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A study of the genus Thaumetopoea (Lepidoptera: Notodontidae) using morphological, ecological, and molecular traits

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Padova, 15 October 2016

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Riassunto

La sottofamiglia Thaumetopoeinae (Notodontidae) comprende circa un centinaio di specie descritte, chiamate comunemente processionarie per il tipico comportamento delle larve durante gli spostamenti in file di vario tipo, organizzate per la ricerca del cibo o dei siti di impupamento. Anche se storicamente considerate in una famiglia distinta, le Thaumetopoeinae sono state recentemente inquadrate come sottofamiglia all'interno dei Notodontidae, sulla base di analisi cladistiche e molecolari. Il gruppo ha una grande importanza forestale perché le loro larve si nutrono delle foglie di alberi e arbusti, sia latifoglie sia conifere, indebolendo le piante e rendendole così più suscettibili all'attacco di altre avversità (i.e. scolitidi). Inoltre esse rappresentano una minaccia per la salute umana e degli animali domestici e di allevamento, a causa del rilascio di setole urticanti da parte delle larve e/o degli adulti. Queste setole, evolutesi come un'efficace strategia di difesa nei confronti di vertebrati predatori, a causa di contatti accidentali con altri vertebrati sono responsabili di reazioni allergiche, anche di forte intensità, sviluppando sintomi che spaziano da semplici dermatiti fino a shock anafilattici, con conseguenze mortali in alcuni casi.

Il genere *Thaumetopoea s.l.*, diffuso in Europa, Mediterraneo e area Iranoturanica, è senza dubbio il più noto ed è stato oggetto di una revisione che ha portato alla suddivisione in tre generi separati (*Helianthocampa, Thaumetopoea, Traumatocampa*). Tuttavia, un recente studio molecolare relativo alle specie più comuni ha chiarito alcune relazioni filogenetiche tra i taxa, mettendo in dubbio la validità della suddivisione e auspicando che uno studio comparato di caratteri morfologici, ecologici e molecolari portasse maggiore chiarezza. Con la presente tesi si è cercato di affrontare tale argomento.

Nella prima parte è stata completata la filogenesi del genere, analizzando 15 specie di *Thaumetopoea s.l.*, considerando anche specie rare rinvenute in diverse collezioni museali. Sono stati utilizzati al proposito 165 caratteri morfologici individuati in testa, torace, addome e genitali maschili. *Thaumetopoea loxostigma* Hacker, descritta all'inizio del 2016, non è stata inclusa in quanto l'unico esemplare descritto (olotipo), non era disponibile. Tuttavia, secondo la descrizione originale *T. loxostigma* è strettamente legata al gruppo *Thaumetopoea apologetica* - *Thaumetopoea jordana*, che necessita di una revisione approfondita. Cinque sottospecie diverse da quelle nominali sono attualmente riconosciute all'interno del genere *Thaumetopoea*, vale a dire: *T. apologetica abyssinica*, *T. herculeana judaea*, *T. processionea pseudosolitaria*, *T. solitaria iranica*, e *T. pityocampa orana*, e sono state incluse nell'analisi. Quando possibile, i campioni sono stati confrontati con il materiale tipico. I caratteri morfologici sono stati infine combinati con 9 geni mitocondriali già noti in letteratura le specie più comuni. Per le altre specie, prevalentemente provenienti da collezioni, ci si è limitati a sequenziare la porzione barcoding del gene *cox1*, date le difficoltà incontrate nell'amplificare DNA antico e frammentato, anche usando primers disegnati *ad hoc*. Le matrici di dati morfologici e molecolari sono state analizzate utilizzando diverse applicazioni informatiche, per valutare approcci diversi. Le analisi sono state condotte sia su matrici separate (morfologica e molecolare), sia combinate (morfologica + molecolare). Infine, i caratteri morfologici sono stati tracciati sull'albero filogenetico di riferimento al fine di identificare apomorfie e cambiamenti omoplastici utili per realizzare una chiave dicotomica per il genere *Thaumetopoea*. Inoltre, sono stati selezionati alcuni caratteri ecologici quali: presenza di setole urticanti sulla larva; sito di impupamento; attività di alimentazione stagionale larvale; tipo di piante ospiti; famiglia di piante ospite, al fine di delineare le caratteristiche fondamentali di un possibile antenato del genere *Thaumetopoea*. I risultati confermano la monofilia del genere e suggeriscono di considerare tutte le specie come appartenenti al genere *Thaumetopoea*.

Nella seconda parte i caratteri morfologici raccolti su un grande numero di campioni sono stati analizzati per costruire una chiave dicotomica e carte di distribuzione del genere *Thaumetopoea*, nell'intento di fornire strumenti per identificare gli esemplari adulti, sia in collezioni museali sia in campo, fornendo contemporaneamente informazioni importanti per le specie di maggiore rilevanza applicata. Nel redigere tale studio, si è reso necessario porre in sinonimia alcuni taxa recentemente descritti, soprattutto per la mancanza di caratteri diagnostici o di incoerenze a livello delle descrizioni originali.

Nella terza parte l'attenzione è stata posta su un particolare gruppo all'interno di *Thaumetopoea*, comprendente le specie eurasiatiche associate alle conifere ma attive come larva nel periodo primaverile-estivo. Le informazioni raccolte, di tipo morfologico ed ecologico, hanno consentito di chiarire la posizione di *T. cheela*, specie nota per pochissimi individui raccolti molti anni fa, che è stata inclusa in questo gruppo e per la quale viene ipotizzata un'associazione con il genere *Cedrus* nelle montagne dell'Himalaya, analogamente ad altre specie afferenti a questo gruppo ma presenti nell'area Mediterranea. Queste osservazioni sostengono l'ipotesi che il genere *Cedrus* potrebbe essere la pianta ospite su cui è avvenuta la speciazione del gruppo.

L'approccio utilizzato nella tesi ha consentito di contribuire alle conoscenze sulla filogenesi del genere *Thaumetopoea*, fornendo informazioni rilevanti per alcuni taxa di interesse applicato, sia per le piante sia per la salute pubblica. Tuttavia ancora molto rimane da fare per chiarire la posizione di taxa attualmente trascurati o individuati da poco tempo grazie agli studi di genetica di popolazione. Appare inoltre interessante espandere il metodo di analisi ad altri generi della sottofamiglia, che apparentemente condividono caratteristiche simili e che stanno minacciando la salute di piante, persone e animali in Africa, Asia e Australia.

Abstract

Thaumetopoeinae include several species called processionary moths, mainly due to their gregarious behaviour to form long single lines to forage and to pupate. Even if historically it has been considered as a separate family, Thaumetopoeinae were recently included as a subfamily of Notodontidae, based on both cladistic and molecular analyses. This groups has a great importance for forestry and landscape because their larvae feed on trees and shrubs, both broadleaved and coniferous, defoliating the canopy and weakening the plants, making them more susceptible to tree killers (i.e. bark beetles). Furthermore, they threaten human and animal health due to the presence of urticating setae in larvae and/or adults that are used as an effective defence strategy against vertebrate predators. In humans, these setae are responsible of allergic reactions, also of strong intensity, from dermatitis to anaphylactic shock; instead in animals, they produce pruritus, necrosis, abortions, anorexia, up to death. *Thaumetopoea* is the most known genus and historically it has been split into three separated genera. A recent molecular study has defined the phylogenetic relationships and the evolution of life history traits for a few taxa of this genus that is mainly distributed in Europe, the Mediterranean and Iranoturanic areas.

In the first study, I completed the phylogeny of the genus analysing all the 15 species of *Thaumetopoea s. lat.*, introducing both rare species found in different museum collections, by defining a set of 165 traits from head, thorax, abdomen, wing and male genitalia. The very recently described *Thaumetopoea loxostigma* Hacker, 2016 could not be included as the only extant specimen, viz. the holotype, was not available. According to the original description, *T. loxostigma* is closely related to the *Thaumetopoea apologetica* – *Thaumetopoea jordana* group, which itself needs revision. Five subspecies other than nominal ones are currently recognised within *Thaumetopoea* , namely *T. apologetica abyssinica*, *T. herculeana judaea*, *T. processionea pseudosolitaria*, *T. solitaria iranica*, and *T. pityocampa orana*, and they were included in the analysis. Whenever possible, the specimens were compared with the types of the various species. Morphological traits were combined with 9 mitochondrial genes already present in literature for some species. For the others, I sequenced the barcoding portion of *cox1* due to the difficulties to amplify old fragmented DNA also using *ad hoc* primers. Matrix was processed using different software, to test different approaches, and the analyses were conducted both on separated matrix (morphological vs. molecular) and in combined ones (morphological + molecular). Finally, morphological traits were plotted on reference tree in order to identify apomorphies and homoplasious changes useful to draw a morphological key for the *Thaumetopoea* genus. Furthermore, I selected ecological and life history traits: presence of urticating setae on larva; pupation site; larval seasonal feeding activity; host plant group; host plant family, in order to outline the traits of a possible ancestor of processionary moths.

In the second study, I use the morphological traits collected and the large number of specimens analysed to draw a morphological key and distribution maps of the whole genus *Thaumetopoea*, which will be helpful to entomologists and foresters to identify the adult specimens both in museum collection and in field. In the meantime, I synonymised some taxa recently described, mainly for

lack of diagnostic characters or inconsistency.

In the third study, I focused on the single clade genus *Thaumetopoea* in order to collect the information about the species of the 'summer' *Thaumetopoea* from Eurasia, feeding on coniferous hosts. Information included morphological and life history traits. Furthermore, the work involved also *T. cheela* that is proposed to be included in this group based on morphology and indirect evidence of life history traits. Although some evidences supported the hypothesis that *Cedrus* could be the host on which most of speciation in the summer clade had happened, more studies have to be made, especially for the less known species.

Although my works complete the phylogeny of the genus of *Thaumetopoea* and provide valid methods to identify the species, which is very important because of the group includes some of the most important forest pests that also affect human and domesticated animal health through the urticating setae, more work is left to do in order to complete the knowledge on some neglected taxa and to expand the analysis to other genera of the subfamily, which apparently share the same traits and are causing similar problems in other continents.

Chapter 1

Introduction to the genus *Thaumetopoea*

Taxonomy

The notodontid genus *Thaumetopoea* Hübner (Table 1) is currently assigned to the nominal subfamily Thaumetopoeinae which, for long time, has been treated as a self-standing family of “urticating” moths composed by three subgroups according to their geographic distribution: Thaumetopoeinae from Palaearctic Region, Anaphinae from Afrotropical Region and Epicominae from Australasian Region (Kiriakoff, 1970). Recently, both cladistic (Miller, 1991) and statistical phylogenetic analyses (Zahiri *et al.*, 2011; 2013) converge in yielding Thaumetopoeinae as monophyletic and nested within Notodontidae. Presently, this subfamily counts about one hundred species in 23 genera (Schintlmeister, 2013) and includes some of the most unpopular and dangerous moths species like the “processionary moths”. In fact, the mature larvae of these species typically move in rows to forage on trees or to reach the pupation site (Roques and Battisti, 2015). All immature Thaumetopoeinae are sub-social, and most of them live in colonies by building silky nests on trees. The genus *Thaumetopoea* is the most thoroughly studied among the Thaumetopoeinae, including species which are mainly spread around the Mediterranean or the west Palaearctic subregions (Schintlmeister, 2013; Simonato *et al.*, 2013; Hacker, 2016; Chapter 2), but with some species widely spread also in the Afrotropics (*Thaumetopoea apologetica*) and northern India (*Thaumetopoea cheela*). This genus was retrieved as monophyletic in all recent phylogenetic analyses based on molecular (Simonato *et al.*, 2013) and both molecular and morphological data (Chapter 2).

Table 1. Currently recognized taxa of the genus *Thaumetopoea s.l.*

Taxon	Author
Thaumetopoea apologetica abyssinica	Strand, 1911
Thaumetopoea apologetica apologetica	Strand, 1909
Thaumetopoea bonjeani	Powell, 1922
Thaumetopoea cheela	Moore, 1883
Thaumetopoea dhofarensis	Wiltshire, 1980
Thaumetopoea herculeana herculeana	(Rambur, 1837)
Thaumetopoea herculeana judaea	Bang-Haas, 1910
Thaumetopoea ispartaensis	Doganlar & Avci, 2001
Thaumetopoea jordana	Staudinger, 1887
Thaumetopoea libanotica	Kiriakoff & Talhouk, 1975
Thaumetopoea loxostigma	Hacker, 2016
Thaumetopoea pinivora	(Treitschke, 1834)
Thaumetopoea pityocampa ENA	—
Thaumetopoea pityocampa orana	(Staudinger, 1901)
Thaumetopoea pityocampa pityocampa	([Denis & Schiffermüller], 1775)
Thaumetopoea processionea processionea	(Linnaeus, 1758)
Thaumetopoea processionea pseudosolitaria	Daniel, 1951
Thaumetopoea sedirica	(Doganlar, 2005)
Thaumetopoea solitaria iranica	Agenjo, 1941
Thaumetopoea solitaria solitaria	(Freyer, 1838)
Thaumetopoea torosica	(Doganlar, 2005)
Thaumetopoea wilkinsoni	Tams, 1925

Applied importance: urticating setae

An important trait shared by all species of *Thaumetopoea* is the occurrence of urticating setae on larvae. These setae are considered to be a protection against vertebrate predators, although all information about them comes from impacts on humans and farmed animals (Battisti *et al.*; Roques and Battisti, 2015).

Urticating action of the setae carried by larval stages of the genus *Thaumetopoea* are known from ancient Greeks where larvae were used to obtain a poison (Battisti *et al.*, 2011). These setae arise dorsally from small areas on abdominal segments which are called “mirrors” due to their property to reflect the light (Démolin, 1963; Battisti *et al.*, 2011). Mirrors consist in a couple of tegumental pockets that are usually kept “closed” with the urticating setae are protected within. Several study conducted by Démolin (1963) showed that mirrors may be actively open when larvae are disturbed and setae are readily released in the environment (Battisti *et al.*, 2011; Moneo *et al.*, 2015). Under scanning electron microscope, these setae are pin-like basally, a few hundreds of micrometres in length (with remarkable intraspecific and interspecific variability), and provided with several pointed barbs which are oriented distally (Battisti *et al.*, 2011; Petrucco Toffolo *et al.*, 2014). These setae are composed by a chitin skeleton and a matrix of several proteins with demonstrated allergenic activity (Lamy *et al.*, 1986; Rodriguez-Mahillo *et al.*, 2012; Berardi *et al.*, 2015). In *Thaumetopoea pityocampa,* urticating setae can reach a density of approx. 60,000 setae/mm^2 (Lamy, 1990; Moneo *et al.*, 2015) with a total of 1,000,000 setae/larva at fifth instar (Moneo *et al.*, 2015). This makes these setae much alike the urticating setae of some American spiders more than to the “spines” of other Lepidoptera (e.g. the larvae of Limacodidae, Megalopygidae and Saturniidae) which they cannot be actively released by the larvae (Battisti *et al.*, 2011). Setae are renewed, and increased in number at each moult, after the third instar (Battisti *et al.*, 2011); the old exuviae are left inside the silky nests, transforming them in a deposit of urticating setae that may be carried by wind over long distances (Lamy, 1990; Battisti *et al.*, 2011) and that remain urticant also after the departure of the colony (Moneo *et al.*, 2015). Urticating setae likely evolved against predators (Battisti *et al.*, 2011) but accidentally they affect also farm animals, pets and humans, who eventually disturb the larvae or live near infested trees.

Urticating setae penetrate into the skin and mucous membranes through the pin like base, then barbs help the penetration. When setae are introduced through the skin, they are exposed to several chitinase-like proteins in order to degrade the foreign bodies, thus, chitin particles and proteins will be exposed to the macrophage receptor that promote and stimulate the immune system (Battisti *et al.*, 2011). Thus, the exposed tissue suddenly show local symptoms like vesicles, due to fluid accumulation, itching and secondary flare and reddening resulting from the inflammatory reaction. Reaction can be delayed up to 24h and generally it is localized in the area of contact (Moneo *et al.*, 2015). Unfortunately, dermatitis could be the slighter reaction on exposed subjects. Indeed, because of their characteristic to be blown by air currents, urticating setae reach eyes, where they cause ocular pathologies, usually aggravated by scratching (Moneo *et al.*, 2015). After the contact, patients show symptoms like conjunctivitis, keratitis and uveitis which could result in cataract, vitritis and retinitis due to the intraocular migration of setae (Battisti *et al.*, 2011; Moneo *et al.*, 2015). Reaching respiratory system, and mucous membranes, the inflammatory reactions involves cough, dyspnea, rhinitis and wheezing (Vega *et al.*, 1999; Shkalim *et al.*, 2008; Moneo *et al.*, 2015). Furthermore, is demonstrated an IgE-mediated hypersensitive reaction due to a sensitization occurred in people which have continuous contacts with urticating setae or in sensible people leading to anaphylactic reaction, in some severe cases (Moneo *et al.*, 2015) At present, at least three proteins involved (thaumetopoein, Tha p 1 and Tha p 2) have been described, and the allergenic potential has been demonstrated for one of them (Tha p 2) (Lamy *et al.*, 1986; Moneo *et al.*, 2003; Berardi *et al.*, 2015).

These reactions affect also farm animals (cattle, goats, horses, sheep) that had come into contact with urticating setae while grazing grass, and pets (dogs, cats) (Moneo *et al.*, 2015) that are probably attracted by the procession of these Lepidoptera. Humans can be affected as well (eye contact, inhalation, ingestion and skin contact) and they show severe symptoms that are described in literature since the nineties with several studies (Moneo *et al.*, 2015).

In particular eye contact leads to develop keratoconjunctivitis, glaucoma and corneal ulceration, causing pain, photophobia and lacrimation. While the contact with oral mucous, cause strong inflammations that induce abundant salivation, and feeding and watering difficult which can lead to anorexia and kidney failure (Moneo *et al.*, 2015). Furthermore, especially in dogs, the resulting coagulation of the tongue blood vessels, produce necrosis of tissues with the following loss of the injured part. Inhalation cause, rhinitis, bronchitis, coughing violently and sometimes in dyspnea, while skin contact produce urticaria, erythematous and itching, as in humans (Moneo *et al.*, 2015). In farm animals, contact with the urticating setae may have even more dramatic effects. In ruminants, the envenomation from *Thaumetopoea* produces fragile vesicles that lead to develop extensive lingual ulcers causing feeding and watering difficulties, with following anorexia, dehydration and bringing the animals to isolate themselves form the herd (Moneo *et al.*, 2015). In horse, urticating setae affect tongue and skins lead to sweating, extended and urticaria, with in some cases intense pruritus that could lead to self-mutilation (Moneo *et al.*, 2015). Moreover, cases of abortion in mare were recorded as a consequence of ingestion of other Thaumetopoeinae (Cawdell-Smith *et al.*, 2012; 2013).

Applied importance: tree defoliation

Processionary moths feed on trees, both broadleaves (e.g. *Thaumetopoea processionea*) and conifers (e.g. *Thaumetopoea pinivora*, *T. pityocampa*), or shrubs (e.g. *Thaumetopoea herculeana*) during their larval stage (Table 2) (Battisti *et al.*, 2015). Larvae may defoliate the whole of a tree, and continue to other neighbouring plants until they reach the fifth instar, eating everything in their path, especially in areas with a high degree of infestation. Often larvae affect juvenile foliage produced during the spring (Battisti *et al.*, 1998) reducing significantly the capacity of photosynthesis of the plant, with important consequences on tree growth (Carus, 2010; Jacquet *et al.*, 2012). Furthermore, repeated defoliations during several years may lead to death, especially in young trees, and make plants more weak, increasing the susceptibility to others pests (e.g. bark beetles) (Jacquet *et al.*, 2012). Besides the economic loss due to death of trees, defoliation has other effects, such as increasing the exposed soil surface to erosion and decreasing the shade, affecting the biodiversity, and the aesthetic value of the landscape.

Table 2. Host plants of the taxa included in the genus *Thaumetopoea*.

Taxon	Host plants
Thaumetopoea apologetica abyssinica	*Maerua* spp.
Thaumetopoea apologetica apologetica	*Maerua* spp.
Thaumetopoea bonjeani	*Cedrus atlantica*
Thaumetopoea cheela	*Pinus* spp. and/or *Cedrus* spp.
Thaumetopoea dhofarensis	Unknown
Thaumetopoea herculeana herculeana	*Cistus* spp., *Erodium* spp., *Helianthemum* spp.
Thaumetopoea herculeana judaea	Unknown
Thaumetopoea ispartaensis	*Cedrus libani*
Thaumetopoea jordana	*Rhus tripartita*
Thaumetopoea libanotica	*Cedrus libani*
Thaumetopoea pinivora	*Pinus* spp.
Thaumetopoea pityocampa ENA	*Cedrus atlantica, Pinus halepensis*
Thaumetopoea pityocampa orana	*Pinus* spp.
Thaumetopoea pityocampa pityocampa	*Cedrus* spp., *Pinus* spp.
Thaumetopoea processionea processionea	*Quercus* spp.
Thaumetopoea processionea pseudosolitaria	*Quercus* spp.
Thaumetopoea sedirica	*Cedrus libani*
Thaumetopoea solitaria iranica	*Pistacia* spp.
Thaumetopoea solitaria solitaria	*Pistacia* spp.
Thaumetopoea torosica	*Pinus brutia*
Thaumetopoea wilkinsoni	*Cedrus libani, Pinus* spp.

Applied importance: climate change, human translocation, and range expansion

Pine processionary moth is recognized as one of the best examples of an insect responding to climate change through range expansion. It is generally known that temperature plays a fundamental role on herbivorous insects, influencing winter survival, and extending the "summer season" at high latitudes, improving growth and reproduction (Bale *et al.*, 2002; Roques *et al.*, 2015). So it is clear to expect that the climate changes in the latest years had provoked an expansion of the natural range of *Thaumetopoea pityocampa*, depending mainly on the warming up of the winter. This results in promoting the survival of the larvae (Battisti *et al.*, 2005; Roques and Battisti, 2015), increasing feeding activity, improving development, and reducing the mortality in early larval stages, that are very sensitive to the variation of temperature (Benigni and Battisti, 1999). The increasing of temperature due to the world climate change allowed to the processionary moths to colonize also higher altitudes and latitudes (Roques and Battisti, 2015), threatening new forest stands. Whether the range of other processionary moth species is closely related to climatic constraints is to be explored (Battisti *et al.*, 2017). In *Thaumetopoea pityocampa* the range has expanded by about 5 km/year in the north of France from 1972 to 2011, resulting in an area of 339,121 km^2 in 2011 (Robinet *et al.*, 2014), with the potential to increase again its range. The same pattern was recorded around Mediterranean Basin and for other species, although the link with climate change should be further explored (e.g. *T. pinivora*, *T. processionea*) (Roques *et al.*, 2015).

An additional factor in the species range expansion is related to humans. In fact, the possibility that the species are taken outside of their range with their host plants as ornamental trees has been shown for *T. pityocampa* and *T. processionea* (Rossi *et al.*, 2016). Commercialized trees do not show specific symptoms but they may carry pupae in the soil surrounding the roots or eggs on the canopy. The latter case could well be that of *T. processionea,* accidentally introduced in London before 2006 (Tomlinson *et al.*, 2015). In new environments, without natural predators, *T. processionea* develop outbreaks in few years and cause several cases of urticaria in children especially in public parks (Gottschling and Meyer, 2006; Clancy, 2008). The range expansion brings the processionary moths to get closer to more densely populated areas (cities, towns etc.) increasing the risk that these Lepidoptera come into contact with more people and animals. This has resulted in the European Union to promote risk assessments and control or disinfestation plans against the processionary moths (EPPO, 2004; EFSA, 2009; FERA, 2015).

In conclusion, considering that forestry are vital for global health providing to increase natural conservation, protecting soil and water, acting as carbon storage (Jactel *et al.*, 2009) and the readiness to conform to the recent climate change, and the health risk for humans and animal, make the genus *Thaumetopoea* a group of species of great interest in order to understand how to carry out suitable measures to reduce their impact and protect humans and animals.

Objectives and contents of the thesis

Most of my work has concerned the phylogeny of *Thaumetopoea* moths combining morphological, ecological and molecular traits in order to (a) create a stable and predictive classification of *Thaumetopoea*, based upon phylogenetic analyses; (b) reconstruct a robust and informative phylogeny of *Thaumetopoea*; (c) elucidate the evolution of the genus *Thaumetopoea* through morphological, ecological, and behavioural traits important for both basic information and applied research.

In this perspective, part of the study focused to the collection of morphological traits about 16 species and 5 subspecies (Schintlmeister, 2013) to produce a complete series of diagnostic traits aimed at building a simple and comprehensive morphological key (Chapter 2). This study was conducted with traditional taxonomic approach, using images of high quality obtained with the process of composite focus-stacking that had allowed to provide a complete description of each species, completed with information on life history, host plants, and larval features obtained from literature.

To reconstruct the phylogeny of the genus (Chapter 2), I used a total evidence approach combining morphological traits and molecular data, mainly obtained from the work of Simonato *et al.* (2013) but also integrated with some barcoding portion of *cox1* sequenced for the current thesis. Morphological characters were coded in a matrix built in specific software to match each species with the proper states of the traits. Phylogeny was inferred with different algorithms and different combination of the dataset. At the same time the evolutionary history of the genus was provided on the total evidence tree using some key life history traits of the group to draw a likelihood profile of the common ancestor of processionary.

Part of my PhD (Chapter 4) was focused on a single clade of *Thaumetopoea*, namely by Démolin (1989) as the “summer” processionary moths due to their behaviour to feed on conifers during the summer season, which includes also the rare *Thaumetopoea cheela*, of which less than 10 specimens preserved in two European museums (Natural History Museum, London, UK and The Bavarian State Collection of Zoology, Munich, Germany) are known. Information on this *Thaumetopoea* clade was collected and related to *T. cheela* in order to deduct the possible host plant and life history of this poorly known species, and to track its origin in relation to that of likely host plant in the genus *Cedrus* (Qiao *et al.*, 2007).

The taxonomic knowledge accumulated during the PhD has allowed to contribute further work on *Thaumetopoea pityocampa* - *wilkinsoni* complex, provided in Chapter S1. This study illustrates the possibilities to produce vital and stable hybrids between the two species under laboratory condition.

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Chapter 2

A total evidence phylogeny for the processionary moths of the genus *Thaumetopoea* (Lepidoptera: Notodontidae: Thaumetopoeinae)

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I contributed to the design of the work, data collection, data analysis, and writing.

A total evidence phylogeny for the processionary moths of the genus *Thaumetopoea* (Lepidoptera: Notodontidae: Thaumetopoeinae)

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Abstract

Processionary moths belong to a group of about 100 species well known for their social behaviour and their urticating systems. The genus *Thaumetopoea s. lat.* includes about 15 species and has been divided into three genera (*Helianthocampa*, *Thaumetopoea s. str*., and *Traumatocampa*) in the last revision, based on key morphological features of the adults and on the host plants of the larvae. We performed a total evidence approach to resolve the phylogeny of the genus *Thaumetopoea s. lat.*, analysing all valid taxa included in this group, plus a broad array of close relatives. *Thaumetopoea* resulted monophyletic and supported by several apomorphies. Further subclades corroborated by synapomorphies were identified. Our phylogeny suggests that *Thaumetopoea* must be regarded as a single genus. The mapping of key life history traits on the total evidence tree allowed to sketch a plausible identikit of the *Thaumetopoea* ancestor and to track the evolution of the genus. The ancestor originated in the eastern Mediterranean area, and used broadleaves as host plants. Successively, a switch to conifers occurred, just once, in a large subclade. The ancestor pupated in the soil, like several current species, but in few taxa this trait was lost, together with the related morphological adaptations.

Keywords: phylogeny, *Thaumetopoea*, morphological traits.

Introduction

Processionary moths belong to a group of insect herbivores well known since the ancient Greek and Roman times for their association with the host plants, their special behaviour, and their urticating and envenomation power (Roques and Battisti, 2015). All processionary moths are now included in Thaumetopoeinae, a clade of Notodontidae (Lepidoptera) (Miller, 1991; Zahiri *et al.*, 2011; 2013). In the past, this group was treated at family rank with three subfamilies: Thaumetopoeinae (mainly Palaearctic), Anaphinae (Afrotropical) and Epicominae (Australasian) (Kiriakoff, 1970). However, this view is now superseded by results obtained from morphological and molecular based phylogenetic analyses (Miller, 1991; Zahiri *et al.*, 2011; 2013). At present, Thaumetopoeinae account for 106 valid species split in 20 genera (Schintlmeister, 2013).

The processionary moths owe their name to the typical processions made by the larvae when they move in lines or groups to forage on trees or to pupation sites. A few species of processionary moths are plant pests and cause outbreaks on trees and shrubs of both broadleaves and conifers in Africa (Wagner and Cobbinah, 2013), Asia (Rahman and Chaudhry, 1992), Australia (Floater and Zalucki, 2000), and Europe (Jacquet *et al.*, 2012). All species are protected against vertebrate predators by urticating setae either as larvae or adults (Battisti *et al.*, in press), and these setae may threaten animal and human health (Battisti *et al.*, 2011). In spite of these problems, in Africa some species are farmed for silk production or used as human food (Huis, 2003; Schabel, 2006).

Most information on life history and taxonomy concerning Thaumetopoeinae relates to the genus *Thaumetopoea*. Hübner ([1820], in 1816-[1826]) erected *Thaumetopoea* to include two species, namely *Phalaena processionea* Linnaeus, 1758, the type species associated with oaks in central and southern Europe, and *Bombyx pityocampa* [Denis & Schiffermüller], 1775, associated with pine in southern Europe and the Mediterranean. More taxa were added later and currently fifteen species are included in this genus (Table 1) (Schintlmeister, 2013). The Palaearctic species of *Thaumetopoea* have been reviewed by Agenjo (1941) and de Freina and Witt (1985, 1987). Agenjo (1941) maintained the split of the genus into the two subgenera *Thaumetopoea* and *Traumatocampa* done by Wallengren (1871) according to the absence/presence of a toothed protuberance on the frontal part of the head (crest) and of foretibia claw (spine) in the adults (*sensu* Hogue, 1963) (Table 2), while de Freina and Witt (1985) raised *Traumatocampa* Wallengren, 1871 to the status of distinct genus. Furthermore, they erected the new genus *Helianthocampa* to accommodate only *Bombyx herculeana* Rambur, 1840 a species feeding on another group of host plants which was previously included in *Traumatocampa* (Agenjo, 1941) (Table 2). Both revisions did not use a phylogenetic approach and the suggested taxonomic changes were not based on rigorous analyses corroborating the monophyly of their genera. In spite of that, the three genera were retained as valid in the most recent catalogue of Notodontidae (Schintlmeister, 2013). However, a recent phylogeny based on nucleotide sequence data on a subset of species indicated that the split of *Thaumetopoea* in three distinct genera is untenable, and provided evidence for inclusion of all the species in a single genus *Thaumetopoea s.lat.* until new revision (Simonato *et al.*, 2013).

We consider here all species of *Thaumetopoea s. lat.* occurring in the Palaearctic and Afrotropical regions (Table 1). They extend altogether over an area ranging from the Atlantic coast of the Iberian Peninsula to the Indian region of Jammu and Kashmir and from Scandinavia to southern Africa, where the species are associated with very diverse habitats and host plants. Our analysis included all taxa that were variously granted with independent status according to revisions and recent papers, for a total of 21 taxa. As outgroups we selected 16 species of other Thaumetopoeinae, encompassing a representative sample of the taxonomic diversity of the subfamily (Table 1, and Table S1) (Schintlmeister, 2013). Thus the final data set contained 37 taxa included in seven genera. We analysed morphological and molecular data separately and in combination. The major aim of our research was to produce a robust phylogeny of *Thaumetopoea s. lat.*, to lay out a framework for a thorough taxonomic revision of the genus. In addition, we aimed at tracking the origin of key life history traits of the group to draw a plausible profile of the common ancestor of processionary moths and to mark the main changes that led to the diversity currently shown by this group.

Table 1. List of Thaumetopoeinae taxa considered in this work

Taxon	Author	Distribution	Host plants
Thaumetopoea processionea processionea	(Linnaeus, 1758)	Europe, Middle East	*Quercus* spp.
Thaumetopoea processionea pseudosolitaria	Daniel, 1951	South - Eastern Europe, Middle East	*Quercus* spp.
Thaumetopoea solitaria iranica	Agenjo, 1941 -	Middle East	*Pistacia* spp.
Thaumetopoea solitaria solitaria	(Freyer, 1838)	South - Eastern Europe, Middle East	*Pistacia* spp.
Thaumetopoea herculeana herculeana	(Rambur, 1837)	South - Western Europe, Northern Africa, Middle East	*Cistus* spp., *Erodium* spp., *Helianthemum* spp.
Thaumetopoea herculeana judaea	Bang-Haas, 1910	Middle East	unknown
Thaumetopoea apologetica abyssinica	Strand, 1911	Eastern Africa	*Maerua* spp.
Thaumetopoea apologetica apologetica	Strand, 1909	Southern and Eastern Africa	*Maerua* spp.
Thaumetopoea dhofarensis	Wiltshire, 1980	Middle East	unknown
Thaumetopoea jordana	Staudinger, 1887	Middle East	*Rhus tripartita*
Thaumetopoea cheela	Moore, 1883	Southern Asia	*Pinus* spp., or *Cedrus* spp.
Thaumetopoea bonjeani	Powell, 1922	North - Western Africa	*Cedrus atlantica*
Thaumetopoea pinivora	(Treitschke, 1834)	Central, Northern and Eastern Europe	*Pinus* spp.
Thaumetopoea libanotica	Kiriakoff & Talhouk, 1975	Middle East	*Cedrus libani*
Thaumetopoea ispartaensis	Doganlar & Avci, 2001	Middle East	*Cedrus libani*
Thaumetopoea sedirica	(Doganlar, 2005)	Middle East	*Cedrus libani*
Thaumetopoea torosica	(Doganlar, 2005)	Middle East	*Pinus brutia*
Thaumetopoea pityocampa pityocampa	([Denis & Schiffermüller], 1775)	Central Europe, Mediterranean region	*Cedrus* spp., *Pinus* spp.
Thaumetopoea pityocampa orana	(Staudinger, 1901)	North - Western Africa	*Pinus* spp.
Thaumetopoea pityocampa ENA	—	North - Eastern Africa	*Cedrus atlantica, Pinus halepensis*
Thaumetopoea wilkinsoni	Tams, 1925	Middle East	*Cedrus libani, Pinus* spp.
Anaphe panda panda	(Boisduval, 1847)	Central - Southern Africa	Polyphagous
Anaphe panda infracta	Walsingham, 1885	Central - Southern Africa	Polyphagous
Anaphe venata	Butler, 1878	Central - Southern Africa	Polyphagous
Anaphe etiennei	Schouteden, 1912	Central - Southern Africa	Polyphagous
Epanaphe nigricincta	(Hulstaert, 1924)	Central - Southern Africa	Polyphagous
Epanaphe subsordida	(Holland, 1893)	Central - Southern Africa	Polyphagous
Epanaphe moloneyi	(Druce, 1887)	Central - Southern Africa	Polyphagous
Epanaphe carteri	(Walsingham, 1855)	Central - Southern Africa	Polyphagous
Gazalina apsara	(Moore, 1859)	Southern Asia	Betulaceae, Fagaceae
Gazalina chrysolopha	(Kollar, 1844)	Southern Asia	Betulaceae, Fagaceae
Gazalina transversa	Moore, 1879	Southern Asia	Betulaceae, Fagaceae
Hypsoides antsianakana	(Oberthür, 1922)	Eastern Africa	Gentianaceae
Hypsoides placidus	(Oberthür, 1923)	Eastern Africa	Gentianaceae
Ochrogaster lunifer	Herrich-Schäffer, 1855	Australia	*Acacia* spp., *Corymbia* spp., *Eucalyptus* spp.
Paradrallia punctigera	Hulstaert, 1924	Central Africa	Fabaceae
Paradrallia rhodesi	Bethune-Baker, 1908	Central Africa	Fabaceae

Materials and methods

Taxonomic sampling

The identification at species level of the specimens was done according to the dichotomous keys provided by Agenjo (1941) and Kiriakoff (1970). When possible, the specimens were compared with the types of various species. All the 15 species of *Thaumetopoea s. lat.* listed by Schintlmeister (2013) were analysed. The very recently described *Thaumetopoea loxostigma* Hacker, 2016 could not be included as the only extant specimen, viz. the holotype, was not available. According to the original description, *T. loxostigma* is closely related to the *Thaumetopoea apologetica* – *Thaumetopoea jordana* group (Hacker, 2016).

Five subspecies other than nominal ones are currently recognised within *Thaumetopoea s. lat.* (Schintlmeister, 2013), namely *T. apologetica abyssinica*, *T. herculeana judaea*, *T. processionea pseudosolitaria*, *T. solitaria iranica*, and *T. pityocampa orana*. These taxa were included in our analysis because they show peculiar habitus and/or separate geographical distributions, as well as differences in habitat preference from the nominal subspecies (Kiriakoff, 1970). Finally, we included in the analysis the informal taxon named by Kerdelhué *et al.* (2009) as *Thaumetopoea pityocampa* ENA (Eastern-North African clade) as it markedly differs from nominal *T. pityocampa* on genetic grounds. Further molecular data, provided by Simonato *et al.* (2013), support the view that *T. pityocampa* ENA actually represents a distinct taxon.

The present study is based on the analysis of specimens deposited in the Department of Agronomy, Food, Natural resources, Animals and Environment of the University of Padua; Museo Civico di Zoologia of Rome; Museo di Zoologia - University of Rome 'Sapienza'; Natural History Museum of London; private collection of A. Schintlmeister (Dresden); Royal Belgian Institute of Natural Sciences of Brussels; Royal Museum of Central Africa of Tervuren; The Bavarian State Collection of Zoology of Munich and Witt Museum of Munich. The complete list of specimens analysed in this paper is provided in Table S1.

Specimens dissection and recording of morphological traits

The abdomen was removed from the specimens listed in Table S1 and processed according to the non-destructive dissection protocol described below, in order to prepare genitalia for successive examination. The abdomens were digested overnight in an incubator kept at 37 °C, immersed, within Eppendorf vial, in 180 µl of ATL buffer and 20 µl Proteinase K and mix. Before and after the incubation the abdomens were vortexed at 350 rpm for 15'. Successively, the abdomen and the solution were processed separately. The abdomens were dissected following the protocol given by Robinson (1976). All forceps and scissors were sterilized before cutting each abdomen. Pipet tips were discarded and changed every time. The genitalia and the abdomens were stained in saturated chlorazol black (75% ethanol) for 30", soaked in absolute ethanol and eventually mounted on slides with Euparal medium. All slides were labelled according to Robinson (1976). Legs and palps were macerated in 10% KOH solution at 50°C for 5-10 minutes, cleaned in water, then stained and mounted on slides as above.

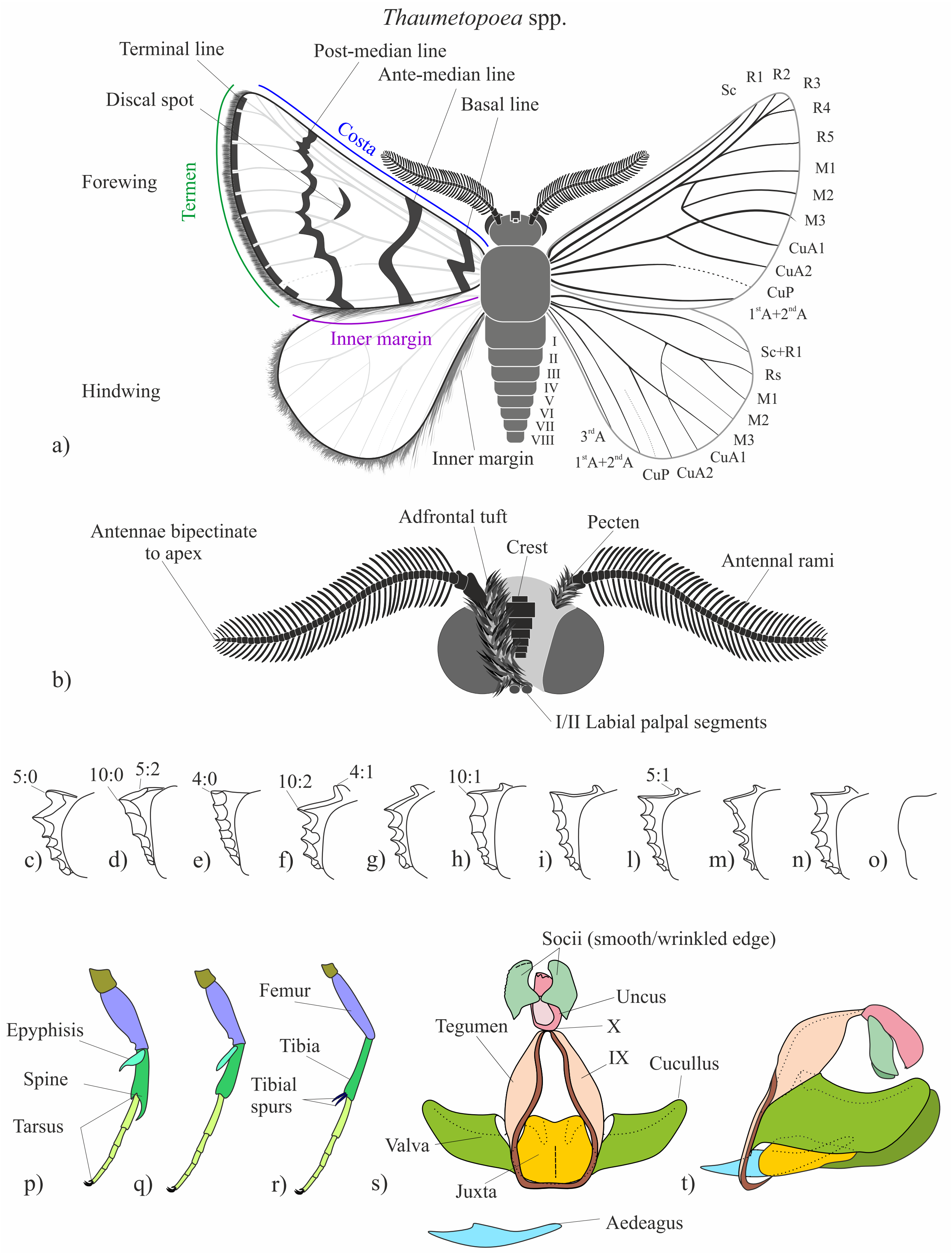


Figure 1. Traits of *Thaumetopoea* spp.. a) Dorsal habitus and veins of wings; b) head traits. Frontal protuberance or crest: c) *T. herculeana*, d) *T. apologetica*, e) *T. dhofarensis* / *T. jordana*, f) *T. pityocampa*, g) *T. wilkinsoni*, h) *T. bonjeani*, i) *T. cheela*, l) *T. libanotica*, m) *T. ispartaensis* / *T. sedirica*, n) *T. pinivora,* o) *T. processionea* / *T. solitaria*. Legs silhouette: p) foreleg of crested *Thaumetopoea,* q) foreleg of non-crested *Thaumetopoea*, r) midleg / hindleg of *Thaumetopoea*. Genitalia of *Thaumetopoea* spp.: s) front view, and t) lateral view.

All morphological observations were taken from pinned specimens of adult moths. Pictures of the morphological characters were taken with a Canon Eos (600D) and a Lumix camera (DMC F200) equipped with additional lens (DMW-LC55) using a led light chamber (Figures 2-3 and S3). Composite focus-stacking images were produced from multiple images captured using AxioCam (MRc5), software Axiovision SE64, (v4.9.1) mounted on a Lumar.v12 Zeiss (Carl Zeiss Microscopy GmbH©) stereomicroscope, and processed with Photoshop CS6 (v13.0) (Adobe System Incorporated©). CorelDraw X5 (v15.1) (Corel Corporation©) was used to create labels and drawings. Traits analysed in the present paper are listed in Figures 2-3 and S3.

Table 2. Association of *Thaumetopoea s. lat.* species-group taxa to genera or subgenera by various authors.

Taxon	Agenjo, 1941	de Freina & Witt, 1987	Simonato et al., 2013	Schintlmeister, 2013
processionea processionea	*Thaumetopoea*	*Thaumetopoea*	*Thaumetopoea*	*Thaumetopoea*
processionea pseudosolitaria	*Thaumetopoea*	—	*Thaumetopoea*	*Thaumetopoea*
solitaria solitaria	*Thaumetopoea*	*Thaumetopoea*	*Thaumetopoea*	*Thaumetopoea*
solitaria iranica	*Thaumetopoea*	—	*Thaumetopoea*	*Thaumetopoea*
herculeana herculeana	*Traumatocampa*	*Helianthocampa*	*Thaumetopoea*	*Helianthocampa*
herculeana judaea	*Traumatocampa*	—	*Thaumetopoea*	*Helianthocampa*
apologetica abyssinica	—	—	*Thaumetopoea*	*Thaumetopoea*
apologetica apologetica	—	—	*Thaumetopoea*	*Thaumetopoea*
dhofarensis	—	—	*Thaumetopoea*	*Thaumetopoea*
jordana	—	—	*Thaumetopoea*	*Traumatocampa*
cheela	—	—	*Thaumetopoea*	*Thaumetopoea*
bonjeani	*Traumatocampa*	*Traumatocampa*	*Thaumetopoea*	*Traumatocampa*
pinivora	*Traumatocampa*	*Traumatocampa*	*Thaumetopoea*	*Traumatocampa*
libanotica	*Traumatocampa*	—	*Thaumetopoea*	*Traumatocampa*
ispartaensis	—	—	*Thaumetopoea*	*Traumatocampa*
sedirica	—	—	*Thaumetopoea*	*Traumatocampa*
torosica	—	—	*Thaumetopoea*	*Traumatocampa*
pityocampa pityocampa	*Traumatocampa*	*Traumatocampa*	*Thaumetopoea*	*Traumatocampa*
pityocampa orana	*Traumatocampa*	—	*Thaumetopoea*	*Traumatocampa*
pityocampa ENA	—	—	*Thaumetopoea*	—
wilkinsoni	*Traumatocampa*	—	*Thaumetopoea*	*Traumatocampa*

Molecular methods

Parallel to the abdomen dissection of single specimens, the total DNA was extracted with QIAGEN© DNeasy blood and tissue kit (Knölke *et al.*, 2005). This procedure did not provide always DNA samples of good quality because of the very different level of tissue conservation of the studied specimens. Thus, molecular analysis was restricted to the best preserved (for molecular purposes) samples.

The DNA extracted during dissection of the genitalia was amplified with universal primers (LCO1490/HCO2198) for the barcode portion of the mitochondrial gene *cox1* (Folmer *et al.*, 1994). Extracts were concentrated with Amicon® Ultra-0.5 Centrifugal Filter Devices, in order to bring all samples to a minimum detectable concentration for the amplification. Each Polymerase Chain Reaction (PCR) contained: 2 µl DNA template (≈15ng/µl), 11.7 µl molecular biology grade water, 4 µl buffer (25 mM), 1.4 µl $MgCl_2$ (5X), 0.2 µl dNTPs mix (2 mM), 0.25 µl forward primer (10 µM), 0.25 µl reverse primer (10 µM), and 0.2 µl GoTaq G2 DNA Polymerase (5 U/µl) (Promega®, 2015) in a total volume of 20 µl. The PCR reactions were performed on a Euroclone thermalcycler under the following conditions: (1) preheated lid at 105°C for 5 min; (2) 30 cycles: 94°C for 30 s, 48 ± 3°C for 45 s, and 72 °C for 45 s; (3) final extension at 72 °C for 5 min. The PCR products were visualised by 1.5% agarose gel electrophoresis. A DNA template to negative and positive control reaction was included in all experiments to test for contamination. The PCR products were not obtained for some species due to the poor quality of the extracted DNA.

The PCR products were purified with ExoSap enzymes (ExoSAP-IT® - USB corporation) and sequenced at the BMR Genomics company (Padua, Italy). The quality of the chromatograms was assessed with the Chromas Lite program (http://technelysium.com.au/wp/chromas/). The final consensus sequence, spanning about 650 base pairs, was assembled using the DNASTAR software (Lasergene® Madison, WI).

The data matrices

One hundred and sixty-five morphological traits were studied in adult moths, divided as follows: head (21), thorax (6), abdomen (9), male and female forewing (56), male and female hindwing (14), male genitalia (59) (Appendix S1). No female genitalia data were included in the present analysis. The hard decision to skip coding characters from the female genitalia was based on the circumstance that a quick screening over representatives of the principal assemblages of *Thaumetopoea* (viz. *T. processionea* and "*Traumatocampa*" *pityocampa*) essentially revealed differences in terms of relative sizes of soft structures or in the degree of sclerotization of pieces, and did not allow to fix definite landmarks or boundaries. Consultation with two notodontid specialists (A. Schintlmeister and T.J. Witt, pers. comm.) confirmed this view and pointed out that traits identified by Agenjo (1941) were unreliable and largely a matter of artefact. It is also worth noting that Miller (1991), in his seminal phylogenetic reconstruction of the Notodontidae, was able to code only 11 female genital characters out of the whole family, whereas 23 were distinguished in the male ones. Further research, however, is being planned to check whether or not the female genitalia may increase resolution of the Thaumetopoeinae phylogeny, at least at the level of the most basal branches of the subfamily, which at this stage were not the major focus of our study.

The traits included in the morphological Matrix (hereafter named Thau.morph see Appendix S3) were mostly those used to identify genera and species of Thaumetopoeinae (Agenjo, 1941; Miller, 1991). The traits were coded following the guidelines provided by Strong and Lipscomb (1999) and Sereno (2007). Thus, 'traits' and the 'variable of the traits' were coded as different characters, in order to split neomorphic from transformational characters, providing a hierarchical order, and maintain the highest phylogenetic signal (Lee and Bryant, 1999).

Morphological wings terminology mainly follows Heath and Emmet (1979) and Heppner (2008). The genitalia terminology follows Berio (1985) and Steinmann and Zombori (1999). For other morphological terminology refer to Fig.1.

The morphological matrix was built in Mesquite v3.04 (Maddison and Maddison, 2015) and

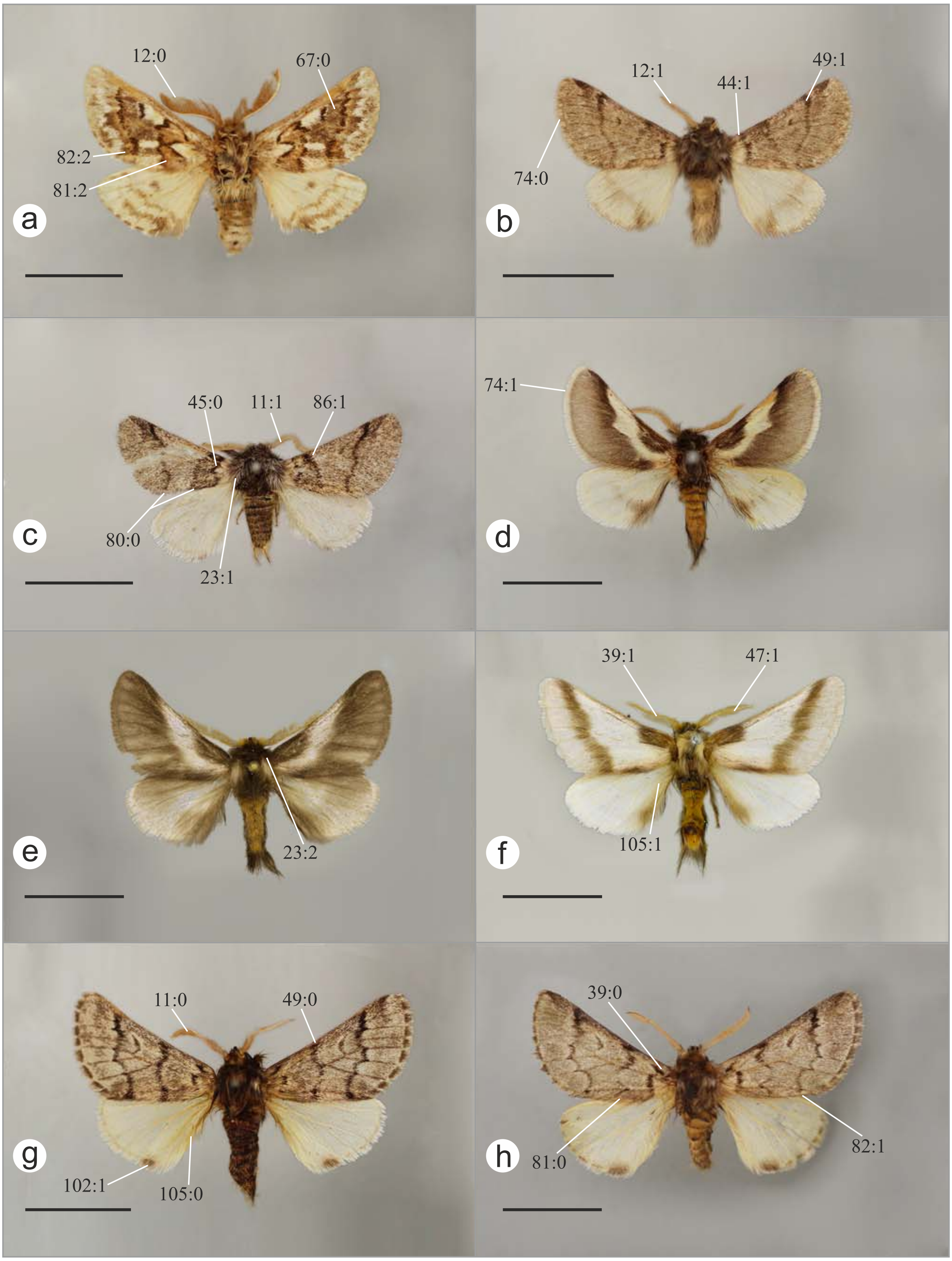


Figure 2. Taxa studied in this work. (a), *Thaumetopoea herculeana herculeana* - Algeria (BMHN(E)_1378597); (b), *Thaumetopoea processionea* - France (BMHN(E)_1378609); (c), *Thaumetopoea solitaria solitaria* - Cyprus (BMHN(E)_1378618); (d), *Thaumetopoea apologetica apologetica* - Kenya (BMHN(E)_1378606); (e), *Thaumetopoea dhofarensis* - Oman (WITT_TH73); (f), *Thaumetopoea jordana* - Palestine (ZSM_TH72); (g), *Thaumetopoea pityocampa pityocampa* - Greece (BMHN(E)_1378583); (h), *Thaumetopoea wilkinsoni* - Cyprus (BMHN(E)_1378572). The collection code associated to the specimen is provided in brackets (see Table S1). Scale bar = 1 cm.

exported as nexus files for subsequent phylogenetic analyses (Appendix S3)

The newly determined sequences were combined with previously available mitochondrial molecular data (Simonato *et al.*, 2013) in order to create a single multigene data set. The alignment of the *cox1* sequences was performed using the MAFFT program implemented in the pipeline TranslatorX (Abascal *et al.*, 2010). This alignment was concatenated with the mitochondrial sequences of Simonato *et al.* (2013) to produce a single multiple alignment (hereafter named Thau. DNA) spanning 6348 positions where unavailable genes were treated as missing data. The list of genes included in Thau.DNA is provided in Table S2.

A total evidence data matrix (hereafter named Thau.tot.comp) was produced by combining morphological and DNA data. The Thau.tot.comp set includes all 37 taxa analysed in this paper. In Thau.tot.comp combined morphological and molecular data are available for 18 species, only morphological traits are present for 18 taxa, and for *T. pityocampa* ENA only molecular data exist (Table S2). Unavailable characters were coded as missing data. Finally, a second total evidence data matrix (hereafter named Thau.tot.red) was created containing only the 18 taxa for which both DNA and morphological data were available.

Phylogenetic analysis

Parsimony analyses (MP) (Fig. S7) were performed with TNT software (v1.5-beta) (Goloboff *et al.*, 2008). The Thau.morph, Thau.DNA, Thau.tot.comp and Thau.tot.red sets were analysed according to the strategy described below. The 'traditional search' algorithm was activated with the following settings: General RAM of 1.0 Gbytes, memory set to hold 1,000,000 trees, setting 1,000 replicates with tree bisection-reconnection (Goloboff, 1999) branch swapping and saving 1,000 trees per replicate. Zero-length branches were collapsed. To evaluate clade support, Bremer support values (Bremer, 1994) were calculated in TNT from 10,000 trees up to 25 steps longer than the most parsimonious trees obtained from 'traditional search' using 'trees from RAM'. A Bootstrap resampling (bt) (Felsenstein, 1985) under equal weights was carried out with 'traditional search' producing 10,000 replicates each of 1,000 random taxa addition; sub-replicates applying TBR, branch swapping and saving 1,000 trees per replication; resampling percentiles were calculated as frequency differences. Consistency index and retention index were calculated in Mesquite v3.04 (Maddison and Maddison, 2015).

The trait changes were mapped in WinClada software (Nixon, 2002) using the following settings: (1) optimization with unambiguous changes only.

Bayesian analysis (BI) were performed using MrBayes (x64, 3.2.5) (Ronquist *et al.*, 2012). The Thau.morph dataset was analysed under Mk1 model (Lewis, 2001) with the following settings: rates= gamma, and coding= variable. The GTR+I+G model was applied to Thau.DNA, and a combination of Mk1 model and GTR+I+G model was used for Thau.tot.comp and Thau.tot. red. Four Markov Chain Monte Carlo (MCMC) chains were run for 10 million generations and sampled every 1,000th generation, with the first 25% of trees discarded as burn-in. Stationarity was considered to be reached when the average standard deviation of split frequencies was less than 0.005.

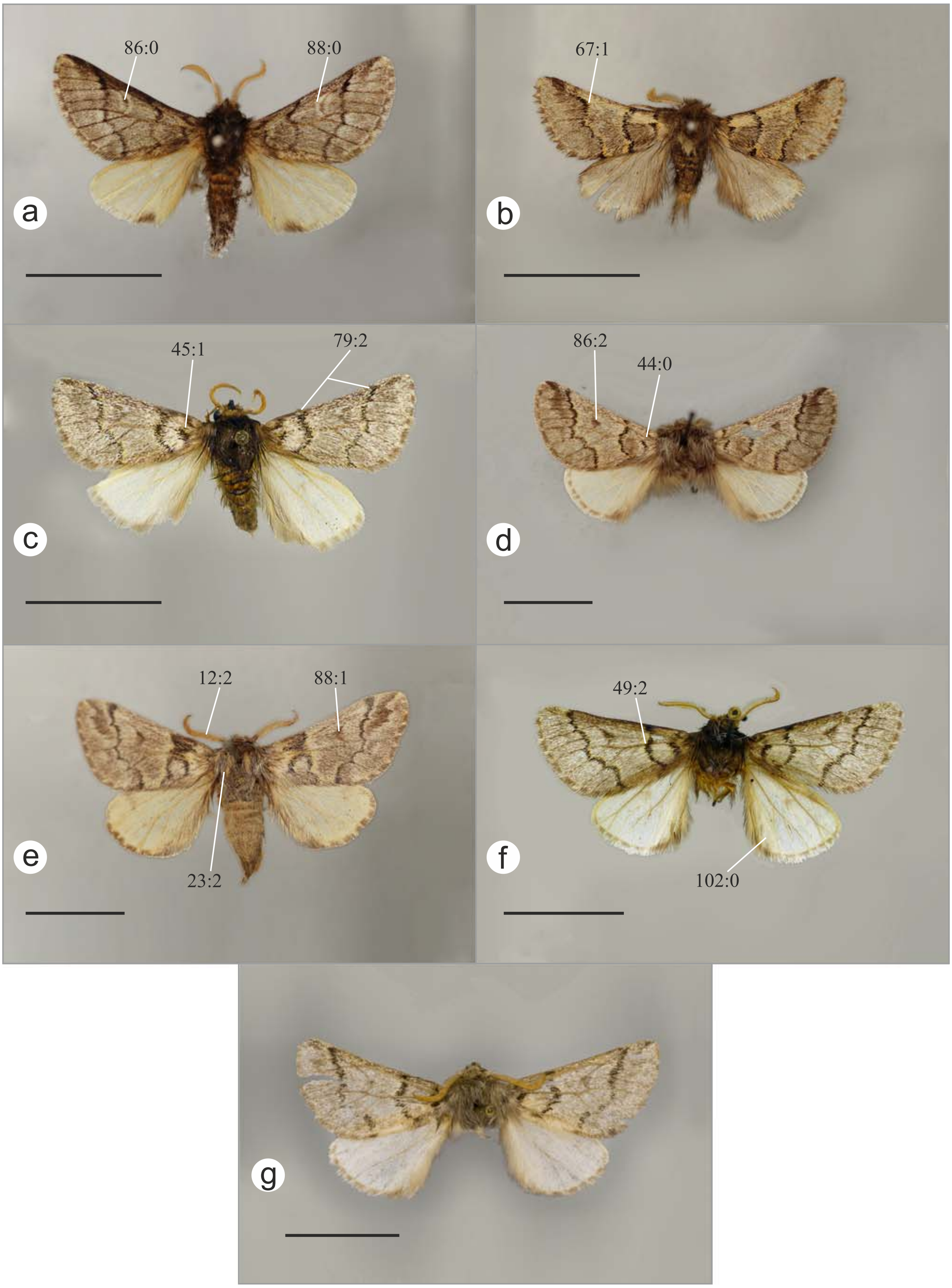


Figure 3. Taxa studied in this work. (a), *Thaumetopoea bonjeani* - Morocco (BMHN(E)_1378573); (b), *Thaumetopoea cheela* ST - India (BMHN(E)_1378635); (c), *Thaumetopoea ispartaensis* - Turkey (DAFNAE_TH30); (d), *Thaumetopoea libanotica* - Lebanon (BMNH(E)_1378640); (e), *Thaumetopoea pinivora* - France (BMHN(E)_1378577); (f), *Thaumetopoea sedirica* PT - Turkey (DAFNAE_TH29); (g), *Thaumetopoea torosica* PT - Turkey (DAFNAE_TH64). The collection code associated to the specimen is provided in brackets (see Table S1). Scale bar = 1 cm.

Maximum likelihood analyses were done using the Iqtree v1.3.13 software (Nguyen *et al.*, 2015). Analyses on Thau.morph dataset were performed with Ordered and MK models (both +FQ+ASC+G4). The GTR+I+G model was applied to Thau.DNA. Finally, combinations of Ordered and MK models plus GTR+I+G were applied to Thau.tot.comp and Thau.tot.red sets.

In every analysis, 50 independent tree searches were performed to minimize the possibility to be entrapped in a local minimum. Ultrafast Bootstrap support (Minh *et al.*, 2013) was calculated for every topology generated with Iqtree v1.3.13 program. In all analyses 10,000 replicates were performed.

The following abbreviations are used in the Results section to describe statistical support to tree topologies: bt, standard bootstrap support, UFB= UltraFastBootstrap support, pp= posterior probability, and Bvs= Bremer value support.

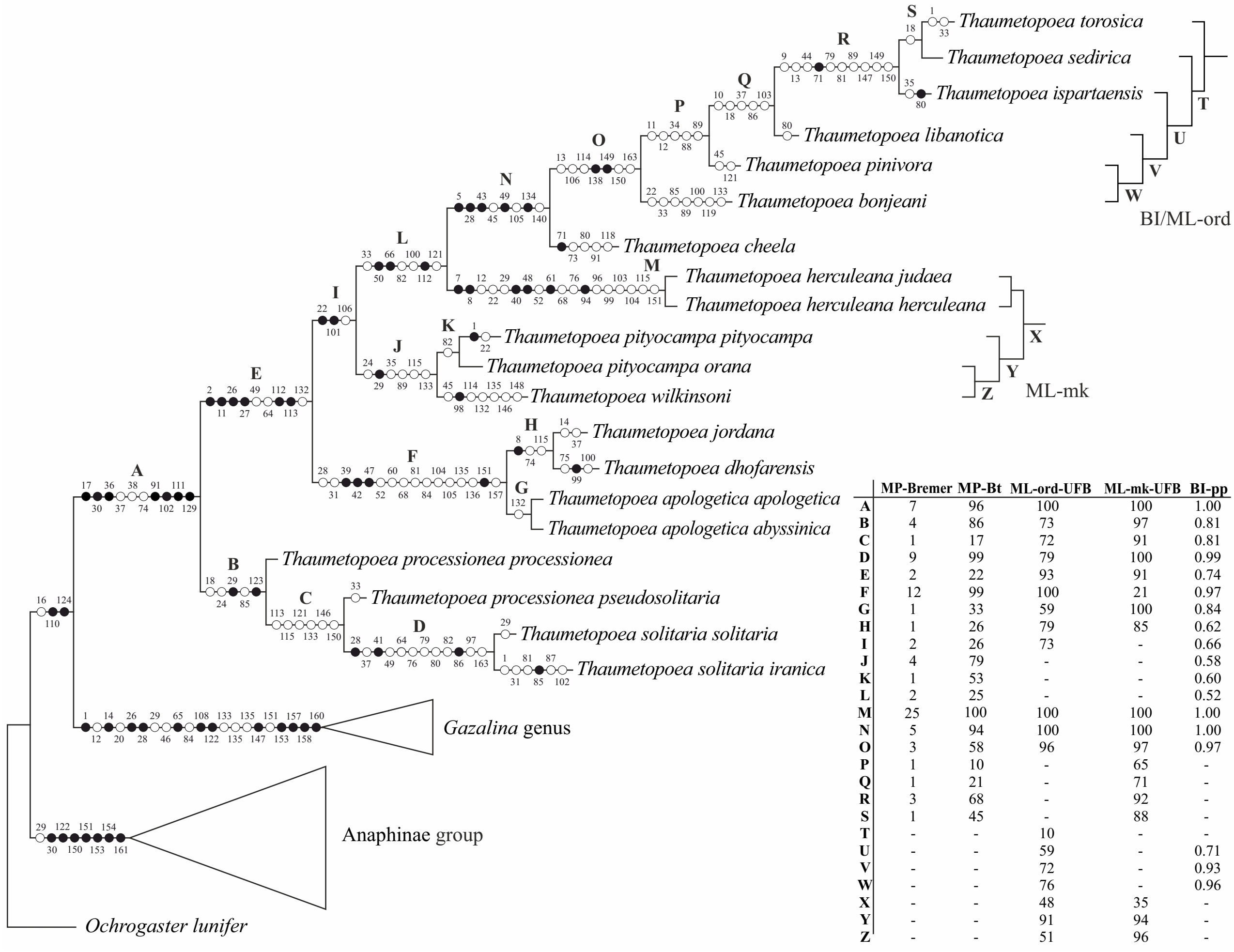
To evaluate alternative phylogenetic hypotheses, alternative topology tests were performed according to the Weighted Shimodaira and Hasegawa test (WSH) (Shimodaira and Hasegawa, 1999) and the Expected Likelihood Weights (ELW) test (Strimmer and Rambaut, 2002). The tests were done with the Iqtree v1.3.13 software (Nguyen *et al.*, 2015).

Tracking the evolution of a selection of characters on the reference tree

The MP tree obtained from Thau.tot.comp set was used as reference topology to track the evolution of six selected characters of genus *Thaumetopoea*.

Firstly, the evolutionary history of five larval morphological, ecological and biological traits (see the Traits description Appendix S1 for the coding strategy) was investigated. These traits are: (1) presence of urticating setae on larva; (2) pupation site; (3) larval seasonal feeding activity; (4) host plant group; (5) host plant family (for nexus format see Appendix S2). The tracking of the transformation pathways was performed with Mesquite 3.04 (Maddison and Maddison, 2015). The character history was traced by the 'Ancestral reconstruction' option based both on maximum parsimony (unordered characters) and the maximum likelihood method of Lewis (Lewis, 2001).

The sixth trait investigated was the reconstruction of the biogeographical patterns of the *Thaumetopoea* species. This analysis was performed with the Statistical Dispersal-Vicariance (S-DIVA) (Yu *et al.*, 2010) (see Results, Fig. 6).



	MP-Bremer	MP-Bt	ML-ord-UFB	ML-mk-UFB	BI-pp
A	7	96	100	100	1.00
B	4	86	73	97	0.81
C	1	17	72	91	0.81
D	9	99	79	100	0.99
E	2	22	93	91	0.74
F	12	99	100	21	0.97
G	1	33	59	100	0.84
H	1	26	79	85	0.62
I	2	26	73	-	0.66
J	4	79	-	-	0.58
K	1	53	-	-	0.60
L	2	25	-	-	0.52
M	25	100	100	100	1.00
N	5	94	100	100	1.00
O	3	58	96	97	0.97
P	1	10	-	65	-
Q	1	21	-	71	-
R	3	68	-	92	-
S	1	45	-	88	-
T	-	-	10	-	-
U	-	-	59	-	0.71
V	-	-	72	-	0.93
W	-	-	76	-	0.96
X	-	-	48	35	-
Y	-	-	91	94	-
Z	-	-	51	96	-

Figure 4. The most parsimonious tree (Length = 534, CI = 0.537, RI = 0.797) obtained from Thau. morph dataset. Optimization: only unambiguous changes are mapped. Black circles, apomorphic characters; white circles, homoplasious changes. Partial topologies show the differences observed in BI and ML trees. MP-Bremer, Bremer support to the node; MP-bt, bootstrap support to the node; ML-ord-UFB, ultrafast bootstrap support to the node (ordered model); ML-mk-UFB, ultrafast bootstrap support to the node (unordered model); BI-pp, posterior probability support to the node.

Results

Morphology-based phylogenetic analysis

The MP tree (Length = 534; CI = 0.537; RI = 0.797) obtained from Thau.morph set is provided in Fig. 4 (see Fig. S4 for the outgroups). Nodes are supported by a variable number of apomorphies and homoplasious changes (Fig. 4; Table 3). The genus *Thaumetopoea* is monophyletic with strong statistical corroboration. The BI and ML topologies are mostly congruent with the MP tree with two noteworthy exceptions listed below.

The arrangement of the species belonging to clade O is different in the BI and ML (ORDERED+FQ+ASC+G4 evolutionary model) trees (Fig. 4; Fig. S5 and S7). In this alternative topology *T. bonjeani* and *T. pinivora* are sister species (clade W), a relationship that receives a very robust statistical support. Moreover, *T. libanotica* is sister taxon of clade W with high, even if not conclusive ($p < 0.95$), statistical corroboration (clade V; BI-pp = 0.93) (Fig. S5). *Thaumetopoea herculeana* is sister taxon of the J clade in the ML analysis based on the MK+FQ+ASC+G4 evolutionary model, but this relationship does not receive statistical corroboration (clade X) (Fig. 4; Fig. S6).

Finally, in all analyses based on morphology the current concept of *T. processionea* turned out as non-monophyletic, as the taxon *T. processionea pseudosolitaria* was recovered as sister to *T. solitaria*, although with no apomorphies supporting this relationship (Fig. 4; Figs. S5-S7).

The test performed on alternative phylogenetic hypotheses provided the results described below. The BI and ML trees topologies obtained from Thau.morph dataset are not rejected (ELW and WSH $p > 0.14$). Their parsimony scores (BI, Length = 543, CI = 0.528, RI = 0.790) (ML, Length = 541, CI = 0.530, RI = 0.791) are comparable with those of MP tree. The cladogram implying the monophyly of *T. processionea* species is not rejected (ELW and WSH > 0.12). This alternative tree exhibits parsimony scores (Length = 535, CI = 0.536, RI = 0.796) almost identical to those of the MP tree (see above).

Conversely, the placement of *T. herculeana* as sister taxon of clade B of Fig. 4 is rejected by ELW test ($p = 0.0306$) but not by WSH ($p = 0.3205$). The parsimony scores for this topology are worse than previous ones (Length = 544, CI = 0.528, RI = 0.789). This latter phylogenetic arrangement was tested because the placement of *T. herculeana* within *Thaumetopoea* is controversial in different analyses (see below).

DNA-based phylogenetic analysis

Availability of molecular data was restricted to 11 species of *Thaumetopoea* (Table S2), due to the failure to obtain DNA for some taxa. The MP cladogram (Tree Length = 4491, CI = 0.492, RI = 0.492) obtained from Thau.DNA set (Fig. S8) shows phylogenetic relationships among *Thaumetopoea* species, which are almost completely mirrored by the BI/ML tree (Fig. S9). This latter topology identifies a statistically supported (1.00/95%) sister taxon relationship between *T. apologetica* and *T. dhofarensis* not observed in the MP tree. The general arrangement of *Thaumetopoea* species relationships in DNA trees broadly agrees with topologies obtained from Thau.morph set (for complete details see Figs. S8-S9). However, the position of *T. herculeana* differs from that obtained from Thau.morph dataset (see node L of Fig 4). In all the DNA-based trees *T. herculeana* belongs to a very well supported clade, which contains also *T. solitaria* and *T. processionea.* In particular, *T. herculeana* reveals a sister species relationship with *T. processionea* with good statistical corroboration (Figs. S8-S9).

The BI/ML tree is not rejected by alternative topology tests (WSH, $p = 0.9868$; ELW, $p = 0.8529$). The parsimony scores are Length = 4490, CI = 0.622 and RI = 0.492. The alternative topology implying the placement of *T. herculeana* as recovered from Thau.morph set (Fig. 4) is rejected by both ELW and WSH tests ($p < 0.0001$). MP scores for this tree (Tree Length = 4634, CI = 0.603, RI = 0.449) are also markedly worse than the MP tree for what concerns the tree length.

Total evidence phylogenetic analysis

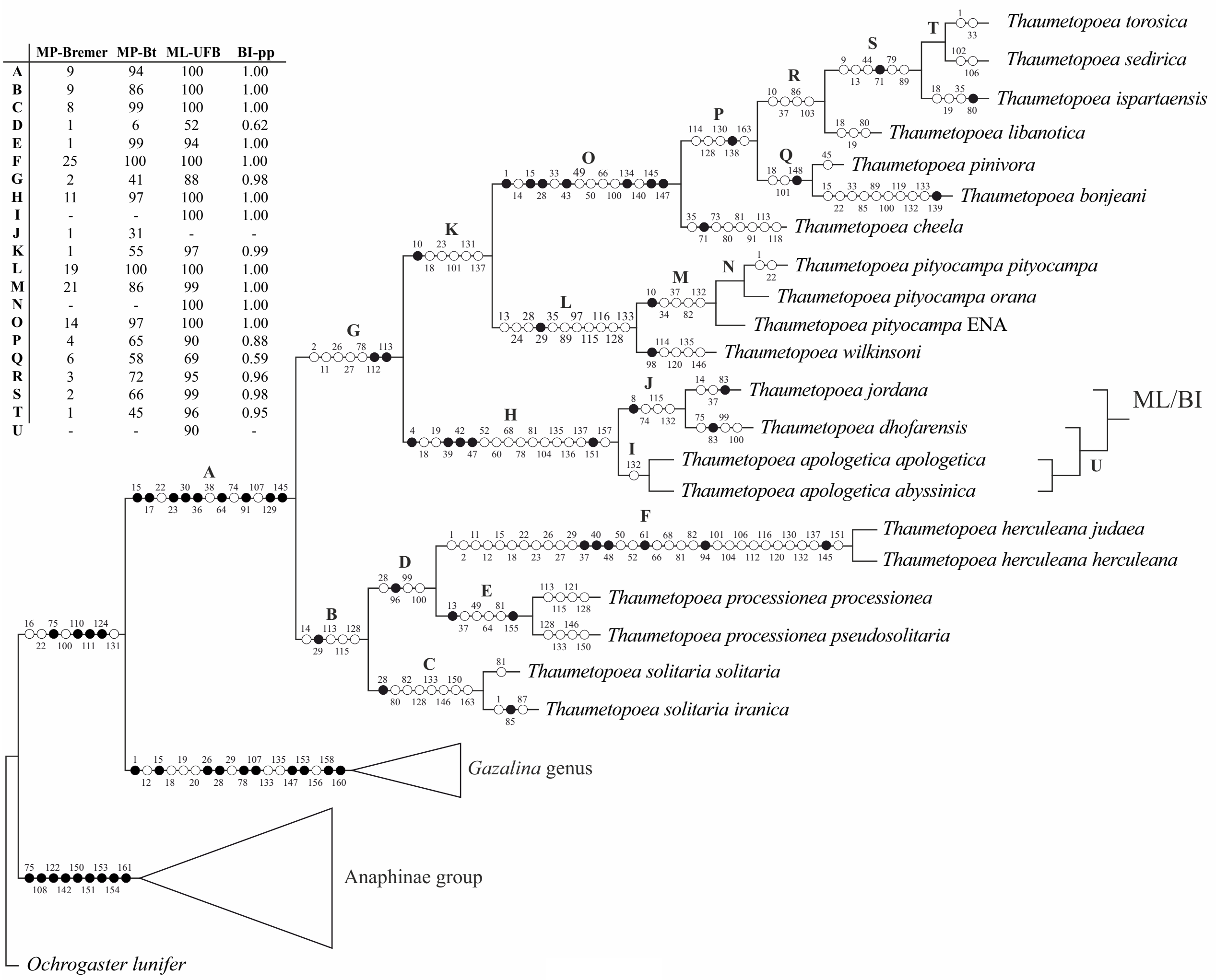
The MP tree obtained from Thau.tot.comp is shown in Fig. 5 (Tree Length = 5046, CI = 0.611, RI = 0.569) (see Fig. S10 for outgroups). The ML and BI trees obtained from the same data set are identical (Fig. S11). Their topology differs from MP cladogram (Figs.5; Fig.S11) by the placement of *T. jordana* and *T. dhofarensis*. These species are sister in the MP cladogram without bt and Bremer support (clade J). Conversely, the alternative BI/ML arrangement receives ML-UFB support (clade U) (Figs. 5 and S11). The vast majority of MP tree nodes receives strong statistical support and are supported by apomorphies and/or homoplasious changes (Fig. 5). Therefore, both the genus *Thaumetopoea* and most of its subclades receive statistical corroboration, but the placement of *T. herculeana* is different from that observed in Fig. 4. Here this moth is sister to *T. processionea* and this relationship receives very robust statistical support. Furthermore, both subspecies of the latter cluster together to form a monophyletic group with strong statistical corroboration.

Table 3. Indication of apomorphic characters and homoplasious changes of total evidence tree showed in Fig. 4.

Node	Apomorphies	Homoplasious changes
A	17:0; 30:0; 36:0; 91:0; 102:1; 111:1; 129:1	37:3; 38:1;74:0
B	29:3; 123:1	18:2; 24:2; 85:1
C	-	113:2; 115:2; 121:1; 133:1; 146:1; 150:1
D	28:3; 41:1; 86:1	37:2; 49:0; 64:0; 76:1; 79:1; 80:0; 82:0; 97:1; 163:1
E	2:0; 11:0; 26:0; 27:0; 112:0; 113:0	49:0; 64:0; 132:0
F	39:1; 42:1; 47:1	28:1; 31:1; 52:0; 60:1; 68:0; 81:3; 84:1; 104:1; 105:1; 135:1; 136:0; 157:2
G	-	132:2
H	8:2	74:0; 115:0
I	22:3; 101:1	106:0
J	29:1	24:0; 35:0; 89:1; 115:0; 133:1
K	-	82:0
L	50:1; 66:1; 112:1	33:1; 82:2; 100:1; 121:1
M	7:1; 8:0; 40:0; 48:0; 61:0; 94:1	12:0; 22:0; 29:0; 52:0; 68:0; 76:1; 96:1; 99:1; 103:1; 104:1; 115:2; 151:2
N	5:1; 28:2; 43:1; 49:2; 134:1	45:1; 140:1
O	138:1; 149:2	13:1; 103:1; 114:1; 150:1; 163:1
P	-	11:1; 12:2; 34:2; 88:1; 89:1
Q	-	10:0; 18:3; 37:3; 86:2; 103:1
R	71:1	9:0; 13:0; 44:0; 79:1; 81:1; 89:1; 147:3; 149:0; 151:0
S	-	18:1

Table 4. Indication of apomorphic characters and homoplasious changes of total morphological tree showed in Fig. 5.

Node	Apomorphies	Homoplasious changes
A	15:1; 17:0; 23:1, 2; 30:0; 36:0; 64:0; 91:0; 129:1; 145:1	22:2,3; 38:1; 74:1; 107:0
B	29:3	14:0; 113:2; 115:2; 128:1
C	28:3	80:0; 82:0; 128:0; 133:1; 146:1; 150:1; 163:1
D	96:1	28:0; 99:1; 100:1
E	13:3	37:3; 49:1; 64:1; 81:1; 155:1
F	-	-
G	112:0; 113:0	2:0; 11:0; 26:0; 27:0; 78:2
H	4:0; 39:1; 42:1; 47:1; 151:0	18:3; 19:2; 52:0; 60:1; 68:0; 78:1; 81:3; 104:1; 135:1; 136:0; 137:3; 157:2
I	-	132:2
J	8:2	74:0; 115:0; 132:0
K	10:1	18:1; 23:0; 101:1; 131:0; 137:0
L	29:1	13:0; 24:0; 28:0; 35:0; 89:1; 97:1; 115:0; 161:1; 128:1; 133:1
M	10:2	34:2; 37:3; 82:0; 132:0
N	-	-
O	1:2; 15:2; 28:2; 43:1; 134:1; 145:0; 147:3	14:1; 33:1; 49:2; 50:1; 66:1; 100:1; 140:1
P	138:1	114:1; 128:4; 130:1; 163:1
Q	148:0	18:0; 101:0
R	-	10:0; 37:3; 86:2; 103:1
S	71:1	9:0; 13:0; 44:0; 79:1; 89:1
T	-	-



	MP-Bremer	MP-Bt	ML-UFB	BI-pp
A	9	94	100	1.00
B	9	86	100	1.00
C	8	99	100	1.00
D	1	6	52	0.62
E	1	99	94	1.00
F	25	100	100	1.00
G	2	41	88	0.98
H	11	97	100	1.00
I	-	-	100	1.00
J	1	31	-	-
K	1	55	97	0.99
L	19	100	100	1.00
M	21	86	99	1.00
N	-	-	100	1.00
O	14	97	100	1.00
P	4	65	90	0.88
Q	6	58	69	0.59
R	3	72	95	0.96
S	2	66	99	0.98
T	1	45	96	0.95
U	-	-	90	-

Figure 5. The most parsimonious tree (Length = 504, CI = 0.611, RI = 0.569) obtained from Thau. tot.comp dataset. Optimization: only unambiguous changes are mapped. Black circles, apomorphic characters; white circles, homoplasious changes. Partial topology shows the difference observed BI and ML trees. MP-Bremer, Bremer support to the node; MP-bt, bootstrap support to the node; ML-ord-UFB, ultrafast bootstrap support to the node (ordered model); ML-mk-UFB, ultrafast bootstrap support to the node (unordered model); BI-pp, posterior probability support to the node.

The analyses performed on Thau.tot.red, the total evidence data set without missing data, provide topologies fully consistent with results obtained from Thau.tot.comp and are not detailed here any further (see Figs. S12-S13).

The BI/ML tree obtained from Thau.tot.comp is not rejected by the topology statistical tests (ELW, p= 0.406; and WSH, p = 0.701). The cladogram implying the placement of *T. herculeana* as sister taxon of clade O of Fig. 5, which mirrors the placement of this species in the Thau.morph analysis (clade L, Fig. 4), is fully rejected by both ELW and WSH tests ($p < 0.0001$).

The evolution of life history traits of Thaumetopoea

The evolutionary pathways of six life history traits were tracked along the MP tree obtained from Thau.tot.comp (Fig. 5). The results of this analysis are summarized in Fig. 6 (see Figs. S14-S15 for details). The inspection of Fig. 6 allows to outline some features that could have characterized the common ancestor of current *Thaumetopoea* moths (clade A). According to Fig. 6, the urticating setae already characterized the larval stage of the ancestor of *Thaumetopoea*, which was active in summer, and used angiosperms as host plants. However, it is unclear which group of angiosperms was the host. Our reconstruction also suggests that the shift from angiosperms to gymnosperms as host plants occurred only once (clade K). Pupation occurred preferably in soil. The ancestral area of the genus was likely the Palaearctic Region and successive colonization of the Afrotropics occurred once/twice in *T. dhofarensis* and *T. apologetica.* Finally, the shift of the larval feeding from summer to winter seems to have occurred in the common ancestor of clade L, and independently in *T. jordana*. The reconstruction for the ancestral state of larval feeding activity at nodes G and K is ambiguously resolved by both MP and ML methods. Thus it could be argued that the shift from summer to winter occurred at node G (slightly preferred by MK1 ML evolutionary model) and successively a secondary reversion to summer activity happened in most of the species of clade O. By considering the global distribution of this character among *Thaumetopoea* species and the effect due to missing data on the reconstruction, this latter interpretation is not very probable in our view.

Discussion

Taxonomic inconsistencies

The monophyly of *Thaumetopoea s. lat.* (clade A, Figs 4-5) is strongly supported in all our analyses, thus corroborating previous studies based on a limited number of taxa (cf. Simonato *et al.*, 2013 and references therein). The phylogenetic relationships within the genus are well resolved and generally consistent with the available classification schemes (Agenjo, 1941; Kiriakoff, 1970), albeit with some important exceptions. All our analyses, based on the combined dataset, identify a clade B (Fig. 5) including the species traditionally assigned to *Thaumetopoea s. str*. (i.e., *T. processionea* and *T. solitaria*), with the addition of *T. herculeana*. Thus, *Thaumetopoea s. str.* is retrieved as paraphyletic. The remaining species of *Thaumetopoea* (clade G), which share a crested frons and a spine on foretibia, form a group corresponding to the (sub)genus *Traumatocampa* (Agenjo, 1941) (Tab. 2.). *Thaumetopoea herculeana* is quite mobile across our phylogenetic reconstructions, depending upon the dataset used. This mirrors its variable taxonomic position, as it has been assigned either to *Traumatocampa* due to the possession of a crested frons and the spine on foretibia (Agenjo, 1941; de Freina and Witt, 1985, 1987) (see also Tab. 2.), or to its own, monotypic genus *Helianthocampa* based upon larval host plant and adult morphology (de Freina and Witt, 1985). However, removing *T. herculeana* from *Traumatocampa* would make this no longer monophyletic on morphological grounds (see above Fig. 4).

Circumscribing Thaumetopoea*,* Traumatocampa*, and* Helianthocampa*: where should the tree be cut?*

The simultaneous presence of frontal crest and foretibia spine supports the monophyly of *Traumatocampa sensu* Agenjo (i.e., clade E of Fig. 4) in our morphology-based analysis (Supplementary material Figs. S4-S7). Frontal crest and foretibia spine have a clear adaptive significance as these species spend the pupal stage deep into the soil (usually between 5 and 20 cm) (Battisti *et al.*, 2015), and newly emerged adults use such anatomical structures to break through to the surface (Démolin, 1969). In contrast, processionary moths that do not have frontal crest and foretibia spine pupate either inside the tent (*T. processionea*) or in the litter (*T. solitaria*), but never deep into the soil.

In contrast to morphology, both molecular and combined datasets recover *T. herculeana* as nested within *Thaumetopoea s. str.* (clade B, Fig. 5), suggesting that the phylogenetic reconstruction is influenced by a strong molecular signal. It must be noted here that for several taxa the DNA sequences are represented solely by the barcoding portion of *cox1* gene (i.e. about 650 base pairs). In the present paper we analysed only mitochondrial genes. Thus it could be argued that the DNA based placement of *T. herculeana* is biased by the origin of the selected markers and does not represent a balanced molecular view. However, the same placement was obtained by Simonato *et al.* (2013), who worked on a DNA multi-genes alignment encompassing both mitochondrial and nuclear markers. Particularly important for our discussion, the positioning of *T. herculeana* (Fig. 5) was recovered also from a data set containing only the nuclear genes: *wingless*, *elongation-factor*

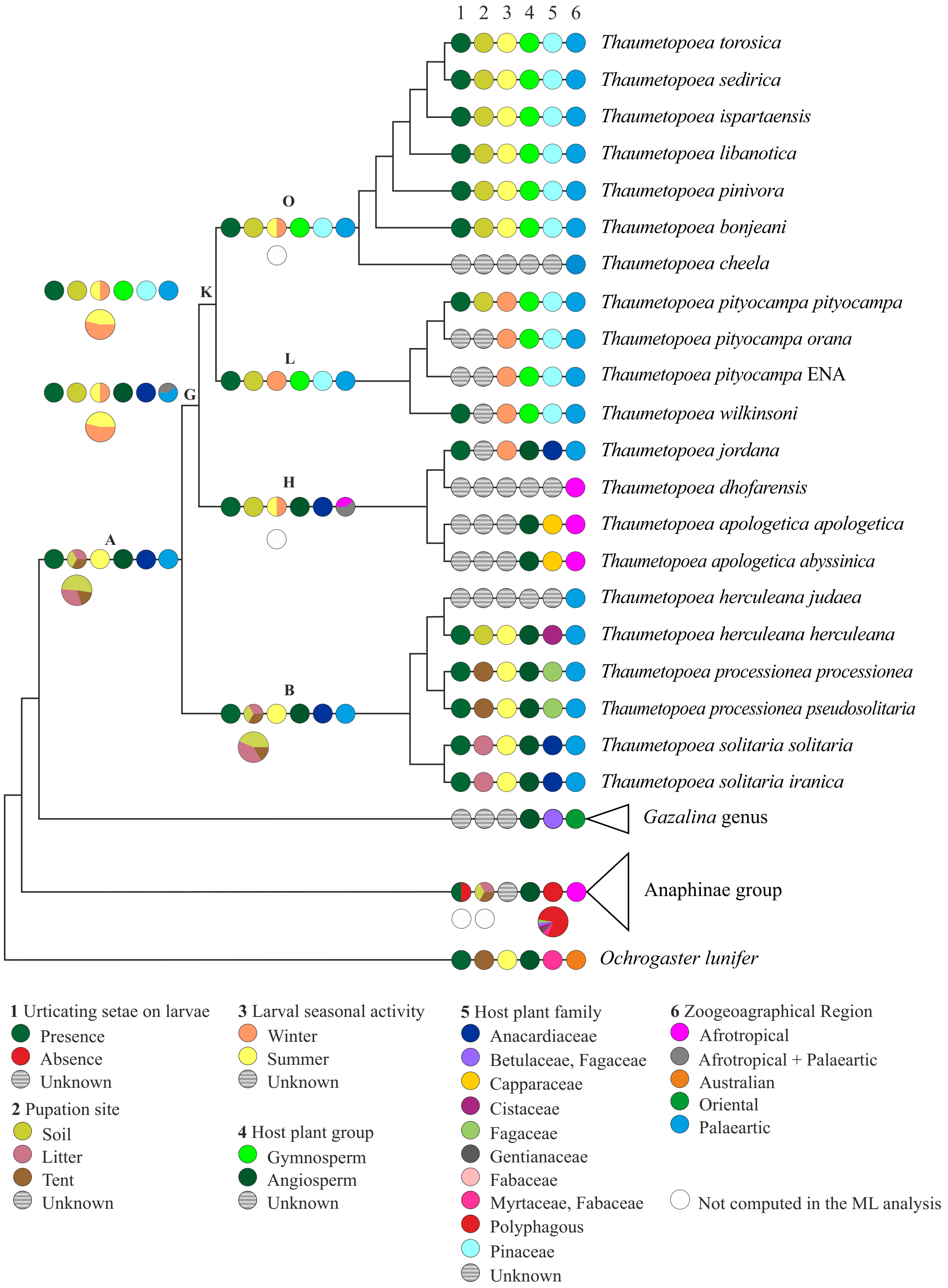


Figure 6. The evolution of life history traits mapped on the MP tree obtained from Thau.tot.comp dataset. The transformational pathways are provided for all terminal nodes plus selected internal nodes (for full details see Figs. S14-S15). Maximum parsimony reconstructions are figured on the branch leading to the selected node. Maximum likelihood reconstructions are provide only for ambiguous results below the reference branch. Traits 1-5 were analysed with the Mesquite software. Trait 6 was mapped with S-DIVA software.

alpha, and *photolyase* (Simonato *et al.*, 2013). This latter result strongly contradicts the hypothesis of the incorrect placement *T. herculeana* due to the mitochondrial origin of molecular markers. Thus, despite the limited gene sampling, compared to the 165 morphological traits, it appears that molecules alone, or in combination with morphology, support a close relationship between *T. herculeana* and *T. processionea*.

Considering the host plant associations, it seems likely that larvae of the ancestor of *Thaumetopoea* fed on angiosperms and that only later a drastic shift to gymnosperms occurred, as observed in clade K (Fig. 6). The reconstruction of the pupation strategy (Fig. 6) supports the view that the soil was probably the pupation site of the *Thaumetopoea* ancestor, even if the proportional likelihood is not conclusive (Fig. 6). It may thence be supposed that soil pupation made the processionary moths better suited to highly variable climatic conditions, such as for instance those occurring in the Mediterranean. Stiff frontal protuberances enabling emerging adults to dig themselves out of hardened soils, either associated with foretibia spines or not, are a common adaptation in several members of the allied family Noctuidae, particularly if living in arid environments, e.g. *Cardepia*, *Craterestra*, *Conicofrontia*, *Grotella*, *Aedophron* (e.g., Janse, 1939; Hogue, 1963; Berio, 1985; Matthews, 1991; Fibiger *et al.*, 2009), but none appears to be configured as those of *Thaumetopoea s. lat.* Thus, it is conceivable that early ancestors of *T. processionea* and *T. solitaria* independently lost both frontal crest and foretibia spine as a consequence of a change in their pupation strategy from soil to litter (*T. solitaria*) and to tent (*T. processionea*). To further substantiate this scenario, it is interesting to note that both *T. processionea* and *T. solitaria* show a pronounced median protuberance on the frons (Fig. 1), whereas in *Gazalina* and other genera of Thaumetopoeinae (e.g., *Anaphe*, *Epicoma, Hypsoides*, and *Ochrogaster*) the frons is flat. The same applies for the foretibia, as both *T. processionea* and *T. solitaria* show a pronounced distal edge (Fig. 1) recalling a "regressed" foretibia spine, which is pretty different from the flat distal edge shared by the remaining Thaumetopoeinae. This is suggestive of the absence of the toothed frontal crest in the two species as being non homologous to that of other genera examined and representing a secondary loss. The toothed crest would therefore be confirmed as a good autapomorphy defining the whole *Thaumetopoea*, not just the *Traumatocampa* branch as supposed by the early authors and corroborated by the morphological dataset alone. Maintained in *T. herculeana*, topology of the total evidence cladogram (Fig. 5) is in accordance with an independent regression of the crest in both *T. processionea* and *T. solitaria*. As a matter of fact, the crest of processionary moths is such a complex structure that following release of the selection on the character it is more parsimonious to admit its loss as a result of some disruption in the underlying developmental gene machinery (e.g., Griffiths *et al.*, 1999; Hottes *et al.*, 2013; Stower, 2013) than assuming its independent arousal in *T. herculeana* and typical *Traumatocampa*.

The conifer feeding taxa: summer vs. winter

All our cladograms agree in reconstructing conifer feeding processionary moths (clade K, Fig. 6) as monophyletic. Monophyly of this clade has been strongly supported also by previous molecular studies (Simonato *et al.*, 2013). Conifer feeding processionary moths split into two subclades (L and O), each being characterized by a number of apomorphic character states (Fig. 5). Species included in clades L and O are also characterized by having different developmental strategies (Démolin, 1989) and diverging sexual pheromone composition (Frérot and Démolin, 1993). Clade L includes taxa whose larvae feed across winter ('winter species') while clade O those feeding across spring and summer ('summer species'). Clades L and O have been recovered as monophyletic in all our analyses, gaining strong statistical support. Clades differ in relation to host plants, as winter taxa usually feed on *Pinus* while summer taxa on *Cedrus*, although notable exceptions exist. In fact, *T. pityocampa* (Clade L) can develop on Cedrus as well as on other conifers (Battisti *et al.*, 2015) whereas *T. pinivora* (Clade O) feeds solely on Pinus, probably as a result of a host shift from a Cedrus-associated ancestor (Cassel-Lundhagen *et al.*, 2013).

The study of the evolution of seasonal feeding of *Thaumetopoea* larvae suggests that a change from summer to winter would have characterized the developmental strategy of the ancestor of clade K, thus triggering allochronic speciation events (e.g., Santos *et al.*, 2007). A winter feeding larval stage has several major advantages in temperate regions, such as a comparatively enemy- and competition-free space, although it can be constrained by winter cold (Battisti *et al.,* 2015). This adaptation, that involves several biochemical and physiological modifications, is a key to understand the response of *T. pityocampa* to former and current climate changes (Battisti *et al.*, 2005), which has likely contributed to the genetic population structure of this taxon (Kerdelhué *et al.*, 2009). Interestingly, under mild oceanic climatic conditions a winter population of *T. pityocampa* in coastal Portugal has recently shifted back to summer feeding, indicating that the trait has great adaptive value (Santos et al., 2007; 2011).

Evolutionary history and conclusions

The study of the evolution of life history traits allows us to sketch an evolutionary scenario for *Thaumetopoea.* The Western Palaearctic Region, and the East Mediterranean-Middle East sub-region in particular, was likely the area from where this group started diversifying from an ancestor developing on angiosperms. It is likely that the host shift from angiosperms to gymnosperms that characterized the ancestor of clade K (Figs. 5-6) occurred during the Tertiary and was further affected during the Quaternary glaciation events. Previous molecular work done on a subset of taxa indicated that the Messinian period is when major genetic diversification of species and clades happened (Salvato *et al.*, 2002; Simonato *et al.*, 2007; Kerdelhué *et al.*, 2009). Pines and cedar trees are documented to occur throughout that period in the West Palaearctic (Richardson, 1998; Qiao *et al.*, 2007), mainly in the mountains. This could have promoted the isolation of the insect populations and the speciation process, as it is evident for the cedar-feeding species in the summer clade (Basso *et al.*, 2016). As the host plant shift appears to be unique, it is not possible to reconstruct with precision its timing and location, although pines are the most likely candidates because of their large predominance in fossil remains of conifers (Richardson, 1998). The host shift drove speciation events on these plants, resulting now in the so-called 'coniferous *Thaumetopoea*' (Simonato *et al.*, 2013) (clade K) (Figs. 5-6), that count almost ten taxa spread mostly in the Mediterranean subregion. Conversely, the ancestors of clades B and H continued to rely on a very diverse number of angiosperms as host plants. A common trait shared by the latter groups is the colonization of dry areas of Middle East and Afrotropical region, which could have been facilitated by the plasticity in the life history. This is remarkably shown by *T. jordana,* which shifted to winter feeding, possibly driven by the necessity to escape from harsh conditions provided by Jordan valley in summer (Trougth, 1954). The spread in the Afrotropical region would ultimately have been achieved by clade H radiating from the Middle East. It would be interesting to compare the special *Thaumetopoea* adaptation to climate and host plants with those of several other genera of Thaumetopoeinae occurring in Africa and Australasia in a number of different habitats, to come up with a comprehensive phylogenetic analysis of the whole group that could help to better understand the evolution of *Thaumetopoea.*

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Supplementary material

Table S1A. Specimens examined in this work. List of collections where specimens are preserved: BMNH: Natural History Museum (formerly British Museum of Natural History), London UK. DAFNAE: Department of Agronomy, Food, Natural resources, Animals and Environment - University of Padua, Padua Italy. Private coll.: Private collection of A. Schintlmeister, Dresden Germany. RBINS: Royal Belgian Institute of Natural Sciences, Brussels Belgium. RMCA: Royal Museum of Central Africa, Tervuren Belgium. MCZR: Museo Civico di Zoologia, Rome Italy. MZUR: Museo di Zoologia, 'Sapienza' University of Rome, Rome Italy. WITT: Witt Museum, Munich Germany. ZSM: The Bavarian State Collection of Zoology, Munich Germany. PT= paratype, ST= syntype.

N°	Ref. slides	Taxon	Locality	Ref. Specimens
1	Noto_2098	*Thaumetopoea apologetica abyssinica*	Ethiopia	BMNH_1378622
2	Noto_2099	*Thaumetopoea apologetica abyssinica*	Ethiopia	BMNH_1378623
3	Noto_2100	*Thaumetopoea apologetica abyssinica*	Ethiopia	BMNH_1378624
4	Noto_2101	*Thaumetopoea apologetica abyssinica*	Ethiopia	BMNH_1378625
5	Noto_298	*Thaumetopoea apologetica abyssinica*	Ethiopia	BMNH
6	Noto_2095	*Thaumetopoea apologetica apologetica*	Arabia	BMNH_1378605
7	Noto_2096	*Thaumetopoea apologetica apologetica*	Arabia	BMNH_1378607
8	Noto_2097	*Thaumetopoea apologetica apologetica*	Arabia	BMNH_1378603
9	Noto_297	*Thaumetopoea apologetica apologetica*	Uganda	BMNH
10	TH 4	*Thaumetopoea apologetica apologetica*	Zimbabwe	Private coll._#29.538
11	TH 5	*Thaumetopoea apologetica apologetica*	Zimbabwe	Private coll._#29.540
12	TH 56	*Thaumetopoea apologetica apologetica*	Zimbabwe	Private coll._#29.537
13	TH 57	*Thaumetopoea apologetica apologetica*	Zimbabwe	Private coll._#29.539
14	TH 58	*Thaumetopoea apologetica apologetica*	Tzaneen	Private coll._#29.541
15	TH 59	*Thaumetopoea apologetica apologetica*	South Africa	Private coll._#29.542
16	TH 6	*Thaumetopoea apologetica apologetica*	Namibia	Private coll._#29.543
17	TH 60	*Thaumetopoea apologetica apologetica*	Zimbabwe	Private coll._#29.544
18	Noto_2083	*Thaumetopoea bonjeani*	Morocco	BMNH_1378573
19	TH 18	*Thaumetopoea bonjeani*	Algeria - Tala Guilef	DAFNAE_TH18
20	TH 19	*Thaumetopoea bonjeani*	Algeria - Tala Guilef	DAFNAE_TH19
21	TH 20	*Thaumetopoea bonjeani*	Algeria - Tala Guilef	DAFNAE_TH20
22	TH 21	*Thaumetopoea bonjeani*	Algeria - Tizi Oujavoub	DAFNAE_TH21
23	TH 22	*Thaumetopoea bonjeani*	Algeria - Tizi Oujavoub	DAFNAE_TH22
24	TH 23	*Thaumetopoea bonjeani*	Algeria - Tizi Oujavoub	DAFNAE_TH23
25	Noto_2111	*Thaumetopoea cheela* ST	India	BMNH_1378635
26	TH 73	*Thaumetopoea dhofarensis*	Oman	WITT_TH73
27	TH 74	*Thaumetopoea dhofarensis*	Oman	WITT_TH74
28	Whiltshire_1911	*Thaumetopoea dhofarensis*	Oman	BMNH
29	Noto_2084	*Thaumetopoea herculeana*	Algeria	BMNH_1378597
30	Noto_2085	*Thaumetopoea herculeana*	Algeria	BMNH_1378596
31	Noto_2086	*Thaumetopoea herculeana*	Morocco	BMNH_1378599
32	Noto_2087	*Thaumetopoea herculeana*	Morocco	BMNH_1378600
33	Noto_2088	*Thaumetopoea herculeana*	Morocco	BMNH_1378601
34	TH 26	*Thaumetopoea herculeana*	Spain	DAFNAE_TH26
35	TH 33	*Thaumetopoea herculeana*	Spain	MZUR_TH33
36	TH 34	*Thaumetopoea herculeana*	Algeria	MZUR_TH34
37	Noto_2089	*Thaumetopoea herculeana judaea*	Palestine	BMNH_1378602
38	Noto_2090	*Thaumetopoea herculeana judaea*	Palestine	BMNH_1378638

Table S1B. Continue from previous page.

N°	Ref. slides	Taxon	Locality	Ref. Specimens
39	TH 30	*Thaumetopoea ispartaensis*	Turkey - Isparta	DAFNAE_TH30
40	TH 61	*Thaumetopoea ispartaensis*	Turkey - Isparta	DAFNAE_TH61
41	Noto_2080	*Thaumetopoea jordana*	Arabia	BMNH_1378574
42	Noto_2082	*Thaumetopoea jordana*	Jordan	BMNH_1378576
43	TH 72	*Thaumetopoea jordana*	Palestine	ZSM_TH72
44	Noto_2081	*Thaumetopoea jordana* *	Jordan	BMNH_1378575
45	Noto_2078	*Thaumetopoea pinivora*	France	BMNH_1378579
46	Noto_2079	*Thaumetopoea pinivora*	Poland	BMNH_1378578
47	TH 24	*Thaumetopoea pinivora*	Germany	WITT_TH24
48	TH 35	*Thaumetopoea pinivora*	Germany	MZUR_TH35
49	Noto_2073	*Thaumetopoea pityocampa orana*	Morocco	BMNH_1378569
50	Noto_2074	*Thaumetopoea pityocampa orana*	Tunisia	BMNH_1378567
51	Noto_2075	*Thaumetopoea pityocampa orana*	Algeria	BMNH_1378565
52	Noto_2076	*Thaumetopoea pityocampa orana*	Algeria	BMNH_1378566
53	Noto_2077	*Thaumetopoea pityocampa orana*	Algeria	BMNH_1378568
54	Noto_2064	*Thaumetopoea pityocampa pityocampa*	Portugal	BMNH_1378589
55	Noto_2065	*Thaumetopoea pityocampa pityocampa*	Portugal	BMNH_1378588
56	Noto_2066	*Thaumetopoea pityocampa pityocampa*	Algeria	BMNH_1378587
57	Noto_2067	*Thaumetopoea pityocampa pityocampa*	Majorca	BMNH_1378585
58	Noto_2068	*Thaumetopoea pityocampa pityocampa*	Republic of Macedonia	BMNH_1378584
59	Noto_2071	*Thaumetopoea pityocampa pityocampa*	Greece	BMNH_1378583
60	Noto_2072	*Thaumetopoea pityocampa pityocampa*	Greece	BMNH_1378582
61	Noto_2112	*Thaumetopoea pityocampa pityocampa*	France	BMNH_1378637
62	Noto_2113	*Thaumetopoea pityocampa pityocampa*	-	BMNH_1378563
63	TH 10	*Thaumetopoea pityocampa pityocampa*	Spain	DAFNAE_TH10
64	TH 12	*Thaumetopoea pityocampa pityocampa*	Italy	DAFNAE_TH12
65	TH 13	*Thaumetopoea pityocampa pityocampa*	Italy	DAFNAE_TH13
66	TH 17	*Thaumetopoea pityocampa pityocampa*	Italy	DAFNAE_TH17
67	TH 32	*Thaumetopoea pityocampa pityocampa*	Croatia	MZUR_TH32
68	TH 46	*Thaumetopoea pityocampa pityocampa*	Italy - Calbarina	DAFNAE_TH46
69	TH 47	*Thaumetopoea pityocampa pityocampa*	Italy - Calbarina	DAFNAE_TH47
70	TH 9	*Thaumetopoea pityocampa pityocampa*	Spain	DAFNAE_TH9
71	TH 27	*Thaumetopoea processionea*	Austria	WITT_TH27
72	Noto_1634	*Thaumetopoea processionea processionea*	France	BMNH
73	Noto_2091	*Thaumetopoea processionea processionea*	Hungary	BMNH_1378610
74	Noto_2092	*Thaumetopoea processionea processionea*	Switzerland	BMNH_1378611
75	Noto_2093	*Thaumetopoea processionea processionea*	France	BMNH_1378613
76	TH 36	*Thaumetopoea processionea processionea*	-	MZUR_TH36
77	TH 37	*Thaumetopoea processionea processionea*	Italy	MCZR_TH37
78	TH 63	*Thaumetopoea processionea processionea*	France	DAFNAE_TH63
79	Noto_2094	*Thaumetopoea processionea pseudosolitaria*	Macedonia	BMNH_1378612
80	TH 65	*Thaumetopoea processionea pseudosolitaria*	Republic of Macedonia	DAFNAE_TH65
81	TH 66	*Thaumetopoea processionea pseudosolitaria*	Italy - Sicilia	ZSM_TH66
82	TH 29	*Thaumetopoea sedirica* PT	Turkey - Sedir	DAFNAE_TH29
83	TH 31	*Thaumetopoea sedirica* PT *	Turkey - Sedir	DAFNAE_TH31
84	Noto_2105	*Thaumetopoea solitaria*	Cyprus	BMNH_1378618

*, specimens without genitalia

Table S1C. Continue from previous page.

N°	Ref. slides	Taxon	Locality	Ref. Specimens
85	Noto_2106	*Thaumetopoea solitaria*	Cyprus	BMNH_1378617
86	Noto_2107	*Thaumetopoea solitaria*	Iraq	BMNH_1378616
87	Noto_2108	*Thaumetopoea solitaria iranica*	Iran	BMNH_1378621
88	Noto_2109	*Thaumetopoea solitaria iranica*	Iran	BMNH_1378620
89	Noto_2110	*Thaumetopoea solitaria iranica*	Iran	BMNH_1378619
90	TH 3	*Thaumetopoea solitaria solitaria*	Pakistan	DAFNAE_TH3
91	TH 38	*Thaumetopoea solitaria solitaria*	Bulgaria	MCZR_TH38
92	TH 39	*Thaumetopoea solitaria solitaria*	Republic of Macedonia	MCZR_TH39
93	TH 64	*Thaumetopoea torosica*	Turkey - Adana	DAFNAE_TH64
94	TH 28	*Thaumetopoea torosica* *	Turkey - Isparta	DAFNAE_TH28
95	Noto_2104	*Thaumetopoea wilkinsoni*	Cyprus	BMNH_1378571
96	TH 11	*Thaumetopoea wilkinsoni*	Dishon - Israel	DAFNAE_TH11
97	TH 48	*Thaumetopoea wilkinsoni*	Israel - Dishon	DAFNAE_TH48
98	TH 49	*Thaumetopoea wilkinsoni*	Israel - Dishon	DAFNAE_TH49
99	TH 50	*Thaumetopoea wilkinsoni*	Israel - Western Negev	DAFNAE_TH50
100	TH 51	*Thaumetopoea wilkinsoni*	Israel - Western Negev	DAFNAE_TH51
101	TH 52	*Thaumetopoea wilkinsoni*	Israel - Judean Foothills	DAFNAE_TH52
102	TH 53	*Thaumetopoea wilkinsoni*	Israel - Judean Foothills	DAFNAE_TH53
103	TH 54	*Thaumetopoea wilkinsoni*	Israel - Southern Judean	DAFNAE_TH54
104	TH 55	*Thaumetopoea wilkinsoni*	Israel - Southern Judean	DAFNAE_TH55
105	Noto_2062	*Thaumetopoea wilkinsoni* [1]	Lebanon	BMNH_1378592
106	Noto_2063	*Thaumetopoea wilkinsoni* [1]	Lebanon	BMNH_1378593
107	Noto_2069	*Thaumetopoea wilkinsoni* [1]	Rhodes	BMNH_1378580
108	Noto_2070	*Thaumetopoea wilkinsoni* [1]	Rhodes	BMNH_1378581
109	Whiltshire_281	*Thaumetopoea wilkinsoni* HT	Cyprus	BMHN(E)_1378629
110	Noto_2102	*Thaumetopoea wilkinsoni* PT	Cyprus	BMNH_1378628
111	Noto_2103	*Thaumetopoea wilkinsoni* PT	Cyprus	BMNH_1378627
112	TH 75	*Anaphe etiennei*	DR of Congo	RMCA_TH75
113	Noto_271	*Anaphe panda*	South Africa	BMNH
114	TH 78	*Anaphe panda*	Tanzania	ZSM_TH78
115	TH 15	*Anaphe panda infracta*	Zambia	DAFNAE_TH15
116	TH 7	*Anaphe panda infracta*	DR of Congo	DAFNAE_TH7
117	TH 14	*Anaphe reticulata*	South Africa	DAFNAE_TH14
118	TH 76	*Anaphe venata*	DR of Congo	RMCA_TH76
119	TH 79	*Anaphe venata*	Congo da Lemba	RMCA_TH79
120	Noto_276	*Epanaphe* (*Anaphe*) *carteri*	Ghana	BMNH
121	Noto_275	*Epanaphe* (*Anaphe*) *moloney*	Zambia	BMNH
122	Noto_302	*Epanaphe* (*Anaphe*) *subsordida*	Nigeria	BMNH
123	TH 70	*Epanaphe carteri*	Angola	ZSM_TH70
124	TH 81	*Epanaphe maynei*	DR of Congo	RMCA_TH81
125	TH 69	*Epanaphe moloney*	Nigeria	ZSM_TH69
126	TH 80	*Epanaphe nigricincta*	Uganda	ZSM_TH80
127	Noto_278	*Epanaphe subsordida*	Cameroon	BMNH
128	TH 82	*Epanaphe subsordida*	DR of Congo	RMCA_TH82
129	TH 16	*Gazalina apsara*	Myanmar	WITT_TH16

[1], labelled as *T. pityocampa pityocampa* in BMNH; *, specimens without genitalia

Table S1D. Continue from previous page.

N°	Ref. slides	Taxon	Locality	Ref. Specimens
130	TH 67	*Gazalina apsara*	Myanmar	WITT_TH67
131	TH 77	*Gazalina apsara*	Pakistan	DAFNAE_TH77
132	TH 2	*Gazalina chrysolopha*	Pakistan	DAFNAE_TH2
133	TH 68	*Gazalina transversa*	Nepal	ZSM_TH68
134	TH 84	*Hypsoides antsianakana*	Madagascar	ZSM_TH84
135	TH 71	*Hypsoides placidus*	-	ZSM_TH71
136	TH 8	*Hypspoides antsianakana* *	Madagascar	DAFNAE_TH8
137	TH 85	*Ochrogaster lunifer*	Australia	DAFNAE_TH85
138	Noto_269	*Ochrogaster lunifer* (*Marane rubricorpus*)	Australia	BMNH
139	Noto_303	*Ochrogaster lunifer* (*ruptimacula*)	-	BMNH
140	Noto_270	*Ochrogaster lunifer* (*Teara contraria*)	Australia	BMNH
141	TH 83	*Paradrallia punctigera*	DR of Congo	RBINS_TH83
142	Noto_299	*Paradrallia rhodesi*	Zambia	BMNH
143	TH 1	*Paradrallia rhodesi*	Zambia	DAFNAE_TH1
144	TH 25	*Paradrallia rhodesi*	Zambia	DAFNAE_TH25

*, specimens without genitalia

Table S2. Mitochondrial genes used.

	cox1-cox2-atp6-atp8-cox3	partial *cob-nad1*	*nad4-nad5*
Thaumetopoea processionea processionea	HE963114	HE956699	HE864329
Thaumetopoea solitaria solitaria	HE963115	HE956700	HE864330
Thaumetopoea herculeana herculeana	HE963108	HE956693	HE864323
Thaumetopoea apologetica apologetica *	LT614824	—	—
Thaumetopoea dhofarensis *	LT614825	—	—
Thaumetopoea bonjeani	HE963107	HE956692	HE864322
Thaumetopoea pinivora	HE963111	HE956696	HE864326
Thaumetopoea libanotica	HE963110	HE956695	HE864325
Thaumetopoea ispartaensis	HE963109	HE956694	HE864324
Thaumetopoea pityocampa pityocampa	HE963112	HE956697	HE864327
Thaumetopoea pityocampa ENA	HE963113	HE956698	HE864328
Thaumetopoea wilkinsoni	HE963116	HE956701	HE864331
Anaphe panda panda *	HM892083	—	—
Anaphe panda infracta *	LT614827	—	—
Epanaphe carteri *	HM892014	—	—
Gazalina chrysolopha *	LT614826	—	—
Hypsoides antsianakana *	LT614829	—	—
Ochrogaster lunifer	AM946601	AM946601	AM946601
Paradrallia rhodesi *	LT614828	—	—

*, sequenced only the barcoding portion of cox1; —, not available; underline, sequences produced for the present work.

Table S3. Data available

	morphology	*cox1*	*cox2*	*cox3*	*atp6*	*atp8*	*cob*	*nad1*	*nad4*	*nad5*
Thaumetopoea processionea processionea	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
Thaumetopoea processionea pseudosolitaria	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Thaumetopoea solitaria solitaria	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
Thaumetopoea solitaria iranica	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Thaumetopoea herculeana herculeana	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
Thaumetopoea herculeana judaea	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Thaumetopoea apologetica apologetica	🟢	🟩	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Thaumetopoea apologetica abyssinica	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Thaumetopoea dhofarensis	🟢	🟩	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Thaumetopoea jordana	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Thaumetopoea cheela	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Thaumetopoea bonjeani	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
Thaumetopoea pinivora	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
Thaumetopoea libanotica	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
Thaumetopoea ispartaensis	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
Thaumetopoea sedirica	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Thaumetopoea torosica	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Thaumetopoea pityocampa pityocampa	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
Thaumetopoea pityocampa orana	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Thaumetopoea pityocampa ENA	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
Thaumetopoea wilkinsoni	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
Anaphe etiennei	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Anaphe panda panda	🟢	🟩	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Anaphe panda infracta	🟢	🟩	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Anaphe venata	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Epanaphe carteri	🟢	🟩	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Epanaphe moloney	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Epanaphe nigricincta	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Epanaphe subsordida	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Gazalina apsara	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Gazalina chrysolopha	🟢	🟩	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Gazalina trasversa	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Hypsoides antsianakana	🟢	🟩	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Hypsoides placidus	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Ochrogaster lunifer	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
Paradrallia punctigera	🟢	🟩	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴
Paradrallia rhodesi	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴

🟢 Complete data, 🟩 Barcoding sequence (about 650 bp), 🔴 Missing data.

Length of genes (bp). *cox1*: 1518 ca.; *cox2*: 679 ca.; partial *cox3*: 126 ca.; *atp6*: 165 ca.; *atp8*: 678 ca.; *cob*: 1062 ca.; *nad1*: 939 ca.; partial *nad4*: 490 ca.; *nad5*: 1509 ca.

Figure S1. Species studied in this work. (a), *Thaumetopoea herculeana herculeana* - Algeria (BMHN(E)_1378597); (b), *Thaumetopoea processionea* - France (BMHN(E)_1378609); (c), *Thaumetopoea solitaria solitaria* - Cyprus (BMHN(E)_1378618); (d), *Thaumetopoea apologetica apologetica* - Kenya (BMHN(E)_1378606); (e), *Thaumetopoea dhofarensis* - Oman (WITT_TH73); (f), *Thaumetopoea jordana* - Palestine (ZSM_TH72); (g), *Thaumetopoea pityocampa pityocampa*- Greece (BMHN(E)_1378583); (h), *Thaumetopoea wilkinsoni* - Cyprus (BMHN(E)_1378572). The collection code associated to the specimen is provided in brackets (see Table S1).

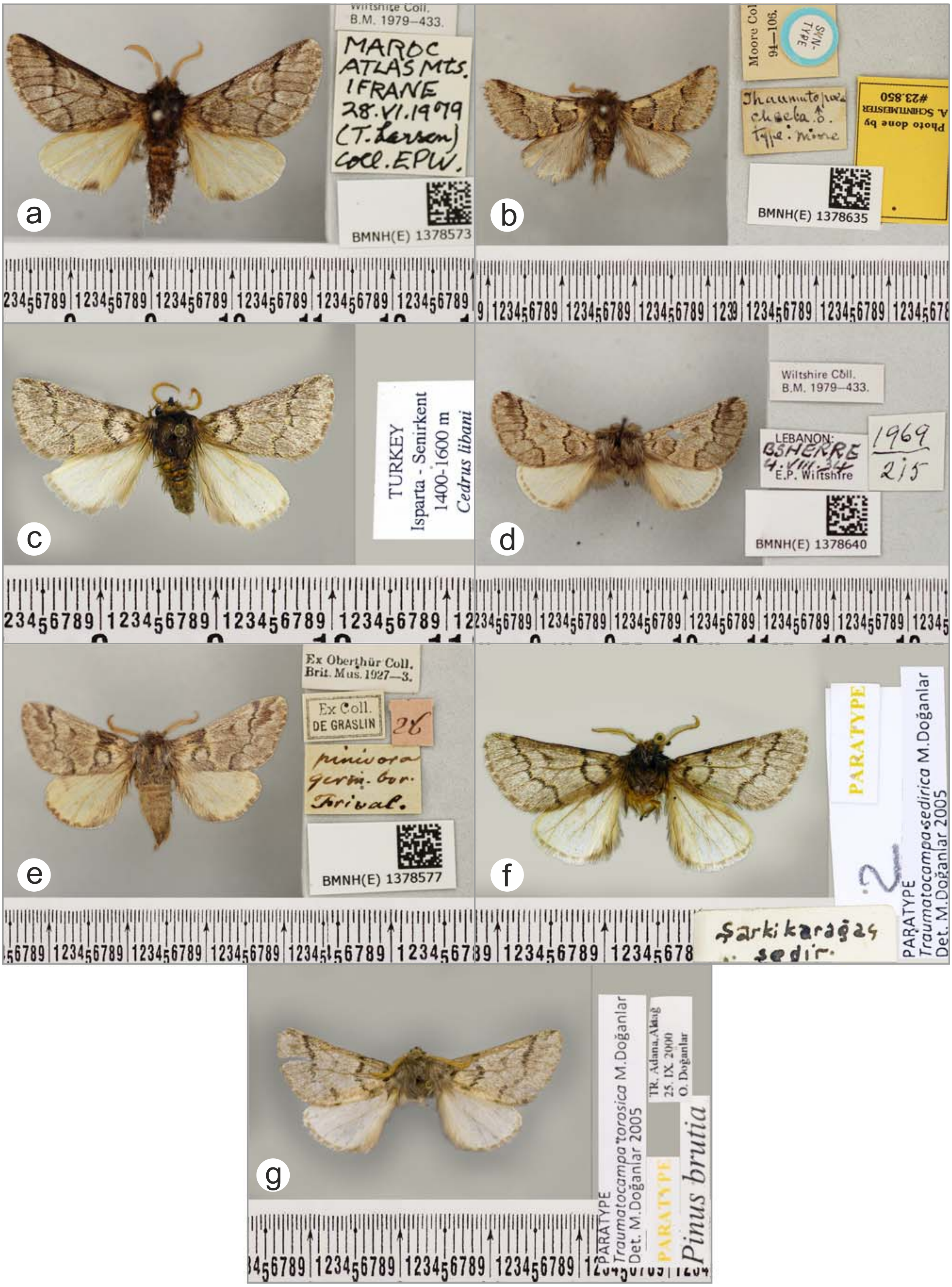

Figure S2. Species studied in this work. (a), *Thaumetopoea bonjeani* - Morocco (BMHN(E)_1378573); (b), *Thaumetopoea cheela* ST - India (BMHN(E)_1378635); (c), *Thaumetopoea ispartaensis* - Turkey (DAFNAE_TH30); (d), *Thaumetopoea libanotica* - Lebanon (BMNH(E)_1378640); (e), *Thaumetopoea pinivora* - France (BMHN(E)_1378577); (f), *Thaumetopoea sedirica* PT - Turkey (DAFNAE_TH29); (g), *Thaumetopoea torosica* PT - Turkey (DAFNAE_TH64). The collection code associated to the specimen is provided in brackets (see Table S1).

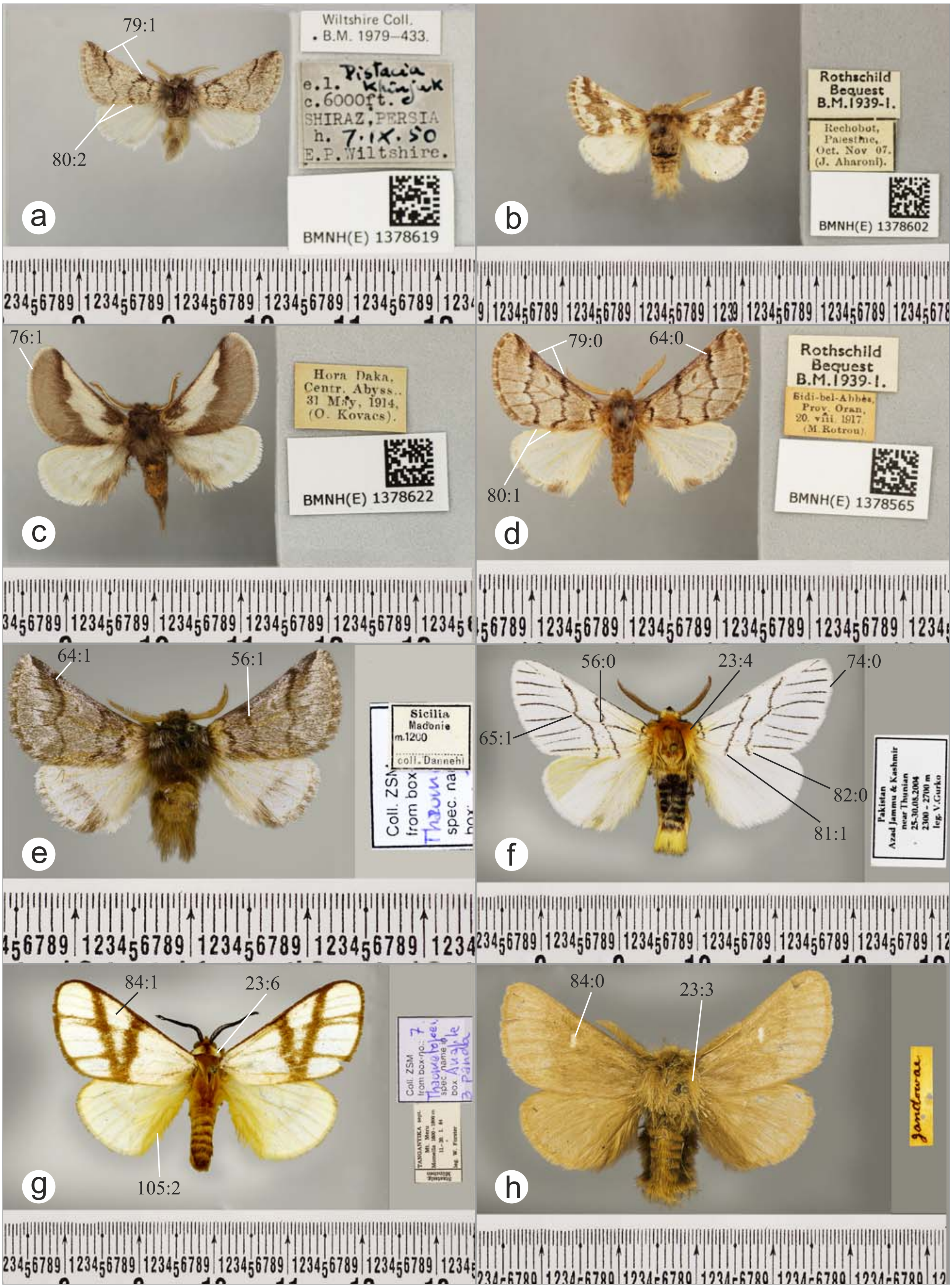

Figure S3. Species studied in this work. (a), *Thaumetopoea apologetica abyssinica* - Abyssinia (BMHN(E)_1378622); (b), *Thaumetopoea herculeana judaea* - Palestine (BMHN(E)_1378602); (c), *Thaumetopoea pityocampa orana* - Algeria (BMHN(E)_1378565); (d), *Thaumetopoea solitaria iranica* - Persia (BMHN(E)_1378619); (e), *Thaumetopoea processionea pseudosolitaria* - Italy (ZSM_TH66); (f), *Gazalina apsara* - Pakistan (DAFNAE_TH77); (g), *Anaphe panda panda* - Tanzania (DAFNAE_TH78); (h), *Ochrogaster lunifer* - Australia (DAFNAE_TH85). The collection code associated to the specimen is provided in brackets (see Table S1).

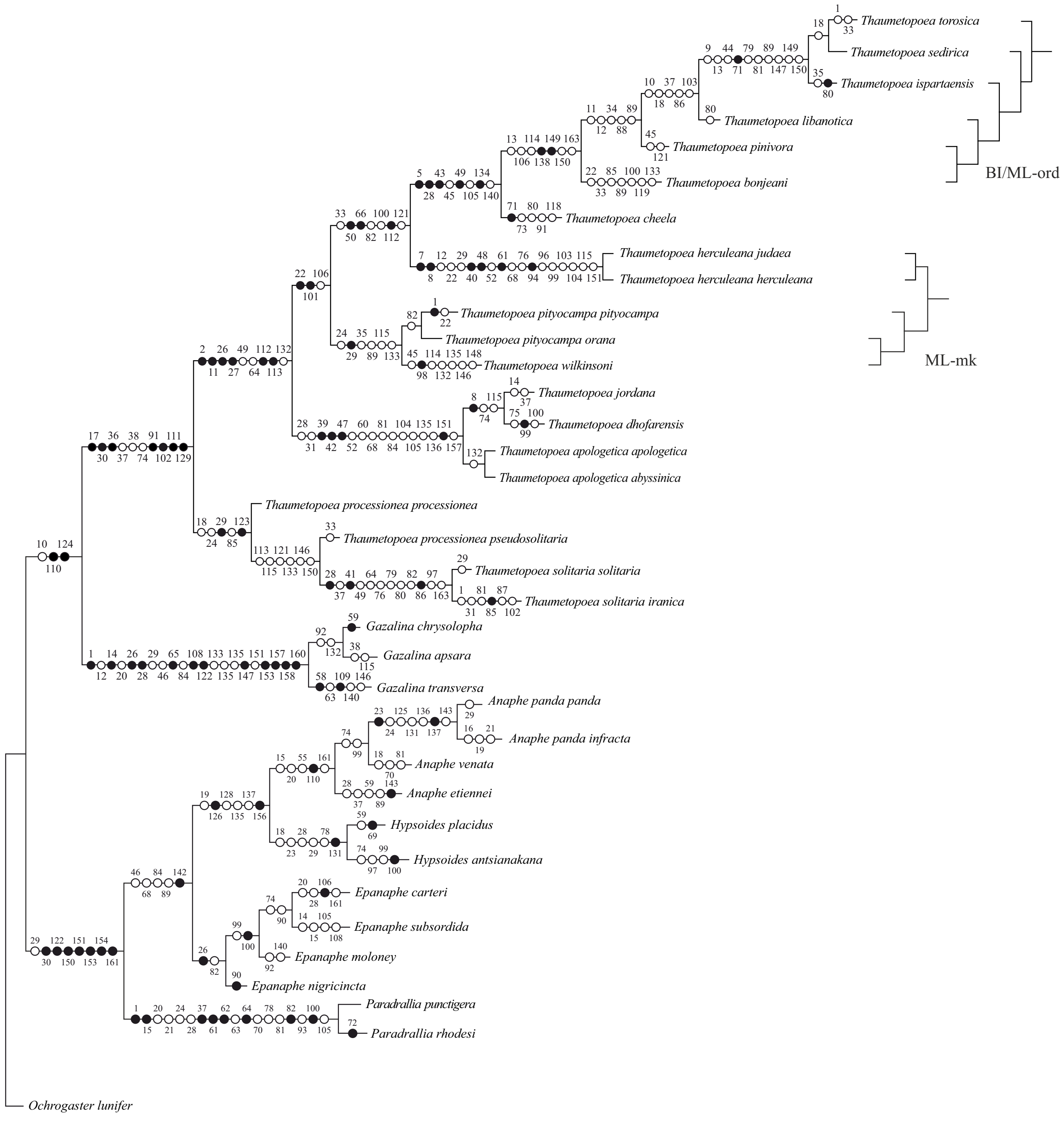

Figure S4. The most parsimonious tree (Length= 534 , CI= 0.537, RI= 0.797) obtained from Thau.morph dataset. Optimization: only unambiguous changes are mapped. Black circles, synapomorphic characters; white circles, homoplasious changes. Partial topology shows the difference with ML and BI trees. MP-Bremer, Bremer support to the node; MP-bt, bootstrap support to the node; ML-ord-UFB, ultrafast bootstrap support to the node, with ordered model; ML-mk-UFB, ultrafast bootstrap support to the node, with unordered model; BI-pp, posterior probability support to the node.

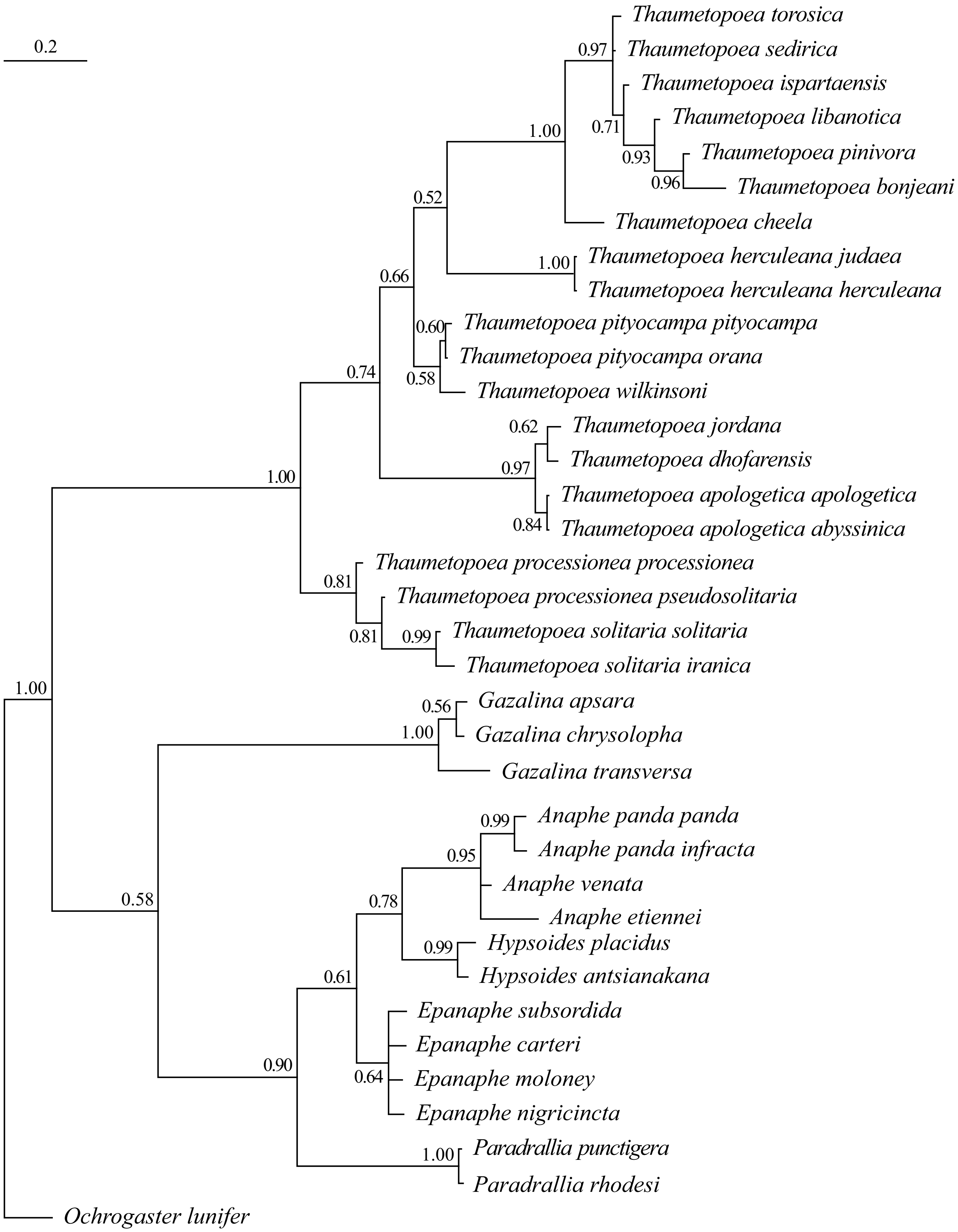

Figure S5. Bayesian tree inferred from Thau.morph dataset. Values at the base of each clade correspond to posterior probabilities.

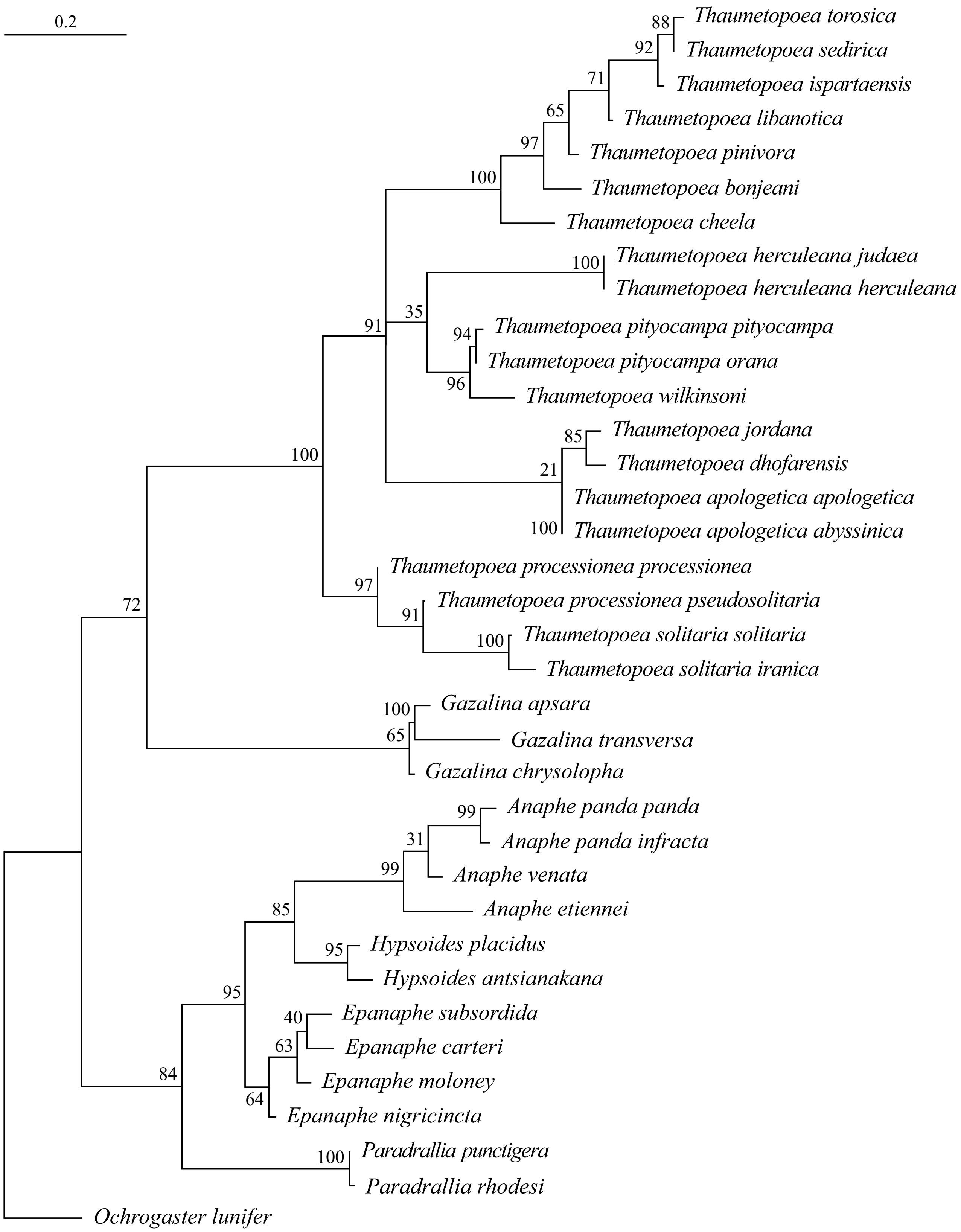

Figure S6. Maximum Likelihood tree (-ln=2971.8323) inferred from Thau.morph dataset performed using MK+FQ+ASC+G4 evolutionary model. Values at the base of each clade correspond to bootstrap support.

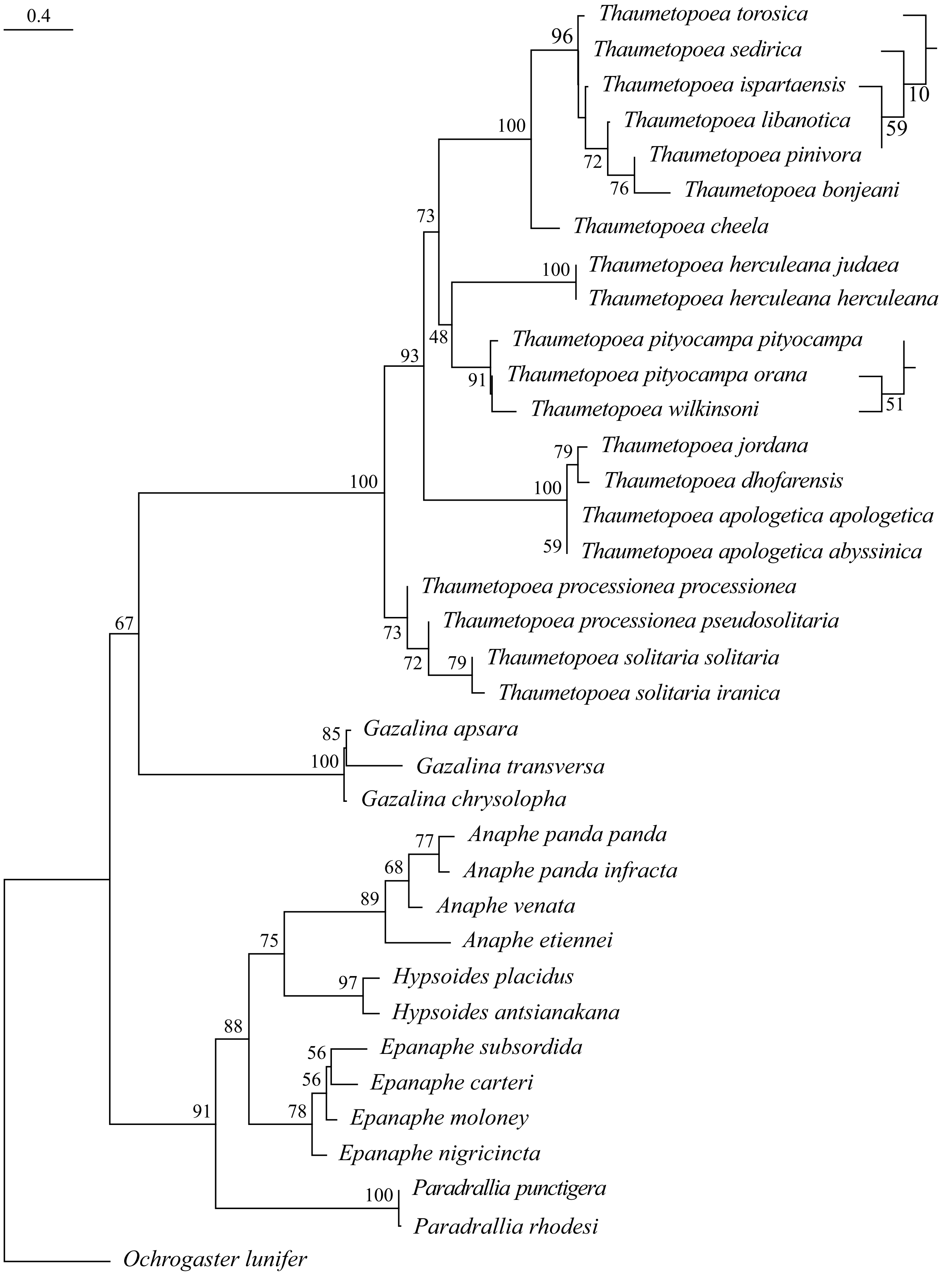

Figure S7. Maximum Likelihood tree (-ln=2536.9751) inferred from Thau.morph dataset performed using ORDERED+FQ+ASC+G4 evolutionary model. Values at the base of each clade correspond to bootstrap support. Partial topologies on right of the main tree to visualize better the relationships in those cases.

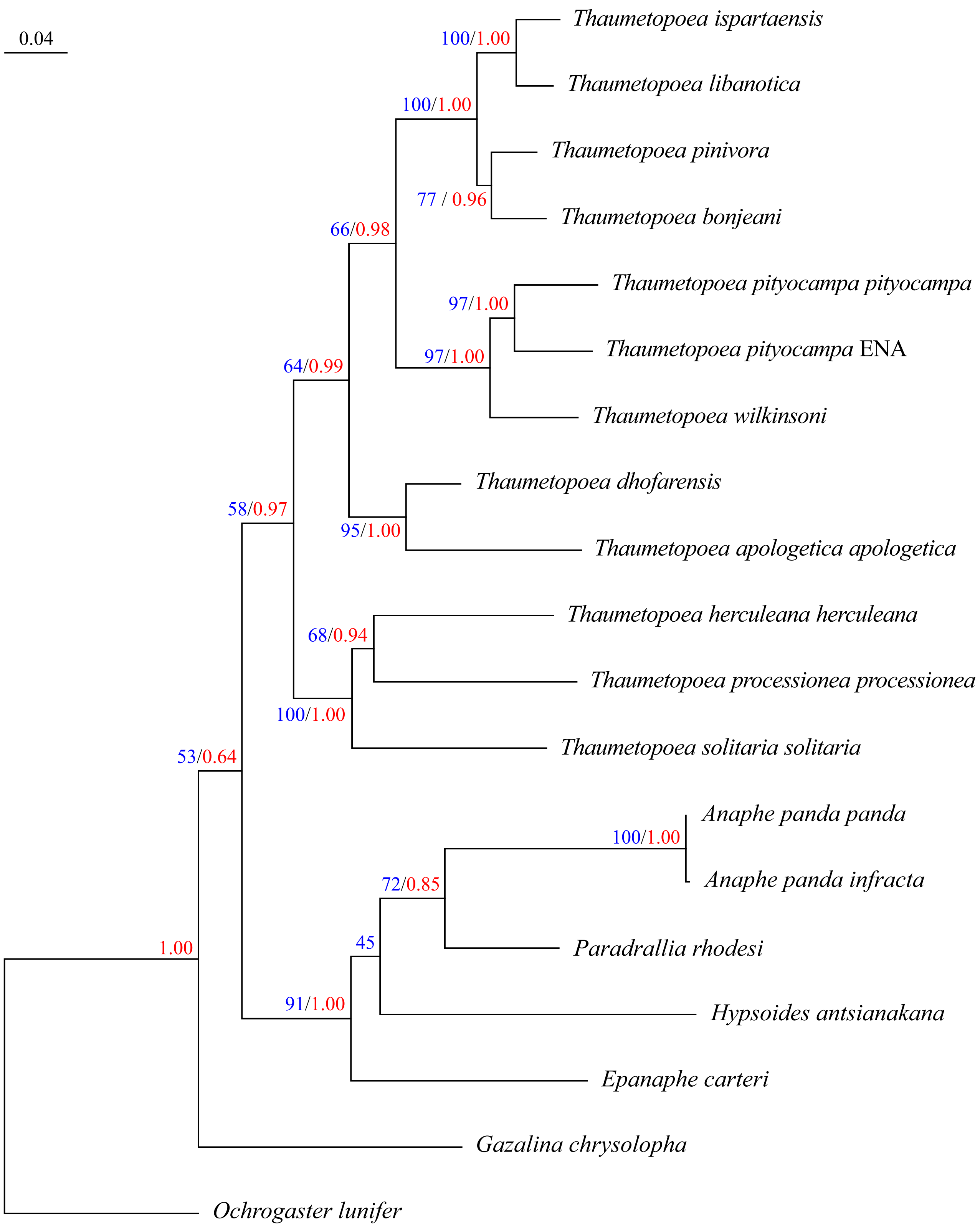

Figure S8. Maximum Likelihood tree (-ln=27774.0440) inferred from Thau.DNA dataset performed using GTR+I+G4 evolutionary model. Values at the base of each clade correspond to bootstrap support (in blue) and posterior probabilities (in red).

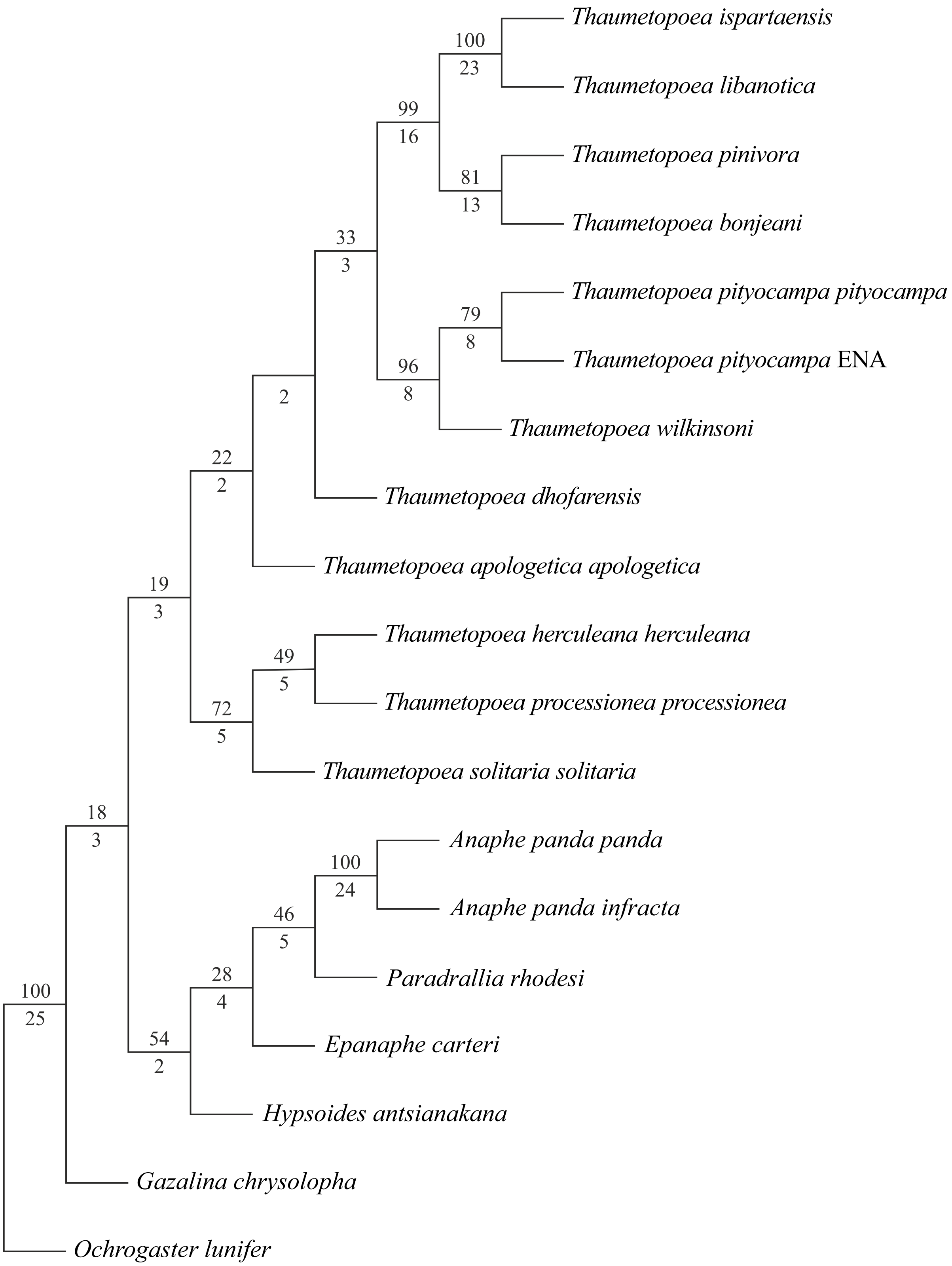

Figure S9. Most parsimonious trees inferred from Thau.DNA dataset (TBR) (Length= 4484, CI= 0.623, RI= 0.494). Values at the base of each clade correspond to bootstrap support (above) and Bremer support (below).

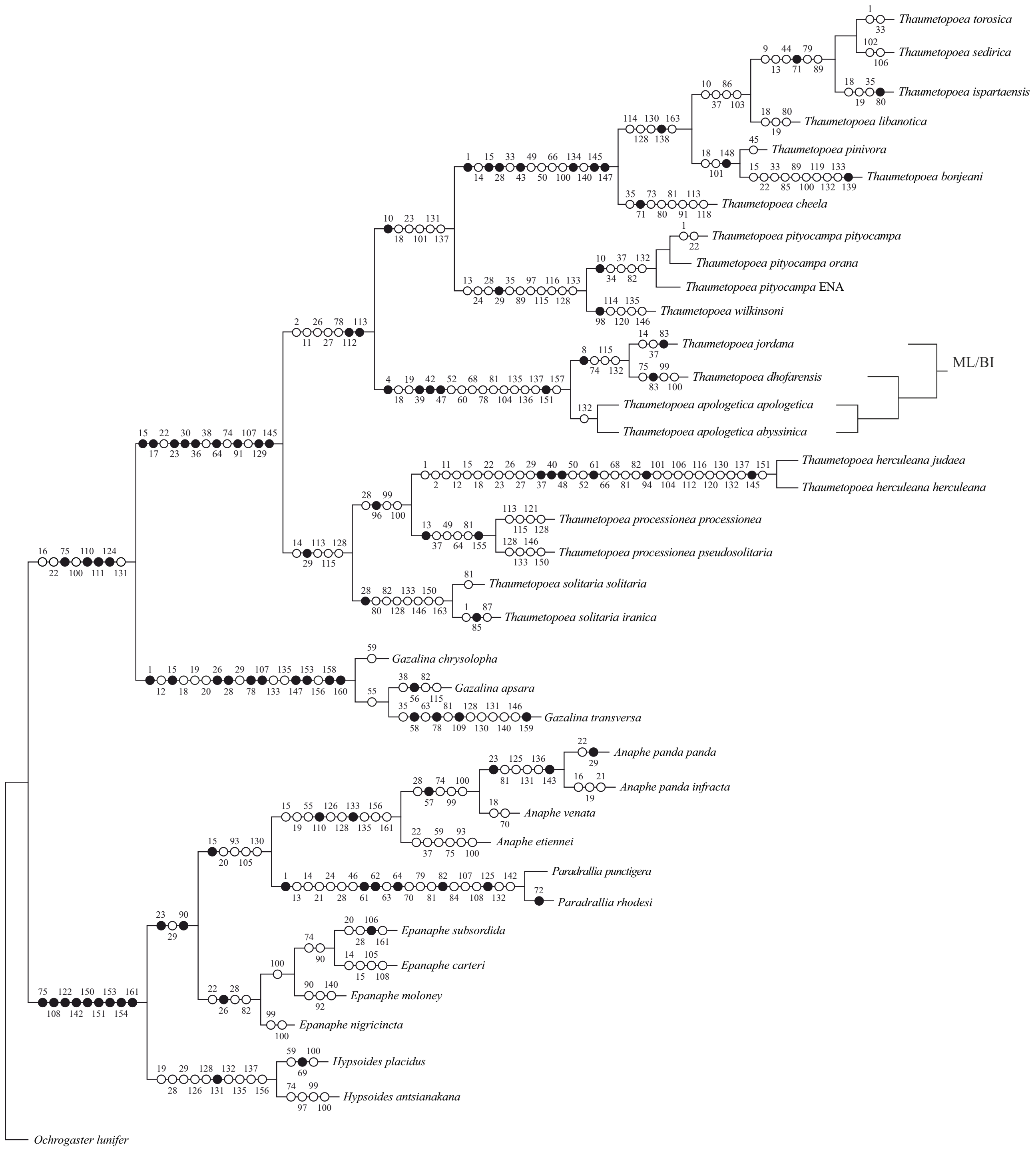

Figure S10. The most parsimonious tree (Length= 5046 , CI= 0.611, RI= 0.569) obtained from Thau.tot.comp dataset. Optimization: only unambiguous changes are mapped. Black circles, synapomorphic characters; white circles, homoplasious changes. Partial topology shows the difference with ML and BI trees. MP-Bremer, Bremer support to the node; MP-bt, bootstrap support to the node; ML-ord-UFB, ultrafast bootstrap support to the node, obtained from ordered model; ML-mk-UFB, ultrafast bootstrap support to the node, obtained from unordered model; BI-pp, posterior probability support to the node.

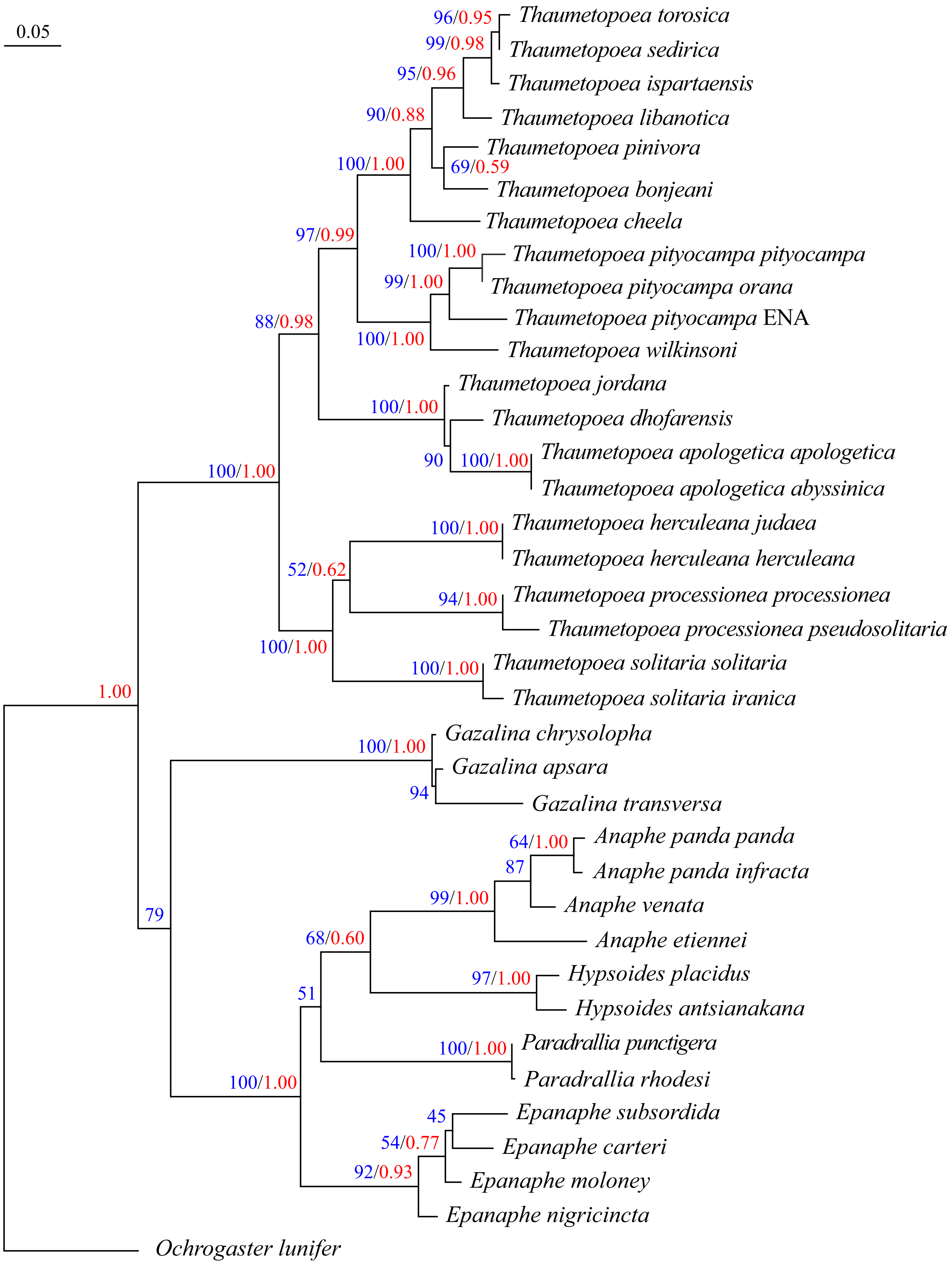

Figure S11. Maximum Likelihood tree (-ln=30433.391857) inferred from Thau.tot.comp dataset, performed using GTR+I+G4 evolutionary model for molecular partition and ORDERED+FQ+ASC+G4 evolutionary model for morphological partition. Values at the base of each clade correspond to bootstrap support (in blue) and posterior probabilities (in red).

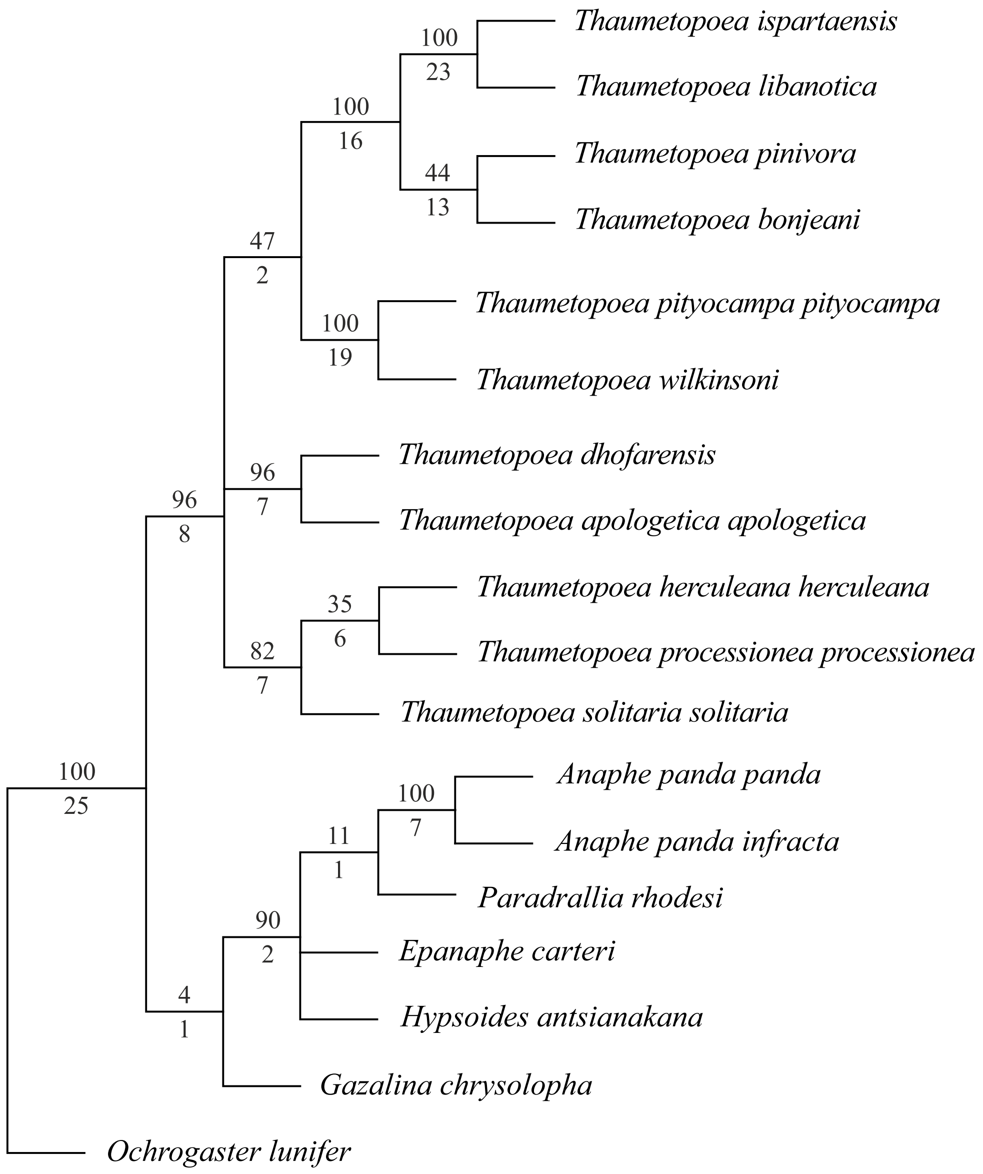

Figure S12. Strict consensus of three most parsimonious trees inferred from Thau.tot.red dataset (TBR) (Length= 4658, CI= 0.641 , RI= 0.511). Values at the base of each clade correspond to bootstrap support (above) and Bremer support (below).

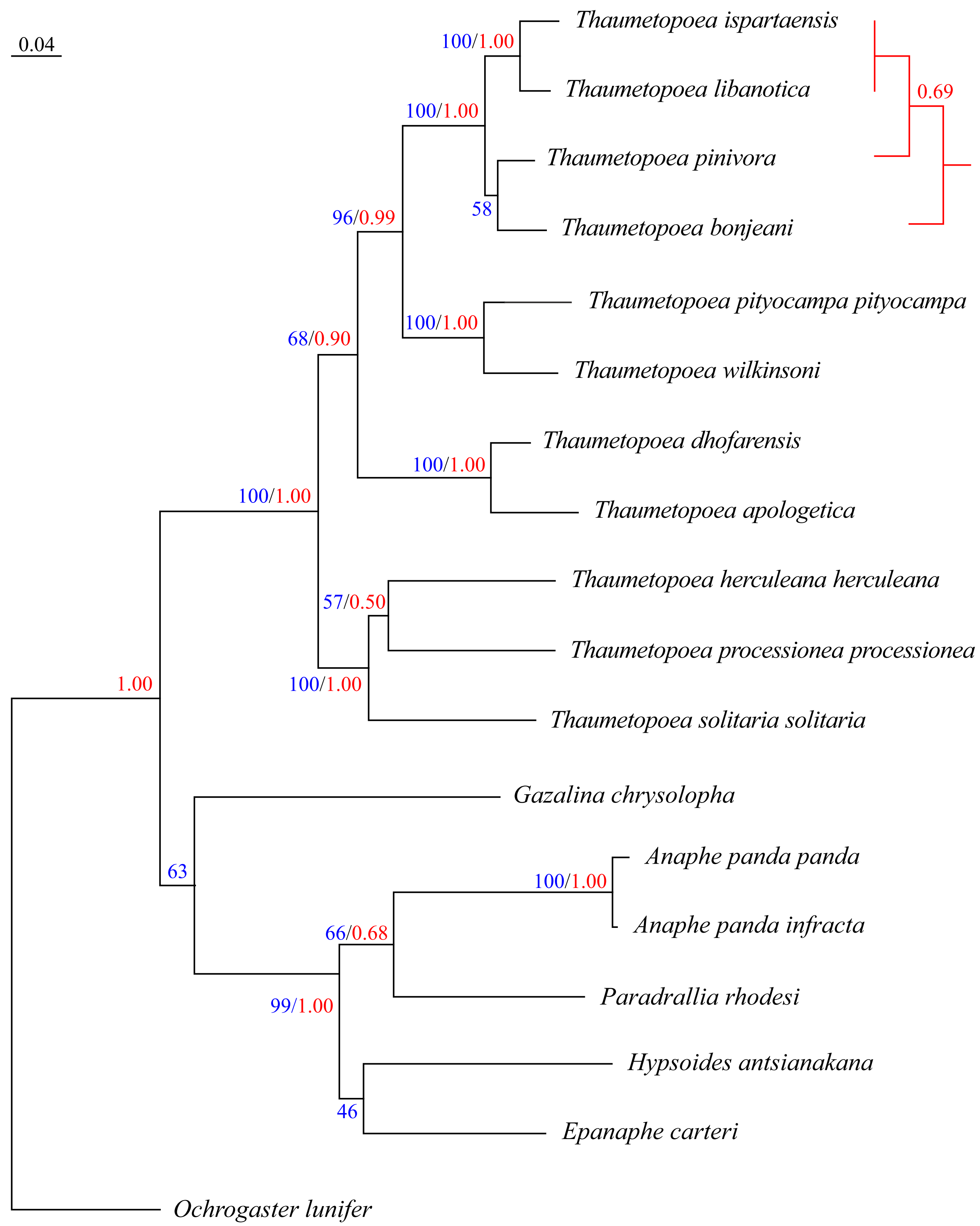

Figure S13. Maximum Likelihood tree (-ln=29835.0572) inferred from Thau.tot.red dataset, performed using GTR+I+G4 evolutionary model with molecular partition and ORDERED+FQ+G4 evolutionary model with morphological partition. Values at the base of each clade correspond to bootstrap support (in blue) and posterior probabilities (in red). Partial topology in red-colour shows the difference with BI tree.

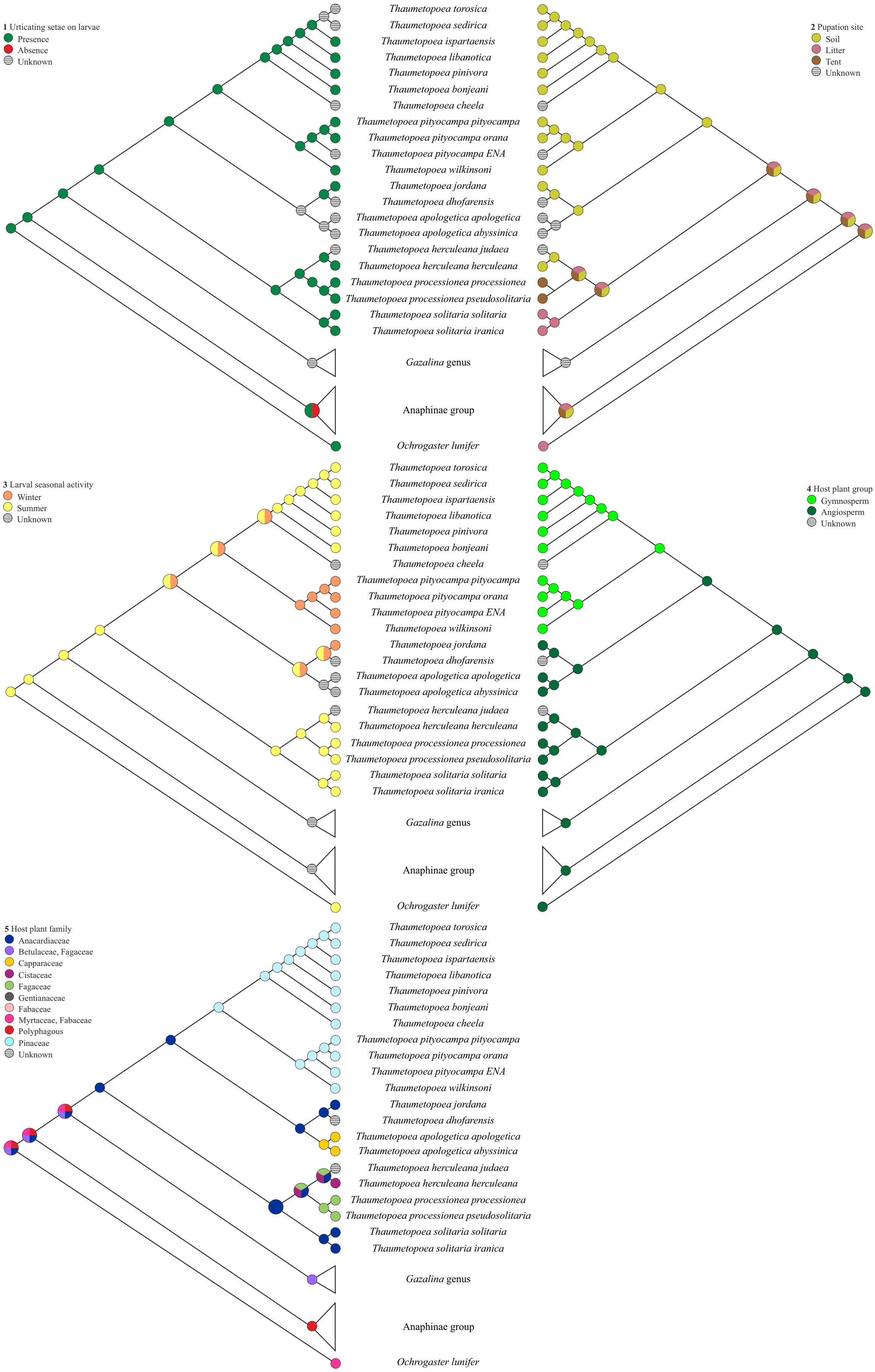

Figure S14. Mapping of biological and ecological traits for each species, reconstructed on reference tree applying maximum parsimony algorithm implemented in Mesquite software. Pie charts are enlarged on ambiguous ancestral nodes to increase clarity.

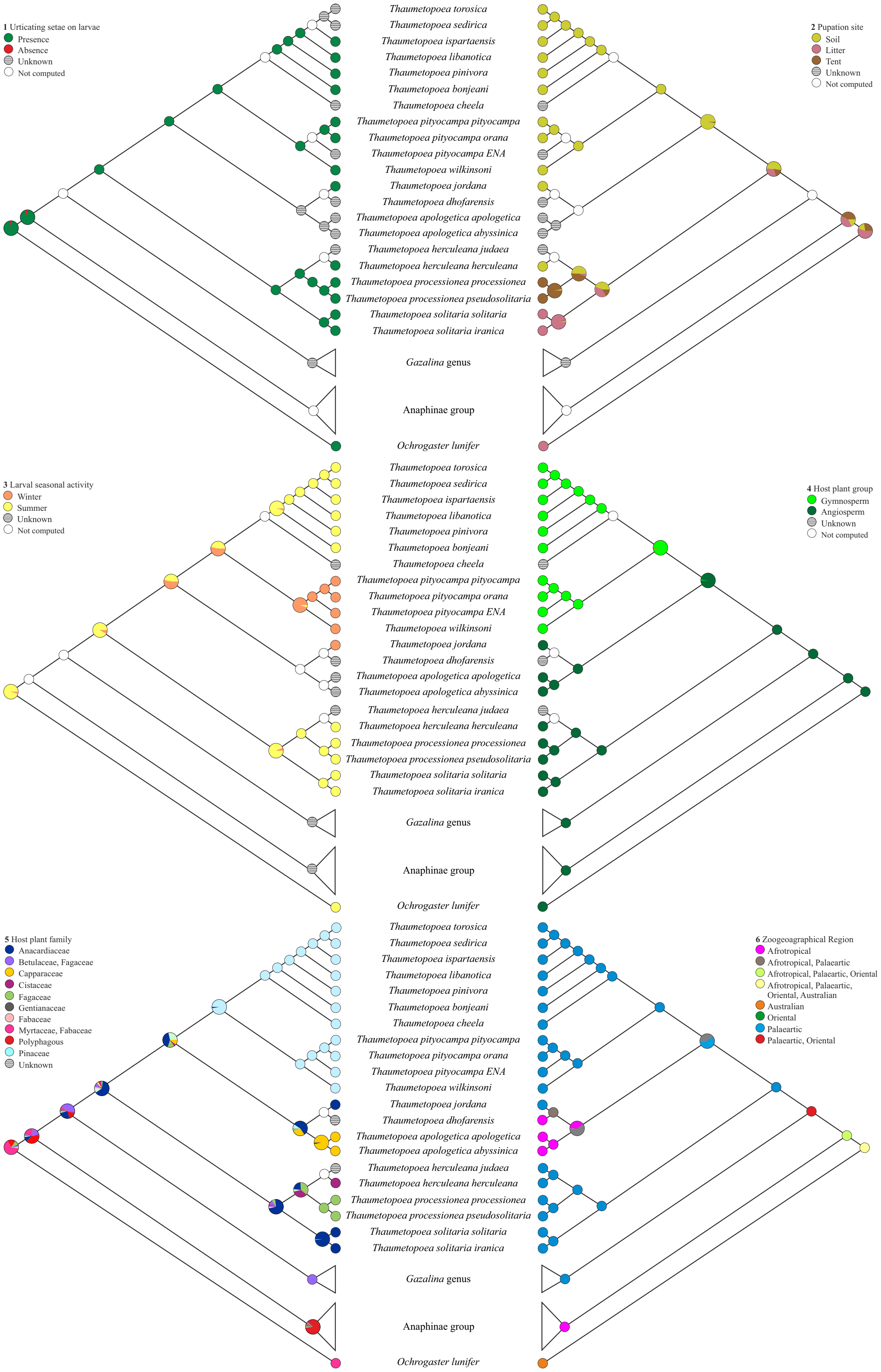

Figure S15. Mapping of biological and ecological traits for each species, reconstructed on reference tree applying maximum likelyhood algorithm implemented in Mesquite software. Pie charts sections represent the proportional likelihood of each character state at the node and are enlarged on ambiguous ancestral nodes to increase clarity. Trait number 6 was mapped with S-DIVA software

Appendix S1

Traits description

Traits and traits-states used in the phylogenetic analysis.

Colours encoded using PANTONE scale (Pantone® Inc., Carlstadt, NJ, USA) as listed below. *Ash grey:* 7536 U, 2327 U, Cool Grey 2 U, Cool Grey 3 C, 414 U/C, 413 U/C, 7535 C, 416 C, 2325 C. *Black:* Neutral Black U, Neutral Black C. *Brownish grey:* 4625 U, 2322 U, 7568 C, 2324 C. *Brown:* 2320 U, 2322 C, 7581 C, 463 C, 469 C, Grey: 422 U/C, 417 U, 2332 C, 5665 U, 425 U, 420 C. *Light brown:* 140 U, 139 U, 873 C, 730 C. *Ochraceous:* 7550 U, 7551 U, 7552 U, 465 C. *Reddish:* 174 U, 175 U, 483 C, 7581 C, 7594 C. *Straw:* 1205 U/C, 1215 U/C, 7402 C, 938 C. *Whitish:* Cool Grey 1 U, 7527 U, Cool Grey 1 C, 7527 C. *Yellowish:* 7406 U, 110 U, 117 U, 124 U, 130 C, 2007 C, 1245 C, 7564 C. *Dark:* Black + Brown.

Head

1. Head hair-like scales colour: brown (0), light brown (1), brownish grey (2), black (3), whitish (4), yellowish (5). Head is covered by hair-like setulae with different interspecific colouration.
2. Frons: crested (0), smooth (1). Sclerotized crest known as frontal protuberance in Lepidoptera (e.g, Hogue, 1963).
3. Crest - number of teeth: six (0), seven (1). Crested frons differs between species for the number of teeth.
4. Apical tooth, position: fore (0) when it grows after the front half of the eye, rear (1) when grows before (Fig.1 e; f).
5. Apical tooth, height: high (0) i.e. when it is well developed and separated from the second tooth; low (1) i.e. when it is small but separated; and flattened (2) i.e. when it is levelled and it is almost fused with the second (Fig.1 c; d; l).
6. Apical tooth, width: large (0), medium (1), narrow (2). Base of apical tooth is more or less wide: more than base of second tooth, as the base, less than half of the base.
7. Length of first and second tooth: equal or subequal (0) disequal (1).
8. Major tooth/teeth: 1st and 2nd pronounced (0), 2nd pronounced (1), 1st pronounced (2). Biggest tooth or teeth pronounced on top of the head, gives to crest a sub-triangular shape.
9. Base of 2nd tooth: wide (0), narrow (1). It expands or narrows basally with edges that diverge or converge, and looks like crest more or less robust.
10. Projection of 2nd tooth: short (0), medium (1), long (2). Second tooth length change in relation with the third tooth, detaching more or less sharply from the third tooth (Fig.1 d; f; h). Ratio between the length of second and third tooth is 2, or between 3 to 4, or more than 4.
11. Antenna length: long (0), short (1). Antenna length change from being long as half of costa to shorter than half of costa (Agenjo, 1941) (Fig.2 c; g).
12. Antennal rami (Fig.1 b) length: long (0) with ratio between 'antenna length' and 'bipectination length' is 3-6, medium (1) with ratio 7-8, short (2) with ratio up to 9. Bipectination length changes as in *T. herculeana* where they are long, or as in *T. pityocampa* where they are

medium elongated, or as in *T. pinivora* where they are short. (Fig.2 a; b. Fig.3 e).

13. Antenna scales colour: ochraceous (0), dark (1), whitish (2), dark and whitish (3).
14. Scape's tuft (Pecten) (Fig.1 b): yellowish (0), ochraceous (1), brownish grey (2), whitish (3), dark (4). Small tuft of hair-like scales is always present at the base of antennae.
15. Adfrontal tuft between eyes and frons (Fig.1 b): ochraceous (0), brownish grey (1), dark (2), whitish (3), yellowish (4), brown (5), black and whitish (6). Tuft of hair-like scales located between eyes and frons.
16. Labial palpus length: short (0), long (1). Total length of palpus is lower or higher than 0.5 mm (Agenjo, 1941).
17. Labial palpal segments: segments are not well articulated (0) when the edge of each segments is indistinct, or articulated (1), with strongly marked edge.
18. Labial palpus: length of first segment: longer than second (0), as long as second (1), shorter than second (2) reaching around ¾ of the second segment, half of the second (3), when its length is half of the second segment (Agenjo, 1941; Miller, 1991).
19. Labial palpus: width of the first segment: wider than the second (0), narrower than the second (1), as wide as the second (2) (Agenjo, 1941).
20. Labial palpus: apical protuberance of the second segment: absent (0), present (1).
21. Labial palpus: apical protuberance of the second segment features: not well delimited (0), well delimited (1).

Thorax

22. Thorax colour hairs: light brown (0), dark (1), ash grey (2), brownish grey (3), whitish (4), yellowish (5), reddish (6).
23. Thorax tuft: 2 white lateral spots in the middle of forewing (0), 2 whitish lateral spots divided by a black line in the middle of root of forewing (1), 2 long whitish lateral spot in the middle of root of forewing (2), 2 long lateral yellow spots (3), long orange spot in centre of thorax (4), 2 long lateral whitish spots that start at the beginning of thorax (5), 2 short lateral white spots that start at the beginning of thorax (6) (Fig.2c; e. Fig.3 e. Fig.S3 f; g; h).
24. Fore-femur (Fig.1 o) robustness: from stout (0) with foretibia wider than 0.85 mm, to medium (1) between 0.7 mm and 0.85 mm, to thin (2) with fore-femur wide less than 0.7 mm (Agenjo, 1941).
25. Epiphysis (Fig.1 o): absent (0), present (1). Epiphysis present in several species of Notodontidae, used to clean the antennae.
26. Epiphysis shape: claw-shape (0), finger-shape (1), acuminate leaf-shape (2), rounded leaf-shape (3).
27. Foretibia spine (Fig.1 o): absent (0), present (1). Spine probably used to emerge from the soil (Agenjo, 1941). This spine is indicate as foretibia 'claws' in other groups of Lepidoptera (e.g, Hogue, 1963).

Abdomen

28. Abdomen colour: ochraceous (0), reddish (1), brown (2), grey (3), black and whitish (4),

yellowish (5).

29. Apical tuft colour: ochraceous (0), brownish grey (1), dark (2), ash grey (3), brown (4), straw (5).
30. Female - Anal tuft composition: with only flat scales (0), only hair-like scales (1), both scales and hair-like scales (2). Character linked to different strategies used to protect egg batches in Thaumetopoeinae.
31. Female - Anal flat scale general pigmentation: dark (0), whitish (1), translucent (2). Due to extreme variation among scales, I analysed scales collected from the medial portion of the anal tuft.
32. Female - Anal flat scale spots: absence (0), presence (1) of pigmentation in scales.
33. Female - Anal flat scale spots spread: localized (0), spread (1). Distribution of scale pigmentation.
34. Female - Anal flat scale width on central portion: from narrow (0) smaller than 0.6 mm, to wide (1) upper than 1.2 mm, and medium (2) between 0.7 mm and 1.1 mm (Agenjo, 1941).
35. Female - Anal flat scale Ratio length/width of terminal portion: < 2 (0), 2 - 3 (1), 3 - 4 (2), > 4 (3) (Simonato *et al.*, 2013).
36. Female - Urticating setae on adult: no (0), yes (1). Hair-like setulae or urticating setae are used to protect eggs batches in Thaumetopoeinae subfamily.

Male and female forewings

37. Forewing scales general colouration: light brown (0), whitish (1), ash grey (2), brown (3), yellowish (4), reddish (5) (Agenjo, 1941; Kiriakoff, 1970).
38. 1.basal line or patch: absent (0), present (1) (Agenjo, 1941).
39. 1.basal line type: line (0), fused on costal patch (1) (Fig.2 f; h).
40. 1.basal line colour: light brown (0), dark (1).
41. 1.basal line shape (Fig.1 a): zigzagged (0), curved (1), straight (2).
42. 1.basal line width: narrow (0), wide (1) up to merge with ante-median line.
43. 1.basal line edges: absent (0), present (1) on both side as yellow edges in ‘summer’ *Thaumetopoea* (Agenjo, 1941; Basso *et al.*, 2016) or as brown edges in *T. herculeana*.
44. 1.basal line from costa to inner margin: incomplete (0), complete (1). Line reaches both edges of forewing or does not (Fig.2 b. Fig.3 d;).
45. 1.basal line continuous: yes (0), no (1). Line interrupted in the middle or not, one or more times (Fig.2 c. Fig.3 c;).
46. 3.ante-median line or patch: absent (0), present (1) (Agenjo, 1941).
47. 3.ante-basal line type: line (0), costal patch (1) (Fig.2 f.)
48. 3.ante-median line colour: light brown (0), brown (1), vague (2), dark yellow (3).
49. 3.ante-median line shape (Fig.1 a): zigzagged (0), straight (1), curved (2), dotted (3) (Fig.2 b; g. Fig.3 f).
50. 3.ante-median line edge: absent (0), present (1) (Agenjo, 1941).
51. 3.ante-median line edge colour: both edges dark (0) in *T. herculeana*, yellow external edge

(1) in ‘summer’ *Thaumetopoea* (Agenjo, 1941; Basso *et al.*, 2016).

52. 3.ante-median line width: narrow (0) wide (1) up to merge with post-median line.
53. 3.ante-median line from costa to inner margin: complete (0), incomplete (1). Line reaches both margins of forewing or does not.
54. 3.ante-median line continuous: yes (0), no (1). Line interrupted in the middle or not, one or more times.
55. 4.median shade: absent (0), present (1).
56. 4.median shade tooth: outwardly on Cu (0), without tooth on Cu (1) (Fig.S3 d; f).
57. 4.median shade width: narrow (0), wide (1), medium (2).
58. 4.median shade from costa to inner margin: complete (0), incomplete (1). Line reaches both edges of forewing or does not.
59. 5.post-median line or patch: absent (0), present (1) (Agenjo, 1941).
60. 5.post-median line type: line (0), patch (1).
61. 5.post-median line colour: light brown (0), dark (1), yellowish (2).
62. 5.post-median line continuous: yes (0), no (1). Line interrupted in the middle or not, one or more times.
63. 5.post-median line from costa to inner margin: complete (0), incomplete (1). Line reaches both edges of forewing or does not.
64. 5.post-median line shape (Fig.1 a): jagged (0), straight (1), dotted (2) (Fig.S3 c; d).
65. 5.tooth on node (Cu1-M3) in post-median line: without outwardly tooth (0), with outwardly tooth (1) (Fig.S3 f).
66. 5.post-median line edge: absent (0), present (1) (Agenjo, 1941).
67. 5.post-median line edge colour: dark internal edge (0), yellow internal edge (1) (Fig.2 a. Fig.3 b).
68. 5.post-median line width: wide (0), narrow (1), medium (2).
69. 6.sub-terminal line: absent (0), present as terminal patch (1).
70. 7.terminal line (Fig.1 a): absent (0) present (1).
71. 7.terminal line colour: dark (0), brownish-grey (1), whitish (2).
72. 7.terminal line from costa to inner margin: complete (0), tornal patch (1). Line is present throughout forewing.
73. 7.terminal line continuous: yes (0), no (1). Line interrupted in the middle or not, one or more times.
74. Fringe: concolorous (0), not concolorous (1) (Fig.2 b; d).
75. Fringe - colour: brown and white (0) where present white patch at the ends of veins, whitish (1), brown (2), yellowish (3), black (4) (Agenjo, 1941).
76. Fringe - series: uniseriate (0), biseriate (1) (Fig.S3 b; f).
77. Fringe distal part silhouette: jagged (0), flat (1). Fringe taken from the apex, between M1-M3 veins. They do have well defined teeth, or do not, to result almost flattened.
78. Fringe - number of teeth in distal part: various (3-8) (0), two (1), three (2), four (3), five (4),

seven (5), nine (6).

79. Costal ends of ante and post-median lines: parallel (0), divergent (1), convergent (2) (Agenjo, 1941; De Freina and Witt, 1987) (Fig.3 c. Fig.S3 a; c;).
80. Inner margin ends of ante and post-median lines: parallel (0), convergent (1), divergent (2), (Agenjo, 1941; De Freina and Witt, 1987) (Fig.2 c. Fig.S3 a; c;).
81. Ante-median line direction junction on inner margin (direction): outwardly (0), inwardly (1), straight (2), does not reach margin (3) (Agenjo, 1941; De Freina and Witt, 1987) (Fig.2 a; h. Fig.S3 f).
82. Post-median line junction on inner margin (direction): outwardly (0), inwardly (1), straight (2) does not reach margin (3) (Agenjo, 1941; De Freina and Witt, 1987) (Fig.2 a; h. Fig.S3 f).
83. End on inner margin of post-median line/patch: reach thorax (0); ends under the middle of basal patch (1), ends under just before basal patch (2).
84. Discal spot: absent (0), present (1) (Agenjo, 1941). A dark spot which can be present on M1-M3 junction (Fig.S3 g; h).
85. Discal spot mark: strong (0), medium (1), faint (2) (Agenjo, 1941) .
86. Discal spot shape: half-moon (0), irregular (1), dot (2) (Fig.2 c. Fig.3 a; d).
87. Discal spot colour: brown (0), whitish (1), black (2).
88. Discal spot ring: absent (0), present (1) (Fig.3 a; e).
89. Forewing marginal lines: absent (0), present (1). Outlines of forewing.
90. Forewing dark marginal lines: completely around wing (0), on costa and inner margin (1), only on costa (2).
91. Female - Elements of forewing pattern: blurred, indistinct (0), sharp, as defined as in males (1).
92. Marked veins: no (0), yes (1). Veins highlighted by dark traits.
93. Male and female hindwing
94. Hindwing scales general colouration: whitish (0), yellowish (1), reddish (2).
95. Ante-median line: absent (0), present (1) vague in *T. herculeana* (Agenjo, 1941).
96. Median line: absent (0), present (1) (Agenjo, 1941).
97. Post-median line: absent (0), present (1) (Agenjo, 1941).
98. Terminal line: absent (0), present (1) (Agenjo, 1941).
99. Terminal line mark: marked brownish (0), vague brownish (1).
100. Fringe: concolorous (0) not concolorous (1).
101. Fringe colour: whitish (0), brownish and white (1), light brown (2), yellowish (3), reddish 4), black (5), black up-white down (6) (Agenjo, 1941).
102. Discal spot: absent (0), present albeit faint (1).
103. Anal spot: absent (0), present (1) (Agenjo, 1941) (Fig.2 g. Fig.3 f).
104. Anal spot - colour intensity: marked, definite (0), vague, faint (1).
105. Anal spot colour: dark (0), light brown (1).
106. Inner margin: whitish (0), brownish grey (1), yellowish (2) (Fig.2 f; g. Fig.S3 g).
107. Underside: as upperside, with faint discal spot (0), as upperside, without pattern (1), as

upperside, with costal dark mark (2).

Male Genitalia

108. Uncus: developed (0), well developed (1), tiny (2) (Kiriakoff, 1970).
109. Uncus shape: claw-like (0) as in *Thaumetopoea s. lat.* it is short and arched with sharp apex, triangular (1) as in *Gazalina* group it is extremely sharp, rounded (2) as in *Anaphe*, *Epanaphe or Hypsoides,* it is short, arched but with large rounded lobes at apex, narrow (3) as in *Paradrallia* group that is arched with narrow lobes (Kiriakoff, 1970).
110. Uncus apical teeth count: single (0), couple (1). Apical teeth/tooth on the apex of uncus, they are more or less sclerotized and sometimes very small.
111. Uncus apical teeth closeness: divided (0), close (1), fused (3). Apical teeth are big but well separated, small or fused.
112. Socii: absent (0), present (1). Socii that arise from centre of ventral side of uncus. In this analysis, typically of *Thaumetopoea s. lat.* (Agenjo, 1941; Kiriakoff, 1970).
113. Socii shape: half-moon (0) wide and rounded, triangular (1) wide and straight, trapezoidal (2) very small and sharp present in *Gazalina* sp. (Kiriakoff, 1970).
114. Socii lateral margin: convex (0), straight (1), concave (2).
115. Socii lateral margin: smooth (0), wrinkled (1).
116. Socii apex shape: rounded (0), narrow (1), sharp (2).
117. Socii apex ribbing: absent (0), present (1). A thickening on the apex of socii.
118. Socii apex ribbing direction: central (0), outer (1). A ribbing inclined towards genitalia or outwards.
119. Socii posterior tooth: absent (0), present (1). Variously sclerotized process that extends in antero-ventral direction.
120. Socii posterior tooth mark: strong, defined (0), vague, faint (1). To identify different degrees of sclerotization.
121. Socii posterior tooth shape: rounded (0) spoon-shaped, sharp (1) and tapered.
122. Socii posterior tooth direction: straight (0), inward (1).
123. Tegumen: developed (0), tiny (1), well developed (2). Different degrees of robustness (Agenjo, 1941; Kiriakoff, 1970). Three different degrees were recognized based on sclerotization, after clarification and during slides mounting.
124. Tegumen central tooth: absent (0), present (1) (Agenjo, 1941). A protuberance on tegumen present in *Thaumetopoea s. srt*. It is well developed in *T. solitaria solitaria*, *T. solitaria iranica* and *T. processionea pseudosolitaria*. It is absent in *Traumatocampa* and *Helianthocampa*. In *T. processionea processionea* this protuberance is extremely reduced and not always visible.
125. Gnathos: absent (0), present (1). Gnathos that arise under uncus, In this analysis, typically of Anaphine group (Kiriakoff, 1970; Heppner, 2008).
126. Gnathos shape: sub-triangular (0), hook-shaped (1), filiform (2).
127. Scaphium: absent (0), present (1). Dorsal sclerotization of tuba analis. Typically in *Anaphe* and *Hypsoides* genera (Kiriakoff, 1970).

128. Subscaphium: absent (0), present (1). Sclerotic membrane between aedeagus and tuba analis. It articulates with the ninth segment via terminal diaphragm. It is typical of *Epanaphe* genus (Kiriakoff, 1970; Heppner, 2008).
129. Valva shape: claw-like (0), reverse claw-like (1), spear-like (2), U-shape (3), S-shape (4), quarter circle (5) (Agenjo, 1941; Kiriakoff, 1970). There are six different shapes of valva based on dimension, apex position, and shape. It was divided in: claw-like if cucullus points downwards, reverse claw-like if it points upwards, spear-like if it is stubby with narrow cucullus, U-shaped if it is stubby with rounded cucullus, S-shaped if it has sinusoidal edges, and quarter circle (5) when valva is wide and with lower edge extremely rounded.
130. Valva baso-dorsal corners process: not sclerotized (0), sclerotized (1) (Kiriakoff, 1970). Due to connection between valva and transtilla that could be sclerotized or not.
131. Valva ventral margin silhouette - basal portion: straight (0), concave (1), convex (2) (Agenjo, 1941). Curvature of proximal half of valva in lower margin.
132. Valva ventral margin silhouette - distal portion: convex (0), extremely convex (1), concave (2), straight (3) (Agenjo, 1941). Curvature of distal half of valva in lower margin.
133. Valva dorsal margin silhouette - basal portion: convex (0), straight (1), concave (2) (Agenjo, 1941). Curvature of proximal half of valva in upper margin.
134. Valva dorsal margin silhouette - distal portion: straight (0), concave (1), convex (2) (Agenjo, 1941). Curvature of distal half of valva in upper margin.
135. Valva dorsal margin junction: not sclerotized (0), sclerotized (1) (Agenjo, 1941).
136. Valva upper ribbing: absent (0), present (1) (Agenjo, 1941). A folding of the upper edge of the valva, typical of *Thaumetopoea s. lat.*, it can show a thickening as in *T. wilkinsoni*.
137. Valva upper ribbing length: short (0) observed only in proximal part of valva, long (1) observed long over the length of valva (Agenjo, 1941).
138. Cucullus shape: rounded (0), narrow (1), sharp (2), broadly rounded (3).
139. Bulge: absent (0), present (1) (Agenjo, 1941). Apical bulge of the valva that it is pressed to produce a visible folding when genitalia are mounted onto slides (Basso *et al.*, 2016).
140. Bulge length: 1/2 of valva (0), 1/4 of valva (1). Measure of bulge size present on cucullus of valva, based on fold observed on slides.
141. Cucullus process: absent (0), present (1).
142. Cucullus process side: upperside (0), underside (1).
143. Saccular process: absent (0), present (1) (Kiriakoff, 1970; Heppner, 2008).
144. Saccular process shape: straight (0), claw-like (1), hook-like (2).
145. Anellus: not sclerotized (0), sclerotized (1) (Kiriakoff, 1970; Heppner, 2008). Variously sclerotized plates that surround the aedeagus; extremely visible in Anaphinae (Kiriakoff, 1970).
146. Juxta shape: pentagonal (0), hexagonal (1), heptagonal (2), triangular (3), diamond (4), bone (5). Main shapes of sclerotized plate ventrally to the aedeagus (Agenjo, 1941).
147. Juxta length/width: as wide as long (0), longer than wide (1) (Agenjo, 1941).
148. Juxta superior margin silhouette: concave (0), convex (1), straight (2) (Agenjo, 1941).

Curvature of upper margin of juxta, that supports aedeagus.

149. Juxta lateral-up margin silhouette: convex (0), straight (1), concave (2) (Agenjo, 1941) Curvature of upper lateral margin of juxta.
150. Juxta lateral-low margin silhouette: convex (0), straight (1), concave (2) (Agenjo, 1941). Curvature of lower lateral margin of juxta.
151. Juxta inferior margin silhouette: convex (0), straight (1), extremely convex (2) (Agenjo, 1941). Curvature of lower margin of juxta.
152. Juxta lateral angle: broadly rounded (0), rounded (1), narrow (2), flat (3). Width of the angle in the middle of lateral margin.
153. Juxta ribbing: absent (0), present (1).
154. Aedeagus length: short (0) when ratio between length and width is below 4, medium (1) with ratio between 4 and 7, and long (2) with ratio up to 7 (Agenjo, 1941; Kiriakoff, 1970).
155. Basal part of aedeagus: completely encircle (0) with lateral opening of ductus copulatorius, as in *Thaumetopoea* genus or not completely encircle (1) where the opening of ductus copulatorius is fully arrear; typical of Anaphine.
156. Process on basal part of aedeagus: absent (0), present (1). Basal process of aedeagus typically of *T. processionea processionea*.
157. Opening on base of aedeagus: flared vertically (0) where widening is on ventral side, flared horizontally (1), circular (2) in base without widening (Kiriakoff, 1970).
158. Aedeagus apex: circular (0), double spiked (1), flared (2) . The apex is simple and circular with an homogeneous sclerotization like in *Thaumetopoea*, or has a localized sclerotization in ventral and dorsal sides, with a membranous conjunctions (*Gazalina* sp.), or it is simple but thickened and slightly flared at the apex (*Anaphe* sp.).
159. Carina of aedeagus: absent (0), present (1) (Heppner, 2008).
160. Carina position: on a single spike (0), on terminal portion (1). Only on one side or all around the apex.
161. Vesica: simple (0) straight without particular lobes, bulbous (1) with several lobes.
162. Cornuti of vesica: absent (0), present (1).
163. Number of cornuti on vesica: one big (0), small several (1).
164. Basal curvature: absent (0), present (1) (Agenjo, 1941). Basal curvature of aedeagus present in some species (i.e. *T. solitaria*).
165. Level of basal curvature: strong (0), gently (1). When curvature is greater or lesser than 45° (Agenjo, 1941)
166. Medial curvature: absent (0), present (1). Medial curvature of aedeagus present in some species, it looks like a narrowing.

Ecological and biological traits

167. Urticating setae on larva: absent (0), present (1).
168. Pupation site: larvae pupate in the soil (0), in the tent (1), or in the litter (2).
169. Larval seasonal feeding: winter (0), summer (1).
170. Host plant group: gymnosperm (0), angiosperm (1).
171. Host plant family: Anacardiaceae (0), Betulaceae, Fagaceae (1), Capparaceae (2), Cistaceae (3), Fagaceae (4), Gentianaceae (5), Leguminosae (6), Myrtaceae, Fabaceae (7), polyphagous (8), Pinaceae (9).
172. Zoogeographical Distribution: Afrotropical (0), Australian (1), Oriental (2), Palaearctic (3).

Appendix S2. Data matrix, in nexus format, of ecological and biological traits.

O_lunifer	121171
T_pityocampa	100093
T_pityocampa_orana	100093
T_pityocampa_ENA	10?093
T_wilkinsoni	100093
T_herculeana	101133
T_herculeana_judaea	?????3
T_solitaria	121103
T_solitaria_iranica	121103
T_processionea	111143
T_processionea_pseudosolitaria	111143
T_bonjeani	101093
T_pinivora	101093
T_libanotica	101093
T_ispartaensis	101093
T_sedirica	?01093
T_torosica	?01093
T_cheela	????93
T_jordana	100103
T_dhofarensis	?????0
T_apologetica	???120
T_apologetica_abyssinica	???120
G_apsara	???112
G_chrysolopha	???112
G_transversa	???112
P_rhodesi	???160
P_punctigera	???160
A_panda	01?180
A_panda_infracta	01?180
A_venata	01?180
A_etiennei	01?180
E_subsordida	???180
E_nigricincta	???140
E_moloney	???140
E_carteri	???140
H_antsianakana	????50
H_placidus	????50

Appendix S3. Data matrix, in nexus format, of morphological traits

```
O_lunifer                          01--------1001511310-6300-0142---?-150----0--10210-0110---10100100--00---02000---1-102100-1020100-0400--1110100----------00110050221200-30-0-0-?51010010100120-00-0-1
T_pityocampa                       30010101020101000100-10010101001020031010001010100-1000---10100000-10100110002010 0-10001120000001100110000001110000111010000-0011000100-00-0-0-01001001110010 0-00-0-0
T_pityocampa_orana                 00010101020101000100-300101010?10200310100010101 00-1000---10100000-101001100020100-10001120000001100110000001110000111010000-0011000100-00-0-0-01001001110010 0-00-0-0
T_pityocampa_ENA                   ??????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????????
T_wilkinsoni                       00010201010101000100-30010101001010021010001110100-1000---10100000-101001100020101-10001120000001000110000001110010111000000-0011001101100-0-0-01102001110010 0-00-0-0
T_herculeana                       10010010000020000000-001101000011020010000000100010 0000---1000000100010011010401 22-100010-000101111111111000011122021110010 00-0021100200-00-0-0-02101002010110 0-00-0-0
T_herculeana_judaea                40010010000020000000-00110100001102001000000010001000 00---1000000100010011010401 22-100010-000101111111111000011122021110010 00-0021100200-00-0-0-02101002010110 0-00-0-0
T_solitaria                        01--------1120100200-21211033001002021011001010100-1000---10100000-10100110104100 0-111010-00000011000110010011122020-1111010-0001011100-20-0-0-01101111010010 0-00-100
T_solitaria_iranica                11--------1120100200-21211033011002021011001010100-1000---10100000-101001101??1010-121110-000000110000--010011122020-1111010-0001011100-20-0-0-01101111010010 0-00-100
T_processionea                     01--------1130100200-21211003001002031010001010110-1000---10100100-10100110004010 1-110010-0000010-1101000100111210 10-1110010-0021001200-10-0-0-01001001010110 0-00-0-0
T_processionea_pseudosolitaria     01--------1130100200-21211003001102031010001010110-1000---10100100-10100110004010 1-110010-0000010-1101000100111220 20-1111010-0001011100-20-0-0-01101111010110 0-00-0-0
T_bonjeani                         20111201110112000000-10110124001001021010011101211 1000---101000011101001100 1?0102-11001120000000-00010001001111011 0-1111000-0041100110-010100-000202111100100-00-110
T_pinivora                         20111201111212200000-30110124001121021010011010121 11000---1010000111010011001 20101-100000-0000000-11010001001111011 0-1010000-0041101210-011100-000202111100100-00-110
T_libanotica                       20111201101212200310-301101240?1??10310100111101211 1000---10100001110100110 01?0002-102000-0000000-11111011001111????????000-00???0?????01?100-000222110100100-00-110
T_ispartaensis                     20111201001202200310-30110124001120031010010110121 11000---1010000111011011001 21211-10200120000000-1111101100111011101011000-0041102210-011100-000320010100100-00-110
T_sedirica                         20111201001102200100-30110124001121031010010110121 11000---1010000111011011001 21210-10200120000000-1110-010001111011101011000-0041102210-011100-000320010100100-00-110
T_torosica                         00???????????2200100-3011?1240010??03101001??1012??1000---1010?00??101101?0?12????-1??0???000????11??????00111011101011000-0041102210-01?100-000320010100100-00-110
T_cheela                           20111201110122200??0-30110124001103021010011110121 11000---1010000111012001 00??0022-100010-1000000-0111001000111 12010-0---000-0021002210-00-100-00032001?100100-00-0-0
T_jordana                          0010--02100122000320-221101140110020 1111-100011100-0100---10100000-000---010010-3120----0-0000000-0000--110011100000-1100000-0031010201030-0-0-01001000010012 0-00-0-0
T_dhofarensis                      0010--02100121000320-221101140110020 1111-100011100-0100---11100000-000---020010-3100----0-0030100-1200--110011100000-1110000-0031010201030-0-0-01001000010012 0-00-0-0
T_apologetica                      00102101100121000320-221101140110020 1111-100011100-0100---11100000-000---110010-3110----0-0000100-0000--110011100010-1010000-0031012201030-0-0-01001000010012 0-00-0-0
T_apologetica_abyssinica           00102101100121000320-221101140110020 1111-100011100-0100---11100000-000---110010-3110----0-0000100-0000--110011100010-1010000-0031012201030-0-0-01001000010012 0-00-0-0
G_apsara                           41--------12133011210441130452 20-0111101200000---0----100010100110-100---010050-10-0----0-1100000-0000--012111121020-0---100-0050012101110-0-0-040111020200211110-0-0
G_chrysolopha                      41--------121330112104?1130452 20-01110----0--0---0----0---0------0--00---01005-----0----0-1100000-0000--012111121010-0---100-0050012101110-0-0-040111020200211110-0-0
G_transversa                       41--------12133011210441130452 20-03110----0--0---0----110110101110-100---010061101-0----0-1000000-0000--01210-121010-0---100-0000221101110-110-041111020200211010-0-0
P_rhodesi                          51--------11004113111552110501-----?40----0--10230-1110---10211200-101011030032133-102200-1010000-0300--2103100----------20100030222200-30-0-0-130011230010200-0110-1
P_punctigera                       51--------11004113111552110501-----?40----0--10230-1110---10211200-101001030032133-102200-1010000-0300--2103100----------20100030222200-30-0-0-13001123001 0??????????
A_panda                            01--------11110113010661110021-----110----0--0---0----112010100100-200---130041101-0----101010100-1200--2112120----------20111020201001010-0-121300112300100 00-00-0-0
A_panda_infracta                   01--------11110013111061110001-----110----0--0---0----112010100100-200---130041101-0----101010100-1200--2112120----------20111020201001010-0-121300112300100 00-00-0-0
A_venata                           01--------11110112010050110001-----110----0--0---0----112010100100-201000130041121-0----101110000-1200--2112120----------20121020221001100-0-101300112300100 00-00-0-0
A_etiennei                         01--------11110113010650110101-----150----0--0---0----11100------0--00---02004-----0----0-1120100-0400--1112120----------20121020221001100-0-111300112300 1?000-00-0-0
E_subsordida                       01--------1111011320-051120001-----110----0--0---0----0---10100100-000---14004---0-0----101000000-1500--2113100----------20120130000200-30-0-101300112300102 00-0100-0
E_nigricincta                      01--------1114511310-051120001-----110----0--0---0----0---10100100-000---04004---0-0----111000000-0000--01????0----------?????????????????-??????????????????????????
E_moloney                          01--------1114511320-051120001-----110----0--0---0----0---10100100-000---04004---0-0----121100000-1500--0112100----------20120130000200-30-1010130011230010200-0100-0
E_carteri                          01--------11145113110051120501-----110----0--0---0----0---10100100-000---14004---0-0----101000000-1500--0212100----------20120130000200-30-0-101300112300102 00-00-0-0
H_antsianakana                     01--------1114511200-530110551-----110----0--0---0----0---11100100-000---14003---2-0----1210000011 1600--0112100----------20121020032101100-0-101300112300100 00-0110-0
H_placidus                         01--------1114511200-530110551-----110----0--0---0----0---0---------10---04?03-----0----121000000-0000--0112100----------20121020032101100-0-101300112300100 00-0110-0
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Chapter 3

A review of the genus *Thaumetopoea* (Lepidoptera: Notodontidae: Thaumetopoeinae)

Manuscript in preparation

I contributed to the design of the work, data collection, data analysis, and writing.
This chapter includes and completes part of the results already published and presented in Chapter 4.

Abstract

Thaumetopoea Hübner, 1820 is a Palaearctic and Afrotropical genus of moths with gregarious larvae living in silk tents on trees and characterized by the occurrence of urticating hairs and the typical movement in lines or groups when foraging or searching for pupation sites, which explain the name of "processionary moths". Since 1900s, the genus Thaumetopoea has been object of intense studies that have allowed to know their life history and ecology, in relation mainly to their applied importance as forest pests or nuisances to human and animal health. This paper provides a review of taxonomy, key life history traits, and distribution maps of all known species and a few selected subspecies. A complete morphological description of adults is provided, adding several new traits that are also used to build a morphological key to discriminate each species.

Keywords: *Thaumetopoea*, processionary moths, taxonomy.

Introduction

Thaumetopoeinae are a small subfamily of Notodontidae that counts about 23 genera widely spread in the Afrotropical, Palaearctic, Oriental and Australasian regions (Schintlmeister, 2013). This groups includes some among the most unpopular and dangerous moths species on Earthwhich are colloquially referred to as the "processionary moths". This name is due to the habit of larvae which form long processions while moving for seeking food or to reach a pupation site (Miller, 1991; Roques and Battisti, 2015). Historically, this subfamily has been devided into three subgroups based on morphological and distributional evidences as follows: the West-Palaearctic genus *Thaumetopoea*, the Afrotropical tribe Anaphini and the Australasian Epicomini (Kiriakoff, 1970) – note that both Anaphini and Epicomini are treated at subfamily rank by Kiriakoff because the Thaumetopoeinae had family status).

Larvae of Thaumetopoeinae are sub-social and usually live in colonies whose members build up a characteristic silky nest on the host tree. Larvae are feared defoliators feeding on shrubs and trees of both conifers and broadleaves (Battisti *et al.*, 2015; Basso *et al.*, 2016). As a dramatic consequence of intense defoliation, host plants reduce growth and are more susceptible to attack by other pathogens (Carus, 2004; Avci and Carus, 2005; Carus, 2010).

The Thaumetopoeinae are also characterized by having urticating setae that can seriously affect humans and animals health (Simonato *et al.*, 2013; Basso *et al.*, 2016; Battisti *et al.*, 2017). These setae can be present in larvae (*Thaumetopoea*, *Epicoma*) or the adults (*Anaphe*, *Hypsoides*, *Gazalina*) and are released by the insects when disturbed as an effective defensive strategy (Battisti *et al.*, 2011). Due to their morphology, floating setae can easily penetrate into the skin and activating inflammatory response and developing serious allergic reactions (pruritus, dermatitis, necrosis) (Vega *et al.*, 1999; Battisti *et al.*, 2011; Moneo *et al.*, 2015).

Thaumetopoea pityocampa and *T. processionea* can develop outbreaks close to human settlements threatening public health, and causing economic losses (Roques, 2015).

During the last 75 years, several taxonomic studies have been conducted on the genus *Thaumetopoea*. These led to remarkable taxonomic and nomenclatural rearrangements, e.g., some names fell into synonymy (Agenjo, 1941; Kiriakoff, 1970), new taxa were proposed and the genus was split into three genera based on weak morphological evidences (Freina and Witt, 1985; 1987; Doganlar and Avci, 2001; Doganlar *et al.*, 2005; Hacker, 2016). Recently, however, phylogenetic studies performed on both molecular and morphological data strongly converge in reconstructing *Thaumetopoea* as monophyletic (Simonato *et al.*, 2013; See Chapter 2).

At present, the genus *Thaumetopoea* counts 16 species and 5 subspecies (Schintlmeister, 2013; Basso *et al.*, 2016; Hacker, 2016) (See Chapter 1, Table 1). Despite the long-standing interest aroused by the processionary moths' ecology and behaviour, a thorough taxonomic revision of these insects are still missing. In this paper, we provide a thorough re-description of all taxa ascribed to *Thaumetopoea* and provide a complete key based on adult morphology.

Materials and Methods

All materials examined consisted of pinned specimens deposited into institutional collections (see Chapter 2, Table S1).

Digital pictures were taken using a led light chamber, with a Canon Eos (600D) for specimens from Natural History Museum of London, and a Lumix camera (DMC F200) equipped with additional lens (DMW-LC55) for other specimens. Observations of the specimens were made using stereomicroscope Lumar v12 Zeiss (Carl Zeiss Microscopy GmbH©, 2013) and multiple images of details were captured using AxioCam (MRc5) (software Axiovision SE64, v4.9.1). The composite focus-stacking images were processed with Photoshop CS6 (v13.0) (Adobe System Incorporated© 2012). CorelDraw X5 (v15.1) (Corel Corporation©, 2010) was used to create labels and drawings.

All dissection of genitalia, legs, and labial palpus, were processed according to Chapter 3 and are resumed briefly below. Abdomen was cut from specimens using sterilized tweezers and it was digested overnight using commercial kit in order to extract DNA. Then, the abdomens were separated by digested solutions and put into potash for about 40’. Legs and palpus was separated using appropriated tweezers or pins and they are digested in potash for 10’. Then, abdomen and genitalia were separated and cleaned up in order to mounting them on slides with Euparal medium such as legs and palpus.

Detailed description provided for all species as well as the habitus and male genitalia photos (Figs. 1-2).

After a preliminary analysis, we decided to not consider female genitalia in the current review. This decision was taken after noticing only small differences in the soft part of the apparatus, in the degree of sclerotization and not in boundaries or shapes. Furthermore, two Notodontidae experts (A. Schintlmeister and T.J. Witt, pers. comm.) suggested that the traits identified by Agenjo (1941) were largely artefacts, resulting of no diagnostic value. Further studies are necessary in order to verify whether female genitalia can be used in the species discrimination and phylogeny of this group.

All the iconographic materials related to the studied taxa is available from the authors.

Taxonomy

Presentation of the genus

Thaumetopoea Hübner, 1820

Type species: *Thaumetopoea processionea processionea* (Linnaeus, 1758) by original designation.

Adult. *Thaumetopoea* is characterized by the combination of the following characters: a body covered by brownish, grey and ochraceous hair-like scales. Valva foliaceous, basal-dorsal sclerotized process and distinctive socii that arise from base of uncus. Juxta that are large and well developed. Vesica simple (Basso *et al.*, in press). Scales on female used to cover eggs batches. Urticating setae that carried only in larval from the third stage and not by adults (Battisti *et al.*, 2011; Basso *et al.*, in press). Furthermore other peculiar traits are: antennae bipectinate to apex, eyes naked, palpus, small and outwardly, proboscis absent, mid-leg and hind-leg with a pair of spur and forewings brownish or grey, rather short but broad without scale tuft on the middle of inner margin (Miller, 1991), tegument well developed. In female ductus bursae not sclerotized and signum present (Kiriakoff, 1970).

Larva. The larvae of *Thaumetopoea* have not been thoroughly studied, with the exception of the urticating apparatus located on the abdominal tergites (Démolin 1963). Pictorial description of the mature larvae (5th or 6th instar) are provided by Battisti et al. (2015). A comparative analysis of the morphology of the urticating setae has been carried out by Petrucco-Toffolo et al. (2014).

Life history. Adults emerge in different periods of the year, depending on the species (Battisti *et al.*, 2015). Females live one or two days while males live quite longer. Females, after copulating, lay 100-350 eggs in batches. Conifer-eating species lay eggs on needles or twigs (Roques, 2015), while broadleaved-eating species lay eggs on the terminal branches that have a diameter below 10-15 mm (Battisti *et al.*, 2015). Eggs are always covered by compact layers of scales (Démolin, 1989). Embryonic development takes about a month, then larvae hatch or may stay in diapause, depending on the species. Larvae are hairy and gregarious and develop among five/six instars. Larvae produce silk that often form a tent more or less thick, where they aggregate in groups of up to hundreds of individuals. After that, they move in a procession (head-to-tail or a diamond-shape) looking for suitable sites to pupate. They pupate in litter, in the soil or in the tent depending on the species (Battisti *et al., 2015; Basso et al., in press).* A number of pupae enter prolonged diapause, which can be extended also for several years. All larvae of the genus *Thaumetopoea* are defoliators of trees and shrubs, and can cause outbreaks of variable intensity (Roques, 2015).

Distribution. Eastern and South Africa, Arabian peninsula, Mediterranean and Europe, Iranoturanic area (Figs 3-4).

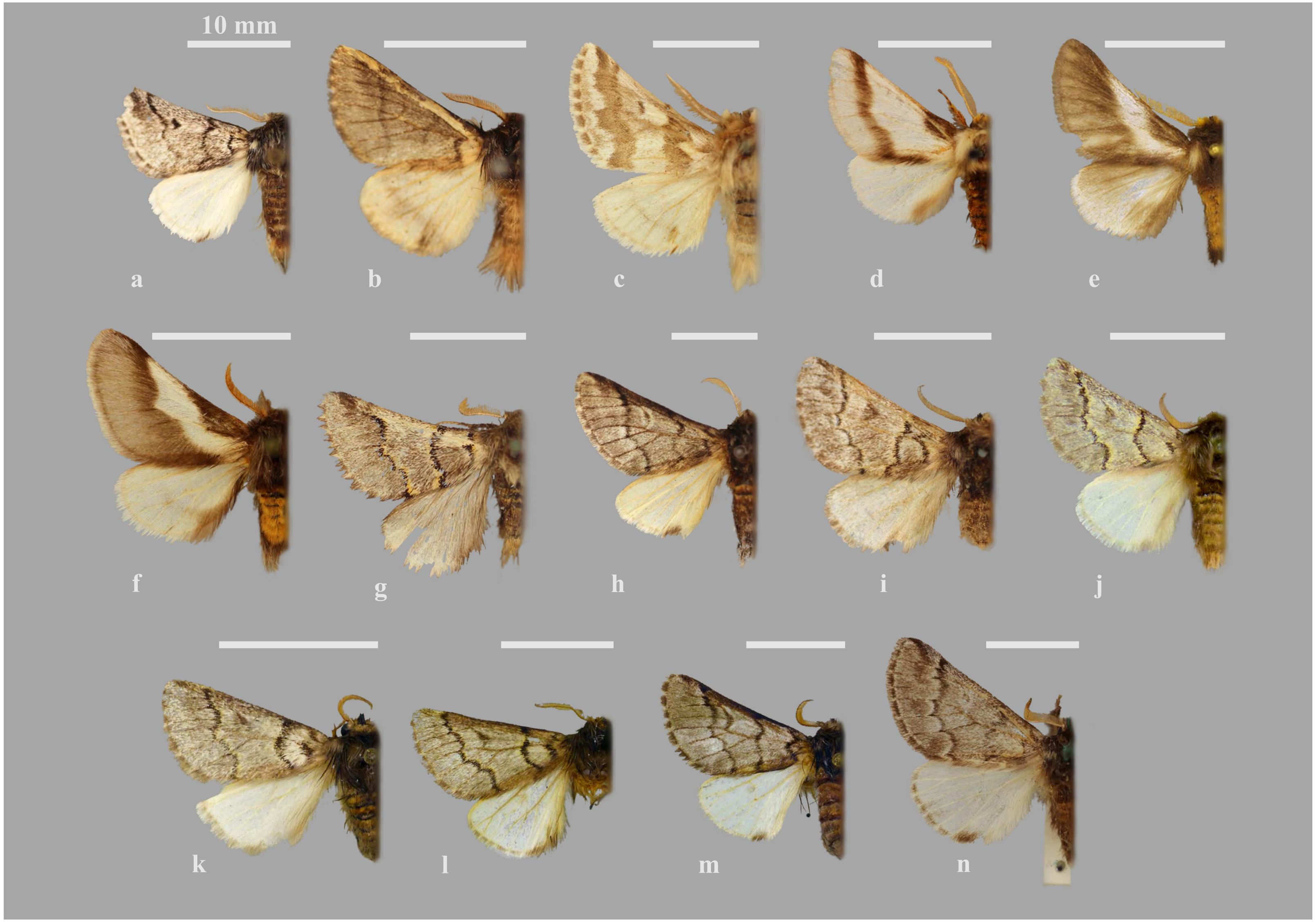

Figure 1. Habitus of adult males. (a), *Thaumetopoea solitaria solitaria*, (b) *Thaumetopoea processionea*; (c), *Thaumetopoea herculeana herculeana*; (d), *Thaumetopoea jordana*; (e), *Thaumetopoea dhofarensis*; (f), *Thaumetopoea apologetica apologetica*; (g), *Thaumetopoea cheela*; (h), *Thaumetopoea bonjeani*; (i), *Thaumetopoea pinivora*; (j), *Thaumetopoea libanotica;* (k), *Thaumetopoea ispartaensis;* (l), *Thaumetopoea sedirica;* (m), *Thaumetopeoea pityocampa pityocampa;* (n), *Thaumetopoea wilkinsoni*. Scale bar = 10 mm.

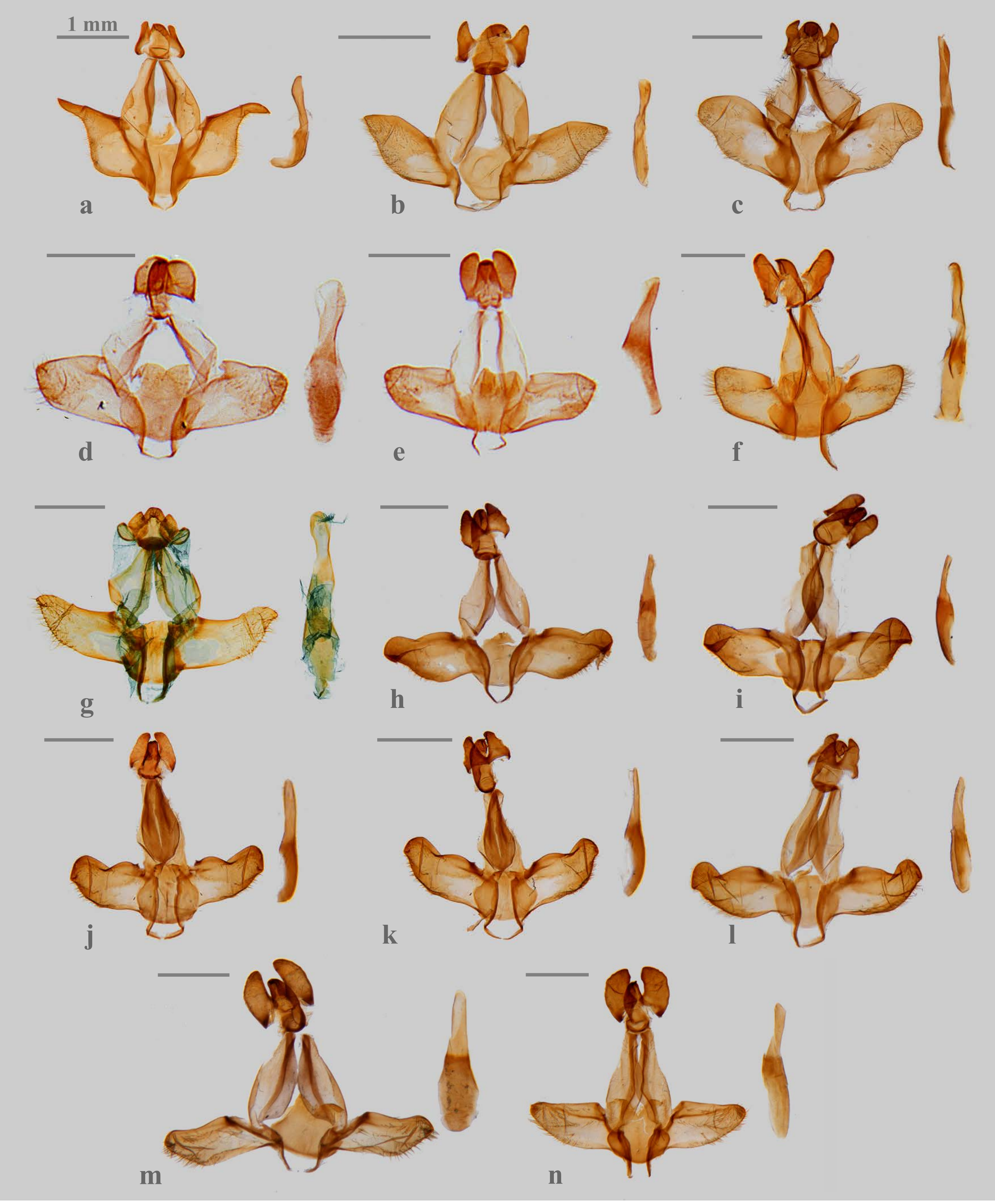

Figure 2. Genitalia of adult males. (a), *Thaumetopoea solitaria solitaria*, (b) *Thaumetopoea processionea*; (c), *Thaumetopoea herculeana herculeana*; (d), *Thaumetopoea jordana*; (e), *Thaumetopoea dhofarensis*; (f), *Thaumetopoea apologetica apologetica*; (g), *Thaumetopoea cheela*; (h), *Thaumetopoea bonjeani*; (i), *Thaumetopoea pinivora*; (j), *Thaumetopoea libanotica;* (k), *Thaumetopoea ispartaensis;* (l), *Thaumetopoea sedirica;* (m), *Thaumetopeoea pityocampa pityocampa;* (n), *Thaumetopoea wilkinsoni*. Scale bar = 1 mm.

Presentation of the taxa

The taxa listed in Table 1 and 2 of Chapter 1 are presented here following the phylogenetic scheme discussed in Chapter 2. A key to the identification of the adult males is provided in Table 1.

Thaumetopoea solitaria solitaria (Freyer, 1838)

Bombyx solitaria Freyer, 1838: 102 (as *Bomb. Solitaria*). Syntypes, males and females (not located). Type locality: European Turkey [as "eurpèaische Turkei"].
Note: The syntypes of Bombyx solitaria Freyer were not found in Senckenberg Forschngsinstitut und Naturmuseum, Frankfurt as stated by Schintlmeister (2013).

Type material examined: no primary types have been available for this study.
Other material examined: ♂, 7.X.1936; Rowandus Kurdistan, IRAQ, E.P. Wiltshire - Ref.: BMNH(E)_1378614; ♂, E.P. Wiltshire 2.X.53, Shaqlawa, 2500ft Kurdistan, IRAQ - Ref.: BMNH(E)_1378616 - Prep.: Not_2107; ♂, Bergama Kurfalli 10.IX.1971, Col.Orhan IJLU, Pistacia vera - Ref.: BMNH(E)_1378615; ♂, E.P. Wiltshire, Prodromos C.4000ft, 26.VIII.1949, CYPRUS - Ref.: BMNH(E)_1378617 - Prep.: Not_2106; ♂, E.P. Wiltshire, Prodromos C.4000ft, 20.VIII.1949, CYPRUS - Ref.: BMNH(E)_1378618 - Prep.: Not_2105; ♂ Cnethocampa solitaria Frr. 266- aris HS 21/2 - Ref.: BMNH(E)_1378644; – British Natural History Museum, London.
♂ Pakistan, Azad Jammu e Kashmir, near Thunian, 2300-2700m 25-30.VIII.2004, leg. V. Gurko - Prep.: TH3; – DAFNAE University of Padua.
♂ Rhodopi Bulgarie, on r. Lukovitza, 15.8.1983, leg. Ganev, Coll. Ex. J. Ganev - Prep.: TH38; ♂ Rhodopi Bulgarie, 8.9.1983, leg. Ganev, Coll. Ex. J. Ganev; ♂ Treska Schlucht Matka, Macedonia, 400-800m 19-29.V.55, leg F. Daniel, ex coll. Proda - Prep.: TH39; – Museo Civico di Zoologia (Rome).
♀, 1.10.78, Israel, Tivon, Palestina, Pistacia, Coll J. Halperin; ♀, Macedonie, Tretska - Matka. Leg. Jos. Thurner; ♂, Zypern, Distr. Nicosia, Kakopetria 700m 12.10.1994 - Prep.: sp.1338; – The Bavarian State Collection of Zoology, Munich.

Diagnosis:

Male. (Fig. 1a). Head generally brown. Antennae covered by whitish scales. Antennae longer less than half of costa, with medium rami (ratio between the length of antenna and rami is from 6 to 8). Pecten yellowish. Adfrontal tuft brownish grey. Frons with a smooth bulge. Palpus small, with first segment shorter than second and wider. Foreleg thin. Spine absent. Epiphysis finger-shaped. Thorax ash grey with 2 whitish lateral spots divided by a black line of hairs. Abdomen grey. Wingspan 25-28 mm. Forewing ground colour ash grey. Three typical narrow dark lines present. Basal line curved, extended from costa to inner margin. Ante-median line zigzagged. Post-median line jagged. Ante and post-median lines divergent on costa and parallel on inner margin. Inner margin last segments of ante-median and post-median line outwardly. Terminal line brown. Discal spot present as a simple spot shape and ringed by an edge of white scales. Fringe, biseriate and not concolorous, alternatively brown and white. Fringe distal margin jagged, with five teeth clearly visible. Hindwing general ground colour and fringe whitish. Terminal vague brownish line present.

Small vague anal spot present. Anal margin whitish.

Male genitalia. (Fig. 2a). Uncus shorter but well developed with 2 apical teeth. Socii narrow with trapezoidal shape and arise basally from uncus. Socii lateral edge smooth and concave, with sharp apex. Posterior tooth from socii vague, sharp and inwardly. Tegumen well developed with rounded tooth in the middle. Valva claw shaped. Lower margin initially straight then extremely convex. Upper margin straight then concave. Cucullus extremely sharp and long. Juxta hexagonal longer than wide, upper edge concave, lateral edges straight, with rounded angle, lower edge almost straight. Aedeagus stout, flared basally and extremely curved basally.

Female. Wingspan 32 mm. Wings ground colour pale and not well defined. Head and thorax grey; abdomen brownish-grey with dark bands. Anal scales in middle narrower than 0.5 mm, straight, and pigmented in centre. Ratio, between length and width around 3-4.

Larva. Larvae are whitish with black spot oval on top of abdominal segment. Larvae are coverd by long grey hairs.

Life history. *Thaumetopoea solitaria* feeds mainly on *Pistacia* spp. but it could be found also on other Anacardiaceae (*Schinus* and *Rhus*) (Halperin, 1983). Eggs are laid in autumn and overwinter to hatch in spring. Larvae feed until May-June during 5 instars without spinning a true tent, and then they pupate into litter (Furth and Halperin, 1979). Prolonged diapause for 1-2 years was observed (Battisti *et al.*, 2015).

Distribution. South-Eastern Europe, Near East (Agenjo, 1941; Roques, 2015) (Fig. 3).

Thaumetopoea solitaria iranica Agenjo, 1941

Thametopoea (Thaumetopoea) solitaria var. iranica Bang-Haas i.l., Agenjo, 1941: 78. Syntypes, 3 males (not located) Iran, Faristan, Fort Sine-Sefid, 2200 m, Strasse Chiraz-Kazeroun. Type locality: Iran, Faristan.

Note: The syntypes of Thaumetopoea solitaria var. iranica Bang-Haas were not found in Museum fur Naturkunde Berlin as stated by Schintlmeister (2013).

Type material examined: no primary types have been available for this study.

Other material examined: ♂, e.l. *Pistacia khinjuk*, h.11.IX.50, Shiraz Mts, Fars Persia, E.P. Wiltshire, c. 6000ft. - Ref.: BMNH(E)_1378621 - Prep.: Not_2108; ♀, h.11.IX.50, e.l. *Pistacia khinjuk*, c. 6000ft., Shiraz Mts, Fars Persia, E.P. Wiltshire - Ref.: BMNH(E)_1378643 - Prep.: E. P. Wiltshire 2147; ♂, e.l. *Pistacia khinjuk*, h. 7.IX.50 Shiraz, Fars Persia, E.P. Wiltshire - Ref.: BMNH(E)_1378620 - Prep.: Not_2109; ♂, e.l. *Pistacia khinjuk*, c.6000ft., Shiraz Persia, h. 7.IX.50, E.P. Wiltshire - Ref.: BMNH(E)_1378619 - Prep.: Not_2110; – British Natural History Museum, London.

Diagnosis:

Male. It shows a variation of habitus of *T. solitaria solitaria* only for ground colour and tonality which is slightly more clear in specimens collected among Iran zone. Wingspan 36-37 mm. In this species forewing discal spot is vague and whitish. We notice also that first segment of ante-median line is extremely inwardly, almost to merge with basal line. Last segment of ante-median line is inwardly. Hindwing without anal spot.

Female. No differences with nominal species was observed.

Larva and **Life history.** Unknown

Distribution. Middle East (Agenjo, 1941) (Fig. 3).

Thaumetopoea processionea processionea (Linnaeus, 1758)

Phalaena Bombyx processionea (Linnaeus, 1758): 500 (as *proceffionea* [*P. Bombix*]). Syntypes: 2♂, ♀, Europe – Linnaean Society of London. Type locality: not indicated (Central and South Europe).

Type material examined: 2♂, ♀, Europe – Linnaean Society of London (material available online at: http://linnean-online.org/view/collection/insects/Phalaena.html. Consulted on October, 2016).

Other material examined: ♂, Basses-Alpes Entrevaux, 12-13-14 Août 1903, R. Powell - Prep: BMNH(E)_1378609; ♂, Predota 1914, Nagy Nyir, Nr. Kecskemet, el.17.VIII - Prep.: Not_2091 - Ref.: BMNH(E)_1378610; ♂, Switzerland Geneva 450m Genthod 17.VIII.63, E. P. Wiltshire - Prep.: Not_2092 - Ref.: BMNH(E)_1378611; ♂, Macedonia, Olympus, 2500ft,1935 (Romei) - Prep.: Not_2094 - Ref.: BMNH(E)_1378612; – British Natural History Museum, London.

♂, [AC 26839] - Prep.: TH36; ♀, Berlin, Z 28/7.00., Postdam; ♀, unknown; ♂, Berlin, Z 30/7.00., Postdam; – Museo di Zoologia, “Sapienza” (Rome).

♂, Ogliata, Roma, 21.VIII.1986, coll. P.Provera; ♂, Cittaducale, Cardito, 5.9.48. Prola; ♂, Rieti, Lazio Italy, Amatrice dint. 1000m, 7/8.9.79, Legit Caponi - Prep.: TH37; – Museo Civico di Zoologia (Rome).

♂, Austria, inf. Maria Ellend, Donau-Auen; 31.VII.1981, leg. Hahn; det. Weth 5.94 - Prep.: TH27; ♀, Iphofen Stelgerwald, c.l. 20.7.1958, Hans Heishmann; – The Bavarian State Collection of Zoology, Munich.

♂, France Bourgogne Avallon Butte de Thill, L1600, 31.VII.2005, Lgt Taverna - Prep.: TH63; ♂, Romagna, Forlì, Predappio m250 lg Fiumi, 1.IX.78. Det. S. Zangheri; – DAFNAE University of Padua.

T. processionea var. *luctifica* Staudinger, 1901: 113. Synonymised with *T. processionea* by Agenjo, 1941.

T. processionea f. *seifersi* Closs, 1917: 147. Synonymised with *T. processionea* by Agenjo, 1941.

T. processionea f. *clausa* Lempke, 1959: 114 (358).

Diagnosis:

Male. (Fig. 1b) Head generally brown. Antennae covered by dark and whitish scales. Antennae shorter than half of costa, with medium rami (ratio between the length of antenna and rami is from 6 to 8). Pecten yellowish. Adfrontal tuft brownish grey. Frons with a smooth bulge. Palpus small, with first segment shorter than second and wider. Foreleg thin. Spine absent. Epiphysis finger-shaped. Thorax ash grey with 2 whitish lateral spots divided by a black line of hairs. Abdomen ochraceous. Wingspan 20-23 mm. Forewing ground colour brown. Three typical dark narrow lines present. Basal line zigzagged extended from costa to inner margin. Ante-median line straight. Post-median line jagged. Ante and post-median lines parallel on costa and convergent on inner margin. Inner margin last segments of ante-median line outwardly and post-median line inwardly. Terminal line brown. Discal half-moon spot present and ringed by an edge of white scales. Fringe, uniseriate and not concolorous, alternatively brown and white. Fringe distal margin jagged, with five teeth clearly visible. Hindwing general ground colour and fringe whitish with brown post-median line and marked anal spot. Anal margin whitish.

Male genitalia. (Fig. 2b). Uncus shorter but well developed with 2 apical teeth. Socii narrow with trapezoidal shape and arise basally from uncus. Socii lateral edge smooth and straight, with narrow apex. Posterior tooth from socii vague, sharp and straight. Tegumen well developed with vague tooth in the middle. Valva spear shaped. Lower margin initially straight then convex. Upper margin straight then convex. Cucullus narrow and stout. Fold present at base of cucullus, across valva. Juxta hexagonal as wide as long, upper edge concave, lateral upper edges straight while lower convex, with rounded angle, lower edge slightly convex. Aedeagus stout, flared basally and straight.

Female. Wingspan 31-36 mm. Wings ground colour pale and not well defined. Head and thorax grey; abdomen brownish-grey with dark bands. Anal scales in middle narrower than 0,5 mm, straight, and pigmented in centre. Ratio, between length and width around 3-4.

Larva. Larvae are blue-blackish in ground colour, with grey lateral band. Long setae are greyish and grouped into 8 tufts per segment.

Life history. *Thaumetopoea processionea* feeds mainly on *Quercus* spp. (Battisti *et al.*, 2015) and occasionally on other species. Eggs laid in the later summer. Eggs overwinter and hatch during spring (April-May) slightly before budbreak. Larvae feed during late spring and summer, and go through six instars (Battisti *et al.*, 2015) at the end of whitch larvae pupate in tent built on trunk.

Distribution. Central Europe, Middle East (Agenjo, 1941; Roques, 2015) (Fig. 3).

Thaumetopoea processionea pseudosolitaria Daniel, 1951

Thaumetopoea processionea pseudosolitaria Daniel, 1951: 27. Holotype: ♂, Mazedonien, Bogdanci; Allotype ♀; Paratype: ♂, ♀, Mazedonien, Bogdanci; – Witt Museum, Munich. Type locality: Mazedonien, Bogdanci.

Type material examined: ♂, Holotype, Mazedonien, Bogdanci; ♀, Co-type, Mazedonien, Bogdanci, coll. Burgeff;– Witt Museum, Munich.

Other material examined: ♂, Sabies d'Olonne Vendée, 16.VIII.1985, B.Goater - Prep.: Not_2093 - Ref.: BMNH(E)_1378613; – British Natural History Museum, London.

♂, Doiransee, coll.Burgeff inf. IX.1916 e.l. - Prep.: TH65; – DAFNAE University of Padua.

♂, Sicilia. Madonie, m1200, coll. Daniel - Prep.: TH66; – The Bavarian State Collection of Zoology, Munich.

Diagnosis:

Male. Head generally brown. Antennae covered by dark and whitish scales. Antennae shorter than half of costa, with medium rami (ratio between the length of antenna and rami is from 6 to 8). Pecten yellowish. Adfrontal tuft brownish grey. Frons with a smooth bulge. Palpus small, with first segment shorter than second and wider. Foreleg thin. Spine absent. Epiphysis finger-shaped. Thorax ash grey with 2 whitish lateral spots divided by a black line of hairs. Abdomen ochraceous and anal tuft ash grey. Wingspan 21-22 mm. Forewing ground colour brown. Three dark narrow lines present. Basal line zigzagged extended from costa to inner margin. Ante-median line straight. Post-median line jagged. Ante and post-median lines parallel on costa and convergent on inner margin. Last segments of ante-median line outwardly and post-median lines on inner margin, inwardly. Terminal line brown. Discal half-moon spot present and ringed by an edge of white scales. Fringe, uniseriate and not concolorous, alternatively brown and white. Fringe distal margin jagged, with five teeth clearly visible. Hindwing general ground colour and fringe whitish with brown post-median line and marked anal spot. Anal margin whitish.

Male genitalia. Uncus shorter but well developed with 2 apical teeth. Socii narrow with trapezoidal shape and arise basally from uncus. Socii lateral edge smooth and concave, with sharp apex. Posterior tooth from socii faint, sharp and inwardly. Tegumen well developed with vague tooth in the middle. Valva spear claw-like. Lower margin initially straight then strongly convex. Upper margin straight then gently concave. Cucullus narrow and sharp. Juxta hexagonal as wide as long, upper edge concave, lateral upper edges straight while lower convex, with rounded angle, lower edge slightly convex. Aedeagus stout, flared basally and straight.

Female. Wingspan 26-30 mm. Wings ground colour pale and not well defined. Head and thorax grey; abdomen brownish-grey with dark bands. Anal scales in middle narrower than 0,5 mm, straight, and pigmented in centre. Ratio, between length and width around 3-4.

Larva and **Life history.** Unknown.

Distribution. Southern Europe (Fig. 3).

Thaumetopoea herculeana herculeana (Rambur, 1840)

Bombix herculeana Rambur, 1840: pl.14. Syntypes, males and females (not located). Type locality: [Spain], Andalusia.

Note: unknown types depository, the illustration was not accompanied by text or type locality as stated by Schintlmeister (2013).

Type material examined: no primary types have been available for this study.

Other material examined: ♂, Maroc, Azrou, Automn, 1920, Ex. Harold Powell - Ref.: BMNH(E)_1378594; ♂, Prov. d'Oran, Geryville, Septembre, 1910, Harold Powell - Ref.: BMNH(E)_1378595; ♂, Guelt-es-Stel, C. Algeria, 20. Septemb.1912, (V. Faroult) - Ref.: BMNH(E)_1378596 - Prep.: Not_2085; ♂, Guelt-es-Stel, C. Algeria, 22. Septemb.1913, (V. Faroult) - Ref.: BMNH(E)_1378597 - Prep.: Not_2084; ♂, Mazagan, Morocco, September 02. (W.Riggenbach) - Ref.: BMNH(E)_1378598; ♂, Mazagan, Morocco, September 02.

(W.Riggenbach) - Ref.: BMNH(E)_1378599 - Prep.: Not_2086; ♂, Rabat, Morocco, (Thery) - Ref.: BMNH(E)_1378600 - Prep.: Not_2087; ♂, Rabat, Morocco, (Thery) - Ref.: BMNH(E)_1378601 - Prep.: Not_2088; – British Natural History Museum, London.

♂ Lusitania, Sammlung Osthelder; ♀, Portugal, ex coll. Jeebold; – Witt Museum, Munich. ♂, Spain - Prep.: TH 26; – DAFNAE University of Padua.

♂, Algeria centr., Guelt-es-Stel, X.1929, Schwingenschuss; ♂, Hassi - Bahbah Algeria, VIII 1929, J. Stattermayer - Prep.: TH 34; ♂, Montarco Pro. Madrid IX.20. F.ESC - Prep.: TH 33; – Museo di Zoologia, "Sapienza" (Rome).

♂, Morocco, Tangier, 150' Oct.2.1934. Querci (*Thaumetopoea colossus*); – Museo Civico di Zoologia (Rome).

T. herculeana ab. *bifasciata,* Spuler, 1908: 105.

T. herculeana var. *colossa,* Bang-Haas, 1910: 31. Synonymised with *T. herculeana* by Kiriakoff, 1970.

T. phosphatophila, Dumont, 1922: 245. Synonymised with *T. herculeana* by Kiriakoff, 1970.

T. herculeana f. *carneades,* Turati, 1924: 49. Defined as subspecies of *T. herculeana* by Agenjo, 1941.

T. herculeana var. *alvarezi,* Agenjo, 1941: 108. Synonymised with *T. herculeana* by Kiriakoff, 1970.

T. herculeana var. *lustrata,* Turati, 1926: 28. Synonymised with *T. herculeana* by Kiriakoff, 1970.

T. herculeana var. *ibarrae,* Agenjo, 1941: 111; pl. 2: 16. Defined as subspecies of *T. herculeana* by Agenjo, 1941; Synonymised with *T. herculeana* by Kiriakoff, 1970.

T. herculeana var. *zernyi,* Agenjo, 1941: 110; pl. 2: 14, 15. Synonymised with *T. herculeana* by Kiriakoff, 1970.

T. herculeana f. *abdallah,* Rungs, 1956: 293.

Diagnosis:

Male. (Fig. 1c) Head light brown. Antennae covered by whitish scales. Antennae longer more than half of costa, with long rami (ratio between the length of antenna and rami is from 3 to 5). Pecten yellowish. Adfrontal tuft ochraceous. Frons crested; with 6 teeth. Apical tooth rear; base larger than width of crest and high. Second one pronounced long as the first. Palpus small, with first segment longer than second and wider. Foreleg stout. Spine present. Epiphysis claw-shaped. Thorax brownish grey with 2 whitish lateral spots divided by a black line of hairs. Abdomen ochraceous. Wingspan 26-35 mm. Forewing ground colour ochraceous or light brown. Three lines present. Basal line narrow, zigzagged, do not reach costa or inner margin. Ante-median wide line zigzagged, with dark edge, both proximal and distal, from costa to inner margin. Proximal edge produce an acute or recto outwardly angle on cubital vein while distal edge straight. Post-median wide line jagged, with proximal dark edge. Ante and post-median lines parallel on costa and convergent on inner margin. Inner margin last segments of ante-median and post-median line straight. Terminal line brown. Discal spot present with half-moon shape and ringed by an edge of white scales. Fringe, biseriate and not concolorous, alternatively brown and white. Fringe distal margin jagged, with five teeth clearly visible. Hindwing general ground colour whitish. Ante-median line present and terminal line vague brownish. Small albeit faint discal spot present, light brown vague anal spot present. Anal margin whitish.

Male genitalia. (Fig. 2c). Uncus shorter but well developed with 2 apical teeth. Socii narrow with trapezoidal shape and arise basally from uncus. Socii lateral edge smooth and concave, with sharp apex. Posterior tooth from socii define, round and inwardly. Tegumen well developed. Valva spear shaped. Lower margin initially concave then convex. Upper margin convex. Cucullus rounded. Juxta heptagonal longer than wide, upper edge concave, lateral edges upper side straight slight inwardly and lower convex, with inwardly angle, lower edges strongly convex. Aedeagus stout, flared basally and, with a process on basal part of aedeagus.

Female. Wingspan 28-40 mm. Wings ground colour ocracheous and not well defined. Head and thorax light brown; abdomen brownish with dark bands. Anal scales in middle narrower than 0,5 mm, straight, and spread pigment. Ratio, between length and width around 3-4.

Larva. Newly larvae are blue-greyish coloured. After third instar they become green-yellowish. Larvae are not reported as urticating (Agenjo, 1941) even if the urticating setae are present (Battisti *et al.*, 2015) and setae proteins responsible of the allergenic activity were identified (Berardi *et al.*, 2015).

Life history. *Thaumetopoea herculeana* feeds on different species of Cistaceae (*Helianthemum vulgare*, *H. croceum* and *Cystus salviaefolius*), and Geraniaceae (*Erodium moschatum* and *E. arborescens*), in open areas (Battisti *et al.*, 2015). Eggs are laid on shrubs and low plants in a period that changes in relation with local weather. Larvae at different stages can be found from October to April. Consequently, adults can be observed from May to October. After the fifth instar they pupate into soil in sunny places (Battisti *et al.*, 2015). They do not spin a tent, stay on vegetation; a silky protection is observed only in central and northern Spain. They move to feed on a triangular procession instead of typical head-to-tail (Battisti *et al.*, 2015).

Distribution. South-Western Europe, North Africa, Near East (Agenjo, 1941; Roques, 2015) (Fig. 3).

Thaumetopoea herculeana judaea Bang-Haas, 1910

Thaumetopoea heculena var. *judaea* Bang-Haas, 1910: 31. Syntypes**:** ♂, ♀ Jerusalem (not located).
Type locality: Jerusalem, Palaestina.
Note: The syntypes of *Thaumetopoea herculeana judaea* Bang-Haas was not found in Museum fur Naturkunde Berlin.

Type material examined: ♂ Palaestina. MNCN_Ent 137894, Labelled as Co-type; ♀ Palaestina. MNCN_Ent 137895, Labelled as Co-type; – Museo National de Ciencias Naturales, Madrid.
Other material examined: ♂, Rechobot, Palestine, Oct. Nov 07., (J. Aharoni) - Ref.: BMNH(E)_1378602 - Prep.: Not_2089; ♂,Umm-el-kilab, S. W. Palestine, 18.7.1920, P. P. Graves 1923-194 - Ref.: BMNH(E)_1378638 - Prep.: Not_2090; – British Natural History Museum,

London.

Diagnosis:

Male. Some specimens show lighter colour than the nominal species. No other morphological difference were identified.

Male genitalia. Specimens analysed show genitalia really similar to the nominal species, except for a cucullus slightly sharper, but it falls within the intraspecific variability of the nominal species.

Female. No differences with nominal species were observed.

Larva and **Life history.** Unknown.

Distribution. Near East (Agenjo, 1941) (Fig. 3).

Remarks:

Even if the identification of *T. herculeana judaea* is possible only basing on the collection site and any other difference are not identify (Basso *et al.*, in press), we prefer to maintain the current subspecies statut because the framented range between it and the nominal species could hide two population genetically well-separated.

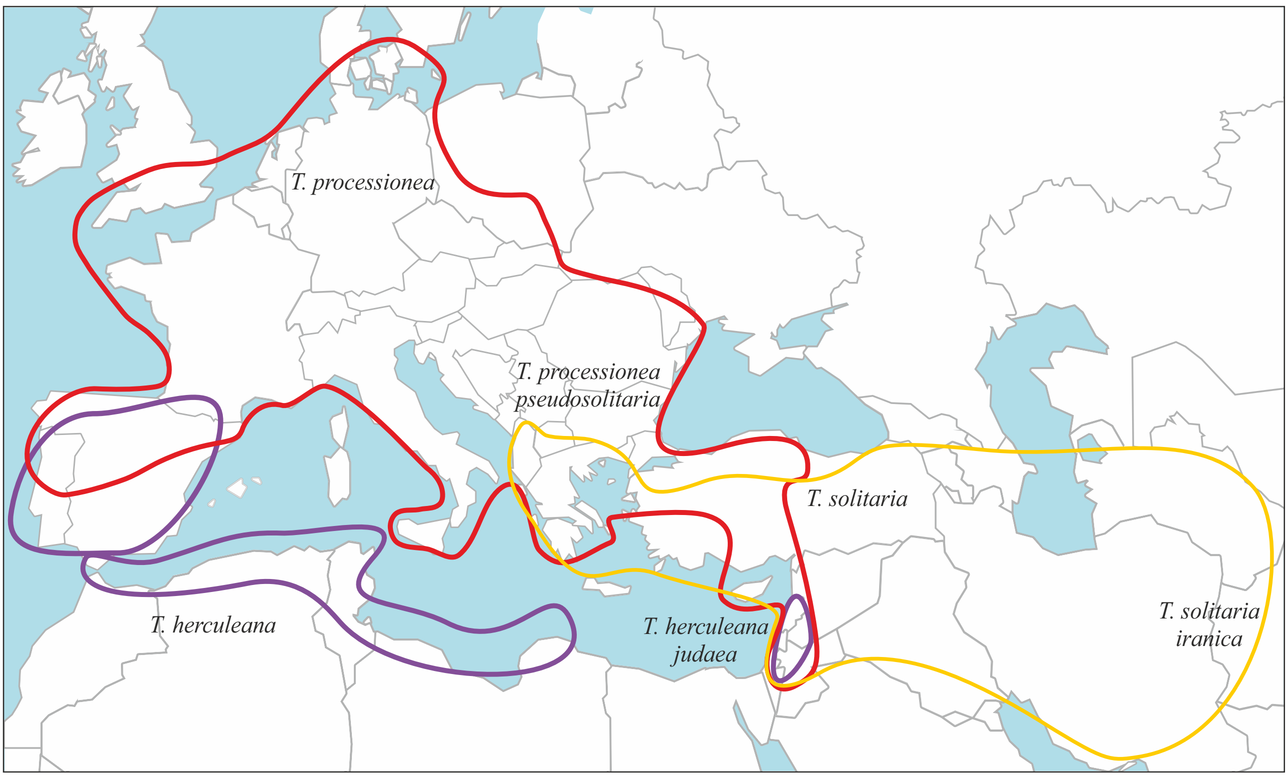

Figure 3. Geographic distribution of the species of the genus *Thaumetopoea* associated with broad-leaves (Clade B, Chapter 2).

Thaumetopoea jordana Staudinger, 1894

Cnethocampa (Thaumatocampa) jordana, Staudinger, 1894: 269. Syntypes: 4 ♂; 4♀ Palestine, Jordanthal – Museum fur Naturkunde Berlin. Type locality: Jordanthal, Palaestina [Israel].

Type material examined: ♂ Jordanthal [Israel], 93. Paulis, ex coll. Staudinger; Syntype; – Museum fur Naturkunde Berlin.

Other material examined: ♂, Arabia: Khauba, 30.I.1937 H. St.J. B. Philby B.M.1937-288 - Ref.: BMNH(E)_1378574 - Prep.: Not_2080; ♂, 29.X.1952, Jordan Valley, Zerqua R. Colony C. 100 m. Below S.L larva on Crataegus, Trevor Trought - Ref.: BMNH(E)_1378575 - Prep.: Not_2081; ♂, 12.X.1952, Jordan Valley, Zerqua R. Colony C. 100 m. Below S.L larva on Crataegus, pupated c 19.3.52, Trevor Trought - Ref.: BMNH(E)_1378576 - Prep.: Not_2082; – British Natural History Museum, London.

♂, Palestine, Jordanthal, Prep.: TH 72; ♀, Palestine, Jerusalem; – The Bavarian State Collection of Zoology, Munich.

Diagnosis:

Male. (Fig. 1d). Head brown. Antennae whitish and longer than half of costa, with medium rami (ratio between the length of antenna and rami is from 6 to 8). Pecten ochraceous. Adfrontal tuft ochraceous. Frons crested with 7 teeth. Apical tooth longitudinal to the body, big, extremely fore and flattened with base large as width of crest. Second one pronounced: reduced and short: ratio between the length of second and third tooth is 2. Palpus small, with first segment long half of second and wide of second. Foreleg medium. Spine present and epiphysis claw shaped. Thorax covered by ash grey hairs with 2 long whitish lateral spots that arise to the root of forewings. Abdomen reddish. Wingspan 26-27 mm. Forewing ground colour whitish. Basal and ante-median lines reduced and fused on a costal brown patch, with straight edge both on basal side and on ante-median side. Post-median line straight, slightly oblique, extended from costa to inner margin, and wide about 2-3 mm. Posterior segment of post-median line ends almost perpendicular on inner margin. Brown stretch on inner margin present, from post-median line to projection of distal portion of costal patch. Discal spot absent. Fringe, uniseriate and concolorous, whitish. Fringe distal margin jagged, with two teeth clearly visible. Hindwing general ground colour and fringe whitish. Medial line brownish. Central spot and anal spot absent. Anal margin brownish reddish.

Male genitalia. (Fig. 2d). Uncus shorter but well developed with 2 apical teeth. Socii narrow with half-moon shape and arise basally from uncus. Socii lateral edge convex and smooth. Apex narrow. Lower teeth very rounded, vague. Tegumen well developed. Valva U-shape. Lower margin initially straight then extremely convex. Upper margin convex, with vague ribbing. Baso-dorsal process on valva sclerotized as other *Thaumetopoea*. Vague bulge on upper margin of costa present. Cucullus broadly rounded. Juxta hexagonal as long as wide, upper edge concave, lateral edges upper straight and then convex, with broadly rounded angle, lower edge convex. Aedeagus stout, flared basally and straight.

Female. Wingspan 30 mm. Wings ground colour brownish pale and not well defined. Head and thorax dark with light hairs; abdomen ochraceous with dark bands. Anal scales whitish, narrow in middle, more or less straight, and pigmented distally. Ratio, between length and width between 3-4.

Larva. First larval instar is yellowish with black head. From third instar larvae become blackish in ground colour. Body start to cover by silver long setae arise from dots. On abdominal segments, they have oval black spots where are located the urticating hairs (Trougth, 1954).

Life history. *Thaumetopoea jordana* is monophagous on *Rhus tripartita* (Furth and Halperin, 1979; Wiltshire, 1982; Battisti *et al.*, 2015). Eggs are laid between October and December and larvae hatch in November-March, starting to feed on the host plant. Between March and April they go to pupate into soil. They do not build a tent. A prolonged diapause of 1-2 years was observed (Battisti *et al.*, 2015).

Distribution: Near East (Agenjo, 1941; Kiriakoff, 1970) (Fig. 4).

Thaumetopoea dhofarensis Wiltshire, 1980

Thaumetopoea dhofarensis, Wiltshire, 1980: 194. Holotype ♂, (Prep. 1911). Dhofar Prov., Khadrafi: 29.IX.I977, (P. GRANVILLE WHITE). Paratypes 3 ♀, 2 ♂, Khadrafi 29.IX.I977; 2 ♂, 2 ♀, Khadrafi: 2.X.I977, (K. GUICHARD). – British Natural History Museum, London by original designation. Type locality: [Dhofar, Oman].

Type material examined: ♂, Dhofar, Khadrafi 670 m. 29.IX.1977 (P.G. White) - Ref.: BMNH(E)_1378630 - Prep.: E.P.Whiltshire 1911, Holotype; ♀, Dhofar 2.100, Khadrafi, 2.X.1977 (P.G. White), - Ref.: BMNH(E)_1378632; Paratype (labelled as holotype); – British Natural History Museum, London.

Other material examined: ♂, Dhofar Kadrafi,29.IX.1977, K. Guichard, - Ref.: BMNH(E)_1378631; – British Natural History Museum, London.♂, Oman, Dhofar prov. Jebel, Samhan Mts. 900-1100 m, Tawi Attair region 3-9.IX.2007, leg. St. Jaki - Prep.: TH 73; ♂, Oman, Dhofar prov. Jebel, Samhan Mts. 900-1100 m, Tawi Attair region 3-9.IX.2007, leg. St. Jaki - Prep.: TH 74; ♀, Oman, Dhofar prov. Jebel, Samhan Mts. 900-1100 m, Tawi Attair region 3-9.IX.2007, leg. St. Jaki; – Witt Museum, Munich.

Diagnosis:

Male. (Fig. 1e). Head brown. Antennae whitish and longer than half of costa, with medium rami (ratio between the length of antenna and rami is from 6 to 8). Pecten ochraceous. Adfrontal tuft ochraceous. Frons crested with 7 teeth. Apical tooth longitudinal to the body, big, extremely fore and flattened with base large as width of crest. Second one pronounced: reduced and short: ratio between the length of second and third tooth is 2. Palpus small, with first segment long half of second and wide of second. Foreleg medium. Spine present and epiphysis claw shaped. Thorax covered by ash grey hairs with 2 long whitish lateral spots that arise to the root of forewings. Abdomen reddish. Wingspan 25-30 mm. Forewing ground colour brown. Basal and ante-median lines reduced and fused on a costal brown patch, with straight edge on basal side and zigzagged on ante-median side. Post-median patch strongly oblique, extended from costa to inner margin, and wide to termen. Segment on inner margin become parallel and reaches thorax. Discal spot absent.

Fringe, uniseriate and concolorous, whitish. Fringe distal margin jagged, with two teeth clearly visible. Hindwing general ground colour brownish with white eye on cell. Fringe whitish. Central spot and anal spot absent. Anal margin brownish grey.

Male genitalia. (Fig. 2e). Uncus shorter but well developed with 2 apical teeth. Socii narrow with half-moon shape and arise basally from uncus. Socii lateral edge convex and smooth. Apex narrow. Lower teeth narrow, vague and straight. Tegumen well developed. Valva U-shape. Lower margin initially straight then extremely convex. Upper margin convex, with vague ribbing. Vague bulge on upper margin of costa present. Cucullus broadly rounded. Juxta hexagonal as long as wide, upper edge concave, lateral edges upper straight and then convex, with broadly rounded angle, lower edge convex. Aedeagus stout, flared basally and straight.

Female. Wingspan 34-38 mm. Wings ground colour brownish pale and not well defined. Head and thorax dark with light hairs; abdomen ochraceous with dark bands. Anal scales whitish, narrow in middle, more or less straight, and pigmented distally. Ratio, between length and width between 3-4.

Larva and **Life history.** Unknown.

Distibution: Southern Arabian peninsula (Fig. 4).

Thaumetopoea apologetica apologetica Strand, 1909

Thaumetopoea apologetica Strand, 1909: 196. Holotype: ♂, D. O. Afrika, Mkalama, 17.II.1905, (v. d. Marwit); Syntypes:2 ♂, Nord-Usambara, Umgegend von MIalo (Holst 1891 - 92) – Natural History Museum, Berlin. Type locality: D. O. Afrika [sub-Saharan Africa].

Type material examined: ♂, D. O. Afrika, Mkalama, 17.II.1905, (v. d. Marwit) Holotype; – Natural History Museum, Berlin.

Other material examined: ♂, Arabia, Suda. 5.VII.1962, G. Popov B.M.1963-130 - Ref.: BMNH(E)_1378605 - Prep.: Not_2095; ♂, Arabia, Suda. 5.VII.1962, G. Popov B.M.1963-130 - Ref.: BMNH(E)_1378607 - Prep.: Not_2096; ♂, Arabia, Suda. 5.VII.1962, G. Popov B.M.1963-130 - Ref.: BMNH(E)_1378603 - Prep.: Not_2097; ♂, Suna, S. Kavirondo, January 1932. (W. Feather) - Ref.: BMNH(E)_1378604; ♂, Suna, S. Kavirondo, January 1932. (W. Feather) - Ref.: BMNH(E)_1378606; ♂, Mt. Elgon, Dec.1932, T.H.E. Jackson - Ref.: BMNH(E)_1378608; ♂, Mt. Elgon, Nov.1932, T.H.E. Jackson - Ref.: BMNH(E)_1378641 - Prep.: Not_297; – British Natural History Museum, London.

♂, S. Rhodesia, Bulawayo - Glenville [Zimbabwe], 7-10.I.1978, Nat. Museum, S.R. Kaster, RF - Ref.: Priv_coll._#29.538 - Prep.: TH 4; ♂, Tvl, Bushman Rock [Zimbabwe], 18.II.1980, Duke, Aj - Ref.: Priv_coll._#29.540 - Prep.: TH 5; ♂, S. W. A., Abachaus, [Namibia] I.1994, Hobohm, G - Ref.: Priv_coll._#29.543 - Prep.: TH 6; ♂, S. Rhodesia, Matsheamhlope, Bulawayo [Zimbabwe], 12.I.1976 , Nat. Museum, S.R. D.K.B. Wheeler - Ref.: Priv_coll._#29.537 - Prep.: TH 56; ♂, Tvl, Bushman Rock [Zimbabwe], 17.II.80, N.J. Duke - Ref.: Priv_coll._#29.539 - Prep.: TH 57; ♂, Tanzeen 19.I.25 A. J. T. Janse - Ref.: Priv_coll._#29.541 - Prep.: TH 58; ♂, Selati Estates, 1-3. II.1976, D. H. Jacobs - Ref.: Priv_coll._#29.542 - Prep.: TH 59; ♂, Bulawayo 15-Z3 Dec 1919, A.

J. T. Janse - Ref.: Priv_coll._#29.544 - Prep.: TH 60; – Private collection.

Diagnosis:

Male. (Fig. 1f). Head brown. Antennae whitish and longer than half of costa, with medium rami (ratio between the length of antenna and rami is from 6 to 8). Pecten ochraceous. Adfrontal tuft ochraceous. Frons crested with 7 teeth. Apical tooth fore and flattened with base large as width of crest. Second one pronounced: well developed but short: ratio between the length of second and third tooth is 2. Palpus small, with first segment long half of second and wide of second. Foreleg medium. Spine present and epiphysis claw shaped. Thorax covered by ash grey hairs with 2 long whitish lateral spots that arise to the root of forewings. Abdomen reddish. Wingspan 26-30 mm. Forewing ground colour brown. Basal and ante-median lines reduced and fused on a costal brown patch, with straight edge on basal side and zigzagged on ante-median side. Post-median patch strongly oblique, extended from costa to inner margin, and wide to termen. Segment on inner margin ends, below the middle of basal patch. Forewing portion between post-median position and termen could brighten up resulting white in some specimens. Discal spot absent. Fringe, uniseriate and not concolorous, whitish. Fringe distal margin jagged, with two teeth clearly visible. Hindwing general ground colour and fringe whitish. Medial line marker or vague brownish. Central spot and anal spot present. Anal margin brownish grey.

Male genitalia. (Fig. 2f). Uncus shorter but well developed with 2 apical teeth. Socii narrow with half-moon shape and arise basally from uncus. Socii lateral edge convex and smooth. Apex narrow. Lower teeth narrow, well define and straight. Tegumen well developed. Valva U-shape. Lower margin initially straight then extremely convex. Upper margin slightly concave then convex, with vague ribbing. Cucullus broadly rounded. Juxta hexagonal as long as wide, upper edge concave, lateral edges upper straight and then convex, with broadly rounded angle, lower edge convex. Aedeagus stout, flared basally and straight.

Female. Wingspan 32 mm. Wings ground colour pale and not well defined. Head and thorax dark with light hairs; abdomen ochraceous with dark bands. Anal scales whitish, narrow in middle, more or less straight, and pigmented distally. Ratio, between length and width between 3-4.

Larva and Life history. Maerua spp. (Gardiner, 1976)

Distribution: South Africa and Tanzania-Uganda (Fig. 4).

Thaumetopoea apologetica abyssinica Strand, 1911

Thaumetopoea abyssinica Strand, 1911: 17. Defined as subpecies of *T. apologetica* by Kiriakoff, 1970. Syntypes: 5♂ Abyssinia, Addis Abeba, April 1908 (Kostlan), – Natural History Museum, Berlin. Type locality: Abyssinia [Ethiopia].

Type material examined: ♂ Abyssinia, Addis Abeba, Alf. Kostlan S., Syntype; – Natural History Museum, Berlin

Other material examined: ♂, Hora Daka, Centr. Abyss., 31 May, 1914 (O. Kovacs) - Ref.: BMNH(E)_1378622 - Prep.: Not_2098; ♂, Abyssinia Harar 8.6.39, R. E. Ellison - Ref.: BMNH(E)_1378623 - Prep.: Not_2099; ♂, Maraquo, Centr. Abyss., May, 1914 (O. Kovacs) -

Ref.: BMNH(E)_1378624 - Prep.: Not_2100; ♂, Abyssinia Harar 7.6.39, R. E. Ellison - Ref.: BMNH(E)_1378625 - Prep.: Not_2101; ♂, Abyssinia, Charada forest, Kajfa, 6000ft. 25.5.05, Ph.C.Zaphiro 1906-78 - Ref.: BMNH(E)_1378642 - Prep.: Not_298; – British Natural History Museum, London.

Diagnosis:

Male. No strongly differences with the nominal species were detected. Wingspan 24-27 mm. Slight differences on wing pattern were detected by Strand (1911b) but we notice that these traits fall into an intraspecific variation of T. apologetica.

Male genitalia. No differences with nominal species was observed.

Female. No differences with nominal species was observed

Larva and **Life history.** Unknown.

Distibution: Ethiopia and Eritrea (Fig. 4).

Thaumetopoea loxostigma Hacker, 2016

Thaumetopoea loxostigma: Hacker, 2016: 146. Holotype: ♂, [Yemen]: South Yemeni/P.D.R.Y, Lahej Governate, Laboos, 2100m, 25./26.VI.1987 (gen.prep. H. HACKER 22728♂) (leg. B. MOLLER) (ex ooll. H. HACKER, ZSM).

Note: species was described only on the holotype, any other material is not available. I check the photos of material available available in the orginal description.

Type material examined: no primary types have been available for this study.

Diagnosis:

Male. From Hacker, (2016) *T. loxostigma* is related to *T. apologetica-jordana* group. Wingspan 27 mm. It differs from the other species, for forewing with a dark post-median lines rather oblique that reach the inner margin in the middle, and whitish external patch.

Male genitalia. From Hacker, (2016), uncus and socii are smaller and less sclerotized than in the other species of the group. Valvae is shorter and basally narrow.

Female. Unknown

Larva and **Life history.** Unknown.

Distibution: Yemen (Fig. 4).

Remarks: by the observation conducted on the photos of *T. loxostigma,* it seems clear that it belongs to the African *Thaumetopoea* (Hacker, 2016; Basso *et al.*, in press). Unfortunately, photos from the original description, are unclear and it is not possible to identify the difference listed by Hacker (2016). In any case, from collection of Schintlmeister we note a specimens collected in Zimbabwe with the same habitus. We also notice that among the *T. apologetica*, there is a high variability, both in the wings pattern and in genitalia. Forewings present always a marked oblique external line, but which it could be of different tonality of brown: from dark-brown to light-brown; also the external forewings patch could be marked or vague. Genitalia show some difference on the apex of socii which varies from sharp to round with several intermediate shape. These intraspecific variation are based only on morphological traits and the large and fragmentary (as we know) distribution of *T. apologetica* could hide a group of species. For these reasons and without a largely sampling in order to study both morphology and DNA, and to not increase the confusion inside the genus, we prefer to reassess the status of *T. loxostigma* when more material will be come available, also for genetical analysis. Furthermore the morphological traits that should split *T. loxostigma* from others species of African clade, are intermediate between *T. jordana* and *T. apologetica*, which could suggest the present of a hybridization zone between this two species.

The identification of *T. apologetica abyssinica* is possible only by the collection site, any other difference are not identified (Basso *et al.*, in press) but considering the large areal of *T. apologetica*, and the possibility to the fragmentation into 3 areas (Fig. 4) and the variability of the environment colonized, we prefer to maintain the current subspecies.

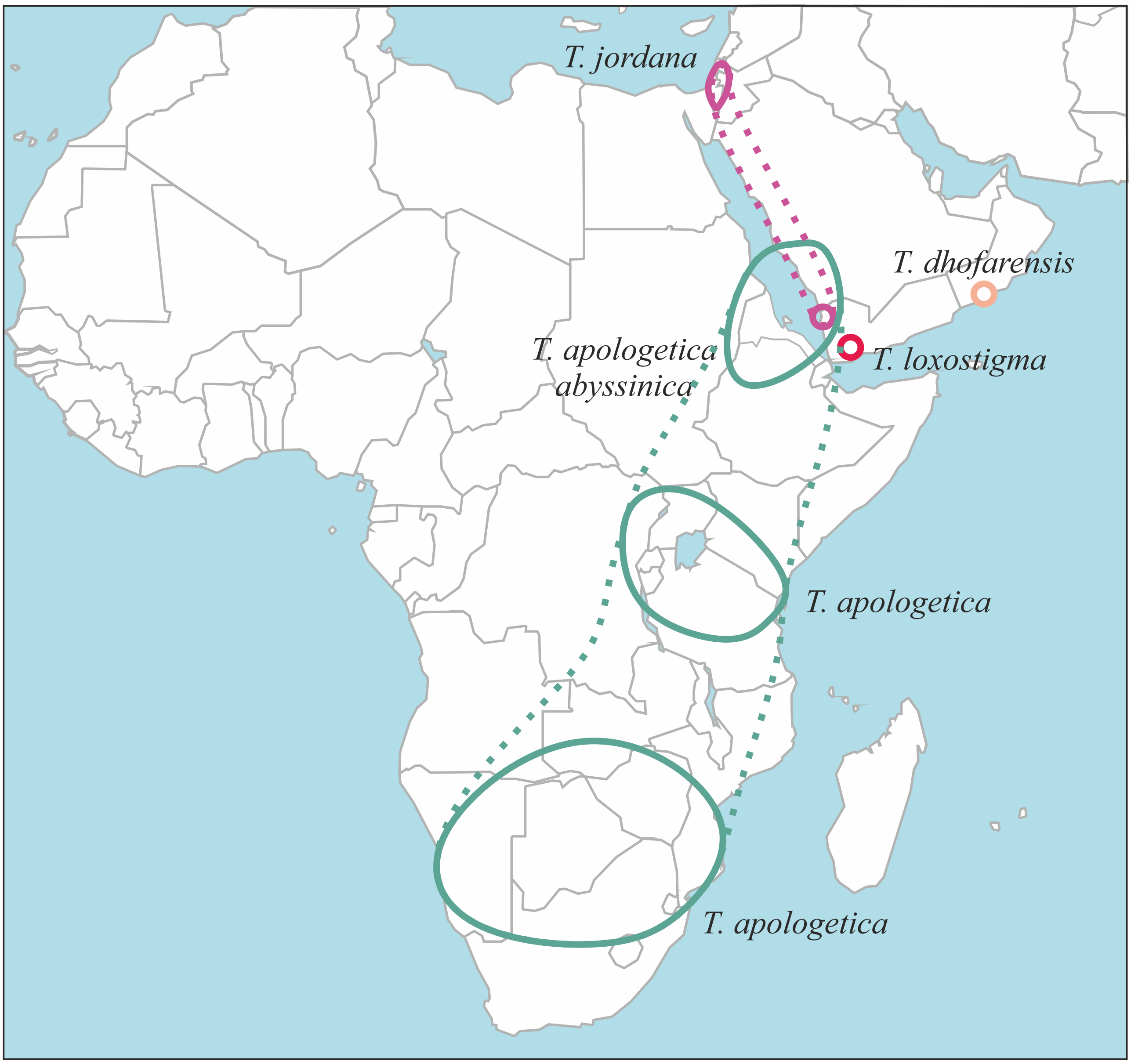

Figure 4. Geographic distribution of the species of the genus *Thaumetopoea* of the *apologetica* group. (Clade H, Chapter 2).

Thaumetopoea cheela Moore, 1883

Thaumetopoea cheela, Moore, 1883: 18; Syntypes: ♂, Umballa district, Cap. Reed - Ref.: BMNH(E)_1378635 - Prep.: Not_2111; ♀, Umballa district Cap. Reed - Ref.: BMNH(E)_1378634; – British Natural History Museum, London. Type locality: Umballa district [North-West India].

Type material examined: ♂, Umballa district - Ref.: BMNH(E)_1378635 - Prep.: Not_2111, Syntype; ♀, Umballa district, Cap. Reed - Ref.: BMNH(E)_1378634, Syntype; – British Natural History Museum, London.

Other material examined: 2 ♂, Sarobi, Afghanistan; – The Bavarian State Collection of Zoology of Munich.

Diagnosis:

Male. (Fig. 1g). Head covered by brownish-grey hairs. Scape tuft brownish-grey. Antennae long, and ochraceous with medium rami (ratio between the length of antenna and rami is from 6 to 8). Pecten brownish grey. Adfrontal tuft dark. Frons crested; with 7 teeth. Apical tooth rear; base narrower than width of crest and low but well separated. Second one pronounced but short. Palpus small. Foreleg medium stout. Spine present and epiphysis claw shaped. Thorax dark dorsally and greyish ventrally, with 2 whitish lateral spots that arise to the root of forewings. Abdomen brownish Wingspan 31 mm. Forewing colour ash grey, ground colour. Three characterized thin dark lines present, from costa to inner margin. Basal line interrupted on medial vein, and zigzagged with marked yellow edges. Ante-median line continuous and curved, with external yellow edge. Post-median line jagged, with internal yellow edge. Ante and post-median lines parallel, on costa and on inner margin. Inner margin last segments of ante-median and post-median line straight. Discal spot present, half-moon shaped, with white edge. Terminal white line present. Fringe, uniseriate and not concolorous, alternatively brown and white. Fringe distal edge almost flat, with three teeth not clearly visible. Hindwing whitish with dark anal spot and brownish-grey anal margin. Fringe, not concolorous, alternatively brown and white. Hindwing underside as upside, with vague central spot.

Male genitalia. (Fig. 2g). Uncus shorter but well developed, with 2 apical small teeth. Socii triangular shape and arise basally from uncus. Socii lateral edge slightly concave and smooth. Lower teeth extremely reduced. The apex of socii narrow. Tegumen well developed. Valva S-shaped. Lower margin initially straight then convex, upper margin slightly concave, with a tiny tip before cucullus. Bulge on cucullus present but reduced, folded for less than ¼ of valva. Bulge causes convexity on upper side and concavity lower side. Upper margin folded initially present strongly junction. Juxta pentagonal and well developed and wide, without rib. Upper angle smooth, lateral edge lower concave and then convex, with rounded angle, lower edge convex. Aedeagus flared basally and straight.

Female. Wingspan 35 mm. Wing ground colour sharp and well define as in male. Anal scales straight, and narrow, with spread pigmentation. Ratio, between length and width, over 4.

Larva and **Life history.** Unknown, but see Chapter 4 for details.

Distribution. Northern India (Moore, 1883), Northern Pakistan, Northern Afghanistan

(Fig. 5).

Thaumetopoea bonjeani (Powell, 1922)

*Cnethocampa bonjeani*Powell, 1922: 188. Syntype: ♀, Morocco, d'Azrou Forest, 500 m to North of Douar de Garde - Ref.: BMNH(E)_1378636; – British Natural History Museum, London. Type locality: Morocco.

Type material examined: ♀, Morocco, d'Azrou Forest, 500 m to North of Douar de Garde - Ref.: BMNH(E)_1378636, Syntype; – British Natural History Museum, London.
Other material examined: ♂ Maroc, Atlas Mts. Ifrane, 28.VI.1979, (T. Larsen), Coll. EPW - Ref.: BMNH(E)_1378573 - Prep.: Not_2083; – British Natural History Museum, London.
6 ♂ Algeria, Tala Guilef, 1825m, N36°38', E4°10' VIII-IX 2014 fresh specimens, C. Kerdelhue. - Preps.: TH18, TH19, TH20; 6 ♂ Algeria, Tizi Oujavoub, 1167m, N36°26', E3°56' VIII-IX 2014 fresh specimens, C. Kerdelhue. - Preps.: TH21, TH22, TH23; – DAFNAE University of Padua.
6 ♀ Algeria, Tala Guilef, 1825m, N36°38', E4°10' VIII-IX 2014 fresh specimens, N. Rahim. 6 ♀ Algeria, Tizi Oujavoub, 1167m, N36°26', E3°56' VIII-IX 2014 fresh specimens, N. Rahim. – DAFNAE University of Padua.

Diagnosis:

Male. (Fig. 1h). Head generally brownish-grey. Scape tuft brownish-grey. Antennae long as or more than half of forewing costa, and ochraceous with medium rami (ratio between the length of antenna and rami is from 6 to 8). Pecten brownish grey. Adfrontal tuft ochraceous. Frons crested; with 7 teeth. Apical tooth rear; base narrower than width of crest and low but well separated. Second one pronounced, from 2 to 3 times bigger than third. Palpus small with first segment longer and wider than second segment. Foreleg medium stout. Spine present and epiphysis claw shaped. Thorax dark dorsally and greyish ventrally, with 2 whitish lateral spots that arise to the root of forewings. Abdomen brownish. Wingspan 30-34 mm. Forewing ground colour ash grey and costal margin marked. Three characterized thin dark lines presents, from costa to inner margin. Basal line interrupted on medial vein, and zigzagged with marked yellow edges. Ante-median line continuous and zigzagged, with external yellow edge. Post-median line jagged, with internal yellow edge. Ante and post-median lines parallel on costa and convergent on inner margin. Inner margin last segments of ante-median line outwardly, while post-median line straight. Discal spot present, half-moon shaped, with white edge. Terminal line present. Fringe, uniseriate and not concolorous, alternatively brown and white. Fringe distal edge almost flat, with three teeth not clearly visible. Hindwing whitish with marked anal spot and brownish fluffy anal margin (Basso *et al.*, 2016). Fringe, concolorous, white. Hindwing underside without pattern.

Male genitalia. (Fig. 2h). Uncus shorter but well developed, with 2 apical teeth. Socii narrow with triangular shape and arise basally from uncus. Socii lateral edge slightly convex and wrinkle. Lower teeth sharp, not well developed and inwardly. The apex of socii narrow and smooth tegumen well developed. Valva S-shaped. Lower margin initially concave then convex, upper angle rounded smooth, with an extremely concavity before cucullus. Bulge on cucullus present, folded for ½ of valva. Bulge causes convexity on upper side and concavity lower side. Upper margin folded initially present strongly junction. Juxta pentagonal as long as wide, ribbing over the length. Upper angel rounded and smooth, lateral edges upper convex and then concave, with rounded angle, lower edge almost straight. Aedeagus flared basally and slightly curved.

Female. Wingspan 34-38 mm. Wings ground colour pale and not well defined. Head and thorax dark with light hairs; abdomen brownish with dark bands. Anal scales straight, and sharp, pigmented in the centre. Ratio, between length and width, between 2 and 3.

Larva. Larvae are blackish in ground colour, with greyish lateral line, a dotted black line is present up to the trachea. Long withish hairs are grouped on tufts, and urticating hairs are located on orange spots.

Life history. *Thaumetopoea bonjeani* is monophagous on *Cedrus atlantica* (Battisti *et al.*, 2015; Basso *et al.*, 2016). Eggs are laid between August and September on underside of cedar branches (Rahim et al. 2016). Eggs overwinter and they hatch during the spring. Larvae make light silky tent and reach the fitfh instar in June-July (Battisti *et al.*, 2015; Basso *et al.*, 2016). They pupate into soil.

Distribution. North-western Africa (Roques, 2015) (Fig. 5).

Thaumetopoea pinivora (Treitschke, 1834)

Gastropacha pinivora Treitschke, 1834: 194. Syntypes: Unspecific number of ♂and ♀, Norddeutschaland [Northern Germany], probably Sternberg, SW of Rostock (ex Bieberteich Neumark) according to Schintlmeister (2013) – Magyar Természettudományi Múzeum, Budapest. Type locality: [Northern Germany].
Note: single Lectotype founded in Magyar Természettudományi Múzeum, Budapest was labelled as “Syntype” by curator Bàlint, 2011-XII.14. (comm. pers.).

Type material examined: ♂, [Northern Germany], Lectotype.
Other material examined: ♂, France, Leech Coll. 1901-173 - Ref.: BMNH(E)_1378579 - Prep.: Not_2078; ♂, Pomerania, ExColl De Grazlin - Ref.: BMNH(E)_1378578 - Prep.: Not_2079; – British Natural History Museum, London.
♂, Bernau, Berlin, Germany - Prep.: TH 35; ♀, unknown; – Museo di Zoologia, “Sapienza” (Rome).
♂, Germany Sachsen Waldheim, IX.1917 e.coll. J.Thurner - Prep.: TH 24; ♀, Ostpreussen e.l. 16.6.09, P 08 Fschhauten, W. Forster; – The Bavarian State Collection of Zoology, Munich.
♂, Germany (Cnethocampa pinivora); – Museo di Storia Naturale “La Specola”.

T. pinivora ab. *nigromarginata,* Petersen, 1899: 245.

T. pinivora ab. *plutonia,* Schultz, 1905: 115.

Diagnosis:

Male. (Fig. 1i). Head and scape tuft brownis-grey. Antennae ochraceous and shorter than half of costa, with short rami (ratio between the length of antenna and rami up to 9). Pecten brownish grey. Adfrontal tuft dark. Frons crested with 7 teeth. Apical tooth rear; base narrower than width of crest and low but well separated. Second one well pronounced but short. Palpus small, first segment longer and wider than second. Foreleg medium stout. Spine present and epiphysis claw shaped. Thorax brownish grey with 2 whitish lateral spots that arise to the root of forewings. Abdomen brownish. Abdomen brownish. Wingspan 27-37 mm. Forewing ground colour ash grey, with 3 typical dark lines in basal, ante-median and post-median positions. Basal line zigzagged, from costa to inner margin, continues. A yellow edge present on both sides. Ante-median line zigzagged with yellow external edge. Post-median line dark and jagged, with internal edge. Ante and post-median lines parallel on costa and convergent on inner margin. Inner margin last segments of ante-median line outwardly, while post-median line inwardly. Discal brown marked spot present with half-moon shape, without edge. Terminal dark line present Fringe, uniseriate and not concolorous, alternatively brown and white. Fringe distal edge almost flat, with three teeth not clearly visible. Hindwing whitish, with brownish grey fringe. Anal mark spot present, anal margin brownish grey. Fringe, not concolorous, alternatively brown and white. Hindwing underside without pattern.

Male genitalia. (Fig. 2i). Uncus claw-like and developed, with 2 apical small teeth. Socii from uncus triangular. Lateral margin convex and wrinkled; apex narrow. Teeth on posterior side of socii sharply marked and straight. Valva S-shaped. Lower margin initially, concave then convex. Upper margin straight on first segment, then convex. Initially folded, without ribbing. Cucullus rounded, with bulge that produce a folding for ¼ or less valva. Juxta pentagonal, well developed and wide, with ribbing. Upper angle rounded and smooth, lateral edge lower concave, then convex, with rounded angle, lower edge straight. . Aedeagus flared basally and basally gently curved.

Female. Wingspan 34-38 mm. wings pale and not well definite. Head and thorax brownish grey. Abdomen anal tuft covered by dark scales, with spread pigmentation. Ratio, between length and width from 2 to 3.

Larva. Larvae are blackish in ground colour, withish laterally, with a black line on spiracles. Larvae are coverd by long withish hairs that aris from dots lacated long body. On abdominal segments, they have oval black spot where are located the urticating hairs (Battisti *et al.*, 2015).

Life history. *Thaumetopoea pinivora* is monophagous on *Pinus sylvestris,* but it coul be occasionally found on *Pinus nigra* and *Pinus mugo* (Hodar *et al.*, 2016). *Thaumetopoea pinivora* was mainly studied in northern Europe where it has a biannual development (Battisti *et al.*, 2015). Eggs are laid in July-August, and overwinter. In late April, they hatch. Larvae group and start to feed on host plant until late July, through five instar. Then they go to pupate, down to the trees in a procession and digging into soil where they overwinters until next July. They do not build tent (Battisti *et al.*, 2015). A portion of pupae enter prolonged diapaused (Battisti *et al.*, 2015).

Distribution. South-western Europe, Northern Europe (Agenjo, 1941; Cassel et al. 2013; Roques, 2015) (Fig. 5).

Thaumetopoea libanotica Kiriakoff and Talhouk, 1975

Thaumetopoea libanotica, Kiriakoff and Talhouk, 1975: Holotype: ♂, Bsherre, Lebanon, A. S. Talhouk coll. emerged Beirut 8.8.74; Allotype: ♀, Bsherre, Lebanon, A. S. Talhouk coll. emerged Beirut 8.8.74 – The Bavarian State Collection of Zoology, Munich. Type locality: Lebanon.

Type material examined: ♂, Bsherre, Lebanon, A. S. Talhouk coll. emerged Beirut 8.8.74, Holotype; ♀, Bsherre, Lebanon, A. S. Talhouk coll. emerged Beirut 8.8.74, Allotype; – The Bavarian State Collection of Zoology, Munich.

Other material examined: ♂, Lebanon, Bsherre, 4.VIII.34 E. P. Wiltshire - Ref.: BMNH(E)_1378639 - Prep.: 250; ♂, Lebanon, Bsherre, 4.VIII.34 E. P. Wiltshire - Ref.: BMNH(E)_1378640 - Prep.: 215; 1♀, Lebanon, Eklosion, Bcharre 4.VIII.34, E. P. Wiltshire – British Natural History Museum, London.

♀, 11.9.1961 e.l. Libanon, 25 Km N von Beirut, & Vartian – The Bavarian State Collection of Zoology, Munich.

Diagnosis:

Male. (Fig. 1j). Head and Scape tuft brownish-grey. Antennae ochraceous and shorter than half of costa, with short rami (ratio between the length of antenna and rami up to 9). Pecten brownish grey. Adfrontal tuft dark. Frons crested with 7 teeth. Apical tooth rear; base narrower than width of crest and low but well separated. Second one well pronounced but short, 2 times longer than third. Palpus small, first segment long as half and narrower than second. Foreleg medium stout. Spine present and epiphysis claw shaped. Thorax brownish grey with 2 whitish lateral spots that arise to the root of forewings. Abdomen brownish. Wingspan 26 mm. Forewing ground colour brown. Three characterized thin dark line presents, extended from costa to inner margin. Basal zigzagged line interrupted on medial vein, extended from costa to inner margin, with both yellow edges. Ante-median zigzagged line with external yellow edge, continuous. Post-median jagged line, with internal yellow edge. Ante and post-median lines parallel on costa and inner margin. Inner margin last segments of ante-median line outwardly, while post-median line straight. Discal brown spot present as dot, without edge. Terminal dark line present. Fringe, uniseriate and not concolorous, alternatively brown and white. Fringe distal edge almost flat, with three teeth not clearly visible. Hindwing whitish, with brownish grey fringe and anal margin. Vague central and anal spots present. Fringe, not concolorous, alternatively brown and white. Hindwing underside without pattern.

Male genitalia. (Fig. 2j). Uncus claw-like and developed, with 2 apical small teeth. Socii from uncus triangular. Valva S-shape slightly elongate, with little bulge. Juxta pentagonal. Upper angle rounded smooth, lateral edge lower and upper side concave, with rounded angle, lower edge straight. Aedeagus medium, with gently basal curve.

Female. Wingspan 32 mm. Wings ground colour pale and not well defined. Head and thorax with greyish hairs; abdomen ochraceous with dark bands. Antennae bipectinated. Abdomen covered by anal scales, spotted in centre. Ratio, between length and width from 2 to 3.

Larva. Larvae are blackinsh in ground colour, with a dorsal series of reddish spot where are located withish long setae. On abdominal segments, they have oval black spot with reddish edge where are located the urticating hairs. Laterally greysh band is interruped by a black dotted line up to the trachea (Kiriakoff and Talhouk, 1975).

Life history. *Thaumetopoea libanotica* is monophagous on *Cedrus libani* (Kiriakoff and Talhouk, 1975; Battisti *et al.*, 2015). Larval information about this species are scarce and limited to a laboratory rearing (Battisti *et al.*, 2015). Kiriakoff and Talhouk (1975) collected a colony of second instar larvae from a cedar tree in May and they rear it until they pupate into soil at the end of June. In August adults emerged (Kiriakoff and Talhouk, 1975; Battisti *et al.*, 2015).

Distribution. Near East (Roques, 2015) (Fig. 5).

Thaumetopoea ispartaensis (Doganlar and Avci, 2001)

Thaumetopoea ispartaensis Doganlar and Avci, 2001: 19. Holotype: ♂, 21.VIII.1999, host: *Cedrus libani*. Allotype and Paratype: 2 ♂; 3 ♀, same data as holotype – Plant Department, Mustafa Kemal University, Antakya, Turkey. Type locality: Turkey, Isparta.

Type material examined: no primary types have been available for this study.
Other material examined: 3♂, Turkey, Isparta, Senirkent 1400-1600m, *Cedrus libani* - Preps.: TH30, TH61; 1♀, Turkey, Isparta, Senirkent 1400-1600m, Cedrus libani; (DAFNAE).

Diagnosis:

Male. (Fig. 1k). Head covered by brownish-grey hairs. Scape tuft brownish-grey. Antennae short, ochraceous, with short rami (ratio between the length of antenna and rami up to 9). Pecten brownish grey. Adfrontal tuft dark. Frons crested, , with 7 teeth. Apical tooth rear; base narrower than width of crest and low but well separated. Second one pronounced but short. Palpus short, with first segment half and narrower than second. Foreleg medium stout. Spine present and epiphysis claw shaped. Thorax dorsally covered by dark brown hairs, with 2 whitish lateral spots that arise to the root of forewings. Ventrally covered by whitish-yellow hairs. Abdomen brown. Wingspan 26-29 mm. Forewing ground colour brown, with three typical dark lines, from costa to inner margin. Costa with mark dark margin. Dark basal line interrupted on medial vein, and zigzagged with marked yellow edges. Ante-median line curved with external yellow edge. Post-median jagged line, with internal yellow edge. Ante and post-median lines divergent on costa and on inner margin. Inner margin last segments of ante-median line and post-median line inwardly. Discal brown spot present as dot, without edge. Terminal line vague line present. Fringe uniseriate and concolorous, alternatively white and brown. Fringe distal edge almost flat, with three teeth not clearly visible. Hindwing whitish, with brownish grey fringe and anal margin. Vague central spot and without anal spots. Fringe, not concolorous, alternatively brown and white. Hindwing underside without pattern.

Male genitalia. (Fig. 2k). Uncus claw-like and developed, with 2 apical small teeth. Socii from uncus triangular with narrow apex with vague rib. Lateral margin convex and wrinkled. Posterior tooth of socii small, sharp and inwardly. Valva S-shaped. Lower margin initially, concave then convex. Upper margin concave on first segment, then convex. Initially folded, without ribbing.

Marked junction present. Cucullus rounded, with bulge that produce a folding for ¼ or less valva. Juxta pentagonal, well developed and wide. Upper angle smooth, lateral edge lower convex, then concave, with rounded angle, lower edge slightly convex. Aedeagus flared basally and basally gently curved.

Female. Wingspan 34-37 mm. Wings ground colour pale and not well defined hindwing dark margin or anal spot absents. Head and thorax dark with light hairs; abdomen brownish with dark bands. Last tergit covered by dark wide scales with pigmentation localized in central part. Ratio, between length and width, less than 2.

Larva. No morphological information are available.

Life history. *Thaumetopoea ispartaensis* is monophagous on *Cedrus libani* (Doganlar and Avci, 2001; Battisti *et al.*, 2015; Basso *et al.*, 2016). Eggs are laid between August and September and overwinter. Eggs hatch around April. They live together into light greyish silk tents until fifth instat that is reached on end of June-July (Basso *et al.*, 2016). After they start to pupate, digging into sunny areas near the cedar forests (Battisti *et al.*, 2015; Basso *et al.*, 2016). Prolonged diapause was not observed (Battisti *et al.*, 2015; Basso *et al.*, 2016).

Distribution. Anatolia (Doganlar and Avci, 2001; Roques, 2015) (Fig. 5).

Thaumetopoea sedirica (Doganlar et al., 2005)

Traumatocampa sedirica Doganlar et al., 2005Doganlar *et al.*, 2005: 229. Holotype: ♂, Turkey, Isparta, Sarkikaragac, 38°02’ 18”N, 31°22’35”E, *Cedrus libani*, 17 August 1968, legit Tosun – Museum of the Agricultural Faculty, Mustafa Kemal University, Hatay, Turkey. Paratypes: 8 ♂, 3 ♀, Turkey, Isparta, Sarkikaragac, same locality as holotype, 17.VIII.-5.ix.l967 – Museum of the Agriculture Faculty, Mustafa Kemal University, Hatay, Turkey. 17 ♂, 2 ♀ Turkey, 1400m, 12.-29. VIII.1968 (Tosun) – Museum of Forest Faculty, Istanbul University, Turkey. Type locality: Turkey, Sedir.

Type material examined: 2 ♂, Sarkikaragac Sedir, 28.VIII.1967, Paratype; Preps.: TH29, TH31; ♂, Sarkikaragac Sedir, 14.VIII.1968; ♂, Sarkikaragac Sedir, 4.VIII.1968, Paratype; ♀, Sarkikaragac Sedir, 14.VIII.1968, Paratype, Det. M. Doğanlar 2005 – DAFNAE University of Padua

Other material examined: ♂, ♀, TR: Isparta, Senirkent, Kapidagi, 21.VIII.1999, M. Avci.

Labelled as T. torosica (Paratype): 4♂, 1♀ Turkey, Adana, Aladag, ex *Pinus brutia*, 25.IX.2000 F. Doğanlar; Det. M. Doğanlar 2005. Preps.: TH28; TH64 – DAFNAE University of Padua, Italy

Thaumetopoea torosica, (*Traumatocampa torosica*), (Doganlar *et al.*, 2005): 233, syn. nov.

Diagnosis:

Male. (Fig. 1l). As *T. ispartaensis* with some exceptions listed below. Wingspan 30-37 mm. Antennae shorter than half of costa, with short rami (ratio between the length of antenna and rami up to 9). First segment of labial palpus as long as and wider than second. Basal line on forewing stopped before costal margin. Ante and post-median lines divergent on costa and convergent on inner margin. Discal spot half-moon shaped. Hindwing with vague anal spot.

Male genitalia. (Fig. 21). No significant differences with *T. ispartaensis* were observed.

Female. Wingspan 33-40 mm. Anal scale dark and wide as in *T. ispartanensis,* with spread pigmentation. Ratio, between length and width, between 2 and 3.

Larva. No morphological information are available.

Life history. *Thaumetopoea sedirica* feeds on *Cedrus libani* (Doganlar *et al.*, 2005). Eggs are laid in cluster on the host bark (Doganlar *et al.*, 2005). No other information are available.

Distribution. Anatolia (Doganlar *et al.*, 2005) (Fig. 5).

Remarks: considering the Turkish species (*T. ispartaensis*, *T. sedirica* and *T. torosica*), we note that the these 3 taxa are spread into a small area, without any finding outside this zone. In the meantime, by recent analysis (Basso *et al.*, in press), *T. torosica* does not have significant apomorphies that allow to discriminate it by *T. sedirica*. Furthermore considering the absence in *T. torosica* paratypes of diagnostic traits reported in the original description, we suggest that *T. torosica* should be treated as a synonym of *T. sedirica*.

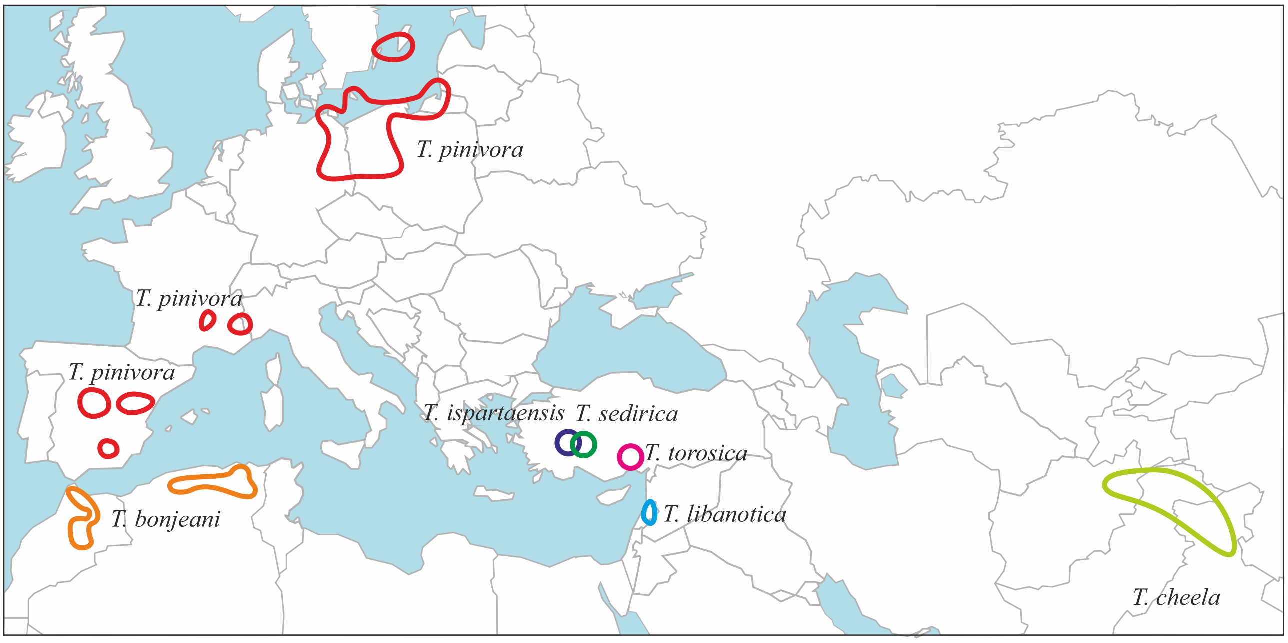

Figure 5. Geographic distribution of the species of the genus *Thaumetopoea* associated with conifers and feeding in summer (Clade O, Chapter 2).

Thaumetopoea pityocampa ([Denis & Schiffermüller], 1775)

Bombyx pityocampa [Denis & Schiffermüller], 1775: 58 (as *B. Pityocampa*). Syntypes, males and females (not located). Type locality: not indicated [Mediterranean Basin].
Note: unknown types depository, or type locality as stated by Schintlmeister (2013).

Type material examined: no primary types have been available for this study.
Other material examined: ♂, Portugal, Serra da Estrela 800m 25.8.1927. Querci, Rothschild Bequest B.M.1939-1 - Ref.: BMNH(E)_1378589 - Prep.: Not_2064; ♂, Portugal, Serra da Estrela 800m 25.8.1927. Querci, Rothschild Bequest B.M.1939-1 - Ref.: BMNH(E)_1378588 - Prep.: Not_2065; ♂, Portugal, Wattison Coll. B.M. 1949-183 (Purchased) - Ref.: BMNH(E)_1378590; ♂, Portugal, Wattison Coll. B.M. 1949-183 (Purchased) - Ref.: BMNH(E)_1378591; ♂, Guelt es Stel Algerie 7.8.1915 - Ref.: BMNH(E)_1378586; ♂, Guelt es Stel Algerie 5.8.1915 - Ref.: BMNH(E)_1378587 - Prep.: Not_2066; ♂, Majorca, C'an Pastilla 14-31.VIII1961 E.W.Classey B.M. 1961-622 - Ref.: BMNH(E)_1378585 - Prep.: Not_2067; ♂, Majorca, C'an Pastilla 14-31. VIII1961 E.W.Classey B.M. 1961-622 - Ref.: BMNH(E)_1378584 - Prep.: Not_2068; ♂, Greece, Thassaloniki, 28.VIII.64, E. Bowers & Mrs Treherne - Ref.: BMNH(E)_1378583 - Prep.: Not_2071; ♂, Greece, Thassaloniki, 28.VIII.64, E. Bowers & Mrs Treherne - Ref.: BMNH(E)_1378582 - Prep.: Not_2072; ♂, Pyrénées-Orientales, Randal; Mariailles; Haut Cady. Juillet et Aout 1909, H.Powell (27) - Ref.: BMNH(E)_1378637 - Prep.: Not_2112; ♂, Higires, aout 1908 - Ref.: BMNH(E)_1378563 - Prep.: Not_2113; – British Natural History Museum, London.
♂, Spain, 2006. Fresh specimen - Prep.: TH 9; ♂, Spain, 2006. Fresh specimen - Prep.: TH 10; ♂, Italy, Friuli V.G. - Moggio Udinese. 700m N46°27'60", E13°11'47", IV.2009. Fresh specimens - Prep.: TH 12; ♂, Italy, Friuli V.G. - Moggio Udinese. 700m N46°27'60", E13°11'47", IV.2009. Fresh specimens - Prep.: TH 13; ♂♂, Italy, Friuli V.G. - Moggio Udinese. 700m N46°27'60", E13°11'47", IV.2009. Fresh specimens - Prep.: TH 17; ♂♂, Calbarina - Italy, N45°16', E11°43'; 136m, 1999. 1151-43. Fresh specimens - Prep.: TH 46; ♂♂, Calbarina - Italy, N45°16', E11°43'; 136m, 1999. 1151-3. Fresh specimens - Prep.: TH 47; ♀♀, Alto Adige, Val venosta, VII 2002, Battisti; – DAFNAE University of Padua.
♂, Orebic - Dalmatia, 7-22.IX.36 leg. DR ZULLICH - Prep.: TH 32; – Museo di Zoologia, "Sapienza" (Rome).

T. pityocampa var. *nigra* Bang-Haas, 1910: 31. Synonymised with *T. pityocampa* by Kiriakoff, 1970.

T. pityocampa f. *obscura* Vorbrodt, 1911: 205.

T. pityocampa ab. *insignipennis* Strand, 1911: 144.

T. pityocampa ab. *convergend* Dannhel, 1925: 6.

T. pityocampa ab. *renegata* Dannhel, 1925: 6.

T. pityocampa f. *bicolor* Reisser, 1928: 17.

T. pityocampa ab. *illineata* Schawareda, 1932: 29.

T. pityocampa ab. *nigrofasciata,* Nitsche, 1933: (21).

T. pityocampa var. *cancioi* Agenjo, 1941: 86. Synonymised with *T. pityocampa* by Kiriakoff, 1970.

T. pityocampa var. *ceballosi* Agenjo, 1941: 88. Synonymised with *T. pityocampa* by Kiriakoff, 1970.

T. pityocampa var. *clara* Agenjo, 1941: 87. Synonymised with *T. pityocampa* by Kiriakoff, 1970.

T. pityocampa var. *pujoli* Agenjo, 1941: 86. Synonymised with *T. pityocampa* by Kiriakoff, 1970.

T. pityocampa var. *vareai* Agenjo, 1941: 88. Synonymised with *T. pityocampa* by Kiriakoff, 1970.

T. galaica Soler, Lanaspa & Pasqual, 1982: 92. Synonymised with *T. pityocampa* by de Freina and Witt, 1985.

Diagnosis:

Male. (Fig. 1l). Head generally black. Antennae ochraceous and longer than half of costa, with medium rami (ratio between the length of antenna and rami is from 6 to 8). Pecten ochraceous. Adfrontal tuft ochraceous. Frons crested with 6 teeth. Apical tooth rear; base large as width of crest and high to be clearly visible. Second one pronounced: well developed and long, 4 times longer than third tooth. Palpus small, with first segment long as second and wider. Foreleg stout. Spine present and epiphysis claw shaped. Thorax covered by dark brown hairs with 2 whitish lateral spots that arise to the root of forewings. Abdomen ochraceous. Wingspan 29-39 mm. Forewing ground colour brown. Costa dark marked. Three dark lines present. Basal and ante-median lines zigzagged and continuous from costa to inner margin. Post-median line jagged producing an angle around 30° with costa. Ante and post-median lines divergent on costa parallel and on inner margin convergent. Inner margin last segments of ante-median line and post-median line outwardly. Discal spot marked with half-moon shape and ringed by an edge of white scales. Terminal line brown. Fringe, uniseriate and not concolorous, alternatively brown and white. Fringe distal margin jagged, with three teeth clearly visible. Hindwing general ground colour and fringe whitish. Terminal vague brownish line present. Central spot and anal spot present. Anal margin whitish.

Male genitalia. (Fig. 2l). Uncus shorter but well developed with 2 apical teeth. Socii narrow with half-moon shape and arise basally from uncus. Socii lateral edge convex and smooth. Apex rounded while lower teeth sharp, well developed and straight. Little ribbing present under the apex. Tegumen well developed. Valva reverse-claw shaped. Lower margin initially straight then convex. Upper margin convex then concave. Cucullus simple and rounded slightly upwards. Juxta hexagonal as long as wide, upper edge concave, lateral edges upper straight and then convex, with rounded angle, lower edge convex. Ribbing in centre. Aedeagus stout, flared basally and straight.

Female. Wingspan 36-49 mm. Wings ground colour pale and not well defined. Head and thorax dark with light hairs; abdomen ochraceous with dark bands. Anal scales in middle wide between 0.7 and 1 mm, more or less straight, and pigmented in centre. Ratio, between length and width under 2.

Larva. Larvae is blackish in ground colour, with grey lateral band. Setae are whitish laterally and reddish on top especially in urticating tergites.

Life history. *Thaumetopoea pityocampa* is oligophagous on *Cedrus* spp. and *Pinus* spp.. *Thaumetopoea pityocampa* is generally univoltine, with the exception of mountains of Corsica Island, where it is semivoltine. Adults emerging from June to September and larvae feeding during the winter. With an exception of population locating in Portugal that feed during summer such as result of allochronic differentiation (Santos *et al.*, 2007, 2011).

Distribution. Southern and central Europe, Middle East, North Africa (Agenjo, 1941; Kiriakoff, 1970; Schintlmeister, 2013; Roques, 2015) (Fig. 6).

Thaumetopoea pityocampa orana (Staudinger, 1901)

Cnethocampa pityocampa var. *orana* Staudinger, 1901: 113. Syntypes: ♂, ♀, Oran, western Mauretania – Museum fur Naturkunde Berlin. Type locality: Oran.

Type material examined: ♂, ♀, Oran, western Mauretania, Syntype – Museum fur Naturkunde Berlin.

Other material examined: ♂, Maroc, foret d'Azrou, Harold Powell, Aoug 1920 - Ref.: BMNH(E)_1378569 - Prep.: Not_2073; ♂, Belvedere, Tunis E.Aug.-Sept. 1915 (M. Blanc) - Ref.: BMNH(E)_1378567 - Prep.: Not_2074; ♂, Sidi-bel-Abbes, Prov.Oran, 20.VIII.1917 (M. Rotrou) - Ref.: BMNH(E)_1378565 - Prep.: Not_2075; ♂, Sidi-bel-Abbes, Prov.Oran, 20.VIII.1917 (M. Rotrou) - Ref.: BMNH(E)_1378566 - Prep.: Not_2076; ♂, Guelt-es-Stel C. Algerie Septemb.1913 - Ref.: BMNH(E)_1378568 - Prep.: Not_2077; ♂, Sebdou, Prov. Oran, 13. Sept.1918 (P. Rotrou) - Ref.: BMNH(E)_1378564; – British Natural History Museum, London.

Diagnosis:

Male. Quite similar to T. pityocampa. they differs only for some colour that in orana are lighter than T. pityocampa. Especially on head, thorax and forewings, but other light specimens was identify also between Spain and France.

Female. No differences with nominal species was observed.

Larva and **Life history.** Unknown.

Distribution. Western Algeria (Oran province) (Fig. 6).

Thaumetopoea wilkinsoni Tams, 1925

Thaumetopoea wilkinsoni Tams, 1925: 293. Holotype: ♂, Cyprus, 29.4.2. *Thaumetopoea wilkinsoni* Tams Holotype, 1924. BRED Pine C. S.N. 31 - Ref.: BMNH(E)_1378629; – British Natural History Museum, London. Paratypes: ♀, *Thaumetopoea wilkinsoni* Tams Paratype, Platres 9/21 - Ref.: BMNH(E)_1378626; ♂, *Thaumetopoea wilkinsoni* Tams Paratype, Platres, Cyprus 5000-6000 feet. 16:9-16:10,1920. Cant. K. J. Hayward. - Ref.: BMNH(E)_1378628 - Prep.: Not_2102; ♂, *Thaumetopoea wilkinsoni* Tams Paratype, Platres, Cyprus 5000-6000 feet. 16:9-16:10,1920. Cant. K. J. Hayward. - Ref.: BMNH(E)_1378627 - Prep.: Not_2103; – British Natural History Museum, London. Type locality: Cyprus.

Type material examined: ♂, Cyprus, 29.4.2. *Thaumetopoea wilkinsoni* Tams Holotype, 1924. BRED Pine C. S.N. 31 - Ref.: BMNH(E)_1378629; – British Natural History Museum, London. ♀, *Thaumetopoea wilkinsoni* Tams Paratype, Platres 9/21 - Ref.: BMNH(E)_1378626; ♂, *Thaumetopoea wilkinsoni* Tams Paratype, Platres, Cyprus 5000-6000 feet. 16:9-16:10,1920. Cant. K. J. Hayward. - Ref.: BMNH(E)_1378628 - Prep.: Not_2102; ♂, *Thaumetopoea wilkinsoni* Tams Paratype, Platres, Cyprus 5000-6000 feet. 16:9-16:10,1920. Cant. K. J. Hayward. - Ref.: BMNH(E)_1378627 - Prep.: Not_2103; – British Natural History Museum, London.

Other material examined: ♂, E. P. Wiltshire, e.l. *Pinus halepensis* h:14.9.1947 Kyrenia CYPRUS - Ref.: BMNH(E)_1378570; ♂, E. P. Wiltshire, e.l. *Pinus halepensis* h:12.9.1947 Kyrenia CYPRUS - Ref.: BMNH(E)_1378571; ♂, Troodos, Cyprus, 2 july 1916 G. F. Wilson. (labeled as *T. pityocampa*) - Ref.: BMNH(E)_1378572; ♂, E. P. Wiltshire, e.l. *Pinus halepensis* h:14.9.1947 Kyrenia CYPRUS - Ref.: BMNH(E)_1378645 - Prep.: E. P. Wiltshire 281;– British Natural History Museum, London. ♂♂, Israel - Dishon, N33°04', E35°31', 6.X.2005, breeding Legnaro - Prep.: TH48; ♂♂, Israel - Dishon, N33°04', E35°31', 6.X.2005, breeding Legnaro - Prep.: TH49; ♂♂, Israel - Western Negev, Qisufim, N31°23'16", E34°24'54", September 2002, *Pinus halepensis*. Fresh specimens - Prep.: TH50; ♂♂, Israel - Western Negev, Qisufim, N31°23'16", E34°24'54", September 2002, *Pinus halepensis*. Fresh specimens - Prep.: TH51; ♂♂, Israel - Judean Foothills, Haruvit, N31°44'17", E34°51'11", September 2002, *Pinus halepensis*. Fresh specimens - Prep.: TH52; ♂♂, Israel - Judean Foothills, Haruvit, N31°44'17", E34°51'11", September 2002, *Pinus halepensis*. Fresh specimens - Prep.: TH53; ♂♂, Israel - Southern Judean, Yahir, N31°20'49", E35°03'03", September 2002, *Pinus halepensis*. Fresh specimens - Prep.: TH54; ♂♂, Israel - Southern Judean, Yahir, N31°20'49", E35°03'03", September 2002, *Pinus halepensis*. Fresh specimens - Prep.: TH55; – DAFNAE University of Padua.

Diagnosis:

Male. (Fig. 1m). Head generally black. Antennae ochraceous and longer than half of costa, with medium rami (ratio between the length of antenna and rami is from 6 to 8). Pecten ochraceous. Adfrontal tuft ochraceous. Frons crested with 6 teeth. Apical tooth rear; base large less than width of crest but high to be clearly visible. Second one pronounced: well developed. 2-3 times longer than third tooth. Palpus small, with first segment long as second and wider. Foreleg stout. Spine present and epiphysis claw shaped. Thorax covered by dark brown hairs with 2 whitish lateral spots that arise to the root of forewings. Abdomen ochraceous. Wingspan 33-38 mm. Forewing ground colour brownish grey. Costa dark marked. Three narrow dark lines present. Basal and ante-median lines zigzagged and not interrupted. Basal line do not reach costa and inner margin. Post-median line jagged producing an angle around 50° with costa. Ante and post-median lines on costa parallel and on inner margin convergent. Inner margin last segments of ante-median line outwardly and post-median line inwardly. Terminal line brown. Discal spot marked with half-moon shape and ringed by an edge of white scales. Fringe, uniseriate and not concolorous, alternatively brown and white. Fringe distal margin jagged, with three teeth clearly visible. Hindwing general ground colour and fringe whitish. Terminal marked brownish line present. Central vague spot and dark anal spot present. Anal margin whitish.

Male genitalia. (Fig. 2m). Uncus shorter but well developed with 2 apical teeth. Socii narrow with half-moon shape and arise basally from uncus. Socii lateral edge wrinkled and convex, with . rounded apex. Posterior tooth from socii well developed, rounded and straight. Little ribbing present under the apex. Tegumen well developed. Valva reverse-claw shaped. Lower margin initially straight then convex. Upper margin straight then concave. Ribbing along all over costal margin. Cucullus simple and rounded slightly upwards. Juxta hexagonal longer than wide, upper edge concave, lateral edges upper concave and then convex, with rounded angle, lower edge convex. Ribbing in centre. Aedeagus stout, flared basally and straight.

Female. Wingspan 41-45 mm. Wings ground colour pale and not well defined. Head and thorax dark with light hairs; abdomen ochraceous with dark bands. Anal scales in middle wider than 1 mm, rounded, and pigmented in centre. Ratio, between length and width under 2.

Larva. Larvae of *T. wilkinsoni* are morphologically indistinguishable from those of *T. pityocampa.*

Life history. *Thaumetopoea wilkinsoni* feeds mainly on *Pinus* spp. even if occasionally it can be found in *Cedrus* spp. (Battisti *et al.*, 2015). It shows a life cycle similar to that of *T. pityocampa* but delayed (Battisti *et al.*, 2015). Eggs start hatching from late August to November and larvae develop during autumn-winter (Battisti *et al.*, 2015). Larvae feed on trees until spring, after that they start procession and going pupate into soil. Diapause can be prolonged up to 9 years (Battisti *et al.*, 2015).

Distribution. Middle East (Agenjo, 1941; Roques, 2015) (Fig 6).

Remarks: the recognition of the *Thaumetopeoa pityocampa* ENA clade that is genetically well separated from the nominal species (Simonato *et al.*, 2007; Kerdelhué *et al.*, 2009), and the possibility of hybridization between *T. pityocampa* and *T. wilkinsoni* (Chapter S1)*,* make hard to define the boundaries of these species. Fortunately, for *T. pityocampa* and *T. wilkinsoni* sampled in the core of their distribution can be recognized by the presence/absence of marked costal ribs on valvae. This traits is present also in boundary population and in hybrids shows an intermediate situation (Chapter S1). However, in most cases the study of the genitalia to identify correctly the species of specimens is necessary. A different scenario is represented by the ENA clade, which does not seem to differ from *T.* pityocampa, although more material needs to be examined. In particular, the status of the subspecies spread in Algeria (*T. pityocampa orana*) has to be reconsidered, so we suggest to maintain the current situation until more information will be available.

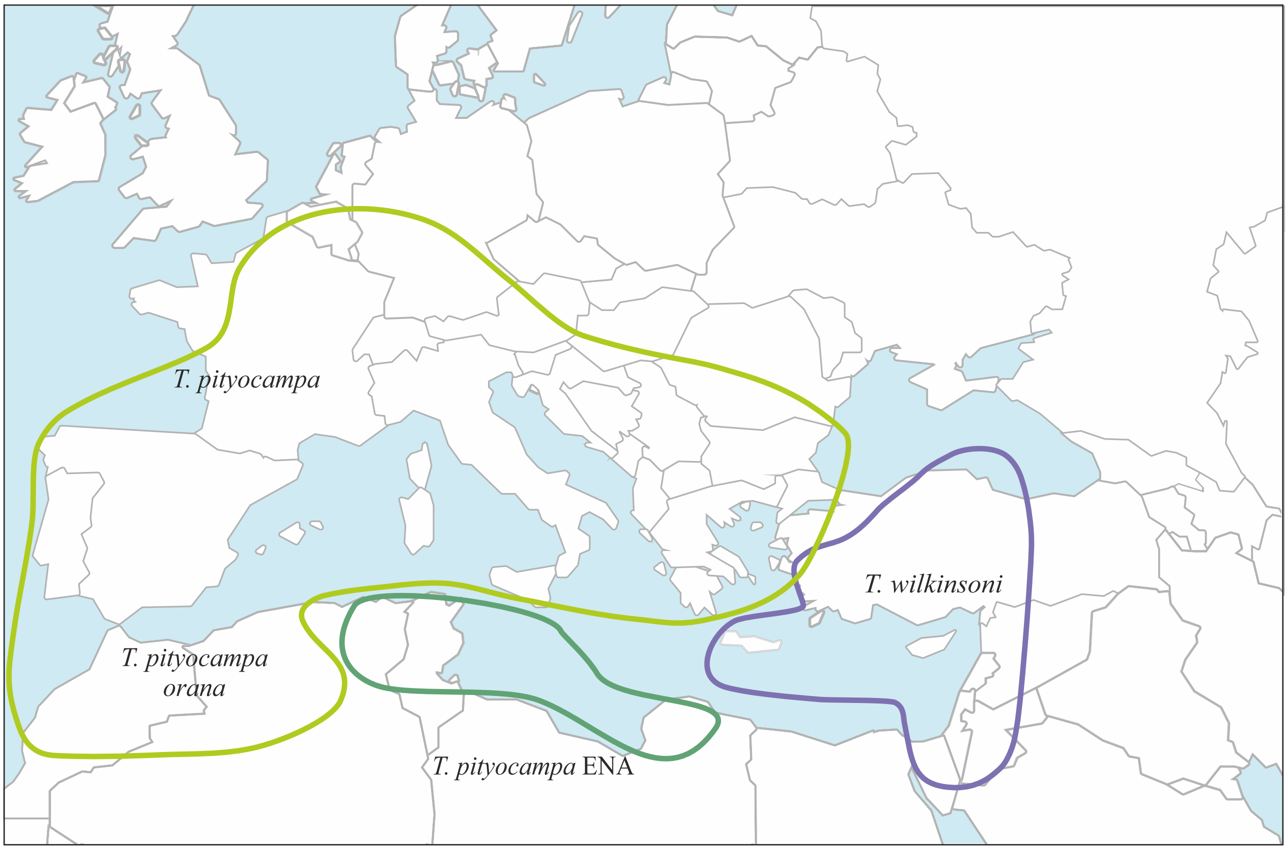

Figure 6. Geographic distribution of the species of the genus *Thaumetopoea* associated with conifers and feeding in winter (Clade L, Chapter 2).

Acknowledgments

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Table 1. Identificantion key of the adult males of the genus *Thaumetopoea*

1	Crest convex and smooth. Foretibia spine absent.	**2**
-	Crest with teeth. Foretibia spine present.	**5**
2	*Forewing:* lines on inner margin are parallel. *Hindwing:* without fascia. *Genitalia:* claw like valva, extremely sharp. *Aedeagus*: strongly curved at the base.	**3**
-	*Forewing:* lines on inner margin are convergent. *Hindwing:* with post-median fascia. *Aedeagus:* basal process present.	**4**
3	*Forewing:* ground colour brownish (Fig. 1a; Fig 2a).	***T. solitaria solitaria***
-	*Forewing:* ground colour light grey.	***T. solitaria iranica***
4	*Genitalia:* spear like valva; with rounded cucullus (Fig. 1b; Fig 2b).	***T. processionea processionea***
-	*Genitalia:* spear like valva; with narrow cucullus.	***T. processionea pseudosolitaria***
5	*Forewing:* ground colour light brownish or whitish.	**6**
-	*Forewing:* ground colour greyish.	**9**
6	Crest with six teeth, first big as second one. *Forewing:* with discal spot. *Genitalia:* spear like valva. Juxta heptagonal (Fig. 1c; Fig 2c).	***T. herculeana herculeana / T. herculeana judaea***
-	Crest with six teeth, first one flattened. *Forewing:* without discal spot; fringe jagged with 2 teeth. *Genitalia:* U-shape valva, cucullus rounded.	**7**
7	*Forewing:* post-median line straight, almost perpendicular on inner margin; marked streak segment on inner margin from post-medial line to the beginning of basal costal patch. *Genitalia:* rounded posterior tooth of socii (Fig. 1d, Fig 2d).	***T. jordana***
-	*Forewing:* post-median patch, more or less marked; streak segment on inner margin from post-medina line to half of basal costal patch.	**8**
8	*Hindwing:* brownish (Fig. 1e; Fig 2e).	***T. dhofarensis***
-	*Hindwing:* whitish; post-medial line vague and not always clearly visible (Fig. 1f; Fig 2f).	***T. apologetica /*** **(*T. loxostigma* to verify)**

9	Crest with six teeth, first one high rear and almost right. *Forewing:* lines without yellow edge; fringe jagged with three teeth. *Genitalia:* socii half-moon shaped. Valva S-shape.	**10**
-	Crest with seven teeth, first one small, rear and almost right. *Forewing:* lines with yellow edge; ante-median line smooth and curved; fringe smooth, with teeth not clearly visible. *Genitalia*: socii triangular shaped. Valva reverse-claw, first segment with marked convexity; cucullus upwardly. Juxta pentagonal.	**11**
10	*Forewing:* first segment (first 2 mm) of post-median line produce an angle of about 35-40° with costa; ante-median line almost straight on first anal vein; Ante-median and post-medina lines outwardly on inner margin. *Genitalia:* valva without costal fold (Fig. 1p; Fig 2p).	***T. pityocampa pityocampa / T. pityocampa orana***
-	*Forewing:* first segment (first 2 mm) of post-median line produce an angle of about 50-55° with costa; ante-median line produce an angle almost right on first anal vein; Ante-median line inwardly and post-medina line outwardly on inner margin. *Hindwing:* brownish terminal fascia. *Genitalia:* valva with marked ribbing over the length of costa. (Fig. 1o; Fig 2o)	***T. wilkinsoni***
11	*Forewing:* ante-median and post-median lines parallel on costa and convergent on dorsum; half-moon discal spot. *Hindwing:* inner margin whitish or vaguely ochraceous.	**12**
-	*Forewing:* ante-median and post-median lines not parallel on costa or not convergent on dorsum; dot discal spot. *Hindwing:* inner margin brownish.	**13**
12	*Forewing:* costa outlined by dark line; discal spot ringed by edge of white scales. *Hindwing:* fringe whitish. *Genitalia:* big bulge (cover almost 1/2 of valva) present on cucullus (Fig 1h; Fig 2h).	***T. bonjeani***
-	*Forewing:* costa not outlined; basal line without interruption in the middle; last segment of ante-median line extremely outwardly and post-median line inwardly on dorsum; discal spot not ringed; Ante-median line produce an obtuse angle on first anal vein. *Hindwing:* fringe white and brown. *Genitalia:* small bulge (cover almost 1/4 of valva) present on cucullus. (Fig. 1e; Fig 2e).	***T. pinivora***

13	*Forewing:* ante-median and post-median lines are parallel on costa and inner margin.	**14**
-	*Forewing:* ante-median and post-median lines are divergent on costa and inner margin. Discal dot without white edge. *Hindwing:* with vague central spot.	**15**
14	*Forewing:* last segments on inner margin are outwardly on ante-median line and straight on post-median line. Genitalia: cucullus with bulge (cover almost 1/4 of valva) (Fig. 1l; Fig 2l).	***T. libanotica***
-	*Forewing:* last segments on inner margin of ante-median and post-median lines are straight. *Genitalia:* lateral margin of socii concave and smooth; cucullus without bulge, but tapered and narrow (Fig. 1g; Fig 2g).	***T. cheela***
15	*Hindwing:* fringe uniseriate; without anal spot (Fig. 1m; Fig 2m).	***T. ispartaensis***
-	*Hindwing:* fringe biseriate; with vague anal spot (Fig. 1n; Fig 2n).	***T. sedirica / T. torosica***

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Chapter 4

The summer *Thaumetopoea* spp. (Lepidoptera: Notodontidae, Thaumetopoeinae) associated with *Cedrus* and *Pinus*

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A review of the "summer" *Thaumetopoea* spp. (Lepidoptera: Notodontidae, Thaumetopoeinae) associated with *Cedrus* and *Pinus*.

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Abstract

The species of the genus *Thaumetopoea* (Lepidoptera, Notodontidae, Thaumetopoeinae) are important from the point of view of forestry and human health as they are defoliators of trees and they release urticating hairs at the larval stage that are a source of allergy. Within the genus, there are two groups associated with conifers which are characterized by larval feeding in winter or in spring-summer, so the groups are called 'winter' and 'summer' processionary moths. This review collects the information about the species of the 'summer' *Thaumetopoea* species from Eurasia. Information includes morphological, biological, and ecological traits of *Th. bonjeani, Th. cheela, Th. ispartaensis, Th. libanotica, Th. pinivora* and *Th. sedirica. Thaumetopoea cheela i*s proposed to be included in this group based on indirect evidence presented in this paper. Information is collected from the literature and checked, where it was possible, with museum and collected specimens. An identification key based on morphological and biological traits is proposed. The conifers of the genus *Cedrus* seem to be associated with the summer *Thaumetopoea*, although more work is needed in order to clarify the evolutionary history of the group.

Keywords: *Thaumetopoea* summer; *Thaumetopoea cheela*; morphological traits.

Introduction

The subfamily Thaumetopoeinae includes several species called processionary moths. These moths are of great importance for ecology and forestry because the larvae feed on trees and shrubs of different families (both broadleaved and coniferous) menacing both growth and survival of trees, especially in the Mediterranean countries (Masutti and Battisti, 1990). Moreover, these species threaten human and animal health because of the presence of urticating setae in larvae and / or adults (Battisti *et al.*, 2011). These hairs are likely used as a defense strategy against vertebrate predators and are responsible for allergic reactions also of strong intensity in humans (Maier et al., 2003).

Historically, the subfamily has been considered as a self-standing family and divided into three subgroups according to their geographic distribution (Kiriakoff, 1970), then treated as a subfamily of Notodontidae based on a cladistics analysis (Miller, 1991). Recent studies, based on morphological (Schintlmeister, 2013) and molecular (Zahiri *et al.*, 2011; 2013) data, confirm the subfamily status of Thaumetopoeinae within the Notodontidae.

In this subfamily, the genus of *Thaumetopoea sensu lato* contains the most studied species, for which a recent molecular analysis has defined the phylogenetic relationships, and the evolution of biological traits, in some Palaearctic species (Simonato *et al.*, 2013), which are mainly distributed in the Mediterranean and Iranoturanic areas (Agenjo, 1941; Kiriakoff, 1970) (Figure 1).

Some authors split this genus in three genera (Freina and Witt, 1987; Schintlmeister, 2013): a) *Traumatocampa* (*bonjeani* Powell, *ispartaensis* Doganlar and Avci, *jordana* Staudinger, *libanotica* Kiriakoff and Talhouk, *pinivora* Treitschke, *pityocampa* Denis and Schiffermüller, *sedirica* Doganlar, *torosica* Doganlar, *wilkinsoni* Tams); b) *Thaumetopoea* (*apologetica* Strand, *cheela* Moore, *dhofarensis* Wiltshire, *processionea* Hübner, *solitaria* Frey), and c) *Heliantocampa* (*herculeana* Rambur). However, this morphological partition is not completely supported from a reassessment of the key morphological traits and by the recent molecular phylogeny of Simonato *et al.* (2013), suggesting that these species should be provisionally treated as part of a single genus.

Within the *Thaumetopoea* species feeding on coniferous trees, Simonato *et al.* (*2013*) identified two clusters based on the timing of larval feeding in winter or in spring-summer, confirming the groups called 'winter' and 'summer' processionary moths by Démolin (1989).

The aims of this paper are to review the information about the summer *Thaumetopoea* species, which include *Th. bonjeani*, *Th. ispartaensis*, *Th. libanotica, Th. pinivora* according to Simonato *et al.* (2013), and two other species (*Th. sedirica* and *Th. cheela*) which were not included in that study. The final aim is to organize and synthetize the knowledge about this group and produce a key based on the wing traits as developed by Agenjo (1941).

Materials and Methods

Systematics

Information about types, collections and number of specimens were obtained from catalogue of Schintlmeister (2013). Descriptions, biogeographic information, and morphological traits were collected from literature and checked with specimens from museum collections. Wings morphology and name of fasciae are based on Heath and Emmet (1979).

Materials

Thaumetopoea bonjeani: 3♂, 2♀: likely from Morocco, Witt Museum, Munich.

Thaumetopoea cheela: 2 ♂: Afghanistan, Surobi district, 1100 m, 07.VII.1961 and 08.VII.1961; legit G. Ebert, 1970 - The Bavarian State Collection of Zoology; Munich.

Thaumetopoea ispartaensis: 3 ♂, 1 ♀: Turkey, Isparta – Senirkent, 1100 – 1600 m. Collected on Cedrus libani and sent by Mustafa Avci from Isparta University to Department of Agronomy, Food, Natural Resources, Animals and the Environment; University of Padua.

Thaumetopoea libanotica: holotype ♂, allotype; Lebanon, Bcharre, Eklosion Beirut, 8.VIII.1974; collection A.S. Talhouk - The Bavarian State Collection of Zoology; Munich.

Thaumetopoea pinivora: 1 ♂: unintelligible collected site; IX.1917; collection Thurner - The Bavarian State Collection of Zoology; Munich. 1 ♀: from Russia, Ostpreussen (Oblast' Kaliningrad), VI.1909; collection Forster - The Bavarian State Collection of Zoology; Munich.

Thaumetopoea sedirica: 1 ♂, 1 ♀: from Turkey, Isparta – Sarkikaragac, 1400m, 38°02' 18"N, 31°22'35"E, 28.VIII.1967; legit (Det.) by M. Doganlar, 2005. Collected on Cedrus libani and sent by M. Doganlar to Department of Agronomy, Food, Natural Resources, Animals and the Environment; University of Padua.

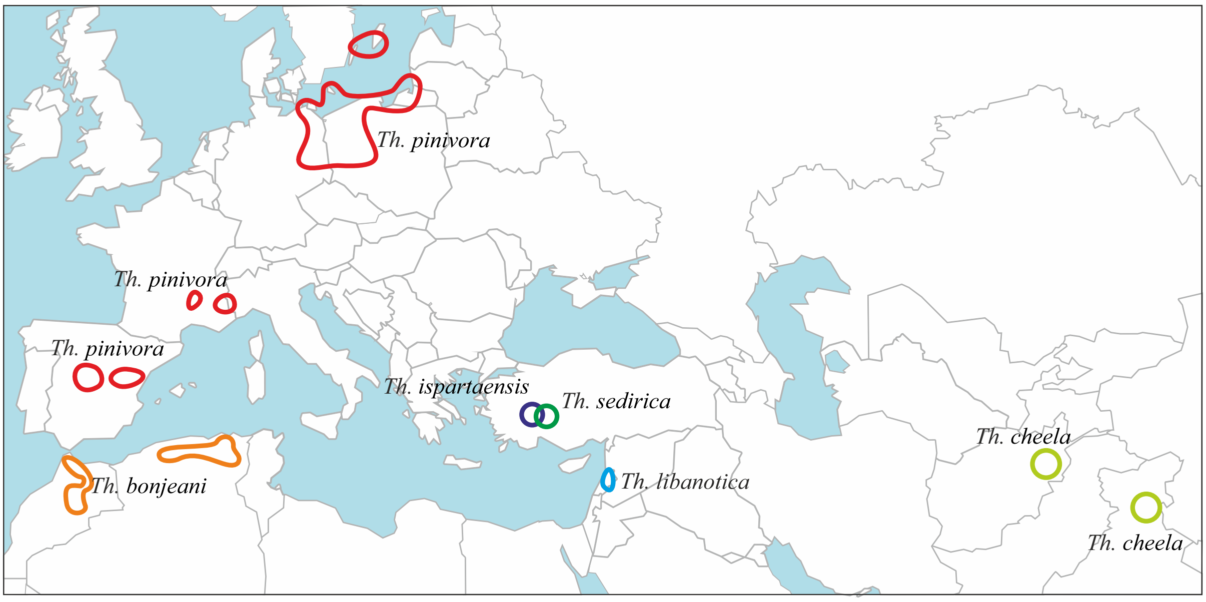


Figure 1. Geographic distribution of the summer *Thaumetopoea* spp.

Results

General morphological traits

Moths of this subfamily are small or medium size. Body covered by grayish hairs (Freina and Witt, 1987). Eyes naked; ocelli absent such as chaetoseme; palpi small and rudimentary, proboscis atrophied (Kiriakoff, 1970). Forewing shape and veins very similar to the Notodontidae ones; without tooth on dorsum and with apices rounded (Freina and Witt, 1987). Vein, using Hampson and Meyrick classification, (Heath and Emmet, 1979) 2 start at 4/5 of cell, 3 and 4 separated, 5 start at middle of discal spot; they are concave (Kiriakoff, 1970; Freina and Witt, 1987). Veins 6, 7, 8 + 9 and 10 are stalked. Width of forewing is about twice hindwing (Kiriakoff, 1970).

Description of species

Thaumetopoea bonjeani (Powell, 1922): 188; (Cnethocampa bonjeani)

Syntype: unspecified number of ♂♂ and ♀♀, Morocco, d’Azrou Forest, 500 m to North of Douar de Garde – The Natural History Museum, London. Not examined.

Description (Figure 2):

According to Agenjo (1941) this species is really similar to *Th. pinivora*. This statement is strongly corroborated by molecular analysis (Simonato *et al.*, 2013).

Male. Antennae bipectinate, long and yellowish in ground color (Agenjo, 1941; Freina and Witt, 1987). Palpus with first segment longer and thicker than second segment (Agenjo, 1941). Canthus present; with 5 teeth and upper side straight (Agenjo, 1941). Upper tooth smaller (Freina and Witt, 1987). Foreleg epiphysis present and well developed. Thorax blackish, with some whitish hairs, which turn into brownish-gray ventrally (Freina and Witt, 1987). Abdomen ochreous (Freina and Witt, 1987) or golden and blackish (Agenjo, 1941). Wingspan 30-34 mm. Forewing ground color ash gray, generally. Three dark fasciae present, with yellow edge (Agenjo, 1941). Basal fascia, more or less straight, from radial vein to inner margin of wing (Agenjo, 1941); this fascia is often barely visible (Freina and Witt, 1987). Shape of ante-median and post-median fasciae clearly distinguish *Th. bonjeani* from *Th. pinivora,* i. e. ante-median fascia forms an obtuse angle outwardly, changing direction straight just before dorsum (Agenjo, 1941). External edge of post-median fascia jagged. Ante and post-median fasciae more or less parallel, especially near costa and dorsum (Agenjo, 1941). A V-shaped discal spot present. Fringes on termen present, alternatively gray and white-yellow (Agenjo, 1941). Hindwing whitish with reddish fluff at margin. Fluff more abundant at anal margin. Fringes alternatively gray and whitish. Anal spot present and well developed (Agenjo, 1941).

Male genitalia. Up to 4 mm when fully stretched. Uncus shorter than gnathos and thin (Doganlar and Avci, 2001). Tegument thin. Valvae basally concave turning into convex apically. Valvae narrow, with a small protuberance on tip. Fultura distinctive, big, with jagged edges similar to that *Th. processionea* (Agenjo, 1941). Aedeagus broad basally (Doganlar and Avci, 2001) and curved, such as *Th. pinivora*, but bigger.

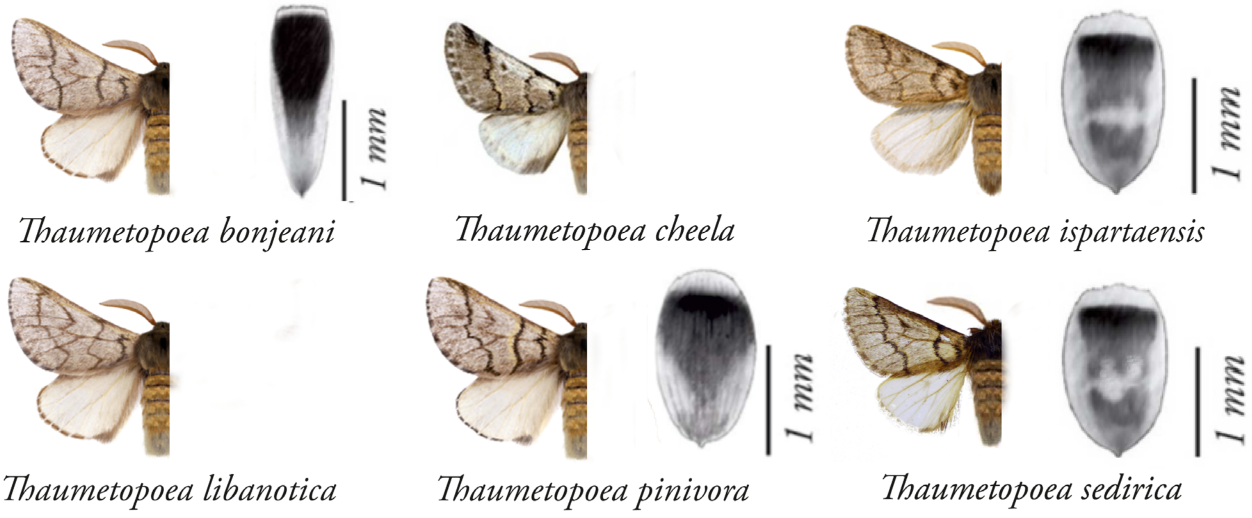
Female. Similar to *Th. pityocampa* and *Th. pinivora* (Agenjo, 1941). Wingspan 34-38 mm, with the wing ground color pale and not well defined (Freina and Witt, 1987). Anal scales straight, long acute, with sharpened base; poorly pigmented at the center (Agenjo, 1941). From literature we known that ratio, between length and width, greater than 2.5 (El Yousfi, 1989; Simonato *et al.*, 2013).

Remarks: head covered by a tuft on scapus of brownish hairs, like in *Th. pinivora*. Canthus small. Discal spot brownish.

Larva. Larva has been described by Démolin (1989) mainly on a comparison with *Th. pityocampa*, from which it can be easily distinguished because of the long white hairs implanted on the trapezoid plates at both sides of the body. In addition, the urticating setae are black instead of orange-red and the anterior edge of the integumental areas carrying them (the ‘mirror’) is characterized by very long white hairs. Démolin (1989) provides a pictorial description of the hairs among the five larval instars (Planche 12 and 12 bis) as well as photographs of the mature larvae (Planche 13 and 14) which are compared to those of *Th. pinivora*.

Life history: Larvae are social and monophagous on *Cedrus atlantica* (Démolin, 1989; El Yousfi, 1989; Rahim *et al.*, in press), preferring low humidity forests (Freina and Witt, 1987). The adults emerge between August and September, mating at night, immediately after emergence. The oviposition occurs on the underside of the cedar branches, during the night. The eggs are then covered with bark-colored scales, making them less visible. The number of eggs per egg batch is extremely variable. After 8 months, between March and April, larvae hatch and group in colonies, building very light silk tents , until to achievement the fifth instar (between June and July) (Rahim *et al.*, in press). Then larvae leave the tree in a procession, in the early hours of the morning and pupate in the soil (El Yousfi, 1989). *Thaumetopoea bonjeani* has an annual development cycle (Démolin, 1989; Rahim *et al.*, in press) (Table 1). The sex pheromone has been identified and results similar to that of *Th. pinivora* and *Th. libanotica* (Frérot, in press).

Distribution: Thaumetopoea bonjeani was found originally in the Atlas Mountains of Morocco (Agenjo, 1941) and later in mountains of Algeria (Freina and Witt, 1987; Rahim *et al.*, in press) (Figure 1).



Thaumetopoea bonjeani *Thaumetopoea cheela* *Thaumetopoea ispartaensis*

Thaumetopoea libanotica *Thaumetopoea pinivora* *Thaumetopoea sedirica*

Figure 2. Males of summer *Thaumetopoea* spp. considered in the study, together with the scales of the anal tuft of female moths, whenever available.

Thaumetopoea cheela Moore, 1883: 18;
Syntype: 1 ♂, 1 ♀, North-West India, Umballa district – The Natural History Museum, London. Not examined.

Description (Figure 2):
Based on the original description of Moore (1883), who defined it as very similar to *Th. pinivora.*

Male. Antennae yellow. Head covered by grayish-brown hairs. Legs thickly clothed with grayish-brown hairs in femur and tibia. Tarsi yellow. Thorax hairs grayish-brown. Ochreous yellow segmental bands present on abdomen. Wingspan 31 mm. Forewing brownish-gray ground color with sparse white scaled; scales disposed longitudinally. Forewings have 3 dark fasciae in basal, ante-median and post-median positions. Post median fascia zig-zagged. Each fasciae bordered with a marked ochreous-yellow edge like in *Th. pinivora.* Fringes alternately gray and white. Hindwing whitish with a dark spot in the anal angle.

*Female. W*ingspan 35 mm. Wing ground color paler than male and not well defined. Head darker brownish-gray; thorax and abdomen have darker brown tuft.

Remarks: dark tuft on scapus. Canthus present and notched, with 6 or 7 teeth. Foreleg epiphysis present. Forewing basal line reduced to some dark points; a brown discal spot bordered present, with white scale; a dark brown pre-apical spot present. Fringes of hindwings more whitish than in the forewing.

No information about genitalia, female scale, larvae and life history is available.

Distribution: from Afghanistan, Surobi district (specimens collected by G. Ebert, see above), to N-W of India, Umballa district (Moore, 1883).

Thaumetopoea ispartaensis Doganlar and Avci, 2001: 20 (*Traumatocampa ispartaensis*);
Holotype: ♂, Turkey, Isparta, Kapidagi: Senirkent (1100-1500 m) – Plant protection Department, Mustafa Kemal University, Antakya, Turkey. Not examined.

Description (Figure 2):
This species is very similar to *Th. bonjeani* (Doganlar and Avci, 2001).

Male. Antennae light brown and bipectinate (Doganlar and Avci, 2001). Palpus thin, with second segment long twice as first (Doganlar *et al.*, 2005). Head covered by dark brown hairs (Doganlar and Avci, 2001). Canthus notched, developed, with 7 teeth, 2 long and 5 small (Doganlar and Avci, 2001). Foreleg epiphysis present (Simonato *et al.*, 2013). Thorax dorsally covered by dark brown hairs (Doganlar and Avci, 2001). Abdomen light brown, with reddish hairs and dark bands laterally (Doganlar and Avci, 2001). Wingspan 26-29 mm. Forewing ground color whitish-gray, with three typical dark brown fasciae. According with Doganlar and Avci (2001), forewing has a really small basal fascia; ante-median and post-median fasciae more or less parallel between costa and dorsum. Post-median fascia more serrated in the middle (Doganlar and Avci, 2001) like in *Th. pinivora*. Discal spot vague with light brown color and halfmoon-shaped (Doganlar and Avci, 2001). Hindwing whitish, without fasciae. Fringes grayish-brown. A vague anal spot sometimes present. Grayish fasciae on anal margin present. Reverse sides of all wings without any particular ground colors (Doganlar and Avci, 2001).

Male genitalia. Uncus long large with curved tip; gnathos long, triangular and broad. Pedunculus, which is inferiorly articulated with the vinculum (Steinmann and Zombori, 1984), well developed and broad in the middle. Valvae with a short apical projection on ventral margin, thicker towards tip. Internal margin of valvae slightly concave while costal margin curved apically; tip of valvae narrow. Ventral side without teeth (Doganlar and Avci, 2001).

Aedeagus slightly longer than valva, curved basally and widen medially. Saccus not developed (Doganlar and Avci, 2001).

Female. Wingspan 34-37 mm. Wing ground color paler than male and not well defined; hindwing without dark margin or anal spot (Doganlar and Avci, 2001). Head and thorax dark with grayish hairs; abdomen yellowish with light brown bands. Last tergit with many scales, pointed with 2 spots (Doganlar *et al.*, 2005). Ratio between length and width near to 2 (Doganlar et al., 2005; Simonato et al., 2013).

Remarks: head with a tuft of whitish-yellow hairs that cover scapus; brown hairs present near canthus. Legs thin with few hairs. A pair of spurs present in the others legs. Thorax covered ventrally by whitish-yellow hairs. Three fasciae on forewing with yellow edges in the same positions such as in *Th. pinivora.* Basal fascia with yellow edge that is most visible in the internal side. Ante-median fascia C shaped with outwardly smooth tip. Ends near dorsum of ante-median and post-median fasciae divergent.

Forewing with vague pre-apical light brown spot and gray fringes. Light grayish band present at anal margin. Hindwing lighter at anal margin and pale on reverse side; a vague light brown spot in the center present; scales with two black spot: one to the apex and a big one near the base. Fultura hexagonal as long as wide.

Life history: Larvae are social and monophagous on *Cedrus libani* (Doganlar and Avci, 2001). Adults emerge between August and September, mating at night, immediately after emergence. Eggs are laid on the underside of cedar branches, during the same night. Eggs are covered with bark-colored scales, such as in *Th. bonjeani.* The number of eggs per egg batch is extremely variable. After almost 7 months, between the end of March and the end of April, larvae hatch and group in colonies, spinning light silk tents, until to achievement the fifth instar (end of June to mid-July). Then, mature larvae pupate on sunny soil near cedar forest. No extended diapause was observed (Avci and İpekdal, in press) (Table 1).

Distribution: Turkey, Isparta region (Doganlar and Avci, 2001) (Figure 1), in cedar stands of the Taurus Mountains (Avci and İpekdal, in press).

Thaumetopoea libanotica Kiriakoff and Talhouk, 1975: 1;
Holotype: ♂, Lebanon, Eklosion, Bcharre – Faculty of Agricultural Sciences, American University of Beirut. Not examined.

Description (Figure 2):

Male. Antennae bipectinate and light brown (Kiriakoff and Talhouk, 1975). Palpus small. Head ochreous-brown dorsally and blackish ventrally; a tuft of whitish-gray hairs cover scapus (Kiriakoff and Talhouk, 1975). Canthus notched, not well developed, with 5 teeth; the upper ones smaller (Doganlar and Avci, 2001). Legs with pale hairs, as well as in thorax ventral side; tarsi yellow (Kiriakoff and Talhouk, 1975). Foreleg epiphysis present (Simonato *et al., 2013).* Thorax dorsally brown with grayish hairs. Abdomen gray, slightly brown with whitish segmental bands. Lateral and anal tufts are grays (Kiriakoff and Talhouk, 1975). Wingspan 26 mm. Forewing ground color brownish-gray, with 3 dark fasciae with pale edges (Kiriakoff and Talhouk, 1975). Fasciae in basal, ante-median and post-median positions. Basal fascia and ante-median fascia V-shaped, with tip outwardly. Basal fascia sometimes discontinued near costa; ante-median fascia with a sharp angle just before dorsum. Post-median fascia continuous and serrated, often with a sharp angle just before dorsum. Upper ends of medians fasciae more or less parallel; instead lower ends more convergent (Kiriakoff and Talhouk, 1975). Discal spot triangular shaped, brownish-gray colored. Vague pre-apical spot present. Fringes whitish, alternate with grayish-brown ones. Hindwing whitish with light brown margin and a vague brownish anal spot. Whitish-gray fringes present. Undersides of wings pale and not well defined (Kiriakoff and Talhouk, 1975).

Male genitalia. Uncus short, curved and tapered distally. Gnathos triangular with rounded corners (Kiriakoff and Talhouk, 1975), bigger than uncus (Doganlar and Avci, 2001); Vinculum narrow. Valvae oval, with tip slightly elongated and rounded, without projection. Aedeagus slightly longer than valvae; wide basally and narrow distally,; slightly curved. Fultura pentagonal (Kiriakoff and Talhouk, 1975). Saccus not developed (Kiriakoff and Talhouk, 1975; Doganlar and Avci, 2001).
Female. Wingspan 32 mm. Wing ground color pale and less defined than male. Antennae described as filiforms by Kiriakoff and Talhouk (Kiriakoff and Talhouk). Head and thorax grayish; abdomen pale with ochreous hairs and dark fasciae (Kiriakoff and Talhouk, 1975). Scale ratio between length and width near to 2 (Simonato *et al.*, 2013).
Remarks: discal spot is similar to halfmoon-shaped. Fultura as long as wide. Antennae in female shortly bipectinate to apex.

Larva. Mature larva is 25-26 mm long, with a background light gray, almost white color. Abdominal tergites have integumental fields carrying urticating setae bordered with red color and intermixed with bundles of long, white hairs (Kiriakoff and Talhouk, 1975).

Life history: Larvae are social and monophagous on *Cedrus libani.* They were found on young trees at high elevation (1900 m) on Lebanon Mountains, in temperate forests, with an average rainfall of 1200 mm/year (Kiriakoff and Talhouk, 1975). Larvae reach the fifth instar at the end of June and they pupate in soil. Adults emerge in August. This data was collected by Kiriakoff and

Talhouk (1975) from a single colony found on a tree and bred in the laboratory (Table 1).
Distribution: was found in the Lebanon forest by Kiriakoff and Talhouk (1975) (Figure 1).

Thaumetopoea pinivora (Treitschke, 1834): 194; (*Gastropacha pinivora*);
Lectotype: ♂, Northern Germany (probably Sternberg, SW of Rostock according to Schintlmeister, 2013) – Magyar Természettudományi Múzeum, Budapest. Not examined.

Infrasubspecific, according to Schintlmeister (2013):

Th. pinivora ab. nigromaculata Peterson, 1899: 245.
Th. pinivora ab. plutonia Schultz, 1905: 115.

Description (Figure 2):
External morphology of *Th. pinivora* is very similar to *Th. pityocampa although showing* significant differences (Agenjo, 1941; Freina and Witt, 1987; De-Gregorio and Redondo, 1994).

Male. Antennae short, bipectinate and more grayish than yellow (Agenjo, 1941). Palpus stretched (Doganlar *et al.*, 2005), with first segment longer and thicker than second sharped segment (Agenjo, 1941). Head covered by brownish hairs (Freina and Witt, 1987). Canthus notched, not well developed (Agenjo, 1941), with 7 teeth, 6 small and 1 big (Freina and Witt, 1987). Leg thin. Foreleg epiphysis present. A pair of spurs in the other legs present. Thorax brown and very hairy; abdomen yellowish (Agenjo, 1941; Freina and Witt, 1987). Wingspan 27-37 mm. Forewing ground color ash gray. Forewings with 3 dark fasciae in basal, ante-median and post-median positions. All of them with yellow edge. In the basal fascia, edge yellow on both sides; in the ante-median fascia yellow edge on external side; in the post-median fascia yellow edge on internal sides (Agenjo, 1941; Freina and Witt, 1987). These edges often discontinuous. Direction of the dark fasciae slightly different from *Th. pityocampa,* in particularly between ante-median and post-median fascia that converge near dorsum (Agenjo, 1941). Forewing marked from a brown halfmoon-shaped discal spot (Freina and Witt, 1987). Fringes alternately brown and white for the entire length of termen. Forewing reverse side pale (Agenjo, 1941; Freina and Witt, 1987). Upper side of hindwings white with dark external margin and with whitish-gray fringes. Anal spot sometimes present. Hindwings reverse side pale (Agenjo, 1941; Freina and Witt, 1987).

Male genitalia. Up to 3,5 mm when fully stretched (Agenjo, 1941). Uncus small, broad and circular, triangular-shaped with thin and straight tips of lateral appendices. Tegument thin. Valvae distinctive, very short and sharp, with straight bottom edge and rounded at the end; upper edge initially perpendicular to the external margin and turning into convex apically. Inner edge almost straight. Outer end of upper edge produces a process with a right angle and a thicker terminal tip. This joins to the inner margin of the distal end. protuberance present, with many bristles, inserted in well-defined cavities (Agenjo, 1941; Freina and Witt, 1987). Fultura pentagonal and very characteristic. Aedeagus not curved (Agenjo, 1941).

Female. Similar to *Th. pityocampa* and *Th. wilkinsoni* (Agenjo, 1941). Wingspan 34-38 mm, with the wing ground color pale and not well defined (Freina and Witt, 1987). Antennae wider but shorter than male (Agenjo, 1941). Head and thorax brown with grayish hairs; abdomen lighter with yellow fasciae (Agenjo, 1941). Anal scales that cover egg batches narrow and short, with base, much pointed. Scales pigmented typically, with wide elliptic dark spot near apex bordered on three sides by a lighter line before the terminal edge (Agenjo, 1941); there are no other black regions except this (Doganlar *et al.*, 2005). Moreover scales ratio between length and width near to 2 (Simonato *et al.*, 2013).

Remarks: head with tuft of whitish-gray hairs that cover scapus, and a line of brown hairs between antennae and canthus. Thorax and legs covered with some whitish-gray hairs. On forewings the fasciae with yellow-whitish margin in the upper portion. Light brown spot between the 2 arms of discal spot often present on forewing. Brown pre-apical spot present at costal margin; it can be extended to half of wing. Forewing reverse side pale and with only a dark spot under costal margin of post-median fasciae. Anal spot on hindwing sometimes vague.

Larva. The only description available refers to Démolin (Démolin) comparison with *Th. bonjeani* (see above). This author considers the larva of *Th. pinivora* not distinguishable from that of *Th. bonjeani*.

Life history: Larvae are highly social (Aimi *et al.*, 2008). In the southern Europe they prefer mountain environments where their host plant growth typically, while in northern Europe, they can be found in the lowland forest (Cassel-Lundhagen *et al.*, 2013). They feed mainly on *Pinus sylvestris* (Freina and Witt, 1987; Cassel-Lundhagen *et al.*, 2013) but occasionally they are found on *Pinus nigra* and *Pinus mugo* (Larsson and Battisti, in press). They prefer slow-growing trees on poor soils (Cassel-Lundhagen *et al.*, 2013). In northern Europe, *Th. pinivora* has a 2 years development cycle. Adults emerge between July and August and mating soon after. Female lays 100-200 eggs on pine needle, from the tip to the base (in contrast to *Th. pityocampa*). Eggs hatch in the early spring. Larvae start to feed on mature needles, mainly during the night, and reach the fifth instar to the end of July. Then they leave the tree in a typical procession to search a suitable site to dig and pupate to a depth of 5-20 cm. Adults emerge in late July of the following year; although a certain proportion of the cocoons have a prolonged diapause (Larsson and Battisti, in press) (Table 1). Larvae do not build thick tents, in contrast to *Th. pityocampa* and *Th. processionea* (Larsson and Battisti, in press).

Distribution: Th. pinivora was originally known from Northern Europe and later from France and central Spain (Cassel-Lundhagen *et al.*, 2013; Larsson and Battisti, in press) (Figure 1).

Thaumetopoea sedirica Doganlar et al., 2005: 231; (*Traumatocampa sedirica*);
Holotype: ♂, Turkey, Isparta, Sarkikaragac – m, 38°02’ 18”N, 31°22’35”E, ex Cedrus libani, 17 August 1968, legit Tosun, Museum of the Agricultural Faculty, Mustafa Kemal University, Hatay, Turkey. Not examined.
Description (Figure 2):
External morphology very similar to *Th. ispartaensis*.

Male. Antennae yellow and bipectinate with ramie (Doganlar *et al.*, 2005). Palpus small, with second segment long as the first segment; first segment swollen outwardly (Doganlar *et al.*, 2005). Head dark brown but with lighter hairs in the upper side. tuft with long dark hairs and white broad scales present to cover scapus (Doganlar *et al.*, 2005); the lines of hairs near canthus pale yellow (Doganlar *et al.*, 2005). Canthus notched, with 3-5 broad teeth (Doganlar *et al.*, 2005); Thorax covered by brownish black hairs. Abdomen with golden hairs (Doganlar *et al.*, 2005). Wingspan 30-37 mm. Forewing with dark brown basal, ante-median and post-median fasciae. Apical ends of ante-median and post-median fasciae divergent on anal margin (Doganlar *et al.*, 2005). A typical light brown discal spot present (Doganlar *et al.*, 2005). Hindwing whitish with apical gray fringe, and without anal-spot (Doganlar *et al.*, 2005).

Male genitalia. Uncus large with curved tip. Valvae broad basally, with a short apical projection on ventral margin that becomes thicker towards the tip. Apex of valva turned almost 95°. Internal margin slightly concave while costal one sharply curved apically; the tip of valva narrow (about 1/3 of valvae). Ventral side without tooth (Doganlar *et al.*, 2005).
Female. Wingspan 33-40 mm. Wing ground color paler than male and less defined. Antennae bipectinate with short ramie (Doganlar *et al.*, 2005). Last tergit with many scales and pointed basally with black dots below apical black spot (Doganlar *et al.*, 2005). Moreover scales ratio between length and width near to 2 (Doganlar *et al.*, 2005).

Remarks: hairs dark. Legs thin. Forewing epiphysis present. A pair of spurs in the other legs present. Thorax ventrally covered by lighter hairs. Forewing ground color in whitish-gray; all fasciae with typically yellow edges, like in *Th. pinivora*. Apical ends of ante-median and post-median fasciae parallel on costal margin. Discal spot with halfmoon-shaped present. Fringes brownish-gray. A darker marker can be present in the anal margin of hindwings. Wings reverse sides pale, without any particular ground colors, with only a vague light brown spot in the center of hindwings.

Larva. No information is available.
Life history: Larvae are social and probably monophagous on *Cedrus libani*. The eggs batches are flat, symmetrical and hexagonal on the host bark (Doganlar *et al.*, 2005).

Distribution: Turkey, Isparta region (Doganlar *et al.*, 2005), (Figure 1).

Key to the identification of the moths of *Thaumetopoea* genus, with special reference to the ‘summer’ feeding species

1	Canthus convex and smooth	to *solitaria* group
-	Canthus with teeth	2
2	Patter of forewing greyish	3
-	Patter of forewing brownish or whitish	4
3	Male with dark fasciae on forewings without yellowish edge; Female with anal scales upper than 2 mm of length or with scales not pointed to proximal part	to *pityocampa* group
-	Male with dark fasciae on forewings with yellowish edge; Female with anal scales lower than 2 mm of length or with scales pointed to proximal part	to *pinivora* group (5)
4	Forewing with discal spot	*herculeana*
-	Forewing without discal spot	to *jordana* group
5	Ante-median fasciae and post-median of forewing much more converged on dorsum than costa; Discontinued basal fasciae	*pinivora*
-	Ante-median fasciae and post-median of forewing are equidistant or divergent on dorsum and costa; Discontinued basal fasciae	6
6	Ante-median fasciae and post- median of forewing are equidistant on dorsum and costa;	*bonjeani*
-	Ante-median fasciae and post-median of forewing are more divergent on dorsum than costa;	7
7	With a strong acute angle on ante-median and post-median fasciae on forewing	*libanotica*
-	Without a strong acute angle on ante-median and post-median fasciae on forewing	8
8	Basal fasciae on forewing probably reduced to some point; discal spot with white edge	*cheela*
-	Basal fasciae on forewing well developed; discal spot without white edge	*ispartaensis*/ *sedirica*

Discussion

The analysis of morphological traits and available data from the literature has identified several similarities between the species considered in this study. Most obvious similarity is based on the wing bands, as all species have 3 thin, dark bands, in basal, ante-median and post-media positions, and they have yellow-whitish edge. Basal fascia is bordered on both internal and external sides; this fascia is discontinuous, often also in *Th. pinivora.* Ante-median fascia is bordered on the external side while the post-median fascia is on the internal site. Moreover a pre-apical spot, more or less marked, is often present. Hindwing has an anal spot, that is visible, although vaguely, also in *Th. ispartaensis.* In fresh specimens probably it could be more marked. Canthus has always teeth, more or less serrated, generally smaller than that of *Th. pityocampa*. Toothed canthus is also present in *Th. cheela*, to give further support to the relatedness of the species to this group. Another important trait could be the shape and pigmentation of the scales used to cover the egg batches; in fact, they are wide and similar in *Th. pinivora*, *Th. ispartaensis* and *Th. sedirica*; while they are narrower and longer in *Th. bonjeani*. Also the analysis of the male genitalia has identified many similarities, especially in the valvae and their apical process but also in fultura that it is pentagonal and similar in *Th. pinivora* and *Th. libanotica*; and it is hexagonal and similar in *Th. ispartaensis* and *Th. bonjeani*. Uncus is small and rounded and aedeagus is generally broader basally and curved, both with a similar shape, although they change in size.

These traits, in addition to those on biology and behavior of larvae, has allowed us to hypothesize that also *Th. cheela* has the same behavior and habits of the other summer *Thaumetopoea*. To further support this hypothesis, we overlapped the collection sites of the specimens and the geographic distribution of *Cedrus deodara* and *Pinus* spp. (*P. gerardiana, P. roxburghii* and *P. wallichiana)* (Critchfield and Little, 1966; Vidakovic, 1982), in order to estimate a possible matching with host plant. As both *Cedrus* and *Pinus* occur in the area where the specimens of *Th. cheela* were collected, we cannot conclude about which one is more likely to be the host plant of this species. Unfortunately, micro-scale data about the sites is not available and the issue can be solved only with new collections, possibly of larvae that have to be searched on these host plants.

In addition, the analysis of specimens of *Th. ispartaensis* and *Th. sedirica* has not revealed substantial differences on wing ground color, or on the fasciae inclination. The traits used in the key by Doganlar *et al.* (2005) did not allow to unambiguously discriminate female scales (Figure 2).

As most of the summer *Thaumetopoea* are associated with *Cedrus,* we compared *Cedrus* phylogeny provided by Qiao *et al.* (2007) with phylogeny analysis developed by Simonato *et al.* (2013) for *Thaumetopoea*, to investigate the evolutionary history of this group in relation to their host plants, as suggested by Wahlberg *et al.* (2013). Qiao *et al.* (2007) state that *C. deodara* was the first species to diverge from the common ancestor, and then the split concerned *C. atlantica, C. libani,* and *C. brevifolia*. With a molecular clock they estimate that the split between *Cedrus deodara* and the others occurred about 60 Mya (millions years ago); *C. atlantica* from North Africa split about 20 Mya and the separation between *C. libani* and *C. brevifolia* occurred 6-7 Mya. Combining data obtained from Simonato *et al.* (2013) with sequences available in Genbank, a multiple alignment including consisting the mitochondrial genes *cox1*, *cox2*, and *atp6* was then used for dating the split events among *Thaumetopoea* species. The substitution rate was set to 0.00022 according to (Gaunt and Miles, 2002). Calculations were performed using Beast 1.8.0 software (Drummond *et al., 2012).* The results obtained from this analysis show that the separation between the summer and the winter *Thaumetopoea* happened about 16.3 Mya (95% HDP interval: 8 to 25). Moreover, the first split in the summer clade between *Th. ispartaensis*/*Th. libanotica* and *Th. bonjeani*/*Th. pinivora* is dated at about 5.5 Mya (95% HPD interval 2.8 to 8.6). The further splits between *Th. pinivora*/*Th. bonjeani* and between *Th. ispartaensis*/*Th. libanotica* occurred about 2.7 Mya (95% HDP interval: 1.2 to 4.4) and about 2.6 Mya (95% HDP interval: 1.2 to 4.2), respectively. As *Th. cheela is* missing from the analysis, we cannot conclude about its possible association with *Cedrus deodara*. Split of the summer clade follows that of *Cedrus atlantica* from the other *Cedrus*, and could be somewhat linked to it. It seems in general that insect species have split later than their host plants, with one of them (*Th. pinivora*) associated with *Pinus* and separated from the sister species *Th. bonjeani* much after the separation of *Cedrus atlantica* from the other *Cedrus*.

Although these results indicate that more studies have to be made, especially for the poorly known species, there are some evidences that *Cedrus* could be the host on which most of speciation in the summer clade has happened. Research should be focused on finding new material on which morphological characters of both adults and larvae have to be tested, together with biological and ecological traits. Moreover, molecular data from this material could complement the evolutionary history and define the phylogenetic relationships within the *Thaumetopoea s.l.*

Table 1. Summary of some biological traits of *Thaumetopoea* spp. ? unknown traits.

Species	Life cycle	Diapause	Flight period	Larval period	Host genus
Th. bonjeani	Univoltine	Egg	August - September	March - July	*Cedrus*
Th. cheela	?	?	?	?	*Cedrus* ?
Th. ispartaensis	?	?	?	?	*Cedrus*
Th. libanotica	Univoltine	Egg	August (rearing)	April - June (rearing)	*Cedrus*
Th. pinivora	Univoltine (south) Biannual (north)	Egg (south), Egg + Pupa (north)	July - August	March - July	*Pinus*
Th. sedirica	?	?	?	?	*Cedrus*

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Chapter 5

Conclusions and future perspectives of the study of the genus *Thaumetopoea*

The genus *Thaumetopoea*

The genus *Thaumetopoea s. lat.* shows some marked morphological and biological differences (i.e. presence/absence of a crest on the front, wing patterns, and larval host-plant) among the species, which were used by de Freina and Witt (1985; 1987) to upgrade two former subgenera to genus (*Thaumetopoea* and *Traumatocampa*) and to create the new genus *Helianthocampa*, implying a polyphyletic origin (Freina and Witt, 1985; 1987). In this thesis it is shown that this view is not supported, based on a thorough analysis of morphological and molecular data.

The monophyly of the genus

The phylogenetic analyses present in Chapter 2 strongly support the monophyly of *Thaumetopoea s. lat.*, as corroborated by previous studies based on a limited number of taxa (Simonato *et al.*, 2013). All my analyses, based on the combined dataset, assigned to the clade of *Thaumetopoea s. str*. the following species: *T. solitaria, T. processionea*, and *T. herculeana*, the latter formerly assigned to the genus *Helianthocampa* (Freina and Witt, 1985; 1987). The remaining *Thaumetopoea s. l.*, which share a crested frons and a spine on foretibia, form a group corresponding to the genus *Traumatocampa* (Agenjo, 1941; Freina and Witt, 1987) and they were divided into 3 clades, i.e. the African (*T. apologetica-jordana* complex), summer (*T. cheela-libanotica* complex), and winter (*T. pityocampa-wilkinsoni* complex) (Démolin, 1989; Basso *et al.*, 2016; Basso *et al.*, in press). According to the morphological view instead, *T. herculeana* is quite mobile in the reconstruction mainly due to the possession of a crested frons, spine on foretibia, and based upon wing pattern (Agenjo, 1941; Freina and Witt, 1985; 1987). However, a bulge on frons and sketch of foretibia spine present in *T. solitaria* and *T processionea* reveal that a crest was anciently present also in the *Thaumetopoea s. str.*, supporting the hypothesis of a double, indipendent loss of these traits. In fact, crest (and the associated spine-traits) are used by the adults to emerge from the ground after the pupation time, and this is not required in *T. processionea* and *T. solitaria* because they pupate in the tent and in the litter, respectively. This hypothesis is corroborated also by the apomorphies present in male genitalia, on fringe shape and presented extensively in the study carried out in Chapter 3. This hypothesis is also supported by the ancestral state reconstruction of the pupation sites (Chapter 2), which suggests that the soil was the pupation site of the ancestors through the Maximum Likelihood reconstruction and so the associated traits (p-value < 0.01) should have been present.

At the meantime, any attempt to move *T. herculena* out from this clade was rejected such as in previous work of Simonato *et al.* (2013), clarifying the position of this species and its morphological inconsistencies noted in several studies throught the years (Agenjo, 1941; Freina and Witt, 1985; 1987).

Furthermore, new organized and complete descriptions provided in Chapter 3 with the analysis of data labels used to draw distribution maps of some less studied species will allow a certain identification of each species of *Thaumetopoea* and lay the groundwork to plan the sampling across

non-European countries, in order to find larvae and/or adults and study their life history.
In conclusion, this thesis analyses the genus *Thaumetopoea* clarifing the main morphological/ molecular inconsistency of *T. herculeana* and setting the base to expand the study to the entire subfamily, with a combined approach and the attention to the traditionally sistematic view. In the meantime, the hypothesis of hybridization that could be occur among sister species produces new questions that only an extensive samplig among the *Thaumetopoea* genus and specific molecular analyses could solve.

Applied importance

This thesis, following rigorous phylogenetic analyses and different methods of ancestral state reconstruction (Chapter 2), clarifies the taxonomic position of species of high applied importance and thus contributes to a better knowledge of their relationships. The best known pest, the pine processionary moth, has been considered for a long time as a complex and with this work it was possible to find discriminating morphological traits, also in the hybrids obtained under laboratory condition. This results has great importance as it will allow entomologists and foresters to correctly identify the taxa they are dealing with, without the need of sequencing the DNA. This is very important, for example, in the case of the introductions associated with trade of trees, as recently observed in Greece and Russia (Battisti et al. 2017). It will also help to better define the contact zone where hybrids are expected to occur, both in Turkey and Algeria. The taxonomic clarification of the summer species of processionary moths feeding on conifers will also contribute to a better understanding of their role in the decline of high elevation forests of cedar in Africa and Asia, where their defoliations exacerbate the already limiting conditions set by climate change. The study also highlights the presence of urticantig setae in all larvae of the genus *Thaumetopoea*, with the only exception of the African clade for which data are still missing. This is clearly another indication of the monophyly and reveals the importance of the defense system for these species, which still represent a threat to humans and domesticated animals in large parts of the world (Battisti *et al.*, 2017). As climate change and global trade is likely to cause range expansion in a number of taxa of the group, the presence of urticating setae has to be considered with extreme care, especially if populations never exposed before to these special types of setae will be affected. Furthermore, traits shared by the ancestors and the time of species differentiation (Chapter 4) allows us to understand the evolution pattern and the reaction of the genus to past climate changes, in oder to develop specific knowledge to anticipate the effects of the range expansion and circumscribe the outbreaks of harmful species whenever possible. It becomes more and more important to spread the information about the risks associated with range expansion of insects, especially if directly harmful to human societies, in order to adopt prevention criteria that are the best approach to contain the expected impacts. In the meantime a new set of morphological traits and an identification key developed for the whole genus (Chapter 3) will allow a quick identification of the adults both in the museum collection and in field, and limit the cases of accidental introductions in potentially risky countries in which this genus is absent (i.e. America).

Future directions

This thesis has demostrated how morphological and molecular traits have a complementary value in phylogenetic reconstruction and are indispensable for solving difficult issues (i.e. T. *herculeana* position). This work lays the basis to complete and expand the knowledge about the Thaumetopoeinae subfamily in order to: (a) infer the sister taxa of the genus *Thaumetopoea*; (b) assess the relationship between the genera from Australia, Africa, and Asia, and the position of the subfamily inside the Notodontidae; (c) complete and implement the morphological and molecular data about Thaumetopoeinae, including the study of tympanal organs, genitalia, and the urticating setae occurring on larvae and adults of Africa, Asia, and Australia, which were preliminary studied also thanks to the present work (Battisti *et al.*, 2017); (d) clarify the position of still unclear taxa, such as *Thaumetopoea pityocampa* ENA (Kerdelhué *et al.*, 2009) and *T. loxostigma* (Hacker, 2016), in order to define valuable morphological features of the adults and a thorough analysis of molecular traits. This seems particularly important for all the species of the *T. apologetica* clade, which are also poorly known in their life history.

These aims have been object of two projects submitted to "Fondazione Aldo Gini" and to the BMNH "Synthesys" grant that, if successful, will allow me to extend and add the information about Thaumetopoeinae, set the foundation to complete this work looking for homologies and diagnostic traits among the African and Australasian genera, applying the tecnhiques successfully used in the thesis.

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Supplementary chapter 1

Evidence of potential hybridization in the *Thaumetopoea pityocampa-wilkinsoni* complex

Paper submitted

I contributed to the morphological analysis and paper writing.

Evidence of potential hybridization in the *Thaumetopoea pityocampa-wilkinsoni* complex

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Abstract

1. The winter pine processionary moth complex includes major defoliating insects of *Pinus* and *Cedrus* forests in southern Europe and the Mediterranean Basin, where they also cause health problems to humans and animals.
2. The complex includes two species recently distinguished based on molecular evidence, *Th. pityocampa* in the west, and *Th. wilkinsoni* in the east of the Mediterranean Basin.
3. Two populations from the core range of *Th. pityocampa* and *Th. wilkinsoni* may produce fertile hybrids when they are given the possibility to breed under controlled conditions, in spite of morphological, phenological, and genetic differences.
4. Hybrid individuals showed intermediate morphological and performance traits, while heterosis for pupal weight was detected in one of the hybrid lines. The genetic analysis confirmed the crosses.
5. Both species have large plasticity and may come into contact at the edge of their range, where they could hybridize.
6. Based on the evidence accumulated so far, it is recommended to maintain the current species designations, although a more careful study of the trait variability is required, especially in the contact zones.

Keywords: Pine processionary moth, hybridization, morphology, genetics, phenology.

Introduction

Interspecific hybridization, although not uncommon between sibling species coming into secondary contact, is usually considered costly, yielding progeny with diminished fitness and, indeed, is thought to be limited in natural systems by reproductive isolating barriers (Mallet, 2005). Barriers to reproduction can be the results of either postzygotic genetic incompatibilities or prezygotic behaviour enhanced by natural selection (Coyne and Orr, 2004). However, hybrids have been recorded in numerous species of insects including Lepidoptera (Descimon and Mallet, 2009). Studies of hybridization between closely related taxa can provide important insights into the nature of species, potential gene flow between taxa, and the process of speciation (Mullen and Shaw, 2014).

The winter pine processionary moth is a major defoliating insect of *Pinus* and *Cedrus* forests in southern Europe and the Mediterranean Basin (Battisti *et al.*, 2015). In addition, the larvae carry urticating setae that represent a threat to domestic animal and human health (Moneo *et al.*, 2015). The effect on forests and vertebrates make the species a pest with high socio-economic impact (Gatto *et al.*, 2009). The winter pine processionary moth complex includes two species. *Thaumetopoea pityocampa* [Denis & Schiffermüller, 1775] (the pine processionary moth *sensu stricto*) occurs from western Maghreb to western Turkey across southern Europe, whereas *Th. wilkinsoni* (Tams, 1925) (the eastern pine processionary moth) occurs from Turkey to Israel as well as in Crete and Cyprus (Roques *et al.*, 2015).

Th. pityocampa was separated from *Th. wilkinsoni* based on subtle morphological and behavioural differences found in some individuals from Cyprus (Tams, 1925). In the revisions of the genus by Agenjo (1941) and de Freina and Witt (1987), the status of *Th. wilkinsoni* as a separate species was confirmed, as well as in the recent catalogue of Notodontidae (Schintlmeister, 2013). The range of *Th. wilkinsoni* has been enlarged by attributing to this species all the Asiatic (Turkey Middle East) populations of pine processionary moth (Halperin, 1990). Démolin (1988) and Frérot and Démolin (1993), however, found that the species have identical sex pheromone and claimed that fertile progeny were obtained from crossing moths of the two species under laboratory conditions, although no further details about these experiment were provided. In addition, Démolin *et al.* (1994) provided an overview of the morphological traits discriminating the two species; based on male genitalia and female tuft scales, they concluded that the two were geographically restricted forms of the same species and that a study of the variability across the range was necessary.

The separation between the two species, however, was supported by molecular data by Salvato *et al.* (2002), Simonato *et al.* (2007), and Kerdelhué *et al.* (2009). The pattern of genetic diversity was typical of species that have experienced marked glaciation cycles with the main divergence times within the complex dating back to the end of the Miocene (Kerdelhué *et al.*, 2009). In addition, a contact zone between species was hypothesized and partly supported by molecular data from north-western Turkey (Ipekdal *et al.*, 2015). Further differences could be identified in the phenology, as *Th. pityocampa* emergence is generally earlier than that of *Th. wilkinsoni*, at least in the core areas, and in the host plants used, although species may share some of the host plants (Battisti *et* *al.*, 2015).

Here we document hybridization between *Th. pityocampa* and *Th. wilkinsoni* as reported earlier. Individuals from populations originating from the core area of each of two species were taken to the laboratory under quarantine conditions and exposed to appropriate temperature in order to obtain simultaneous emergence. Once the hybrids were obtained, they were reared to check their viability, and analysed by mitochondrial and nuclear DNA markers to confirm hybridization. In addition, key morphological parameters used in the past to discriminate between the two species, namely male genitalia, and the abdominal scales of the female moths, as well as wing pattern, were compared in pure and hybrid lines. Finally, the performance of the pure and hybrid lines was compared in laboratory bioassays to detect potential heterosis or hybrid depression.

Materials and methods

Crossing experiment

Two populations were selected from the core range of *Th. pityocampa* and *Th. wilkinsoni* in Italy and Israel, respectively. These should be far enough apart to be sure we have pure lines for each species. About 30 colonies of the *Th. pityocampa* population were collected from a stand of *Pinus nigra* from Veneto Calbarina (45°16'N, 11°43'E, 136 m). Colonies were reared on the same host in the campus of the Padova University in outdoor cages until pupation in spring 2004. The *Th. wilkinsoni* population were from a stand of *Pinus brutia* and *Pinus halepensis* from Dishon (33°05'N, 35°31'E, 441 m), reared on these hosts in the campus of the Agricultural Research Organization at Bet-Dagan in outdoor cages until pupation in spring 2004. Pupae were then taken from each rearing, separated from the cocoon and sexed based on genitalia slits until a total of 350 males and 350 females was obtained for each species. The pupae of *Th. wilkinsoni* were then sent from Israel to Italy, where the pupae of both populations were kept in sand (four boxes with 350 individuals in each) under controlled conditions in a quarantine-safe laboratory.

As each population displays a different emergence time in their natural habitat (Battisti *et al.*, 2015), a temperature manipulation was carried out in order to have at least partly overlapping adults emergence, as done in a similar experiment (Branco *et al.*, in press). The pupae of *Th. pityocampa*, for which the adult emergence was expected in August, were kept at 15°C, which is about 5°C less than the average temperature of the soil in the original habitat, until one month before the expected emergence time for the *Th. wilkinsoni* population (September). The *Th. wilkinsoni* population was then taken to higher temperatures (22-25°C) for 2 weeks in order to speed up their development. At the end of this treatment, the boxes with the pupae were put in two large cages (1.5x1.5x1.5 m) under naturally fluctuating temperature from August 5th to September 25st (average 23.8°C, SD 9.8). In one cage there were two boxes, one with 350 female pupae of *Th. pityocampa* and another with 350 male pupae of *Th. wilkinsoni*, and vice versa in the other cage. Moth emergence and mating occurred in the cages. When at least 20 hybrid egg batches were obtained for each cage (see below), boxes with male and female pupae of the same species were allocated to each of two clean cages in order to get egg batches of the respective species to be used as a control.

To facilitate mating, several wooden sticks about 20 cm long were inserted into sand on the bottom of the boxes to allow emerging moths to climb, unfold wings and take off, as well as a place for females to start pheromone release. Three potted mountain pines (*Pinus mugo*) about 1 m high were used to allow mated females to lay eggs. The pine species was selected because it is suitable for oviposition of *Th. pityocampa* (Stastny *et al.*, 2006). In addition, those female moths that did not oviposit during the first night following emergence were taken from the cages and isolated with one male from the same cage in darkness inside a 100 ml jar (6x10 cm) containing a wooden stick to facilitate moth hanging and oviposition (Démolin, 1969). The number of moths emerged and egg batches laid were checked every day from the first emergence, counting live individuals and removing both the egg batches and dead moths. Egg batches from the four F1 lines were designated as: PW (hybrid line of *Th. pityocampa* female and *Th. wilkinsoni* male), WP (hybrid line of *Th.*

wilkinsoni female and *Th. pityocampa* male), PP (pure line of *Th. pityocampa*), WW (pure line of *Th. wilkinsoni*).

The egg batches were labelled and maintained individually at 24±2°C until hatching. When a sufficient number of eggs hatched, subsets of individuals from each batch were taken for running starvation and performance bioassays, respectively. Neonate larvae taken individually from different egg batches were used as replicates. In the starvation bioassay, 10 non-sib larvae were kept without food in Petri dishes at 24±2°C, noting daily the number of dead larvae until total mortality was observed. The performance bioassay consisted of rearing two subsets of 20 larvae each in Petri dishes on needles of *P. halepensis* and *P. nigra* as described by Stastny *et al.* (2006). The larvae were weighed before the start and when they moulted to the second instar to estimate the relative growth rate (RGR, $mg \cdot mg^{-1} \cdot d^{-1}$), calculated as $RGR = [\ln(M_f) - \ln(M_i)]/T$, where ln is the natural logarithm, M_f and M_i are the final and initial mass of larvae, and T is the elapsed time in days (Gordon, 1968). The mortality of the larvae was assessed and a larva was considered dead when it did not react to mechanical disturbance with a brush.

Surviving larvae of the four lines and those hatched from egg batches not used in the bioassays were reared on fresh cut branches of *P. halepensis* and *P. nigra* in 8 separate cages under natural fluctuating temperature between September 2004 and March 2005. Pupae were retrieved from the soil on the bottom of the cage and a sample of 15 females and 15 males for each line and host plant species were weighed individually. Due to limitations in the capacity to maintain rearing lines under quarantine conditions, pupae belonging to the two pure lines were discarded while those of the two hybrid lines were maintained in two separate cages to obtain the F2, called PWPW and WPWP. The egg batches of the F2 were maintained under laboratory conditions as for the F1, and once the egg hatching was observed and the larval viability tested on *P. nigra* needles, the experiment was terminated and the insect material disposed as requested by quarantine measures.

Table 1. Number of moths emerging from the pupae used for the experiment (350 males and 350 females for each of the two species) and number of egg batches obtained for the four lines of the F1 generation. First letter indicates female. P= *Th. pityocampa*, W= *Th. wilkinsoni*.

	PP	WW	PW	WP
Female x Male moths	91 x 80	28 x 27	92 x 130	181 x 37
Egg batches	41	8	39	22
Egg batches yield (%)	45.1	28.6	42.4	12.2

Genetics

Both mitochondrial and nuclear markers were used to assess the identity of the individuals obtained from the crossing experiment, *i.e.* to check whether they were actual hybrids and to exclude possible errors in the sexing of pupae. As female moths mate only once and lay all their eggs in one batch (Battisti *et al.*, 2015), one individual per colony was used. Mitochondrial markers were tested on 53 larvae of the F1 lines (16, 11, 18, and 8 from PP, WW, PW, and WP, respectively) and 12 larvae of the F2 lines (9 and 3 for PWPW and WPWP, respectively). Nuclear markers were tested on 38 larvae of the F1 lines (10, 10, 10, and 8 from PP, WW, PW, and WP, respectively) and 9 larvae of the F2 lines (6 and 3 PWPW and WPWP, respectively). Each larva was preserved in ethanol 70% and stored at − 20°C before DNA extraction, which followed the salting-out procedure (Patwary *et al.*, 1994).

A mitochondrial DNA fragment of the COI gene was amplified from all individuals and examined through single-strand conformation polymorphism (SSCP) analysis, as described in Salvato *et al.* (2002). For each mobility class, one to five individuals were sequenced directly by dideoxy chain termination method at BMR Genomics sequencing service (BMR Genomics srl, Padova, Italy), in order to check for the accuracy of the SSCP analysis and to determine the corresponding haplotypes. The amplified fragment length polymorphism (AFLP) protocol (Vos *et al.*, 1995) was used with three primer combinations yielding 155 polymorphic loci on 47 individuals analysed. Approximately 50 ng of DNA were digested with Eco RI and Mse I restriction enzymes and ligated to specific AFLP adapters. Each sample was subsequently diluted 10-fold and used as template for preselective and selective (Eco RI AAC/ Mse I-CAT, Eco RI-AGC/ Mse I-CAT, Eco RI-AAG/ Mse I-CAC) PCR amplifications. AFLP products were run in an ABI PRISM 3700 DNA Analyser (Applied Biosystems). Band scoring was performed with Genotyper version 3.7 (Applied Biosystems) considering bands in the range 50–400 bp. The intensity of each individual peak was normalized on the basis of the total signal intensity and the peak was considered only if its intensity exceeded a fixed threshold of 100 fluorescent units. AFLP profiles were recorded in a matrix as presence or absence of bands for each individual.

Morphology

The following key morphological traits were considered: the male genitalia, the scales of female moth abdominal tuft, and the wing pattern according to Basso *et al.* (in press). Genitalia of 10 males from each pure and hybrid lines were analysed. The abdomen was removed and placed in 10% KOH solution for 20-25 minutes. Following maceration, abdomen and genitalia were cleaned and mounted on slides to be examined under the microscope following the protocol given by Robinson (1976). Scales of female abdominal tuft were gently picked up with forceps from the central part of the tuft and mounted on slides for microscopic observation. Pictures and measurements of male genitalia and female scales were captured using AxioCam (MRc5) mounted on Stereoscopy Lumar. v12 Zeiss (Carl Zeiss Microscope GmbH©, 2013) and with the software AxioVision SE64 (Rel. 4.9.1).

Data analyses

Anova was employed to compare the fresh weight of neonate larvae, the RGR of the first instar, and pupal weight. If available, sex was used as a factor as well as the host plant. Anova was also used to compare the ratio length/width of the tuft scales of the female moths. Tukey's test was used for pairwise comparison of means. The Kaplan–Meier product limit survival curve (Log Rank Test) was used to examine the resistance to starvation in fasting bioassays. Significance level was P=0.05. Separate GLMs (quasibinomial distribution, logit link) were used to analyse the effect of lines and host on larval mortality in the performance bioassay. For the genetics data, the COI sequence chromatograms were visualized and aligned with *Th. pityocampa* and *Th. wilkinsoni* COI haplotypes retrieved from Salvato *et al.* (2002) and Simonato *et al.* (2007), using the software MEGA 7 (Kumar *et al.*, 2016). AFLP dataset was analysed through a Principle Coordinate Analysis (PCoA) (Huff *et al.*, 1993) implemented in GenAlEx version 6.1 (Peakall and Smouse, 2007).

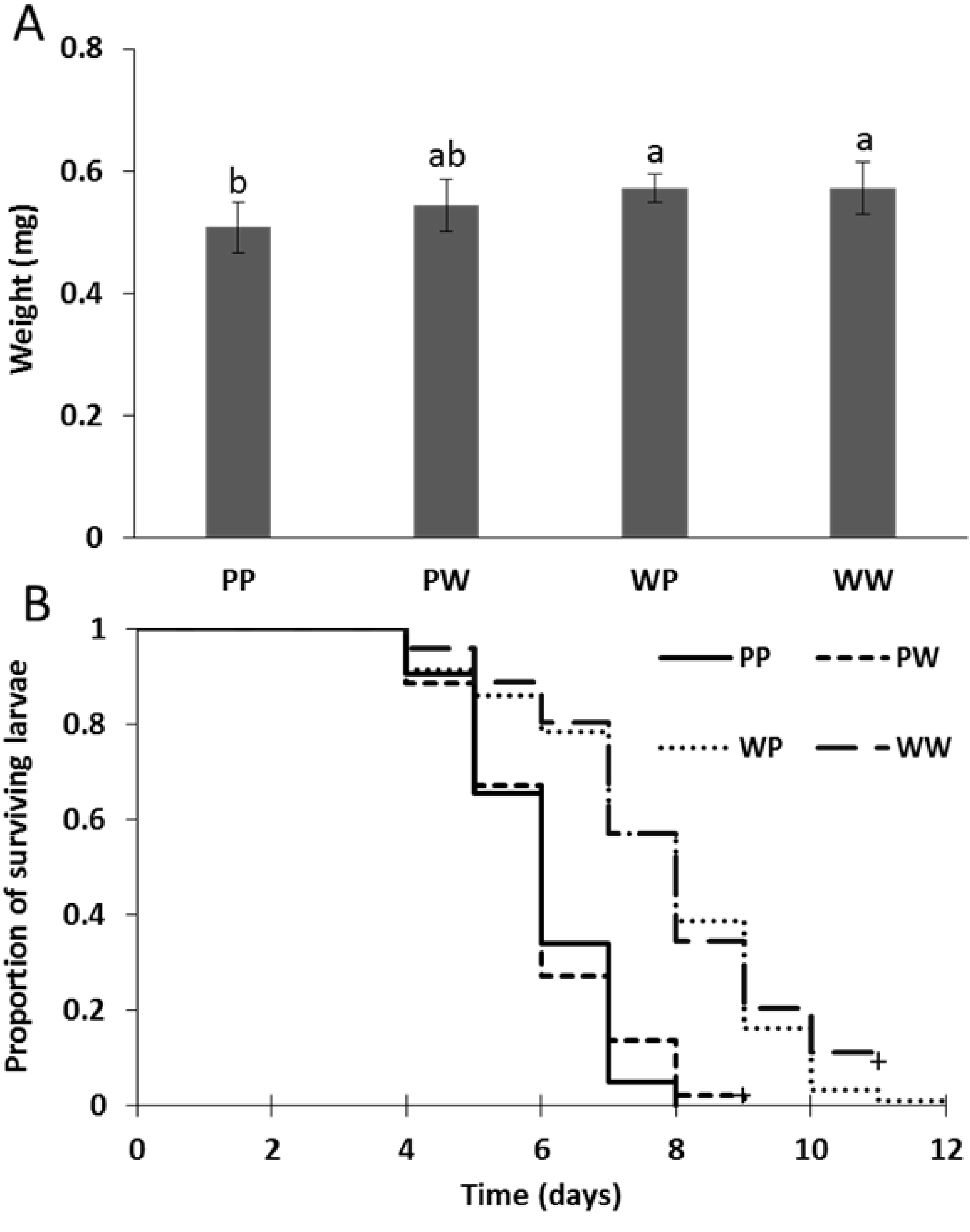

Figure 1. A) Individual fresh weight (mean ± SE) of neonate larvae of the four F1 lines. Different letters indicate significant differences in pairwise comparison of means (Tukey's test, $p<0.05$). Fig. 1 B) Larval survival during the fast bioassay with first instar larvae of the four F1 lines. First letter indicates female (P= *Th. pityocampa*, W= *Th. wilkinsoni*).

Results

Crossing experiment

The 350 pupae for each sex and species used to start the experiment yielded variable numbers of moths, *i.e.* 33.4% (males of *Th. pityocampa*), 44.9% (males of *Th. wilkinsoni*), 52.2% (females of *Th. pityocampa*), and 59.6% (females of *Th. wilkinsoni*) while the remaining pupae died. The moths of the two species overlapped in their emergence time (from August 10th to September 20th) and could mate and lay eggs (Table 1). The egg laying yield clearly depended on the availability of males and females of the two species that emerged on any particular day (see Supplementary material 1).

All the egg batches hatched normally. The larvae of WW were significantly heavier than those of PP (Fig. 1A), with the hybrid WP identical to WW and the hybrid PW at intermediate level between the pure lines ($F_{3,56}$=10.11, P<0.001). The starvation bioassay showed significant difference with a higher resistance to starvation of hybrid WP and pure line WW than that of hybrid PW and pure line PP (P<0.001, Log Rank Test) (Fig. 1B).

The relative growth rate of the first instar larvae was significantly higher for WW, on both host plants, with hybrid lines showing intermediate values ($F_{3,110}$= 5.716, P<0.01) (Fig. 2). The host plant did not affect the RGR of the first instar larvae ($F_{1,112}$=0.39, P=0.43). The mortality at the end of the first instar in the performance bioassay was very low, *i.e.* less than 2.7%, with no significant difference among lines and between host (LR test in GLM, quasibinomial error, log link, $F_{4,104}$=7.02, P=0.21 for the line and $F_{1,107}$=7.46, P=0.36 for the host plant). Pupal weight, however, was higher for individuals reared on *P. nigra* than on *P. halepensis* (female $F_{1,114}$= 41.64 P<0.001, male $F_{1,114}$=19.32 P<0.001), and the hybrid WP was heavier for both females ($F_{3,113}$= 15.57 P<0.001) and males ($F_{3,114}$= 21.52 P<0.001), providing evidence of heterosis in this line (Fig. 3).

The adults of the F1 hybrid lines emerged in the second half of August 2005. A limited number of moths was obtained (6 females and 12 males for PW, 3 females and 2 males for WP) and they were given the chance to mate and lay eggs, which resulted in 5 and 3 egg batches for PWPW and WPWP, respectively. All the egg batches hatched normally and the larvae were able to feed on *P. nigra* needles, when the experiment was terminated.

Genetics

The SSCP analysis clearly distinguished two haplotypes for the COI fragment, which were then confirmed by sequencing. All the individuals analysed for the PP and PW lines were assigned to the COI haplotype 1, the most common COI haplotype of *Th. pityocampa* in Italy (Salvato *et al.*, 2002), whereas the individuals sampled for the WP and WW lines were assigned to the haplotype 5, the only COI haplotype of *Th. wilkinsoni* found in Israel (Simonato *et al.*, 2007).

A total of 155 polymorphic bands were obtained by AFLP analysis. Among these, 43 and 40 bands were specific for *Th. pityocampa* and *Th. wilkinsoni*, respectively, whereas 57 bands were shared between the two species. Principal Coordinate Analysis, whose first three components accounted for 28.2% of the total genetic variability in the dataset, showed that the lines PP and WW were well differentiated, and that all the F1 and F2 lines fell between the parental lines, with F2 (PWPW and WPWP) showing a higher diversity than the F1 lines (PW and WP) (Fig. 4).

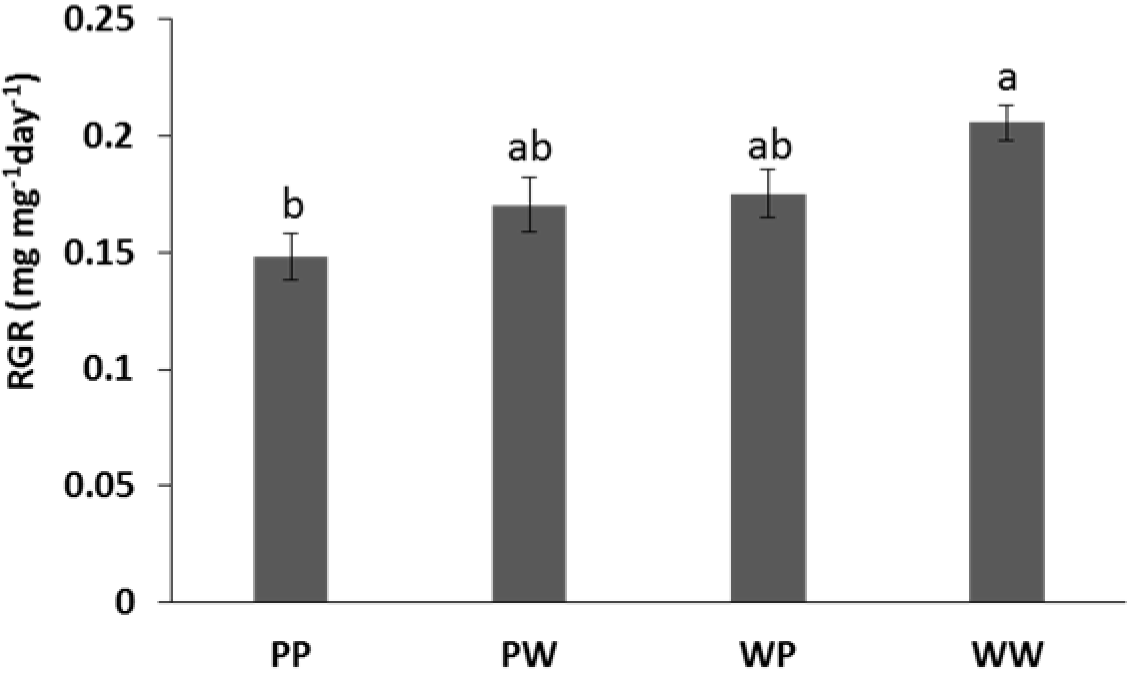

Figure 2. Relative growth rate of the first instar larvae of the four F1 lines in the performance bioassays (mean values ± SE). Different letters indicate significant differences in pairwise comparison of means (Tukey's test, $p<0.05$). First letter indicates female. P= *Th. pityocampa*, W= *Th. wilkinsoni*.

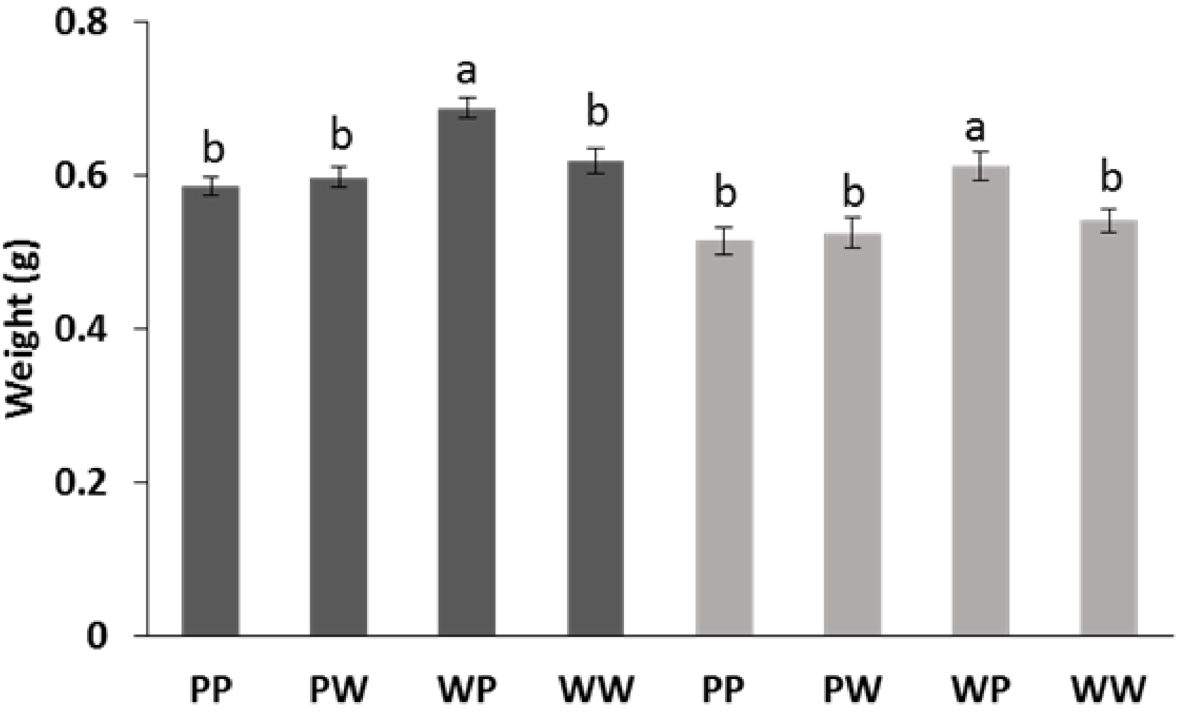

Figure 3. Fresh weight of female pupae of the four F1 lines (mean values ± SE), in black fed with *P. nigra* and in grey with *P. halepensis*. Different letters indicate significant differences in pairwise comparison of means within host plants (Tukey's test, $p<0.05$). First letter indicates female. P= *Th. pityocampa*, W= *Th. wilkinsoni*.

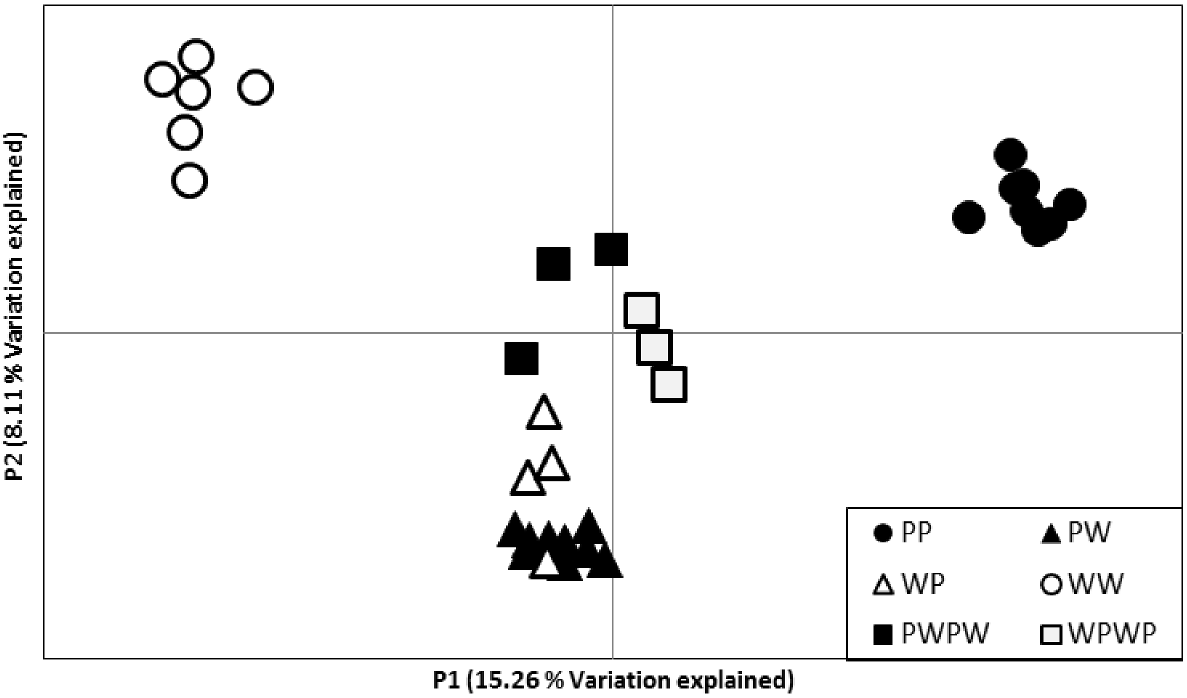

Figure 4. Principal Coordinates Analysis clustering of the AFLP genotypes analysed for the four F1 lines and the two F2 lines (PWPW, WPWP). First letter indicates female. P= *Th. pityocampa*, W= *Th. wilkinsoni*.

Morphology

The two species were distinguishable based on traits (male genitalia and the abdominal tuft scales of female moths) presented in Fig. 5 and the hybrids could be discriminated from the pure lines and from each other. A general description of the hybrid moths is provided in the Supplementary material 2. The male genitalia of the hybrid lines showed traits that were intermediate between the two species. Shape of socii were as in WW, but with lateral edge less wrinkled. Valvae, the most distinctive trait used for species discrimination, were also intermediate. In fact, ribbing on the entire length of the valvae, more defined in WW and characterizing this species (Tams, 1925), in hybrid lines was vague and present only in the first third of the valvae. A careful analysis of genitalia discriminated between hybrid types. Difference between PW and WP specimens was identified in the upper margin of valvae and in the apex of socii. Indeed, in PW specimens valvae had an extremely convex angle just before cucullus, and socii were a little wider than in WP specimens, with a ribbing on apex that lacks in WP specimens (see arrows in Fig. 5).

The ratio (length/width) of the abdominal tuft scales of female moths showed significant difference among lines ($F_{3,51}$=11.99, P<0.01). The scales were larger and shorter in WW (1.64±0.041, mean±SE) than in PP (2.08±0.054), while the shape of both hybrids was intermediate with a ratio of 1.70±0.05 in PW and 1.90±0.12 in WP (Fig. 5). The pairwise comparison was statistically significant only between the pure lines.

Discussion

The two populations from the core range of two species of pine processionary moth, *Th. pityocampa* and *Th. wilkinsoni*, produced fertile hybrids when they were given the possibility to breed in the laboratory, as already stated by Frérot and Démolin (1993) who unfortunately did not document their experimental procedure in a thorough way. The successful breeding occurred in spite of a large genetic divergence between the taxa (Salvato *et al.*, 2002; Simonato *et al.*, 2007; Kerdelhué *et al.*, 2009; Simonato *et al.*, 2013; Kerdelhué *et al.*, 2015) and morphological differences in male genitalia and female tuft scales that have been used to discriminate the species (Tams, 1925; Wilkinson, 1926; Agenjo, 1941; de Freina and Witt, 1987; Démolin *et al.*, 1994; Basso *et al.*, in press). However, it was necessary to modify the emergence time of the two species because they are largely divergent in their core range, at least when taken from very distant sites (Battisti *et al.*, 2015).

Hybrid zone are common in insects (Zhao *et al.*, 2005; Mallet *et al.*, 2007; Descimon and Mallet, 2009; Gompert *et al.*, 2010) and can entail complex interactions between the species (Hewitt, 1988; Bridle and Vines, 2007). In the winter pine processionary moth complex, the occurrence of hybrid zones has been hypothesized by Kerdelhué *et al.* (2009) in the contact zones between the three major genetic clades, i.e. *Th. pityocampa* and *Th. wilkinsoni* in western Turkey and between the two main *Th. pityocampa* mitochondrial clades in Algeria. Recently, Ipekdal *et al.* (2015) and El Mokhefi *et al.* (2016) explored these contact zones in Turkey and Algeria, respectively, identifying signs of introgression.

The cross between species, obtained as in the present study with laboratory temperature manipulation in order to get simultaneous occurrence of adults, has been done for a number of insect taxa, e.g. the bush cricket *Eupholidoptera* (Allegrucci *et al.*, 2013) and the mealybugs *Planococcus* spp. (Kol-Maimon *et al.*, 2014), and does not invalidate the species designations *per se*. There could be a number of reasons for species to maintain reproductive compatibility in spite of a long separation in time and space. Based on mitochondrial DNA evidence, *Th. pityocampa* and *Th. wilkinsoni* diverged a few million years ago on the opposite sides of the Mediterranean Basin. They came only relatively recently into contact (Kerdelhué *et al.*, 2009 and 2015), so that nowadays one may consider that the range of the winter pine processionary moth complex is continuous throughout the Mediterranean (Roques *et al.*, 2015). In addition, the two species maintain an identical sexual pheromone (Frérot and Démolin, 1993).

Although differences in male genitalia are usually considered as a pre-zygothic barrier (Shapiro and Porter, 1989), they did not seem to be determinant in the experimental conditions of this study. Moreover, the male genitalia could be used as a morphological tool to identify hybrid lines from material collected in the field. The abdominal tuft scales also displayed an intermediate shape between the pure lines, however the high variance observed at regional level in both species (Frerot and Démolin 1993) suggests that this trait is unreliable for hybrids identification (Ipekdal *et al.*, 2016).

No signs of outbreeding depression were observed, and most hybrid fitness measures were intermediate between the parental lines; heritability of most studied traits showed maternal imprinting and only one showed heterosis (weight of pupae was higher in the WP hybrid). Mortality in the hybrid lines reared to obtain the F2 was very high and probably was related to the rearing conditions, as observed in other studies with pure lines (Berardi *et al.*, 2015), or to lethality sometimes observed in hybrids (Maheshwari and Barbash, 2011). Differences in starvation assays of the first instar larvae were found between the two pure lines, and they may be linked to variation in egg size due to different ecological conditions existing in the original sites. For example, the larger eggs of *Th. wilkinsoni* may produce larvae that cope better with the semi-arid conditions of its range, and especially with tough needles, as it has been observed for a population of *Th. pityocampa* (Ruschioni *et al.*, 2015). Larger egg size involves a cost in terms of fecundity, which is generally lower in *Th. wilkinsoni* (Avci *et al.*, 2015) and in the southern populations of *Th. pityocampa* (Pimentel *et al.*, 2010; Zamoum *et al.*, 2015). To confirm the pattern, the weight of hybrid neonate larvae, which can be taken as a proxy of body and egg size, was higher in WW pure line and WP hybrid line than in PP pure line, whereas the other hybrid PW showed an intermediate value. Although the RGR at the end of first instar of the two hybrid lines was intermediate, the overall performance of the larvae, as revealed by the pupal weight, was higher in the hybrid WP than in all the other lines, indicating that the initial differences alone did not determine weight differences in the successive developmental stages, providing further evidence of the plasticity of the species. Surprisingly, *Th. wilkinsoni* did better on *P. nigra* than on its native host *P. halepensis*, confirming that host specialization is not a major issue in this species (Kerdelhué *et al.*, 2006). Indeed *Th. wilkinsoni* can naturally develops on *P. nigra* in its native range (Avci *et al.*, 2015; Ipekdal *et al.*, 2015).

The clustering of pure and hybrid lines revealed by developmental and morphological analyses was confirmed by the neutral genetic markers, which shows that genetic tools will be useful to identify hybrids in the field. It would be interesting now to assess what happens in the contact zones, for both developmental and morphological traits, to infer the amount of introgression. Based on the evidence accumulated so far on several traits of the *Th. pityocampa-wilkinsoni* complex and the latest update about the debate on species definition (Mullen and Shaw, 2014), it is recommended to maintain the current species as designated (e.g. Lushai *et al.*, 2005). To date, a low frequency of hybrids between the *Th. pityocampa-wilkinsoni* has been observed in nature. A more intensive study of trait variability would be required, especially in the contact zones, for the evaluation of possible presence of reproductive barriers apart from phenology. Whether these hybrids can become important in pest management issues is still uncertain, although such a scenario should be taken into account since the pine processionary moth is expanding its range naturally (Battisti *et al.*, 2005) and through humans accidentally introducing it with plant material from sites located far away (Robinet *et al.*, 2012; Avtzis *et al.*, 2016).

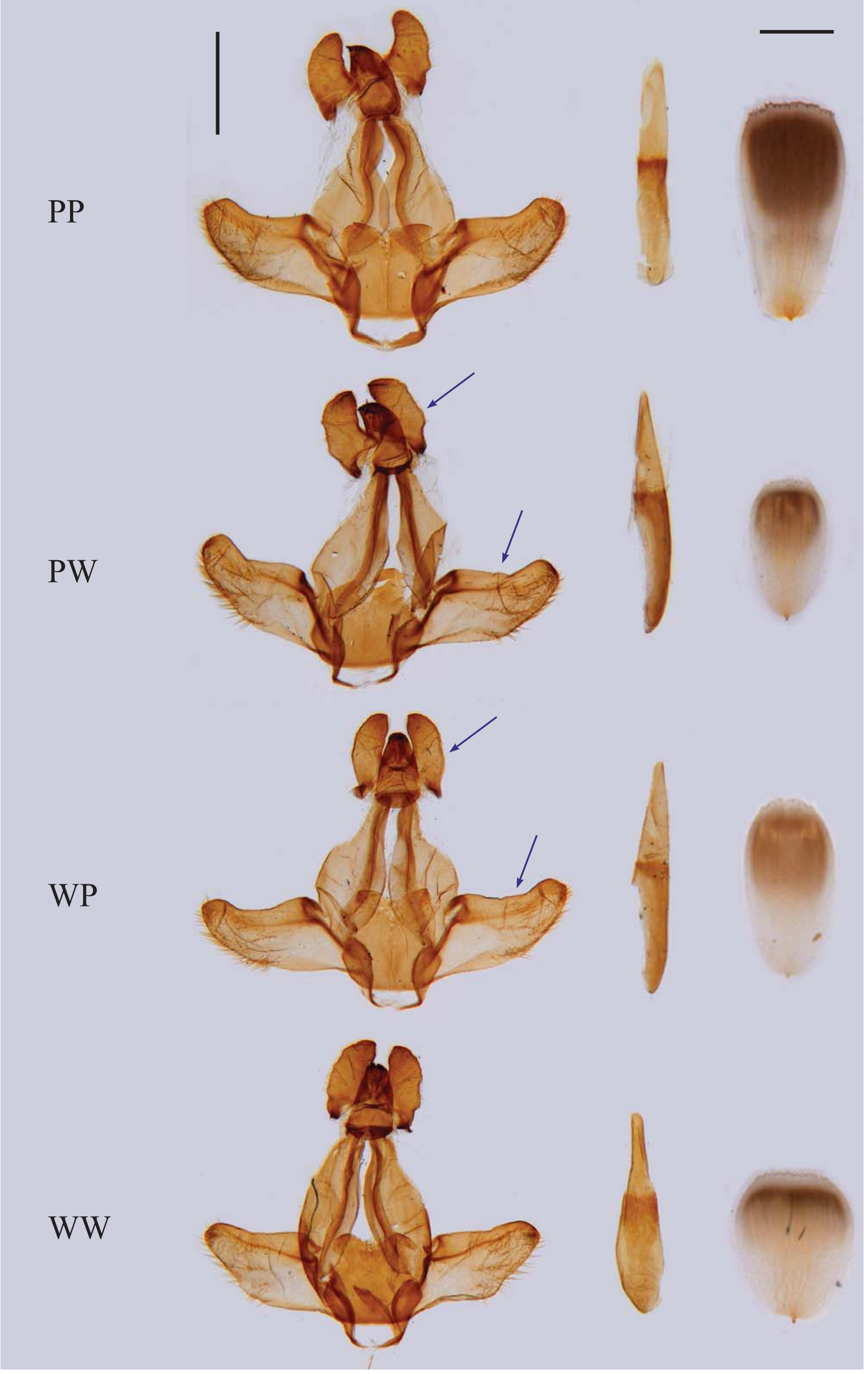

Figure 5. Male genitalia and abdominal tuft scales of female moths of the four F1 lines. Arrows indicate the discriminating traits between the hybrid lines discussed in the text. Bar scale = 1 mm. First letter indicates female. P= *Th. pityocampa*, W= *Th. wilkinsoni*.

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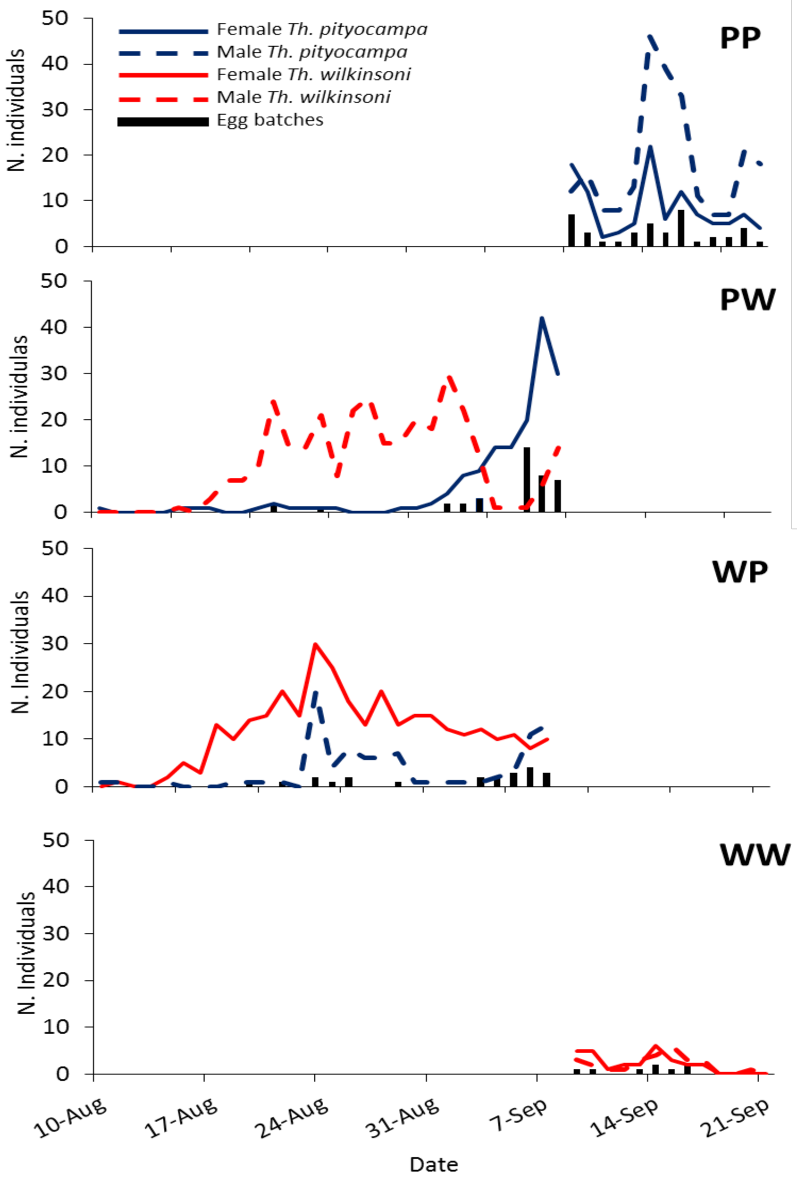
Supplementary material 1

Emergence and oviposition of moths of *Th. pityocampa* and *Th. wilkinsoni* under controlled laboratory conditions during the summer 2004, and their combinations in order to obtain pure and hybrid lines. First letter indicates female. P= *Th. pityocampa*, W= *Th. wilkinsoni*.



Supplementary material 2

Morphological description of the adult moths obtained from the cross of *Th. pityocampa* and *Th. wilkinsoni*. The two hybrid lines PW and WP (first letter indicates female; P= *Th. pityocampa*, W= *Th. wilkinsoni*) were distinguishable only based on male genitalia (see Fig. 5 and relevant text).

Male. Head brown, with light brown tuft between eyes and crest. Antennae bipectinate to apex, with ochraceous tuft on scape, long half of costa and ochraceous in ground colour. Crest present, with six teeth: first apically medium, and narrow, second well pronounced and long. Palpus rudimentary and outwardly, with first segment long as second segment and wider. Thorax dark brown or greyish with two dots of whitish hairs laterally, where forewings fit. Legs stout. Forelegs with well-developed spine and claw-shape epiphysis. A couple of spurs on each other legs. Abdomen ochraceous with brown-greyish hairs on anal apex. Wingspan: 34-39 mm. Forewing ground colour generally brown with three narrow dark fascia. Basal line zigzagged often incomplete (not touching costa) and continuous. Ante-median line vague zigzagged, complete and continuous. Post-median line jagged, complete and continuous. Ante-median and post-median line parallel on costal margin and convergent on dorsum. Terminal line dark complete and discontinuous. Silhouette of fringe, with clear visible three teeth on distal side, alternatively brown and white. Marked dark half-moon spot present in discal position, surrounded by white scales. Pre-apical vague streak present after post-median line. a vague whitish terminal line present. Hindwing whitish in ground colour, without any line except a vague alternate brown and white one in terminal position. Fringe white. Anal spot developed and anal margin fluffy white. Underside wings pattern paler and not defined. *Male genitalia.* Around 3.5 mm when fully stretched. Uncus well developed and short, with two apical teeth. Socii wide with half-moon shape. Apex rounded. Lateral edge convex slightly wrinkled and lower teeth well developed and sharp. Tegumen developed. Valvae without any apical process. Inner margin almost straight, lower margin initially straight and then convex, upper margin initially straight and then concave, folded over the length, with a vague ribbing, marked only in first third. Cucullus simple, with rounded apex. Juxta hexagonal well developed, as wide as long with two projections folded to support the aedeagus, with rib in lower part of juxta. Upper edge concave, lateral edge basally convex and apically straight, forming rounded angle. Lower edge convex. Aedeagus long, broad basally, slightly convex ventrally. Vesica simple.

Female. Bigger than male. Antennae as in male, but with shorter pectination. Wingspan: 43-46 mm. Wing ground colour pale and not well defined, generally more whitish than male. Anal scales straight, long and wide with sharpened base, pigmented at distal portion. Ratio (length/width) 1.7-1.9.

Supplementary chapter 2

Further activities carried out during the PhD period that concluded works started during my master's thesis

Is It an Ant or a Butterfly? Convergent Evolution in the Mitochondrial Gene Order of Hymenoptera and Lepidoptera

Babbucci, M.*, Basso, A.*, Scupola, A., Patarnello, T., Negrisolo, E., 2014. Is It an Ant or a Butterfly? Convergent Evolution in the Mitochondrial Gene Order of Hymenoptera and Lepidoptera. *Genome Biol Evol*, 6, 3326-3343.
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Abstract

Insect mitochondrial genomes (mtDNA) are usually double helical and circular molecules containing 37 genes that are encoded on both strands. The arrangement of the genes is not constant for all species, and produces distinct gene orders (GOs) that have proven to be diagnostic in defining clades at different taxonomic levels. In general, it is believed that distinct taxa have a very low chance of sharing identically arranged GOs. However, examples of identical, homoplastic local rearrangements occurring in distinct taxa do exist. In this study, we sequenced the complete mtDNAs of the ants *Formica fusca* and *Myrmica scabrinodis* (Formicidae, Hymenoptera) and compared their GOs with those of other Insecta. The GO of *F. fusca* was found to be identical to the GO of Dytrisia (the largest clade of Lepidoptera). This finding is the first documented case of an identical GO shared by distinct groups of Insecta, and it is the oldest known event of GO convergent evolution in animals. Both Hymenoptera and Lepidoptera acquired this GO early in their evolution. Using a phylogenetic approach combined with new bioinformatic tools, the chronological order of the evolutionary events that produced the diversity of the hymenopteran GOs was determined. Additionally, new local homoplastic rearrangements shared by distinct groups of insects were identified. Our study showed that local and global homoplasies affecting the insect GOs are more widespread than previously thought. Homoplastic GOs can still be useful for characterizing the various clades, provided that they are appropriately considered in a phylogenetic and taxonomic context. Key words: mitochondrial genomics, gene order analysis, gene order evolution, convergent evolution, Hymenoptera, Lepidoptera.

The highly rearranged mitochondrial genomes of the crabs *Maja crispata* and *Maja squinado* (Majidae) and gene order evolution in Brachyura

Basso, A.*, Babbucci, M.*, Pauletto, M., Riginella, E., Mazzoldi, C., Patarnello, T., Negrisolo, E., submitted.

* These authors contributed equally to this work.

Abstract

We sequenced the mitochondrial genomes of the spider crabs *Maja crispata* and *Maja squinado* (Majidae, Brachyura). Both genomes contain the whole set of 37 genes characteristic of Bilaterian genomes, encoded on both α- and β-strands. Both species exhibit the same gene order, which is unique among known animal genomes. In particular, all the genes located on the β-strand form a single block. This gene order was analysed together with the other nine gene orders known for the Brachyura. Our study confirms that the most widespread gene order (BraGO) represents the plesiomorphic condition for Brachyura and was established at the onset of this clade. All other gene orders are the result of transformational pathways originating from BraGO. The different gene orders exhibit variable levels of genes re-arrangements, which involve only tRNAs or all types of genes. Local homoplastic arrangements were identified, while complete gene orders remain unique and represent signatures that can have diagnostic value. Brachyura appear to be a hot-spot of gene order diversity within the phylum Arthropoda. Our analysis, allowed to track, for the first time, the fully evolutionary pathways producing the Brachyuran gene orders. This goal was achieved by coupling sophisticated bioinformatic tools with phylogenetic analysis.