

First Description of the Wild-Collected First Crabs of *Etisus* sp. (Crustacea: Brachyura: Xanthidae) from the Saudi Arabian Red Sea

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Abstract. No first crab of the genus *Etisus* has been described yet. In this study, thirty-five megalops of an *Etisus* sp. were collected from Obhur Creek, Jeddah, Saudi Arabia and reared in the laboratory up to first crab stage. These first crabs were identified using molecular barcode methods and morphologically described herein and compared with those of the two species of xanthid crabs, *Eurytium limnosum* and *Leptodius exaratus*. Apart from some minor variations, three characters differentiate the first crabs of the present study with those of the other two species including presence of several minute spines on the basal segment of the peduncle of antennule, presence of crista dentate on the endopod of third maxilliped and serrated lateral margins of third abdominal segment.

Keywords: COI gene, *Etisus*, First crab, Morphological description, Red Sea.

1. Introduction

Etisus of Xanthidae has 24 species since the publication of the worldwide checklist of Brachyura (Ng *et al.* 2008) and some more new additions have recently been made – *Etisus guinotae* Felder and Thoma 2010 (Felder and Thoma, 2010), and *Etisus evamuelleriae* Hyžný, Van Bakel and Guinot, 2014 (Hyžný *et al.* 2014). This crab's genus is found in the intertidal and sub-tidal zones of the Indo-West Pacific Sakai (1976). They dwell under the rocks or in the sponges and other sessile invertebrates, and feed on algae or detritus (Lee *et al.* 2014). In this genus, the carapace is feebly convex, both longitudinally and transversely and the frontal margin is generally feebly developed in front of the supra-orbital angles, and tips of the fingers of chelipeds are hoofed (Sakai, 1976; Serène, 1984).

Morphological descriptions of the larvae of this genus are not many. In case of *Etisus* the

zoael morphological features are known for the pre-zoea to zoea IV of *Etisus laevimanus* Randall, 1840 (Suzuki, 1978) and the first zoeas of *E. anaglyptus*, *E. electra*, *E. frontalis* (Dana 1852) and an *Etisus* sp. (Al-Haj and Al Aidaroos, 2017). In this genus morphological description for megalopal stage is available for only *E. laevimanus* (Suzuki, 1978) and *E. odhneri* (Marco-Herrero *et al.* 2021) and no such descriptions for first crabs. In the present study, the morphological features of the first crabs of an ocean-collected unknown species of this genus are described for the first time. Identification is done through COI gene sequences.

2. Materials and Methods

Meroplanktons attracted by an underwater torch light of 320 lumens were collected using a plankton net of 150 µm mesh size, at dusk on 18 March 2021 from the jetty of the Faculty of Marine Sciences, King

Abdulaziz University, Obhur, Saudi Arabia. (21°42'34.30"N 39° 5'45.44"E). Thirty-five megalops were collected. Ten megalops were kept in 2 litre containers holding 1.5 L of seawater. The following environmental factors were maintained during the culture, 40 PSU salinity, 30 °C and 12:12 h photoperiods. The larvae were fed with artemia and fish flakes (Aquafin gold fish flakes). Water exchanged every day. Within 2-10 days, 19 megalops metamorphosed into first crabs. Ten specimens of them were preserved in 70% alcohol for dissection and 9 specimens in 100% alcohol for molecular analysis. Dissections were carried out in polyvinyl lactophenol with a Leica M80 stereo microscope. Using a Leica 6000B phase contrast stereomicroscope fitted with a *camera lucida* their appendages were drawn and setae were counted. The following measurements were made for the specimens: carapace length (CL), distance between anterior and posterior margins of carapace; the greatest width of carapace (CW), distance between the first anterolateral teeth of either side; frontal length (FL), the distance across the inner orbital angles.

Isolation and sequencing of COI gene was performed based on the procedures given in Chen and Shih (2018). Three primers were used; LCO1490, HCO2198 and COL14, and the nucleotides were deposited in GenBank (accession number: OP457187.1). The sequence was blasted on GenBank nucleotides and better match was obtained to identify the species (Fig 1).

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor, 1969) and are in the units of the number of base substitutions per site. The analysis involved 12 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 588 positions in the final dataset. Evolutionary analyses were conducted in MEGA 7 (Kumar *et al.* 2016).

3. Results

Taxonomy

Family Xanthidae MacLeay, 1838

Subfamily: Etisinae Ortman, 1893

Genus *Etisus* H. Milne Edwards, 1834

Etisus sp.

(Figures 1-6)

First crab

Size: CW = 1.12 ± 0.03 mm; CL = 1.23 ± 0.1 mm; FL = 0.75 ± 0.06 .

Carapace (Fig. 1a). Slightly longer than broad; front bilobed, serrated; eyes stalked, internal orbital margin serrated, notched; anterolateral margin cut into four teeth including external orbital angle, margin spinulated, regions of carapace moderately defined.

Antennule (Fig. 1b). Peduncle 3-segmented with 14-16, 9, 9-10 setation; endopod 2-segmented with 1, 5 setae; exopod with 6 segments, proximal segment naked, second segments with 9 aesthetascs, third segment with 1 seta; fourth segments with 2 setae, fifth and sixth segments with 1 and 2 setae, respectively.

Antenna (Fig. 1c). Three-segmented peduncle with 5 (2 plumose, 3 simple), 3, 2 setation; flagellum 6-segmented with 0, 3, 4, 0, 4, 3 setation, all simple.

Mandible (Fig. 2a). Palp 3 segmented with 0, 0, 11 plumose setation.

Maxillule (Fig. 2b). Coxa with 17-18 plumose, 1 simple setae; basis with 21-24 plumose setae; endopod 2-segmented; first segment with 2 setae proximally and one seta distally; second segment with 2 setae terminally; exopod with 4 long plumose setae.

Maxilla (Fig. 2c). Coxa with 10+6-7 setae; basis with 6+11-13 plumose setae; endopod unsegmented with 6 setae (5 plumose, 1 simple); scaphognathite with 53-55 plumose setae; several simple lateral setae present.

First maxilliped (Fig. 3a). Coxa with 13-15 setae; basis with 21-23 setae; endopod with 9 setae (3 plumose, 6 simple); exopod 2-segmented with 2, 5 plumose setae on first and second segments, respectively; epipod with 15-17 long setae.

Second maxilliped (Fig. 3b). Coxa and basis undifferentiated with 2 distal setae; endopod 4-segmented with 9-11, 1, 7-8, 13 setae; exopod 2-segmented with 12-13 (10-11 plumose, 2 simple) and 6 (5 plumose, 1 simple) setae, respectively.

Third maxilliped (Fig. 4a) Coxa and basis undifferentiated with 17-19 setae; endopod 5-segmented with a crista dentate, ischium with 22-26 setae; merus with 13-15 setae; carpus with 13 setae, propodus and dactylus with 7-9 and 5 setae respectively; exopod 2-segmented, proximal segment with 7-9 and 6 (5-6 plumose, 1 simple) setation; epipod with 22-25 setae.

Pereiopods (Figs. 4b–5a-c, 6a). All segments of chelipeds and ambulatories with several setae (plumose, simple) and spines, spines several in distal two segments; chelae with several simple plumose setae and few simple setae; cutting edges of fingers with well-developed teeth, distal end hoofed.

Abdomen (6b). Seven segments with several simple, and few plumose setae; third segment winged with serrated margin, distal segment lanceolated.

The mitochondrial COI gene sequence of the present study matched with the similar sequence of a specimen collected from the Red Sea Egyptian Coast of the Red Sea (accession number: ON260936.1) (Amer *et al.*, 2022) with 99.47% accuracy (Fig. 7).

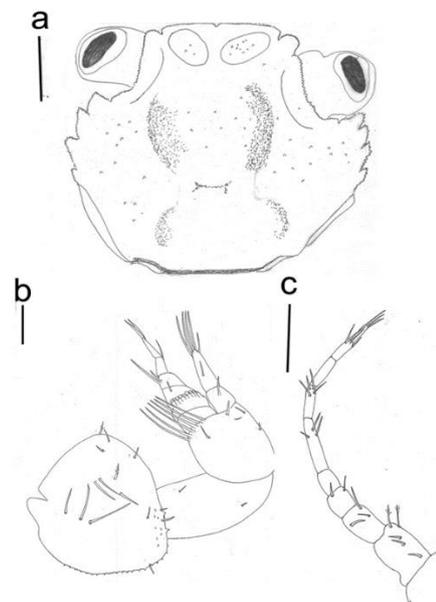


Fig. 1. First crab of *Etisus* sp. a) carapace (unit = 0.25 cm); b) antennule (unit = 0.1 cm); c. antenna (0.2 cm).

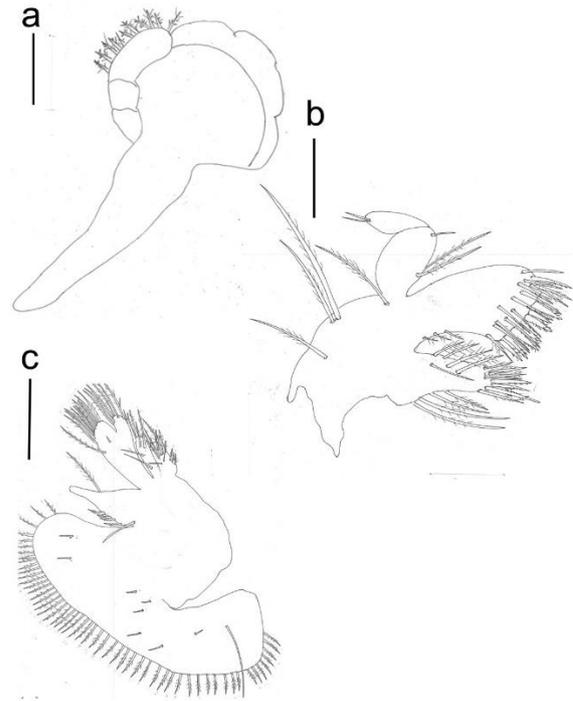


Fig. 2. First crab of *Etisus* sp. a) mandible (unit = 0.1 cm);
b) maxillule (unit = 0.1 cm); c. maxilla (0.1 cm).

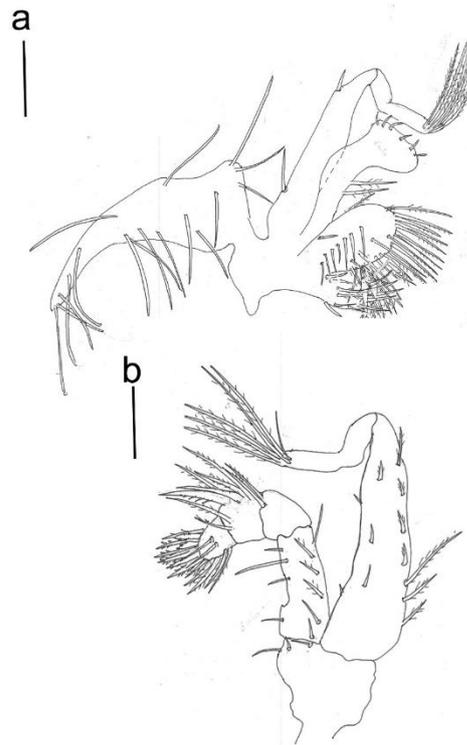


Fig. 3. First crab of *Etisus* sp. a) first maxilliped (unit = 0.2 cm); b) second maxilliped (unit = 0.1 cm).

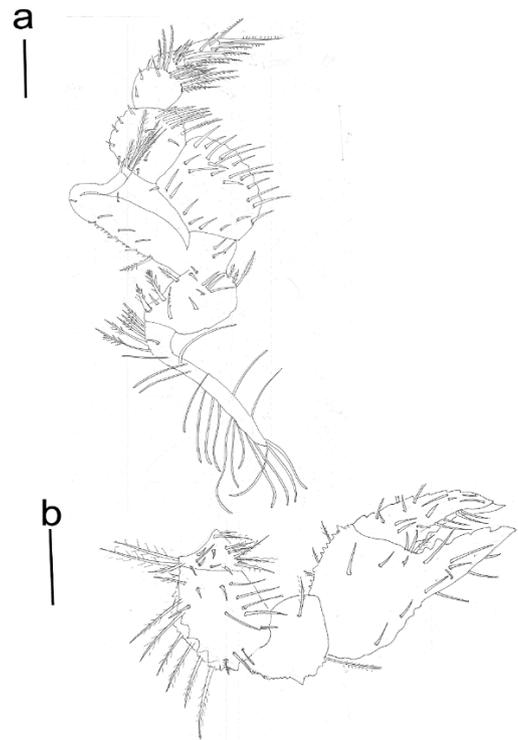


Fig. 4. First crab of *Etisus* sp. a) third maxilliped (unit = 0.2 cm); b) cheliped (unit = 0.5 cm).

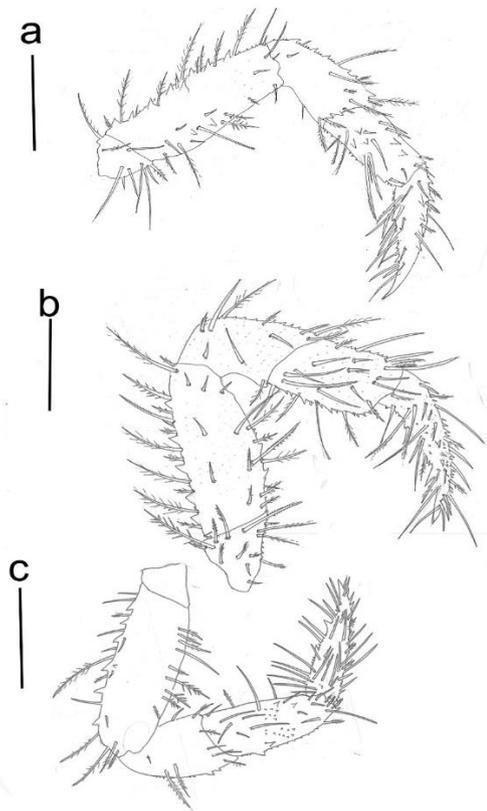


Fig. 5. First crab of *Etisus* sp. a) first ambulatory leg (unit = 0.2 cm); b) second ambulatory leg (unit = 0.2 cm); c) third ambulatory leg (unit = 0.25 cm).

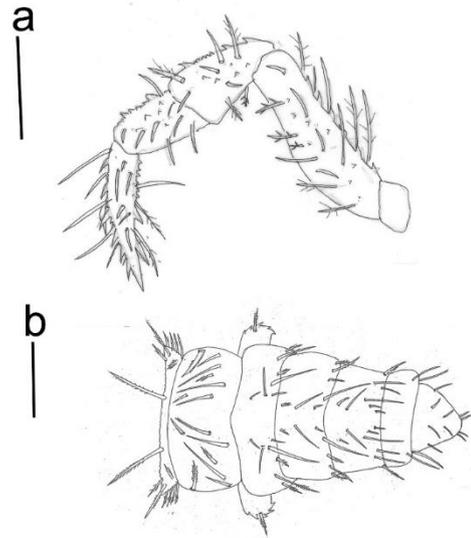


Fig. 6. First crab of *Etisus* sp. a) fourth ambulatory leg (unit = 0.2 cm); b) abdomen (unit = 0.2 cm).

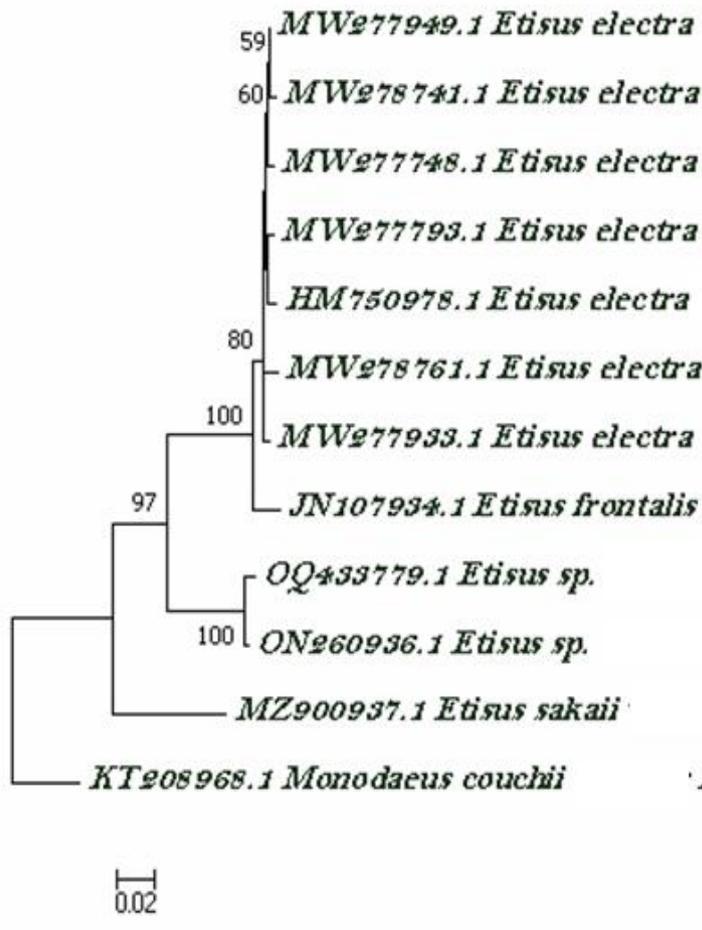


Fig. 7. The phylogenetic tree (based on mt COI) showing the evolutionary relationship in NCBI database between different species *Etisus*.

Table 1. The morphological comparison of the first crab of *Etisus* sp. (present study) with other xanthoid species.

Species/Characters		<i>Etisus</i> sp.	<i>Eurythium limosum</i>	<i>Leptodius exaratus</i>	
References		<i>Present study</i>	Guimaraes and Fransozo (2005)	Lwin et al. (2007)	
Antennule	Peduncle	3 SEG (14-16),2,9-10S)	3 SEG (15,3,9S)	3 SEG (14-16,3,13S)	
	Endopod	2 SEG (1,5S)	2 SEG (0,3S)	2SEG (0,7S)	
	Exopod	6 SEG (0,9A, 1S,2S,1S, 2S)	5 SEG (6AE,5AE+S, 4AE+S,1S,1S)	5SEG(6A,5A+1S,4A,4A,3 S)	
Antenna	Peduncle	3 SEG (5, 3, 2S)	3 SEG (4S,3S,1S)	3SEG (4,2,2S)	
	Flagellum	6 SEG (0,3,4,0,4,3S)	7 SEG (0,3,0,4,0,4,3S)	7 SEG (0,2,0,5,0,3,3S)	
Mandible	Palp	3 SEG (0,0.11 S)	2 SEG (0, 8S)		
Maxillule	Coxa	17-18S	14S	13S	
	Basis	21-24S	22S	20S	
	Endopod	2 SEG (3,2S)	UNSEG (3)	2SEG (5,5S)	
Maxilla	Coxa	10+6-7S	4+6S	8+7S	
	Basis	6+11-13S	7+9S	6+8S	
	Sch	53-55S	49-56S	50-56S	
	Ls	Several	9-12S	7-9S	
First Maxilliped	Coxa	13-15S	11S	19S	
	Basis	21-23S	25S	32S	
	Endopod	9S	UNSEG (8 OR 9S)	12S	
	Exopod	2 SEG (2,5S)	2SEG(2,6S)	2SEG(0-2,6)	
	Epipod	15-17S	15S	18-21S	
	Second Maxilliped	Coxa & Basis	3	1S	ND
		Endopod	4 SEG (9-11,1,7-8,13S)	4SEG(9,1,6,10S)	4SEG (12,2,10,10S)
Exopod		2 SEG (12,6S)	2SEG(10,5S)	2SEG (9,6S)	
Third Maxilliped	Coxa & Basis	17-19S	NUM S	NUM S	
	Endopod	5 SEG (22-26,13-15,13,7-9,5S)	5SEG(19,21,11,10,6S)	5 SEG (20-31,10-12,14,11,9S)	
	Exopod	2 SEG (7-9,6S)	2SEG(5,6S)	2 SEG (8,6S)	
	Epipod	22-25S	28S	27S	

Abbreviations: A= aesthetascs; LS= lateral setae; ND= no data; NUM= numerous; S= seta; SCH= scaphgnathite; SEG= segment

4. Discussion

The morphological identification of brachyuran megalops and first crabs from plankton samples is nearly impossible at genus or species level, for their morphological features cannot be matched with the adult forms (Marco-Herrero *et al.* 2015). Nevertheless, these larval groups can be identified by DNA barcodes (Pardo *et al.* 2009; Brandao *et al.* 2016; Marco-Herrero, *et al.* 2014; Spiridonov, *et al.* 2014), if their COI or 16S rRNA gene

sequences are available in Genbank or BOLD (Marco-Herrero *et al.* 2021).

This is the first description of the first crab of *Etisus* spp., therefore intrageneric morphological comparison is not possible, and therefore this species is compared with those of the first crabs of two xanthoid species, *Eurythium limosum* and *Leptodius exaratus* (Lwin *et al.* 2007; Guimaraes and Negreiros-Fransozo, 2005). Several similarities are found between the first crabs of this species and the other two xanthoidean crabs (Table 1): setations

of the first and distal segments of the flagellum of antenna (0 and 3 setae, respectively), setation of the first segment of the palp of mandible (0), presence of lateral setae on the scapagnathite of maxilla and the setation of the distal segment of the exopod of third maxilliped (6).

The noteworthy difference between this species and the other two xanthoidean crabs are: the number of segments of the exopod of antennule (6 vs 5) and number of the segments

of the flagellum of antenna (6 vs 7). In the basal segment of the peduncle of the present species has several minute spines and such spines are absent in the other two species (Fig. 1b). The 5-segmented endopod of maxilliped has crista dentate and this is absent in the other two xanthoidean species. The lateral wings of the third abdominal segment have serrated lateral margins, whereas in the other two species this margin is smooth. The later three characters may found only in the first crabs of *Etisus* sp. But more first crabs from this genus should be studied to validate the taxonomic implications of these characters.

5. Conclusion

No morphological descriptions are available for the first crabs of the genus *Etisus*. In the present study plankton-based first crab from this genus is morphologically described for the first time. Three characters are found to be unique for the first crabs of this genus: presence of several minute spines on the basal segment of the peduncle of antennule, presence of crista dentate in the endopod of third maxilliped and serrated lateral margins of third abdominal segment. The other species of the first crabs of this genus should be studied to validate the taxonomic implications of these characters.

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الوصف الأول للسلطعون الأول من جنس *Etisus* sp. (القشريات: السرطانات البحرية-
زانثيدى) والذي تم جمعه من ساحل البحر الأحمر السعودي

ندى ناقور، وأناند جيو كومار *

قسم الأحياء البحرية، كلية علوم البحار، ص ب ٨٠٢٠٧ جدة، جامعة الملك عبد العزيز،

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المستخلص. لم يتم حتى الآن وصف السلطعون الأول من جنس *Etisus*. في هذه الدراسة، تم جمع خمسة وثلاثين *dvrn* مجالوب من نوع *Etisus* sp. من خور أبجر، جدة، المملكة العربية السعودية وتم تربيتها في المختبر حتى مرحلة السلطعون الأول. تم تعريف السرطانات الأولى باستخدام طرق الباركود الجزيئية وتم وصفها شكليا هنا ومقارنتها مع نوعين من سرطانات الزانثيد، *Eurytium* و *limnosum*. بصرف النظر عن بعض الاختلافات الطفيفة، هناك ثلاث سمات تفرق بين السرطانات الأولى في الدراسة الحالية وتلك الخاصة بالنوعين الآخرين، بما في ذلك وجود عدة أشواك دقيقة على الجزء القاعدي من سويقة الهوائي، ووجود كريستا مسننة على نهاية الأرجل الداخلية للفك العلوي الثالث. وكذلك الحواف الجانبية المسننة للعقلة البطنية الثالثة. الكلمات المفتاحية: جين COI، *Etisus*، السلطعون الأول، الوصف الظاهري، البحر الأحمر.