

# SHORT COMMUNICATION

## Correct diagnosis of early zoeal stages of *Athanas nitescens* (Leach, 1814) (Decapoda, Caridea, Alpheidae) using laboratory-raised larvae

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*The morphology of the first two larval stages of Athanas nitescens (Leach, 1814), reared under laboratory conditions, is redescribed. The present data are compared with previous works, since a clarification of the morphological characters of the first two larval stages of A. nitescens is needed, in order to avoid misidentification of these stages in the future.*

### INTRODUCTION

*Athanas nitescens* (Leach, 1814) is a widely distributed species, occurring from southern Scandinavia to Congo, Madeira, Canaries and Cape Verde Islands and the Mediterranean Sea (d'Udekem d'Acoz, 1999). Although several authors have pointed to the existence of morphological variations between Atlantic and Mediterranean specimens (e.g. Nouvel, 1941; Holthuis, 1951; Holthuis and Gottlieb, 1958), a study analysing morphological and genetic differences is still missing (d'Udekem d'Acoz, 1999).

Several larval stages of *A. nitescens* (Leach, 1814) have been first described in some detail, from plankton samples, by Sars (Sars, 1906). Sars noted that the first larval stage of this species presented morphological characters generally attributed to a more advanced caridean larval stage (e.g. telson separated from the sixth abdominal somite and the presence of uropods) (Sars, 1906). In 1921, Webb described, from plankton samples, what she considered to be the first two zoeal stages of

*A. nitescens* stating that the larval stage figured by Sars as the first therefore corresponds to the third one (Webb, 1921). Unfortunately, Webb provided no illustrations of these early larval stages (Webb, 1921). Later, Lebour hatched the larvae of *A. nitescens* in the laboratory and noticed that the newly hatched larvae had some unexpected larval characters, namely a short rostrum and stalked eyes, and that the next larval stage was considered as a 'typical third stage' (Lebour, 1932). Taking into account Webb's (Webb, 1921) previous work, Lebour considered that the 'normal second stage had been skipped' (Lebour, 1932). Besides *A. nitescens* larval studies, and although the genus *Athanas* is spread over the world, only the early larval stages of four other species are known: *Athanas djiboutensis* by Gurney (Gurney, 1927), *Athanas dimorphus* by Gurney and Bhuti *et al.* (Gurney, 1927; Bhuti *et al.*, 1977), and *Athanas japonicus* and *Athanas parvus* by Yang (Yang, 2003a,b).

The purposes of the present work are to present a standardized description of the two first zoeal stages of

*A. nitescens* hatched under laboratory conditions and to compare them with previous works, since the clarification of the morphological characters of these larval stages will avoid misidentification of these stages in the future.

## RESULTS AND DISCUSSION

Five *A. nitescens* ovigerous females were collected during September 2001 using baited traps at Cape Raso (38°42' N; 09°29' W), 30 km west of Lisbon, Portugal. The females were kept in the laboratory individually in 2000 mL beakers provided with aeration, at 20°C and in darkness until hatching. Thirty larvae from each female were reared individually in small plastic containers (~20 mL each) and were fed with the microalgae *Nannochloropsis minuta*, supplied at a final density of  $50 \times 10^6$  cell·L<sup>-1</sup>. Ten larvae, from each female, at the first and second zoeal stage were randomly sampled, fixed with 4% formalin and preserved in 70% ethanol.

Drawings and measurements were made with the aid of a camera lucida on a binocular Wild M8. Setal observations and drawings were made using a Zeiss microscope with camera lucida. Setal counts and other morphological features are described according to Clark *et al.* (Clark *et al.*, 1998). The spent females and larval stages have been deposited in the Instituto Nacional de Investigação Agrária e Pescas (IPIMAR) in Lisbon, Portugal (number IPIMAR/A/An/11. 2001).

The general morphological features of *A. nitescens* first and second larval stage are as follows:

### Zoea I

Measurements: TL = 1.73–1.82 mm; CL = 0.55–0.58 mm.

Carapace (Fig. 1A): Smooth, with anterior region broad, displaying a spine at anterior angle; pointed rostrum, not reaching the end of the antennule peduncle; eyes large and stalked.

Antennule (Fig. 1B): Peduncle two segmented, with 2,2 distal setae; exopod with two thick aesthetascs and two small setae terminally; endopod smaller, with one long plumose terminal seta.

Antenna (Fig. 1C): Protopod with a small spine on the inner posterior side; endopod with one apical seta; exopod with three distal segments, with nine plumose setae on inner side and two plumose setae on outer side, plus a simple small seta on apex.

Mandibles (Fig. 1D): Incisor process smaller than molar process; palp absent.

Maxillule (Fig. 1E): Coxal endite with four setae; basal endite with four setae; endopod unsegmented with two distal setae; exopod absent.

Maxilla (Fig. 1F): Coxal endite unilobed, with three setae; basal endite unilobed, with three setae; endopod unsegmented with four marginal setae and minute spines sparsely distributed; exopod with four marginal plumose setae.

First maxilliped (Fig. 1G): Coxa and basis without setae; endopod five segmented with one apical seta on distal segment; exopod three segmented with 1,1,4 plumose distal setae.

Second maxilliped (Fig. 1H): Basis without setae; endopod four segmented with 0,0,1,2+1 setae; exopod two segmented, with 1,4 distal plumose setae.

Third maxilliped (Fig. 1I): Basis without setae; endopod four segmented, with 0,0,2,3 setae; exopod three segmented, with 1,1,4 plumose terminal setae.

First pereopod (Fig. 1J): Biramous bud.

Second to fourth pereopods (Fig. 1A): Absent.

Fifth pereopod (Fig. 1K): Uniramous long bud reaching the first maxilliped.

Abdomen (Fig. 1A): Six abdominal somites without any spines; the sixth longer and fused with telson; anal spine absent.

Pleopods (Fig. 1A): Absent.

Uropods (Fig. 1A): Absent.

Telson (Fig. 1L): Narrow, with very small median cleft and 7 + 7 processes posteriorly, being the innermost pair very small.

### Zoea II

Measurements: TL = 1.87–1.94 mm; CL = 0.55–0.60 mm.

Carapace (Fig. 2A): Unchanged.

Antennule (Fig. 2B): Peduncle two segmented, with one small plumose seta on inner margin and two plumose setae distally; second segment with two distal long setae on ventral side, and a lobe with four small setae on dorsal side; otherwise unchanged.

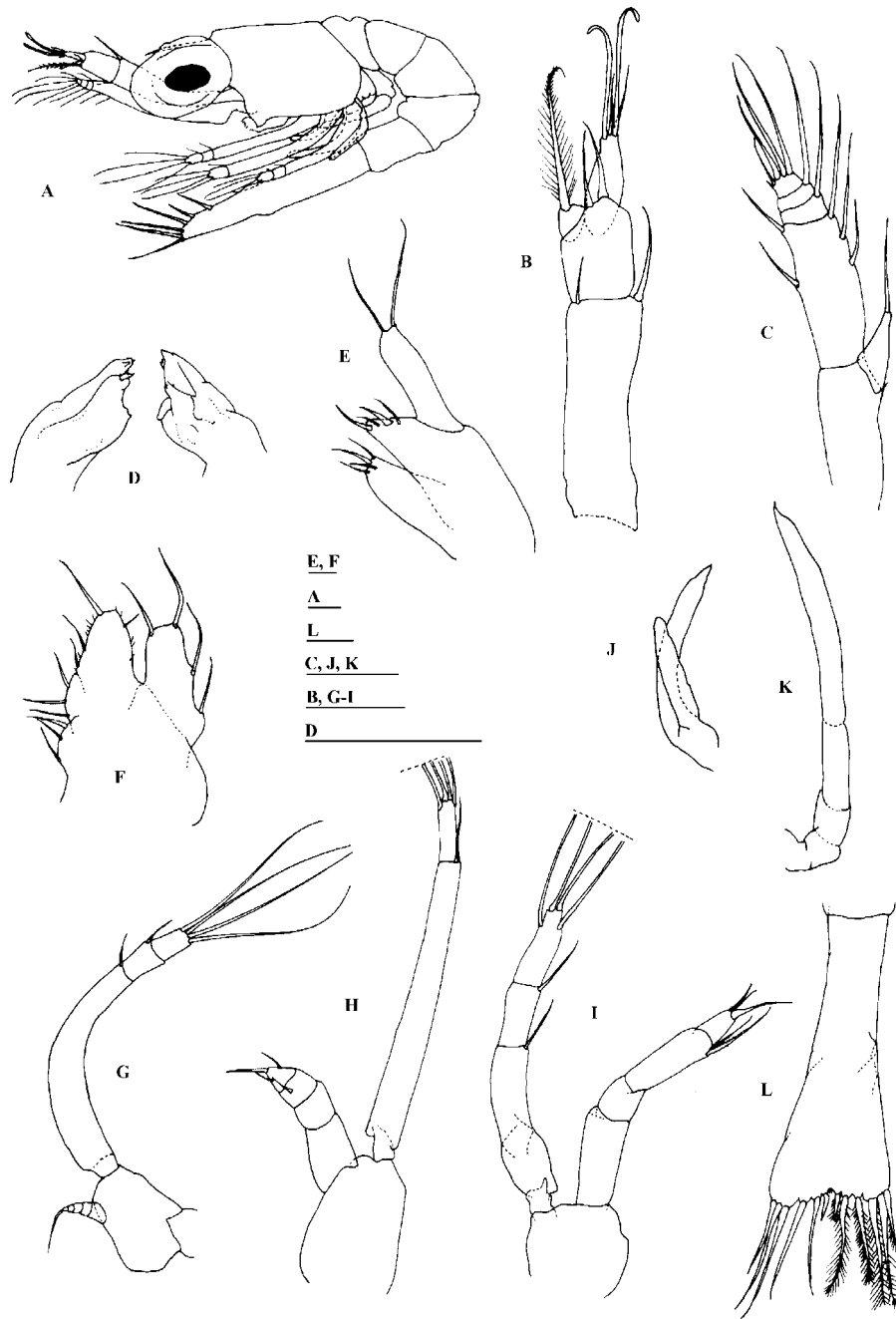
Antenna (Fig. 2C): Protopod without setae; exopod with one distal segment, with one plumose setae on outer side and 10 plumose setae on inner side, plus a simple small seta on apex; otherwise unchanged.

Mandibles (Fig. 2D): Incisor process smaller than molar process; palp absent; right mandible with a strong tooth in median part.

Maxillule (Fig. 2E): Coxal endite with five setae; otherwise unchanged.

Maxilla (Fig. 2F): Coxal endite unilobed, with two setae; basal endite unilobed, with three setae; unsegmented bilobed endopod with six marginal setae arranged as figured; exopod with five marginal plumose setae.

First maxilliped (Fig. 2G): Basis without setae; endopod four segmented with one sub-apical seta and one



**Fig. 1.** First zoeal stage of *Athanas nitescens* (Leach, 1814). **A**, lateral view; **B**, antennule; **C**, antenna; **D**, mandibles; **E**, maxillule; **F**, maxilla; **G**, first maxilliped; **H**, second maxilliped; **I**, third maxilliped; **J**, first pereiopod; **K**, fifth pereiopod; **L**, telson. Scale bars: **A–D** and **G–L**, 100 µm; **E–F**, 10 µm.

apical seta on distal segment; exopod three segmented with 1,1,4 plumose distal setae.

Second maxilliped (Fig. 2H): Basis with two simple setae; exopod three segmented, with 1,1,4 distal plumose setae; otherwise unchanged.

Third maxilliped (Fig. 2I): Basis with one seta; exopod four segmented, with 0,0,2,3 setae;

exopod three segmented, with 1,1,4 plumose terminal setae.

First pereiopod (Fig. 2J): Basis with one simple seta; endopod four segmented, with 0,0,2,2 (one subterminal and one terminal) setae; exopod two segmented, with 1,4 plumose terminal setae.

Second to fourth pereiopods (Fig. 2A): Absent.



**Fig. 2.** Second zoeal stage of *Athanas nitescens* (Leach, 1814). **A**, lateral view; **B**, antennule; **C**, antenna; **D**, mandibles; **E**, maxillule; **F**, maxilla; **G**, first maxilliped; **H**, second maxilliped; **I**, third maxilliped; **J**, first pereopod; **K**, fifth pereopod; **L**, telson and uropods. Scale bars: **A-L**, 100  $\mu$ m.

Fifth pereopod (Fig. 2K): Five segmented, with the last segment shaped as a long and slender stylet, displaying a serrated extremity (as figured).

Pleopods (Fig. 2A): Absent.

Uropods (Fig. 2L): Biramous; exopods well developed not reaching the end of telson, with six plumose setae; endopod small without any setae.

Telson (Fig. 2L): More rectangular, with an almost imperceptible median cleft.

*Athanas nitescens* first larval stage presents the typical form of the genus, namely rounded eyes, telson rather narrow, slightly indented, and body double bent with dorsal connection between the carapace and the abdomen making a 90° angle. However, some of the features

displayed are not commonly found in the first zoeal stage of this genus. The first zoea of *A. nitescens* already presents a short rostrum and stalked eyes rather than the usual sessile eyes of other *Athanas* species first larval stage (Gurney, 1927, 1938; Bhuti *et al.*, 1977; Yang, 2003a,b). Another uncommon feature of the first zoeal stage is the antennular peduncle segmented when in all other *Athanas* larvae it is still unsegmented. The second larval stage of this species also presents some more advanced morphological characters, namely the telson already separated from the sixth abdominal somite and the presence of uropods, which are only found in the third zoeal stage of other *Athanas* species.

These uncommon characters were also pointed out by Lebour's description of the first zoeal stages of *A. nitescens* (Lebour, 1932). Her description agrees with the present one, even in the presence of stalked eyes, only differing in the shape of the telson (figured as more triangular). Nevertheless, as previously mentioned, Lebour (Lebour, 1932) considered that her laboratory results did not correspond to the correct larval series of this species in the plankton. According to Lebour (Lebour, 1932), this might be due to laboratory artefacts, while in Sars description (Sars, 1906) it could be a possible abbreviation in the development of *A. nitescens* in the Christiania Fjord where his specimens were collected. These conclusions were made based on Webb's (Webb, 1921) work.

Webb's (Webb, 1921) larval description refers to the two earliest larval stages that should appear before the first larval stage figured by Sars (Webb, 1921). Since Webb was referring to a 'normal' caridean first larval stage (Webb, 1921), it was assumed by Lebour that the eyes described were sessile and not stalked (Lebour, 1932). Besides this feature, we also noticed that the antennule protopod described by Webb (Webb, 1921) is a simple unjointed process, which in the present description is two segmented. In our larvae, the telson is narrow and presents a very small median cleft, while the one described by Webb (Webb, 1921) has the shape of a flattened triangular swimming plate. The mandible palp is absent in our description and all maxillipeds present four long plumose setae on the exopod, rather than two or three setae. All these differences lead us to conclude that the larvae described by Webb (Webb, 1921) are not *A. nitescens* larvae but probably another caridean larvae common in Plymouth area. Webb's (Webb, 1921) larvae were collected from the plankton and the actual parental species of those larvae remains unknown, since no larvae were reared to the postlarval stage.

From the present description, we conclude that Lebour (Lebour, 1932) has described the first larval stage of *A. nitescens*, while the first larval stage of

*A. nitescens* described by Sars (Sars, 1906) corresponds to the second zoeal stage of the present work. We agree with Sars (Sars, 1906) when he suggests that he may have skipped the first stage (saying that he has not witnessed its escape from the ova). Lebour (Lebour, 1932) considered Sars's (Sars, 1906) first larva as the third due to its resemblance with the stage she considered as the third, believing that she skipped the typical second stage in her study.

The thought that the larvae described by Webb (Webb, 1921) actually belongs to the species *A. nitescens*, and Lebour's (Lebour, 1932) assumption on the validity of Webb's laboratory study on *A. nitescens* larvae, could have led researchers working with plankton samples to consider the first and second zoeal stages of this species as the second and third stages, respectively. This misidentification of larval stages is probably the reason of the absence of the first larval stage of this species from plankton samples in European waters (e.g. see Barnich, 1996). Although the present larvae were not reared until metamorphosis to megalopa, it is reasonable to assume that the larval development of *A. nitescens* could pass through eight stages instead of nine zoeal stages as generally considered (e.g. González-Gordillo *et al.*, 2001). Therefore Sars (Sars, 1906) Fig. 6, Plate 1 probably represents stage 3, Fig. 7 represents stage 7, and Fig. 5 is probably illustrating the eighth and last larval stage changing to a young form.

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## REFERENCES

- Barnich, R. (1996) The larvae of the Crustacea Decapoda (Excl. Brachyura) in the plankton of the French Mediterranean coast (Identification Keys and Systematic Review). PhD Dissertation. Cuvillier Verlag, Göttingen, Germany, 189 pp.
- Bhuti, G. S., Shenoy, S. and Sankolli, K. N. (1977) Laboratory reared alpheid larvae of the genera *Automate*, *Athanas* and *Synalpheus* (Crustacea Decapoda, Alpheidae). In *Proceedings of the Symposium on Warm Water Zooplankton*. Spec. Natl. Inst. Oceanogr., Goa, India, pp. 588–600.
- Clark, P. F., Calazans, D. and Pohle, W. (1998) Accuracy and standardization of brachyuran larval descriptions. *Invertebr. Reprod. Dev.*, **33**, 127–144.
- González-Gordillo, J. I., dos Santos, A. and Rodríguez, A. (2001) Checklist and annotated bibliography of decapod crustacean larvae from the southwestern European coast (Gibraltar Strait area). *Sci. Mar.*, **65**, 275–305.
- Gurney, R. (1927) Cambridge expedition to the Suez Canal, 1924. XV. Report on the larvae of Crustacea Decapoda. *Trans. Zool. Soc. Lond.*, **22**, 231–286.

- Gurney, R. (1938) The larvae of the decapod Crustacea Palaemonidae and Alpheidae. *Sci. Rep. Gl. Barrier Reef Exp. (1928-1929)*, **6**, 1-60.
- Holthuis, L. B. (1951) The caridean Crustacea of tropical West Africa. *Atlantide Report*, **2**, 7-187.
- Holthuis, L. B. and Gottlieb, E. (1958) An annotated list of the Decapod Crustacea of the Mediterranean coast of Israel, with an appendix listing the Decapoda of the eastern Mediterranean. *Bull. Res. Council. Isr.*, **7B**, 1-126.
- Lebour, M. V. (1932) The larval stages of the Plymouth Caridea. IV. The Alpheidae. *Proc. Zool. Soc. Lond.*, **1932**, 463-469.
- Nouvel, L. (1941) Contribution à l'étude systématique des *Athanas* (Crustacés décapodes nageurs) des côtes de France. *Bull. Inst. Océanogr. Monaco*, **806**, 1-15.
- Sars, G. (1906) Postembryonal development of *Athanas nitescens*, Leach. *Arch. Math. Nat.*, **27**, 1-29.
- d'Udekem d'Acoz, C. (1999) Inventaire et distribution des crustacés décapodes de l'Atlantique nord-oriental, de la Méditerranée et des eaux continentales adjacentes au nord de 25°N. *Collection Patrimoines Naturels (M.N.H.N./S.P.N.)*, **40**, 1-383.
- Webb, G. (1921) The larvae of the Decapoda Macrura and Anomura of Plymouth. *J. Mar. Biol. Ass. U. K.*, **12**, 385-425.
- Yang, H. J. (2003a) Early zoeas of *Athanas japonicus* Kubo, 1936 (Decapoda, Caridea, Alpheidae) reared in the laboratory. *Crustaceana*, **76**, 443-452.
- Yang, H. J. (2003b) Early zoeas of *Athanas parvus* De Man, 1910 (Decapoda: Caridea: Alpheidae) reared in the laboratory. *Proc. Biol. Soc. Wash.*, **116**, 710-718.