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Polymorphism and synonymy of *Brookesia antakarana* and *B. ambreensis*, leaf chameleons from Montagne d'Ambre in north Madagascar

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Abstract. We examine the taxonomic status of two Malagasy leaf chameleon taxa, *Brookesia antakarana* Raxworthy & Nussbaum, 1995 and *B. ambreensis* Raxworthy & Nussbaum, 1995, integrating morphological and genetic evidence. Specimens assigned to these species occur in syntopy in Montagne d'Ambre, northern Madagascar, and were originally described based on differences in the shape of their pelvic shields. We found that the shape of these shields falls on a continuous spectrum, and detected only weak differences between the two taxa in a few other morphological features, all of which were correlated with shield length. Members of the two taxa (as assigned based on pelvic shield morphology) also showed extensive haplotype sharing in one nuclear and one mitochondrial marker. We conclude that at present there is no convincing evidence that these species are distinct, and act as first revisers in the sense of the International Code of Zoological Nomenclature to place *B. ambreensis* into the synonymy of *B. antakarana*.

Key words. Squamata, Chamaeleonidae, morphology, molecular genetics, taxonomy, Amber Mountain, Antsiranana.

Introduction

In their work on the diversification patterns of Madagascar's dwarf chameleons (genus *Brookesia* Gray, 1865), Townsend et al. (2009) showed that specimens from Montagne d'Ambre in northern Madagascar assigned to the two locally endemic species *Brookesia antakarana* Raxworthy & Nussbaum, 1995 and *B. ambreensis* Raxworthy & Nussbaum, 1995 were highly similar genetically. Their reconstruction of the phylogeny of these chameleons found the two taxa to be interdigitated, and characterised by comparatively low genetic divergences. In light of this finding, it is pertinent to review the taxonomy of these species, and the question of whether or not they are synonymous.

In their original descriptions, Raxworthy & Nussbaum (1995) considered these two species to be strongly differentiated, predominantly on the basis of the 'pelvic shield', which is 'diamond shaped' in *B. antakarana* versus 'no well-defined pelvic shield' in *B. ambreensis*. Additionally, the number of dorsolateral pointed tubercles (here termed 'dorsolateral spines') would be a candidate distinguishing character according to the key of Raxworthy & Nussbaum (1995), but the values given in the diagnoses of the species (12 in *B. ambreensis* vs. 12–13 in *B. antakarana*) do not agree with those given in their key (11 in *B. ambreensis* vs. 12–13 in *B. antakarana*), leaving the value of this character unclear. A further character, the number of dorsolateral spines on the tail, was mentioned in the descriptions of the holotypes of both species and the diagnosis of *B. ambreensis*, but was not included in the diagnosis of *B. antakarana* or in the key; its potential relevance was highlighted however in Glaw & Vences (2007). Finally, colouration was apparently different between the two taxa, with *B. ambreensis* described by Raxworthy & Nussbaum (1995) as dorsally 'unmarked, or with a thin dark brown vertebral line,' while *B. antakarana* was described as dorsally having 'three, dark brown, blunt chevrons'.

Given the syntopic occurrence of these two species (altitudinal ranges of 650–1200 m a.s.l. in *B. antakarana* and 650–1150 m a.s.l. in *B. ambreensis* according to Raxworthy & Nussbaum 1995, and found in syntopy by us), and the evidence of Townsend et al. (2009) that they are genetically non-assortative, a revision of their taxonomic status is necessary.

Materials and methods

Sampling and terminology

Specimens were sought at night in Montagne d'Ambre along trails and within the forest on thin branches and leaves by torchlight. Field identification of individuals was based primarily on the condition of the pelvic shield. Some individuals were collected as specimens, and euthanized with concentrated MS 222, fixed in 90% ethanol and stored in 70% ethanol. Tissue samples (muscle) were placed in 96% ethanol for genetic study. Specimens were deposited at the collection of the Université d'Antananarivo, Mention Zoologie et Biodiversité Animale, Madagascar (UADBA) and in the Zoologische Staatssammlung München, Germany (ZSM). Reference is made to specimens in the University of Michigan, Museum of Zoology (UMMZ). Field numbers refer to Ronald A. Nussbaum (RAN), Frank Glaw and Miguel Vences (FG/MV, FGZC).

Morphology and morphometrics

Callipers were used to measure the following variables to the nearest 0.1 mm: Snout–vent length (SVL), body height (BH), tail length (TAL), eye diameter (ED), orbital crest to eye distance (OC), mouth length (ML), upper arm length (UAL), lower arm length (LAL), thigh length (TL), shank length (SL), pelvic shield width (SHW), pelvic shield length (SHL), and pelvic shield diagonal (SHD). Relative measures used in statistics discussed below were calculated by dividing these measures by SVL. Furthermore, the following scale counts were done: scales around midbody (SAR), number of supraocular crest spines (SCS), scales along posterior casque limit (CLS), infralabial scales (ILS), scales from casque to eye (SCE), number of dorsolateral spines including the sacral spine following Raxworthy & Nussbaum (1995) (DLS), and number of hand scales between finger tips (NHS). Finally, we coded presence (+) or absence (–) of tail spines (TLS); these were not counted because they decrease in size toward undetectability posteriorly, and are generally either present over the entire length of the tail or wholly absent. For a schematic diagram of investigated characters, see Figure 1. Counts and measurements were carried out by student assistants (and some repeated and verified by MDS). Each count or measurement was carried out by a single person over 1–2 days, without a priori knowledge of the identity of the specimens, to avoid inter-individual differences in count interpretations. Morphometric and meristic data were analysed in R 3.4.3 (R Core Team 2014). Homogeneity of variance of variables was assessed with Levene's tests. Residuals of Generalised Linear Models (GLMs) were assessed for normality with Q-Q plots and Shapiro-Wilk tests. GLM denominator degrees of freedom were calculated based on Satterthwaite's approximation. Principal Component Analyses were conducted with scaled and centred log-transformed data to reduce magnitude and skew biases.

DNA sequencing and analysis of sequences

DNA was extracted following standard salt extraction protocols, using proteinase K digestion in a concentration of 10 mg/ml (Bruford et al. 1992). For analyses of mitochondrial divergence, we targeted fragments of one mitochondrial gene: NADH dehydrogenase subunit 2 (ND2). To understand concordance of mitochondrial and nuclear DNA differentiation, we focused on one nuclear gene segment of the recombination-activating gene 1 (Rag-1). For primers and protocols, see Townsend et al. (2009) and Tolley et al. (2013) for ND2, and Rakotoarison et al. (2015) for Rag-1 (nested approach). PCR products were sequenced directly using an automated DNA sequencer (ABI 3130 XL, Applied Biosystems). Quality control of sequences was carried out using CodonCode Aligner (Codon Code Corporation). For sequence alignment, as well as calculation of uncorrected p-distances between sequences, we used MEGA7 (Kumar et al. 2016). Newly determined sequences were deposited in GenBank with the following accession numbers: MH683055–MH683089.

The two markers were analysed separately because our primary objective was to obtain evidence from unlinked loci (mitochondrial versus nuclear) for genetic differentiation of lineages, which would provide support for their status as distinct species. Haplotypes of the Rag-1 fragment were inferred using the PHASE algorithm (Stephens et al. 2001) implemented in DnaSP software, Version 5.10.3 (Librado & Rozas 2009). From the phased Rag-1 sequences and the unphased ND2 sequences, we reconstructed a Maximum Likelihood tree with Jukes-Cantor substitution model (the most simple available model, chosen to avoid over-parametrisation considering the very few mutations in the data set) in MEGA7 (Kumar et al. 2016) and entered this tree together with the alignment in the software Haploviewer, written by G. B. Ewing (http://www.cibiv.at/~greg/haploviewer), which implements the methodological approach of Salzburger et al. (2011).

Results

Morphological analyses

Voucher specimens available from our fieldwork consisted of eight individuals morphologically identified as *Brookesia ambreensis* (four males and four females) and 11 individuals identified as *B. antakarana* (nine males and two females), all preserved in the ZSM (Table 1). Three additional individuals from the UADBA collection, identified in the field as either *B. ambreensis* (two specimens) or *B. antakarana* (one), were included in our molecular analysis but were not available for morphological analysis. Overall, ND2 sequences were obtained for six *B. ambreensis* and eight *B. antakarana*, whereas Rag-1 sequences were obtained for seven *B. ambreensis* and eight *B. antakarana* (Table 1). In addition, morphometric measurements were also taken from sympatric *B. stumpffi* for comparative purposes (see revised diagnosis, below).

Morphological analysis of the main character diagnosing *B. ambreensis* and *B. antakarana*, the pelvic shield, revealed strong differences in the individuals assigned to these two species (Fig. 2), because the morphology of the pelvic shield was the primary feature used to assign specimens to either species. Specimens assigned to *B. antakarana* have a clearly defined rhomboid of roughly equivalent length and width, whereas in *B. ambreensis*, the 'shield area' was more elongated posteriorly, extending onto the tail, and thus often not discernible as a clear shield. The holotypes of the two species fell into these two categories, in agreement with the original description (Raxworthy & Nussbaum 1995). However, several individuals had intermediate character states, e.g. ZSM 1504/2008 (Fig. 2).

We quantified the length and width of the pelvic shield by three morphometric measurements (SHL, SHW, SHD). As expected given their trigonometric relationship (see Fig. 1), SHL and SHD were very strongly correlated (Pearson's product-moment correlation, correlation = 0.98, $p < 0.0001$), but both were also moderately cor-

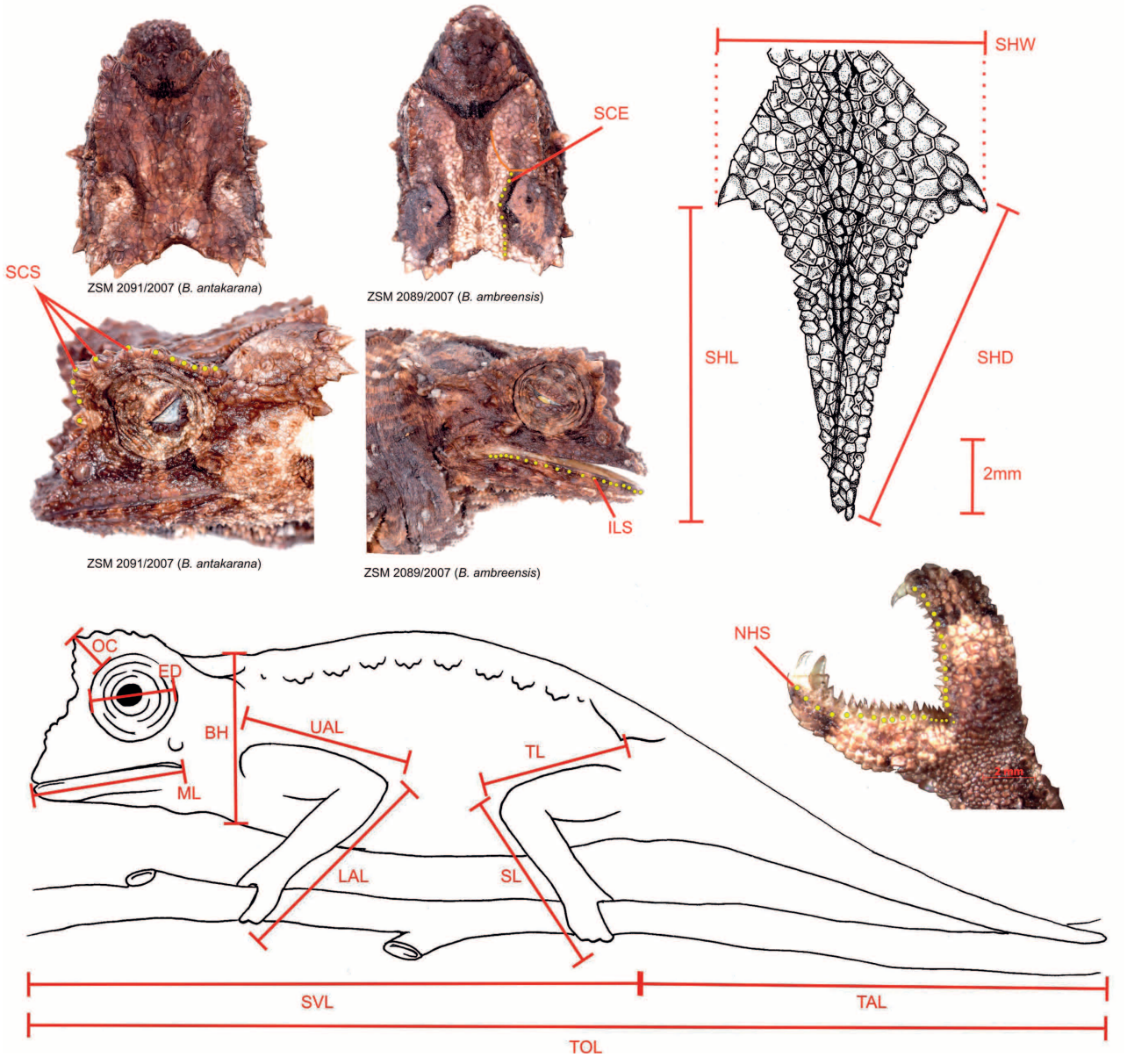

Figure 1. Morphometric measurements and scale counts taken for specimens of *Brookesia*. Abbreviations: BH – body height, ILS – infralabial scales, LAL – lower arm length, ML – mouth length, OC – orbital crest to eye distance, SCE – scales from casque to eye, SCS – number of supraocular crest spines, SHL – pelvic shield length, SHD – pelvic shield diagonal, SHW – pelvic shield width, NHS – number of hand scales between finger tips, ED – eye diameter, SL – shank length, SVL – snout–vent length, TAL – tail length, TL – thigh length, TOL – total length, UAL – upper arm length.

Table 1. Morphological measurements (in mm) of *Brookesia* species from Montagne d'Ambre National Park (including former Forêt d'Ambre Special Reserve). See Materials and Methods for abbreviations. GenBank accession numbers are provided for ND2 and Rag-1 (marker not sequenced if accession number missing).

Catalogue number	Field number	Morphological species identification	ND2	Rag-1	SVL	TAL	ED	OC	ML	UAL	LAL	TL	SL	SHW	SHL	SHD
Males																
ZSM 1029/2003		*B. ambreensis*	–	–	47.0	35.0	4.8	1.7	9.5	10.8	13.3	8.6	11.7	7.2	5.6	6.5
ZSM 1030/2003	FG/MV 2002.3086	*B. ambreensis*	MH683056	MH683088	49.7	35.0	4.5	1.7	9.7	8.2	12.2	8.7	11.1	7.1	7.8	8.6
ZSM 2090/2007	FGZC 1071	*B. ambreensis*	MH683061	MH683077	46.2	30.2	4.3	1.8	8.6	7.5	11.2	8.7	10.5	7.3	5.4	6.8
ZSM 226/2004	FGZC 445	*B. ambreensis*	FJ975197	MH683078	43.1	27.6	4.2	0.5	7.9	8.0	10.2	7.4	9.6	5.8	6.4	7.0
ZSM 1031/2003	FG/MV 2002.2374	*B. antakarana*	–	MH683079	49.2	32.4	4.5	1.8	9.7	7.8	11.4	9.5	10.4	6.9	2.4	4.1
ZSM 1032/2003	FG/MV 2002.2375	*B. antakarana*	MH683065	MH683080	47.4	29.1	5.6	1.3	9.1	8.9	11.5	8.3	10.2	6.5	3.2	4.2
ZSM 1003/2003	FG/MV 2002.3018	*B. antakarana*	MH683055	MH683081	43.1	31.0	4.6	1.6	9.2	8.2	10.8	8.2	10.2	7.1	3.9	4.9
ZSM 1034/2003	FG/MV 2002.3087	*B. antakarana*	MH683058	MH683086	37.6	28.1	3.9	1.3	8.2	7.9	11.5	8.1	9.4	6.4	3.9	5.2
ZSM 2092/2007	FGZC 1074	*B. antakarana*	MH683063	MH683082	45.5	28.3	4.1	1.5	8.8	7.3	11.3	8.2	9.5	6.1	3.9	4.7
ZSM 2093/2007	FGZC 1075	*B. antakarana*	MH683064	MH683083	45.7	26.2	4.5	1.0	8.7	6.2	10.8	7.6	10.0	6.6	3.4	4.5
ZSM 225/2004	FGZC 444	*B. antakarana*	FJ975195	MH683084	45.1	29.3	4.9	0.9	8.9	8.9	11.0	7.7	10.8	6.8	3.8	4.7
ZSM 234/2004	FGZC 456	*B. antakarana*	FJ975196	MH683085	54.3	33.3	5.3	0.9	9.4	8.8	11.8	7.5	10.7	6.9	3.8	4.9
ZSM 1661/2012	FGZC 4913	*B. antakarana*	–	–	48.6	29.7	4.5	0.9	8.0	7.6	10.6	7.3	8.7	6.6	3.0	4.5
ZSM 2095/2007	FGZC 1079	*B. stumpffi*	MH683071	–	49.8	33.4	5.7	1.5	9.7	8.7	11.3	9.6	12.4	6.4	4.7	5.8
ZSM 1681/2012	FGZC 4920	*B. stumpffi*	–	–	41.5	29.7	3.8	1.4	7.6	7.2	9.8	8.7	10.0	6.0	4.4	5.2
Females																
ZSM 1028/2003	FG/MV 2002.2378	*B. ambreensis*	MH683057	MH683075	46.7	40.2	4.4	2.1	10.8	10.4	12.5	11.3	12.3	10.6	9.4	10.8
ZSM 2088/2007	FGZC 1068	*B. ambreensis*	MH683059	MH683089	51.2	33.0	4.5	1.4	10.5	9.3	12.0	9.5	11.5	8.0	5.9	7.3
ZSM 2089/2007	FGZC 1070	*B. ambreensis*	MH683060	MH683076	51.8	37.0	4.6	1.7	11.0	10.2	13.0	10.6	13.3	8.2	6.7	8.0
ZSM 1504/2008	FGZC 1858	*B. ambreensis*	–	MH683087	45.9	31.0	5.2	1.6	10.2	9.9	13.9	10.9	12.2	8.3	6.9	7.5
ZSM 1033/2003	FG/MV 2002.2380	*B. antakarana*	–	–	60.8	39.6	5.1	1.5	11.1	10.4	14.1	10.0	12.8	8.5	3.5	5.5
ZSM 2091/2007	FGZC 1073	*B. antakarana*	MH683062	–	48.1	37.0	4.6	1.4	10.6	9.2	12.8	8.2	11.0	8.6	3.2	5.2
ZSM 2094/2007	FGZC 1077	*B. stumpffi*	MH683070	–	51.8	38.1	4.1	1.9	9.9	10.7	12.9	11.1	12.4	7.3	6.3	7.3
ZSM 2165/2007	FGZC 1234	*B. stumpffi*	–	–	47.3	33.9	4.2	1.6	9.4	9.0	13.1	10.4	12.2	6.9	4.6	5.7
ZSM 2166/2007	FGZC 1235	*B. stumpffi*	–	–	54.0	37.7	4.2	2.0	11.3	9.9	13.0	10.2	12.3	7.0	5.0	6.1

Catalogue number	Morphological species identification	SAR	SCS	CLS	ILS	SCE	DLS	TLS	NHS
Males									
ZSM 1029/2003	*B. ambreensis*	165	13	29	19	11.5	13	–	31.0
ZSM 1030/2003	*B. ambreensis*	200	16	24	22	15.0	12	–	31.5
ZSM 2090/2007	*B. ambreensis*	181	11	23	19	11.0	12	–	25.5
ZSM 226/2004	*B. ambreensis*	210	16	24	20	11.0	12	–	26.0
ZSM 1031/2003	*B. antakarana*	221	14	22	20	9.0	13	+	26.0
ZSM 1032/2003	*B. antakarana*	189	13	21	18	10.0	12	–	23.0
ZSM 1003/2003	*B. antakarana*	184	13	25	19	11.0	12	+	26.0
ZSM 1034/2003	*B. antakarana*	199	12	21	19	10.5	12	–	25.0
ZSM 2092/2007	*B. antakarana*	194	14	22	20	9.0	12	+	28.0
ZSM 2093/2007	*B. antakarana*	205	10	22	18	9.0	12	+	24.5
ZSM 225/2004	*B. antakarana*	242	13	26	21.5	9.0	12	+	28.5
ZSM 234/2004	*B. antakarana*	206	14	22	20	12.0	12	+	21.0
ZSM 1661/2012	*B. antakarana*	214	14	19	19	9.0	11	–	30.0
ZSM 2095/2007	*B. stumpffi*	193	15	22	19	13.0	10	+	25.0
ZSM 1681/2012	*B. stumpffi*	196	14	20	18.5	11.5	9	+	21.5
Females									
ZSM 1028/2003	*B. ambreensis*	227	12	16	20	9.0	12	–	25.5
ZSM 2088/2007	*B. ambreensis*	226	15	29	19.5	12.0	13	+	31.5
ZSM 2089/2007	*B. ambreensis*	235	14	18	20	12.0	12	+	33.5
ZSM 1504/2008	*B. ambreensis*	228	12	27	23	11.0	12	–	24.5
ZSM 1033/2003	*B. antakarana*	204	13	23	21	11.0	12	–	25.0
ZSM 2091/2007	*B. antakarana*	204	12	24	19	8.0	12	+	26.5
ZSM 2094/2007	*B. stumpffi*	227	15	23	20.5	14.5	9	+	24.0
ZSM 2165/2007	*B. stumpffi*	203	14	27	22	10.0	10	+	21.0
ZSM 2166/2007	*B. stumpffi*	198	14	21	22	13.0	10	+	23.0

related with SHW (Pearson's product-moment correlation, SHW~SHD = 0.59, p = 0.0075; SHW~SHL = 0.51, p = 0.0273), suggesting that the length of the pelvic shield (of which both SHL and SHD are metrics) is related to its width (SHW). Statistical analysis comparing the differences in characters between specimens assigned to *B. antakarana* and *B. ambreensis* with sex as a random factor (generalised linear model [GLM]: Character ~ Species + (1|Sex)) yielded highly significant results in SHL and SHD (SHL: $F_{1,17.00}$ = 51.674, p = 1.515e-06; SHD: $F_{1,17.00}$ = 47.74, p = 2.523e-06) but not SHW ($F_{1,16.61}$ = 0.6356, p = 0.4366). The same results were found under non-parametric Wilcoxon U-tests between males of the two species (insufficient female specimens to perform direct comparisons), which remained significant after Bonferroni correction for multiple comparisons (both SHL and SHD: W = 36, p = 0.0364). Again, this finding is unsurprising given our a priori assignment of specimens to either species based on the shape of the pelvic shield.

Relative shank length (SL) was also found to be significantly different ($F_{1,16.95}$ = 5.8017, p = 0.02768), and relative thigh length (TL) and upper arm length (UAL) were close to statistical significance ($F_{1,16.70}$ = 3.7672, p = 0.06904 and $F_{1,16.40}$ = 3.2903, p = 0.08803, respectively) between specimens assigned to either species in GLM analysis; in each case, specimens assigned to *B. ambreensis* tended to have higher values than *B. antakarana*. Each of these measures correlated significantly with SHL and thereby also SHD (Pearson's product-moment correlations: SL~SHL, correlation = 0.65, p = 0.0026; UAL~SHL, correlation = 0.50, p = 0.028; TL~SHL, correlation = 0.62, p = 0.0046). None of these differences were highlighted as significant in Wilcoxon U-tests between males of the two species after Bonferroni correction. No relationship was found between assigned species and the number of dorsolateral spines (chi-square test, χ^2 = 1.497, df = 2, p = 0.4731); both groups had individuals with 12 and 13 dorsolateral spines, though one specimen assigned to *B. antakarana* also had 11 dorsolater-

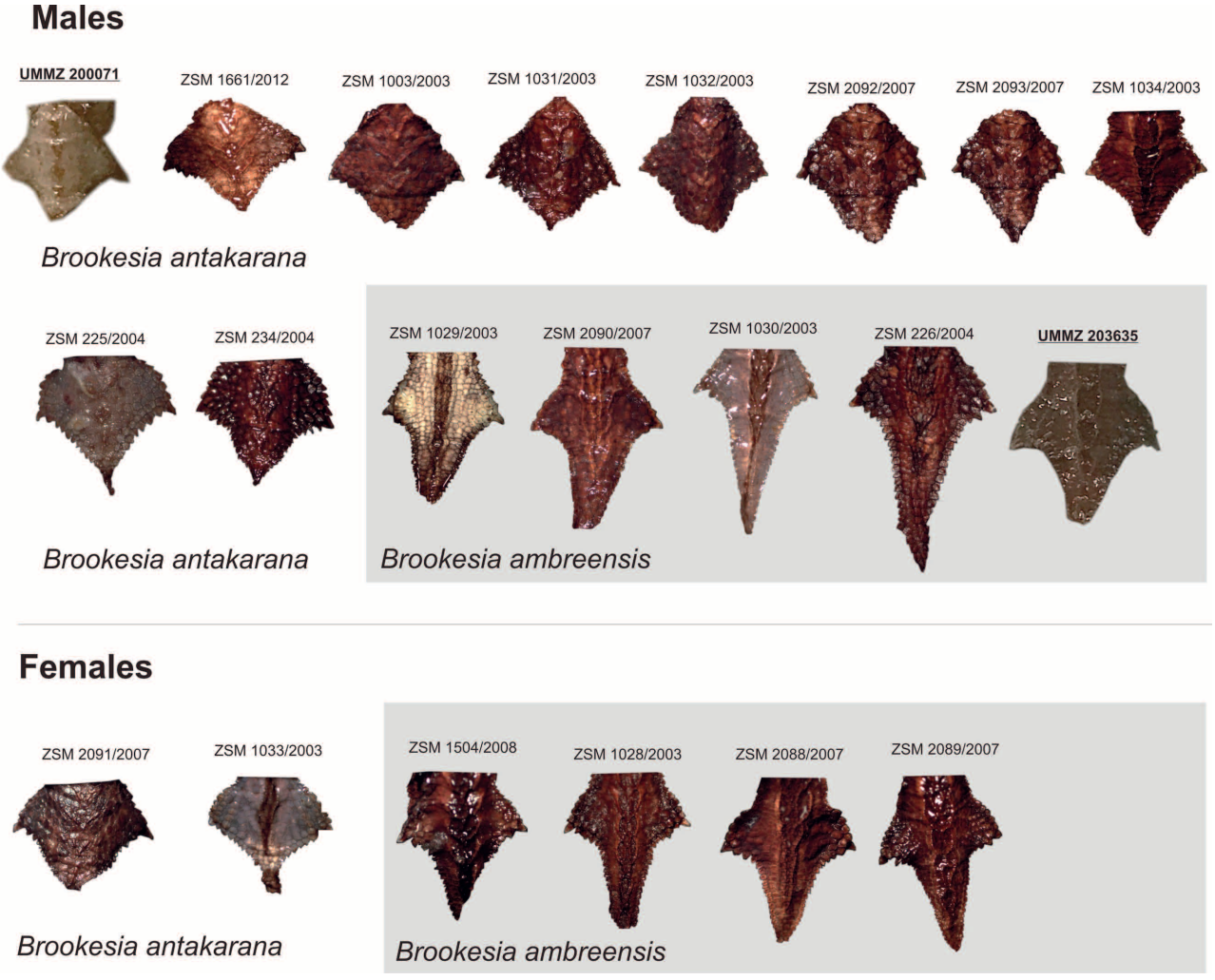

Figure 2. Pelvic shield region of specimens assigned to *B. ambreensis* and *B. antakarana*, separately for males and females. Bold and underlined voucher numbers (from the UMMZ collection) refer to the holotypes of the respective species.

al spines. Furthermore, specimens assigned to either species had dorsolateral spines on their tails, although these were present in more specimens of *B. antakarana* (6/10) than *B. ambreensis* (2/8).

Principal component analysis (PCA) of scaled and centred log-transformed measurement and count data (SVL, TAL, ED, OC, ML, UAL, LAL, TL, SL, SHW, SHL, SHD, SAR, SCS, CLS, ILS, SCE, DLS, NHS) showed limited displacement between specimens assigned to either taxon on the first through third principal components (PCs; Fig. 3). Together these three PCs accounted for 66% of the variation in the data (Table 2); eight PCs were required to account for over 95%. The first PC was size related, most strongly loaded by tail length, but the second and third were loaded most strongly by pelvic shield measurements, scale counts, ED, OC, and SVL (Table 2). What clustering there was (no overlap between convex hulls in PC1 vs. PC2, Fig. 3) was thus apparently related largely to body size and especially tail length, as well as pelvic shield shape. Any semblance of assortment disappeared completely when pelvic shield characters were excluded from the analysis (Fig. 3).

Table 2. Principal component loadings, standard deviation, and proportion of variance, for principal component analysis including shield-related values.

	PC1	PC2	PC3
Standard Deviation	2.6010	1.8075	1.5515
Proportion of Variance	0.3560	0.1719	0.1267
Cumulative proportion	0.3560	0.5280	0.6546
SVL	0.15297	0.13788	-0.43115
TAL	0.34646	0.01036	-0.16909
ED	0.12717	0.24622	-0.37442
OC	0.22297	0.26572	0.26007
ML	0.30086	0.24223	-0.13377
UAL	0.25849	-0.02008	-0.12847
LAL	0.32142	0.11852	0.07269
TL	0.22741	0.20187	0.19722
SL	0.33953	0.08293	-0.03436
SHW	0.26571	0.19746	0.00134
SHL	0.19576	-0.40691	0.18435
SHD	0.21154	-0.39300	0.18255
SAR	-0.17557	-0.09299	-0.41640
SCS	0.07802	-0.31595	-0.34363
CLS	0.24353	-0.14910	0.11555
ILS	0.14853	-0.31884	-0.28586
SCE	0.27027	-0.23952	0.01547
DLS	0.03426	0.13170	0.18110
NHS	0.12987	-0.25061	0.10509

Chromatic variation

Examination of photos in life of specimens involved in this study (Fig. 4) shows that two colour morphs are recognisable, namely one in which clear, almost right-angled chevrons are present on the dorsum and the overall colouration is mottled and greyish (Figs 4a, c, e), and a second in which the vertebral stripe is light in colour with either longer, acute chevrons, or lacking chevrons entirely, and the overall body colouration is more uniformly brown (Figs 4b, d, f–h). The right-angled chevron morph matches the description of *B. antakarana*, while the more uniform colour morph with a light vertebral stripe is similar to that described for *B. ambreensis*. However, these colour morphs are unrelated to pelvic shield morphology; Fig. 4f shows a specimen with a well-defined diamond-shaped pelvic shield with highly acute chevrons on a light dorsal stripe; that is, the pelvic shield morphology of *B. antakarana* with the colouration of *B. ambreensis*.

Genetic results

The analysed ND2 fragment, after trimming of stretches at the beginning and end with missing data, had a length of 411 base pairs. All sequences were very similar, with a maximum number of eight nucleotide substitutions. Uncorrected pairwise distances were 0.0–1.7% within *B. ambreensis*, 0.0–1.7% within *B. antakarana*, and 0.0–1.7% between *B. ambreensis* and *B. antakarana*. The haplotype network (Fig. 5) reflects this situation and groups all ND2 sequences into a single network, with a maximum of eight steps, and with four of six haplotypes shared between *B. ambreensis* and *B. antakarana*. A similar situation was observed in Rag-1, with a maximum number of 11 steps between haplotypes in the network (Fig. 5) and sharing in the two most common out of nine haplotypes.

A reanalysis of the DNA sequence data provided by Raxworthy et al. (2002) agreed with this intriguing picture. In the mitochondrial ND4 gene the distances between samples of *B. antakarana* and *B. ambreensis* amounted to only 0.8–1.7% uncorrected p-distance, corresponding to 7–16 substitutions in the 922 bp fragment sequenced by Raxworthy et al. (2002). In contrast, this gene typically shows quite high inter-specific divergences among many squamate species, for instance 9–19% uncorrected pairwise distances among various *Brookesia* species on the basis of sequences from Raxworthy et al. (2002). One sequence of a *B. ambreensis* paratype (AF443238; field number RAN 38125, corresponding to UMMZ 200074) differed by only 7 substitutions from the single sequence of a *B. antakarana* paratype (AF443249; RAN 38057 = UMMZ 203636) but by 11 substitutions from a second *B. ambreensis* paratype (AF443240; RAN 38476 = UMMZ 200077).

Discussion

It is clear from our results that there are no concordant morphological, chromatic, or genetic differences among the two taxa *Brookesia ambreensis* and *B. antakarana*; al-

though there was a significant difference in the length and width of the pelvic shield, these differences are a result of a priori use of this character to assign specimens to each taxon, and do not therefore constitute independent evidence of morphologically distinct groups of individuals. Other weak differences between these species correlate with the length of the pelvic shield, suggesting that some characteristics may be linked to variation in this structure, possibly through pleiotropic genetic effects. The taxa did not cluster strongly in principal component analyses, and lost any clustering when pelvic shield characters were omitted. The differences in pelvic shield length cannot be explained by sexual dimorphism of a single species, as they are unrelated to sex of the specimens (see Fig. 2).

As mentioned above, the number of dorsolateral spines has been suggested to be useful for distinguishing *B. antakarana* and *B. ambreensis*, but the values given in the diagnosis (and holotype description) of *B. ambreensis* do not match those given in the key of Raxworthy & Nussbaum (1995). We found no relationship between the pelvic shield morphology and number of dorsolateral spines, and conclude that this is not a useful character for distinguishing these taxa. However, we note that there is a clear segregation between these two taxa and the partially sympatric *B. stumpffi*, which has 9–10 dorsolateral spines (though we note that our sample size of *B. stumpffi* is small at n = 5; Table 1). Tail spines also appear to be polymorphic in specimens assigned to either taxon, and are not assortative.

The lack of genetic differentiation in nuclear and mitochondrial genes, combined with (i) weak morphological differentiation that is strongly related to a priori methods used in species assignment, which (ii) occurs on a continuous gradient and not two absolute classes as originally proposed, suggests that the specimens we have examined belong to a single species with variable pelvic shield morphology. Further support for the lack of distinction between the two taxa comes from the three other potentially diagnostic characters mentioned in the introduction: (1) the number of dorsolateral spines does not differ between them, (2) the presence and absence of spines on the tail is not diagnostic, and (3) colouration is independent of pelvic shield morphology.

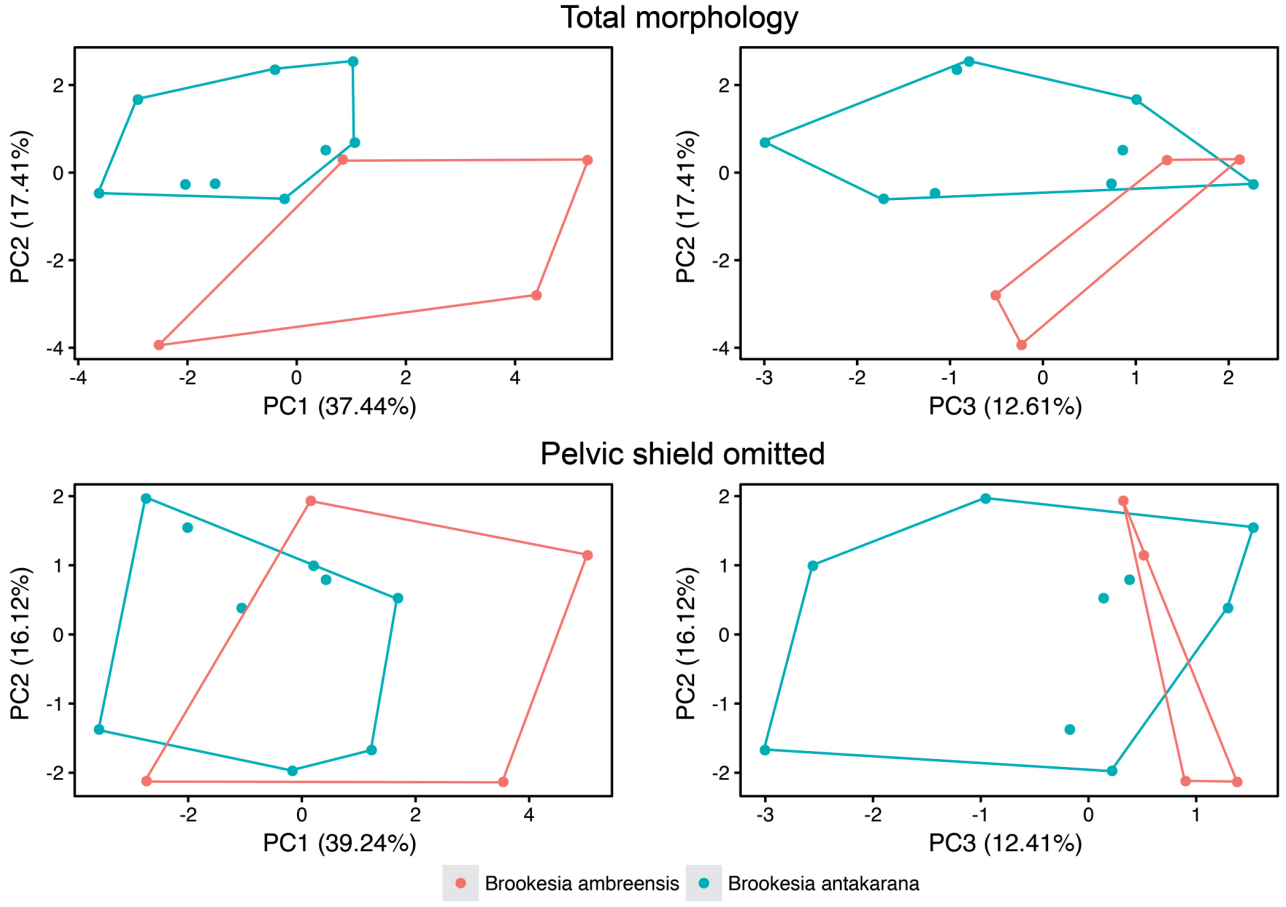

Figure 3. Principal component analysis of the morphology of male specimens assigned to *Brookesia antakarana* and *B. ambreensis*. Parenthetical values indicate the percentage of the variation explained by the principal component. Weak segregation of *B. antakarana* and *B. ambreensis* is evident in PCs 1–3 when total morphology is considered (above), but vanishes when pelvic shield characters are omitted (below).

In summary, it is apparent that these two names represent only a single species, and therefore require synonymisation. As the two names were erected together in the same paper, they are seen as having been published 'simultaneously' in the sense of the International Code of Zoological Nomenclature Article 24.2.1 with regard to their priority (ICZN 1999). We here act as 'first revisers' in the sense of the Code Article 24.2.1, and designate *B. ambreensis* as junior synonym of *B. antakarana*. We take this decision on the basis that several species endemic to Montagne d'Ambre

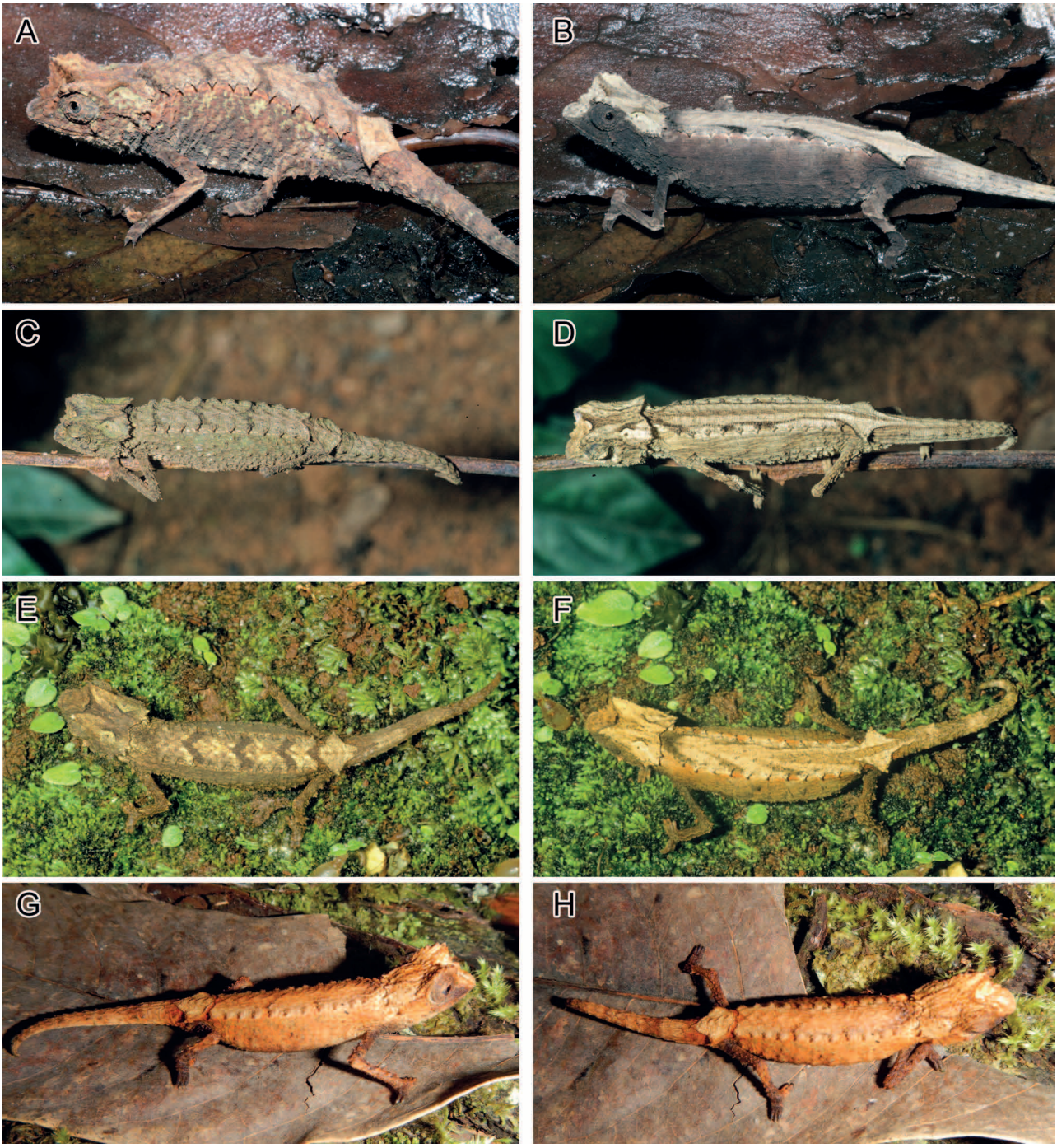

Figure 4. Specimens of *Brookesia antakarana/ambreensis* photographed in Montagne d'Ambre between 1994 and 2012. (A) Probably ZSM 2091/2007 (FGZC 1073); (B) unidentified individual photographed in 2007; (C) unidentified individual photographed in 2003; (D) probably ZSM 2030/2003 (FGMV 2002-3086); (E–F) uncollected individuals photographed in 1994; (G–H) unidentified individual photographed in 2012.

and the surrounding area carry the specific epithet '*ambreensis*' or a variation thereof, e.g. the chameleons *Calumma ambreense* (Ramanantsoa, 1974) and *C. amber* Raxworthy & Nussbaum, 2006, and the frog *Mantidactylus ambreensis* Mocquard, 1895. On the contrary, no other reptiles or amphibians yet have the name '*antakarana*', and in consequence the risk of confusion is considerably lower with this name than the alternative. We consider this decision to be in keeping with Recommendation 24A of the Code in providing a greater universality of nomenclature.

Based on this decision, we here revise the diagnosis of *B. antakarana*, based on the original description of Raxworthy & Nussbaum (1995): A *Brookesia* species with a complete series of 11–13, dorsolateral pointed tubercles on the body; a pelvic shield in the sacral region can be well-defined or not well-defined, and varies in shape; no dorsal ridge (keel); presence or absence of small pointed tubercles on the tail, absence of prominent pointed tubercles on chin or around the cloaca; supraocular cone rounded and does not project further forward than nostril; the horizontal distance between the snout tip and anterior margin of eye is less than the eye diameter; colouration highly variable, it can include a light dorsolateral stripe or dark chevrons; SVL up to 37.6–54.3 mm in males, 45.9–60.8 mm in females.

Brookesia antakarana can be distinguished from the generally similar species *B. brygooi, B. decaryi, B. bonsi, B. stumpffi, B. griveaudi, B. valerieae,* and *B. lineata* as follows: from *B. brygooi* by absence of enlarged tubercles around cloaca (vs. presence) and small supranasal cones (vs. prominent); from *B. decaryi* and *B. bonsi* by absence of enlarged tubercles around cloaca (vs. presence); from *B. stumpffi* by more dorsolateral spines (11–13 vs. 9–10); from *B. griveaudi* by rounded supraocular cone (vs. pointed) and by more dorsolateral spines (11–13 vs. 9–10); from *B. valerieae* by more dorsolateral spines (11–13 vs. 9–10) and rounded supraocular cone (vs. pointed); from *B. lineata* by absence of pointed chin tubercles (vs. four tubercles) and typically more dorsolateral spines (11–13 vs. 11; 11 spines in *B. antakarana* is a rare condition).

At present, the two taxa *B. antakarana* and *B. ambreensis* are both listed as Near Threatened in the IUCN Red List

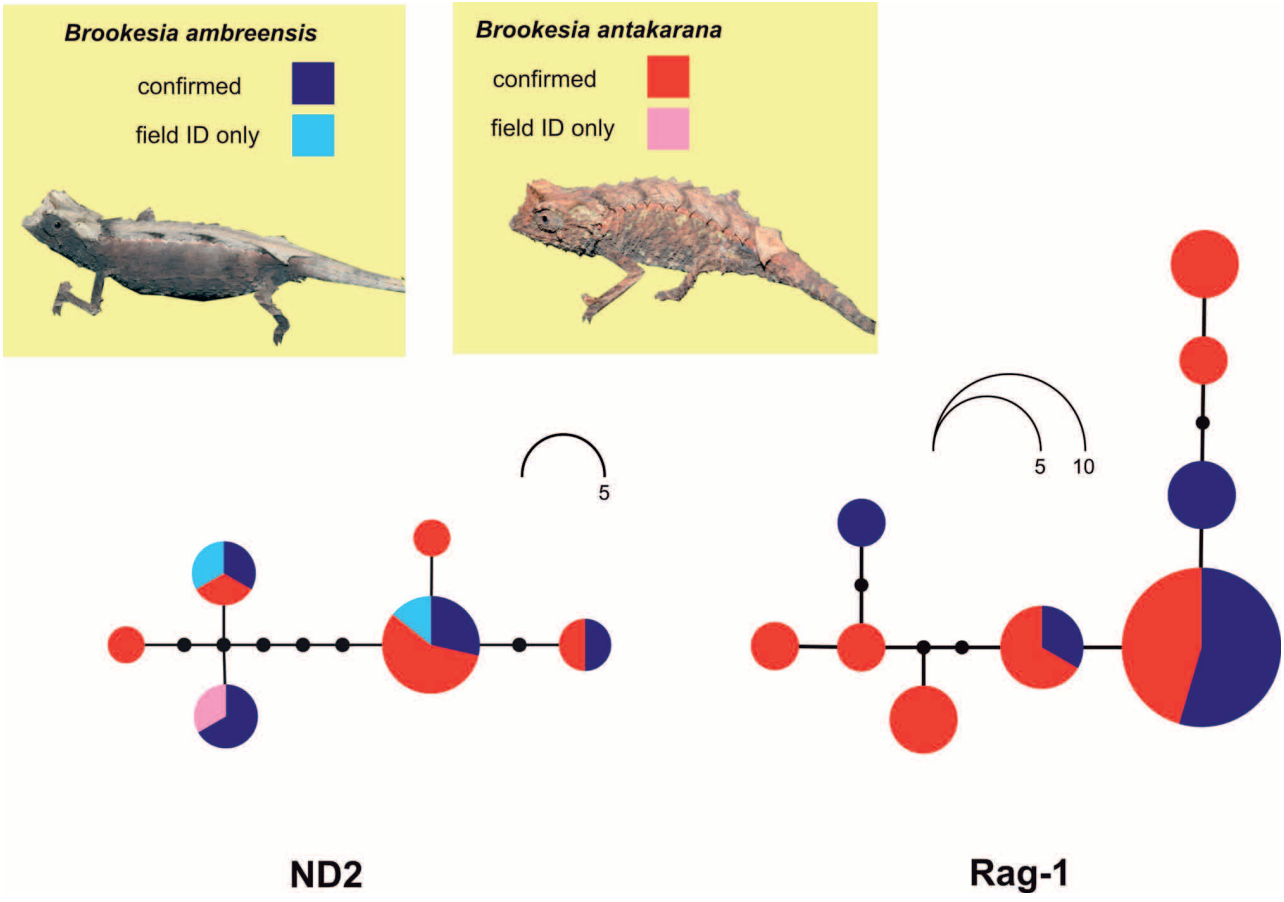

Figure 5. Haplotype networks of the mitochondrial ND2 and the nuclear Rag-1 genes in *Brookesia* specimens assigned morphologically to *B. ambreensis* or *B. antakarana* based on pelvic shield form (see typical morphology in inset photos). Dark blue and dark red colours denote sequences referring to specimens for which detailed morphological data were available (Tables 1–2), light red and light blue colours refer to sequences of three individuals for which only field identification was available. Rag-1 haplotypes were inferred by the Phase algorithm and the network thus reconstructed with two sequences per individual.

(Jenkins et al. 2011a, b). We take this opportunity to briefly revisit the conservation status of this single species following our taxonomic revision. Recently, the borders of the Montagne d'Ambre National Park were redrawn to include a larger area and to integrate the Forêt d'Ambre Special Reserve into the National Park (according to documents of the Direction des Aires Protégées Terrestres of Madagascar, S. M. Goodman pers. comm.). Despite this improvement, the lower reaches of the park have on-going illegal logging activity, and are used as a thoroughfare for local people moving between villages. In places, this has led to extensive habitat degradation. These areas are largely outside the range of *B. antakarana* in terms of elevation, but it can be found at low density in and around some of the higher areas of disturbance (MDS, pers. obs.). We therefore conclude that the status of Near Threatened remains appropriate for this species; should there be any dramatic decline in the implementation of protection in these forests, the species could rapidly qualify as Endangered under criterion D2 (IUCN 2012).

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