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A New *Paralepetopsis* Limpet from a South China Sea Seep Hints at a Paraphyletic Neolepetopsidae

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Neolepetopsidae is a little-studied true limpet family only known from deep-sea chemosynthetic ecosystems, containing just over a dozen species in three genera: *Neolepetopsis*, *Paralepetopsis*, and *Eulepetopsis*. Although considered monophyletic by a recent phylogenetic analysis, a lack of *Paralepetopsis* sequence linked to morphology casts some uncertainty. Here, we discovered a new species of *Paralepetopsis* from the Haima methane seep in the South China Sea, described as *Paralepetopsis polita* sp. nov. The new species is distinct from all other described *Paralepetopsis* by its smooth and semi-transparent shell, combined with a radula exhibiting pluricuspid teeth with two cusps. We tested its relationship with other neolepetopsids using a molecular phylogeny reconstructed from the mitochondrial *COI* gene, revealing a surprising position nested within Lepetidae, a family with a very different radula morphology. The clade containing lepetids and our new species was recovered sister to other neolepetopsids with sequence data available. This hints at a paraphyletic Neolepetopsidae, and suggests the neolepetopsid-type radula might not be exclusive to one monophyletic group of limpets.

Key words: Cold seep, Deep sea, Gastropoda, Mollusca, Phylogenetics.

BACKGROUND

The true limpet family Neolepetopsidae is a small, little-studied group containing just 16 species (Chen et al. 2021), and is the only patellogastropod family restricted to chemosynthesis-based ecosystems (McLean 1990 2008; Warén and Bouchet 2001). The family has contained three genera since the original description, including *Neolepetopsis*, *Eulepetopsis*, and *Paralepetopsis*. *Neolepetopsis* is mostly known from inactive sulfides at the periphery of active vent fields, though there is one species known from whale bones and a record is present from a seep off Peru (Van Dover 2019; Warén and Bouchet 2001; Chen et al. 2021).

Paralepetopsis inhabits a wide-range of environments from hydrothermal vents to hydrocarbon seeps to whale bones (Beck 1996; McLean 2008), while *Eulepetopsis* is only known from active chimneys near high-temperature venting (Warén and Bouchet 2001; Chen et al. 2021). *Eulepetopsis* is characterised in having a low-profile shell that is fully transparent due to the lathic calcite composition (McLean 1990); *Neolepetopsis* and *Paralepetopsis* are more similar but the shell of *Neolepetopsis* carries strongly cancellate sculptures (McLean 1990).

The most distinctive feature of Neolepetopsidae among Patellogastropoda is the unusual radula with articulate shafts, containing a robust central tooth, two

dissimilar inner laterals, a strong and broad pluricuspid formed by the fusing of multiple outer laterals, and two marginal teeth (McLean 1990 2008). The radula is variable in the extent of mineralisation, ranging from barely mineralised (typical for *Paralepetopsis*) to relatively well-mineralised (more common in *Neolepetopsis*). The condition of the neolepetopsid-type radula in having a well-developed rachidian tooth is only mirrored in Patellidae among patellogastropod families, but patellids have three pairs of marginals and the teeth are usually strongly mineralised (McLean 1990). This radula type is shared among all three genera and is considered to be an evidence of monophyly for the family (McLean 1990). Nevertheless, some differences are observed among genera, such as the outermost marginal in *Eulepetopsis* being extremely reduced compared to the other genera and the lateral cusps being typically narrow and sharply pointed in *Neolepetopsis* but overhanging with broad, blunt cusps in *Paralepetopsis* (McLean 2008).

The phylogenetic position of Neolepetopsidae, its monophyly, and the relationships among the three genera remain unsettled. Earlier work recovered the family as sister to Acmaeidae using a fragment of the *18S* rRNA gene (Harasewych and McArthur 2000), whereas later work with better taxon sampling using the mitochondrial cytochrome oxidase *c* subunit I (*COI*) gene and the nuclear histone *H3* gene recovered it as sister to Lepetidae (Nakano and Sasaki 2011). A recent phylogenetic reconstruction (Chen et al. 2021) was the first to include supposed members from all three neolepetopsid genera, recovering a monophyletic Neolepetopsidae and again a sister relationship to Lepetidae, though not with strong support. However, the two sequences of *Paralepetopsis* included in these phylogenetic studies lack morphological support (Aktipis and Giribet 2010; Goffredi et al. 2017) and are only provisionally assigned to this genus; whether they truly represent members of *Paralepetopsis* remains questionable. As such, molecular data from a specimen morphologically confirmed as *Paralepetopsis* is much needed to confirm the phylogenetic position of that genus.

Paralepetopsis currently contains seven described species, the type species being *P. floridensis* McLean, 1990 from a hydrocarbon seep in Florida Escarpment (McLean 1990). Two further seep species are known, including *Paralepetopsis lepichoni* Warén & Bouchet, 2001 from Nankai Trough, Japan and *Paralepetopsis sasakii* Warén & Bouchet, 2009 from a West African seep. Three vent species have been described, including *Paralepetopsis rosemariae* Beck, 1996 from the Edison Seamount in the western Pacific, *Paralepetopsis ferrugivora* Warén & Bouchet, 2001 from Mid-Atlantic Ridge, and *Paralepetopsis tunnicliffae* McLean, 2008 from Juan de Fuca Ridge. The last species, *Paralepetopsis clementensis* McLean, 2008, was found on a whale fall off California.

Haima seep is a cold seep located on the north-western slope of the South China Sea, with a lush chemosynthetic ecosystem fuelled by rich deposits of methane hydrates (Chen et al. 2018; Feng et al. 2018). During a research cruise to the Haima seep, a hitherto undescribed species of *Paralepetopsis* was found living on the shells of the large vesicomyid clam *Archivesica marissinica* Chen et al., 2018. Here, we describe and characterise this new species, and test the monophyly of Neolepetopsidae using a molecular phylogeny with the first reliable genetic data from *Paralepetopsis*.

MATERIALS AND METHODS

Sample Collection

Specimens of limpets were collected with several individuals of the vesicomyid clam *Archivesica marissinica* Chen et al., 2018 in September 2020 from the Haima cold seep (16°41.6561'N, 110°23.8165'E, 1361 m depth; Fig. 1) by the remotely operated vehicle (ROV) *Haima II* using a scoop-net, on-board R/V *Haiyang #6*. The clams were immediately placed in a -80°C deep-freezer after the ROV returned on deck. Later in the shore-based laboratory, limpets were removed from the frozen clams and placed into 99% ethanol before further investigation.

Morphology

Limpet specimens were observed and dissected under a stereo dissecting microscope (Olympus SZX9), with shell lengths (SL), shell widths (SW), and shell heights (SH) measured using digital callipers to the nearest one decimal point. Soft parts were carefully removed from its shell using tungsten needles and rehydrated in a graded ethanol-MilliQ water series (99%, 90%, 70%, 50%, 30%, 10%, 0% ethanol) for one hour each and left to sit in MilliQ water overnight prior to observation. Detailed anatomical investigation was not possible due to the poor preservation of tissue. Specimens investigated herein were deposited into either the Tropical Marine Biodiversity Collections of the South China Sea, Chinese Academy of Sciences, Guangzhou, China (TMBC) or the National Museum of Nature and Science, Tsukuba, Japan (NSMT).

For scanning electron microscopy (SEM) of the radula, fine forceps and tungsten needles were used to dissect the radula ribbon from a limpet, which was then
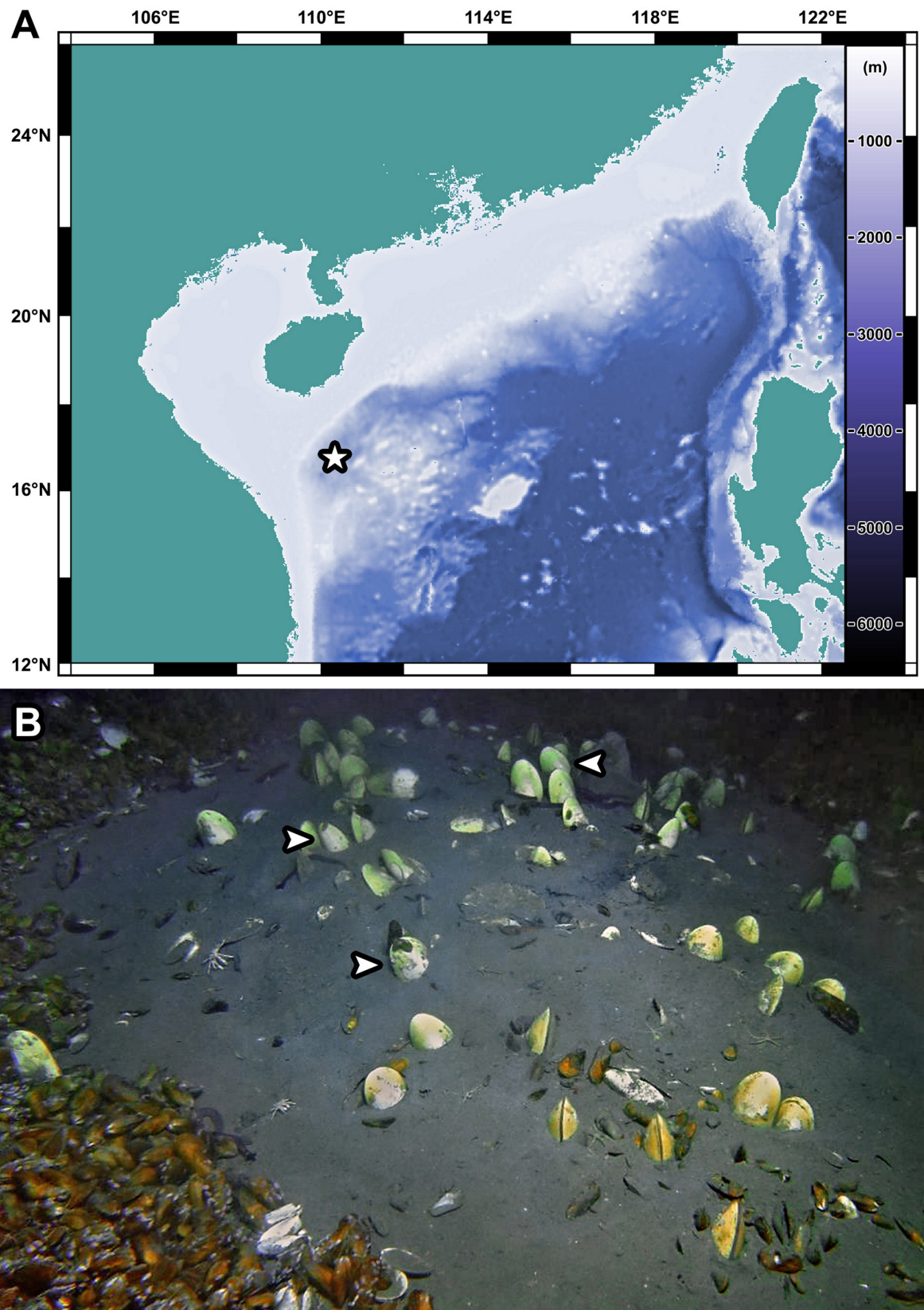
Fig. 1. The Haima methane seep in the South China Sea. A: Map showing the location of Haima seep southeast off Hainan Island. B: Photograph of the type locality showing *Paralepetopsis polita* sp. nov. (arrows) on the shells of the vesicomyid clam *Archivesica marissinica*.

placed in diluted commercial bleach (1:10 dilution) for cleaning. As neolepetopsid radulae are prone to disintegration (McLean 1990), the radula was monitored using the dissecting microscope and immediately moved into MilliQ water upon sufficient cleaning. The radula was washed in fresh MilliQ water and again in 99% ethanol before being mounted onto a SEM stub using carbon tape. To observe the surface sculpture of the shell, the shell of a limpet was placed into the same diluted commercial bleach after removing the soft parts, cleaned briefly using soft brushes, then washed twice in MilliQ water and twice in 99% ethanol before being mounted onto SEM stubs using carbon tape. The SEM stubs were observed using a Hitachi TM-3000 table-top SEM uncoated at 15 kV.

Genetics

Two limpet specimens were used for DNA sequencing. Genomic DNA was extracted using the DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA) following manufacturer's protocols. The barcoding fragment of the mitochondrial *COI* gene was amplified and sequenced using the universal primer pair HCO2198–LCO1490 (Folmer et al. 1994), using methods as reported in previous studies (Zhou et al. 2018; Chen et al. 2021). Geneious R11 (https://www.geneious.com) was used to assemble the consensus sequence for each limpet individual, prior to downstream analyses. New sequences generated herein have been deposited into NCBI GenBank under the accession numbers ON810774–ON810775.

Bayesian inference was used for phylogenetic reconstruction as implemented in the software MrBayes v3.2.6 (Ronquist et al. 2012), using a 472 bp alignment of the *COI* gene following a previous study (Chen et al. 2021). A selection of patellogastropod *COI* sequences was downloaded from GenBank, including representatives of all eight currently recognised families: Acmaeidae, Eoacmaeidae, Lepetidae, Lottiidae, Nacellidae, Neolepetopsidae, Patellidae, and Pectinodontidae (Nakano and Ozawa 2007; Nakano and Sasaki 2011; Chen et al. 2021). Two vetigastropod sequences from Fissurellidae were selected as the outgroup, following Nakano and Ozawa (2007) and Chen et al. (2021). These sequences were aligned in Geneious R11. As the third codon position of *COI* was suggested to be problematic for the group from a previous comprehensive phylogenetic analysis of Patellogastropoda (Nakano and Ozawa 2007), this position was removed from downstream analyses. The most suitable substitution model was found to be GTR+I+G for both the first and second codon positions using the PartitionFinder v.2.1.1 software (Lanfear et al.

2017). In MrBayes, Metropolis-coupled Monte Carlo Markov chains were run for one million generations with topologies sampled every 100 generations. Tracer v1.6 (Rambaut et al. 2013) was used to monitor and select a suitable burn-in, determined as 3000 in our case.

RESULTS

SYSTEMATICS

Subclass Patellogastropoda Lindberg, 1986
Family Neolepetopsidae McLean, 1990
Genus *Paralepetopsis* McLean, 1990

Type species: *Paralepetopsis floridensis* McLean, 1990 (by original designation).

Paralepetopsis polita sp. nov.
(Figs. 2–4)
urn:lsid:zoobank.org:act:B202762C-E2B1-42E1-8644-7B7DFCC556BF

Type locality: On the shell of *Archivesica marissinica*, Haima methane seep (16°41.6561'N, 110°23.8165'E, 1361 m depth) in the South China Sea (Fig. 1B).

Type material: Holotype (TMBC031004; Fig. 2A); SL 7.9 mm, SW 5.6 mm, SH 2.4 mm; a piece of mantle removed for DNA sequencing. Paratype 1 (NSMT Mo-79367; Fig. 2B); SL 7.0 mm, SW 4.7 mm, SH 2.8 mm. Paratype 2 (NSMT Mo-79368; Fig. 2C); SL 6.4 mm, SW 4.3 mm, SH 2.3 mm. Paratype 3 (NSMT Mo-79369; Fig. 2D); SL 5.6 mm, SW 4.0 mm, SH 2.0 mm. Paratype 4 (TMBC031005; Fig. 3); SL 5.7 mm, SW 4.0 mm, SH 1.6 mm; a piece of foot used for DNA sequencing, shell and radula used for SEM. All collected from the type locality, taken using a scoop-net by ROV *Haima II* in September 2020 on-board R/V *Haiyang #6* during cruise HYDZ6-202005. They were originally frozen in -80°C while still attached to *Archivesica* clams and later transferred into 99% ethanol.

Etymology: 'Politus' (Latin), polished or smooth, named for its smooth teleoconch surface devoid of major concentric or radial sculpture.

Diagnosis: A medium-sized *Paralepetopsis* (SL up to 7.9 mm) with semi-transparent shell lacking any significant sculpture except very fine concentric growth lines, apex located on the mid-line at the anterior third of the shell. Central tooth with finely pointed cusp, other teeth with quadrangular cusps. All teeth exhibiting sturdy, well-developed shaft; each teeth significantly descend in position relative to the central tooth.

Description: Shell (Figs. 2, 3A–B) thin, semi-transparent, with a very thin layer of transparent periostracum where not corroded. Maximum shell length among specimens available 7.9 mm (holotype). Elliptic in outline, narrower at anterior end than posterior end, especially obvious in large specimens (Fig. 2A). Apex on midline, located at approximately one-third from anterior edge. Apex strongly corroded, inner surface of protoconch sealed. Protoconch unknown. Shell profile moderately high, margin aligned along one plane in specimens examined. Anterior margin straight to concave (Fig. 2A–B), posterior margin convex. Shell surface nearly completely smooth except very fine concentric growth lines. Muscle scars indistinct.

Radula (Fig. 3C–D) with two laterals, pluricuspid tooth, two marginals on either side of rachidian, no clear evidence of significant mineralisation. Each tooth from rachidian outwards descent significantly in position, each outer tooth diverging posteriorly, resulting in teeth on each row aligned like inverted 'V'. Rachidian narrow but sturdy, well-developed, with finely pointed cusp. Shaft of rachidian slender with narrow lateral ridge on each side, narrower than cusp where they connect. First inners lateral twice as broad as rachidian, with broad, overhanging, truncated quadrangular cutting edge. Shaft of first inner lateral sigmoidal in outline on outer side. Second inner lateral similar but with strong lateral ridge near base to accommodate base of first inner lateral. Pluricuspid robust, twice as broad as inner laterals, with one very broad, overhanging rounded inner cusp plus one small, narrow, poorly formed outer cusp. Shaft with lateral mid-shaft projection from where shaft tapers towards base. Inner marginals with narrow shaft, broadening apically to form rounded, spoon-like overhanging cusps. Shaft of inner marginals most
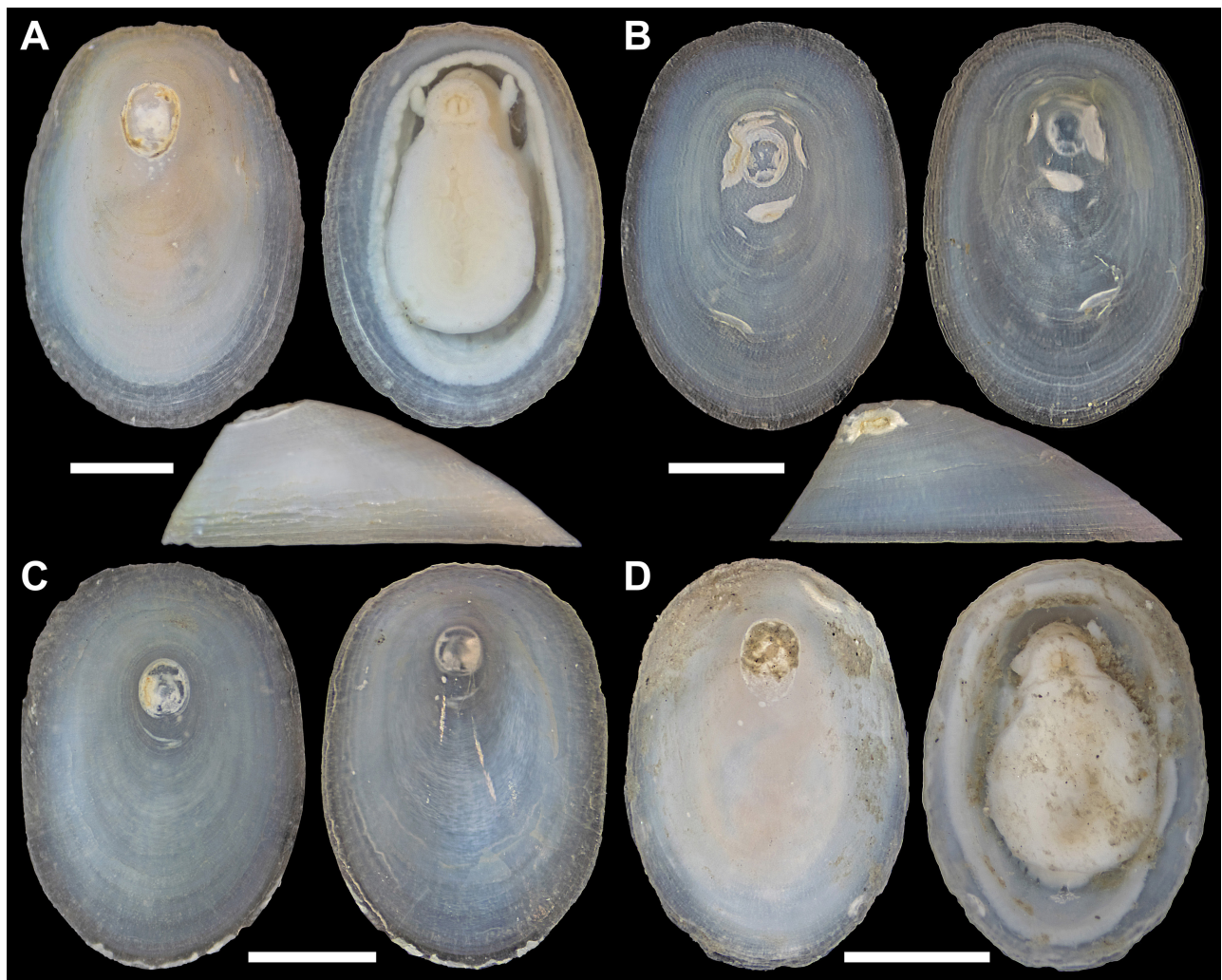

Fig. 2. *Paralepetopsis polita* sp. nov.. A: Holotype (TMBC031004). B: Paratype 1 (NSMT Mo-79367). C: Paratype 2 (NSMT Mo-79368). D: Paratype 3 (NSMT Mo-79369). Scale bars = 2 mm.

narrow at centre, with lateral projection near base. Outer marginals two-thirds as long as inner marginals, with broader shafts. Cusps spoon-like, less developed than inner marginals.

Soft parts (Fig. 4). Cephalic tentacles simple, conical, tapered, lacking appendages. Eyes appear lacking from external examination. Oral disc with muscular outer lip, labial lobe poorly developed. Clear groove separates oral disc from foot. Foot sole oval, large, with unciliated rim, epipodium lacking. Shell muscle U-shaped, comprising numerous muscle bundles along posterior third of body, length of bundles increases posteriorly. Mantle edge with numerous papillae, presumably sensory. Mantle cavity extending to just over one-third of body length. Gonad located along mid-body ventrally posterior or pericardium, partly visible through mantle roof. Ctenidium lacking. Intestine and stomach embedded within digestive glad which extends extensively as seen through mantle roof. Intestine loops twice prior to emerging as rectum. Anus located on right side of mantle roof, just left of urogenital papillae. Right kidney sizeable, at posterior end near shell muscles.

Distribution: Only known from the Haima methane seep area in the South China Sea.

Remarks: The shell lacking cancellate sculpture, as well as a radula with broad, overhanging lateral cusps lacking noticeable mineralisation places *P. polita* sp. nov. in *Paralepetopsis* rather than *Neolepetopsis*. Two features together separate *P. polita* sp. nov. from all other described *Paralepetopsis* species, including the semi-transparent shell lacking any significant sculpture and the pluricuspid tooth with two cusps. Although species such as *P. tunnicliffae* and *P. rosemariae* also exhibit little shell sculpture, they still show weak radial striae (Beck 1996; McLean 2008) which are lacking in *P. polita* sp. nov. *Paralepetopsis clementensis* is the only described congener lacking radial sculpture,
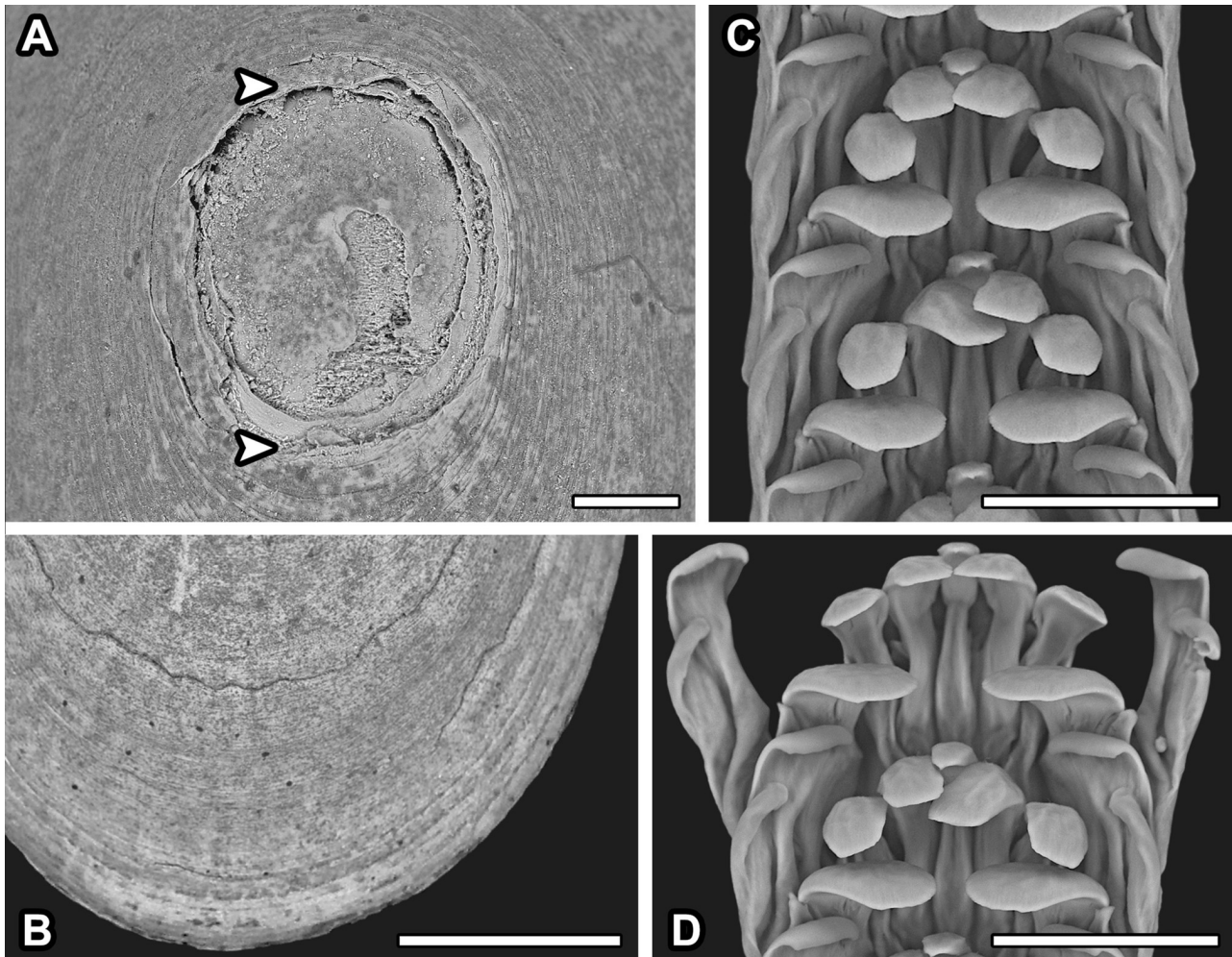

Fig. 3. *Paralepetopsis polita* sp. nov., scanning electron micrographs of paratype 4 (TMBC031005). A: Apex with arrows indicating the region lost by corrosion. B: Shell surface. C–D: Radula. Scale bars: A = 200 µm, B = 1 mm, C–D = 50 µm.

but that species has a clearly convex anterior margin (McLean 2008) as opposed to straight to concave in *P. polita* sp. nov. The only other neolepetopsid exhibiting a pluricuspid with two clearly separated cusps is *Neolepetopsis gordensis* McLean, 1990 which also has a much larger, major inner cusp and a smaller outer cusp (McLean 1990 2008). Although the cutting edge of the pluricuspid tooth in *P. ferrugivora* also exhibits two to three blunt tubercles, they are not separated out to form individual cusps (Warén and Bouchet 2001). A key to the described *Paralepetopsis* species, with information on the known distribution of each, is shown in table 1.

Molecular Phylogeny

We used the first and second codon positions of the *COI* gene to reconstruct the phylogeny of Patellogastropoda and to assess the systematic position of *Paralepetopsis polita* sp. nov., with the consensus tree shown in figure 5. In our tree, the two sequences of *P. polita* sp. nov. included (from the holotype and paratype 4) were recovered together with full support (Bayesian posterior probability, BPP = 1). They were, however, not clustered with other sequences assigned to Neolepetopsidae which formed a weakly supported clade (BPP = 0.6) – including two described species of *Neolepetopsis* and two described species of *Eulepetopsis*, as well as two sequences of undescribed preliminarily assigned to *Paralepetopsis* but without published data on their morphology (Aktipis and Giribet 2010; Goffredi et al. 2017). Within this clade, the two *Neolepetopsis* species formed a strongly supported clade (BPP = 0.98), as did the two *Eulepetopsis* species (BPP = 0.97); these two genera were recovered as
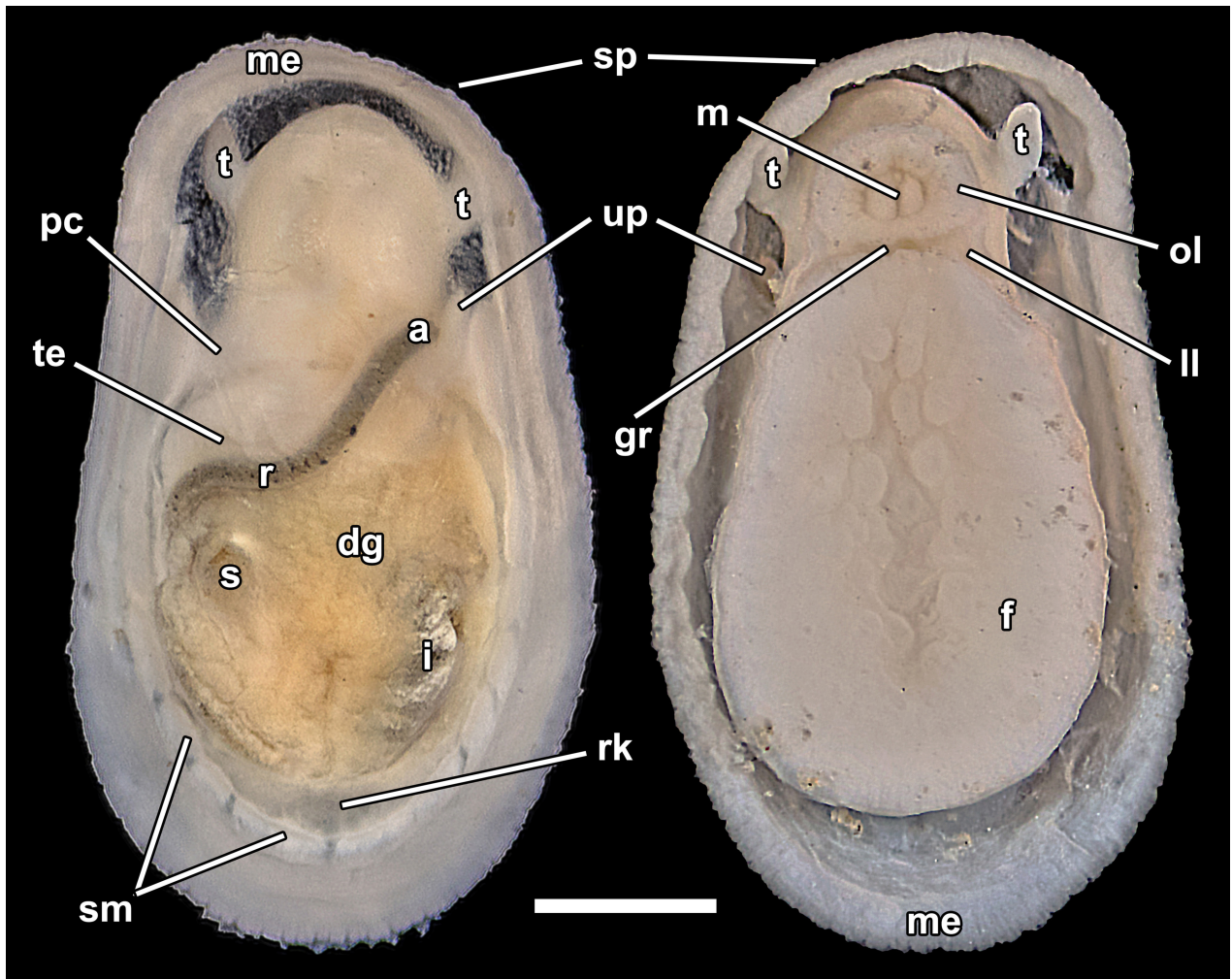

Fig. 4. *Paralepetopsis polita* sp. nov. (paratype 1, NSMT Mo-79367), external anatomy. Left: dorsal view after removing the shell, right: ventral view. Abbreviations: a, anus; dg, digestive gland; f, foot; gr, groove separating the foot's sole from peripheral flange; i, intestine; ll, labial lappet; m, mouth; me, mantle edge; ol, outer lip of the mouth; pc, pericardium; r, rectum; rk, right kidney; s, stomach; sm, shell muscles; sp, sensory papillae; t, cephalic tentacle; te, testis; up, urinogenital papilla.
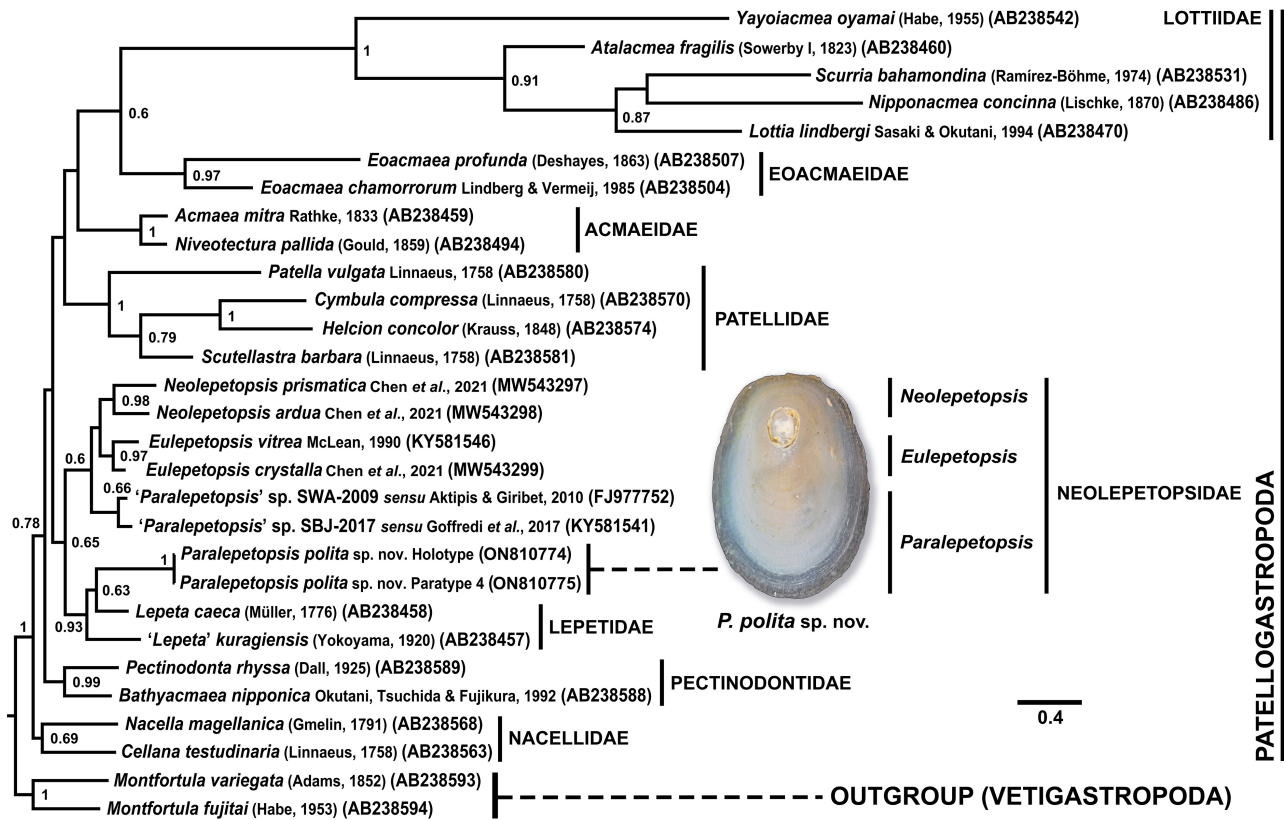
Fig. 5. Reconstructed consensus tree of Patellogastropoda using Bayesian inference, based on 472 bp of the mitochondrial *COI* gene. Node values indicate Bayesian posterior probabilities, only values > 0.6 are shown. GenBank accession numbers of the sequences are indicated in parentheses the species names.

Table 1. Key to the described *Paralepetopsis* species, including information on their distribution

1	a	Shell with radial sculpture	Go to 2
	b	Shell lacking any significant sculpture	Go to 7
2	a	Radial sculpture consisting of fine striation	Go to 3
	b	Radial sculpture consisting of strong ribs	Go to 4
3	a	Rachidian and laterals with triangular cusps	*P. floridensis* (Atlantic, Florida Escarpment, 3270 m, seep)
	b	Rachidian and laterals with square cusps	*P. rosemariae* (Pacific, Edison Seamount, 1483 m, vent)
4	a	Shell surface with raised nodules where ribs cross	Go to 5
	b	Shell surface lacking raised nodules	Go to 6
5	a	Rachidian and laterals with triangular cusps	*P. lepichoni* (Pacific, Nankai Trough, 2140 m, seep)
	b	Rachidian and laterals with round cusps	*P. sasakii* (Atlantic, West Africa off Congo River, 3150 m, seep)
6	a	Shafts of marginals well-developed	*P. ferrugivora* (Atlantic, Mid-Atlantic Ridge, 1665–1728 m, vent)
	b	Shafts of marginals not differentiated from base	*P. tunnicliffae* (Pacific, Juan de Fuca Ridge, 2145 m, vent)
7	a	Anterior slope of shell convex	*P. clementensis* (Pacific, Off California, 1800 m, whale fall)
	b	Anterior slope of shell straight to concave	*P. polita* sp. nov. (Pacific, South China Sea, 1361 m, seep)

sisters with weak support (BPP = 0.43). This clade was in turn sister to a moderately supported clade (BPP = 0.66) containing the two undescribed species assigned to *Paralepetopsis*, but only with moderate support (BPP = 0.66).

Surprisingly, we found *P. polita* sp. nov. nested within two sequences of species in Lepetidae, where it was sister to *Lepeta caeca* (Müller, 1776) with moderate support (BPP = 0.63). These two species were in turn found to be closely related to *Lepeta kuragiensis* (Yokoyama, 1920) with strong support (BPP = 0.93). The clade containing these three species was found to be sister of the clade containing the abovementioned six neolepetopsid species, a relationship that was moderately supported (BPP = 0.65). The other six currently accepted patellogastropod families were all recovered as monophyletic with various levels of support (BPP = 0.69–1), the relationships among which were the same as a previously published phylogeny by Chen et al. (2021).

DISCUSSION

The phylogenetic position of *Paralepetopsis polita* sp. nov. recovered in our phylogenetic analysis (Fig. 5) is surprising, and suggests a paraphyletic Neolepetopsidae where *Paralepetopsis* is paraphyletic, with *P. polita* sp. nov. nested within species currently assigned to Lepetidae with strong support. Though *P. polita* sp. nov. appeared to nest within *Lepeta*, that genus has been suggested to be paraphyletic (Nakano and Sasaki 2011). The phylogenetic position of *P. polita* sp. nov. is in strong contrast to the morphology where *P. polita* sp. nov. is clearly assignable to Neolepetopsidae on the basis of the characteristic radula type (McLean 1990 2008). Lepetidae is a strictly subtidal family, representing another little-studied deep-water group of true limpets. Lepetids, including *Lepeta*, are characterised by a very different radula type lacking the rachidian and with fused inner laterals as well as the loss of outer laterals, resulting in a narrow radula with only two laterals and two marginals being present (McLean 1990; Nakano and Ozawa 2007). This is strikingly different from the radula of *P. polita* sp. nov.

Considering that Neolepetopsidae and Lepetidae have been repeatedly recovered as sister families in molecular phylogenies (Nakano and Sasaki 2011; Chen et al. 2021), a condition which we also recovered in our tree, it appears likely that the two are indeed the most closely related groups among living patellogastropods. Since the articulating teeth and the presence of radula are considered to be plesiomorphic characters of Patellogastropoda (Fretter 1990), it seems likely that the shared ancestor of Neolepetopsidae and Lepetidae possessed a neolepetopsid-type radula. If this is the case, perhaps *Paralepetopsis* represents a lineage of Lepetidae retaining the plesiomorphic radula type, and in this scenario Neolepetopsidae, as currently considered, would indeed be paraphyletic.

Another possibility is that the *Paralepetopsis*-like radula may be present in two evolutionarily distinct lineages, one in Neolepetopsidae and one in Lepetidae. This is suggested by the two sequences of '*Paralepetopsis*' in Aktipis and Giribet (2010) and Goffredi et al. (2017), which clustered with other neolepetopsids, unlike *P. pollita* sp. nov. – but as these sequences are not linked to described species or morphological data it is unclear whether they truly are *Paralepetopsis*. Testing these hypotheses would require sequences from other described neolepetopsid species and especially *P. floridensis*, the type species of *Paralepetopsis*. If *P. floridensis* is clustered with other neolepetopsids, then *P. polita* sp. nov. may actually represent a new genus of Lepetidae with an unusual radula. Either way, our present results strongly suggest the clade containing Neolepetopsidae and Lepetidae requires revision. Lacking genetic data to test the relationship with *P. floridensis*, we retain *P. polita* sp. nov. in *Paralepetopsis* for the time being. An improved phylogenetic reconstruction of Neolepetopsidae, using multiple genetic markers and including data from the type species of each genus, is warranted in the future.

In addition to both being deep-water clades, Neolepetopsidae and Lepetidae also share a number of characters such as the reduction of mantle tentacles, the lack of eyes, and interestingly the absence of basal plate in the radula (McLean 1990; Nakano and Ozawa 2007). Unfortunately, the specimens of *P. polita* sp. nov. available herein were first frozen and then transferred to high-grade ethanol, rendering the tissue unsuitable for detailed examination of fine internal anatomy. Future collections of fresh individuals fixed in a manner suitable for anatomical investigations, such as formalin or glutaraldehyde fixation, would be very useful in clarifying the systematic position of *P. polita* sp. nov. from an anatomical point of view.

CONCLUSIONS

A new limpet discovered from the Haima methane seep in the South China Sea morphologically matched the true limpet genus *Paralepetopsis* in the family Neolepetopsidae and is described as *P. polita* sp. nov. Phylogenetic reconstruction of Patellogastropoda including the new species revealed a surprising position where it was nested within Lepetidae instead

of other sequences assigned to Neolepetopsidae. As this is the first sequence of the genus *Paralepetopsis* with reliable morphological identification, our results point to the possibility that Neolepetopsidae may be paraphyletic. Whether this is true or alternatively *P. polita* sp. nov. represents a separate lineage from the nominal *Paralepetopsis* requires future molecular data from other described species, especially the type species *P. floridensis*. Our results also indicate that the neolepetopsid-type radula is perhaps shared by more than one evolutionary lineage, adding to the evidence that radula may not be the most reliable morphological character in assessing the systematic relationships and monophyly of true limpet groups (Fretter 1990; Chen et al. 2019).

Authors' contributions: CC conceived the project and designed the study. J-WQ participated on the research cruise and collected the specimens used herein. CC carried out morphological investigation of the specimens. ZZ and JS conducted DNA sequencing, the resulting data were analysed by CC, ZZ, and JS. CC interpreted the data and drafted the original manuscript. All authors contributed to the manuscript and gave final approval for its submission and publication.

Competing interests: We have no competing interests.

Availability of data and materials: Genetic sequences generated in this study were deposited into NCBI GenBank under the accession numbers ON810774-ON810775. All specimens examined were deposited at either the Tropical Marine Biodiversity Collections of the South China Sea, Chinese Academy of Sciences, Guangzhou, China (TMBC) or the National Museum of Nature and Science, Tsukuba, Japan (NSMT) for permanent storage.

Consent for publication: N/A, paper does not involve personal data.

Ethics approval consent to participate: N/A, paper does not involve human subjects or laboratory animals.

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