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A new gastropod associated with a deep-sea whale carcass from São Paulo Ridge, Southwest Atlantic

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Abstract

An unfamiliar gastropod was collected from a deep-sea whale carcass at the base of the São Paulo Ridge in the Southwest Atlantic by the manned research submersible *Shinkai 6500*, and is here described as a new species of the abyssochrysoidean genus *Rubyspira*, *R. brasiliensis* **sp. nov.**, following morphological and molecular phylogenetic examinations. There are only two other known species in the genus, which occur together in the Monterey Submarine Canyon off California. The present new species was shown by the molecular analysis to be closer to one of the Californian species than the other. It was found aggregated on and around a whale carcass at a depth of 4204 m, which represents the deepest record of whale-fall ecosystems ever discovered.

Key word: taxonomy, Abyssochrysoidea, new species, Gastropoda, *Rubyspira*, shell morphology, external anatomy

Introduction

During the *Quelle 2013* Expedition, an around-the-world research expedition conducted by the Japan Agency for Marine-Earth Science & Technology (JAMSTEC) using the manned research submersible *Shinkai 6500*, several dives were carried out in the Southwest Atlantic off Brazil. A sunken whale carcass was discovered there on April 24, 2013 at a depth of 4204 m during Dive #1334. Among foraging and scavenging animals in the community, an unusual gastropod was found aggregated on and around the whale bones. Based on morphological observation and molecular phylogenetic analysis, we reached the conclusion that this is an undescribed species of the abyssochrysoidean genus *Rubyspira*, which was recently established by Johnson *et al.* (2010) to accommodate two species collected together from the same whale carcass in Monterey Bay, northern California at a depth of 2893 m. It is described as a new species in the present paper, representing the first record of this genus away from the type locality.

Material and methods

Materials. All the examined specimens were collected from the same whale carcass discovered on the São Paulo Ridge, Southwest Atlantic (28°31.12′S, 41°39.41′W), at a depth of 4204 m during two manned submersible dives: YK13-04 leg.1, *Shinkai* 6500 Dive # 1334, April 24, 2013 (JAMSTEC 1130037537); YK13-04 leg.1, *Shinkai* 6500 Dive # 1336, April 26, 2013 (JAMSTEC 1130037539) (Fig. 1). They were directly fixed in 99% ethanol on board. Twenty specimens in total, including empty shells, were available for the present study. The holotype and paratypes (#1–3 and #4–5) were selected from the former lot and deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP), National Museum of Nature and Science, Tsukuba (NSMT) and JAMSTEC, respectively.

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Morphology. Morphological observations, including the examination of external anatomy, were made on ethanol-fixed specimens. The radula was extracted from the buccal mass, cleaned in x10 diluted bleach (Kitchen Haitaa, Kao Co. Ltd.), rinsed in distilled water, and observed with a scanning electron microscope (JEOL JSM-6380LV) with gold/palladium alloy coating. Light microscope photographs of the shells, operculum and soft parts were taken with a Nikon D300 digital camera coupled to either a Micro-Nikkor 60mm lens or to reversely attached Nikkor 20mm lens. Images were combined through focus stacking and processed using Adobe Photoshop ®. Shell dimensions were obtained by a combination of direct measurement of shell length with a vernier caliper to 0.1 mm (Table 1), and calculation from digital images (Fig. 5A), for shell length (L), shell width (W), apertural length (LA) and spire length (LS), according to the definition by Reid & Ozawa (2016: 6). Because the apical part and/or the aperture of some specimens were broken during the sampling procedure, anomalous relative shell length (L2) and width (W2) were measured as shown in Fig. 5A to compare proportions of some abnormal specimens (Fig. 6).

DNA analysis. A deep-frozen specimen was used for DNA sequencing. DNA was extracted from the foot. The specimen was thoroughly washed in autoclaved and filtered seawater to eliminate surface contaminants. DNA extraction was conducted using the DNeasy Tissue Kit (Qiagen Japan, Tokyo, Japan).

The cytochrome c oxidase subunit I (*COI*) gene was amplified by PCR using the Ex Taq PCR Kit (Takara, Kyoto, Japan). Two oligonucleotide primers (1 μM each) and <1 μg of DNA template were added to the reaction mixtures. Thermal cycling was as follows: denatured at 96°C for 20 s; annealed at 55°C for 45 s; and extended at 72°C for 2 min for a total of 35 cycles. The oligonucleotide primer sequences used for this amplification were LCO1490 and HCO2198 (Folmer *et al.* 1994). The molecular size of the PCR products was checked with 1.2% Agarose S (Nippon Gene, Toyama, Japan) gel electrophoresis.

DNA sequencing of the amplified *COI* genes was performed using the BigDye Terminator Cycling Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA, USA). The LCO1490 and HCO2198 primers were used in sequencing reactions. Sequencing was performed using a genetic analyzer (ABI PRISM 3100, Applied Biosystems). The sequence reported here has been deposited in the DDBJ database under accession number LC106304.

A nearly complete sequence of the *COI* gene (621 bp) was analyzed using the gapped-BLAST search algorithm (Altschul *et al.* 1997; Benson *et al.* 2000) to estimate the degree of similarity to other *COI* gene sequences. The database used for similarity analyses was the non-redundant nucleotide sequence database from GenBank. All *COI* gene sequences from the selected operational taxonomic units were manually aligned and phylogenetic analysis was restricted to nucleotide positions that were unambiguously alignable in all sequences. The alignments of *COI* genes were tested for optimal fit of various nucleotide substitution models using the MEGA7 software (Kumar *et al.* 2016). The base frequencies, proportion of invariable sites and a gamma distribution were estimated from the dataset. The HKY85 model (Hasegawa *et al*. 1985) incorporating variable sites (G + I) was selected by MEGA. The maximum likelihood (ML) analysis with MEGA of this dataset was performed using an input tree generated by BIONJ (Gascuel 1997) with the model. Bootstrap trees (1000 replicates) were constructed using the same parameters as the ML tree.

Minimum genetic distances based on Kimura 2 parameters (K2P) model were calculated between *Rubyspira* species using MEGA7 software. These distances were calculated using the *COI* alignment of all the *Rubyspira* sequences deposited in the DDBJ database without gaps.

TABLE 1. Shell dimensions of type series of *Rubyspira brasiliensis* **sp. nov.**, in mm except for L/W and LS/LA (%).

Type status	Registration	Condition	L	W	L/W	LA	LS	LS/LA
Holotype	MZUSP 141885	99% ethanol	38	33.1	1.15	22.6	15.5	0.69
Paratype #1	NSMT-Mo 78943	99% ethanol	33.6	31.7	1.06	22.5	11.1	0.49
Paratype #2	NSMT-Mo 78944	99% ethanol	34.2	30.0	1.14	22.2	12.0	0.54
Paratype #3	NSMT-Mo 78945	dry + SEM stub	31.2	-	-	22.3	8.9	0.40
Paratype #4	JAMSTEC No. 1130057451	99% ethanol	-	-	-	18.8	12.1	0.64
Paratype #5	JAMSTEC No. 1130057452	99% ethanol	31.5	28.3	1.11	20.0	11.5	0.57
Mean			33.7	30.8	1.12	21.4	11.8	0.56

Taxonomy

Superfamily Abyssochrysoidea Tomlin, 1927

Remarks. Johnson *et al*. (2010) demonstrated, by using three mitochondrial and four nuclear genes, that *Rubyspira* falls in a clade containing four other genera: *Desbruyeresia*, *Alviniconcha* and *Ifremeria*, which are usually classified in the family Provannidae, and *Abyssochrysos* in the Abyssochrysidae. The clade was then shown to be sister to another clade containing species of *Provanna*, suggesting that the Provannidae is paraphyletic and including Abyssochrysidae in the clade. The precise systematic position of *Rubyspira* in the superfamily has thus not been settled yet (see also Johnson *et al*. 2015).

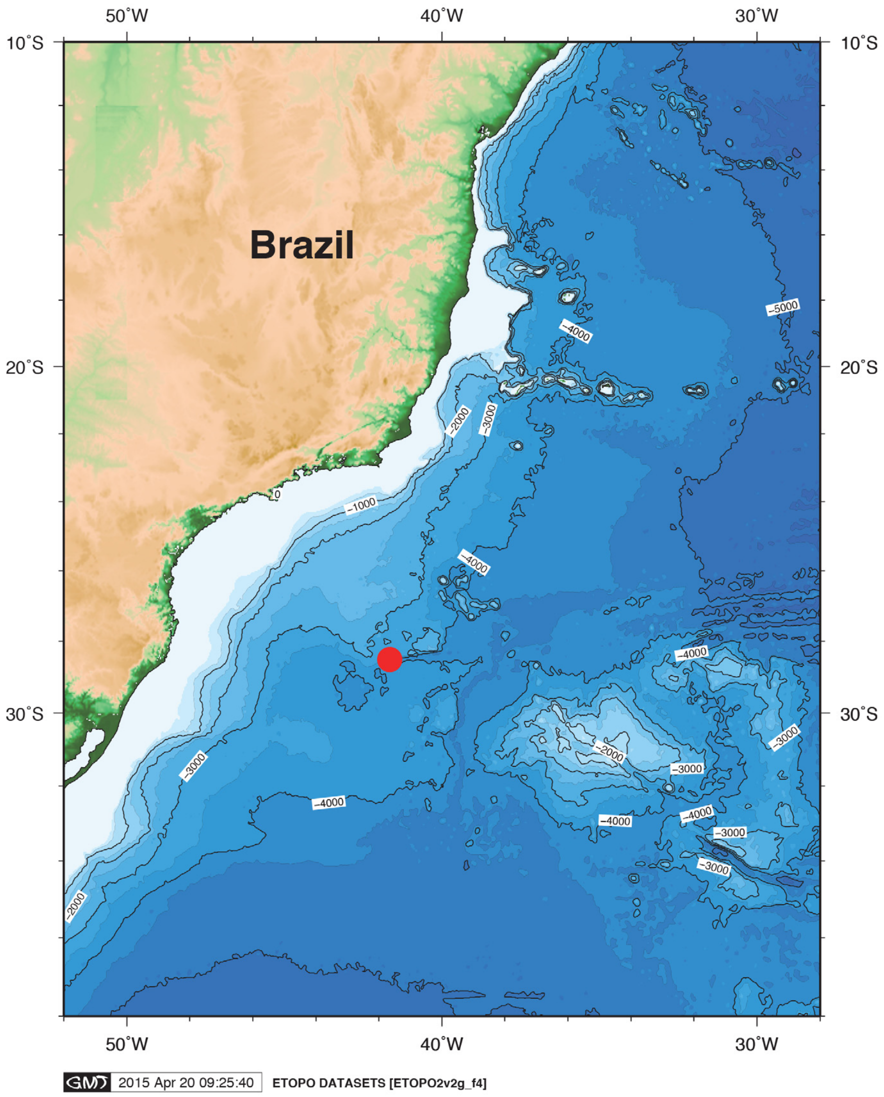

FIGURE 1. A map showing the location of the deep-sea whale carcass at the São Paulo Ridge, where the present new species was collected.

Genus *Rubyspira* Johnson *et al.*, 2010

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

Rubyspira brasiliensis Hasegawa, Fujiwara & Okutani sp. nov.

(Figs 2–5)

Type material. Holotype MZUSP 141885 (Figs 2A–E). Paratypes, #1 NSMT-Mo 78943 (Figs 2F–H); #2 NSMT-Mo 78944; #3 NSMT-Mo 78945 (Figs 3, 4); #4 JAMSTEC No. 1130057451; #5 JAMSTEC No. 1130057452. All the specimens are stored in 99% ethanol, except paratype #6, which was used for the examination of the soft parts, including the radula. Shell dimensions and depository of the type series are summarized in Table 1.

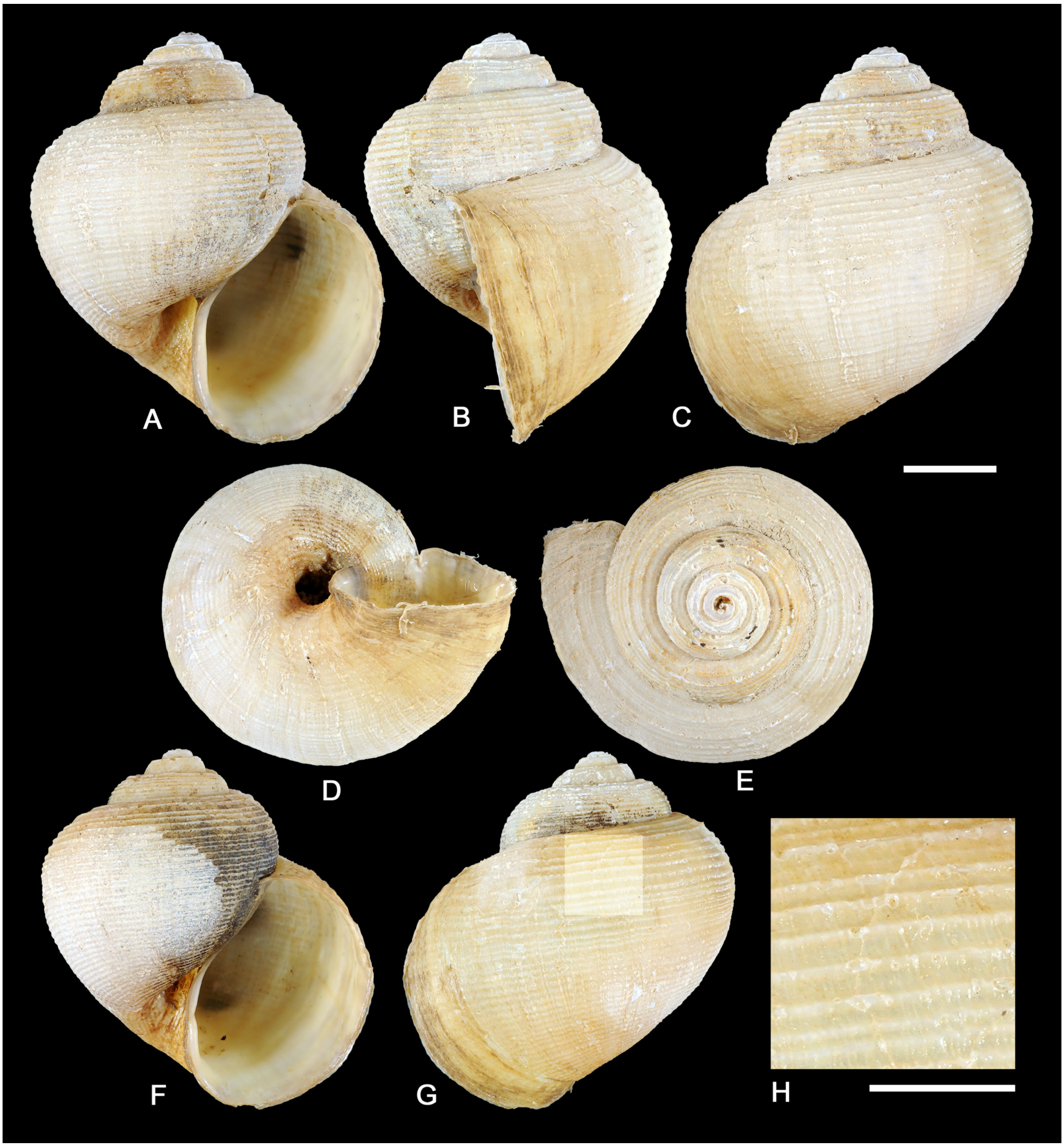

FIGURE 2. Shell of *Rubyspira brasiliensis* **sp. nov.** A-E: holotype, MZUSP 141885. F-H: paratype #1, NSMT-Mo 78943; H, enlarged image of the square area shown in G to show the detail of sculpture. Scales: A-G = 10 mm; H = 5 mm.

Additional material examined: MZUSP 141885 (4 exs).

Type locality. São Paulo Ridge, Southwest Atlantic (28°31.12´S, 41°39.41´W), at a depth of 4204 m, on and around a whale carcass (see Material section for detail).

Diagnosis. Shell globose, with an *Ampullaria*-like appearance, sculptured by fine, crowded spiral cords all over. Umbilicus deeply open. Radula taenioglossate; central, lateral and marginal teeth smooth, completely lacking denticles.

Description. *Shell* (Figs 2, 5) rather thin, globose, with *Ampullaria*-like appearance, covered by pale straw-colored, filmy periostracum, which occasionally bears weak axial lamellae near labral margin. Protoconch missing in all specimens examined due to corrosion. Teleoconch consisting of at least four roundly inflated whorls with moderately constricted suture. Initial teleoconch whorls with sharp angulation at shoulder, which becomes indistinct on lower whorls. Last whorl large, occupying 89% of shell length. Surface sculptured by fine, crowded spiral cords with narrower interspaces all over. Spiral cords rather uniform in thickness and crowded in basal part of last whorl, but becoming stronger and more irregular adapically, especially on sutural ramp area, where strongest spiral cord sometimes forms weak shoulder keel. 15 or more cords on penultimate whorl, some 40–50 on last whorl. Umbilicus narrow but deep, encircled by indistinct fasciole; inner wall ornamented by weak spiral cords. Aperture semi-circular, entire with no anterior sinus or anal notch. Outer lip smooth, neither reflected nor thickened. Inner lip weakly calloused, continuing to nearly straight columellar lip and barely reflected over umbilicus.

Animal (Fig. 3). One probably immature female specimen was examined. Head with relatively large and extremely broad snout and rather blunt cephalic tentacles, lacking pigmented eyes; foot small with propodium and metapodium, separated by narrow lateral groove. Mantle edge simple with small but distinct notch at left corner and simple (not annulate) pallial tentacle in notch in front of anus. Ctenidium well developed, covering nearly one third of pallial roof, and consisting of triangular leaflets. Osphradium relatively small, leaf-like and situated near left corner of pallial roof. Posterior esophagus swollen with soil-like contents, seen through epithelium of mantle floor. Stomach large, comprising large part of ventral side of visceral mass. Rectum also considerably packed with soil-like contents, and sharing most part of right side of pallial roof, especially in its proximal part, leaving little space for gonoduct.

Radula (Fig. 4A) taenioglossate, 2-1-1-1-2. Central tooth with ovo-quadrate base and triangular cusp, smooth. Lateral tooth with broad shaft and gently incurved, blunt cusp. Inner and outer marginal teeth gently incurved, with blunt tip; shaft carrying low, lateral wing. All teeth distinctly smooth, with no trace of denticles.

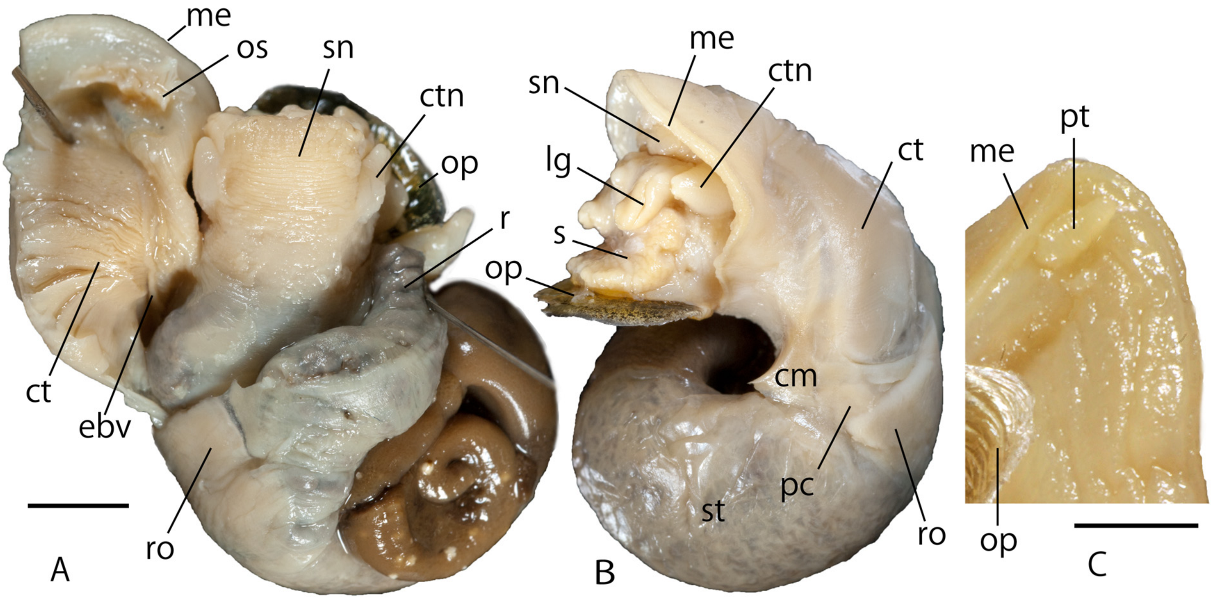

FIGURE 3. External anatomy of *Rubyspira brasiliensis* **sp. nov.**, paratype #3, NSMT-Mo 78945. A: dorsal view with mantle longitudinally cut along the right side of ctenidium. B: ventral view. Abbreviations: cm, columellar muscle; ct, ctenidium; ctn, cephalic tentacle; ebv, efferent branchial vessel; lg, lateral groove; me, mantle edge; mp, metapodium; op, operculum; os, osphradium; pc, pericardium; pt, pallial tentacle; r, rectum; ro, renal organ; s, sole; sn, snout; st, stomach. Scales: A, B = 5 mm; C = 1 mm.

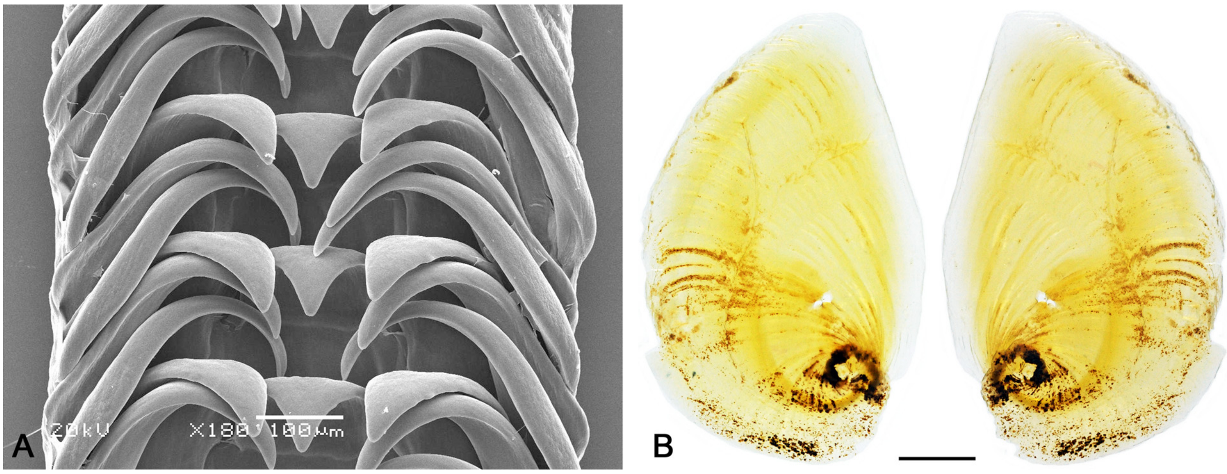

FIGURE 4. Radula and operculum of *Rubyspira brasiliensis* **sp. nov.** , paratype #3, NSMT-Mo 78945, same specimen as the one illustrated in Figure 3. A. radula, a scanning electron micrograph. B: internal and external views of operculum. Scales: A = 100 µm; B = 1 mm.

Operculum (Fig. 4B) leaf-like in shape, yellowish brown, horny, paucispiral, with indistinct spiral ridge in center. Rather small relative to size of apertural opening.

Variation (Figs 5, 6). All the specimens larger than 30 mm in shell length (19 examples) were rather uniform both in general shape and sculpture, except for some minor variations mentioned in the description. Only three empty shells in the present material were small (less than 30 mm in length), and they differ considerably from the larger ones in being more narrowly elongate in shape and possessing finer and more crowded spiral ribs. Because of the absence of live-collected specimens of the latter form, both are provisionally regarded herein as forms or growth stages of a single species.

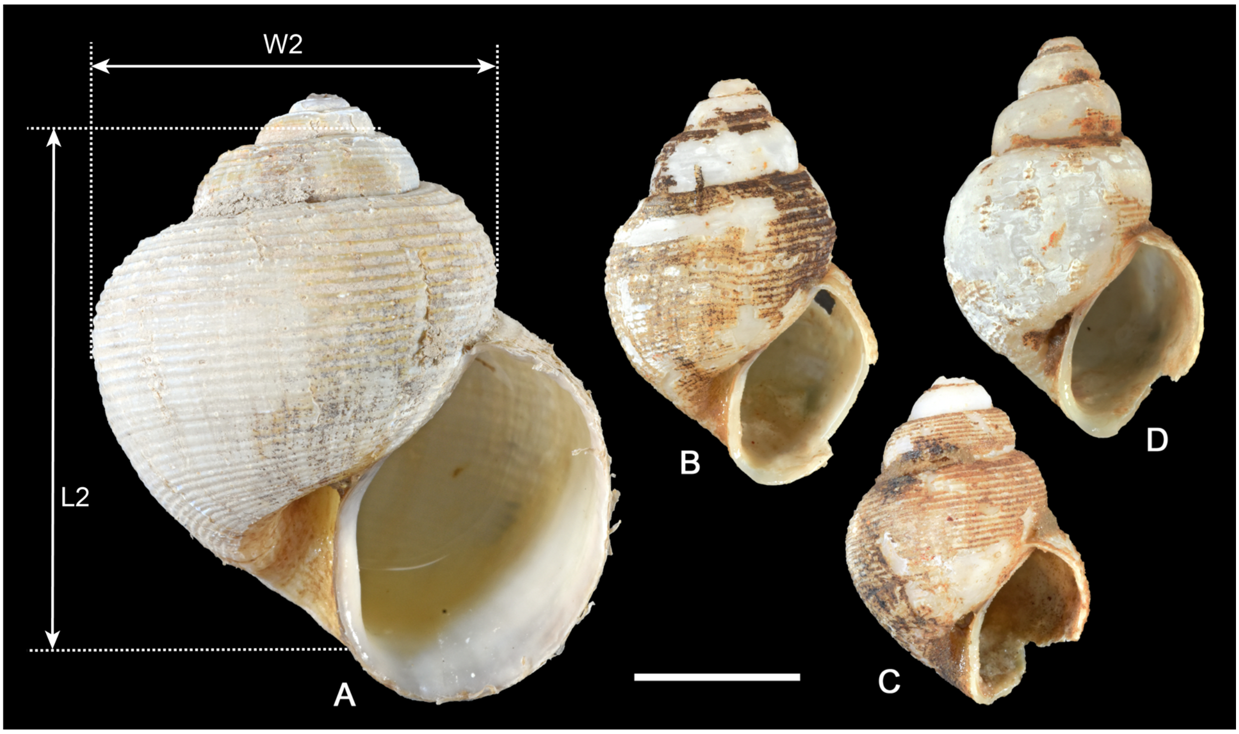

FIGURE 5. Comparison of shell morphology of normal (A) and aberrant (B-D) specimens of *Rubyspira brasiliensis* **sp. nov.** A. holotype. B-D. aberrant specimens from the type locality obtained together with the holotype, NSMT-Mo 78945. All at the same scale. Scale = 1 mm. Anomalous relative shell length (L2) and width (W2) were measured as shown in A to compare the proportions of these specimens (Figure 6) because of the incomplete conditions of the apical part and outer lip of some specimens. L2 represents the length between the apical margin of the penultimate whorl and the intersection of the basal part of body whorl and aperture in the frontal view.

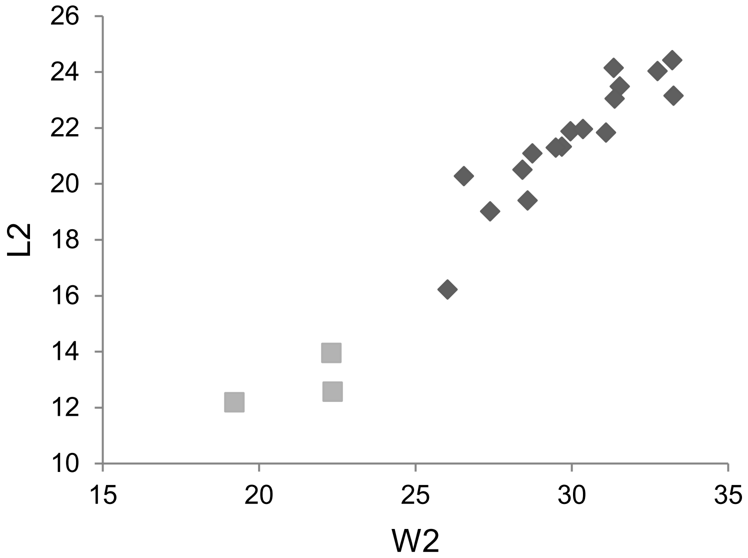

FIGURE 6. Relationship between shell length and shell width of "normal" (rhomboids), and "aberrant" (gray squares) specimens. Because of the imperfect conditions of some specimens, shell length and width were represented by the parameters indicated in the Figure 5, as L2 and W2, respectively.

Etymology: The specific epithet *brasiliensis* refers to the type locality, the São Paulo Ridge, which is located off Brazil.

Remarks. Only two Recent species (*R. osteovora* and *R. goffrediae*) have previously been known in the genus *Rubyspira* (Johnson *et al*. 2010). The present new species is readily distinguished from both by its *Viviparus*-like globose shell with a widely perforate umbilicus. No other conchologically comparable species in other systematic groups has been found from deep-sea chemosynthetic communities worldwide, including whale falls.

Phylogeny

Molecular phylogenetic analysis using the cytochrome c oxidase subunit I (*COI*) gene sequence performed by the maximum likelihood (ML) method placed *R. braziliensis* **sp. nov.** within a clade containing two other species of the genus *Rubyspira* (Fig. 7). The present new species was shown to be most closely related to *R. osteovora* by a bootstrap value of 95%, and it formed a monophyletic clade with other *Rubyspira* gastropods in ML supported by a bootstrap value of 82%.

COI genetic distances between *R. brasiliensis* **sp. nov.** and *R. osteovora* are 0.105–0.108, which are greater than intraspecific values in *R. osteovora* (0.000–0.002) and in *R. goffrediae* (0.000–0.006). Genetic distances between *R. braziliensis* **n. sp.** and *R. goffrediae* for the *COI* ranged from 0.219 to 0.228.

Discussion

At first glance, the present new species differs greatly from other species in the genus *Rubyspira*, including the type species *R. osteovora,* which possess a *Turritella*-like elongate conical shell with a narrow umbilical chink and an elongate aperture. It is actually too different to be considered congeneric with the others based on traditional comparison of conchological features. Nevertheless, the present species was shown by molecular analysis to belong in the genus, as stated above. This generic allocation is also supported by anatomical features such as the broad snout with stubby cephalic tentacles, simple pallial margin without papillae (papillae are present in *Alviniconcha* spp.: Warén & Bouchet 1993), the fleshy foot with a distinct lateral groove, and the non-annulated pallial tentacle (distinctly annulated in *Provanna* spp.: Warén & Ponder 1991). Furthermore, the new species has a distinctive radula, characterized by smooth cusps with no trace of denticles, which is closely similar to that of *R.*

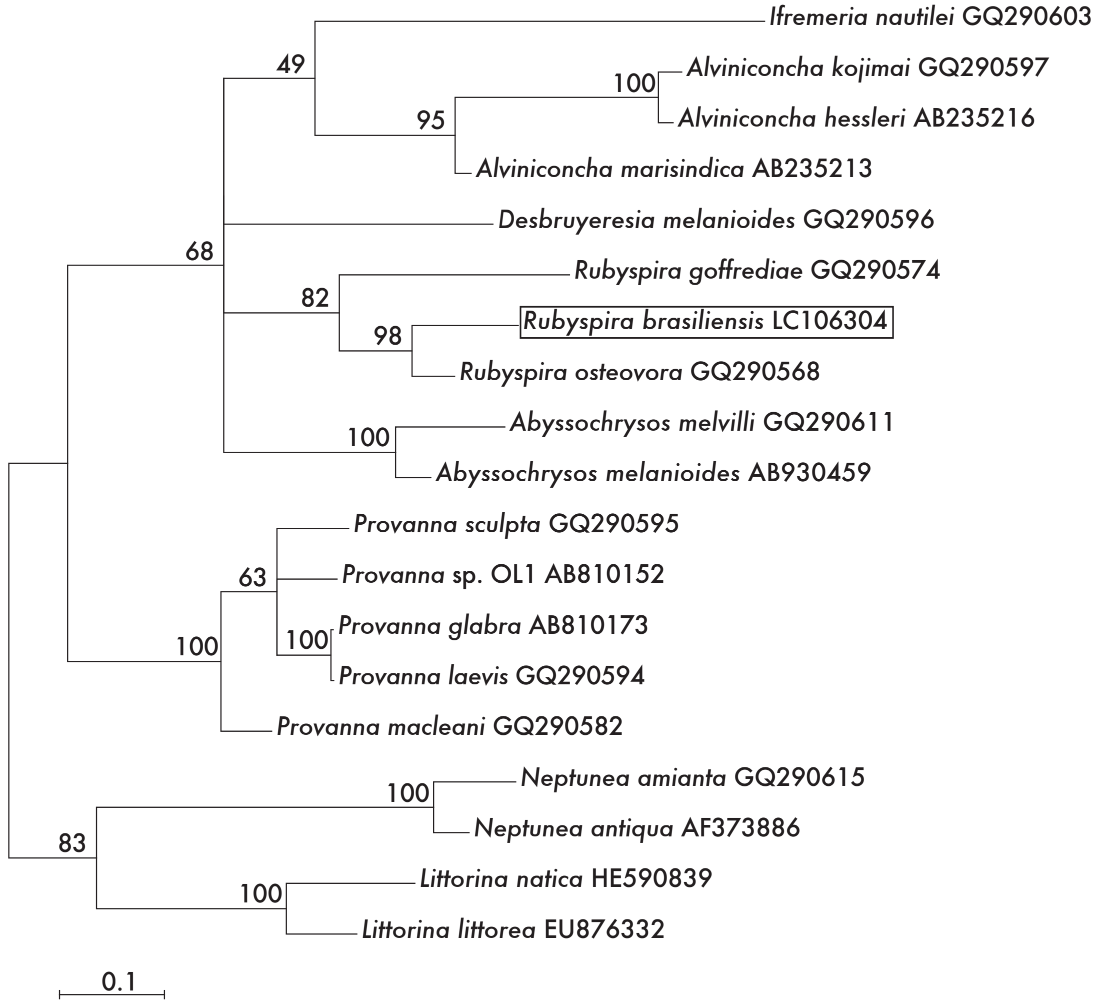

FIGURE 7. Phylogenetic placement of *Rubyspira brasiliensis* **sp. nov.** within gastropods based on the cytochrome c oxidase subunit I (*COI*) gene sequences. The maximum-likelihood tree is shown. Scale bar represents 0.1 nucleotide substitution per sequence position. The percentage of 1000 bootstrap resamplings in ML is shown for each branch. The *Rubyspira* gastropod examined in this study is highlighted. The accession numbers used for this study are following the operational taxonomic unit names.

osteovora (Johnson *et al*. 2010: fig. 2A). It is noteworthy that *R. goffrediae*, which inhabits sympatrically with *R. osteovora*, considerably differs from these species in the morphology of radula, in possessing vestigial central teeth and distinct cusps on each tooth. This is consistent with the fact that the present new species is more closely related to *R. osteovora* than *R. goffrediae* in the molecular analysis.

In establishing the genus *Rubyspira,* Johnson *et al*. (2010) discussed their biology, including evolution, nutrition, and symbiotic bacteria, besides taxonomical descriptions and phylogeny. Regarding nutrition intake, the two previously known species have shown to differ based on the differences in their body sizes, the morphology of radula and their microhabitats: *R. osteovora* is a sediment eater, while *R. goffrediae* scrapes fragments from intact bone. The present new species may be a sediment eater, because of the similarity in the body size and morphology of radula to those of *R. osteovora*, as well as its occurrence not only on whale bones but also on the adjacent seafloor. The digestive system of this species (esophagus, stomach and rectum) are actually filled with soil-like material, but it contains a small amount of hard particles, probably fragments of whale bones, suggesting a direct intake of bone material at least in part.

Concerning the geographical distribution, species of the genus *Rubyspira* are currently known only from the Monterey Submarine Canyon off California (Johnson *et al*. 2010) and off Brazil (present study). Both areas are separated by the Isthmus of Panama, which was formed in the Middle Pliocene, 3.5 Ma (e.g. Coates *et al.* 1992) or between 25 and 23 Ma (Farris *et al.* 2011). Johnson *et al*. (2010) demonstrated in the molecular analysis with fossil calibrations that the two Californian species diverged ca. 30Ma ago. This is consistent with the fact that the present new species is phylogenetically closer to one of the Californian species, *R. osteovora*, than the other, suggesting that divergence of *R. osteovora* + *R. brasiliensis* **sp. nov.** and *R. goffrediae* occurred before the closure of the Isthmus of Panama.

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