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MOLECULAR PHYLOGENY OF BRAZILIAN *MABUYA* (REPTILIA, SQUAMATA, SCINCIDAE) OF THE *AGILIS/CAISSARA/HEATHI* COMPLEX

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Phylogenetic relationships among neotropical skinks of the genus *Mabuya* are currently unknown. Three species, *Mabuya agilis*, *M. caissara* and *M. heathi* are morphologically very similar and appear to be closely related, but their relationships have never been studied. Here we examine their phylogenetic relationships using partial sequences of the mitochondrial 12S and 16S rRNA genes. Nine populations of *M. agilis*, four of *M. heathi* and one of *M. caissara* were sampled, as well as one population from four other South American *Mabuya* species. Results of both maximum likelihood and maximum parsimony analyses reveal a strongly supported monophyletic group comprising all populations of *agilis, caissara* and *heathi*, but relationships of this clade with the other *Mabuya* species were not resolved. Genetic distances among members of the *agilis/caissara/heathi* clade ranged from 0.0% to 2.6%, whereas distances between its members and the other four congeners ranged from 6.0% to 8.6%. Genetic distances and the internal tree structure of the *agilis/caissara/heathi* clade are somehow consistent with the geographic location of the sampled populations. Based on our results, we suggest that this complex may represent a single species, though more data are needed to verify this.

Key words: Brazil, lizard, mitochondrial DNA, phylogenetics, skink

INTRODUCTION

Intercontinental relationships within the circumtropical genus "*Mabuya*" Fitzinger (Scincidae: Lygosominae), are far more complex than previously thought (Mausfeld *et al.*, 2002; Carranza & Arnold, 2003). A molecular analysis of the genus recently carried out by Mausfeld *et al*. (2002) demonstrated that *"Mabuya"* consists of four separated and deep evolutionary lineages, representing distinct and well-supported monophyletic radiations. To reflect the independent origins of the American, Asian, Afro-Malagasy and Cape Verdian groups, the genus *"Mabuya"* has been partitioned into four genera, keeping the name *Mabuya* for the neotropical species.

However, the systematics and taxonomy of neotropical *Mabuya* are still in an unstable state and remain poorly understood. Since Dunn's (1935) work, no other comprehensive revision of New World *Mabuya* has been carried out. Several subsequent contributions have added to or modified Dunn's original taxonomic arrangement, including the description of new species, the revalidation of old names previously in the synonymy of *M. mabouya* (see Rodrigues, 2000 for a review), the raising of a subspecies to specific status (Mausfeld & Lötters, 2001) and the restriction of the name *M. mabouya* to populations of the southern Lesser Antilles (Miralles, 2005). Nevertheless, the situation is still far from satisfactory. Indeed, even the recently proposed taxonomic arrangement of Avila-Pires (1995) concerning the two most common Amazonian species has not been universally accepted (see Rodrigues, 2000). Currently, the genus *Mabuya* is represented in continental South America by 17 species, most of which occur in Brazil (Rodrigues, 2000; Mausfeld & Lötters, 2001; Miralles *et al.*, 2005), but the phylogenetic relationships among them remain unstudied.

Recently, Rodrigues (2000) divided the South American *Mabuya* species into four phenetic groups, but cautioned that such groups were 'only convenient and based on similarity'. One of those groups comprised 'small species with normal (i.e. not prominent or acuminate) snout, paired frontoparietals and vertebral stripes on body' and included five species: *M. dorsivittata*, *M. guaporicola*, *M. heathi*, *M. agilis* and *M. caissara*. The former two (which Dunn [1935] believed to be closely related to each other) are rather distinctive in colour pattern and in some characteristics of scalation and morphology (*M. dorsivittata* normally has three supraoculars, instead of four as in the remaining species; *M. guaporicola* has comparatively short limbs and a high number of presacral vertebrae [Dunn, 1935; Greer *et al.*, 2000]), and do not occur in coastal areas. The latter three species, which are common in open habitats along the Atlantic rainforest biome of eastern Brazil (though *M. heathi* also occurs throughout the semi-arid caatingas), are very similar to each other. They all share the same general colour pattern, with a dark lateral band (extending from the snout to the tip of the tail) bordered below by a vivid white stripe that extends from the lower lip to the groin, and a mid-dorsal stripe with irregular dark borders (which can also be described as a pair of parallel irregular dark stripes, as in Schmidt & Inger's [1951)] description of *M. heathi*), which is usually

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TABLE 1. List of voucher specimens for each species included in the present study, with their respective localities, collection numbers and accession numbers (12S, 16S). All samples are from Brazil, except those of *Eumeces obsoletus* and *Mabuya* sp. Brazilian state codes are: BA - Bahia; CE - Ceará; DF - Distrito Federal; ES - Espírito Santo; RJ - Rio de Janeiro; RN - Rio Grande do Norte; SP - São Paulo.Acronyms: CHUNB - Coleção Herpetológica da Universidade de Brasília; MNRJ - Museu Nacional, Rio de Janeiro; UFC - Universidade Federal do Ceará; ZFMK - Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn.

Species	Locality	Collection number	Accession number
Eumeces obsoletus	USA	ZFMK 77248	AF548781, AF549169
Mabuya sp.	Tobago	ZFMK 62603	AY070339, AY070357
M. dorsivittata	Cunha, SP	MNRJ 9338	AY070346, AY070363
M. macrorhyncha	Queimada Grande island, SP	MNRJ 7287	AF548782, AF549170
M. nigropunctata	Brasília, DF	CHUNB 9624	AF548783, AF549171
M. heathi	Crateús (Serra das Almas), CE	UFC L2288	AY070330, AY070349
M. heathi	Natal, RN	MNRJ 8361	AF548784, AF549172
M. heathi	Abrolhos archipelago (Siriba island), BA	MNRJ 6663	AF548786, AF549174
M. heathi	Abrolhos archipelago (Sueste island), BA	MNRJ 6655	AF548785, AF549173
M. agilis	Prado, BA	MNRJ 9337	AY070326, AY070347
M. agilis	Guriri, ES	MNRJ 9543	AF548790, AF549178
M. agilis	Setiba, ES	MNRJ 9524	AF548791, AF549179
M. agilis	Praia das Neves, ES	MNRJ 9514	AF548789, AF549177
M. agilis	Carapebus (Jurubatiba restinga), RJ	MNRJ 9508	AF548792, AF549180
M. agilis	Massambaba, RJ	MNRJ 9560	AF548794, AF549182
M. agilis	Maricá (Barra de Maricá restinga), RJ	MNRJ 9561	AF548796, AF549184
M. agilis	Rio de Janeiro (Grumari beach), RJ	MNRJ 9491	AF548795, AF549183
M. agilis	Ilha Grande, Angra dos Reis, RJ	MNRJ 9494	AF548793, AF549181
M. caissara 1	Caraguatatuba (Massaguassu beach), SP	MNRJ 9476	AF548787, AF549175
M. caissara 2	Caraguatatuba (Massaguassu beach), SP	MNRJ 9485	AF548788, AF549176

lighter in colour than the rest of the metallic-brown dorsum. Such characters are mentioned, in varying detail, in the descriptions of each of the three species (Raddi, 1823; Schmidt & Inger, 1951; Rebouças-Spieker, 1974). Rodrigues (1990) implicitly suggested that *M. agilis* and *M. heathi* are conspecific and closely related to *M. caissara,* but did not comment the subject any further. Rocha (2000) also suggested that *M. caissara* and *M. agilis* should be closely related phylogenetically, given their strong morphological similarity. The geographic distributions of the three species appear to be contiguous in eastern Brazil: *Mabuya heathi* ranges from Fortaleza, in Ceará state, across the semi-arid "caatinga" habitats (see Eiten, 1992) of north-eastern Brazil and along the coast, south to Salvador, in Bahia state (Vanzolini *et al.* 1980; Peters & Donoso-Barros 1986), with an insular population recently reported from the Abrolhos archipelago (Dutra & Vrcibradic, 1998); *Mabuya agilis* occurs along the coastal lowlands and slopes of adjacent mountain ranges, from southern Bahia state to Rio de Janeiro state (Vanzolini, 1988; Rocha, 2000; Rocha *et al*., 2002*a*); *M. caissara* is apparently restricted to the northern coast of São Paulo state, from Ubatuba to Bertioga (Rebouças-Spieker, 1974; Vanzolini, 1988).

In the present study we examine the relationships among *Mabuya agilis*, *M. caissara* and *M. heathi*, based on an analysis of mtDNA sequences, to assess if they are reciprocally monophyletic. We include four other neotropical *Mabuya* species in the analysis in order to assess whether those three species form a monophyletic group.

MATERIALS AND METHODS

To examine the genetic variation within and among *Mabuya agilis*, *M. caissara* and *M. heathi*, liver or muscle tissue samples were collected from nine populations of *M. agilis*, one population of *M. caissara*, and four populations of *M. heathi* (Table 1). Among them are samples from the type localities of *M. agilis* (Rio de Janeiro municipality) and of *M. caissara* (Massaguassu beach, Caraguatatuba). We could not obtain samples from the type locality of *M. heathi* (Fortaleza, Ceará state), but we got material from an inland locality (Crateús) within the semi-arid "caatinga" domain in Ceará state, about 340 km SW of Fortaleza (which is a coastal locality). We sampled one individual for each population except for *M. caissara* from Caraguatatuba, in which case samples from two individuals were used (Table 1). Sampled localities for specimens of the *agilis/caissara/heathi* complex used in this study are depicted in Fig. 1.

Tissue samples of four other neotropical congeners, *M. dorsivittata , M. macrorhyncha, M. nigropunctata* and *Mabuya* sp. (a specimen from Tobago; the taxonomic status of *Mabuya* populations from that island requires confirmation [A. Miralles, pers. comm.]) were also included in the analysis (Table 1). The latter three taxa were once part of Dunn's (1935) *M. m. mabouya* together with members of the *agilis/caissara/heathi*

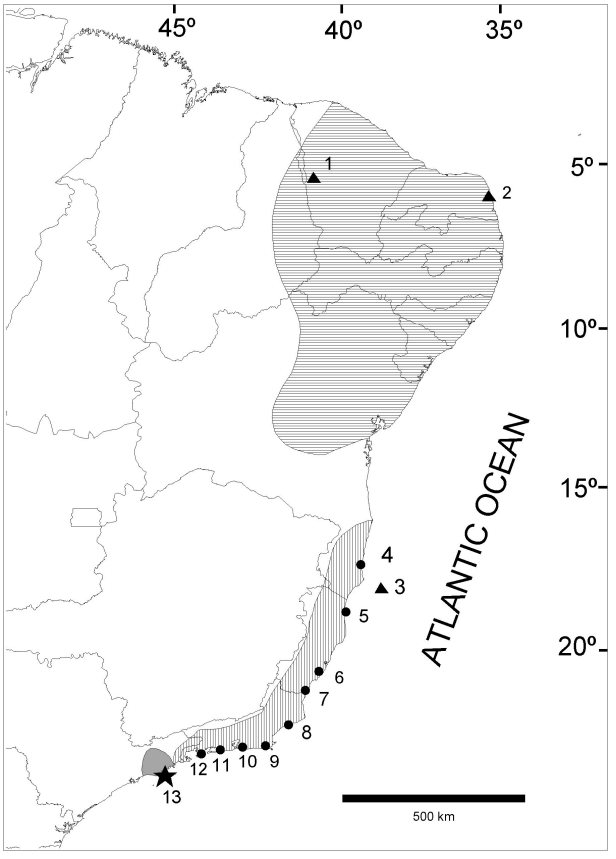

FIG. 1. Map of eastern Brazil showing the localities from which samples of *Mabuya heathi* (triangles), *M. agilis* (dots) and *M. caissara* (star) used in this study were obtained: 1 - Crateús, CE; 2 - Natal, RN; 3 - Abrolhos archipelago, BA; 4 - Prado, BA; 5 - Guriri, ES; 6 - Setiba, ES; 7 - Praia das Neves, ES; 8 - Carapebus, RJ; 9 - Massambaba, RJ; 10 - Maricá, RJ; 11 - Grumari, RJ; 12 - Ilha Grande, RJ; 13 - Caraguatatuba, SP. Brazilian state codes are: BA - Bahia; CE - Ceará; ES - Espírito Santo, RJ - Rio de Janeiro; RN - Rio Grande do Norte; SP - São Paulo. Approximate geographic distributions for each species, given as horizontal stippling (*M. heathi*), vertical stippling (*M. agilis*) and shading (*M. caissara*), are based on the literature and on data from material deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP).

complex, whereas *M. dorsivittat*a was included by Rodrigues (2000) in a phenetic group that also contained *M. agilis, M. caissara*, *M. heathi* and *M. guaporicola* (unfortunately, we could not obtain tissue samples from this last species). The North American scincine *Eumeces obsoletus* was used as outgroup.

Individuals from populations of the *agilis/caissara/heathi* complex included in our study are virtually indistinguishable in general appearance, except for *M. heathi* from the Abrolhos archipelago. The latter differ from members of other populations of the complex in being slightly lighter in coloration, with a caramel-brown to tan (rather than bronze) dorsum and a brown (rather than black) lateral dark band. Individuals from Praia das Neves and Setiba show some tendency towards larger body size than those from the other localities, but are otherwise identical to them (see Rocha *et al*., 2002*a*). All voucher specimens used in the present study are listed in Table 1, with their collection locality, voucher numbers, and GenBank accession numbers.

DNA was extracted from the tissue samples using QiaAmp tissue extraction kits (Qiagen). We used the primers 16sar-L (light chain; 5' - CGC CTG TTT ATC AAA AAC AT - 3') and 16sbr-H (heavy chain; 5' - CCG GTC TGA ACT CAG ATC ACG T - 3') of Palumbi et al. (1991) to amplify a section of the mitochondrial 16S ribosomal RNA gene. PCR cycling procedure was as follows. Initial denaturation step: 90 s at 94°C; 33 cycles: denaturation 45 s at 94°C, primer annealing for 45 s at 55°C, extension for 90 s at 72°C. Additionally, we used the primers 12SA-L (light chain; 5' - AAA CTG GGA TTA GAT ACC CCA CTA T - 3') and 12SB-H (heavy chain; 5' - GAG GGT GAC GGG CGG TGT GT - 3') of Kocher et al. (1989) to amplify a section of the mitochondrial 12S ribosomal RNA gene. Cycling procedure was as follows: 35 cycles: denaturation 45 s at 94°C, primer annealing for 60 s at 50°C, extension for 120 s at 74°C (12S).

PCR products were purified using Qiaquick purification kits (Qiagen). Sequences were obtained using an automatic sequencer (ABI 377). The obtained sequences (lengths referring to the aligned sequences including gaps) comprised 550 bp (16S), and 398 bp (12S). Sequences have been submitted to GenBank (Table 1).

Sequences were aligned using the computer program ClustalX (Thompson *et al*., 1997; default parameters). Alignment was subsequently adjusted manually using the computer program Se-Al 1.0a1 (Rambaut, 1996). We explored the quality of our alignment by varying alignment gap opening cost (6, 9, 12) and comparing between all three different alignments. In the 12S data set no ambiguous sites could be detected, in the 16S data set one ambiguously aligned region of 12 bp was found; these sites were excluded from the analysis (Gatesy *et al*., 1993; Milinkovitch & Lyons-Weiler, 1998). The complete alignment is available from the authors on request.

Confidence in the phylogenetic signal for the data-set was assessed using two different methods implemented in PAUP*4.0b8 (Swofford, 2002). The presence of a significant phylogenetic signal was estimated using the g1 statistic (Hillis & Huelsenbeck, 1992) estimated from 100,000 randomly generated parsimony trees (excluding the outgroup), and the permutation-tailed-probability (PTP) test (heuristic search with random sequence addition and 10 replicates; randomized ingroup taxa only) as suggested by Faith & Cranston (1991), with 100 replicates.

Prior to phylogenetic reconstruction, we tested for homogeneity of base frequencies among taxa using the χ^2 test as implemented in PAUP*4.0b8 (which ignores correlation due to phylogenetic structure): (1) over all sites, (2) over parsimony-informative sites only, (3) without constant sites (parsimony-uninformative and constant sites will mislead the χ^2 test (Misof *et al*.,

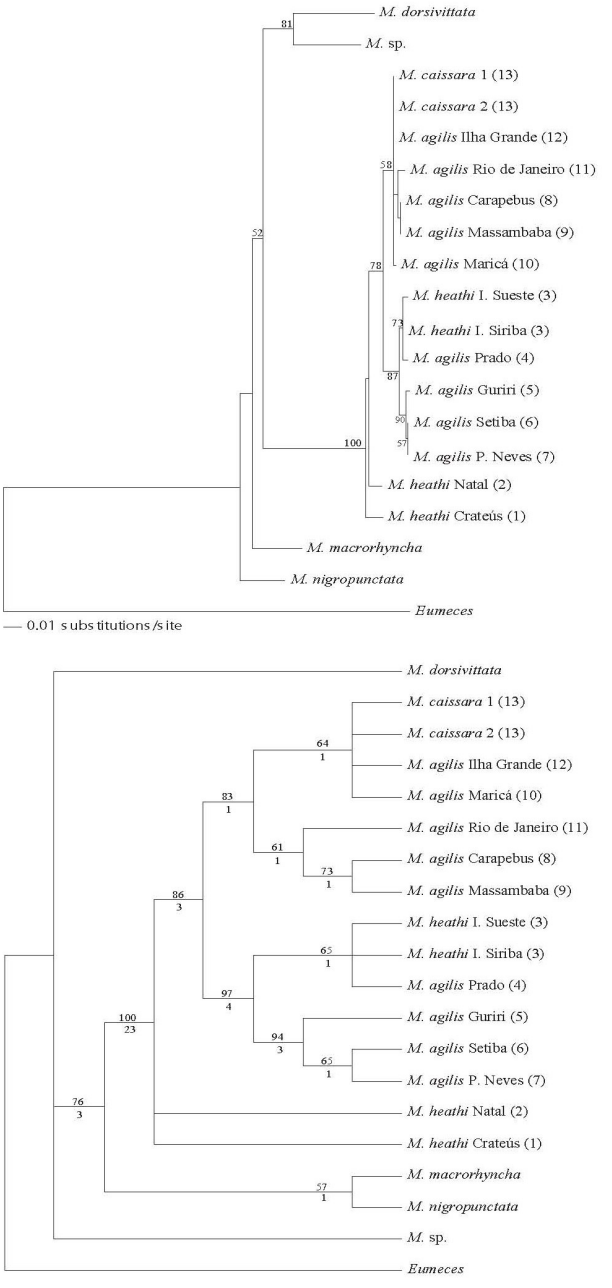

FIG. 2. Phylogram of the maximum likelihood tree (top) and the cladogram of the strict-consensus maximum parsimony tree (bottom) obtained from PAUP* searches using *Eumeces obsoletus* as outgroup. Numbers above nodes represent bootstrap proportions for 2000 and 100 pseudoreplicates for parsimony and likelihood analyses, respectively. Boostrap proportions less than 50% are not shown. Numbers below nodes in MP tree represent decay indices.

2001). All phylogenetic reconstructions were conducted on alignment unambiguous characters only, with the combined data set of the 16S and 12S gene fragments, and were performed with PAUP*4.0b8 (Swofford, 2002). We performed maximum-parsimony (MP) and maximum likelihood (ML) reconstructions. Parsimony reconstructions were performed with heuristic searches on parsimony-informative characters only, with 10 random stepwise addition replicates, and tree bisection-reconnection (TBR) branch-swapping. Gaps were treated as a 5th character state (Giribet & Wheeler, 1999; Simmons & Ochoterena, 2000; Simmons *et al*., 2001). When more than a single tree was found, a strict consensus tree was generated.

MODELTEST 3.06 (Posada & Crandall, 1998, 2001) was used to select the best-fit model for nucleotide substitution for our data set. Parameters of the model (substitution parameters, shape of gamma distribution, proportion of invariable sites) were estimated from the data set, without sites containing gaps (Aguinaldo *et al*., 1997), using a neighbour-joining starting-tree with p-distance. The ML tree was calculated with the parameter estimates obtained under the best-fit model. A heuristic search was made with 10 replicates of random stepwise addition and tree bisection-connection (TBR) branch swapping. A matrix of pairwise sequence differences for the combined 16S and 12S rRNA genes was calculated using the p-distance.

The relative branch support of the phylogenetic analysis was evaluated with 2000 bootstrap pseudoreplicates (heuristic search, with 10 replicates of random stepwise addition, TBR branch-swapping, parsimony-informative characters only) for MP, and 100 replicates for ML analysis (heuristic search, 10 replicates of random stepwise addition, TBR branch-swapping). As an alternative measure of nodal support, decay indices (Bremer, 1994) were calculated by running heuristic searches (100 random addition replicates, with TBR branch-swapping, and saving trees one step longer in each run) using TreeRot, version 2 (Sorenson, 1999) and PAUP*4.0b8.

RESULTS

The analyzed sequences from the 16S and 12S rRNA genes constitute a matrix of 936 characters. Of the 936 analyzed character sites analyzed, 117 sites were variable, and 88 were parsimony-informative. The matrix for the absolute number of DNA-sequence differences and uncorrected p-distances for all nucleotide sites is presented in Table 2. Phylogenetic signal is clearly present in the data set ($g1$=-1.2306, P=0.01; PTP test, P=0.01). When all characters were included, we found no significant deviation from the homogeneity of base frequencies among taxa (χ^2=6.710, P=1.00, df=57). The same was true for the parsimony-informative sites only (χ^2=25.167, P=0.9999, df=57) and without constant sites (χ^2=35.568, P=0.988, df=57).

The MP analysis produced eight equally most-parsimonious trees (tree length=191; CI=0.639; RI=0.766, RC=0.489). The comparison between the different likelihood scores for each model showed that the TrN + I + G model (Tamura & Nei, 1993) was determined to be the appropriate model for our data set. This model incorporates unequal base frequencies [$\pi_{(A)}$=0.339, $\pi_{(T)}$=0.219, $\pi_{(C)}$=0.255, $\pi_{(G)}$=0.187, a proportion of invariable sites (I=0.5431), and a gamma distribution shape parameter (G=0.523).

Both the strict consensus of the optimal MP trees and the best ML tree are shown in Fig. 2. Both MP and ML methods produced very similar topologies (with a few

TABLE 2. Summary of the absolute number of DNA-sequence differences (above the diagonal) and uncorrected p-distances (below the diagonal)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	*Eumeces*		128	135	133	128	154	146	150	149	150	148	148	148	149	151	152	152	152	149
2	*Mabuya* sp.	0.143		52	54	54	65	64	67	67	65	66	66	66	65	68	64	67	67	65
3	*M. dorsivittata*	0.151	0.058		58	59	73	68	77	52	74	75	75	77	73	78	76	75	75	73
4	*M. nigropunctata*	0.149	0.060	0.064		40	58	62	58	59	63	61	61	63	64	59	58	59	59	64
5	*M. macrorhyncha*	0.142	0.060	0.065	0.043		58	56	58	59	59	57	57	60	58	59	57	58	58	58
6	*M. heathi* Crateús	0.172	0.072	0.081	0.062	0.062		15	21	23	17	21	21	21	18	21	24	23	23	18
7	*M. heathi* Natal	0.163	0.071	0.075	0.067	0.060	0.016		22	24	17	19	19	17	16	22	21	22	22	16
8	*M. heathi* I. Siriba	0.167	0.075	0.085	0.063	0.062	0.023	0.024		2	15	15	15	15	14	2	7	6	6	14
9	*M. heathi* I. Sueste	0.166	0.075	0.085	0.064	0.064	0.025	0.026	0.002		17	17	17	17	16	4	9	8	8	16
10	*M. agilis* Maricá	0.167	0.072	0.082	0.068	0.064	0.018	0.018	0.016	0.018		4	4	6	1	15	18	17	17	1
11	*M. agilis* Massambaba	0.165	0.073	0.083	0.066	0.061	0.023	0.020	0.016	0.018	0.004		0	4	3	15	18	17	17	3
12	*M. agilis* Carapebus	0.165	0.073	0.083	0.066	0.061	0.023	0.020	0.016	0.018	0.004	0.000		4	3	15	18	17	17	3
13	*M. agilis* Rio de Janeiro	0.165	0.073	0.085	0.068	0.065	0.023	0.018	0.016	0.018	0.007	0.004	0.004		5	15	18	17	17	5
14	*M. agilis* Ilha Grande	0.166	0.072	0.081	0.069	0.062	0.019	0.017	0.015	0.017	0.001	0.003	0.003	0.005		14	17	16	16	0
15	*M. agilis* Prado	0.168	0.076	0.086	0.064	0.064	0.023	0.024	0.002	0.004	0.016	0.016	0.016	0.016	0.015		9	8	8	14
16	*M. agilis* Guriri	0.169	0.071	0.084	0.062	0.061	0.026	0.023	0.008	0.010	0.019	0.019	0.019	0.019	0.018	0.010		3	3	17
17	*M. agilis* P. Neves	0.169	0.075	0.083	0.064	0.062	0.025	0.024	0.007	0.009	0.018	0.018	0.018	0.018	0.017	0.009	0.003		0	16
18	*M. agilis* Setiba	0.169	0.075	0.083	0.064	0.062	0.025	0.024	0.007	0.009	0.018	0.018	0.018	0.018	0.017	0.009	0.003	0.000		16
19	*M. caissara*	0.166	0.072	0.081	0.069	0.062	0.019	0.017	0.015	0.017	0.001	0.003	0.003	0.005	0.000	0.015	0.018	0.017	0.017	

minor differences regarding *Mabuya* species outside the *agilis/caissara/heathi complex*). Both trees show strong bootstrap support (100%) for a monophyletic group containing all *Mabuya agilis*, *M. caissara* and *M. heathi.* Within this group, however, *M. agilis* and *M. heath*i were not recovered as monophyletic by neither MP nor ML analyses. Genetic differences within the *agilis/caissara/heathi* clade varied from 0% (between the two *M. caissara* samples, between *M. caissara* and *M. agilis* from Ilha Grande, between *M. agilis* from Massambaba and Carapebus, and between *M. agilis* from Setiba and Praia das Neves) to 2.6% (between *M. heathi* from Crateús and *M. agilis* from Guriri, and between individuals of *M. heathi* from Natal and Sueste island, Abrolhos) (Table 2). The lowest genetic difference between a member of the *agilis/caissara/heathi* group and any other *Mabuya* species was 6.0% (between *M. heathi* from Natal and *M. macrorhyncha*) (Table 2). The *agilis/caissara/heathi* clade itself was found (in both MP and ML analyses) to consist of a southern *agilis/caissara* group (populations from the states of Rio de Janeiro and São Paulo; this cluster was, however, only weakly supported in the ML tree), a northern *agilis/heathi* group (populations from the states of Bahia and Espírito Santo), and the two northernmost *heathi* specimens from Natal and Crateús (Fig. 2). These latter two *M. heathi* samples were sister taxa to the clade formed by the southern and northern groups in the ML tree, whereas in the MP tree they form a basal polytomy (Fig. 2).

The relationships of the *agilis/caissara/heathi* clade with respect to the other neotropical *Mabuya* sampled for this study were not resolved. In the ML tree (see Fig. 2) a moderately supported (81% bootstrap) *M. dorsivittata-M.* sp. clade is sister to the *M. agilis/caissara/heathi* clade, although the bootstrap support is very low. In the MP analysis (see Fig. 2), a weakly supported (57% bootstrap) *M. nigropunctata-M. macrorhyncha* clade appears as sister to the *agilis/caissara/heathi* complex with moderate (76%) bootstrap support (Fig. 2).

DISCUSSION

The *Mabuya agilis/caissara/heathi* complex forms, in both MP and ML analyses, a well-supported monophyletic group with rather small genetic distances among its members (0.0-2.6%). The clade contains two well-defined sub-groups: a southern *M. agilis/caissara* group, and a northern *M. agilis*/heathi group, though the former has only weak bootstrap support (58%) in the ML tree. Basal to these two sub-groups are the two northernmost *M. heathi* specimens. Non-monophyly of *M. agilis* is indicated by the fact that some of its populations are closely related to *M. caissara* whereas others are closely related to *M. heathi.* Non-monophyly of *M. heathi* is revealed by the fact that the insular populations are closely related to northern *M. agilis* populations whereas other populations are more distantly related to all other members of the complex.

Even though our results do not allow recognition of the sister group to the *agilis/caissara/heathi* clade (due to the lack of a high bootstrap support for the respective node), they definitely show that *M. dorsivittata*, *M.* sp., *M. macrorhyncha* and *M. nigropunctata* are all substantially divergent from it. Although Rodrigues (2000) included *M. dorsivittata, M. agilis*, *M. caissara* and *M. heathi* in the same phenetic group (together with *M. guaporicola*), our analyses did not support a clade uniting *dorsivittata* with the other three species. Besides, among the sampled ingroup taxa, *Mabuya dorsivittata* was the one that showed the greatest genetic distances (7.5-8.6%) from members of the *agilis/caissara/heathi* clade. This and the fact that neither the MP nor the ML analysis indicate that *M. dorsivittata* is the sister taxon to the *agilis/caissara/heathi* complex suggests that Rodrigues' (2000) tentatively division of South American species into phenetic groups based mainly on superficial similarities may not reflect the actual phylogenetic relationships among these species, as cautioned by himself. Nevertheless, a more comprehensive analysis including *M. guaporicola* and other species is needed to properly assess this issue.

In both trees, topotypic *Mabuya caissara* clustered with the *M. agilis* populations from Rio de Janeiro state, with *M. caissara* genetically differing from them by only 0.0-0.5%. This suggests that *M. agilis* is paraphyletic with respect to *M. caissara*, and that both species should be considered conspecific. Rebouças-Spieker (1974) considered *M. caissara* to be more divergent from the southern São Paulo coast and Rio de Janeiro (referred to as Guanabara) forms (*M. macrorhyncha* and *M. agilis*, respectively) than the latter two are to each other. However, the data and illustrations in her work suggest a greater overall similarity between *M. caissara* and her samples from Rio de Janeiro (i.e. *agilis*). Rebouças-Spieker (1974) described a hypothetical parapatric speciation scenario, in which *M. caissara* would have diverged from an ancestor resembling the populations from southern São Paulo (*M. macrorhyncha*) and from Rio de Janeiro (*M. agilis*) during a period in which the northern São Paulo coastal area was isolated due to sea level variations. Our molecular data also indicate that *M. macrorhyncha* is not closely related to *M. agilis/caissara*, though its true affinities within the sampled ingroup taxa remain unclear. Rebouças-Spieker's (1974) hypothesis that the three forms originated from a common ancestor along the south-eastern Brazilian coast is thus not supported by our results. At that time, Rebouças-Spieker (1974) apparently believed that *M. macrorhyncha* occurred only along the southern coast of São Paulo and adjacent small islands, thus it is not surprising that she assumed that *M. macrorhyncha* must have originated in that region. *Mabuya macrorhyncha* is now known to have an extensive distribution in eastern Brazil (Rodrigues, 2000) where, except for the São Paulo populations, it is broadly sympatric with *M. agilis* and *M. heathi* (e.g. Araújo, 1994; Freire, 1996; Rocha, 2000). It is interest-

ing to note that Rebouças-Spieker (1974) pointed out that South American *Mabuya* "need to be studied comprehensively" and that "numerous detailed regional studies, backed by genetical and biochemical methods will be needed before a broad understanding comes within reach". In the present study, our genetic data did not support Rebouças-Spieker's hypothesis regarding evolution of south-eastern coastal Brazilian *Mabuya,* which further stresses the importance of detailed studies.

Mabuya heathi from the Abrolhos archipelago was found to be closely related to mainland *M. agilis* from Espírito Santo and Bahia states. Also, in both trees the Abrolhos populations clustered (though with relatively low bootstrap support, i.e. 65-73%) with the population of Prado, which is the mainland population that is geographically closest to the archipelago, among those included in our study. Genetic distances between the two Abrolhos samples (from Sueste and Siriba islands) and between the Siriba island and mainland Prado samples are very low (0.2-0.4%). This indicates that the Abrolhos populations may have originated from the mainland ones in southern Bahia, which presumably reached the archipelago quite recently, via overwater dispersal, considering that those islands are of volcanic origin and were never connected to the mainland (Martin *et al.*, 1980). Besides the fact that the Abrolhos *M. heathi* show such a low genetic variation from mainland *M. agilis* from southern Bahia, both the MP and the ML tree indicate that northern mainland heathi populations (Natal and Crateús) are not closely related to the Abrolhos archipelago individuals. Thus, the Abrolhos populations should be referred to as *M. agilis,* a step we have already taken in recent studies (Rocha, 2000; Rocha *et al.*, 2002*b*).

The two most divergent samples within the complex come from Crateús and Natal, the two northernmost and most distant localities. The Crateús and Natal populations differ genetically from each other by 1.6% and from the other *agilis/caissara/heathi* populations by 1.8-2.6% and by 1.7-2.6%, respectively. Walker & Avise (1998) used mtDNA divergence to point out that, in turtles, patterns of divergence broadly correspond to biotic entities. Specifically, they found that 90% of the putative sister species show mtDNA sequence divergence greater than 2%. However, the data of Mead *et al.* (2001) indicates that taxa that have differentiated to even greater degrees, like the two northernmost *Mabuya heathi* forms, could remain reproductively compatible and exhibit complex patterns of gene exchange. Unfortunately, we do not have sequences from populations of *M. heathi* between Natal and the presumed southern limit of the species in the mainland at Salvador, Bahia state. Analyses of such sequences could evidence if there is a tendency for genetic divergence between *heathi* and northern *agilis* populations to decrease with geographic proximity. In any case, genetic distances between *M. heathi* and *M. agilis/caissara* are relatively small, and the apparent continuous distribution of the *agilis/heathi* complex along the Brazilian coast suggests that gene flow may occur. This phylogenetic pattern appears to correspond to "phylogeographic category III" of Avise (2000), in which 'most or all haplotypes are closely related, yet are localized geographically. The implication is that contemporary gene flow has been low enough in relation to population size to have permitted lineage sorting and random drift (or, perhaps, diversifying selection) to promote genetic divergence among populations that nonetheless were in historical contact recently'.

To sum up, our analyses support a monophyletic *Mabuya agilis/caissara/heathi* clade, within which the nominal species *agilis* and *heathi* are paraphyletic. The *agilis/caissara/heathi* clade possibly represents a single widespread species. Nevertheless, a detailed revision of those three taxa including morphological analyses and comparisons is desirable before taxonomic alterations are formally proposed. Also, further molecular analyses including sequences from more populations of *heathi* and from other *Mabuya* species could help to improve our understanding of the relationships within the *agilis/caissara/heathi* complex and between it and other congeners.

Note added in proof . While this paper was in press, an article by Whiting *et al*. (2006) focusing on the molecular phylogeny of New World *Mabuya* has appeared in print. As in our study, these authors found strong support for a clade consisting of *M. agilis* and *M. heathi* populations (*M. caissara* was not included in their analysis), with these two species not being reciprocally monophyletic. Also, their analysis supported a sister-group relationship between a *M. macrorhyncha/M. agmostich*a clade and the *M. agilis/M. heathi* cluster.

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