



# A new genus and species of Stygarctidae (Heterotardigrada: Arthrotardigrada) from Yaku-Shin-Sone bank (Northwest Pacific)

Shinta Fujimoto<sup>1</sup> · Susumu Ohtsuka<sup>2</sup>

Received: 19 February 2019 / Revised: 9 May 2019 / Accepted: 24 June 2019 / Published online: 26 July 2019  
© Senckenberg Gesellschaft für Naturforschung 2019

## Abstract

A marine armoured tardigrade, *Hansenarctus toyoshiomaruae* gen. et sp. nov., is described from Yaku-Shin-Sone bank, south of Yakushima island, Northwest Pacific. It belongs to the family Stygarctidae Schulz, 1951 for its five-lobed head, cuticular plates, and non-digitate claws, but a combination of the following character states warrants the erection of the new genus: the semi-globular secondary clavae, sub-divided lateral lobes of the head, antero-dorsal, postero-dorsal, and latero-dorsal processes on the dorsal plates except for the caudal plate, two tapering lateral processes (one pair) on each body plate, four lateral processes (two pairs) on the caudal plate, intersegmental regions between dorsal plates, looped seminal receptacles without additional cuticular structures at the openings, and four claws with the internal ones bearing dorsal spurs. Its phylogenetic position is briefly discussed based on morphology and the following topology was suggested for Stygarctidae: (*Megastygarctides*, (*Neostygarctus*, (*Faroestygarctus*, *Hansenarctus* gen. nov., (*Mesostygarctus*, *Pseudostygarctus*), (*Parastygarctus*, *Prostygarctus*, *Stygarctus*))). Stygarctid genera (excluding *Megastygarctides* and *Neostygarctus*) share a synapomorphic state: the presence of exteriorly projected apophyses in the pharyngeal bulb.

**Keywords** Japan · Marine meiofauna · Phylogeny · Systematics · Tardigrada

## Introduction

The majority of marine tardigrades are accommodated in Arthrotardigrada Marcus, 1927, a paraphyletic order of the class Heterotardigrada Marcus, 1927 (e.g. Jørgensen et al. 2010; Fujimoto et al. 2017). Currently, the order is mainly characterised by the presence of the median cirrus (e.g.

Fontoura et al. 2017). However, it was originally established as a group of species with highly telescopic legs (Marcus 1927) and it was when Schulz (1951) created the family Stygarctidae Schulz, 1951 for *Stygarctus bradypus* Schulz, 1951, that the median cirrus was explicitly used as a diagnostic character. Similar to this, the family Stygarctidae was originally diagnosed as an arthrotardigrade with four claws directly inserted on legs (Schulz 1951), in contrast to our present recognition as an armoured family (e.g. Hansen et al. 2012). Renaud-Debyser (1965) inexplicitly used the cuticular plates covering the body ('cuirasse' in original text) as a diagnostic character of Stygarctidae when she described the second genus, *Parastygarctus* Renaud-Debyser, 1965. The plates (and also sensory organs) were implemented in the familial diagnosis by McKirdy et al. (1976) when they described stygarctid taxa, including two genera, *Pseudostygarctus* McKirdy, Schmidt & McGinty-Bayly, 1976 and *Megastygarctides* McKirdy, Schmidt & McGinty-Bayly, 1976, a unique genus that was later assigned to its own subfamily by Bello & de Zio Grimaldi (1998). Since then, *Mesostygarctus* Renaud-Mornant, 1979, *Neostygarctus* Grimaldi de Zio, D'Addabbo

This article is registered in ZooBank under <http://zoobank.org/8F323ACC-B117-4DE2-8539-9B213A96EE7A>

Communicated by S. Gollner

✉ Shinta Fujimoto  
shinta.f@water-bears.com

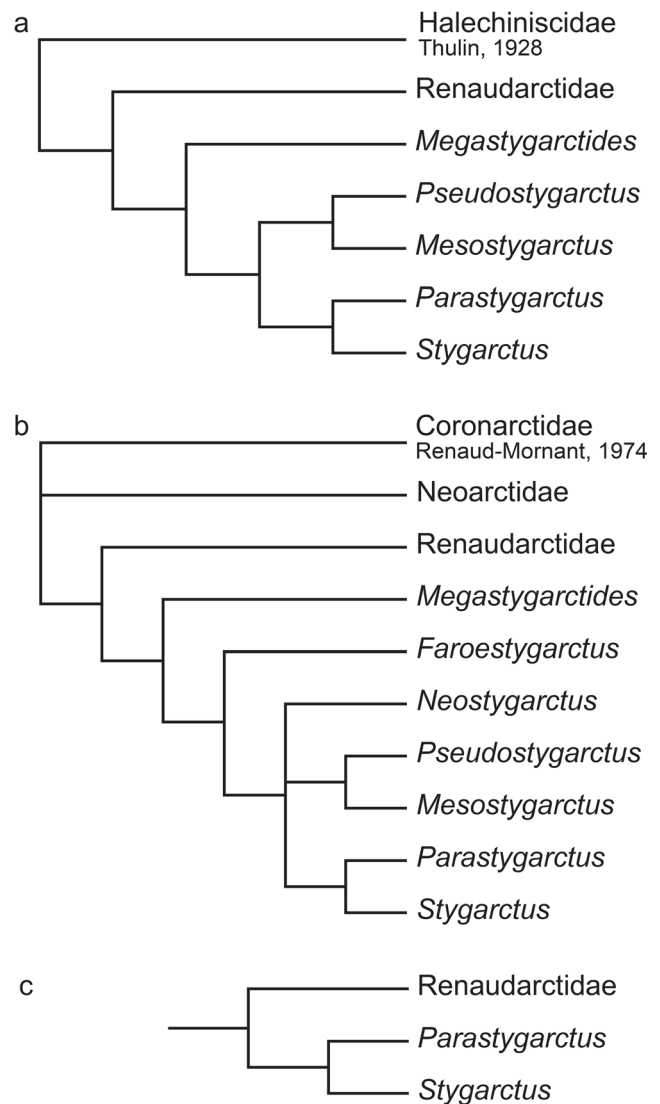
<sup>1</sup> Research Center for Marine Biology, Graduate School of Life Sciences, Tohoku University, 9 Sakamoto, Asamushi, Aomori 039-3501, Japan

<sup>2</sup> Takehara Station, Setouchi Field Science Center, Graduate School of Integrated Sciences for Life, Hiroshima University, Takehara, Hiroshima 725-0024, Japan

Gallo & Morone De Lucia, 1982, *Faroestygarctus* Hansen, Kristensen & Jørgensen, 2012, and *Prostygarctus* Rubal, Veiga, Fontoura & Sousa-Pinto, 2013 have been described as genera of this family. Renaud-Mornant's (1979) *Mesostygarctus* was included in *Pseudostygarctus* by Gallo D'Addabbo et al. (2001) and reinstated by Hansen et al. (2012). The taxonomic status of *Neostygarctus* has been frequently revised: Soon after its description by Grimaldi de Zio et al. (1982), it was transferred to Renaudarctidae Kristensen & Higgins, 1984 by Kristensen and Higgins (1984). Subsequently, its own family, Neostygarctidae Grimaldi de Zio, D'Addabbo Gallo & Morone De Lucia, 1987, was erected by Grimaldi de Zio et al. (1987) and supported by Bello and de Zio Grimaldi (1998), reinstated to Stygarctidae by Hansen et al. (2012), placed in its own family again by Kristensen et al. (2015), and recently considered a stygarctid genus in two studies (Fontoura et al. 2017; Tchesunov 2018). Another genus, *Neoarctus* de Zio Grimaldi, D'Addabbo Gallo & Morone De Lucia, 1992, was originally described as a stygarctid in its own subfamily (de Zio Grimaldi et al. 1992) but subsequently elevated to family rank, Neoarctidae de Zio Grimaldi, D'Addabbo Gallo & Morone De Lucia, 1992, and removed from Stygarctidae by Bello & de Zio Grimaldi (1998) for its unique features.

The phylogeny of these stygarctid genera have been discussed in some studies (e.g. Kristensen and Higgins 1984; Morone De Lucia et al. 1984; Bello and de Zio Grimaldi 1998; Hansen et al. 2012). Bello and de Zio Grimaldi (1998) conducted the first phylogenetic analysis based on 30 morphological characters (Fig. 1 a). Subsequently, Hansen et al. (2012) reconstructed the phylogenetic tree with their dataset of 81 morphological characters of all described armoured marine species (Fig. 1 b). However, with the exception of the monophyly of each genus and one clade consisting of *Mesostygarctus* and *Pseudostygarctus*, their topology received low statistical support values or was not recovered in the Bayesian inference (Hansen et al. 2012). The two studies, by Bello and de Zio Grimaldi (1998) and Hansen et al. (2012), respectively, used different unarmoured marine families erected by Thulin (1928) and Renaud-Mornant (1974) as out groups, but their topologies do not conflict except for the position of *Neostygarctus*. Taking into account that Hansen et al. (2012) also conducted a likelihood-based test and confirmed *Neostygarctus* as a part of Stygarctinae, their hypothesis is more likely to be true than that of Bello and de Zio Grimaldi (1998). So far, no molecular phylogenetic analyses have tested to support this hypothesis. Owing to the small number of stygarctid representatives used, molecular phylogenetic analyses (Fujimoto et al. 2017) inferred only limited relationships of the taxa under study (Fig. 1c).

Herein, we describe a new genus and species of the family Stygarctidae discovered from the oceanic bank off Yakushima island, Kyushu, Japan, and discuss its

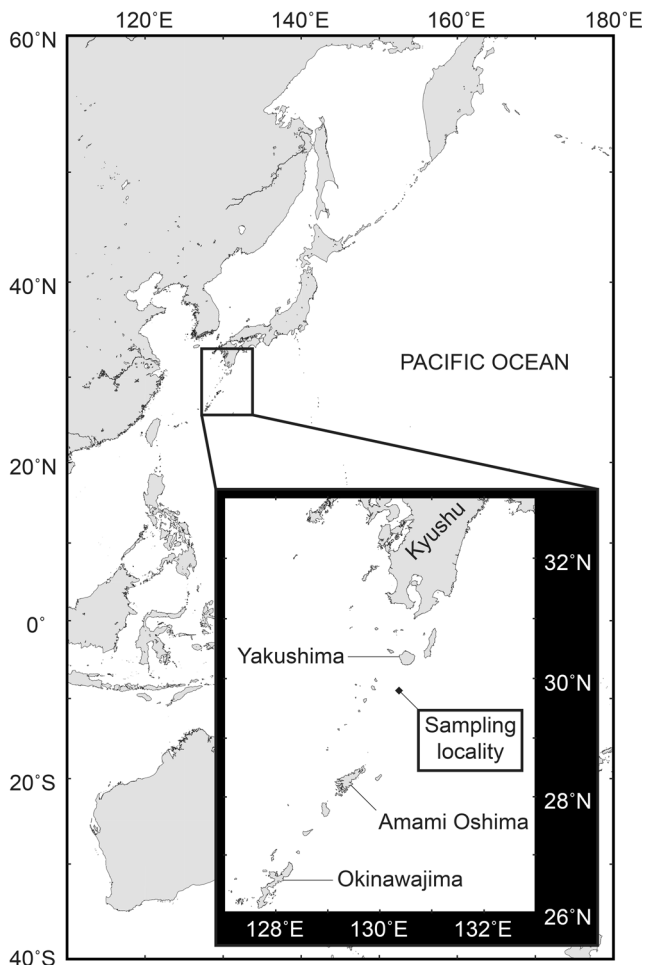


**Fig. 1** Phylogenetic trees of Stygarctidae. **a** Morphological phylogeny of Bello and de Zio Grimaldi (1998); **b** morphological phylogeny of Hansen et al. (2012); **c** a clipping from the molecular phylogeny of Fujimoto et al. (2017). Stygarctids and non-stygarctids were represented by genera and families, respectively

phylogenetic position in the family based on some selected morphological characters mentioned in Hansen et al. (2012).

## Material and methods

A sediment sample was collected by a biological dredge at Yaku-Shin-Sone bank (vessel coordinates: from 29° 48.047' N, 130° 22.102' E to 29° 47.959' N, 130° 22.099' E; depth 177 m) on 21 Oct. 2016 during the TR/V *Toyoshiomaru* cruise (Fig. 2). For extraction of meiofauna, a part of the sediment sample was processed with freshwater (Kristensen 1983) using a 30- $\mu$ m mesh



**Fig. 2** Map of sampling locality (Yaku-Shin-Sone bank)

net and fixed in ca. 4% buffered formaldehyde on board. After the cruise, the meiofauna extract was processed with Ludox® HS-40 colloidal silica (Sigma-Aldrich Co., St. Louis) (e.g. Giere 2009) using a 32- $\mu$ m opening mesh net and specimens were sorted out under a stereomicroscope. The two specimens of the new genus and species were mounted first in distilled water for observation and then in glycerol as permanent preparations for a detailed observation. All observations were conducted using a differential interference contrast microscope (Olympus BX53). The holotype and paratype are deposited in the Zoological Collection of Kyoto University (KUZ). Terminology basically follows that in Hansen et al. (2012).

Morphological characters were measured using Adobe Illustrator CS6 (Adobe Systems Incorporated.). The map (Fig. 2) was illustrated using GMT version 5.4.4 (Wessel et al. 2013) and Adobe Illustrator CS6. Other figures were prepared using Adobe Illustrator CS6 and Photoshop CS6.

## Results

### Systematics

Order Arthrotardigrada Marcus, 1927

Family Stygarctidae Schulz, 1951

Subfamily Stygarctinae Schulz, 1951

Genus *Hansenarctus* gen. nov.

<http://zoobank.org/D715C4E5-FF1F-49BB-A67C-E15B0AC9485B>

**Diagnosis.** Stygarctinae with punctate cuticle; dorsally-plated cephalic region (head) divided into anterior medial lobe with pair of internal cirri, pair of medio-lateral lobes each with dorsal semi-globular secondary clava and ventral external cirrus, and pair of lateral lobes each subdivided into antero-lateral and postero-lateral lobes with anterior lateral cirrus and posterior ovoid primary clava on each of latter lobe, and interior to five lobes, antero-dorsal process with median cirrus, postero-dorsal process, and pair of weak latero-dorsal processes present; body plates I–III each with antero-dorsal and postero-dorsal processes, pair of latero-dorsal processes, and pair of tapering lateral processes; caudal plate with pair of antero-lateral processes each with proximally accordion-articulated, tapering cirrus E, pair of postero-lateral processes, and caudal incision; intersegmental regions present between all dorsal plates; ventral plates absent; in female, seminal receptacles open ventrally, postero-exteriorly apart from rosette-like gonopore with long, sinuous ducts each forming large loop terminating exteriorly in small vesicle supported by ventral swelling; in male, oval gonopore open immediately anterior to anus; apophyses as exteriorly projected bars with distal swelling present in pharyngeal bulb; legs each with four non-digitate claws; internal claws each with dorsal spur; leg IV sensory organs ovoid.

**Type species.** *Hansenarctus toyoshiomaruae* gen. et sp. nov.

**Etymology.** The new genus is dedicated to Dr. Jesper Guldberg Hansen for his contribution to tardigradology. *Hansen* + *arctus* (Latinised Greek), meaning Hansen's bear.

**Remarks.** Among the three armoured marine tardigrade families (Neoarctidae, Renaudarctidae, and Stygarctidae), the new genus clearly belongs to Stygarctidae because of the presence of the lateral cirri and primary clavae on the cephalic plate (whereas those of Neoarctidae are on body plate I), and because of the four non-digitate claws on the legs (whereas Neoarctidae has three non-digitate claws and Renaudarctidae has digitate claws) (Bello and de Zio Grimaldi 1998; Hansen et al. 2012; Fujimoto and Yamasaki 2017). In Stygarctidae, the new genus better matches the diagnosis of the subfamily Stygarctinae Schulz, 1951 by its subdivision of the head into five lobes and the presence of four claws on all legs, rather than Megastygartidinae Bello & de Zio Grimaldi, 1998 with a maximum of four head lobes and four claws on legs I–III and two claws on legs IV (Bello and de Zio Grimaldi 1998; Hansen and Kristensen 2006). In Stygarctinae, *Neostygartus* clearly differs from the new genus

by character states such as cephalic region with long lateral lobes, the presence of dorsal spines and cuticular spines, claws with digits, and robust cirri E (Tchesunov 2018). *Prostygarctus* also has digits and in addition to this difference, it differs from the new genus by the character states such as the subdivided anterior medial lobe, dorsal spines at the posterior margins of most plates, bent secondary clavae, and cirri E's ball and socket articulation (Rubal et al. 2013) (*Pseudostygarctus* also has the last character state (Hansen et al. 2012)). Among the remaining five genera of Stygarctinae, the new genus resembles *Faroestygarctus*, *Mesostygarctus*, and *Pseudostygarctus* by its semi-globular secondary clavae and claws with dorsal spurs, instead of *Parastygarctus* and *Stygarctus* with elongate secondary clavae and long accessory filaments (Bello and de Zio Grimaldi 1998; Hansen et al. 2012). However, the new genus is distinguished from *Faroestygarctus* by its clearly defined five-lobed head and legs bearing four claws, whereas *Faroestygarctus* has lesser developed semi-circular head with three lobes and legs bearing two claws (Hansen et al. 2012). The absence of cuticular membranes on the head and body plates, absence of cuticular structures at the seminal receptacle openings, and presence of small seminal receptacle vesicles in the new genus reject its placement in the other two genera (Hansen et al. 2012). In addition to these disagreements to the diagnoses of described genera, the new genus is unique in having dorsal plates with dorsal and latero-dorsal processes. Therefore, the erection of a new genus for the new species is justified.

*Hansenarctus toyoshiomaru* gen. et sp. nov.

<http://zoobank.org/BD80228C-B93B-41BF-B77B-99FC5424EEF5>

**Diagnosis.** Same as for genus.

**Material examined.** Holotype: KUZ Z2161, adult female. Paratype: KUZ Z2162, adult male. Collected together from type locality and mounted in glycerol.

**Type locality.** Yaku-Shin-Sone bank (vessel coordinates: from 29°48.047'N, 130°22.102'E to 29°47.959'N, 130°22.099'E; depth 177 m).

**Etymology.** The specific epithet (noun, genitive case) refers to TR/V *Toyoshiomaru* of School of Applied Biological Science, Hiroshima University.

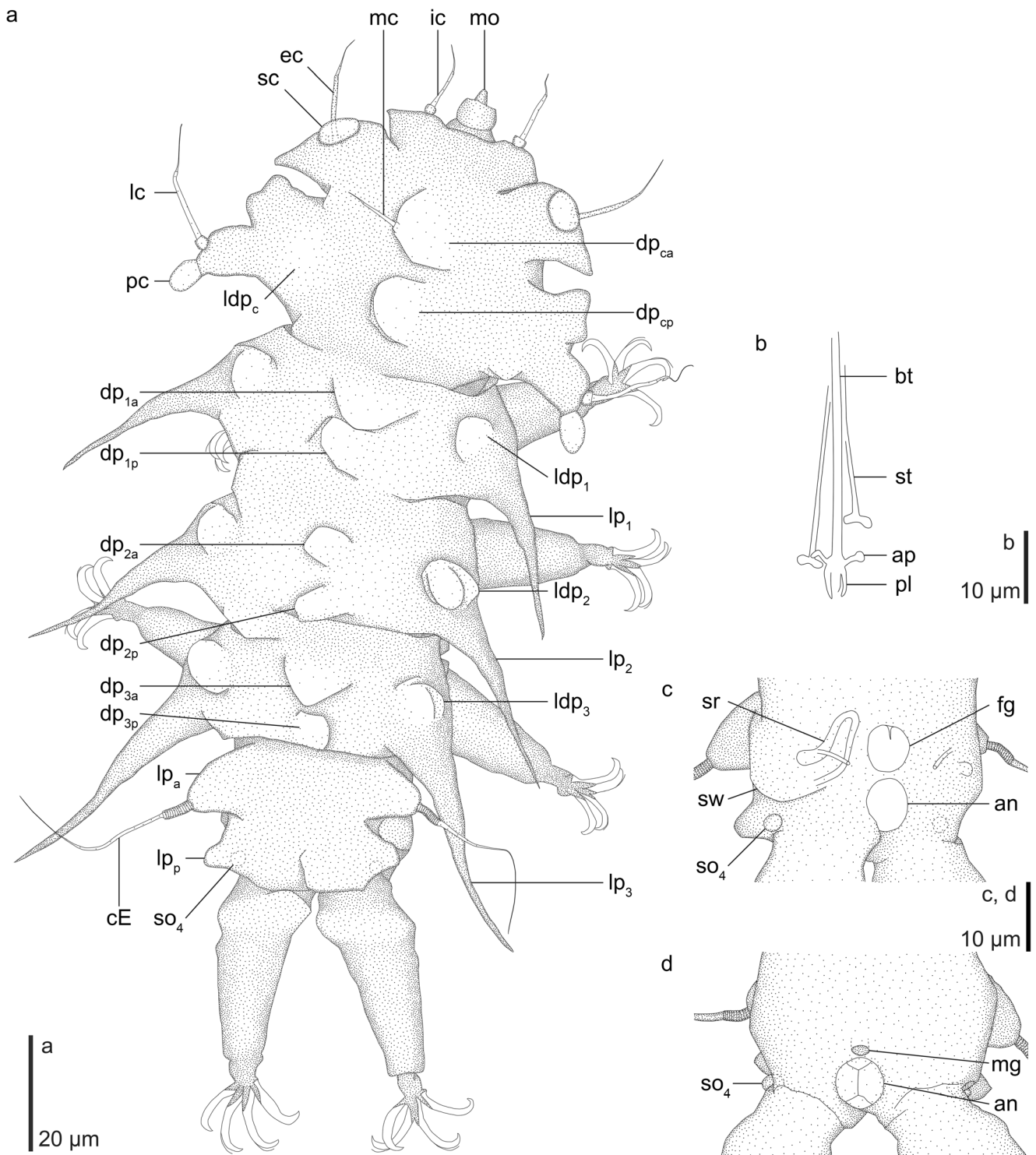
**Description of holotype** (KUZ Z2161, Figs. 3a–c and 4). Adult female with body length (from medio-anterior margin of medial lobe to medio-posterior margin of caudal plate) of 140 µm (Figs. 3a and 4a). Body divided into cephalic (head) region with dorsal cephalic plate, three dorsally plated body segments, and caudal region with dorsal caudal plate. Dorsal intersegmental regions recognised between dorsal plates. Ventral plates absent. Body surface covered with coarse, granular sculptures lined by epicuticular pillars except for smooth surface of ventral side of trunk.

Cephalic region (head) (Figs. 3a and 4b) with dorsal plate divided into five lobes: medial lobe, pair of medio-lateral lobes,

and pair of lateral lobes. Anterior medial lobe having pair of anteriorly directed weak processes each with internal cirrus (13 µm) and exterior acute edge. Paired medio-lateral lobes each carrying globular secondary clava (height 4 µm, width 7 µm) in dorsal position at anterior margin, external cirrus (20 µm) in ventral position slightly posterior from anterior margin, and acute edge in postero-exterior position. Paired lateral lobes each subdivided into angular antero-lateral lobe and postero-lateral lobe. Postero-lateral lobe with lateral cirrus (22 µm) in antero-lateral position and ovoid primary clava (length 6 µm, width 4 µm) in postero-lateral position. Medial lobe ventral to medio-lateral and lateral lobes. Antero-dorsal and postero-dorsal processes and latero-dorsal process present interior to five lobes. These processes dome-shaped without sharp tips. Antero-dorsal process with median cirrus (10 µm) on its peak situated medially at level of medio-lateral lobes. Postero-dorsal process situated medially at level of lateral processes. Weak latero-dorsal process situated at base of one of lateral lobe and one process missing from base of other lobe (for further observation see: **Remarks on paratype male**). All cephalic cirri each consisting of short scapus, tubular portion, and flagellum. Ventral to medial lobe, anteriorly directed mouth cone present. By brief observation of specimen in distilled water at × 400 magnification before permanent preparation, bucco-pharyngeal apparatus morphology recognised: Thin, long buccal tube terminating in apophyses as exteriorly projecting bars (only two recognised but three likely) each with distal swelling and three placoids in pharyngeal bulb not reaching level of body segment I and paired stylets each terminating in bifurcate furca (Figs. 3b and 4c; due to lack of detailed observation at × 1000 magnification, no measurements were made). Detail of bucco-pharyngeal apparatus dissolved and contour of pharyngeal bulb became indistinct after permanent preparation.

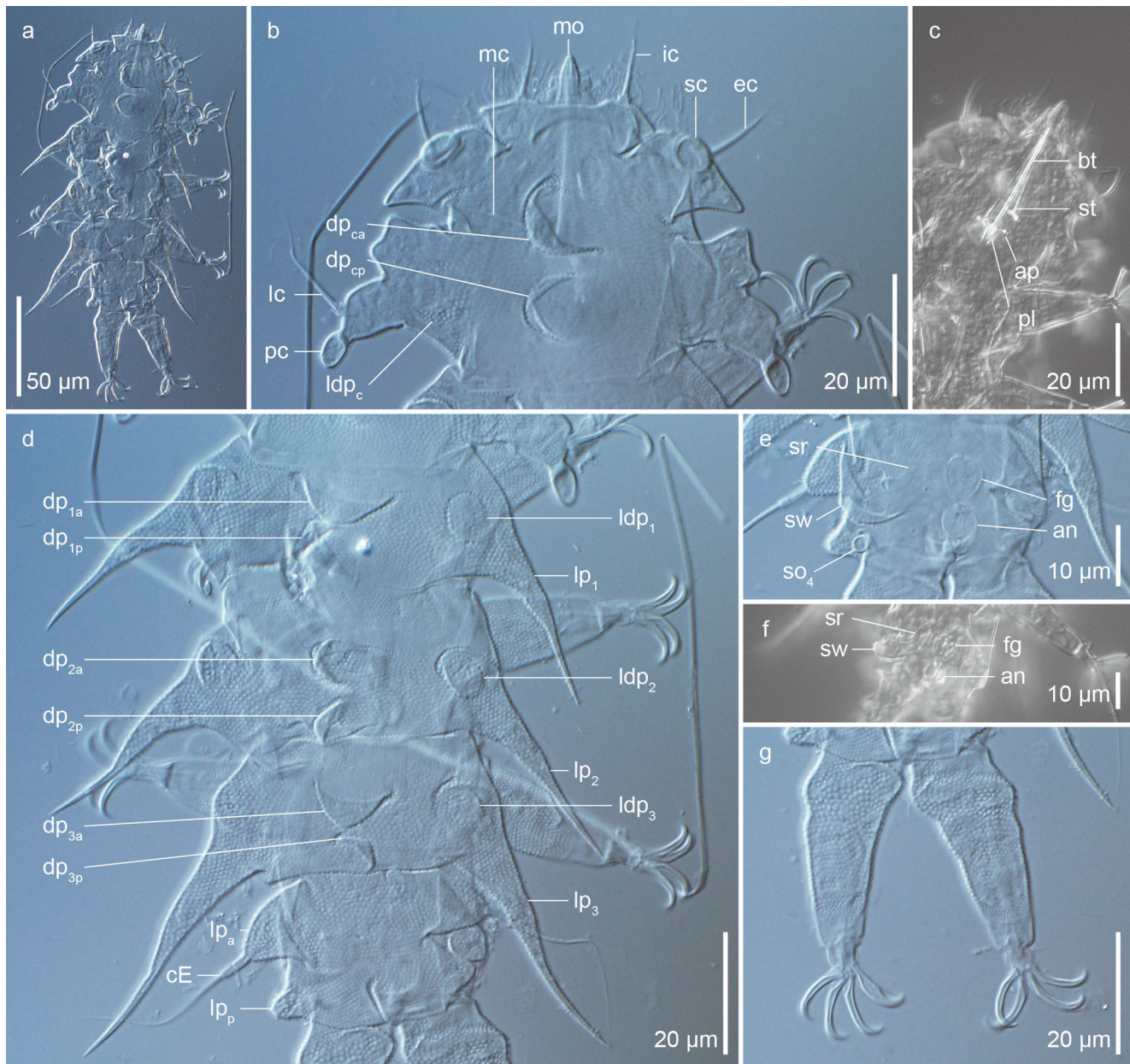
Body segments I–III (Figs. 3a and 4d) having dorsal plates (body plates I–III). Body plates each carrying medial antero-dorsal and postero-dorsal processes, pair of latero-dorsal processes, and pair of tapering lateral processes directed postero-laterally. Antero-dorsal, postero-dorsal, and latero-dorsal processes dome-shaped without sharp tip as those on cephalic plate. Antero-dorsal process and pair of latero-dorsal processes situated at anterior-most position of each body plate. Postero-dorsal process situated at posterior-most position of each body plate.

Caudal segment having caudal plate with pair of antero-lateral processes and smaller, conical postero-lateral processes, and caudal incision (Figs. 3a and 4d). Paired cirri E (31 µm) with proximal accordion-like articulations on antero-lateral processes. Ventrally, caudal segment having gonopore, paired seminal receptacles, and anus (Figs. 3c and 4e,f). Detail of gonopore and anus not recognisable after permanent preparation (Figs. 3c and 4e). Brief observation of specimen in distilled water at × 400 magnification before permanent preparation showed rosette-like gonopore (length 7 µm, width 5 µm) surrounded by cuticular ring 1 µm anterior



**Fig. 3** Drawings of *Hansenarctus toyoshiomaruae* gen. et sp. nov. **a** Dorsal view of holotype female, KUZ Z2161; **b** holotype female’s bucco-pharyngeal apparatus, KUZ Z2161 (incomplete drawing based on micrograph taken at  $\times 400$  magnification); **c** holotype female’s genital structure, KUZ Z2161 (detail of gonopore and anus not recognised); **d** paratype male’s genital structure, KUZ Z2162. an, anus; ap, apophysis; bt, buccal tube; cE, cirrus E; dp<sub>ca</sub>, cp, 1a, 1p, 2a, 2p, 3a, 3p, antero- and postero-

dorsal processes of cephalic plate and body plates I–III; ec, external cirrus; fg, female gonopore; ic, internal cirrus; lc, lateral cirrus; ldp<sub>c</sub>, 1–3, latero-dorsal process of cephalic plate and body plates I–III; lp<sub>1–3</sub>, lateral process of caudal plate; mc, median cirrus; mg, male gonopore; mo, mouth cone; pc, primary clava; pl, placoid; sc, secondary clava; so<sub>4</sub>, leg IV sensory organ; sr, seminal receptacle; st, stylet; sw, ventral swelling



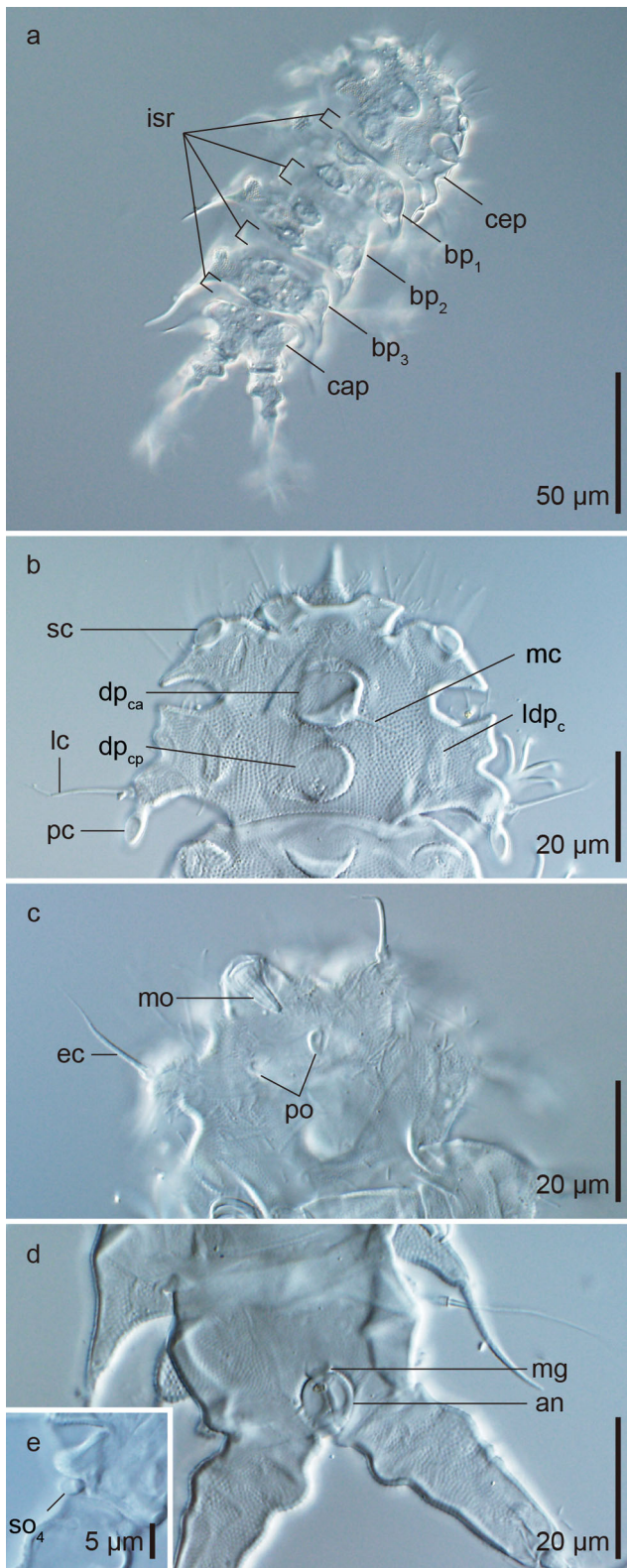
**Fig. 4** Differential interference contrast micrographs of *Hansenarctus toyoshiomaruae* gen. et sp. nov. (holotype female, KUZ Z2161). **a** habitus; **b** dorsal view of cephalic plate; **c** bucco-pharyngeal apparatus observed before permanent preparation; **d** body plates and caudal plate; **e** ventral view of caudal segment; **f** genital structure observed before permanent preparation; **g** legs IV. an, anus; ap, apophysis; bt, buccal tube; cE, cirrus E; dp<sub>ca</sub>, cp, 1a, 1p, 2a, 2p, 3a, 3p, antero- and postero-dorsal processes of

cephalic plate and body plates I–III; ec, external cirrus; fg, female gonopore; ic, internal cirrus; lc, lateral cirrus; ldp<sub>c</sub>, 1–3, latero-dorsal process of cephalic plate and body plates I–III; lp<sub>1–3</sub>, lateral process of body plates I–III; lp<sub>a</sub>, p, antero- and postero-lateral processes of caudal plate; mc, median cirrus; mo, mouth cone; pc, primary clava; pl, placoid; sc, secondary clava; so<sub>4</sub>, leg IV sensory organ; sr, seminal receptacle; st, stylet; sw, ventral swelling

to anus (length 7 μm, width 5 μm) consisting of paired lateral lobes and two smaller anterior and posterior lobes (Fig. 4f). Paired seminal receptacles (Figs. 3c and 4e,f) open postero-externally from gonopore. Seminal receptacle ducts each exteriorly directed, forming longitudinally elongated loop, and terminating in exteriorly situated small vesicle. Exact tracks of ducts where loops begin not confirmed (this section of the

duct is not drawn in Fig. 3c). Ventral cuticle of caudal segment has pair of swellings supporting seminal receptacles.

Between cephalic plate and body plate I, body plates I–II, body plates II–III, and body plate III and caudal plate, intersegmental regions present. No apparent margins or folds delimiting boundaries of dorsal plates and intersegmental regions. However, intersegmental regions



**Fig. 5** Differential interference contrast micrographs of *Hansenarctus toyoshiomaruae* gen. et sp. nov. (paratype male, KUZ Z2162). **a** Dorsal view with focus on plates; **b** dorsal view of cephalic plate; **c** ventral view of cephalic segment; **d** genital structure; **e** leg IV sensory organ. an, anus; bp<sub>1–3</sub>, body plates I–III; cap, caudal plate; cep, cephalic plate; dp<sub>ca</sub>, cp, antero- and postero-dorsal processes of cephalic plate; ec, external cirrus; isr, intersegmental region; lc, lateral cirrus; mc, median cirrus; mg, male gonopore; mo, mouth cone; pc, primary clava; po, pore; sc, secondary clava; so<sub>4</sub>, leg IV sensory organ

between cephalic plate and body plate I recognised as region with gradually depressing cuticle that subducts beneath cuticle anterior to body plate I. Intersegmental region between body plate III and caudal plate completely overlapped by plates and not recognizable (for further observation on intersegmental regions see **Remarks on paratype male**).

Leg subdivisions indistinct from coxa to tibia. Leg cuticle sculptures distally finer, apparently smooth from possible boundary between femur and tibia to tarsus. Legs IV (Fig. 4g) having coarser cuticle sculptures than other legs. Each leg carrying four directly inserted claws (claw lengths (heights): legs I internal and external claws both 8 µm; legs IV internal claws 11 µm, external claws 8 µm). Internal claws each with dorsal spur. Leg I–III sensory organs absent. Ovoid leg IV sensory organs (length 3 µm, width 2 µm) (Figs. 3a,c and 4e) at base of leg IV.

**Remarks on paratype male** (KUZ Z2162, Figs. 3d and 5). The paratype male has a body length of 134 µm, which is smaller than that of the holotype female. In water-mounted deep preparation (specimen not squeezed), the dorsal plates and intersegmental regions were easily distinguished by the depressed state of the latter regions and the steep slopes between the plates and the intersegmental regions (Fig. 5a). However, when the specimen was squeezed as in the holotype female described above, the intersegmental regions became difficult to delimit margins and also appeared differently depending on how the dorsal cuticle overlapped or subducted. Because of this flexibility of the dorsal cuticle between the segments (at least for this species), we preferred to use the term intersegmental “regions” rather than intersegmental “plates.”

The latero-dorsal processes of the cephalic plate (Fig. 5b) are more evident in the paratype male than in the holotype female. They are more like short longitudinal ridges than the other dome-shaped latero-dorsal processes of the body segments I–III.

The primary clavae (length 7 µm, width 4 µm), secondary clavae (height 6 µm, width 4 µm), and all cephalic cirri (median cirrus 13 µm, internal cirri 13 µm, external cirri 23 µm, lateral cirri 22 µm) do not exhibit any apparent sexual dimorphism (Fig. 5b). A pair of pores that do not penetrate to the

recognizable as regions between postero-dorsal processes of anterior plates and antero-dorsal processes of posterior plates and also as regions with finer sculptures compared to dorsal plates. Intersegmental region

dorsal side, with their openings reinforced by thick cuticles, is present on the ventral surface posterior to the base of the mouth cone (Fig. 5c). Hansen et al. (2012) reported a similar structure in a female of *Faroestygarcus dezioae* Hansen, Kristensen & Jørgensen, 2012; as such, although these pores were observed only in males, we do not consider these to be sexually dimorphic characters. It is not clear why these pores are not recognised in the holotype female.

The caudal segment exhibits sexual dimorphism. The paratype male has an oval gonopore just anterior to the anus (length 8  $\mu\text{m}$ , width 7  $\mu\text{m}$ ) and the paired swellings present in the holotype female are absent (Figs. 3d and 5d). Sexual dimorphism was not recognised in the cirri E (34  $\mu\text{m}$ ) and the lateral processes. The leg IV sensory organs (length 4  $\mu\text{m}$ , width 3  $\mu\text{m}$ ) of the paratype male (Fig. 5e) seem more ovoidal than that of the holotype female, but the possibility of an unsuitable orientation is not ruled out.

## Discussion

Here, we briefly discuss a possible phylogenetic position of *Hansenarctus toyoshiomaruae* gen. et sp. nov. in Stygarctinae based on the phylogeny of stygarctids proposed by both Bello and de Zio Grimaldi (1998) and Hansen et al. (2012). First, both studies inferred the affinity of two genera, *Mesostygarcus* and *Pseudostygarcus*. The synapomorphy proposed by Bello and de Zio Grimaldi (1998), the “ball and socket” articulation, is regarded as absent in *Mesostygarcus* by Hansen et al. (2012), but Hansen et al. (2012) generally provided the following synapomorphies shared by these two genera: the presence of two pairs of lateral processes on each body plate, presence of cuticular membranes on the lateral processes, seminal receptacle ducts looped near terminal vesicles, seminal receptacle openings overlapping the gonopore, seminal receptacle openings with cuticular bars and pockets, and long tapering cirri E situated on large lateral processes. Since the position of cirri E is the only affinity between these two genera and the new genus, it is not likely that the three genera consist the same clade.

Bello and de Zio Grimaldi (1998) also hypothesised the clade *Parastygarcus* + *Stygarcus* and proposed the presence of caudal spikes, elongate, club-shaped secondary clavae, and the presence of long filaments on the internal claws as synapomorphies. Hansen et al. (2012) recovered this topology but it was not strongly supported by statistics and they did not mention this clade in their conclusion. According to Hansen et al. (2012), this clade’s synapomorphies could be summarised as follows: the presence of posterior acute points on the lateral head lobes, presence of long filaments on the internal claws, claws on legs IV slightly larger than those on other legs, and elongate, club-shaped secondary clavae. The relative claw length is the only similarity to *Hansenarctus toyoshiomaruae* gen. et sp. nov. This clade

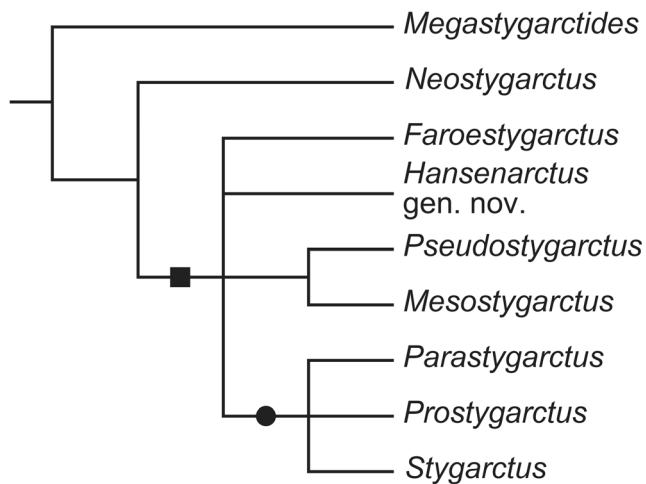
shows closer affinity to *Prostygarcus* in the erect, interiorly bent secondary clavae (Rubal et al. 2013) rather than to the new genus. The unique digitate claws of *Prostygarcus* may have been newly acquired by this clade (see Kristensen et al. (2015) for discussion on digits).

Outside the two clades, *Mesostygarcus* + *Pseudostygarcus* and *Parastygarcus* + *Prostygarcus* + *Stygarcus*, lies *Faroestygarcus*, *Hansenarctus toyoshiomaruae* gen. et sp. nov., and *Neostygarcus*. Hansen et al. (2012) inferred *Faroestygarcus* as a sister group to the rest of Stygarctinae. However, we suggest *Neostygarcus* to be the sister group to the rest of Stygarctinae owing to the character state of the apophyses. Although apophyses dissolve after permanent preparation, a unique character state, exteriorly projected apophyses inserted at the base of the placoids in the pharyngeal bulb, is known in Stygarctidae (for character state distribution, see Table 1). Although the information on the apophyses is of paucity, we suggest the character state to be consistent within each stygarctid genus. In addition, based on the two relationships we discussed above, *Mesostygarcus* + *Pseudostygarcus* and *Parastygarcus* + *Prostygarcus* + *Stygarcus*, we hypothesise the presence of the exteriorly projected apophyses in *Pseudostygarcus* and *Prostygarcus*. With these assumptions, we propose the exteriorly projected apophyses as a synapomorphy of a clade consisting of *Faroestygarcus*, *Hansenarctus* gen. nov., *Mesostygarcus*, *Parastygarcus*, *Prostygarcus*, *Pseudostygarcus*, and *Stygarcus*. Hansen et al. (2012) interpreted the character state polarity in the opposite way and suggested the exteriorly projected apophyses to have been lost in the outgroup taxa and *Neostygarcus* and *Megastygarcidides*. Since this character state is not known outside Stygarctidae, our interpretation is reasonable than that of Hansen et al. (2012).

**Table 1** Number of species with or without exteriorly projected apophyses in the pharyngeal bulb in stygarctid genera. Data from: Hansen et al. (2012), Fujimoto and Miyazaki (2013), Rubal et al. (2013), Fujimoto (2014), Kristensen et al. (2015), Tchesunov (2018), and Ürkmez et al. (2018)

Genus	Present	Absent	Unknown	Total
Megastygarcidinae				
<i>Megastygarcidides</i>	0	4	2	6
Stygarcinae				
<i>Faroestygarcus</i>	1	0	0	1
<i>Hansenarctus</i> gen. nov.	1	0	0	1
<i>Mesostygarcus</i>	1	0	1	2
<i>Neostygarcus</i>	0	4	0	4
<i>Parastygarcus</i>	3	0	4	7
<i>Prostygarcus</i>	0	0	1	1
<i>Pseudostygarcus</i>	0	0	5	5
<i>Stygarcus</i>	4	0	2	6





**Fig. 6** Updated phylogenetic hypothesis of stygarctid genera amended from Hansen et al. (2012). Black circle: a synapomorphy ‘elongate, club-shaped secondary clavæ’; black square: a synapomorphy ‘exteriorly projected apophyses in the pharyngeal bulb’

The relationship between *Faroestygarcus* and *Hansenarctus* gen. nov. is difficult to discuss only with the available morphological information owing to both genera’s monotypy and *Faroestygarcus*’s progenetic morphology (Hansen et al. 2012). However, the similar pores in the cephalic region of *Faroestygarcus* (originally described as ‘internal and highly refractive cuticular funnel-shaped structures’ (Hansen et al. 2012)) and *Hansenarctus* gen. nov. could imply synapomorphy. The cuticular sculptures of the two genera are also similar under light microscopy but we would need to conduct scanning electron microscopy of *Hansenarctus* gen. nov. before further discussion (scanning electron micrographs of *Faroestygarcus* are provided by Hansen et al. (2012)).

The updated phylogeny we suggest for stygarctid genera is shown in Fig. 6. This hypothetical topology is a brief extension of that by Bello and de Zio Grimaldi (1998) and Hansen et al. (2012) based on limited characters. To understand the relationships of *Faroestygarcus*, *Hansenarctus* gen. nov., *Mesostygarcus* + *Pseudostygarcus*, and *Parastygarcus* + *Prostygarcus* + *Stygarcus*, and to settle the position of *Neostygarcus*, we strongly recommend conducting molecular phylogenetic analyses.

**Acknowledgements** We thank the captain and crew of TR/V *Toyoshimaru* of Hiroshima University and the research members of the cruise no. 2016-25 for their assistance in collection on board.

### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

**Sampling and field studies** All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities.

**Data availability** The material observed in this study, KUZ Z2161 and KUZ Z2162, is deposited in the Zoological Collection of Kyoto University.

### References

- Bello G, de Zio Grimaldi S (1998) Phylogeny of the genera of the Stygarctidae and related families. (Tardigrada: Heterotardigrada). *Zool Anz* 237:171–183
- de Zio Grimaldi S, D’Addabbo Gallo M, Morone De Lucia MR (1992) *Neoarctus primigenius* n. g., n. sp., a new Stygarctidae of the Tyrrhenian Sea (Tardigrada, Arthrotardigrada). *Boll Zool* 59:309–313. <https://doi.org/10.1080/11250009209386687>
- Fontoura P, Bartels PJ, Jørgensen A, Kristensen RM, Hansen JG (2017) A dichotomous key to the genera of the Marine Heterotardigrades (Tardigrada). *Zootaxa* 4294(1):1–45. <https://doi.org/10.11646/zootaxa.4294.1.1>
- Fujimoto S (2014) A new *Stygarcus* (Arthrotardigrada: Stygarctidae) from Japan, with entangled seminal receptacle ducts. *Zootaxa* 3784(2):187–195. <https://doi.org/10.11646/zootaxa.3784.2.8>
- Fujimoto S, Miyazaki K (2013) *Neostygarcus lovedeluxe* n. sp. from the Miyako Islands, Japan: The first record of Neostygarcidae (Heterotardigrada: Arthrotardigrada) from the Pacific. *Zool Sci* 30(5):414–419. <https://doi.org/10.2108/zsj.30.414>
- Fujimoto S, Yamasaki H (2017) A new genus and species of Renaudarctidae (Heterotardigrada: Arthrotardigrada) from Ryukyu Archipelago, Japan. *Mar Biol Res* 13(3):288–299. <https://doi.org/10.1080/17451000.2016.1257809>
- Fujimoto S, Jørgensen A, Hansen JG (2017) A molecular approach to arthrotardigrade phylogeny (Heterotardigrada, Tardigrada). *Zool Scr* 46:496–505. <https://doi.org/10.1111/zsc.12221>
- Gallo D’Addabbo M, de Zio Grimaldi S, Sandulli R (2001) Heterotardigrada of two submarine caves in S. Domino Island (Tremiti Islands) in the Mediterranean Sea with the description of two species of Stygarctidae. *Zool Anz* 240:361–369. <https://doi.org/10.1078/0044-5231-00043>
- Giere O (2009) Meiobenthology: the microscopic motile fauna of aquatic sediments. Springer-Verlag, Berlin Heidelberg
- Grimaldi de Zio S, D’Addabbo Gallo M, Morone De Lucia MR (1982) *Neostygarcus acanthophorus*, n. gen. n. sp. nuovo Tardigrado marino del Mediterraneo. *Cah Biol Mar* 23:319–324
- Grimaldi de Zio S, D’Addabbo Gallo M, Morone De Lucia MR (1987) Adaptive radiation and phylogenesis in marine Tardigrada and the establishment of Neostygarcidae, a new family of Heterotardigrada. *Boll Zool* 54:27–33. <https://doi.org/10.1080/11250008709355552>
- Hansen JG, Kristensen RM (2006) The ‘hyena female’ of tardigrades and descriptions of two new species of *Megastygarcoides* (Arthrotardigrada: Stygarctidae) from Saudi Arabia. *Hydrobiologia* 558:81–101. <https://doi.org/10.1007/s10750-005-1409-5>
- Hansen JG, Kristensen RM, Jørgensen A (2012) The armoured marine tardigrades (Arthrotardigrada, Tardigrada). *Sci Danica Ser B Biol R Dan Acad Sci Lett* 2:1–91
- Jørgensen A, Faurby S, Hansen JG, Møbjerg N, Kristensen RM (2010) Molecular phylogeny of Arthrotardigrada (Tardigrada). *Mol Phylogenet Evol* 54:1006–1015. <https://doi.org/10.1016/j.ympev.2009.10.006>
- Kristensen RM (1983) Loricifera, a new phylum with Aschelminthes characters from the meiobenthos. *Z Zool Syst Evol* 21:163–180. <https://doi.org/10.1111/j.1439-0469.1983.tb00285.x>

- Kristensen RM, Higgins RP (1984) A new family of Arthrotardigrada (Tardigrada: Heterotardigrada) from the Atlantic coast of Florida, U.S.A. *Trans Am Microsc Soc* 103:295–311. <https://doi.org/10.2307/3226191>
- Kristensen RM, Sørensen MV, Hansen JG, Zeppilli D (2015) A new species of *Neostygarctus* (Arthrotardigrada) from the Condor Seamount in the Azores, Northeast Atlantic. *Mar Biodivers* 45(3): 453–467. <https://doi.org/10.1007/s12526-015-0323-y>
- Marcus E (1927) Zur Anatomie und Ökologie mariner Tardigraden. *Zool Jahrb Abt Syst Ökol Geogr Tiere* 53:487–558
- McKirdy DJ, Schmidt P, McGinty-Bayly M (1976) Interstitielle Fauna von Galapagos XVI. Tardigrada Mikrofauna des Meeresbodens 58: 409–449
- Morone De Lucia MR, Grimaldi de Zio S, D'Addabbo Gallo M (1984) Description of *Parastygarctus biungulatus* n. sp. and hypothesis of phylogeny in the Stygarctidae family (Heterotardigrada: Arthrotardigrada). *Oealia* 10:85–94
- Renaud-Debyser J (1965) *Parastygarctus higginsi* n. g., n. sp., Tardigrade marin Interstitiel de Madagascar. *C R Acad Sci Paris* 260:955–957
- Renaud-Mormant J (1974) Une nouvelle famille de tardigrades marins abyssaux: les Coronarctidae fam. nov. (Heterotardigrada). *C R Acad Sci Paris* 278:3087–3090
- Renaud-Mormant J (1979) Tardigrades marins de Madagascar. II. Stygarctidae et Orellidae, III. Considérations écologiques générales. *Bull Mus nat His Nat Paris 4e Sér 1 Sect A(2)*:339–351
- Rubal M, Veiga P, Fontoura P, Sousa-Pinto I (2013) A new intertidal arthrotardigrade, *Prostygarctus aculeatus* gen. nov., sp. nov. (Tardigrada: Heterotardigrada) from the North of Portugal (Atlantic ocean). *J Limnol* 72(s1):8–14. <https://doi.org/10.4081/jlimnol.2013.s1.e2>
- Schulz E (1951) Über *Stygarctus bradypus* n. g. n. sp., einen Tardigraden aus Küstengrundwasser und seine phylogenetische Bedeutung. *Kiel Meeresforsch* 8:86–97
- Tchesunov AV (2018) A new tardigrade species of the genus *Neostygarctus* Grimaldi de Zio et al., 1982 (Tardigrada, Arthrotardigrada) from the Great Meteor Seamount, Northeast Atlantic. *Eur J Taxon* 479:1–17. <https://doi.org/10.5852/ejt.2018.479>
- Thulin G (1928) Über die Phylogenie und das System der Tardigraden. *Hereditas* 11:207–266. <https://doi.org/10.1111/j.1601-5223.1928.tb02488.x>
- Ürkmez D, Ostrowska M, Roszkowska M, Gawlak M, Zawierucha K, Kristensen RM, Kaczmarek Ł (2018) Description of *Megastygarctides sezginii* sp. nov. (Tardigrada: Arthrotardigrada: Stygarctidae) from the Turkish Black Sea coast and a key to the genus *Megastygarctides*. *Mar Biol Res* 14:1–16. <https://doi.org/10.1080/17451000.2017.1342845>
- Wessel P, Smith WHF, Scharroo R, Luis J, Wobbe F (2013) Generic mapping tools: improved version released. *EOS Trans AGU* 94(45):409–410. <https://doi.org/10.1002/2013EO450001>

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.