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Chromosome Data for Malagasy Poison Frogs (Amphibia: Ranidae: *Mantella*) and Their Bearing on Taxonomy and Phylogeny

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ABSTRACT—We compared chromosome morphologies for 11 species of Malagasy poison frogs, genus *Mantella*, and three outgroup taxa (genus *Mantidactylus*) using conventional and fluorescence staining techniques. All species studied had a karyotype of 2n=26, with five larger and eight smaller chromosome pairs. The 11th pair was acrocentic in *Mantella nigricans* which represents the first such observation in the genus. The nucleolus organizer region (NOR) was located at secondary constrictions on chromosome pair 2 in all *Mantella* studied and in *Mantidactylus grandisonae* (while located on other chromosomes in all other species of *Mantidactylus* studied so far). Heterochromatin distribution was highly variable among *Mantella* species; C-bands positively staining with DAPI and CMA_3 were observed. The possible structure of these bands, seemingly containing both A+T rich and C+G rich heterochromatin, is discussed. Phylogenetic reconstruction using chromosomal characters provided very little information. Evolution of the characters studied is probably either too fast (heterochromatin arrangement) or too slow (NOR location) to match the main cladogenetic events among *Mantella* species groups.

INTRODUCTION

Madagascar is famous for its organismal diversity and high degree of endemism. Among the most speciose vertebrate clades are the mantellines. This lineage, including *Mantella* and *Mantidactylus* (Glaw and Vences, 1994; Glaw *et al*., 1998) has been considered as subfamily Mantellinae of the cosmopolitan frog family Ranidae (Blommers-Schlösser, 1993) or as separate family Mantellidae (Dubois, 1992). Mantellines are characterized by a specialized mating behavior involving absence of a strong mating amplexus (Blommers-Schlösser, 1993; Glaw *et al*., 1998). They are a monophyletic group as supported by morphological and molecular studies (Glaw *et al*., 1998; Richards and Moore, 1998; Richards *et al*., 2000).

Currently more than 65 nominal species of *Mantidactylus* are known (Glaw and Vences, 1999, 2000; Glaw *et al*., 2000). They are highly diverse, ranging from large and semiaquatic to minute and scansorial, and from species with fairly generalized tadpoles to highly specialized species with direct development (Blommers-Schlösser and Blanc, 1991; Glaw and Vences, 1994). Molecular data demonstrated that *Mantidactylus* is not monophyletic: the molecular study of Richards *et al.* (2000) supported relationships of *Mantella* to species of *Mantidactylus* belonging to the subgenera *Blommersia*, *Guibemantis*, and *Pandanusicola*.

In contrast, the genus *Mantella* is a well defined monophyletic unit (Vences *et al*., 1998a, b) containing about 17 morphologically poorly differentiated species (Vences *et al*., 1999). *Mantella* are attractive, small diurnal frogs which accumulate skin alkaloids, most probably by uptaking arthropod prey (Daly *et al*., 1996; 1997), and characterized by aposematic coloration. Hypotheses of intrageneric relationships have been proposed based on a number of different character sets (Pintak *et al*., 1998; Vences *et al*., 1998b, c).

The chromosomes of mantelline frogs have been described thus far mainly by Blommers-Schlösser (1978). She

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described general chromosome morphology in 24 species of *Mantidactylus* and *Mantella aurantiaca*, *M. betsileo*, and *M. haraldmeieri*. In addition, Pintak *et al.* (1998) provided data on *Mantella aurantiaca*, *M. baroni*, *M. betsileo*, *M. expectata*, *M. haraldmeieri*, *M. laevigata*, and *M. viridis*. The present study complements these earlier contributions by adding new species of *Mantella* to the data set. Besides general chromosome morphology, we also studied the distribution and composition of heterochromatin and the location of nucleolus organizer regions (NORs). Our goals were (1) to test recent *Mantella* classification by searching for chromosomal differences between closely related taxa, and (2) to obtain a new set of data to test hypotheses of *Mantella* phylogeny.

MATERIALS AND METHODS

We examined a total of 36 specimens of *Mantella* belonging to 11 different species (see appendix for voucher specimens), which belong to the *M. aurantiaca* group (*M. aurantiaca*), *M. betsileo* group (*M. betsileo, M. cf. betsileo, M. expectata, M. viridis*), *M. cowani* group (*M. baroni, M. cowani, M. nigricans*), *M. laevigata* group (*M. laevigata*) and *M. madagascariensis* group (*M. madagascariensis, M. pulchra*) as defined by Vences *et al.* (1999). Six specimens of three species of *Mantidactylus* were used for outgroup comparisons. Voucher specimens have been deposited at the Museo Regionale di Scienze Naturali, Torino (MRSN) and the Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn (ZFMK). Each specimen was treated with 0.01 ml per g body weight of a 0.5 mg/ml colchicine solution. Four hr later, animals were sacrificed using 0.1% tricaine metasulfonate (MS-222). Intestines, spleens, lungs, and gonads were removed and incubated for 30 min in a 0.7% sodium citrate solution. Chromosomes were obtained by air drying and scraping as described by Olmo *et al.* (1986). Besides conventional staining (5% Giemsa at pH 7), the following techniques were applied: (1) $AgNO_3$-banding of NORs following Howell and Black (1980); (2) staining with the C+G specific fluorochrome chromomycin A_3 (CMA_3) according to Sahar and Latt (1980), with a reduced exposure (a few seconds) to the non fluorescent dye, methyl green; (3) the A+T specific fluorochrome, DPI/distamycin (DA), and CMA_3/DA (DAPI: Schweizer 1976); (4) C-banding as described by Sumner (1972), incubating the slides for 5 min at 45°C in $Ba(OH)_2$; (5) in situ digestion with *Alu* I endonucleases (compare Mezzanotte *et al.* 1983). Suitable results were achieved by staining, either separately or sequentially, with CMA_3 and DAPI after hydrolysis in $Ba(OH)_2$ or digestion with *Alu* I.

Metaphase chromosomes were stained with Giemsa; $AgNO_3$ and C-banding/Giemsa were viewed on a Zeiss PHOM III phase contrast microscope, whereas the fluorochrome-stained metaphases (CMA_3 and DAPI) were viewed on a Leitz epifluorescent microscope. Of each taxon, at least four Giemsa-stained metaphases and two metaphases stained with each of the banding methods used were studied. Images were digitized using a scanner. Karyotypes were constructed using Adobe Photoshop 3.0. Measurements to determine relative chromosome length (rl; percentage ratio between the length of each chromo-

Table 1. Relative chromosome lengths (rl) of chromosomes 1–13 in the species studied. Data are mean values with standard deviations.

Species	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)
Mantella aurantiaca	16.3±0.5	13.0±0.8	12.0±0.3	11.5±0.5	9.9±0.4	7.1±0.3	5.9±0.3	5.5±0.2	5.2±0.3	5.0±0.3	4.7±0.4	4.6±0.3	4.2±0.5
Mantella baroni	16.1±0.6	13.3±0.7	12.2±0.8	10.5±0.5	10.2±0.3	6.8±0.2	5.6±0.3	5.6±0.4	5.1±0.3	4.8±0.2	4.3±0.3	4.5±0.4	3.9±0.2
Mantella betsileo	16.7±0.8	14.3±0.5	12.1±0.6	10.6±0.6	9.9±0.3	6.3±0.7	5.7±0.4	4.9±0.4	4.8±0.3	4.4±0.4	3.8±0.3	3.6±0.2	3.3±0.4
Mantella cf. *betsileo*	15.3±0.3	13.4±0.3	12.8±0.5	10.8±0.8	9.8±0.1	6.8±0.3	6.6±0.8	4.5±0.3	3.9±0.5	4.4±0.3	3.9±0.1	3.8±0.1	3.4±0.5
Mantella cowani	14.2±0.8	12.8±0.5	11.8±0.3	11.3±0.1	10.1±0.4	6.8±0.2	5.8±0.3	5.4±0.3	5.2±0.3	5.4±0.8	4.0±0.4	3.9±0.7	3.8±0.8
Mantella expectata	15.7±0.6	13.2±0.7	11.9±0.4	10.8±0.5	9.9±0.4	5.9±0.3	5.7±0.3	5.4±0.2	5.2±0.3	4.4±0.2	4.2±0.3	3.8±0.4	3.7±0.2
Mantella laevigata	14.8±0.6	13.3±0.4	11.7±0.3	11.3±0.2	10.1±0.5	6.0±0.5	5.7±0.2	5.6±0.3	5.2±0.3	4.7±0.2	4.3±0.1	3.8±0.4	3.7±0.2
Mantella madagascariensis	14.3±1.0	12.1±0.9	11.1±0.7	11.0±0.5	10.0±0.2	6.7±0.2	5.6±0.5	5.5±0.5	5.4±0.7	4.9±0.5	4.4±0.5	4.3±0.1	3.7±0.7
Mantella nigricans	16.3±0.1	14.1±0.3	10.8±0.6	11.0±0.1	9.9±0.1	7.1±0.3	6.0±0.5	5.2±0.2	5.3±0.4	4.8±0.3	4.4±0.6	4.2±0.2	3.9±0.4
Mantella pulchra	14.3±0.9	12.9±0.7	12.8±0.8	10.8±0.2	10.2±0.3	7.2±0.4	5.4±0.2	5.3±0.1	5.0±0.1	4.6±0.1	4.2±0.5	4.1±0.3	4.0±0.2
Mantella viridis	16.4±0.4	13.2±0.4	11.8±0.3	11.2±0.3	10.0±0.4	6.1±0.4	5.6±0.1	5.3±0.2	5.1±0.1	4.6±0.3	3.9±0.4	3.9±0.3	3.5±0.3
Mantidactylus grandisonae	18.3±0.6	15.8±0.7	15.3±0.5	12.5±0.5	9.1±0.4	5.7±0.3	5.5±0.4	4.6±0.2	4.1±0.4	3.8±0.3	3.7±0.4	3.3±0.3	3.0±0.2
Mantidactylus bicalcaratus	14.5±0.6	13.1±0.4	11.6±0.7	11.4±0.9	9.3±0.4	6.4±0.2	6.0±0.5	5.5±0.3	5.2±0.4	4.9±0.5	4.5±0.6	4.1±0.6	3.8±0.3
Mantidactylus cf. *punctatus*	15.3±0.7	12.9±0.1	12.1±0.3	11.8±0.7	9.8±0.5	7.0±0.3	6.5±0.3	5.4±0.8	5.1±0.3	5.0±0.3	4.6±0.3	4.3±0.5	3.6±0.4

Table 2. Centromer indices (ci) of chromosomes 1–13 in the species studied. Data are mean values with standard deviations.

Species	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)
Mantella aurantiaca	46.9±2.0	39.6±3.0	32.3±3.3	40.3±2.7	42.5±1.9	30.7±2.3	45.9±4.0	45.3±2.2	44.1±3.7	44.6±2.8	40.0±2.8	44.0±3.5	43.9±3.3
Mantella baroni	45.1±2.2	39.5±2.9	31.9±1.6	40.4±2.0	40.7±2.3	30.9±2.7	45.9±3.4	44.9±3.4	42.9±4.0	44.6±2.8	40.0±.8	42.7±2.4	39.9±2.1
Mantella betsileo	46.0±2.0	38.9±2.2	30.9±3.3	38.6±1.7	43.8±1.6	45.8±1.2	44.5±5.6	44.0±3.2	42.3±4.0	45.8±2.2	40.3±2.1	42.0±3.3	43.0±3.2
Mantella cf. *betsileo*	45.4±2.3	36.8±3.1	31.5±3.1	39.0±1.1	42.0±3.1	29.9±2.3	45.3±2.7	42.4±3.2	43.0±2.1	46.1±2.1	40.0±1.3	41.2±3.2	42.8±2.7
Mantella cowani	46.2±2.8	38.6±2.5	32.9±1.8	40.8±2.0	43.1±1.8	30.0±2.6	43.3±2.9	44.8±2.1	46.1±3.1	45.8±2.1	40.0±2.4	41.1±2.5	43.1±3.3
Mantella expectata	45.9±2.1	38.6±4.3	31.7±2.1	39.1±4.1	39.7±1.4	30.2±4.1	44.7±2.0	40.7±2.6	44.0±1.8	41.5±3.5	39.3±2.7	42.6±4.8	42.7±3.2
Mantella laevigata	45.5±3.5	39.4±3.4	32.0±4.4	37.3±3.1	41.7±1.5	33.2±2.8	40.8±4.3	44.4±2.5	43.2±3.9	47.6±2.6	39.5±1.9	43.2±2.9	42.4±4.4
Mantella madagascariensis	44.1±3.4	39.1±2.3	31.1±3.7	39.3±2.5	42.9±4.1	29.8±2.9	42.9±2.8	42.4±3.8	44.2±1.9	45.7±2.2	41.2±1.6	42.3±3.4	43.1±2.9
Mantella nigricans	44.2±4.1	39.6±2.9	31.8±4.0	38.6±3.1	41.3±3.0	32.2±3.0	44.2±1.5	43.5±3.1	42.0±4.0	46.2±2.2	0.09±1.8	43.1±1.5	42.7±0.2
Mantella pulchra	45.3±1.2	38.2±4.0	31.9±1.5	37.3±2.5	41.2±3.1	31.3±2.2	43.4±2.1	44.8±3.1	43.9±4.0	45.2±2.7	40.2±1.7	42.6±1.2	43.0±2.1
Mantella viridis	45.7±1.8	38.7±2.7	32.2±4.8	38.6±2.2	41.8±2.1	33.2±1.8	41.6±3.1	45.7±2.4	46.1±2.2	45.1±1.9	42.5±2.9	42.0±3.2	44.1±4.4
Mantidactylus grandisonae	39.0±5.0	33.3±5.3	38.0±4.6	41.2±4.0	41.6±3.8	31.8±3.3	40.9±3.9	45.9±4.0	40.6±3.5	33.3±3.0	42.8±3.7	46.2±4.0	41.7±3.6
Mantidactylus bicalcaratus	44.0±2.2	41.1±2.0	31.3±3.8	30.9±3.0	39.9±2.8	34.1±2.5	42.3±4.0	41.9±2.1	39.1±1.9	40.3±1.7	43.2±2.9	40.5±1.5	40.1±1.9
Mantidactylus cf. *punctatus*	43.9±3.2	40.2±2.3	34.0±2.8	30.2±1.6	41.0±2.1	35.0±2.3	43.4±3.6	40.6±2.0	39.1±1.9	40.5±2.0	41.6±3.1	39.7±1.8	41.1±1.2

some to the total length of all the chromosomes) and centromer index (ci; ratio between short arm and total length of each chromosome) were carried out using the digitized images. In the following accounts, DAPI-positive bands are referred to simply as "DAPI+", CMA_3-positive bands as "CMA+".

Cladistic analysis was carried out using PAUP*, version 4 beta (Swofford, 1998). We calculated Maximum parsimony and Neighbor-joining (NJ) trees based on total character differences, and tested the trees by running 2000 bootstrap replicates. An interspecific principal component analysis (PCA) of mean rl and ci values was performed with SPSS for Windows, version 6.1.2.

RESULTS

All species of *Mantella* and *Mantidactylus* examined had

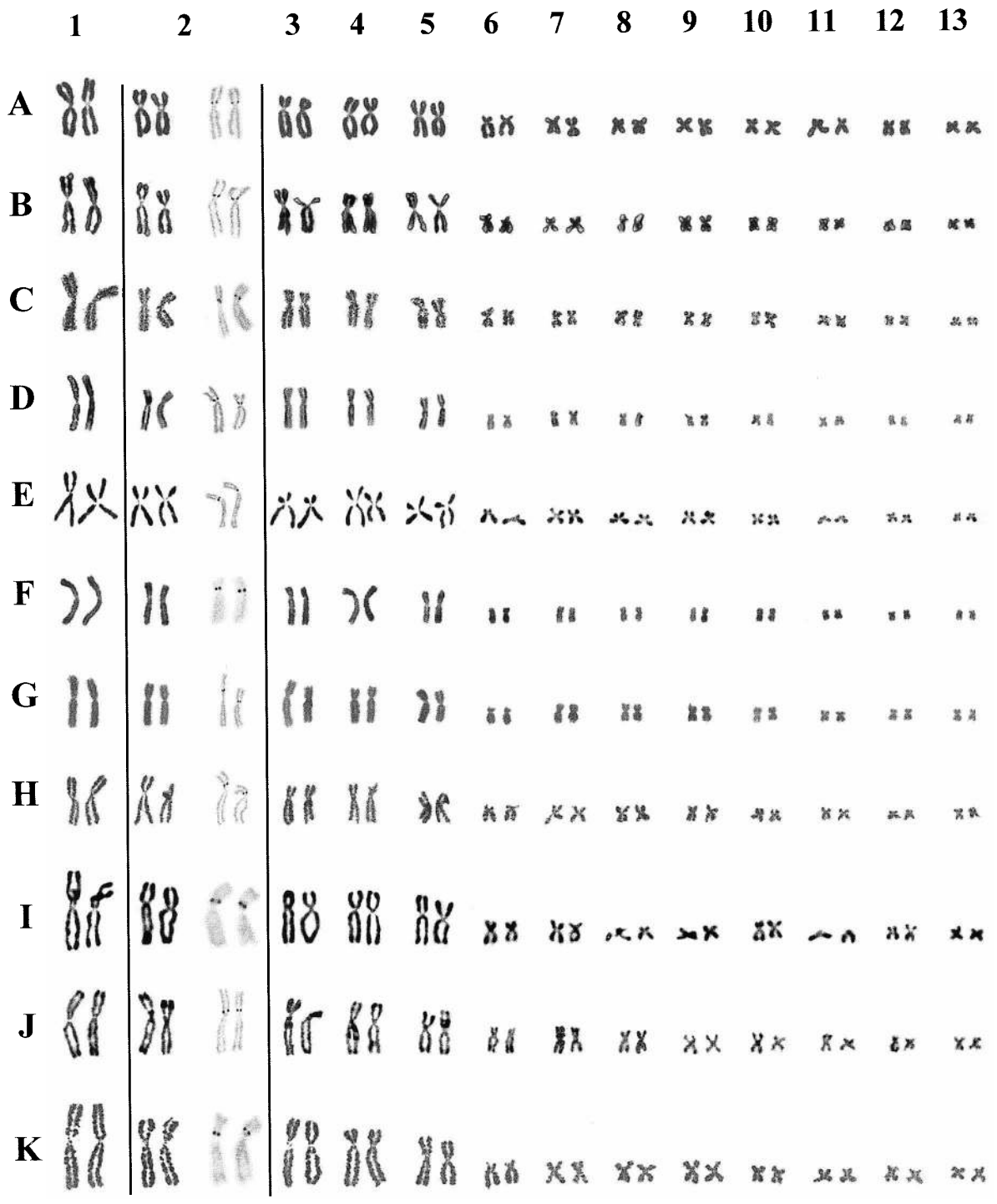

Fig. 1. Giemsa stained karyotype of *Mantella* taxa studied: *M. aurantiaca* (**A**), *M. baroni* (**B**), *M. betsileo* (**C**), *M.* cf. *betsileo* (**D**), *M. cowani* (**E**), *M. expectata* (**F**), *M. laevigata* (**G**), *M. madagascariensis* (**H**), *M. nigricans* (**I**), *M. pulchra* (**J**), *M. viridis* (**K**). The $AgNO_3$ stained NOR bearing 2nd chromosome pair is also reported.

a karyotype of 2n = 26 chromosomes with five larger and eight smaller chromosome pairs. All chromosomes were metacentric except for the submetacentric third and sixth pair (Table 1 and 2; Fig. 1). The only deviations from this pattern were exhibited by *M. betsileo* which showed a metacentric sixth pair, and *M. nigricans* in which the 11th pair was acrocentric (Table 1 and 2). All *Mantella* species as well as *Mantidactylus grandisonae* had a secondary constriction near the centromere on the short arm of the 2nd chromosome pair; this secondary constriction was selectively stained by the $AgNO_3$ and the CMA_3/MG staining, indicating that it corresponds to the NOR. In *Mantidactylus* cf. *punctatus* and *M. bicalcaratus*, the NOR was interstitial on the short arm of the 1st chromosome pair (Fig. 1).

Eight principal component factors with an Eigenvalue >10 were obtained by PCA. The first and second principal component factors together explained 56.8% of the observed total variation. The first factor was mainly influenced by relative chromosome lengths: although the highest principal component loading was that of the centromer index of chromosome 2, the four next highest loadings were those of the relative lengths of chromosomes 2, 3, 10, and 13. The second factor,

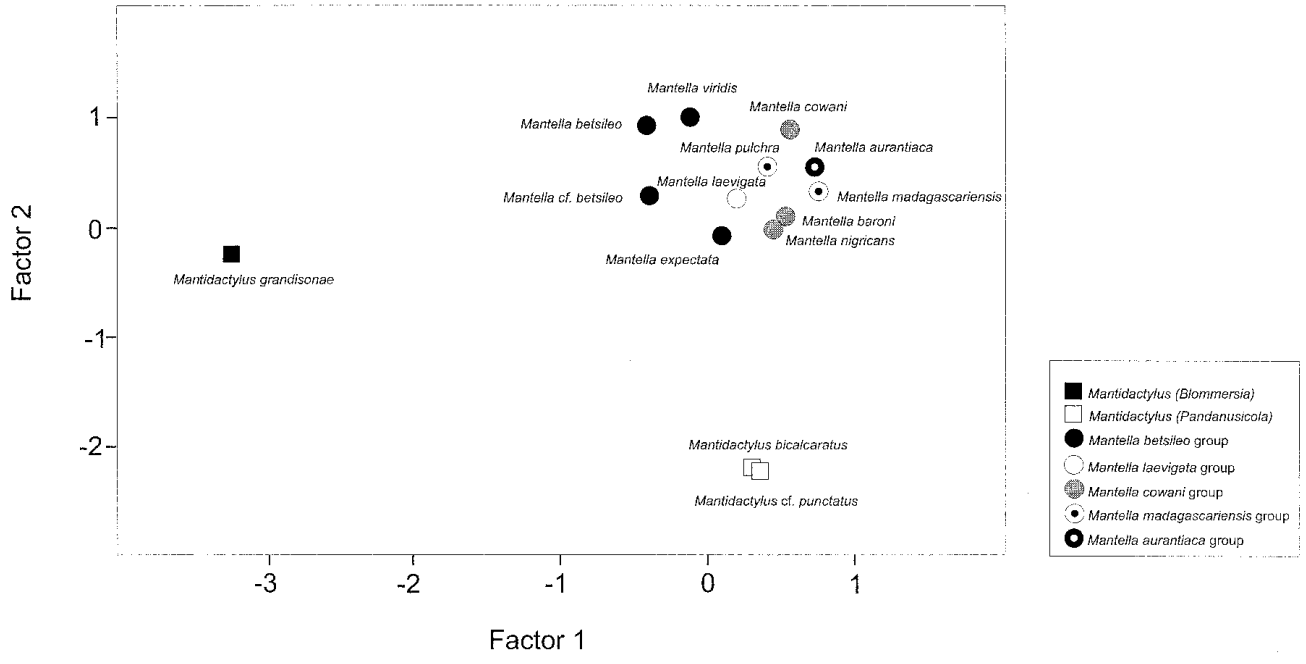

Fig. 2. Scatterplot of first and second factors of a PCA of morphometric chromosome data as given in Table 1 and 2 (ci and rl).

Table 3. Distribution of centromeric, telomeric, and peritelomeric heterochromatin in *Mantella* and *Mantidactylus* species studied. For each species, we listed numbers of chromosomes on which a respective band was observed, followed by C and/or D if the bands stained positively with CMA_3 and/or DAPI, respectively. Chromosome numbers in brackets refer to faint staining. The telomeric heterochromatin of *M. betsileo* was located on the long arm of the first chromosome and the short arm of the second chromosome; the peritelomeric band of *M. laevigata* was located on the short arm of the second chromosome.

	Centromeric heterochromatin	Telomeric heterochromatin	Peritelomeric heterochromatin
Mantella aurantiaca	1–5 (C, D)	1–5 (C)	6, 9, 10, 11 (C, D)
Mantella baroni	1–13	–	6
Mantella betsileo	1–13 (D)	1,2 (C)	–
Mantella cf. *betsileo*	1–13 (C, D)	1 (D)	–
Mantella cowani	1–3 (C)	1–13 (C), 4 (D)	–
Mantella expectata	1–9, 12–13 (D)	6, 10, 11 (C)	–
Mantella laevigata	1–9, 12–13 (D)	8–13 (C)	2 (C)
Mantella madagascariensis	[1–5]	1, 8, 9 (C)	6, 10, 11 (D)
Mantella nigricans	1, 3, 11 (C, D)	1–10, 12–13 (C, D)	11 (C, D)
Mantella pulchra	[1–13?]	1–13 (C)	6, 10, 11 (C, D)
Mantella viridis	1–5 (D)	6–13 (C)	–
Mantidactylus bicalcaratus	[6, 12] (C, D)	1–13 (C)	3 (C)
Mantidactylus grandisonae	1–13 (C, D)	1–13 (C)	–
Mantidactylus cf. *punctatus*	1–13 (C, D)	1–13 (C)	3 (C)

Table 4. Karyological character states used for phylogenetic analysis: (1) Configuration of 6th chromosome. 0 submetacentric; 1 metacentric. (2). Configuration of 11th chromosome. 0 submetacentric; 1 acrocentric; 2 metacentric. (3) Intensity of centromeric C-bands. 0 distinct; 1 faint or very faint. (4) Presence of centromeric C-bands. 0 present on all chromosomes; 1 present on all chromosomes except 10th and 11th; 2 present on chromosomes 1–5; 3 present on chromosomes 1-3; 4 present on chromosomes 1, 3, 11; 5 present on chromosomes 6, 12. (5) DAPI-staining of centromeric C-bands. 0 DAPI-negative; 1 DAPI-positive. (6) CMA-staining of centromeric C-bands. 0 CMA-negative; 1 CMA-positive. (7) Presence of telomeric C-bands. 0 absent; 1 scattered on only a few (up to three) chromosomes; 2 mainly present on chromosomes 1–5; 3 mainly present on chromosomes 6–8; 4 present on all or almost all chromosomes. (8) DAPI-staining of telomeric chromosomes. 0 DAPI-negative; 1 DAPI-positive. (9) CMA-staining of telomeric chromosomes. 0 CMA-negative; 1 CMA-positive. (10) Large peritelomeric or telomeric C-band present on long arm of 6th chromosome. 0 absent; 1 present. (11) Large peritelomeric C-bands on 10th chromosome. 0 absent; 1 present. (12) Large peritelomeric C-band on 11th chromosome. 0 absent; 1 present. (13) Paracentromeric bands on 1st and 3rd chromosome. 0 absent; 1 present. (14) NOR localization. 0 interstitial on short arm of the 1st chromosome pair; 1 on short of the 2nd chromosome pair. (15) Peritelomeric band on the long arm of the 3rd chromosome pair: 0 absent; 1 present.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Mantella aurantiaca	0	0	0	2	1	1	2	0	1	1	1	1	0	1	0
M. baroni	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0
M. betsileo	1	0	0	0	1	0	1	0	1	0	0	0	0	1	0
M. cf. *betsileo*	0	0	0	0	1	1	1	1	0	0	0	0	0	1	0
M. cowani	0	0	0	3	0	1	4	0/1	1	0	0	0	0	1	0
M. expectata	0	0	0	1	1	0	1	0	1	0	0	0	1	1	0
M. laevigata	0	0	0	1	1	0	3	0	1	0	0	0	1	1	0
M. nigricans	0	1	0	4	1	1	4	1	1	0	0	1	0	1	0
M. madagascariensis	0	0	1	2	0	0	1	0	1	1	1	1	0	1	0
M. pulchra	0	0	1	?	0	0	4	0	1	1	1	1	0	1	0
M. viridis	0	0	0	2	1	0	3	0	1	0	0	0	0	1	0
Mantidactylus bicalcaratus	1	0	1	5	1	1	4	0	1	0	0	0	0	0	1
M. grandisonae	0	0	0	0	1	1	4	0	1	0	0	0	0	1	0
M. cf. *punctatus*	1	0	0	0	1	1	4	0	1	0	0	0	0	0	1

on the other hand, was mainly influenced by the centromer indices; the five highest loadings were those of the ci values of the chromosomes 9, 4, 13, 8, and 5. In the corresponding scatterplot, the three species of *Mantidactylus* appeared widely separated from all *Mantella* species (Fig. 2). Among *Mantella*, the analysis clustered the species of the *M. betsileo* group away from the remaining species (along factor 1), while the other *Mantella* species did not show a clustering pattern consistent with their attribution to species groups.

Heterochromatin staining resulted in a wide array of band distribution and staining patterns. Beside the centromeric, telomeric and peritelomeric heterochromatin bands summarized in Table 3, paracentromeric bands were found in *M. expectata* on chromosomes 1, 3 and 5, and in *M. laevigata* on chromosomes 1 and 3. These consisted of DAPI-positive bands bordered by CMA_3-positive bands.

In order to use the karyological data to assess phylogenetic relationships, we defined 15 characters based on the results presented above: Maximum parsimony analysis of the data summarized in Table 4 (using the three *Mantidactylus* species as outgroups; all characters unordered) failed to resolve relationships among *Mantella* species. A strict consensus of the most parsimonious cladograms resulted in a basal polytomy in which *Mantidactylus grandisonae* clustered together with the ingroup species. In the Neighbor-joining analysis (Fig. 6), bootstrap support >50% was found for the following groupings: a clade containing *Mantella laevigata* and *M. expectata*, the sister group relationship of this clade to *M. viridis*, and a clade containing *M. pulchra* and *M. madagascariensis*.

DISCUSSION

The results largely support previous data on *Mantella* karyology (Blommers-Schlösser, 1978; Pintak *et al.*, 1998). Generally, chromosome morphology in *Mantella* is rather uniform (all species have 2n = 26, with five larger and eight smaller chromosome pairs). However, several of the interspecific differences observed may bear taxonomic relevance.

The presence of an acrocentric chromosome pair in *Mantella nigricans*, unique in the genus, supports the hypothesis (Vences *et al.,* 1999) that this taxon stands on its own at the species level. The differentiation found between *Mantella betsileo* and *M.* cf. *betsileo* regarding the morphology of the sixth chromosome (metacentric vs. submetacentric) and the important differences in heterochromatin distribution support the hypothesis that these two forms may be not conspecific. The affinities of the species of the *M. betsileo* group to each other and to *M. laevigata* is supported by their general chromosome morphology (Fig. 2).

Heterochromatin distribution is quite different among *Mantella* species. Actually, at least faint differences were observed between each species included in our study. Hence, the results have to be interpreted with some caution, as we studied only a limited number of specimens of each species, and too few data are available on differentiation between conspecific populations of *Mantella*. However, as we studied both males and females of most species (see appendix), we can exclude at least that the differences described here are erroneous interpretations of sexual dimorphism.

According to our analyses of *Mantella*, the same heterochromatic band can be DAPI-positive (thus rich in A+T) or

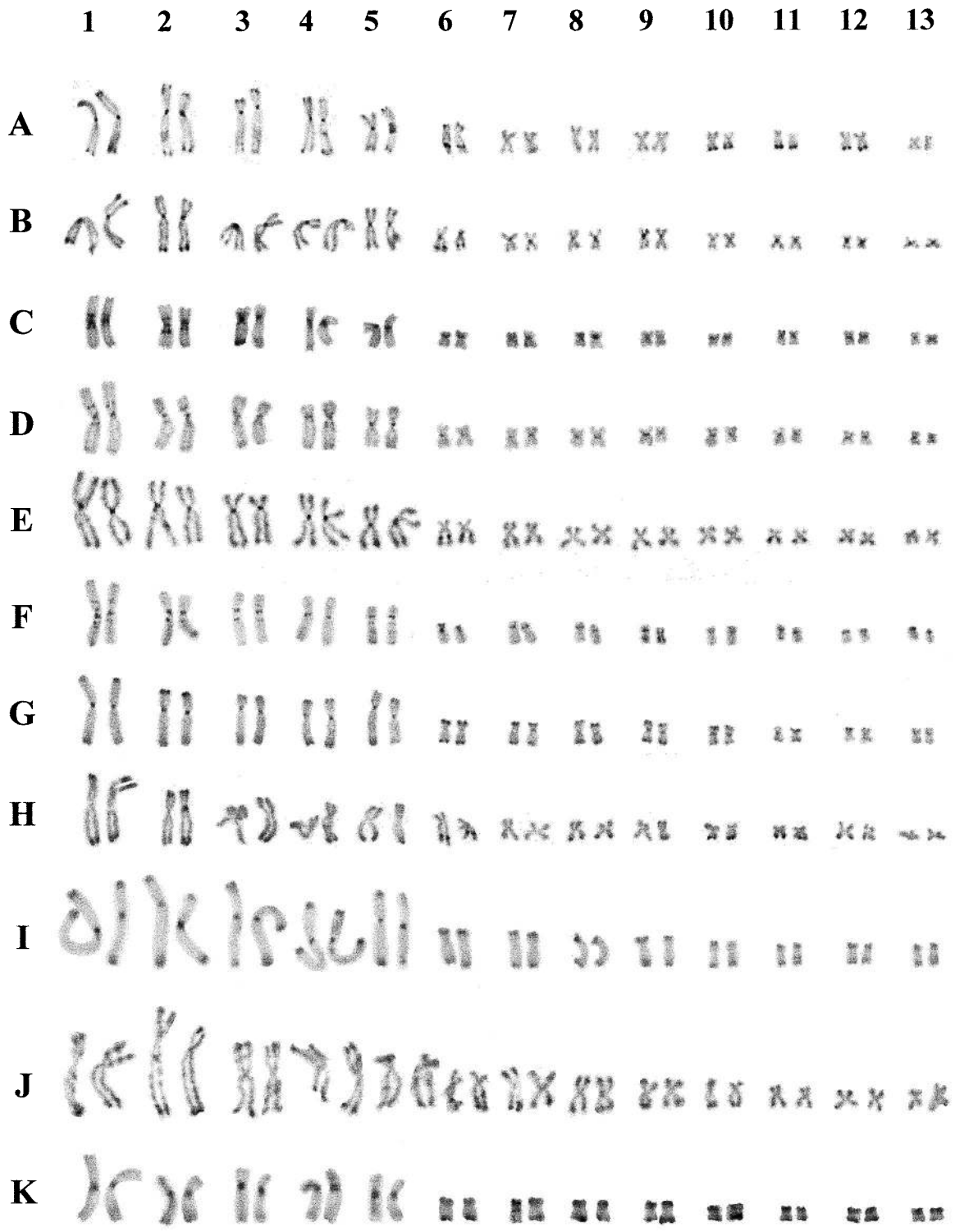

Fig. 3. C-banded karyotype of the 11 *Mantella* taxa studied: *M. aurantiaca* (**A**), *M. baroni* (**B**), *M. betsileo* (**C**), *M.* cf. *betsileo* (**D**), *M. cowani* (**E**), *M. expectata* (**F**), *M. laevigata* (**G**), *M. madagascariensis* (**H**), *M. nigricans* (**I**), *M. pulchra* (**J**), *M. viridis* (**K**) .

CMA_3-positive (thus rich in G+C). So far, in different plant and animal species, the positive staining of a heterochromatin band has been observed to be limited to either DAPI or CMA_3 (John, 1988; Schmid and Guttenbach, 1988). Molecular studies of the organization of sex-linked satellite DNA in chicken W chromosomes has shown that specific satellite families are included in different chromomeres on the W lampbrush chromosome (Solovei *et al.*, 1998). The arrangement in different chromomeres is a known character in heterochromatic bands (Okada and Comings, 1974). We therefore hypothesize the

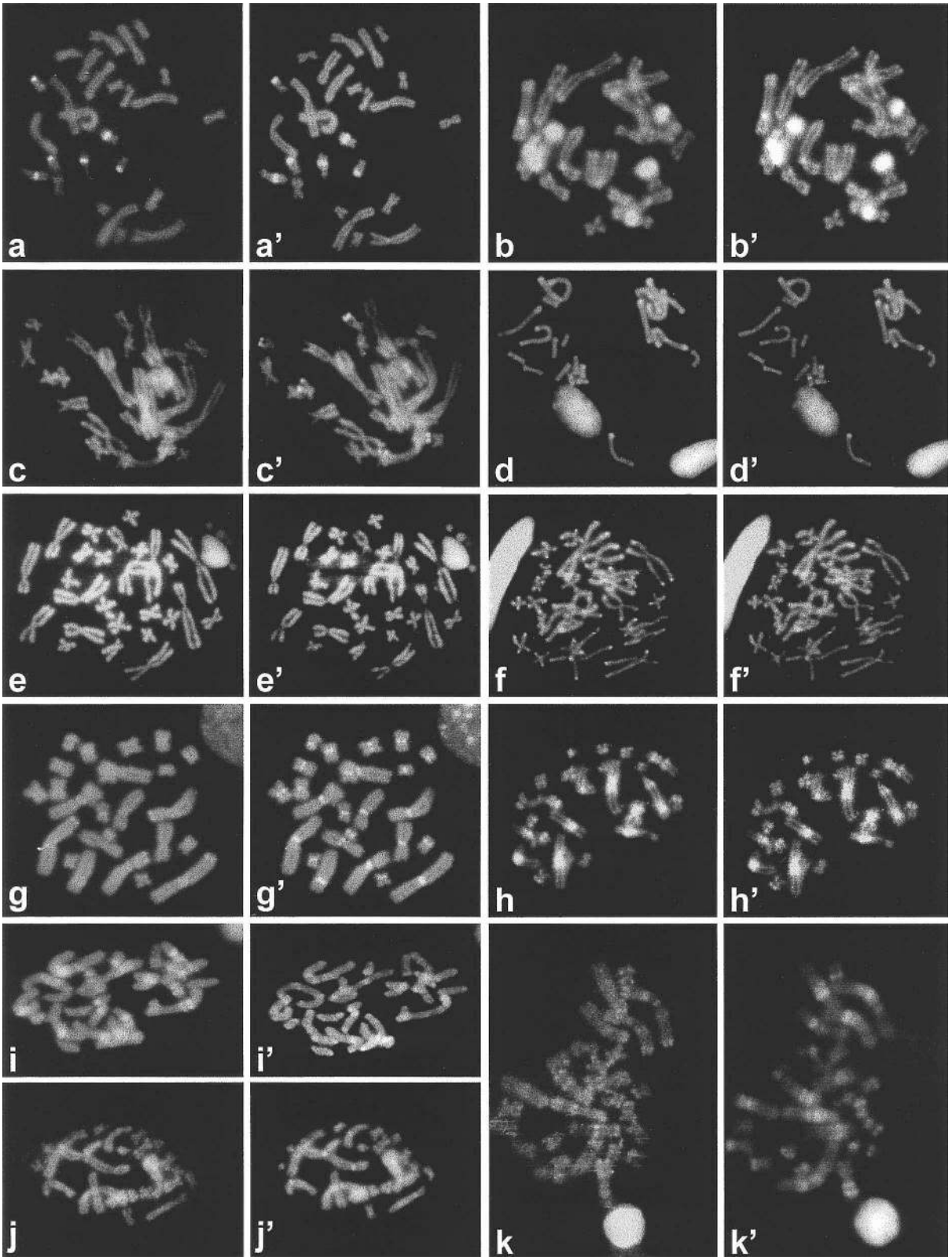

Fig. 4. C-banded matephase plates of *M. pulchra* (**a** and **a'**), *M. aurantiaca* (**b** and **b'**), *M. madagascariensis* (**c** and **c'**), *M. nigricans* (**d** and **d'**), *M. baroni* (**e** and **e'**), *M. cowani* (**f** and **f'**), *M. betsileo* (**g** and **g'**); *M.* cf. *betsileo* (**h** and **h'**), *M. expectata* (**i** and **i'**), *M. viridis* (**j** and **j'**) and *M. laevigata* (**k** and **k'**) successively stained with Chromomycin A_3 (simple cases) and DAPI (marked cases).

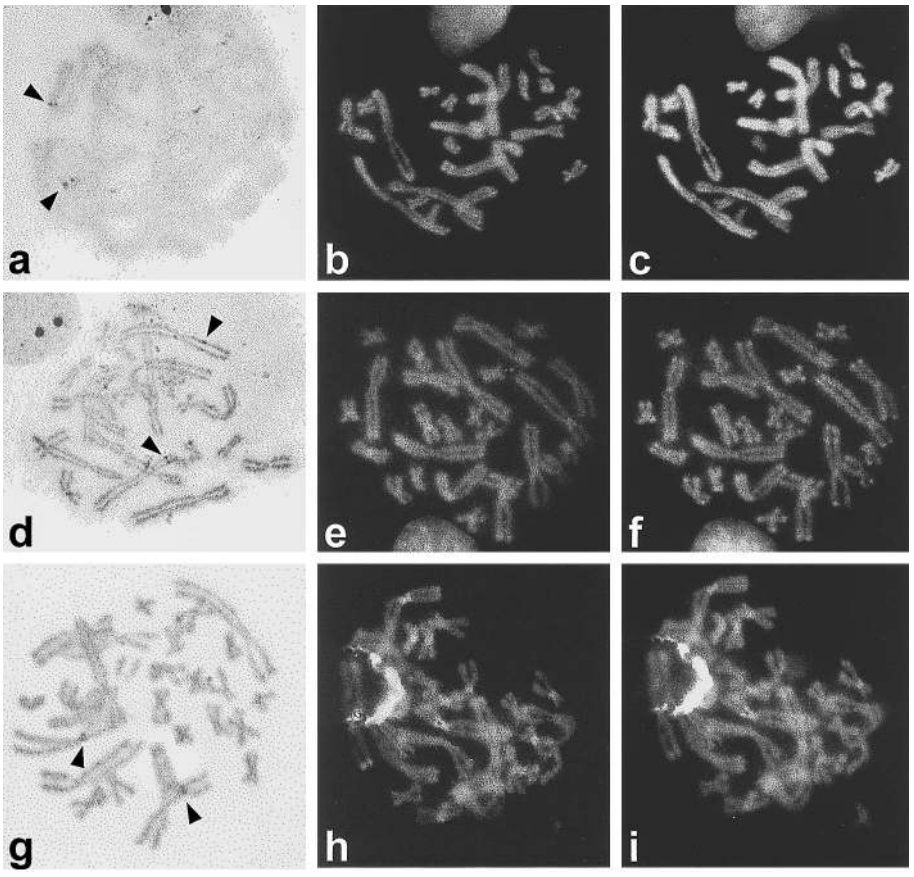

Fig. 5. $AgNO_3$ stained metaphase plates (left row), and the same metaphase plates successively stained with Chromomycin A_3 (medium row) and DAPI (right row) of *Mantidactylus bicalcaratus* (**a**, **b**, and **c**), *M. punctatus* (**d**, **e**, and **f**) and *M. grandisonae* (**g**, **h**, and **i**). The arrows point to NORs.

presence of different families of highly repeated DNA sequences (one rich in A+T and another one in G+C) in *Mantella*, which are each arranged in distinct chromomere units. In different species, selective amplification of these units may lead (1) to bands containing either mainly A+T rich chromomere units or G+C rich chromomere units (and thus staining either DAPI+ or CMA+, as in *M. betsileo*, *M. madagascariensis*, and *M. viridis*); (2) to bands containing both types of units in comparable proportion (and thus staining DAPI+ and CMA+ as in *M. aurantiaca*, *M.* cf. *betsileo*, and *M. pulchra*). A similar situation was observed in other Malagasy anuran genera belonging to the superfamily Ranoidea (*Mantidactylus*, *Boophis, Heterixalus*), and may therefore be widespread among ranoid anurans (Odierna, pers. obs.).

Taxonomic and limited phylogenetic relevance within *Mantella* may be attributed to the existence of specific locations in which the accumulation of heterochromatic material is possible or excluded. Indeed, within *Mantella*, two main groups of species can be distinguished regarding the heterochromatin distribution. In one, heterochromatin was mainly found in the telomeric or peritelomeric regions of the chromosomes 6-13 and was largely absent or scarce in the centromeric regions of these elements (*M. aurantiaca*, *M. baroni*, *M. cowani*, *M. madagascariensis*, *M. nigricans*, *M. pulchra*, and *M. viridis*). In the other, the centromeric regions of the chromosomes 6-13 were richer in heterochromatin than their telomeric and peritelomeric regions (*M. betsileo*, *M.* cf. *betsileo*, *M. expectata*, and *M. laevigata*).

Several further groupings are possible based on the heterochromatin distribution patterns. *Mantella aurantiaca*, *M. madagascariensis*, and *M. pulchra* share the presence of distinct peritelomeric bands on the 6th, 10th, and 11th chromosome. *Mantella aurantiaca* additionally has a peritelomeric band on the 9th chromosome. This largely corresponds to the data of Pintak *et al.* (1998) who have found peritelomeric bands on the 6th, 11th, and 12th chromosome in *M. aurantiaca* (correct ordering of the small chromosomes is often difficult and may vary according to the method used). Pintak *et al.* (1998) have further observed peritelomeric bands on the 6th and 11th chromosome of *M. crocea*, indicating that it is also closely related to *M. aurantiaca*, *M. madagascariensis*, and *M. pulchra*. This is also corroborated by allozyme data which strongly support a monophyletic group containing these four species (Vences *et al.*, 1998c). A peritelomeric C-band on the 6th chromosome also occurred in *M. baroni*, as indicated by the present data and data in Pintak *et al.* (1998). In contrast, allozyme data and osteology clearly indicate that *M. baroni* is part of a monophyletic group containing also *M. cowani* and *M. nigricans* (Vences *et al.*, 1998b,c). Thus, the band on the 6th chromosome may be not homologous in *M. baroni* and in the *M.*

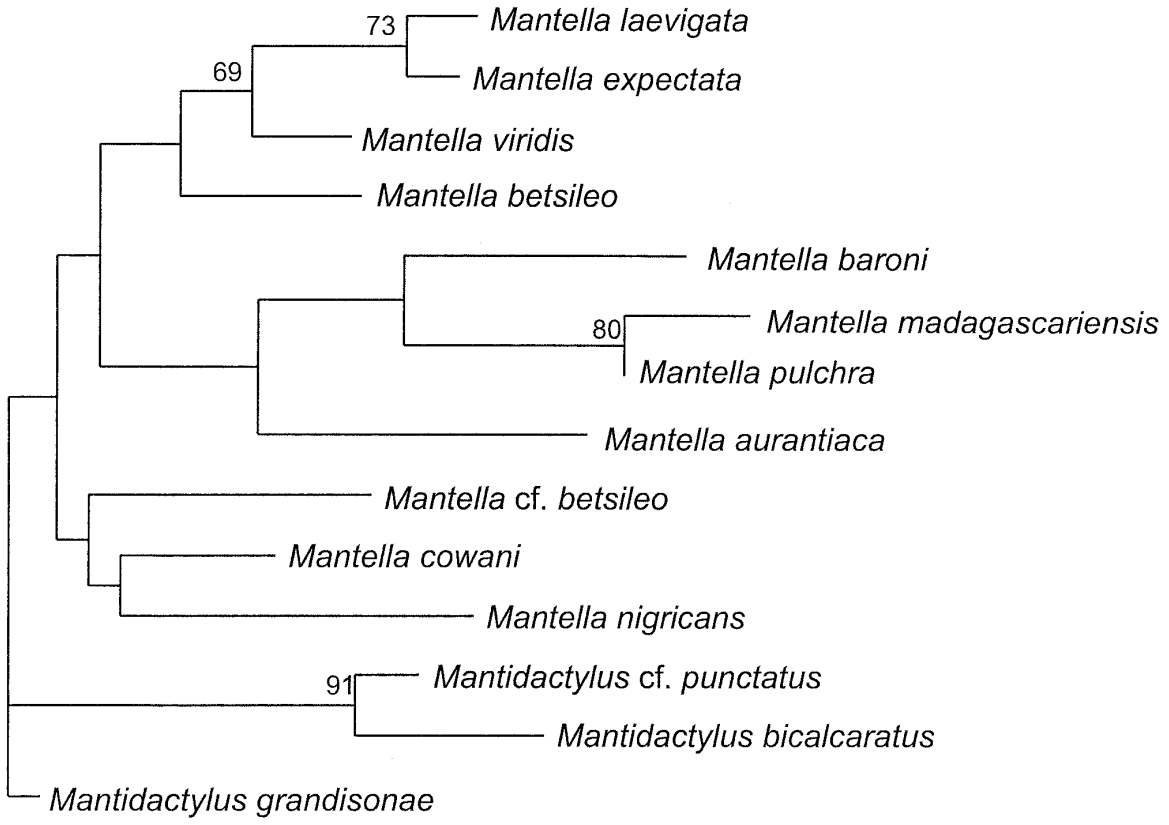

Fig. 6. Neighbor-joining tree based on analysis of data in Table 4. Numbers are bootstrap values in percent (2000 replicates).

madagascariensis and *M. aurantiaca* groups (as also supported by the different reaction to CMA_3 and DAPI stainings of the band in the three groups). Alternatively, the band may have been lost in other members of the *M. baroni* group.

Another species pair with similar heterochromatin distribution comprised *M. laevigata* and *M. expectata*. These are the only species which showed adjacent separate bands in pericentromeric areas which were either CMA+ or DAPI+. *Mantella expectata* belongs to the *M. betsileo* group which also contains *M. viridis*, whereas *M. laevigata* has an isolated position within the genus (Vences *et al.*, 1998c, 1999). Both *M. laevigata* and the *M. betsileo* group are thought to be basal groups within *Mantella* (Vences *et al.*, 1998b,c), but a sister-group relationship of *M. laevigata* and *M. expectata* is contradicted by allozyme data (Vences *et al.*, 1998c).

The location of the NOR has been demonstrated to be of phylogenetic and taxonomic validity in many animal groups including amphibians, reptiles, and fish (Amemiya and Gold, 1990; King, 1990; Olmo *et al.*, 1993). In the genus *Mantidactylus*, Aprea *et al.* (1998) have found variability of the NOR location among different species groups and subgenera but, on the other hand, a constant state within these groups. The state occurring in *Mantella* is similar to *Mantidactylus grandisonae* (subgenus *Blommersia*) following the data presented herein. However, it differs from the states in the subgenera *Brygoomantis* (*Mantidactylus alutus*, Aprea *et al.*, 1998) *Gephyromantis* (*Mantidactylus luteus*, Aprea *et al.*, 1998; *M. silvanus*, Odierna, unpubl.), *Pandanusicola* (*Mantidactylus bicalcaratus*, *M.* cf. *punctatus*, data herein), and *Phylacomantis* (*Mantidactylus redimitus*, Aprea *et al.*, 1998). Vences *et al.* (1998b) have hypothesized that the subgenera *Guibemantis*, *Blommersia*, and *Pandanusicola* may be the closest extant relatives to *Mantella*. The chromosomal data indicate that *Blommersia* probably is a better candidate for the sister group of *Mantella* than *Pandanusicola*. No NOR data are available so far for *Guibemantis*.

The analysis of intrageneric *Mantella* relationships based on karyological characters did not provide adequate phylogenetic resolution (Fig. 6). The karyological characters studied may have evolved either too slow (NOR) or too fast (heterochromatin distribution) to match the main cladogenetic events within *Mantella*. The NOR data appear to be more informative for rather old splits (e.g., the separation of the *Blommersia-Mantella* clade from other *Mantidactylus*) whereas the heterochromatin is so quickly re-distributed that only presumably very young groups (such as the clade containing *Mantella aurantiaca*, *M. madagascariensis*, and *M. pulchra* which are genetically extremely similar according to Vences *et al.*, 1998c) conserve some common, slightly informative patterns.

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APPENDIX: MATERIAL EXAMINED

Material without locality data was obtained through the pet trade; some specimens examined were destroyed for the analysis and thus not preserved, accounting for minor discrepancies between number of examined and catalogued specimens.

Mantella aurantiaca, one male and four females, ZFMK 72001–72004, 72143; *M. baroni*, two males and two juveniles, ZFMK 72008-72009, 72146; *M. betsileo*, two males and three females, ZFMK 72017–72020, 72002; one male and one female, ZFMK 72017 and 72020, captive-bred from a stock from Nosy Be, NW-Madagascar; *M.* cf. *betsileo*, one female, ZFMK 72024, from near Morondava, W Madagascar; *M. cowani*, two males, ZFMK 72014, 72149; *M. expectata*, one male and two juveniles, ZFMK 72147, 72021, 72023; *M. laevigata*, three males and one juvenile, ZFMK 72010–72013; *M. madagascariensis*, one male, one female, and two juveniles, ZFMK 72005–72007, 72148; *M. nigricans*, one male, ZFMK 72015; *M. pulchra*, one male and one female, ZFMK 72142; *M. viridis*, one male and one female, ZFMK 72016, 72145; *Mantidactylus bicalcaratus*, one male and two females, MRSN A1977.1, from Ambolokopatrika Rainforest (between Anjanaharibe-Sud and Marojejy), Andranomadio (campsite 2), 14°32 S, 49°26' E, 860 m; *M. grandisonae*, one male, MRSN A1975.1 (FN 7806), from Foret de Beanjada, Masoala National Park, 15°17' S, 49°60' E, 620 m; *M.* cf. *punctatus*, two females, MRSN A1976.1, Ambolokopatrika Rainforest (between Anjanaharibe-Sud and Marojejy), Andranomadio (campsite 2), 14°32' S, 49°26' E, 860 m.