Genoa University

School of Mathematics, Physics and Natural Sciences

PhD course in Sciences and Technologies for the Environment and the Territory

Marine Sciences

Taxonomy and distribution of Italian and Antarctic Pycnogonida, with identification keys to genera and species

Reviewers

Prof. Loris Galli Candidate

Prof.ssa Daniela Pessani Elisa Colasanto

Prof. Roberto Sandulli

Contents

1. List of publications	5
2. Summary	5
3. Introduction on Pycnogonida	6
4. Morphological characters	7
4.1 Cephalon	
4.1.1 Proboscis	
4.1.2 Cheliphores	11
4.1.3 Palps	
4.1.4 Ovigers	
4.1.5 Ocular tubercle	14
4.2 Trunk	
4.3 Internal anatomy	
4.3.1 Alimentary canal	
4.3.2 Circulatory system	
4.3.3 Nervous system	
4.3.4 Reproductive system	
4.4 Life cycle	
4.5 Feeding	20
4.6 Phylogeny	21
5. Research project	
6. Pycnogonids of Ligurian Sea	24
7. Pycnogonids of Tyrrhenian Sea	26
8. Checklist of Italian Pycnogonids and identification key	28
8.1 Checklist of Italian Pycnogonids	28
8.2 DISTAV Pycnogonida database	30
8.3 Preparation of the identification key	
9. Identification key of Italian Pycnogonida	
9.1 Key to genera	36
9.2 Key to species	
9.2.1 Genus Achelia	
9.2.2 Genus Ammothea	41
9.2.3 Genus Ammothella	
9.2.4 Genus Anoplodactylus	48
9.2.5 Genus Ascorhynchus	57
9.2.6 Genus Callipallene	62
9.2.7 Genus <i>Endeis</i>	71
9.2.8 Genus Neopallene	74

9.2.9 Genus Neotrygaeus	75
9.2.10 Genus Nymphon.	76
9.2.11 Genus Paranymphon	79
9.2.12 Genus Pycnogonum	80
9.2.13 Genus Rhynchothorax	83
9.2.14 Genus Tanystylum	85
9.3 Critical issues.	87
10. Antarctic Pycnogonida	89
10.1 DNA data and morphological observations	89
10.1.1 Nymphon and Pentanymphon	92
10.1.2 Austropallene	94
10.1.3 Ammothea and Achelia	95
10.1.4 Pallenopsis	96
10.1.5 Endeis and Anoplodactylus	98
10.1.6 Decolopoda	99
10.1.7 Colossendeis	101
10.1.8 Samples selected for DNA analysis	101
10.2 Updated Checklist of Antarctic and sub-Antarctic Pycnogonida	104
10.3 Key to Antarctic genera	112
10.4 Key to Antarctic species (incomplete)	115
10.4.1 Genus Achelia	115
10.4.2 Genus Ammothea	116
10.4.3 Genus Austroraptus	116
10.4.4 Genus Cilunculus.	117
10.4.5 Genus Dromedopycnon	117
10.4.6 Genus Sericosura.	118
10.4.7 Genus Tanystylum	118
10.4.8 Genus Ascorhynchus	120
10.4.9 Genus Eurycyde	121
10.4.10 Genus Colossendeis	121
10.4.11 Genus Decolopoda	122
10.4.12 Genus <i>Dodecolopoda</i>	122
10.4.13 Genus Austropallene	123
10.4.14 Genus Callipallene	124
10.4.15 Genus Cheilopallene	124
10.4.16 Genus Oropallene	124
10.4.17 Genus Pseudopallene	125
10.4.18 Genus Seguanallene	

	10.4.19 Genus Heteronymphon	. 125
	10.4.20 Genus Nymphon	. 126
	10.4.21 Genus Pentanymphon	. 127
	10.4.22 Genus Sexanymphon	. 128
	10.4.23 Genus Pallenopsis	. 128
	10.4.24 Genus Endeis	. 128
	10.4.25 Genus Anoplodactylus	. 129
	10.4.26 Genus Phoxichilidium	. 130
	10.4.27 Genus Pentapycnon	. 130
	10.4.28 Genus Pycnogonum	. 131
	10.4.29 Genus Rhynchothorax	. 132
	10.4.30 Genus Austrodecus	. 132
	10.4.31 Genus Pantopipetta	. 135
11.	Pycnogonida and Citizen Science	. 136
12.	Conclusions	. 137
13.	References	. 139

1. List of publications

Galli L, Colasanto E, Betti F and Capurro M. (2019). Pycnogonids (Arthropoda: Pycnogonida) of Portofino, Ligurian Sea (North-Western Mediterranean Sea). *The European Zoological Journal*, 86:1, 241-248. https://doi.org/10.1080/24750263.2019.1637951

Colasanto E and Galli L. (2021). The Pycnogonids (Arthropoda, Pycnogonida) of Torrevaldaliga (Tyrrhenian Sea), Italy, with data on *Endeis biseriata*, new record for the Mediterranean Sea. *The European Zoological Journal*, 88:1, 622-631. https://doi.org/10.1080/24750263.2021.1910741

Colasanto E and Galli L. (2021). People's contribution to the knowledge of Pycnogonida: citizen science in the case of a "problematic" taxon. *Biogeographia – The Journal of Integrative Biogeography*, 36. http://dx.doi.org/10.21426/B636053543

Colasanto E and Galli L. (2021). Pycnogonida. In: Bologna M.A., Zapparoli M., Oliverio M., Minelli A., Bonato L., Cianferoni F., Stoch F. (eds.), Checklist of the Italian Fauna. Version 1.0. Last update: 2021-05-31. Available at: https://www.lifewatchitaly.eu/en/initiatives/checklist-fauna-italia-en/checklist/

2. Summary

This thesis provides a description of the projects carried out in the three-year period 2018-2021 as part of the study of the pycnogonids. The procedures that led to the drafting of the papers in these three years are then described in detail.

A general overview of the class Pycnogonida (Arthropoda, Chelicerata) is reported, highlighting the main characteristics of these organisms.

Subsequently, the updated checklist of the Italian pycnogonids (Colasanto and Galli, 2021a) is proposed with the detailed distribution of the species, generated eleven years after that of Bartolino and Chimenz (2010). The dichotomous key proposed for the species identification refers to these documents. The key was obtained through bibliographic investigations on the morphological characteristics of the species, integrating with information taken from keys already present in the literature (in particular, Munilla and Soler-Membrives, 2014) and laboratory analysis of samples.

Parallel to the study of the Italian pycnogonids, specimens preserved at the National Museum of Antarctica were examined and the barcoding DNA of some of them was sequenced. Identification keys to genera and species were sketched, based on morphological characters. Therefore, the updated checklist of Antarctic and sub-Antarctic Pycnogonida is also reported.

Finally, some considerations on the possibility to involve Scuba-divers on pycnogonids monitoring through citizen science projects are reported.

3. Introduction on Pycnogonida

Pycnogonida (Latreille, 1810) are a poorly known class of marine benthic Arthropoda Chelicerata, represented by nearly 1350 species and 80 genera (Bamber et al., 2022).

The name Pycnogonida was given to these animals in 1810, but they were also called Podosomata and Pantopoda, respectively by Leach and Gerstaecker in later times (King, 1973). Podosomata is currently no longer used; the entire Pycnogonida group includes the extinct specimens, while Pantopoda is used to indicate the still living organisms.

These organisms are widespread worldwide: they have been collected in all seas all over the world from equator to poles (Bamber et al., 2022), ranging from littoral zone to more than 6000 m depth (King, 1973; Munilla and Soler-Membrives, 2014). Due to their morphology resembling that of terrestrial spiders, Pycnogonida are called commonly "sea spiders". Their body size ranges from few mm to 75 cm of leg span (for example in some species of *Colossendeis* genera) (Child, 1995b; Munilla and Soler-Membrives, 2014; Bamber et al., 2022).

The ecology of these animals is very variable. There are species that can be found in association with algae, phanerogams, others on detrital bottoms, still others are interstitial or bathy-pelagic; also species associated with Porifera, Cnidaria, Mollusca and Echinodermata, as parasites or predators (Arnaud and Bamber, 1987), are known (Fig. 1).



Figure 1. The pycnogonid
Anoplodactylus virescens under a
stereoscope.
Elisa Colasanto's photo

4. Morphological characters

Like all arthropods, pycnogonids have a chitinous cuticle and articulated legs (usually four pairs). The body can be slender or almost rounded, and it can be divided into three parts: cephalon, trunk and abdomen. The morphological organization of their body, therefore, is different from that of terrestrial spiders (Fig. 2).

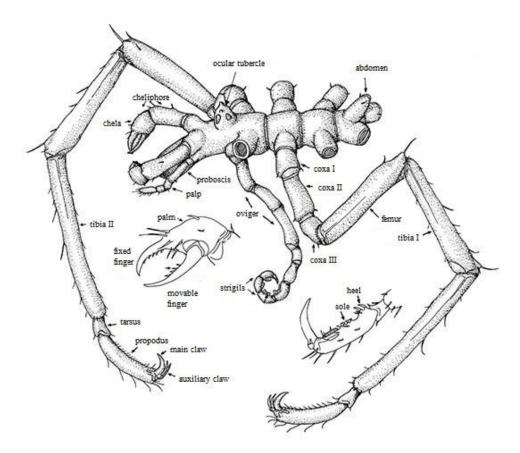


Figure 2. Body structures of a generic pycnogonid. From Munilla and Soler-Membrives 2014, mod.

The trunk is normally composed by three somites, between which the suture lines may be more or less visible; on the last segment there is the abdomen (this is usually a small vertical bump with the anus on top). Each somite can bear different types of ornamentation such as dorsal spurs, spines, tubercles or long setae (Arnaud and Bamber, 1987).

Each segment bears also a pair of locomotory legs, as well as the cephalon that bears one pair of legs. Although these organisms usually have eight legs, it is possible to find exceptions: for example, some Antarctic genera have ten or twelve legs (*Decolopoda* Eights, 1835, *Pentanymphon* Hodgson, 1904, *Pentapycnon* Bouvier, 1910, *Dodecolopoda* Calman & Gordon, 1933 and *Sexanymphon* Hedgpeth & Fry, 1964) (King, 1973; Child 1995a, b; Munilla and Soler-Membrives, 2014).

Besides, rare specimens show abnormal shapes due to anomalies in the development of trunk and/or appendages (Brenneis and Scholtz, 2020). Some individuals, then, can have less than four pairs of legs. These abnormalities and, in particular, the presence of extra segments is related with the ability of pycnogonids to regenerate limbs after injuries (King, 1973). Organisms with anomalies related to other parts of the body can also be recorded, such as, for example, double ocular tubercle, malformation of the claws or

somites with asymmetrical development (Arnaud and Bamber, 1987; Scholtz and Brenneis, 2016; Galli et al., 2019).

The total body length of the pycnogonids can range from less than 1 mm (in some *Rhynchothorax* species) up to a length of 10 cm for deep-sea *Colossendeis* species (Arnaud and Bamber, 1987). Within a single genus, the size may vary between species and within the same species the females are often larger than the males (Arnaud and Bamber, 1987) (Fig. 3).

It is difficult to determine which factors influence the size the most. In general, the littoral species are small: large forms with elongated legs would be too fragile for high-energy environments. The larger species are typical of deep and polar waters where organisms are characterized by low metabolic rate and a tendency to gigantism (Arnaud and Bamber, 1987).

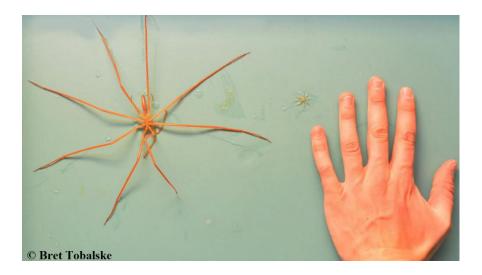


Figure 3. Size difference between two species of pycnogonids.

Bret Tobalske's photo

Generally, the colours of these animals are yellowish-brown, white or transparent; despite this, some species have a variable chromatic range and evident colours.

Some species of Antarctic *Colossendeis* appear yellow, orange or red; *Anoplodactylus evansi*, *Meridionale harrisi*, *Pycnogonum aurilineatum*, *Stylopallene cheilorhynchus* and *S. tubirostris* are examples of species with striking liveries (respectively red and violet, red and yellow, black with a yellow-white stripe on the trunk and both *Stylopallene* are black and yellow). Probably, these colours have a defense function as aposematic stains (Arnaud and Bamber, 1987).

In most cases, the coloration shown by the species reflects that of the intestinal contents: an example from Italian fauna is *Anoplodactylus virescens*, which often shows green and pink colours (as can be seen in figure 4). In such cases body colouring warrants good mimesis in the natural environment.



Figure 4. Dorsal view of an Anoplodactylus virescens, showing a general greenish colour; parts of the cuticle with pink pigmentation are also noted.

Elisa Colasanto's photo

Moreover, some morphological adaptations to the various living environments are known. Abyssal forms are often anophthalmic; those species living in environments with high hydrodynamism are stocky and robust; the infaunal species are recognized, among other characters, by the lack of pigments, flattened body and not ornamented cuticle (Arnaud and Bamber, 1987).

4.1 Cephalon

The front part of the body, also called cephalon or cephalothorax, has no external segmentation and can be compact (as in the genus *Tanystylum*) or developed in length (as in the genus *Callipallene*), but it can also vary within the same genus. The cephalon between the insertion of cheliphores and the first lateral processes is called "neck": this is the part that can be more or less elongated, with the ocular tubercle in different positions depending on the genus or species considered.

Various organs and appendages are associated with this first body segment: a proboscis, the ocular tubercle, cheliphores, palps and ovigers (three pairs of appendages which in some cases may be absent in the adult phase, with specific functions). These three pairs of appendages prove to be fundamental for the identification, being the main characters used in the dichotomous keys to both the Italian and Antarctic genera.

The hind part of the cephalon shows two lateral processes bearing the first pair of legs and, as for the trunk, also the cephalon can have different ornamentation and thorniness (Arnaud and Bamber, 1987).

4.1.1 Proboscis

In the antero-ventral position there is a mobile proboscis at the distal end of which the mouth opens. Proboscis is involved in the ingestion of food and shows variations in shape and proportion in the different families. This organ is made up of three longitudinal elements called antimers, each ending with a chitinous lip: for this reason, the proboscis has a particular tri-rayed symmetry (Arnaud and Bamber, 1987).

The uptake of food occurs thanks to the combined action of the muscularized jaws and pharynx that allow scraping and sucking of nutrients (Arnaud and Bamber, 1987; King, 1973).

The proboscis has different morphologies, cuticle ornamentations and freedom of movement, based on the different eating habits of each species (Fig. 5). In species that have well developed cheliphores, the movements of the proboscis are very limited: it is the task of the cheliphores to bring the food (especially parts of sponges and hydroids) towards the mouth area. While in species with atrophied or missing cheliphores (*Achelia* and *Endeis*) the proboscis is able to make large movements in the region of the collar (the area in which it is articulated to the cephalon), assuming different inclinations (King, 1973).

The proboscis usually has setae or spines with a tactile or chemoreceptive function for food detection (Lehmann et al., 2017).



Figure 5. Detail of the proboscis of Ascorhynchus simile.

Elisa Colasanto's photo

4.1.2 Cheliphores

The cheliphores, when present, are located in the frontal position in the anterior part of the cephalon, above the proboscis (Fig. 6).

They consist of a basal segment (called scape), one or two median articles and, in most cases, a terminal claw that intervenes in the alimentary process, consisting of a fixed and a mobile finger jointed together.

In some species the fingers of the claw are very elongated and have a notable internal dentition, with the function of manipulating the food and perhaps also holding the substrate (Arnaud and Bamber, 1987). In general, the complexity of their structure depends on dietary habits: as already mentioned, in the genera that have a good development of the proboscis, the cheliphores may be absent (for example, in the genera *Colossendeis, Endeis* and *Pycnogonum*) or reduced (genera *Achelia, Neotrygaeus* and *Tanystylum*) (King, 1973).

These appendages, which have chemoreceptors for food detection (Lehmann et al., 2017), are always present and functional in the juvenile stage of protonymphon (Munilla and Soler-Mmebrives, 2014) but can regress with the advent of the adult stage and the consequent change in diet.



Figure 6. Detail of cheliphores of Callipallene tiberi; on movable and fixed finger on top lots of small teeth can be observed.

Elisa Colasanto's photo

4.1.3 Palps

The palps are a couple of appendages placed laterally to the proboscis and to the cheliphores; their function is generally sensory (Lehmann et al., 2017), alimentary and/or cleansing, due to the more or less marked presence of thorns or setae on their surface (King, 1973; Arnaud and Bamber, 1987).

They are usually multi-segmented appendages, in which the number of articles varies from 1 to 20 (King, 1973). In many genera they are absent (for example, in *Pycnogonum* and *Endeis*); in others they are reduced (genus *Pallenopsis*) or vestigial (genus *Phoxichilidium*) (King, 1973). Due to this variability, they are one of the most important diagnostic characters (Fig. 7).

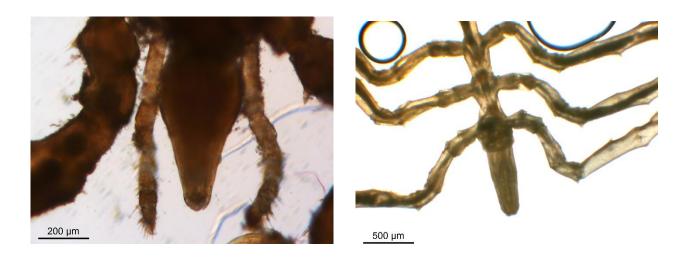


Figure 7. Left: detail of palps of Achelia echinata, on each side of proboscis. Right: Endeis spinosa: both palps and cheliphores are absent.

Elisa Colasanto's photos

4.1.4 Ovigers

The ovigers are the third pair of cephalic appendages, exclusive to the Pycnogonida: they are located ventrally, between the insertion of the proboscis and the first lateral process (first leg's joint) (Fig. 8). They are multi-segmented (with a variable number of articles, also used as an identification character), and can be characterized by a terminal claw.

In most males they carry out the fundamental function of transporting the fertilized ovigerous sacs and the larvae after hatching. In some species also the females possess ovigers, but the task of keeping the eggs is left to the male. The ovigers have also another function in both males and females: these can be wrapped around the other appendages in order to clean up them and remove the debris or parasites from their surface (King, 1973; Arnaud and Bamber, 1987). The shape and development of ovigers can also be an important taxonomic character. For example, there are genera in which females lack ovigers (*Anoplodactylus, Endeis* and *Pycnogonum*) (Arnaud and Bamber, 1987).

The last four (or five) articles of the ovigers are called strigils (Child, 1979) and are provided with simple or compound bristles, usually distributed in two rows. The abundance and morphology of these has great taxonomic importance, allowing, in some cases the identification to the species level based on the so called "strigilar formula", i.e. the number of bristles on each article of strigils.

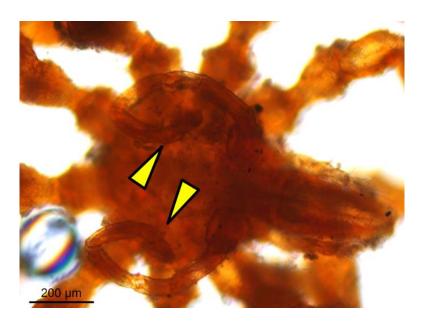


Figure 8. Ventral view of Tanystylum conirostre: ovigers originates at the base of the proboscis.

Elisa Colasanto's photo

4.1.5 Ocular tubercle

The dorsal zone of the cephalon, along its midline, hosts the ocular tubercle. This is usually characterized by the presence of four simple pigmented eyes and, at times, of different sizes (the anterior ones larger), although some deep waters species may be blind or with de-pigmented or reduced eyes (Lehmann et al., 2017) (Fig. 9).

Each eye is made up of a semi-circular retina and a single lens, separated by a certain number of photoreceptive cells. Although the lenses of these sense organs are not joined, their surface covers much of the lateral surface of the ocular tubercle, allowing for multidirectional sensitivity to light. Presumably, in fact, the eyes of the pycnogonids cannot lead to the formation of images, but only detect the light intensity (King, 1973).

The lateral organs of Sokolow (small and poorly pigmented) can be observed near the eyes: over time, various functions have been associated with these structures (Lehmann et al., 2017) probably in charge of thermoception or chemoreception (Richter, 1982; Arnaud and Bamber, 1987).

The ocular tubercle can be placed anteriorly or posteriorly on the cephalon and can vary in shape according to the species considered (it can be elongated, compact, rounded, mucronate, conical, etc...).

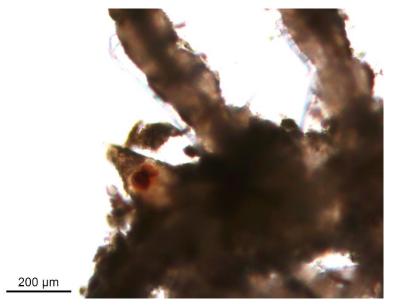


Figure 9. Detail of the conical ocular tubercle with pigmented eyes of Ascorhynchus castelli.

Elisa Colasanto's photo

4.2 Trunk

The trunk is made up of three mutually articulated metamers (and the first articulated to the cephalon).

Laterally, each segment has a pair of lateral processes, to which the legs are articulated, for a total of eight legs, including the pair already mentioned on the cephalon. These processes, as well as the entire trunk, may have variable dorsal ornamentation: thorns, tubercles, setae or arborescent processes, etc. The function of these elements is not clear (King, 1973).

The ambulacral legs are made up of nine segments (Fig. 10): starting from the proximal area ca be found the coxa I (articulated to the lateral process), coxa II, coxa III, femur, tibia I, tibia II, tarsus, propodus and terminal claw. Tibia and femur are usually the largest articles, while the tarsus is the smallest. Generally, the propodus has a curved shape and a variable number of spines of different sizes; the main claw can be accompanied by a pair of auxiliary claws of variable length.

The legs can vary in length and appearance, being devoid of ornaments or rich of tubercles, thorns, setae, which can have a certain taxonomic importance.

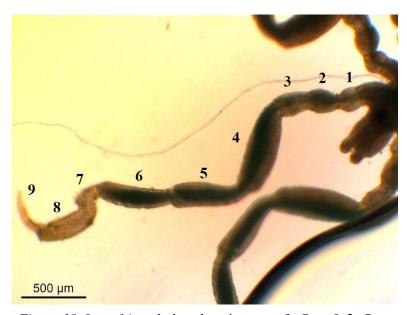


Figure 10. Leg of Anoplodactylus virescens. 1. Coxa I; 2. Coxa II; 3. Coxa III; 4. Femur; 5. Tibia I; 6. Tibia II; 7. Tarsus; 8. Propodus; 9. Terminal claw.

Elisa Colasanto's photo

The larval and juvenile pycnogonids have a considerable regenerative capacity of the limbs: following the scarring of the missing appendix, the new rudimentary limb is reconstituted within the remaining cuticle and completed at the time of moulting. "Reconstructed" limbs can be identified in adults due to their smaller size (King, 1973).

Inside the legs there are the digestive caeca and the ducts of the reproductive system: the genital pores generally open on the ventral surface of the coxae II, in all the legs in the females and only on the third and fourth pair of legs in the males (Fig. 11). The female orifice is usually larger in diameter and mature females may show enlarged femora in which the eggs are contained before laying (Arnaud and Bamber, 1987).

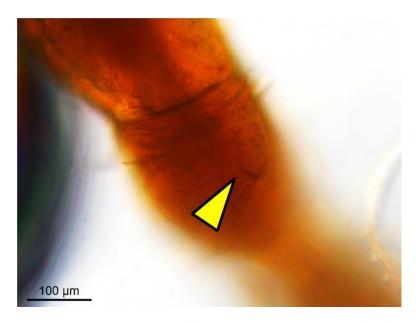


Figure 11. Detail of a female leg of Neotrygaeus communis; the genital pore is on the ventral region of coxa II.

Elisa Colasanto's photo

The femora of males have from one to several pores in which the cement glands open. These are secretory glands that produce a substance capable of retaining the eggs received by the female in cohesive sacks. (Munilla and Soler-Membrives, 2014). The pores of these glands are variable in morphology, number and arrangement.

The trunk ends with a dorsal abdomen, often fused with the fourth segment. The abdomen can be of variable size and length and can carry spines or tubercles that recall the ornamentation of the back. The term "segment" is not attributed to this body element since it is not innervated by a nerve ganglion (Munilla and Soler-Membrives, 2014). The abdomen ends with the anal opening.

The locomotion in these animals can take place actively, walking or swimming, or passively, letting oneself be carried away by the currents. They usually advance slowly over the substrate and the distance travelled probably depends only on the spatial distribution of the substrate where they need to go to deposit the juvenile individuals (King, 1973; Arnaud and Bamber, 1987). When they are moving in the water column, it is clear that pycnogonids do not have structures and appendages suitable for swimming (King, 1973).

4.3 Internal anatomy

These organisms do not have a respiratory and excretory system and gas exchanges take place via the body surface, through the thinnest areas of the integument (body joints) (Arnaud and Bamber, 1987).

4.3.1 Alimentary canal

The digestive tract extends from the mouth, located in the proboscis, to the anus, at the apex of the abdomen. The digestive canal can be divided into three parts: the front part (stomodeus) consists of the mouth with a trimer section, surrounded by a variable number of setae and variously sclerotic, based on the individual's eating habits. The mouth is in communication with a pharynx inside the proboscis; there are setae and filtering and shredding teeth that lead the food up to the oesophagus. The internal membrane of this anatomical part carries a "sieve" made up of long setae, known as "oyster basket", with the function of maceration and ingestion of food; the oesophagus ends with a tripartite valve, connecting to the intestine. The second part (mesodium) branches following the profiles of the legs, culminating with several blinds that can also reach the tibia and tarsus (in some cases they also branch inside the proboscis or cheliphores, given the small size of the trunk); the absorption of nutrients takes place through epithelial cells. Finally, the third part (proctodeus) consists of a small canal that ends with the anal opening (King, 1973; Arnaud and Bamber, 1987).

4.3.2 Circulatory system

The circulatory system of the pycnogonids is open, without blood vessels, and occupies the internal cavities of the body. The only structure attributable to a primitive vessel is represented by the heart, located dorsally to the alimentary canal: the movement of the fluid inside it is caused by the peristaltic contractions of the underlying intestine. The haemolymph from the heart spreads towards the proboscis, from which ventrally it reaches the trunk and legs, and then returns to the heart (King, 1973; Arnaud and Bamber, 1987).

Haemolymph performs various functions: reaching the organs of the body, it is able to transport nutrients and hormones; it is also essential to mediate breathing through the areas where the cuticle is thinner and could play a fundamental role during moulting periods to balance the pressure inside the body. This fluid is colourless and rich in different types of cells: granulocytes, leukocytes and amoebocytes (King, 1973).

4.3.3 Nervous system

The central nervous system of these organisms consists of a metameric ventral ganglion chain: these ganglia, almost always fused, form two cephalic thickenings (supra-esophageal and sub-esophageal). These cerebral ganglia innervate the ocular tubercle, the cheliphores, the palps and ovigers, and the proboscis, respectively. On the other hand, the ventral trunk ganglia correspond to the appendages in position and number (King, 1973; Arnaud and Bamber, 1987).

In addition to the ocular tubercle, the existence of other sense organs, in particular chemoreceptors, has been demonstrated. These are usually located on the palps, but it has been shown that even species without these appendages (*Endeis* for example) are able to recognize nutrient-rich waters (Arnaud and Bamber, 1987). In fact, these receptors may be found on the epidermal bristles, on the distal part of the trunk, or on the legs (Lehmann et al., 2017).

4.3.4 Reproductive system

Generally, the pycnogonids have separate sexes: the reproductive organs consist of a pair of ovaries or testes located above the intestinal canal, near both sides of the haemocele. In most species, the eggs mature inside the femora and it is not uncommon to be able to observe them under the stereoscope. Each genital diverticulum communicates with the outside through a duct located on the second coxa: the opening of the genital pores occurs through an operculum and can often be located at the apex of a conical or tubular tubercle (for example, in the genera *Achelia* and *Anoplodactylus*) (Arnaud and Bamber, 1987).

Following the maturation of the eggs, a fibrous layer rich in proteins, lipids and polysaccharides is formed between them, capable of carrying out an adhesive function: in this way, the eggs are kept cohesive and can be taken from the male after the exit from the second coxa of the female (King, 1973; Arnaud and Bamber, 1987).

4.4 Life cycle

The pycnogonids, as already mentioned, are gonochoric with external fertilization, primitive parental care by the male and sexual dimorphism. Despite this, some specimens of the genus *Anoplodactylus* were found to be gynandromorphic, that is, with both male and female characteristics (Arnaud and Bamber, 1987); moreover, the species *Ascorhynchus corderoi* (Marcus, 1952) is the only known species of hermaphroditic pycnogonid so far (King, 1973).

Regarding sexual dimorphism, it is necessary to remember the presence of cement glands in the femora of males: these secrete their content, an agglutinating and cementing substance, in order to preserve and compact the sacs of fertilized eggs, and then attach them on the ovigers (Munilla and Soler-Membrives, 2014).

The number of ovigerous sacs that the male carries depends on the number of females he has fertilized: each egg sac adhering to the male's ovigers comes from a single female gonopore; consequently, eggs belonging to different females are not kept in the same pouch. If there are several sacs, these are arranged chronologically along the ovigers: in the proximal part there are those acquired first and the more recent ones in the distal part; for this reason, it is possible to observe males carrying larvae and eggs at the same time (King, 1973; Munilla and Soler-Membrives, 2014).

The practice of transporting eggs and larvae involves greater biological costs, since these individuals are more easily identifiable by predators, perform slower movements and are often attacked by a significant number of epibionts (Munilla and Soler-Membrives, 2014).

Littoral species have seasonal ovulation cycles while offshore species frequently release eggs throughout the year (King, 1973).

Pycnogonids are characterized by anamorphic development: initially the larva has few metamers which, over time, tend to increase in number until they reach that of adults (Munilla and Soler-Membrives, 2014).

Once the eggs have hatched, the larva that emerges can be of different types, based on the family or genus they belong to. Without going into detail and considering the typical protonymphon (name by which the larva is defined in its early stages of development) we recognize three phases for growth of the species *Tanystylum orbiculare* shown in figures 12, 13 and 14:

I. Protonymphon: just after hatching, the individual has a rounded body, reduced cheliphores (but with a claw already present) and sketches of the palps. These proto appendages possess a long distal spine, probably useful for maintaining some stability while the male carries the larvae.

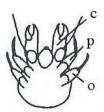


Figure 12. Protonymphon. Body structures: **c**. cheliphores; **o**. ovigers; **p**. palps. From Munilla and Soler-Membrives 2014, mod.

II. Post-larva: following the first moults, the individual begins to develop legs (one pair at a time), the previously sketched appendages acquire articles, and the body begins to lengthen.

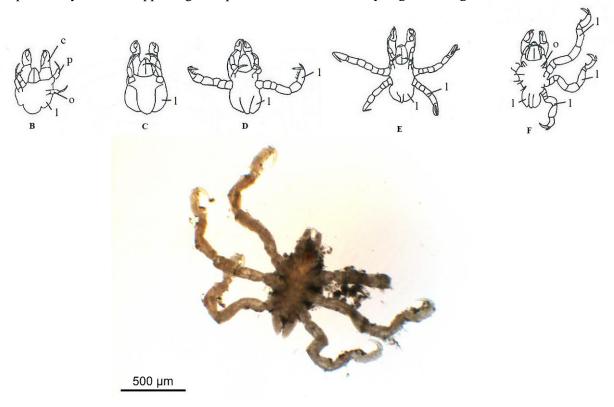


Figure 13. Post-larva. Paw development occurs in several stages, one pair at a time (D-F). Body structures: **c**. cheliphores; **l**. legs; **o**. ovigers; **p**. palps. From Munilla and Soler-Membrives 2014, mod.

Photo: post-larva still without the last pair of legs. Elisa Colasanto's photo

III. Juvenile individual: the legs complete their development, the abdomen appears, the palps are defined, the ovigers appear and, based on the species, the structure of the cheliphores is defined (in some cases through a partial or complete regression).

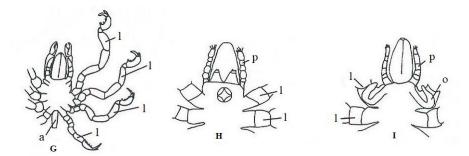


Figure 14. Juvenile. Development of the fourth pair of legs, palps and ovigers. In this species, the regression of the cheliphores is observed (H). Body structures: **a**. abdomen; **l**. legs; **o**. ovigers; **p**. palps. From Munilla and Soler-Membrives 2014, mod.

4.5 Feeding

In general, the eating habits of these animals are largely unknown. The data obtained so far reveal species able to feed on detritus, algae, sessile invertebrates or those with little or no ability to move such as hydrozoans, anthozoans, bryozoans, sponges and molluscs (King, 1973).

The nutrition of pycnogonids is mainly based on sucking, exercised with the three lips present at the apex of the proboscis and with the pharyngeal muscles. This operation can be assisted by the intervention of the cheliphores (or of the first pair of legs in the species without these appendages) and, thanks to the "oyster basket", the components that could damage the tissues of the intestine are shredded (Arnaud and Bamber, 1987).

The diet of the different species is conditioned by the living environment and seasonal availability. Consequently, the conformation of the proboscis also varies from species to species, based on adaptation. In some cases, the species may vary their eating habits over the course of the year, using different seasonal trophic strategies. *Ammothella longipes*, for example, can lead a mainly carnivorous diet in spring and early summer (when invertebrate larvae are abundant), and then switch to a detritivorous diet in autumn and winter (Soler-Membrives et al., 2011).

There are also many species endoparasitic and ectoparasitic of molluscs, octoorals and polychaetes. Hydroid and bivalves in particular can be parasitized by encysted larvae that spend the entire period of development within their tissues (King, 1973).

4.6 Phylogeny

The pycnogonids are a group of marine Arthropods of uncertain affinity, frequently associated with the Chelicerata. The main morphological characteristics and autapomorphies consist of an external proboscis, a pair of ventral appendages (ovigers), a reduced trunk (which consequently causes the extension of various organs inside the legs) and an abdomen reduced to a single article. These exclusive characters belong to all representatives of the taxon, denoting a clear monophyletism. Another aspect that supports the monophyletic hypothesis is the presence in different lineages of polymeric species with one or two more body segments and, therefore, five or six pairs of legs (Munilla and Soler-Membrives, 2014).

In general, the texts and the informative-didactic works have a simplistic approach in defining the pycnogonids as sea spiders, based only on their superficial resemblance to the terrestrial spiders (Araneae) (King, 1973).

Recent molecular, morphological and paleontological analyses differ from each other in the placement of this taxon within the Arthropods. Several studies propose to separate the pycnogonids from the chelicerates, considering them as a primitive lineage (Munilla and Soler-Membrives, 2014). Some authors have always considered the pycnogonids as an isolated group of arthropods (King, 1973; Waloszek and Dunlop, 2002) while others, instead, approached them to crustaceans (Krøyer, 1840; Waloszek and Dunlop, 2002).

On the other hand, studies of just over ten years ago position the pycnogonids within the Chelicerata, as the sister-group of the Euchelicerata (Arachnida and Xifosura) (Waloszek and Dunlop, 2002; Munilla and Soler-Membrives, 2014). This hypothesis is based on the fact that both groups possess a first pair of chelated appendages (synapomorphy), in addition to the homology between palps and pedipalps, the subdivision of the body into two regions (prosoma and opisthosoma) and the lack of antennae and chewing oral appendages (Munilla and Soler-Membrives, 2014).

According to a study proposed by Arango and Wheeler (2007), based on morphological and molecular characteristics, the following can be stated:

- * The most primitive families are: Austrodecidae, Colossendeidae and Pycnogonidae (three families whose members are devoid of cheliphores), to which the species Phoxichilidium femoratum is added.
- ❖ The family *Ammotheidae* includes the genus *Tanystylum* and it is closely linked to the family *Rhynchothoracidae*, both without cheliphores.
- ❖ The genera *Eurycyde* and *Ascorhynchus* are separated by *Ammotheidae*, forming a separate family (*Ascorhynchidae*).
- ❖ Callipallenidae and Nymphonidae, both with well-developed cheliphores, form a single clade each.
- ❖ The genus *Endeis* is related to family *Phoxichilidiidae*.
- ❖ The genus *Pallenopsis* constitutes a family itself (*Pallenopsidae*).

5. Research project

This research is aimed at deepening the taxonomic study of a marine fauna group of particular interest: the pycnogonids. Much of the project is related to the study of samples stored in the entomology laboratory of the DISTAV, including many hundreds of specimens related to different collection stations in the Mediterranean. It should be emphasized that almost all of the samples were only provided with labels with codes of reference to certain research projects. Therefore, a preliminary work of reconstruction of the history of each individual sample was necessary in order to be able to bring it back to a well-defined date and collection site. Then, approximately 200 indeterminate Antarctic specimens in the collection of the National Museum of Antarctica were examined. Wherever possible, genetic analyses (barcoding DNA) and insights were associated with systematic analysis, aimed at defining the differences between different geographically contiguous communities, as well as the historical evolution of the populations of individual sites monitored for several years.

The three-year research (2018-2021) was articulated as follows.

First stage (2018-2021): bibliographic analysis and examination of the material available for the identification of Mediterranean and Antarctic pycnogonids.

- ❖ Initial phase of collection of bibliographic data concerning Pycnogonida, with particular interest for articles on taxonomy and fauna of the geographical areas of interest.
- Preparation of a keys for the identification of pycnogonids.
- Analysis of available samples to verify the reliability of the keys in order to identify specimens at the level of genus and species. Specimens, when possible, were also sexed and classified according to they stage of development.
- ❖ Organization of samples and data storage. Each sample was labelled indicating the collection site, date and all the information necessary for the characterization of the site.

Second stage (2019-2020): statistical analysis of the Ligurian and Tyrrhenian Sea samples.

- Quantitative study at population level where samples are available at the same location at different times to estimate the trend, taking into account the variation in environmental conditions over the period considered.
- Determination of sex-ratio and phenology for each species and analysis of possible fluctuations of population over time.

Third stage (2021): focus on the possibility to plan citizen science projects on pycnogonids.

- Analysis of the images found on various naturalistic websites and verification of the reliability of the identifications.
- ❖ Assessment of the amount of reliable previously collected data and shared on the net by divers.
- ❖ Identification of possible strategies to improve the yield of amateur observations and the limits/opportunities of possible citizen-science projects in the future.

The main objective of this study was to build keys of the Italian pycnogonids (not already existing) and Antarctic ones as accurate as possible, to allow the identification of genera and, when possible, species.

More generally, this survey could contribute to increase the knowledge about these organisms. The bibliography relating to this group of marine animals is very extensive but fragmentary, with particular reference to the Antarctic and Iberian species, but the data collected in Italy are very scarce.

To this purpose, some identification keys of the Antarctic species (see for instance Child, 1995a, b; Cano-Sánchez and López-González, 2014, 2019; Švara and Melzer, 2016) are available as a basis to work on, but a comprehensive one has not yet been produced. Given the enormous variability of Antarctic species (for example genera such as *Colossendeis* and *Nymphon*, with 36 and 76 species, respectively), the more recent keys available in the literature are incomplete and do not mention all the species recorded so far in the Antarctic waters (Munilla and Soler Membrives, 2009).

6. Pycnogonids of Ligurian Sea

Deep studies on these organisms are not frequent for Italian Seas: excluding the published checklist of 2010 (Bartolino and Chimenz, 2010), specific papers were focused on Central and Southern Italy (Krapp, 1973; Chimenz Gusso et al., 1979; Chimenz et al. 1979, 1993; Chimenz and Cottarelli, 1986; Piscitelli and Barone, 2000). Our study on Ligurian Sea (Galli et al., 2019) was published 19 years after the last paper on field surveys, integrating some new results and updating the few researches done in this area (Faraggiana, 1940; Krapp, 1975; Arnaud, 1987; Chimenz Gusso 2000).

Pycnogonids' fresh specimens (499 individuals) were collected monthly from September 2017 to August 2018 by the marine biologist Dr. Federico Betti. The sampling area was in the Portofino Marine Protected Area (MPA) from the coastal strip of the Portofino promontory (Genoa) (WGS 84 coordinates of the sampling site: 44° 17′ 55.70" N, 9° 13′ 7.15" E), in the Eastern part of Ligurian Sea. Sampling activities were performed at 5 metres depth and main macroalgae and hydroids were collected by visually oriented sampling. Every month around 113 g of organisms were collected, covering a surface of about 900 cm² (Galli et al., 2019).

Some other pycnogonids (417 specimens) from the same area found in unstudied historical collections (30-40 years aged - kindly donated by Prof. Mario Mori) were used to increase the studied material.

Samples were caught at a maximum depth of 15 metres, fixed in formaline 4% and then in ethanol 70% to be analysed.

The collected pycnogonids (499 specimens) were studied under a stereoscope and, sometimes, an interference contrast microscope (Leica DM LB2). 42 of them remained unidentified or identified only to genus level, while 457 were assigned to 10 different species: *Achelia echinata* Hodge, 1864, *Ascorhynchus castelli* (Dohrn, 1881), *Neotrygaeus communis* (Dohrn, 1881), *Tanystylum conirostre* (Dohrn, 1881), *Anoplodactylus angulatus* (Dohrn, 1881), *A. petiolatus* (Kröyer, 1884), *A. pygmaeus* (Hodge, 1864), *A. virescens* Hodge, 1864, *Callipallene phantoma* (Dohrn, 1881) and *C. tiberi* (Dohrn, 1881).

The additional 417 specimens confirmed the presence in the study area of nine of the species mentioned above (except *A. virescens*). Besides, two other species could be added to the list: *Pycnogonum pusillum* Dohrn, 1881 and *Endeis spinosa* (Montagu, 1808).

Through this study, the phenology of the five more abundant species was outlined and three species were registered as new for the Ligurian Sea (*Callipallene phantoma*, *C. tiberi* and *Pycnogonum pusillum*).

For completeness, the list of species known from the biogeographical sector corresponding to the Ligurian Sea (north of Piombino and Capo Corso, part of the north-western sector of the Mediterranean) is shown in table I.

Table I. Checklist of pycnogonids of Ligurian Sea, updated in 2019.

* Species previously unknown for Ligurian Sea

Family	Species
Ammotheidae	Achelia echinata Hodge, 1864
	Ammothella appendiculata (Dohrn, 1881)
	A. longioculata (Faraggiana, 1940)
	A. longipes (Hodge, 1864)
	Neotrygaeus communis (Dohrn, 1881)
	Paranymphon spinosum Caullery, 1896
	Tanystylum conirostre (Dohrn, 1881)
Ascorhynchidae	Ascorhynchus arenicola (Dohrn, 1881)
	A. castelli (Dohrn, 1881)
Callipallenidae	C. phantoma (Dohrn, 1881) *
	C. tiberi (Dohrn, 1881) *
Nymphonidae	Nymphon gracile Leach, 1814
Endeidae	E. charybdaea (Dohrn, 1881)
	E. spinosa (Montagu, 1808)
Phoxichilidiidae	Anoplodactylus angulatus (Dohrn, 1881)
	A. petiolatus (Krøyer, 1844)
	A. pygmaeus (Hodge, 1864)
	A. virescens (Hodge, 1864)
Pycnogonidae	P. plumipes Stock, 1960
	P. pusillum Dohrn, 1881 *

For further information, see the article:

Galli L, Colasanto E, Betti F and Capurro M. (2019). Pycnogonids (Arthropoda: Pycnogonida) of Portofino, Ligurian Sea (North-Western Mediterranean Sea). *The European Zoological Journal*, 86:1, 241-248. https://doi.org/10.1080/24750263.2019.1637951

7. Pycnogonids of Tyrrhenian Sea

As mentioned in the previous paragraph, there were few studies available on the Italian Pycnogonida; among these, the pycnogonid fauna of Civitavecchia was studied by Chimenz et al. (1979) and Arnaud (1987). They found 15 species from which they drawn some faunal considerations (Chimenz Gusso and Lattanzi, 2003).

Examining an old collection from the Torrevaldaliga Power Station (North-Tyrrhenian Sea), attributable to 1979-1980, we updated the species list of this area.

Totally, 315 specimens were analysed and only three of them were identified only at the genus level, while the others were assigned to 6 different species: *Ammothella appendiculata* (Dohrn, 1881), *Anoplodactylus californicus* Hall, 1912, *Endeis biseriata* Stock, 1968, *E. spinosa* (Montagu, 1808), *Nymphon gracile* Leach, 1814 and *Tanystylum conirostre* (Dohrn, 1881).

Thanks to this study, it was possible to produce an updated checklist of Central Tyrrhenian pycnogonids (Table II) belonging to the northern section of the central-western area of the Mediterranean Sea (Area 2, based on Bianchi, 2004).

The data shown in the table have been obtained from the previous checklist of Italian Pycnogonida (Bartolino and Chimenz, 2010) and from various publications relating to the following Tyrrhenian areas: Central Tyrrhenian Sea (Chimenz et al., 1979; Arnaud, 1987; Chimenz Gusso and Lattanzi, 2003; Lehmann et al., 2014), South Tyrrhenian Sea (Chimenz and Cottarelli, 1986; Arnaud, 1987), Gulf of Naples (Dohrn, 1881; Bouvier, 1923; Chimenz and Cottarelli 1986; Arnaud, 1987) and Sardinia (Bouvier, 1923; Lehmann et al., 2014).

Table II. Checklist of pycnogonid species in the Tyrrhenian Sea, with some indications of their distribution. Legend: CT, Central Tyrrhenian Sea; GN, Gulf of Naples; NT, North Tyrrhenian Sea; SAR, Sardinia; ST, South Tyrrhenian Sea.

Family	Species	CT	GN	NT	SAR	ST
Ammotheidae	Achelia echinata Hodge, 1864	X	X	X		X
	A. langi (Dohrn, 1881)	X	X	X		X
	A. vulgaris (Costa, 1861)		X			
	Ammothella appendiculata (Dohrn, 1881)	X	X	X		X
	A. biunguiculata (Dohrn, 1881)	X	X			
	A. longioculata (Faraggiana, 1940)	X		X		X
	A. longipes (Hodge, 1864)	X	X	X		X
	A. uniunguiculata (Dohrn, 1881)	X	X	X		X
	Neotrygaeus communis (Dohrn, 1881)	X	X		X	X
	Paranymphon spinosum Caullery, 1896	X	X	X		
	Tanystylum conirostre (Dohrn, 1881)	X	X	X		X
	T. orbiculare Wilson, 1878	X		X		
Ascorhynchidae	Ascorhynchus arenicola (Dohrn, 1881)	X	X	X		X
	A. castelli (Dohrn, 1881)	X	X	X		X
	A. pudicus Stock, 1970	X				
	A. simile Fage, 1942	X				
Callipallenidae	Callipallene acribica Krapp, 1975	X				
	C. emaciata (Dohrn, 1881)	X	X	X		
	C. phantoma (Dohrn, 1881)	X	X	X		
	C. producta (Sars, 1888)	X				
	C. spectrum (Dohrn, 1881)	X	X	X		
	C. tiberi (Dohrn, 1881)	X	X			_

Family	Species	CT	GN	NT	SAR	ST
Nymphonidae	Nymphon gracile Leach, 1814	X	X	X		
Endeidae	Endeis biseriata Stock, 1968	X				
	E. charybdaea (Dohrn, 1881)	X	X	X		X
	E. spinosa (Montagu, 1808)	X	X	X		
Phoxichilidiidae	Anoplodactylus angulatus (Dohrn, 1881)	X	X			X
	A. californicus Hall, 1912	X				X
	A. massiliensis Bouvier, 1916	X				
	A. petiolatus (Krøyer, 1844)	X	X	X		X
	A. pygmaeus (Hodge, 1864)	X	X	X		X
	A. virescens (Hodge, 1864)	X	X			X
Pycnogonidae	Pycnogonum nodulosum Dohrn, 1881	X	X		X	
	P. plumipes Stock, 1960	X				
	P. pusillum Dohrn, 1881	X	X	X		
Rhynchothoracidae	Rhynchothorax alcicornis Krapp, 1973	X	X			
	R. mediterraneus Costa, 1861	X	X			

An important contribution to the pycnogonids' fauna knowledge has been the record of *Endeis biseriata* Stock, 1968, new for the Mediterranean Sea. This species was found in West New Guinea (Stock, 1968), later in the Red Sea (Stock, 1970) and other areas of Southeast Asia; it was recently registered in the Gulf of Oman (Lucena et al., 2018). Two hypotheses were made about the presence of this species in the Mediterranean: the most accredited is that according to which the dispersion may have occurred through fouling, but the possibility of a lessepsian migration cannot be excluded.

The identification of *Endeis biseriata* near Torrevaldaliga allowed us to update the previous checklist reporting one more species of *Endeis* in the Italian waters.

For further information, see the article:

Colasanto E and Galli L. (2021). The Pycnogonids (Arthropoda, Pycnogonida) of Torrevaldaliga (Tyrrhenian Sea), Italy, with data on *Endeis biseriata*, new record for the Mediterranean Sea. *The European Zoological Journal*, 88:1, 622-631, DOI: 10.1080/24750263.2021.1910741

8. Checklist of Italian Pycnogonids and identification key

8.1 Checklist of Italian Pycnogonids

During 2020 we have been involved in compiling the checklist of Italian Pycnogonida (Colasanto and Galli, 2021a) as part of the project on the New Checklist of the Italian Fauna. This project is coordinated by the Scientific Committee for Italian Fauna and implemented with the support of the Italian National Research Council and LifeWatch Italy and involved about 160 professional taxonomists or amateurs.

This new checklist updates the last one published by Minelli et al. (1993-1995) and enriched in the next years. Differently from that, the new one excludes protozoans and is accessible exclusively online, making its update and fruition easier. This new checklist contains terrestrial, freshwater and marine species of the Italian fauna, including more than 25000 species and subspecies (https://www.lifewatchitaly.eu/en/initiatives/checklist-fauna-italia-en/).

Every scientific name is related to the author and the year of description and other information are often given, such as the distribution, the status of endemic or allochthonous species.

The previous checklist of Italian pycnogonids was published in 2010 (Bartolino and Chimenz, in Relini, 2010). We have been able to update it thanks to our research and other recent papers (Lehmann et al., 2000; Montoya Bravo et al., 2006; Occhipinti-Ambrogi et al., 2011; Soler-Membrives and Munilla, 2015; Lucena et al., 2018; Galli et al., 2019; Colasanto and Galli, 2021b).

Table III shows the presence of 45 pycnogonid species in the Italian seas, divided by the different sectors (based on Bianchi, 2004).

Table III. Checklist of pycnogonids of the Italian seas (updated in 2021).

° Alien species; * Endemic species; ? species with doubtful data

Family	Species	1	2	3	4	5	6	7	8	9
Ammotheidae	Achelia echinata Hodge, 1864	X	X	X				X	X	X
	Achelia langi (Dohrn, 1881)		X	X				X	X	?
	Achelia simplex (Giltay, 1934)								X	X
	Achelia vulgaris (Costa, 1861)		X				X			
	Ammothea hilgendorfi (Böhm, 1879) °									X
	Ammothella appendiculata (Dohrn, 1881)	X	X	X						X
	Ammothella biunguiculata (Dohrn, 1881)		X	X				X		X
	Ammothella longioculata (Faraggiana, 1940)	X	X				X	X		X
	Ammothella longipes (Hodge, 1864)	X	X	X				X		X
	Ammothella uniunguiculata (Dohrn, 1881)		X	X			X	X		
	Neotrygaeus communis (Dohrn, 1881)	X	X	X						X
	Paranymphon spinosum Caullery, 1896	X	X	X						
	Tanystylum conirostre (Dohrn, 1881)	X	X	X			X	X	X	X
	Tanystylum orbiculare Wilson, 1878		X	X			X			X
Ascorhynchidae	Ascorhynchus arenicola (Dohrn, 1881)	X	X	X						

Family	Species	1	2	3	4	5	6	7	8	9
	Ascorhynchus castelli (Dohrn, 1881)	X	X	X						
	Ascorhynchus pudicus Stock, 1970		X							
	Ascorhynchus simile Fage, 1942		X					X		
Callipallenidae	Callipallene acribica Krapp, 1975		X							
	Callipallene brevirostris (Johnston, 1837)								X	?
	Callipallene emaciata (Dohrn, 1881)		X	X					X	X
	Callipallene phantoma (Dohrn, 1881)	X	X	X			X	X		X
	Callipallene producta (Sars, 1888)		X	X						
	Callipallene spectrum (Dohrn, 1881)		X	X			X		X	X
	Callipallene tiberi (Dohrn, 1881)	X	X	X			X			X
	Neopallene campanellae Dohrn, 1881 *			X						
Nymphonidae	Nymphon gracile Leach, 1814	X	X	X						
	Nymphon parasiticum Merton, 1906 *			X						
	Nymphon puellula Krapp, 1973						X			
Endeidae	Endeis biseriata Stock, 1968 °		X							
	Endeis charybdaea (Dohrn, 1881)	X	X	X						X
	Endeis spinosa (Montagu, 1808)	X	X	X				X		X
Phoxichilidiidae	Anoplodactylus angulatus (Dohrn, 1881)	X	X	X			X	X	X	X
	Anoplodactylus californicus Hall, 1912°		X	X						
	Anoplodactylus compositus Chimenz, Cottarelli & Tosti, 1991 *							X		
	Anoplodactylus massiliensis Bouvier, 1916		X							
	Anoplodactylus petiolatus (Krøyer, 1844)	X	X	X			X	X	X	X
	Anoplodactylus pygmaeus (Hodge, 1864)	X	X	X			X	X		X
	Anoplodactylus robustus (Dohrn, 1881)			X						X
	Anoplodactylus virescens (Hodge, 1864)	X	X	X				X		
Pycnogonidae	Pycnogonum nodulosum Dohrn, 1881		X	X						
	Pycnogonum plumipes Stock, 1960	X	X					X		
	Pycnogonum pusillum Dohrn, 1881	X	X	X			X	X	X	
Rhynchothoracidae	Rhynchothorax alcicornis Krapp, 1973		X	X				X		
	Rhynchothorax mediterraneus Costa, 1861		X	X				X		

Based on our studies, we found information about three alien species (marked by the symbol °): *Ammothea hilgendorfi* is considered by Krapp and Sconfietti (1983) an alien species with an established population. Occhipinti-Ambrogi et al. (2011) do not exclude its lessepsian nature and its diffusion by shipping fouling.

Endeis biseriata was described as pantropical species probably with an original Indo-Pacific distribution, previously known also from the Western coasts of the Atlantic Ocean and the Red Sea (Lucena et al., 2018). We found this species in the North Thyrrhenian Sea: the first record in the Mediterranean Sea (Colasanto and Galli, 2021b).

Anoplodactylus californicus was found in the waters of Latium and Norther Sicily (Chimenz et al., 1979; Chimenz Gusso, 2000). Occhipinti-Ambrogi et al. (2011) attributed its spread through shipping fouling.

We have also recorded three endemic species (marked by the symbol *): *Neopallene campanellae*, a single specimen found at Punta Campanella (Dohrn, 1881); *Nymphon parasiticum*, a single juvenile specimen found in the Gulf of Naples (Merton, 1906); *Anoplodactylus compositus*, found only once in Brindisi (Chimenz et al., 1991).

Two species have incomplete data regarding the ninth biogeographical sector: *Achelia langi* was registered on Croatia's coasts, near Cape Savudrija by Montoya Bravo et al. (2006) and Lehmann et al. (2014) and also near of Pula and Brijuni National Park (Croatia) by Lehmann et al. (2021). So far this species has never been found on Italian coasts, but this record of *Achelia langi* confirms its presence in the biogeographical area 9 (for this reason we indicated the presence with "?"). *Callipallene brevirostris* was found in the Lagoon of Venice in 1944-1945 (Stock,1952) but we can not be sure about its presence in this area because of the data's ages and the complexity of identification of the species belonging to this genus.

8.2 DISTAV Pycnogonida database

Data on the pycnogonids preserved in the entomology laboratory of DISTAV (Corso Europa 26, Genova, Italy) as part of the Genoa University Zoology Museum were archived in a dedicated database. These specimens collected in different Italian places (most of them come from Portofino, others from the Tyrrhenian Sea and still others from the Adriatic) were used to test step by step the identification keys we have processed.

The periods and localities in which they were collected are very variable since they are separate collections made by different marine biologists (just think that the Torrevaldaliga samples are dated 1979-1980).

Totally 1635 specimens were examined. Only 63 of them remained indetermined, being larvae, post-larvae of juveniles, due to the scarse knowledge of the postembrional development of several species and to the radical change of body characteristics during growth.

A summarized list of the material mentioned above is shown in the table IV. The province of origin of the samples identified at species level will be indicated.

It can be noted that in the various collections *Tanystylum conirostre* (Dohrn, 1881) is a very widespread and always very numerous species.

Table IV. DISTAV Pycnogonida database. Species divided according to the province of collection

Province	N.er of specimens	Genus	Identified ONLY to genus level	Species	Identified to species level
Ancona	11	Ammothella	2		
		Endeis		E. spinosa	7
		Tanystylum		T. conirostre	2
Genova	1148	Achelia	53	A. echinata	121
		Ammothella	1	A. longipes	1
				A. uniunguiculata	3
		Anoplodactylus	7	A. angulatus	5
				A. petiolatus	7
				A. pygmaeus	106
				A. virescens	15
		Ascorhynchus	3	A. castelli	47
		•		A. simile	1
		Callipallene	16	C. phantoma	36
		•		C. tiberi	126
		Endeis	1	E. charybdaea	5
				E. spinosa	30
		Neotrygaeus		N. communis	140
		Pycnogonum	1	P. pusillum	1
		Tanystylum		T. conirostre	422
Grosseto	1	Paranymphon		P. spinosum	1
Lecce	2	Anoplodactylus		A. virescens	1
		Tanystylum		T. conirostre	1
Livorno	10	Achelia	1		
		Anoplodactylus		A. petiolatus	2
		Ascorhynchus		A. castelli	1
				A. simile	4
		Paranymphon		P. spinosum	1
		Pycnogonum		P. nodulosum	1
Macerata	2	Tanystylum		T. conirostre	2
Napoli	3	Ascorhynchus		A. castelli	3
Roma	327	Achelia	1		
		Ammothella	2	A. appendiculata	4
		Anoplodactylus		A. californicus	183
		Ascorhynchus		A. simile	1
		Endeis		E. biseriata	23
				E. spinosa	9
		Nymphon		N. gracile	1
		Tanystylum		T. conirostre	103
Siracusa	1	Pycnogonum		P. pusillum	1
Savona	66	Achelia		A. echinata	2
		Anoplodactylus		A. petiolatus	1
				A. pygmaeus	1
		Endeis	1	E. spinosa	3
		Tanystylum		T. conirostre	58
Venezia	1	Ammothea		A. hilgendorfi	1

8.3 Preparation of the identification key

Concerning the preparation of the identification key, an in-depth bibliographic analysis was initially conducted in search of taxonomic descriptions and redescriptions of pycnogonids present within the Italian fauna.

As a starting point, we referred to the first checklist of Italian species (Bartolino & Chimenz, 2010), part of a wider list of marine species of the Italian fauna (Relini, 2010). Subsequently, following the update of the checklist (Colasanto and Galli, 2021a), the key was also modified and enriched.

The 2010 list reported 44 species of the 56 known for the Mediterranean Sea (Lehmann et al., 2014) while the final list (updated in July 2020) recorded 45 species due to the already mentioned addition of *Endeis biseriata*.

I think it is quite likely that the real number of Italian species is greater than known. Some species are small in size (and are not detected during the sorting procedures), others have limited distribution in areas with particular climatic or hydrological conditions and still others are widespread on the bathyal level (where, however, very rare surveys are carried out). Furthermore, pycnogonids are very little studied in Italy, so the information completeness on the biodiversity of this group will always be lower than that of other groups of main interest.

After the examination of the species widespread in the Italian seas, an even more in-depth bibliographic search was necessary, in order to find the original articles or documents relating to the description of individual genera or species, and also articles concerning taxonomic revisions.

Subsequently, the identification key of the 14 genera of Italian pycnogonids was created: thanks to the information acquired and the illustrations often present within the taxonomic papers, the characteristics with greater diagnostic importance were isolated and, through the methodological use of dichotomy, the different genera have gradually been discriminated against. This key directly identifies genera, without intermediate steps to families for greater convenience and to keep it valid regardless of the system followed.

The characters necessary to distinguish the different genera are quite general and mainly related to the presence, absence and morphology of the appendages (cheliphores, palps and ovigers).

Focusing on more specific characteristics (for example: ornamentation of the integument, auxiliary claws, conformation of the ocular tubercle, strigilar formula, etc.) it was also possible to draw up a key for the identification of the 45 Italian species. The procedure was more complex, as it is not always easy to understand which characters are reliable from the diagnostic pont of view (in many cases there is a considerable intraspecific variability); furthermore, not all descriptions are always accurate and precise.

For these reasons, the possibility to test the effectiveness of our key by identifying "fresh" samples was crucial in allowing it to be continuously improved. To achieve the goal, it was also necessary and useful to examine the already existing keys, such as that of King (1986) for the British Isles and that of Munilla and Soler-Membrives (2014) for the Iberian Peninsula.

The tools used for the samples' identification and cataloguing are as follows:

- ❖ Petri dishes: the samples were placed in these to be analysed (Fig. 15).
- ❖ Alcohol diluted to 70% and/or glycerine: useful for analysing and then storing the samples (for this only alcohol).
- **Stereoscope** (Fig. 15).
- ❖ Leica DM LB2 optical microscope with Leica DFC 295 camera and Leica Application Suite (Vers. 3.8).
- Tweezers.
- Needle mounted.
- ❖ Paper labels with indication of the sampling location, sampler, depth, date of collection and scientific name of the identified specimen.
- **!** Eppendorf tubes.
- ❖ Identification key (updated from time to time).





Figure 15. Left: part of the laboratory equipment used to identify samples. Right: petri dish containing the label related to two Anoplodactylus virescens specimens. Elisa Colasanto's photos

9. Identification key of Italian Pycnogonida

The class Pycnogonida includes four orders, three of these host the fossil species with segmented abdomen, while the fourth (order Pantopoda) includes the living species (Munilla and Soler-Membrives, 2014).

A list of the 45 Italian species, for which the analytical key has been prepared, is reported below, divided by families.

Order PANTOPODA

Family Ammotheidae Dohrn, 1881

Genus Achelia Hodge, 1864

Achelia echinata Hodge, 1864

Achelia langi (Dohrn, 1881)

Achelia simplex Giltay, 1934

Achelia vulgaris (Costa, 1861)

Genus Ammothea Leach, 1814

Ammothea hilgendorfi (Bohm, 1879)

Genus Ammothella Verrill, 1900

Ammothella appendiculata (Dohrn, 1881)

Ammothella biunguiculata (Dohrn, 1881)

Ammothella longioculata (Faraggiana, 1940)

Ammothella longipes (Hodge, 1864)

Ammothella uniunguiculata (Dohrn, 1881)

Genus Neotrygaeus Munilla & Alonso-Zaragaza, 2014

Neotrygaeus communis (Dohrn, 1881)

Genus Paranymphon Caullery, 1896

Paranymphon spinosum Caullery, 1896

Genus Tanystylum Miers, 1879

Tanystylum conirostre (Dohrn, 1881)

Tanystylum orbiculare Wilson, 1878

Family Ascorhynchidae Hoek, 1881

Genus Ascorhynchus Sars, 1877

Ascorhynchus arenicola (Dohrn, 1881)

Ascorhynchus castelli (Dohrn, 1881)

Ascorhynchus pudicum Stock, 1970

Ascorhynchus simile Fage, 1942

Family Callipallenidae Hilton, 1942

Genus Callipallene Flynn, 1929

Callipallene acribica Krapp, 1975

Callipallene brevirostris (Johnston, 1837)

Callipallene emaciata (Dohrn, 1881)

Callipallene phantoma (Dohrn, 1881)

Callipallene producta (Sars, 1888)

Callipallene spectrum (Dohrn 1881)

Callipallene tiberi (Dohrn, 1881)

Genus Neopallene Dohrn, 1881

Neopallene campanellae Dohrn, 1881

Family Nymphonidae Leach, 1815

Genus Nymphon Fabricius, 1794

Nymphon gracile Leach, 1814

Nymphon parasiticum Merton, 1906

Nymphon puellula Krapp, 1974

Family Endeidae Norman, 1908

Genus Endeis Philippi, 1843

Endeis charybdaea (Dohrn, 1881) Endeis biseriata Stock, 1968

Endeis spinosa (Montagu, 1808)

Family Phoxichilidiidae Sars, 1891

Genus Anoplodactylus Wilson, 1878

Anoplodactylus angulatus (Dohrn, 1881) Anoplodactylus californicus Hall, 1912

Anoplodactylus compositus Chimenz, Cottarelli & Tosti, 1991

Anoplodactylus massiliensis Bouvier, 1916

Anoplodactylus petiolatus (Krøyer, 1844)

Anoplodactylus pygmaeus (Hodge, 1864)

Anoplodactylus robustus (Dohrn, 1881)

Anoplodactylus virescens (Hodge, 1864)

Family Pycnogonidae Latreille, 1806

Genus Pycnogonum Brünnich, 1764

Pycnogonum nodulosum Dohrn, 1881

Pycnogonum plumipes Stock, 1960

Pycnogonum pusillum Dohrn, 1881

Family Rhynchothoracidae Thompson, 1909

Genus Rhynchothorax Costa, 1861

Rhynchothorax alcicornis Krapp, 1973

Rhynchothorax mediterraneus Costa, 1861

9.1 Key to genera

	Rounded body (circular or oval) without surface segmentationgenus <i>Tanystylum</i> Segmented body
2	Cheliphores and palps both present
3	Palps reduced to "buds" (present only in males)
1	Cheliphores reduced to one-articled scape (without chela)
5	5-segmented palpsgenus <i>Nymphon</i> Palps with 6 or more articles
<u>.</u>	6-articulated palps; well-developed cheliphoresgenus <i>Paranymphon</i> Palps 8–10-segmented. Small cheliphores' chela not passing the tip of proboscis
7	Palps with 9 articles, the 3rd of which is relatively short; 3-segmented cheliphores
3	Cheliphores with a very short rounded basal article and a distal spinegenus <i>Ammothea</i> Cheliphores with elongated proximal article9
)	8-segmented palps
10	Cheliphores present, missing palps
11	10-segmented ovigers in both sexes
12	Ovigers with terminal claw and apophysis with spines on the fifth segmentgenus Neopallene Ovigers without terminal claw
13	Palps present, missing cheliphores
	Slender body; legs twice the length of the body; auxiliary claws present; 7-segmented ovigers present only in males

9.2 Key to species

9.2.1 Genus Achelia

1	The first three segments separated by sutures and the 3rd and 4th segment fused. Lateral processes and legs with spiny projections
-	All segments separated by sutures (the one between the 3rd and the 4th segment weakly visible). Lateral processes without spines
2	Ovoid proboscis. Auxiliary claws 3/4 the length of the main one. Fourth lateral processes without dorsal-distal tubercles. Ocular tubercle a little higher than wild. Strigilar formula 2:2:1:2-3
-	Piriformis and pointed proboscis. Auxiliary claws 1/2 or 2/3 the length of the main one
-	Coxae II of males with three pairs of setate lateral tubercles. All lateral processes with dorsal-distal tubercles; ocular tubercle much higher than wide; tibia II longer than the femur, about 7 times longer than broad; strigilar formula 2:2:2:2
	about 4 times longer than broad; strigilar formula 0-1:1:1:2

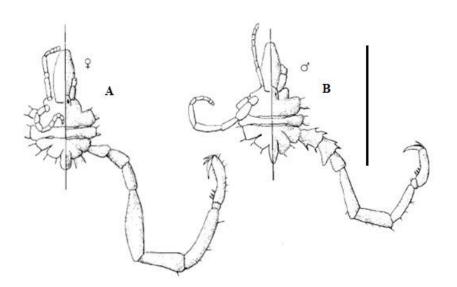


Figure 16. Achelia simplex – A. ventral and dorsal view of a female, with details of palp, cheliphore and leg; B. ventral and dorsal view of a male, with details of palp, oviger and leg.

Scale bar: 1 mm. From King 1986, mod.

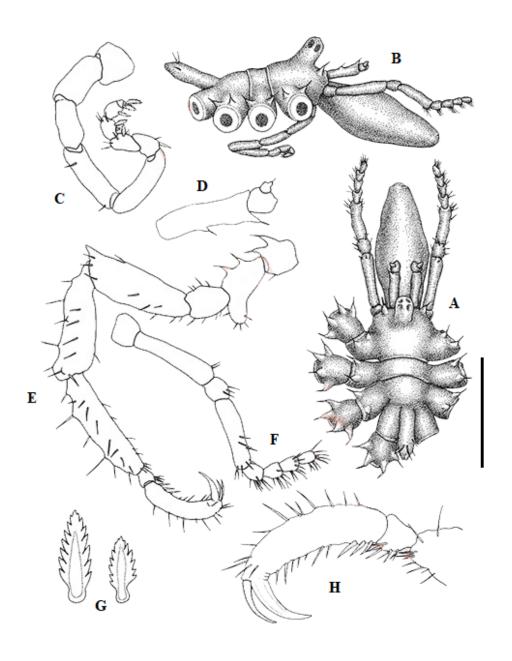


Figure 17. Achelia langi – A. dorsal view; B. lateral view; C. oviger; D. cheliphore; E. male leg; F. palp; G. strigils detail; H. distal part of the leg (tarsus, propodus and terminal claws). Scale bar: 1 mm, C-H are enlarged. From Munilla and Soler-Membrives 2014, mod.

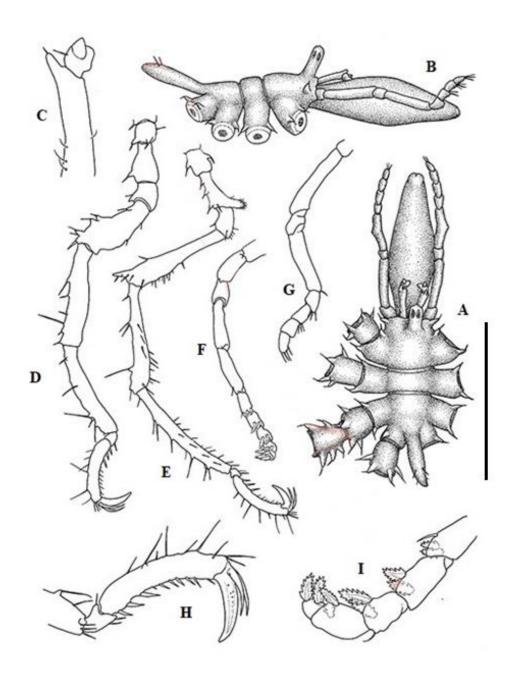


Figure 18. Achelia vulgaris – A. dorsal view; B. lateral view; C. cheliphore; D. female leg; E. male leg; F. ovigers; G. palp; H. distal part of the leg (tarsus, propodus and terminal claws); I. strigils detail. Scale bar: 1 mm, C-H are enlarged. From Munilla and Soler-Membrives 2014, mod.

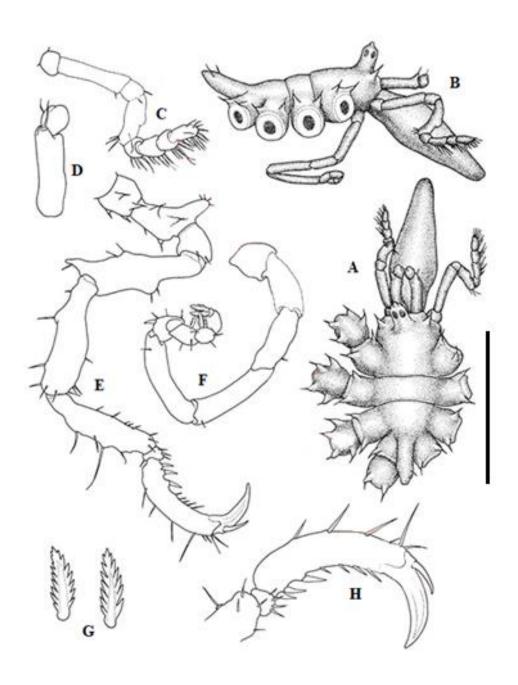


Figure 19. Achelia echinata – A. dorsal view; B. lateral view; C. palp; D. cheliphore; E. male leg; F. oviger; G. strigils detail; H. distal part of the leg (tarsus, propodus and terminal claws). Scale bar: 1 mm, C-H are enlarged. From Munilla and Soler-Membrives 2014, mod.

9.2.2 Genus Ammothea

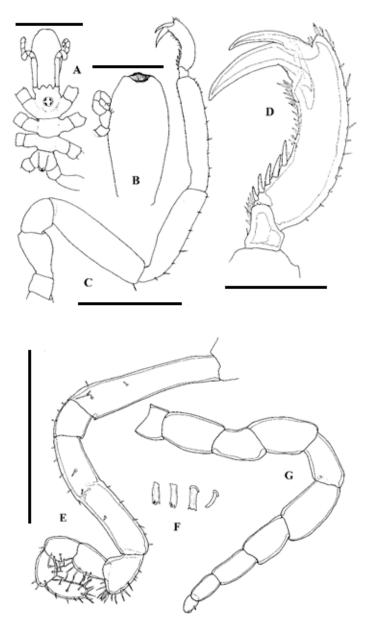


Figure 20. Ammothea hilgendorfi – A. dorsal view, scale bar: 2 mm; B. proboscis and last articles of palp detail, scale bar: 4 mm; C. leg, scale bar: 2 mm; D. detail of the distal part of the leg (tarsus, propodus and auxiliary claws), scale bar: 0,5 mm; E. palp; F. detail of some strigilar spines; G. oviger. Scale bar E-G: 1 mm. From Krapp and Sconfietti 1983, mod.

9.2.3 Genus Ammothella

Key mainly based on the strigilar formula:

1	Presence of at least two dorsal segmentation sutures of the body
2	Presence of only two dorsal sutures. Last two segments fused
3	10-jointed ovigers; strigils with composed spines according to the formula (1:1:1:2)
-	Propodus with two short and curved terminal claws (main claw reduced or missing). Ovigers made of 10 articles of which 2nd, 4th and 5th longer

Alternative key to genus Ammothella based on claws and proboscis:

1	Absence of auxiliary claws and presence of only one dorsal suture between the first and second segment of the body
2	Main claw reduced or absent
3	Lateral processes of the trunk without dorsal-distal tubercles, abdomen very long and inclined in the middle
4	Proboscis ovoid, chela reduced to a stump in adults, strigilar formula (1:1:1:2)
-	Fusiform proboscis, chela reduced to a stump in adults showing sometimes the residue of the movable finger; strigilar formula (0:2:1:2)

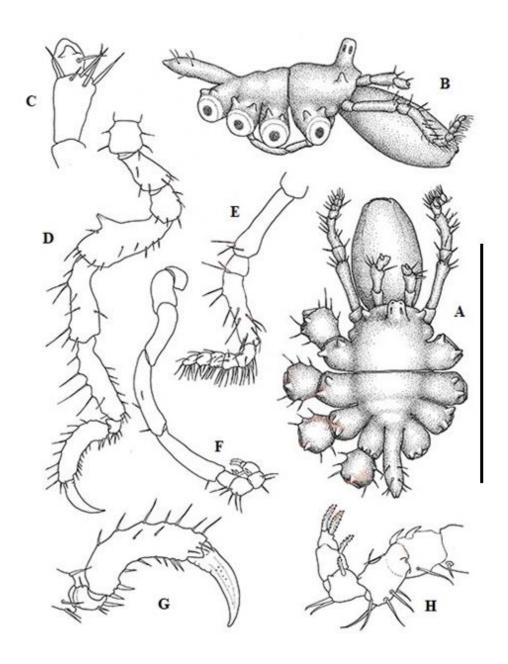


Figure 21. Ammothella uniunguiculata – A. dorsal view; B. lateral view; C. cheliphore; D. leg; E. palp; F. oviger; G. detail of the distal part of the leg (tarsus, propodus and terminal claw); H. detail of strigils. Scale bar: 2 mm, C-H are enlarged. From Munilla and Soler-Membrives 2014, mod.

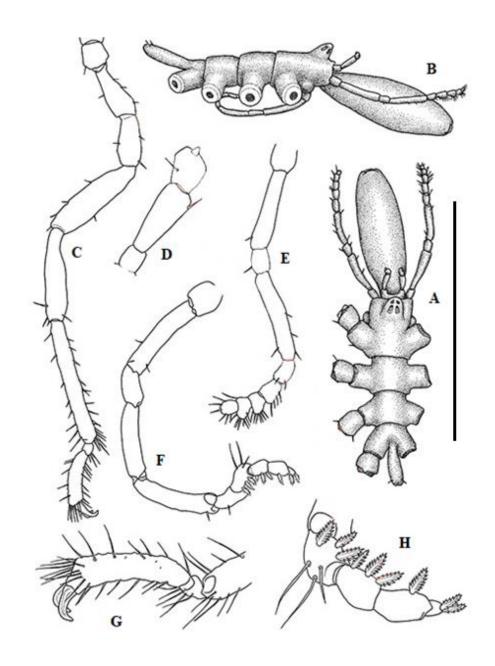


Figure 22. Ammothella biunguiculata – A. dorsal view; B. lateral view; C. leg; D. cheliphore; E. palp; F. oviger; G. detail of the distal part of the leg (tarsus, propodus and terminal claws); H. detail of strigils. Scale bar: 2 mm, C-H are enlarged. From Munilla and Soler-Membrives 2014, mod.

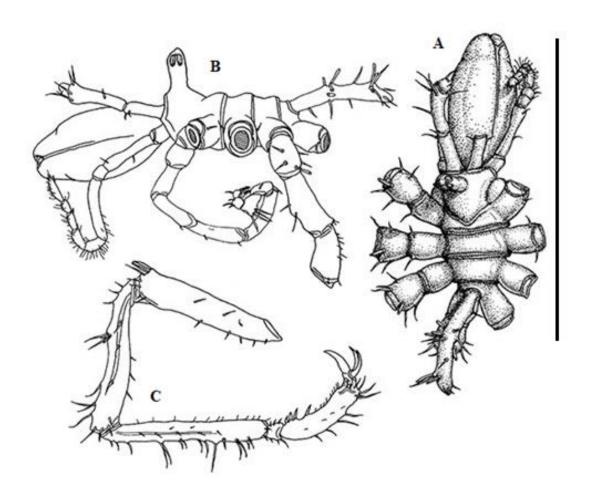


Figure 23. Ammothella appendiculata – A. dorsal view; B. lateral view; C. leg (without coxae). Scale bar: 2 mm, C is enlarged. From Child 1992a, mod.

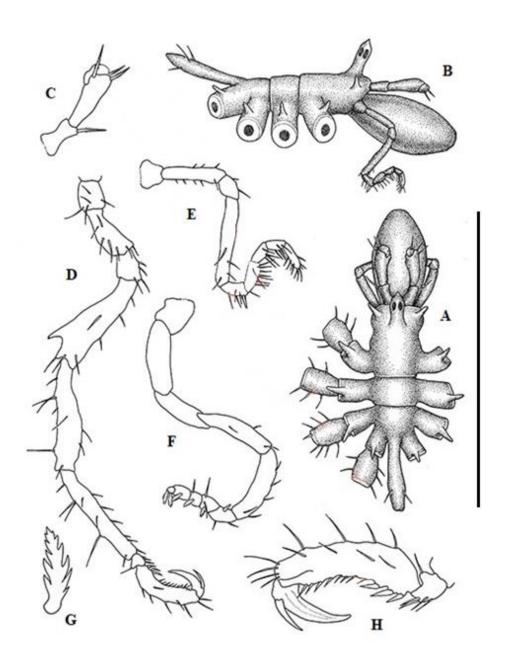


Figure 24. Ammothella longioculata – A. dorsal view; B. lateral view; C. cheliphore; D. leg; E. palp; F. oviger; G. detail of a strigilar spine; H. detail of the distal part of the leg (tarsus, propodus and auxiliary claws). Scale bar: 1 mm, C-H are enlarged. From Munilla and Soler-Membrives 2014, mod.

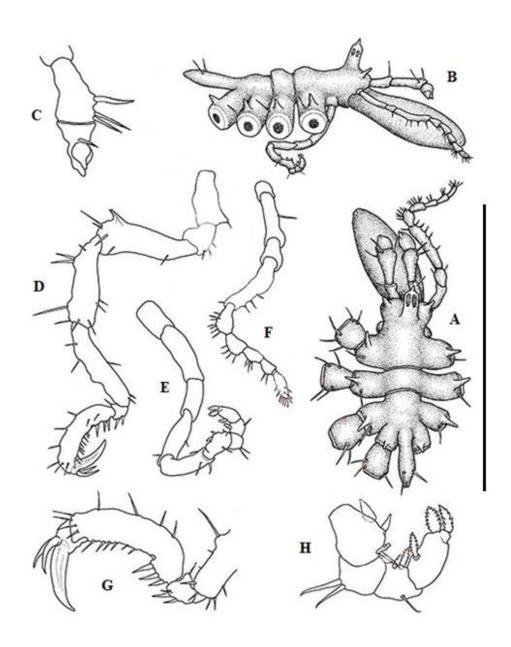


Figure 25. Ammothella longipes – A. dorsal view; B. lateral view; C. cheliphore; D. leg; E. oviger; F. palp; G. detail of the distal part of the leg (tarsus, propodus and terminal claws); H. detail of strigils. Scale bar: 2 mm, C-H are enlarged. From Munilla and Soler-Membrives 2014, mod.

9.2.4 Genus Anoplodactylus

1	Every lateral process of the trunk with a dorsal tubercle in distal position
2	Ocular tubercle with rounded apex. Unsegmented trunk. Claws of cheliphores without internal teeth. Propodus with 2 or 3 strong spines in the proximal part, followed by 5 curved spines. Main claw almost as long as the propodus and presence of very small lateral auxiliary claws (1/7 of the main one)
-	Mucronate ocular tubercle. Dorsal side of the trunk partially segmented. Chelae of the cheliphores with internal denticles. Propodus with 2 strong spines in the proximal part flanked by 2 or 3 thinner spines. Terminal claw almost as long as the propodus and without auxiliary claws
3	Proboscis with angled lateral margins4
-	Proboscis anteriorly truncated with blunt margins
-	Lateral processes touching each other for all their length. Trunk without superficial segmentation and cheliphores claws with a seta for each finger. Last segment of the ovigers characterized by two external rows of 6 cylindrical spines
5	Strongly angled proboscis. Claws of cheliphores with internal teeth (from 3 to 6) both on the fixed finger and on the movable finger. High conical ocular tubercle, with well visible eyes
-	Blunter proboscis. Cheliphores claws with a thorny seta on the external side and 6 spines on the internal margin of the movable finger; fixed finger without thorns. Low ocular tubercle, with little evident eyes and two small lateral-distal tubercles
-	Barely visible trunk segmentation sutures. Males with rudimentary ovigers. Females with "wing" appendages at the base of the ventral side of the proboscis. Well-developed cheliphores claws covered with some short spines
	by two rows of external spines and a single internal spine. Claws of cheliphores with one or two basal setae

^{*}Anoplodactylus massiliensis (Fig. 33) is missing - Without tubercles; claws of cheliphores without internal teeth (?); body with complete intersegmental sutures. Propodus less than half the length of the main claw, with 2 or 3 thorny bristles on the proximal ventral margin (Bouvier, 1916). Notes: Bouvier's original description (1916) is very approximate and the drawing reported on Bouvier (1923) is too stylized to obtain useful detailed information.

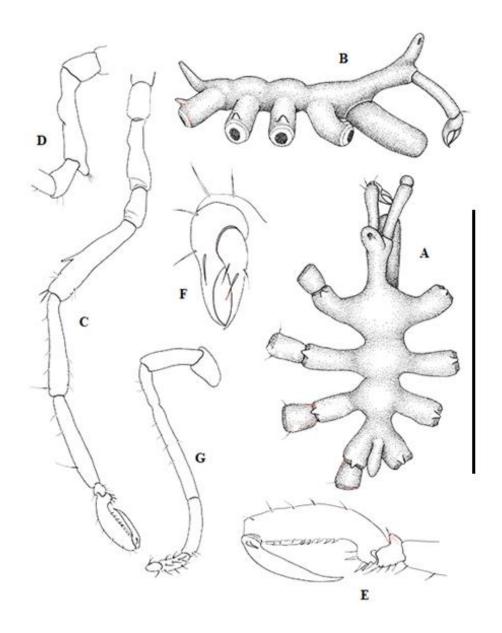


Figure 26. Anoplodactylus petiolatus – A. dorsal view; B. lateral view; C. leg; D. proximal part of the leg; E. detail of the distal part of the leg (tarsus, propodus and terminal claws); F. chela; G. oviger. Scale bar: 1 mm, C-G are enlarged. From Munilla and Soler-Membrives 2014, mod.

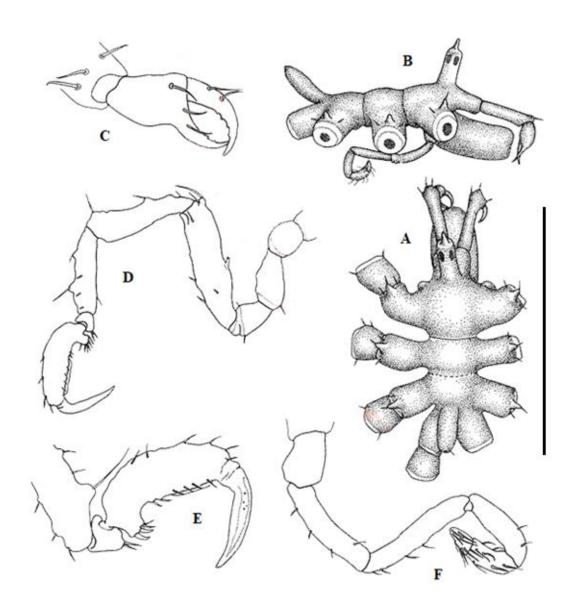


Figure 27. Anoplodactylus pygmaeus – A. dorsal view; B. lateral view; C. chela; D. leg; E. detail of the distal part of the leg (tarsus, propodus and terminal claw); F. oviger. Scale bar: 1 mm, C-F are enlarged. From Munilla and Soler-Membrives 2014, mod.

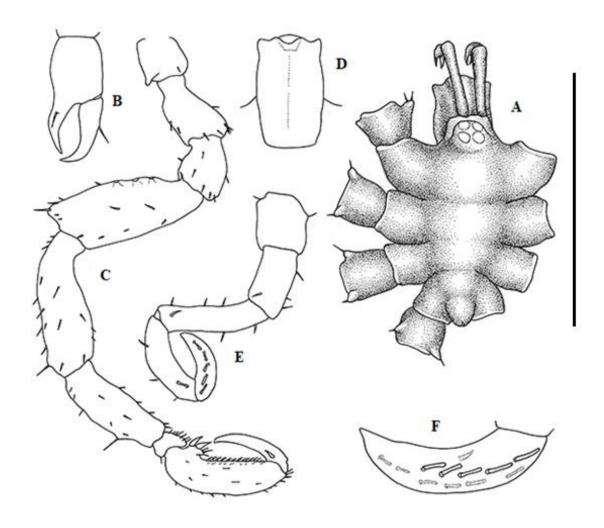


Figure 28. Anoplodactylus robustus – A. dorsal view; B. chela; C. leg; D. ventral view of the proboscis; E. oviger; F. detail of the last segment of strigils. Scale bar: 1 mm, B-F are enlarged. From Munilla and Soler-Membrives 2014, mod.

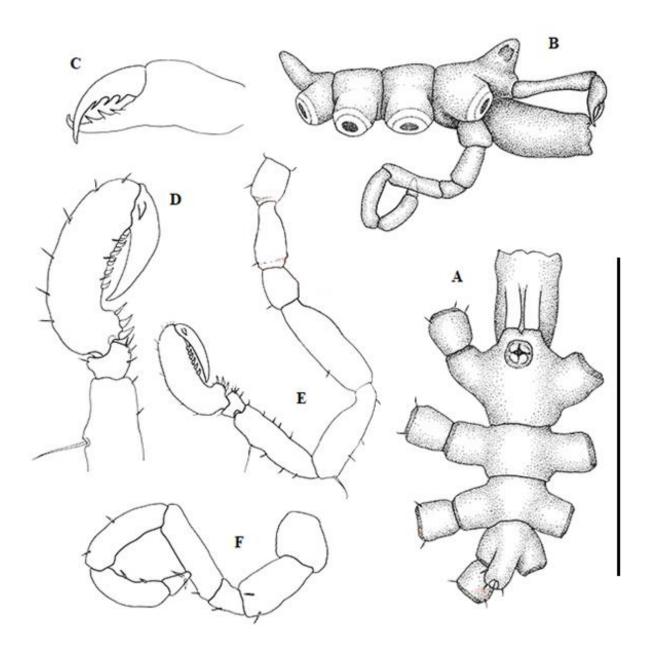


Figure 29. Anoplodactylus angulatus – A. dorsal view; B. lateral view; C. chela; D. detail of the distal part of the leg (tarsus, propodus and terminal claws); E. leg; F. oviger. Scale bar: 2 mm, C-F are enlarged. From Munilla and Soler-Membrives 2014, mod.

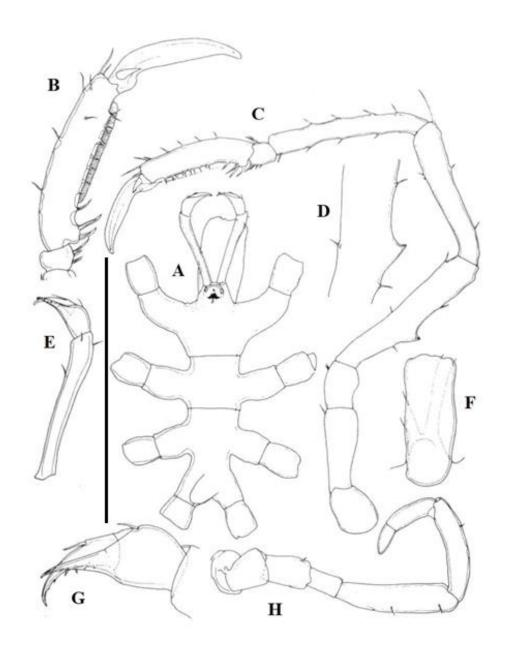


Figure 30. Anoplodactylus compositus – A. dorsal view; B. detail of the distal part of the leg (tarsus, propodus and terminal claws); C. leg; D. duct of cement gland; E. cheliphore; F. ventral view of the proboscis; G. chela; H. oviger. Scale bar: 1 mm, B-H are enlarged. From Chimenz et al. 1991, mod.

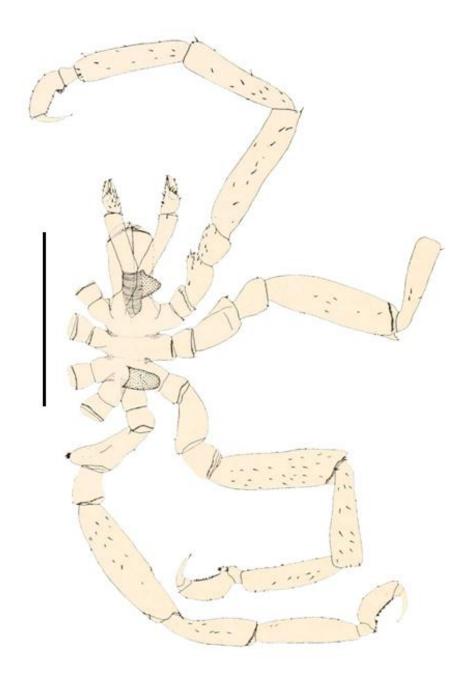


Figure 31. Anoplodactylus californicus – dorsal view; ovigers are missing, in this species they are rudimental. Scale bar: 2 mm. From Hall 1912, mod.

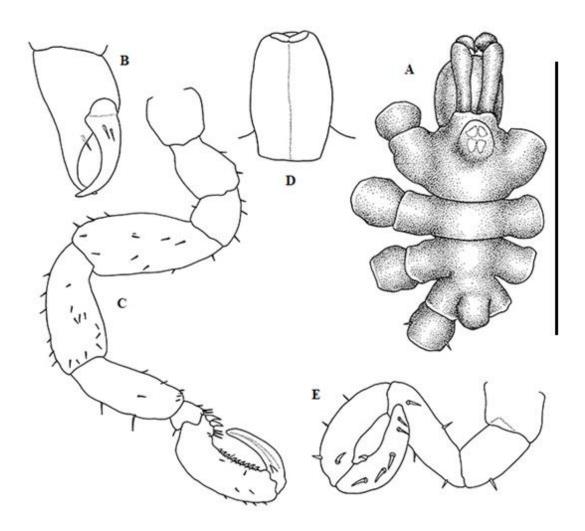


Figure 32. Anoplodactylus virescens -A. dorsal view; B. chela; C. leg; D. proboscis; E. oviger. Scale bar: 2 mm, B-E are enlarged. From Munilla and Soler-Membrives 2014, mod.

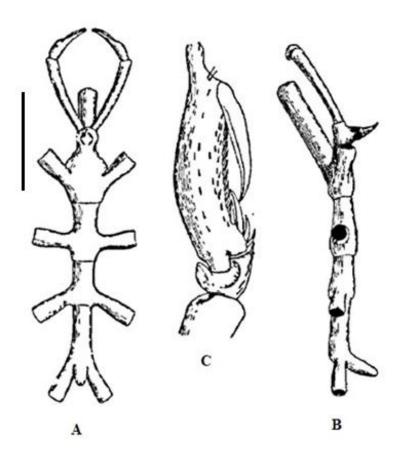


Figure 33. Anoplodactylus massiliensis – A. dorsal view; B. lateral view; C. detail of the distal part of the leg (tarsus, propodus and terminal claw). Scale bar: 2 mm, C is enlarged. From Bouvier 1923, mod.

9.2.5 Genus Ascorhynchus

1	Presence of spiny dorsal tubercles, one for each segment of the trunk. Lateral processes and legs without evident setae
-	Trunk segments without dorsal tubercles. Lateral processes and legs carrying numerous setae in dorsal and lateral position
2	Dorsal tubercles with no setae. Lateral processes with well-developed dorsal tubercles, similar to segmental ones. Ovigers with the 2nd joint with a ventral tubercle and the 4th longer than the others. Strigils with enlarged spines arranged according to the formula 8(7):5(5):3(5):4(2)
-	Dorsal tubercles with setae. Lateral processes with reduced or completely absent dorsal tubercles. Femur and tibia I with a distal tubercle. Ovigers with strigils having pinnate spines arranged in two rows according to the formula 6(7):4(5):3(3):5(2)
3	Dorsal-distal tubercles on lateral processes and a rounded protuberance on the dorsal part of the coxae II
-	Lateral processes without dorsal-distal tubercles and without any rounded protuberance on the dorsal part of the coxae II

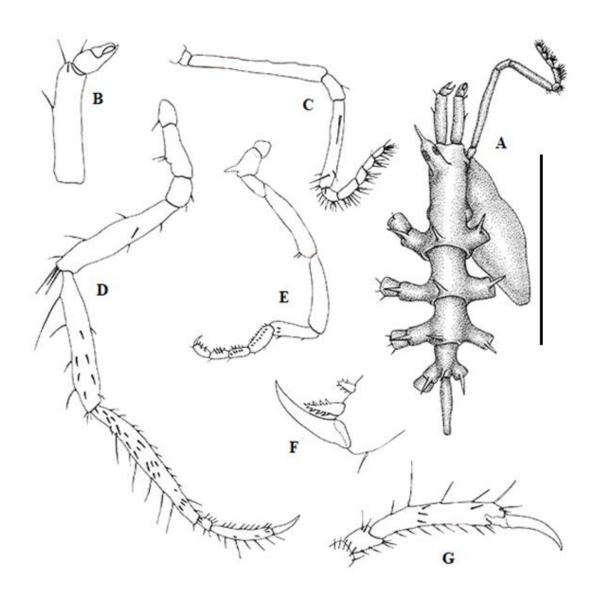


Figure 34. Ascorhynchus pudicus – A. dorsal view; B. cheliphore; C. palp; D. leg; E. oviger; F. detail of the last strigilar article; G. detail of the distal part of the leg (tarsus, propodus and terminal claw). Scale bar: 2 mm, B-G are enlarged. From Munilla and Soler-Membrives 2014, mod.

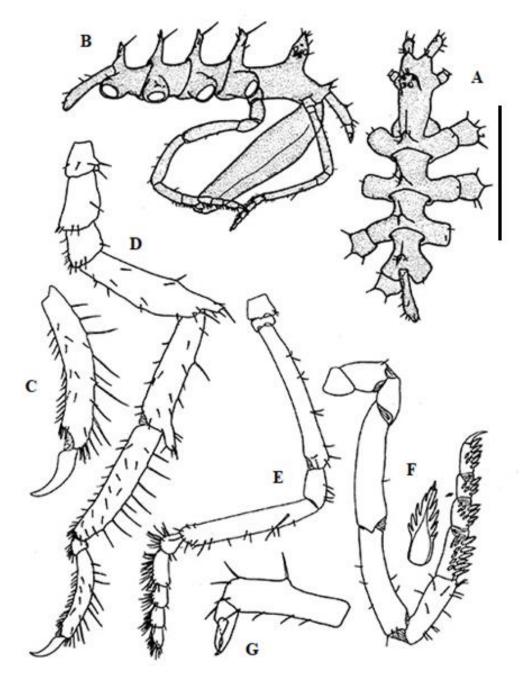


Figure 35. Ascorhynchus castelli – A. dorsal view; B. lateral view; C. detail of the distal part of the leg (propodus and terminal claw); D. leg; E. palp; F. oviger with detail of strigils seta; G. cheliphore. Scale bar: 2 mm, C-G are enlarged. From Munilla and Soler-Membrives 2014, mod.

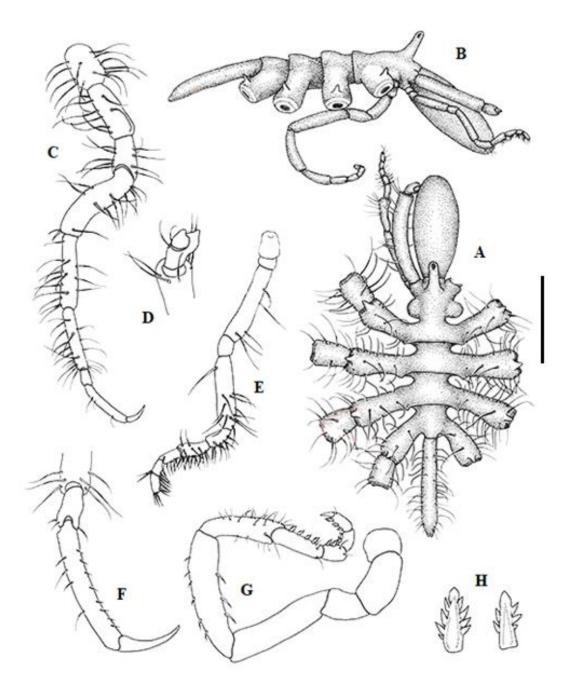


Figure 36. Ascorhynchus simile – A. dorsal view; B. lateral view; C. leg; D. cheliphore; E. palp; F. detail of the distal part of the leg (tarsus, propodus and terminal claw); G. oviger; H. detail of strigilar setae. Scale bar: 2 mm, C-H are enlarged. From Munilla and Soler-Membrives 2014, mod.

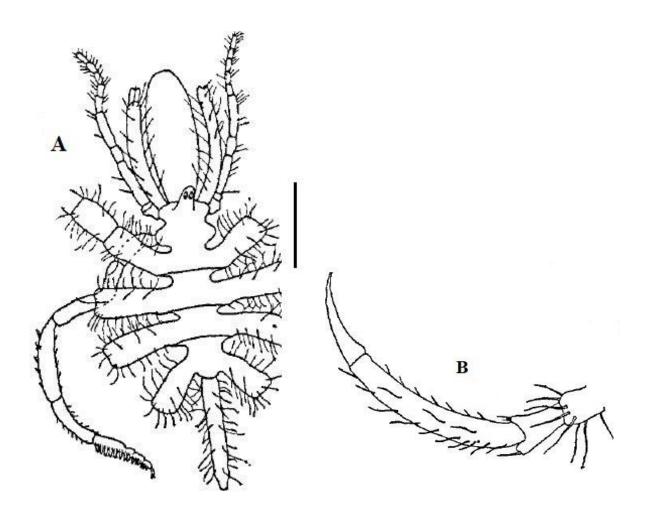


Figure 37. Ascorhynchus arenicola -A. dorsal view with an oviger; B. detail of the distal part of the leg (tarsus, propodus and terminal claw). Scale bar: 2 mm, B is enlarged. From Bouvier 1923, mod.

9.2.6 Genus Callipallene

1	Totally segmented trunk (light but visible suture between the last two segments)2
-	Partially segmented trunk, the last two segments fused
2	Straight propodus. Main claw 2/3 the length of the propodus and auxiliary claws 1/3 the length of the main one (or even less in males). Elongated and slender cephalon which exceeds the length of the rest of the body
-	Strongly curved propodus. Main claw half the length of the propodus and auxiliary claws half the length of the main one. Moderately long cephalon
3	Proboscis with rounded apex. Lateral trunk processes smooth, half their diameter apart. 10-jointed ovigers with strigils having setae arranged according to the formula (4-5:6:4:6). Propodus with 3-4 strong proximal spines
-	Proboscis with angled apex. Lateral processes well-spaced which may carry one, two or no distal spines. 10-jointed ovigers with strigils having setae arranged according to the formula (6:5:5:6). Tubercle of the fifth ovigeral article of the male with apical bristle. II coxa as long as the sum of I and III. Propodus with 4-5 strong proximal spines
-	Propodus with two strong proximal spines, followed by 4 medium length spines and 8 very short spines. Auxiliary claws thinner than the main one but almost as long. Ovigers with strigils having setae arranged according to the formula (6:6:6:5)
5	8 or 9 spines in the last segment of the strigils; auxiliary claws at least 2/3 the length of the main one
-	5 or 6 spines in the last segment of the strigils6
6	Mucronate ocular tubercle. Ovigers with strigils following the formula (6:5:4:5). Propodus with 5 thick proximal spines. Main claw rather long, auxiliary claws about 2/3 the length of the main one
-	Ovigers with strigils following the formula (6:5:6:6). Propodus curved externally with 4-5 thick proximal spines. Main claw rather long, auxiliary claws about half the length of the main one

^{*}Callipallene brevirostris – reported in the Venice lagoon; the identification of the species could be erroneous and can be traced back to *C. emaciata*. The species is distributed in the Atlantic and in the North Sea, but it is not reported in the Mediterranean (Child, 1992a).

Genus Callipallene (alternative key)

1	Totally segmented trunk; suture between the las two segments light but visible
-	Partially segmented trunk, the last two segments fused
2	Very long neck, straight sole of the propodus, auxiliary claws 1/3 longer than the main one (or even less in males)
-	Variable long neck, curved sole of the propodus, auxiliary claws half the length of the main claw
-	Proboscis with rounded apex. Lateral processes smooth, half their width apart. Strigilar formula (4-5:6:4:6). Propodus with 3-4 strong proximal spines
4	Rounded ocular tubercle, lateral processes as long as wide <i>Callipallene brevirostris*</i> (Fig. 41) Mucronate ocular tubercle, lateral processes longer than wide
5	Auxiliary claws about half the length of the main one
6 -	Propodus with five strong spines on the heel, auxiliary claws 2/3 long of the main one, proboscis with an angled profile in ventral view

^{*}Callipallene brevirostris – reported in the Venice lagoon, the identification of the species could be erroneous and can be traced back to *C. emaciata*. The species is distributed in the Atlantic and in the North Sea, but it is not reported in the Mediterranean (Child, 1992a).

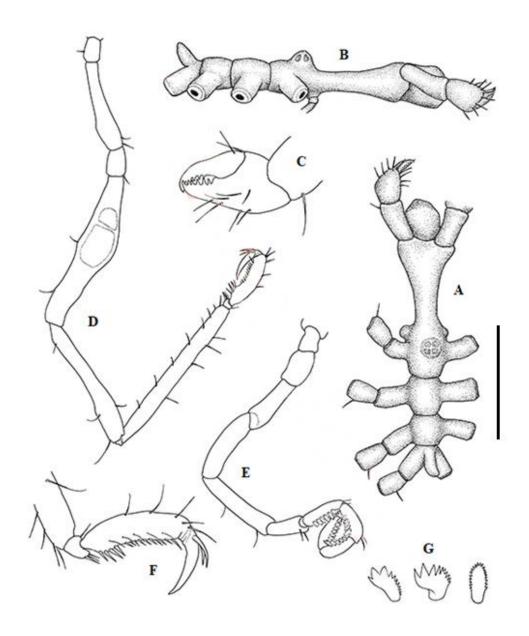


Figure 38. Callipallene phantoma – A. dorsal view; B. lateral view; C. chela; D. leg; E. oviger; F. detail of the distal part f the leg (tarsus, propodus and terminal claws); G. detail of the strigilar setae. Scale bar: 2 mm, C-G are enlarged. From Munilla and Soler-Membrives 2014, mod.

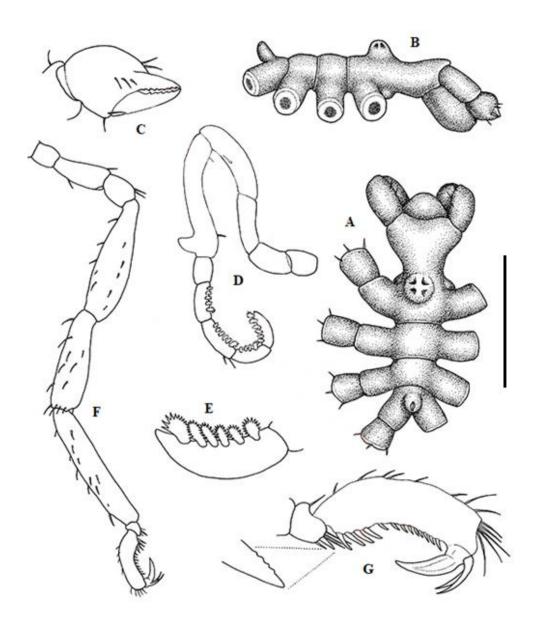


Figure 39. Callipallene emaciata – A. dorsal view; B. lateral view; C. chela; D. male oviger; E. detail of the distal part of the oviger (composed strigilar setae); F. leg; G. detail of the distal part of the leg (tarsus, propodus and terminal claws. Scale bar: 1 mm, C-G are enlarged. From Munilla and Soler-Membrives 2014, mod.

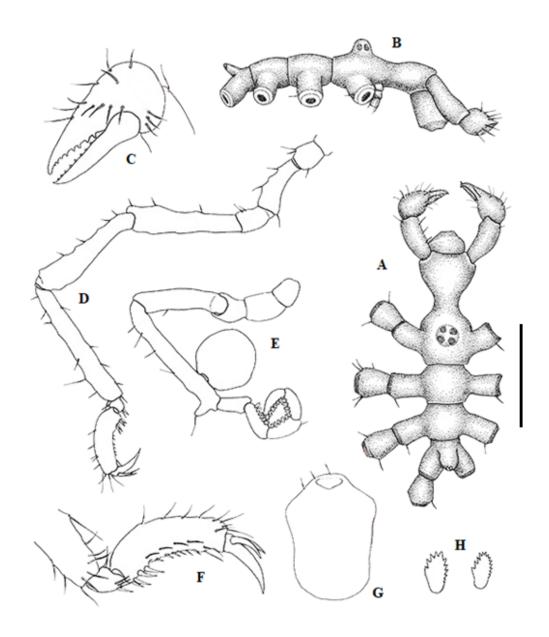


Figure 40. Callipallene tiberi – A. dorsal view; B. lateral view; C. chela; D. leg; E. oviger; F. detail of the distal part of the leg (tarsus, propodus and terminal claws); G. ventral view of the proboscis; H. detail of strigilar setae. Scale bar: 1 mm, C-H are enlarged. From Munilla and Soler-Membrives 2014, mod.

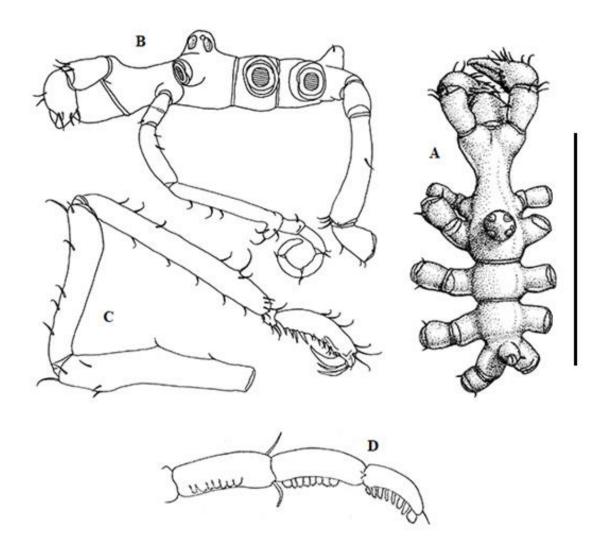


Figure 41. Callipallene brevirostris – A. dorsal view; B. lateral view; C. leg (without coxae); D. detail of strigils. Scale bar: 2 mm, C-D are enlarged. From Munilla and Soler-Membrives 2014, mod.

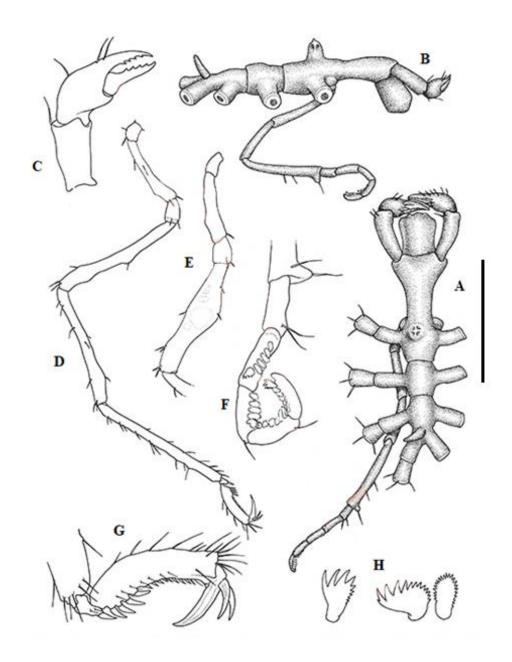


Figure 42. Callipallene spectrum – A. dorsal view; B. lateral view; C. chela; D. leg; E. proximal part of a female's leg; F. distal part of the oviger; G. detail of the distal part of the leg (tarsus, propodus and terminal claws); H. detail of strigilar setae. Scale bar: 1 mm, C-H are enlarged. From Munilla and Soler-Membrives 2014, mod.

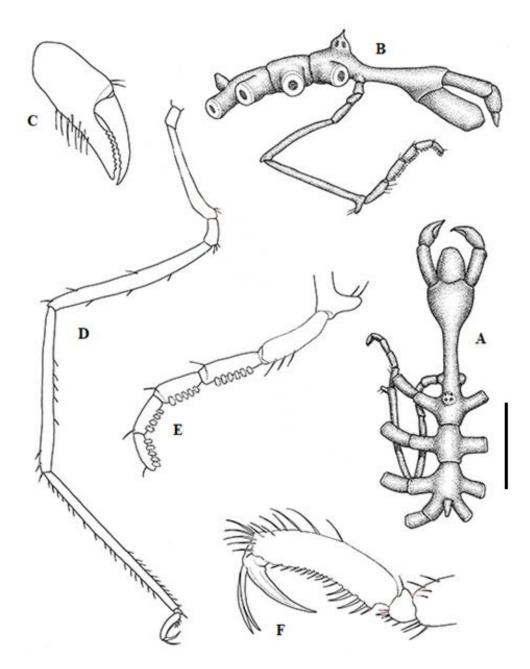


Figure 43. Callipallene producta – A. dorsal view; B. lateral view; C. chela; D. leg; E. distal part of the oviger with detail of strigilar setae; F. detail of the distal part of the leg (tarsus, propodus and terminal claws). Scale bar: 2 mm, C-F are enlarged.

From Munilla and Soler-Membrives 2014, mod.

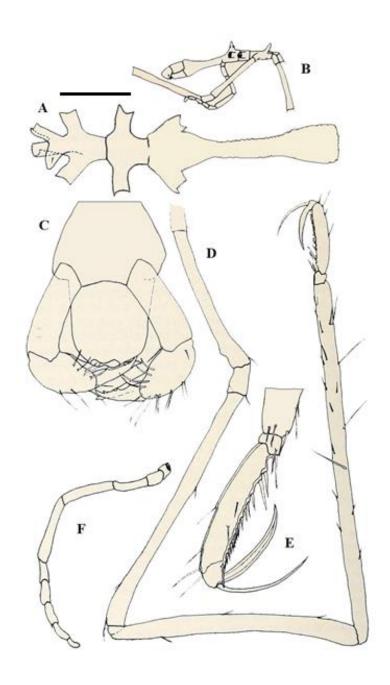


Figure 44. Callipallene acribica – A. dorsal view; B. lateral view; C. cheliphores and proboscis; D. leg; E. detail of the distal part of the leg (tarsus, propodus and terminal claws); F. oviger. Scale bar: 1 mm, C-F are enlarged. From Krapp 1975, mod.

9.2.7 Genus Endeis

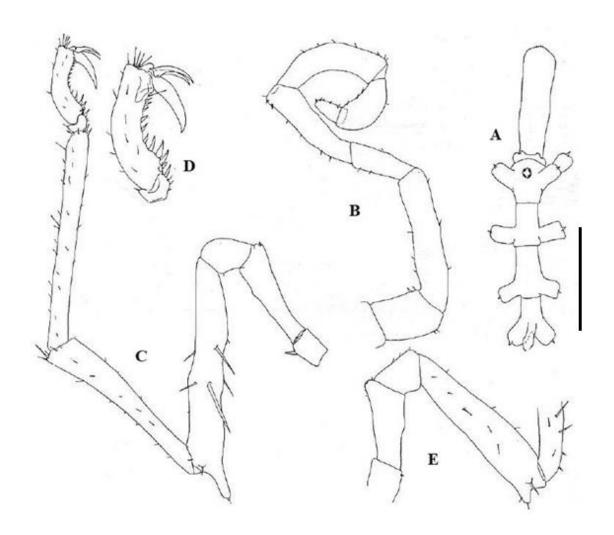


Figure 45. Endeis biseriata – A. dorsal view; B. oviger; C. leg; D. propodus with detail of spines and auxiliary claws; E. proximal segment of a male leg. Scale bar: 2 mm, B-E are enlarged. From Stock 1968, mod.

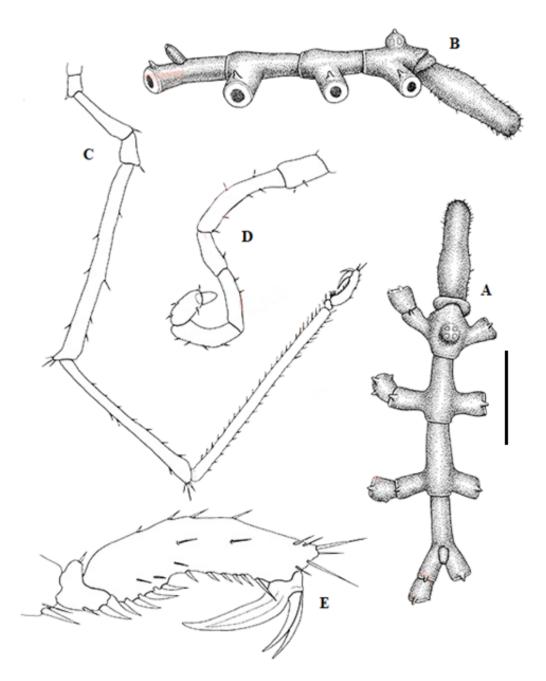


Figure 46. Endeis charybdaea – A. dorsal view; B. lateral view; C. leg; D. oviger; E. detail of the distal part of the leg (tarsus, propodus and auxiliary claws). Scale bar: 2 mm, C-E are enlarged. From Munilla and Soler-Membrives 2014, mod.

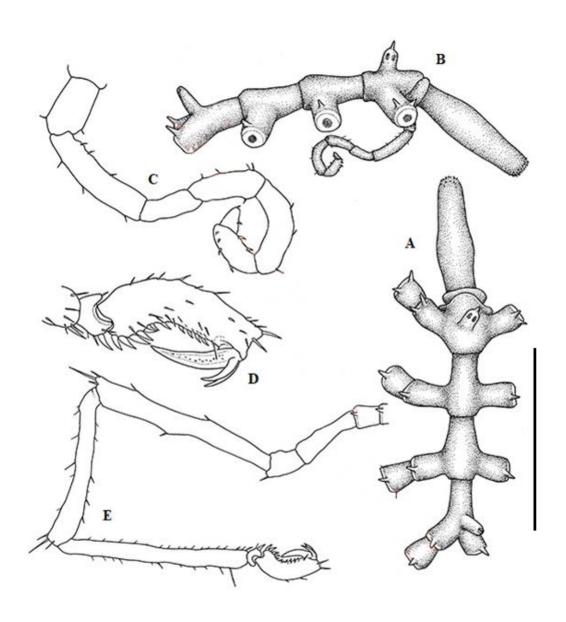


Figure 47. Endeis spinosa – A. dorsal view; B. lateral view; C. oviger; D. detail of the distal part of the leg (tarsus, propodus and terminal claws); E. leg. Scale bar: 2 mm, C-E are enlarged. From Munilla and Soler-Membrives 2014, mod

9.2.8 Genus Neopallene

Tapered and fully segmented body. Cheliphores armed with spiny teeth. Palps absent in females and reduced to a "gem" in males. Pair of spines in the distal part of the lateral processes and the coxa I of all legs. Auxiliary claws slightly exceeding half the length of the main one..........Neopallene campanellae (Fig. 48)

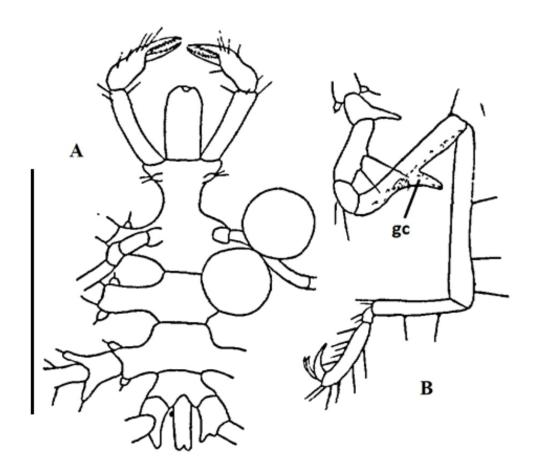


Figure 48. Neopallene campanellae – A. ventral view: the chelae are equipped with denticles; B. leg, in evidence a tubercle linked to the cement gland (gc). Scale bar: 2 mm, B is enlarged. From Bouvier 1923, mod.

9.2.9 Genus Neotrygaeus

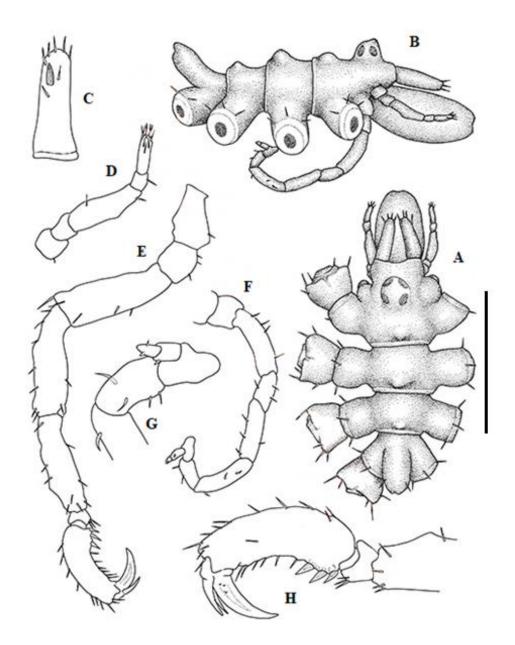


Figure 49. Neotrygaeus communis – A. dorsal view; B. lateral view; C. cheliphores; D. palp; E. leg; F. male oviger; G. detail of strigils; H. detail of the distal part of the leg (tarsus, propodus e terminal claws). Scale bar: 2 mm, C-H are enlarged. From Munilla and Soler-Membrives 2014, mod.

9.2.10 Genus Nymphon

1	Propodus	with	auxiliary	claws.	Compos	sed	spine	s on o	ovigers	strigils	arrange	dacco	ording t	o the
	formula	(14:11)	1:9:13).	Terminal	l claw	of	the	oviger	rs short	and	crested	in its	distal	part
											Nympho	n grac	ilis (Fig	g. 50)

- Nymphon parasiticum (?) (Fig. 52) insufficient description and not based on adult individuals. In the more mature specimen used for the description, the auxiliary claws are absent (Merton, 1906).

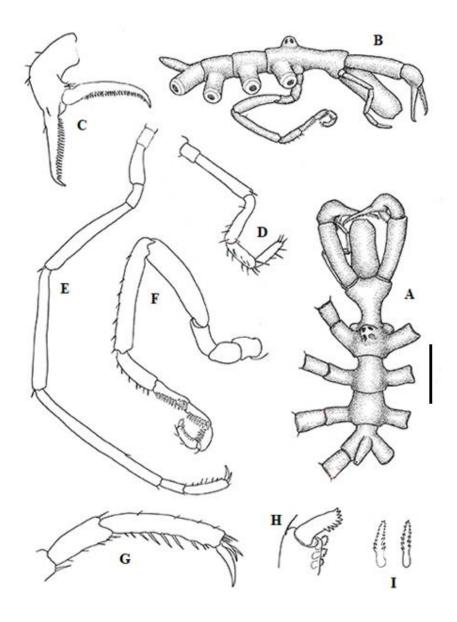


Figure 50. Nymphon gracilis — A. dorsal view; B. lateral view; C. chela; D. palp; E. leg; F. oviger; G. detail of the distal part of the leg (tarsus, propodus and terminal claws); H. detail of the ovigeral claw; I. detail of strigilar setae. Scale bar: 2 mm, C-I are enlarged. From Munilla and Soler-Membrives 2014, mod.

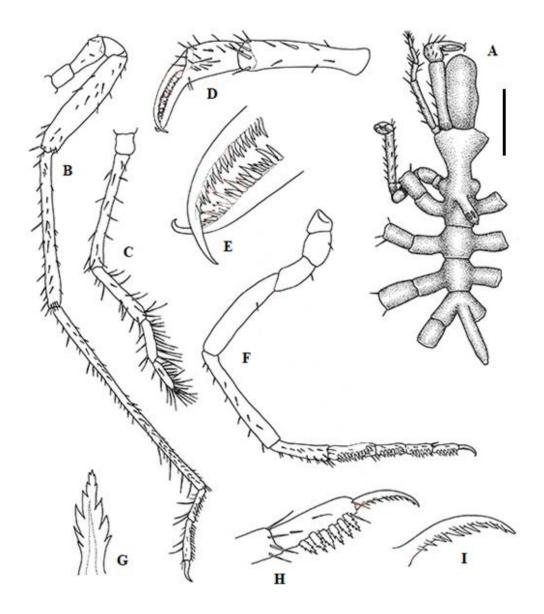


Figure 51. Nymphon puellula – A. dorsal view; B. leg; C. palp; D. cheliphore; E. chela; F. oviger; G. detail of a composed strigilar seta; H. detail of the last article of strigils; I. detail of the ovigeral claw. Scale bar: 2 mm, B-I are enlarged. From Munilla and Soler-Membrives 2014, mod.

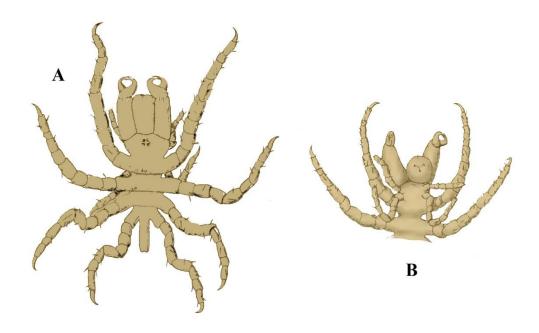


Figure 52. Nymphon parasiticum -A. dorsal view of immature individual in which can be observed a complete trunk segmentation and no auxiliary claws; B. ventral view. From Merton 1906, mod.

9.2.11 Genus Paranymphon

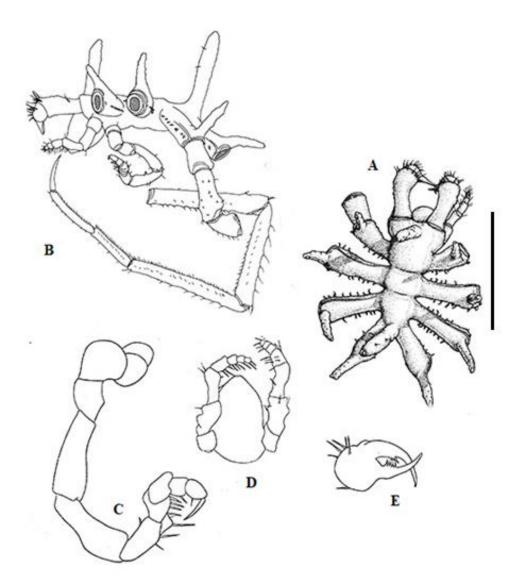


Figure 53. Paranymphon spinosum – A. dorsal view; B. lateral view; C. oviger; D. ventral view of palps and proboscis; E. chela. Scale bar: 2 mm, B-E are enlarged. From Munilla and Soler-Membrives 2014, mod.

9.2.12 Genus Pycnogonum

1	Auxiliary claws missing
-	Auxiliary claws present
2	Tegument strongly covered by small warts (grainy appearance); proboscis without dorsa
	tubercles
-	Tegument only weakly warty; proboscis with two small dorsal tubercles in the central par
	Pycnogonum plumipes (Fig. 56)

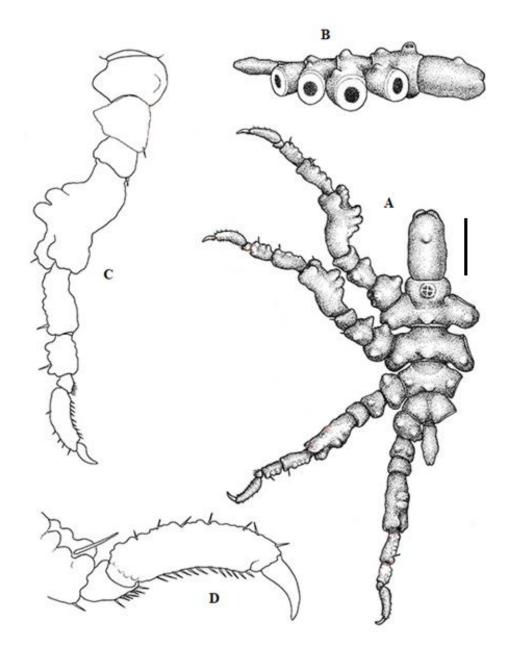


Figure 54. Pycnogonum nodulosum – A. dorsal view; B. lateral view; C. leg; D. detail of the distal part of the leg (tarsus, propodus and terminal claw). Scale bar: 2 mm, C-D are enlarged. From Munilla and Soler-Membrives 2014, mod.

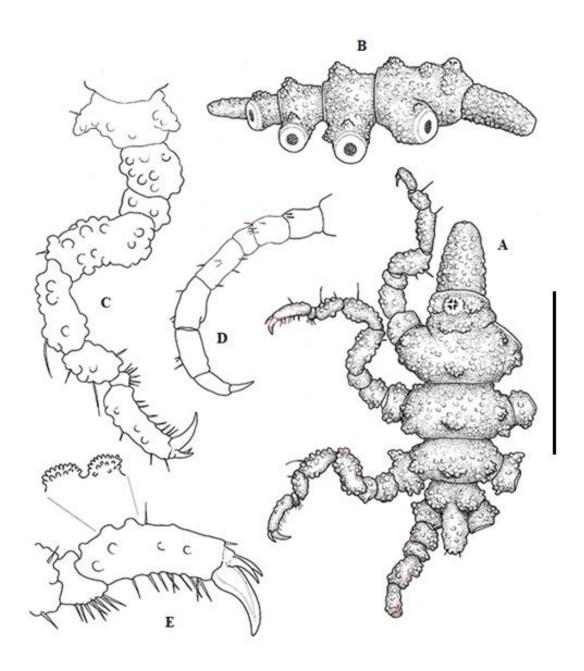


Figure 55. Pycnogonum pusillum – A. dorsal view; B. lateral view; C. leg; D. oviger; E. detail of the disatl part of the leg (tarsus, propodus and terminal claws). Scale bar: 2 mm, C-E are enlarged. From Munilla and Soler-Membrives 2014, mod.

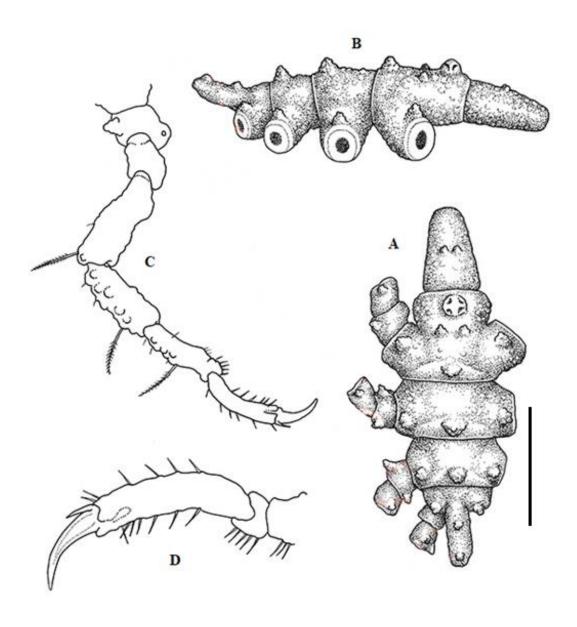


Figure 56. Pycnogonum plumipes – A. dorsal view; B. lateral view; C. leg; D. detail of the distal part of the leg (tarsus, propodus and terminal claw). Scale bar: 2 mm, C-D are enlarged. From Munilla and Soler-Membrives 2014, mod.

9.2.13 Genus *Rhynchothorax*

- 1 Totally segmented trunk. Palps with penultimate article having a distal tubercle. Ovigers of 10 articles; the fourth and the sixth longer than the others, the seventh bearing two compound and two simple spines; the eight with four compound and only one simple spines; the nineth and the tenth each with two compound and one simple spines; last article provided with serrated lamina. claw Propodus with a terminal and two evident auxiliary Partially segmented trunk (last two segment fused). Palps of 5 articles, the 2nd and 3rd with a

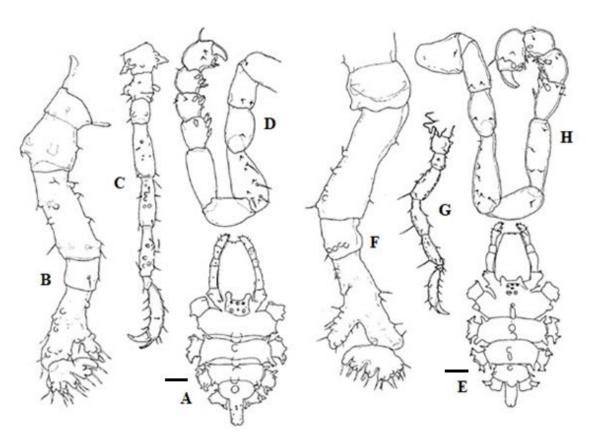


Figure 57. Rhynchothorax alcicornis – A-D: male; A. dorsal view; B. palp; C. oviger; D. leg. E-H: female; E. dorsal view; F. palp; G. oviger; H. leg. Scale bars A and E: 0,1 mm, B-D and F-H are enlarged. From Chimenz et al. 1993, mod.

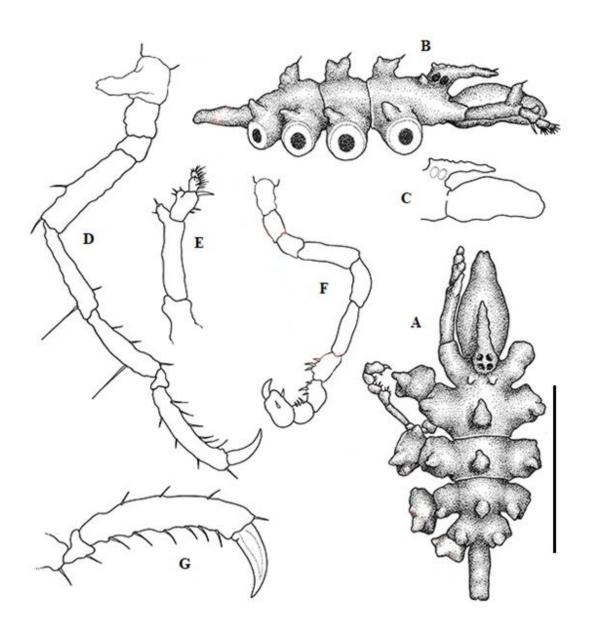


Figure 58. Rhynchothorax mediterraneus – A. dorsal view; B. lateral view; C. proboscis' profile; D. leg; E. palp; F. oviger; G. detail of the distal part of the leg (tarsus, propodus and terminal claw). Scale bar: 1 mm, C-G are enlarged. From Munilla and Soler-Membrives 2014, mod.

9.2.14 Genus Tanystylum

1	Palps	with	4	articl	es,	the	2nd	of	which	is	the	longest.	Tibiae	with	three	dorsal	tubercles
				· • • • • • • •									T	anysty	lum co	onirostr	e (Fig. 59
-	Palps	with	1	from	4	to	6	artic	les, t	he	3^{rd}	longest.	Tibiae	wit	hout	dorsal	tubercles
													Ta	nysty	lum or	biculare	e (Fig. 60)

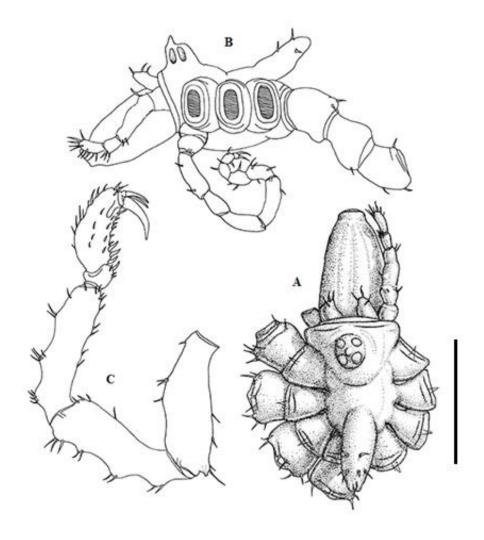


Figure 59. Tanystylum conirostre – A. dorsal view; B. lateral view; C. leg. Scale bar: 2 mm, C is enlarged. From Munilla and Soler-Membrives 2014, mod.

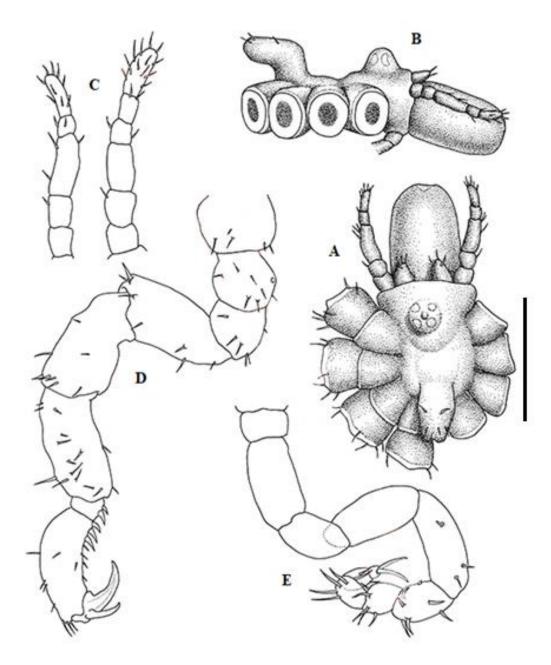


Figure 60. Tanystylum orbiculare – A. dorsal view; B. lateral view; C. palps (with five or six articles); D. leg; E. oviger. Scale bar: 2 mm, C-E are enlarged. From Munilla and Soler-Membrives 2014, mod.

9.3 Critical issues

This identification key is very simple and generally based on evident morphological features. In fact, palps, cheliphores and ovigers are the main characters to observe to identify a genus or a species. Despite this, the intraspecific variability is often wide, and single specimens can sometimes not fit the general description of their species.

In general, it was possible to notice that the strigilar formula is not a reliable feature: it is often difficult to count the exact number of spines (for example in ovigerous males) and this number is often variable.

In the following some examples of the above issues are mentioned. To overcome this problem, it would be necessary to observe a large number of samples from different areas and combine morphological observations with those related to DNA.

Genus Achelia

Achelia echinata and A. vulgaris differ mainly by the number of coxae's tubercles. The other features cited in the key are not so easy to detect and very variable. Besides, the tubercles are present only in male specimens: in case of a female, the identification remain uncertain.

Genus Callipallene

This genus is one of the most critical: the differences between species are few and confused. Some years ago, Staples completed a revision of the similar genus *Pseudopallene* (Staples, 2014) belonging to the same family (Callipallenidae). The genus *Callipallene* needs a revision too.

The sutures between body segments are very variable, as well as the length of the neck. For this reason, we tried to build an alternative key to consider these characters but in different order, in the hope to make the discrimination more effective.

Genus Nymphon

This genus is very wide as number of species, but only three of them have been recorded in the Italian seas. The problematic one is *N. parasiticum*: the original description (Merton, 1906) is very rough and based on a juvenile, with still many characters to develop. For this reason, we can not confirm safely what are the main diagnostic features for this species.

Genus Tanystylum

The only two species belonging to this genus create some difficulties during identification. The number of palps' joint is variable and the characteristic shape of the abdomen of *T. orbiculare* is not cited in literature (except for Munilla and Soler-Membrives, 2014). During the observation of samples, we found some individuals with an erect abdomen and others with an abdomen laying down. Moreover, some *T. conirostre* samples do not have such evident tubercles on the legs: one wonders how reliable this diagnostic character is.

10. Antarctic Pycnogonida

10.1 DNA data and morphological observations

In the National Museum of Antarctica (DISTAV, Corso Europa 26, Genoa, Italy), henceforth referred to in the text as MNA, 382 pycnogonid specimens are preserved.

Among them, 281 samples were examined during 2020-2021 under a stereomicroscope and identified at genus or species level (Tab. V).

A batch of 81 specimens have been previously identified by specialists and non-specialists (many do not report data on the responsible of the identification) at least down to the genus level. These specimens have been taken into consideration only for possible comparisons.

Only 20 specimens remained completely not identified.

Table V. Examined samples of MNA divided for genus.

Genus	N.er of specimens	Identified ONLY to genus level	Species	Identified to species level
Achelia	15	6	A. spicata	9
Ammothea	109	36	A. australiensis	1
			A. carolinensis	40
			A. clausi	15
			A. glacialis	6
			A. hesperidensis	1
			A. meridionalis	1
			A. minor	8
			A. spinosa	1
Austroraptus	2		A. juvenilis	1
<u>-</u>			A. praecox	1
Colossendeis	38	38		
Decolopoda	3		D. australis	2
-			D. sp. nov.	1
Austropallene	21	1	A. brachyura	1
			A. bucera	3
			A. calmani	11
			A. tcherniai	1
			A. tibicina	3
			A. calmani	1
Nymphon	21	13	N. charcoti	6
			N. mendosum	2
Pentanymphon	28		P. antarcticum	28
Pallenopsis	18	18		
Endeis	12	1	E. australis	11
Anoplodactylus	3		A. australis	3
Pycnogonum	1		P. gaini	1
Rhynchothorax	2		R. australis	2
Austrodecus	8		A. glaciale	8

As can be seen from the table V and as will be highlighted in the following paragraphs, there are genera that are more critical than others: the identification of the species of *Colossendeis*, *Nymphon* and *Pallenopsis* is particularly problematic, making dichotomous keys difficult to set up (especially for the first two genera).

After a traditional morphological analysis, hundred samples preserved in MNA were chosen for a barcoding DNA analysis in order to verify identifications and, for undetermined specimens, to try to reach a better insight.

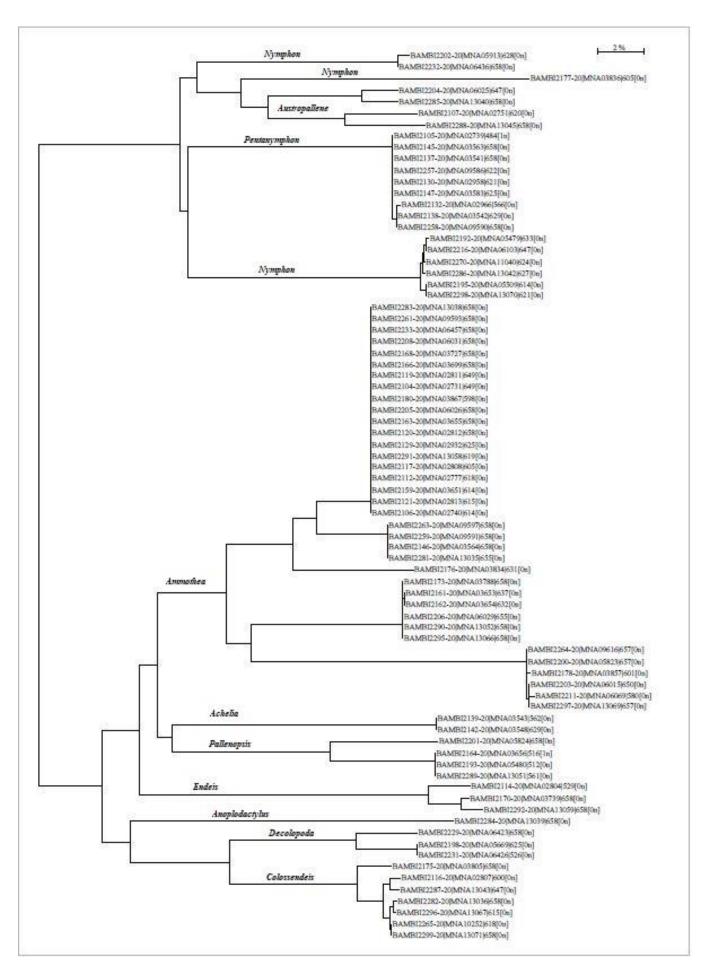
DNA barcoding can help to solve identification problems that in some cases arise when the morphological approach is adopted but, in theory, it is suitable and reliable only at the species or genus level (CBG, 2021).

This type of analysis is conducted on a sequence of 648-base pair of the mitochondrial gene cytochrome c oxidase subunit I (COI), which has different characteristics that make it suitable for this purpose, such as the absence of introns, the maternal inheritance only, the possibility to be extracted even from damaged samples or in the presence of little amount of tissue and the ability to highlight differences within species and between very close species thanks to its high variability (CBG, 2021).

Our samples were preserved in 70% ethanol and Dr. Matteo Cecchetto (member of staff of MNA) took care of the sampling of small body parts (usually the propodus of the paw) to send to the Canadian Center for DNA Barcoding (CCDB).

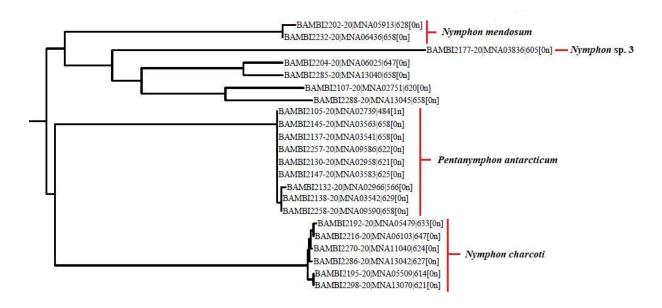
Out of 100 specimens sent, 78 proved to be analysable and provided information on the assumed species based on their barcode DNA sequence.

The tree derived from the comparison of the DNA sequences is shown below, with the indication on each branch of the genus to which the specimens were supposed to belong. In the following part of this thesis, it has been broken up to examine more in depth each cluster.



Pentanymphon antarcticum Hodgson, 1904

Nine specimens [MNA 2739, MNA 2958, MNA 2966, MNA 3541, MNA 3542, MNA 3563, MNA 3583, MNA, 9586, MNA 9590], morphologically identified as *Pentanymphon antarcticum* Hodgson, 1904 lay close to each other on the tree based on DNA and BLAST identification supports that based on morphology.



Close to the branch with these specimens, two other branches can be evidenced on the barcoding DNA tree related to nine specimens morphologically ascribed to genus *Nymphon*. Based on DNA, it seems they should belong to three different species of such genus.

Nymphon charcoti Bouvier, 1911

Six samples were found to belong to a single taxon that, based on the results of the BLAST, shows high percentages of compatibility with the following three species: *Nymphon charcoti*, *Nymphon unguiculatum* and *Nymphon australe*.

After a careful morphological analysis, it has been observed that all the specimens share the following characteristics.

Description [MNA 05479, MNA 05509, MNA 06103, MNA 11040, MNA 13042, MNA 13070]

Slender body with lateral processes spaced one or more times their diameter (Bouvier, 1911; Gordon, 1932; Child, 1995b; Weis et al., 2011), articles 2-3-4-5 of the palps of subequal length (Gordon, 1932; Child, 1995b; Weis et al., 2011), bridge exposed at the ventral insertion of the proboscis (Child, 1995b), 5th ovigeral segment curved and thinned in the proximal part (Gordon, 1932), presence of spines on the lateral processes (Weis et al., 2011), tarsus longer than the propodus (Child, 1995b), absence of auxiliary claws (Bouvier, 1911; Gordon, 1932; Child, 1995b; Weis et al., 2011), tibia II segment of greater length (Gordon, 1932), presence of setae on all legs (according to Gordon 1932, *N. lanare* has got setae but Bamber specifies that this species is blind, so we exclude it from the analysis) (Bamber, 2011).

These characteristics lead to the conclusion that these six specimens belong to the species *Nymphon charcoti* Bouvier, 1911.

Nymphon mendosum (Hodgson, 1907)

The sequences obtained from two samples were found to have high percentages of compatibility with *Nymphon mendosum*.

Description [MNA 5913, MNA 6436]

Lateral processes close together, even if they do not have contact with each other, with a pair of distal dorsal spines (sometimes three), hairless trunk with fused third and fourth segments. The last two articles of the palps almost sub-equal but the fourth longer than the fifth and setose, the scape of the cheliphores about as long as the proboscis, each finger of the chelae with about twenty denticles (40 in each chela). Two long spines at the insertion of the cheliphores and a very thorny scape (with long thin spines), palm shorter than the chela. Short neck. Rounded ocular tubercle of medium height with well evident eyes. Presence of distal and lateral dorsal spines also on the coxae (at least two pairs per coxa increasingly thin towards the third). Fifth ovigeral segment not swollen and insertion of the ovigers in contact with the first pair of lateral processes. Presence of rows of setae on the longer segments of the legs and thorns near the joints, femur > tibia I and femur > tibia II. Very small auxiliary claws, propodus > tarsus. Main claw less than half the length of the propodus, abdomen carried horizontally that exceeds the end of the coxae I and narrows in the distal part with two or four distal lateral bristles.

These characteristics correspond to the original description of *N. mendosum* (Hodgson, 1907; Child, 1995b; Weis et al, 2011 - from photo).

Note: despite the long and evident spines on the scape of the cheliphores, these are never named in the dichotomous keys (Gordon, 1932; Child, 1995b). There are not so many robust and stocky species of genus *Nymphon* with spines on the lateral processes, coxae, cheliphores and legs. Therefore, this detail should be added to the already existing keys.

We have compared these specimens with those already identified as *Nymphon proximum* in the National Museum of Antarctica collection because Weis et al. (2011) reports the confusion between *N. mendosum* and this species. They are very similar but *N. proximum* is much stockier and has spiny tubercles also on the trunk; moreover, its lateral processes are much closer together (even in contact to each other in some cases).

In conclusion, our sample corresponds to the original description of Nymphon mendosum (Hodgson, 1907).

Nymphon sp. 3

As regards the sample with the identification code MNA 03836, no correspondence was obtained with BLAST. The morphological description is reported here but the species has remained identified only as *Nymphon* sp.3.

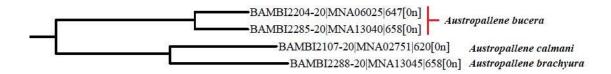
Description [MNA 03836]

Glabrous trunk completely segmented, lateral processes spaced apart more than their diameter, short abdomen which reaches just the end of the lateral processes. Low and rounded ocular tubercle with depigmented eyes, presence of two papillae on the top. Scape of the cheliphores shorter than the proboscis, chelae with some short setae especially near the insertion of the mobile finger. Notches on the mobile finger in less quantity than those on the fixed finger. Palps 5-segmented of which the second longer, 4^{th} and $5^{th} > 3^{rd}$ and slightly setose. Coxa II > coxa I + coxa III. Tibia longer major segment. Tarsus > propodus. Main claw less than half the length of the propodus and auxiliary claws about 1/3 of the main one. Tarsus with few

thorns on the ventral part, propodus with about ten thorns clearly visible along the sole. Visible genital pores on all paws. Ovigers with 5th article much elongated and narrower in the proximal part. Strigils with denticulated bristles and terminal ovigeral article with 5-7 very small denticles.

10.1.2 Austropallene

Only four specimens belonging to this problematic genus were genetically analysed: three different species were found and identified with the dichotomous key proposed in this thesis. For two of these specimens no matches were obtained by BLAST and the only match found turned out to be inaccurate.



Austropallene bucera Pushkin, 1993

For these two specimens [MNA 6025, MNA 13040] the BLAST gives a correspondence greater than 98% for *Austropallene cornigera* (Pushkin, 2011). However, the careful comparison of several morphological characters (the setose tubercles on coxae I, the shape of the sole and heel, the movable finger of cheliphores) with those described by the author have led us to a conclude they must be identified as *A. bucera*.

Austropallene calmani Gordon, 1944

For this specimen [MNA 2751] there were no correspondences in the BLAST. Despite this, it is unmistakable as it has thorny tubercles on the scape of the cheliphores: it is the only species of the genus with this characteristic (Pushkin, 1993).

Austropallene brachyura (Bouvier, 1911)

This specimen [MNA 13045] is very different from the others especially in the shape of the cheliphores: very thin and elongated and with rounded ends. Thanks to the table of the species and the key (Pushkin 1993, 2011), it has been identified as *Austropallene brachyura*; also in this case no correspondence was found by BLAST.

10.1.3 Ammothea and Achelia

The identification of 36 specimens belonging to genus *Ammothea* was quite simple thanks to the availability of a complete and recent key to its species (Cano-Sánchez and López-González, 2014).

Ammothea carolinensis Leach, 1814

[MNA 2731, MNA 2740, MNA 2777, MNA 2808, MNA 2811, MNA 2812, MNA 2813, MNA 2932, MNA 3651, MNA 3655, MNA 3699, MNA 3727, MNA 3867, MNA 6026, MNA 6031, MNA 6457, MNA 9593, MNA 13038, MNA 13058]

19 individuals were identified as *Ammothea carolinensis* (their affinity was confirmed by DNA barcoding and BLAST supported the identification).

Ammothea clausi Pfeffer, 1889

[MNA 3653, MNA 3654, MNA 3788, MNA 6029, MNA 13052, MNA 13066]

Six individuals correctly identified.

Ammothea glacialis (Hodgson, 1907)

[MNA 3564, MNA 9591, MNA 9597, MNA 13035]

These 4 specimens, initially identified as *Ammothea bentartica*, turned out to be *A. glacialis* thanks to the identification made by BLAST and a subsequent review in the laboratory. Three of these specimens were juveniles and it is quite common that juvenile stages of close species are similar to each other.

Ammothea minor (Hodgson, 1907)

[MNA 3857, MNA 5823, MNA 6015, MNA 6069, MNA 9616, MNA 13069]

Six individuals analysed, with correct identification even before the genetic analysis.

Ammothea spinosa (Hodgson, 1907)

[MNA 3834]

One individual analysed, with correct identification even before the genetic analysis.

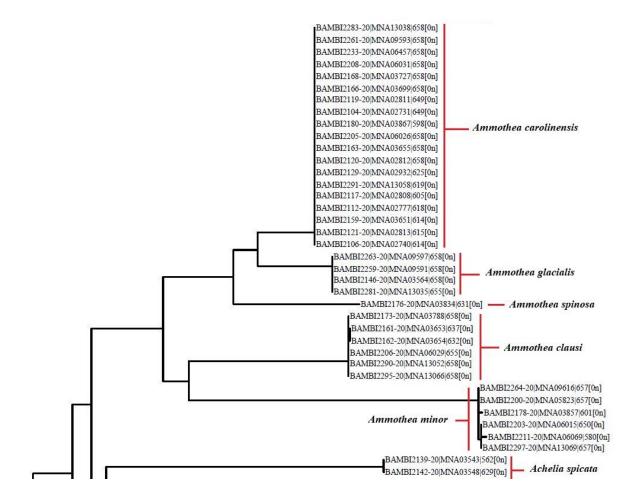
Achelia spicata (Hodgson, 1915)

[MNA 3543, MNA 3548]

Two samples of *Achelia* were identified in the laboratory by traditional morphological observation and this identification was then confirmed by genetic analysis.

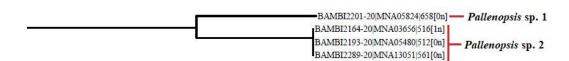
Note: according to Child (1995a) the auxiliary claws should be at least half as long as the main one or longer, but our specimens do not fit this description. Our observations are however supported by Calman (1915) who drafted a key to some species *Achelia*.

The other morphological characteristics match with *Achelia spicata* description: it is a very slender species, with well separated lateral processes and with clearly visible trunk sutures (especially between the first three segments). Child (1995a) mentions that more or less stocky individuals of this species may exist and that these two morphologies are often found simultaneously in the same sampling.



10.1.4 Pallenopsis

Four specimens of this genus were attributed to two different species and thanks to the genetic analysis different indications are provided, which however do not correspond to the traditional morphological observations. During the laboratory activity the recent key proposed by Cano-Sánchez and López-González (2019) was used.



Pallenopsis sp. 1

Description [MNA 5824]

Cheliphores with non-curved chela touching each other along their entire length, lateral processes detached from each other, auxiliary claws present and about half the length of the main one, trunk/proboscis ratio less than 2.4, cylindrical proboscis, propodus/main claw ratio greater than 1.3, coxa II longer than the sum of coxa I and coxa III, setose pad on mobile finger.

Our specimen seems to correspond to *Pallenopsis gracilis* Cano-Sánchez and López-González, 2019 except for propodus configuration (Cano-Sánchez and López-González, 2019).

Furthermore, tiny tubercles seem to be on the lateral processes that would bring our sample closer to *Pallenopsis hiemalis* Hodgson, 1907, to which, however, the configuration of the propodus and the tubercles on the coxae I do not correspond (Cano-Sánchez and López-González, 2019). According to Hodgson (1907) *P. hiemalis* has a bulge on the distal part of each lateral process, the anterior eyes should be larger than the posterior ones and the ovigers should be without denticulate spines.

The specimen examined could be similar to *Pallenopsis boehmi* Schimkewitsch, 1930 but the ocular tubercle is pointed and not truncated (Cano-Sánchez and López-González, 2019).

Besides, there are too many inconsistencies (ocular tubercle, auxiliary claw length and propodus configurations) with *Pallenopsis buphtalmus* Pushkin, 1993 suggested by BLAST based on barcode DNA, but with only 97.6% correspondence. The main claw/auxiliary claw ratio should be greater than 3 but, in our sample, it is less (Cano-Sánchez and López-González, 2019).

Analysing also *Pallenopsis kupei* Clark, 1971 the auxiliary claws should be more than half the length of the main one and on the heel there should be three sturdy spines followed by finer spines on the sole (Clark, 1971b): our sample has auxiliary claws half as long as the main one and there is no distinction between heel and sole.

Given the inconsistencies with most of the species known and with that suggested by the BLAST (*P. buphtalmus*), the MNA 5824 sample remains identified as "*Pallenopsis* species 1".

Pallenopsis sp. 2

The other three *Pallenopsis* specimens [MNA 3656, MNA 5480, MNA 13051] obtained 100% correspondences with *Pallenopsis patagonica* (Hoek, 1881) and *P. kupei* Clark, 1971 on the BLAST (sequences inserted by Claudia Arango): despite this, the morphological characteristics do not correspond.

P. patagonica should have lateral processes in contact in the proximal area (in our species lateral processes are not touching) while *P. kupei* should have a different propodus configuration. A similarity with *P. hiemalis* is noted, but this species has got tubercles on lateral processes (Cano-Sánchez and López-González, 2019).

Furthermore, 100% compatibility was obtained on BOLD system also with four "*Pallenopsis* sp." identified by an expert of this group.

Due to all these inconsistencies, these three specimens remain unidentified and associated with the name "Pallenopsis species 2".

This is a typical situation in which genetic analysis do not prove to be helpful to morphological one and which, on the contrary, prove to be completely useless.

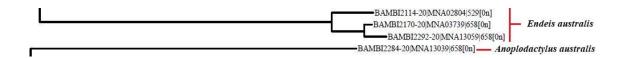
10.1.5 Endeis and Anoplodactylus

Endeis australis (Hodgson, 1907)

Description [MNA 2804, MNA 3739, MNA 13059]

Trunk about 4 mm long, absence of the collar on the proximal part of the proboscis, ocular tubercle mucronate, coxae II with low glandular and widened tubercle in the dorsal position, tarsus with a robust spine, propodus not very curved with three to five robust spines of heterogeneous size, auxiliary claws about half the length of the main one.

Based on these characteristics the three specimens were identified as *Endeis australis* (Hodgson, 1907). BLAST did not give matches.



Anoplodactylus australis (Hodgson, 1914)

The only specimen morphologically identified as *Anoplodactylus australis* (Hodgson, 1914) [MNA 13039] has got a 100% compatibility with *Anoplodactylus* cfr. *cribellatus* by BLAST. This species is recorded in India and New Caledonia (Calman, 1923; Bamber, 1997) and therefore it is clearly not an Antarctic species.

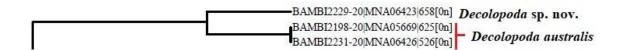
Considering Hodgson's original description, the main feature of *A. australis* should be that it has pointed ventral-distal tubercles on its proboscis (Hodgson, 1914). This character is present in the MNA specimen. However, the pores of the cement glands in *A. australis* should be 7 or 8 (Calman 1915; Child, 1995b; Chimenz Gusso and Gravina, 2001) while on our sample there are from 10 to 16. This character seems to be more similar to *A. cribellatus* which lateral processes have a single long seta and more than ten cement gland pores (up to 18 "cribellate" cement gland pores) (Bamber, 1997).

In this case the genetic analysis was not useful too, on the contrary these reveal 100% compatibility with a species whose range and ecology are incompatible with Antarctica. Morphological characters also leave some doubts.

Therefore, the specimen is identified as *Anoplodactylus* cfr *australis*.

10.1.6 Decolopoda

Tree specimens of genus *Decolopoda* were chosen for DNA barcoding analysis.



Decolopoda australis Eights, 1835

Two sample were morphologically identified as *Decolopoda australis* [MNA 5669, MNA 6426] and this identification is confirmed by genetic data (BLAST).

Decolopoda sp. nov.

Although BLAST gives a 100% correspondence with *D. australis*, specimen [MNA 6423] shows some morphological differences from that species and from the other species known from the Antartica *D. qasimi*. Besides, the genetical distance from this specimen and those also morphologically identified ad *D. australis* is rather high. This specimen is supposed to belong to a new species.

Description [MNA 6423]

Considerable size, circular and unsegmented trunk, long and thin abdomen ending beyond the coxae I. Ocular tubercle slightly blunt but not very high with four evident eyes. Proboscis that starts thin and widens towards in the middle while at three quarters it curves downwards, trilobed apex of the proboscis truncated, spines on the distal half of the proboscis. Palps of 9 articles of which the second and fourth of greater length. Round palps insertion. Bi-segmented cheliphores' scape, the first article long up to the middle of the proboscis, the second much shorter and facing downwards. Chela pointing downwards without internal teeth and very slender, fingers that come together to form a circumference. Very light small spines on the lateral processes and on the coxae I in the dorsal position. Ovigers of 10 articles with strigilar bristles arranged in three or four longitudinal rows and terminal claw. Round insertion of ovigers with groove in the distal part. Main claw almost as long as the propodus. Tarsus longer than propodus. Propodus without thorns. Absence of auxiliary claws. Presence of sporadic small spines on the longer segments of the legs but generally glabrous. Genital orifices present on all legs. Tarsus with radial spines around the distal insertion (in contact with the propodus) in the ventral area; 4-5 similar spines also present in the ventral-distal part of the tibia II.

In order to clarify the main differences between the Antarctic species of this genus, the basic characteristics (Eights, 1835; Sree et al., 1993; Child, 1995b) are listed in table VI:

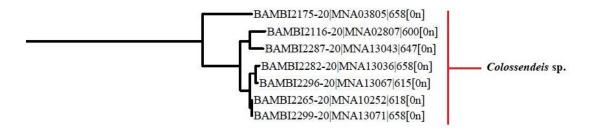
Table VI. Main differences between Decolopoda australis, D. qasimi and the sample stored in MNA, supposed to be a new species (D. sp. nov.)

D. australis	D. qasimi	D. sp. nov.
Propodus with 5-8 robust spines	Legs entirely covered by setae.	Propodus without spines.
on ventral side and 4-5 spines		Tarsus glabrous, with only 4-5
near claw insertion.		small spines on the insertion of
Tarsus with more than 10 ventral		propodus.
spines and 5-6 spines near the		Body glabrous with the presence
insertion of propodus.		of sparse small spines on the
Small spines on ventral side of		longer segments of the legs.
tibia II, on dorsal side of tibia I		
and femur.		
Abdomen exceeding the end of	•	Abdomen exceeding the end of
the coxa I.	(but even longer according to the	the coxa I.
	drawing it seems even longer –	
0-1	Sree et al., 1993).	0-110
Ovigers 10-segmented with big	Ovigers 9-segmented with	
setae arranged on 3-4 rows.	denticulate spines arranged on formula 5:4:4:3.	and numerous setae arranged on
Low and blunt conical ocular		3-4 rows. Low and blunt conical ocular
tubercle.		
tubercie.	small protuberances on each side	tubercie.
Eyes visible.	near the apex.	Eyes visible.
3	Faintly visible eyes.	†
Cheliphores' chela forming an	<i>'</i>	Cheliphores' chela forming a
oval when closed.		circle when closed.

Despite the many similarities to *D. australis*, the main difference concerns the spines on legs, tarsus and propodus: usually these features are diagnostic, so we hypothesise that the specimen examined belongs to a new species.

10.1.7 Colossendeis

These is a very critical group: the only seven samples genetically analysed remained identified only at genus level.



Most of the specimens [MNA 2807, MNA 10252, MNA 13036, MNA 13043, MNA 13067, MNA 13071] show very high BLAST affinities with samples identified as *Colossendeis* sp. and with sequences of *C. avidus*, *C. glacialis*, *C. scoresbii* and *C. drakei*.

Given the difficulty in finding all the original descriptions and given the ambiguity and the inconsistencies in the literature on the species of this genus, we preferred to identify the specimens to the genus level.

Further analysis and observations should be carried out on these specimens and on the others preserved in MNA.

10.1.8 Samples selected for DNA analysis

In the table VII the identifications of the 78 specimens selected for barcoding DNA analysis is summarized (specimens are listed in ascending MNA code order).

Table VII. Checklist of the 78 specimens selected for barcoding analysis

Catalogue number	Family	Genus	Scientific name
MNA 02731	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814
MNA 02739	Nymphonidae	Pentanymphon	Pentanymphon antarcticum Hodgson, 1904
MNA 02740	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814
MNA 02751	Callipallenidae	Austropallene	Austropallene calmani Gordon, 1944
MNA 02777	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814
MNA 02804	Endeidae	Endeis	Endeis australis (Hodgson, 1907)
MNA 02807	Colossendeidae	Colossendeis	Colossendeis sp.
MNA 02808	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814
MNA 02811	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814

Catalogue number	Family	Genus	Scientific name
MNA 02812	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814
MNA 02813	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814
MNA 02932	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814
MNA 02958	Nymphonidae	Pentanymphon	Pentanymphon antarcticum Hodgson, 1904
MNA 02966	Nymphonidae	Pentanymphon	Pentanymphon antarcticum Hodgson, 1904
MNA 03541	Nymphonidae	Pentanymphon	Pentanymphon antarcticum Hodgson, 1904
MNA 03542	Nymphonidae	Pentanymphon	Pentanymphon antarcticum Hodgson, 1904
MNA 03543	Ammotheidae	Achelia	Achelia spicata (Hodgson, 1915)
MNA 03548	Ammotheidae	Achelia	Achelia spicata (Hodgson, 1915)
MNA 03563	Nymphonidae	Pentanymphon	Pentanymphon antarcticum Hodgson, 1904
MNA 03564	Ammotheidae	Ammothea	Ammothea glacialis (Hodgson, 1907)
MNA 03583	Nymphonidae	Pentanymphon	Pentanymphon antarcticum Hodgson, 1904
MNA 03651	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814
MNA 03653	Ammotheidae	Ammothea	Ammothea clausi Pfeffer, 1889
MNA 03654	Ammotheidae	Ammothea	Ammothea clausi Pfeffer, 1889
MNA 03655	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814
MNA 03656	Pallenopsidae	Pallenopsis	Pallenopsis sp. 2
MNA 03699	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814
MNA 03727	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814
MNA 03739	Endeidae	Endeis	Endeis australis (Hodgson, 1907)
MNA 03788	Ammotheidae	Ammothea	Ammothea clausi Pfeffer, 1889
MNA 03805	Colossendeidae	Colossendeis	Colossendeis sp.
MNA 03834	Ammotheidae	Ammothea	Ammothea spinosa (Hodgson, 1907)
MNA 03836	Nymphonidae	Nymphon	Nymphon sp. 3
MNA 03857	Ammotheidae	Ammothea	Ammothea minor (Hodgson, 1907)
MNA 03867	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814
MNA 05479	Nymphonidae	Nymphon	Nymphon charcoti Bouvier, 1911
MNA 05480	Pallenopsidae	Pallenopsis	Pallenopsis sp. 2
MNA 05509	Nymphonidae	Nymphon	Nymphon charcoti Bouvier, 1911
MNA 05669	Colossendeidae	Decolopoda	Decolopoda australis Eights, 1835
MNA 05823	Ammotheidae	Ammothea	Ammothea minor (Hodgson, 1907)
MNA 05824	Pallenopsidae	Pallenopsis	Pallenopsis sp. 1
MNA 05913	Nymphonidae	Nymphon	Nymphon mendosum (Hodgson, 1907)
MNA 06015	Ammotheidae	Ammothea	Ammothea minor (Hodgson, 1907)
MNA 06025	Callipallenidae	Austropallene	Austropallene bucera Pushkin, 1993
MNA 06026	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814
MNA 06029	Ammotheidae	Ammothea	Ammothea clausi Pfeffer, 1889
MNA 06031	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814
MNA 06069	Ammotheidae	Ammothea	Ammothea minor (Hodgson, 1907)

Catalogue number	Family	Genus	Scientific name
MNA 06103	Nymphonidae	Nymphon	Nymphon charcoti Bouvier, 1911
MNA 06423	Colossendeidae	Decolopoda	Decolopoda sp. nov.
MNA 06426	Colossendeidae	Decolopoda	Decolopoda australis Eights, 1835
MNA 06436	Nymphonidae	Nymphon	Nymphon mendosum (Hodgson, 1907)
MNA 06457	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814
MNA 09586	Nymphonidae	Pentanymphon	Pentanymphon antarcticum Hodgson, 1904
MNA 09590	Nymphonidae	Pentanymphon	Pentanymphon antarcticum Hodgson, 1904
MNA 09591	Ammotheidae	Ammothea	Ammothea glacialis (Hodgson, 1907)
MNA 09593	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814
MNA 09597	Ammotheidae	Ammothea	Ammothea glacialis (Hodgson, 1907)
MNA 09616	Ammotheidae	Ammothea	Ammothea minor (Hodgson, 1907)
MNA 10252	Colossendeidae	Colossendeis	Colossendeis sp.
MNA 11040	Nymphonidae	Nymphon	Nymphon charcoti Bouvier, 1911
MNA 13035	Ammotheidae	Ammothea	Ammothea glacialis (Hodgson, 1907)
MNA 13036	Colossendeidae	Colossendeis	Colossendeis sp.
MNA 13038	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814
MNA 13039	Phoxichilidiidae	Anoplodactylus	Anoplodactylus australis (Hodgson, 1914)
MNA 13040	Callipallenidae	Austropallene	Austropallene bucera Pushkin, 1993
MNA 13042	Nymphonidae	Nymphon	Nymphon charcoti Bouvier, 1911
MNA 13043	Colossendeidae	Colossendeis	Colossendeis sp.
MNA 13045	Callipallenidae	Austropallene	Austropallene brachyura (Bouvier, 1911)
MNA 13051	Pallenopsidae	Pallenopsis	Pallenopsis sp. 2
MNA 13052	Ammotheidae	Ammothea	Ammothea clausi Pfeffer, 1889
MNA 13058	Ammotheidae	Ammothea	Ammothea carolinensis Leach, 1814
MNA 13059	Endeidae	Endeis	Endeis australis (Hodgson, 1907)
MNA 13066	Ammotheidae	Ammothea	Ammothea clausi Pfeffer, 1889
MNA 13067	Colossendeidae	Colossendeis	Colossendeis sp.
MNA 13069	Ammotheidae	Ammothea	Ammothea minor (Hodgson, 1907)
MNA 13070	Nymphonidae	Nymphon	Nymphon charcoti Bouvier, 1911
MNA 13071	Colossendeidae	Colossendeis	Colossendeis sp.

10.2 Updated Checklist of Antarctic and sub-Antarctic Pycnogonida

Based on the new data and on in-depth bibliographic research an updated checklist of the pycnogonids of Antarctic and sub-Antarctic waters is inserted here.

A list of 31 genera, 284 species and two subspecies is reported below, divided by families. The order of appearance of the families was taken from PycnoBase: World Pycnogonida Database (Bamber et al., 2022). Some species are marked with the following symbols: ° Species in MNA collection; * Species identified during this study; § Species not in the last checklist (Munilla and Soler Membrives, 2009).

In the table VIII the number of species by genus is summarised.

Order PANTOPODA

Family Ammotheidae Dohrn, 1881

Genus Achelia Hodge, 1864

Achelia assimilis (Haswell, 1875) °

Achelia communis (Bouvier, 1906) °

Achelia dohrni (Thompson, 1884)

Achelia hoekii (Pfeffer, 1889)

Achelia lagena Child, 1994

Achelia parvula (Loman, 1923) °

Achelia quadridentata (Hodgson, 1910)

Achelia serratipalpis (Bouvier, 1911)

Achelia spicata (Hodgson, 1915) °*

Achelia sufflata Gordon, 1944

Achelia transfuga Stock, 1954

Genus Ammothea Leach, 1814

Ammothea adunca Child, 1994

Ammothea allopodes Fry & Hedgpeth, 1969

Ammothea antipodensis Clark, 1972

Ammothea armentis Child, 1994

Ammothea australiensis (Flynn, 1919) °*§

Ammothea bentartica Munilla, 2001°

Ammothea bicorniculata Stiboy-Risch, 1992 §

Ammothea bigibbosa Munilla & Ramos, 2005

Ammothea calmani Gordon, 1932

Ammothea carolinensis Leach, 1814 °*

Ammothea childi Cano & López-González, 2013 §

Ammothea clausi Pfeffer, 1889 °*

Ammothea gigantea Gordon, 1932

Ammothea glacialis (Hodgson, 1907) °*

Ammothea gordonae Child, 1994

Ammothea hesperidensis Munilla, 2000 °*

Ammothea isabellae Cano & López-González, 2014 §

Ammothea longispina Gordon, 1932

Ammothea magniceps Thompson, 1884 §

Ammothea meridionalis Hodgson, 1915 °*

Ammothea minor (Hodgson, 1907) °*

Ammothea pseudospinosa Cano & López-González, 2013 §

Ammothea sextarticulata Munilla, 1991

Ammothea spinosa (Hodgson, 1907) °*

Ammothea striata (Möbius, 1902)

Ammothea stylirostris Gordon, 1932°

Ammothea tetrapora Gordon, 1932

Ammothea tibialis Munilla, 2002

Ammothea uru Clark, 1977 §

Ammothea victoriae Cano & López-González, 2007 °

Genus Austroraptus Hodgson, 1907

Austroraptus calcaratus Gordon, 1944°

Austroraptus juvenilis Calman, 1915 °*

Austroraptus polaris Hodgson, 1907

Austroraptus praecox Calman, 1915 °*

Austroraptus sicarius Fry & Hedgpeth, 1969

Genus Cilunculus Loman, 1908

Cilunculus acanthus Fry & Hedgpeth, 1969

Cilunculus cactoides Fry & Hedgpeth, 1969

Cilunculus kravcovi Pushkin, 1973

Cilunculus spinicristus Child, 1987

Genus Dromedopycnon Child, 1982

Dromedopycnon acanthus Child, 1982

Genus Sericosura Fry & Hedgpeth 1969

Sericosura mitrata (Gordon, 1944)

Genus Tanystylum Miers, 1879

Tanystylum antipodum Clark, 1977

Tanystylum brevicaudatum Fage & Stock, 1966

Tanystylum brevipes (Hoek, 1881)

Tanystylum beuroisi Arnaud, 1974

Tanystylum cavidorsum Stock, 1957

Tanystylum pfefferi Loman, 1923

Tanystylum neorhetum Marcus, 1940 °

Tanystylum oedinotum Loman, 1923

Tanystylum ornatum Flynn, 1928

Tanystylum styligerum (Miers, 1875)

Family Ascorhynchidae Hoek, 1881

Genus Ascorhynchus Sars, 1877

Ascorhynchus antipodus Child, 1987

Ascorhynchus cooki Child, 1987

Ascorhynchus cuculus Fry & Hedgpeth, 1969

Ascorhynchus inflatus Stock, 1963

Ascorhynchus simplex Nakamura & Child, 1991 §

Ascorhynchus ornatus (Helfer, 1938)

Genus Eurycyde Schiödte, 1857

Eurycyde antarctica Child, 1987

Family Colossendeidae Jarzynsky, 1870

Genus Colossendeis Jarzynsky, 1870

Colossendeis adelpha Child, 1998

Colossendeis angusta Sars, 1877

Colossendeis australis Hodgson, 1907 °*

Colossendeis avidus Pushkin, 1970

Colossendeis belekurovi Pushkin, 1993

Colossendeis brevirostris Child, 1995

Colossendeis colossea Wilson, 1881

Colossendeis concedis Child, 1995

Colossendeis drakei Calman, 1915

Colossendeis elephantis Child, 1995

Colossendeis enigmatica Turpaeva, 1974

Colossendeis ensifer Child, 1995

Colossendeis fragilis Pushkin, 1993

Colossendeis glacialis Hodgson, 1907°

Colossendeis grassus Pushkin, 1993

Colossendeis hoeki Gordon, 1944

Colossendeis insolitus Pushkin, 1993

Colossendeis korotkevitschi Pushkin, 1984

Colossendeis kurtchatovi Turpaeva, 1993

Colossendeis leniensis Pushkin, 1993

Colossendeis leptorhynchus Hoek, 1881

Colossendeis longirostris Gordon, 1938

Colossendeis macerrima Wilson, 1881

Colossendeis media Hoek, 1881

Colossendeis megalonyx Hoek, 1881°

[+ subsp. Colossendeis megalonyx arundirostris Fry & Hedgpeth, 1969] §

Colossendeis mica Pushkin, 1970

Colossendeis notialis Child, 1995

Colossendeis pseudochelata Pushkin, 1993

Colossendeis robusta Hoek, 1881°

Colossendeis scoresbii Gordon, 1932

Colossendeis scotti Calman, 1915°

Colossendeis stramenti Fry & Hedgpeth, 1969

Colossendeis tenuipedis Pushkin, 1993

Colossendeis tethya Turpaeva, 1974

Colossendeis tortipalpis Gordon, 1932

Colossendeis wilsoni Calman, 1915°

Genus Decolopoda Eights, 1835

Decolopoda australis Eights, 1835 °*

Decolopoda qasimi Sree, Sreepada & Parulekar, 1993

Genus Dodecolopoda Calman & Gordon, 1933

Dodecolopoda mawsoni Calman & Gordon, 1933

Family Callipallenidae Hilton, 1942

Genus Austropallene Hodgson, 1915

Austropallene brachyura (Bouvier, 1911) °*

Austropallene bucera Pushkin, 1993 °*

Austropallene calmani Gordon, 1944 °*

Austropallene cornigera (Möbius, 1902) °*

Austropallene cristata (Bouvier, 1911)

Austropallene gracilipes Gordon, 1944

Austropallene spinicornis Pushkin, 1993

Austropallene tcherniai Fage, 1952 °*

Austropallene tenuicornis Pushkin, 1993

Austropallene tibicina Calman, 1915 °*

Genus Callipallene Flynn, 1929

Callipallene margarita (Gordon, 1932)

Genus Cheilopallene Stock, 1955

Cheilopallene gigantea Child, 1987

Cheilopallene trappa Clark, 1972 §

Genus Oropallene Schimkewitsch, 1930

Oropallene dimorpha (Hoek, 1898)

Oropallene dolichodera Child, 1995

Oropallene metacaula Child, 1995

Genus Pseudopallene Wilson, 1878

Pseudopallene centrotus Pushkin, 1990

Genus Seguapallene Pushkin, 1975

Seguapallene insignatus Pushkin, 1975

Family Nymphonidae Wilson, 1878

Genus Heteronymphon Gordon, 1932

Heteronymphon exiguum (Hodgson, 1927)

Heteronymphon krappi Munilla & Soler-Membrives, 2015 §

Genus Nymphon Fabricius, 1794

Nymphon aculeatum Child, 1994 §

Nymphon adareanum Hodgson, 1907

Nymphon andriashevi Pushkin, 1993

Nymphon arcuatum Child, 1995°

Nymphon articulare Hodgson, 1908 °

Nymphon australe Hodgson, 1902

[+ subsp. N. australe caecum Gordon, 1944] §

Nymphon banzare Gordon, 1944 §

Nymphon biarticulatum (Hodgson, 1907)

Nymphon bicornum Arnaud & Child, 1988 §

Nymphon bouvieri Gordon, 1932

Nymphon brachyrhynchum Hoek, 1881

Nymphon brevicaudatum Miers, 1875

Nymphon bucuspidum Child, 1995

Nymphon chaetochir Utinomi, 1971

Nymphon charcoti Bouvier, 1911 °*

Nymphon clarencei Gordon, 1932

Nymphon compactum Hoek, 1881

Nymphon eltaninae Child, 1995°

Nymphon femorale Fage, 1956 §

Nymphon forticulum Child, 1995

Nymphon frigidum Hodgson, 1907

Nymphon galatheae Fage, 1956

Nymphon gerlachei Giltay, 1935

Nymphon glabrum Child, 1995

Nymphon gracilipes Miers, 1875

Nymphon granulatum Arnaud & Child, 1988 §

Nymphon gruzovi Pushkin, 1993

Nymphon hadale Child, 1982

Nymphon hamatum Hoek, 1881

Nymphon hampsoni Child, 1982 §

Nymphon hiemale Hodgson, 1907

Nymphon inferum Child, 1995

Nymphon inornatum Child, 1995

Nymphon isaenki Pushkin, 1993

Nymphon lanare Hodgson, 1907

Nymphon lomani Gordon, 1944

Nymphon longicollum Hoek, 1881

Nymphon longicoxa Hoek, 1881

Nymphon macquariensis Child, 1995

Nymphon macrochelatum Pushkin, 1993

Nymphon mendosum (Hodgson, 1907) °*

Nymphon microgracilipes Pushkin, 1993

Nymphon monothrix Child, 1995°

Nymphon multidens Gordon, 1932

Nymphon multituberculatum Gordon, 1944

Nymphon nakamurai Munilla & Soler-Membrives, 2015 §

Nymphon neelovi Pushkin, 1993

Nymphon neumayri Gordon, 1932

Nymphon orcadense (Hodgson, 1908)

Nymphon pagophilum Child, 1995

Nymphon paucidens Gordon, 1932

Nymphon paucituberculatum Gordon, 1944

Nymphon pfefferi Loman, 1923

Nymphon phasmatodes Bohm, 1879

Nymphon premordicum Child, 1995

Nymphon primacoxa Stock, 1968 §

Nymphon proceroides Bouvier, 1911

Nymphon procerum Hoek, 1881

Nymphon profundum Hilton, 1942 §

Nymphon proximum Calman, 1915 °

Nymphon pseudogracilipes Pushkin, 1993

Nymphon punctum Child, 1995

Nymphon residuum Stock, 1971 §

Nymphon rybakovi Pushkin, 1993

Nymphon sabellum Child, 1995

Nymphon scotiae Stock, 1981

Nymphon subtile Loman, 1923

Nymphon tenuimanum Hodgson, 1915

Nymphon tenuipes Bouvier, 1911

Nymphon trituberculum Child, 1995

Nymphon tubiferum Stock, 1978 §

Nymphon typhlops (Hodgson, 1915)

Nymphon unguiculatum Hodgson, 1915

Nymphon villosum (Hodgson, 1907)

Nymphon walvisense Stock, 1981 §

Nymphon zundianum Pushkin, 1993

Genus Pentanymhpon Hodgson, 1904

Pentanymhpon antarcticum Hodgson, 1904 °*

Genus Sexanymphon Hedgpeth & Fry, 1964

Sexanymphon mirabilis Hedgpeth & Fry, 1964

Family Pallenopsidae Fry, 1978

Genus Pallenopsis Wilson, 1881

Pallenopsis boehmi Schimkewitsch, 1930

Pallenopsis buphtalmus Pushkin, 1993

Pallenopsis candidoi Mello-Leitao, 1949

Pallenopsis gracilis Cano & López-González, 2019 °§

Pallenopsis gurjanovi Pushkin, 1993

Pallenopsis hodgsoni Gordon, 1938 °§

Pallenopsis kupei Clark, 1971

Pallenopsis latefrontalis Pushkin, 1993

Pallenopsis lateralia Child, 1995°

Pallenopsis lattina Pushkin, 1993

Pallenopsis leiopus Pushkin, 1993

Pallenopsis macronyx Bouvier, 1911

Pallenopsis obliqua (Thomson, 1884)

Pallenopsis patagonica (Hoek, 1881) °

Pallenopsis pilosa (Hoek, 1881) °

Pallenopsis rotunda Cano & López-González, 2019 °§

Pallenopsis spicata Hodgson, 1915

Pallenopsis tumidula Loman, 1923

Pallenopsis vanhoeffeni Hodgson, 1915

Pallenopsis villosa Hodgson, 1907

Pallenopsis yepayekae Weis, 2014 §

Family Endeidae Norman, 1908

Genus Endeis Philippi, 1843

Endeis australis (Hodgson, 1907) °*

Endeis viridis Pushkin, 1976

Family Phoxichilidiidae Sars, 1891

Genus Anoplodactylus Wilson, 1878

Anoplodactylus australis (Hodgson, 1914) °*

Anoplodactylus californicus Hall, 1912

Anoplodactylus laciniosus Child, 1995

Anoplodactylus laminifer Arnaud, 1974

Anoplodactylus petiolatus (Kröyer, 1844)

Anoplodactylus speculus Child, 1995

Anoplodactylus typhlops Sars, 1888

Anoplodactylus vemae Child, 1982

Anoplodactylus virescens (Hodge, 1864)

Genus Phoxichilidium Milne Edwards, 1840

Phoxichilidium pyrgodum Child, 1995

Family Pycnogonidae Wilson, 1878

Genus Pentapycnon Bouvier, 1910

Pentapycnon bouvieri Pushkin, 1993

Pentapycnon charcoti Bouvier, 1910

Genus Pycnogonum Brünnich, 1764

Pycnogonum calculum Bamber, 1995

Pycnogonum diceros Marcus, 1940 §

Pycnogonum gaini Bouvier, 1910 °*

Pycnogonum gordonae Pushkin, 1984

Pycnogonum magellanicum Hoek, 1898

Pycnogonum magnirostrum Möbius, 1902

Pycnogonum paragaini Munilla, 1990

Pycnogonum platylophum Loman, 1923

Pycnogonum sivertseni Stock, 1955

Family Rhynchothoracidae Thompson, 1909

Genus Rhynchothorax Costa, 1861

Rhynchothorax articulatus Stock, 1968 §

Rhynchothorax australis Hodgson, 1907 °*

Rhynchothorax oblongus (Pushkin, 1977)

Rhynchothorax percivali Clark, 1976

Rhynchothorax philopsammum Hedgpeth, 1951

Family Austrodecidae Stock, 1954

Genus Austrodecus Hodgson, 1907

Austrodecus breviceps Gordon, 1938

Austrodecus calcaricauda Stock, 1957

Austrodecus cestum Child, 1994

Austrodecus crenatum Child, 1994

Austrodecus curtipes Stock, 1957 °

Austrodecus elegans Stock, 1957

Austrodecus enzoi Clark, 1971 §

Austrodecus fagei Stock, 1957

Austrodecus frigorifugum Stock, 1954 §

Austrodecus (Microdecus) fryi Child, 1994

Austrodecus glabrum Stock, 1957

Austrodecus glaciale Hodgson, 1907 °*

Austrodecus goughense Stock, 1957

Austrodecus kelpi Pushkin, 1977

Austrodecus longispinum Stock, 1957

Austrodecus macrum Child, 1994

Austrodecus (Microdecus) minutum Clark, 1972 §

Austrodecus nausinoos Švara & Melzer, 2016 §

Austrodecus profundum Stock, 1957

Austrodecus pushkini Child, 1994

Austrodecus serratum Child, 1994

Austrodecus simulans Stock, 1957
Austrodecus sinuatum Stock, 1957
Austrodecus tristanense Stock, 1955
Austrodecus varum Child, 1994
Genus Pantopipetta Stock, 1963
Pantopipetta australis (Hodgson, 1914)
Pantopipetta buccina Child, 1994
Pantopipetta lata Stock, 1981
Pantopipetta longituberculata (Turpaeva, 1955)

Table VIII. Summary table of the number of species by genus

Family	Genus	N.er of species
Ammotheidae	Achelia	11
	Ammothea	30
	Austroraptus	5
	Cilunculus	4
	Dromedopycnon	1
	Sericosura	1
	Tanystylum	10
Ascorhynchidae	Ascorhynchus	6
	Eurycyde	1
Colossendeidae	Colossendeis	36 + 1 subsp.
	Decolopoda	2
	Dodecolopoda	1
Callipallenidae	Austropallene	10
-	Callipallene	1
	Cheilopallene	2
	Oropallene	3
	Pseudopallene	1
	Seguapallene	1
Nymphonidae	Heteronymphon	2
	Nymphon	76 + 1 subsp.
	Pentanymphon	1
	Sexanymphon	1
Pallenopsidae	Pallenopsis	21
Endeidae	Endeis	2
Phoxochilidiidae	Anoplodactylus	9
	Phoxichilidium	1
Pycnogonidae	Pentapycnon	2
	Pycnogonum	9
Rhynchothoracidae	Rhynchothorax	5
Autrodecidae	Austrodecus	25
	Pantopipetta	4

10.3 Key to Antarctic genera

1	Twelve legs
2	Slender legs. Lateral processes of the trunk smooth, separated by a distance equal to their diameter. Ocular tubercle in anterior position with respect to the height of the first lateral processes. Big eyes (the front pair is largest). Tarsus slightly longer than propodus, vestigial auxiliary
-	claws
3	Ten legs
4	Cheliphores and palps absent genus Pentapycnon Cheliphores and palps present 5
5 -	With auxiliary claws genus Pentanymphon Without auxiliary claws genus Decolopoda
6 -	Cheliphores and palps both present
7	Round or discoid body in dorsal view, lateral processes in contact or slightly separated, unisegmented scape of cheliphores, palps of 4-8 articles
8	Palps of 8 articles with frequent ventral projection in the last 4, pyriform or swollen proboscis in the median part with narrowing in the proximal part
9	Round body, absent dorsal segmentation, cheliphores reduced to a stump and without claws. Palps of 4-7 articles
10 -	Palps with 5 or less articles
11	Palps reduced to a bud, cheliphores with uni-segmented scape, absence of auxiliary claws
-	Palps with 4 or 5 segments, or uni-segmented (but in this case the scape of the cheliphores is biarticulated)
12	Uni-segmented palps in both sexes, bi-articulated scape, ovigers without terminal claw genus <i>Pallenopsis</i> Palps with 4 or 5 articles
13	Palps with 4 articles, uni-articulated scape, ovigers with terminal claw genus <i>Oropallene</i> 3

-	Cheliphores exceeding the length of the proboscis, with a uni-articulated scape and with chela provided with evident denticles. Ocular tubercle set back with respect to the insertion of the cheliphores, positioned anterior to the first pair of lateral processes
15 -	Without auxiliary claws
16 -	Palps of 10 articles and ovigers with terminal claw
17 -	Palps 7-segmented, blind ocular tubercle, uni-segmented scape as broad as long, chela reduced to a stump ¹ . Cement gland pores in the proximal part of the femur genus <i>Sericosura</i> Trunk with or without evident dorsal tubercles, fully formed or atrophied chelae, eyes present or absent. Cement glands with tubercle located in the distal half of the femur
18	Cephalon having an extended front hood that hides the insertion of the cheliphores. Cement gland forming a long cone in the distal half of the femur
19	Complete trunk sutures, trunk segments each having only one dorsal tubercle or none
-	Suture between the last two segments of the trunk not visible, cephalon with a pair of very evident dorsal tubercles placed on the sagittal midline, first and second segment of the trunk each with only one dorsal tubercle, strigils with a few toothed spines
20	Cheliphores present, palps missing
21	With auxiliary claws
22	Ovigers of 10 articles23Ovigers 5-7segmented in males, absent in females25
23	Ovigers without terminal claw genus Callipallene Ovigers with terminal claw 24
24	Slender body with elongated cephalon and lateral processes at least as distant as their diameter (except in O. dimorpha, in which however the spines of the strigils are typically lamellar); the
-	longest segment of the ovigers is the fifth
25 -	Vestigial auxiliary claws placed laterally to the main claw
26	Narrow proboscis with pointed distal part, smooth cheliphores' chelae, ovigers without terminal claw

¹ Females with tibiae having long setae in ventral position (absent in males).

-	Wide and short proboscis, chelae of crenulate or indented cheliphores, ovigers with terminal claw genus Cheilopallene
27	Cheliphores missing, palps present
28	Trunk without segmentation, palps 9-10segmented
29	Proboscis ovoid, palps 4-6segmented, ovigers 9-10segmented
30	Trunk slender, often devoid of tubercles, slender lateral processes, ovigers 10-segmented in both sexes
31	Slender body, legs twice as long as the body, 7-segmented ovigers ³ genus <i>Endeis</i> Stocky body, short legs slightly longer than the body, 9-segmented ovigers ⁴ genus <i>Pycnogonum</i>

Remarks: given the tools and resources at our disposal, and even more given the incompleteness and contradiction of the literature regarding many genera, we have succeeded in producing a complete and fairly simple key of the genera, based largely on the most well-known morphological characters (cheliphores, palps and ovigers). On the contrary, the keys to the species are often incomplete and may in some cases result ineffective.

_

² Ovigers 1-6segmented or rudimentary or absent in the males of some species

³ Ovigers missing in females of this species

⁴ Ovigers missing in females of this species

10.4 Key to Antarctic species (incomplete)

10.4.1 Genus Achelia

1	Distal articles of the palps markedly serrated
2	Absence of auxiliary claws, lateral processes with a small distal spine, proboscis that narrows considerably in the distal part
3	Lateral processes without dorsal tubercles or with tubercles only on the last three
4	Palps 8-segmented with asymmetrical 7th segment, very low ocular tubercle, auxiliary claws usually less than half the length of the main one
5 -	Legs with setose tubercles everywhere
6	Legs with several spiny or setose tubercles, some higher than their diameter, trunk usually but not always with two tubercles or slender spines in a dorsal-median position, cheliphores and palps with few dorsal tubercles
7	Trunk devoid of tubercles with setae, coxae I with only two dorsal-distal setose tubercles, proboscis with typical elongated shape
-	Trunk with anterolateral setose tubercles, coxae I with four dorsal-distal and lateral setose tubercles, proboscis with typical or downward curved shape
8	Proboscis with the typical elongated shape, with marked swelling near the median area <i>A. parvula</i> Atypical proboscis, with marked swelling more or less in the middle of its length, with the distal half slender and curved downwards

Starting from the dichotomous key of Child (1995a) and adding information from other descriptions (the original ones, when possible) an almost complete key was obtained.

Achelia sufflata Gordon, 1944 is the only species missing. The original description is unavailable and not enough information was found to include it in the key.

Achelia megacephala Hodgson, 1915 was present in the previous checklist (Munilla and Soler-Membrives, 2009) but was excluded from the current one as "nomen dubium" (WoRMS, 2022).

Achelia quadridentata (Hodgson, 1910) is also a critical taxon: in the original description it has only one tubercle on coxae I and it seems to be the only feature that can discriminate it from A. spicata. Despite this, other more recent descriptions report coxae I with two tubercles (one very evident and one smaller) in different positions (Barnard, 1954; Munilla, 1988) or three setose tubercles (Arnaud, 1974). Since these descriptions are shown to be inconsistent, it was decided to rely on the original one and not take into account the other characters: for example, tubercles on the cephalic area that appear only in Munilla's description (1988).

10.4.2 Genus Ammothea

This genus is already covered by a useful key, published by Cano-Sánchez and López-González (2014) with all 30 species registered in Antarctic and sub-Antarctic waters. This key is very complete because it uses some morphometric features in addition to qualitative characters.

Based on that key (Cano-Sánchez and López-González, 2014), seven species have been added to the previous checklist: *Ammothea australiensis* (Flynn, 1919), *A. bicorniculata* Stiboy-Risch, 1992, *A. childi* Cano-Sánchez and López-González, 2013, *A. isabellae* Cano-Sánchez and López-González, 2014, *A. magniceps* Thompson, 1884, *A. pseudopsinosa* Cano-Sánchez and López-González, 2013 and *A. uru* Clark, 1977.

On the contrary, three have been eliminated. *A. cooki* Child, 1987 is unaccepted (probably belonging to another genus). *A. dubia* (Hedgpeth, 1950) is unaccepted and considered belonging to genus *Boehmia*. *A. gibbosa* Bouvier, 1913 is unaccepted because junior synonym of *A. carolinensis* (WoRMS, 2022).

Out of the specimens we examined, 109 were ascribed to the genus *Ammothea* and, among them, 75 were identified at species level (see the paragraph 10.1.3).

10.4.3 Genus Austroraptus

A key to the five species of this genus was created by Fry & Hedgpeth (1969). Another similar key (only for four species) can be found in Child (1995a), where the only one missing was *Austroraptus sicarius* which has 8-segmented palps and fingers may be functional in adults (Fry & Hedgpeth, 1969).

10.4.4 Genus Cilunculus

1	Trunk devoid of conspicuous dorsal-median tubercles, short cheliphores sometimes vestigial, apparently one-segmented scape or incomplete suture between first and second segment, no eyes
-	Trunk with conspicuous dorsal-median tubercles, slender cheliphores, bi-segmented scape
2	Lateral processes with a long dorsal seta, pointed and slender ocular tubercle, ovigers with terminal claw
-	Trunk and lateral processes with rows of spines, ovigers without terminal claw C. spinicrista
-	Dorsal-median tubercles of the trunk with long setae each coming from small tubercles, lateral processes with some similar dorsal-distal setose tubercles, short cheliphores, first segment hardly longer than its diameter, auxiliary claws longer than half on the main one

This key was prepared based on Child (1995a): except for the scape's number of segments, it was easier to split the four species on the base of presence or absence of eyes.

10.4.5 Genus *Dromedopycnon*

This is the only species of this genus known for Antarctic and sub-Antarctic waters (Child, 1982 and 1995a) and a more complete description is reported to highlight its features.

10.4.6 Genus Sericosura

This species was described first as an *Achelia* by Gordon in 1944 (WoRMS, 2022) and then was moved to a new genus (Fry & Hedgpeth, 1969). A good description of this species can be found in Fry and Hedgpeth (1969) and in Child (1982 and 1995a).

10.4.7 Genus Tanystylum

1	Trunk without tubercles or other ornaments except the short thorns on the lateral processes
2	4-segmented palps, very short cheliphores, pointed and tubular proboscis
3	Tapered proboscis with a broad base, oblique-angled abdomen, usually 6-segmented palps, sometimes with seven segment, legs with conspicuous short pointed spines
4	Distal barrel-shaped proboscis with proximal constriction, abdomen carried horizontally, 7-segmented palps, legs with flimsy short blunt spines
5 -	Cylindrical proboscis, ovigers of 10 articles
6	Base of the abdomen without dorsal-median tubercle
7	Trunk with 3-4 dorsal-median spines, lateral processes without tubercles but with 2-3 distal spines
-	Trunk without dorsal-median thorns but with two horns at the anterior corners of the cephalon, on each of the lateral processes and on each coxa I
8	Barrel-shaped proboscis, as long as the trunk, abdomen extending beyond the first coxae, the 4th pair of legs with a broad, bulbous and setose base as high as the lower ocular tubercle

The point 5 of the previous key is quite uncertain: another identification key for this genus is then proposed:

1 -	Absent cheliphores
2	Terminal segment of the palps about twice as long as its width
3	Horizontal abdomen
4	Palps with 5 articles, terminal segment of the palps more than five times longer than wide <i>T. brevipes</i> Palps with 6-7 articles
5	Trunk with 2 or 3 spines placed anteriorly to the abdomen
5	Setae on the legs uniformly narrow up to have pointed apexes, trunk and coxae I with numerous spinules, 2nd segment of the palps of greater length
7	Trunk with conspicuous spines above a tubercle anterior to the abdomen
8	Final part of the lateral processes and coxae I divided into 2-4 lobes, abdomen extending beyond the coxae I
9	Palps 4-segmented, female ovigers with bi- or tri-toothed spines

T. brevipes appears twice in the key, since it is likely to find specimens without cheliphores (Clark, 1977).

Although *T. styligerum* is often described with 7-segmented palps (Clark, 1977, Child, 1955a), in the original description Miers (1875) wrote: "second pair [of appendages] five-jointed". For this reason, in our key the number of palp articles is not used for its identification.

Both keys must be considered incomplete since they do not include *Tanystylum pfefferi* Loman, 1923. This species is sometimes considered a synonym of *T. neorhetum* Marcus, 1940 and *T. oedinotum* Loman, 1923 (Clark, 1977, Müller, 1993). Despite this, *Tanystylum pfefferi* Loman, 1923 is currently considered a valid species with "accepted" status (WoRMS, 2022).

10.4.8 Genus Ascorhynchus

1	the insertion of the ovigers, denticulated ovigeral spines in several spatially close rows, very short terminal claw
-	Uni-segmented scape, ovigeral spines denticulated in one or two rows only
2	Ocular tubercle and eyes completely missing, hairless lateral processes, long slender and hairless abdomen, small and hairless chelae, denticulated spines of strigils arranged in two rows with formula 8:7:6:6 with hairless terminal claw and almost as long as the last ovigeral article, tarsus long the middle of the propodus, claws about 0.43 the length of the propodus
-	Ocular tubercle present
3	Scape length shorter than scape diameter, ocular tubercle positioned on the anterior edge of the cephalic segment, a pair of very small tubercles placed dorsally at the insertion of the cheliphores, very long abdomen extending beyond the coxae II of the fourth pair of legs, very long proboscis wide in the distal area and with a flat oral surface, very setose legs on their distal part
-	Short abdomen, whose length not exceeding the line of the 4th pair of lateral processes, broad conical tubercles placed dorsally at the insertion of the cheliphores, ocular tubercle wider than high with several protuberances at the apex, proboscis with marked proximal constriction and marked distal narrowing, legs almost hairless
5	Cylindrical ocular tubercle higher than wide, strigilar formula 4:2:1:3, main claw variable but usually 5/6 long of propodus

The preparation of this key was quite simple: in Child (1995a) a key for three of these species (*Ascorhynchus cooki*, *A. cuculus* and *A. simplex*) is available and, through the original descriptions of the other species, it was possible to make it complete. In particular, after having divided the species according to the segmentation of the scape, it was possible to discriminate the remaining ones based on the presence or absence of the ocular tubercle. *Ascorhynchus antipodus* is the only species without ocular tubercle and sensory papillae (Child, 1987).

Although Child cited *A. simplex* Nakamura & Child, 1991, this species was not included in the previous checklist (Munilla and Soler-Membrives, 2009); on the contrary, the species *A. hedgpethi* Turpaeva, 1974 is excluded because "junior synonym" of *A. cuculus* Fry & Hedgpeth, 1969 (WoRMS, 2022).

The information about *A. ornatus* and a clear drawing of this species can be found in Stock (1953), where the author specifies some unclear features of Helfer's original description. Furthermore, this species is actually considered to belong to the genus *Ascorhynchus* instead of the previous *Ainigma* (Helfer, 1938) (Stock, 1953). Lastly, *Ascorhynchus inflatus* can be easily recognized for its tall ocular tubercle very similar to its dorsal tubercles (Stock, 1963) and without eyes (Stock, 1963; Child, 1992b).

10.4.9 Genus Eurycyde

This is the only species of this genus known for Antarctic and sub-Antarctic waters and a more complete description is reported to highlight its particular features (Child, 1987).

10.4.10 Genus Colossendeis

This is the second genus for abundance, with 36 different species and one subspecies. Although it is an easily recognizable genus because of the large size, colour and prowess of the proboscis, there is no comprehensive key to the Antarctic and sub-Antarctic species.

Child (1995b) reported different species of *Colossendeis* and a key of "only" 18 species. Starting from this, various attempts have been made but it has not been possible to produce a complete key.

This is a problematic genus due to the very high number of species differing from each other for minute features (Child, 1995b) or morphologically very similar and differing only by DNA (see the paragraph 10.1.7).

During various attempts for the construction of the key, understanding which characters were actually diagnostic was the greatest obstacle accompanied by the difficulty in finding descriptions (original and otherwise) that were as precise as possible. The last attempt at the key left out 10 species, resulting not so much reliable (for this reason it is not reported here).

For example, the little information about *Colossendeis enigmatica* Turpaeva 1974 and *C. tethya* Turpaeva 1974 was obtained from the drawings available in the original papers (Turpaeva, 1974). Besides, it was impossible to add *Colossendeis perforata* Turpaeva, 1993 in the checklist because the sampling location is not clear, due to the original description of the author, all completely in Russian (Turpaeva, 1993).

Another difficulty is to face up to the probable three subspecies of *Colossendeis megalonyx* Hoek, 1881 (Fry and Hedgpeth, 1969; WoRMS, 2022) and its synonyms (WoRMS, 2022). For example: *Colossendeis megalonyx arundirostris* Fry and Hedgpeth, 1969 was inserted in the checklist of Munilla and Soler-Membrives (2009) as *Colossendeis arundirostris*; this name is now not accepted (WoRMS, 2022) and it must be considered a subspecies of *Colossendeis megalonyx* Hoek, 1881.

A key to identify the different subspecies of *Colossendeis megalonyx* is reported in a recent work from the Weddell Sea and adjacent waters (Turpaeva and Rajsky, 2013) but these have not been added to the checklist, given the variability and difficulty in studying this taxonomic group.

In the end, to quote Child (1995b): "The Antarctic species of this genus need a thorough revision, but I leave this difficult task to a future student of the Pycnogonida".

10.4.11 Genus Decolopoda

These two species are easily recognizable from each other (as the genus too). For this reason, it was considered necessary to mention the observation of a specimen of this genus, which however has characteristics that do not match with those of the two aforementioned species: a probably new species (paragraph 10.1.6).

10.4.12 Genus Dodecolopoda

This species is very rare (Child, 1995b) but it is very easily recognizable due to the six pair of legs, the proboscis downcurved and cheliphores with long scape. It is interesting to note that the first classified specimen probably possessed some regenerated articles of the fifth right leg, as they were smaller than the others (Calman and Gordon, 1933); another proof of this ability in pycnogonids.

10.4.13 Genus Austropallene

1	Absence of dorsal tubercles on the trunk segments
2	Marked heel in the proximal part of the propodus
3	Setose tubercles on the first coxa
4	Lateral processes separated by less than half their diameter, distal tubercles on fixed finger close together, femoral glands present
5 -	Movable finger with tooth on the inner surface
6 -	Rounded fixed finger of cheliphores
7	Cheliphores' fingers having the same length and converging when closed
8	Internal surface of the cheliphores' fingers concave and the tips of the fingers with a similar conformation, scape having a pair of pointed tubercles
9	Cheliphores' scape with several spines, proboscis almost completely cylindrical, narrow only in the final part

The present key was obtained from the one published by Pushkin (2011), translating his key from Russian and from morphological observation of the samples at our disposal.

The recognition at the genus level is very easy, given the particular morphology of the cheliphores and the proboscis.

10.4.14 Genus Callipallene

This is the only species of this genus known for Antarctic and sub-Antarctic waters and a more complete description is reported to highlight its particular features (Child, 1995b).

10.4.15 Genus Cheilopallene

Cheilopallene trappa Clark, 1972 was added in the new checklist, because it was sampled in the Snares Islands (Campbell Plateau, New Zealand) (Clark, 1971a) and it was defined, together with *C. gigantea*, as a sub-Antarctic species (Staples, 2015).

10.4.16 Genus Oropallene

Two of these species (*Oropallene dolichodera* and *Oropallene metacaula*) were described by Child (1995b) and this key also inserts the species described by Hoek (1898) (*Oropallene dimorpha*). In general, they are easily recognizable from each other thanks to a few simple identifiable characters.

10.4.17 Genus Pseudopallene

It was decided to exclude the species *Pseudopallene glutus* Pushkin, 1975 cited in the original checklist (Munilla and Soler-Membrives, 2009) as it is defined taxon inquirendum (WoRMS, 2022) because it was defined as incompatible with this genus (Staples, 2014). This species has lateral processes long and widely spaced, very long tarsus and absence of the palps bud: the latter character is typical of genus *Pseudopallene*. Arnaud and Branch (1991) confirmed that *Pseudopallene glutus* has no palps.

10.4.18 Genus Seguapallene

General information about this genus taken from Pushkin (1975), especially from the drawings; specific characters found in Stock (1991).

10.4.19 Genus Heteronymphon

Before 2015, *Heteronymphon exiguum* (Hodgson, 1927) was the only species of this genus registered for the southern hemisphere (Child, 1995b). In any case, the differences between this species and *H. krappi* Munilla and Soler-Membrives, 2015 are many (Munilla and Soler-Membrives, 2015) and very evident, so the identification should not be particularly complicated.

10.4.20 Genus Nymphon

This is the first genus for abundance, with 76 different species and one subspecies. As genus *Colossendeis*, this genus is easily recognizable thanks to its general morphology; despite this, there is no comprehensive key to the Antarctic and sub-Antarctic species of *Nymphon*.

Child (1995b) reported different species of *Nymphon* and two different keys: due to the large variability of this genus, for a long time, attempts have been made to divide it into "groups" to make the classification of the species easier.

In the past, some species were classified under genus *Chaetonymphon* to underline the morphological differences with *Nymphon* species (Child, 1995b) but during the redescription of some species, these were reunited under genus *Nymphon* (Gordon, 1932).

For this reason, it is possible to find two different groups of species: the *Australe* group and the *Hamatum* group. The differences between these two categories can be found in Child (1995b), accompanied by two keys, respectively for 19 and 18 species (and one subspecies).

For now, these are the most complete identification keys for this found in the literature; starting from this, various attempts have been made but it has not been possible to produce a trusted key (as for *Colossendeis* species).

The division into two groups, however, does not always simplify recognition: for example, it is common to find individuals attributed to the species *N. australe*, which later turned out to be synonyms or subspecies (Child, 1995b; WoRMS, 2022). In fact, this species is the most captured in Antarctic and sub-Antartic waters and the consistent variation observed in it is due to the great number of specimens to compare (Child, 1995b).

The *Hamatum* group is composed by species characterized by blindness (with reduced or absent ocular tubercle) and absence of auxiliary claws (Child, 1995b). Despite the simple features, the variability of the species is very high.

Besides, some species whose characteristics do not fall within the canons of the two groups, are considered separately; such Antarctic species are also many (Child, 1995b) and this increases the difficulty in identifying the samples and, above all, create a complete key. In fact, despite the DNA analyses carried out on three *Nymphon* species, one of these remained identified only at the genus level.

Our updated list of species differs from the previous checklist (Munilla and Soler-Membrives, 2009) for the addition of 12 species listed in the table IX.

Table IX. Species of Nymphon included in the current checklist of Antarctic Pycnogonida and absent in the previous one (Munilla and Soler-Membrives, 2009)

Species	Notes
Nymphon aculeatum Child, 1994	Cited in Child (1995b) (Hamatum group)
Nymphon banzare Gordon, 1944	Diagnosed and discussed in Child (1995b) and inserted in his key (<i>Australe</i> group)
Nymphon bicornum Arnaud & Child, 1988	Cited in Child (1995b) (Hamatum group)
Nymphon femorale Fage, 1956	Cited in Child (1995b) (Hamatum group)
Nymphon granulatum Arnaud & Child, 1988	Cited in Child (1995b) (Hamatum group)
Nymphon hampsoni Child, 1982	Cited in Child (1995b) (Australe group)
Nymphon nakamurai Munilla & Soler-Membrives, 2015	Discovered six years later the publication of checklist (2009)
Nymphon primacoxa Stock, 1968	Cited in Child (1995b) (Hamatum group)
Nymphon profundum Hilton, 1942	Cited in Child (1995b) (Hamatum group)
Nymphon residuum Stock, 1971	Cited in Child (1995b) (Hamatum group)
Nymphon tubiferum Stock, 1978	Cited in Child (1995b) (Hamatum group)
Nymphon walvisense Stock, 1981	Cited in Child (1995b) (Hamatum group)

Besides, based on the Child's keys (Child, 1995b) it was decided to add the subspecies *N. australe caecum* Gordon, 1944 to the list as well.

There are also two species that it was not possible to include in the checklist, since the sampling location is not clear, due to the original description of the author, completely in Russian (Turpaeva, 1993): *Nymphon filatovae* Turpaeva, 1993 and *N. petri* Turpaeva, 1993.

Four species of the original key were removed. *Nymphon stylops* Bouvier, 1913 and *Nymphon isabellae* Turpaeva, 2000 are synonymized, respectively to *N. australe* Hodgson, 1902 and *N. gerlachei* Giltay, 1935. *Nymphon longisetosum* Hodgson, 1915 and *Nymphon polare* Hodgson, 1915 are marked with "nomen dubium" (WoRMS, 2022).

10.4.21 Genus Pentanymphon

Species very easily to recognize because of the five pairs of legs (as the genera *Decolopoda* and *Pentapycnon*).

10.4.22 Genus Sexanymphon

Species very rare (Child, 1995b) and easily to recognize because of the six pairs of legs (as the genus *Dodecolopoda*).

10.4.23 Genus Pallenopsis

This genus is already covered by a useful key, published by Cano-Sánchez and López-González (2019) with all 24 species registered in Antarctic and sub-Antarctic waters. This key is very complete because it uses some morphometric features in addition to qualitative characters. This is a particularly difficult genus and, as for others, the lack of detailed information on the species is frequent and many are considered a complex species (Cano-Sánchez and López-González, 2019).

The species of *Pallenopsis* in our checklist are 21. We insert *P. hodgsoni* Gordon 1938 (absent in the previous checklist but cited in Child, 1995b) and remove *P. longiseta* Turpaeva, 1957 because it is now considered as an "alternate representation" of the homonym species *Bathypallenopsis longiseta* (Turpaeva, 1957) (WoRMS, 2022). The other two species missing from the article of Cano-Sánchez and López-González (2019) are *P. hiemalis* Hodgson, 1907 and *P. meridionalis* Hodgson, 1914, both considered "unaccepted" by WoRMS (WoRMS, 2022).

Eighteen of our samples are identified as *Pallenopsis* but only for seven a specific (insecure) identification was assumed. Through DNA analysis we were able to verify the presence of certainly two different species, that remained unidentified (see the paragraph 10.1.4).

10.4.24 Genus Endeis

- Proboscis equipped with a collar at the base; curved propodus with four identical spines...... E. viridis

It is strange that Child did not mention *E. viridis* and even defined *E. australis* as "the only one in this genus to occur in Antarctic waters" (Child, 1995b). Another main difference between the two species concerns the body size and the trunk length: *E. viridis* is smaller in size than *E. australis* (Pushkin, 1976), which has got up to 5 mm of trunk length (Chimenz Gusso and Gravina, 2001).

10.4.25 Genus Anoplodactylus

1	Missing eyes
2	Ocular tubercle missing or as a low bump
3	Ovigers 6-segmented 4 Ovigers 5-segmented 8
4 -	All or some lateral processes with a small dorsal tubercle
5 -	Trunk completely segmented
6	Ocular tubercle as a low cone. ♂ Presence of sexual pores carried on long tubercles and articles of particularly shaggy strigils. ♀ Presence of wing appendages on the ventral surface of the proboscis.
-	Rounded or conical and narrow ocular tubercle (mucronate)
7	Rounded ocular tubercle, 2nd segment of ovigers 5 times greater than its diameter
8	Lateral processes with dorsal-distal bridges having 1-2 setae each, coxa I with small dorsal-distal tubercles carrying 1-2 setae, sometimes presence of two pairs of auxiliary claws
9	Cylindrical proboscis with spurs on the ventro-distal corners

This key was based on Child's one (Child, 1995b) which contains seven species; the two species added are *A. laminifer* and *A. virescens*. Differently from the original key, it was decided to discriminate the species first with respect to the presence/absence of eyes and subsequently on the basis of the segmentation of the ovigers. Besides, *A. petiolatus* is mentioned twice as tubercles on the lateral processes may sometimes be absent (Child, 1995b).

This type of key, despite being simple and based on easily identifiable characteristics, has the drawback not to allow the easy identification of the females of this genus which have no ovigers.

10.4.26 Genus Phoxichilidium

The only species of this genus widespread in Antarctic and sub-Antarctic waters, precisely described by Child (1995b).

10.4.27 Genus Pentapycnon

Many other differences between these two species can be found in the original description of *P. bouvieri* (Child, 1995b).

10.4.28 Genus Pycnogonum

-	cular tubercle, ovigers of 9 articles and terminal claw, propodus' claw about half the length of the article
2	Proboscis with ornaments or protuberances
3	Proboscis with two dorsal tubercles having two papillae at the apex, trunk having three large dorsal tubercles, ovigers of 9 segments, terminal claw of the oviger shorter than the last article, considerable dimensions (trunk + proboscis: 23-24 mm)
4	Coxae I with 2-4 dorsal-distal tubercles 5 Coxae I without tubercles 6
5	Cylindrical proboscis with rounded oval apex, conical tubercles of trunk and lateral processes, 3rd and 4th ovigeral article of comparable length with the 5th
6 -	Femur with a proximal-ventral protuberance 7 Femur without a ventral protuberance 8
7 -	Auxiliary claws missing
8	Ovigers of 8 articles, trunk with little dorsal tubercles, two small rounded protuberances behind the ocular tubercle

Child (1995b) made a key for four species only while this key tries to be a useful tool for identifying all 9 species.

Differently from the original checklist (Munilla and Soler-Membrives, 2009), we inserted *Pycnogonum diceros* Marcus, 1940 in place of *P. rhinoceros* Loman, 1923 considered unaccepted (WoRMS, 2022).

The most problematic taxon is *Pycnogonum platylophum* Loman, 1923: in Arnaud & Branch (1991) each of the lateral processes has a dorsal-distal tubercle, but in other two keys the processes are smooth (Marcus, 1940; Child, 1995b). As with other genera or species, we believe that Child's considerations are more reliable.

10.4.29 Genus Rhynchothorax

1	Without tubercles on the trunk, ocular tubercle or eyes
2	Lateral processes and coxae I with tubercles
3	Low ocular tubercle, with a slender anterior extension twice the height of the tubercle itself, auxiliary claws present
-	Coxae I with low dorsal tubercles, lateral processes without tubercles, ocular tubercle slightly higher than wide, sometimes with a small anterior extension with vertical or oblique angle as well as trunk tubercles

In the original checklist (Munilla and Soler-Membrives, 2009) *Rhynchothorax articulatus* Stock, 1968 was not cited, although Child (1995b) inserted it in an almost complete key of Antarctic and sub-Antarctic species.

The only addition to the Child's (1995b) key is *Rhynchothorax oblongus* Pushkin, 1977, of which we have a description in Russian (Pushkin, 1977): the little information necessary for its placement in the key derives from the drawings by Pushkin.

10.4.30 Genus Austrodecus

1	Ovigers 5 or 6-segmented and propodus with auxiliary claws
2	Coxae I with tubercles ordered from anterior to posterior coxae according to the formula 1,2,2,1 3 Coxae I with tubercles ordered in other sequences
3	Abdomen with a small distal tubercle, terminal segments of the palps fused in the shape of a knob, very short auxiliary claws, less than 0.3 times the length of the main one
-	Trunk with tall and slender median tubercles, long and slender ocular tubercle, cement gland as a low and wide cone
5	Tibiae I longest segments, orifice of the cement gland as a straight slender cone, terminal segment of the ovigers longer than the 4th segment

-	terminal segment of the ovigers of equal length or longer than the 4th segment
6	Orifice of the cement gland as a very slender and partly concave cone, terminal segment of the ovigers as long as the 4 th segment
-	Orifice of the cement gland as a low rounded bump, terminal segment of the ovigers longer than the 4 th one
7 -	Coxae I and coxae II with a single tubercle each
8	Trunk with few papillae, devoid of dorsal-median tubercles, lateral processes separated by a length equal to their diameter or greater, abdomen devoid of distal tubercle
9	Dorsal-median tubercles of the trunk and abdominal higher than their basal diameter, very long ocular tubercle, with eyes, very long proboscis, equal to the length of the trunk, the two terminal segments of the palps hardly longer than their diameter
10 -	Coxae I with a single tubercle, the other six coxae with two distinct tubercles
-	Dorsal-median tubercles of the trunk high, pointed, parallel for most of their length, robust and very close lateral processes, moderately short ocular tubercle with a bulbous base, flat at the apex in dorsal view, femoral cement gland as a small proximal protuberance
12	Ocular tubercle usually high, very narrow, well separated lateral processes that never touch, propodus with very few spines on the sole, cement gland as a broad cone as long as the femoral diameter or longer, with a distal pore
13	Trunk with dorsal-median tubercles with broad base and tapering at the tip, ocular tubercle low, blunt, with a small narrowing, propodus with some spines on the sole, cement gland orifice as a very small cone with a lateral pore, auxiliary claws almost half of the main one
14 -	All coxae I with two dorsal-distal tubercles
15	Trunk devoid of tubercles but with dorsal-median papillae, tall and slender ocular tubercle, with no excessively broad base, slender propodus, cement gland as high cone equal to the diameter of the femur, auxiliary claws less than half the length of the main one
16 -	Moderately low ocular tubercle, roughness present on coxae II, coxae III of all legs with a single dorsal-distal tubercle, abdomen without dorsal spur on the terminal part

-	proximal area and broad at the apex, coxae I with a high tubercle and a second tiny one on the 1 st and 4 th pair of legs respectively, two on the 2 nd and 3 rd pair
18	Ovigers 6-segmented and auxiliary claws missing
19	Compact trunk with wide and low tubercles, lateral processes in contact without tubercles, last two segments of the palps with a typical unfused shape, tubercles on the coxae I that follow the course 1,2,2,1
20	Trunk and tubercles of the lateral processes very setose, proboscis slightly longer than the palps, two distal segments of the palps of equal length, segment of the legs of greater length with some small dorsal tubercles, tibiae I are the longest segments, terminal article of the ovigers with some tiny papillae and a few spines
21	Ovigers 4-segmented, with or without auxiliary claws, cement gland, where known, on all legs22 Ovigers 1-segmented, with auxiliary claws, cement gland on the fourth pair of legs only (subgenus <i>Microdecus</i>)
22	Auxiliary claws absent, terminal segments of palps short and forming a sub-chela, very high dorsal-median tubercles, all coxae I with a single tubercle
23	Strong trunk, lateral processes close in contact, ocular tubercle and abdomen very short, dorsal-median sub-tubular tubercles much longer than their diameter, 3 rd segment of the palps slightly longer than 3 times its diameter
-	Slender trunk, non-contacting lateral processes, ocular tubercle and abdomen long to very long, dorsal-median tubercles with small slender spines or low bumps
24	Trunk with dorsal-median tubercles with small points, ocular tubercle about 4-5 times longer than the diameter at the apex, coxae III with low tiny dorsal tubercles, large auxiliary claws longer than half of the main one
-	Trunk with dorsal-median tubercles with small pointed bumps, ocular tubercle at least 8 times longer than the diameter at the apex, coxae III with slender dorsal tubercles as long as the diameter of the segment, tiny auxiliary claws smaller than the diameter of the main one
25	Trunk elongated with four slender dorsomedial tubercles, well separated lateral processes, ocular tubercle and moderately long slender leg segments, coxae I with long slender tubercles following the sequence 1,2,2,1 from front to back, abdomen almost cylindrical

This key was prepared on the base of dichotomous key of Child (1995a) and Švara and Melzer (2016) which cited almost all species. The only one left excluded is *Austrodecus kelpi* Pushkin, 1977.

Most of the information about *A. kelpi* were obtained from Child (1995a), who considers this species very close to *A. pushkini* Child, 1994 and as the main difference the author mentioned is the tubercles present on the back of the coxae I of the fourth pair of leg. Unfortunately, the original description is written entirely in Russian, and the drawing is not particularly detailed (Pushkin, 1977) so for the key purposes we followed Child's (1995a) notes and remarks, trying to insert the species in the key in a coherent way.

10.4.31 Genus Pantopipetta

This key was created by Child (1995a), containing all the four species of the genus.

As before, for already published keys, please refer to the competent authors.

11. Pycnogonida and Citizen Science

In parallel with the study of the Italian and Antarctic pycnogonids, it was possible to conduct a research related to citizen science. Seven websites were examined, where photographs of these organisms were available. The aim was to verify the possibility of conducting citizen science investigations on this group of marine animals. 384 observations were considered, most of them also containing information regarding the location and date of sampling.

In recent years, several citizen science projects have involved the naturalistic field (Silvertown, 2009) and as regards marine biology, most of these have focused on coastal environments, to signal the presence or absence of certain species (Earp and Liconti, 2019).

Pycnogonids have never been the subject of a study of this kind, and it was our interest to understand if it was possible to carry out a project of this type and above all to verify what impact this almost completely unknown taxon can have on the population of divers and common people.

Most of the observations were obtained from iNaturalist since it is a particularly rich site with very active users; the observations considered reach up to April 2021. Practically all the observations were made by non-specialists and not all the identifications reached the species level.

Considering the observations of this website (327), these were attributable to 50 genera and 48 species (of which only four genera 5 species identified incorrectly). Despite this, many inconsistencies were found and very often different genera were confused.

In any case, the most easily recognizable species are those with a particular and very evident colouring such as *Anoplodactylus evansi*, *Meridionale harrisi*, *Pycnogonum aurilineatum*, *Stylopallene cheilorhynchus* and *S. tubirostris*.

Given the difficulty in identifying these animals even by specialists, it is believed that this group of animals is not particularly suitable for citizen science projects. However, nothing excludes those qualitative studies may use the help of expert divers: some of them they may be interested in the recognition of these species.

For further information, see the article:

Colasanto E and Galli L. (2021). People's contribution to the knowledge of Pycnogonida: citizen science in the case of a "problematic" taxon. *Biogeographia – The Journal of Integrative Biogeography*, 36. http://dx.doi.org/10.21426/B636053543

12. Conclusions

As regards the dichotomous key of the Italian Pycnogonids, it will be necessary to carry out a continuous updating through the identification of "fresh" specimens to improve the quality of the descriptions.

Future investigations related to particular specimens will help to identify the most reliable diagnostic characters: there are particularly insidious genera from the identification point of view such as *Achelia*, *Ascorhynchus*, *Callipallene* and *Tanystylum*.

Within the *Achelia* genus, the diagnostic unreliability of the strigilar formulas has been verified several times, although they are valid diagnostic characters for other genera. Identification is simple in the case of males, but it is necessary to find functional diagnostic characters regardless of sex.

As regards the genus *Ascorhynchus*, on the other hand, the determination of sex is complex, in the absence of morphological characteristics associated with dimorphism and given the difficulty in identifying the genital pores (for this reason, for all the specimens identified in this study, the sex was not specified). In addition, it is also necessary to review the iconography relating to the species *A. castelli*: some characteristics, such as the long bristles at the apex of the dorsal tubercles were never observed during the examination of the specimens. The description of Munilla & Soler Membrives (2014) makes a vague reference: a doubt therefore arises as to their diagnostic weight.

The genus *Callipallene* includes species whose strigils do not differ significantly; for that reason, it is necessary to observe characters such as the curvature of the propodus and the length of the auxiliary claws, which are not always easy to interpret with objectivity. Moreover, it is necessary to have a large quantity of samples of the various species to verify or not the diagnostic importance of the length of the neck. From what we have been able to observe in the laboratory, within different samples of *C. tiberi*, this is very variable.

Finally, although the genus *Tanystylum* in our seas includes only two species, it would be necessary to verify the diagnostic reliability of all the characters mentioned in the literature. Furthermore, it could be interesting to re-study the samples stored at DISTAV also from a molecular point of view.

To increase knowledge on Italian Pycnogonids it would be interesting to plan further research and continue to collect data on the species (*Endeis biseriata* may not be the only "new" species within our seas). However, based on our means, we were able to give a significant contribution with our 1572 specimens identified.

In addition to a further collection of bibliographic data, it would be necessary to continuously update the analytical key and analyse the samples not only by way of the instrumentation used during these years, but to support the reliability of the identifications through SEM and molecular analysis (to achieve more accurate classifications).

Although the collection of pycnogonids is always a corollary of other major environmental investigations, the preliminar study on upper infralittoral zone fauna at Portofino add to the knowledge of these animals. It would be interesting to extend these collections to different areas of Italy (and therefore to different substrates) to verify the presence of other species. For example, as regards Liguria, it could be interesting to collect material also in the *Posidonia oceanica* prairie to obtain data on the species related to this habitat.

Thanks to a more targeted and frequent collection, it will be possible to obtain a number of samples such as to be able to conduct quantitative studies at the population level, taking into account the influence of the various environmental parameters. Likewise, it will be possible to acquire more information on the phenology of individual species and, consequently, to provide a more detailed view of their life cycle.

Finally, these surveys will allow a qualitative and quantitative comparison of the unpublished data with the historical ones obtained from bibliographic surveys. This contribution highlights the importance of the proper and accurate preservation of labelled samples obtained from faunal and/or ecological research and it demonstrates how even old forgotten collections can turn out to be rich in new information.

Regarding the Antarctic pycnogonids key, we hope to provide further information and tools regarding the multitude of species found in those areas.

The specific criticalities of each genus emerged during the drafting of the key: two particularly problematic genera emerged (*Colossendeis* and *Nymphon*) that deserve a review and, given the specific Antarctic richness, it cannot be excluded that in the next years other species will be discovered and will be added to our checklist.

On the other hand, for some genera (*Austroraptus* and *Pantopipetta*) the identification keys corresponded with those already present in the literature and approved by experts, confirming our work.

It was possible to identify 281 specimens preserved at the MNA and a future goal could be to complete the identification of the collection, adopting classical morphological techniques combined with genetic confirmation. Given the difficulties encountered, it is inadvisable to base the identification of species only on DNA analysis.

More specifically, DNA proved to be fundamental for *Ammothea glacialis* and *Nymphon mendosum*, providing information that cast doubt on the morphological interpretation (only six samples out of 78). In all other cases the genetic correspondences were found to be useless for various reasons: they were found to be inconsistent (samples with very high compatibility with different species – *Colossendeis* sp. and *Pallenopsis* sp.) or misleading (the Antarctic species *A. australis* 100% compatible with an Indian species).

It is necessary to repeat how only one of these two techniques (classical morphology and DNA), used independently, can be misleading in the identification of the species.

Finally, we were positively surprised by the diffusion of pycnogonid images through non-specialists and enthusiasts: despite being a little-known category of organisms, some species are particularly suitable for photographing in the natural environment.

Despite this, as it was easy to guess, these species are not the easiest group of animals for citizen science studies: the difficulty in specific identification (even by experts) is a huge obstacle to carry out projects of this type.

Nevertheless, for some species data on the presence of identified families or genera could be useful for more general studies at community level and photographs can also improve the knowledge about the eco-ethology of this group.

One way to bring non-specialists closer to this group is to increase the relationship between institutions and scuba-diving centers and provide simple identification keys but, as discussed in this thesis, identification is not easy even for specialists.

13. References

Arango CP and Wheeler WC. (2007). Phylogeny of the sea spiders (Arthropoda; Pycnogonida) based on Direct Optimization of six loci and morphology. *Cladistics. International Journal of the Willi Hennig Society*. 16: 155-203.

Arnaud F. (1974). Nouveaux Pycnogonides des îles Saint Paul et Amsterdam (Océan Indien). *Téthys*, 5 (4): 779-790.

Arnaud F. (1987). Les pycnogonides (Chelicerata) de Méditerranée: distribution écologique, bathymetrique et biogéographique. *Mésogée* 47:37–58.

Arnaud F and Bamber RN. (1987). The biology of Pycnogonida. Advances in Marine Biology. 24: 1-96.

Arnaud F and Branch ML. (1991). The Pycnogonida of subantarctic Marion and Prince Edward Islands: Illustrated keys to the species. S. Afr. J. Antarct. Res. Vol. 21 (1): 65-71.

Bamber RN. (1997). Two pycnogonids (Arthropoda) from the Coral Sea region: a new *Callipallene* and a reassessment of the *Anoplodactylus cribellatus* complex. *Species Diversity*, 2(1), 43-50.

Bamber RN. (2011). The sea-spiders (Arthropoda: Pycnogonida) of Admiralty Bay, King George Island. *Polish Polar Research*, 27-38.

Bamber, RN, El Nagar A and Arango CP. (Eds.) (2022). Pycnobase: World Pycnogonida Database. Available: http://www.marinespecies.org/pycnobase. Accessed 15 January 2022. DOI: 10.14284/360

Barnard KH. (1954). South African Pycnogonida. Ann. S. Afr. Mus. 41 (3): 81-158.

Bartolino V and Chimenz C. (2010). Pycnogonida. Biol. Mar. Mediterr. 17 (suppl. 1): 394-397.

Bianchi CN. (2004). Proposta di suddivisione dei mari italiani in settori biogeografici. *Notiziario SIBM* 46: 57–59.

Bouvier EL. (1911). Les Pycnogonides du "Pourquoi Pas?". C.R. Séanc. Hebd. Acad. Sci., Paris, 152: 1136-1141.

Bouvier EL. (1916). Sur un Nymphonomorphe nouveau capturé par le Travailleur, dans les mers européenes au course de sa campagne de 1881. *Bulletin du Muséum National d'Histoire Naturelle, Paris*. (1) 22: 14-16.

Bouvier EL. (1923). Pycnogonides. Faune de France. 7: 1-69.

Brenneis G and Scholtz GA. (2020). A postlarval instar of *Phoxichilidium femoratum* (Pycnogonida, Phoxichilidiidae) with an exceptional malformation. *Journal of Morphology*. 1–3. https://doi.org/10.1002/jmor.21303

Calman WT. (1915). Pycnogonida. Brit. Antarct. (Terra Nova) Exped., 1910, Zool., 3 (1): 1-74.

Calman WT. (1923). Pycnogonida of the Indian museum. *Records of the Zoological Survey of India*, 25(3), 265-299.

Calman WT and Gordon I. (1933). A dodecapodous pycnogonid. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character*, 113(781), 107-115.

Cano-Sánchez E and López-González PJ. (2014). New findings and a new species of the genus *Ammothea* (Pycnogonida, Ammotheidae), with an updated identification key to all Antarctic and sub-Antarctic species. *Helgoland Marine Research*, 68(1), 155-168.

Cano-Sánchez E and López-González PJ. (2019). Two new species and new findings in the genus *Pallenopsis* (Pycnogonida: Pallenopsidae) with an updated identification key to Antarctic and sub-Antarctic species. *Zootaxa*, 4585(3).

Centre for Biodiversity Genomics, University of Guelph (2021). The Global Taxonomy Initiative 2020: A Step-by-Step Guide for DNA Barcoding. *Technical Series No. 94. Secretariat of the Convention on Biological Diversity, Montreal*, 66 pages [CBG in text].

Child CA. (1979). Shallow-water Pycnogonida of the Isthmus of Panamá and the coasts of Middle America. *Smiths. Contr. Zool., Washington.* 293: 1-86.

Child CA. (1982). Deep-sea Pycnogonida from the North and South Atlantic Basins. *Smiths. Contr. Zool.*, 349: 1-54.

Child CA. (1987). New and little known Pycnogonida from Antarctic and Subantarctic Waters. *Proc. biol. Soc. Wash.*, 100 (4): 902-916.

Child CA. (1992a). Shallow-water Pycnognida of the Gulf of Mexico. *Memoirs if the Hourglass Cruisses*. 9: 1-86.

Child CA. (1992b). Pycnognida of the Southeast Pacific Biological Oceanographic Project (SEPBOP). *Smithson. Contr. Zool.* 526.

Child CA. (1995a). Antarctic and Subantarctic Pycnogonida: Ammotheidae and Austrodecidae. *Antarctic Research Series* (63) 99 pp.

Child CA. (1995b). Antarctic and Subantarctic Pycnogonida: Nymphonidae, Colossendeidae, Rhynchothoraxidae, Pycnogonidae, Endeididae, and Callipallenidae. *Antarctic Research Series* (69) 165 pp.

Chimenz Gusso C, Brignoli PM and Basciano G. (1979). Pantopodi del porto di Civitavecchia e Dintorni (Italia Centrale). *Cah. Biol. Mar.* 20: 471-497.

Chimenz C and Cottarelli V. (1986). Soft bottom Pycnogonida from the Gulf of Salerno (Italy). *Oebalia* 13(n.s.):137–146.

Chimenz C, Cottarelli V and Tosti M. (1991). Researches on the Mediterranean Pycnogonida. 1. *Anoplodactylus compositus* n. sp. (*Arthropoda, Chelicerata*). *Fragm. Ent.* 23 (1): 7-13.

Chimenz C, Tosti M and Cottarelli V. (1993). Taxonomical and ecological observations on Pycnogonida from Apulian coasts (southern Italy). *Bolletino di zoologia*. 60:3, 339-347 DOI: 10.1080/11250009309355834.

Chimenz Gusso C and Lattanzi L. (2003). Mediterranean Pycnogonida: faunistic, taxonomical and zoogeographical considerations. *Biogeographia* 24: 251–262.

Chimenz Gusso C, Fresi E, Cinelli F, Mazzella L, Pansini M and Pronzato R. (1978). Evoluzione delle biocenosi bentoniche di substrato duro contro un gradiente di luce in una grotta marina superficiale. II. Pantopodi. *Memorie di Biologia Marina e Oceanografia* 8: 91–103.

Chimenz Gusso C. (2000). Picnogonidi delle coste italiane: Quadro delle conoscenze (Pycnogonida). *Memorie della Società Entomologica Italiana* 78:541–574.

Chimenz Gusso C and Gravina MF. (2001). Faunistic and biological traits of some Antarctic Pycnogonida. *Italian Journal of Zoology*, 68(4), 335-344.

Clark WC. (1971a). Pycnogonida of the Snares islands. New Zealand Journal of Marine and Freshwater Research. 5:2, 329-341. DOI: 10.1080/00288330.1971.9515386

Clark WC. (1971b). Pycnogonida of the Antipodes Islands. *New Zealand Journal of Marine and Freshwater Research*, 5:3-4, 427-452, DOI: 10.1080/00288330.1971.9515396

Clark WC. (1977). The genus *Tanystylum* Miers, 1879 (Pycnogonida) in the Southern oceans. *Journal of the Royal Society of New Zealand*, 7:3, 313-338, DOI: 10.1080/03036758.1977.10419431

Colasanto E and Galli L. (2021a). Pycnogonida. In: Bologna M.A., Zapparoli M., Oliverio M., Minelli A., Bonato L., Cianferoni F., Stoch F. (eds.), *Checklist of the Italian Fauna. Version 1.0.* Last update: 2021-05-31. Available at: https://www.lifewatchitaly.eu/en/initiatives/checklist-fauna-italia-en/checklist/

Colasanto E and Galli L. (2021b). The Pycnogonids (Arthropoda, Pycnogonida) of Torrevaldaliga (Tyrrhenian Sea), Italy, with data on *Endeis biseriata*, new record for the Mediterranean Sea. *The European Zoological Journal*, 88:1, 622-631, DOI: 10.1080/24750263.2021.1910741

Dohrn A. (1881). Die Pantopoden des Golfes von Neapel. Fauna und Flora des Golfes von Neapel 3:1-252.

Earp HS and Liconti A. (2019). Science for the future: the use of citizen science in marine research and Conservation. *In YOUMARES 9 - The oceans: our research, our future*. (ed by S. Jungblut, V. Liebich & M. Bode-Dalby), 1–20. Springer.

Eights J. (1835). Description of a new animal belonging to the Arachnides of Latreille; discovered in the sea along the shores of the New South Shetland Islands. *Boston J. Nat. Hist.*, 1 (2): 203-206.

Faraggiana R. (1940). Pantopodi del Mar Ligure. *Bollettino dei Musei di Zoologia e Anatomia comparata di Torino* (ser. 3) 48:145–158.

Fry WG and Hedgpeth JW. (1969). Pycnogonida 1. Colossendeidae, Pycnogonidae, Endeidae and Ammotheidae (Fauna of the Ross Sea, 7). *Mem. NZ Ocean. Inst.* 49:1–139.

Galli L, Colasanto E, Betti F and Capurro M. (2019). Pycnogonids (Arthropoda: Pycnogonida) of Portofino, Ligurian Sea (North-Western Mediterranean Sea), *The European Zoological Journal*, 86:1, 241-248. https://doi.org/10.1080/24750263.2019.1637951).

Gordon I. (1932). XI.—Re-description of some type-specimens of Pycnogonida of the genus *Nymphon*. *Annals and Magazine of Natural History*, 9(49), 97-120.

Hall HVM. (1912). Studies in Pycnogonida, I. First Annual Report of Laguna marine Laboratory. 1: 91-99.

Hodgson TV. (1907). Pycnogonida. National Antarctic Expedition 1901-1904. Rep. Natn. Antarct. Exped. 1901-1904, Nat. Hist., 3: 1-72, 10 pls.

Hodgson TV. (1914). Preliminary report on the Pycnogonida of the German Southpolar Expedition, 1901-1903. *Zoologische Anzeiger*, 45(4), 158-165.

Hoek PPC. (1898). On four pycnogonids dredged during the cruise of the Challenger. *Tijdschrift der Nederlansche Dierkundige Vereeniging*, 5, 190-301.

King PE. (1973). Pycnogonids. *Hutchinson & Co. (Publishers)*, London, pp.144.

King PE. (1986). Sea Spiders: A revised key to the adults of littoral Pycnogonida in the British Isles. *Field Stud.* 6 (3): 493-516.

Krapp F. (1973). Pycnogonida from Pantelleria and Catania, Sicily. Beaufortia. 21 (277): 55-74.

Krapp F. (1975). Bathyale und zirkalittorale Pantopoden (Pycnogonida) aus dem Adriatischen und Ligurischen Meer, mit *Callipallene acribica* n.sp. *Bonn. zool. Beitr.* 26 (1-3): 280-290.

Krapp F and Sconfietti R. (1983). *Ammothea hilgendorfi* (Böhm, 1879), an adventitious pycnogonid new for the Mediterranean Sea. *Marine Ecology*, 4(2), 123-132.

Krøyer H. (1840). Om Pycnogonidernes Forvandlinger. Naturhistorik Tidsskrift, Kjobenhavn 1 (3), 299-306.

Lehmann T, Heß M and Melzer RR. (2014). Common littoral pycnogonids of the Mediterranean Sea. *Zoosyst. Evol.* 90 (2), 163–224. DOI: 10.3897/zse.90.7520

Lehmann T, Heß M and Melzer RR. (2017). Sense organs in Pycnogonida: A review. *Acta Zool.* 2018; 99:211–230. https://doi.org/10.1111/azo.12207

Lehmann T, Spelda J, Melzer R and Buršić M. (2021). Pycnogonida (Arthropoda) from Northern Adriatic *Corallina officinalis* Linnaeus, 1758 belts. *Mediterranean Marine Science*, 22(1), 102-107. https://doi.org/10.12681/mms.23223

Lucena RA, Fatemi Y and Christoffersen ML. (2018). Checklist of sea spiders (Arthropoda: Pycnogonida) from the Persian Gulf and the Gulf of Oman with new record of *Endeis biseriata* (Böhm, 1879) for the region. *Turkish Journal of Zoology* 42.6: 709-714.

Marcus E. (1940). Os Pantopoda brasileiros e os demais sul-americanos. *Boletins da Faculdade de Philosophia, Sciencias e Letras, Universidade de São Paulo. Zoologia*, 4(4), 3-179.

Merton H. (1906). Eine auf Tethys leporina parasitisch lebende Pantopodenlarve (*Nymphon parasiticum* n. sp.). *Mitt Zool. Station Neapel*. 18: 136–141.

Miers EJ. (1875). Descriptions of new species of Crustacea collected at Kerguelen's Island by the Rev. A.E. Eaton. *Ann. Mag. Nat. Hist.*, (4) 16: 73-76.

Minelli A, Ruffo S and La Posta S. (1993-1995). Checklist delle Specie della Fauna d'Italia. *Edizioni Calderini, Bologna*: vol. 1-24 (fasc. 1-110).

Montoya Bravo MF, Meltzer L, Meyer R and Melzer RM. (2006). Pycnogonids under infralitoral stones at Cape Savudrija, Northern Adriatic Sea (Pantopoda, Ammotheidae). *Spixiana* 29.1: 87.)

Müller HG. (1993). World Catalogue and bibliography of the recent Pycnogonida. H.G. Müller. *Wetzlar*. 388 pp.

Munilla T. (1988). A collection of pycnogonids from Namibia/South West Africa. *Monogr. Zool. Marino*, 3: 177-204.

Munilla T and Soler-Membrives A. (2009). Check-list of the pycnogonids from Antarctic and sub-Antarctic waters: zoogeographic implications. *Antarctic Science* 21 (2), 99–111 (2009) & *Antarctic Science Ltd* 2009. doi:10.1017/S095410200800151X

Munilla T and Soler-Membrives A. (2014). Pycnogonida. In: *Fauna Iberica*. Vol. 39. Ramos, M.A. et al. (eds). *Museo Nacional de Ciencias Naturales*. CSIC. Madrid. pp 296.

Munilla T and Soler-Membrives A. (2015). Pycnogonida from the Bellingshausen and Amundsen seas: taxonomy and biodiversity. *Polar Biology*, 38(3), 413-430.

Occhipinti-Ambrogi A, Marchini A, Cantone G, Castelli A, Chimenz C, Cormaci M, Froglia C, Furnari G, Gambi MC, Giaccone G, Giangrande A, Gravili C, Mastrototaro F, Mazziotti C, Orsi-Relini L and Piraino S. (2011). Alien species along the Italian coasts: an overview. *Biological invasions* 13.1: 215-237.

Piscitelli G and Barone G. (2000). Prima nota sui Picnogonidi delle Isole Tremiti. *Biologia Marina Mediterranea* 7:718–722.

Pushkin AF. (1975). New species of Callipallenidae (Pantopoda) from the Subantarctic. *Zoologicheskii Zhurnal*, 54(9), 1402-1406. [in Russian, English summary]

Pushkin AF. (1976). New species of Endeidae (Pantopoda) from Subantarctic. *Zoologicheskii Zhurnal*, 55(6), 923-925. [in Russian, English summary]

Pushkin AF. (1977). New species of Austrodecidae from Antarctic. *Explorations of the Fauna of the Seas*, 21, 54-59. [in Russian, English summary]

Pushkin AF. (1993). The pycnogonids fauna of the South Ocean: biological results of the soviet Antarctic expeditions. *Russian Academy of Sciences: Exploration of the Fauna of the Seas St Petersburg/Portorosa*. [in Russian]

Pushkin AF. (2011). Redescription of three species of Pycnogonida of the genus *Austropallene* from the Southern Ocean with the key for identifications of all Antarctic species of the genus. *ПРОБЛЕМЫ АРКТИКИ И АНТАРКТИКИ*, 4(90): 63-75. [in Russian, English summary]

Relini G. (2010). Checklist della Flora e della Fauna dei mari italiani – Checklist of the Flora and Fauna of Italian seas. Parte II. *Biologia Marina Mediterranea*. 17 (Suppl. 1): V-IX.

Richter S. (1982). Zur Ultrastruktur der seitlichen Sinnesorgane am Augenhügel von *Anoplodactylus pygmaeus* (Pycnogonida). *Helgoland Marine Research*, 35, 465–478.

Scholtz G and Brenneis G. (2016). The pattern of a specimen of *Pycnogonum litorale* (Arthropoda, Pycnogonida) with a supernumerary leg can be explained with the "boundary model" of appendage formation. *The Science of Nature* 103:13 (9 pp). DOI: 10.1007/s00114-016-1333-8.

Silvertown J. (2009). A new dawn for citizen science. *Trends in Ecology and Evolution*, 24, 467–471. DOI: 10.101 6/j.tree.2009.03.017

Soler-Membrives A, Arango CP, Cuadrado M and Munilla T. (2011). Feeding biology of carnivore and detritivore Mediterranean Pycnogonids. *Journal of the Marine Biological Association of the United Kingdom*. 93(3): 635-643.

Soler-Membrives A and Munilla T. (2015). PYCNOIB: Biodiversity and Biogeography of Iberian Pycnogonids. *PLoS ONE* 10(3): e0120818. doi:10.1371/journal.pone.0120818

Sree VJ, Sreepada RA and Parulekar AH. (1993). An unusual giant pycnogonid (Pycnogonida—Colossendeidae) *Decolopoda qasimi* sp. nov. from Antarctic waters. *Current Science*, 179-181.

Staples DA. (2014). A revision of the callipallenid genus *Pseudopallene* Wilson, 1878 (Pycnogonida, Callipallenidae). *Zootaxa*, 3765(4), 339-359.

Staples DA. (2015). A reassessment of the pycnogonid genus *Cheilopallene* (Arthropoda, Callipallenidae) with description of a new species from Papua New Guinea. *Zootaxa*, 3995(1), 51-57.

Stock JH. (1953). Re-description of some of Helfer's pycnogonid type-specimens. *Beaufortia*, 4 (35): 33-45.

Stock JH. (1968). Pycnogonida collected by the Galathea and Anton Bruun in the Indian and Pacific Oceans. *Vidensk Medd Dansk Naturh Foren* 137: 7-65.

Stock JH. (1963). South African deep-sea Pycnogonida, with descriptions of five new species. *Ann. S. Afr. Mus.*, 46 (12): 321-340.

Stock JH. (1970). A new species of *Endeis* and other pycnogonid records from the Gulf of Aqaba. *Bull. Zool. Mus.* 2: 1-4.

Stock JH. (1991). Deep-water Pycnogonida from the surroundings of New Caledonia. *In: A. CROSNIER* (ed.), Résultats des Campagnes MUSORSTOM, Volume 8. Mém. Mus. natn. Hist, not., (A), 151: 125-212.

Švara V and Melzer RR. (2016). A new species of *Austrodecus* Hodgson, 1907 (Pycnogonida, Austrodecidae) from the Chilean fjords. *Helgoland Marine Research*, 70(1), 1-7.

Turpaeva EP. (1974). The pycnogonida of the Scotia Sea and surrounding waters. *Trudy Instituta Okeanologii PP Shirshov, Akademiya Nauka USSR*, 98, 277-305. [in Russian, English summary]

Turpaeva EP. (1993). Picnogonida collected during "Dmitri Mendeleev" 43rd cruise to the South Atlantic basins. *Transactions of PP Shirshov Institute of Oceanology*, 127, 159-175. [in Russian, English summary]

Turpaeva EP and Rajsky AC. (2013). Sea Spiders of the Genus *Colossendeis* (Colossendeidae, Pycnogonida) from the Weddell Sea and Adjacent Waters. *Journal of Siberian Federal University*. *Biology* 2 (2013 6) 130-149. [in Russian, English summary]

Waloszek D and Dunlop JA. (2002). A larval sea spider (Arthropoda: Pycnogonida) from the Upper Cambrian 'Orsten' of Sweden, and the phylogenetic position of pycnogonids. *Palaeontology*, 45(3), 421-446.

Weis A, Friedrich S and Melzer RR. (2011). Antarctic Pycnogonida housed at the Bavarian State collection of zoology. *Zoosystematics and Evolution*, 87(2), 297-317.

WoRMS Editorial Board. (2022). World Register of Marine Species. Available from https://www.marinespecies.org at VLIZ. Accessed 2022-01-04. doi:10.14284/170