

Comparative chromosome morphology of Malagasy poison frogs (Amphibia: Ranidae: *Mantella*)

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Abstract. Karyotypes of 7 species of *Mantella* are described, and are used to draw hypotheses on interspecific relationships. All studied species, *M. betsileo*, *M. viridis*, *M. laevigata*, *M. haraldmeieri*, *M. baroni*, *M. crocea* and *M. aurantiaca*, had a chromosome complement of $2n = 26$, with 5 pairs of large chromosomes and 8 pairs of small chromosomes. We performed phenetic analysis of relative chromosome lengths (rl) and centromer indices (ci) as well as phylogenetic analysis of c-banding patterns and some distinct rl and ci differences. All results indicated close relationships of *M. aurantiaca* and *M. crocea*. By phenetic analysis, *M. betsileo* and *M. viridis* are closely grouped together, as are *M. laevigata* and *M. baroni*. By phylogenetic analysis, *M. haraldmeieri*, *M. betsileo* and *M. viridis* are the most basal species, whereas *M. laevigata* and *M. baroni* sequentially split off from a clade leading to *M. aurantiaca* and *M. crocea*. These results are largely in agreement with other data sets. However, the position of *M. laevigata*, which is difficult to assess due to its large number of autapomorphies, and of *M. haraldmeieri*, are partially conflicting with results from osteology, bioacoustics, colouration and allozyme differentiation.

Key words: Anura: Mantellinae, Madagascar, karyotypes, phylogeny

Introduction

The ranoid frog subfamily Mantellinae, endemic to Madagascar, currently consists of two genera, *Mantella* and *Mantidactylus* (Glaw & Vences 1994). *Mantidactylus* is a speciose genus containing 62 described species with very different morphology and breeding biology (Vences et al. 1997, Glaw & Vences 1997), whereas *Mantella* is a well-defined, monophyletic group of currently about 17 known species (Vences et al. 1998). Malagasy poison frogs are small, largely diurnal and often colourful anurans with alkaloid skin toxins (Daly et al. 1996).

Karyotypes of Malagasy ranids and hyperoliids have been described by Blommers-Schlösser (1978) who included 23 species of *Mantidactylus* and 3 species of *Mantella* in her analysis. Chromosome number was $2n = 26$ in most species, with a reduction to $2n = 24$ in *Mantidactylus aglavei* and in species of the subgenus *Brygoomantis*. Acrocentric chromosomes occurred in species of the subgenera *Gephyromantis*, *Hylobatrachus*, *Chonomantis* and *Ochthomantis*. A phenetic comparison of Blommers-Schlösser's (1978) data on relative chromosome lengths and centromer indices (Glaw et al. 1998b) demonstrated that karyological differentiation between most Malagasy mantellines and rhacophorines is rather low. The three species of *Mantella* (*M. haraldmeieri* [as *M. cowani*

from Fort Dauphin], *M. aurantiaca* and *M. betsileo*) studied by Blommers-Schlösser (1978) clustered most closely to *Mantidactylus* species currently assigned to the subgenera *Blommersia*, *Guibemantis* and *Pandanusicola*. These groups are probably the closest relatives of *Mantella* as indicated by external characters, osteology and tadpole morphology (Blommers-Schlösser & Blanc 1993; also M. Vences, personal observation).

The aim of the present paper is to describe the karyotypes of 7 species of *Mantella*, and to use these data to draw a hypothesis on the interspecific relationships.

Material and Methods

Most studied specimens were supplied by the pet trade, and their localities are therefore not completely reliable: (1) *M. aurantiaca*, 6 specimens from near Andasibe (partly collected by T. Pintak), and 2 specimens from an uncertain locality given as „500 km south of Andasibe“; (2) *Mantella crocea*, 8 specimens from near Andasibe (partly collected by T. Pintak); (3) *M. baroni*, 8 specimens from near Moramanga (partly collected by T. Pintak); (4) *M. haraldmeieri*, 6 specimens from near Tolagnaro; (5) *M. betsileo*, 7 specimens from Nosy Be; (6) *M. viridis*, 7 specimens; and (7) *M. laevigata*, 2 specimens. We recently discovered (e.g. Vences et al. 1998) that the taxon *Mantella madagascariensis* as understood in the past actually consists of two well differentiated but externally very similar species. Since not all specimens used for chromosomal preparations were preserved as vouchers, we can not totally exclude that the *M. baroni* series included some *M. madagascariensis*; however, this species is so far not known from the Moramanga locality, and by photographs of several studied specimens, these can clearly be attributed to *M. baroni*.

Chromosomal preparations and measurements were all made by T. Pintak. Tissue was extracted without killing specimens by first cutting a skin piece (ca. 1 x 2 mm) from live specimens, and extracting the regenerating skin after 8-14 days. Wounds were treated with antibiotics to avoid infections. Extracted tissue was prepared following methods described by Macgregor & Varley (1988) for amphibian gut epithelium cells. Metaphase chromosomes were photographed under a microscope (x 1000). Photographs were xeroxed to a final amplification of about x 8500, and these copies used to measure relative chromosome lengths (rl) and centromer indices (ci). 16 metaphases of several specimens of each species were studied (*M. laevigata*: 10 metaphases). C-banding was carried out using standard protocols (Macgregor & Varley 1988), using 6 metaphases of at least 3 specimens of each species (*M. laevigata*: 6 metaphases of 2 specimens).

These data were submitted to phenetic analysis using the SPSS software package (version 5.0.2) for Windows. Mean relative lengths and centromer indices of all chromosome pairs were used, giving a total number of 26 characters. Relative lengths were included after multiplication by 10, in order to make them comparable in relative weight to the centromer indices. Euclidian distances between all taxa were calculated, and used to construct a phenetic UPGMA-dendrogram. Characters coded into discrete character states were further analyzed with Hennig86 software (Farris 1988). *Mantidactylus* species of the subgenera *Blommersia*, *Guibemantis* and *Pandanusicola* were used as the outgroup.

Results

All species studied had a karyotype of $2n = 26$, with 5 pairs of large chromosomes and 8 pairs of small chromosomes (Fig. 1).

A secondary constriction was noted in all species on chromosome 2, and additionally in *M. laevigata* on chromosome 6. By c-banding, one band was noted on chromosome 6 in *M. aurantiaca*, *M. crocea* and *M. baroni*, one on chromosome 11 in *M. aurantiaca* and

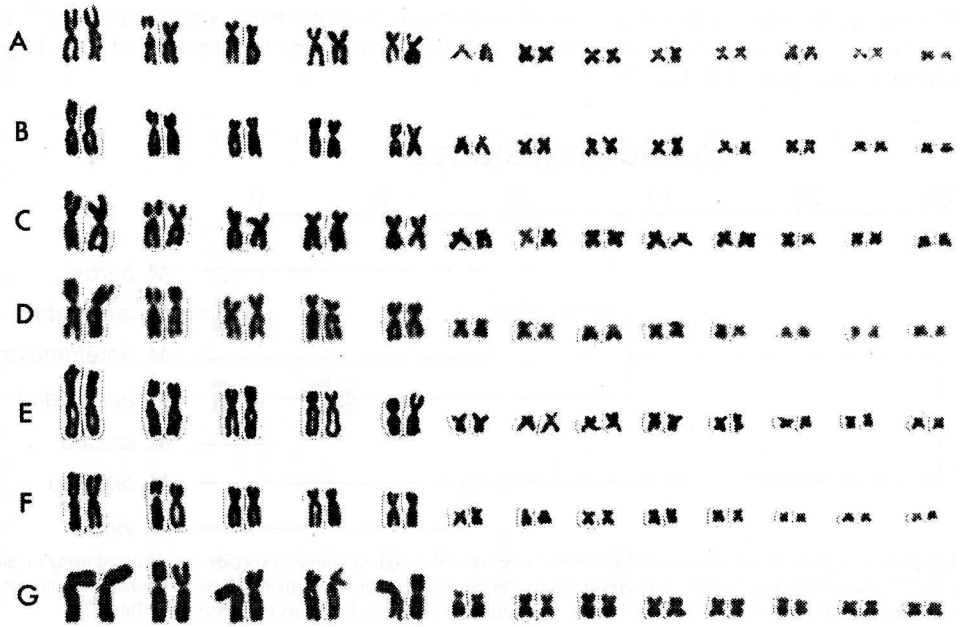


Fig. 1. Karyograms of 7 studied *Mantella* species. A *M. aurantiaca*, male; B *M. crocea*, female; C *M. baroni*, female; D *M. haraldmeieri*, male; E *M. betsileo*, female; F *M. viridis*, female; G *M. laevigata*, male. Scale: 10 μ m.



Fig. 2. Karyograms showing the c-banding results in 6 studied *Mantella* species (*M. haraldmeieri* not shown). A *M. aurantiaca*; B *M. crocea*; C *M. baroni*; D *M. betsileo*; E *M. viridis*; F *M. laevigata*. Scale: 10 μ m.

M. crocea, one on chromosome 12 in *M. aurantiaca*, and two bands on chromosome 7 in *M. laevigata*. Additionally, all species had bands near the centromers of the large chromosomes (pairs 1-5; Fig. 2).

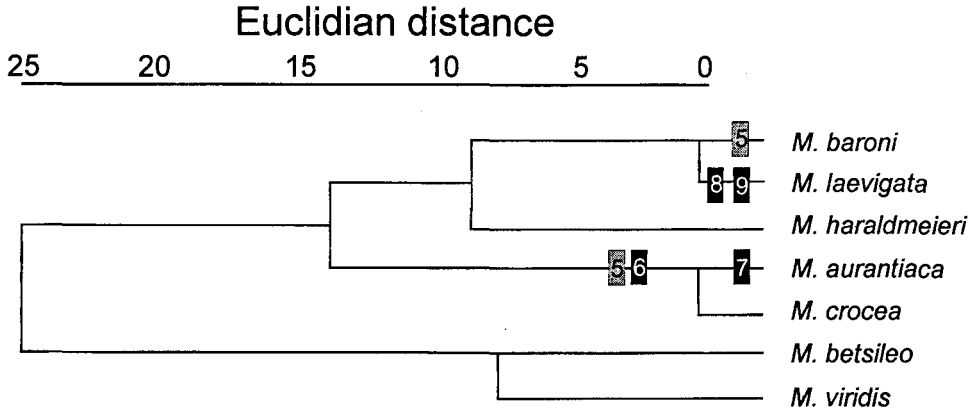


Fig. 3. Dendrogram drawn using euclidian distances between the studied *Mantella* species as calculated using ci and rl (x10) values. Presence of secondary constrictions and c-bands is represented by black (single transition according to phenetic data) and grey (convergent transition according to phenetic data) bars (numbers as in Table 2).

Three species pairs had a similar chromosome morphology when only ci and rl values were considered (Table 1, Fig. 3). *M. baroni* and *M. laevigata*, as well as *M. aurantiaca* and *M. crocea* clustered very closely together. *M. betsileo* and *M. viridis* also clustered together, but at a relatively larger distance. *M. haraldmeieri* had a rather isolated position; this taxon was grouped with the *baroni/laevigata* cluster. A similar grouping was achieved when only ci values were compared, except for *M. haraldmeieri*, which in this case was grouped with the *betsileo/viridis* cluster (dendrogram not shown). Comparing only rl values, the *aurantiaca/crocea* and *baroni/laevigata* clusters remained without changes, but *M. haraldmeieri* was grouped with *M. viridis* whereas the position of *M. betsileo* was very isolated and far from all other species (dendrogram not shown).

No comparative data of suited outgroup taxa were available for c-bands. Our data on secondary constrictions differed from those obtained by Blommers-Schlösser (1978), so that a comparison in this respect, too, did not seem to be reasonable. Polarization of these characters was therefore not possible. In the unrooted dendrogram shown in Fig. 3, the characters were included *a posteriori*, according to their presence in the studied species. However, they should not be seen as synapomorphies, since the direction of evolutionary change is not certain, and the loss of c-bands or secondary constrictions could also be derived.

However, it was possible to include data on c-bands and secondary constrictions without polarization in a phylogenetic analysis. Some of the ci and rl values could be coded into discrete character states and were thus also included. Polarization of the observed states, in these cases, was sometimes possible by comparison with the data of Blommers-Schlösser (1978). Data were available for the outgroup taxa *Mantidactylus blommersae*, *M. wittei*, *M. liber*, *M. depressiceps*, *M. tornieri*, *M. bicalcaratus* and *M. pulcher*.

Character states were coded in arabic numbers (see Table 2). We consistently coded the outgroup state as "0" to make reading of character states and presumed state transitions easier. States of characters with either contradictory, lacking or insufficient outgroup data were coded with capital letters to allow their immediate recognition. These characters were included without outgroup data in the analysis. Character numbers correspond to those in Table 2.

Table 1. Relative lengths (rl) and centromer indices (ci) of the chromosomes 1-13 of the haploid karyotype of the studied *Mantella* species. Values are given as mean \pm standard deviation.

	rl(1)	rl(2)	rl(3)	rl(4)	rl(5)	rl(6)	rl(7)	rl(8)	rl(9)	rl(10)	rl(11)	rl(12)	rl(13)
<i>M. aurantiaca</i>	14.7 \pm 0.4	12.5 \pm 0.3	11.4 \pm 0.4	10.8 \pm 0.4	9.4 \pm 0.4	6.2 \pm 0.2	5.8 \pm 0.1	5.5 \pm 0.1	5.3 \pm 0.2	4.9 \pm 0.3	4.9 \pm 0.4	4.8 \pm 0.3	4.2 \pm 0.6
<i>M. crocea</i>	14.2 \pm 0.8	11.8 \pm 0.2	11.5 \pm 0.3	10.8 \pm 0.4	9.9 \pm 0.4	6.3 \pm 0.2	5.9 \pm 0.2	5.6 \pm 0.2	5.3 \pm 0.1	5.1 \pm 0.2	4.8 \pm 0.4	4.5 \pm 0.2	4.3 \pm 0.3
<i>M. baroni</i>	14.5 \pm 0.6	12.8 \pm 0.3	11.8 \pm 0.8	10.8 \pm 0.5	10.2 \pm 0.3	6.0 \pm 0.3	5.6 \pm 0.3	5.5 \pm 0.2	5.2 \pm 0.2	4.8 \pm 0.2	4.1 \pm 0.2	4.0 \pm 0.2	3.9 \pm 0.2
<i>M. haraldmeieri</i>	15.7 \pm 0.6	13.2 \pm 0.8	12.0 \pm 0.4	11.3 \pm 0.5	10.1 \pm 0.4	5.7 \pm 0.3	5.5 \pm 0.3	5.4 \pm 0.2	5.2 \pm 0.2	4.4 \pm 0.3	4.0 \pm 0.3	3.9 \pm 0.2	3.5 \pm 0.2
<i>M. betsileo</i>	16.7 \pm 0.8	14.3 \pm 0.5	12.5 \pm 0.6	11.6 \pm 0.6	9.6 \pm 0.5	5.7 \pm 0.5	5.3 \pm 0.4	4.9 \pm 0.3	4.8 \pm 0.4	4.4 \pm 0.4	3.8 \pm 0.4	3.6 \pm 0.4	3.3 \pm 0.4
<i>M. viridis</i>	16.4 \pm 0.4	13.2 \pm 0.4	11.8 \pm 0.3	11.2 \pm 0.3	10.0 \pm 0.3	5.7 \pm 0.2	5.5 \pm 0.1	5.3 \pm 0.2	5.0 \pm 0.1	4.6 \pm 0.3	3.9 \pm 0.3	3.8 \pm 0.3	3.5 \pm 0.2
<i>M. laevigata</i>	14.8 \pm 0.6	13.3 \pm 0.4	11.7 \pm 0.4	11.3 \pm 0.3	10.1 \pm 0.8	6.0 \pm 0.3	5.7 \pm 0.2	5.6 \pm 0.3	5.2 \pm 0.4	4.7 \pm 0.2	4.3 \pm 0.2	3.8 \pm 0.2	3.7 \pm 0.2
	ci(1)	ci(2)	ci(3)	ci(4)	ci(5)	ci(6)	ci(7)	ci(8)	ci(9)	ci(10)	ci(11)	ci(12)	ci(13)
<i>M. aurantiaca</i>	43.4 \pm 1.5	40.4 \pm 3.0	31.7 \pm 3.0	41.0 \pm 2.1	43.0 \pm 2.8	29.9 \pm 1.5	43.5 \pm 2.2	47.4 \pm 2.4	44.9 \pm 2.0	48.6 \pm 1.5	38.3 \pm 1.8	44.4 \pm 2.8	45.9 \pm 3.0
<i>M. crocea</i>	46.3 \pm 1.8	37.2 \pm 1.5	31.9 \pm 1.5	41.8 \pm 1.7	44.3 \pm 1.6	29.0 \pm 2.6	43.3 \pm 3.0	44.5 \pm 2.2	46.6 \pm 2.6	46.8 \pm 1.3	39.4 \pm 2.4	39.1 \pm 2.4	43.9 \pm 3.9
<i>M. baroni</i>	45.1 \pm 2.2	39.5 \pm 2.9	40.4 \pm 2.0	31.9 \pm 1.6	40.7 \pm 2.3	30.9 \pm 2.7	45.9 \pm 2.4	43.8 \pm 4.4	41.9 \pm 6.9	45.1 \pm 3.4	41.9 \pm 6.6	42.7 \pm 2.4	36.9 \pm 2.1
<i>M. haraldmeieri</i>	44.2 \pm 2.1	36.3 \pm 4.3	37.0 \pm 3.3	35.0 \pm 4.1	39.7 \pm 1.4	42.6 \pm 5.4	43.7 \pm 2.0	33.0 \pm 2.6	44.5 \pm 2.0	41.5 \pm 3.5	34.4 \pm 2.7	42.9 \pm 4.8	42.4 \pm 3.2
<i>M. betsileo</i>	46.0 \pm 2.0	38.9 \pm 2.2	34.9 \pm 3.3	33.3 \pm 3.7	43.8 \pm 1.6	47.2 \pm 2.0	40.0 \pm 5.6	40.9 \pm 5.5	43.8 \pm 4.0	46.8 \pm 2.2	43.7 \pm 4.1	43.8 \pm 4.3	42.7 \pm 3.2
<i>M. viridis</i>	45.7 \pm 1.8	38.7 \pm 2.7	39.5 \pm 2.8	31.5 \pm 5.0	41.8 \pm 2.0	45.7 \pm 3.2	28.1 \pm 4.6	45.7 \pm 2.4	46.1 \pm 2.2	45.1 \pm 1.9	44.5 \pm 4.9	40.4 \pm 3.2	46.3 \pm 2.9
<i>M. laevigata</i>	45.5 \pm 2.2	39.4 \pm 2.2	39.7 \pm 2.4	33.3 \pm 1.1	41.7 \pm 1.5	33.2 \pm 1.8	40.6 \pm 2.4	44.4 \pm 2.5	43.2 \pm 3.9	47.6 \pm 2.6	39.5 \pm 1.9	43.2 \pm 2.9	42.4 \pm 4.4

Table 2. Characters used for phylogenetic analysis and their states in the studied *Mantella* species. Characters are explained in the text. *Mantidactylus* species of the subgenera *Blommersia*, *Guibemantis* and *Pandanusicola* were used as outgroup, and outgroup data taken from Blommers - Schlösser (1978). States found in the outgroup (plesiomorphic states) are coded as 0, derived states as 1. In characters without available, or with contradictory outgroup data, states are coded in capital letters (A-B). CB = c-band, SC = secondary constriction, C = chromosome.

Character	1	2	3	4	5	6	7	8	9
	rl(1)	rl(2)	rl(6-13)	ci(6)	CB on C6	CB on C11	CB on C12	2 CB on C7	SC on C7
<i>M. aurantiaca</i>	A	0	1	1	B	B	B	A	A
<i>M. crocea</i>	A	0	1	1	B	B	A	A	A
<i>M. baroni</i>	A	0	0	1	B	A	A	A	A
<i>M. haraldmeieri</i>	B	0	0	0	A	A	A	A	A
<i>M. betsileo</i>	B	1	0	0	A	A	A	A	A
<i>M. viridis</i>	B	0	0	0	A	A	A	A	A
<i>M. laevigata</i>	A	0	0	1	A	A	A	B	B
OUTGROUP	A/B	0	0	0	0/(1)	?	?	?	?

Characters used are as follows:

(1) The relative length of chromosome 1 was largest in *M. betsileo*, *M. viridis* and *M. haraldmeieri*. The largest gap in the successively arranged mean values of the studied species separated these three from the remaining taxa. The outgroup data were contradictory, and the states were thus not polarized: A (rl[1]<15.5), B (rl[1]>15.5).

(2) The large relative length of chromosome 2 was an autapomorphy of *M. betsileo*, which was separated by the largest gap. No *Mantidactylus* had a similar large value. States: 0 (rl[2]<13.7), 1 (rl[2]>13.7).

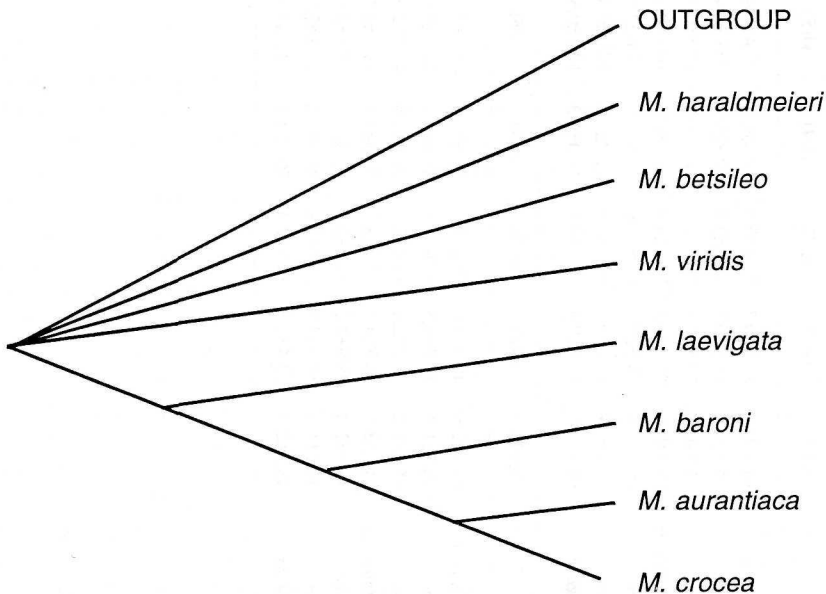


Fig. 4. Strict consensus cladogram of the two most parsimonious cladograms (consistency index 100, retention index 100) resulting from analysis of characters in Table 2.

- (3) The sum of relative lengths of the small chromosomes (6-13) were largest in *M. crocea* and *M. aurantiaca*, which were separated by the largest gap from the other species. All outgroup taxa except one (*Mantidactylus depressiceps*) had lower values. States: 0 (rl[6-13]<40.2), 1 (rl[6-13]>40.2).
- (4) A submetacentric sixth chromosome was found in *M. laevigata*, *M. baroni*, *M. crocea* and *M. aurantiaca*. Such a state was not found in the outgroup; in the whole genus *Mantidactylus* it is only found in *M. femoralis* and *M. peraccae*. States: 0 (ci[6]>37), 1 (ci[6]<37).
- (5) C-band on chromosome 6: A (absent), B (present).
- (6) C-band on chromosome 11: A (absent), B (present).
- (7) C-band on chromosome 12: A (absent), B (present).
- (8) Two c-bands on chromosome 7: A (absent), B (present).
- (9) Secondary constriction on chromosome 6: A (absent), B (present).

Phylogenetic analysis grouped *M. crocea* and *M. aurantiaca* as a monophyletic unit. *M. baroni* was the sister group of this clade, and *M. laevigata* was the sister group of the *baroni/crocea/aurantiaca* clade (Fig. 4). Relationships between the *laevigata/baroni/crocea/aurantiaca* clade and the remaining species (*M. haraldmeieri*, *M. betsileo* and *M. viridis*) were not satisfactorily resolved.

Discussion

Comparing c-banding and secondary constriction data to the phenetic analysis of ci and rl values, a major conflict regards the evolution (or loss) of a c-band on chromosome 6. This character infers close relationships between *M. baroni*, *M. aurantiaca* and *M. crocea*, and may be taken as a rather strong argument to see these three species as a natural group. In fact, this clade was also supported by phylogenetic analysis.

The most unequivocal grouping was that of *M. crocea* with *M. aurantiaca*; close relationships between these two species were supported by c-bands on chromosomes 6 and 11, as well as by centromer indices and relative lengths both analyzed together and separately. They also formed a monophyletic group according to the phylogenetic analysis.

Although *M. baroni* and *M. laevigata* clustered closely together in the phenetic analysis, their distinctness was emphasized by the presence of a secondary constriction and two C bands in *M. laevigata* which were absent in *M. baroni*. Also, *M. betsileo* and *M. viridis* clustered together in the phenetic analysis, but the phenetic distance between them was relatively large. This was mainly due to the enlarged second chromosome of *M. betsileo*. This state was corroborated by the data of Blommers-Schlösser (1978), who found a similarly large second chromosome in *M. betsileo*. Analysis of her data on three *Mantella* resulted in a close cluster that contained *M. aurantiaca* and *M. haraldmeieri*, largely separated from the isolated *M. betsileo* (M. Venes, personal observation). By phylogenetic analysis, there was no unequivocal chromosomal character inferring a monophyletic grouping of *M. betsileo* and *M. viridis*.

Relationships of *M. haraldmeieri*, too, were not satisfactorily solved by the chromosomal data. Phenetic analysis grouped the species with the *baroni/laevigata* cluster, whereas the phylogenetic analysis did not allow for definite conclusions. However, a different coding of character 1 (the second largest value gap separated only *M. betsileo* and *M. viridis* from the remaining taxa) would have placed *M. haraldmeieri* in the *laevigata/baroni/crocea/aurantiaca* clade.

The general grouping inferred from the chromosome data largely agrees with osteology (Venes et al. 1998), bioacoustics, colouration and genetic differentiation (M. Venes, personal observation). These data sets agree in placing, among others, *M. betsileo*, *M. viridis* and *M. laevigata* as most basal species, whereas *M. aurantiaca* and *M. crocea* show many synapomorphic traits.

In several data sets, *M. laevigata* has many autapomorphies, making assessment of its relationships difficult. Its breeding biology (single eggs deposited in water-filled tree holes; Glaw & Vences 1992) and parental care (feeding of eggs to tadpoles; Glaw et al. 1998a) are unique in the genus *Mantella*. Bioacoustically, the double-click-call of *M. laevigata* is a state shared with *M. betsileo* and *M. viridis*. Beside chromosome morphology, the only other indication of relationships between *M. baroni* and *M. laevigata* is their lack of a horse-shoe marking on the black throat, a pattern which is generally also absent in *M. haraldmeieri*, *M. cowani*, and *M. nigricans*. However, *M. laevigata* lacks the unforked sternum, which is an apomorphic state shared by *M. baroni*, *M. haraldmeieri*, *M. cowani*, and *M. nigricans* (Vences et al. 1998), as well as the single-click call, which is also a state only found in these species. On the other hand, as indicated by unforked sternum, general colour pattern, and call, *M. haraldmeieri* is most probably closely related to *M. baroni*, although chromosomal data do not support this conclusion.

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