Zoeal stages of Hiplyra variegata (Rüppell, 1830) (Crustacea: Brachyura: Leucosiidae) reared in the laboratory and collected from plankton at Al-Kharrar creek, central Red Sea

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ABSTRACT
The zoeal stages of Hiplyra variegata are described and illustrated for the first time. Zoea I was obtained from an ovigerous female, and the zoea II and zoea III stages were captured in the plankton (their identity was confirmed by rearing some of them from earlier stages). Comparisons are made with the only other species of the genus of which larvae are known, i.e. H. platycheir. The presence of a lateral spine on the carapace of zoea I is one of the most important characters that can be used for easy recognition of H. variegata. It is important to note that the third zoea of H. variegata has four small spines at each posterolateral margin of the carapace, in contrast to three spines in other species.

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Introduction
The family Leucosiidae Samouelle, 1819 comprises about 476 species in some 66 genera (Ng et al. 2008; Naderloo and Apel 2012). Zoeal and megalopal stages of Leucosiidae are important components of the meroplankton (Quintana 1984). However, the larval morphology is only known for 66 species, including 15 genera; the relevant literature up to 2012 was listed by Al Haj (2012) and Hirose et al. (2012).

Leucosiidae larvae that are known can be distinguished from others by: (1) telson a more or less triangular plate with the posterolateral margins toothed, smoothly round, acute or square; (2) telson fork absent with three pairs of setae in all stages; (3) reduction or absence of the antenna, (4) endopod of the maxillule often unsegmented; (5) setae of the maxillar endopods numbering four or, more frequently three; (6) endopod of the second maxilliped unsegmented or incompletely segmented and with the setation reduced; (7) reduction of setation of the endopod of the first maxilliped; (8) pleon with five pleomeres in all stages (Rice 1980a, 1980b; Hirose et al. 2012).

Crabs of the family Leucosiidae are common in dredging-grounds on sandy bottoms (in littoral and sublittoral soft sediment), although some species can be found at depths of approximately 2500 m (Lebour 1928; Schembri 1982). They are one of the most diverse brachyuran families (Naderloo and Apel 2012). In females, the egg mass is not
visible externally. Eggs are completely covered, protected and hidden by the large pleon which fits tightly under the carapace within a deeply excavated sternum (Lebour 1928; Ng et al. 2008).

Five species of leucosiid larvae have been identified and illustrated in detail in the central Red Sea to date. These are *Hipyra variegata*, *Leucosia anatum*, *Philyra* sp. 1, *Philyra* sp. 2 (which might be *P. granigera*) and *Arcania* sp. (possibly *A. brevifrons*) (Al Haj 2012). Leucosiid larvae are among the most abundant brachyuran larvae in the northern and central Red Sea (Echelman and Fishelson 1990; Al Haj 2012) and their highest densities clearly appeared at inshore stations of the central Red Sea (Al Haj 2012).

Galil (2009) re-examined the old genus *Philyra* and divided it into eight genera. Among those, *Hipyra* comprises six species distributed in the Indo-West Pacific: *H. elegans* Gravier, 1920; *H. longimana* A. Milne Edwards, 1874; *H. michellinae* Galil, 2009; *H. platycheir* De Haan, 1841; *H. sagitta* Galil, 2009; and *H. variegata* (Rüppell, 1830). Recently a new species, *H. ramli*, was described from the Gulf of Oman by Naderloo and Apel (2012). Only *H. variegata* has been recorded from the Red Sea (Galil 2009).

Under laboratory conditions, *Philyra* and *Hipyra* complete their larval development from zoea I to megalopal stages within at least 12 days, in two, two, three and four days, with respect to the individual larval stages (Hashmi 1968; Terada 1979, 1984; Krishnan and Kannupandi 1990; Ko 1996, 2000, 2001). The duration of the megalopal stages was recorded as being five days (Hashmi 1968; Krishnan and Kannupandi 1990; Ko 2000), while Quintana (1986a) found that the development of the megalopa to the early first crab stages took one month.

*Hipyra variegata* is distributed in the western Indian Ocean, East Africa (Mombasa, Kenya), Red Sea, Gulf of Aden, Arabian Gulf and Gulf of Oman (Naderloo and Türkay 2012). This small-sized species was briefly described and illustrated by Rüppell (1830) from the Red Sea. A more elaborate description and illustration is given by Galil (2009).

For one species of *Hipyra*, *H. platycheir* (De Haan, 1841) (as *Philyra platycheir*), the larvae have been described from material obtained by rearing and from plankton (Quintana 1986a, 1986b; Ko 2000; Ghory and Siddiqui 2008; Sakhaie et al. 2010) from Korean, Iranian and Pakistani waters, respectively. The aim of the present study was hatching, rearing and describing the zoeal stages of *H. variegata* for the first time.

**Material and methods**

An ovigerous female specimen of *H. variegata* was collected by hand dredge from a sublittoral soft sediment habitat on 28 February 2012 for rearing and describing the zoea I stage. Other stages (zoea II and zoea III) were collected from Al Kharrar creek, located between latitudes 22°45’ and 23°00’ N and longitudes 38°45’ and 39°00’ E, northwest of Rabigh city and 169 km north of Jeddah, central Red Sea in March 2012 using a plankton net of 342 µm mesh size.

The planktonic samples (400 larvae) were sorted in a plastic container (2 l). Active larvae (20) from each zoeal stages of *H. variegata* were separated and reared in 800 ml bowls under laboratory conditions. This allowed them to grow and metamorphose into the next zoeal stages (Quintana 1986b).

The ovigerous crab was kept in a small aquarium (200 ml); the seawater was changed every day until hatching took place. The larvae hatched on 2 March 2012. Soon after
hatching, the healthy and actively swimming larvae (100 larvae) were removed and separated into five large bowls (20 larvae in 800 ml of seawater). The temperature was kept constant at 27°C, salinity at 37‰ and photoperiod at 12 h light and 12 h dark (Al Haj and Al Aidaroos 2014). The larvae were subjected to moderate aeration and fed with the rotifer Brachionus sp., together with algae.

Some randomly selected active larvae and planktonic samples were kept and preserved in formaldehyde 4% for dissection. The dissection was done in polyvinylelactophenol using a WILD stereo-microscope. Drawings of appendages were made using a camera lucida attached to an Olympus BH-2 microscope equipped with differential interference contrast (DIC).

As suggested by Clark et al. (1998) at least five replicates of each appendage were drawn to determine any variations. The individual zoal stages were described and fully illustrated. The sequence of the zoal descriptions is based on the malacostracan pleomere plan from anterior to posterior. Setal armature of appendages was described from proximal to distal segments and in order of endopod to exopod. The long antennular aesthetascs, and the long plumose natatory setae of the first and second maxillipeds were drawn truncated (Clark et al. 1998). Figures were made to scale with aid of a camera lucida and ocular micrometer.

Measurements (nearest to 0.01 mm) were based mainly on five specimens of each larval stage. A micrometer was used for measurement of carapace length (CL) from the base of the rostral spine to the posterolateral carapace margin.

The female of Hiplura variegata was deposited in the collection of the Senckenberg-Museum (Frankfurt, Germany) under the catalogue number SMF 40833.

Results

The larvae hatched on 2 March 2012 after three days of collection. High mortality rates were observed in zoal hatching. After three days, all larvae had died. Zoea I was compared with zoa I captured in the plankton and all characters were confirmed. From the collected plankton sample, live H. variegata larvae of each stage were isolated and transported to bowls (20 larvae in 800 ml of sea water) with medium aeration. After one day, some of the zoa I and zoa II had metamorphosed into zoa II and zoa III respectively. The successfully moulted zoa I and zoa II had metamorphosed into zoa II and zoa III respectively. The successfully moulted zoal stages had the same characters as larvae captured in the plankton. Zoea III did not successfully moult and thus did not reach the megalopa stage.

Description of the larvae

Zoea I

Size of CL (mean ± SD): 0.3 ± 0.02 mm.

Carapace (Figure 1a, d): globose, dorsal spine absent, rostral spine short, lateral spines short, ventral margin without setae. Eyes sessile.

Antennule (Figure 1g): uniramous, endopod absent, exopod unsegmented with 3 (2 long and 1 shorter) aesthetascs and one terminal simple seta.

Antenna (Figure 1h): a reduced lobated bud, endopod and exopod absent.
Figure 1. *Hiplyra variegata* (Rüppell, 1830), lateral view of carapace: (a) zoea I; (b) zoea II; (c) zoea III. Dorsal view of rostrum spine: (d) zoea I; (e) zoea II; (f) zoea III. Antennule: (g) zoea I; (i) zoea II; (k) zoea III. Antenna: (h) zoea I; (j) zoea II; (l) zoea III.
Mandible: endopod palp absent.

Maxillule (Figure 2a): coxal endite with 6 plumodonticulate setae, basial endite with 5 (4 cuspidate and 1 plumodonticulate) setae, endopod 2-segmented, proximal segment without setae; distal segment with 4 plumose terminal setae, exopod plumose seta present.

Maxilla (Figure 2b): coxal endite not bilobed with 3 + 2 plumodonticulate setae, basial endite bilobed with 4 + 4 plumodonticulate setae, endopod unsegmented, not bilobed with two long terminal and one subterminal plumodonticulate setae, exopod (scaphognathite) with 4 plumose marginal setae and a long stout distal process.

First maxilliped (Figure 3a): coxal segment without setae, basis with 8 plumodonticulate setae arranged 2 + 2 + 2 + 2, endopod 5-segmented with 2, 2, 1, 2, 1 + 4 (1 simple subterminal and 4 plumodonticulate terminal) setae, exopod 2-segmented, distal segment with 4 long terminal plumose natatory setae.

Second maxilliped (Figure 3b): coxal segment without setae; basis with 4 plumose setae arranged 1 + 1 + 1 + 1, endopod unsegmented with 3 (1 longer plumodonticulate subterminal and 2 shorter plumose terminal) setae, exopod 2-segmented, distal segment with 4 long terminal plumose natatory setae.

Pereiopods: absent.

Pleon (Figure 4c): 5 pleomeres, the second pleomere with a pair of dorsolateral processes, the third pleomere with a pair of dorsolateral processes. Pleomeres 2–5 each with 1 pair of posterodorsal plumose setae, pleopods absent.

Telson (Figure 4c): round and triangular plate with 3 small spines at each posterolateral margin; posterior margin concave, with 3 pairs of serrulate setae arranged in a single row, innermost 2 setae approximately twice longer than outer seta, telson forks absent.

**Zoea II**

Size of CL (mean ± SD): 0.4 ± 0.2 mm.

Carapace (Figure 1b, e): eyes stalked, otherwise unchanged.

Antennule (Figure 1i): exopod with two longer and three shorter aesthetascs, otherwise unchanged.

Antenna (Figure 1j): unchanged besides size.

Mandible: unchanged besides size.

Maxillule (Figure 2c): basial endite with 7 (5 cuspidate and 2 plumodonticulate) setae, otherwise unchanged.

Maxilla (Figure 2d): scaphognathite with 9 plumose marginal setae and the long stout posterior process now much reduced in size. Otherwise unchanged.

First maxilliped (Figure 3c): exopod distal segment with 6 long terminal plumose natatory setae, otherwise unchanged.

Second maxilliped (Figure 3d): exopod distal segment with 6 long terminal plumose natatory setae, otherwise unchanged.

Pereiopods (Figure 4a): buds, elongated, incompletely segmented, chela present.

Pleon (Figure 4d, g): the first pleomere with 2 dorsomedial plumose setae. Pleomeres 2–5 with pleopod buds, otherwise unchanged.

Telson (Figure 4d): unchanged besides size.
Figure 2. *Hiplura variegata* (Rüppell, 1830), maxillule: (a) zoea I; (c) zoea II; (e) zoea III. Maxilla: (b) zoea I; (d) zoea II; (f) zoea III.
Figure 3. *Hiplyyra variegata* (Rüppell, 1830), first maxilliped: (a) zoea I; (b) zoea II; (c) zoea III. Second maxilliped: (d) zoea I; (e) zoea II; (f) zoea II; Third maxilliped: (g) zoea III.
Figure 4. *Hiplyra variegata* (Rüppell, 1830), pleopod: (a) zoea II; (b) zoea III. Pereiopod: (c) zoea II; (d) zoea III. Dorsal view of pleon: (e) zoea I; (f) zoea II; (g) zoea III. Dorsal view of telson: (h) zoea III.
**Zoea III**

Size of CL (mean ± SD): 0.5 ± 0.02 mm.

Carapace (Figures 1c, f): unchanged besides size.

Antennule (Figure 1k): exopod unsegmented with 5 (2 longer and 3 shorter) aesthetascs.

Antenna (Figure 1l): unchanged besides size.

Mandible: unchanged besides size.

Maxillule (Figure 2e): basial endite with 8 (6 cuspidate and 2 plumodenticulate) setae, otherwise unchanged.

Maxilla (Figure 2f): scaphognathite with 12 plumose marginal setae, otherwise unchanged.

First maxilliped (Figure 3e): exopod distal segment with 6 long terminal plumose natatory setae, otherwise unchanged.

Second maxilliped (Figure 3f): exopod distal segment with 6 long terminal plumose natatory setae, otherwise unchanged.

Third maxilliped (Figure 3g): arthrobranch gill bud absent, epipod present, endopod well developed and longer than exopod.

Pereiopods (Figure 4b): well developed and partly segmented.

Pleon (Figure 4e, h): the first pleomere with 5 dorsomedial plumose setae. Pleomeres 2–5 with developed pleopod buds, otherwise unchanged.

Telson (Figure 4f): round and triangular plate with 4 small spines at each posterolateral margin; otherwise unchanged.

**Discussion**

Recently, through the work of Galil (2009), the taxonomy of *Hiplyra* species has changed. *Hiplyra platycheir* is considered an exclusively eastern Asian species while, for the Indian Ocean material, a new species *H. sagitta* Galil, 2009 has been introduced. Therefore, the larval descriptions hitherto published under the name of *P. platycheir* certainly belong to two species. Only Ko (2000) and Quintana (1986a, 1986b) refer to *H. platycheir* proper, while specimens from the Gulf (Sakhaie et al. 2010) and Pakistan (Ghory and Siddiqui 2008) presumably refer to *H. sagitta* Galil, 2009. The morphological features of the first zoal stage of *H. variegata* correspond to those that define the zoeas of Leucosiidae according to Rice (1980a, 1980b) and Hirose et al. (2012).

Characteristics of zoa I of *H. variegata* reared for the present study differ somewhat from those described for *H. platycheir* (Ko 2000 [in Korea]), presumed *H. sagitta* (Sakhaie et al. 2010 [in Iran]) and *H. aff. platycheir* (presumably also *H. sagitta* Galil 2009 or something different from that) (Ghory and Siddiqui 2008 [in Pakistan]) (Table 1). One of the important characters of *H. variegata* that can be used to distinguish it from related species is the presence of a lateral spine on the carapace which is absent in other species.

There are some differences in the morphology of zoa I of *H. platycheir* reported by Ko (2000) and the presumed *H. sagitta* described by Sakhaie et al. (2010) and by Ghory and Siddiqui (2008). The number of aesthetascs of the antennule and the distal segment of the endopod of the first maxilliped are different. The specimens from the Gulf have
four aesthetasc and one seta, while in the Pakistan specimens there are two aesthetasc and two setae (three aesthetasc and one seta in *H. variegata*), also the Gulf specimens have only four setae on the endopod of the first maxilliped while five setae are present in the other specimens (Table 1). Hirose et al. (2012) mention that the types of setae in each structure (such as the maxillule, maxilla, and the first and second maxillipeds) are the main distinguishing characteristics of the four species of *Persephona* (Leach, 1817), but there are no large differences between the setae types of the four species of *Hiplyra*, except for the basial endite of the maxillule in zoea II (Tables 1, 2).

The lateral swollen protuberance of the carapace appears in zoea II of *H. platychira* (Ko 2000). Also the numbers of aesthetasc and seta of the antennule were different in the case of *H. variegata* (the present study) (6 + 1 versus 5) and also the plumose setae of the exopod (scaphognathe) maxilla have different numbers (12 versus 9) (Table 2).

There are three important differences in the morphology of the carapace, telson and pleon of zoea III (Table 2). The swollen protuberance is developed into a lateral spine in zoea III of *H. platychira* (Ko 2000), while it is absent in the same species described by Quintana (1986b) from Japan and the presumed *H. sagitta* (Ghory and Siddiqui 2008)

### Table 1. A comparison between the first stage zoeas of the genus *Hiplyra*.

| Characters       | H. variegata | H. platychira | Presumed H. sagitta | H. aff. platychira |
|------------------|--------------|---------------|---------------------|-------------------|
| Carapace         | +            | (s)           | –                   | –                 |
| Lateral spine    |               |               |                     |                   |
| Setae            | +            | +             | –                   | +                 |
| Antennule        | Aesthetasc, 3,1 | 4,1           | 4,1                 | 2,2               |
| simple seta      | Me            |               |                     |                   |
| Maxillule        | Coxa         | 4pse          | 5 pse               | 5 pse             |
| Basal            | 4csp + 1pse  | 5 csp         | 5 csp               | 3 csp + 2pse      |
| Maxilliped 1     | Endopod: 5seg. | 2, 2, 1, 2, 4 + 1 | 2, 2, 1, 2, 4 + 1 | 2, 2, 1, 2, 4 pse* |
|                 | pse + ss     | pse + ss      |                     | 2, 2, 1, 2, 5 pse |
| Maxilliped 2     | Basis        | 4pse          | 4pse                | 4pse              |
|                 | Endopod      | 2ps + 1pse    | 2ps + 1pse          | 3pse              |
| Reference        | Present study | Ko (2000)     | Sakhaie et al. (2010)| Ghory and Siddiqui (2008) |

Abbreviations: – absent; + present; (s) swelling; ps, plumose seta; pse, plumodenticulate seta; csp, cuspidate; ss, simple seta; *observation from original drawing.

### Table 2. A comparison between the zoea II and zoea III of the genus *Hiplyra*.

| Characters: Zoea II | H. variegata | H. platychira | H. variegata | H. platychira | H. aff. platychira |
|---------------------|--------------|---------------|--------------|---------------|-------------------|
| Carapace:           | +            | (s)           | –            | –             |                   |
| Lateral spine       |               |               |              |               |                   |
| Antennule           | 5 aes, 0 ss  | 6 aes, 1 ss   | 6 aes, 0 ss  | 6 aes, 1 ss   | 4 aes, 2 ss       |
| Setae               | 4 aes, 1 ss  | 4 aes, 2 ss   | 6 aes, 1 ss  | 4 aes, 2 ss   | 4 aes, 1 ss       |
| Maxillule           | 5csp + 2pse  | 6pse + 1ss    | 6csp + 2pse  | 6pse + 1ss    | 6csp + 2pse       |
| Basal               | 0, 2 + 2ps   | 0, 2 + 2ps    | 0, 2 + 2ps   | 0, 2 + 2ps    | 0, 2 + 2ps        |
| Endopod             | 9ps           | 12ps          | 12ps         | 23ps          | 23ps              |
| Maxilla:            | 12ps          | 23ps          | 23ps         | 3ps*          | 4pss              |
| Scaphognathite      | 2ps           | 1ps*          | 5ps          | 3ps*          | 4pss              |
| Pleon: pleomere I   | 3             | 3             | 4            | 3             | 3                 |
| Telson: posterior   |               |               |              |               |                   |
| lateral spins       |               |               |              |               |                   |
| Reference           | Present study | Ko (2000)     | Present study | Ko (2000)     | Quintana (1986b)  |
|                     |               |               |               |               | Ghory and Siddiqui (2008) |

Abbreviations: – absent; + present; (s), swelling; aes, aesthetasc; ps, plumose seta; pse, plumodenticulate seta; csp, cuspidate; ss, simple seta; *observation from original drawing.
from Pakistan. It is important to note that zoea III of *H. variegata* has four small spines at each posterolateral margin, in comparison with three spines in the other species. The dorsomedial setae on the first pleomere of the Pleon can be used to distinguish between the different presumed species (three in Korea and Japan, four in the presumed *H. sagitta* from Pakistan) and *H. variegata* (five in the Red Sea). Also the numbers of aesthetascs of the antennule and plumose setae of the exopod (scaphognathite) of the maxilla were different in the two species of *Hiplyra* (Table 2). These differences could also point towards the Pakistan specimens not belonging to the presumed *H. sagitta* at all, but to another unknown species.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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