

Out of Asia: Mitochondrial DNA Evidence for an Oriental Origin of Tiger Frogs, Genus *Hoplobatrachus*

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Most examples of intercontinental dispersal events after the Miocene contact between Africa and Asia involve mammal lineages. Among amphibians, a number of probably related groups are known from both continents, but their phylogenies are so far largely unresolved. To test the hypothesis of Miocene dispersal against a Mesozoic vicariance scenario in the context of Gondwana fragmentation, we analyzed fragments of the mitochondrial 16S rRNA gene (572 bp) in 40 specimens of 34 species of the anuran family Ranidae. Results corroborated the monophyly of tiger frogs (genus *Hoplobatrachus*), a genus with representatives in Africa and Asia. The African *H. occipitalis* was the sister group of the Asian *H. crassus*, *H. chinensis*, and *H. tigerinus*. *Hoplobatrachus* was placed in a clade also containing the Asian genera *Euphlyctis* and *Nannophrys*. Combined analysis of sequences of 16S and 12S rRNA genes (total 903 bp) in a reduced set of taxa corroborated the monophyly of the lineage containing these three genera and identified the Asian genus *Fejervarya* as its possible sister group. The fact that the African *H. occipitalis* is nested within an otherwise exclusively Asian clade indicates its probable Oriental origin. Rough molecular clock estimates did not contradict the assumption that the dispersal event took place in the Miocene. Our data further identified a similar molecular divergence between closely related Asian and African species of *Rana* (belonging to the section *Hylarana*), indicating that Neogene intercontinental dispersal also may have taken place in this group and possibly in rhacophorid treefrogs. © 2001 Elsevier Science

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INTRODUCTION

According to plate tectonic schemes (Barron *et al.*, 1981), Asia and Africa—fragments of the Mesozoic su-

percontinents Laurasia and Gondwana, respectively—remained isolated by the Tethys Sea through most of the Cenozoic. Although limited faunal exchanges may have taken place in the early Paleogene (Krause and Maas, 1996), the Tethys apparently acted as a rather effective dispersal barrier, possibly reinforced by a climatic filter (Rage, 1988). The Neogene contact between both continents through the Arabian peninsula led to an intensive faunal exchange. This is apparent from the distribution patterns of extant taxa (e.g., Balke, 1995). It is especially well documented in the fossil record of mammalian faunas (Bernor *et al.*, 1987; Flynn and Winkler, 1994). However, few relevant data are available for amphibians, a second group with a limited capacity of oversea dispersal.

Frogs of the family Ranidae are a speciose group of controversial systematics with centers of diversity and endemism in Asia and Africa (Duellman and Trueb, 1985). Major disputes involve groups of unresolved relationships. For example, it is disputed whether rhacophorid and mantellid treefrogs, phylogenetically nested within the Ranidae, should be classified as subfamilies or families (Laurent, 1951, 1986; Dubois, 1981, 1987, 1992; Duellman and Trueb, 1985; Channing, 1989; Blommers-Schlösser, 1993; Marmayou *et al.*, 2000; Vences and Glaw, 2001). Due to the unresolved phylogeny, biogeographic hypotheses in the ranid radiation so far remain largely speculative. Comprehensive treatments are rare as most workers focused only on either African or Asian ranid taxa (e.g., Clarke, 1981; Emerson and Ward, 1998; Emerson *et al.*, 2000a; see discussion in Dubois, 1981, 1987, 1992).

Recent molecular studies challenged ranid classification by questioning the monophyly of several genera (Richards and Moore, 1998; Marmayou *et al.*, 2000; Bossuyt and Milinkovitch, 2000; Emerson *et al.*, 2000b). Oriental ranids are generally thought to have originated by ancestors that reached Asia on the Indian continental plate (Duellman and Trueb, 1985; Bossuyt and Milinkovitch, 2001). However, the genus

Tomopterna, with representatives in Africa, Asia, and Madagascar, was demonstrated to consist of three unrelated geographic lineages and thus was uninformative in regard to vicariance or dispersal patterns in ranid biogeography (Vences *et al.*, 2000a).

A further ranid subgroup present in Africa, Asia, and Madagascar is *Hoplobatrachus* (*sensu* Dubois, 1992). This genus contains large, conspicuous frogs, common in swamps and rice fields, which often are consumed by humans. They have usually been named bullfrogs, but to avoid confusion with other, unrelated, "bullfrog" species such as *Pyxicephalus adspersus*, *Rana catesbeiana*, or *Leptodactylus pentadactylus*, we will refer to the genus as tiger frogs. This name makes reference to the carnivorous habits of their tadpoles and to the scientific name of *H. tigrinus*, one of the first described species of the genus.

Close relationships of the single African tiger frog species *H. occipitalis* (see Appendix 2) to the Asian taxa have long been assumed and were generally not explicitly questioned (e.g., Clarke, 1981). However, no phylogenetic data demonstrating these relationships have been published. *H. tigrinus* has been reported as being introduced into Madagascar by Guibé (1953), but neither the circumstances of the introduction nor the specific identities of the Malagasy populations have been clarified since (Blommers-Schlösser and Blanc, 1991). In the present study we assessed the phylogenetic relationships of all nominal species of tiger frogs and of potentially related ranid lineages using mitochondrial DNA sequences. Our main purpose was to test the hypothesis of amphibian dispersal between Africa and Asia and thus to contribute to the understanding of the regional historical biogeography.

MATERIALS AND METHODS

Tissue samples (muscle; either fresh or preserved in 98% ethanol) were available from 40 specimens belonging to 34 ranid and 1 outgroup species. Voucher specimens are listed in Appendix 1. DNA was extracted with QIAamp tissue extraction kits (Qiagen). We used the primers (of Palumbi *et al.*, 1991) 16SA-L (light chain; 5'-CGC CTG TTT ATC AAA AAC AT-3') and 16SB-H (heavy chain; 5'-CCG GTC TGA ACT CAG ATC ACG T-3') to amplify a section of the mitochondrial 16S ribosomal RNA gene, the primers 12SA-L (light chain; 5'-AAA CTG GGA TTA GAT ACC CCA CTA T-3') and 12SB-H (heavy chain; 5'-GAG GGT GAC GGG CGG TGT GT-3') to amplify a section of the mitochondrial 12S ribosomal RNA gene, and the primers L14841 (light chain; 5'-CTC CCA GCC CCA TCC AAC ATC TCA GCA TGA TGA AAC TTC G-3'), modified from Kocher *et al.* (1989), and CB3-H (heavy chain; 5'-GGC AAA TAG GAA GTA TCA TTC TG-3'), modified from Palumbi *et al.* (1991), to amplify a section of the mitochondrial cytochrome *b* (cyt *b*) gene.

PCR conditions followed Vences *et al.* (2000b) and Mausfeld *et al.* (2000). PCR products were purified with QIAquick purification kits (Qiagen) and sequenced with an automatic sequencer (ABI 377). Sequences (see Appendix 1 for GenBank accession numbers) were aligned manually with the computer program SEQUENCE NAVIGATOR (Applied Biosystems). An additional sequence from GenBank (*H. occipitalis*; AF261263) was further added to the alignment.

To assess whether the different gene fragments could be submitted to combined analysis, we tested all possible combinations using the partition homogeneity test (parsimony method of Farris *et al.*, 1995), as implemented in PAUP*, version 4b8 (Swofford, 2001). Prior to phylogenetic reconstruction, we explored which substitution model best fit our sequence data. We applied a hierarchical likelihood ratio test for testing the goodness-of-fit of nested substitution models. Using the program MODELTEST (Posada and Crandall, 1998), we calculated the test statistic $\delta = 2 \log \Lambda$, with Λ being the ratio of the likelihood of the null model divided by the likelihood of the alternative model (for details see Huelsenbeck and Crandall, 1997).

Phylogenetic analyses were carried out with PAUP*. We calculated maximum-parsimony (MP) trees with gaps treated as a fifth character and neighbor-joining (NJ) and maximum-likelihood (ML) trees with gaps treated as missing data. In the MP analyses we conducted heuristic searches with initial trees obtained by simple stepwise addition, followed by branch swapping using the TBR (tree bisection-reconnection) routine implemented in PAUP*. Only minimal-length trees were saved and zero-length branches were collapsed. We carried out searches by unweighted parsimony, based on transversions only and by weighting transversions 2:1 and 3:1 against transitions. The NJ method was conducted under the HKY85 model of character substitution (Hasegawa *et al.*, 1985) with base frequencies and gamma distribution parameter for site heterogeneity estimated from the data set. The ML trees were obtained with heuristic searches with settings as in MP, with the substitution model proposed by MODELTEST for each data subset.

Following Hedges (1992), 2000 bootstrap replicates (Felsenstein, 1985) were run in all analyses except ML, for which only 100 bootstrap replicates were run in the combined analysis due to computational constraints. Additionally, the robustness of nodes was tested by Kishino-Hasegawa tests (Kishino and Hasegawa, 1989) as implemented in PAUP* (RELL bootstrap, 1000 replicates, one-tailed test). All gene fragments were checked for clock-like behavior with the program TREE PUZZLE 5.0 (Schmidt *et al.*, 2000).

RESULTS

The 41 aligned 16S sequences of 34 ranid and 1 bufonid outgroup species had a total length of 572 bp. Of these, 287 were constant, 64 variable and parsimony uninformative, and 221 variable and parsimony informative.

The assumption of a molecular clock resulted in a tree with a log-likelihood significantly worse than that of the more complex tree without a clock ($\log L_{\text{with clock}} = -6605.80$, $\log L_{\text{without clock}} = -6509.55$; likelihood ratio test statistic $\Delta = 192.50$). The simpler (clocklike) tree was rejected on the 0.05 significance level.

MODELTEST proposed a general time-reversible substitution model (GTR + G + I) as best fitting the 16S data, with a proportion of invariable sites of $I = 0.4182$, a gamma distribution shape parameter of 0.7219, empirical base frequencies (A: 0.3542; C: 0.1965; G: 0.1839; T: 0.2654), and substitution rates ($\text{rate}_{[A-C]} = 5.98$, $\text{rate}_{[A-G]} = 12.45$, $\text{rate}_{[A-T]} = 7.12$, $\text{rate}_{[C-G]} = 1.25$, $\text{rate}_{[C-T]} = 33.85$, all other rates = 1.0) estimated from the data set. These parameters were used for ML analysis.

The resulting ML tree (not shown) agreed well with the strict consensus of four equally most parsimonious trees as obtained by MP analysis (not shown; tree length 1500 steps; consistency index 0.351) and the NJ tree (Fig. 1) in all main aspects. Use of only transversions for MP analysis or differential weighting of transitions and transversions (2:1, 3:1) did not result in relevant differences of topology.

Hoplobatrachus was a monophyletic group, supported by moderate bootstrap values (60 and 75%, respectively, in NJ and MP analyses); the African *H. occipitalis* was the sister group of the three Asian species *H. crassus*, *H. tigrinus*, and *H. chinensis*. The Malagasy specimen was grouped with *H. tigrinus* from India. Bootstrap support for these intrageneric groupings was moderate to high (74–97%). *Hoplobatrachus* was placed in a monophyletic group with two other Asian genera, *Nannophrys* and *Euphlyctis* (93 and 65%).

Several further aspects of the obtained trees are noteworthy. *Strongylopus* and *Tomopterna* were grouped with *Cacosternum*, although the support of this topology was low (70 and 39%). European, African, and Asian species of *Rana* were grouped as a monophylum (60 and 89%). The African *Ptychadena* and *Pyxicephalus* were grouped as the most basal taxa.

For the 12S rRNA sequences (331 bp) in a reduced set of 15 taxa, MODELTEST proposed a Tamura–Nei substitution model (TrN + G), with no invariable sites, a gamma distribution shape parameter of 0.3299, empirical base frequencies (A: 0.3603; C: 0.2216; G: 0.1564; T: 0.2618), and substitution rates ($\text{rate}_{[A-G]} = 3.84$, $\text{rate}_{[C-T]} = 7.55$, all other rates = 1.0) estimated from the data set. MP, NJ, and ML analyses placed *H.*

occipitalis as sister group of *H. chinensis* (MP and NJ: 65 and 62%), *Euphlyctis* as sister group of *Nannophrys* (82 and 58%), and these three taxa as a monophylum (76 and 43%).

Substitutions in the cytochrome *b* analysis were best explained by a Tamura–Nei model (TrN + I + G) according to MODELTEST, with a proportion of invariable sites of $I = 0.4445$ and a gamma distribution shape parameter of 0.4357. Empirical base frequencies (A: 0.2915; C: 0.3238; G: 0.0995; T: 0.2807) and substitution rates ($\text{rate}_{[A-G]} = 9.40$ and $\text{rate}_{[C-T]} = 20.69$; all other rates = 1.0) were used for further analysis. The separate analysis of cytochrome *b* sequences (454 bp) resulted in very low bootstrap support of the obtained topology. The only clade with a bootstrap value larger than 50% was the *Euphlyctis*–*Nannophrys* lineage (MP and NJ: 65 and 58%). This coding gene fragment thus was not informative in regard to the origin of African *Hoplobatrachus* and probably is in general not suitable for the analysis of higher-level phylogeny in amphibians (Graybeal, 1993).

The partition homogeneity test revealed that the 12S and 16S structural gene partitions were suitable for combination (homogeneity not rejected; $P = 0.23$), but not together with the coding *cyt b* fragment (for the combination of all three fragments, for 12S/*cyt b*, and for 16S/*cyt b*, fragment homogeneity was rejected; $P = 0.01$).

For the combined 12S/16S data set, a general time-reversible substitution model (GTR + G + I) fitted best, with a proportion of invariable sites of $I = 0.3841$ and a gamma distribution shape parameter of 0.6591 estimated from the data set. Empirical base frequencies (A: 0.3645; C: 0.2199; G: 0.1462; T: 0.2694) and substitution rates ($\text{rate}_{[A-G]} = 3.88$ and $\text{rate}_{[C-T]} = 6.91$; all other rates = 1.0) were used for further analysis. The combined analysis of 16S and 12S rRNA gene fragments largely corroborated the results outlined above (Fig. 2). Clocklike behavior of the combined sequences was rejected ($P < 0.05$). In the MP analysis, 505 of the 903 characters were constant, 117 were variable and parsimony uninformative, and 281 were variable and parsimony informative. Two equally most parsimonious trees were found (length 2210 steps, consistency index 0.481). The two included *Hoplobatrachus* species were a monophyletic group in the MP bootstrap consensus tree (bootstrap support 41%) and in the NJ and the ML analyses (bootstrap support 81 and 85%), but not in the most parsimonious trees. *Euphlyctis* and *Nannophrys* were sister groups (bootstrap support 85, 92, and 83%, respectively, in NJ, MP, and ML analyses). The group composed of these two genera and of *Hoplobatrachus* was strongly supported (100, 90, and 66%). *Fejervarya* was identified as a possible sister group of this clade (54, 66, and 88%). Trees

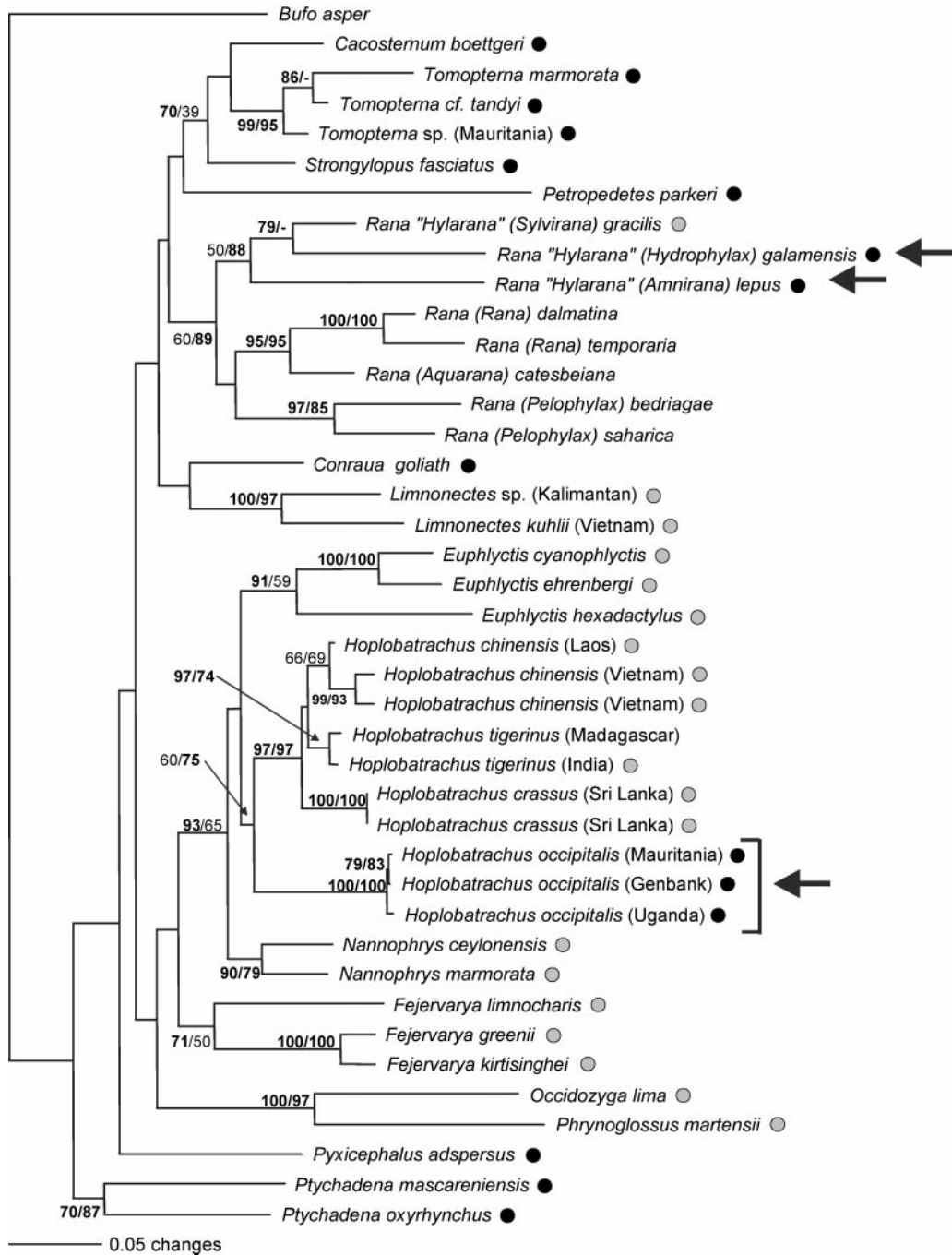


FIG. 1. Neighbor-joining phylogram (HKY85 distances; gamma shape factor 0.7219) of taxa studied based on 572-bp sequences of the 16S rDNA gene. *Bufo asper* (Bufonidae) was used as outgroup. Numbers are bootstrap values in percentage (2000 replications) for NJ and MP analyses, respectively. No values are given for nodes which received support below 50% in both analyses. Values above 70% are printed in boldface. A hyphen refers to cases in which the corresponding topology was not present in the respective bootstrap consensus tree. Black circles represent subsaharan African species; gray circles represent Asian species; species from Europe, North Africa, and North America are not marked. Boldface arrows mark African species with a likely origin by dispersal from Asia.

(not shown) obtained from combined MP analysis with only transversions and weighting transitions and transversions (2:1, 3:1) agreed with these results.

Additionally, the obtained tree topologies (16S and 12S/16S combined) were tested by Kishino–Hasegawa

tests against all possible trees that would indicate a non-Asian origin of African tiger frogs (by placement of the *H. occipitalis* clade as sister group to the African species included). Kishino–Hasegawa test revealed that all of the tested topologies were significantly

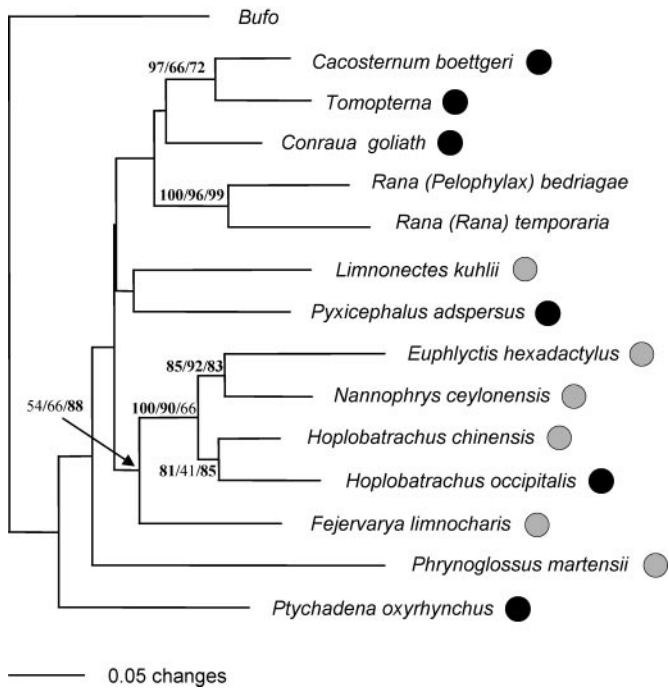


FIG. 2. Neighbor-joining phylogram (HKY85 distances; gamma shape factor 0.6591) of a reduced set of taxa based on the combined analysis of 572-bp sequences of the 16S rRNA gene and 331-bp sequences of the 12S rRNA gene. For *Tomopterna*, we combined sequences of *T. marmorata* (16S) and *T. cf. tandyi* (12S). *Bufo asper* (Bufonidae) was used as outgroup. Numbers are bootstrap values in percentage (2000 replications for NJ and MP analyses, 100 for ML analyses). See legend of Fig. 1 for additional explanations.

worse than the original tree (the null hypothesis was refuted with $P < 0.01$).

DISCUSSION

This study provides, for the first time, genetic indications for monophyly of a ranid genus occurring in Asia and in subsaharan Africa. Although the MP analysis failed to support unambiguously the relationships between *H. chinensis* and *H. occipitalis* in the 12S/16S combined analysis, we consider the available support for monophyly of tiger frogs rather convincing. In addition to the mitochondrial evidence presented here, the African *H. occipitalis* shares with the Asian tiger frogs many similarities in morphology and osteology (Bolkay, 1915; Boulenger, 1918; Deckert, 1938; Laurent, 1950; Clarke, 1981). Unambiguous synapomorphies are found in the derived tadpole morphology (Pope, 1931; Dubois, 1987, 1992; Fei *et al.*, 1991) with shared detailed structures, including double keratodont rows (unique in ranids; see Noble, 1927, pp. 68–69), a strongly developed horny beak, and large keratinized “supplementary teeth” on the buccal roof (Fig. 3).

Even better supported by the genetic data was the

identification—among the genera included in the study—of *Euphlyctis* and *Nannophrys* as sister groups of *Hoplobatrachus*. These three genera may be related to *Fejervarya*, as indicated by the combined tree. Another Asian genus possibly related to this assemblage is *Sphaerotheca* (Marmayou *et al.*, 2000; Vences *et al.*, 2000a). In contrast, close relationships of *Hoplobatrachus* to African, European, and North American ranid genera could significantly be excluded. This also applies to several robust forms which due to their large body size may appear as superficially similar to tiger frogs, i.e., the African *Pyxicephalus* and *Conraua* and the North American bullfrog *R. catesbeiana*. It can therefore be assumed that the current distribution of *Hoplobatrachus* is due to intercontinental dispersal. The alternative explanation, a Mesozoic Gondwanan origin of *Hoplobatrachus* ancestors, who then could have reached Asia on the drifting Indian plate (see Duellman and Trueb, 1985), can be rejected because (a) *Hoplobatrachus* is nested within a clade of otherwise exclusively Asian frogs, (b) no endemic *Hoplobatrachus* are present on Madagascar which was part of an isolated Madagascar–India continent between 130 and 90 million years (my) before present (Barron *et al.*, 1981), and (c) such an ancient divergence between African and Asian species is not in accordance with the low amount of genetic differentiation.

The pairwise 16S rRNA differentiation between *H. occipitalis* and the Asian tiger frogs is 8–10% (2–4% with transversions only) (Table 1). It is conspicuous that the divergence level between the Asian *Rana (Sylvirana) gracilis* on the one hand and the African *Rana (Amnirana) lepus* and *Rana (Hydrophylax) galamensis* on the other hand (all belonging to the section “*Hylarana*,” sensu Dubois, 1992) is rather similar to that found in *Hoplobatrachus* (11%; 4% with transversions only). A further example of African ranoid anurans related to Asian taxa is found within the rhacophorids

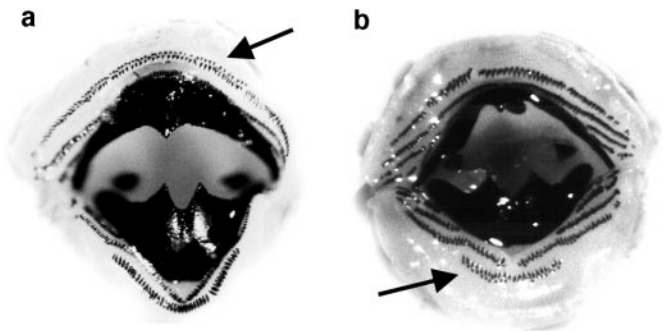


FIG. 3. Photographs of mouth parts of tadpoles of (a) *H. occipitalis* (MNHN 2000.2911; Ivory Coast) and (b) *H. chinensis* (MNHN 1999.425; Vietnam) showing the probably synapomorphic arrangement of keratodonts in double rows; this state is, as far as is known, unique among ranids and has so far been largely ignored in attempts to assess ranid relationships (but see Dubois, 1987, 1992; Fei *et al.*, 1991).

TABLE 1

Pairwise Sequence Divergences in the 16S rRNA Gene Fragment Studied among Probable African-Asian Ranid Sister Groups

Species pair	16 S divergence (all substitutions, including indels)	16 S divergence (transversions only)
<i>Hoplobatrachus occipitalis</i> - <i>H. tigrinus</i>	56 Substitutions (10%)	19 Substitutions (4%)
<i>Hoplobatrachus occipitalis</i> - <i>H. chinensis</i>	47 Substitutions (8%)	13 Substitutions (2%)
<i>Hoplobatrachus occipitalis</i> - <i>H. crassus</i>	57 Substitutions (10%)	14 Substitutions (2%)
<i>Rana galamensis</i> - <i>R. gracilis</i>	63 Substitutions (11%)	22 Substitutions (4%)
<i>Rana lepus</i> - <i>R. gracilis</i>	61 Substitutions (11%)	22 Substitutions (4%)
<i>Chiromantis xerampelina</i> - <i>Buergeria robusta</i>	66 Substitutions (12%)	33 Substitutions (6%)
<i>Chiromantis xerampelina</i> - <i>Polypedates leucomystax</i>	71 Substitutions (12%)	33 Substitutions (6%)
<i>Chiromantis xerampelina</i> - <i>Rhacophorus nigropalmatus</i>	88 Substitutions (15%)	31 Substitutions (5%)

Note. The last three rows contain rhacophorid treefrogs, which are considered either a ranid subfamily or a separate family. As the sister group of the African *Chiromantis* has not been unambiguously identified, we compared it with representatives of three genera. The fact that *Chiromantis* shows the lowest differentiation to *Buergeria* is probably due to decreased substitution rates in this latter genus, which is thought to contain the most basal rhacophorids.

(Richards and Moore, 1998). However, the genetic Asiatic-African divergence in this case is distinctly higher (12–15%; 5–6% with transversions only).

With the genetic allozyme distances of Beerli *et al.* (1996) and their assumed clock rate, the divergence between *Rana bedriagae* and *R. saharica* can be calculated at 10–8 million years before present. With the 16S rRNA divergence of 8.7% between these two species, this would yield a rate of ca. 1% pairwise sequence divergence per my. Rates assumed for the 16S and 12S rRNA genes in other amphibians range between 0.4 and 0.7%/my (Caccone *et al.*, 1997; Veith *et al.*, 1998). For transversions, rates of 0.10–0.16%/my have been estimated (Caccone *et al.*, 1997). Application of these rates would place the African-Asian *Hoplobatrachus* divergence at 25–8 my before present (40 my with the lowest transversion rate and the highest transversion divergence). Also, the corresponding divergences between Asian and African *Rana* of the *Hylarana* section may be estimated within this interval, assuming a slightly accelerated molecular clock in this group. Such small rate differences are in accordance with the non-clock behavior of the gene fragments studied and with the longer branches of *Rana* species compared to *Hoplobatrachus* (Figs. 1 and 2). In contrast, the dispersal of rhacophorids may have occurred earlier.

The large spread between minimum and maximum estimates is caused by uncertainties in the application of the different calibrations available. Only such very rough estimates are possible until reliable ranid calibrations become available. Nevertheless, it can be stated that the results of this conservative approach are in accordance with a more or less parallel intercontinental dispersal of several anuran groups between Asia and Africa in the Neogene. It is sound to assume that these took place at the moment of contact between the Arabian peninsula and the continent of Africa in the Miocene, at 20–17 my (Bernor *et al.*, 1987). The

dispersal ability of the involved lineages is also emphasized by the fact that *Hoplobatrachus* and *Chiromantis* contain savannah inhabitants able to colonize rather arid habitats. The same is true for at least some *Rana* of the *Hylarana* section (such as *R. galamensis*).

The molecular data positioned the African *H. occipitalis* in a lineage that, except for *Hoplobatrachus*, contains three Asian genera: *Nannophrys*, *Euphlyctis*, and *Fejervarya*. The assumption of an African origin of this lineage would imply three dispersal events, namely of the ancestors of *Fejervarya*, of the *Nannophrys/Euphlyctis* lineage, and of the Asian *Hoplobatrachus*, to Asia. In contrast, a single dispersal event (of the *H. occipitalis* ancestor into Africa) would suffice to explain an Asian origin of the lineage, which therefore appears as the most parsimonious hypothesis. No conclusive statement can be made on the *Hylarana* section of *Rana* as only a small subset of this group was included in this study. However, according to Dubois (1992), the largest number of species and subgenera of this section are found in Asia, and an Oriental origin of its African representatives may therefore be taken into consideration. Rhacophorids are represented in Africa only by the genus *Chiromantis* (three species), but contain a much larger diversity at the genus and species levels in Asia (Duellman and Trueb, 1985). *Chiromantis* is nested within the Asian lineage, close to the genera *Buergeria*, *Rhacophorus*, and *Polypedates* (Richards and Moore, 1998 and our own data), which makes dispersal of its ancestors from Asia likely (Laurent, 1951).

The phylogeny among deeper ranid lineages is largely unresolved (e.g., Dubois, 1992; Blommers-Schlösser, 1993; Bossuyt and Milinkovitch, 2000, 2001; Emerson *et al.*, 2000a,b; Marmayou *et al.*, 2000; Vences *et al.*, 2000a,c). The centers of diversity of the group, both at the generic and at the species level, are Africa and Asia (and Madagascar, if rhacophorid and mantel-

lid treefrogs are considered part of the ranid lineage) (Duellman and Trueb, 1985). Only a reduced number of groups of the genus *Rana* occur in North America, and only a few species of that genus dispersed into the Neotropics (Hillis and Davis, 1986; Dubois, 1992). Although vicariance scenarios have been proposed to explain the presence of ranids in Asia (Duellman and Trueb, 1985), the lack of deep endemic lineages in the Neotropics may indicate a radiation posterior to Gondwana fragmentation (Feller and Hedges, 1998). Ranid fossils are known since the Eocene of Europe (Rage, 1984), although unpublished data indicate the possible existence of remains from the Late Cretaceous (Sanchiz, 1998). In several published molecular phylogenies and in the trees presented here, African and Asian ranid lineages were grouped at basal positions of the tree. However, none of these topologies was sufficiently corroborated. While the present paper was in review, Bossuyt and Milinkovitch (2001) proposed a scenario of Early Tertiary "Out-of-India" dispersal of ranids. Their hypothesis requires the assumption of

Late Mesozoic or Early Tertiary exchanges between ancestral African, European, and Asian faunas. Convincing evidence for such multiple intercontinental radiations, however, is yet to be found. Currently, the genus *Hoplobatrachus* and probably the *Hylarana* section of *Rana* and the rhacophorid genus *Chiromantis* are the only corroborated examples for Tertiary Asiatic-African dispersal among ranids.

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APPENDIX 1

Specimens Examined

Species	Origin	Collection No.	Genbank accession 16S	Genbank accession 12S	Genbank accession Cyt <i>b</i>
<i>Bufo asper</i>	Tanak Masa island, West Sumatra (16S)	—	AF124109	U52733*	—
<i>Bufo macrocristatus</i>	—	—	—	—	AY008250
<i>Cacosternum boettgeri</i>	Bredell, South Africa (12S); Hardap, Namibia (16S)	ZFMK 66727	AF215414	AF124096/ AF215208	AY014384
<i>Conraua goliath</i>	Cameroon	ZFMK 64829	AF215388	AF215190	AY014385
<i>Euphlyctis cyanophlyctis</i>	Cochin, India	MNHN 2000.650	AY014366	—	—
<i>Euphlyctis ehrenbergi</i>	Yemen	MNHN 2000.649	AY014367	—	—
<i>Euphlyctis hexadactylus</i>	Unawatuna, Sri Lanka	not collected	AF215389	AF215191	AY014386
<i>Fejervarya greenii</i>	Nuwara Eliya, Sri Lanka	MNHN 2000.617	AY014378	—	—
<i>Fejervarya kirtisinghei</i>	Laggalla, Sri Lanka	MNHN 2000.620	AY014380	—	—
<i>Fejervarya limnocharis</i>	Laos	MNHN 1997.3932	AF215416	AF215210	AY014387
<i>Hoplobatrachus chinensis</i>	Ky Thuong, Vietnam	ZFMK TZ55	AF285208	—	—
<i>Hoplobatrachus chinensis</i>	Vietnam	ZFMK TZ301	AY014372	—	—
<i>Hoplobatrachus chinensis</i>	Laos	MNHN 1997.4900	AY014368	AY014362	AY014389
<i>Hoplobatrachus crassus</i>	Unawatuna, Sri Lanka	not collected	AY014375	—	—
<i>Hoplobatrachus crassus</i>	Sri Lanka	—	AY014369	—	—
<i>Hoplobatrachus occipitalis</i>	Uganda	ZFMK 65186	AY014374	AY014361	AY014388
<i>Hoplobatrachus occipitalis</i>	Mauritania	ZFMK 23WB01	AY014373	—	—
<i>Hoplobatrachus occipitalis</i>	Comoe NP, Ivory Coast	FMNH 257224	AF261263*	—	—
<i>Hoplobatrachus tigerinus</i>	South India	—	AY014371	—	—
<i>Hoplobatrachus tigerinus</i>	Antsiranana, Madagascar	ZSM 503/2000	AY014370	—	—
<i>Limnonectes kuhlii</i>	Laos	MNHN 1997.3904	AF215415	AF215209	AY014390
<i>Limnonectes</i> sp.	Kalimantan	MV 245	AY014379	—	—
<i>Nannophrys ceylonensis</i>	Kitulgala, Sri Lanka	MNHN 2000.627	AF215394	AF215197	AY014391
<i>Nannophrys marmorata</i>	Laggalla, Sri Lanka	MNHN 2000.621	AF215395	—	—
<i>Occidozyga lima</i>	Philippines	ZMB 50910	AF215398	—	—
<i>Petropedetes parkeri</i>	Nguti, Cameroon	not preserved	AF124132	—	—
<i>Phrynoglossus martensii</i>	Laos	MNHN 1997.3948	AF215401	AY014364	AY014393
<i>Ptychadena mascareniensis</i>	Moramanga, Madagascar	ZFMK 66683	AF215407	—	—

APPENDIX 1—Continued

Species	Origin	Collection No.	Genbank accession 16S	Genbank accession 12S	Genbank accession Cyt b
<i>Ptychadena oxyrhynchus</i>	Kwambonambi, South Africa	ZFMK uncatalogued	AF215403	AF215205	AY014394
<i>Pyxicephalus adspersus</i>	KwaMbonambi, South Africa	ZFMK 66446	AF215505	AY014365	AY014395
<i>Rana (Amnirana) lepus</i>	Cameroon	ZFMK 64830	AY014377	—	—
<i>Rana (Aquarana) catesbeiana</i>	—	—	X12841*	—	—
<i>Rana (Hydrophylax) galamensis</i>	Kibungo, Kito, Rwanda	—	AY014382	—	—
<i>Rana (Pelophylax) bedriagae</i>	Antalya, Turkey	not preserved	AF215422	AY014363	AY014392
<i>Rana (Pelophylax) saharica</i>	La Pecherle, Tunisia	not preserved	AF215426	—	—
<i>Rana (Rana) temporaria</i>	Koblenz, Germany	ZFMK 69883	AF124135	AF124103	AY014396
<i>Rana (Rana) dalmatina</i>	Altube, Spain	ZFMK 68392	AY014381	—	—
<i>Rana (Sylvirana) gracilis</i>	Belihuloya, Sri Lanka	MNHN 2000.614	AY014376	—	—
<i>Strongylopus fasciatus</i>	Little Brak, South Africa	ZFMK 66444	AF215412	—	—
<i>Tomopterna marmorata</i>	Mt. Meru, South Africa	not preserved	AF215507	—	AY014397
<i>Tomopterna cf. tandyi</i>	Khorixas, Namibia	ZFMK 66403	AF215419	AF215213	—
<i>Tomopterna sp.</i>	Mauritania	ZFMK WB02	AY014383	—	—

Note. Collection abbreviations: FMNH, Field Museum, Chicago; MNHN, Muséum National d'Histoire Naturelle, Paris; MV, field number of Michael Veith, specimens to be catalogued in the Field Museum, Chicago; ZFMK, Zoologisches Forschungsinstitut und Museum A. Koenig, Bonn; ZMB, Zoologisches Museum der Universität, Berlin; ZSM, Zoologische Staatssammlung, München. GenBank accession numbers marked with an asterisk refer to sequences obtained by other authors.

APPENDIX 2

Taxonomic and Nomenclatural Notes

Considering the controversial classification of ranid frogs, it appears necessary to provide justifications for several generic assignments which implicitly were followed throughout the present paper. Generally, the taxonomy employed follows the proposals of Dubois (1992), with a few modifications.

(1) Dubois (1981, 1987, 1992) considered *H. demarchii* a second African member of *Hoplobatrachus*. However, examination of a syntype of *Rana demarchii* Scortecci, 1929 (The Natural History Museum, BMNH 1930.2.1.2) showed that *demarchii* belongs to the Palearctic subgenus *Rana (Pelophylax)*. This is well corroborated by its general morphology, light dorsal coloration with distinct dark markings, and unforked omosternum as revealed by X-ray pictures. On the other hand, evidence exists that at least two different species are currently mixed under the name *Hoplobatrachus occipitalis* (see Bogart and Tandy, 1976; Dubois, 1981).

(2) Evidence will be presented elsewhere that *Hoplobatrachus chinensis* (Osbeck, 1765) is the valid name for the species called *Rana rugulosa* or *Hoplobatrachus rugulosus* by various authors (A. Dubois and A. Ohler, unpublished data).

(3) Our results (Fig. 2) demonstrate that the African clade of *Hoplobatrachus* (i.e., *H. occipitalis*) is distinct from the clade comprising the three Asian representatives (*H. chinensis*, *H. crassus*, *H. tigerinus*). Given a probable Miocene age of separation of the two clades as

discussed above, it seems probable that detailed examinations will also reveal morphological and osteological differences between them, which may justify a separation at the subgenus level. In this case, as pointed out by Dubois (1981, p. 240), the name *Hoplobatrachus* Peters, 1863 (type species by monotypy, *Hoplobatrachus ceylanicus* Peters, 1863, junior subjective synonym of *Rana crassa* Jerdon, 1853) would apply to the Asian species, and *Ranosoma* Ahl, 1924 (type species by original designation, *Ranosoma schereri* Ahl, 1924, junior subjective synonym of *Rana occipitalis* Günther, 1859) would be available to name the African lineage.

(4) Reasons for considering *Fefervarya* a distinct genus were provided by Dubois and Ohler (2000); the present data confirm this interpretation.

(5) *Strongylopus* has been considered as a subgenus of *Rana* by Dubois (1992), but as a separate genus by other authors (e.g., Passmore and Carruthers, 1995). Molecular data presented here do not indicate close relationships of *Strongylopus* to *Rana*; the taxon is therefore considered as a distinct genus.

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