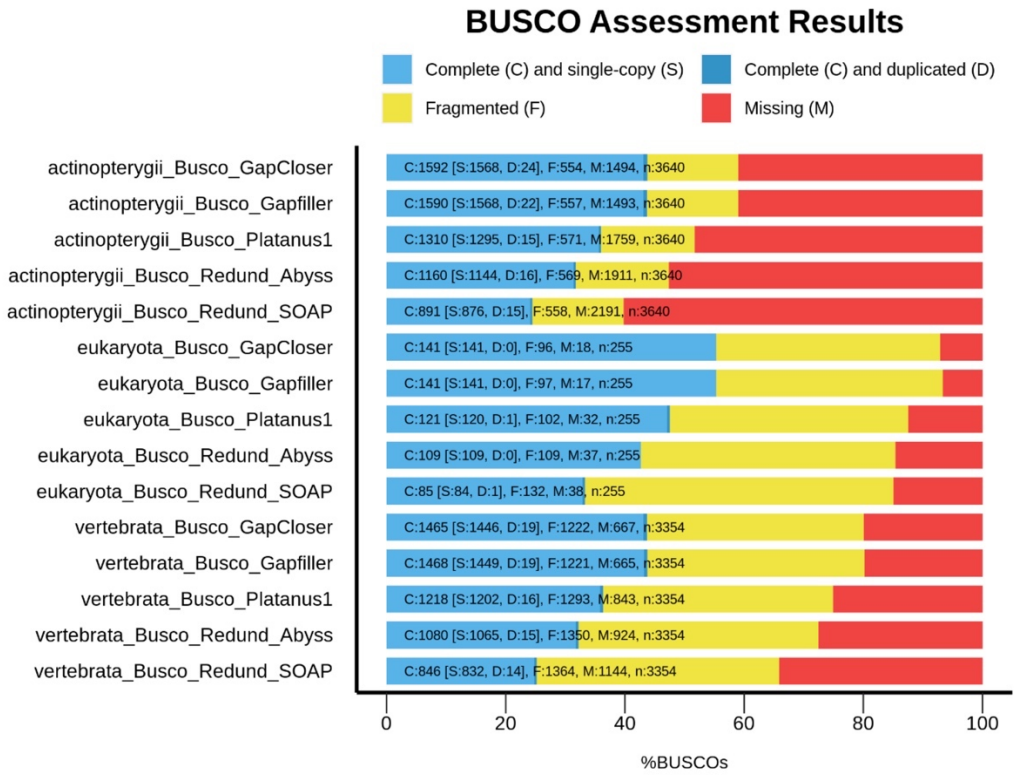


Figure 17. BUSCO analysis of *Z. cristatus* whole genome assemblies draft obtained from the Illumina data with different assemblers.

3.3.2 - Nanopore reads

Nanopore raw data are represented by long reads, and their analysis requires a first essential step which is named basecalling, commonly not performed with short reads data. This process allows the conversion from raw data to nucleic acid sequences and could be performed by the MinION itself. Despite this, commonly the researchers use external pipelines because currently Nanopore basecalling itself is considered more complex and less effective qualitatively. In our study, after completion of sequencing, the raw signal intensity data was used for base calling using Guppy basecaller (v3.1.5), an external software from Oxford Nanopore [391]. Reports were created with NanoPlot online [380]. Reads with a mean qscore (quality) greater than 8 and a read length greater than 8 kb were used and trimmed for adaptor sequences using Porechop (v0.2.4), a total of reads 4,981,468 cleaned reads were used for the genome assembly. The Nanopore reads' bioinformatics workflow is shown in Figure 18.

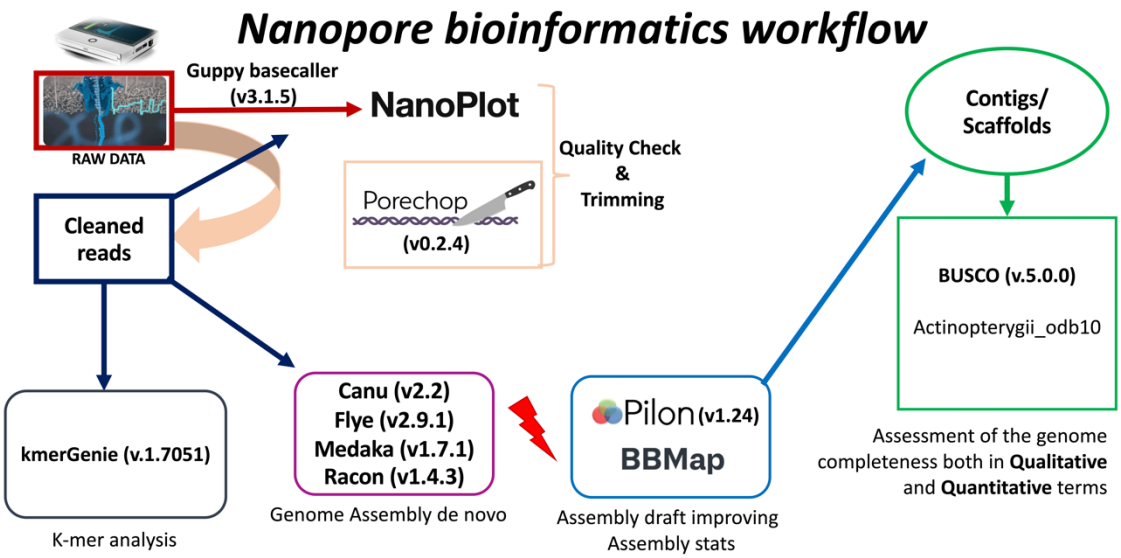


Figure 18. Nanopore bioinformatics workflow resuming scheme.

The K-mer frequency analysis was carried out employing kmerGenie software (v.1.7051) [392]. Raw nanopore sequencing reads were corrected using the program Canu (v2.2) [393], and some *de novo* genome assembly trials were performed using Flye assembler (v2.9.1) [393], a *de novo* assembler for single-molecule sequencing reads. , The initial draft assemblies were polished for three rounds using the raw nanopore reads with Racon (v1.4.3) [394] and one round with Medaka Medaka (v1.7.1) [395] from Oxford Nanopore Technologies. Afterward, reads from Illumina sequencing were used by bwa-mem to align to the draft genome assemblies. The alignment files were then used by Pilon (v1.24) [396] for three rounds of polishing, which identified inaccuracies between the input genome and the evidence in the reads through alignment analysis.

Genome assembly statistics were calculated using the bbmap stats.sh script from the BBTools suite [397] and are shown in Table 9.

Table 9. Report of the *Z. cristatus* Nanopore whole genome assembly draft obtained from first run Nanopore reads with Canu assembler. All statistics are based on contigs of size >= 500 bp, unless otherwise noted (e.g., "# contigs (>= 0 bp)" and "Total length (>= 0 bp)" include all contigs).

Features	N°
Contigs (>= 0 bp)	77549
Contigs (>= 1000 bp)	75523
Contigs (>= 5000 bp)	41632
Contigs (>= 10000 bp)	19582
Contigs (>= 25000 bp)	3408
Contigs (>= 50000 bp)	436

Total length (>= 0 bp)	626188371
Total length (>= 1000 bp)	624788580
Total length (>= 5000 bp)	522486028
Total length (>= 10000 bp)	364701853
Total length (>= 25000 bp)	125578673
Total length (>= 50000 bp)	27133039
Contigs	77328
Largest contig	138440
Total length	626095177
GC %	45.02
N50	11942
N75	3720
L50	14856
L75	51037
N's per 100 kbp	0.00

The completeness of this genome assembly draft was evaluated using BUSCO (v.5.0.0) [390] by searching for universal single-copy orthologs in the related lineage-specific dataset Actinopterygii_odb10. The results of this analysis are shown in Figure 19.

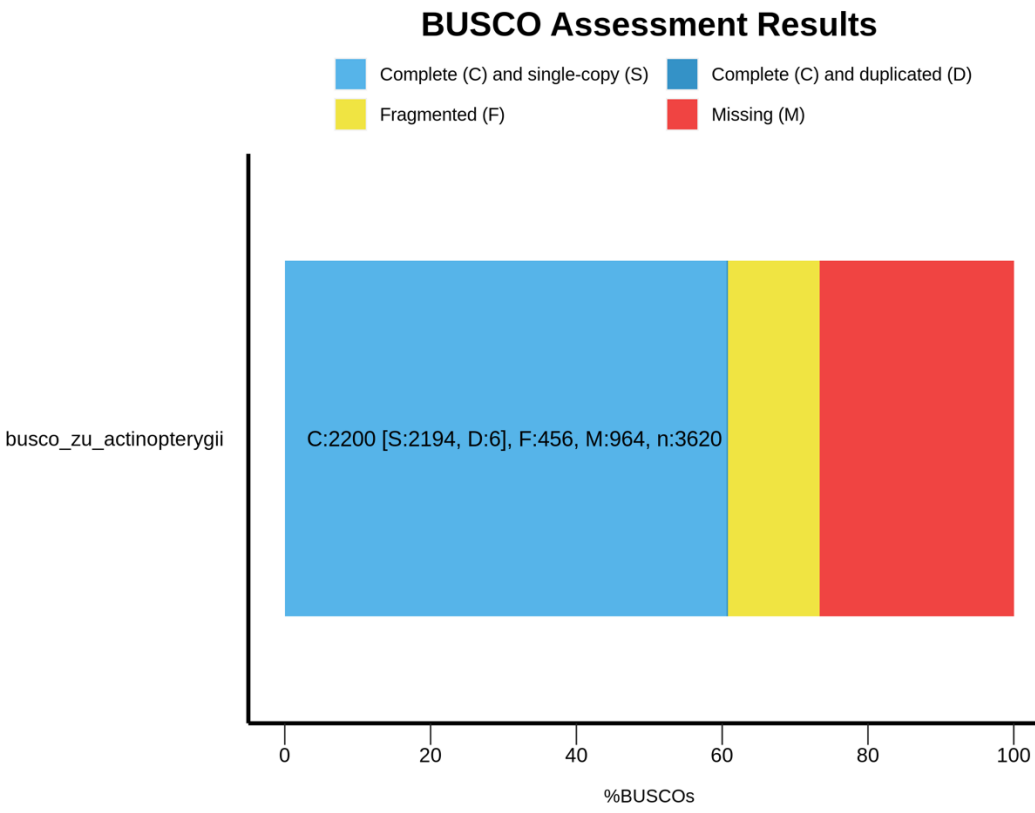


Figure 19. BUSCO analysis of *Z. cristatus* whole genome assembly draft obtained from the Nanopore data with Flye assembler.

3.3.3 - Hybrid assembly

Considering the low level of predicted genome completeness of the first two assemblies trial based on the use of Illumina and Nanopore reads separately, a hybrid assembly merging these two datasets was performed, to obtain a better result on the final genome draft. The raw long reads produced by the first run of MinION Mk1c were assembled with the CANU software (v.2.2) [393], using its algorithm to performs also the self-correction of reads. This assembly was then aligned and merged with the best assembly obtained by the short reads only using the quickmerge software (v.0.3) [398]. To correct potentially misassembled contigs we executed the longstitch workflow using the homonymous tool (v.1.0.3) [399]. Table 10 shows the final statistics of this hybrid assembly trial output.

Table 10. Report of the *Z. cristatus* hybrid whole genome assembly draft obtained from the merging of Illumina and Nanopore reads with Canu assembler. All statistics are based on contigs of size >= 500 bp, unless otherwise noted (e.g., "# contigs (>= 0 bp)" and "Total length (>= 0 bp)" include all contigs).

Features	N°
Contigs (>= 0 bp)	19153
Contigs (>= 1000 bp)	19153
Contigs (>= 5000 bp)	16594
Contigs (>= 10000 bp)	13059
Contigs (>= 25000 bp)	7351
Contigs (>= 50000 bp)	3889
Total length (>= 0 bp)	750136548
Total length (>= 1000 bp)	750136548
Total length (>= 5000 bp)	741464000
Total length (>= 10000 bp)	715337745
Total length (>= 25000 bp)	622021039
Total length (>= 50000 bp)	498985378
Contigs	19153
Largest contig	1702472
Total length	750136548
GC %	44.71
N50	87110
N75	36337
L50	1994
L75	5380
N's per 100 kbp	473.55

The completeness of this hybrid genome assembly draft was quantitatively reviewed with BUSCO (v.5.0.0) [390] by searching for universal single-copy orthologs in the related lineage-specific dataset Actinopterygii_odb10. The results of this analysis are shown in Figure 20.

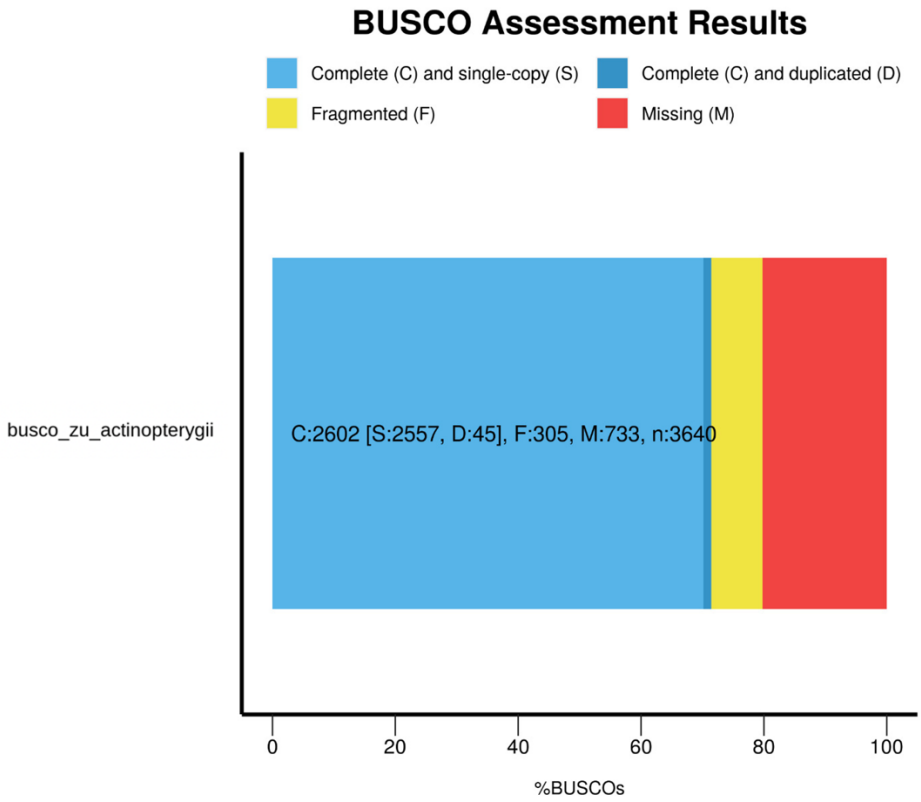


Figure 20. BUSCO analysis of *Z. cristatus* hybrid whole genome assembly draft obtained from the merging of Illumina and Nanopore reads with Canu assembler.

3.4 Remarks on the whole genome assemblies obtained in this study

Despite the difficulties encountered during the *Z. cristatus* DNA isolation phases, by trying to achieve the best possible quality, the process has been educational and instructive to understand better the tips of these processes, which escape based only on the use of standard kits. The result of that first part of the process was essential for the success of the second sequencing run to the MinION. As evidenced by the data shown in Table 8, the result of this second Nanopore sequencing was significantly better than the first, particularly for what was our goal after the first two sequencings and genome assembly tests. Indeed, the

use of Illumina's short reads and the first, not entirely satisfactory, Nanopore reads has not led us to have sufficient completeness of the draft of the genome to be able to undertake studies on genes and other end applications, such as the annotation of the first whole genome of *Z. cristatus* (Figures 17 and 19).

The subsequent hybrid assembly, instead, allowed us to obtain a better final quality of the genome draft, which allowed us to undertake the final stages of the project, which concern the following chapters (Figure 20). However, it is notable how the BUSCO analyses on the completeness of the genome have provided during the steps, increasing values of about 36%, 61%, and 72%, for Illumina, Nanopore first run, and hybrid assemblies, respectively (including only the single and duplicated complete copy of genes comparing the draft with the related lineage-specific dataset "Actinopterygii_odb10").

These values are due to the partial fragmentation of the assemblies, identifiable by the metrics of the three assemblies. Indeed, despite a decreased amount of contigs was registered passing from the Illumina, Nanopore, and hybrid assemblies, 179698, 77328, 19153, respectively, only this third draft showed an acceptable level of fragmentation compared to the relative sizes of 751753356 bp, 626095177 bp, 750136548 bp, respectively, allowing further investigations. This result confirms the validity of this approach in using hybrid reads to achieve a greater final output, compared to the use of only short reads libraries in the assembly of big sized genomes, such as the teleost ones.

However, despite the hybrid assembly draft showing a total completeness percentage of about 82%, we decided to make a new run on the MinION to obtain a better long reads result starting from better initial material; that is why thorough processing of the sample. The very encouraging results obtained from the output of this second sequencing will undoubtedly be able to guarantee us, in the next steps of the project, a better quality of the draft of the genome (>90%, expected), allowing us to reach the final annotation. Indeed, we reached a significantly higher mean of reads length (6718.5 vs. 1922.4) due to a higher total amount of bases (20,911,327,694.0 vs. 9,576,318,637.0) and the contemporary a smaller number of total reads (3,112,478.0 vs. 4,981,468.0), which means a less fragmented raw

output which will undoubtedly lead to a final version of the assembly qualitatively superior and contiguous.

4. MITOCHONDRIAL DNA EXTRACTION

Given the ecological relevance of mesopelagic and bathypelagic organisms and the complexity of deep-sea environments, the phylogeny, genetic adaptations, and distribution triggers of rare mesopelagic species, require broader and more complete knowledge.

The study of mitochondrial DNA is of fundamental importance from a phylogenetic point of view. Indeed, often it is used to review and establish the proper phylogenetic relations in teleost and cartilaginous fishes [400–402]. The features of this molecule, makes it essential in population biology and stock assessment. Moreover, the functional role of several mitochondrial genes covers essential aspects of bony fish adaptation to bathymetrical gradients or extreme environments, such as thermal tolerance or respiratory capacity in oxygen-limited conditions [12].

The mitochondrial genome (mtDNA) structure of bony fishes is constituted by a small circular double-stranded DNA molecule (about 15-20 kb in length), containing 37 functional subunits, of which 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA), and two ribosomal RNA (rRNA) [15]. Moreover, this molecule contains a particular control region called "D-loop", representing the origin of heavy-strand (H-strand) replication. Highly repeated sequences characterize it, contents in Adenine and Thymine and high variability in the teleost, having a mutation rate five times higher than the other mitogenome [403]. For this reason, the D-loop sequence is used more in DNA barcoding techniques to fast and accurate identification of the organisms from the different matrices, and while the high conservation rate and absence of recombinant processes give some mitogenome PCGs and rRNA a principal role as the primary markers used for conducting evolutionary and taxonomic studies in teleost species [16]. Moreover, studying the polymorphisms of these sequences could lead to interesting insights from an adaptative point of view when comparing organisms from different habitats. The increasing use of mtDNA gene sequences in populations and evolutive studies led to the MitoFish database [404] to collect all the annotated mitochondrial sequences of fishes in a single database.

Starting from the Sanger sequencing technique, which allowed the sequence of these relatively short genomes in some weeks, the next-generation methods, such as Illumina platforms, boost this research field by improving timing and accuracy.

Within Lampriformes, the annotations of whole-genome or complete mtDNA are very scarce [129]. Regarding the scalloped ribbonfish, the literature presents just one annotation, made by Miya and colleagues in 2001 [16], which reports the first contribution of this species as an incomplete sequence of mitochondrial DNA (without D-loop). For this reason, the importance of other specimen annotations, possibly complete, is essential to enrich the literature on Lampriformes order and the Trachipteridae family and for the in-deep study of this fascinating species.

4.1 Isolation, assembly, and annotation

A total of 169,273,456 and 219,941,430 raw reads were obtained from the sequencing of the 300 bp and 550 bp insert-size libraries with the Illumina HiSeq 4000, respectively. After quality filtering and trimming, over 93% (149,168,979 and 176,325,972 reads from the 300 bp and 550 bp library, respectively) of the total reads were used for the genome assembly, which resulted in a single, circular DNA molecule of 17,450 bp in length, with a mean coverage of 1370.98X (standard deviation: ±237.78X). The cleaned reads (Phred-score ≥25) were then assembled by MitoFinder software v.1.4.1 [405] using the *Z. cristatus* incomplete mitogenome (GenBank Accession: AP002926.1) as reference.

To determine the genome coverage, clean reads were mapped back against the assembled mitochondrial genome using the bwa algorithm (v. 0.7.17.r1188) [406] and then Qualimap 2 software [407] was used to evaluate alignment data and determine mapping statistics and other genome metrics. Hence, the mitochondrial genome was annotated using the MitoFish web server [404]. Finally, the sequence was deposited on GenBank database as follow, BioSample: SAMN28862047; Sample name: Zu_crist_UME_1; SRA: SRS13301390; Accession: PRJNA845808. GenBank "Mitochondrial genome of *Zu cristatus* (Bonelli, 1819)": Zucrist_UME1_MT ON695781.

4.2 Investigations on the mitochondrial genome of *Zu cristatus*

4.2.1 - Zu cristatus mitogenome structure

A total of 37 genes (22 tRNAs, 13 protein-coding genes, and 2 rRNAs), including the D-loop region, never annotated for this species, were identified in the circular double-stranded mtDNA molecule on 17,450 bp (Table 11). This reference represents the first complete mitochondrial DNA for *Z. cristatus*. Furthermore, this molecule revealed similar patterns of gene positioning in the J (Forward) and N (Reverse) strands, comparably to the mtDNA structure of other teleost species, both freshwater and saltwater, with annotated complete sequences [408,409].

Table 11. Organization of *Z. cristatus* mitochondrial genome.

Gene	Strand	Position		Size (bp)	IGN	Start/Stop Codons
		Start	End			
Phe (F)	J	1	50	50	0	-
rRNA12S	J	51	1007	957	0	-
Val (V)	J	1008	1080	73	0	-
rRNA16S	J	1081	2757	1677	0	-
Leu (L)	J	2758	2831	74	0	-
ND1	J	2832	3800	969	0	**ATG/TAG**
ILE (I)	J	3810	3879	70	9	-
GLN (Q)	N	3879	3949	71	-1	-
Met (M)	J	3949	4017	69	-1	-
ND2	J	4018	5062	1045	0	**ATG/T**
Trp (W)	J	5063	5135	73	0	-
Ala (A)	N	5266	5334	69	130	-
Asn (N)	N	5335	5407	73	0	-
Cys (C)	N	5416	5480	65	8	-
Tyr (Y)	N	5481	5548	68	0	-
COI	J	5550	7100	1551	1	**GTG/TAG**
Ser (S)	N	7101	7171	71	0	-
Asp (D)	J	7175	7243	69	3	-
COII	J	7257	7947	691	13	**ATG/T**
Lys (K)	J	7948	8021	74	0	-
ATPase 8	J	8023	8190	168	1	**ATG/TAA**
ATPase 6	J	8181	8863	683	-10	**ATG/TA**
COIII	J	8864	9648	785	0	**ATG/TA**

Gly (G)	J	9649	9717	69	0	-
ND3	J	9718	10066	349	0	**ATG/T**
Arg (R)	J	10067	10135	69	0	-
ND4L	J	10136	10432	297	0	**ATG/TAA**
ND4	J	10426	11806	1381	-7	**ATG/T**
His (H)	J	11807	11875	69	0	-
Ser (S)	J	11876	11942	67	0	-
Leu (L)	J	11949	12021	73	6	-
ND5	J	12022	13854	1833	0	**ATG/TAA**
ND6	N	13850	14371	522	-5	**ATG/AGG**
Glu (Q)	N	14372	14440	69	0	
Cyt b	J	14444	15584	1141	3	**ATG/T**
Thr (T)	J	15585	15655	71	0	
Pro (P)	N	15655	15723	69	-1	

A graphical reconstruction of the molecule generated via the online tool MitoFish (v3.75) [404] is shown in Figure 21. An in-depth analysis of the *Z. cristatus* mtDNA isolated in this study was conducted to elucidate its features.

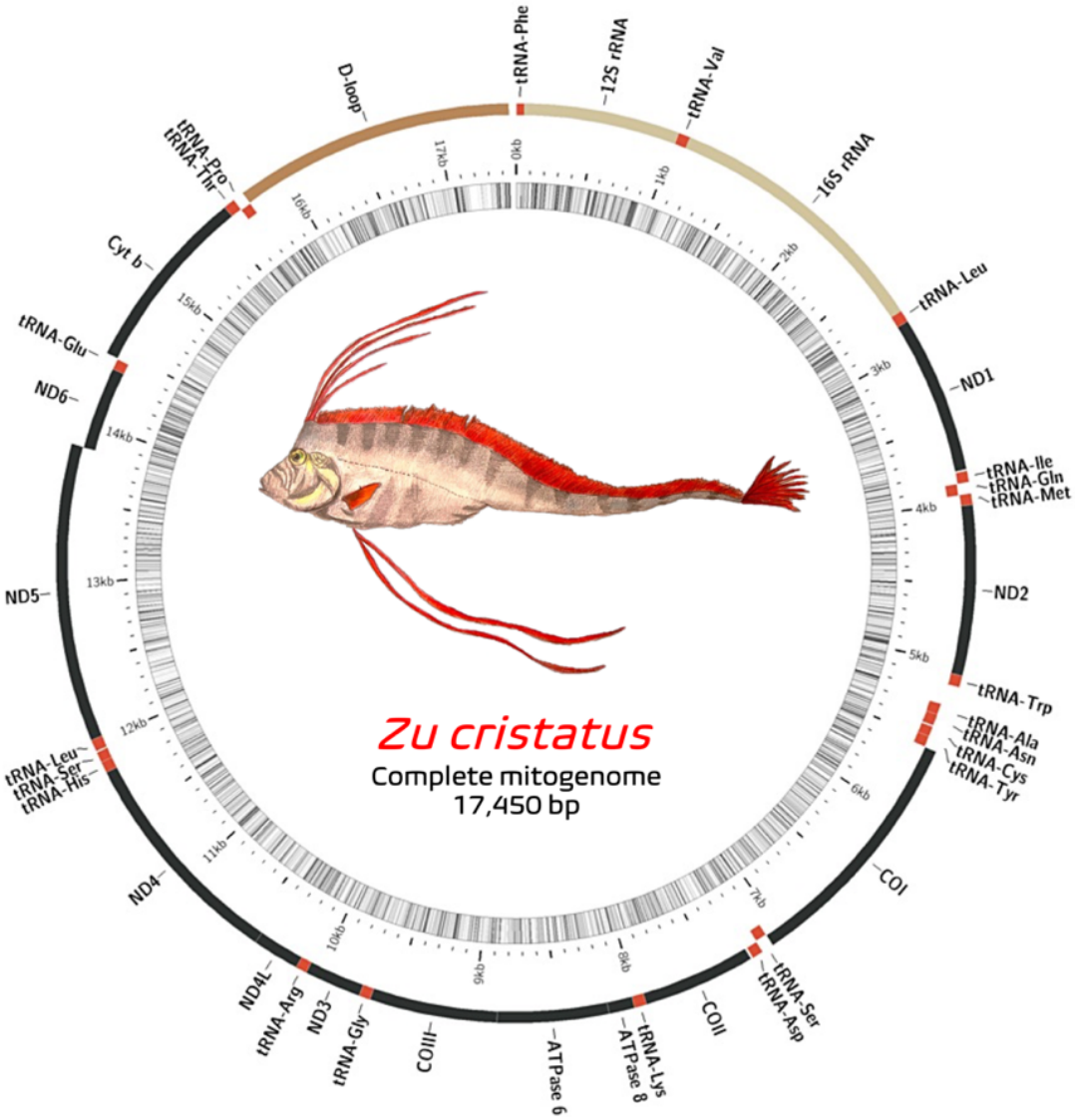


Figure 21. Structure of the mitochondrial genome of *Z. cristatus* isolated during this study. A total of 37 genes (22 tRNAs, 13 protein-coding genes and 2 rRNAs) were identified in the assembly, including the D-loop region. The internal circle represents G+C content per every 5 bp (darker lines show the higher G+C content). The external circle represents the two mtDNA strands (J-Forward eternally, N-Reverse internally). The red, black and avana blocks indicate tRNAs, PCGs, and rRNAs, respectively. The D-loop region is colored brown. The genome graphical representation was created using Mitofish, *Z. cristatus* illustration was made by Prof. Serena Savoca.

4.2.2 - Mitogenome features

Each sequence's nucleotide content values were obtained using MEGA X [410]. The composition bias based on the asymmetry values of all the sequences were estimated using the formulas: AT-skew = (A% - T%)/(A% + T%), and GC-skew = (G% - Ci%)/(G% + C%) [411]. Analyzing the asymmetry of the *Z. cristatus* whole mitochondrial genome, we found

negatives AT-skews (- 0.0814) and negative GC-skews (- 0.1898) (Table 12), showing the content of A and C (Adenine and Cytosine) in greater proportions in the majority chain compared to the content of T and G (Thymine and Guanine), respectively. Those findings follow the pattern of nucleotides composition observed in other teleost species as *Cyprinodon rubrofluviatilis* and *Kryptolebias marmoratus,* which showed both negative AT- and GC-skews [412], but contrast with some others like the Sciaedidae species *Johnius belangerii* and *Argyrosomus japonicus* which showed negative AT-skews and positive GC-skews and vice versa, respectively [413]. The strand asymmetry of mostly metazoans mitogenomes often exhibits positive AT-skew values, which reveal more A than T on the strand, and negative GC-skew values, which indicate more C than G [414,415]. Despite this, it is not rare that this strand bias is inversed, with negative AT skews and positive GC skews on the majority strand, or as in our case, both negative. This trend is attributable to an alteration, often an inversion, of the replication origin (ROI) located in the control region (D-loop), resulting in an altered strand asymmetry [416].

While, mainly due to the low shared contents of G, it seems to be a generalized feature of fish species mitogenome to having a greater AT percentage than GC, and in the same manner among AC and TG [417].

Table 12. Total lengths, compositions, and skewness of sequenced *Z. cristatus* whole mitogenome and concatenated PCGs, tRNAs, rRNAs and D-loop (Control region).

	Size (bp)	A%	T%	G%	C%	AT%	GC%	AT-SKEW	GC-SKEW
Whole mitogenome	17450	25.9	30.5	17.7	26.0	56.4	43.6	-0.0814	-0.1898
PCGs	11415	23.1	33.6	17.2	26.1	56.7	43.3	-0.1850	-0.2070
tRNAs	1460	27.9	27.1	23.8	21.2	55.0	45.0	0.0137	0.0563
rRNAs	2634	30.3	23.7	22.1	23.8	54.1	45.9	0.1222	-0.0380
dLOOP	1727	30.7	28.7	17.3	23.3	59.4	40.6	0.0341	-0.1481

The total length of PCGs resulted in 11,415 bp, and the AT content resulted in 56.4% (Table 11). The asymmetry analysis of these concatenated regions resulted in negative AT-skews (-0.1850) and negative GC-skews (- 0.2070) (Table 11); a similar trend recently showed for *Pseudocaranx dentex* by Li and colleagues [418]. The almost equal proportions of T and G to A and C in this region follow the values of asymmetry obtained in several mitogenome fish

studies of various ecological and biological habits representing a generalized trend [418–420].

Individually evaluated, PCGs presented average AT contents ranging from 49.2% (ND6) to 60.07% (ATPase8) (Table 13). Also, all PCGs had negative AT-skews and, in most cases, negative GC-skews, except ND6. These data are interestingly in accordance with studies on species with similar habits of *Z. cristatus* [421], while contrasting with some studies on Anguilliformes fish of the Muraenidae family [422,423], highlighting different skewness patterns concerning their behaviors.

Table 13. Sizes, compositions, and skewness of sequenced *Z. cristatus* PCGs.

PCGs	Size (bp)	A%	T%	G%	C%	AT%	GC%	AT-SKEW	GC-SKEW
ATPase 6	683	24.7	35.9	12.6	26.8	60.6	39.4	-0.1836	-0.3606
ATPase 8	168	29.8	31.0	10.7	28.6	60.7	39.3	-0.0196	-0.4545
COI	1551	23.6	33.1	18.7	24.6	56.7	43.3	-0.1682	-0.1356
COII	691	26.8	31.5	16.9	24.7	58.3	41.7	-0.0819	-0.1875
COIII	785	23.3	33.0	17.8	25.9	56.3	43.7	-0.1719	-0.1837
Cyt b	1141	22.0	34.8	16.7	26.5	56.8	43.2	-0.2253	-0.2252
ND1	969	20.4	35.0	16.8	27.8	55.4	44.6	-0.2626	-0.2454
ND2	1045	21.4	33.3	14.6	30.6	54.7	45.3	-0.2168	-0.3531
ND3	349	20.3	36.4	15.5	27.8	56.7	43.3	-0.2828	-0.2848
ND4	1381	23.7	33.7	16.1	26.6	57.3	42.7	-0.1742	-0.2462
ND4L	297	17.5	34.0	18.2	30.3	51.5	48.5	-0.3203	-0.2500
ND5	1833	25.5	33.2	15.6	25.7	58.7	41.3	-0.1301	-0.2444
ND6	522	18.0	31.2	35.4	15.3	49.2	50.8	-0.2685	0.3962

The mitogenome of *Z. cristatus* presented a set of 22 tRNAs, with a value in total length of 1460 bp, AT contents of 55.0%, and positive AT/GC-skews (Tab 11), similarly to recently reported data for freshwater species as *Gobiobotia naktogensis* [424], but contrasting findings on *Sinorhodeus microlepis* which reported an unusual negative AT-skew on tRNAs [425]. Individually, the tRNA AT content ranged from 40.8% (tRNA-Thr) to 66.0% (tRNA-Phe) (Tab 13), and the lengths ranged from 50 bp (tRNA-Phe) to 74 bp (tRNA-Leu and tRNA-Lys) as shown in Table 13. This range value of tRNAs length resulted wider than normally reported for teleost, particularly due to the abnormal short length of tRNA-Phe of 50 bp, which contrast with common values of 68-70 bp reported for this value in several species

[426,427]. Further insights are needed to deepen this strange feature of *Z. cristatus* mitogenome by representing the lowest length value for this tRNA in bony fishes.

Table 14. Sizes, compositions, and skewness of sequenced *Z. cristatus* tRNAs.

tRNAs	Size (bp)	A%	T%	G%	C%	AT%	GC%	AT-SKEW	GC-SKEW
tRNA-Ala	69	27.5	33.3	23.2	15.9	60.9	39.1	-0.0952	0.1852
tRNA-Arg	69	31.9	33.3	17.4	17.4	65.2	34.8	-0.0222	0.0000
tRNA-Asn	73	27.4	28.8	24.7	19.2	56.2	43.8	-0.0244	0.1250
tRNA-Asp	69	31.9	29.0	20.3	18.8	60.9	39.1	0.0476	0.0370
tRNA-Cys	65	24.6	24.6	30.8	20.0	49.2	50.8	0.0000	0.2121
tRNA-Gln	71	23.9	31.0	29.6	15.5	54.9	45.1	-0.1282	0.3125
tRNA-Glu	69	24.6	34.8	26.1	14.5	59.4	40.6	-0.1707	0.2857
tRNA-Gly	69	34.8	30.4	17.4	17.4	65.2	34.8	0.0667	0.0000
tRNA-His	69	24.6	27.5	26.1	21.7	52.2	47.8	-0.0556	0.0909
tRNA-Ile	70	22.9	24.3	28.6	24.3	47.1	52.9	-0.0303	0.0811
tRNA-Leu	73	34.2	28.8	19.2	17.8	63.0	37.0	0.0870	0.0370
tRNA-Leu	74	24.3	21.6	27.0	27.0	45.9	54.1	0.0588	0.0000
tRNA-Lys	74	35.1	25.7	17.6	21.6	60.8	39.2	0.1556	-0.1034
tRNA-Met	69	31.9	27.5	15.9	24.6	59.4	40.6	0.0732	-0.2143
tRNA-Phe	50	38.0	28.0	18.0	16.0	66.0	34.0	0.1515	0.0588
tRNA-Pro	69	26.1	29.0	29.0	15.9	55.1	44.9	-0.0526	0.2903
tRNA-Ser	67	25.4	28.4	22.4	23.9	53.7	46.3	-0.0556	-0.0323
tRNA-Ser	71	21.1	28.2	29.6	21.1	49.3	50.7	-0.1429	0.1667
tRNA-Thr	71	19.7	21.1	28.2	31.0	40.8	59.2	-0.0345	-0.0476
tRNA-Trp	73	31.5	21.9	21.9	24.7	53.4	46.6	0.1795	-0.0588
tRNA-Tyr	68	23.5	19.1	30.9	26.5	42.6	57.4	0.1034	0.0769
tRNA-Val	73	27.4	19.2	24.7	28.8	46.6	53.4	0.1765	-0.0769

The subunits rRNA 16S and rRNA 12S concatenated had a length of 2,634 bp and AT contents of 54.1%) as reported in Table 11, with positive AT-skews and negative GC-skews, values in the normal range for marine teleost. Individually, the subunit 12S shows AT content of 51.7% and a length of 957 bp, while 16S resulted in 55.4% AT content with a length of 1,677 bp (Table 15). Also, in this case, literature studies reported comparable values for marine teleost [418,428], while freshwater species commonly report relatively higher rRNA AT content values [429,430].

Table 15. Sizes, compositions, and skewness of sequenced *Z. cristatus* rRNAs.

rRNAs	Size (bp)	A%	T%	G%	C%	AT%	GC%	AT-SKEW	GC-SKEW

12S rRNA	957	28.2	23.5	23.2	25.1	51.7	48.3	0.0909	-0.0390
16S rRNA	1677	31.5	23.9	21.5	23.1	55.4	44.6	0.1389	-0.0374

The control region (D-loop) of *Z. cristatus* had a length of 1,727 bp and an AT content of 59.4%, with a positive AT-skew and a negative GC-skew (Table 12). These data were interesting; indeed, the control region of the few bony fishes for which this sequence is annotated shows a quite higher AT content. Moreover, the length of the D-loop region sequenced in our study resulted among the longest in literature comparable to the *Dicentrarchus labrax* one, firstly reported at about 2,5 kb by Cecconi et al. in 1995 [403], and more recently by Tine and colleagues with a length of 1950 bp [431]. Several other authors have reported very short control regions compared to these values, ranging on average values of 900 bp [432–435], with rare cases of longer sequences as in the case of *Pagrus major* reported by Xia et al. [428] or *Epinephelus epistictus* recently reported by Vella N. and Vella A. [436], together with related species that had shorter lengths of this sequence. One factor not to be underestimated is that *P. major* represents the species most similar from a biological and ecological point of view to *Z. cristatus* among the cited literature.

Moreover, *D. labrax* has aspects of the life cycle that allow it to adapt to somewhat different environments, even if, in this case, mainly for salinity variations, in a similar way to our species. Hence, it is significant that these two species have control region length values that are comparable to *Z. cristatus*. However, further investigation is needed, as very few mitogenomes in fish are complete in D-loop sequences, so data is still lacking. As reported by Cecconi et al., these variations could be attributable to the high rate of repetitive regions of this mitogenome part [403].

The mitochondrial sequence of *Z. cristatus* presented 15 small, non-coding, intergenic nucleotide regions (IGN), with sizes varying from 1 to 130 nucleotides, totaling 199 nucleotides (Table 11), with an average length of 13.27 nucleotides. These data resulted in uncommonly concerning the big sizes of the Ala's IGN (130 nucleotides), which altered the total length and the size range of these regions. Indeed, IGN sizes range commonly from 1 to 20 bp, with not uncommon cases of 40-50 of maximum [413,437,438]. However, due to

their assessed variability and polymorphisms, influenced by many factors like the sex of the specimen, even for these features, a vast basin of annotated sequences could reveal interesting insights [439].

Data on AT content of the 37 mitochondrial genes and AT- and GC-skews are graphically shown in Figures 22 and 23.

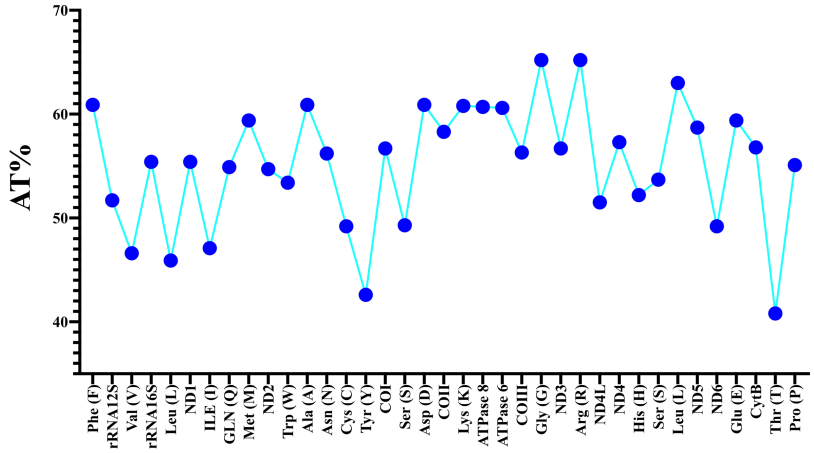


Figure 22. Information on AT content (%) of *Z. cristatus* mitogenome genes. This figure was generated using *GraphPad Prism 8.0.1* [440].

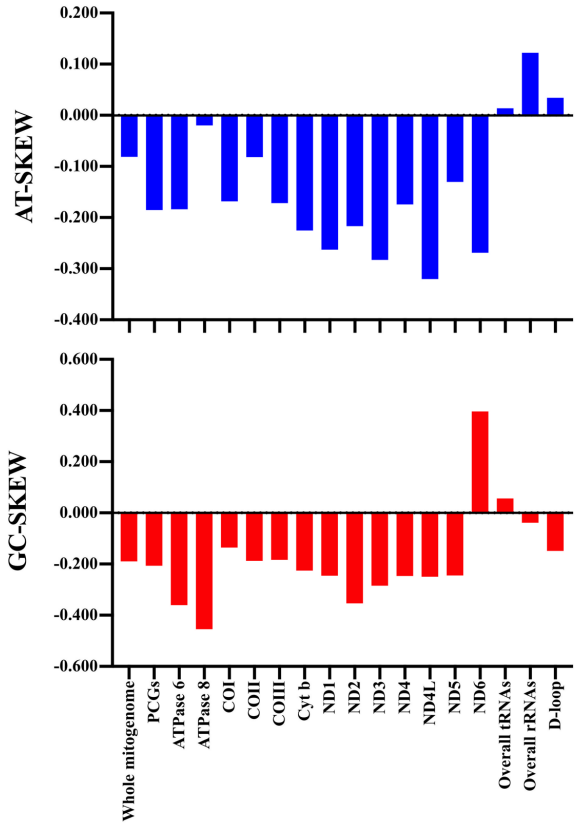


Figure 23. Information on AT- and GC-skews values of *Z. cristatus* whole mitogenome, PCGs concatenated and singularly, tRNAs, rRNAs and D-loop. This figure was generated using *GraphPad Prism 8.0.1* [440].

4.2.3 - Characteristics of protein-coding genes (PCGs)

The mitogenome of *Z. cristatus* shows twelve PCGs (ND1, ND2, COI, COII, ATP8, ATP6, COIII, ND3, ND4L, ND4, ND5, and CYT B) showed a sense of transcription on the J strand (Forward), and just ND6 on N strand (Reverse), the same pattern reported for several bony fishes sequences [408,409]. A total of 3,710 codons, excluding the stop codons, are used in *Z. cristatus* mitogenome. Analyzing the PCGs start codons, most genes show ATG as the standard start codon, except for COI, which used GTG as reported in Table 11, like that reported in other studies on bony fish of freshwater [412] and saltwater [421]. Indeed, uncommon start codons are very rare in bony fish, as the CCT recently reported in *Mobula tarapacana* by Chandrasekaran et al. [441]. On the contrary, the stop codons are more variable in the teleost, despite the complete stop codon TAA in the most common among PCGs. It is estimated that the expression of complete TAA stop codons is due to post-transcriptional polyadenylation events. However, the recording of incomplete stop codons (TA/T) is typical

in bony fishes, as confirmed by our data on *Z. cristatus,* which shows five T (ND2, COII, ND3, ND4, and CYT B) and two TA (ATP6 and COIII) stop codons on PCGs, and following several other authors [412,421]. Also, TAG is a common stop codon shared by several species on ND1 [417,435] and more rarely in COI [442], confirming our findings on *Z.cristatus.* Some other authors reported the TAG stop codon even for ND3, ND5, and ND6 in other species [408,437]. Our data shows that the stop codon AGG on ND6 is rarely reported in bony fishes and is more frequent in freshwater species such as Caracidae [430], and in *M. tarapacana* [441]. Another rare stop codon is represented by the CCT one, recently reported for ND4 in *M. tarapacana* by Chandrasekaran et al. [441].

The relative synonymous codon usage (RSCU) was estimated using MEGA X [410]. The analysis of the RSCU (Table 16) showed that almost all codons are present in our sequenced mitogenomes, except for AGA, which synthesizes the Arginine amino acid.

Table 16. Relative synonymous codon usage (RSCU) in sequenced *Z. cristatus* whole mitogenome.

Amino Acid	Codon	Count	RSCU
Alanine (A)	**GCU**	118	1.35
	GCC	120	1.37
	GCA	91	1.04
	GCG	21	0.24
Arginine (R) AGN	**AGA**	0	0
Arginine (R) CGN	**CGU**	13	1.01
	CGC	23	1.79
	CGA	30	2.34
	CGG	10	0.78
Aspartate (D)	**GAU**	39	1.07
	GAC	34	0.93
Asparagine (N)	**AAU**	70	1.22
	AAC	45	0.78
Cysteine (C)	**UGU**	10	0.74
	UGC	17	1.26
Glutamine (Q)	**CAA**	68	1.4
	CAG	29	0.6
Glutamate (E)	**GAA**	59	1.22
	GAG	38	0.78
Glycine (G)	**GGU**	80	1.32
	GGC	60	0.99
	GGA	40	0.66
	GGG	63	1.04

Histidine (H)	**CAU**	52	1.03
	CAC	49	0.97
Isoleucine (I)	**AUU**	175	1.47
	AUC	81	0.68
	AUA	102	0.85
Leucine (L) UUN	**UUA**	146	1.3
	UUG	39	0.35
Leucine (L) CUN	**CUU**	225	2.01
	CUC	97	0.87
	CUA	127	1.13
	CUG	38	0.34
Lysine (K)	**AAA**	43	1.12
	AAG	34	0.88
Methionine (M)	**AUG**	59	1
Phenylalanine (F)	**UUU**	139	1.14
	UUC	105	0.86
Proline (P)	**CCU**	82	1.53
	CCC	92	1.71
	CCA	31	0.58
	CCG	10	0.19
Serine (S) AGN	**AGU**	27	0.61
	AGC	27	0.61
Serine (S) UCN	**UCU**	74	1.66
	UCU	60	1.35
	UCU	63	1.42
	UCU	16	0.36
Threonine (T)	**ACU**	102	1.56
	ACC	70	1.07
	ACA	74	1.13
	ACG	16	0.24
Tryptophan (W)	**UGG**	29	1
Tyrosine (Y)	**UAU**	65	1.2
	UAC	43	0.8
Valine (V)	**GUU**	88	1.47
	GUC	43	0.72
	GUA	73	1.22
	GUG	35	0.59
Stop	**UAA(*)**	3	0.1
	UGA(*)	87	2.84
	UAG(*)	2	0.07
	AGG(*)	1	0.08

Thus, the most frequently used codons were, in decreasing order, CGA (Arg) (RSCU value 2.34), CUU (Leu) (RSCU value 2.01), CGC (Arg) (RSCU value 1.79), while AGG (Arg) (RSCU value 0.08), CCG (Pro) (RSCU value 0.19), and ACG (Thr) and GCG (Ala) (RSCU value 0.24) were rarely used. The frequencies of the RSCU are graphically represented in Figure 24.

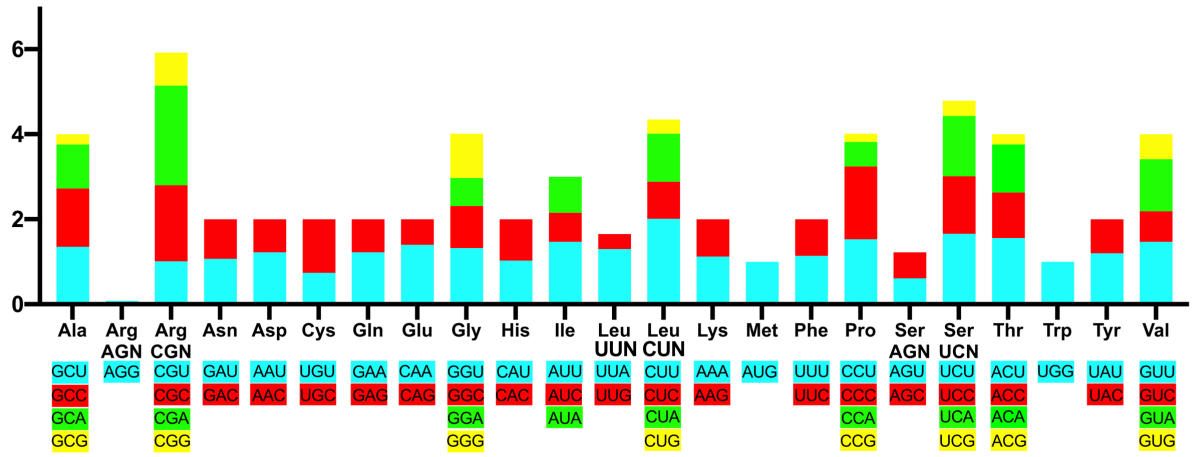


Figure 24. Relative synonymous codon usage (RSCU) of *Z. cristatus* mitogenome. RSCU values are represented on the y-axis, and families of synonymous codons and their respective amino acids are indicated on the x-axis. This figure was generated using *GraphPad Prism 8.0.1* [440].

The RSCU trend of *Z. cristatus* shows a similar trend with Carangiformes [418], Pleuronectiformes [419], and also freshwater species like Cipriniformes [443].

UGA represents the most used in our sequenced genome regarding stop codon usage. This codon that generally is related to W synthesis, sometimes in fish species, represents a stop codon [444,445].

4.2.4 - Pairwise distance

Fish mitogenome DNA studies have led to significant innovations in species identification and population [16]. Most of the studies carried out in this field used MT-genes as markers for species identification; specifically, the most used following main species-specific DNA sequences are ribosomal 16S and 12S subunits, cytochrome b (CYT B or *Cyt b*), and cytochrome c oxidase I (COI or *mt-co1*) [446–449]. COI has been widely used thanks to its moderate variability in nucleotide composition and gained the role of "DNA barcode" for species identification [430]. Despite this, several studies demonstrated that may limit the use of only COI in strictly related species [450,451]. COI markers may slowly evolve, resulting in low nucleotide sequence distances in some taxonomic groups, thus preventing specific discrimination of closely related species [452,453] when the gene does not contain effective regions for barcoding applications [454]. Therefore, COI barcodes are not enough to indiscriminate species identification results, especially in some cases [455]. In these terms,

it is evident how important it is to identify MT markers or conduct a multiple markers approach.

This situation has led to the formulation of the proposal to study and analyse the complete mtDNA sequence to identify mitochondrial markers or multiple marker approaches [170] with higher and more inter-specific divergence.

Data on mtDNA are scarce and incomplete for the Lampriformes order, making species identification difficult. To create a baseline of suitable markers for Lampriformes ID, we performed the following analyses on the 13 PCGs of the *Z. cristatus* mtDNA sequenced.

The pairwise distance method lies at the basis of the inter-specific divergence and then phylogenetic tree reconstruction. The value of the p-distance index gives information on affinity among tree-like compared organisms. The higher the p-distance value is, the farther the relation between the organisms, and vice-versa. The kinship between species, specimens or populations is enhanced by genetic distance. Studying the pairwise distance among different specie using single mitochondrial PCGs can help define the most suitable marker for drawing phylogenetic trees. We have used PCGs sequences of *Z. cristatus* and other 42 species to evaluate the pairwise distances among these species. Species have been selected in relation to life habits, habitat features and taxonomical distances (Supplementary Table 1).

GenBank Accession IDs, to obtain the single PCGs, are listed in supplementary table 2. Each of the thirteen *Z. cristatus* PCGs sequences (ATP6, ATP8, COI, COII, COIII, CYTB, ND1, ND2, ND3, ND4, ND4L, ND5, ND6) has been firstly aligned with the correspondent of the other 42 species.

Analyses were conducted using the Maximum Composite Likelihood model [456]. This analysis involved 43 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Evolutionary analyses were conducted in MEGA11 [457]. DNA conservation sequence analyses have been carried out using DnaSP (v.6) [458].

The number of base substitutions per site from between sequences is shown in Table 17 and in Figure 25.

Table 17. Estimates of evolutionary divergence between sequences. Green and red highlighting refer minimum and maximum pairwise distance detected for each gene among the species respectively.

Zu cristatus MT-genes													
Species/Gene	**ATP6**	**ATP8**	**COI**	**COII**	**COIII**	**CYTB**	**ND1**	**ND2**	**ND3**	**ND4**	**ND4L**	**ND5**	**ND6**
Ataxolepis apus	0.304	0.460	0.253	0.266	0.257	0.324	0.400	0.519	0.405	0.442	0.267	0.440	0.533
Ateleopus japonicus	0.400	0.579	0.244	0.247	0.257	0.358	0.383	0.523	0.455	0.445	0.353	0.414	0.504
Benthosema pterotum	0.346	0.428	0.251	0.316	0.303	0.351	0.378	0.480	0.429	0.439	0.279	0.414	0.546
Cetomimus sp.	0.314	0.468	0.261	0.261	0.267	0.320	0.398	0.510	0.413	0.435	0.255	0.445	0.557
Cetostoma regani	0.352	0.401	0.255	0.258	0.259	0.329	0.388	0.523	0.422	0.456	0.323	0.400	0.465
Chlorophthalmus agassizi	0.352	0.439	0.258	0.250	0.269	0.307	0.381	0.494	0.461	0.435	0.304	0.424	0.656
Chlorophthalmus nigromarginatus	0.356	0.555	0.268	0.255	0.282	0.297	0.368	0.496	0.411	0.437	0.318	0.422	0.634
Danacetichthys galathenus	0.339	0.412	0.281	0.265	0.286	0.348	0.391	0.502	0.491	0.469	0.299	0.481	0.559
Danio rerio	0.324	0.499	0.270	0.294	0.294	0.370	0.430	0.609	0.512	0.436	0.342	0.580	0.646
Dentex dentex	0.376	0.464	0.254	0.258	0.271	0.354	0.351	0.525	0.440	0.451	0.278	0.415	0.634
Dentex gibbosus	0.379	0.401	0.246	0.257	0.279	0.361	0.351	0.523	0.424	0.446	0.285	0.422	0.656
Diaphus splendidus	0.354	0.428	0.245	0.292	0.284	0.337	0.357	0.528	0.371	0.443	0.230	0.414	0.517
Electrona carlsbergi	0.363	0.428	0.250	0.314	0.308	0.339	0.374	0.499	0.394	0.439	0.276	0.408	0.554
Eutaeniophorus festivus	0.311	0.460	0.259	0.266	0.259	0.322	0.401	0.523	0.409	0.434	0.259	0.434	0.533
Eutaeniophorus sp.	0.352	0.401	0.255	0.258	0.259	0.329	0.388	0.521	0.422	0.456	0.323	0.400	0.465
Gyrinomimus myersi	0.303	0.419	0.264	0.263	0.260	0.342	0.401	0.537	0.392	0.441	0.275	0.444	0.547
Gyrinomimus sp.	0.305	0.474	0.257	0.261	0.260	0.326	0.408	0.515	0.427	0.454	0.267	0.448	0.556
Harpadon microchir	0.328	0.522	0.244	0.237	0.272	0.349	0.370	0.518	0.421	0.441	0.302	0.419	0.449
Ijimaia dofleini	0.364	0.594	0.238	0.256	0.271	0.336	0.382	0.529	0.455	0.427	0.322	0.392	0.474
Lampadena atlantica	0.331	0.497	0.264	0.299	0.327	0.339	0.386	0.490	0.442	0.468	0.290	0.404	0.518
Lampris guttatus	0.320	0.605	0.260	0.271	0.288	0.320	0.399	0.456	0.405	0.417	0.255	0.409	0.437
Myctophum affine	0.368	0.460	0.265	0.283	0.298	0.345	0.349	0.521	0.383	0.449	0.233	0.410	0.475
Neoscopelus macrolepidotus	0.335	0.457	0.253	0.268	0.262	0.339	0.369	0.451	0.426	0.454	0.281	0.393	0.511
Pagellus acarne	0.389	0.512	0.266	0.268	0.267	0.370	0.338	0.533	0.422	0.482	0.261	0.432	0.636
Pagellus bogaraveo	0.416	0.496	0.270	0.251	0.265	0.342	0.350	0.516	0.402	0.463	0.277	0.425	0.637
Parataeniophorus gulosus	0.354	0.411	0.253	0.258	0.259	0.330	0.386	0.521	0.422	0.456	0.323	0.401	0.465
Procetichthys kreffti	0.325	0.394	0.257	0.223	0.260	0.321	0.326	0.499	0.398	0.449	0.245	0.397	0.437
Regalecus glesne	0.279	0.316	0.236	0.192	0.219	0.256	0.319	0.406	0.347	0.360	0.191	0.359	0.324
Salmo salar	0.287	0.480	0.259	0.244	0.270	0.335	0.368	0.489	0.459	0.437	0.260	0.400	0.433
Sarda sarda	0.351	0.434	0.244	0.266	0.274	0.362	0.341	0.512	0.433	0.433	0.264	0.417	0.449
Saurida undosquamis	0.329	0.513	0.265	0.250	0.260	0.343	0.361	0.498	0.490	0.428	0.281	0.417	0.489
Saurida wanieso	0.318	0.526	0.248	0.231	0.265	0.363	0.363	0.510	0.473	0.442	0.324	0.421	0.482
Scopelengys tristis	0.334	0.468	0.254	0.261	0.291	0.329	0.351	0.449	0.383	0.448	0.249	0.389	0.485
Seriola dumerili	0.324	0.434	0.260	0.254	0.285	0.341	0.356	0.518	0.430	0.440	0.313	0.422	0.487
Stylephorus chordatus	0.331	0.527	0.249	0.268	0.262	0.345	0.365	0.496	0.368	0.403	0.286	0.463	0.474
Synodus variegatus	0.357	0.534	0.261	0.273	0.294	0.355	0.405	0.565	0.458	0.491	0.343	0.478	0.544
Thunnus alalunga	0.338	0.443	0.250	0.265	0.282	0.333	0.340	0.527	0.456	0.436	0.286	0.403	0.465
Thunnus thynnus	0.347	0.443	0.252	0.263	0.272	0.330	0.341	0.524	0.438	0.431	0.283	0.399	0.465
Trachipterus trachypterus	0.242	0.259	0.198	0.196	0.196	0.239	0.273	0.362	0.262	0.314	0.191	0.323	0.298
Trachurus japonicus	0.313	0.386	0.265	0.267	0.294	0.317	0.344	0.480	0.396	0.448	0.281	0.394	0.492
Trachurus trachurus	0.310	0.385	0.265	0.265	0.300	0.324	0.352	0.472	0.381	0.459	0.277	0.388	0.473
Triphoturus mexicanus	0.375	0.495	0.249	0.289	0.277	0.332	0.376	0.534	0.416	0.446	0.254	0.402	0.490

As expected, and confirmed by phylogenetical analyses (see following sections), the minimum pairwise distances recorded were between *Z. cristatus* and *T. trachypterus* for all mitochondrial PCGs (green highlighting in Table 17). While the maximum pairwise distance site detected for each gene among the species selected resulted from a highly variable, higher distanced pairwise PCGs resulted between *Z. cristatus* and *D. rerio*.

The analysis of the p-genetic distance among all PCGs showed that the NAD group genes display more sequence distance than COI and CYTB genes (Figure 25).

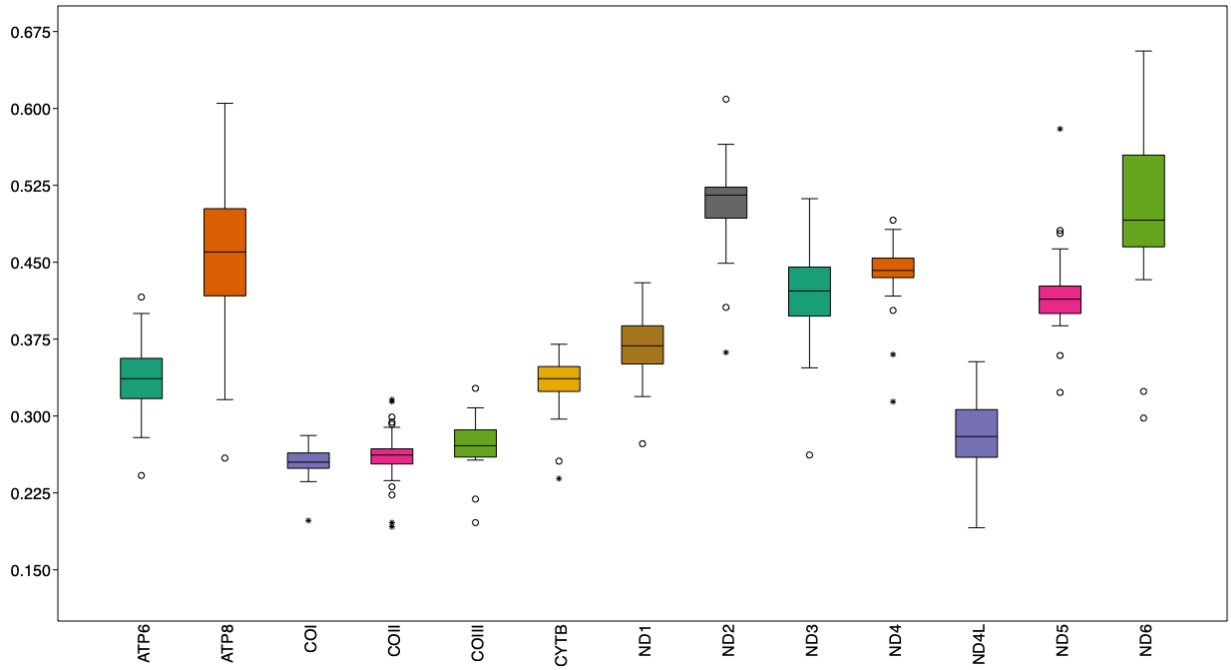


Figure 25. Overall mean p-genetic distance ±SD in PCGs comparison of *Z. cristatus* and other 42 species selected. Number of base substitutions per each site from averaging over all sequence pairs is shown. *p* distances are on the y axes, while single PCGs are on the x axes. Circles and asterisks identify the outliers.

ND genes showed a higher degree of sequence variability among studied species. Still, only ND3 and ND4 showed a higher sequence conservation rate, 0.401 and 0.460 (p-distance), respectively, which are helpful to be targeted for primer design evaluation.

Thus, ND3 and ND4 genes appeared more appropriate for barcoding as it provides species-level information on selected species. This highlights the necessity to explore better potential markers for species identification especially regarding ND genes. It has been reported, in

fact, for the Sparidae family that useful markers for precise species identification are ND2 and ND5 genes [455].

Our results corroborate the importance of whole mtDNA analyses in creating unequivocal and compelling identification markers in teleost species.

4.3 Phylogeny of the mitochondrial genome of *Zu cristatus*

Taxonomy and systematics of the order Lampriformes are still affected by the absence of a wide data basin, especially from a molecular point of view, which currently represent the main goal of the research in regard [129]. Particularly for the rarest families, the few or incomplete descriptions joined to the morphological variation within, and similarity between Lampriformes species leads to some uncertainty on phylogenetic relationships [200]. The molecular analysis will be essential to assess better the taxonomic status of these taxa, which probably will pass through revisions.

4.3.1 - Phylogenetic relationships of Lampriformes based on mt-co1 sequences

Here we report the results of the phylogenetic reconstruction of Lampriformes based on the currently available cytochrome oxidase subunit 1 (*mt-co1*) gene sequences revealed, with high posterior probability support, that the topology of the tree is partially following the currently accepted taxonomic relationships amongst the families (Table 1). The analysis comprises all the currently available sequences on NCBI of 21 species of the order annotated on the GenBank database (Supplementary Table 2). Although we have chosen the most common gene used for this kind of analysis in teleost, the remaining six species included in the order (*L. guntheri, L. machadoi, R. elongatus, R. kessinger, T. fukuzakii, T. ishikawae*) still have no annotated *mt-co1* sequences, denoting the strong lack of data on the subject and the need to deepen this topic. Particularly, no available data was present for the entire family of Radiicephalidae. Hence, it was not possible to include them in our analysis. On the contrary, all the species of the Lampridae, Veliferidae, and Regalecidae families showed the *mt-co1* sequences available in NCBI.

The coding sequences of mt-co1 from 21 Lampriformes species were aligned with Muscle and then trimmed with Gblocks. A total of 542 positions were selected for Bayesian phylogenetic reconstruction using the GTR+I nucleotide substitution model. The consensus tree was built after burning 25% of the trees from 500,000 generations. Bayesian posterior probabilities are represented as percentages. This analysis was performed with the pipeline NGPhylogeny.fr [459] (Figure 26).

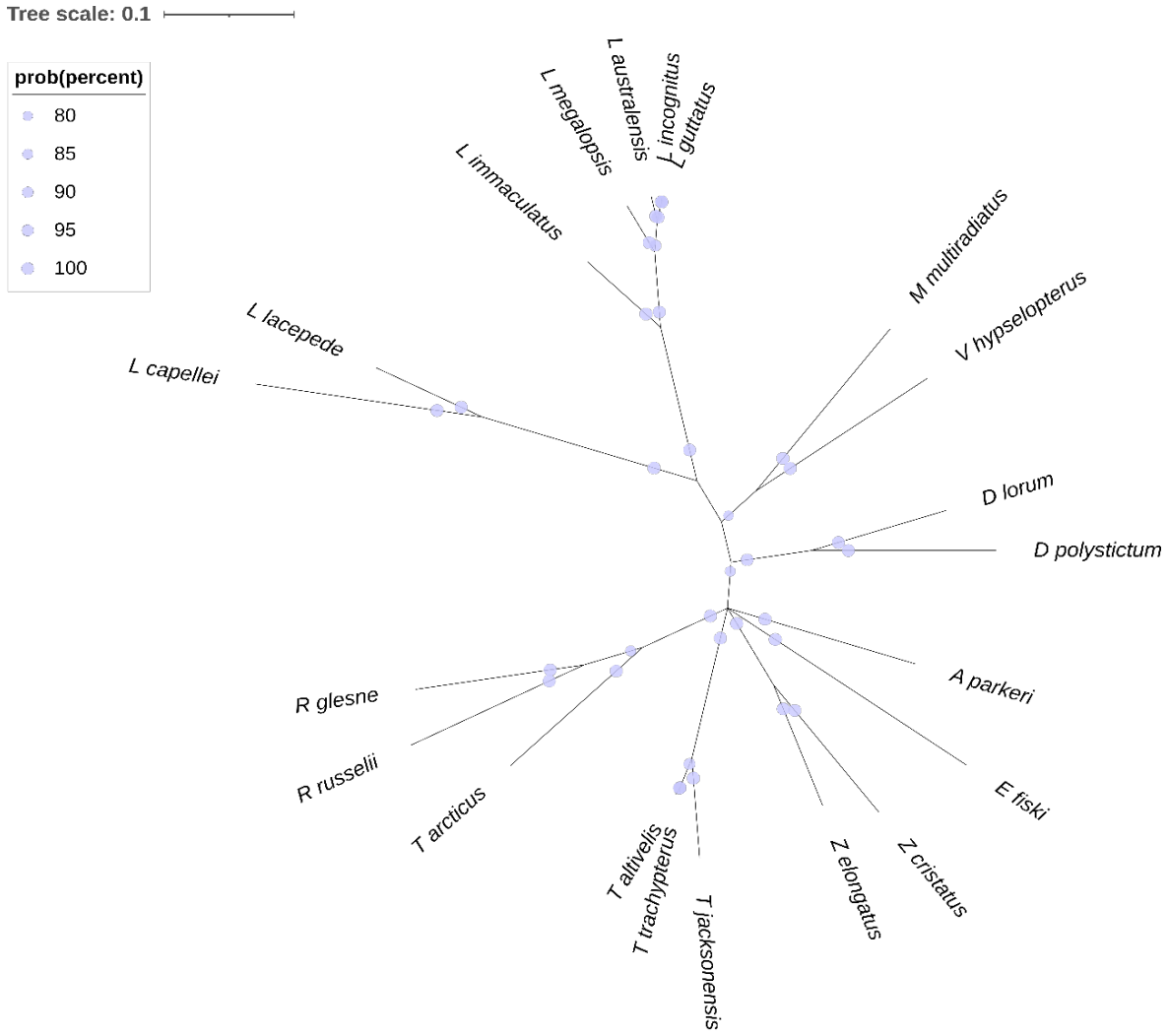


Figure 26. Unrooted radiation tree illustrating the phylogenetic relationships of the Lampriformes species based on the currently available mt-co1 sequences. The scale bar corresponds to an estimated evolutionary distance of 0.1. The tree was shown using the iTOL utility.

From our analysis, the members of the first two taxa clustered in accordance with the current taxonomy on two monophyletic branches, not distant from each other. Differently, the three species of the Regalecidae family were grouped in two separate branches from the same node, one related to the two species of the Regalecus. At the same time, the other one

consisted only of *A. parkeri*. The other three branches originated from the same node, two of whom related to the Trachipteridae genera *Trachipterus* and *Zu*, correctly grouped, and the other one concerning the species *E. fiski* resulted separately from the other Lophotidae. Indeed, the species of the genus *Lophotus* grouped somewhat distant from *E. fiski*, in a branch near the Lampridae one, as a well-defined clade. This grouping represents the most important difference between our analysis and the accepted phylogeny of the order.

Similarly, the two species of the genus *Desmodema* clustered separately, but in this case, not too far from the rest of the Trachipteridae genera. It is also interesting to note how *T. arcticus* grouped separately from the congeneric, on a shared branch with *Regalecus* species, originated from the same node as the rest of *Trachipterus*. We can resume the most interesting insight from our analysis of the position within the Lophotidae and Trachipteridae families. Further and more complex analyses are needed to better assess these relationships, especially those between quite different organisms also morphologically and biologically, such as Lophotidae [158].

4.3.2 - Phylogenetic relationship between Zu cristatus and related taxa based on the mitogenome sequences

For phylogenetic inference evaluation of our sequencing, our complete *Z. cristatus* mitogenome was compared with a subset of 44 fish mitochondrial genomes downloaded from the MitoFish database (Supplementary Table 3). Our dataset comprises all major ray-finned fish lineages and major taxa, such as Teleostea, Clupeocephala, Euteleostea, Neoteleostea, Acanthomorpha, and Percomorpha. Based on this variety, we provided our analysis to expand the phylogenetic relationships among *Z. cristatus*, the other Lampriformes species with annotated mtDNA genome, and the rest of the related groups with similar biological and ecological habits in most cases, plus two outgroups chosen between two taxa of fresh and brackish water (*D. rerio* and *S. salar*).

This analysis was performed using the JolyTree utility v.1.1b.191021ac [460]. The resulting tree was visualized with iTOL v6.5.6 [461] (Figure 27).

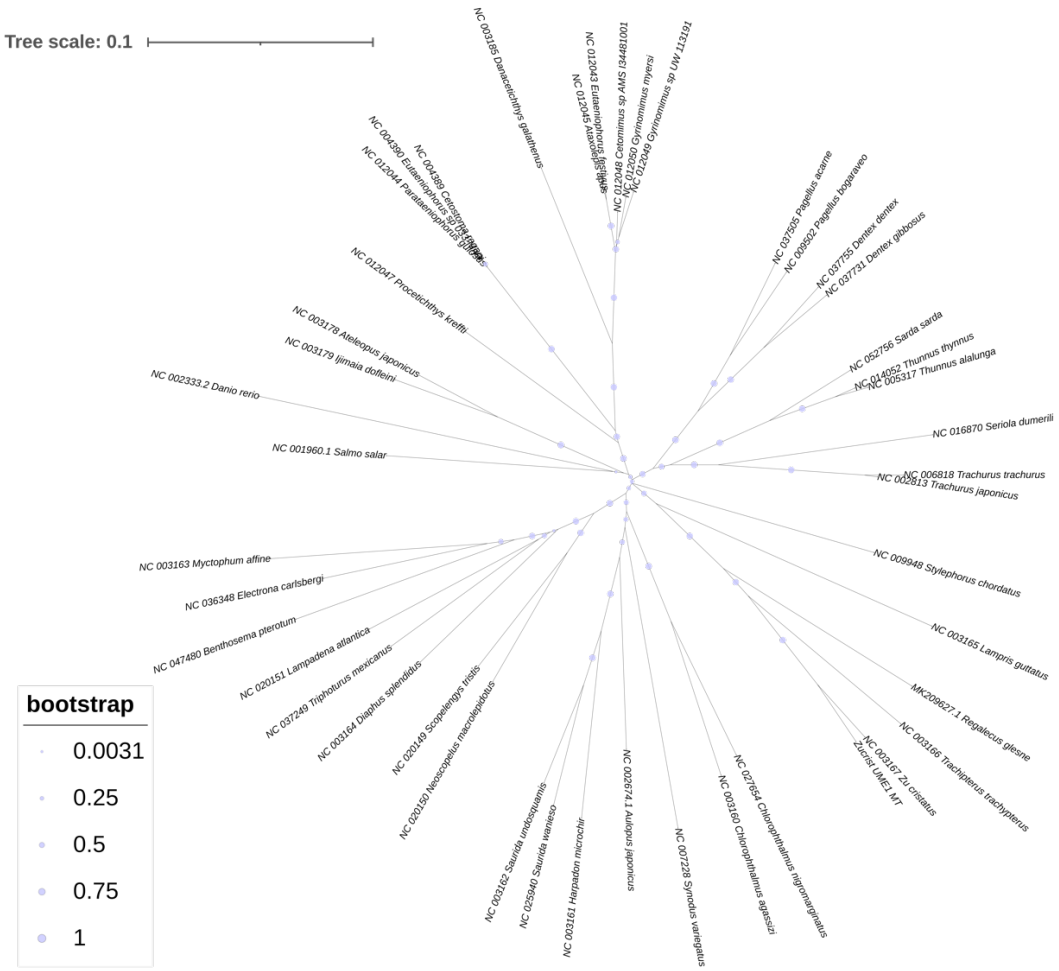


Figure 27. Unrooted radiation tree inferred by JolyTree using 44 mitogenomes downloaded from the MitoFish database (Supplementary Table 2). The scale bar corresponds to an estimated evolutionary distance of 0.1. The tree was shown using the iTOL utility.

Based on this analysis, it is interesting to note how the results of phylogenetics relationships within Lampriformes order are comparable to the previous one referred to mt-co1 sequences and reported in Figure 26. Indeed, the trachipterids *Z. cristatus* and *T. trachypterus* are grouped, with high bootstrap values, in the same branch, followed in order by *R. glesne* and *L. guttatus,* respectively, less closely related. These results align with previously reported classifications for the order [132,156,271], particularly under the most recent one made by Yu and colleagues in 2019 related to the *R. glesne* mtDNA annotation. The difference between the two *Z. cristatus* sequences analyzed is attributable to the presence in our new annotation of the D-loop portion, which on the contrary, was absent in the annotation of Miya et al. 2001 [16].

Very interesting is the position of *S. chordatus,* currently a species of the order Stylephoriformes but once part of the current order of Lampriformes as a separate family [1]. Our analysis confirms the position of the Stylephoridae, and despite its group as the nearest taxon to Lampriformes, distance assigns it to a different order [2]. Similarly interesting was to analyze other taxa once entered by old classifications in the order Lampriformes, such as the existing Cetomimidae family of Bercyformes order and the Atelopodidae family of Atelopodiformes order [153]. About the first, previously classified as the Mirapinnidae family within the Lampriformes order, the ten species analyzed showed strict relation among them as a well-assessed taxon within the tree. Indeed, the strict groupings of species of the genera *Gyrinomimus* and *Cetomimus* were in line with the classification of Colgan et al. [462] based on the 16S rDNA data, as well as the distance with the genus *Cetostoma*. Our data also according with the bGMYC analysis of Weber (2020) [10], which reported in their maximum made credibility tree for the family the genera *Danacetichthys, Cetostoma*, and *Procetichthys* grouped respectively in three separated branches in this order far from the rest of Cetomimids. About the Ateleopodidae family, our analysis clearly showed the relationships within the taxon for the genera *Ateleopus* and *Ijimaia*, which grouped nearest the Cetomimidae species and far from Lampriformes ones. These findings contrast with the older classifications [153], mainly based on morphological evidence, such as those reported by Sasaki and colleagues in 2005 [463]. They studied the structure of the ethmoid region and proposed a strict relation between Ateleopodidae and Lampriformes taxa. Following our results, several molecular pieces of evidence have recently contradicted these findings on morphological bases [178,464].

Regarding the other taxa inserted in our analysis, the three order Carangiformes, Scombriformes, and Spariformes, are all commercially important orders in the Mediterranean basin, sometimes captured with any rare specimen of Lampriformes. These genera have clustered accordingly with the existing classification with high bootstrap values, both within the three orders and with the rest of the groups analyzed in our tree [2,464,465]. About the order Aulopiformes, our findings are in accordance with Tan and colleague [466] about recent *mt-co1*-based relationships of the genus *Saurida;* in the same

manner, the results of Zhang and Xian [467] confirmed the relationships within the *Saurida* genus and the ones with *Harpadon* and *Synodus*, that in our analysis grouped accordingly with their classification. The slight distance found in our analysis between these genera and the *Chlorophthalmus* species supported the classification of Ota et al. [468] proposed in 2000 for the Order and based on *Cyt b* sequences.

The last considered order of Mictophiformes, as for Aulopiformes species, comprises meso- and bathypelagic fish involved in the same trophic webs of Lampriformes. Our analysis showed a correct grouping of these species within them, supported by high bootstrap values, partially in accordance with the previous classification based on the morphological position of photophores, a species-specific feature of these fishes, proposed by Denton and Adams in 2015 [469]. Indeed, they proposed a stricter phylogenetics relation between the genera *Benthosema* and *Myctophum*, while our analysis revealed a stricter relationship among the genera *Myctophum* and *Electrona*; however, the distances are very small in both cases, and these three genera are, in accordance with the two reconstructions, grouped quite far from *Diaphus.* The position of the genera *Triphoturus* and *Lampadena* are in accordance with the reconstruction of Paxton [470], while the grouping of *Scopelengys* and *Neoscopelus* genera requires more analysis to be confirmed.

The clustering of the species *D. rerio* and *S. salar* employed as outgroups confirmed the validity of our analysis.

5. OPSIN GENE FAMILY INVESTIGATION

Enhancing the knowledge of the distribution and diversity of aquatic organisms is essential to evaluate their conservation status [82,199]. The current state of art is affected by the limitation of professional fisheries focusing on a restricted number of commercially interesting species. These limitations led to a scarcity of data on many ecologically essential species with no commercial value [5,471]. Among these, Lampriformes species have a confirmed ecological role in the mesopelagic trophic webs. They arouse the scientific community's interest concerning their adaptations to life in deep-sea environments. Indeed, the process that led several Lampriformes species to pass from the shallow waters in which they live in juvenile stages to the high-depth oceans in adult life hides adaptive mechanisms still largely unknown [8,231].

The light regime in the water column led to separate mesopelagic, bathypelagic, bathyal, and abyssal zones. Until 1000m, the mesopelagic zone is characterized by even more attenuated and monochromatic sunlight, in which long wavelengths are absorbed while the short ones are scattered, leaving feeble blue-green light (470–480 nm) [28]. Over the 1000m depth the sun-derived luminous radiation is absent; hence the bathypelagic, bathyal, and abyssal zones are characterized by darkness. The only light radiations at these depth come from the intrinsic and extrinsic bioluminescence produced by living organisms [119].

The surrounding environment influences the visual system features of the organisms and their ecology and morpho-functional adaptations. In function of the depth and photic zone that fishes inhabit predominantly, they evolved these sensory systems, and all linked the morphological structure and physiological functions [472].

5.1 Opsin proteins characteristics and functions

The rhodopsin-like G protein-coupled receptor (GPCR) family represents a broad group of membrane-bound proteins that, among the others (e.g., olfactory receptors, hormones, and neurotransmitter receptors), includes opsin proteins [473]. Biochemically, the opsin proteins are bound with a vitamin A-derived chromophore at a lysine residue on position 296 [474].

Responding to light radiation absorbance, this chromophore isomerizes, inducing a conformational modification in the protein that causes intracellular G-protein-mediated transduction. This signal provokes hyperpolarization or depolarization of the membrane by activating the ciliary or rhabdomeric cells [475].

Although the isolation of these retinal pigments was obtained more than a century ago [476], the isolation of the first opsin gene sequence occurred only in 1983, the rhodopsin (RH1) of *Bos taurus* [477], followed one year after by the isolation of the RH1 of *Homo sapiens* [478]. Rhodopsin gene's function is related to vision in dim or scotopic light conditions and is expressed in rod cells. Differently, cone cells are used for bright light or photopic vision and expressed by short wavelength sensitive (SWS) and long wavelength sensitive (LWS) opsins [479]. Currently, the opsins are divided into five subfamilies: the long wave- or red-sensitive (LWS), two medium wave- or green-sensitive (MWS or Rhodopsins 1 and 2, RH1 and RH2), and two short wave- or blue-sensitive (SWS1 and SWS2) [98,475]. Davies and colleagues reported in 2007 about all these five opsins subfamilies in the lamprey *Geotria australis*, highlighting that this gene family organization was ancestral in vertebrates' evolution [480].

The origin of the subdivision of the opsin genes family seems to be attributable to the consequences of whole genome duplication events. Indeed, a tandem duplication appears in the vertebrate evolutive history to produce the LWS opsin and a second gene that gave rise to all the SWS1, SWS2, RH1, and RH2 opsins. Moreover, after analysis of several species, it is known how fewer opsins are present in mammals compared to their early vertebrate ancestors (SWS2 and RH2 genes were lost) [481,482]. Regarding actinopterygians, several PCR-based surveys and whole genome sequencing results have demonstrated that these species possess more opsins variants compared to the vertebrate ancestor, probably due to additional whole genome duplication events that occurred in this group [195].

5.2 *Zu cristatus* opsin-like gene products identification and insights

To identify opsin family proteins in our genome draft, an investigation was conducted on the Uniprot database [483] to create a dataset of related bony fish species opsins previously

annotated (Supplementary Table 4). Hence, full-length opsins were first processed using cd-hit to reduce query sequences' redundancy and computational time [484]. The results from unique opsins were searched on the *Z. cristatus* genome using exonerate software (v.2.54.1) [485]. Using ad hoc awk scripting for each blast match, we derivates the genomic coordinates extending it by 10,000 bp upstream and downstream of the match. The resulting loci were extracted using the samtools utility (v. 1.10) [486] and further computationally scanned by the Augustus algorithm (v.3.3.3) for the ab initio gene prediction [487].

Comparing the resulting 1057 raw predicted proteins with the original query opsins employing OrthoFinder software (v.2.3.11) for the classification of the *Z. cristatus* opsin-like gene products [488] reached 17 groups of protein with well-assessed structure and identity compared to the original queries. This groups contains the 20 best predicted opsin-like proteins found in the *Z. cristatus* genome draft. To better relate each protein sequence to its family, was performed an identity analysis using the BLAST online platform [489] for all these 20 opsins with a reliable structure for future annotation. Pairwise alignment analysis was performed using ClustalW [490], among the most interesting sequences found during this investigation in the main three opsin families (SWS, MWS, and LWS). Representative results of this data analysis are shown in Figures 28, 29 and 30.

```
g907                    MDSLSLSVNGVSYTEAADISK----PDDPFNGPIKSIAPWNFTILAILMFVVTSLSLGEN
A0A8C5B519_VerAn_La     MDTVSLSVNGVSYTEAPKIYEAADLPDDPFTGPIKSLAPWNFTFLAVLMFVVSSLSLSEN
                        **::************..* :    *****.*****:******:**:*****:****.**

g907                    FVVMFVTFKYKQLRQPLNYVIVNLALADFLVSLTGGVISFLTNARGYFFLGTWACVLEGF
A0A8C5B519_VerAn_La     FLVMFVTFKFKQLRQPLNYIIVNLAIADFLVSLTGGLISFLTNARGYFFLGTWACVLEGF
                        *:*******:*********:*****:**********:**********************

g907                    AVTFFGIVALWSLAVLSLERFFVICRPLGNIRLRGN------MHCWDCCSSGPFP-----
A0A8C5B519_VerAn_La     AVTFFGIVALWSLAVLSFERFFVICRPLGNVRLRGKHSMLGLLFVWTFSFVWTFPPVLGW
                        ****************:************:****:      :. *  .   .**

g907                    ------------------SSD----------------LPL-----CW--------------
A0A8C5B519_VerAn_La     NSYTVSKIGTTCEPNWYSTNMVDHSYIITFFVTCFILPLGVIIFCYGKLLQKLKKVSHGR
                                       *::                ***     *:

g907                    ------------------------------------------------------------AG
A0A8C5B519_VerAn_La     LATARKPERQVTRMVVVMIVAFMVGWTPYAAFSIVVTACPTIQLDPRVGAIPAFFSKTAA
                                                                                    *.

g907                    IATP--------------------------------          Score:  79
A0A8C5B519_VerAn_La     VYNPVIYVYMNKQVAVARGLLRRFGDCNRIGAHGLVR
                        : .*
```

Figure 28. Pairwise alignment of "vertebrate ancient long opsin a" sequences. g907 and A0A8C5B519 are our predicted *Z. cristatus* and *Gadus morhua,* respectively.

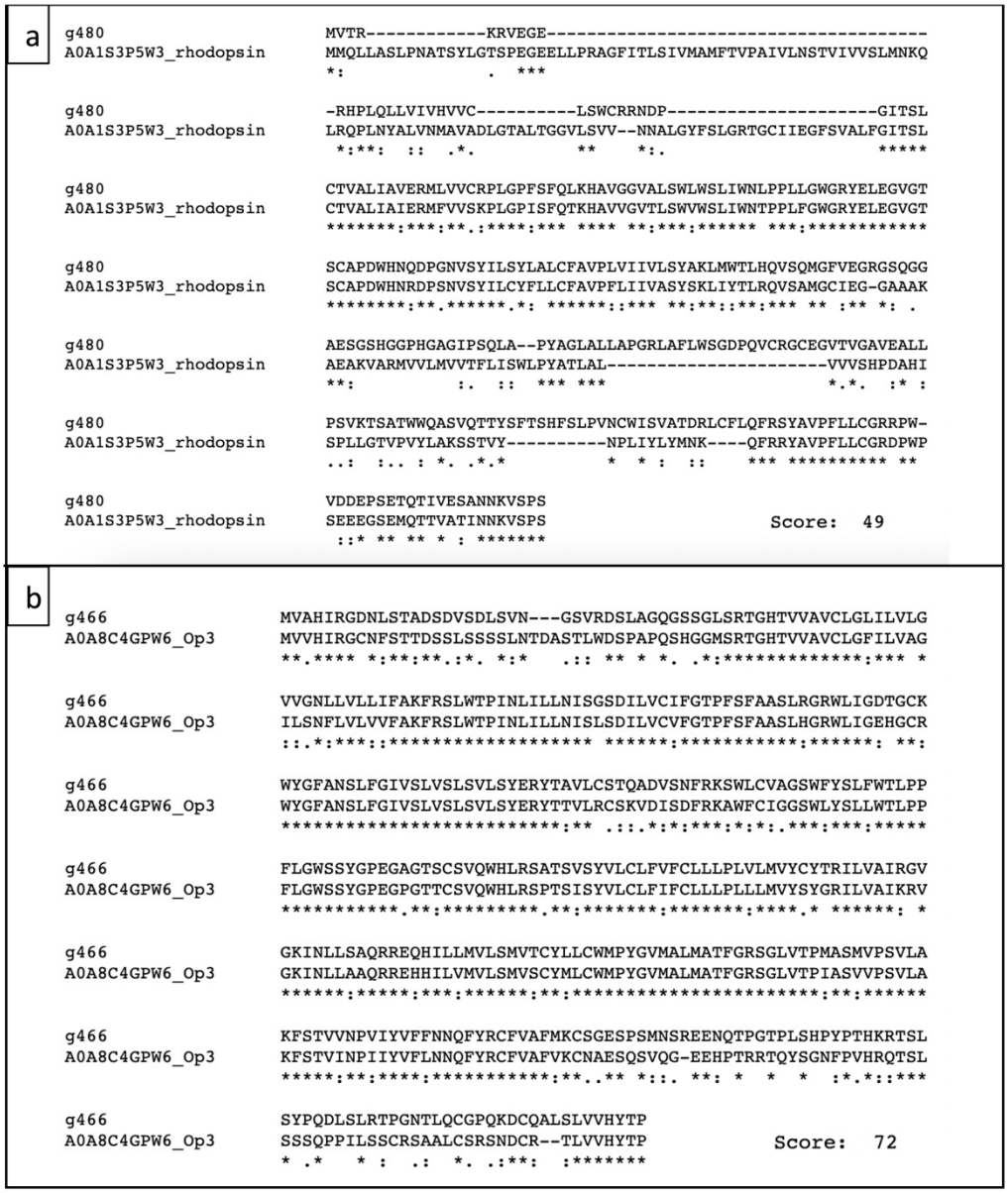


Figure 29. Pairwise alignment of MWS opsins family sequences. **a)** g480 and A0A1S3P5W3 are Rhodopsin of our predicted *Z. cristatus* and *Salmo salar,* respectively. **b)** g466 and A0A8C4GPW6 are Opsin 3 of our predicted *Z. cristatus* and *Dicentrarchus labrax*.

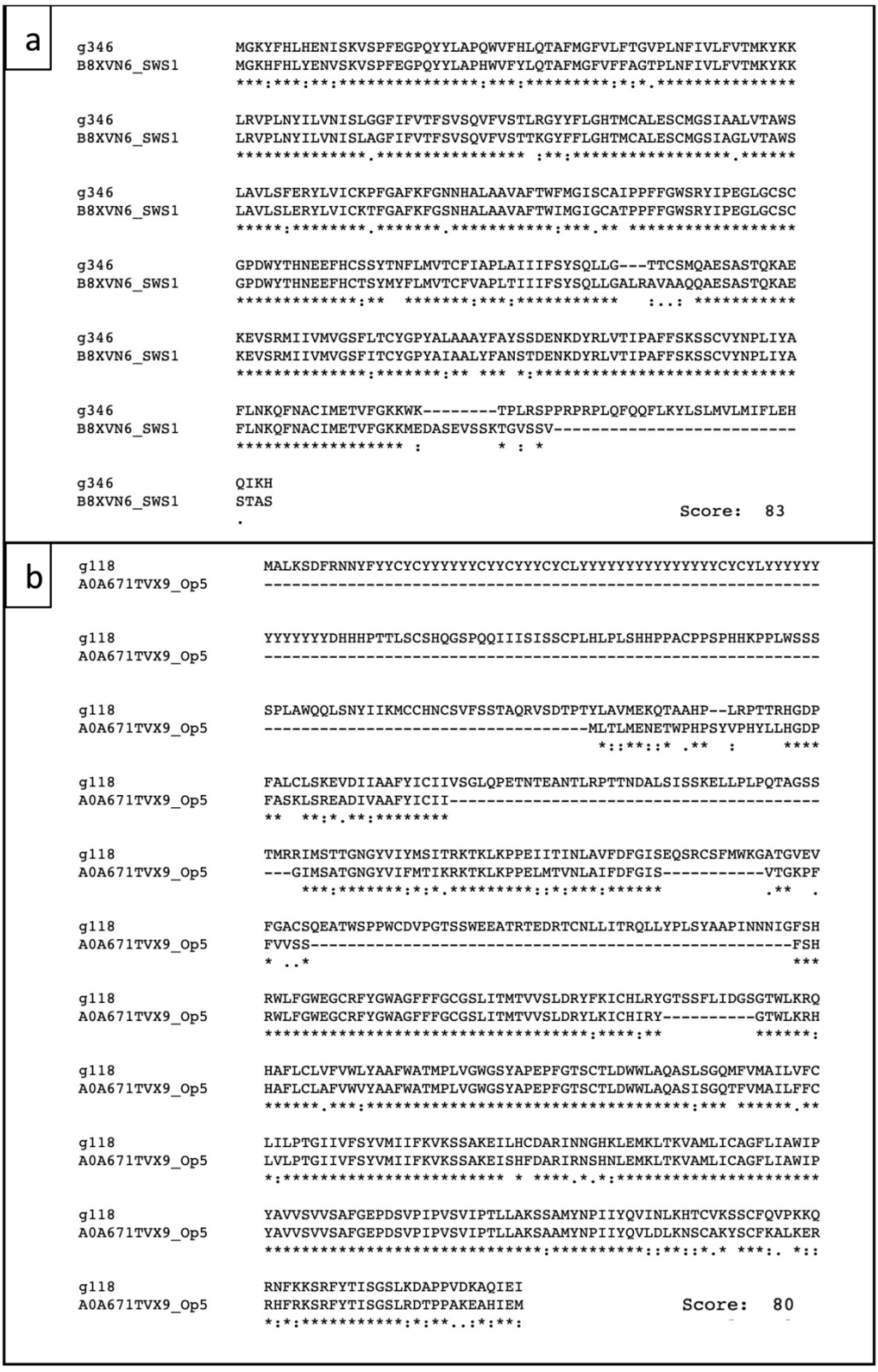


Figure 30. Pairwise alignment of SWS opsins family sequences. **a)** g346 and B8XVN6 are SWS1 of our predicted *Z. cristatus* and *Stenobrachius leucopsarus,* respectively. **b)** g118 and A0A671TVX9 are Opsin 5 of our predicted *Z. cristatus* and *Sparus aurata.*

Compared to other vertebrates, bony fishes show wide gene repertoires due to duplication events, which lead to several proteins for each family [475]. Moreover, when mutations or substitution have involved the key site of these sequences, variation in function or inactivation takes place [491]. For these reasons, an increased basin of genomic data is important to investigate and better assess the articulate structure of the protein families concerning their functions. Our analysis was carried out using our current best version of the *Z. cristatus* genome, obtained with a hybrid assembly including the reads from the Illumina and Nanopore platforms.

Regarding the opsins family, the key sites of modifications were related to the modulation of maximal wavelength absorption [475], a very important function considering bathymetrical migrators species such as *Z. cristatus*. Despite tandem duplication events producing several opsin gene copies in bony fishes, none of these appear to be related to the '3R' whole genome duplication event, which took place in the ancestor of teleost [361]. This event is confirmed by the presence in opsin gene families of some ancient proteins like our analyzed "vertebrate ancient long opsin a". Indeed, our investigation of *Z. cristatus* genome revealed the presence of some copies of this opsin-like polypeptide, one of these showed very high identity with this previously annotate ancient opsin Pairwise analysis against a vertebrate ancient long opsin a of *G. morhua* resulted in a well-conserved sequence with a high alignment score Figure 28. This could be due to the habits of the species that shared a mesopelagic lifestyle, as well as trophic habits partly overlapping. However, from an environmental light exposure point of view, these species shared the same condition, considering the habitude of living in more shallow water during the early life stage compared to the adult [492,493]. It is therefore understandable how this ancient protein, a precursor of the current opsins, has been well preserved in these two compared species.

Multiple gene copies are most prevalent in the RH2 and LWS opsin subfamilies, commonly expressed in double cones. Our investigation has not revealed evidence of LWS opsins' presence in *Z. cristatus* genome at this stage. Probably, this is due to the draft nature of our assembly and will be assessed in the next step of the project, until the final annotation of the whole genome. But it is interesting to note how, in contrast with the phylogenetic history

influence on the teleost opsin gene family size, it seems that biological features, such as feeding or reproductive behaviors and morphology, have not a great influence on opsin expression and differentiation [475]. Differently, light transmission in water has a significant effect on this evolutive process. Indeed, opsin gene expression differences have been associated with the variable spectral life environment in killifish [494] or migratory species as *S. salar* and *Anguilla anguilla* [495,496]. In contrast, some findings in species that live in more homogeneous habitats, such as the deep-sea and glacial marine environments, show fewer expression levels of these genes, especially those related to the LWS family [497–500]. These observations highlight the importance of considering the environmental influence and bathymetrical gradient on broad opsin repertoires evolution of teleost.

Regarding MWS, such as Rhodopsins 1 and two, our findings reveal some interesting insights. Indeed, several opsin-like sequences found on the *Z. cristatus* genome draft have shown high identity with the opsins of this family. Remarkably, the pairwise analysis results shown in Figure 29 highlight the good alignment score from the analysis against *D. labrax* opsin 3, also known as encephalopsin [501]. On the contrary, a *Z. cristatus* rhodopsin-like alignment with the same protein of the Salmoniformes *S. salar* showed a low score value. This could be explained by the different habits of the compared species, as *S. salar* is a famous euryhaline species that, during his life, passed through extreme migrations among the cold freshwater river and open sea saltwater. During these migrations, the illumination condition and absorption change, leading to extreme variability within the opsin gene family [502]. On the contrary, the more depth nature of *Z. cristatus*, coupled with his habitude to living in epi- and mesopelagic zones in early life stages, probably led to a marked ability to capture the medium light wavelength, more abundant in deep waters. This could explain the average similarity with the MWS of a species with a similar habitude of *S. salar,* regarding life at shallow depths, like *D. labrax* [195].

Interestingly, our analysis revealed a massive presence of SWS opsins related to vision in the deep-sea environment or, for terrestrial vertebrates, in low-illuminated habitats [480]. Analyzing the SWS opsin-like sequences found in the *Z. cristatus* genome draft, we report in Figure 30 a representative example of a high alignment score among abyssal species and

Z. cristatus SWS1 opsin-like gene product. Indeed, Mictophiformes species are linked throughout their life cycle to bathypelagic and abyssal environments, as confirmed by the evolution of specific structures adapted to life in dark environments such as photophores [472]. Hence, this high percentage of identity in the short wavelength light visual protein is reasonable. On the contrary, it is surprising to report a similar value among opsin 5 of *Sparus aurata* and the predicted one of *Z. cristatus.* Indeed, this is a euryhaline species commonly found in coastal relative shallow waters [503]. It should, however, considered that, as for *S. salar*, these species facing very different environments have a very high variability within the opsin family. Moreover, *S. aurata* is a species with very marked nocturnal eating habits, especially when it lives on sandy bottoms, which may have led it to develop an even more varied opsin asset during evolution. More related studies will undoubtedly be necessary and exciting.

Considering that our *Z. cristatus* genome still represents a draft, the reported results will be better evaluated when the genome will be complete and annotated in further steps. So here are shown some of the most exciting and reliable insights, although the amount of data is already much more extensive.

CONCLUSIONS AND PERSPECTIVES

The research question of this study originated from the status of current knowledge about the biology, distribution, genetic features, and phylogenetic relationships of the order Lampriformes. Indeed, despite their ecological importance, the literature about this bony fish order is still fragmented and mainly constituted by the synthetic report on the specimens' occurrence, often with little detailed information inserted as short contribution in collective articles. This scarcity of data especially affects the Mediterranean basin, where, based on our literature review, these fishes are probably more common than expected. Despite this, their low commercial value and relative rarity, if compared to other Mediterranean mesopelagic species, have made their ecological role and importance have never been in-depth investigated. More information would be essential to assess their role in marine mesopelagic and bathypelagic trophic webs and the features of their life cycle, also considering their peculiar life-history traits, that make them actors at different trophic levels in the various stages of their life cycle. From these regards, this contribution would help to enhance the consideration of Lampriformes species in marine scientific research, also considering rare features showed by some species, as for instance the homeothermy of *L. guttatus*, the possession of an ink glands of some Lophotidae and Radiicephalidae, the characteristic head-up swimming style of Trachipteridae, and the autotomy capacity of *R. glesne*.

Nonetheless, the presence of few genomic and mitochondrial sequences annotated for this taxon does not allow to deepen knowledge about their bathymetrical migration and consequential adaptations to the different conditions between shallow and deep marine environments. Moreover, considering their mentioned uncommon morphology and physiology, deepening the molecular basis of these features could highlight some exciting insights both from a genetic and ecologic point of view, especially considering these features in relation to their lifestyle. Providing the first whole genome draft of *Z. cristatus*, this contribution opens new perspectives from these points of view, adding important references to the few annotated in the online databases about Lampriformes species. The

annotation of the first complete mitogenome of the species, with in-depth analysis of its features, also allow us to review the phylogeny of the taxon and provide interesting new insights.

The present thesis has developed over five main chapters, each dedicated to improving one of these deficient above exposed aspects.

Chapter one provides the results of a thorough review of the literature concerning the Lampriformes in the Mediterranean Sea to gather information on their geographical distribution in this area. This aspect, never investigated before, will be essential to increase the attention of researchers to the areas where these species are less present, trying to understand better if their distribution is due to the gap in the data or to the ecological features.

In chapter two, a *Z. cristatus* occurrence in the Ionian Sea was described, providing an in-depth analysis of this adult specimen's morphometric and meristic data. These data were compared to all the other Mediterranean Sea occurrences, trying to resume these fragmented contributions. Representing the most detailed report in the literature, this contribution will undoubtedly provide essential insights into the morphology and biogeography of the species.

Chapter three describes the experimental procedures that led to the production of the first whole genomes draft of *Z. cristatus*. Trying to obtain an excellent quality output, a careful work of DNA isolation from *Z. cristatus* muscle tissue, with different methods and processing, as well as the use of two technologies (Illumina for short reads and Nanopore for long reads) during the sequencing phases. The assembly was carried out with three different workflows using and comparing numerous software, and a final draft was realized by combining the reads obtained by the two different technologies in a hybrid assembly. This method gave the best results, leading to the possibility to create a baseline data set for further analysis of the genome.

Chapter four concerns the isolation of the first complete mitochondrial DNA genome of *Z. cristatus*. In comparison with other species, extensive analysis has been carried out to describe the structure and functions of this mitochondrial DNA genome. Moreover, the

phylogenetic relationships within the Lampriformes order were evaluated, confirming the existing classification. The mitogenome has been annotated in the GenBank database and will be very useful for researchers in this field for phylogenetic, adaptive, and population studies.

Chapter five describes the first application on the whole genome of *Z. cristatus*. The analysis of the opsin-like proteins found in the genome draft provided exciting insights related to this functionally important family of peptides, which in meso- and bathypelagic fish is significant.

Further investigations will be necessary to better understand the functional mechanisms behind the adaptations that this species has undergone during its evolution. To realize this, it will be necessary to obtain the final annotation of the whole genome, reaching an even greater level of completeness of the assembly than the current ones, for which the last sequencing carried out has laid an outstanding expectation to realize it in the following months. Once this is done, the complete genome will be available to all researchers in this field and will provide essential insights and a basis to deepen the knowledge of these fascinating organisms, still poor known.

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Appendix 1 (Supplementary Tables)

Supplementary Table 1. Features of 43 species used for mitochondrial gene by gene pairwise comparisons.

Species	Order	Depth range (m)	Areal distribution
Trachurus japonicus	Carangiformes	0-275	Pacific Ocean
Chlorophthalmus agassizi	Aulopiformes	50-1000	Atlantic Ocean and Mediterranean Sea
Harpadon microchir	Aulopiformes	50-1000	Indo-Pacific Ocean
Saurida undosquamis	Aulopiformes	1-350	Indian Ocean
Myctophum affine	Myctophiformes	0-600	Atlantic Ocean
Diaphus splendidus	Myctophiformes	0-8000	Atlantic and Indo-Pacific Ocean
Lampris guttatus	Lampriformes	0-500	Worldwide
Trachipterus trachypterus	Lampriformes	100-600	Atlantic and Pacific Ocean, Mediterranean Sea
Zu cristatus	Lampriformes	0-2000	Worldwide
Ateleopus japonicus	Ateleopodiformes	140-600	Pacific Ocean
Ijimaia dofleini	Ateleopodiformes	70-1281	Pacific Ocean
Danacetichthys galathenus	Beryciformes	0-1330	Atlantic, Indian and Pacific Ocean
Cetostoma regani	Beryciformes	0-2250	Atlantic Ocean
Eutaeniophorus sp.	Beryciformes	0-200	Worldwide
Thunnus alalunga	Scombriformes	0-600	Worldwide
Trachurus trachurus	Carangiformes	0-1050	Atlantic Ocean and Mediterranean Sea
Synodus variegatus	Aulopiformes	3-121	Indo-Pacific Ocean
Pagellus bogaraveo	Eupercaria *incertae sedis*	150-700	Atlantic Ocean and Mediterranean Sea
Stylephorus chordatus	Stylephoriformes	300-800	Atlantic Ocean
Eutaeniophorus festivus	Beryciformes	0-200	Worldwide
Parataeniophorus gulosus	Beryciformes	0-1400	Indian and Atlantic Ocean
Ataxolepis apus	Beryciformes	1464-?	Pacific Ocean
Procetichthys kreffti	Beryciformes	0-2200	Atlantic Ocean
Cetomimus sp.	Beryciformes	0-1800	Pacific Ocean
Gyrinomimus sp.	Beryciformes	0-1400	Southern Oceans
Gyrinomimus myersi	Beryciformes	1280-2791	Atlantic Ocean
Thunnus thynnus	Scombriformes	0-985	Atlantic Ocean and Mediterranean Sea
Seriola dumerili	Carangiformes	1-360	Worldwide
Scopelengys tristis	Myctophiformes	400-1830	Atlantic, Indian and Pacific Ocean
Neoscopelus macrolepidotus	Myctophiformes	300-1180	Atlantic and Pacific Ocean
Lampadena atlantica	Myctophiformes	60-1000	Atlantic Ocean
Saurida wanieso	Aulopiformes	1-350	Indo-Pacific Ocean
Chlorophthalmus nigromarginatus	Aulopiformes	184-285	Indo-Pacific Ocean
Electrona carlsbergi	Myctophiformes	1-1008	Worldwide
Triphoturus mexicanus	Myctophiformes	25-180	Pacific Ocean
Pagellus acarne	Eupercaria *incertae sedis*	0-500	Atlantic Ocean and Mediterranean Sea
Dentex gibbosus	Eupercaria *incertae sedis*	20-220	Atlantic Ocean and Mediterranean Sea

Dentex dentex	Eupercaria *incertae sedis*	0-200	Atlantic Ocean and Mediterranean Sea
Benthosema pterotum	Myctophiformes	10-300	Atlantic and Indo-Pacific Ocean
Sarda sarda	Scombriformes	80-200	Atlantic Ocean and Mediterranean Sea
Danio rerio	Cypriniformes	0-20	Asia
Salmo salar	Salmoniformes	0-210	Atlantic Ocean

Supplementary Table 2. Dataset of 21 Lampriformes mt-co1 sequences available on NCBI database used for phylogenetic reconstruction of the relationships within the taxon.

GenBank Accession ID	Scientific name	COI length (bp)
MN123259.1	*Agrostichthys parkeri*	648
GU440303.1	*Desmodema lorum*	652
MN117725.1	*Desmodema polystictum*	658
BBG74528.1	*Eumecichthys fiski*	516
JF931925.1	*Lampris australensis*	655
GU992944.1	*Lampris guttatus*	662
MN123398.1	*Lampris immaculatus*	645
JF931946.1	*Lampris incognitus*	655
LC521832.1	*Lampris megalopsis*	685
MN123409.1	*Lophotus cappellei*	645
KR086866.1	*Lophotus lacepede*	665
EU366588.1	*Metavelifer multiradiatus*	782
MN123480.1	*Regalecus glesne*	645
KU943114.1	*Regalecus russelii*	552
GU440558.1	*Trachipterus altivelis*	652
MT323519.1	*Trachipterus arcticus*	629
HQ564197.1	*Trachipterus jacksonensis*	652
OM527150.1	*Trachipterus trachypterus*	652
GU673640.1	*Velifer hypselopterus*	652
MT323474.1	*Zu cristatus*	632
MN123529.1	*Zu elongatus*	651

Supplementary Table 3. Dataset of 44 fish mitochondrial genomes downloaded from MitoFish used for phylogenetic inference evaluation.

GenBank Accession ID	Scientific name	MT genome length
NC_002813	*Trachurus japonicus*	16559
NC_003160	*Chlorophthalmus agassizi*	16221
NC_003161	*Harpadon microchir*	16061
NC_003162	*Saurida undosquamis*	15737
NC_003163	*Myctophum affine*	16239
NC_003164	*Diaphus splendidus*	15985

NC_003165	*Lampris guttatus*	15598
NC_003166	*Trachipterus trachypterus*	16162
NC_003167	*Zu cristatus*	15987
NC_003178	*Ateleopus japonicus*	16650
NC_003179	*Ijimaia dofleini*	16645
NC_003185	*Danacetichthys galathenus*	16555
NC_004389	*Cetostoma regani*	16508
NC_004390	*Eutaeniophorus sp 033-Miya*	16508
NC_005317	*Thunnus alalunga*	16527
NC_006818	*Trachurus trachurus*	16559
NC_007228	*Synodus variegatus*	16448
NC_009502	*Pagellus bogaraveo*	16941
NC_009948	*Stylephorus chordatus*	15894
NC_012043	*Eutaeniophorus festivus*	16528
NC_012044	*Parataeniophorus gulosus*	16494
NC_012045	*Ataxolepis apus*	16528
NC_012047	*Procetichthys kreffti*	16527
NC_012048	*Cetomimus sp AMS I34481001*	16532
NC_012049	*Gyrinomimus sp UW 113191*	16570
NC_012050	*Gyrinomimus myersi*	16568
NC_014052	*Thunnus thynnus*	16527
NC_016870	*Seriola dumerili*	16530
NC_020149	*Scopelengys tristis*	16768
NC_020150	*Neoscopelus macrolepidotus*	16609
NC_020151	*Lampadena atlantica*	17807
NC_025940	*Saurida wanieso*	16552
NC_027654	*Chlorophthalmus nigromarginatus*	17663
NC_036348	*Electrona carlsbergi*	18282
NC_037249	*Triphoturus mexicanus*	18012
NC_037505	*Pagellus acarne*	16486
NC_037731	*Dentex gibbosus*	16771
NC_037755	*Dentex dentex*	16656
NC_047480	*Benthosema pterotum*	18052
NC_052756	*Sarda sarda*	16506
Zucrist_UME1	***Zu cristatus***	**17450**
MK209627 (NCBI)	*Regalecus glesne*	16,536
NC_002333	*Danio rerio*	16596
NC_001960	*Salmo salar*	16665

Supplementary Table 4. Dataset of the annotated or predicted teleost opsins used as query during our opsin-like protein identification in the whole genome of *Z. cristatus*.

Uniprot Accession ID	Scientific name	Opsin family or subfamily

P35359	*Danio rerio*	Rhodopsin
Q9W6A7	*Danio rerio*	Red-sensitive opsin-1
Q9W6A5	*Danio rerio*	Green-sensitive opsin-1
Q9W6A6	*Danio rerio*	Green-sensitive opsin-4
Q9W6A8	*Danio rerio*	Opsin-1, short-wave-sensitive 2
Q9W6A9	*Danio rerio*	Opsin-1, short-wave-sensitive 1
Q8AYM7	*Danio rerio*	Green-sensitive opsin-3
Q8AYM8	*Danio rerio*	Green-sensitive opsin-2
Q8AYN0	*Danio rerio*	Red-sensitive opsin-2
Q801U8	*Danio rerio*	Rhodopsin
A0A0N9NXZ8	*Danio rerio*	Rhodopsin
Q8AV67	*Danio rerio*	Rhodopsin
Q6P981	*Danio rerio*	Opsin 1 sws1
A0A0N9N7J4	*Danio rerio*	Long-wavelength-sensitive-1 cone opsin
A8E5J4	*Danio rerio*	Opsin 1 (Cone pigments), short-wave-sensitive 2
A0A0N9P0E6	*Danio rerio*	Long-wavelength-sensitive-2 cone opsin
Q7SZY0	*Danio rerio*	Opsin 1 (Cone pigments), long-wave-sensitive, 1
Q6NW86	*Danio rerio*	Opsin 1 (Cone pigments), short-wave-sensitive 1
A0A0R4IMF0	*Danio rerio*	Opsin-1, short-wave-sensitive 2
Q9YGZ4	*Dicentrarchus labrax*	Rhodopsin
E6ZFQ3	*Dicentrarchus labrax*	Rhodopsin
E6ZFQ2	*Dicentrarchus labrax*	Rhodopsin
A0A8C4GPW6	*Dicentrarchus labrax*	Teleost multiple tissue opsin 3a
A0A8C4GU75	*Dicentrarchus labrax*	Opsin 5
A0A8C4H795	*Dicentrarchus labrax*	Opsin 4xb
Q9YH02	*Sparus aurata*	Rhodopsin
A0A671UDI1	*Sparus aurata*	Rhodopsin
A0A671VTD4	*Sparus aurata*	Rhodopsin
A0A671TSR5	*Sparus aurata*	Rhodopsin
A0A671TVX9	*Sparus aurata*	Opsin 5
A0A671YCV0	*Sparus aurata*	Opsin 4a (melanopsin)
A0A671Y7G0	*Sparus aurata*	Opsin-5-like
A0A671VKY1	*Sparus aurata*	Teleost multiple tissue opsin 3b
A0A2R3ZCJ1	*Hippoglossus hippoglossus*	Rhodopsin
Q8QGQ0	*Hippoglossus hippoglossus*	Blue opsin
Q8QGP9	*Hippoglossus hippoglossus*	Red opsin
Q8QGR3	*Hippoglossus hippoglossus*	UV opsin
A0A0B4WV39	*Lampris guttatus*	Short-wavelength sensitive opsin 2B
P32310	*Carassius auratus*	Blue-sensitive opsin
P32313	*Carassius auratus*	Red-sensitive opsin
P32311	*Carassius auratus*	Green-sensitive opsin-1
P32313	*Carassius auratus*	Red-sensitive opsin
Q90309	*Carassius auratus*	Ultraviolet-sensitive opsin

P32309	*Carassius auratus*	Rhodopsin
O13227	*Conger conger*	Blue-sensitive opsin
Q9YGZ3	*Salaria pavo*	Rhodopsin
O42604	*Zeus faber*	Rhodopsin
A0A0B4WWR9	*Zeus faber*	Short-wavelength sensitive opsin 2B
P79848	*Poecilia reticulata*	Rhodopsin
A0A3P9P9F4	*Poecilia reticulata*	Opsin 4
A0A3P9Q6E7	*Poecilia reticulata*	Opsin 5
A0A3P9N3I9	*Poecilia reticulata*	Opsin 3
A0A140JTJ6	*Poecilia reticulata*	SWS1 opsin
G0XNX9	*Poecilia reticulata*	LWS A180 opsin
U6BY11	*Poecilia reticulata*	LWS-4 opsin
A0A140JTI9	*Poecilia reticulata*	LWS-2 opsin
Q0H3C3	*Poecilia reticulata*	Violet/blue-sensitive opsin
A0A140JTJ5	*Poecilia reticulata*	SWS2-B opsin
A0A140JTJ4	*Poecilia reticulata*	SWS2-A opsin
U6BY76	*Poecilia reticulata*	Short wave sensitive-2B opsin
F1JYM7	*Poecilia reticulata*	Short-wavelength sensitive opsin type 2A
U6BY90	*Poecilia reticulata*	Long wave sensitive-2 opsin
A0A140JTJ0	*Poecilia reticulata*	LWS-3
Q0H3C2	*Poecilia reticulata*	Opsin 1, short wave sensitive
U6BY12	*Poecilia reticulata*	Long wave sensitive-3 opsin
U6BY75	*Poecilia reticulata*	LWS-1
O13018	*Salmo salar*	Vertebrate ancient opsin
A0A1S3SP35	*Salmo salar*	Visual pigment-like receptor peropsin
Q6XR07	*Salmo salar*	SWS2 opsin
A0A1S3N1U7	*Salmo salar*	Melanopsin isoform X1
A0A1S3P5W3	*Salmo salar*	Rhodopsin
A0A1S3L0Y6	*Salmo salar*	Melanopsin isoform X1
A0A1S3QTC5	*Salmo salar*	Red-sensitive opsin-like
Q6XR08	*Salmo salar*	SWS1 opsin
Q6XR10	*Salmo salar*	LWS opsin
A0A1S3L7R3	*Salmo salar*	Opsin-5-like isoform X2
A0A1S3L868	*Salmo salar*	Opsin-5-like isoform X1
I1W5U9	*Salmo salar*	Melanopsin
A0A1S3LVX3	*Salmo salar*	Opsin-3-like isoform X3
A0A1S3RFH2	*Salmo salar*	Opsin-5-like
A0A1S3MCD5	*Salmo salar*	Opsin-5
Q804X9	*Gadus moruha*	Melanopsin-A
Q804Q2	*Gadus moruha*	Melanopsin-B
A0A8C5F4Y4	*Gadus moruha*	Teleost multiple tissue opsin 3a
Q5K6I6	*Gadus moruha*	Blue-sensitive pigment
A0A8C4ZTQ7	*Gadus moruha*	Opsin 7, group member b

A0A8C5FAF1	*Gadus moruha*	Teleost multiple tissue opsin 2a
Q5K6H7	*Gadus moruha*	Rhodopsin
A0A8C5B519	*Gadus moruha*	Vertebrate ancient long opsin a
A0A8C4ZJM1	*Gadus moruha*	Opsin 4xa
A0A8C5BQF9	*Gadus moruha*	Teleost multiple tissue opsin b
A0A8C4ZRG9	*Gadus moruha*	Blue-sensitive opsin-like
A0A8C5ABC6	*Gadus moruha*	Opsin 4b
A0A8C5BBG8	*Gadus moruha*	Opsin 4
A0A8C4ZCK7	*Gadus moruha*	Opsin 9
A0A8C4ZDA8	*Gadus moruha*	Opsin 8, group member a
A0A8C4ZUK3	*Gadus moruha*	Opsin 5
A0A8C5FX53	*Gadus moruha*	Opsin 8, group member c
A0A8C4Z2B2	*Gadus moruha*	Retinal pigment epithelium-derived rhodopsin homolog
A0A8C5B589	*Gadus moruha*	Opsin 7, group member a
A0A8C4Z134	*Gadus moruha*	Vertebrate ancient opsin-like
A0A8C4Z866	*Gadus moruha*	Opsin 3
A0A8C5CB49	*Gadus moruha*	Opsin 6, group member a
Q9YGZ9	*Mugil cephalus*	Rhodopsin
Q9YGZ8	*Chelon labrosus*	Rhodopsin
Q9YGZ6	*Chelon auratus*	Rhodopsin
Q9YGZ9	*Mugil cephalus*	Rhodopsin
Q9YGZ7	*Chelon saliens*	Rhodopsin
Q9YH00	*Lithognathus mormyrus*	Rhodopsin
Q9YH03	*Sarpa salpa*	Rhodopsin
Q9YH05	*Diplodus annularis*	Rhodopsin
Q9YH04	*Diplodus vulgaris*	Rhodopsin
A0A0B4WX63	*Spondyliosoma cantharus*	Rhodopsin
A0A0B4WWN6	*Spondyliosoma cantharus*	Rhodopsin
B8XVN6	*Stenobrachius leucopsarus*	SWS1 opsin
D2IV74	*Stenobrachius leucopsarus*	Rhodopsin
D2IV75	*Stenobrachius leucopsarus*	Rhodopsin
D2IV76	*Stenobrachius leucopsarus*	Rhodopsin
B4YA22	*Stenobrachius leucopsarus*	Rhodopsin