

Molecular Phylogeny of the Genus *Acanthosaura* (Agamidae)

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Abstract: A 900 bp fragment of mitochondrial cytochrome b was sequenced from 63 specimens of the arboreal agamid lizard genus *Acanthosaura* from Vietnam, Laos, Myanmar (Burma), Thailand and Malaysia, representing all four currently recognized species. A hypothesis of maternal relationships was generated based on a maximum parsimony analysis of 44 different haplotypes. *Pseudocalotes* and *Calotes* were used as outgroup taxa. The genealogical analyses of the cytochrome b sequences recovered four lineages represented by *A. armata*, *A. capra*, *A. crucigera*, and *A. lepidogaster*. A fifth lineage consisted of one specimen from Ngoc Linh, Vietnam that was considered to be *A. lepidogaster*; however, recognizing it as such would render *A. lepidogaster* paraphyletic. *Acanthosaura crucigera* consisted of two clades. One of these clades contains cysteine in a portion of its cytochrome b, and is the sister group of all other species of *Acanthosaura*. In turn, the second clade of *A. crucigera* formed the sister group of *A. armata*, *A. capra*, and *A. lepidogaster*. A clade containing *A. armata*, *A. capra* and the sample from Ngoc Linh, Vietnam was the sister group to *A. lepidogaster*.

Key words: *Acanthosaura*; Agamidae; Vietnam; Thailand; Laos; Molecular phylogeny; Cytochrome b

INTRODUCTION

Acanthosaura Gray 1831 is a genus of arboreal agamids distributed in southern China,

Vietnam, Thailand, western Malaysia, including Pulau Pinang and Pulau Tioman, Laos, Cambodia, Myanmar, and Indonesia (Sumatra and Anamba) (Manthey and Crossmann, 1997). These lizards are usually restricted to evergreen forests at elevations up to 1800 m, and prefer riparian habitats.

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The genus contains four species. The relatively most common species, *A. lepidogaster*, occurs in northern Thailand, Vietnam, Cambodia, Myanmar, Laos, and southern China (Yunnan, Guangxi, Guangdong, Fujian, Hainan). *A. capra* is known from Cambodia, Laos and Vietnam. *A. armata* is distributed in southern Thailand north to the Nakhon Si Thammarat Province, western Malaysia as well Palau Pinang and Pulau Tioman, and Indonesia (Sumatra). Finally, *A. crucigera* was recorded from Myanmar, Thailand, Central Vietnam (Annam), West Malaysia, and Cambodia (Boulenger, 1885; Smith, 1935; Taylor, 1963; Manthey and Grossmann, 1997). The genus has not undergone any recent taxonomic re-evaluation. The phylogenetic relationships of the species have never been evaluated.

The taxonomic status of the genus *Acanthosaura* is unclear. Some authors have treated it as a junior synonym of *Gonocephalus* (Smith, 1935); and others have referred some species to the genus *Japalura* (Boulenger, 1890). Several described species have been synonymized with *Acanthosaura lepidogaster* (Wermuth, 1967). Recently, most herpetologists have recognized *Acanthosaura* as containing the four species *armata*, *capra*, *crucigera*, and *lepidogaster* (Manthey and Schuster, 1992; Ananjeva, 1997). Although this association was partly supported by recent molecular data (Macey et al., 2000), the taxonomy of the genus and relationships among its species remain obscure. The problem derives from the great anatomical similarity of the taxa. Variability in the diagnostic characters, such as length of the nuchal and postorbital spines, and length of the diastema between nuchal and dorsal crests, causes much taxonomic confusion. The only species easily diagnosed is *A. capra*. It differs from the other species of *Acanthosaura* by having only one pair of postorbital spines and no nuchal spines.

Relatively few specimens of *Acanthosaura* (except *A. lepidogaster*) exist in museum collections because of a limited historical collecting. The paucity of specimens precludes

statistically based analyses of morphological variation and relationships within this genus. Consequently, we pursued a molecular evaluation of the relationships of the matriarchic lineages among samples of *Acanthosaura*. Mitochondrial DNA (mtDNA) is commonly used in population and evolutionary systematics (Hillis et al., 1996). We chose cytochrome b (cytb) because of its applicability in relatively recent divergences (Wilson et al., 1985; Moritz et al., 1987; Harrison, 1989). Our investigation crosses the boundary between micro- and macroevolution because we investigated multiple individuals from many collecting sites, and all four recognized species.

MATERIALS AND METHODS

Blood or liver tissues from 63 individuals representing all four currently recognized species were obtained from throughout the range of *Acanthosaura* (Fig. 1; Appendix). The agamids *Pseudocalotes brevipes* and *Calotes versicolor* were used as the outgroup taxa. Choice of outgroup taxa is based on phylogenetic relationships revealed by Macey et al. (2000).

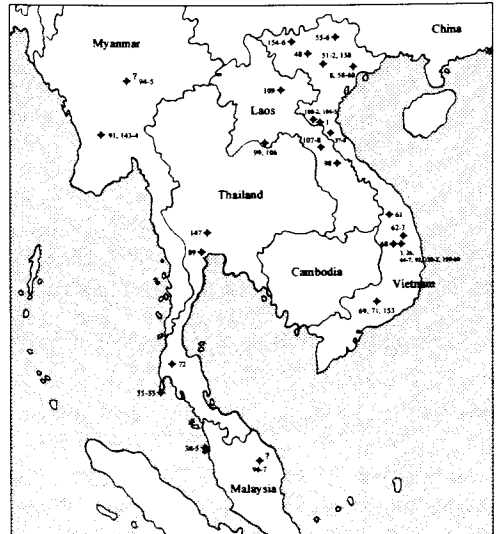


FIG. 1. Map showing the distribution of the genus *Acanthosaura* and localities sampled in this study.

Laboratory protocols

DNA was extracted from liver and blood tissues following standard proteinase k and phenol chloroform protocols (Sambrook et al., 1989). A fragment of mtDNA containing most of *cyt-b* and tRNA^{threonine} was amplified from 1–6 µl aliquots of isolated DNA using the polymerase chain reaction (PCR). The light and heavy strand primers used were modified versions of those given by Kocher et al. (1989) (Table. 1). Amplification conditions were as follows: after an initial denaturation at 94 C for 300 s, 31 cycles followed with a denaturation at 94 C for 45 s, annealing at 42–45 C for 45 s, and extension at 70 C for 120 s. Cycle sequencing reactions used a two step program: 15 cycles followed with denaturation at 94 C for 45 s, annealing at 47–53 C for 45 s, extension at 70 C for 60 s, and 15 cycles of denaturation at 94 C for 45 s and extension at 60 C for 60 s. Three sequencing primers were used (the light strand primer *mt-a*, and two heavy strand primers, *smt-f* and *mt-b2*) to obtain sequences of both strands using an automatic sequencer (ALF Express). Sequencing was performed for 5–11 hr depending on the length of the fragment. Each sequence was verified by sequencing from heavy and light strand primers with a large overlap of the segments and from different PCR amplification products.

Phylogenetic analysis

Alignment of the *cyt-b* sequences was

achieved manually. The base composition, transition/transversion ratio, genetic distances, and phylogenies were estimated using PAUP* (ver. 4.0b10; Swofford, 2000).

Phylogenetic relationships are generated by maximum parsimony (MP) (Siddall and Kluge, 1997; Kluge, 2002). All trees were calculated using PAUP*, and were constructed using heuristic searches with tree bisection-reconnection (TBR) branch swapping and saving all most parsimonious trees (MPTs). Addition-sequence replication was performed 1000 times holding 10 trees at each step. Only potentially phylogenetically informative sites were used in the analysis, and the starting tree was obtained by random stepwise addition. All characters were evaluated as unweighted. Constraint trees were constructed using MacClade (ver. 4.00; Maddison and Maddison, 2000).

Nodal consistency was assessed by decay analysis (Bremer, 1994) and nonparametric bootstrap analysis with 10,000 pseudoreplicates. Decay analysis was calculated using AutoDecay ver. 4.0 (Eriksson, 1998). For each bootstrap replicate, two heuristic searches were performed with closest addition of taxa.

A suite of preferred trees was obtained by using successive approximations weighting (Farris, 1969; Carpenter, 1994). Reweighting was based on the retention index (Farris, 1989) using the best fit (maximum value) and a base weight of 1. The data were reweighted until a consistent tree length was obtained. Following

TABLE 1. Primer sequences (5'–3') of cytochrome *b* used for amplifying DNA from agamid lizards of the genus *Acanthosaura*. Positions in the chicken genome corresponding to the 3' end positions of primers are given in parentheses. L and H are heavy-strand and light-strand DNA, respectively.

Primer	Sequence
smt A (L-14995)	5'-CAACATCTCAGCATGATGAAACTTCG-3'
mt A-new (L-14995)	5'-TCCCAGCCCCATCCAACATCTCAGCATGATGAAACTTCG-3'
mt C (L-15311)	5'-GCAAGTCTTCTACCATGAGGACAAATATC-3'
mt E (H-15698)	5'-AATAGGAAGTATCATTCCGGTTTTGT-3'
mt F-new (H-16060)	5'-AGGGTGGAGTCTTCAGTTTTTGGTTTACAAGACCAATG-3'
smt F (H-16060)	5'-TCAGTTTTTGGTTTACAAGACCAATG-3'
mt B2 (H-15298)	5'-GCCCAGAakGATATTTGTCTCA-3'

analysis, data weights were reset to 1, and the suite of trees was sorted for length. Consensus trees (strict and 50% majority rule) were constructed from the suite of equally most parsimonious trees.

RESULTS

A 900 bp fragment of *cyt-b* was obtained from most of the 63 samples of *Acanthosaura*. Multiple specimens represented most sampling localities. Some individuals had identical sequences. These were combined for analysis resulting in 44 different mtDNA haplotypes from *Acanthosaura*. Homologous sequences from *Calotes versicolor* and *Pseudocalotes brevipes* were obtained and used for the analyses. The sequences are deposited in GenBank (Accession Numbers AY572869–AY572930). Although the fragment was completely sequenced for most individuals, 716–720 bp were obtained for *A. capra* (A150–152, A159–160), 688–720 bp from *A. crucigera* (A69, A71, A153), and 684–704 bp from *A. lepidogaster* (A154–156, A158).

Authenticity of the mtDNA

None of the sequences contained premature stop codons, insertions, or deletions, and therefore do not appear to be nuclear copy pseudogenes. The amino acid sequences identified regions that are conserved among vertebrates. These regions corresponded to those of other animals (Howell, 1989; Irwin et al., 1991), suggesting that these sequences represent functional copies that encode a protein. The strong bias against guanine on the light strand found in all analyzed sequences (A=28.3–30.9%, C=30.1–32.0%, T=25.4–27.6%, G=12.0–14.3%) was characteristic of the mitochondrial genome (Kocher et al., 1989).

Substitutions and genetic variation

Among the 900 bp of *cyt-b* sequenced, 483 positions were variable and 381 were potentially phylogenetically informative. As expected, the most variable sites occurred in the third

codon position ($n=274$), less variation occurred at the first position ($n=127$), and little variation was observed at the second position ($n=82$).

Absolute pairwise genetic distances of potentially phylogenetically informative sites only were calculated for all sequences of *Acanthosaura*. The amount of substitution varied from no site changes between individuals of a population to 118–219 sites between species. Between *Acanthosaura* and the outgroup taxa, differences occurred at 209–270 sites. The sequences of *Calotes* and *Pseudocalotes* differed at 252 sites. The transition/transversion ratio among the species of *Acanthosaura* varied from 12:1 to near 1:1, although the mean was 2.48:1. As expected, the transition/transversion ratio was higher among taxa near the terminal branches of the tree, and lower near the base of the tree.

Phylogenetic analysis

The unweighted maximum parsimony analysis of 1000 random additions of taxa resolved 562 trees, each with a length of 1296 steps, CI=0.48, RI=0.80, and RC=0.38. A 50% majority rule consensus tree is shown in Fig. 2. Most nodes were consistently resolved; 26 of 36 nodes had a BSP of 100%, seven ranged from 74–97%, and only three were supported by 52–55%. The data appeared to have significant structure.

Successive approximation weighting achieved a stable tree length after nine iterations. The maximum parsimony evaluation of the reweighted data resolved 231 trees. When character weights were reset to a value of one, tree lengths varied from 1296–1308 steps. Fifty-four trees had the same length as those obtained in the unweighted analysis; the strict consensus and the 50% majority rule consensus trees based on these trees were identical to each other, and to the 50% majority rule consensus tree based on the unweighted data (Fig. 2), except that the node uniting *A. lepidogaster* A55–56 with the clade (A8, A58, A60, A59) collapsed. Weighting first and second codon positions twice as much as the

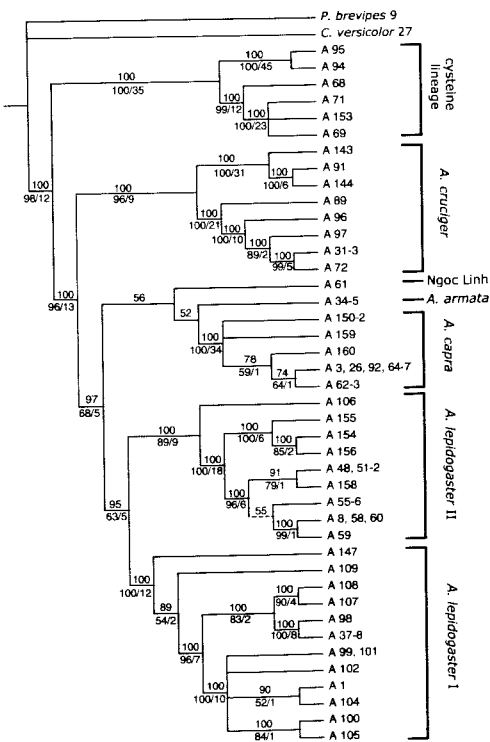


FIG. 2. A majority-rule consensus tree of 562 maximum parsimony trees depicting the maternal lineages among populations of lizards of the genus *Acanthosaura* derived from the analysis of cytochrome b sequence data. Numbers above the line are the consensus values and those below are decay values and bootstrap proportions. The dashed line connecting specimens within a clade of *A. lepidogaster* represents the single collapsed node obtained from successive approximations weighting, and from weighting first and second codon positions twice as much as the third. Specimen numbers refer to individuals in the Materials and Methods section of this paper.

third obtained the identical result of 54 most parsimonious trees and the same consensus trees.

Multiple individuals from the same sampling locality always clustered together, reflecting a shared historical maternal lineage. As expected, there was a high degree of concordance between geography and maternal history.

Within *Acanthosaura*, several maternal lineages could be clearly defined. Four lineages

represented *A. crucigera*, *A. lepidogaster*, *A. armata*, and *A. capra*. A fifth lineage consisted of *A. lepidogaster* from Ngoc Linh, Vietnam (specimen A61). This sample from the Central Highlands made *A. lepidogaster* paraphyletic. A sixth lineage was formed by paraphyletic *A. "crucigera"* (Fig. 2). One lineage of *A. crucigera* (A68-69, A71, A94-95, and A 153) was the only group to have the amino acid cysteine in a portion of its cyt-b; this "cysteine lineage" is the sister group of all other *Acanthosaura*. The other lineage of *A. crucigera* is, in turn, the sister group of *A. lepidogaster*, *A. capra* and *A. armata* (Fig. 2).

The decay analysis found substantial support for nodes at the base of the tree. For example, 35 steps supported monophyly of the cysteine group, and 13 additional steps were required to break the monophyly of *Acanthosaura* exclusive of the cysteine group. Whereas the base of the tree exhibited great stability, more terminal groups that consisted of multiple specimens from nearby collecting sites did not receive much support.

High bootstrap proportions supported many nodes. These included all nodes resolved within the cysteine lineage of *A. crucigera*, the monophyly of *A. capra*, and the two groups of *A. lepidogaster*. Monophyly of *A. capra*, and the cysteine lineage was supported by a BSP of 100% (Fig. 2). A monophyletic *A. crucigera* (exclusive of the cysteine lineage) was supported by a BSP of 96%. A monophyletic *A. lepidogaster*, exclusive of A61, was supported by a BSP of 63%, and both maternal lineages of *A. lepidogaster* received high BSP supports of 100% and 89% (Fig. 2). Both "A61" and *A. armata* were each represented by a single unique sequence. Thus, their monophyly cannot be evaluated.

Acanthosaura lepidogaster, exclusive of A61, consists of two distinct subgroups. One, "lepidogaster I", includes specimens from Laos, north-central Vietnam, and Thailand. The second subgroup, "lepidogaster II", includes specimens from northern and north-eastern Vietnam. The maternal genealogy resulted in limited geographic mixing. One

specimen of *A. lepidogaster* (A106) from Bolikhamxay Province, Laos (Fig. 1) consistently clusters with "*lepidogaster* II" (Fig. 2).

A single specimen of *A. lepidogaster*, A61, usually maintained its association with the clade of *A. armata* and *A. capra*. Specimen A61 did not cluster with other *A. lepidogaster* in any of the MPTs, even when it was combined with *A. armata*. However, a monophyletic lineage of *A. lepidogaster*, including A61 and excluding *A. armata*, requires only three additional steps on the MPT. This reduces to two additional steps if A61 and *A. armata* are a clade that forms the sister group to *A. lepidogaster*.

Acanthosaura crucigera, apart from the cysteine lineage, is clearly monophyletic. The genetically most distinctive population evaluated is from northern Myanmar (Bago Yoma, A91 and A143). Forcing *A. crucigera* to be a monophyletic species, while allowing for all other possible arrangements to occur on the tree, requires nine additional steps on the MPT.

DISCUSSION

Phylogenetic relationships within the genus Acanthosaura

All maximum parsimony evaluations resolved extremely similar branching patterns. Macey et al. (2000) based on an analysis of sequences from several mtDNA genes, including ND1, ND2, COI and eight intervening tRNA genes, found that the two species of *Acanthosaura* in their investigation shared a common ancestor. Their survey of taxa was not sufficient to document the monophyly of all species of *Acanthosaura*. Unfortunately, the diversity of outgroup species in our dataset was insufficient to cladistically test this hypothesis. However, given the observed distances among our ingroup and outgroup taxa, we are very confident in the monophyly of *Acanthosaura*.

We consistently resolved a single ancestral maternal lineage for the four, long-recognized species and at least two not previously known. A single specimen from Ngoc Linh, Vietnam

that strongly resembles *A. lepidogaster*, A61, represents one newly discovered lineage. The sixth, the "cysteine lineage," is the sister group of all other *Acanthosaura*. Conservatively, each of the six lineages represents at least one species. Support for monophyly of the six lineages was considerable. High BSPs supported the four lineages represented by multiple specimens. In addition, each of the six major lineages was supported by long branch lengths on the trees.

The cladogenic pattern among species in the "*crucigera*" lineage reflect a classic pattern of dispersal (Murphy and Aguirre-Léon, 2002), in this instance southward. The oldest cladogenic event isolated maternal lineages in northern Myanmar (A91, A1443-144, Bog Yoma) and more recent events occurred further south.

The extent of sequence divergence within *A. lepidogaster* forms an interesting problem for future investigation. Specimen A61, from Ngoc Linh Mountain, Vietnam, did not cluster with other specimens of this species. Although geographically closer to some other specimens, it was very divergent in its cyt-b sequences (ca. 16% divergence, or an average of 144 nucleotide sites). Certainly, percent divergence cannot be used as an objective arbitrator for defining species (Frost and Hillis, 1990; Wake and Schneider, 1998). However, this high level of sequence divergence is indicative of speciation. Equally interesting, the two most divergent clades of *A. lepidogaster* exclusive of A61 also differ substantially. The average divergence of 12% generally exceeds that known to occur among individuals of other vertebrate species. This suggest that either two additional cryptic species have been combined in the name *A. lepidogaster*, or these lizards are a goldmine for future studies of evolutionary genetics. Sampling in the contact zone of the two haplotypes is critical.

The *A. capra* and *A. armata* lineages are well defined by the sequence data. They differ from one another at an average of 16% of the total nucleotide sites. The absence of clear cladistic relationships among samples of *A.*

capra reflects restricted sample sites—all 15 specimens are from two adjacent localities.

Taxonomic implications

The cysteine lineage includes *A. "crucigera"* from Krong Pa, Vietnam (A68), Cat Tien National Park, Vietnam (A69, 71 and 153), and pet trade specimens reputed to be from Myanmar (A94 and A95). The cysteine lineage can be diagnosed from other species of *Acanthosaura* using molecular characters; for example, it differs from other species by more than 20% of the amino acids in the sequenced fragment. Monophyly and the ability to diagnose are required operational criteria for recognition of species under any phylogenetic based species concept (e.g., Cracraft, 1989; Nixon and Wheeler, 1990; Brooks and McLennan, 1999, 2002). Our preliminary evaluations reveal that morphological differences separate the specimens of the two major "cysteine" subgroups from other species of *Acanthosaura*. Furthermore, the two specimens of *Acanthosaura* putatively from Myanmar cannot be morphologically associated with any known species. The cysteine lineage could consist of at least three species, considering the extent of divergence that occurs within Vietnam alone.

Although specimen "A61" from Ngoc Linh, Vietnam was initially identified as *A. lepidogaster*, this determination is now questionable. In most trees based on equally weighted data, it formed the sister group to *A. armata* plus *A. capra* (Fig. 2). These cladogenic resolutions require recognizing this population as different from *A. lepidogaster*. In support of this conclusion, some morphological characteristics separate A61 from other species of *Acanthosaura*. However, additional specimens from Ngoc Linh are desirable before describing the species. This finding is not particularly surprising given the high level of endemism that characterizes the fauna of primary forests of the Annam Mountains, including Ngoc Linh. (Darevsky and Orlov, 1994, 1997).

The two genetically distinct maternal lineages of *A. lepidogaster* are not diagnosable

morphologically. Even though the maternal lineages are distinct, they might not be separate species. Gene flow may be ongoing where the two lineages come in contact. The extent of mtDNA divergence does not necessarily indicate an absence of gene flow. For example, in the lizard genus *Urosaurus*, historical lineages in Baja California, Mexico differ by 13% divergence in *cyt-b* (Johan Lindell, personal communication), and yet a cladistic allozymic study infers ongoing gene flow among the maternal lineages (Aguirre-Léon et al., 1999). Indeed, in the genetically best-studied geographic region, the central region of the peninsula of Baja California, Mexico, multiple species average 7–9% divergence in mtDNA without any indication of the absence of gene flow (Murphy and Aguirre-Léon, 2002; Riddle et al., 2000). Consequently, in the absence of morphological differentiation, nuclear gene analysis is required to document the presence of multiple cryptic species of *A. lepidogaster*. On a broader scale, our evaluation of mitochondrial *cyt-b* sequences revealed that at least seven lineages of *Acanthosaura* could be considered as discrete species, including two species within the cysteine lineage. Detailed morphological work or nuclear DNA data is needed to independently corroborate the hypothesis that these lineages represent separate species.

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APPENDIX

List of vouchers used in this study and GenBank accession numbers of sequences. ZISP=Zoological Institute, St. Petersburg, Russia; ROM=Royal Ontario Museum, Toronto, Canada; CAS=California Academy of Science, San Francisco, USA; FMNH=The Field Museum, Chicago, USA; HLMD=Hessisches Landesmuseum, Darmstadt, Germany; PCUM=private collection of Ulrich Manthey, Berlin, Germany; PCUJ=private collection of Ulrich Joger; IRSNB=Royal Belgian Institute of Natural Sciences (Institut Royal des Sciences Naturelles de Belgique).

Acanthosaura armata

A34, A35-PCUM, Pulau Pinang (=Penang), Penang State, West Malaysia: 5°54'N, 100°12'E.

Acanthosaura capra

A3-ROM32160, A26-ROM32167, A64-ROM31984, A65-ROM32154, A66-ROM32155, A67-ROM32160, A92-ROM31983, A150-ROM32161, A151-ROM32152, A152-ROM32162, A159-ROM32143, A160-ROM32166, Krong Pa, Gia Lai Province, Vietnam: 14°20'29"N, 108°28'40"E.; A62-30627, A63-ROM30628, Tram Lap, Gia Lai Province, Vietnam: 14°26'24"N, 108°22'59"E.

Acanthosaura crucigera

A31, A32, A33-PCUM, Khao Lak, District (Amphoe) Takua Pa, Province Phang Nga, Thailand: 8°55' 8°65'N, 98°14' 98°45' E. A72-IRSNB15141, Phang Nga Wildlife Breeding Center, Tabon Tahk Daed, Thai Muang District, Phang Nga Province, Southern Thailand: 8°72'N, 98°27'E. A89-Kaeng Krachan, Kao Yoi Districts, Phetchaburi Province, Central Thailand: 13°99'N, 100°25'E (tissue sample and photo from Peter P. Van Dijk). A91-CAS206626, A143-CAS208426, Bago Division, Bago Yoma, E. Myanmar: 18°52'59"N, 95°52'44.9"; A144-CAS206626, Bago Division, Bago Yoma, E. Myanmar: 18°81'67"N, 95°08'50.4"; A96, A97-per trade, Malaysia.

Acanthosaura lepidogaster

A1-ROM26328, Khe Moi River, 24 km (by road) W of Con Cuong Village, Nghe An Province, Vietnam: 18°56'18"N, 104°53'01"E; A8-ROM31954, A58-ROM31957, A59-ROM31960, A60-ROM35038, Chi Linh, Hia Duong Province, Vietnam: 21°12'44"N, 106°28'39"E; A-37-ZISP20753-1, A38-ZISP20753-2, Northern Annam, Vu Quang, Ha Tinh Province, Vietnam: 18°16'N, 105°15'E; A48-ROM30503, A51-ROM30720, A52-ROM30694. A158-ROM30693, Tam Dao, Vinh Phu Province, Vietnam: 21°27'25"N, 105°38'48"E; A55-ROM36073, A56-ROM36075, Quang Thanh, Cao Bang Province, Vietnam: 22°37'43"N, 105°54'46"E; A98-FMNH255481, Boualapha District, Khammouane Province, Laos: 17°17'N, 105°41'E; A99-FMNH255582, A101-FMNH255583, A104-FMNH255581, Nghe An, Con Cuong District, Vietnam: 18°58'N, 104°48'E; A100-FMNH255585, A102-FMNH255587, A105-FMNH255584, Nghe An, Tuong Duong

District, Vietnam: 19°03'N, 104°37'E; A103-FMNH255490, A109-FMNH255489, Vieng Tong District, Hauphan Province, Laos: 20°14'N, 103°12'E; A106-FMNH255491, Thaphabat District, Bolikhamxay Province, Laos: 18°27'N, 103°10'E; A107-FMNH255488, A108-FMNH255487, Nakai District, Khammouane Province, Laos: 17°53'N, 104°55'E; A147-PCUM, Khao-Yoi, Thailand: 13°22'N, 99°81'40"E; A154-ROM38117, vicinity of Sa Pa, ~4 km W of Sa Pa Village., on tributary of Golden River (=Suoi Vang), Lao Cai Province, Vietnam: 22°18'59"N, 103°49'16"E.; A155-ROM38115, A156-ROM38116—vicinity of Sa Pa, ~5 km SW of Sa Pa Village., Lao Cai Province, Vietnam: 22°18'56"N, 103°49'35"E.

Acanthosaura sp.

A61-ROM37082, Ngok Linh, Kon Tum Province, Vietnam: 15°05'08"N, 107°55'42"E.

A153-ROM42241, A71-ROM42240, A69-ROM37083, Dong Nai, Cat Tien National Park, Dong Nai Province, Vietnam: 11°25'23"N, 107°25'42"E.

A68-ROM31985, Krong Pa, Gia Lai Province, Vietnam: 14°20'29"N, 108°28'40"E.

A94, A95-HLMD-RA2969-26970. per trade, Myanmar.

Calotes versicolor

A27-HLDM 57—per trade, Vietnam

Pseudocalotes brevipes

A9-ROM 30515, Pac Ban, Na Hang Nature Reserve, Tuyen Quang Province, Vietnam: 22°25'05"N, 105°38'05"

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