Specific distinctness and biogeography of the dwarf chameleons *Brookesia minima*, *B. peyrierasi* and *B. tuberculata* (Reptilia: Chamaeleonidae): evidence from hemipenial and external morphology

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Abstract

In a recent paper Raxworthy & Nussbaum (1995) included the Madagascan dwarf chameleons *Brookesia peyrierasi* and *B. tuberculata* in the synonymy of *B. minima*, regressing known hemipenial differences between *B. tuberculata* and *B. peyrierasi* as artifacts. Our studies demonstrate that the later paper's description of *B. tuberculata* by Brygoo & Domergue (1975) was largely correct and referred to anatomical but real structures. The enormous differences in the hemipenis morphology of *B. tuberculata* (hemipenis of six specimens examined) and *B. peyrierasi* (hemipenis of five specimens examined) strongly suggest they are not conpecific. Furthermore, morphological differences in the arrangement of head crests and expression of supraocular spines were noted between these two taxa and *B. minima*. The differentiation of the three taxa is furthermore corroborated by both univariate and multivariate analyses of morphometric data. We therefore propose to consider *Brookesia minima*, *B. peyrierasi*, and *B. tuberculata* as distinct species. This conclusion corroborates the validity of biogeographic regions as suggested from the analysis of distribution patterns of other *Brookesia* species.

Key words: Reptilia, Chamaeleonidae, *Brookesia minima* group, systematics, genital morphology, external morphology, biogeography

INTRODUCTION

In his monograph on the dwarf chameleons of the genus *Brookesia*, endemic to Madagascar, Brygoo (1978) listed five very small species (total length up to 45 mm) which were characterized by the absence of a continuous row of dorsal-lateral spines, and by the absence of a pelvic shield. *Brookesia dentata*, *B. minima*, *B. peyrierasi*, *B. ramanantsoa* and *B. tuberculata*. Brygoo & Domergue (1975) created a *Brookesia minima* group to accommodate these species. Each was only known from a single or few localities, and in no instance sympatric occurrence of two species of the group was recorded (Brygoo, 1978). According to Brygoo & Domergue (1975) and Brygoo (1978), species distinction within the group was mainly based on genital morphology; hemipenes were found to be largely different between *B. peyrierasi* (Brygoo & Domergue, 1975) (type locality Nosy Mangabe), *B. tuberculata* (Mocquard, 1884) (type locality Montagne D'Ambre) and *B. ramanantsoa* (Brygoo & Domergue, 1975) (type locality Toari d'Ambatohioato, according to the original description, resp. Toari d'Ambatohioato according to Brygoo, 1978) but remained unknown for *B. minima* (Boettger, 1895) (type locality Nosy Be) and *B. dentata* (Mocquard, 1880) (type locality Suberbieville). *Brookesia dentata* and *B. ramanantsoa* were distinguishable from the other three species by having three pairs of dorso-lateral spiny tubercles.

In 1995, Raxworthy & Nussbaum published an extensive review of the *Brookesia* species of northern Madagascar. Besides the description of six new species, five taxa were synonymized: both *B. tuberculata* and *B. peyrierasi* were included in the synonymy of *B. minima*, *B. ramanantsoa* was included in the synonymy of *B. dentata*, *B. anosy* was included in the synonymy of *B. ilida*, and *B. legendrei* was included in the synonymy of *B. ebenaui*.

Recently, some authors have raised doubts as to the justification of some of these synonymizations. Brady et al. (1996) continued considering *B. anosy* as a valid species. Schimmann & Jesus (1996) described a new species of the *minima* group (*B. eburnia* from the Tsingy de Bemaraha in western Madagascar) and resurrected *B. peyrierasi* and *B. tuberculata* based on several morphological differences.
In the present paper we present strong evidence for the specific distinctness of *B. tuberculata* and *B. perrieri* based on hemipenis morphology. We provide evidence for the validity of all three taxa (*B. tuberculata*, *B. perrieri*, and *B. tuberculata*) based on differences in external morphology and provide considerations on the biogeography of *Brookesia* in northern Madagascar.

**MATERIAL AND METHODS**

Eversion of hemipenes in fresh specimens was first done by finger pressure on the hemipenal pockets at the ventral tail base in cranial direction, subsequently by injecting the organs with 70% alcohol, in order to get the maximum turgidity (see also Bohne, 1982). Only partially evaginated hemipenes of already fixed and preserved specimens were brought to full eversion by using a fine forceps for further inserting the retractor muscle into the not fully evaginated organs (see also Zugler & Bohne, 1997). The method recently applied by Poant (1994) and Zugler & Bohne (1997) for eversion of the inverted hemipenes of already fixed and preserved specimens was not successfully used due to the inadequacies of the genital organs. Terminology of genital morphology follows Klauer & Bohne (1986) and Bohne (1988): the proximal part of the hemipenis is termed pedicel, followed by the trapezoid (inner part) and the apex (distal part). HPL is used as abbreviation for hemipenis length, measured from clasper base to tip of apex. Museum abbreviations used are as follows: MNHN = Muséum National d'Hist. Naturelle, Paris; MRSN = Muséum Regional de Ciêncas Naturais, Teto; SMSF = Smithsonian Institution und Naturmeum, Senckenberg, Frankfurt; UMZ = University of Michigan, Museum of Zoology, Ann Arbor; ZFMK = Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn; ZSM = Zoologisches Staatsmuseum, München.

Of the total of 70 examined specimens, we took morphometric measurements in a total of 61 specimens: six juveniles and two females from the type specimen. Because no conclusive data exists for the specific attribution of this population, the specimens were excluded from further analysis; morphometric comparisons were carried out using 53 adult specimens. Variables measured were TL = total length; TaL = tail length; HW = head width; HH = head height; E = horizontal diameter of eye; Arm = arm length; body length (BL) was calculated by subtraction of TaL from TL. All measurements were made by the same person (M.V.).

**RESULTS**

Descriptions of hemipenes

*Brookesia perrieri* (SMF 65899; Fig. 1). HPL: 4-5 mm (partially evaginated in the fresh specimen; terminal structures brought to maximum eversion posterior to fixation). Hemipenes strong and slightly elongate. Suckers spermatic ducts forked at the base of the apex, its branches surrounding the distal lobes. Suckers lips broad. Apex consists of two semicircular lobes, each showing four microlateral cones at its terminal end. The inner cones insert more on the aseptic surface of the respective lobes, slightly separated from each other of the three cones, which are connected at their base.

Additionally, genital preparations of four species of *B. perrieri* were available: MNHN 1968.875 (Fig. 2); MNHN 1968.875 (Fig. 3), MNHN 1974.236 (HPL 4.5 mm); and ZFMK 60758. The left hemipenis of ZFMK 60758 was partially evaginated and was brought to full eversion posterior to fixation. Hemipenes of the specimens correspond excellently to the evaginated genital organs of SMF 65899. On each lobe of the hemipenes four cones were recognized; only the lobes of the right hemipenis of MNHN 1974.236 bear a very small sixth cone. In one further specimen (MNHN 1974.243), the tip of the hemipenis (less than one mm) can be seen outside the clasper; even in this virtually unevaginated organ, the conical cones of the hemipenis are visible.

*Brookesia tuberculata* (ZFMK 6124; Figs 4 & 5). TaL: 14 mm; HPL: 4-5 mm (freshly evaginated); Hemipenes strong and curved towards the central axis of the specimen. Pedicel slender, trunci with conspicuous lateral bulges. Suckers spermatic ducts forked at the base of the apex, its branches leading laterally to the end of the apex. Outer suckers lip slightly longer than inner suckers lip. Apices laterally enlarged, consisting terminally of two slight bulges and a hardened, probably mineralized crown-like structure at the centre of the aseptic surface. Both terminal bulges comprise an elongate sphen-like structure. The crown-like structure, which is open towards the aseptic surface, consists of few rounded tips. Both crown-like and sphen-like structures (Figs 4 & 5) are connected to the retractor muscle. There are no mineralized cones.

Additionally, evaginated hemipenes of five specimens from the same area were available: ZFMK 62149 (HPL 3.5 mm), MNHN 1968.870 (HPL 4.5 mm), MNHN 1968.873 (HPL 5 mm); MNHN 1969.873 (HPL 5 mm), MNHN 1969.873 (HPL 5 mm); MNHN 1969.873 (HPL 5 mm). The hemipenes partly appear only slightly curved and conspicuously slender and elongate with very slight lateral bulges at the apex. However, both the elongate sphen-like structures within the terminal bulges, as
well as the crown-like structure at the terminal ascidiate surface are well recognizable. The crown-like structures consist of several small and rounded tips. The main structures thus correspond well to the genital preparations of ZFMK 61342.

External morphology

Careful examination of the available material of the three taxa considered yielded several characters which can be used for their distinction.

(a) Brookesia pygmaea (Fig. 7) is significantly larger (Table 2) and has a more spiny head and body ornamentation than B. minima and B. tuberculata.

(b) Brookesia minima (Fig. 8) differs from the remaining two taxa by details of head ornamentation; the ridge system which is found dorsally on the head has a symmetrical, rather complex but regular pattern in B. tuberculato and especially in B. pygmaea, but is largely reduced in B. minima (Fig. 9). Especially transversal patterns are lacking in most specimens of B. minima, the ridge systems being confined to irregular elements arranged longitudinally. The inner longitudinal ridges are mostly confluent to the supraneal crest in B. minima, whereas in B. tuberculata and B. pygmaea they...
Fig. 12. Scatterplot of tail length (TL) and body length (BL) in *Brookesia minima*, *R. tuberculata* and *B. pyrrhopygia*. Closed symbols, males; open symbols, females.

Table 2. Morphological and differential characters in *Brookesia minima*, *R. tuberculata* and *B. pyrrhopygia*. Measurements and ratios are given in millimeters and range (mean ± SD) or parentheses. Morphometric values calculated from data in Table 1 (individuals excluded from analysis). Body length (BL) in mm calculated as TL. TL.

<table>
<thead>
<tr>
<th>Character</th>
<th><em>B. minima</em></th>
<th><em>R. tuberculata</em></th>
<th><em>B. pyrrhopygia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>(3 males, 3 females)</td>
<td>16.7 (±1.9 ± 1.9)</td>
<td>18.0 (±3.9 ± 2.8)</td>
<td>16.4 (±1.9 ± 2.1)</td>
</tr>
<tr>
<td>(7 males, 1 female)</td>
<td>16.7 (±3.9 ± 3.9)</td>
<td>18.0 (±3.9 ± 2.8)</td>
<td>16.4 (±1.9 ± 2.1)</td>
</tr>
<tr>
<td>(15 males, 15 females)</td>
<td>17.5 (±4.0 ± 4.0)</td>
<td>19.1 (±4.0 ± 4.0)</td>
<td>19.1 (±4.0 ± 4.0)</td>
</tr>
</tbody>
</table>

A discriminant analysis was performed on data from Table 1, grouping species according to morphological characters. The results of the analysis are presented in Table 3. Discriminant analysis correctly group membership for all species with a probability > 99.99%, and for males and females with a probability > 99.99% when sexes were separately analysed.

Table 3. Standardised canonical discriminant function coefficients from a multiple discriminant function analysis of morphological and physical characters in *Brookesia minima*, *R. tuberculata* and *B. pyrrhopygia*. Data are given for separate analysis of males and females. The two characters with the highest evalue for each function are marked by a superscripted 1.

Fig. 13. Scatterplot of the first and second discriminant function in *Brookesia minima* (●), *R. tuberculata* (■) and *B. pyrrhopygia* (▲) females (a) and males (b), based on a multivariate discriminant analysis of data in Table 1.

DISCUSSION

Hemipenal differentiation

Raxworthy & Nussbaum (1995) regarded *Brookesia pyrrhopygia* and *R. tuberculata* as synonyms of *B. minima* due to the presumed lack of differential characters, concluding that there is no evidence to diagnose more than one minima group *Brookesia* in northern Madagascar. However, Brygoo & Domergue (1969, 1974) provided a diagnosis of both taxa based on genital morphology. Raxworthy & Nussbaum (1995: 528) quoted the published differences as follows: The hemipenis of *Brookesia tuberculata* from Montagne d'Ambre has a single apex bearing three short horns (Brygoo & Domergue, 1975), while the hemipenises of *B. pyrrhopygia* from Nosy Mangabe and Fartanea (north of Maroantsetra) has a b-lobed apex each bearing a papilla crested with 4–6 papillae (Brygoo & Domergue, 1970).

The hemipenes of their material (specimens UMMS 2007-17-8 and 2007-17-9, from the eastern localities Marojejy and Makodra, respectively) they observed similarities to the hemipenes of *B. pyrrhopygia*. On the contrary, they disregard the differences to the hemipenes of *R. tuberculata* as they were described by Brygoo & Domergue (1975, 1974), stating that the organs of the pertinent specimens (MNNH 1868, 1872) are badly deformed and do not appear to be fully evolved. The unusual structures of the hemipenes of *R. tuberculata* had previously been discussed by Brygoo & Domergue (1975) and Klaver & Böhme (1986) who also considered the possibility that they constitute artifacts.

The new material described in the present study allows for the first time definitive clarifications about the genital morphology of *R. tuberculata*. The hemipenises preparations of ZFMK 01242 are totally evaginated and fully developed, whereas those described by Brygoo & Domergue (1975) are obviously slender and not completely turned (MNNH 1868, 1872). Nevertheless, the most important structural characters are clearly recognizable in the litter (Fig. 6), and were largely correctly described and figured by Brygoo & Domergue (1975). This

lather by relatively shorter arms (P < 0.001) and tail (P < 0.05).

Within taxa, intersexual differences were found in *R. tuberculata* (ArmL: P < 0.05; HH: P < 0.005; Tal. Tal: P < 0.005). One constant sexually dimorphic pattern found in all three taxa (although not statistically significant in *B. minima*), H dives tail length. Tal. were relatively shorter in females (Fig. 12) except for one male specimen of *R. pyrrhopygia* (MNNH 1974-242) which possibly had a multilobed tail. Females were generally larger than males, although this difference only significant in *B. pyrrhopygia* (BL: i.e., 1.74 ± 0.001).

A discriminant analysis using data from Table 1 was performed separately on the sexes. Significant results were achieved both for males and females when the sexes were separated (Fig. 12), and for a combined analysis of both sexes, indicating that the interspecific differences are more important than sexual dimorphism.

Table 2. Morphology and differential characters in *Brookesia minima*, *R. tuberculata* and *B. pyrrhopygia*. Measurements and ratios are given in millimeters and range (mean ± SD) or parentheses. Morphometric values calculated from data in Table 1 (individuals excluded from analysis). Body length (BL) in mm calculated as TL. TL.
regards the lateral trunal bulges (transverse hemispherically), the two terminal spheniform structures, and the crown-like structure at the centre of the terminal sulcal surface (one zone apical, confluence division in the elements does not include the elongated groups in the trunks). A difference is that in the hemispheres of FZMK, 61242; the crown-like structure shows a more rounded tips than in Brygova & Domercq (1975) figures. This may reflect real individual differences, whereas the elongated general appearance (the corps... is an irregular cylinder) and the relatively strongly projecting terminal structures of the hemispheres of MNHN 1968, 872 and ZMUK 62994 are probably artefacts due to the lack ofreniform link. The left hemispheres of MNHN 1968, 872 as figured by Brygova & Domercq (1975) shows an apical swelling not recognizable in ZMUK 62942 which may be an artefact as often occurs when liquid is directly injected into the tail base without prior manual expansion of the organs (Ziegler & Böhm, 1997). The swelling may also represent the apically shifted lateral bulges.

The detailed description of the hemispheres of B. perieiatus (Brygova & Domercq, 1969, Fig. 3) is largely corroborated by our observations except for the number of terminal cones 'chaquet dents portes dres' of the groups of apical epimorphs postemergence of a long tail in the two groups. The demic-geometric telforam has developed, est composé de six à quatre épines verticales, le demic-geometric telfor will être suivis d’rangle, également verticales. According to the data presented herein, each of these terminal cones bears a large mineralized area of terminal structures or an occurrence. In one of our small fifth cone on both lobes of one hemipenis (MNHN 1974, 236).

Summarizing, there are very distinct hemipenial differences between B. perieiatus and B. tuberculata. The hemispheres of B. perieiatus are apically largely characterized by two semicircular lobes with mineralized areas at their terminal ends, whereas those of B. tuberculata show terminal spheniform structures within the terminal bulges, and the head structure at the centre of the terminal sulcal surface

It can also be observed that the differences are due to seasonal variation. Material studied includes B. perieiatus from collections in the dry season (June 1970 and September 1970), November 1971, and March 1972. An explanation of the observed differences due to seasonal variation is also not probable, however, very probable mineralized or calcified structures on the hemispheres of B. perieiatus (Brygova & Domercq, 1969) and probably also B. tuberculata (Böhm, 1997) whereas occurrence of seasonal modifications of mineralized or calcified structures is not probable (see also Ziegler & Böhm, 1997).

According to Klauer & Böhm (1986), the apical ornamentation of Malagasy [Brookesia] species consists of two papillae or denticulate crests or crested lobes, somewhat unequal in size. However, perieiatus is the most observed variation within the genus, whereas the unusual structure of B. tuberculata may represent a derived state.

Morphological differentiation

It is a general problem in Brookesia systematics and morphological differentiation between species is often low and moreover sometimes difficult to describe. In the tiny species of the B. minor group, many characters are largely reduced in which B. tuberculata can be used. B. perieiatus shows features. (Dentiform spinules, pelvic shield, head structure).

One peculiarity of many tetrapod species at the lower size limit is the retention of paedomorphic features, which often results in a reduced development of external characters such as fingers and toes. (Hanken & Wolke, 1993). With up to 34 mm long, 35 mm total length (Table 1). B. minima and B. tuberculata are included among the smallest reptiles in the world. (Glis & Venes, 1994). The lack of denticulate spinules, pelvic shield and distinct head crests in the B. minima group species can be seen as retained paedomorphic features. In fact, Brookesia species with distinct body ornamentations show a pronounced reduction of adult characters in their juvenile stage (F. pers., obs. for B. stumfigull). This is reflected by the fact that in two cases, specimens belonging to the B. minima group may have been misidentified as juveniles of larger species.

Glis & Venes (1994) stated that one paratype of B. karawari (MNHN 1993, 159) possibly belongs to a species of the B. minima group, and Raxworthy & Vordermeier (1995) found that the two paratypes of B. madagascariensis (MNHN 1968, 193) is in fact a specimen of B. danae.

Increased maintenance of paedomorphic characters may explain why the small B. minima shows distinct head ridges than B. perieiatus, and the larger epimorph features and spinules and tubercles in the tuberculata in this scheme to be related to its larger size. However, the large superciliaries of B. tuberculata (not found in the larger B. perieiatus) contradict this explanation and indicate that maintenance of paedomorphic characters has affected the three taxa in a different way.

This also points to another fact which may be considered in the context of the large hemipenial differences between B. perieiatus and B. tuberculata. Until present, phylogenetic analysis of the genus Brookesia has been published, and no derived characteristics except the small size are known which would support a monophyletic origin of the B. minima group. Although unexpected to us, it is possible that this group of species the group evolved independently from larger ancestors by retention of paedomorphic features, as has already been demonstrated in miniature animals (Hanken & Wolke, 1993).

Biogeographical considerations

Although new data are necessary to clarify definitely the distribution of Brookesia minima, B. perieiatus and B. tuberculata, at current state (Fig. 14) the known localities (after Glis & Venes, 1994 and Raxworthy & Vordermeier, 1995) can be compared as follows to the type locality of B. minima: Nosy Be, and possibly Mananarivo. B. tuberculata: Montagne d'Argent, B. perieiatus: Nosy Mangabe, Fantanomiasa, Masoala, Marojejy, and possibly Sambava and Andranomavelo. The species from Mantondra belongs to B. perieiatus based on the
Table 4. Number of forest-dwelling amphibian and reptile species recorded at the type localities of *Brookesia minima* and *B. peripheria* (Nosy Mangabe), and number of species not known from the type localities.

<table>
<thead>
<tr>
<th>Location</th>
<th>Amphibians</th>
<th>Reptiles</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nosy Be</td>
<td>16 (9)</td>
<td>34 (17)</td>
<td>50 (26)</td>
</tr>
<tr>
<td>Montagne d’Ambre</td>
<td>34 (18)</td>
<td>48 (27)</td>
<td>82 (45)</td>
</tr>
<tr>
<td>Nosy Mangabe</td>
<td>23 (12)</td>
<td>38 (21)</td>
<td>61 (33)</td>
</tr>
</tbody>
</table>

Table 5. Similarity coefficients between the forest herpetofaunal type localities of *Brookesia minima* (Nosy Be), *B. peripheria* (Montagne d’Ambre) and *B. peripheria* (Nosy Mangabe) based on species numbers in Table 4.

<table>
<thead>
<tr>
<th>Amphibians</th>
<th>Nei’s D̄</th>
<th>Nei’s D̄</th>
<th>Nei’s D̄</th>
<th>Nei’s D̄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nosy Be</td>
<td>0.20</td>
<td>0.38</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>Montagne d’Ambre</td>
<td>0.38</td>
<td>0.46</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td>Nosy Mangabe</td>
<td>0.21</td>
<td>0.24</td>
<td>0.21</td>
<td>0.21</td>
</tr>
</tbody>
</table>

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REFERENCES


