



First record of Foulassi Screeching Frog, *Arthroleptis adelphus* (Perret, 1966) (Anura, Arthroleptidae, Arthroleptinae), from Nigeria, with notes on its phylogenetic position

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Abstract

We report the first known occurrence of the Foulassi Screeching Frog, *Arthroleptis adelphus* (Perret, 1966), from Nigeria. A specimen of *A. adelphus* was collected during herpetological survey work conducted in Cross River National Park, south-eastern Nigeria. Morphometrics and mitochondrial 16S rRNA gene confirm identity of the specimen. Matrilineal genealogy reveals a sister relationship of *A. adelphus* from Nigeria with individuals from south-western Cameroon. Genetic analysis further shows geographic structuring and divergence among populations of *A. adelphus* from the Guineo–Congolian forest region. We offer updates to the IUCN geographic range of *A. adelphus*.

Keywords

Cross River National Park, Guineo–Congolian forest region, range extension, sub-Saharan Africa

Academic editor: Ross MacCulloch | Received 13 January 2019 | Accepted 21 February 2019 | Published 1 March 2019

Citation: Nneji LM, Adeola AC, Okeyoyin A, Onadeko AB, Eniang EA, Adeyi AO, Oladipo SO, Adedeji BE, Usongo JY, Andem AB, Ugwumba OA, Ugwumba AAA, Olory C (2019) First record of Foulassi Screeching Frog, *Arthroleptis adelphus* (Perret, 1966) (Anura, Arthroleptidae, Arthroleptinae), from Nigeria, with notes on its phylogenetic position. Check List 15 (2): 253–258. <https://doi.org/10.15560/15.2.253>

Introduction

Species of the genus *Arthroleptis* Smith, 1849 are found in tropical sub-Saharan Africa (Blackburn 2008, Frost 2019). Commonly referred to as screeching frogs or squeakers, they are mostly terrestrial breeders, feeding on a range of arthropods including ants and termites

(Blackburn 2008). Currently, 48 species are known to occur in sub-Saharan Africa (Frost 2019). In Nigeria, this genus is represented by 3 species: *Arthroleptis palava* (Blackburn, Gvoždík & Leaché, 2010), *Arthroleptis poecilnotus* (Peters, 1863), and *Arthroleptis variabilis* (Matschie, 1893) (AmphibiaWeb 2019). There has been no record of *Arthroleptis adelphus* (Perret, 1966) in

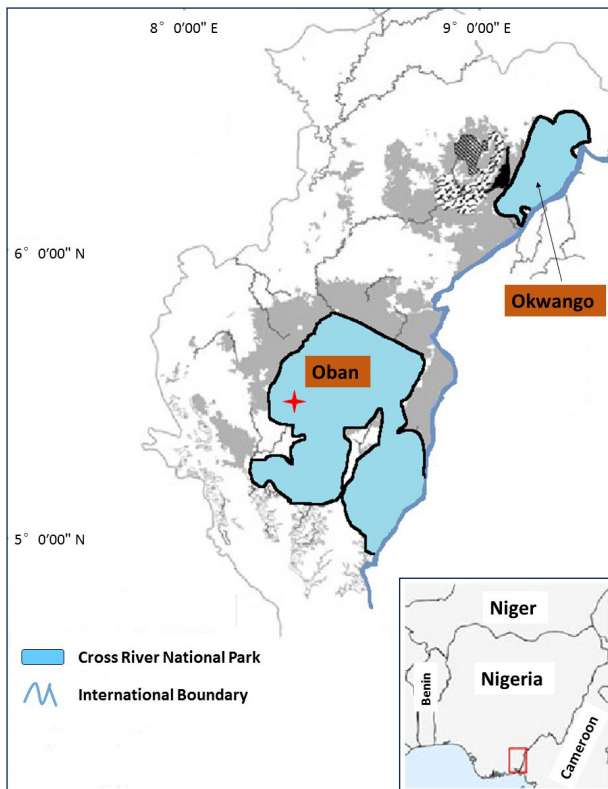


Figure 1. Map showing the sampled locality, Cross River National Park, Cross River, Nigeria. The new record of *Arthroleptis adelphus* was observed and collected from locality indicated by a red star.

Nigeria, although it occurs nearby in the forests of southern Cameroon, Equatorial Guinea (including Bioko), Gabon, and possibly southwestern Central African Republic and northwestern Republic of the Congo (Frost 2019). *Arthroleptis adelphus* is known to inhabit leaf litter within lowland forests, often avoiding marshy areas (Burger et al. 2006, IUCN 2014, Larson and Zimkus 2018). Although this species is classified by the IUCN as Least Concern, forest loss associated with agriculture, logging and human settlement could negatively impact its survival and distribution (IUCN 2014).

Herein, we report the first record of *A. adelphus* in Nigeria and offer updates to the IUCN geographic range for *A. adelphus*.

Methods

Specimen collection. Ethical approval for the field survey (NPH/GEN/121/XXV/461) was obtained from National Park Service, Abuja, Nigeria. The herpetological survey was conducted in Cross River National Park (CRNP) (Fig. 1) from 18 June to 15 December 2018. Using visual encounter survey and opportunistic observation, 5 individuals of *A. adelphus* were observed in the field by BEA and JYU during the day. However, only 1 juvenile individual was collected as the others escaped before capture. Information such as habitat type and geographic coordinates were documented. The specimen was euthanized humanely with hydrous chlorobutanol. A tissue (muscles) sample was collected and stored in

95% ethanol. Subsequently, the voucher specimen was fixed with 4% formalin and preserved in 75% ethanol for long-term storage. The voucher and tissue samples were deposited in the museum of the Department of Zoology, University of Ibadan, Ibadan, Nigeria.

Preliminary species identification and morphometrics. Preliminary species identification followed primary literature of Zimkus and Blackburn (2008). In addition, the morphometrics of the voucher specimen were recorded. Measurements were made by BEA using a digital caliper with a precision of 0.01 mm. Twenty-three linear measurements were taken as follows: SVL (snout-vent length), HW (head width), HL (head length), TRL (trunk Length), SL (snout length), ED (eye diameter), TYD (tympanum outer diameter), ETD (eye to tympanum length), IOD (interorbital distance), FAL (fore-arm length), FL1 (first finger length), FL2 (second finger length), FL3 (third finger length), FL4 (fourth finger length), HiL (hind Limb length), TL (tibia length), SL (shank Length), FOL (foot length), TL1 (first toe length), TL2 (second toe length), TL3 (third toe length), TL4 (fourth toe length), and TL5 (fifth toe length).

Molecular laboratory protocol. Total genomic DNA of the ethanol preserved tissue was extracted following phenol-chloroform extraction procedure (Sambrook and Russell 2001). Matrilineal genealogy was inferred using mitochondrial gene fragment 16S rRNA, which is commonly used as a DNA barcoding marker for African anurans (Nneji et al. 2019). An approximately 540 bp segment of 16S rRNA gene was amplified with primers designed by Bossuyt and Milinkovitch (2000). The PCR cycle profiles were as follow: 5 min initial denaturation at 94 °C, followed by 35 cycles of 1 min at 94 °C, annealing for 45 s at 55 °C, extension for 1 min at 72 °C; final extension for 10 min at 72 °C. Purified PCR products were directly sequenced in both forward and reverse directions with an automated DNA sequencer (ABI 3730). The nucleotide sequence was viewed and confirmed manually using SeqManTMII (DNASTAR Lasergene 7). Furthermore, the amplified sequence was submitted to BLAST search in NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to verify for sequence similarity.

Genetic data analysis. To further confirm the identity of the specimen and examine its phylogenetic position, our sequence was compared with others obtained from GenBank. A total of 101 16S rRNA sequences of 7 species belonging to the genus *Arthroleptis* from West and Central Africa were downloaded as follows: *A. adelphus* ($n = 16$), *A. palava* ($n = 11$), *A. poecilnotus* ($n = 34$ including *Arthroleptis* cf. *poecilnotus*), *A. variabilis* ($n = 15$), *Arthroleptis perreti* (Blackburn, Gonwouo, Ernst & Rödel, 2009) ($n = 5$), *Arthroleptis sylvaticus* Laurent, 1954 ($n = 15$) and *Arthroleptis taeniatus* (Boulenger, 1906) ($n = 5$) (Appendix Table A1). Sequences of 3 closely related species were downloaded to include as outgroup taxa: *Arthroleptis formosus* (Rödel, Kouame, Doumbia

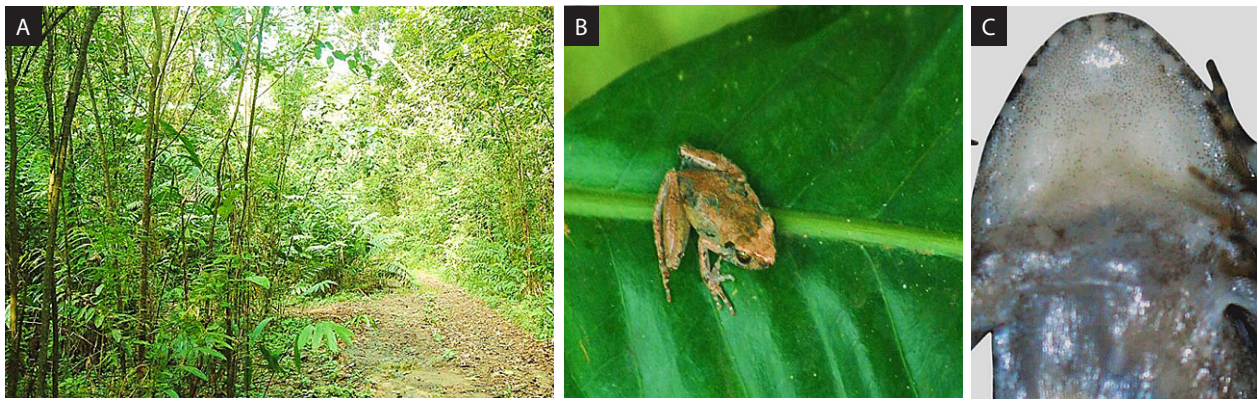


Figure 2. A. Habitat where *Arthroleptis adelphus* CRNPA0049 was collected. B. CRNPA0049 in life. C. Ventral view of the preserved specimen.

& Sandberger, 2011), *Arthroleptis aureoli* (Schjötz, 1964), and *Arthroleptis langeri* (Rödel, Doumbia, Johnson & Hillers, 2009). Nucleotide sequences were aligned in MEGA 6.0 using ClustalW (Tamura et al. 2013) with default parameters. Hypervariable regions that could not be reliably aligned were manually excluded from the analysis, resulting in the exclusion of approximately 100 bp. The best-fit model of the nucleotide substitution was selected as GTR+I+G using the Akaike Information Criterion as implemented in jModeltest 3.7 (Posada and Crandall 1998). The maximum likelihood (ML) was performed with RAXML v. 7.0.3 (Stamatakis 2006) using the GTR + G model with 100 random addition replicates and per partition branch lengths (Townsend et al. 2011). Reliability of the ML tree was assessed by bootstrap analysis (Felsenstein 1985) including 1000 replications. The matrilineal genealogy was visualized using FigTree v. 1.4.2 (Rambaut 2012). Nodes were considered strongly supported if they received ML bootstrap proportions > 70%.

Results

Arthroleptis adelphus (Perret, 1966)

Figure 2A–C

New record. Nigeria, Cross River State, Calabar, Cross River National Park, Oban East Range, Erokut Camp (05°21'89" N, 008°26'46" E), BEA and JYU, 3 August 2018 (1 specimen, juvenile).

Our herpetological survey of CRNP discovered that *Arthroleptis adelphus* occurs within the lowland forest of Erokut Camp of Oban East Range, CRNP (Fig. 2A). Individuals of *Arthroleptis adelphus* were commonly encountered within the leaf litter of lowland forest and on vegetation 0.5–1.0 m above the ground. The specimen of *Arthroleptis adelphus* from CRNP represents the first record from Nigeria, extending this species' geographic distribution west by approximately 149.9 km from its nearest location in south-western Cameroon. The voucher specimen was deposited in the museum of the Department of Zoology, University of Ibadan, Nigeria, under reference number CRNPA0049. The 16S

rRNA sequence was submitted to GenBank under accession number MK377154.

Identification. The voucher specimen was morphologically identified as a member of the genus *Arthroleptis* by the presence of moderate pedal webbing, and the absence of tarsal tubercle, outer metatarsal tubercle, heel tubercle (located at the proximal end of the tarsus) and circum-marginal groove at manual and/ or pedal digit tips. The individual was identified as juvenile because of its small body size and lack of secondary sexual characteristics. The morphological measurements of the voucher are as follows: SVL = 19.10 mm, HW = 6.20 mm, HL = 7.00 mm, TRL = 8.80 mm, SL: 1.40 mm, ED: 2.0 mm, TYD = 1.20 mm, ETD = 0.8 mm, IOD = 1.40 mm, FAL = 8.70 mm, FL1 = 2.40 mm, FL2 = 2.70 mm, FL3 = 3.90 mm, FL4 = 2.10 mm, HiL = 10.90 mm, TL = 6.90 mm, SHL = 9.00 mm, FOL = 15.2 mm, TL1 = 2.90 mm, TL2 = 3.40 mm, TL3 = 4.20 mm, TL4 = 6.70 mm, and TL5 = 3.60 mm. The individual could not be assigned to a species using morphology alone; thus, molecular data was used to determine the species identity.

The BLAST search for 16S rRNA sequence of our specimen showed 99% DNA sequence similarity with *A. adelphus* FJ151093 and FJ151092 from Cameroon. Sequence data showed that *A. adelphus* CRNPA0049 from Nigeria clustered genetically in a clade consisting of other *A. adelphus* from Central Africa (Figure 3). ML analysis revealed that CRNPA0049 formed sister group relationship with specimens FJ151093 and FJ151092 from Ejagham Forest Reserve, Southwest Cameroon (ML bootstrap proportion = 97%, Fig. 3).

Distribution, natural history and conservation. *Arthroleptis adelphus* is now known from lowland forests in southeastern Nigeria. Its documented range now extends from lowland forests of south-eastern Nigeria to southern Cameroon, Equatorial Guinea (including Bioko), and Gabon. The most common syntopic anurans in CRNP were *Sclerophrys gracilipes* (Boulenger, 1899) and *Sclerophrys maculata* (Hallowell, 1854). Considering the relatively large geographic distribution of *A. adelphus* across Central Africa, we do not consider *A.*

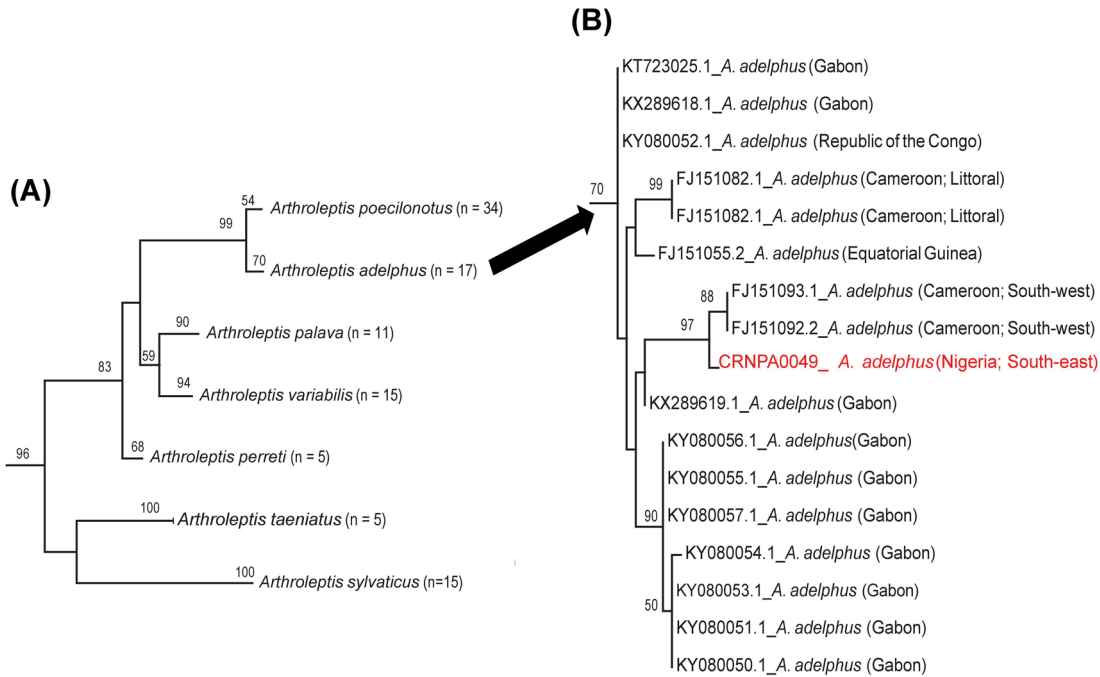


Figure 3. Matrilineal genealogy. **A.** Collapsed ML phylogram of some Central and West African species of the genus *Arthroleptis* inferred based on mtDNA 16S rRNA. **B.** Expanded ML Phylogram showing the matrilineal relationships within the *Arthroleptis adelphus* species group. Numbers at each node represent the ML bootstrap values; nodes with bootstrap values above 50% are shown. The new record of *A. adelphus* from Nigeria is in red.

adelphus to be threatened. However, increased deforestation and agricultural practices in the region pose threats to this species. Additional field studies appear necessary to document population density and status.

Discussion

Our study documents the occurrence of *A. adelphus* from the lowland forest of south-eastern Nigeria. Previously, its distribution was thought to span across southern Cameroon, Equatorial Guinea (including Bioko), Gabon, and possibly in southwestern Central African Republic and northwestern Republic of the Congo (Burger et al. 2006, Larson and Zimkus 2018, Frost 2019). The proximity of CRNP to the border of southwestern Cameroon and habitat of the lower Guineo–Congolian forest region support the possibility of *A. adelphus* occurrence in south-eastern Nigeria. Our findings corroborate the previous study of Nneji et al. (2018) that highlighted similarities of Nigerian and Cameroonian herpetofauna due to shared environmental conditions. Our observation, therefore, strengthens the importance of extensive survey of CRNP and other regions in south-eastern Nigeria to reveal patterns of biogeographic relatedness of amphibians in the lower Guineo–Congolian forest region.

Consistent with the report of the IUCN (2014), our recent survey revealed that *A. adelphus* is closely associated with lowland forest habitats threatened by deforestation, slash and burn agricultural practices, habitat conversion, and logging. Amphibians are highly sensitive to comparatively minor forest degradation (Ernst

and Rödel 2005, Ernst et al. 2006), and the destruction of habitat poses devastating effects on the population status and survival of *A. adelphus* across Central and West Africa. This calls for a concerted approach for the conservation of this species.

The phylogeny generated as part of this study was not only informative with respect to confirmation of the identity of our specimen from CRNP, but also had implications for the possibility of cryptic diversity within *A. adelphus*, as proposed by Deichmann et al. (2017). Divergence among populations of *A. adelphus* is evident, and CRNPA0049 is more closely related to specimens from south-western Cameroon than it is to specimens from elsewhere (Fig. 3). The type locality for *A. adelphus* is “Foulassi”, near Sangmelima, South Cameroon (Frost 2019), and CRNPA0049 was collected near the type locality. This, therefore, provides additional confirmation regarding the validity of our specimen as *A. adelphus*. Thus, we offer recommendations for the IUCN update of the geographic range of this species.

Acknowledgements

This study was funded by National Geographic Society Grant (EC-357C-18). We are grateful to the rangers at Cross River National Park for assistance in sampling. We thank the management of National Park Service in Abuja, Nigeria for providing permission and technical assistance for the field survey. We also acknowledge the 3 anonymous reviewers and academic editor for helpful suggestions that improved this manuscript.

Authors' Contributions

LMN designed the study; BEA and JYU collected and preserved the specimen; BEA measured the specimen; LMN performed the genetic and morphological identification; ACA, AOO, AOA, ABO, EAE, SOO, OAU, ABA, AAU and CO provided technical assistance for the study and field collections; LMN, BEA, SOO and ACA wrote the initial draft of the manuscript, reviewed and finalized the manuscript; all authors read and approved the final manuscript.

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Appendix

Table A1. Information of Central and West African species of the genus *Arthroleptis* used in the matrilineal genealogy.

Species	GenBank Accession Numbers	Reference	Species	GenBank Accession Numbers	Reference
Ingroup					
1 <i>Arthroleptis palava</i> 1	HM238195	Blackburn et al. (2010)	53 <i>A. variabilis</i> 8	DQ283081	Frost et al. (2006)
2 <i>A. palava</i> 2	HM238194	Blackburn et al. (2010)	54 <i>A. variabilis</i> 9	FJ151058	Blackburn (2008)
3 <i>A. palava</i> 3	HM238193	Blackburn et al. (2010)	55 <i>A. variabilis</i> 10	FJ151147	Blackburn (2008)
4 <i>A. palava</i> 4	HM238189	Blackburn et al. (2010)	56 <i>A. variabilis</i> 11	FJ151091	Blackburn (2008)
5 <i>A. palava</i> 5	HM238188	Blackburn et al. (2010)	57 <i>A. variabilis</i> 12	FJ151086	Blackburn (2008)
6 <i>A. palava</i> 6	HM238187	Blackburn et al. (2010)	58 <i>A. variabilis</i> 13	AF124107	Vences et al. (2006)
7 <i>A. palava</i> 7	HM238186	Blackburn et al. (2010)	59 <i>A. variabilis</i> 14	FJ151083	Blackburn (2008)
8 <i>A. palava</i> 8	FJ151150	Blackburn (2008)	60 <i>A. variabilis</i> 15	KX289620	Larson & Zimkus (2018)
9 <i>A. palava</i> 9	FJ151143	Blackburn (2008)	61 <i>Arthroleptis adelphus</i> 1	KX289619	Larson & Zimkus (2018)
10 <i>A. palava</i> 10	FJ151142	Blackburn (2008)	62 <i>A. adelphus</i> 2	FJ151093	Blackburn (2008)
11 <i>A. palava</i> 11	FJ151132	Blackburn (2008)	63 <i>A. adelphus</i> 3	KY080057	Deichmann et al. (2017)
12 <i>Arthroleptis cf. poecilonotus</i> 1	KY080067	Deichmann et al. (2017)	64 <i>A. adelphus</i> 4	KY080056	Deichmann et al. (2017)
13 <i>A. cf. poecilonotus</i> 2	KY080066	Deichmann et al. (2017)	65 <i>A. adelphus</i> 5	FJ151082	Blackburn (2008)
14 <i>A. cf. poecilonotus</i> 3	KY080065	Deichmann et al. (2017)	66 <i>A. adelphus</i> 6	FJ151081	Blackburn (2008)
15 <i>A. cf. poecilonotus</i> 4	KY080064	Deichmann et al. (2017)	67 <i>A. adelphus</i> 7	KY080055	Deichmann et al. (2017)
16 <i>A. cf. poecilonotus</i> 5	KY080063	Deichmann et al. (2017)	68 <i>A. adelphus</i> 8	KY080054	Deichmann et al. (2017)
17 <i>A. cf. poecilonotus</i> 6	KY080062	Deichmann et al. (2017)	69 <i>A. adelphus</i> 9	KY080053	Deichmann et al. (2017)
18 <i>A. cf. poecilonotus</i> 7	KY080061	Deichmann et al. (2017)	70 <i>A. adelphus</i> 10	KY080052	Deichmann et al. (2017)
19 <i>A. cf. poecilonotus</i> 8	KY080060	Deichmann et al. (2017)	71 <i>A. adelphus</i> 11	KY080051	Deichmann et al. (2017)
20 <i>A. cf. poecilonotus</i> 9	KY080059	Deichmann et al. (2017)	72 <i>A. adelphus</i> 12	KY080050	Deichmann et al. (2017)
21 <i>A. cf. poecilonotus</i> 10	KY080058	Deichmann et al. (2017)	73 <i>A. adelphus</i> 13	KT723025	Larson (unpublished)
22 <i>Arthroleptis poecilonotus</i> 1	KX671725	Portik et al. (2016)	74 <i>A. adelphus</i> 14	FJ151092	Blackburn (2008)
23 <i>A. poecilonotus</i> 2	KX671724	Portik et al. (2016)	75 <i>A. adelphus</i> 15	FJ151055	Blackburn (2008)
24 <i>A. poecilonotus</i> 3	KX671723	Portik et al. (2016)	76 <i>A. adelphus</i> 16	KX289618	Larson & Zimkus (2018)
25 <i>A. poecilonotus</i> 4	KX492599	Portik & Blackburn (2016)	77 <i>A. adelphus</i> 17	MK377154	This study
26 <i>A. poecilonotus</i> 5	JX564853	Zhang et al. (2013)	78 <i>Arthroleptis perreti</i> 1	FJ151139	Blackburn (2008)
27 <i>A. poecilonotus</i> 6	FJ151114	Blackburn (2008)	79 <i>A. perreti</i> 2	FJ151138	Blackburn (2008)
28 <i>A. poecilonotus</i> 7	FJ151113	Blackburn (2008)	80 <i>A. perreti</i> 3	FJ151095	Blackburn (2008)
29 <i>A. poecilonotus</i> 8	FJ151112	Blackburn (2008)	81 <i>A. perreti</i> 4	FJ151094	Blackburn (2008)
30 <i>A. poecilonotus</i> 9	FJ151111	Blackburn (2008)	82 <i>A. perreti</i> 5	KY080068	Deichmann et al. (2017)
31 <i>A. poecilonotus</i> 10	FJ151110	Blackburn (2008)	83 <i>Arthroleptis sylvaticus</i> 1	KY080084	Deichmann et al. (2017)
32 <i>A. poecilonotus</i> 11	FJ151109	Blackburn (2008)	84 <i>A. sylvaticus</i> 2	KY080083	Deichmann et al. (2017)
33 <i>A. poecilonotus</i> 12	FJ151108	Blackburn (2008)	85 <i>A. sylvaticus</i> 3	KY080082	Deichmann et al. (2017)
34 <i>A. poecilonotus</i> 13	FJ151090	Blackburn (2008)	86 <i>A. sylvaticus</i> 4	KY080081	Deichmann et al. (2017)
35 <i>A. poecilonotus</i> 14	FJ151089	Blackburn (2008)	87 <i>A. sylvaticus</i> 5	KY080080	Deichmann et al. (2017)
36 <i>A. poecilonotus</i> 15	FJ151085	Blackburn (2008)	88 <i>A. sylvaticus</i> 6	KY080079	Deichmann et al. (2017)
37 <i>A. poecilonotus</i> 16	FJ151084	Blackburn (2008)	89 <i>A. sylvaticus</i> 7	KY080078	Deichmann et al. (2017)
38 <i>A. poecilonotus</i> 17	HM238191	Blackburn et al. (2010)	90 <i>A. sylvaticus</i> 8	KY080077	Deichmann et al. (2017)
39 <i>A. poecilonotus</i> 18	HM238190	Blackburn et al. (2010)	91 <i>A. sylvaticus</i> 9	KY080076	Deichmann et al. (2017)
40 <i>A. poecilonotus</i> 19	HM238192	Blackburn et al. (2010)	92 <i>A. sylvaticus</i> 10	KY080075	Deichmann et al. (2017)
41 <i>A. poecilonotus</i> 20	KU166812	Rockney et al. 2015	93 <i>A. sylvaticus</i> 11	KX492600	Portik & Blackburn (2016)
42 <i>A. poecilonotus</i> 21	KU166811	Rockney et al. 2015	94 <i>A. sylvaticus</i> 12	DQ283078	Frost et al. (2006)
43 <i>A. poecilonotus</i> 22	KU166807	Rockney et al. 2015	95 <i>A. sylvaticus</i> 13	FJ151087	Blackburn (2008)
44 <i>A. poecilonotus</i> 23	KU166807	Rockney et al. 2015	96 <i>A. sylvaticus</i> 14	FJ151148	Blackburn (2008)
45 <i>A. poecilonotus</i> 24	KU166806	Rockney et al. 2015	97 <i>A. sylvaticus</i> 15	FJ151106	Blackburn (2008)
46 <i>Arthroleptis variabilis</i> 1	AY948739	Roelants et al. 2007	98 <i>Arthroleptis taeniatus</i> 1	KY080089	Deichmann et al. (2017)
47 <i>A. variabilis</i> 2	EU350213	Ernst et al. (2008)	99 <i>A. taeniatus</i> 2	KY080088	Deichmann et al. (2017)
48 <i>A. variabilis</i> 3	EU350212	Ernst et al. (2008)	100 <i>A. taeniatus</i> 3	KY080087	Deichmann et al. (2017)
49 <i>A. variabilis</i> 4	FJ151070	Blackburn (2008)	101 <i>A. taeniatus</i> 4	KY080086	Deichmann et al. (2017)
50 <i>A. variabilis</i> 5	FJ151069	Blackburn (2008)	102 <i>A. taeniatus</i> 5	KY080085	Deichmann et al. (2017)
51 <i>A. variabilis</i> 6	AY322301	Roelants et al. (2004)	Outgroup		
52 <i>A. variabilis</i> 7	KX289621	Larson & Zimkus (2018)	1 <i>Arthroleptis formosus</i>	JN408726	Rödel et al. (2011)
			2 <i>Arthroleptis aureoli</i>	JN408731	Rödel et al. (2011)
			3 <i>Arthroleptis langeri</i>	JN408724	Rödel et al. (2011)