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Rediscovery after 25 years – first photographic documentation and DNA barcoding of the deep-sea pycnogonid species *Ascorhynchus hippos* Turpaeva, 1994 (Chelicerata, Pycnogonida, Ascorhynchidae) from the Kuril-Kamchatka Trench

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Abstract

The female specimen of *Ascorhynchus hippos* Turpaeva, 1994 was collected in 2015 during the Russian-German deep-sea expedition SokhoBio (Sea of Okhotsk Biodiversity Studies) at the abyssal western slope of the Kuril-Kamchatka Trench at a depth of 4469 m using a camera-epibenthic sledge. It is the first record of this species since the discovery of one female holotype and one male paratype in 1990. *Ascorhynchus hippos* is easily distinguishable from its congeners by the two prominent tubercles above the chelifore insertions, the absence of the eye tubercle and eyes, and the tubercles on the mid-dorsal trunk segments and the lateral processes. Here we present the first photographic documentation of all three known specimens of *A. hippos* and the COI barcode of the new specimen is also provided.

Key Words

COI Barcode, Northwest Pacific Ocean, sea spiders, SokhoBio Expedition 2015

Introduction

The genus *Ascorhynchus* Sars, 1877 is characterized by a trunk with segment borders which have high flaring posterior rims, and which sometimes have median tubercles. In addition, the lateral processes are often found with dorsodistal tubercles or small lateral tubercles and the trunk is mostly smooth, without setae or spines (Fry and Hedgpeth 1969; Child 1992). Perhaps the most characteristic feature of the genus is the large proboscis which usually has 1 or 2 constrictions and is highly mobile (Arnaud and Bamber 1987).

To date, 78 *Ascorhynchus* species are described (Bamber et al. 2022). The genus is often included in Ammotheidae Dohrn, 1881, but it has recently been removed from this family and transferred to Ascorhynchidae Hoek, 1881 (Arango and Wheeler 2007). However, the placement, monophyly, and composition of Ascorhynchidae are still uncertain (Sabroux et al. 2017; Ballesteros et al. 2021).

After 25 years, the species *Ascorhynchus hippos* Turpaeva, 1994 was rediscovered during the SokhoBio Expedition in 2015 about 1000 km southwest from the type locality. The Kuril-Kamchatka Trench, where all three specimen known so far (holotype, paratype, and new specimen) were found, is one of the deepest trenches of the World Ocean with a maximum depth of 10542 m (Angel 1982). Compared to other trenches, the bottom fauna of the Kuril-Kamchatka Trench is relatively well studied. Main studies were performed by Russian scientists since 1949 using materials from several expeditions with the

R/V Vityaz (e.g., (Kamenev 2019); Turpaeva (1971a); (Turpaeva 1971b) or the R/V Akademik Mstislav Keldysh (e.g. Turpaeva (1994)). Following these footsteps, the two Russian-German deep-sea expeditions KuramBio (Kurile Kamchatka Biodiversity Studies) in 2012 and SokhoBio (Sea of Okhotsk Biodiversity Studies) in 2015 were realised (Brandt and Malyutina 2015; Malyutina et al. 2018).

Material and methods

Specimen from the SokhoBio Expedition 2015

1 female (gravid); R/V "Akademik M.A. Lavrentyev", 71st cruise, sampling event 10-7; NW Pacific, south off Kuril Island Simushir; 46°07.80'N, 152°10.30'E – 46°07.30'N, 152°11.50'E; 4469 m depth; 29.07.2015; camera epibenthic sledge (C-EBS); deposited in the SNSB – Bavarian State Collection of Zoology, Arthropoda varia section, ZSMA20171084.

Holotype

1 female; R/V "Akademik Mstislav Keldysh", 22nd cruise, station 2325; NW Pacific, east off Kamchatka; 53°27.70'N, 160°59.30'E – 53°24.97'N, 160°57.17'E; 3106–2992 m; 12.08.1990; Sigsbee trawl; deposited in the Shirshov Institute of Oceanology Russian Academy of Science, Ocean Benthic Fauna Lab. Collection, INV0000793.

Paratype

1 male; R/V "Akademik Mstislav Keldysh", 22nd cruise, station 2323; NW Pacific, east off Kamchatka; 53°05.40'N, 161°55.20'E – 53°07.00'N, 161°56.12'E; 4890–4984 m; 10.08.1990; Sigsbee trawl; deposited in the Shirshov Institute of Oceanology Russian Academy of Science, Ocean Benthic Fauna Lab. Collection, INV0000792.

Image stacks

Photo series were taken either with a NIKKOR 85 mm f/3.5G lens mounted on a Nikon D7000 camera combined with a Cognisys STKS-C-StackShot apparatus or with a Nikon V1 camera mounted on a Leica Z 16 APO stereo microscope. Up to 28 photos were combined into a single composite image with a greater field of depth using HELICON FOCUS 5.3 (HeliconSoft).

DNA barcoding

Right leg 3 with muscle tissue was taken for DNA barcoding from the female specimen from the SokhoBio Expedition. DNA extraction, amplification, and sequencing of the COI gene were carried out by AIM – Advanced Identification Methods GmbH (Leipzig, Germany). The DNA sequence is available from GenBank under the accession number MW916507.

Results

Comparison with the holotype

The female specimen from the SokhoBio Expedition 2015 generally resembles the female holotype of *A. hippos* (Figs 1–3). However, some features differ. The shape of the two prominent horn-like tubercles above the chelifore insertions varies. In the specimen from the SokhoBio Expedition they are more U-shaped and in the holotype V-shaped (Fig. 1C, F). In the male paratype, the right tubercle is stunted but they also seem to be V-shaped (Fig. 1I). Moreover, the proboscis of the specimen from the SokhoBio Expedition is, in relation to the trunk length, longer than in the holo- and paratypes (Fig. 1B, E, H). Altogether, it seems that the specimen from the SokhoBio Expedition belongs to *A. hippos*. Nevertheless, more specimens are needed to decide if these differences are just an intraspecific variation or may imply that, in fact, these are two different species.

Comparison with other species

There are seven species of deep-sea *Ascorhynchus* known from the Kuril-Kamchatka Trench so far: *A. bucerus* Turpaeva, 1971, *A. mariae* Turpaeva, 1971, *A. losinalosinskii* Turpaeva, 1971, *A. inflatus* Stock, 1963, *A. japonicum* Ives, 1891, *A. levivani* Turpaeva, 1994, and *A. hippos* Turpaeva, 1994 (Turpaeva 1971a, b; 1994). In contrast to *A. hippos* (Figs 1A, B, 2A, 3A, B) and *A. levivani*, all others bear an ocular tubercle. However, only *A. inflatus*, *A. losinalosinskii* and *A. japonicum* have (rudiments of) eyes whereas *A. mariae* and *A. bucerus* have a reduced ocular tubercle without eyes (Stock 1963; Turpaeva 1971a, b). One of the most characteristic features of *A. hippos* is certainly the two prominent horn-like tubercles above the chelifore insertions (Figs 1A, C, 2A, 3A, B), a feature which this species shares with *A. inflatus* and *A. bucerus*. However, in comparison to *A. hippos*, these tubercles are small in *A. inflatus* and slender and pointed in *A. bucerus* (Stock 1963; Turpaeva 1971a, b). Besides these significant differences, in *A. inflatus* the tubercles on the mid-dorsal trunk segments and the lateral processes are more pointed and taller than in *A. hippos* (Figs 1A, 2G, 3A–C). In *A. losinalosinskii*, the tubercles on the mid-dorsal trunk segments are taller and on the lateral processes, they are smaller than in *A. hippos*. In *A. japonicum*, tubercles are present on the mid-dorsal trunk but are absent on the lateral processes. Lastly,

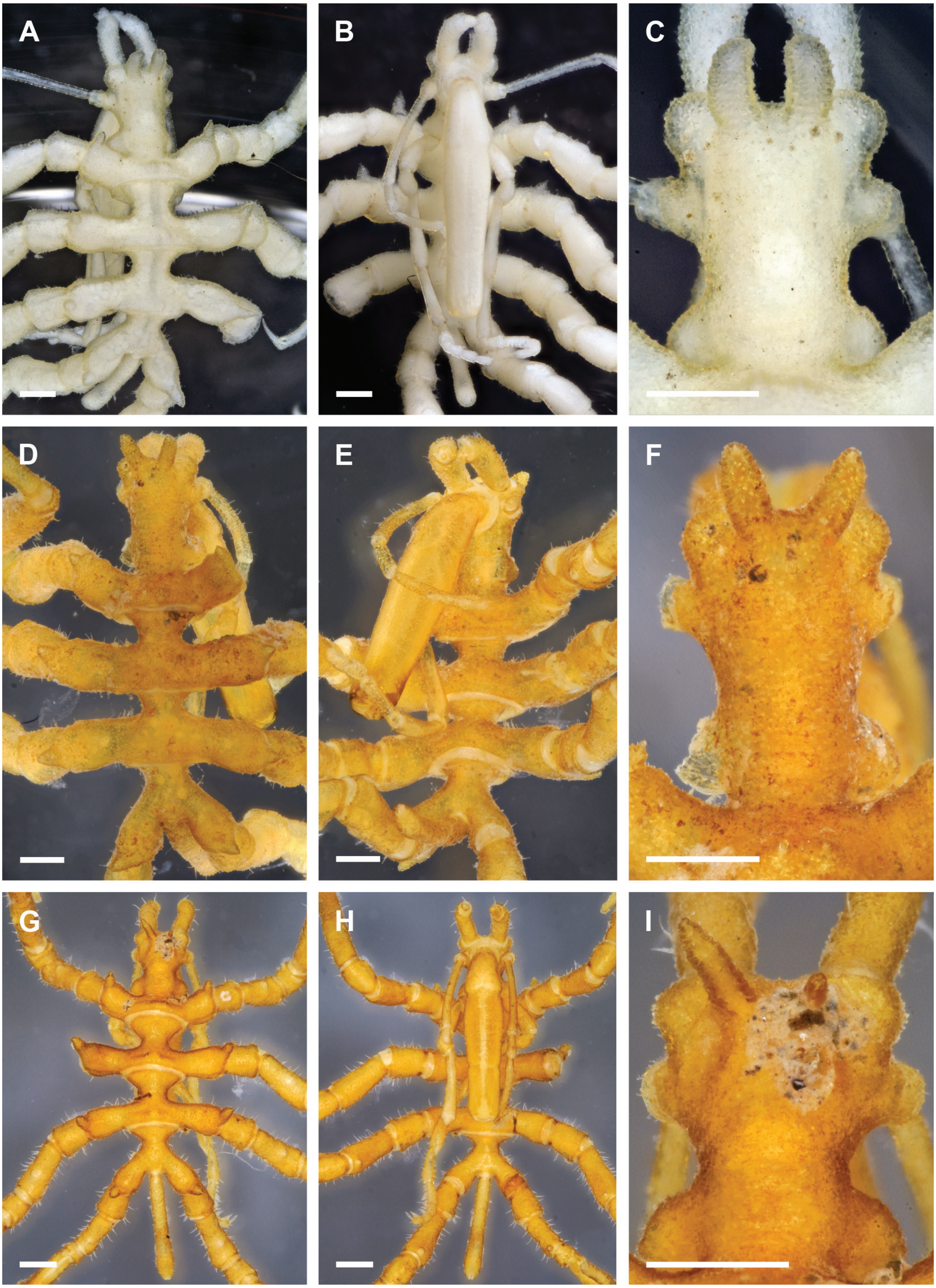

Figure 1. *Ascorhynchus hippos*, specimen from the SokhoBio Expedition 2015 (**A–C**), female holotype (**D–F**) and male paratype (**G–I**). Each with dorsal view (**A, D, G**), ventral view (**B, E, H**), and detail of the two prominent tubercles above the chelifore insertions (**C, F, I**). Scale bars: 500 µm.

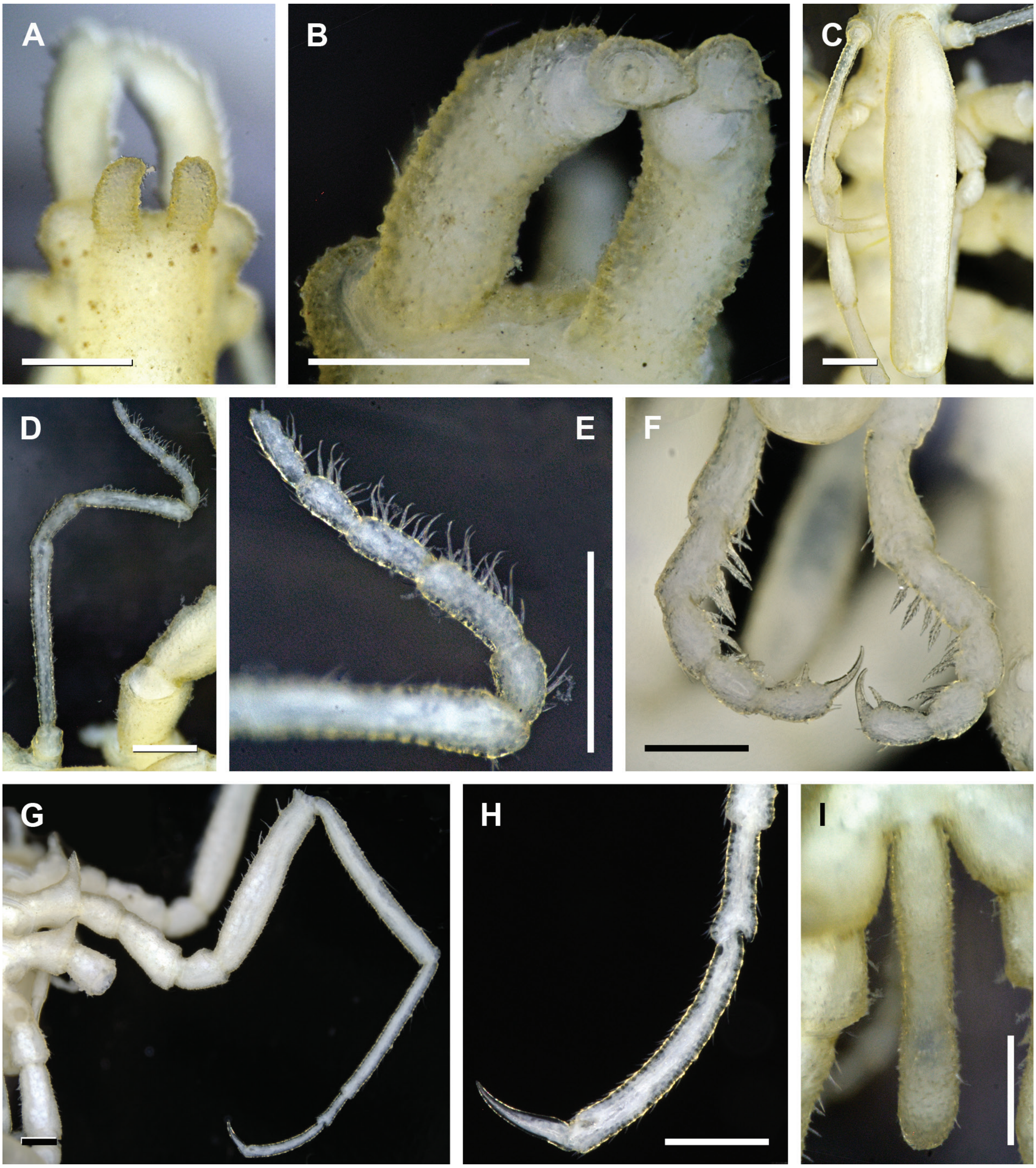

Figure 2. Details of *Ascorhynchus hippos* specimen from the SokhoBio Expedition 2015. **A.** Two prominent tubercles above the chelifore insertions; **B.** Chelifores; **C.** Proboscis; **D.** Palp; **E.** Distal articles of palp; **F.** Distal articles of oviger; **G.** Right leg 2; **H.** Tarsus, propodus, and claw of right leg 2; **I.** Abdomen. Scale bars: 500 µm.

A. mariae, *A. bucerus* and *A. levivani* do not bear any tubercles on the mid-dorsal trunk or on the lateral processes (Ives 1891; Stock 1963; Turpaeva 1971a, b, 1994).

Of all other deep-sea species of *Ascorhynchus* of the World Ocean, *A. hippos* most resembles *A. antipodus* Child, 1987 and *A. extenuata* (Calman, 1938). However, *A. hippos* is easily distinguishable from these two species. *Ascorhynchus antipodus* is found in the area of the Antipodes Islands (South Pacific) at a depth of 5340 m, lacks an eye tubercle, and bears two anterolateral tubercles that hang over the chelifores (Child 1987). These tubercles differ from the horn-like tubercles of *A. hippos* in their conical, pointed shape, their much smaller size, and they are set much wider apart. In addition, the long chelifores with chelae and the absence of dorsal trunk tubercles help differentiate it from *A. hippos*. In *A. extenuata*, present in the Zanzibar area at 925–2926 m depth (Calman 1938), the tubercles are conical and pointed and are also set further apart than in *A. hippos*. Additional

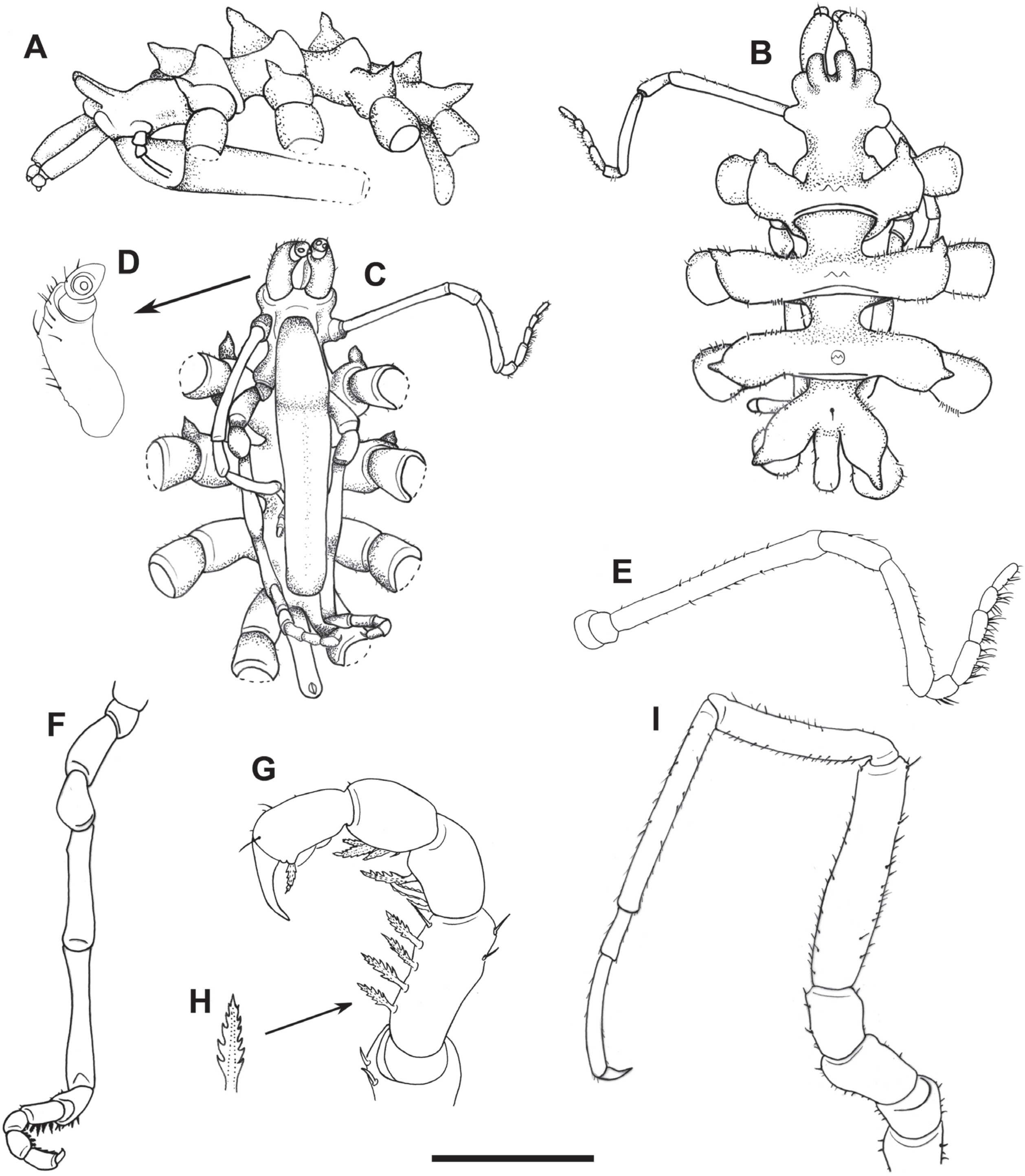

Figure 3. Details of *Ascorhynchus hippos* specimen from the SokhoBio Expedition 2015. **A.** Lateral view; **B.** Trunk dorsal view; **C.** Trunk ventral view; **D.** Left chelifore; **E.** Palp; **F.** Oviger; **G.** Strigilis with terminal claw; **H.** Compound spine of oviger; **I.** Second leg; Scale bars: 1.8 mm (**A**); 2.3 mm (**B**); 1.9 mm (**C**); 0.9 mm (**D**); 1 mm (**E**); 1.3 mm (**F**); 0.3 mm (**G**); 0.2 mm (**H**); 1.6 mm (**I**).

characters, which separate it from *A. hippos*, include the scapes composed of two articles with chelae, the different shape of the proboscis, and also the different mid-dorsal spines or tubercles on the trunk segments.

Another two blind species of deep-sea *Ascorhynchus* with horn-like tubercles near the frontal margin were found in New Caledonia: *A. fragilis* Stock, 1991 and *A. pilipes* Stock, 1991 (Stock 1991). However, *A. fragilis* and *A. pilipes* have, as their names indicate, generally a much thinner and more setose, respectively, appearance.

COI barcode sequence

DNA sequence is available from GenBank (accession number MW916507).

ATAAGAATTTTAATTCGAACAGAATTAGGTA-CACCTTCTTCCTTAATTGGTGATGATCAAATC-TATAATGTAATCGTTACTTCCCATGCATTTAT-TATAATTTTTTTTATAGTTATACCTATAATAATCG-GAGGATTTGGAAATTGATTAGTCCCTTTAATA-ATCGGAGCTCCTGATATAGCTTTTCCACGAATA-AATAATATAAGATTTTGGCTACTACCTCCTTCTTT-GACTCTTCTATTAACTTCATCCTTAATTGAAA-GAGGAAGGGGAACAGGATGAACAATTTATC-CCCCTTTATCTTCAAATATCTCTCATTCTG-GATCTTCAGTAGACTTAACTATTTTTTTCTTTA-CATCTCGCAGGCGCTTCTTCAATTTTAGGAG-CAATTAATTTTATCACTACCATTGTAAATATAC-GTTCTCCTGGTATAACTTTAGAACAAAT-TCCTTTATTTGTATGAAGAGTTATAATTACAG-CCATTTTATTATTATTATCTTTACCTGTTTTAG-CAGGAGCTATTACTATACTTCTTACTGATC-GGAATTTTAATACATCTTTCTTTGACCCAG-CAGGAGGAGGAGACCCAATTTTATATCAA-CATTTATTTTGATT

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