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DISTRIBUTION AND BIOLOGY OF LIGHTIELLA MAGDALENINA (CRUSTACEA, CEPHALOCARIDA)

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.....AI MIEI NONNI !!!!!

L'Homme et la Mer

Homme libre, toujours tu chériras la mer!
La mer est ton miroir, tu contemples ton âme
Dans le déroulement infini de sa lame
Et ton esprit n'est pas un gouffre moins amer.

Tu te plais a plonger au sein de ton image;
Tu l'embrasses des yeux et des bras, et ton coeur
Se distrait quelquefois de sa propre rumeur
Au bruit de cette plainte indomptable et sauvage.

Vous êtes tous les deux ténébreux et discrets;
Homme, nul n'a sondé le fond de tes abîmes;
O mer, nul ne connaît tes richesses intimes,
Tant vous êtes jaloux de garder vos secrets!

Et cependant voilà des siècles innombrables
Que vous vous combattez sans pitié ni remords,
Tellement vous aimez le carnage et la mort,
O lutteurs éternels, O frères implacables!

Charles Baudelaire

1 – ABSTRACT

Lightiella magdalenina is the more recently cephalocarid species described (La Maddalena Archipelago, Sardinia). This first important finding in the Mediterranean sea fills a gap in the distribution of the genus and of the entire class. The aim of the present study is to report some information about distribution and biology of this cephalocarid species. *L. magdalenina*, like most cephalocarid species, shows a pronounced anamorphic mode of development with a gradual and sequential addition of segment and limbs throughout more of 17 stages of development. It is currently known from a single locality where 55 specimens have been found during more than nine years of sampling. The *Type locality* is characterised by muddy sand bottom, very rich in organic matter with leaf fragments of *Posidonia oceanica*; grain size analysis, has underlined that sand is a very fine sand with an organic component of 95%. Zoobenthos was composed of 11 different taxa; Cletopsyllidae and Normanellidae Copepoda are unknown for Italy. A new species of family Cletopsyllidae, *Isocletopsyllus* sp. nov. has been identified. Up to present, only one species *Hutchinsoniella macracantha*, have been studied at molecular level. We report the partial sequences of two mitochondrial genes of relevant importance for phylogenetic analysis (cytochrome c oxidase I and cytochrome b) from *Lightiella magdalenina.* A reduced median network analysis clarified the genetic relationships between the two cephalocarid species.

2 - INTRODUCTION

2.1 - CEPHALOCARIDA

Cephalocarida are an ancient lineage of small benthic crustaceans distributed from the intertidal to approximately 1550 m deep. The first species, *Hutchinsoniella macracantha*, was discovered by Howard L. Sanders (1955) from the muddy bottom of Long Island Sound, N. Y. (Unites States). Subsequently studies, in various parts of the world, have contributed to described other ten species belonging to four genera, i.e. *Lightiella, Sandersiella, Hampsonellus* and *Chiltoniella*.

These species include *Lightiella serendipita* Jones, 1961, from San Francisco Bay; *L. incisa* Gooding, 1963, from Barbados and in the other localities in the Caribbean Sea; *L. monniotae* Cals & Delamare Deboutteville, 1970, from New Caledonia; *L. floridana* McLaughlin, 1976 from the west coast of Florida; *L. magdalenina* Carcupino, Floris, Addis, Castelli, Curini-Galletti, 2006 from La Maddalena Archipelago (Sardinia, Italy); *Sandersiella acuminata* Shiino, 1965 from Japan; *S. calmani* Hessler & Sanders, 1973 from the coast of Perù; *S. bathyalis* Hessler & Sanders, 1973, from off Walvis Bay, southwest Africa; *Hampsonellus brasiliensis* Wakabara & Mizoguchi, 1976 Brazil and *Chiltoniella elongata*, Knox & Fenwick 1977, from New Zealand.

At present, the genus *Lightiella* Jones, 1961, is the most speciose of the cephalocarid genera and most records are known for the Gulf of Mexico and Caribbean area (Gooding, 1963; Sanders & Hessler, 1964; McLaughlin, 1976; Saloman, 1978; Stoner, 1981; Heard & Goeke, 1982; De Troch *et al*., 2000;

Schiemer & Ott, 2001; Martin *et al*., 2002). Only four additional records of this genus are known for San Francisco Bay, California (Jones, 1961); Biscayne Bay, Florida (Hessler & Sanders, 1973); Saint Vincent Bay, New Caledonia (Cals & Delamare Deboutteville, 1970) and La Maddalena Archipelago, Italy (Carcupino *et al.*, 2006). *L. magdalenina* is the most recently cephalocarid species described and its first finding in the Mediterranean sea fills a gap in the distribution of the genus and of the entire class. No species had been previously reported from Europe.

2.1.1 EXTERNAL ANATOMY

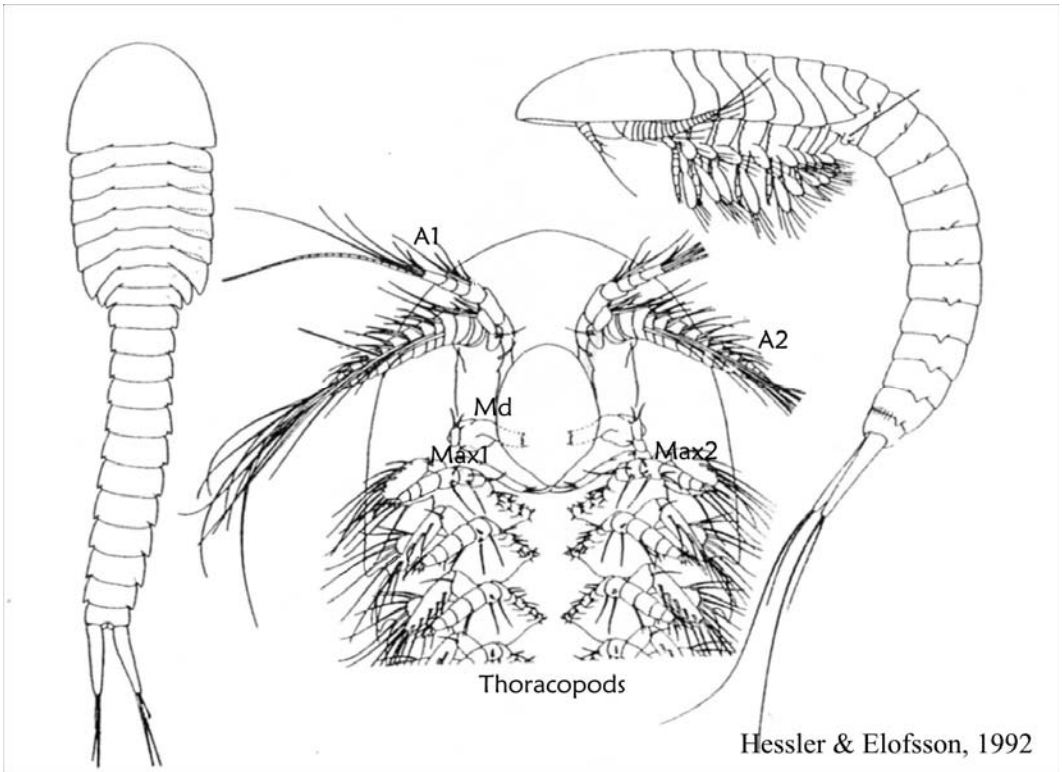

Fig. 1: Adult specimens of Hutchinsoniella macracantha, Sanders, 1955. A1: *first antenna;* ***A2:*** *second antenna;* ***Md:*** *mandible;* ***Max1:*** *first maxilla;* ***Max2:*** *second maxilla.*

The Cephalocarids are small crustaceans about 4 mm in adult length. Their body comprises a cephalon and a trunk of 20 articulated segments (9 thoracic and 11 abdominal segments including telson) (Fig. 1). The horseshoe-shaped cephalon is short and about as broad as long; no eyes have been observed both in the adult

that in the larval stages. First eight trunk segments with rounded lateral pleura and carrying each a pair of thoracopods; 9-19 trunk segments without pleura and thoracopods bearing lateral spinose processes. Penultimate abdominal segment bears a ventral comb except that in the genus *Lightiella.* The anal segment (telson) bearing two caudal rami characterized by a ventral comb of strong teeth and with two well developed dorsal spines with rounded edge. Caudal rami equalling the width of telson and bearing four terminal setae (Sanders, 1955; 1963; Hessler & Elofsson, 1992; Carcupino *et al.,* 2006). Labrum is large, broadly rounded anteriorly, covering a well-developed mouth oral cavity leading to an esophagus visible anteriorly. The first antenna, six-segmented, is unbranched and bearing a apical distal sensory setae, including the aesthetasc. The second antenna is large and biramous; exopod is composed by nineteen segments while the endopod is short and is composed by only two segment that bear few setae. The mandible lacks a palp; its gnathal lobe extends into the oral atrium, just posterior to the mouth. The first maxilla has a three-segmented endopod, a paddle-like exopod with distal setae and a long gnathal lobe which brings food forward to the mandible. The second maxilla and first five thoracopods, biramous, with about the same length and morphology. A large, flattened protopod (coxa plus basis) bears medially a series of setose endites that serve to pass food forward along the midline to the mouth. The protopod bearing three rami: a six or five-segmented endopod, a two segmented paddle-like exopod and a unsegmented paddle-like epipod. Both exopod and epipod bearing distal setae (Sanders, 1955; 1963; Hessler & Elofsson, 1992; Carcupino *et al.,* 2006).

Thoracopods 6–7 are slightly smaller than the previous legs. Thoracopod 6 is very similar to the others but which bears a genital pore on the posterior surface (Hessler *et al.,* 1970; 1995; Hessler & Wakabara, 2000; Carcupino *et al.,* 2006), Only *Chiltoniella* seems to bear the genital pore on thoracopod 9 (Knox & Fenwick, 1977).

Thoracopod 8, lost in the genus *Lightiella*, lacks an endopod. Finally, the ninth limb is a rudimentary to which the brooded egg is attached (Sanders, 1955; 1963; Hessler & Elofsson, 1992).

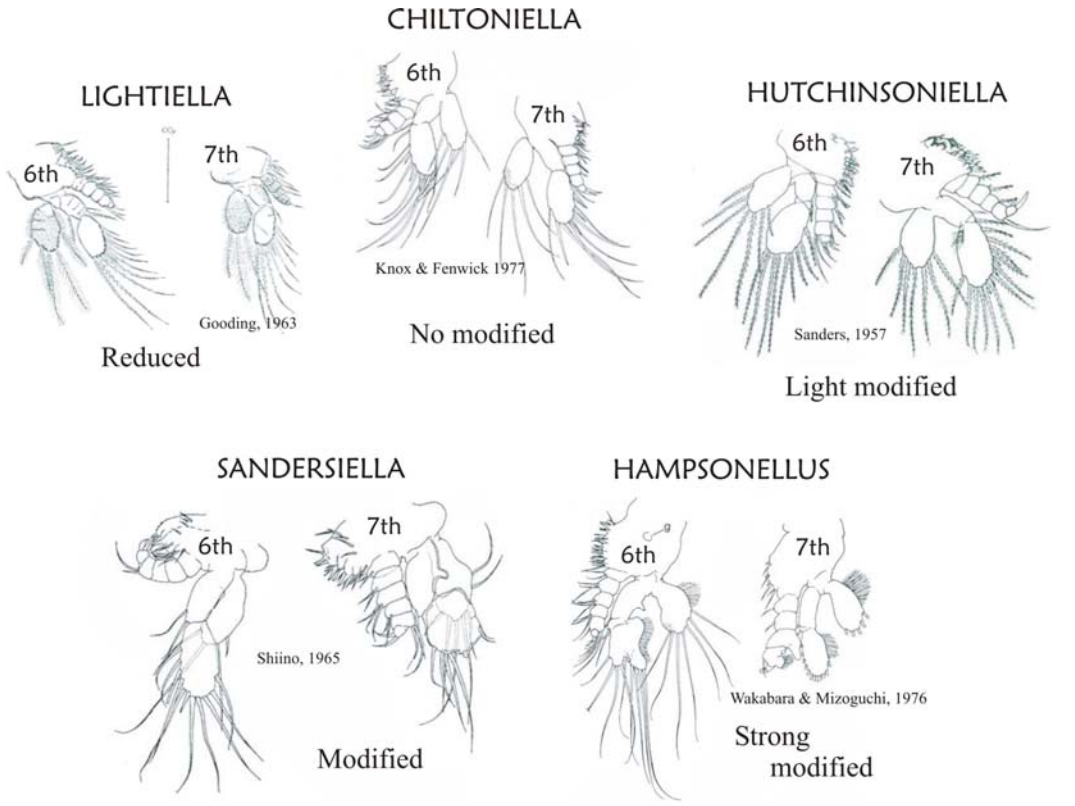

Fig. 2: *Sixth and seventh modified thoracopods of cephalocaris species.*

The most important diagnostic characters between cephalocarid genera are the peculiar morphological differences of thoracopods 6 and 7 (Fig. 2). In the genus *Chiltoniella*, thoracopods 6 and 7 are considered to be unmodified, or at least less modified than in the other genera (Knox & Fenwick, 1977). Thoracopod 6 has an endopod with two, instead of three, terminal, claws; segment 2 of the exopod bears several rows of minute denticles on the latero-proximal quarter. The endopod of thoracopod 7 is reduced in length and, similar to the previous one, has two terminal claws.

In *Hutchinsoniella*, the only claw of thoracopod 7 is bluntly rounded and some of the flexor muscles of the epipod of thoracopod 6 are larger than in the other thoracopods (Hessler, 1964).

In *Sandersiella* (Shiino, 1965; Hessler & Sanders, 1973) and *Hampsonellus* (Hessler & Wakabara, 2000) the distal segment of the exopod of the thoracopod 6 is highly modified in the same way. It is divided into two lobes, each provided with its own set of setae. The three *Sandersiella* species and *Hampsonellus brasiliensis*, however, are easily discriminated on the basis of specific details.

Moreover, thoracopod 7 is significantly modified only in *Hampsonellus brasiliensis*.

Finally, *Lightiella* differs from all other genera in the reduction of segment 8, which also lacks thoracopods and pleura. In this genus, thoracopods 6 and 7 are smaller than, but very similar to, the other limbs (Jones, 1961; Gooding, 1963; Cals & Delamare Deboutteville, 1970; McLaughlin, 1976; Carcupino *et al.*, 2006).

2.1.2 INTERNAL ANATOMY

Cephalocarida is a poorly known group, with most of the morphological and molecular data regarding only one species, *Hutchinsoniella macracantha* Sanders, 1955. Extensive studies have been made of skeletomusculature (Hessler, 1964; Read *et al*., 1994), the central nervous system (Helofsson 1992; Helofsson & Hessler, 1990; 1992), the excretory system (Hessler & Helofsson, 1991), antennary sensilla (Helofsson & Hessler, 1990; 1991), the digestive system (Elofsson *et al*., 1992), body sensilla (Helofsson & Hessler, 1994), postcephalic excretory organs (Hessler & Helofsson, 1995) and reproductive system (Hessler *et al*., 1970; 1995).

2.1.3 REPRODUCTION

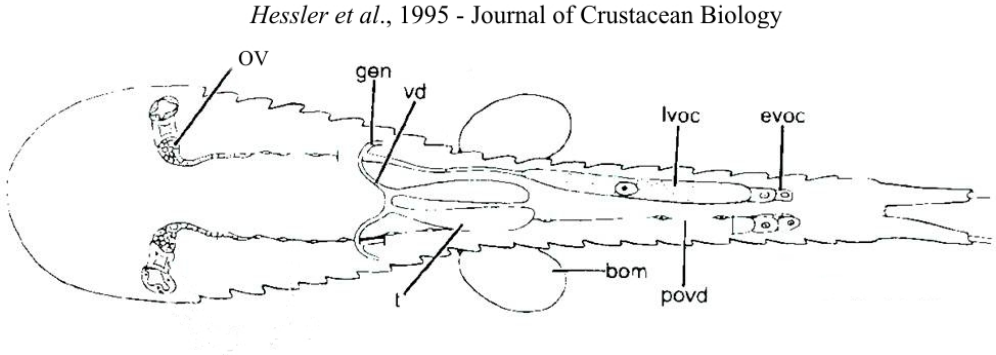

Fig. 3: Reproductive system of *H. macracantha*. *OV* ovary; *gen* common genital duct; *vd* vas deferent; *t* testis; *bom* brooding ovum; *povd* posteriorly extending oviduct; *lvoc* oocyte in late stage of vitellogenesis; *evoc* oocyte in earty stage of vitellogenesis.

Cephalocarids present very specialization in their reproductive biology (they are simultaneous hermaphrodites with probable self-fertilization) (Hessler *et al.*, 1970; 1995).

H. macracantha is a true hermaphrodite with entirely separate functional ovaries and testes. Ovaries are located next to the second maxilla while oviducts run posteriorly through the thorax, continue of the abdomen, but then loops forward in the eighteenth trunk segment; it continue forward to the sixth thoracic segment, where it joins the vas deferens (Fig. 3). Testis, sausage-shaped, are located in the seventh and twelfth trunk segment. After joining, the oviduct and vas deferens descend into six thoracopod as a compact unit whose gonopore is located halfway down the posterior face of protopod (Hessler *et al.*, 1970; 1995) (Fig. 3). Among the free-living crustaceans are very few cases of hermaphroditism in which both sexes function simultaneously.

Cephalocarids have never been observed mating. It has been assumed that the modified endopod of the seventh thoracopod is a clasping organ; yet the separation of the genital pore from the ninth postcephalic segment, which bears the brooded egg, suggests that this modification may actually sever in directing the egg as it is laid to its place of attachment. Cephalocarid spermis non-mobile, which indicates that individuals participating in cross-fertilization must come into intimate contact. There is no evidence of the formation of spermatophores (Brown & Metz, 1967; Hessler *et al*., 1970).

H. macracantha is ovigerous from June through September and carry two ovisacs each attached by a short stalk to the limb rudiments of the genital segment while a single egg sac was reported in the genus *Lightiella* by Gooding (1963), De Troch *et al.* (2000) and Martin *et al.* (2002). Each ovisac contains a single embryo. Observations in the laboratory indicate that a female can bear only three broods or six embryos during the breeding season (Sanders, 1957; Sanders & Hessler, 1964).

2.1.4 LARVAL DEVELOPMENT

Larval development of cephalocarids shows a pronounced anamorphic mode of growth characterized by a high number of postembryonic stages (metanaupliars

and juveniles), with a gradual and sequential addition of segment and limbs. The transformation from the metanauplair stage to the juvenile one is marked by the complete lost of the enditic process of the second antenna. A free-living metanauplius with a three-segmented trunk and further 18 developmental stages have been reported for *H. macracantha* (Sanders, 1963). A 4-segmented late embryo, which probably represents the first free-living larva and another 12 stages have been reported for *L. incisa* (Sanders & Hessler, 1964), whereas only 3 late larval stages are known for *L. serendipita* (Jones, 1961). Larval development appears characterized by a first phase, during which two segments are added at each moult, followed by a the second phase, in which only one segment is added. Indeed, the authors hypothesized that, in all the above-mentioned species, the number of observed stages very likely representing an incomplete developmental series and which are probably much more numerous larval stages than actually known (Sanders, 1963; Sanders & Hessler, 1964).

2.1.5 SYSTEMATIC

Class	**CEPHALOCARIDA**
Order	**BRACHYPODA**
Family	**HUTCHINSONIELLIDAE**
Genus	
Hutchisoniella	*macracantha* (Sander, 1955) USA.
Lightiella	*serendipita* (Jones, 1961) California.
	incisa (Gooding, 1963) West Indies.
	monniotae (Cals & Deboutteville, 1970) New Caledonia.
	floridana (McLaughin, 1976) Florida.
	magdalenina (Carcupino *et al.*, 2006) Sardinia, Italy.
Sandersiella	*acuminata* (M. Shiino, 1965) Japan.
	calmani (Hesser, & Sander, 1973) Perù.
	bathyalis (Hesser, & Sander, 1973) S.W. Africa.
Hampsonellus	*brasiliensis* (Wakabara & Mizoguchi, 1976) Brazil.
Chiltoniella	*elongata* (Knok & Fenwick, 1977) New Zealand.

2.1.6 DISTRIBUTION AND ECOLOGY

At present, Cephalocarida is one of the least speciose of the crustacean classes. Only eleven species, belonging to five genera, have hitherto been reported from North and South America (Sanders, 1955; Jones, 1961; Gooding, 1963; Sanders & Hessler, 1964; Wakabara, 1970; Hessler & Sanders, 1973; McLaughlin, 1976; Saloman, 1978; Stoner, 1981; Heard & Goeke, 1982; De Troch, *et al*., 2000; Hessler & Wakabara, 2000; Schiemer & Ott, 2001; Martin *et al*., 2002), Africa (Hessler & Sanders, 1973), Japan (Shiino, 1965), New Zealand (Knox & Fenwick, 1977), New Caledonia (Cals & Delamare Deboutteville, 1970) and Europe (Carcupino *et al.,* 2006).

Ecological information about microhabitat and associate benthic fauna on cephalocarids is very sparse. Only three species (*H. macracantha, L. incisa* and *L. floridana*) have been reported in abundant numbers while the other species are found from a very small number of specimens obtained only once (De Troch *et al.*, 2000). Cephalocarids occur in substrates that are bare or covered with seagrass, in intertidal as well as in deep sea habitats, and over a wide range of temperature. The one common factor in these habitats has been the flocculent nature and high organic content of the surface sediments (Gooding, 1963; Sanders, 1963; Sanders & Hessler 1964; McLaughing, 1976; Saloman, 1978; De Troch *et al.,* 2000; Carcupino *et al.,* 2006). A lot of cephalocarid specimens are reported for sediment with seagrass beds, it's act as a "sediments trap" that allows fine particles and low density matter to settle out of suspension and form a flocculent-like sediment that cephalocarids could feed in any other substratum (Sanders & Hessler, 1964; De Troch *et al.,* 2000). *L. incisa* live in the anoxic deeper sediment, oxygenated by bioturbation (Polychaeta), rather than to live in the oxygenated top layers and what possible is that the itself animal provides an oxygenated tube to live in (De Troch *et al.,* 2000; Schiemer & Ott, 2001).

The main sediment characteristics are similar for the different seagrass species; the preferred median grain size was between 230 and 260 μm. The maximum density of *L. incisa* was found lowest NO_3–N concentration (< 60 μg/l NO_3 –N) and an intermediate NO_2^-–N concentration (< 15 μg/l NO_2 –N); lowest concentration NH_4^+-N (<10 mg/l NH_4^+-N). The same was true for silicate (SiO_2),

with highest *L. incisa* numbers at the lower concentration (between 600 and 1000 µg/l). The opposite trend was found for phosphate: maximal densities corresponded to the highest concentration $PO_4^{3-}P$ (between 145 and 160 µg/l). The data on total organic matter (% TOM) showed only minor variation with depth with an intermediate value (4-4.5 % TOM) (De Troch *et al.*, 2000).

✶ WORLD'S DISTRIBUTION OF THE CEPHALOCARIDS

:

Hutchinsoniella macracantha

- 8 specimens from the soft ooze in Long Island Sound (41°13.6'N 72°50.6'W) at 10-29 meters depth (Sanders, 1955).
- 1000 specimens from soft flocculent sediment in Buzzards Bay, Massachusetts (41°28.5'N 70°53.0'W) at 14-21 meters depth (Hessler & Sanders, 1964).
- Some specimens in the soft muds covered by *Zostera* in Hadley Harbor (41°30.5'N 70°42.4'W) at 1-3 meters depth (Hessler & Sanders, 1973).
- 4 specimens in organic-rich muddy in Southern New England (39°58.4'N 70°40.3'W) at 300 meters depth (Hessler & Sanders, 1964).

- 1 specimen from a muddy ooze bottom Southern New England (40°32.7'N 70°51.2'W) at 69 meters depth (Hessler & Sanders, 1973).
- 1 specimen from a muddy sand bottom in North Carolina (34°33.0'N 76°33.0'W) at 10 meters depth (Hessler & Sanders, 1973).
- Some specimens from a muddy sand bottom in Chesapeake Bay (37°35.3'N 75°37.5W) at 6 meters depth (Seth & Van Engel, 1969).
- 1 specimen from a silt-clay botton in Angra dos Reis, Brazil (23°03.6'S 44°13.6'W) at 50 meters depth (Wakabara, 1970).

Lightiella serendipita

- 7 specimens in muddy sand in San Francisco bay, California (37°54.2'N 122°23.1'W) at almost two meters depth (Jones, 1961).

Lightiella incisa

- 2 specimens from an intertidal *Thalassia* bed of Barbados (13°04.5'N 59°36.5'W) (Gooding, 1963).
- 2 specimens from an *Thalassia* bed on the La Parguera, Puerto Rico (17°58.2'N 67°02.05'W) (Gooding, 1963).
- 119 specimens (58 adults and 61 larval stages) near La Parguera, Puerto Rico (17°58.2'N 67°02.05'W) at 0.5-2.5 meters depth (Sanders & Hessler, 1964).
- 1 larval specimen in Biscayne Bay, Florida (25°43.5'N 80°09.0'W) (Hessler & Sanders, 1973).
- 131 specimens in seagrass bed in the Caribbean coast of Yucatan Peninsula, Mexico (19°47.10'N 87°28.13'W) at 0,5-2 meters depth (De Troch *et al.,* 2000).
- Some specimens in Carrie Bow Cay, Belize (Schiemer & Ott, 2001).
- 1 specimens in British Virgin Islands (18°28.55'N 64°34.5'W) (Martin *et al.,* 2002).

Lightiella monniotae

- 1 specimens from organic-rich detrital sand in Saint Vincent bay, New Caledonia (22°00.5'N 135°56.8'W) at 4 meters depth. (Cals & Delamare Deboutteville, 1970).

Lightiella floridana

- 58 specimens in fine quartz covered with organic matter and sea grass beds in Anclote Anchorage, Florida (28°10.5'N 82°48.87'W) 1 meter depth (McLaughlin, 1976).
- 12 specimens in very soft silty bottoms as well as exoposed limestone with coral and sponges in Pinellas County, Florida (Saloman, 1978).

Lightiella sp.

- Some specimens in the Northeast Gulf of Mexico (Stoner, 1981).
- Some specimens in the Mobile Bay, Alabama (Heard & Goeke, 1982).

Lightiella magdalenina

- 28 specimens in muddy sand bottom, rich in organic material mostly consisting of leaf fragments of *Posidonia oceanica* in La Maddalena Archipelago, Sardinia (41° 11,41' N; 09°23,5'E) at 15–20 meters depth (Carcupino *et al.*, 2006).

Sandersiella acuminata

- 1 specimens in muddy bottom covered by *Zostera* in Tomioka Bay, Japan (32°32.0'N 130°02.0'E) at 2.1 meters depth (Shiino, 1965).
- 20 specimens in the same area reported by Kikuchi (1968).
- 27 specimens in soft muddy bottom in Seto Inland Sea, Japan (34°10.0'N 153°17.0'E) at 9-32 meters depth (Kikuchi, 1968).

Sandersiella calmani

- 2 specimens from organic-rich mud off the coast of Perù (15°04.0'S 75°28.0'W) at 85 meters depth (Hessler & Sanders, 1973).

Sandersiella bathyalis

- 1 specimens in Walvis Bay, Southwest Africa (23°05.0'S 12°31.5'E) at 1546-1559 meters depth (Hessler & Sanders, 1973).
- 1 specimens in Walvis Ridge Southwest Africa (19°57.0'S 11°02.0'E) at 1227 meters depth (Hessler & Sanders, 1973).

Hampsonellus brasiliensis

- 1 specimens in San Paulo, Brazil (23°01.6'S 46°19'W) (Wakabara & Mizoguchi, 1976).
- 6 specimens in fine sand in Bertoga Cove, San Paulo, Brazil (23°53.0'S 46°10.0'W) (Hessler & Wakabara, 2000).
- 6 specimens in very fine sand in Ubatumirim Cove, San Paulo, Brazil (23°23.0'S 44°55.75'W) at 17 meters depth (Hessler & Wakabara, 2000).
- 19 specimens in silt-clay in Rio de Janeiro, Brazil (23°53.0'S 46°10.0'W) at 5-15 meters depth (Hessler & Wakabara, 2000).

Chiltoniella elongata

- 2 specimens in highly organic mud in Hawke Bay (39°34.2'S 176°59.1'E) at 13-16 meters depth (Knox & Fenwick, 1977).

2.1.7 PHYLOGENY

Since their first description (Sanders, 1955), Cephalocarida have been considered the most primitive living crustaceans (Sanders, 1963; Hessler, 1964, 1984, 1992; Hessler & Newman, 1975; Abele, 1982; Hessler & Elofsson, 1992). Although they present specialization in their reproductive biology (they are simultaneous hermaphrodites with probable self-fertilization) (Hessler *et al*., 1970; 1995). Cephalocarida seem to preserve several features in their external morphology and development that remind those of the hypothetical ancestral crustacean. These features are: 1) post-antennal head limbs and trunk segments all bearing series of very similar limbs; 2) very gradual development to the adult stage, not differing greatly from the larva. At present, Cephalocarida remains a poorly known group, with most of the morphological and molecular data referred to only one species, *Hutchinsoniella macracantha*, and its phylogenetic position is strongly debated. It has been considered: a sister group to Branchiopoda (Schram, 1986) or to a higher taxon that includes Branchiopoda (Branchiopoda + Malacostraca, Hessler, 1992) or (Branchiopoda + Maxillopoda, Walossek, 1993;

1999) or directly linked to maxillopod crustaceans (Ito, 1989; Spears & Abele, 1999; Lavrov *et al.*, 2004).

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A new species of the genus *Lightiella*: the first record of Cephalocarida (Crustacea) in Europe

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A new species of Cephalocarida belonging to the genus *Lightiella* is described. Like all known species of *Lightiella*, the new species is characterized by reduction of trunk segment 8, which also lacks both pleura and thoracopods. The diagnostic characters of the species are: (1) one seta on the inner distal corner of the penultimate endopodal segment of second maxilla and thoracopods 1–5; (2) only one claw on the distal segment of the endopod of thoracopod 6. A cladistic analysis of 27 morphological characters was used to estimate the phylogeny of all species of *Lightiella*, with all other cephalocarid species used as outgroups. The discovery of this species in the Mediterranean fills a gap in the distribution of the genus and of the entire class. © 2006 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2006, **148**, 209–220.

ADDITIONAL KEYWORDS: cladistic analysis – Mediterranean – thoracopod morphology – systematics.

INTRODUCTION

Since their first description (Sanders, 1955), Cephalocarida have been considered the most primitive living crustaceans (Sanders, 1963; Hessler, 1964, 1984, 1992; Hessler & Newman, 1975; Hessler & Elofsson, 1992). Together with a specialization in their reproductive biology (they are simultaneous hermaphrodites with probable self-fertilization), Cephalocarida seem to preserve several features of their external morphology and development which are similar to those of the hypothetical ancestral crustacean. These features are: (1) postantennal cephalic limbs and trunk segments all bearing series of very similar limbs; (2) very gradual development to the adult stage, not differing greatly from the larva. Despite their phylogenetic importance, Cephalocarida remain a poorly known group, with most of the morphological and molecular data regarding only one species, *Hutchinsoniella macracantha* Sanders, 1955 (Sanders, 1957, 1963; Hessler, 1964, 1992; Brown & Metz, 1967; Hessler, Hessler & Sanders, 1970; Hessler & Newman, 1975; Elofsson & Hessler, 1990, 1991, 1992; Elofsson, Hessler & Hessler, 1992; Hessler & Elofsson, 1992; Read, Hessler & Govind, 1994; Hessler, Elofsson & Hessler, 1995; Spears & Abele, 1999; Regier & Shultz, 2001; Richter, 2002; Lavrov, Brown & Boore, 2004).

At present, Cephalocarida is one of the least speciose of the crustacean classes. Only ten species, belonging to five genera, have hitherto been reported from North and South America (Sanders, 1955; Jones, 1961; Gooding, 1963; Sanders & Hessler, 1964; Wakabara, 1970; Hessler & Sanders, 1973; McLaughlin, 1976; Saloman, 1978; Stoner, 1981; Heard & Goeke, 1982; De Troch, Fiers & Vincx, 2000; Hessler & Wakabara, 2000; Schiemer & Ott, 2001; Martin, Cadien & Zimmerman, 2002), Africa (Hessler & Sanders, 1973), Japan (Shiino, 1965), New Zealand (Knox & Fenwick, 1977) and New Caledonia (Cals & Delamare Deboutteville, 1970), occurring from the intertidal to a depth of about 1550 m. No species have been reported thus far from Europe.

All the described genera are so similar in general morphology that only one family seems to be justified. However, the genus *Lightiella* Jones, 1961 differs from the others in the reduction of trunk segment 8, which

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also lacks thoracopods. Most records of *Lightiella* are known for the Gulf of Mexico and Caribbean (Gooding, 1963; Sanders & Hessler, 1964; McLaughlin, 1976; Saloman, 1978; Stoner, 1981; Heard & Goeke, 1982; De Troch *et al*., 2000; Schiemer & Ott, 2001; Martin *et al*., 2002). Only three additional records of this genus are known for San Francisco Bay, California (Jones, 1961), Biscayne Bay, Florida (Hessler & Sanders, 1973) and Saint Vincent Bay, New Caledonia (Cals & Delamare Deboutteville, 1970). Moreover, it is the most speciose of the cephalocarid genera, with four species: *L. serendipita* Jones, 1961, *L. incisa* Gooding, 1963, *L. floridana* McLaughlin, 1976 and *L. monniotae* Cals & Delamare Deboutteville, 1970.

In the present paper, we describe a new species of the genus *Lightiella*, which represents the eleventh cephalocarid species to be recognized and the first species known from Europe. In addition, to estimate the phylogeny of all species of *Lightiella*, including the new one described here, we use a cladistic analysis of 27 morphological characters, with all other cephalocarid species used as outgroups.

MATERIAL AND METHODS

Samples

Specimens were collected at 15–20 m depth from a muddy sand bottom, rich in organic material mostly consisting of leaf fragments of *Posidonia oceanica*, on the southern shore of the isle of S. Stefano, part of the La Maddalena Archipelago. Three samplings were carried out by hand using SCUBA in October 1999, July 2004, and October 2004, respectively. Twenty-eight specimens (13 adults and 15 larvae) were found.

Four adults were prepared and mounted for light and scanning electron microscopic (SEM) analyses.

Light microscopy

Two specimens were dissected to isolate the cephalic and trunk appendages. Each appendage was mounted on a separate slide using Aquamount medium.

Scanning electron microscope (SEM)

Two samples were fixed in formalin 4%, dehydrated in a graded ethanol series, dried in a Polaron Jumbo critical-point drier, sputter-coated with gold in an Edwards SI5A unit and observed with a ZEISS DMS 962 scanning electron microscope of the Electron Microscopy Center, Sassari University.

Cladistic analysis

The analysis was based on 11 taxa and 27 morphological characters with 70 character states. As ingroup, we used the five *Lightiella* species known at present (including the new species): *L. serendipita*, *L. incisa*, *L. floridana*, *L. monniotae*, and *L. magdalenina* sp. nov. All other cephalocarid species were used as outgroups: *Hutchinsoniella macracantha*, *Chiltoniella elongata*, three species of the genus *Sandersiella*, *S. acuminata*, *S. calmani*, and *S. bathyalis*, and *Hampsonellus brasiliensis*.

Character coding was based on the original descriptions and figures reported in: Sanders (1957) for *Hutchinsoniella macracantha*; Jones (1961) for *L. serendipita*; Gooding (1963) for *L. incisa*; Shiino (1965) for *S. acuminata*; Hessler & Sanders (1973) for *S. calmani* and *S. bathyalis*; McLaughlin (1976) for *L. floridana*; Knox & Fenwick (1977) for *C. elongata*; Cals & Delamare Deboutteville (1970) for *L. monniotae*; Hessler & Wakabara (2000) for *Hampsonellus brasiliensis*.

Characters and character states (shown in bold) were (Table 1):

1. Thoracic segment 8, pleura: present (**0**); reduced to a spinose process (**1**); absent (**2**).
2. Thoracic segment 8, limbs: present (**0**); absent (**1**).
3. Abdominal segments, pleura: prominent but smaller than those of segments 1–7 (**0**); small but distinct (**1**); reduced to a spinose process (**2**).
4. First antenna, knoblike structure on the second segment: present and jointed (**0**); present and unjointed (**1**); absent (**2**); unknown (**?**).
5. Second antenna, knoblike structure on the second protopod segment: present and setose (**0**); present and naked (**1**); absent (**2**).
6. First antenna, length ratio formula of 4th- 5th segments: 1 : 1 (**0**); 1 : 2 (**1**); 2 : 3 (**2**); unknown (**?**).
7. First antenna, length ratio formula of 3rd- 6th segments: 5 : 10 (**0**); 5 : 4 (**1**); 1 : 1 (**2**); 2 : 3 (**3**); unknown (**?**).
8. Labrum, shape: anteriorly and posteriorly pointed (**0**); pointed only posteriorly (**1**); both anteriorly and posteriorly rounded (**2**); unknown (**?**).
9. Mandible, incisor process: one tooth (**0**); two teeth (**1**); unknown (**?**).
10. First maxilla, endopod: 3-segmented (**0**); 4-segmented (**1**); unknown (**?**).
11. First maxilla, setae on the endopod distal segment: 4 (**0**); 3 (**1**); unknown (**?**).
12. First maxilla, gnathobase: jointed (**0**); unjointed (**1**).
13. First maxilla, setae on the gnathobase: 5 (**0**); 4 (**1**); 3 (**2**); 2 (**3**).
14. First maxilla, setae on the exopod: 14 (**0**); 11 (**1**); 9 (**2**); 8 (**3**); 7 (**4**).
15. Second maxilla, endopodal segments: 6 (**0**); 5 (**1**).
16. Second maxilla, number of claws on the endopod distal segment: 4 (**0**); 3 (**1**).

17. Second maxilla, setae on the epipod: 6–10 (**0**); 5 (**1**); 4 (**2**).
18. Thoracopods 1–4, number of claws on the endopod distal segment: 4 (**0**); 3 (**1**).
19. Thoracopod 5, number of claws on the endopod distal segment: 4 (**0**); 3 (**1**).
20. Thoracopod 6, number of claws on the endopod distal segment: 3 (**0**); 2 (**1**); 1 (**2**).
21. Thoracopod 7, number of claws on the endopod distal segment: 1 (**0**); 2 (**1**).
22. Thoracopods 1–5, setae on the epipod: 6–10 (**0**); 5 (**1**); 4 (**2**).
23. Thoracopod 6, exopod: unmodified (**0**); strongly modified (**1**).
24. Second maxilla and thoracopods 1–5, small seta on the inner distal corner of the penultimate endopodal segment: present (**0**); absent (**1**).
25. Second maxilla and thoracopods 1–7, number of spines or short setae between the long setae on the last segment of exopod: one (**0**); more than one (**1**).
26. Penultimate abdominal segment, ventral comb: present (**0**); absent (**1**).
27. Telson, spinose processes on the dorsal caudal margin: present (**0**); absent (**1**).

The data matrix was edited in MacClade (Maddison & Maddison, 1992) and the parsimony analysis was performed in PAUP (Swofford, 1993). An exhaustive search (with collapse option in effect) was applied and all minimal trees were retained. Clade support was assessed by bootstrap and jack-knife (1000 replicates).

RESULTS

FAMILY HUTCHINSONIELLIDAE SANDERS, 1955
GENUS *LIGHTIELLA* JONES, 1961
***LIGHTIELLA MAGDALENINA* SP. NOV.** (FIGS 1–5)

Holotype: One adult kept in ethanol, October 1999, S. Stefano isle, La Maddalena Archipelago, deposited in the Swedish Natural History Museum, Stockholm (SNMH) (accession number: SMNH Type 6141).

Type locality: Italy, Sardinia, S. Stefano isle, La Maddalena Archipelago, water depth 14 m, very fine muddy sand with shells and organic material (mostly leaves of *Posidonia oceanica*).

Paratypes: Serial slides of cephalic appendages, trunk appendages and telson of 1 adult, October, 1999, from the type locality, deposited in the Swedish Natural History Museum, Stockholm (SMNH) (accession numbers: SMNH Type 6142).

Serial slides of cephalic appendages, trunk appendages and telson of 1 adult, October, 1999 (accession numbers DIZABceph1.1); 1 whole gold-coated adult, mounted on a stub for SEM observation and 1 dissected gold-coated adult mounted on two stubs, July, 2004 (accession numbers DIZABceph1.2); 9 adults (accession numbers DIZABceph1.3) and 15 larvae (accession numbers DIZABceph1.4) kept in an aqueous solution of 4% formalin, July and October, 2004. All these specimens are deposited in the zoological collection of the Department of Zoology and Biological Anthropology (DIZAB), Sassari University.

Etymology: The species is named after the locality where it was collected: La Maddalena Archipelago (from lat. 'Magdalena').

Diagnosis: This species is distinguished from congenerics on the basis of the following characters: (1) one small seta on the inner distal corner of the penultimate endopodal segment of second maxilla and thoracopods 1–5; (2) only one claw on the distal segment of the endopod of thoracopod 6.

Description

Adult (body length up to 2.6 mm) (Fig. 1A–D). Holotype. Trunk 20-segmented (including telson) and 5 times as long as cephalon. Trunk segments 1–7 with terga produced latero-ventrally forming well developed and overlapping pleura with rounded free edges (Fig. 1A). Trunk segment 8 reduced and without pleura and legs (Fig. 1B). Trunk segment 9 with highly modified legs (see detailed description below) and tergum with lateral spines (Fig. 1B). Trunk segments 10–19 without legs, and with pleura developed into strong spinose processes (Fig. 1A). Telson bearing two caudal rami, and characterized by a ventral comb of strong teeth and with two well developed dorsal spines with rounded edges (Figs 1A, C, D). Caudal rami equalling the width of telson and bearing one or two short, and two long, terminal setae (Fig. 1A).

First antenna: (Fig. 2A). 6-segmented. Length ratio formula of 3rd–6th segments: 3-1-2-3. Setal formula (from base to tip); 0; 2; 4; 0; 0; 7 + 1 aesthete.

Second antenna: (Fig. 2B). Protopod 2-segmented. Endopod 2-segmented with 2 setae on the distal margin of the first segment and three setae and two spines on the second segment. Exopod 19-segmented with setal formula: 2; 2; ?; ?; 0; 1; 1; 2; 0; 1; 1; 1; 1; 2; 1; 1; 1; 1; 4.

Labrum: (Fig. 2C–E). Large, broadly rounded anteriorly, acutely triangular posteriorly. Postero-ventral surface with thin setae randomly distributed.

Mandible: (Figs 3A, B). Without palp. Incisor processes bearing two teeth with one small seta in between. The molar processes with numerous small teeth.

First Maxilla: (Figs 2C, 3C–F). Biramous. Protopod with an elongate and unsegmented gnathobase bear-

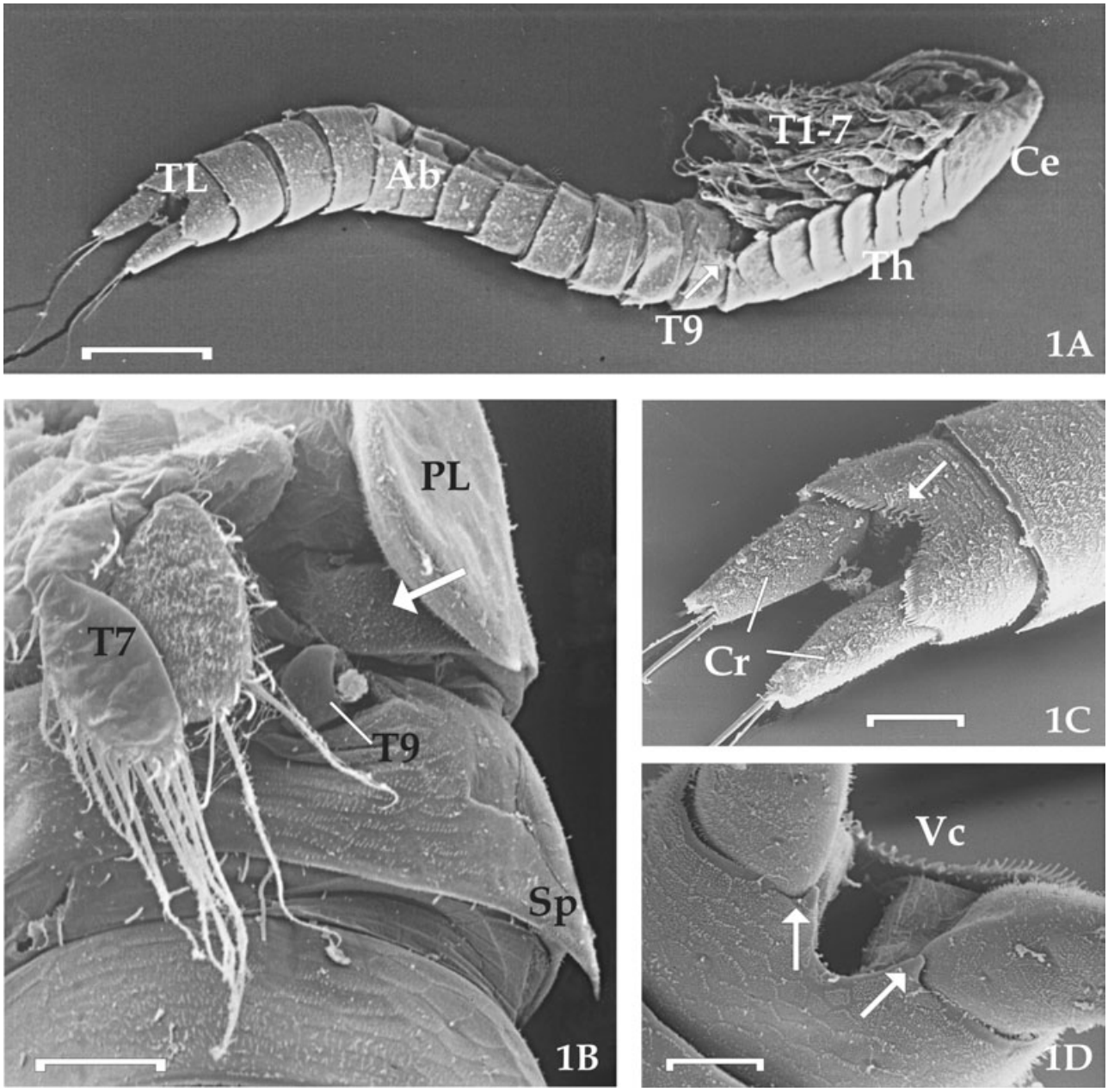

Figure 1. Scanning electron micrographs of ***Lightiella magdalenina* sp. nov.** A, ventral view of an adult showing the horseshoe shaped cephalon (Ce), 9-segmented thorax (Th) with thoracopods 1–7 (T1-7) and reduced thoracopod 9 (T9), 11-segmented abdomen (Ab), including telson (TL). Scale bar = 300 μm. B, high magnification of segments 7–9 showing: segment 7 with pleura (PL) and limb (T7); segment 8 reduced and lacking pleura and limb (arrow); segment 9 with tergum reduced to a spine (Sp) and limb highly modified (T9). Scale bar = 14 μm. C, ventral view of the last portion of the abdomen showing the ventral comb on the telson (arrow) and caudal rami (Cr). Scale bar = 90 μm. D, dorsal view of telson characterized by two spinose processes with rounded edges (arrows); ventral comb (Vc). Scale bar = 10 μm.

ing three indented spines and two plumose setae (Fig. 3E–F). Endopod 3-segmented. Each segment bears a small seta on its inner corner. In addition to this small seta, the last segment bears two other setae which are long and plumose (Fig. 3C, D) (for setal formula see Table 2). Exopod with 7/8 marginal plumose setae (Fig. 3C).

Second maxilla and thoracopods 1–5: (Fig. 4A–D). Biramous, with about the same length and morphol-

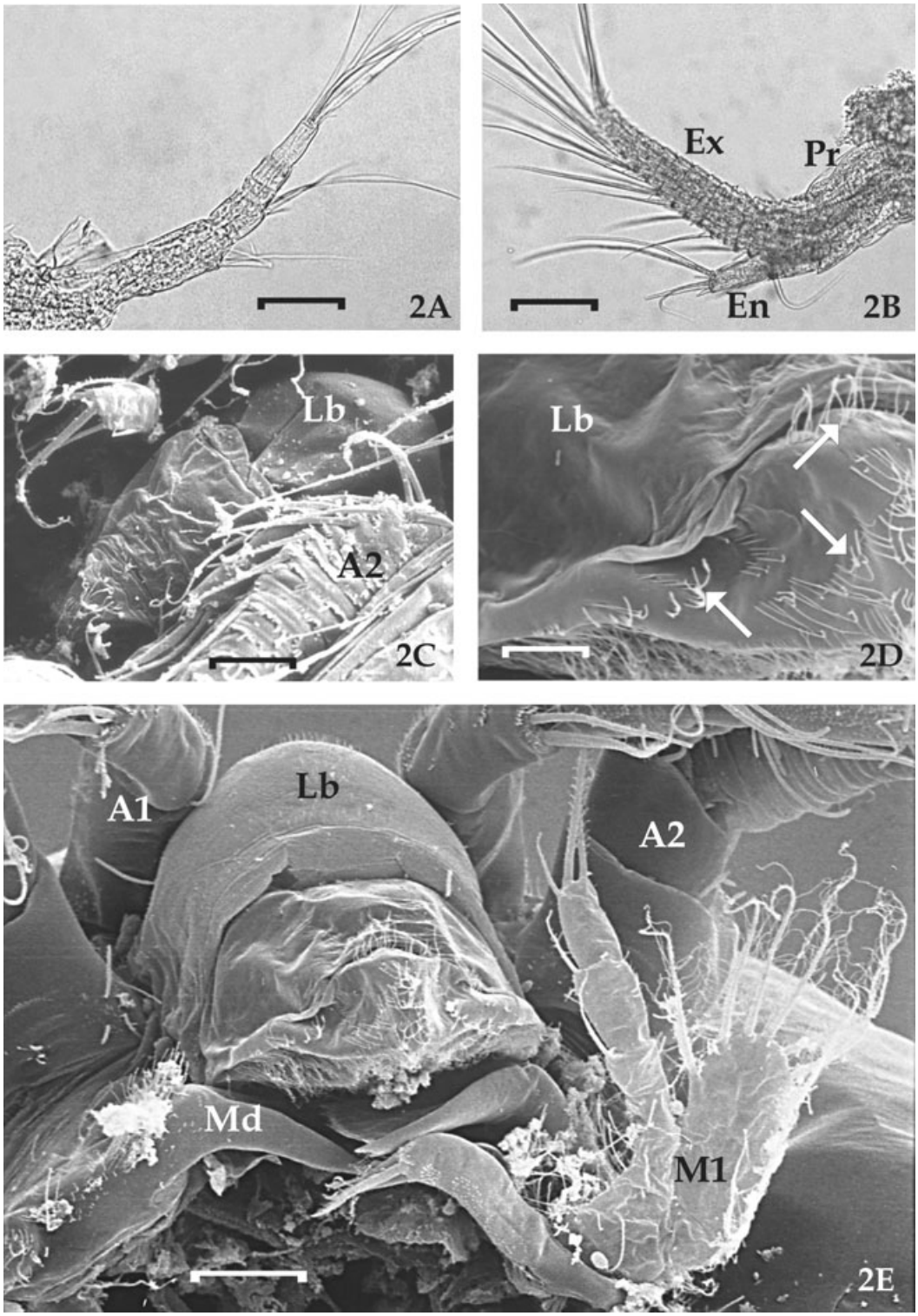

Figure 2. Light and scanning electron micrographs of first and second antennae and labrum of ***Lightiella magdalenina* sp. nov.** A, 6-segmented first antenna. Scale bar = 70 μm. B, second antenna with 2-segmented protopod (Pr), 2-segmented endopod (En) and 19-segmented exopod (Ex). Scale bar = 80 μm. C, ventral view of labrum (Lb), which appears rounded anteriorly and acutely triangular posteriorly; second antenna (A2). Scale bar = 45 μm. D, detail of the postero-ventral surface of labrum (Lb) covered by thin setae randomly distributed (arrows). Scale bar = 3 μm. E, posterior view of cephalon separated by the remaining part of the body at the level of the second maxilla. First antenna (A1), second antenna (A2), labrum (Lb), unsegmented mandible (Md), first maxilla (M1). Scale bar = 10 μm.

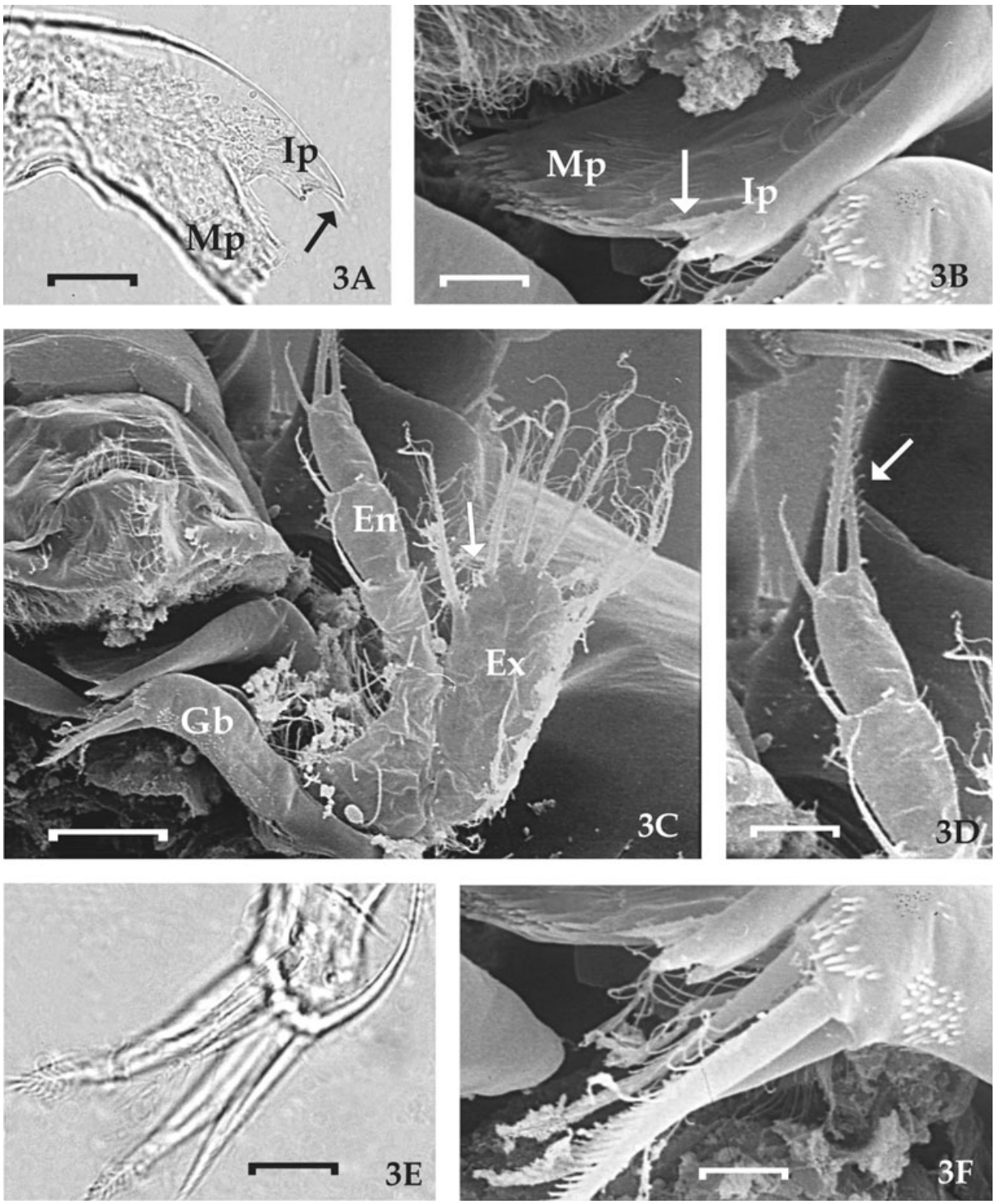

Figure 3. Light and scanning electron micrographs of mandible and first maxilla of ***Lightiella magdalenina*** **sp. nov.** A, B, mandible with incisor process (Ip) bearing two teeth with one small seta in between (arrow) and molar process (Mp) with numerous small teeth. Scale bars: A, 35 μm; B, 2 μm. C, first maxilla with unsegmented protopodal gnathobase (Gb), 3-segmented endopod (En) and unsegmented exopod (Ex). Exopod bears 8 long plumose setae, one of which is broken (arrow). Scale bar = 10 μm. D, detail of the endopod showing the small seta on the inner corner of each segment and the two longer plumose setae of the last segment (arrow). Scale bar = 5 μm. E, F, detail of gnathobase bearing three indented spines and two plumose seta. Scale bars: E, 10 μm; F, 2 μm.

Table 1. Character matrix for all described cephalocarid species. Question mark denotes unknown character state

Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Hutchinsoniella macracantha	0	0	1	0	0	2	0	0	0	1	0	0	1	2	0	1	1	1	1	0	1	1	0	0	1	0	1
Sandersiella acuminata	0	0	0	1	2	?	?	2	0	1	0	1	1	2	0	1	1	1	1	1	1	1	1	0	1	0	1
Sandersiella calmani	0	0	0	?	2	?	?	2	?	1	0	1	1	2	0	1	0	1	1	1	1	0	1	0	1	0	1
Sandersiella bathyalis	0	0	0	?	2	?	?	2	?	1	0	1	1	0	0	1	0	1	1	1	1	0	1	0	1	0	1
Hampsonellus brasiliensis	0	0	0	2	2	?	?	2	0	0	0	1	2	1	1	1	0	1	1	1	0	0	1	0	1	0	1
Chiltoniella elongata	0	0	0	0	1	0	3	?	?	1	1	1	3	2	1	1	1	0	1	1	1	1	0	0	1	0	1
Lightiella serendipita	2	1	2	2	2	1	2	2	0	?	?	0	2	4	1	1	2	0	1	0	0	2	0	1	0	1	1
Lightiella incisa	2	1	2	2	2	2	1	1	0	0	0	1	1	3	1	0	1	0	1	1	0	1	0	1	0	1	0
Lightiella monniotae	1	1	2	2	2	1/2	1	1	1	0	1	1	0	4	1	0	1	0	1	1	0	1	0	1	0	1	0
Lightiella floridana	1	1	2	2	2	0	2	1	1	0	1	1	1	3	1	0	1	0	1	1	0	1	0	1	0	1	0
***Lightiella magdalenina* sp. nov.**	2	1	2	2	2	1	2	1	1	0	1	1	0	3	1	0	2	0	0	2	0	2	0	0	0	1	0

Table 2. Setae, claws and number of protopodal endites on adult limbs. Claws (roman numbers); number of protopodal endites (NPe); endopodal segments (En1–5); exopodal segments (Ex1–2); epipod (Ep)

	NPe	En1	En2	En3	En4	En5	Ex1	Ex2	Ep
1° maxilla		1	1	1 + 2			7/8		
2° maxilla	6	2	3	2	3 + 1	IV	2 + 1	14	4
1° thoracopod	6	2/3	3/4	3	3 + 1	IV	2 + 1	15	4
2° thoracopod	6	3	4/3	3	3 + 1	IV	2 + 1	12/15	4
3° thoracopod	6	2/2	4/3	3	3 + 1	IV	2 + 1	13/15	4
4° thoracopod	6	3	3	3	4 + 1	IV	3 + 1	14	4
5° thoracopod	6	3/4	3/4	3	3 + 1	IV	2 + 1	13/14	4
6° thoracopod	6	2/3	3/4	2	2 + 1	I	1 + 1	11/12	4
7° thoracopod	3	1	1	1	0	I	0 + 1	10/11	4

ogy. Protopod 1-segmented, bearing 6 enditic processes on the latero-internal margin (Fig. 4C), and with 1-segmented epipod on its outer distal corner (Fig. 4A, B). Endites are armed with spines and setae. Epipod with four long-terminal setae (Fig. 4A, B). Endopod 5-segmented. Segments 1–3 bearing from 1 to 5 setae on the inner corner (see Table 2 for setal formula). Segment 4 with one seta on the inner corner and a group of three or four setae on the outer corner (Fig. 4A, B, D). Distal segment with four claws. Three of these are large, indented and decreasing in size medially. The last one is small, smooth and located on the medial side of the base of the outermost claw (Fig. 4D). Exopod 2-segmented; for setal formula, see Table 2. Segment 2 bears from 12 to 15 long setae and one spine. The latter divides the setae into two groups, with the distal group always consisting of four setae (Fig. 4A, B).

Thoracopods 6–7: (Fig. 5A–E). Slightly smaller than the previous legs. Thoracopod 6 is very similar to the others with the exception of the distal endopodal segment, which bears only one claw (Fig. 5A), and the protopod, with a genital pore on the posterior surface. The genital pore is oval, with the major axis parallel to the protopodal endites. Its opening is covered by a convex plug-like membrane and its lateral margin is covered by short thin setae (Fig. 5B, C). Thoracopod 7 similar to the previous one except for the reduced protopod, bearing only 3 endites (Fig. 5D, E).

Thoracopod 8: Absent.

Thoracopod 9: (Figs 1B, 5F). Highly modified. Inserted on the ventro-lateral surface of segment 9 and comprised of two parts: an apical part, consisting of a short cylindrical process, emerging from the lateral concave surface of a subspherical basal part.

Cladistic analysis

The analysis yielded 8 most parsimonious trees (tree length 59, consistency index 0.7288, retention index

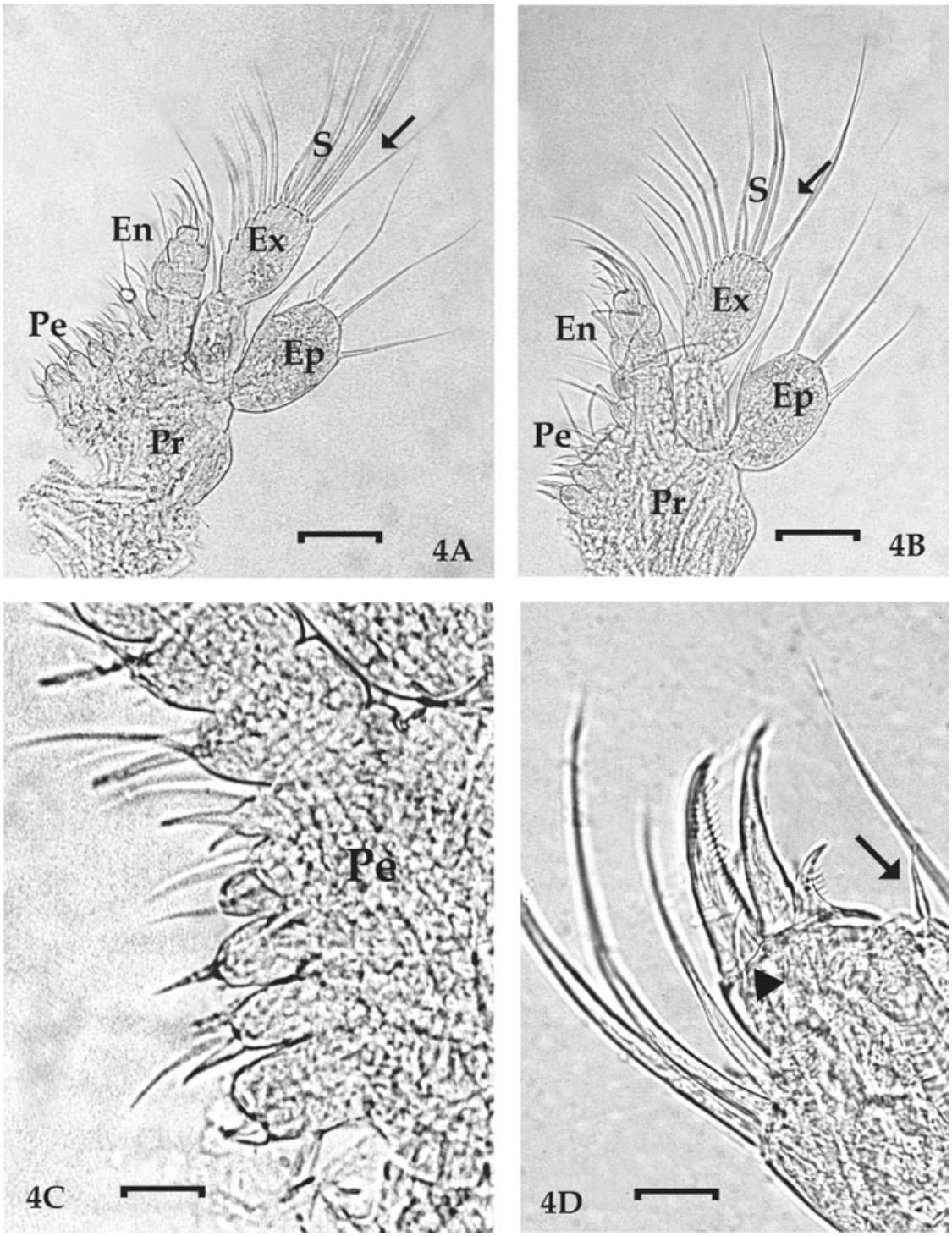

Figure 4. A, B, light micrographs of second maxilla (A) and thoracopod 5 (B) of ***Lightiella magdalenina* sp. nov.** showing the same morphological organization. Protopod (Pr) characterized by 6 enditic processes (Pe) and 1-segmented epipod (Ep) with 4 long plumose setae. 5-segmented endopod (En) and 2-segmented exopod (Ex) with a spine (S) that distinguishes the distal group of 4 long setae (arrow). Scale bars: A, 56 μm; B, 60 μm. C, high magnification of protopodal endites (Pe) armed with spines and setae. Scale bar = 25 μm. D, detail of the last two segments of endopod. Segment 4 characterized by the small seta on the inner corner (arrow) and 3 long setae on the outer corner. The last segment bears 4 claws, three of which are large and indented; the last one is small, smooth and located on the medial side of the base of the outermost claw (arrowhead). Scale bar = 15 μm.

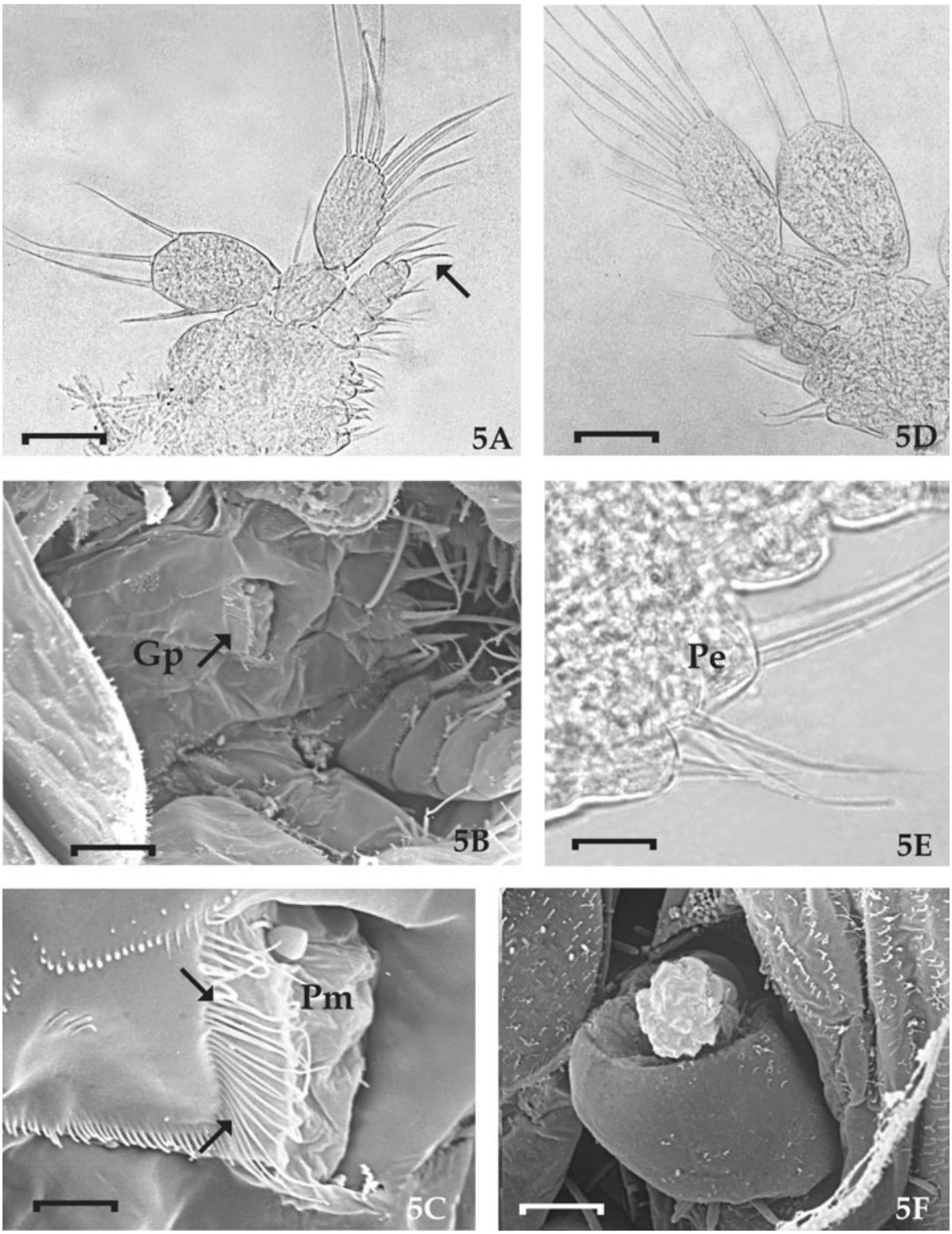

Figure 5. Light and scanning electron micrographs of thoracopods 6, 7 and 9 of ***Lightiella magdalenina* sp. nov.** A, general view of thoracopod 6 with only one claw (arrow) on the distal endopodal segment. Scale bar = 58 μm. B, posterior surface of protopod of thoracopod 6 with genital pore (Gp). Scale bar = 5 μm. C, high magnification of genital pore showing the convex plug-like membrane (Pm) and the short thin setae (arrows) covering its lateral margin. Scale bar = 2 μm. D, general view of thoracopod 7 which appears similar to the preceding one, except for the reduced protopod characterized by only three enditic processes. Scale bar = 52 μm. E, detail of protopod of thoracopod 7 showing only three enditic processes (Pe). Scale bar = 14 μm. F, high magnification of thoracopod 9. Scale bar = 3 μm.

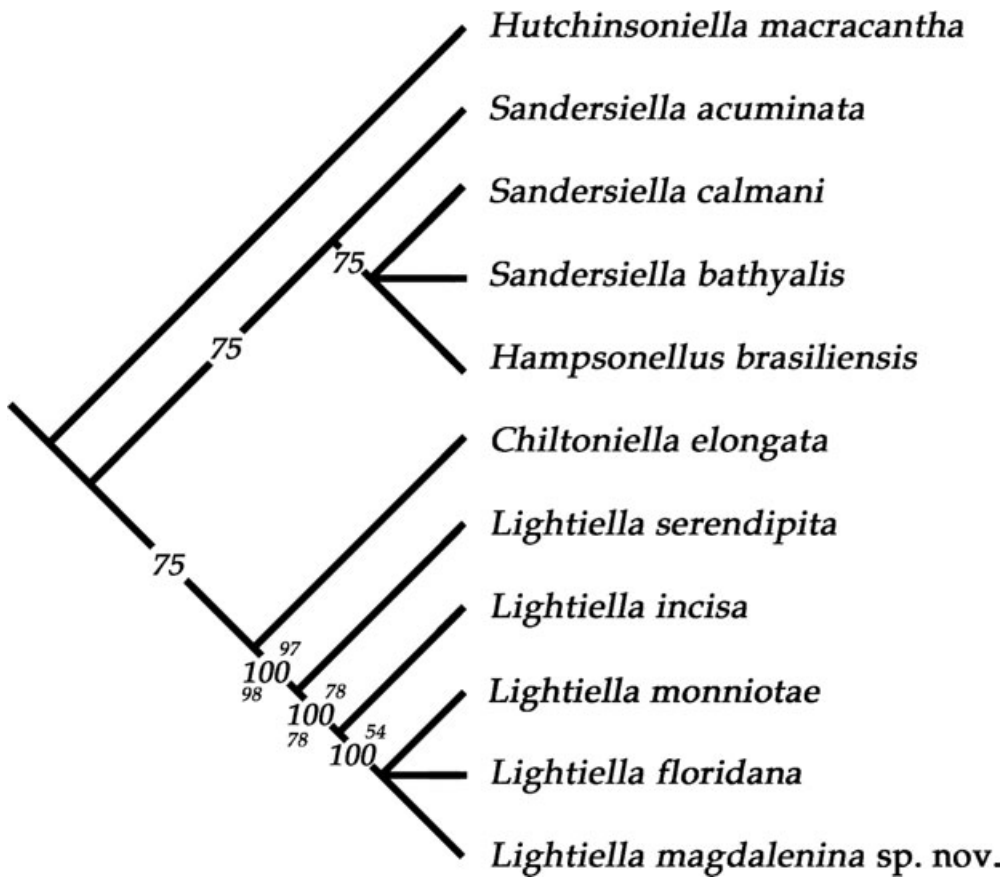

Figure 6. Majority–rule consensus tree from 8 primary trees.

0.7746). The analysis performed without characters 6 and 7, which are indeterminate in most of the outgroup species, yielded the same number of trees (tree length 51), same tree topography and similar values of consistency index (0.7451) and retention index (0.8088). Similarly, the use of all other cephalocarid species or *H. macracantha* alone as outgroup yielded identical results. Support values were generally low, strongly supporting only the monophyly of the genus *Lightiella* and the basal position of *L. serendipita*. The new species appears as a derived taxon within the genus *Lightiella*, nested within an unresolved and weakly supported clade including *L. monniotae* (from New Caledonia) and *L. floridana* (from Florida) (Fig. 6).

DISCUSSION

All cephalocarid species resemble each other in gross morphology, particularly the horseshoe-shaped cephalon, the 20-segmented trunk (9 thoracic and 11 abdominal segments including telson) and the similar morphology of second maxilla and thoracopods 1–7. Thoracopods 8 and 9 are the only limbs strongly reduced and/or modified. In all known species, thoracopod 9 is always strongly modified into a small subspherical appendage, whereas thoracopod 8 (which can be absent, as in the *Lightiella* species) is reduced in size and lacks an endopod. All other limbs, including the second maxilla and thoracopods 1–7, are biramous and consist of a basal protopod from which a multisegmented endopod and a bisegmented exopod emerge. The protopod is also characterized by several enditic processes on the latero-internal margin, provided with spines and setae, and one epipod on the outer distal corner, bearing several long setae. Setae of different length, spines and claws are also present on the segments of both endopod and exopod.

The most important diagnostic characters between cephalocarid genera are the peculiar morphological differences of thoracopods 6 and 7.

In the genus *Chiltoniella*, thoracopods 6 and 7 are considered to be unmodified, or at least less modified than in the other genera (Knox & Fenwick, 1977). Thoracopod 6 has an endopod with two, instead of three, terminal, claw-like setae; segment 2 of the exopod bears several rows of minute denticles on the latero-proximal quarter. The endopod of thoracopod 7 is

reduced in length and, similar to the previous one, has two terminal claw-like setae.

In *Hutchinsoniella*, the only claw of thoracopod 7 is bluntly rounded and some of the flexor muscles of the epipod of thoracopod 6 are larger than in the other thoracopods (Hessler, 1964). However, information on the latter character is not available for the other cephalocarid taxa.

In *Sandersiella* (Shiino, 1965; Hessler & Sanders, 1973) and *Hampsonellus* (Hessler & Wakabara, 2000) the distal podomer of the exopod of the thoracopod 6 is highly modified in the same way. It is divided into two lobes, each provided with its own set of setae. The three *Sandersiella* species and *Hampsonellus brasiliensis*, however, are easily discriminated on the basis of specific details. Moreover, thoracopod 7 is significantly modified only in *Hampsonellus brasiliensis*.

Finally, *Lightiella* differs from all other genera in the reduction of segment 8, which also lacks thoracopods and pleura. In this genus, thoracopods 6 and 7 are smaller than, but very similar to, the other limbs (Jones, 1961; Gooding, 1963; Cals & Delamare Deboutteville, 1970; McLaughlin, 1976).

The reduction of trunk segment 8 and the absence of both thoracopod 8 and the ventral comb of the penultimate abdominal segment support the attribution of the new species described here to the genus *Lightiella*.

Lightiella magdalenina sp. nov. differs from the other *Lightiella* species in the presence of: (1) one small seta on the inner distal corner of the penultimate endopodal segment (never reported for any other *Lightiella* species); (2) only one claw on the distal segment of the endopod of thoracopod 6 (whereas *L. incisa*, *L. floridana*, *L. monniotae* and *L. serendipita* all have two claws).

Like *Hutchinsoniella macracantha* (Hessler *et al*., 1995) and *Hampsonellus brasiliensis* (Hessler & Wakabara, 2000), *L. magdalenina* shows a genital pore on the posterior face of thoracopods 6. Only *Chiltoniella* seems to bear the genital pore on thoracopod 9 (Knox & Fenwick, 1977). All the modifications of thoracopods 6–8, as well as that of thoracopod 9, are considered to be related to reproductive function. In particular, the reduction or absence of thoracopod 8 seems to facilitate egg transfer from thoracopod 6 to thoracopod 9.

In the best known *Hutchinsoniella macracantha*, two large eggs, laid during each reproductive event, emerge from the genital pores and are then carried and cemented on thoracopods 9 (Hessler *et al*., 1995). In *Lightiella*, two eggs seems to be laid only occasionally. Two egg sacs were only found in 1 of 17 ovigerous specimens examined (Sanders & Hessler, 1964). A single egg sac was also reported in *Lightiella* by Gooding (1963), De Troch *et al*. (2000) and Martin *et al*. (2002).

Reconstruction of the phylogenetic relationships within the Cephalocarida is severely hampered by the lack of support of the resulting trees, mostly due to the number of poorly described taxa. However, monophyly of the genus *Lightiella*, as well as the basal position of *L. serendipita*, appears well supported. The close relationship between the new species and *L. monniotae* (from the Pacific Ocean) and *L. floridana* (from Florida) suggests an ancient Tethyan origin for the clade and speciation by means of allopatric divergence after the closure of the Tethys Sea.

Interestingly, the results of the phylogenetic analysis challenge the monophyly of the genus *Sandersiella*, as understood at present, and its relationships with *Hampsonellus brasiliensis* (Wakabara & Mizoguchi, 1976).

L. magdalenina sp. nov. is thus far known only for a very restricted site, about 15–20 m deep on the southern shore of the tiny island of S. Stefano. Samples of nearby benthic communities at comparable depths failed to yield specimens of the new species. It is noteworthy that the animals are not particularly inconspicuous, and the lack of previous reports from the Mediterranean may point to a very narrow distribution. This seems to be the case with Cephalocarida taxa. Indeed, most of them are only known for a single locality, and even within that locality they appear to be exceedingly rare. Therefore, it is fortunate that the only station where the new species has been found lies within the boundaries of the La Maddalena Archipelago National Park, which should ensure protection of its habitat.

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RESEARCH ARTICLE

Larval development of *Lightiella magdalenina* (Crustacea, Cephalocarida)

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Abstract The larval development of the newly discovered cephalocarid from Mediterranean sea, *Lightiella magdalenina*, was analysed using light and scanning electron microscopy. Twenty-nine larval specimens, divided into 15 metanaupliar and 2 juvenile stages of development, were found. The first six metanaupliar stages had an even number (6, 8, 10, 12, 14 and 16) of trunk segments including telson. The condition of 20 trunk segments, typical of the adult, was reached after another 4 stages by the addition of a single segment per stage. At this tenth stage, the larvae had an incomplete number of trunk limbs. Another five stages were needed to complete the cephalic appendage development, passing from the metanaupliar to the juvenile stage, characterized by loss of the naupliar enditic process of the second antenna. Trunk limbs development was completed during the last two juvenile stages. According to the ontogenetic data reported for *Hutchinsoniella macracantha* and *Lightiella incisa*, these seventeen stages probably do not represent the complete developmental series. Nevertheless, they allow us to clarify the main features of cephalocarid ontogeny and show specific differences in the development of both the trunk segments and appendages.

Introduction

Cephalocarids are small benthic crustaceans distributed from the intertidal to approximately 1,550 m deep. The first species described was *Hutchinsoniella macracantha* Sanders, 1955. Studies in various parts of the world subsequently led to the description of another ten species belonging to four genera, i.e. *Lightiella, Sandersiella, Hampsonellus*, and *Chiltoniella* (Jones 1961; Gooding 1963; Shiino 1965; Cals and Delamare Deboutteville 1970; Hessler and Sanders 1973; McLaughlin 1976; Knox and Fenwick 1977; Hessler and Wakabara 2000; Carcupino et al. 2006).

The genus *Lightiella* Jones, 1961, as all cephalocarids species, shows the following morphological features: horseshoe-shaped cephalon; 20-segmented trunk (9 thoracic and 11 abdominal segments including telson); abdomen without limbs; similar morphology of second maxilla and thoracopods; thoracopods biramous; thoracopods 9 strongly modified into small and subspherical appendages. Moreover, it differs from all the other genera in the reduced size of trunk segment eight, which also lacks thoracopods. At present, it is the most speciose of the cephalocarid genera, with five species: *L. serendipita* Jones, 1961 (from California), *L. incisa* Gooding, 1963 (from the Caribbean area to the northern Gulf of Mexico), *L. floridana* McLaughlin, 1976 (from the west coast of Florida), *L. monniotae* Cals and Delamare Deboutteville, 1970 (from New Caledonia) and *L. magdalenina* Carcupino, Floris, Addis, Castelli and Curini-Galletti, 2006 (from Sardinia island, Italy). The last species is the first cephalocarid reported from Europe and its discovery in the Mediterranean Sea fills a gap in the distribution of the genus and of the entire class.

Together with a specialization in their reproductive biology (they are simultaneous hermaphrodites with probable self-fertilization) and nervous system, Cephalocarida preserve several features similar to those of the hypothetical ancestral crustacean. Thus, since their first description, they have been considered the most primitive living crustaceans (Sanders 1957, 1963; Hessler 1964, 1984, 1992; Hessler

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and Newman 1975; Hessler and Elofsson 1992). The phylogenetic position of Cephalocarida is now debated. It has been considered: a sister group to Branchiopoda (Schran 1986) or to a higher taxon that includes Branchiopoda (Branchiopoda + Malacostraca, Hessler 1992) or (Branchiopoda + Maxillopoda, Walossek 1993, 1999, 2003a) or directed linked to maxillopod crustaceans (Ito 1989; Spears and Abele 1999; Lavrov et al. 2004).

One of the most primitive features was considered to be the very gradual development to the adult stage, which does not differ greatly from the larva. A free-living metanauplius with a three-segmented trunk and further 17 developmental stages have been reported for *H. macracantha* (Sanders 1963). A 4-segmented late embryo, which probably represents the first free-living larva, and another 12 stages have been reported for *L. incisa* (Sanders and Hessler 1964), whereas only 3 late larval stages are known for *L. serendipita* (Jones 1961). These findings revealed a pronounced anamorphic mode of growth characterized by a high number of postembryonic stages, which are probably much more numerous than actually known. Indeed, the authors hypothesized that, in all the above-mentioned species, the number of observed stages very likely representing an incomplete developmental series.

Different developmental series among species, as well as different numbers of trunk segments in the first free-living stages, could have taxonomic and phylogenetic value. Therefore, to clarify this matter and better understand the ontogenetic process of Cephalocarida, we examined larval and juvenile stages of *L. magdalenina* by means of light and electron microscopy.

Materials and methods

During the period October of 1999, June 2001, May–October 2004 and 2005, we collected 29 larvae and 1 ovigerous adult of *L. magdalenina*. Specimens were collected at 15–20 m depth from a muddy sand bottom, rich in organic material mostly consisting of leaf fragments of *Posidonia oceanica*, on the southern shore of the isle of S. Stefano, part of the La Maddalena Archipelago (Sardinia, Italy). Samplings were carried out by hand using SCUBA. In particular, two specimens were collected in June 2001; one in May 2005, three in September 2005 and all the other in October 1999, 2004 and 2005. Light and scanning electron microscopic (SEM) analyses were performed.

Light microscopy

Specimens were dissected to isolate the cephalic and trunk appendages. Each appendage was mounted on a separate slide using Faure medium. Micrographs were taken with a WELL AXIOSTAR PLUS microscope and a CANON POWER SHOT G6 digital camera. Each original micrograph was transformed into a drawing-like image with the option photocopy function of Microsoft PhotoDraw V 2.

Scanning electron microscopy (SEM)

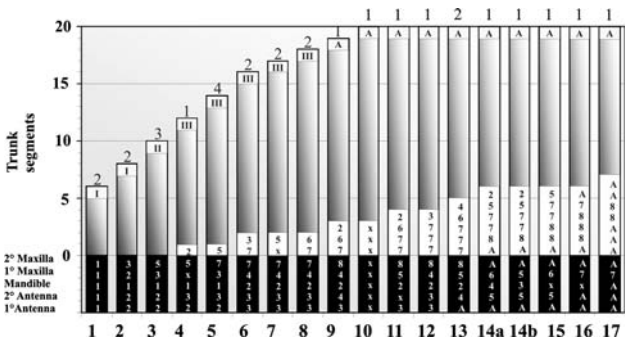
Specimens were fixed in formalin 4%, dehydrated in a graded ethanol series, dried in a Polaron Jumbo critical-point drier, sputter-coated with gold in an Edwards SI5A unit and observed with a ZEISS DMS 962 scanning electron microscope of the Electron Microscopy Centre, Sassari University.

Results

Seventeen developmental stages are identified among the 29 larval specimens collected. Many of the stages are known only from a single specimen (see Table 1).

A single ovigerous adult bearing only one egg on its right ninth limb has been found (October 2001) (Fig. 1a). The first 15 free-living larval stages have a metanaupliar appearance characterized by a well-developed naupliar enditic process on the second antenna. The last two stages are juveniles, in which the naupliar enditic process is no longer evident. Stages 1–6 are characterized by an even number of trunk segments including telson: 6, 8, 10, 12, 14 and 16 segments respectively (Fig. 1b–g). In the other four stages, the larvae have 17, 18, 19 and 20 trunk segments (Fig. 1h–m). At stage 10 (Fig. 1m), the larvae have only three pairs of trunk limbs. Another five stages are needed to complete the cephalic appendage development, passing from the metanaupliar to the juvenile stage. Trunk limbs

Table 1 Diagram in which is reported the number of larval stages (in abscissa), cephalic and trunk segments (in ordinate), specimens founded for each stage (numbers on the columns), developmental stages of each appendage (numbers 1–8, inside the columns) developmental stages of telson (roman numbers I-III, inside the columns). The vowel **A,** inside the columns, indicates the last developmental stage of both appendages and telson. The black portion of the columns is referred to the cephalic appendages, the white one to the trunk limbs



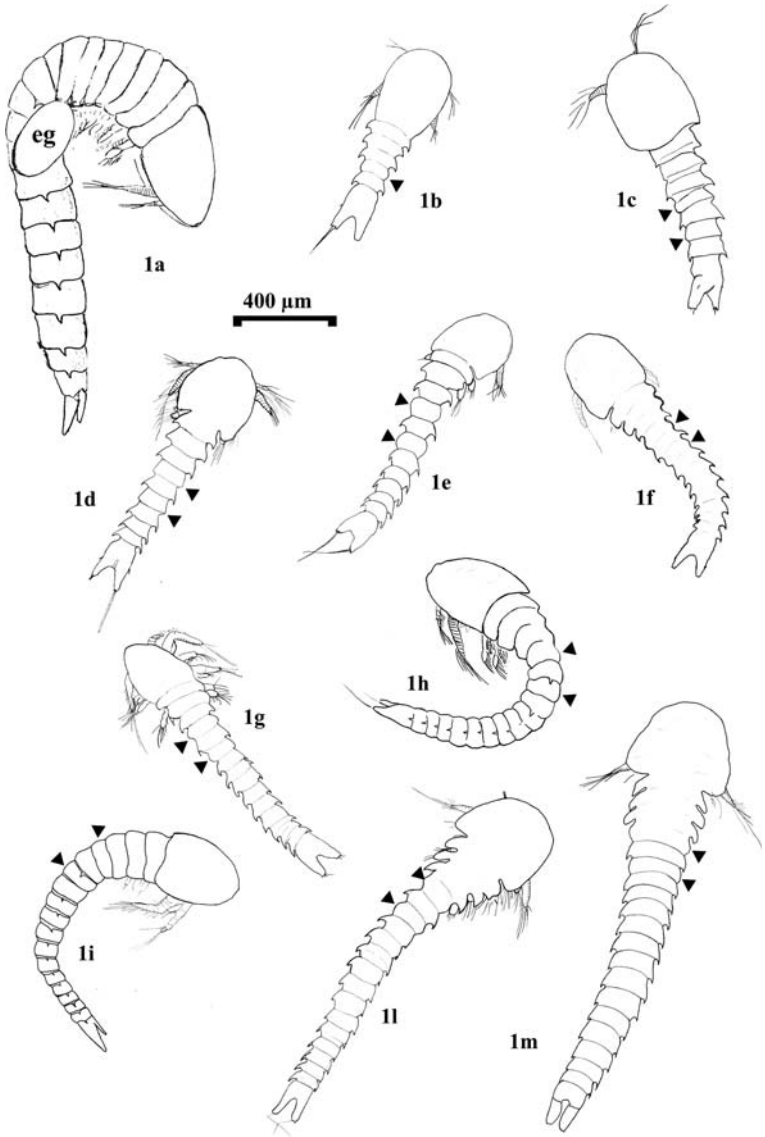


Fig. 1 Adult and larvae of *Lightiella magdalenina*. **a** Ovigeous adult bearing one egg (*eg*) on its ninth limb. Metanaupliar stages with 6 (**b**), 8 (**c**), 10 (**d**), 12 (**e**), 14 (**f**) 16 (**g**), 17 (**h**), 18 (**i**), 19 (**l**) and 20 (**m**) trunk segments, respectively. *Arrowhead* trunk segments without spines

development is completed during the juvenile stages, although the second juvenile stage that we found still has a well-developed eighth segment and lack of thoracopods 9.

To better schematize each larval stage, their description will be preceded by a detailed analysis of the ontogeny of both telson and appendages (cephalic and postcephalic limbs). The developmental stages of each appendage were reconstructed from the modification of its morphological features, without consideration of the larval stage to which it belonged. In this way, we considered the first stage of growth to be the one in which each appendage had the morphology most different from the adult form (for the adult morphology, see the original description of the species, Carcupino et al. 2006).

Telson: developmental stages 1–4

(**1**) Telson with two lateral small setae on the lateral margins; ventral comb and dorsal spinose processes absent (Fig. 2a). (**2**) As in preceding stage but a ventral comb present and small (Fig. 2b). (**3**) Lateral setae absent and ventral comb more developed (Fig. 2c). (**4**) As in adult with ventral comb completely developed and the two dorsal spinose processes present (Fig. 2d–e).

First antenna: developmental stages 1–4

(**1**) Six-segmented as in adult but setal formula: 0; 1; 1; 0, 1, 3 + 1 aesthetasc (Fig. 2f). (**2**) As in preceding stage but setal formula: 0; 2; 2; 0; 1; 3 + 1 aesthetasc (Fig. 2g). (**3**) As in preceding stage but setal formula: 0; 2; 3; 0; 1; 3 + 1 aesthetasc (Fig. 2h). (**4**) As in adult with setal formula: 0; 2; 4; 0; 0; 7 + 1 aesthetasc (Fig. 2i).

Second antenna: developmental stages 1–6

(**1**) Two-segmented endopod; 13-segmented exopod and 1-segmented finger-like enditic process. Enditic process, arising on the coxal segment of protopod, bears 1 lateral and 3 distal setae (Fig. 3a). Proximal segment of endopod with two or three distal setae; distal segment with two spines and three setae as in adult (Fig. 3a, inset). Basiopod

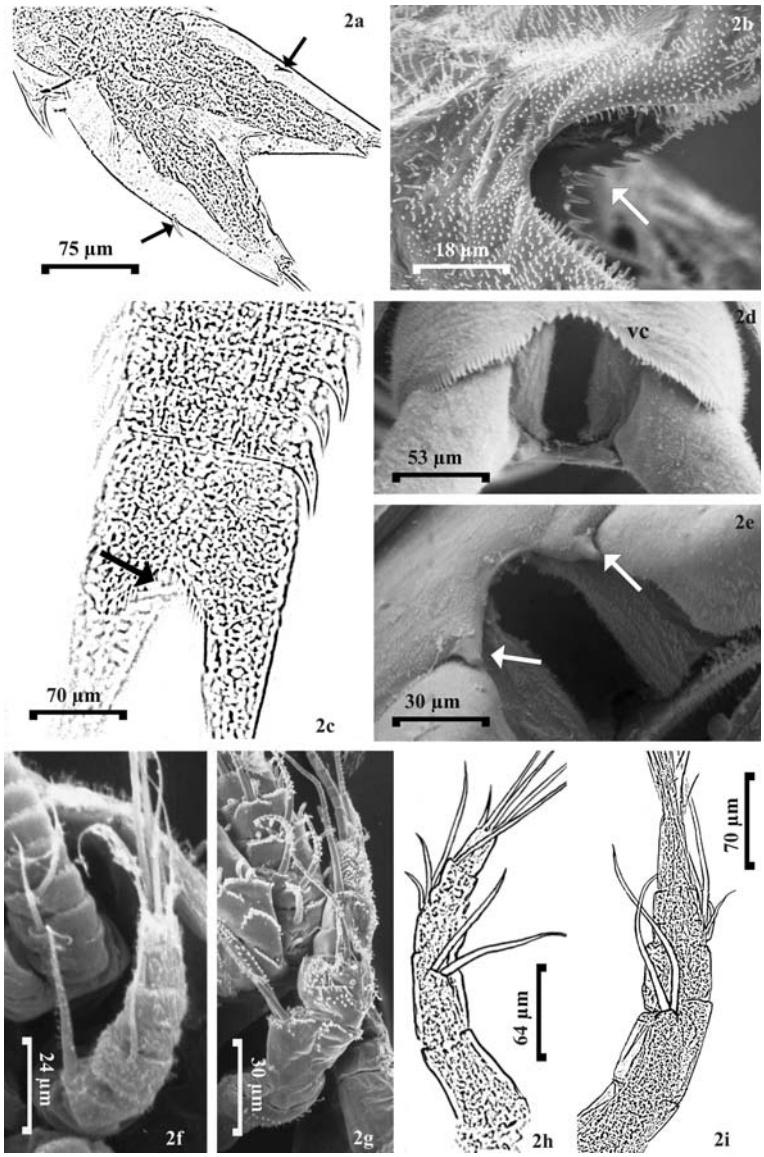


Fig. 2 Developmental stages of telson (**a–e**) and first antenna (**f–i**). **a** stage 1 lateral small setae (*arrows*) present; ventral comb and dorsal spinose processes absent. Stages 2 (**b**) and 3 (**c**) lateral setae not more visible, ventral comb (*arrow*) present but not completely developed, dorsal spinose processes still absent. **d–e** Stage 4 as adult morphology with both ventral comb (*vc*) and spinose processes (*arrows*) completely developed. Stages 1–4 6-segmented as in adult but setal formula of: 0; 1; 1; 0,1, 3 + 1 aesthetasc (**f**); 0; 2; 2; 0; 1; 3 + 1 aesthetasc (**g**); 0; 2; 3; 0; 1; 3 + 1 aesthetasc (**h**); 0; 2; 4; 0; 0; 7 + 1 aesthetasc (**i**)

with two setae (Fig. 3a). (**2–4**) As preceding stage but exopod of 14 (Fig. 3b), 15 (Fig. 3c) and 16 (Fig. 3d) segments, respectively. (**5**) As preceding stage but exopod with 18 segments and enditic process reduced and bearing only a distal seta (Fig. 3e). (**6**) As in adult with enditic process and setae on the basiopod absent (Fig. 3f).

Mandible: developmental stages 1–5

(**1**) Well developed palp consisting of a seven-segmented exopod and a large bilobate endopod (Fig. 4a). Proximal segments one and two of exopod without setae, segments 3–6 bearing one long seta on their inner corner and distal segment with two long apical setae (Fig. 4a). Distal lobe of endopod with three apical setae consisting of a large, smooth and conical basal part and a longer thin and plumose distal part (Fig. 4b). Two small and smooth setae are located on the anterior face of the palp at the base of the above mentioned setae. One more additional seta is also present on the superior margin of the lobe (Fig. 4a, b). Proximal lobe as distal one but the additional seta on the lobe margin (Fig. 4d). Basis with six setae on its inner margin. Gnathic process with well distinguished incisor and molar processes. Incisor process bearing one big pointed tooth and one small plumose seta (Fig. 4c). An additional seta, which persists in the following stages except in adult, is located on the anterior margin of the incisor process (Fig. 4e–f). Molar process as in adult with numerous small teeth (Fig. 4a, c). (**2**) Palp as in preceding stage but exopod six-segmented and bearing three setae on the distal segment (Fig. 4d). (**3**) Palp as in preceding stage but exopodal distal segment with only two setae (Fig. 4e) and proximal lobe of endopod with only two setae (Fig. 4e). (**4**) Palp very reduced; exopod and endopod no longer distinguishable. Incisor process as in preceding stages (Fig. 4f). (**5**) As in adult: palp absent; incisor processes lacking of the anterior

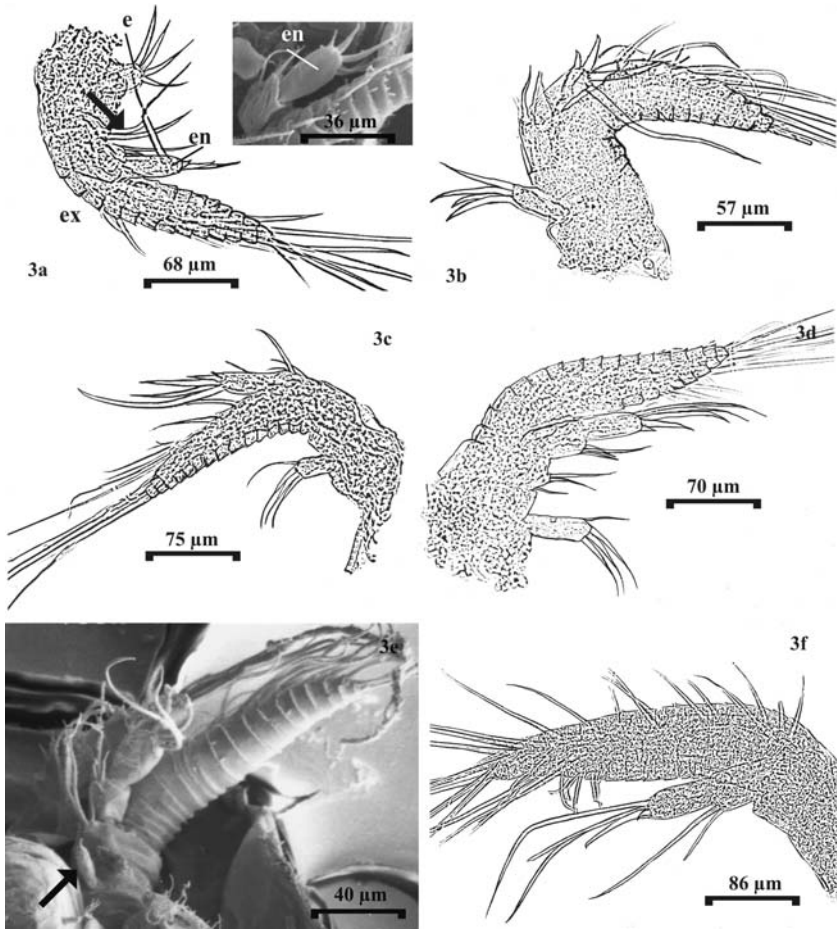


Fig. 3 Developmental stages of second antenna. **a** Stage 1 13-segmented exopod (ex) 2-segmented endopod (en) (better visible in the *inset*), well developed enditic process **e** bearing four apical setae and basis of protopod with two setae (*arrow*). **b-d** Stages 2–4 as preceding stage but exopod of 14 (**b**) 15 (**c**) and 16 (**d**) segments, respectively. **e** Stage 5 exopod 18-segmented, enditic process (*arrow*) reduced and bearing only one distal seta. **f** As in adult; exopod 19-segmented, enditic process and setae on the protopod basis absent

seta and bearing two teeth with one small seta in between (Fig. 4g).

First maxilla: developmental stages 1–7

(**1**) One-segmented exopod with eight distal setae of different lengths: three long setae on the outer margin, two long setae apically located, and three setae of decreasing size on the inner margin (Fig. 5a). Endopod externally subdivides in three segments, the second of which appears internally divided into two parts. Proximal segments one and two bear two lateral setae each. Distal segment with two apical claws (Fig. 5a). Protopod with two endites bearing two or three setae each (Fig. 5a). (**2**) Exopod with eight setae of about equal length (two of which have been lost in the specimen of Fig. 5b). Four-segmented endopod. Segments 1 and 3 bear two setae, segment 2 has one seta and distal segment has two apical claws. Protopodal endites bearing four or five setae (Fig. 5b). (**3**) As preceding stage but the first three segments of endopod bear two setae each (Fig. 5c). (**4**) As preceding stage but with three claws on distal segment of endopod (Fig. 5d). (**5**) Very similar to the preceding stage but a small developing gnathobase with two distal setae starts to be visible (Fig. 5e). (**6**) Gnathobase more developed and bearing four apical setae, exopod more elongated than preceding stage, segments 1 and 2 of endopod with only one seta (Fig. 5f). (**7**) Adult-like morphology with a three-segmented endopod but segment 3 with three apical and one lateral setae (Fig. 5g). Gnathobase well developed as in adult (Fig. 5h).

Second maxilla and thoracopods 1–5: developmental stages 1–9

(**1**) Rudimentary three-lobate structure with endopod and exopod unsegmented. Pseudepipod small and triangular in shape. Protopod without endites (Fig. 6a). (**2**) Endopod still unsegmented with only small apical claw. Exopod with two not well-distinguished segments; distal segment with only three or four setae, proximal segment without setae. Pseudepipod with only one small distal claw-like seta. Protopod as in preceding stage (Fig. 6b). (**3**) Endopod as in preceding stage, exopod better segmented and with distal segment bearing six apical setae of different length. Pseudepipod with two setae. Protopod as in preceding stage (Fig. 6c). (**4**) Endopod three-segmented; proximal segments 1 and 2 without setae and much smaller than distal segment which bears one claw. Exopod two-segmented; distal segment with seven setae (one of which have been lost in the specimen of Fig. 6d), proximal segment as in preceding stage. Pseudepipod with three setae (Fig. 6d). (**5**) Endopod five-segmented as in adult but with only one claw on distal

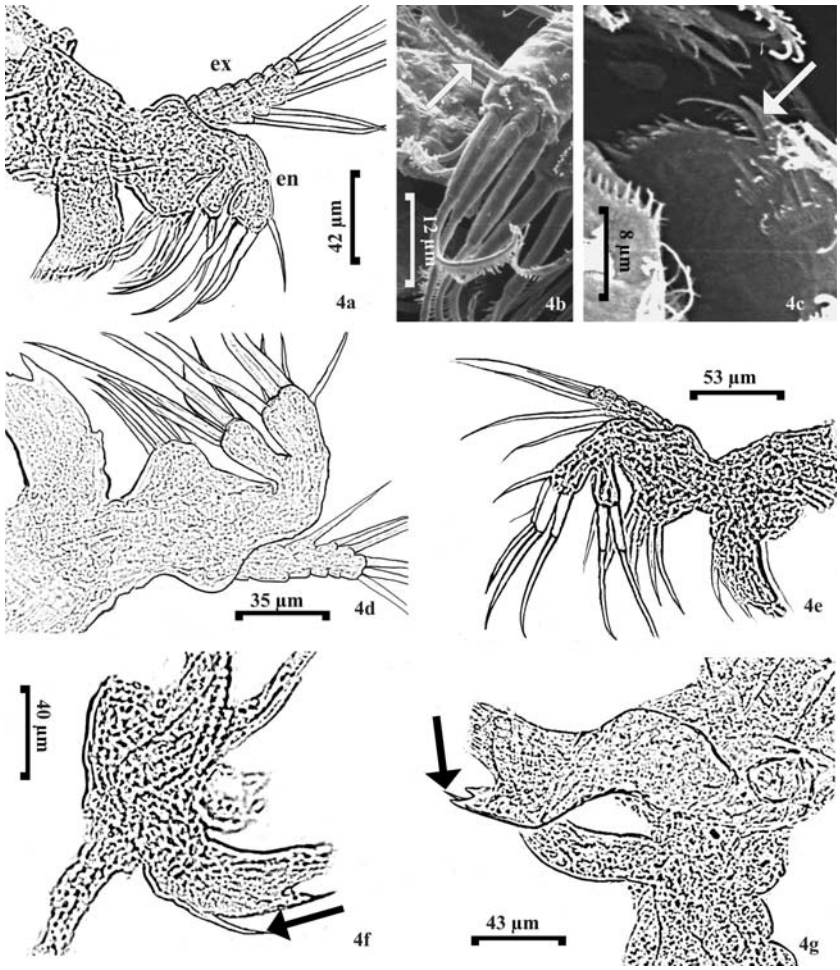


Fig. 4 Developmental stages of mandible. **a-c** Stage 1 palp well developed and consisting of a large bilobate endopod (*en*) and a 7-segmented exopod (*ex*), bearing one long seta on segments 3–6 and two setae on distal segment. **b** Scanning electron micrograph of distal lobe of endopod showing the three complex setae and one small seta (*arrow*). **c** Scanning electron micrograph of incisor process with only one tooth (*arrow*) and one plumose seta. **d** Stage 2 as in preceding stage but exopod 6-segmented in which the distal segment bears three setae. **e** Stage 3 as preceding stage but exopod bears only five setae and proximal lobe of endopod with only two complex setae. **f** Palp very reduced with exopod and endopod not more distinguishable and gnathic process as preceding stage. The *arrow* shows the long seta of the incisor process anterior margin, which is present but not clearly visible in the figures of the all preceding stages. **g** Stage 4 as in adult: palp absent; incisor process lacking of anterior seta and bearing two teeth with one small seta in between (*arrow*)

segment. Exopod as in preceding stage. Pseudepipod with four setae as in adult. Protopodal endites start to be formed, but they do not bear setae (Fig. 6e). (**6**) Endopodal proximal segments 1–3 bearing one or two setae on their inner corner. Segment 4 with one seta on its outer corner and segment 5 with one apical claw. Exopodal distal segment with only seven or eight setae. At this stage, the fifth seta is smaller than the others (as in the adult), so that the distal group of four setae is evidenced. Proximal segment with one seta on the inner margin. Protopodal endites developed and bearing setae (Fig. 6f). (**7**) Distal segment of exopod with 9–11 setae, proximal exopod segment with one seta on both inner and outer lateral margins. Distal endopod segment with two claws, segment 4 with two setae on its outer corner and one small seta on its inner corner (Fig. 6g). (**8**) As in preceding stage but with three apical claws on distal segment of endopod (Fig. 6h). (**9**) As adult morphology, with four apical claws on distal segment of endopod and 12–15 apical setae on distal segment of exopod (Fig. 6i).

Thoracopod 6; developmental stages 1–6

This limb is slightly smaller than the previous legs. Moreover its adult morphology is characterized by only one claw on the distal segment of endopod, so that its development lack of stages 7–9.

Thoracopod 7

Because in the only specimen in which the seventh limb was present, it had an adult morphology, no information are available on its developmental stages.

Larval stages

For each of the 17 larval stages, we report the specimen's number, data of sampling, number of postcephalic segments, total length and trunk segment morphology. The developmental stage of the cephalic and postcephalic appendages and telson are summarized in Table 1.

Metanaupliar stages with incomplete number of trunk segments

Stage 1. Two specimens; September 2005, October 2005. About 0.82 and 0.80 mm in total length, respectively. Six postcephalic segments. Trunk segment without limbs and

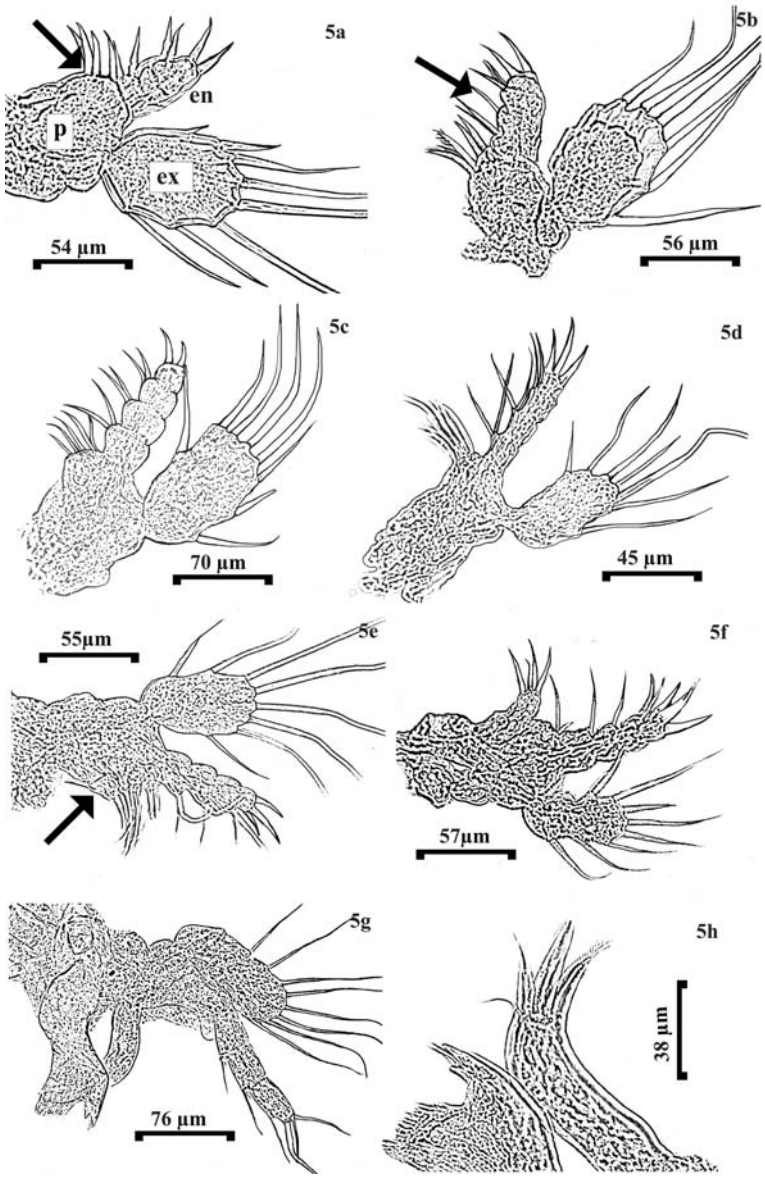


Fig. 5 Developmental stages of 1° maxilla. **a** stage 1 protopod (*p*) with two endites (*arrow*), gnathobase absent. 1-segmented exopod (*ex*) and 3-segmented endopod (*en*). Segments 1, 2 bear two setae, distal segment bears two claws. **b** Stage 2 as preceding stage but 4-segmented endopod. Segment 2 bears only one setae (*arrow*). **c** Stage 3 as preceding stage but segments 2 of endopod bears two setae. **d S**tage 4 as preceding stage but with three claws on distal segment of endopod. **e** Stage 5 a small gnathobase (*arrow*) with two distal setae stars to be visible. **f** Stage 6 as preceding stage but with gnathobase more developed and bearing four apical setae; exopod more elongated. **g** Stage 7 like adult morphology with three-segmented endopod. First two segments bear one seta, third segment with four setae. Gnathobase well developed, protopodal endites absent. **h** High magnification of gnathobase

pleurae. Trunk segments 1, 3 and 5 with a pair of lateral large spines. Trunk segments 2 and 4 with small spines and without spines respectively (Fig. 1b).

Stage 2. Two specimens; September 2005, October 2005. About 1.1 mm in total length. Eight postcephalic segments. Trunk segment without limbs and pleurae. Trunk segments 1, 3, 5 and 7 with a pair of lateral large spines. Trunk segments 2 and 4 as in preceding stage. Trunk segment 6 without spines (Fig. 1c).

Stage 3. Three specimens; October 1999, 2004 and 2005. About 1.13, 1.18 and 1.24 mm in total length, respectively. Ten postcephalic segments. Trunk segments without limbs and pleurae. Trunk segments 1, 3, 5, 7–9 with large spines. Trunk segments 2, 4 and 6 as in preceding stage (Fig. 1d).

Stage 4. One specimens; October 2004. About 1.2 mm in total length. Twelve postcephalic segments. First trunk segment with limbs and pleurae. Trunk segments 3, 5, 7–11 with spines. Trunk segments 2, 4 and 6 as in preceding stage (Fig. 1e).

Stage 5. Four specimens; 3 in September 2005, 1 in October 2004. About 1.2, 1.0, 1.3. and 1.25 mm in total length. A total of 14 postcephalic segments. First trunk segment with limbs and pleurae. Trunk segments 3, 5, 7–13 with spines. Trunk segments 2, 4 and 6 as in preceding stage (Fig. 1f).

Stage 6. Two specimens; October 2004. About 1.25 and 1.3 mm in total length. A total of 16 postcephalic segments. First two trunk segments with limbs and pleurae. Trunk segments 3, 5, 7–15 with spines. Trunk segments 4 and 6 without spines (Fig. 1g).

Stage 7. Two specimens; June 2001, October 2004. About 1.4 and 1.35 mm in total length. A total of 17 postcephalic

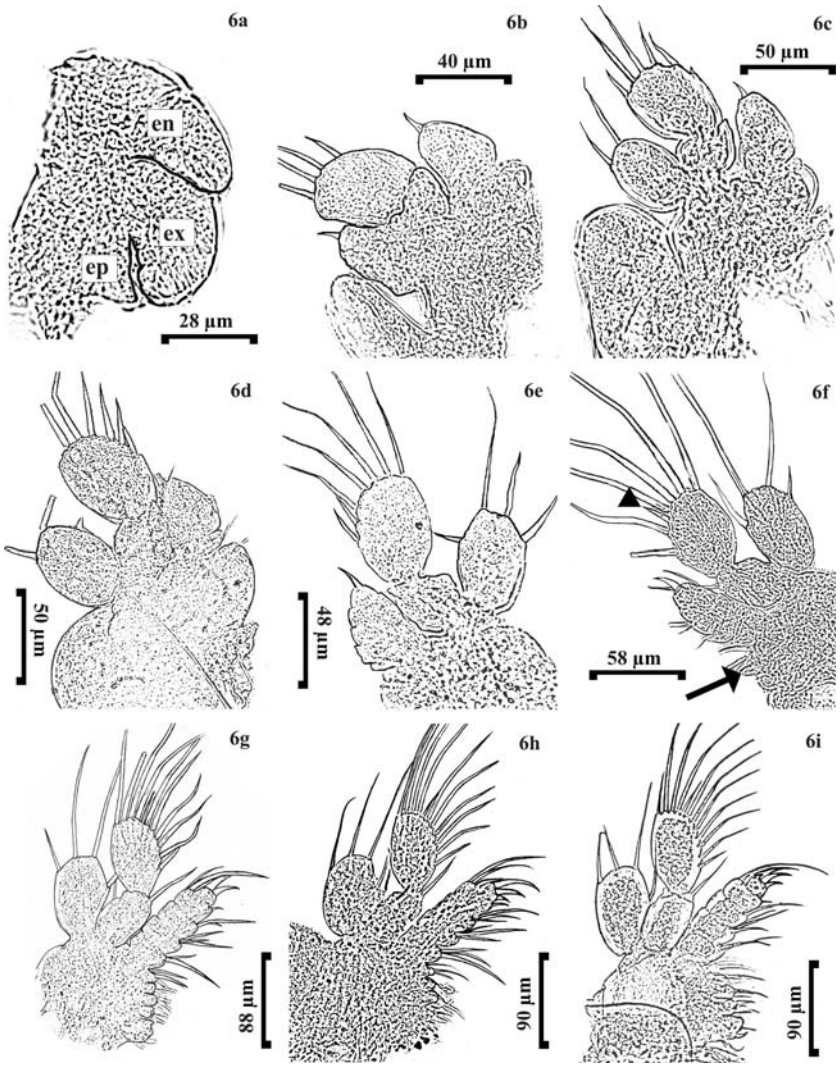


Fig. 6 Developmental stages of 2° maxilla and thoracopods 1–5. **a** Stage 1 Endopod (*en*) and exopod (*ex*) 1-segmented and without setae. Small triangle-like shaped pseudepipod (*ep*). Protopod without endites. **b** Stage 2 endopod 1-segmented with one small claw-like seta. Exopod with two but not well distinguished segmentes; distal segment with four setae. Pseudepipod with one small distal seta. **c** Stage 3 endopod as preceding stage; exopod better segmented and distal segment with 6 setae. Pseudepipod with two setae. **d** Stage 4 endopod three-segmented with one claw on distal segment. Exopod two-segmented; distal segment with seven setae. Pseudepipod with 3 setae. **e** Stage 5 Endopod five-segmented as in adult but with only one claw on distal segment. Exopod as preceding stage. Pseudepipod with four setae. Protopodal endites star to be formed. **f** Stage 6 endopod as preceding stage. At this stage the fifth distal seta (*arrowhead*) of exopod appears smaller than the others. Pseudepipod as in adult. Protopodal endites better developed and bearing setae (*arrow*). **g** Stage 7 as preceding stage but exopod with more numerous setae on distal segment, and endopod with only two claws on distal segment. **h** Stage 8 as preceding stage but with three claws on endopod distal segment. **i** Stage 9 as adult morphology

segments. First two trunk segments with limbs and pleurae. Trunk segments 3, 5, 7–16 with spines. Trunk segments 4 and 6 without spines (Fig. 1h).

Stage 8. Two specimens; June 2001, October 2004. About 1.55 mm in total length. A total of 18 postcephalic segments. First two trunk segments with limbs and pleurae. Trunk segments 3, 5, 7–17 with spines. Trunk segments 4 and 6 without spines (Fig. 1i).

Stage 9. One specimen; October 2004. About 1.7 mm in total length. A total of 19 postcephalic segments. First three trunk segments with limbs and pleurae. Trunk segments 5, 7–18 with spines. Trunk segments 4 and 6 without spines (Fig. 1l).

Metanaupliar stages with 20 trunk segments as in adult but enditic process of the 2nd antenna still present

Stage 10. One specimen; May 2001. 1.86 mm in total length. First three segments with trunk limbs and pleurae. Trunk segments 5, 7–19 with spines. Trunk segments 4 and 6 without spines. The cephalic and trunk appendages were too damaged to provide data (Fig. 1m).

Stage 11. One specimen; October 2004. About 1.76 mm in total length. First four segments with trunk limbs and pleurae. Trunk segments 5, 7–19 with spines. Trunk segment 6 without spines.

Stage 12. One specimen; October 2004. About 1.9 mm in total length. As preceding stage but limbs 4 in a more advanced stage of development (Table 1).

Stage 13. Two specimens; October 2004. About 1.73 and 1.9 mm in total length. First five segments with trunk limbs and pleurae. Trunk segments 7–19 with spines. Trunk segment 6 without spines.

Stage 14a, b. One specimen each; October 2004. About 1.90 mm in total length. Both specimens have the first six trunk segments with limbs and pleurae and the trunk segments 7–19 with spines. However, they differ in the developmental stage of mandible and first maxilla (Table 1).

Stage 15. One specimen; October 2004. About 1.7 mm in total length. As the preceding stages but limbs 5 and 6 in a more advanced stage of development (Table 1).

Juveniles stages with 20 trunk segments as in adult and enditic process of the 2nd antenna absent

Stage 16. One specimen; October 2004. About 1.90 mm in total length. First six trunk segments with limbs and pleurae. Segments 7–19 with spines.

Stage 17. One specimen; October 2004. About 1.90 mm in total length. First seven trunk segments with limbs and pleurae. Eighth trunk segment still well developed but without spine and trunk limb 9 not present. Trunk segments 9–19 with spines.

Discussion and conclusions

Cephalocarida species seem to be exceedingly rare and have a very narrow distribution, with the exception of *H. macracantha* and *L. incisa*, which are known for a large number of specimens and for several localities (Wakabara 1970; Hessler and Sanders 1973; Saloman 1978; Stoner 1981; Heard and Goeke 1982; De Troch et al. 2000; Martin et al. 2002). *L. magdalenina*, like most cephalocarid species, is currently known from a single locality, S. Stefano Isle in the La Maddalena Archipelago. At this locality, less than 50 specimens (larvae and adults) have been found during more than 2 years of sampling. Thus, the 17 ontogenetic stages reported here are described for a few specimens. Despite this low number of individuals, all stages found show the same abundance, so that each stage can be considered to represent a real step in the development of the species. Therefore, these results help to clarify both major and minor aspects of the mode of development of Cephalocarida, for which previously reported data only regard *H. macracantha* (Sanders 1963), *L. serendipita* (Jones 1961) and *L. incisa* (Sanders and Hessler 1964). However, the low number of specimens found, and their concentration in only 1 month of the year (October), probably due to an occasional and particularly fortunate sampling, does not permit any conclusion on the reproductive period and life cycle of *L. magdalenina.*

Larval stages with 3, 5, 7, 9, 11, 14, 15, 16, 17, 19 and 20 trunk segments have been described in *H. macracantha* and with 8, 14, 16, 17, 18 and 20 in *L. incisa*. Three late larvae all with 20 segments are only known for *L. serendipita.* Moreover, in all three species, the condition of 20 segments, typical of adults, is reached in larvae with an incomplete series of limbs. Therefore, numerous other stages, all with 20 segments, are recognizable.

Two main hypotheses about Cephalocarida ontogeny have been proposed on the basis of these findings. The first involves the addition of two segments at each moult during the early stages of development, followed by other stages characterized by the addition of a single segment, until the achievement of the 20 segments typical of adults (Sanders 1963). Later, Sanders and Hessler (1964) considered the addition of only one segment as an occasional event and supported a normal mode of development characterized at each moult by the addition of two segments.

In *L. magdalenina*, the occurrence of metanaupliar stages with 6, 8, 10, 12, 14 and 16 trunk segments, including telson, confirms that the larval stages with 6, 10 and 12 segments hypothesized in *L. incisa* by Sanders and Hessler (1964), really exist. Moreover, the total absence of larvae with 5, 7, 9, 11, 13 and 15 segments in both species suggests that at least the first developmental stages of Cephalocarida are really characterized by the addition of two segments at each moult. As mentioned above, two segments were also reported to be added during the first developmental stages of *H. macracantha* (Sanders 1963). In this species, however, the first five metanaupliar stages show a different number of segments (3, 5, 7, 9 and 11 segments including telson). This difference is likely due to a different first free-living stage, reported to be three-segmented in *H. macracantha* and four-segmented in *L. incisa* (Sanders 1963; Sanders and Hessler 1964).

The larval development of *L. magdalenina* continues with the addition of a single segment, so that larvae with 17, 18, 19 and 20 segments are recognizable. Larvae with 15, 16, 17 and 19 segments, preceding those with 20 segments, were also reported for *H. macracantha* (Sanders 1963). These data appear to demonstrate that the addition of a single segment is not an occasional event in Cephalocarida development, as proposed by Sanders and Hessler (1964).

Moreover, in *L. incisa* the trunk limbs development is described to occur in only two steps: (1) rudimentary and (2) completely developed (Sanders and Hessler 1964). On this base, Sanders and Hessler (1964) hypothesized at each moult is "the addition of two postcephalic segments, the completion of one rudimentary limb, and the beginning of the next". Although in *L. magdalenina* each limb does not show a regular sequence of development including all nine morphological stages here described, they need much more than two moults to reach the adult morphology (as shown in Table 1). More than two moults are also needed to complete development of *H. macracantha* limbs. In this species, the second maxilla and all trunk limbs are reported to develop in three main steps: (1) bifurcate lobe devoid of spines and setae; (2) like the adult, but with incomplete number of both endites and setae of protopod and endopod; (3) fully developed. However, an additional stage, consisting of three-ramous appendages with both exopod and endopod unsegmented, is reported for trunk limbs 6 and 7 (Sanders 1963).

From these data, we can infer that the ontogenetic process of the known Cephalocarida species, including *L. magdalenina*, is longer and much more gradual than previously described. In *H. macracantha*, there is likely an additional stage with 13 segments, between stage 5 (with 11 segments) and stage 6 (with 14 segments), as well as another larval stage with 18 segments is between stage 9 (with 17 segments) and stage 10 (with 19 segments). Both these additional stages were hypothesized by Sanders (1963). The occurrence in *L. incisa* of a late embryo with 4 trunk segments (Sanders and Hessler 1964) suggests that the metanauplius with 6 segments could be preceded by a first stage with 4 segments also in *L. magdalenina*. The larval stage with 19 segments, reported here for *L. magdalenina* but not found in *L. incisa*, should also be present in the latter species. According to these data, *H. macracantha* should have at least 20 stages, two more than the 18 described, *L. incisa* should have at least 17 stages, 5 more than the 12 described, and *L. magdalenina* should have much more than 17 stages here reported. In this latter species in fact, the seventh limb is present only in the second juvenile stage, where it is completely formed. The existence of additional stages with this limb incompletely developed is therefore likely. Moreover, the second juvenile stage still has a well-developed segment 8 and lack the ninth pair of limbs. One or more additional stages characterized by the reduction of segment 8 and formation of ninth limbs must be present.

The same basic developmental pattern is also shown by the delayed anlagen of thoracopods which appear, in all species, several stages later than their corresponding segments.

In *H. macracantha*, the first pair of trunk limbs appears at the third stage of development, corresponding to larvae with seven trunk segments (Sanders 1963). In *L. magdalenina*, the first trunk limbs appear at stage 5, in larvae with 12 segments while in *L. incisa*, these limbs are first reported in larvae with 14 segments; however, since larvae with 12 segments were not found in this latter species (Sanders and Hessler 1964), it is arguable that the first pair of limbs appears at the same 12-segmented stage in both *Lightiella* species.

All the above mentioned data show that within a general developmental scheme, major and minor differences can be observed at both the genus and species levels. One of the most obvious differences among genera involves the first free-living stage, which is three-segmented in *H. macracantha* while is supposed to be 4-segmented in both *L. magdalenina* and *L. incisa*. Both the higher number of trunk segments at hatching and the delayed anlagen of limbs could be related to the minor numbers of trunk appendages that have to be formed. In fact, *Lightiella* species have one less pair of limbs than *H. macracantha*. This hypothesis could be verified by data on the larval development of other species, such as those of the genera *Sandersiella, Hampsonellus and Chiltoniella*, which share with *H. macracantha* eight pairs of well-developed trunk limbs.

Other differences at the genus level seem to be the number of eggs laid and the number of trunk segments bearing lateral spines.

Two eggs are reported for *H. macracantha* (Sanders 1963), whereas two eggs seem to be laid only occasionally by *Lightiella* species. A single egg sac was reported in *L. incisa* by Gooding (1963), De Troch et al. (2000) and Martin et al. (2002). Two egg sacs were only found in 1 of 17 ovigerous specimens of the same species examined by Sanders and Hessler (1964). In *L. magdalenina*, the single ovigerous specimen collected had one egg. Moreover, in *L. incisa* the single ovisac seems to appear to the opposite size of the only one developed ovary (Sander and Hessler 1964).

In *H. macracantha*, all trunk segments show lateral spines, which are replaced by pleurae when the limbs develop, whereas not all segments bear spines in *L. incisa*, *L. magdalenina* and *L. serendipita*. The absence of spines seems to have a species-specific value, as there is a different status in the three *Lightiella* species. For *L. serendipita*, Jones (1961) reported the absence of spines on segments 6 and 8 and hypothesized their absence on segments 2 and 6. Sanders and Hessler (1964) considered Jones' hypothesis justified by the documented lack of spines in segments 2, 4, and 6 in *L. incisa*. However, the two species differ in segment 8: it lacks spines or pleurae in both larvae and adults of *L. serendipita*, whereas it initially shows spines (then replaced by pleurae) in *L. incisa*. Finally, in *L. magdalenina*, segment 2 has a small spine and segments 6 and 4 are smooth; as in *L. incisa*, segment 8 has spines, which are then replaced by pleurae.

Differences at the species level regard the ontogeny of the mandibular palp exopod and first maxillary endopod. In *L. incisa* and *L. magdalenina*, the development of these structures is characterized by reduction of the segment numbers, whereas in *H. macracantha* they seem to have a fixed number of segments. The latter species has the first maxilla with a four-segmented endopod in all stages. The mandibular palp maintains a six-segmented exopod in all the stages preceding its disappearance (Sanders 1963). In *L. incisa*, a six-segmented exopod is present only in the early metanaupliar stages, after which it is reduced to five segments in the middle and late stages (Sanders and Hessler 1964). In *L. magdalenina*, the exopod shows a reduction from seven to six segments.

Comparing the Cephalocarida anamorphic development with that of some extant and fossil taxa, such as anostracans (Benesch 1969; Fryer 1983), copepods (Dahms 1987, 1990, 1992, 2000) and, in particular, with the "Orsten" branchiopodan *Rehbachiella kinnekullensis* Müller, 1938 (Walossek 1993, 1995; Walossek and Maas 2005), it is apparent that it shows considerable deviation from a regular anameric pattern.

Cephalocarida show a strong reduction of the early stages. They hatch with a fairly advanced free-living stage respect to the orthonauplius (bearing only three pairs of appendages) showed by most of the crustacean taxa (Dahms 2000). The early phase of the "Orsten" *R. kinnekullensis* comprised four naupliar instars, starting with a (ortho)nauplius, and ending, after a series of three successive instars, at the metanauplius three, in which the segment of maxilla is still free from the shield (Walossek 1993, 1995). Anostracans, like *Artemia salina* L., show a slight reduction of early naupliar stages, with the addition of three postmandibular segments at the second moult, whereas the first maxilla bud, together with one additional segment, is developed at the third moult (Benesch 1969). In the postembryonic phase of copepods, like *Drescheriella glacialis* Dahms and Dieckmann, 1987 and other harpaticoids, one additional pair of appendages are added at each consecutive five stages (naupliar stages N I–VI). From a orthonauplius, the maxillule precursors develop at N II, maxillae at N III and, before the first copepodid stage, one pair of appendages are developed at each naupliar stage (Dahms 1987 1990, 1992, 2000).

Reduction in the Cephalocarida ontogeny is also recognizable in the somites addition, that occurs, at least in the first phase, in "jumps" (two segment at each moult). Jumping in somites and limbs addition is also present in other crustacean taxa such as Malacostraca, but in a quite different way. Eumalacostracans taxa (e.g. decapoda) show different larval phases, in which sets of somites and limbs are added simultaneously (Cockcroft 1985). Walossek and Maas (2005) interpreted these developmental "jumps" as an independent abbreviation of ontogeny, during which original larval instars were skipped for reaching the adult phase more rapidly. This interpretation could be correct for malacostracans, but not for cephalocaridans, where the adult phase is reached after a very gradual development, particularly after the formation of 13 trunk segments. Moreover, addition and functionality of limbs is extremely delayed. When the addition of both thoracic and abdominal segments is finished, only three trunk limbs are present. So, it is apparent and noteworthy that in Cephalocarida the abdominal and thoracic somites form in a parallel way. Moreover, at least in *L. magdalenina*, the second maxille and the first five pairs of limbs need of nine developmental instars to reach their adult morphology and, likely, their functionality.

No delay or jump can be recognized in the formation of in both segments and limbs, in the "Orsten" *R. kinnekullensis*. At the end of the naupliar phase and because of the formation of somites in two steps, in this species, 13 trunk segments (12 of them bearing limbs) developed after a series of 26 successive moults. During the same series, limbs appeared at the second developmental step of the corresponding segment (Walossek 1993, 1995). Only one somites is added per each moult in *A. salina* and in copepods. Both these taxa show some delay in the development of postmandibular limbs, and the abdominal somites develop only after the formation of all thoracomers and their limbs. In particular, in *A. salina*, at the end of the larval phase that is characterized by the formation of 13 thoracomers (all bearing limbs), a subsequent postlarval phase occurs. During this latter phase, six abdominal segments form, the naupliar limbs modify and the genital segments acquire their sexual features.

A postlarval phase has been supposed to exist even in the fossil *R. kinnekullensis*. The latter instar (30th stage) found and described is still considered to be a larval stage. Moreover, the absence of larger and adult records has been correlated to the selectivity of the "Orsten"-type preservation, that only catches small size meiofaunal biota (Walossek 1993, 1995, 2003a, 2003b; Mass et al. 2006).

In conclusion the present data allow us to reject the hypothesis of the addition of only one segment as an occasional event in the Cephalocarida development, and confirm a general scheme of growth with two different phases. An initial phase characterized by the addition of two segments per stages, followed by a second phase during which only a single segment is added. Our data also demonstrate that the Cephalocarida low and anamorphic development consists of more numerous stages than those previously reported, even if the exact number of instars remain still unknown. In spite of that, several features such as the numerous "jumps" in the somites addition and the contemporary formation of both thoracic and abdominal somites, makes it as not the longest, most complete and pleisomorphic developmental series among Crustacea.

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Running head: Molecular data of *Lightiella magdalenina*

Molecular data on two mitochondrial genes of a newly discovered crustacean species (*Lightiella magdalenina*, Cephalocarida).

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ABSTRACT

Cephalocarids are a rare and poorly known class of small benthic crustaceans, consisting of only eleven species belonging to five genera. Up to present, only one species *Hutchinsoniella macracantha,* have been studied at molecular level. We report the partial sequences of two mitochondrial genes of relevant importance for phylogenetic analysis (cytochrome c oxidase I and cytochrome b) from the newly discovered Mediterranean species. *Lightiella magdalenina.* A reduced median network analysis clarified the genetic relationships between the two cephalocarid species.

Cephalocarids are a class of small benthic crustaceans distributed from the intertidal zone to approximately 1550m depth. At present, only eleven species belonging to five genera have been described.. *Lightiella magdalenina* is the most recently described species from La Maddalena Archipelago (Sardinia, Italy)(41°13'N; 9°25'E), (41^{o} 11'24.61" N; 09^{o}23'55.44"E) characterized by a elevated degree of endemism. No other species have been reported so far in Europe, and its finding in the Mediterranean sea (Carcupino *et al.,* 2006) fills a gap in the world distribution of the entire class. Its type locality is characterized by a muddy sand bottom very rich in organic matter with little seagrass beds. Since its first description (Sanders, 1955), Cephalocarida was considered the most primitive living crustacean class, but, at present, it remains a poorly known taxon, and its phylogenetic position is debated. Molecular data regard only one species, *Hutchinsoniella macracantha*, and refer to a complete mitochondrial genome (Lavrov *et al.,* 2004), to two mitochondrial genes (Giribet *et al.,* 2001) and to six nuclear genes (Spears & Abele, 1997; Colgan *et al.,* 1998; Regier & Shultz, 1998; 2001; Shultz & Regier, 2000; Giribet *et al.,* 2001; Richter *et al.,* 2007). However, molecular analysis did not provide unquestionable results in terms of phylogenetic relationships, since, even limiting the survey to mtDNA, some Authors tentatively related Cephalocarida with Remipedia (Giribet *et al.,* 2001), with Maxillopoda and Pentastomida (Lavrov *et al.,* 2004), and with Copepoda (Hassanin, 2006).

The aim of this short communication is to provide to the scientific community molecular data on the new species of this rare and poorly known

crustacean class, by sequencing two mitochondrial genes selected for their value in phylogenetic analysis: the cytochrome c oxidase subunit I (COI) and cytochrome b (Cyt-b). The first gene has been proposed by Hebert *et al.* (2003) as a sort of genetic "barcode", demonstrating that it can serve as the core of a global bioidentification system for animals. The second gene, widely used in vertebrate evolutionary studies, was confirmed particularly effective also for the reconstruction of molecular phylogeny in invertebrates (Simmons & Weller, 2001).

To carry out PCR amplifications of a partial region of both genes, we used primers designed by Folmer *et al.* (1994) (COI) and Boore & Brown (2000) (Cyt-b). DNA was extracted from the whole body of the specimen of about 4 mm length using standard procedures. The DNA yield was enhanced using the GenomiPhi® commercial kit according to the supplier's specifications. The PCR amplification mix contained: 0.4 µM of each primer; 2.5 U of Taq DNA Polymerase; 2.5 mM of $MgCl_2$; 200 µM of dNTPs. The PCR profile consisted of 35 cycles (denaturation: 1' at 94°C; annealing: 1' at 52°C; extension: 1'30'' at 72°C).

The PCR amplifications yielded a product of 644bp for the COI gene, and 381bp for the Cyt-b gene. The sequences of the two partial genes are reported in Genbank, accession numbers XX000000 and XX000000. The two sequences were compared with those of *H. macracantha*, showing 144 changes (67 transitions and 77 transversions) for COI and 131 (46 transitions, 85 transversions, and 3 deletions belonging to the same codon) for Cyt-b. These mutations leaded to 38 aminoacidic changes over 214 for the COI enzyme, and to 50 aminoacidic changes and the deletion of one aspartate over 126 for the Cyt-b enzyme.

A Median Joining Network analysis (Bandelt *et al.*, 1999) was carried out by the software Network 4.5.0.0 (http://www.fluxus-engineering.com), using the sequences of *L. magdalenina* and those of 8 representative species of Pancrustacea, whose complete genome was reported in Lavrov *et al.* (2004). The median joining networks is reported in Fig. 1 for COI and Cyt-b genes. As expected, *L. magdalenina* resulted consistently associated with *H. macracantha*. For the COI gene, the cephalocarid clade is separated by the basal median vector

by 54 changes that represent the plesiomorphic status for the class. Among the 144 nucleotide differencies between the two species, for 35 of them the program cannot univocally assign the ancestral status (resulting in a triangular reticulation of the network), 62 changes are apomorphic for *L. magdalenina* and 47 changes for *H. macracantha*. As to the Cyt-b gene, 40 changes differentiates Cephalocarida from other taxa, and, among the 131 differences between the two species, 26 are unresolved, and 67 and 39 are derivate in *L. magdalenina* and *H. macracantha* respectively. The equality of the evolutionary rate between the two species was tested for both genes with the method proposed by Tajima (1993) using the Mega4 software (Tamura *et al.*, 2007). In spite of the observed differences in the number of apomorphic nucleotides, the Tajima relative rate test of neutrality resulted non significant for both genes, whatever other sequence was used as outgroup. Mega4 was also used to draw a Neighbor-Joining tree, that confirms the relationships between the two species (Fig. 2).

The network analysis showed the independent separation of Cephalocarida and other Pancrustacea classes, a marked divergence of Branchiura, and a possible correlation between Pentastomida and Remipedia. A discussion on arthropod evolution is by far beyond the aim of this short note, nevertheless we consider useful to contribute with molecular data on a newly discovered species, which increase the knowledge on the genetic variation within Cephalocarida, for addressing further research in this strongly debated field.

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FIGURE AND LEGENDS

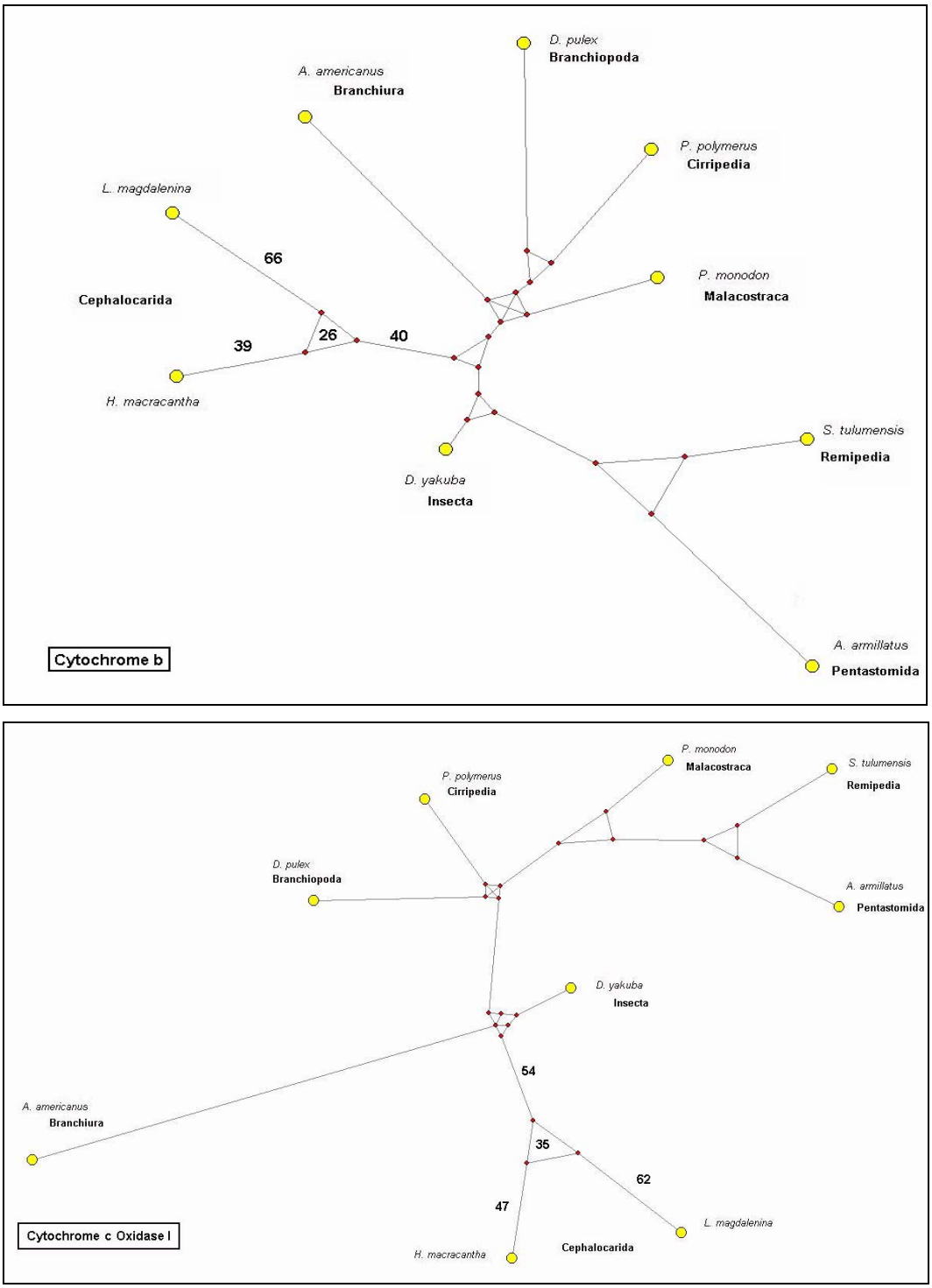

Fig. 1. *Median Joining Network of Citochrome b (above) and Citochrome c Oxidase I (below) sequences from L. magdalenina and 8 Pancrustacea species.*

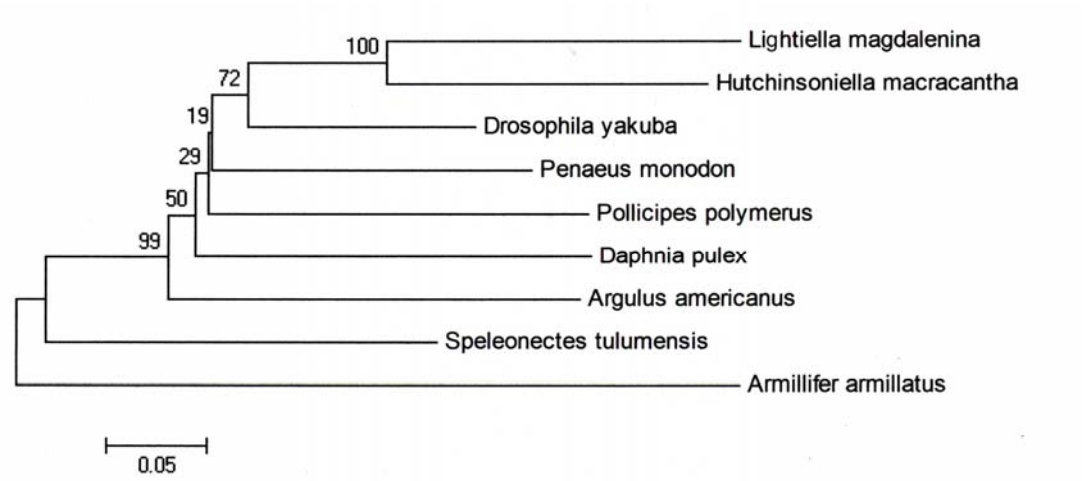

Fig. 2. *Neighbor-Joining tree and bootstrap values (1000 replicates) of Citochrome b and Citochrome c Oxidase I genes from L. magdalenina and 8 Pancrustacea species.*

5.3 DISTRIBUTION AND MICROHABITAT NOTES FOR *LIGHTIELLA MAGDALENINA* (CEPHALOCARIDA): SEDIMENT AND BENTHIC COMMUNITY ANALYSIS

Although Cephalocarids have a world–wide distribution and have been reported in shallow intertidal localities to > 1500 deep (Schram, 1986), they are very difficult to found. Specimens of *L. magdalenina* have been obtained several time during the last ten years, but their total number is however very low (means density of 0.018 individuals/10 cm^3), and all of them are reported from a single locality (*Type locality*). Although the low total number of specimens, an equal number of larvae and adults were found in each samples (see Table 1). This data indicate that the species is in its natural habitat represented by a reproductive population of small size.

The small number of live specimens, the temporal discontinuity (2 specimens in 2006, 11 specimens in 2007) with which they have been retrieved and their different developmental stages, have not allowed to set up organic and comparable experiments of breeding and their maintenance had a negative conclusion after few days.

Ecological information about cephalocarids microhabitat are very few. Its occur in substrates that are bare or covered with seagrass, in intertidal as well as in deep sea habitats, and over a wide range of temperature. The one common factor in these habitats seems to be the flocculent organic matter of the superficial sediment (Gooding, 1963; Sanders, 1963; Sanders & Hessler 1964; McLaughing, 1976; Saloman, 1978; De Troch *et al.,* 2000; Carcupino *et al.,* 2006).

On these bases, samples of benthic communities from eight different Mediterranean sites have been analyzed. All site were at a comparable depth and had bottom characteristics similar to that one of the *Type locality* (muddy sand bottom, 15-20 meters depths, very rich in organic matter mostly consisting of leaf fragments of *Posidonia oceanica*). Five sites were localized in the North Sardinian (2 near Alghero, 1 in Stintino, 1 near Golfo Aranci, 1 near Loiri - Porto San Paolo) and three

in the South-East France (near Marseille). Samples analysis failed to yield specimens of *L. magdalenina*. It is noteworthy that animals are not particularly inconspicuous, and the lack of both previous and present reports from the Mediterranean may point to a very narrow distribution. This seems to be the case with Cephalocarida taxa. Indeed, with the exception of *H. macracantha* and *L. incisa*, which are known for a large number of specimens and for several localities (Wakabara 1970; Hessler & Sanders 1973; Stoner 1981; Heard & Goeke 1982; De Troch *et al.*, 2000; Martin *et al.*, 2002), most of cephalocarids, are only known from a single locality, and even within that locality they appear to be exceedingly rare.

In order to better characterized the type and unique locality in which *L. magdalenina* is at moment reported, both grains-size analysis and benthic community analysis have been performed. For sediment analysis, three macrocores (diameter 8 cm) at different depth (15 and 18 meters) were taken. Sediment was first observed at the light microscope for a quantitative estimation of quartz grain. Then, sediment was dried for 24 h at 60 °C, separated using a standard sieve piles with half phi interval, in silt (<64 µm), sand (64-200 µm) and gravel (>200 µm). Grain size analysis has underlined that the sediment is a very fine sand with an organic component of 95%. All the samples are composed of 70% fragments of rhizomes of *Posidonia oceanica* and of 30% of fragments of bivalve and gastropods shells. Grain size of these organic fragments belong for 75% to the sands (200-64 µm) and for 15-20% to the silts (Fig.1).

Data on sediment characteristics are only reported from two different locality (Punta Allen, Messico and Carrie Bow Cay, Belize) belonging to the range zone of *L. incisa* (De Troch *et al*., 2000; Schiemer & Ott, 2001). In both locality *L. incisa* was present with an high density (thousands individuals per square meter) and show an high vertical mobility. In particular *L. incisa* was found to have the maximum density between 4-5 cm in fine sediment (median grain size between 230 and 260µm) (De Troch *et al.*, 2000) and between 12-15 cm in coarse sand (median grain size of 1000 µm) (Schiemer & Ott, 2001). This kind of vertical distribution suggested that *L. incisa* inhabits oxygenated microhabitat below the redox potential discontinuity

(RPD) layer created by burrowing macrofauna organisms. Three of the four specimens of *L. incisa* obtained by Gooding (1963) were from water aspirated from callianassinid and thallasinid burrows. De Troch *et al.* 2000 reported *L. incisa* closely associated to Polychaeta.

As shown the analysis of the other organisms sorted in the same samples and periods of *L. magdalenina*, Polychaeta are well represented in this sediment (see Table 2). Moreover, as shown in Table 1, the first specimens of *L. magdalenina* was obtained in 1999, during an Environmental Impact Analysis (E.I.A) carried out with a quantitative and qualitative benthic community analysis in which burrowing Polychaeta such as *Amphiglena mediterranea, Armandia cirrosa, Cossura soyeri, Paradoneis lyra*, were present (see Table 3) (Castelli *et al.*, 1999).

Copepod harpacticoids clearly dominated the benthic fauna of this locality (table 2). In total, seven families (Ameiridae, Canuellidae, Cletopsyllidae, Normanellidae, Canthocamptidae, Porcellidiidae and Diosaccidae) were determined. The family Ameiridae is the most abundant (represented with percentages among 60%) while all other families have a very inferior percentage (see Table 4). Among the latter, Normanellidae and Cletopsyllidae were unknown for Italy. A new species of family Cletopsyllidae, *Isocletopsyllus sardus* sp. nov. (Addis *et al.*, submitted) has been describe.

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FIGURES AND TABLES

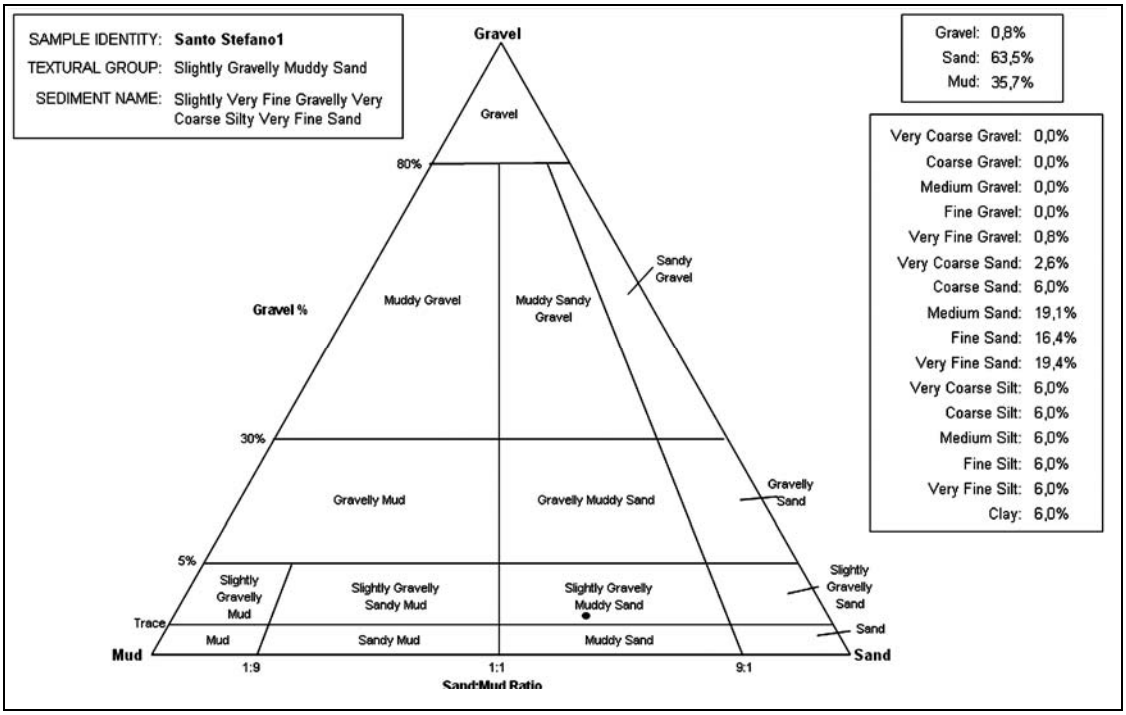

Fig.1: *Grain size analysis*

	1999	2000	June 2001	July 2004	October 2004	May 2005	September 2005	October 2005	June 2006	November 2006	November 2007	June 2008
ADULT	3	3	-	3	8	2	-	-	1	0	3	0
LARVAL STAGE	2	-	2	-	16	2	5	2	1	0	2	0
TOTAL	5	3	2	3	24	4	5	2	2	0	5	0
TOTAL YEAR	5	3	2	27		11			2		5	0

Table 1. Number of L. magdalenina specimens reported since 1999 to 2008.

SAMPLINGS		2004 / 2007
NEMATODA		312
ECHIURIDA		31
GASTEROPODA		30
BIVALVA		25
POLYCHAETA		466
OSTRACODA		9
COPEPODA		1817
MALACOSTRACA		215
OPHIURIDEA		7
ASTEROIDEA		3
CEPHALOCARIDA		34
Taxa numbers		10
Individuals numbers		2949

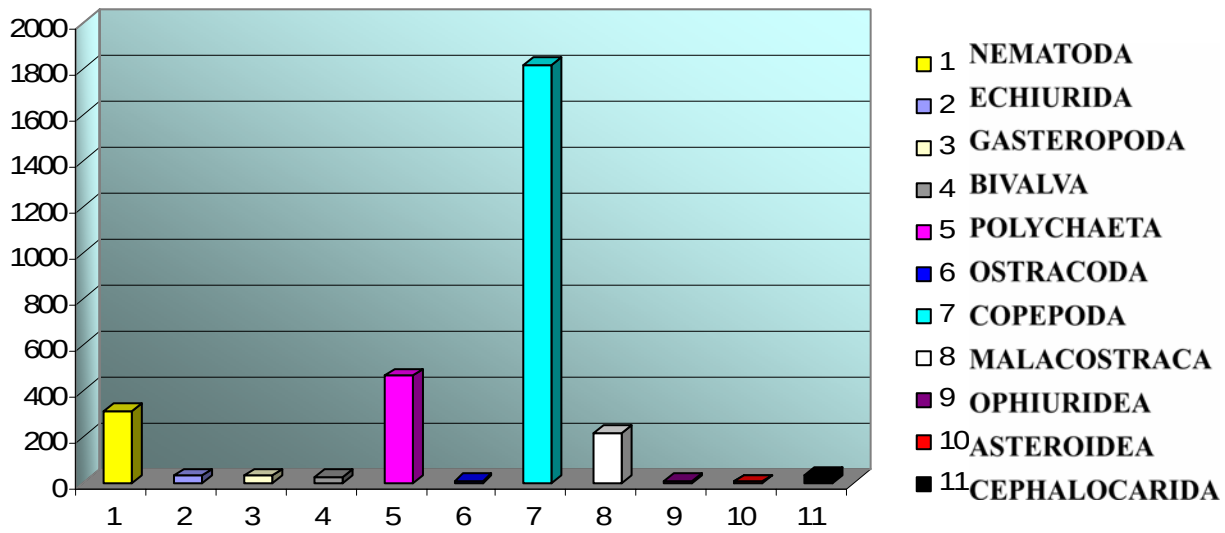

Table 2: List of benthic taxa from Type locality, La Maddalena Archipelago.

	P^ta. San. Giorgio 5 mt.		P^ta. S. Giorgio 15 m.	
Nemertea				
Nemertea n.c.		19	6	1
Nematoda				
Nematoda n.c.		2	2	2
Annelida Polychaeta				
Amphiglena mediterranea	1	2	1	
Aricidea assimilis			1	
Armandia cirrhosa		1		1
Chrysopetalum debile		1		
Cossura soyeri			1	1
Eunice vittata			1	
Exogone naidina	1	1		
Grubeosyllis limbata		5		
Gyptis sp.		2		
Harmothoe sp.		1		1
Heteromastus filiformis		1		
Levinsenia oculata			2	1
Magelona rosea			1	
Monticellina dorsobranchialis				1
Nephtys sp.	1			1
Paradoneis lyra		1	2	1
Parapionosyllis elegans		2		
Pettiboneia urciensis		4		
Pista cristata		1		
Prionospio malmgreni			1	1
Protodorvillea kefersteini		6	1	2
Schistomeringos rudolphii	2	2		
Sphaerosyllis cryptica	1	1		
Sphaerosyllis pirifera		4		
Sphaerosyllis taylori	6	2		
Sphaerosyllis tetralix		1		
Spionidae n.c.	1		1	
Syllis prolifera		1		
Syllis truncata cryptica		4		
Xenosyllis scabra				1
Annelida Oligochaeta				
Oligochaeta n.c.		2	2	2
Sipunculida				
Sipunculida n.c.			1	3
Mollusca				

Mitrella gervillei	1			
Balcis incurva		1		
Mantellum hians		2		
Crustacea				
Cephalocarida n.c.				1
Cumacea n.c. sp.A	1			
Copepoda Harpacticoida n.c. sp.A	1		1	
Amphipoda n.c. sp.A		1		
Amphipoda n.c. sp.B	2			
Amphipoda n.c. sp.G		1		
Caprella sp.		4		
Tanaidacea n.c. sp.A		2		
Decapoda Hyppolitidae n.c. sp.B		1		
Decapoda Macrura n.c.		2		
Echinodermata				
Amphipolis squamata			1	

Table 3. *List of benthic taxa from Type locality, La Maddalena Archipelago reported by Castelli et al., 1999.*

SAMPLINGS		2004 / 2007
Copepods number		1817
COPEPODA-Harpac.	Ameiridae	1167
COPEPODA-Harpac.	Canuellidae	246
COPEPODA-Harpac.	Cletopsyllidae	68
COPEPODA-Harpac.	Normanellidae	11
COPEPODA-Harpac.	Canthocamptidae	10
COPEPODA-Harpac.	Porcellidiidae	106
COPEPODA-Harpac.	Diossacidae	34
COPEPODA-Harpac.	N.C.	30
COPEPODA	CALANOIDA	145

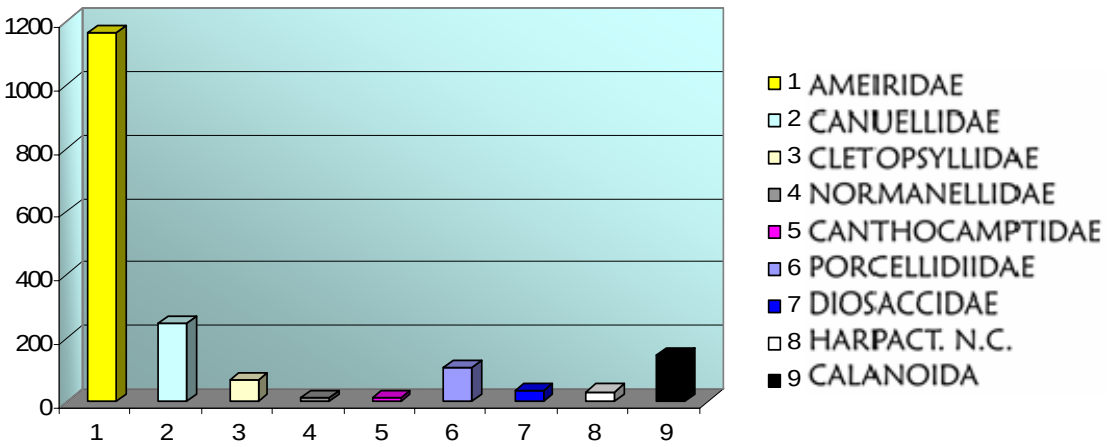

Table 4: *Copepoda composition of samplings (2004-2007) from Type locality, La Maddalena Archipelago.*

Running head: A new species of *Isocletopsyllus*

A new species of the genus *Isocletopsyllus* (Harpacticoida, Cletopsyllidae)

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ABSTRACT

A new harpacticoid species, *Isocletopsyllus sardus* sp. nov. (Cletopsyllidae), is reported from S. Stefano isle (La Maddalena Archipelago, Sardinia, Italy). The new species lives in organic mud at a depth of about 20 m, and it is distinguishable from the other species of the genus by: 1) antennary exopod 1-segmented with 2 setae of different length on distal edge, 2) apical segment of P1 bearing 2 inner setae and 2 apical geniculate setae, and 3) sexual dimorphism of P2 and caudal rami. This is the first record of the family Cletopsyllidae from Italy and the third one for the Mediterranean Sea.

INTRODUCTION

Cletopsyllids are extremely rare marine benthic copepods. Most of the nine species currently allocated to this family are known from a small number of specimens, and three of them (*Cletopsyllus papillifer*, *Retrocalcar secundus*, *Isocletopsyllus quartus*) from only a single female (Huys & Lee, 1998/99). They live in various sediment types down to a depth of 460 m, although most occur in shallow waters (Boxshall & Halsey, 2003).

The type genus *Cletopsyllus* Willey, 1935, together with the genera *Normanella* Brady, *Pseudocleta* Lang and *Laophontopsis* Sars, was first attributed to the Normanellinae, in the family Laophontidae (Lang, 1944). Later, Nicholls (1945) upgraded Normanellinae to the family level, with the only genera *Normanella* and *Cletopsyllus*. The family Normanellidae was first refused by Lang (1948) and then reproposed by Huys & Willems (1989). The latter authors allocated two subfamilies to Normanellidae: Normanellinae, for the genus *Normanella*, and Cletopsyllinae, for the genera *Cletopsyllus* and *Pseudocletopsyllus* Vervoort, 1964. At that time, the genus *Cletopsyllus* comprised nine species and *Pseudocletopsyllus* only one (see Lee *et al.*, 2003).

The subfamily Cletopsyllinae was later removed from the Normanellidae and upgraded to the family level (Huys & Lee, 1998/99). Moreover, the same authors described a new species and divided Cletopsyllidae into four different lines, represented by the genera:

Cletopsyllus with the species *C. papillifer* Willey, 1935 (type species) from the Atlantic Ocean, *C. bacescui* Marcus, 1976 from East Mbvakumi, and *C. rotundifera* Fiers, 1986 from the West Indian Islands.

Retrocalcar with *R. brattstroemi* (Geddes, 1981) from Great Egg Island, Bahamas, *R. secundus* (Nicholls, 1945) from Western Australia, and *R. sagamiensis* (Itô, 1971) from Japan.

Isocletopsyllus with *I. tertius* (Por, 1964) from the Israeli Mediterranean coast, and *I. quartus* (Soyer, 1966) from Banyuls sur Mer, France.

Bathycletopsyllus with only one species *B. hexarthra* Huys & Lee, 1998/99 from the Indian Ocean, off La Réunion.

Pseudocletopsyllus Vervoort, 1964 was considered very similar to the V copepodid stage described by Itô for *R. sagamiensis*, and thus regarded as *genus inquirendum* in the family Cletopsyllidae (Huys & Lee, 1998/99).

In this paper, we describe a new species of the genus *Isocletopsyllus*, which represents the first Cletopsyllidae known from Italy. It was discovered during a study of meiofauna associated with *Lightiella magdalenina*, the first cephalocarid species reported from Europe (Carcupino *et al.*, 2006).

MATERIALS AND METHODS

During the periods October 2004, and October 2006-2007, we collected 50 adults and 3 copepodite stages at 15–20 m depth from a muddy sand bottom rich in organic material on the southern shore of S. Stefano isle, part of the La Maddalena Archipelago (Sardinia, Italy). Samplings were carried out by hand using SCUBA. Light and scanning electron microscopic analyses were performed.

Light microscopy (LM)

Specimens were dissected to isolate the cephalic and trunk appendages. Each appendage was mounted on a separate slide using Faure medium. Micrographs were taken with a WELL AXIOSTAR PLUS microscope and a CANON POWER SHOT G6 digital camera. Each original micrograph was transformed into a drawing-like image with the photocopy function of Microsoft PhotoDraw V 2.

Scanning electron microscopy (SEM)

Specimens were fixed in formalin 4%, dehydrated in a graded ethanol series, dried in a Polaron Jumbo critical point drier, sputter-coated with gold in an Edwards SI5A unit and observed with a ZEISS DMS 962 scanning electron microscope of the Electron Microscopy Centre, Sassari University.

RESULTS

SYSTEMATICS

Order HARPACTICOIDA Sars, 1903

Family CLETOPSYLLIDAE Huys & Willems, 1989

Genus *ISOCLETOPSYLLUS* Huys & Lee, 1998/99

Isocletopsyllus sardus **sp. nov.**

(Figures 1-5)

Type material

Holotype: adult female kept in formalin 5%, 1360 µm in length (S. Stefano isle, La Maddalena Archipelago, 41°11'17.57"N 09°24'26.03"E; water depth: 20 m; very fine muddy sand rich in organic material mostly consisting of leaf fragments of *Posidonia oceanica*) (SMNH type .....). Collected by A. Addis, 22 October 2006.

Paratypes: adult female, 1540 µm in length, completely dissected and mounted on 10 slides (S. Stefano isle, La Maddalena Archipelago, 41°11'17.57"N 09°24'26.03"E; water depth: 20 m; very fine muddy sand rich in organic material mostly consisting of leaf fragments of *Posidonia oceanica*) (SMNH type .....). Collected by A. Addis, 22 October 2006.

Adult male, 1760 µm in length, partially dissected, with first antenna, P2-P5 and caudal rami mounted on 6 slides (S. Stefano isle, La Maddalena Archipelago, 41°11'17.57"N 09°24'26.03"E; water depth: 20 m; very fine muddy sand very rich in organic material mostly consisting of leaf fragments of *Posidonia oceanica*) (SMNH type .....). Collected by A. Addis, 22 October 2006.

Fifty additional specimens (29 adult males, 18 adult females and 3 copepodites) are kept in formalin and deposited in the zoological collection of the Department of Zoology and Evolutionary Genetics, Sassari University.

Etymology

The species is named after the locality where it was recorded: Sardinia (adjective *sardus*: from Sardinia)

Diagnosis

Isocletopsyllus with 2 setae of different length on distal edge of antennary exopod; apical segment of P1 endopod bearing 2 inner setae and 2 apical geniculate setae; caudal ramus about 7.5 times as long as greatest width, and almost equal length in both sexes (slightly shorter in male).

Description of adult female

Body length, measured from anterior margin of rostrum to posterior margin of caudal rami, varying from 1340 µm to 1600 µm (mean±sd 1490±115 µm) (Fig. 1a). Nauplius eye present. Rostrum (Figs 1b-c) large, triangular and about 1.6 times as long as greatest width; bifid at tip and with 2 small lateral setae. Ventral side with a median small tube below the two apical lobes (Fig. 1c, insert). Body elongate, slightly tapering posteriorly. Somites clearly defined. Dorsal and ventral posterior edges of each body segment with spinous formations of different size. Anal somite (Figs 1d-e) bearing 2 lateral setae dorsally and long setules bordering the anal opening ventrally; additional spinules around ventral and dorsal posterior margin. Anal operculum prominent, semi-circular, bearing many slender spinules at margin. Caudal rami (Fig. 1e) well developed, 221.2±38 µm in length (about 7.5 times as long as greatest width), with inner apical edge slightly concave and bearing 2 small spines laterally; 2 additional small setules on the apical dorsal surface. All setae present on and near the distal end: 1 inner seta, articulated at base, 3 outer setae, distal one smaller; 3 terminal setae, innermost one shorter.

Antennule (Fig. 2a): 4-segmented; first segment short, with some thin spinules on posterior margin; 1 inner hairy seta and 1 outer spiniform projection apically. Second segment as long as first, with 2 outer strong cylindrical projections, each bearing 1 bare seta on apical end; 6 additional setae are present: 3 on the inner margin, 1 on the outer edge and 2 on the dorsal surface. Third segment about twice as long as second, with outer margin smooth (not modified).

Inner edge with 7 or 8 bare setae. Distal inner edge with two projections, innermost larger and bearing a long slender aesthetasc and 2 or 3 apical setae, outermost bearing 1 small seta. Fourth segment with 8-10 setae on distal and lateral edges. Apical acrothek consisting of 2 setae.

Antenna (Figs 2b-c): allobasis elongate with incomplete original segmentation; abexopodal margin with two groups of spinules and with 1 small abexopodal seta; spiniform projection near distal inner edge. Endopod bearing some spinules along inner margin; inner distal armature consisting of 2 pinnate spines laterally, 2 curved pinnate spines and 3 geniculate setae apically. A spiniform projection, with minute hairs, on outer distal edge (Fig. 2c). Exopod 1-segmented with 2 setae of different length on distal edge.

Mandible (Fig. 2d): palp biramous and well developed; basis with 3 pinnate setae, endopod with 1 lateral pinnate and 3 distal bare setae, exopod small, 1-segmented, with 1 apical seta. Gnathobase with one row of spinules at base of palp; with 2 strong teeth, several multicuspidate blades, and an additional long pinnate seta at dorsal corner (lost in the specimen of Fig. 2d).

Maxillula not shown.

Maxilla (Fig. 2e): syncoxa with long spinules around outer margin, short spinules medially and with 3 endites. Proximal endite smallest, with 1 long pennate seta; middle endite with 1 pectinate spine and 1 bare seta; distal endite with similar armature but 1 more bare seta. Allobasis drawn out into a pectinate claw; accessory armature consisting of 2 bare setae and 1 pinnate spine. Endopod as a minute rudiment bearing 3 setae of increasing size from inner to outer side.

Maxilliped (Figs 2f-h): subchelate 3-segmented; syncoxa with 3 pinnate setae. Basis unarmed; with row of spinules along palmar margin and a few spinules along outer margin. Endopod represented by an apically curved claw, minutely pinnate in distal half; accessory armature consisting of 1 long bare seta.

P1 (Fig. 3a): coxa well developed. Inner portion of basis produced into a cylindrical pedestal for endopod; 1 outer bipinnate seta, 1 spine at the inner corner and a spinous process on distal margin between exopod and endopod. Exopod 3-segmented; first segment with 1 long outer spine; second segment bearing 1 outer and 1 inner spine; third segment bearing 2 outer spines and 2 apical geniculate

setae. Endopod 2-segmented, prehensile; first segment nearly 3 times as long as second segment and with a long seta on middle inner edge. Distal segment with 2 minute setae along inner margin, 1 pinnate spine and 1 geniculate distal apical seta.

P2 (Fig. 3b): coxa almost the same as in P1. Basis without pedestal for endopod but similarly armed. Exopod 3-segmented; first segment with 1 outer spine; second segment bearing 1 inner and 1 outer long spine; third segment with 3 outer spines, 2 terminal and 1 inner pinnate setae. Endopod 2-segmented; first segment with 1 hairy inner seta on middle margin and a small spine on distal outer corner; second segment with hairy outer margin and armed with 1 outer, 2 apical and 4 inner hairy setae.

P3 (Fig. 3c): coxa almost the same as in preceding legs. Basis with a long outer seta (lost in the specimen of Fig. 3c). Exopod 3-segmented; first and second segment with 1 outer spine and 1 inner long hairy seta; third segment bearing 3 outer spines, 2 apical and 2 inner hairy setae. Endopod 2-segmented; first segment with 1 hairy inner seta on middle margin and a small spine on distal outer corner; second segment with hairy outer margin and bearing 1 outer, 2 apical and 3 inner hairy setae.

P4 (Fig. 3d): coxa and base well developed, base with long outer articulated seta. Exopod 3-segmented; first and second segment with 1 outer spine and 1 inner long seta; third segment bearing 3 outer spines, 2 apical setae and 2 inner setae. Endopod 2-segmented; first segment with 1 inner hairy seta on middle margin and a small spine on distal outer corner; second segment with hairy outer margin and bearing 1 outer, 2 apical and 3 inner hairy setae.

P5 (Fig. 3e): baseoendopod with an elongate, triarticulate seta on outer distal corner (lost in the specimen of Fig. 3e); endopodal lobe triangular with 3 setae on inner margin and 2 apical setae; distal portion distinctly bilobate with inner lobe of spine-like shape and outer lobe rounded. Exopodal segment elongate, rectangular, with 1 inner, 1 apical and 4 outer setae.

Genital field (Fig 3f): Genital field located in the middle of genital somite and with two genital apertures. Female bearing an egg sac with 15-20 eggs (Fig. 3f, insert).

Description of adult male

Body length, measured from tip of rostrum to caudal rami, varying from 1400 to 1760 µm (mean±sd 1560±124 µm) (Fig. 4a). Nauplius eye present. Body with somatic ornamentation similar to that of female. Sexual dimorphism present in: a) genital and first abdominal somites separated; b) caudal rami, c) antennule and thoracic limb morphology (P2-P6).

Caudal rami (Figs 4b-c) well developed, 219.5±19.6 µm in length (about 7.3 times as long as greatest width), inner apical edge straight and without setae.

Antennule (Fig. 5a) 7-segmented, subchirocer with geniculation between segments 5 and 6. First segment bearing 1 outer spine and 1 inner long seta on apical margin; second segment with 2 processes along posterior margin: proximal process conical and bearing 1 seta at its base; distal process cylindrical and with 1 apical seta. Nine additional setae are present (two groups of 3 setae each on inner margin, 1 seta on apical outer corner and 2 setae on dorsal surface). Third segment reduced. Fourth segment represented by a small sclerite along anterior margin and with 4 or 5 lateral setae. Fifth segment very large and swollen with convex outer margin and with 1 inner seta; distal process bearing a long aesthetasc and 2 setae. Sixth segment unarmed, seventh segment bearing outer setae and apical acrothek consisting of 2 small setae (Fig. 5a, insert).

P2 (Fig. 5b): second endopodal segment modified with respect to female and bearing an outer small pinnate spine, an apical long hairy seta, and an inner small bare seta.

P3 (Fig. 5c): second endopodal segment weakly modified, with an outer dentate seta and 2 apical long hairy setae.

P4 (Fig. 5d): third segment of exopod weakly modified, with an outer pectinate setae.

P5 (Fig. 5e): baseoendopod as in female, with elongate, triarticulate seta and an outer protopodal seta; endopodal lobe triangular with 1 basal and 2 apical hairy setae. Exopodal segment elongate, rectangular, and with hairy inner margin; 1 inner hairy seta and 1 apical and 3 outer bare setae.

P6 (Fig. 5f): 1-segmented with only 1 apical seta.

DISCUSSION

Isocletopsyllus sardus, like all Cletopsyllidae species, is characterized by the following morphological features:

- female antennules with two distinctive conical processes on the outer margin of the second segment (because of the presence of an apical seta, the conical processes are not considered homologous with the non-setiferous hook-like process commonly found in the Laophontoidea and Tetragonicipitidae) (Huys, 1990; Huys & Lee, 1998/99);

- male antennules typically subchirocer with geniculation between segments 5 and 6 and a proximal spinous outgrowth, homologous with the proximal conical process of the female (Huys & Lee, 1998/99), on the outer margin of segment 2;

- antenna with allobasis in which the original segmentation is marked by incomplete superficial sutures on either surface. Exopod typically small and with 2 apical setae;

- P1 exopod with inner seta on exp-2, and 4 elements on exp-3;

- P1 endopod 2-segmented and inserted on a cylindrical outgrowth of the basis;

- P2-P4 endopod 2-segmented;

- baseoendopod of P5 represented by a very long extension bearing an outer basal seta typically 3-articulated and composite, which (like the other basal setae) has been considered to have a mechanoreceptory function (Huys & Lee, 1998/99);

- female genital field characterized by a paired arrangement, considered the most primitive state within the Harpacticoida (Huys & Lee, 1998/99);

- sexual dimorphism in the last segment of P2 endopod.

Regarding the last character, considered one of the most diagnostic characters of the family (Huys & Lee, 1998/99), males of *I. sardus* have a spine with simple tip instead of an apophysis at the inner distal corner. However, re-examination of the original figures and descriptions shows that an apophysis completely fused at the base, as reported by Huys & Lee (1998/99), is present and well developed in only four species (*C. rotundifera, B. hexarthra, R. sagamiensis* and *R. brattstroemi*) (Geddes, 1981; Fiers, 1986; Huys & Lee, 1998/99). A spine with simple or bifid tip was reported in *C. bacescui* (Marcus, 1976) and *I.*

quartus sensu Marcus (1976), respectively. Finally, neither apophysis nor spine seems to be present in *I. tertius* (Por, 1964). In the last species, however, the sexual dimorphism of the P2 endopod was overlooked in the original description, as also noted by Huys & Lee (1998/99).

The morphological features of the new cletopsyllid species described here, namely (1) body somites with dentate or crenulated posterior margin, but without spinous process dorsally, (2) female antennule 4-segmented and with posterior margin of segment 3 smooth, (3) P4 exopod with unilateral pectinate spines, and (4) P5 exopod of male with 5 setae, match those of the genus *Isocletopsyllus*, except for the dimorphic length of the caudal rami. Indeed, *I. sardus* sp. nov. has caudal rami of approximately equal length in both sexes (slightly shorter in the male), and therefore not markedly longer in males than in females as indicated in the genus diagnosis by Huys & Lee (1998/99). However, the latter condition was clearly observed in *I. tertius* (Por, 1964), the only *Isocletopsyllus* species with the description of both sexes (one male and one female). Only one female is known for *I. quartus* (Soyer, 1966), the second species currently included in the genus. The description of the *I. quartus* male *sensu* Marcus (1976) was rejected and considered a *species inquirenda* in *Isocletopsyllus* by Huys & Lee (1998/99). In fact, the latter authors considered Marcus's description of the caudal rami, i.e. much shorter than in the female, "an unlikely discrepancy within a single genus". The length of the caudal rami of *I. sardus* suggest that this feature can be considered a diagnostic character at the species level but not at the genus level.

The diagnosis of *I. sardus* sp. nov. is based on the following characters:

1) 2 setae of different length on distal edge of antennary exopod (2 equal setae in both *I. tertius* and *I. quartus*);

2) apical segment of P1 endopod bearing 2 inner setae and 2 apical geniculate setae (1 inner seta and 2 apical geniculate setae reported in *I. tertius*, only 2 apical geniculate setae in *I. quartus*).

3) female caudal ramus 7.5 times as long as greatest width (6 times as long as greatest width in *I. tertius*, more than 10 times as long as greatest width in *I. quartus*);

4) caudal rami of approximately equal length in both sexes, slightly shorter in male (longer in male in *I. tertius*, unknown in *I. quartus*).

Moreover, *I. sardus* sp. nov. differs from *I. tertius* in (1) rostrum bifid at tip (rostrum trifid at tip in *I. tertius*), (2) anal segment without dorsal process (anal segment with peculiar dorsal process in *I. tertius*), and it differs from *I. quartus* in (1) all body segments with a spinous process on posterior edges (first and second segment without spinous process in *I. quartus*), (2) maxilla with 3 endites (4 endites in *I. quartus*), 3) maxilliped syncoxa bearing 3 setae (2 in *I. quartus*), 4) base of P1 with distal spinous process (base of P1 without distal process in *I. quartus*).

Key for Cletopsyllidae species

For the introduction of *I. sardus* sp. nov., the previous key for the identification of cletopsyllid species (Huys & Lee, 1998/99) has been modified as follows:

1 Female antennule 6 segmented…….*Bathycletopsyllus hexarthra*

- Female antennule 4-segmented……..…….....2

2 Third segment of female antennule with crenulate posterior margin; male fifth leg with 4 setae/spines on exopodal segment.……(*Cletopsyllus*)..3

- Third segment of female antennule with smooth posterior margin; male fifth leg with 5 setae/spines on exopodal segment…….....5

3 Baseoendopodal lobe of both sexes with long curved terminal process; second endopodal segment of first leg with 2 inner setae; rostrum trifid or bifid at tip……4

- Baseoendopodal lobe without (male) or with short rounded terminal process (female); second endopodal segment of first leg with 1 inner seta; rostrum with rounded apex…….....….....…….....*C. rotundifera*

4 Inner seta on first endopodal segment of leg 1 inserted at 55% of segment length; baseoendopodal lobe of female fifth leg elongate, rectangular

……*C. bacescui*

- Inner seta on first endopodal segment of leg 1 inserted at 66% of segment length; baseoendopodal lobe of female fifth leg short, triangular….*C. papillifer*

5 Female caudal rami with outer proximal margin produced into lobate expansion bearing a spur-like process posteriorly and secondary process dorsally…….....(*Retrocalcar*)..6

- Female caudal rami without lobate expansion; ramus markedly longer in male …...……....(*Isocletopsyllus*)..8

6 Second endopodal segment of first leg with 1 inner seta……...*R. secundus*

- Second endopodal segment of first leg with 2 inner setae.....7

7 Caudal ramus 3.6 (male) and 6.5 (female) times as long as greatest width; outer spines on third exopodal segment of leg 3 modified in male...*R. brattstroemi*

- Caudal ramus 5.3 (male) and 9.0 (female) times as long as greatest width; outer spines on third exopodal segment of leg 3 not modified in male ……...*R. sagamiensis*

8 Caudal ramus 6 times as long as greatest width; rostrum trifid at tip …….....*I. tertius*

- Caudal ramus more than 6 times as long as greatest width; rostrum bifid at tip 9

9 Antennary exopod with 2 setae of equal length; distal margin on base of leg 1 without spinous process between exopod and endopod……*I. quartus*

- Antennary exopod with 2 setae of different length; distal margin on base of leg 1 with spinous process between exopod and endopod……*I. sardus*

Most Cletopsyllidae species are known from a very low number of individuals and none of the nine species currently allocated to this family has been recorded again since its original description, with the doubtful exception of *I. tertius* (see Huys & Lee, 1998/99). Therefore, *I. sardus*, with a total of 53

individuals, is the most abundant species of the family and its discovery represents the first record of Cletopsyllidae from Italy.

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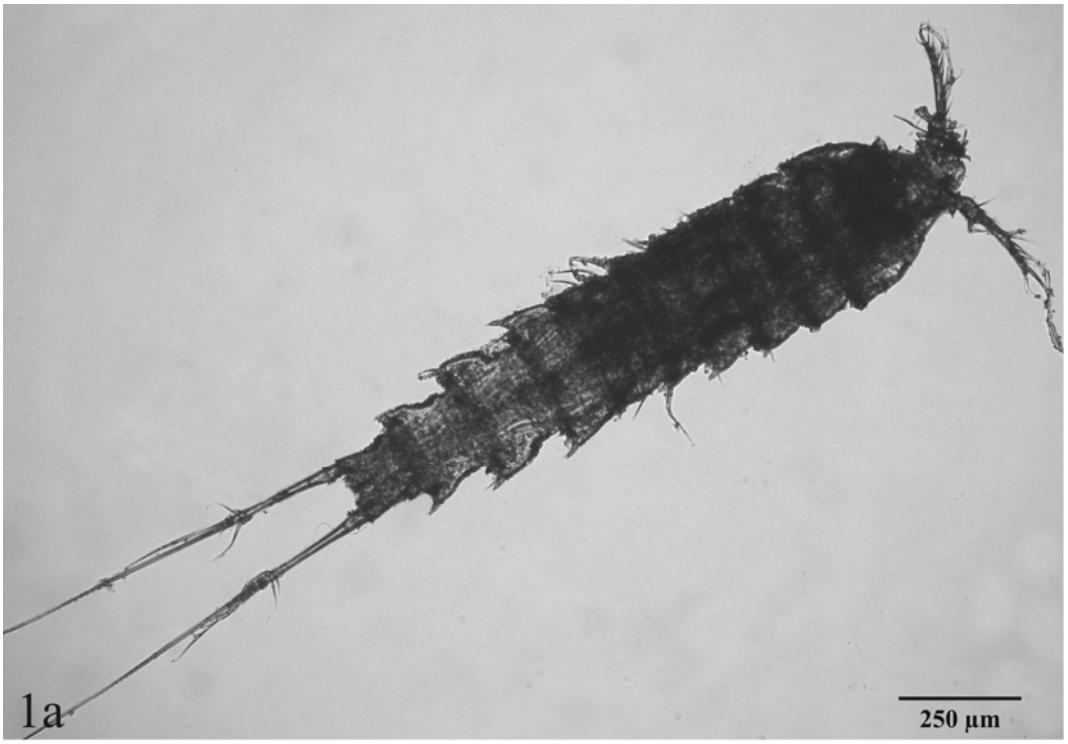
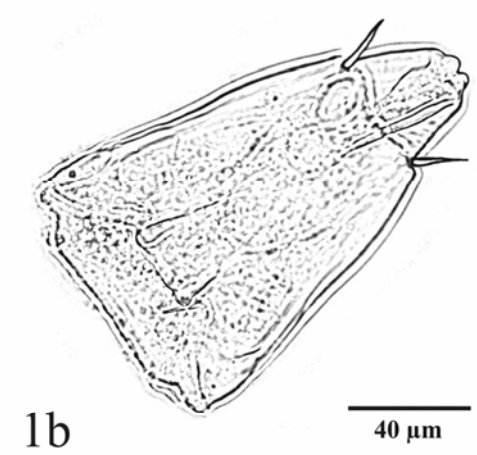
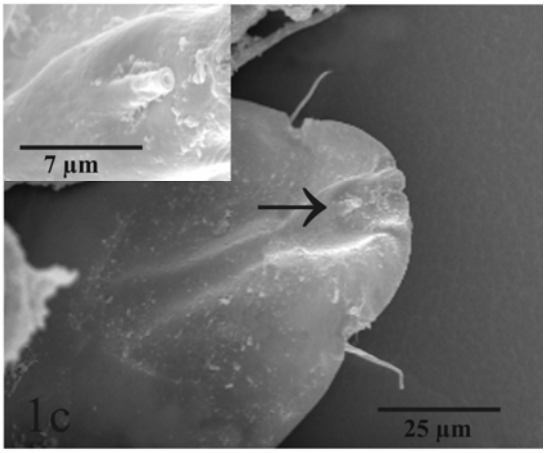
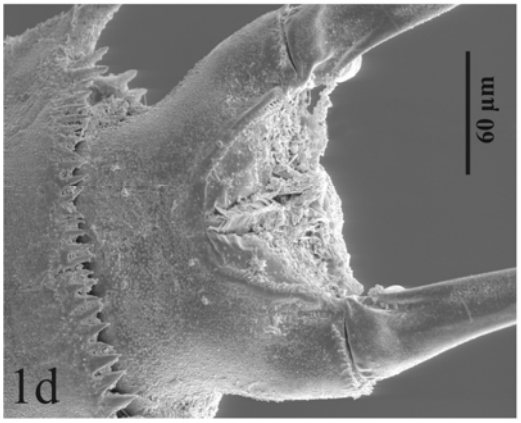
FIGURE AND LEGENDS









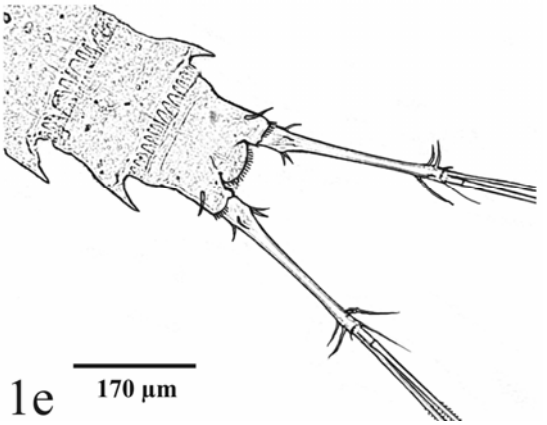


Fig. 1. *Female of Isocletopsyllus sardus sp. nov.* ***A.*** *Dorsal view. Scale bar = 250 µm.* ***B-C.*** *Rostrum with the two apical lobes and 2 lateral setae. Dorsal side (B), ventral side (C). Median small tube (arrow). B, scale bar = 40 µm; C, scale bar = 25 µm;* ***insert****, median small tube, scale bar= 7 µm.* ***D.*** *Ventral side of anal somite with setules bordering the anal opening. Scale bar = 60 µm.* ***E.*** *Dorsal side of anal somite with anal operculum, semi-circular and bearing many slender spinules at margin. Caudal rami with inner apical edges slightly concave. Scale bar = 170 µm.*

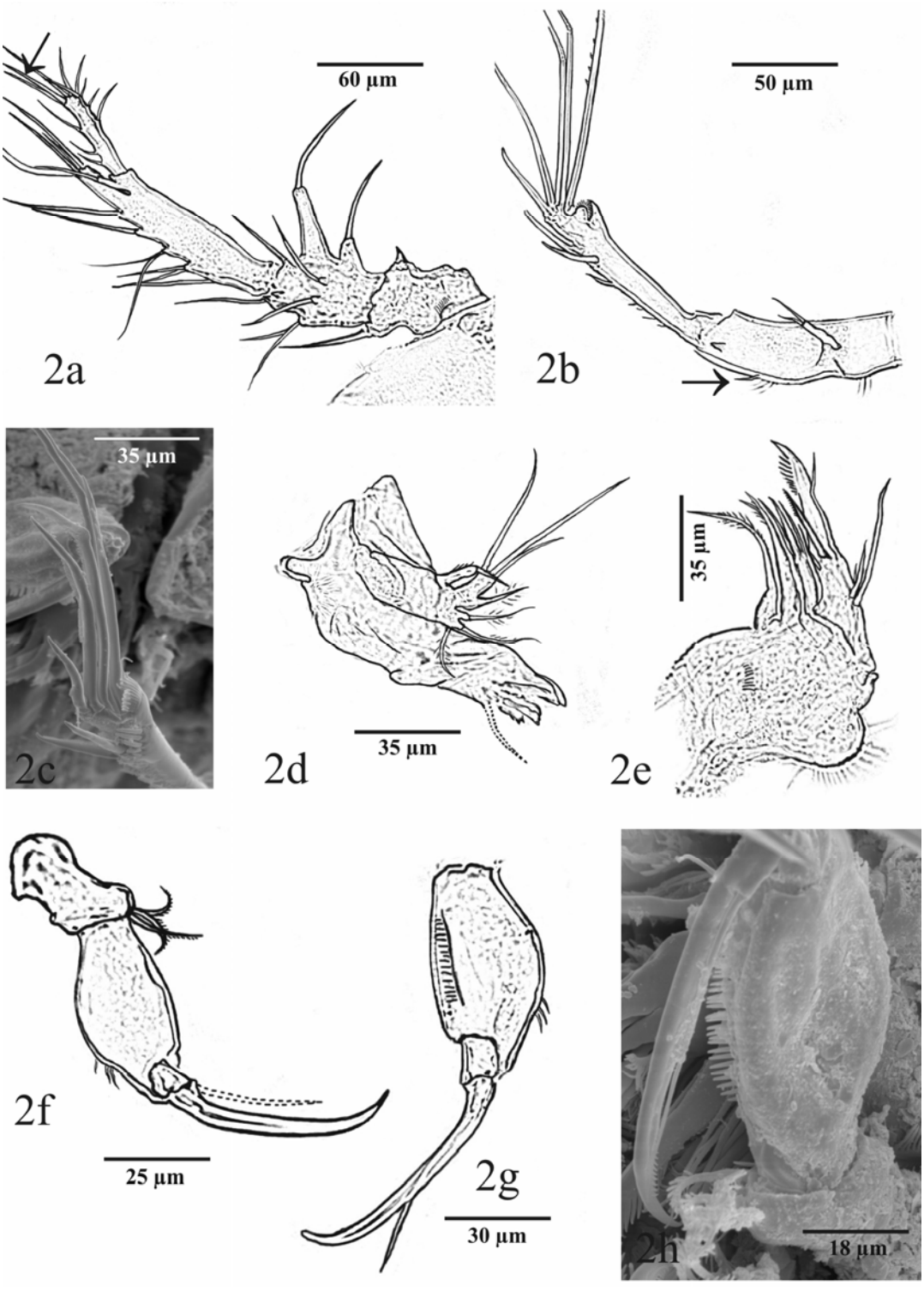

60 µm
2a
50 µm
2b
35 µm
2c
35 µm
2d
35 µm
2e
2f
25 µm
2g
30 µm
2h
18 µm

Fig. 2. *Female of Isocletopsyllus sardus sp. nov.* ***A.*** *First antenna (A1) 4-segmented. Apical acrothek (arrow). Scale bar = 60 µm.* ***B.*** *Second antenna (A2). Exopod bearing 2 apical setae of different length; abexopodal margin with 1 small abexopodal seta (arrow) Scale bar = 50 µm.* ***C.*** *Scanning electron micrograph of second antenna distal armature. Scale bar = 35 µm.* ***D.*** *Mandible. Scale bar = 35 µm.* ***E.*** *Maxilla. Scale bar = 35 µm.* ***F-H.*** *Maxilliped subchelate 3-segmented.* ***F*** *scale bar = 25 µm;* ***G*** *scale bar = 30 µm;* ***H*** *scale bar = 18 µm.*

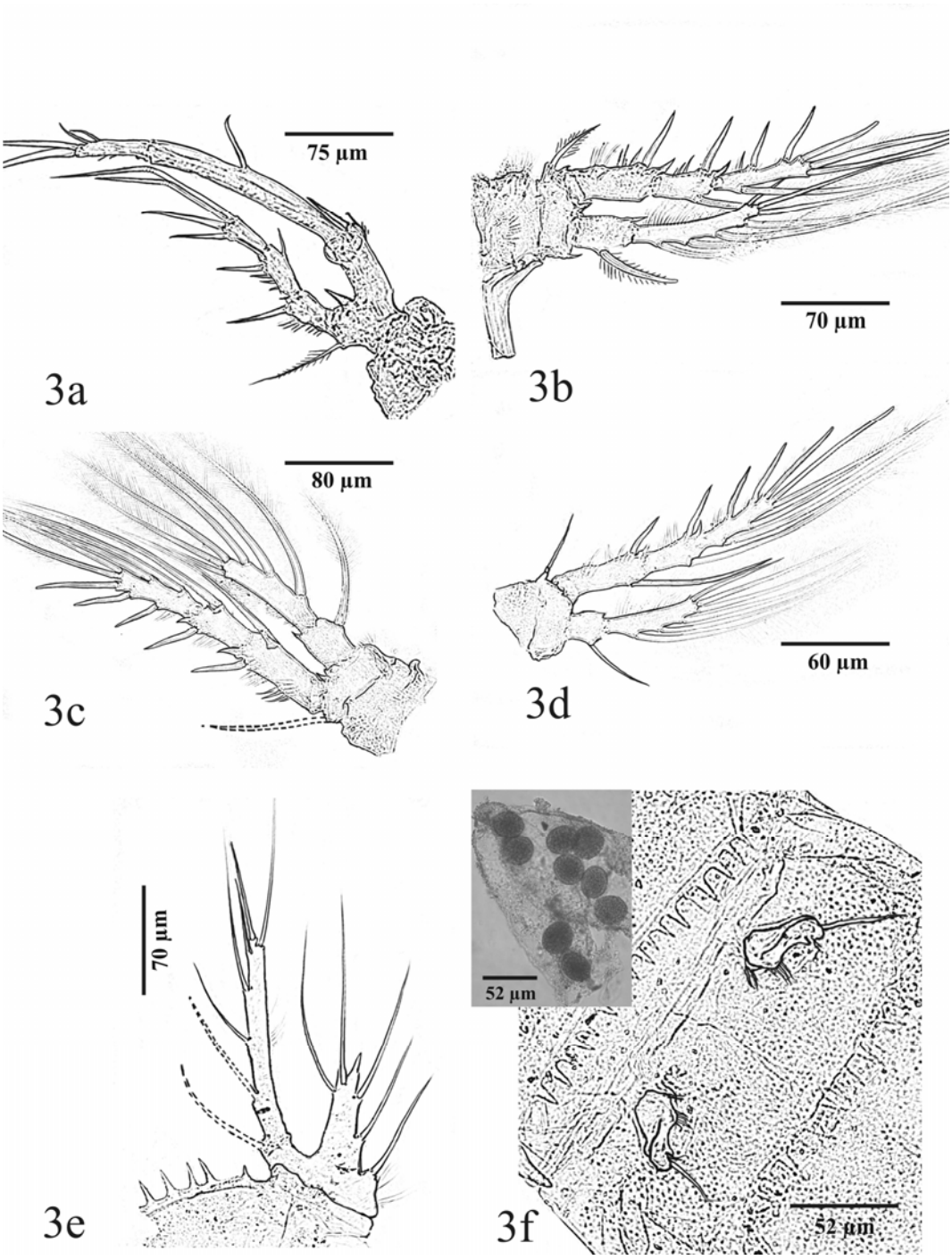

75 µm
3a
70 µm
3b
80 µm
3c
60 µm
3d
70 µm
3e
52 µm
52 µm
3f

Fig. 3. *Female of Isocletopsyllus sardus sp. nov.* ***A.*** *P1 with 3-segmented exopod and 2-segmented endopod. Basis with a spinous process on distal margin, between exopod and endopod. Distal segment of endopod bearing 2 inner minute setae, and two distal geniculate setae. Scale bar = 75 µm.* ***B.*** *P2. Scale bar = 70 µm.* ***C.*** *P3. Scale bar = 80 µm.* ***D.*** *P4. Scale bar = 60 µm.* ***E.*** *P5. Baseoendopod with an elongate, triarticulate seta lost in this image; endopodal lobe with 3 inner and 2 apical setae; exopodal segment elongate, rectangular, with 1 inner, 1 apical and 4 outer setae. Scale bar = 70 µm.* ***F.*** *Genital field. Scale bar = 52 µm.* ***Insert****. Egg sac. Scale bar = 52 µm.*

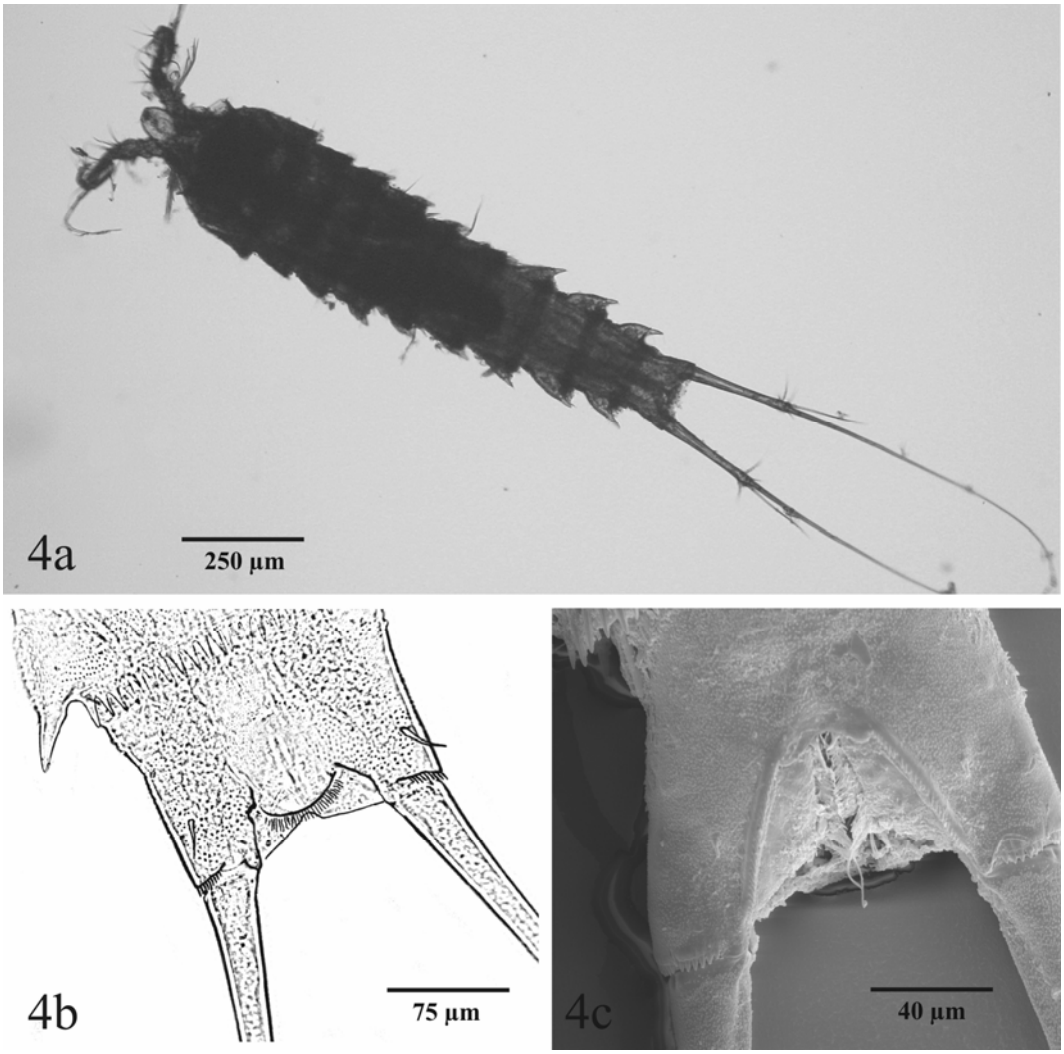


Fig. 4. *Male of Isocletopsyllus sardus sp. nov.* ***A.*** *Dorsal view. Scale bar = 250 µm.* ***B.*** *Dorsal side of anal somite. Caudal rami with inner apical edge straight and without setae. Scale bar = 75 µm.* ***C.*** *Ventral side of anal somite. Scale bar = 40 µm.*

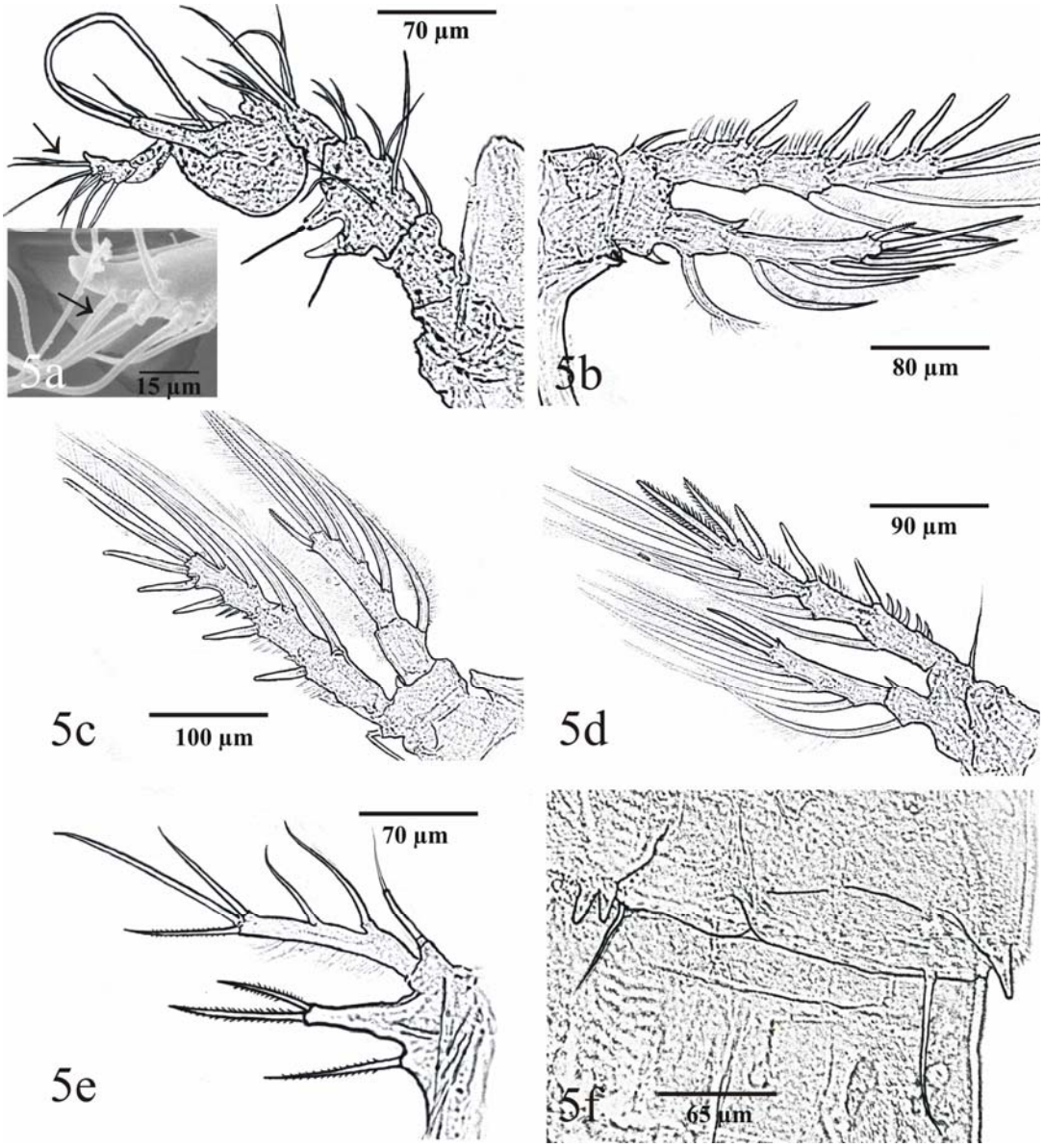


Fig. 5. *Male of Isocletopsyllus sardus sp. nov.* ***A.*** *First antenna. Apical acrothek (arrow). Scale bar = 70 µm.* ***Insert****, apical acrothek (arrow). Scale bar = 15 µm.* ***B.*** *P2. Scale bar = 80 µm.* ***C.*** *P3. Scale bar = 100 µm.* ***D.*** *P4. Scale bar = 90 µm.* ***E.*** *P5. Baseoendopod with an elongate, triarticulate seta; endopodal lobe triangular with 1 basal and 2 apical hairy setae. Exopodal segment with 1 inner hairy seta and 1 apical and 3 outer bare setae. Scale bar = 70 µm.* ***F.*** *P6. Scale bar = 65 µm.*

6 – ACKNOWLEDGEMENTS

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7 – RIASSUNTO

Nel seguente lavoro sono riportate alcune informazioni riguardo alla distribuzione e alla biologia di *Lightiella magdalenina* (Carcupino, Floris, Addis, Castelli, Curini-Galletti, 2006), scoperta di recente nelle acque dell'Arcipelago di La Maddalena (Sardegna, Italia). Questa rappresenta l'ultima specie descritta per i crostacei cefalocaridi e la prima ed unica segnalazione di questo taxon nel Mediterraneo. Questo ritrovamento, quindi, riempie un vuoto nella distribuzione del genere e dell'intera classe.

Al fine di determinare con maggior precisione l'areale della specie, sono stati effettuati numerosi campionamenti nella costa Nord della Sardegna (2 nel Comune di Alghero, 1 a Stintino, 1 nei pressi di Golfo Aranci, 1 a Cala Finanza nel Comune di Loiri Porto San Paolo) e nelle acque antistanti Marsiglia (Francia). Tutti i siti presi in esame non hanno portato al ritrovamento di nessun nuovo esemplare, mettendo in evidenza come questa specie, al pari della maggior parte dei cefalocaridi, sia molto rara e con un areale piuttosto limitato e puntiforme.

Per quanto riguarda lo sviluppo larvale, *L. magdalenina* al pari delle altre specie, ha uno sviluppo molto lento ed anamorfico con l'aggiunta di segmenti corporei ed appendici ad ogni muta. In particolare sono stati individuati 17 differenti stadi di sviluppo (15 metanaupliari e 2 giovanili). I primi 6 stadi metanaupliari presentano un numero pari (6, 8, 10, 12, 14 e 16) di segmenti corporei incluso il telson. La condizione di 20 segmenti tipica dello stadio adulto è raggiunta con ulteriori quattro stadi a 17, 18, 19 e 20 segmenti, mediante l'aggiunta di un singolo segmento per muta. Il numero completo di appendici viene raggiunto attraverso ulteriori 5 stadi, sempre a 20 segmenti corporei. Dopo i primi 15 stadi di sviluppo si assiste al passaggio dalla forma metanaupliare a quella giovanile, caratterizzata dalla perdita del processo enditico naupliare della 2° antenna. Infine, lo stadio adulto viene raggiunto durante gli ultimi due stadi giovanili. La presenza di due serie di sviluppo caratterizzate rispettivamente da un numero di segmenti corporei pari a 6, 8, 10, 12, 14 e 17, 18, 19, 20 conferma che l'aggiunta di un segmento per muta, nelle fase tardiva, non rappresenta un evento occasionale, come precedentemente ipotizzato, ma bensì una caratteristica dello sviluppo dei cefalocaridi. L'analisi delle caratteristiche morfologiche delle

appendice nei diversi stadi di sviluppo ha permesso di individuare, per ognuna di esse, una differente serie di sviluppo. La seconda mascella e le prime cinque appendici toraciche hanno mostrato la serie più lunga, con nove differenti stadi, a dimostrazione di uno sviluppo particolarmente lento e graduale, mentre l'appendice a sviluppo più rapido è rappresentata dalla prima antenna, con una serie di sviluppo ridotta a quattro stadi. La scarsità degli esemplari ed il loro reperimento quasi interamente concentrato in un unico mese (Ottobre, 2004), forse a causa di un occasionale e fortunato campionamento, non hanno consentito di ottenere dati sul ciclo vitale e sul periodo riproduttivo.

L'analisi genetica ha messo in evidenza come *L. magdalenina* risulti saldamente associata con *H. macracantha* per i due geni mitocondriali COI e Cyt-b. Questi dati sono stati quindi allineati e confrontati con i corrispondenti, presenti in letteratura, e relativi ad alcune specie appartenenti a diverse classi del clade Pancrustacea (Insecta, Cirripedia, Branchiura, Branchiopoda, Malacostraca, Remipiedia, Pentastomida). I data-set così creati sono stati utilizzati per la costruzione di Network rivelatisi utili nell'evidenziare la stretta correlazione filogenetica esistente tra le due specie di cefalocaridi e la netta divergenza che separa tale classe dagli altri taxa.

La *type locality* è caratterizzata da un fondale sabbio fangoso, ricco di materia organica rappresentata principalmente da *matte* morta di *Posidonia oceanica*. L'esame granulometrico del sedimento evidenzia che la componente organica è presente con valori superiori al 95%. Tutti i campioni risultano infatti composti per il 70% da frammenti di rizomi di *Posidonia oceanica* e dal 30% da frammenti di bivalvi e gasteropodi; le dimensioni di questi frammenti organici ricadono per il 75% nella classe delle sabbie (200-64 µm) e per il 15-20% nella classe dei fanghi (<64 µm).

La comunità zoobentonica rinvenuta risultata caratterizzata da 11 differenti taxa. Tra questi i copepodi arpacticoidi risultano essere il gruppo dominante. Nell'ambito di questo taxon sono state identificate sette famiglie: Ameiridae, Canuellidae, Canthocamptidae, Porcellidiidae, Diosaccidae, Normanellidae e Cletopsyllidae. Queste due ultime famiglie non erano mai state segnalate per l'Italia e nell'ambito dei cletopsyllidi, è stata individuata una probabile nuova

specie appartenete al genere *Isocletopsyllus.* La nuova specie differisce dalle altre due del genere attualmente conosciute per: 1) esopodite della 2° antenna con due setole di differente lunghezza 2) il segmento apicale del P1 che porta due setole interne e due apicali 3) dimorfismo sessuale sul P2 e sui rami caudali. Questa risulta la prima segnalazione per la famiglia in Italia e la terza per il Mediterraneo.