

An integrative approach distinguishes three new species of Abyssochrysoidea (Mollusca: Caenogastropoda) associated with organic falls of the deep south-west Atlantic

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Two new species of *Rubyspira* and one of *Cordesia* (Gastropoda: Abyssochrysoidea) are described morphologically and genetically, based on specimens collected from whale bones and wood parcels artificially implanted in the deep south-west Atlantic Ocean, at c. 1500 and 3300 m depths. *Rubyspira pescaprae* sp. nov. and *R. elongata* sp. nov. occur preferentially on whale bones. *Cordesia atlantica* sp. nov. is dominant on wood parcels. Distribution of *Cordesia* was hitherto only associated with hydrocarbon seeps off West Africa. Stable isotopes and gut content analyses were carried out to check possible trophic pathways. Both *Rubyspira* species are bone eaters, while *Cordesia atlantica* seems to rely on a wider range of food sources, including juveniles of wood-specialized xylophagid bivalves. Morphological and genetic evidence suggest that *Rubyspira* and *Cordesia* are more closely related to *Abyssochrysos* than to any other Abyssochrysoidea snails. In the present study, *Cordesia* is considered part of the family Abyssochrysididae based on molecular and morphological evidence, such as the presence of a penis and pallial tentacles arrangement.

ADDITIONAL KEYWORDS: *Cordesia* – deep-sea snails – gastropod – phylogeny – *Rubyspira* – taxonomy – whale falls – wood falls.

INTRODUCTION

The discovery of deep-sea hydrothermal vents in the Galápagos Rift in the late 1970s caught the attention of scientists to an environment with higher biomass compared to surrounding areas receiving meager organic resources (Lonsdale, 1977; Corliss *et al.*, 1979). The same patterns applied to cold seeps, first discovered in the Florida Escarpment (Paull, *et al.*, 1984). However, chemosynthesis was not restricted only to those habitats, since large organic falls, such

as whale carcasses, may also induce the appearance of a chemosynthetic biota in deep areas owing to a large, localized input of organic carbon. Similar to what occurs in other chemosynthesis-based habitats, a suite composed of generalist and specialist organisms colonize these areas (Smith *et al.*, 1989). The fauna found in whale falls can exploit resources on different successional stages (Smith *et al.*, 2002, 2015, Smith & Baco, 2003, Fujiwara *et al.*, 2007, Lundsten *et al.*, 2010b).

Wood parcels may also congregate specialists, such as xylophagid bivalves that feed on the substrate through symbiosis (Turner, 1973). Feeding activities of wood specialists generate detrital organic matter enriching surrounding sediments, while decreasing

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substrate availability. Substrate can last for years, depending on size, and may create a sulphophilic stage (Bernardino *et al.*, 2010; Bienhold *et al.*, 2013). However, Kalenitchenko *et al.* (2018) showed that chemosynthetic activity is not dependent on xylophagid species and can happen even before their settlement. This sulphophilic stage is characterized by anaerobic microbial decomposition of lipids, emitting sulphides during the degradation, enabling the arrival of animals that exploit sulphides and also from higher trophic levels (Smith *et al.*, 1989; Smith & Baco, 2003; Fujiwara *et al.*, 2007; Amon *et al.*, 2013)

Organic falls are important environments increasing deep-sea biodiversity and connecting chemosynthesis-dependent fauna among cognate habitats (Smith *et al.*, 1989; Distel *et al.*, 2000; Sumida *et al.*, 2016). Despite being known to be rich, the biodiversity and biogeography of these type of reducing environment are still poorly comprehended compared to seeps and vents. Moreover, most organic falls studies, either natural or implanted, are from the north-east Pacific Ocean basin (e.g. Smith *et al.*, 1989; Smith & Baco, 2003; Goffredi *et al.*, 2004; Smith, 2006; Lundsten *et al.*, 2010a, b). Faunal patterns in this basin may not be representative of other areas for which biogeographical, populational and evolutionary data are wanting (Lundsten *et al.*, 2010a). Few studies have been carried out in other ocean basins, such as the north-western Pacific (Fujiwara *et al.*, 2007), the Southern Ocean (Amon *et al.*, 2013; Smith *et al.*, 2014) and the south-west Atlantic (Sumida *et al.*, 2016; Saeedi *et al.*, 2019).

Gastropod molluscs are important animals in chemosynthetic environments, with different ecological roles related to their feeding strategies, and are present in most natural and artificially implanted whale falls (Smith *et al.*, 1989, 2014; Fujioka *et al.*, 1993; Smith & Baco, 2003; Braby *et al.*, 2007; Fujiwara *et al.*, 2007; Johnson *et al.*, 2010; Lundsten *et al.*, 2010a, 2010b; Amon *et al.*, 2013, 2017; Sumida *et al.*, 2016). Many deep-sea gastropods are endemic to these habitats (Sasaki *et al.*, 2010). Until the late 2000s, about 100 genera and more than 200 species of gastropods were found only in hydrothermal vents and cold seeps of the Indo-Pacific, Indian and Atlantic Oceans, making gastropods the most species-rich group of vent animals known to date (Desbruyères *et al.*, 2006; Sasaki *et al.*, 2010).

The superfamily Abyssochrysoidea Tomlin, 1927 are caenogastropods, closely related to Littorinidae, supported by morphological features (Warén & Ponder, 1991) and molecular data (Colgan *et al.*, 2007). Abyssochrysoidea, known for inhabiting solely the deep sea, is composed of two families: Abyssochrysidae Tomlin, 1927, with the single genus *Abyssochrysos*

Tomlin, 1927, and Provannidae Warén & Ponder, 1991, exclusive of chemosynthetic environments and composed of five genera (Sasaki *et al.*, 2010). Abyssochrysoidea also include the deep-sea snail *Rubyspira* Johnson *et al.*, 2010, which is not yet assigned to any known family and was first discovered on a whale carcass in the deep, north-eastern Pacific basin at 2893 m deep (Johnson *et al.*, 2010).

All *Rubyspira* species are whale-fall specialists that can consume relatively large pieces of bone or the enriched sediment around whale carcasses (Johnson *et al.*, 2010). Bacteria have also been found in their gills, but details of this possible symbiotic relationship are still unknown (Johnson *et al.*, 2010). Three *Rubyspira* species have been formally described: *R. osteovora* Johnson *et al.*, 2010 and *R. goffrediae* Johnson *et al.*, 2010 from the north-east Pacific basin, and *Rubyspira brasiliensis* Hasegawa *et al.*, 2019 from the south-west Atlantic Ocean (Johnson *et al.*, 2010; Hasegawa *et al.*, 2019). Most of these species, excepted *R. goffrediae*, are abundant in whale falls, representing a significant biomass of the fauna (Johnson *et al.*, 2010; Sumida *et al.*, 2016; Hasegawa *et al.*, 2019). No *Rubyspira* species resemble extant abyssochrysid snails, but their shell morphology is similar to fossils of *Atresius liratus* Gabb 1869, found in Cretaceous hydrocarbon seeps (Johnson *et al.*, 2010). They were found to be closely related to *Abyssochrysos*, an abyssochrysid gastropod that shares some morphological characteristics, such as the arrangement of pallial tentacles, the presence of a pedal furrow and similar paraspermatozoa, as well as genetic traits (Johnson *et al.*, 2010; Hasegawa *et al.*, 2019). However, the genus was not placed in Abyssochrysidae due to the lack of a penis and differences in radular morphology. The arrangement of this genus within Abyssochrysoidea is still awaiting improvement, pending the analysis of more taxa and/or more morphological and genetic data.

The remaining five Abyssochrysoidea genera are currently assigned to Provannidae. Phylogenetic analyses often recover the clade *Abyssochrysos* + *Rubyspira* (Johnson *et al.*, 2010; Chen *et al.*, 2016a, 2019), which is sister to a group that includes *Alviniconcha* Okutani & Ohta, 1988, *Desbruyeresia* Warén & Bouchet, 1993 and *Ifremeria* Bouchet & Warén, 1991. These genera together form a clade that is the sister-group of *Provanna* Dall, 1918. Therefore, Provannidae is not monophyletic (Johnson *et al.*, 2010; Chen *et al.*, 2016a, b, 2019; Hasegawa *et al.*, 2019; Linse *et al.*, 2019).

Cordesia Warén & Bouchet, 2009 was not included in these analyses, mostly because this genus was described only from a few specimens of *C. provannoides* Warén & Bouchet, 2009 from a hydrocarbon seep off Congo and off the Florida Escarpment. These samples

were previously fixed in formalin and, therefore, are not suitable for genetic studies. Warén & Bouchet (2009) diagnosed the genus as ‘similar to *Provanna* but male with a penis; two right pallial tentacles, one much smaller’. They also pointed out that these diagnostic characters are only found in abyssochrysid; these similarities opened the question if *Cordesia* should be classified as an abyssochrysid snail or if Provannidae should be placed in Abyssochrysidae. However, Warén & Bouchet (2009) associated this species to the provannids based on feeding behaviour, type of habitat (i.e. chemosynthetic environments) and morphological traits, such as characters of the larval shell and radula. The loss of a penis led Warén & Bouchet (2009) to suggest that some conditions found in Abyssochrysoidea are plesiomorphic, and are modified in *Alviniconcha*, *Desbruyeresia*, *Ifremeria* and *Provanna*. This hypothesis remains to be tested, with a parallel discussion about the arrangement of these families among abyssochrysoideans and, as a result, how they became tied to chemosynthesis-based environments. Molecular data of *C. provannoides* are much sought after in order to help resolve these questions.

In this study we use integrative taxonomy to distinguish and describe three new Abyssochrysoidea species, two in the genus *Rubyspira* and one in *Cordesia*, collected from the deep south-west Atlantic. They were found dwelling on artificially implanted organic falls in five sites with depths ranging from 1413 to 3358 m (Fig. 1; Table 1). A detailed description of their larval and adult shells plus soft parts was performed. Direct observations of gut content, and carbon and nitrogen stable isotopes, were used to understand their nutrition, because *Rubyspira* species described to date are considered bone specialists and little is known about feeding preferences of *Cordesia*. Mitochondrial molecular markers, *COI* and 16S, were used to investigate their phylogenetic position within Abyssochrysoidea. Molecular studies were also used to better understand the phylogenetic relationships of *Rubyspira*, which is still unassigned at family level, and the position of *Cordesia*, since the present study provides the first molecular data for the genus.

MATERIAL AND METHODS

STUDY SITE AND SAMPLING

Organic substrates were deployed in the deep south-west Atlantic along the 1500 and 3300 m isobaths using free-fall landers at six sites (Fig. 1; Table 1). Landers are independent experimental structures that remain on the seabed to attract colonizing organisms. Structures were constructed of aluminium and equipped with three boxes containing whale

vertebrae, three boxes with wood parcels and three boxes containing inorganic material for control (see lander design in: Saeedi *et al.*, 2019). The position of each substrate in each box was randomly chosen. The boxes were open at their upper parts and contained a 500- μ m mesh, which allowed the exchange of water around the substrates. Each lander was equipped with deep-sea glass buoys and a Teledyne Benthos 866A acoustic releaser for recovery. The lids were kept open until they left the bottom upon retrieval.

The study area is located at the continental slope and the abyssal plain of the south-west Atlantic Ocean. The landers were bathed by the southern part of the North Atlantic Deep Water (NADW) at around 1500 m depth and by the Antarctic Bottom Water (AABW) at deeper sites. NADW present temperatures around 3–4 °C and AABW temperatures are lower than 2 °C, and both have high dissolved oxygen contents (De Madron & Weatherly, 1994; Silveira *et al.*, 2000).

Landers were deployed with R/V *Alpha Crucis* on May/June 2013. The lander at SP1500 site was recovered after 16 months on the seafloor on board the R/V *Alpha Delphini*, on October 2014. Later, in May 2015, the other landers were recovered on board the Polar R/V *Almirante Maximiano* after 23 months left in the deep sea (lander RJ1500 was lost). The substrates were immersed in cold seawater (4 °C) after recovery and individuals sorted onboard were preserved in molecular-grade ethanol for molecular genetic analyses and in 4% buffered formaldehyde for morphological identifications. All the remaining organisms and substrates were preserved in 96% ethanol for long-term storage.

DNA EXTRACTION AND PHYLOGENETIC ANALYSES

DNA was extracted from the pedal muscle of at least ten individuals of each species, using the DNeasy Blood and Tissue Extraction Kit (QIAGEN). Around 650 bp of *COI* were amplified by PCR using the primers HCO-2198 and LCO-1490 (Folmer *et al.*, 1994) and around 550 bp of 16S rRNA with 16SAR and 16SBR primers (Palumbi, 1996). The PCR was carried out in a 12.5- μ L reaction, including 2 μ L of DNA template, 0.1 μ L of both reverse and forward primers (20 μ M), 6.25 μ L Gotaq Mastermix dNTP mixture 2x and 4.05 μ L sterilized deionized water. The thermocycling protocol was initialized at 95 °C for 1 min for denaturation, followed by 35 cycles of 45 s at 94 °C, annealing at 50 °C for 1 min, an extension step at 72 °C for 1 min, ending with 72 °C for 7 min. Then the amplification was confirmed with a 1.2% agarose gel electrophoresis. The purification was conducted with QIAGEN Purification Kit. Sequencing was carried out by Myleus Biotecnologia with Sanger method. Fragments of each gene were bidirectionally sequenced and assembled in GENEIOUS v.11.0.3 (Kearse *et al.*, 2012). Sequences were deposited in GenBank and accession

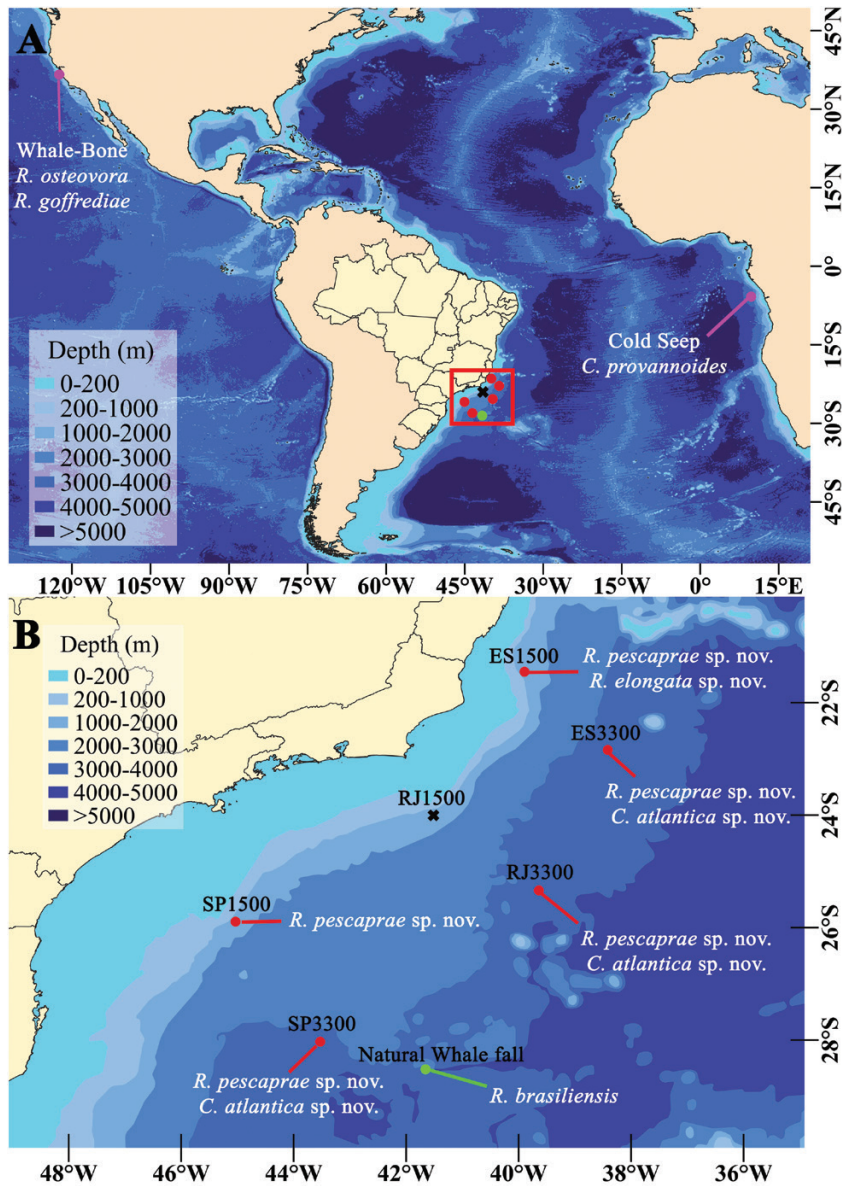


Figure 1. World-wide distribution of *Rubyspira* and *Cordesia* species. A, previous records from the north-east Pacific and West Africa. B, *Rubyspira brasiliensis* from a natural whale-fall on the Brazilian shelf. Lander deployment sites implanted during the BioSuOr Project and new data (this study) from the south-west Atlantic. RJ1500 was lost.

Table 1. Coordinates and physical-chemical characteristics of stations when landers were implanted during the BioSuOr project at the SW Atlantic

Station BioSuOr	Latitude	Longitude	Depth (m)	T °C (surface)	T °C (bottom)	Sal (surface)	Sal (bottom)
ES1500	21°27'00.5"S	39°53'47.4"W	1491	25.76	3.58	37.36	34.76
ES3300	22°50'27.1"S	38°24'58.8"W	3322	25.11	2.42	37.15	34.90
RJ1500	24°00'12.6"S	41°30'54.6"W	1413	25.44	3.52	37.36	34.62
RJ3300	25°20'18"S	39°38'28.3"W	3285	23.74	2.45	36.92	34.91
SP1500	25°53'38.4"S	45°02'05.4"W	1508	24.52	3.64	37.23	34.67
SP3300	28°01'42.4"S	43°31'46.8"W	3358	22.94	1.03	36.82	34.75

numbers are given in the phylogenetic tree (Fig. 2; Supporting Information, Table S1).

The sequences were aligned using MAFFT (Katoh et al., 2002) with G-INS-I and E-INS-I options for COI and 16S, respectively, implemented in GENEIOUS v.11.0.3 (Kearse et al., 2012). The alignments of COI, 16S and both genes concatenated were carried out using the resulting sequences and the abyssochrysoid sequences available for both genes on GenBank (Fig. 2; Supporting information, Table S1): *Littorina littorea* Linnaeus, 1758,

(Caenogastropoda), the neogastropods *Neptunea amianta* Dall, 1890 and *N. antiqua* Linnaeus, 1758 (after Johnson et al., 2010) were selected as outgroups and trees were rooted on the *Neptunea* clade. Gastropod species with only COI datasets available, such as *Desbruyeresia* species (excepted for *D. melanioides* Warén & Bouchet, 1993) and *Rubyspira brasiliensis*, were also included for better resolution on concatenated analysis. The model selected was GTR+I+G using JModelTest 2 (Guindon & Gascuel, 2003; Darriba et al., 2012) with Bayesian information criteria.

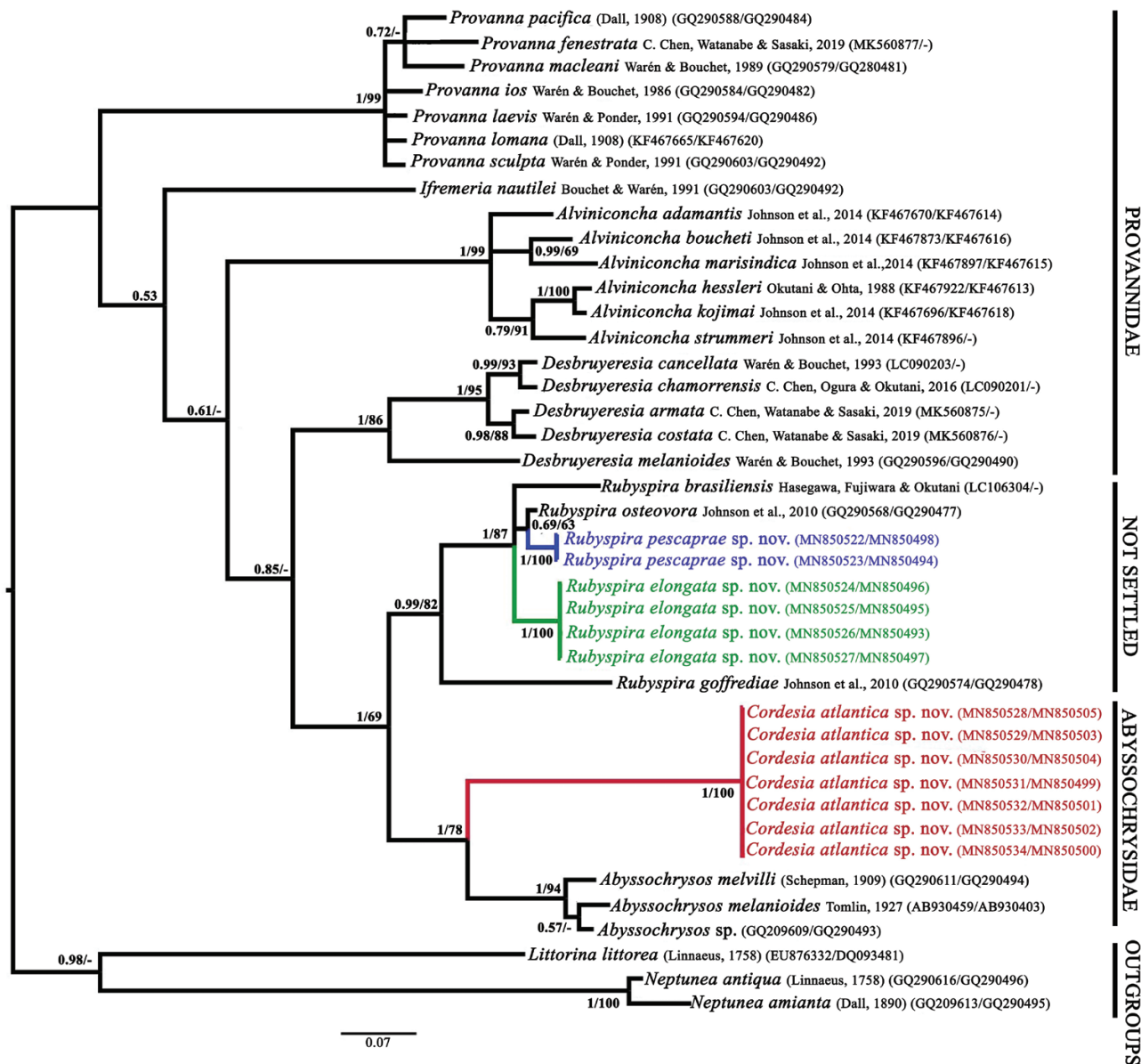


Figure 2. Phylogenetic concatenated tree reconstruction of COI and 16S of abyssochrysoid gastropods. Showing the systematic position of the new species of *Cordesia* and *Rubyspira*. Bayesian inference (GTR+G+I) nodes values and maximum likelihood (GTR+G+I) bootstrap support are shown, respectively. Support with less than 50% for BI and 60% for ML are not shown. Genbank accession number are given next to species (COI/16S), species without sequenced genes are expressed as '-'. Family memberships based on current study are given on the right side.

The phylogenetic reconstruction was performed using Bayesian inference (BI) and maximum likelihood (ML). Bayesian inference analysis was conducted in MrBayes v.3.2.6 (Ronquist *et al.*, 2012) for each locus individually and for the final concatenated phylogenetic tree. Chains were run for 10^7 generations and topologies were sampled every 500 generations, discarding the first 2×10^5 trees. Maximum likelihood reconstruction was performed in RAxML 8.2 (Stamatakis, 2014) and the values for gamma shape parameters was 0.789 and 0.544 as proportion of invariants. Statistical ML node supports were obtained by 10 000 bootstraps. Phylogenetic trees were edited with FigTree v.1.4.3 (Rambaut, 2010) for better visualization. Genetic distances for *COI* and 16S were given by the mean of sequenced genes of the three new species compared to the selected dataset in GENEIOUS v.11.0.3 (Kearse *et al.*, 2012).

TROPHIC ANALYSES

Individuals stored only in 70% ethanol were used for dissection of the digestive system under a Nikon SMZ800 stereomicroscope. The gut content of ten individuals of *Rubyspira pescaprae* and of ten *Cordesia atlantica* were removed, dried and put on a double-sided carbon tape on a JEOL JSM-5800LV scanning electron microscope.

Stable isotope analyses of carbon and nitrogen were carried out using pedal muscle of ethanol-preserved individuals of *Rubyspira pescaprae* and *Cordesia atlantica*. Possible food sources, such as whale bones, wood, bacterial mats and xylophagaid bivalves (see below), were also selected for this analysis. Samples were frozen and freeze-dried for 24 h, weighed and placed on tin capsules. At least three specimens of each species were selected for this procedure, with the exception of *Rubyspira elongate*, which is a smaller and less abundant species and it would be necessary to use too many individuals to reach the minimum weight. Each organism was removed from different landers and substrates (i.e. whale bones, wood parcels and inert material) to check for differences in diet. Samples were sent to the School of Biological Sciences of Washington State University and analysed with a Eurovector Elemental Analyser.

The results are expressed in delta notation:

$$\delta = 1000 \times [(R_{\text{sample}}/R_{\text{standard}}) - 1]$$

where R is the ratio of heavy to light isotope, the standard referential in this study was atmospheric N_2 for nitrogen and Peedee Belemnite for carbon. The effects of ethanol preservation were considered negligible for the $\delta^{15}N$ analyses of the species (Fanelli *et al.*, 2010; Lau *et al.*, 2012). No correction factor was applied for $\delta^{13}C$ values, but ethanol effects are variable between taxa and can affect results on mollusc species

(Fanelli *et al.*, 2010; Lau *et al.*, 2012). However, only two species were considered in this work and the high standard deviations seem to be more related to the diversity of food sources.

Results were compared to available stable isotope data for species of *Rubyspira* and *Osedax*, the latter a known bone specialist (Johnson *et al.*, 2010; Alfaro-Lucas *et al.*, 2018). There are no available stable isotope data for *Cordesia provannoides*.

MORPHOLOGICAL ANALYSES

Morphological investigation and dissection were carried out with a Zeiss SteREO Discovery V8 stereomicroscope using individuals preserved in 70% ethanol or in buffered formalin. Specimens were sorted, photographed and growth series of species were made.

For each species, radulae were dissected and tissues around them were dissolved in 10% NaClO, washed in distilled water and mounted on stubs with double-sided carbon tape. They were metallized with gold, using a Balzers SCD-050 sputter coater, and observed under a JEOL JSM-5800LV scanning electron microscope (SEM) at Instituto de Biologia da Universidade de Campinas. SEM electromicrographs were also made for adult individuals with detailed views of the larval and adult shells.

RESULTS

MOLECULAR PHYLOGENY

Molecular phylogenetic analysis using maximum likelihood and Bayesian inference place both new *Rubyspira* species within their genus in both individual and concatenated trees of *COI* and 16S ribosomal RNA (Fig. 2; Supporting information, Table S1). Both *Rubyspira* form a monophyletic clade with *R. goffrediae* that is strongly to moderately supported (BI: 0.99, ML: 79%; Fig. 2). However, relationships with other species were not well resolved. The new species *Cordesia atlantica* is recovered as sister to the *Abyssochrysos* clade (BI: 1, ML: 74%) and *Rubyspira* is recovered as a sister-group of *Abyssochrysos* + *Cordesia* (BI: 1, ML: 61%). The clade formed by these three genera is a sister-group of *Desbruyeresia*.

NUTRITION

Light microscopy and SEM reveals that the gut content of both new species of *Rubyspira* are filled with a mixture of sediment and bone fragments of many different sizes. Many large bone pieces are observed mainly in the stomach of *R. pescaprae* (Fig. 3E–G), while in *R. elongata* only small pieces of bone are present. However, only few specimens of *R. elongata* were analysed due to the low number of individuals available.

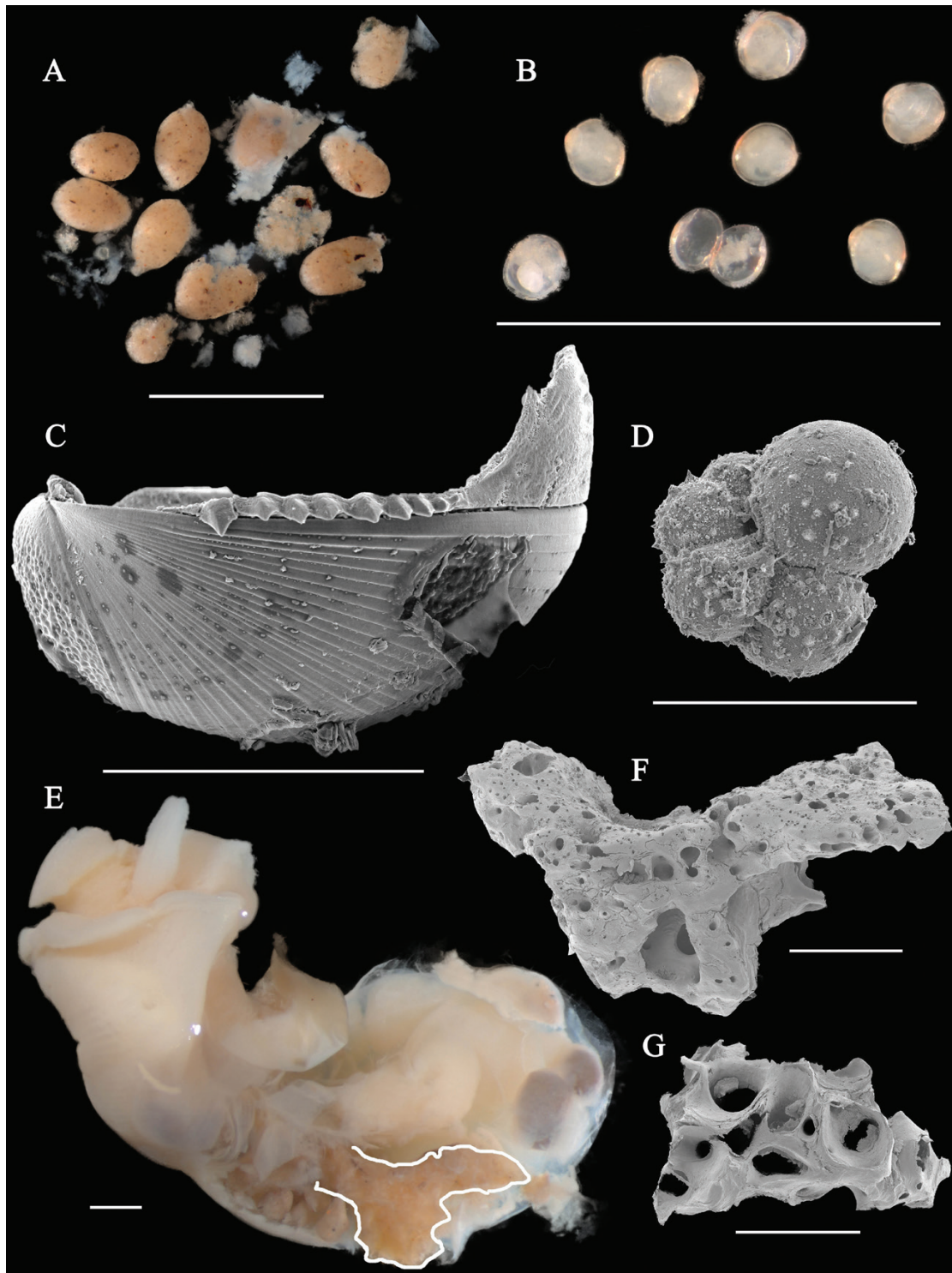


Figure 3. Digestive tract content of *Cordesia atlantica* (A, B, C, D) and *Rubyspira pescaprae* (F, G). Photograph under stereomicroscope (A, B, E) and scanning electron microscopy (C, D, F, G). A, *Cordesia atlantica* faecal pellets. B, Xylophagaidae bivalves found in *C. atlantica* pellets (A). C, D, juvenile Xylophagaidae bivalve and foraminifera, respectively, found on *Cordesia* specimen digestive tract. E, *Rubyspira pescaprae* removed from its shell showing a large piece of whale bone within its dissected stomach (highlighted in white). F, the same bone fragment shown on E. G, bone fragment. Scale bars are 1000 μm for A, B, E, F, G and 100 μm for C, D.

Direct observation of gut content of *Cordesia atlantica* shows a mixture of wood fragments, sediment and mineral particles, with few organic matter particles. In addition, juvenile shells of Xylophagidae bivalves and Foraminifera were observed in the faecal pellets inside the specimens (Fig. 3A–D).

Considering food resources, the $\delta^{15}\text{N}$ presents high variations on whale bones, wood parcels and bacterial mats, and $\delta^{13}\text{C}$ is much more variable on bones. The highest mean values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are both on bones, and the lowest on bacterial mats and woods, respectively. Most individual values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are also variable. *Rubyspira pescaprae* presents an enrichment around 3 for $\delta^{13}\text{C}$ and 0.5 for $\delta^{15}\text{N}$ when compared to whale bones. For *Cordesia atlantica*, the enrichment is higher than 4 for $\delta^{13}\text{C}$ and 1.5 for $\delta^{15}\text{N}$ considering *Xylophaga* spp., the closest possible food source analysed (Fig. 4; Supporting information, Table S2).

TAXONOMY

PHYLUM: MOLLUSCA LINNAEUS, 1758

CLASS: GASTROPODA CUVIER, 1797

SUBCLASS: CAENOGASTROPODA COX, 1959

[Unplaced to Order]

SUPERFAMILY: ABYSSOCHRYSOIDEA TOMLIN, 1927

[Unplaced to Family]

GENUS: *RUBYSPIRA* JOHNSON ET AL., 2010

Type species: *Rubyspira osteovora* Johnson et al., 2010 (by original designation).

Diagnosis: Tall adult shell, composed by various, spirally sculptured whorls covered by a periostracum forming small bristles, and bearing no siphonal canal; the multispiral protoconch was considered as reflecting a planktotrophic development sealed with a calcareous plug. Two pallial tentacles are present, there being a large one on the right corner of the snout and a single smaller one on the left.

RUBYSPIRA PESCAPRAE SP. NOV.

(FIGS 5A–O, 6, 7A–C)

LSID: zoobank.org:act:276AD8F9-303F-46CD-B3DC-E5F3ABB6ABC9.

Type locality: Off Rio de Janeiro, Brazil, 3285 m, 25°20'18"S, 39°38'28.3"W, on and around organic falls (whale bones and wood parcels).

Holotype: ZUEC-GAS 7913 (Fig. 5A, E, L), sta. RJ3300 (25°20'18"S, 39°38'28.3"W) off Rio de Janeiro State, Brazil – ‘BioSuOr Project’ coll., 23 May 2015, depth 3285 m. Measurements: shell height: 28.9 mm; shell width: 13.0 mm.

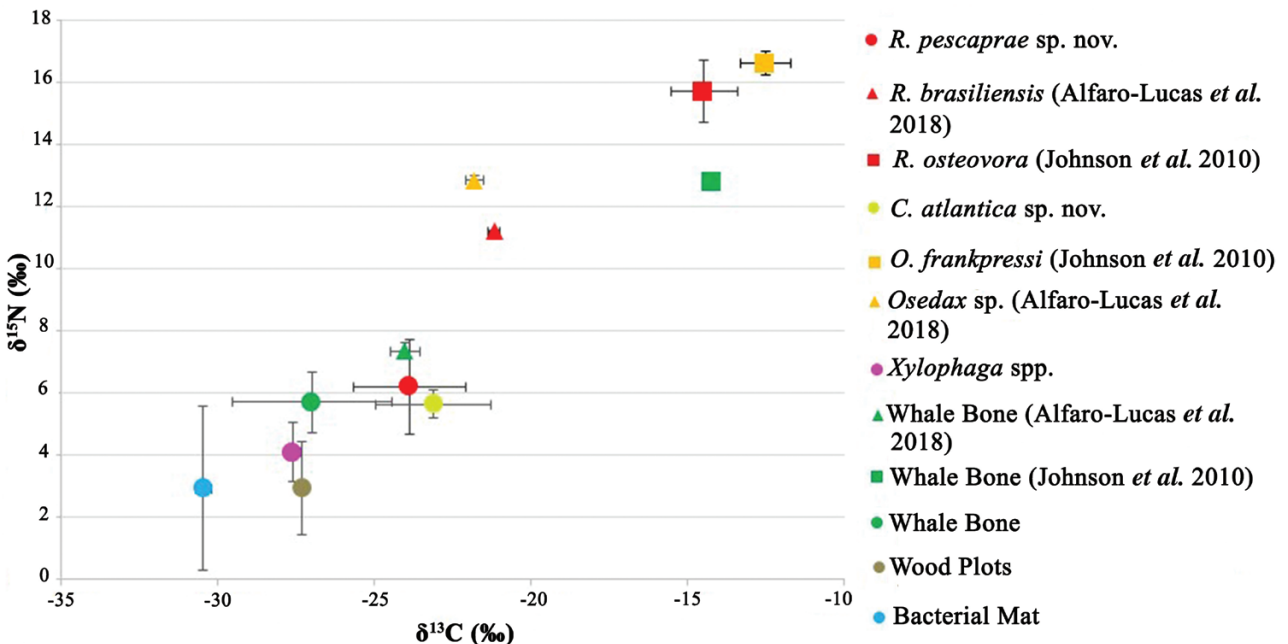


Figure 4. Stable ^{13}C and ^{15}N isotope analysis for *Rubyspira pescaprae* and *Cordesia atlantica*, along possible food sources. Data of previous works were added for a better view of bone specialists expected enrichment (Johnson et al., 2010; Alfaro-Lucas et al., 2018).

Paratypes: #1 ZUEC-GAS 7914, sta. same as holotype (total of 23 specimens, three of them in Fig. 5B–C, F, M, N); #2 ZUEC-GAS 7915, sta. ES1500 (21°27'00.5"S, 39°53'47.4"W) off Espírito Santo State, May 2015, depth 1491 m (two specimens, one of them in Fig. 5G, H); #3 ZUEC-GAS 7916, sta. ES3300 (22°50'27.1"S, 38°24'58.8"W) off Espírito Santo State, May 2015, depth 3322 m (six specimens); #4 ZUEC-GAS 7917, sta. SP1500 (25°53'38.4"S, 45°02'05.4"W) off São Paulo State, October 2014, depth 1508 m (11 specimens); #5 MZUSP 151714, sta. same as holotype (8 specimens); #6 MNRJ 23581, sta. same as holotype (six specimens).

Other materials examined: c. 880 specimens stored at ColBIO (Coleção Biológica Prof. Edmundo F. Nonato) at IOUSP. Collected in all sites of the present study. 25°54'S, 45°01.8'W, 1508 m depth (SP1500), October 2014, 21°27'S, 39°54'W, 1491 m depth (ES1500); 22°50.4'S, 38°25.2'W, 3322 m depth (ES3300); 25°20.4'S, 39°38.4'W, 3285 m depth (RJ3300); 28°01.8'S, 43°31.8'W, 3358 m depth (SP3300), May/June 2015.

Etymology: From *pes* (Latin), foot, and *capra* (Latin), goat. It refers to the similarity between the radular marginal teeth and a crowbar, which in Portuguese is called 'pé de cabra', 'goat foot'. Used as a feminine adjective.

Diagnosis: Teleoconch tall, multispiral, with well-marked, sulcate suture; surface creamy white, smooth except basally where it is covered by flat, shallow, spiral ridges; periostracum thin, translucent, forming fine spiral lines. Protoconch whitish, with 2.5 whorls, with a cancellate sculpture. Radula taenioglossate (formula 2 + 1 + C + 1 + 2), with a central tooth bearing a smooth, pointed cusp, two hook-like laterals bearing small rounded denticles, and marginal teeth slender and incurved, with blunt, bifid cusp like a crowbar.

Shell: Adult shell large, thick, tall, width about 45% of height; up to 28.9 mm in height and 13.0 mm in width (measurements of the holotype). Multispiral, with teleoconch formed by up to about seven whorls, each one with a slightly and uniformly rounded profile and delimited by a well-marked, shallow sulcate suture; body whorl about half of shell height (Fig. 5A–C, E, F, L, M). Umbilicus closed. Surface creamy white, often corroded, smooth except basally where about 18 flat, shallow spiral ridges are present and partially covered by the preceding whorl. Periostracum thin, translucent, forming fine, spiral lines (Fig. 5D). Aperture orthocone, nearly one-third of shell height in fully developed individuals (Fig. 5A) (a half in smaller ones: Fig. 5G–I, N), teardrop-shaped, with smooth lips, the outer thin, with a convex and uniformly rounded profile, vertical

in position in its median portion; inner lip sinuous. Siphonal and anal canal absent. Apex often eroded, without the protoconch and first teleoconch whorls, sealed with a calcareous plug. Protoconch whitish (Fig. 5G, H), with 2.5 whorls (Fig. 5J), maximum diameter 0.54 mm, with a cancellate sculpture composed by both distinct, well-defined spiral and axial cords (Fig. 5G–K), the former ones absent in the upper-third of each whorl (Fig. 5K); transition between the proto- and teleoconch well defined (Fig. 5J). Operculum horny, thin, yellowish brown, paucispiral, with distinct growth lines and nucleus 20–25% height, width two-thirds the height of the operculum (Fig. 5O); small relative to the size of the aperture.

Radula: About 1.4 mm long; length five times as broad in adult specimens (Figs. 6A, B). Taenioglossate, formula 2 + 1 + C + 1 + 2 (Fig. 6C). Central tooth with quadrate base and triangular, pointed cusp, smooth (Fig. 6C, D). Lateral teeth narrower but longer than central, hook-like, with incurved cusp, bearing small rounded denticles in both edges (Figs. 6C, D). Both marginal teeth with same size and shape, slender and incurved, with blunt, bifid cusp like a crowbar; small rounded denticles may be present (Fig. 6E).

Soft parts: The head has a large, broad and flat snout bearing a ventro apical mouth and large cephalic tentacles without eye lobes (Fig. 7A). The foot is relatively small as compared to the snout, and has a furrow separating an anterior propodium from the posterior metapodium (Fig. 7A, B). The simple mantle edge bears one large pallial tentacle on the right side of the snout, and one smaller on the left corner of the mantle; in most specimens, these tentacles are strongly contracted (Fig. 7B, C). Male aphyllid.

Remarks: Specimens of *R. pescaprae* mostly resemble *R. osteovora* regarding the shell shape and radula. However, in the former, the spiral sculpture is smoother. Moreover, the marginal teeth of *R. pescaprae* have a crowbar-like distal end, while *R. osteovora* bears rounded, marginal teeth. Juvenile specimens of both *R. pescaprae* and *R. elongata* are easily confused; larger adult specimens are easily distinguished by the deeper suture and the more rounded profile of the adult shell whorls, characteristics that are only present in *R. pescaprae*. Traits of the larval shell, such as the numbers of whorls and strong axial and spiral sculpture, can possibly indicate a planktotrophic development, characteristic for other species of this genus.

Rubyspira brasiliensis and *R. goffrediae* have a more globose shell and so are distinguished from *R. pescaprae*, in which it is taller. As highlighted by

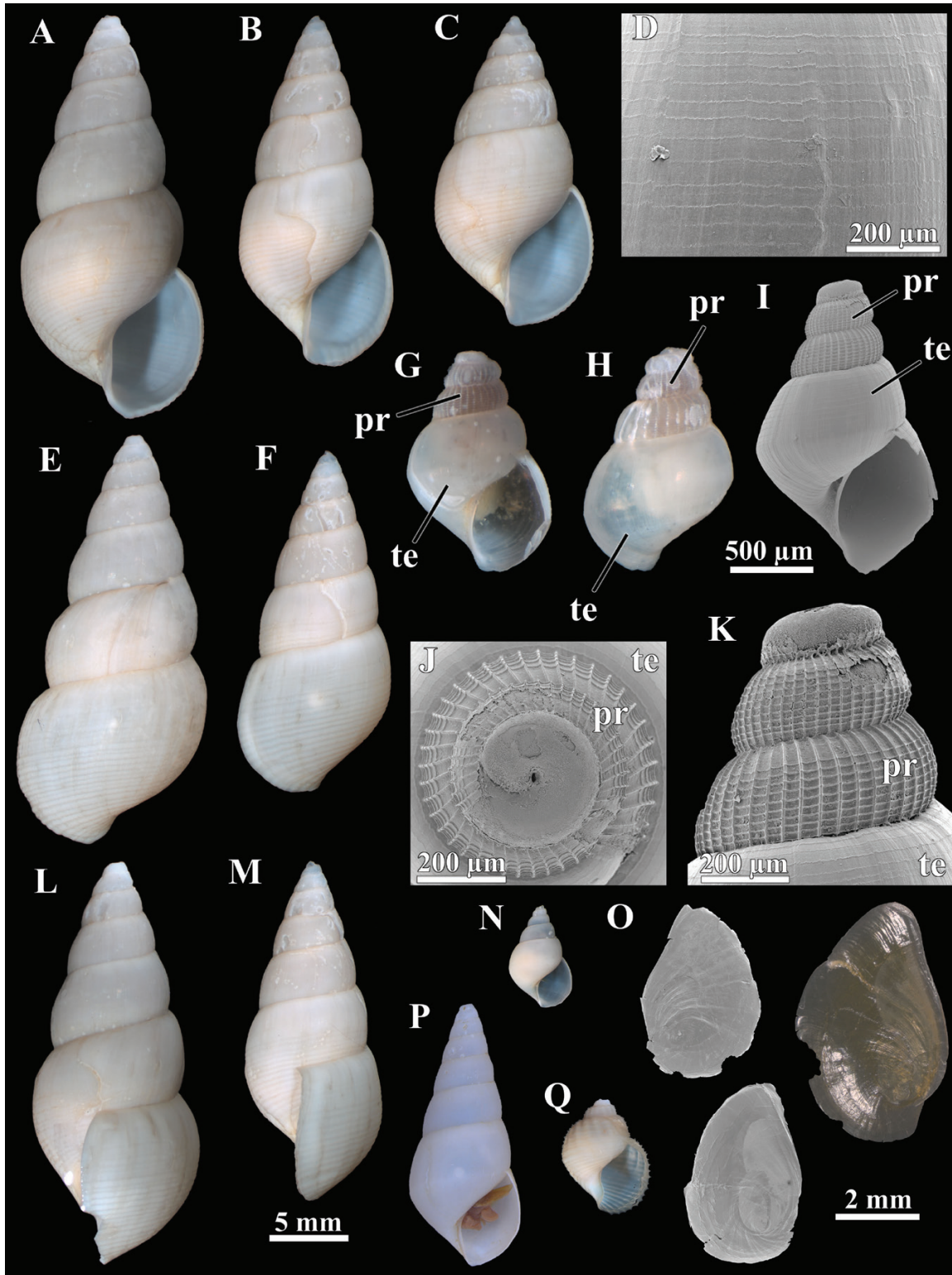


Figure 5. *Rubyspira pescaprae* (except P and Q). Shell features, as viewed by the stereomicroscope (A–C, E–H, L–O) and SEM (D, I–K). In the same scale, the holotype is shown in A, E, L, and four paratypes in B, F, M (all these three from the same individual), C, G, H (these two from the same individual) and N. D, a detail view of the teleoconch surface shows the periostracum forming spiral lines. I, a small specimen (as in G and H; these three shells are in the same scale) was sorted

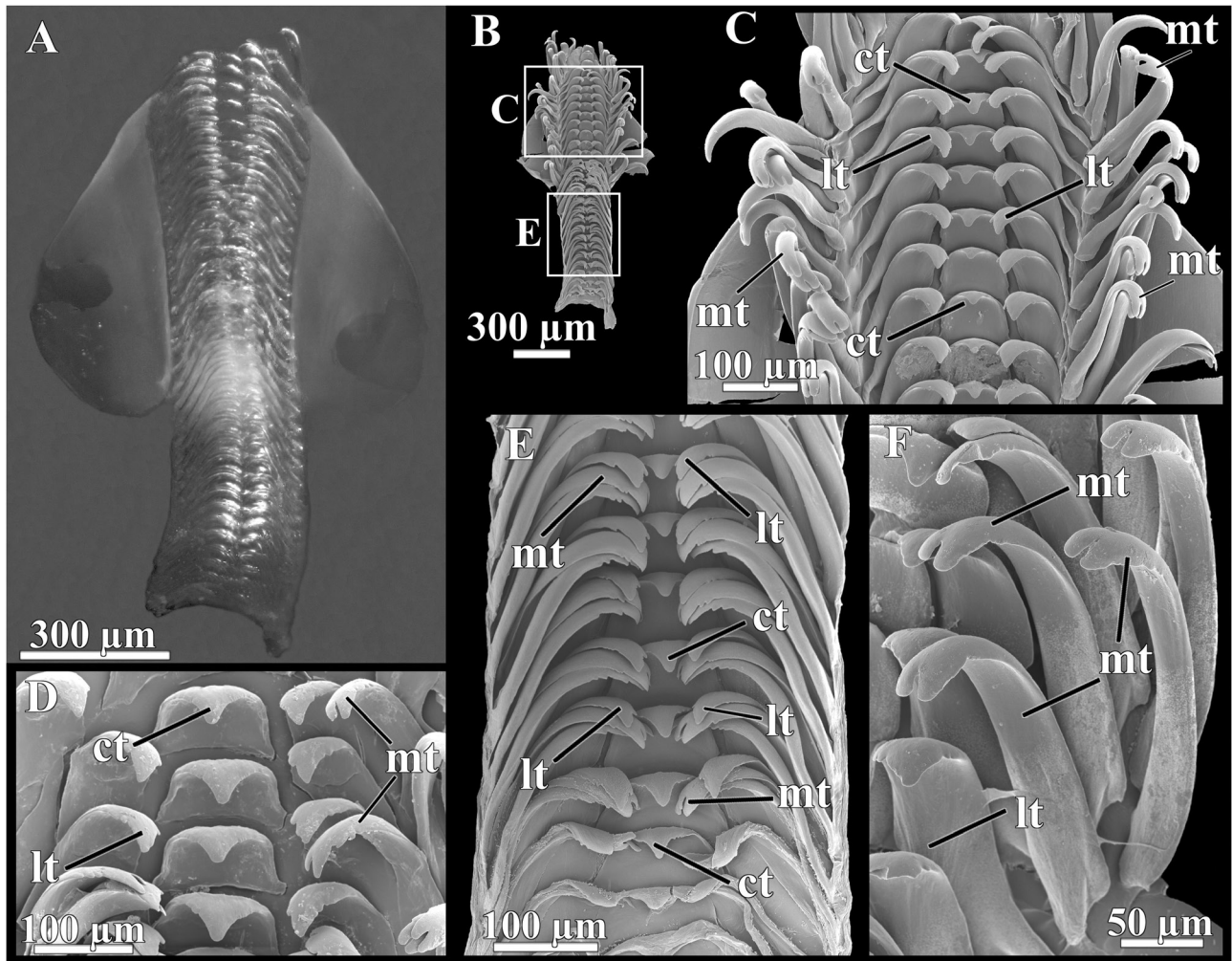


Figure 6. *Rubyspira pescaprae*. The radula, as viewed by the stereomicroscope (A) and SEM (B–F). It is complete in A and B. B, the boxes highlight two different parts of the radula, which are shown in detail in C and D. The teeth are viewed spread apart in C, and shrunken in D. The central and lateral teeth are shown in more detail in E, and the marginals in F. ct, central tooth; lt, lateral tooth; mt, marginal tooth.

Hasegawa *et al.* (2019), all the teeth of the radula of *R. brasiliensis* are smooth, and so distinct from the ones of *R. pescaprae*. The bifid cusp of the marginals is characteristic of *R. pescaprae* new species, being distinctive from all other *Rubyspira* species.

Intraspecific divergences were lower than 1% and around 2% for COI and 16S, respectively. The lowest interspecific COI divergence of *R. pescaprae* was against *R. osteovora* (4.8%) and the highest was with *R. goffrediae* (16%). *Rubyspira pescaprae* is also less

distant to *R. osteovora* on 16S, with 2.9% divergence from each other, while the most distant is *R. goffrediae* (~10%) (Table 2).

Distribution: Only known from the studied area, the Brazilian south-eastern coast (south-west Atlantic); 1491 to 3358 m depth. This new species was abundant in all sites with different sizes and developmental stages, except in SP3300, where only four juvenile specimens were collected. Most animals were found

to observe a well-preserved protoconch, which is also shown in detail in apical (J) and lateral views (K). J, the protoconch can be observed as having about 2.5 whorls; the first whorl is eroded and then its cancellate sculpture is not preserved. O, operculum: external view in the upper left, and internal view in the other two. P, Q, the holotypes of *Rubyspira elongata* and *Cordesia atlantica* are showed in the scale as in A–C, E, F and L–N (the scale is shown below M). pr, protoconch; te, teleoconch.

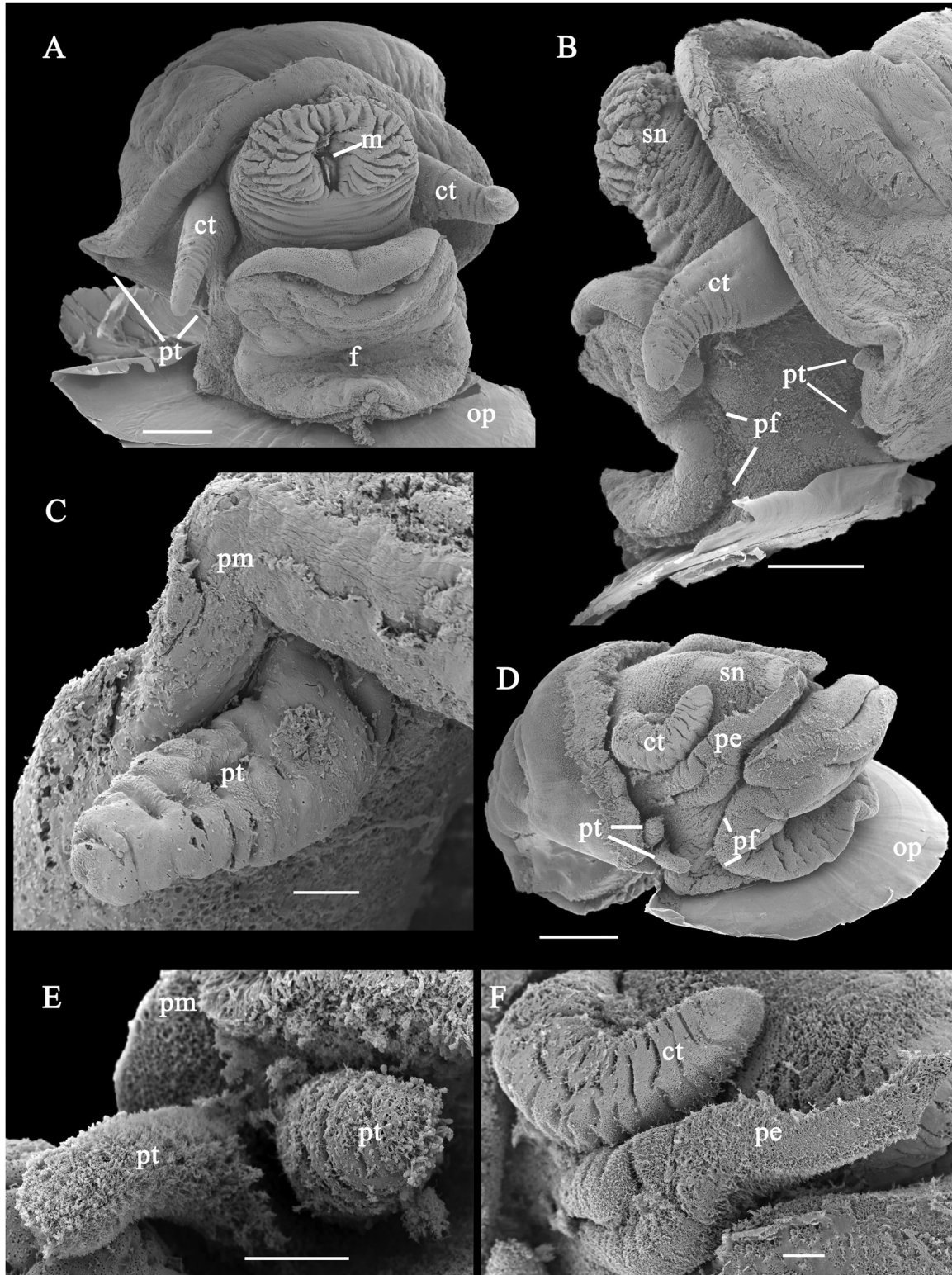


Figure 7. Scanning electron microscopy of critical dried soft parts. (A, B, C) *Rubyspira pescaprae*: A, head-foot, frontal view; B, head-foot and mantle, right side; C, right corner of pallial margin with detail of bigger pallial tentacle. D, E, F, *Cordesia atlantica*: D, head-foot, right side; E, right corner of pallial margin, detailed view of both pallial tentacles; F, detailed view of cephalic tentacle and penis. Ct: cephalic tentacle; m: mou th; op: operculum; pe: penis; pf: pedal furrow; pm: pallial margin; pt: pallial tentacle; sn: snout. Scale bars are 500 μm for A, B, D and 100 μm for C, E, F.

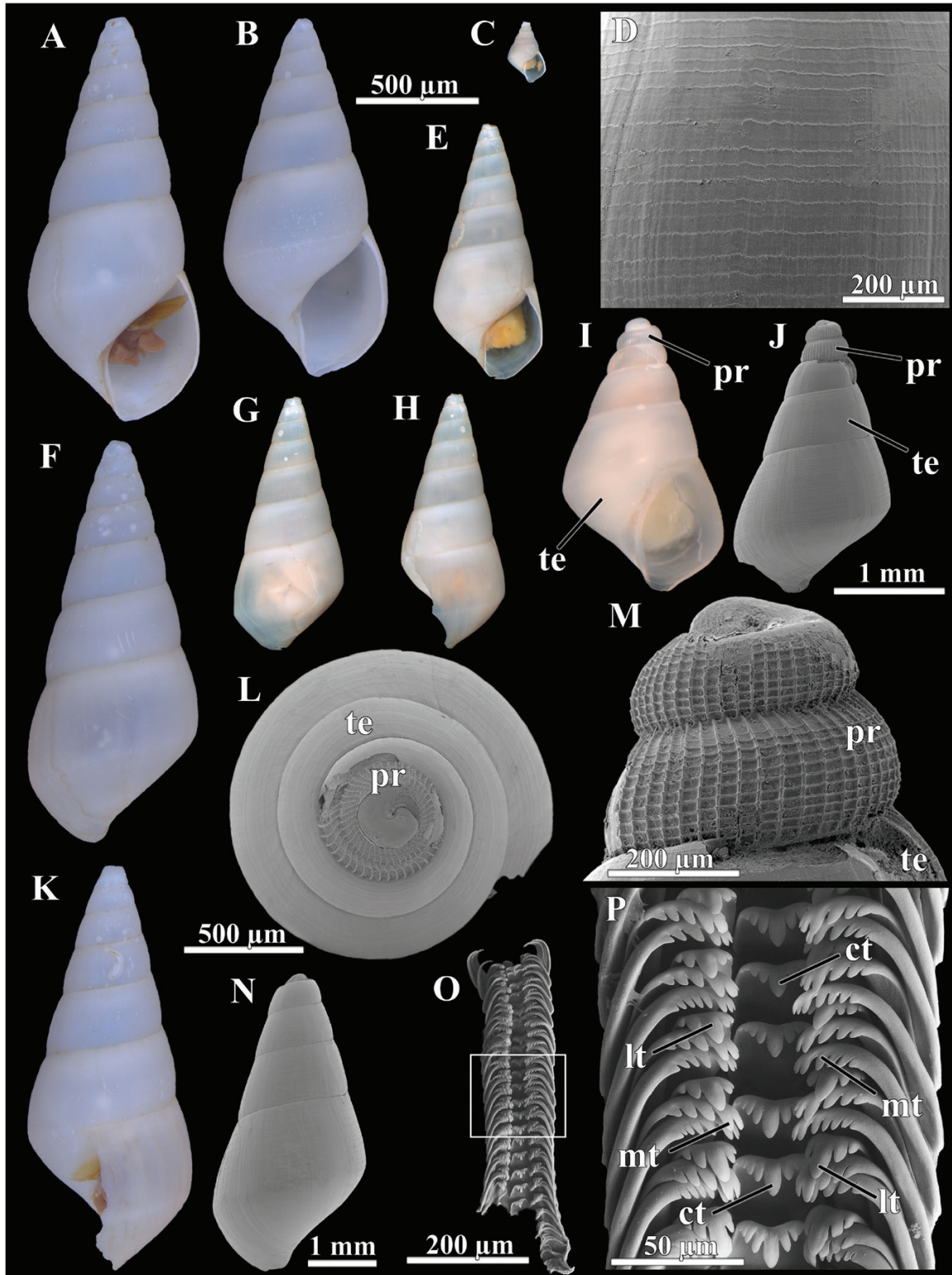


Figure 8. *Rubyspira elongata*. Shell and radula features, as viewed by the stereomicroscope (A–C, E–I, K) and SEM (D, J, L–P). In the same scale, the holotype is shown in A, F, K, and three paratypes in B, C, E, G and H (these latter three from the same individual). D, a detail view of the teleoconch surface shows the periostracum forming spiral lines. I, J, a small specimen (as in N) was sorted to observe a well-preserved protoconch, which is also shown in detail in apical (L) and lateral

a straight profile in its upper, almost vertical half, shouldered in the confluence with the lower, straight half; inner lip formed by two straight upper and lower parts, the latter one vertical. Siphonal and anal canal absent. Apex often eroded, without the protoconch and first teleoconch whorls, sealed with a calcareous plug (Fig. 8N). Protoconch whitish (Fig. 8C, I), with 2.5 whorls (Fig. 8L), maximum diameter 0.53 mm, with a cancellate sculpture composed by both distinct, well-defined spiral and axial cords (Fig. 8I, J, L, M), the former ones absent in the upper-fourth of each whorl (Fig. 8M); transition between the proto- and teleoconch well defined (Fig. 8J, L, M). Operculum horny, thin, yellowish brown, paucispiral, with distinct growth lines and nucleus 35–40% height, width two-thirds the height; small relative to the size of the aperture.

Radula: About 0.5 mm long; length 5.5 times as broad in adult specimens (Fig. 8O). Taenioglossan, formula $2 + 1 + C + 1 + 2$ (Fig. 8O, P). Central, lateral and marginal stout teeth, all with rounded denticles on cusps, larger in middle, decreasing in periphery (Fig. 8P). Central tooth with quadrate base and triangular; lateral narrower. Both marginal teeth with same size and shape, longer and narrower than the lateral tooth.

Soft parts: Ventrally-apical mouth, flat and small snout. Cephalic tentacles without eye lobes. Males aphylic. One large, pallial tentacle on the right and a smaller one on the left corner of the pallial margin near the end of the mantle.

Remarks: The shell of *Rubyspira elongata* resembles that of *R. pescaprae* and *R. osteovora*, but it is much more slender, bearing a more plane suture and smooth surface. This tall shell is easily distinguishable from the more globose ones of *R. brasiliensis* and *R. goffredie*. The juveniles resemble those of *R. pescaprae*, and the same traits that differentiate adult shell morphology is the best way to sort them. *Rubyspira elongata* radula markedly differs from other species of *Rubyspira*, mainly regarding its rounded denticles, which are present in all teeth. The larval shell morphology, with many whorls and a well-marked axial and spiral sculpture, suggest a planktotrophic development. Only few supposed fully grown specimens of *R. elongata* were found, most collected specimens were juveniles.

The intraspecific divergence of *R. elongata* is lower than 0.5% for *COI* and 16S. Considering other

Rubyspira species, *R. elongata* was least distant from *R. osteovora* (lower than 6%) and *R. pescaprae* (3%), for *COI* and 16S, respectively, while the most divergent species was *R. goffredie* for both genes (*COI*: 14.5%; 16S: 10%) (Table 2).

Distribution: Only known from the studied area, off the south-eastern coast of Brazil (south-west Atlantic); at 1491 m deep. Most collected specimens were juveniles and all the animals came from the same site, found both on whale bones (~50%) and wood parcels (~50%).

FAMILY ABYSOCHRYSIDAE TOMLIN, 1927

Diagnosis (emended): Gastropods of this family present turreted or globose shells, with prominent axial ribs and covered with a brownish periostracum. Lack of eye globe and two annulated, pallial tentacles that arise from the mantle. Radula is taenioglossate, with central teeth with multiple or a single cusp and a robust lateral tooth.

Remarks: Abysochrysidae snails are known for inhabiting deep-sea environments (Tomlin, 1927; Houbrick, 1979; Bouchet, 1991; Killeen & Oliver, 2000). The relation to other caenogastropods is uncertain, but they share similarities with Provannidae (superfamily Abysochrysoidea), such as the annulated, pallial tentacles and internal anatomy (Warén & Ponder, 1991). Molecular reconstructions put Littorinidae as a sister-group of the clade Abysochrysoidea (Colgan *et al.*, 2007; and after Johnson *et al.*, 2010).

Formerly, only the genus *Abysochrysos* was included in the family Abysochrysidae, presenting six known species with deposit-feeding behaviour, carnivory and parasitism (Houbrick, 1979; Warén & Bouchet, 2009). In this study, we consider *Cordesia* as part of this family based on molecular and morphological data obtained in this work (see further below), adding two more species to the group, one of them being described as follows.

GENUS *CORDESIA* WARÉN & BOUCHET, 2009

Type species: *Cordesia provannoides* Warén & Bouchet, 2009 (by original designation).

Diagnosis: Males of this genus possess a cylindrical penis. Protoconch sealed with a calcareous plug and

views (M). L, the protoconch can be observed as having about 2.5 whorls; the first whorl is eroded and then its cancellate sculpture is not preserved. The whole radula is shown in O, where the box indicates the position of the detailed view in P. A–C, E, F–H and K are in the same scale (shown in between B, C and E). ct, central tooth; lt, lateral tooth; mt, marginal tooth; pr, protoconch; te, teleoconch.

with planktotrophic larval development traits. Two right pallial tentacles. Radula with long and slender marginal teeth that resemble those of *Provanna*. *Cordesia* species have planktotrophic development that could be observed by many larval shell whorls and a complex sculpture on protoconch. These deep-sea gastropods are associated with chemosynthetic environments, such as cold seeps and organic falls.

***CORDESIA ATLANTICA* SP. NOV.**

(Figs 9, 10)

LSID: zoobank.org:act:AEB78F45-7D70-462E-BE65-D234D850DFD4.

Type locality: Off Rio de Janeiro, Brazil, 3285 m, 25°20'18"S, 39°38'28.3"W, on and around organic falls (whale bones and wood parcels).

Holotype: ZUEC-GAS 7920 (Fig. 9A, D), sta. RJ3300 (25°20'18"S, 39°38'28.3"W) off Rio de Janeiro State, Brazil – 'BioSuOr Project' coll., 23 May 2015, depth 3285 m. Measurements: shell height: 7.05 mm; shell width: 4.9 mm.

Paratypes: #1 ZUEC-GAS 7921, sta. same as holotype (total of 17 specimens, two of them in Fig. 9B, E); #2 ZUEC-GAS 7922, sta. SP3300 (28°01'42.4"S, 43°31'46.8"W) off São Paulo State, May 2015, depth 3358 m (total of five specimens, one of them in Fig. 9F); #3 ZUEC-GAS 7923, sta. ES3300 (22°50'27.1"S, 38°24'58.8"W) off Espírito Santo State, May 2015, depth 3322 m (six specimens); #4 MZUSP 151716, sta. SP3300 (28°01'42.4"S, 43°31'46.8"W), May 2015, depth 3358 m (five specimens); #5 MZUSP 151718, sta. ES3300 (22°50'27.1"S, 38°24'58.8"W) off Espírito Santo State, May 2015, depth 3322 m (six specimens); #6 MNRJ 23583, sta. ES3300 (22°50'27.1"S, 38°24'58.8"W) off Espírito Santo State, May 2015, depth 3322 m (six specimens).

Other materials examined: c. 380 specimens stored at ColBIO (Coleção Biológica Prof. Edmundo F. Nonato) at IOUSP. Collected in 22°50.4'S, 38°25.2'W, 3322 m depth (ES-3300); 25°20.4'S, 39°38.4'W, 3285 m depth (RJ-3300); 28°01.8'S, 43°31.8'W, 3358 m depth (SP-3300), May/June 2015.

Etymology: Named after the Atlantic Ocean where this species was found. Used as a feminine adjective.

Diagnosis: Shell thin, short and broad, with teleoconch formed by up to three whorls bearing a uniformly rounded profile, delimited by shallow sulcate suture; surface brownish, sculptured by seven to eight spiral

cords at the end of penultimate whorl and nine to ten more well-defined ones on the basal surface of the body whorl; 22 broad axial ribs present in the upper part of body whorl; periostracum thin, translucent, forming small bristles and axial fine lamellae; broad aperture with a narrow and short siphonal channel. Protoconch whitish, with two whorls, with a cancellate sculpture. Radula taenioglossate (formula $2 + 1 + C + 1 + 2$), with a central tooth triangular-shaped and a pointed apical part set off, laterals with apical cusp bearing one or two lateral denticles and another strong denticle well below, and marginals with apical and lateral denticles, larger and less abundant in the inner marginal.

Shell: Adult shell of medium size, thin, short and broad, width about 70% of height; up to 7.05 mm in height and 4.9 mm in width (measurements of the holotype). Teleoconch formed by up to three whorls of rapid increasing diameter, each one with uniformly rounded profile, delimited by a well-marked, shallow sulcate suture; body whorl about 80% of shell height (Fig. 9A, B, E, F, I). Umbilicus closed. Surface brownish, sculptured by seven to eight spiral cords at the end of the penultimate whorl and nine to ten a little bit more well-defined ones on the basal surface of the body whorl; in the upper part of body whorl these spiral cords are intersected to right angle by about 22 broad axial ribs. Periostracum thin, translucent, forming small bristles over the spiral sculpture and axial fine lamellae (Fig. 9C). Opisthocline, broad aperture, about 60% of shell height, with a distinct but narrow and short siphonal channel; outer lip thin, ornamented by the periostracum bristles, with a convex and uniformly rounded profile in larger animals, slightly expanded in lower part (Fig. 9A, B, E) (in some smaller ones, almost straight in the upper portion and slightly shouldered posteriorly; Fig. 9F); inner lip sinuous. Columella marked by a shallow furrow, curved to the right and forwards anteriorly. Anal canal absent. Apex often eroded, without the protoconch and first teleoconch whorls, sealed with a calcareous plug. Protoconch whitish (Fig. 9F), with two whorls, with a cancellate sculpture composed by both distinct, well-defined spiral and axial cords (Figs. 9H, J, K, M), the former ones absent in the upper fourth of each whorl (Fig. 9M); transition between the proto- and teleoconch well defined. Operculum horny, thin, yellowish brown, paucispiral, with distinct growth lines and nucleus 25% height, width two-thirds the height of the operculum (Fig. 9N); small relative to the size of the aperture. Specimens with 1.6 mm to 7.2 mm in shell height and 1.1 mm to 4.9 mm in width.

Radula: About 1.8 mm long; narrow, length 12 times as broad in adult specimens (Fig. 10A). Taenioglossate, formula $2 + 1 + C + 1 + 2$ (Fig. 10B, C). Central tooth

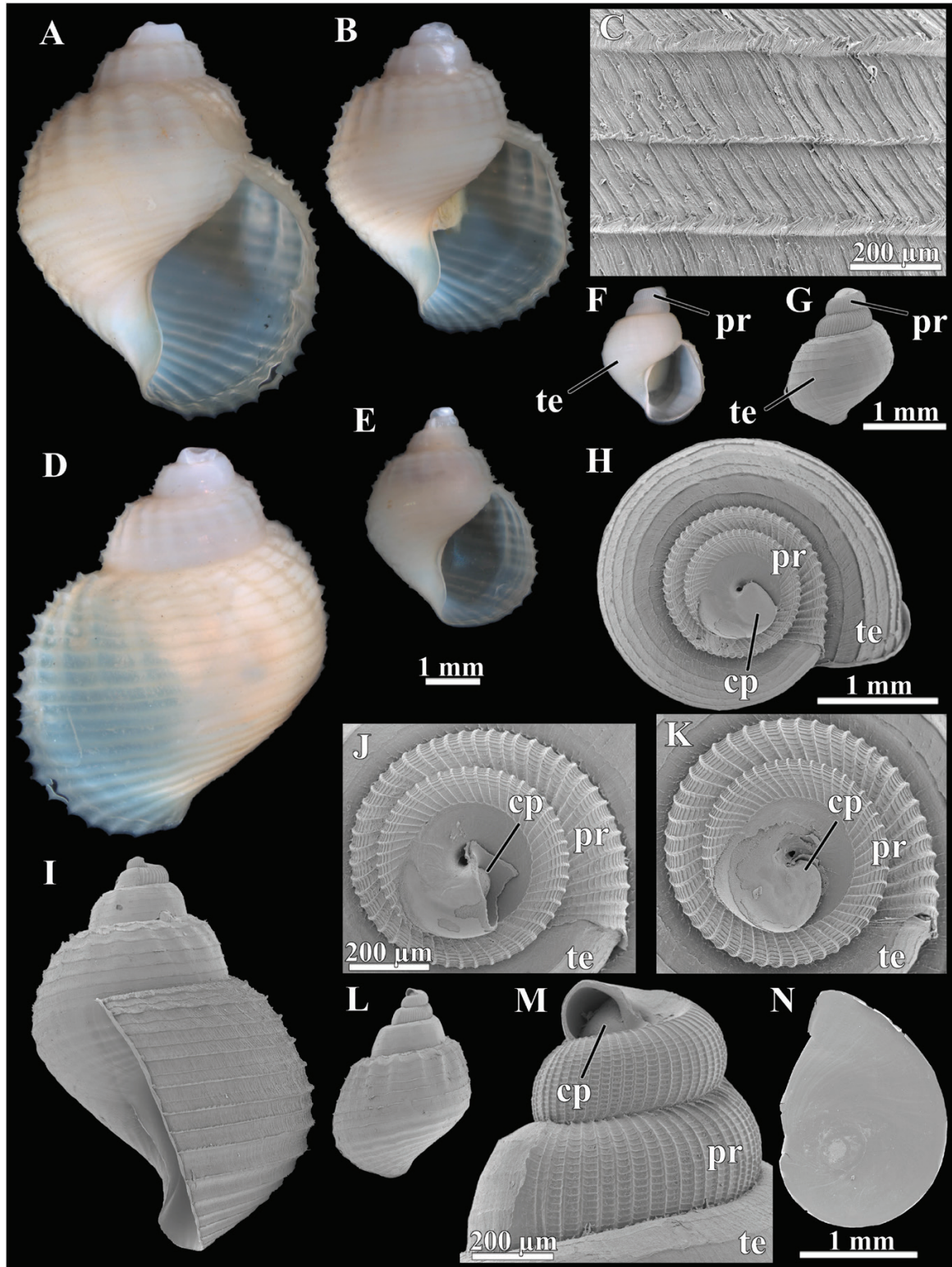


Figure 9. *Cordesia atlantica*. Shell features, as viewed by the stereomicroscope (A, B, D–F) and SEM (C, G–N). In the same scale, the holotype is shown in A and D, and three paratypes in B, E, and F. C, a detail view of the teleoconch surface shows the periostracum forming spiral bristles and axial fine lamellae. F, G, two small specimens are observed in the same scale, showing the protoconch and the first whorls of the teleoconch. H, J, K, the calcareous plug is viewed replacing the

with expanded base, triangular-shaped; apical part set off, pointed (Fig. 10C, D). Lateral tooth robust, with apical cusp bearing one strong central denticle and one or two lateral denticles on outer side (Figs. 10E, F); another strong denticle is also present well below. Both marginal teeth long, with apical and lateral denticles, larger and less abundant in the inner marginal (Figs. 10C, G).

Soft parts: Males possess a cylindrical penis, that goes slender on distal side (Fig. 7D, F). Two large, right, annulated pallial tentacles with almost similar sizes (Fig. 7D, E). Head-foot of normal size for the group, with a flat and large snout. Large and smooth cylindrical cephalic tentacles without eye-lobes (Fig. 7D). Ventro-apical mouth. Soft parts covered with small sensory cilia.

Remarks: The shell and the radula of *C. atlantica* are similar to those of *C. provannoides*, which was described based on three specimens. The holotype of *C. provannoides* appears to be worn, and its shell looks a little bit narrower (Warén & Bouchet, 2009: fig. 9E) than that of the new species and the siphonal channel is slightly wider in the latter than in the former. The same arrangement of pallial tentacles is observed on both *Cordesia* species, but both are of the same size on *C. atlantica*, while one is much smaller in *C. provannoides*. The penis is also cylindrical, but it becomes thinner in the new species. More precise comparisons among these species are necessary, this pending on the availability of well-preserved specimens of *C. provannoides*.

Intraspecific divergences of *Cordesia atlantica* are lower than 2% for 16S and around 1% for COI. There are no molecular data for *C. provannoides* to compare with the new species. For COI, *C. atlantica* is less distant from the genus *Abyssochrysos* than other genera (13.4% mean distance) and for 16S it is closer to *Rubyspira* species (13.2% mean distance) (Table 2).

Distribution: Only known from the sites of this study. South-west Atlantic deep-sea, in organic falls from 3285 to 3358 m. Most individuals were collected on the control substrate (rubber mat) (~44%), but they were also abundant on wood (~39%) and, less frequently, on whale bones (17%). It was the first time *Cordesia* was found on organic falls. The presence of gastropods of different sizes and stages of development indicates a probable recruitment in the South-west Atlantic

deep-sea throughout the year. The high number of whorls, axial and spiral sculptures on the larval shell suggests a planktotrophic development, like that of *C. provannoides*.

DISCUSSION

The first two species of *Rubyspira* were found in the Monterey Canyon, California, living on a grey whale carcass at 2893 m depth (Johnson *et al.*, 2010). Later, a third one was recovered from the south-west Atlantic at 4204 m depth (Sumida *et al.*, 2016; Hasegawa *et al.*, 2019). The two new species increase the number of *Rubyspira* species in the south-west Atlantic to three, surpassing the amount of species at the type locality of the genus. It is also the shallowest site where *Rubyspira* species were collected (1491 m depth at ES1500). The presence of specimens of *R. pescaprae* at different stages of development and size suggests a continuous recruitment, but it is not clear if this is from different larval pools or autorecruitment of the established assemblage. There were only juveniles and in low abundance on SP3300, which was probably due to the influence of a distinct colder water mass (AABW) from the other deeper sites (RJ3300 and ES3300), which were bathed by mixed waters (AABW + NADW). The distribution of the bone-eating worm *Osedax* also seems to be influenced by the water masses and their direction flow in south-west Atlantic (Shimabukuro & Sumida, 2019). *Rubyspira elongata* was a much rarer species and it was only found at ES1500, one of the shallower sites. Besides, only few adult specimens were collected, suggesting a later recruitment. Both new *Rubyspira* species present the expected arrangement of pallial tentacles of the genus, as well as shell traits, pedal furrow, no siphonal canal and planktotrophic development (Table 3).

For the first time *Cordesia* was found outside the type locality and from organic falls. The type species, *C. provannoides*, was previously described from a hydrocarbon seep off West Africa, Congo, at 3150 m depth (Warén & Bouchet, 2009). *Abyssochrysoidea* snails are known for inhabiting mainly chemosynthetic environments, but, formerly, only the genera *Provanna* and *Desbruyereia* were found in other habitats. *Provanna* inhabits vents, seeps and organic falls (Warén & Ponder, 1991; Sasaki *et al.*, 2008; Lundsten *et al.*, 2010a; Sasaki *et al.*, 2010; Chen *et al.*, 2016a; Sasaki *et al.*, 2016), whereas *Desbruyeresia* was

most beginning eroded part of the protoconch; J, K, are in the same scale, and show different phases of erosion, with the calcareous plug just beginning to be exposed in J. L, a small specimen is observed through its abapertural view, and its protoconch is viewed in detail in M. N, operculum in external view. A, B, D, E, I and L are in the same scale (shown below E). cp, calcareous plug; pr, protoconch; te, teleoconch.

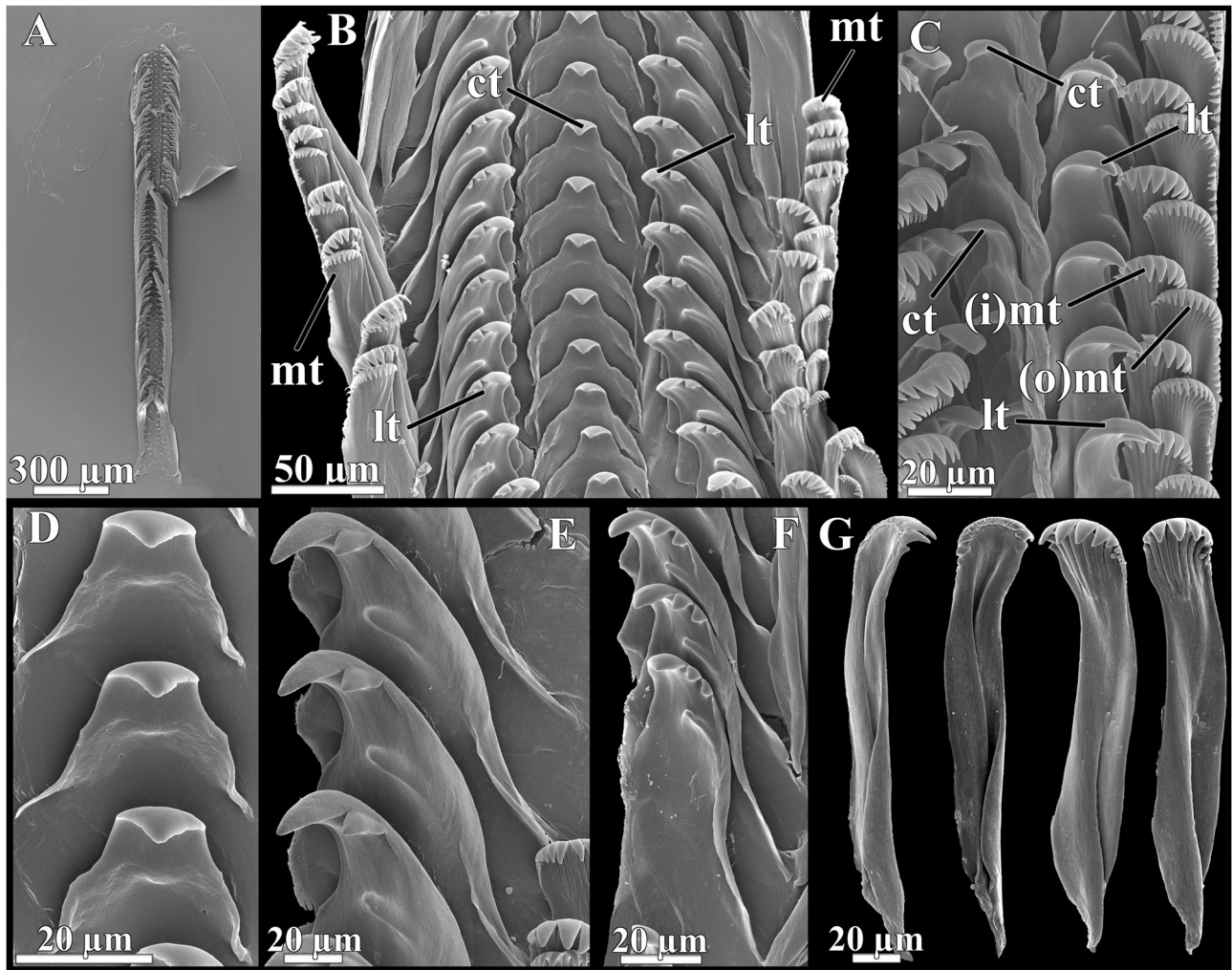


Figure 10. *Cordesia atlantica*. The radula as viewed by the SEM: A, it is complete; B, a part of it is shown, with the teeth spread apart, also in detail in C; D, central teeth; E, F, lateral teeth, with one and two lateral denticles, respectively; G, marginal teeth. ct, central tooth; lt, lateral tooth; (i)(o) mt, (inner or outer) marginal tooth.

collected only in seeps and vents (Warén & Bouchet, 1993; Chen *et al.*, 2016b, 2019). *Cordesia* is the first Abyssochrysoidea that dwells both on food falls and cold seeps, but not on hydrothermal vents (such as *Provanna* that is present on these three chemosynthetic habitats) (Table 3). This may be related to a relatively low tolerance to reducing environments compared to other snails of this superfamily or because there are still other animals of this group to be found in vents.

Regarding the trophic preferences, *R. pescaprae* and *R. elongata* rely on whale bones. According to radular morphology, *R. goffrediae* scrapes fragments directly from whale bones, while *R. osteovora* and *R. brasiliensis* eat bone-enriched sediments around whale falls (Johnson *et al.*, 2010; Sumida *et al.*, 2016; Hasegawa *et al.*, 2019). *Rubyspira pescaprae* presented large fragments of whale bones in its stomach, which can

indicate straight use of bones, but its radula and body size resemble those found on *R. osteovora*. Moreover, sediment was also found alongside the large bone fragments. Only a few (~10%) and small individuals were collected on wood plots and inert material. Even though no *in situ* observations were made in this study, these morphological traits and distribution may indicate that this new species may have both feeding behaviours during its life: eating enriched sediment when juvenile and then bones when adult. In contrast, *R. elongate* has a smaller radula relative to its size and also had smaller bone fragments in its digestive tract. It was equally collected on whale bones and woods, probably indicating a dietary reliance on enriched sediment around bones.

The direct observation of *C. atlantica* gut contents showed a mix of sediment and small pieces of

Table 3. Ecological and morphological features of Abyssochrysoidea known genera investigated in this study

Classification	Habitat	Feeding Strategies	Morphological Traits				
			Radula	Larval Shell	Pallial Tentacles	Penis	Pedal Furrow
Provannidae							
<i>Provanna</i>	Hydrothermal vents, cold seeps and organic falls	Grazers	Marginal teeth long and flat with many denticles, lateral teeth with 2–5 denticles, triangular central tooth	Lecithotrophic development traits	One pallial tentacle	Absent	Absent
<i>Ifremeria</i>	Hydrothermal vents	Endosymbiosis	Marginal teeth long and flat with many denticles, lateral teeth with 2–5 denticles, triangular central tooth	Planktotrophic development traits	Absent	Absent	Absent
<i>Alviniconcha</i>	Hydrothermal vents	Endosymbiosis	Marginal teeth long and flat with many denticles, lateral teeth with 2–5 denticles, triangular central tooth	Planktotrophic development traits	Absent	Absent	Absent
<i>Desbruyeresia</i>	Hydrothermal vents and cold seeps	Grazers	Marginal teeth long and flat with many denticles, lateral teeth with 2–5 denticles, triangular central tooth	Planktotrophic development traits	One pallial tentacle	Absent	Absent
Not settled							
<i>Rubyspira</i>	Organic falls	Bone eaters	Diverse features, simplified but strong teeth	Planktotrophic development traits	Two pallial tentacles, one smaller	Absent	Present
Abyssochrysidae							
<i>Cordesia</i>	Cold seeps and organic falls	Carnivorous and grazers	Marginal teeth long and flat with many denticles, lateral teeth with 2–5 denticles, triangular central tooth	Planktotrophic development traits	Two pallial tentacles; same size or one smaller	Present	Present
<i>Abyssochrysos</i>	Deep sea, but not on chemosynthetic environments	Carnivorous and parasitic	Strong marginal and lateral teeth with denticles, central tooth half-moon shaped	Lecithotrophic development traits	Two pallial tentacles; same size or one smaller	Present	Present

wood, which was expected since the genus was considered a sediment and bacterial mat grazer (Warén & Bouchet, 2009). However, foraminifera and juvenile bivalves of the wood-specialist family Xylophagaidae were found inside the digestive tract of *Cordesia*, also indicating a probable carnivorous behaviour. These Foraminifera may indicate a direct use of organic matter from sediment. Therefore, it is possible that *C. atlantica* relies on diverse food resources, facilitating this species to occur in wide habitat spectra.

Stable isotopes exhibit a large standard deviation in food source samples due to a pool of bacterial mats and a variety of wood and vertebrae, consequently it is also variable in organisms of this study. Nevertheless, the results confirm a dietary reliance mainly on whale bones for *R. pescaprae*, as also observed by Johnson *et al.* (2010) for *R. osteovora* and *R. goffrediae* and by Alfaro-Lucas *et al.* (2018) for *R. brasiliensis* (Fig. 4). For *C. atlantica*, stable isotopes indicated that the Xylophagaidae juvenile bivalves are possibly the main food source, suggesting a preference for a carnivorous diet rather than bacterivorous as previously stated by Warén & Bouchet (2009). This behaviour can only be associated with *Abyssochrysos* among Abyssochrysoidea. However, it is likely that these gastropods also rely on bacterial mats for nutrition, suggesting an omnivorous strategy. There is no isotopic evidence that *Cordesia atlantica* feeds on wood, even though few pieces of wood were observed in guts.

Phylogenetic analyses clearly indicate that *Cordesia* is more closely related to *Abyssochrysos* than any other provannid genera. Previous reconstructions showed that *Rubyspira* and *Abyssochrysos* are sister-clades (see also: Johnson *et al.*, 2010; Hasegawa *et al.*, 2019), but these analyses did not include molecular data of *Cordesia*, of which its position within Abyssochrysoidea was unknown until now. In this work, *Rubyspira* forms a strongly supported clade with *Cordesia* and *Abyssochrysos*, and this clade is closely related to *Desbruyeresia*. As reported in previous studies, *Provanna* appears to be the most basal genus of Abyssochrysoidea, but it is not strongly supported in this work (Johnson *et al.*, 2010; Chen *et al.*, 2016a, 2019).

As pointed out by Warén & Bouchet (2009), and in the present description, *Cordesia radula* and shell traits, such as tall and flat marginal teeth and the protoconch, are more similar to those of Provannidae. Nevertheless, the presence of a penis, a pedal furrow and the arrangement of pallial tentacles are only comparable to Abyssochrysoidea. Warén & Bouchet (2009) considered the presence of a penis a plesiomorphic feature in Provannidae to include *Cordesia* within this family, but lost in all other provannid genera. However, based on the new phylogenetic reconstruction, it

is more plausible that the presence of penis is an apomorphic trait in Abyssochrysoidea not present in other Abyssochrysoidea genera.

The similarities of feeding mode and habitat preference were also used to include *Cordesia* within Provannidae (Warén & Bouchet, 2009). The fact that species of *Abyssochrysos* are not known from chemosynthetic environments and are not grazers like provannids is relevant, but does not seem enough to define families. Besides, Provannidae genera seem to dwell on a variety of food sources. For example, *Ifremeria* and *Alviniconcha* have endosymbionts, while *Desbruyeresia* and *Provanna* are grazers and deposit feeders. In fact, our trophic analyses showed that *Cordesia* does not seem to be a grazer, but feeds on a wide variety of resources, including juvenile xylophagid bivalves. Considering the similarities between *Cordesia* and *Abyssochrysos*, and that both form a strongly supported clade, we regard the genus *Cordesia* as an Abyssochrysoidea (Table 3). Therefore, *Cordesia* is the first genus of Abyssochrysoidea found in chemosynthetic environments.

As observed here and by Johnson *et al.* (2010), *Rubyspira* exhibits morphological characteristics that resemble those of Abyssochrysoidea (i.e. pallial tentacle arrangement and pedal furrow), but males are aphylic (Table 3). Our phylogenetic reconstruction shows a moderate to strong support of *Rubyspira* + Abyssochrysoidea clade. However, *Rubyspira* is still not settled at family level due to the absence of a penis.

The phylogenetic analysis of Abyssochrysoidea presented in this study did not completely clarify the relationship of this superfamily, but it helps to elucidate the phylogenetic position of *Cordesia* based on new molecular data. It is clear that to resolve where *Rubyspira* should be inserted, a global study of Abyssochrysoidea, including a morphological review of all genera and a comprehensive molecular dataset, need to be conducted.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Table S1. Genbank accession numbers of dataset of *COI* and 16S used on molecular analyses in this study. Species without sequenced genes are expressed as '-'.
Table S2. Stable Isotopes analysis results of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with standard deviation for *Rubyspira pescaprae* and *Cordesia atlantica* along possible food sources.