

Molecular phylogeny of the Indochinese freshwater mussel genus *Scabies* Haas, 1911 (Bivalvia: Unionidae)

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ABSTRACT.— Molecular phylogeny of the Indochinese freshwater mussel genus *Scabies* Haas, 1911 was studied. The examined taxa included all seven recognized species of the genus *Scabies* (*S. phaselus*, *S. anceps*, *S. scobinatus*, *S. crispata*, *S. mandarinus*, *S. nucleus* and *S. songkramensis*) and two closely related Indochinese species currently assigned to the genus *Radiatula* Simpson, 1900 (*R. humilis* and *R. pilata*). Maximum likelihood (ML) and Bayesian Inference (BI) phylogenetic trees based on the concatenated sequences of two mitochondrial genes (cytochrome oxidase c subunit 1 and 16S ribosomal RNA) and one nuclear gene (28S ribosomal RNA) fragments (1,586 bp) revealed a polyphyletic relationship in both genera compared to the outgroups of other genera from the tribe Indochinellini. *Scabies songkramensis* was clustered separately from the congeners, whereas *R. humilis* and *R. pilata* were grouped within the *Scabies* clade with high support (88% bootstrap support for ML and a Bayesian posterior probability of 1 for BI), and separated from other members of *Radiatula* from western Indochina (Myanmar and India). Based on these phylogenetic results, in combination with morphological and biogeographic data, these two Indochinese *Radiatula* species were transferred to *Scabies*, as *S. humilis* (Lea, 1856) comb. nov. and *S. pilata* (Lea, 1866) comb. nov. The genus *Radiatula* s.s. should be implied only to taxa endemic to the western Indochinese region, while the genus *Scabies* are distributed in the Chao Phraya and Mekong River basins and other rivers that drain into the Gulf of Thailand.

KEY WORDS: Indochina, freshwater mussels, *Radiatula*, taxonomy, Unionidae

INTRODUCTION

Freshwater mussels in the family Unionidae consist of more than 680 known species described worldwide (Graf, 2013; Graf and Cummings, 2007). These animals are especially diverse in tropical Asia, which has been hypothesized to be their center of radiation (Bogan, 2008; Bolotov et al., 2017a, b; Graf and Cummings, 2007). Taxonomic members of the family occur in all types of aquatic environments and play many important ecological roles. They influence water chemistry and quality

according to their filter feeding strategy, serve as food for predators, and provide physical structure in the local habitat to other organisms (Strayer, 2008).

Freshwater mussels have recently become one of the most severely endangered groups of animals, with many species under threat and some being severely endangered, due to human activities, such as habitat destruction, introduction of invasive species and loss of host fishes (Bogan, 2008; Haag and Williams, 2014; Zieritz et al., 2016). However, the status and rate of extinction of

freshwater mussels is poorly understood at this time, because of their uncertain taxonomic classification and deficient data (Köhler et al., 2012). Although, many recent integrative works have focused on the freshwater mussels of the Indotropical region, and have greatly improved our knowledge on the taxonomy and biogeography of these animals (Bolotov et al., 2017a, b, 2018; Jeratthitikul et al., 2019; Konopleva et al., 2017, 2019; Pfeiffer et al., 2018), many taxa remain poorly attended.

The genus *Scabies* Haas, 1911 is a medium size freshwater mussel group, belonging to the tribe Indochinellini Bolotov et al., 2018, subfamily Parreysiinae Henderson, 1935 (Pfeiffer and Graf, 2015). The genus is distinguishable from others by having a rather solid but not very thick shell that is generally sculptured with brownish zigzag-lines in a V- or W- pattern (Brandt, 1974). The genus was first introduced as a subgenus of the genus *Nodularia* Conrad, 1853, and later raised to the generic level by Haas (1969). Currently, seven species are recognized in the genus *Scabies*, namely: *S. crispata* (Gould, 1843), *S. phaselus* (Lea, 1856), *S. nucleus* (Lea, 1856), *S. songkramensis* Kongim & Panha, 2015, *S. anceps* (Deshayes, 1874), *S. scobinatus* (Lea, 1856) and *S. mandarinus* (Morelet, 1863) (Kongim et al., 2015; Pfeiffer et al., 2018). Note that the last three species were recently raised up to the species level by Pfeiffer et al. (2018). All of these seven species are distributed in the mainland southeast Asia, endemic to the Chao Phraya and Mekong River Basins and other rivers that drain into the Gulf of Thailand, except for *S. crispata*, which is recorded from the Tavoy River, Myanmar (Brandt, 1974; Graf and Cummings, 2019).

Other members of the tribe Indochinellini that occur in Indochinese Rivers are the genera *Indonaia* Prashad, 1918, *Harmandia* Rochebrune, 1881, *Unionetta* Haas, 1955 and *Radiatula* Simpson, 1900 (Konopleva et al., 2019; Pfeiffer et al., 2018). The first three genera are monotypic, whereas the genus *Radiatula* contains two ascribed species found in this region, *R. pilata* (Lea, 1866) and *R. humilis* (Lea, 1866) (Brandt, 1974; Pfeiffer et al., 2018).

The present study aimed to reveal the phylogenetic relationship of the genus *Scabies* based on multilocus data of two mitochondrial genes, cytochrome c oxidase subunit I (COI) gene and the 16S ribosomal RNA (16S), and one nuclear gene, 28S ribosomal RNA (28S).

MATERIAL AND METHODS

Taxon and Specimen sampling

The examined taxa in present study included all valid species of the genus *Scabies*: *S. phaselus*, *S. anceps*, *S. scobinatus*, *S. crispata*, *S. mandarinus*, *S. nucleus* and *S. songkramensis*, plus two closely related taxa currently ascribed to the genus *Radiatula*; *R. pilata* and *R. humilis* (Bolotov et al., 2018). Sequences from other taxa representing all the genera within the tribe Indochinellini, and some taxa from other tribes within the subfamily Parreysiinae were retrieved from the GenBank database (Bolotov et al., 2017a, b, 2019; Pfeiffer and Graf, 2015; Pfeiffer et al., 2018; Whelan et al., 2011), and used for phylogenetic reconstruction in this study. The list of the taxa analyzed, with the locality name, voucher specimen ID and GenBank accession number, are listed in Table 1.

TABLE 1. List of taxa analyzed in the present study with the locality name, voucher specimen ID and Genbank accession number.

Taxon	Locality	Voucher ID	COI	16S	28S	Source
<i>Scabies phaselus</i>	Tron Stream, Uttaradit, Thailand	MUMNH:UNI0042	MG288593	MG552733	MG552785	This study
	Kho Lueak, Phetchabun, Thailand	MUMNH:UNI0403	MG288596	MG552736	MG552788	This study
	Phra Phloeng River, Nakhon Ratchima, Thailand	MUMNH:UNI1050	MG288589	MG552729	MG552781	This study
	Hua Thale, Nakhon Ratchasima, Thailand	MUMNH:UNI1098	MG288595	MG552735	MG552787	This study
	Mun River, Satuek, Buri Ram, Thailand	MUMNH:UNI1279	MG288590	MG552730	MG552782	This study
		MUMNH:UNI1280	MG288592	MG552732	MG552784	This study
	Mun River, Tha Tum, Surin, Thailand	MUMNH:UNI1119	MG288598	MG552738	MG552790	This study
		MUMNH:UNI1125	MG288599	MG552739	MG552791	This study
		MUMNH:UNI1243	MG288597	MG552737	MG552789	This study
		MUMNH:UNI1253	MG288604	MG552744	MG552796	This study
	Xe Don River, Muang Khongxedon, Laos	MUMNH:UNI1257	MG288605	MG552745	MG552797	This study
		MUMNH:UNI1029	MG288591	MG552731	MG552783	This study
	Yang Stream, Watthana Nakhon, Thailand	MUMNH:UNI1064	MG288601	MG552741	MG552793	This study
	Yang Stream, Sa Kao, Thailand	MUMNH:UNI1072	MG288600	MG552740	MG552792	This study
	Wang Chik Stream, Sa Kao, Thailand	MUMNH:UNI1006	MG288602	MG552742	MG552794	This study
<i>Scabies scobinatus</i>	Sai Khao River, Chanthaburi, Thailand	MUMNH:UNI1009	MG288603	MG552743	MG552795	This study
		MUMNH:UNI0801	MG288594	MG552734	MG552786	This study
	Phraphui Stream, Chanthaburi, Thailand	MUMNH:UNI1081	MG288612	MG552752	MG552804	This study
	Huai Luang River, Udon Thani, Thailand	MUMNH:UNI1057	MG288611	MG552751	MG552803	This study
	Yam River, Sakon Nakhon, Thailand	MUMNH:UNI0366	MG288606	MG552746	MG552798	This study
	Nam Don River, Tha Khek, Khammouane, Laos	MUMNH:UNI1391	MG288610	MG552750	MG552802	This study
	Xe Nou River, Savannakhet, Laos	MUMNH:UNI1432	MG288609	MG552749	MG552801	This study
	Huay Thahao Stream, Savannakhet, Laos	MUMNH:UNI1312	MG288608	MG552748	MG552800	This study
	Xe Bang Nouan River, Savannakhet, Laos	MUMNH:UNI1219	MG288607	MG552747	MG552799	This study
	Kratie – Stung Treng, Cambodia	UF 507385 (ICH-00428)	MH350928	-	MH350977	Pfeiffer et al. (2018)
<i>Scabies anceps</i>		UF 507394 (ICH-00456)	MH350929	-	MH350978	Pfeiffer et al. (2018)

TABLE 1. Continued

Taxon	Locality	Voucher ID	COI	16S	28S	Source
<i>Scabies crispata</i>	Kratie – Stung Treng, Cambodia	UF 507431 (ICH-00583)	MH350936	-	MH350985	Pfeiffer et al. (2018)
	Mae Lai River, Phrae, Thailand	MUMNH:UNII0084	MG288632	MG552772	MG552824	This study
	Yom River, Nakhon Sawan, Thailand	MUMNH:UNII123	MG288634	MG552774	MG552826	This study
	Mae Klong River, Ratchaburi, Thailand	MUMNH:UNII0141	MG288633	MG552773	MG552825	This study
<i>Scabies mandarinus</i>	In Trai Stream, Prachin Buri, Thailand	MUMNH:UNII0398	MG288631	MG552771	MG552823	This study
	Phrom Hot River, Aranyaprathet, Thailand	MUMNH:UNII0961	MG288620	MG552760	MG552812	This study
	Phrom Hot River, Watthana Nakhon, Thailand	MUMNH:UNII1023	MG288622	MG552762	MG552814	This study
	Serei Sophorn River, Cambodia	MUMNH:UNII0988	MG288621	MG552761	MG552813	This study
	Cambodia	UMMZ:304646	KP795023	KP795048	KP795006	Pfeiffer and Graf (2015)
<i>Scabies nucleus</i>	Mekong Delta, Vietnam	UF 507434 (ICH-00593)	MH350937	-	MH350986	Pfeiffer et al. (2018)
	Mekong Delta, Vietnam	UF 507450 (ICH-00630)	MH350939	-	MH350988	Pfeiffer et al. (2018)
	Xe Don River, Muang Khongxeton, Laos	MUMNH:UNII264	MG288613	MG552753	MG552805	This study
	Mun River, Ban Pasart, Surin, Thailand	MUMNH:UNII266	MG288614	MG552754	MG552806	This study
<i>Scabies humilis</i> comb. nov.	Mun River, Surin, Thailand	MUMNH:UNII0928	MG288616	MG552756	MG552808	This study
	Dom Yai River, Ubon Ratchathani, Thailand	MUMNH:UNII490	MG288617	MG552757	MG552809	This study
		MUMNH:UNII491	MG288618	MG552758	MG552810	This study
	Xe Don River, Pakse, Laos	MUMNH:UNII368	MG288615	MG552755	MG552807	This study
<i>Scabies pilata</i> comb. nov.		MUMNH:UNII375	MG288619	MG552759	MG552811	This study
	Mekong River, Nakhon Phanom, Thailand	MUMNH:UNII0514	MG288623	MG552763	MG552815	This study
	Nam Hin Boun River, Khammouane, Laos	MUMNH:UNII172	MG288627	MG552767	MG552819	This study
	Nam Pakan River, Khammouane, Laos	MUMNH:UNII215	MG288628	MG552768	MG552820	This study
	Xe Nou River, Savannakhet, Laos	MUMNH:UNII422	MG288626	MG552766	MG552818	This study
	Xe Bang Fai River, Savannakhet, Laos	MUMNH:UNII151	MG288625	MG552765	MG552817	This study
	Banghiang River, Phosay, Savannakhet, Laos	MUMNH:UNII436	MG288624	MG552764	MG552816	This study
		MUMNH:UNII440	MG288630	MG552770	MG552822	This study
	Banghiang River, Savannakhet, Laos	MUMNH:UNII414	MG288629	MG552769	MG552821	This study

TABLE 1. Continued

Taxon	Locality	Voucher ID	COI	16S	28S	Source
<i>Scabies songkramensis</i>	Songkhram River, Udon Thani, Thailand	MUMNH:UNIO0329	MG288635	MG552775	MG552827	This study
	Yam River, Sakon Nakhon, Thailand	MUMNH:UNIO0365	MG288638	MG552778	MG552830	This study
	Songkarm River, Tha Uthen, Thailand	MUMNH:UNIO0405	MG288637	MG552777	MG552829	This study
	Songkhram River, Bueng Kan, Thailand	MUMNH:UNIO0805	MG288639	MG552779	MG552831	This study
	Bung River, Na Kae, Thailand	MUMNH:UNIO0520	MG288636	MG552776	MG552828	This study
<i>Indonaiia caerulea</i>	Fish pond, Krishna River basin, India	RRel	KT869141	-	-	GenBank
<i>Indonaiia andersoniana</i>	Lake Indawgyi, Myanmar	RMBH:biv_108_1	KX865835	KX865709	KX865606	Bolotov et al. (2017a)
<i>Indonaiia lima</i>	Nagarjuna Sagar, Krishna River, India	RR11	KP268827	-	-	GenBank
<i>Indochinella pugio</i>	Lake Nant Phar, Myanmar	RMBH:biv_258_1	MF352261	MF352325	MF352386	Bolotov et al. (2017b)
	Tavoy River, Myanmar	RMBH:biv_147_3	KX865852	KX865623	KX865724	Bolotov et al. (2018)
		RMBH:biv_148_7	KX865856	KX865627	KX865728	Bolotov et al. (2018)
		RMBH:biv_148_15	KX865857	KX865628	KX865729	Bolotov et al. (2018)
<i>Radiatula mouloti</i>	Sittang River near Taungoo, Myanmar	RMBH:biv_248_1	MF352234	MF352305	MF352363	Bolotov et al. (2017b)
<i>Radiatula bonneaudii</i>	Tar Pein River, Myanmar	RMBH:biv_260_5	MF352266	MF352330	MF352390	Bolotov et al. (2017b)
<i>Radiatula nyitkyinae</i>	A tributary of Lake Indawgyi, Myanmar	RMBH:biv_107_1	KX865838	KX865609	KX865710	Bolotov et al. (2017a)
<i>Unionetta fabogina</i>	Kratie – Stung Treng, Cambodia	UF 507399 (ICH-00489)	MH350930	-	MH350979	Pfeiffer et al. (2018)
	Kratie – Stung Treng, Cambodia	UF 507399 (ICH-00490)	MH350931	-	MH350980	Pfeiffer et al. (2018)
	Kratie – Stung Treng, Cambodia	UF 507399 (ICH-00491)	MH350932	-	MH350981	Pfeiffer et al. (2018)
<i>Harmandia sombriensis</i>	Khorat Plateau, Thailand	UF 507791 (ICH-00810)	MH350952	-	MH351001	Pfeiffer et al. (2018)
	Khorat Plateau, Thailand	UF 507791 (ICH-00811)	MH350953	-	MH351002	Pfeiffer et al. (2018)
	Khorat Plateau, Thailand	UF 507831 (ICH-00889)	MH350959	-	MH351008	Pfeiffer et al. (2018)
<i>Coelatura aegyptiaca</i>	Nile River, Egypt	ANSP-416304	JN243894	KP795045	JN243872	Pfeiffer and Graf (2015); Whelan et al. (2011)
<i>Parreysia corrugata</i>	Periyar River, India	ParCor1	KJ872809	-	-	GenBank
<i>Leoparreyisia canefrii</i>	Sittang River near Taungoo, Myanmar	RMBH: biv_249	MF352237	MF352307	MF352365	Bolotov et al. (2017b)
<i>Trapezidens exolescens</i>	Tavoy River, Myanmar	RMBH:biv_145_3	KX230532	KX230548	KX230559	Bolotov et al. (2017a)
<i>Lamellidens marginalis</i>	Unnamed puddle, Kaladan River, Myanmar	RMBH:biv_153	KX230547	KX230558	KX230569	Bolotov et al. (2017a)

The newly obtained specimens in this study were collected by hand from different localities in Thailand and Laos plus one locality in Cambodia (Fig. 1A). After euthanization by two-step methods following the AVMA Guidelines for the Euthanasia of Animals (AVMA, 2013), part of the foot or mantle tissue was cut and preserved in 95–100% (v/v) ethanol and then stored at -80 °C for long-term storage. Other soft body and shell specimens were preserved in 70% (v/v) ethanol for the vouchers, and deposited at Department of Biology, Faculty of Science, Mahidol University (MUMNH hereafter). Species/subspecies identification was performed based on morphological characters with attention to the structure of the pseudocardinal and lateral teeth, muscle attachment scars, shell sculpture and shell shape, following the original description and previous works (Brandt, 1974; Kongim et al., 2015).

DNA extraction, PCR and sequencing

Total genomic DNA was extracted from small pieces of tissue using a DNA extraction kit for animal tissue (NucleoSpin Tissue kit, MACHEREY-NAGEL) following the standard procedure of the manual. Fragments of two mitochondrial genes (COI and 16S) and one nuclear gene (28S) were PCR amplified. The primer sets used were L1490 and H2198 (Folmer et al., 1994) for COI, 16sar-L-myt and 16sbr-H-myt (Lydeard et al., 1996) for 16S, and C1 and D2 (Jovelín and Justine, 2001) for 28S. Each PCR was performed using a T100™ thermal cycler (BIO-RAD) with a final reaction volume of 20 µL (15 µL of EmeraldAmp® GT PCR Master Mix, 1.5 µL of each primer, 10 ng of template DNA and distilled water up to 20 µL total volume). Thermal cycling conditions for the PCR were: 94 °C for 3 min followed by 34

cycles of 1 min at 94 °C, 1 min at 50 °C (COI), 52 °C (16S) or 52°C (28S), and 90 s at 72 °C, and then followed by a final 5 min at 72 °C. Amplification of PCR products was confirmed by resolution through 1.5% (w/v) agarose gel electrophoresis before purification by PEG precipitation. Purified PCR products were commercially sequenced in both directions (forward and reverse) using an automated sequencer (ABI prism 3730XL) at Bio Basic Inc., Canada. Nucleotide sequences were deposited in the GenBank database under submission numbers: MG288589–MG288639 and MG552729–MG552831, and listed in Table 1.

Phylogenetic analyses

Sequences were aligned using Clustal W, implemented in MEGA7 (Stecher et al., 2016). The best-fit model of nucleotide substitution was estimated for each gene partition by KAKUSAN4 (Tanabe, 2011). Phylogenetic tree was inferred by maximum likelihood (ML) and Bayesian inference (BI) methods using the concatenated dataset of the COI + 16S + 28S genes fragment sequences. The ML analysis was performed using RAXML 8.2.10 (Stamatakis, 2014), with the GTRGAMMA nucleotide substitution model (Silvestro and Michalak, 2012) and 1,000 ML bootstrap replications.

The BI analysis, using the Markov chain Monte Carlo technique (MCMC), was performed by MrBayes 3.2.6 (Ronquist et al., 2012). The best-fit models based on the Akaike Information Criterion (AIC: Akaike, 1974) were applied: GTR+G for COI and 16S partitions and HKY85+G for the 28S partition. Ten million generations were run with a random starting tree. The result trees were sampled every 1000th generation and were used to estimate the Bayesian posterior probabilities (bpp) for each branch after the first 25% of obtained trees were discarded

as burn-in. The effective sample size (ESS) value sampled from the MCMC analysis was greater than 5,000 in all parameters. Both ML and BI analyses were implemented on the CIPRES portal (Miller et al., 2010).

Genetic divergences between interspecific taxa and intraspecific within each taxa were also calculated using uncorrected pairwise genetic distances, based on the COI dataset, as implemented in MEGA7 (Stecher et al., 2016).

RESULTS

DNA sequences of the COI, 16S and 28S gene fragments were successfully obtained from all 52 specimens newly sequenced in this study. The final matrix contained 78 individuals and an alignment of 1,586 base pairs (bp), as 658 bp for COI, 496 bp for 16S and 432 bp for 28S. For the COI data set, a total of 242 (36.78%) variable sites were identified, 201 (30.54%) of which were parsimony-informative sites.

The phylogenetic trees reconstructed using the ML and BI methods were highly congruent for almost major clades in both analyses. The ML phylogenetic tree based on the concatenated COI + 16S + 28S dataset is shown in Figure 1B. All of the nominal species were supported as a monophyletic clade (87–100% bootstrap support for ML and 0.88–1 bpp for BI). However, a polyphyletic relationship among members of the genera *Scabies* and the Indochinese *Radiatula* (*R. humilis* and *R. pilata*) was revealed. *Scabies songkramensis* was placed separate from the other congeners at the basal part of the tree. Next to *S. songkramensis*, there was a large supported clade of *H. somboriensis*, *U. fabagina*, the Indochinese *Radiatula* and

other *Scabies* species. Within this clade *H. somboriensis* and *U. fabagina* were placed at the basal part, while the two Indochinese *Radiatula* species were grouped together with other *Scabies* with high support (88% bootstrap support for ML and 1 bpp for BI). However, the relationship among them was uncertain. Whereas, other members of the genus *Radiatula* from the western Indochina were clustered separately as a monophyletic clade and placed next to the *S. songkramensis* clade at the basal portion.

The average uncorrected p-distance pairwise divergence of the COI gene is shown in Table 2. Among *Scabies* species, the average distance values ranged from 2.04–10.87%. *Scabies songkramensis* showed the highest divergence from the other *Scabies* species, ranging from 9.42–10.87%. The average distance among the three closely related taxa of *S. phaselus*, *S. anceps* and *S. scobinatus* were relatively low, ranging from 2.04–2.58 %. Interestingly, the two Indochinese *Radiatula* species showed a closer relationship to those from the genus *Scabies* (4.35–7.33%) than to the other congeners from the genus *Radiatula* (8.88–9.80%).

DISCUSSION

The multi-locus phylogeny based on the concatenated COI + 16S + 28S sequences revealed non-monophyly in *Scabies* and *Radiatula*. This polyphyly is consistent with previous works (Pfeiffer et al., 2018). A strongly supported clade of *S. songkramensis* was resolved as distant from the other *Scabies* species, but with the clades of *H. somboriensis* and *U. fabagina* placed between them (Fig. 1B). The average intraspecific divergences between *S. songkramensis* and the other *Scabies*

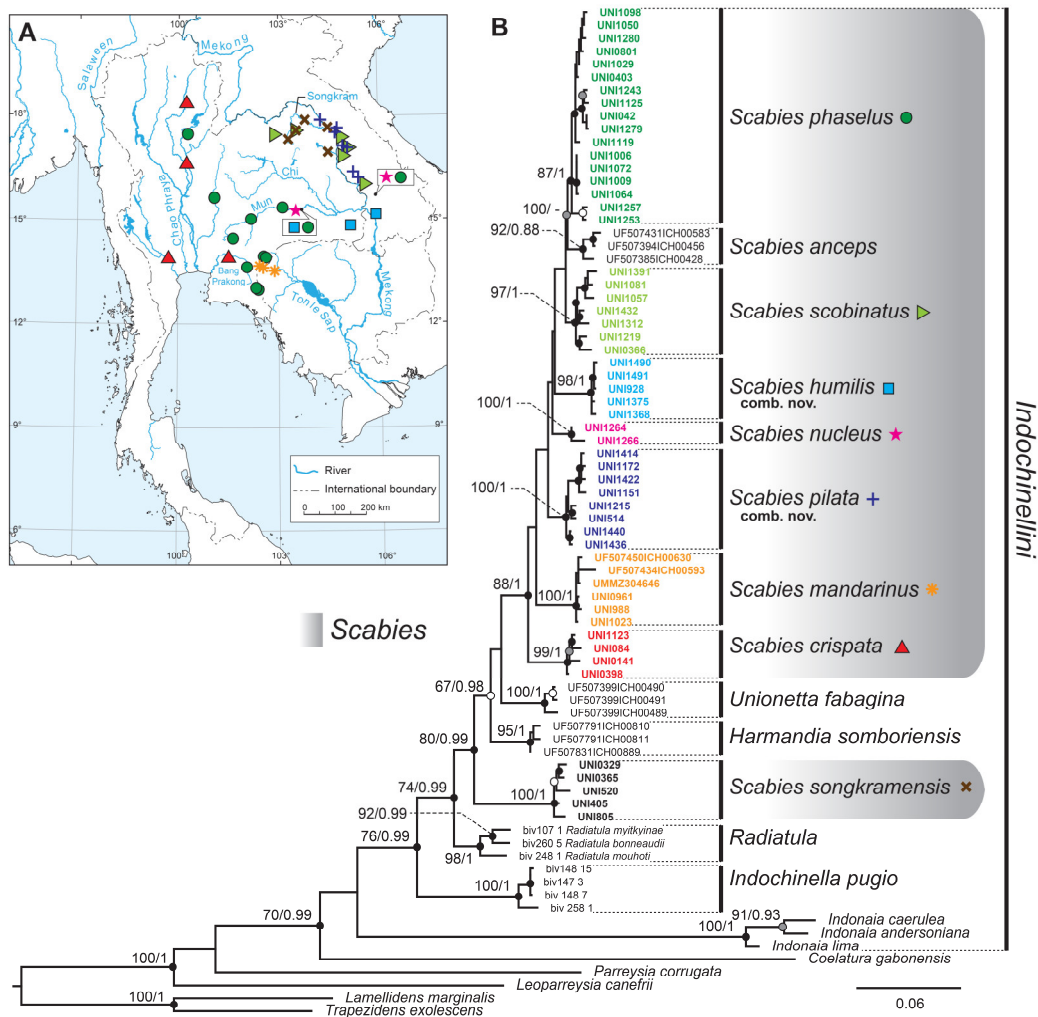


FIGURE 1. (A) Map of Indochina showing the main river systems with sampling locations for the specimens used for DNA sequence analysis. Symbols refer to *Scabies* species/clades as suggested by the phylogenetic analyses. **(B)** Phylogenetic relationships of *Scabies* species and related taxa based on ML analysis of the concatenated COI + 16S + 28S sequence dataset. Bootstrap values from ML and bpp from BI analysis of major nodes are shown (ML/BI). Nodes with 0.95 or higher bpp and/or 70% or higher bootstrap value were regarded as sufficiently supported and marked with black circles (supported by both BI and ML), white circles (supported only by BI) or grey circles (supported only by ML).

members were high (9.42–10.87%). In addition, Kongim et al. (2015) noted a karyotypic differentiation between *S. songkramensis* and *S. crispata*. These data question the taxonomic placement of *S. songkramensis* in the genus *Scabies*.

However, its distinguishable conchological characters are not sufficiently different to clearly separate it from the genus *Scabies* and confirm its generic classification. Further extensive morphological study, including examination of the soft body part

TABLE 2. Average inter- and intra-specific uncorrected p-distance (% \pm S.E.) for the 658 bp COI gene sequences between *Scabies* species and related taxa. Average intraspecific within each taxon are in bold.

Taxa	1.	2.	3.	4.	5.	6.	7.	8.	9.	12.	13.	14.
1. <i>S. phaselus</i>	1.05\pm0.22											
2. <i>S. anceps</i>	2.04 \pm 0.45	0.31\pm0.21										
3. <i>S. scobinatus</i>	2.58 \pm 0.43	2.41 \pm 0.48	1.55\pm0.30									
4. <i>S. crispata</i>	6.15 \pm 0.88	6.02 \pm 0.90	6.53 \pm 0.88	0.99\pm0.26								
5. <i>S. mandarinus</i>	5.19 \pm 0.75	5.25 \pm 0.80	5.69 \pm 0.80	6.17 \pm 0.88	0.68\pm0.20							
6. <i>S. nucleus</i>	3.71 \pm 0.64	3.68 \pm 0.70	4.10 \pm 0.65	6.19 \pm 0.92	4.82 \pm 0.76	1.22\pm0.41						
7. <i>S. humilis</i>	5.19 \pm 0.77	4.75 \pm 0.76	5.19 \pm 0.77	7.33 \pm 0.94	5.90 \pm 0.88	4.65 \pm 0.77	0.30\pm0.13					
8. <i>S. pilata</i>	4.35 \pm 0.64	4.34 \pm 0.67	5.13 \pm 0.70	6.24 \pm 0.88	4.95 \pm 0.75	4.20 \pm 0.69	5.58 \pm 0.80	1.29\pm0.28				
9. <i>S. songkramesis</i>	10.01 \pm 1.09	10.87 \pm 1.15	10.36 \pm 1.06	9.42 \pm 1.10	9.97 \pm 1.11	10.24 \pm 1.11	10.40 \pm 1.06	10.43 \pm 1.09	1.06\pm0.24			
10. <i>R. mouhoti</i>	9.19 \pm 1.09	9.89 \pm 1.16	9.49 \pm 1.08	8.66 \pm 1.10	10.44 \pm 1.20	8.89 \pm 1.13	9.03 \pm 1.09	9.80 \pm 1.10	9.33 \pm 1.09	N/A		
11. <i>R. bonneauidi</i>	8.57 \pm 1.06	9.28 \pm 1.15	9.03 \pm 1.07	8.70 \pm 1.11	9.34 \pm 1.14	8.97 \pm 1.15	8.88 \pm 1.10	9.63 \pm 1.13	8.57 \pm 1.02	4.41 \pm 0.75	N/A	
12. <i>R. mytkyinae</i>	8.47 \pm 1.05	9.13 \pm 1.12	9.05 \pm 1.06	7.71 \pm 0.99	9.47 \pm 1.17	8.51 \pm 1.10	9.03 \pm 1.13	9.56 \pm 1.12	9.67 \pm 1.18	4.71 \pm 0.83	3.19 \pm 0.65	N/A

and detailed study of its glochidia larva, need to be examined and compared with those of other *Scabies* species and other genera in the tribe Indochinellini.

The validity at the species level of all the other all *Scabies* species and the two Indochinese *Radiatula* species was confirmed by their monophyly in the present study (Fig. 1B). However, three sister species (*S. phaselus*, *S. anceps* and *S. scobinatus*) still require further investigation for their taxonomic status. Although, there are clear shared patterns in their shell morphology and their distribution (Figs. 1 and 2), they shared a rather low molecular divergence from each other (2.04–2.58 %), where these values were lower than the generally accepted average intraspecific divergence of 5% for barcoding in mussels (Boyer et al., 2011), but were still higher than the 2% divergence accepted for species delineation in animals (Hebert et al., 2003).

Nevertheless, the biogeographic distribution pattern of these three species is interesting. *Scabies phaselus* were found in the Chao Phraya River basin and the former Sunda River systems (i.e. Siam River System; Voris, 2000), and many tributaries of the Mekong River in the Khorat Plateau (Fig. 2A). The Mun and Chi Rivers were hypothesized to formerly have been connected to the Chao Phraya River prior to the middle Pleistocene, before the flow direction changed eastwards into the Mekong River (Hutchison, 1989). *Scabies scobinatus* and *S. anceps* are distributed in the Mekong River basin, but *S. scobinatus* is endemic to the Songkram River basin in the northern part of the Khorat Plateau and some tributaries of the middle Mekong River in Laos, whereas *S. anceps* can be found in the lower Mekong River basin, such as the Mekong Delta in Vietnam and Stung Treng, Cambodia (Pfeiffer et al.,

2018). A phylogeographic and population genetic study based on specimens from all the drainage systems of their distribution ranges should be investigated in order to better reveal the evolutionary history of the freshwater mussels in the region.

The multi-locus phylogeny of this study also supported the polyphyly of the genus *Radiatula*, recovering *R. humilis* and *R. pilata* among the species of *Scabies* with significant support, whereas the three *Radiatula* species from the western Indochina were resolved as a separate monophyletic clade (Fig. 2). This result leads to the suggestion of transferring *R. humilis* and *R. pilata* to the genus *Scabies*, in order to reflect their realistic evolutionary history of a shared common ancestor among them. Here, we provide additional support for their taxonomic transfer to *Scabies*, as *S. humilis* comb. nov. and *S. pilata* comb. nov., in terms of their biogeographic distribution and morphology. The genus *Radiatula* s.s. should be implied to only taxa endemic to the western Indochinese region, while the genus *Scabies* are distributed in the Chao Phraya and Mekong River Basins and other rivers that drain into the Gulf of Thailand.

In Indochina, the Salween/Mekong River drainages divide the freshwater biogeographic division into two subregions, the Western Indochina and the Sundaland subregion, with different freshwater mussel assemblages endemic at the generic level for each subregion (Bolotov et al., 2018). Distribution data for specimens of all *Scabies* species collected and examined in this study, together with previous records, generally supported the endemism of these animals to drainages in the Chao Phraya and Mekong River Basins and other rivers that drain into the Gulf of Thailand (Graf and Cummings, 2019; Pfeiffer et al., 2018). In particular, *S.*

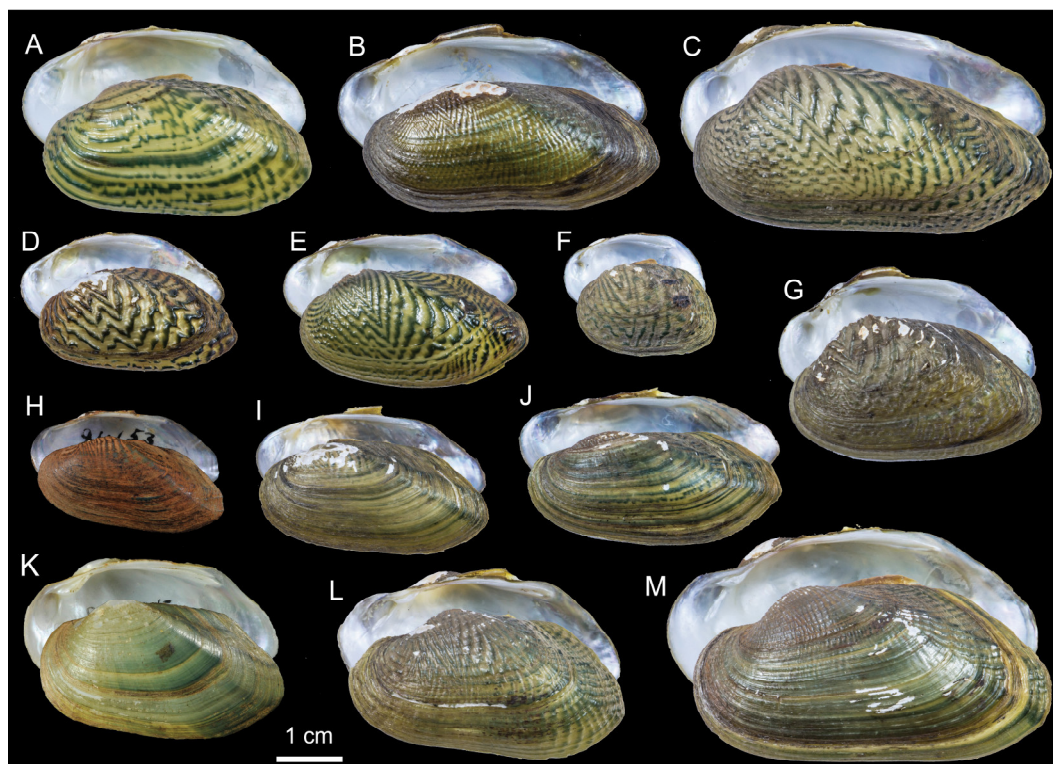


FIGURE 2. Shells of **A.** *Scabies phaselus* MUMNH:UNI1243 from Surin. **B.** *Scabies scobinatus* MUMNH:UNI0366 from Sakon Nakhon. **C.** *Scabies crispata* MUMNH:UNI0141 from Ratchaburi. **D, E.** *Scabies mandarinus* **D.** MUMNH:UNI0981 from Krong Serei Sophorn, Cambodia and **E.** MUMNH:UNI1021 from Sa Kaeo. **F.** *Scabies nucleus* MUMNH:UNI1266 from Surin. **G.** *Scabies songkramensis* MUMNH:UNI0365 from Sakon Nakhon. **H–J.** *Scabies humilis* **comb. nov.**, **H.** Lectotype USNM 84153 from Siam (=Thailand), **I.** Specimen MUMNH:UNI1368 from Pakse, Laos and **J.** Specimen MUMNH:UNI1490 from Ubon Ratchathani. **K–M** *Scabies pilata* **comb. nov.**, **K.** Holotype USNM 85195 from Siam (=Thailand), **L.** Specimen MUMNH:UNI1490 from Savannakhet, Laos and **M.** Specimen MUMNH:UNI1172 from Khammouane, Laos.

humilis **comb. nov.** and *S. pilata* **comb. nov.** seem to be endemic to the Mekong River and its tributaries in Thailand, Laos and Cambodia (Brandt, 1974) and have clearly distinct ranges from other *Radiatula* species. In addition, no specimens of these two species were collected from the Chao Phraya River basin between eastern Myanmar to the Khorat Plateau (Brandt, 1974; Graf and Cummings, 2019). The other four *Radiatula* s.s. species are abundant

throughout Myanmar in the western Indochina region (Bolotov et al., 2017b). One species, *Radiatula bonneaudii* (Eydoux, 1838), has expanded its distribution range into northern India and Nepal (Bolotov et al., 2017b; Graf and Cummings, 2019).

Morphological data provides additional support for the systematic position of *S. humilis* **comb. nov.** and *S. pilata* **comb. nov.** in the genus *Scabies*. They share zigzag-

lines in a V- or W-pattern of sculptured shell characteristics with other members, although this pattern does not present in a brownish colour. The examined specimens of *S. scobinatus*, *S. crispata*, *S. nucleus* and *S. songkramensis* also lacked this brownish zigzag line, such as in the example shells shown in Fig. 2. In *S. humilis* comb. nov. the zigzag-sculpture covers only the umbonal region (Fig. 2H-J), which usually cannot be seen in eroded specimens (Fig. 2I). In *S. pilata* comb. nov., this sculpture is much denser (Fig. 2M). The dense sculpture pattern also appears in individuals of *S. phaselus* collected from the northern part of the Korat Plateau (Fig. 2B).

The sculptured pattern varies within individuals of *S. pilata* comb. nov., ranging from covering the entire shell to covering half of the shell, to being present as zigzag wrinkles in umbonal region. Some specimens from the Nam Pakan River, Khammouane, Laos showed a heavy and broader sculptured shell, similar to those of *S. songkramensis* and *S. crispata*. All representative individuals of *S. pilata* comb. nov. variants were sequenced and included in this study, and they were recovered as a strongly supported clade (Fig. 1B). *Radiatula crispisulcata* (Benson, 1862), the type species of the genus *Radiatula*, also bears a dense sculptured pattern, but it radiates from the umbo rather than representing a zigzag pattern. The smooth shell of *S. pilata* comb. nov. resembles that of *R. bonneaudii* from Myanmar, which may have led to its misclassification. However, the molecular phylogenetic analysis showed a clear separation between them.

Moreover, *S. pilata* comb. nov. and *S. humilis* comb. nov. also share an elongated shell shape, which is often concave in the middle part or with an almost straight

ventral margin (Fig. 2), rather than the ventrally convex shell in *Radiatula* s.s. from western Indochina, although some individuals of *R. bonneaudii* have a somewhat concaved ventral margin (Graf and Cummings, 2019). The characteristic of a ventrally concaved shell can be easily seen in mature specimens of *S. scobinatus* (Fig. 2B), *S. crispata* (Fig. 2C) and *S. pilata* comb. nov. (Fig. 2K-M). Brandt (1974) also mentioned the similarity in the shell shape between *S. phaselus* and *S. humilis* comb. nov. (as *R. humilis*).

The other important character is the pseudocardinal teeth in the right valve, which consist of ‘a high, triangular tooth that is deeply incised’ (Brandt, 1974). This character can be found in all *Scabies* members, but it is less present in *S. humilis*. Pseudocardinal teeth of *S. humilis* are compressed, lamelliform, low and sharp. Some specimens of *S. phaselus* in this study exhibited a similar shape of pseudocardinal teeth, but were rather high and triangular.

Taxonomy

Family Unionidae Fleming, 1828

Genus *Scabies* Haas, 1911

Type species: *Unio scobinatus* Lea, 1856

Scabies humilis (Lea, 1856) comb. nov.

(Fig. 2H-J)

Unio humilis Lea, 1856: 93. Type locality: Siam [=Thailand].

Unio humilis—Lea, 1857: 296, pl. 26, fig. 10.

Unio humilus [sic.]—Woodward, 1969: 54.

Material examined.—Lectotype USNM_84153 (Fig. 2H) from “Siam” [Thailand]. Chi River, Koeng, Mueang, Maha Sarakham, Thailand [16° 13' 55.5" N 103° 16' 23.8" E], 6 shells, MUMNH:UNI2245-2450; Mun River, Tha

Tum, Surin, Thailand [15° 19' 28.7" N 103° 40' 28.2" E], 1 shell, MUMNH:UNI0928; Dom Yai River, Detch Rdom, Ubon Ratchathani, Thailand [14° 53' 55.3" N 103° 05' 58.4" E], 9 shells, MUMNH:UNI1490-1499; Xe Don River, Muang Khong Xe Don, Pakse, Laos [15° 54' 46.7" N 105° 48' 42.5" E], 5 shells, MUMNH:UNI1361-1365; Xe Don River, Ban Nong Du, Pakse, Laos [15° 21' 37.7" N 105° 49' 52.9" E], 2 shells, MUMNH:UNI2245-2246; Xe Don River, Ban Photok, Pakse, Laos [15° 07' 54.0" N 105° 48' 37.2" E], 9 shells, MUMNH:UNI1367-1374.

Remarks.— *Scabies humilis* comb. nov. is transferred from *Radiatula* to *Scabies* based on the multi-locus molecular data (Fig. 2B). It can be distinguished from other *Scabies* species by having compressed hinge teeth and the zigzag-line sculptured pattern is restricted to only the umbononal region. This species is similar to *S. scobinatus*, but the latter species has a more concaved ventral margin of the shell and the entire shell is covered by a dense sculptured pattern.

Distribution.— Mekong River and its tributaries in Thailand, Laos, Cambodia and probably Vietnam (Brandt, 1974).

***Scabies pilata* (Lea, 1866) comb. nov.**

(Fig. 2K–M)

Unio pilatus Lea, 1866: 18: 133. Type locality: Siam [=Thailand].

Unio pilatus Lea, 1868: 281, pl. 38, fig. 95.

Material examined.— Holotype USNM 85195 (Fig. 2K) from “Siam” [Thailand]. Mekong River, Ban Tha Krai, Bueng Kan, Thailand [18° 20' 21.2" N 103° 41' 53.7" E], 4 shells, MUMNH:UNI2217-2220; Mekong River, Wat Tha Phou, Bueng Kan, Thailand [18° 20' 19.8" N 103° 43' 23.7" E], 1 shell, MUMNH:UNI2260; Mekong River, Ban

Phaeng, Nakhon Phanom, Thailand [17° 50' 56.1" N 104° 17' 00.7" E], 5 shells, MUMNH:UNI0514-0518; Mekong River, Nam Kam, That Phanom, Nakhon Phanom, Thailand [16° 48' 41.5" N 104° 44' 07.3" E], 10 shells, MUMNH:UNI1773-1782; Bang-I River, Pho Sai, Don Tan, Mukdahan, Thailand [16° 20' 44.5" N 104° 53' 25.1" E], 3 shells, MUMNH:UNI2253-2255; Mekong River, Khong Chiam, Ubon Ratchathani, Thailand [15° 21' 04.0" N 105° 27' 47.5" E], 9 shells, MUMNH:UNI1584-1593; Nam Ou River, Hat Gna, Luang PraBang, Laos [20° 05' 11.3" N 102° 15' 51.0" E], 1 shell, MUMNH:UNI2221; Nam Xeung River, Pak Xeung, Luang PraBang, Laos [19° 58' 42.8" N 102° 14' 51.4" E], 7 shells, MUMNH:UNI2270-2276; Nam Khan River, Xiang Ngeun, Luang PraBang, Laos [19° 45' 57.4" N 102° 10' 55.7" E], 6 shells, MUMNH:UNI2386-2391; Mekong River, Tha Deua, Sainyabuli, Laos [19° 25' 35.1" N 101° 50' 46.5" E], 1 shell, MUMNH:UNI2269; Nam Phoun River, Ban Phoun Savat, Sainyabuli, Laos [18° 24' 59.9" N 101° 29' 42.6" E], 3 shells, MUMNH:UNI2429-2431; Nam Pakan River, Khammouane, Laos [17° 39' 52.2" N 104° 37' 37.0" E], 2 shells, MUMNH:UNI1215-1216; Nam Hin Boun River, Khammouane, Laos [17° 35' 46.8" N 104° 37' 04.9" E], 2 shells, MUMNH:UNI1171-1172; Nam Don River, Thakek, Khammouane, Laos [17° 26' 32.9" N 104° 51' 22.2" E], 1 shell, MUMNH:UNI0330; Xe Bang Fai River, Savannakhet, Laos [17° 04' 40.3" N 104° 59' 12.4" E], 11 shells, MUMNH:UNI1150-1160; Xe Nou River, Ban Dong Mak Fai, Savannakhet, Laos [17° 04' 22.34" N 105° 02' 54.9" E], 11 shells, MUMNH:UNI1420-1429, 1431; Banghiang River, Ban Lahanam, Savannakhet, Laos [16° 16' 37.5" N 105° 16' 32.6" E], 18 shells, MUMNH:UNI1386, 1404-1419, 2297; Banghiang River, Ban Phosay,

Savannakhet, Laos [16° 05' 55.5" N 105° 22' 32.0" E], 10 shells, MUMNH: UNI1433-1442; Mekong River, Pakkse, Laos [15° 06' 02.5" N 105° 49' 37.1" E], 1 shell, MUMNH: UNI1685; Xe Khong River, Ban Keang Luang, Laos [15° 26' 33.2" N 106° 43' 29.6" E], 6 shells, MUMNH: UNI 1767-1772; Xe Khong River, Xekong, Laos [15° 19' 54.0" N 106° 42' 03.0" E], 7 shells, MUMNH: UNI 2238-2244; Xe Kaman River, Ban Samakkee, Xe Kong, Laos [14° 47' 30.5" N 106° 50' 48.9" E], 1 shell, MUMNH: UNI2242; Mekong River, Ban Song Pluey, Champasak, Laos [14° 26' 44.1" N 105° 52' 14.2" E], 12 shells, MUMNH: UNI1829-1840; Mekong River, Ban Houa Khong, Don Khong, Laos [14° 10' 07.1" N 105° 47' 46.6" E], 7 shells, MUMNH: UNI1750-1756.

Remarks.— *Scabies pilata* comb. nov. is transferred from *Radiatula* to *Scabies* based on the multi-locus molecular data (Fig. 1B). It can be distinguished from other *Scabies* species by having a much higher posterior and moderately inflated umbo. The smooth individuals of *S. pilata* comb. nov. resemble *R. bonneaudii* from Myanmar, but they are distinguished by the stronger posterior slope, smaller umbonal region and location of the umbo, which are placed in the anterior third of the shell and not in the middle of the shell.

Distribution.— Mekong River and its tributaries in Thailand, Laos and Cambodia.

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