

The genus *Jorunna* (Nudibranchia: Discodorididae) in Europe: a new species and a possible case of incipient speciation

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ABSTRACT

To investigate the conspecificity of different morphotypes of *Jorunna tomentosa* (Cuvier, 1804) (type species of genus *Jorunna* Bergh, 1876), we studied specimens sampled from across part of the geographical distribution of the species, using a combination of morphoanatomical characters and molecular phylogenetics. Bayesian and maximum likelihood phylograms were inferred based on the mitochondrial genes cytochrome *c* oxidase subunit I (COI) and 16S ribosomal RNA, and the nuclear gene histone H3. We used the automatic barcode gap discovery method to aid in species delimitation. COI genetic uncorrected p-distances were estimated between and within species. Animals were dissected and the reproductive system, radulae and labial cuticles were examined; scanning electron microscopy was employed to study ultrastructural elements of anatomical characters. The results revealed the presence of a new species (*Jorunna artsdatabankia* n. sp.) and a possible case of incipient speciation in *J. tomentosa* with our COI data indicating the presence of two morphoanatomically indistinct lineages that are separated from each other by distances of 3.2–5.0%. The genetic distance between *J. artsdatabankia* n. sp. and its sister species *J. tomentosa* was 9.0–12.3%; the former species is characterized by a plain white to yellow background colour with irregularly placed small brown spots, smooth radular teeth and a longer vas deferens, wider vagina and a longer copulatory spine (up to 600 µm longer) than the latter. A diagnostic comparison of all species of European *Jorunna* is included, as well as a discussion of the assignment of *J. lemchei* to the genus *Gargamella*.

INTRODUCTION

Understanding diversity patterns is of paramount importance for conservation biology and to address theoretical questions of speciation and biogeography (Bickford *et al.*, 2007; Marrone *et al.*, 2013; Korshunova *et al.*, 2017b; Sørensen *et al.*, 2020). Over the past two decades, integrative taxonomic approaches combining classical morphoanatomical studies with modern DNA analytical methods have largely challenged our understanding of biological diversity. Consequently, many cryptic species (genetically distinct species that are similar in appearance and thus difficult to discriminate based on their external morphology or species exhibiting variable colour pattern that is traditionally assumed to be intraspecific natural variability) have been detected (Sørensen *et al.*, 2020). Sea slugs of the order Nudibranchia are no exception and several studies have revealed the occurrence of cryptic lineages. These

studies include those by Gosliner & Fahey (2011) on *Dermatobranchus* van Hasselt, 1824; Carmona *et al.* (2014) on *Anteaeolidiella* M.C. Miller, 2001; Pola, Roldán & Padilla (2014) on *Okenia* Menke, 1830; Ekimova *et al.* (2015) on *Dendronotus* Alder & Hancock, 1845; Wilson & Burghardt (2015) on *Pteraeolidia* Bergh, 1875; Kienberger *et al.* (2016) on *Aeolidia* Cuvier, 1798; Korshunova *et al.* (2017a, 2019, 2020) on Flabellinidae Bergh, 1889, *Trinchiesia* Ihering, 1879 and *Eubranchus* Forbes, 1838, respectively; and Sørensen *et al.* (2020) on *Polycera* Cuvier, 1816.

Despite the variability observed in some species of the nudibranch genus *Jorunna* Bergh, 1876 (Discodorididae Bergh, 1891), the possible occurrence of cryptic or pseudocryptic species (*sensu* Hoover *et al.*, 2015; Korshunova *et al.*, 2019) has never been suggested in the peer-reviewed literature. However, Goodwin *et al.* (2011a,b) reported and illustrated two morphotypes of *Jorunna* from Northern Ireland and stressed that one of these likely corresponded

to a new taxon, which seems to be conspecific with the new species we describe here (see the 'Results' section). Nudibranchs of the genus *Jorunna* are characterized by an oval-elongate body with background colours varying from white and greyish-white to yellowish-orange, reddish-brown and pinkish-purple. The notum may be covered with large dark brown blotches, dark rings and irregularly distributed spots that vary in colour (ranging from pale brown to almost black), colour intensity and size (see Camacho-García & Gosliner, 2008: fig. 1; Edmunds, 2011: fig. 9a, b; Alvim & Pimenta, 2013: figs 1, 2; Ortea *et al.*, 2014: fig. 10; Ortea & Moro, 2016: figs 4–6, 9; Tibiriçá, Pola & Cervera, 2017: fig. 18). The body is lined with numerous, tightly spaced caryophyllidia tubercles carrying four to seven vertical spicules arranged in a circular crown that surrounds a spherical ciliated knob (Kress, 1981; Foale & Willan, 1987; Gosliner, 1994).

Twenty-one species of *Jorunna* are recognized worldwide (MolluscaBase, 2020a) and 11 of them have been recorded in the Atlantic Ocean, with 4 occurring in the western Atlantic [*J. coloradilla* Ortea & Moro, 2016; *J. davidbowieii* Ortea & Moro, 2016; *J. spazzola* (Er. Marcus, 1955); and *J. spongiosa* Alvim & Pimenta, 2013] and 7 in the eastern Atlantic [*J. efe* Ortea, Moro & Caballer in Ortea *et al.*, 2014; *J. evansi* (Eliot, 1906); *J. ghanensis* Edmunds, 2011; *J. glandulosa* Edmunds, 2011; *J. lemchei* Ev. Marcus, 1976; *J. onubensis* Cervera, García-Gómez & García, 1986; and *J. tomentosa* (Cuvier, 1804)]. The species *J. lemchei*, which was described on the basis of two specimens from Ballyvaughan Bay, western Ireland, was reassigned to the genus *Gargamella* Bergh, 1894 by Ortea *et al.* (2014) due to the presence of penial hooks (see the 'Discussion' section). Of particular interest among European *Jorunna* is the type species of the genus, *J. tomentosa*. This species is characterized by variable colouration, with body colour ranging from shades of greyish-white and cream-yellow to pale orange. The notum may be plain or blotched with light brown to chocolate brown spots of varying size; the spots are distributed irregularly, in two longitudinal rows aligned with the rhinophores or in a combination of these two arrangements (Bergh, 1893; Thompson & Brown, 1984; Thompson, 1988; Picton & Morrow, 1994; Malmberg & Lundin, 2015) (Fig. 1). The size spans from 10 to 55 mm, with most specimens being between 20 and 30 mm (Odhner, 1907; Hunnam & Brown, 1975; Thompson & Brown, 1984; Hayward & Ryland, 2017; personal observations).

Jorunna tomentosa has a wide bathymetric range that extends from depths of a few metres down to possibly more than 400 m (Grieg, 1912; Hunnam & Brown, 1975; Ev. Marcus, 1976; Camacho-García & Gosliner, 2008; Cordeiro *et al.*, 2015). This species has been reported to feed on heteroscleromorph sponges, such as *Halichondria panicea*, *Haliclona oculata* and *Haliclona cinerea* (Millott, 1937; Swennen, 1961; Wolter, 1967; Bloom, 1976; Todd, 1981; Thompson & Brown, 1984; Thompson, 1988; McDonald & Nybakken, 1997). It occurs from Finnmark in northern Norway (this study), southwards along the European Atlantic coastline (Swennen, 1961; Ev. Marcus, 1976; Evertsen & Bakken, 2005), British Isles and French coast (Pruvot-Fol, 1954; Thompson & Brown, 1984; Picton & Morrow, 1994) down to the Iberian Peninsula, including the archipelagos of the Azores and Canary Islands (Malaquias & Morenito, 2000; Cervera *et al.*, 2004; Domènech, Avila & Ballesteros, 2006), and as far as the Mediterranean Sea off Turkey (Saltik, 2005; Ballesteros, Madrenas & Pontes, 2016; Zenetos *et al.*, 2016; Prkić *et al.*, 2018; Furfaro *et al.*, 2020). In addition, the species has been recorded from Cape Province, South Africa, but to date this has not been confirmed by molecular data (Camacho-García & Gosliner, 2008).

Except for the study by Goodwin *et al.* (2011a,b), it was never questioned whether the various colour morphs attributed to *J. tomentosa* could in fact represent putative cryptic lineages instead of being part of the intraspecific variability of the species, as currently assumed. This is an issue that has to be addressed because *J. tomentosa* is the type species of the genus. Using an integrative approach combining molecular phylogenetics with morphoanatom-

ical characters, we here investigate the taxonomic status of the colour morphs currently placed under *J. tomentosa*. In addition, we revise the key features important for the diagnosis of European species of *Jorunna*, including the elusive *Gargamella lemchei*.

MATERIAL AND METHODS

Taxon sampling

Live specimens were obtained by snorkelling and SCUBA diving between February 2018 and December 2019. The animals were photographed *in situ* or inside a small aquarium with a black background using a digital SLR camera equipped with macro lens and external flashlights. The total length (TL; mm) of each individual was measured. Specimens were frozen in seawater for *c.* 12–24 h, after which they were defrosted and preserved in absolute ethanol (>96% conc.). This enables the body of the animals to become fully extended, easing later anatomical work. All samples are deposited in the collections of the University Museum of Bergen (ZMBN). Additional specimens were also studied; these consisted of material from the ZMBN collection and specimens obtained through donations and loans from the NTNU University Museum, Norwegian University of Science and Technology, Trondheim (Bakken, Hørsaker & Daverdin, 2020) and the California Academy of Sciences. One sample (ZMBN 125946) was obtained from a bottom trawl during a research cruise with the University of Bergen in October 2018 on board the research vessel F/F G.O. Sars. In total, 61 specimens were studied for their morphology and DNA: 37 from Norway, 6 from Northern Ireland, 5 from Ireland, 1 from France, 2 from Spain, 8 from Portugal, including the Azores archipelago, and 2 from South Africa (for details, see 'Material examined' in the 'Results' section). The geographic distribution of the European species of *Jorunna* was inferred on the basis of the specimens studied and literature records.

DNA extraction, amplification and sequencing

DNA was extracted from a small sample of foot tissue using the Qiagen DNeasy® Blood and Tissue Kit (Qiagen, California, USA; catalogue no. 69506), following the manufacturer's protocol for "Purification of Total DNA from Animal Tissues" but repeating step 9 with only 100 µl of AE buffer. Three gene regions were used for this study: the mitochondrial markers cytochrome *c* oxidase subunit I (COI) and 16S ribosomal RNA (16S rRNA), and the nuclear histone H3 (H3). For amplification and sequencing, we used the primers used by the following authors: COI, Folmer *et al.* (1994) (forward primer LCO1490: GGTCAACAAATCATAAA-GATATG; reverse primer HCO2198: TAAACTTCAGGGT-GACCAAAAATCA); 16S rRNA, Palumbi *et al.* (1991) (forward primer 16S ar-L: CGCCTGTTTATCAAAAACAT; reverse primer 16S br-H: CCGGTCGTAACCTCAGATCAGCT); and H3, Colgan *et al.* (1998) (forward primer H3AD5'3': ATGGCTCG-TACCAAGCAGACVGC; reverse primer H3BD5'3': ATATCCT-TRGGCATRATRGTTGAC). PCR amplification was carried out in a Bio-Rad C1000 thermal cycler with a total reaction volume of 50 µl for all three genes. For COI and 16S rRNA, PCR solutions contained 17.5 µl Sigma-Aldrich water, 5 µl buffer (Qiagen OneStep RT-PCR Buffer), 5 µl dNTP, 5 µl Q-solution, 7 µl MgCl₂, 2 µl of each primer, 0.5 µl TAQ and 1 µl DNA; for H3, we used 20.5 µl Sigma-Aldrich water and 4 µl MgCl₂. Apart from the annealing temperature, PCR thermal cycling conditions were the same for all three markers. The thermal cycling conditions were as follows: initial denaturation at 95 °C for 3 min; 39 cycles of denaturation at 94 °C for 45 s, annealing at 45 °C (COI), 40 °C (16S rRNA) or 50 °C (H3) for 45 s and extension at 72 °C for 2 min; and a final extension at 72 °C for 10 min. Both a positive and a negative control were included in each run to check for successful amplification



Figure 1. Chromatic variability in *Jorunna tomentosa* s.l., as shown by photographs taken in the field (date when image was taken is shown within parentheses after the locality). **A.** *Jorunna artsdatabankia* n. sp., Kristiansund, Møre og Romsdal, Norway (2018), ZMBN 127749, TL = 40 mm. **B.** *Jorunna tomentosa* lineage A, Ballyhenry Island, Northern Ireland (2013), CAS-IZ 193035. **C–H.** *Jorunna tomentosa* lineage B. **C.** Kristiansund, Møre og Romsdal, Norway (2017), ZMBN 125644, TL = 15 mm. **D.** Mosteiros, São Miguel Island, Azores (2011), ZMBN 87955, TL = 30 mm. **E.** Drøbak, Akershus, Norway, ZMBN 125553 (2018), TL = 25 mm. **F.** Averøy, Møre og Romsdal, Norway, ZMBN 125591 (2018), TL = 28 mm. **G.** Brattøya, Møre og Romsdal, Norway (2017), ZMBN 125632, TL = 35 mm. **H.** Kristiansund, Møre og Romsdal, Norway (2018), ZMBN 125651, TL = 26 mm. Image credits: **A, C, F–H,** N. Aukan; **B,** T.M. Gosliner; **D,** M.A.E. Malaquias; **E,** T. Kinn Kvamme.

and to rule out contamination. Samples that did not readily yield successful PCR results with the standard protocols were run adding 2 or 4 μ l DNA (decreasing the respective volume of Sigma-Aldrich water) and reducing the volume of $MgCl_2$ to 1.75 μ l (increasing the respective volume of Sigma-Aldrich water).

The quality and quantity of PCR products were assessed using gel electrophoresis. PCR product (4 μ l) with Ficoll 5 \times loading dye (1 μ l) was run on a 1.2% agarose gel containing the staining agent GelRed covered in TAE 1 \times buffer. To quantify and estimate the length of amplified DNA fragments, 5 μ l FastRuler was used as a ladder. The gel was run for 20 min at 80 V and then analysed under UV light (Syngene, Cambridge, UK). GeneSnap (v. 7.01) and GeneTools (v. 4.0; Syngene) were used for images and manual band quantification. Successful PCR products were purified using the EXO-SAP method with exonuclease 1 (EXO; 10 units/ μ l) and shrimp alkaline phosphatase (SAP; 1 unit/ μ l, USB C) in 10- μ l reactions (EXO 0.1 μ l, SAP 1.0 μ l, Sigma-Aldrich water 0.9 μ l, PCR product 8 μ l). Reactions were run on a thermal cycler at 37 $^{\circ}$ C for 30 min (incubation) followed by 15 min at 80 $^{\circ}$ C (enzyme inactivation). Samples that contained high concentrations of DNA were diluted after the manual band quantification by combining 1 μ l PCR product with 7 μ l Sigma-Aldrich water.

Successfully amplified PCRs were prepared for sequencing using the BigDye $^{\text{R}}$ Terminator v3.1 Cycle Sequencing Kit protocol (Applied Biosystems $^{\text{TM}}$). The total reaction volume for each sample was 10 μ l: 1 μ l DNA (10 ng), 1 μ l sequencing buffer, 1 μ l BigDye, 1 μ l of each primer (3.2 mM) and 7 μ l Sigma-Aldrich water. The reactions were run in a thermal cycler at 96 $^{\circ}$ C for 5 min (initial denaturation), followed by 25 cycles at 96 $^{\circ}$ C for 10 s (denaturation), 50 $^{\circ}$ C for 5 s (annealing) and finally at 60 $^{\circ}$ C for 4 min. After the thermal reactions, 10 μ l of Sigma-Aldrich water was added to the samples to obtain a final volume of 20 μ l before submitting to the sequencing laboratory facility at the Department of Biological Sciences, University of Bergen, Norway. All sequencing reactions were run on the capillary-based Applied Biosystems 3730XL DNA Analyzer.

Sequence editing, alignment, phylogenetic and species delimitation analyses

A total of 113 novel sequences were generated from 61 specimens of the genus *Jorunna* for the three markers COI, 16S rRNA and H3. In addition, 4 sequences of *J. tomentosa sensu lato*, 3 sequences of *J. funebris* (Kelaart, 1859) and 42 sequences of 14 cryptobranched species belonging to the dorid genera *Chromodoris* Alder & Hancock, 1855 (2 species); *Discodoris* Bergh, 1877 (2 species); *Felimida* Ev. Marcus, 1971 (2 species); *Geitodoris* Bergh, 1891 (1 species); *Glossodoris* Ehrenberg, 1831 (1 species); *Halgerda* Bergh, 1880 (4 species); *Peliodoris* Bergh, 1880 (1 species); and *Rostanga* Bergh, 1879 (1 species) were obtained from GenBank (Table 1). Following Valdés' (2002) suggestion that the family Chromodorididae Bergh, 1891 is sister to the Discodorididae, the chromodorid nudibranch *Glossodoris hikuensis* (Pruvot-Fol, 1954) was used to root the phylogenetic trees.

Chromatograms of forward and reverse DNA strands were edited and assembled using the software Geneious R11 v. 11.0.5 (Biomatters, Auckland, New Zealand; Kearse et al., 2012). To test for potential contamination, all sequences were individually checked using BLAST, as implemented in Geneious. The DNA sequences of the two protein-coding genes (COI and H3) were translated into amino acid sequences using the invertebrate mitochondrial genetic code to check for the presence of stop codons. Sequences were aligned using the programme MUSCLE (Edgar, 2004) implemented in Geneious under default settings (i.e. a maximum of eight iterations). Single-gene alignments were trimmed at both ends to a position at which at least half of the sequences contained nucleotide information. Blocks of ambiguous data in the 16S rRNA region were identified using Gblocks Server

0.91b (Castresana, 2000) with both stringent and relaxed settings (Table 2). Saturation was tested for the first, second and third codon positions of the protein-coding genes by plotting the total number of transitions and transversions against uncorrected pairwise p-distances between sequences. Intraspecific and interspecific minimum and maximum COI uncorrected genetic distances (p-distances) were calculated for species of *Jorunna* using the programme MEGA X (Kumar et al., 2018) (Table 3).

Best-fit models of evolution were estimated using the Akaike information criterion (Sakamoto, Ishiguro & Kitagawa, 1986) implemented in jModelTest v. 2.1.10 (Guindon & Gascuel, 2003; Darriba et al., 2012). The selected models were GTR + I + G for COI, TVM + I + G for 16S rRNA, TPM1uf + I + G for 16S rRNA stringent (S16S rRNA), TIM1 + I + G for 16S rRNA relaxed (R16S rRNA) and GTR + I for H3. Single-gene phylogenetic analyses for the COI, H3, 16S rRNA, S16S rRNA and R16S rRNA alignments were performed using Bayesian inference (BI) with the software MrBayes v. 3.2.1 (Huelsenbeck & Ronquist, 2001); we used three parallel runs of 5 million generations, sampling every 100 generations. Convergence of independent runs was examined in Tracer v. 1.7 (Rambaut et al., 2018) with a burn-in of 25%. The single-gene datasets were concatenated in Geneious for taxa with sequences available for at least two markers. Two concatenated gene datasets were assembled (mitochondrial genes combined and all genes combined) and phylogenies reconstructed using BI and maximum likelihood (ML). The Bayesian concatenated alignments were run in MrBayes using three parallel runs of 15 million generations each, sampling every 100 generations; for the ML analyses, we used RAxML v. 8.2.12 (Stamatakis, 2014) with random starting trees and 1,000 bootstrap replicates. All phylogenetic analyses were run on the CIPRES Science Gateway v. 3.3 (Miller, Pfeiffer & Schwartz, 2010). Branch support was assessed using posterior probability (PP) values for BI and nonparametric bootstrap support (BS) values for ML. Only branches with PP values ≥ 0.95 and BS values $\geq 75\%$ were considered highly supported (Felsenstein, 1985; Huelsenbeck et al., 2001). Consensus trees were edited in FigTree v. 1.4.4 (Rambaut, 2018) and Gravit Designer v. 2020-1.2.1 (Corel Corporation, 2020).

Species were delimited using the molecular-based automatic barcode gap discovery (ABGD) (Puillandre et al., 2012) method, with all analyses conducted on the ABDG web server (<https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>). The COI alignment (both including and excluding the outgroup) was used as the input file and analysed with the three evolutionary models available (Jukes-Cantor, Kimura and simple distance models) default settings.

Examination of morphoanatomical characters

Morphoanatomical characters of 12 specimens of *J. tomentosa sensu lato*, representing the three lineages recognized by the molecular phylogenetic hypotheses and the ABGD method, were examined. Dissections were performed using a Nikon SMZ 1500 stereo microscope equipped with a Nikon D5100 digital camera and a camera lucida. Oral tentacles, rhinophoral lamellae, branchial leaves and mantle structures, such as the caryophyllidia, were studied prior to dissections. Animals were dissected by dorsal incision and the digestive parts were separated from the buccal mass (by cutting the oesophagus) and the reproductive system (by cutting the hermaphroditic duct). The buccal mass was dissolved in a 10% sodium hydroxide solution until the labial cuticle and radula had been freed from their surrounding tissue (this took c. 24 h). The structures were then rinsed with distilled water and examined with the aid of an Olympus CX31 light microscope using the software cellSens v.1.18 (Olympus Cooperation). Each reproductive system was studied in detail and drawn using a camera lucida. Penial structures and copulatory spines were isolated for further examination by light microscopy and scanning electron microscopy (SEM).

Table 1. List of specimens used for DNA sequencing, with sampling localities and voucher and GenBank acc. nos.

Species	Locality	Voucher no.	GenBank acc. no.		
			COI	16S rRNA	H3
<i>Jorunna funebris</i>	Guam: Mariana Islands	CPIC00633	KP871645*	KP871693*	KP871669*
<i>J. onubensis</i>	Spain: Huelva	ZMBN 125474	MW784171	MW784483	MW810587
<i>J. artsdatabankia</i> n. sp.	Norway: Frøya	NTNU-VM-58891	MW784174	MW784486	MW810589
<i>J. artsdatabankia</i> n. sp.	Norway: Kristiansund	ZMBN 127749	MW784172	MW784487	–
<i>J. artsdatabankia</i> n. sp.	Norway: North Sea	ZMBN 125946	MW784173	MW784485	MW810590
<i>J. tomentosa</i> lineage A	Norway: Gulen	ZMBN 127710	MW784177	MW784490	MW810611
<i>J. tomentosa</i> lineage A	Northern Ireland: Ballyhenry Island	ZMBN 127711	MW784180	MW784489	MW810603
<i>J. tomentosa</i> lineage A	Northern Ireland: Ballyhenry Island	CAS-IZ 193035	MW784176	MW784491	MW810607
<i>J. tomentosa</i> lineage A	Ireland: Ringhaddy	ZMBN 127707	MW784178	MW784488	MW810605
<i>J. tomentosa</i> lineage A	France: La Rochelle	ZMBN 125512	MW784175	MW784492	MW810597
<i>J. tomentosa</i> lineage A	Portugal: Parque Natural da Arrábida	CAS-IZ 176820	MW784179	–	MW810602
<i>J. tomentosa</i> lineage B	Sweden: Kristineberg	–	AJ223267*	AJ225191*	–
<i>J. tomentosa</i> lineage B	Sweden: Kattegatt	Gastr 8965V	MG935216*	–	–
<i>J. tomentosa</i> lineage B	Spain: Bay of Biscay	–	KU697718*	–	–
<i>J. tomentosa</i> lineage B	Norway: Finnmark	NTNU-VM-75953	MW784205	–	–
<i>J. tomentosa</i> lineage B	Norway: Finnmark	NTNU-VM-75975	MW784188	–	–
<i>J. tomentosa</i> lineage B	Norway: Finnmark	NTNU-VM-76040	MW784207	–	–
<i>J. tomentosa</i> lineage B	Norway: Lofoten	NTNU-VM-213	MW784220	–	MW810588
<i>J. tomentosa</i> lineage B	Norway: Frøya	NTNU-VM-58888	MW784204	MW784502	MW810600
<i>J. tomentosa</i> lineage B	Norway: Trondheim	NTNU-VM-66872	MW784227	MW784496	MW810591
<i>J. tomentosa</i> lineage B	Norway: Trondheim	NTNU-VM-66871	MW784195	–	–
<i>J. tomentosa</i> lineage B	Norway: Agdenes	NTNU-VM-67968	MW784221	–	–
<i>J. tomentosa</i> lineage B	Norway: Trondheim	NTNU-VM-66873	MW784213	–	–
<i>J. tomentosa</i> lineage B	Norway: Kristiansund	ZMBN 125644	MW784217	–	–
<i>J. tomentosa</i> lineage B	Norway: Kristiansund	ZMBN 125651	MW784206	–	–
<i>J. tomentosa</i> lineage B	Norway: Kristiansund	ZMBN 125632	MW784222	–	–
<i>J. tomentosa</i> lineage B	Norway: Kristiansund	ZMBN 127775	MW784181	–	–
<i>J. tomentosa</i> lineage B	Norway: Averøy	ZMBN 125591	MW784182	–	–
<i>J. tomentosa</i> lineage B	Norway: Gjemnes	ZMBN 127730	MW784209	–	–
<i>J. tomentosa</i> lineage B	Norway: Gulen	NTNU-VM-66876	MW784191	–	–
<i>J. tomentosa</i> lineage B	Norway: Gulen	NTNU-VM-66874	MW784216	–	–
<i>J. tomentosa</i> lineage B	Norway: Gulen	NTNU-VM-68525	MW784215	–	–
<i>J. tomentosa</i> lineage B	Norway: Gulen	NTNU-VM-66875	MW784228	–	–
<i>J. tomentosa</i> lineage B	Norway: Gulen	ZMBN 127712	MW784219	–	–
<i>J. tomentosa</i> lineage B	Norway: Haugesund	ZMBN 125878	MW784196	–	–
<i>J. tomentosa</i> lineage B	Norway: Egersund	ZMBN 127553	MW784208	–	–
<i>J. tomentosa</i> lineage B	Norway: Egersund	ZMBN 127567	MW784214	–	–
<i>J. tomentosa</i> lineage B	Norway: Egersund	ZMBN 127568	MW784210	–	–
<i>J. tomentosa</i> lineage B	Norway: Drøbak	ZMBN 125038	MW784201	MW784494	MW810596
<i>J. tomentosa</i> lineage B	Norway: Drøbak	ZMBN 125553	MW784211	MW784497	MW810593
<i>J. tomentosa</i> lineage B	Norway: Drøbak	ZMBN 127603	MW784197	MW784503	MW810592
<i>J. tomentosa</i> lineage B	Norway: Drøbak	ZMBN 125057	MW784218	–	–
<i>J. tomentosa</i> lineage B	Norway: Drøbak	ZMBN 125563	MW784212	–	–
<i>J. tomentosa</i> lineage B	Norway: Drøbak	ZMBN 125581	MW784223	–	–
<i>J. tomentosa</i> lineage B	Norway: Drøbak	ZMBN 127577	MW784194	–	–
<i>J. tomentosa</i> lineage B	Norway: Drøbak	ZMBN 127593	MW784189	–	–
<i>J. tomentosa</i> lineage B	Norway: Drøbak	ZMBN 125090	MW784198	–	–
<i>J. tomentosa</i> lineage B	Northern Ireland: Ballyhenry Island	ZMBN 127709	MW784190	MW784501	MW810594
<i>J. tomentosa</i> lineage B	Northern Ireland: Rathlin Island	ZMBN 127708	MW784224	MW784504	MW810608
<i>J. tomentosa</i> lineage B	Northern Ireland: Ballyhenry Island	ZMBN 127704	MW784192	–	–
<i>J. tomentosa</i> lineage B	Northern Ireland: Stangford	ZMBN 127706	MW784183	–	–
<i>J. tomentosa</i> lineage B	Ireland: Connemara	ZMBN 127705	MW784199	MW784500	MW810612
<i>J. tomentosa</i> lineage B	Ireland: Connemara	ZMBN 127714	MW784225	MW784505	MW810609
<i>J. tomentosa</i> lineage B	Ireland: Connemara	ZMBN 127713	MW784200	–	–
<i>J. tomentosa</i> lineage B	Ireland: Connemara	ZMBN 127715	MW784226	–	–
<i>J. tomentosa</i> lineage B	Spain: Pontevedra, Galicia	ZMBN 132446	MW784193	MW784507	MW810599

Table 1. Continued.

Species	Locality	Voucher no.	GenBank acc. no.		
			COI	16S rRNA	H3
<i>J. tomentosa</i> lineage B	Portugal: Parque Natural da Arrábida	CAS-IZ 176819	MW784229	–	–
<i>J. tomentosa</i> lineage B	Azores: Faial Island	CAS-IZ 175753	MW784202	MW784506	MW810610
<i>J. tomentosa</i> lineage B	Azores: Faial Island	CAS-IZ 175752	MW784185	MW784508	MW810604
<i>J. tomentosa</i> lineage B	Azores: Faial Island	CAS-IZ 175757	MW784184	MW784495	MW810606
<i>J. tomentosa</i> lineage B	Azores: Faial Island	CAS-IZ 175761	MW784203	MW784493	MW810595
<i>J. tomentosa</i> lineage B	Azores: Faial Island	ZMBN 81683	MW784186	MW784499	MW810601
<i>J. tomentosa</i> lineage B	Azores: São Miguel Island	ZMBN 87955	MW784187	MW784498	MW810598
<i>J. tomentosa</i> lineage B	South Africa: Eastern False Bay	SAMC-A089801	MW784230	MW784484	–
<i>J. tomentosa</i> lineage B	South Africa: Knysna Lagoon	SAMC-A089803	MW784231	–	–
Outgroup species					
<i>Chromodoris striatella</i>	Australia: Shoalwater Bay	AM C415149D	MG883327*	MG883021*	MG873227*
<i>Chromodoris willani</i>	Japan: Ie Island	UF352011A	MG883374*	MG883069*	MG873242*
<i>Discodoris cebuensis</i>	Hawaii: Maalea Bay	CAS-IZ 185141	KP871639*	KP871687*	KP871663*
<i>Discodoris hummelincki</i>	Jamaica: St. James	CPIC00654	KU950019*	KU949949*	KU950062*
<i>Felimida binza</i>	Portugal: Madeira Island	MMFHN29959	KX262409*	KX262442*	KX279317*
<i>Felimida clenchi</i>	Brazil: Cabo Frio	MZSP97531	KX262390*	KX262429*	KX279311*
<i>Geitodoris heathi</i>	United States: California	CAS-IZ 181314	KP871642*	KP871690*	KP871666*
<i>Glossodoris hikuerensis</i>	Mozambique: Vamizi Island	MB28-0050001	MK994107*	MK994159*	MK994133*
<i>Halgerda carlsoni</i>	Philippines: Batangas	CAS-IZ 177575	KP871643*	KP871691*	KP871667*
<i>Halgerda dichromis</i>	South Africa: KwaZulu-Natal	MHN-VFI	MH578088*	MH578116*	MH578152*
<i>Halgerda nuarrensensis</i>	Mozambique: Nuarro	MB28-004874	MH578102*	MH578115*	MH578132*
<i>Halgerda wasinensis</i>	Mozambique: Pomene	MB28-004918	MH578091*	MH578129*	MH578140*
<i>Peltodoris atromaculata</i>	–	–	AF120637*	DQ280054*	DQ280013*
<i>Rostanga elandsia</i>	South Africa: Olifantsbos Bay	CAS-IZ 176110	KP871651*	KP871699*	KP871674*

Sequences downloaded from GenBank are marked with an asterisk.

Table 2. Gblocks masking parameters for the relaxed and stringent 16S rRNA alignments.

Parameters	Relaxed	Stringent
Minimum number of sequences for a conserved position	22	22
Maximum number of sequences for a flanking position	22	35
Maximum number of contiguous nonconserved positions	8	4
Minimum length of block	5	10
Allowed gap position	Half	None
Gblocks alignment	464 bp (93% of 497 bp in original alignment)	382 (76% of 497 bp in original alignment)

Table 3. Interspecific and intraspecific uncorrected COI p-distances (%) for *Jorunna*.

		1	2	3	4	5
1	<i>J. onubensis</i>	–				
2	<i>J. funebris</i>	16.9	–			
3	<i>J. artsdatabankia</i> n. sp.	12.6–12.7	16.9	0.15		
4	<i>J. tomentosa</i> lineage A	10.0–10.3	18.0–19.1	10.3–10.8	0.0–0.68	
5	<i>J. tomentosa</i> lineage B	10.0–12.0	18.0–20.0	9.0–12.3	3.2–5.0	0.0–0.26

Intraspecific p-distances are in bold font.

Caryophyllidia, labial cuticles, penises and copulatory spines were critical point dried transferring the structures from absolute ethanol to a dry dish and adding one drop of hexamethyldisilazane. After c. 30 min, the dried structures, together with the radulae, were mounted on stubs, sputter coated with gold or gold–palladium and studied by SEM (machines used: Hitachi S-3000N and FEI QuantaTM FEG 450).

Institutional abbreviations

CAS-IZ California Academy of Sciences, California, USA
 MNHN Muséum national d'Histoire naturelle, Paris, France
 NHMUK Natural History Museum, London, UK
 NTNU-VM Norwegian University of Science and Technology, NTNU University Museum, Trondheim, Norway

SAMC South African Museum Collection, Iziko South African Museum, Cape Town, South Africa
 ZMBN Department of Natural History, University Museum of Bergen, University of Bergen, Norway

RESULTS

Molecular phylogenetic analysis

DNA was successfully amplified for 61 of 78 specimens, yielding 113 novel sequences for the three markers COI (61 sequences; 581–658 bp), 16S rRNA (26 sequences; 464–483 bp) and H3 (26 sequences; 252–339 bp). In addition, 49 sequences from GenBank were included in the final phylogenetic analyses (Table 1). Of the single-gene alignments, the COI (658 bp; 79 sequences) and R16S rRNA (464 bp; 42 sequences) ones yielded better resolved phylogenies than the H3 (350 bp; 41 sequences), S16S rRNA (382 bp; 42 sequences) and 16S rRNA (497 bp; 42 sequences) datasets (Fig. 2; Supplementary Material Figs S1–S4). Therefore, from the three 16S rRNA datasets analysed, we selected the R16S rRNA alignment for the concatenation-based analyses.

While the COI phylogenetic analysis showed maximal support for *Jorunna artsdatabankia* sp. nov. (PP = 1, BS = 100%) and high support for *J. tomentosa* lineage A (PP = 0.98, BS = 98%), *J. tomentosa* lineage B was only moderately supported in the BI analysis (PP = 0.90) and not supported in the ML analysis (BS = 48%) (Fig. 2). The 16S rRNA tree recovered the monophyly of *J. artsdatabankia* sp. nov. with marginally high support (PP = 0.94), and clustered all representatives of *J. tomentosa* with marginally high support (PP = 0.92), but sequences of *J. tomentosa* A formed a highly supported clade (PP = 0.99) (Supplementary Material Fig. S1). The S16S rRNA analysis showed two clusters, one consisting of all the sequences of *J. artsdatabankia* sp. nov. (PP = 0.76) and one with all representatives of *J. tomentosa* lineages A and B (PP = 0.97) (Supplementary Material Fig. S2). In the R16S rRNA analysis, *J. artsdatabankia* sp. nov. was only moderately supported (PP = 0.91, BS = 71%), while a clade with all the sequences of *J. tomentosa* A and B was highly supported in the BI analysis but not by ML (PP = 0.95, BS = 57%). All representatives of *J. tomentosa* A clustered together in a highly supported clade (PP = 0.99, BS = 94%; Supplementary Material Fig. S3). The H3 tree provided high support for the monophyly of *J. artsdatabankia* sp. nov. (PP = 0.99), but showed only weak support (PP = 0.58) for the clustering of sequences of *J. tomentosa* lineages A and B (Supplementary Material Fig. S4).

The phylogenetic analysis of the concatenated mitochondrial gene alignments (COI + R16S rRNA) showed maximal support (PP = 1, BS = 100%) for the clade of *J. artsdatabankia* sp. nov. and high support for *J. tomentosa* lineage A (PP = 1, BS = 99%), whereas the cluster of sequences representing *J. tomentosa* lineage B was not highly supported (PP = 0.70, BS = 59%) (Fig. 3). The concatenated analysis of the three genes (COI + R16S rRNA + H3) yielded maximal support for *J. artsdatabankia* sp. nov. (PP = 1, BS = 100%). In this analysis, the sequences of *J. tomentosa* lineages A and B clustered together (PP = 1, BS = 47%), with the representatives of *J. tomentosa* A forming a highly supported clade (PP = 1, BS = 99%; Fig. 4).

The COI uncorrected p-distances between all sequenced species of *Jorunna* are given in Table 3. The largest genetic distance was observed between *J. funebris* and *J. tomentosa* (18–20%). The estimated genetic distance between *J. artsdatabankia* sp. nov. and *J. tomentosa* lineage A ranges from 10.3% to 10.8%, and between *J. artsdatabankia* sp. nov. and *J. tomentosa* lineage B from 9.0% to 12.3%. Between *J. tomentosa* lineage A and *J. tomentosa* lineage B, the estimated genetic distance was substantially lower, ranging between 3.2% and 5.0%.

The ABGD analyses, both with and without outgroup species, were run with the prior maximum divergence of intraspecific diversity (*P*) ranging from 0.001 to 0.1 for each of the evolutionary models and resulted in up to ten partitions, ranging from 2 to 35 groups (Supplementary Material Fig. S5). The partitions that yielded four

and five groups (excluding outgroup species) were congruent with the results of the phylogenetic analyses. Between *P* values of 0.02 and 0.06, the analyses with all three evolutionary models suggested four lineages, namely *J. funebris*, *J. onubensis*, *J. artsdatabankia* sp. nov. and the lineage of *J. tomentosa* A + *J. tomentosa* B. Between *P* values of 0.005 and 0.01, the analyses treated *J. tomentosa* A and *J. tomentosa* B as two distinct species, with *J. funebris*, *J. onubensis* and *J. artsdatabankia* sp. nov. being the other species recognized. Partitions that retrieved less than four or five groups (*P* > 0.06) were considered unreliable as all species except *J. funebris* were lumped as a single taxonomic unit, even though morphological and molecular evidence clearly separates these species. On the other hand, partitions exceeding four or five groups (*P* < 0.003) likely result from splitting artefacts caused by lower *P* values (Puillandre *et al.*, 2012), and were not consistent with the phylogenetic hypotheses (Figs 2–4) and morphological data (see the ‘Systematic Descriptions’ section).

Overall, the molecular phylogenetic and ABGD analyses are consistent with the presence of an undescribed species, here called *J. artsdatabankia* sp. nov. These analyses also indicate the presence of two lineages within *J. tomentosa*, suggesting a possible case of incipient speciation (see the ‘Systematic Descriptions’ and ‘Discussion’ sections).

SYSTEMATIC DESCRIPTIONS

Superfamily DORIDOIDEA Rafinesque, 1815

Family DISCODORIDIDAE Bergh, 1891

Genus *Jorunna* Bergh, 1876

Jorunna Bergh, 1876: 414 (type species *Doris johnstoni* Alder & Hancock, 1845 [= *Jorunna tomentosa* (Cuvier, 1804), by monotypy]).

Kentrodoris Bergh, 1876: 413 (type species *Kentrodoris rubescens* Bergh, 1876 [= *Jorunna rubescens* (Bergh, 1876); subsequent designation (Ev. Marcus, 1976)]).

Audura Bergh, 1878: 567 (type species *Audura maima* Bergh, 1878 [= *Jorunna maima* (Bergh, 1878), by monotypy]).

Centrodoris P. Fischer, 1880–1887 (1883): 522 (unjustified emendation of *Kentrodoris* Bergh, 1876).

Awuka Er. Marcus, 1955: 155 (type species *Awuka spazzola* Er. Marcus, 1955 [= *Jorunna spazzola* (Er. Marcus, 1955); subsequent designation (Ev. Marcus, 1976)]).

Taxonomic history: See Valdés & Gosliner (2001) and Camacho-García & Gosliner (2008) for a taxonomic history of the genus *Jorunna*.

Diagnosis: Adult size 1–20 cm. Body depressed, oval-elongate. Background colour white, greyish-white, yellowish-orange, reddish-brown or purple. Notum with large brown blotches, dark rings or brownish speckles. Notum velvety in appearance; densely covered with caryophyllidia with long conical base, long spicules and rounded, ciliated tubercle. When present, mantle glands white, distributed around mantle edge. Rhinophores up to 20 lamellae, fully retractile into low sheaths; with apical knob. Gills retractile into low sheath; up to 14 uni- to tripinnate branchial leaves encircling anal papilla. Rhinophores and gills may be speckled with white, brown or black spots. Foot narrow, anteriorly notched, grooved. Oral tentacles slender digitiform, bulbous or triangularly flattened. Labial cuticle smooth or armed with jaw elements. Radula formula 14–35 × 33–13.0.13–33, except for *J. pardus* that exhibits a much higher number of lateral teeth (41 × 80.0.80 for a specimen with TL = 20 mm). Lateral teeth hook-shaped, larger in middle of half-row, with outermost laterals slender, sometimes denticulated; rachidian tooth absent. Reproductive system triaular; ampulla large; prostate massive, differentiated; penis and vagina unarmed. Large accessory gland with copulatory spine. Distributed worldwide, from boreal waters to the tropics; occurring

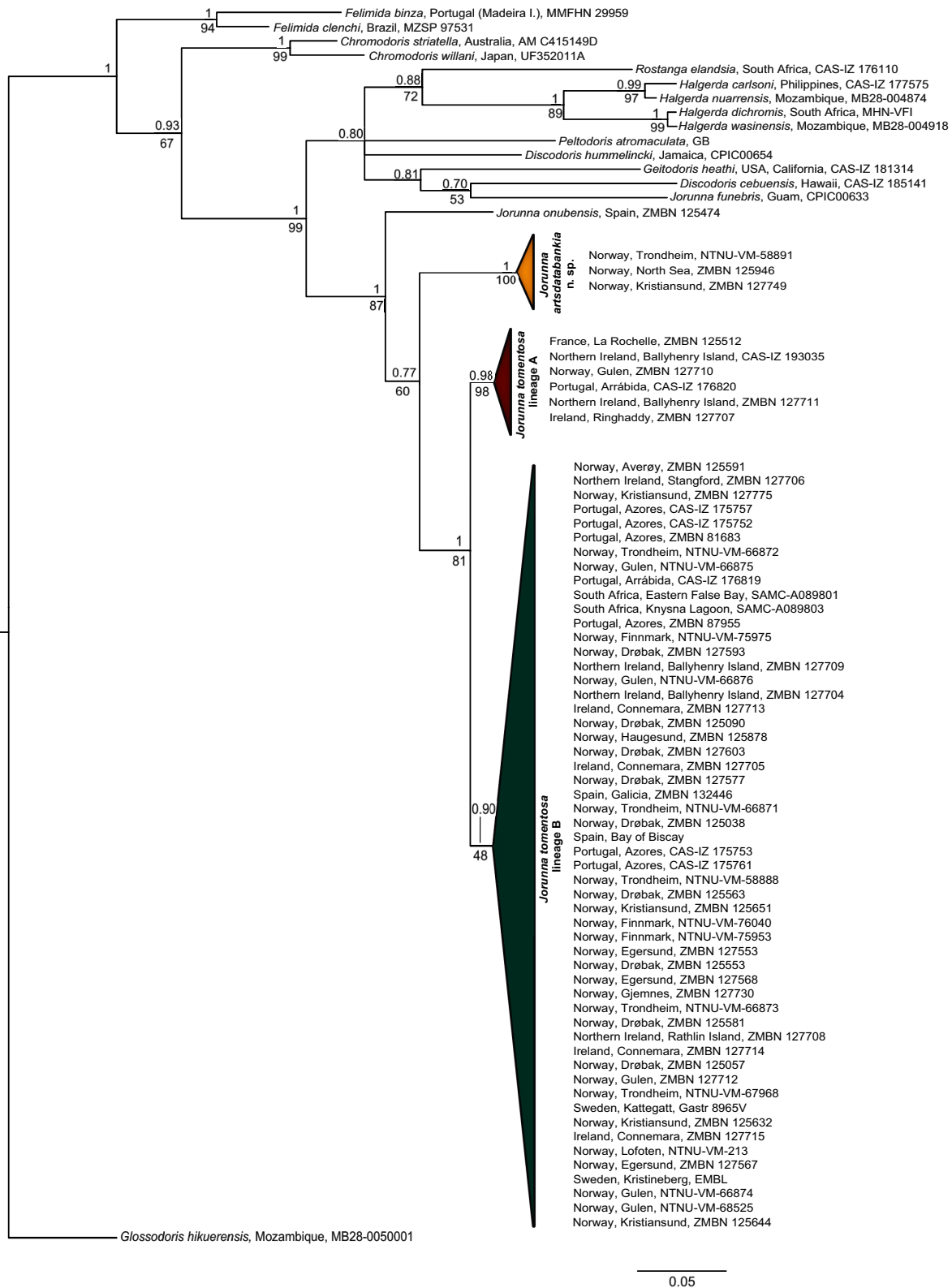


Figure 2. Bayesian phylogeny based on the COI dataset. PP and BS values are shown above and below branches, respectively. BS values <50% are not shown. The tree is rooted on *Glossodoris hikuensis*. Scale bar indicates substitutions per site.

SYSTEMATICS OF *JORUNNA*

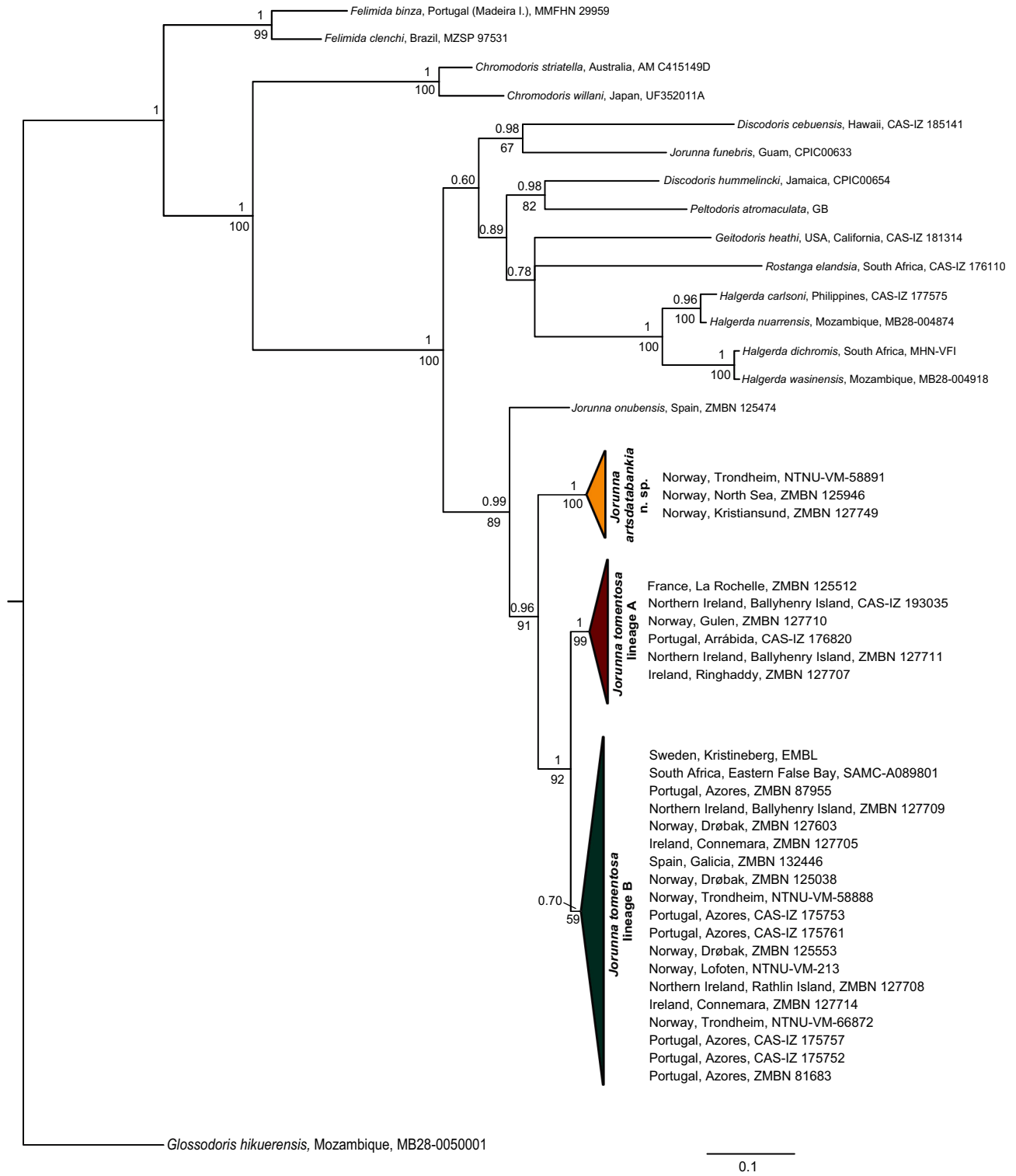


Figure 3. Bayesian phylogeny based on COI + R16S rRNA dataset. PP and BS values are shown above and below branches, respectively. BS values <50% are not shown. The tree is rooted on *Glossodoris hikuensis*. Scale bar indicates substitutions per site.

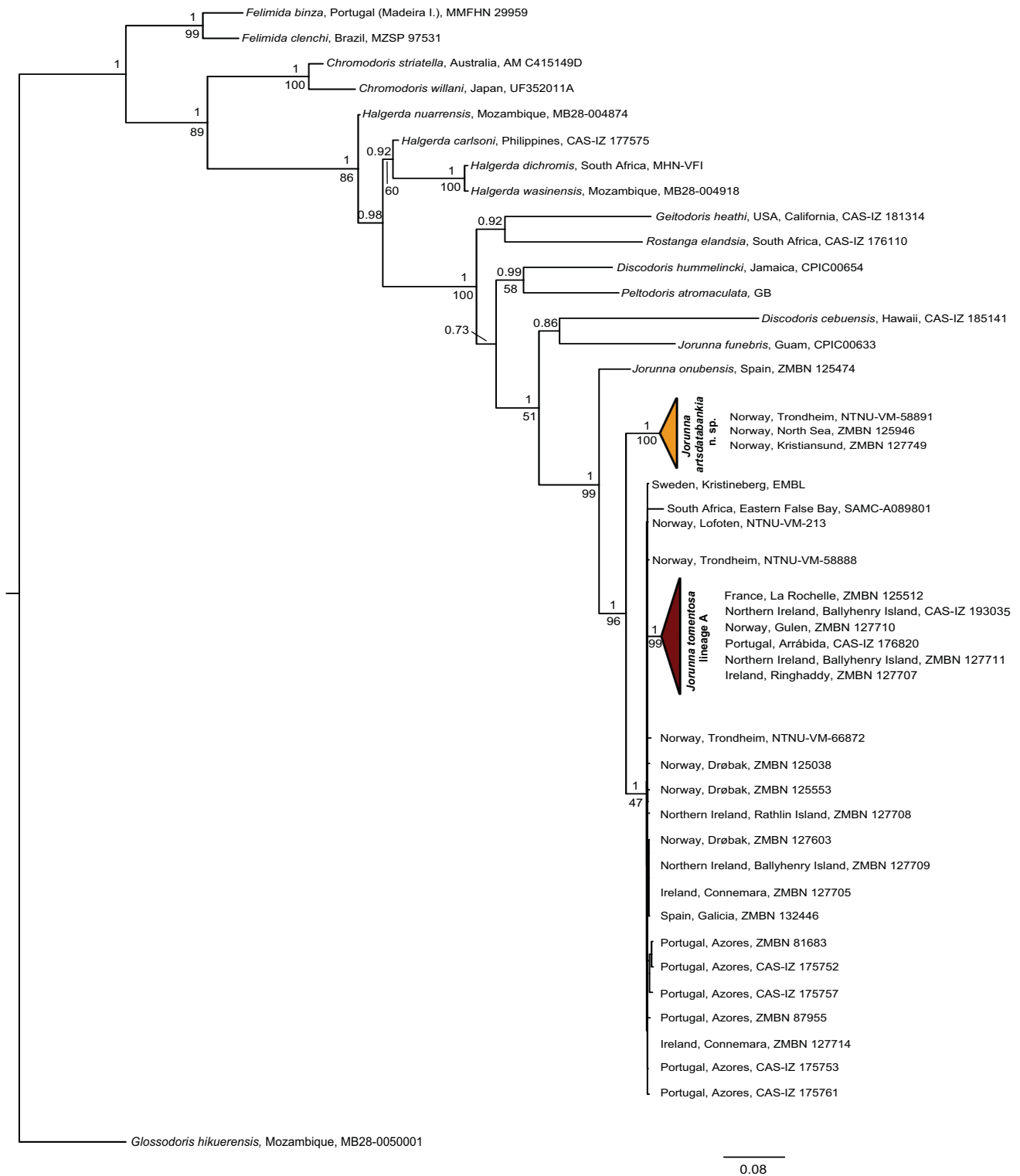


Figure 4. Bayesian phylogeny based on the COI + R16S rRNA + H3 dataset. PP and BS values are shown above and below branches, respectively. The tree is rooted on *Glossodoris hikuensis*. Scale bar indicates substitutions per site.



Figure 5. Live images of *Jorunna tomentosa* lineage B from Connemara, western Ireland, the type locality of *J. lemchei*. **A.** ZMBN 127714 (fixed), TL = 14 mm. **B.** ZMBN 127713 (fixed), TL = 13 mm. **C.** ZMBN 127715 (fixed), TL = 13 mm. **D.** ZMBN 127705 (fixed), TL = 25 mm.

mostly in shallow waters (Ev. Marcus, 1976; Valdés & Gosliner, 2001; Camacho-García & Gosliner, 2008; Edmunds, 2011; Alvim & Pimenta, 2013; Ortea *et al.*, 2014; Ortea & Moro, 2016; Zenetos *et al.*, 2016; Tibiriça *et al.*, 2017; Furfaro *et al.*, 2020).

***Jorunna tomentosa* (Cuvier, 1804)**
(Figs 1, 5–12)

Doris tomentosa Cuvier, 1804: 470 [La Rochelle; neotype MNHN-IM-2000-35690, designated by Camacho-García & Gosliner (2008)].

Jorunna tomentosa—Iredale & O'Donoghue, 1923: 227. Odhner, 1939: 35, fig. 18. Pruvot-Fol, 1954: 274, fig. 109. Swennen, 1961: 196. Ev. Marcus, 1976: 20, figs 9–19. García-Gómez, 1983: 43. Cervera *et al.*, 2004: 44, 82. Camacho-García & Gosliner, 2008: 144–148, figs 1a, b, 2, 3. Moen & Svensen, 2020: 369. Malmberg & Lundin, 2015: 38. Prkić *et al.*, 2018: 222.

Doris johnstoni Alder & Hancock, 1845: fam. 1, pl. 5, figs 1–8 (Cullercoats, North Shields, UK; syntype NHMUK 1858.5.28.203). Alder & Hancock, 1851: fam. 1, pl. 2, figs 8–10. Hancock & Embleton, 1852: 215, pl. 12, figs 2, 10, pl. 14, fig. 10, pl. 15, fig. 1, pl. 17, fig. 2. Alder & Hancock, 1855: pl. 46, fig. 4.

Jorunna johnstoni—Bergh, 1876: 414. Bergh, 1880: 47, 117; pl. 8, fig. 19; pl. 9, figs 1–11. Bergh, 1881: 114, pl. K, figs 20–28. Bergh, 1884: 683, pl. 70, figs 21–23. Cuénnot, 1904: 17. Hoffmann, 1926: 10. Odhner, 1926: 23. Labbé, 1933: 214, figs 2, 3. Nobre, 1938: 51.

Jorunna johnstoni var. *alba* Bergh, 1881: 119; pl. J, figs 17–21; pl. K, figs 29–36. Bergh, 1884: 683–685, pl. 70, fig. 20.

Jorunna atypha Bergh, 1881: 125, pl. J, figs 22–25 (Trieste, Italy, Adriatic Sea; type material untraceable, probably lost).

Taxonomic history: Cuvier (1804) established the species *Doris tomentosa* on material received from the naturalist Louis Benjamin Fleuriau de Bellevue; the type locality was La Rochelle, France. Cuvier described the species as having a curved body with a semi-translucent white to grey background colour and a mantle exceed-

ing the foot; he also indicated that the animal was “a little woolly to the touch, ... covered by small rounded tubercles in elongated cones” (Cuvier, 1804: 472). Cuvier's (1804) reference ‘woolly’ texture is what botanists refer to as hairy (Latin: *tomentosa*).

Johnston (1838) identified a small specimen from Berwick Bay, UK, as *Doris obvelata* O. F. Müller, 1776 [today considered a synonym of *Cadlina laevis* (Linnaeus, 1767); see MolluscaBase, 2020b], but Alder & Hancock (1845) examined this same specimen, identifying it as new species, which the authors named after George Johnston as *Doris johnstoni* Alder & Hancock, 1845. Fischer (1869) suggested that *D. johnstoni* was a junior synonym of *D. tomentosa* and, since then, Fischer's view has been generally accepted (e.g. Ev. Marcus, 1976; Valdés & Gosliner, 2001; Camacho-García & Gosliner, 2008; Alvim & Pimenta, 2013; Ortea & Moro, 2016).

Bergh (1876), based on the original description of *D. johnstoni*, proposed the new combination *Jorunna johnstoni*, which was adopted, for example, by Cuénnot (1904). Later, Bergh (1881) described three white specimens from Trieste, Italy, as *J. johnstoni* var. *alba*. This variety was considered to be a junior synonym of *J. tomentosa* by Ev. Marcus (1976), who at the same time suggested the possibility that these specimens could belong to a distinct species. Bergh (1881) also described the species *J. atypha* from a single specimen from Trieste, Italy, and this resembles the greyish-white morphotypes that we detected for *J. tomentosa* lineage B. After having contacted the curators at the Natural History Museum of Denmark, University of Copenhagen (formerly the Zoological Museum of the University of Copenhagen), we concluded that the type specimen of *J. atypha* is most likely lost. We provisionally list *J. atypha* as a junior synonym of *J. tomentosa*. Ideally, future studies should include material from Trieste for comparison.

Although Iredale & O'Donoghue (1923), based on Fischer (1869) and Bergh (1876), were the first to use the combination *J. tomentosa*, several authors continued to refer to the species as *J. johnstoni* (e.g. Hoffmann, 1926; Odhner, 1926; Labbé, 1933; Nobre, 1938). Kay & Young (1969) and Edmunds (1971) considered *J. tomentosa* to be distributed worldwide, but Ev. Marcus (1976) assigned the specimens studied from Hawaii by Kay & Young (1969) and from Tanzania by Edmunds (1971) to the new species *J. alisonae* Ev. Marcus,

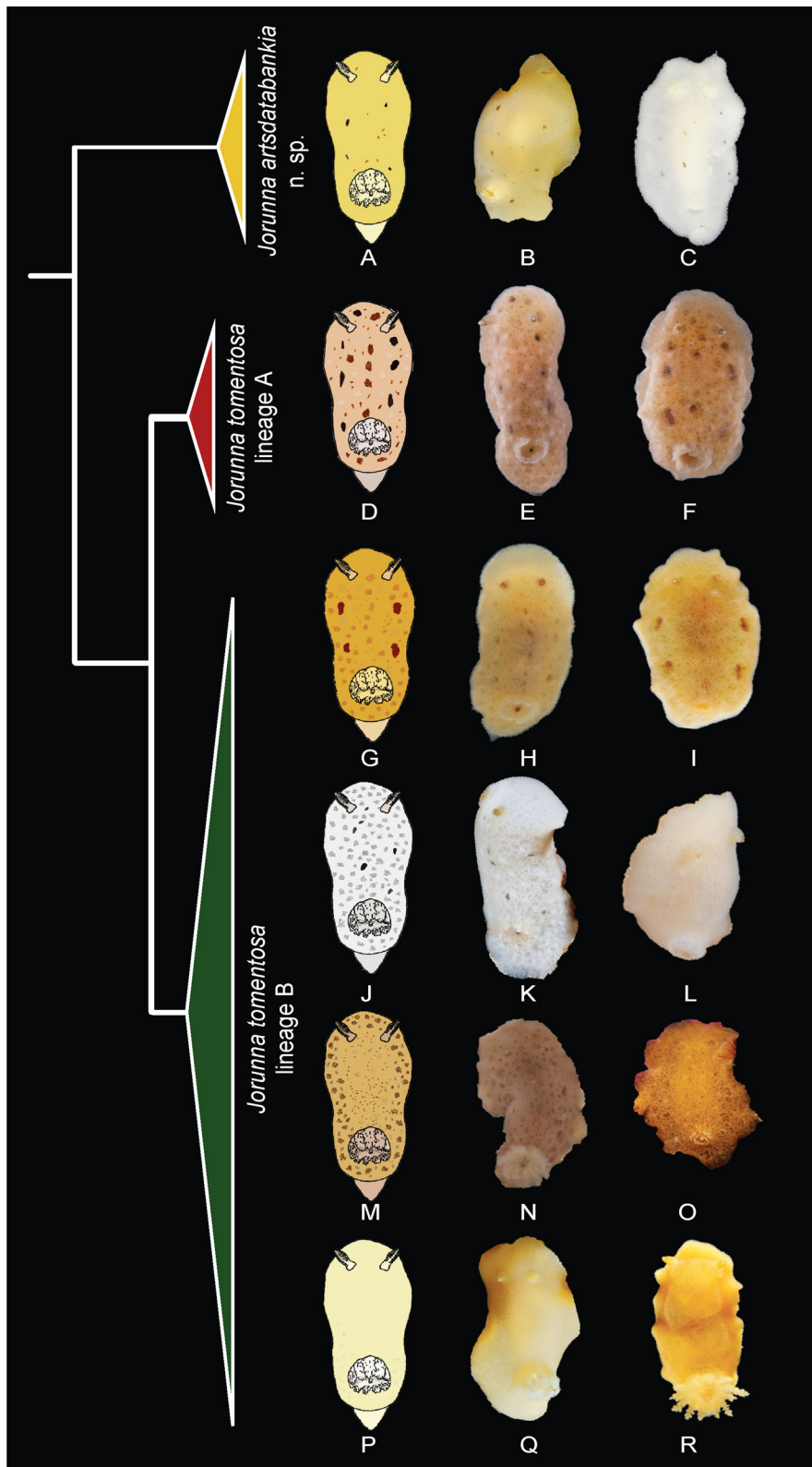


Figure 6. Drawings and live images representing the main colour morphs of *Jorunna artdatabankia* n. sp. (A–C), *J. tomentosa* lineage A (D–F) and *J. tomentosa* lineage B (G–R). **B.** ZMBN 127749. **C.** NTNU-VM-58891. **E.** ZMBN 127711. **F.** ZMBN 127707. **H.** ZMBN 127712. **I.** ZMBN 127567. **K.** ZMBN 125591. **L.** ZMBN 125632. **N.** ZMBN 87955. **O.** ZMBN 125553. **Q.** ZMBN 125651. **R.** ZMBN 125038.

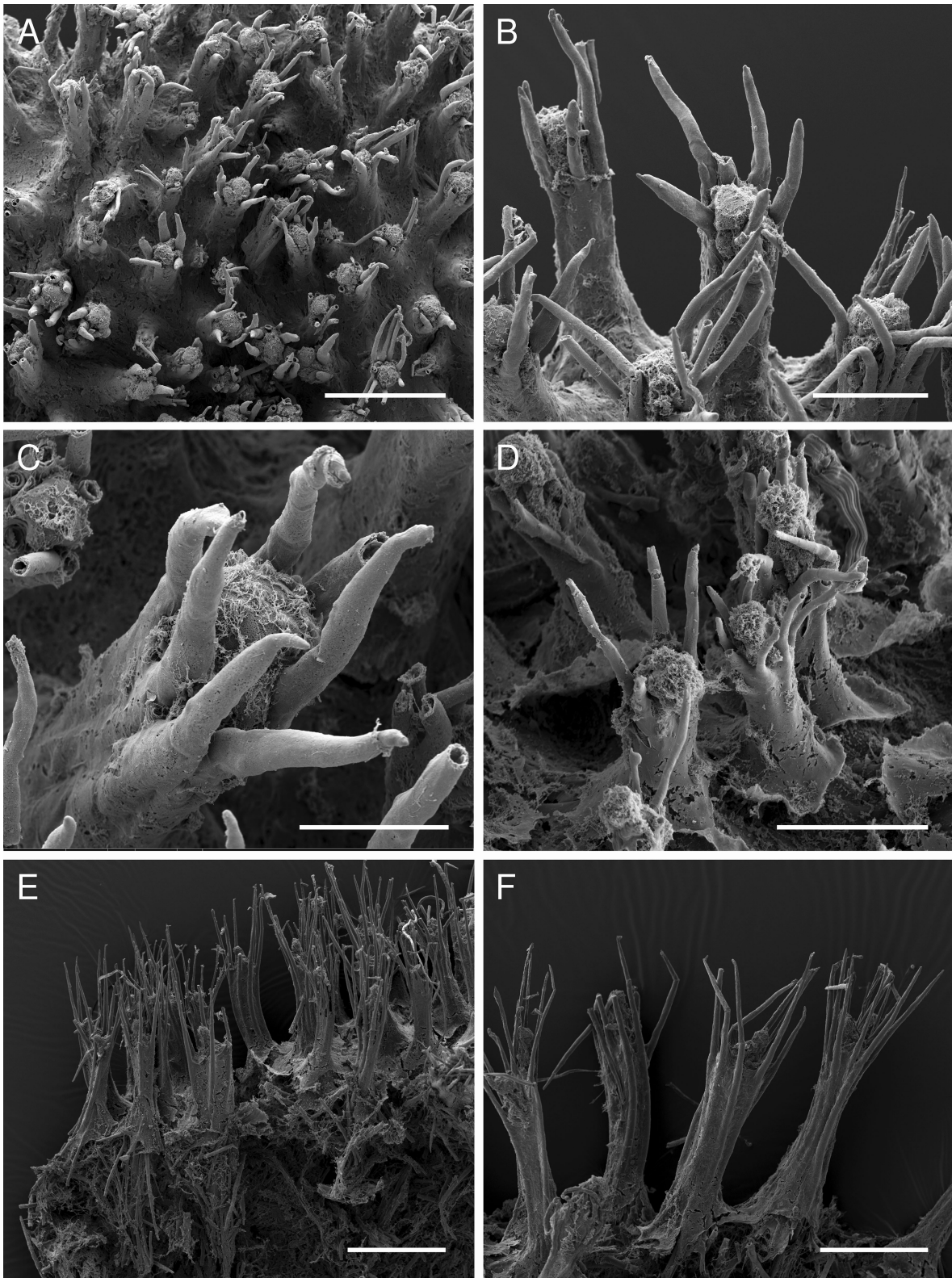


Figure 7. *Jorunna tomentosa*, SEM images of caryophyllidia. **A–C.** Caryophyllidia with up to seven spicules surrounding the ciliated knob *J. tomentosa* lineage B (NTNU-VM-213). **D.** Detailed view of caryophyllidia in *J. tomentosa* lineage B (ZMBN 127705). **E, F.** Caryophyllidia with long, slender spicules in *J. tomentosa* lineage A (ZMBN 127711). Scale bars: **A, E** = 250 µm; **B** = 100 µm; **C** = 50 µm; **D** = 100 µm; **F** = 150 µm.

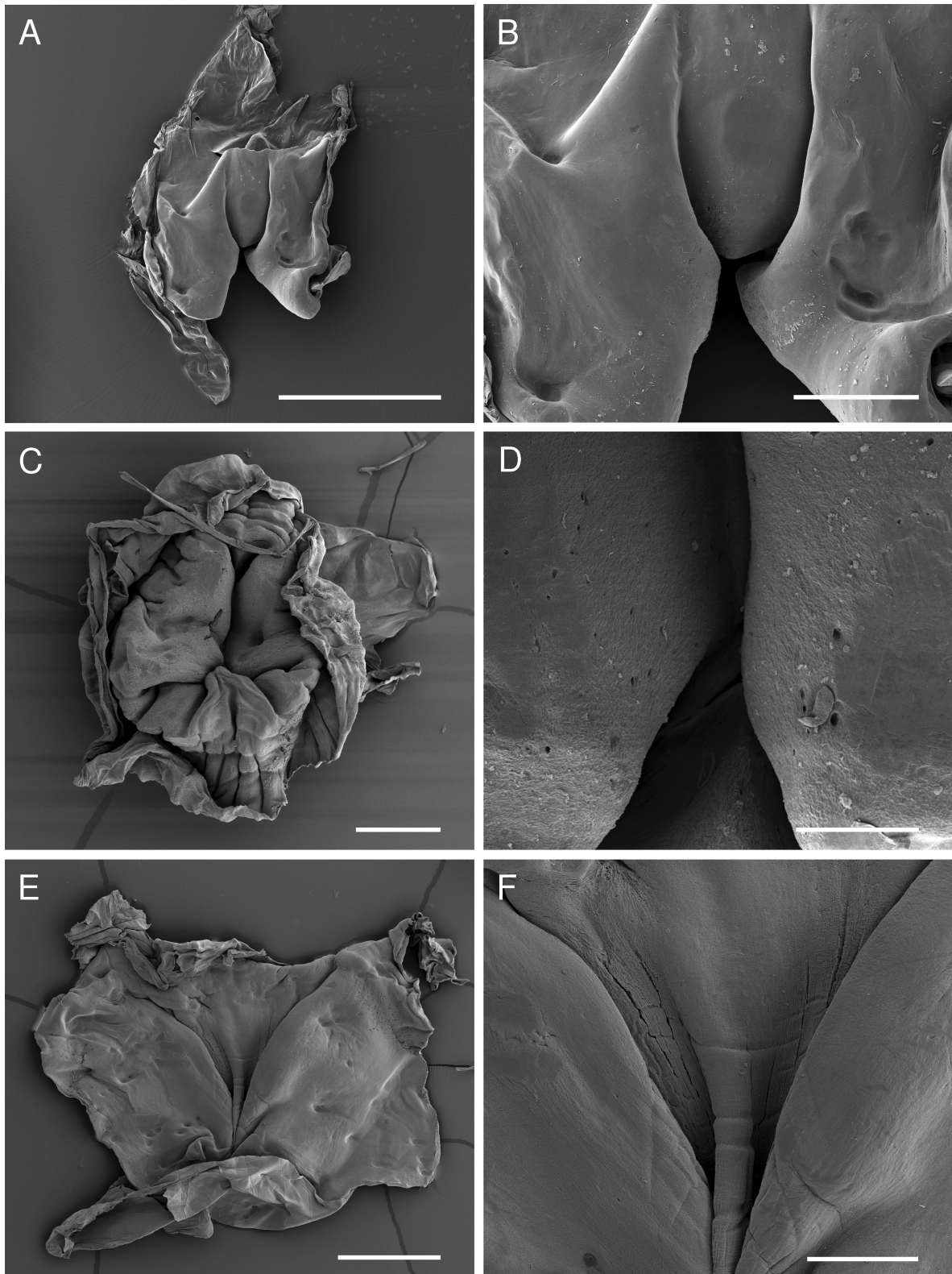


Figure 8. *Jorunna tomentosa*, SEM images of labial cuticles. **A, B.** Opened labial cuticle of *J. tomentosa* lineage A (ZMBN 127707). **C, D.** Labial cuticle (not opened) of *J. tomentosa* lineage B (ZMBN 87955). **E, F.** Opened labial cuticle of *J. tomentosa* lineage B (ZMBN 125553). Scale bars: **A** = 500 μm ; **B** = 50 μm ; **C, E** = 500 μm ; **D** = 250 μm ; **F** = 150 μm .

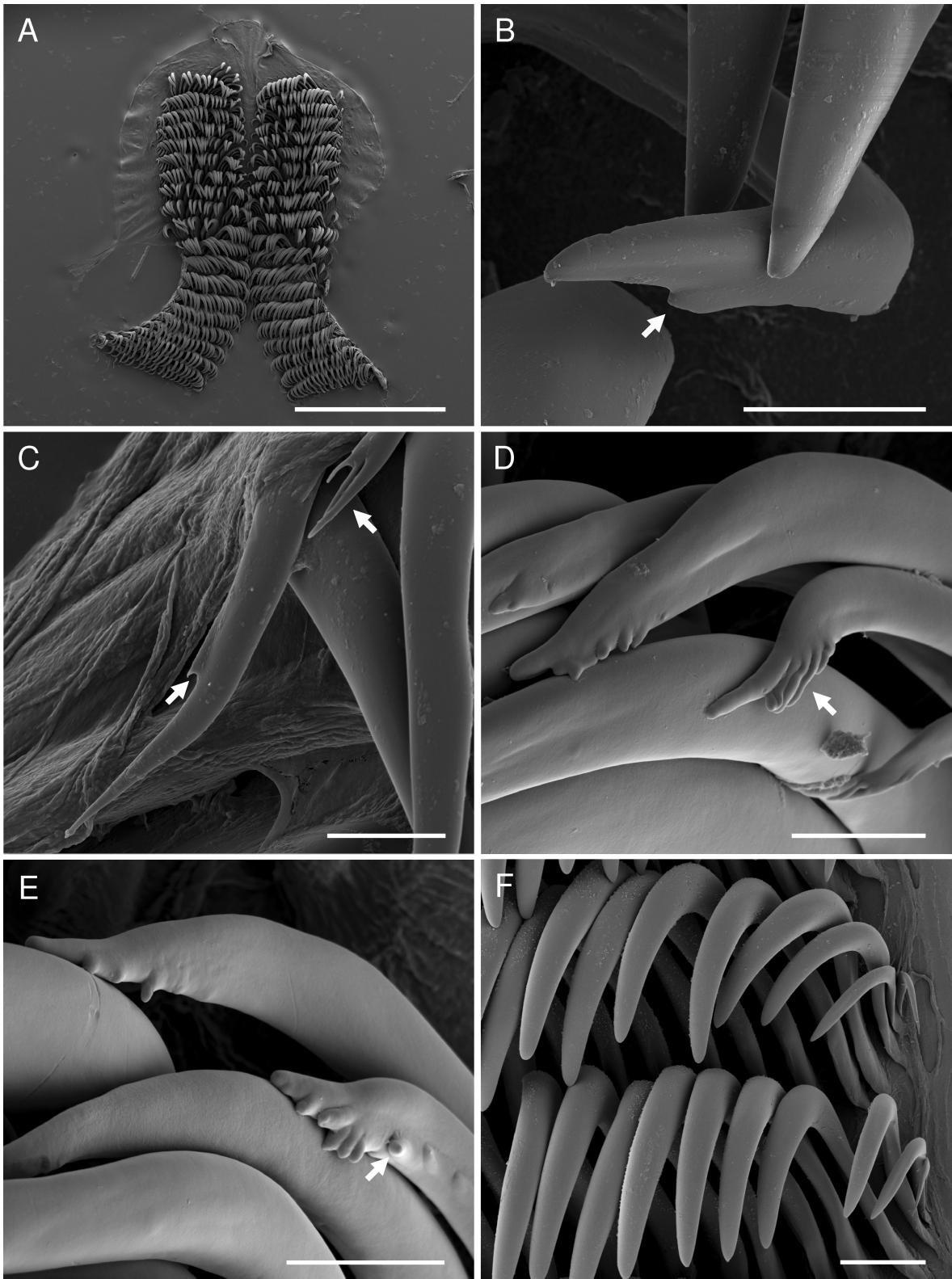


Figure 9. *Jorunna tomentosa*, SEM images of the radula. **A.** General view of the radula in *J. tomentosa* lineage A ($21 \times 23.0.23$; ZMBN 127710). **B.** Detail of innermost lateral carrying one denticle (see arrow) in *J. tomentosa* lineage A (ZMBN 127710). **C.** Detailed view of outermost sickle-shaped laterals with denticles (see arrows) in *J. tomentosa* lineage A (ZMBN 127710). **D, E.** Detail of outermost lateral teeth in *J. tomentosa* lineage B, showing that up to eight denticles can be either knob-shaped or finger-like (see arrows) (ZMBN 125038). **F.** Detail of outermost sickle-shaped laterals in *J. tomentosa* lineage B, showing the lack of denticles (ZMBN 127603). Scale bars: **A** = 1 mm; **B** = 20 μm ; **C** = 10 μm ; **D** = 10 μm ; **E** = 10 μm ; **F** = 50 μm .

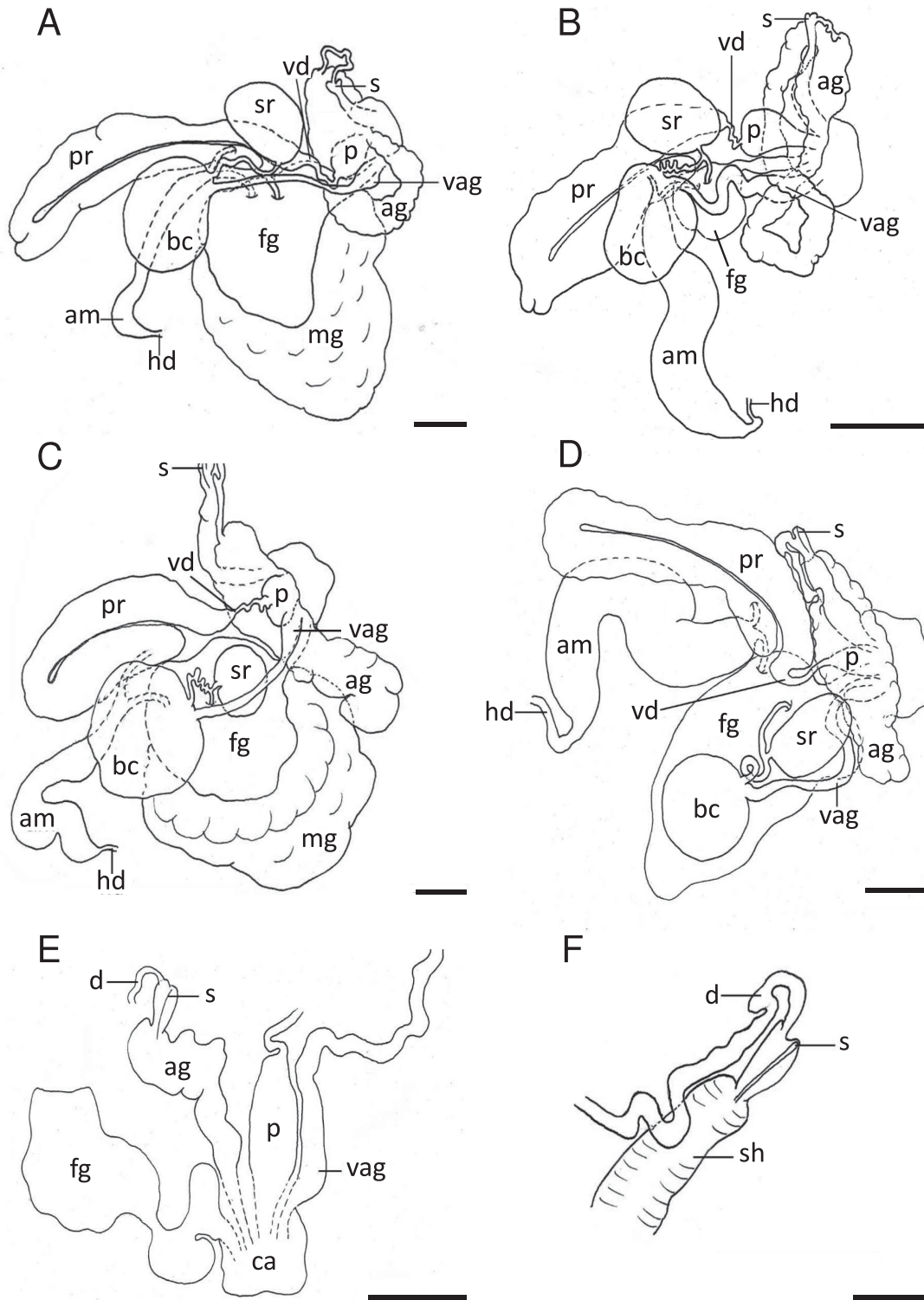


Figure 10. *Jorunna tomentosa* lineages A and B, reproductive organs. **A.** Reproductive system of *J. tomentosa* lineage B (ZMBN 87955), showing large mucous gland. **B.** Reproductive system of *J. tomentosa* lineage B (ZMBN 127705), showing small female gland (mucous gland removed for clarity). **C.** Reproductive system of *J. tomentosa* lineage A (ZMBN 127710), showing large female and mucous glands. **D.** Reproductive system of *J. tomentosa* lineage A (ZMBN 127711; mucous gland removed for clarity). **E.** *Jorunna tomentosa* lineage A (ZMBN 127707). Female gland, accessory gland, penis and vagina converging in the common atrium. **F.** *Jorunna tomentosa* lineage A (ZMBN 127711). Detail of muscular spine sheath with attached duct connecting to accessory gland. Abbreviations: ag, accessory gland; am, ampulla; bc, bursa copulatrix; ca, common atrium; d, duct connecting ag with spine sheath; fg, female gland; hd, hermaphroditic duct; mg, mucous gland; p, penis; pr, prostate; s, copulatory spine; sh, spine sheath; sr, seminal receptacle; vag, vagina; vd, vas deferens. Scale bars: **A–E** = 1 mm; **F** = 500 μ m.

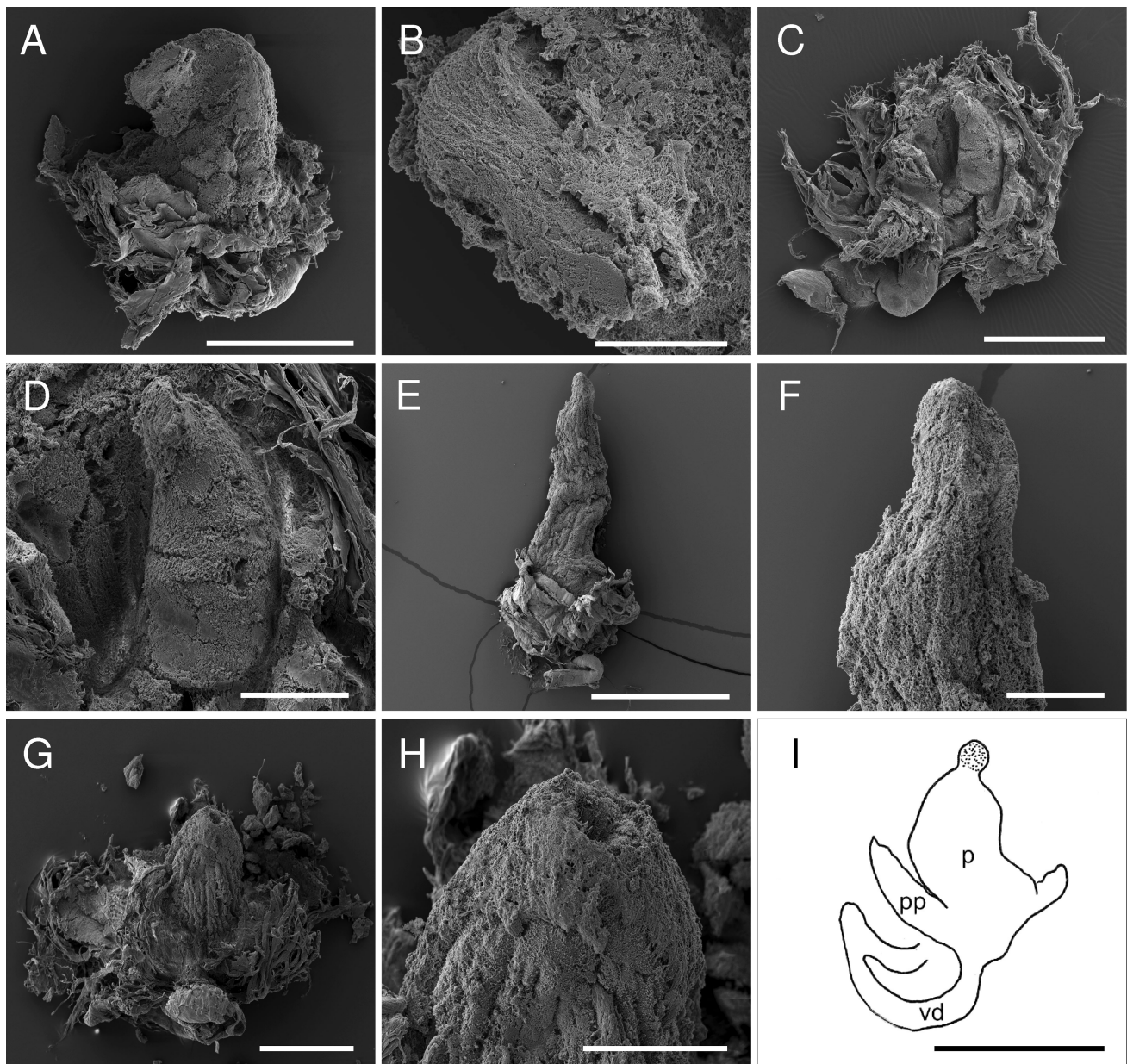


Figure 11. *Jorunna tomentosa* lineages A and B, SEM images and drawings of the penis. **A.** General view of the penis and penial tip in *J. tomentosa* lineage A (ZMBN 127710). **B.** Detailed view of penial tip in *J. tomentosa* lineage A (ZMBN 127710). **C.** General view of penis in *J. tomentosa* lineage A (ZMBN 127711), showing penis embedded in the penial bulb. **D.** Detailed view of the penis in *J. tomentosa* lineage A (ZMBN 127711). **E.** General view of the penis in *J. tomentosa* lineage B (ZMBN 125553). **F.** Detailed view of the penis and penial tip in *J. tomentosa* lineage B (ZMBN 125553). **G.** General view of the penis and penial tip in *J. tomentosa* lineage B (ZMBN 87955). **H.** Detailed view of the penis and penial tip in *J. tomentosa* lineage B (ZMBN 87955). **I.** Illustration of penis in *J. tomentosa* lineage B (ZMBN 87955), prior to preparation for SEM, showing the circular knob at the tip of the penis (lost in the preparation for SEM). Abbreviations: p, penis; pp, tissue of penial bulb; vd, vas deferens. Scale bars: **A, C** = 500 μ m; **B, H** = 100 μ m; **D, F** = 150 μ m; **E** = 1 mm; **G** = 250 μ m; **I** = 500 μ m.

1976 and *J. malcolmi* Ev. Marcus, 1976, respectively. Following these changes, the distribution of *J. tomentosa* could no longer be considered to be cosmopolitan.

Material examined: All specimens have been fixed and sequenced, unless stated otherwise. NORWAY. Kråka, Borgvær, Vestvågøy, Nordland (68.334701, 13.813291), 1 spec., dissected, NTNU-VM-213 (TL = 20 mm; lineage B). Sarnespollen, Magerøya, Finnmark (70.988712, 25.747425), 1 spec., not fixed, NTNU-VM-75953 (lineage B). Engelskmanntsjæret, Bøkfjorden, Finnmark (69.744444, 30.086295), 2 specs, NTNU-VM-75975, NTNU-VM-76040 (both

lineage B). Slettvik, Agdenes, Trøndelag (63.592309, 09.540685), 2 specs: NTNU-VM-66871, NTNU-VM-67968 (both lineage B). Aursøya Brygge, Frøya, Trøndelag (63.792438, 8.89163), 1 spec., NTNU-VM-58888 (TL = 10 mm; lineage B). NTNU Biological Station, Trondheim, Trøndelag (63.441109, 10.348831), 2 specs: NTNU-VM-66873 (TL = 13 mm; lineage B); NTNU-VM-66872 (TL = 14 mm; lineage B). Brattøya, Kristiansund, Møre og Romsdal (63.062076, 7.695494), 4 specs: ZMBN 125651 (TL = 26 mm; lineage B); ZMBN 125644 (TL = 15 mm; lineage B); ZMBN 125632 (TL = 25 mm; lineage B); ZMBN 127775 (TL = 35 mm; lineage B). Stavnes, Averøy, Møre og

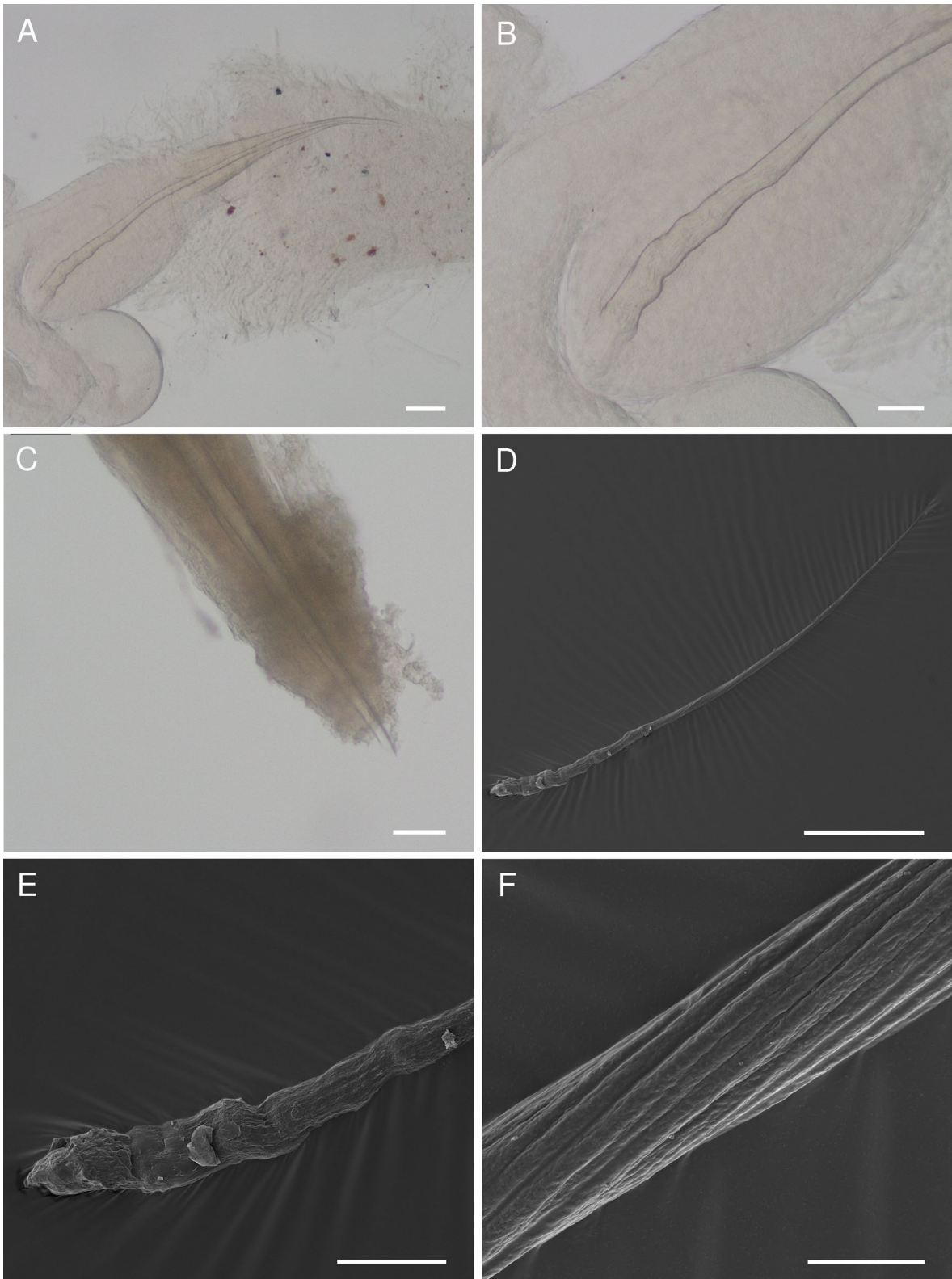


Figure 12. *Jorunna tomentosa* lineages A and B, light microscopy (A–C) and SEM (D–F) images of the copulatory spine. **A.** Copulatory spine embedded in muscular tissue in *J. tomentosa* in lineage A (ZMBN 127710). **B.** Detail of spine base embedded in muscular tissue in *J. tomentosa* in lineage A (ZMBN 127710). **C.** Tip of spine protruding from tissue in *J. tomentosa* in lineage B (ZMBN 87955). **D.** Micrograph showing complete copulatory spine in *J. tomentosa* in lineage A (ZMBN 127710). **E.** Detail of spine base in *J. tomentosa* in lineage A (ZMBN 127710). **F.** Micrograph showing detail of spine texture in *J. tomentosa* in lineage B (ZMBN 87955). Scale bars: **A–C, F** = 50 µm; **D** = 25 µm; **E** = 250 µm.

Romsdal (63.114832, 7.662235), 1 spec., ZMBN 125591 (TL = 28 mm; lineage B). Krifast, Bergsøya, Gjemnes, Møre og Romsdal (62.973522, 7.784554), 1 spec., ZMBN 127730 (TL = 12 mm; lineage B). Glossvika, Gulen, Vestland (60.960225, 5.128899), 5 specs: ZMBN 127710 (TL = 23 mm; lineage A); NTNU-VM-66874 (TL = 12 mm; lineage B); NTNU-VM-66876 (TL = 12 mm; lineage B); NTNU-VM-68525 (TL = 17 mm; lineage B); NTNU-VM-66875 (TL = 12 mm; lineage B). Gylte Brygge, Drøbak, Viken (59.682436, 10.623525), 7 specs: ZMBN 125553 (TL = 25 mm; lineage B); ZMBN 127603 (TL = 25 mm; lineage B); ZMBN 125038 (TL = 30 mm; lineage B); ZMBN 125563 (TL = 23 mm; lineage B); ZMBN 125581 (TL = 18 mm; lineage B); ZMBN 127577 (TL = 17 mm; lineage B); ZMBN 127593 (TL = 12 mm; lineage B). Færgestad, Hurum, Viken (59.664458, 10.600886), 1 spec., ZMBN 125057 (TL = 26; lineage B). Sandholmen, Haugesund, Rogaland (59.408210, 5.377251), 1 spec., ZMBN 125878 (TL = 13 mm; lineage B). Tingelsædet, Egersund, Rogaland (58.417110, 5.998327), 3 specs: ZMBN 127553 (TL = 15 mm; lineage B); ZMBN 127567 (TL = 18 mm; lineage B); ZMBN 127568 (TL = 12 mm; lineage B). NORTHERN IRELAND. Ballyhenry Island, Strangford Lough (54.393969, -5.578313), 4 specs: ZMBN 127704 (TL = 32 mm; lineage B); ZMBN 127709 (TL = 29 mm; lineage B); ZMBN 127711 (TL = 30 mm; lineage A); CAS-IZ 193035 (lineage A). Rathlin Island (55.31138, -6.256670), 1 spec., ZMBN 127708 (TL = 17 mm; lineage B). Ringhaddy, Strangford Lough (54.451046, -5.631184), 1 spec., ZMBN 127707 (TL = 30 mm; lineage A). IRELAND. Strangford Lough (54.537024, -5.615899), 1 spec., ZMBN 127706 (TL = 21 mm; lineage B). Inishdegil More, Connemara (53.636815, -9.919531), 4 specs: ZMBN 127715 (TL = 13 mm; lineage B); ZMBN 127713 (TL = 13 mm; lineage B); ZMBN 127714 (TL = 14 mm; lineage B); ZMBN 127705 (TL = 25 mm; lineage B). FRANCE. Vieux Passage, Plouhinec (47.671968, -3.209786), 1 spec., ZMBN 125512 (lineage A). SPAIN. San Vicente do Grove, Pontevedra, Galicia (42.455301, -8.922588), 1 spec., ZMBN 132446 (TL = 10 mm; lineage B). PORTUGAL. Faial Island, Azores (38.590668, -28.697813), 5 specs: ZMBN 81683 (TL = 9 mm; lineage B); CAS-IZ 175753 (TL = 9 mm; lineage B); CAS-IZ 175752 (lineage B); CAS-IZ 175757 (TL = 12 mm; lineage B); CAS-IZ 175761 (TL = 9 mm; lineage B). São Miguel Island, Mosteiros, Azores (37.898156, -25.821991), 1 spec., dissected ZMBN 87955 (TL = 30 mm; lineage B). Parque Natural da Arrábida, Arrôr (38.439806, -9.053361), 2 specs: CAS-IZ 176820 (lineage A); CAS-IZ 176819 (lineage B). SOUTH AFRICA. Eastern False Bay (-34.182600, 18.821896), 1 spec., SAMC-A089801 (lineage B). Knysna Lagoon (-34.049100, 23.048600), 1 spec., SAMC-A089803 (lineage B).

Diagnosis: Background colour grey-white, yellow-cream, dark yellow, pale orange or orange-brown; caryophyllidia uniform, dense, sometimes tilted towards each other forming slightly elevated whitish patches; notum plain or mottled with small, pale brown spots, often combined with 4–9 large, dark brown blotches aligned along lateral and median lines. Mantle glands present. Rhinophores with 9–12 lamellae, uppermost with brown pigmentation. Nine to 14 gills, slightly brighter than background colour, with brown pigmentation on some leaves, cup-shaped. Foot visible when animal in motion. Oral tentacles digitiform. Radular formula 19–25 × 28–19.0.19–28. Up to 8 slender, sickle-shaped outermost lateral teeth. Outermost laterals predominantly denticulated (up to 8 denticles), sometimes smooth. Labial cuticle smooth. Bursa copulatrix up to 3 times larger than seminal receptacle. Copulatory spine 0.55–1.1 mm in length.

External morphology (Figs 1, 5–7): TL = 20–30 mm. Coloration of notum orange-brown (lineage A; Figs 1, 6D–F) or varying from grey-white to yellow, yellow-orange and orange-brown (lineage B; Figs 1, 5, 6G–R); notum with 4–9 dark brown blotches aligned

along lateral and median lines (lineage A; Figs 1, 6D–F) or mottled with small, pale brown spots, sometimes in combination with dark brown blotches or lacking spots and blotches (lineage B; Figs 5, 6G–I). Caryophyllidia densely spaced, some tilted towards each other forming white patches (Fig 7). Rhinophores with 9–12 lamellae, slightly brighter than dorsum, pigmented at the tips. Nine to 14 bi- to tripinnate gills, slightly brighter than dorsum, encircling pigmented anal pore. Foot of same colour as dorsum, posteriorly visible when gliding, somewhat pointed at end. Oral tentacles pale yellow, slender.

Labial cuticle (Fig 8): Labial cuticle smooth.

Radula (Fig 9): Radular formula of smallest studied specimens 21–22 × 23.0.23 (TL = 23 mm; ZMBN 125038, lineage B; ZMBN 127710, lineage A) and largest studied specimens 19–25 × 28–25.0.25–28 (TL = 30 mm; ZMBN 87955, lineage B; ZMBN 127707, lineage A; ZMBN 127711, lineage A). Radula broad. Rachidian tooth absent; lateral teeth simple, hook-shaped with broad base and rounded cusp; mid-lateral teeth larger than inner laterals; innermost laterals smooth or with 1 denticle or round swelling; 3–8 slender, sickle-shaped outermost lateral teeth; outermost lateral teeth smooth or bearing up to 8 denticles.

Reproductive system (Figs 10–12): Hermaphroditic duct slender, emerging from digestive gland. Ampulla long, curved, divided into short oviduct entering upper mass of female gland and connective duct entering prostate. Prostate large, tubular, differentiated into 2 portions; narrows into short coiled vas deferens leading to penial bulb situated within common atrium. Penis cylindrical, smooth. Vagina wider and longer than deferent duct; without hooks, enters common atrium. Bursa copulatrix rounded, up to 2 times as large as oval seminal receptacle, connected by a short, coiled duct to bursa. Uterine duct thin, connecting distally with female gland mass, entering common atrium. In mature specimens, hardened female gland mass is surrounded by large mucous gland; immature specimens possess small, soft female gland and lack mucous gland. Accessory gland large, convoluted; emerges into long, coiled duct connecting to heart-shaped ovate sac bearing a straight copulatory spine with rounded base; ovate sac embedded in muscular pouch emptying into common atrium; copulatory spine placed within a lining membrane that forms a protective sheath and protrudes from posterior end of ovate sac beyond tip of spine.

Ecology: Common in shallow rocky subtidal zones (Cordeiro *et al.*, 2015; Moen & Svensen, 2020; this study), where it is often found on top of or next to sponges on which it is known to feed, such as *Halichondria panicea*, *Haliclona oculata* and *Haliclona cinerea* (Swennen, 1961; Wolter, 1967; Bloom, 1976; McDonald & Nybakken, 1997; Moen & Svensen, 2020). Nevertheless, field observations seem to indicate that *Jorunna* feeds only on haplosclerid sponges, such as those of the genus *Haliclona* (order Haplosclerida), and that records of feeding on *Halichondria* spp. (order Suberitida) are likely to be misidentifications. Furthermore, *Jorunna tomentosa* has been found crawling upon ascidians and other rock-associated fauna (personal observations).

Grieg (1912) reported specimens with a white background colour (no reference to speckles or blotches) from depths between 110 and 450 m in the North Sea, off Norway and the Faroe Islands. Although difficult to confirm, based on the depth and colour pattern, it is possible that the specimens studied by Grieg (1912) were examples of the new species described here.

Distribution: In the north, from Bøkfjorden, Troms og Finnmark and Borgvær, Vestvågøy, Nordland (this study), southwards along Trøndelag, Møre og Romsdal, Vestland, Viken and Rogaland in Norway, the Kattegat in Sweden (Hansson, 1998; Evertsen & Bakken, 2002, 2005, 2013), Helgoland, Germany (Ev. Marcus,

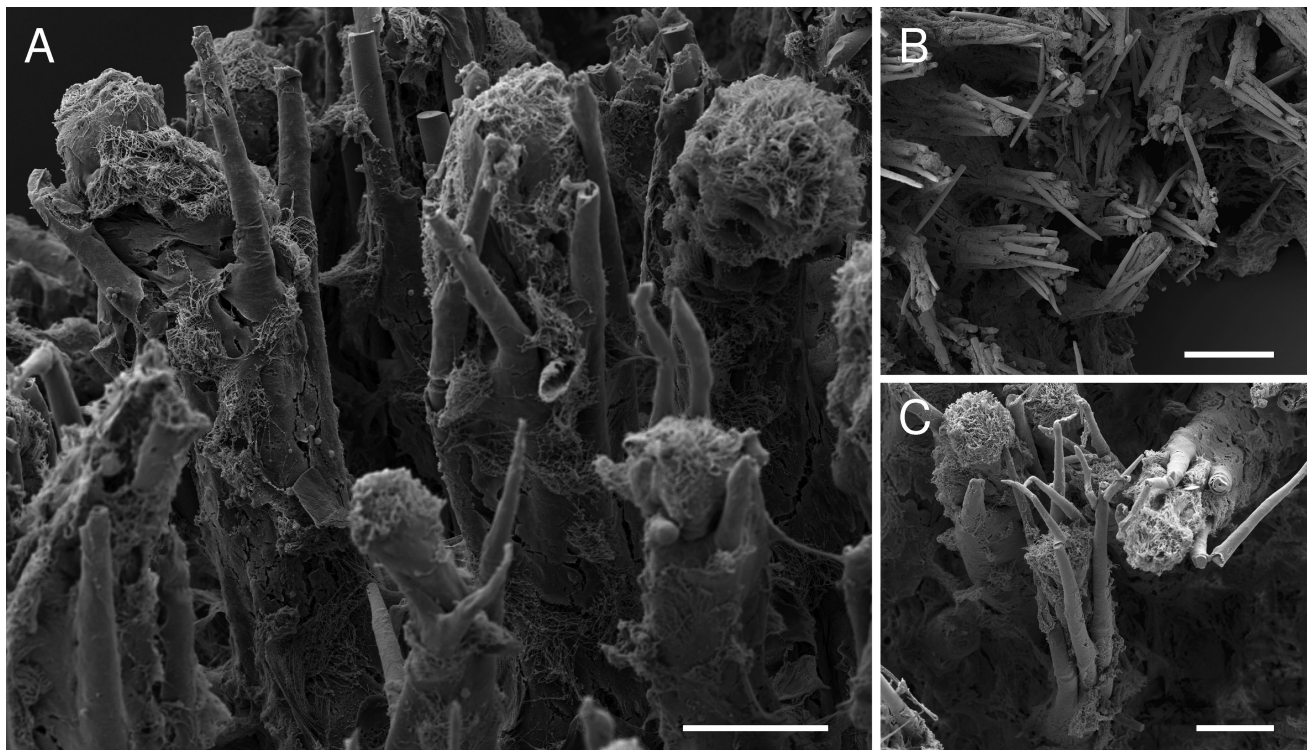


Figure 13. *Jorunna artsdatabankia* n. sp., SEM images of caryophyllidia. **A.** Detail of caryophyllidia (NTNU-VM-58891). **B.** General view of caryophyllidia (ZMBN 127749; structures slightly damaged). **C.** Detail of spicules surrounding the ciliated tubercle (NTNU-VM-58891). Scale bars: **A** = 250 µm; **B**, **C** = 50 µm.

1976), the Netherlands (Swennen, 1961), British Isles (Thompson & Brown, 1984; Picton & Morrow, 1994; Moen & Svensen, 2020), Atlantic coast of France where the type locality, La Rochelle, is located (Camacho-García & Gosliner, 2008) to the Iberian Peninsula including the archipelagos of the Azores and Canary Islands (Ros, 1978; Malaquias, 2001; Cordeiro *et al.*, 2015; Ortea & Moro, 2016). In the Mediterranean Sea, the species is distributed along the coasts of Spain (Camacho-García & Gosliner, 2008; Ballesteros *et al.*, 2016), Italy (Furfaro *et al.*, 2020), Slovenia (Zenetos *et al.*, 2016), Croatia (Prkić *et al.*, 2018) and Turkey (Saltik, 2005). The species is also reported from South Africa, between Elands Bay on the Atlantic coast to Knysna on Indian Ocean coast (Gosliner, 1987; Camacho-García & Gosliner, 2008; this study).

Remarks: The systematic revisions by Ev. Marcus (1976) and Camacho-García & Gosliner (2008) included comprehensive morphoanatomical data on *Jorunna tomentosa*. However, the present study is the first to recognize the existence of two putative lineages within *J. tomentosa* (here designated A and B), suggesting a possible case of incipient speciation. Specimens of lineage A correspond to the morphotype originally described for *J. tomentosa* (Cuvier, 1804; Alder & Hancock, 1845; Pruvot-Fol, 1954; Ev. Marcus, 1976), which is characterized by an orangish-brown notum covered with dark brown blotches (Figs 1B, 6D–F). Specimens of lineage B are more variable in both notal coloration and blotchiness (Figs 1C–H, 6G–R). Genetically, the single-gene COI alignment (Fig. 2) suggests the existence of the two lineages, although lineage B is only marginally supported (lineage A: PP = 0.98; lineage B: PP = 0.90). However, the 16S rRNA data showed that the two lineages cluster together (PP = 0.95) with a subset of lineage A specimens forming a cluster within the larger clade (Supplementary Material Figs S1–S3). The H3 gene data (Supplementary Material Fig. S4) showed that while specimens of lineages A and B clustered together, this was not supported (PP = 0.58). These findings, in addition to the

COI uncorrected p-distances between lineages A and B (3.2–5.0%), which are substantially lower than the distances recorded between the well-established species of the genus (Table 3), suggest a possible case of incipient speciation (i.e. are not yet consistent with complete lineage sorting and the occurrence of two valid species). Moreover, our detailed study of the anatomy of these two lineages did not reveal the presence of clear anatomical differences (Figs 7–12).

There are discrepancies in the data available for the number of gills in *J. tomentosa*. For example, Thompson & Brown (1984: 219, pl. 21) and Hayward & Ryland (2017: fig. 10.26), reported 17 branchial leaves in specimens of 40 and 55 mm, but only 9 and 11 leaves are visible in their illustrations. Among the material examined here, a maximum of 14 branchial leaves were counted and this is assumed to correspond to the maximum number of gills in this species (see Table 4). According to Ev. Marcus (1976) and Camacho-García & Gosliner (2008), the number of denticles on the outermost lateral teeth is a variable character both among and within species of *Jorunna*. This observation was confirmed for both lineages of *J. tomentosa* examined here: Both may bear up to 8 denticles or may have the outermost lateral radular teeth entirely smooth.

Jorunna artsdatabankia new species (Figs 1, 6, 13–17)

Jorunna sp. nov.—Goodwin *et al.*, 2011b: 39. Goodwin *et al.*, 2011a: 55.

Jorunna tomentosa—Anderson, 1999–2020 (in part, records from Scotland). Picton & Morrow, 2016.

Type material: Holotype (sequenced and dissected; TL of fixed specimen = 40 mm), Brattøya, Kristiansund, Møre og Romsdal, Norway (63.062076, 7.695494), ZMBN 127749. Paratype (sequenced and dissected, TL of fixed specimen = 15 mm), Skogsøya, Frøya, Trøndelag, Norway (63.845076, 8.631778), NTNU-VM-

Table 4. Synoptic table of diagnostic characters of the currently recognized and valid species of *Jorunna* in Europe, including *J. artsdatabankia* n. sp. and *Gargamella lemchei*.

	<i>J. artsdatabankia</i> n. sp.	<i>J. tomentosa</i> (Cuvier, 1804)	<i>J. efe</i> Ortea, Moro & Caballer in Ortea <i>et al.</i> , 2014	<i>J. evansi</i> (Elliott, 1906)	<i>J. onubensis</i> Cervera, García-Gómez & García, 1986	<i>J. spazzola</i> (Er. Marcus, 1955)	<i>G. lemchei</i> (Ev. Marcus, 1976)
Dorsum	White to yellow with irregular small brown spots.	Grey-white, yellow-cream, orange or reddish-brown. Dark blotches in two lateral rows, combined with dark spots scattered across notum. Some specimens lack spots.	Pale pink, orange or reddish. Darker spots scattered across notum.	Violet to violet-cream. Dark blotches of various size scattered across notum, parted by whitish circles.	Light brown to pink. Dark blotches of varying size scattered across notum.	Purplish to whitish-grey. Brown spots on each side of notum forming a row, or spots near margin irregularly arranged.	Cream to pale brown. May carry minute brown spots.
Caryophyllidia	c. 200 μm long, dense and uniform	c. 150 μm long, dense and uniform	c. 400 μm long, dense and uniform	c. 200 μm long, dense and uniform	c. 180 μm long, dense and uniform	c. 300 μm long, dense and uniform	c. 200 μm long, dense; some tilted towards each other forming whitish, slightly raised spots
Mantle glands	Present	Present	Present	Present	Present	Present	Not known
Rhinophores	White to yellow-cream, 9–12 lamellae. Not dotted. Apex of knob protruding.	Yellow-cream to grey, 9–12 lamellae, dotted in upper part. Apex of knob protruding.	Yellow-cream to pale pink, 12–14 lamellae, dotted. White apex of knob protruding.	Violet to violet-cream, 10–12 lamellae, dotted in lower part. White upper part to knob; apex protruding.	Light brown to pink, 9–15 lamellae, dotted. Apex of knob protruding.	Light grey, 8–10 lamellae, dotted. Opaque white tips with apex of knob protruding.	Creamy-whitish, up to 20 lamellae, lacking dots. Apex of knob protruding.
Gills	9–14 bi- to tripinnate leaves. Similar in colour to dorsum; not dotted.	9–14 bi- to tripinnate leaves forming a cup. Yellow-cream to grey, sometimes dotted.	9 bi- to tripinnate leaves arranged in a circle. Similar to dorsal colour, with lighter apices.	10 uni- to bipinnate leaves arranged in a circle. Similar to dorsal colour, with white apices.	9–12 bi- to tripinnate leaves arranged in a circle. Light brown to transparent appearance, dotted.	5–10 bi- to tripinnate leaves arranged in a circle. Light grey, speckled with minute brown spots.	11–12 bi- to tripinnate leaves arranged in a circle, cup-like. Whitish, slightly dotted.
Foot	Grooved and notched anteriorly.	Posteriorly visible when animal is in motion. Grooved and notched anteriorly.	Posteriorly visible when animal is in motion. Grooved and notched anteriorly.	Posteriorly visible when animal is in motion. Grooved and notched anteriorly.	Posteriorly visible when animal is in motion. Grooved and notched anteriorly.	Grooved and notched anteriorly.	Grooved and notched anteriorly.
Oral tentacles	Digitiform	Digitiform	Digitiform	Bulbous with pointy tip	Digitiform, slender	Triangular, flattened	Triangular, flattened
Radular formula (shown in relation to TL)	15–40 mm: 19–25 × 21–18.0.18–21	16–30 mm: 19–25 × 28–19.0.19–28	22–30 mm: 20–22 × 25–22.0.22–25	14 mm: 19 × 20.0.20	14–18 mm: 18–21 × 24–18.0.18–24	7 mm: 22 × 13.0.13; TL (?): 15 × 20.0.20	12 mm: 31 × 35.0.35; TL (?): 26 × 32.0.32

Table 4. Continued.

	<i>J. artsdatabankia</i> n. sp.	<i>J. tormentosa</i> (Cuvier, 1804)	<i>J. efe</i> Ortea, Moro & Caballer in Ortea et al., 2014	<i>J. evansi</i> (Eliot, 1906)	<i>J. onubensis</i> Cervera, García-Gómez & García, 1986	<i>J. spazzola</i> (Er. Marcus, 1955)	<i>G. lemchei</i> (Ev. Marcus, 1976)
Radular teeth	Hook-shaped with single cusp. Both innermost and outermost laterals lack denticles.	Hook-shaped with single cusp. Outermost laterals slender, with up to 8 denticles, or smooth.	Hook-shaped with single cusp. Outermost 3 laterals slender and sickle-shaped; with up to 2 denticles.	Hook-shaped with single cusp. Outermost 6 laterals slender, with up to 5 denticles.	Hook-shaped with single cusp. Outermost 4–5 laterals slender, with small denticles.	Hook-shaped with single cusp. Innermost laterals with up to 4 denticles. Outermost slender, with finger-like projections.	Hook-shaped with single cusp. Outermost 4 laterals slender. Teeth smooth.
Labial cuticle	Smooth	Smooth	With jaw elements	With jaw elements	With jaw elements	With jaw elements	Smooth
Copulatory spine	Straight, round base, 1.6–1.7 mm in length	Straight, round base, 550 μm to 1.1 mm in length	Present	Curved, rounded base, c. 300 μm	Straight spine, c. 100 μm	Straight base, c. 165 μm	Straight, c. 500 μm
Bursa copulatrix and seminal receptacle	Bursa copulatrix slightly larger than seminal receptacle	Bursa copulatrix up to three times larger than seminal receptacle	Bursa copulatrix up to three times smaller than seminal receptacle	Bursa copulatrix up to twice the size of seminal receptacle	Bursa copulatrix up to three times larger than seminal receptacle	Bursa copulatrix about twice the size of seminal receptacle	Bursa copulatrix slightly larger than seminal receptacle
Penial hooks	Absent	Absent	Absent	Absent	Absent	Absent	Present
Deferent duct	Shorter than vagina, convoluted. Longer when compared to <i>J. tormentosa</i> .	Shorter than vagina, convoluted.	Approximately three times longer than vagina, highly convoluted.	Approximately twice the length of vagina, convoluted.	Over three times longer than vagina, highly convoluted; connected to shorter, convoluted nonprostatic deferent duct.	Shorter than vagina, convoluted.	Shorter than vagina, slightly convoluted.
Geographic range	Norwegian coast and offshore North Sea grounds	Norwegian coast, Faroe Islands, Britain, France, Spain, Azores, Canary Islands, Italy, Slovenia, Croatia, Algeria, South Africa	Lanzarote, Tenerife, Azores	Cape Verde Islands (São Vicente) and Italy (Naples)	Spain, Canary Islands, Algarve, Portugal	Mediterranean, Barbados, Brazil, Mexico, Costa Rica, Bahamas	Western Ireland (Ballyvaughan Bay)
Type locality	Brattøya, Kristiansund, Norway	La Rochelle, France	El Reducto, Lanzarote	São Vicente, Cape Verde Islands	El Portil, Huelva, Spain	Ilha de São Sebastião, São Paulo, Brazil	Ballyvaughan Bay, western Ireland
Key references	This study	Marcus (1976); Camacho-García & Gosliner (2008)	Ortea et al. (2014)	Eliot (1906); Rudman & Avern (1989); Ortea & Moro (2016)	Cervera, García-Gómez & García (1986); Malaquias & Morenito (2000); Cervera et al. (2004)	Marcus (1955); Alvim & Pimenta (2013)	Marcus (1976); Just & Edmunds (1985); Camacho-García & Gosliner (2008)

Data on *J. tormentosa* are from the published literature and this study.

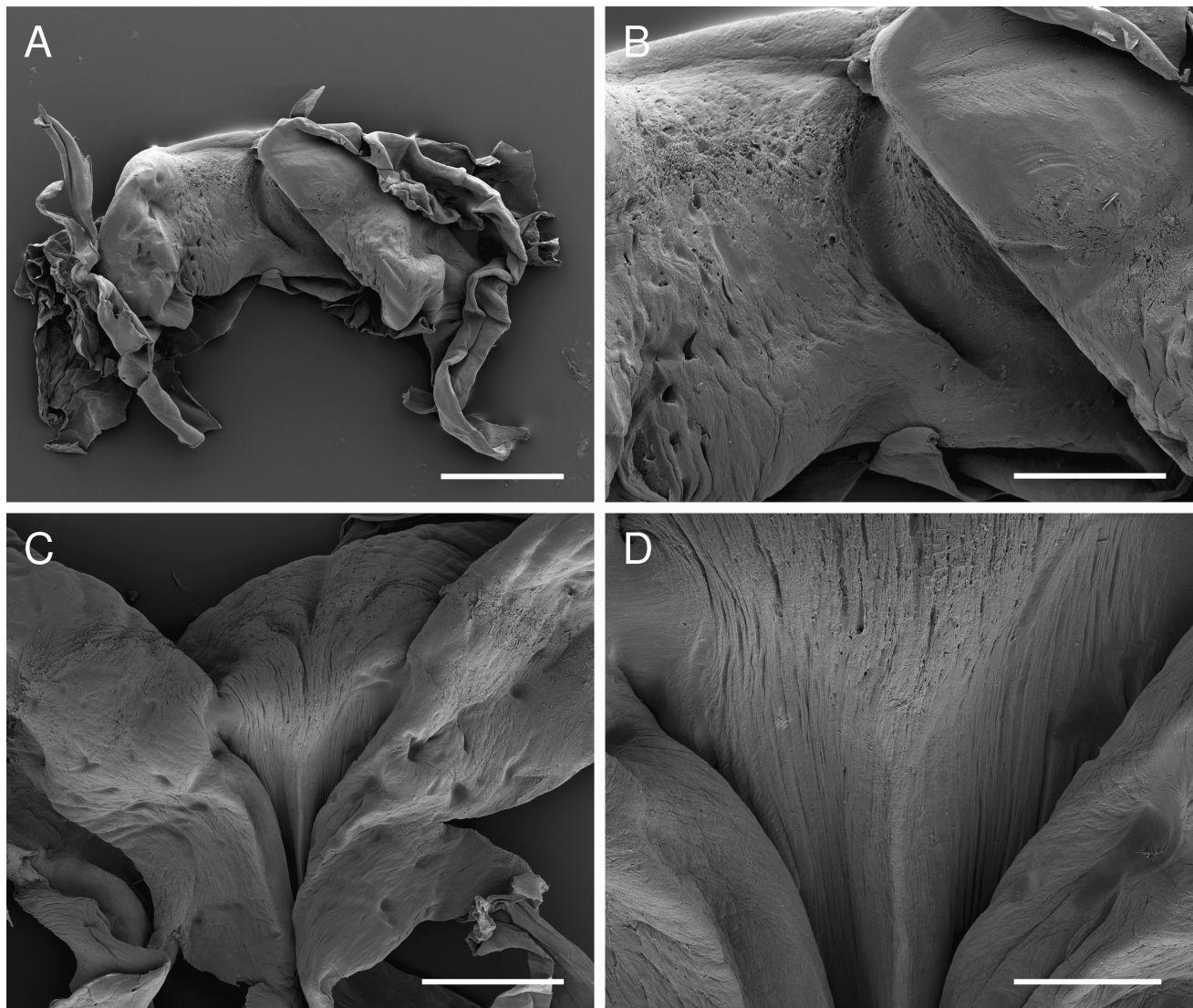


Figure 14. *Jorunna artsdatabankia* n. sp., SEM images of labial cuticles. **A.** General view of labial cuticle (ZMBN 125946). **B.** Detail of labial cuticle (ZMBN 125946). **C.** General view of labial cuticle (ZMBN 127749). **D.** Detail view of labial cuticle (ZMBN 127749). Small indentations visible in **A–C** were caused by forceps. Scale bars: **A, C** = 500 μm ; **B** = 200 μm ; **D** = 150 μm .

58891. Paratype (sequenced and dissected; TL of fixed specimen = 30 mm), North Sea (60.726944, 0.505371), ZMBN 125946.

Etymology: Named after the Norwegian government body Artsdatabanken (Norwegian Biodiversity Information Centre) that has a major role in supporting the study of biodiversity in Norway.

ZooBank registration: urn:lsid:zoobank.org:act:A7A9DC44-3B4D-4D8E-9351-863553EEC11D

Diagnosis: Background colour plain yellow to white; caryophyllidia uniform, densely arranged; notum irregularly speckled with brown spots of varying size and number. Mantle glands present. Rhinophores with 9–12 lamellae. Nine to 14 gills; slightly brighter than background colour and lacking pigmentation. Oral tentacles digitiform. Radular formula 19–25 \times 21–18.0.18–21. Three to 6 slender, sickle-shaped outermost smooth lateral teeth. Labial cuticle smooth. Bursa copulatrix only slightly larger than seminal receptacle. Copulatory spine 1.6–1.7 mm in length.

External morphology (Figs 1, 6, 13): TL = 15–40 mm. Dorsum plain yellow to white, speckled with irregularly distributed brown spots;

covered with numerous, densely packed caryophyllidia tubercles. Rhinophores with 9–12 lamellae, slightly brighter than dorsum, similar colour as notum. Nine to 14 bi- to tripinnate gills, slightly brighter than dorsum, encircling anal pore. Foot of same colour as dorsum, somewhat pointed at end. Oral tentacles of same colour as foot, digitiform.

Labial cuticle (Fig. 14): Smooth.

Radula (Fig. 15): Radular formula 19 \times 18.0.18 (TL = 15 mm, NTNU-VM-58891), 25 \times 20.0.20 (TL = 30 mm, ZMBN 125946), 23 \times 21.0.21 (TL = 40 mm, ZMBN 127749). Radula broad. Rachidian tooth absent; lateral teeth simple, hook-shaped with broad base and rounded cusp; mid-lateral teeth larger than inner laterals; inner laterals with smooth edges; 3–6 slender, smooth, sickle-shaped outermost laterals.

Reproductive system (Figs 16, 17): Hermaphroditic duct slender. Ampulla long, curved, varying in width; divided into short oviduct entering upper mass of female gland and connective duct entering prostate. Prostate large, tubular, differentiated into 2 portions;

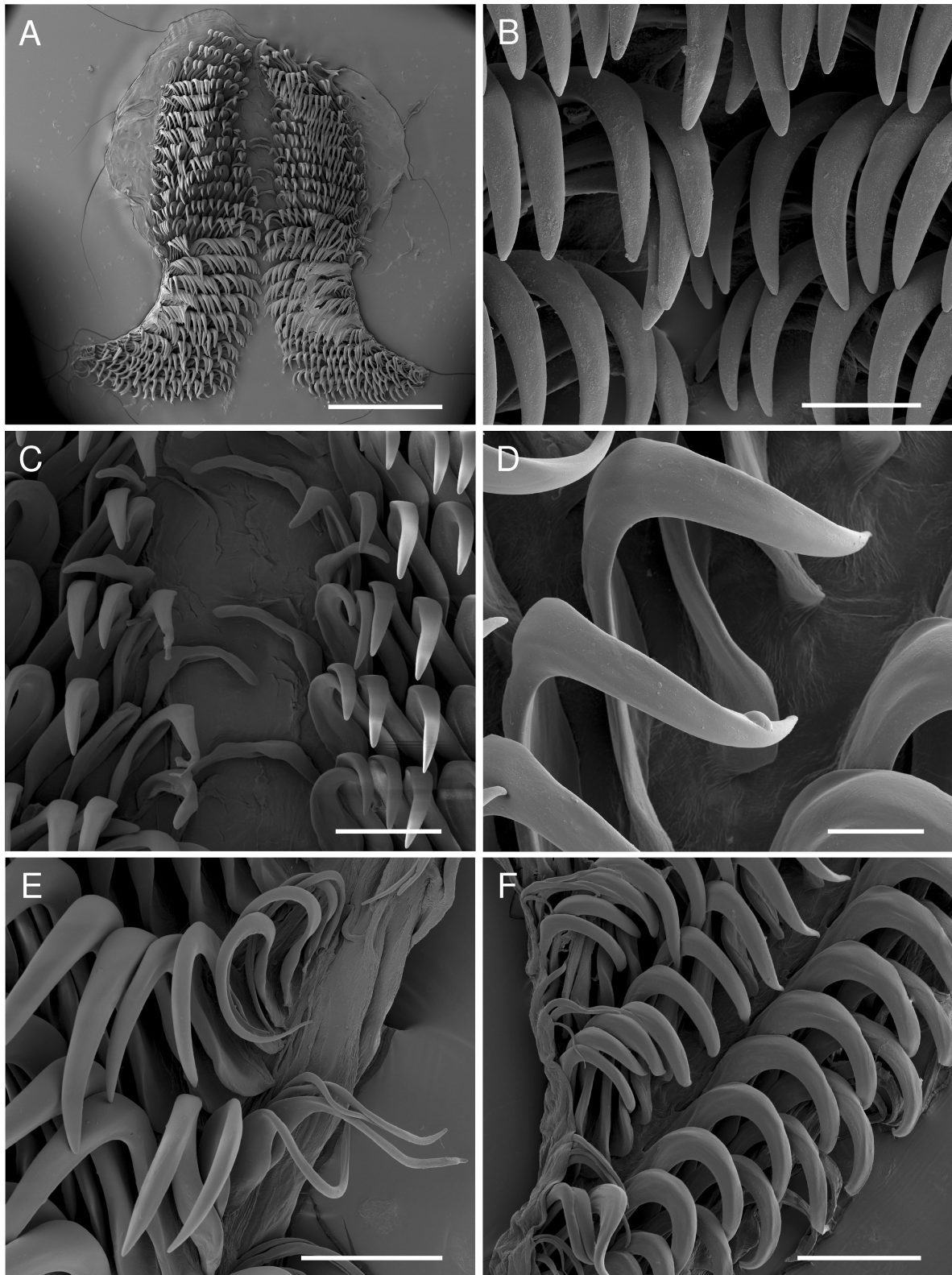


Figure 15. *Jorunna artsdatabankia* n. sp., SEM images of radula. **A.** General view ($25 \times 20.0.20$) (ZMBN 125946). **B.** Innermost lateral teeth with smooth edges (NTNU-VM-58891). **C.** Detail of innermost lateral teeth with smooth edges; rachidian tooth absent (ZMBN 125946). **D.** Detailed view of the only known case of an innermost lateral with swelling (ZMBN 127749). **E.** Detailed view of outermost lateral teeth with smooth edges (ZMBN 125946). **F.** Outermost lateral teeth with smooth edges (ZMBN 127749). Scale bars: **A** = 1 mm; **B, D** = 50 μ m; **C, F** = 150 μ m; **E** = 100 μ m.

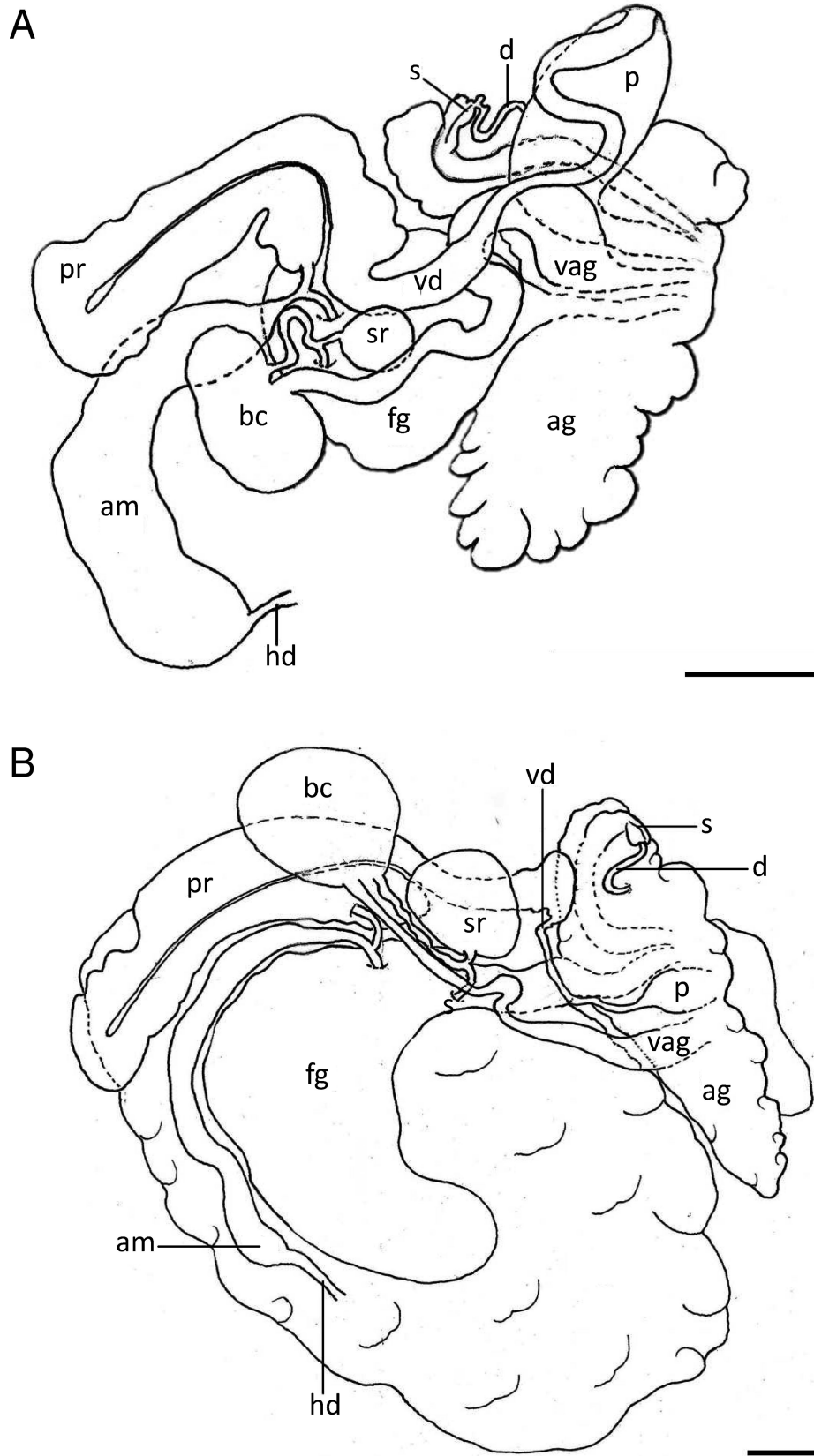


Figure 16. *Jorunna artsdatabankia* n. sp., reproductive organs. **A.** Reproductive system with mucous gland removed (NTNU-VM-58891). **B.** Reproductive system with mucous gland *in situ* (ZMBN 127749). Abbreviations: ag, accessory gland; am, ampulla; bc, bursa copulatrix; d, duct connecting with the accessory gland; fg, female gland; hd, hermaphroditic duct; mg, mucous gland; p, penis; pr, prostate; s, copulatory spine; sr, seminal receptacle; vag, vagina; vd, vas deferens. Scale bars: **A** = 500 μm; **B** = 1 mm.

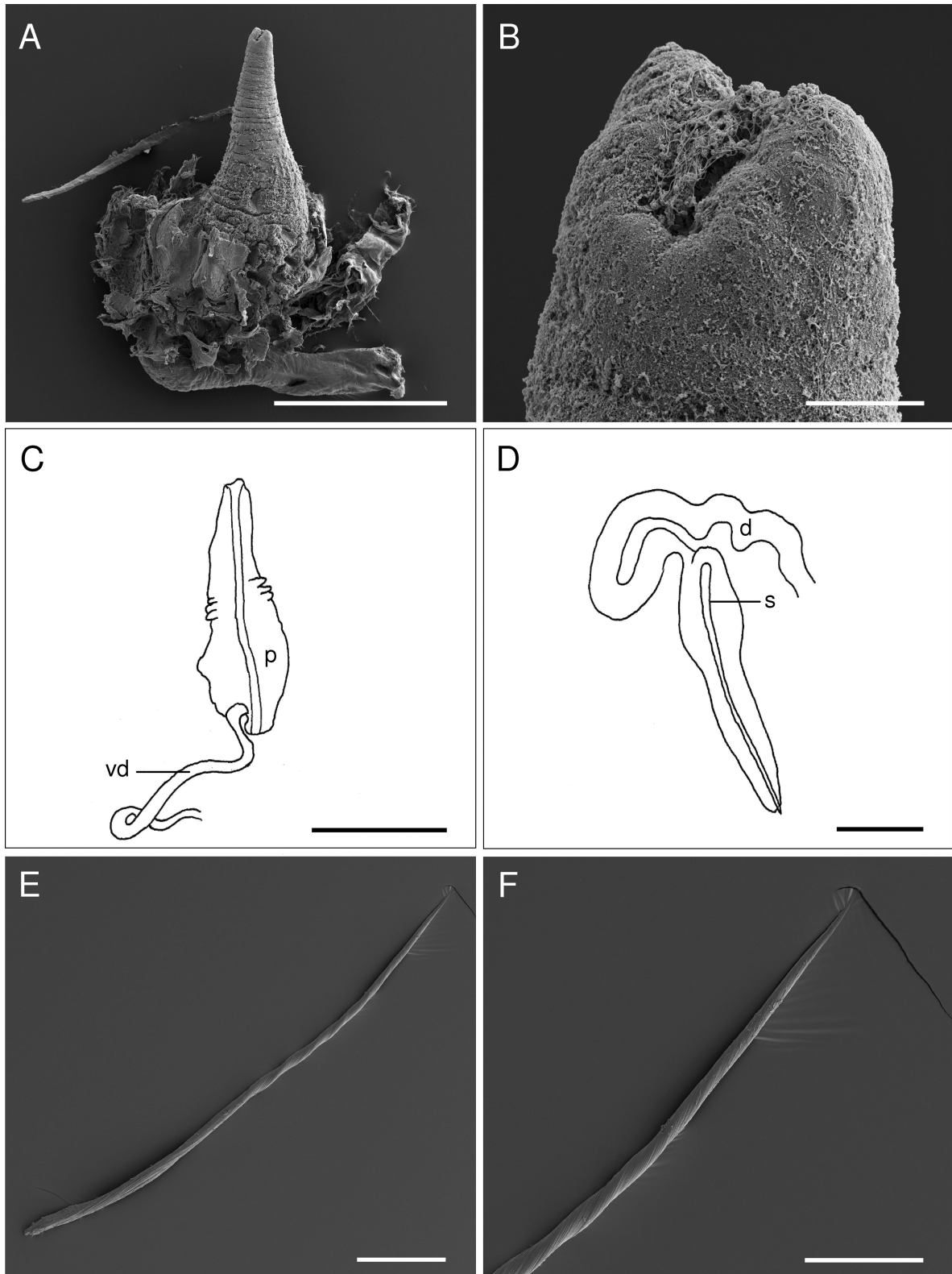


Figure 17. *Jorunna artsdatabankia* n. sp., SEM images and drawings of penial structures and copulatory spines. **A.** General view of penis (NTNU-VM-58891). **B.** Detailed view of penial tip (NTNU-VM-58891). **C.** Drawing of penis with deferent duct (ZMBN 125946). **D.** Drawing of spine embedded in tissue with attached duct connecting to accessory gland (ZMBN 125946). **E.** Entire copulatory spine (ZMBN 125946). **F.** Upper part of copulatory spine (ZMBN 125946). Abbreviations: d, duct connecting to accessory gland; p, penis; s, copulatory spine; vd, vas deferens. Scale bars: **A, D** = 500 μ m; **B** = 25 μ m; **C** = 1 mm; **E** = 250 μ m; **F** = 150 μ m.

narrows into long, narrow vas deferent duct that leads into penial bulb situated within common atrium. Penis smooth, with rounded base and elongated tip. Vagina long, wider than deferent duct, without hooks; entering common atrium. Bursa copulatrix rounded, larger than seminal receptacle. Seminal receptacle globose, connected to bursa copulatrix by duct. Uterine duct thin, connecting distally with female gland mass. Female gland mass enters common atrium. Accessory gland large, convoluted; emerges into long, coiled duct connecting to heart-shaped ovate sac bearing a long copulatory spine with rounded base; ovate sac embedded in muscular pouch emptying into common atrium; copulatory spine held in lining membrane forming protective sheath, protruding from posterior end of ovate sac beyond tip of spine.

Ecology: Little is known about the ecology of this new species. The image included here in Figure 1A shows a living specimen crawling among ascidians, polychaete tube worms (family Sabellidae) and sponges similar to those found in the typical habitat of *J. tomentosa* in Norway (e.g. *Haliclona* spp. and *Halichondria* spp.). This suggests a possible diet overlap between *J. tomentosa* and the new species. The new species was found at depth range of 27 to about 350 m (this study).

Distribution: We can confirm that the new species occurs at three localities along the western coast of Norway, that is Frøya, Trøndelag; Kristiansund, Møre og Romsdal; and the offshore grounds in the North Sea, between the city of Bergen in Norway and the Shetland Islands, UK. Based on images of live specimens (Goodwin *et al.*, 2011a,b; Picton & Morrow, 2016), this species likely also occurs in Northern Ireland (Maidens; Damicornis Bay, Duncan's Bay and Picton Reef on Rathlin; and north of the Storks on the Skerries) and Scotland (Anderson, 1999–2020).

Remarks: The species *J. artsdatabankia* n. sp. has been figured under the names *J. tomentosa* by Picton & Morrow (2016) for the British Isles and *Jorunna* sp. nov. by Goodwin *et al.* (2011a,b) from the Maidens, Rathlin and Skerries in Northern Ireland. Externally, *J. artsdatabankia* n. sp. differs from the two lineages of *J. tomentosa* (lineages A and B) by having a plain notal background colour with small, irregularly placed brown spots (Figs 1A, 6A–C). While the illustration of *D. johnstoni* (= *Jorunna johnstoni*; see the 'Remarks' section of *J. tomentosa*) by Alder & Hancock (1845: pl. 5, figs 1–3) resembles *J. artsdatabankia* n. sp., the description of the colour pattern ("It is blotched with pale brownish patches, ...") excludes the possible conspecificity of both taxa, since the new species, as mentioned above, always has a plain background colour, and if spots are present, they are few and far between.

The examined radulae had fewer teeth per row compared to specimens of *J. tomentosa* lineages A and B that were of comparable body length (Figs 9, 15). None of the examined specimens of *J. artsdatabankia* n. sp. carried denticles on the outermost lateral teeth. Denticulation on either the innermost or outermost lateral teeth was detected in six of eight studied radulae of *J. tomentosa* lineages A and B. In two specimens from lineage B, however, the outermost teeth lacked denticles (Fig. 9F). The vagina and the vas deferens of *J. artsdatabankia* n. sp. were found to be longer compared to *J. tomentosa* lineages A and B (Figs 10, 16). The copulatory spines in *J. artsdatabankia* n. sp. were 1.6 and 1.7 mm in specimens measuring 3 and 4 cm in length, respectively. In *J. tomentosa* lineages A and B, individuals ranging from 2 to 3 cm carried spines of 0.55–1.1 mm, so were c. 600 µm shorter compared to those in *J. artsdatabankia* n. sp. While the genetic distance between *J. artsdatabankia* n. sp. and *J. tomentosa* is 9.0–12.3% and therefore substantial (Table 3), the new species shows subtle but detectable morphological differences from *J. tomentosa*. For example, the new species has a distinctive notal coloration pattern and shows clear differences in the radula and reproductive system.

DISCUSSION

New species and a possible case of incipient speciation

Prior to this work, the possibility of there being cryptic lineages under the name *Jorunna tomentosa* had not been suggested (Alder & Hancock, 1845; Ev. Marcus, 1976; Valdés & Gosliner, 2001; Camacho-García & Gosliner, 2008). Our results have revealed the presence of one new species *J. artsdatabankia* sp. nov. and a possible case of incipient speciation involving partially unresolved lineages that are here referred to as *J. tomentosa* A and *J. tomentosa* B.

The species *J. artsdatabankia* sp. nov. could be considered pseudocryptic (*sensu* Hoover *et al.*, 2015; Korshunova *et al.*, 2019) due to the occurrence of clear morphological differences between it and *J. tomentosa*. Its external coloration (Figs 1A, 6) is distinct from *J. tomentosa* (Cuvier, 1804; Alder & Hancock, 1845; Ev. Marcus, 1976; Camacho-García & Gosliner, 2008; this study), but this was overlooked in previous scientific studies. A survey of the technical literature and dedicated websites shows that this species not only is present off the western coast of Norway, but also likely occurs off Northern Ireland and Scotland (Anderson, 1999–2020; Picton & Morrow, 2016; Goodwin *et al.*, 2011a,b). The examined radulae of *J. artsdatabankia* sp. nov. lack denticles on the outermost lateral teeth (Fig. 15), a character usually present in *J. tomentosa*. However, according to Ev. Marcus (1976) and Camacho-García & Gosliner (2008), the presence of denticles in the outermost teeth may vary within a single radula in specimens of *J. tomentosa*. This variability was also found in the material examined for this study and similar variability has also been reported from other European species of *Jorunna* (Fig. 9; Table 4).

The species *J. tomentosa* and *J. artsdatabankia* sp. nov. are sympatric, with overlapping distributions in Norway. According to the phylogenetic species concept, the phylogenetic structure in sympatric species is assumed to be the result of the lack of interbreeding between the sympatric taxa, followed by lineage sorting over time (Mayr, 1942; Avise & Wollenberg, 1997; Wheeler & Meier, 2000; Bickford *et al.*, 2007; de Queiroz, 2007). This is supported by the anatomical differences in the reproductive systems of the two species. For example, *J. artsdatabankia* sp. nov. has a copulatory spine that is c. 600 µm longer (Figs 12, 17; Table 4) than *J. tomentosa*, suggesting prezygotic reproductive isolation, since the spine is believed to have a function in mating. The vagina and deferent duct are also longer in *J. artsdatabankia* sp. nov. (Figs 10, 16).

The two putative lineages of *J. tomentosa* (A and B) recognized in this study were not highly supported in any of our phylogenetic analyses (Figs 2–4; Supplementary Material Figs S1–S4) and the genetic distance between them was lower (3.2–5.0%) than those for other studied species in the genus (10.3–16.9%; Table 3). Perhaps, the phylogenetic structure evident in our analyses reflects a case of incipient speciation, due to insufficient time for reproductive isolation to have been achieved. The result of this may be incomplete lineage sorting, with mating still likely occurring between these two lineages.

In nudibranchs, interspecific genetic distances between sister species can be relatively low. For example, Carmona *et al.* (2013) found a minimum cut-off value of 5.5% (COI uncorrected p-distance) in Aeolidiidae Gray, 1827 and Sørensen *et al.* (2020) found a minimum genetic distance of 4.3–5.8% in Polyceridae Alder & Hancock, 1845. Tibiriçá, Pola & Cervera (2018) supported the delimitation of two species of *Halgerda* Bergh, 1880 with a divergence of 3.6%. The genetic divergence observed between *J. tomentosa* lineages A and B (3.2–5.0%) falls within these ranges. However, the inferred genetic distances between all other pairs of sister species of *Jorunna* are higher (10.3–16.9%) and none of our phylogenetic analyses showed the two lineages to be monophyletic. Moreover, no distinct morphoanatomical differences were found between these two lineages.

The external coloration of *J. tomentosa* lineage A (Fig. 6, row 2) is closer to the original colour pattern described for the species (e.g. Alder & Hancock, 1845; Ev. Marcus, 1976; Picton & Morrow, 1994) and one specimen from near the type locality in La Rochelle, France (ZMBN 125512) clustered within lineage A (Figs 2–4). Specimens in lineage B are chromatically more variable with respect to both the notal background colour and the blotchy pattern (Fig. 6G–R).

Taxonomic status of elusive European species of Jorunna

In comparison to other European species of *Jorunna*, both *J. efe* and *J. onubensis* have distinctive morphoanatomical characters and are thus taxonomically valid species (Cervera, García-Gómez & García, 1986; Ortea et al., 2014; Table 4). In *J. efe*, the bursa copulatrix is up to three times smaller than the seminal receptacle, a ratio that is usually inverted in all other species of *Jorunna* (Ortea et al., 2014; Table 4). *Jorunna onubensis* is the only member of the genus with a deferent duct over three times longer than the vagina; this duct is highly convoluted and connected to a nonprostatic deferent duct (Cervera et al., 1986; Table 4).

On the other hand, the validity of the species *J. evansi* and *J. spazzola* has been highly debated (Ev. Marcus, 1976; Rudman & Avern, 1989; Camacho-García & Gosliner, 2008; Alvim & Pimenta, 2013). Although *J. evansi* was originally assigned to the genus *Rostanga* Bergh, 1879 (Eliot, 1906), Rudman & Avern (1989) proposed the new combination *J. evansi* due to its colour pattern, a dorsum covered with caryophyllidia and the narrow radula with 20 or fewer teeth in a half row (this last character is diagnostic of *Jorunna*). Because of similarities in coloration and radular morphology, Rudman & Avern (1989) suggested that *J. evansi* and *J. spazzola* from Brazil were synonyms. Camacho-García & Gosliner (2008) supported this view and stressed that the only difference between the two species was the consistent absence of a denticle on the innermost lateral teeth in *J. evansi*; this denticle is sometimes present in *J. spazzola*. On the other hand, Ortea & Moro (2016) considered *J. spazzola* and *J. evansi* to be valid species due to the absence of denticles on the inner laterals of *J. spazzola*. This character, however, has been regarded as being of doubtful value for separating species by Camacho-García & Gosliner (2008) and, as we have shown here, may be potentially variable within species.

Several authors have commented on the possible synonymy of *J. spazzola* and *J. luisae* Ev. Marcus, 1976 (Ev. Marcus, 1976; Camacho-García & Gosliner, 2008; Alvim & Pimenta, 2013; Ortea & Moro, 2016). Ev. Marcus (1976) described the species *J. luisae* based on ten preserved specimens from Naples, Italy. While he stressed the minor differences between this species and the Brazilian *J. spazzola*, he regarded both as being valid because of their disjunct geographical distributions. Camacho-García & Gosliner (2008), after comparing material of *J. spazzola* from Costa Rica with published descriptions of *J. luisae* from Naples, suggested that the latter was a synonym of the former. On the other hand, Alvim & Pimenta (2013) considered both *J. spazzola* and *J. luisae* to be valid species on the basis of differences in the reproductive system. The authors compared illustrations of the holotype of *J. luisae* (from Camacho-García & Gosliner, 2008) with the original description of *J. spazzola* (Ev. Marcus, 1955) and their own material of *J. spazzola* from Brazil, and concluded that *J. luisae* has an accessory gland that is convoluted, short and wide and a thin deferent duct, whereas in *J. spazzola* the accessory gland is tubular, long and thin, and the deferent duct is thick. However, a comparison of the characters detailed by Alvim & Pimenta (2013) with the original work by Ev. Marcus (1976: figs 24, 39) does not show such clear differences. The original drawings of both *J. spazzola* and *J. luisae* show that the vagina is thin close to where it originates from the bursa copulatrix and thickens towards the common atrium. Padula (2015) listed *J. luisae* as a synonym of *J. spazzola* without further remarks, whereas Ortea & Moro

(2016) synonymized *J. luisae* with *J. evansi* because of similar radular morphology and the occurrence in both taxa of jaw elements on the labial cuticle.

The grey-white morphotype found in *J. tomentosa* lineage B (Figs 1F, G, 6J–L) resembles Bergh's (1881) description of *J. atypha*, which was based on a single specimen collected in Trieste, Italy. Therefore, while we here consider *J. atypha* to be a junior synonym of *J. tomentosa*, we stress that future confirmation by comparison with material from the type locality Trieste is desirable.

Generic assignment of Gargamella lemchei

Ev. Marcus' (1976) concept of *J. lemchei* was based on the presence of penial hooks ("spines") in the male atrium and penial papilla (absent in *J. tomentosa*). Despite the absence of notal spots in the two specimens examined by her, she stressed that *J. lemchei* and *J. tomentosa* were externally very similar. In addition, Ev. Marcus (1976) mentioned that although *J. lemchei* lacked denticulation on the outermost lateral teeth, this character occurred irregularly in *J. tomentosa*. Thompson & Brown (1984) considered *J. lemchei* to be a synonym of *J. tomentosa*, stating that specimens from western Ireland were indistinguishable, despite acknowledging the absence of dorsal spots in *J. lemchei*. Surprisingly, they did not discuss the presence of penial hooks in *J. lemchei*. On the other hand, Just & Edmunds (1985), based on the absence of notal spots and differences in the reproductive system, considered *J. lemchei* to be a valid species. Valdés & Gosliner (2001) and Camacho-García & Gosliner (2008) also regarded *J. lemchei* to be a valid species because of the presence of penial hooks and lack of denticulation on the outermost lateral teeth.

Ortea et al. (2014) were the first authors to propose the reassignment of *J. lemchei* to the genus *Gargamella*, based on the fact that no other species of *Jorunna* carries penial hooks. However, they did not discuss the presence of a copulatory spine [referred by Ev. Marcus (1976: 52, fig. 47) as the vestibular stylet with a length of 500 µm], a character absent in all other species of *Gargamella* (Bergh, 1894; Ortea, Pérez & Llera, 1982; Garovoy, Valdés & Gosliner, 1999; Valdés & Gosliner, 2001; Moro & Ortea, 2015). The genus *Gargamella* differs from *Jorunna* in possessing penial hooks, vaginal hooks or a combination of the two and in lacking a copulatory spine. Thus, if the reassignment of *J. lemchei* to the genus *Gargamella* is confirmed, this would imply that *G. lemchei* is the only species bearing a copulatory spine in the genus.

In an attempt to test the phylogenetic position of '*J. lemchei*' (type locality: Ballyvaughan Bay, western coast of Ireland), four specimens from Connemara close to the type locality were included in this study (Fig. 5; Table 1). The phylogenetic analyses showed that all four grouped with *J. tomentosa* lineage B (Figs 2–4). The coloration of these four specimens varied from pale yellow to grey-white and all but one had two to five larger brown blotches (Fig. 5). Moreover, a dissected specimen (ZMBN 127705) lacked penial hooks, further supporting its conspecificity with *J. tomentosa* and confirming the absence of representatives of '*J. lemchei*' in our dataset. Therefore, the validity and generic assignment of *J. lemchei* remain elusive and only the collection of new specimens matching the description of *J. lemchei* from the vicinity of the type locality could shed light on this ongoing controversy.

This study represents another example of the importance of integrative taxonomic approaches to study marine biodiversity. The line between characters that represent intraspecific variability and those associated with already distinct lineages can be a challenge to define. In these cases, the study of several genes with different evolutionary rates can often help to define the taxonomic value of morphological characters. As the taxonomic validity of several species described from the Mediterranean Sea and the Macaronesian archipelagos of Cape Verde and the Canary Islands is still uncertain, future work on European *Jorunna* should include better sampling from these areas.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Molluscan Studies* online.

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