

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*, regarding known hemipenial differences between *B. tuberculata* and *B. peyrierasi* as artefacts. Our studies demonstrate that the hemipenis description of *B. tuberculata* by Brygoo & Domergue (1975) was largely correct and referred to unusual but real structures. The enormous differences in the hemipenis morphology of *B. tuberculata* (hemipenes of six specimens examined) and *B. peyrierasi* (hemipenes of five specimens examined) strongly suggest that they are not conspecific. 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 dorso-lateral spines, and by the absence of a pelvic shield: *Brookesia dentata*, *B. minima*, *B. peyrierasi*, *B. ramanantsoai* 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 syntopic 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, 1894) (type locality Montagne d'Ambre) and *B. ramanantsoai* (Brygoo & Domergue, 1975) (type locality 'forêt d'Ambohiboataba' according to the original description, resp. 'forêt d'Ambohiboatavo' according to

Brygoo, 1978) but remained unknown for *B. minima* (Boettger, 1893) (type locality Nosy Be) and *B. dentata* (Mocquard, 1900) (type locality Suberbieville). *Brookesia dentata* and *B. ramanantsoai* 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. Beside 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. ramanantsoai* was included in the synonymy of *B. dentata*, *B. antoetrae* was included in the synonymy of *B. thielei*, and *B. legendrei* was included in the synonymy of *B. ebenauui*.

Recently, some authors have raised doubts as to the justification of some of these synonymizations. Brady *et al.* (1996) continued considering *B. antoetrae* as a valid species. Schimmenti & Jesu (1996) described a new species of the *minima* group (*B. exarmata* 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. peyrierasi* based on hemipenis morphology. We also discuss evidence for the validity of all three taxa (*B. minima*, *B. peyrierasi*, 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 hemipenial 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 Böhme, 1988). Only partially everted 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 everted organs (see also Ziegler & Böhme, 1997). The method recently applied by Pesantes (1994) and Ziegler & Böhme (1997) for eversion of the inverted hemipenes of already fixed and preserved specimens was not successfully used due to the delicateness of the genital organs. Terminology of genital morphology follows Klaver & Böhme (1986) and Böhme (1988): the proximal part of the hemipenis is termed pedicel, followed by the truncus (mesial part) and the apex (distal part). HPL is used as abbreviation for hemipenis length, measured from cloacal base-point to tip of apex. Museum abbreviations used are as follows: MNHN = Muséum National d'Histoire Naturelle, Paris; MRSN = Museo Regionale di Scienze Naturali, Torino; SMF = Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt; UMMZ = University of Michigan, Museum of Zoology, Ann Arbor; ZFMK = Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn; ZSM = Zoologische Staatssammlung, München.

Of the total of 70 examined specimens, we took morphometric measurements in a total of 61 specimens: six juveniles and two females from Sambava, because no conclusive *a priori* evidence existed for the specific attribution of this population, 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; Eye = horizontal diameter of eye; ArmL = arm length; body length (BL) was calculated by subtraction of TaL from TL. All measurements were made by the same person (M. V.).

Data were processed with the software package SPSS for Windows; we carried out analyses of covariance (ANCOVAs) on the measurements of adult specimens. BL was used as covariate to eliminate the size factor. Males and females were analysed separately. We also performed a multivariate discriminant analysis, comparing males and females both separately and together.

Collectors are given for the type specimens only; collecting dates only for those specimens for which the

corresponding collection catalogues and labels contained unequivocal information; more detailed data on the other specimens are available in the MNHN, MRSN, SMF, and ZFMK catalogues, and on request from the authors. The following specimens were examined:

Brookesia minima

SMF 16512 (lectotype), SMF 16513 (paralectotype), and ZSM 17/1921 (paralectotype), all from Nosy Be, collected by C. Ebenau and A. Stumpff, 1880–1885; SMF 16514 (Nosy Be); MNHN 1884.582 and 1886.25 (Nosy Be); ZFMK 48215–48222, 51390, 51525, and 60664 (all from Nosy Be); ZFMK 53965–53969 (Nosy Be, collected on VII. 1993).

Brookesia tuberculata

MNHN 1893.183 (holotype) from Mararaomby, Montagne d'Ambre, collected by Alluaud and Belly; MNHN 1986.870, 871, and 873 (all from Montagne d'Ambre, collected on 10. VI. 1972); MNHN 1986.874 (locality given as 'Diego' [=town near Montagne d'Ambre]; MRSN R1640 (Montagne d'Ambre, collected on IV. 1990); ZFMK 61242 and 62194–62195 (all from Montagne d'Ambre, collected on 25. XI. 1995).

Brookesia peyrierasi

MNHN 1968.190 (holotype), 1968.184, 1968.186–189, and 1968.191 (paratypes), all from Nosy Mangabe, collected by A. Peyrieras on I. 1968; MNHN 1974.236–247 and 1974.249–250 (all from Nosy Mangabe); MNHN 1989.3748 (Nosy Mangabe according to attached label); MNHN 1974.251–252 (Sambava, collected on XI. 1968); MNHN 1986.875 (forêt de Fantanendra, north of Maroantsetra, collected on 21. II. 1969); SMF 65898–65901 (Nosy Mangabe); ZFMK 52477–52478 (Nosy Mangabe), ZFMK 60753–60756 and 61242 (all from Nosy Mangabe, collected on 16. VI. 1995); MRSN R1641.1–2 (Nosy Mangabe, collected on IV. 1990).

RESULTS

Descriptions of hemipenes

Brookesia peyrierasi (SMF 65899; Fig. 1). HPL: 4–5 mm (partly everted in the fresh specimen; terminal structures brought to maximum eversion posterior to fixation). Hemipenes strong and slightly elongate. Sulcus spermaticus distinctly forked at the base of the apex, its branches surrounding the distal lobes. Sulcal lips broad. Apex consists of two semicircular lobes, each showing

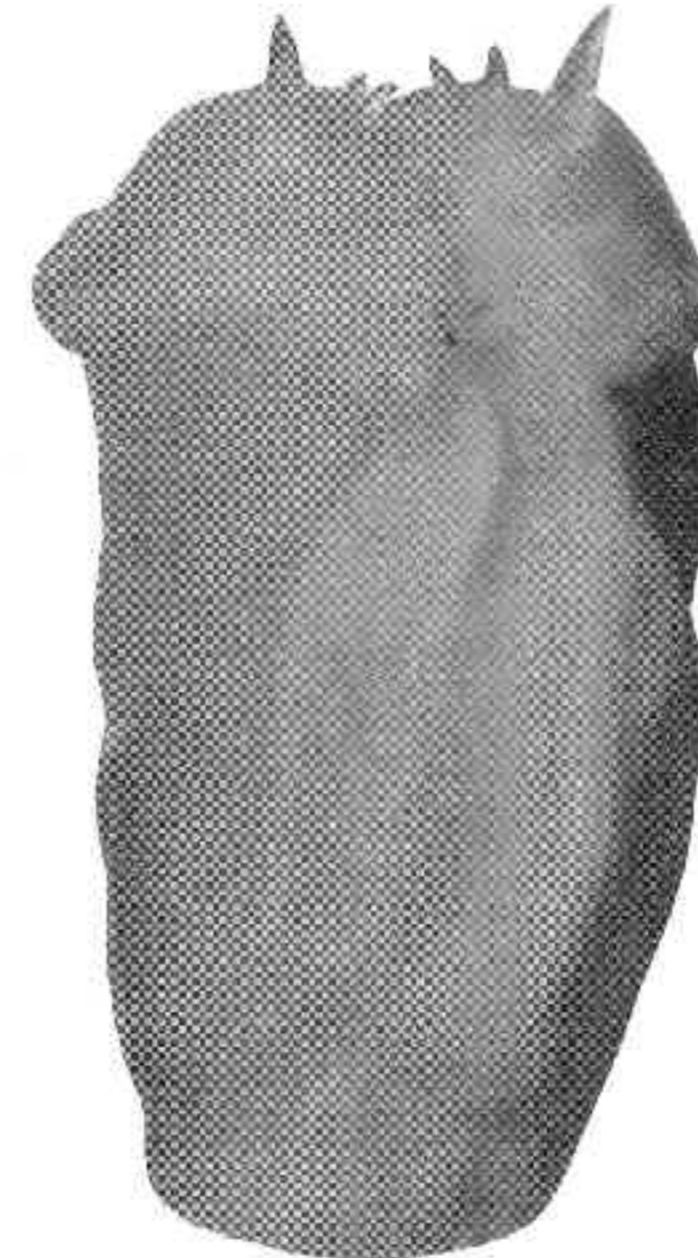


Fig. 1. Sulcal view of the left hemipenis of *Brookesia peyrierasi* (SMF 65899); scale: 1.5 mm.

four mineralized cones at its terminal end. The inner cones insert more on the asulcate surface of the respective lobe, slightly separated from each of the outer three cones, which are connected at their base.

Additionally, genital preparations of four specimens of *B. peyrierasi* were available: MNHN 1986.875 (Fig. 2), MNHN 1968.190 (holotype of *B. peyrierasi*; HPL 4.5 mm; Fig. 3), MNHN 1974.236 (HPL 5 mm) and ZFMK 60756. The (left) hemipenis of ZFMK

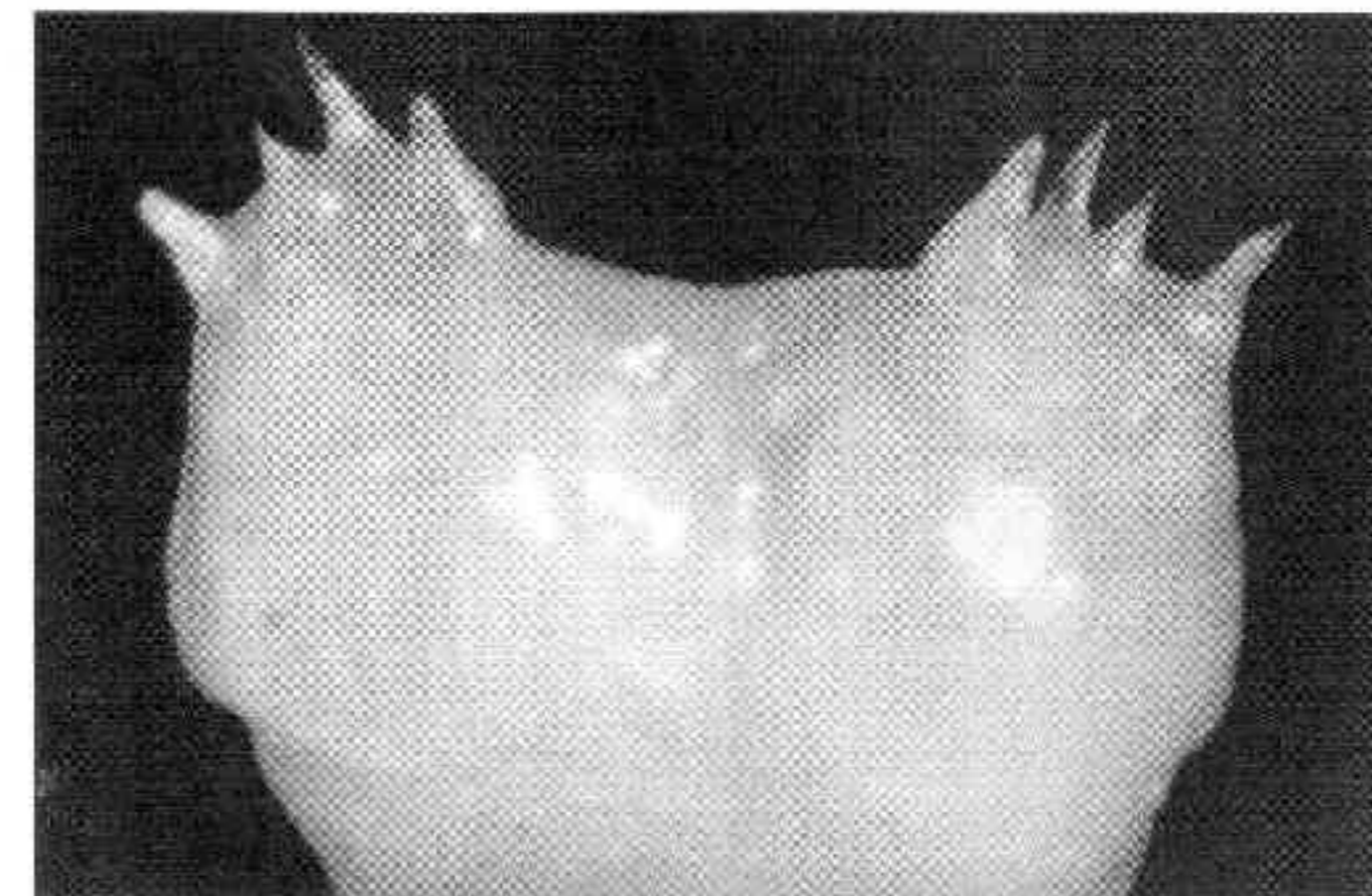


Fig. 2. Asulcal view of the apex of the hemipenis of *Brookesia peyrierasi* (MNHN 1986.875). Photograph by R. Kernchen.

60756 was only partially everted and was brought to full eversion posterior to fixation. Hemipenes of the specimens correspond excellently to the everted 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 fifth cone. In one further specimen (MNHN 1974.242), the tip of the hemipenis (less than one mm) can be seen outside the cloaca; even in this virtually uneverted organ, the mineralized cones of one lobe are visible.

Brookesia tuberculata (ZFMK 61242; Figs 4 & 5). TaL: 14 mm; HPL: 4–5 mm (freshly everted). Hemipenes strong and curved towards the central axis of the specimen. Pedicel slender, truncus with conspicuous lateral bulges. Sulcus spermaticus distinctly forked at the base of the apex, its branches laterally leading to the end of the apex. Outer sulcal lip slightly stronger than inner sulcal lip. Apex laterally enlarged, consisting terminally of two slight bulges and a hardened, possibly mineralized crown-like structure at the centre of the asulcate surface. Both terminal bulges comprise an elongate siphon-like structure. The crown-like structure, which is open towards the sulcate surface, consists of few rounded tips. Both crown-like and siphon-like structures (Figs 4 & 5) are connected with the retractor muscle. There are no mineralized cones.

Additionally, everted hemipenes of five specimens from the same area were available: ZFMK 62194 (HPL 3 mm), MNHN 1986.870 (HPL 5.5 mm), MNHN 1986.871 (HPL 5.5 mm), MNHN 1986.873 (HPL 5 mm; Fig. 6), and MNHN 1986.874 (HPL 5.5 mm). Because of the partly poor preparations (no maximum turgidity in the genital preparations of ZFMK 62194 and MNHN 1986.870, 871 and 873; distended and partly destroyed apex in those of MNHN 1986.871 and 874) the hemipenes partly appear only slightly curved and conspicuously slender and elongate with very slight lateral bulges at the truncus. However, both the elongate siphon-like structures within the terminal bulges, as

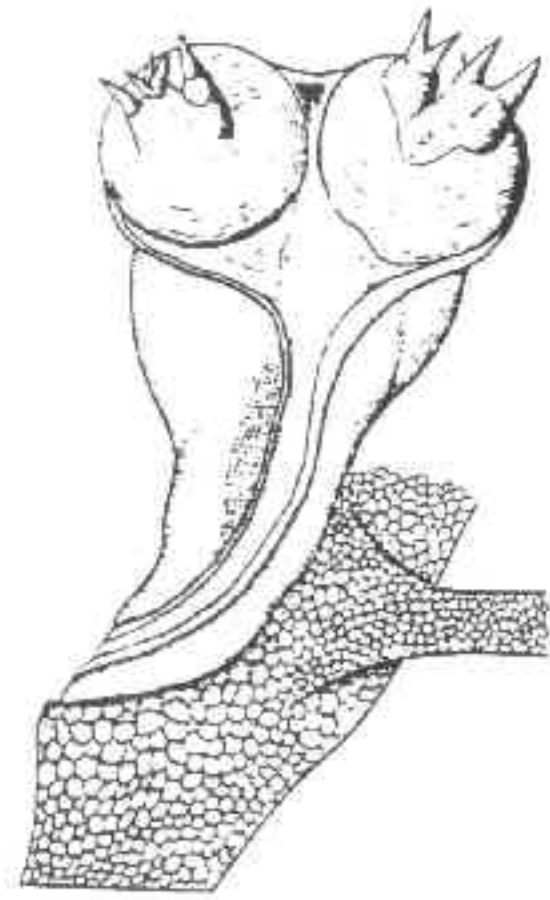


Fig. 3. Sulcal view of the hemipenis of *Brookesia peyrierasi* (holotype MNHN 1968.190) after Brygoo & Domergue (1969); scale: 1.5 mm.

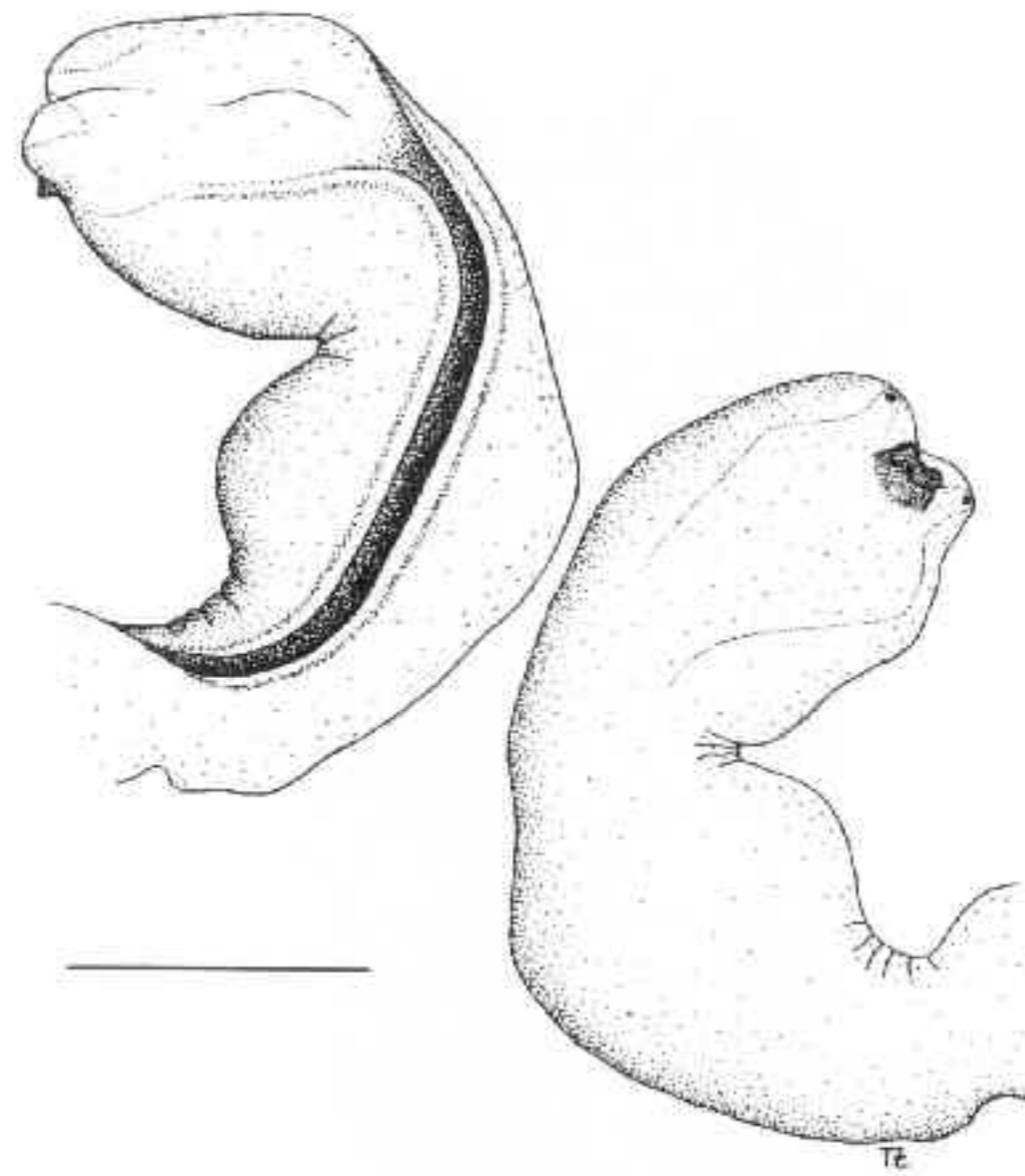


Fig. 5. Left hemipenis of *Brookesia tuberculata* (ZFMK 61242); scale: 1.5 mm. Left sulcal, right asulcal view. Due to a slightly different perspective as compared to Fig. 4, not all terminal tips of the crown-like structure are discernible. External openings of siphon-like structures are symbolized by a black spot (compare Fig. 4). Drawing by T. Ziegler.

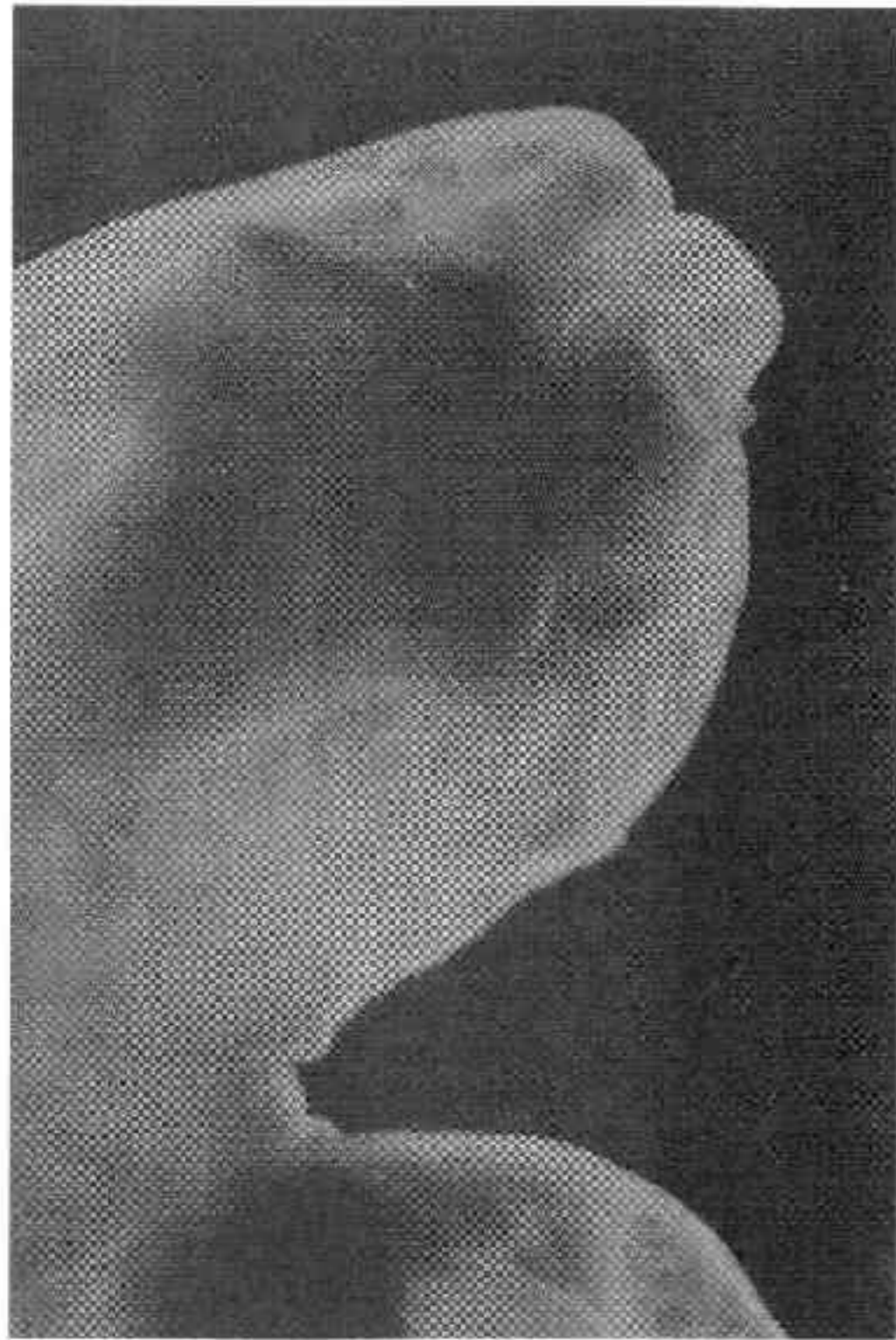


Fig. 4. Asulcal view of the apex of the left hemipenis of *Brookesia tuberculata* (ZFMK 61242).

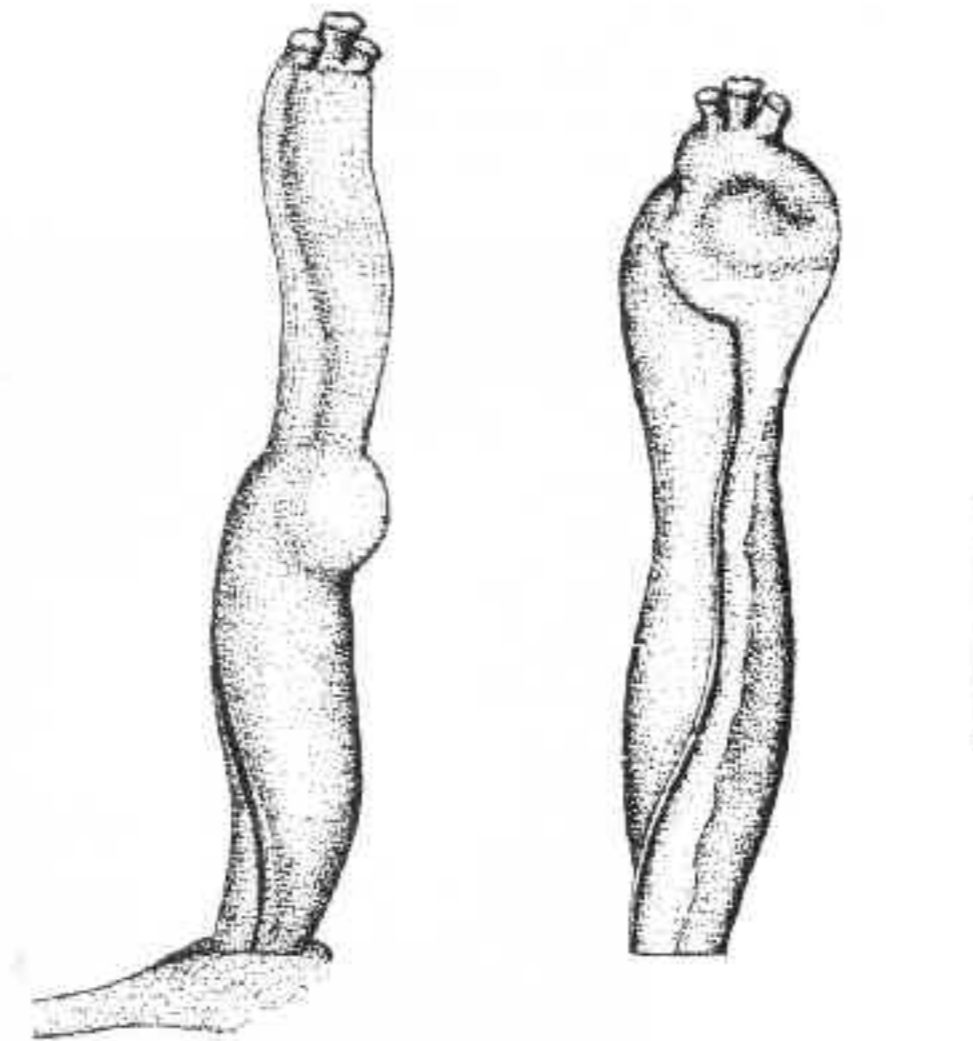


Fig. 6. Right and left hemipenes of *Brookesia tuberculata* (MNHN 1986.873) after Brygoo & Domergue (1975); scale: 1.5 mm. Left asulcal, right sulcal view.

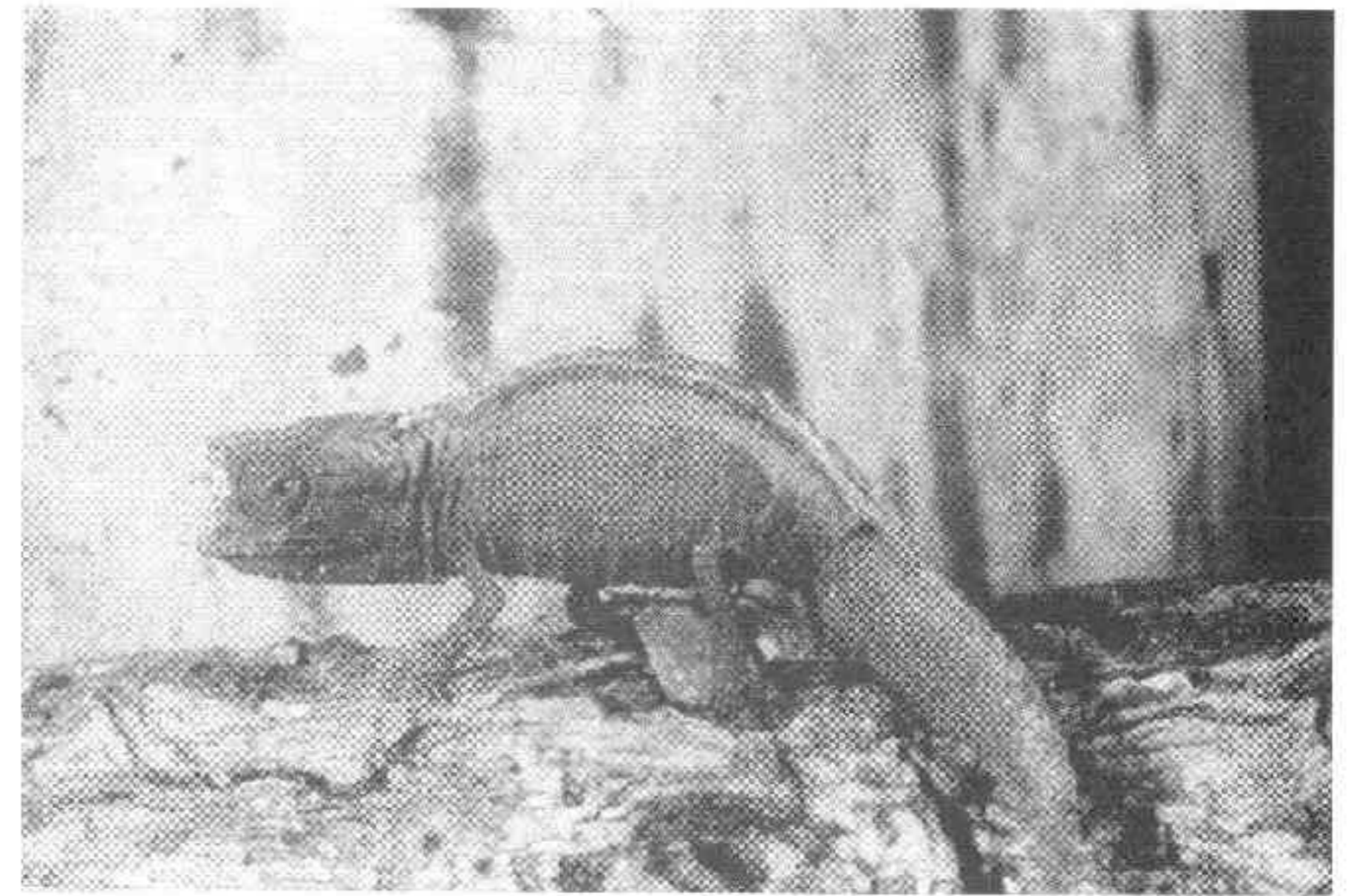


Fig. 7. Adult male of *Brookesia peyrierasi* from Nosy Mangabe.

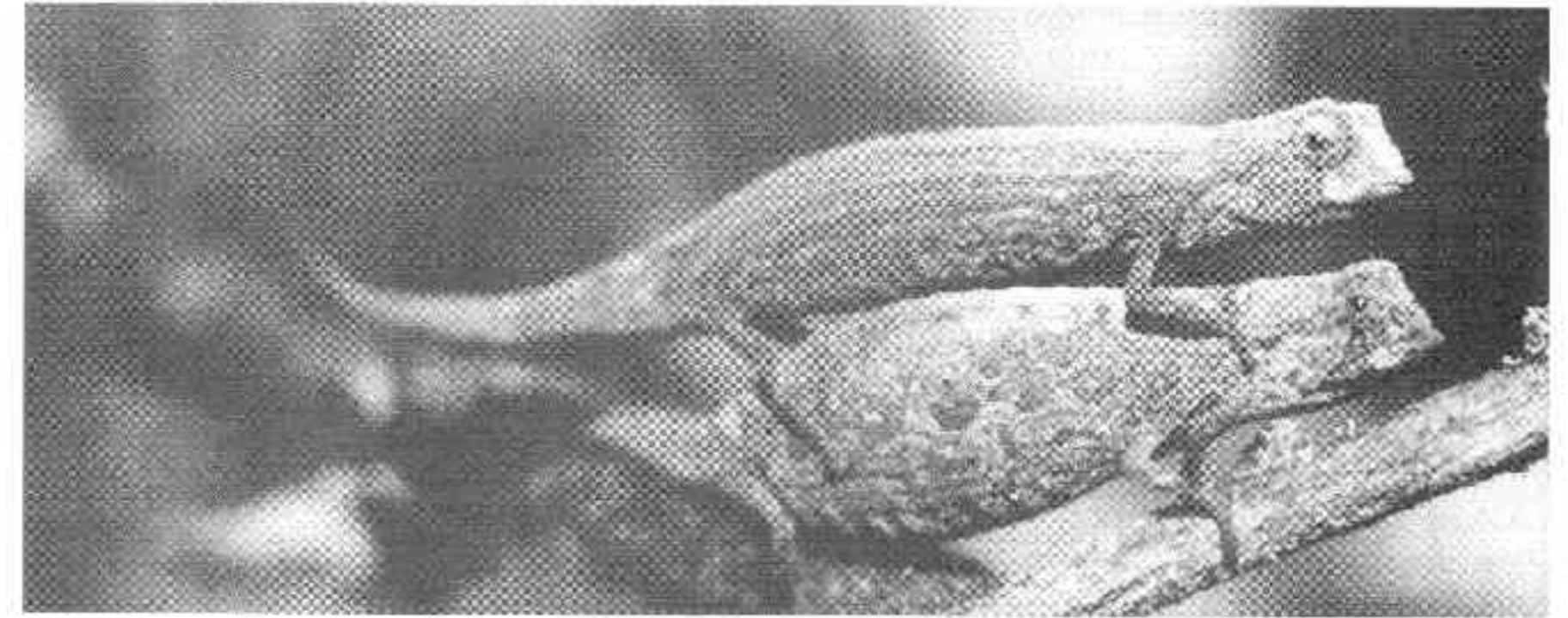


Fig. 8. Mating couple (male above) of *Brookesia minima* from Nosy Be. Note the slender body and the thin legs of both specimens, and the lack of supraocular spines in the male. Photograph by Wolfgang Schmidt.

well as the crown-like structure at the terminal asulcate 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 61242.

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 peyrierasi* (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. tuberculata* and especially in *B. peyrierasi*, 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 supraocular crest in *B. minima*, whereas in *B. tuberculata* and *B. peyrierasi* they

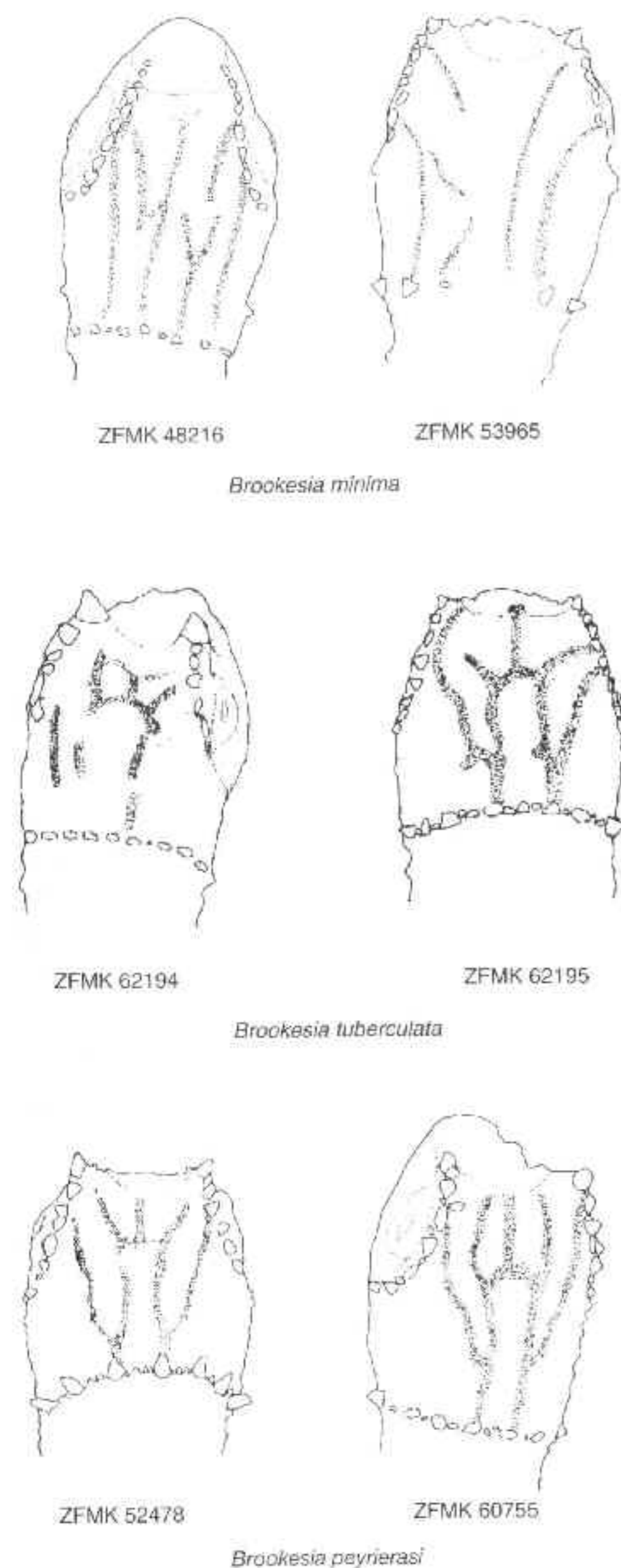


Fig. 9. Schematic drawings of typical structures of dorsal head surface in *Brookesia minima*, *B. peyrierasi* and *B. tuberculata*. Shaded lines represent elevated ridge structures. Scales and tubercles are only shown along supraocular crest and neck. Not to scale.

Table 1. Morphometric measurements (in mm) of examined specimens of *Brookesia minima*, *B. tuberculata* and *B. peyrierasi*. Used abbreviations: M = male, F = female, J = juvenile; TL = total length; TaL = tail length; HW = head width; HH = head height; Eye = horizontal diameter of eye; ArmL = arm length

	Sex	TL	TaL	HW	HH	Eye	ArmL
<i>Brookesia minima</i>							
ZFMK 51525	M	28.6	11.8	2.8	2.5	1.7	5.5
ZFMK 53966	M	30.3	12.0	3.0	3.0	1.7	6.4
ZFMK 53969	M	26.0	11.0	2.9	2.7	1.7	5.1
MNHN 1884.582	F	29.0	11.5	2.9	2.7	1.6	6.0
MNHN 1886.25	F	26.0	10.5	2.7	2.4	1.4	5.2
SMF 16512	F	31.5	11.9	3.3	2.8	1.7	6.5
SMF 16513	F	27.8	11.5	2.7	2.5	1.5	5.6
SMF 16514	F	31.8	12.9	3.3	3.1	1.7	6.7
ZFMK 48215	F	31.3	12.0	3.0	3.0	1.5	6.8
ZFMK 48216	F	33.8	12.7	3.3	3.0	1.4	6.1
ZFMK 51390	F	29.7	11.0	3.2	3.0	1.7	6.2
ZFMK 53965	F	32.3	12.1	3.2	3.2	1.6	6.5
ZFMK 53967	F	33.8	14.5	3.3	3.1	1.8	6.1
ZFMK 60664	F	30.0	11.7	3.0	3.0	1.6	6.3
ZFMK 48217	J	24.9	10.0	2.6	2.2	1.4	5.4
ZSM 17/1921	F	33.6	12.7	3.2	3.1	1.8	6.7
<i>Brookesia tuberculata</i>							
MNHN 1893.183	M	30.2	12.7	3.7	3.0	1.7	5.8
MNHN 1986.870	M	32.8	14.7	3.6	2.9	1.6	5.5
MNHN 1986.871	M	32.6	14.8	3.6	2.8	1.6	6.2
MNHN 1986.873	M	34.8	16.2	3.7	2.9	1.7	5.8
MNHN 1986.874	M	31.5	13.0	3.4	3.1	1.8	5.7
MRSN R1640	M	32.7	15.3	3.6	2.9	1.4	5.8
ZFMK 62194	M	25.2	10.8	3.0	2.7	1.4	4.8
ZFMK 62195	F	29.9	10.8	4.0	3.8	1.7	4.7
<i>Brookesia peyrierasi</i>							
MNHN 1968.188	M	36.6	15.7	3.8	3.4	1.8	7.1
MNHN 1968.189	M	37.1	16.4	3.9	3.4	1.9	6.9
MNHN 1968.190	M	38.0	16.5	4.2	3.6	1.9	7.4
MNHN 1974.236	M	39.2	17.1	4.2	3.4	1.6	7.6
MNHN 1974.242	M	34.9	12.6	4.0	3.6	2.0	7.8
MNHN 1974.243	M	36.1	16.4	3.7	3.3	1.9	7.4
MNHN 1974.244	M	39.3	17.7	4.3	3.7	1.8	7.8
MNHN 1974.246	M	39.8	17.4	3.9	3.6	1.8	7.6
MNHN 1974.247	M	35.3	15.5	3.8	3.4	1.8	6.8
MNHN 1974.249	M	36.8	16.9	3.9	3.7	1.8	7.5
MNHN 1986.875	M	38.8	17.7	3.8	3.5	1.8	7.3
MNHN 1989.3748	M	38.5	17.6	4.1	3.7	1.8	7.7
SMF 65899	M	37.8	18.1	4.1	3.5	2.0	7.5
ZFMK 60755	M	36.4	15.7	3.9	3.5	1.8	7.3
ZFMK 60756	M	36.4	16.3	4.2	3.6	2.0	7.2
MNHN 1968.184	F	36.1	14.9	4.2	3.6	1.7	7.1
MNHN 1968.186	F	36.9	13.8	4.5	4.0	1.8	7.4
MNHN 1968.187	F	39.2	15.4	4.1	3.7	1.9	7.8
MNHN 1968.191	F	41.8	16.8	4.7	4.2	2.1	8.0
MNHN 1974.237	F	39.6	16.5	3.8	3.6	1.8	7.9
MNHN 1974.240	F	38.9	15.3	4.5	3.9	2.1	7.5
MNHN 1974.241	F	34.7	14.3	4.0	3.9	1.8	7.8
MNHN 1974.245	F	35.9	14.0	3.9	3.8	1.7	7.9
MNHN 1974.250	F	34.5	14.2	4.1	3.8	1.8	7.7
MNHN 1974.251	F	43.6	16.2	4.4	3.9	2.0	9.0
MNHN 1974.252	F	41.0	15.4	4.6	4.2	2.0	8.3
MRSN R1641.1	F	38.8	15.8	4.6	4.1	1.9	7.8
MRSN R1641.2	F	32.2	13.1	3.9	3.5	1.9	7.7
SMF 65898	F	39.8	15.8	4.4	4.5	2.0	7.7
ZFMK 60753	F	39.0	15.1	4.6	4.3	2.1	7.5
ZFMK 60754	F	41.0	16.3	4.1	3.9	2.0	8.3
ZFMK 52478	F	42.0	17.7	4.5	4.1	2.0	8.1
MNHN 1974.238	J	29.5	11.5	3.6	3.2	1.6	7.3
MNHN 1974.239	J	27.1	11.3	3.3	2.9	1.7	6.3
SMF 65900	J	31.9	14.0	3.6	3.5	1.9	5.9
SMF 65901	J	25.0	10.2	3.1	3.0	1.4	5.5
ZFMK 52477	J	30.1	12.9	3.5	3.2	1.5	6.3

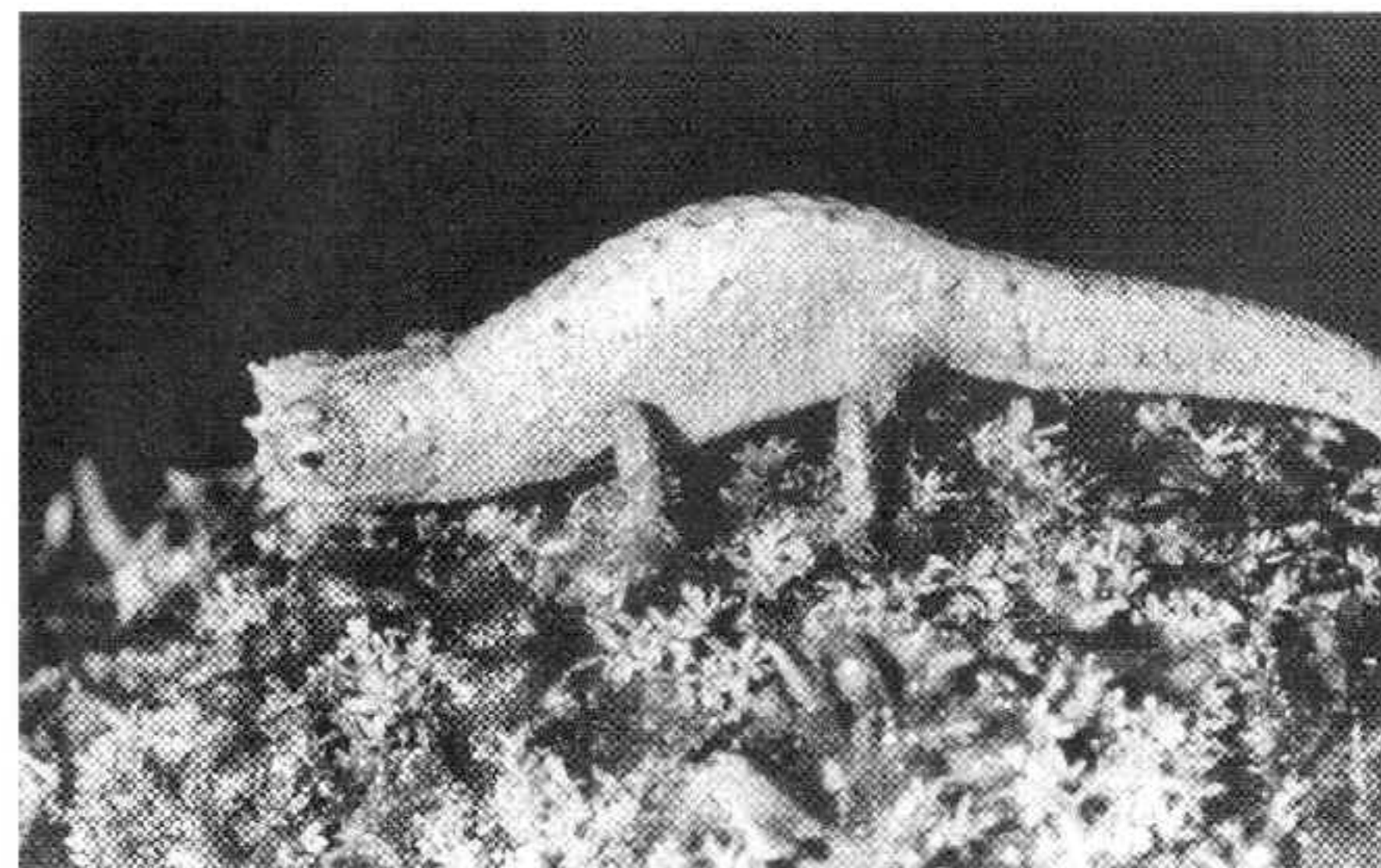


Fig. 10. Adult male of *Brookesia tuberculata* from Montagne d'Ambre. Note the distinct supraocular spines, the clumsy body shape and the thick legs.

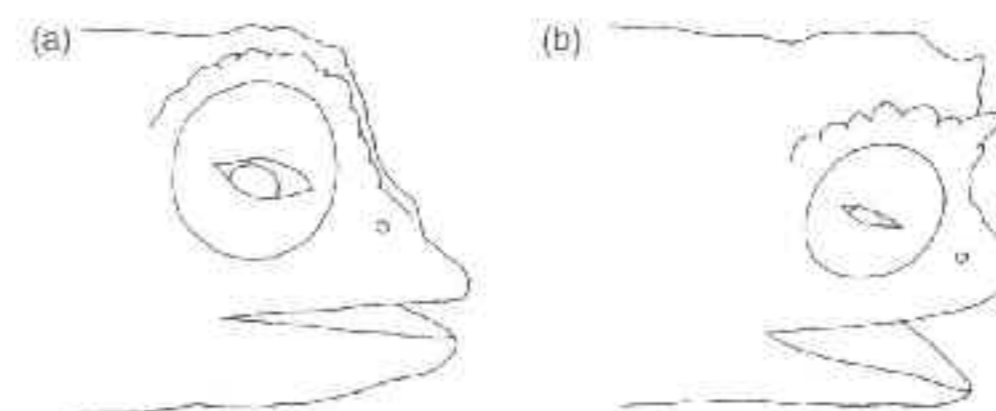


Fig. 11. Drawings of head profiles: (a) *Brookesia minima* male (ZFMK 53966); (b) *B. tuberculata* male (ZFMK 62194), showing supraocular spine.

generally curve anteriorly towards the centre. No *B. minima* specimen was observed with the regular and distinct head crest ornamentation pattern typical for *B. tuberculata* and *B. peyrierasi* as shown in Fig. 9. About one-fifth of the *B. peyrierasi* specimens (mostly males) show a weak expression of this pattern, but all *B. tuberculata* and all except one *B. peyrierasi*, had either recognizable transversal elements or longitudinal crests which at the front curve towards the centre. One single *B. peyrierasi* specimen (MNHN 1974.247) could not be distinguished from *B. minima* by head crest ornamentation alone.

(c) *Brookesia tuberculata* males (Fig. 10) differ in having one distinct, protruding spine on each supraocular crest (Fig. 11; absent in females), which are clearly shorter in *B. peyrierasi* and much shorter in *B. minima*. Additionally, *B. tuberculata* differs from *B. minima* by more robust and clumsy body shape and thicker legs (see Figs 8 & 10). The thicker constitution of the legs is

obvious in all photographs of live specimens available to us, and was verified in additional specimens observed in the field. However, due to the extremely small size of the species, this difference is very difficult to be reliably measured in preserved specimens.

Morphometrically, there are significant differences between the three taxa. Table 1 shows measurements of studied specimens, whereas differential characters are summarized in Table 2.

Brookesia peyrierasi differed from the remaining taxa by a constantly larger size, without overlap in BL values in males, and very little overlap in female values (Table 2). Size differences of males and females of *B. peyrierasi* were significant (sexes tested separately; *t*-test; $P < 0.001$) to those of *B. minima* and *B. tuberculata* except in the female comparison *B. peyrierasi* *B. tuberculata* (only one female of the latter available; $P < 0.07$).

By pairwise ANCOVA comparisons, differences between males were as follows: *B. minima* differed from *B. tuberculata* by a relatively narrower head ($P < 0.005$), and relatively shorter tail ($P < 0.01$). *Brookesia minima* differed from *B. peyrierasi* by a relatively narrower and lower head ($P < 0.005$), shorter arms ($P < 0.05$) and tail ($P < 0.005$), and larger eyes ($P < 0.05$). *Brookesia tuberculata* differed from *B. peyrierasi* by relatively shorter arms ($P < 0.001$) and lower head ($P < 0.001$).

Differences between females were as follows: *B. minima* differed from *B. tuberculata* by a relatively narrower and lower head ($P < 0.001$), and relatively longer arms ($P < 0.005$). *Brookesia minima* differed from *B. peyrierasi* by a relatively narrower and lower head ($P < 0.001$), and shorter arms ($P < 0.001$) and tail ($P < 0.05$). *Brookesia tuberculata* differed from *B. peyr-*

