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Morphology of the Zoeal Stages of *Hexapus sexpes* Fabricius, 1798 (Decapoda, Brachyura, Hexapodidae) Reared in the Laboratory

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Abstract: The present information is based on the study of the zoeal stages of *Hexapus sexpes*. A single ovigerous female of *Hexapus sexpes* was collected from the North West Persian Gulf (29°56'14"S, 49°02'28"W) in April 2009. Female was maintained in aquarium containing natural sea water at 25°C until the eggs hatched. The present study describes the zoeal stages of *Hexapus sexpes*. A complete description and illustration of larval features is provided and a comparison with closely related species (*Spiroplax spiralis* and *Lambdophallus anfractus*) showing known larval development is presented. The zoeal stages of *H. sexpes* are very similar to that of *L. anfractus*. The zoeal of two species, however, could separate by the number of setae on the maxillule and maxilla.

Key words: Brachyura, Hexapodidae, *Hexapus sexpes*, Persian Gulf, zoeal stages

INTRODUCTION

Melo (1996) reported that there are approximately 5000 species of brachyuran crabs world-wide, representing over half the number of decapod species presently recognized. But the later estimates ranged from 5000 to 10000 (Ng, 1998; Martine and Davis, 2001; Yeo *et al.*, 2008).

There have been relatively few taxonomic studies on the Brachyuran of the Persian Gulf region, despite recent ecological focus on the area due to its economic importance (Price and Robinson, 1993).

Apel (2001) collected some specimens of crabs on the Arabian side of the Persian Gulf, revised previous collected material and subsequently presented a total of 200 species of crabs for the Persian Gulf. The important work of Apel (2001) is the last key reference for the Persian Gulf crabs. Apart from the family Penaeidae, there has been no attempt to identify the larvae of Persian Gulf decapods beyond generic level. In recent surveys of the planktonic decapoda of the Western Gulf (Al-Aidaros, 1993), descriptions of the first Zoea of six Brachyurans from the Persian Gulf (Al-Khayat and Jones, 1996).

The Hexapodidae is a small family (13 genera, 22 species) of Atlantic and Indo-Pacific brachyuran crab (Ng *et al.*, 2008). But the larval development is only known for 2 species. *Spiroplax spiralis* (Lago, 1988) and

Lambdophallus anfractus (Matsuo, 1971). Only 2 species of Hexapodidae have been recorded from the Persian Gulf (*Hexapus sexpes* Fabricius, 1798) and (*Lambdophallus sexpes* Alcock, 1900) (Apel, 2001). The present work describes the zoeal stages of *Hexapus sexpes*.

MATERIALS AND METHODS

A single ovigerous female of *H. sexpes*, 15 mm carapace length, was collected by van Veen grab (0.027 m²) from the North West Persian Gulf (29°56'14"S, 49°02'28"W) on 19th April 2009. The female crab was held in an aquarium (50×20×25 cm) until the eggs hatched after 4 days. Individual larvae were also reared in glass containers of 500 mL capacity (approximately 20 larvae/container) to characterize developmental stages. Mean daily water temperature in the tank and glass container was 25±1°C and average salinity was 37±1 ppt. Newly hatched larvae were fed with fresh *Artemia* nauplii. Approximately 40% water in each glass container was exchanged daily. The larvae were fixed and preserved in 10% neutral Formalin for later use (Ko, 1995).

Larval specimens were separated using a stereoscope. Specimens were dissected in 25% lactic acid solution and drawn with the aid of a camera lucida attached to an Olympus CH-2 microscope. All measurements were made with an ocular micrometer.

Illustrations are based on a sample of 3 individuals and represent an average animal with the features most commonly occurring in the sample. Drawing techniques, nomenclature and the formulation of tables follow standards by Rice (1979).

RESULTS

The larvae of *H. sexpes* hatched out early in the morning on 23th April 2009 and passed through three zoeal stages. Mean intermolt duration for each stage which molted was calculated from individual culture data: zoea I, 4 days, zoea II, 5 days. The larva died 5 days after reaching zoea III, without molting to the megalopa (Fig 1a-c). Measurements of larval features are summarized in Table 1. The major characteristics of the larvae are described below.

First zoeal stage (Fig. 2):

- **Carapace (Fig. 2a):** Dorsal spine long, distally slightly curved, longer than rostral spine; rostral spine straight and slightly longer than antennal

protopod; lateral spines present and shorter than dorsal spine; anterodorsal setae absent; ventral margins without setae; eyes sessile

- **Antenna (Fig. 2b):** Protopod slightly shorter than rostral spine and distally spinulate; exopod elongated, almost as long as protopod, with one medial setae; endopod absent
- **Antennule (Fig. 2c):** Uniramous; endopod absent; exopod unsegmented, with 3 long aesthetascs and 1 small setae, all terminal
- **Maxillule (Fig. 2d):** Coxal endite with four plumose setae and one simple setae; basal endite with 5 setal processes; endopod 2-segmented, proximal segment with 1 seta, distal segment with four terminal setae
- **Maxilla (Fig. 2e):** Coxal endite bilobed, with 2+3 setae; basal endite bilobed, with 3+3 setae; endopod bilobed, with 2+4 setae; exopod (scaphognathite) margin with 3 plumose setae
- **First maxilliped (Fig. 2f):** Coxa without seta; basis with 7 setae (5 plumose + 2 simple); endopod 5-segmented, with 2, 2, 1, 2, 5 (1 subterminal+4 terminal) setae; exopod with 4 plumose natatory setae

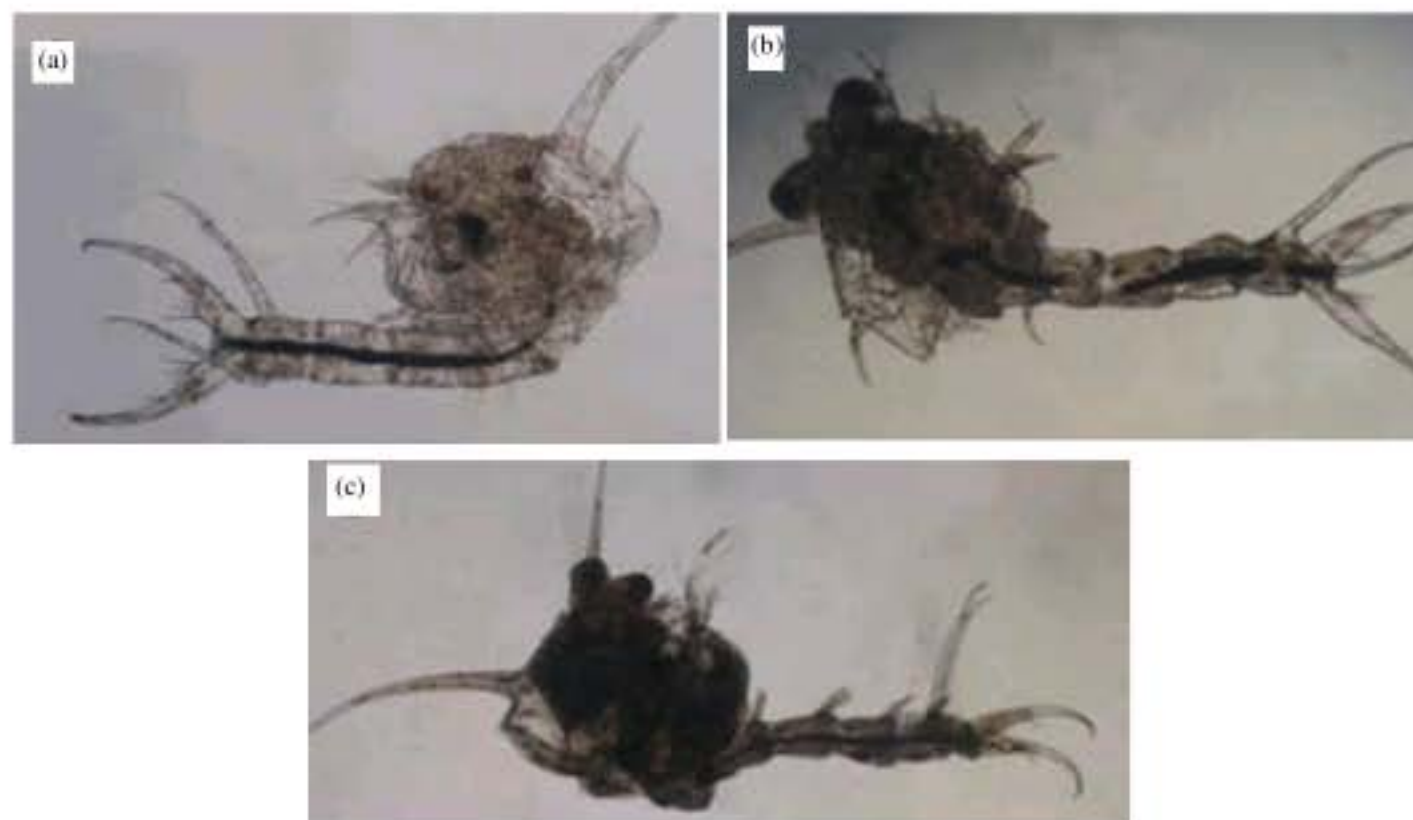


Fig. 1: Larval stages of *Hexapus sexpes*. (a) first zoeal stage; (b) second zoeal stage and (c) third zoeal stage

Table 1: Morphological features of zoeal stages of *Hexapus sexpes**

| Measurements | Zoea I | Zoea II | Zoea III |
|---|------------|------------|------------|
| Length of carapace (CL) | 0.645±0.01 | 0.702±0.02 | 0.780±0.02 |
| Length of dorsal spine on carapace (DL) | 0.672±0.01 | 0.728±0.01 | 1.053±0.03 |
| Length of rostrum (RL) | 0.288±0.03 | 0.401±0.01 | 0.605±0.01 |
| Length of lateral spine on carapace | 0.160±0.02 | 0.215±0.10 | 0.234±0.02 |
| Length of antennal protopodite (AP) | 0.214±0.03 | 0.322±0.03 | 0.531±0.01 |
| Length of antennal exopodite (AEx) | 0.213±0.02 | 0.321±0.01 | 0.531±0.10 |
| Length of abdominal somite | 0.176±0.01 | 0.224±0.02 | 0.312±0.02 |
| Length of dorsal spine on first abdominal somite | 0.121±0.10 | 0.156±0.20 | 0.216±0.03 |
| Length of lateral spine on fifth abdominal somite | 0.384±0.10 | 0.546±0.01 | 0.648±0.01 |
| Length of telson | 0.456±0.02 | 0.585±0.02 | 0.632±0.30 |
| Length of fork telson | 0.384±0.03 | 0.480±0.01 | 0.521±0.10 |

*All measurements as Mean±SD (N = 3) expressed in mm

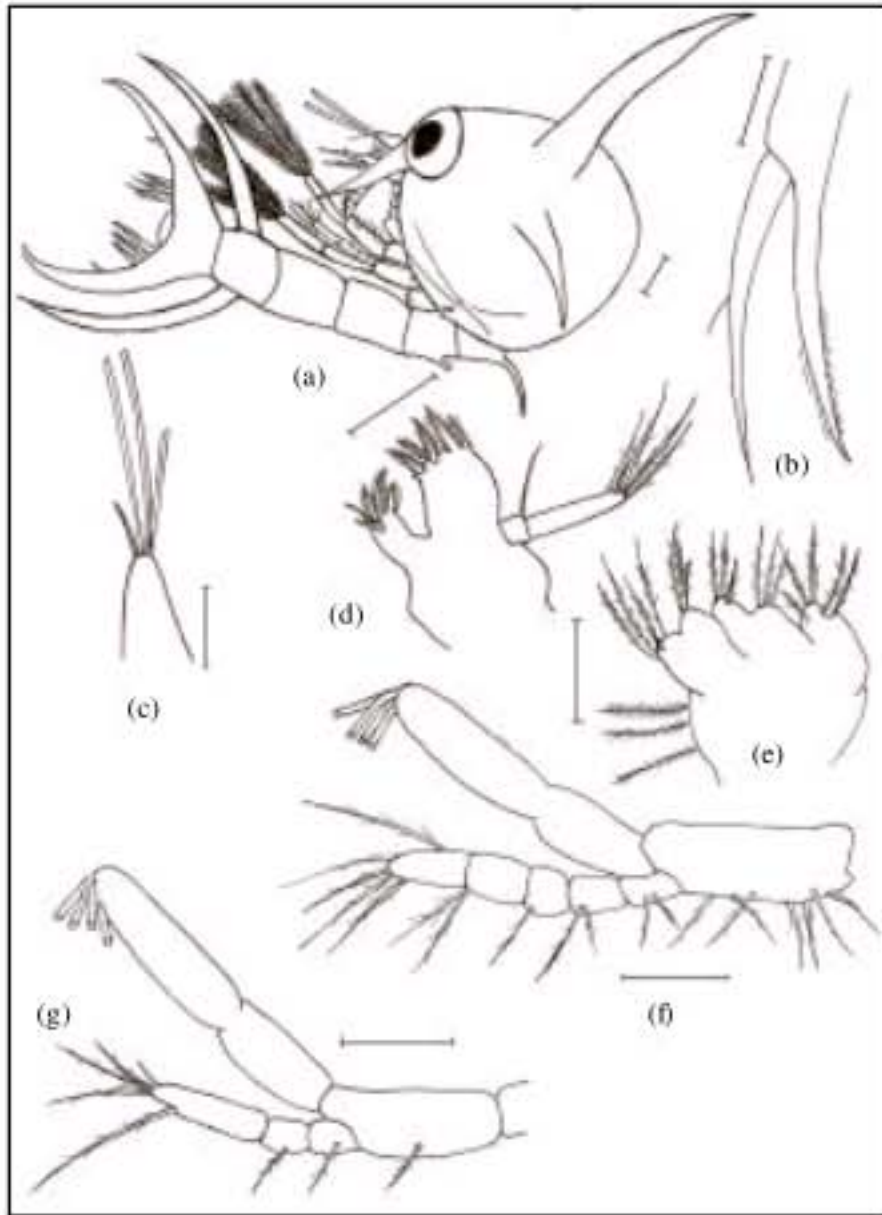


Fig. 2: *Hexapus sexpes* (a) first zoeal stage; (b) lateral view; (c) antenna; (d) antennule; (e) maxillule; (f) maxilla and (g) first maxilliped; second maxilliped. Scale bars, 0.1 mm

- **Second maxilliped (Fig. 2g):** Coxa without seta; basis with 1 setae; endopod 3-segmented, with 1, 1, 5 (1 subterminal + 4 terminal) setae respectively; exopod 2-segmented, distal segment with 4 terminal natatory plumose setae
- **Abdomen (Fig. 2a):** Abdomen consisting of five somites and telson: One curved dorsal spine present on somite 1; one pair of dorsolateral knobs present on somite 2; one pair of lateral spine on somite 5 (lateral spine as long as telson). Pleopods absent. Telson with slender cornua and wide median notch. One pair of dorsal medial spines; Three stout long spines medially at base of cornua; most internal spines longer than others

Second zoeal stage (Fig. 3):

- **Carapace (Fig. 3a):** Eyes stalked; one plumose setae on posterolateral margins of carapace; otherwise unchanged
- **Antenna (Fig. 3b):** Endopod bud developing; otherwise unchanged

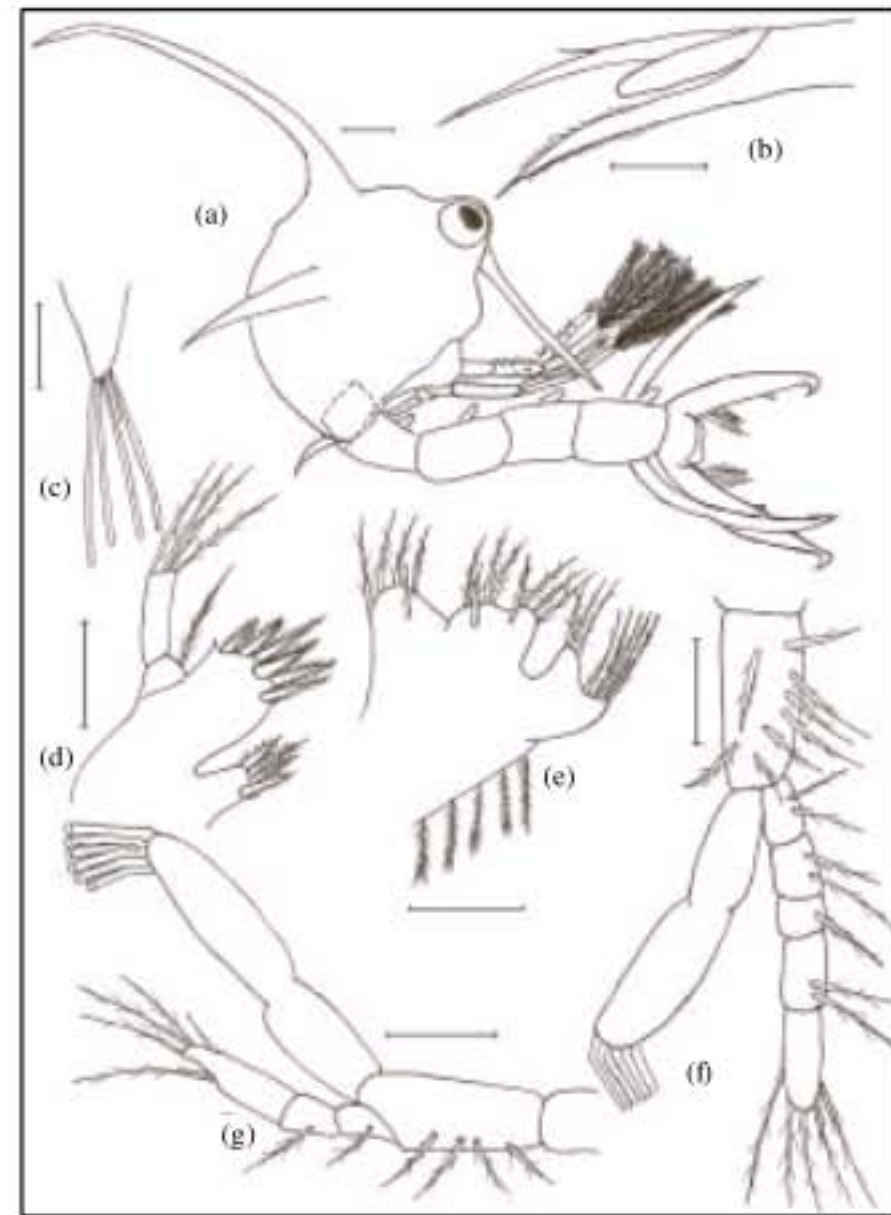


Fig. 3: *Hexapus sexpes* (a) second zoeal stage; (b) lateral view; (c) antenna; (d) antennule; (e) maxillule; (f) maxilla and first maxilliped second maxilliped. Scale bars, 0.1 mm

- **Antennule (Fig. 3c):** Exopod now with 4 long aesthetascs; otherwise unchanged
- **Maxillule (Fig. 3d):** Basial endite with 6 setal processes; otherwise unchanged
- **Maxilla (Fig. 3e):** Basial endite bilobed, with 4+4 setae; endopod bilobed, with 3+5 setae; exopod (scaphognathite) margin with 5 plumose setae; otherwise unchanged
- **First maxilliped (Fig. 3f):** Basis with 8 setae; exopod with 6 plumose natatory setae; otherwise unchanged
- **Second maxilliped (Fig. 3g):** Basis with 4 setae; exopod with 6 plumose natatory setae ; otherwise unchanged.
- **Abdomen (Fig. 3a):** Somites 2-5 each with pleopods present, endopods absent; otherwise unchanged.
- **Telson (Fig. 3a):** Each fork long, gradually curved, not spinulated; otherwise unchanged

Third zoeal stage (Fig. 4):

- **Carapace (Fig. 4a):** Eyes stalked; three plumose setae on posterolateral margins of carapace; otherwise unchanged

DISCUSSION

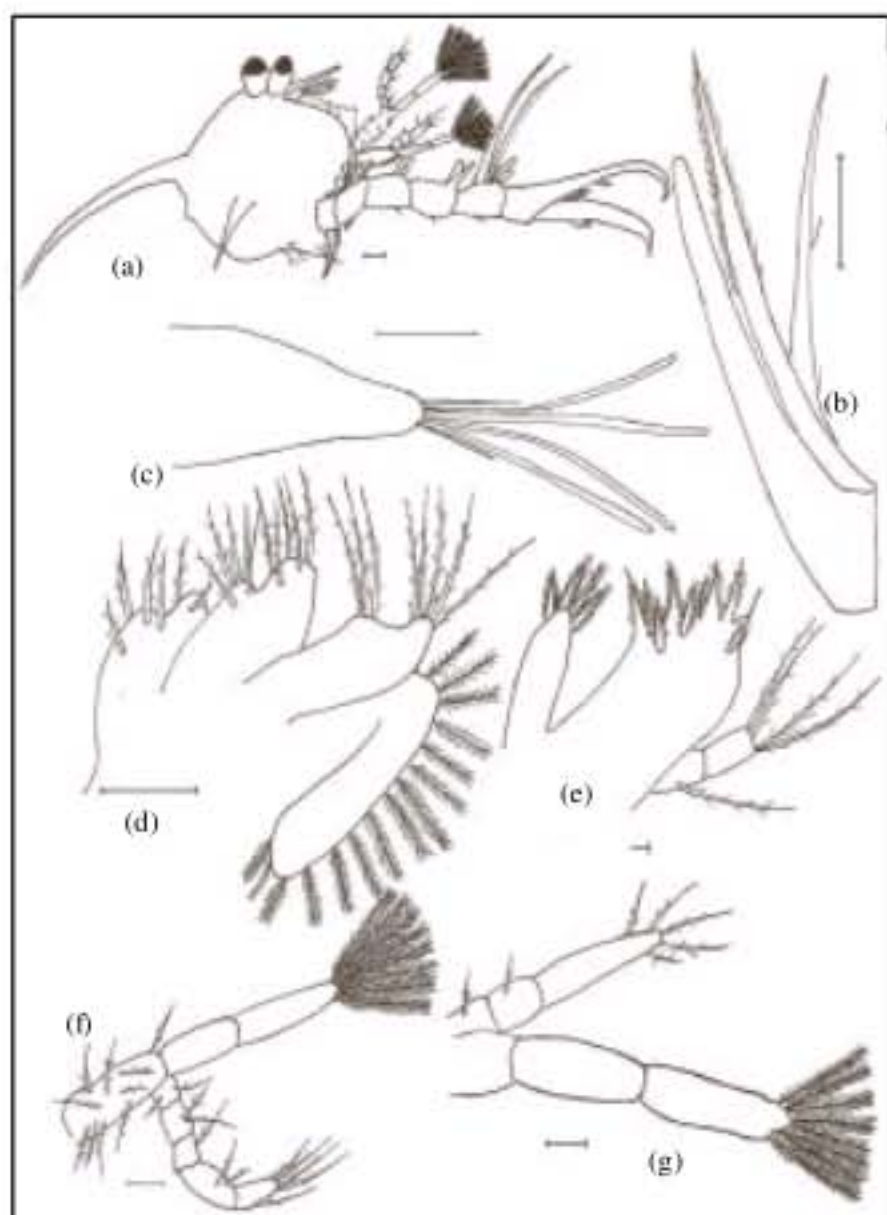


Fig. 4: *Hexapus sexpes* (a) third zoeal stage; (b) lateral view; (c) antenna; (d) antennule; (e) maxillule; (f) maxilla and (g) first maxilliped second maxilliped. Scale bars, 0.1 mm

- **Antenna (Fig. 4b):** Endopod bud approximately 73% length of exopod; otherwise unchanged
- **Antennule (Fig. 4c):** Exopod now with 4 long aesthetascs and 2 setae; otherwise unchanged
- **Maxillule (Fig. 4d):** Basial endite with 8 setal processes; otherwise unchanged
- **Maxilla (Fig. 4e):** Coxal endite bilobed, with 3+3 setae; basial endite bilobed, with 5+5 setae; exopod (scaphognathite) margin with 15 plumose setae; otherwise unchanged
- **First maxilliped (Fig. 4f):** Basis with 10 setae; exopod with 8 plumose natatory setae; otherwise unchanged.
- **Second maxilliped (Fig. 4g):** Exopod with 8 plumose natatory setae; otherwise unchanged
- **Abdomen (Fig. 4a):** Pereiopods 1-4 further developed, showing beginning of segmentation; somite 3, 4, 5 with 2 dorsomedial setae; pleopod buds further developed showing beginning of endopodite segmentation; otherwise unchanged
- **Telson (Fig. 4a):** Each fork long, gradually curved, not spinulated; One pair of dorsal medial spines gradually curved; otherwise unchanged

The subfamily Hexapodinae was established by Miers in 1886 within the family Pinnotheridae that included the genera *Hexapus*, *Amorphopus*, *Thaumastoplax* and *Asthenognathus*. Subsequently, the subfamily was placed within the family Goneplacidae by Alcock in 1900 (Lago, 1988). Guinot (1978) created the superfamily Hexapodoidea with the single family Hexapodidae. Manning and Holthuis (1981) confirmed Guinot's (1978) creation by stating that the absence of the fifth pair of pereopods in the Hexapodinae was a character which warranted the recognition of the group as a separate family, allied but distinct from the goneplacids.

Manning and Holthuis (1981) recognized 11 genera, 19 species of Atlantic and Indo-Pacific and clarified the identity of the type genus *Hexapus* De Haan, 1835. They recognised 18 species, but Manning (1982) subsequently argued that *Hexapus estuarinus* (Sankarankutty, 1975) is a junior synonym of *H. sexpes* (Fabricius, 1798).

Identification of the species of family Hexapodidae is often very difficult from adult specimens and there is still confusion regarding the taxonomic position of *Hexapus* and *Lambdophallus*. Some records suggested that *H. sexpes* and *L. anfractus* are synonymous. Stephensen (1946) confirmed the opinion Tesch, 1918 that *L. anfractus* Rathbun, 1909 is synonymous with *H. sexpes* Fabricius, 1798.

Campbell and Stephenson (1970) reported that although sternal grooves have thus been demonstrated in both *Lambdophallus* and *Hexapus*, the continued existence of *Lambdophallus* as a separate genus is justified because in *Lambdophallus* the eyestalks are fixed and the sternal grooves extend laterally from below the junction of the sixth and seventh abdominal segments, running parallel and close to the posterior margin of the first sternite, in *Hexapus* they run anterolaterally towards the bases of the maxillipeds (Alcock and McArdle, 1902). Because of this, Rathbun's *L. anfractus* must, as Tesch 1918 suggested, be transferred to the genus *Hexapus*.

In spite of the observation of Stephenson (1946) and Campbell and Stephenson (1970) some records *L. anfractus*, is not a synonym of *H. sexpes* for example Serene and Soh (1976) and Apel (2001). Apel (2001) reported *H. sexpes* and *L. sexpes* as two types from the Persian Gulf. He recognized *H. sexpes* distribution in all the Persian Gulf while *L. sexpes* only from the Strait of Hormuz. They also have the expression that the taxonomic position and the distribution of the various types of *Hexapus* genus are unclear and needs urgent revision. Finally, Ng *et al.* (2008) reported that *H. anfractus* is synonymous with *Lambdophallus*. Adult specimens

Table 2: Comparison of the first zoea in the family Hexapodidae

| Species | Spiroplax spiralis | Lambdophallus anfractus | Hexapus sexpes |
|------------------------|--------------------|-------------------------|----------------|
| Carapace spine | | | |
| DS | + | + | + |
| LS | + | + | + |
| RS | + | + | + |
| Antennule | | | |
| Aes | 3 | 3 | 3 |
| Se | 2 | 1 | 1 |
| Antenna | | | |
| E/P | 1.03 | 1 | 1 |
| Maxillule | | | |
| end. | 4 | 3 | 4 |
| bas. | 5 | 5 | 5 |
| cox. | 4 | 3 | 5 |
| Maxilla | | | |
| end. | 4+3 | 3+3 | 4+2 |
| bas. | 5+7 | 4+3 | 3+3 |
| cox. | 6 | 6 | 5 |
| First abdominal somite | | | |
| DS | - | + | + |
| Fifth abdominal somite | | | |
| LS | - | + | + |
| Telson | | | |
| OS | + | - | - |
| Sources | Lago (1988) | Matsuo (1971) | Present study |

DS: Dorsal spine; RS: Rostral spine; LS: Lateral spine; OS: Outer spine; Aes.: Aesthetascs; Se.: Setae; E/P: Ratio exopodite/protopodite; end.: Endopod; bas.: Basial endite; cox.: Coxal endite; +: Present; -: Absent

which used in this research had characteristics alike as *H. sexpes* in Stephensen (1946), thus we used *H. sexpes* as name of specimens. There are few papers on morphology of Hexapodidae so it is difficult to compare *H. sexpes* larval stage with other species and *H. sexpes* larval stages have not studied yet. The present work is the first study of the zoeal stages of *H. sexpes* based on reared in the laboratory.

A comparison of the first zoeal stage among *S. spiralis*, *L. anfractus* and *H. sexpes* particularly as regards their abdominal appendages, is presented in Table 2.

The larval stages in *H. sexpes* are very similar to *L. anfractus*. Some of the common characteristics are: Dorsal spine long, distally slightly curved; lateral spines present and shorter than dorsal spine. The number of swimmerets of the exopodite of maxilliped increases as 4-6-8 as it grows. There is a curved spine on the dorsal of the 1st abdominal segments and one pair of dorsolateral knobs present on somite 2 and a long spine on either lateral side of the fifth segment. Comparison between *H. sexpes* and *L. anfractus* show some differences (Table 2). The abdomen is composed of six distinct segments in the zoeal stages of *L. anfractus* as opposed to most brachyuran. In *H. sexpes* the zoeal stages never have more than 5 abdominal segments, the endopod of the maxillule has four setae in *H. sexpes* (three setae in *L. anfractus*), the coxal endite of the maxillule has five setae in *H. sexpes* (three setae in *L. anfractus*), the

endopod of the maxilla has 4+2 setae in *H. sexpes* (3+3 setae in *L. anfractus*), the basial endite of the maxilla has 3+3 setae in *H. sexpes* (4+3 setae in *L. anfractus*) and the coxal endite of the maxilla has five setae in *H. sexpes* (six setae in *L. anfractus*).

Larval biology is not only an intrinsic part of lifehistory studies, but contributes essential information also to various other biological disciplines, including the broad area of crustacean research. Inferring from heritable ontogenetic patterns, comparative studies of larval morphology also aid the identification of phylogenetic relationships within and among higher taxa (Anger, 2006).

Thus considering the morphological similarity between the members of Hexapodidae and the confusion regarding its taxonomy larval studies of the species may help in correct identification of genera and species of the family.

CONCLUSION

The identification of the species of family Hexapodidae is often very difficult from adult specimens. The larval study of the species may help in correct identification. The Larval characters as discussed in this paper to support the separation of *H. sexpes* Fabricius, 1798 and *L. anfractus* Rathbun, 1909. The zoeal stages of *H. sexpes* to be very similar to that of *L. anfractus*. The zoeal of two species, however, could separate by the number of setae on the maxillule and maxilla (Table 2).

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