Article

Morpho-taxonomic study of first zoeas of *Atergatis integerrimus* (Lamarck, 1801) and *A. floridus* (Linnaeus, 1767) (Crustacea: Decapoda: Brachyura: Xanthidae) reared in the laboratory

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Abstract
Laboratory studies were carried out on an ovigerous female of *Atergatis integerrimus* (Lamarck, 1801) and *A. floridus* (Linnaeus, 1767) collected from Buleji (Karachi, Pakistan). After 9 days larvae were hatched out as first zoeal stage at room temperature 29^{o}C-30^{o}C and water temperature 28^{o}C-29^{o}C in filtered seawater with a salinity of 35-37 parts per thousand and pH 7.5-7.9. The larvae were fed *Artemia* nauplii. A description, illustration, and comparison of its first zoeal stages with the descriptions of its congeners are provided.

Keywords Crustacea; Decapoda; brachyuran; Xanthidae; larvae.

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1 Introduction

In the coastal ecosystems, brachyuran crabs form one of the major animal groups, with 6793 varieties (Ng, Guinot and Davie, 2008). As a result of their high adaptability and ability to survive in extreme habitats, brachyuran crabs are extremely tolerant to natural or anthropogenic environmental changes (Guinot and Hurtado, 2003; Cannicci et al., 2009; Klaus and Plath, 2011; Fusi et al., 2015). The brachyuran crab is an important part of the aquatic ecosystem's trophic web, both as a primary consumer or as a secondary predator (Dye and Lasiak, 1986; Klaus and Plath, 2011; Miller and Morgan, 2015).

The Xanthidae represent the most diverse group of crab families in terms of species and genera, especially in the tropical areas. Most of them inhabit rocky shores at the littoral, where they occupy every possible niche.
Identification of species can be challenging, and a large number of synonyms have surfaced over the years (Odhner, 1925). It has not been possible to successfully divide the family into subfamilies (Rathbun, 1930). In their multigene study, Lai et al. (2011) identified numerous problems with current xanthid classification and systematics. A number of zoeal characters were incorporated into their analysis, and they found that these characters were phylogenetically significant, especially when combined with other evidence like DNA sequences and adult morphology. Nevertheless, they believe that the Xanthidae still require a radical systematic reorganization.

During the latter half of the 19th century, the taxonomy and systematics of the Xanthidae changed substantially. There have been several studies that have attempted to integrate traditional classifications of adult xanthids with groupings based on zoeal morphology and setotaxy (Wear, 1970; Rice, 1980; Martin, 1984; Martin et al., 1985; Clark and Galil, 1988 and Terada, 1990). Clark (2007) studied first stage zoeae from 48 species representing 11 subfamilies. According to his analyses, larval and adult based systematics for the Xanthidae were incompatible. The analysis of first stage zoeas did not support the existing adult-based classification of the 11 subfamilies studied. Finally, he found that on the basis of first stage zoeal antennal types, the xanthids could be divided into five groups that did not correspond to any of the adult-based classifications. All these groupings will be changed now as accepted classification of adult xanthids is reordered (Lai et al., 2011). The molecular results obtained by these authors were consistent with phylogenetic relationships implied by adult characters including sternal characters, position of genital openings and morphology of the first zoea. Many species formerly included in the family Xanthidae have since been moved to new families. Setal morphology is conservative for a number of xanthid characters (lateral and dorsal carapace spines, antennule, maxillule , maxilla, maxilliped1, mxilliped 2 with the exception of the distal endopod segment, the setation on abdominal somites 2–5 and setation of the telson margin and these generally provide little phylogenetic information Lai et al. (2011).

In the present paper the first zoeal stages of *Atergatis integerrimus* (Lamarck, 1801) and *A. floridus* (Linnaeus, 1767) are described, illustrated and compared with diagnostic larval characters of its congener's larvae given earlier. As a result, this study aims to improve our understanding of brachyuran larvae collected from planktonic samples.

2 Materials and Methods

2.1 Collection sites

An ovigerous females of *A. integerrimus* (Lamarck, 1801) and *A. floridus* (Linnaeus, 1767) were obtained from Buleji (long 66°49' 12" E, lat 24°50' 12" N). Buleji is rocky cum sandy shore and it has tide pools, at low tide provide home for a wide variety of animals (Fig. 1).

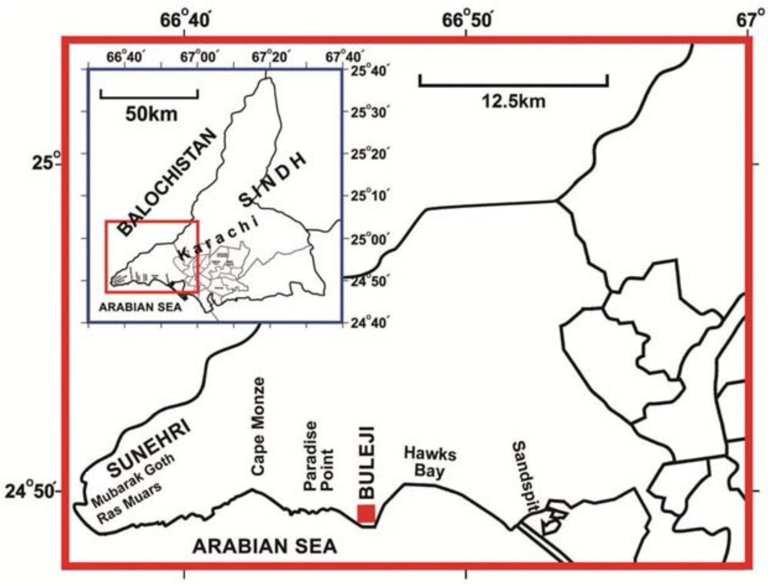

Fig. 1 Map showing sampling sites (solid circles).

2.2 Collection maintains technique in aquaculture laboratory

The ovigerous females were kept in the laboratory in filtered seawater with a salinity of 35-37‰ and pH 7.5-7.9 at room temperature 29°C-30°C and water temperature 28°C-29°C. The larvae were hatched after 9 days. Newly hatched larvae were segregated and placed, five larvae per beaker (500 ml), containing filtered seawater of salinity 35-37‰ and temperature 28°C-29°C. *Artemia* nauplii were offered as food. Each beaker was examined daily for exuviae and dead larvae. Temporary slides were made by using glycerin plus 5% formalin (3:1).

Fig. 2 Maintain newly hatched larvae.

2.3 Microscopic observations (Measurement, Identification and Analysis)

The specimens were dissected by using tungsten needle under a binocular microscope (Nikon) with 10x/21 magnifications. The illustrations were made with the help of Olympus BX51 microscope (magnifications WHN10X/22 x10, 20 and 40) with Nomarski interference contrast and camera lucida attachment. Measurements (millimeter = mm) of illustrated specimens were made by using stage micrometer. The total length (TL) was determined from the tip of the rostrum to the mid posterior border of the telson. The spent female and the remaining larvae were preserved in formalin and housed in the Marine Reference Collection and Resource Centre, University of Karachi (Fig. 2).

3 Genus *Atergatis* De Haan, 1833

Cancer (*Atergatis*) de Haan, 1833-1849 (1835): 17.

Atergatis Dana, 1852: 148, 157; Milne Edwards A., 1862: 49; Milne Edwards A., 1865: 234; Kossmann, 1877: 19; Ortmann, 1893: 460; Alcock, 1898: 94; Klunzinger, 1913: 147; Balss, 1922: 123; Odhner, 1925: 83; Ward, 1934: 13; Sakai, 1939: 441(key), 446; Barnard, 1950: 206; Buitendijk, 1960: 267; Sakai, 1965: 131; Guinot, 1967: 261; Sakai, 1976: 408; Serène, 1984: 138(keys), 144; Wada, 1995: 396(key), 397.

Key to the species of the genus *Atergatis* is as follows:

1- Anterolateral margin of carapace forming a tooth at junction with posterolateral margin. Upper margin of propodus of cheliped keeled ……………………………………………………..**2**

2a- Carapace with even surface, without indications of regions…………………***intergerrimus.***

2b- Carapace with somewhat lumpy surface; variegated with spots and confluent blotches………………………………………………………………….……***floridus.***

4 Results

4.1 Description of the larvae (Fig. 3)

Fig. 3 *Atergatis integerrimus* (Lamarck, 1818).

4.2 *Atergatis integerrimus* (Lamarck, 1818) (Fig. 3)

Zoea I (Fig. 4A - J)

Size.- TL = 1.63mm - 1.87mm

Diagnostic Features.-

Carapace (Figs. 4A,B).- Rostral, dorsal and lateral spines present; eyes sessile.

Antennule (Fig. 4C).- Uniramous with 2 terminal aesthetascs and 3 termianl setae.

Antenna (Fig. 4D).- Protopod long bearing bilateral rows of spinules; exopod with 3 small setae.

Mandible (Fig. 4E).- With well-developed incisor and molar processes.

Maxillule (Fig. 4F).- Coxal endite with 6 plumodenticulate setae; basial endite with 3 cuspidate and 3 plumodenticulate setae; endopod 2-segmented with 1,4 (2 subterminal + 4 terminal) setae, respectively.

Maxilla (Fig. 4G).- Coxal endite bilobed with 4+3 plumodenticulate setae; basial endite bilobed with 3 + 3 plumodenticulate setae; endopod bilobed with 2+4 plumodenticulate setae, respectively; exopod (scaphognathite) with 4 marginal plumose setae and 1 long stout plumose process.

Maxilliped I (Fig. 4H).- Coxa naked; basis with 10 plumodenticulate setae arranged 2,2,3,3 on medial margin; endopod 5-segmented with 3,2,1,2,5 (1 subterminal + 4 terminal) plumodenticulate setae, respectively; exopod 2-segmented, distal segment with 4 terminal plumose natatory setae.

Maxilliped II (Fig. 4I).- Coxa naked; basis with 4 plumodenticulate setae; endopod 3-segmented with 1,1,5 (3 terminal + 2 subterminal) plumodenticulate setae, respectively; exopod 2-segmented, distal segment with 4 terminal plumose natatory setae.

Abdomen (Fig. 4J).- Five somites; somite 2 with 1 pair of dorsolateral processes directed anteriorly; somite 3 with 1 pair of dorsolateral processes directed posteriorly; somites 3-5 with pointed posteriolateral angle;

somites 2-5 with 1 pair of posteriolateral setae.

Telson (Fig. 4J).- Bifurcated; each furca bearing 1 lateral spine and 1 dorsal spine; poserior margin of telson with 3 pairs of spinulate setae.

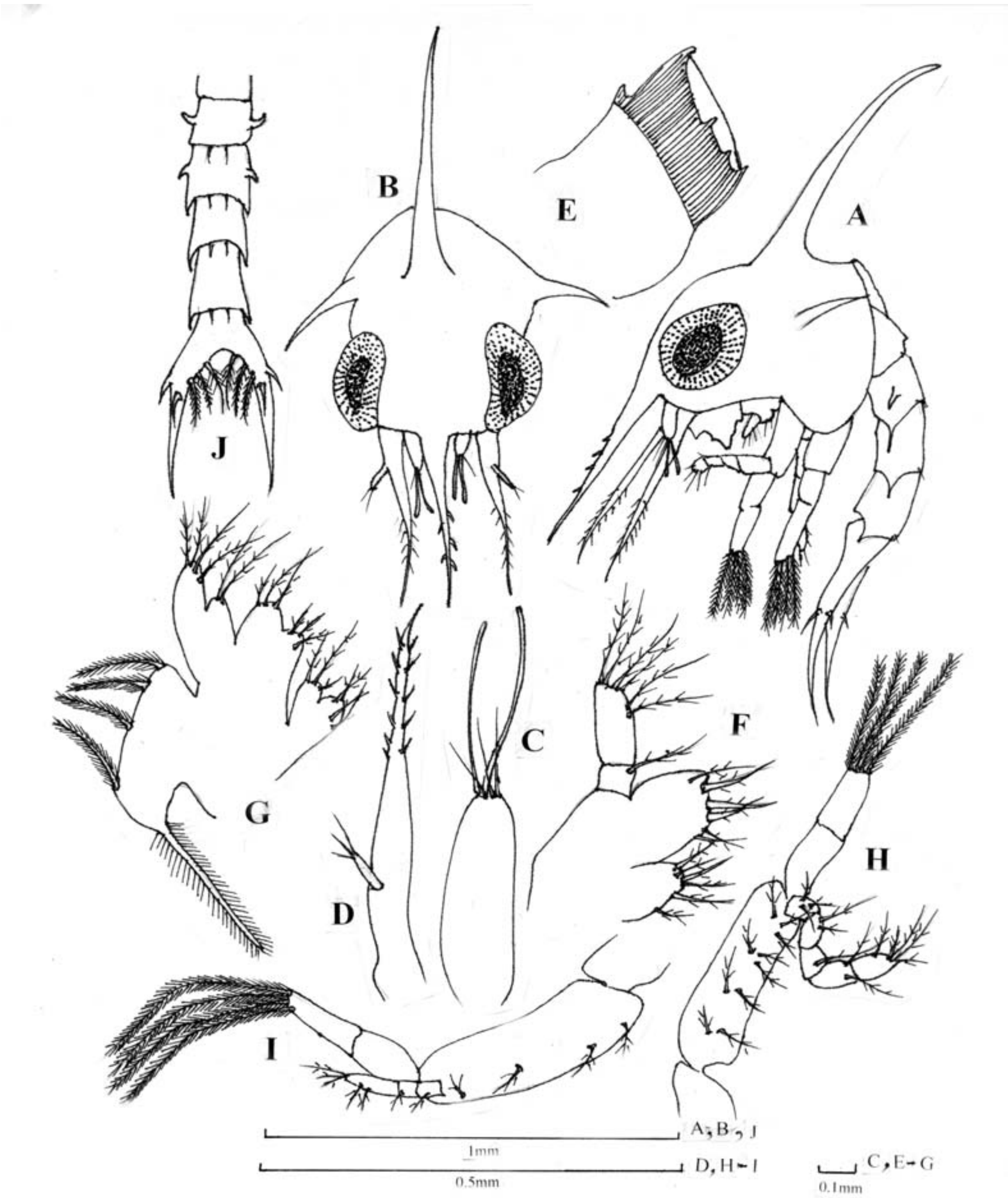

Fig. 4 *Atergatus integerrimus* (Lamarck, 1818). Zoea I: A, entire, lateral view; B, dorsofrontal view; C, antennule; D, antenna; E, mandible; F, maxillule; G, maxilla; H - I, maxillipeds I - II; J, abdomen with telson, dorsal view.

Fig. 5 *Atergatis floridus* (Linnaeus, 1767).

4.3 ***Atergatis floridus*** **(Linnaeus, 1767) (Fig. 5)**

Zoea I (Fig. 6A - I)

Size.- TL = 1.62mm -1.81mm

Diagnostic Features.-

Carapace (Figs. 6A).- Rostral, dorsal and lateral spines present; eyes sessile.

Antennule (Fig. 6B).- Uniramous with 2 terminal aesthetascs and 2 termianl setae.

Antenna (Fig. 6C).- Protopod long bearing bilateral rows of spinules; exopod with 3 small setae.

Mandible (Fig. 6D).- With well-developed incisor and molar processes.

Maxillule (Fig. 6E).-Coxal endite with 6 plumodenticulate setae; basial endite with 2 cuspidate and 3 plumodenticulate setae; endopod 2-segmented with 1,4 (2 subterminal + 4 terminal) setae, respectively.

Maxilla (Fig. 6F).- Coxal endite bilobed with 3+3 plumodenticulate setae; basial endite bilobed with 3 + 3 plumodenticulate setae; endopod bilobed with 5+4 plumodenticulate setae, respectively; exopod (scaphognathite) with 4 marginal plumose setae and 1 long stout plumose process.

Maxilliped I (Fig. 6G).- Coxa with 1 seta; basis with 9 plumodenticulate setae arranged 2,2,2,3 on medial margin; endopod 5-segmented with 3,2,1,2,5 (1 subterminal + 4 terminal) plumodenticulate setae, respectively; exopod unsegmented, with 4 terminal plumose natatory setae.

Maxilliped II (Fig. 6H).- Coxa with 1 seta; basis with 4 plumodenticulate setae; endopod 3-segmented with 0,1,4 (3 terminal + 1subterminal) plumodenticulate setae, respectively; exopod unsegmented, with 4 terminal plumose natatory setae.

Abdomen (Fig. 6I).- Five somites; somite 2 with 1 pair of dorsolateral processes directed anteriorly; somite 3 with 1 pair of dorsolateral processes directed posteriorly; somites 3-5 with pointed posteriolateral angle; somite one with single posteriolateral seta, and somites 2-5 with 1 pair of posteriolateral setae.

Telson (Fig. 6I).- Bifurcated; each furca bearing 1 lateral spine and 1dorsal spine; poserior margin of telson with 3 pairs of spinulate setae.

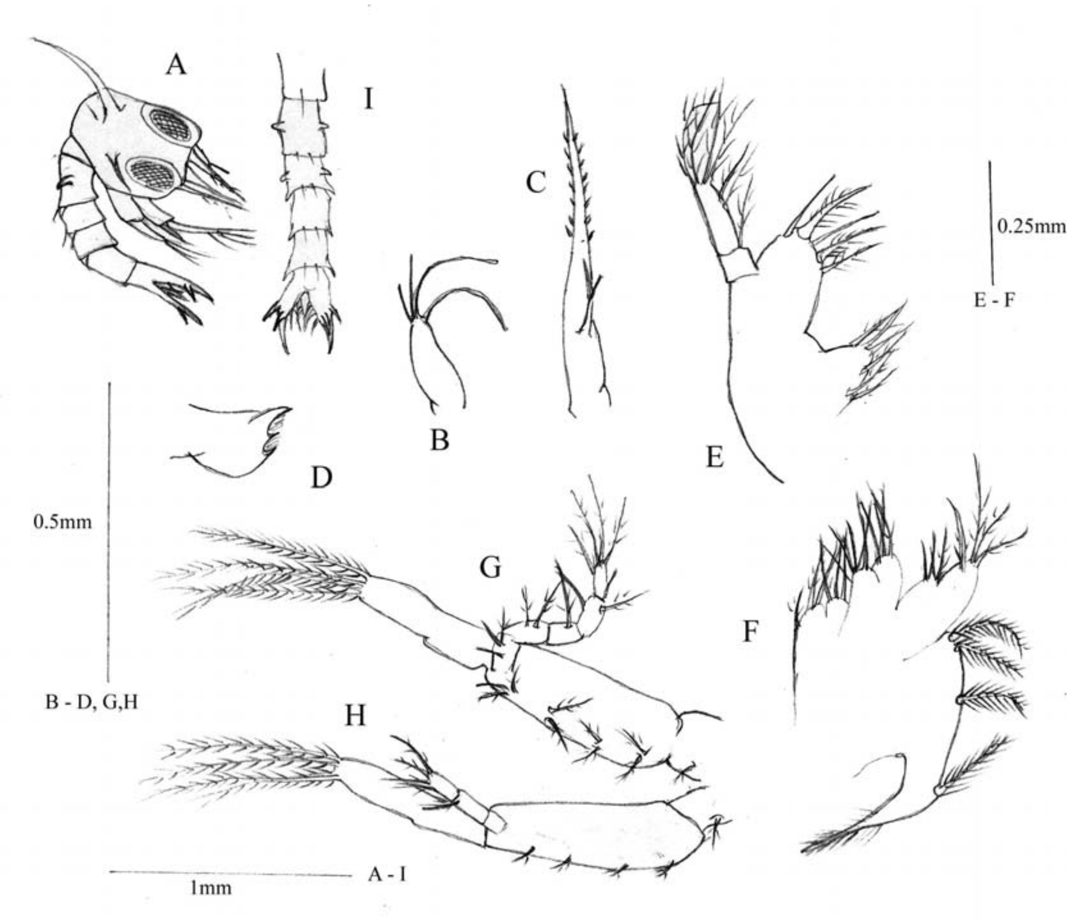

Fig. 6 ***Atergatus floridus*** **(Linnaeus, 1767)**. **Zoea I:** A, entire, lateral view; B, antennule; C, antenna; D, mandible; E, maxillule; F, maxilla; G - H, maxillipedsI - II; I, abdomen with telson, dorsal view.

5 Discussions

There is no detailed description of most of the brachyuran crab larvae, or the descriptions of early authors do not meet the modern requirements for accurate comparative studies (Clark et al., 1998). Marine biologists have been unable to identify the majority of plankton species due to a lack of reliable larval descriptions (Clark and Paula, 2003). In the past few years, a number of adequate descriptions have been made, which enable us to develop a more consistent methodology for identifying larvae in the Indian Ocean.

As far as I know, the present *Atergatis* larvae are the first ones raised from Pakistan. From previous studied world over larval descriptions of the genus *Atergatis* are known in *A. integerrimus* by James and Thirumilu (1991) and Tanaka et al. (2009, 2010) (not available) Goh (2015); *A. reticulatus* by Terada (1980); *A. floridus* by Tanaka and Konishi (2001) and Clark et al. (2004); *A. subdentatus* by Clark et al. (2004); *Atergatis floridus* is believed to have four zoeal stages (Clark and Paula, 2003). *A. floridus* from northern and western Indian Ocean can be referred *A. ocyroe* (Ng and Davie, 2007).

Table 1 An analysis of laboratory-reared zoea I of *A. integerrimus* and *A. floridus* (present study) and their congeners.

Characters	***A.integerrimus* present study**	***A. floridus* present study**	***A. reticulatus* Terada (1980)**	***A. floridus* Tanaka & Konishi (2001)**	***A. floridus* Clark et al (2004)**	***A. subdentatus* Clark et al (2004)**
Carapace:						
posteriodorsal setae	absent	present	absent	present	present	present
rostral spine	distally spinulate	distally spinulate	absent	absent	distally spinulate	distally spinulate
Antennule:	2 aesthetascs + 3 setae	2 aesthetascs + 2 setae	4 aesthetascs	4 aesthetascs	4 aesthetascs + 1 seta	4 aesthetascs + 1 seta
Antenna:						
endopod setae	absent	absent	absent	absent	present	present
exopod setae	3 setae	3 setae	3 setae	2 setae	3 setae	3 setae
Maxillule:						
coxalendite	6 setae	6 setae	7 setae	7 setae	7 setae	7 setae
basialendite	6 setae	5 setae	-	-	5 setae	5 setae
Maxilla:						
coxalendite	4+3 setae	3+3 setae	-	-	4+4 setae	4+4 setae
basialendite	3+3 setae	3+3 setae	5+4 setae	-	5+4 setae	5+4 setae
endopod	2+4 setae	5+4 setae	-	-	3+5 setae	3+5 setae
Maxilliped I:						
coxa	setae absent	1 seta	-	-	1 seta	1 seta
Maxilliped II:						
endopod	1,1,5 setae	0,1,4 setae	1,1,6 setae	-	1,1,6 setae	1,1,6 setae
Telson:						
lateral spine	1 pair	1 pair	2 pairs	-	2 pairs	2 pairs

Zoea I of *A. integerrimus* differed from previously described zoeae of the congeners by the combination of following characters: dorsal carapace spine, spinulated rostral spine nearly as long as protopod, antenna in the first zoea, and other differences seen in antenna, maxillule, maxilla, maxillipeds and telson (Table 1). It has been reported that there is some significant geographical variation in the length of zoeal carapace spines of *A. floridus* between specimens from Singapore and Japan (Shirley et al., 1987; Tanaka and Konishi, 2009). This could be due to the effect of some environmental factors.

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