Folia Zool. - 47(4): 261–274 (1998)

Allozyme differentiation in the genus *Mantella* (Amphibia: Anura: Mantellinae)

Miguel VENCES[1], Axel HILLE[1] and Frank GLAW[2]

[1] *Zoologisches Forschungsinstitut und Museum Alexander Koenig, Adenauerallee 160, D-53113 Bonn, Germany; e-mail: m.vences@link-lev.de*
[2] *Zoologische Staatssammlung, Münchhausenstraße 21, D-81247 München, Germany; e-mail: kld1127@mail.lrz-muenchen.de*

Received 1 December 1997; Accepted 12 November 1998

A b s t r a c t . Allozyme electrophoresis was carried out on a total of 120 specimens belonging to 12 species of Malagasy poison frogs of the genus *Mantella*. Allele frequencies computed for 17 scored loci were used to analyze intrageneric relationships. Genetic distances between *Mantella aurantiaca*, *M. crocea* and *M.* sp. 3 were very low, and their specific status is thus questionable. Relatively low genetic distances were also found between (1) *M. betsileo* and *M. viridis*, (2) *M. baroni* and *M. cowani*, and (3) *M. pulchra* and *M. madagascariensis*, respectively. *M. bernhardi* and *M. laevigata* showed relatively high genetic distances to all other taxa. A phylogenetic analysis of our data largely confirmed the phenetic clustering; two main lineages could be distinguished, one consisting of *M. bernhardi*, *M. expectata*, *M. viridis* and *M. betsileo*, and a second one containing the remaining taxa studied. Positioning of *M. bernhardi*, *M. laevigata* and *M. pulchra*, however, is problematic as revealed by jacknife analysis. Analyses by frequency parsimony revealed that a more parsimonious tree topology is achieved by both changing the position of *M. bernhardi* in the tree, and by placing *M. laevigata* as a sister species to all other taxa. The main phylogenetic conclusions drawn from this study are largely in accordance with other results from independent data sets. The allozyme data show that some very differently coloured taxa are virtually identical genetically, whereas other taxa with nearly identical colour patterns are genetically well differentiated. The genus may therefore be a good model for studies of aposematism and colour pattern evolution in amphibians. As a general trend, *Mantella* species endemic to the rainforests of central eastern Madagascar belong to few complexes of closely related forms, whereas most of the genetically well differentiated species are found in the northern part of the island. The differentiation of the main lineages in the genus may thus have largely occurred in northern Madagascar.

Key words: Malagasy poison frogs, enzyme electrophoresis, phylogeny, taxonomy, biogeography, Madagascar

Introduction

Madagascar, the fourth largest island in the world, harbours a large diversity of anuran amphibians with a high level of endemism at species (>99%), genus (83%) and subfamily (43%) levels (data from G l a w & V e n c e s 1994). The origin and affinities of most Malagasy amphibian groups are insufficiently known; the largest number of species is currently included in the Ranidae, a family of controversial and unresolved systematics (D u e l l m a n & T r u e b 1986, D u b o i s 1992, B l o m m e r s - S c h l ö s s e r 1993). The most prominent group of Malagasy anurans is the genus *Mantella* which contains about 13 species according to G l a w & V e n c e s (1994) and 15-17 species according to V e n c e s et al. (submitted). *Mantella* are small and colourful frogs of scarce interspecific morphological differentiation. Their classification, in the past, was mostly based on colour patterns of preserved specimens, and thus largely dependent on subjective perceptions of

levels of intraspecific colour variability by the respective workers (e. g. Guibé 1964, 1978, Busse 1981, Blommers-Schlösser & Blanc 1991). Since many species of *Mantella* are very attractive to the pet trade business, locally restricted and rare taxa may be vulnerable to excessive collecting. A reliable assessment of distribution and abundance of species, needed for a proper definition of conservation priorities, is only possible on the basis of a stable taxonomy and clear species diagnoses.

Allozyme studies in various amphibian groups have proved to be useful both to recognize cryptic species and to resolve species complexes (e. g. Capula et al. 1985, Lanza et al. 1986, Highton 1989) and phylogenetic relationships between species (e. g. Hillis et al. 1983, Hillis 1988, Duellman & Hillis 1987). In the present paper we study allozyme differentiation of 12 *Mantella* taxa. Our aims are mainly (1) to describe genetic differentiation within the genus and (2) to use the allozyme data to draw a hypothesis of intrageneric phylogeny.

Material and Methods

Most specimens were obtained alive through the pet trade, and localities are therefore not exactly and reliably known. Taxonomy follows Glaw & Vences (1994) except for (1) *M.* sp. 3, which corresponds to a *M. aurantiaca*-like morph pictured in Glaw & Vences (1994) on colour plate 52, (2) *M. baroni* which is used for the form named *M. madagascariensis* in Glaw & Vences (1994) (see also Daly et al. 1996), and (3) *M. madagascariensis* which is used for the form named *M. „loppei"* by Glaw & Vences (1994). Justifications of these changes are given in Vences et al. (submitted). Specimens were sacrificed, and muscle and liver tissues extracted and frozen at -80°C for electrophoresis. Specimens were preserved in 70% ethanol and deposited in the collection of the Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn (ZFMK), corresponding to the numbers ZFMK 62685-62796.

Table 1. Enzymes analyzed, electrophoretic conditions and tissue types used. Buffers are abbreviated as follows: **TC II pH 8** Tris-Citrate: 12% starch gel, 16 h, 100V, ca. 25 mA, ca. 4V/cm; **TVB pH 8,0** (Tris-Borate-EDTA): 12% starch gel, 18 h, 80 V; **AC pH 7,6** Amine-Citrate: 12% starch gel, 16 h, 100 V; **PHOS pH 6,7** Phosphate-Citrate: 12% starch gel, 16 h, 100 V. See Hille & Meinig (1996) for sources of stain and buffer protocols. * substrate was leucile-alanine.

Enzyme	E. C. Code	Locus	Direction	Tissue	Buffer
a-Glycerophosphate dehydrogenase	1.1.1.8	Gpdh	anodal	muscle	TC II pH 8
Lactate dehydrogenase	1.1.1.27	Ldh-1, Ldh-2	anodal	muscle	AC pH 7,6
Malate dehydrogenase	1.1.1.37	Mdh-1	anodal/cathodal	muscle	TC II pH 8, PHOS pH 6,7
NADP-Malate dehydrogenase (Malic enzyme)	1.1.1.40	Me	anodal	muscle	PHOS pH 6,7
Isocitrate dehydrogenase	1.1.1.42	Idh-1, Idh-2	anodal	muscle	TCII pH 8
Adenylate kinase	2.7.4.3	Ak	anodal	muscle	TC II pH 8
Phosphoglucomutase	2.7.5.1	Pgm-1	anodal	muscle	TC II pH 8
Esterase	3.1.1.-	Est-3	anodal	liver	TVB pH 8
Peptidase	3.4.11.-	Pep-LA*	anodal	muscle	TC II pH 8
Guanine deaminase	3.5.4.3	Gda	anodal	liver	TVB pH 8
Adenosine deaminase	3.5.4.4	Ada	anodal	muscle	TC II pH 8
Aconitase	4.2.1.3	Acon	anodal	liver	TC II pH 8
Mannose phosphate isomerase	5.3.1.8	Mpi	anodal	muscle	AC pH 7,6
Glucose phosphate isomerase	5.3.1.9	Gpi	anodal/cathodal	muscle	PHOS pH 6,7
Serum protein	-	Prot-3	anodal	liver/blood	TVB pH 8

Several taxa showed colour variability within the studied samples: *M. aurantiaca* (red and orange morphs), *M.* sp. 3 (red and orange morphs), *M. crocea* (yellow and green morphs), and *M. cowani* (red-black and yellow-black morphs). No relevant allozyme differentiation was found between the morphs attributed to one taxon, respectively (data not shown), and they were therefore included together in further analysis.

Allozyme electrophoresis followed standard techniques; a detailed description of the method used (vertical starch gel electrophoresis) and sources for buffer and stain protocols are provided by Hille & Meinig (1996). Electromorphs of 17 scorable loci were assigned to alleles and coded in alphabetical order according to increasing anodal mobility. Enzymes analyzed as well as buffer systems used are given in Table 1.

Genotypes per locus of each specimen are listed in the M.-Sc. thesis of the senior author (Vences 1996 [unpublished]: Untersuchungen zur Phylogenie der madagassischen Anurengattung *Mantella*, unter Integration biochemischer und morphologischer Merkmale) which is deposited in the ZFMK.

Data were processed with BIOSYS-1 software (Swofford & Selander 1981) to calculate allele frequencies, polymorphism and heterozygosity. Standard genetic distances (D) between populations (Nei 1972) were estimated employing DISPAN (Ota 1993).

We used phenetic clustering to get an overview of genetic differentiation. Phenograms were constructed from the D matrix by using the unweighted pair group-method with arithmetic mean (UPGMA) (Sneath & Sokal 1973), and the Neighbor-Joining (NJ) method (Saitou & Nei 1987). Bootstrap tests (Efron 1982, Felsenstein 1985) for these trees were performed by 1000 replications with loci as units.

A phylogenetic analysis of the data was carried out with HENNIG86 software (Farris 1988), using one species of *Mantidactylus* (*M. biporus*) as outgroup (*Mantidactylus* and *Mantella* together form the subfamily Mantellinae according to Glaw & Vences 1994). Each allele was coded as one character, polarized by its presence or absence in the outgroup (as regularly done in analysis of electrophoretic results, e. g. Veith 1996a; but see Meier 1994 and Lipscomb 1994 for critizisms and restrictions of this method). HENNIG86 commands used were mh* in combination with bb*, and implicit enumeration (ie) for exhaustive tree finding.

As complement of the cladistic analysis, the statistical method of jacknife analysis (Lanyon 1985; see Siddall 1995) was applied for estimation of "tree confidence". This data resampling technique was selected as a means to discover the effect that taxon removal may have on the stability of the results obtained from parsimony analysis. We used two options of the programme LANYON.EXE in RANDOM CLADISTICS (M. Siddall 1995: Random Cladistics 3.0 - random.exe and random.doc). Option 1 results in a table summarizing the effects of taxa removal. Critical taxa are those that induce the discovery of many more equally parsimonious trees or depresse retention indices when they are deleted. Problematic taxa (taxa difficult to place in the topology) increase the retention index when deleted and shorten considerably the tree lengths. Executing option 3 invokes the calculation of an index of clade stability, the Jacknife Monophyly Index (JMI) which assigns a value to each clade according to its frequency of occurrence in jacknife pseudoreplicates.

To better deal with allele polymorphism, we also constructed several trees derived from gene frequencies by frequency parsimony as implemented in the FREQPARS routine (Swofford & Berlocher 1987). Frequency arrays for the allozyme data were taken from BIOSYS-1 output. Frequency parsimony searches for trees that minimize the total

amount of allelic change as measured by the Manhattan distance. The method accommodates polymorphism without the need for coding strategies that may be implicitly ambiguous, it remarkably reduces the impact of sampling errors (e.g., the failure to down-weight rare alleles in taxa) and optimally uses all frequency information that is otherwise lost in presence/absence coding schemes (Berlocher & Swofford 1997).

Results

In Table 2, allele frequencies of the studied species are summarized. Table 3 informs about estimates of mean heterozygosity and allele polymorphism. Alleles fixed for certain species occurred at relatively few loci, and no diagnostic alleles were found. In all species, mean observed heterozygosity was lower than expected at most loci (Table 3).

The UPGMA-phenogram (Fig. 1) based on Nei's (1972) standard genetic distance (D; see Table 4), as calculated from allele frequencies in Table 2, shows three clusters of species separated by a distance equal to or less than 0.22: (1) *Mantella betsileo* and *M. viridis*; (2) *M. cowani* and *M. baroni*; (3) *M. madagascariensis, M. pulchra, M. aurantiaca, M. crocea* and *M.* sp. 3. The latter three cluster very close to each other (D<0.05). On the other hand, three species remain rather distant to the others (D >0.3): *M. bernhardi, M. laevigata* and *M. expectata*. Bootstrap values (≥90) highly support the groupings 1-3 as described above; the lowest bootstrap values characterize the positions of *M. laevigata* and *M. bernhardi* in the phenogram. The Neighbor-Joining tree (Fig. 2) is largely in accordance with the UPGMA phenogram results, except for (1) the joint clustering of *M. viridis* and *M. expectata*, and (2) the different position of *M. laevigata*.

Results of the cladistic analysis are shown in Fig. 3. Two main clades are distinguished: *M. betsileo, M. viridis, M. expectata* and *M. bernhardi* form one lineage, whereas the remaining taxa form a second lineage. *M. baroni* and *M. cowani* appear as a monophyletic unit, as does the group containing *M. pulchra, M. madagascariensis, M. aurantiaca, M. crocea* and *M.* sp. 3. The latter three forms are grouped as a paraphyletic assemblage.

Table 5 shows the effects of jacknifing taxa, i. e. systematically leaving taxa out of the data set in the cladistic analysis (option 1 in LANYON.EXE). Of the 12 ingroup taxa, *M. madagascariensis* is critical since its exclusion induces the discovery of two equally parsimonious trees. Similarly, *M. pulchra, M. laevigata,* and *M. bernhardi* are most problematic; with the exclusion of either, the retention index rises because of its sensitivity to topological character distribution (Farris 1989). Almost all monophyletic subclades in the tree retained high jacknife support (JMI=100%; option 3 in LANYON.EXE), indicating the generally high stability of clades within and among the most parsimonious trees found. Only the clade comprising *M. crocea, M. pulchra* and *M. madagascariensis* is supported by a lower JMI (95%).

Summarizing these results, the position of two taxa (*M. laevigata* and *M. bernhardi*) is not sufficiently corroborated by bootstrap values of UPGMA- and Neighbor-Joining trees. Additionally, they were identified as problematic taxa in the cladistic analysis. We therefore used FREQPARS to test whether alternative joining of these taxa may result in a more parsimonious topology. However, as noted by Swofford & Berlocher (1987), the implementation of FREQPARS has only limited ability to search for the shortest tree. We therefore used the USERTREE option to specify favourable alternatives. The shortest alternative (total branch length 92.33) found out of six trials is shown in Fig. 4. *M. laevigata* is placed as sister group of all other taxa, whereas *M. bernhardi* appears as a sister group of the *aurantiaca-crocea*-sp.3-*pulchra-madagascariensis* cluster.

Table 2. Allele frequencies and sample sizes in the *Mantella* species studied. Abbreviations: bet = *M. betsileo*, vir = *M. viridis*, exp = *M. expectata*, pul = *M. pulchra*, mad = *M. madagascariensis*, cow = *M. cowani*, bar = *M. baroni*, lae = *M. laevigata*, ber = *M. bernhardi*, cro = *M. crocea*, aur = *M. aurantiaca*, sp3 = *M.* sp. 3, OUT = outgroup (*Mantidactylus biporus*). For abbreviation of loci see Table 1. (N) = number of specimens scored for a given locus and species.

	bet	vir	exp	pul	mad	cow	bar	lae	ber	cro	aur	sp3	OUT
Gpdh													
(N)	*11*	*5*	*6*	*13*	*7*	*4*	*4*	*1*	*8*	*9*	*4*	*5*	*3*
A	0.09	0	0	0.04	0	0	0	0	0.56	0.11	0	0	0
B	0.91	1	1	0.92	0.57	1	1	1	0.38	0.83	1	1	1
C	0	0	0	0.04	0.43	0	0	0	0.06	0.06	0	0	0
Ldh1													
(N)	*14*	*5*	*6*	*13*	*10*	*5*	*6*	*3*	*7*	*7*	*3*	*3*	*2*
A	0.57	0.60	0.67	0.92	0.90	1	1	1	1	1	1	1	1
B	0.43	0.40	0.33	0.08	0.10	0	0	0	0	0	0	0	0
Ldh2													
(N)	*16*	*6*	*7*	*16*	*10*	*7*	*6*	*3*	*10*	*9*	*9*	*5*	*2*
A	0	0	0	0.03	0	0	0	0	0	0	0	0	0
B	0	0.08	0.21	0	0	0	0	0	0.75	0	0.11	0	0
C	0	0	0	0.13	0.05	0.07	0	0	0	0	0	0.10	0
D	0.72	0.33	0.79	0.84	0.95	0.93	1	1	0.25	0.89	0.89	0.90	0
E	0.28	0.58	0	0	0	0	0	0	0	0.11	0	0	1
Mdh1													
(N)	*13*	*3*	*4*	*10*	*8*	*5*	*5*	*1*	*3*	*6*	*4*	*3*	*2*
A	0	0	0	0	0	0	0	0	0	0.17	0.25	0.67	0
B	0.92	1	0	1	1	1	1	1	1	0.83	0.75	0.33	1
C	0.08	0	1	0	0	0	0	0	0	0	0	0	0
Me													
(N)	*12*	*3*	*5*	*11*	*8*	*4*	*5*	*1*	*3*	*6*	*3*	*3*	*2*
A	0	0	0	0	0	0	0	0	0	0	0	0	1
B	0	0	0	0.46	0.19	0	0	0	1	0.33	0	0.17	0
C	1	1	1	0.55	0.81	1	1	0	0	0.67	1	0.83	0
D	0	0	0	0	0	0	0	1	0	0	0	0	0
Idh1													
(N)	*17*	*6*	*7*	*16*	*10*	*7*	*5*	*3*	*12*	*10*	*9*	*5*	*2*
A	1	1	1	0.03	0	0	0	0.83	1	0	0	0.10	1
B	0	0	0	0.88	1	0.29	0.20	0.17	0	0.95	1	0.80	0
C	0	0	0	0	0	0.71	0.80	0	0	0.05	0	0	0
D	0	0	0	0.06	0	0	0	0	0	0	0	0.10	0
E	0	0	0	0.03	0	0	0	0	0	0	0	0	0
Idh2													
(N)	*10*	*6*	*7*	*16*	*10*	*7*	*6*	*2*	*9*	*8*	*7*	*5*	*0*
A	0.40	0	0	0.34	0.45	1	1	1	0	1	1	1	-
B	0	0	0.07	0	0	0	0	0	1	0	0	0	-
C	0.60	1	0.86	0.63	0.55	0	0	0	0	0	0	0	-
D	0	0	0.07	0.03	0	0	0	0	0	0	0	0	-

Table 2. Continued

Ak													
(N)	*13*	*3*	*5*	*11*	*8*	*6*	*5*	*1*	*5*	*7*	*6*	*3*	*2*
A	1	0.67	0.10	0	0	0.08	0	0	0.10	0.07	0	0	0
B	0	0.33	0.90	1	1	0	0	1	0.90	0.93	1	1	1
C	0	0	0	0	0	0.92	1	0	0	0	0	0	0
Pgm1													
(N)	*14*	*6*	*7*	*16*	*8*	*6*	*6*	*2*	*9*	*8*	*8*	*5*	*3*
A	0	0	0	0	0	0	0	0	0	0	0	0	0.5
B	0	0	0	0	0.06	0	0	0	0	0.25	0.19	0.20	0.5
C	0.93	1	1	0.94	0.94	0.92	0.17	1	1	0.75	0.81	0.80	0
D	0.07	0	0	0.06	0	0.08	0.83	0	0	0	0	0	0
Est3													
(N)	*9*	*5*	*2*	*12*	*7*	*3*	*3*	*3*	*1*	*7*	*4*	*3*	*2*
A	0	0	0	0.13	0.29	0	0	0	0	0.21	0.50	0.67	0
B	0.11	0	0	0	0	0	0	0	0	0	0	0	0
C	0	0	0	0.58	0.36	0	0	0	0	0.79	0.50	0.33	1
D	0	0.80	1	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0.29	0.36	0	0	0	0	0	0	0	0
F	0.83	0.20	0	0	0	1	1	1	1	0	0	0	0
G	0.06	0	0	0	0	0	0	0	0	0	0	0	0
Pep-LA													
(N)	*15*	*5*	*4*	*14*	*10*	*7*	*6*	*3*	*5*	*7*	*3*	*3*	
A	0	0	0	0	0.15	0	0.33	0	0	0.29	0	0.17	0
B	0	0	0	1	0.85	0	0.08	0	0	0.71	1	0.83	0
C	0	0	0	0	0	1	0.58	1	1	0	0	0	0
D	0.10	0	1	0	0	0	0	0	0	0	0	0	0
E	0.80	1	0	0	0	0	0	0	0	0	0	0	0
F	0.10	0	0	0	0	0	0	0	0	0	0	0	1
Gda													
(N)	*8*	*5*	*3*	*11*	*8*	*6*	*3*	*2*	*4*	*8*	*4*	*4*	*2*
A	0	0	0	0	0	0.42	0.50	0	0	0	0	0	0
B	0	0	0	0.46	0.69	0.58	0.50	1	0	1	1	1	0
C	0	0	0	0.55	0.31	0	0	0	0	0	0	0	0
D	0.75	0.10	0.33	0	0	0	0	0	1	0	0	0	1
E	0.25	0.70	0.67	0	0	0	0	0	0	0	0	0	0
F	0	0.20	0	0	0	0	0	0	0	0	0	0	0
Ada													
(N)	*9*	*2*	*4*	*11*	*8*	*7*	*5*	*1*	*3*	*7*	*6*	*2*	*1*
A	0.11	0	0	0.05	0.31	0.21	0.70	0	0.50	0.43	0.42	0.50	0
B	0.89	0.50	0.25	0.32	0.69	0.50	0.30	1	0.50	0.57	0.58	0.50	1
C	0	0.50	0.75	0.64	0	0.29	0	0	0	0	0	0	0
Acon													
(N)	*5*	*5*	*4*	*7*	*6*	*3*	*5*	*2*	*1*	*6*	*4*	*3*	*1*
A	0	0	0	0	0	0	0	0	0	0.08	0	0	0
B	0	0	0	0	0.08	0	0	0	0	0.42	0	0.17	0
C	0.40	0.40	1	0.14	0.25	0	0.40	1	0	0	0	0	0
D	0.60	0.60	0	0	0	0	0	0	1	0	0	0.33	0
E	0	0	0	0.57	0.50	0.33	0.20	0	0	0.50	0.75	0.17	0
F	0	0	0	0.29	0.17	0.67	0.40	0	0	0	0.25	0.33	0
G	0	0	0	0	0	0	0	0	0	0	0	0	1

Table 2. Continued

Mpi													
(N)	*14*	*3*	*3*	*12*	*8*	*4*	*4*	*1*	*3*	*8*	*3*	*4*	*1*
A	0	0	0	0	0	0	0	0	0	0	0	0	1
B	0	0	0	0	0	0.13	0	0	0	0	0	0	0
C	0.36	0	1	0.04	0.06	0	0.25	0	0.50	0	0	0	0
D	0.39	0.17	0	0.08	0	0.88	0.75	0	0	0	0	0	0
E	0.14	0.67	0	0.88	0.75	0	0	0	0.50	1	1	1	0
F	0.11	0.17	0	0	0.06	0	0	0	0	0	0	0	0
G	0	0	0	0	0.13	0	0	1	0	0	0	0	0
Gpi													
(N)	*15*	*3*	*5*	*10*	*8*	*5*	*5*	*1*	*4*	*5*	*4*	*3*	*2*
A	0	0	0	0.45	0	0	0	0	0	0.08	0	0.17	0
B	0	0	0	0.50	0.69	0.20	0.60	0	0.75	0.33	0.50	0.50	1
C	0.20	0.50	0.10	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0.05	0.31	0	0	0	0.25	0.33	0.38	0.33	0
E	0.80	0.50	0.90	0	0	0.80	0.40	1	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0.25	0.13	0	0
Prot-3													
(N)	*4*	*2*	*1*	*9*	*7*	*4*	*4*	*2*	*1*	*8*	*5*	*3*	*0*
A	0	0	0	0	0.21	0	0	0	0	0	0	0	-
B	0	0	0	0	0.36	0	0.25	0	0	0	0.10	0.33	-
C	0	0	0	1	0.43	0	0	0	0	0.25	0	0	-
D	1	1	1	0	0	1	0.75	0	1	0.75	0.90	0.67	-
E	0	0	0	0	0	0	0	1	0	0	0	0	-

Table 3. Parameters of genetic variability in studied *Mantella* species. A locus was considered as polymorphic if the frequency of the most common allele was below 95%; ** Unbiased estimate (Nei 1978).

	mean sample size	mean allele number	% polymorphic loci	mean heterozygosity observed	mean heterozygosity Hdy-Wbrg-prediction **
1. *M. betsileo*	11.7±0.9	2 ±0.2	76.5	0.105±0.035	0.278±0.053
2. *M. viridis*	4.3±0.4	1.7±0.2	52.9	0.129±0.069	0.29 ±0.07
3. *M. expectata*	4.7±0.4	1.5±0.2	41.2	0.04 ±0.019	0.146±0.048
4. *M. pulchra*	12.2±0.7	2.4±0.2	76.5	0.205±0.047	0.294±0.057
6. *M. madagascariensis*	8.3±0.3	2.2±0.2	82.4	0.208±0.046	0.352±0.061
7. *M. cowani*	5.3±0.4	1.6±0.1	52.9	0.14 ±0.053	0.191±0.056
8. *M. baroni*	4.9±0.2	1.6±0.2	52.9	0.151±0.058	0.26 ±0.065
8. *M. laevigata*	1.9±0.2	1.1±0.1	5.9	0.02 ±0.02	0.02 ±0.02
9. *M. bernhardi*	5.2±0.8	1.4±0.1	35.3	0.105±0.04	0.165±0.06
10. *M. crocea*	7.5±0.3	2 ±0.2	76.5	0.141±0.045	0.298±0.057
11. *M. aurantiaca*	5.1±0.5	1.5±0.2	47.1	0.088±0.041	0.198±0.059
12. *M.* sp. 3	3.6±0.2	1.9±0.2	64.7	0.184±0.057	0.322±0.071

Discussion

Mean heterozygosity as detected by us in *Mantella* is clearly lower than it would be expected in a Hardy-Weinberg equilibrium. Such a result is not uncommon in electrophoretic studies, the heterozygosity values found being either lower (e. g. Kaiser et al. 1994, Wood 1996) or higher ("fixed" heterozygosity, e. g. Picariello et al. 1990) than expected. Deviations from the Hardy-Weinberg equilibrium may be caused by demographic events like

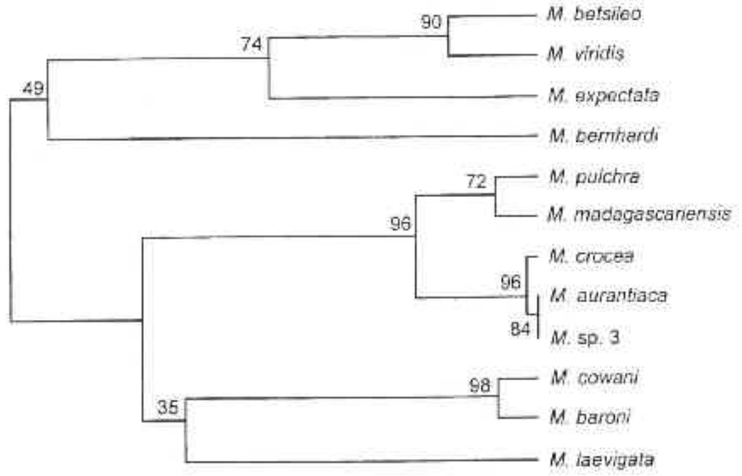


Fig. 1. UPGMA-phenogram of the *Mantella* species studied, constructed from N e i ' s standard genetic distances as given in Table 4. Numbers are bootstrap values in per cent (1000 replications).

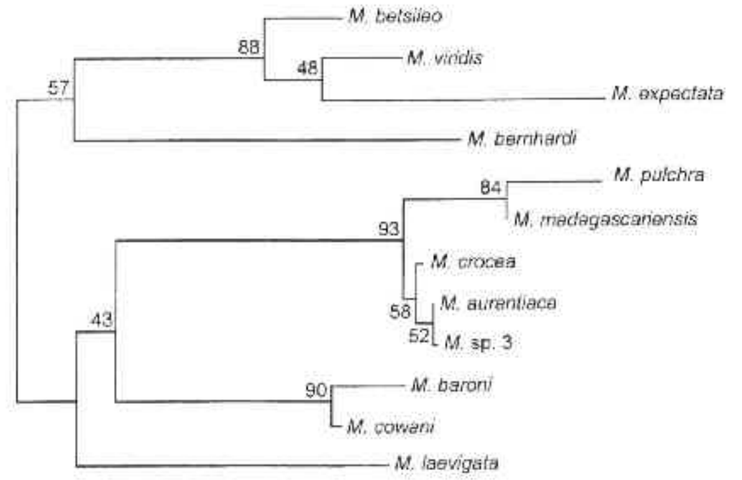


Fig. 2. Neighbor-Joining tree of the *Mantella* species studied, constructed from N e i ' s standard genetic distances as given in Table 4. Numbers are bootstrap values in per cent (1000 replications).

migration, selection against hybrids or assortative mating. Also a non-Mendelian basis of zymogram variation can not be excluded (M u r p h y et al. 1996). In the present study, we tend to consider the deviation as a result of a conservative approach to zymogram interpretation. Atypical heterozygote banding patterns are known in many enzymes (R i c h a r d s o n et al. 1986), and we preferred to score an atypical banding pattern as homozygotic expression of known alleles, rather than as heterozygotes or "rare" different alleles. Actually interpreting a heterozygote pattern (e. g. ab) as a homozygote of one of its alleles (aa or bb) may result in strong effects on genetic distance measures, but generally has no influence on the cladistic analysis. In the present study, results of the cladistic and the phenetic analysis (the latter based on distance measures) are largely concordant (Figs. 1-3), and a relevant influence of a possible unbiased interpretation of heterozygotes can thus be excluded.

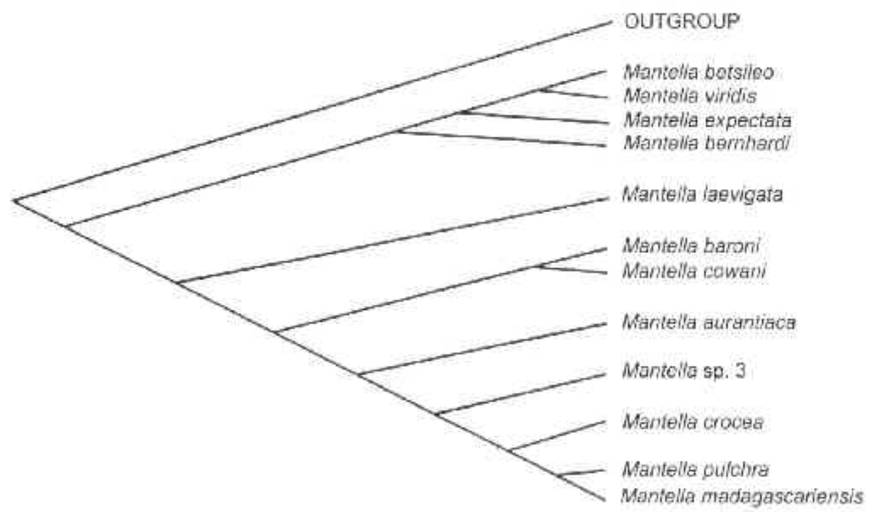


Fig. 3. Most parsimonious cladogram resulting from cladistic analysis of allozyme data. *Mantidactylus biporus* was used as outgroup. Length is 151 steps, consistency index is 50.

Table 4. Matrix of N e i ' s (1972) standard genetic distances between *Mantella* species, based on allozyme data of all 17 loci studied as given in Table 2. Abbreviations of species as in Table 2.

	bet	vir	exp	pul	mad	cow	bar	lae	ber	cro	aur
bet	0										
vir	0.1190	0									
exp	0.4103	0.3074	0								
pul	0.8067	0.6328	0.8014	0							
mad	0.7022	0.6441	0.8219	0.0713	0						
cow	0.4089	0.6071	0.7946	0.6339	0.5649	0					
bar	0.5010	0.7738	0.9072	0.7068	0.5613	0.0702	0				
lae	0.5553	0.7503	0.7515	0.6946	0.5615	0.4371	0.5465	0			
ber	0.4973	0.6306	0.8321	0.8016	0.7503	0.6856	0.7683	0.6719	0		
cro	0.6912	0.6763	0.8997	0.1702	0.0961	0.4576	0.4820	0.5713	0.7032	0	
aur	0.6764	0.6557	0.8276	0.1942	0.0935	0.4207	0.459	0.6084	0.7591	0.0126	0
sp3	0.6809	0.6628	0.8025	0.2200	0.1109	0.4590	0.4692	0.5816	0.6995	0.0229	0

Table 5. Effect of jacknifing on the phylogenetic data used for construction of Fig. 3. The effect of removal of one taxon in turn on the number of equally most parsimonious trees, tree length, and retention index is shown. Abbreviations of species as in Table 2.

deleted	trees	length	ri
(none)	1	151	56
aur	1	148	55
sp3	1	146	55
vir	1	145	54
exp	1	139	56
pul	1	138	57
mad	2	145	53
cow	1	145	55
bar	1	146	55
lae	1	143	57
ber	1	141	58
cro	1	142	56

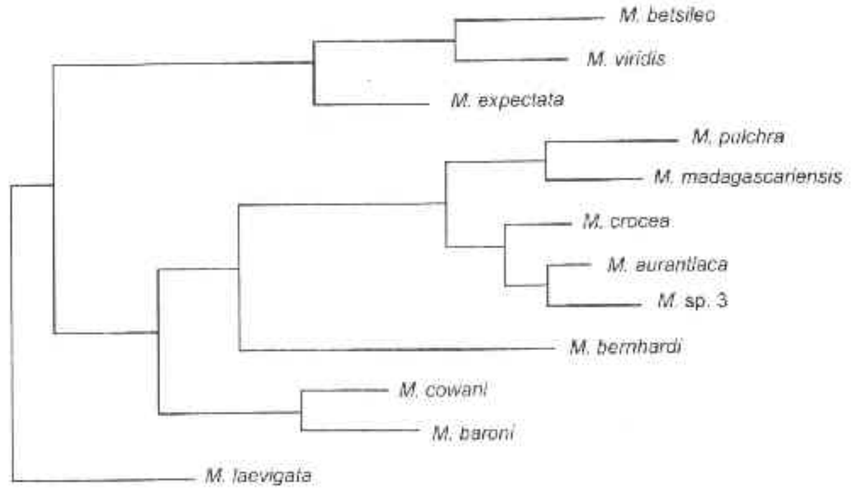


Fig. 4. Shortest tree found by frequency parsimony, using the USERTREE option of FREQPARS to test for improved positions of *M. bernhardi* and *M. laevigata* in the tree.

High levels of allelic polymorphism as found in our study are not unknown in anurans. Hitchings & Beebee (1997) found 10 alleles at the MPI locus in populations of *Rana temporaria* from Brighton, United Kingdom, with 3-5 alleles occurring in any one population. In the case of our *Mantella* samples, it must be considered that most specimens were supplied by the pet trade. In Madagascar, animal wholesalers keep high numbers of *Mantella* specimens in large outdoor terraria, and specimens belonging to the same species, but originating from different localities and collectors are put into the same cage (M. Vences & F. Glaw, pers. obs.). Thus, our series probably do not represent single populations but possibly include specimens from different localities, which may partly explain the high allele polymorphism in the samples studied. Also, it can not be excluded that hybridization between closely related species may occur in the wild, with allele introgression and a further increase in polymorphism.

Electrophoretic data presented herein are largely in accordance with osteological, karyological and bioacoustic characters (Vences et al. in press, Pintak et al. 1998, pers. obs.). The groupings *betsileo-viridis-expectata*, *cowani-baroni*, and *madagascariensis-pulchra-crocea-aurantiaca*-sp.3 are supported by all data sets.

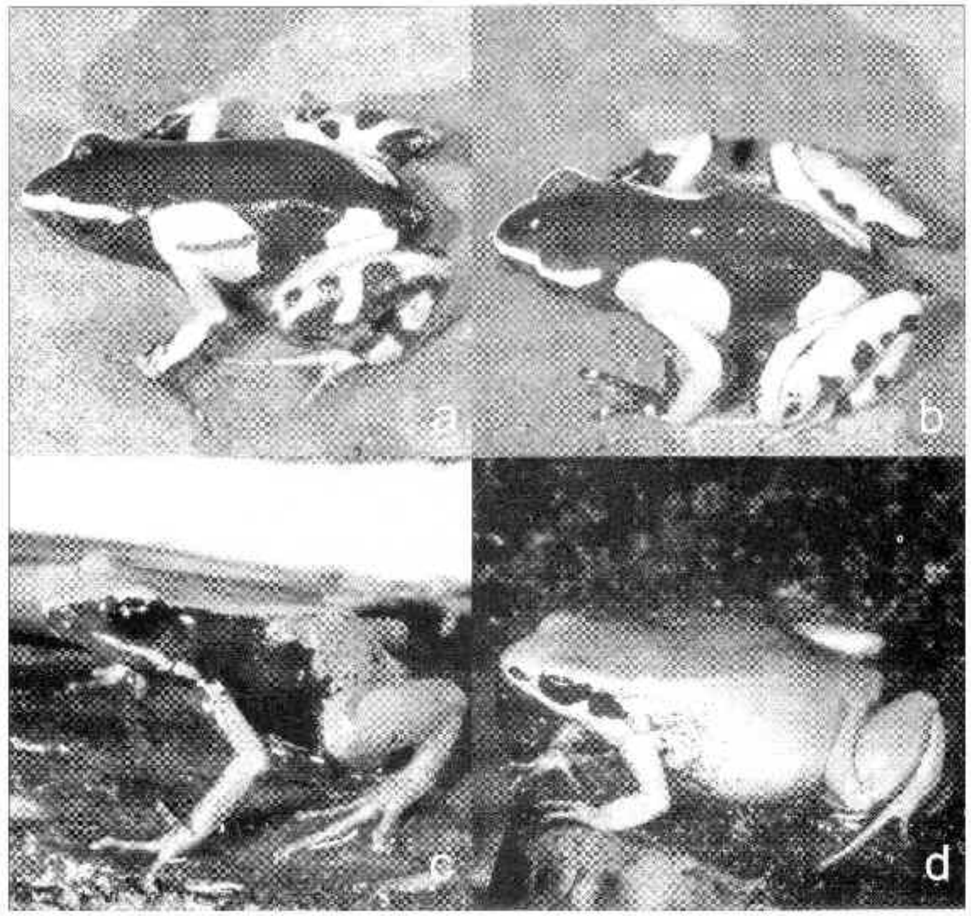
M. bernhardi is very similar to *M. madagascariensis* and *M. pulchra* in several colour patterns. Its positioning by electrophoretic data is problematic, and electrophoretic data are not unequivocal (compare Figs. 1-3 with Fig. 4). However, a position distant from *M. betsileo*, *M. viridis* and *M. expectata* (as in Fig. 4) is a more parsimonious solution, which is in accordance with other character sets. Bioacoustically, *M. bernhardi* differs from all other *Mantella* by its trill calls (Glaw & Vences 1994, pers. obs.), which seems to correspond well with its large genetic distance (D>0.49) from other *Mantella* species.

The position of *M. laevigata* is rather basal in all cladograms produced by different data sets, but its large number of autapomorphies makes assessment of relationships difficult. Its relatively large genetic differentiation (D>0.5) emphasizes this isolated position. It is interesting that the most parsimonious positioning of this species is a sister group relationship to all other *Mantella* species (Fig. 4), but further statements are not possible due to the low

number of specimens of *M. laevigata* (mean sample size 1.9, Table 3). Clearly, further investigations are necessary to produce a definitive conclusion.

Our data, for the first time, provide a proper basis for estimating genetic differentiation in the genus *Mantella*, and allow us to correlate it with colour pattern variability. Surprisingly, our results demonstrate that both (1) large intraspecific variability and (2) interspecific similarities of colour patterns exist within the genus (Pl. 1): (1) The *M. aurantiaca* complex (*M. aurantiaca*, *M. crocea*, *M.* sp. 3) is an example of relatively large colour pattern variability in a genetically largely undifferentiated group, and even single species within the complex (e. g. *M. crocea*) show pattern variation (Pl. 1). (2) *M. baroni* and *M. madagascariensis* are relatively distant genetically (D=0.56), but in some *madagascariensis* populations, specimens are virtually indistinguishable from (syntopic) *M. baroni* by dorsal colouration (pers. obs.; see Pl. 1).

These examples may indicate that (especially dorsal) colouration of *Mantella* species is influenced by strong selective pressures, as is to be expected in diurnal, toxic and aposematic animals. The observed degree of colour variation seems to be partly independent from genetic



Pl. 1. Examples of interspecific colour pattern similarity and intraspecific pattern variation in *Mantella*. (a) *M. madagascariensis*, (b) *M. baroni*. These two species are extremely similar in dorsal colour pattern but well differentiated genetically (D=0.56). (c) *M. crocea*, typical morph with yellowish back and largely black flanks, (d) *M. crocea*, greenish morph with largely uniform pattern. No relevant allozyme differences were detected between specimens belonging to these two morphs (D<0.01).