Can you find me? A new sponge-like nudibranch from the genus *Jorunna* Bergh, 1876 (Mollusca, Gastropoda, Discodorididae)

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Abstract

The nudibranch diversity of the western Indian Ocean is comparatively one of the least studied in the world. In this paper a sponge-like Discodorididae nudibranch *Jorunna liviae* sp. nov. is described. The description is based on integrative anatomy, including molecular analysis of two genes (the mitochondrial COI and the nuclear H3), dissections, electron microscopy (SEM) of buccal elements, micro tomography of the spicule’s arrangements and ecological observations. This study provides the first ever molecular data of *Jorunna* species from the western Indian Ocean, helping to fill the gap to further understand this apparent paraphyletic genus.

Key Words

biodiversity, Heterobranchia, Mozambique, new species, phylogeny, sea slugs

Introduction

The systematic of the genus *Jorunna* was revised by Camacho-García and Gosliner (2008) based on morphological characters. These authors examined 246 specimens (including 30 type specimens) and described two new species. The genus *Jorunna* Bergh, 1876 is widely distributed with species found in the Indo-Pacific, Mediterranean, Atlantic and Eastern Pacific. Alvim and Pimenta (2013) revised the anatomy of the family Discodorididae Bergh, 1891 from Brazil and added a new species for the genus (*Jorunna spongiosa* Alvim & Pimenta, 2013). Recently, Neuhaus et al. (2021) provided a molecular and morphological review of the European species and described a new species (*Jorunna artsdbankia* Neuhaus, Rauch, Bakken, Picton, Pola & Malaquias, 2021). Currently, 22 *Jorunna* species are accepted as valid (MolluscaBase eds. 2022). Nevertheless, there are still many undescribed species, particularly in the Indo-Pacific. In the field-guide of sea slugs of the Indo-Pacific, Gosliner, Valdés and Behrens (2015) illustrated 16 species of *Jorunna* from which only six are described: *Jorunna funebris* (Kelaart, 1859), *Jorunna labialis* (Eliot, 1908), *Jorunna ramicola* Miller, 1996, *Jorunna rubescens* (Bergh, 1876), *Jorunna parva* (Baba, 1938) and *Jorunna alisonae* Marcus, 1976. Nevertheless, the authors did not include two Australian species: *Jorunna hartleyi* (Burn, 1958) and *Jorunna pantherina* (Angas, 1864).

Despite current research efforts to raise the biodiversity knowledge of nudibranchs from the western Indian Ocean (e.g. Manson-Parker 2015; Tibiriçá et al. 2017a, b, 2018, 2020), this region remains comparatively far less studied than other areas of the Indo-West Pacific. The high number of undescribed species often hampers comprehensive biogeographic studies. Thus, the discovery of new species is of primary importance to advance our global knowledge on how biodiversity is formed and
how species diversity spreads across oceans. Moreover, the lack of molecular data from the western Indian Ocean (WIO) limits phylogenetic studies. Of all specimens sequenced from the genus *Jorunna* so far, none are from the WIO. The present study contributes to fill this gap by providing a description of a new *Jorunna* species from Mozambique, including molecular, morphological and ecological data.

**Methods**

**Sample collection**

Six specimens were collected by scuba diving in Ponta do Ouro (26°51'26"S, 32°53'46"E), Mozambique by J. Ström-voll & Y. Tibiriçá. All specimens were found on sponge *Amphimedon brevispicalifera* (Dendy, 1905), four on the reef ‘Doodles’ (26°51'21"S, 32°53'46"E) and two on the ‘Steps Reef’ (26°49'30"S, 32°53'46"E), between 15–18m depth. Sponge identification was based on a porifera assessment study conducted in the same area (Calcínai et al. 2020). Specimens were photographed in situ and in a tank and individually measured. The animals were then relaxed by freezing and preserved in ethanol 96%. Samples were deposited in the Museu Nacional de Ciencias Naturales de Madrid (MNCN) and Museu de História Natural de Maputo (MHNM).

**Morphological study**

Specimens were dissected by dorsal incision under a dissecting microscope Nikon SMZ18. Their reproductive system was separated, examined and drawn under a dissecting microscope Leica 80 with an attached camera lucida. Surrounding radula tissue was removed by immersing in 10% sodium hydroxide for about 8 hours or on a solution containing 180 mL of the tissue lysis buffer ATL with 20 mL of proteinase K-solution incubated in 56 °C for 48h (Holznagel 1998). Labial cuticle and radula were then mounted for electron microscopy (SEM) examination. Imagines were obtained under a FEI NanoSEM 450 scanning microscope at the Servicios Centrales de la Ciencia y Tecnologia de la UCA (MEB), Universidad de Cadiz. Microcomputed tomography (µCT) was carried out to inspect the spicules arrangement by the Servicio de Técnicas No Destructivas del Museo Nacional de Ciencias Naturales de Madrid (MNCN-CSIC). This technique uses x-ray attenuation of biological tissues in three different planes allowing for 2D and 3D image reconstructions (Ziegler et al. 2018). Images were reconstructed using VGSTUDIO MAX 2.2 and visualized in myVGL by Volume Graphics (https://www.volumegraphics.com). Spicules sizes were measured in the µCT images using the distance instrument tool. Measurements were taken from spicules that were clearly visible and from different parts of the body.

**DNA extraction, amplification and sequence**

DNA extraction and amplification were conducted by the peripheral services of the Instituto Universitario de Investigacion Marina (INMAR–UCA). Genomic DNA was extracted from a small sample of foot tissue using the Qiagen DNeasy Blood & Tissue extraction kit, following the manufacturer’s instructions. One mitochondrial gene cytochrome c oxidase subunit (COI) and one nuclear gene histone H3 (h3) were amplified by polymerase chain reaction (PCR), using the universal primers LCO1490 and HCO2198 (Folmer et al. 1994) and H3AD-F and H3BD-R (Colgan et al. 2003), respectively. We tried to amplify the gene 16S using the 16S universal primers 16Sar-L and 16Sbr-H (Palumbi et al. 2002) but all attempts were unsuccessful. PCRs were performed in 25-μl reactions with 2 μl of DNA template. COI amplifications were performed with an initial denaturation for 3 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 46 °C and 1 min at 72 °C with a final extension of 5 min at 72 °C. H3 amplifications were performed with an initial denaturation for 3 min at 95 °C, followed by 25 cycles of 45 s at 94 °C, 45 s at 50 °C (annealing temperature) and 2 min at 72 °C, with a final extension of 10 min at 72 °C. Once completed, successful PCR products were sent to Macrogen, Inc. (Madrid, ES) for purification and sequencing.

All sequences were revised and examined in Geneious v.10.2.4 (Kearse et al. 2012). Possible contamination was verified using the Basic Local Alignment Search tool (BLAST) web server (https://blast.ncbi.nlm.nih.gov/Blast.cgi, Altschul et al. 1990). New sequences were uploaded to Genbank, NCBI and accession numbers are provided in Appendix 1. Outgroup sequences and other *Jorunna* spp. sequences were obtained from GenBank. The outgroup selection followed Neuhaus et al. (2021). Additionally, one species of each available genus of Discodorididae Bergh, 1891 from GenBank was included in the analysis with preference given for type species. When available, up to three sequences of each morpho-species of *Jorunna* from GenBank, NCBI were included in the phylogeny. Preference was given to specimens with COI and H3. Sequences were aligned in Geneious (https://www.geneious.com) using Muscle and default settings.

**Phylogenetic analysis**

Maximum likelihood (ML) and Bayesian inference (BI) were used to infer evolutionary relationships. Analyses were conducted for individual genes as well as for the concatenated COI+16S. JModeltest was used to estimate the best fit-evolutionary model by applying the Akaike information criterion (AIC) for each gene. The model chosen was the GTR+I+G for COI and H3. Bayesian inference was performed via MrBayes v.3.2.6 (Ronquist and Huelsenbeck 2003) and run for 5,000,000 generations and four chains, with unlinked parameters, partitioned by genes and a burn-in of 25%. Node support was assessed based on the posterior probability (PP) and considered strongly supported when PP
Species delimitation

Three molecular species delimitation analyses were conducted to aid the species hypothesis. Firstly, Species by Automatic Partitioning (ASAP) was performed on the ingroup COI dataset applying the Kimura two Parameter (K2P) and the default setting parameters (Puillandre et al. 2021). Secondly, the Poisson Tree Processes model (bPTP) was implemented in the bPTP web server (https://species.h-its.org) applying default setting parameters (Puillandre et al. 2021). Secondly, ic Partitioning (ASAP) was performed on the in-group COI to aid the species hypothesis. Firstly, Species by Automatic Partitioning (ASAP) was performed on the ingroup COI dataset applying the Kimura two Parameter (K2P) and the default setting parameters (Puillandre et al. 2021). Secondly, the Poisson Tree Processes model (bPTP) was implemented in the bPTP web server (https://species.h-its.org) applying default setting parameters (Puillandre et al. 2021); and, third, the minimum COI p-distance was calculated applying default settings on Mega X version 10.2.4 (Kumar et al. 2018).

Results

Systematics

Order Nudibranchia Cuvier, 1817
Superfamily Doridoidae Rafinesque, 1815
Family Discodorididae Bergh, 1891
Genus Jorunna Bergh, 1876

Jorunna liviae Tibiričić, Strömvoll & Cervera, sp. nov.
https://zoobank.org/5B6809CC-32EE-456F-8302-4B8A3D4E0513


Material examined. Holotype: MNCN15.05/200187 (dissected and sequenced), 12.04.2022, Doodles, Ponta do Ouro, Mozambique, depth 15 m, length 20 mm. Paratypes: MNCN15.05/200188 (dissected and sequenced), 12.04.2022, Doodles, Ponta do Ouro, Mozambique, depth 17 m, length 11 mm. MNCN15.05/94693 (sequenced and tomography), 12.04.2022, Doodles, Ponta do Ouro, Mozambique, depth 15 m, length 20 mm., size 5 mm. MNCN15.05/200189 (dissected and sequenced), 14.04.2022, Doodles, Ponta do Ouro, Mozambique, depth 18 m, length 13 mm. MHN.MOL.2022002, (2 specs.), 23.06.2022, Steps Reef, Ponta do Ouro, Mozambique, depth 16 m, length 30 mm (both).

Type locality. Ponta do Ouro, Mozambique (26°51'26"S, 32°53'4"E).

Habitat. Specimens were collected on submerged subtropical compressed sandstone reefs in Ponta do Ouro, Mozambique.

Diagnosis. Body elongate-ovulated. Dorsum pale gray to pink, covered on highly dense caryophyllidia; rhinophores short, with up to nine lamellae, ending in a knob apex; six to nine bipinnate branchial leaves encircling the anal pore. Radula with five to seven very thin pectinated outermost teeth bearing long bundled fibrous denticles. Labial cuticle smooth. Copulatory spine with bifid apex.

Etymology. This species is dedicated to Livia Renée Cornelius, daughter of the second author of this paper.

Description. External morphology (Figs 1, 2). Length varied from 11 to 30mm. Body elongate-ovulated, with gritty texture (Fig. 1A). Mantle covered on highly dense caryophyllid, evenly distributed on the dorsum (Fig. 2A). Caryophyllidia elongated, formed by five to eight spicules, projecting over tip, forming a crown of approximately 140 µm on the dorsum, taller on the margin of gill sheath (≈ 280 µm). Rhinophoral and branchial sheaths long, margin covered by caryophyllidia (Fig. 1D). Rhinophores short, retractable, with six to eight diagonal lamellae with a knob protruding apex (Fig. 1E). Gill with six to nine retractile, bipinnate branchial leaves, held vertically and forming a closed circle around the anal pore (Fig. 1F). Foot narrower than mantle, bilabiately, upper lip bifurcate at center (Fig. 1B). Side of the foot covered by spicules (= 60 µm), spicules absent on foot sole (Figs 1B, C, 2B, C). Feet do not project beyond mantle in natural crawling position. Oral tentacles small and conical. Dorsum color pale pink to gray. Some specimens covered by pinkish-brown minute dots forming spots distributed on the notum. Gill and rhinophores translucent pinkish-white. Oral tentacle white. Upper lip translucent white with brownish dots. Foot pinkish-white.

Internal morphology. (Figs 3, 4) The visceral mass is enveloped by a translucent-white tissue covered by brownish dots. Eye spots are visible by transparency.

Digestive system. Smooth labial cuticle (Fig. 3A). Oral tube long, about twice the size of oral bulb, with a pair of retractor muscles (Fig. 4A). Buccal bulb ovate, short, radular sac small and ovate, protruding ventrally, with a pair of strong retractor muscles (Fig. 4A).

Radular formula difficult to determinate as outermost teeth are very thin and overlapping each other (Fig. 3B). Approximate radular formula is: 24 × 5–7.22.0.22.5–7 for the 13 mm specimen MNCN15.05/200189 and 38 × 6–7.26.0.26.6–7 for the 20 mm specimen MNCN15.05/200187. Rachidian tooth absent. Innermost and lateral teeth are single cusped, hamate, lacking denticles (Fig. 3C). Lateral teeth gradually increase in size from the inner teeth (= 25 µm) toward the external margin (outermost teeth = 100 µm). Five to seven outermost teeth highly differentiated, very thin, pectinate, bearing 5 to 9 long bundled fibrous denticles (Fig. 3D, E).

Oesophagus passing through nerve ring, where it folds. Pair of salivary glands, relatively short, uniform, near the base of oesophagus (4A). Oesophagus connects to oval stomach. Intestine about half of oesophagus diameter. Caecum locate ventrally to stomach.
Figure 1. *Jorunna liviae* sp. nov. (MNCN15.05/200187) external morphology. A. Dorsal view; B. Ventral view; C. SEM photography of dorsal caryophyllids; D. Rhinophores sheath details; E. Rhinophore; F. Gill branches.
Figure 2. Microcomputed tomography (µCT) of *J. liviae* sp. nov. (MNCN15.05/94693). A. Exterior view: dorsal (top) and anterior (bottom); B. Internal arrangement of the spicules: dorsal (top) and ventral (bottom); C. General internal arrangement of spicules: green = top right; blue = middle right; red = bottom right.
Figure 3. SEM photographs of *Jorunna livae* sp. nov. A. labial cuticle (MNCN15.05/200188); B. entire view of the radula; C. Half-row of posterior part of the radula; D. Outermost teeth; E. Detail of the outermost teeth; F. Copulatory spine.
Digestive gland cone-shaped, occupying approximately 30% of visceral mass. Anus opening at the center of gill circle.

**Central nervous system.** Central nervous system partially covered by blood gland. This is divided into two parts, anterior part about half the size of posterior part. Cerebral ganglia about half the size of pleural ganglia. Cerebral ganglia and pleural ganglia fused. Pedal ganglia ventrally located connected by a simple pedal commissure. Buccal ganglia short, ventrally located. Rhinophoral ganglia bulb-shaped, about 30% the size of cerebral ganglia. Eyes connected to cerebral gland by short rhinophoral nerve (Fig. 4B).

**Reproductive system.** Hermaphroditic duct leading to an ampulla long and convoluted, located between female gland and accessory gland. Ampulla branching into short oviduct and prostate. Flattened and ovulated prostate narrowing into a thin deferent duct, expanding into ejaculatory portion. Penis unarmed. Accessory gland size and shape varied according to the specimen, from pear-shaped and similar size to the female gland MNCN15.05/200187 to elongated and half of the size MNCN15.05/200189; in all specimens it narrows into a very thin, highly convoluted tube. Copulatory spine in accessory gland of approximately 1.25 mm (Fig. 3F). Vagina with similar length and width than deferent duct, leading to an oval bursa.

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**Figure 4. Jorunna livae** sp. nov. internal anatomy. A. Oral mass; mo – mouth; rm – retractor muscles; ob – oral bulb; oe – oesophagus; ot – oral tube; rs – radular sac; sg – salivary gland; B. Central system (blood gland removed); cg – cerebral ganglia; cp – pedal commissure; ey – eye; gp – pedal ganglia; pl – pleural ganglia; rg – rhinophoral ganglia; C. Reproductive system; ag – accessory gland; amp – ampulla; bc – bursa copulatrix; cs – copulatory spine; dd – deferent duct; pr – prostate; sr – seminal receptacle; ud – uterine duct; vag – vagina.
Natural history. This species has only been seen associated with the sponge *A. brevispiculifera*, on which the species is very cryptic (Fig. 5A, B). They are usually found at the base of the sponge branches but they have also been seen on other parts. When removed from the host sponge, the *Jorunna liviae* sp. nov. stretches the body curling the mantle toward the middle of the foot, similar to what Miller (1996) observed for *J. ramicola*. Perhaps this behavior aims to protect the sole of the foot which lacks caryophylliid. The white egg mass is also found on the same sponge and forms a close spiral ribbon of approximately five coils (Fig. 5F). A likely undescribed species of nudibranch egg-eater *Favorinus* sp. has been seen feeding on the *J. liviae* sp. nov. egg mass (Fig. 5C, D). Curiously, most of the time the egg ribbons are found on the tip of the sponge. Perhaps this strategy provides some protection against encrusting organisms due the higher water flux in this part of the sponge. Mating has been observed through July between specimens of different sizes of the species is very cryptic (Fig. 5A, B). Perhaps this behavior aims to protect the sole of the foot which lacks caryophylliid. The white egg mass is also found on the same sponge and forms a close spiral ribbon of approximately five coils (Fig. 5F). A likely undescribed species of nudibranch egg-eater *Favorinus* sp. has been seen feeding on the *J. liviae* sp. nov. egg mass (Fig. 5C, D). Curiously, most of the time the egg ribbons are found on the tip of the sponge. Perhaps this strategy provides some protection against encrusting organisms due the higher water flux in this part of the sponge. Mating has been observed through July between specimens of different sizes. Mating has been observed through July between specimens of different sizes. Prefer sandy reefs with predominantly hydroids, soft corals and tonalities (Fig. 5E).

Molecular study and phylogeny. We successfully amplified the gene COI and H3 of four *Jorunna liviae* sp. nov. specimens. The phylogenetic trees constructed by BI and ML analyses of single gene datasets (Suppl. material 1) were not conflictive but differed in the ability to resolve phylogenetic relationships. The single gene H3 analysis retrieved the lowest resolution and the concatenate dataset the highest. Nevertheless, all *Jorunna* species were recovered with more than 50% support in all analysis. In general, the BI analysis better solved the relationship between species, while the ML analysis appears to reflect population structure. Therefore, the results discussed below are based on the concatenated analysis (Fig. 6), except when stated otherwise.

The family Discodorididae formed a large polytomy. The genus *Jorunna* was divided in two paraphyletic clades, one containing all specimens of *J. funebris* (PP = 1; BS = 94) and another clade with the remaining *Jorunna* species (PP = 0.99; BS = 74).

The COI inter-specific variation (uncorrected $p$-distance) within the genus varied from 9.08% between *J. tomentosa* lineage B (LB) and *J. artsdatabankia* to up 16.92% between *J. funebris* and *J. tomentosa* lineage A (LA) (Table 1). The COI intra-specific variation of *Jorunna liviae* sp. nov. ranged from 0.16% to 1.08%. The closest species to *Jorunna liviae* sp. nov. was *J. tomentosa* lineage B with a minimum $p$-distance of 13.06%. ASAP retrieved 10 partitions, in both analysis (COI and concatenate) the partitions with higher score (asap-score 1.50–3) *Jorunna liviae* sp. nov. was retrieved as a distinct taxonomic unit. Curiously, *J. funebris* were retrieved as a species complex in all possible partitions.

Table 1. COI inter- and intraspecific uncorrected $p$-distances.

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<th><em>Jorunna</em>&lt;br&gt;artsdatabankia</th>
<th><em>Jorunna</em>&lt;br&gt;tomentosa&lt;br&gt;LA</th>
<th><em>Jorunna</em>&lt;br&gt;tomentosa&lt;br&gt;LB</th>
<th><em>Jorunna</em>&lt;br&gt;liviae&lt;br&gt;sp. nov.</th>
<th><em>Jorunna</em>&lt;br&gt;onubensis</th>
<th>Intraspecific</th>
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<td><em>Jorunna</em>&lt;br&gt;artsdatabankia</td>
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<td>0–0.15%</td>
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<td><em>Jorunna</em>&lt;br&gt;tomentosa&lt;br&gt;LA</td>
<td>10.30</td>
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<td>0.15–0.68%</td>
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<tr>
<td><em>Jorunna</em>&lt;br&gt;tomentosa&lt;br&gt;LB</td>
<td>9.08</td>
<td>3.65</td>
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<td></td>
<td></td>
<td>0.15–0.92%</td>
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<td><em>Jorunna</em>&lt;br&gt;liviae&lt;br&gt;sp. nov.</td>
<td>14.29</td>
<td>14.74</td>
<td>13.06</td>
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<td>0.16–1.08%</td>
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<tr>
<td><em>Jorunna</em>&lt;br&gt;onubensis</td>
<td>12.61</td>
<td>10.74</td>
<td>10.45</td>
<td>12.31</td>
<td></td>
<td>N/A</td>
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<td><em>Jorunna</em>&lt;br&gt;funebris</td>
<td>16.92</td>
<td>17.78</td>
<td>17.78</td>
<td>16.92</td>
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<td>0.46–14.18%</td>
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Discussion

The phylogenetic relationships within the family Discodorididae are poorly solved. Most of the type species of Discodorididae genera are not sequenced which hinders our capacity to further understand the family. *Jorunna* is one of the few genera of the Discodorididae family which has its type species (*J. tomentosa*) sequenced. However, a recent study based on three genes (COI+16S+H3) reveals that it is uncertain if *J. tomentosa* represents two distinct lineages (Neuhaus et al. 2021). In our phylogenetic analysis, *J. tomentosa* is divided into two sub-clades (lineage A and B), which form a clade which is sister of *J. artsdatabankia* and related to *J. onubensis* and *J. liviae* sp. nov. Additionally, the genus *Jorunna* appears paraphyletic as *J. funebris* did not nest within the large *Jorunna* clade. In Camacho-Garcia and Gosliner’s (2008) morphological study, *J. funebris* nested on a clade together with *J. rubescens*, *J. parva* and *J. pardus*, which is sister of the clade containing the remaining *Jorunna* species studied by the authors. Unfortunately, to date no other species from the *J. funebris* clade has been sequenced. Consequently, the lack of molecular data from several *Jorunna* species hampers any further conclusion about the phylogeny of the genus. Nevertheless, it is clear that the species here described belongs to the genus *Jorunna*, as it forms a clade with the type species. In addition, the new species fits all the morphological diagnosis characters of the genus (see Camacho-Garcia and Gosliner 2008). Interesting, all the species delimitation analyses suggest that *J. funebris* is a species complex, or alternatively, as proposed by Ip et al. (2019), there is an identification error in their sequences. *Jorunna liviae* sp. nov. is similar in appearance to the Atlantic species *J. spongiosa* and *J. tomentosa*. This
Figure 5. *Jorunna liviae* sp. nov. *in situ*. A. Hosting sponge *Amphimedon brevispiculifera* (Dendy, 1905); B. *Jorunna liviae* sp. nov. resting on sponge; C. *Jorunna liviae* sp. nov. near its egg mass, and *Favorinus* sp. feeding on it; D. Close-up of *Favorinus* sp.; E. *Jorunna liviae* sp. nov. mating; F. Details of *Jorunna liviae* sp. nov. egg mass.
latter is typically found in European waters (Atlantic and Mediterranean), but few records exist from South Africa and none of them from the Indian Ocean side (Camacho-García and Gosliner 2008; Neuhaus et al. 2021). Apart from the geography and genetic distance, these three species can be clearly distinguished by their radulae, in particular by the shape of the outermost teeth. These are very thin and pectinate in *J. liviae* sp. nov., hooked with small branches on *J. spongiosa* and slender hamate with up to 8 short denticles in *J. tomentosa*. In fact, the outermost pectinate teeth of *Jorunna liviae* sp. nov. are quite unique, and only similar to *J. parva*, a species also found in the WIO but easily distinguishable by the yellow background and dark caryophyllidia. Camacho-García and Gosliner (2008) provided detailed anatomical descriptions and comparative tables of *Jorunna* species by region. To better illustrate the differences between the species described in this study, we adapted and updated Camacho-García and Gosliner’s (2008) comparative table of the Indo-Pacific *Jorunna* species, including recent distribution and morphological data observed by us, as well as the species *J. liviae* sp. nov. and *J. labialia* (Table 2). This latter species is found in the western Indian Ocean and Red Sea but was under ‘Mediterranean and Western Atlantic’ species in Camacho-García and Gosliner’s (2008) comparative tables.

The Indo-Pacific species that most resembles *J. liviae* sp. nov. is *J. ramicola*; a species first described from New Zealand and likely occurring in Mozambique (Tibiriçá et al. 2017a).

![Figure 6. Bayesian inference tree based on the concatenate sequence dataset (COI+H3) collapsed (PP< 0.5). Numbers at the top of nodes indicate Bayesian Posterior probability (PP) and on the bottom bootstrap support from the maximum likelihood analysis (BS). Colored bars on the right represent the results of the species delimitation analyses on the *Jorunna* spp., from left to right: ASAP on COI dataset, PTP on COI dataset, bPTP on COI+H3 dataset.](image)
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<tr>
<td>Dorsal color</td>
<td>White to yellow, cream, dark brown rings of different sizes</td>
<td>Purple to brown to pale orange with dark patches</td>
<td>Green to yellow with brown spots, orange, horizontal black stripes</td>
<td>Dark orange to dark brown, carposphyllid to dark brown</td>
<td>Pale pink, large brown patches encrusted with white or dark purple spots</td>
<td>Pale grey, dark grey to spotted grey spots, light brown to brown without rhinophores to gill</td>
<td>Pale grey to light brown with patches of similar color</td>
<td>White to dark dull grey with light brown spots</td>
<td>Pinkish grey, darker spots sometimes present</td>
</tr>
<tr>
<td>Rhinophores</td>
<td>14–20 lamellae</td>
<td>16 lamellae, terminal knob</td>
<td>23–25 lamellae</td>
<td>13–15 lamellae</td>
<td>7–8 lamellae</td>
<td>= 10 lamellae</td>
<td>short, up to 13 lamellae, terminal knob</td>
<td>wide, 8–11 lamellae</td>
<td>short, 6–8 lamellae, terminal knob</td>
</tr>
<tr>
<td>Gill color</td>
<td>White with dark black defoliations</td>
<td>Same than mantle</td>
<td>Base white, dark brown rhacides</td>
<td>Light yellow, base dark brown, tips light yellow, dark brown rhacides</td>
<td>White</td>
<td>Grey to brown with cream glandular spots</td>
<td>Dark grey</td>
<td>White to pinkish</td>
<td></td>
</tr>
<tr>
<td>Foot sole color</td>
<td>Dark spots around the margin, dark translucent white</td>
<td>Margins sparsely speckled, speckled white, translucent white</td>
<td>Black spots on sole and lateral, white or cream</td>
<td>Dark spots on sole and lateral, white or cream</td>
<td>Pink</td>
<td>Pale grey</td>
<td>?</td>
<td>Whitish-pink</td>
<td></td>
</tr>
<tr>
<td>Upper lip color</td>
<td>White to yellow</td>
<td>Cream yellow, speckled, yellowish, with brown spots on each side of the lip</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>White</td>
<td></td>
</tr>
<tr>
<td>Oral tentacles color</td>
<td>White to cream, with brown spots in some specimens</td>
<td>Speckled</td>
<td>Light white to yellow</td>
<td>Yellowish, with brown spots on each side of the lip</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>White-pinkish</td>
<td></td>
</tr>
<tr>
<td>Mantle Glands</td>
<td>White, distributed around the mantle edge</td>
<td>Dorsally visible when the animal is in motion</td>
<td>Dorsally visible when the animal is in motion</td>
<td>Dorsally visible when the animal is in motion</td>
<td>Dorsally visible when the animal is in motion</td>
<td>?</td>
<td>?</td>
<td>White, distributed around the mantle edge</td>
<td>?</td>
</tr>
<tr>
<td>Foot</td>
<td>Dorsally visible when the animal is in motion</td>
<td>Dorsally visible when the animal is in motion</td>
<td>Dorsally visible when the animal is in motion</td>
<td>Dorsally visible when the animal is in motion</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>Rarely visible dorsally</td>
<td></td>
</tr>
<tr>
<td>Radula</td>
<td>21 × (21.0.21) in 20mm-long preserved specimen</td>
<td>20 × (28.0.28) in 18mm-long preserved specimen</td>
<td>26 × (18.1.18) in 13mm-long preserved specimen</td>
<td>25 × (15.0.15) in 18mm-long preserved specimen</td>
<td>21 × (23.0.23) in 18mm-long preserved specimen</td>
<td>16 × (17.0.17) in 20mm-long preserved specimen</td>
<td>14 × (8.0.18) in 5mm-long preserved specimen</td>
<td>19 × (17.0.17) in 12mm-long preserved specimen</td>
<td>8 × (7.26.0.26.6–7) in 20mm specimen</td>
</tr>
<tr>
<td>Innermost teeth</td>
<td>Hamate, longer and thinner than midlateral teeth, lacking denticles</td>
<td>Hamate, small, lacking denticles</td>
<td>Hamate, blunt, lacking denticles</td>
<td>Hamate, elongated, lacking denticles</td>
<td>Hamate, pointed, lacking denticles, more than 5 denticles</td>
<td>Hamate, pointed, lacking denticles, more than 3 denticles</td>
<td>Hamate, pointed, lacking denticles, more than 3 denticles</td>
<td>Hamate, pointed, lacking denticles, more than 3 denticles</td>
<td>Hamate, pointed, lacking denticles, more than 3 denticles</td>
</tr>
<tr>
<td>Midlateral teeth</td>
<td>Hamate, lacking denticles</td>
<td>Hamate, lacking denticles</td>
<td>Hamate, elongated, blunt, lacking denticles</td>
<td>Hamate, elongated, lacking denticles</td>
<td>Hamate, pointed, elongated, up to 3 denticles near the cusp</td>
<td>Hamate, pointed, elongated, up to 3 denticles near the cusp</td>
<td>Hamate, pointed, elongated, up to 3 denticles near the cusp</td>
<td>Hamate, pointed, elongated, up to 3 denticles near the cusp</td>
<td>Hamate, pointed, elongated, up to 3 denticles near the cusp</td>
</tr>
<tr>
<td>Outermost teeth</td>
<td>Hamate, lacking denticles, curved pointed, elongated, pointed</td>
<td>Hamate, lacking denticles</td>
<td>Hamate, elongated, pointed, lacking denticles</td>
<td>Hamate, elongated, pointed, lacking denticles</td>
<td>Hamate, pointed, elongated, up to 3 denticles</td>
<td>Hamate, pointed, elongated, up to 3 denticles</td>
<td>Hamate, pointed, elongated, up to 3 denticles</td>
<td>Hamate, pointed, elongated, up to 3 denticles</td>
<td>Hamate, pointed, elongated, up to 3 denticles</td>
</tr>
<tr>
<td>Labial cuticle</td>
<td>Smooth</td>
<td>With jaw elements</td>
<td>Smooth</td>
<td>Smooth</td>
<td>With jaw elements</td>
<td>With jaw elements</td>
<td>With jaw elements</td>
<td>With jaw elements</td>
<td>Smooth</td>
</tr>
<tr>
<td>Accessory gland and spine</td>
<td>Present, curved spine = 717 µm long</td>
<td>Present, long pointed spine</td>
<td>Present, curved spine = 3.7 mm long</td>
<td>Present, spine = 477 µm long</td>
<td>Present, spine = 198 µm long</td>
<td>Present, curved spine = 2.25 mm long</td>
<td>Present, curved spine = 0.60 mm long</td>
<td>Present, curved spine = 1.25 mm long</td>
<td>Present, curved spine = 1.25 mm long</td>
</tr>
</tbody>
</table>

Table 2. Comparative morphology of valid *Jorunna* species from the Indo-Pacific Ocean.

In *J. ramicola* were denticulated, while in *J. liviae* sp. nov. they are simple hamate. In addition, the labial cuticle in *J. ramicola* bears jaw elements (Camacho-Garcia and Gosliner 2008), while in *J. liviae* sp. nov. it is smooth. Externally, they can be easily separated by the color of the rhinophores, which in *J. ramicola* is dark pigmented and in *J. liviae* sp. nov. whitish pink. Additional differences are provided in the comparative Table 2.
Conclusions

Based on morphological and genetic data there is no doubt that _J. liviae_ sp. nov. is a newly discovered species. Here we provide the first sequence of _Jorunna_ species to the WIO. We recommend further efforts to sequence other _Jorunna_ species in order to clarify the monophyly of the genus and phylogenetic relationships. In addition, _J. funebris_ specimens from different geographic regions should be morphologically and genetically examined as they may represent a species complex.

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References


Tibricá, Y. et al.: A new sponge-like nudibranch from the genus _Jorunna_
Supplementary maximum credibility tree of the COI and 16S sequence alignments for *Jorunna liviae* sp. nov.

Authors: Yara Tibiriçá, Jenny Strömvoll, Juan Lucas Cervera

Data type: phylogenetic

Explanation note: Bayesian maximum credibility tree of the COI and 16S sequence alignments. Posterior or probabilities (PP) are indicated above each and bootstrap values (BS) are indicated below each branch. Branch lengths indicate the proportion of substitutions. PP < 0.5 and BS < 50 are not shown.

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