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Morphology of the larval stages of a Mediterranean population of the allochthonous Say's mud crab, *Dyspanopeus sayi* (Decapoda: Brachyura: Panopeidae)

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SUMMARY: In this study the complete larval development (including four zoeal stages and a megalopa stage) of *Dyspanopeus sayi* is described and illustrated in detail based on larvae reared in the laboratory. Ovigerous females were collected in Alfacs Bay, Ebro Delta (NW Mediterranean) where the species was recently introduced. These larval stages were compared with others collected in the plankton from the same area, the larval stages described previously for this species and the larval stages of the two other Panopeidae that inhabit the Iberian Peninsula: *Panopeus africanus* and *Rhithropanopeus harrisii*. Differences were found in both zoeal and megalopal larval characters between *D. sayi* and the other two species, which could raise doubt about their placement in the same family.

Keywords: Panopeidae, *Dyspanopeus sayi*, allochthonous species, larval stages, zoea, megalopa.

RESUMEN: Morfología de los estadios larvarios de una población mediterránea del cangrejo alóctono, *Dyspanopeus sayi* (Decapoda: Brachyura: Panopeidae). – En este estudio se describe e ilustra en detalle el desarrollo larvario (4 estadios zoea y un estadio megalopa) de *Dyspanopeus sayi*, a partir de larvas cultivadas en el laboratorio. Las hembras ovígeras fueron capturadas en la Bahía de Alfacs (NO Mediterráneo), donde la especie ha sido recientemente introducida. Los estadios larvarios descritos han sido comparados con larvas del plancton capturadas en la misma zona, con descripciones previas de esta especie y con descripciones de los estadios larvarios de las otras dos especies de Panopeidae que habitan en la Península Ibérica: *Panopeus africanus* y *Rhithropanopeus harrisii*. Se han encontrado diferencias destacables en algunos caracteres de los estadios, zoea y megalopa, lo cual podría poner en duda la posición de estas especies dentro de la misma familia.

Palabras clave: Panopeidae, *Dyspanopeus sayi*, especie alóctona, estadio larvario, zoea, megalopa.

INTRODUCTION

The native distribution of Say's mud crab, *Dyspanopeus sayi* (Smith 1869), encompasses the Atlantic coast of North America from Florida to Canada (Nizinski 2003). It is considered an invasive species in other parts of the world as a result of human activities and has probably been accidentally transported in ballast water like many other marine invertebrates (Davidson and Simkanin 2012). Currently, outside of its native range, *D. sayi* has been recorded from southwest England, Queens Dock, Swansea (Wales) (Ingle 1980, Clark 1986), on the French and Dutch coasts of the North Sea (Vaz *et al.* 2007), and more recently in the Black Sea (Micu *et al.* 2010). It is also present in the Mediterranean Sea, where it has been collected in Venice, the Marano and Varano lagoons, and in the Po River Delta (western Adriatic Sea) (Froglia and Speranza 1993, Mizzan 1995, Florio *et al.* 2008). The most recent record is from the western Mediterranean, which constituted the first record for the coast of the Iberian Peninsula (Schubart *et al.* 2012). Figure 1 shows the current distribution of *Dyspanopeus sayi* worldwide.

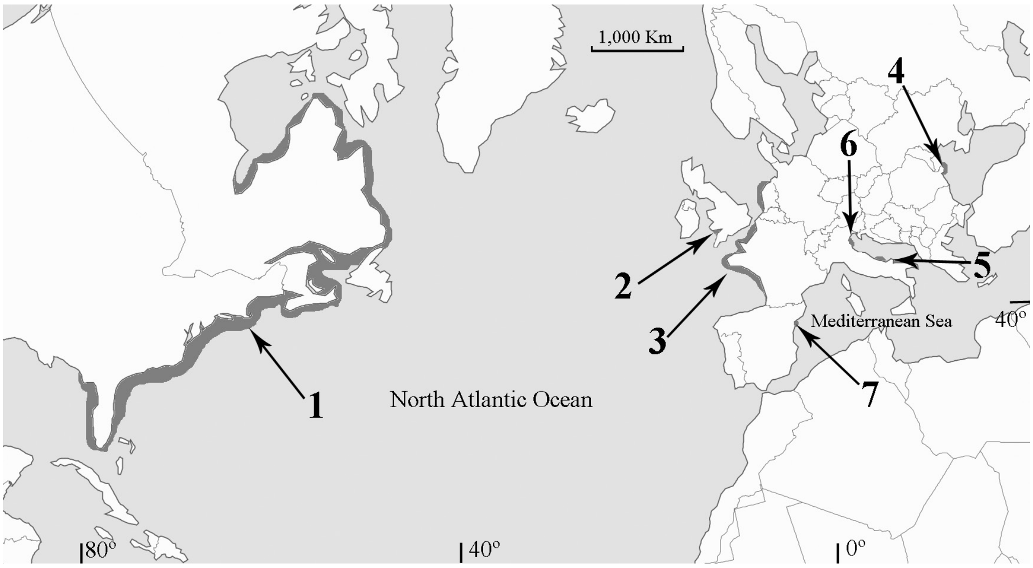


Fig 1. – Worldwide distribution of Say's mud crab *Dyspanopeus sayi* (Smith, 1869). References: 1, Nizinski (2003); 2, Ingle (1980), Clark (1986); 3, Vaz *et al.* (2007); 4, Micu *et al*. (2010); 5, Florio *et al*. (2008); 6, Froglia and Speranza (1993), ICES (2005); 7, Schubart *et al.* (2012).

Dyspanopeus sayi is a euryhaline and eurythermic species that inhabits estuaries and shallow coastal marine waters (see Schubart *et al*. 2012). This species is the second Panopeidae that has established large populations in estuarine habitats of the Iberian Peninsula. The first species was *Rhithropanopeus harrisii* (Gould 1841), reported for the first time in the Guadalquivir River (south Atlantic coast of Spain) (Cuesta *et al.* 1991). Another panopeid inhabiting the Iberian Peninsula is *Panopeus africanus* A. Milne-Edwards, 1867, which is an endemic crab with a wide distribution from Angola to Portugal (Rodríguez and Paula 1993).

A number of authors have partially or completely studied the larval development of the three species: Rodríguez and Paula (1993) described *Panopeus africanus*; Connolly (1925), Hood (1962), Chamberlain (1962), and Kurata (1970) studied *Rhithropanopeus harrisii* and Marco-Herrero *et al.* (2012) recently redescribed the megalopa stage; and Birge (1883), Hyman (1925), Chamberlain (1957, 1961), Kurata (1970), Clark (2007) and Schubart *et al.* (2012) studied *Dyspanopeus sayi*. With the exception of the zoea I, the descriptions of the remaining three zoeal stages and the megalopa of *Dyspanopeus sayi* are incomplete, not detailed and poorly illustrated according to the modern standardization of brachyuran larval descriptions (Clark *et al.* 1998).

In the present study the complete larval development (four zoeal stages and a megalopa) of *Dyspanopeus sayi* is described and illustrated in detail based on larvae reared in the laboratory. We compared these larval stages with those collected in the plankton, and the larval stages described previously for the other two Panopeidae that inhabit the Iberian Peninsula, namely *P. africanus* and *R. harrisii.*

MATERIALS AND METHODS

Three ovigerous females of *Dyspanopeus sayi* of 16 to 18 mm cephalothorax length were collected by beam trawls from sandy-muddy bottoms of Alfacs Bay, Ebro Delta (40°40'N, 0°40'E) covered by the seagrass *Cymodocea nodosa* and the alga *Caulerpa prolifera* (see Pérez and Camp 1986, Fusté 1988), in August 2011. Specimens were transported to the laboratories of the IRTA (Institut de Recerca i Tecnologia Agroalimentàries) in Sant Carles de la Ràpita. The females were kept in 40-L aquariums. After hatching, actively swimming zoea I were transferred to 500-mL beakers (n=5). A total of 20 larvae were placed in each beaker. The subsequent rearing cultures were conducted at a constant salinity of 34, a temperature of 18±1°C and a natural photoperiod of *ca*. 12 h light per day (early spring condition). After the water had been changed, the Chlorophyceae *Tetraselmis chuii* and *Artemia* sp. naupii were provided daily as feed.

A qualitative plankton survey was carried out on 24 and 25 September 2012 in the Alfacs Bay, Ebro Delta using a plankton net with a mouth opening of 0.25 m^2 and mesh size of 250 μm. Samples were taken during daytime, fixed in ethanol (96%), and later sorted in the laboratory under a Wild MZ6 compound microscope.

For easier observation of larvae structures and setation under microscope, a digestion-stain procedure (Marco-Herrero *et al*. 2012) was carried out. Initially, entire specimens were placed for 10 minutes in a watch glass with 2 ml of heated lactic acid. Immediately after, 3 drops of Clorazol Black stain (0.4 g Clorazol Black powder dissolved in 75 ml 70% ethanol) were added to the heated solution. After 5-10 minutes, the specimen was removed from the solution and placed on a slide

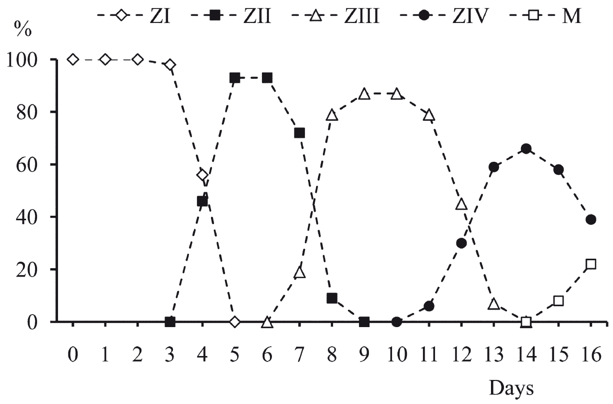


FIG 2. – Percentage survival and duration of larval stages of *Dyspanopeus sayi* reared under laboratory conditions (18±1°C and 34±1 salinity). ZI-IV, zoea I-IV; M, megalopa.

with lactic acid, in order to proceed with the dissection of the appendages.

Drawings and measurements were made using a Wild MZ6 and Zeiss compound microscope with Nomarski interference, both equipped with a *camera lucida*. All measurements were made by an ocular micrometer. Descriptions and measurements of different larval stages were based on at least 10 specimens of each stage from culture larvae. In Figure 7 first and second maxilliped of zoea I are drawn without exopod, and plumose natatory setae of the maxillipeds exopods of zoeae II-IV are drawn truncated. Description and figures are arranged according to the standards proposed by Clark *et al.* (1998).

Measurements taken for the zoeal stages were: rostrodorsal length (RDL), distance from the tip of the rostral spine to the tip of the dorsal spine; cephalothorax length (CL) between eyes (base of the rostrum) to the posterolateral carapace margin; cephalothorax width (CW) as the distance between the tips of lateral spines; rostral spine length (RL) from base of eye to tip of rostral spine; and antennal length (AL) from base of eye to tip of the protopod. Measurements for the megalopa included: CL measured from the frontal to posterior margin of carapace; CW as the carapace maximum width.

A larval series has been deposited at the Biological Collections of Reference of the Institut de Ciències del Mar (ICM-CSIC) in Barcelona under accession numbers ICMD13022501-3.

RESULTS

In the plankton samples a total of 9 zoeae I, 2 zoeae II and 1 zoea IV of *Dyspanopeus sayi* were identified and used for morphological and meristical comparison with those reared in the laboratory.

The larval development of *Dyspanopeus sayi* consists of four zoeal stages and a megalopa. At 18±1°C and 34 salinity the zoeal development was completed in a minimum of 15 days (appearance of the megalopa). The duration and survival of each larval stage is shown in Figure 2. The first zoeal stage is redescribed (see Schubart *et al*. 2012) and only the main differences in subsequent stages are described.

Larval description

Dyspanopeus sayi (Smith, 1869)

Zoea I

(Figs 3A, G; 5 A, E; 6 A, E; 7 A, E; 8 A, D)

Size: RDL=1863.5±12.4 μm; CL=514.4 ± 31.9 μm; CW=612.5±31.5 μm; RL=786.0±12.2 μm; AL=832.3±50.0 μm; N=10.

Cephalothorax (Fig. 3A, G): dorsal spine straight and well developed with small tubercles over the surface; rostral spine straight and slightly longer than dorsal spine; ventral caparace margin without setae, 1 pair of posterodorsal simple setae; eyes sessile.

Antennule (Fig. 5A): uniramous, unsegmented and conical; endopod absent; exopod with 4 terminal aesthetascs (two long and two shorter) and 1 simple seta.

Antenna (Fig. 5E): protopod long, equal in length to rostral spine, with rounded tip and without spines; exopod reduced to a minute bud with 1 small terminal simple seta; endopod absent.

Mandible: incisor and molar process developed; palp absent.

Maxillule (Fig. 6A): coxal endite with 7 plumodenticulate setae; basial endite with 5 terminal setae (3 cuspidate and 2 plumodenticulate); endopod 2-segmented with 1,2 subterminal + 4 terminal sparsely plumose setae respectively; epipod and exopod setae absent.

Maxilla (Fig. 6E): coxal endite bilobed with 4+4 plumodenticulate setae; basial endite bilobed with 5+4 plumodenticulate setae; unsegmented endopod bilobed, with 3 and 2 subterminal + 3 terminal sparsely plumose setae, respectively; exopod (scaphognathite) with 4 marginal plumose setae plus one stout plumose process.

First maxilliped (Fig. 7A): coxa with 1 sparcely plumose seta; basis with 10 medial sparsely plumodenticulate setae arranged as 2+2+3+3; endopod 5-segmented, longer than exopod, with 3,2,1,2,5 (1 subterminal + 4 terminal) sparcely plumodenticulate setae; exopod 2-segmented with 4 terminal plumose natatory setae.

Second maxilliped (Fig. 7E): coxa without setae; basis with 4 sparsely plumodenticulate setae arranged 1+1+1+1; endopod 3-segmented, with 1, 1, 5 (2 subterminal + 3 terminal) setae; exopod 2-segmented with 4 terminal plumose natatory setae.

Third maxilliped: absent.

Pleon (Fig. 8A, D): five pleonites. Pleonite 1 without setae; pleonites 2-5 with a pair of minute simple setae on posterodorsal margin; pleonite 2 with pair of forwardly directed dorsolateral processes and pleonite 3 with smaller dorsolateral processes backward directed.

Pleopods: absent.

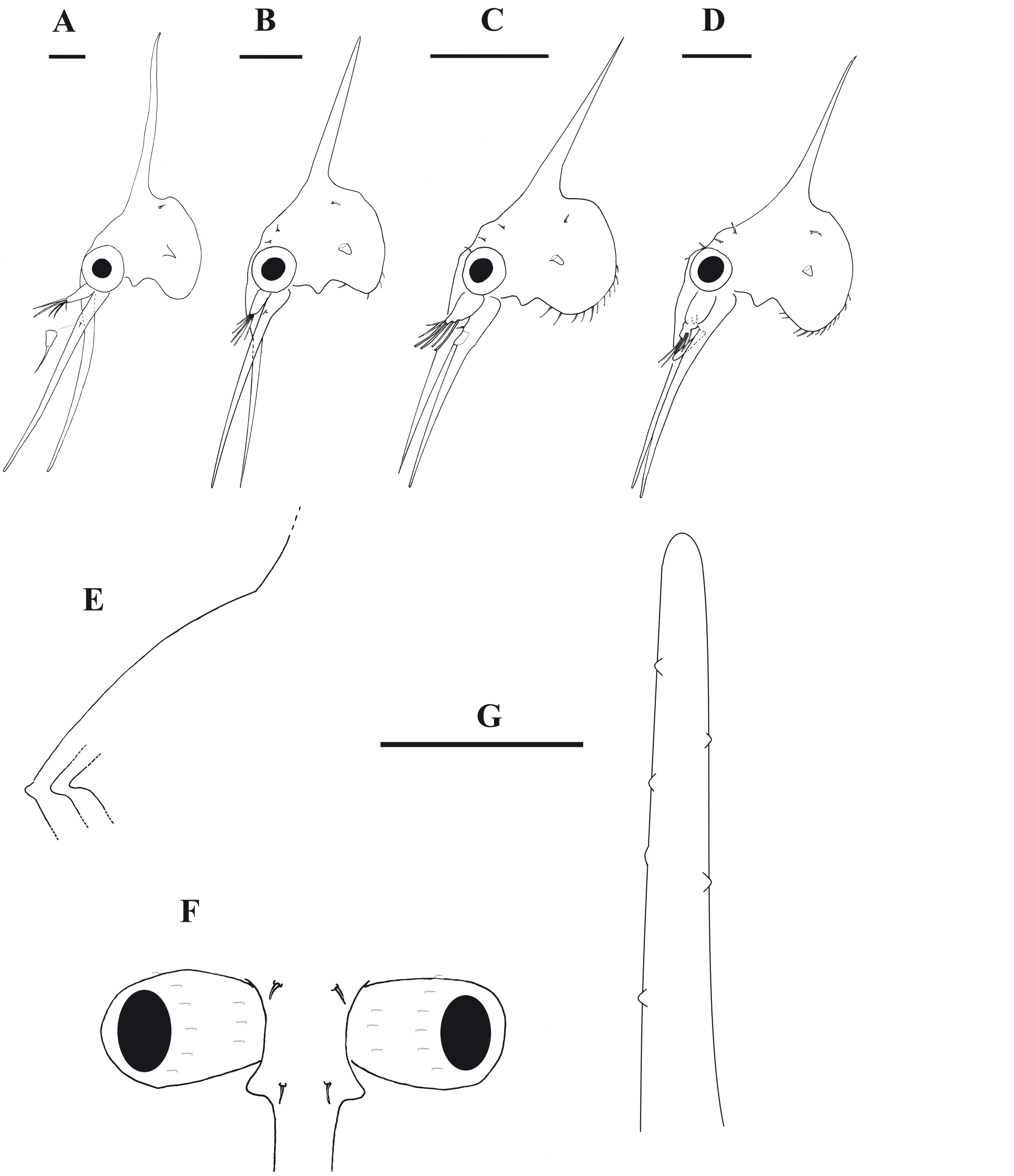


FIG 3. – *Dyspanopeus sayi*. Cephalothorax, lateral view. A, zoea I; B, zoea II; C, zoea III; D, zoea IV; E, cephalothorax, lateral spine, zoea II-IV; F, detail of the frontal view of rostrum of zoea IV; G, detail of distal part of dorsal spine of zoea I. Scale bars = 0.5 mm.

Telson (Fig. 8A, D): bifurcated, with deep median cleft; 2 pairs of 3 serrulate setae on posterior margin, medial setae longest; furcae with dorsal spine on mid part, and not spinulated.

Zoea II
(Figs 3B, E; 6B; 8E)

Size: RDL=2033±28.8 µm; CL=582.6±20.6 µm; CW=593±41.6 µm; RL=830±60.3 µm; AL=889.3±39 µm, N=10.

Cephalothorax (Fig. 3B, E): anteromedian ridge more pronounced than zoea I; 2 pairs of anterodorsal simple setae; 4 setae on ventral margin including 1 plumose anterior seta and 3 sparsely setose posterior setae; eyes stalked and movable.

Antennule: exopod with 5 terminal aesthetascs (2 long and 3 shorter) plus one small seta.

Antenna: Endopod present as a small bud.

Maxillule (Fig. 6B): basial endite with 8 setae (4 terminals cuspidate, 3 subterminal plumodenticulate and 1 proximal plumose seta); exopodal seta present.

Maxilla: scaphognathite with 11 plumose marginal setae.

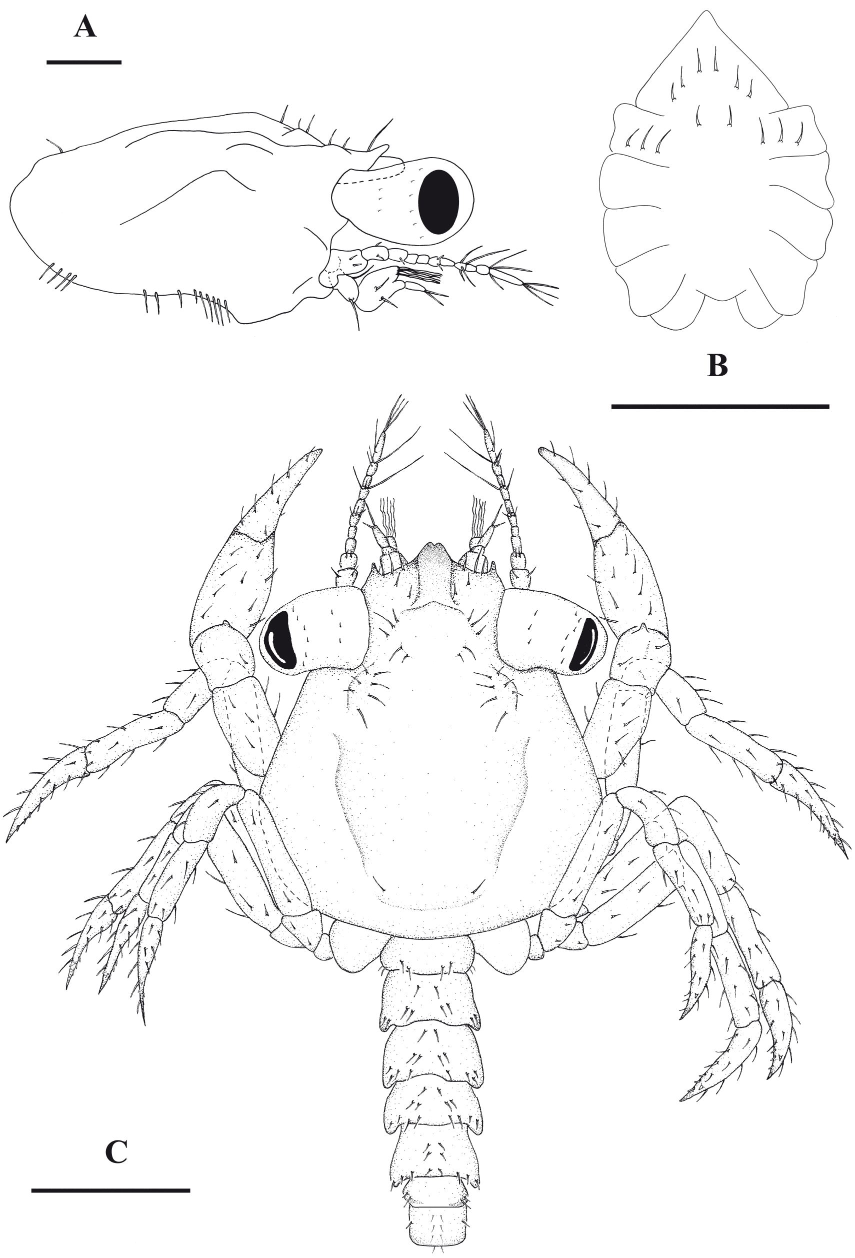


FIG 4. – *Dyspanopeus sayi*. Megalopa. A, cephalothorax, lateral view; B, sternum; C, whole dorsal view. Scale bars = 0.5 mm.

First maxilliped: exopod with 6 terminal plumose natatory setae.

Second maxilliped: exopod with 6 terminal plumose natatory setae.

Third maxilliped: present as undifferentiated bud.

Pleon (Fig. 8E): pleonite 1 with 1 mid-dorsal seta.

Pleopods: all present as undifferentiated buds.

Zoea III
(Figs 3C, E; 5F; 6F; 7B; 8F)

Size: RDL=2644±57.9 µm; CL=862.5±17.6 µm; CW=756.5±35 µm; RL=988.3±72.3 µm; AL=1051.3±74.1 µm, N=10.

Cephalothorax (Fig. 3C, E): anteromedian ridge more pronounced than zoea II; 3 pairs of anterodorsal simple setae; 11 setae on ventral margin including 1 plumose anterior seta and 10 sparsely setosed posterior setae.

Antennule: exopod with 6 aesthetascs (4 terminal and 2 subterminal).

Antenna (Fig. 5F): endopod more elongated.

Maxillule: coxal endite with 8 terminal plumodenticulate setae; basial endite with 9 setae (3 subterminal plumodenticulate setae, 5 terminal cuspidate setae and 1 proximal plumose seta).

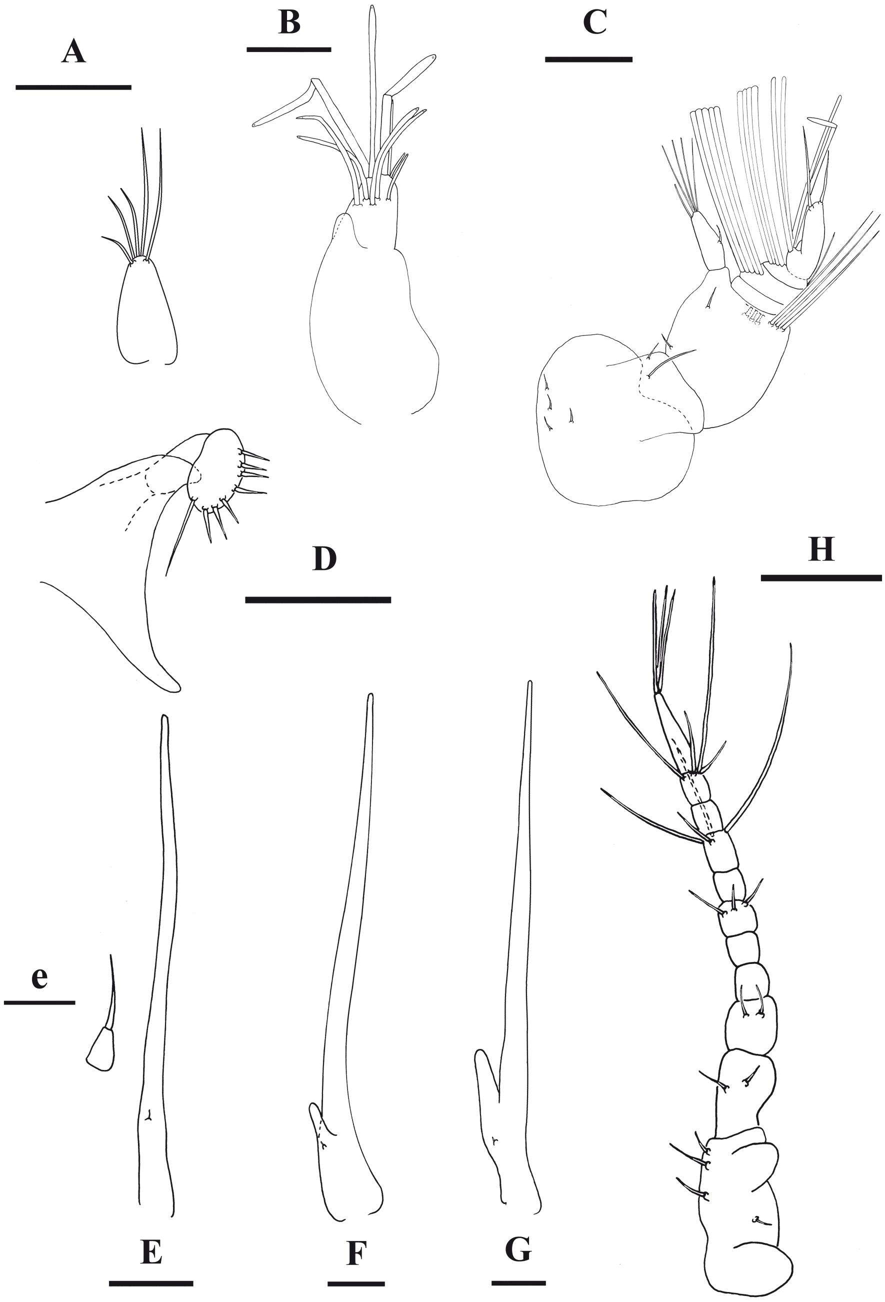


FIG 5. – *Dyspanopeus sayi*. Antennule. A, zoea I; B, zoea IV; C, megalopa. Mandible, D, megalopa. Antenna, E, zoea I; e, detail of exopod; F, zoea III; G, zoea IV; H, megalopa. Scale bars = 0.1 mm.

Maxilla (Fig. 6F): basial endite with 5+5 plumodenticulate setae; scaphognatite with 18-19 plumose marginal setae.

First maxilliped (Fig. 7B): endopod segment 5 with 6 (2 subterminal +4 terminal) sparsely plumodenticulate setae; exopod with 8 terminal plumose natatory setae.

Second maxilliped: exopod with 9 terminal plumose natatory setae.

Third maxilliped: unsegmented and without setae, differentiated in endopod and exopod buds.

Pereiopods: unsegmented and without setae. First pair bilobed (cheliform), pereiopod 2-5 as elongated buds.

Pleon (Fig. 8F): six pleonites plus telson.

Pleopods (Fig. 8F): present on pleonites 2-6 as small buds, endopods absent.

Zoea IV

(Figs 3D-F; 5B, G; 6C, G; 7C, F; 8B, G)

Size: RDL=3080.5±51.6 µm; CL=1059.0±14.1 µm; CW=927.5±24.7 µm; RL=1312.0±35.3 µm;

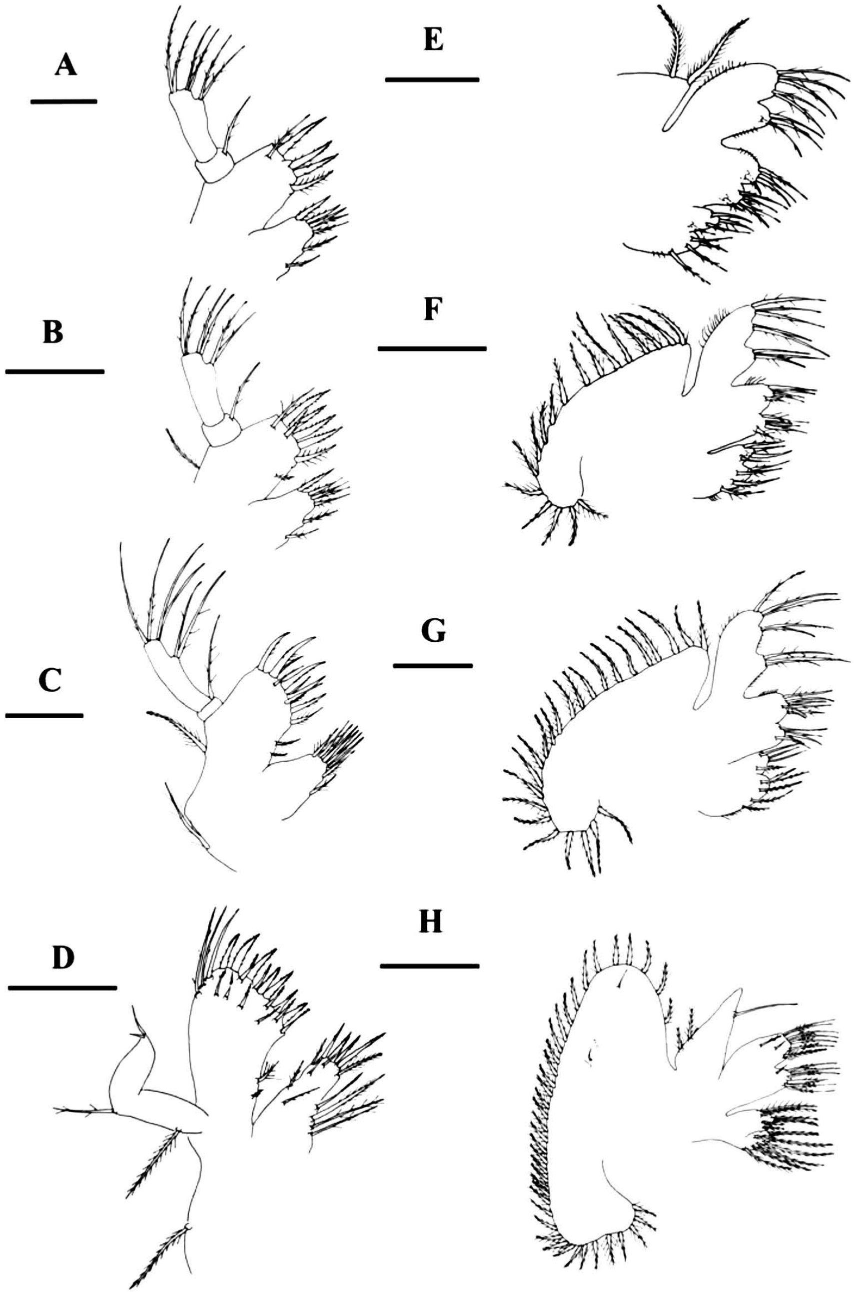


Fig 6. – *Dyspanopeus sayi*. Maxillule. A, zoea I; B, zoea II; C, zoea IV; D, megalopa. Maxilla, E, zoea I; F, zoea III; G, zoea IV; H, megalopa. Scale bars = 0.1 mm.

AL=1378.0±31.7 µm, N=10.

Cephalothorax (Fig. 3D-F): anteromedian ridge more pronounced than zoea III; 4 pairs of anterodorsal simple setae; 16 setae on ventral margin including 1 plumose anterior seta and 15 sparsely setosed posterior setae.

Antennule (Fig. 5B): endopod present; exopod with 10-11 aesthetascs (5 subterminal and 5-6 terminal).

Antenna (Fig. 5G): endopod more elongated.

Maxillule (Fig. 6C): coxal endite with 9-10 plumodenticulate setae; basial endite with 11-12 setae (4-5 subterminal plumodenticulate, 5 terminal cuspidate, 2 proximal plumose seta); epipodal seta present.

Maxilla (Fig. 6G): coxal endite with 5+4 plumodenticulate setae; basial endite with 5-6+6-7 plumodenticulate setae; scaphognatite with 24-27 plumose marginal setae.

First maxilliped (Fig. 7C): coxa with 2 sparsely plumose setae; exopod with 9 terminal plumose natatory setae. Epipodite present.

Second maxilliped (Fig. 7F): exopod with 11 terminal plumose natatory setae.

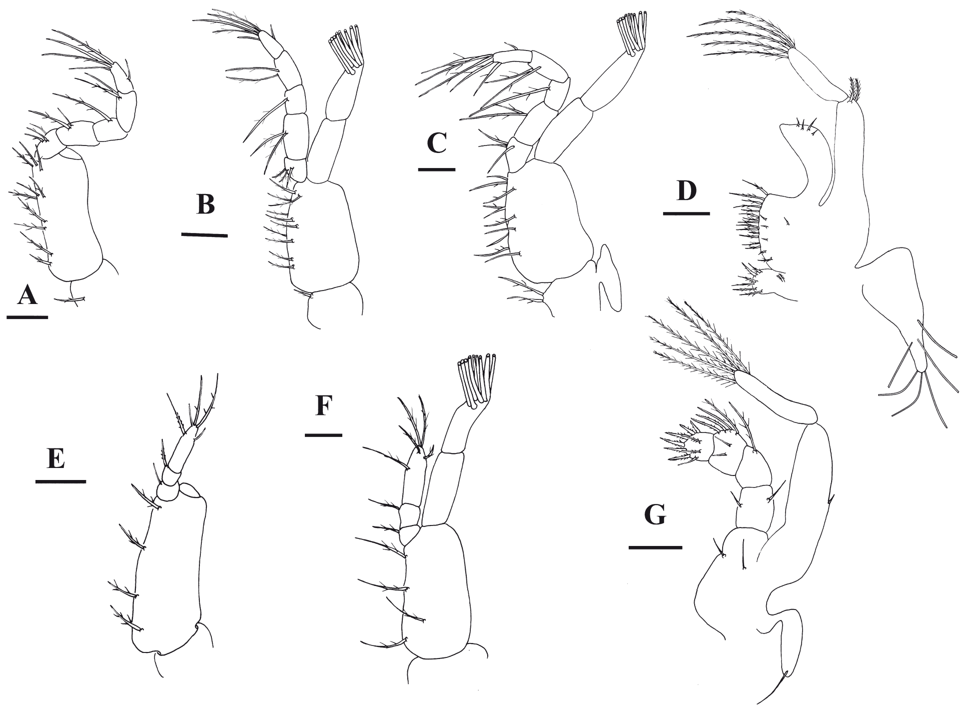


Fig 7. – *Dyspanopeus sayi*. First maxilliped: A, zoea I; B, zoea III; C, zoea IV; D, megalopa. Second maxilliped: E, zoea I; F, zoea IV; G, megalopa. Scale bars = 0.1 mm.

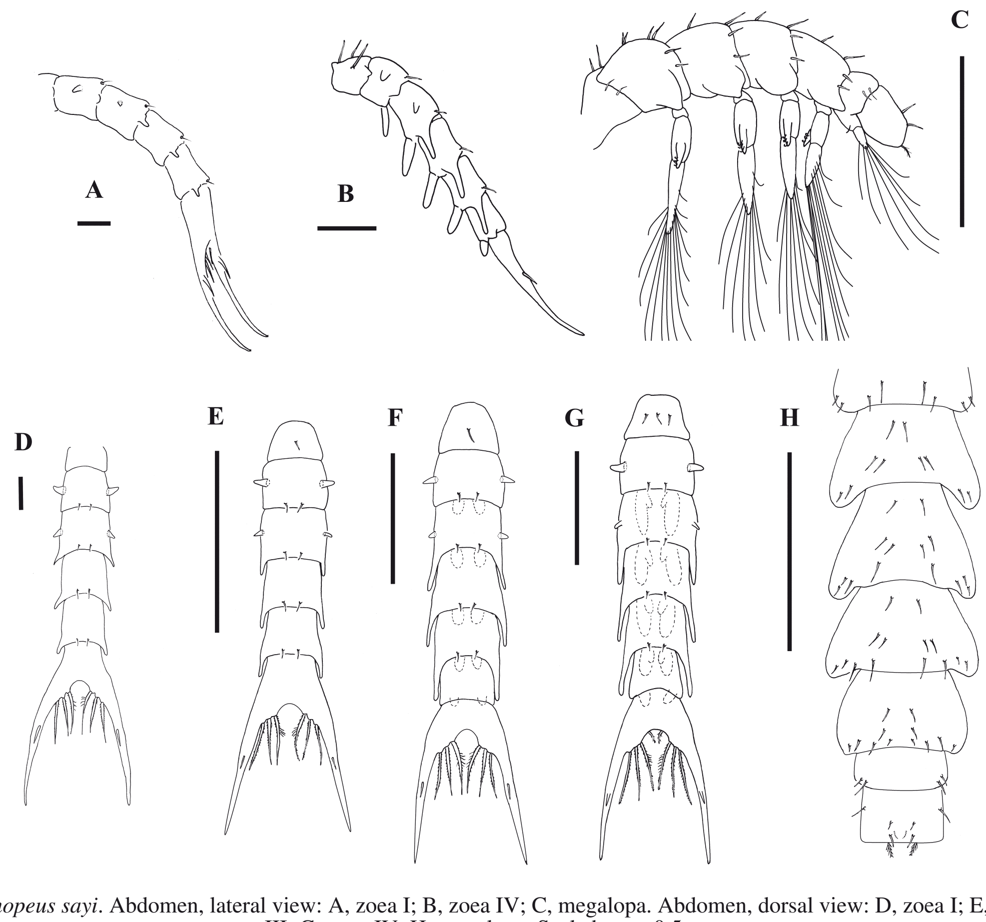
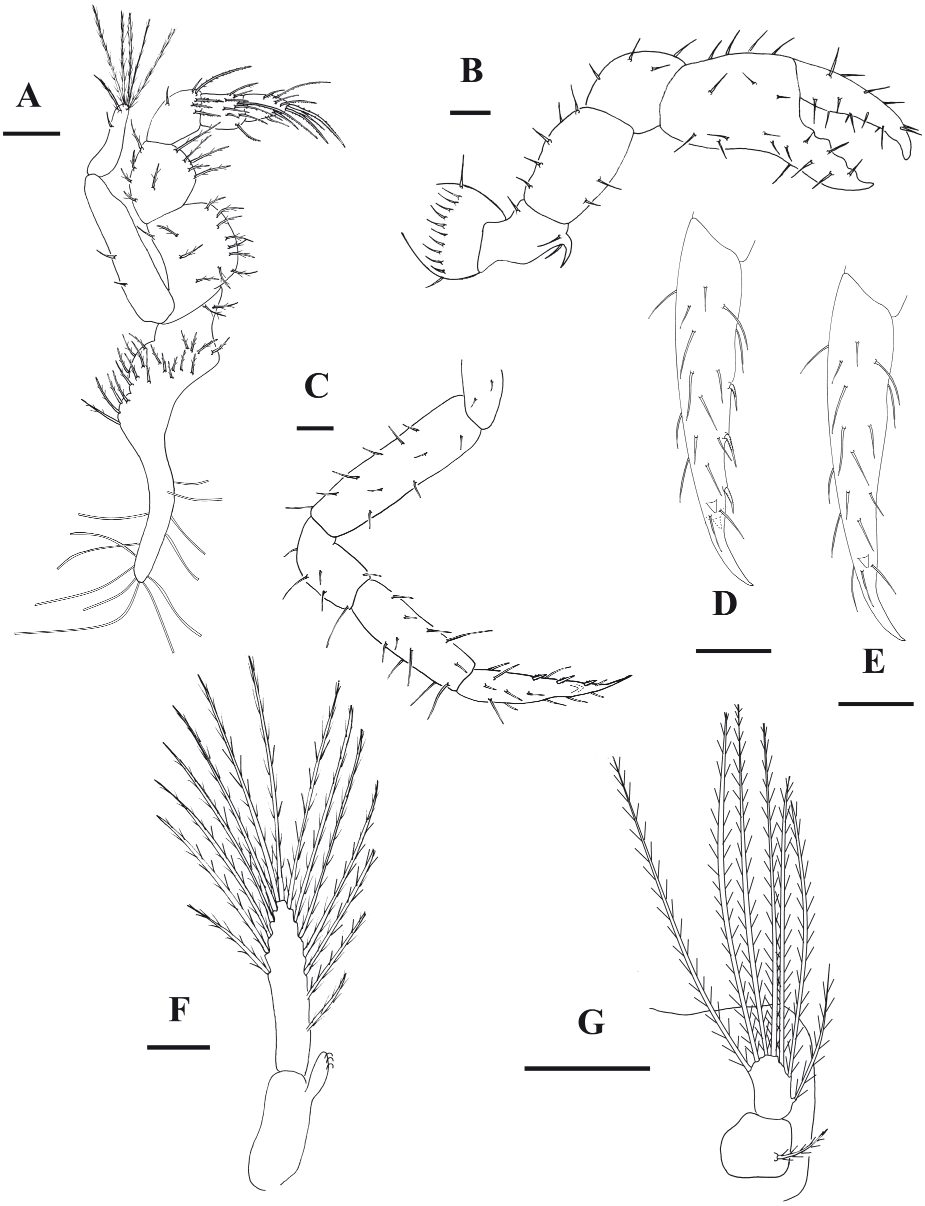


Fig 8. – *Dyspanopeus sayi*. Abdomen, lateral view: A, zoea I; B, zoea IV; C, megalopa. Abdomen, dorsal view: D, zoea I; E, zoea II; F, zoea III; G, zoea IV; H, megalopa. Scale bars = 0.5 mm.



Fɪɢ 9. – *Dyspanopeus sayi*. Megalopa. A, third maxilliped; B, cheliped; C, pereiopod 3; D, dactylus pereiopod 4; E, dactylus pereiopod 5; F, pleopod 2; G, uropod. Scale bars = 0.1 mm.

Third maxilliped: rudimentary, slightly segmented, without setae.

Pereiopods: all slightly segmented, without setae.

Pleon (Fig. 8B, G): Pleonite I with 3 mid-dorsal setae.

Pleopods (Fig. 8B, G): biramous buds now with endopod present more elongated than previous stage.

Telson (Fig. 8B, G): one extra pair of short setae on inner posterior margin.

Megalopa

(Figs 4 A-C; 5C, D H; 6D, H; 7D, G; 8C, H; 9)

Size: CL=1130±100 µm; CW=1060±100 µm; N=15

Cephalothorax (Fig. 4A-C): frontal margin is obliquely downward with 2 lateral spines, rostrum ending in a median triangular notch with bifid tip; the peduncle of eyes with 9 small setae on dorsal part; 1 pair of protogastric and mesobranchial protuberance present; setation as shown.

Antennule (Fig. 5C): peduncle 3-segmented, with 4,2,2 plus 2 pairs of 3 long plumodenticulate setae, respectively; endopod unsegmented with 1 medial, 1 subterminal and 4 terminal setae; exopod 4-segmented, with 0,5,4,4 aesthetascs and 0,0,1,2 setae.

Antenna (Fig. 5H): peduncle 3-segmented with 4,2,2 setae; flagellum 8-segmented with 0,0,3,0,4,0,4,3 setae, respectively.

Mandible (Fig. 5D): palp 2-segmented, with 8 terminal setae on distal segment.

Maxillule (Fig. 6D): coxal endite with 14 plumose setae; basial endite with 2 setae on lower margin, 10 subterminal sparsely plumodenticulate setae and 8 terminal cuspidate setae; endopod unsegmented with 1 proximal and 2 terminal setae.

Maxilla (Fig. 6H): coxal endite with 7+6 terminal plumose setae; basial endite with 7+8 sparsely plumodenticulate setae; endopod unsegmented with 1 long simple seta; scaphognathite with 38-40 marginal plumose setae plus 2 small simple setae on each lateral surface.

First maxilliped (Fig. 7D): coxal endite with 2 proximal and 6 terminal plumodenticulate setae; basial endite with 13 plumodenticulate terminal setae plus 7 proximal simple setae; endopod unsegmented with 4 setae on distal part; exopod 2-segmented, with 2 terminal plumodenticulate setae on proximal segment, and 5 plumose setae on distal segment; epipod well developed with 6 setae.

Second maxilliped (Fig. 7G): protopod without setae; endopod 5-segmented with 2 simple, 2 simple, 1 plumodenticule, 2 simple + 4 plumodenticulate, and 3 proximal simple + 6 terminal plumodenticulate setae, respectively; exopod 2-segmented, with 1 medial simple seta on proximal segment and 5 terminal plumose setae on distal segment; epipodite reduced with 1 terminal seta.

Third maxilliped (Fig. 9A): protopod with 19 plumodenticulate setae; endopod 5-segmented, with 12,10,6,9,9 plumodenticulate setae respectively; exopod 2-segmented with 2 medial simple setae on proximal segment and 5 terminal plumose setae on distal segment plus 1 subterminal simple seta; epipodite well development with 4 proximal plumodenticulate setae and 12 long setae on distal part.

Pereiopods (Fig. 9B-E): cheliped sparsely setose as shown, prominent curved spine on ischium; pereiopods 2-5 thin and setose, inner margin of dactyli with 3 stout spines and 2 shorter lateral spines, except dactylus of pereiopod 5 with no spines on inner margin and only one shorter lateral spine.

Sternum (Fig. 4B): maxilliped sternites completely fused with 8 setae, cheliped sternites with 3 setae each, sternal sutures are interrupted medially.

Pleon (Fig. 8C, H): six pleonites plus telson; setation as shown.

Pleopods (Figs 8C, 9F, G): biramous, present on pleonites 2-5; endopod with 3 cincinuli in all four pairs; exopod with 12-14 long plumose natatory setae; uropods uniramous with 1, 7 long plumose natatory setae on proximal and distal segment respectively.

Telson (Fig. 8C, H): rectangular, truncated with a pair of lateral setae, and posterior margin with 2 sparsely plumose setae, 2 pairs of dorsal setae, and 1 ventral pair.

DISCUSSION

There is a well-established population of *Dyspanopeus sayi* in the area of Alfacs Bay, Ebro Delta, NW Mediterranean, as evidenced by the collection of mature males and ovigerous females since 2005 (see Schubart *et al*. 2012; Guerao pers. obs.) and by the occurrence of larval stages in the plankton of the bay in September 2012. The presence of zoea I, zoea II and zoea IV in these samples from the inner part of the bay suggests that this species carries out its complete larval development in the bay, independently of the possibility of larval dispersal offshore. The scattered populations of this species in the Mediterranean compared with the continuous distribution long the French and Dutch coastline (see Fig. 1) is due to the low number and location of appropriate habitats in the Mediterranean, as there are no estuaries with a gradient of mixed salinities. The most similar habitats are lagoons, deltas, and closer bays, which are ecosystems with brackish waters; however, these habitats are not abundant in the Mediterranean basin. This scarcity of available habitats also explains the low number of populations of the other panopeid *Rhithropanopeus harrisii* introduced into the Mediterranean, in contrast with its wider distribution on the European Atlantic coasts (Projecto-García *et al*. 2010).

The zoeal stages collected in the Alfacs Bay plankton were measured and dissected. No significant differences were found in size, morphology or setation with respect to the cultured material. This is important information for examining morphological descriptions of larvae because meristic data obtained from specimens reared in the laboratory can be considered as valid, regardless of possible anomalies due to culture conditions (González-Gordillo and Rodríguez 2000, Wehrtmann and Albornoz 2003, Marco-Herrero *et al*. 2012).

According to previously published data, the duration of zoeal development of *D. sayi* ranges from 14 days at 21°C to 27 days at 14°C (Chamberlain 1957). Kurata (1970) reported complete zoeal development in a minimum of 15 days (April culture) and 14 days (May culture) but did not give data on temperature. Data obtained in the present study, 15 days at 18°C, fall within the range mentioned above and corroborate the well-known relationship between temperature and duration of decapod larval development (Anger 2001).

Previous descriptions of larval stages of *D. sayi,* except the zoea I described by Clark (2007) and Schubart *et al.* (2012), are incomplete, brief or inaccurate. Illustrations are also incomplete and in some cases are of low quality. The present study provides, for the first time, data on cephalothorax setation for zoea II-IV, in addition to information on the right setation pattern of mouthparts. Setation is described for the megalopa stage and illustrations of the sternum are provided for the first time, in addition to illustrations of the ischial spine of the cheliped, spinulation and the dactyli of the pereiopods. The following are some of the noteworthy differences: a fourth pair of inner serrulated setae was observed on the telson margin in zoea IV in the present study rather than in zoea III as reported by Chamberlain (1961); and the short “feeler” on the dactylus of pereio-

Table 1. – Main morphological and meristic differences between larval stages of *Dyspanopeus sayi*, *Panopeus africanus* and *Rhithropanopeus harrisii*. Abbreviations: dsl, cephalothorax dorsal spine length; rsl, cephalothorax rostral spine length; sp., spines; plp, posterolateral process; seg., number of segment; s., setation; sbls, subterminal long setae.

	Dyspanopeus sayi	*Panopeus africanus*	*Rhithropanopeus harrisii*
Reference	Present study	Rodríguez and Paula, 1993	Kurata (1970), Marco-Herrero *et al.* (submitted)
Zoeal stages			
dsl/rsl ratio	dsl < rsl	dsl ≥ rsl	2 dsl < rsl
Antennal protopod sp.	absent	present (ZI-III)	present (minute)
Antennal protopod tip	rounded	acute	acute
Pleonite 3 dorsolateral process	present	present	absent
Pleonite 5 plp / pleonite length	plp ≤ pleonite length	plp ≤ pleonite length	plp > 2 pleonite length
Telson lateral sp.	absent	2	absent
Megalopa stage			
Spines on frontal margin	present	present	absent
Antennal flagellum seg. (s.)	8(0,0,3,0,4,0,4,3)	8(0,0,2-4,0,3-4,0,4,3-4)	6(0,0,0,0,0,0)
Mandibular palp seg. (s.)	2 (0,8)	3 (0,0,8-9)	2 (0,5)
Cheliped ischial spine	present (curved)	present (curved)	sometimes (never curved)
Maxilliped sternite s.	8	6*	6
Cheliped sternite s.	3	3*	4-6
Pereiopods 2-5 sternites s.	0	0*	1-4
Pereiopod 5, dactylus sp/sbls	1/0	0/3	0/0
Uropods s.	1, 7	1, 7-8	0, 3-4

*Marco-Herrero *et al.* (unpublished)

pod 5 described by Kurata (1970) was not observed in the present study. Other differences in the setation pattern (fewer setae) could be due to miscounts.

Panopeidae in its present composition (see Ng *et al.* 2008) is a complex family with high heterogeneity between larval forms (Martin 1988). In some cases, this variability in larval morphology has been used as evidence to support taxonomic changes in the family, such as the recent establishment of the genus *Acantholobulus* Felder and Martin 2003, for some species of *Panopeus* H. Milne-Edwards, 1843. The larval stages of *D. sayi* fit in Group I of the classification of the Xanthid larvae by Martin (1984), which also includes *R. harrisii* and *Panopeus africanus;* however, it should be pointed out that other panopeid species have been placed in other groups of this classification based on larval morphology. This may suggest that this family needs to be studied further, taking into account other evidence based on adult morphology, and using techniques such as DNA analysis combined with larval morphology. Although they are all in Martin's Group I, the zoeal stages of the three Iberian panopeids show clear differences that allow easy identification. They also show differences in the megalopa stage, a larval phase which is not considered in the classification by Martin (1984). The main differences observed in the zoeal stages are observed in the ratio between the dorsal and rostral spine lengths, as well as in the antennal and pleonal morphology (see Table 1). Zoeal stages of *Panopeus africanus* have well-developed dorsal and rostral spines that are similar in size in zoea I; however, the dorsal spine is longer than the rostral spine in the other three zoeal stages. In *R. harrisii* and *D. sayi*, both cephalothoracic spines are well developed, but the rostral spine is longer than the dorsal spine, and in *R. harrisii* it is more than twice as long as the dorsal spine. The antenna of the zoeae of the three species are characterized by a reduced exopod, although it is less reduced in *Panopeus africanus*, which even has a fourth zoeal stage that shows two terminal setae instead of one, as in the rest of the zoeal stages and in the other two species. In all cases the protopod is as long as the rostral spine, but the tip is acute in *P. africanus* and *R. harrisii* and rounded in *D. sayi* with no spinulation. *Panopeus africanus*, however, has strong spines increasing in size towards the tip in zoea I and with fewer spinules in subsequent stages; in *R. harrisii* there are only minute spinules in the distal part. Differences were also observed in the pleon morphology, such as the number of dorsolateral processes, which were only present in pleonite 2 in *R. harrisii* and in pleonites 2 and 3 in *P. africanus* and *D. sayi*. This is one of the features characterizing Group I; therefore, *R. harrisii* must be considered an exception with respect to this character. Other differences in pleonal features are shown in Table 1.

The megalopa stage also shows clear differences between the three species (see Table 1). The rostrum is similar in *P. africanus* and *D. sayi*, although the spines at the basal angles, called "horns" in some papers, are more developed and acute in *P. africanus*. The megalopa of *R. harrisii* does not have these spines on the rostrum. The chelipeds are also similar in *P. africanus* and *D. sayi* but the strongly curved ischial spine is not present in *R. harrisii* (in some cases there is a small spine but it is never curved). The main difference in the megalopa stage is the number of segments of the antennal flagellum: there are eight in *P. africanus* and *D. sayi*, and six in *R. harrisii*. Other differences are seen in the mandibular palp, sternum, the dactyli of pereiopods, and uropods, and are shown in Table 1.

These larval differences allow larvae of the three panopeids inhabiting Iberian Peninsula waters to be accurately identified; however, the fact that species belonging to the same family may show large differences could raise doubts about whether they really belong to the same family. Future studies using molecular techniques in combination with larval morphology could shed light on the real phylogenetic relationships in this complex brachyuran family.

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