Additional file: The first endemic West African vertebrate family – a new anuran family highlighting the uniqueness of the Upper Guinean biodiversity hotspot

Michael F. Barej^{a,*}, Andreas Schmitz^b, Rainer Günther^a, Simon P. Loader^c, Kristin Mahlow^a, Mark-Oliver Rödel^a

^aMuseum für Naturkunde, Leibniz Institute for Research on Evolution and Biodiversity, Invalidenstrasse 43, D-10115 Berlin, Germany
^bNatural History Museum of Geneva, Department of Herpetology and Ichthyology, C.P. 6434, 1211 Geneva 6, Switzerland
^cUniversity of Basel, Department of Environmental Sciences (Biogeography), Klingelbergstr.
27, Basel 4056, Switzerland

*Corresponding author: <u>michael@barej.de</u>

Content:

1. Molecular analyses	
1.1 GenBank accession numbers	2
1.2 Molecular dating	4
1.3 Topology test	5
2. Osteological analyses	
2.1 Odontobatrachus osteology	6
2.2 Osteological characters distinguishing Petropedetidae and	40
Odontobatrachidae fam. nov.	10
3. Amphibian diversity in African realms	25
4. References	26

page:

1. Molecular analyses

1.1 GenBank accession numbers

Previous studies of African and Asian ranids lacked representatives across all families and genera. This study aimed to provide as comprehensive a sampling as possible to resolve the appropriate taxonomic rank of Odontobatrachus. Initial analyses tentatively indicated the grouping of Odontobatrachus and the family Dicroglossidae (Hoplobatrachus; occurring in Africa and Asia). A second member of Dicroglossidae family, belonging to Limnonectes, was generated as a chimaera (Limnonectes poilani and L. laticeps) to form a clade of the family Dicroglossidae, to more thoroughly test the grouping of Odontobatrachus. Where possible, two distinct genera or distantly related species of one genus within one family have been included. Widely distributed anuran families with genera of Asian and African origin have been included in our analyses. Museum abbreviations of voucher and tissue samples included are as follows: The Natural History Museum, London, United Kingdom (BMNH); California Academy of Sciences, San Francisco, USA (CAS); Natural History Museum of Geneva, Switzerland (MHNG); Staatliches Museum für Naturkunde, Stuttgart, Germany (SMNS); Vietnam National Museum of Nature, Hanoi, Vietnam (VNMN); Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK); Museum für Naturkunde, Berlin, Germany (ZMB); Zoological Natural History Museum, Addis Ababa University, Ethiopia (ZNHM-AAU). Remaining material (Be, JR) refers to field collections. Remarks: (1) Sequences of Limnonectes *poilani* (x¹) were not assigned individual collection numbers; respective publications are provided [1,2] and listed in the references. (2) A BLAST search in GenBank [3] of specimen CAS 246787 revealed highest similarity with "Ingerana" baluensis (Ceratobatrachidae) and is consequently maintained as "Ingerana" sp. All GenBank numbers are provided in Table A1.

Table A1 List of taxa included in this study. List of taxa included in this study including voucher ID and locality data (country codes: CI = Cote d'Ivoire, CM = Cameroon, BJ = Benin, ET = Ethiopia, GA = Gabon, GN = Guinea, ID = Indonesia, IN = India, KH = Cambodia, LR = Liberia, MG = Madagascar, MM = Myanmar, TZ = Tanzania, VN = Viet Nam). Sequences marked in italic letters were extracted from GenBank and refer to deviating vouchers. New accession numbers added upon acceptance of the manuscript.

family	genus	species	country	coll #	12S	16S	cytb	BDNF	SIA	rag1
Arthroleptidae	Leptodactylodon	bicolor	CM	ZMB 78460	KF991244	KF991264	KF991306	KF991283	KF991343	KF991325
Arthroleptidae	Trichobatrachus	robustus	CM	ZMB 77508	KF991245	KF991265	KF991307	KF991284	KF991344	KF991326
Brevicipitidae	Callulina	kisiwamsitu	TZ	BMNH 2002.45	AY531840	AY531863	FJ998380	KF991285	KF991345	KF990043
Brevicipitidae	Probreviceps	macrodactylus	TZ	BMNH 2002.754	FN81100	FN811049	FN811094	KF991286	KF991346	KC632525
Ceratobatrachidae	"Ingerana"	sp.	MM	CAS 246787	KF991246	KF991266	KF991308	KF991287	KF991347	KF991327
Ceratobatrachidae	Platymantis	papuensis	ID	ZMB 70131	KF991247	KF991267	KF991309	KF991288	KF991348	KF991328
Conrauidae	Conraua	alleni	GN	ZMB 78433	KF693285	KF693389	KF693669	KF693487	KF693549	KF693609
Conrauidae	Conraua	robusta	CM	ZMB 78427	KF693281	KF693385	KF693665	KF693483	KF693545	KF693605
Dicroglossidae	Hoplobatrachus	occipitalis	GN	ZMB 79256	KF991248	KF991268	KF991310	KF991289	KF991349	KF991329
Dicroglossidae	Limnonectes	laticeps/poilani			DQ283378	DQ283378	AB488856	AB489065	DQ282841	AB488960
Hemisotidae	Hemisus	marmoratus	TZ	BMNH 2005.1385	AY531831	AY531854	FN811075	AB612014	KF991350	AB612013
Hyperoliidae	Hyperolius	ocellatus	CM	MHNG 2715.58	KF693275	KF693379	KF693659	KF693477	KF693539	KF693599
Limnodynastidae	Lechriodus	melanopyga	ID	ZMB 79235	KF991249	KF991269	KF991311	KF991290	KF991351	KF991330
Mantellidae	Boophis	quasiboehmei	MG	ZMB 79257	KF991250	KF991270	KF991312	KF991291	KF991352	KF991331
Mantellidae	Mantidactylus	grandidieri	MG	JR153	KF991251	KF991271	DQ235432	KF991292	KF991353	KF991332
Micrixalidae	Micrixalus	fuscus	IN	no Voucher	KF991252	KF991272	KF991313	KF991293		KF991333
Microhylidae	Phrynomantis	microps	CI	SMNS 9773	AY531855	AY531832	FN563039	AB611980	KF991354	AB611977
Nyctibatrachidae	Nyctibatrachus	sp.	IN	no Voucher	KF991253	KF991273	KF991314	KF991294	KF991355	KF991334
Odontobatrachidae	Odontobatrachus	natator	GN	ZMB 78211	KF693286	KF693390	KF693670	KF693488	KF693550	KF693610
Odontobatrachidae	Odontobatrachus	sp.	GN	ZMB 78317	KF693290	KF693394	KF693674	KF693492	KF693554	KF693614
Petropedetidae	Arthroleptides	martiensseni	TZ	ZFMK 77306	KF693292	KF693396	KF693676	KF693494	KF693556	KF693616
Petropedetidae	Arthroleptides	yakusini	TZ	BMNH 2005.567	JX546941	JX546965	KF693680	KF693502	KF693563	KF693622
Petropedetidae	Ericabatachus	baleensis	ET	ZNHM-AAU-A2013-001	KF938362	KF938365	KF991315	KF991295	KF991356	KF938370
Petropedetidae	Petropedetes	johnstoni	CM	ZFMK 87710	KF693314	GU256028	KF693690	KF693515	KF693576	KF693635
Petropedetidae	Petropedetes	juliawurstnerae	CM	MHNG 2713.19	KF693323	KF693419	KF693693	KF693517	KF693578	KF693637
Petropedetidae	Petropedetes	palmipes	GA	NCSM 76813	KF693329	KF693424	KF693695	KF693519	KF693580	KF693639
Phrynobatrachidae	Phrynobatrachus	africanus	CM	MHNG 2715.45	KF693278	KF693382	KF693662	KF693480	KF693542	KF693602
Phrynobatrachidae	Phrynobatrachus	auritus	CM	MHNG 2715.89	KF693277	KF693381	KF693661	KF693479	KF693541	KF693601
Ptychadenidae	Hildebrandtia	ornata	BJ	ZMB 79258	KF991254	KF991274	KF991316	KF991296	KF991357	KF991335
Ptychadenidae	Ptychadena	aequiplicata	LI	ZMB 79259	KF991255	KF991275	KF991317	KF991297	KF991358	KF991336
Pyxicephalidae	Aubria	subsigillata	LI	ZMB 79260	KF991256	KF991276		KF991298	KF991359	KF991337
Pyxicephalidae	Pyxicephalus	cf. edulis	BJ	Be119	KF991257	KF991277	KF991318	KF991299	KF991360	KF991338
Ranidae	Hylarana	galamensis	GN	ZMB 79262	KF991258	KF991278	KF991319	KF991300	KF991361	KF991339
Ranidae	Pelophylax	lateralis	KH	ZFMK 92555	KF991259	KF991279	KF991320	KF991301	KF991362	EF088273
Ranixalidae	Indirana	sp.1	IN	no Voucher	KF991260	KF991280	KF991321	KF991302	KF991363	KF991340
Ranixalidae	Indirana	sp.2	IN	no Voucher	KF991261	KF991281	KF991322	KF991303	KF991364	KF991341
Rhacophoridae	Chiromantis	rufescens	CM	ZFMK87811	KF991262	KF991282	KF991323	KF991304	KF991365	GQ204605
Rhacophoridae	Theloderma	bicolor	VN	VNMN 1394	KF991263	JX046475	KF991324	KF991305	KF991366	KF991342

1.2 Molecular dating

Calibrations points used were based on data extracted from GenBank sequences, respective GenBank numbers are provided in Table A2. Tentative divergence time estimates provided an age estimate of the West African family Odontobatrachidae fam. nov. of 90.10 Ma [95% confidence interval (CI Ma) 84.19-97.06 Ma, Figure A1]. The split between the families Pyxicephalidae and Petropedetidae has been calculated as 81.25 Ma (CI Ma: 79.32-83.15 Ma). The origin of the family Odontobatrachidae dates back to major splits within the higher-level taxon Ranoidae [4]. Interestingly, molecular timescales could provide a means to estimate taxonomic rank for amphibians [5]. Avise and Liu' [5] estimation for amphibians fits well within the taxonomic rank of family for Odontobatrachidae.

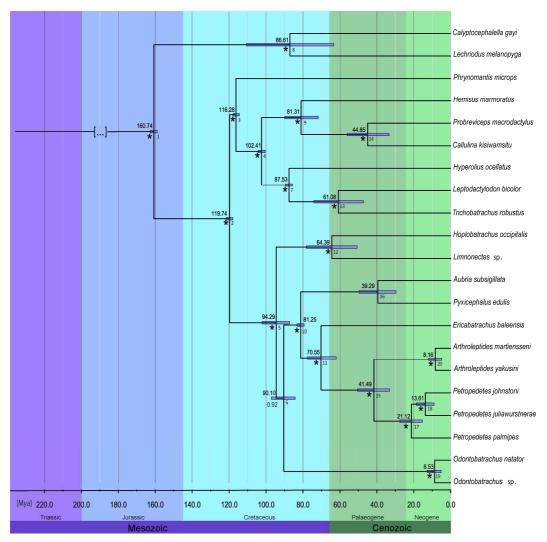


Figure A1 Timetree of Ranoidea. Timetree of the Ranoidea based on mitochondrial and nuclear sequences. Given are mean estimate values (above the 95% congruence interval bar, CI), posterior probabilities (below CI) and node numbers behind the respective node. Divergence times are provided in Table A2. The tree is rooted with hierarchical outgroups Sarcopterygii (*Latimeria*), Caudata (Cryptobranchidae, Hynobiidae); outgroups not shown (for GenBank numbers of outgroups see Table A3).

Node	CI MA
1	158.84-162.73
2	117.97-121.55
3	114.48-118.00
4	100.49-104.28
5	87.32-102.10
6	84.19-97.06
7	85.64-89.46
8	63.34-110.63
9	71.74-89.94
10	79.32-83.15
11	62.00-77.52
12	50.63-78.15
13	47.29-74.13
14	33.41-55.96
15	33.14-50.42
16	29.60-49.65
17	15.31-27.55
18	8.99-18.66
19	5.18-12.75
20	4.89-12.08

 Table A2 Divergence time estimate of Ranoidea. Divergence time estimate, 95% confidence/credibility

 interval in million years (CI MA). Node numbers refer to Figure A1.

Table A3 List of outgroup taxa included in the timetree. List of GenBank sequences applied for molecular dating estimates.

	family	genus	species	mitochondrial genes	BDNF	SIA	rag1
Osteichthyes: Sarcopterygii	Latimeriidae	Latimeria	menadoensis	AP006858	JQ073170		JQ073290
Caudata	Cryptobranchidae	Andrias	japonicus / davidianus	NC004926	EU275889		AY583346
Caudata Anura	Hynobiidae Limnodnastidae	Hynobius Calyptocephalella	yiwuensis gayi	NC020649 JF703228	HM037750 JF703236	 DQ282893	HM037725 EF107334

1.3 Topology tests

Likelihood values of seven alternative topologies were tested against the unconstraint optimal tree (Fig. 1). Results of the AU-test and the SH-test are provided in Table A4. The genus *Odontobatrachus* was placed either basal to the Petropedetidae (Table A4.1), sister to any genus within the Petropedetidae (Table A4.2-A4.4) or sister to a clade comprising Central and East African torrent-frog genera (*Petropedetes* + *Arthroleptides*; Table A4.5). All placements related to the family Petropedetidae were clearly rejected. However, due to a weak resolution in basal nodes (lacking resolved relationships within the Natatanura), topologies placing Odontobatrachidae fam. nov. sister to other families cannot be excluded completely – as shown in the potential grouping with Conrauidae, which cannot be statistically significantly rejected.

However, alternative topologies show lower probability values and were not shown to have any strong support in any phylogenetic analyses (compare Table A4.1, A4.6 and A4.7).

#	Alternative topologies	-In <i>L</i>	AU-Test	SH-Test
	unconstrained topology	38414.13	0.97	0.99
1	Odontobatrachidae fam. nov. sister to Petropedetidae (<i>Ericabatrachus</i> (<i>Petropedetes, Arthroleptides</i>))	38428.86	<0.05	<0.05
2	Odontobatrachidae fam. nov. sister to Ericabatrachus	38842.84	<0.001	<0.01
3	Odontobatrachidae fam. nov. sister to Arthroleptides	38576.46	<0.001	<0.001
4	Odontobatrachidae fam. nov. sister to Petropedetes	38568.83	<0.001	<0.001
5	Odontobatrachidae fam. nov. sister to Petropedetidae s.str. (<i>Petropedetes, Arthroleptides</i>)	38441.99	<0.001	<0.001
6	Odontobatrachidae fam. nov. sister to Pyxicephalidae (<i>Aubria, Pyxicephalus</i>)	38427.52	<0.05	<0.05
7	Odontobatrachidae fam. nov. sister to Conrauidae (<i>Conraua</i>)	38419.45	0.15	0.14

Table A4 Log-likelihoods and P values of AU test and SH test of the unconstrained tree and seven alternative topologies, with focus on the family Petropedetidae.

2. Osteological analyses

2.1 Odontobatrachus osteology

We provide a detailed analysis of osteological characters based on CT scans (mainly ZMB 78203; supplemented with ZMB 78216, ZMB 78222, ZMB 78243) and double stained (ZMB 78222) material of *Odontobatrachus natator*.

Skull: Paired frontoparietals rectangular, their posterior ends fused and affiliated to the otoccipital (*sensu* Trueb [6]), frontoparietals width 51% of length, anterior margins fragmented, without contact to the nasals (Figure A2a); sphenethmoid in dorsal view visible between the anterior margin of the frontoparietals and the posterior margin of the nasals; paired nasals large and roofing entire forepart of the skull (Figure A2a), posterolateral process strongly expressed and with a dorsal groove; nasals not reaching the maxillary at any point; occipital condyles roundish, well developed and distant from one another; otic crest and epiotic eminence clearly pronounced; pars media plectra long and rod-shaped, the pars interna plectra clubbed; pterygoid triradiate, its anterior ramus long, not reaching the neopalatine, its medial ramus rather short and robust with its roundish end attached to the braincase, pterygoid not in contact with the alary process of the parasphenoid, posterior ramus long, narrow and attached to the quadrate (Figure A2c, A13a); squamosal strongly built, zygomatic ramus long and tapered, otic ramus shorter, wider and not firmly connected to the crista parotica (Figure A2c); quadratojugal well developed; angle between the ventral ramus of the squamosal and the quadratojugal about 40°;

maxillary exhibits a conspicuous pre-orbital process consisting of a small and pointed anterior part and a longer flap-like posterior part attending but not reaching palatinum dorsolaterally (not discernable in the cleared and stained preparations; only recognizable in X-ray computed micro tomography); maxillaries bear about 40 conical teeth with pointed tips, fore teeth remarkably longer, more robust, more pointed and more curved than the shorter and straighter rear ones (Figure A2c, A9a); premaxillaries also comprising long, robust, curved and pointed teeth, 5 on the left and 6 on the right side (Figure A2d); alary process in the middle of the premaxillary directed anterodorsally, its dorsolaterally oriented ends bifurcate, palatal shelf moderately wide and proximal and distal palatal processes strongly pronounced; anterior palatal shelf (flange) of the maxillary posterodorsally oriented, expanding strongly, ending hook-like; bony septomaxillaries (Figure A2c) with strikingly thin and pointed posteroventral processes; mentomeckelian on its border to the dentary with a conspicuous, posteriorly curved tusk-like odontoid tooth; angulosplenial with rounded coronoid process; alary processes of the parasphenoid long, narrow and posteriorly directed (Figure A2b); angle between cultriform process and alary process about 110°; cultriform process half as wide as posterior sphenethmoid, reaching up to the middle of the sphenethmoid anteriorly; a prominent posteromedial process of the parasphenoid reaching almost the foramen magnum; posterior margin of the neopalatine elevated; shape of the vomer complex (Figure A2b; A8a), its posteromedial ramus (dentigerous process) covering the proximal part of the neopalatine, five teeth present; posterior process of vomer well developed, connecting to main mass of vomer.

Vertebral column and ilia: Vertebral column (Figure A2f) consisting of seven procoelus presacral vertebrae, eights vertebra rather amphicoelous; atlas in dorsal view compact with cuspidal cervical cotyles, in ventral view centrum of atlas clearly wider than centra of all other vertebrae; relative length of the transverse processes: III>IV \approx V \approx VI \approx VII \approx VIII>II, those of vertebra II slightly anteriorly directed, those of III, IV, V and VI slightly posteriorly oriented and those of vertebra VII straight; sacral diapophyses scarcely dilated, posteriorly directed; angle between the central axis of the urostyle and the central axis of a sacral diapophyse about 58°, a roof-like protuberance from their proximal ends to the neural arch; neural arches and even the neural processes of all vertebrae do not overlap (nonimbricate state); vertebrae II-VII with well-developed neural processes, vertebrae I and VIII without such neural processes; urostyle with dorsal crest, crest proximally with an elongate knot and cleft dorsally over most of its length, urostyle possessing bicondylar articulation to the sacrum; ilium with well pronounced dorsal crest (about as high as the ilial shaft) that reaches almost to the end of the bone.

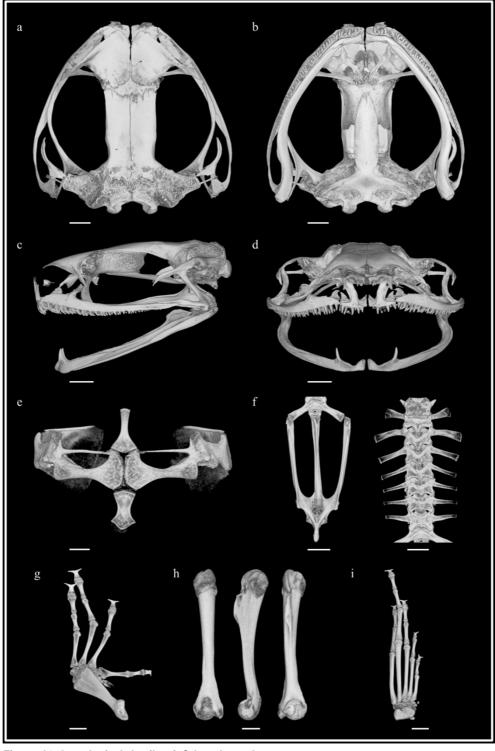


Figure A2 Osteological details of *Odontobatrachus natator*. Osteological details of *Odontobatrachus natator* (ZMB 78203); virtually isolated. a: dorsal view of the skull, scale bar = 2mm; b: ventral view of the skull, scale bar = 2mm; c: lateral view of left side the skull, lower jaw virtually rotated to open the mouth, scale bar = 2mm; d: frontal view of the skull, lower jaw virtually rotated to open the mouth, scale bar = 2mm; e: ventral view of pectoral girdle, scale bar = 1.5mm; f: axial skeleton in dorsal view (left = pelvic girdle, scale bar = 3mm; right = vertebral column, scale bar = 2mm); g: left hand in dorsal view, unguals virtually positioned in plane, scale bar = 2mm; h: left humerus (left = dorsal view; middle = lateral view; right = ventral view), scale bar = 1.5mm; i: left foot in dorsal view, unguals virtually positioned in plane, scale bar = 2.5mm.

Pectoral girdle: pectoral girdle firmisternal (Figure A2e); coracoids robust, perpendicular to each other and fused with the scapulae, their proximal ends not overlapping, proximal and distal epiphyses of the same size; omosternum completely ossified, base of omosternum convex and not divided as in many other African ranids [7,8]; episternum cartilaginous with shape of transversal section of a mushroom whose "head's" centre is slightly concave and whose stipe extends anteriorly; clavicle with broad base that is united with the pars acromialis of the scapula, proximal ends of clavicles lie close to one another, not fused to the coracoids; scapula and cleithrum with adjacent areas ossified, only posterior part of the suprascapula moderately cartilaginous; procoracoids small, cartilaginous, rod-shaped elements, distally invested to the scapulae and proximally terminating in the half length of the posterior side of the clavicles (in 3 of 4 cases: right procoracoid of ZMB 78203 reaches up to the proximal tip of the clavicle); distal parts of procoracoids stronger developed than proximal parts (proximal part of the procoracoids are more strongly developed in most other anurans); bony metasternum short and wide, its lateral surfaces concave, its distal margin almost triangular; cartilaginous and bilobate xiphisternum somewhat smaller than episternum.

Extremities: humerus bears a short and rounded crista on its proximal anterior half; large cristae on male humerus absent (Figure A2h); phalangeal formula of the hand 2-2-3-3 (Figure A2g); terminal phalanx of every finger and toe T-shaped and downward oriented; no peculiarities in the shape or structure of the long bones of the hind limb; phalangeal formula of the foot 2-2-3-4-3 (Figure A2i); metacarpal of digit II not differing in shape.

Hyolaryngeal apparatus (Figure A3): hyoid plate wider than long; hyale without a free flange towards jaw, medial element of anterior process (anterior horn) of hyale small and hook-like; hyoglossal sinus deeper than anterior border of base of alary processes; anterolateral process T-shaped with a broad base, the posterolateral processes long, reaching up to the middle of the posteromedial processes; distance between posteromedial processes less than one time the width of proximal expansion of posteromedial processes apart; small cartilaginous bridge between the enlarged anterior ends of the posteromedial processes; calcifications or ossifications only in posteromedial processes present.

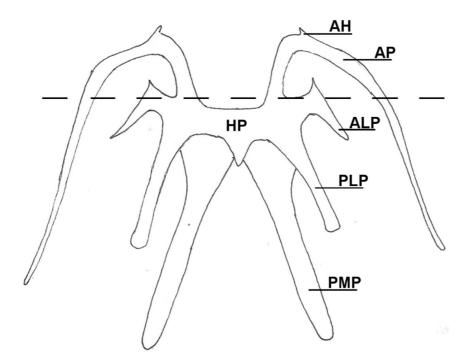


Figure A3 Hyoid of *Odontobatrachus natator*. Hyoid of *Odontobatrachus natator* (ZMB 78222); AH = anterior horn, ALP =anterolateral process, AP = anterior process, HP = hyoid plate, PLP = posterolateral process, PMP = posteromedial process (thyrohyal), dashed line showing to hyoglossus sinus.

2.2 Osteological characters distinguishing Petropedetidae and Odontobatrachidae fam. nov.

Using micro-tomographic analysis we differentiated *Odontobatrachus* from formerly assumed close relatives of the genera *Petropedetes*, and *Arthroleptides*. Applied scanning specifications as in Material and Methods section. Skeletal features of *Odontobatrachus* have been compared to following taxa: *Arthroleptides martiensseni* (ZMB 21793; male holotype), *A. yakusini* (ZMB 48472; female), *Petropedetes cameronensis* (ZMB 8222; female holotype, ZMB 27159 male syntype of *P. obscurus* = *P. cameronensis* see Barej et al. 2010), *P. euskircheni* (ZMB 73693; male paratype), *P. perreti* (ZMB 73735; male), *P. vulpiae* (ZMB 73692; male paratype); *P. juliawurstnerae* (ZMB 73694; male paratype). In her simultaneous analysis of molecular and morphological characters in ranid frogs, Scott (2005) provided osteological characters of the family Petropedetidae based on X-rays photos and cleared and stained preparations. Her material consisted of *Arthroleptides martiensseni*, *Petropedetes* (now: *Odontobatrachus*) *natator*, *Petropedetes cameronensis*, *P. parkeri* and *P. newtoni*. In their revision of Central African torrent frogs Barej et al. [9] synonymised *P. newtoni* with *P. johnstoni* and described three new taxa. Vouchers examined by Scott [8] were not available to us; hence, species assignments cannot be reviewed to ensure comparison with our material. Recognized

differences between Odontobatrachidae fam. nov and Petropedetidae are summarized on genus level in Table A5 and illustrated in Figures A3-A15, respectively.

 Table A5 Osteological characters in African torrent-frogs (part1). Distinguishing osteological characters

 of the families Petropedetidae and Odontobatrachidae fam. nov. Characters assessed by CT-scans. Continued on

 next page.

.

	Odontobatrachidae fam. nov.	Petropedetidae		
	Odontobatrachus	Petropedetes	Arthroleptides	see figure
	axi	al skeleton and pectoral gi	rdle	
clavicle, thickness	thin, tapering medially and meeting the procoracoid cartilage medially	thin or of moderate thickness, not tapering medially meeting the procoracoid cartilage medially	approximately equal in width along entire length, of moderate thickness, meeting the procoracoid cartilage medially	A4
medial edges of coracoids	not overlapping	not overlapping	overlapping	A4
posterior section of metasternum, shape	wide, triangular and weakly serrated	wide, triangular with distinctly serrated posterior edge	narrow, not serrated, hardly triangular or straight	A4
ossification of suprascapular cartilage	limited to moderate (only ZMB 78243) ossification	considerable ossification	moderate to considerable ossification	A4
omosternum, shape of proximal end (base)	convex	concave to bifurcated	bifurcated	A5
		<u>skull</u>		
ventral sphenethmoid, extent of forward expansion of mineralized anterior portion	considerable expansion	no expansion	no expansion	A6
parasphenoid alae, in ventral view	clearly angled posteriorly	perpendicular to body axis (lateral)	perpendicular to body axis (lateral)	A6
pterygoid, anterior ramus in relation to neopalatines and planum antorbitale	not reaching neopalatines and planum orbitale	reaching neopalatines and planum orbitale	reaching neopalatines and planum orbitale	A7
neopalatines	medially not broaded	distinctly broadend	medially not broaded	A8
vomers, development and position	not reduced	reduced and seperated in two parts	reduced and solely anterior part present	A8
vomer, posterior process	well developed, connected to main mass of vomer	separate from main mass of vomer	absent	A8
vomerine teeth	present	present	absent	A8
pre- and maxillary teeth	distinct, pointed and curved backwards, of tooth-like shape	straight or slightly backwards oriented cone- like processes	straight or slightly backwards oriented cone- like processes	A9
tusk-like odontoid on mandible	present in males and females	absent	absent	A10

	4 Osteological characters i	n Anican torrent-hogs (pai	12 . Continue of Table A4		
	Odontobatrachidae fam. nov.	Petropedetidae			
	Odontobatrachus	Petropedetes	Arthroleptides	see figure	
		skull (continued)	-		
nasals, median contact	in median contact, with exception of one individual	widely separate, not in contact	widely separate, not in contact	A11	
nasals, shape	broad, triangular to rectangular, with short rear process	slender triangular, with prolonged rear process	slender triangular, with prolonged rear process	A11	
frontoparietals, anterior margins	medial edges extend forward nearly as much as lateral edges	medial edges do not extend forward as much as lateral edge	medial edges do not extend forward as much as lateral edge	A12	
premaxilla, angle of pars facialis (alary process), transverse plane	inclined laterally outwards away from midline	dorsally, perpendicular to pars dentalis	dorsally, perpendicular to pars dentalis		
squamosal, length of zygomatic ramus relative to length of otic ramus	zygomatic ramus longer than otic ramus	zygomatic ramus approximately equal in length or shorter than otic ramus	zygomatic ramus shorter than otic ramus	A13	
		extremities			
bifurcate cristae on distal dorsal surface of humerus in males	absent	present (not all species)	present (not all species)	A14	
metacarpal of digit II in breeding males forming a spike	absent	present (not all species)	present (not all species)	A15	



Figure A4 Pectoral girdle in African torrent-frogs. Ventral view of the pectoral girdle; virtually isolated; scale bar = 1mm. a: *Odontobatrachus natator* (ZMB 78203); b: *Arthroleptides martiensseni* (ZMB 21793), coracoids disarticulated; c: *Petropedetes cameronensis* (ZMB 8222), posterior section of metasternum incomplete; d: *P. perreti* (ZMB 73735); e: *P. juliawurstnerae* (ZMB 73694); f: *P. euskircheni* (ZMB 73693); g: *P. vulpiae* (ZMB 73692).



Figure A5 Omosternum in African torrent-frogs. Omosternum in ventral view; virtually isolated; scale bar = 0.5mm. Species assignments as in Figure A4.

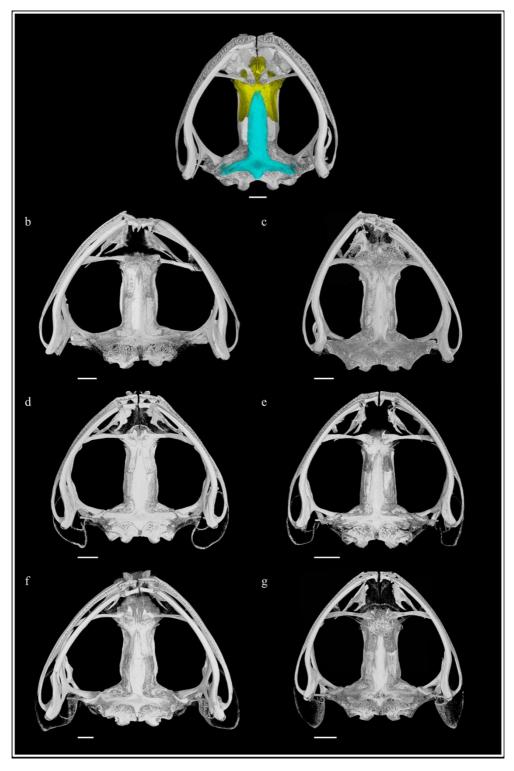


Figure A6 Ventral view of skull in African torrent-frogs. Ventral view of the skull; virtually isolated; scale bar = 2mm. Segmentation: yellow = sphenethmoid; blue = parasphenoid. Species assignments as in Figure A4.

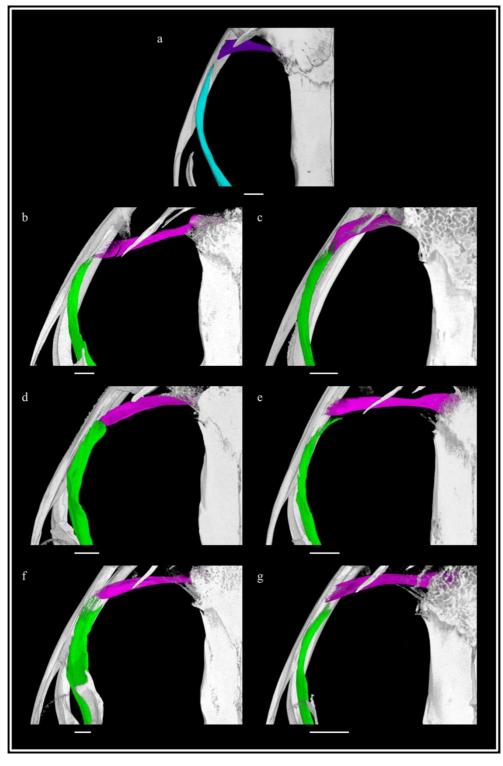


Figure A7 Neopalatine and pterygoid in African torrent-frogs. Close up of the left side of the skull in dorsal view; scale bar = 1mm. Segmentation: violet and red = neopalatine; blue and green = pterygoid. Species assignments as in Figure A4.

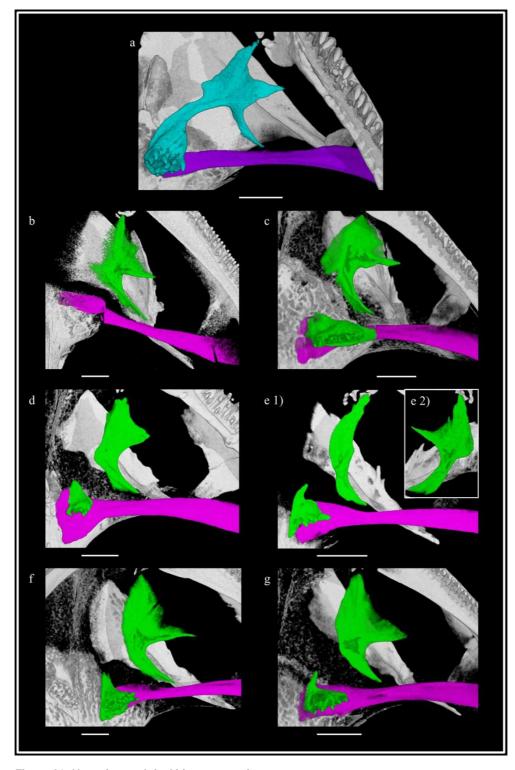


Figure A8: Vomerine teeth in African torrent-frogs. Close up of the left side of the skull in ventral view, exceptionally e2): right side; scale bar = 1mm. Segmentation: violet and red = neopalatine; blue and green = vomer and if available vomerine teeth. Species assignments as in Figure A4. Remark: e1): broken vomer of the left side of the skull of *Petropedetes juliawurstnerae* (ZMB 73694); e2): complete vomer of the right side of the skull of the same specimen.

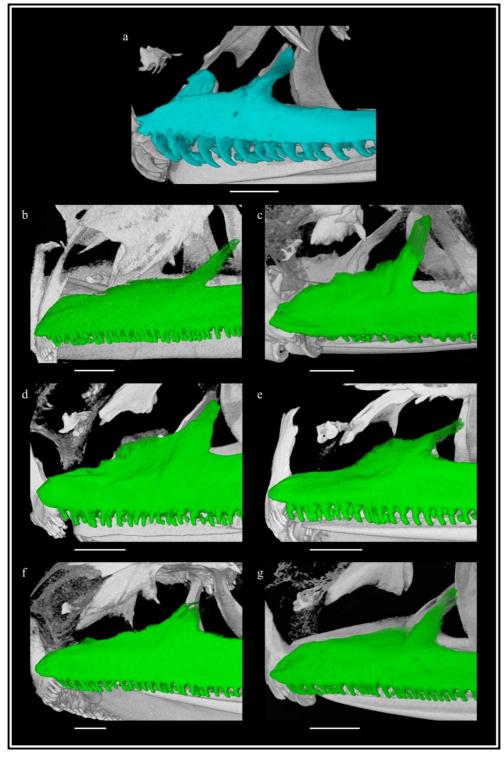


Figure A9 Maxillary teeth in African torrent-frogs. Close up of the anterior part of the skull in lateral view, left side of the skull; scale bar = 1 mm. Segmentation: blue and green = the anterior third of the maxilla. Species assignments as in Figure A4.

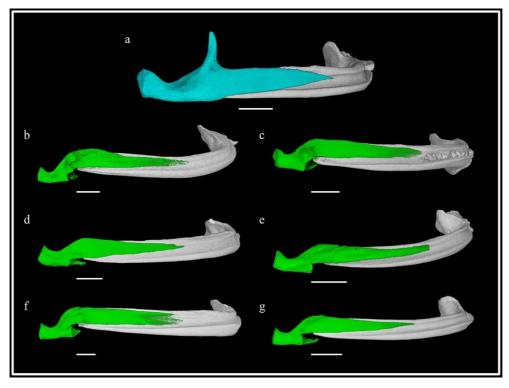


Figure A10 Left mandible in African torrent-frogs. Left mandible in anterior view; virtually isolated; scale bar = 1mm. Segmentation: blue and green = mentomeckelian and dentary bone. Species assignments as in Figure A4.

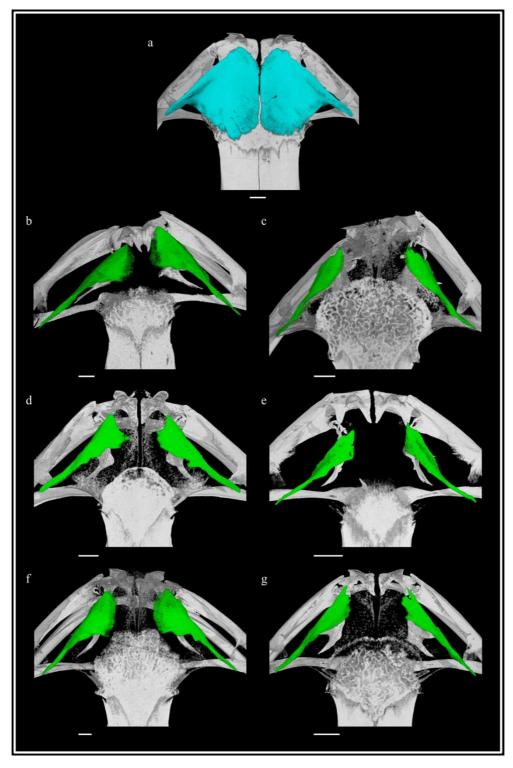


Figure A11 Shape of nasal bones in African torrent-frogs. Close up of the anterior part of the skull in dorsal view; scale bar = 1mm. Segmentation: blue and green = nasalia. Species assignments as in Figure A4.

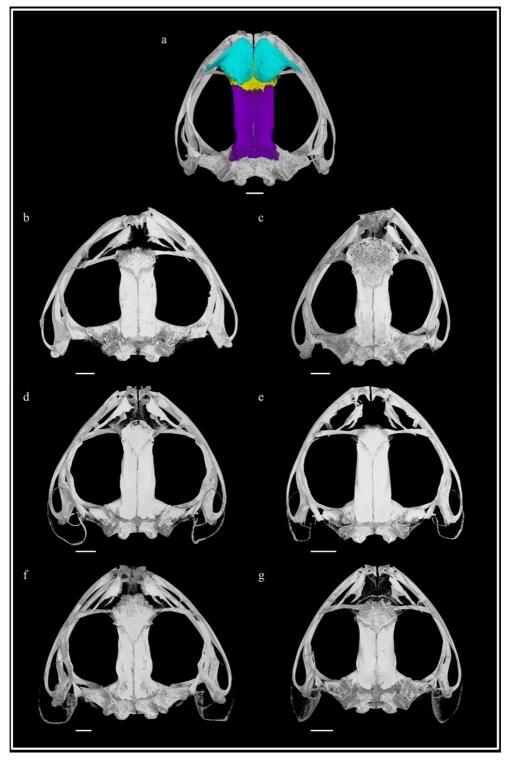


Figure A12 Skull in dorsal view in African torrent-frogs. Skull in dorsal view; virtually isolated; scale bar = 2mm. Segmentation: blue = nasalia; yellow = sphenethmoid; violet = frontoparietals. Species assignments as in Figure A4.

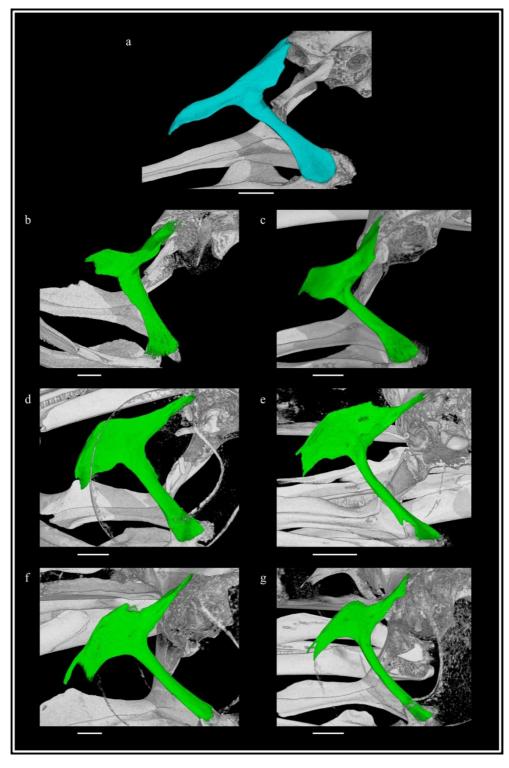


Figure A13 Squamosal in African torrent-frogs. Close up of the left side of the skull; anterolateral view, until the squamosal reaches the maximum expansion; scale bar = 1mm. Segmentation: blue and green: squamosal. Species assignments as in Figure A4.



Figure A14 Humerus in African torrent-frogs. Humerus virtually isolated, left = dorsal view, right = lateral view, positioned anterior to posterior from top to bottom, scale bar = 2mm. Species assignments as in Figure A4; *Petropedetes cameronensis* (ZMB 27159). *P. cameronensis* lacks the enlarged crista as brachial hypertrophy is not present in the species [9]; comparable to the on-related genus *Leptodactylodon* [10], in which males of species with brachial hypertrophy likewise possess enlarged cristae on the humerus.

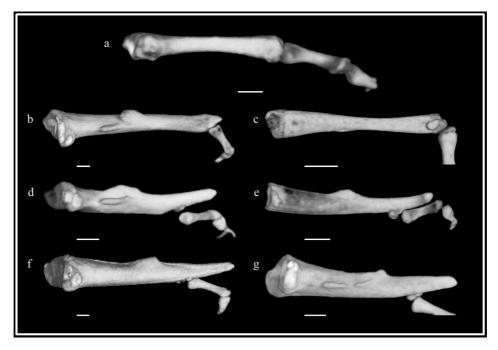


Figure A15 Metacarpal of Digit II in African torrent-frogs. Close up of the left hand, metacarpal of Digit II in lateral view; virtually isolated, scale bar = 0.5 mm. Species assignments as in Figure A4; *Petropedetes cameronensis* (ZMB 27159). *P. cameronensis* lacks a spike [9].

3. Amphibian diversity in African realms

In order to get an overview of overall diversity and number of endemic taxa, numbers of families and genera occurring in the Upper Guinea forests, we compared endemicity on different systematic levels in the four African biogeographic regions of western, central, eastern and southern Africa as recognized in published literature [11-16]. We refer to rough geographic sub-divisions, as present knowledge is incomplete, differing between vertebrate groups and applied data [16,17]. Sub-division is as follow: western Africa: Senegal to west of Cross River, central Africa: east of Cross River, eastwards to the Albertine Rift, southwards to Angola and north-western Zambia, eastern Africa: east of the Albertine Rift, southwards to Mozambique, southern Africa: south of central and eastern Africa, northwards to Ethiopia and Eritrea. Borders of recognized areas of central, eastern and southern Africa have not been demarcated strictly in the literature however such border areas do not contain endemics above the species level. Table A6 provides data on shared higher taxa between at least two biogeographic regions, endemics and total numbers at generic and family level are given (Table A6). Data extracted from Frost [18].

 Table A6 Amphibian diversity in African biogeographic regions.
 Amphibian diversity (on family and genus level)

 in African realms based on Frost [18].

	L - J			
	western Africa	Central Africa	eastern Africa	southern Africa
<u>genera (shared)</u>	24	28	27	24
genera (endemic)	3	17	13	11
genera (total)	27	45	40	35
families (shared)	13	14	15	13
families (endemic)	1	0	0	1
families (total)	14	14	15	14

4. References

- Kotaki M, Kurabayashi A, Matsui M, Khonsue W, Hon Djong T, Tandon M, Sumida M: Genetic divergences and phylogenetic relationships among the *Fejervarya limnocharis* complex in Thailand and neighboring countries revealed by mitochondrial and nuclear genes. *Zool Sci* 2008, 25:381-390.
- Kotaki M, Kurabayashi A, Matsui M, Kuramoto M, Hon Djong T, Sumida M: 2010.
 Molecular phylogeny of the diversified frogs of genus *Fejervarya* (Anura: Dicroglossidae). *Zool Sci* 2010, 27:386-395.
- 3. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW: GenBank. *Nucleic Acids* 2013, 41: D36-D42.
- 4. Bossuyt F, Roelants K: **Frogs and toads** (**Anura**). In *The time-tree of life*. Edited by Hedges SB, Kumar S Oxford University Press; 2009: 357-364.
- 5. Avise JC, Liu J-X: On the temporal inconsistencies of Linnean taxonomic ranks. *Biol J Linn Soc* 2011, **102**:707-714.
- Trueb L: Bones, frogs, and evolution. In: Evolutionary biology of the anurans: contemporary research on major problems. Edited by Vial JL. University of Missouri Press, Columbia, Missouri, 1973:65-132.
- 7. Deckert K: Beiträge zur Osteologie und Systematik ranider Froschlurche. Sber Ges naturf Freunde Berl 1938, 4–7:127-184.
- Scott E: A phylogeny of ranid frogs (Anura: Ranoidea: Ranidae), based on a simultaneous analysis of morphological and molecular data. *Cladistics* 2005, 21:507-574.
- Barej MF, Rödel M-O, Gonwouo NL, Pauwels OSG, Böhme W, Schmitz A: Review of the genus *Petropedetes* Reichenow, 1874 in Central Africa with the description of three new species (Amphibia: Anura: Petropedetidae). *Zootaxa* 2010, 2340:1-49.
- Amiet J-L: Révision du genre Leptodactylodon Andersson (Amphibia, Anura, Astylosterninae). Ann Fac Sci Yde 1980, 27:69-224.
- 11. Channing A: Amphibians of Central and Southern Africa. Ithaka: Cornell University Press; 2001.
- Channing A, Howell KM: *Amphibians of East Africa*. Ithaka: Cornell University Press; 2006.
- du Preez L, Carruthers V: A complete guide to the frogs of southern Africa. Cape Town: Striuk nature; 2009.

- 14. Largen M, Spawls S: *The amphibians and reptiles of Ethiopia and Eritrea*. Frankfurt/M: Chimaira Edition; 2010.
- Penner J, Wegmann M, Hillers A, Schmidt M, Rödel M-O: A hotspot revisited a biogeographical analysis of West African amphibians. *Divers Distrib* 2011, 17:1077-1088.
- Poynton JC: Distribution of amphibians in Sub-Saharan Africa, Madagascar, and Seychelles. In Patterns of distribution of amphibians, a global perspective. Edited by Duellman WE: Johns Hopkins University Press, Baltimore; 1999:483-539.
- Linder HP, de Klerk HM, Born J, Burgess ND, Fjeldså J, Rahbek C: The partitioning of Africa: statistically defined biogeographical regions in sub-Saharan Africa. J Biogeograph 2012, 39:1189-1205
- Frost DR: Amphibian Species of the World: an Online Reference. Version 5.6. [http://research.amnh.org/herpetology/amphibia/index.html] 2013.