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A new Antarctic heterobranch clade is sister to all other Cephalaspidea (Mollusca: Gastropoda)

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For a long time, Diaphanidae has been considered a basal family within Cephalaspidea, based on the presence of plesiomorphic morphological features within this taxon. Traditionally, the family contained the genera *Bogasonia*, *Colobocephalus*, *Colpodaspis*, *Diaphana*, *Newnesia*, *Toledonia* and *Woodbridgea*. Some phylogenetic analyses of several of these genera support the basal position of Diaphanidae within Cephalaspidea *sensu stricto*. However, the family is presently confirmed to be a polyphyletic taxon in which only the genus *Diaphana* is included. Several genera previously embraced within the family, such as the monotypic *Newnesia*, have never been previously analysed in molecular studies. Here, we provide an extensive morphological, anatomical and histological description of a new species of *Newnesia* from Antarctic deep waters (967–1227 m depth) in the Drake Passage. We also discuss the similarities to the traditional Diaphanidae genera to try to shed light into this phylogenetic conundrum. We sequenced cytochrome *c* oxidase subunit I, 16S rRNA, 28S rRNA and histone H3 markers of *Newnesia antarctica* and *Newnesia joani* n. sp. We analysed a comprehensive dataset of sequenced genera to evaluate the placement of both *Newnesia* species within the cephalaspidean families. Maximum-likelihood and Bayesian phylograms support the monophyly of *N. joani* n. sp. and suggest cryptic speciation in *N. antarctica* specimens. *Newnesia* is recovered as the most basal offshoot of Cephalaspidea, suggesting the establishment of a new family restricted to Antarctic waters, named Newnesiidae n. fam., to hold both species. The possible Antarctic origin of Cephalaspidea is discussed.
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Introduction

Heterobranch sea slugs and snails are traditionally grouped into the paraphyletic group "Opisthobranchia" (e.g. Wägele *et al.* 2014). Among them, monophyletic Cephalaspidea is a taxon distributed worldwide (OBIS 2016), usually found from shallow to deep muddy bottoms, but some species live in association with seagrasses, algae or sessile invertebrates (Gosliner *et al.* 2008). The original diagnostic character of Cephalaspidea is the presence of a cephalic shield. This, together with sessile eyes and posterior tentacular folds, are characteristic features related mostly to their burrowing habits, other than true synapomorphies (Mikkelsen 2002). The diagnostic characters of the Cephalaspidea *sensu stricto* (without Runcinacea and Acteonoidea; Mikkelsen 1996; Malaquias *et al.* 2009) are the presence of three hardened oesophageal gizzard plates, flexed ciliated strips

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in the mantle cavity, a prepharyngeal nerve ring (i.e. located anterior to the pharynx), and the genital ganglion located on the visceral nerve loop (Mikkelsen 1996). Later, Mikkelsen (2002) recognized only the two-first characters as valid autapomorphies, rejecting the other two.

Diaphanidae Odhner, 1914 (Amphisphyridae Gray, 1857) has been for a long time considered a basal family within Cephalaspidea, because they exhibit plesiomorphic morphological features (Jensen 1996). For instance, they present a fully formed shell, cephalic tentacles, and, although having an armed oesophagus, they lack distinct gizzard plates (Schiøtte 1998). The family was first erected to embrace the genera *Diaphana* Brown, 1827, *Toledonia* Dall, 1902 (described under the name *Ptisanula* Odhner, 1913), and provisionally *Newnesia* Smith, 1902 (Odhner 1914). Diaphanidae was primarily defined on negative characters: absence of parapodia, jaws and gizzard plates (Eliot 1906; Odhner 1914; Thiele 1931). Its members also present rudimentary oral tentacles, a narrow radula and an external sperm groove. Jensen (1996) stated that these were autapomorphies or symplesiomorphies, rather than synapomorphic characters. Therefore, the apparent resemblances were interpreted as homoplastic adaptations to epifaunal habits and suctorial feeding. Consequently, the family became a wastebasket taxon, where several genera have been included since then (see below). Phylogenetic analyses of some of its genera supported the basal position of the family Diaphanidae within Cephalaspidea *s. s.*, although only *Diaphana* retrieved basal, while the other diaphanids included in these studies appeared polyphyletic (Thollesson 1999; Malaquias *et al.* 2009; Jörger *et al.* 2010; Oskars *et al.* 2015).

The genus *Bogasonia* Warén, 1989 was later described based on dried specimens, and its resemblances to *Toledonia* (i.e. volute shell and three-seriate radula) lead Warén (1989) to suggest the new subfamily Toledoniinae. This separation was corroborated by recent molecular analyses, which, however, suggested to place *Toledonia* (and subsequently *Bogasonia*) into the Cylichnidae (Oskars *et al.* 2015). The subfamily Diaphaniinae Odhner, 1914, thus, included *Diaphana*, *Newnesia* and *Woodbridgea* Berry, 1953. The latter was described only from a unique shell and was never found again (Berry 1953). The genera *Colpodaspis* M. Sars, 1870, with two nominal species, and the monotypic *Colobocephalus* M. Sars, 1870 were included into Diaphanidae based on shell characters (Garstang 1894; Odhner 1939). Lately, a more accurate description of live specimens of these three species (Brown 1979; Ohnheiser & Malaquias 2014), together with phylogenetic analyses, placed both genera in the new family Colpodaspididae Oskars *et al.* 2015; far away from Diaphanidae *s. s.* (Oskars *et al.* 2015). Moreover, the genus *Rhinodiaphana* was also considered to be a diaphanid, but it has been recently transferred to Philinidae (Ohnheiser & Malaquias 2013). Additionally, the controversial family Notodiaphanidae Thiele, 1931, previously considered parent of Diaphanoidea, is considered *incertae sedis* within the Cephalaspidea (Ortea *et al.* 2013; Oskars *et al.* 2015). Therefore, several families have been designed subsequently to include most genera of Diaphanidae *sensu lato*. However, the relationships of the Antarctic genus *Newnesia* and the elusive *Woodbridgea*, which in former times were also included in the Diaphanidae, remain so far untested.

The monospecific genus *Newnesia* was first described by Smith (1902) based on four specimens of *N. antarctica* collected in Cape Adare (Ross Sea). The description included shell and radula features. Later, Eliot (1906) re-described the same specimens and gave a short description of the internal soft organs. Strebel (1908) described a new genus and species named *Anderssonia sphinx* from Paulet Island (north of the Antarctic Peninsula), later synonymized with *N. antarctica* by Odhner (1926). Jensen (1996) gave an accurate and comparative description of the internal anatomy of *N. antarctica*. This species is currently restricted to Antarctic and Subantarctic circumpolar waters at depths ranging from 16 to 655 m (Aldea & Troncoso 2008).

In this study, we aim (i) to describe a new *Newnesia* species from Antarctic deep waters by using morphological and molecular characters; (ii) to compare the morphology of the new species to the rest of the Diaphanoidea *s. l.* genera; (iii) to provide a phylogenetic hypothesis for the position of the genus *Newnesia* within Cephalaspidea; and (iv) to evaluate the ancestral features of this genus in a phylogenetic context.

Material and methods

Sample collection

Samples of *Newnesia joani* n. sp. (Fig. 2) were collected with Agassiz trawl in muddy bottoms at the Drake Passage, north of King George Island (Antarctica), during the Antarctic cruise ANT XV/3 of the R/V Polarstern (Gutt & Arntz 1999). All specimens were collected in a single dredge operation (48/336) on 19th of March 1998, at a 967–1227 m depth range from 61°27.6'S, 58°4.1'W to 61°26.5'S, 58°7.4'W (Fig. 1). Twenty-seven specimens were collected; eight were preserved in 70% ethanol for anatomical and histological analyses, the rest were frozen and two of these were transferred to absolute ethanol for genetic extraction. Specimens of *N. antarctica* were collected during different campaigns. During ANT XXI/2, December the 24th, 2003 (PS65/259-1), *N. antarctica* (1) was collected from the Austasen Bank in the eastern Weddell Sea (70° 57' S, 10° 33.02' W) with a bottom trawl, at 333 m depth. During Andeep I, ANT XIX, January the

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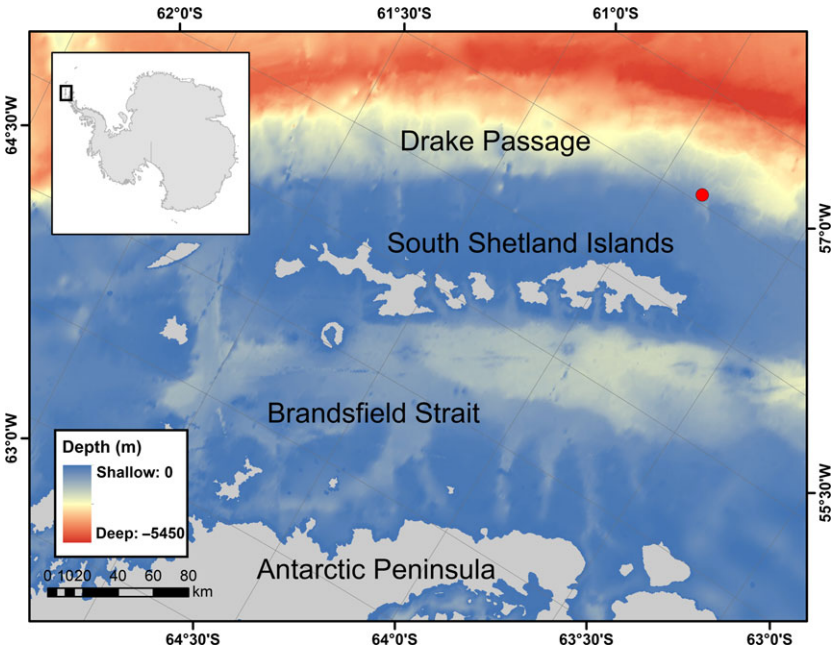

Fig. 1 Map of the South Shetland Islands and surrounding waters showing the position of the station AGT 48/336 (red dot), where *Newnesia joani* n. sp. was collected.

30th, 2002 (PS61/046-7), *N. antarctica* (2; voucher n° ZSMMoll20021145) was collected from north of the South Scotia Ridge (start 60°39.19'S, 53°56.85'W; end 60°38.06'S, 53°57.51'W) at 2889–2893 m depth with an epibenthic sledge.

Additionally to the four sequenced *Newnesia* specimens, sequences of 38 cephalaspidean species and 13 outgroup taxa were obtained from GenBank (see Supplementary Material 1). Taxon sampling was designed to cover representatives of all available sequenced cephalaspidean families. Outgroups consisting of 13 species representing seven Heterobranchia clades of similar ranking to that of Cephalaspidea (Jörger *et al.* 2010) were included in the analyses (i.e. Acochlidia, Acteonoidea, Anaspidea, Nudibranchia, Runcinacea, Sacoglossa and Umbraculida). The trees were rooted with the nudibranch species *Aldisa smaragdina*, a sister lineage to the Tectipleura (Euopisthobranchia + Panpulmonata) molluscs (Zapata *et al.* 2014). In total, this study includes 154 sequences.

Morphological analysis

Three specimens of *N. joani* n. sp. were dissected under a stereomicroscope for anatomical analysis. Both buccal masses and shells were immersed in potassium hydroxide for up to three hours to dissolve the organic tissues, and then rinsed with distilled water. Shells and radulae were mounted on metallic stubs with bioadhesive carbon sticky tabs and coated with carbon for scanning electron microscopy (SEM). One individual was dehydrated in an ethanol series and embedded in HEMA for histological analysis (Kulzer method; see Wägele 1997). Serial sections (2.5 μm thick) were stained with Toluidine blue, which specifically stains acid mucopolysaccharides red to violet, and neutral mucopolysaccharides and nucleic acids, as well as proteins in various blue shades.

DNA amplification

Total genomic DNA was extracted from small pieces of foot tissue for most samples, using DNeasy Tissue Kit (Qiagen, Valencia, CA, USA). Molecular markers included three fragments of the mitochondrial genes cytochrome *c* oxidase subunit I (COI), 16S rRNA and 28S rRNA, and the nuclear gene histone H3. A fragment of *ca.* 720 bp of the mitochondrial protein-encoding gene COI was amplified using the primers LCO1490 and HCO2198 (Folmer *et al.* 1994). A fragment of *ca.* 465 bp of the 16S rRNA gene was amplified using the primer pair 16Sar-L and 16Sbr-H (Palumbi *et al.* 2002). A fragment of *ca.* 746 bp of the 28S gene was amplified using the primer pairs LSU5-F (Littlewood *et al.* 2000) and LSU1600-R (Williams *et al.* 2003). A fragment of *ca.* 318 bp of the protein-encoding gene histone H3 was amplified using the primer pair H3AD5'3' and H3BD5'3' (Colgan *et al.* 1998). PCR amplifications were carried out in a 24 μL-reaction volume, including 18.25 μL Sigma dH_2O, 2.5 μL CoraLLoad buffer, 1.25 μL MgCl, 0.5 μL dNTP, 0.5 μL of each primer, 0.5 μL Taq and 0.5 μL of genomic DNA. Polymerase chain reaction (PCR) program for COI and 16S rRNA involve an initial denaturing step (95 °C for 15 min) followed by 25 cycles of denaturation (94 °C for 45 s),

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annealing (40–55°C for 1:30 min) and extension (72 °C for 1:30 min), with a final extension step at 72 °C for 10 min. For 28S rRNA and histone H3, the PCR started with an initial denaturation step at 95°C for 3 min followed by 35 cycles including denaturation at 94 °C for 45 s, annealing at 50–52 °C for 45 s, and extension at 72 °C for 2 min, with a final extension step at 72 °C for 10 min. Amplified products were purified using microCLEAN (Microzone Ltd., Sussex, UK) and sequenced at the UB Scientific and Technological Centres (CCiT-UB) on an ABI 3730XL DNA Analyser (Applied Biosystems).

Phylogenetic analysis

Chromatograms were visualized and sequences were assembled in Geneious Pro 8.1.5 (Drummond *et al.* 2010). These were compared against the GenBank nucleotide database with the BLAST algorithm (Altschul *et al.* 1997) to check for contamination. Alignments were trimmed to a position at which more than 50% of the sequences had nucleotides and missing positions at the ends were coded as missing data. All new sequences have been deposited in GenBank (see Supplementary Material 1 for accession numbers). We used GBlocks 0.91b on the final trimmed alignment for identifying and excluding blocks of ambiguous data in single, non-codifying gene alignments (16S and 28S) with relaxed settings (Talavera & Castresana 2007).

Bayesian inference (BI) was performed on the concatenated alignment of the four genes, using MrBayes ver. 3.2.5 (Ronquist *et al.* 2011) with a GTR model of sequence evolution (Tavaré 1986), corrections for a discrete gamma distribution, and a proportion of invariant sites (GTR + Γ + I; Yang 1996) specified for each gene partition, as selected in jModelTest ver. 2.1.7 (Posada 2008) under the Akaike Information Criterion (Posada & Buckley 2004). Two runs, each with three hot chains and one cold chain, were conducted in MrBayes for 20 million generations, sampling every 2,000th generation, using random starting trees. The analysis was performed twice, and 25% of the runs were discarded as burn-in after checking for stationarity with Tracer v.1.6. (Rambaut *et al.* 2014). The remaining trees were combined to find the maximum *a posteriori* probability estimate of phylogeny.

Maximum-likelihood (ML) analyses were conducted using RAxML ver. 8.1.2 (Stamatakis 2014). For the maximum-likelihood searches, a GTR model of sequence evolution with corrections for a discrete gamma distribution (GTR + Γ; Yang 1996) was specified for each data partition, and 500 independent searches were conducted. Nodal support was estimated via the rapid bootstrap algorithm (1000 replicates) using the GTR-CAT model (Stamatakis *et al.* 2008). Bootstrap resampling frequencies were thereafter mapped onto the optimal tree from the independent searches. Additionally, we assessed saturation by constructing a tree without the third codon position of the protein coding genes COI and H3, and, as there were no differences, we used the alignment with the third position.

COI uncorrected *P*-distances were calculated using MEGA 7 for all species of the dataset which had more than one congener (Table 1).

Results

Systematic description

In the present work, a new family Newnesiidae, and a new species, *Newnesia joani* (Figures S2–6), are established. The extensive diagnosis and description (including shell, radula, external morphology, internal anatomy and histology) of these new taxa are provided in Supplementary Material 2. In summary, *N. joani* n. sp. differs from *N. antarctica* in having an internal, instead of the external shell; a three-seriate radula with lamellate laterals, instead of a monoseriate radula; a distinct left anterolateral funnel connected to a complex repugnatorial gland, instead of the smooth mantle rim; and a distinct parietal ganglion in the visceral loop. Morphological and molecular characters clearly separate the new species from *N. antarctica*, since *P*-distances based on the COI sequences between *N. joani* n. sp. and both *N. antarctica* specimens were 12.9 ± 1.5% and 9.2 ± 1.2% respectively.

Phylogenetic analysis

The total dataset contained 40 cephalaspidean species, corresponding to all families sequenced hitherto, and 13 outgroup taxa. The concatenated alignment consisted of 2,203 characters, of which COI had 614 characters, 16S 352 characters, 28S 928 characters and H3 had 309 characters. ML and BI analysis recovered a tree with strong support for monophyletic Newnesiidae n. fam. (PP = 1; BS = 100), composed by both *N. antarctica* and *N. joani* n. sp., which was in turn the earliest branching Cephalaspidea *s. s.* group (Fig. 3). In general, the topology of the phylogenetic tree is in accordance to previous studies including the same taxa (Oskars *et al.* 2015).

Discussion

A new species of cephalaspidean mollusc from deep waters in the Drake Passage (Antarctica; 967–1227 m) is described here under the name *N. joani* n. sp. (Fig. 2). *Newnesia joani* n. sp. was found to be related to *N. antarctica* using both morphological and molecular analyses (Smith 1902; Odhner 1926; Jensen 1996), although specific morphological traits of the new species clearly separate both species. The genus *Newnesia* forms a distinct lineage at the base of the Cephalaspidea (PP = 1, BS = 100), and we thus consider it to represent a discrete family named Newnesiidae n. fam.

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Table 1 Matrix for COI uncorrected *P*-distances ± standard deviation for the genera with several species included in the phylogenetic analyses

	N. joani n. sp. (1)	*N. joani* n. sp. (2)	*N. antarctica* (1)	*N. antarctica* (2)	*B. striata*	*B. ampulla*	*P. babai*	*P. indisticta*	*Philinorbis* sp. A	*Philinorbis* sp. B	*L. quadrata*	*L. ventricosa*	*Colinatys* sp. A (1)	*Colinatys* sp. A (2)	*D. globosa*	*Diaphana* sp. EED
Newnesia joani n. sp. (2)	0 ± 0															
Newnesia antarctica (1)	12.9 ± 1.5	12.9 ± 1.5														
Newnesia antarctica (2)	9.2 ± 1.2	9.2 ± 1.2	11.4 ± 1.4													
Bulla striata	21.6 ± 1.8	21.6 ± 1.8	22.5 ± 1.8	22.3 ± 1.8												
Bulla ampulla	22.1 ± 1.8	22.1 ± 1.8	24.0 ± 1.9	23.3 ± 1.8	17.4 ± 1.7											
Philine babai	17.8 ± 1.7	17.8 ± 1.7	19.9 ± 1.7	20.1 ± 1.7	20.6 ± 1.8	19.9 ± 1.7										
Philine indisticta	20.6 ± 1.7	20.6 ± 1.7	21.2 ± 1.7	19.5 ± 1.6	21.6 ± 1.8	19.5 ± 1.7	15.6 ± 1.5									
Philinorbis sp. A	21.6 ± 1.8	21.6 ± 1.8	22.0 ± 1.8	19.9 ± 1.7	23.6 ± 1.9	23.1 ± 1.9	18.4 ± 1.7	19.1 ± 1.7								
Philinorbis sp. B	21.0 ± 1.8	21.0 ± 1.8	22.5 ± 1.9	20.6 ± 1.8	23.1 ± 1.9	22.7 ± 1.9	18.4 ± 1.8	16.9 ± 1.6	6.6 ± 1							
Laona quadrata	19.3 ± 1.7	19.3 ± 1.7	19.1 ± 1.7	19.7 ± 1.7	22.3 ± 1.9	22.0 ± 1.8	15.4 ± 1.6	20.5 ± 1.7	18.6 ± 1.7	19.3 ± 1.7						
Laona ventricosa	19.5 ± 1.7	19.5 ± 1.7	23.1 ± 1.8	21.0 ± 1.7	21.0 ± 1.7	22.0 ± 1.7	19.3 ± 1.8	20.3 ± 1.7	18.6 ± 1.7	19.9 ± 1.8	18.9 ± 1.6					
Colinatys sp. A (1)	20.6 ± 1.8	20.6 ± 1.8	19.7 ± 1.8	21.6 ± 1.8	19.3 ± 1.8	20.3 ± 1.8	18.2 ± 1.7	18.0 ± 1.7	19.3 ± 1.8	19.5 ± 1.9	20.3 ± 1.8	20.8 ± 1.8				
Colinatys sp. A (2)	20.6 ± 1.8	20.6 ± 1.8	19.7 ± 1.8	21.6 ± 1.8	19.3 ± 1.8	20.3 ± 1.8	18.2 ± 1.7	18.0 ± 1.7	19.3 ± 1.8	19.5 ± 1.9	20.3 ± 1.8	20.8 ± 1.8	0 ± 0			
Diaphana globosa	17.4 ± 1.6	17.4 ± 1.6	19.1 ± 1.7	17.1 ± 1.6	24.0 ± 1.8	23.8 ± 1.8	19.9 ± 1.7	19.1 ± 1.6	21.2 ± 1.8	22.9 ± 1.8	19.9 ± 1.7	21.0 ± 1.7	21.0 ± 1.7	21.0 ± 1.7		
Diaphana sp. EED	18.0 ± 1.7	18.0 ± 1.7	19.7 ± 1.7	17.6 ± 1.6	23.5 ± 1.8	22.9 ± 1.8	20.3 ± 1.8	18.8 ± 1.6	21.4 ± 1.8	22.9 ± 1.8	20.3 ± 1.7	21.4 ± 1.7	21.6 ± 1.8	21.6 ± 1.8	1.7 ± 0.6	
Diaphana minuta	17.6 ± 1.6	17.6 ± 1.6	18.4 ± 1.7	18.0 ± 1.6	23.1 ± 1.7	22.5 ± 1.8	18.8 ± 1.7	19.1 ± 1.6	19.5 ± 1.7	20.3 ± 1.7	18.2 ± 1.6	20.8 ± 1.7	18.6 ± 1.6	18.6 ± 1.6	13.5 ± 1.5	13.9 ± 1.5

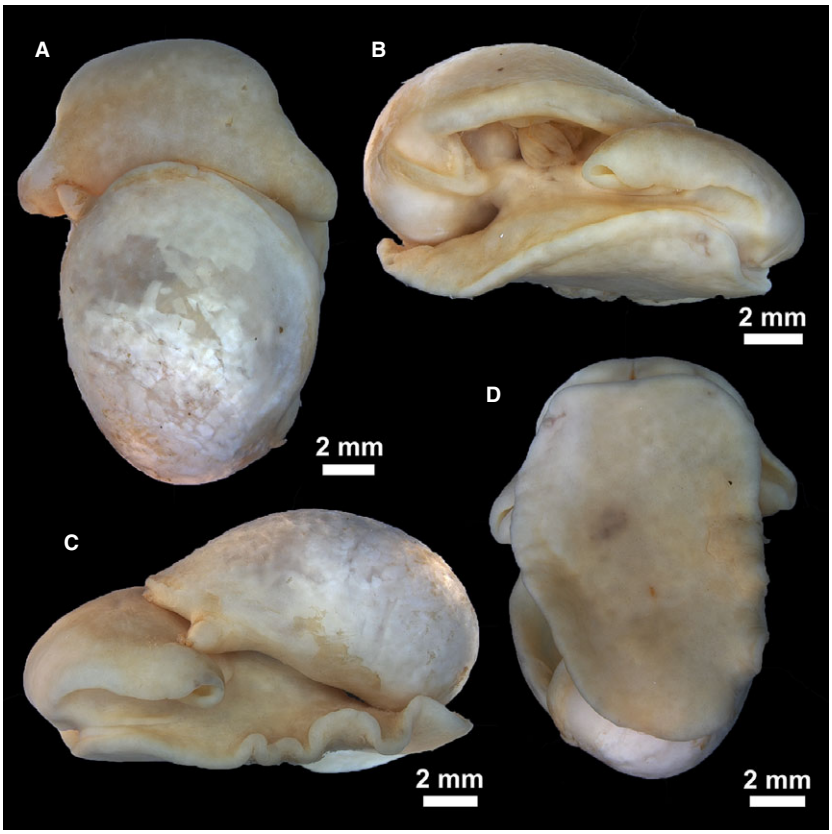

Fig. 2 External view of *Newnesia joani* n. sp. preserved holotype. –A. Dorsal view. –B. Right lateral view. –C. Left lateral view.–D. Ventral view.

separated from Diaphanidae. Molecular markers show a clear differentiation between *N. joani* n. sp. and the two specimens of *N. antarctica* (Fig. 3). Moreover, COI *P*-distances of 9.2–12.9% between both *Newnesia* species indicate cryptic speciation of *N. antarctica* specimens (Table 1). In fact, both *N. antarctica* specimens analysed here were collected at very distant locations (eastern Weddell Sea and Scotia Ridge). Similarly, cryptic speciation has been shown in other heterobranchs of Antarctic circumpolar waters (Wilson *et al.* 2009, 2013). However, a thorough taxon sampling of *N. antarctica* from additional locations is needed to corroborate this hypothesis.

Here, Diaphanidae *s. l.* is recovered polyphyletic and we found further support on the families described recently by Oskars *et al.* (2015). However, we included an additionally basal lineage to Cephalaspidea *s. s.*, the new family Newnesiidae. The basal position based on molecular analyses is also reflected by the presence of such a broad array of plesiomorphic morphological features not found again within other cephalaspidean groups: e.g. the presence of a well-developed cephalic shield, the absence of anterior tentacular processes and gizzard plates. The simple cuticle lining in the oesophagus and stomach of *Newnesia* (as well as in *Toledonia*) may constitute a precursor of the complex gizzard plates of some cephalaspidean groups. Further plesiomorphic features in euthyneuran heterobranchs are the lateral position of the mantle cavity with the gonopore opening posteriorly, the prepharyngeal position of the nerve ring, as well as the long visceral nerve loop (Wägele *et al.* 2014). Moreover, in *Newnesia* as well as in *Diaphana*, the cerebral and pleural ganglia are still separated by a distinct commissure (Huber 1993). However, only *N. joani* n. sp. has a pentaganglionate visceral loop with a distinct parietal ganglion (Fig. S4d). The pentaganglionate condition has been proposed as a synapomorphy of Euthyneura (=Pentaganglionata; Haszprunar 1985), only present in 'basal' taxa of all major Heterobranchia *s. l.* clades (Brenzinger *et al.* 2013a). Eliot (1906) described a second gill-like organ that he considered to be an osphradium in *N. antarctica*, but histological sections herein demonstrated that this is a true gill (Fig. S5d), which together with the primary gill typically form a plicatidium (Morton 1972). This is similar to those of other heterobranchs with burrowing habits, such as *Akera* or *Acteon* (Fretter & Graham 1954; Morton 1972).

The new family Newnesiidae is characterized by an unusual big trapezoidal cephalic shield with folded posterior cephalic lobes. Cephalic lobes might act as chemosensory

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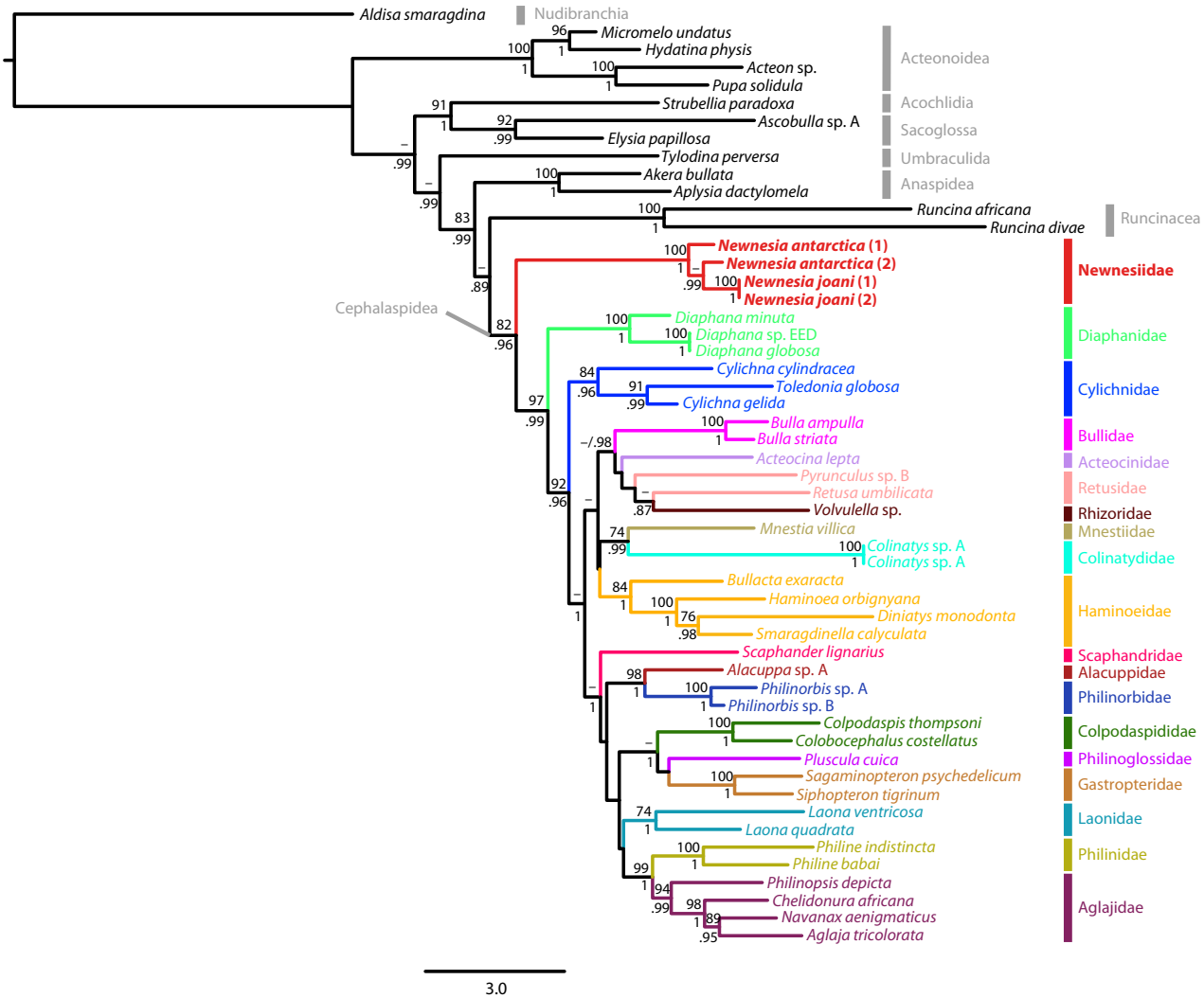

Fig. 3 Phylogenetic tree of the Cephalaspidea based on the combined COI, 16S, 28S and H3 genes using maximum-likelihood (ML) and Bayesian inference (BI). Numbers on nodes indicate bootstrap support values (ML) and posterior probability values (BI). Cephalaspidean families are marked in colours corresponding to families at the right side, while outgroup clades are in grey.

organs since neuronal follicles were ubiquitously seen (Fig. S6b). The presence of two follicular and multicellular repugnatorial glands is another defining characteristic of the family. These repugnatorial glands might represent modified Blochmann's glands, a gland type that is seen in other heterobranch species too (Brenzinger *et al.* 2013b). These glandular organs are surrounded by musculature helping to release the contents outside (Fig. S5a, b), probably in a similar way as in the mantle dermal formations (MDFs) of doridoideans (Avila & Durfort 1996), some cladobranchs (Moles *et al.* 2016), and other heterobranchs (Wägele *et al.* 2006). This mechanism seems to be improved in the frontal gland of *N. joani* n. sp. since it is connected through a funnel to the exterior (Fig. S4b). However, its follicular arrangement and the presence of distinct secreting ducts lead to conclude these are not MDFs, in contrast to previous interpretations (Wägele *et al.* 2006), but a distinct glandular organ only found in the family Newnesiidae to date.

Newnesiidae n. fam. presents some shared morphological characters to the genera originally assigned to the family Diaphanidae (see Table 2). The new family bears a globose shell similar to that of *Colpodaspis*, *Colobocephalus*, and some species of the genus *Diaphana*, although it is internal only in the Colpodaspididae (Ohnheiser & Malaquias 2014; see Fig. S2a, b) and in *N. joani* n. sp. The radula, however, differs considerably: the genera *Colpodaspis*, *Colobocephalus* and *Diaphana* present long hooked

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Table 2 Comparative table of diagnostic characters of the former Diaphanidae genera compared to the Newnesiidae n. fam.

	Newnesia joani n. sp.	*Newnesia antarctica*	*Diaphana*	*Toledonia*	*Bogasonia*	*Woodbridgea*	*Colpodaspis*	*Colobocephalus*
Shell	Internal	External	External	External	External	External	Internal	Internal
Shape	Globose	Globose	Globose-elongate	Elongate	Elongate	Globose	Globose	Globose
Radula	1.1.1	0.1.0	0-1.1.1.1.1-0	0-1.0-1.1.0-1.1-0	1.1.1	?	1.0.1	1.0.1
Rachidian	Unicuspid	Unicuspid	Bilobed	Unicuspid	Unicuspid	?	Absent	Absent
1st lateral	Lamellate	Absent	Hook shaped	Absent	Lamellate	?	Hook shaped	Hook shaped
Tentacular processes	Absent	Absent	Present	Present	Present	?	Present	Present
Prostate	Undivided	Absent?	Divided or undivided	Undivided	?	?	Undivided	Undivided
Family	Newnesiidae n. fam.	Newnesiidae n. fam.	Diaphanidae	Cylichnidae	Cylichnidae?	?	Colpodaspididae	Colpodaspididae
Reference	Present study	Jensen (1996)	Schiøtte (1998)	Marcus (1976)	Warén (1989)	Berry (1953)	Brown (1979)	Ohnheiser & Malaquias (2014)

?: Unknown.

laterals and lack a rachidian in both colpodaspidids, while it is bilobed in *Diaphana* (Brown 1979; Schiøtte 1998). Lateral teeth are very thin and likely vestigial in *N. joani* n. sp. (Fig. S2c, d), while *N. antarctica* lacks them. Dissection of *N. antarctica* (1) from the Weddell Sea revealed the typical radular formula of 25 × 0.1.0, although having four denticles along its right border and three denticles in the left border of the rachidian teeth (see Fig. S1). This has never been reported before for *N. antarctica* and it was not observed in the specimens of *N. joani* n. sp. The radula of *Newnesia* resembles that of *Toledonia* and *Bogasonia*, since they also present a unicuspid rachidian and sometimes thin lamellate laterals (Warén 1989), as for *N. joani* n. sp. However, the uniseriate radula with unicuspid teeth (together with a muscular and voluminous pharynx) may be an adaptation to suctorial feeding rather than a homology (Jensen 1996). Both *Toledonia* and *Bogasonia* present a shell with an elongated spire (Marcus 1976; Warén 1989), and therefore morphologically differ from *Newnesia*. In fact, morphological evidence lead Odhner (1926) and Warén (1989) to propose several subfamilies within Diaphanidae *s.l.*, some of which have been supported as distinct families in recent molecular phylogenies (Oskars *et al.* 2015). Therefore, the apparent similarities clustering the primal Diaphanidae *s. l.* genera may be interpreted as homoplastic adaptations to epifaunal habits and suctorial feeding (Jensen 1996). For instance, the pedal gland is present in different species with epifaunal habits, thus it might be either a plesiomorphy or a homoplasy of *Toledonia*, *Colpodaspis* and *Newnesia* (Jensen 1996). Further studies should ascertain these questions in the future.

There are approximately 80 species of heterobranchs described in Antarctica, being Cephalaspidea (~25) one of the most speciose groups (De Broyer *et al.* 2016). Interestingly, several families and genera are found only in Antarctic waters, and they are crucial for the phylogenetic comprehension of the evolution of heterobranch lineages. In fact, basal members of some major Nudipleura (Nudibranchia + Pleurobranchomorpha) linages are exclusively Antarctic. This, together with molecular clock analyses, suggested a possible Antarctic origin of nudibranchs and pleurobranchomorphs (Wägele *et al.* 2008; Martynov & Schrödl 2009; Göbbeler & Klussmann-Kolb 2010). Giving the data presented here, we also propose an Antarctic origin for the Cephalaspidea. Likewise to Nudipleura species, cephalaspideans may have dispersed through deep-sea waters; due to the Antarctic Bottom Water (Stepanjants *et al.* 2006). Migration through deep waters to the Atlantic and Pacific Ocean basins might have occurred during glacial maxima, similarly to what happens in other benthic phyla, such as cnidarians, crustaceans and echinoderms, among others (Vinogradova 1997; Stepanjants *et al.* 2006). This is also supported by the occurrence of other basal lineages such as *Diaphana* and *Toledonia* in Antarctic and deep-water areas (Marcus 1976; Schiøtte 1998). Further molecular clock analyses should shed light on the geographical origin of Cephalaspidea.

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reject Orthogastropoda. *Proceedings of the Royal Society of London B: Biological Sciences*, *281*, 20141739.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Data of the species included in the phylogenetic analyses and information considered in this study.

Appendix S1. Supplemental material

Fig. S1. Scanning electron microscopy (SEM) micrographs of the rachidian teeth of *Newnesia antarctica* (1) from the Weddell Sea. Uneven number of denticles observed.

Fig. S2. Scanning electron microscopy (SEM) micrographs of *Newnesia joani* n. sp.

Fig. S3. External view of *Newnesia joani* n. sp.

Fig. S4. Schematic representation of *Newnesia joani* n. sp.

Fig. S5. Histological sections of *Newnesia joani* n. sp.

Fig. S6. Histological sections of *Newnesia joani* n. sp.

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A new Antarctic heterobranch clade is sister to all other Cephalaspidea (Mollusca: Gastropoda)

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Running title: **A new basal family of Cephalaspidea**

Supplemental material 1.

***Newnesia joani* n. sp. and Newnesiidae n. fam.**

Systematic description

Gastropoda Cuvier, 1795

Cephalaspidea Fischer, 1883

Newnesiidae Moles, Wägele, Schrödl & Avila n. fam.

http://zoobank.org/NomenclaturalActs/6650E66C-F4F1-4606-929E-8821C2372FF1

Diagnosis: Shell external or internal, globose, thin; apical area flattened, with large aperture. Radular formula: 0.1.0 or 1.1.1 (see Fig. S1). Sharp unicuspidated rachidian teeth with denticles along borders. Broad cephalic shield, posterolateral cephalic lobes present. Tentacular processes absent. Jaws and gizzard plates absent. Cuticularized and spinous stomach. External sperm groove present, running laterally on right side of body from gonopore to penial pore. Parapodia absent. Two gills lying in roof and floor of mantle cavity, respectively. Two repugnatorial glands present: one placed on left antero-lateral side, and one on right postero-lateral side right after mantle cavity (infrapallial lobe).

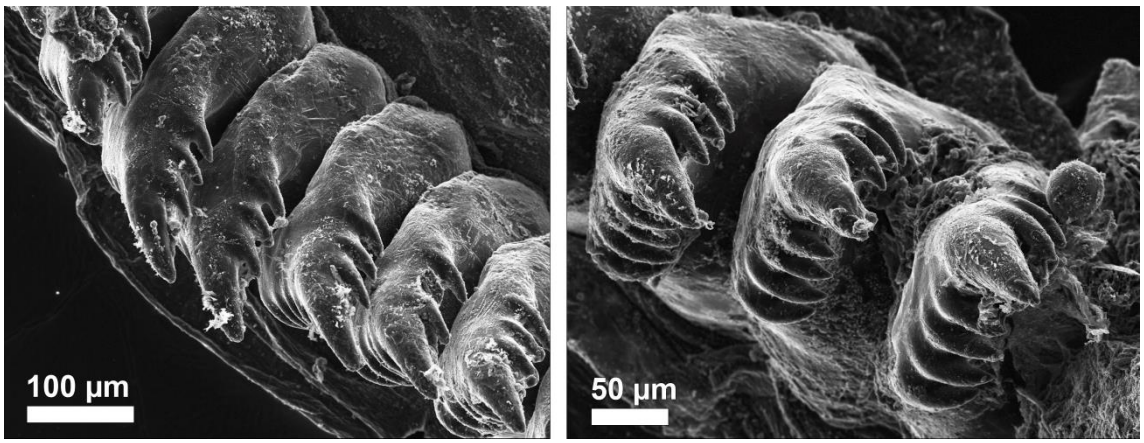

Figure S1. Scanning electron microscopy (SEM) micrographs of the rachidian teeth of *Newnesia antarctica* (1) from the Weddell Sea. Uneven number of denticles observed.

Geographical distribution: from 16 to 1227 m depth, endemic to Antarctic and Subantarctic waters.

Type genus: *Newnesia* Smith, 1902; **Type species**: *Newnesia antarctica* Smith, 1902; by monotypy; Ross Sea.

***Newnesia joani* n. sp.**

Figures S2–6

http://zoobank.org/NomenclaturalActs/0B175ACB-D90D-4203-8FDE-ED11218A2CFF

Holotype (Fig. S3a–d): 15.7 mm, preserved in 70 % ethanol. Deposited in SNSB Zoologische Staatssammlung München (Catalog number ZSM Moll 20150456).

Paratypes: (1) 21 mm, dissected; (2) 19 mm, dissected; (3) 18 mm, dissected; (4) 10.7 mm, sectioned; (5) 10.4 mm, preserved in 70 % ethanol; (6) 8.5 mm, preserved in 70 % ethanol. Dissected and un-dissected specimens are deposited at SNSB Zoologische Staatssammlung München (Catalog number ZSM Moll 20150456). The sectioned individual, as well as radula and shell SEM preparations, are deposited at the University of Barcelona. Paratype (1) is deposited at the CRBA (Centre de Recursos de Biodiversitat Animal, http://www.ub.edu/crba/english/index.htm) under the Catalog number CRBA2024.

Shell (Fig. S2a, b): Maximum height 16.5 mm; maximum width 12 mm. Internal, thin, white; concave, slightly globose in shape, composed of 2.5 whorls, presenting wide aperture strongly oblique to shell axis. Shell covering whole viscera. Protoconch not protruding. Apical area flat, apex barely acute. Surface ornamentation consisting of faint parallel spiral lines with some thin transverse lines producing a reticulate pattern, sometimes thinner lines alternating with wider ones. Umbilicus absent. Lip present, thin, not ornamented, parietal callus absent. Periostracum external, thin, translucent, yellowish, and elastic.

Radula (Fig. S2c–e): Radular formula 19–21 x 1.1.1. Three-seriated, composed by large denticulate teeth with large, hollow, partly overlapping bases. Rachidian teeth with a central sharp cusp, one small denticle at each side positioned in an angle of 45º to central cusp. 5–6 further denticles along rachidian border, each one having sharp

cusp curved towards inner edge; these gradually decreasing in size towards base. Lateral teeth thin, lamellate, with strongly convex anterior margin; placed with their basal edges in longitudinal direction, having concave outer surface.

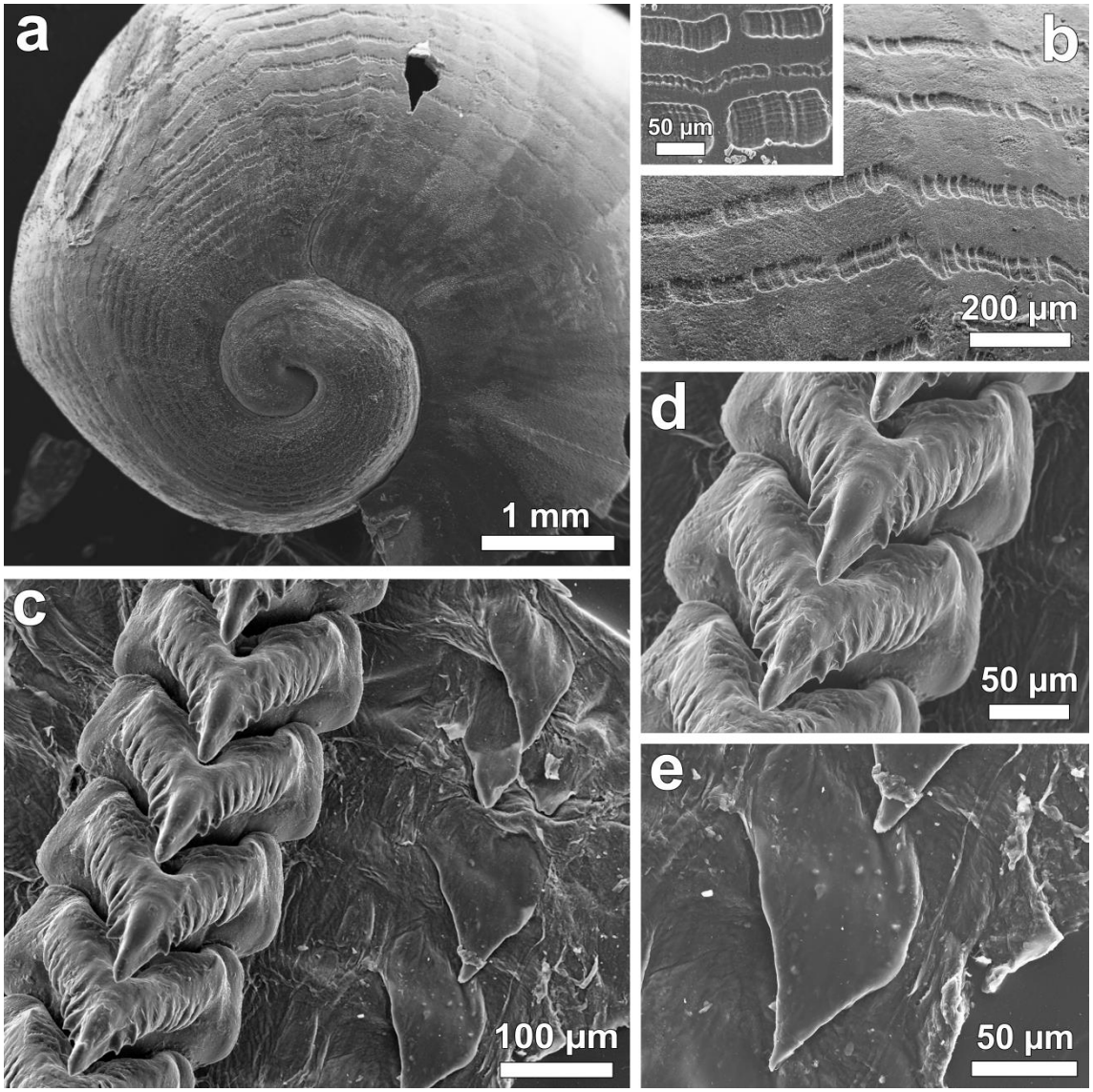

Figure S2. Scanning electron microscopy (SEM) micrographs of *Newnesia joani* n. sp. **a** – shell, apical area. **b** – shell microsculpture, close up showing distinct ornamentation in the same shell. **c** – general view of the radula. **d** – detail of the rachidian tooth. **e** – detail of the lateral tooth.

External morphology (Fig. S3): Live specimens beige to light brown in color, beige and whitish when fixed. A picture of the live animal can be seen in Rauschert & Arntz (2015; plate 41, page 48). Body oval shaped, margin only interrupted by two posterior

cephalic lobes, when looking from dorsal view. Cephalic shield broad, thickened, trapezoidal; mouth opening lying ventrally; eyes shining through transparent notal tissue, located in mid-anterior lateral edges; head with two large, folded, postero-lateral orientated velar lobes displaying ciliated grooves; penial opening placed in the right anterior notch under cephalic lobe. Foot broad, not overpassing body perimeter; propodium squared and slightly lobulated, metapodium oval. Pedal gland opening in the middle foot, visible as an ovate furrow. Conical funnel in frontal left side of notum, lying above left cephalic lobe. Mantle cavity placed on right side and partially covered by shell; inside with prominent, plicate, primary gill; anus opening posteriorly on right side of body close to edge of mantle cavity in small anal papilla (Fig. S4a). Kidney forming a dorsal bulge in mantle cavity, which is partly covered by plicated accessory gill. Accessory gill smaller than primary, which is placed directly underneath.

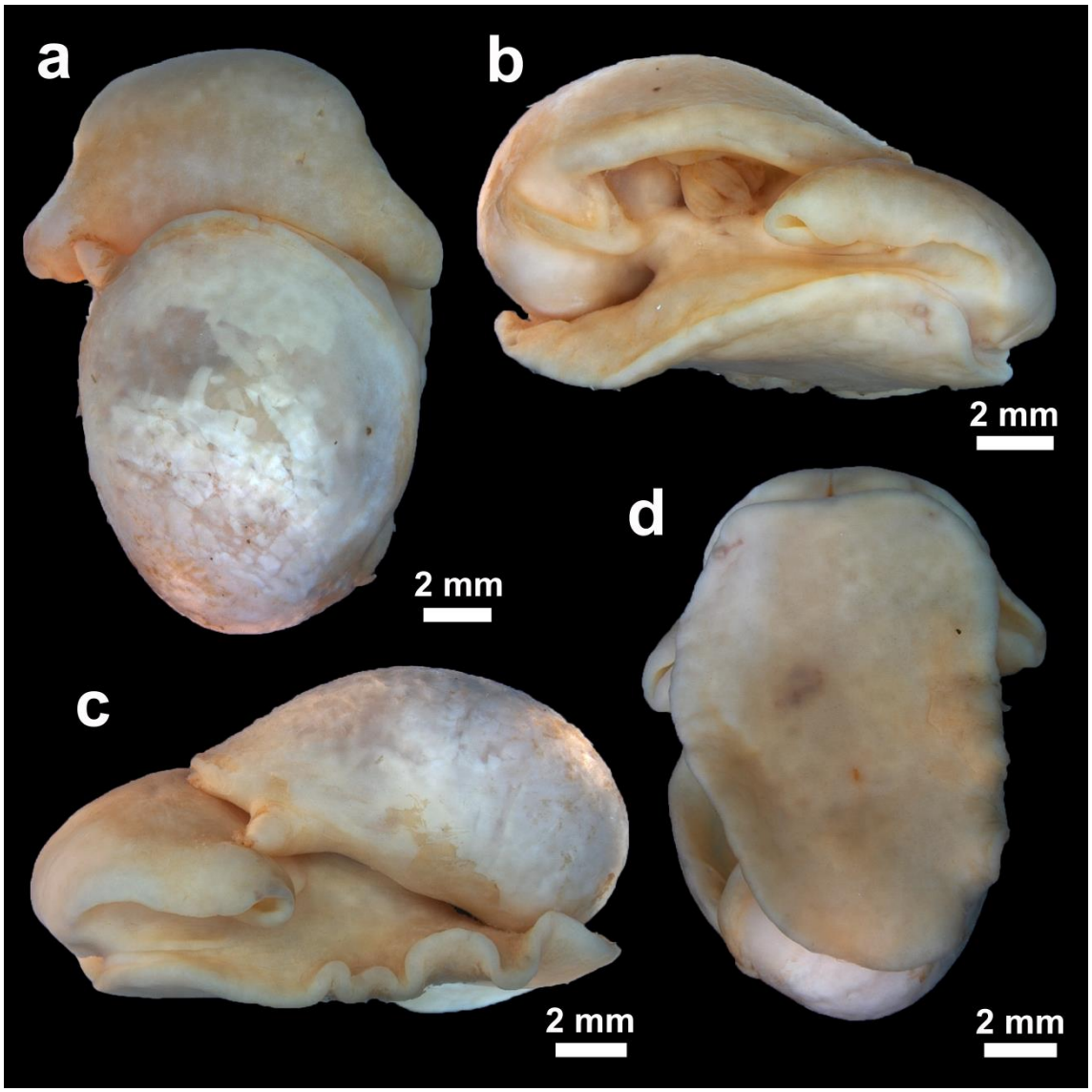

Figure S3. External view of *Newnesia joani* n. sp. preserved holotype. **a**– dorsal view. **b** – right lateral view. **c** – left lateral view. **d** – ventral view.

Digestive system (Fig. S4b): Mouth lying above horizontal furrow between propodium and anterior cephalic shield. Oral glands subepidermal, follicular, containing acid and neutral mucopolysaccharides, opening directly at each side into oral tube, without distinct tube (Fig. S6c). Anterior pharynx elongated and lined with thin cuticle, later on covered with knobbed or spiniform cuticular structures lying on thicker cuticle layer. Posterior pharynx containing odontophore, lined with smooth cuticle. Few denticulate processes, only observed next to radula. Posterior pharynx surrounded by thick muscle layers, whereas anterior pharynx exhibiting fewer muscles. No jaws detected. Salivary glands open into pharynx through thin multiciliated paired ducts. Salivary glands

sausage shaped with narrow section, lying close to oesophagus and stomach while extending until medium body length. Oesophagus running to left side, widening posteriorly, and entering stomach on left side; anterior region with thin cuticle, “T” shaped in cross section, and presenting multiple folds. Epithelium composed of large macrovacuolated cells with bluish, fibrillar content and columnar cells, all having basal nucleus (Fig. S6d); these were not seen in rest of digestive tract epithelium; thin cuticle lining oesophagus. Stomach lying in mid-left section of the animal, anteriorly presenting interior cuticle with knobs, and larger spines posteriorly (Fig. S5e). Gizzard plates absent. Digestive gland occupying most of visceral whorl; composed of numerous diverticula, connected by continuous and expansive lumen. Digestive gland epithelium composed of at least three cell types (Fig. S5f): (1) digestive cells containing spherical, pinkish food vacuoles; (2) microtubule-containing cells with large vacuoles of fibrillar content; and (3) secretory cells containing reddish vacuoles, see (Kress *et al.* 1994). Intestine originating from stomach, on left side, and running dorsally towards right side, just in front of digestive gland. Rectum cells multiciliated, containing acidic mucopolysaccharides.

Juvenile specimens had similar digestive system arrangement compared to adults, but also presenting two large digestive gland diverticula. First one, reaching far into mid-ventral cephalic region and connecting posteriorly to digestive tract. Second one, extending anteriorly in visceral mass and under shell, right behind anterior repugnatorial gland into mid-right section, occupying almost entirely transversal section of animal. Both diverticula composed by four cell types: (1) columnar multiciliated cells close to reduced lumen, (2) globular cells with large nucleus, (3) cells aggregated into follicles, and (4) bluish granulated cells (Fig. S6e). Diverticula surrounded by transversal and longitudinal muscular fibers; longitudinal muscular fibers found at both sides of ventral digestive gland diverticulum.

Reproductive system (Fig. S4c): Monaulic. Gonad (ovotestis) large, slightly lobulated, granular; intermingling with digestive gland, reaching into body whorls; connecting

directly to nidamental glands by tiny duct. Albumen gland elongated, lobulated, connecting separately to other two parts of nidamental glands (capsule and membrane glands). Capsule gland plicated, convoluted, connecting to membrane gland of similar arrangement, but with different texture. Nidamental glands directly enter vagina. Receptaculum seminis wide, globose, entering proximally vagina by short duct. Bursa copulatrix thin, saccular, opening distally into vagina by long duct. Gonopore situated mid-laterally under primary gill, connecting to distinct, external sperm groove (Fig. S5c), leading into opening of highly muscular penial sheath, under right cephalic lobe. Penis unarmed, retractile, connecting directly into single, tubular prostate gland.

Nervous system (Fig. S4d): Composed of prepharyngeal nerve ring connected to visceral ring loop, reaching far back along digestive system. Two cerebral ganglia situated above prepharyngeal region, connected by distinct long commissure. Optical nerves short, leading to small optical ganglion. Distal optical nerve long, up to four times longer than diameter of eye. Eyes with lens, vitreous humor, and retina. Rhinophoral ganglion bilobed, sending nerves forward anteriorly and laterally; one nerve running to small ganglion from where posterior cephalic lobes are innervated. Sensory neuronal cells organized into highly innervated follicles (Fig. S6b), thus chemosensory function is assumed. Each follicle with cortical layer of arranged neuronal cells; each of these with dendrites leading into center of follicle, far into the epidermis; tip of dendrite with several cilia lying outside. These cells are organized into cephalic sensory organ, called lip organ, in the anterior part of cephalic lobe, and Hancock's organ in posterior part. Two small buccal ganglia located below pharynx at the base of salivary ducts and near oesophagus, separated by small commissure and connected by connectives to cerebral ganglia.

Pedal ganglia placed below pharynx and connected to cerebral and pleural ganglia by one relatively long connective nerve. Statocyst with several ovate otogonia, close to pedal ganglia. Right pleural ganglion connected to supraintestinal ganglion, this in turn connected to small genital ganglion, while left pleural ganglion only

connected to smaller distinct parietal ganglion, connected in turn to subintestinal ganglion; this and supraintestinal ganglion connect to visceral (=abdominal) ganglion.

Circulatory, excretory, and respiratory systems: Pericardial complex (composed of one auricle and ventricle within pericardium) aligning transversely across longitudinal axis of body, lying in mid-anterior region under shell. Kidney large, saccular, occupying anterior right part of visceral mass, lying under shell in mantle cavity roof, attached to right side of pericardium (Fig. S5d), as well as to accessory gill, which has thin lamellae. Primary gill larger than accessory gill.

Glandular organs: Huge bluish glandular cells – probably containing neutral mucopolysaccharides – placed at cephalic and propodium edges (Fig. S6a), missing in notch between two cephalic lobes. Smaller glandular cells widespread in epidermis, commonly staining blue in cephalic region and purple-reddish ventrally in foot. Funnel located anterior to left part of visceral mass, connecting to follicular organ through duct paved with columnar multiciliated cells. This organ consisting in up to twenty follicles of 593.9 µm ± 112 µm (mean ± sd) surrounded by layer of 83.6 µm ± 34.6 µm of muscles (Fig. S5a). Each follicle containing two types of glandular cells, all leading into common lumen (Fig. S5b); first type stained purple, containing acid mucopolysaccharides; second one with macrovacuole occupying the entire cytoplasm, staining light blue; maximum size of these cells 90.83 µm ± 12.8 µm. Both cell types also leading into lumen of funnel. Similar follicles also placed at posterior right side between shell and protuberated notum rim; each one leading individually to outside with distinct duct. There is no thick muscle lining in this posterior lying organ, only some muscle fibres. Pedal gland opening ventrally in middle part of foot; pyriform, composed only by follicles of glandular cells containing acid mucopolysaccharides.

Ecology: Twenty-seven animals of different sizes, including juveniles and reproductive adults, were found in muddy bottoms dominated by asteroids, ophiuroids, polychaetes, echiurids, and the dendronotid nudibranch, *Tritoniella belli* Eliot, 1907. Usually, the digestive tract was mainly empty; however in some animals the stomach and intestine

contained sand particles, sclerotized structures, and spicules of, probably, soft corals. Occasionally, cellular structures of unidentified origin were found. Broad and thick cephalic shield together with habitat suggests burrowing habits. Moreover, a sectioned specimen presented six different endoparasites (*i.e.*, copepods and/or nematodes) in the cephalic lobes and foot (Fig. S6f).

Etymology: *Newnesia joani* n. sp. is named after Joan Giménez, a cetacean biologist and esteemed colleague, in recognition of his support and friendship.

Type locality: Between 967–1227 m depth in the Drake Passage, north of King George Island (South Shetland Islands, Antarctica).

Remarks: *N. joani* n. sp. is mainly characterized by the presence of: (1) internal and globose shell; (2) three-seriate radula with sharp unicuspid rachidian tooth and lamellate laterals; (3) broad cephalic shield and posterolateral tentacular lobes; (4) left anterolateral repugnatorial gland (with a distinct funnel) and right posterolateral repugnatorial gland; (5) presence of distinct parietal ganglion. Uncorrected COI *p*-distances between both specimens of *N. joani* n. sp. was zero, and 12.9 ± 1.5%, and 9.2 ± 1.2%, respectively between *N. joani* n. sp. and the two specimens of *N. antarctica.*

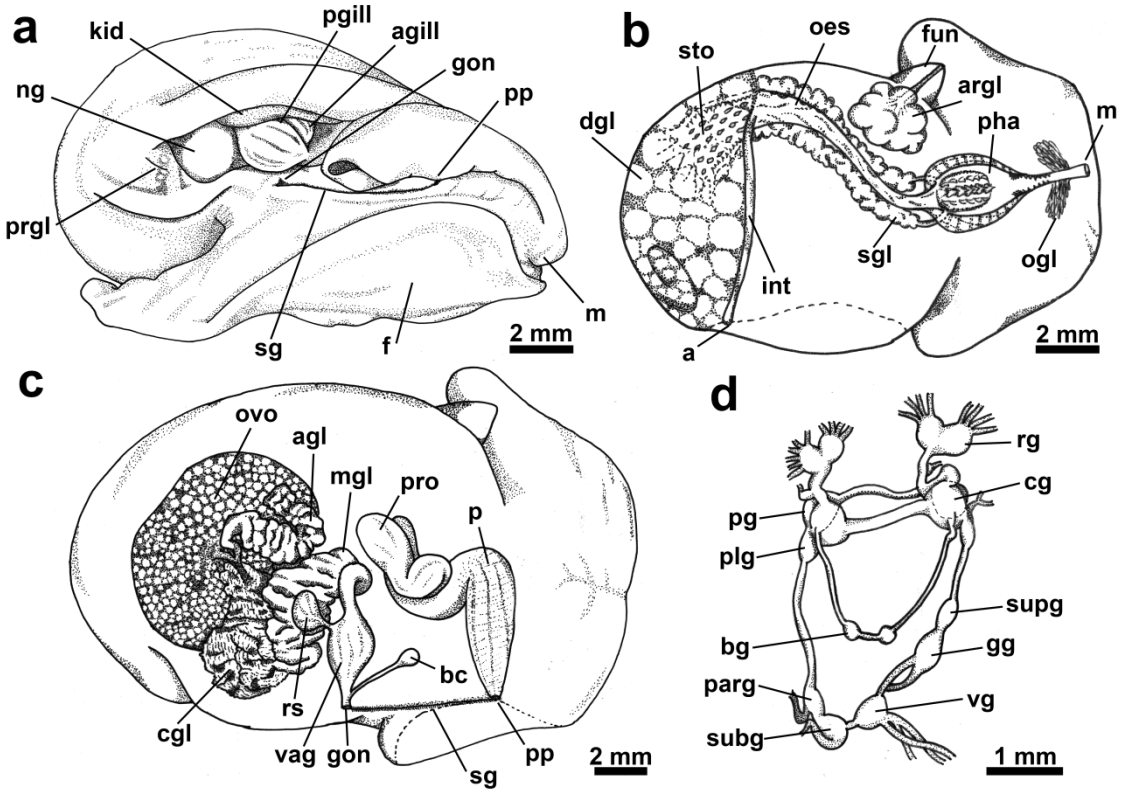

Figure S4. Schematic representation of *Newnesia joani* n. sp. **a** – external view of the right side of the body showing mantle cavity organs. *agill* accessory gill; *f* foot; *gon* gonopore; *kid* kidney; *m* mouth; *ng* nidamental glands; *pgill* primary gill; *pp* penial pore; *prgl* posterior repugnatory gland; *sg* sperm groove. **b** – dorsal schematic view of the digestive system. *a* anus; *argl* anterior repugnatorial gland; *dgl* digestive gland; *fun* funnel; *int* intestine; *m* mouth; *oes* oesophagus; *ogl* oral glands; *pha* pharynx; *sgl* salivary gland; *sto* stomach. **c** – dorsal schematic view of the reproductive system. *agl* albumen gland; *bc* bursa copulatrix; *cgl* capsule gland; *gon* gonopore; *mgl* membrane gland; *ovo* ovotestis; *p* penis; *pp* penial pore; *pro* prostate; *rs* receptaculum seminis; *sg* sperm groove; *vag* vagina. **d** – nervous system showing the prepharyngeal and visceral nerve loops. *bg* buccal ganglion; *cg* cerebral ganglion; *gg* genital ganglion; *parg* parietal ganglion; *pg* pedal ganglion; *plg* pleural ganglion; *subg* subintestinal ganglion; *supg* supraintestinal ganglion; *rg* rhinophoreal ganglion; *vg* visceral ganglion.

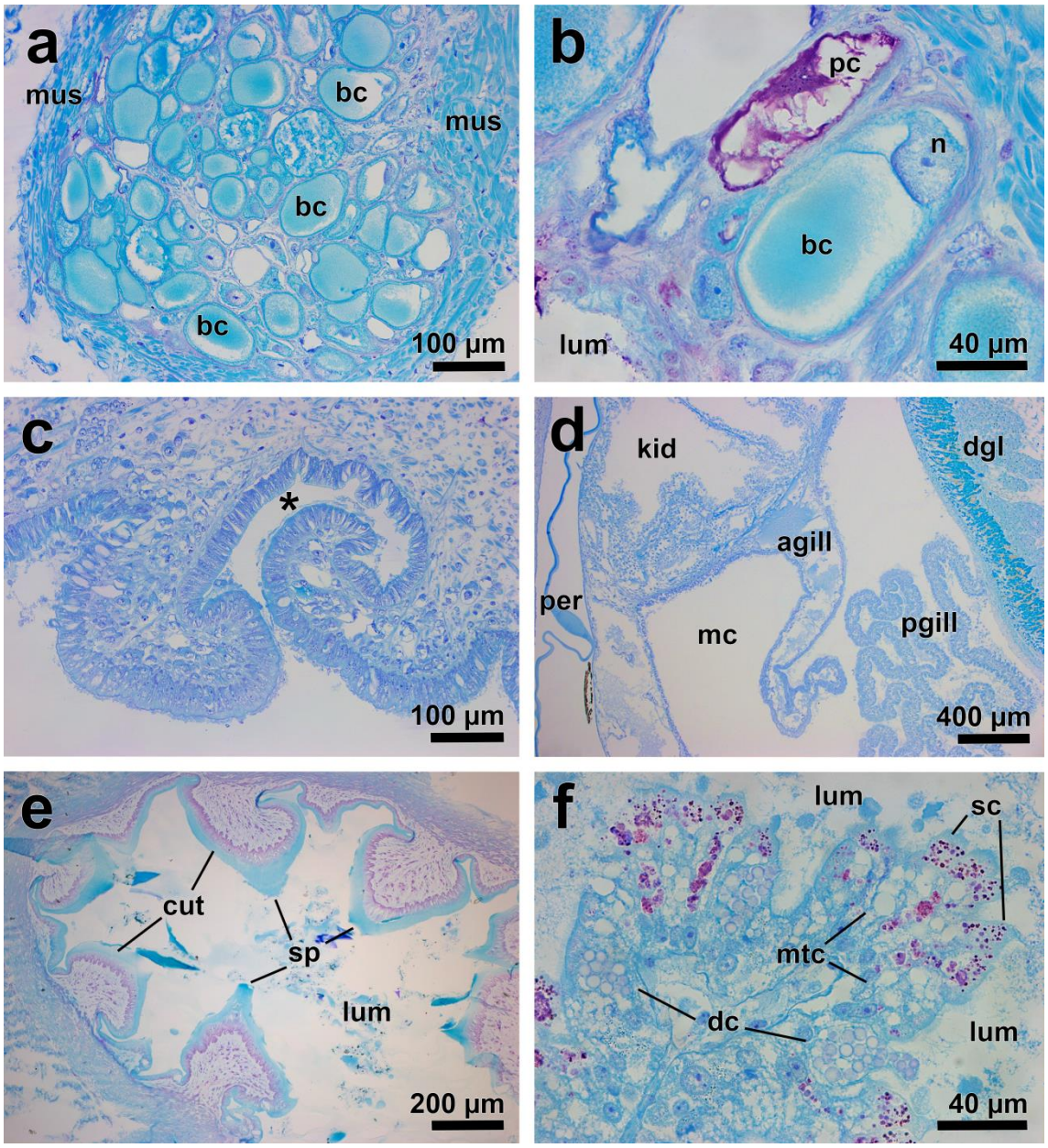

Figure S5. Histological sections of *Newnesia joani* n. sp. **a** – follicle of the anterior repugnatorial gland composed by blue staining cells (*bc*) and surrounded by muscular fibers (*mus*). **b** – detail of the lumen (*lum*), purple (*pc*) and blue macrovacuolar (*bc*) glandular cells and its nucleus (*n*), of the anterior repugnatorial organ. **c** – cross section of the sperm groove showing sperm groove (asterisk). **d** – detail of the mantle cavity roof (*mc*) where kidney (*kid*), accessory (*agill*) and primary *(pgill*) gills are found; digestive gland (*dgl*) and shell periostracum (*per*) are also seen. **e** – cross section of the stomach lumen (*lum*) delimitated by spines (*sp*) with a thick cuticle (*cut*). **f** – detail of the digestive gland cells (*dc*), microtubule-containing cells (*mc*), and secretory cells (*sc*); all surrounding the digestive gland lumen (*lum*).

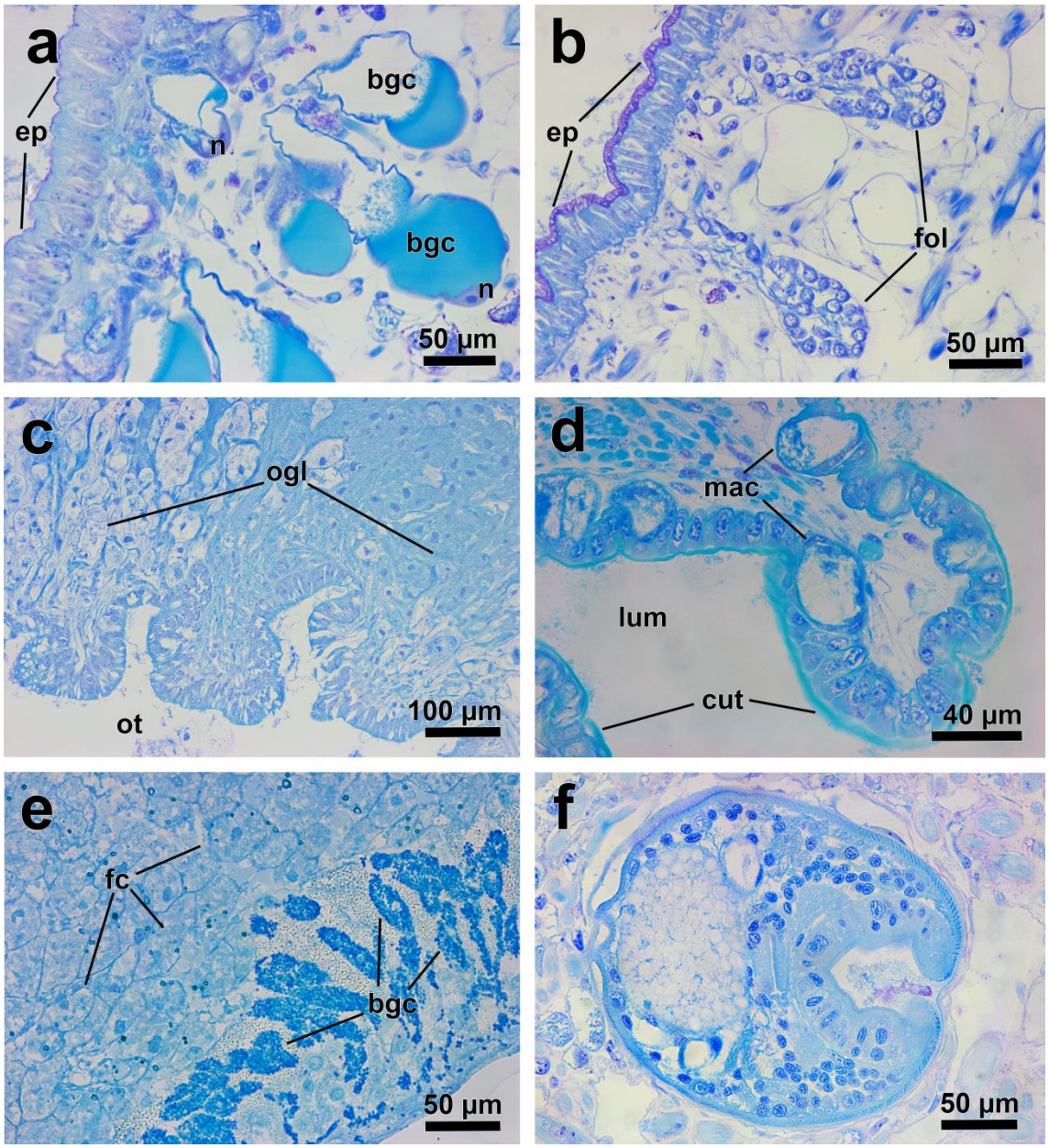

Figure S6. Histological sections of *Newnesia joani* n. sp. **a** – cephalic epithelium (*ep*) containing huge blue glandular cells (*bgc*) and nuclei (*n*). **b** – sensory neuronal cells organized into follicles (*fol*) near the cephalic lobe's epithelium (*ep*). **c** – oral glands (*ogl*) found near the oral tube (*ot*). **d** – oesophagus epithelium folded and composed of large macrovacuolated cells with bluish, fibrillar content (*mac*) and columnar cells (*cc*); these are lined by a thin cuticle (*cut*) in contact to the lumen (*lum*) of the digestive tract. **e** – detail of the digestive reservoir glands of juveniles showing follicular cells (*fc*) and bluish granulated cells (*bgc*). **f** – cross section of a parasite found in the right cephalic lobe.

Reference:

Rauschert, M. & Arntz, W. E. (2015). Antarctic Macrobenthos – a field guide of the invertebrates living at the Antarctic sea_oor. Arntz & Rauschert Selbstverlag: Wurster Nordseeküste (pp. 1–143). ISBN 978-3-00-049890-9.

A new Antarctic heterobranch clade is sister to all other Cephalaspidea (Mollusca: Gastropoda)

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Running title: **A new basal family of Cephalaspidea**

Supplementary Table 1 Data of the species included in the phylogenetic analyses and information considered in this study. Voucher accession numbers [SNSB Zoologische Staatssammlung München] are given along the text for the species sequenced herein, and GenBank accession numbers for all the genes included in the analyses, being the sequences generated for this study in bold letters.

Higher taxa	Family	Species	COI	16S	28S	H3
Cephalaspidea	Acteocinidae Dall, 1913	*Acteocina lepta* Woodring, 1928	KF992197	KJ022827	KJ023022	KJ022891
	Aglajidae Pilsbry, 1895	*Aglaja tricolorata* Reiner, 1807	AM421902	AM421854	AM421950	–
		Chelidonura africana Pruvot-Fol, 1953	DQ974654	KJ022777	DQ927216	KJ022928
		Navanax aenigmaticus (Bergh, 1893)	JN402059	JN402144	–	JN402117
		Philinopsis depicta (Renier, 1807)	AM421892	AM421831	AM421954	–
	Bullidae Gray, 1827	*Bulla ampulla* Linnaeus, 1758	DQ986524	DQ986584	DQ986647	KJ022885
		Bulla striata Bruguière, 1792	DQ986565	DQ986630	DQ986692	KJ022886
	Colpodaspididae Oskars, Bouchet & Malaquias, 2015	*Colobocephalus costellatus* Sars, 1870	KJ023013	KJ022873	KF992207	KJ02286
		Colpodaspis thompsoni Brown, 1979	KF992158	KJ022774	DQ927222	KJ022947
	Colinatydidae Oskars, Bouchet & Malaquias, 2015	*Colinatys* sp. A	DQ974665	KJ022776	DQ927223	KJ022946
		Colinatys sp. A	DQ974666	KJ022783	DQ927224	KJ022939
	Cylichnidae Adams & Adams, 1854	*Cylichna cylindracea* (Pennant, 1777)	KF992159	K022779	KJ23057	KJ022943
		Cylichna gelida (Smith, 1907)	–	EF489326	EF489374	–
		Toledonia globosa Hedley, 1916	EF489395	EF489327	EF489375	–

Diaphanidae Odhner, 1914	*Diaphana globosa* (Lovén, 1846)	KF992162	KJ022791	KJ23056	KJ022930
	Diaphana minuta Brown, 1827	KF643345	AJ223404	–	–
	Diaphana sp. EED	EF489394	EF489325	EF489373	–
Gastropteridae Swainson, 1840	*Sagaminopteron psychedelicum* Carlson & Hoff, 1974	DQ974667	KJ022787	DQ927225	KJ022934
	Siphopteron tigrinum Gosliner, 1989	DQ974668	KJ022788	DQ927226	KJ022933
Haminoeidae Pilsbry, 1895	*Bullacta exarata* (Philippi, 1849)	GQ332576	KJ022800	HM100714	KJ022920
	Diniatys monodonta (Adams, 1850)	KF992178	KJ022809	KJ023040	KJ022912
	Haminoea orbignyana (Férussac, 1822)	KF615813	KJ022794	KF615776	KJ022927
	Smaragdinella calyculata (Broderip & Sowerby I, 1829)	KF992185	KJ022815	KJ023034	KJ022905
Laonidae Pruvot-Fol, 1954	*Laona quadrata* (Wood, 1839)	JX944809	KJ022793	KJ023010	KJ022952
	Laona ventricosa (Jeffreys, 1865)	JX944803	KJ022831	KJ023008	KJ022978
Mnestiidae Oskars, Bouchet & Malaquias, 2015	*Mnestia villica* (Gould, 1859)	KF992161	KJ022789	DQ927236	KJ022931
Newnesiidae Moles, Wägele, Schrödl & Avila, 2016	*Newnesia joani* n. sp. Moles, Wägele, Schrödl & Avila, 2016 (1)	**XXX**	KU939089	–	**XXX**
	Newnesia joani n. sp. Moles, Wägele, Schrödl & Avila, 2016 (2)	**XXX**	KU939090	KU939091	**XXX**
	Newnesia antarctica Smith, 1902 (1)	**XXX**	KU939085	KU939087	**XXX**
	Newnesia antarctica Smith, 1902 (2)	**XXX**	KU939086	KU939088	**XXX**
Philinidae Gray, 1850	*Philine babai* Valdés, 2008	KF877702	KJ022854	KJ022989	KJ022968
	Philine indistincta Ohnheiser & Malaquias, 2013	JX944798	KJ022832	–	KJ022950
Philinoglossidae Hertling, 1932	*Pluscula cuica* Marcus, 1953	KF992203	KJ022837	KJ023016	KJ022881

	Philinorbidae Oskars, Bouchet & Malaquias, 2015	*Philinorbis* sp. A	KF877715	KJ022869	KJ022999	KJ022960
		Philinorbis sp. B	KF877716	KJ022853	KJ022990	KJ022979
	Retusidae Thiele, 1925	*Pyrunculus* sp. B	DQ974678	KJ022773	DQ927237	KJ022948
		Retusa umbilicata (Montagu, 1803)	KF992163	KJ022792	KJ023055	KJ022929
	Rhizoridae Dell, 1952	*Volvulella* sp.	DQ974684	KJ022785	DQ927244	KJ022937
	Scaphandridae Sars, 1878	*Sabatia* sp. A	KF992204	KJ022863	KJ023015	KJ022876
		Scaphander lignarius (Linnaeus, 1758)	KC351563	KC351526	KC351545	KJ094553
Acteonidea	Acteonidae d'Orbigny, 1843	*Acteon* sp.	DQ974648	KJ022782	DQ927213	KJ022940
		Pupa solidula (Linnaeus, 1758)	DQ238006	EF489319	AY427481	EF133483
	Aplustridae Gray, 1847	*Hydatina physis* (Linnaeus, 1758)	DQ986572	DQ986637	DQ986699	–
		Micromelo undatus (Bruguière, 1792)	DQ974653	KJ022778	DQ927214	KJ022944
Acochlidia	Acochlidiidae Küthe, 1935	*Strubellia paradoxa* (Strubell, 1892)	HQ168457	HQ168419	HQ168445	–
Anaspidea	Akeridae Mazzarelli, 1891	*Akera bullata* Müller, 1776	KF992164	KJ022795	KJ023054	KJ022926
	Aplysiidae Lamarck, 1809	*Aplysia dactylomela* Rang, 1828	KF992168	KJ022798	KJ023050	KJ022921
Nudibranchia	Cadlinidae Bergh, 1891	*Aldisa smaragdina* Ortea, Pérez & Llera, 1982	KF992175	KJ022806	KJ023043	KJ022914
Runcinacea	Runcinidae Adams & Adams, 1854	*Runcina africana* Pruvot-Fol, 1953	DQ974680	KJ022780	DQ927240	KJ022942
		Runcina divae (Marcus & Marcus, 1963)	KF992195	KJ022825	KJ023024	KJ022893
Sacoglossa	Plakobranchidae Gray, 1840	*Elysia papillosa* Verrill, 1901	HQ616844	HQ616815	–	HQ616869
	Volvatellidae Pilsbry, 1895	*Ascobulla* sp. A	DQ974683	KJ022781	DQ927243	KJ022883
Umbraculida	Tylodinidae Gray, 1847	*Tylodina perversa* (Gmelin, 1791)	KF992172	KJ022803	KJ023046	KJ022917