POLYPHYLY OF *TOMOPTERNA* (AMPHIBIA: RANIDAE) BASED ON SEQUENCES OF THE MITOCHONDRIAL 16S AND 12S rRNA GENES, AND ECOLOGICAL BIOGEOGRAPHY OF MALAGASY RELICT AMPHIBIAN GROUPS

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ABSTRACT.- Frogs currently attributed to the ranid genus *Tomopterna* are known from sub-saharan Africa, southern Asia and Madagascar. Molecular studies of mitochondrial 12S rRNA and 16S rRNA gene sequences indicate that each of these geographic clusters are more closely related to other locally occurring anuran taxa than they are to each other. The Malagasy *T. labrosa* (hitherto subgenus *Laliostoma*) belongs to a monophyletic group including *Aglyptodactylus, Boophis,* and the mantellines; the southern Asian species (hitherto subgenus *Sphaerotheca*) appear to be related to ranids of the genus *Limnonectes* (subgenus *Feyervarya*); and most surprisingly, the African species clustered with cacosternine ranids. These results suggest that the Asian *Sphaerotheca* and the Malagasy *Laliostoma* should be raised to generic rank. As a consequence, the distribution pattern of *Tomopterna* are not informative regarding continental drift and biogeographic Madagascar-India relationships. Phylogenetic data on Malagasy anurans indicate that old evolutionary lineages and relict forms (e. g., *Laliostoma, Aglyptodactylus, Scaphiophryne*) breed mainly in lentic waterbodies, and often occur in savannah habitats. In *Boophis* and mantellines, evidence exists that the most basal groups are rather generalized pond breeders, whereas the speciose groups of forest brook breeders may be monophyletic groups partly of more recent origin. If corroborated by further evidence, this trend would indicate an originally relatively dry and open habitat type in Madagascar at the time of differentiation of the main lineages of Malagasy anurans, which may have taken place in the late Mesozoic or early Cenozoic.

KEY-WORDS.- Anura, Ranidae, Raninae, Cacosterninae, Mantellinae, Rhacophorinae, *Laliostoma, Sphaerotheca, Tomopterna,* Molecular phylogeny, Madagascar, India, South Africa, Continental drift, Reproductive modes

RESUME.- Les amphibiens rattachés au genre *Tomopterna* sont connus de l'Afrique au dessous du Sahara, du sud de l'Asie et de Madagascar. Des études moléculaires à partir des séquences des gènes 12S rARN et 16S rARN montrent que chacun de ces groupes géographiques d'espèces est plus affine avec d'autres anoures de la même région qu'avec des groupes d'espèces d'autres régions. L'espèce malgache *T. labrosa* (cf. sous-genre *Laliostoma*) appartient a un groupe monophylétique comprenant, *Aglyptodactylus, Boophis* et les Mantellinae. Les espèces du sud de l'Asie (cf. sous-genre *Sphaerotheca*) semblent associées au genre *Limnonectes* (sous-genre *Feyervarya*), et, plus surprenant encore les espèces africaines sont associées aux Cacosterninae. Ces résultats suggèrent que *Sphaerotheca* et *Laliostoma* devraient être élevés au rang de genre. En conséquence, le modèle de distribution des *Tomopterna* ne nous apprend rien sur la dérive des continents ou sur les relations biogéographiques entre Madagascar et l'Inde. Les données

phylogénétiques sur les amphibiens malgaches montrent que les lignées anciennes ou les formes relictuelles (e.g., *Laliostoma, Aglyptodactylus, Scaphiophryne*) se reproduisent dans des cours d'eau lents, et souvent se trouvent dans des milieux savanicoles. De toute évidence, chez *Boophis* et les Mantellinae, les groupes le plus primitifs semblent généralistes pour la reproduction, tandis que les groupes forestiers correspondent à des lignées monophylétiques plus récentes. Si ce modèle se confirmait, il indiquerais qu'un habitat original de type ouvert et sec a pu exister à Madagascar au moment de la différentiation des grandes lignées d'amphibien malgaches, qui a eu lieu entre la fin du Mésozoïque et le début du Cénozoïque.

MOTS-CLES.- Anura, Ranidae, Raninae, Cacosterninae, Mantellinae, Rhacophorinae, *Laliostoma, Sphaerotheca, Tomopterna,* phylogénie moléculaire, Madagascar, Inde, Afrique du Sud, Dérive continentale, Modèles de reproduction

INTRODUCTION

The genus *Tomopterna* currently contains 15 stout, medium-sized frog species (see Glaw *et al.* 1998b; Schleich & Anders 1998) which mainly inhabit arid regions of sub-saharan Africa, southern Asia, and Madagascar. *Tomopterna* species are burrowing frogs, similar in general appearance to several other xerophilic anurans, such as *Pelobates, Scaphiopus* and *Spea* (Pelobatidae), *Notaden* (Myobatrachidae), and *Pyxicephalus* (Ranidae).

Tomopterna is considered to belong to the family Ranidae, an amphibian group with controversial and chaotic systematics (Duellman & Trueb 1986). Subfamilial classification of ranids differs largely between authors (e. g. Clarke 1981; Duellman & Trueb 1986; Dubois 1992; Blommers-Schlösser 1993; Glaw *et al.* 1998a). *Tomopterna* is part of the subfamily Raninae according to Blommers-Schlösser (1993) and Glaw *et al.* (1998a), whereas Dubois (1992) placed the genus within its own subfamily Tomopterninae.

Despite detection of intrageneric osteological differences (Clarke 1981), different opinions on subfamilial placement, and an unusual distribution pattern, recent authors generally have not questioned the monophyletic origin of the genus.

In the last several years molecular and morphological evidence has accumulated to show that the current classification of *Tomopterna* is in need of revision (Glaw *et al.* 1998a; Richards & Moore 1998; Scott & Channing, pers. comm.; Dubois, pers. comm.). In the present paper, we present molecular data showing that the three geographic lineages currently included in *Tomopterna* are not monophyletic, and are therefore wrongly classified in a single genus. We also review some relevant morphological characters in these lineages and their assumed relatives, and discuss the ecological biogeography of Malagasy anurans in the light of the new classification. In the following accounts, the name *Tomopterna* will only be used in a strict sense referring to the African species. When referring to genus *Tomopterna* as hitherto understood, we will use the name *Tomopterna* sensu lato (s.l.) or will write « *Tomopterna* » (in quotation marks).

MATERIALS AND METHODS

For molecular analyses, DNA was extracted from tissue samples (hindlimb muscle, either fresh or preserved in 96% pure ethanol; or toes cut off from specimens in the field which were subsequently released). We used the primers 16Sar-L and 16Sbr-H

(Palumbi *et al.* 1991) to amplify a section of the mitochondrial 16S ribosomal RNA gene and the primers 12SA-L and 12SB-H (Palumbi *et al.* 1991) to amplify a section of the mitochondrial 12S ribosomal RNA gene. The obtained sequences (lengths referring to the aligned sequences including gaps) comprised 550 bp (16S) and 361 bp (12S) homologous to the bp positions 4000-4563 (16S) and 2554-2913 (12S) of the *Xenopus laevis* mitochondrial genome (Roe *et al.* 1985). They were analyzed using PAUP, version 4 (Swofford 1998). We calculated maximum parsimony (MP) trees with gaps treated as a fifth character, and neighbor-joining (NJ) trees based on the Jukes-Cantor distance (Jukes & Cantor 1969), with gaps treated as missing data. 2000 bootstrap replicates (Felsenstein 1985) were run in all analyses following Hedges (1992). Only topologies with a bootstrap support of 70% and higher were considered in taxonomic and phylogenetic conclusions, according to Hillis & Bull (1993) who found a 95% probability of correct topology in branches supported by such bootstrap values. *Kassina senegalensis* (Hyperoliidae) was used as outgroup.

Details of primers, cycling protocols and methodology of analyses are given in Vences et al. (in press). Voucher specimens are listed in Table I. Collection acronyms used are: Muséum National d'Histoire Naturelle, Paris (MNHN); National Museum of Natural History, Smithsonian Institution, Washington, D.C. (USNM); Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn (ZFMK); Zoölogisch Museum Amsterdam (ZMA); Zoological Survey of India, Kolkata (ZSI); Zoologische Staatssammlung, München (ZSM); and TZ (field numbers of Thomas Ziegler; specimens to be deposited in ZFMK). Sequences have been submitted to GenBank (see Table I for accession numbers). Osteological comparisons are based on specimens listed in Glaw *et al.* (1998a) and on *Tomopterna natalensis* (ZFMK 68815); *Tomopterna tuberculosa* (ZMA 5860), *Cacosternum boettgeri* (ZFMK 33116-33117), «*Tomopterna*» (*Sphaerotheca*) *breviceps* (ZSM 270/1979), « *Tomopterna* » (*Laliostoma*) *labrosa* (ZMA 6769), *Limnonectes limnocharis* (TZ 526, ZFMK 49215). Keratodont formula of tadpoles is set out according to Dubois (1995).

RESULTS AND DISCUSSION

MOLECULAR PHYLOGENY OF *TOMOPTERNA* SENSU LATO

Neighbor-joining analysis of the 550 base pairs of the sequenced 16S rRNA gene (Fig. 1) did not group the included species of *Tomopterna* s. l. as a monophyletic unit. Instead, the Malagasy *labrosa* was grouped with *Mantella, Mantidactylus, Boophis* and *Aglyptodactylus* (supported by a bootstrap value of 69%); the Asian *breviceps* and *dobsonii* were grouped with *Limnonectes limnocharis* (65%) and the African *natalensis, cryptotis, marmorata,* and cf. *tandyi* were grouped with *Anhydrophryne* and *Cacosternum* (96%). Similar results were achieved in this and the following analyses by a maximum parsimony (MP) approach, but the bootstrap supports were slightly lower.

12S sequences were available for a lower number of taxa (Table I), but their analysis reinforced the 16S data (results not shown). The Malagasy *labrosa* was grouped with *Aglyptodactylus* (72%) and *Mantella* and *Mantidactylus* (73%), the Asian *breviceps* and *dobsonii* were grouped with *Limnonectes limnocharis* (86%), and the African cf. *tandyi* was grouped with *Cacosternum* (73%).

A combined analysis of both data sets resulted in an increase of the bootstrap values (Fig. 2). The polyphyly of *Tomopterna* was supported by high values of 97%, 100%, and 95%.

Additionally, we attempted to amplify and analyse sequences of the mitochondrial cytochrome *b* gene. As different primers did not result in successful amplification in several of the taxa, only a limited data set was finally available. The preliminary analysis of 280 bp failed to resolve significantly the relationships between most of the included clades, but did not contradict the 12S and 16S topology: the African cf. *tandyi* and *marmorata* were sister taxa supported by 90% and did not cluster with the Malagasy *labrosa*; no sequences of Asian *Tomopterna* s. l. species were obtained (Vences, Kosuch & Veith, unpublished).

CLASSIFICATION

The molecular data strongly support the polyphyletic nature of *Tomopterna* s. l. (Glaw *et al.* 1998a) and the molecular data On the other hand, both morphological presented in this paper support monophyly of the three geographic clusters currently included in the genus, which correspond to the subgenera *Tomopterna* (Africa), *Sphaerotheca* (Asia), and *Laliostoma* (Madagascar). We therefore propose to raise these three taxa to generic rank. The new classification of the currently known species of *Tomopterna* s. l. (Glaw *et al.* 1998b) is therefore as follows:

(1) Genus *Tomopterna* Duméril & Bibron, 1841

Type species: *Pyxicephalus delalandii* Tschudi, 1838

Included species: *Tomopterna cryptotis* (Boulenger, 1907); *Tomopterna delalandii* (Tschudi, 1838); *Tomopterna krugerensis* Passmore & Carruthers, 1975; *Tomopterna natalensis* (Smith, 1849); *Tomopterna tandyi* Channing & Bogart, 1996; *Tomopterna tuberculosa* (Boulenger, 1882).

Distributed in sub-saharan Africa. Known at least from Angola, Botswana, Cameroon, Eritrea, Ethiopia, Kenya, Malawi, Mozambique, Namibia, Niger, Nigeria, Senegal, Somalia, South Africa, Sudan, Tanzania, Uganda, Zambia, Zimbabwe (see Rödel 1996).

(2) Genus *Sphaerotheca* Günther, 1859

Type species: *Sphaerotheca strigata* Günther, 1859

Included species: *Sphaerotheca breviceps* (Schneider, 1799); *Sphaerotheca dobsonii* (Boulenger, 1882); *Sphaerotheca leucorhynchus* (Rao, 1937); *Sphaerotheca rolandae* (Dubois, 1983); *Sphaerotheca maskeyi* (Schleich & Anders, 1998); *Sphaerotheca swani* (Myers & Leviton, 1956). *Sphaerotheca strachani* (Murray, 1884); *Sphaerotheca* sp. by A. K. Sarkar A further species from Goa, western India, is about to be described (pers. comm.). *Tomopterna rufescens* (Jerdon, 1853) was transferred to the genus *Limnonectes* by Dubois (1987).

Distributed in South Asia. Known from Bangladesh, India, Myanmar, Nepal, Pakistan, Sri Lanka.

(3) Genus *Laliostoma* Glaw, Vences & Böhme, 1998

Type species: *Tomopterna labrosa* Cope, 1868

Included species: *Laliostoma labrosum* (Cope, 1868)

Distributed in Madagascar.

OTHER CHARACTERS

Molecular data suggest phylogenetic affinities of *Tomopterna* to the cacosternines, of *Sphaerotheca* to *Limnonectes*, and of *Laliostoma* to *Aglyptodactylus*. To find additional support for these relationships, we reviewed a number of key characters in these groups.

Tadpoles. - According to the drawings of Wager (1965), keratodont formula of *Tomopterna* is 1:2+2/3 to 1:3+3/3, sometimes with an interrupted first lower tooth row (1:3+3/1+1:2). The tadpoles of cacosternines (*Cacosternum* and *Microbatrachella*) have a similar arrangement of keratodonts (1:1+1/3 to 1:3+3/1+1:2; Wager 1965). According to Glaw & Vences (1994), *Laliostoma labrosum* has a keratodont formula of 1:5+5/3, which is similar to that of *Aglyptodactylus madagascariensis* (1:5+5/1+1:2 to 1:6+6/1+1:2; Blommers-Schlösser 1979; Glaw & Vences 1994). According to Kirtisinghe (1957), *Sphaerotheca breviceps* has a formula of 1:1+1/3 and a broad upper gap in the marginal papillae. The same buccal morphology is found in *Limnonectes limnocharis* and other South Asian members of this genus (Kirtisinghe 1957). Arrangement of marginal papillae is similar in the taxa considered, all having a broad upper gap in the row of papillae.

Breeding biology. - As far as known, *Tomopterna*, *Laliostoma* and *Sphaerotheca* are generalized pond breeders which lay a rather large number of single eggs into shallow, often temporary pools. *Limnonectes limnocharis* lays large clutches in lentic habitats (pers. obs.). Cacosternines partly show derived breeding modes, *Anhydrophryne* and *Arthroleptella* having direct development. *Cacosternum* lays its eggs in small clumps attached to stones or vegetation of relatively shallow waters (Wager 1965).

Chromosomes. - All taxa considered have a karyotype of 2n = 26, except for the tetraploid *Tomopterna tandyi* which has 4n = 52, and *Sphaerotheca*, in which the number is unknown (Blommers-Schlösser 1978; King 1990; Channing & Bogart 1996; Prakash 1998).

Ilium. - According to Clarke (1981), African *Tomopterna* are characterized by a derived shape of the dorsal ilium processes which is lacking in *Laliostoma* and *Sphaerotheca*. We have been unable to distinguish the states observed by Clarke (1981) in our material; the states identified by Glaw *et al.* (1998a) probably refer to different structures (Clarke pers. comm. 1998). Thus, ilium shape remains to be studied in Asian and Malagasy ranids.

Tarsals. - *Laliostoma* and *Aglyptodactylus* are characterized by the presence of only two free distal tarsals, a state not found in other ranids but typical for many rhacophorines (Blommers-Schlösser 1993). The plesiomorphic condition, three free distal tarsals, is found in *Tomopterna* and *Sphaerotheca* (Glaw *et al.* 1998a), as well as in *Cacosternum* and *Arthroleptella* (Blommers-Schlösser 1993; pers. obs.) and in *Limnonectes limnocharis* (pers. obs.).

Pectoral girdle. - Typical for cacosternines is the reduced ossification of pectoral girdle elements: the omosternal style and the procoracoid clavicular bar are cartilaginous or slightly ossified in several genera (Blommers-Schlösser 1993). Neither *Tomopterna* nor the other groups regarded here show a similar reduction. The omosternum is moderately forked in *Laliostoma*, *Aglyptodactylus*, and *Sphaerotheca* (Glaw *et al.* 1998a); broadly forked in *Limnonectes limnocharis* (pers. obs.), unforked or only slightly forked in *Tomopterna* (Clarke 1981; Glaw *et al.* 1998a), apparently unforked in cacosternines in which this element is largely reduced and only visible as cartilaginous rudiment (pers. obs. in *Cacosternum*).

Digital subarticular sesamoids. - According to Blommers-Schlösser (1993), these elements should be absent in ranids. We observed, however, distinct sesamoids in one

specimen of *Tomopterna*, in *Sphaerotheca*, and in *L. limnocharis*. We also saw sesamoids in a single specimen of *Cacosternum*, although this seems to be an exceptional state in cacosternines (Scott, pers. comm.). Obviously, a more comprehensive survey of presence or absence of these structures among ranids is necessary. We did not see sesamoids in any of the studied specimens of *Laliostoma* or *Aglyptodactylus*, nor in any of about 25 studied representatives Malagasy mantellines and rhacophorines.

Nuptial pads. - Distinct black nuptial pads during the breeding period are typical for *Laliostoma* and *Aglyptodactylus* (Glaw *et al.* 1998a). Only very faint pads are visible in *Tomopterna* (pers. obs.). Pads are present but colourless in *Cacosternum* and *Arthroleptella hewitti* (Scott, pers. comm). Distinct, grey velvety pads are visible in breeding *Limnonectes limnocharis* (pers. obs.). Dark nuptial pads are not recognizable in two *Sphaerotheca breviceps* (ZSM 270/1979/1 and 270/1979/2) although these specimens have enlarged and partially dark vocal sacs, indicating that they were breeding males (pers. obs.).

Relative finger length. - Typical for many ranids is a relatively short second finger as compared with the first finger. This state is present in *Laliostoma* and *Aglyptodactylus* and distinguishes both genera from Malagasy rhacophorines and mantellines (Glaw *et al.* 1998a). It is also present in *Tomopterna* and *Sphaerotheca* (Glaw *et al.* 1998a), and in *Limnonectes limnocharis* (pers. obs.). Finger 1 is shorter than finger 2 in cacosternines (pictures in Passmore & Carruthers 1995; pers. obs.).

Except for the similar general appearance, there is no morphological character state known to be unique to species of *Tomopterna*, *Laliostoma* and *Sphaerotheca*. Morphology thus does not support a monophyletic group containing these three taxa alone. It is difficult to polarize most of the informative characters regarded here. However, in a purely phenetic comparison, the assumed relationships of *Tomopterna* to cacosternines are supported by similarities in tadpole morphology (number of keratodont rows) and by lack of a distinctly forked omosternum (although the reduction of this element in cacosternines renders a homology assessment difficult). Relationships of *Laliostoma* to *Aglyptodactylus* are supported by their reduced number of tarsals, larger number of tadpole keratodont rows, presence of distinct black nuptial pads, and lack of digital subarticular sesamoids. Relationships of *Sphaerotheca* to *Limnonectes limnocharis* are supported by the reduced number of keratodont rows of their tadpoles.

AFFINITIES AND BIOGEOGRAPHY OF *LALIOSTOMA*, *SPHAEROTHECA* AND *TOMOPTERNA*

According to Glaw *et al.* (1998a), *Laliostoma labrosum* is similar in morphology, osteology, and reproductive biology to the genus *Aglyptodactylus*. Genetically, *Laliostoma* was found to be the sister group of *Aglyptodactylus* in the analysis of 12S data and in the combined analysis. Despite its rather large genetic differentiation to all taxa included, we consider it as almost certain that *Laliostoma* is closely related to *Aglyptodactylus*; either, both taxa are sister groups, or their clades split off paraphyletically from the lineage leading towards *Boophis*, *Mantidactylus* and *Mantella*.

The close relationship of *Laliostoma* and *Aglyptodactylus* (already detected by Glaw *et al.* 1998 based on morphological characters) contradict their inclusion in two different families (*Aglyptodactylus* in the Rhacophoridae and *Tomopterna* in the Ranidae) as was done by most earlier authors (e. g. Frost 1985; Duellman & Trueb 1986; Blommers-Schlösser & Blanc 1991). This was the reason to remove *Aglyptodactylus* from the rhacophorines and to include it in the Ranidae (Glaw *et al.* 1998). The molecular results of Richards & Moore (1998) and those presented in this paper contradict this conclusion and favour the inclusion of *Laliostoma* and *Aglyptodactylus* in the rhacophorines. However, this classification would also be

tentative, until a more robust classification of the ranid-rhacophorine-complex will be achieved.

The relationships of *Sphaerotheca* are enigmatic, especially due to the unsatisfactory state of knowledge on the phylogeny and systematics of Asian ranids. In our cladogram, they were grouped with *Limnonectes limnocharis*. On the other hand, the second *Limnonectes* included, *L. kuhli*, did not appear as sister group of *L. limnocharis*, indicating that further generic rearrangements may be needed. According to Dubois (1992), *L. kuhli* is the type species of *Limnonectes* whereas *L. limnocharis* is the type species of the subgenus *Feyervarya*; the latter appears to merit generic rank according to our results.

Most surprising is the clustering of African *Tomopterna* with cacosternine ranids. This grouping appears to be well supported by the high bootstrap values. Affinities between both groups were also observed in molecular studies of cacosternine phylogeny by E. Scott and A. Channing (Scott, pers. comm.). Cacosternines are a group of small frogs restricted to southern Africa, with a center of diversity and endemism in the Cape Region. They have been included in the Petropedetinae, but were resurrected as subfamily by Blommers-Schlösser (1993). At first impression, they appear to be rather different from the African *Tomopterna* species which, however, show a similar distributional pattern; most species occurring in South Africa.

African *Tomopterna* were in the past considered as members of the genus *Pyxicephalus* (e.g. Wager 1965; Dubois 1981). Our results confirm the decision of Clarke (1981) to exclude it from that genus; we did not detect close affinities between them.

Considering the enormous diversity of ranid frogs, we may not have identified in our analysis the direct sister groups of *Laliostoma* and, especially, *Sphaerotheca* and *Tomopterna*. It is certain, however, that the three genera are each more closely related to ranid lineages occurring in their geographic areas than they are to each other. These frogs (*Tomopterna* s. l.) clearly do not display distribution patterns resulting from continental drift in the Indian Ocean as was suggested by Duellman & Trueb (1986). The splitting of *Tomopterna* s. l. into three unrelated genera reduces the number of examples of extant vertebrate groups showing biogeographic affinities between Madagascar and India.

RELICT AND BASAL AMPHIBIAN GROUPS IN MADAGASCAR

The phylogeny and classification proposed above transfer *Laliostoma labrosum* from a rather speciose to a monotypic genus. *Laliostoma* is genetically distinctly differentiated from other Malagasy ranid lineages, and is largely restricted to the xeric areas of western and southern Madagascar. Only 15 of the 182 currently recognized Malagasy amphibian species are known exclusively or mainly from arid western and southern Madagascar. Similar to *Laliostoma*, most of these belong to basal groups with low species diversity: the microhylid genus *Dyscophus* (*D. insularis* in the west, *D. antongili* and *D. guineti* in the east; Glaw & Vences 1994) which, within the Microhylidae, has primitive characters such as an ossified omosternum and maxillary teeth (Parker 1934); the genus *Scaphiophryne* (three species in the west and south, two species in savannah highland areas, and one species in the east; Glaw & Vences 1994) which was considered to be a possible "living fossil" by Wassersug (1984) due to its unique tadpoles; and the genus *Aglyptodactylus* (three species, two of them restricted to the west) which lacks several of the apomorphies of Malagasy rhacophorines and mantellines (Glaw *et al.* 1998a).

All these groups are characterized by a similar reproductive mode according to the classification of Duellman & Trueb (1986). As typical explosive breeders, they lay a large number of small pigmented eggs into shallow, often temporary, water bodies. The tadpoles are active feeders and generally complete metamorphosis within a few weeks (Blommers-Schlösser & Blanc 1991; Glaw & Vences 1994; Vences *et al.* 1999). This reproductive mode is rare in Malagasy rain forest areas (generally occurring in less than 25% of the species; Glaw & Vences 1997), but clearly dominant in arid western Madagascar. In Kirindy, it probably occurs in 11 of 12 anuran species (92%; Glaw & Vences 1997). It is also a common mode among African savannah anurans (Rödel 1996).

Species of the genera mentioned above which are distributed in humid eastern Madagascar (*Aglyptodactylus madagascariensis*; *Scaphiophryne marmorata, S. madagascariensis, S. pustulosa; Dyscophus antongili, D. guineti*) do not differ in reproductive mode from their western relatives. Although the continuous rainfall in the east would allow prolonged reproduction, at least *Aglyptodactylus* and *Scaphiophryne* show an explosive breeding behaviour mostly restricted to the beginning of the rainy season.

A similar reproductive mode is also found in the two species of *Boophis* which are known to occur in the arid regions of western Madagascar: *B. xerophilus* and the widely distributed generalist *B. tephraeomystax* (Blommers-Schlösser & Blanc 1991; Glaw & Vences 1997). Both species belong to the *B. tephraeomystax* group (Glaw & Vences 1997). According to our osteological observations, *B. tephraeomystax* is characterized by two plesiomorphic characters: presence of the anterolateral hyoid process, and presence of a (small) third free tarsal. All other *Boophis* so far examined lack the anterolateral hyoid process (Glaw *et al.* 1998a) and have two free tarsals (Blommers-Schlösser 1993), possibly qualifying *B. tephraeomystax* as the most basal *Boophis* species. The brook-breeding forest species included in the *B. goudoti* group, *B. microtympanum* group, *B. rappiodes* group, *B. luteus* group and *B. difficilis* group probably form a monophylum based on these two characters.

The endemic Malagasy subfamily Mantellinae (genera *Mantella* and *Mantidactylus*) is characterized by derived reproductive modes; nearly all species have rather large eggs which are deposited outside water (Glaw *et al.* 1998a; Vences *et al.* 1999). Due to their generalized tadpole and chromosome morphology, and their femoral gland structure, species of the subgenera *Blommersia* and *Guibemantis* can be considered the most basal of the mantellines. These groups differ from other *Mantidactylus* in being largely pond and swamp breeders; the mainly brook-dwelling groups (subgenera *Brygoomantis, Chonomantis, Hylobatrachus, Mantidactylus, Ochthomantis*) probably form a monophyletic group (pers. obs.).

Among the anuran genera restricted to the eastern rainforests the reproductive mode typical for western groups is rare. Only the monotypic *Paradoxophyla* (with unclarified affinities to the Scaphiophryninae or Microhylinae; Blommers-Schlösser & Blanc 1991, 1993) deposits a large number of small eggs in stagnant water (but does not seem to be an explosive breeder), and can be considered as relict form endemic to the east.

ECOLOGICAL BIOGEOGRAPHY OF MALAGASY ANURANS

Laliostoma, Aglyptodactylus, Dyscophus and *Scaphiophryne* appear to be basal amphibian groups adapted to arid regions. We assume that their phylogenetic origin was in western Madagascar, and that only more recently species of *Aglyptodactylus, Dyscophus* and *Scaphiophryne* colonized eastern Madagascar. As these genera show a low species diversity, they can be considered relicts (see Blommers-Schlösser & Blanc,

1993: 391). The most basal species of *Boophis* are also distributed in western Madagascar and exhibit a reproductive mode typical for arid environments. Except for *Paradoxophyla,* there are no clear relict groups endemic to the eastern rainforests.

A similar situation can be found in the reptiles. Definitive relicts are those genera with a low species diversity and which can not easily be derived from ancestors rafting from Africa through the Mozambique Channel: boas (*Sanzinia,* one species; *Acrantophis,* two species), « iguanas » (*Chalarodon,* one species; *Oplurus,* six species), and podocnemine turtles (*Erymnochelys,* one species). Except *Sanzinia* and *Acrantophis madagascariensis,* which are also common in eastern rainforest, all these genera are largely restricted to arid western and southern Madagascar.

These patterns indicate a xeric environment in Madagascar at the time of differentiation of the main lineages of Malagasy anurans. Madagascar separated from Africa at ca. 130 million years before present (Storey 1995). According to Storey *et al.* (1995), Madagascar was connected to the east to the Indian landmass which separated 88 million years before present and drifted northwards during the late Cretaceous and early Cenozoic. Mesozoic climate was at least locally rather dry according to current paleoclimatic hypotheses (e.g. Spicer *et al.* 1994). The trade winds which are responsible for Madagascar's present humid climate probably did not blow before the opening of the Indian Ocean. Rainforest may thus have been temporarily rare or even absent on Madagascar. After the emergence of rainforest, a few groups (mantellines, *Boophis,* cophyline microhylids) underwent large radiations into this new habitat, which led to the enormous diversity in species numbers and reproductive modes observed in the Malagasy anurans today. Whether their ancestors were already present on Madagascar since its separation from Africa, or arrived more recently over Cenozoic land bridges (with probably rather dry and hostile environments for amphibians) can not be determined at present.

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Table I. List of specimens used for analysis. Familial and subfamilial classification according to Blommers-Schlösser (1993). A star marks released specimens (no voucher collected).

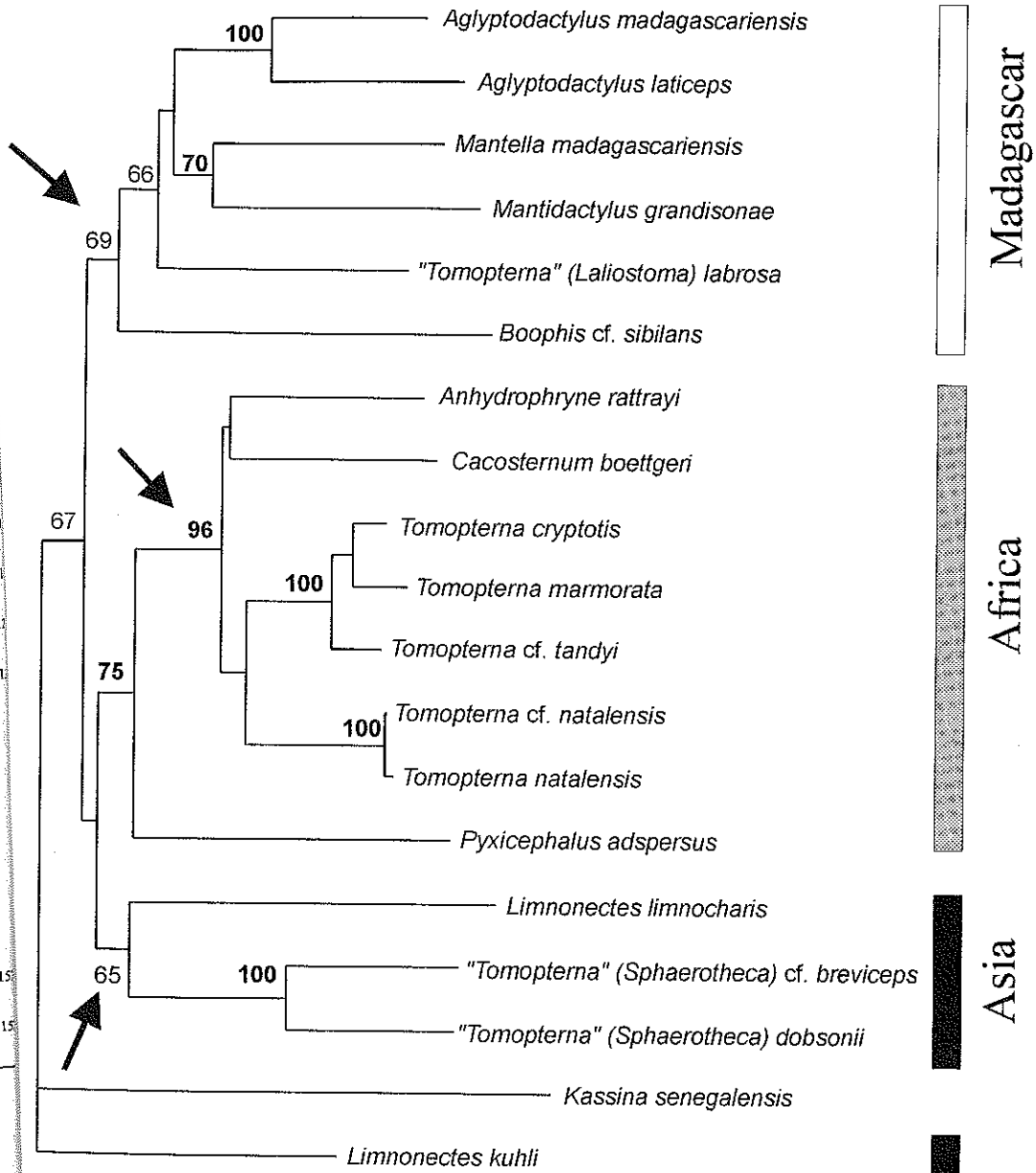| Family (Subfamily)	Species	Origin	ZFMK	GenBank 16S	GenBank 12S
Hyperoliidae	Kassina senegalensis	St. Lucia, South Africa*	-	AF215445	AF124097
Ranidae (Mantellinae)	Mantella madagascariensis	Madagascar	ZFMK 62732	AF124131	AF124101
Ranidae (Mantellinae)	Mantidactylus grandisonae	Ambato, Madagascar	ZFMK 66669	AF215315	AF215149
Ranidae (Rhacophorinae)	Boophis cf. sibilans	Ranomena, Madagascar	ZFMK 62797	AF215338	-
Ranidae (Rhacophorinae)	Aglyptodactylus madagascariensis	Andasibe, Madagascar	ZFMK 64137	AF215330	AF215179
Ranidae (Rhacophorinae)	Aglyptodactylus laticeps	Kirindy, Madagascar	ZFMK 64135	AF215329	AF215180
Ranidae (Cacosterninae)	Cacosternum boettgeri	Bredell, South Africa (12S)*;	---	---	AF124096
Ranidae (Cacosterninae)	Cacosternum boettgeri	Hardap, Namibia (16S)	ZFMK 66727	AF215414	---
Ranidae (Cacosterninae)	Anhydrophryne rattrayi	near Alice, South Africa	ZFMK 66448	AF215504	-
Ranidae (Raninae)	Laliostoma labrosum	Kirindy, Madagascar	ZFMK 66698	AF215278	AF215178
Ranidae (Raninae)	Sphaerotheca cf. breviceps	Myanmar	USNM 520490	AF215417	AF215211
Ranidae (Raninae)	Sphaerotheca dobsonii	Pataravakkam Hill, India	ZSI A9074	AF215418	AF215212
Ranidae (Raninae)	Tomopterna cf. tandyi	Khorixas, Namibia	ZFMK 66403	AF215419	AF215213
Ranidae (Raninae)	Tomopterna natalensis	Itala Park, South Africa*	-	AF215508	-
Ranidae (Raninae)	Tomopterna cf. natalensis	Port St Johns, South Africa	ZFMK 68815	AF215509	-
Ranidae (Raninae)	Tomopterna marmorata	Mt. Meru, South Africa*	-	AF215507	-
Ranidae (Raninae)	Tomopterna cryptotis	Bredell, South Africa*	-	AF215506	-
Ranidae (Raninae)	Pyxicephalus adspersus	KwaMbonambi, South Africa	ZFMK 66446	AF215505	-
Ranidae (Raninae)	Limnonectes limnocharis	Laos	MNHN 1997.3932	AF215416	AF215...
Ranidae (Raninae)	Limnonectes kuhli	Laos	MNHN 1997.3904	AF215415	AF215...

Fig. 1. Neighbor-joining tree based on 550 bp of mitochondrial 16S ribosomal RNA gene sequences. Numbers are bootstrap values in percent (2000 replicates; values below 50% not given; values over 70% bold). *Kassina senegalensis* was used as outgroup. Arrows indicate nodes supporting non-monophyly of *Tomopterna* s. l.
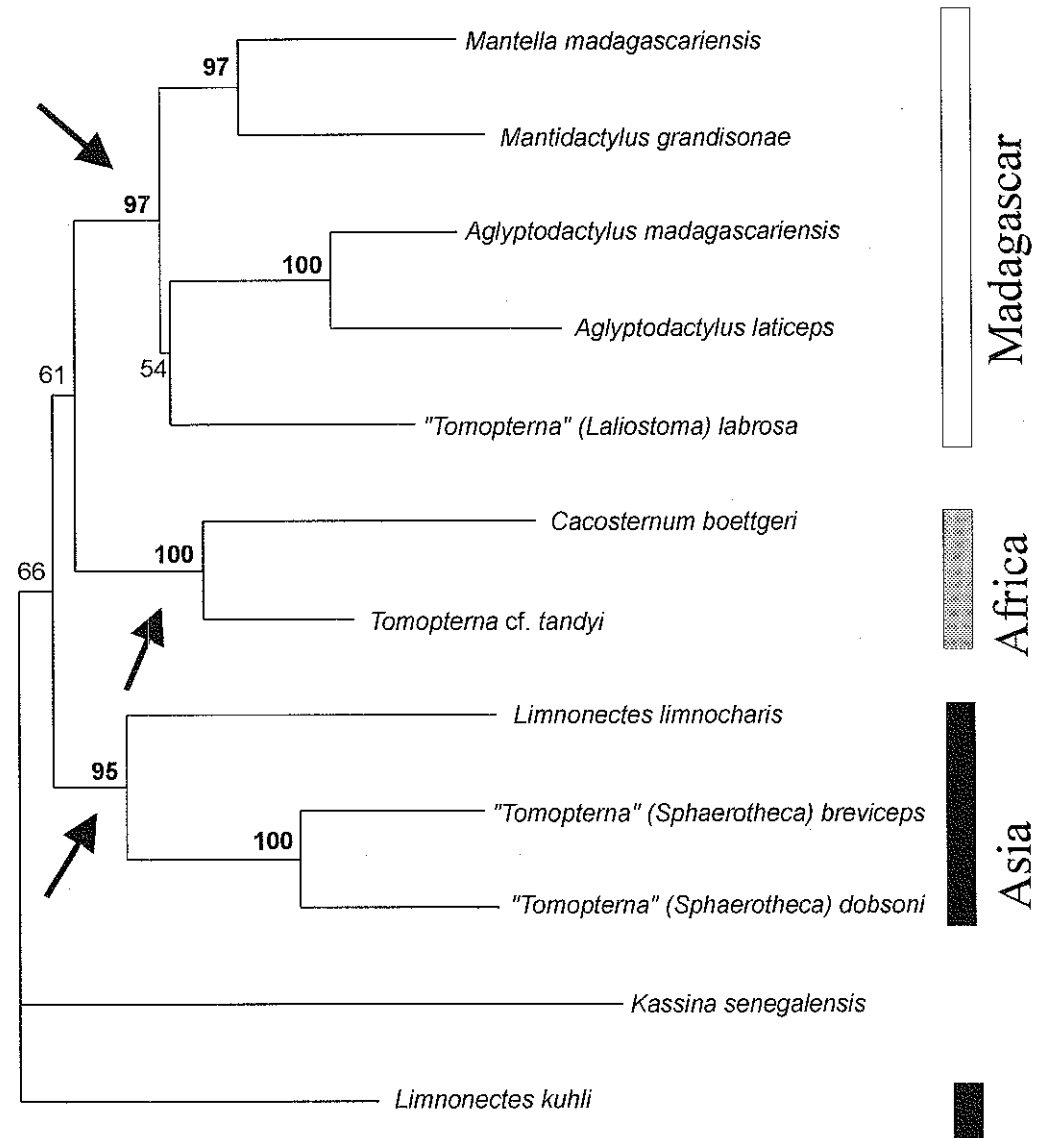
Fig. 2. Neighbor-joining tree based on a combined analysis (909 bp) of the 12S and 16S sequences. Numbers are bootstrap values in percent (2000 replicates; values below 50% not given; values over 70% bold). *Kassina senegalensis* was used as outgroup. Arrows indicate nodes supporting non-monophyly of *Tomopterna* s. l.