

Systematic relationships of Chinese freshwater semisulcospirids (Gastropoda, Cerithioidea) revealed by mitochondrial sequences

Li-Na Du^{1,2,3,#}, Jun Chen^{3,#}, Guo-Hua Yu^{2,*#}, Jun-Xing Yang^{1,4,*}

¹ Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming Yunnan 650223, China

² Key Laboratory of Ecology of Rare and Endangered Species and Environmental Protection (Guangxi Normal University), Ministry of Education, Guilin Guangxi 541004, China

³ Key Laboratory of the Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China

⁴ Yunnan Key Laboratory of Plateau Fish Breeding, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming Yunnan 650223, China

ABSTRACT

The systematics of Semisulcospiridae in China is revised here based on morphological characters and mitochondrial phylogenetics. Phylogenetic relationships within the Chinese semisulcospirids were assessed via DNA sequences from mitochondrial analysis (cytochrome c oxidase I and 16S rRNA). This research contains most morphospecies of semisulcospirids previously recorded in China. Based on these results, the family of Chinese Semisulcospiridae is represented by three genera: i. e., viviparous *Semisulcospira* Böttger, 1886, oviparous *Hua* Chen, 1943 and *Koreoleptoxis* Burch and Jung, 1988. These genera can be distinguished from each other by reproductive anatomy, reproductive mode, and radula features. Species of *Hua* are mainly distributed in southwest China and Guangxi, whereas *Koreoleptoxis* and *Semisulcospira* are mainly distributed in south and northeast China.

Keywords: Phylogeny; China; *Semisulcospira*; *Hua*; *Koreoleptoxis*

Open Access

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Copyright ©2019 Editorial Office of Zoological Research, Kunming Institute of Zoology, Chinese Academy of Sciences

INTRODUCTION

Semisulcospirids are an ecologically important and taxonomically challenging family of freshwater snails distributed in lakes, rivers, and streams in eastern Asia, including Far East Russia, Japan, Korean peninsula, and China, and in western North America (Köhler, 2016, 2017; Liu et al., 1993; Strong & Köhler, 2009; Yen, 1939). Basic knowledge on freshwater gastropods in China was first established in the late nineteenth and early twentieth centuries (e. g., Dautzenberg & Fischer, 1906, 1908; Fulton, 1914; Heude, 1888), with all cerithioidean freshwater gastropods designated under the single genus *Melania* Lamarck, 1799 (junior synonym of *Thiara* Röding, 1798). Heude (1888, 1889 (1882 – 1890)) recorded 24 species of

Received: 30 January 2019; Accepted: 16 July 2019; Online: 21 August 2019

Foundation items: This research was funded by the National Natural Science Foundation of China (31301865), Special Funds for Young Scholars of Taxonomy of the Chinese Academy of Sciences (ZSBR-011), Key Laboratory of the Zoological Systematics and Evolution of the Chinese Academy of Sciences (Y229YX5105), Strategic Priority Research Program of the Chinese Academy of Sciences (XDA23080502), National Natural Science Foundation of China-Yunnan Joint Fund (U1702233) and Sino BON-Inland Water Fish Diversity Observation Network

#Authors contributed equally to this work

*Corresponding authors, E-mail: yangjx@mail.kiz.ac.cn; yugh2018@126.com

DOI: 10.24272/j.issn.2095-8137.2019.033

Melania in south China, especially in the Yangtze River Basin. Yen (1939) subsequently identified Chinese freshwater snails from the Naturmuseum Senckenberg, Germany, and recorded 12 Chinese species of Thiaridae and a new genus, *Senckenbergia* Yen, 1939, for the type species *Melania pleuroceroides* from Sichuan. Chen (1943) later described two genera, *Wanga* (type species *Melania henriettae* Gray, 1833) and *Hua* (type species *Melania telonaria* Heude, 1888), into which were placed eight and eleven nominal species, respectively. In dispute, Morrison (1954) proposed *Wanga* and *Hua* to be synonyms of *Brotia* H. Adams 1866 and *Oxytrema* Rafinesque 1819, respectively, although the suggestion of *Hua* being a synonym of *Oxytrema* was not accepted by subsequent authors (Du et al., 2019; Köhler, 2016, 2017; Strong & Köhler, 2009). Abbott (1948) described a new genus, *Namrutua*, for the type species *Melania ningpoensis*. However, *Senckenbergia* and *Namrutua* were primarily ignored by most Chinese researchers, with all species instead placed in *Semisulcospira* Böttger, 1886 (Liu et al., 1979, 1993; Xu, 2007; Zhang et al., 1997). Several species of *Semisulcospira* were subsequently described in China, including *S. inflata* Tchang & Tsi 1949 recorded in Lake Dianchi, Yunnan, and *S. marica* Li, Wang & Zhang 1994 and *S. crassicosta* Liu, Wang & Zhang 1994 recorded in Guizhou Province (Liu et al., 1994; Tchang & Tsi, 1949). Liu et al. (1994) also described two new species of *Paludomus*, *P. qianensis* Liu Zhang & Duan 1994 and *P. cinctus* Liu, Zhang & Duan 1994, in Guizhou Province. Xu (2007) stated that there were 53 species of Semisulcospiridae found in China, including 49 species of *Semisulcospira*, two species of *Hua*, one species of *Paludomus*, and one species of *Potodoma*. However, Xu (2007) did not revise the validity of these species. The first systematic revision of semisulcospirids was provided by Strong & Köhler (2009) based on morphological characters and molecular phylogenetic analysis. They showed *Hua* to be a valid genus and distinguishable from *Semisulcospira* by distinct features of the digestive, reproductive, and nervous systems. Du et al. (2017, 2019) designated *Semisulcospira trivolvus* Yen 1939 as synonym to *S. paludiformis* Yen 1939 and further stated that it should be assigned to the genus *Sulcospira* Troschel, 1858 (Pachychilidae Fischer & Crosse, 1892). More recently, Du et al. (2019) studied semisulcospirids of southwest China by morpho-anatomy and mitochondrial phylogenetics and supported *Namrutua* and *Senckenbergia* as viviparous and as synonyms of *Semisulcospira*. Additionally, the taxonomic status of several species were discussed, in particular *M. cancellata* Benson 1842 and *M. suifuensis* Chen 1937 were treated as junior synonyms to *S. ningpoensis* (I. Lea, 1856), and *M. dulcis* Fulton 1904, *M. lauta* Fulton 1904, and *S. inflata* Tchang 1949 were treated as junior synonyms to *Hua textrix* (Heude, 1888). However, several semisulcospirid species were not contained in Du et al. (2019), including *H. kweichouensis* (Chen, 1937) (synonym to *H. intermedia* (Gredler, 1885) in this study), *Paludomus minensis* Chen 1943 (synonym to *Lithogryphus pallens* Bavay & Dautzenberg 1910, valid species name is *H. pallens* in this study), and *P.*

rotundata (Heude, 1888). Furthermore, most semisulcospirids were recorded in the Yangtze River. Thus, based on previous literature, 52 species of semisulcospirids have been recorded in China (Du & Yang, 2019; Du et al., 2019; Liu et al., 1993; Xu, 2007). However, the systematic and phylogenetic relationships of semisulcospirids remain poorly understood.

From 2014 to 2017, specimens of semisulcospirids were collected from 14 Chinese provinces, including Heilongjiang, Jilin, Liaoning, Anhui, Jiangxi, Hunan, Zhejiang, Fujian, Guangdong, Guangxi, Guizhou, Sichuan, Chongqing, and Yunnan. Based on morphological and molecular analyses, the specimens were used to clarify the phylogenetic and systematic relationships among Chinese semisulcospirids.

MATERIALS AND METHODS

Taxon sampling

Specimens were collected from 2014 to 2017 by L.N. Du et al. in 14 Chinese provinces (Figure 1). The foot muscle tissues were preserved in 99% ethanol. All voucher specimens were deposited at the Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ, CAS) (Table 1). The identification of specimens included verification of origin descriptions and topotypic specimen photos.

We examined 225 sequenced samples from 41 species, including 95 sequenced samples from 22 species of *Hua* from Yunnan, Chongqing, Guangxi, and Guizhou, 90 sequenced samples from 12 species of *Koreoleptoxis* from Heilongjiang, Jilin, Hunan, Jiangxi, Fujian, Zhejiang, and Anhui, and 40 sequenced samples from seven species of *Semisulcospira* from Hunan, Jiangxi, Guangxi, Chongqing, Zhejiang, and Liaoning (Table 1).

Morphology

Measurements and descriptions, including shell dimensions, radulae, and shell features, followed Strong & Köhler (2009) and Du et al. (2019). Radulae and embryonic shells were cleaned enzymatically with proteinase K for 30 min, after which they were sonicated for 60 s, mounted on aluminum specimen stubs with adhesive tabs, and coated with gold palladium for analysis via scanning electron microscopy (Hitachi S-3000, Japan) at 15 kV. Soft body anatomy was observed using a microscope (Leica S6D, 6.3–40x) and was described following the terminology used by Strong & Köhler (2009) and Köhler (2017).

Molecular analyses

Genomic DNA was purified via standard phenol-chloroform isolation and ethanol precipitation. Partial sequences of mitochondrial cytochrome c oxidase subunit I (*COI*, 772 bp) and 16S ribosomal RNA (16S, 856 bp) were amplified using primers F2 and R2 (Du et al., 2019) for *COI*, and 16S-F (Wilson et al., 2004) and H3059 (Palumbi et al., 1991) for 16S. Homologous sequences of other semisulcospirids were obtained from GenBank, with all new sequences deposited in same under accession Nos. MK944096–MK944245, MK968961–MK969107, MK991781–MK991783, MN013828–

Table 1 Materials used in this study with voucher numbers, localities, GenBank accession Nos. and synonym

Taxon name	Locality	Voucher No.	Synonym	GenBank accession No.		
				16S rRNA	COI	
<i>Hua aristarchorum</i>	Huize County, Yunnan	D870, D872,	<i>Melania aristarchorum</i>	MK944142–43	MK969006–07	
	Songming County, Yunnan	D109, D201–D203, D263–D265, D272, D274, D275, D281, D646, D648–D649, D1497, D1503, D1505		MK251646, MK251648–50, MK251651–52, MK944096, MK251659, MK251663, MK251665–68, MK944238–40	MK251733–34, MK251735–36, MK968961, MK251741, MK251743, MK251745–47, –	
		Libo County, Guizhou	D899–D901, D903	<i>M. aubryana</i> , <i>Semisulcospira aurbryana</i>	MK251683–85	MK251762–65
		Xundian County, Yunnan	D268, D269, D282	<i>M. bailleti</i> , <i>Oxytrema bailleti</i>	MK251653–54, MK251664	MK251737–38, MK251744
<i>H. funingensis</i>	Funing County, Yunnan	D919, D920, D922	–	MK251689–90	MK251766–68	
<i>H. intermedia</i>	Anshun City, Guizhou	D1471, D1472, D1475	<i>M. praenotata intermedia</i> , <i>Paludomus (Hemimitra)</i> <i>kweichouensis</i> , <i>H. kwei-</i> <i>chouensis</i> , <i>H. praenotata</i> <i>intermedia</i>	MK944231–33	MK969096–98	
<i>H. kunmingensis</i>	Songming County, Yunnan	D636, D639, D641–D644	–	MK251632, MK251634, MK251636–39	MK251720, MK251721, MK251723–26	
<i>H. liuii</i>	Chongqing City	D866–D869	–	MK251679–82	MK251758–61	
<i>H. pallens</i>	Chongqing City	D1048–D1049, D1051	<i>Lithogryphus pallens</i> , <i>P. minensis</i>	MK944148–50	MK969012–14	
<i>H. rotundata</i>	Anshun City, Guizhou	D1481, D1486	<i>M. rotundata</i>	MK944234–35	MK969099–100	
<i>H. srupea</i>	Chenggong County, Yunnan	D189, D190	<i>M. srupea</i> , <i>S. srupea</i> , <i>Wanga srupea</i>	MK251608–09	–, MK251699	
<i>H. tchangsii</i>	Songming County, Yunnan	D286–D288	–	MK251625–27	MK251714–16	
<i>H. telonaria</i>	Songming County, Kunming, Yunnan	D640, D1492, D1496, D1526, D1528	<i>M. telonaria</i> , <i>M. telonaria</i> , <i>M. leprosa</i> , <i>S. telonaria</i> , <i>O. telonaria</i>	MK251635, MK944236–37, MK944242–243	MK251722, MK969101–02, MK969104–05	
	Jinning County, Kunming, Yunnan	D194–DD198, D860, D1578	<i>M. textrix</i> , <i>M. textoria</i> , <i>M. dulcis</i> , <i>M. lauta</i> , <i>S. dulcis</i> , <i>S. lauta</i> , <i>S. inflata</i>	MK251612–16, MK944141, MK944245	MK251701–05, MK969005, MK969107	
<i>H. vultuosa</i>	Jinning County, Yunnan	D856, D858–D859	<i>M. vultuosa</i> , <i>S. vultuosa</i>	MK251676–78	MK251755–57	
<i>Hua</i> sp.1	Lingyun County, Guangxi	D1253 D1257, D1268–D1272	–	MK944205–06, MK944217–21	MK969070–71, MK969082–86	
<i>Hua</i> sp2	Tian'e County, Guangxi	D248, D250, D252–253	–	MK251640–44	MK251727–30	
<i>Hua</i> sp2	Lingyun County, Guangxi	D1258–D1267	–	MK944207–16	MK969072–81	
<i>Hua</i> sp3	Zhunyi County, Guizhou	D256, D258	–	MK251623–24	MK251712–13	

GenBank accession No.					
Taxon name	Locality	Voucher No.	Synonym	16S rRNA	COI
<i>Hua</i> sp4	Luquan County, Yunnan	D1522, D1527	–	MK944241, MK944243	MK969103, MK969105
<i>Koreoleptoxis amurensis</i>	Mudanjiang City, Heilongjiang	D456, D460, D461, D462–D466	<i>M. amurensis</i> , <i>S. amurensis</i> , <i>Parajuga amurensis</i>	MK944097–99, MK251617–21	MK968962–64, MK251706–10
<i>K. bicinctus</i>	Wuyuan County, Jiangxi	D1142, D1144, D1145, D1149, D1151, D1172, D1173, D1178, D1179	<i>H. bicinctus</i>	MK944174, MK944176–77, MK944180, MK944182, MK944200–03	MK969038, MK969040–41, MK969044, MK969046, MK969064–67
<i>K. davidi</i>	Wuyuan County, Jiangxi	D1143, D1147, D1150	<i>M. davidi</i> , <i>S. davidi</i>	MK944175, MK944179, MK944181	MK969039, MK969043, MK969045
<i>Ko. diriminuta</i>	Ningguo City, Anhui	D729		MK944120	MK968984
<i>K. dolichostoma</i>	Lishui City, Zhejiang	D546, D549, D551, D552, D553, D816, D820	<i>S. dolichostoma</i>	MK944100–04, MK944132–33	MK968965–69, MK968996–97
<i>K. friniana</i>	Wuyuan County, Jiangxi	D1146, D1152, D1154, D1155, D1164	<i>M. friniana</i> , <i>H. friniana</i>	MK944178, MK944183, MK944185–16, MK944194	MK969042, MK969047, MK969049–50, MK969058
<i>K. pacificans</i>	Wuyuan County, Jiangxi	D1122–D1125, D1127–D1128, D1132–D1135, D1137–D1140, D1153, D1157–D1161, D1167–D1171, D1193, D1196	<i>M. pacificans</i> , <i>S. pacificans</i> , <i>P. minbeiensis</i>	MK944159–62, MK944164–73, MK944184, MK944187–91, MK944195–99, MK944204, –	MK969023–26, MK969028–37, MK969048, MK969051–55, MK969059–63, MK969068, MK969069
<i>K. pacificans</i>	Ningguo City, Anhui	D711, D715, D717, D718, D720, D723, D726		MK944111–15, MK944117–18	MK968976–80, MK968982
<i>K. praenotata</i>	Lishui City, Zhejiang	D810, D1163, D1403	<i>M. resinacea</i>	MK944131, MK944193	MK968995, MK969057
	Daweishan County, Hunan	D728	<i>M. schmackeri</i>	MK944227	MK969092
	Ningguo City, Anhui	D1112, D1113, D1115, D1116	<i>M. theaeapotes</i>	MK944119	MK968983
<i>K. terminalis</i>	Wuyuan County, Jiangxi	D1126, D1162	<i>M. terminalis</i> , <i>S. terminalis</i>	MK944155–58	MK969019–22
	Wuyuan County, Jiangxi			MK944163, MK944192	MK969027, MK969056
<i>K. terminalis</i>	Ningguo City, Anhui	D721		MK944116	MK968981
	Huangshan City, Anhui	D766, D768–D771, D774–D777		MK944122–30	MK968986–94
	Ningpo City, Zhejiang	D838–D840		MK944138–40	MK969002–04
<i>S. calculus</i>	Kuandian County, Liaoning	D1592–D1594	<i>M. calculus</i> , <i>M. paucicincta</i>	MK991781–83	MN013828–30
<i>S. cinnamomea</i>	Tongren City, Guizhou	D1455, D661, D662, D664, D665	<i>M. gredleri</i> var. <i>cinnamomea</i>	MK944230, MK944106–07, MK944109–10	MK969095, MK968971–72, MK968974–75
<i>S. gredleri</i>	Hezhou City, Guangxi	D1052–D1055	<i>M. gredleri</i>	MK944151–54	MK969015–18

GenBank accession No.					
Taxon name	Locality	Voucher No.	Synonym	16S rRNA	COI
	Hengyang City, Hunan	D1381-D1385		MK944222-26	MK969087-91
	Tongren City, Guizhou	D1453-D1454		MK944228-29	MK969093-94
	Zhangshu City, Jiangxi	D663, D666-D667		MK944108, MK251602-03	MK968973, MK251693-94
	Ningguo City, Anhui	D734		MK944121	MK968985
<i>S. ningpoensis</i>	Neijiang City, Sichuan	D1022-D1024	<i>M. cancellata</i> , <i>M. ningpoensis</i> , <i>M. bensoni</i> , <i>M. fortune</i> , <i>M. suiifuensis</i> , <i>S. cancellata</i> , <i>Melanoides ningpoensis</i> , <i>H. (Namrutua) ningpoensis</i>	MK944145-47	MK969009-11
	Zhangshu City, Jiangxi	D656, D672, D682, D224, D227		MK944105, MK251606-07, MK251604-05	MK968970, MK251697-98, MK251695-96
<i>S. pleuroceroides</i>	Chongqing	D1042-D1043, D1045-D1046	<i>M. pleuroceroides</i>	MK251669-72	MK251748-51
<i>Semisulcospira</i> sp.	Ningpo City, Zhejiang	D828, D831, D834, D836	-	MK944134-37	MK968998-9001

-: Not available.

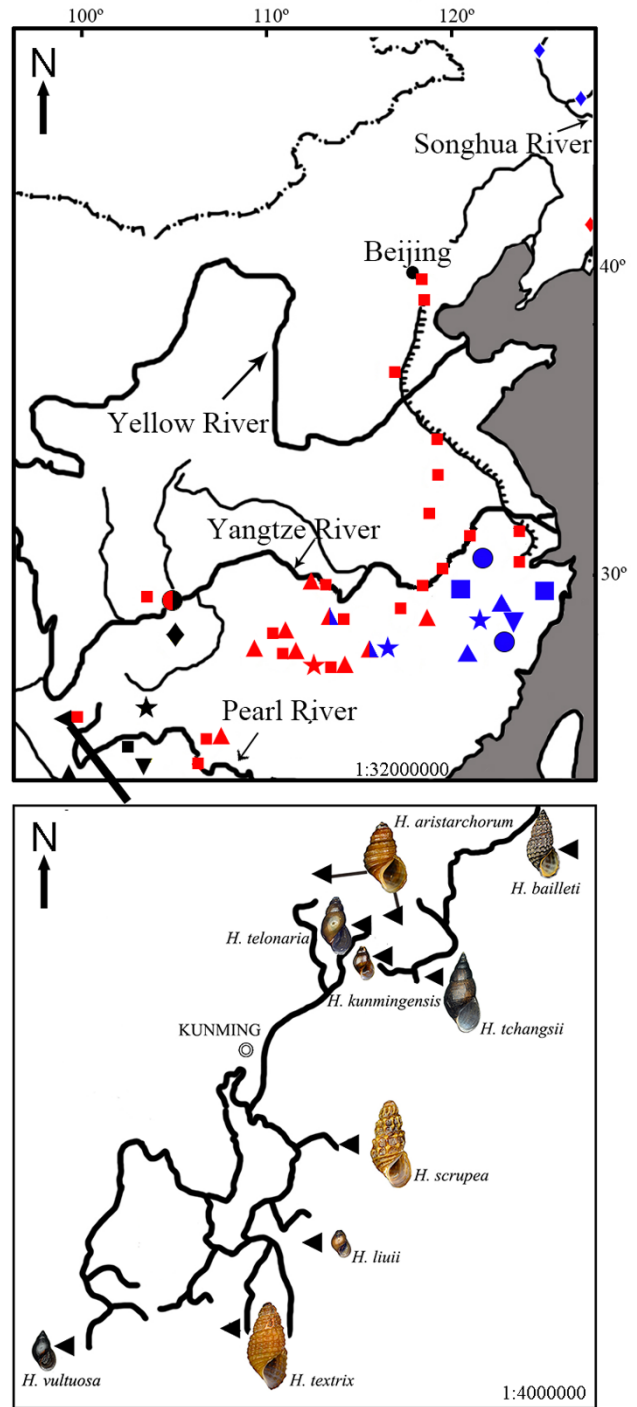


Figure 1 Sampling localities of semisulcospirids in China

Colors indicate collection sites of samples belonging to clades delimited in Figure 2 and Figure 3. Black: ●: *Hua pallens*; ▲: *H. jacqueti*; ■: *H. aubryana*; ▼: *H. funingensis*; ◆: *H. sp3*; ★: *H. intermedia*; ◀: Species from Dianchi Basin. Red: ●: *Semisulcospira pleuroceroides*; ■: *S. ningpoensis*; ▲: *S. gredderi*; ★: *S. cinnamomea*; ◆: *S. calcutus*. Blue: ●: *Koreoleptoxis pacificans*; ■: *K. terminalis*; ▲: *K. praenotata*; ★: *K. peregrinorum*; ▼: *K. dolichostoma*; ◆: *K. amurensis*.

MN013830 (Table 1). We also acquired various sequences from GenBank for use as the ingroup following Du et al. (2019), including one *Koreoleptoxis* species (*K. amurensis*), three *Semisulcospira* species (*S. pleuroceroides*, *S. gredleri* (Böttger, 1886), *S. ningpoensis*), and thirteen *Hua* species (*H. aristarchorum* (Heude, 1888), *H. telonaria* (Heude, 1888), *H. aubryana*, *H. bailleti* (Bavay & Dautzenberg, 1910), *H. tatrix*, *H. vultuosa* (Fulton, 1914), *H. scruposa* (Fulton, 1914), *H. funingensis* Du, Köhler, Yu, Chen & Yang, 2019, *H. kunmingensis* Du, Köhler, Yu, Chen & Yang, 2019, *H. liuii* Du, Köhler, Yu, Chen & Yang, 2019, *H. tchangsi* Du, Köhler, Yu, Chen & Yang, 2019, and two undescribed) (MK251600 – MK251768); *Elimia clenchi*, *E. catenaria*, and *Melanopsis* sp. were also obtained from GenBank as the outgroup to root the phylogeny in accordance with the trees in Strong & Köhler (2009) and Köhler (2017).

Sequence alignment of 16S rRNA and *COI* was performed with default parameters using Mafft v7.305 (Katoh et al., 2002) and the CIPRES Science Gateway (Miller et al., 2010). Pairwise distances between species were determined using MEGA 7 (Kumar et al., 2016) and the optimal substitution model was selected by corrected Akaike Information Criterion using jModeltest v2.1.10 (Posada, 2008). MRBAYES v3.2.6 (Ronquist et al., 2012) and RAxML-HPC v.8.2.10 (Stamatakis, 2014) were used for Bayesian inference and maximum likelihood analyses, respectively, in reference to the selected model of sequence evolution. Bayesian posterior probabilities (BPPs) of nodes were determined using Metropolis-coupled Markov chains (one cold chain) for five million generations, with sampling every 100 generations. Node support for maximum likelihood analysis was determined using 1 000 rapid bootstrap replicates.

RESULTS

Sequence analysis and mitochondrial phylogeny

The final concatenated dataset contained 221 16S sequences and 220 *COI* sequences. The 16S alignment contained 567 nucleotide positions after pruning alignment ends and removing poorly aligned portions. The *COI* dataset had 762 nucleotide positions and a total length of 1 339 bp, including 553 variable sites and 497 phylogenetically informative sites. The GTR+I+G model was selected as the best-fit model of nucleotide substitution. The dataset contained sequences from previously published studies, which were obtained from GenBank, as well as the new sequences, which were deposited in GenBank (accession Nos. shown in Table 1). Sequences of Western North America *Juga*, type species of the genera *Semisulcospira libertina* and *Koreoleptoxis globus ovalis*, and other published sequences of *K. nofilila* and *S. reiniana* from GenBank were included to check the phylogenetic positions of the Chinese semisulcospirids.

Both phylogenetic analyses produced identical, usually well-supported topologies consisting of two main clades (A and B), which contained sequences of various nominal species previously recorded in China. Clade A could be divided into

four subclades: subclade A1 contained species *P. rotundata*, *P. minensis*, and *L. pallens*; subclade A2 contained one undescribed species from Nanpanjiang River, *Hua* sp. 3, *H. aristarchorum*, and species of *Wanga* as orphans without genus affiliation from southwest China and Guangxi Zhuang Autonomous Region; subclade A3 contained *H. funingensis* and an undescribed species, *Hua* sp. 2, from Nanpanjiang River; subclade A4 contained *H. telonaria* (type species), species of *Hua* from southwest China and Vietnam, and two undescribed species (Figure 2). Clade B could be divided into three subclades; subclades B1 and B2 contained species of viviparous *Semisulcospira* from Japan and China; subclade B3 contained oviparous *K. globus ovalis*, *K. nofilila*, and *K. amurensis*, and species from the lower Yangtze River (Figure 3). These clades revealed an approximate phylogeographic structure, which roughly adhered to the major drainage systems in China (Figure 1). Results indicated that clade A (black) occurred predominantly in southwest China and drainages of the upper Yangtze and Pearl rivers, whereas subclades B2 (red) and B3 (blue) mainly occurred in the middle and lower Yangtze River, with a few specimens collected from northeast China (Figure 1).

Comparative morphology

We identified several patterns largely consistent with the phylogenetic clustering in the mitochondrial trees shown in Figure 2 and Figure 3. Subclades B1 and B2 are viviparous and mature females do not possess an ovipositor pore or egg-laying groove below the right cephalic tentacle; furthermore, the lower margin of the central teeth is straight in both subclades. Clade A and subclade B3 are both oviparous, but show differences in reproductive anatomy; specifically, mature females in clade A possess an ovipositor pore or egg-laying groove below the right cephalic tentacle, whereas mature females in subclade B3 possess both an ovipositor and egg-laying groove. In addition, the lower margin of the central teeth is w-shaped in clade A, but triangular in subclade B3.

Regarding operculum features, the nucleus is obvious and located in the lower base of the operculum in all subclades, except subclade A1, in which it is not obvious (Figure 4A–B). In subclade A3, the nucleus is very small and located in the lower one sixth of the operculum (Figure 4C–D); in subclades A2 and A4 and clade B, the operculum shows a clear spiral growth line and the nucleus is located in the lower one third of the operculum (Figure 4E–H).

In clade A, species of subclades A1 and A4 (except *H. aubryana* and *H. jacqueti*), as well as *H. sp. 3*, and *H. funingensis*, possess smooth shells, whereas all other species possess a sculptured shell. In *P. minensis*, *L. pallens*, *H. liuii*, and *H. funingensis*, the lateral teeth exhibit a single prominent squarish cusp without small denticles at either side (Figure 5B), whereas, in all other species, the lateral teeth exhibit two to four small denticles at each side (Figure 5A). In *P. minensis*, *P. rotundata*, *L. pallens*, *H. sp. 3*, and *H. tatrix*, the mature females possess an egg-laying groove, whereas mature females in all other species possess an oviparous pore. In

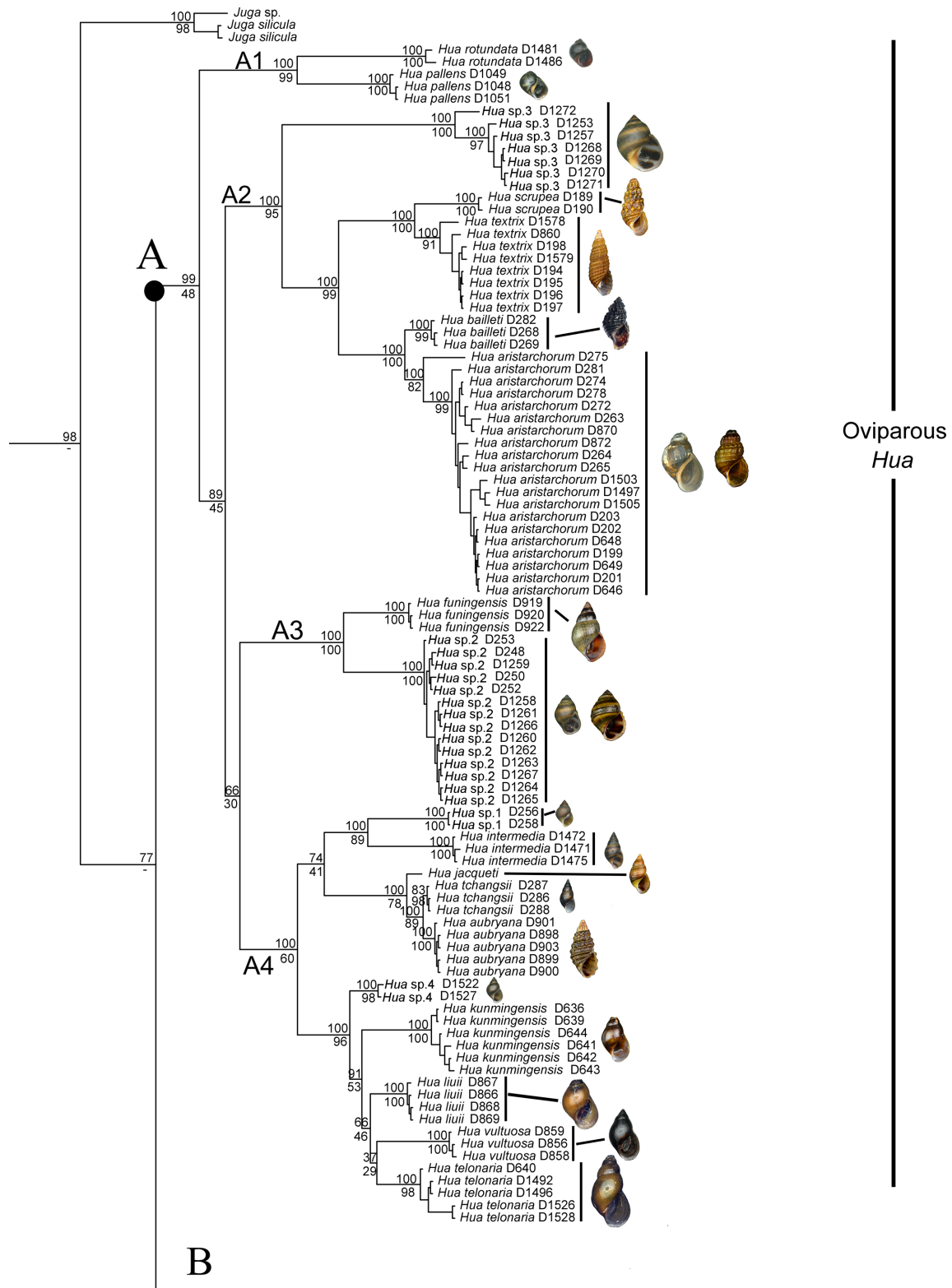


Figure 2 Bayesian phylogram of clade A based on analysis of concatenated dataset of mitochondrial cytochrome c oxidase subunit I (COI) and 16S rRNA sequences

Colored circles indicate origin of samples as indicated on map in Figure 1.

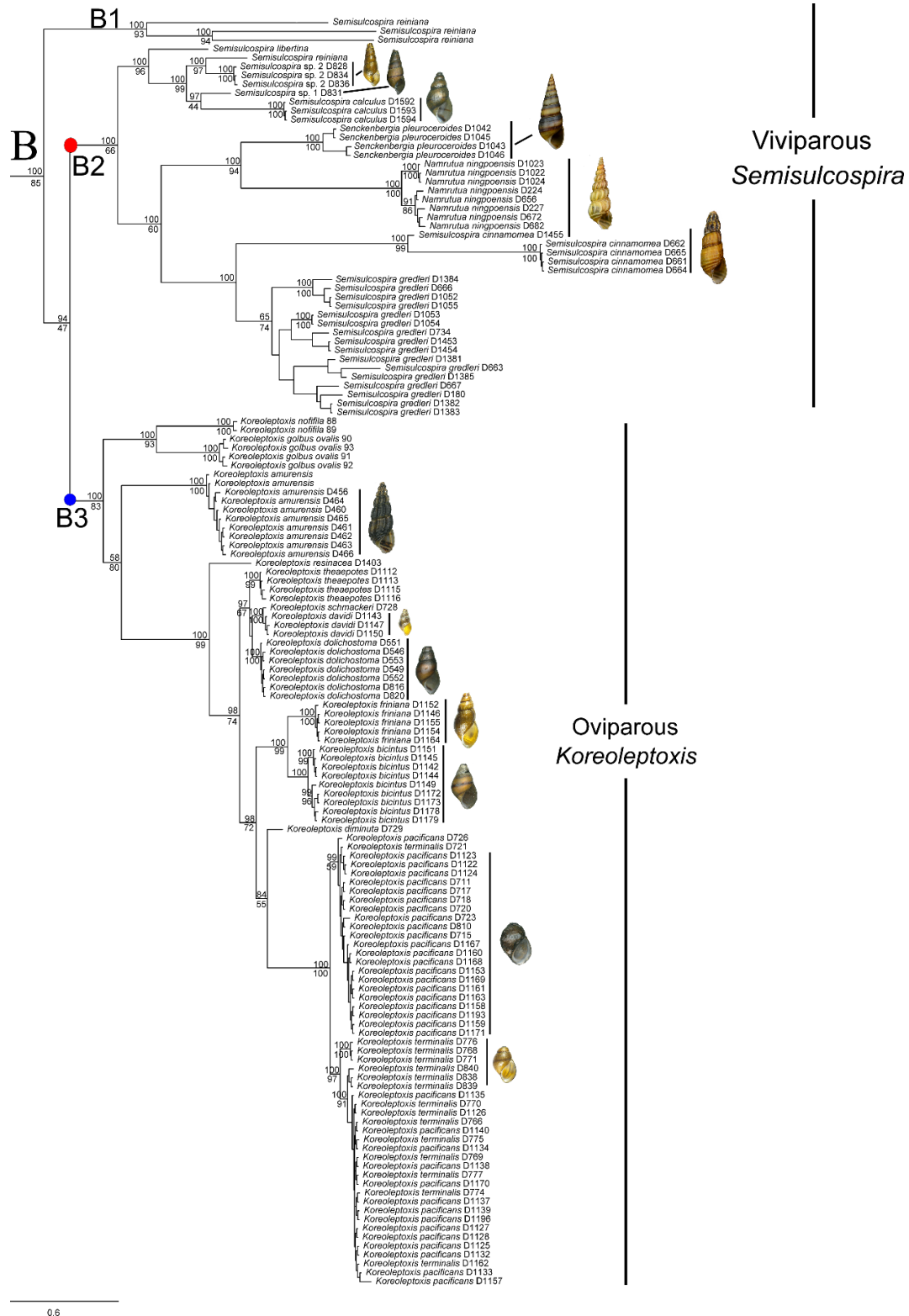


Figure 3 Bayesian phylogram of clade B based on analysis of concatenated dataset of mitochondrial cytochrome c oxidase subunit I (COI) and 16S rRNA sequences.

Colored circles indicate origin of samples as indicated on map in Figure 1.

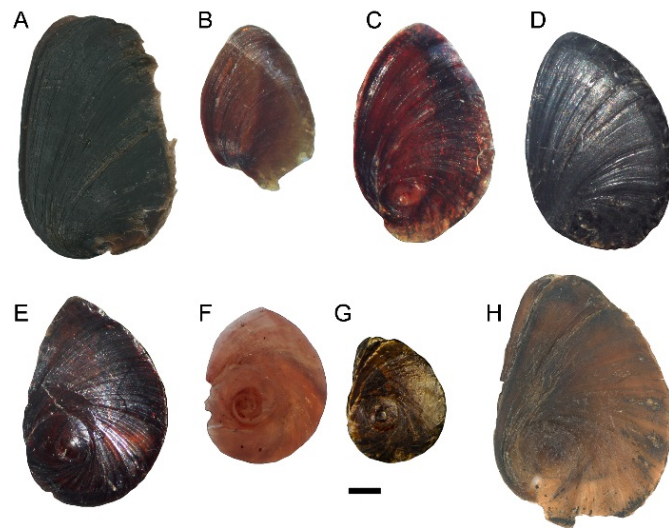


Figure 4 Opercula of semisulcospirids from China

A: *Hua pallens*; B: *H. rotundata*; C: *H. funingensis*; D: *H. sp.2*; E: *H. telonaria*; F: *Semisulcospira pleuroceroides*; G: *N. ningpoensis*; H: *K. amurensis*. Scale bar: 1 mm.

subclade B2, *S. ningpoensis*, *S. gredleri*, and *S. cinnamomea* (Gredler, 1887) have well-sculptured shells, whereas all other Chinese species possess a smooth shell. In subclade B3, *K.*

amurensis and *Melania pacificans* Heude, 1888 have well-sculptured shells, whereas all other Chinese species have a smooth shell.

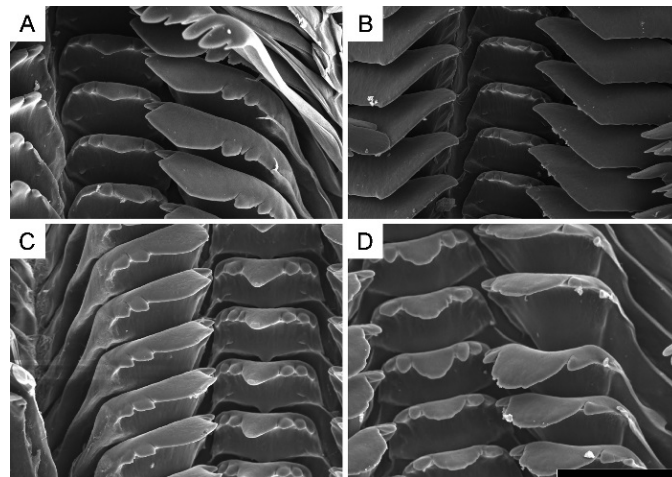


Figure 5 Radulae of semisulcospirids from China

A: *Hua telonaria*; B: *H. funingensis*; C: *Koreoleptoxis*; D: *Semisulcospira*. Scale bar: 100 μ m.

Mitochondrial differentiation

Pairwise comparisons of *COI* distances revealed that clade A and subclades B2 and B3 differed from each other by average uncorrected *P*-distances of 14.6% to 14.7% (average 14.6%). Species within clade A differed by an average uncorrected *P*-distance of 0.8% to 14.5% (average 11.2%). Species within subclade B2 differed by an average uncorrected *P*-distance of 10.0% to 14.9% (average 12.0%). Species within subclade B3 differed by an average uncorrected *P*-distance of 1.2% to 11.2% (average 6.6%).

DISCUSSION

In this study, the Chinese semisulcospirids were divided into two main clades, i. e., clades A and B. Minton & Lydeard (2003) reported maximum intergeneric differences of 15.5% for *COI* in Semisulcospiridae. Here, we observed a maximum intergeneric divergence of 14.7%. Hence, clade A, together with subclades B2 and B3, represented the genera of *Hua*, *Koreoleptoxis*, and *Semisulcospira*, respectively. Morphologically, the three genera can be distinguished from

each other by reproductive mode, radula, and female reproductive features.

Liu et al. (1993) and Xu (2007) assigned *Senckenbergia pleuroceroides* and *Namrutua ningpoensis* into *Semisulcospira*, but did not provide a reason for this decision. Du et al. (2019) treated *Namrutua* and *Senckenbergia* as junior synonyms to *Semisulcospira* based on morphological and phylogenetic evidence. In addition to three Chinese species, i. e., *S. gredleri*, *S. ningpoensis*, and *S. pleuroceroides*, four other species, i. e., *S. cinnamomea* (Gredler, 1887) from Yangtze River, *S. calculus* (Reeve, 1860) from Liaoning Province in northeast China, and two undescribed species from Zhejiang Province were contained in this study. *Semisulcospira cinnamomea* was initially described as a subspecies of *S. gredleri* (Gredler, 1887); however, it is treated as a valid species in this study as it can be distinguished from *S. gredleri* by its six to nine whorls (vs. four to five) and body whorl not inflated (vs. inflated). Furthermore, *M. calculus* and *M. paucicincta* Martens 1894 are virtually identical (shell size, proportion, rate of whorl expansion, and color bands), and are here treated as synonyms, with *Semisulcospira calculus* considered as the valid species name due to the description of *M. calculus* predating that of *M. paucicincta*. The Chinese species of *Semisulcospira* can be distinguished from each other by their shell features (sculptured or smooth, body whorl inflated or not, number of whorls) and radulae.

Several species were originally described as members of *Paludomus*, including *P. kweichouensis*, *P. minensis*, *P. qianensis*, and *P. cinctus* (Chen, 1937, 1943; Liu et al., 1994). Although the Chinese *Paludomus* species are similar to *P. conica* Gray (type species of *Paludomus*, type location in India) in operculum features, Abbott (1948) stated that the mantle margin in *P. conica* contains 20 to 25 papillae, whereas the mantle margin in Chinese *Paludomus* is smooth. Furthermore, in the current study, phylogenetic evidence indicated that Chinese *Paludomus* species recorded in southwest China could belong to *Hua* (Semisulcospiridae). In addition, *P. minensis* and *L. pallens*, which come from the same locality, are virtually identical, except that the body whorl in *L. pallens* is more inflated than that in *P. minensis*; thus, here, they are deemed to be synonyms, with *Hua pallens* considered as the valid species name as the description of *L. pallens* predates that of *P. minensis*. Furthermore, *P. kweichouensis* is treated as a synonym of *M. intermedia* Gredler, 1884 due to their virtually identical shells and same locality, with *Hua intermedia* considered as the valid species name due to the description of *M. intermedia* predating that of *P. kweichouensis*.

Chen (1943) originally described *Wanga* (containing eight species) and *Hua* (containing eleven species). In this study, however, the species of *Wanga* and *Hua* from southwest China and Guangxi are placed in *Hua*, which therefore contains 19 species in China, i. e., *H. aristarchorum*, *H. aubryana*, *H. baillei*, *H. funingensis*, *H. intermedia*, *H. jacqueti*, *H. kunmingensis*, *H. liuii*, *H. pallens*, *H. rotundata*, *H. scrupea*, *H. textrix*, *H. telonaria*, *H. tchangsii*, *H. vultuosa*, and

four undescribed species (Du et al., 2019); other species from south of the middle and lower Yangtze River are placed into *Koreoleptoxis*, which therefore contains *K. diminuta* (Boettger, 1887), *K. friniana* (Heude, 1888), *K. joretiana* (Heude, 1890), *K. naiadarum* (Heude, 1890), *K. oreadarum* (Heude, 1888), *K. parenotata* (Gredler, 1884), *K. schmackeri* (Boettger, 1886), and *K. toucheana* (Heude, 1888).

Within *Hua*, species can be distinguished from each other by the shell (sculptured or smooth, elongated or ovate), operculum, radula, and female reproductive features. Additionally, within *Koreoleptoxis*, species can be distinguished from each other by the shell (sculptured or smooth, elongated or ovate) and radula features. Although *K. pacificans* and *K. terminalis* are not monophyletic in the phylogenetic tree, *K. pacificans* can be distinguished from *K. terminalis* by morphological characteristics: i. e., shell solid and sculptured (vs. thin and smooth) and same number of inner and outer margin teeth (vs. different). Hence, *K. pacificans* and *K. terminalis* are treated as valid species in this study. Incongruence between mtDNA phylogeny and morphospecies is not a rare phenomenon in semisulcospirids (Köhler, 2016, 2017). Retention of ancestral polymorphisms, introgression, heteroplasmy, recombination, and the presence of morphologically cryptic species or taxonomic misidentification may explain why mtDNA phylogenies do not accurately reflect the relationships of their host organisms (Ballard & Whitlock, 2004; Edwards & Beerli, 2000; Funk & Omland, 2003; Köhler & Deen, 2010; Köhler, 2016; White et al., 2008).

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

L.N.D. and J.X.Y. designed the study. J.C. checked the specimens of *Koreoleptoxis* and *Semisulcospira* that preserved in Institute of Zoology, CAS. G. H. Y. supervised the molecular analyses. L. N. D. extracted genomic DNA and wrote the manuscript with the other authors' input. L.N.D. and G.H.Y. sequenced mitochondrial DNA and submitted to GenBank. L.N.D. and G.H.Y. revised the manuscript. All authors read and approved the final version of the manuscript.

ACKNOWLEDGEMENTS

We are grateful to G.H. Cui, S.S. Shu, S.W. Liu, X.A. Wang, Y.E. Jiang, C. Yuan, X.F. Pan, T. Qin (Kunming Institute of Zoology, Chinese Academy of Sciences), J. Yang (Nanning Normal University, China) and J.H. Lan (Du'an Fishery Technique Popularization Station) for specimens collection. Special thanks are due to Dr. V. Héros (Muséum National d'Histoire Naturelle, Paris, France), Dr. A. Hänggi (Naturhistorisches Museum, Basel, Switzerland), Dr. E. Strong (National Museum of Natural History in Washington DC, USA), and Dr. J. Ablett (Natural History Museum, London, UK) for providing photographs of type specimens. We are grateful to Dr. C. Watts for polishing this manuscript.

REFERENCES

- Abbott RT. 1948. Handbook of medically important mollusks of the Orient and the western Pacific. *Bulletin of the Museum of Comparative Zoology*, **100**(3): 245–328.
- Ballard JWO, Whitlock MC. 2004. The incomplete natural history of mitochondria. *Molecular Ecology*, **13**(4): 729–744.
- Chen SF. 1937. Four new species of freshwater molluscs from China. *Journal of the Washington Academy of Sciences*, **27**: 444–448.
- Chen SF. 1943. Two new genera, two new species, and two new names of Chinese Melaniidae. *The Nautilus*, **57**(1): 19–21.
- Dautzenberg P, Fischer H. 1906. Contribution à la faune malacologique de l'Indo-Chine. *Journal de Conchyliologie*, **54**: 145–226. (in Germany)
- Dautzenberg P, Fischer H. 1908. Liste de mollusques récoltés par M. Mansuy en Indo-Chine et description d'espèces nouvelles. *Journal de Conchyliologie*, **56**: 169–217. (in Germany)
- Du LN, Chen XY, Yang JX. 2017. Morphological redescription and neotype designation of *Sulcospira paludiformis* (Yen, 1939) from Hainan, China. *Molluscan Research*, **37**: 66–71.
- Du LN, Köhler F, Yu GH, Chen XY, Yang JX. 2019. Comparative morpho-anatomy and mitochondrial phylogeny of the Semisulcospiridae of Yunnan, southwestern China, with description of four new species (Gastropoda, Cerithioidea). *Invertebrate Systematics*, doi: 10.1071/IS18084.
- Du LN, Yang JX. 2019. A review of *Sulcospira* (Gastropoda: Pachychilidae) from China, with description of two new species. *Molluscan Research*, **39**(3): 241–252.
- Edwards SV, Beerli P. 2000. Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution*, **54**(6): 1839–1854.
- Fulton HC. 1904. On some new species of *Melania* and *Jullienia* from Yunnan and Java. *Journal of Malacology*, **11**: 51–52.
- Fulton HC. 1914. Descriptions of new species of *Melania* from Yunnan, Java, and the Tsushima Islands. *Journal of Mollusca Studies*, **11**(3): 163–164.
- Funk DJ, Omland KE. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics*, **34**: 397–423.
- Heude PM. 1882–1890. Mémoires concernant l'histoire naturelle de l'empire chinois par des pères de la Compagnie de Jésus. Notes sur les Mollusques terrestres de la vallée du Fleuve Bleu. Chang-Hai: Mission Catholique, 188. (in Germany)
- Heude PM. 1888. Diagnoses molluscorum novarum in Sinis. *Journal de Conchyliologie*, **36**: 305–309. (in Germany)
- Katoh K, Misawa K, Kuma KI, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, **30**(14): 3059–3066.
- Köhler F. 2016. Rampant taxonomic incongruence in a mitochondrial phylogeny of *Semisulcospira* freshwater snails from Japan (Cerithioidea: Semisulcospiridae). *Journal of Molluscan Studies*, **82**(2): 268–281.
- Köhler F. 2017. Against the odds of unusual mtDNA inheritance, introgressive hybridization and phenotypic plasticity: systematic revision of Korean freshwater gastropods (Semisulcospiridae, Cerithioidea). *Invertebrate Systematics*, **31**: 249–268.
- Köhler F, Deen G. 2010. Hybridisation as potential source of incongruence in the morphological and mitochondrial diversity of a Thai freshwater gastropod (Pachychilidae, Brotia H. Adams, 1866). *Zoosystematics and Evolution*, **86**(2): 301–314.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33**(7): 1870–1874.
- Liu YY, Zhang WZ, Wang EY. 1979. Freshwater molluscs. In: Economic Fauna of China. Beijing: Science Press. (in Chinese)
- Liu YY, Zhang WZ, Wang YX. 1993. Medical Malacology. Beijing: China Ocean Press. (in Chinese)
- Liu YY, Zhang WZ, Wang YX, Duan YH. 1994. Eight new species of freshwater molluscs in southwest China (Gastropoda: Bivalvia). *Acta Zootaxonomica Sinica*, **19**(1): 25–36.
- Miller MA, Pfeiffer W, Schwartz T. 2010. "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA, pp 1–8.
- Morrison JPE. 1954. The relationships of old and new world melanians. *Proceedings of the United States National Museum*, **103**(3325): 357–394.
- Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G. 1991. The Simple Fool's Guide to PCR. University of Hawaii, Honolulu.
- Posada D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**(7): 1253–1256.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**(3): 539–542.
- Stamatakis A. 2014. RAxML Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **30**(9): 1312–1313.
- Strong EE, Köhler F. 2009. Morphological and molecular analysis of '*Melania*' *jacqueti* Dautzenberg and Fischer, 1906: from anonymous orphan to critical basal offshoot of the Semisulcospiridae (Gastropoda: Cerithioidea). *Zoologica Scripta*, **38**(5): 483–502.
- Tchang S, Tsi CY. 1949. Liste des mollusques d'eau douce recueillis pendant les années 1938–1946 au Yunnan et description d'espèces nouvelles. *Contributions from the Institute of Zoology, National Academy of Peiping*, **5**: 205–220. (in French)
- White DJ, Wolff JN, Pierson M, Gemmell NJ. 2008. Revealing the hidden complexities of mtDNA inheritance. *Molecular Ecology*, **17**: 4925–4942.
- Wilson AB, Glaubrecht M, Meyer A. 2004. Ancient lakes as evolutionary reservoirs: evidence from the thalassoid gastropods of Lake Tanganyika. *Proceedings of the Royal Society B, Biological Sciences*, **271**(1538): 529–536.
- Xu XF. 2007. Classification and Morphology on Pleuroceridae (Gastropoda: Mesogastropoda) of Some Area in Middle Reaches of Changjiang River. Ph. D. dissertation, Nancang University, China. (in Chinese)
- Yen TC. 1939. Die chinesischen Land- und Süßwasser-Gastropoden des Natur-Museums Senckenberg. *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft*, **444**: 51–59. (in Germany)
- Zhang NG, Hao TX, Wu CY, Chen YX, Zhang W, Li JK, Zhang Y. 1997. A research of freshwater Gastropoda from Yunnan. *Studia Marina Sinica*, **39**: 15–25.