

# Molecular phylogenetic evidence for paraphyly of the genus *Sooglossus*, with the description of a new genus of Seychellean frogs

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Received 31 October 2005; accepted for publication 10 August 2006

The Seychelles harbour an endemic frog family, the Sooglossidae, currently containing two genera: *Sooglossus*, with three species, and *Nesomantis*, with one species. These unique frogs are generally considered to be basal neobatrachians, although their relationships to other neobatrachian taxa, except the Nasikabatrachidae, remain unresolved. Our molecular phylogeny based on a dataset consisting of fragments of the nuclear rag-1 and rag-2 genes, as well as mitochondrial 16S rRNA in representatives of the major neobatrachian lineages, confirmed the previously postulated Sooglossidae + Nasikabatrachidae clade and the placement of the South American *Caudiverbera* with the Australian Myobatrachidae, but did not further resolve the position of sooglossids. Our results do, however, unambiguously show sooglossids to be monophyletic but the genus *Sooglossus* to be paraphyletic, with the type species *Sooglossus sechellensis* being more closely related to *Nesomantis thomasseti* than to *Sooglossus gardineri* and *Sooglossus pipilodryas*, in agreement with morphological, karyological, and bioacoustic data. As a taxonomic consequence, we propose to consider the genus name *Nesomantis* as junior synonym of *Sooglossus*, and to transfer the species *thomasseti* to *Sooglossus*. For the clade composed of the species *gardineri* and *pipilodryas*, here, we propose the new generic name *Leptosoglossus*. A significant genetic differentiation of 3% was found between specimens of *Sooglossus thomasseti* from the Mahé and Silhouette Islands, highlighting the need for further studies on their possible taxonomic distinctness. © 2007 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2007, 91, 347–359.

**ADDITIONAL KEYWORDS:** Amphibia – Anura – biogeography – *Leptosoglossus* – molecular phylogeny – *Nesomantis* – new genus – Sooglossidae – *Sooglossus*.

## INTRODUCTION

The frogs of the basal neobatrachid family Sooglossidae are restricted to mossy mountain forests on two

islands of the Seychelles archipelago: Mahé and Silhouette. These granitic islands on the Mascarene plateau are highly isolated Gondwanan fragments in the Indian Ocean, 1000 km south of India (Briggs, 2003). The Seychelles became isolated from other landmasses approximately 67–47 Mya, although the islands may have been connected to each other during the last glaciation (Badyukov, Demidenko & Kaplin,

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1989). The family Sooglossidae consists of four species divided into two genera; *Sooglossus*, with three small-sized species, and *Nesomantis*, with a single medium-sized species. All four species are listed as 'vulnerable' on the IUCN Red List (<http://www.globalamphibians.org>; accessed 9 July 2005) due to their restricted ranges, being associated with moist upland rainforests. *Sooglossus sechellensis* (Boettger, 1896) and *Sooglossus gardineri* (Boulenger, 1911) are leaf-litter inhabiting species, whereas *Sooglossus pipilodryas* Gerlach & Willi, 2003 is arboreal and usually lives in the axils of endemic palms and banana trees. *Nesomantis thomasseti* Boulenger, 1909 is associated with rock overhangs. *Sooglossus gardineri* is one of world's smallest tetrapods with a snout-vent length of only 9.8 mm in adult males.

The relationships of Sooglossidae to other frogs have been a subject of continuing debate. Except for their placement with the Pelobatidae (Griffiths, 1963), they usually were classified with a modern lineage of frogs that several authors (Nussbaum, 1982; Hay *et al.*, 1995; Feller & Hedges, 1998; Van der Meijden, Vences, Hoegg & Meyer, 2005) referred to as Neobatrachia. Partly following the classification of Dubois (2005), who proposed discontinuing the use of this name as the formal name of a suborder of frogs, here, we use only the name 'neobatrachians' informally to refer to this clade. Within neobatrachians, Sooglossidae have been grouped with the ranoids (Savage, 1973), the Microhyloidea (Blommers-Schlösser, 1993), and in the hyloid superfamily sister to Myobatrachinae (Lynch, 1973; Duellman & Trueb, 1986; Ford & Cannatella, 1993). The last comprehensive review of the taxonomic position of this family was provided more than 20 years ago by Nussbaum (1984).

Recent molecular studies based on analysis of different markers have also failed to provide convincing evidence allying Sooglossidae with any other neobatrachian group, with the notable exception of their strongly-supported sister group relationship to the newly-discovered Nasikabatrachidae from India (Biju & Bossuyt, 2003). Phylogenies using DNA sequences coding for 12S and 16S rRNA (Hay *et al.*, 1995), 12S, 16S and the valine tRNA, together with rhodopsin and single exon fragments of rag-1 and CXCR-4 (Biju & Bossuyt, 2003), rag-1 and rag-2 (Hoegg, Vences, Brinkmann & Meyer, 2004) and a larger fragment of rag-1 (San Mauro, Vences, Alcobendas, Zardoya & Meyer, 2005) placed the sooglossids, or sooglossids plus nasikabatrachids (Biju & Bossuyt, 2003), either as an isolated clade basal in the neobatrachians, or related to other neobatrachian clades with very low phylogenetic support.

A phylogenetic pattern that became obvious from recent molecular studies on deep anuran relationships (Biju & Bossuyt, 2003; Hoegg *et al.*, 2004; Roelants &

Bossuyt, 2005; San Mauro *et al.*, 2005) is the existence of two well-defined and very species-rich neobatrachian clades, ranoids and hylids, and of several other, more basal lineages of largely unclarified relationships. Besides the sooglossids and nasikabatrachids, these basal assemblages include the heleophrynids from South Africa, the myobatrachids from Australia, and the South American *Caudiverbera* which previously was believed to belong to the Leptodactylidae. Firmly established relationships among these taxa would be highly informative for the reconstruction of anuran biogeography and the effect of the fragmentation of Gondwana (Biju & Bossuyt, 2003). However, recent phylogenetic studies did not include sooglossids together with representatives of all other basal lineages, and all used only *Nesomantis* to represent the Sooglossidae clade. A molecular test for the monophyly of sooglossids is so far missing. At the level of intrafamilial relationships in the Sooglossidae, it has long been suspected that the genus *Sooglossus* might be paraphyletic with respect to *Nesomantis* (Noble, 1931). Additional morphological evidence for paraphyly of *Sooglossus* has since been accumulated (Griffiths, 1963; Gerlach & Willi, 2002).

Here, we provide the first intrafamilial molecular phylogenetic hypothesis of the Sooglossidae, based on two nuclear genes and one mitochondrial ribosomal RNA gene. Our study also includes representatives of all deep neobatrachian lineages identified to date to test for monophyly and relationships of the Sooglossidae.

## MATERIAL AND METHODS

### SELECTION OF TAXA

All four currently described species of the Sooglossidae were included in this study. We used the following specimens: *S. gardineri* (4-2003, collected by R. Boistel, collection of the Muséum National d'Histoire Naturelle, Paris, MNHN 2003.3410, Silhouette, Jardin Marron, 400 m a.s.l.); *S. pipilodryas* (4-2003, collected by R. Boistel, MNHN 2003.3411, Silhouette, Jardin Marron 350 m a.s.l.); two specimens of *S. sechellensis* (4-2003, collected by R. Boistel, MNHN 2003.3412–3413, Silhouette, Jardin Marron 450 m a.s.l.); and two specimens of *N. thomasseti* (5-7-2001, collected by L. Chong Seng & A. Ohler, and MNHN 2001.0269, Mahé; 4-2003, collected by R. Boistel, MNHN 2003.3414, Silhouette, Jardin Marron 350 m a.s.l.). We also selected representatives of several hyloid and ranoid families available from GenBank (Table 1) to place the clade formed by the Sooglossidae and *Nasikabatrachus* with its closest taxon within the neobatrachians. We included *Nasikabatrachus sahyadrensis*, although no rag-2 sequence is available for this species. Of the ranoid superfamily, we selected *Rana temporaria* as a

**Table 1.** Taxa included in this study and their GenBank accession numbers

Species	Family	16S	Rag-1	Rag-2
<i>Agalychnis callidryas</i>	Hylidae	AY330890	AY323765	AY323780
<i>Bufo regularis</i>	Bufoidea	AY330891	AY323763	AY323784
<i>Heleophryne regis</i>	Heleophrynidae	AF432230*	AY323764	AY323786
<i>Hyperolius viridiflavus</i>	Hyperoliidae	AF215441	AY323769	AY323789
<i>Kaloula pulchra</i>	Microhylidae	AY330893	AY323772	AY323790
<i>Lechriodus melanopyga</i>	Myobatrachidae	DQ872915	AY583341	DQ872908
<i>Caudiverbera caudiverbera</i>	Leptodactylidae (?)	DQ872913	AY583337	DQ872909
<i>Leptodactylus fuscus</i>	Leptodactylidae	AY263226	AY323770	AY323791
<i>Nasikabatrachus sahyadrensis</i>	Nasikabatrachidae	AY364381	AY364225	NA
<i>Pipa parva</i>	Pipidae	AY333690	AY323761	AY323799
<i>Rana temporaria</i>	Ranidae	AF249048	AY323776	AY323803
<i>Nesomantis thomasseti</i> 'Mahé'	Sooglossidae	AY330889	AY323778	AY323798
<i>Nesomantis thomasseti</i> 'Mahé'	Sooglossidae	DQ872914	–	–
<i>Nesomantis thomasseti</i>	Sooglossidae	AY364373	–	–
<i>Nesomantis thomasseti</i> 'Silhouette'	Sooglossidae	DQ872920	–	–
<i>Nesomantis thomasseti</i> 'Silhouette'	Sooglossidae	X86288	–	–
<i>Sooglossus sechellensis</i>	Sooglossidae	DQ872916	DQ872921	DQ872910
<i>Sooglossus sechellensis</i>	Sooglossidae	DQ872917	–	–
<i>Sooglossus gardineri</i>	Sooglossidae	DQ872919	DQ872923	DQ872911
<i>Sooglossus pipilodryas</i>	Sooglossidae	DQ872918	DQ872922	DQ872912

\*Sequence of *Heleophryne purcelli* used.

NA, not applicable.

representative ranid, *Kaloula pulchra* representing the Microhylidae, and the hyperoliid *Hyperolius viridiflavus*. The superfamily Hyloidea was represented by the bufonid *Bufo regularis*, the hylid *Agalychnis callidryas*, and the leptodactylids *Leptodactylus fuscus* and *Caudiverbera caudiverbera*. Also included were the basal neobatrachians *Heleophryne regis* of the Heleophrynidae, and *Lechriodus melanopyga* representing the Myobatrachidae. The pipid frog *Pipa parva* was used as the outgroup.

#### SEQUENCING AND ALIGNMENT

DNA was extracted from toe clips fixed in 99% ethanol. Tissue samples were digested using proteinase K (final concentration 1 mg mL<sup>-1</sup>), homogenized, and subsequently purified following a high-salt extraction protocol (Bruford *et al.*, 1992). Primers for rag-1 and rag-2 were from Hoegg *et al.* (2004) as reported in Chiari *et al.* (2004). Primers for the fragment of the 16S rRNA gene were 16SA-L and 16SB-H of Palumbi *et al.* (1991). Polymerase chain reaction (PCR) was performed in 25- $\mu$ L reactions containing 0.5–1.0 units of REDTaq DNA Polymerase (Sigma), 50 ng genomic DNA, 10 pmol of each primer, 15 nmol of each dNTP, 50 nmol additional MgCl<sub>2</sub> and the REDTaq PCR reaction buffer (in final reaction solution: 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.1 mM MgCl<sub>2</sub> and 0.01% gelatine). For rag-1 and rag-2, cycle conditions were

adapted from a long range PCR protocol (Barnes, 1994), with an initial denaturation step at 94 °C for 5 min, followed by ten cycles with 94 °C for 30 s, annealing temperatures increasing by 0.5 °C per cycle from 52 to 57 °C and extending for 3 min at 68 °C. Additionally, 20 cycles were performed with 94 °C for 10 s, 57 °C for 40 s, and 68 °C for 3 min. The final extension was performed at 68 °C for 5 min. For 16S, the denaturation step was followed by 35 cycles of denaturation at 94° for 30 s, annealing at 50° for 30 s, and extension at 72° for 90 s.

PCR products were purified via spin columns (Qiagen). Sequencing was performed directly using the corresponding PCR primers (forward and reverse). DNA sequences of both strands were obtained using the BigDye Terminator cycle-sequencing ready reaction kit (Applied Biosystems Inc.) on an ABI 3100 capillary sequencer in accordance with the manufacturer's instructions. New sequences were combined with existing sequences taken from GenBank in the final dataset. These sequences were deposited in GenBank; for accession numbers, see Table 1.

Chromatograms were checked by eye using Sequencher (Gene Codes Corp.) or Chromas, version 1.45 (Technelysium Pty Ltd) and the sequences were subsequently aligned. Rag-1 and rag-2 sequences were aligned by hand using the Mega3 alignment editor (Kumar, Tamura & Nei, 2004). The 16S sequences were aligned using Clustal W (Thompson, Higgins &

Gibson, 1994) and subsequently edited by hand. Gapped and hypervariable sections of the 16S alignment were removed from the full alignment. Hypervariable regions were included in the dataset containing only *Nasikabatrachus* and the sooglossids.

#### DATA ANALYSIS

A homogeneity partition test (Farris *et al.*, 1994), as implemented in PAUP\* (Swofford, 2002), rejected homogeneity of the different markers ( $P = 0.06$ ). Besides a pooled analysis of the combined data set, we therefore also performed separate analyses of each of the various genes. Transitions and transversions were plotted against F84 distances (Felsenstein, 1984) for the separate gene alignments. None of the datasets showed signs of saturation.

Phylogeny reconstruction based on the separate and combined datasets was performed using maximum likelihood (ML) and Bayesian inference (BI) methods. The best fitting models of sequence evolution were determined by the AIC criterion in Modeltest, version 3.06 (Posada & Crandall, 1998). ML tree searches were performed using PhyML, version 2.4.4 (Guindon & Gascuel, 2003). Bootstrap branch support values were calculated with 500 replicates. The Bayesian analyses of the combined and separate datasets was conducted with MrBayes, version 2.0 (Huelsenbeck & Ronquist, 2001), using models estimated with Modeltest under the AIC criterion, with 250 000 generations, sampling trees every tenth generation (and calculating a consensus tree after omitting the first 3000 trees).

The parphyly of the genus *Sooglossus* in our analysis theoretically could have been caused by introgression phenomena or sample contamination regarding the clade containing *S. sechellensis* and *Nesomantis*. To test for this possibility, and to assess the differentiation among individuals of *Nesomantis* from different populations, we sequenced the 16S rRNA gene from a second specimen of *S. sechellensis* and from a *Nesomantis* specimen from Silhouette, added three *Nesomantis* sequences from GenBank, and submitted this 16S data set to a separate ML analysis with *Nasikabatrachus* as outgroup.

#### MICROTOMOGRAPHY

Microtomographic analyses were carried out in the European Synchrotron Radiation Facility (ESRF) at Grenoble, France. We used the following adult specimens: four *N. thomasseti*, three *S. sechellensis*, four *S. gardineri*, and three *S. pipilodryas*. The animals were deposited in a small tube of polypropylene. Microtomography in absorption-based form (amplitude contrast and phase contrast), consists of recording several

hundreds of radiographs, with the sample slightly rotated between exposures, and it uses a standard filtered back-projection algorithm to perform the three-dimensional (3D) reconstruction. The detector uses a FReLoN 1024 × 1024 and 2048 × 2048 camera (Labiche *et al.*, 1996), and involves an optical microscope assembly between the X-ray sensitive converter and the CCD. The effective pixel size is varied by altering the visible-light part of the assembly. Three different sets of experiments were performed on the imaging beamline ID19 of the ESRF, with pixel sizes, respectively, of 7.5 μm at a sample-to-detector distance of 40 mm, 10 μm at 40 mm specimen-to-detector distance and 30 μm at 40 mm specimen-to-detector distance. For phase contrast, samples were scanned at a sample-detector distance of 300 mm. The experiment was performed to obtain a high resolution 3D image of the skeleton and soft tissue. It was performed with the synchrotron radiation monochromatized to 17, 20, and 20.5 keV by a double-crystal silicon monochromator operating in the vertical plane. The flux at the level of the sample for a beam current of 60–180 mA and a wiggler as X-ray source is approximately  $8 \times 10^9$  photons  $s^{-1} mm^{-2}$ . Image visualization was performed using Amira software, version 3.1, from TGS and the public domain ImageJ program developed at the United States National Institute of Health (<http://rsb.info.nih.gov/ij/>). Supplementary data on osteology such as colour plates are available on the web site ([http://indigene.ibaic.u-psud.fr/rubrique.php3?id\\_rubrique=41](http://indigene.ibaic.u-psud.fr/rubrique.php3?id_rubrique=41)) of the Université d'Orsay.

#### RESULTS

The final combined dataset consisted of 1447 bp of rag-1, 810 bp of rag-2, and 435 bp of 16S rRNA, resulting in a combined alignment of 2692 bp. The 16S rRNA alignment had 118 parsimony informative sites, and 256 conserved sites. Rag-1 and rag-2 had 445 and 325 parsimony informative, and 815 and 337 conserved sites, respectively.

Basal neobatrachian relationships were poorly resolved with all datasets. In both the ML and BI analyses, the rag-1 and rag-2 datasets resolved the hylid and ranoid clades and a separate myobatrachid clade, whereas the 16S rRNA dataset provided poor resolution at this level. All separate and combined analyses were congruent in the resolution of a clade representing the Sooglossidae with high support. All datasets using both phylogeny reconstruction methods resolved identical relationships within the Sooglossidae with high support. Sooglossids were split in two clades: *S. gardineri* and *S. pipilodryas* were sister taxa, as were *N. thomasseti* and *S. sechellensis*.

Sooglossids were placed sister to *Nasikabatrachus* in all analyses, and *Caudiverbera* clustered with the

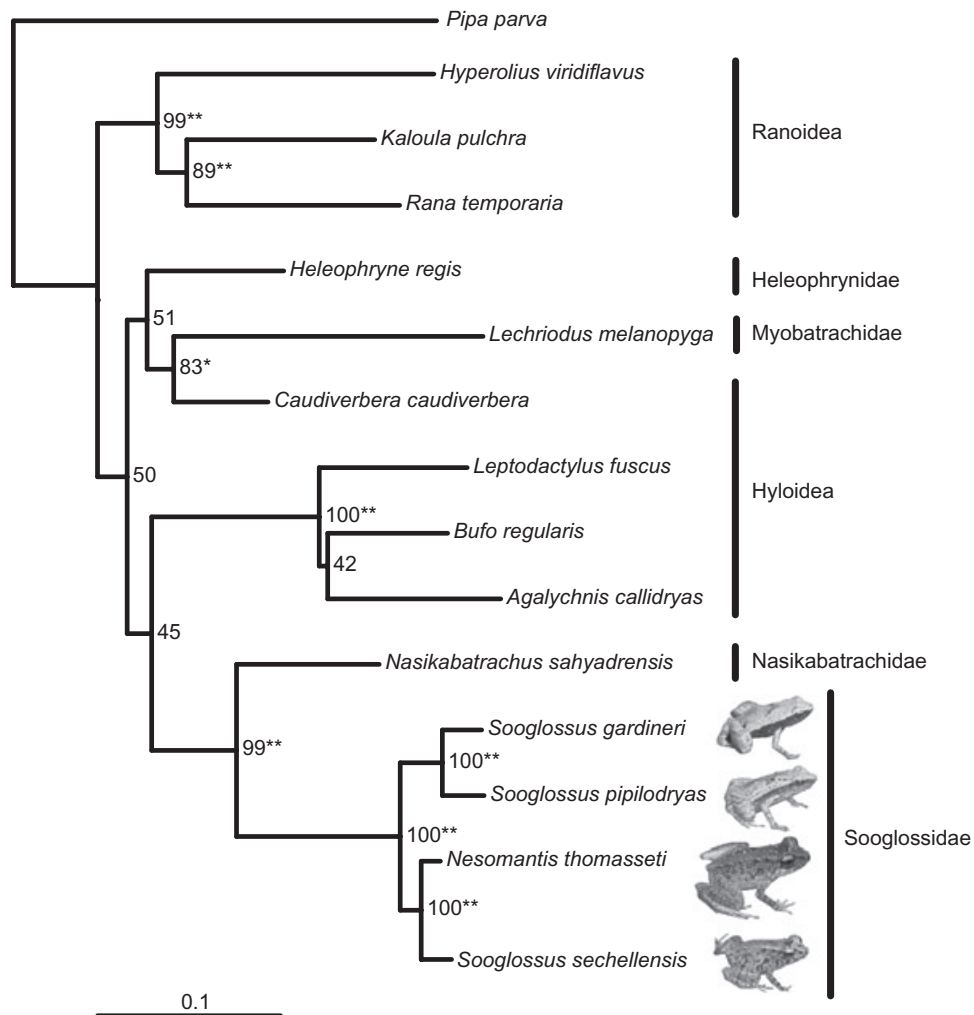


myobatrachid *Lechriodus* based on both nuclear datasets. This sister relationship of *Caudiverbera* and *Lechriodus* was not supported by the 16S dataset, which grouped *Caudiverbera* with *Heleophryne*, albeit with only low support values (68% Bayesian posterior probability and 66% ML bootstrap support). *Heleophryne* was weakly associated with the clade formed by *Caudiverbera* and *Lechriodus* in the combined analyses (Fig. 1).

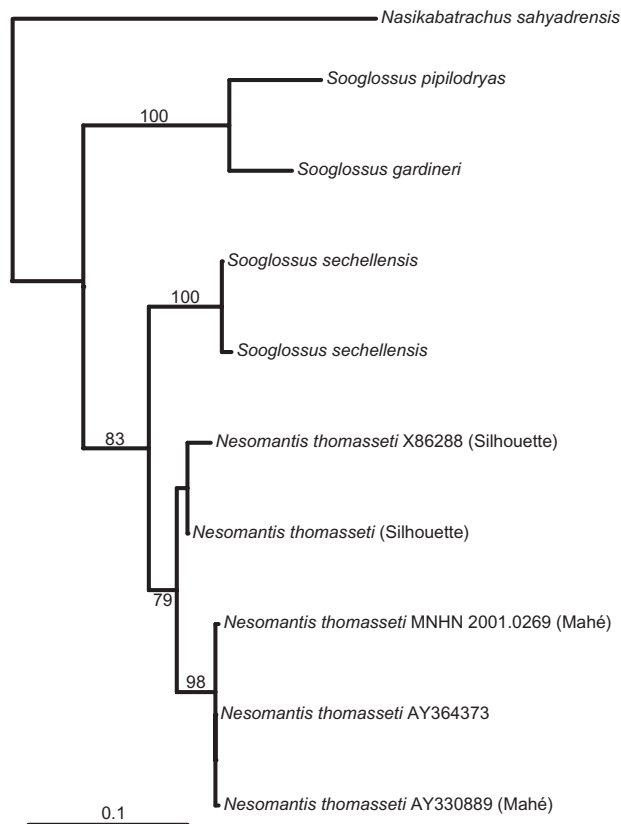
To be able to include published data on additional sooglossid individuals, we performed a second analysis based on the 16S rRNA gene only. This dataset included the two *N. thomasseti* samples (from Mahé and Silhouette) available to us, as well as three further sequences of this species from GenBank, two individuals of *S. sechellensis*, single individuals of *S. gardineri* and *S. pipilodryas*, and *Nasikabatrachus*

as the outgroup. The obtained tree (Fig. 2) provided the same arrangement of taxa as the combined analysis and furthermore placed sequences of *N. thomasseti* from Mahé and Silhouette, respectively, in two separate subclades.

Genetic differentiation between sooglossid taxa was of similar levels as known for other amphibians. Pairwise Jukes–Cantor (JC) corrected distance (Jukes & Cantor, 1969) between *S. gardineri* and *S. pipilodryas* based on 16S was 5.7%, whereas the distance between *S. sechellensis* and *N. thomasseti* was 4.4%. The rag-1 and rag-2 fragments showed a similar pattern; the JC corrected pairwise distances between *S. gardineri* and *S. pipilodryas* were 4.0% for both rag-1 and rag-2, and 2.3% and 2.5% between *S. sechellensis* and *N. thomasseti*, respectively. The mean 16S rRNA-based JC distance between the Silhouette and Mahé



**Figure 1.** Maximum likelihood phylogram of the combined dataset of rag-1, rag-2, and 16S rRNA. Numbers indicate bootstrap support percentages of 500 replicates. A single asterisk indicates a Bayesian posterior probability of over 0.97. Two asterisks indicate a Bayesian posterior probability of 1.0. Sizes of insets are not shown to scale.



**Figure 2.** Maximum likelihood phylogram based on a reduced dataset (16S rDNA only). Numbers indicate bootstrap percentages of 500 replicates. Different 16S rDNA sequences of *Nesomantis* available through GenBank were included: X86288 (Hay *et al.*, 1995), AY364373 (Biju & Bossuyt, 2003), and AY330889 (Hoegg *et al.*, 2004).

specimens of *N. thomasseti* was 3.0%. The mean 16S rRNA based distance between the *S. gardineri*/*S. pipilodryas* clade and the *N. thomasseti*/*S. sechellensis* clade was 16.6%.

## DISCUSSION

### RELATIONSHIPS AMONG BASAL NEOBATRACHIANS

The phylogenetic relationship of the clade formed by the Sooglossidae and *Nasikabatrachus* to other neobatrachians could not be resolved in our analyses. Although correct assignment of most species to the larger superfamilies Ranoidea and Hyloidea is usually unproblematic, the current work, similar to previous studies using molecular characters (Hay *et al.*, 1995; Biju & Bossuyt, 2003; Hoegg *et al.*, 2004; San Mauro *et al.*, 2005), failed to provide resolution among the more basal neobatrachian taxa. Like the sooglossids, most of these basal taxa are species-poor, and have

only relictal distributions. Sooglossids are restricted to the Seychelles, *Nasikabatrachus* is highly localized in India as are heleophrynids in South Africa, and *Caudiverbera*, which is possibly closely related to the Australian myobatrachids, in South America. The global scattering of their small distributions, suggests that these taxa might be remnants of an ancient neobatrachian radiation predating the breakup of Gondwana (San Mauro *et al.*, 2005). Lack of basal resolution among neobatrachian groups might be because inadequate markers were used in these studies. Alternatively, the initial radiation of neobatrachians could have occurred too fast to be reconstructable from present DNA sequences. Nevertheless, the data published previously and those that are included in the present work allow for three conclusions regarding the relationships of these frogs: (1) the Sooglossidae are a monophyletic group, which (2) is confirmed to be sister to the Indian *Nasikabatrachus*, thereby validating the biogeographical scenario of Biju & Bossuyt (2003); (3) the placement of *Caudiverbera*, which is typically included with the Leptodactylidae, sister to the myobatrachid *Lechriodus* rather than with the leptodactylid *Leptodactylus*, receives further support in addition to the phylogeny of San Mauro *et al.* (2005) that was based on only rag-1 sequences. By contrast to the other lineages of basal neobatrachians, Myobatrachidae is a species-rich taxon with 124 species (including Rheobatrachidae and Limnodynastidae; AmphibiaWeb.org, as of August 2005), and a more comprehensive sampling of these Australian taxa in the future will be crucial to fully understand the phylogenetic and biogeographical pattern of the various clades in the initial neobatrachian radiation.

A striking character of sooglossids is their lack of extensible external vocal sacs (R. Boistel, pers. observ.) and the absence of middle ear ossicles (Parker, 1934). A single internal vocal sac with very small vocal slits (1 mm length, Tyler, 1985) is present. The middle ear is considered an adaptation to hearing in air (Allen, 1985) and the external vocal sacs are a common solution for the need for communication over greater distances in anurans. It has been assumed that frogs lacking such structures are deaf and voiceless, which is contradicted by the fact that at least some sooglossids emit advertisement calls (Gerlach & Willi, 2002). The closely related *Nasikabatrachus* shares the absence of the tympanum (see absence of bone columella in Biju & Bossuyt, 2003: fig. 1e, f; Dutta *et al.*, 2004). Of the 5157 described species of anurans placed in 32 families (AmphibiaWeb.org), approximately 6% are earless (Boistel, 2003). Although the Sooglossidae and Nasikabatrachidae form one of the basal clades of the neobatrachians, the majority of neobatrachians do have a middle ear and its loss is most probably secondary (R. Boistel, unpubl. data).

## INTRAFAMILIAL PHYLOGENY AND THE CLASSIFICATION OF SOOGLOSSIDS

The paraphyly of the genus *Sooglossus* as shown by our data corroborates the earlier findings based on morphology (Noble, 1931; Griffiths, 1963; Gerlach & Willi, 2002), vocalizations (Nussbaum, Jaslow & Watson, 1982), genetic distance data (Green, Nussbaum & Datong, 1988), and karyology (Nussbaum, 1979). The high support that this placement receives (based on the separate and combined molecular datasets irrespective of phylogeny reconstruction method) and the low genetic distance between *N. thomasseti* and *S. sechellensis*, suggests a need for further taxonomic reconsideration of the genus *Nesomantis*. This robust arrangement may also provide a basis for the study of the evolution of reproductive modes and other features of the biology of these genetically highly distinctive frogs.

Originally, *Sooglossus* was described by Boulenger (1906) and assigned to the family Ranidae in order to separate *Arthroleptis sechellensis* Boettger, 1896 from the African *Arthroleptis*. The new genus was defined by the presence of an entire, elliptical tongue which Boulenger (1906) lists as the sole distinctive character. When he discovered a second species (*N. thomasseti*) from the Seychelles Islands, Boulenger (1909) described it as a member of a new ranid genus mainly distinguished by the presence of vomerine teeth and the shape of the digits. The characters used by Boulenger (1882) to redefine *Nectophryne*, particularly the presence of a fleshy web, allow us to understand his generic allocation of a new species *Nectophryne gardineri* Boulenger (1911) as a member of the Bufonidae. Thus, the frogs from the Seychelles were historically classified as members of two distinct families, grouping *S. sechellensis* with *N. thomasseti* as corroborated by the morphological study of Gerlach & Willi (2002). Available information on reproductive modes also shows some differences within the genus *Sooglossus*: *S. sechellensis* deposits eggs in a terrestrial nest. These hatch into nonfeeding tadpoles that are transported on the back of the male until metamorphosis (Nussbaum, 1984). Also, eggs of *N. thomasseti* are deposited in a terrestrial nest, and hatch into nonfeeding tadpoles (R. Boistel, unpublished data). By contrast, *S. gardineri* lays terrestrial eggs that hatch into froglets without a free tadpole stage. The breeding habits of *S. pipilodryas* are unknown.

Summarizing, except for body size, there is no convincing morphological difference supporting the recognition of a separate genus *Nesomantis* to place the species *thomasseti* separate from *sechellensis*. The genetic distance between these species is relatively low for frogs (4.4% in the 16S rRNA gene). In contrast, the species *gardineri* and *pipilodryas* form a

genetically highly distinct clade, supported by molecular data and morphological and behavioural evidence. We suggest that these findings should be reflected in the taxonomy by including both *sechellensis* and *thomasseti* in a single genus *Sooglossus*, with the generic name *Nesomantis* being a junior synonym, and by describing a new genus *Leptosoglossus* to accommodate the two remaining sooglossid species, *gardineri* and *pipilodryas*.

## SOOGLOSSIDAE NOBLE, 1931

*SOOGLOSSUS* BOULENGER, 1906

*Sooglossus* Boulenger, 1906: *Type species by monotypy: Arthroleptis sechellensis* Boettger, 1896.

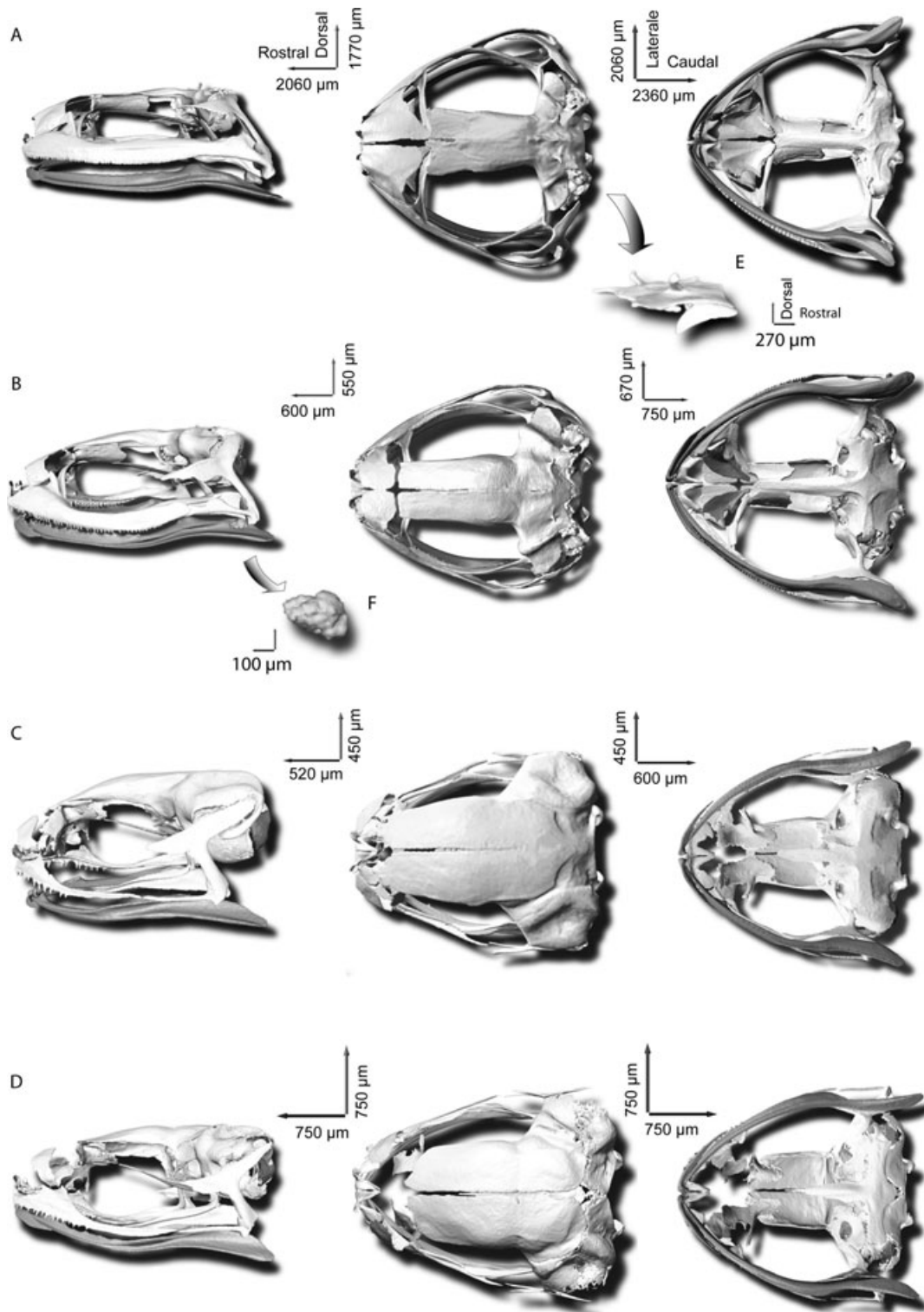
*Nesomantis* Boulenger, 1909: *Type species by monotypy: Nesomantis thomasseti* Boulenger, 1909.

**New synonym.**

*Species included: Sooglossus sechellensis* (Boettger, 1896); *Sooglossus thomasseti* (Boulenger, 1909).

*Diagnosis:* Small to medium sized sooglossids (16–45 mm snout–vent length) with protruding nostrils, widely separated metacarpal tubercles, fingers with pointed toe pads, and tubercular skin on dorsum; toes free, without web.

*Cranium (Fig. 3):* Dorsum of braincase with frontoparietals narrowly separated anteriorly and sutured through greater most of their lengths, and overlapping otic capsule (exoccipital), but not fused to it, extended laterally with medial margins of epiotic eminence and posteriorly at anterior margin of exoccipital; exotosis producing a pair of spines on frontoparietals. Nasals narrowly separated medially and close to preorbital process of maxilla. Presence of neopalatine, its lateral end distinctly wider than medial end, expanded or not on sphenethmoid, absence of anterolateral ossification of sphenethmoid; two halves of sphenethmoid separated dorsomedially and fused ventromedially in their posterior part on 4/5 of their length or not, but forming posterior nasal cavity and olfactory foramina. Maxillary arcade complete bearing teeth, composed of maxilla articulated anteriorly with premaxilla and posteriorly with quadratojugal. Pars facialis of maxilla broad, teething starting just after terminus of zygomatic ramus of squamosal; anterior end of maxilla with pointed process overlapping premaxilla at superior part of pars dentalis; jaw articulation largely posterior of operculum. Squamosals T-shaped, otic ramus of squamosal forming a slender plate, incurved posteromedially, otic plate absent; zygomatic ramus well developed and deflected medially; otic ramus shorter than zygomatic ramus; ventral ramus inclined anteriorly, investing lateral



**Figure 3.** Volume rendering of X-ray microtomography of skulls of Sooglossidae species. A, *Sooglossus thomasseti*, MNHN 2001.0269, Mahé, resolution 30 µm; B, *Sooglossus sechellensis*, MNHN 1984.2371, Mahé, resolution 7.5 µm; C, *Leptosoglossus gardineri*, MNHN 1984.2369, Mahé, resolution 7.5 µm; D, *Leptosoglossus pipilodryas*, RBSS 2003.0007, Silhouette, resolution 7.5 µm; E, detailed latero-posterior view of otoccipital region with exostosis producing a pair of processes in *S. thomasseti*; F, detail of lateral view of sesamoid bone of articulation of maxillary mandibular of *S. sechellensis*. Left, lateral view from left; middle, dorsal view; right, ventral view.



surface of palatoquadrate and distinctly separated from quadratojugal. Pterygoid triradiate slender and gracile; anterior ramus of perygoid extends anterolaterally from otic capsule to articulation with groove of maxillae formed by the partes palatina and facialis; posterior ramus investing medial surface of palatoquadrate; medial ramus terminating vertically on anterior margin of otic capsule and not in contact with prootic and parasphenoid alary process; medial ramus longer than posterior ramus. Medio-ventral part of braincase closed by bone; parasphenoid T-shaped with alary process, orientated laterally but slanted slightly posterolaterally; cultriform process of parasphenoid extending anteriorly to level of antero-ventral margins of sphenethmoid; anterior terminus of cultriform process of irregular shape and non-acuminate; parasphenoid not fused with sphenethmoid and otic capsule; posterior process of parasphenoid acuminate. Operculum entirely ossified. Denticulate serration on dentary present; a sesamoid at maxillo-mandibular articulation present.

*Postcranium* (Fig. 4): Eight procoelous, not imbricate presacral vertebrae; presacral I and II fused or not; atlantal condylar type I (Lynch, 1971), widely separated; neural arches of vertebrae II–VIII bearing a single, posteriorly directed spinous process, overlapping succeeding vertebra or not. Sacro-coccygeal articulation monocondylar; sacral diapophyses dilated. Anterior end of urostyle bearing a pair of vestigial processes; transverse processes of third and fourth presacral vertebrae much wider than width of sacral diapophyses.

*Arciferal pectoral girdle*: Cleithrum and suprascapula distinguishable; suprascapula with proximal section ossified, Y-shaped; cleithrum partially ossified distally. Coracoid, scapula, procoracoids and clavicle not synostotically united but linked by cartilage, partially ossified. Omosternum and sternum ossified or partially ossified.

Terminal phalanges simple, sharply pointed, ending in a very small knob, no intercalary cartilage between penultimate and ultimate phalanges of fingers and toes.

*Phylogenetic definition*: The clade stemming from the most recent common ancestor of *S. sechellensis* (Boettger, 1896) and *S. thomasseti* (Boulenger, 1909).

*Reproductive behaviour*: In *S. sechellensis*, eggs are laid on the ground; after hatching, nonfeeding (endotrophic) tadpoles will climb on the back of adult and are carried until metamorphosis. Females of *S. thomasseti* are known to have large and pigmentless ovarian eggs, and they hatch into endotrophic tadpoles (R. Boistel, pers. observ.).

*Etymology*: Composed of the Classical Greek terms *soos*, safe, sound, unscathed, unwounded; *glossa*, tongue.

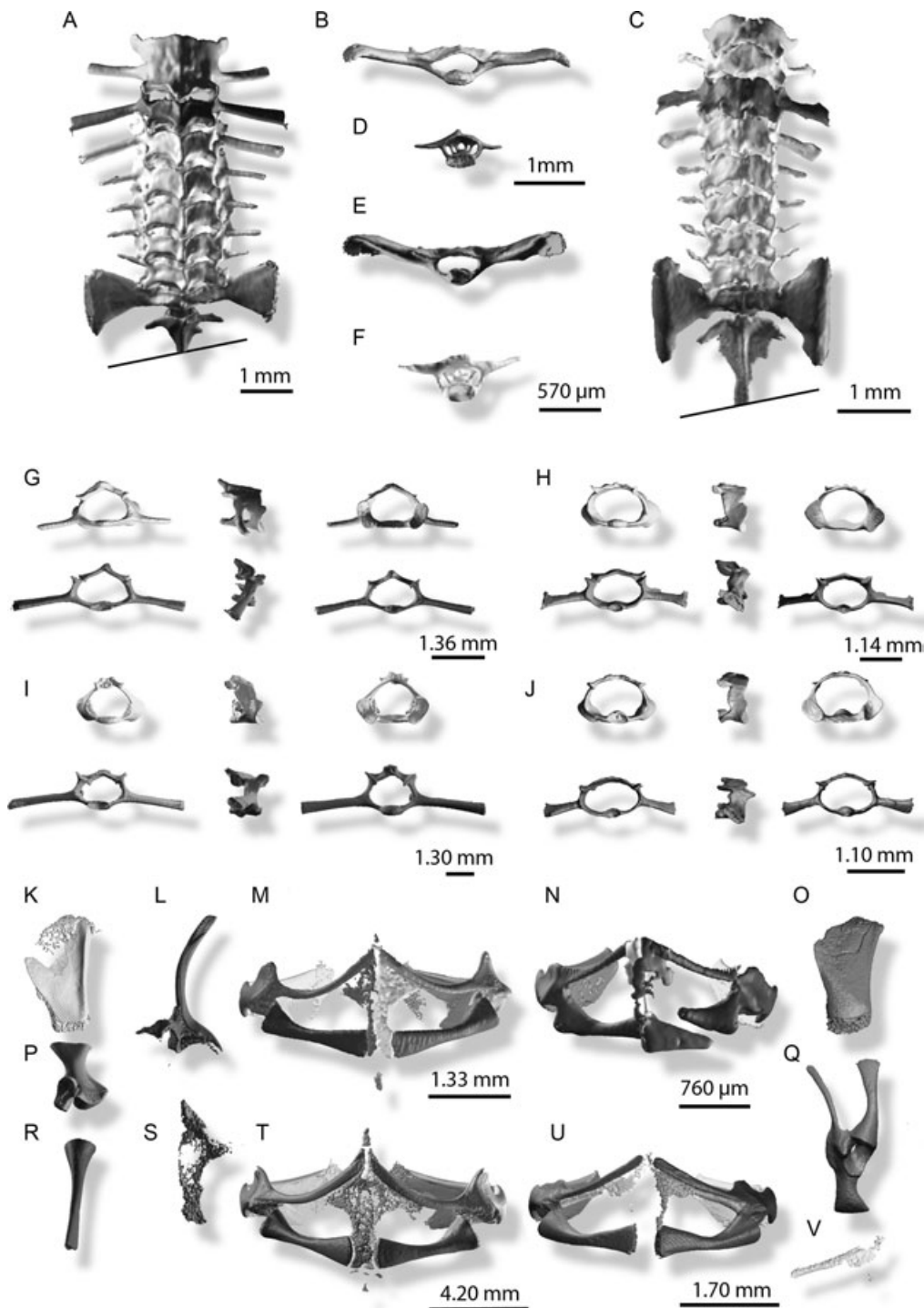
#### **LEPTOSOOGLOSSUS GEN. NOV.**

*Type species by present designation*: *Nectophryne gardineri* Boulenger, 1911.

*Species included*: *Leptosooglossus gardineri* (Boulenger, 1911); *L. pipilodryas* (Gerlach & Willi, 2002).

*Diagnosis*: Small sized sooglossids (9.3–16.4 mm snout–vent length) with nonprotruding nostrils; reduced metacarpal tubercles, reduced toe pads (pointed on feet only or on digit III only), and a smooth skin except for rows of well-defined tubercles on dorsum; toes with fleshy webs.

*Cranium* (Fig. 3): Dorsum of braincase with frontoparietals narrowly separated anteriorly and fused through posterior half of their lengths or not and overlapping otic capsule (exoccipital), but fused or not, extended laterally with medial margins of epiotic eminence and posteriorly at anterior margin of exoccipital; no exostosis producing a pair of spine on frontoparietals. Nasals widely separated medially and not in contact with preorbital process of maxilla. Neopalatine reduced or absent; if absent, presence of anterolateral ossification of sphenethmoid in ventral region of planum antorbitale; two halves of sphenethmoid separated dorsomedially and fused ventromedially on 1/6 of length or not, but forming posterior nasal cavity and olfactory foramina. Maxillary arcade incomplete, bearing teeth, composed of maxilla articulated anteriorly with premaxilla but posteriorly not articulated with quadratojugal. Pars facialis of maxilla slender, teething beginning at level of posteroventral margin of sphenethmoid; anterior part of maxilla with pointed process, overlapping premaxilla at inferior part of pars dentalis; jaw articulation at level of operculum or just posterior. Squamosals T-shaped, otic ramus of squamosal forming slender plate, parallel or deflected laterally to medial plan; otic plate absent; zygomatic ramus well developed and deflected laterally; length of otic ramus larger than zygomatic ramus; ventral ramus inclined anteriorly, investing lateral surface of palatoquadrate, close but separated from quadratojugal. Pterygoids triradiate, slender and gracile; anterior ramus of perygoid extending anterolaterally from otic capsule to articulation with maxillae formed by pars palatine; posterior ramus investing medial surface of palatoquadrate; medial ramus terminating vertically on anterior margin of otic capsule and not in contact with prootic and parasphenoid alary process; medial ramus longer or smaller than posterior ramus; medial ramus of pterygoid not expended, articulating



**Figure 4.** Volume rendering of X-ray microtomography of postcranial skeleton of Sooglossidae species. A, B, D, G, M, *Sooglossus sechellensis*, MNHN 1984.2371, Mahé, resolution 7.5 µm; C, E, F, J, N, *Leptosooglossus gardineri*, MNHN 1984.2369, Mahé, resolution 7.5 µm; H, O, Q, W, X, *Leptosooglossus pipilodryas*, RBSS 2003.0007, Silhouette, resolution 7.5 µm; I, K, L, P, R, S, T, *Sooglossus thomasseti*, RBSS 2003.0001, Silhouette, resolution 7.5 µm. A, C, vertebral column, dorsal view; B, E, Sacrum, posterior view; D, F, urostyle, anterior view; G, H, I, J (upper row), atlas vertebrae, posterior (left), lateral (middle), and anterior (right) view; G, H, I, J (lower row), third presacral vertebrae (left), lateral (middle), and anterior (right) view; M, N, T, W, pectoral girdle, ventral view; K, O, suprascapula, dorsal view; P, scapula, lateral view; L, clavicle, dorsal view; R, coracoid, anterior view; S, X, ossification of epi- and precoracoid cartilage; Q, scapula, clavicle, coracoid and procoracoid, synosteotically united, ventro-lateral view. N, the coracoid of *L. gardineri* is fractured.

vertically with anterodorsal edge of optic capsule. Medioventral part of braincase not closed by bone; parasphenoid T-shaped with alary process, orientated slightly posterolateral; cultriform process of parasphenoid extending anteriorly to level of postero-ventral margins of sphenethmoid; anterior terminus of cultriform process pointed and acuminate; parasphenoid fused or not with sphenethmoid and otic capsule; posterior process of parasphenoid is truncate. Operculum partly ossified. Denticulate serration on dentary absent, but a single toothlike process on each dentary; sesamoid at maxillo-mandibular articulation absent.

*Postcranium (Fig. 4)*: Eight procoelous vertebrae or vertebrae III to VIII procoelous and vertebra I with posteriorly concave centrum and vertebra II with biconvex centra; non-imbricate presacral vertebrae; presacral I and II not fused; atlantal condylar type I (Lynch, 1971), widely separated; neural arches of vertebra II–VIII without neural spine. Sacro-coccygeal articulation monocondylar; sacral diapophyses further dilated. Anterior end of urostyle bearing a pair of vestigial processes; transverse processes of third and fourth presacral vertebrae wider than the width of sacral diapophyses.

*Pseudo-arciferal pectoral girdle*: Cleithrum and suprascapula entirely ossified. Coracoid, scapula, procoracoids and clavicle synosteotically united. Omosternum and sternum cartilaginous.

Terminal phalanges simple, sharply pointed, ending in a very small knob, no intercalary cartilage between penultimate and ultimate phalanges of fingers and toes.

*Phylogenetic definition*: The clade stemming from the most recent common ancestor of *L. gardineri* (Boulenger, 1911) and *L. pipilodryas* (Gerlach & Willi, 2002).

*Reproductive behaviour*: Females of *L. gardineri* sit on top of eggs laid in hidden terrestrial sites. Fully metamorphosed froglets of 3–4 mm will hatch out of these eggs. No tadpole carrying. Reproduction of *L. pipilodryas* is not yet known.

*Etymology*: Composed by the Classical Greek terms *lepton*, small, fine; *soos*, safe, sound, unscattered, unwounded; *glossa*, tongue.

#### NOTE ADDED IN PROOF

While the current paper was in press, several relevant papers on amphibian phylogeny have been published. Most relevant of these is the paper by Frost *et al.* (2006), in which new classifications were proposed for some of the taxa included in the current study. Although the classification of the Sooglossidae was not

changed, the unique position of the genus *Caudiverbera* was acknowledged by Frost *et al.* (2006) by placing it in the family Batrachophrynidae together with *Telmatobufo*. The removal of *Batrachophrynus* from this family subsequently required the renaming of the family containing the remaining taxa to Calyptocephalellidae. Despite the inclusion by Frost *et al.* (2006) of a large molecular as well as morphological dataset in their analysis, the branching order in the basal part of the neobatrachian group remains largely unsupported also in their study.

#### ACKNOWLEDGEMENTS

We are very grateful to the Seychelles Bureau of Standards for permission (SBS) to work at the Silhouette Island, Republic of Seychelles, the R. Gerlach family (Nature Protection Trust of Seychelles), and L. Chong Seng for assisting during fieldwork. Also, many thanks to M. Géze and M. Dellinger, for facilitating and accommodating the use of the graphic workstation at CEMIM (MNHN, Paris). Many thanks to the Ministry of Environment of Seychelles and the Directory General of Conservation, Mr D. Dogley, for delivering collecting permits used for the specimens mentioned in this paper. Finally, thanks to A. Dubois, J. M. Boistel, M. Boistel, M. Goyon, T. Aubin, A. Mazabraud, P. Cloetens, E. Boller, P. Tafforeau, J.-F. Aubry, J.-Y. Tinevez, and the teams of id19 in ESRF and Laboratoire des Reptiles et Amphibiens (MNHN) for their help. The European Synchrotron Radiation Facility (ESRF) is acknowledged for providing beamtime in the framework of proposal SC-1593 and MD179. This work was financially supported by the Deutsche Forschungsgemeinschaft (grant ME1725/10-1).

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