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Record of deep-sea *Nephasoma constricticervix* (Sipuncula, Golfingiidae) from the Guinea Basin (South-East Atlantic)

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Abstract

Six specimens of *Nephasoma constricticervix* are reported from the Guinea Basin from 5141–5171 m depth, collected during the Diva II expedition. This species was reported earlier from this region as "unpublished record". Most characteristic are the long introvert hooks (up to 123 µm in our specimens), which decrease in size posteriorly and a constriction between introvert and trunk. An SEM investigation of the hooks shows their fine structure and the presence of papillae associated with the hooks.

Key Words

Deep-sea, distribution, Golfingiidae, morphology, SEM

Introduction

Sipunculans commonly occur in deep-sea sediments, but we know much less about the deep-water species compared to species from shallower waters (Kędra and Shields 2011; Saiz et al. 2018). About one third of the approximately 150 species of sipunculans occur in deep water (Saiz et al. 2018). They are mainly deposit feeders in soft sediments (Murina 1984; Kędra et al. 2018). At least some species produce deep burrows and are therefore important ecosystem engineers (Romero-Wetzel 1987; Shields and Kędra 2009). The deep-sea sipunculan fauna of northern oceans has been described better than that of tropical or southern oceans (e.g. Kędra and Murina 2007; Kędra and Shields 2011; Kędra et al. 2018), but Saiz et al. (2018) have identified hotspots of deep-sea sipunculan diversity in different regions, including the Eastern part of the South Atlantic.

A genus with several deep-sea species is *Nephasoma* Pergament, 1940. Species are often very small and difficult to dissect; taxonomically important characters are few or difficult to observe. Therefore, the genus has a vivid taxonomic history with discussions about the validity of synonyms (Cutler and Cutler 1986; Kędra and Shields 2011). Currently, 25 species are regarded as valid. 23 of these species go back to a review of the genus by Cutler and Cutler (1986), two further species were described later by Kawauchi and Rice (2009). Most literature about *Nephasoma* species concerns taxonomy and distribution. Morphological characters are usually documented by drawings and rarely by fine structural documentation such as scanning electron microscopy (SEM). Such images are only available from *N. pellucidum* (Keferstein, 1865) (Schulze and Rice 2009), *N. columbaris* Kawauchi & Rice, 2009 (Kawauchi and Rice 2009) and in a short comparison of hooks from *N. abyssorum* (Koren & Danielssen, 1875), *N. diaphanes* (Gerould, 1913) and *N. lilljeborgi* (Danielssen & Koren, 1880) (Kędra and Shields 2011).

Nephasoma is part of the Golfingiidae, which has changed a bit over time. Traditional classification included *Golfingia*, *Nephasoma* and *Thysanocardia* in the family (Cutler and Gibbs 1985). Now the family also includes the genera *Phascolion*, *Onchnesoma* and *Themiste*, which makes the golfingiids the richest in genera and species within the Sipuncula (Schulze et al. 2005; Kawauchi et al. 2012; Lemer et al. 2015).

Here is made the formal record of *Nephasoma constricticervix* from the Guinea Basin, including microscopical and SEM observations. The specimens were

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collected during the DIVA II expedition. The expedition report (Türkay and Pätzold 2009) mentions the presence of about 15 specimens of sipunculans and echiurids in box-core samples, including a large *Nephasoma* speciemen of 10 cm length. The small specimens reported here were originally overlooked and sorted out later from bulk-fixed sediment samples.

Material and methods

Six specimens were collected from samples taken during the Meteor ME 63-2 DIVA II expedition to the South-East Atlantic. Sediment was taken with a box corer and bulk fixed in 4% formalin. Fauna was sorted out later by the German Center for Marine Biodiversity Research (DZMB: Deutsches Zentrum für Marine Biodiversitätsforschung) and transferred to 70% ethanol.

Specimens were first photographed with a digital microscope using the stacking function (Keyence VHX-7000). For scanning electron microscopy (SEM), specimens were dehydrated in a series of increasing ethanol concentrations, critical point dried in a Leica EM CPD300, sputter coated with platinum in a Polaron SC7640 Sputter Coater and investigated with a LEO SEM 1524. Specimens are stored as SEM objects in the Museum for Nature of the LIB (Leibniz Institute for the Analysis of Biodiversity Change), Hamburg. Accession numbers, station number, dates, depth and coordinates are given below in the results section. One specimen (ZMH-ANN-031325) was lost during preparation for SEM.

Results

Golfingiidae Stephen & Edmonds, 1972

Nephasoma constricticervix Cutler, 1969

Nephasoma constricticervix Cutler, 1969: 215–217, fig. 4 (type locality: Western North Atlantic, 33°57'N, 65°47'W).

Material examined. 6 specimens were collected at 5 stations of the DIVA II expedition Meteor ME 63-2 from the Guinea Basin:

South Atlantic Ocean • 1 specimen; Guinea Basin; 00°50.04'N, 005°35.03'W; 5,141 m depth; 19 Mar. 2005; St. 81; no number (specimen lost). • 1 specimen; Guinea Basin; 00°50.07'N, 005°35.04'W; 5141 m; 19. Mar. 2005; St. 82; ZMH-ANN-031326. • 1 specimen; Guinea Basin; 00°50.00'N, 005°34.97'W; 5144 m; 19. Mar. 2005; St. 83; ZMH-ANN-031327. • 1 specimen; Guinea Basin; 00°37.23'N, 006°28.13'W; 5171 m; 24. Mar. 2005; St. 101; ZMH-ANN-031328. • 2 specimens; Guinea Basin; 00°37.25'N, 006°28.13'W; 5171 m; 24. Mar. 2005; St. 102; ZMH-ANN-031329 and ZMH-ANN-031330.

Description. Specimens measure between approximately 2.3 mm (smallest specimen, Fig. 1a) and 16.3 mm (longest specimen, Fig. 1c). Specimens were generally not in very good condition and the trunk was partly injured, making it sometimes difficult to recognize whether specimens are complete or fragments. Some specimens have a bulbous introvert (Fig. 1a), in others the introvert is more slender (Fig. 1b, c). There can be further thickenings of the body at irregular positions (e.g. Fig. 1c). Some, but not all specimens had a constriction at the transition between introvert and trunk (Fig. 1b, c, e, g). The length of the trunk is roughly three to four times the length of the introvert (Fig. 1b, c, e), but the introvert is not fully everted in all specimens. Specimens are yellowish to light brownish and transparent. Few details of the trunk could be recorded, for example the coiling intestine (Fig. 1d) and a sediment-filled gut (Fig. 1h) in specimen ZMH-ANN-031326 and oocytes in specimen ZMH-ANN-031330 (Fig. 1e, f). The trunk surface was not well preserved, but there are no indications of local thickenings of the cuticle (pseudoshields).

None of the specimens had their tentacles everted, but the introvert shows slender hooks up to the anterior tip (Fig. 2a, b) or starting slightly further posterior (Fig. 2c, d). In all specimens hook length decreases from anterior to posterior. The anterior hooks are longest in specimen ZMH-ANN-031329 and measure about 123 µm, in other specimens they measure 93 µm (ZMH-ANN-031328), 75 µm (ZMH-ANN-031330), 68 µm (ZMH-ANN-031326) and 40 µm (ZMH-ANN-031327). They decrease to about 20 µm length in all specimens. Hook arrangement appears to be in a spiral in specimen ZMH-ANN-031330 (Fig. 2a), but in other specimens it appeared more unordered (Fig. 2c, f, h). One specimen (ZMH-ANN-031328) had anterior hooks followed by a region free of hooks and further posterior hooks (Fig. 2g, h). The region free of hooks included several semispherical elevations (Figs 2h, 3b). In one specimen (ZMH-ANN-031329) the introvert is injured (Fig. 2e, f). Because the introvert is not fully everted, it can not be excluded that further hooks are present in the anterior, still inverted part of the introvert.

There is a sharp border between hooks and the surface of the introvert (Fig. 3a–e). Each hook sits on the frontal side of an elevation of the introvert epidermis (Fig. 3a, d), which is called here socket. The elevations in the hook-free region of specimen ZMH-ANN-031328 appear to be these basal sockets of the hooks. One detached hook was observed in this specimen lying with the tip pointing in frontal direction (Fig. 3b).

The basal sockets of the hooks carry one or two papillae (Fig. 3a, d–f). Papillae are semispherical and have a round tip structure (Fig. 3d, f). Mostly there is one papilla per hook, but sometimes there are two, a regular pattern concerning the number of papillae could not be observed. Further papillae are present anterior of the hooks. The shape of the hooks is slender and elongate, slightly backwards curved in the upper part. The lower side of the hooks is set off against the remaining hook and has a slight extension towards the socket (Fig. 3a, d).

Figure 1. General shape and body regions. **a.** Specimen ZMH-ANN-031328, anterior fragment showing trunk (tr) and introvert (in) with hooks (ho) anterior and posterior of a hook-free region; **b.** Specimen ZMH-ANN-031327; **c.** Specimen ZMH-ANN-031326; **d.** Magnification from **c** showing the coiled intestine in an inflated body region; **e.** Specimen ZMH-ANN-031330; **f.** Magnification of oocytes (oo) from **e**; **g.** Constriction between introvert and trunk in specimen ZMH-ANN-031326; **h.** Sediment-filled intestine in specimen ZMH-ANN-031326.

Discussion

Because the preservation of the trunk is far from optimal, a comparison of the newly reported Guinea Basin specimens with already described species is based on shape, size and arrangement of the hooks, general body form, the constriction between introvert and trunk and the absence of a pseudoshield. The Guinea Basin specimens have comparably long and slender hooks. Some *Nephasoma* species have no hooks; most have quite short hooks and few have longer hooks (Cutler and Cutler 1986). With up to 123 µm the Guinea Basin specimens are comparable in length to *N. abyssorum* (Koren & Danielssen, 1875), *N. constricticervix* Cutler, 1969, *N. cutleri* (Murina, 1975) and *N. vitjazi* (Murina, 1964). *Nephasoma abyssorum* has characteristic hooks with a broad base, dark staining and spiral arrangement (see Cutler and Cutler 1986; see also SEM image in Kędra and Shields 2011, which is not very informative). *Nephasoma constricticervix* and *N. vitjazi* both have very long and slender hooks, they are up to 250 µm in *N. constricticervix* and up to 280 µm in *N. vitjazi* (Murina 1964; Cutler 1969; Cutler and Cutler 1986). *Nephasoma vitjazi* has local thickenings of the cuticle known as pseudoshield (Murina 1964), which is not present in the Guinea Basin specimens. *Nephasoma cutleri* has hooks up to 150 µm length, but it has a longer intro-

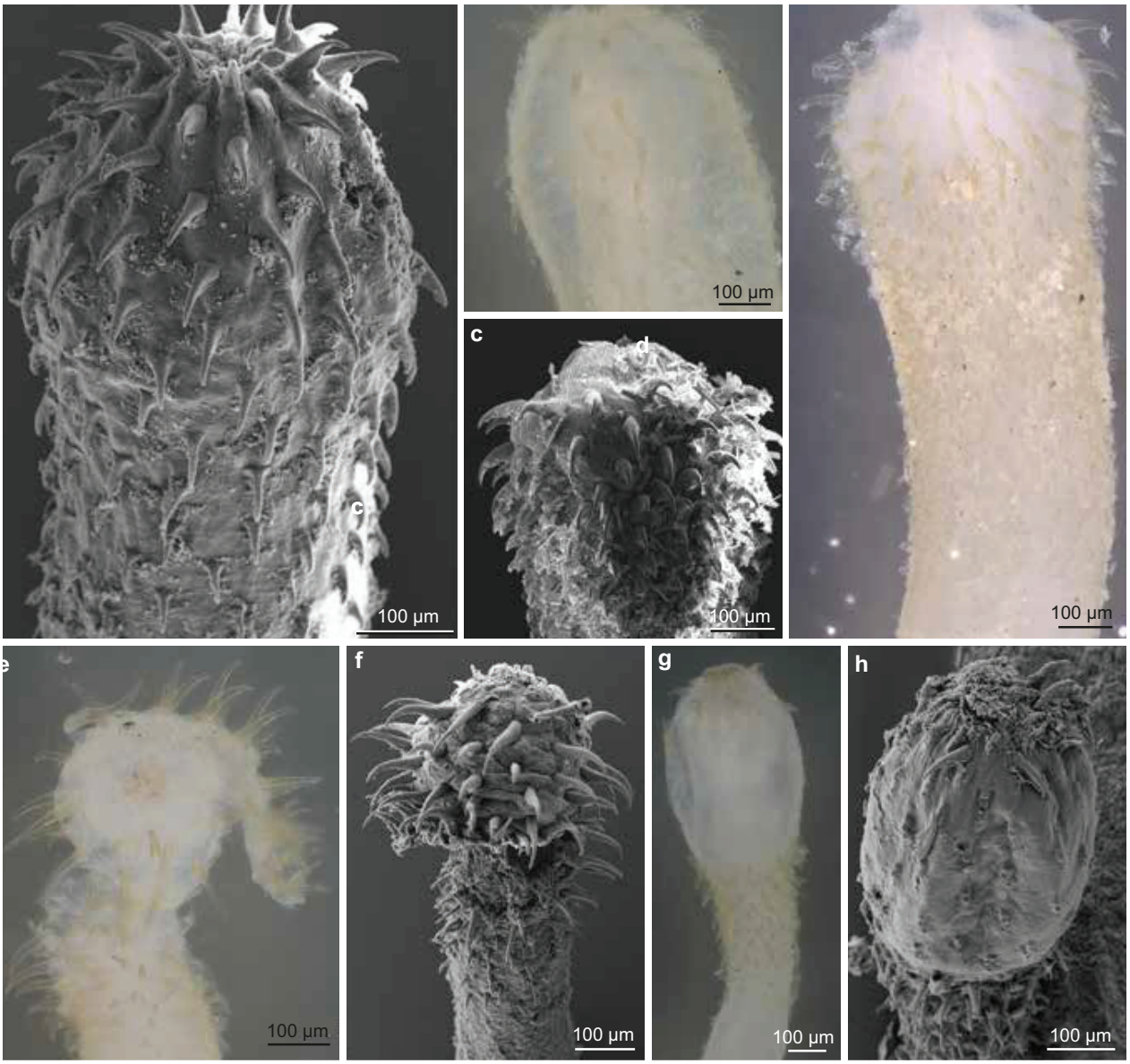

Figure 2. Anterior introvert, each introvert is shown by SEM and by digital microscopy. **a, b.** Specimen ZMH-ANN-031330; **c, d.** Specimen ZMH-ANN-031326; **e, f.** Specimen ZMH-ANN-031329; **g, h.** Specimen ZMH-ANN-031328.

vert compared to the trunk length than the Guinea Basin specimens, at least in the observed state of introvert eversion. Some specimens have a constriction between between introvert and trunk, this has been reported only for *N. constricticervix* and *N. constrictum* (Southern, 1913). *Nephasoma constrictum* differs from the Guinea Basin specimens from being flask-shaped and having pigmented, raised papillae (see Cutler and Cutler 1986). Therefore, from the species mentioned above, the Guinea Basin specimens resemble closest *N. constricticervix*, although their hook length is shorter than the reported values for *N. constricticervix* (Cutler and Cutler 1986). Longer hooks, however, may be present in the uneverted part of the introvert in the Guinea Basin specimens. Additionally, the Guinea Basin likely matches the reported distribution of *N. constricticervix*. It has been reported from the Western and (rarely) from the Eastern North Atlantic: close to Bermuda in 1500–4815 m depth (Cutler 1969), Puerto Rico in 5499 m depth (Cutler and Cutler 1980a), the Gulf of Gascogne in 1985–2360 m depth (Cutler and Cutler 1980b) and off West Africa (21°02'07"N, 21°33'06"W; Murina 1993) in 4284 m depth. Murina (1993) additionally reports one specimen from the Antarctic Weddell Sea in 4535 m depth (70°40'06"S, 15°47'08"W) and mentions further unpublished records from the southern Pacific Ocean. Cutler and Cutler (1980a) and Cutler and Cutler (1986) mention unpublished records from the Eastern South Atlantic until 22°S. It is not completely clear why these southeastern records were published in this vague way. Probably the specimens were not obvious enough (introvert not fully extended, bad preservation etc.). The other species with longer hooks differ in their distribution: *N. abyssorum* is a species with broad distribution, but most abundant in the Northeast Atlantic, *N. constrictum*

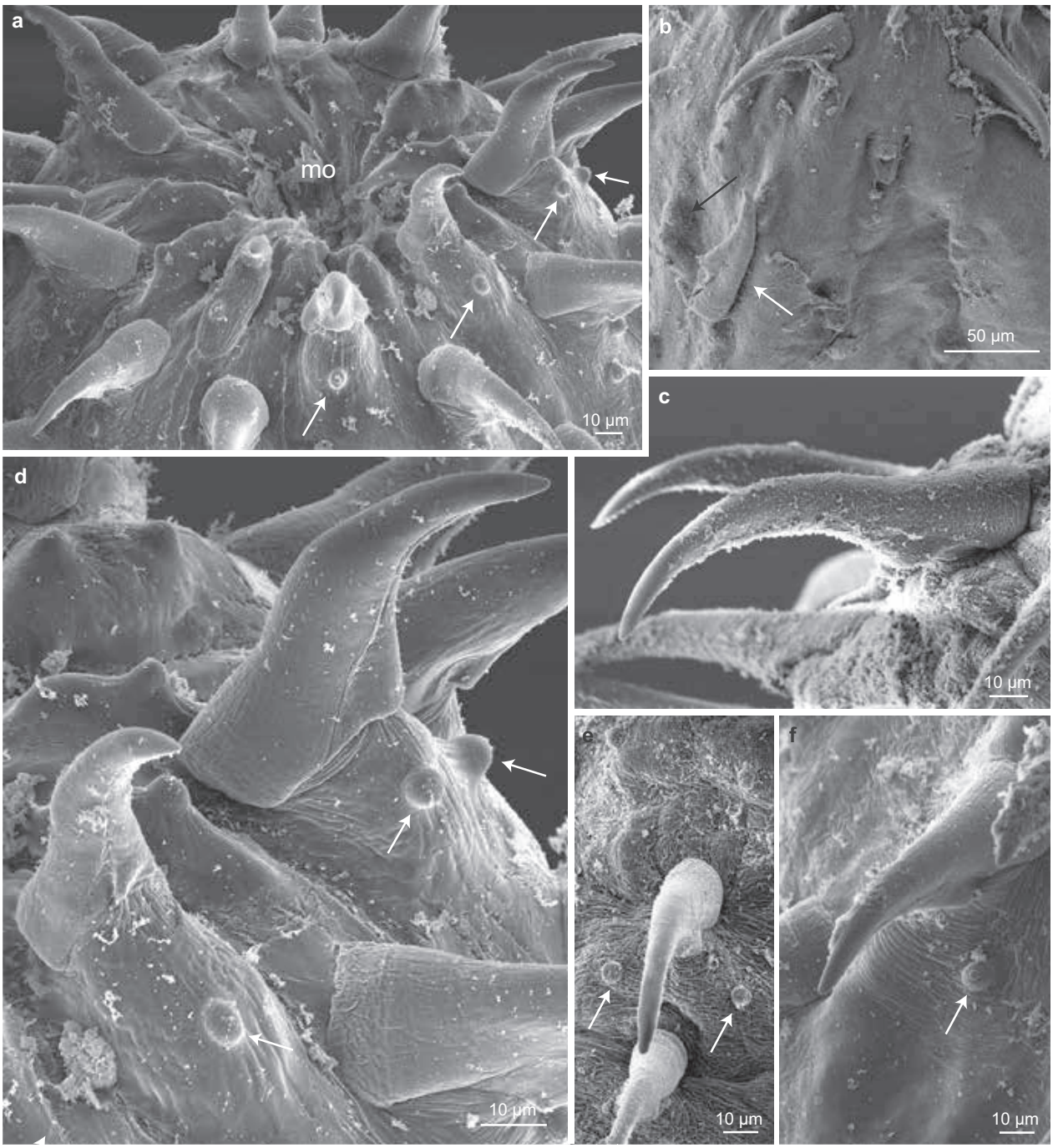

Figure 3. Details of the introvert, all images by SEM. **a.** Anterior end of specimen ZMH-ANN-031330. The hooks surround the introvert opening, which leads to the mouth (mo). Arrows point to the papillae on the basal sockets of the hooks; **b.** Transition between region with and without hooks. The black arrow points at a socket, the white arrow points at a detached hook. Specimen ZMH-ANN-031328; **c.** Shape of the hooks, from specimen ZMH-ANN-031329; **d.** Magnification from **a** showing more details on the fine structure of hooks, socket and papillae (arrows); **e.** Hooks and papillae in specimen ZMH-ANN-031329; **f.** Hook and papilla in specimen ZMH-ANN-031330.

is also a Northeast Atlantic species and *N. cutleri* and *N. vitjazi* are Pacific species (see Cutler and Cutler 1986). In summary, it is concluded that the Guinea Basin specimens represent *N. constricticervix*, both in morphology and distribution, although the measured hook size is under the reported values for this species.

It is striking that one of the investigated specimens has a region free of hooks on the introvert. The similarity of the elevations present in this region with the basal socket of hooks in other specimens makes it very likely that hooks were present, but were shed off for unknown reason. The presence of one obviously detached hook, which may be held in position by a very small tissue connection, is further support to this hypothesis. The sharp border between the hook cuticle and the introvert cuticle might make it possible that hooks can detach and break off the

cuticle. Cutler and Cutler (1986) assume that hooks may get lost during development in some species, but clear evidence for this remains to be shown.

For some species, the arrangement of hooks on the introvert is regarded as characteristic. For example, *N. abyssorum* (Koren & Danielssen, 1875) has hooks arranged in spirals and *N. rimicola* (Gibbs, 1973) has hooks arranged in rings. Among the Guinea Basin specimens, hooks appeared spirally arranged in one specimen, but such pattern could not be observed in others. Therefore, the arrangement of hooks in the new specimens remains uncertain.

A character that is known, but has received little attention are the papillae close to the hooks. Papillae are usually described from the trunk. To my knowledge, papillae associated with the hooks were only figured for *N. columbaris* (Kawauchi and Rice 2009; their fig. 2e and f) and for *N. diaphanes diaphanes* (Kędra and Shields 2011; their fig. 8c). In all cases (including the Guinea Basin specimens) papillae are semicircular structures with an apical set-off center, which could be a receptive or secretive opening. Neither in *N. columbaris* nor in *N. diaphanes diaphanes* (Gerould, 1913) the hooks sit on elevated sockets as in the Guinea Basin specimens and the papillae are loosely distributed among the hooks (*N. columbaris*) or anterior and between the hooks (*N. diaphanes diaphanes*) (Kawauchi and Rice 2009; Kędra and Shields 2011).

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Additional information

Conflict of interest

The author has declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Artificial Intelligence (AI) use

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Author contributions

The author solely contributed to this work.

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.