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Rapid Communication

A record of *Arcania brevifrons* Chen, 1989 (Crustacea; Decapoda; Leucosiidae) from the Mediterranean coast of Israel

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Abstract

Two adult specimens of *Arcania brevifrons*, a leucosiid crab native to the Indo-West Pacific Ocean, were recently collected off the southern Israeli coast, at the southeastern Mediterranean Sea. Molecular analyses based on the mitochondrial barcoding gene cytochrome oxidase sub unit I (COI) revealed two closely related haplotypes and suggest the species has entered the Mediterranean on at least two separate occasions. This is the fourth Erythraean leucosiid species recorded in the Mediterranean Sea. The presence of an ovigerous female may indicate the existence of an established population.

Key words: Erythraean invasion, Mediterranean, Suez Canal, Red Sea, non-indigenous species, barcoding

Introduction

The Levantine shelf biota has an increasing component of Erythraean aliens (Galil et al. 2016b). Of the 41 non-indigenous decapod species recorded from the Israeli coast, all but two, the vessel-transported Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 (Portunidae) and the Atlantic brown shrimp *Penaeus aztecus* Ives, 1891 (Penaeidae) have been introduced through the Suez Canal. Three Erythraean leucosiid crabs have established populations in the Mediterranean Sea: *Myra subgranulata* Kossmann, 1877, first recorded in 1929 off Jaffa (Monod 1930), *Ixa monodi* Holthuis and Gottlieb, 1956 in 1955 off Mersin, Turkey (Holthuis and Gottlieb 1956), *Coleusia signata* (Paul'son, 1875) in 1953 Tel Aviv, Israel (Holthuis and Gottlieb 1958) – all three species have since spread across the Levant – from Egypt to Greece (http://www.ciesm.org/atlas/appendix2.html, viewed 21 July 2017).

Five species of the genus *Arcania* Leach, 1817 have been recorded in the Red Sea or in nearby waters: *A. brevifrons* Chen, 1989, *A. erinacea* (Fabricius, 1787), *A. gracilis* Henderson, 1893, *A. septemspinosa* (Fabricius, 1787) and *A. tuberculata* Bell, 1855 (Galil 2001; Naderloo and Sari 2005; Galil et al. 2012).

Here, we report the occurrence of a previously unrecorded *Arcania* species off the Mediterranean coast of Israel. Its colour pattern and shape of the vulvae identify it as *A. brevifrons* Chen, 1989, previously recorded from the Red Sea (Galil 2001). Mitochondrial cytochrome oxidase sub unit I (COI) were compared with leucosiid sequences in BoLD system and confirm the species' placement in *Arcania*. The new find is the fourth introduced leucosiid species recorded in the Mediterranean Sea.

Material and methods

Sampling was carried out off Ashdod, Israel by a commercial 240 hp bottom trawler (net opening 12 m wide, 1.2 m high, mesh at cod-end 42 mm, net equipped with two sweeplines for 70 m effective width). Trawling was conducted on 11 December, 2016, at 60 m depth, on silt and clay sediment.

The specimens were transported to the National Institute of Oceanography, Haifa, photographed, measured and tissue samples removed for DNA extractions. The Israeli specimens were photographed using Nikon SMZ1000 stereomicroscope with DeltaPix camera. MNHN specimens were photographed using Canon EOS 60D, with a 50 mm objective and Kenko extension tubes (20 and 36 mm). The specimens, preserved in 70% EtOH, are deposited in the Crustacea collection at the Steinhardt Museum of Natural History, Tel Aviv University, Israel (SMNH).

Molecular analysis

DNA extraction

Total genomic DNA was extracted from ethanol-preserved leg muscles, using the QIAamp® DNA Micro Kit (QIAGEN), following the manufacturer's instructions.

PCR amplification

For each amplification, 2 µl of diluted DNA (1:10) from each specimen were added to a PCR reaction mixture in a total solution volume of 50 µl that consisted of VWR® Taq DNA Polymerase 2X Master Mix and 0.1 µM of forward and reveres primers, LCO1490f (5'-GGTCAACAAATCATAAAGATAT TGG-3') and HCO2198r (5'-TAAACTTCAGGGTG ACCAAAAAATCA-3'), following Folmer et al. (1994). The thermocycle profile consisted of 94 °C for 2 minutes, 35 cycles of 94 °C for 30 s, 52 °C for 40 s, and 72 °C for 1 minute, with a final extension at 72 °C for 10 minutes (Ivanova et al. 2007). The PCR products were screened on 1.5% agarose gel. The same PCR primers were then used for direct sequencing of the PCR products (Macrogen Inc., South Korea).

Sequence analyses

Forward and reverse sequences of the PCR products were aligned and corrected using DNA baser 4.12.0 (DNA Baser Sequence Assembler v4 [2013], Heracle BioSoft, http://www.DnaBaser.com) and BioEdit (Hall 1999). The corrected COI sequences were compared to the BoLD (Barcode of Life Data Systems) identification system (http://www.boldsystems.org/views/idrequest.php) and to the NCBI data base (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Molecular analysis

The two COI sequences from Israel present two different but closely related haplotypes (differing in 9/597 nucleotides; 98.49% identity), indicating that both specimens belong to a single taxon and that more than a single founder was introduced to the Mediterranean Sea. Sequences were uploaded to http://www.boldsystems.org with BoLD ID: BIM 527-17 and BIM 526-17 for our specimen no AP-043 and AP-042, respectively, and to the NCBI (accession numbers: MF488953 for AP-042 and MF488954 for AP-043). This is the first BoLD record for *A. brevifrons*. Comparison of the two Israeli specimens to the most similar sequences in the BoLD database revealed 96.31–95.41%, 90.17–89.14% and 89.54–88.51% to COI sequences of a single "private" voucher (details unavailable) and to two published sequences of *Arcania* sp. (both from Mozambique, MAINBAZA cruise vouchers MNHN-IU-2008-12673 (= MNHN-B31784) and MNHN-IU-2008-12677 (= MNHN-B31788), respectively, Muséum national d'Histoire Naturelle, Paris), support the placement of the Mediterranean specimens in the genus *Arcania* as presently defined (Galil 2001). Due to various logistical reasons we were unable to sequence the tissues of additional specimens of *A. brevifrons* we used for our morphological study. Nevertheless, we felt it was useful to have the present Mediterranean material barcoded for the record and possible future comparisons.

Systematics

LEUCOSIOIDEA Samouelle, 1819
Leucosiidae Samouelle, 1819
Arcania Leach, 1817
Arcania brevifrons Chen, 1989
(Figures 1–3)

Arcania brevifrons Chen, 1989: 204, figs 31f, 32e–f, pl. 5, fig. 6; Galil 2001: 172, fig. 1A, 4A; Kumar et al. 2013: 543.

Material examined: Israel. Off Ashdod, 31°49.81′N; 34°30.296′E to 31°46.03′N; 34°27.592′E, 60 m, 11 December 2016, 1 female ovigerous cl 30.0 mm (SMNH AR29689); same data, 1 female cl 27.7 mm (SMNH AR29707).

Comparative material: Philippines. MUSORSTOM 3 stn 141, 11°44.6′N; 122°45.35′E, 40–44 m, 06 June 1985, det. H. Chen, 1 male cl 20 mm, badly damaged, holotype [MNHN-IU-2008-10961 (= MNHN-B18079)]; 1 male immature cl 10 mm, 1 female immature cl 11 mm, paratypes [MNHN-IU-2008-10961 (= MNHN-B18080)] same data as holotype.

Madagascar. CREVETTIERE 1973 NW coast, 12°49.5′S; 48°30.0′E, 55 m, 02 August 1973, coll. A. Crosnier, det. H. Chen, 1 female cl 28.6 mm [MNHN-IU-2014-11970 (= MNHN-B18472)].

Fiji. MUSORSTOM 10, stn CP 1323, 17°16.1′S; 177°45.7′E, 143–173 m, 07 August 1998, coll. Bouchet, Richer, 3 females cl 28.6, 25.1, 17.9 mm MNHN-IU-2016-10932 (= MNHN-B27434). BORDAU 1 stn CP

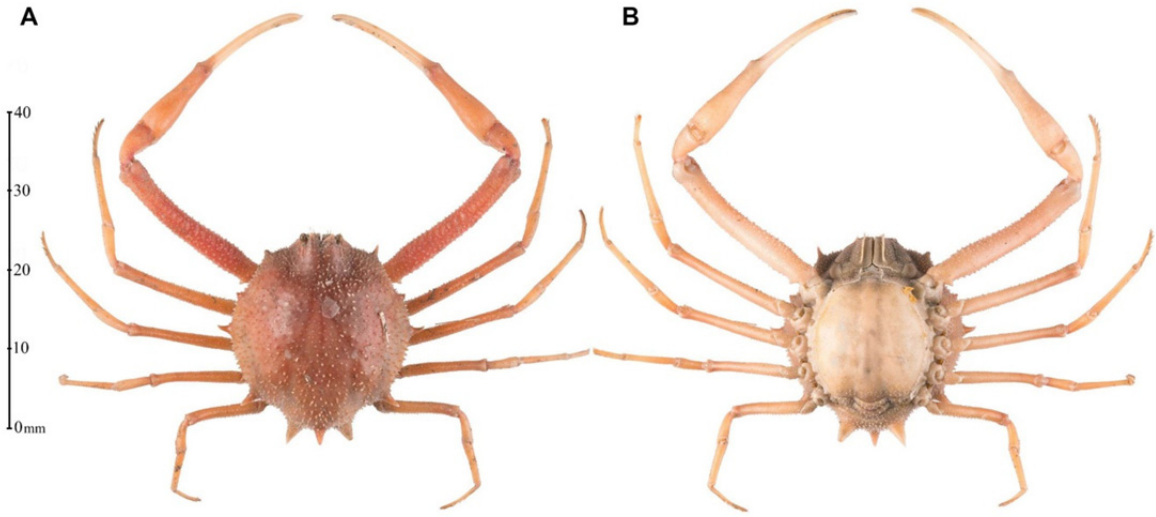

Figure 1. *Arcania brevifrons* Chen, 1989. SMNH AR29689, ovigerous female, cl 30.0 mm, Ashdod, Israel. **A**. habitus, dorsal view, **B**. habitus, ventral view. Photographed by G. Paz.

Figure 2. *Arcania brevifrons* Chen, 1989. SMNH AR29689, ovigerous female, cl 30.0 mm, Ashdod, Israel. Cephalothorax frontal view. Photographed by N. Mollaret.

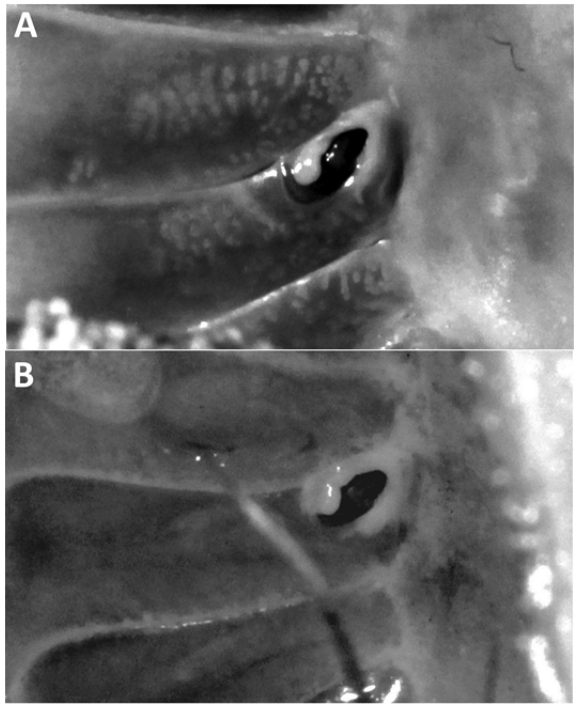

Figure 3. *Arcania brevifrons* Chen, 1989. Right vulva. **A**. SMNH AR29689, ovigerous female, cl 30.0 mm, Ashdod, Israel. **B**. MNHN-IU-2014-11970 (= MNHN-B18472), female, cl 28.6 mm, NW coast, Madagascar. Photographed by N. Mollaret.

1437, 17°11′S; 178°46′W, 160–177 m, 02 March 1999, coll. P. Bouchet et al., 1 female cl 26.4 mm MNHN-IU-2016-10933 (= MNHN B27435).

Description: Carapace globose, nearly rounded in adult female (Figure 1A, B), dorsal surface densely, uniformly covered with columnar granules. Front divided into 2 triangular lobes by wide triangular gap, lobes squat, minutely granulate. Margins of carapace with 11 spines: one spine each on subhepatic, anterolateral, midlateral, posterolateral and posterior margins, single spine on intestinal region. Anterolateral spines smallest; midlateral, posterolateral, intestinal spines

upcurved, granulate; posterior spines dorsoventrally flattened, granulate; distal end of intestinal spines not reaching those of posterior spines. Branchiogastric grooves indistinct, intestinal region moderately inflated.

Antennular fossae oblique, basal antennular segment with acutely triangular lobe laterally. Mesial end of infraorbital margin forming elongate triangular spine. Anterolateral margin of buccal cavern with 2 sharp spines. Eyes fitting within orbit, only corneal part exposed. Mxp3 covered with conical granules, save for the mesial margin of ischium, merus with prominent conical granules anterolaterally; ischium more than twice as long as merus; ischium with longitudinal row of setae. Exopod narrowed at level of proximal half of merus, gap between narrowed part of exopod, merus thus fitting triangular spine of anterolateral margin of buccal cavern (Figure 2).

Thoracic sternites covered with rounded granules; granulation about equal to pterygostomial region. Vulva on somite 6, on mesial end of thoracic suture 5/6; Vulva tadpole shaped, wider mesially, sternal cover on sublateral part of anterior margin (Figure 3A). Abdomen with basal somites granulate, shield-like fused somites smooth, laciniate telson minutely granulate.

Cheliped long, slender, subequal. Cheliped merus nearly as long as carapace, thickly set with conical granules on upper surface, minute granules on lower surface, with tubercle proximally on posterior margin. Carpus, palm with minute granules on upper surface, granules smaller, sparser on lower surfaces. Fingers slender, longer than palm, their cutting margins unevenly denticulate throughout. Ambulatory legs slender, with minute conical granules throughout, sparser on lower margins, granulation most prominent on posteriormost legs. Dactylus keeled on mesial, lateral margins, setose.

Colour in life: Carapace dorsally dark pink with irregular network of dark reddish lines medially on patterned with reddish lines, abdomen bone coloured; cheliped merus red-orange, fingers pale, ambulatory legs pale orange (Figure 1).

Remarks: Unfortunately, the holotype of *A. brevifrons* preserved in the MNHN is in poor condition and the paratypes are immature individuals with carapace length one third as long as that of the adult female collected off the Israeli coast. However, comparison with specimens collected in Fiji and Madagascar revealed close similarity, including in the characteristic shape of the vulvae, an essential diagnostic feature, with a taxonomic value comparable to that of the gonopods (Figure 3A, B). *Arcania brevifrons* is morphologically similar to *A. tropicalis* Naruse, 2014, but can be easily distinguished from the latter in its G1 distally bent at right angle (sinuously curved in *A. tropicalis*), the shape of the vulvae, and the colour pattern (cf. Naruse 2014: fig. 18a).

Distribution: Originally described from the Philippines (Chen 1989), it has been recorded in the Red Sea, Seychelles, Madagascar, Mozambique Channel, Pakistan, India, Indonesia, Fiji (Galil 2001, Kumar et al. 2013). Newly recorded in the Mediterranean Sea.

Discussion

The Suez Canal is the most significant introduction pathway into the Mediterranean Sea (Galil et al. 2016b, 2017). The Canal's typical cross-sectional area that was 3600 m² in 2000, is at present 5200 m². The last expansion increased its depth to 24 m to allow passage of vessels up to draught of 66 ft, and the Suez Canal Authority is already conducting feasibility studies with the aim to increase its depth in order to allow passage of vessels with draught to 72 ft (http://www.suezcanal.gov.eg, viewed June 3, 2017). The implications of a deeper, wider canal on transport of Erythraean biota through the Canal are obvious: "a steeply increasing invasion of Red Sea animals into the Mediterranean can be expected" (Thorson 1971: 846). Although no concerted effort was undertaken to study the Erythraean bionvasion, seven non-indigenous decapod species have been newly recorded between 2008 and 2015 along the Israeli coastline, all but one introduced through the Suez Canal (De Grave et al. 2012; Karhan et al. 2013; Galil and Mendelson 2013; Rothman et al. 2013; Levitt et al. 2014; Gönülal et al. 2016; Galil et al. 2016a).

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