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DESCRIPTION OF THE DECAPODID STAGE OF *PLESIONIKA NARVAL* (FABRICIUS, 1787) (DECAPODA: CARIDEA: PANDALIDAE) IDENTIFIED BY DNA BARCODING

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ABSTRACT

The morphology of the decapodid stage of *Plesionika narval* (Fabricius, 1787) is described and illustrated based on larvae collected in the Canary Islands waters (NW Africa). Mitochondrial DNA analysis of the barcoding gene COI sequences confirmed the identity of the larvae specimens. Decapodid development of *P. narval* is compared with other pandalid and related genera *Pandalus*, *Pandalopsis*, *Procletes*, *Stylopandalus*, and *Icotopus*. Based on their morphological similarities we concluded that the nomina dubia genus *Icotopus* is a synonym of *Plesionika* and herein selected *Plesionika* over *Icotopus* as the valid name for the genus.

KEY WORDS: decapodid, DNA barcoding, *Icotopus*, Pandalidae, *Plesionika narval*

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INTRODUCTION

Most crustacean decapods have life histories that include a pelagic larval phase. This period is a critical time in which the larvae are highly vulnerable to starvation, predation, and advection away from suitable juvenile habitat and survival rates may be near zero (Pechenik, 1999). Consequently, planktonic studies have been applied in order to investigate the population fluctuations for ecosystem assessment and fisheries management. However, identification of decapod larvae from plankton samples is normally a very difficult task (Anger, 2006). Their processing is time-consuming and requires a well-trained human expert capable of distinguishing subtle morphological features. This problem usually increases because complete descriptions of larval ontogeny, obtained from ovigerous females, are not available for many species (see González-Gordillo et al., 2001). Particularly difficult is the larval rearing of deep-sea species and species with extended pelagic larval duration that complicate the collection of the complete series of larval stages for morphological descriptions. Fortunately, the development of molecular-based-identification or the DNA barcoding techniques is increasing our possibilities to match adults with the juvenile or larvae of these problematic specimens collected in the field. As example, a recent phylogenetic study has provided definitive evidence that the larva of *Cerataspis monstrosa* Gray, 1828, whose adult identity had remained a mystery for over 180 years, is actually an early larval form of the deep-sea shrimp *Plesiopenaeus armatus* (Bate, 1881) (Bracken-Grissom et al., 2012). Similarly, De Grave et al. (2010) applied DNA analysis to clarify the systematic status of the caridean superfamily, Galatheacaridoidea. For species with long larval duration such as lobsters, which can spend up to two years in the plankton (Anger, 2006), DNA barcoding techniques are particularly helpful for the correct identification of their phyllosoma. Molecular techniques have also made possible the morphological description of the previously unknown final-stage phyllosoma larva of *Panulirus echinatus* Smith, 1869 (by Konishi et al., 2006), *Scyllarus pygmeanus* (Bate, 1888) (by Palero et al., 2008) and *Scyllarus arctus* (Linnaeus, 1758) (by Palero et al., 2011) collected in the plankton.

Plesionika, currently represented by about 92 recognized species, stands as the most diverse genus of Pandalidae (De Grave and Fransen, 2011). It is primarily distributed at low latitudes, and contains a number of large-bodied shrimps of current or potential economic importance (Holthuis, 1980). They have also been subject of numerous biological and fishery studies (see Vafidis et al., 2005). In spite of that, there has been no significant progress on the study of their larvae. In fact, details of larval morphology have remained almost unknown. The only reliable larval descriptions based on laboratory reared material are for *Plesionika acanthonotus* (Smith, 1882) (by Bourdillon-Casanova, 1960), *Plesionika edwardsii* (Brandt, 1851) (by Landeira et al., 2009a) and *Plesionika narval* (Fabricius, 1787) (by Landeira et al., 2009b). However, these descriptions are limited to the early zoeal morphology due to problems in larval rearing. The most successful study obtained seven zoeal stages after 20 days of culture (Landeira et al., 2009a). At that stage, the zoeae of *P. edwardsii* still lack third pereiopods

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and pleopods implying a long series of larval stages; a "primitive" feature of species of *Plesionika* as suggested by Lebour (1940). However, there is no information at all on the decapodid of *Plesionika*. Decapodid is the first postzoeal stage of decapods (Kaestner, 1970). This stage constitutes the settlement larval form, and therefore, is a critical period in the life cycle of decapods (Anger, 2001). In general, great changes occur in the larval traits from zoea to decapodid and give rise to distinct larval forms. Thus, the term decapodid includes many names of terminal larvae: glaucothoe for Anomura, megalopa for Brachyura, puerulus for Palinuroidea, or mastigopus for Sergestoidea (Felder et al., 1985). However, in some carideans the morphological and behavioural transitions between stages are gradual rather than metamorphic (Anger, 2001, 2006). Consequently, it is sometimes rather difficult to distinguish successive larval stages such as between late zoeae and decapodid or between late decapodid and early juveniles. Nevertheless, following Anger (2006) the beginning of the decapodid stage can be delimited by the presence of functional pleopods, whereas it ends when the pereiopodal exopods disappear and/or loss their main natatory function.

The present study provides a complete description and illustration of the late decapodid stage of *Plesionika narval* collected in the Canary Islands waters, constituting the first description of this terminal larval stage in the genus *Plesionika*. The identity of the larvae was confirmed by molecular analysis. This work also compares the decapodid of *Plesionika* with those of the other pandalid genera *Pandalus*, *Pandalopsis*, *Procletes*, and *Stylopandalus*, as well as with the nomina dubia *Icotopus* attributed to *Plesionika* by Lebour (1940).

Materials and Methods

Sample Collection

The material studied in this work was obtained during the CETOBAPH cruise, carried out from 4 to 20 April, 2012 on board R.V. Cornide de Saavedra. The cruise took place in the Canary Islands (NW Africa) and sampling stations were located in the open ocean, north of the archipelago (28°48′N, 16°01′W) and SW of El Hierro (27°39′N, 18°03′W), La Palma (28°32′N, 18°00′W) and Tenerife (28°04′N, 16°48′W) islands. Specimens were collected between 100-700 m depth, using a mid-water trawl (total length 101.6 m, mouth opening 20 m, and cod-end mesh size 10 mm). After a trawling time of 1 hour, decapods were sorted from the total catch and preserved in 80% ethanol. In the laboratory, decapodids of *P. narval* were extracted and preserved in 100% ethanol for DNA analysis.

Morphological Analysis

Drawings and measurements were made with the aid of a camera lucida on a binocular (ZEISS Stemi SV6), following the method proposed by Clark et al. (1998). The specimens were in good conditions except for the loss of pereiopods, many setae and broken rostrums. Hence, the setation pattern described here should be considered with caution. The late decapodid stage is fully described, but only discrete remarks on earlier decapodid and early juvenile stages are described. Carapace length (CL) was measured from the posterior orbital margin to the mid-dorsal end of the posterior carapace margin. The specimens examined in this study are deposited in the National Taiwan Ocean University (catalogue numbers from NTOU M01785-NTOU M01792) and in the Museum of Natural Science of Tenerife (catalog number TFMCZP/3137; DL/746).

Molecular Analysis

One specimen of each of the early decapodid, late decapodid and early juvenile stages was used for the barcoding gene mitochondrial COI sequencing (657 bp). The identification of the larvae was further confirmed by sequence comparison of two mitochondrial genes, COI and 16S rRNA. Analysis of two genetic markers can help to avoid the misleading result from the possible presence of nuclear mitochondrial pseudogenes (numts). The primer set for COI was LCO1490/HCO2198 (Folmer et al., 1994) and 16S rRNA was 16Sar (Simon *et al.*, 1994)/16S1472 (Crandall and Fitzpatrick, 1996). Crude genomic DNA was extracted from the fourth and fifth pleopods of the studied specimens by the Qiagen® DNeasy® Blood and Tissue Kit following the protocol. PCR reaction was performed in totally 25 μl reactions with 50-250 ng of the DNA templates, 2.5 μl of 10× polymerase buffer (TaKaRa *Taq*™), 0.5 μl of 25 mM magnesium chloride ($MgCl_2$, TaKaRa *Taq*™), 0.5 μl of 2.5 mM of deoxyribonucleotide triphosphate mixture (dNTPs, TaKaRa *Taq*™), 0.5 μl of 10 μM for each primer (MDBio), 0.5 U of *Taq* polymerase (5 U/μl, TaKaRa *Taq*™), and additional 0.5 μl of 1% bovine serum albumin (BSA; stock concentration 0.5 mg/μl) for the COI gene. The PCR cycling condition was as followed: 5 min at 95°C for initial denaturation, then 40 cycles of 30 s at 94°C, 30 s at 48.0°C for the COI gene and 45.5°C for the 16S rRNA gene, 30 s at 72°C, and final extension for 7 min at 72°C. 1% agarose gel for electrophoresis was used for checking the size and quality of PCR products before the use of High Pure PCR Product Purification kit (Roche Applied Science) to purify the PCR products for sequencing. Sequencing products were run (forward and reverse directions) with the same PCR primer set on an ABI 310 Genetic Analyzer (Applied Biosystems) by the commercial bio-company. Sequences were assembled, cleaned and edited from two strands sequences to obtain a consensus sequence by the computer program SeqMan Pro™ (LASERGENE®, DNASTAR).

The COI sequences (657 bp) obtained from the early decapodid, late decapodid and early juvenile (GenBank accession Nos KJ670308-670310, respectively) were blasted into GenBank for species matching. The closest match (98.7-100% similarity) of these sequences is *Plesionika narval* (Pandalidae), which has six COI sequences (554-657 bp: JN412729, JQ306288, JQ305932, JQ305933, JQ305934, JQ306289) in the GenBank. The sequence similarity suggests that the larvae are possibly member of *Plesionika*. Therefore, the 21 species of *Plesionika* available in the GenBank with COI sequences more than 554 bp were downloaded for nucleotide divergence comparisons and Bayesian inference (BI) phylogenetic tree construction. Other than *P. narval*, there is also a positive matching of one of the two COI sequences of *Stylopandalus richardi* (Coutière, 1905) available in the GenBank (JN412730: 600 bp, JQ306317, 575 bp), though these two sequences have 25.9% nucleotide divergence. Thus, these two *S. richardi* sequences were also included in the analysis. The dataset of all sequences was aligned by BioEdit v.7.1.3 (Hall, 1999), and translated into the corresponding amino acids to check the stop codon by MEGA v.6. (Tamura et al., 2013). This computer program was also used to calculate the pairwise divergence. A best-fit model of DNA substitution and model parameters were selected and estimated based on the Akaike's criterion (AIC) by jModelTest v.2.1.3 (Darriba et al., 2012). MrBayes v.3.2 (Huelsenbeck and Ronquist, 2001) was used to construct the Bayesian inference (BI) phylogenetic tree from the dataset for relating the early decapodid, late decapodid and early juvenile to the known species. Two independent BI runs were performed with 5,000,000 generations each sampled every 1000 generations. A 50% majority-rule consensus tree was obtained from all post-burn-in sampled trees.

Results

DNA Barcoding Identification

The dataset comprises of 81 sequences of COI from 21 species of *Plesionika* (including four of the eight species reported from the Canary Islands, see González-Pérez, 1995), one *Stylopandalus* (*S. richardi*), an early decapodid, a late decapodid and an early juvenile. There is no stop codon existed in this COI dataset after checking of translation. The best-fit model of COI dataset estimated by jModelTest (selected with corrected AIC) was TIM2 + I + G evolutionary model ($-\ln L = 7686.0935$, $G = 0.5220$, $I = 0.4940$).

The early decapodid, late decapodid, and early juvenile form a stable clade (Pp = 1.0) with all the six *P. narval* sequences and one of the two *S. richardi* sequences (JN412730) available in GenBank (Fig. 1), with only 0.0-

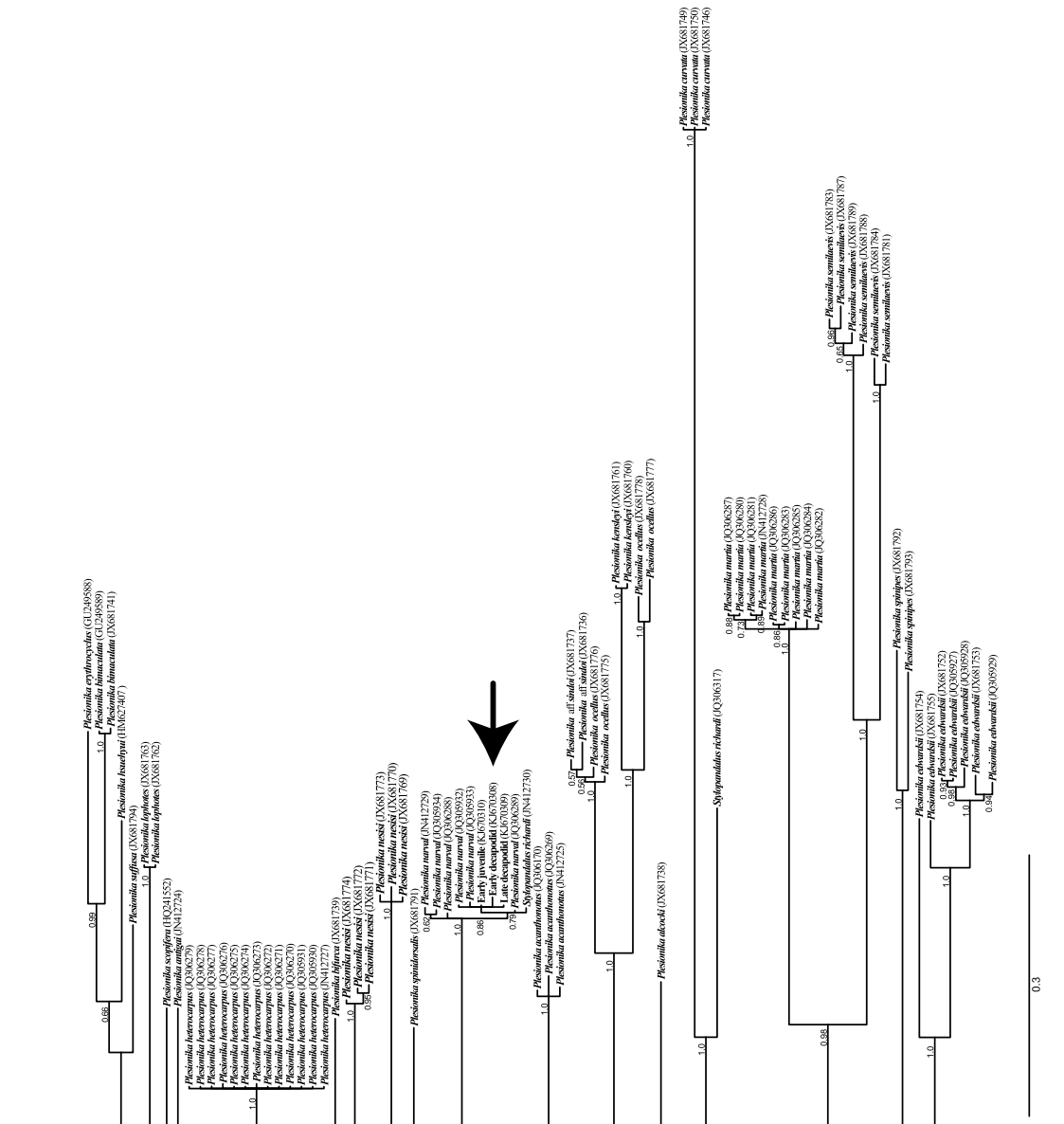

Fig. 1. Bayesian phylogenetic tree from partial sequences (554-657 bp) of COI gene amongst early decapodid, late decapodid, early juvenile (indicated by an arrow), and species of *Plesionika* and *Stylopandalus richardi* downloaded from the GenBank. Posterior probability estimated by Bayesian inference shown on branches.

1.3% uncorrected pairwise distances (*p*-distance) divergence. The *p*-distance amongst the early decapodid, late decapodid, and early juvenile only have 0-0.3% sequence divergence, while the nucleotide divergence amongst the six *P. narval* COI sequence from the GenBank are 0.0-0.9%. The JN412730 COI sequence of *S. richardi* differs from the present larvae and the GenBank sequences for *P. narval* in merely 0.4-0.9% and 0.2-0.9% nucleotide divergence, respectively. However, the other COI sequence of *S. richardi* (JQ306317) in the GenBank is at a very different clade (Fig. 1) and has a nucleotide divergence of 25.9%, 21.1-21.3% and 21.1-21.5% from the JN412730 *S. richardi*, the present larvae and the GenBank sequences for *P. narval*, respectively. The 16S rRNA sequences (529-539 bp, GenBank accession nos. KJ670311-670313) of the early decapodid, late decapodid, and early juvenile are nearly identical (nucleotide divergence 0.2-0.4%). There are three 16S rRNA sequences available for *P. narval* in the GenBank (451 bp: JN412689, JN412690, JN412691; with JN412690 from the same specimen that provided the JQ306289 COI sequence) and they are all identical and with only 0.0-0.2% nucleotide divergence from the present larvae. There is no 16S rRNA sequence available for *S. richardi* in the GenBank. Nevertheless, a specimen of *S. richardi* from Taiwan (NTOU M01306, identified by the second author TYC) succeeded in generating the 16S rRNA sequence (540 bp, GenBank accession no. KJ670314) though it failed in COI sequencing. The 16S rRNA segment of the specimen from Taiwan of *S. richardi* has large nucleotide divergences of 18.7-18.9% and 18.7% from the present larvae and *P. narval* in the GenBank, respectively. Thus, the 16S rRNA data generally concurs with the COI dataset and suggests that there is a misidentification of the specimen for the JN412730 COI segment in the GenBank. Both *P. narval* and *S. richardi* bear many teeth on the dorsal and ventral margins of the rostrum, and previously they were included under the same genus *Parapandalus* Borradaile, 1899 (see Chace, 1985). The body size of *S. richardi* is rather small and therefore small specimens of *P. narval* can be easily mixed up with *S. richardi* (also see Discussion below).

The low sequence divergences of 0.0-1.3% and 0.0-0.2% in the COI and 16S rRNA genes, respectively, are generally considered as intraspecific variations in decapod crustaceans (see Shih et al., 2004; Matzen da Silva et al., 2011, 2012; Robe et al., 2012). Moreover, of the eight species of *Plesionika* reported from the Canary Islands (González-Pérez, 1995), only *P. edwardsi* is somewhat similar to *P. narval* (previously these two species were included under the same genus *Parapandalus*, see Chace, 1985). *Plesionika edwardsi* is included in the COI phylogenetic tree and it occupies a position far away from the present larvae (Fig. 1, with 22.4-24.3% nucleotide divergence). The other *Plesionika* reported from the Canary Islands are all very different and not belong to the *P. narval*-species group (see Chan and Cronsier, 1991). Thus, it can be regarded that the early decapodid, late decapodid and early juvenile in the present study all belong to the same species, which is *P. narval*.

Larval Distribution

From a total of 39 trawl tows, 12 resulted in the captured of 32 decapodids and 1 early juvenile of *P. narval*. Most of the larvae were collected in La Palma, with 22 specimens; whereas in Tenerife and El Hierro only 7 and 4 larvae were found respectively. The only juvenile specimen was collected in Tenerife. No larvae were captured in the oceanic trawls, northern Tenerife. In general, the abundance of the larvae of *P. narval* was low, constituting <2% of the total abundance of crustacean decapods collected. The most abundant mesopelagic shrimps collected were: *Oplophorus spinosus* (Brullé, 1839), *Systellaspis debilis* (A. Milne-Edwards, 1881a), *Acanthephyra purpurea* A. Milne-Edwards, 1881b, *Deosergestes corniculum* (Krøyer, 1855), *Sergia grandis* (Sund, 1920), and *Funchalia villosa* (Bouvier, 1905).

Morphological Description *Plesionika narval*

Late Decapodid

Carapace (Fig. 2A-D).—CL = 7.72 ± 1.33 mm. Carapace smooth with anterior and posterior dorsomedian tubercles. Gastrofrontal carina present. Rostrum straight and relatively long (1.24 times as long as carapace), overreaching antennal scale. Dorsal edge of the rostrum and anterior part of the carapace armed dorsally with 60-64 spines, ventrally armed with 30-37 spines along mid-distal section. Spines disposed relatively close to each other and size progressively reduced distally. Stout supraorbital spine. Sharp antennal and pterygostomial spines. Short pterygostomial carina with marginal plumose setae. Anteroventral margin of carapace with 6-7 small denticles. Eyes stalked with cornea well developed. In early decapodid the rostrum is shorter (same length of carapace length) with only 11 spines dorsally and widely spaced, and small antennal spines. In the early juvenile stage, the dorsomedian tubercles become smaller, supraorbital spines disappear, the rostrum is longer (around two times carapace length) and eyes have a distinct ocellus.

Antennule (Fig. 3A).—Peduncle 3-segmented with 69, 14, 10 plumose setae. Stylocerite slender, acute and overreaching middle of proximal segment. Long and segmented inner and outer flagella.

Antenna (Fig. 3B).—Basis with lateral and dorsal spines. Scaphocerite approximate as long as carapace, rather narrow, with sharp distolateral spine clearly overreaching blade, inner margin with 57-62 plumose setae.

Mandible (Fig. 3C, D).—Incisor and molar process developed, palp absent. Early juvenile stage with rudimentary, unsegmented palp.

Maxillule (Fig. 3E, F).—In early decapodid coxal endite unilobed with 10 plumodenticulate and 22 plumose setae. Basial endite with 13 plumodenticulate and 23 plumose setae. Endopod unsegmented with 11 sparsely plumose setae. In late decapodid, coxal endite unilobed with 8 plumodenticulate setae, 4 short spiniform setae and numerous small, thin simple setae and microtrichias. Basial endite with 13 short spiniform setae and numerous small, thin simple setae and microtrichias. Endopod unsegmented with 1 simple and 1 sparsely plumose setae.

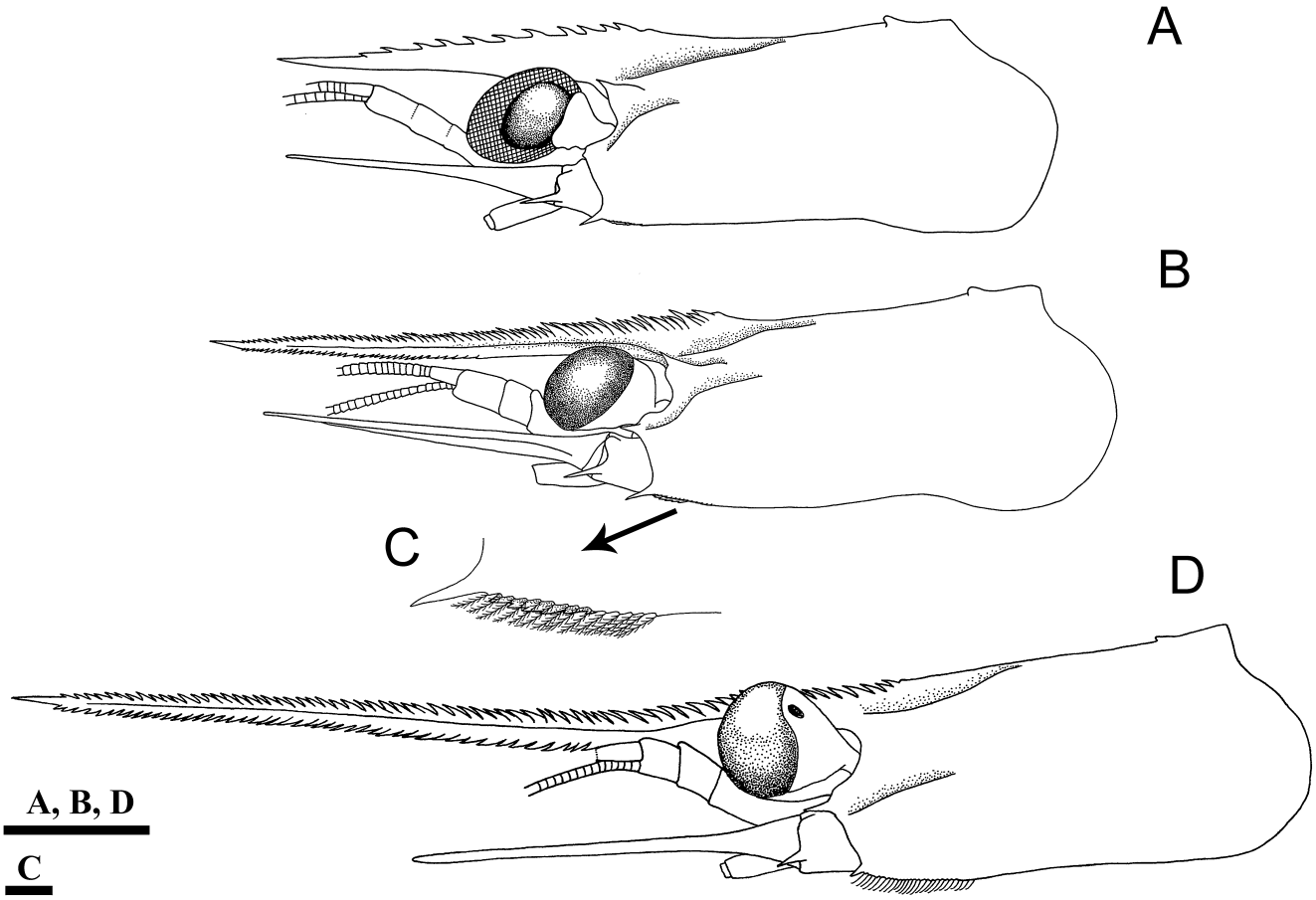

Fig. 2. *Plesionika narval*. Lateral view of carapace. A, early decapodid; B, late decapodid; C, anteroventral magin of carapace; D, early juvenile. Scale bars = 1 mm.

Maxilla (Fig. 3G, H).—In early decapodid, length of 2.4 mm. Coxal endite bilobed with 27 plumose setae. Basial endite bilobed, with 14 plumose and 1 sparsely plumose setae, and 19 plumose and 1 sparsely plumose setae. Endopod with 41 plumose setae. Scaphognathite with 108 marginal plumose setae, about 2.7 times longer than broad. In late decapodid, length of 3 mm. Coxal endite bilobed with 18 plumose setae. Basial endite bilobed, with 12 and 14 plumose setae each. Endopod with 11 long plumose setae. Scaphognathite with 126 marginal plumose setae, about 3.6 times longer than broad. In early juvenile stage unchanged.

First Maxilliped (Fig. 3I).—Coxal endite with at least 8 short setae. Basial endite with about 41 short setae. Endopod 3-segmented with (3, 9, 5 + 4) sparsely plumose setae. Exopod with 32 plumose setae proximally, distally flagellated with plumose setae. Large bilobate epipod present.

Second Maxilliped (Fig. 3J).—Endopod 5-segmented, extending beyond middle of exopod, with (2 + 3, 4 + 10, 3, 13, 5) plumodenticulate setae. Exopod long, flagellated with plumose setae. Epipode bearing well-developed podobranch.

Third Maxilliped (Fig. 3K).—Slender, endopod slightly longer than exopod. Endopod length 8.28 mm in early decapodid, 9.50 mm in late decapodid and 16.13 mm in early juvenile. Endopod 6-segmented. First, second, third and forth segments with (9 + 9, 2 + 3, 5 + 1, 13 + 2) plumodenticulate setae. Fifth segment with 10 + 8 plumodenticulate setae and 2 serrulate setae distally. Sixth segment with 4 terminal simple setae. Exopod flagellated with long plumose setae. Small epipod bearing podobranch from late decapodid onwards.

Pereiopod 1 (Fig. 4A, B).—Biramous, endopod as long as expopod. Endopod with numerous plumodenticulate setae along entire length. Distal carpus and proximal propodus sections with patch of serrulate setae. Small dactylus with simple terminal setae. Exopod flagellated with long plumose setae. Epipod absent.

Pereiopod 2 (Fig. 4C, D).—Biramous, endopod as long as expopod. Endopod with numerous plumodenticulate setae along its entire length. Carpus two-divided (not multidivided) with crown of serrulate setae distally. Chela with blunt fingers bearing tufts of distal simple and serrulate setae, cutting edges not developed. Dactylus distinctly longer than fixed finger (approx. 1.3 times length of finger). Exopod flagellated with long plumose setae. Epipod absent.

Pereiopod 3 (Fig. 4E).—Missing due to damage in early decapodid specimens. Biramous, longer than pereiopod 2. Endopod length 13.73 mm in late decapodid and 33.06 mm in early juvenile. Endopod 1.7 times expopod length. Endo-

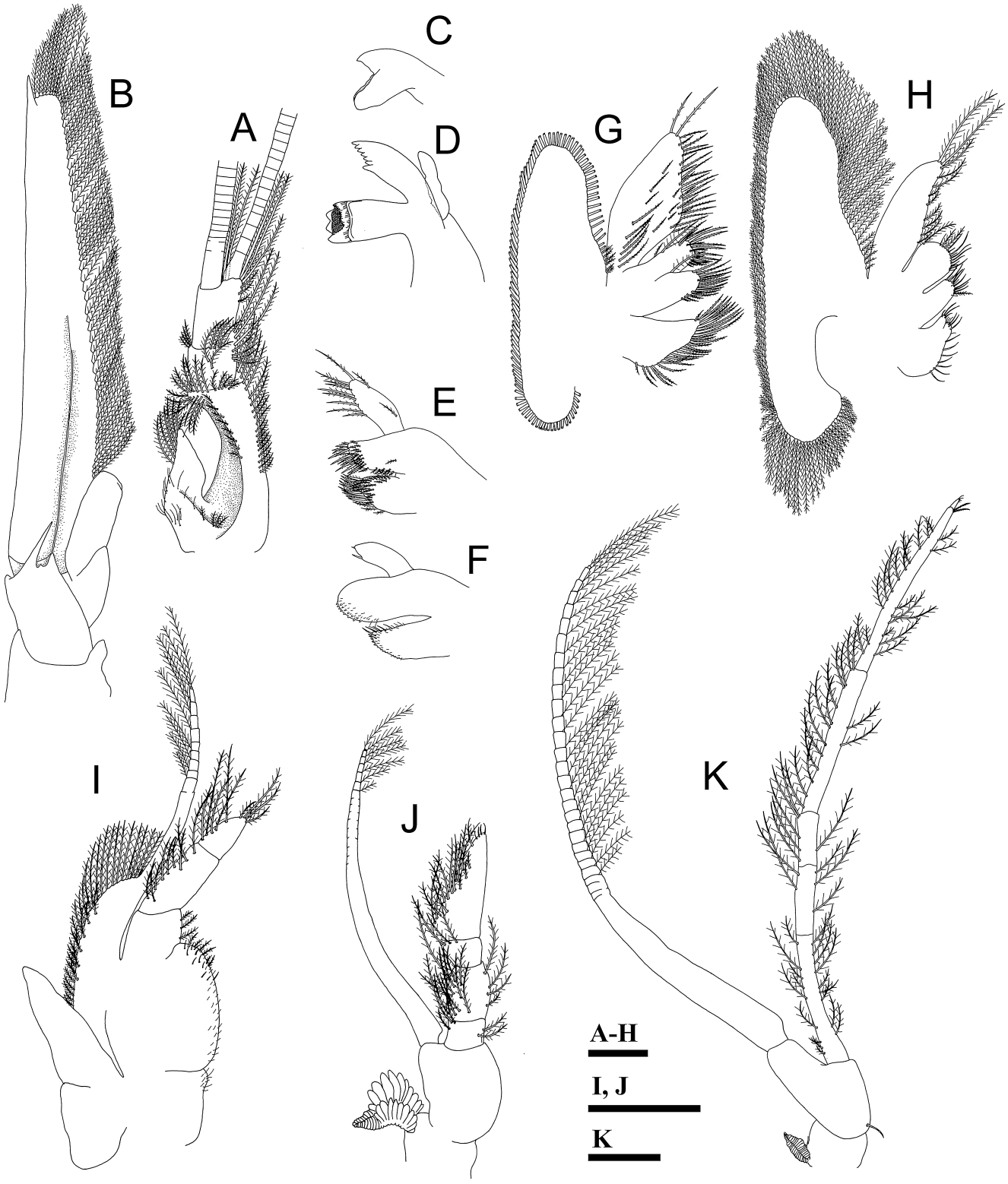

Fig. 3. *Plesionika narval*. A, antennule (late decapodid); B, antenna (late decapodid); C, mandible (late decapodid); D, mandible (early juvenile); E, maxillule (early decapodid); F, maxillule (late decapodid); G, maxilla (early decapodid); H, maxilla (late decapodid); I, first maxilliped (late decapodid); J, second maxilliped (late decapodid); K, third maxilliped (late decapodid). Scale bars A, B, I-K = 1 mm; C-H = 0.5 mm.

pod with numerous plumodenticulate setae along its entire length. Without chela. Exopod flagellated with long plumose setae. Epipod absent.

Pereiopod 4 (Fig. 4F).—Missing due to damage in early decapodid specimens. Biramous, longer than pereiopod 3. Endopod length 15.04 mm in late decapodid and 34.81 mm

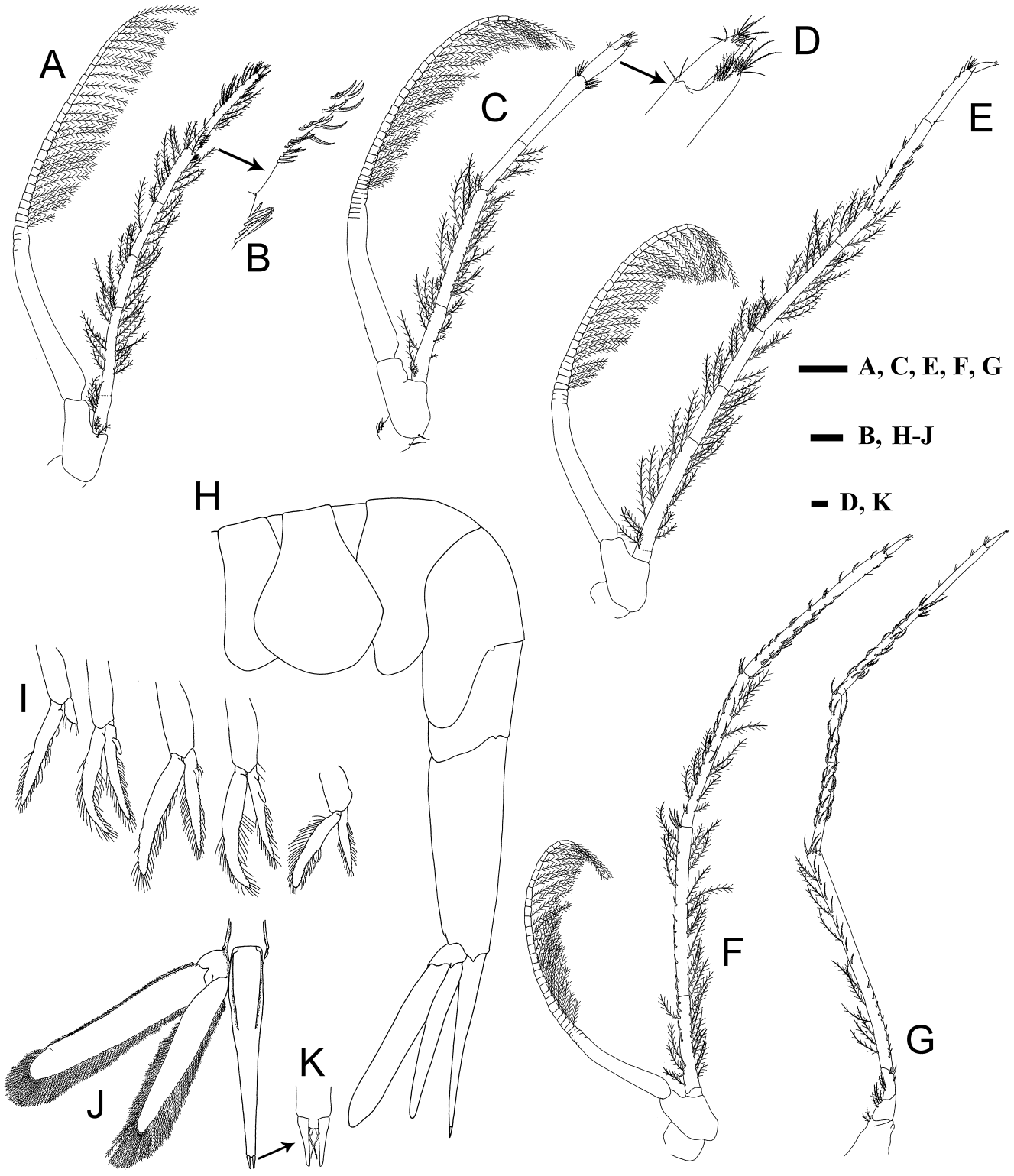

Fig. 4. *Plesionika narval*, late decapodid. A, pereiopod 1; B, detail of setation; C, pereiopod 2; D, detail of chela; E, pereiopod 3; F, pereiopod 4; G, pereiopod 5; H, abdomen; I, pleopods; J, telson and uropoods; K, detail of distal spines of telson. Scale bars A, C, E, F-J = 1 mm; B, D, K = 0.1 mm.

in early juvenile. Endopod 2.1 times expopod length. Setation of mid-proximal and mid-distal endopod dominated by plumodenticulate setae and serrulate setae respectively. Ischium and merus with row of spiniform seate. Exopod flagellated with long plumose setae. Epipod absent.

Pereiopod 5 (Fig. 4G).—Missing due to damage in early decapodid specimens. Without exopod. Slightly longer than pereiopod 4 (16.21 mm in late decapodid and 36.45 mm in early juvenile) and with similar setation pattern. Epipod absent.

Pleon (Fig. 4H).—Smooth. Segmented, with 6 somites. Somites 1-5 with a pair of pleopods. Somite 4 rounded, somite 5 slightly pointed.

Pleopods (Fig. 4I).—Biramous and unsegmented. Endopod and exopod with plumose setae. Presence of appendix interna in endopod of second through fifth pleopods.

Uropods (Fig. 4H, J).—Slender and just extending beyond tip of telson. Exopod about 6 times longer than wide, about as long as endopod, with small diaeresis spine.

Telson (Fig. 4J, K).—Slender and slightly shorter than length of sixth pleonite. Distal margin with 1 outer pair of stout setae and 1 inner pair of simple setae.

Discussion

The zoeae of *Plesionika* share several, common morphological characters of Pandalidae, summarized as follows: dorsal connection between carapace and abdomen almost at an 180° angle; eye peduncle narrowed at base; antennular peduncles strongly concave; rostrum well developed since first stage and with dorsal spines in later stages; supraorbital spines present and pereiopod 5 without exopod (Lebour, 1940; Pike and Williamson, 1964; Landeira et al., 2009b). However, the morphology of advanced larvae or decapodid stage was completely unknown up to now. In the present study, molecular analysis facilitated the specific identification of decapodids collected with mesopelagic trawls as *P. narval*. Therefore, this is the first description of the decapodid morphology for the genus.

The caridean decapodid stage generally has both the larval and juvenile morphological traits (Felder et al., 1985; Anger, 2006). The following five characters are typical of the larva of *Plesionika*: carapace with anterior and posterior dorsomedian tubercle; supraorbital spines present; mandible without palp; first four pereiopods with exopods; carpus of pereiopod 2 not multi-articulated. The morphology of the early juvenile specimen studied here confirmed that the rudimentary mandibular palp and the multi-articulation on the carpus of pereiopod 2 only appear from this stage onwards. On the contrary, the stout supraocular spines and the exopods of pereiopods 1-4 are features that disappear. The dorsomedian protuberances on the carapace also seem to disappear but more gradually. In the early juvenile stage they are still present as smaller protuberances, but will almost disappear in the adult stage as described in Chan and Crosnier (1991). The gradual morphological transition from zoea to decapodid is evident in the development of pereiopodal exopods. Decapodids of *P. narval* retain the periopodal exopods (a zoeal character) that still perform natatory functions. They should persist but tend to disappear gradually over several juvenile moults.

These shifts in functional morphology of pereiopods are accompanied by equally gradual transitions of behaviour, from fully pelagic swimming to a mixture of near-bottom swimming and crawling during the settlement of decapodid stages, and eventually to walking on benthic surfaces in late decapodids and early juveniles (Anger, 2006). The rostrum of *P. narval* undergoes a striking development in length from larva to adult. The rostrum of zoea I is unarmed, slender, straight but slightly pointed downward at the tip. Also, at this stage the rostrum is long, reaching the antennular exopod. However, the length decreases significantly from this stage to at least the zoea V, when it is just longer than frontal lobe (Landeira et al., 2009b). In the decapodid stages the rostrum approaches the adult form in shape, length and number of spines. Also, the rostrum of the earlier decapodid (shorter and with less and more separated dorsal spines) points out that the development of the rostrum is progressive, even inside the decapodid phase. Nevertheless, it is still unknown how the rostrum grows between the zoea V and decapodid periods.

Lebour (1940) described the larval development of *Stylopandalus richardi* (as *Parapandalus richardi*), suggesting it should be similar to *Plesionika*. She observed a similar grow pattern in the rostrum. It decreases until zoea VI stage when the rostrum begins to lengthen and reaches nearly half the length of the eyes. From this stage the rostrum of *S. richardi* rapidly elongates and acquires the two basal spines typical of the adult form. At zoea VI of *S. richardi* the pereiopods lack exopods and pleopods are bud. At this larval stage *P. edwardsii* is less developed showing the pereiopod 2 still as bud and absence of pereiopod 3 and pleopods (Landeira et al., 2009b). The result of the present study not only suggests that the rostrum elongates later in *Plesionika*, but also that this genus has a longer series of zoeal stages than *Stylopandalus*; probably not less than 12 stages. In any case, the morphology of decapodid stage of *S. richardi* resembles that of *P. narval* described above. As adults, these species also show close morphological affinities since they had been treated under the same genus *Parapandalus* before (though this genus is now no longer recognized, see Chace, 1985).

Regarding the other pandalid genera, unfortunately descriptions of decapodid morphology are only available for *Pandalina*, *Pandalus* and *Pandalopsis*. For *Pandalina*, Pike and Williamson (1964) described the complete larval series of *Pandalina brevirostris* (Rathke, 1843) obtained mainly from plankton specimens. Despite their accurate description of zoeal stages, they provided a very short description of the decapodid stage without illustrations that prevents a proper comparison with *P. narval*. The telson shape (narrower distally) and the presence of exopods on the pereiopods are the only similarities between both species noted in that description.

Pandalus and *Pandalopsis* are the best studied pandalid genera, in relation with their larval morphology (Lee et al., 2007). In general these genera contain species with shorter and abbreviated larval development. For example, *Pandalopsis* completes the development in only 3-5 zoeal

stages, whereas in *Pandalus* the number of zoeae varies among species, as reviewed by Komai (1999): *P. montagui* Leach, 1814 (5-7 zoeae); *P. stenolepis* Rathbun, 1902 (6 zoeae); *P. hypsinotus* Brandt, 1851 (3-6 zoeae); *P. platyceros* Brandt, 1851 (2, 4 zoeae). Decapodid morphology of both genera is close to their respective adult phase, thus some distinct characters of adults can be used to separate decapodids of *Pandalopsis* and *Pandalus*. Accordingly, *Pandalopsis* has longer antennules and posses a laminate expansion with ventral spinules on the merus of the third maxilliped and on the ischium of the first pereiopod. Unlike *P. narval*, the decapodid of *Pandalus* and *Pandalopsis* show a developed mandibular palp (but not for *Pandalopsis dispar* Rathbun, 1902, Park et al., 2004), absence or reduction of exopods on the pereiopods and more distal spines on the telson. Furthermore, the telsons of *Pandalus* and *Pandalopsis* are U-shaped, whereas in *P. narval* the telson is more slender and strongly narrowing distally, V-shaped.

The description of the decapodid stage of *Procletes levicarina* (Bate, 1888) is also available (see Gopala Menon, 1972, as *Heterocarpus levicarina*) if we accept its identification as valid. A detailed description of the larval development of this species was based on material derived from plankton samples obtained during the International Indian Ocean Expedition (1960-1965). The decapodids of *Procletes levicarina* appear to be different from decapodids of *P. narval* in less slender body shape; bearing distinct dorsal, lateral and dorsomedian carinae on the carapace; abdomen with dorsomedian carina on all somites; second and third abdominal somites with anterodorsal protuberance.

On the other hand, the decapodid stage of *P. narval* shows a striking similarity with some advanced larval stages collected during the late nineteenth century. Specifically *P. narval* resembles with some specimens placed within the genus *Icotopus* by Bate (1888) and Coutière (1907). In 1888, Bate created *Icotopus* based on a late zoeal stage taken off Cape Howe, Australia, that was named *Icotopus arcurostris*. Later, Coutière (1907) described two other species from decapodid stages collected in the Atlantic Ocean: *Icotopus amplisimus* Coutière, 1907, and *Icotopus approxima* Coutière, 1907. All these larval species share common morphological features with the decapodid stage of *P. narval*. As an experienced worker on decapod larvae, Lebour (1940) hypothesized that *Icotopus* may be a synonym of *Plesionika*. The present study, using both molecular identification and morphological comparisons, strongly supports Lebour's view on *Icotopus*. As both the names *Icotopus* and *Plesionika* were simultaneously published in the same paper (Bate, 1888), these two taxon names do not have priority over one another but require a first revisor action to select the valid name (International Code of Zoological Nomenclature, Article 24.2.2). We here select the genus name *Plesionika* over *Icotopus* as the former name is much more widely used nowadays. Amongst the three described forms of *Icotopus*, *I. amplisimus* is most similar to the less developed decapodid described in the present study, suggesting that they may belong to the same species. These two forms have the same rostrum type (as long as carapace length with 11 spines only dorsally and widely spaced) and their carapace lengths are similar, around 6-7 mm. Moreover, the type localities of *I. amplisimus* are the Canary Islands and the Balearic Islands, which are in the same areas where the present decapodids collected. Nevertheless, the high diversity of *Plesionika* in these archipelagos (González Pérez, 1995; d'Udekem d'Acoz, 1999) and the brief description and illustrations given by Bate (1888) makes the relationships amongst *I. arcurostris*, *I. amplisimus* and *P. narval* very difficult to resolve.

It may need to be pointed out that the size of the decapodids in the present work is particularly large. The carapace length of the decapodids of *P. narval* is not only longer than the decapodids of the other genera but also the earlier juvenile stages of the same species. González et al. (1997) studied the biology of *P. narval* over a 20-year period and found that the minimum size was 2 mm carapace length, which is much smaller than our decapodid size (CL = 6-7 mm). The transition from the pelagic larval phase to the adult benthic habitat is often achieved through behavioural changes and morphological transformations, prompted by specific habitat cues. If the last zoeal stage larvae does not detect such cues, it may delay the transition to the following stage. The larvae may then pass through several instars that enables the larvae to grow but without significant morphological differences (Gebauer et al., 2003). For *P. narval*, being a benthic-suprabenthic species, the larvae hatch at the bottom and then migrate to the surface as zoea I. As in the other deep-sea pandalids such as *Pandalus borealis* Krøyer, 1838 (see Ouellet and Lefaivre, 1994), it is likely that throughout the development the larvae of *P. narval* gradually lose their positive phototaxis and tend to swim to the bottom to settle. It is possible that the present specimens underwent this phenomenon but were not able to perform such ontogenetic vertical migration. This delayed their transition from zoea to decapodid and also from decapodid to juvenile with their sizes greatly increased.

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