A Noteworthy New Species of the Family Vesicomyidae from the South China Sea (Bivalvia: Glossoidea)

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Abstract: “Calyptogena” marissinica n. sp. is described from the Haima cold seep on the northwestern slope of the South China Sea, China, at depths of 1,372 m and 1,398 m. Phylogenetic reconstruction using the cytochrome oxidase c subunit I (COI) gene shows that it is sister to Calyptogena similaris but distinct from that species by a pairwise distance of 3.9%, larger than the average intraspecific variation within the genus Calyptogena sensu lato. Morphologically, the new species is distinguished from C. similaris in having a less elongate shell, with nearly straight ventral margin, and no apparent anterior lateral tooth.

Keywords: new species, deep-sea, cold seep, Calyptogena, C. similaris

Introduction

Calyptogena (sensu lato) is the most diverse group of deep-sea vesicomyid bivalves in the western Pacific region and its marginal seas. Its distribution and diversification have been the focus of recent research (e.g., Kojima et al., 2004; Audzijonyte et al., 2012; Decker et al., 2012; Johnson et al., 2017). More than forty species have been described around the world (Okutani et al., 2000; Cosel & Olu, 2009; Krylova & Sahling, 2010; Krylova & Cosel, 2011; Huber, 2015) in several “genera” recently established or revived, of which 19 are known from the western Pacific region (Okutani et al., 2009; Fujikura et al., 2012; Okutani et al., 2013; Okutani, 2017). Large vesicomyid clams recently collected from a methane seep southeast off Hainan Island, China, are judged to represent an undescribed taxon on the grounds of morphological characters and molecular phylogeny. In a molecular phylogenetic reconstruction this species was shown to be sister to C. similaris Okutani, Kojima & Ashi, 1997, hitherto known from several sites within the Nankai Trough at depths of 1,900–2,100 m. The description of the new species is given below.

Materials and Methods

Sample collection

Specimens studied herein were collected by the ROV Haima (Guangzhou Marine Geological Survey, China) from the recently discovered “Haima” methane seep between 1,372 m and 1,398 m depth off southern Hainan Island in the northern sector of the South China Sea. Within the Haima
seep, clams were collected in two areas: a shallower one at 1,372 m deep and another slightly deeper at 1,398 m. Figure 1 shows a map of the collecting site. The collections were made on two occasions, one in March 2015 (the shallower site) and another in April 2016 (both sites) (Liang et al., 2017).

**Molecular analysis**

Genomic DNA of two specimens (the holotype and paratype #3) was extracted using a DNeasy Tissue Extraction Kit (QIAGEN, U.S.A.), following the manufacturer’s standard protocol. The cytochrome c oxidase I (COI) barcoding gene was first obtained for DNA barcoding and phylogenetic reconstruction using the universal primer pair LCO1490 and HCO2198 (Folmer et al., 1994) and sequenced using Sanger sequencing in the BGI Hong Kong sequencing center. The sequences obtained were aligned and checked by eye using the programme Geneious v10.0.9 (Kearse et al., 2012). The two newly generated sequences are deposited in GenBank under the accession numbers MH006690–MH006691.

For phylogenetic analyses, available COI sequences of described *Calyptogena* species were obtained from GenBank. In the case where more than two sequences were available, one was chosen randomly. Two bivalves, including *Mercenaria mercenaria* (Linnaeus, 1758) and *Lucina pensylvanica* (Linnaeus, 1758), as well as one gastropod *Littorina littorea* (Linnaeus, 1758) were used as outgroups. Table 1 lists the taxa and sequences used, with their GenBank accession numbers.

Fig. 1. Map of the South China Sea showing the location of Haima methane seep, indicated by a star symbol. Depth contours are in 1,000 m intervals. This map was generated using the GEBCO_08 bathymetry grid (British Oceanographic Data Centre, 2010) with the WGS-84 Equidistant Cylindrical projection.
Table 1. Taxa used in the phylogenetic analysis, with GenBank accession numbers. *Calyptogena sensu lato* species are represented under genus *Calyptogena*.

<table>
<thead>
<tr>
<th>Class</th>
<th>Family</th>
<th>Taxa</th>
<th>Accession Number</th>
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<tr>
<td>Gastropoda</td>
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<td><em>Calyptogena gigas</em> (Dall, 1896)</td>
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<td></td>
<td>“<em>Calyptogena</em>” mariassinica n. sp. Paratype #3</td>
<td>MH006691</td>
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</table>

The total aligned sequence length used in the reconstruction was 526 bp. Prior to phylogenetic analyses, the most suitable evolutionary model was selected with the program PartitionFinder 2 (Lanfear et al., 2016) using the greedy algorithm and Bayesian Information Criterion (Lanfear et al., 2012) and start tree estimated by PhyML 3.0 (Guindon et al., 2010). The best substitution scheme selected for all three codon positions was the Hasegawa-Kishino-Yano model with gamma distribution. Phylogenetic reconstruction was then carried out with Bayesian inference using the program MrBayes 3.2 (Ronquist et al., 2012). Metropolis-coupled Monte Carlo Markov chains were run for 1,000,000 generations with topologies being sampled every 100 generations. The first 25% were discarded as “burn-in” to ensure that chains had converged; Tracer v. 1.6 (Rambaut et al., 2013) was used to check for convergence.

**Taxonomy**

Family Vesiomyidae Dall & Simpson, 1901

Genus *Calyptogena* Dall, 1891

Type species: *Calyptogena pacifica* Dall, 1891, by monotypy.
“Calyptogena” marissinica n. sp.  
(Figs 2–3)

**Type locality:** Haima methane seep, 1,372 m deep, off southern Hainan Island, northern sector of the South China Sea. Paratypes #1 and #3 were collected at the same location as the holotype, whereas paratype #2 was collected at a slightly deeper area (1,398 m deep) within the same seep.

**Type specimens:** Holotype: Hong Kong Baptist University, Department of Biology Specimen Collection (HKBU Mol-2018010001). Paratypes #1–3: National Museum of Nature and Science, Tsukuba (NSMT-Mo 79001 to 79003). For details, see Measurement section below.

**Description:** Shell thin, rather lightly-built, chalky, elongate-oval in profile, slightly attenuated in front and somewhat flared posteriorly (Fig. 2). Shell height about a quarter of shell length. Umbo situated at about 20% anteriorly, with weakly prosogyrous beaks. External surface covered by straw-colored, varnished periostracum, which is occasionally commarginally lamellate, particularly in front of umbral region and marginal region. Periostracum corroded behind umbral region suggesting presence of boundary between buried portion and exposed portion. Round anterodorsal and anterior margins gently continuous to sub-straight ventral margin. No lunule present. Posterior margin also round. A low, obscure radial ridge running from umbo to postero-ventral corner. Escutcheon not demarcated, dorso-posterior margin weakly convex, representing greatest position in shell height, and generating nearly flat area between dorsal margin and dorsal rim of radial ridge. Ligament amphidetic, strong, extending about half length from umbo to posterior tip. Calcareous fibrous layer rather weak.

Internal surface porcellaneous white, with weak luster. Obsolete radial lines present all over, somewhat more distinct in postero-marginal region. Anterior adductor scar broad comma-shaped, while posterior one oval with top protruding. Pallial line starting from ventro-posterior corner of anterior adductor scar, smooth, ending in acute pallial horn of shallow sinus. No secondary attachment scar present.

Hinge plate (Fig. 3) rather weak. In right valve, central cardinal tooth (1) deltoid, horizontally

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**Fig. 2.** “Calyptogena” marissinica n. sp. A. Holotype (HKBU Mol-2018010001), SL = 103.7 mm. B. Paratype #1 (NSMT-Mo 79001), SL = 149.6 mm. C. Hinge armature of holotype. D. Internal view of the right valve of paratype #1. Scale bars: 20 mm. See text for abbreviations.
protruding, bearing two to three low radial ribs on roof; anterior ramus of subumbonal cardinal tooth (3a) very low; posterior ramus (3b) strong, nearly parallel to dorsal margin, creating an arch of about 150° with 3a, overhanging tooth 1 at proximal part, bearing minute dentition on top; deep socket present in front; anterior pedal retractor scar present behind 3a; nymph narrow with elongate nymphal ridge (nr); neither lateral teeth nor subumbonal pit present. Of left valve, central cardinal tooth bifurcated, minute notch present on bridge between anterior ramus (2a) and posterior one (2b), which curves in \( \wedge \)-shape; anterior ramus of subumbonal cardinal tooth indistinct, posterior ramus of the same (4b) rib-like, disposed in radial position almost parallel to nymphal ridge (nr).

**Etymology:** After the type locality, meaning “Chinese Sea”.

**Measurements:** Holotype (HKBU Mol-20180100001): SL 103.7 mm, SH 54.2 mm, SW 35.9 mm. GenBank accession number: MH006690. Paratype #1 (NSMT-Mo 79001): SL 146.9 mm, SH 73.7 mm, SW 52.6 mm (Empty shell, with cracks on left valve). Paratype #2 (NSMT-Mo 79002): SL 188.0 mm, SH 90.0 mm, SW 52.6 mm (Empty shell, with cracks on left valve). Paratype #3 (NSMT-Mo 79003): Not measurable (Only dorsal half of right valve); GenBank accession number: MH006691.

**Distribution:** Hitherto known only from the type locality: Haima methane seep, south off Hainan Island. 1,372–1,398 meters depth.

**Discussion**

**Morphology**

As the present new species was proved to be most closely related to *Calyptogena similaris* in the phylogenetic reconstruction using the mitochondrial COI gene, morphological comparison will firstly be made between these species. As Krylova & Sahling (2010) did not give the generic assignment for *C. similaris* in their thorough taxonomic review of the Vesticomyidae, it is more appropriate to place the new taxon in *Calyptogena sensu lato* pending future revision of the family,
than to assign it to any of the current “genera”.

The shell of *C. similaris*, from 2,000 m deep in the Nankai Trough, is far more elongate than “*C.* marissinica” n. sp. (SL/SH 2.66 in *C. similaris* vs 2.00–2.08 in “*C.* marissinica”; see Okutani *et al.*, 1997) with a concave ventral margin. The tooth 1 of the right valve in *C. similaris* is almost vertical, in comparison to the oblique tooth 1 in the new species. This results in a similar difference in the direction of the socket between tooth 2a and 2b of the left valve. The cardinal armature of *C. similaris* bears a certain similarity to that of the new species, but carries an apparent anterior lateral tooth (AI and AII in Okutani *et al.*, 1997; PI & PII loc. cit. are defined as “nymphal ridge” in the present description).

“*Calyptogena*” marissinica n. sp. superficially resembles *Calyptogena* (*Phreagena*) edisonensis Okutani, Kojima & Kim, 2004 from the Edison Seamount in the New Ireland Basin, Southwest Pacific. *Calyptogena* (*P.*) edisonensis has a similar tooth configuration to “*C.* marissinica” n. sp., but differs from this new species in having an apparent subumbonal pit. Furthermore, the two species can be separated by their external forms, in that the radial ridge running from umbo to postero-ventral corner is more significant in “*C.* marissinica” (particularly in young specimens) and that the dorsal-posterior margin is more raised in “*C.* marissinica”.

One congeneric species, *Calyptogena nanshaensis* Xu & Shen, 1991, has been reported from the South China Sea, at 6°04´N, 113°37´E, 2,626 m depth (photo in Lutaenko & Xu, 2008). According to its original description, 2a and 2b are well fused on top with lamellar 4b; 3a and 3b are situated apart in a wide angle, 3b overlying 3a (Xu & Shen, 1991). Tooth 1 was not mentioned.

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**Fig. 4.** Reconstructed phylogenetic tree of *Calyptogena sensu lato* using a 526 bp fragment of mitochondrial COI gene. Node values indicate Bayesian posterior probability, only those above 0.7 are shown. GenBank accession numbers of the COI sequences used are shown in parentheses.
there. *Calyptogena nanshaensis* is also different from the present new species in having a straight dorsal margin and a bluntly acute posterior end. Furthermore, *C. nanshaensis* is much smaller (holotype 66.4 mm in shell length) than “*C.* marissinica n. sp.

**Molecular phylogeny**

The consensus tree obtained from the Bayesian phylogenetic analysis is shown in Fig. 4. “*Calyptogena* marissinica n. sp. is clearly nested within a fully supported (Bayesian posterior probability, BPP = 1.00) monophyletic clade corresponding to *Calyptogena sensu lato* consisting of all included *Calyptogena* (sensu lato) species. The two specimens of the new species analyzed had identical sequence. Among the described species included, the new species was placed sister to *C. similis* (BPP = 1.00). The pairwise distance between these two species was 3.9\% (20/513 bases difference). This is higher than the pairwise distance generally found between other vesicomyid species, which is < 2.0\% (Baco et al., 1999; Peek et al., 2000; Martin & Goffredi, 2012), and does not contradict with recognizing the present taxa as a new species. The “*C.* marissinica n. sp. – *C. similis* pair was placed sister (but with weak support, BPP = 0.72) to the fully supported (BPP = 1.00) *C. laubieri* and *C. gigas* pair.

**In situ observations at the Haima seep**

“*Calyptogena* marissinica n. sp. is the second vesicomyid discovered to inhabit chemosynthetic ecosystems in the northern sector of the South China Sea. It is one of the two most visually dominating benthic species in the Haima methane seep area. Another dominant species is the mytilid *Bathymodiolus platifrons* Hashimoto & Okutani, 1994, representing a westward range extension of this species in the South China Sea (Feng et al., 2015; Hashimoto & Okutani, 1994; Wong et al., 2015; Li, 2017; Sun et al., 2017; Xu et al., 2017). “*Calyptogena* marissinica n. sp. was also found associated with a number of other molluscs at the Haima seep area. These include *Acharax* cf. *johnsoni* (Dall, 1891) (although only dead shells were seen and none were collected), small clusters of a conoidean gastropod strongly resembling *Phymorhynchus buccinoides* Okutani, Fujikura & Sasaki, 1993 (none were collected), and two specimens of a small white limpet that were found attached to the shell of paratype #2 of “*C.* marissinica n. sp. Observation of radula indicate the limpet likely belongs to the Neolepetopsidae.

**Acknowledgements**

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A New Species of Vesicomyidae from the South China Sea


南シナ海北部から発見されたオトヒメハマグリ科の
1新種ハイナシシロウリガイ（新称）

陳 充・奥谷喬司・梁 前勇・邱 建文

要 約

南シナ海北部、中国海南島南西沖で新しく見つかったメタン渦水域“海馬渦水”（水深1,372 m 〜1,398 m）からシロウリガイ類の未記載種が発見されたので新種として記載した。

“Calyptogena” marissinica n. sp. ハイナシシロウリガイ（新種・新称）

ホロタイプの殻長は103.7 mm であるが、パラタイプの一つ（死殻）は殻長188.0 mm に達する。この類としてはやや太短く、殻高は殻頂の後方で最大となり殻長の20％前後。殻皮は光沢のある茶色で、成長脈が著しい。月面も幅面も無い。触感は後背縁の1/2 に達する。右殻の中央主歯（Fig. 3: 1；以下同様）は三角錐状で殻頂下主歯前歯（3a）は短いが後歯（3b）は長く、前歯と15° をなす。左殻の中央主歯は二叉（2a, 2b）、殻頂下前主歯は不明瞭であるが、後主歯（4b）は放射状に配置する。歯丘（nr）はよく発達する。殻頂下洞は無い。

備考：本種のミトコンドリア COI 領域のデータから、本種は南海トラフの水深2,084 m から記載されたニヨリシロウリガイ Calypptogena similis Okutani, Kojima & Ashi, 1997 と同じクレードに入ることが明らかである。ニヨリシロウリガイとは一層細長く渦入した股縁を持つことなどから一見して区別ができるが、現在いわゆる広義の Calypptogena は形態よりも分子系統解析によって属が細分化されつつあるにも拘らず、ニヨリシロウリガイは何れの既存の“属”に対しても配置されていない現状から、本新種の属位は敢えて Calypptogena のままとして扱った。