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**I. The Taxonomic Status of the Genus *Nesionixalus* Perret,  
1976 (Anura: Hyperoliidae), Treefrogs of São Tomé and  
Príncipe, with Comments on the Genus *Hyperolius***

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The endemic hyperoliid treefrogs of São Tomé and Príncipe currently recognized as *Nesionixalus thomensis* (Bocage, 1986) and *N. mollerii* (Bedriaga, 1892) are re-examined. The results of two molecular analyses indicate that these taxa are closely related to each other (monophyletic) but nest within the genus *Hyperolius*. A comparison of the morphological character states used by Perret (1976; 1988) to erect the genus *Nesionixalus* from within *Hyperolius* Rapp with a broad range of *Hyperolius* species reveals that most of these are not unique to the island endemics; all except the characters of size and digital tip shape are distributed among a number of other species within the latter genus. *Nesionixalus* Perret, 1976 is returned to the synonymy of *Hyperolius* Rapp and *Nesionixalus thomensis* and *N. mollerii* to *Hyperolius thomensis* Bocage and *H. mollerii* Bedriaga, respectively.

The islands of São Tomé and Príncipe (which together comprise the tiny African Republic of São Tomé e Príncipe) lie along a magmatic geological feature known as the Guinea Line (or Cameroon Volcanic Line), which is a flaw (or hotline — Meyers et al. 1998) in the African tectonic plate between 1000–1500 km long (Simkin et al. 1994; Burke 2001) that has served as a channel for magmas for millions of years. The Guinea Line extends across the ocean-continent transition, and magmatic extrusions up through it have given rise to major oceanic and continental topographic relief extending from southwest to northeast including the oceanic islands of Annobón (Pagalu; 4.9 my), São Tomé (13+ my), Príncipe (31+ my), the recent continental island of Bioko (known in colonial times as Fernando Poo and now part of the Republic of Equatorial Guinea), and the mainland features of Mount Cameroon, the various ranges that comprise the Cameroon Highlands, and the Jos Plateau of Nigeria (Lee et al. 1994). The islands of Annobón, Príncipe and São Tomé are oceanic islands in the sense of Darlington (1957), Carlquist, (1965) and MacArthur and Wilson (1967) and long known for the high levels of endemism in their biota; some taxa are shared between the latter two. The three islands are separated from each other and from the West African coastlines of Equatorial Guinea and Gabon by ocean depths in excess of 3000 m. Príncipe is geologically the oldest and only about 130 km<sup>2</sup> in area, but it is well-watered and has significant relief provided by a central volcanic peak of just under 1000 m. It is about 220 km southwest of Bioko, the only continental and most northerly island of the chain, about 220 km from the African

mainland, and approximately 146 km northeast of São Tomé. The latter is geologically younger than Príncipe and considerably larger, at 836 km<sup>2</sup>, with the central Pico do São Tomé rising to above 2000 m. São Tomé is situated 280 km off the mainland and 180 km northeast of the southern-most of the oceanics, tiny (17 km<sup>2</sup>) Annobón or Pagalu, which is part of the Republic of Equatorial Guinea. (Juste et al. 1994).

From late March to early June 2001, the California Academy of Sciences conducted a large, multi-disciplinary research expedition to both islands of the Republic of São Tomé e Príncipe (see Drewes 2002 for a popular account). Voucher specimens and tissues were collected of all of the amphibian and reptile species. This material allows us to address questions pertinent to both taxonomy and biogeography such as the status of the genus *Nesionixalus* relative to other genera in the family Hyperoliidae as well as to add natural history information based on our field observations. In this paper, we address the first issue.

The large, flamboyant treefrog, hitherto known as *Nesionixalus thomensis* (Bocage, 1886), is endemic to the island of São Tomé (Fig. 1A); a second, recently elevated species, *N. malleri* (Bedriaga 1892), is a smaller treefrog (Fig. 1B) present and widespread on both São Tomé and Príncipe. *Nesionixalus thomensis* was originally described as a member of the genus *Hyperolius* by Bocage (1886), the type material, five specimens from Roça Saudade (ca. 800 m), Rio Quiza (probably Rio Quija in SW part of the island; exact locality unknown), and Ile São Tomé was housed in the Museu Bocage in Lisbon. There the series was re-examined in the mid 1970s by Perret, who placed the species in a new genus, *Nesionixalus*, based on large snout-vent length (males: 30–35 mm; females: 42–47 mm), shape of digital tips (oval, broader than long), dorsal skin of males covered by fine spinosities (smooth in females), absence of vocal sac in males (m. interhyoideus undifferentiated), absence of buccal vocal sac openings in males, and large size but small number of eggs (Perret 1976). In 1978, the Museu Bocage was destroyed by fire and all herpetological material was lost (E.G. Crespo, *pers. commun.*).

In the course of his phylogenetic analysis of the Hyperoliidae which included 63 species of 15 genera (including *Semnodactylus* as *Kassina wealei*), Drewes (1984) located what appeared to be the only four museum specimens of *N. thomensis* still in existence, a male and female in London (BM 1951.1.1.91 and 98.3.30.39) and a pair in Vienna (NHMW 3695–6). The male specimens

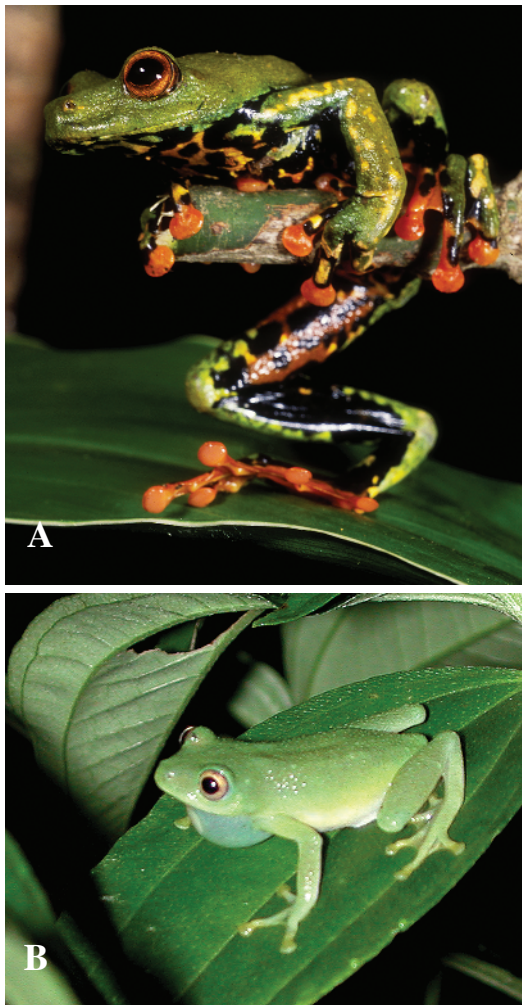


Figure 1A–B. (A) *Nesionixalus* (=Hyperolius) *thomensis* ♀; CAS 218934, São Tomé Id: Macambrara. Photo by. D. Lin. (B) *Nesionixalus* (=Hyperolius) *malleri* ♂; CAS 219203-07, Príncipe Id: Baie das Agulhas. Photo by R.C. Drewes.

exhibited vocal sacs consistent with Drewes' character 21, state 1 (e.g. sac simple; fibers of m. interhyoideus parallel) and buccal vocal sac openings consistent with character 20, state 2 (anterior slits). The four specimens were x-rayed but no additional character states were observed to support *Nesionixalus* as a separate genus within the context of Drewes' largely myo-skeletal data set, and he returned *Nesionixalus thomensis* to the synonymy of *Hyperolius thomensis* Bocage.

Four years later, Perret (1988) resurrected the genus *Nesionixalus*; he re-examined the Vienna material and while conceding the presence of the vocal sac and vocal sac openings as observed by Drewes (1984), he reiterated large snout-vent length, male integumental spinosities, transversely enlarged toe pads, and large size but small number of eggs (which he considered indicative of a specialized reproductive niche) as diagnostic. He added that the testes were large in size, the gular gland narrow and not conspicuous, and terminal phalanx obtuse or weakly notched ("*status 2 de Drewes, non hyperolien*"). He did not indicate which terminal phalanx nor how this latter observation was made. In the same work, Perret (1988) described three additional genera to accommodate Cameroonian species formerly accepted as members of the genus *Hyperolius* Rapp 1842: *Alexteroon* to include *Hyperolius obstetricans* Ahl, 1931, *Arlequinus* for *H. krebsi* Mertens, 1938, and *Chlorolius* for *H. koehleri* Mertens, 1940.

In 1992, Loumont reported on the results of field work undertaken in July 1988 and January 1990 on São Tomé and Príncipe; she revised the known amphibian fauna of both islands and provided the first chromosome data and advertisement calls for the endemic anurans. She recognized three hyperoliid tree frogs: *Leptopelis palmatus* (Peters 1868) known only from Príncipe; *Nesionixalus thomensis* (Bocage, 1886), endemic to São Tomé, and *Hyperolius molleri* Bedriaga, 1892, a green, moderate-sized treefrog which is numerous, widespread and endemic to both islands. Loumont (1992:51) placed *H. molleri* in the genus *Nesionixalus* along with *N. thomensis*, citing Perret's 1988 diagnostic characters. She also listed a number of character states in *N. molleri* that were more similar to typical *Hyperolius* than to *N. thomensis*. Loumont evidently collected no tissue samples and did not deal with anuran larvae — the tadpoles of the anuran endemics remain undescribed. In the same year, Loumont joined Schätti in a publication on the herpetofauna of São Tomé Island (Schätti and Loumont 1992).

In his additions and corrections to Frost's (1985) *Amphibian Species of the World*, Duellman (1993) recognized the genus *Nesionixalus* (*N. thomensis* and *N. molleri*) as well as the genera *Alexteroon*, *Arlequinus* and *Chlorolius* of Perret. Schiøtz (1999) included these taxa in his book on African treefrogs as well. In his generic description Schiøtz (1999, p.309) reiterates Perret's absence of vocal sac and vocal sac openings character states as diagnostic of the genus in spite of Drewes' (1984) demonstration of their presence in *Nesionixalus thomensis*, and Perret's (1988) subsequent acknowledgment that such is the case. Following his treatment of *N. thomensis*, *Nesionixalus molleri* is described as with a large gular sac (Schiøtz 1999:311).

To date, none of the species of *Nesionixalus*, *Alexteroon*, *Arlequinus* or *Chlorolius* has been tested or confirmed in a published molecular and/or morphological phylogenetic analysis.

## MATERIALS AND METHODS

Five species of *Hyperolius* and the two species of *Nesionixalus* (*N. thomensis* and *N. molleri* from both São Tomé and Príncipe) were used as the in-group in an initial analysis. *Leptopelis palmatus* was designated as the outgroup based on results from previous studies indicating that *Leptopelis* is at least basal to all other hyperoliid genera (Liem 1970; Drewes 1984; Channing 1989; Richards and Moore 1996; Emerson et al. 2000; Vences et al. 2003).

An approximately 2 kb sequence that includes part of the 12S and 16S ribosomal genes and

the t-RNA for valine was amplified and sequenced with the primers and protocols in Wilkinson et al. (1996) and Wilkinson (1997). All sequences produced for this study were deposited in GenBank (see Appendix, Table 1 for a list of sequences, their sources, and accession numbers).

The sequences were aligned following the procedure of Kjer (1993) using a putative secondary structure for *Rana catesbeiana* as a standard (Nagae 1988). Because of insertion and deletion events in variable regions, those bases considered to have ambiguous alignments were removed from the analyses.

We performed a maximum unweighted parsimony analysis using an exhaustive search. We also performed a bootstrap analysis (Felsenstein 1985) under maximum parsimony with 1000 replicates using the branch-and-bound search, and calculated Bremer's decay indices (Bremer 1994) to evaluate the amount of support for the resulting clades. The decay index analysis was facilitated by the program Autodecay (Eriksson 1998).

We performed a second analysis on approximately 500 b of only the 16S ribosomal gene in which, in addition to the species used in the first analysis, included DNA sequences retrieved from GenBank and Richards and Moore (1996) for two species each from the genera *Afrixalus*, *Heterixalus*, *Kassina*, the species *Tachycnemis seychellensis*, two more species and one more subspecies of the genus *Hyperolius*, and one more species from the genus *Leptopelis* (see Appendix, Table 1). Because of the additional taxa in this analysis, we used a heuristic search with 100 random stepwise additions followed by TBR branch swapping. We also performed a bootstrap analysis under maximum parsimony with 1000 replicates using the heuristic search with 10 random stepwise additions followed by TBR branch swapping and calculated Bremer's decay indices on this second data set. Museum symbolic codes used herein follow Leviton et al. (1985).

## RESULTS AND DISCUSSION

The first parsimony analysis resulted in one cladogram of 1167 steps with a consistency index (excluding uninformative characters) of 0.691, a retention index of 0.662, and a  $g_i$  statistics (Huelsenbeck and Hillis 1993) of -0.648 for the distribution of all possible trees in the exhaustive search. The second analysis resulted in four most parsimonious cladograms of 865 steps long with a consistency index (excluding uninformative characters) of 0.494 and a retention index of 0.613.

In both analyses *Nesionixalus thomensis* and *N. mollerii* formed a well-supported clade nested within the genus *Hyperolius*, with *H. cinnamomeoventris* as the sister taxon to the *Nesionixalus* clade (Figs. 2

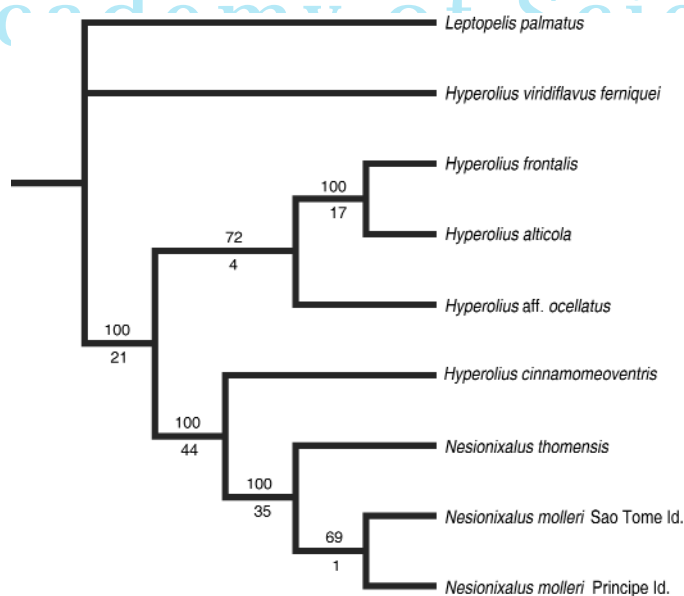


FIGURE 2. The most parsimonious cladogram resulting from an exhaustive search in a maximum parsimony analysis on approximately 2000 bases for five of the species of *Hyperolius* and two species of *Nesionixalus* in Table 1. Numbers above branches represent bootstrap support for the respective clade, while numbers below branches are decay indices.



and 3). In the second analysis, the genus *Hyperolius* formed a well-supported clade separate from all other genera in the analysis except *Nesionixalus* (Fig. 3). Also, *N. mollerii* (CAS 218848) from São Tomé formed a clade with *N. thomensis* instead of *N. mollerii* (CAS 219125) from Príncipe in two of the four most parsimonious cladograms, indicating that the reduced number of characters in this analysis could not separate *N. mollerii* from *N. thomensis*.

Our molecular results indicate two possible conclusions: either (1) *Nesionixalus* is not a valid genus (Drewes 1984), and both *N. thomensis* and *N. mollerii* are members of the genus *Hyperolius*, or (2) the species of *Nesionixalus* are a clade deserving of generic recognition, and their inclusion in the genus *Hyperolius* as currently recognized renders the latter paraphyletic.

In light of the results of the analyses above, we reexamined the characters used by Perret (1976, 1988) to define the genus *Nesionixalus*. We relied heavily on an unpublished M.A. dissertation by L.G. Wilson (2000). This work is important to a discussion of the genus *Hyperolius* for at least three reasons: (1) it is the first attempt at a phylogenetic analysis of the genus (Wieczorek et al. [2001] focused on a subset — the *H. viridiflavus* complex), (2) it includes the largest sample size (31) of *Hyperolius* species studied to date (fifty-one morphological characters were employed, and outgroup species included four of the genus *Afraxalus*, three of the Malagasy *Heterixalus*, and *Cryptothylax greshoffi*); and (3) it is the first cladistic analysis that includes three out of four of Perret's (1988) new genera, *Alexteroon*, *Chlorolius* and *Nesionixalus*. Specimens of *Arlequinus* have been unavailable for study thus far.

#### Perret's Diagnostic Characters

Most of the characters used by Perret (1976, 1988) to define *Nesionixalus thomensis* are equivocal with the exception of the transverse oval shape of the finger and toe tips:

#### Size

Snout-vent lengths in the lost type series were given as 30.0 and 35.0 mm for the two adult

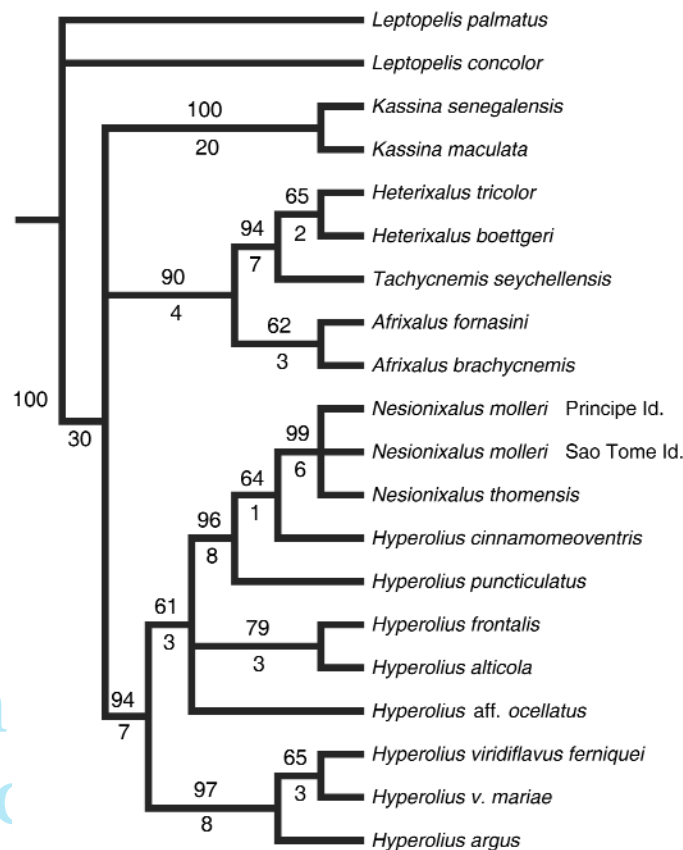


FIGURE 3. Strict consensus of four most parsimonious cladograms resulting from an heuristic search in a maximum parsimony analysis on approximately 500 bases for all species used in the analysis (Table 1). Numbers above and below branches are as in Fig. 2.

males, and 42.0 and 47.0 mm for the females; the fifth specimen of the series was evidently a juvenile at 18.0 mm (Perret, 1976). The 18 specimens in Loumont's (1992) collection were of snout-vent lengths 27.0–35.0 mm in males and 36.0–41.0 mm in females. CAS material includes two females at 47.5 and 49.2 mm and 11 adult males ranging from 35.4–41.1 mm; one male, likely subadult, of 31.0 mm was collected as a singleton (see Appendix, Table 2). Snout-vent lengths of *Nesionixalus thomensis* females do indeed exceed published records for members of the genus *Hyperolius*, but the females of a number of African mainland species attain or exceed 40.0 mm snout-vent lengths, including *Hyperolius torrentis*, *H. balfouri*, *H. sankuruensis*, *H. major* and *H. horstocki* with *H. bobirensis* of Ghana attaining snout-vent lengths of 47.0 mm (Schjøtz 1999). The overall large size of *N. thomensis* may be attributed to the phenomenon of island gigantism, especially in light of the fact that there are many taxa endemic to São Tomé and/or Príncipe that exhibit the same phenomenon. For instance, the distantly related hyperoliid frog (*sensu* Drewes 1984), *Leptopelis palmatus*, endemic to the tiny island of Príncipe is the largest member of that genus and the largest African treefrog, with females reaching 110 mm snout-vent length. Another frog endemic to São Tomé, the ranine *Ptychadena newtoni* is the largest member of its genus (Loumont 1999). The gekkonid lizard, *Hemidactylus greeffii*, endemic to both islands, is the largest African species in the genus (Loveridge 1947) and the giant sunbird of São Tomé, *Dreptes thomensis*, is substantially larger than all other species in the Afro-Asian family Nectariniidae (Cheke et al. 2001). Among the angiosperms endemic to São Tomé is the giant, 2-m tall, *Begonia baccata*, a paleoendemic considered to be at least 3 million years old (Plana et al. 2004).

#### **Dorsal epidermal asperities**

Males of both *Nesionixalus thomensis* and *N. mollerii* (*sensu* Loumont, 1992) possess fine asperities (“*spinosités cornées*” of Perret 1988) which are relatively evenly spaced on dorsally exposed surfaces of the body. These structures are visible with little or no magnification; some, but not all of these asperities are black in *Nesionixalus* and the dorsal skin of females of both species lack them. This character is not unique to the island species; it is also shared by a number of mainland species of *Hyperolius*. Males of *H. spinigularis* have dorsal asperities, although these are not always pigmented (Schjøtz 1975; 1999). All males in a large series of *H. alticola* (see Appendix, Table 2) from southwestern Uganda exhibit uniformly distributed spinosities (non-pigmented) which are absent in the females. Wilson (2000) coded the character state of dorsal spinules of *N. thomensis* and *N. mollerii* as the same in *Hyperolius bobirensis*, *H. endjami*, *H. laurenti*, *H. spinigularis*, *H. sylvaticus*, *H. tannerorum*, and *H. viridigulosus*, as well as in *Chlorolius koehleri* and *Alexteroon obstetricans*.

#### **Gular gland**

Perret (1988) described the gular gland of *N. thomensis* as narrow (“*mince*”), not obvious (“*peu apparente*”) and not very projecting [posteriorly?] (“*ne faint pas saillie*”). Wilson (2000) coded states of three characters of the male gular gland: size (four states based upon percent of gular area covered by the gland), thickness (four states based on gland thickness relative to that of surrounding, non-gland gular skin) and overlapping or free margins of the gland. The latter character, modified from Liem (1970), is a measurement of how much of the lateral and posterior borders of the gland cover distinct folds of distensible skin of the vocal pouch (Drewes 1984). The male gular glands of *N. thomensis* and *N. mollerii* occupy one-half or less of the total gular area, a character state shared by *Hyperolius laurenti*, *H. montanus*, *H. occidentalis*, *H. sylvaticus* and *Alexteroon obstetricans*; the thickness of the glands is the same as that of the glands of 24 of the

31 species of *Hyperolius* examined by Wilson (2000), and the extent of the glands free margins is the same as in *Hyperolius guttulatus*, *H. lamottei*, *H. laurenti*, *H. montanus*, *H. ocellatus* and *H. v. viridiflavus*.

#### **Terminal Phalanx**

In the second description, Perret (1988) describes the terminal phalanx of *N. thomensis* as obtuse or weakly notched, as in Drewes (1984) state 2 [of character 14] and thus “non hyperolien.” Perret does not indicate which terminal phalanx nor the method of preparation, but this description is in error. All terminal phalanges in our cleared and stained material of both taxa are slender, and peniform with basal constriction, consistent with Drewes character 14, state 1, found in nine of the 12 species of *Hyperolius* he examined (Drewes, 1984, Fig. 10C; *H. balfouri* — not coded, *H. argus* and *H. phantasticus* had claw-shaped terminal phalanges — 14(0)). This character is of doubtful intergeneric utility as Wilson (2000) found three states of this character among species of *Hyperolius*. The condition of the terminal phalanges of both *N. thomensis* and *N. mollerii* (MHNG specimens utilized by Loumont) was comparable to that in 22 of the 31 *Hyperolius* species cited in her study.

#### **Size of testes**

Perret (1976, 1988) was impressed with the large size of the testes of the two males in the type series of *N. thomensis* (7 and 8 mm) and presented these data as a defining character in both the original and second description. Loumont (1992) reiterated this trait, noting that testes of *H. mollerii* were also large (to 6.7 mm) and suggested this character together with the dorsal asperities justified placement of *H. mollerii* in *Nesionixalus* Perret. In both descriptions, Perret (1976, 1988) added large size of eggs and small clutch size as further distinguishing *N. thomensis*. Wilson (2000) examined male testis size in *Hyperolius* and relatives and coded four states of this character based on testis length expressed as a percentage of total snout-vent length. *Nesionixalus thomensis* and *N. mollerii* grouped with the eastern *Hyperolius tuberilinguis* at 19–21% of SVL and *H. bobirensis* and *H. montanus* had even larger testes at 25% and 34% SVL, respectively. All remaining *Hyperolius* fell within the 6–17% SVL range. Obviously the validity of this character depends upon the male frogs being at the peak of reproductive activity with testes fully enlarged; the fact that male *Hyperolius* are almost always located and collected while sexually active, together with Wilson's large sample size of males of 31 species of *Hyperolius*, suggest that these observations are reasonably accurate and that the testis size in *Nesionixalus* does not serve to differentiate the genus from *Hyperolius*.

#### **Eggs and clutch size**

Comparative data regarding size of eggs and clutches among hyperoliid species are scarce. Members of the genus *Hyperolius* are generally described as having small eggs (0.8–1.5 mm) which in a few species are deposited in water or typically on vegetation above the water surface in clutches of 100 to 500 (Channing, 2001). Perret (1976) found 25 large (2.0–2.5 mm) presumably ovarian eggs in each of two of the three females in type series of *N. thomensis*; in erecting the genus he suggested that the small number and large size of the eggs might indicate a specialized reproductive mode “un cycle biologique particulier.” Loumont (1992) added new data for *H. thomensis* (50 to 60 ovarian [?] eggs of less than 0.5 mm diameter in two females) and *H. mollerii* (70+ mature eggs per clutch, 1.5 mm diameter). However, Fahr (1993) presented a figure of an *H. mollerii* egg mass, which included at least 110 eggs. Egg and clutch size are of questionable taxonomic value

for hyperoliid treefrogs; there are almost no data available on the former except perhaps scattered references in the experimental literature and relative to the number of species recognized, data are very sparse for the latter. In his comprehensive book, Schiøtz (1999) treated 85 species and species groups within the genus *Hyperolius* but was able to provide clutch size data for only 14 of them. Of these, five are known to deposit fewer than 50 eggs per clutch: *H. frontalis*, *H. mitchelli*, *H. mosaicus*, *H. pusillus* and *H. semidiscus*. Channing (2001) added clutch size data for four additional species including *H. pickersgilli* with 50 eggs.

### ***Finger and toe tips***

The size and nature of expanded digital finger and toe tips was examined by Liem (1970); Drewes (1984) did not employ the character in his hyperoliid work. With respect to this character, Channing (1989) re-coded and used Liem's data on rhacophorids only. All of our specimens of *Nesionixalus thomensis* exhibit toe tips that are as previously described: enlarged, oval and wider in the horizontal plane than they are long — consistent with Liem's character 33, state 4 which he found common in a number of rhacophorid genera but not in any hyperoliid species. As Perret correctly observed (1976), the condition of *N. thomensis* digital expansions is very similar to that in *Acanthixalus spinosus* (see Appendix, Table 2), a West African hyperoliid not examined by Liem (1970). In addition, nearly identically-shaped digital tips are found in *Kassina maculifer* (*K. parkeri* of Scortecci 1932; Drewes 1984, 1985; Tandy and Drewes 1985; Appendix, Table 2 herein) of the arid Somali Horn. On the other hand, the expanded digital tips of all of our *N. mollerii* from both São Tomé and Príncipe Islands are disc-shaped, consistent with Liem's (1970) character 33, state 2, which he found in all *Hyperolius* and the majority of other hyperoliid genera he examined.

We did not make a broad survey of hyperoliid digital pads and concede that there are no species of *Hyperolius* known with digital tips similar to those of *N. thomensis*. However, this single character state is not sufficient to support recognition of a distinct genus; moreover, this state is not shared by the purported congener, *N. mollerii*. Other than toe pad shape and body size, all of the morphological characteristics used by Perret (1976, 1988) to define *Nesionixalus* and Loumont (1992) to subsequently reassign *H. mollerii* are shared by other species of *Hyperolius* or attributable to island effects. This coupled with the results of our molecular analysis indicate that the genus *Nesionixalus* Perret is not a lineage separable from *Hyperolius* and that *N. thomensis* and *N. mollerii* should be relegated to the synonymies of *Hyperolius thomensis* Bocage, 1886 and *Hyperolius mollerii* Bedriaga, 1892 respectively.

In all molecular studies to date, *Hyperolius* has been shown to be monophyletic with respect to other hyperoliid genera; however, sample sizes have been extremely limited (Richards and Moore 1996, three species; Emerson et al. 2000, three species; Wiczorek 1999, 14 species and 16 subspecies of the *H. viridiflavus* complex, but with *Afrixalus* as lone outgroup; Vences et al. 2003, six species). Similarly, all morphology-based phylogenetic studies support the monophyly of *Hyperolius* (Liem 1970, 14 *Hyperolius* species examined; Drewes 1984, 12 examined; Channing 1989, data from Drewes, *op. cit.* and Liem, *op. cit.*) and widely-cited, regional works have assumed it (e.g., Schiøtz 1967, 1975, 1999; Rödel 1996; Channing 2001). *Hyperolius* species can be extremely difficult to determine, especially after preservation; however, the genus has, until the recent descriptions of *Nesionixalus*, *Chlorolius*, *Arlequinus* and *Alexteroon* (Perret 1988; Amiet 2000) been rather easy to discern by initial determination of pupil shape (Drewes 1984:45–46). For instance, if the specimen in hand has horizontally-oval pupils (Fig. 4 B), is obviously arboreal with expanded digital tips (as opposed to *Chrysobatrachus*, which is terrestrial [Laurent, 1964]) and has a single gular gland in males (as opposed to *Acanthixalus*, in which gular glands are paired), it is a member of the genus *Hyperolius*.



At this time, we offer no taxonomic judgments on the validity of Perret's (1988) *Alexteroon*, *Chloroliulus* and *Arlequinus*. We lack sequence data for these taxa, and morphological work suggesting the first two be returned to the genus *Hyperoliulus* is unpublished (Wilson 2000). The morphological definitions for *Alexteroon* and *Chloroliulus* do not seem to include any synapomorphies that would serve to distinguish them unequivocally from *Hyperoliulus*, and in all cladograms presented by Wilson (2000), *Alexteroon*, *Chloroliulus* and *Nesionixalus* nested within *Hyperoliulus* regardless of outgroup (*Afrixalus*, *Cryptothylax*). Diagnostic morphological character states used in the past (Liem 1970; Drewes 1984) would suggest that *Arlequinus* is most likely to have a separate evolutionary history from *Hyperoliulus*. *Arlequinus* shares the quadratic pupil shape with *Acanthixalus*, *Afrixalus*, *Heterixalus*, *Opisthothylax* and *Cryptothylax* within the Hyperoliidae (Fig. 4).

Monophyly in the genus *Hyperoliulus* has been either assumed by researchers or demonstrated (either morphologically or molecularly) on the basis of very small sample sizes relative to the number of currently recognized species. We contend that to make taxonomic decisions on member taxa in the absence of robust knowledge of relationships within the largest hyperoliid genus runs the risk of adding to an already confused situation. A modern revision of the genus *Hyperoliulus* based on both molecular and morphological data is a daunting but long overdue task. We do recognize that *H. malleri* and *H. thomensis* is a molecularly highly supported clade (as shown in Figs. 2 and 3). Moreover, both species share a chromosome number of  $2n = 26$  (Loumont 1992; Schätti and Loumont 1992; Fahr 1993), a condition rare among known *Hyperoliulus* species but not unique (Morescalchi 1968). However, to designate this clade a genus separate of *Hyperoliulus* would render *Hyperoliulus* paraphyletic, a result not supported by morphological analysis in this study. An intriguing

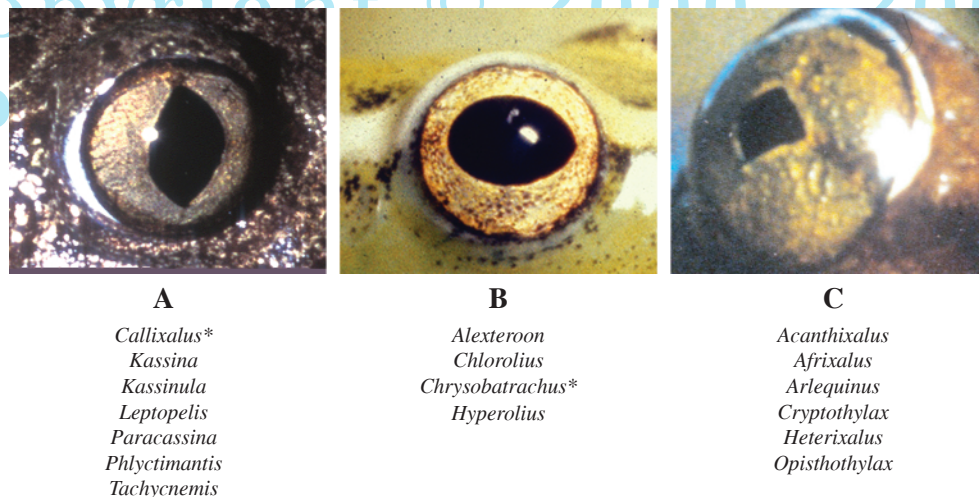


FIGURE 4. Pupil shapes within the Hyperoliidae. Liem (1970) and Drewes (1984) both recognized three states of pupil-shape among hyperoliid frogs, but both combined two of these shapes, the vertical ellipse and rhomboidal/quadrangular, into a single character state; Channing (1989) followed suit, utilizing the same data. Schiøtz (1999:43) provided a key that attempts to clarify pupil-shape in both living and preserved specimens: "1a. Pupil horizontal, square or (after preservation) round", and "2b. Pupil vertically elliptical or rhomboidal." We feel that these treatments mask states which are probably not homologous. Here we present the range of hyperoliid pupil shapes based on photographs of living specimens.

A. vertical (vertical ellipse). *Kassina maculata* (CAS 184054; Photo by J. Vindum); B. horizontal (horizontal oval, round). *Hyperoliulus parkeri* (CAS 154572-72; Photo by J. Vindum); C. rhomboidal (quadrangular, square). *Cryptothylax greshoffii* (MHNG specimen; Photo by J.-L. Perret).

\*To our knowledge, members of these genera have never been photographed in life.

ing result of this study is the fact that this clade is more closely related to *H. cinnamomeoventris* than any other member of the genus studied; in fact, the support for this relationship is higher than within the *mollerithomensis* clade itself (a Bremer's decay index of 44 as opposed to 35; Fig. 2), and to our *Hyperolius* aff. *ocellatus* sample from Bioko, the northern-most member of the island chain, which might be expected due to geographic proximity. *Hyperolius cinnamomeoventris*, usually readily identifiable, is among the most widespread member of the genus and one of the few species that inhabits both savannah and forest (Schjøtz 1999). Such questions as the possible common ancestry of *H. cinnamomeoventris* and the island endemics can only be determined by a much more broadly representative sample of *Hyperolius* and other hyperoliid genera. So far, GenBank has sequence data for only 15 identified species of *Hyperolius* — along with 26 subspecies of the *H. viridiflavus* complex and four unidentified entities. Not counting *Nesionixalus*, *Arlequinus*, *Alexteroon* and *Chlorolius*, this represents about 12% of the species currently recognized in *Hyperolius* that are distributed throughout sub-Saharan Africa. There are undoubtedly tissues in museums that have not yet been sequenced, or sequences not yet entered into GenBank. At CAS, we have unsequenced tissues and vouchers for six such species and with the increase in fieldwork during the past decade especially in West Africa, there are no doubt many more at other institutions.

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## APPENDIX

TABLE 1. Sources for DNA sequences used in this study.

Species	Source	GenBank Accession Number
<i>Afrixalus brachynemesis</i>	Richards and Moore (1996)	
<i>Afrixalus fornasinii</i>	GenBank	AFU22071
<i>Heterixalus boettgeri</i>	GenBank	HBV24672
<i>Heterixalus tricolor</i>	Richards and Moore (1996)	
<i>Hyperolius argus</i>	Richards and Moore (1996)	
<i>Hyperolius alticola</i>	CAS 207322	AY603984
<i>Hyperolius cinnamomeiventris</i>	CAS 202493	AY603985
<i>Hyperolius frontalis</i>	CAS 201986	AY603986
<i>Hyperolius</i> aff. <i>ocellatus</i>	CAS 207321	AY603988
<i>Hyperolius punctulatus</i>	Richards and Moore (1996)	
<i>Hyperolius viridiflavus ferniquei</i>	CAS 191296	AY603987
<i>Hyperolius v. mariae</i>	GenBank	HVU22064
<i>Kassina maculata</i>	GenBank	KMU22072
<i>Kassina senegalensis</i>	Richards and Moore (1996)	
<i>Nesionixalus malleri</i>	CAS 219125	AY603990
<i>Nesionixalus malleri</i>	CAS 218848	AY603989
<i>Nesionixalus thomensis</i>	CAS 218925	AY603991
<i>Leptopelis concolor</i>	GenBank	LCU22079
<i>Leptopelis palmatus</i>	CAS 219177	AY603992
<i>Tachynemesis seychellensis</i>	GenBank	TSU22080

TABLE 2. Preserved specimens examined.

Species	Museum Number	Locality
<i>Acanthixalus spinosus</i>	CAS 153799–800	Cameroon: Sangmelima, Foullassi, Ngam
<i>Hyperolius alticola</i>	CAS 180449–481	Uganda: Rukungiri Dist: Bwindi Impenetrable NP: Munyaga Rv.
<i>Nesionixalus</i> (= <i>Hyperolius</i> ) <i>thomensis</i>	CAS 218925–937; 219404	São Tomé e Príncipe: São Tomé Id: Macambrara.
<i>Kassina maculifer</i>	CAS 140351; 140353–354; 140356–361	Kenya: Wajir Dist: Wajir