Morphological identification and molecular confirmation of the deep-sea blue and red shrimp *Aristeus antennatus* larvae

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The early life stages of the blue and red shrimp *Aristeus antennatus* (Decapoda: Dendrobranchiata: Penaeoidea: Aristeidae) were described by Heldt in 1955 based on plankton samples, larval rearing and assumptions of species habitat. Even with adequate keys, identification of its first larval stages remained a difficult task due to the lack of specific morphological characters which would differentiate them from other Penaeoidea species. Larvae of *A. antennatus* were collected in the continental slope off the Spanish Mediterranean coast in August 2016 with a neuston net and preserved in ethanol 96%. DNA from the larvae was extracted and the molecular markers COI and 16S rDNA were sequenced and compared to that of adults with the objective of confirming the previous morphological description. Then, we present additional information to the morphological description of *A. antennatus* larval stages through scanning electron microscopy and molecular analysis. This represents the first documented occurrence of *A. antennatus* larvae off the Catalan coast and sets the grounds for further work on larval ecology and population connectivity of the species, which is an important contribution to a more sustainable fishery.
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Abstract. The early life stages of the blue and red shrimp *Aristeus antennatus* (Decapoda: Dendrobranchiata: Penaeoidea: Aristeidae) were described by Heldt in 1955 based on plankton samples, larval rearing and assumptions of species habitat. Even with adequate keys, identification of its first larval stages remained a difficult task due to the lack of specific morphological characters which would differentiate them from other Penaeoidea species. Larvae of *A. antennatus* were collected in the continental slope off the Spanish Mediterranean coast in August 2016 with a neuston net and preserved in ethanol 96%. DNA from the larvae was extracted and the molecular markers COI and 16S rDNA were sequenced and compared to that of adults with the objective of confirming the previous morphological description. Then, we present additional information to the morphological description of *A. antennatus* larval stages through scanning electron microscopy and molecular analysis. This represents the first documented occurrence of *A. antennatus* larvae off the Catalan coast and sets the grounds for further work on larval ecology and population connectivity of the species, which is an important contribution to a more sustainable fishery.
1. Introduction

The deep-sea blue and red shrimp *Aristeus antennatus* (RISSO 1816; Decapoda: Dendrobranchiata: Penaeoidea: Aristiidae) is one of the most valuable fishing resources in the Mediterranean Sea. It is the main target of bottom trawlers along the coasts of Northwestern Africa, Portugal, Spain, France, Italy and Malta (www.fao.org). In the Spanish Mediterranean coast, it can represent up to 50% of the economical benefits for the fishermen associations (MAYNOU 2008, DGPAM 2017). Mature females aggregate at the continental shelf break in the summer (SARDÀ et al. 1994, 1997). Despite its social and economic relevance in the area, the knowledge about the species’ life cycle remains incomplete as its larval stages are still scarcely known. As this fishery progresses towards more integrative methods of stock assessment and management, filling the knowledge gap about the early life stages of *A. antennatus* is a crucial step in the study of its dispersal and population connectivity.

In dendobranchiate shrimps, the first larval stage hatching from the egg is usually a free-living nauplius. This stage has up to 6 substages depending on the species. It is followed by a variable number of zoeal stages often referred to as protozoa (early zoeae, with natatory antennules and antennae) and mysis (late zoeae, where the natatory function is assured by the pereiopods). The last mysis then metamorphoses into the first decapodid, which after a number of moults will come to settle in the adult habitat (ANGER 2001).

The description of the larval stages of *A. antennatus* (HELDT 1955) was based on 35 individuals caught in a plankton survey around the Balearic archipelago. The assumptions leading to the attribution of the larvae to *A. antennatus* were based on the author’s extensive knowledge of Penaeoid larvae in the Mediterranean Sea. This morphological identification has never been confirmed since. To present date, occurrence of *A. antennatus* larvae has only been detected in low numbers in plankton surveys off the Algerian and Portuguese Atlantic coasts, the Canary Islands and in the Balearic Sea, identified using Heldt’s larval descriptions (SERIDIJI 1971; DOS SANTOS, 1998, CARBONELL et al. 2010, LANDREIRA 2010, TORRES et al. 2013). The morphological description of a second mysis stage (TORRES et al. 2013), also based on larvae caught in plankton, was the most recent addition to the larval series, with the last larval stages still remaining unknown. Rearing of larvae in the laboratory from berried females is a usual technique to accomplish the description of a complete larval cycle (e.g. DI MUZIO et al. 2018). This is not possible in the case of *A. antennatus* due to the particularity of dendobranchiate shrimps releasing their eggs directly to the water column, as opposed to being carried by females. To our knowledge, only one deep-sea Penaeoid larva from a plankton survey has been molecularly identified (BRACKEN-GRISSOM et al. 2012). The available descriptions of Penaeoid larvae from laboratory studies correspond only to species inhabiting shallow waters – as for instance the caramote prawn *Penaeus kerathurus* (TORKMEN 2003) – since females are easily cultured in tanks and eggs can be collected from the water upon release. No study has yet reported the culture of females of any deep-sea Penaeoid species.

The morphological identification of Penaeoidea first larval stage, the protozoa I (PZI), is often a particularly difficult task due not only to the small size of the specimens but also to the fact that in some cases different species share the same larval morphology (MARTIN et al. 2014). It is only in later stages that the different larval series can be more easily distinguished based on morphological traits visible at the optical stereomicroscope, such as the presence and number of rostrum spines, supraorbital and/or pterygostomian spines, etc. On this matter, the early larval stages of *A. antennatus* are no exception. In fact, the description of the PZI was based on a single individual captured in the plankton of Balearic waters, in the Western Mediterranean Sea (HELDT 1955). The morphological characters that distinguish *A. antennatus* PZI from other Penaeoid species present in
our study area such as *Sicyonia carinata*, *Parapenaeus longirostris* or *Funchalia woodwardii* are generally clear (DOS SANTOS & LINDLEY 2001). However, the distinction between Aristeidae and Benthesicymidae PZI stage is more difficult because the only morphological character that allows their differentiation is a small endopod on the third maxilliped (mxp3), present in *A. antennatus* and absent in *Gennadas* spp. (HELDT 1955, GURNEY 1924). In the case of *A. antennatus*, the mxp3 is biramous, with 2 long plumose setae and 1 small simple seta on the exopod. In the case of *Gennadas* spp., the mxp3 is uniramous with 2 long plumose terminal setae. The reproductive period of the genus *Gennadas* has not yet been studied, but larvae caught in the plankton all year round have been classified as *Gennadas* spp. according to available information (FUSTÉ 1982, 1987; TORRES et al. 2014). The reproductive period of *A. antennatus* in the Mediterranean Sea is strictly seasonal in the summer (COMPANY et al. 2003). With both larval types occurring concurrently during the summer, their morphological differentiation becomes a key issue in the study of decapod larval communities and of *A. antennatus* larval distribution in particular.

The available descriptions of penaeoid larvae from plankton surveys have generally based their identification on an extensive knowledge of the adult morphology and ecology (HELDT 1938, 1955, GURNEY 1924). Although morphological identification is an essential first step, in some cases it can be insufficient and lead to misidentifications (PALERO et al. 2008, SULLIVAN & NEIGEL 2017). In this context, the use of molecular markers can be particularly useful in the confirmation of visual identification of specimens, in complement to keys and descriptions based on morphological characters (OLSON et al. 1991, WEBB et al. 2006). Previous studies on decapod crustacean larvae have used this technique either to confirm existing descriptions or as a complement to the descriptions of new stages (RAUPACH & RADULOVICI 2015, LANDEIRA et al. 2014, BRACKEN-GRISsom et al. 2012, PAN et al. 2008).

The objective of this study was to examine the morphology of the first protozoea of the deep-sea blue and red shrimp *A. antennatus* in order to find useful characters to distinguish it from *Gennadas* spp. larvae and to use molecular techniques to confirm the identification of all its known larval stages.

## 2. Materials and methods

### 2.1. Sampling and morphological identification of *A. antennatus* and *Gennadas* spp. larvae and adult specimens

In order to obtain both *A. antennatus* and *Gennadas* spp. larval types, we performed 2 plankton samplings, one in the summer when *A. antennatus* is at its peak reproductive period, and one in the winter, when *A. antennatus* does not reproduce but *Gennadas* spp. larvae are likely to be found. Nevertheless, the presence of *Gennadas* spp. larvae has been reported in the summer in the Balearic Sea (TORRES et al. 2014) and we were aware that we could encounter a mix of both species when aiming to collect *A. antennatus* PZI larvae in the summer.

Summer sampling took place during a deep-sea cruise from mid-July to the end of August 2016 on board the research vessel *Garcia del Cid* in various locations off the Spanish Mediterranean coast (Fig. 1). Plankton samples were taken using a 0.5 m²-mouth neuston net with a 300-µm mesh between 0.5 and 1 m depth over bottoms of 123 to 1626 m. The samples were rinsed with distilled water and preserved in 96% ethanol. Samples were sorted in the laboratory using a Leica Wild M6 stereomicroscope and all larvae morphologically identified as *A. antennatus* following the available
descriptions (HELDT 1955, TORRES et al. 2013) were stored individually in 96% ethanol.

Winter sampling took place from mid-February to early March 2017 during a deep-sea cruise on board the same research vessel off the NW Mediterranean coast (Fig. 1). Plankton samples were taken in integrated oblique tows using a 60-cm diameter bongo with a 300-µm mesh net between 500 m depth and the surface, over bottoms of 1952 and 1790 m. They were sorted on board using an Olympus SZ stereomicroscope and larvae morphologically identified as _Gennadas_ spp. PZ I following the available description (GURNEY 1924) were rinsed with distilled water and preserved individually in 96% ethanol.

Identifications of larvae through DNA barcoding are only reliable when the obtained sequences are compared to those of adult specimens of known species. Adults of _A. antennatus_ from commercial trawling vessels had been previously identified and cross-checked with the available literature (ZARIQUIEY-ÁLVAREZ 1968). They had been collected, preserved and their DNA amplified and the resulting sequences were therefore available for comparison. The GenBank accession numbers are EU977139-40 for 16S rDNA (SARDÀ et al. 2010) and EU908514 for COI (ROLDÁN et al. 2009). In the case of _Gennadas elegans_, two adult individuals were selected from a sampling cruise in May 2010 in the Mediterranean Sea. They had been previously identified by Dr. Pere Abelló at the Institut de Ciències del Mar according to the available literature (ZARIQUIEY-ÁLVAREZ 1968) and preserved in 96% ethanol. For the purpose of this paper, we extracted DNA from their abdominal tissue and sequenced the product following the same method as for _Gennadas_ spp. larvae to provide genetic information about the species (GenBank accession numbers MH605176 and MH605177).

2.2. Analysis of the protozoea I pool

2.2.1. Morphological analysis

In order to closely examine the morphology of the PZI larvae from the summer sampling, Scanning Electron Microscopy was used for 10 randomly selected individuals identified as _A. antennatus_ PZI. Also, 3 individuals from the winter sampling, morphologically identified as _Gennadas_ spp. PZI, were randomly selected. Both sets of larvae were immersed in a graded acetone series (25, 50, 75 and 100%), dried to critical point, mounted on stubs with self-adhesive carbon stickers and coated in gold. They were observed under a Hitachi S-3500N scanning electron microscope.

Furthermore, measurements of carapace length, telson rami length, telson angle and length of last somite of pleon were taken for all remaining individuals from the winter sampling identified as _Gennadas_ spp. (n = 9). Also, the same measurements were carried out for 10 individuals from the summer sampling, identified as _A. antennatus_. To do this, we used a Leica M205 C stereomicroscope and ImageJ image analysis software.

2.2.2. Molecular analysis

In order to confirm the identity of a representative sample of the protozoea I pool found in the summer sampling, we randomly selected 24 PZI individuals attributed to _A. antennatus_, from a total of 527 found. Selection was done according to spatial criteria with the objective of covering the whole study area. A maximum of 3 larvae per station were selected where the total number found was highest. We also randomly selected 4 PZI larvae from the winter sampling, morphologically attributed to _Gennadas_ spp., from a total of 11 individuals found. Information about larvae analyzed and their GenBank accession numbers are shown in Table 1.

DNA isolations were carried out using a commercial kit optimized for small samples (Quick-DNA
Microprep Plus kit, Zymo Research) and resuspended in a final volume of 10 µL. A negative control that contained no sample was included in every isolation round to check for contamination during the experiments.

For larvae morphologically identified as *A. antennatus*, a 617-base pair fragment of the mitochondrial gene Cytochrome Oxidase I (COI) was amplified by polymerase chain reaction (PCR) using the primer pair COILAa (5’ GGT GAC CCA GTC CTT TAC CA 3’) and COIHa (5’ GTC TGG ATA ATC AGA ATA CCG AC 3’) (Roldán et al. 2009), specific for *A. antennatus*. For larvae and adult individuals identified as *Gennadas* spp., a 658-base pair fragment of the COI gene was amplified using the primer pair CrustDF1 (5’ GGT CWA CAA AYC ATA AAG AYA TTG G 3’) (Steinke et al. 2016) and HCO-2198 (5’ TAA ACT TCA GGG TGA CCA AAA AAT CA 3’) (Folmer et al. 1994). PCRs were carried out in a final volume of 25 µL, containing 12.50 µL of Supreme NZY Taq Green PCR Master Mix (NZYTech), 0.5 µM of each primer, 2.5 µL of the template DNA solution, and PCR-grade water up to 25 µL. The thermal cycling conditions were as follows: an initial denaturation step at 95 ºC for 5 min, followed by 35 cycles of denaturation at 95 ºC for 30 s; annealing at 53 ºC (COILAa and COIHa) or at 49 ºC (CrustDF1 and HCO-2198) for 30 s; extension at 72 ºC for 45 s; and a final extension step at 72 ºC for 5 min. A negative control that contained no DNA was included in every PCR round to check for cross-contamination.

PCR products were run on a 1 % agarose gel stained with Real Safe (Durviz) and imaged under UV light, to verify amplicon size. PCR products were bidirectionally sequenced using the PCR primers. Electropherogram analysis and overlapping was conducted in Geneious 8.1.8 (Biomatters Ltd.). During electropherogram analysis, the primer annealing regions and the low quality regions at both ends of each electropherogram were trimmed (error probability limit of 0.03). Sequence reads were manually checked for sequencing errors or ambiguous base calls. The positions with double peaks were coded using the IUPAC ambiguity code (e.g., R: G or A). In order to check for possible pseudogenes, the sequences were aligned with BioEdit and no insertions or deletions were detected. Then, all sequences were translated into proteins with online software ExPASY (Gasteiger et al. 2003) and no stop codons were detected in the appropriate reading frame. The resulting nucleotide sequences were compared to available information in GenBank using Basic Local Alignment Search Tool (BLASTN 2.8.0, Zhang Z. et al. 2000).

### 2.3. Analysis of all known larval stages of *A. antennatus*

For the molecular confirmation of the rest of known larval stages of *A. antennatus*, we randomly selected 3 PZII, 1 PZIII, 3 mysis I (MI) and 1 mysis II (MII) and followed the same procedure as in section 2.2.1 (Table 2).

In addition, a molecular analysis with ribosomal gene 16S rDNA was conducted for all known larval stages. To do this, we randomly selected 5 PZI, 6 PZII, 5 PZIII, 6 MI and 2 MII (Table 2). Genomic DNA isolation from whole larvae (HotSHOT) was performed following Montero-Pau et al. (2008) with slight modifications. Polymerase chain reaction (PCR; Saiki et al. 1988) methods for amplification of the mitochondrial 16S rDNA gene followed the procedures outlined in Roldán et al. (2009). Standard precautions were adopted to detect contamination and related problems. PCR products were verified on 1% agarose gel with ethidium bromide (0.5mg/ml) and were purified for sequencing by treating with exonuclease I and shrimp alkaline phosphatase (Werle et al. 1994). DNA sequencing reactions were carried out with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer’s instructions. Primers used for sequencing were the same as those employed for PCR amplifications. Finally, labelled fragments were loaded
onto an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems) at the Laboratori d'Ictiologia Genètica, Universitat de Girona, Spain.

Nucleotide sequences were aligned and edited in Geneious v7.1.4 (KEARSE et al. 2012). In order to confirm the identification of the larvae, simultaneous comparisons were done with reference sequences from adults of six species of dendrobranchiate decapod crustaceans: Aristeus antennatus, Aristaemorpha foliacea, Genadas elegans, Gennadas valens, Parapenaeus longirostris and Penaeus (Melicertus) kerathurus. The corresponding GenBank accession numbers are: EU977139-40 (SARDÀ et al. 2010), MF496984-86 (ROLDÁN et al. 2017), JX403858.1 (BRACKEN-GRISSOM et al. 2012), KU324845.1 (ZITARI-CHIATTI et al. 2009) and EF589715.1 (PASCOAL et al. 2008) respectively.

3. Results

3.1. Analysis of the protozoea I pool

3.1.1. Morphological analyses

The morphology of the third maxilliped (mxp3) of the PZI larvae selected was studied under SEM. For the summer larvae examined (expected to be A. antennatus), the mxp3 was not visible in 2 of the 10 selected individuals due to the specimens’ position. In the other eight, the mxp3 was clearly biramous and showed 2 long plumose setae on the exopod (Fig. 2). No small simple seta was observed on the exopod of the mxp3 in any of the individuals. For the winter larvae examined (then designed as Gennadas spp. since A. antennatus only reproduces in summer), the mxp3 was clearly visible in 2 of the 3 individuals and it was biramous in both of them (Fig. 3). The endopod in Gennadas spp. is smaller and less conspicuous than that of A. antennatus, but differences among individuals do not allow the use of this character for its taxonomical identification. Other morphological characters were observed under SEM, such as the presence of frontal organs and the telson angle, with no conclusive distinctive traits between both species. A comparison of all morphological characters observed is presented in Table 3.

3.1.2. Molecular analyses

The COI sequences from the 24 PZI larvae from the summer sampling revealed 8 different haplotypes and all retrieved only A. antennatus sequences when analyzed in BLAST (GenBank accession numbers MH605140 to MH605163). To our knowledge, this is the first record of A. antennatus PZI stage since its description from a single individual (HELDT 1955). On the other hand, all 4 larvae morphologically identified as Gennadas spp. from the larval description showed an average resemblance of 99.38% to the sequences from the 2 Gennadas elegans adult individuals analyzed (GenBank accession numbers MH605172 to MH605175 for larvae; MH605176 and MH605177 for adults).

3.2. Molecular analysis of all known larval stages of A. antennatus

All known stages of A. antennatus were genetically identified with two markers: COI and 16S rDNA (Table 2). For marker COI, all 8 larvae analyzed corresponding to the four remaining known larval stages of A. antennatus were successfully sequenced (617 bp) and were identified as A. antennatus. The sequences corresponded to 2 different haplotypes (GenBank accession numbers
MH605164 to MH605171). For marker 16S rDNA, all 24 larvae analyzed from the five known larval stages of the species were successfully sequenced (300 bp) and all were also undoubtedly identified as *A. antennatus*. The sequences corresponded to 4 different haplotypes (GenBank accession numbers MH433629, MH433630, MH433631 and MH433632). Overall, we obtained a 309 bp global alignment with 84 variable positions that allow the discrimination of *A. antennatus* from other Mediterranean Dendrobranchiata species.

4. Discussion

The larvae collected in the present study are the highest number of *A. antennatus* larvae ever found in plankton samples, and analysis of these data (under preparation) could shed light on the species’ larval distribution and connectivity for the Western Mediterranean Sea. All collected larval stages of *A. antennatus* were identified with the available morphological descriptions made by HELDT (1955) and TORRES et al. (2013) and confirmed by molecular methods.

From a morphological point of view, there are still some difficulties in identifying the first prozoea of *A. antennatus*, since this stage is strikingly similar to that of *Gennadas* spp. In this study, even using SEM it was not possible to confirm the previous description of these two species. The available description for the first prozoea of *Gennadas* spp. (GURNEY 1924) notes an uniramous third maxilliped, whereas in our case two of the three *G. elegans* PZI showed a small endopod on the mxp3. The presence of a biramous mxp3 in *G. elegans* PZI stage may eliminate the possibility of distinguishing it from *A. antennatus* using morphological characters. The examination of other morphological traits such as telson invagination and anal spines structure did not yield any concluding results on features to differentiate both species despite the slight differences noted. On the other hand, the observed *A. antennatus* PZI larvae invariably showed only 2 long plumose setae on the exopod of the mxp3. The description appointed, in addition, one small simple seta on the exopod of the mxp3 (HELDT 1955), which was not observed in any of the individuals examined.

This suggests that the small simple seta described may appear later in the development of this stage and that newly molted PZI of *A. antennatus* would only present the 2 long plumose setae. Nevertheless, we have examined all 527 *A. antennatus* PZI individuals collected and all of them show only the 2 long plumose setae on the exopod of the mxp3 when observed at the stereomicroscope. The presence of a small simple seta on the exopod of the mxp3 is often used as a distinctive character of *A. antennatus* PZI when the endopod is not clearly visible. However, in the light of our results we recommend that this feature not be used to distinguish the PZI of *A. antennatus* and *G. elegans*.

Our results show that 100% of the analyzed PZI larvae with the shared morphology of *A. antennatus/Gennadas* spp. were molecularly identified as *A. antennatus*. Nevertheless, the presence of *Gennadas* spp. older larval stages in this sampling collection (CARRETÓN et al. unpubl. data) calls for caution during morphological identification. Until a new and standardized description of both larval types is completed and a morphological character is found to tell both species apart, molecular techniques remain the most reliable method for a correct species identification.

According to the descriptions of HELDT (1995), the larval series of *A. antennatus* is also morphologically very similar to that of *Aristaeomorpha foliacea* (RISSO 1827). Both species keep a smooth abdomen and carapace throughout their larval cycle, and it is possible that their PZIs can be confused as well in areas where both species coexist abundantly (Central and Eastern Mediterranean). Unfortunately, there is still no available description of the PZI of *A. foliacea* that
we can use to clarify this matter. In our study area, records of *A. foliacea* adult individuals have been decreasing and the species has been considered locally extinct in the Northwestern Mediterranean (CAU et al. 2002, CARTES et al. 2011). Nevertheless, a more thorough examination of the available bibliography is being carried out to clarify the morphological differences among these larval forms in the hopes of facilitating a more accurate identification (CARRETÓN et al., in prep).

5. Conclusions

Molecular analysis performed for all known larval stages of *A. antennatus* caught in the Western Mediterranean Sea proved that available descriptions for *A. antennatus* larval series are accurate. Molecular tools used clearly differentiate PZI of *A. antennatus* and *Gennadas elegans*. From the morphological examination of the first protozoea stage, and contrary of what was expected, the third maxilliped is similar for both *A. antennatus* and *Gennadas elegans*, which makes this character not suitable for use in morphological identification. The absence of a small simple seta on the exopod of the third maxilliped of *A. antennatus* first protozoae examined indicates that this character is not suitable for use in the morphological differentiation of *A. antennatus* and *Gennadas elegans* first protozoea.

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Figure 1

Stations where larvae were selected.

Red dots: summer sampling. Blue dots: winter sampling. Bathymetry is shown every 200 m.
Figure 2

General view (A) and detail of a third maxilliped (B) of an *Aristeus antennatus* protozoea I.
Figure 3

General view (A) and detail of a birramous third maxilliped (B) of a *Gennadas elegans* protozea I.
**Table 1** (on next page)

List of protozoea I larvae analyzed by station.

PZ – protozoea. N – number of individuals analyzed.
| Stage | Station | Lon (ºE) | Lat (ºN) | Bottom depth (m) | Sampling depth (m) | N | Putative species |
|-------|---------|----------|----------|------------------|-------------------|---|-----------------|
| PZI   | 56      | 0.7158   | 38.9593  | 858              | 0.5 – 1           | 1 | A. antennatus   |
|       | 60      | 0.4811   | 39.0395  | 800              | 0.5 – 1           | 1 | A. antennatus   |
|       | 98      | 1.3945   | 40.7777  | 404              | 0.5 – 1           | 2 | A. antennatus   |
|       | 105     | 1.8895   | 40.958   | 1143             | 0.5 – 1           | 2 | A. antennatus   |
|       | 112     | 2.5133   | 41.298   | 695              | 0.5 – 1           | 1 | A. antennatus   |
|       | 113     | 2.5473   | 41.1702  | 1038             | 0.5 – 1           | 1 | A. antennatus   |
|       | 120     | 3.0187   | 41.2557  | 1473             | 0.5 – 1           | 3 | A. antennatus   |
|       | 126     | 3.1165   | 41.4957  | 331              | 0.5 – 1           | 1 | A. antennatus   |
|       | 123     | 2.9417   | 41.508   | 507              | 0.5 – 1           | 3 | A. antennatus   |
|       | 122     | 2.9698   | 41.4127  | 642              | 0.5 – 1           | 1 | A. antennatus   |
|       | 124-2   | 2.8203   | 41.5606  | 378              | 0.5 – 1           | 1 | A. antennatus   |
|       | 133-1   | 3.3415   | 41.8232  | 650              | 0.5 – 1           | 1 | A. antennatus   |
|       | 133-3   | 3.3538   | 41.9002  | 298              | 0.5 – 1           | 1 | A. antennatus   |
|       | 145     | 3.4609   | 42.2327  | 123              | 0.5 – 1           | 1 | A. antennatus   |
|       | 145-1   | 3.3855   | 42.3362  | 462              | 0.5 – 1           | 1 | A. antennatus   |
|       | 148     | 3.6878   | 41.8280  | 1626             | 0.5 – 1           | 1 | A. antennatus   |
| TOTAL |         |          |          |                  |                   | 24|                 |
| PZI   | A09     | 3.4130   | 41.2550  | 1952             | 0 - 500           | 2 | Gennadas spp.   |
|       | B05     | 2.8811   | 41.3418  | 1790             | 0 - 500           | 2 | Gennadas spp.   |
| TOTAL |         |          |          |                  |                   | 4 |                 |
Table 2 (on next page)

List of larvae analyzed for DNA regions COI (top) and 16S rDNA (bottom) by station.

Putative species is A. antennatus in all cases. PZ – protozoea; M – mysis. N – number of individuals analyzed.
| Stage | Station | Lon (°E)  | Lat (°N)  | Bottom depth (m) | Sampling depth (m) | N |
|-------|---------|-----------|-----------|------------------|--------------------|---|
| COI   |         |           |           |                  |                    |   |
|       | PZI     | 96        | 1.4862    | 40.6578        | 940                | 0.5 – 1 | 1 |
|       |         | 138       | 3.5032    | 41.4412        | 1380               | 0.5 – 1 | 1 |
|       |         | 145       | 3.4609    | 42.2327        | 1626               | 0.5 – 1 | 1 |
|       | PZII    | 57        | 0.9092    | 38.9843        | 728                | 0.5 - 1 | 1 |
|       | MI      | 57        | 0.9092    | 38.9843        | 728                | 0.5 - 1 | 2 |
|       | MII     | 162       | 0.5502    | 38.9512        | 776                | 0.5 - 1 | 2 |
|       | TOTAL   |           |           |                  |                    | 8   |
| 16 rDNA| PZI     | 112       | 2.5133    | 41.298          | 695                | 0.5 - 1 | 3 |
|       |         | 113       | 2.5473    | 41.1702        | 1038               | 0.5 - 1 | 2 |
|       | PZII    | 124-1     | 2.8917    | 41.6367        | 200                | 0.5 - 1 | 1 |
|       |         | 143b      | 3.4248    | 41.9788        | 187                | 0.5 - 1 | 2 |
|       |         | 145       | 3.4609    | 42.2327        | 123                | 0.5 - 1 | 1 |
|       |         | 133       | 3.2760    | 41.8762        | 600                | 0.5 - 1 | 2 |
|       | PZIII   | 57        | 0.9092    | 38.9843        | 728                | 0.5 - 1 | 2 |
|       | MI      | 53        | 0.5502    | 38.9512        | 776                | 0.5 – 1 | 1 |
|       |         | 144       | 0.9008    | 38.6558        | 528                | 0.5 - 1 | 4 |
|       | MII     | 162       | 0.5502    | 38.9843        | 728                | 0.5 - 1 | 2 |
|       | TOTAL   |           |           |                  |                    | 24  |
**Table 3** (on next page)

Comparison of relevant morphological characters of the first protozoea stage examined.

Mxp3 – third maxilliped.
| Morphological characters | Aristeus antennatus Heldt (1955) | Aristeus antennatus Present study | Gennadas sp. Gurney (1924) | Gennadas elegans Present study |
|--------------------------|----------------------------------|----------------------------------|-----------------------------|----------------------------------|
| Total length (TL; mm)    | 1.55                             | 1.20±0.05                        | 0.98                        | 1.10±0.10                        |
| Carapace length (CL; mm) | NA                               | 0.42±0.03                        | NA                          | 0.38±0.04                        |
| Mxp3                     | birramous                        | birramous                        | uniramous                   | birramous                        |
| Number of setae exop. Mxp3 | 2 long plumose, 1 small simple | 2 long plumose                   | 2 long plumose              | 2 long plumose                   |
| Frontal organs           | small, round                     | small, round                     | small, round                | small, round                     |
| Length of the last somite of the pleon with telson (mm) | NA | 0.38±0.07 | NA | 0.32±0.04 |
| Length of the telson rami (mm) | NA | 0.11±0.01 | NA | 0.11±0.02 |
| Proportion: length of last somite of pleon with telson / length of telson rami | NA | 0.30±0.05 | NA | 0.35±0.05 |
| Telson angle (°)         | NA                               | 35.73±8.32                      | NA                          | 35.84±7.67                      |