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Mitochondrial diversity of the white-toothed shrews (Mammalia, Eulipotyphla, *Crocidura*) in Vietnam

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Abstract

We explored the patterning of genetic diversity within white-toothed shrews of the genus *Crocidura* from 14 localities across Vietnam. An analysis of cytochrome oxidase *c* subunit I (COI) and cytochrome *b* (*cytb*) gene fragments from 185 specimens of white-toothed shrews of the genus *Crocidura* from 14 localities across Vietnam revealed six deeply divergent (p-distance for both COI and *cytb* >10%) lineages, corresponding to the morphological species *C. sokolovi*, *C. zaitsevi*, *C. phuquocensis*, *C. indochinensis*, *C. tanakae* and, *C. phanluongi. Crocidura sokolovi* was distinct from all other Vietnamese white-toothed shrews (~13% on average for both *cytb* and COI). In addition to demonstrating the genetic separation of previously described species, substantial cryptic genetic diversity was revealed. *Crocidura zaitsevi* and *C. tanakae* each included two subgroups that corresponded to geographically remote localities, while *C. indochinensis* contained two distinct subgroups that exhibited co-varying patterns of morphological and ecological differentiation, suggesting that the individuals from Sa Pa represent a separate species (provisionally named *Crocidura* sp. AB1). Mitochondrial data generated for the type specimens of *C. phanluongi* from Yok Don and Bu Gia Map supported the validity of the species while an additional specimen from Binh Chau, South Vietnam originally referred to *C. phanluongi* exhibited a deep genetic split (*cytb*: 8.4%; COI: 8.7%) from a neighbouring population in Yok Don. We propose that the specimen from Binh Chau also represents an undescribed species (provisionally named *Crocidura* sp. AB2). Our *cytb* data were then compared to the sequences of 28 species of *Crocidura* from Southeast Asia available in GenBank, suggesting that three more species occur in northern Vietnam, namely C. *wuchihensis*, *C. attenuata* and *C. fuliginosa/C. dracula*. The discovery of fairly deep genetic divergences among Vietnamese *Crocidura* illustrates that the understudied and largely undescribed diversity of white-toothed shrews in Southeast Asia requires deeper scrutiny. It also shows the useful insights of mitochondrial markers as to the taxonomic resolution of this enigmatic group of mammals.

Key words: DNA barcodes, molecular biodiversity, Soricidae, Southeast Asia, molecular diagnostics, cryptic species

Introduction

The white-toothed shrews (*Crocidura*) have a broad distribution across the Old World tropics and are one of the most speciose genera of mammals (Hutterer 2005). Despite their remarkable alpha-taxonomic diversity, these shrews are morphologically rather uniform, which contributes to their extreme taxonomic complexity. The evolutionary relationships of the species within this genus are the subject of serious debate. Most studies involving protein, chromosomal, and recent molecular data suggest the evolutionary division of *Crocidura* into Afrotropical and Asian lineages (Maddalena 1990; Maddalena & Ruedi 1994; Ruedi 1998; Bannikova *et al*. 2006; Dubey *et al*. 2008; Lavrenchenko *et al*. 2009); however, the monophyly of these clades is still insufficiently supported. Yet both karyological and molecular data strongly suggest that East Asian *Crocidura* comprise a mixture of southern and

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northern faunal elements (Motokawa *et al*. 2000, 2005; Dubey *et al*. 2008) and their relationship with Afrotropical species is very complex due to several transcontinental faunal exchanges hypothesized between Eurasia and Africa (Dubey *et al*. 2007).

Vietnam occupies a key geographic position within Southeast Asia, as it spans a majority of the region's ecosystems. However, white-toothed shrews are among the most poorly known mammals in the country and only five species of *Crocidura* were historically documented therein (Heaney & Timm 1983; Huynh *et al*. 1994; Lunde *et al.* 2004; Kuznetsov 2006). More recently, a series of focused collecting trips and in-depth morphological studies have led to the description of several new species and further enhanced the resolution of species' distributions (Lunde *et al*. 2003, 2004; Jenkins *et al*. 2007, 2009, 2010; Abramov *et al*. 2008a). The total number of described *Crocidura* species recorded in Vietnam now stands at eleven: *C. attenuata*, *C. fuliginosa*, *C. indochinensis*, *C. kegoensis*, *C. sokolovi*, *C. phuquocensis*, *C. wuchihensis*, *C. zaitsevi*, *C. guy*, *C. annamitensis* and *C. phanluongi* (Abramov *et al*. 2008a; Can *et al*. 2008; Jenkins *et al*. 2009; Jenkins *et al*. 2010). Although some clarifications regarding the diagnostic morphological features of these species have been provided, the patterning of genetic diversity both among and between them remains largely unexplored.

The purpose of this study represents a first step toward breaching this gap, by employing a combination of two mitochondrial markers. The standardized 657 base pair segment of the 5' "DNA barcode" region of cytochrome *c* oxidase subunit I gene (COI; Hebert *et al*. 2003) was targeted in an effort to document alpha-taxonomic diversity as part of the Barcode of Life initiative. This marker has proven effective in evaluating the genetic diversity of taxonomically complex mammal groups posing difficulties in morphological discrimination (Clare *et al*. 2007; Borisenko *et al*. 2008; Francis *et al*. 2010). Full or partial cytochrome *b* (*cytb*) sequences were also generated from exemplar lineages highlighted by barcoding in order to place our results within an historical context provided by comparing them with the existing *Crocidura cytb* sequences available in GenBank, thus allowing broader comparisons with existing molecular data. Although valuable, GenBank data is known to be error-ridden (Harris 2003) and typically lacks reference to the actual specimens examined (Ruedas et al. 2000), making the data problematic for molecular diagnostic applications.

Material and methods

Cytochrome oxidase *c* subunit 1 (COI) and cytochrome *b* (*cytb*). A total of 185 *Crocidura* specimens were analyzed. Pieces of muscle and/or internal organs (liver, heart, kidney) fixed in 96% ethanol were used as sources for DNA extraction. The DNA barcode region of COI was sequenced from 180 specimens and partial or complete *cytb* gene sequences were recovered from an additional 32 specimens. All the specimens were collected between 2002–2009 from among 14 localities surveyed across Vietnam and one locality in Laos (Fig. 1). Included were the holotypes and/or parts of the type series of four recently described species (*C. zaitsevi*, *C. sokolovi*, *C. phuquocensis*, and *C. phanluongi*). Morphological voucher specimens are deposited in the Zoological Institute of the Russian Academy of Sciences (ZIN, St. Petersburg), and the Zoological Museum of Moscow University (ZMMU, Moscow).

The list of Vietnamese specimens analysed including collection localities, museum catalogue numbers, GenBank accession numbers and BOLD process ID is provided in Tables 1 and 2. Besides, seven original sequences obtained from *C. suaveolens*, *C. sibirica* and *C. lasiura* for *cytb* (Ac No HM586991-97) and 38 sequences of *C. suaveolens*, *C. sibirica*, *C. shantungensis*, *C. lasiura*, *C. olivieri* and *C. macmillani* for COI (BOLD No SKMZM365-08, SKMZM366-08, SKMZM371-08, SKMZM372-08, SKMZM486–488-08, SKMZM490-08, SKMZM493–496-08, SKMZM498-08, SKMZM500–504-08, SKMZM800–805-09, SKMZM811–814-09, SKMZM816-09, SKMZM817-09) were used to assess genetic diversity. We also included *cytb* sequence data from several published sources (Ruedi *et al*. 1998; Bannikova *et al*. 2006, 2009; Ohdachi *et al*. 2004, 2006; Dubey *et al*. 2008; Esselstyn *et al*. 2009; Lavrenchenko *et al*. 2009) to place the shrews within Vietnam into a regional phylogeographic context.

DNA isolation, PCR amplification and sequencing. COI. Tissues were submitted to the Canadian Centre for DNA Barcoding, Biodiversity Institute of Ontario, University of Guelph (Guelph, Canada) pre-arrayed into 96-well microplates following standard sampling instructions (Borisenko *et al*. 2008; Borisenko *et al.* 2009). Approximately 5 mg or 2 mm^3 of tissue was sampled from each specimen. Prior to DNA extraction, each plate well was

filled with 50 µl of lysis buffer with Proteinase K and the plates were incubated for 12–18 h at 56°C, followed by a robotic DNA extraction protocol as described in Ivanova *et al*. (2006). Standard barcoding protocols for PCR amplification and sequencing were employed, following PCR and sequencing with regular and M13-tailed universal vertebrate primer cocktails (Clare *et al*. 2007; Ivanova *et al*. 2006). In cases where a full length barcode was not recoverable using standard primers, the internal primer RonM (Pfunder *et al*. 2004) and its M13-tailed modification (Borisenko *et al*. 2008) were used to amplify a shorter COI fragment (421 bp). The standard CCDB protocol with 1/24 BigDye dilution (Ivanova & Grainger 2007) was used for sequencing. PCR products were visualized on a 2% agarose gel using an E-Gel96 Pre-cast Agarose Electrophoresis System (Invitrogen) as described in DeWaard *et al* (2008). Products were labelled by using the BigDye© Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) as described in (Hajibabaei et al. 2005) using a standard1/24 BigDye dilution (Ivanova & Grainger 2007) and sequenced bidirectionally using an ABI 3730XL capillary sequencer following manufacturer's instructions. Sequences were assembled from raw sequencer trace files using SeqScape v 2.1.1 (Applied Biosystems) and verified by eye. Specimen provenance and barcode sequence data were stored and analyzed using the Barcode of Life Data System — BOLD (Ratnasingham and P.D.N. Hebert 2007) in the publicly accessible project title/code through the online interface at http://www.barcodinglife.org.

TABLE 1. List of the specimens analysed for the cytochrome oxidase *c* subunit 1 (COI) gene. Holotypes are marked in bold.

species	BOLD Accession No	Catalogue number and field specimen code	Specimen code on Fig. 2–3	Collecting locality
C. sokolovi	ABMIV152 08	**ZIN 91232, CVN179**	1	Vietnam, Kon Tum Province, Ngoc Linh Mt.; 15º05´N, 107º57´E
	ABMIV083 08	ZIN 96404, CVN12	2	
	ABMIV082 08	ZIN 96396, CVN4	3	
	ABIOW052 08	ZIN 91233, CVN180	4	
	ABMIV081 08	ZIN 96394, CVN2	5	
C. zaitsevi A	ABMIV091 08	ZIN 96343, CVN43	1	Vietnam, Kon Tum Province, Ngoc Linh Mt.; 15º05´N, 107º57´E
	ABMIV093 08	ZIN 96348, CVN48	2	
	ABMIV149 08	ZIN 91219, CVN172	3	
	ABMIV146 08	ZIN 91214, CVN167	4	
	ABMIV097 08	ZIN 96379, CVN79	5	
	ABMIV095 08	ZIN 96359, CVN59	6	
	ABIOW119 08	ZIN 96345, CVN45	7	
	ABIOW108 08	ZIN 96358, CVN58	8	
	ABIOW115 08	ZIN 96340, CVN40	9	
	ABIOW114 08	ZIN 96339, CVN39	10	
	ABIOW100 08	ZIN 96364, CVN64	11	
	ABIOW092 08	ZIN 96372, CVN72	12	
	ABIOW120 08	ZIN 96346, CVN46	13	
	ABIOW117 08	ZIN 96342, CVN42	14	
	ABMIV151 08	ZIN 91225, CVN178	15	
	ABMIV094 08	ZIN96354, CVN54	16	
	ABIOW088 08	ZIN 96377, CVN77	17	
	ABMIV088 08	ZIN 96331, CVN31	18	
	ABMIV150 08	**ZIN 91224, CVN177**	19	
	ABMIV085 08	ZIN 96319, CVN19	20	
	ABMIV098 08	ZIN 96380, CVN80	21	
	ABIOW041 08	ZIN 97511, CVN213	22	Vietnam, Quang Binh Province, Phong Nha - Ke Bang National Park; 17º38´N, 106º06´E

continued next page

TABLE 1. (continued)

species	BOLD Accession No	Catalogue number and field specimen code	Specimen code on Fig. 2–3	Collecting locality
C. zaitsevi B	ABMIV120 08	ZIN 96413, CVN121	1	Vietnam, Khanh Hoa Province, Hon Ba Mt.; 12º07´N, 108º57´E
	ABMIV121 08	ZIN 96414, CVN122	2	
	ABMIV159 08	ZMMU S-175185, CVN187	3	
	ABIOW149 09	ZIN 97629, CVN215	4	Vietnam, Lam Dong Province, Bi Doup Mt.; 12º11´N, 108º41´E
	ABIOW166 09	ZIN 97643, CVN232	5	
	ABIOW172 09	ZIN 97635, CVN238	6	
	ABIOW173 09	ZIN 97648, CVN239	7	
	ABIOW203 09	ZIN 97659, CVN 270	8	
	ABIOW195 09	ZIN 97636, CVN262	9	
	ABIOW198 09	ZIN 97639, CVN265	10	
	ABIOW133 09	ZIN 98949, CVN338	11	
	ABIOW165 09	ZIN 97642, CVN231	12	
	ABIOW130 09	ZIN 98946, CVN335	13	
	ABIOW153 09	ZIN 97630, CVN219	14	
	ABIOW171 09	ZIN 97634, CVN237	15	
	ABIOW169 09	ZIN 97646, CVN235	16	
	ABIOW170 09	ZIN 97647, CVN236	17	
C. phuquocensis	ABMIV158 08	ZIN 96662, CVN186	1	Vietnam, Kien Giang Province, Phu Quoc Isl.; 10°23'N, 104°00'E
	ABMIV155 08	ZIN 96659, CVN183	2	
	ABMIV157 08	ZIN 96661, CVN185	3	
	ABMIV156 08	**ZIN96660, CVN184**	4	
Crocidura sp.AB1	ABMIV111 08	ZIN 96276, CVN104	1	Vietnam, Lao Cai Province, Sa Pa District, 22º20`N, 103º50`E
	ABMIV106 08	ZIN 96269, CVN99	2	
	ABMIV109 08	ZIN 96274, CVN102	3	
	ABIOW222 09	ZIN 97797, CVN289	4	
	ABIOW051 08	ZIN 98250, CVN58 27	5	
	ABIOW217 09	ZIN 97792, CVN284	6	
	ABIOW075 08	ZIN 96434, CVN109	7	
	ABIOW072 08	ZIN 96499, CVN120	8	
	ABIOW216 09	ZIN 97791, CVN283	9	
	ABIOW240 09	ZIN 97815, CVN307	10	
	ABIOW230 09	ZIN 97805, CVN297	11	
	ABIOW014 08	ZIN 98248, CVN31 60	12	
	ABIOW220 09	ZIN 97795, CVN287	13	
	ABIOW049 08	ZIN 98260, CVN74 124	14	
	ABIOW050 08	ZIN 98267, CVN93 142	15	
	ABIOW233 09	ZIN 97808, CVN300	16	
	ABIOW221 09	ZIN 97796, CVN288	17	
	ABIOW223 09	ZIN 97798, CVN290	18	
	ABIOW015 08	ZIN 98249, CVN32 85	19	
	ABIOW069 08	ZIN 96442, CVN117	20	
	ABMIV108 08	ZIN 96271, CVN101	21	

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TABLE 1. (continued)

species	BOLD Accession No	Catalogue number and field specimen code	Specimen code on Fig. 2–3	Collecting locality
	ABMIV101 08	ZIN 96264, CVN94	22	
	ABMIV117 08	ZIN 96438, CVN113	23	
	ABMIV110 08	ZIN 96275, CVN103	24	
	ABMIV100 08	ZIN 96262, CVN93	25	
	ABMIV118 08	ZIN 96439, CVN114	26	
	ABMIV116 08	ZIN 96436, CVN111	27	
	ABMIV114 08	ZIN 96433, CVN108	28	
	ABIOW225 09	ZIN 97800, CVN292	29	
	ABIOW074 08	ZIN 96432, CVN107	30	
	ABIOW232 09	ZIN 97807, CVN299	31	
	ABIOW219 09	ZIN 97794, CVN286	32	
Crocidura sp. AB2	ABMIV171 08	ZIN 97089, CVN201		Vietnam, Ba Ria – Vung Tau Province, Binh Chau - Phuoc Buu Nature Reserve; 10º32´N, 107º29´E
C. indochinensis	ABIOW202 09	ZIN 97672, CVN269	1	Vietnam, Lam Dong Province, Bi Doup Mt.; 12º11´N, 108º41´E
	ABIOW199 09	ZIN 97670, CVN266	2	
	ABIOW200 09	ZIN 97671, CVN267	3	
	ABIOW145 09	ZIN 98962, CVN350	4	
	ABIOW177 09	ZIN 97668, CVN243	5	
	ABIOW206 09	ZIN 97673, CVN273	6	
	ABIOW207 09	ZIN 97674, CVN274	7	
C. tanakae A	ABMIV145 08	ZIN 91230, CVN165	1	Vietnam, Kon Tum Province, Ngoc Linh Mt.; 15º05´N, 107º57´E
	ABMIV142 08	ZIN 91226, CVN161	2	
	ABMIV143 08	ZIN 91227, CVN162	3	
	ABMIV144 08	ZIN 91229, CVN164	4	
	ABIOW063 08	ZIN 91228, CVN163	5	
	ABIOW062 08	ZIN 91231, CVN166	6	
	ABMIV129 08	ZIN 96412, CVN138	7	Vietnam, Khanh Hoa Province, Hon Ba Mt.; 12º07´N, 108º57´E
	ABIOW040 08	ZIN 97510, CVN212	8	Vietnam, Quang Binh Province, Phong Nha - Ke Bang National Park; 17º38´N, 106º06´E
	ABIOW128 09	ZIN 98933, CVN333	9	Vietnam, Lam Dong Province, Bi Doup Mt.; 12º11´N, 108º41´E
	ABIOW143 09	ZIN 98960, CVN348	10	
	ABIOW150 09	ZIN 97606, CVN216	11	
	ABIOW179 09	ZIN 97615, CVN245	12	
	ABIOW144 09	ZIN 98691, CVN349	13	
	ABIOW161 09	ZIN 97613, CVN227	14	
	ABIOW158 09	ZIN 97610, CVN224	15	
	ABIOW160 09	ZIN 94612, CVN226	16	
	ABIOW183 09	ZIN 97625, CVN249	17	
	ABIOW174 09	ZIN 97623, CVN240	18	
	ABIOW164 09	ZIN 97622, CVN230	19	

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TABLE 1. (continued)

species	BOLD Accession No	Catalogue number and field specimen code	Specimen code on Fig. 2–3	Collecting locality
	ABIOW175 09	ZIN 97624, CVN241	20	
	ABIOW155 09	ZIN 97608, CVN221	21	
	ABMIV164 08	ZMMU S-173391, CVN192	22	
	ABMIV168 08	ZMMU S-173397, CVN198	23	
	ABMIV165 08	ZMMU S-173392, CVN193	24	
	ABIOW138 09	ZIN 98940, CVN343	25	
	ABIOW135 09	ZIN 98937, CVN340	26	
	ABIOW136 09	ZIN 98938, CVN341	27	
	ABIOW140 09	ZIN 98942, CVN345	28	
	ABIOW137 09	ZIN 98939, CVN342	29	
	ABIOW139 09	ZIN 98941, CVN344	30	
	ABIOW154 09	ZIN 97607, CVN220	31	
	ABIOW181 09	ZIN 97651, CVN247	32	
	ABIOW201 09	ZIN 97620, CVN268	33	
	ABIOW178 09	ZIN 97614, CVN244	34	
	ABIOW193 09	ZIN 97619, CVN260	35	
	ABIOW021 08	ZIN 97504, CVN207	36	Vietnam, Quang Tri Province, Huong Hoa Nature Reserve; 16º56´N, 106º35´E
	ABIOW022 08	ZIN 97505, CVN208	37	
	ABIOW024 08	ZIN 97507, CVN210	38	
	ABIOW023 08	ZIN 97506, CVN209	39	
	ABIOW039 08	ZIN 97508, CVN211	40	
	ABIOW126 09	ZIN 98935, CVN331	41	Vietnam, Binh Phuoc Province, Bu Gia Map National Park; 12º11'N, 107º12'E
	ABIOW127 09	ZIN 98936, CVN 332	42	
C. tanakae B	ABIOW033 08	ZIN 91195, CVN145	43	Vietnam, Lao Cai Province, Van Ban District; 21º58´N, 104º02´E
	ABIOW064 08	ZIN 91209, CVN159	44	
	ABMIV131 08	ZIN 91190, CVN140	45	
	ABMIV140 08	ZIN 91208, CVN158	46	
	ABMIV137 08	ZIN 91204, CVN154	47	
	ABIOW036 08	ZIN91198, CVN148	48	
	ABIOW032 08	ZIN91193, CVN143	49	
	ABMIV135 08	ZIN 91201 CVN151	50	
	ABMIV141 08	ZIN 91210, CVN160	51	
	ABMIV138 08	ZIN 91205, CVN155	52	
	ABMIV136 08	ZIN 91202, CVN152	53	
	ABMIV139 08	ZIN 91207, CVN157	54	
	ABMIV133 08	ZIN 91194, CVN144	55	
C. phanluongi	ABMIV172 08	ZIN 97090, CVN202	1	Vietnam, Dak Lak Province, Yok Don National Park; 12º58´N, 107º49´E
	ABIOW019 08	**ZIN 97092, CVN204**	2	
	ABIOW018 08	ZIN 97091, CVN203	3	
	ABIOW020 08	ZIN 97093, CVN205	4	
	ABIOW124 09	ZIN 98930, CVN329	5	Vietnam, Binh Phuoc Province, Bu Gia Map National Park; 12º11'N, 107º12'E

TABLE 2. List of specimens analysed for the cytochrome *b* (*cytb*) gene. Holotypes are marked in bold. The sequences retrieved from GenBank are given with asterisks.

species	GenBank Accession No	Catalogue number and/or field specimen code	Specimen code (on Fig. 3 if present)	Collecting locality
C. sokolovi	HM586998	ZIN 96393, CVN1	1	Vietnam, Kon Tum Province, Ngoc Linh Mt.; 15º05´N, 107º57´E
	HM586999	ZIN 96394, CVN2	2	
	HM587000	ZIN 96396, CVN4	-	
C. zaitsevi A	HM587002	ZIN 96319, CVN19	1	Vietnam, Kon Tum Province, Ngoc Linh Mt.; 15º05´N, 107º57´E
	HM587004	ZIN 96321, CVN21	2	
	HM587003	ZIN 96320, CVN20	-	
C. zaitsevi B	HM587021	ZIN 97628, CVN217	1	Vietnam, Lam Dong Province, Hon Giao Mt.; 12º11´N, 108º48´E
	HM587025	ZIN 97642, CVN231	2	Vietnam, Lam Dong Province, Bi Doup Mt.; 12º11´N, 108º41´E
	HM587026	ZIN 97643, CVN232	-	
	HM587013	ZIN 96413, CVN121	-	Vietnam, Khanh Hoa Province, Hon Ba Mt., 12º07´N, 108º57´E
	HM587014	ZMMU S-175185, CVN187	-	
C. phuquocensis	HM587011	**ZIN 96660, CVN184**	ZIN 96660	Vietnam, Kien Giang Province, Phu Quoc Isl.; 10°23'N, 104°00'E
Crocidura sp. AB1	HM587006	ZIN 96264, CVN94	ZIN 96264	Vietnam, Lao Cai Province, Sa Pa District, 22º20`N, 103º50`E
	HM587005	ZIN 96262, CVN93	-	
	HM587007	ZIN 96265, CVN95	-	
C. indochinensis	HM587023	ZIN 97669, CVN261	1	Vietnam, Lam Dong Province, Bi Doup Mt.; 12º11´N, 108º41´E
	HM587024	ZIN 97670, CVN266	2	
C. tanakae	AB175081*		1	Taiwan, Nantou Co.
	AB175080*		2	
	HM587017	ZMMU S-173392, CVN193	3	Vietnam, Lam Dong Province, Bi Doup Mt.; 12º11´N, 108º41´E
	HM587022	ZIN 97627, CVN218	4	Vietnam, Lam Dong Province, Hon Giao Mt.; 12º11´N, 108º48´E
	HM587012	ZIN 91190, CVN140	-	Vietnam, Lao Cai Province, Van Ban District, 21º58´N, 104º02´E
	HM587001	ZIN 96409, CVN17	-	Vietnam, Kon Tum Province, Ngoc Linh Mt.; 15º05´N, 107º57´E
	HM587010	ZIN 96411, CVN139	-	
		ZIN 96412, CVN138	-	Vietnam, Khanh Hoa Province, Hon Ba Mt.; 12º07´N, 108º57´E
	FJ814038*	ROM115021	5	China, Hunan
	FJ814037*	ROM11505	6	
	FJ814036*	ROM11504	7	
	FJ814035*	ROM114960	8	
	FJ814033*	ROM111317	9	Vietnam
	FJ814032*	ROM111293	10	
	HM587027	ZIN 98935, CVN331	11	Vietnam, Binh Phuoc Province, Bu Gia Map National Park; 12º11'N, 107º12'E
	HM587028	ZIN 98936, CVN 332	12	
	HM587031	ZIN 99026, Laos317	13	Laos, Khammouane Province, Ban Doy Area; 17º33′N, 104º49′E
	HM587032	ZIN 99028, Laos319	14	

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TABLE 2. (continued)

species	GenBank Accession No	Catalogue number and/or field specimen code	Specimen code (on Fig. 3 if present)	Collecting locality
	EU122211	MVZ185237	15	Vietnam, Vinh Phu Province, Vin Yen District, Tam Dao; 21º27`, N, 105º38`E
	FJ814031*	ROM107661	16	Vietnam
	FJ814047*	ROM116443	17	China, Hunan
	FJ814042*	ROM116114	18	
	FJ814044*	ROM116366	19	
	FJ814046*	ROM116432	20	
	FJ814045*	ROM116426	21	
	HM587029	ZIN 98937, CVN340	22	Vietnam, Lam Dong Province, Bi Doup Mt.; 12º11´N, 108º41´E
	HM587030	ZIN 98938, CVN341	23	
Crocidura sp.AB2	HM587018	ZIN 97089, CVN201	ZIN 97089	Vietnam, Ba Ria – Vung Tau Province, Binh Chau - Phuoc Buu Nature Reserve; 10º32´N, 107º29´E
C. phanluongi	HM587019	ZIN 97091, CVN203	-	Vietnam, Dak Lak Province, Yok Don National Park; 12º58´N, 107º49´E
	HM587020	**ZIN 97092, CVN204**	ZIN 97092	
C. attenuata	AB175083*	AMNH101493	1	Vietnam, Ha Giang Province, Mt. Tay Con Linh II; 22°45'N, 104°50'E
	AB175082*	AMNH101492	2	
	FJ814039*	ROM116033	3	China, Guangxi,
	FJ814034*	ROM114916	4	China, Hunan,
C. wuchihensis	AB175084*	AMNH101499	1	Vietnam, Ha Giang Province; Mt. Tay Con Linh II; 22°45'N, 104°50'E
	AB175085*	AMNH101508	2	
	FJ814043*	ROM116129	3	China, Guangxi
	FJ814041*	ROM116095	4	
	FJ814040*	ROM116090	5	
	EU122212*	MVZ186404	6	Vietnam, Vinh Phu Province, Vin Yen District, Tam Dao; 21º27`N, 105º38`E
C. dracula	AB175079*	AMNH101526	1	Vietnam, Ha Giang Province, Mt. Tay Con Linh II; 22°45'N, 104°50'E
C. fuliginosa	FJ813925*	IZEA3753	2	Peninsular Malaysia, Cameron Highland
	FJ813924*	IZEA3553	3	

Cytb. Genomic DNA was isolated from ethanol-fixed liver, kidney or muscles by proteinase K digestion, phenol-chloroform deproteinization and isopropanol precipitation (Sambrook *et al*. 1989). The mitochondrial H-strand region containing the complete *cytb* gene (1140 bp) was amplified by PCR with the forward/reverse primer combination L14728_Cr/H1310_Cr or L14723_Cr/H1310_Cr. In cases when DNA was degraded, fragments of *cytb* were amplified in two PCR reactions using L363A/ Cro_481b which was also used for sequencing. All primers were designed specifically for this study (Table 3). The PCR reaction conditions for *cytb* amplification included the initial denaturation at 94°C for 3 min, 35 cycles of 94°C for 30 s, annealing at 57–60°C for 1 min, and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min, and indefinite storage at 4°C. PCR products were visualized on 1% agarose gel and then purified using DEAE Watman or NH4EtOH. Approximately 10–40 ng of the purified PCR product was used for sequencing with each primer by the ABI 3100-Avant autosequencing system using ABI PRISM®BigDyeTM Terminator v. 3.1.

FIGURE 1. Distribution of sampling localities in Vietnam: 1—Lao Cai Province, Sa Pa District; 2—Lao Cai Province, Van Ban District; 3—Ha Giang Province; Mt. Tay Con Linh II (data from GenBank); 4—Vinh Phu Province, Vin Yen District, Tam Dao (data from GenBank); 5—Quang Binh Province, Phong Nha - Ke Bang National Park; 6—Quang Tri Province, Huong Hoa Nature Reserve; 7—Kon Tum Province, Ngoc Linh Mt.; 8—Dak Lak Province, Yok Don National Park; 9—Binh Phuoc Province, Bu Gia Map National Park; 10—Lam Dong Province, Bi Doup Mt.; 11—Lam Dong Province, Hon Giao Mt.; 12—Khanh Hoa Province, Hon Ba Mt; 13—Ba Ria–Vung Tau Province, Binh Chau - Phuoc Buu Nature Reserve; 14—Kien Giang Province, Phu Quoc Isl.; and in Laos: 15—Khammouane Province, Ban Doy Area.

Analyses of sequences. *Cytb* and COI gene sequences were aligned by eye using BioEdit v.7.0.5.3 (Hall 1999). The final alignment of the mitochondrial regions included 1140 bp for *cytb* and 657 bp for COI. Patterns of evolutionary sequence diversification for both COI and *cytb* were assessed using neighbour joining methods (NJ) implemented on PAUP* version 4.0b10 (Swofford 1998), based on pairwise p-distance matrix. To assess nodal support, 1000 bootstrap pseudoreplicates were generated. In order to cluster sequences likely belonging to different species we used the approach of Francis *et al.* (2010). To assess the genetic independence of species, we first

defined genetically distinct haplotype clusters on the NJ tree, using a combination of genetic distances and bootstrap support. We then calculated the mean genetic distance from each cluster to other clusters both within and among species. We defined the minimum interspecific distance as the minimum distance between specimens belonging to different species.

TABLE 3. Primers designed for amplification and sequencing of cytochrome b (*cytb*) gene in the Vietnamese species of *Crocidura.*

primer	sequence (5'-3')
L14728_Cr	GACATGAAAAATCATCGTTGTTCTTCAAC
L14723_Cr	CCTATGACATGAAAAATCATCGTTGTT
H1310_Cr	GAATATCAGCTTTGGGTGYTGATGGTGG
Cro_481b	ACGGAAAAGCCTCCTCAGATTCATTCTAC
L363A	CGCAGTTATAGCCACCGCCTTTATAGG

Results

The results of the NJ analysis of the COI and *cytb* p-distance matrices are presented on Figs. 2–3, and in Tables 4–5. The different interpretations from COI and *cytb* are due to the differences in the comparative context available for these two markers: COI includes significantly more specimens analyzed in this study, while there is a greater taxonomic sampling of *cytb* in GenBank.

The NJ tree for COI (Fig. 2) contains 180 specimens of shrews from Vietnam which split into six highly divergent (p-dist > 10%) lineages. These haplogroups correspond to the morphological species *C. sokolovi, C. zaitsevi, C. phuquocensis, Crocidura* ex. gr. *indochinensis, C.* cf. *tanakae,* and *C. phanluongi*. Four of these clusters are further subdivided into subgroups with varying levels of genetic differentiation. The haplogroups containing *C. zaitsevi* and *C. tanakae* each include two subgroups: *C. zaitsevi* A and B and *C.* cf. *tanakae* A and B separated by a p-distance of 1.5% and 2.3%, accordingly. *C. phanluongi* is represented by two similar, but strongly divergent (8.7%) lineages. The cluster identified here as *C.* ex gr. *indochinensis* contains two subgroups with fairly deep genetic divergence (p-dist = 4.0%). Each haplogroup and subgroup is supported with high bootstrap values; however, their relationships remain unclear.

The *cytb* dendrogram (Fig. 3) contains fewer specimens of Vietnamese shrews but a broader set of sequences of *Crocidura* species from extralimital regions of Southeast Asia. All groups seen on the COI dendrogram are present and supported by high bootstrap values in the *cytb* dendrogram as well. *C. zaitsevi* is the nearest neighbour to the *C. indochinensis*/*C. wuchihensis* group and, together with it, clusters with the *C. dsinezumi/C. attenuata/C. kurodae/C. lasiura* branch. The similarity of two lineages referred to *C. phanluongi* sensu lato (p-dist = 7.9%) is more obvious, compared to COI, with a bootstrap support of 94%. Mean p-distances between the Vietnamese species range between 7.75% (*C. wuchihensis*/*C. indochinensis* s.l.) and 15.5% (*C. sokolovi/C. phuquocensis*).

Discussion

The patterning of genetic diversity within and among Vietnamese *Crocidura* obtained using mitochondrial markers enabled us to discriminate all described morphospecies and to highlight hidden genetic divergences that may taxonomic refinements. Specific details are discussed below.

Crocidura sokolovi and *C. zaitsevi*

Both COI and *cytb* mitochondrial sequence data confirmed the genetic authenticity of *C. sokolovi* and *C. zaitsevi* recently described from central Vietnam (Jenkins *et al.* 2007). The relative amount of separation from the respective closest groups (COI/*cytb* of 11.5/11.1% and 9.4/9.9%, correspondingly) falls within the range of other species-level divergences observed from our data and the literature. Interestingly, the mitochondrial DNA data further highlight the fact that *C. sokolovi* from high altitudes of Ngoc Linh Mountain is not only distinct, but also

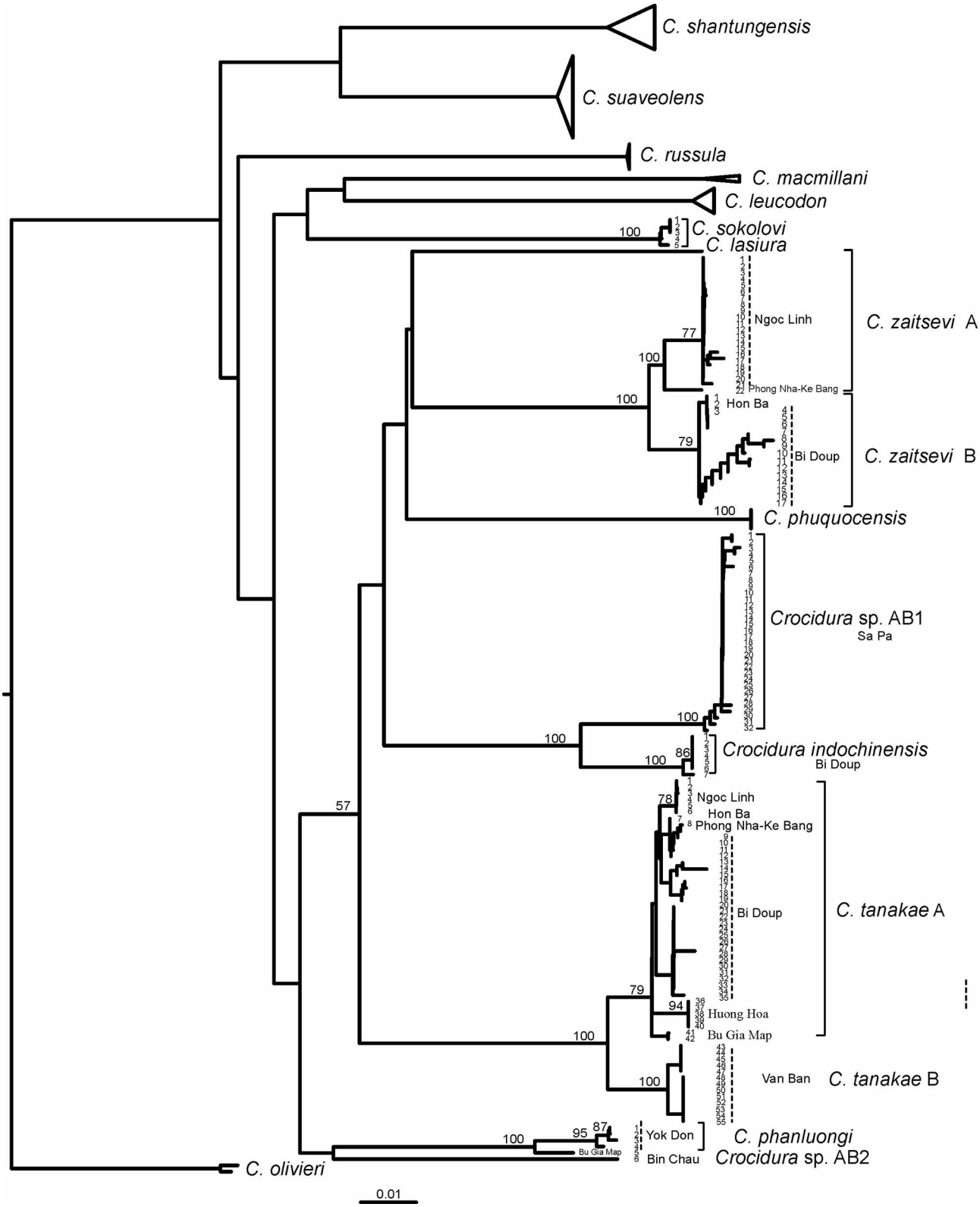

FIGURE 2. The Neighbor-Joining tree for the cytochrome oxidase *c* subunit 1 (*COI*) gene fragment. The bootstrap values (≥ 50 %) obtained from 1000 pseudoreplications are presented above the branches. *Crocidura olivieri is* used as outgroup.

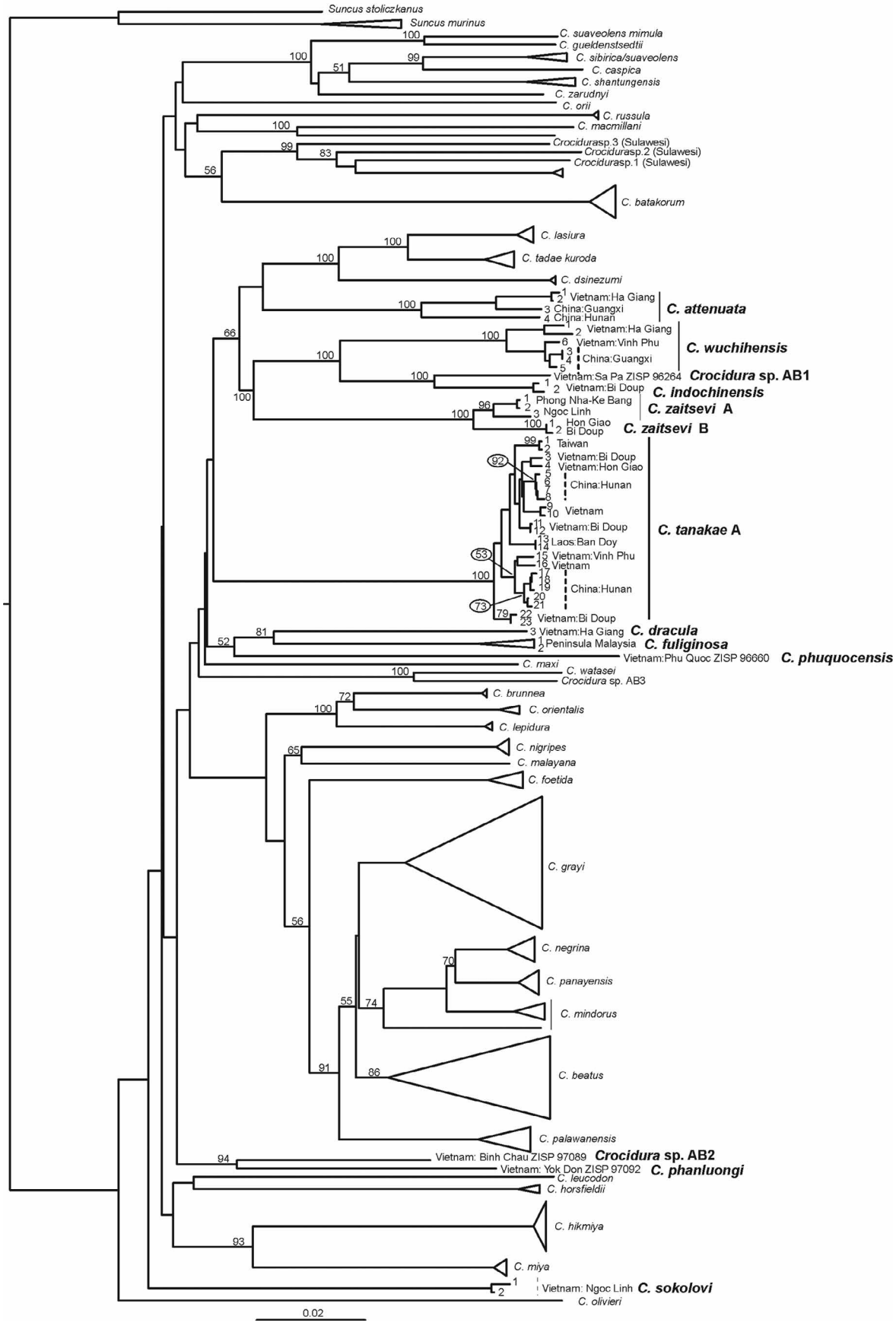

FIGURE 3. The Neighbor-Joining tree for the cytochrome *b* (*cytb*) gene fragment. Designations as on the Fig. 2. *Suncus murinus* and *S. stoliczkanus* are used as outgroup.

TABLE 4. Comparison of intraspecific vs. interspecific p-distances (%) using the cytochrome oxidase *c* subunit 1 (COI) gene. Average /maximum intragroup distances are given on the diagonal; average/minimum distances between groups or species are shown under the diagonal.

	C. lasiura	*C. sokolovi*	*C. zaitsevi* A	*C. zaitsevi* B	*C. zaitsevi* combined	*Crocidura* sp. Sapa	*C. indochinensis*	*Crocidura* sp. Sapa +*C. indochinensis*	*C. tanakae* A	*C. tanakae* B	*C. tanakae* combined	*C. phanluongi*	*Crocidura* sp. Bihn Chau	*C. phanluongi* + *Crocidura* sp. Bihn Chau
C. sokolovi	12.81/12.71	0.15/0.30												
C. zaitsevi A	9.21/8.71	13.82/13.46	0.18/1.50											
C. zaitsevi B	9.59/9.37	14.36/13.70	1.50/1.00	0.17/0.71										
C. zaitsevi combined	9.37/8.71	14.06/13.46	-	-	0.84/2.20									
Crocidura sp. Sapa	10.79/10.57	12.51/12.23	10.94/8.49	10.80/8.81	10.88/8.49	0.10/0.50								
C. indochinensis	10.60/10.51	13.87/13.67	11.27/10.19	11.12/9.19	11.2/9.2	4.00/3.83	0.90/0.30							
Crocidura sp. Sapa + C. indochinensis	10.75/10.51	12.75/12.23	11/8.49	10.86/8.81	10.94/8.49	-	-	1.28/4.28						
C. tanakae A	10.72/9.71	12.83/12.16	10.95/10.25	11.43/10.24	11.16/10.25	11.32/9.38	10.27/8.09	11.13/8.09	0.61/1.65					
C. tanakae B	10.64/10.57	13.30/13.15	10.18/9.94	10.82/9.94	10.46/9.94	11.69/11.16	9.94/9.63	11.38/9.63	2.35/1.98	0.23/0.46				
C. tanakae combined	10.7/9.71	12.94/12.16	10.77/9.94	11.28/9.94	10.99/9.94	11.41/9.39	10.19/8.09	11.19/8.09	-	-	1.23/3.63			
C. phanluongi	11.96/10.86	11.53/10.86	12.15/11.77	12.61/11.62	12.35/11.62	12.88/10.78	12.63/10.86	12.84/10.78	10.08/10.01	11.20/10.70	10.9/10.13	0.80/1.97		
Crocidura sp. Bihn Chau	11.49/11.49	12.01/11.93	11.59/11.04	12.07/11.77	11.8/11.04	12.4/12.21	12.53/12.39	12.42/12.21	11.31/10.68	11.19/11.11	11.28/10.68	8.73/8.50	-	
C. phanluongi + *Crocidura* sp. Bihn Chau	11.88/10.86	11.61/10.86	12.06/11.04	12.52/11.62	12.26/11.04	12.8/10.78	12.62/10.86	12.77/10.78	10.89/10.13	11.2/10.7	10.96/10.13	-	-	3.45/8.89
C. *phuquocensis*	10.41/10.41	12.74/12.63	10.35/9.55	10.12/8.43	10.25/8.43	11.02/10.47	10.21/9.94	10.87/9.94	11.47/10.87	11.79/11.72	11.55/10.87	11.37/11.16	11.11/11.11	11.33/11.11

TABLE 5. Average/maximum intragroup distances and average/minimum distances between closest haplogroups of Vietnamese *Crocidura* using the cytochrome *b* (*cytb*) gene.

	Within group variation	Closest group in the NJ dendrogram	Average distance to the closest group (according to the NJ topology)	Minimum distance between haplotypes belonging to different groups
C. zaitsevi A	1.60/2.44	*C. zaitsevi* B`	2.28	2.03
C. zaitsevi B	0	*C. zaitsevi* A		
C. zaitsevi combined	-	*C. wuchichensis*+*C. indochinensis*+ *Crocidura* sp. Sapa	10.91	9.30
C. wuchichensis	1.31/2.54	*C. indochinensis*+ *Crocidura* sp. Sapa	7.75	7.36
C. indochinensis	0.28	*Crocidura* sp. Sapa	4.09	4.03
Crocidura sp. Sapa	-	*C. indochinensis*		
C. tanakae A	0.88/1.58	*C. lasiura*+*C. dsinezumi*+*C. tadae*+*C. zaitsevi* +*C. attenuate* + *C. wuchichensis*+*C. indochinensis*+ *Crocidura* sp. Sapa	11.7	9.91
C. phanluongi	-	*Crocidura* sp. Bihn Chau	7.93	7.93
Crocidura sp. Bihn Chau	-	*C. phanluongi*		
C. *phuquocensis*	-	*C. dracula* + *C. fuliginosa*	12.61	12.02
C. fuliginosa	1.74	*C. dracula*	9.28	9.27
C. dracula	-	*C. fuliginosa*		
C. sokolovi	0.38	all studied species exept *C. olivieri*	13.34	10.90

deeply divergent from all other Vietnamese white-toothed shrews: ~13% on average (for both *cytb* and COI). While it has been supposed that *C. zaitsevi* is endemic to Ngoc Linh Mountain (Jenkins *et al*. 2007, 2009), our data suggest this species to be more widely distributed across South Vietnam. Two haplogroups (p-dist = 2.3%, *cytb* and 1.5%, COI) were found within *C. zaitsevi*, which correspond to two geographically remote localities: haplogroup A containing specimens from the type locality (Ngoc Linh Mt., Central Vietnam), and haplogroup B found in Hon Ba Mt. and Bi Doup Mt., South Vietnam (Fig. 2–3). The haplogroup from the Phong Nha—Ke Bang area in central Vietnam is associated with haplogroup A; however, it is distinct (p-dist = 1.3%, COI) and may represent a separate subgroup. Thus, the intraspecific differentiation of *C. zaitsevi* is relatively high and comparable with that of *C. wuchihensis* and *C. tanakae*.

Crocidura indochinensis and *C. wuchihensis*

The group containing *C. indochinensis* and *C. wuchihensis* appears on the *cytb* tree with 100% bootstrap support.

Originally described from Langbian Mountain, Dalat Plateau (South Vietnam), *C. indochinensis* occurs in Myanmar, Yunnan China and Vietnam (Hutterer 2005). Our dataset includes a small series of *C. indochinensis* from the vicinity of Bi Doup Mountain, about 25 km from its type locality. These are medium-sized shrews with a relatively long tail. This species is poorly represented in collections, which led Jenkins *et al*. (2009) to suggest that it has a disjunct distribution. Aside from the Da Lat Plateau in southern Vietnam, *C. indochinensis* has also been recorded from the north of the country (Can *et al*. 2008; Jenkins et al. 2009), particularly in the vicinity of Sa Pa (specimen FMNH 39029 at the Field Museum of Natural History, Chicago – see Jenkins *et al*. 2009). Nonetheless, we did not find any northern members of this haplogroup, either in our collection or among GenBank sequences. On both the *cytb* and COI trees specimens from Sa Pa are separated from *C. indochinensis* by p-distances of 4.1 % (*cytb*) and 4.0% (COI). While the shrews from Bi Doup and Sa Pa may be treated as genetically divergent populations of the same species because they are nearest neighbours on mtDNA trees, morphological data support their separation as different species. In our sample, specimens from Sa Pa differ from the Bi Doup of *C. indochinensis* in

smaller size and shorter tail and thus resemble *C. wuchihensis* (sensu Jenkins *et al*. 2009, 2010). Furthermore, there are significant discrepancies in habitat preferences and altitudinal distribution of these two haplogroups (Abramov *et al*. 2008b, 2010). Thus, we suggest that specimens from Sa Pa could be specifically distinct and propose a temporary designation under *Crocidura* sp. AB1, pending a formal species description.

The haplogroup on Fig. 3 assigned to *C. wuchihensis* includes shrews from Tay Con Linh II Mountain, Ha Giang Province, northern Vietnam (see Lunde *et al*. 2003) and those from China, Guangxi, sequenced by Esselstyn & Brown (2009). At this point, no genomic data are available for *C. wuchihensis* from the type locality (Hainan Is., China); however, specimens from the most proximate mainland area (Guangxi Province) have been referred to as *C. wuchihensis* (Esselstyn & Brown 2009), which makes this name the best available for this haplogroup. The specimen MVZ186404 from Vinh Phu Province, northern Vietnam (code 6 on Fig.3) was deposited in GenBank as *C. fuliginosa* (EU122212, Meegaskumbura *et al*. 2007); however, its position within the *C. wuchihensis* haplogroup suggests an identification error, a problem not uncommon with GenBank sequence submissions (Harris, 200X). Jenkins *et al*. (2009) state that *C. wuchihensis* is widely distributed across Vietnam, including the provinces Lao Cai, Ha Giang and Lang Son in the north and Ha Thinh and Quang Nam in Central Vietnam. Our mtDNA data contradict this assertion, suggesting that the distribution of *C. wuchihensis* is restricted to areas east and north of the Red River (Fig.1). Furthermore, no genetically similar haplotypes were detected among specimens from Lao Cai Province (Van Ban and Sa Pa areas). Within northern Vietnam, *C. wuchihensis* splits into two lineages (p-distance of 2.1% for *cytb*) which probably represent distinct geographic populations.

Crocidura fuliginosa and *C. dracula* group

Crocidura fuliginosa Blyth, 1855 was among the first species of *Crocidura* documented from Vietnam (Heaney & Timm 1983). GenBank holds two complete *cytb* sequences assigned to this species from Cameron Highland, Peninsular Malaysia (codes 1, 2 on Fig. 3) and one from northern Vietnam, Ha Giang Province (code 3 on Fig. 3). Specimens from these two localities are separated by a p-distance of 9.3% for *cytb*, suggesting that they are specifically distinct. GenBank also holds several short sequences that were not included in our dendrograms. Three of them (AF003759, EF524752, EF524708) are part of the Malayan cluster while sequences from Yunnan (EF524792, EF524775) are neighbours with the specimen from northern Vietnam. Moreover, the analysis of nuclear BRCA1 and ApoB genes (Dubey *et al*. 2008) also supports the distinction between *C. fuliginosa* of Peninsular Malaysia and the specimens from Yunnan which are named there as *C. dracula*. The name *C. dracula* was used to define shrews from South China (Ellerman & Morrison-Scott 1951), until Jenkins (1976) proposed it to be a subspecies of *C. fuliginosa*—a notion later followed by others (Heaney & Timm 1983; Jiang & Hoffmann 2001; Hutterer 2005). Comparison of mtDNA data suggests that specimens from northern Vietnam and southern China (close to the type locality of *C. dracula*) belong to the same haplogroup, which is deeply divergent from Malayan specimens. *Crocidura fuliginosa* was described from Shwegyin (=Schwegyin), central Burma (Corbet and Hill 1992), so biogeographically it is unclear whether this name can be applied to the Malayan haplogroup. Recognising that this question requires resolution, we provisionally apply the name "*fuliginosa*" to shrews from the southern part of Southeast Asia, including Malaysia and South Myanmar. Following the above interpretation, the occurrence of *C. fuliginosa* s. str. in Vietnam, to this end, has not been confirmed genetically. In their latest review of Southeast Asian white-toothed shrews, Jenkins *et al.* (2009) provide records of *C. fuliginosa* only from northern Vietnam and southern China. The record of *C. fuliginosa* (*s.lato*) from Kon Tum and Lam Dong Provinces in Central Vietnam (Can *et al*. 2008) is not supported by collection material. It is still possible that *C. fuliginosa* s.str extends its range into southern Vietnam. A ZMMU specimen S-144368 from Bai Canh Is. off the coast of southern Vietnam was identified as *C. fuliginosa* (Paulina Jenkins, pers. comm., 2009). Furthermore, Van Peenen *et al.* (1970) recorded a specimen of large shrew from the neighbouring island of Con Son (Con Dao Archipelago), identified as *C. fuliginosa* (USNM 357348, Smithsonian Institution).

Comparison of *cytb* and COI sequences obtained from *C. phuquocensis* with available reference data corroborate the specific status of this taxon recently described from Phu Quoc Island (Abramov *et al*. 2008a). On the *cytb* tree, this haplogroup is the nearest neighbour to the *C. fuliginosa–C. dracula* group, although with low bootstrap support.

Crocidura phanluongi

C. phanluongi is a species recently described from the lowlands of southern Vietnam and north-eastern Cambodia (Jenkins *et al*. 2010). Our *cytb* and COI data for the type series of *C. phanluongi* from Yok Don and Bu Gia

Map provide genetic support for its species status. The specimen ZIN 97089 from Binh Chau, Ba Ria—Vung Tau Province, southern Vietnam, which was also referred to *C. phanluongi* by Jenkins *et al*. (2010), is deeply divergent from it nearest neighbours collected in Yok Don (p-distance=8.4% for *cytb* and 8.7% for COI), suggesting that it may represent a separate, undescribed species, provisionally defined here as *Crocidura* sp. AB2. The corresponding voucher specimen from Binh Chau also has a slightly shorter tail (61% of head and body length), compared to *C. phanluongi* from other localities (68–83%; see Jenkins *et al*. 2010).

Crocidura attenuata and *Crocidura tanakae*

The genetic analysis presented here of the most common and widely distributed Vietnamese shrew *C. attenuata* revealed an unexpected and previously undocumented pattern. Comparison of original *cytb* sequence information with GenBank data suggests that specimens from Vietnam are genetically similar to *C. tanakae* described from Taiwan. Because *C. tanakae* is currently recognized as a separate species (Motokawa *et al*. 1997, 2001; Hutterer 2005), our results call for the reappraisal of the taxonomic status of Vietnamese shrews previously referred to as *C. attenuata*. Until this is done, we propose to apply the name *C. tanakae* to the specimens that are genetically similar to the Taiwanese haplogroup. Such specimens have been collected across Vietnam, suggesting broad geographic distribution of this species on the mainland. In contrast, *C. attenuata* proper appears to be found only in the northernmost part of Vietnam (Ha Giang Province; Tay Con Linh II Mountain). Similar to *C. wuchihensis* (in its present interpretation), it is restricted to areas to the north and east of the Red River (Fig. 1) and is replaced by *C. tanakae* elsewhere.

Our relatively limited *cytb* sample shows little structuring among Chinese and Southesast Asian *C. tanakae*; however, the broader COI data demonstrates two clearly defined haplogroups within Vietnam (p-dist = 2.3%): one of them (*A*, Fig. 1) occurs in Central and South Vietnam (Huong Hoa, Phong Nha—Ke Bang, Ngoc Linh, Hon Ba, Bi Doup), while the other (*B*, Fig. 1) is restricted to the northern part of the country (Hoang Lien Mountains, Van Ban District).

Other *Crocidura* species

Three available GenBank sequences of *C. horsfieldii* included in the present analysis suggest that the sequence AB175078 of a specimen from Thailand forms a highly supported cluster with *C. watasei* but not with specimens of *C. horsfieldii* from Sri Lanka and India. Since the name *C. horsfieldii* Tomes, 1856 was described from Sri Lanka (Corbet & Hill 1992), we find it hardly applicable to the Thai specimen referred to *C. horsfieldii* in Dubey *et al.* (2008), Esselstyn *et al.* (2009), and Esselstyn & Brown (2009). We propose that it should be regarded as a yet unidentified species, provisionally named *Crocidura* sp. AB3.

The inclusion of available *Crocidura cytb* sequences from GenBank in our study revealed a disturbing trend, colloquially referred to as the "taxonomic impediment" (Carvalho *et al.* 2007). Over time, more and more DNA sequences are submitted to GenBank that are attributed to species names that appear to be assigned without proper taxonomic verification. At the other extreme, a growing pool of publications highlights the significant increase in the number of species of *Crocidura* described from Southeast Asia since Ruedi's (1996) work—both within the region on the whole (Dubey *et al*. 2008; Esselstyn *et al*. 2009) and within Vietnam in particular (Lunde *et al*. 2003, 2004; Jenkins *et al*. 2007, 2009, 2010; Abramov *et al*. 2008a). The discovery of more new species in this area is very likely. Several recently described species of *Crocidura* reported from Vietnam based on morphological data are known only from type localities (*C. rapax*, *C. kegoensis*, *C. annamitensis*, and *C. guy*) with no published sequence data available for them, causing further confusion. The data standards associated with barcoding (Hanner 2009) represent a significant improvement to the staus quo, particularly if new species descriptions include barcodes, a move that could help to disambiguate the application of names going forward.

As an example, several specimens from Gia Lai and Lam Dong provinces in southern Vietnam formerly reported as *C. indochinensis* (Heaney & Timm 1983) were recently re-identified as *C. rapax* (Jenkins *et al*. 2009). However, a targeted study of a large series (96 specimens) of shrews from the Dalat Plateau, Lam Dong Province (Abramov *et al*. 2010) did not reveal any individuals matching the morphological diagnosis of this species. The question remains open whether this was due to the actual differences in the species diversity sampled or due to the differential interpretation of morphological diagnoses.

While our pilot study of Southeast Asian *Crocidura* is insufficient to draw phylogenetic conclusions or to make a substantial taxonomic revision of this genus, it highlights some key points related to our assessment of the

alpha-taxonomic diversity and species diagnostics of its Vietnamese members. In particular, this study characterized the genetic profiles of several recently described species: *C. sokolovi*, *C. zaitsevi*, *C. phuquocensis*, and *C. phanluongi*. It also suggested the existence of two possibly undescribed species: *Crocidura* sp. AAB1 from Sa Pa and *Crocidura* sp. AB2 from Binh Chau. Finally, it helped formulate working hypotheses related to the taxonomic status and distribution patterns of species groups *C. attenuata/C. tanakae*, *C. fuliginosa/C. dracula*, and *C. indochinensis/C. wuchihensis*. These questions and others, such as the taxonomic status of the "northern" and "southern" forms of *C. tanakae* and *C. zaitsevi* are in need of further research.

This study underpins the need for an integrative approach towards the systematics and diagnostics of speciose and morphologically cryptic taxa, such as the genus *Crocidura*. Further progress would be aided by a more in-depth phylogenetic study, one involving not only mitochondrial, but also nuclear markers, a suite of morphological and karyological characters, as well as ecological peculiarities and distributional patterns. We hope that the approach used here and the presented data will contribute towards building a more comprehensive picture of the taxonomic and phylogeographic diversity of Southeast Asian white-toothed shrews.

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APPENDIX

The list of non-Vietnamese specimens used in DNA barcoding and sequences retrieved from GenBank.

COI

Crocidura russula: AY332675, AY332676, AY769263, AY769264, NC006893. *C. shantungensis:* SKMZM500 08-SKMZM503 08, SKMZM494 08-SKMZM496 08, SKMZM498 08. *C. suaveolens*: SKMZM490 08, SKMZM493 08, SKMZM802 09, SKMZM804 09, SKMZM805 09, SKMZM807 09, SKMZM808 09, SKMZM811 09, SKMZM814 09, SKMZM816 09, SKMZM817 09, SKMZM486 08-SKMZM488 08. *C. leucodon*: SKMZM800 09, SKMZM801 09, SKMZM803 09, SKMZM812 09, SKMZM813 09. *C. lasiura*: SKMZM504 08.*C. olivieri*: SKMZM365 08, SKMZM366 08. *C. macmillani*: SKMZM371 08, SKMZM372 08.

Cytb

S. murinus: DQ630386, DQ630390. *Suncus stoliczkanus*: AB175077. *Crocidura orii:* AB175087. *C. russula:* AY769263, AY769264. *C. shantungensis:* AB077082, AB077078, EU742592-EU742594, EU742584-EU742585, EU742589, EU742590, EU742586, EU742587, EU742589. *C. suaveolens:* AB077280, AB077288, EU742613, AY994379. *C. guel-denstaedtii:* AY994376. *C. sibirica:* EU742583, AB077279. *C. caspica:* AY994370. *C. zarudnyi:* AY925211. *C. lasiura*: AB077071, AB077072. *C. leucodon*: DQ065609. *C. olivieri:* EU742597. *C. macmillani:* EU742601. *Crocidura*. cf. *lucina:* EU742603. *C. tadae kurodai*: AB057420, AB062686, AB115557. *Crocidura* sp.1: FJ814025. *Crocidura* sp.2: FJ814026. *Crocidura* sp.3: FJ814027. *C. maxi:* FJ814024. *C. dsinezumi:* AB077274, AB077277. *C. batakorum:* FJ813972-FJ813974, FJ813976, FJ813977. *C. musseri:* FJ813927, FJ813929. *C. lasiura:* AB077071-AB077072. *C. maxi:* FJ814024. *C. watasei:* ABO77074. *Crocidura* sp.AB3: AB175078. *C. brunnea:* DQ630385. FJ814030. *C. orientalis:* FJ814029. *C. lepidura:* FJ814023, FJ814022. *C. nigripes:* DQ630384, FJ13926, FJ13928. *C. malayana:* DQ630381. *C. foetida:* FJ814053-FJ814055. *C. grayi:* FJ814051, FJ814049, FJ813900, FJ813967, FJ813942, FJ813830, FJ813865, FJ813867, FJ813880, FJ813930, FJ813872, FJ813839, FJ813898, FJ813895, FJ813856, FJ813848, FJ813857. *C. negrina:* FJ813961, FJ813962, FJ813951, FJ813952. *C. panayensi:* FJ813944-FJ813947.*C. mindorus:* FJ813940-FJ813943. *C. beatus:* FJ813980, FJ813884, FJ813844, FJ813847, FJ813838, FJ813837, FJ813879, FJ813846, FJ814019, FJ814010, FJ814015. *C. palawanensis:* FJ813978, FJ813902, FJ813915, FJ813912. *C. horsfieldii:* EU122213, FJ814028. *C. hikmiya:* EU122219-21, EU122217, EU122218, EU122222, EU122223. *C. miya:* EU122214-EU122216.