

Description of a new species of the genus *Cultellus* Schumacher, 1817 (Bivalvia, Pharidae) from the South China Sea, based on integrative taxonomy

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Abstract

The present study describes a new species within the genus *Cultellus* Schumacher, 1817 collected from the South China Sea. An integrative taxonomic approach incorporating morphological comparisons, geometric morphometrics and genetic analyses was used to identify and differentiate the new species. *Cultellus exilis* sp. nov. is distinguished from its congeners by its slender, fragile and translucent valves, curved posteroventral margins and relatively large protractor scars. The geometric morphometric analyses, based on outlines data, indicated that samples of *Cultellus exilis* sp. nov. clustered together and were distinctly separated from other species. Multiple species delimitation results, based on the mitochondrial COI gene, support the separation of *Cultellus exilis* sp. nov. from its related congeners. Phylogenetic analyses of a nuclear (28S rRNA) and two mitochondrial (COI, 16S rRNA) genes using Maximum Likelihood and Bayesian Inference methods revealed that the species belongs to the genus *Cultellus*. The superfamily Solenoidea Lamarck, 1809, which includes the families Solenidae Lamarck, 1809 and Pharidae H. Adams & A. Adams, 1856, exhibits closer affinity to the family Hiatellidae Gray, 1824 than to Solecurtidae d'Orbigny, 1846. Furthermore, we found that the genus *Siliqua* was clustered alongside the genera *Ensiculus* and *Phaxas* as a sister clade, which contradicts the current systematics of the subfamily within the family Pharidae. This work highlights the utility of integrative taxonomy for species identification, recognition and phylogenetic investigation.

Key Words

geometric morphometrics, integrative taxonomy, Pharidae, phylogeny, Solenoidea, species delimitation

Introduction

The superfamily Solenoidea Lamarck, 1809 including two families, namely Solenidae Lamarck, 1809 and Pharidae H. Adams & A. Adams, 1856, constitutes a collective of benthic bivalves that belong to the order Adapedonta Cossmann & Peyrot, 1909 (Cosel 1993; Bieler et al. 2010; Carter et al. 2011). These molluscs are commonly referred to as “razor clams” or “jackknife clams” due to their narrow-elongated shells and sharp edges (Saeedi et al. 2013; Giacomino and

Signorelli 2021). Solenidae was regarded as a family by H. & A. Adams, containing two subfamilies Soleninae (including *Solen*, *Solena*, *Ensis*) and Pharinae (including *Pharus*, *Pharella*, *Cultellus*, *Siliqua*, *Macha* = *Solecurtus*, *Azor* = *Azorinus*, *Siliquaria* = *Tenagodus*, *Novaculina*) (Adams and Adams 1858). Cosel (1993) elevated the superfamily Solenoidea and allocated two families Solenidae and Pharidae within it, based on the number of central teeth. Solecurtidae d'Orbigny, 1846 and Psammobiidae J. Fleming, 1828 were currently taken as families within the superfamily Telli-

noidea Blainville, 1814 (Bieler et al. 2010). Huber (2010) documented that the family Solenidae encompasses the genera *Neosolen*, *Solen* and *Solena*, while the family Pharidae comprises 14 genera including *Afrophaxas*, *Cultellus*, *Ensiculus*, *Ensis*, *Phaxas*, *Sinocultellus*, *Orbicularia*, *Pharella*, *Sinonovacula*, *Novaculina*, *Nasopharus*, *Pharus*, *Sinupharus* and *Siliqua*. Furthermore, *Novaculininae* Ghosh, 1920 was considered a junior synonym of *Pharellinae* within the family Pharidae by Bolotov et al. (2018). At present, this systematics has been widely accepted.

The genus *Cultellus* was initially proposed by Schumacher (1817) to accommodate the jackknife clam *Cultellus magnus* Schumacher, 1817 = *C. maximus* (Gmelin 1791). The shells of this genus are of medium to large size, exquisitely elongated and slender, with the umbos located closer to the anterior end. Coen (1933) established the subgenus *Cultellus* (*Cultrensis*) Coen, 1933 to accommodate *Cultellus* (*Cultrensis*) *adriaticus* Coen, 1933. This species is characterised by its straight dorsal margin and two lines extending from the umbo to the posterior end (Thiele 1992), whereas this species was regarded as a junior synonym of *Phaxas pellucidus* (Pennant, 1777) (McKay and Smith 1979). Adams (1861) observed that *Cultellus cultellus* (Linnaeus, 1758) exhibits significant differences in characters compared to other species within the genus *Cultellus*. It has more curved margins and a shorter internal rib. Hence, he proposed a new genus *Ensiculus* H. Adams, 1861 within the family Pharidae for this species. Huber (2010) suggested that the genus *Cultellus* currently comprises five valid species, *C. attenuatus* Dunker, 1862, *C. hanleyi* Dunker, 1862, *C. maximus*, *C. subellipticus* Dunker, 1862 and *C. vitreus* Dunker, 1862. Three of these species are found along the coast of China: *C. maximus* (Gmelin 1791) reported from Taiwan island, *C. subellipticus* Dunker, 1862 distributed in the South China Sea and *C. attenuatus* Dunker, 1862 widespread in the Yellow Sea, the East and South China Seas (Liu 2008; Xu and Zhang 2008). Nonetheless, the generic allocations have yet to be validated by molecular analysis.

Razor clams are highly valued for their delectable taste and nutrition richness. In recent years, there has been an increasing interest amongst scholars in investigating the artificial breeding of these species (Zeng et al. 2010; Xu et al. 2013; Jiang et al. 2017; Li et al. 2022). However, the phylogeny of the genus *Cultellus* and even the superfamily Solenoidea has received limited attention. Previous studies that utilised single gene fragments or Random Amplified Polymorphic DNA (RAPD) methods consistently indicated that *Cultellus* belongs to Pharidae, a family closely related to Solenidae (Chen et al. 2005; Wu et al. 2008; Bolotov et al. 2018). Phylogenetic analysis, based on the mitochondrial genes, revealed a close relationship between the genera *Cultellus* and *Sinonovacula*, indicating their membership in the same family (Yuan et al. 2012; Yu et al. 2016). Analysis of two nuclear genes (18S and 28S) unveiled that the superfamilies Solenoidea and Hiatelloidea formed sister clades (Taylor et al. 2007). Bieler et al. (2014) demonstrated a well-supported clade between Hiatellidae and Solenoidea by utilising more genes (COI, 16S, 28S, 18S, H3) for

constructing the phylogenetic tree. A recent mitochondrial phylogenomic study further indicated a closer relationship between Solenoidea and Hiatelloidea compared to Solecurtidae (Li et al. 2022). Given that the aforementioned studies were conducted on a limited number of taxonomic groups, the phylogenetic relationships of the taxa within the Solenoidea remain inadequately investigated.

In May 2021, a previously unknown jackknife clam was collected from the South China Sea using the Agassiz trawl and subsequently preserved at the Institute of Oceanology, Chinese Academy of Sciences (IOCAS). In this study, an integrative taxonomic approach incorporating molecular and geometric morphometrics was employed to identify and differentiate this species. Additionally, historical specimens collected in the last century and currently housed at the Marine Biological Museum, Chinese Academy of Sciences (MBMCAS) were also identified as this species. This methodology enables a more comprehensive understanding of the evolutionary relationships between species and a more reliable delimitation of species. Morphological examination and genetic analyses revealed that the specimen represented a hitherto undescribed species that belongs to the genus *Cultellus*. Herein, we formally describe and illustrate the new species, which adds to the known species diversity of the genus *Cultellus* from Chinese waters.

Materials and methods

Specimen collection and morphological analyses

The specimen was collected from the shallow water in the South China Sea using the Agassiz trawl (Fig. 1). Ocean Data View software version 5.4.0 (<http://odv.awi.de>) was used to plot the collection locations. The newly-sampled specimen was preserved in 75% ethanol solution and deposited in the Marine Biological Museum, Chinese Academy of Sciences (MBMCAS), Qingdao, China, alongside other historical shell specimens. The shells were observed under a Zeiss SteREO Discovery.V12 stereomicroscope (Zeiss, Wetzlar, Germany) and photographs were captured using a Canon EOS6D camera. Shell measurements were taken to the nearest 0.01 mm using a Vernier caliper. Abbreviations used in this study: L, shell length; H, shell height; W, width of right valve.

Geometric image acquisition

Each sample was assigned to a uniform numbering and the right inner surface of each shell was photographed using a Sony ILCE-7RM4. To minimise random errors caused by factors like angle and lighting, the camera was securely fixed on a camera stand during photography. The type specimen images of *C. attenuatus*, *C. hanleyi*, *C. subellipticus* and *C. vitreus* were provided by the Natural History Museum, London. The outline data used for morphometric analysis were captured by ImageJ v.2 (Schneider et al. 2012) software to ensure consistency in

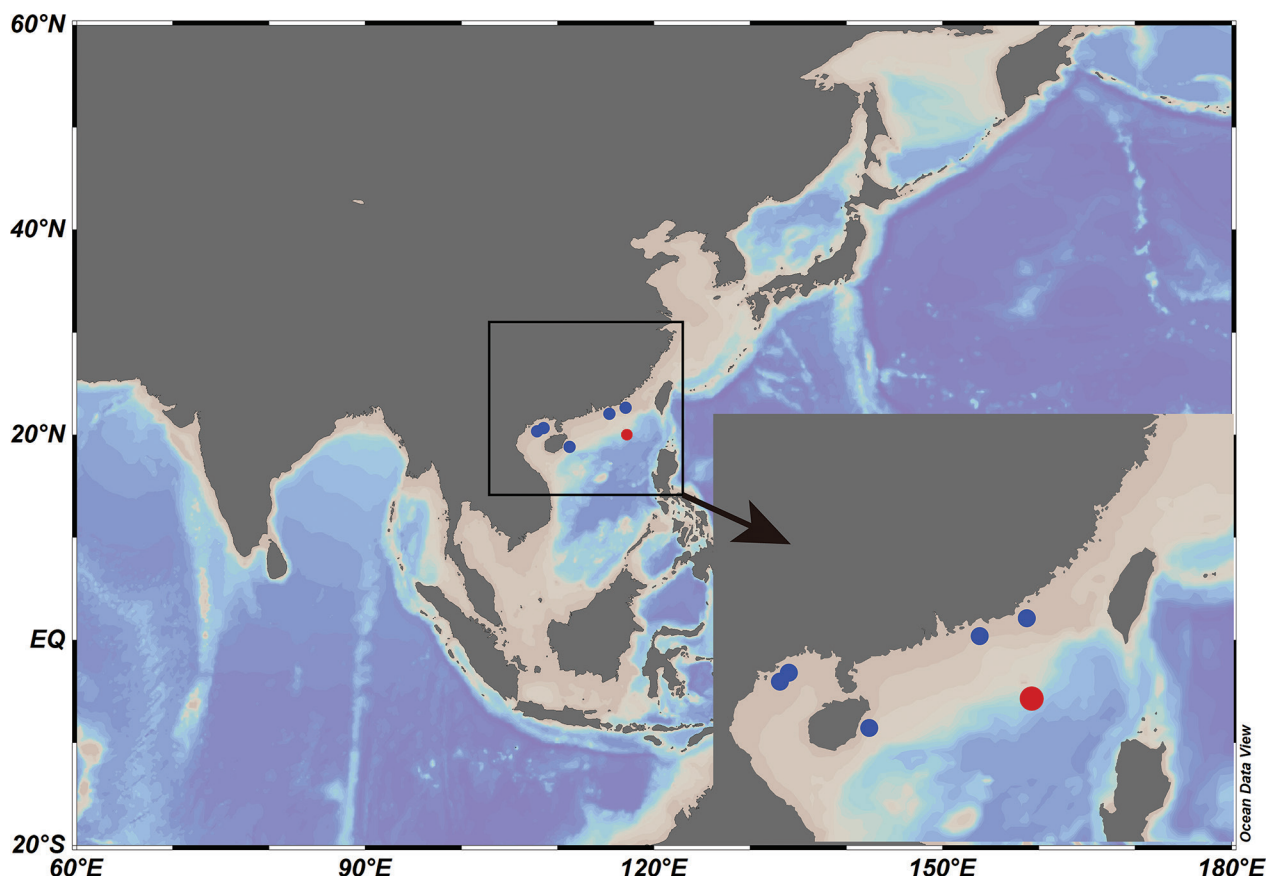


Figure 1. The location of *Cultellus exilis* sp. nov. in the South China Sea. The red dot represents holotype and the blue dots represent paratypes.

point selection direction. Subsequently, the above data were imported into Mathematica v.13.2 and uniformly sampled using the Polly.Morphometrics.12.4 package to obtain 150 semi-landmarks for each sample.

Geometric morphometric analyses

Initially, Generalised Procrustes Analysis (GPA) was conducted on the obtained data, in order to eliminate the influence of non-shape differences caused by sample size, photo size, position etc. Subsequently, Thin Plate Splines analysis (TPS) and Principal Component Analysis (PCA) were performed on the transformed data to convert the shape variation of all data points into a smaller number of uncorrelated principal component variation indicators. Then, the first three principal components were selected as representatives of morphological variation and analysed using Linear Discriminant Analysis (LDA). All the above data analyses were executed using Past v.4.12 software (Hammer et al. 2001).

DNA extraction and PCR amplification

Genomic DNA of specimens used in the present study was extracted from the foot of the animals using the Marine Animal Genomic DNA Extraction Kit (Tiangen Biotech, Beijing, China) following the manufacturer's instructions

and frozen at -20°C . The mitochondrial DNA cytochrome c oxidase subunit I (COI) region was amplified using universal primers LCO1490 and HCO2198 (Folmer et al. 1994). The mitochondrial 16S rRNA gene was amplified using 16Sa (Xiong and Kocher 1991) and 16Sb (Edgecombe et al. 2002) and the nuclear 28S rRNA gene was amplified using 28Sa and 28Sb (Whiting et al. 1997). Polymerase chain reaction (PCR) amplification was carried out in a total reaction volume of 25 μl , consisting of 12 μl of 2 $\times$ Es TaqMasterMix (Dye) (CWBio Co., Ltd, Beijing, China), 1 μl of each primer, 2 μl of template DNA and 9 μl of DNase-free ddH₂O. The amplification conditions consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 46°C for 30 s (52°C for 28S rRNA), extension at 72°C for 1 min and a final extension at 72°C for 10 min.

Sequencing and data analysis

Amplification products were detected using agarose gel electrophoresis and subsequently sequenced by Sangon Biotech (Shanghai, China). The obtained sequences were uploaded and compared to the existing sequences in GenBank (www.ncbi.nlm.nih.gov/Genbank) using the Basic Local Alignment Search Tool (BLAST) provided by the National Center for Biotechnology Information. To conduct phylogenetic analyses, available sequence

data for the superfamily Solenoidea were retrieved from GenBank (Suppl. material 1: table S1). *Hiatella arctica* (Linnaeus, 1767) and *Solecurtus divaricatus* (Lischke, 1869) were regarded as the outgroup taxa. The sequences of each gene fragment were independently aligned using MAFFT v.7 (Katoh and Standley 2013) with the G-INS-i and Q-INS-i algorithms for the protein-coding and ribosomal regions, separately. The sequences of three genes from the same individual were concatenated into a single sequence in SequenceMatrix v.1.8 (Vaidya et al. 2011). The best-fitting evolutionary model of the concatenated dataset was selected using the Akaike Information Criterion, as implemented in jModelTest 2.1.10 (Darriba et al. 2012). Phylogenetic trees were constructed based on the Maximum Likelihood (ML) using IQ-TREE v. 2.1.3 with bootstrap values for 2000 replicates (Minh et al. 2020). Bayesian Inference (BI) analysis was performed using MrBayes v.3.2.7, with three parallel runs of five million generations each, sampling every 1000 generations and burn-in set to 25% (Ronquist et al. 2012).

Multiple species delimitation methods were used to investigate the hypothesis that the specimen represents a distinct species. The COI data of 30 homologous sequences were analysed using Automated Barcode Gap Discovery (ABGD) carried out by the web-based interface (available at <https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html/>) (Puillandre et al. 2012) using the Kimura 2 parameter substitution model (TS/TV = 2.0), prior for the maximum value of intraspecific divergence ranging between 0.001 and 0.1, 10 recursive steps and a relative gap width (X) of 1.0. Bayesian implementation of the Poisson Tree Processes (bPTP) species delimitation model was analysed (Zhang et al. 2013) at the web server of the Heidelberg Institute for Theoretical Studies, Germany (<http://species.h-its.org/>) using ML phylogenetic trees as input data. Markov Chain Monte Carlo (MCMC) runs were performed for 100,000 steps and sampled every 100 steps with burn-in set to 0.1. Removing the outgroup in initial runs did not affect delimitation results. The General Mixed Yule Coalescent (GMYC) model (Pons et al. 2006) was used in PyR8S of iTaxoTools v.0.1 (Vences et al. 2021) with default parameters to determine the species of analysed individuals, based on ultrametric time trees derived from single-locus data. Pairwise comparisons of the *p*-genetic distances, based on COI genes, were also calculated by MEGA X (Kumar et al. 2018).

Results

Systematics

Superfamily Solenoidea Lamarck, 1809

Family Pharidae H. Adams & A. Adams, 1856

Genus *Cultellus* Schumacher, 1817

Type species. *Solen maximus* Gmelin, 1971 (by monotypy).

Cultellus exilis sp. nov.

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Figs 2, 3, 4F

Type specimens. *Holotype*: MBM229032, one complete individual, collected on 28 May 2021 by Agassiz trawl on the research vessel “TAN KAH KEE” (muddy bottom, depth 55 m). *Paratype*: MBM264485, one complete specimen, collected from the Beibu Gulf, China, January 1962 (muddy bottom, depth 55 m); MBM264488, two complete specimens, collected from the Beibu Gulf, China, December 1959 (Habitat unknown); MBM264497, one complete specimen, collected from Haimen, Guangdong Province, China, March 1954 (Habitat unknown); MBM264500, one complete specimen, collected from Shanwei, Guangdong Province, China, January 1995 (Habitat unknown); MBM229040, one complete specimen, collected from Hainan Province, China, January 1959 (muddy bottom, depth 91.5 m).

Type locality. Neritic zone of the South China Sea (depth 55 m, 20°1'13.44"N, 117°10'45.84"E); Muddy bottom.

Etymology. The specific epithet “*exilis*” is derived from the Latin, referring to its slender shell, which is a remarkable difference from other species in this genus.

Description. Shell medium in size, flattened, elongate, fragile, glazed, translucent, equivalve, inequilateral. Some specimens are covered with various-sized bubbles on their surface (Figs 2A–D, 3A–D). Umbo depressed, weakly prosogyrous, situated at anterior 2/9 of shell. Anterior area short, elliptical, posterior area elongated, both ends gaping, anterior end slightly turned-up, posterior end slightly more pointed than anterior end; antero-dorsal margin downward sloping, postero-dorsal margin almost straight, ventral margin rather arcuate, postero-ventral margin more curved than antero-ventral, middle-ventral margin almost straight. Periostracum of valve surface yellowish; shell surface with fine, dense, regular and non-isometric co-marginal growth lines, without radial lines; lunule and escutcheon absent. Ligament short, but strong, dark brown and situated opisthodontic.

Interior shell off-white, with yellowish periostracum in margin. Each valve with one white, strong, thin, straight, internal radial rib extending from umbonal area to anterior end, forming ca. 21° angle with antero-dorsal margin (Fig. 2H–J). Anterior adductor muscle scar subtriangular, obvious, near internal rib; posterior adductor scar falciform, almost straight near the dorsal margin; pedal retractor scars rhombic, situated at the corner between umbo and internal rib; pallial sinus shallow, pallial lines connected to adductor scars. Left valve with three strong and projecting cardinal teeth, central tooth bifid at the top and confluent at the base, without lateral tooth (Fig. 2H); right valve with two long and strong cardinal teeth, anterior protruding downwards, posterior pointing to the back end, without lateral tooth (Fig. 2I).

Siphons short and bifurcated, situated at posterior area; gills transversely folded; foot strong, depressed, truncated, situated at anterior area.

Measurement. Holotype: MBM229032: L = 34.66 mm, H = 10.35 mm, W = 2.27 mm. Paratype: MBM264485: L =

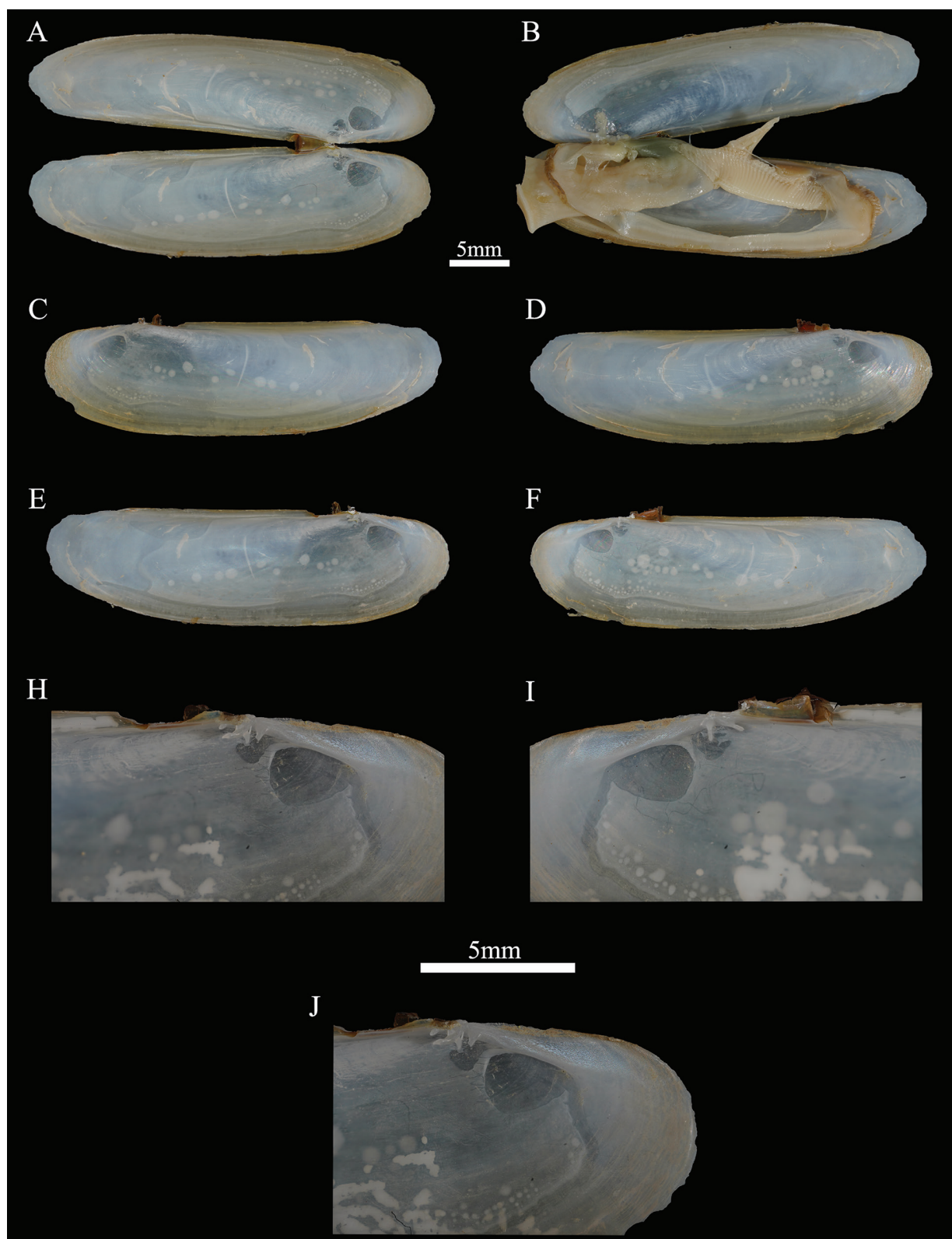


Figure 2. *Cultellus exilis* sp. nov. (holotype) **A, B.** MBM229032, L = 34.66 mm; **C, D.** Exterior views of left and right valve; **E, F.** Interior views of left and right valve; **H.** Hinge of left valve; **I.** Hinge of right valve; **J.** Internal radial rib of left valve.

45.42 mm, H = 12.65 mm, W = 2.37 mm; MBM264488: L = 61.51 mm, H = 16.26 mm, W = 3.73 mm; MBM264497: L = 77.05 mm, H = 21.14 mm, W = 4.72 mm; MBM264500: L = 73.30 mm, H = 19.74 mm, W = 4.52 mm; MBM264601: L = 44.32 mm, H = 12.42 mm, W = 2.33 mm.

Remarks. The shells of *Cultellus* are relatively less elongated compared to those of the other genera of Solenoidea. Typically, the shells of *Cultellus* have rounded anterior and posterior ends, an anteriorly located umbo, strong internal ribs and three cardinal teeth on the left

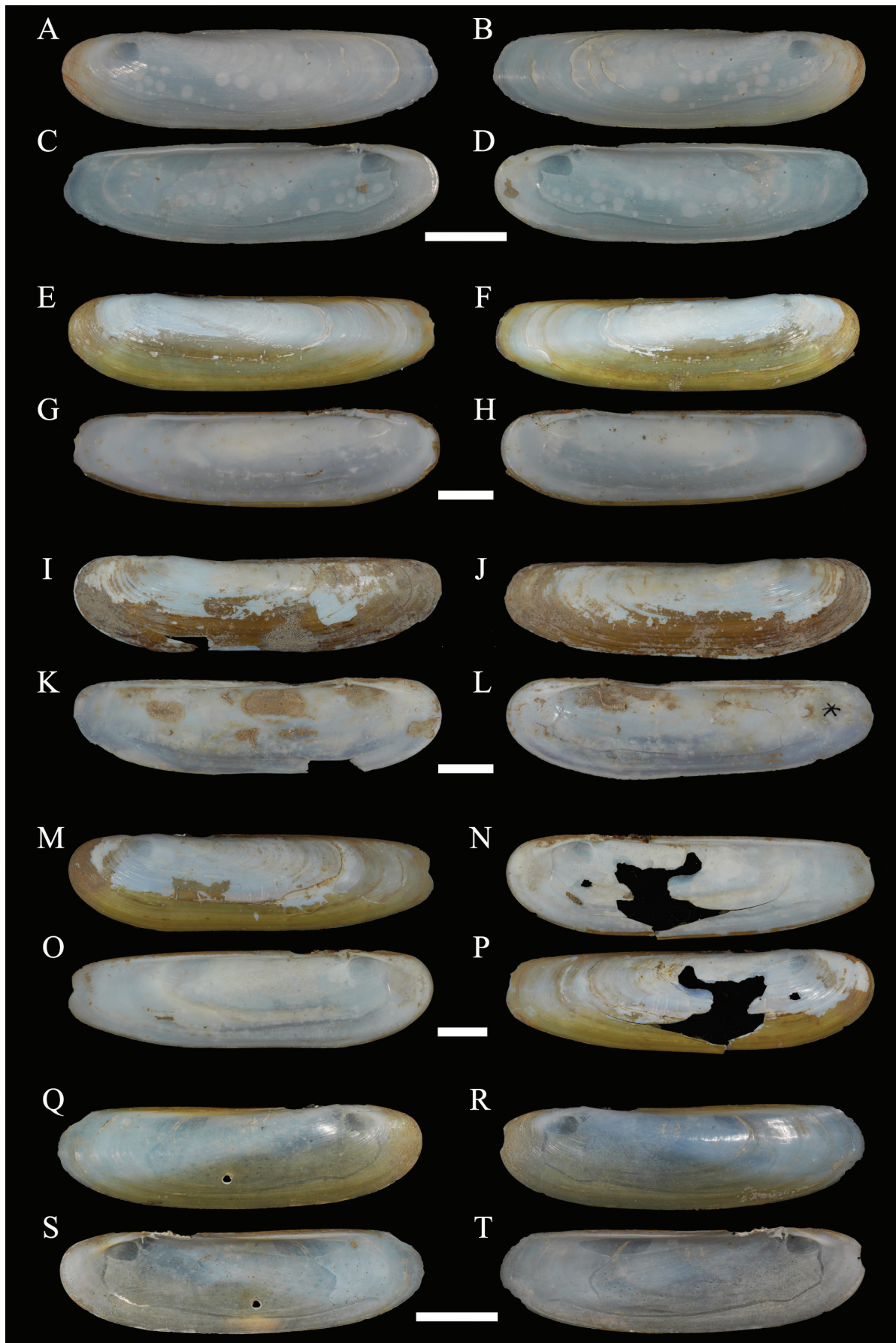


Figure 3. *Cultellus exilis* sp. nov. (paratypes) **A–D.** MBM264485; **E–H.** MBM264488; **I–L.** MBM264497; **M–P.** MBM264500; **Q–T.** MBM229040. Scale bars: 10 mm.

valve and two on the right (Thiele 1992). The remarkable morphologic similarity amongst *Cultellus* members makes species identification challenging. The distinguishing features that separate *Cultellus exilis* sp. nov. from other species in this genus are its particularly slender, fragile and translucent shells. Additionally, the new species differs from the *C. maximus* (Fig. 4E) in that its posterior end is notably narrower than its anterior end (Gmelin 1791). It closely resembles *C. attenuatus* (Fig. 4A) and *C. vitreus* (Fig. 4D). However, *C. attenuatus* (Fig. 4A) differs from the new species by the anterior area of the shell, which is obviously wider than the posterior part. Additionally, the posterior end of *C. vitreus* is slightly truncated (Fig. 4D), whereas *Cultellus exilis* is more curved. *C. subellipticus* (Fig. 4C) differs from the new species by the posterior area of the shell, which is much wider than the anterior part (Dunker 1862; Clessin 1888). Compared to this new species, the length-to-height ratio of *C. hanleyi* is 3.3–3.4:1 (Fig. 4B), whereas the ratio of *Cultellus exilis* is 3.5–3.8:1.

Geometric morphometrics of shell outlines. Principal Component Analysis (PCA) was conducted on the Procrustes alignment outline data of 32 samples representing six species. The results revealed that the first three principal components accounted for a cumulative contribution rate of 96.88% (PCA1 80.79%, PCA2 13.97% and PCA3 2.12%), indicating that they can represent the major morphological differences amongst the samples. According to the extreme distortion state of the thin plate spline plots, the main difference of all samples along the PCA1 axis occurs in the relative height of the shell (Fig. 5B, C). Differences in the PCA2 axis mainly involve the length-to-height ratio of the shells. Shells were increasingly elongated from the PCA2+ to PCA2- direction (Fig. 5A, D). Considering the distortion observed along both the PCA1 and PCA2 axes, the main variation in the outlines of the six species within the genus *Cultellus* is the relative length and height of the shell. Discriminant analysis of all data indicated that the first two variance values explain 96.98% of the overall variation. A Discriminant analysis plot clearly demonstrated that *Cultellus exilis* sp. nov. is distinctly separated from the other species, forming its own cluster (Fig. 6). Therefore, the geometric morphometric analysis results, based on outlines, support distinguishing the new species from other species within the genus *Cultellus*.

Species delimitation analyses. All species delimitation analyses, namely ABGD, bPTP and GMYC, conducted on the COI sequences, resulted in the delimitation of eleven species in the superfamily Solenoidea. The analysis confirmed that *Cultellus exilis* sp. nov. is a distinct species from other *Cultellus* species. Sequences of the genus *Cultellus* were delimited into four species, i.e. *C. attenuatus*, *C. subellipticus*, *C. maximus* and *Cultellus exilis* sp. nov. (Fig. 7). In addition, based on the available molecular data, the analysis of a 581-bp fragment of the COI gene yielded a 12.2%–13.9% pairwise distance between *Cultellus exilis* sp. nov. and other congeners, a divergence higher than the intraspecific variation (0–1.7%) of the genus *Cultellus* (Suppl. material 1: table S2).

Phylogenetic analyses. The best-fitting evolutionary model of the concatenated dataset (COI, 16S and 28S) was selected as GTR+G+I using the Akaike Information Criterion implemented in jModelTest 2.1.10. The evolutionary relationships amongst the razor clams were depicted on a phylogenetic tree constructed using the ML and BI methods. These trees exhibited highly similar topologies (Fig. 8). Phylogenetic trees obtained from the datasets were completely resolved and highly supported, as indicated by posterior probability (PP) or bootstrap scores (BS). *Cultellus exilis* sp. nov. and the two other *Cultellus* species formed a clade within the family Pharidae. The sequences of *Cultellus exilis* sp. nov. recovered a well-supported lineage distinct from the other congeners. Notably, *Ensiculus cultellus* and *Phaxas pellucidus* did not cluster together with the genus *Cultellus*. Instead, they occupied different branch positions in both ML and BI trees. On the ML tree, *Ensiculus cultellus* clustered with *Phaxas pellucidus* and formed a sister clade with the genus *Siliqua*. While on the BI tree, *Ensiculus cultellus* grouped with the genus *Siliqua*, constituting a sister lineage to *Phaxas pellucidus*. All phylogenetic results strongly supported the close relationship between pharids and solenids (PP = 0.99; BS = 92). Furthermore, the family Hiatellidae was found to be closely related as a sister to the superfamily Solenoidea (PP = 1; BS = 100). The best-fitting evolutionary model of the COI sequence was GTR+G. The phylogenetic tree inferred using ML criteria, based on single gene COI (Fig. 8), showed a similar overall topology.

Discussion

In this paper, we present a new species of razor clam that was confirmed using an integrative taxonomic approach involving shell morphological comparisons, geometric morphometrics and genetic analysis. This species can be morphologically distinguished from other congeners by its slender valve, more curved posteroventral margin and relatively larger protractor scar (Fig. 4). The geometric morphometric analyses, based on outline data, revealed that samples of *Cultellus exilis* sp. nov. were clustered together and clearly separated from other species of this genus (Fig. 6). The molecular analysis demonstrated that this species belongs to the genus *Cultellus*, but is genetically distinct from other known *Cultellus* species (Figs 7, 8).

The genus *Cultellus* currently encompasses five valid extant species that inhabit the waters of the Indo-West Pacific, with most ranging from tropical to temperate seas (Okutani 2000; Swennen et al. 2001; Min et al. 2004; Thach 2005; Thach 2007; Xu and Zhang 2008; Huber 2010; Coan and Petit 2011; Poppe 2011). The new species, *Cultellus exilis* sp. nov., was also found from this region (Fig. 1). The paratype specimens were previously misidentified as *C. attenuatus*, but our study corrected the misidentifications using integrative taxonomy. Geometric morphometrics has been used to quantify morphological changes in the process of biological evolution since

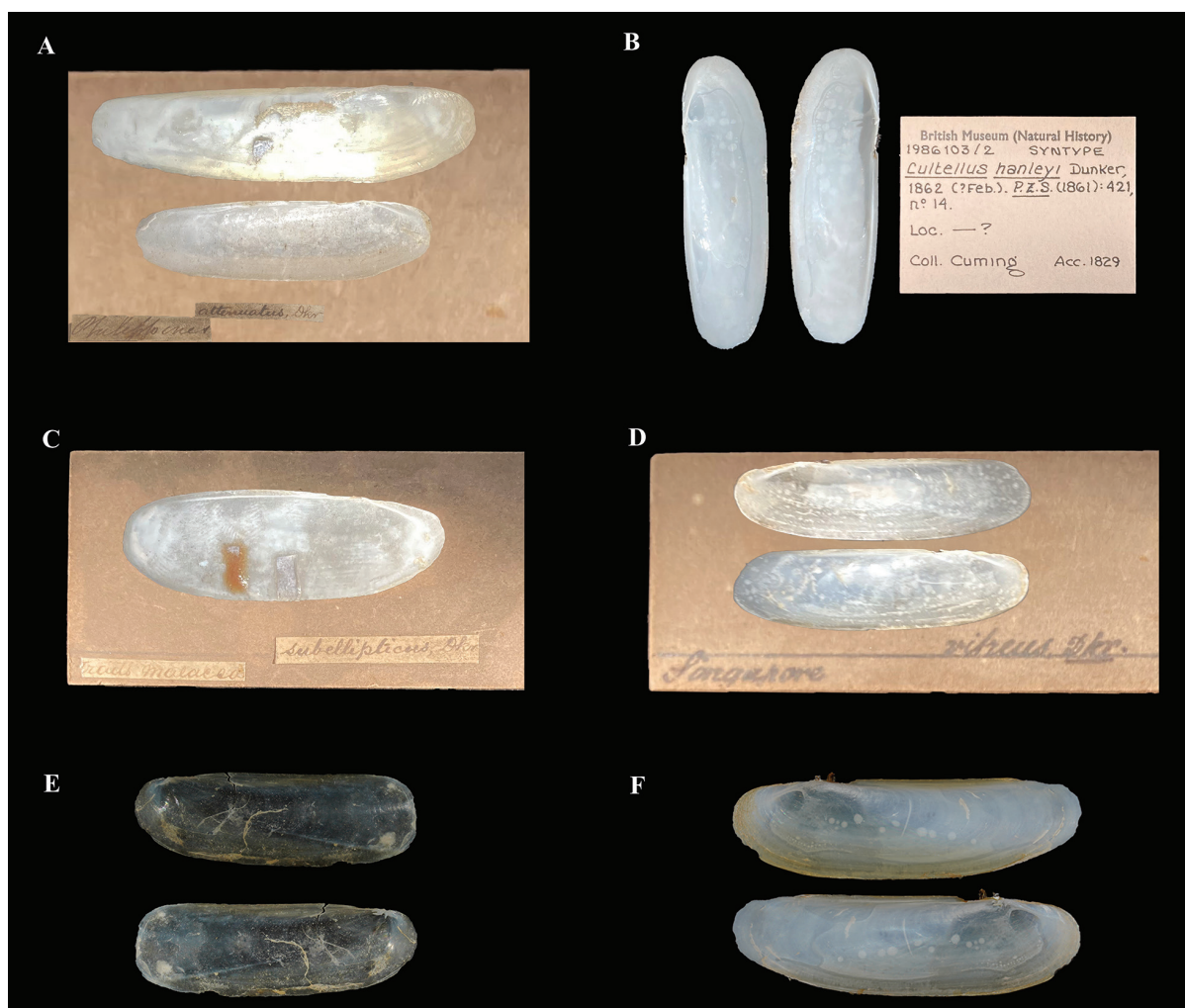


Figure 4. **A.** *Cultellus attenuatus* Dunker, 1862. Two syntypes, NHMUK 20240145: L = 54.7 mm, H = 13.9 mm; L = 45.2 mm, H = 12.2 mm; **B.** *Cultellus hanleyi* Dunker, 1862. One syntype, NHMUK 1986103: L = 54.1 mm, H = 16.1 mm; **C.** *Cultellus subellipticus* Dunker, 1862. One syntype, NHMUK 20240146: L = 46.3 mm, H = 16.0 mm; **D.** *Cultellus vitreus* Dunker, 1862. One syntype, NHMUK 20240147: L = 39.5 mm, H = 10.9 mm; **E.** *Cultellus maximus* (Gmelin, 1791). MBM264615: L = 33.6 mm, H = 11.3 mm; **F.** *Cultellus exilis* sp. nov. MBM229032: L = 34.66 mm, H = 10.35 mm.

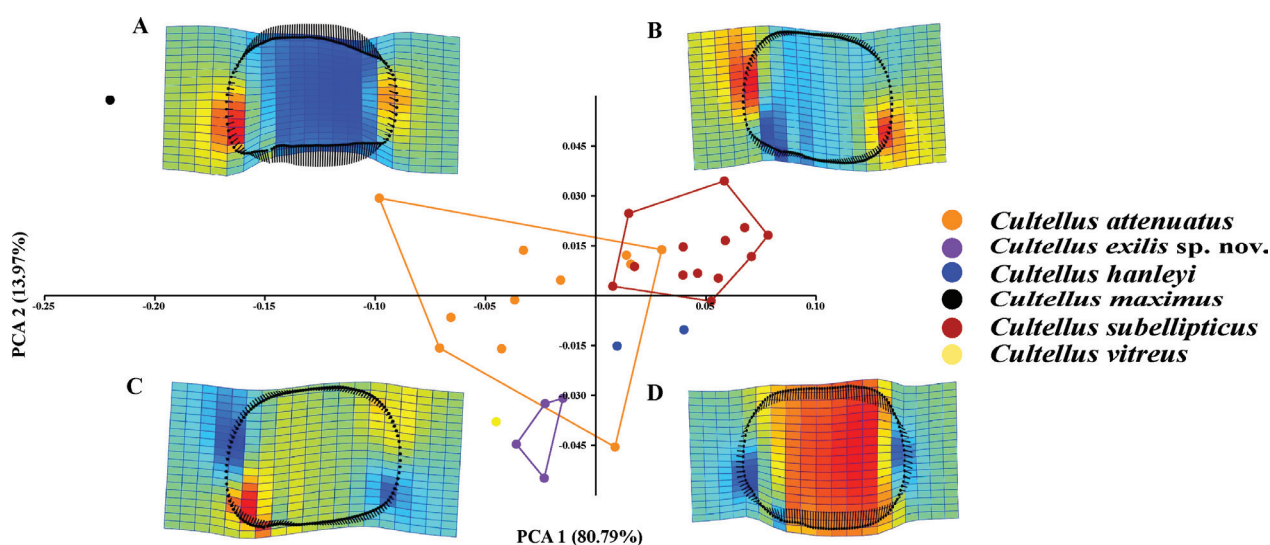


Figure 5. Principal component and thin plate spline analyses, based on outlines of the genus *Cultellus*. **A.** The extreme distortion of the outlines in the negative of PC2; **B.** The extreme distortion of the outlines in the positive of PC1; **C.** The extreme distortion of the outlines in the negative of PC1; **D.** The extreme distortion of the outlines in the positive of PC2. Each colour of the dot in the principal component analysis diagram represents a species.

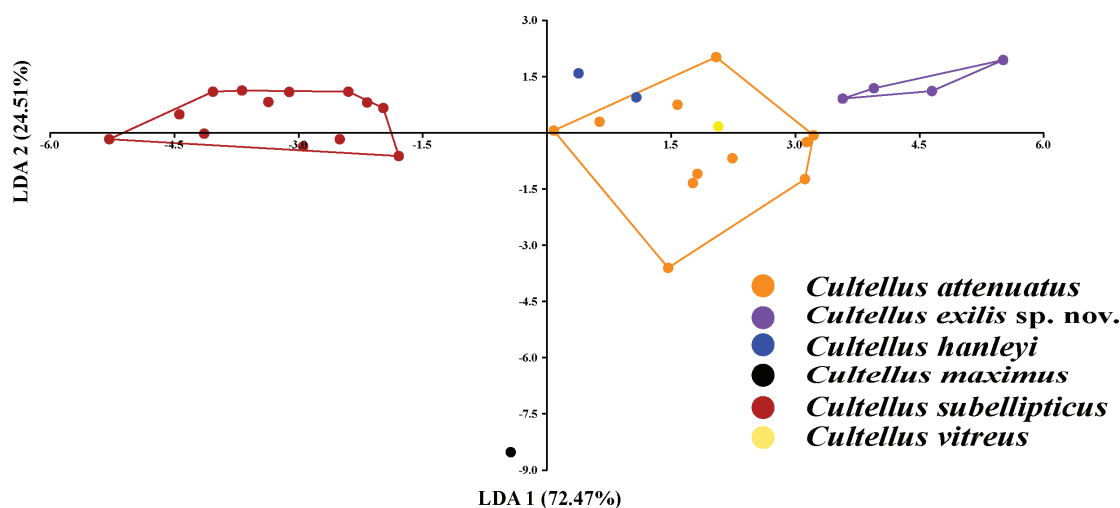


Figure 6. Scatter plot of discriminant analysis of the genus *Cultellus*. Each colour of the dot in the principal component analysis diagram represents a species.

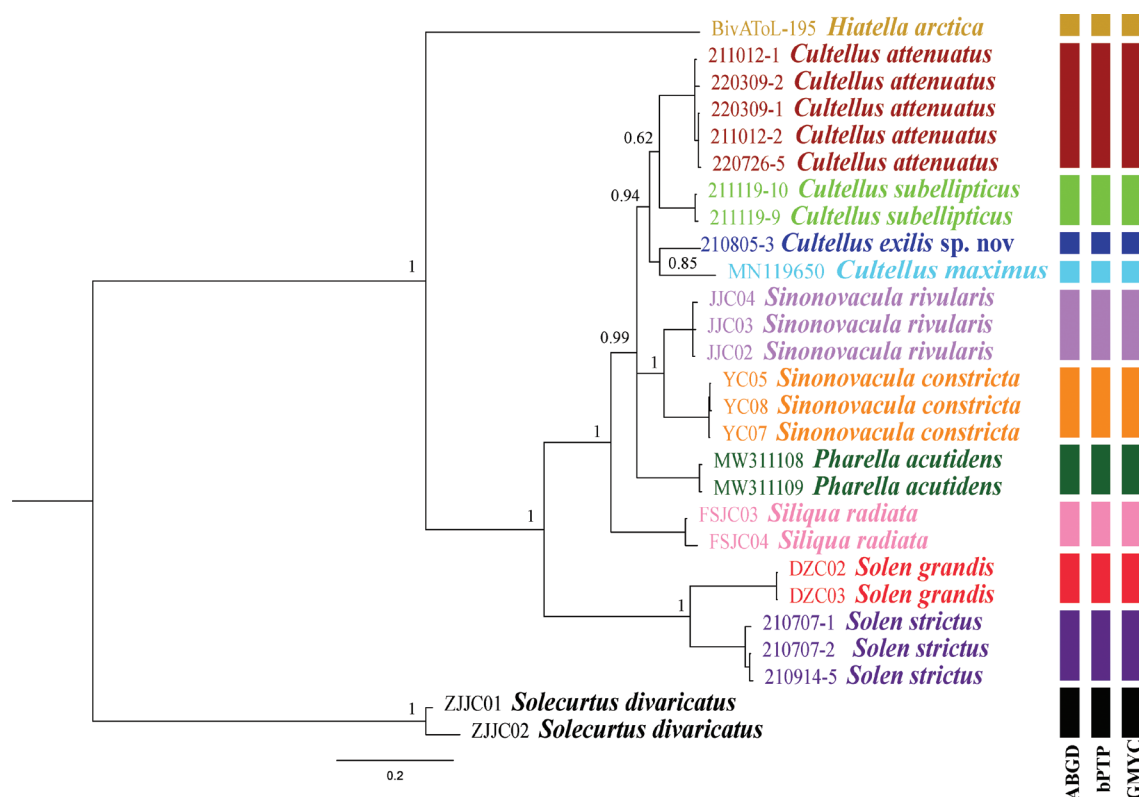


Figure 7. Phylogenetic tree obtained by the Maximum Likelihood (ML), based on COI gene sequences. Numbers adjacent to nodes refer to ML bootstrap scores (BS < 50 represented by “*”). The results of three species delimitation methods are shown on the right of the figure (Each species is represented by a single colour).

its proposal (Jacobson and Moyers 1993). Although it is not as widely used in molluscs as other methods, some studies have shown that geometric morphometrics analysis is suitable for explaining the convergent evolution of bivalves (Serb et al. 2011; Sherratt et al. 2016), discovering taxonomic information for species identification (Marinho and Arruda 2021) etc. This study highlights the importance of using integrative taxonomy for species identification and phylogenetic studies. In recent years, integrative taxonomy has been proven to be more reliable for biological taxonomy and species identification, especially for morphologically similar or indistinguishable

species (Zhang et al. 2018a, 2018b; Sørensen et al. 2020; Zhang et al. 2020). Furthermore, integrative taxonomy facilitates phylogenetic analysis and exploration of evolutionary relationships amongst closely-related species (Sun et al. 2016; Liao et al. 2021). Integrative taxonomy will play a significant role in the taxonomy and phylogeny of marine organisms in the future.

The findings of this study are largely consistent with the previous molecular research on the phylogenetic relationships of the superfamily Solenoidea. Our phylogenetic trees, constructed using mitochondrial (COI, 16S) and nuclear (28S) genes, revealed that *Cultellus*,

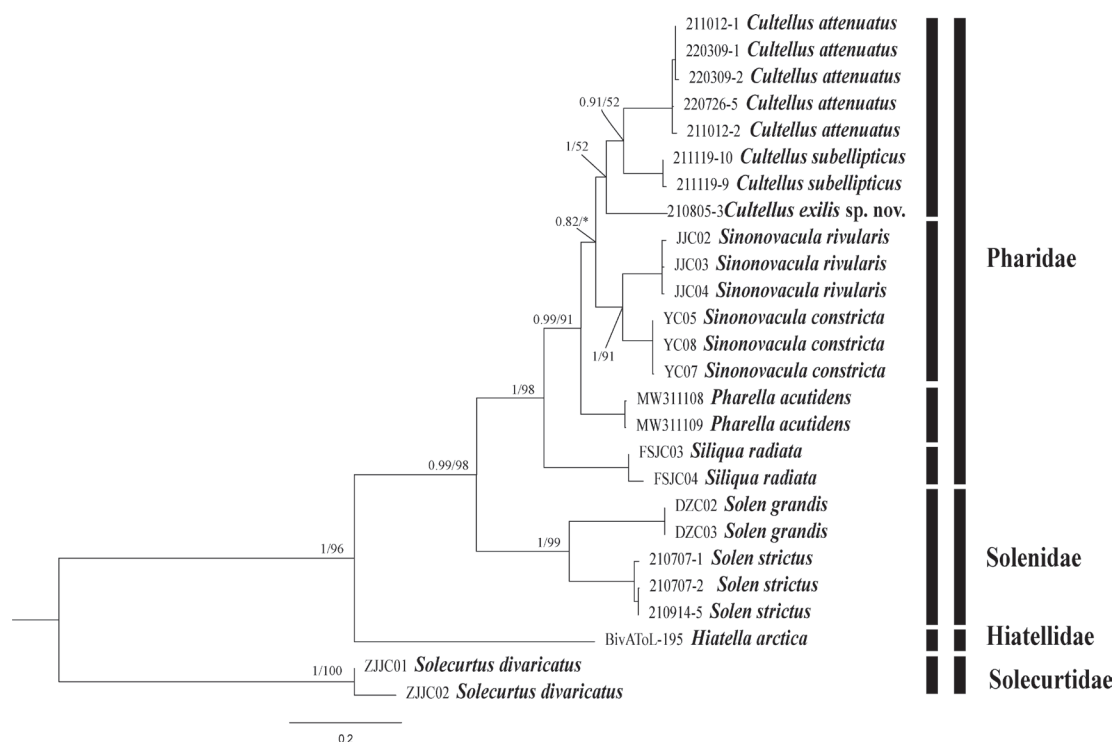


Figure 8. Phylogenetic tree inferred by Bayesian Inference analysis (BI) and Maximum Likelihood (ML), based on concatenated dataset of COI, 16S and 28S genes. Bayesian posterior probability and Maximum Likelihood bootstrap scores (left and right, respectively. “-” represents different branch position on ML and BI trees) are shown above the branch.

Sinonovacula, *Siliqua*, *Pharella*, *Ensiculus* and *Phaxas* belong to the family Pharidae. Interestingly, it appears that *Cultellus cultellus* (= *Ensiculus cultellus*) and *Cultellus* (*Cultrensis*) *adriaticus* (= *Phaxas pellucidus*) do not actually belong to the genus *Cultellus*, validating the morphological views proposed by Adams (1861) and McKay and Smith (1979). Both BI and ML results demonstrated that Pharidae and Solenidae formed a sister group. Furthermore, Pharidae, Solenidae and Hiatellidae clustered into a monophyletic group (Fig. 8), indicating that Solenoidea and Hiatellidae belong to the order Adapedonta. Bieler et al. (2014) pointed out the common characteristics amongst the species of Adapedonta, including the absence of the oesophageal lip, a simple transverse ridge pattern of the right wall sorting area and the presence of a ridge posteriorly bordering the right wall sorting area. Compared to previous studies, our study incorporated more molecular information and taxonomic groups, further verifying the reliability of the current systematics (Taylor et al. 2007; Bieler et al. 2014; Bolotov et al. 2018; Li et al. 2022).

Moreover, Bolotov et al. (2018) found that the genus *Cultellus* clustered together with *Sinonovacula* and *Pharella* in a phylogenetic tree of the family Pharidae, based on the concatenation of three genes (COI+16S+28S). They classified the genera *Novaculina*, *Sinonovacula*, *Pharella* and *Cultellus* as the subfamily Pharellinae. In our study, we observed that the genus *Siliqua* formed a sister clade with the genera *Ensiculus* and *Phaxas*. These findings collectively suggest that the current taxonomy of the family Pharidae is indeterminate and needs to be verified and reassessed by taking into account additional information.

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Supplementary material 1

Additional information

Authors: Yanan Yu, Yingyi Jiao, Junlong Zhang

Data type: docx

Explanation note: **table S1**. List of species and GenBank accession numbers of sequences used in the present study. **table S2**. Pairwise comparisons of the p-genetic distances based on COI sequences.

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