ZOOLOGICAL RESEARCH

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

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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*

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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

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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 descried 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 trivolvis* 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

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

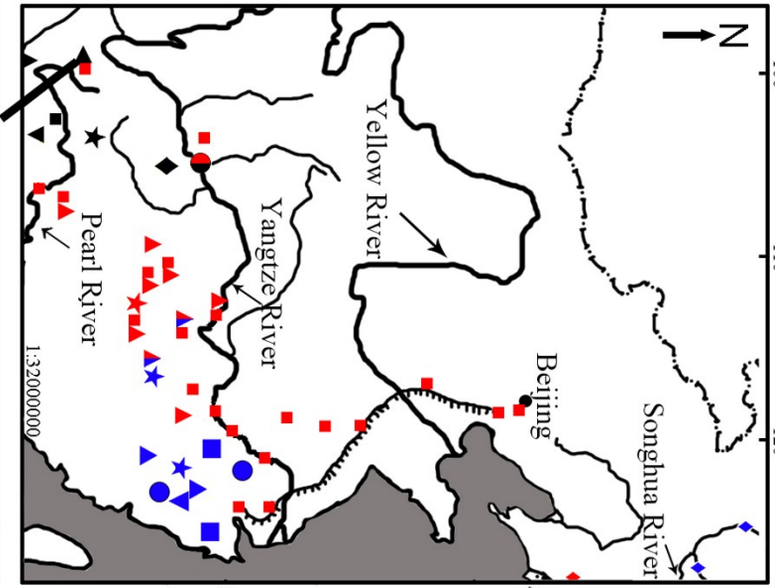
Continued

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

Continue

Taxon name	Locality	Voucher No.	Synonym	GenBank accession No.	
				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
S. ningpoensis	Neijiang City, Sichuan	D1022–D1024	*M. cancellata, M. ningpoensis, M. bensoni, M. fortune, M. suifuensis, S. cancellata, Melanoides ningpoensis, H. (Namrutua) ningpoensis*	MK944145–47	MK969009–11
	Zhangshu City, Jiangxi	D656, D672, D682, D224, D227		MK944105, MK251606–07, MK251604–05	MK968970, MK251697–98, MK251695–96
S. pleuroceroides	Chongqing	D1042–D1043, D1045–D1046	*M. pleuroceroides*	MK251669–72	MK251748–51
Semisulcospira sp.	Ningpo City, Zhejiang	D828, D831, D834, D836	–	MK944134–37	MK968998–9001

–: Not available.



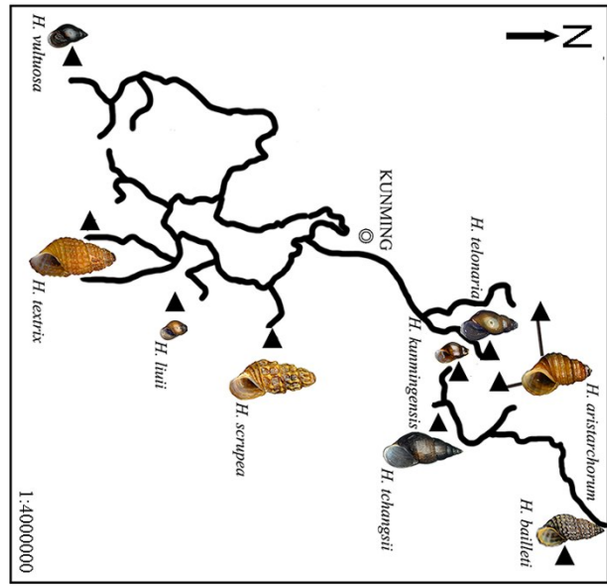


Figure 1 Sampling localities of semisulcospirids in China

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. gredleri*; ★: *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. textrix*, *H. vultuosa* (Fulton, 1914), *H. scrupea* (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. tchangsii* 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. nofifila* 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. pallen*s; 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. nofifila*, 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. textrix*, the mature females possess an egg-laying groove, whereas mature females in all other species possess an oviparous pore. In

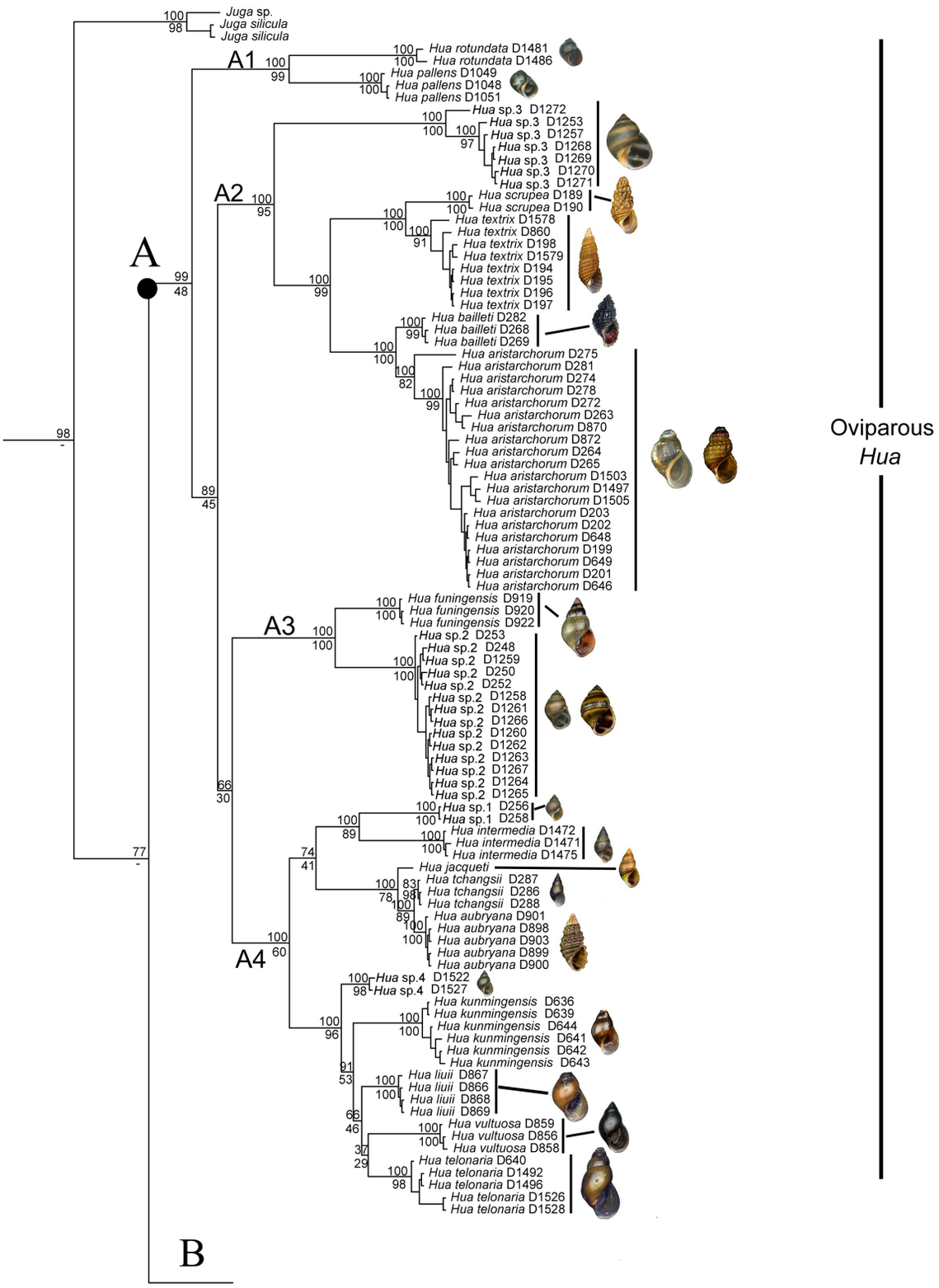


Figure 2 Bayesian phylogram of clade A based on analysis of concatenated dataset of mitochondrial cytochrome c oxidase subunit I (*COI*) and 16S rRNA sequences

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

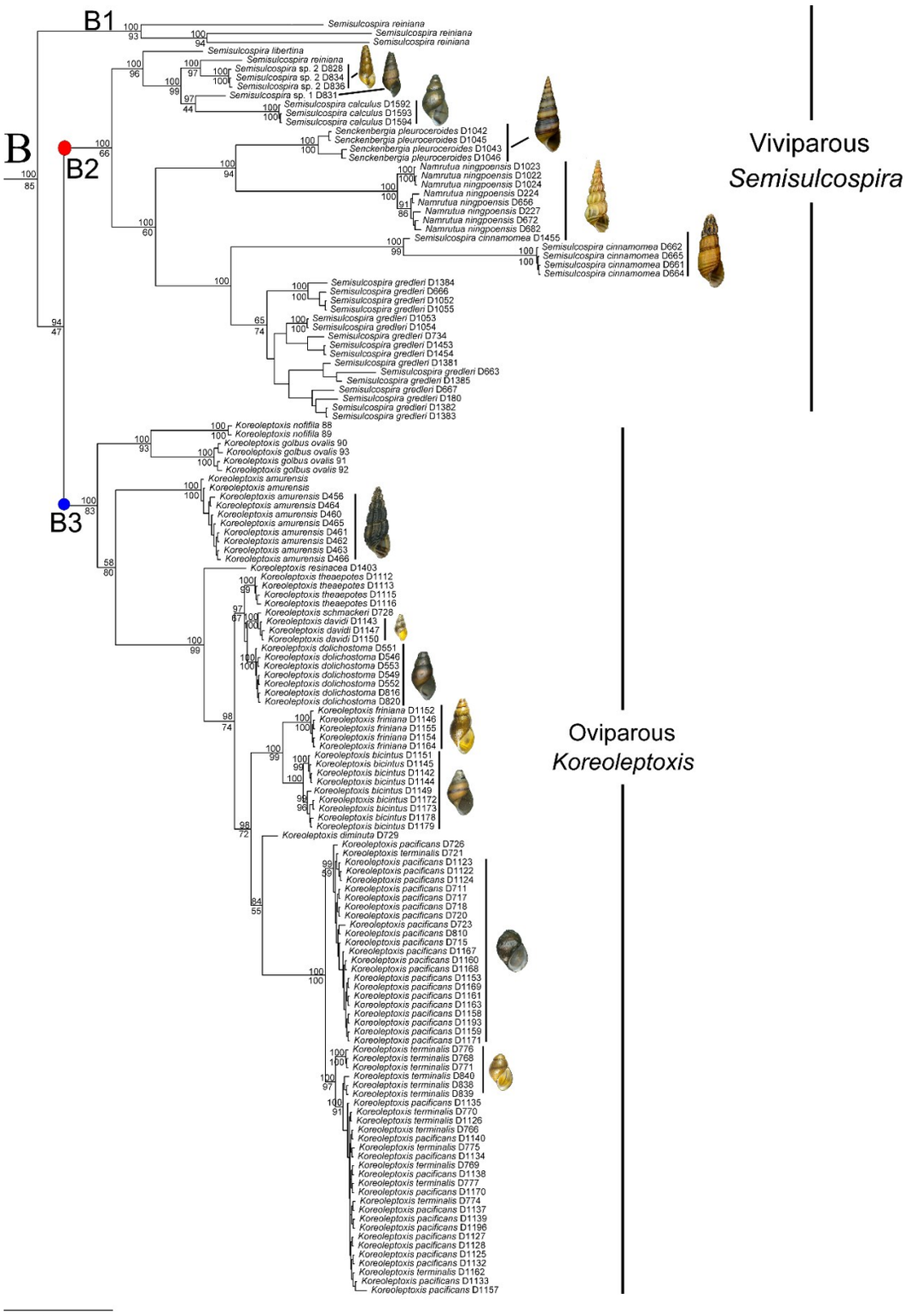


Figure 3 Bayesian phylogram of clade B based on analysis of concatenated dataset of mitochondrial cytochrome c oxidase subunit I (*COI*) and 16S rRNA sequences.

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

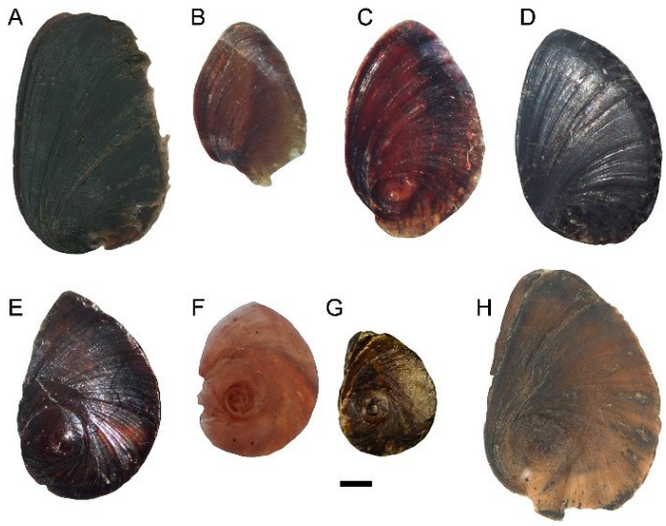


Figure 4 Opercula of semisulcospirids from China
A: *Hua pallens*; B: *H. rotundata*; C: *H. funingensis*; D: *H.* sp. 2; E: *H. telonaria*; F: *Semisulcospira pleuroceroides*; G: *N. ningpoensis*; H: *K. amurensis*. Scale bar: 1 mm.

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.

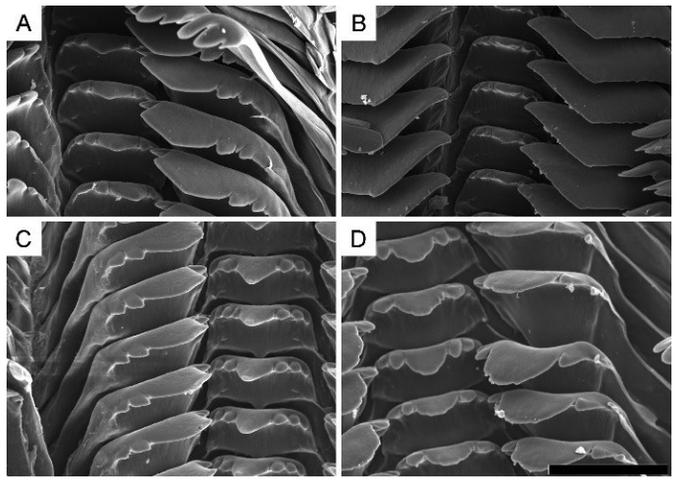


Figure 5 Radulae of semisulcospirids from China
A: *Hua telonaria*; B: *H. funingensis*; C: *Koreoleptoxis*; D: *Semisulcospira*. Scale bar: 100 μm.

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. bailleti*, *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 & Deein, 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.

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