DOI: https://doi.org/10.3897/arphapreprints.e64141

Molecular phylogeny suggests synonymy of *Sandalia bridgesi* within *Sandalia triticea* (Gastropoda: Ovulidae)

Qiong Wu, BingPeng Xing, Mao Lin, GuangCheng Chen, ChunGuang Wang

ARPHA Preprints Not peer-reviewed, not copy-edited manuscript posted on February 08, 2021. DOI: https://doi.org/10.3897/arphapreprints.e64141

Molecular phylogeny suggests synonymy of *Sandalia bridgesi* within *Sandalia triticea* (Gastropoda: Ovulidae)

Wu Qiong[1]. Email: wuqiong@tio.org.cn
Xing BingPeng[1,2]. Email: bluprin@tio.org.cn
Lin Mao[1]. Email: linmao@tio.org.cn
Chen GuangCheng[1,2]. Email: chenguangcheng@tio.org.cn
Wang ChunGuang[1]✉. Email: wangchunguang@tio.org.cn
[1] Third Institute of Oceanography, Ministry of Natural Resources, PRC. 178#, Daxue Road, Siming District, Xiamen, Fujian, China, 361005
[2] Observation and Research Station of Coastal Wetland Ecosystem in Beibu Gulf, Ministry of Natural Resources, Beihai, China, 536015
Corresponding author: Wang ChunGuang Email: wangchunguang@tio.org.cn

Run head: Synonymy of Sandalia bridgesi with Sandalia triticea

Abstract
The Ovulidae are the most ecologically diverse family of the order Littorinimorpha. And the *Sandalia* is a small genus containing only three species. In the present study, we collected 44 specimens of Ovulidae (Gastropoda: Cypraeacea), belonging to six genera and eight species, including 20 *Sandalia bridgesi* and 2 *Sandalia triticea* individuals. The cytochrome c oxidase I gene (COI), 16S rRNA, and ITS1-5.8S-ITS2 sequences were obtained from the specimens and compared with sequences downloaded from GenBank to calculate genetic distances and construct phylogenetic trees. The sequences of *S. bridgesi* and *S. triticea* exhibited a high degree of similarity, and the *S. bridgesi* does not formed a separate clade, supporting the proposition that *S. bridgesi* should be considered a synonym of *S. triticea*.

Key Words: molecular phylogeny, Ovulidae, *Sandalia*, synonymy, COI, ITS, 16S

Introduction
Sandalia is a genus belonging to the family Ovulidae. First described by C. N. Cate in 1973 (Lorenz and Fehse 2009), the genus is mainly characterised by shells that are broadly ovate, a wide aperture, and denticulate labrum.

According to data from the World Register of Marine Species (WoRMS) (Tran 2009) and Worldwide Mollusc Species Data Base (WMSDB) (Liggia 2020), only three *Sandalia* species have been described as of December 2020, namely *S. bridgesi* Lorenz, 2009, *S. meyeriana* (C. N. Cate, 1973), and *S. triticea* (Lamarck, 1810). All species of the genus are distributed in the West Pacific region. *S. triticea* was first described in 1810 by Lamarck (Lorenz and Fehse 2009) and *S. bridgesi* was first described in 2009 by German zoologist Dr Felix Lorenz (Lorenz 2009). The latter species differs from its congeners by the obvious and striking transparency of the dorsum in contrast to the calloused labrum and terminals. As described by Lorenz, *S. bridgesi* and *S. triticea* are highly similar, with the main differences being as follows: *S. triticea* has lower

transparency and usually possesses a red or purple shell and light-coloured callosities. The middle portion of the dorsal side is normally light coloured, and the shell is roughly pear-shaped. Under ultraviolet (UV) light, yellow fluorescence can be seen in the terminal collars, callosities, dorsal mid-portion, and sometimes the entire shell. By contrast, *S. bridgesi* has a relatively uniformly coloured dorsal portion and a wider anterior part, and only exhibits fluorescence in a small area on either end of the shell under UV light.

DNA barcoding, which involves using a short DNA sequence for species classification, has received widespread attention since its advent (Meier et al. 2006). First proposed by Hebert et al. (2003), DNA barcoding was initially used as a tool for species identification. In the present study, we sequenced the cytochrome c oxidase subunit I (COI) gene, 16S rRNA and ITS1-5.8S-ITS2 (ITS) region for the construction of phylogenetic trees to elucidate the relationship between *S. bridgesi* and *S. triticea*. We obtained the sequence data for the internal transcribed spacer (ITS) regions of Ovulidae for the first time.

Material and methods

Specimen collection

We collected 44 specimens of Ovulidae from depths of 0–6 m during spring low tides in Beihai City, Guangxi Province, and the cities of Xiamen and Quanzhou in Fujian Province between July and November 2020. Detailed information of the collected specimens is shown in Table 1. Specimens of the coral hosts in which the ovulids resided were also collected. All specimens were morphologically identified by experts and taxonomists in accordance with the identification keys published in the Fauna Sinica (Ma 1997), Cowries and Their Relatives of China (Zhang and Wei 2011), The Living Ovulidae: A Manual of the Families of Allied Cowries: Ovulidae, Pediculariidae and Eocypraeidae (Lorenz and Fehse 2009), Conchylia 40 (2009): 38-44. (Lorenz 2009), Worldwide Mollusc Species Data Base (Liggia 2020), and Hardy's Internet Guide to Marine Gastropods (http://www.gastropods.com) (Hardy 2020a).

The specimens were observed and photographed on the dorsal, ventral, and lateral sides under a Leica S9D stereomicroscope (Fig. 1 & Fig. 2). Specimens of *Sandalia* were preserved in 95% alcohol, and coral specimens were preserved using methods for the preparation of dried specimens.

DNA extraction and sequencing

Amplification was performed on three gene regions in each specimen, namely the mitochondrial markers 16S rRNA and cytochrome c oxidase subunit (COI) and the ITS region.

DNA was extracted from each specimen's muscle tissue using the DNeasy Blood & Tissue Kit (QIAGEN, China) following the corresponding protocol for animal tissues. Nucleic acid concentration in the DNA extracts was measured using BioDrop (BioDrop, UK). Due to the presence of inhibitors in the specimen tissues, all DNA extracts were diluted 50–500 fold before PCR amplification (Reijnen and van der Meij 2017; 2019). Our experimental results indicated that the appropriate concentration for the diluted

DNA extracts was approximately 0.2 µg/mL.

Each PCR had a reaction volume of 50 µL and contained the following: 25 µL PCR mixture [Taq plus Master Mix II (Dye Plus)], 2 µL of each primer (10 µM), 5 µL (diluted) DNA extract, and 16 µL extra pure water.

Table 2 shows the details of the PCRs performed for the three gene regions.

Not all markers were successfully amplified for all specimens, successfully amplified COI, 16S rRNA, and ITS samples were sent to Sangon Biotech Co., Ltd (Shanghai, China) for PCR cleaning and sequencing. The sequences were submitted to GenBank (http: //www.ncbi.nlm.nih.gov) (Table 1).

DNA data processing and molecular analyses

Joining and alignment of the sequences and trimming of ends with low signal strength were performed using DNAMAN v. 9 (Lynnon Biosoft, Canada) and SeqMan v. 7.1.0 (DNAStar, U.S.A.). The sequences were then converted to the .fas format using the EditSeq module in Lasergene (DNAStar, U.S.A.) (Burland and G 2000). Multiple sequence alignment was performed using the ClustalW tool in MEGA X (Kumar et al. 2018).

Phylogenetic trees were constructed, and genetic distances were calculated using MEGA X, with the precision of calculations set to eight decimal places. Models with the lowest Bayesian information criterion (BIC) scores, considered to best describe the substitution patterns, were determined for all sequences using the Find best DNA models (ML) option in MEGA X (Nei and Kumar 2000; Tamura et al. 2011). Phylogenetic trees were also constructed using the Kimura 2-parameter (K2P) method (Kimura 1980). All the data were set to 1000 bootstraps in the test for inferred phylogenies to estimate the internal stability of the tree nodes. As the results from the two different phylogenetic reconstructions were congruent, only the phylogenetic trees generated from models with the lowest BIC scores have been shown in the present paper.

Using pairwise distance data exported in table form from MEGA X, heatmaps were created with Microsoft Excel (Microsoft Office 2016) and Adobe Illustrator 2020.

Results

Morphological data

Based on the photographs and descriptions provided by Lorenz (Lorenz 2009; Lorenz and Fehse 2009) and Hardy (2020a), the 44 specimens were identified as belonging to six genera and eight species. Twenty-two of the specimens were *Sandalia* species, among which 20 were identified as *S. bridgesi,* and two were identified as *S. triticea* based on differences in shell transparency, external appearance and colour.

White coral specimens were morphologically identified as *Hicksonella guishanensis,* and red coral specimens were morphologically identified as *Melithaea japonica* (Ma 1997; Mao and Jiang 1994). Table 1 shows the information and identification outcomes of the collected specimens.

Molecular data

COI

Forty-one COI sequences were successfully amplified. After editing, the consensus length of all barcode sequences was 615 bp, and no stop codons, insertions, or deletions were observed in any of the sequences. All analysed sequences were larger than 600 bp. The sequences were compared with the COI sequence of *S. rhodia* [synonym: *S. triticea* (Sartori 2014)] downloaded from the National Center for Biotechnology Information (NCBI) (GenBank-MG450349). Phylogenetic tree construction was performed using the HKY+G+I model (Tamura 1992) (Fig. 3), and the root location was confirmed by selecting the COI sequence of *Mauritia arabica* (GenBank-JF693392) as the outgroup. The data processing methods used for the amplified sequences were also adopted for the two sequences downloaded from NCBI.

The greatest and smallest genetic distances between *S. bridgesi* and *S. triticea* among our specimens were 0.0214% (20200920-T2 and20201117-H19) and 0% (20200722-T1 and 20200722-H7), respectively. The smallest and greatest interspecific genetic distances among specimens other than *S. bridgesi* and *S. triticea* were 0.1028% (20200920-H1 and 20201117-H2, 20200722-H5) and 0.2133% (20200722-HL and 20200920-T2), respectively.

16S rRNA

Thirty-nine 16S rRNA sequences with lengths of approximately 520 bp were successfully amplified. After trimming, segments with lengths of 460 bp were obtained and compared with the 16s rRNA sequence of *Cypraea gracilis* downloaded from NCBI (GenBank MH571331) to find the best model using the Bayesian approach. Results indicated that the T92 (Tamura 3-parameter model)+I (Tamura et al. 2011) model was the most optimal evolutionary model with the lowest BIC score (Fig. 4).

The greatest genetic distance between *S. triticea* and *S. bridgesi* was 0.0064% (20200722-T1 and 20201117-H7). By contrast, the smallest interspecific genetic distance among the other specimens was 0.0325% (20200722-H2 and 20201117-H6).

ITS1-5.8S-ITS2

The amplified ITS sequences had lengths of 1200–1300 bp before trimming and approximately 1200 bp after trimming. Results of analysis using the Bayesian approach showed that the K2+G model (Kimura 1980) had the lowest BIC score. The ITS sequences' phylogenetic tree was unrooted due to the unavailability of outgroup sequences (Fig. 5). The greatest and smallest genetic distances between *S. triticea* and *S. bridgesi* were 0.0051% (20200722-T2 and 20200722-H1) and 0% (20200722-T1 and 20201117-H2), respectively, and the smallest genetic distance among the other specimens was 0.0980% (20201117-H6 and 20201117-H13).

Discussion

Morphological data

S. bridgesi is a new species established based on morphological characters (Lorenz 2009). It differs from *S. triticea* mainly in shell transparency and length-to-width ratio. However, in our collected specimens, we observed the presence of a continuous

transition in the length-to-width ratio and variations in transparency with observation angle, light intensity, and individual differences. Additionally, we noticed the presence of coevolution effects between Ovulidae and Gorgonacea. Shell and mantle colour also show a high degree of variability due to influences by various environmental factors and therefore, cannot be used as marker characteristics to distinguish between species (Schiaparelli et al. 2005). For instance, *Diminovula culmen* (Lorenz and Fehse 2009), *Serratovolva dondani,* and *Crenavolva striatula* (Hardy 2020c) exhibit diverse shell colour changes. In certain species such as Crenavolva aureola, coloured bands on the shell cannot be regarded as stable traits (Hardy 2020b). During our species identification process, both S. bridgesi and S. triticea specimens were irradiated with UV light with wavelengths of 395, 365, and 254 nm, but the yellow fluorescence reported by Lorenz (2009) could not be observed. Therefore, fluorescence may not serve as a stable trait in Ovulidae. Given the subjectivity and instability involved in morphological identification, the use of molecular data for taxa identification may be an effective method for resolving the issues described above.

Molecular data

COI barcoding has been widely applied in identifying species belonging to the class Gastropoda (Hou et al. 2013; Layton et al. 2014; QUINTERO-GALVIS and RAQUEL-CASTRO 2013; Stothard and Rollinson 1997). Research evidence has shown that COI is more capable of reflecting geographical differences than shell characters in certain taxa (Simison and Lindberg 1999). As Ovulidae evolve more rapidly than other Gastropoda taxa, they possess high phenotypic plasticity (Lorenz 2020; Reijnen and van der Meij 2017; Sánchez et al. 2016), leading to greater difficulties in classification solely based on morphology. In recent years, researchers have utilised COI and 16S rRNA in investigating the phylogeny of Ovulidae and found that both are capable of distinguishing specimens at the species level (Reijnen and van der Meij 2019; Sánchez et al. 2016; Schiaparelli et al. 2005), resulting in the discovery of synonymy among ovulid species (Reijnen 2015). Meyer and Paulay (2005) utilised barcoding in the analysis of the sequences of more than 2000 individuals in 263 taxa of the family Cypraeidae, the sister group to Ovulidae (Meyer 2003; 2004; Rosenberg 1992), and found that identification of unknowns was 98% accurate with a neighbour-joining approach against an evolutionary significant unit (ESU) phylogeny. The correspondence between ESU definitions and traditional morphological taxonomy was high, with 255 ESUs (97%) recognised previously at either the specific or subspecific level, indicating that an ESU is a taxonomic unit equivalent to or smaller than a species. Therefore, traditional taxonomy within Cypraeidae at the species or subspecies level is supported by molecular data in addition to independent morphological criteria.

From the phylogenetic tree constructed using COI sequences (Fig. 3), it can be observed that the sequences of S. bridgesi and S. triticea were clustered on the same clade, indicating the absence of significant genetic differentiation between the COI sequences of these specimens. Other clades were also well-supported, which is in agreement with the findings of Meyer et al. The smallest genetic distance among the COI sequences of specimens other than *S. bridgesi* and *S. triticea* was approximately

4.8 times that of the greatest genetic distance between *S. bridgesi* and *S. triticea*, clearly demonstrating the high degree of similarity between the COI sequences of *S. bridgesi* and *S. triticea*.

The phylogenetic tree constructed from the 16S rRNA sequences showed that different specimens could be clearly distinguished at the species level using 16s rRNA(Fig. 4). A study by Schiaparelli et al. (2005) showed that the minimum and maximum interspecific divergence values (obtained using the Jukes-Cantor model) of the 16S rRNA distance matrix between ovulid species were 0.03 and 22.3%, respectively. In the present study, the smallest genetic distance among species other than *S. triticea* and *S. bridgesi* was 0.0325%, supporting the findings reported by Schiaparelli et al. The greatest genetic distance between *S. triticea* and *S. bridgesi* was 0.0064%, which was only approximately one-fifth of the smallest genetic distance among other specimens. Therefore, the 16S rRNA data further support the synonymy between *S. triticea* and *S. bridgesi*.

Being a non-transcribed spacer region, the ITS region is subject to smaller selective pressures and generally undergoes rapid evolution (Odorico and Miller 1997). It is commonly used for analysis at the population and species level because of its high degree of sequence variation (Harris and Crandall 2000; Hillis and Dixon 1991). Therefore, ITS1-5.8S-ITS2 provides higher discriminating power at lower taxonomic levels. Among the ITS sequences obtained in the present study, the smallest genetic distance among specimens other than *S. triticea* and *S. bridgesi* was approximately 19 times that of the greatest genetic distance between *S. triticea* and *S. bridgesi*, representing a significantly larger intraspecific-interspecific genetic distance ratio compared with COI and 16S rRNA. This indicates that genetic differentiation did not occur even in the rapidly evolving ITS1-5.8S-ITS2 gene region between *S. triticea* and *S. bridgesi*. In the ITS phylogenetic tree (Fig. 5), S. triticea was convincingly clustered with *S. bridgesi* while the other clades were well supported.

Conclusion

In conclusion, the COI, 16S rRNA, and ITS1-5.8S-ITS2 data of the ovulid specimens collected in the present study indicated the absence of genetic differences between *S. bridgesi* and *S. triticea*. Both the phylogenetic trees and heatmaps (Fig. 6-Fig. 8) showed a high degree of similarity between *S. bridgesi* and *S. triticea*, suggesting that the morphological differences between the two species may be caused by phenotypic plasticity than genetic differences, which is not unusual among molluscs. As the extremely high level of phenotypic plasticity in ovulid species results in much ambiguity in morphology-based classification criteria, the analysis of species evolution through molecular approaches is of great significance to the elucidation of evolutionary history (Schiaparelli et al. 2005). Based on the present knowledge, it is evident that striking phenomena of convergence and homoplasy characterise shell morphology in Ovulidae and that a molecular framework is necessary to recognise phylogenetically related groups.

Acknowledgements

We are very grateful to H.Y.D. This work was supported by The Scientific Research Foundation of Third Institute of Oceanography MNR (22020017), The Global climate change and ocean atmosphere interaction research, The Marine Biological Sample Museum Upgrade and Expansion, Biological classification system research, Monitoring and Protection of the Seamount Ecosystem in the Western Pacific Ocean (DY135-E2-2-04), and The National Natural Science Foundation of China (41406216).

References

Burland, G T (2000) DNASTAR's Lasergene sequence analysis software. Bioinformatics methods and protocols. Springer, 71-91. doi:https://doi.org/10.1385/1-59259-192-2:71

Hardy's Internet Guide to Marine Gastropods (& Near Classes). http://www.gastropods.com/3/Shell_68033.shtml [accessed 12.2020]

Hardy's Internet Guide to Marine Gastropods (& Near Classes)-Crenavolva aureola. http://www.gastropods.com/5/Shell_14115.shtml [accessed 12.2020]

Hardy's Internet Guide to Marine Gastropods (& Near Classes)-Crenavolva striatula striatula. http://www.gastropods.com/5/Shell_8225.shtml [accessed 12.2020]

Harris DJ, Crandall KA (2000) Intragenomic variation within ITS1 and ITS2 of freshwater crayfishes (Decapoda: Cambaridae): implications for phylogenetic and microsatellite studies. Molecular Biology and Evolution 17: 284-291. doi:https://doi.org/10.1093/oxfordjournals.molbev.a026308

Hebert PD, Ratnasingham S, De Waard JR (2003) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London Series B: Biological Sciences 270: S96-S99. doi:https://doi.org/10.1098/rsbl.2003.0025

Hillis DM, Dixon MT (1991) Ribosomal DNA: molecular evolution and phylogenetic inference. The Quarterly review of biology 66: 411-453. doi:https://doi.org/10.1086/417338

Hou L, Dahms H-U, Dong C, Chen Y, Hou H, Yang W, Zou X (2013) Phylogenetic positions of some genera and species of the family Buccinidae (Gastropoda: Mollusca) from China based on ribosomal RNA and COI sequences. Chinese Science Bulletin 58: 2315-2322. doi:https://doi.org/10.1007/s11434-013-5922-z

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of molecular evolution 16: 111-120. doi:https://doi.org/10.1007/BF01731581

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35: 1547-1549. doi:https://doi.org/10.1093/molbev/msy096

Layton KK, Martel AL, Hebert PD (2014) Patterns of DNA barcode variation in Canadian marine molluscs. PloS one 9: e95003. doi:https://doi.org/10.1371/journal.pone.0095003

Worldwide Mollusc Species Data Base-Genus: Sandalia. https://www.bagniliggia.it/WMSD/SearchSpecies.php?freeText=&inputFamily=&inputGenus=sandalia&inputSpecies=&inputVariety=&inputAuthor=&B1=Search [accessed 12.2020]

Lorenz F (2009) Two new species of Ovulidae from the Western Pacific (Gastropoda: Ovulidae). Conchylia 40: 38-44

Lorenz F (2020) The "Black morph Cyphoma " from the Netherlands Antilles (Gastropoda: Ovulidae). Acta Conchyliorum 19: 69-75

Lorenz F, Fehse D (2009) The living Ovulidae: a manual of the families of allied cowries: Ovulidae, Pediculariidae and Eocypraeidae. ConchBooks, pp.

Ma X (1997) Class Gastropoda, Order Mesogastropoda, Superfamily Cypraeacea: a major project of the National Natural Science Foundation of China in the Period of the Eighth Five-Year Plan. Science Press, pp.

Mao X, Jiang W (1994) Marine fauna of Zhoushan Zhejiang People's Publishing House, HangZhou, China, pp.

Meier R, Shiyang K, Vaidya G, Ng PK (2006) DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. Systematic biology 55: 715-728. doi:https://doi.org/10.1080/10635150600969864

Meyer CP (2003) Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. Biological Journal of the Linnean Society 79: 401-459. doi:https://doi.org/10.1046/j.1095-8312.2003.00197.x

Meyer CP (2004) Toward comprehensiveness: increased molecular sampling within Cypraeidae and its phylogenetic implications. MALACOLOGIA-PHILADELPHIA- 46: 127-156

Meyer CP, Paulay G (2005) DNA barcoding: error rates based on comprehensive sampling. PLoS biol 3: e422. doi:https://doi.org/10.1371/journal.pbio.0030422

Nei M, Kumar S (2000) Molecular evolution and phylogenetics. Oxford university press, pp. doi:https://doi.org/10.1017/S0016672301219405

Odorico D, Miller D (1997) Variation in the ribosomal internal transcribed spacers and 5.8 S rDNA among five species of Acropora (Cnidaria; Scleractinia): patterns of variation consistent with reticulate evolution. Molecular Biology and Evolution 14: 465-473. doi:https://doi.org/10.1093/oxfordjournals.molbev.a025783

QUINTERO-GALVIS J, RAQUEL-CASTRO L (2013) Molecular phylogeny of the Neritidae (Gastropoda: Neritimorpha) based on the mitochondrial genes cytochrome oxidase I (COI) and 16S rRNA. Acta Biológica Colombiana 18: 307-318

Reijnen BT (2015) Molecular data for Crenavolva species (Gastropoda, Ovulidae) reveals the synonymy of C.chiapponii. ZooKeys: 15-26. doi:https://doi.org/10.3897/zookeys.501.9144

Reijnen BT, van der Meij SE (2017) Coat of many colours—DNA reveals polymorphism of mantle patterns and colouration in Caribbean Cyphoma Röding, 1798 (Gastropoda, Ovulidae). PeerJ 5: e3018. doi:https://doi.org/10.7717/peerj.3018

Reijnen BT, van der Meij SE (2019) Systematics of the subfamily Aclyvolvinae (Caenogastropoda: Ovulidae) based on molecular and morphometric analyses. Journal of Molluscan Studies 85: 336-347. doi:https://doi.org/10.1093/mollus/eyz020

Rosenberg G (1992) An introduction to the Ovulidae (Gastropoda: Cypraeacea). American Conchologist 20: 4-7

Sánchez JA, Fuentes-Pardo AP, Almhain ÍN, Ardila-Espitia NE, Cantera-Kintz J, Forero-Shelton M (2016) The masquerade game: marine mimicry adaptation between egg-cowries and octocorals. PeerJ 4: e2051. doi:https://doi.org/10.7717/peerj.2051

World Register of Marine Species: WoRMS-Species: Sandalia rhodia (A. Adams, 1854). http://www.marinespecies.org/aphia.php?p=taxdetails&id=819783 [accessed 12.2020]

Schiaparelli S, Barucca M, Olmo E, Boyer M, Canapa A (2005) Phylogenetic relationships within Ovulidae (Gastropoda: Cypraeoidea) based on molecular data from the 16S rRNA gene. Marine Biology 147: 411-420. doi:https://doi.org/10.1007/s00227-005-1566-0

Simison WB, Lindberg DR (1999) Morphological and molecular resolution of a putative cryptic species complex: a case study of Notoacmea fascicularis (Menke, 1851)(Gastropoda: Patellogastropoda). Journal of Molluscan Studies 65: 99-109. doi:https://doi.org/10.1093/mollus/65.1.99

Stothard J, Rollinson D (1997) Partial DNA sequences from the mitochondrial cytochrome oxidase subunit I (COI) gene can differentiate the intermediate snail hosts Bulinus globosus and B. nasutus (Gastropoda: Planorbidae). Journal of Natural History 31: 727-737. doi:https://doi.org/10.1080/00222939700770361

Tamura K (1992) Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+ C-content biases. Mol Biol Evol 9: 678-687. doi:https://doi.org/10.1093/oxfordjournals.molbev.a040752

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28: 2731-2739. doi:https://doi.org/10.1093/molbev/msr121

World Register of Marine Species: WoRMS-Genus: Sandalia. http://www.marinespecies.org/aphia.php?p=taxdetails&id=430624 [accessed 12.2020]

Zhang S, Wei P (2011) Cowries and their relatives of China. China Ocean Press, pp.

ARPHA Preprints Not peer-reviewed, not copy-edited manuscript posted on February 08, 2021. DOI: https://doi.org/10.3897/arphapreprints.e64141

Table 1. Specimens used in the analyses, including locality, date, and GenBank accession data

Date collected	Collection number	Species	Locality	GenBank accession numbers		
				COI	16S rRNA	ITS
20200721	20200721-H1	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410824	MW411391	-
	20200721-H2	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410825	MW411390	-
	20200721-H3	*Phenacovolva* sp.	China, FuJian, XiaMen	-	-	-
	20200721-H4	*Phenacovolva* sp.	China, FuJian, XiaMen	-	MW411396	-
	20200721-H5	*Phenacovolva* sp.	China, FuJian, XiaMen	MW410821	MW411395	-
20200722	20200722-H1	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410826	-	MW411417
	20200722-H2	*Primovula formosa*	China, FuJian, XiaMen	MW410819	MW411393	MW411419
	20200722-H3	*Primovula formosa*	China, FuJian, XiaMen	MW410820	MW411392	MW411418
	20200722-H4	*Cuspivolva bellica*	China, FuJian, XiaMen	MW410804	MW411371	MW411405
	20200722-H5	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410827	MW411389	MW411416
	20200722-H6	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410828	MW411388	MW411415
	20200722-H7	*Cuspivolva bellica*	China, FuJian, XiaMen	-	MW411370	MW411404
	20200722-H8	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410829	MW411387	MW411414
	20200722-H9	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410830	MW411386	MW411413
	20200722-H10	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410831	MW411385	MW411412
	20200722-HL	*Phenacovolva tokioi*	China, FuJian, XiaMen	MW410823	-	-
	20200722-T1	*Sandalia triticea*	China, FuJian, ShiShi	MW410843	MW411373	MW411407
20200920	20200920-T2	*Sandalia triticea*	China, FuJian, ShiShi	MW410844	MW411372	MW411406
	20200920-H1	*Cuspivolva queenslandica*	China, FuJian, ShiShi	MW410817	-	-
	20200920-H2	*Crenavolva trailli*	China, GuangXi, BeiHai	MW410818	-	-

ARPHA Preprints Not peer-reviewed, not copy-edited manuscript posted on February 08, 2021. DOI: https://doi.org/10.3897/arphapreprints.e64141

Table 1. Specimens used in the analyses, including locality, date, and GenBank accession data (Continued)

20201117	20201117-H1	*Phenacovolva* sp.	China, FuJian, XiaMen	MW410822	MW411394	-
	20201117-H2	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410832	MW411384	MW411411
	20201117-H3	*Cuspivolva bellica*	China, FuJian, XiaMen	MW410805	MW411369	MW411403
	20201117-H4	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410833	MW411383	MW411410
	20201117-H5	*Cuspivolva bellica*	China, FuJian, XiaMen	MW410806	MW411368	MW411402
	20201117-H6	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410834	MW411382	MW411409
	20201117-H7	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410835	MW411381	-
	20201117-H8	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410836	MW411380	-
	20201117-H9	*Cuspivolva bellica*	China, FuJian, XiaMen	MW410807	MW411367	-
	20201117-H10	*Cuspivolva bellica*	China, FuJian, XiaMen	MW410808	MW411366	-
	20201117-H11	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410837	MW411379	-
	20201117-H12	*Cuspivolva bellica*	China, FuJian, XiaMen	MW410809	MW411365	MW411401
	20201117-H13	*Cuspivolva bellica*	China, FuJian, XiaMen	MW410810	MW411364	MW411400
	20201117-H14	*Cuspivolva bellica*	China, FuJian, XiaMen	MW410811	MW411363	-
	20201117-H15	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410838	MW411378	-
	20201117-H16	*Cuspivolva bellica*	China, FuJian, XiaMen	MW410812	MW411362	-
	20201117-H17	*Cuspivolva bellica*	China, FuJian, XiaMen	MW410813	MW411361	-
	20201117-H18	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410839	MW411377	-
	20201117-H19	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410840	MW411376	MW411408
	20201117-H20	*Cuspivolva bellica*	China, FuJian, XiaMen	MW410814	MW411399	-
	20201117-H21	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410841	MW411375	-
	20201117-H22	*Sandalia bridgesi*	China, FuJian, XiaMen	MW410842	MW411374	-
	20201117-H23	*Cuspivolva bellica*	China, FuJian, XiaMen	MW410815	MW411398	-
	20201117-H24	*Cuspivolva bellica*	China, FuJian, XiaMen	MW410816	MW411397	-

Table 2. Details of gene regions and associated primer pairs used in the study

Gene region	Fragment size (bp)	Primers	Annealing temperature	Reference
COI	~680	Lco1490/Hco2198	45℃, +0.5℃/cycle,15cycle, 49℃, 20cycle	Vrijenhoek, 1994
16S	~550	16SAR/16SBR	52℃	Reijnen & van der Meij, 2019
ITS1-5.8S-ITS2	~1200	GastF/GastR	56℃	Hoy & Rodriguez, 2013

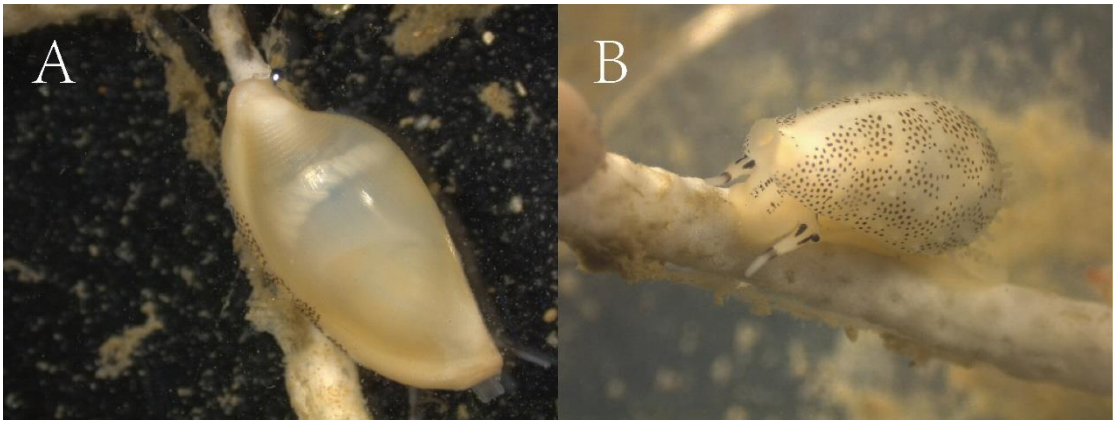

Figure-1: Living animals. **A**. *S. bridgesi* Lorenz, 2009 (20201117-H19) **B**. *S. bridgesi* Lorenz, 2009 (20201117-H6)

ARPHA Preprints Not peer-reviewed, not copy-edited manuscript posted on February 08, 2021. DOI: https://doi.org/10.3897/arphapreprints.e64141

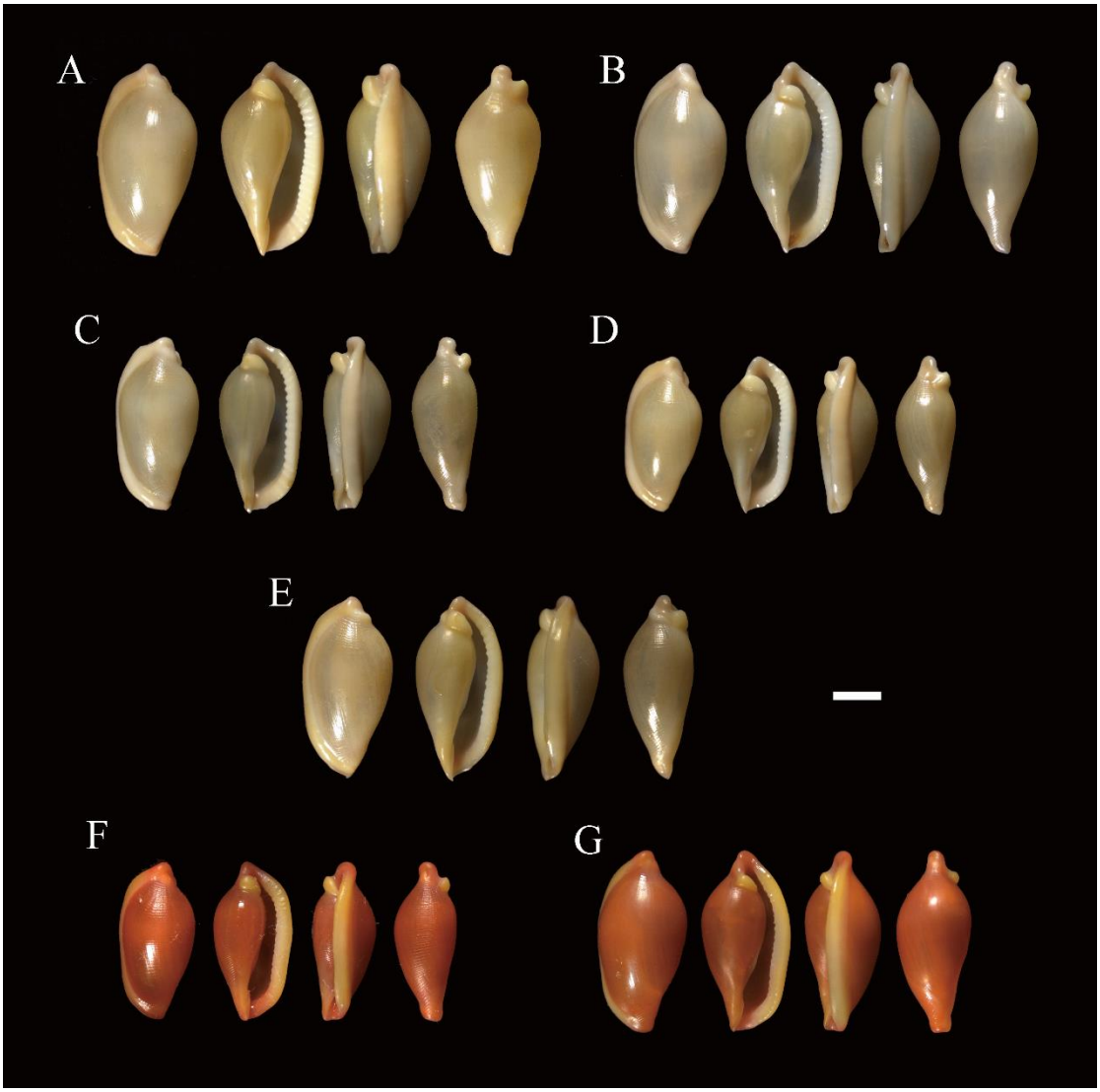

Figure-2: Dorsal, ventral, and lateral sides views of shells. **A**. *S. bridgesi* Lorenz, 2009 (20200722-H1) **B**. *S. bridgesi* Lorenz, 2009 (20200722-H5) **C**. *S. bridgesi* Lorenz, 2009 (20200722-H6) **D.** *S. bridgesi* Lorenz, 2009 (20200722-H9) **E.** *S. bridgesi* Lorenz, 2009 (20200722-H10) **F.** *S. triticea* (Lamarck, 1810) (20200722-T1) **G**. *S. triticea* (Lamarck, 1810) (20200722-T2) Scale bars =2mm

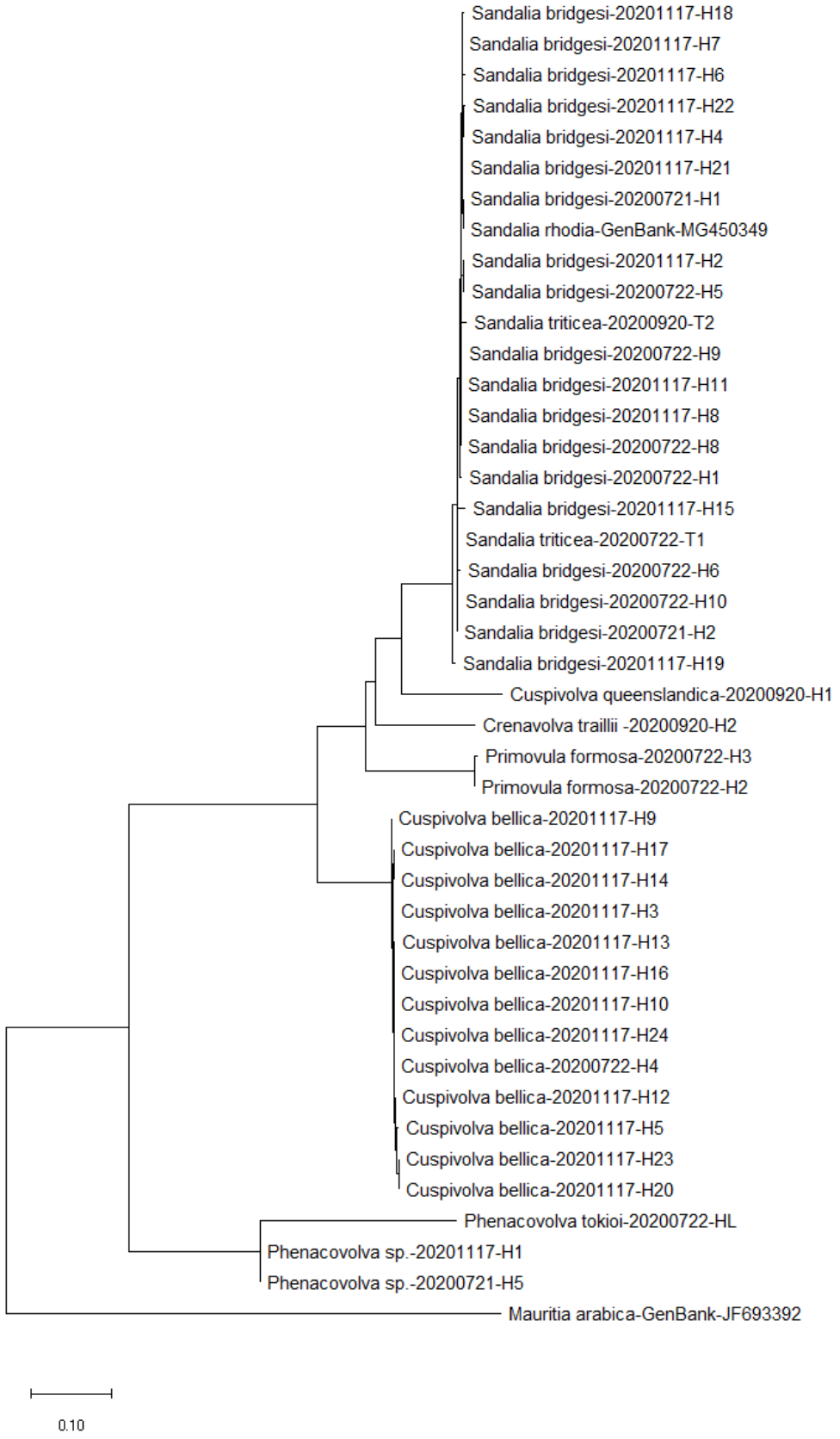

Figure-3: Phylogenetic tree constructed from alignments of COI sequences using the HKY+G+I model for 41 Ovulidae species.

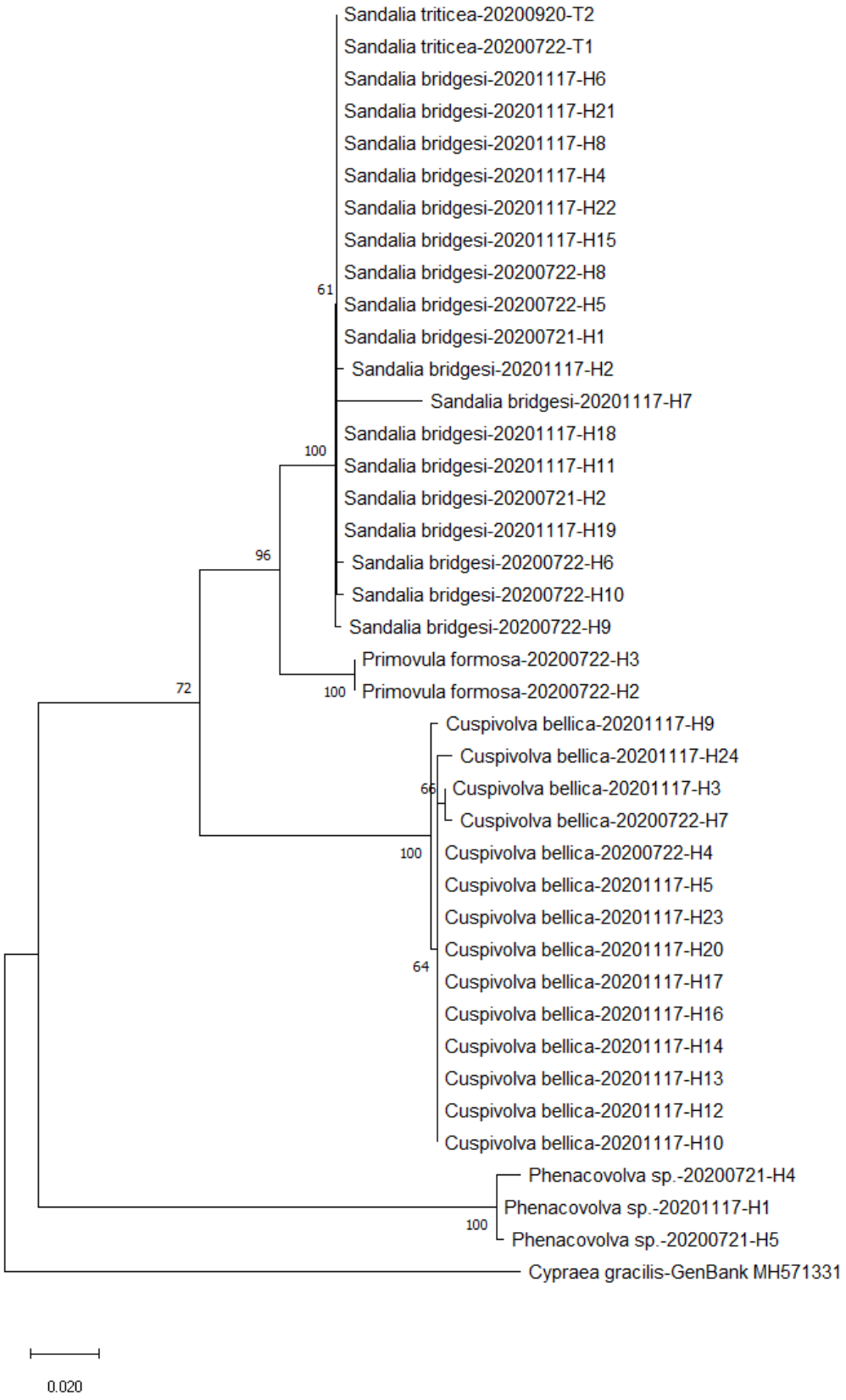

Figure-4: Phylogenetic tree constructed from alignments of 16S rRNA sequences using the Tamura 3-parameter+I model for 39 Ovulidae species.

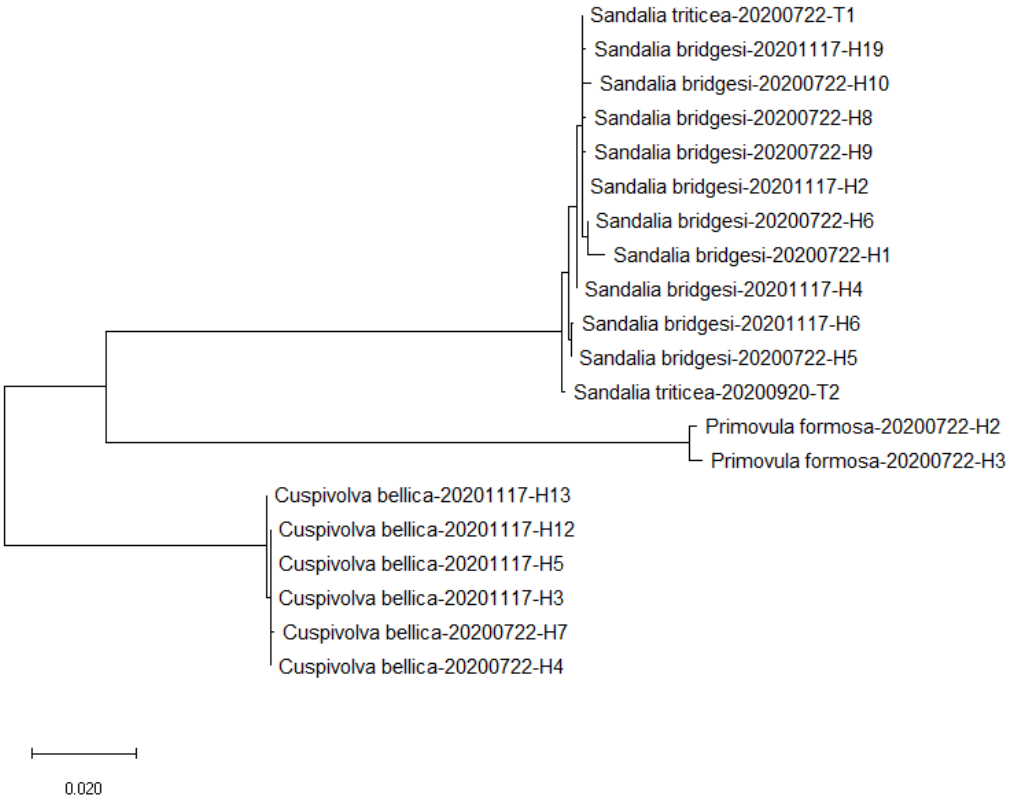

Figure-5: Phylogenetic tree constructed from alignments of ITS region sequences using the K2+G model for 20 Ovulidae species.

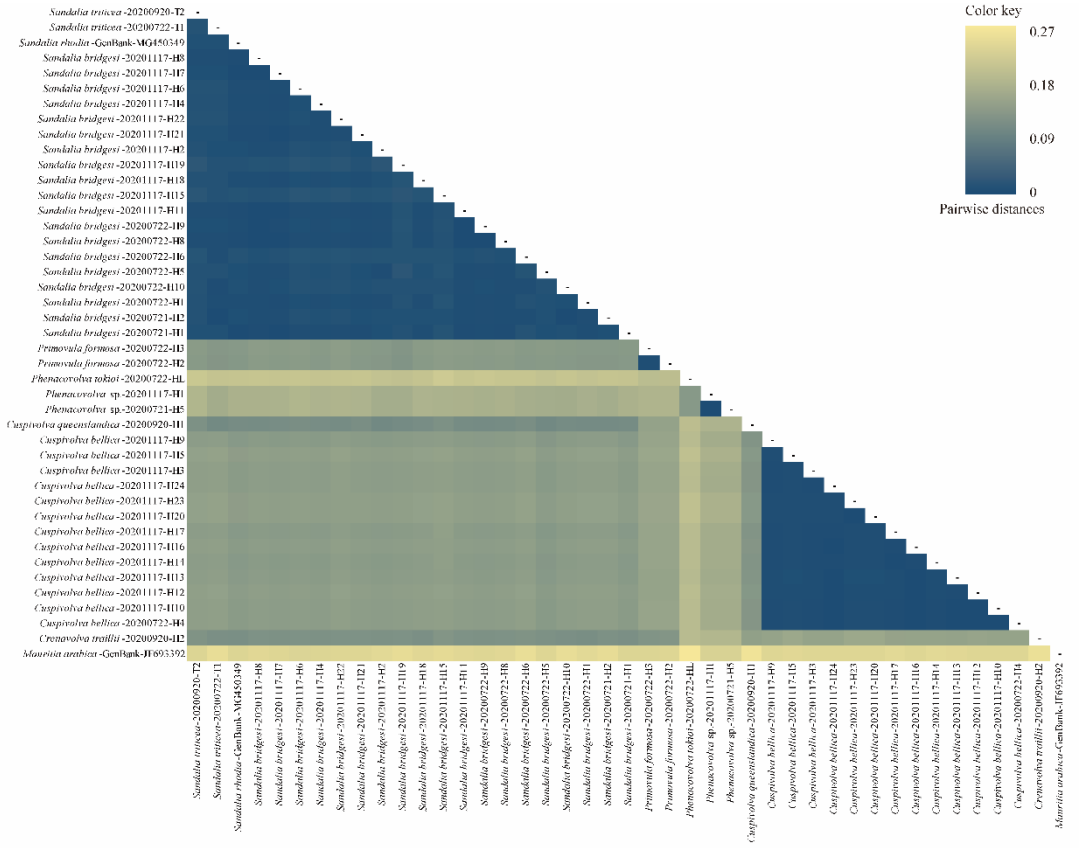

Figure-6: Heatmap showing distribution of pairwise genetic distances estimated from COI sequences for 41 Ovulidae species based on the Jukes-Cantor model.

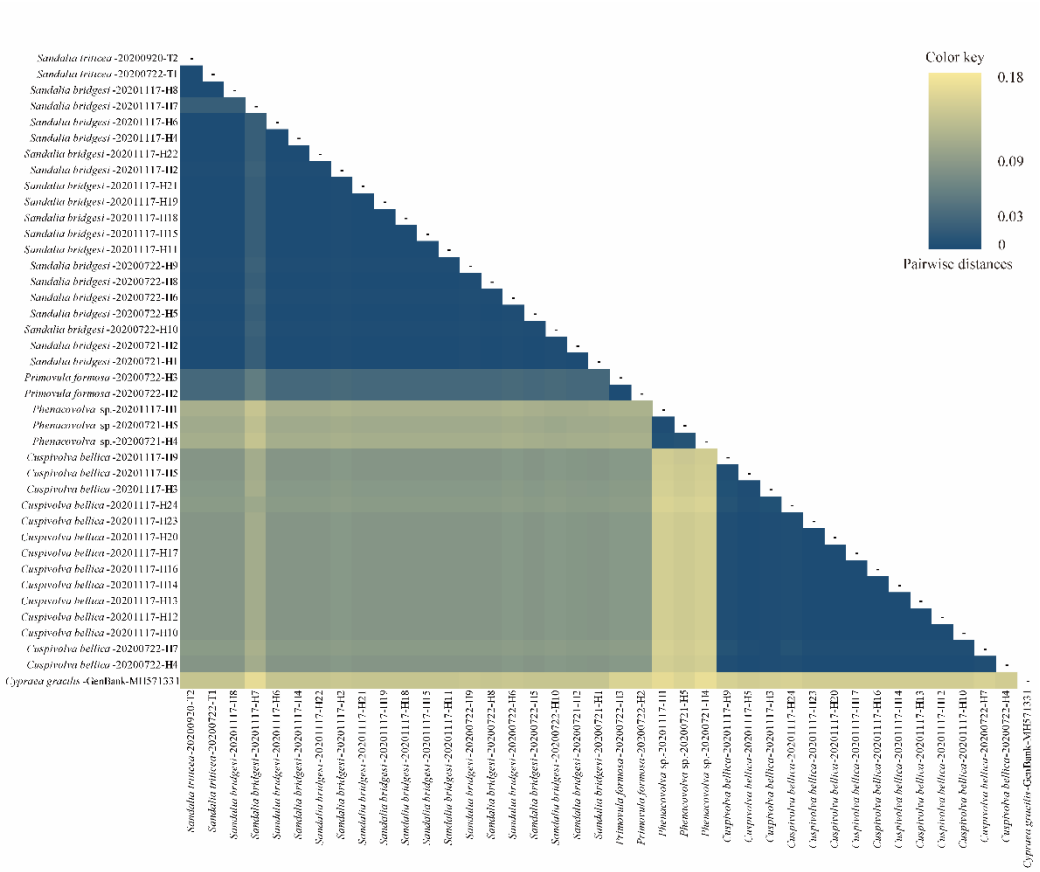

Figure-7: Heatmap showing distribution of pairwise genetic distances estimated from 16S rRNA sequences for 39 Ovulidae species based on the Jukes-Cantor model.

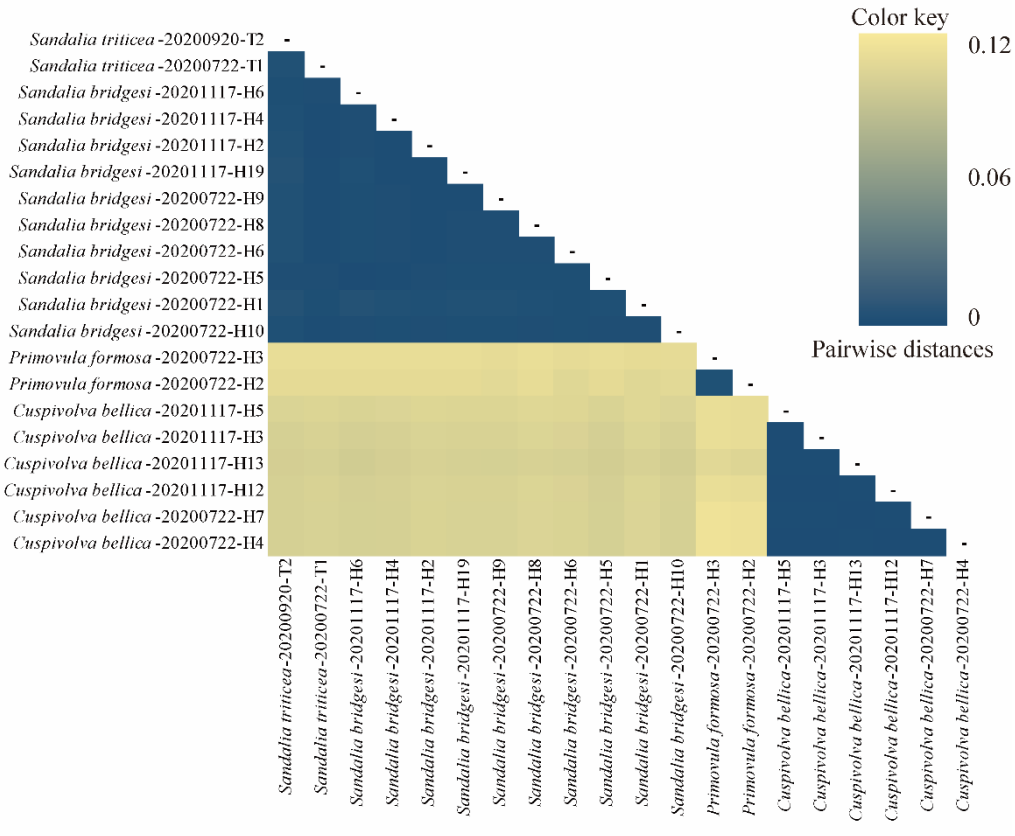

Figure-8: Heatmap showing distribution of pairwise genetic distances estimated from ITS region sequences for 20 Ovulidae species based on the Jukes-Cantor model.