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A phylogeny and evolutionary natural history of mesoamerican toads (Anura: Bufonidae: *Incilius*) based on morphology, life history, and molecular data

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Abstract

We combine mitochondrial and nuclear DNA sequence data with non-molecular (morphological and natural history) data to conduct phylogenetic analyses and generate an evolutionary hypothesis for the relationships among nearly every species of Mesoamerican bufonid in the genus *Incilius*. We collected a total of 5,898 aligned base-pairs (bp) of sequence data from mitochondrial (mtDNA: 12S–16S, cyt b, ND2–CO1, including tRNAs$^{TRP–TYR}$ and the origin of light strand replication; total 4,317 bp) and nuclear (CXCR4 and RAG1; total 1,581 bp) loci from 52 individual toads representing 37 species. For the non-molecular data, we collected 44 characters from 29 species. We also include *Crepidophryne*, a genus that has not previously been included in molecular analyses. We present results of parsimony and Bayesian analyses for these data separately and combined. Relationships based on the non-molecular data were poorly supported and did not resolve a monophyletic *Incilius* (*Rhinella marina* was nested within). Our molecular data provide significant support to most of the relationships. Our combined analyses demonstrate that inclusion of a considerably smaller dataset (44 vs. 5,898 characters) of non-molecular characters can provide significant support where the molecular relationships were lacking support. Our combined results indicate that *Crepidophryne* is nested within *Incilius*; therefore, we place the former in the synonymy of the latter taxon. Our study provides the most comprehensive evolutionary framework for Mesoamerican bufonids (*Incilius*), which we use as a starting point to invoke discussion on the evolution of their unique natural history traits.

Key words: Amphibia, *Crepidophryne*, natural history, phylogeny, taxonomy

Resumen

Combinamos datos moleculares de DNA mitocondrial y nuclear con datos morfológicos y de historia natural para realizar análisis filogenéticos y proponer hipótesis para esclarecer las relaciones filogenéticas entre especies de bufonidae dentro del género *Incilius* provenientes de Mesoamérica. Colectamos un total de 5,898 pares de bases (bp) de secuencias alineadas de loci mitocondriales (mtDNA: 12S–16S, cyt b, ND2–CO1, incluyendo tRNAs$^{TRP–TYR}$ y el origen de la replicación de la cadena liviana; totales 4,317 bp) y nucleares (CXCR4 y RAG1; totales 1,581 bp), obtuvimos loci de 52 ejemplares de sapos que representan 37 especies. Para los datos no moleculares, registramos 44 caracteres de 29 especies. Incluimos al género *Crepidophryne*, que nunca ha sido usado en análisis moleculares previos. Presentamos los resultados de los análisis Bayesianos y de parsimonia que realizamos combinando los datos y luego separándolos. Las relaciones resultantes basadas sólo en datos no-moleculares no son claras y no presentan al género *Incilius* como un grupo monofilético (*Rhinella marina* resulta dentro del grupo). Nuestros datos moleculares muestran un soporte significativo para varias de las relaciones filogenéticas. Y la combinación de ambos datos demuestra que al incluir la pequeña base de datos (44 vs. 5,898 caracteres) de caracteres no-moleculares ayuda significativamente a hacer más fuertes las relaciones que parecían débiles en el análisis con solo datos moleculares. Los resultados del análisis combinado indican que *Crepidophryne* se incluye dentro de *Incilius*; además, proponemos esta forma como un sinónimo del taxón mas reciente. Nuestro estudio proporciona el más comprensivo marco evolucionario para los bunonidos de Mesoamérica (*Incilius*), en el cual se empieza a discutir la importancia de la evolución de caracteres únicos de historia natural.

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Introduction

Mesoamerica, here considered to be the continental area south of the United States and north of Colombia, is a complex region characterized by geographic and climatic extremes and a correspondingly diverse biota (reviewed by Campbell, 1999, and Savage, 2002). There is general agreement that the region has clear biogeographical influences from both North and South America, but these influences are relatively minimal in light of the predominant endemic radiations (Savage, 1982). Not surprisingly, Mesoamerica has attracted the attention of many biogeographic studies (e.g., Stuart, 1954; Savage, 1982) and was the conceptual birthplace for the field of modern vicariance biogeography (Rosen, 1978). Exemplar studies have examined relationships among species in endemic highland squamate radiations (e.g., Crother *et al.*, 1992; Campbell and Frost, 1993), phylogeographic patterns among allopatric highland populations of rodent species (e.g., Sullivan *et al.*, 2007; Harris *et al.*, 2000), lowland fishes (e.g., Hulsey *et al.*, 2004), beetles (e.g., Marshall and Liebherr, 2000), and frogs (e.g., Zaldívar-Riverón, 2004). In this paper we present the most extensive phylogenetic study for the majority of bufonid toads from this region, and we take the opportunity to review the varied natural histories of these anurans in an explicit evolutionary framework.

A review of the amphibians of Mesoamerica (Campbell, 1999) listed 389 species of anurans; that list excluded part of the Central Plateau of Mexico, which certainly has its faunal associations with North America. Since that review, many additional species have been described and, relevant to this work, there currently are 62 species of bufonids recognized from Mesoamerica (Frost, 2010). These are allocated to the primarily North American clade *Anaxyrus* (which has many species occuring in Mexico), the primarily South American clades *Atelopus*, *Rhinella* and *Rhaebo* (which have relatively few species in lower Central America), and the autochthonous clade *Incilius*, which currently has 35 species known from across the entire Mesoamerican region. Our efforts here are focused on the relationships among species referred to *Incilius.*

Graybeal (1997: fig. 13) and all subsequent studies have found a monophyletic Bufonidae comprising two major groups. One is a large monophyletic, but nameless, group containing the species formerly referred to *Bufo*, plus various genera that rendered that problematic taxon paraphyletic; the taxonomy of this group has continued to undergo revision since the initial efforts by Frost *et al.* (2006). The other group is a non-monophyletic assemblage usually referred to informally as the "atelopodids" (including the familiar Harlequin frogs—*Atelopus*, and a variety of other generally smaller montane toads such as *Osornophryne*). Frost *et al.* (2006a) provided an important review of Graybeal's (1997) work. Additional efforts (Pauly *et al.*, 2004; Frost *et al.*, 2006a; Pramuk *et al.*, 2006; Pramuk *et al.*, 2008; Van Bocxlaer *et al.*, 2009; Van Bocxlear *et al.*, 2010) have found differing relationships among three major clades of New World bufonids. Pauly *et al.* (2004), Frost *et al.* (2006a), and Pramuk *et al.* (2006, 2008) and Pyron & Wiens (2011) found *Rhinella, Anaxyrus,* and *Incilius* to form a monophyletic group (albeit in differing positions with respect to one another). Van Bocxlaer *et al.* (2009, 2010) incorporated broader taxonomic and geographic sampling of bufonids and discovered *Anaxyrus* and *Incilius* as sister taxa that were sister to a clade containing a monophyletic *Rhinella* plus a large variety of Old World taxa. All studies agree with the placement of the South American clade *Rhaebo* lying outside any arrangement of *Rhinella*, *Anaxyrus,* and *Incilius.* The conflicting results of these broad-based studies represent an important problem in bufonid systematics and biogeography (e.g., Pramuk *et al.*, 2008). Our study was not designed to address these issues, nor to test the existing hypotheses of relationships among these genera, but rather to elucidate relationships among species of *Incilius.*

The majority of species currently referred to *Incilius* were historically allocated to a *Bufo valliceps* group that has been presented in myriad of different forms; some treatments also included a *Bufo coccifer* group. However, there has never been agreement on the content of these various groups, and none were based on results of phylogenetic analyses. Given that these groups have been mentioned so frequently in the literature, a brief review is appropriate. Firschein (1950) proposed the *Bufo valliceps* group (content: *Incilius cristatus* and *I. valliceps*) and the *Bufo cristatus* group (content: *Incilius cavifrons* and *I. cristatus*). Firschein (1950) did not consider the relationships of several other crested toads (e.g., *I. mazatlanensis*) in Middle America and his taxonomic arrangement is problematic because he placed *I. cristatus* simultaneously in two different groups. Subsequently Blair (1959, 1961) alluded to a *Bufo valliceps* group, but did not define it. Based on osteology, Tihen (1962) provided an explicit proposal of the content of the *Bufo valliceps* group and divided it into "South American" and "Mexican" sections. Blair (1966) disagreed with Tihen, claiming that he (Blair, 1959, 1963) already had proposed the content of a *Bufo valliceps* group—a claim that is not justified in Blair's earlier papers. Blair (1966) provided a summary of the group whose

content somewhat matches that of Tihen's (1962) "Mexican Section." Porter (1962, 1964) provided a thorough review of the species then recognized in Mexico. Martin (1972) provided definitions, based on osteology, for the following: *B. valliceps* group, *B. alvarius* group, *B. coccifer* group, *B. canaliferus* group, *B. occidentalis* group, *B. marmoreus* group, *B. bocourti* group, *B. periglenes* group, and *B. holdridgei* group; considered altogether, these groups all include species currently referred to *Incilius* (discounting recently described species). In major works on the *Bufo valliceps* group since 1950, 20 species have been assigned to the group by one or more authors; the most recent formal application of the concept of a *B. valliceps* group was that of Duellman & Schulte (1992), who provided a definition but did not list the content of the group. The current phylogenetic concept of the clade *Incilius* is based on the works of Pauly *et al.* (2004), Frost *et al.* (2006a), and Van Bocxlaer *et al.* (2010). Frost *et al.* (2006a) resurrected *Cranopsis* Cope, 1875, for the Mesoamerican toads, in error, corrected to *Ollotis* Cope, 1875 by Frost *et al.* (2006b), and later to *Incilius* Cope, 1863, by Frost *et al.* (2009a). Frost *et al.* (2006a) provided both a diagnosis and content for this clade. Most previous studies (cited above) have found *Incilius* to be monophyletic. Van Bocxlaer *et al.* (2010) found *Incilius* to be non-monophyletic because of the placement of the taxon *bocourti* as sister to *Anaxyrus.* Pauly *et al.*'s (2004) parsimony analysis found *bocourti* sister to *Rhinella* + *Anaxyrus* with weak support; their likelihood analyses resolved a monophyletic *Incilius* (including *I. bocourti*) with strong support. Pyron & Wiens (2011) found *I. bocourti* within *Incilius*, with low support (58% bootstrap).

Since the efforts by Firschein (1950) and Porter (1962, 1964), many new species in this complex have been described or resurrected from synonymy: Mendelson (e.g., 1994, 1997a, b), Mulcahy & Mendelson (2000), McCranie & Wilson (2000), and O'Neill & Mendelson (2004), Mendelson & Mulcahy (2010), and Mendelson *et al.* (*in press*), and Santos-Barrera & Flores Villela (2011). Although the aforementioned recent phylogenetic studies including bufonids (e.g., Pramuk *et al.*, 2008) included species of crested toads that have been historically referred to some concept of an *Incilius* (= *Bufo*) *valliceps* group, the only studies specific to the group were Mulcahy & Mendelson (2000) and Mulcahy *et al.* (2006). In these latter papers, a clade containing a group of species ecologically associated with mostly upland moist forests was discovered and informally referred to as the "Forest toads," along with the well supported lowland species pair *I. valliceps* + *I. nebulifer.* Mendelson *et al.* (2005) reviewed and revised the *Incilius* (= *Bufo*) *coccifer* group, including descriptions of several new species, and presented a preliminary phylogeny for some of the species in the group.

Considered together, species of *Incilius* show a remarkable ecological and biogeographical diversity that arguably exceeds that of any comparable clade of Neotropical amphibians. These toads include micro-endemic species fully restricted to undisturbed cloud forest habitat (e.g., *I. spiculatus;* Mendelson, 1997b; Mendelson *et al.*, 1999) and widespread lowland species that prefer disturbed habitats (e.g., *I. valliceps;* Mendelson, 1998; Mendelson *et al.*, 1999; McCranie & Wilson, 2002). The genus includes species largely restricted to subhumid habitats (e.g., *I. luetkenii;* Savage, 2002), rainforests (e.g., *I. campbelli;* Mendelson, 1994), or upland pine-oak forests (e.g., *I. cycladen;* Mendelson *et al.*, 2005). Collectively, the group has representatives in every major biogeographic region of Mesoamerica (Campbell, 1999; Duellman & Sweet, 1999), although the Central Plateau of Mexico is only peripherally occupied by *I. occidentalis* and *I. mccoyi.* The anuran faunas of North America and Mesoamerica share remarkably few species, a pattern illustrated roughly by the few species that occur in both USA and Mexico (Campbell, 1999; Duellman & Sweet, 1999; Mulcahy & Mendelson, 2000). It is noteworthy then that only three species of *Incilius* straddle the Neotropical/Nearctic boundary—viz., *I. alvarius* in the Sonoran Desert, *I. mazatlanensis* along the Pacific Coast of Mexico, and *I. nebulifer* along on the Gulf Coast of Mexico and the USA. Similarly, to the South, only the species *I. coniferus* penetrates the South American continent, occurring in the Choco region of Colombia and Ecuador. As future endeavors to reconstruct the bewildering complexity of Mesoamerican biological evolution proceed, we offer *Incilius* as a group comparable to both highland clades with restricted species' distributions, such as squamates in the genera *Bothriechis*, *Atropoides*, and *Abronia* (Crother *et al.*, 1992; Chippindale *et al.*, 1998; Castoe *et al.*, 2009), and clades of more widespread lowland species, such as cichlid fishes (Hulsey *et al.*, 2004). Using data from morphology, natural history, and both nuclear and mtDNA sequence data, we here contribute a phylogenetic hypothesis of the relationships among species of *Incilius,* including nearly all extant species. We then use this phylogenetic hypothesis as a basis for a brief discussion of the evolutionary natural history and biogeography of these toads.

Material and methods

In this paper we follow the taxonomic recommendations of Frost *et al.* (2006a), Pramuk *et al.* (2008), Frost *et al.* (2008), and Frost *et al.* (2009a). Marginal disagreement on bufonid taxonomy was reviewed by Frost *et al.* (2009b).

Taxon sampling. In order to examine the phylogenetic relationships among Mesoamerican bufonids, we examined two datasets: I) 44 non-molecular characters from morphology and life history; and II) 5,898 base-pairs (bp) of mitochondrial and nuclear sequence data. For the molecular data, we sampled a total of 52 individuals representing 37 species (with outgroups), including most species of Mesoamerican bufonids (excluding *Atelopus*). However, no tissues samples were available for five taxa (*Incilius gemmifer, I. mccoyi, I. holdridgei, I. periglenes,* and *I. peripatetes).* Where possible, we sampled multiple individuals for species with broad geographic ranges (e.g., *I. valliceps).* For outgroup taxa, we used *Anaxyrus boreas* from the "North American" clade (Pauly *et al.*, 2004) and five species [*Rhinella marina, R. festae R. schneideri, R. margaritifera* (= *typhonius*), and *Rhaebo haematiticus*] from the "South American" clade discussed by Pramuk (2006). Note: we mention the invalid name *R. typhonius* here because our tissue sample is listed by some authors in GenBank under that name. We rooted all of our trees post-analyses between *Incilius* and the outgroup taxa *Rhinella marina, R. margaritifera, Rhaebo haematiticus,* and *Anaxyrus boreas.* We did not designate *Rhinella* (= *Rhamphophryne*) *festae* nor *Crepidophryne* as outgroups, so as to test their phylogenetic position with respect to the other included taxa (see below). The non-molecular dataset contained 29 species; osteological specimens for some species were not available (e.g., some recently described species such as *I. signifer).* In the non-molecular dataset, we included four South American taxa (*Rhinella marina, R. festae, R. margaritifera,* and *Rhaebo haematiticus*) and *Anaxyrus boreas* as outgroups. We first present the molecular and non-molecular data separately, followed by combined analyses. The combined analyses of both datasets include one representative from each of the 37 species. We were unable to obtain all data for some specimens and some sequences were taken from GenBank for taxa that were already available from the same specimens used in our study (Pauly *et al.,* 2004; Pramuk *et al.*, 2008). Table 1 shows all samples used in the molecular analyses, including voucher information (see Appendix I for GenBank accession numbers, and Appendix II for non-molecular vouchers). Because of specimen and tissue availability, we had to use a specimen of *Crepidophyrne chompipe* for molecular data and *C. epiotica* for non-molecular data; our original sampling efforts occurred prior to the taxonomic revision by Vaughan & Mendelson (2007), when all populations were referred to *C. epiotica.* Thus, in our phylogenetic analyses we use the terminal "*Crepidophryne*" to represent what we consider a monophyletic group (see Discussion).

TABLE 1. Voucher specimens used for molecular analyses. Note that our sample of *Rhinella margaritifera* is listed by some in GenBank as "*Bufo cf. typhonius*" and *Rhinella festae* is listed as "*Rhamphophryne*" in GenBank.

out	Locality	Museum No.
Outgroup:		
A. boreas	USA: California: Los Angeles Co., San Dimas Canyon	MVZ 223292
Rhaebo haematiticus	Costa Rica: Cartago: I.C.E. Plant in Rio Macho	MVZ 164805
Rhinella marina	Ecuador: Loja, Vilcabamba	KU 217482
R. schneideri	Paraguay: Parque Nacional San Luis de la Sierra	KU 289057
R. margaritifera (= *typhonius*)	Peru: Madre de Dios: Cuzco Amazonico, 15 km E Puerto Maldonado	KU 215146
R. festae	Ecuador: Pastaza: Petrolera Garza 1, NE Montalvo	KU 217501
Mesoamerican bufonids:		
Incilius alvarius	USA: Arizona: Cochise County	UTA A-53924
*I. aucoinae*_1	Costa Rica: Golfito, Quebrada Canaza	UCR 14323
*I. aucoinae*_2	"	UCR 14324
I. bocourti	Guatemala: Escuintla	UTA A-50920
*I. campbelli*_1	Belize: Toledo: El Tigre/Columbia River FR	USNM 326155

continued next page

TABLE 1. (continued)

out	Locality	Museum No.
*I. campbelli*_2	"	USNM 326161
*I. campbelli*_3	Guatemala: Izabal; Sierra de Caral, San Miguelito	KU 221203
*I. campbelli*_4	Guatemala: Izabal: Montanas del Mico, Las Escobas	UTA A-50902
I. canaliferus	Guatemala: Escuintla: Palín, Finca Medio Monte	UTA A-47640
I. cavifrons	Mexico: Veracruz: Sierra de los Tuxtlas, Volcan San Martin	UTA A-ENS 10384
*I. coccifer*_1	El Salvador: Morazan	KU 290030
*I. coccifer*_2	Costa Rica: San Jose: Montanas Jamaic	TCWC 83998
I. coniferus	Costa Rica: Prov. Cartago: Cabina Tapanti	MVZ 203775
Crepidophryne chompipe	Costa Rica: Cerro Dantas, Cordillera Volcanic Central	UCR 16075
I. cristatus	Mexico: Puebla: Municipio de Zacapoaxtla, Apulco	EBUAP 544
I. cycladen	Mexico: Guerrero: near Agua de Obispo	UTA A-54847
I. fastidiousus	Costa Rica: Puntarenas: Rio Coton below La Casita	MVZ 217438
*I. ibarrai*_1	Honduras: Ocotepeque	UTA A-53662
*I. ibarrai*_2	Guatemala: Quiche	UTA A-52528
I. karenlipsae	Panama: Cocle: Parque Nacional G. D. Omar Torrijos	UTA-A-59522
*I. leucomyos*_1	Honduras: Atlantida: La Ceiba, Cordillera Nombre de Dios	UTA A-50642
*I. leucomyos*_2	Honduras: Olancho: Quebrada, El Pinol, Parque Nacional La Muralla	USNM 559731
*I. leucomyos*_3	Honduras: Francisco Morazan	UTA A-MEA 892
*I. luetkenii*_1	Guatemala: Zacapa: S Teculutan	UTA A-50877
*I. macrocristatus*_1	Mexico: Oaxaca: Santa Maria Chimalapa	MZFC-EPR 37
I. *macrocristatus*_2	Mexico: Oaxaca: Santa Maria Chimalapa	MZFC-259
*I. macrocristatus*_3	Mexico: Chiapas: 10.0 mi NW Pueblo Nuevo Solistahuacan	UTA-JAC 7993
I. marmoreus	Mexico: Oaxaca: 0.7 mi NE Tapanatepec	UTA A-13032
I. mazatlanensis	Mexico: Sonora: Alamos	MVZ 132967
I. melanochlorus	Costa Rica: Prov. Heredia: La Selva Biological Station	MVZ 229635
*I. nebulifer*_1	USA: Louisiana: Tangipahoa Parish; Hammond	UTA A-52489
*I. nebulifer*_2	Mexico: Veracruz: road to Hueytepec	UTA A-54860
I. porteri	Honduras: Francisco Morazan: Reserva Biologica Cerro Uyuca, Cabot Biological Station	UF-JHT 2249
I. occidentalis	Mexico: Oaxaca: El Tejacate	UTA A-13543
I. perplexus	Mexico: Guerrero: Rio Zopilote, N of Zumpango de Rio	UTA A-54851
I. pisinna	Mexico: Michoacan: Hwy 37 S of Lombardia	UTA A-JAC 26118
I. signifer	Panama: Cocle: El Cope	UTA A-JRM 4968
I. spiculatus	Mexico: Oaxaca: S of Vista Hermosa	UTA A-54853
I. tacanensis	Mexico: Chiapas: Colonia Talquian, Union Juarez, Volcan Tacana	MVZ 170329
*I. tutelarius*_1	Mexico: Oaxaca: Cerro Baul	MZFC 5262
*I. tutelarius*_2	Mexico: Oaxaca: Cerro Baul	MZFC 5277
*I. valliceps*_1	Mexico: Veracruz: Catemaco	MZFC JRM-3868
*I. valliceps*_2	Honduras: Cortes: Tegucigalpita	USNM 530601
I. **sp. nov.**_1	Guatemala: Huehuetenango: Nenton, Aldea Yalambojoch	UTA A-52597
I. **sp. nov.**_2	Guatemala: Huehuetenango: Nenton, Aldea Yalambojoch	UTA A-52591
I. **sp. nov.**_3	Guatemala: Huehuetenango, on Ridge ca. 2km NW Barillas	MVZ 143380

Frost *et al.* (2006a) noted several potential morphological synapomorphies between *Crepidophryne* and South American toads of the genus *Rhamphophryne*—a taxon later placed in the synonymy of *Rhinella* by Chapparo *et al.* (2007; see also Pramuk, 2006, and Pramuk *et al.* 2008; and Van Bocxlaer *et al.*, 2010). Indeed, all data, including both molecules and morphology, that have been brought to bear on *Rhamphophryne* and *Rhinella* suggests a close relationship (see Discussion); however, our study is the first to include *Crepidophryne.* Therefore, we included *Rhinella* [= *Rhamphophryne*] *festae* to test the relationship between *Crepidophryne* and *Rhamphophryne.* For the molecular data, we used sequences of *Rhinella* (= *Rhamphophryne*) *festae* (KU 217501) from GenBank [CXCR4: DQ306521 (Pramuk *et al.*, 2008); RAG-1: DQ158349 and 12S–16S: DQ158423 (Pramuk, 2006).

Additionally, Van Bocxlaer *et al.* (2010) included three samples of the widespread species *I. valliceps*, and one sample from its putative sister-taxon *I. nebulifer* (all from GenBank). Their analyses found *I. valliceps* to be non-monophyletic, with one sample from Honduras (USNM 534129) placed as sister to the Mexican–Guatemalan species *I. macrocristatus*, and the other two samples as sister to *I. nebulifer.* Likewise, Pyron & Wiens (2011) included the 12S–16S, CXCR4, and RAG1 data from this individual as well, and recovered a chimeric "*I. valliceps*" sister to a *I. macrocristatus* + *I. campbelli* clade. Two previous analyses (Mulcahy & Mendelson, 2000; Mulcahy *et al.*, 2006), with greater geographic sampling, have recovered a monophyletic *I. valliceps* as sister to *I. nebulifer.* Thus, we compared the 12S–16S (DQ158493), CXCR4 (DQ306545.1), and RAG1 (DQ158409.1) sequences of USNM 534129 with our data and examined the specimen (USNM 534129) to verify its identity.

TABLE 2. Primers used in this study.

Locus	Name	Sequence '5 to '3
cyt b	MVZ43	GAGTCTGCCTWATYGCYCARAT
cyt b	CB3H	GGCAAATAGGAARTATCATTC
16S	16Sar	CGCCTGTTTATCAAAAACAT
16S	16Sbr	CCGGTCTGAACTCAGATCACGT
12S	12StPhe	AAAGCACRGCACTGAAGATGC
12S	12Se	GTRCGCTTACCWTGTTACGACT
ND2	metF6	AAGCTTTCGGGCCCATACC
ND2	CO1r1	AGRGTGCCAATGTCTTTGTGRTT
ND2	IncAsnF1	AAACGCTCAATCCAGCGAGCT
ND2	IncAsnR1	AGCTCGCTGGATTGAGCGTTT
ND2	IncND2f1	TGCYCAAGAAATARTTAAACA
ND2	IncND2r1	TGTTTAAYTATTTCTTGRGCA
CO1	dgLCO-1490	GGTCAACAAATCATAAAGAYATYGG
CO1	dgHCO-2198	TAAACTTCAGGGTGACCAAARAAYCA
CXCR4	CXCR4C	GTCATGGGCTAYCARAAGAA
CXCR4	CXCR4F	TGAATTTGGCCCRAGGAARGC
RAG1	RAG1F	AGYCARTAYCATAARATGTA
RAG1	RAG1R	GCRTTNCCDATRTCRCARTG

Molecular data sampling. We collected sequence data from six mitochondrial gene regions: 12S, 16S, cyt b, ND2, $tRNA^{TRP}$, $tRNA^{ALA}$, $tRNA^{ASN}$, OL (origin of light strand replication), $tRNA^{CYS}$, and $tRNA^{TYR}$ (all treated as one partition), and CO1. In addition, we collected sequence data from two nuclear loci (CXCR4 and RAG1) for a total of eight markers. The protein-encoding nuclear loci were previously used in a world-wide bufonid study and we followed their protocols for PCR and sequence reactions (Pramuk *et al.*, 2008). The mitochondrial genes 12S–16S are frequently used in anuran studies (Pauly *et al.*, 2004; Pramuk *et al.*, 2008; Frost *et al.*, 2006) and cyt b is used in studies focused on *Incilius* (Mulcahy & Mendelson, 2000; Mendelson *et al.*, 2005; Mulcahy *et al.*, 2006). These loci were collected using primers and protocols similar to our previous studies referenced above. The ND2 and tRNA regions are frequently used in general amphibian and reptile studies (e.g., Macey *et al.*, 1997; 1998) and

were collected using their primers as well as internal primers designed specifically for *Incilius*, and CO1 is used in the DNA Barcode of Life Project (e.g., Crawford *et al.*, 2010), and was obtained using primers from Meyer (2003). A complete list of primers used for this study is shown in Table 2. Profiles for PCR reactions were run similar to Mulcahy & Mendelson (2000) with annealing temperature varying from 45–51.0° C for the mitochondrial DNA, while the nuclear loci were obtained with the following profile: step 1: 94.0° C for 2:45 min; step 2: 94.0° C for 0:15 s, step 3: 51.0* C for 0:20 s (where * reduces the temp by 0.3° C each cycle); Step 4: 72.0° C for 1:00 min, back to step 2, for 35 cycles, and a final elongation of 72.0° C for 7:00 min. Products of PCR were purified using Millipore microplates, sequence reactions were conducted in both directions with PCR primers, using BigDye® Terminator v3.1 Cycle Sequencing Kit using manufacturers protocols. Sequenced products were purified using Sephadex columns and run out on an ABI 3730xl sequencer at the BYU DNA Sequencing Center. Complimentary chromatograms were assembled and annotated in Sequencher™ v4.7, alignments were done by eye and adjusted in MacClade v4.08 by translated amino acid sequence for protein encoding loci. The ribosomal (12S and 16S) regions were aligned using the ClustalW (v1.4) default option in MacVector. This method uses an open gap penalty of 10, extended gap penalty 5, delay divergent 40%, and weighted transitions in order to minimize gaps. The tRNAs were aligned by secondary structure (Macey *et al.*, 1997). There were no insertions or deletions in the protein-encoding loci, and very few (<40) in the ribosomal and tRNA gene regions, gaps were treated as missing data.

Phylogenetic analyses. Phylogenetic analyses of non-molecular data. We examined phylogenetic relationships among Mesoamerican bufonids using parsimony (separately and combined) and Bayesian analyses of the combined datasets (molecular and non-molecular). We chose these methods because parsimony offers simple, unweighted analyses of the data and Bayesian analyses offer more complex model for the molecular data, while maintaining a simple model for the non-molecular data (Felsenstein, 2004). Based partly on the analysis presented by Mendelson (1997c) and information in Mendelson *et al.* (1999), we identified and scored 44 characters drawn from osteology, soft tissue, larval morphology, and natural history (Appendix III). The non-molecular data matrix was evaluated using a maximum parsimony analysis using PAUP* v4.0b10 (Swofford, 2000). Heuristic searches (1000 random addition replicates) were performed using tree-bisection-reconnection branch swapping, saving all minimal length trees at each replicate; the starting seed used was 1. The tree was rooted between the outgroup taxa and *Incilius*, because of the ambiguity involved in the sister group to *Incilius* (Pauly *et al.*, 2004; Frost *et al.*, 2006a; Pramuk *et al.*, 2008; Van Bocxlaer *et al.*, 2009; Van Boxclaer *et al.*, 2010). Agreement among the shortest trees was assessed by strict consensus. Bremer/decay indices (Bremer, 1994) were measure by keeping step-by-step longer trees, and taking a strict consensus of each run, until all nodes were collapsed, and recording the number of steps required to collapse nodes. Bootstrap analyses were conducted to test nodal support, based on 100 replicates, each with 100 random step-wise additions per replicate. Characters 2, 7, 8, and 37 were treated as ordered, following the reasoning proposed by Wilkinson (1992) and Campbell & Frost (1993); exceptions to this treatment are discussed with the character descriptions (Appendix III). All characters were equally weighted. When possible, multiple specimens were examined in order to assess individual variation. In the few cases in which multiple character states were observed among individuals, the character was coded as polymorphic to account for all observed conditions. Variation in character states among specimens examined were coded as polymorphic in the data matrix (e.g., 0/1; see Wiens, 2000). In almost every case, we had only one or two skeletal specimens available, so we were unable to employ any frequency-based parsimony methods (e.g., Smith & Gutberlet, 2001). The complete non-molecular data matrix appears in Table 3**;** descriptions of each character are presented in Appendix III. Missing data were coded as "?" in all analyses.

Phylogenetic analyses of molecular data. Analyses of the molecular data were based on parsimony and Bayesian inference because of the simplistic model in parsimony, particularly for the less-informative nuclear loci, and Bayesian for the convenience of combining the non-molecular data. Parsimony analyses were conducted in PAUP* using the heuristic search options with 100 random, step-wise additions, tree bisection-reconnection branch swapping algorithm, saving multiple best-trees. Bootstrap analyses were conducted to test nodal support, based on 100 replicates, each with 10 random step-wise additions per replicate for the mtDNA and nuclear data separately. The nuclear data were set to have maximum number of trees saved at 10,000 for both heuristic and bootstrap searches. For the combined molecular data, parsimony bootstrap analyses were based on 1000 replicates, each with 100 random step-wise additions per replicate. Bayesian analyses were conducted in MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). Each partition was analyzed in MrModeltest v2.2 (Nylander, 2004) to determine the best model of nucleotide substitution under the Akaike Information Criterion, because this method penalizes increased parameters, thus favors a more simplistic model than the hierarchical likelihood ratio test (Felsenstein, 2004). The

TABLE 3. Non-molecular data matrix used in this study. Characters coded as series indicate taxa with multiple states for that character (i.e., polymorphisms, '0–1' stands for 0 and 1). See Appendix III for character descriptions.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
Anaxyrus boreas	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0
Crepidophryne	1	2	0	3	1	0	1	0	1	1	1	0	?	?	?	?	1	0	1	0	0	0
Incilius aucoinae	1	1	0	1	1	0-1	1	1	1	1	0-1	1	0	1	0	1	1	0	0	0	0	1
I. alvarius	1	2	0	1	0	1	1	0	1	0	0	0	1	2	0	1	1	0	1	1	2	0
I. boucourti	1	2	0	2	0	1	1	1	1	0	0	0	0	1	0	1	0	0	1	0	0	1
I. campbelli	1	1	1	1	1	0-1	1	1	1	1	0-1	1	0	1	0	1	1	0	1	0	0	1
I. canaliferus	0	1	0	1	0	0	1	0	1	0	0	0	1	0	0	1	0	0	1	1	0	1
I. cavifrons	1	0-1-2	0	1	1	0-1	2	2	1	1	0-1	1	1	1	0	1	1	0	1	0	0	1
I. coccifer	1	2	0	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0	1	1	0	1
I. coniferus	1	2	0	1	1	1	1	1	1	1	1	1	0	1	0	1	1	0	0	1	0	1
I. cristatus	1	2	0	3	1	0-1	2	2	1	1	0-1	1	0	1	0	1	1	0	1	0	0	1
I. fastidiosus	0	2	0	1	0	0	0	0	1	1	0	0	1	0	0	1	1	0	0	1	0	0
I. ibarrai	1	2	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	1	0	1
I. leucomyos	1	1	1	3	1	1	3	1	1	1	0-1	1	0	1	0	1	0	0	1	0	0	1
I. luetkeni	1	2	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	0	1
I. macrocristatus	1	2	0	1	1	0-1	2	2	1	1	0-1	1	1	1	0	1	1	0	1	0	0	1
I. marmoreus	0	1	0	1	0	1	0	0	1	1	0	0	1	1	0	1	0	0	0	0	0	1
I. mazatlanensis	1	2	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1	1	0	1
I. melanochlorus	1	1	0	1	1	0-1	1	1	1	1	0-1	1	0	1	0	1	1	0	0	0	0	1
I. nebulifer	1	2	0	1	1	1	1	1	1	1	1	1	1	1	0-1	1	1	0	1	0	0	1
I. occidentalis	0	2	0	0	0	0	1	0	1	0	0	0	1	1	1	1	0	0	0	0	0	0
I. perplexus	0	2	0	2	0	0	1	0	1	0	1	0	1	1	0	1	0	1	0	0	1	1
I. spiculatus	1	2	0	3	1	0-1	3	1	1	1	0	1	0	1	0	1	1	0	1	1	0	1
I. tutelarius	1	2	0	3	1	1	1	1	1	1	0-1	1	0	1	0	1	1	0	1	0	0	1
I. valliceps	1	2	0	1	1	1	1	1	1	1	1	1	1	1	0-1	1	1	0	1	0	0	1
Rhaebo haematiticus	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Rhinella marina	1	2	0	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	0	1	1	2
Rhinella margaritifer	1	1	0	3	0	0	3	0	0	1	0	0	0	0	0	0	1	0	1	0	1	2
Rhinella festae	1	2	0	3	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	2

continued next page

TABLE 3. (continued)

	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
Anaxyrus boreas	1	1	0	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
Crepidophryne	1	0	2	1	0	?	1	0	0	3	1	0	2	?	?	?	1	1	1	1	1	1
Incilius aucoinae	0	1	1	0	1	0	1	1	2	1	1	0	1	1	?	?	0	0	0	0	0	0
I. alvarius	0	1	0	1	1	1	1	0	2	1	0	1	0	0	?	2	0	0	0	0	0	0
I. boucourti	0	0	1	1	0	?	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
I. campbelli	0	1	1	0	1	0	1	1	2	1	1	0	1	1	1	0	0	0	0	0	0	0
I. canaliferus	0	1	2	1	1	0	0	0	1	1	1	0	0	0	?	?	0	0	0	0	0	0
I. cavifrons	0	0	1	0	1	0	1	1	2	1	1	0	1	1	0	0	0	0	0	0	0	0
I. coccifer	1	0	1	1	1	0	1	0	1	3	0	1	0	0	2	1	0	0	0	0	0	0
I. coniferus	1	1	1	1	1	1	1	0	1	2	1	0	0	0	2	0	0	0	0	0	0	0
I. cristatus	0	0	1	0	1	0	1	1	0	1	1	0	1	1	0	0	0	0	0	0	0	0
I. fastidiosus	1	0	2	1	0	?	0	0	0	3	1	0	0	0	1	0	0	0	1	?	0	0
I. ibarrai	1	1	1	1	1	0	1	0	1	1	0	1	0	0	2	0	0	0	0	0	0	0
I. leucomyos	0	1	1	0	1	0	1	1	2	1	1	0	1	1	1	0	0	0	0	0	0	0
I. luetkeni	1	1	1	1	1	1	1	1	1	2	0	1	0	0	2	2	0	0	0	0	0	0
I. macrocristatus	0	1	1	0	1	0	1	1	2	1	1	0	1	1	1	0	0	0	0	0	0	0
I. marmoreus	0	1	1	1	1	0	0	0	1	1	0	1	0	0	1	2	0	0	0	0	0	0
I. mazatlanensis	0	1	1	1	1	0	1	1	1	1	0	1	0	0	2	1	0	0	0	0	0	0
I. melanochlorus	0	1	1	0	1	0	1	1	2	1	1	0	1	1	?	?	0	0	0	0	0	0
I. nebulifer	1	1	1	0	1	0	1	1	2	1	0	1	0	0	2	1	0	0	0	0	0	0
I. occidentalis	0	1	0	1	1	0	1	0	2	0	0	1	0	0	?	?	0	0	0	0	0	0
I. perplexus	1	1	1	1	0	0	0	1	2	0	1	?	?	?	?	?	0	0	0	0	0	0
I. spiculatus	0	0	1	0	1	0	1	1	0	1	1	0	1	1	1	0	0	0	0	0	0	0
I. tutelarius	0	0	1	0	1	0	1	1	0-1-2	1	1	1	1	1	1	0	0	0	0	0	0	0
I. valliceps	1	1	1	0	1	0	1	1	2	1	0	1	0	0	2	1	0	0	0	0	0	0
Rhaebo haematiticus	1	0	0	1	1	1	1	1	1	1	1	0	1	0	1	0	0	0	0	0	0	0
Rhinella marina	0	1	1	1	1	1	1	0	2	1	1	1	0	0	2	2	0	0	0	0	0	0
Rhinella margaritifer	1	0	1	0	1	0	1	0	2	1	1	0	1	0	2	0	0	0	0	0	0	1
Rhinella festae	0	0	2	1	0	?	1	0	0	1	1	0	2	?	?	?	1	1	1	1	1	1

molecular data were separated into 18 partitions, one for each codon position of each protein-encoding loci (CXCR4, RAG1, cyt b, ND2, and CO1 because a different model was selected for each codon, demonstrating they are evolving under different models) and one each for the RNA 12S, 16S, and tRNA/OL region. Two analyses were run for 50 million generations each, saving trees every 1000, with four heated chains (user defaults). Stationarity was assessed by the average standard deviation of split frequencies (ASDSF < 0.01) and visual plots of log-likelihood by generation in Tracer v1.2 (Rambaut and Drummond, 2004); the first 10,000 trees (of 50,000) were discarded as the burn-in. A 50% majority-rule with compatible groups ("allcompat") consensus was taken from the remaining trees and posterior probabilities of 0.95 or above were considered significant.

Phylogenetic analyses of molecular and non-molecular data. The combined datasets were analyzed under parsimony and Bayesian conditions. Parsimony analyses were conducted in PAUP* under the same conditions as the molecular data alone (see above), with 1000 bootstrap replicates, each with 100 random additions per replicate, with the morphological characters 2, 7, 8, and 37 coded as ordered, all others unordered. Bayesian analyses were conducted in MrBayes with 19 partitions, 18 for the molecular data (as above) and the 19th for the morphological data with characters 2, 7, 8, and 37 coded as ordered, all others unordered, and using the Mk model (Lewis, 2001) with a parameter (Γ) for rate variation among characters. Two analyses were run for 50 million generations each, saving trees every 1000, with four heated chains (user defaults). Stationarity was assessed by the ASDSF (<0.01) and by plotting log-likelihood by generation Tracer; the first 10,000 trees were discarded. An "allcompat" consensus was taken from the remaining trees and posterior probabilities of 0.95 or above were considered significant. Alignments were deposited in the Dryad Repository (doi:10.5061/dryad.t1r37b7v).

Results

Phylogenetic analyses of non-molecular data. Parsimony analysis of the 44 non-molecular data discovered 149 equally most parsimonious trees (170 steps; CI = 0.353; RI = 0.637). A strict consensus of these trees failed to recover a monophyletic *Incilius,* with the taxon *Rhinella marina* being placed therein. Among the outgroups, the following taxa formed a clade: *Rhaebo haematiticus, Rhinella margaritifera,* plus the taxon pair *Crepidophryne* + *Rhinella festae* (Fig. 1). A clade containing all of the "Forest toad" species (sensu Mendelson *et al.*, 1999, and Mulcahy & Mendelson, 2000) was recovered with 68% bootstrap support. The majority of other species traditionally referred to the *valliceps* and *coccifer* groups, and *Rhinella marina* were in a polytomy, including the Forest toad clade. The remaining species of *Incilius* were placed in poorly resolved basal clades, with the most basal divergence being *I. fastidiosus.*

Phylogenetic analyses of molecular data. We obtained 5,898 aligned base pairs (bp), 4,317 bp of mtDNA and 1,581 bp of nuclear DNA from 52 individual specimens, characteristics of each locus, including size and number of parsimony-informative sites can be found in Table 4. Parsimony analyses of the mtDNA recovered 27 equally parsimonious trees, each 6,827 steps. A strict consensus of these trees (Appendix IV, Fig. A) recovered a monophyletic *Incilius*, with *I. bocourti* as the most basal divergence, with a clade containing *I. tacanensis,* and *I. alvarius* + *I. occidentalis* as the next most basal divergence. The *I. valliceps* group was resolved as monophyletic and weakly supported as sister to a (*marmoreus* (*canaliferus* + *perplexus*)) clade. *Crepidophryne* was nested within *Incilius*. The *coccifer* group is strongly placed sister to a (*fastidiosus* (*Crepidophryne* (*coniferus* +*karenlipsae*))) clade—the *I. coniferus* group. Parsimony analyses of the nuclear loci (CXCR4 and RAG1) recovered 33,275 equally parsimonious trees of 529 steps, with generally poor resolution. A strict consensus tree (Appendix IV, Fig. B) showed most of the *valliceps* group as monophyletic with the exception of a clade containing *luetkenii* + *mazatlanensis* and *melanochlorus* + *aucoinae*, which was placed in a basal polytomy among all other clades. *Crepidophryne* was again nested within *Incilius*, and placed sister to *I. coniferus*. Parsimony analyses of the 5,898 aligned bp from combined nuclear and mtDNA data contained 1,413 parsimony-informative characters and resulted in two trees, 7,388 steps in length. A strict consensus tree (Fig. 2) has a topology with *I. bocourti* as the first divergence in *Incilius*, then a clade consisting of *I. tacanensis, I. alvarius* + *I. occidentalis* that is sister to all remaining species. Clades referable to *I. coniferus, I. coccifer,* and *I. valliceps* groups were recovered, with *I. coniferus* group and *I. coccifer* group sister to one another. A (*canaliferus* (*marmoreus* + *perplexus*)) clade was also found, in a basal polytomy with the *I. coniferus* + *I. coccifer* groups, and the *I. valliceps* group. Within the *I. valliceps* group, Forest toads were rendered paraphyletic with respect to the Lowland toads (Fig. 2).

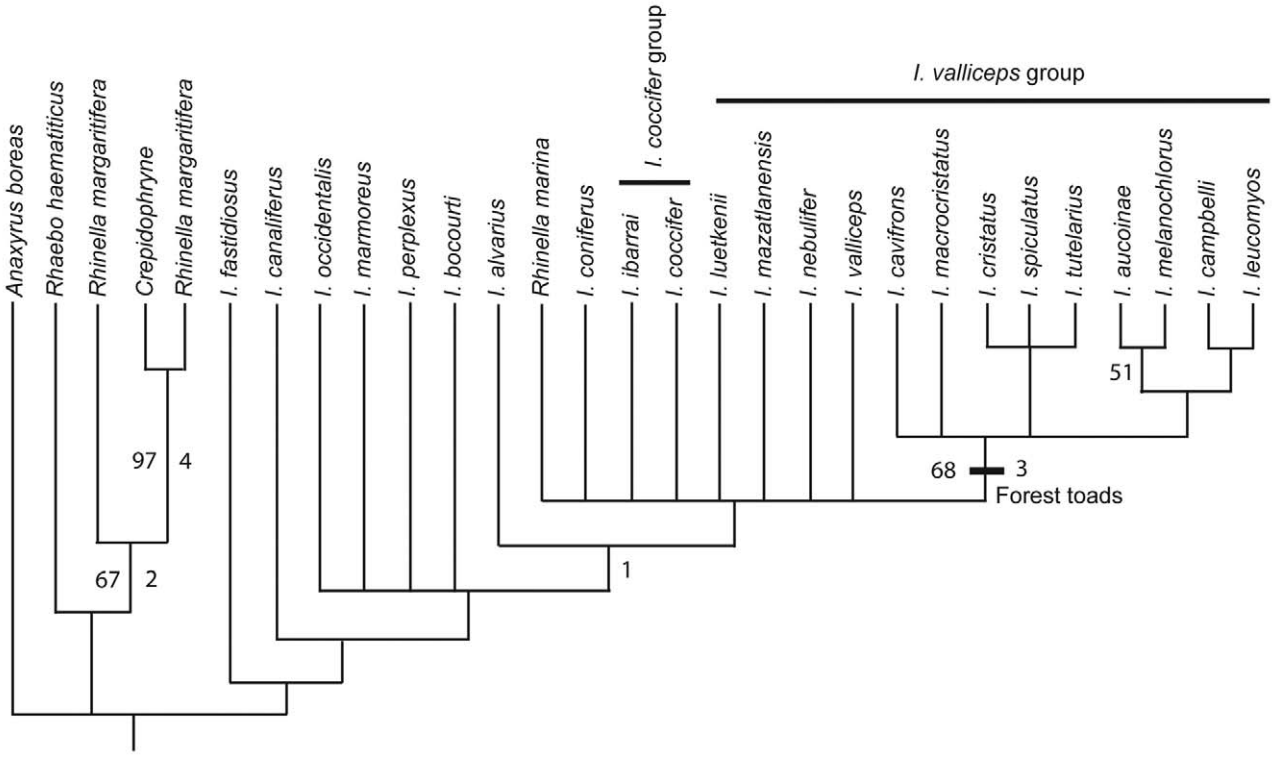

FIGURE 1. Parsimony analysis of the non-molecular data (44 transformation series; Appendix III). Shown here is the strict consensus tree of the 149 equally most parsimonious trees (170 steps; CI = 0.353; RI = 0.637). Bootstrap values are shown above nodes, decay indices are shown below. We note the lack of basal resolution within the clade containing the "Forest toads" (e.g., *Incilius campbelli*, *I. macrocristatus*, etc.), and especially the position of *Rhinella marina* that renders *Incilius* paraphyletic.

Bayesian analyses of the combined nuclear and mtDNA reached convergence after 500,000 generations. However, the first 10,000 trees (of 50,000) were discarded as a conservative measure. The ASDSF < 0.0004. A 50% "allcompat" majority consensus of the remaining trees is shown in Figure 3, and an average –ln*L* = 41400.47 score was obtained post-burn-in. The topology was overall very similar to the parsimony analyses, but with the (*canaliferus* (*marmoreus* + *perplexus*)) clade as sister to the *I. coniferus* + *I. coccifer* groups. The *I. valliceps* group was monophyletic, but the Forest toads and Lowland clades were paraphyletic with respect to one another. The lower Central American species of Forest toads (*I. melanochlorus* + *I. aucoinae*) were placed sister to the Pacific Versant *I. mazatlanensis* + *luetkenii* lowland clade, although with weak support, and the Atlantic Versant *I. valliceps* + *I. nebulifer* lowland clade was placed sister to the Nuclear Central American Forest toads (Fig. 3).

Our analysis of the specimen *I.* cf. *valliceps* (USNM 534129) that rendered *I. valliceps* paraphyletic in the analyses of Van Bocxlaer *et al.* (2010) revealed the 12S–16S sequences of USNM 534129 (DQ158493) are 6–7 bp different from our samples of *I. leucomyos* 2 and 3, while both the CXCR4 (DQ306545.1) and RAG1 (DQ158409.1) sequences are identical to our sample *I. leucomyos* 2. Our examination of the specimen USNM 534129 also confirmed its identity as *I. leucomyos*.

Phylogenetic analyses of molecular and non-molecular data combined. Parsimony analyses of the combined non-molecular and molecular data (5,942 characters) contained 1404 parsimony informative characters, and resulted in two trees of 7,451 steps in length (Fig. 4). The only difference was with the placement of an undescribed species (*I.* **sp. nov.**), which was sister to *I. macrocristatus* in one tree, and sister to the (*I. macrocristatus* (*I. campbelli* + *I. leucomyos*)) clade in the other. *Incilius* was found to be monophyletic with *I. bocourti* as sister to all other Mesoamerican species. The (*I. tacanensis* (*I. alvarius* + *I. occidentalis*)) clade was recovered as the next most basal divergence within *Incilius*, the *I. coniferus* + *I. coccifer* groups were sister to one another, and weakly placed sister to the *I. valliceps* group. Within the *I. valliceps* group, the Lowland and Forest toad clades were both monophyletic with respect to each other, albeit each weakly supported. Bayesian analyses of the combined data reached stationar-

ity by the first 500,000 generations (ASDSF = 0.0006), as a conservative measure, the first 10,000 trees were discarded (from 50,000) as the burn-in process. A 50% "allcompat" majority consensus topology (Fig. 5; avg. –ln*L* = 40414.31) recovered *I. bocourti* as the most basal divergence in *Incilius*, with an (*I. tacanensis* (*I. alvarius* + *I. occidentalis*)) clade being sister to the remaining species. Within the remaining species were two clades, one containing the *I. valliceps* group, and the other containing all other remaining Mesoamerican species. Within the *I. valliceps* group, the lowland species formed a clade and the Forest toads formed a clade, albeit weakly supported (post. prob. = 0.63; Fig. 5).

TABLE 4. Characteristics of loci used in this study.

Loci	No. of characters (pars. inform.)	Partition	Sub. Model
mtDNA	4,317 (1259)		
cyt b	675 (252)	pos 1	SYM+I+Γ
		pos 2	F81+I
		pos 3	GTR+I+Γ
ND2	1,032 (435)	pos 1	GTR+I+Γ
		pos 2	GTR+I+Γ
		pos 3	GTR+Γ
CO1	732 (236)	pos 1	GTR+I+Γ
		pos 2	GTR
		pos 3	GTR+I+Γ
12S	933 (164)	12S	GTR+I+Γ
16S	568 (126)	16S	GTR+I+Γ
tRNAs	376 (45)	tRNAs	HKY+I+Γ
nuclear	1,581 (153)		
CXCR4	717 (69)	pos 1	K80+I
		pos 2	F81
		pos 3	HKY+Γ
RAG1	864 (84)	pos 1	GTR+Γ
		pos 2	F81+I
		pos 3	HKY+Γ

There were no non-molecular synapomorphies unique to any single clade. The *canaliferus-marmoreus-perplexus* clade was supported by absence of a supraorbital flange on the frontoparietal (Char. 1), the cultriform process of the parasphenoid reaching to the level of the planum antorbitale (Char. 17), broad contact between the medial ramus of the pterygoid and parasphenoid ala (Char. 22), the space between the zygomatic and ventral rami of the squamosal filled with bone (Char. 24), and also having a small, free xiphisternum (Char. 29). Both the *I. coccifer* and *I. valliceps* groups are supported by the presence of the canthal, preorbital, parietal, and suborbital crests (Chars. 5, 6, 8, 12), although this could also be interpreted simply as the loss of these crests in the *I. coniferus* group. The *I. coccifer* group is supported by having cultriform process of the parasphenoid reaching to the level of the planum antorbitale (Char. 17), a robust quadratojugal (Char. 20), and broad contact between the medial ramus of the pterygoid and parasphenoid ala (Char. 22). The sister relationship between the *I. coccifer* and *I. coniferus* groups is supported by the long zygomatic ramus of the squamosal (Char. 23). The *I. valliceps* group is supported by the space between the zygomatic and ventral rami of the squamosal filled with bone (Char. 24; but reversed in most of the Forest toads), the straight shape of the nasals (Char. 26; but reversed in *I. luetkenii* + *I. mazatlanensis*), and presence of an omosternum (Char. 30). The *I. coniferus* group is supported by the absence of inguinal fat bodies (Char. 34). The Forest toads are supported by the behaviors of depositing eggs in streams during the dry season (Chars. 35, 36).

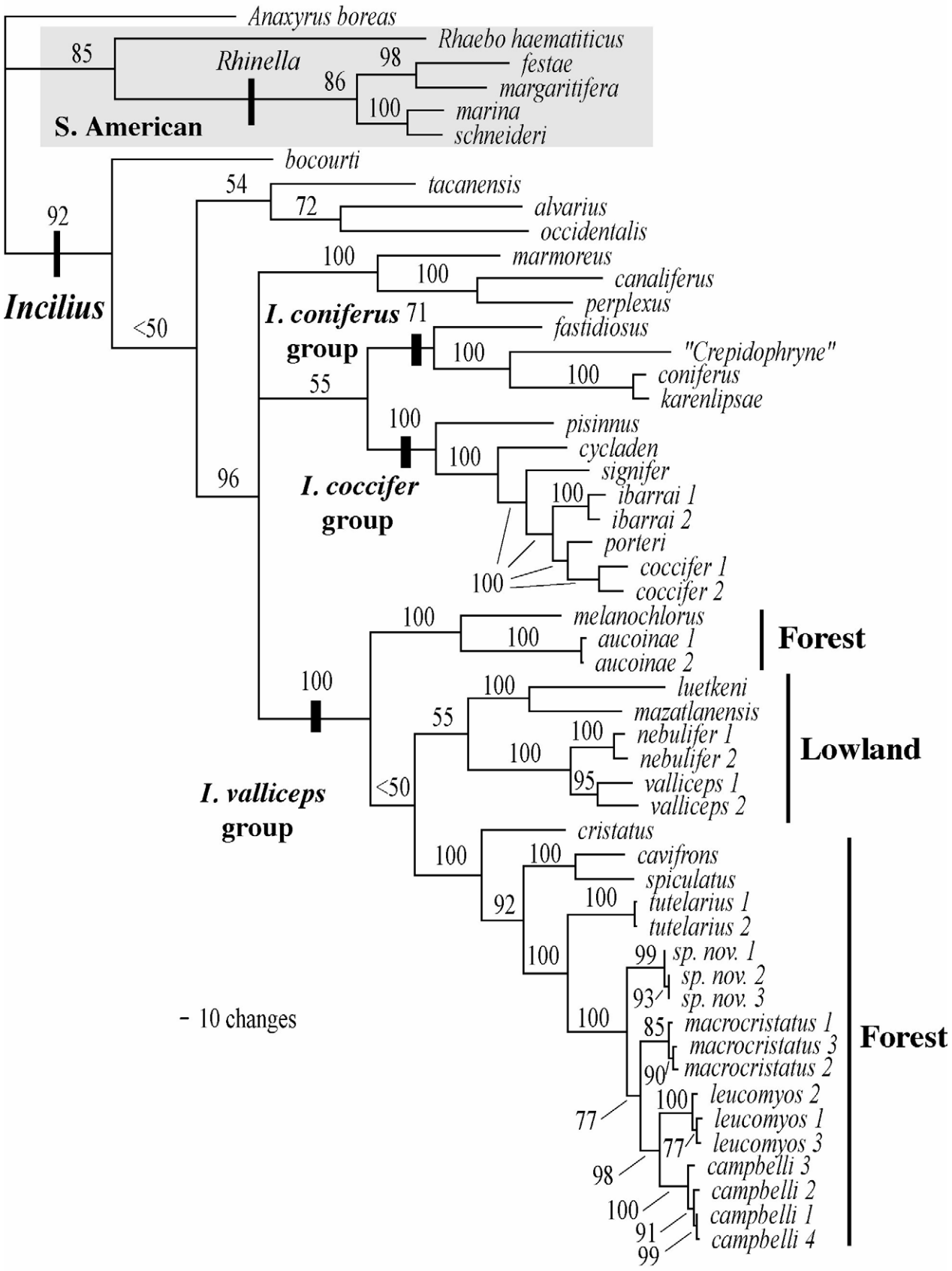

FIGURE 2. Parsimony analyses of the combined mtDNA and nuclear data (5,898 bp). A strict consensus of two trees is shown, with bootstrap values > 50 based on 1000 replicates, each with 100 random additions per replicate. The taxon *Incilius* **sp. nov.** is described by Mendelson *et al.* (in press).

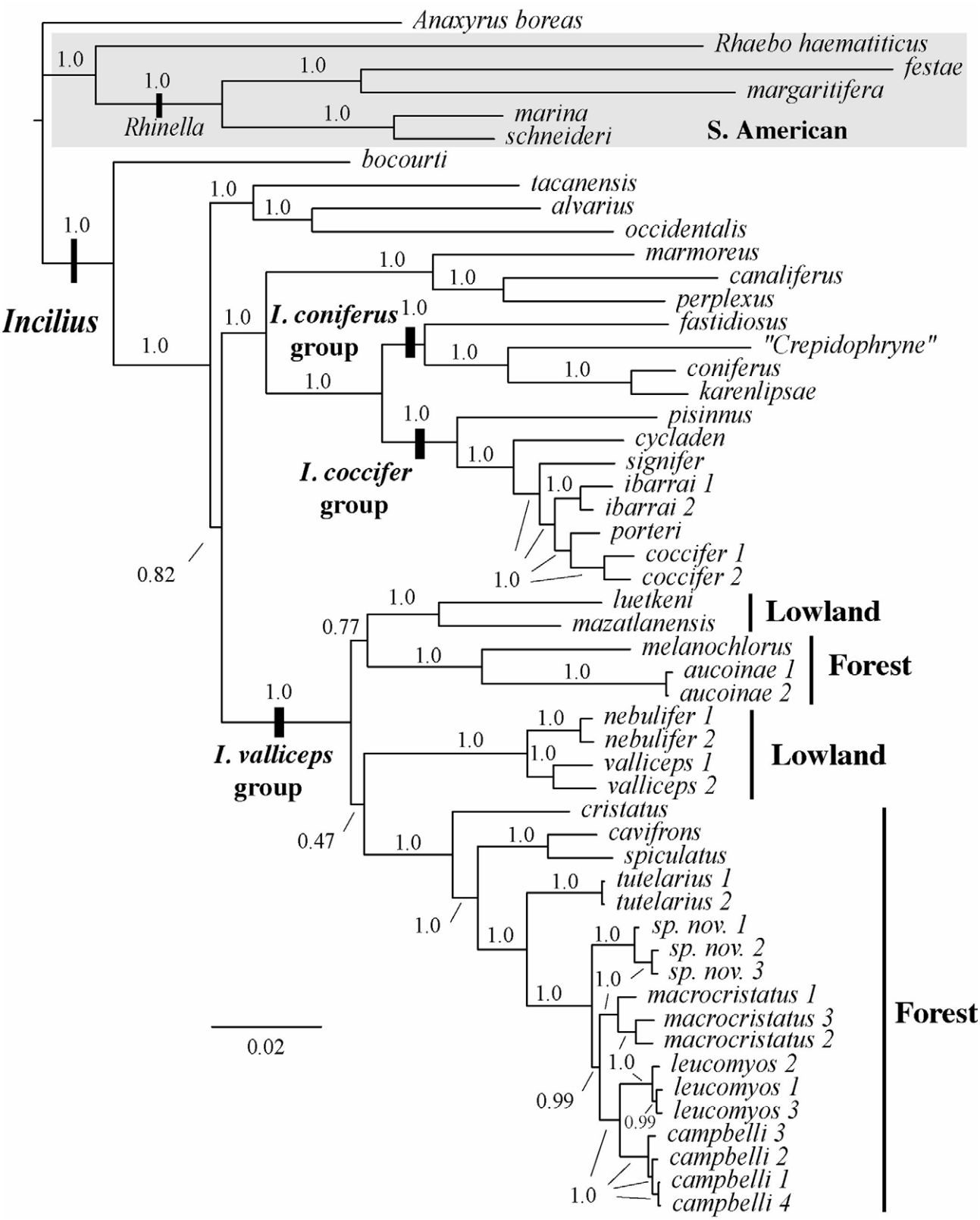

FIGURE 3. Bayesian consensus of the combined mtDNA and nuclear data (5,898 bp). Analyses were based on 50 x 10^6 generations, sampling every 1000, with the first 10,000 trees discarded as burn-in, posterior probabilities are shown for branches supported by > 0.50. The taxon *Incilius* **sp. nov.** is described by Mendelson *et al.* (in press).

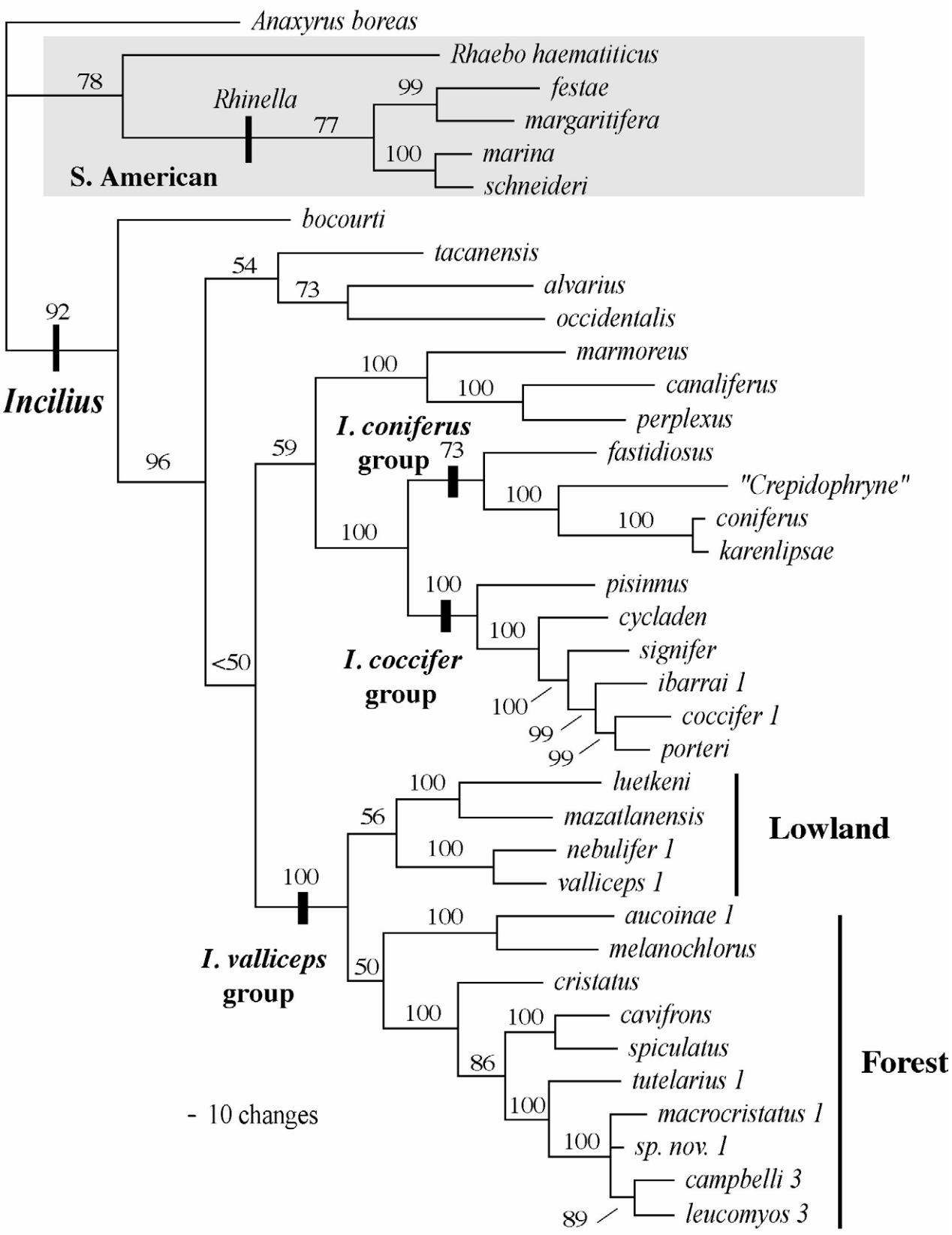

FIGURE 4. Parsimony analyses of the combined non-molecular (44 characters) and molecular data (5,898 bp). A strict consensus of two trees is shown, with bootstrap values > 50 based on 1000 replicates, with 100 random additions per replicate. The taxon *Incilius* **sp. nov.** is described by Mendelson *et al.* (in press).

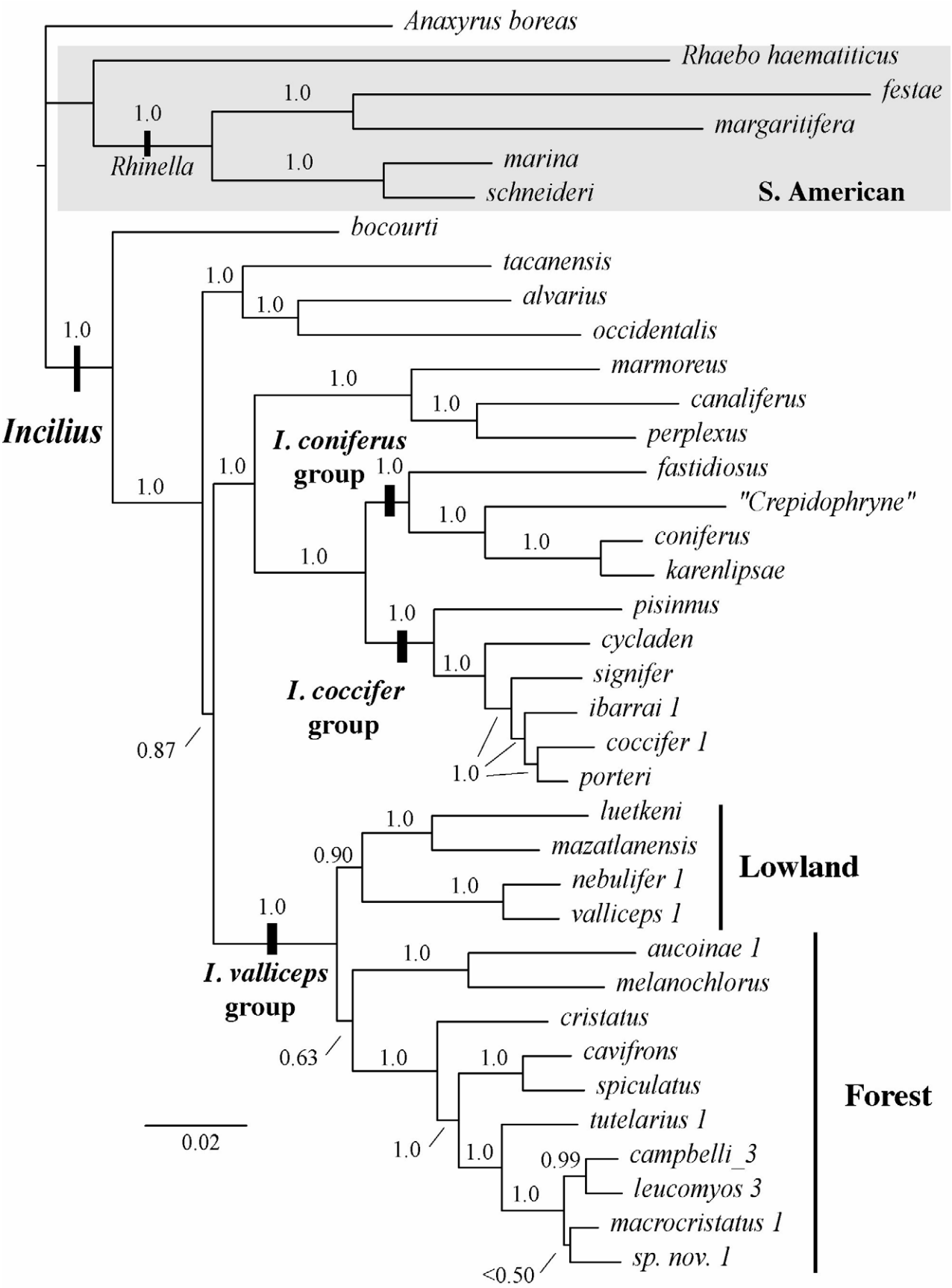

FIGURE 5. Bayesian analyses of the non-molecular (44 characters) and molecular data (5,898 bp) combined. Analyses were run for 50 x 10^6 generations, sampling every 1000, with the first 10,000 trees discarded as burn-in, posterior probabilities are shown for branches supported by > 0.50. The taxon *Incilius* **sp. nov.** is described by Mendelson *et al.* (in press).

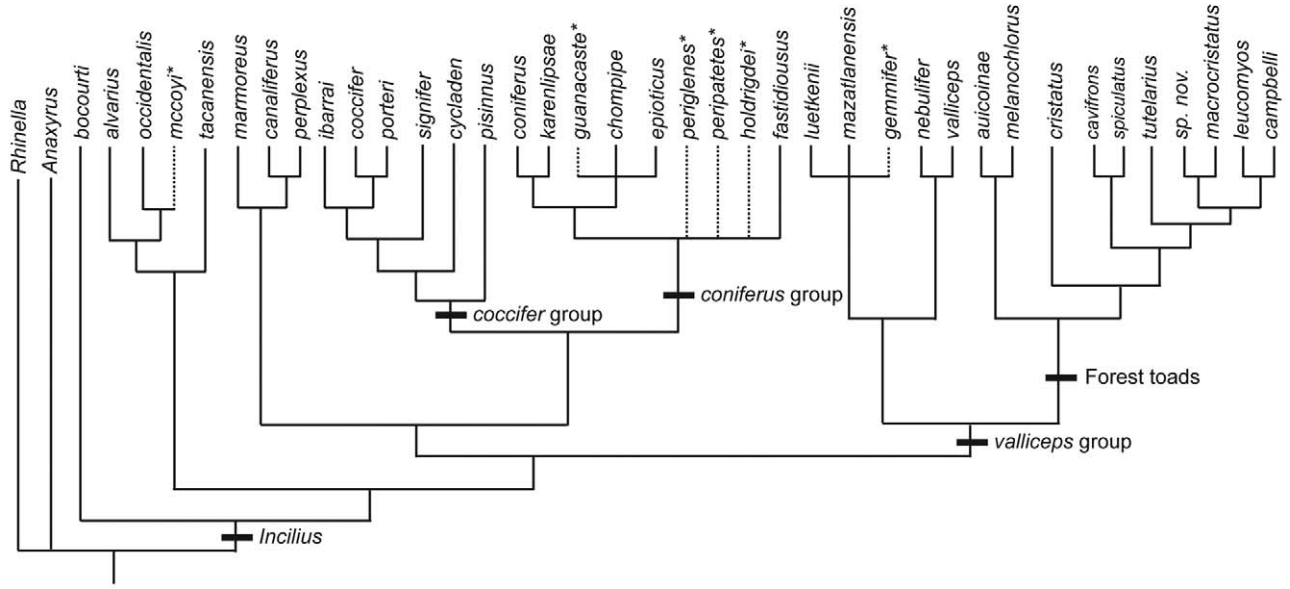

FIGURE 6. Summary hypothesis for the phylogenetic relationships among all known species of *Incilius.* Taxa indicated by an asterisk (*) and dashed lines were not included in our analyses because of lack of material available; their positions shown here are tentative, based on other lines of evidence (see Discussion). We hope that samples of these missing taxa may become available in the future, so that this hypothesis may be tested. The taxon *Incilius* **sp. nov.** is described by Mendelson *et al.* (in press).

Discussion

Phylogeny of *Incilius*. Our analyses that included molecular data are in general agreement regarding the phylogenetic position of most of the included species of *Incilius* (Figs. 2–5). The montane species *I. bocourti*, from the highlands of Nuclear Central America (Guatemala, and Chiapas, Mexico, specifically) was recovered within *Incilius* in all analyses with strong support and as the most basal divergence within *Incilius* with weak (parsimony <50% bootstrap; Fig. 2 molecular data only) to strong support (96%) in the parsimony analysis of combined data (Fig. 4) and in the Bayesian analyses (1.0 post. probs., Figs. 3, 5). Van Bocxlaer *et al.* (2010) recovered *I. bocourti* as sister to *Anaxyrus*, but this relationship was poorly supported in their results, and likely was caused by the lack of data for *I. bocourti* in their study. Van Bocxlaer *et al.*'s (2010) analyses included data for only one (12S–16S) of the three loci they considered. Our data for *I. bocourti* included all of our sampled loci. We note that the data for *I. bocourti* used by Van Bocxlaer *et al.* (2010) are from the work of Pauly *et al.* (2004), in which the taxon was also placed outside of *Incilius* (in their parsimony analysis only) and that our sample differs from that sample by only one base-pair in the 12S gene, and is identical for 16S. Therefore, we believe both samples to represent legitimate *I. bocourti* specimens, and the failure of previous studies to recover *I. bocourti* within *Incilius* was caused by low amounts of data.

Monophyletic assemblages that could reasonably be called the *I. coccifer, I. coniferus,* and *I. valliceps* groups are well supported in all analyses. The taxon *Crepidophryne* renders *Incilius* paraphyletic, being placed in a clade with another small species, *I. fastidiosus*, and as the sister to *I. coniferus* + *karenlipsae* in molecular analyses. In no analyses including molecular data was *Crepidophryne* found to be closely related to *Rhinella* (= *Rhamphophryne*) *festae*.

Within the *I. valliceps* group, sister taxa were recovered for lowland species both on the Pacific (*I. luetkenii* + *I. mazatlanensis*) and Atlantic (*I. valliceps* + *I. nebulifer*) versants; though relationships between these pairs of lowland species and the Forest toads were sometimes poorly resolved. The most notable disagreement between the parsimony and Bayesian molecular analyses are in the placement of the species pair *I. melanochlorus* + *I. aucoinae*, which were placed sister to the lowland species and the remainder of the Forest toads using parsimony (<50%; Fig. 2), or sister to the lowland Pacific Versant clade (*I. luetkenii* + *I. mazatlanensis*) in the Bayesian analysis (0.77 post. probs., Fig. 3). It is noteworthy that the addition of non-molecular data strengthened the position of *I. melanochlorus* + *I. aucoinae* as members of the Forest toads. The molecular data alone did not resolve the Forest toads as

monophyletic under parsimony (Fig. 2) nor Bayesian analyses (Fig. 3), while in combination with the non-molecular data, under both parsimony and Bayesian conditions, the Forest toads were resolved as monophyletic with weak support (50% bootstrap, 0.63 post. probs.; Figs. 4, 5, respectively). The general morphology and the ecology of the adults and larvae of *I. melanochlorus* and *I. aucoinae* are similar to that of the Forest toads, suggesting that their placement among the Forest toads may be correct (Fig. 1). The Bayesian and parsimony analyses of the combined molecular and non-molecular data are otherwise in general agreement with one another, with the primary differences being the placement of the new species (*I.* **sp. nov.**); note one of the two equally parsimonious trees had a topology identical to the Bayesian analyses. These combined analyses agree closely with the general topology of the Bayesian analysis of molecular data alone, identifying clades referable to the *I. coccifer* and *I. valliceps* groups, the Forest toads, the basal divergence of *I. bocourti*, and the clade (*I. fastidiosus* (*Crepidoprhyne* (*I. coniferus* + *I. karenlipsae*))). We consider the combined data (molecular and non-molecular) Bayesian analyses as our best hypothesis for relationships among species of *Incilius* (Fig. 5). This approach contains the most data in a combined analysis with variable, independent (unlinked) complex models of nucleotide evolution (i.e. GTR + I + ; Table 4), while maintaining a simple model for the non-molecular data (Mk + Γ, Lewis; 2001).

The non-molecular data showed high levels of homoplasy, similar to the case in the analyses of Pramuk (2006). Morphological features such as auditory apparati and unilateral vs. bilateral vocal slits show no clear patterns of evolution in these toads. Presence vs. absence of some of the salient cranial crests that typify *Incilius* lend support to some clades, and the *I. valliceps* group is nearly unique (in our analysis) by having an omosternum (also seen in *I. perplexus* and *Rhaebo haematiticus*). The monophyly of the Forest toads is partially supported by their unique breeding behaviors (discussed below). That group tends also to have a unique coloration that anuran biologists sometimes refer to as a "dead-leaf" pattern of boldly contrasting hues of black, blue-gray, and brown. Among the Forest toads, this pattern is not well developed in *I. tutelarius* and is usually sexually dimorphic, being more developed in females. We could not devise a character-coding scheme to capture these subtle details (but see Pramuk, 2006: p. 452).

Unfortunately, because of the lack of available tissue samples or prepared skeletal preparations for some species, our sampling of *Incilius* was incomplete. In an effort to provide a phylogenetic hypothesis that is more complete—and therefore useful for subsequent testing and conservation efforts—we offer an admittedly speculative cladogram in Fig. 6. This tree is based on the results of our combined Bayesian analyses (Fig. 5), with the placement of taxa for which we only had molecular data based on the Bayesian analysis (Fig. 3). We tentatively place seven species not included in our analyses—viz., *I. mccoyi, I. guanacaste, I. epioticus, I. holdridgei, I. periglenes, I. peripatetes,* and *I. gemmifer.* The taxa *guanacaste* and *epioticus* formerly were included, along with *chompipe,* in the genus *Crepidophryne* (see Vaughan & Mendelson, 2007), and we assume that they form a monophyletic group based on their nearly identical morphology. Their phylogenetic position as the sister group to *I. coniferus* (Figs. 2–5) is based on our inclusion of *I. chompipe* in our molecular analyses. The placement of *I. peripatetes* and *I. holdridgei* in the clade containing *I. fastidiosus* is based on our agreement with the argument for the close relationship among these species put forward by Savage (2002: p.195). The placement of *I. mccoyi* is based on the close relationship with *I. occidentalis* implied by Santos-Barrera & Flores-Villela (2011). We place *I. gemmifer* in the clade with *I. luetkenii* and *I. mazatlanensis* based on our admittedly speculative assessment of overall similarity and biogeography. The recently described species *I. karenlipsae* (Mendelson & Mulcahy, 2010) is placed as sister to *I. coniferus* based on cyt b and 16S mtDNA data. Based on the analyses presented by Graybeal (1995; 1997), we place the extinct species *I. periglenes* in the clade containing *Crepidophryne, I. coniferus, I. karenlipsae, I. fastidiosus, I. holdridgei,* and *I. peripatetes.* We note also that the geographic distribution of *I. periglenes* is in agreement with the overall biogeographic pattern of this clade (see discussion below; Fig. 7). We note that the taxon *I. holdridgei,* which was long assumed also to be extinct, was rediscovered as this project was reaching its conclusion (Abarca *et al.*, 2010); we urge future studies to attempt to include this species in phylogenetic analyses.

Van Bocxlaer *et al.* (2010) incorporated three samples identified as *I. valliceps,* and found the species to be non-monophyletic, with a sample from Honduras (USNM 534129) positioned as sister to *I. macrocristatus* (one of the Forest toads). This result is problematic because the taxon *I. valliceps* has been well studied and this arrangement conflicts with the results of Mulcahy & Mendelson (2000) and Mulcahy *et al.* (2006); these latter studies incorporated multiple samples referable to *I. valliceps* from throughout its extensive range. Van Bocxlaer *et al.* (2010) used data from 12S, 16S, CXCR4 genes that are posted on GenBank; the mitochondrial data are from Pramuk (2006) and the nuclear data are from Pramuk *et al.* (2008). Based on examination of the voucher specimen and comparison/reanalysis of the sequence data, we determined that the paraphyly of *I. valliceps* in Van Bocxlaer *et al.* (2010) is the result of a misidentification; USNM 534129 is *I. leucomyos,* not *I. valliceps.* This misidentification is

corroborated by our comparison of the molecular data from GenBank with our data (described above). The scope of the studies and sampling of Pramuk (2006) and Pramuk *et al.* (2008) were such that this misidentification was not evident and it does not affect conclusions reached therein. This misidentification also does not affect the general conclusions presented by Van Bocxlaer *et al.* (2010) regarding evolution of bufonids, but it does explain their non-monophyly of *I. valliceps* illustrated in their Figure S1. Otherwise, with respect to the sampling and topology presented by Van Bocxlaer *et al.* (2010:fig. S1), the position of USNM 534129 (listed there as "*I.* cf. *valliceps*") as sister to *I. macrocristatus* (listed there as "*I macrocristata*") is fully expectable, as *I. macrocristatus* and *I. leucomyos* are both well established to be in the clade of Forest toads. The misidentification of this specimen also explains the non-monophyly of *I. valliceps* and *I. nebulifer* and why *I. valliceps* (including data from this individual) was placed sister to the Forest toads in the study by Pyron & Wiens (2011). This record of *I. leucomyos* is from Quebrada Machin, Colon, Honduras, which represents a noteworthy range extension beyond the documented range of the species (McCranie & Wilson, 2002).

Taxonomic issues. The phylogenetic position of *Crepidophryne* in our results renders *Incilius* paraphyletic. Accordingly, we refer the taxon *Crepidophryne* Cope, 1889, to the synonymy of *Incilius* Cope, 1863. In doing so, we refer the three known species (sensu Vaughan & Mendelson, 2007) to *Incilius—I. chompipe, I. epioticus*, and *I. guanacaste*. Frost *et al.* (2006a: p. 217) discussed some putative morphological synapomorphies suggesting that *Rhamphophryne* may be closely related to *Crepidophryne.* All previous molecular analyses that have included species of *Rhamphophryne* (Frost *et al.*, 2006a; Pramuk, 2006, Chapparo *et al.*, 2007; Pramuk *et al.* 2008, Van Bocxlaer *et al.*, 2009; Van Bocxlaer *et al.*, 2010; Pyron & Wiens, 2011) have found it to be nested well within *Rhinella.* The study by Pramuk (2006) also included an extensive morphological dataset that corroborated the molecular placement of *Rhamphophryne* within *Rhinella.* Chapparo *et al.* (2007) and Pramuk *et al.* (2008) referred the genus *Rhamphophryne* to the synonymy of *Rhinella*. We present novel analyses here in that our data set is the first to include molecular and non-molecular data for both *Crepidophryne* and "*Rhamphophryne*" (*Rhinella festae*). We included the molecular data available in GenBank for "*Rhamphophryne festae"* and included this taxon in our non-molecular dataset. The molecular data reflected all other studies and did not support a close relationship between *Rhamphophryne* and *Crepidophryne.* However, this question deserves further attention in the form of an analysis that includes all species formerly referred to *Rhamphophryne*. In a review, Graybeal & Cannatella (1995) found no support for the monophyly of this group. Frost el al. (2006a: p. 131) mentioned the morphological similarities between *Rhamphophryne* and *Rhinella margaritifera* (sister taxa, in their analyses), but later (Frost *et al.*, 2006a, p. 217) highlighted the morphological differences between those taxa, and highlighted some morphological similarities between *Rhamphophryne* and *Crepidophryne.* The review of *Rhamphophyrne* by Trueb (1971) indicated variation with respect to potential phylogenetically informative characters such as vertebral number, presence of parotoid glands, and even the extensively webbed feet that Frost *et al.* (2006a: p. 217) listed as characteristic. We agree with the taxonomic proposition by Chapparo *et al.* (2007) to place *Rhamphophyrne* in the synonymy of *Rhinella,* as it is consistent with every analysis conducted to date, including the present study. However, that taxonomy assumes the monophyly of *Rhamphophryne* sensu Trueb (1971) and such monophyly has yet to be demonstrated, although the three taxa (*R. rostrata, R. festae,* and *R. macrorhina)* included by Van Bocxlaer *et al.* (2010) and Pyron & Wiens (2011) did form a monophyletic group in those analyses. Nonetheless, it remains possible that some, but likely not all, species of *Rhamphophryne* may be closely related to *Crepidophryne* and consequently would be included in *Incilius.*

Our non-molecular dataset recovered "*Crepidophryne"* and "*Rhamphophryne"* to be sister taxa, outside of the clade *Incilius.* However, our much larger dataset incorporating molecular and non-molecular data indicated that the evident morphological similarities between species of *"Crepidophryne"* and most species of *"Rhamphophryne"* discussed by Frost *et al.* (2006a) such as producing small clutches of large, unpigmented eggs (and likely direct development), protruding snouts, unusual bi-lobed livers, reduced auditory apparati, and fleshy webbing on the digits are convergent and not synapomorphies, as was suggested as possible by Frost *et al.* (2006a).

Prior to Mendelson (2001) and Mendelson *et al.* (2005), all populations represented by our sampling of the *I. coccifer* group were referred to the taxon *I. coccifer* (Cope, 1866). Mendelson (2001) demonstrated the validity of *I. ibarrai* (Stuart, 1954) and Mendelson *et al.* (2005) demonstrated the validity of *I. cycladen* (Lynch and Smith, 1966). Our colleague J. R. McCranie, and various co-authors, have repeatedly argued against the recognition of some species in the *I. coccifer* group, though they have brought no data to dispute them. Specifically in reference to *I. ibarrai*, as recognized by Mendelson (2001), McCranie & Wilson (2002, p.175) argued that "*…only one species of the* B. coccifer *complex,* B. coccifer*, is represented by the Honduran material."* Subsequently, an additional species

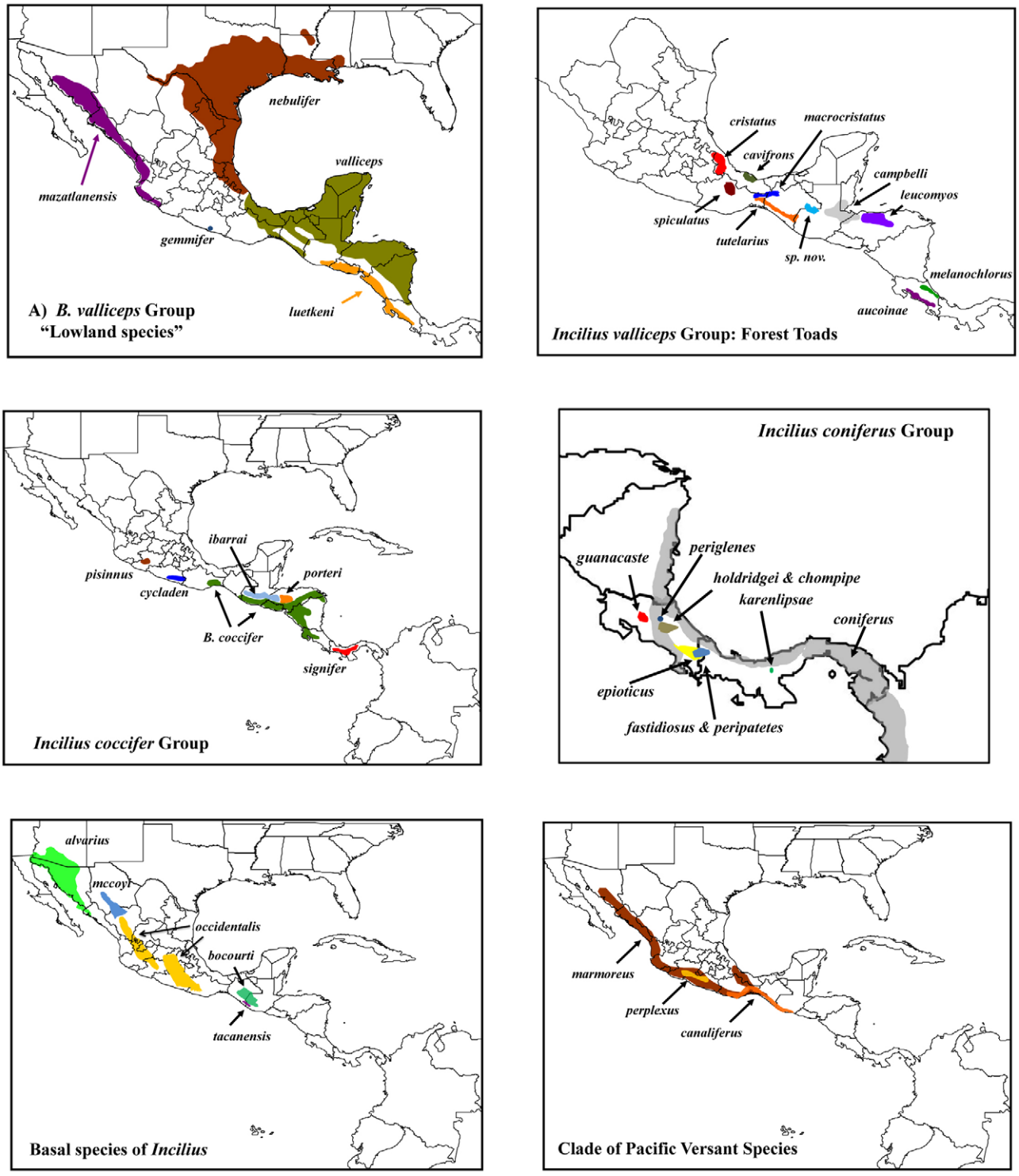

FIGURE 7. Generalized distributional patterns of the species of *Incilius*, organized by clade. This figure highlights the varying geographic scales over which evolution in each clade has proceeded.

from the complex was described from Honduras: *I. porteri* (Mendelson, Williams, Sheil, & Mulcahy, 2005). McCranie and Castañeda (2007) refer to an unpublished manuscript to claim that *I. porteri* is not valid and, by implication, neither is *I. ibarrai.* These same sentiments were repeated by McCranie (2009). Mendelson *et al.* (2005) presented morphological diagnoses of *I. porteri* and *I. ibarrai*, as well as additional new species in this complex and a preliminary molecular analysis of relationships within the group. The results of a more complete sampling of the group presented here are consistent with the recognition of multiple species in the *I. coccifer* group. Our results of the Bayesian analyses go further to suggest that the Guatemalan and Honduran samples of *I. ibarrai*

represent two species. Inasmuch as McCranie (2009) has continued to recognize *I. leucomyos* as a species distinct from the very similar and geographically proximal species *I. campbelli* (a distinction that we do not contest), we note that the morphological and molecular differences between *I. coccifer, I. ibarrai,* and *I. porteri* are evidently greater than the differences between *I. leucomyos* and *I. campbelli* (see Mendelson *et al.*, 2005, and branch lengths illustrated here in Figs. 2–5).

Historical studies of bufonids usually have incorporated names for various groups (e.g., "*Bufo valliceps* group"). Because of this history and the arguable convenience of such terms, we continue to use these terms informally to identify the *I. valliceps* and *I. coccifer* groups as well as the term "Forest toads" that we have used (Mulcahy & Mendelson, 2000) to refer to that ecologically unique subset of the *I. valliceps* group (see below); in addition we suggest informal recognition of the *I. coniferus* group. The opportunity exists to formally designate taxonomic names (e.g., genera or subgenera) to these clades, however we choose not to do so. We do not find the clade *Incilius* to be so large and cumbersome that such taxonomic steps are necessary. In general, we find subgenera to be of little taxonomic utility and generally unpopular in the systematics literature (see Frost *et al.*, 2009b). Furthermore, it is likely that additional species of *Incilius* remain to be discovered, either through field work in poorly documented regions, or through careful reviews of currently recognized species with widespread and/or naturally allopatric distributions (e.g., *I. mazatlanensis* and *I. occidentalis,* respectively). Our decision to include the Barcode of Life gene region (CO1) in this study was intended to aid surveys of any sort (e.g., biodiversity or conservation projects) in the identification of various species of *Incilius*, especially in cases where traditional morphology may be difficult (e.g., larval or subadult individuals).

Evolutionary natural history of *Incilius*. New World toads are not known for their extensive diversity of reproductive strategies. Similarly, most species generally are considered to be somewhat of habitat generalists, readily tolerating moderate to extensive human disturbance of their habitats. So, it is remarkable that some clear evolutionary shifts in reproductive ecology of toads have arisen within such a moderately speciose genus as *Incilius.* The familiar reproductive strategy of placing eggs in temporary or permanent puddles and ponds during the rainy season clearly is the plesiomorphic condition for *Incilius*, and indeed this strategy is most common among the included species. The clade we refer to as the Forest toads represent a distinct switch in these behaviors. All species of Forest toads place their eggs in small streams in the dry season (Mendelson *et al.*, 1999). Some bufonids (e.g., *Atelopus* spp., *Rhinella chrysophora*) have tadpoles with morphologies evidently adapted for life in streams, such as large oral discs with numerous tooth rows and abdominal suckers for maintaining position on rocks in swiftly flowing water. Although the Forest toad larvae develop in such environments, they lack anything similar in terms of specialized morphologies associated with streams. It can be interpreted that breeding in streams during the dry season is an adaptive strategy to avoid scouring events caused by heavy rainfall that can have the effect of washing eggs and larvae far downstream, if not killing them outright. Nonetheless, atypical rain patterns during the dry season can have this same effect. For example, the eggs and larvae of entire cohorts of *I. cavifrons* can be destroyed during occasional "*Norte*" winter storm systems that move through the Sierra de los Tuxtlas, Veracruz, Mexico (Shannon & Werler, 1955; JRM pers. observation); McCranie & Wilson (2002: p. 183) described a similar observation of *I. leucomyos* in the month of June in Honduras. In such situations, there are apparent negative consequences in the lack of specialized stream-adapted mouth parts and abdominal suckers.

The other derived condition with respect to reproduction is in the case of three species formerly referred to the genus *Crepidophryne*. Vaughan & Mendelson (2007) reviewed the evidence to suggest that these three species are endotrophic, having direct development wherein the eggs are deposited in leaf litter on the forest floor and the young hatch out as fully formed toadlets, with no intervening free-living aquatic tadpole stage. It is important to note, however, that this aspect of their biology has never been confirmed through direct observations of these elusive toads, but rather is inferred based on indirect evidence (see Vaughan & Mendelson, 2007, and citations therein). If these toads do have direct development, then this stands as a rather surprising evolutionary shift in that direct development mirroring that known (or suspected) in the relatively unrelated (Pyron & Wiens, 2011) bufonids *Oreophrynella* and *Osornophyrne* (Wells, 2007). Relevant to earlier discussions in this paper, endotrophy is also suspected in the species formerly referred to *Rhamphophryne* (Thibaudeau & Altig, 1999). Mendelson & Mulcahy (2010; see also Novak & Robinson, 1975) reviewed the possibility that the reproductive behavior of inguinal amplexus (versus axillary amplexus), which is rare in hyloid frogs, may possibly occur in multiple species in the *I. coniferus* group, including the species formerly referred to *Crepidophryne*. However, Bozo-Oviedo & Solano-Barquero (2009) reported *C. epiotica* to have a slightly modified version of axillary amplexus.

Our field experiences with most of the species of *Incilius*, as well as reviews of the literature and especially field notes, indicate that the species are associated with available habitats in differing ways. Most are relatively tolerant of human habitat disruption, and individuals of almost all species may be found in a variety of disturbed habitats including towns, agricultural lands, roadside ditches, and every variety of secondary growth. The species pair *I. nebulifer* and *I. valliceps* has been described as "weedy" as they appear to be far more common in disturbed habitats than in primary forests (Mendelson, 1994; Lee, 1996; Mendelson *et al.*, 1999; McCranie & Wilson, 2002). In contrast, the small-sized species of the *I. coniferus* group (i.e., *I. chompipe, I. guancaste,* and *I. epioticus*, and *I. fastidiosus)* are leaf-litter specialists typically associated with primary forest habitats (Lips & Krempels, 1995; Savage, 2002). All of the Forest toads inhabit wet forests in the form of rainforest (e.g., *I. campbelli;* Mendelson, 1994) or cloudforest (e.g., *I. macrocristatus;* Mendelson, 1997a) and appear to be highly intolerant of even relatively minor habitat disruption. In some areas where agricultural clearings abut large tracts of undisturbed forest, an endemic species of Forest toad may occur in sharp ecological parapatry with invasive (if native) species such as *I. valliceps* (Mendelson, 1990; 1994), while in other areas it would appear that the invasive species has replaced the endemic in relatively small patches of "interior" forest (Urbina-Cardona *et al.*, 2006). The latter study found *I. valliceps* instead of *I. cavifrons* in patches of primary forest in a general area where the latter formerly was common (Firschein, 1950; Mendelson, 1997b). In any case, virtually never are species of Forest toads found in syntopy with other bufonids. Although one cannot "code" for a character such as "ecological sensitivity to habitat disturbance" to be included in a phylogenetic analysis, our review indicates that this feature of their biology typifies the entire clade of Forest toads. We note that five out of the nine Forest toad species are listed as Endangered or Critically Endangered (www.iucnredlist.org; verified 1 November 2011), suggesting such sensitivity to change has predetermined this clade for endangerment or extinction in the wake of human activities such as land-use change and climate change.

The phenomenon of hybridization among species of bufonids—especially those species referred to the genus *Bufo* prior to the work of Frost *et al.* (2006a)—was extensively studied by W. F. Blair and students (see Blair, 1972, for review; Tandy & Keith, 1972). In nature, some bufonids hybridize relatively readily, although this phenomenon has been best documented among species of *Anaxyrus* (e.g., Sullivan, 1986; Green, 1996; Masta *et al.*, 2002). Nonetheless, the general perception remains that non-atelopodid bufonids are "rampant hybridizers" both in the lab and in the wild. It seems noteworthy then that examination of thousands of specimens of *Incilius* (see Mendelson, 1997c) plus innumerable field observations has not revealed a single specimen from Mesoamerica that reasonably could be identified as a hybrid, based on data from either molecules and/or morphology. Mulcahy *et al.* (2006) identified sharp parapatry in the wild for the species pair *I. nebulifer* + *I. valliceps*, with no evidence of hybridization, although their sample size was small and they used only mtDNA. Further, we are not aware of any published report of wild-caught hybrids involving two species of *Incilius*, nor between a species of *Incilius* and any species of either *Rhinella* or *Rhaebo.* While many species of *Incilius* may be broadly sympatric, or even syntopic with *Rhinella marina*, few species of *Incilius* are sympatric with one another. Although some of these species may appear to be sympatric (e.g., Fig. 7), they are often narrowly separated ecologically (see above). These lines of evidence, of course, do not indicate that such hybridization does not occur, but are suggestive that it may be rare and/or that these species may be subject to strong negative pre- or post-zygotic selection. The exception to the generality about *Incilius* occurs at the northeastern end of their distribution—where *I. nebulifer* ranges into Texas and Louisiana, USA. In this region *I. nebulifer* is known to hybridize with *A. fowleri* (JRM pers. obs.; Vogel & Johnson, 2008). In studies in Louisiana, USA, Vogel & Johnson (2008) posited that habitat disturbance, coupled with the invasive nature of *I. nebulifer,* has resulted in both the ecological opportunity and the reality of hybridization between *I. nebulifer* and *A. fowleri.* The offspring of these crosses may reach adult size in the wild (JRM pers. obs.) but typically are sterile (Vogel & Johnson, 2008). Thus, despite apparent opportunities for hybridization within *Incilius* and among other genera, the only instances occur with *Anaxyrus* spp. in southeastern North America, as was duly noted by Blair (1972, p. 205): "*The* americanus *group is the only other North American species group with which metamorphosis is known in both reciprocals of crosses with the* valliceps *group*." A point elegantly quantified by an Index of Postzygotic Isolation, based on Blair's own data, by Malone & Fontenot (2008).

Because of its broad distribution and evident evolution in both lowland and upland areas, as well as in both humid and subhumid habitats, the genus *Incilius* offers an interesting model for studies in the historical biogeography of Mesoamerica. Although some biogeographical patterns seem evident in our topologies, we prefer to await a tree with stronger support and more complete resolution before invoking detailed scenarios related to cladogenesis

in this group. Nevertheless, a review of Figure 7 suggests that each of the major clades of *Incilius* has experienced speciation in remarkably different spatial and ecological contexts. The basal lineage comprising *I. alvarius*, *I. occidentalis*, and *I. tacanensis* includes species occurring, respectively, in the Sonoran Desert, the Sierra Madrean and Trans-Mexican Volcanic cordilleras, and a small section of the Pacific volcanic chain straddling the Mexico–Guatemala border. This unusual biogeographic association, coupled with the unique morphology of *I. alvarius* (e.g., smooth skin, tibial glands, etc.) may represent an old lineage that has experienced substantial extinction over time. The *I. coccifer* group is a stark contrast to its sister clade (the *I. coniferus* group). The *I. coccifer* group is scattered across most of Mesoamerica, occurring in both humid and subhumid habitats and at a large variety of elevations. The *I. coniferus* group, however, contains one widespread lowland species (*I. coniferus*) and a series of highland endemics partitioned at a very fine scale along the Talamanca Cordillera of Lower Central America.

Lastly, the *I. valliceps* group presents basal divergences, in an unresolved order, resulting in North–South paired taxa on both the Pacific and Atlantic versants, and then a clade of Forest toads that occur mostly on isolated sky islands in southern Mexico and Nuclear Central America, with a basal Atlantic–Pacific species pair in Lower Central America. Based on simple relative divergences, we can infer that the Atlantic–Pacific divergence in lowland species of the *I. valliceps* group preceded subsequent divergences along the respective versants. Our results also suggest that the biogeographic history of the Forest toads may be quite similar to that of the viperid snakes recently studied by Castoe *et al.* (2009). This may be particularly so with the snakes *Bothriechis* and *Atropoides*, where basal lineages occur in the Talamanca Cordillera in Lower Central America, and more recent divergences occur in Nuclear Central America. We hope that our results presented here offer a basis for more complete sampling, and more refined studies of this group of toads, because indeed they appear to offer a remarkable system for the study of evolution and biogeography in the complex regions of Mesoamerica.

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APPENDIX I. GenBank Accession numbers for voucher specimens used in this study (pending).

Taxon	Voucher No.	CXCR4	RAG1	16S	12S
Anaxyrus boreas	MVZ 223292	DQ306499[a]	HM563973	HM563856	DQ158436[b]
Rhaebo haematiticus	MVZ 164805	HM563888	HM563974	HM563857	HM563815
Rhinella marina	KU 217482	DQ306544[a]	DQ158393[b]	DQ415571[c]	DQ158474[b]
Rhinella schneideri	KU 289057	DQ306528[a]	DQ158399[b]	DQ415572[c]	DQ158480[b]
Rhinella margaritifer	KU 215146	HM563889	HM563975	HM563858	HM563816
Rhinella festae	KU 217501	DQ306521	DQ158349	DQ158423	DQ158423
Incilius alvarius	UTA A-53924	HM563891	HM563977	HM563860	HM563818
Incilius aucoinae_1	UCR 14323	HM563892	HM563978	HM563861	HM563819
Incilius aucoinae_2	UCR 14324	HM563893	HM563979	HM563862	HM563820
Incilius bocourti	UTA A-50920	HM563894	HM563980	HM563863	HM563821
Incilius campbelli_1	USNM 326155	HM563895	HM563981	HM563864	HM563822
Incilius campbelli_2	USNM 326161	HM563896	HM563982	HM563865	HM563823
Incilius campbelli_3	KU 221203	HM563897	HM563983	AY008253[d]	HM563824
Incilius campbelli_4	UTA A-50902	HM563898	HM563984	HM563866	HM563825
Incilius canaliferus	UTA A-47640	HM563899	HM563985	HM563867	HM563826
Incilius cavifrons	UTA A-ENS 10384	HM563900	HM563986	HM563868	HM563827
Incilius chompipe	UCR 16075	HM563890	HM563976	HM563859	HM563817
Incilius coccifer_1	KU 290030	DQ306526[a]	DQ158366[b]	AY927856[e]	DQ158443[b]
Incilius coccifer_2	TCWC 83998	HM563901	HM563987	AY929302[e]	HM563828
Incilius coniferus	MVZ 203775	HM563902	HM563988	AY927859[e]	HM563829
Incilius cristatus	EBUAP 544	HM563903	HM563989	HM563869	HM563830
Incilius cycladen	UTA A-54847	HM563904	HM563990	AY927858[e]	HM563831
Incilius fastidiousus	MVZ 217438	-	-	HM563870	AY680248[f]
Incilius ibarrai_1	UTA A-53662	HM563905	HM563991	AY927854[e]	HM563832
Incilius ibarrai_2	UTA A-52528	HM563906	HM563992	AY927855[e]	HM563833
Incilius karenlipsae	UTA-A-59522	-	-	GU552454	-
Incilius leucomyos_1	UTA A-50642	HM563907	HM563993	HM563871	HM563834
Incilius leucomyos_2	USNM 559731	HM563908	HM563994	HM563872	HM563835
Incilius leucomyos_3	UTA A-MEA 892	HM563909	HM563995	HM563873	HM563836
Incilius luetkeni	UTA A-50877	HM563910	HM563996	HM563874	HM563837
Incilius macrocristatus_1	MZFC-EPR 37	HM563911	HM563997	AY008251[d]	HM563838
Incilius macrocristatus_2	MZFC-259	HM563912	HM563998	HM563875	HM563839
Incilius macrocristatus_3	UTA-JAC 7993	-	-	JN867995	-
Incilius marmoreus	UTA A-13032	HM563913	HM563999	HM563876	HM563840
Incilius mazatlanensis	MVZ 132967	HM563914	HM564000	HM563877	HM563841
Incilius melanochlorus	MVZ 229635	HM563915	HM564001	HM563878	HM563842
Incilius nebulifer_1	UTA A-52489	HM563916	HM564002	AY008169[d]	HM563843
Incilius nebulifer_2	UTA A-54860	HM563917	HM564003	DQ415599[c]	HM563844
Incilius occidentalis	UF-JHT 2249	HM563918	HM564004	HM563879	HM563845
Incilius perplexus	UTA A-13543	HM563919	HM564005	HM563880	HM563846
Incilius pisinnus	UTA A-54851	HM563920	HM564006	HM563881	HM563847

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APPENDIX 1. (continued)

Taxon	Voucher No.	CXCR4	RAG1	16S	12S
Incilius porteri_1	UTA A-JAC 26118	HM563921	HM564007	HM563882	HM563848
Incilius signifer	UTA A-JRM 4968	HM563922	HM564008	HM563883	HM563849
Incilius spiculatus	UTA A-54853	HM563923	HM564009	HM563884	HM563850
Incilius tacanensis	MVZ 170329	HM563924	HM564010	HM563885	HM563851
Incilius tutelarius_1	MZFC 5262	HM563925	HM564011	HM563886	HM563852
Incilius tutelarius_2	MZFC 5277	HM563926	HM564012	HM563887	HM563853
Incilius valliceps_1	MZFC JRM-3868	HM563927	HM564013	AY008211[d]	HM563854
Incilius valliceps_2	USNM 530601	HM563928	HM564014	AY008227[d]	HM563855
I. **sp. nov.**_*1*	UTA A-52597	JN867949	-	JN867996	-
I. **sp. nov.**_*2*	UTA A-52591	JN867950	-	JN867997	-
I. **sp. nov.**_*3*	MVZ 143380	-	-	JN867998	-

continued.

Taxon	Voucher No.	cyt b	ND2	tRNAs	CO1
Anaxyrus boreas	MVZ 223292	HM563929	JN868005	JN868043	JN867951
Rhaebo haematiticus	MVZ 164805	HM563930	JN868019	JN868054	JN867969
Rhinella marina	KU 217482	DQ415597[c]	-	-	-
Rhinella schneideri	KU 289057	DQ415598[c]	-	-	-
Rhinella margaritifer	KU 215146	HM563931	JN868028	JN868063	JN867978
Rhinella festae	KU 217501	-	-	-	-
Incilius alvarius	UTA A-53924	HM563933	JN868006	JN868044	JN867952
Incilius aucoinae_1	UCR 14323	HM563934	JN868007	-	JN867953
Incilius aucoinae_2	UCR 14324	HM563935	JN867999	JN867999	JN867954
Incilius bocourti	UTA A-50920	HM563936	JN868008	JN868045	JN867955
Incilius campbelli_1	USNM 326155	HM563937	JN868009	JN868046	JN867956
Incilius campbelli_2	USNM 326161	HM563938	JN868010	JN868047	JN867957
Incilius campbelli_3	KU 221203	HM563939	JN868011	JN868048	JN867958
Incilius campbelli_4	UTA A-50902	HM563940	JN868012	JN868049	JN867959
Incilius canaliferus	UTA A-47640	HM563941	JN868013	-	JN867960
Incilius cavifrons	UTA A-ENS 10384	HM563942	JN868014	JN868050	JN867961
Incilius chompipe	UCR 16075	HM563932	JN868000	JN868000	JN867962
Incilius coccifer_1	KU 290030	HM563943	JN868015	JN868051	JN867963
Incilius coccifer_2	TCWC 83998	HM563944	JN868016	JN868052	JN867964
Incilius coniferus	MVZ 203775	HM563945	JN868001	JN868001	JN867965
Incilius cristatus	EBUAP 544	HM563946	JN868017	JN868053	JN867966
Incilius cycladen	UTA A-54847	HM563947	JN868018	-	JN867967
Incilius fastidiousus	MVZ 217438	HM563948	JN868002	JN868002	JN867968
Incilius ibarrai_1	UTA A-53662	HM563949	JN868020	JN868055	JN867970
Incilius ibarrai_2	UTA A-52528	HM563950	JN868021	JN868056	JN867971
Incilius karenlipsae	UTA-A-59522	GU552455	-	-	-
Incilius leucomyos_1	UTA A-50642	HM563951	JN868022	JN868057	JN867972
Incilius leucomyos_2	USNM 559731	HM563952	JN868023	JN868058	JN867973
Incilius leucomyos_3	UTA A-MEA 892	HM563953	JN868024	JN868059	JN867974

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APPENDIX 1. (continued)

Taxon	Voucher No.	cyt b	ND2	tRNAs	CO1
Incilius luetkeni	UTA A-50877	HM563954	JN868025	JN868060	JN867975
Incilius macrocristatus_1	MZFC-EPR 37	HM563955	JN868026	JN868061	JN867976
Incilius macrocristatus_2	MZFC-259	HM563956	JN868027	JN868062	JN867977
Incilius macrocristatus_3	UTA-JAC 7993	JN867945	-	-	-
Incilius marmoreus	UTA A-13032	HM563957	JN868003	JN868003	JN867979
Incilius mazatlanensis	MVZ 132967	HM563958	JN868029	JN868064	JN867980
Incilius melanochlorus	MVZ 229635	HM563959	JN868030	JN868065	JN867981
Incilius nebulifer_1	UTA A-52489	HM563960	JN868031	JN868066	JN867982
Incilius nebulifer_2	UTA A-54860	HM563961	JN868032	JN868067	JN867983
Incilius occidentalis	UF-JHT 2249	HM563962	JN868033	-	JN867984
Incilius perplexus	UTA A-13543	HM563963	JN868034	-	JN867985
Incilius pisinnus	UTA A-54851	HM563964	JN868004	JN868004	JN867986
Incilius porteri_1	UTA A-JAC 26118	HM563965	JN868035	-	JN867987
Incilius signifer	UTA A-JRM 4968	HM563966	JN868036	JN868068	JN867988
Incilius spiculatus	UTA A-54853	HM563967	JN868037	JN868069	JN867989
Incilius tacanensis	MVZ 170329	HM563968	JN868038	JN868070	JN867990
Incilius tutelarius_1	MZFC 5262	HM563969	JN868039	JN868071	JN867991
Incilius tutelarius_2	MZFC 5277	HM563970	JN868040	JN868072	JN867992
Incilius valliceps_1	MZFC JRM-3868	HM563971	JN868041	JN868073	JN867993
Incilius valliceps_2	USNM 530601	HM563972	JN868042	JN868074	JN867994
I. **sp. nov.***_1*	UTA A-52597	JN867946	-	-	-
I. **sp. nov.***_2*	UTA A-52591	JN867947	-	-	-
I. **sp. nov.***_3*	MVZ 143380	JN867948	-	-	-

Footnote for Table in Appendix I. [a]Pramuk *et al.*, 2008; [b]Pramuk 2006; [c]Mulcahy *et al.*, 2006; [d]Mulcahy and Mendelson, 2000; [e]Mendelson *et al.*, 2005; [f]Pauly *et al.*, 2004.

APPENDIX II. Specimens examined for non-molecular data..

Anaxyrys boreas: United States: Colorado: Gunnison Co.: Gothic, 10,000 ft. (KU 135222–23).
Incilius alvarius: United States: Arizona: Pima Co.: 16 mi S Tucson (KU 14082); Santa Cruz Co.: 5 mi N Tucumcari (KU 14081).
Incilius aucoinae: Costa Rica: Puntarenas: 2 km NW Dominical, 10 m (KU 91667).
Incilius bocourti: Guatemala: Huehuetenango: Laguna de Vecha (KU 117369, 117371).
Incilius campbelli: Guatemala: El Peten: 11 km NNW Chinaja (KU 55898).
Incilius canaliferus: Guatemala: Suchitepequez: Volcan Zuníl (CAS 70560, 70619).
Incilius cavifrons: Mexico: Veracruz: S slope Volcan San Martin (KU 58287).
Incilius coccifer: Costa Rica: Puntarenas: 4 km WNW Esparta (KU 68147–49).
Incilius coniferus: Costa Rica: Cartago: Moravia de Turriabla, 1200 m (KU 68150–51); Tapanti, 1200 m (KU 91814–15).
Incilius cristatus: Mexico: Puebla: Tezuitlán (KU 39587).
Incilius epioticus: Panama: Bocas del Toro: N slope Cerro Pando, 1450 m (KU 117383, 107394).
Incilius fastidiosus: Panama: Bocas del Toro: N slope Cerro Pando, 1810 m (KU 117372–73).
Incilius holdridgei: Costa Rica: Heredia: Rama Sur Río Las Vueltas, 2100 m (KU 117377–79).
Incilius ibarrai: Guatemala: Jalapa: Jalapa (TNHC 54532).
Incilius leucomyos: Costa Rica: Atlantida: 15 km E La Ceiba, Corozal Mtns (LACM 47308).
Incilius luetkenii: Costa Rica: Puntarenas: 2.4–3.0 km NW Esparta (KU 68153); Nicaragua: Managua: Tipitapa (KU 84928–29); Rivas: 1.5 km N Moyogalpa (KU 84926).
Incilius occidentalis: Mexico: Puebla: 14.4 km W Huachinango (KU 59871).
Incilius macrocristatus: Mexico: Chiapas: 6. 2 km S Rayon Mescalapa, 1690 m (KU 58302).

Incilius marmoreus: Mexico: Guerrero: 5.6 km S San Andreas de la Cruz, 420 m (KU 84893–94); Oaxaca: 4.5 km W Tehuantepec (KU 59865).

I. mazatlanensis: Mexico: Sinaloa: 6 km NE El Fuerte, 150 m (KU 78985); Sonora: between Alamos and Minas Nuevas, 427 m (KU 186792).

Incilius melanochlorus: Costa Rica: Turriabla, IAIA (KU 28356); Turriabla, IAIA, Río Reventazon (KU 32819); jct Rio Tuis and Rio Reventazon (KU 65530); Esquinas Bridge at Turriabla (KU 32809); Pavones, Turriabla (KU 139994, 139999–40000).

Incilius nebulifer: USA: Texas: Bexar Co.: Somerset (KU 20422).

Incilius perplexus: Mexico: Guerrero: 20 km S Iguala (KU 186795); Morelos: 3.5 km W Cuautlixco, 1300 m (KU 84896, 84898).

Incilius spiculatus: Mexico: Oaxaca: Vista Hermosa, 1600 m (KU 86671); 2.8 km S Vista Hermosa, 1570 m (KU 137523).

Incilius tutelarius: Mexico: Oaxaca: Colonia Rodulfo Figueroa, S of Cerro Baul, 20.0 km W Rizo de Oro (UTA A-4184). *Incilius valliceps:* Mexico: Chiapas: 14.4 km SW Las Cruces, 700 m (KU 68155–56); Oaxaca: 6 km N Palomares (KU 59874–76).

Rhaebo haematiticus: Panama: Darien: Cerro Quia, 740 m (KU 96160, 96162); Tacaruna, 550 m (KU 77659).

Rhinella festae: Peru: Amazonas: vicinity of Galilea, on Rio Santiago, 200 m (USNM 568751–53,568764, 568766).

Rhinella margaritifera: Ecuador: Napo: Santa Cecilia, 340 m (KU 152909).

Rhinella marina: Nicaragua: Managua: 3 mi SW Managua (KU 42566); Rivas: Lago de Nicaragua, Isla de Ometepe (KU 84938).

APPENDIX III. Non-molecular character descriptions.

Illustrations and full descriptions of Characters 1–34 were presented by Mendelson (1997c). Terminology used for cranial crests follows that used by Mendelson (1994). Following the logic put forward by Wilkinson (1992) and Campbell and Frost (1993), multistate Characters 2, 7, 8, and 37 were treated as ordered. The remaining multistate characters were treated as unordered. For taxa lacking certain osteological elements; e.g., *Incilius (= Crepidophryne) epioticus* lacks the neopalatines), characters relating to those elements were coded as unknown, using the symbol "?."

Character Transformation Series

1. **Supraorbital flange on frontoparietals:**
   0: absent (frontoparietal does not enter orbit)
   1: present (frontoparietal enters orbit)
2. **Frontoparietal-nasal contact:**
   0: no contact
   1: lateral contact only
   2: full contact
3. **Contact between frontoparietals:**:
   0: medial contact through entire length
   1: frontoparietals divergent anteriorly
4. **Occipital artery pathway:**
   0: open (entirely)
   1: partially covered (open anteriorly)
   2: partially covered (open posteriorly)
   3: fully covered
5. **Canthal crest (on nasal):**
   0: absent
   1: present
6. **Preorbital crest (on maxillary process of nasal):**
   0: absent
   1: present
7. **Supraorbital crest (on frontoparietal):**
   0: absent
   1: present
   2: present, hypertrophied, thick
   3: present, flared vertically, thin
8. **Parietal crest (on frontoparietal):**
   0: absent
   1: present
   2: present, hypertrophied
9. **Postorbital crest (on frontoparietal and squamosal):**

0: absent
1: present

10. Supratympanic crest (on otic ramus of squamosal):
0: absent
1: present

11. Pretympanic crest (on zygomatic ramus of squamosal):
0: absent
1: present

12. Suborbital crest (on pars facialis of maxilla):
0: absent;
1: present.

13. Extent of medial separation between neopalatines:
0: widely separated; contact sphenethmoid only marginally
1: nearly in contact at midline of sphenethmoid

14. Ventral ridge on neopalatine:
0: ridge absent
1: ridge present
2: bearing odontoids

15. Width of medial head of neoplatine:
0: same width as at mid-length
1: broader than width at mid-length

16. Width of lateral head of neopalatine:
0: same with as at mid-length
1: broader than width at mid-length

17. Anterior extent of cultriform process of parasphenoid:
0: reaches level of planum antorbitale
1: does not reach level of planum antorbitale

18. Ventral crest on alae of parasphenoid:
0: absent
1: present

19. Pterygoid process on pars palatina of maxilla:
0: absent
1: present

20. Shape of quadratojugal:
0: slender
1: robust

21. Nature of maxilla-quadratojugal overlap:
0: maxilla lateral to qudratojugal
1: maxilla ventral to quadratojugal
2: maxilla dorsal to quadratojugal

22. Medial ramus of pterygoid:
0: abuts parasphenoid ala
1: broad contact, or overlap, with parasphenoid ala, along shared lateral margins
2: overlaps parasphenoid ala ventrally

23. Length of zygomatic ramus of squamosal:
0: short
1: long, nearly in contact with maxilla

24. Relationship between zygomatic and ventral rami of squamosal:
0: space between zygomatic and ventral rami filled with bone
1: space between zygomatic and ventral rami not filled with bone

25. Shape of anterior tip of nasals:
0: short, broad
1: elongate, narrow
2: elongate, broad

26. Angle of profile of nasals:
0: straight
1: curved ventrally

27. Stapes:
0: stapes absent
1: stapes present

28. Shape of stapes:

0: blade-shaped
1: rod-shaped

29. Xiphisternum:
0: free, small
1: free, large

30. Omosternum:
0: absent
1: present

31. Vocal slits:
0: absent
1: unilateral
2: bilateral

32. Parotoid glands:
0: present, distinct, parallel to midline, oblong or ovoid
1: present, distinct, divergent, ovoid or triangular
2: present, distinct, small, round
3: present, indistinct, tiny cluster of pores at corner of cranial crests

33. Lateral descending row of tubercles on skin:
0: absent
1: present

34. Inguinal fat bodies:
0: absent;
1: present.

35. Breeding site:
0: ponds
1: streams
2: leaf litter

36. Breeding season:
0: wet season
1: dry season

37. A-2 gap in larval mouthparts:
0: absent
1: present, narrow
2: present, wide

38. Coloration of caudal musculature of tadpole:
0: uniformly dark
1: dark, with pale dorsal saddles
2: pale, with dark mottling

39. Development of offspring
0: larval
1: direct

40. Eggs
0: numerous, small, pigmented
1: few, large, unpigmented

41. Webbing of hand and foot
0: thin
1: thick, fleshy

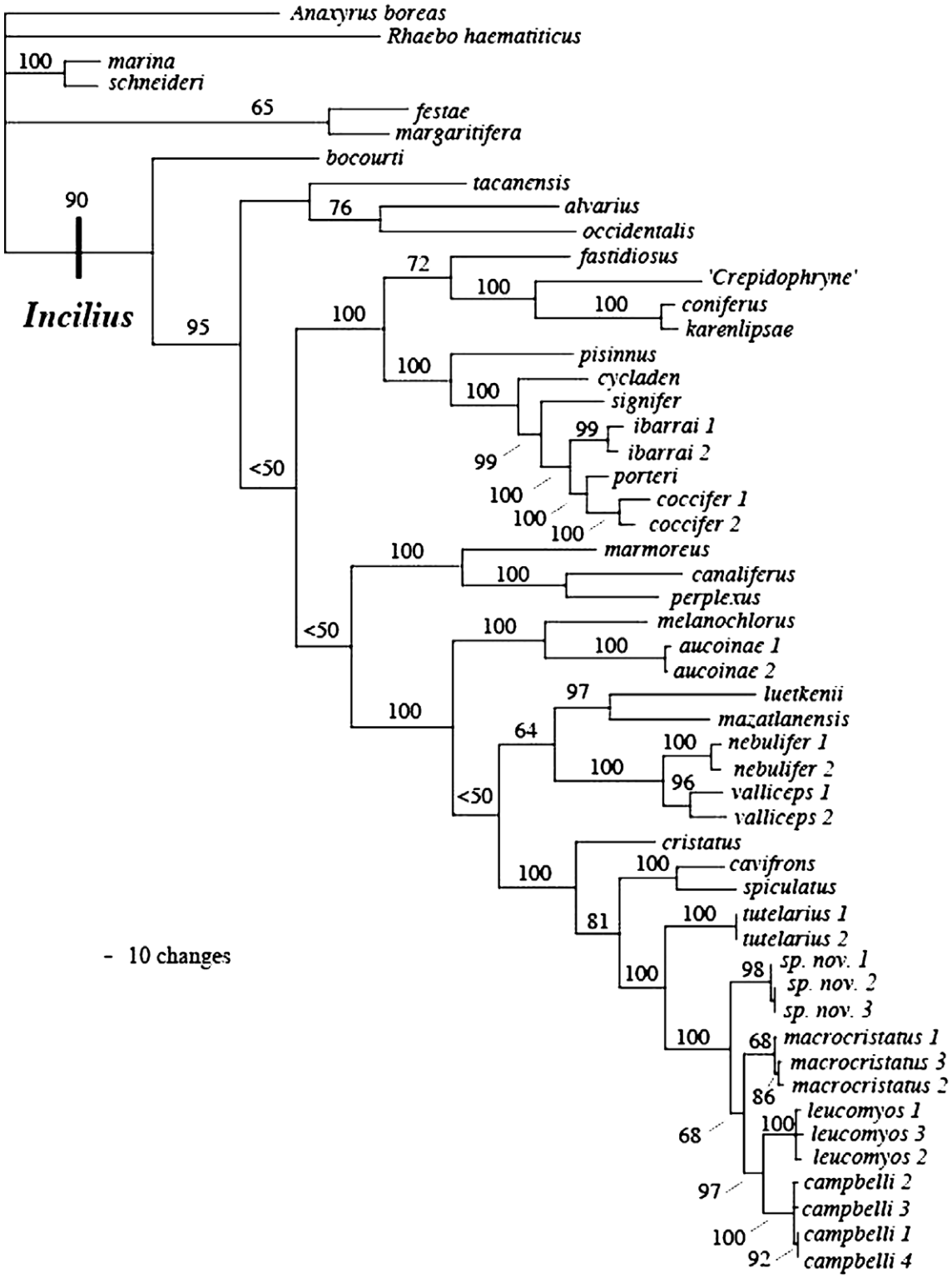
42. Liver
0: trilobed, left side enlarged
1: bilobed, right side greatly enlarged

43. Number of vertebrae
0: eight
1: seven

44. Alary processes of maxillae
0: oriented posteriorly
1. oriented anteriorly, projecting beyond margin of maxillae

APPENDIX IV. Supplementary tree figures.

APPENDIX IV FIGURE A. Parsimony analyses of mtDNA data (4,316 bp). A strict consensus of 27 trees (6,827 steps) is shown, with bootstrap scores >50 based on 100 replicates (10 random additions per replicate).



APPENDIX IV FIGURE B. Parsimony analyses of nuclear data (1,581 bp). A strict consensus of 33,275 trees, (529 steps) is

shown, with bootstrap scores >50 based on 100 replicates (10 random additions per replicate). Both searches held 10,000 maximum trees at each replication.