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[1]*Museo de Zoología 'Alfonso L. Herrera', Facultad de Ciencias, Universidad Nacional Autónoma de México, México, Distrito Federal, México;* [2]*Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, México, Distrito Federal, México*

Phylogeny and evolution of dorsal pattern in the Mexican endemic lizard genus *Barisia* (Anguidae: Gerrhonotinae)

A. Zaldivar-Riverón[1], A. Nieto-Montes de Oca[1] and J. P. Laclette[2]

Abstract

The phylogeny of the Mexican lizard genus *Barisia* was assessed using an 878 bp fragment of the mtDNA ND4 gene and a section of associated tRNA genes, as well as 16 external morphological characters. The terminal taxa comprised the currently recognized members of *Barisia*, including the four subspecies of the polytypic *Barisia imbricata* and individuals from different populations of the widespread *B. i. imbricata* and *Barisia i. ciliaris*, although for *Barisia levicollis* only morphology could be examined. The 'step-matrix frequency' and the 'step-matrix gap-weighting' coding approaches were employed simultaneously for the morphological data set, and three different scaling methods were evaluated for the last approach. Maximum parsimony (MP) analyses were performed for the separate and combined data sets and Bayesian analysis was also performed for the mtDNA sequence data. The hypothesis obtained from the simultaneous MP analysis strongly supports the monophyly of *Barisia*, but the 'exclusivity' of *B. imbricata* as well as of *B. i. imbricata* and *B. i. ciliaris* were not recovered. Moreover, inclusion of the morphological data showed *B. levicollis* nested within a clade together with the taxa assigned to *B. i. ciliaris*. This, together with the genetic distances and geographic concordance among the haplotypes examined, confirms that *B. imbricata* represents several species, although the actual species limits in this composite taxon are still unclear. Applying previously published rates of molecular evolution to the mtDNA data gives ages of divergence similar to the times proposed for some Pleistocene–Miocene geological and climatic phenomena that occurred in the Mexican territory. Variation of the dorsal pattern within *Barisia* was mapped onto the simultaneous morphological and molecular phylogeny, indicating that the two main states present in the taxa assigned to *B. imbricata*, an adult dorsal pattern present in females and absent in males and the absence of any pattern in both sexes, occur each in separate lineages. This suggests a possible scenario, where sexual dichromatism within *Barisia* has been repeatedly lost in different lineages.

Key words: mtDNA – morphology – ND4 – gap-weighting coding method – species boundaries – sexual dichromatism

Introduction

The Mexican, endemic genus *Barisia* Gray, 1838 contains four species that mainly inhabit temperate regions of moderately high elevation (Guillette and Smith 1982; Good 1988; Zaldivar-Riverón and Nieto-Montes de Oca 2001, 2002). Two of these species, *Barisia herrerae* Zaldivar-Riverón and Nieto-Montes de Oca, 2002 and *Barisia rudicollis* (Wiegmann, 1828), have small geographical distributions restricted to some areas in the central part of the Transvolcanic Belt (Zaldivar-Riverón and Nieto-Montes de Oca 2001, 2002). In contrast, *Barisia levicollis* Stejneger, 1890 and *Barisia imbricata* (Wiegmann, 1828) have much wider distributions, the former occurring in western, north-western, and north-central Chihuahua, and the latter ranging from southern Chihuahua south and east to central and southern Oaxaca (Guillette and Smith 1982; Good 1988; Lemos-Espinal et al. 2000) (Fig. 1). Additionally, geographic morphological variation in *B. imbricata* has resulted in its recognition of four subspecies, which also have considerable differences in their geographic ranges. *Barisia i. jonesi* Guillette and Smith, 1982 and *Barisia i. planifrons* (Bocourt, 1878) are restricted to some areas of Michoacán and Oaxaca, respectively, whereas *B. i. imbricata* (Wiegmann, 1828) occurs along the Transvolcanic Belt and adjacent regions, and *Barisia i. ciliaris* (Smith, 1942) occupies different areas of the Sierra Madre Occidental, Sierra Madre Oriental, and Mexican Plateau (Tihen 1949b; Guillette and Smith 1982).

The validity and composition of *Barisia* have been subjects of several morphological studies (e.g. Tihen 1949a,b; Guillette and Smith 1982; Good 1987, 1988). Nevertheless, the taxonomic status of *B. levicollis* and the subspecies of *B. imbricata* remain unclear. In one of these studies, Tihen (1949b) divided the genus into three groups based on several apparently exclusive features. Two of these groups included the members of the currently recognized genus *Mesaspis* Cope, 1877 and a third one, named the *imbricata* group, contained *B. imbricata*, *B. levicollis*, and *B. rudicollis*. In this work, three subspecies of *B. imbricata* were recognized: *B. i. imbricata*, *B. i. ciliaris*, and *B. i. planifrons*. However, in the first of these subspecies atypical features were noticed in some populations from the southern Distrito Federal, Michoacán and Veracruz. Decades later, Guillette and Smith (1982) conducted a taxonomic revision of their *B. imbricata* complex, composed of *B. levicollis* and four subspecies of *B. imbricata*, one of which was described therein. Due to the lack of consistent, diagnostic external morphological features and the scarcity of information about geographic relationships, these authors retained the taxonomic status of those taxa. However, several non-fixed differences in scale characters, body size, colour, and pattern were observed among the complex members, leaving their taxonomic status uncertain. More recently, Smith et al. (2002) elevated the subspecies of *B. imbricata* to a specific level, but no further justification than the previously published morphological information.

One of the characteristics traditionally used to distinguish among the members of the '*imbricata* complex' is the presence/absence of adult dorsal pattern. In all members of *Barisia*, and apparently most of the gerrhonotine species, newborns and juveniles of both sexes possess a dorsal pattern, which is usually composed of a series of dark transverse bands on the body (Stebbins 1958; Guillette and Smith 1982; Vial and Stewart 1989; Campbell and Frost 1993). However, this pattern

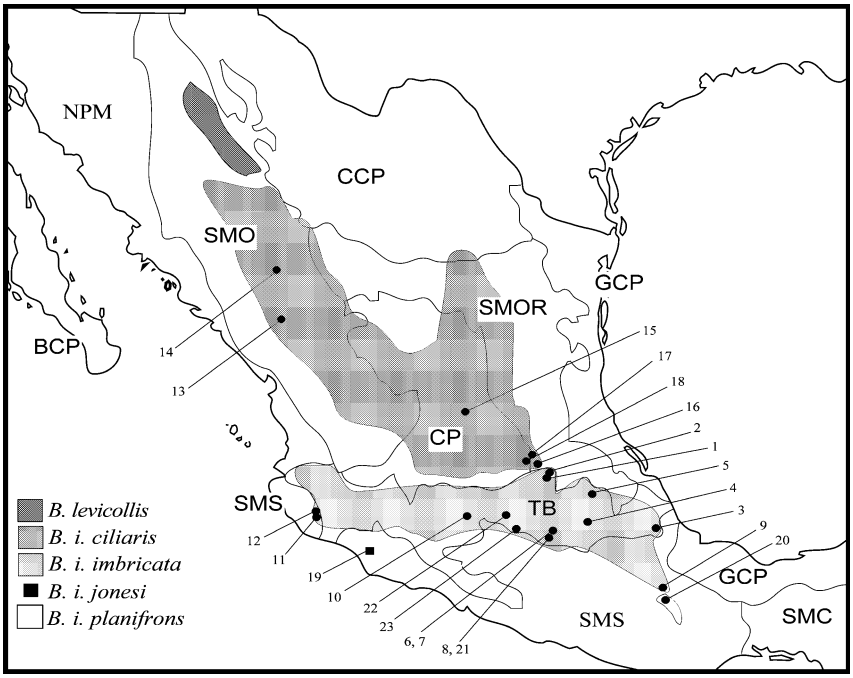

Fig. 1. Distribution of *B. levicollis* and the subspecies of *B. imbricata* based on museum specimens and literature records. Black dots represent the localities sampled for the haplotypes of *Barisia* included in this study except for *B. i. jonesi*, whose locality sampled and known geographic distribution is represented by a black square; numbers correspond to the species assignment listed in Table 1. Mexican physiographic provinces: BCP, Baja California Province; NPM, Northwest Plains and Mountain Ranges; SMO, Sierra Madre Occidental; CCP, Chihuahua and Coahuila Plateaus and Mountain Ranges; SMOR, Sierra Madre Oriental; GCP, Gulf Coastal Plain; CP, Central Plateau; TB, Transvolcanic Belt; SMS, Sierra Madre del Sur; SMC, Sierra Madre de Chiapas

shows intra- and interspecific variation in adults within the genus. Based on outgroup comparisons, Good (1988) argued that the ancestral adult dorsal pattern in the Gerrhonotinae is the presence of a broad dorsal stripe flanked by darker sides in both sexes. If this hypothesis is correct, the loss of this dorsal pattern in one or both sexes would be a derived condition within *Barisia*. A reconstruction of the evolutionary history of the genus may help to clarify not only the taxonomic status of its taxa, but also to determine whether the above feature has appeared independently in separate lineages. Unfortunately, the only phylogenetic analysis performed within *Barisia*, which was based on morphological information, did not divide *B. imbricata* into its four recognized subspecies but included it as a single terminal taxon (Good 1988).

In this study, mtDNA sequence and external morphological data were employed separately and in combination to reconstruct the evolutionary relationships among the species and subspecies of *Barisia*. Several populations of the widespread *B. i. imbricata* and *B. i. ciliaris* were sampled. Using the phylogenetic hypotheses obtained, we re-evaluate the taxonomic status of the members of the *imbricata* complex (*sensu* Guillette and Smith 1982). In addition, we use the phylogeny derived from the simultaneous analysis to make inferences about biogeography and evolution of coloration and sexual dichromatism within the genus.

Materials and Methods

Taxon sampling

A total of 18 ingroup terminal taxa including representatives of the seven recognized species and subspecies of *Barisia* were initially selected. Of these terminal taxa, four and nine represented *B. i. ciliaris* and *B. i. imbricata*, respectively. For these subspecies, mtDNA sequences were obtained from specimens collected in different localities across their geographic distribution (Fig. 1), and their morphological information was scored from specimens collected in the same or geographically contiguous localities. Those terminal taxa represented by more than one haplotype from the same or a nearby locality were only included once in the morphological and simultaneous analyses. Although we lack sequence data for *B. levicollis*, this species was included in the combined morphological and molecular analysis.

In a phylogenetic analysis of morphological data (Good 1988), the (*Abronia* Gray, 1838 + *Mesaspis*) clade appeared as the sister taxon to *Barisia*, followed below by *Elgaria* Gray, 1838. However, a phylogenetic analysis of morphological and molecular data showed *Abronia* as the sister taxon to the (*Barisia* + *Mesaspis*) clade (Chippindale et al. 1998). Thus, monophyly of *Barisia* and its relationships with other gerrhonotine genera were assessed here including two species of *Abronia* [*Abronia chiszari* Smith and Smith, 1981 and *Abronia graminea* (Cope, 1864)] and one of *Mesaspis* [*M. gadovii gadovii* (Boulenger, 1838)] in the analysis, and using *E. kingii* Gray, 1913 to root our trees. Specific and subspecific designations, localities, voucher numbers, and GenBank accession numbers of the sequenced specimens are given in Table 1. The specimens examined for the morphological data set are those listed in Zaldivar-Riverón and Nieto-Montes de Oca's (2002) study, except for the specimens assigned to *B. i. imbricata* and *B. i. ciliaris* and for the species of *Abronia*, *Mesaspis*, and *Elgaria*, which are listed in Appendix A. The Mexican physiographic provinces and subprovinces referred to in the analysis of biogeography are those defined by Ferrusquía–Villafranca (1998).

Morphological data

A total of 16 external morphological characters were examined from 205 alcohol-preserved museum specimens (Appendix B). Fifteen of these characters describe variation in squamation and one was morphometric. Nomenclature of scales followed Good (1988) and Campbell and Frost (1993) in the case of the occipital scales.

Despite the small size of the samples examined for most terminal taxa, several of them showed considerable variation in most of the examined characters. Moreover, some of these characters are meristic (*e.g.* number of longitudinal dorsal scale rows) or morphometric (maximum adult snout-vent length). There is evidence that these characters can contain important phylogenetic structure, and that this information is best retrieved analysing them as quantitative. Thus, the potential phylogenetic information from these characters was incorporated in subsequent analysis using the step-matrix frequency (Wiens 1995; Berlocher and Swofford 1997) and the step-matrix gap-weighting (Wiens 2001a; Wiens and Etheridge 2003) methods for the polymorphic, qualitative and the quantitative characters, respectively. The use

Table 1. Specific and subspecific designations, localities, and voucher and GenBank accession numbers of the examined specimens in this study. All localities are from Mexico

Number	Species	Locality	Voucher number	GenBank accession number
1	*B. i. imbricata* 1	Zacualtipán, Hidalgo	MZFC FMQ 2606	AY605108
2	*B. i. imbricata* 1a	0.5 km NE of Tianguistengo junction, Hidalgo	MZFC FMQ 2601	AY660756
3	*B. i. imbricata* 2	Pico de Orizaba, Veracruz	MZFC AZR 315	AY605109
4	*B. i. imbricata* 3	San Tadeo Huexoyucan, Tlaxcala	MZFC 12563	AY605110
5	*B. i. imbricata* 4	km 97 on Huauchinango–Apizaco Road, Puebla	MZFC 12544	AY605111
6	*B. i. imbricata* 5	2 km W of Albergue El Pino, Delegación Magdalena Contreras, Distrito Federal	MZFC 11663	AY605112
7	*B. i. imbricata* 5a	Delegación Magdalena Contreras, Distrito Federal	MZFC 11662	AY660757
8	*B. i. imbricata* 6	Parque Nacional Lagunas de Zempoala, Morelos	MZFC 1195	AY605113
9	*B. i. imbricata* 7	Peña Verde, Cañada de Cuicatlán, Oaxaca	MZFC 12545	AY605114
10	*B. i. imbricata* 8	Mil Cumbres, Michoacan	MZFC AZR 309	AY605115
11	*B. i. imbricata* 9	23 km from El Terrero, Manantlán, Jalisco	MZFC 6001	AY605116
12	*B. i. imbricata* 9a	Tapalpa, Jalisco	NA	AY605117
13	*B. i. ciliaris* 1	El Salto, Durango	MZFC 12547	AY605105
14	*B. i. ciliaris* 1a	E of Santiago Papasquiaro, Durango	MZFC EMD 26	AY660758
15	*B. i. ciliaris* 2	2 km W Alvarez, Municipality of Díaz Zaragoza, San Luis Potosi	MZFC 11082	AY605106
16	*B. i. ciliaris* 3	Municipality of Cadereyta, 1 km NE El Doctor, Queretaro	MZFC 8430	AY605107
17	*B. i. ciliaris* 3a	Municipality of Pinal de Amoles, 6 km NW Rancho Los Velázquez, Queretaro	MZFC 9252	AY660759
18	*B. i. ciliaris* 4	Municipality of Colón, Pinal de Zamorano, Queretaro	MZFC 9402	AY660760
19	*B. i. jonesi*	Coalcomán, Michoacan	MZFC UGV 4116	AY605118
20	*B. i. planifrons*	Sierra de Juárez, Yuvila, Oaxaca	MZFC 12546	AY605119
21	*B. herrerae*	Approximately 4 km E Ocuilan, Estado de Mexico	MZFC 9580	AY605120
22	*B. rudicollis* 1	Municipality of Tuxpan, El Pinal, Michoacan	MZFC 9594	AY605121
23	*B. rudicollis* 1a	Avándaro, Valle de Bravo, Estado de Mexico	MZFC 12541	AY660761
24	*A. graminea*	Puerto de la Soledad, Oaxaca	MZFC 4830	AY605102
25	*A. chiszari*	20 km NE of Bastonal, Los Tuxtlas, Veracruz	MZFC uncatalogued molt	AY605101
26	*M. g. gadovii*	Omiltemi, Guerrero	MZFC 10206	AY605104
27	*E. kingii*	Cascada de Basaseachic, Chihuahua	MZFC 5754	AY605103

of these methods represents an objective alternative to solving the problems of character definition and character state delimitation in morphological phylogenetics (Wiens 1995, 1999, 2001a; Wiens and Servedio 1997; Wiens and Etheridge 2003). Only one meristic character (character 4) was coded using the traditional (qualitative) approach because it consists of only two states, which are invariant within the terminal taxa examined. For construction of the step matrices, the maximum cost of a transformation used for the polymorphic, meristic and morphometric characters and the weight of the fixed qualitative character were both set to 1000. Some of the scale characters varied bilaterally in some individuals (e.g. number of superciliars). In these cases, sample size was considered as twice the number of examined specimens. Frequencies of polymorphic qualitative characters and means of quantitative characters are shown in Appendix C. Coding of all the characters is presented in Appendix D.

One of the problems associated with the use of the step-matrix gap-weighting coding method is how to weight or scale the different types of characters relative to each other. Three different scaling approaches have been proposed to adjust the weight of quantitative characters: the between-characters, between-states, and mixed (which is a combination of the previous two) scalings (Wiens 2001a; Wiens and Etheridge 2003). However, it has been observed that these approaches may produce very different phylogenetic trees (Wiens 2001a; Wiens and Etheridge 2003). Therefore, we analysed our morphological data set with these three scaling methods. For the mixed scaling, meristic characters with a range of mean-taxa trait values < 5.0 were coded with the between state scaling, and those with ranges > 5.0 with the between-character scaling. The congruence between the three morphological and the mtDNA topologies was then evaluated using the consensus fork index of Colless (1980), which calculates the propotion of nodes in common between two trees. In order to make topologies comparable, this index was calculated excluding *B. levicollis* and the terminal taxa represented by more than one haplotype in the morphological and the mtDNA phylogenies, respectively. The performance of these scaling approaches was also assessed calculating the consistency (CI; Kluge and Farris 1969), retention (RI; Farris 1989), and data decisiveness (DD; Goloboff 1991) indices of their most parsimonious trees (MPTs). An approximation of the mean length of all possible trees, necessary for calculation of the DD, was obtained from 100 000 randomly generated trees using the equiprobable model.

Mitochondrial DNA sequences

Total genomic DNA was extracted from liver and/or muscle tissue previously stored at −70 °C using the QIAamp tissue kit (Qiagen, Inc., Valencia, CA, USA), except for *A. chiszari*, which was extracted from a piece of shed skin following the DNA extraction protocol given by Clark (1998). The mtDNA fragment sequenced included a portion of the ND4 protein-coding gene and the adjacent section comprising the histidine, serine, and part of the leucine tRNA genes. This fragment has been successfully used to investigate evolutionary relationships among closely related species in different reptile groups (e.g. Arévalo et al. 1994; Wiens et al. 1999; Flores-Villela et al. 2000; Doan and Castoe 2003). PCR reactions were carried out in a 50 µl volume consisting of 2 µl of template DNA, 1 µl of each primer (5 µM), 5 µl dNTPs (0.8 mM), 5 µl of 10X PCR reaction buffer, 2.5 µl of $MgCl_2$ (1.5 mM), 0.5 µl of *Taq* DNA polymerase (5 U/µl), and 33 µl of H_2O. The following primers designed by Arévalo et al. (1994) were employed: ND4 (forward: 5′ TGACTACCAAAAGCTCATGTAGAAGC3′); Leu (reverse: 5′ TRCTTTTACTTGGATTTGCACCA 3′). Additionally, we designed an internal primer to corroborate the sequences obtained for some of the samples (light strand primer Barisia: 5′GAACGCACGAACTCACG3′). The PCR program for all amplifications had an initial 5 min denaturation at 94 °C, followed by 35 cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. A 5 min extension followed the final cycle. PCR products were cleaned with Wizard PCR Prep™ columns (Promega, Inc., Madison, WI,

USA) and then sequenced using the dideoxy terminator cycle sequencing kit (Applied Biosystems, Inc., Foster City, CA, USA) and an ABI 373A automated DNA sequencer. Sequences were edited and aligned manually using the program Bioedit version 5.0.6 (Hall 1999). Indels were found in only one position in the alignment, and this was treated as missing data in subsequent analyses. Base frequencies, pairwise sequence divergences, and all statistical tests were executed with PAUP* version 4.0b10 (Swofford 1998).

Phylogenetic analysis

Maximum parsimony (MP) analyses were performed for the separate and the simultaneous data sets using PAUP*. A branch and bound search was performed for the different morphological analyses, whereas a heuristic search with tree bisection and reconnection (TBR) branch swapping and 10 000 random sequence addition replicates was executed for the mtDNA and the combined data sets. Characters were treated as unordered and equally weighted for the mtDNA analysis, and weight of nucleotide characters in the simultaneous analysis was set to 1000 to make them comparable with the maximum cost of a transformation used for the morphological characters (see above). Clade support was evaluated using a non-parametric bootstrap (BTP; Felsenstein 1985) with 1000 pseudoreplicates, each consisting of a heuristic search with 20 random taxon addition replicates and TBR branch swapping, and considering those clades with values ≥70% as strongly supported (Hillis and Bull 1993). Bremer support (BS) was also obtained for all the internal branches recovered from analysis of the mtDNA data set using TreeRot version 2 (Sorenson 1999) with its default parameters.

A Bayesian MCMC analysis was additionally performed for the mtDNA data using MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001). In order to decrease the chance of reaching apparent stationary on local optima, two separate analyses were conducted, each one consisting of four chains, random starting trees, and uniform prior distribution of parameters. The chains were run for four million generations, sampling trees every 1000 generations. The HKY + Γ model of sequence evolution was used in the Bayesian analyses for the tRNA genes, whereas an alternative model similar to the TrN + Γ and compatible with MrBayes 3.0b4, the GTR + Γ, was used for the ND4 gene. These two models were determined as the most appropriate for the two fragments based on the program Modeltest version 3.06 (Posada and Crandall 1998). The analysis was run considering four different partitions, one for tRNA genes and three for the ND4 gene based on its codon positions. Stationary was determined by eye and the burn-in trees were discarded. The remaining trees were used to estimate Bayesian posterior probabilities (BPP), considering the clades as significantly supported if they were present in ≥95% of the sampled trees (Huelsenbeck and Ronquist 2001; Wilcox et al. 2002).

The only currently available parsimony-based test of topologies, the Templeton test (Templeton 1983; Felsenstein 1985), cannot be used to compare the MPT (s) derived from the data to one or more *a priori*-specified trees (Goldman *et al*. 2000). Therefore, we only investigated for significant differences between the likelihood of the Bayesian mtDNA phylogeny and those calculated for four alternative topologies using the Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa 1999) with a full likelihood optimization and 1000 bootstrap replicates. Heuristic MP searches as mentioned above were carried out in order to obtain the shortest alternative trees using the GTR + Γ model to set the parameters of the MP topologies in the SH-tests. One of the compared alternative topologies constrained *Barisia* as non-monophyletic, forcing into a single clade the two species exclusive of the *imbricata* complex (*B. herrerae* and *B. rudicollis*) with the species of *Abronia*. The remaining three alternative topologies constrained *B. imbricata* as well as its subspecies *B. i. ciliaris*, and *B. i. imbricata* as 'exclusive' [using the term 'exclusive' instead of monophyletic below the species level according to De Queiroz and Donoghue (1990)]. In order to test whether there was significant rate heterogeneity among linages, a Maximum Likelihood ratio test (Felsenstein 1981, 1988) was performed on the Bayesian mtDNA tree. Specifically, PAUP* was used to estimate the likelihood of the GTR + Γ model with and without a molecular clock assumption, given the data and tree, and the significance of differences was tested using a likelihood ratio test and a chi-square table.

Evolution of dorsal pattern

The evolution of dorsal coloration and sexual dichromatism within *Barisia* was investigated by coding the observed variation into the three following unordered character states and then mapping them on the combined MP phylogeny using MacClade version 4.0 (Maddison and Maddison 2000): (0) presence of a series of dorsal and/or lateral dark transverse bands in adults of both sexes; (1) presence of a series of dorsal and/or lateral dark transverse bands only in adult females (adult males without any apparent dorsal pattern); (2) absence of any dorsal pattern in adults of both sexes. Assignment of character states for all the terminal taxa was based on the adult specimens examined for the morphological data set and on personal observations made in the field by AZR. The SH-test was performed as above to test for significant differences between the phylogeny derived from the Bayesian mtDNA analysis and an alternative topology that assumes that the adult dorsal pattern present in females and absent in males and its absence in both sexes have each evolved only once within *Barisia*.

Results

Morphological data

All 16 selected morphological characters were parsimony informative using the three scaling methods. The between-states, between-characters, and mixed scaling analyses recovered one, three, and four MPTs, respectively (tree length = 173 093.8, 22 172, and 35 669.28; Fig. 2a–c), and most of their clades are weakly supported. The strict consensus of the MPTs obtained by the between-characters scaling and the mixed scaling are mostly similar in their relationships, although the second one is less resolved, and both differ considerably with respect to the between-states MPT. Among the most relevant relationships recovered, monophyly of *Barisia* is weakly supported in the between-characters and the mixed consensus trees (BTP = 60 and 65, respectively), with the clade *B. rudicollis* + *B. herrerae* (BTP = 98 and 99, respectively) placed at the base. Moreover, the exclusivity of *B. imbricata*, as well as of *B. i. imbricata* and *B. i. ciliaris*, are not recovered by any of the three scaling methods. In all the trees, *B. levicollis* is nested within a weakly supported clade containing the taxa assigned to *B. i. ciliaris* (between-states: BTP = 64; between-characters: BTP < 50; mixed; BTP < 50) and appearing as the sister taxon of the northern-most population of *B. i. ciliaris* (between-states: BTP = 67; between-characters: BTP = 67; mixed: BTP = 64).

None of the relationships recovered between the between-state scaling and the mtDNA trees are similar (consenus fork index = 0), and only one relationship, the monophyly of *Barisia*, is shared between the latter and the between-character scaling and mixed scaling trees (consenus fork index = 0.04 in both cases). On the other hand, the performance values of the three different scaling methods were very similar, with the mixed MPTs having the highest CI and the between-states MPT the highest RI and DD values (between-states: CI = 0.633, RI = 0.771, DD = 0.738; between-characters: CI = 0.644, RI = 0.755, DD = 0.730; mixed: CI = 0.648, RI = 0.761, DD = 0.0.734). Thus, based on its highest consensus fork index and CI values, we chose the strict consensus of the mixed scaling MPTs as our preferred morphological phylogeny.

Molecular and combined data

Sequence length for most of the 27 haplotypes obtained varied from 877 to 878 bp, 715 of which correspond to the ND4 and

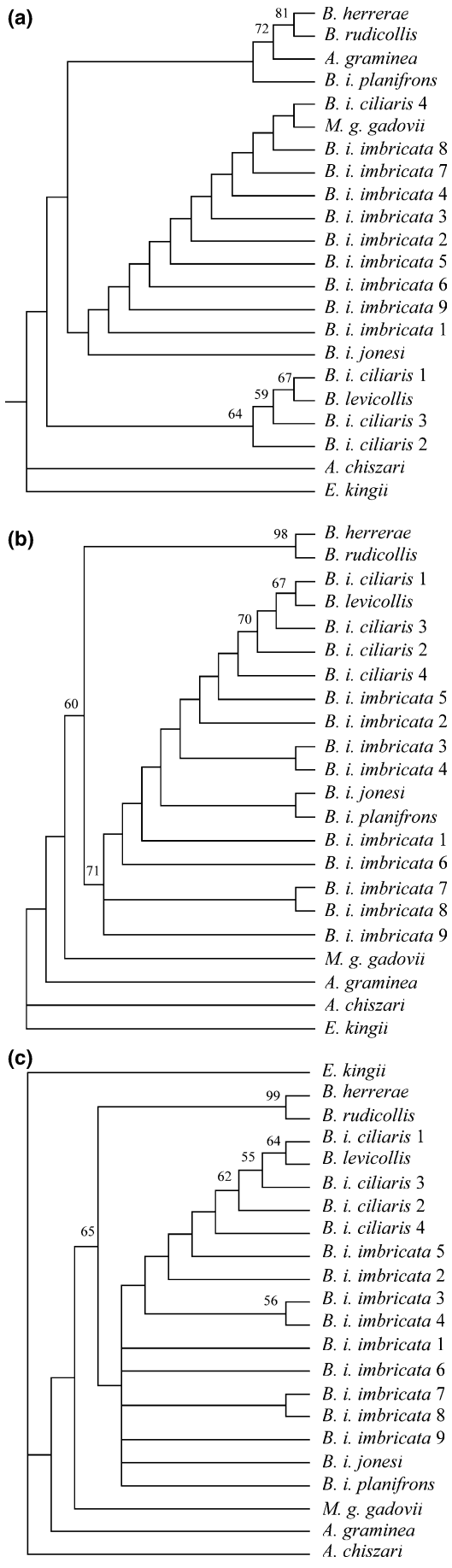

Fig. 2. Phylogenetic relationships within *Barisia* based on the 16 selected morphological characters using three different scaling methods for the quantitative characters with the step-matrix gap-weighting approach. (a) Single MPT recovered using the between-states scaling. (b) Strict consensus of the three MPTs recovered using the between-character scaling. (c) Strict consensus of the four MPTs recovered using the mixed scaling. Numbers above branches refer to clades supported by bootstrap values > 50%

162–163 to the tRNA genes. However, in five sequences 12–55 bp near the 5′ end of the fragment could not be obtained, and 93 bp of the middle of the ND4 gene could not be sequenced for the *A. chiszari* haplotype. A total of 351 characters are variable and 243 are parsimony informative. As with previous studies with other groups of reptiles (e.g. Castoe et al. 2003; Doan and Castoe 2003), base composition of the gene fragment sequenced was found to be strongly negatively G biased ($A = 0.32$, $C = 0.27$, $G = 0.13$, $T = 0.28$).

The MP analysis recovered two equally MPTs (length = 767, CI excluding uninformative characters = 0.581, RI = 0. 0.677). The burn-in in the two Bayesian analyses occurred after 20 000 generations. The topologies of the 50% majority rule consensus trees derived from these two analyses are identical. Therefore, all the trees recovered after burn-in by both analyses were pooled in a single 50% majority rule consensus (including other compatible groups), which had a mean -ln *L* score of 5214.88.

The strict consensus of the two MPTs and the 50% majority rule consensus tree of the two Bayesian analyses are mostly similar (Fig. 3a,b), only differing in two relationships (see below). The clades recovered in both analyses are in most cases geographically continuous but do not reflect the current taxonomy of the taxa involved. Monophyly of *Barisia* is strongly supported by both tree-building methods (BTP = 100, BS = 41, BPP = 1.0) with *B. i. planifrons* poorly supported at the base (BTP < 50, BS = 1; BPP = 0.73). In the consensus tree of the MP analysis there is a polytomy composed of a clade with the two haplotypes of *B. rudicollis* (BTP = 100, BS = 8) and two major clades that contain the remaining haplotypes of *Barisia*. This polytomy appears resolved but weakly supported in the Bayesian topology, where the *B. rudicollis* clade is placed at the base of the two major clades (BPP = 0.31).

One of the two major clades, which is strongly supported only in the Bayesian topology (BTP < 50, BS = 2, BPP = 1.0), is composed of two strongly supported subclades. One of these contains the haplotypes of *B. i. ciliaris* from the Sierra Madre Oriental (*B. i. ciliaris* 3 + *B. i. ciliaris* 3a; BTP = 100, BS = 16, BPP = 1.0). The second one (BTP = 100, BS = 6, BPP = 1.0) has two additional, inconsistently supported subclades, one with the haplotypes of *B. i. ciliaris* from the Central Plateau and *B. i. imbricata* two from Pico de Orizaba in the Transvolcanic Belt (BTP = 64, BS = 2, BPP = 1.0), and the other one with the two haplotypes of *B. i. ciliaris* from the Sierra Madre Occidental (BTP = 100, BS = 10, BPP = 1.0) appearing as the sister group of a strongly supported subclade (BTP = 100, BS = 5, BPP = 1.0) with the haplotypes of *B. i. imbricata* and *B. i. jonesi* from the Pacific Mountain Ranges and Cuestas subprovince in the Sierra Madre del Sur (BTP = 80, BS = 1, BPP = 0.89). In the second major clade (BTP < 50, BS = 1, BPP = 0.53), *B. herrerae* is weakly supported as the sister taxon of a subclade with the

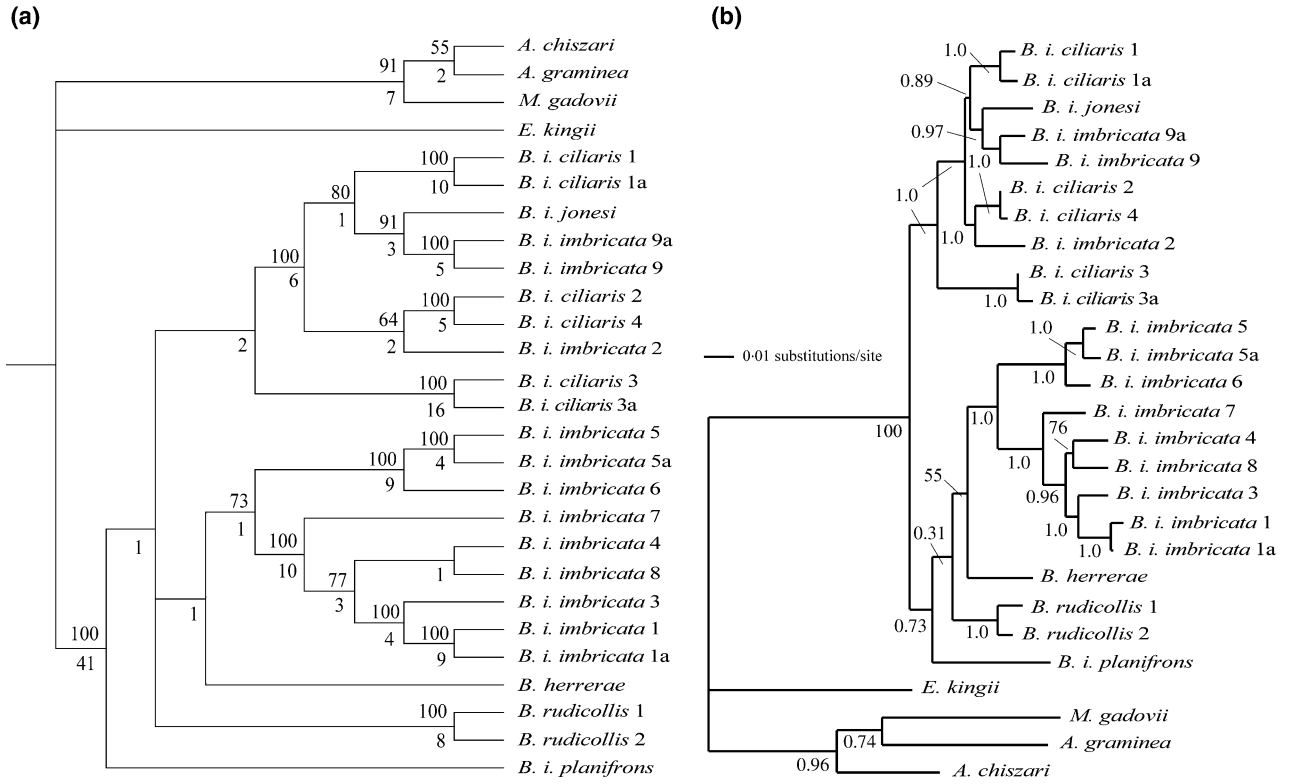

Fig. 3. Consensus trees recovered by the two different phylogenetic reconstruction methods employed for the mtDNA data. (a) Strict consensus of the two MPTs recovered by the MP analysis. Bootstrap values >70% and Bremer support values are shown above and below branches, respectively. (b) 50% Majority rule consensus tree obtained pooling the trees recovered after burn-in from the two Bayesian analyses. Clade credibility values >50% are shown above branches

remaining haplotypes of *B. i. imbricata*, which is supported by high BTP (73), and BPP (1.0) but not by BS (1) values. Eight of the haplotypes within this subclade came from the Transvolcanic Belt and the remaining one from the Tehuacan–Cuicatlan–Quiotepec infraprovince (Oaxaca and Puebla Highlands subprovince) in the Sierra Madre del Sur.

The haplotypes of *Abronia* and *Mesaspis* comprise a strongly supported clade in both MP and Bayesian trees (BTP = 91, BS = 7, BPP = 0.96). However, the relationships among them are poorly supported and differ between the Bayesian and MP analyses. In the MP topology, *M. gadovii* is at the base of a clade with the two species of *Abronia* (BTP = 55, BS = 2). In contrast, in the Bayesian topology *A. chiszari* is the sister taxon of the (*A. graminea* + *M. gadovii*) clade (BPP = 0.74).

The MP simultaneous analysis of the mtDNA and morphological data sets recovered a single MPT (length = 769 544.52, CI excluding uninformative characters = 0.593, RI = 0.621) (Fig. 4). Most of the relationships in this tree were similar to those recovered by the MP and Bayesian mtDNA analyses, although its clade support values generally decreased. Two distinct relationships, however, are revealed with the combination of the two data sets. *Barisia rudicollis* and *B. herrerae* appear strongly supported as sister species (BTP = 93), whereas *B. levicollis*, which was not included in the mtDNA analyses, appears strongly supported as the sister taxon of the northernmost population of *B. i. ciliaris* (*B. i. ciliaris* 1) (BTP = 79).

The SH-tests significantly reject the mtDNA alternative topologies explored ($p < 0.01$), except for the test that constrained *B. imbricata* as exclusive ($p = 0.384$). The Maximum Likelihood ratio test revealed that there is significant rate heterogeneity among the taxa included ($p = 0.006$). However, the rate heterogeneity is only marginally significant when the outgroups are pruned from the tree that enforces a molecular clock using the most basal haplotype of *Barisia* (*B. i. planifrons*) for rooting the tree ($p = 0.036$).

Evolution of dorsal pattern

Among the examined populations of *Barisia*, an adult dorsal pattern consisting of dark transversal bands is present in both sexes in *B. herrerae* and *B. rudicollis* (Fig. 5a,b, respectively); is absent in both sexes in *B. i. ciliaris*, *B. levicollis*, and few populations assigned to *B. i. imbricata* (Fig. 5e); and is present only in females in *B. i. jonesi*, *B. i. planifrons*, and most of the populations assigned to *B. i. imbricata* (Fig. 5c,d). In the latter taxa, adult males are distinguished by lacking any dorsal pattern and having instead uniform brown to olive green dorsal colour, whereas in some populations of *B. i. imbricata* from the Transvolcanic Belt adult females have a broad middorsal band flanked by darker bands. Moreover, in taxa that lack any dorsal pattern, males often have white flecking along the body (e.g. Fig. 5e). Sexual dichromatism has also been recorded in *B. rudicollis* and *B. herrerae* (Zaldivar-Riverón and Nieto-Montes de Oca 2001, 2002), as well as in some species of *Mesaspis* and *Elgaria* (Taylor 1956; Stebbins 1958; Karges and Wright 1987; Knight and Duerre 1987; Vial and Stwart 1989). However, in these species both males and females have a series of dorsal and/or lateral dark stripes along

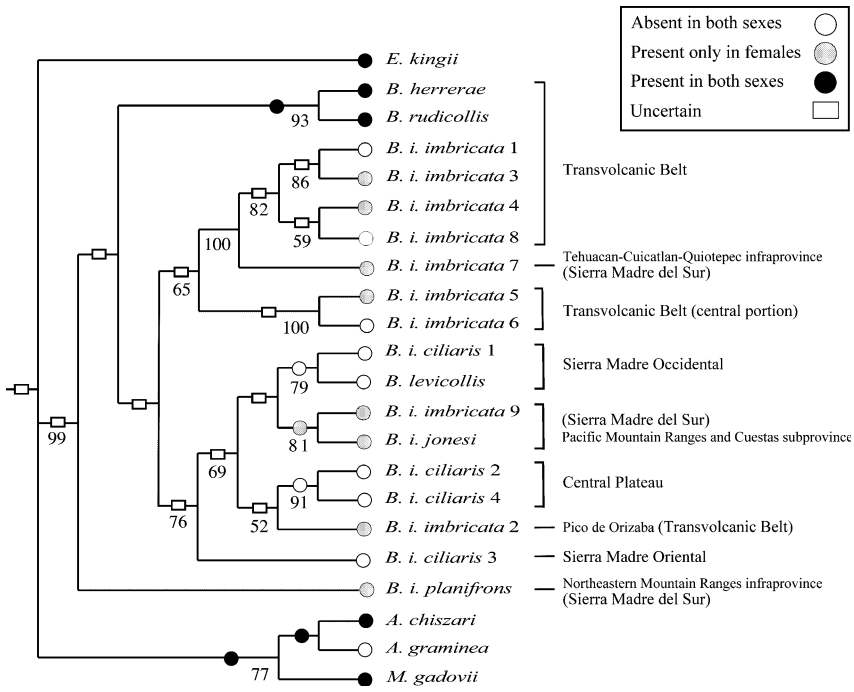

Fig. 4. Single MPT recovered by the simultaneous analysis of the mtDNA and morphological data. Bootstrap values > 70% are shown below branches, The Mexican provinces and subprovinces corresponding to the haplotypes localities are indicated at the right of the tree. The ancestral nodes are mapped and the three different adult dorsal pattern states scored for the examined terminal taxa are shown at the tip of the branches. A detailed definition of these states is given in the text

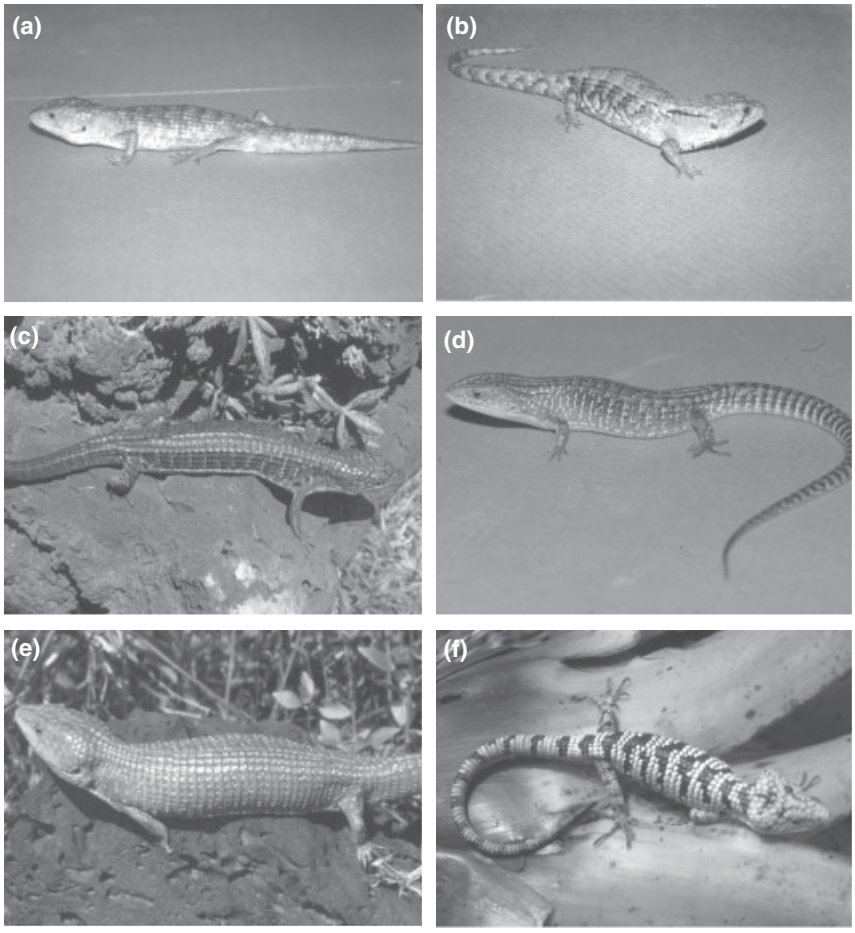

Fig. 5. Variation of adult dorsal pattern in *Barisia*. (a) *B. herrerae* (male, MZFC 9588); (b) *B. rudicollis* (female MZFC 12541); (c) *B. i. planifrons* (female, MZFC 12546); (d) *B. B. i. imbricata* (female, MZFC 11663); (e) *B. i. ciliaris* (male, MZFC AZR s/n); (f) *A. taeniata* (Wiegmann, 1828) (male, MZFC 11090)

the body and tail. Although a dorsal pattern in adults of both sexes appears in most species of the other gerrhonotine genera (e.g. Fig. 5f), its absence in both sexes is known to occur in *A. graminea* and also in other species of *Abronia* with bright green coloration like *A. matudai* (Hartweg and Tihen, 1946) and *A. ochoterenai* (Martín del Campo, 1939) (Good 1988).

Mapping the selected states of dorsal pattern onto the simultaneous MP phylogeny fails to estimate the ancestral state for *Barisia* and for the two major clades recovered within the genus (Fig. 4). However, the mapping of the three dorsal pattern states shows that two of them, the absence of adult pattern in both sexes and an adult dorsal pattern present in females and absent in males, appear intermingled in the two major clades. The SH-test indicated that the Bayesian mtDNA phylogeny showing these different independent origins for the above states is a significantly better explanation than the alternative hypothesis grouping taxa with the same conditions into single clades ($p = 0.0001$).

Discussion

Separate and simultaneous phylogenies

Our mtDNA and preferred morphological (mixed scaling) phylogenies differ considerably in many of their relationships. However, as expected in intraspecific phylogenetic analyses (Wiens and Penkrot 2002), the mtDNA phylogenies are much more robust than the morphological one, as can be appreciated from the noticeable difference in their numbers of well-supported clades. Nonetheless, there are some interesting relationships in the preferred morphological phylogeny. As with the mtDNA phylogenies, the morphological phylogeny does not recover the exclusivity of *B. imbricata* or *B. i. ciliaris*. Moreover, in the latter phylogeny the relationships of *B. levicollis*, which could not be included in the mtDNA analysis, suggest that this taxon is more closely related to the northern populations of *B. i. ciliaris* than they are to other populations assigned to this subspecies. This may receive some support from the geographic proximity of *B. levicollis* and the haplotypes of *B. i. ciliaris* 1 and 1a. In addition, as suggested in a previous study (Zaldivar-Riverón and Nieto-Montes de Oca 2002), *B. herrerae* and *B. rudicollis* are strongly supported as sister species (BTP = 99; characters 1, 7–10, CI = 1.0, 0.87, 0.78, 0.47, and 1.0, respectively).

In our simultaneous analysis combination of the mtDNA and morphological information in intraspecific phylogenies generally yielded the mtDNA haplotype tree, which is caused by the weak phylogenetic signal normally found in morphological data (Wiens and Penkrot 2002). Inclusion of morphological data in our simultaneous analysis, however, helped to improve a weakly recovered part of the two mtDNA topologies (the placement of *B. rudicollis* and *B. herrerae* as sister taxa) and to reconstruct other one that could not be assessed by the mtDNA data set alone (the placement of *B. levicollis* as sister taxon of the northernmost population of *B. i. ciliaris*). Thus, despite a decrease in most of the clade support values found in the simultaneous MPT, we considered this MPT as our best estimate of phylogeny, but also take into account the clade support values found in the similar relationships recovered by the MP and Bayesian analyses.

The strong support found for the monophyly of *Barisia* contradicts the proposed close relationship between *B. rudicollis* and the members of *Abronia* based on some anatomical similarities (Tihen 1949b; González-Romero and López-Gonzalez 1990). Monophyly of *Barisia* based on its presumably derived possession of fused supranasal and upper postnasal scales and one to four superciliaries also was previously suggested (Zaldivar-Riverón and Nieto-Montes de Oca 2002) and, not surprisingly, these two characters helped to recover the monophyly of the genus in the mixed scaling analysis (character 2, CI = 1.0; character 7, CI = 0.87). An exclusive *B. imbricata* was not recovered, although the relationships that indicate this were not strongly supported and the alternative mtDNA hypothesis of an exclusive *B. imbricata* was not statistically rejected. Further molecular phylogenetic studies including *B. levicollis* are necessary to confirm whether *B. imbricata* is paraphyletic with regard to the former species. Whether *B. imbricata* is exclusive or not, however, the haplotypes assigned to the widespread *B. i. imbricata* and *B. i. ciliaris* were non-exclusive with respect to each other or *B. i. jonesi*. Also, the SH-tests rejected the mtDNA alternative hypotheses of their exclusivity.

Species delimitation

Previous phylogenetic studies with other reptile groups based on the gene markers used herein have found inter- and intraspecific genetic divergences ranging from 5.5 to 30% and from < 1 to 8%, respectively (Kraus et al. 1996; Forstner et al. 1998; Scott and Keogh 2000; Castoe et al. 2003; Doan and Castoe 2003). However, the highest of these intraspecific divergences (8%) was regarded conservatively as intraspecific in a case likely involving more than one species (Doan and Castoe 2003). Herein, genetic distances among the four subspecies of *B. imbricata* and within *B. i. imbricata* and *B. i. ciliaris* are in some cases higher than the highest intraspecific distances aforementioned (2.85–10.43%, 0.06–10.38%, and 1.25–6.47%, respectively; Table 2).

Application of the tree-based approach of Wiens and Penkrot (2002) to delimit species for the well-supported, geographically continuous groups of taxa recovered in our simultaneous MPT suggests that *B. imbricata* is composed of several evolutionary lineages (*sensu* Wiley 1978). Unfortunately, because of the small number of sampled populations of *B. i. ciliaris* and *B. i. imbricata*, the actual number of species contained in each of them and in *B. imbricata* remains uncertain. However, several taxonomic considerations can be made based on our results.

The number of longitudinal dorsal scale rows has been regarded as a highly subjective character in some anguid genera because of the difficulty to distinguish between the lateral fold and the dorsal scales (Campbell and Frost 1993). Nonetheless, *B. i. imbricata* has been traditionally distinguished by the presence of 12–14 longitudinal dorsal scale rows versus 16 rows found in the remaining subspecies of *B. imbricata* (Tihen 1949b; Guillette and Smith 1982; Smith et al. 2002). However, the phylogenetic relationships, genetic distances, and geographic distribution of the taxa assigned to *B. i. imbricata* suggest that they represent several evolutionary lineages, even though most of these lineages have no known morphological diagnostic features. Regarding the clade with most of the taxa assigned to *B. i. imbricata*, its two subclades, which have separate geographic distributions in the Transvolcanic Belt and an adjacent region in the Sierra Madre del Sur, might represent two different evolutionary lineages. The genetic distances within these subclades (1.27–3.99%) are within the range of intraspecific distances, whereas distances between them (6.72–8.25%) are similar to the distances between the taxa in each of these subclades and the remaining taxa of *Barisia* (6.04–10.38%). Also, populations of the Distrito Federal and Morelos subclade are characterized by the unique, non-fixed presence of 12 longitudinal dorsal scale rows.

Table 2. Number of nucleotide substitutions (above diagonal) and *p* uncorrected sequence divergences (below diagonal) among the haplotypes examined in this study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1 *B. i. ciliaris* 1	–	11	28	49	54	29	77	67	36	72	62	69	75	76	68	70	39	33	36	78	67	59	58	153	134	154	150
2 *B. i. ciliaris* 1a	0.0125	–	28	48	53	30	76	66	36	71	61	69	73	74	65	67	40	34	37	77	68	58	55	155	138	155	152
3 *B. i. ciliaris* 2	0.0319	0.0319	–	43	47	3	77	67	25	70	62	63	69	67	67	68	37	30	32	74	67	55	53	142	130	151	152
4 *B. i. ciliaris* 3	0.0558	0.0547	0.0490	–	5	43	83	73	55	78	70	69	73	72	76	75	60	51	55	85	72	59	57	148	133	156	150
5 *B. i. ciliaris* 3a	0.0647	0.0636	0.0564	0.0060	–	48	83	78	58	79	73	70	75	74	78	76	62	52	58	87	73	60	59	141	125	148	146
6 *B. i. ciliaris* 4	0.0354	0.0367	0.0036	0.0523	0.0584	–	69	64	25	63	56	61	66	61	61	61	37	29	32	68	59	49	46	134	121	142	144
7 *B. i. imbricata* 1	0.0877	0.0866	0.0877	0.0945	0.0997	0.0846	–	5	78	25	35	71	71	70	40	29	91	77	84	84	67	68	63	167	140	167	164
8 *B. i. imbricata* 1a	0.0792	0.0781	0.0792	0.0863	0.0932	0.0781	0.0060	–	70	21	27	61	63	63	33	25	79	64	73	75	58	59	55	155	129	156	152
9 *B. i. imbricata* 2	0.0410	0.0410	0.0285	0.0626	0.0696	0.0304	0.0888	0.0827	–	73	72	68	70	74	71	75	47	41	44	84	79	63	60	148	135	158	157
10 *B. i. imbricata* 3	0.0820	0.0809	0.0797	0.0888	0.0950	0.0774	0.0285	0.0251	0.0831	–	28	63	63	62	34	28	86	72	76	80	65	60	59	161	138	160	162
11 *B. i. imbricata* 4	0.0706	0.0695	0.0706	0.0797	0.0876	0.0686	0.0399	0.0321	0.0820	0.0319	–	59	59	61	34	24	79	65	70	77	53	55	51	160	142	157	157
12 *B. i. imbricata* 5	0.0786	0.0786	0.0718	0.0786	0.0840	0.0743	0.0809	0.0721	0.0774	0.0718	0.0672	–	11	20	61	65	74	64	63	79	59	53	54	151	130	159	153
13 *B. i. imbricata* 5a	0.0870	0.0847	0.0801	0.0847	0.0898	0.0804	0.0825	0.0744	0.0813	0.0732	0.0685	0.0127	–	21	59	65	80	69	70	79	62	53	54	153	133	159	152
14 *B. i. imbricata* 6	0.0866	0.0843	0.0763	0.0820	0.0887	0.0743	0.0797	0.0745	0.0843	0.0706	0.0695	0.0228	0.0243	–	60	65	81	72	70	78	59	53	54	148	127	150	151
15 *B. i. imbricata* 7	0.0774	0.0740	0.0763	0.0866	0.0938	0.0750	0.0456	0.0393	0.0809	0.0387	0.0387	0.0695	0.0686	0.0683	–	35	81	69	72	77	60	59	54	163	142	161	162
16 *B. i. imbricata* 8	0.0797	0.0763	0.0774	0.0854	0.0913	0.0748	0.0330	0.0297	0.0854	0.0319	0.0273	0.0740	0.0754	0.0740	0.0399	–	81	66	79	79	58	60	55	167	146	166	165
17 *B. i. imbricata* 9	0.0445	0.0456	0.0422	0.0684	0.0744	0.0453	0.1038	0.0936	0.0536	0.0981	0.0901	0.0844	0.0929	0.0924	0.0924	0.0924	–	25	39	83	76	65	64	150	137	161	156
18 *B. i. imbricata* 9a	0.0376	0.0388	0.0342	0.0582	0.0624	0.0352	0.0878	0.0756	0.0468	0.0821	0.0741	0.0730	0.0802	0.0821	0.0787	0.0753	0.0285	–	31	74	65	57	55	148	131	153	154
19 *B. i. jonesi*	0.0410	0.0422	0.0365	0.0627	0.0697	0.0392	0.0958	0.0865	0.0502	0.0867	0.0798	0.0719	0.0814	0.0798	0.0821	0.0901	0.0445	0.0353	–	81	65	63	66	143	130	155	156
20 *B. i. planifrons*	0.0888	0.0877	0.0843	0.0968	0.1043	0.0828	0.0957	0.0887	0.0957	0.0911	0.0877	0.0900	0.0917	0.0888	0.0877	0.0900	0.0946	0.0844	0.0924	–	68	63	62	163	133	163	158
21 *B. herrerae*	0.0763	0.0774	0.0763	0.0820	0.0875	0.0716	0.0763	0.0684	0.0900	0.0740	0.0604	0.0672	0.0719	0.0672	0.0683	0.0661	0.0867	0.0741	0.0741	0.0774	–	47	45	150	126	149	154
22 *B. rudicollis* 1	0.0672	0.0661	0.0626	0.0672	0.0718	0.0594	0.0774	0.0697	0.0718	0.0683	0.0626	0.0604	0.0614	0.0604	0.0672	0.0683	0.0741	0.0650	0.0718	0.0718	0.0535	–	14	156	130	158	148
23 *B. rudicollis* 1a	0.0670	0.0636	0.0613	0.0659	0.0706	0.0558	0.0730	0.0649	0.0694	0.0684	0.0591	0.0625	0.0627	0.0625	0.0627	0.0637	0.0741	0.0636	0.0765	0.0717	0.0520	0.0162	–	153	130	157	146
24 *M. g. gadovii*	0.1743	0.1765	0.1617	0.1686	0.1690	0.1628	0.1902	0.1831	0.1686	0.1834	0.1822	0.1720	0.1776	0.1686	0.1856	0.1902	0.1710	0.1687	0.1631	0.1856	0.1708	0.1777	0.1767	–	101	117	141
25 *A.chiszari*	0.1707	0.1757	0.1659	0.1696	0.1686	0.1658	0.1779	0.1707	0.1719	0.1753	0.1805	0.1657	0.1731	0.1619	0.1802	0.1853	0.1747	0.1673	0.1659	0.1693	0.1609	0.1659	0.1682	0.1291	–	94	121
26 *A. gramínea*	0.1754	0.1765	0.1720	0.1777	0.1773	0.1728	0.1902	0.1844	0.1800	0.1822	0.1788	0.1811	0.1847	0.1708	0.1834	0.1891	0.1836	0.1745	0.1768	0.1856	0.1697	0.1800	0.1816	0.1333	0.1206	–	132
27 *E.kingii*	0.1708	0.1731	0.1731	0.1708	0.1748	0.1748	0.1868	0.1794	0.1788	0.1845	0.1788	0.1743	0.1765	0.1720	0.1845	0.1879	0.1779	0.1756	0.1779	0.1800	0.1754	0.1686	0.1688	0.1606	0.1559	0.1503	–

The remaining taxa assigned to *B. i. imbricata* were intermingled with those of *B. i. ciliaris* and *B. i. jonesi*. One of these, *B. i. imbricata* 9, is the sister taxon of *B. i. jonesi*, both coming from the same subprovince, the Pacific Mountain Ranges and Cuestas subprovince in the Sierra Madre del Sur. Genetic distances between haplotypes belonging to these taxa (3.53–4.44%) were intermediate between the clearly interspecific and intraspecific distances in this study. Therefore, whether the populations assigned to *B. i. imbricata* from the latter subprovince and *B. i. jonesi* represent the same lineage needs further investigation, emphasising the study of their apparent morphological differences. On the other hand, our morphological study revealed that the specimens assigned to *B. i. ciliaris* from the Sierra Madre Occidental have one unfixed feature that appears to occur in a higher frequency than in the remaining populations of *Barisia* (one canthal, 44.4%). Also, these specimens are evidently bigger and exhibit a lighter dorsal coloration than specimens assigned to *B. i. ciliaris* from the remaining provinces. Furthermore, Guillette and Smith (1982) characterized *B. i. ciliaris* by having a light brown to cream coloured dorsum in adults of both sexes. However, of the 45 specimens assigned to *B. i. ciliaris* examined in this study, 41 came from northern localities situated in Chihuahua, Durango and Nuevo Leon, whereas only four came from central and southern localities in Zacatecas and Hidalgo, respectively. Thus, confirmation of the three aforementioned potentially diagnostic features for the *B. i. ciliaris* populations from the Sierra Madre Occidental will require comparison of additional material that covers the entire distribution of this putative subspecies.

The genetic distances found within the (*B. i. imbricata* 2 and *B. i. ciliaris* 2 and 4) clade, which was strongly supported by BPP but weakly supported by BTP and BS values, were surprisingly within the range of intraspecific distances (2.85–3.6%). This suggests that this clade represents a separate evolutionary lineage, despite the fact that *B. i. imbricata* 2 came from the same province as the clade with most of the taxa assigned to *B. i. imbricata* (but see below). However, the possible conspecificity of the members of this clade needs further investigation. Finally, the taxon assigned to *B. i. ciliaris* from the Sierra Madre Oriental in Querétaro is strongly supported as the sister taxon to the large clade containing the (*B. i. imbricata* 9 + 9a + *B. i. jonesi*, and possibly *B. i. ciliaris* 1 + 1a) and (*B. i. imbricata* 2 + *B. i. ciliaris* 2 + 4) clades. If the latter clades are conceived as conspecific, *B. i. cilaris* 3 could also belong to the same lineage, provided this is not contradicted by significant morphological differentiation. However, if these represent different lineages, *B. i. ciliaris* 3 would also represent a separate species. The genetic distances between the haplotypes assigned to *B. i. ciliaris* 3 and the other haplotypes (4.9–7.44%) are in some cases intermediate, and might be regarded either as inter- or intraspecific. Further investigation is needed in this whole clade.

Despite its unresolved relationships and absence of known morphological autapomorphies, the considerable molecular divergence of *B. i. planifrons* from the remaining members of *B. imbricata* (8.28–10.43%), reported allopatry with respect to the remaining members of the genus (Guillette and Smith 1982), and provenance from an area with high levels of endemism (Flores-Villela and Gerez 1994; Nieto-Montes de Oca 2003) suggests that it represents a distinct species. Finally, two consistent morphological autapomorphies, the presence of only one superciliary and 57–63 transversal dorsal rows of

scales (mixed scaled analysis: CI = 0.87 and 0.49, respectively), define *B. levicollis* as a separate species.

Divergence times and phylogeographic inferences

The molecular clock hypothesis has a long history of controversy, mainly because of its accuracy and the mechanism of evolution involved; however, the use of approximate estimates of divergence times could serve as an important tool for understanding evolutionary processes (see Nei and Kumar 2000 for a review of the topic). We therefore calculated rough estimates of divergence times among the haplotypes of *Barisia* based on a rate of molecular evolution employed by Macey et al. (1999) for the mtDNA ND1, ND2, COI and their associated tRNA genes in five gerrhonotine genera. This rate of molecular evolution, which ranges from 0.65–0.69% change per lineage per million years before present (Myr BP), resulted in divergence times for the gerrhonotine genera that varied between 8.55 and 12.08 Myr BP. Using the same evolution rates for the genetic marker sequenced here resulted in slightly higher divergence times among the four gerrhonotine genera included (9.35–14.63 Myr BP). We thus scaled the molecular clock estimates for the aforementioned genes to the ND4 and associated tRNA genes calculating the average ratio estimates between the haplotypes of two taxa present in Macey et al.'s (1999) and our study, *B. i. imbricata* from the Tranvolcanic Belt (excluding *B. i. imbricata* 2, 5 and 6) and *E. kingii*.

The scaling factor obtained, which was of 0.85% change per lineage per Myr BP, gave divergences that ranged from 0.35 to 3.8 Myr BP among the haplotypes of *B. i. ciliaris* from the Sierra Madre Oriental, Central Plateau, and Sierra Madre Occidental. On the other hand, divergences ranged from 4.0 to 5.86 Myr BP between haplotypes from the aforementioned regions and the haplotypes of *B. i. imbricata* from Tehuacan–Cuicatlan–Quiotepec infraprovince (Sierra Madre del Sur) and the Transvolcanic Belt with the exclusion of the one from Pico de Orizaba. Interestingly, the two haplotypes of *B. i. imbricata* from the southern Mexico City mountain ranges are more divergent from the rest of haplotpyes from the Transvolcanic Belt than these haplotypes are from each other (3.95–4.85 and 0.35–2.68 Myr BP, respectively). Finally, divergences between the haplotype of *B. i. planifrons* from Sierra de Juarez, and between the haplotypes of *B. herrerae* and *B. rudicollis*, which come from the central part of the Transvolcanic Belt, and the remaining haplotypes of *Barisia* ranged from 4.21 to 6.13 and 3.28 to 3.82 Myr BP, respectively.

The ages of divergence proposed here are congruent with the transition from the Sierra Madre Occidental to the Transvolcanic Belt during the middle to late Miocene 11–7 Myr BP (Ferrari et al. 1994, 1999). Thus, this geological event could have separated the *B. i. imbricata* populations from the Transvolcanic Belt from the populations of the remaining regions. Furthermore, the glaciations during the Pliocene–Pleistocene, which resulted in several expansion/contraction cycles of the temperate biota in Central and North America at that time (Toledo 1982; Graham 1989, 1993, 1999), concord with a more recent genetic divergence among the populations of *B. i. imbricata* from the Transvolcanic Belt and the Tehuacan–Cuicatlan–Quiotepec infraprovince. The relationships found among the haplotypes from the Sierra Madre Occidental, Sierra Madre Oriental, and the Pacific Mountain Ranges subprovince also suggest a close faunistic affinity among these zones to the exclusion of the Tehuacan–Cuicatlan–Quiotepec and the North-eastern Montain Ranges regions in the Sierra Madre del Sur and Transvolcanic Belt, except for the Pico de Orizaba locality. The Pico de Orizaba, also known as Citlaltépetl, is a volcano currently considered to be at the eastern end of the Transvolcanic Belt (Carrasco-Nuñez and Rose 1995; Carrasco-Nuñez 1999). However, until a few decades ago this volcano and other mountain ranges from southeast Puebla and the adjacent regions of Veracruz and Oaxaca were believed to be part of the Sierra Madre Oriental (e.g. De Cserna 1961; Murray 1961). The relationships found here among the haplotypes included agree more with this last proposal.

Evolution of dorsal pattern

The presence/absence of adult dorsal pattern has been traditionally used as one of the diagnostic features for distinguishing among the different members of the *B. imbricata* complex (Tihen 1949b; Guillette and Smith 1982). In this study, we demonstrate that a dorsal pattern of dark transverse bands present in adult females but absent in males, as well as the absence of pattern in adults of both sexes, have each evolved in several separate lineages within *Barisia*. In addition, intraspecific variation in female adult dorsal pattern also appears to occur in at least some of the suggested distinct lineages in our mtDNA phylogenies; viz. the populations assigned to *B. i. imbricata* from the Transvolcanic Belt (Fig. 4). Thus, the current classification of *Barisia* based on this feature does not reflect the evolutionary history of the genus.

Wiens (2001b) proposed four scenarios for the gain and loss of sexual dimorphism within a population. According to this author, sexual dimorphism could arise through the gain of a trait in males alone (but see Andersson 1994), or its gain in both sexes with the subsequent loss of the trait in females. On the other hand, sexual dimorphism could be lost by either the loss of the male trait or by its gain in females. Unfortunately, our best estimate of phylogeny could not recover most of the ancestral dorsal pattern states and thus we are not able at this stage to propose with confidence any of the aforementioned hypotheses to explain the variation in the presence of sexual dichromatism within *Barisia*. However, acceptance of Good's (1988) hypothesis of the presence of dorsal pattern as the ancestral condition in the Gerrhonotinae would imply that the adult sexual dichromatism within *Barisia* arose by the gain of dorsal pattern in both sexes and then its loss in males, and that this sexual dichromatism also has been repeatedly lost by the subsequent loss of the trait in females. The presence of a dorsal pattern in juveniles of the unpatterned taxa assigned to *B. imbricata* (A. Zaldivar-Riverón, unpublished data) supports this state as the ancestral condition within the genus.

Colour features are among the morphological character systems probably more influenced by both historical causes and selective pressures (Thorpe 2002; Thorpe and Stenson 2003). Specifically, sexual selection has been argued to be the most important factor causing sexual dichromatism, whereas ecological selection regimes are likely to influence colour and pattern variation in some populations regardless of their shared evolutionary trajectory. In the case of anguids and other lizards, sexual dichromatism usually consists of a distinctive colour and/or pattern in males, which is generally acquired at sexual maturity accompanied by an increase in head width relative to body size (Stebbins 1958; Karges and Wright 1987; Vial and Stwart 1989; Zaldivar-Riverón and

Nieto-Montes de Oca 2001, 2002). It has been hypothesized that these features could develop in response to two possible factors: (1) sexual selection pressures probably associated with agonistic and/or courtship behaviours (e.g. Vitt and Cooper 1985; Vial and Stwart 1989; Formanowicz et al. 1990), or (2) 'intraspecific niche divergence', where the sexes differentiate morphologically in order to reduce intraspecific competition (e.g. Griffith 1991). On the other hand, intraspecific variation in colour and pattern has been shown to be determined in some species of lizards by local adaptation to environmental conditions such as temperature, precipitation, or type of vegetation (e.g. Gübitz et al. 2000; Thorpe and Stenson 2003). Future ecological and behavioural studies will help to identify the factors responsible for sexual dichromatism and intraspecific variation in colour pattern in some of the suggested lineages of *Barisia*.

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Zusammenfassung

Phylogenie und Evolution des dorsalen Musters in der endemischen mexikanischen Gattung Barisia (Anguidae: Gerrhonotinae)

Wir untersuchten die Phylogenese der mexikanischen Eidechsengattung *Barisia* anhand von 878 Basenpaaren eines Fragmentes der mtDNA des ND4 Gens und eines Abschnitts assoziierter tRNAs sowie 16 äußeren morphologischen Merkmalen. Terminale Taxa waren die akzeptierten Arten der Gattung *Barisia* inklusive der vier Unterarten der polytypischen *B. imbricata* und Individuen verschiedener Populationen der weitverbreiteten *B. i. imbricata* und *B. i. ciliaris*. Für *B. levicollis* konnten nur die morphologischen Eigenschaften untersucht werden. Der 'Step-matrix-frequency' und der 'Step-matrix-gap-weighting' Kodierungsansatz wurden simultan für die morphologischen Daten eingesetzt. Für das 'Step-matrix-gap-weighting' wurden drei verschiedene Skalierungsmethoden verwendet. Eine Maximum-Parsimony-Analyse wurde für beide Datensätze einzeln und in Kombination durchgeführt. Die von einer kombinierten Maximum-Parsimony-Analyse generierte Hypothese unterstützt die Monophylie von *Barisia*, aber die 'Exklusivität' von *B. imbricata* sowie *B. i. imbricata* und *B. i. ciliaris* konnten nicht wieder gefunden werden. Zusammen mit der genetischen Distanz und der geographischen Übereinstimmung der untersuchten Haplotypen bestätigt dies, dass *B. imbricata* mehrere Arten representiert, doch ist die Abgrenzung der Arten innherhalb dieses Taxons unklar. Der Einsatz bereits publizierter Raten molekularer Evolution in den mtDNA Daten ergibt ähnliche Zeitwerte wie die vermuteten Zeitwerte für die geologischen und klimatischen Phänomene, welche im mexikanischen Territorium im Pleistozän-Miozän stattfanden. Das Kartieren der ausgewählten Ausprägungen der dorsalen Muster auf der Bayesischen Topologie zeigt, dass sich die beiden Hauptausprägungen, die in den Taxa, die *B.imbricata* zugeordnet werden, vorkommen (ein nur in weiblichen Adulten vorkommendes dorsales Muster und das Fehlen von Mustern in beiden Geschlechtern) in mindestens drei beziehungsweise vier Fällen eigenständig entwickelt hatten. Dieses deutet auf ein Szenario, in dem der sexuelle Dichromatismns wiederholt in verschiedenen Linien von *Barisia* verloren ging.

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Authors' addresses: A. Zaldivar-Riverón (present address and address for correspondence) Department of Biological Sciences, Imperial College London, Silwood Park Campus, Ascot, Berkshire SL5 7PY, UK. E-mail: alejandro.zaldivar-riveron@imperial.ac.uk; A. Nieto-Montes de Oca, Museo de Zoología 'Alfonso L. Herrera', Facultad de Ciencias, Universidad Nacional Autónoma de México, México, Distrito Federal 04510, México, E-mail: anmo@hp.fciencias.unam.mx; J. P. Laclette, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, México, Distrito Federal 04510, México.

Appendix A

Specimens examined for the morphological data

Specimens assigned to *B. herrerae*, *B. i. jonesi*, *B. i. planifrons* and *B. rudicollis* are listed in Zaldivar-Riverón and Nieto-Montes de Oca's (2002) study. Acronyms for museum collections follow Leviton *et al.* (1985), except for MZFC (Universidad Nacional Autónoma de México) and EBUAP (Escuela de Biología, Universidad Autónoma de Puebla).

Barisia imbricata ciliaris 1 ($n = 18$): MEXICO: *Durango*: El Salto (MZFC 12547–48); 5 mi ENE El Salto (LSUMZ 30668); 10 mi E El Salto (AMNH 68357); 10 mi W El Salto (AMNH 98164); 56 mi W Durango (LACM 92674); Navajas (LACM 121928); Navajas, *c.* 40 mi W El Salto (LACM 121314); Hacienda Los Coyotes, 3 mi S El Salto (LACM 92675); 3 mi NW Los Coyotes (LACM 136860); 10 mi S rancho Santa Bárbara (LACM 133639); 6 mi SE Llano Grande (LACM 121927); Navios (AMNH 118078); Rancho Las Canoas, Municipality of Suchil, Reserva de la Biosfera (IBH 2503); Rancho Temascal, Municipality of Suchil (IBH 7299); Reserva de la Michilía, Municipality of Suchil (IBH 7300–01); Vicinity of Palo Gordo (CM 90195).

Barisia imbricata ciliaris 2 ($n = 3$): MEXICO: *San Luis Potosí*: Cerro Conejo, between Llano de Conejo and Llano de Garzas (LSUMZ 341); 2 km W Alvarez, Municipality of Díaz Zaragoza (MZFC 11082); 10 mi NE Bledos (CM 41516). *Barisia imbricata ciliaris* 3 ($n = 5$): MEXICO: *Querétaro*: Municipality of Cadereyta, 1 km NE El Doctor (MZFC 8430); Vicinity of Pinal de Amoles (MZFC 6875); San Joaquín (MZFC 7551); Municipality of Pinal de Amoles, 6 km NW Rancho Los Velázquez (MZFC 9252–53). *Barisia imbricata ciliaris* 4 ($n = 5$): MEXICO: *Querétaro*: Municipality of Colón, Pinal de Zamorano (MZFC 9401–03, 9752–53). *Barisia imbricata imbricata* 1 ($n = 2$): MEXICO: *Hidalgo*: Zacualtipán (MZFC FMQ 2606); Atotonilco El Grande (FMNH 106156). *Barisia imbricata imbricata* 2 ($n = 11$): MEXICO: *Veracruz*: 2 mi ESE Las Vigas (LSUMZ 28687, 31053); 1.5 mi S Puerto del Aire (LSUMZ 10978); 4 km W Puerto Morelos (MZFC 727); 1–5 km W Xometla (LACM 131443, 131445–47); SE slope Pico de Orizaba (LACM 135557); Orizaba, Texmalaquilla (LACM 121929); Pico de Orizaba (MZFC AZR 315). *Barisia imbricata imbricata* 3 ($n = 2$): MEXICO: *Tlaxcala*: Volcán La Malinche (MZFC 12564); San Tadeo Huexoyucan (MZFC 12563). *Barisia imbricata imbricata* 4 ($n = 20$): MEXICO: *Mexico*: Xalatlaco, El Capulín (MZFC 3242, 3384–85); Parque Nacional; Popocatépetl (MZFC 3413); Zoquiapan, Cañada del Quesero (MZFC 3352, two specimens); NE extreme Llano Grande (MZFC 982, two specimens); Parque Nacional Zoquiapan (MZFC 3207, seven specimens); *Puebla*: km 97 on Huauchinango–Apizaco road (MZFC 12544); Santa Rita Tlahuapan (EBUAP 460); Colonia El Cerrito, Puebla City (EBUAP 463). *Barisia imbricata imbricata* 5 ($n = 9$): MEXICO: *Distrito Federal*: Ejido San Nicolás Totolapan, 2 km W of Albergue El Pino, Delegación Magdalena Contreras, 3320 m (MZFC 11663; GenBank accession no. AY605112); Serranía del Ajusco (MZFC 282–84); Ajusco, Llano Cieneguillas (MZFC 279–80); Ajusco, W of Valle Monte Alegre (MZFC 281); approximately 200 m E. P.C.V.U. (MZFC 4326).

Barisia imbricata imbricata 6 ($n = 7$): MEXICO: *Morelos*: Parque Nacional Lagunas de Zempoala (MZFC 2333, 1195–96, 12565; IBH 1671, 3159, 3665). *Barisia imbricata imbricata* 7 ($n = 1$): MEXICO: *Oaxaca*: Peña Verde, Cañada de Cuicatlán (MZFC 12545). *Barisia imbricata imbricata* 8 ($n = 1$): Mexico: *Michoacán*: Mil Cumbres (MZFC AZR 309). *Barisia imbricata imbricata* 9 ($n = 9$): MEXICO: *Jalisco*: Manantlán, Laboratorio Natural Las Joyas (MZFC 4714); 23 km from El Terrero, Manantlán (MZFC 6001); Cerro Grande, El Terrero, near El Tapeixtle (MZFC 6002, 6019, 6773–74); El Tapeixtle, 3 km N El Terrero; El Tapeixtle (MZFC 8138–40). *Abronia graminea* ($n = 9$): MEXICO: *Oaxaca*: Puerto de la Soledad (MZFC-4294–95, 4830). *Puebla*: Puerto Morelos (MZFC-3256, 6331); Mpio. de Ozumbilla, Tepeyolulco (MZFC-7816). *Veracruz*: Acultzingo, 1 km S Puerto del Aire (MZFC-4124); La Joya (MZFC-5032).

Abronia chiszari ($n = 1$): MEXICO: *Veracruz*: Santa Marta volcanic range, near Bastonal, 800 m (UNAM-LT 3151).

Elgaria kingii ($n = 9$): MEXICO: *Aguascalientes*: Mpio. de Calviño, Arroyo Ibañez (MZFC-11318–19). *Chihuahua:* close to El Divisadero station (MZFC-2165); Cascada de Basaseachic. *Jalisco*: Autlán, Rincón de Manantlán (MZFC-6003). Colima: Minatitlán, 2.2 km road to El Terrero (MZFC-6813).

Mesaspis gadovii gadovii ($n = 10$): Guerrero: Omiltemi (MZFC-2710, 2714–15, 2718, 2721, 2731, 2733, 2739, 2754, 10206).

Appendix B

Morphological characters

The consistency index obtained of each character for the between-states, between-characters, and mixed scaling methods are given after the character states description, respectively. Numbers for the character states given for the qualitative characters do not refer to polarity. Range of mean taxa values is indicated for the meristic and morphometric characters.

1. *Nasal-rostral contact*: absent (0) (Fig. 6a); present, with anterior internasals present (1) (Fig. 6b). Polymorphic and qualitative. (CI = 1.0, 1.0, 1.0). A nasal-rostral contact occurs in all species of *Elgaria*, but this is due to the absence of the anterior internasals (Good 1988). Thus, the latter condition was coded as 0 in this study.
2. *Supranasal-upper postnasal fusion*: (0) absent; (1) present (Fig. 6a,b). Fixed and qualitative (CI = 0.33, 1.0, 1.0).
3. *Postnasal-anterior loreal fusión*: (0) absent; (1) present (Fig. 6a,b). Polymorphic qualitative (CI = 1.0, 1.0, 1.0).
4. *Number of loreals*: (0) two (Fig. 6b); (1) one (Fig. 6a). Meristic, only two fixed states (CI = 1.0, 1.0, 1.0; range of mean taxa values = 1.0).
5. *When one loreal is present, then canthal*: (0) absent (Fig. 6a); (1) present. Polymorphic and qualitative (CI = 0.519, 0.604, 1,0).
6. *Frontonasal*: (0) present (Fig. 6d); (1) absent (Fig. 6c). Polymorphic and qualitative (CI = 0.281, 0.643, 0.604).
7. *Superciliars*: Meristic (CI = 0.509, 0.868, 0.868; range = 1–6. Four superciliars: Fig. 6a,b).
8. *Occipitals*. Mersitic (CI = 0.781, 0.781, 0.781; range = 1–3. One occipital: Fig. 6c; three: Fig. 6d). The elements herein mentioned as occipitals were those considered by Good (1988) as the interoccipitals + occipitals.
9. *Number of longitudinal nuchal rows*. Meristic (CI = 0.577, 0.467, 0.467; range = 4–10).
10. *Lateral nuchals*: (0) moderately keeled, not extended laterally (Fig. 6e); (1) strongly keeled and projected laterally (Fig. 6f). Fixed and qualitative (CI = 1.0, 1.0, 1.0).
11. Postmentonal: (0) divided; (1) undivided. Polymorphic and qualitative (CI = 0.666, 0.666, 0.66).

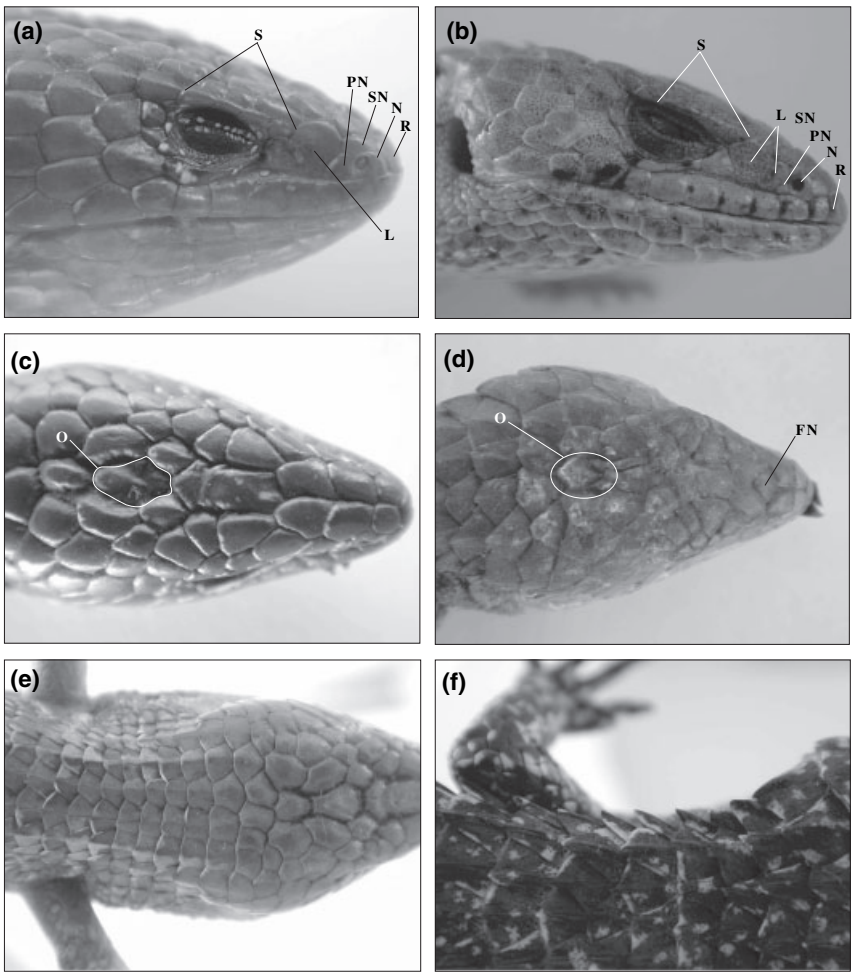

Fig. 6. Lateral and dorsal views of the head of selected specimens of *Barisia*. (a) Lateral view of the head of *B. i. imbricata* (MZFC 281). (b) Lateral view of the head of *B. rudicollis* (MZFC 12541). (c) Dorsal view of the head of *B. i. planifrons* (MZFC 12546). (d) Dorsal view of the head of *B. rudicollis* (MZFC 12541). (e) Lateral view of the head of *B. i. imbricata* (MZFC 281) showing the smooth, non-laterally projected nuchal scales. (f) Lateral view of the head of *B. rudicollis* (MZFC 9586) showing strongly keeled and laterally projected nuchal scales. R, rostral; N, nasal; L, loreal; SN, supranasal; PN, postnasal; C, canthal; S, superciliar series; O, occipitals

12. *Number of transversal dorsal rows*. Meristic (CI = 0.557, 0.447, 0.433; range of mean taxa values = 27.0). The number of transversal dorsal rows was counted starting from the scale posterior to the occipital until the level of the anal cavity.
13. *Number of longitudinal dorsal rows*. Meristic (CI = 0.46, 0.49, 0.49; range of mean taxa values = 6.0).
14. *Number of transversal ventral rows*. Meristic (CI = 0.437, 0.479, 0.437; range of mean taxa values = 16.0).
15. *Number of longitudinal ventral rows*. Meristic (CI = 1.0, 0.552, 0.552; range of mean taxa values = 2.0).
16. *Maximum snout-vent length in adults*. Morphometric (CI = 0.845, 0.47, 0.455; range of mean taxa values = 83.3).

Appendix C

Trait frequencies and mean values for the polymorphic, qualitative and the meristic and morphometric characters, respectively

Taxon	1	3*	5*	6	7*	8	9	11	12	13	14	15	16
Barisia herrerae	96%	39.6%	—	0	3.91	3	4.86	0	31.04	14	53.04	14	125
Barisia i. ciliaris 1	0	0	44.4%	0	3.10	1.11	8.11	16.7%	41.56	16	57.81	12	146.3
Barisia i. ciliaris 2	0	0	16.6%	0	2.83	1	8.66	33.3%	40.66	16	54.33	12	129
Barisia i. ciliaris 3	0	0	0	0	3.1	1	8	0	40.66	16	54.75	12	134.5
Barisia i. ciliaris 4	0	0	10%	0	2.8	1	8	0	40.2	16	54.6	12	100.5
Barisia i. imbricata 1	0	0	0	0	3	1	8.5	0	39	16	54.5	12	116.3
Barisia i. imbricata 2	0	0	5%	0	2.92	1	8.8	0	38.6	14	55	12	112.7
Barisia i. imbricata 3	0	0	0	0	3	1	8	0	39	14	56	12	110.6
Barisia i. imbricata 4	0	0	2.5%	0	3	1.33	8	0	38.13	14	55.78	12	108.3
Barisia i. imbricata 5	0	0	7.1%	0	2.88	1	8	0	38.21	13.3	55.12	12	113.6
Barisia i. imbricata 6	0	0	0	0	3	1	8	0	37.5	14	54.5	12	114.5
Barisia i. imbricata 7	0	0	0	0	3	1	8	0	39	14	54	12	109
Barisia i. imbricata 8	0	0	0	0	3	1	8	0	38	14	53	12	106.8
Barisia i. imbricata 9	0	0	0	0	3	1	8	0	38.11	14	53.77	12	114.7
Barisia i. jonesi	0	0	0	0	3	1.09	8	0	38.63	14	55.17	12	118
Barisia i. planifrons	0	0	0	0	3.07	1	8	0	36	14	54.9	12	120.1
Barisia levicollis	0	0	0	0	1	1.14	9.92	0	48.21	16	60.5	12	151.3
Barisia rudicollis	100%	50%	—	44.4%	4.03	3	5.51	0	29.35	14	50.17	14	124.2
Abronia chiszari	0	0	—	100%	6	1	8	0	46	16	58	12	?
Abronia graminea	0	0	—	100%	5.87	1	6	0	27.5	14.5	50.87	13.62	116.7

Appendix C. Continued.

Taxon	1	3*	5*	6	7*	8	9	11	12	13	14	15	16
Mesaspis g. gadovii	0	0	—	100%	4.14	1	9.37	0	47.42	17.1	53.71	12	102
Elgaria kingii	0	0	—	100%	6	1	10	0	55	14	58.8	12	124.4

*Characters whose sample size was considered to be the double of the examined specimens due to they varied bilaterally.

Appendix D

Data matrix of the 16 selected morphological characters

	Characters															
Terminal taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
B. herrerae 1	1	1	1	0	?	2	4	5	0	1	0	2	1	3	2	g
B. i. ciliaris 1	0	1	0	1	6	2	5	2	4	0	1	e	3	h	0	j
B. i. ciliaris 2	0	1	0	1	5	2	9	0	6	0	2	d	3	7	0	h
B. i. ciliaris 3	0	1	0	1	0	2	5	0	3	0	0	d	3	a	0	i
B. i. ciliaris 4	0	1	0	1	4	2	b	0	3	0	0	c	3	9	0	1
B. i. imbricata 1	0	1	0	1	0	2	7	0	5	0	0	b	1	8	0	a
B. i. imbricata 2	0	1	0	1	2	2	8	0	7	0	0	9	1	c	0	6
B. i. imbricata 3	0	1	0	1	0	2	7	0	3	0	0	b	1	g	0	5
B. i. imbricata 4	0	1	0	1	1	2	7	4	3	0	0	7	1	f	0	3
B. i. imbricata 5	0	1	0	1	3	2	a	0	3	0	0	8	0	d	0	7
B. i. imbricata 6	0	1	0	1	0	2	7	0	3	0	0	4	1	8	0	8
B. i. imbricata 7	0	1	0	1	0	2	7	0	3	0	0	b	1	6	0	4
B. i. imbricata 8	0	1	0	1	0	2	7	0	3	0	0	5	1	2	0	2
B. i. imbricata 9	0	1	0	1	0	2	7	0	3	0	0	6	1	5	0	9
B. i. jonesi	0	1	0	1	0	2	7	1	3	0	0	a	1	e	0	c
B. i. planifrons	0	1	0	1	0	2	6	0	3	0	0	3	1	b	0	d
B. levicollis	0	1	0	1	0	2	c	3	9	0	0	h	3	k	0	k
B. rudicollis	2	1	2	0	?	1	3	5	1	1	0	1	1	0	2	e
A. chiszari	0	0	0	0	?	0	0	0	8	0	0	f	3	i	0	?
A. graminea	0	0	0	0	?	0	1	0	2	0	0	0	2	1	1	b
M. g. gadovii	0	0	0	0	?	0	2	0	3	0	0	g	4	4	0	0
E. kingii	0	0	0	0	?	0	0	0	a	0	0	i	1	j	0	f