ELECTRONIC SUPPLEMENTARY MATERIAL for Stuart, Inger & Voris

Sampling

Specimens were collected in the field in Cambodia, Thailand, and Borneo by the authors and shortly preserved in 10% buffered formalin after fixing pieces of liver in 95% ethanol or 20% DMSO-salt saturated storage buffer, or freezing pieces of heart, liver, and muscle in liquid nitrogen. Specimens and tissue samples were later deposited at The Field Museum (FMNH), Chicago, and specimens were transferred to 70% ethanol upon arrival there. Voucher specimens and tissue samples from additional localities in Vietnam, Thailand, Peninsular Malaysia, and Sumatra were borrowed from the holdings of the FMNH and other institutions (Table 1).

Extraction, Amplification, and Sequencing of DNA from Fresh Tissues

Total genomic DNA was extracted from fresh tissues (tissues that were preserved at the time of collection for the purpose of genetic analysis) using PureGene Animal Tissue DNA Isolation Protocol (Gentra Systems, Inc.). Primers for amplifying and sequencing mitochondrial DNA were designed from amphibian sequences in GenBank (Table 2). A fragment of mitochondrial DNA that encodes part of the cytochrome oxidase c subunit III (COXIII) gene, the complete tRNA glycine, the complete NADH dehydrogenase subunit 3 (ND3) gene, and part of the tRNA arginine was amplified by PCR (the polymerase chain reaction; 94°C 45s, 49°C 30s, 72°C 1 min) for 35 cycles using the primer pair L-COXIII/Arg-HND3III. A fragment of mitochondrial DNA that encodes part of the 16S ribosomal RNA (16S) gene was amplified by PCR (94°C 45s, 60°C 30s, 72°C 1 min) for 35 cycles using the primer pair L-16SRanaIII/H-16SRanaIII. An additional fragment of mitochondrial DNA that encodes part of the tRNA methionine, the complete NADH deydrogenase subunit 2 (ND2) gene, and part of the tRNA tryptophan was amplified from samples of *O. livida* by PCR (94°C 45s, 49°C 30s, 72°C 1 min) for 35 cycles using the primer pair Met-LND2/Trp-HND2.

PCR products were electrophoresed in a 1% low melt agarose TALE gel stained with ethidium bromide and visualized under ultraviolet light. The bands containing DNA were excised and agarose was digested from bands using GELase (Epicentre Technologies). PCR products were sequenced in both directions by direct double strand cycle sequencing using Big Dye version 3 chemistry (Perkin Elmer). The amplifying primers, and in the case of ND2 a forward and reverse internal primer, were used in the sequencing reactions. Cycle sequencing products were precipitated with ethanol, 3 M sodium acetate, and 125 mM EDTA, and sequenced with a Prism 3100 Genetic Analyzer (ABI) or 3730 DNA Analyzer (ABI). Sequences were edited and aligned using Sequencher v. 4.1 (Genecodes).

The aligned *O. livida* dataset used in the phylogenetic analyses contained 2,150 mitochondrial DNA characters, consisting of 1,431 protein-coding, 623 rRNA, and 96 tRNA characters. The aligned *R. chalconota* dataset contained 1,082 mitochondrial DNA characters, consisting of 393 protein-coding, 615 rRNA, and 74 tRNA characters. Sequences were deposited in GenBank (accession numbers DQ650352-DQ650632).

Extraction, Amplification, and Sequencing of Historical DNA from Museum Type Specimens

A small piece of abdominal muscle or liver was removed from a syntype female of *O. chloronota* (BMNH 1947.2.28.6; collected prior to 1875) and the neotype female of *O. livida* (BMNH 1889.3.25.48; collected in 1887). DNA was extracted from this tissue using a protocol modified from Kearney & Stuart (2004). Extractions were performed using UV-sterilized supplies inside a Purifier PCR Enclosure (Labconco) in a separate room from where fresh tissues of the *O. livida* group had been previously extracted and amplified. Tissues were washed three times (2 h, 2 h, 12 h) with 1.5 mL of GTE Buffer to bind excess formalin (Shedlock et al., 1997) and twice in 1 mL dH₂0, and then incubated at 56°C for 5 days in 300 µL of TNES Buffer (10 mM Trizma Base, 100

mM NaCl, 10mM EDTA, 2% SDS, 39mM DTT) with daily additions of 300 μ g of proteinase-K. The remaining extraction procedure followed the DNeasy Tissue Kit (Qiagen) protocol for animal tissues, with these modifications: 300 μ L AL Buffer and 400 μ L of 100% ethanol were used rather than 200 μ L of each, two spins of 500 μ L of the extraction product through the DNeasy mini column was necessary to accommodate the larger extraction volume, a second spin was added for 1 minute at full speed after discarding the Buffer AW2 flow-through fluid, and 75 μ L of Buffer AE was added to the DNeasy membrane rather than 100-400 μ L, after which the membrane was incubated at 65°C for 5 min before centrifuging.

Five fragments of 181-339 nucleotide basepairs (bp; after primer sequences were trimmed) of 16S were amplified by PCR (94°C 45s, 54°C 30s, 72°C 1 min) for 40 cycles using the primer pairs L-16SRanaIII/H-R20016S, L-R14016S/H-R37516S, L-R14016S/H-R48016S, and L-R43016S/H-16SRanaIII (Table 2). Primers were designed so that the resulting fragments overlapped by 42-265 bp (after primer sequences were trimmed). This measure ensured that fragments of contaminant DNA were not concatenated into chimeric sequences that might be erroneously judged to be authentic (Olson & Hassanin, 2003). The 25 μ I PCR reactions utilized 4 μ I of bovine serum albumin (BSA; New England BioLabs) to prevent PCR inhibitors, a relatively large amount (4 μ I) of DNA template to overcome low extraction yield, the high-quality Taq polymerase AmpliTaq Gold (Roche), and extra cycles (40 total) of the PCR reaction. PCR products were sequenced, edited, and aligned as described above.

A total of 361 bp was obtained from the *O. chloronota* syntype. A total of 600 bp was obtained from the *O. livida* neotype, and this represented the complete fragment of 16S that was obtained from fresh tissues (above).

Phylogenetic Analyses

Phylogenies were reconstructed using the maximum parsimony optimality criterion and mixed-model Bayesian inference. The *O. livida* dataset was rooted with *Amolops* cf. *chapaensis* and *O. bacboensis*, and the *R. chalconota* dataset was rooted with *R. cubitalis* and *R. erythraea*.

Maximum parsimony analyses were performed using PAUP* 4.0b10 (Swofford, 2002). A heuristic search of the *O. livida* dataset was performed with equal weighting of nucleotide substitutions, stepwise addition with 100 random addition replicates, and TBR branch swapping limited to 1,000 trees per replicate. The search recovered >70,000 equally most parsimonious trees (L=1966) and strict consensus showed that these trees differed only by the arrangement of individuals within clades. A heuristic search of the *R. chalconota* dataset was performed with equal weighting of nucleotide substitutions, stepwise addition with 1000 random addition replicates, and TBR branch swapping. The search recovered 541 equally most parsimonious trees (L=846) and strict consensus showed that these trees differed only by the arrangement of individuals within clades. Nodal support was evaluated with 500 nonparametric bootstrap pseudoreplications (Felsenstein, 1985) using the heuristic search option with TBR branch swapping limited to 10,000,000 rearrangements per replicate.

Mixed-model Bayesian analyses were performed using MrBayes 3.1 (Ronquist & Huelsenbeck, 2003). The datasets were separated into first codon position, second codon position, third codon position, rRNA, and tRNA data partitions. The model of sequence evolution that best described each of the five data partitions was inferred using the Akaike Information Criterion as implemented in Modeltest 3.7 (Posada & Crandall, 1998). The models selected by Modeltest for the *O. livida* dataset were GTR+G for the first codon position partition, GTR+I+G for the second codon position and rRNA partitions, TIM+I+G for the third codon position partition, and TVM+G for the tRNA partition. The models selected by Modeltest for the *R. chalconota* dataset were SYM+G for the first codon position partition, TVM+I for the second codon position partition, TrN+G for the third codon position partition, GRT+G for the rRNA partition, and HKY+G for the tRNA partition. Some of these models are not implemented in MrBayes 3.1, and so the next more complex model available in the program was used for those partitions. Four independent

Bayesian analyses were performed on each dataset. In each analysis, six chains were run for 10,000,000 generations using the default priors, trees were sampled every 5000 generations, and the first 25% of trees were discarded as 'burn-in.' A 50% majority rule consensus of the sampled trees was constructed to calculate the posterior probabilities of the tree nodes. The *O. livida* and *R. chalconota* analyses resulted in standard deviations of split frequencies among the four runs of 0.008995 and 0.007749, respectively. Both analyses also had relatively stationary trace plots of clade probabilities, as viewed using AWTY (Wilgenbusch *et al.*, 2004). These two measures suggest that the four runs of each analysis had sufficiently converged and that topologies were sampled in proportion to their true posterior probability distribution.

National Park; NR = Nature Reserve; Prov. = Province; WS = Wildlife Sanctuary. Institutional abbreviations used are AMNH = American Museum of Natural History, New York; BMNH = The Natural History Museum, London; FMNH = Field Museum of Natural History, Chicago; FRIM = Forest Research Institute Malaysia, Kuala Lumpur; ROM = Royal Ontario Museum, Toronto; UTA = University of Texas at Arlington. Brackets indicate Table 1. Tissue samples sequenced in this study. Locality abbreviations used are BCA = Biodiversity Conservation Area; Dist. = District; NP = coordinates were determined by the author rather than by the original collector.

Voucher	Field/Tissue No.	Locality
Odorrana bacboensis		
FMNH 255611 paratype Odorrana cf. chapaensis	HKV 62713	Vietnam, Nghe An Prov., Con Cuong Dist., Pu Mat NR, 18°56'N 104°45'E
AMNH 163775	AMCC 106479	Vietnam, Ha Giang Prov., Yen Minh Dist., Du Gia Commune, 22°52′21″N 105°13′50″E
Odorrana banaorum		
FMNH 262874	HKV 65476	Cambodia, Ratanakiri Prov., Ta Veng Dist., Virachey NP, 14°11'16.3"N 107°17'36.1"E
FMNH 262875	HKV 65477	Cambodia, Ratanakiri Prov., Ta Veng Dist., Virachey NP, 14°11'16.3"N 107°17'36.1"E
FMNH 262878	HKV 65480	Cambodia, Ratanakiri Prov., Ta Veng Dist., Virachey NP, 14°11'16.3"N 107°17'36.1"E
FMNH 262889	HKV 65865	Cambodia, Mondolkiri Prov., O'Rang Dist., Seima BCA, 12°18'08.4"N 107°03'08.1"E
FMNH 262796	HKV 65570	Cambodia, Mondolkiri Prov., O'Rang Dist., Seima BCA, 12°16'24.6"N 107°03'53.1"E
FMNH 262800	HKV 65839	Cambodia, Mondolkiri Prov., O'Rang Dist., Seima BCA, 12°18'58"N 107°05'59"E
FMNH 262888	HKV 65841	Cambodia, Mondolkiri Prov., O'Rang Dist., Seima BCA, 12°18'58"N 107°05'59"E
FMNH 262801	HKV 65840	Cambodia, Mondolkiri Prov., O'Rang Dist., Seima BCA, 12°18'58"N 107°05'59"E
FMNH 262890	HKV 65867	Cambodia, Mondolkiri Prov., O'Rang Dist., Seima BCA, 12°18'08.4"N 107°03'08.1"E
FMNH 262802	HKV 65866	Cambodia, Mondolkiri Prov., O'Rang Dist., Seima BCA, 12°18'08.4"N 107°03'08.1"E
FMNH 262891	HKV 65868	Cambodia, Mondolkiri Prov., O'Rang Dist., Seima BCA, 12°18'08.4"N 107°03'08.1"E
FMNH 262740	HKV 65905	Cambodia, Mondolkiri Prov., O'Rang Dist., Seima BCA, 12°18'58"N 107°05'59"E
FMNH 262804	HKV 65918	Cambodia, Mondolkiri Prov., O'Rang Dist., Seima BCA, 12°18'08"N 107°03'08"E
FMNH 262900	HKV 65919	Cambodia, Mondolkiri Prov., O'Rang Dist., Seima BCA, 12°18'08"N 107°03'08"E
FMNH 262901	HKV 65920	Cambodia, Mondolkiri Prov., O'Rang Dist., Seima BCA, 12°18'08"N 107°03'08"E
ROM 39862	•	Vietnam, Gia Lai Prov., An Khe Dist., Krong Pa, 14°20'38"N 108°28'45"E
ROM 39901 paratype	•	Vietnam, Gia Lai Prov., An Khe Dist., Tram Lap, 14°26'39"N 108°32'97"E
ROM 39913 paratype	•	Vietnam, Gia Lai Prov., An Khe Dist., Tram Lap, 14°26'39"N 108°32'97"E
Odorrana chloronota		
BMNH 1947.2.28.6 syntype	•	India, Darjeeling, [26°45'00"N 88°15'00"E]
FMNH 268916	20887	Thailand, Krabi Prov., Khao Phanom Bencha NP, 08°14'26"N 98°54'51"E
THNHM uncataloged	50942	Thailand, Krabi Prov., Khao Phanom Bencha NP, 08°14'26"N 98°54'51"E
FMNH 263418	THNHM 4451	Thailand, Prachuap Kirikhan Prov., Hua Hin Dist., Kaeng Krachan NP, 12°32′16″N 99°27′41″E
FIMINE 2034.17	1 HINHIM 4452	i naliand, Prachuap Kiriknan Prov., Hua Hin Dist., Kaeng Krachan NP, 12 32 16 N 99 2/41 E

Locality	Thailand, Prachuap Kirikhan Prov., Hua Hin Dist., Kaeng Krachan NP, 12°32'16"N 99°27'41"E Thailand Prachuan Kirikhan Prov. Hua Hin Dist. Kaeng Krachan NP, 12°32'16"N 99°27'41"F	יומומונט, ו ומכוומקר אוואומון ו יסיי, ווממ יווו בוסני, ואמכוק ואמכומון או , וב טב וטוא 30 בי דו ב	Thailand, Loei Prov., Phu Rua Dist., Phu Luang WS, 17°20'02.8"N 101°30'32.4"E	Loei Prov., Phu Rua Dist., Phu Luang WS, 17°15'32.4"N	Loei Prov., Phu Rua Dist., Phu Luang WS, 17°15'32.4"N	Phu Rua	Loei Prov., Phu Rua	Thailand, Loei Prov., Phu Rua Dist., Phu Luang WS, 17°21'05.5"N 101°30'13.4"E	Thailand, Loei Prov., Phu Rua Dist., Phu Luang WS, 17°21'05.5"N 101°30'13.4"E		Thailand, Krabi Prov., Khao Phanom Bencha NP, 08°14'26"N 98°54'51"E	Thailand, Krabi Prov., Khao Phanom Bencha NP, 08°14'26"N 98°54'51"E	Thailand, Ranong Prov., Namtok Ngao NP, [09°56′N 98°43′E]	Thailand, Ranong Prov., Namtok Ngao NP, [09°56′N 98°43′E]	Thailand, Ranong Prov., Namtok Ngao NP, [09°56'N 98°43'E]	Ranong	Prov., Namtok Ngao NP,	Prov., Namtok Ngao NP,	Prov., Namtok Ngao NP, [09°56'N	Prov., Namtok Ngao		Thailand, Ranong Prov., Namtok Ngao NP, [09°56′N 98°43′E]	Thailand, Prachuap Kirikhan Prov., Hua Hin Dist., Kaeng Krachan NP, 12°32'16"N 99°27'41"E	Thailand, Prachuap Kirikhan Prov., Hua Hin Dist., Kaeng Krachan NP, 12°32'16"N 99°27'41"E	Myanmar, Thagata Juwa, [16°11′00"N 98°31′39"E]		Loei Prov., Phu Rua Dist., Phu Luang WS, 17°16'48.7"N	Phu Rua Dist., Phu Luang WS, 17°16'48.7"N	Thailand, Loei Prov., Phu Rua Dist., Phu Luang WS, 17°16'48.7"N 101°31'07.5"E	Thailand, Loei Prov., Phu Rua Dist., Phu Luang WS, 17°15′32.4"N 101°30′22.8"E	[⊃] hu Luang WS, 17°15'32.4"N		Vietnam, Gia Lai Prov., An Khe Dist., Buon Loi, 14°20'N 108°36'E	Vietnam, Gra Lai Flov., All Kije Dist., Buom Loi, 14 Zoly 100 30 E Cambodia, Ratanakiri Prov., Ta Veng Dist., Virachey NP, 14°11'16.3"N 107°17'36.1"E
Field/Tissue No.	THNHM 4493 THNHM 4495		HKV 65996	HKV 66219	HKV 66222	HKV 66258	HKV 66273		HKV 66278		50903	50904	66734	66735	66739	66761	66763	66764	66765	29299		6219	THNHM 4453	THNHM 4494			HKV 66206	HKV 66207	HKV 66209	HKV 66217	HKV 66220		HKV 60014	HKV 65441
Voucher	FMNH 263416 THNHM 09970	Odorrana cf. chloronota	FMNH 265929	FMNH 265931	FMNH 265932	FMNH 265934	FMNH 265939	FMNH 265942	FMNH 265944	Odorrana hosii	THNHM uncataloged	FMNH 268778	FMNH 268244	FMNH 268247	FMNH 268253	FMNH 268246	FMNH 268252	FMNH 268250	FMNH 268249	FMNH 268245	Odorrana livida	FMNH 268251	FMNH 263415	THNHM 09971	BMNH 1889.3.25.48 neotype	Odorrana cf. livida	FMNH 265919	FMNH 265920	FMNH 265922	FMNH 265923	FMNH 265925	Odorrana moratkai	FMNH 253837 topotype	FMNH 262871

Locality	Malaysia, Sarawak, Bukit Sarang, 02°39'12.2"N 113°03'09"E		Indonesia, Sumatra Barat, Padang, Limau Manis, 00°55'S 100°28'E	Indonesia, Sumatra Barat, Padang, Limau Manis, 00°55'S 100°28'E	Indonesia, Sumatra Barat, Padang, Limau Manis, 00°55'S 100°28'E	Indonesia, Sumatra Barat, Padang, Limau Manis, 00°55'S 100°28'E	Indonesia, Sumatra Barat, Padang, Sikayan Ubi, 00°53'S 100°28'E	Indonesia, Sumatra Barat, Padang, Sikayan Ubi, 00°53'S 100°28'E		Malaysia, Kedah, Gunung Jerai, Lower Tupah River, 05°47'N 100°26'E	Malaysia, Kedah, Gunung Jerai, Lower Tuupah River, 05°47'N 100°26'E	Malaysia, Kedah, Gunung Jerai, Perigi Cascade, 05°47'N 100°26'E		Malaysia, Kedah, Gunung Jerai, Batu Hampar River, 05°47'N 100°26'E	Malaysia, Kedah, Gunung Jerai, Batu Hampar River, 05°47'N 100°26'E	Malaysia, Kedah, Gunung Jerai, Batu Hampar River, 05°47'N 100°26'E	Malaysia, Kedah, Gunung Jerai, Batu Hampar River, 05°47'N 100°26'E		Malaysia, Sarawak, Bukit Sarang, 02°39'12.2"N 113°03'09"E								
Field/Tissue No.	51640	51216	51234	51242		15560	15568	15575	15664	15987	15994		JS 00327	JS 00330	JS 00497		JS 00381	JS 00390	JS 00408	JS 00393		51158	51198	51244	51259	51261	51310
Voucher	FMNH 268983	FMNH 267822	FMNH 267824	FMNH 267825	Rana cf. chalconota B5	FMNH 268573	FMNH 268574	FMNH 268575	FMNH 268580	FMNH 268585	FMNH 268586	Rana labialis	FRIM 1539	FRIM 1735	FRIM 829	Rana cf. Iabialis	FRIM 1736	FRIM 1418	FRIM 826	FRIM 1401	Rana raniceps	FMNH 267958	FMNH 267960	FMNH 267961	FMNH 267962	FMNH 267963	FMNH 267964

Table 2. Oligonucleotide primers used to amplify and sequence frog mitochondrial DNA in this study. 'L' and 'H' refer to light and heavy strands, respectively. 'A' and 'S' refer to amplifying and sequencing, respectively.

Primer	Product	Use	Sequence
L-COXIII	ND3	A, S	5'-CCGCATGATACTGACACTT-3'
Arg-HND3III	ND3	A, S	5'-AACTGTCTTTTTTGGACTAGC-3'
Met-LND2	ND2	A, S	5'-CAATGTTGGTTAAAATCCTTCC-3'
Trp-HND2	ND2	A, S	5'-AGGCTTTGAAGGCCTTTGGTC-3'
L-ND2am	ND2	S	5'-TTATAGCCTTCTCCTCCATCG-3'
H-ND2bana	ND2	S	5'-CTATGATTTTTCGAAGTTGAG-3'
H-ND2dhsxs	ND2	S	5'-ATTCATCCTAGGTGGCCGAT-3'
L-ND2hos	ND2	S	5'-ACTTCGGAAAATTATAGCCTTCTC-3'
H-ND2hos	ND2	S	5'-GAGAAATATGGCGGTTGTCAT-3'
L-ND2pdd	ND2	S	5'-CGGAATTGGCCAAACTCAAC-3'
H-ND2pdd	ND2	S	5'-TTTGGGTCATGAGGTTGATA-3'
L-ND2thai	ND2	S	5'-CAACTTCGAAAGATCATAGC-3'
L-ND2xs	ND2	S	5'-GACTGATCTTATCGACCTGAC-3'
L-16SRanaIII	16S	A, S	5'-GAGTTATTCAAATTAGGCACAGC-3'
H-16SRanaIII	16S	A, S	5'-CATGGGGTCTTCTCGTCTTAT-3'
H-R20016S	16S	A, S	5'-TTCTTGTTACTAGTTCTAGCA-3'
L-R14016S	16S	A, S	5'-AATTACTAAAACATCCACTAACCC-3'
H-R37516S	16S	A, S	5'-TAAAATTTGCCGAGTTCCTTC-3'
H-R48016S	16S	A, S	5'-CAAGTGATTATGCTACCTTCG-3'
L-R21516S	16S	A, S	5'-AGTAACAAGAACTTGCCATTC-3'
L-R43016S	16S	A, S	5'-CTGTTTACCAAAAACATCGCCT-3'

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