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Neostygarctus lovedeluxe n. sp. from the Miyako Islands, Japan: The First Record of Neostygarctidae (Heterotardigrada: Arthrotardigrada) from the Pacific

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A new species of the previously monospecific marine tardigrade family Neostygarctidae is described. *Neostygarctus lovedeluxe* n. sp. was found from a submarine cave in Miyako Islands, Japan. This is the first record of Neostygarctidae from the Pacific. The new species is easily distinguished from the previously known *N. acanthophorus* by its number of dorsal spines, as *N. lovedeluxe* has two spines each on the three dorsal body plates in contrast to one in *N. acanthophorus*. Furthermore the morphology of the two clawed juvenile is reported for the first time in Neostygarctidae, providing new insights into the common sequence in some ontogenic traits.

Key words: Miyako Islands, submarine cave, taxonomy, phylogeny, Tardigrada, meiobenthos

INTRODUCTION

Marine tardigrades are members of the marine meiobenthos inhabiting various types of sediments. Most of these marine species are grouped into Arthrotardigrada, an order considered to be the basal group of the class Heterotardigrada (Kristensen and Higgins, 1984). Among the Arthrotardigrada, the monospecific family Neostygarctidae has attracted tardigradologists' attention for its unique morphology, and its phylogenetic position has been discussed frequently (Moreno de Lucia et al., 1984; Kristensen and Higgins, 1984; Grimaldi de Zio et al., 1987a; Bello and De Zio Grimaldi, 1998).

Neostygarctus acanthophorus Grimaldi de Zio, D'Addabbo Gallo and Morone De Lucia, 1982, the only previously described Neostygarctidae species, has been reported from the Mediterranean Sea and the Indian Ocean (Grimaldi de Zio et al., 1982a; Grimaldi de Zio et al., 1990; De Zio Grimaldi and Gallo D'Addabbo, 2001; Gallo et al., 2007). In the present study, we describe the second species of the Neostygarctidae from a submarine cave in the Miyako Islands of the Ryukyu Arc, which is the first record of this family from the Pacific Ocean. The common sequence of some ontogenic traits is also discussed. The present study is also the first investigation of submarine cave tardigrades in the Northwestern Pacific, previously only recorded in Mediterranean (Grimaldi de Zio et al., 1982b; Villora-Moreno, 1996; Sandulli et al., 1999; Gallo D'Addabbo et al., 2001) and Australian waters (Boesgaard and Kristensen, 2001).

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MATERIALS AND METHODS

Sediment samples were collected from the entrance of the submarine cave (Twin Cave) off Shimoji Island, in the Miyako Islands by SCUBA diving (Fig. 1). The surface of the collection site was covered with broken pieces of corals (> 20 mm) and beneath it was a layer of sand. The sediment was carefully collected using a transplanting trowel. The collected sample was treated by the freshwater shock technique (Kristensen, 1983), and the supernatant was decanted through a 32-µm mesh net. The sample was treated with a graded ethanol series from 20% and preserved in 70%. Tardigrades were then sorted under a stereomicroscope. After a detailed observation on the bucco-pharyngeal apparatus, the tardigrade specimens were mounted in Hoyer's medium for permanent slide preparation. All observations were performed using a differential interference contrast microscope (Nikon ECLIPSE E800). The type series is deposited in the Seto Marine Biological Laboratory (SMBL), Kyoto University, Shirahama, Wakayama, Japan.

RESULTS

Systematics

Order **ARTHROTARDIGRADA** Marcus, 1927

Family **Neostygarctidae** Grimaldi de Zio, D'Addabbo Gallo, De Lucia Morone, 1987

Diagnosis (emended). Arthrotardigrades having five dorsal plates each with dorsal and marginal spines; cephalic region subdivided into six lobes, anterior pair bears internal cirri, stout median cirrus near base of anterior pair, anterolateral pair bears external cirri and secondary clavae, lateral pair bears lateral cirri and primary clavae; three body plates with two conical lateral processes; caudal plate with two lateral swollen lobes; ventral plates reduced to folds; complete set of cephalic cirri; primary clavae slightly elongated; secondary clavae globular and thick-walled; cirrus E with accordion-plated articulation; legs with four tubular claws terminating in triangular solid hooks, two medial ones with dorsal bristle; stylet supports absent; seminal receptacles as cuticular pouches with long sinuous ducts opening close to female gonopore.

Genus ***Neostygarctus*** Grimaldi de Zio, D'Addabbo Gallo, Morone De Lucia, 1982

Diagnosis. As the family.

Neostygarctus lovedeluxe **n. sp.**

(Figs. 2–4; Table 1)

Diagnosis. *Neostygarctus* with one medial dorsal spine on cephalic and caudal plates; two dorsal spines on each of three body plates.

Type material. All the specimens were collected at the entrance of a submarine cave (Twin cave), off Shimoji Island, Okinawa, Japan (24°48′11.7″N, 125°9′0.4″E; 15 m in depth) on 28 June 2012. Holotype: SMBL Type 456, adult female. Paratypes: SMBL Type 457, four-clawed juvenile, sex undetermined; SMBL Type 458, two-clawed juvenile, sex undetermined.

Etymology. The specific epithet, *lovedeluxe*, refers to 'Love Deluxe', the name of a supernatural power enabling one to have complete control over one's hair, which appeared in *JoJo's Bizarre Adventure Part 4: Diamond Is Unbreakable*, a famous Japanese manga written and illustrated by Hirohiko Araki. The hairy appearance of the new species appears as if affected by the power of 'Love Deluxe'.

Description of holotype. Body 130 μm in length, with five segments (Figs. 2, 3); each segment having dorsal plate with dorsal and marginal spines; surface of dorsal plates covered with numerous minute spikes; ventral surface smooth, with segmental folds.

Cephalic region subdivided into three pairs of lobes: anterior pair protruding, basally bearing internal cirri (24 μm long); anterolateral pair less-protruding, bearing external cirri (39 μm long) with base and globular thick-walled secondary clavae (7 μm wide × 5 μm long); lateral pair strongly protruding, bearing lateral cirri (38 μm long) and primary clavae (4 μm long) on long, robust cirrophorus (14 μm long). Median cirrus (37 μm long) with cirrophorus directed dorsally inserted anteriorly on cephalic plate. Robust mid dorsal spine (47 μm long) on medial lobe, positioned posteriorly on cephalic plate. On caudal edge of plate, pair of roundish lateral lobes with spines present. Eyes absent.

Mouth cone anteroventrally protruded on ventral side of cephalic segment. Bucco-pharyngeal apparatus (Fig. 4A) consisting of buccal tube, three placoids, pharyngeal bulb, and pair of stylets with furca. Anterior portion of bucco-pharyngeal apparatus not suitable for observation. Stylet supports absent.

Three body segments, each with pair of dorsal spines and pair of conical lateral processes. Pair of dorsal spines (first 17 μm long,

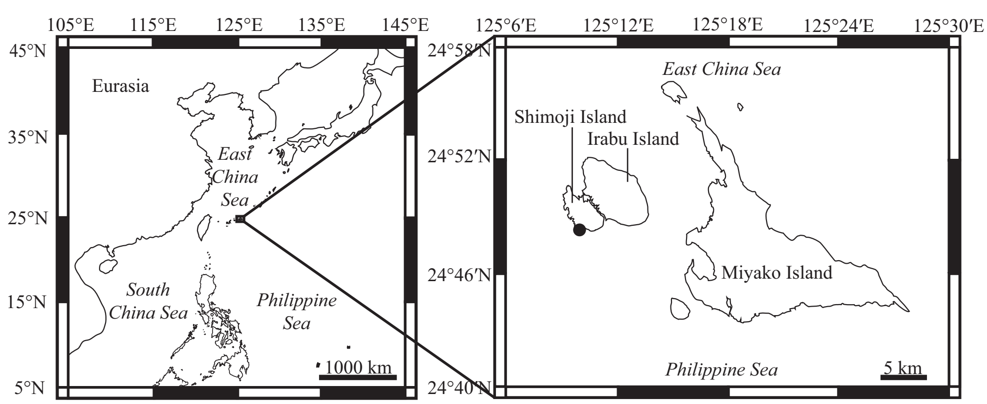

Fig. 1. Map of the sampling locality (solid circle).

Table 1. Morphometrics of the type series (in μm). Dashes (–) indicate unmeasured traits.

	HOLOTYPE	PARATYPE	PARATYPE
Slide no.	SMBL Type 456	SMBL Type 457	SMBL Type 458
Status	ADULT FEMALE	FOUR-CLAWED JUVENILE	TWO-CLAWED JUVENILE
Body length	130	108	86
Median cirrus	37	23	23
Internal cirrus	24	23	9
External cirrus	39	36	12
Lateral cirrus	38	44	27
Primary clava	4	4	4
Secondary clava (width × length)	7 × 5	7 × 5	3 × 3
Dorsal spine I	47	36	19
Dorsal spines II	17	10	22
Dorsal spines III	24	22	20
Dorsal spines IV	–	–	15
Dorsal spine V	15	7	5
Cirrus E	101	96	43
Leg IV appendage	6	5	6

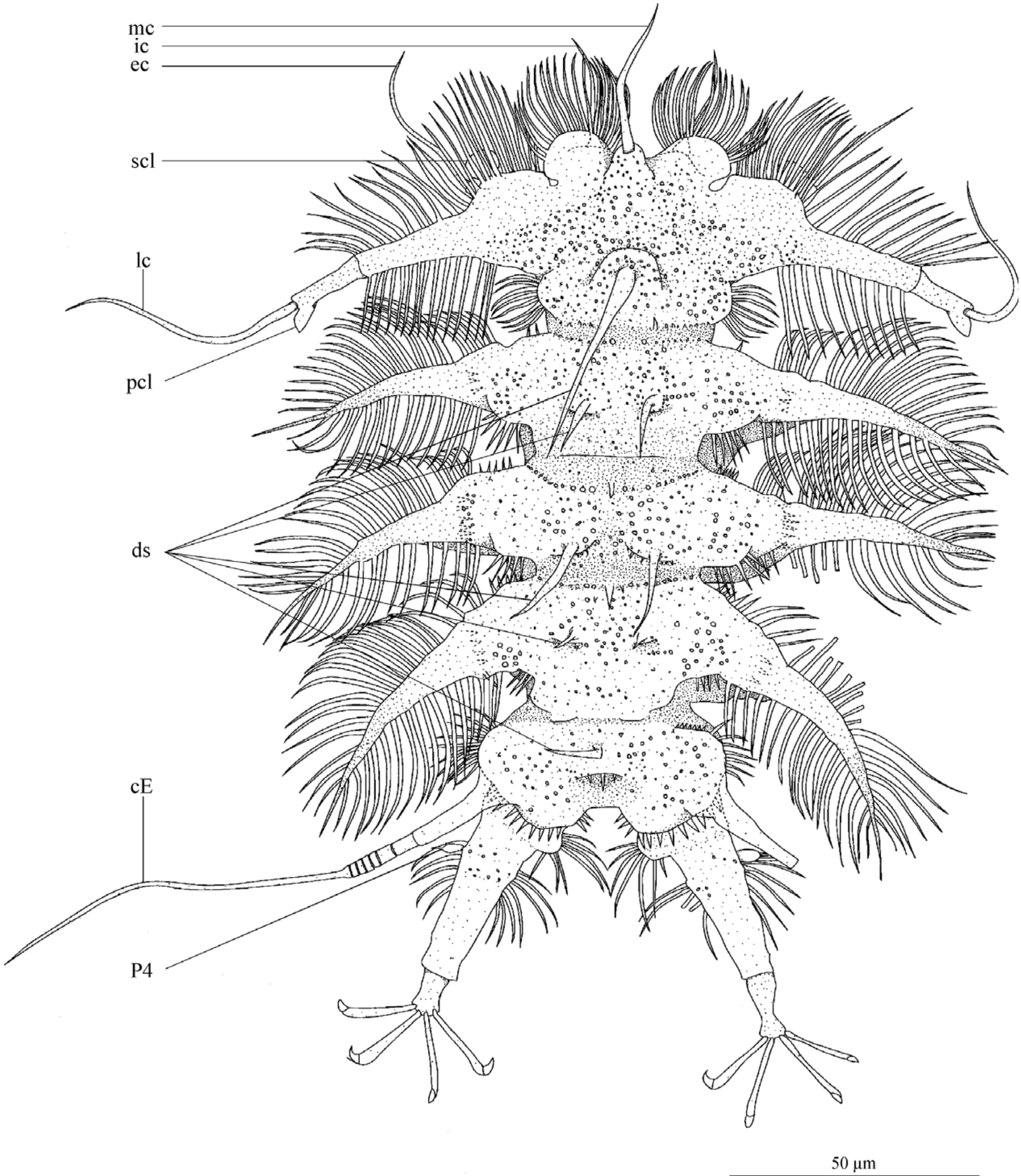

Fig. 2. *Neostygarctus lovedeluxe* n. sp. Dorsal view of the holotype. Abbreviations: cE, cirrus E; ds, dorsal spines; ec, external cirrus; ic, internal cirrus; lc, lateral cirrus; mc, median cirrus; pcl, primary clava; P4, leg IV appendage; scl, secondary clava.

second 24 µm long, third not measured) each inserted on slight lobe. Dorsal posterior portion of lateral process's base lobed with spines. Base of lateral process with small spikes on ventral side.

Caudal region with mid-dorsal spine (15 µm long), pair of lateral lobes, and pair of cirri E (101 µm long). Cirrus E with accordion-plated articulation inserted on robust cirrophorus.

No spines or appendages on legs I–III. Leg IV with proximal and medial spines. Leg IV having slightly elongated papillae with short terminal spine.

Legs with four tubular claws terminated in triangular solid hooks, two medial ones with dorsal bristle (Fig. 4B).

Rosette-like female gonopore positioned 3 µm anterior to anus (Fig. 4C). Two oval seminal receptacles with long sinuous ducts present.

Remarks on juveniles. A four-clawed juvenile [SMBL Type 457] and a two-clawed juvenile [SMBL Type 458] were collected from the same sample as the holotype. The four-

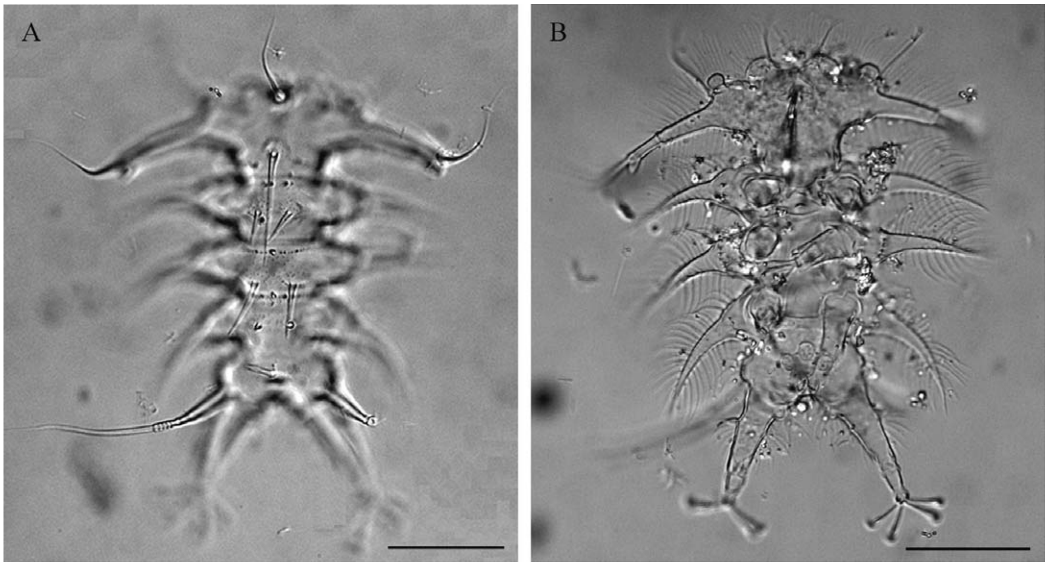

Fig. 3. *Neostygarctus lovedeluxe* n. sp. DIC micrograph of the holotype. **(A)** Dorsal view. **(B)** Ventral view. Scale bar = 50 µm.

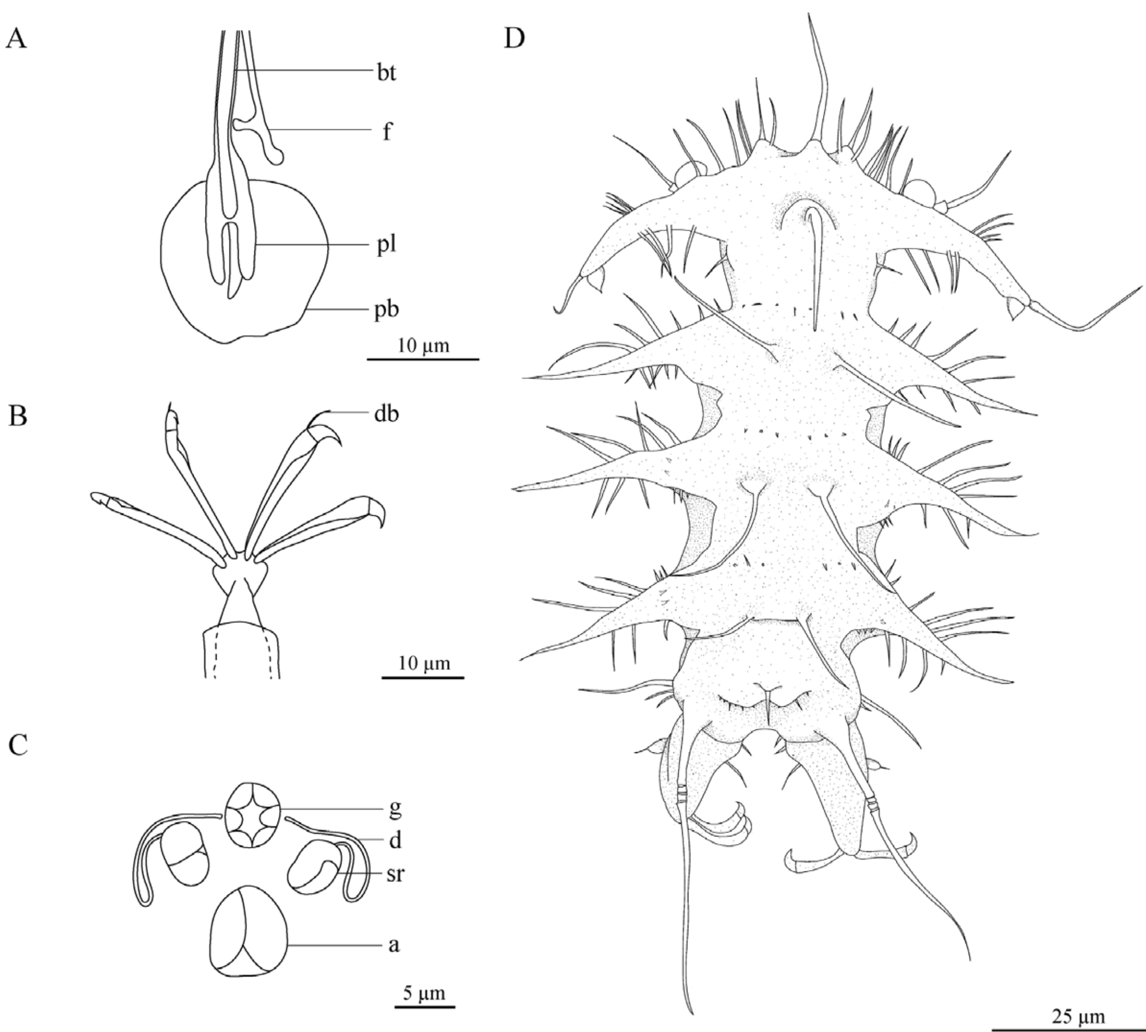

Fig. 4. *Neostygarctus lovedeluxe* n. sp. **(A)** Bucco-pharyngeal apparatus of the holotype. **(B)** Claws of the fourth pair of legs of the holotype. **(C)** Female gonopore, seminal receptacles and anus of the holotype. **(D)** Dorsal view of the paratypic two-clawed juvenile. Abbreviations: a, anus; bt, buccal tube; d, duct; db, dorsal bristle; f, furca; g, gonopore; pb, pharyngeal bulb; pl, placoid; sr, seminal receptacle.

clawed juvenile resembles the adult morphology, but without a gonopore, seminal receptacles, or an anus. The two-clawed juvenile (Fig. 4D) resembles the four-clawed one in general morphology, but differs in the following aspects: the number of claws; the subdivision of the cephalic region, especially in the weakly developed anterior and the anterolateral lobes; the sparsely distributed marginal spines of the dorsal plates; the sparsely distributed minute spikes on the dorsal surface; the sparsely distributed spines on leg IV; the indistinct dorsal plates; and the indistinct boundary of the lateral lobe and the cirrophorus bearing the lateral cirrus and the primary clava.

DISCUSSION

Differential diagnosis

Neostygarctus lovedeluxe n. sp. differs from *N. acanthophorus* by the number of robust dorsal spines on the five plates: they show a 1-2-2-2-1 arrangement in *N. lovedeluxe*, but a 1-1-1-1-1 arrangement in *N. acanthophorus*. Another difference is the deeper incision between the anterior and the anterolateral lobes in the new species.

Evolutionary significance of the ontogenic traits

The taxonomic position of *Neostygarctus* has been a controversial subject since its discovery; it was originally placed in the Stygarctidae by Grimaldi de Zio et al. (1982a), moved to the Renaudarctidae by Kristensen and Higgins (1984), and then Grimaldi de Zio et al. (1987a) established the new family Neostygarctidae for this genus. The phylogenetic position of the family has been discussed based on the adult morphology (Kristensen and Higgins, 1984; Grimaldi de Zio et al., 1987a; Bello and De Zio Grimaldi, 1998), but it remains unsettled.

A two-clawed juvenile of *N. acanthophorus* has been reported from the Indian Ocean, but lacks a morphological description (Gallo et al., 2007). In the present study, the morphology of the two-clawed juvenile of Neostygarctidae is described for the first time. Among the possible related families (Neoarctidae, Renaudarctidae, and Stygarctidae), ontogenic information including the morphology of the two-clawed juvenile has been obtained only in Stygarctidae (Renaud-Mornant and Anselme-Moizan, 1969; McKirdy et al., 1976; Grimaldi de Zio et al., 1987b; Gallo D'Addabbo et al., 2001). We observe the common sequence in ontogenic traits between the new species and the members of Stygarctidae.

Cephalic lobes. In contrast to the later development stages, the two-clawed juvenile of the new species has a less-protruding anterior, and anterolateral lobes lacking an incision between them. In Stygarctidae, the lateral lobes of the cephalic region of *Parastygarctus renaudae* grow longer during development (Grimaldi de Zio et al., 1987b). This ontogenic sequence in the cephalic lobes agrees with one of the evolutionary lines suggested in a cladistic analysis using adult morphology by Bello and De Zio Grimaldi (1998), in which the lobes protrude from a *Neostygarctus*-like state (the lateral lobes more protruded than the anterior and the anterolateral lobes) to a *Parastygarctus*-like state (all lobes protrude to the same degree). *Pseudostygarctus rugosus* displays, however, two opposing directions of sequence in postembryonic development: One confirms the discussed sequence in the sense that its division between the anterolateral and lateral lobes are less evident in the two-clawed juvenile. The second contradicts it in the sense that the two-clawed juvenile has deep incisions between the anterior and anterolateral lobes, but which disappear in later development due to the fusion of lobes, leaving two closed rings (Gallo D'Addabbo et al., 2001).

Lateral processes. A common ontogenic sequence in the lateral processes of body segments can be detected from the following observations on the postembryonic development. The new species preserves a pair of conical lateral processes for each body segment throughout the development. *Parastygarctus renaudae* has a similar developmental pattern with the increasing length of the processes during the development (Grimaldi de Zio et al., 1987b). In *Stygarctus bradypus*, the conical lateral processes of the two-clawed juvenile change into the funnel-shaped ones in later developmental stages (Renaud-Mornant and Anselme-Moizan, 1969). *Pseudostygarctus triungulatus* has small, and *Stygarctus lambertii* has no lateral processes in the two clawed juvenile, but these become well-developed in later stages (McKirdy et al., 1976; Grimaldi de Zio et al., 1987b). These ontogenic traits suggest the following sequence for lateral processes: The most basic state is the absence of lateral processes (in *Megastygarctides*) followed by the possession of a pair of conical ones (in some species of *Parastygarctus* and Neostygarctidae), and the funnel-shaped processes (in *Stygarctus*) or the processes with thin short membranes (in *Pseudostygarctus*) represent a more advanced state. Bello and De Zio Grimaldi (1998) suggested, however, a reverse sequence, based on their cladistic analysis of adult morphology.

Body plates. The body plates of the two-clawed juvenile in the new species are indistinct in comparison to those of the later stages. In Stygarctidae, the two-clawed juvenile of *Stygarctus bradypus* has no distinguishable plates (Renaud-Mornant and Anselme-Moizan, 1969) and that of *S. lambertii* lacks intersegmental plates (Grimaldi de Zio et al., 1987b). These observations suggest that the presence of distinct plates is an advanced state. Guldberg and Kristensen (2006) indicated the same sequence of traits based on the intrageneric comparison of *Megastygarctides* adults, whereas Kristensen and Higgins (1984) and Bello and De Zio Grimaldi (1998) indicated the reverse, also based on the adult morphology. The former study dealt with Heterotardigrada, and the latter with Neoarctidae, Neostygarctidae, Renaudarctidae, Stygarctidae and *Halechiniscus* (a member of Halechiniscidae used as an outgroup).

Studies on the ontogeny and phylogeny of tardigrades are still at an early stage, and the common sequence of traits suggested in the present study are often in disagreement with those in previous studies. Accumulation of morphological and ontogenic data as well as a more reliable molecular phylogenetic tree with broader taxon sampling are required to unravel the evolution and phylogeny of the Neostygarctidae and other tardigrades.

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