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A new species of Puddle Frog, genus *Phrynobatrachus* (Amphibia: Anura: Phrynobatrachidae) from Ghana

CALEB OFORI-BOATENG[1,2], ADAM D. LEACHÉ[3], BRIGHT OBENG-KANKAM[1], N'GORAN GERMAIN KOUAMÉ[4], ANNIKA HILLERS[5] & MARK-OLIVER RÖDEL[6,7]

[1]*CSIR-Forestry Research Institute of Ghana, Kumasi, Ghana*
[2]*Wildlife and Range Management Department, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*
[3]*Department of Biology and Burke Museum of Natural History and Culture, University of Washington, Seattle, WA 98195-1800, USA*
[4]*Jean Lorougnon Guédé University, Department of Biology and Animal Physiology, Daloa, BP 150, Côte d´Ivoire*
[5]*Wild Chimpanzee Foundation, FDA Compound, Whein Town, Mt. Barclay, Montserrado County, Liberia*
[6]*Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, 10115 Berlin, Germany*
[7]*Corresponding author. E-mail: mo.roedel@mfn-berlin.de*

Abstract

We describe a new species of *Phrynobatrachus* from the eastern part of the Upper Guinea forest region, Ghana, West Africa. Morphologically, the new species can be distinguished from all of its congeners by the combination of a slender body, short and pointed snout, a relatively warty dorsum, a black-spotted throat in both sexes, a gular flap in males, a dark spotted chest, a white-greyish venter with occasional blackish spots, rudimentary pedal webbing, none to slightly dilated finger tips and strongly delated toe tips, presence of both inner and outer metatarsal tubercles and absence of a dark face mask, eyelid tubercles and longer dorsal ridges. We collected mitochondrial DNA (mtDNA) sequence data from the 16S rRNA gene to measure the genetic diversity of the new species, and to estimate phylogenetic relationships. The new species is a distinct and monophyletic evolutionary lineage most closely related to *Phrynobatrachus gutturosus*, *P. fraterculus* and *P. maculiventris.* The discovery of this new species highlights that the biodiversity of West African forests is still incompletely known and that the few remaining forests need urgent protection.

Key words: Biodiversity hotspot, *Phrynobatrachus afiabirago* **sp. nov**., *Phrynobatrachus latifrons,* Upper Guinea Forests, Ghana

Introduction

The Upper Guinea forests of West Africa support high levels of species richness and endemism, and are severely threatened at the same time. They are therefore listed among the world's biodiversity hotspots (Mittermeier *et. al.* 2004). The richness of endemic species also applies to amphibians, which however, is still not completely known. In the last 10 years (2007–2017) many new amphibian species have been described and more are still awaiting formal descriptions (e.g. Rödel *et al*. 2011a, 2012a). Remarkable new discoveries include for instance the giant *Arthroleptis krokosua* from Ghana (Ernst *et al*. 2008), a new tree-frog genus and species, *Morerella cyanophthalma,* from Ivory Coast (Rödel *et al*. 2009b), and a new frog family with various new species (Barej *et al*. 2014, 2015). Apart from these eye-catching frogs, new species have also been discovered in less conspicuous genera, i.e. the puddle frogs, *Phrynobatrachus* Günther, 1862 (Hillers *et al*. 2008; Rödel *et al*. 2009a, 2010, 2012b).

Phrynobatrachus species are small to medium sized and endemic to sub-Saharan Africa (Zimkus *et al*. 2010). The genus currently comprises about 90 species that occur in a variety of habitats from dry savannah to rainforest, and from lowland to montane habitats (Zimkus *et al*. 2010; Frost 2017). Most species have aquatic egg deposition and exotrophic tadpoles (Rödel 2000; Channing 2001; Channing *et al*. 2012). Exceptions are species with terrestrial egg deposition and exotrophic larvae (e.g.; Rödel & Ernst 2002b; Rödel *et al*. 2004) or terrestrial egg deposition and non-hatching, endotrophic (non-feeding) larvae (Rödel & Ernst 2002a).

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Frost (2017) currently list 23 *Phrynobatrachus* species for West Africa, west of the Dahomey Gap. Two of these species (*P. albolabris* and *P. vogti*) are however synonyms of *P. latifrons* (Rödel *et al*. 2005, and unpubl. data). The majority of the 21 valid species live in different forest habitats (Guibé & Lamotte 1963; Perret 1988). Because of their species-specific habitat requirements, several studies have used *Phrynobatrachus* as a model system to evaluate the impacts of human-induced changes on rain forest ecosystems (e.g. Ernst & Rödel 2005; Adum *et al*. 2012; Ofori-Boateng *et al*. 2012). From Ghana the occurrence of 13 *Phrynobatrachus* species has been confirmed; one of them, *P. intermedius,* seems to be endemic to the country's south-western forest region (Rödel *et al*. 2009a).

Previous amphibian surveys in Ghana have focused on protected areas such as national parks and forest reserves (Rödel & Agyei 2003; Leaché *et al*. 2006; Kouamé *et al*. 2007; Hillers *et al*. 2009), while only few have focused on unprotected sites (Leaché 2005; Hillers *et al*. 2009). However, these poorly explored sites may still harbour undescribed diversity.

During field surveys in June 2006 and September 2007, COB, NGK and AH collected a putative undescribed species of *Phrynobatrachus* from south-western Ghana. Later, Zimkus and colleagues included one of these specimens in a phylogenetic analysis of the genus, and confirmed that it is one of several new species (Zimkus *et al*. 2010). The new species was part of a clade further containing *P. gutturosus*, *P. maculiventris,* and *P. fraterculus*. In May 2011 and April 2016, COB and AL collected additional specimens of this new species in two localities in the Atewa Range, south-eastern Ghana. The undescribed small species has a distinct ventral colour pattern, and morphologically most closely resembles *P. gutturosus, P. maculiventris*, and *P. fraterculus*. Here, we describe the new species and report on a mitochondrial DNA based phylogenetic analysis (mtDNA) to confirm its phylogenetic relationships. We further provide first data about the distribution and ecology of the new species.

Materials and methods

Morphology. We examined 14 specimens of the undescribed *Phrynobatrachus* from two different localities in the Atewa Range (Fig. 1). A single male specimen (ZMB 73708), collected from a lowland locality near the Kakum National Park in Ghana, showing the same haplotypes as the Atewa vouchers, and a female (ZMB 73709) from the same locality, were also examined for intra-specific variations. Tissue samples were taken from most specimens (ZMB 73708; UWBM 5924–5925, 9041–9046), and were used for the molecular phylogenetic analyses (see below). The specimens were euthanized in a chlorobutanol solution and fixed in 10% buffered formalin, stored in 70% ethanol, and deposited at the University of Washington Burke Museum of Natural History and Culture, USA (UWBM) and the Museum für Naturkunde Berlin, Germany (ZMB).

Because the new species exhibited a combination of morphological characters that readily distinguishes it from all other species of *Phrynobatrachus,* we compared it mainly to the species which are most similar, morphologically and genetically, i.e. *P. gutturosus, P. fraterculus* and *P. maculiventris*. A complete list of the specimens examined is provided in Appendix I. Institutional abbreviations for museums and collections follow Sabaj-Pérez (2010), except for the Burke Museum of the University of Washington (UWBM), MOR (field number M.-O. Rödel; tissue sample only) and COB (working collection of C. Ofori-Boateng).

Nomenclature of measurements follows Rödel & Ernst (2002b) and Rödel *et al*. (2009a). Measurements (see Table 1) were taken with digital callipers (± 0.1 mm) and with the aid of a dissecting microscope. Measurements were collected by COB and MOR. Photographs of preserved specimens were taken with an amplified digital imaging system (BK Plus Lab System, Dun Inc.) at the UWBM.

Molecular analyses. We collected new DNA sequence data for seven samples of the new species. The phylogenetic analysis conducted for 60+ *Phrynobatrachus* species by Zimkus *et al*. (2010) included one sample of the new species described here, and found support for a clade containing the new species, *P. maculiventris, P. pintoi, P. gutturosus,* and *P. fraterculus*. Therefore, we combined our new data with available *Phrynobatrachus* sequences for these species, published by Zimkus *et al*. (2010) and Rockney *et al*. (2015). One sample of *Phrynobatrachus dispar* was included as an out-group to root the tree, and was selected based on the genus-level phylogenetic analysis conducted by Zimkus *et al*. (2010). For convenience we also compare herein genetic similarity of the new species with all 20 West African *Phrynobatrachus* species for which genetic data are available (Table 2).

TABLE 1. Measurements (mm) of type specimens of *Phrynobatrachus afiabirago* **sp. nov**.; C = collection; # = accession number, f = female, m = male, SVL = snout-vent-length, HW = head width, FL = femur length, TL = tibia length, FLT = foot length incl. longest toe, ED = eye diameter, TD = tympanum diameter, IOD = interorbital distance, EN = distance eye-nostril, ES = distance eyes snout-tip; * = holotype.

C	UWBM								ZMB								
#	5924	**5925***	9041	9042	9043	9044	9045	9046	86154	86155	86156	86157	86158	86159	86160	73708	73709
Sex	f	**m**	f	f	m	m	m	m	f	m	m	m	m	m	m	m	f
SVL	23.4	**18.4**	23.2	21.8	18.3	18.1	17.2	18.8	22.7	19.7	18.7	19.0	17.9	20.3	19.4	16.3	22.5
HW	8.1	**7.1**	8.2	7.2	5.9	5.9	5.8	5.6	7.3	6.3	6.0	6.4	6.2	6.6	5.8	5.1	6.3
FL	11.6	**8.2**	11.4	11.6	9.2	7.2	8.0	7.5	11.5	9.6	8.8	8.4	8.6	10.3	8.7	8.0	10.0
TL	13.3	**10.9**	12.3	12.6	11.2	8.1	10.0	10.5	12.9	10.2	10.0	10.6	10.0	11.2	9.7	8.9	11.5
FLT	19.3	**14.8**	18.8	16.9	14.7	13.8	15.7	15.5	16.8	15.2	16.0	15.4	15.2	15.7	16.3	-	16.7
ED	2.3	**2.0**	2.6	2.5	1.8	1.4	2.1	2.6	2.3	2.3	2.2	2.3	2.0	2.3	2.2	1.7	2.5
TD	–	**0.9**	1.7	1.1	1.2	1.5	1.3	–	1.4	1.3	1.1	0.8	0.8	0.9	0.7	0.8	1.3
IOD	2.4	**3.1**	2.7	2.1	2.3	2.0	1.1	2.3	2.0	1.7	1.6	1.6	1.8	1.9	2.1	1.6	1.8
EN	2.1	**1.7**	1.8	1.8	0.9	1.7	0.2	1.6	1.8	1.9	1.6	1.9	1.6	1.9	1.5	1.5	2.1
ES	2.9	**2.2**	3.1	3.4	3.2	3.2	2.8	2.8	2.8	2.8	2.5	2.8	2.2	2.9	2.4	2.0	2.9

We PCR-amplified and sequenced a fragment of the 16S rRNA mitochondrial DNA gene. Standard methods of DNA extraction and PCR amplification were used (Rockney *et al*. 2015). Purified PCR products were sequenced using an ABI 3730 automated sequencer. All new sequences are deposited in GenBank (accession numbers: MF167602–MF167608). Sequences were edited using Geneious, v10.1.3 (Kearse *et al*. 2012) and multiple sequence alignments were generated using Muscle v3.8.31 (Edgar 2004).

Maximum likelihood (ML) analyses were conducted with RAxML v7.0.4 (Stamatakis 2006) with the GTRCAT model of nucleotide substitution without rate variation. All ML analyses executed 100 rapid bootstrap replicates followed by a thorough ML search under the specified model.

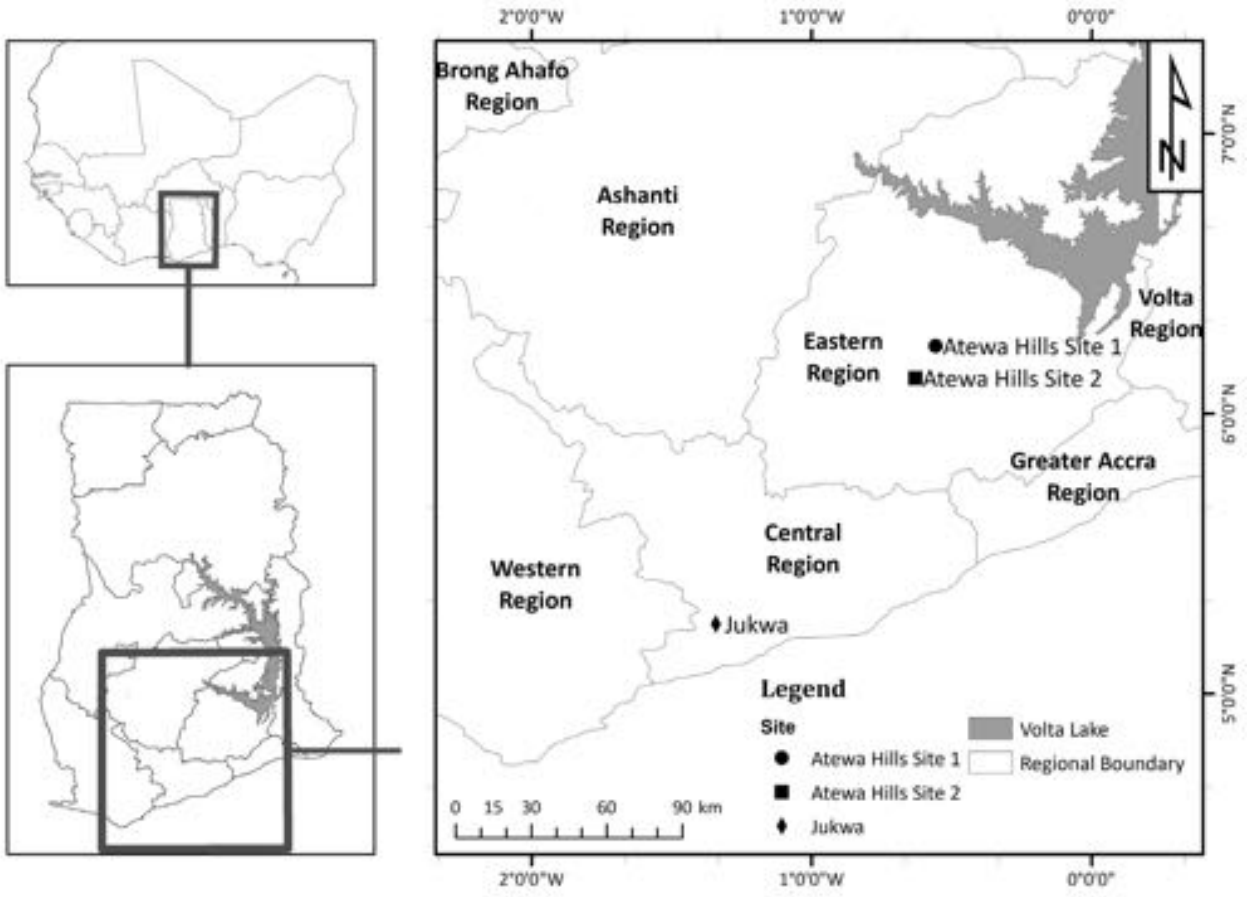

FIGURE 1. Distribution of *Phrynobatrachus afiabirago* **sp. nov.** in Ghana. The type locality is indicated by a circle, square and diamond mark localities where other individuals of the type series were collected; position of Ghana within West Africa (upper left) and the area within Ghana (lower left) where the new species occurs (right).

Results

Description of the new species.

***Phrynobatrachus afiabirago* sp. nov.**
Figs 2–3

Holotype. UWBM 05925, field number: ADL 3999), a male from Atewa Range, Ghana, 6.24246°N, -0.5571°E (date = WGS84), 769 m asl, 26 May 2011, coll. C. Ofori-Boateng & A.D. Leaché.

Paratypes (15). UWBM 9042–9046; ZMB 86155–86159 (males) and UWBM 9041, UWBM 5924, ZMB 86154 (females) collected from north-eastern (6°14'32.856"N, 0°33'25.56"W) and north-western part of the Atewa Range (6°7'40.7634"N, 0°37'44.04"W) respectively, April 2016, coll. C. Ofori-Boateng & A.D. Leaché; ZMB

86160 (male), Atewa range, Atiwiredu, 06°12'22.7"N, 0°34'39.2"W, 817 m asl, 7 June 2006, coll. N.G. Kouamé & C. Ofori-Boateng; ZMB 73708 (male) and ZMB 73709 (female), Jukwa forest, south-western Ghana, 5°14'44.13"N, 1°22'42.31"W, coll. C. Ofori-Boateng & A. Hillers.

Diagnosis. The molecular results place the new species within *Phrynobatrachus.* Morphologically this genus is distinguished from the similar looking and often syntopic *Arthroleptis* and juvenile *Ptychadena* by the presence of a tarsal tubercle (absent in *Arthroleptis* and *Ptychadena*); presence or absence of webbing (always absent in *Arthroleptis*); lack of a black spot in the tympanal region (present in *Arthroleptis*); lack of a median dorsal skin raphe (present in *Arthroleptis*); and the lack of parallel dorsal ridges (present in *Ptychadena*).

The new species is a small frog (< 25 mm) with a short, pointed snout and warty skin; warts most prominent on back; snout-vent length (SVL) of adult males 17.2–20.3 mm, adult females measure 22.5–23.4 mm; mean index head width/SVL is 0.3 (0.3–0.4; N = 17); eyelid tubercles absent; tympanum present but indistinct; fingers without webbing; rudimentary pedal webbing; tips of fingers not or slightly enlarged; toes distinctly enlarged to discs; small inner metatarsal tubercle present, larger than outer metatarsal tubercle; distinct tarsal tubercle; males with a subgular vocal disc, extending to anterior chest, dusted in black or covered with dark black blotches; males with large white nuptial pads on dorsal surface of first finger, femoral glands absent; belly of both sexes with minute black dots to large black blotching on white background.

The new species differs genetically from other West African *Phrynobatrachus* by 5–19% in the investigated part of the 16S gene (see below). Superficially the new species resembles *P. latifrons*, but can be told apart by the more pronounced pedal webbing and the yellow throat of breeding males in the latter species. Genetically and morphologically closest to the new species are three other West African species: *P. gutturosus*, *P. fraterculus* and *P. maculiventris. P. afiabirago* **sp. nov.** males of the new species share with *P. maculiventris* a throat, that is either completely covered with blackish spots or completely dusted in black (Figs. 2, 3, 4F); in *P. gutturosus* the black coloration of the throat in males is typically restricted to the anterior throat and does not extend onto the chest (Fig. 4B; Chabanaud 1921; Rödel 2000). The throat of *P. fraterculus* males is dirty yellowish grey (Fig. 4D). The belly of *P. afiabirago* **sp. nov.** always shows some dark patterning, ranging from very few small black dots to being densely beset with large dark blotches (Fig. 3). The belly of adult *P. gutturosus* is usually white or only exhibiting very few minute black dots (Fig. 4B); in *P. fraterculus* the belly is greyish white with few larger and smaller black dots along the edges and in the pectoral region (Fig. 4D); the belly of *P. maculiventris* is densely covered with huge black blotches, separated by white lines (Fig. 4F). Furthermore *P. fraterculus* and *P. maculiventris* differ from the new species by having a smooth or slightly granular back skin (Figs. 4C, E), in contrast to the presence of many distinct warts in *P. afiabirago* **sp. nov.** (Fig. 2). The most distinctive feature, differentiating *P. afiabirago* **sp. nov.** from *P. gutturosus, P. fraterculus* and *P. maculiventris,* is the lack of femoral glands in males of the new species, present in males of the other three species.

From other West African *Phrynobatrachus* species *P. afiabirago* **sp. nov.** differs morphologically (for genetic differences see below) by small size (SVL <25 mm; *versus* adults equal or larger than 25 mm in: *P. intermedius, P. liberiensis, P. plicatus, P. natalensis*); absence of a black lateral face mask (black face mask present in *P. intermedius, P. plicatus*); absence of a spiny eyelid tubercle (present in *P. annulatus, P. calcaratus, P. pintoi, P. taiensis, P. villiersi*); presence of distinct black lateral bands on flanks (shared with most *P. latifrons* and some other, but smaller species); throat of males with black markings, but not entirely black (males with yellow throat: *P. alleni, P. latifrons*; uniform black or grey: *P. liberiensis, P. plicatus, P. calcaratus, P. pintoi, P. villiersi, P. guineensis, P. phyllophilus, P. ghanensis, P. francisci, P. natalensis, P. tokba*; white or greyish: *P. annulatus*; clear with darker stippling and without obvious vocal sac: *P. brongersmai*); males with disc-like vocal sac skin extending to anterior chest (only shared with *P. gutturosus, P. fraterculus, P. maculiventris, P. pintoi*); most individuals with larger black spots on white belly (shared with *P. taiensis, P. ghanensis, P. pintoi, P. annulatus*—at least some spots round with white centre, *P. villiersi*—black blotches on clear blue belly; some other *Phrynobatrachus* may have few small black spots); rough warty back skin (back skin smooth in *P. fraterculus, P. maculiventris, P. hieroglyphicus*; sometimes smooth in breeding *P. latifrons, P. tokba*); males without femoral glands (femoral glands present in males of *P. gutturosus, P. fraterculus, P. maculiventris, P. taiensis, P. phyllophilus*); no longer or X-shaped scapular or dorsal ridges (scapular ridges long and converging towards mid-body, almost X-shaped: *P. alleni, P. plicatus*); distinct but rudimentary webbing (webbing more developed in *P. alleni, P. latifrons, P. francisci, P. natalensis, P. intermedius, P. liberiensis, P. plicatus, P. rainerguentheri*).

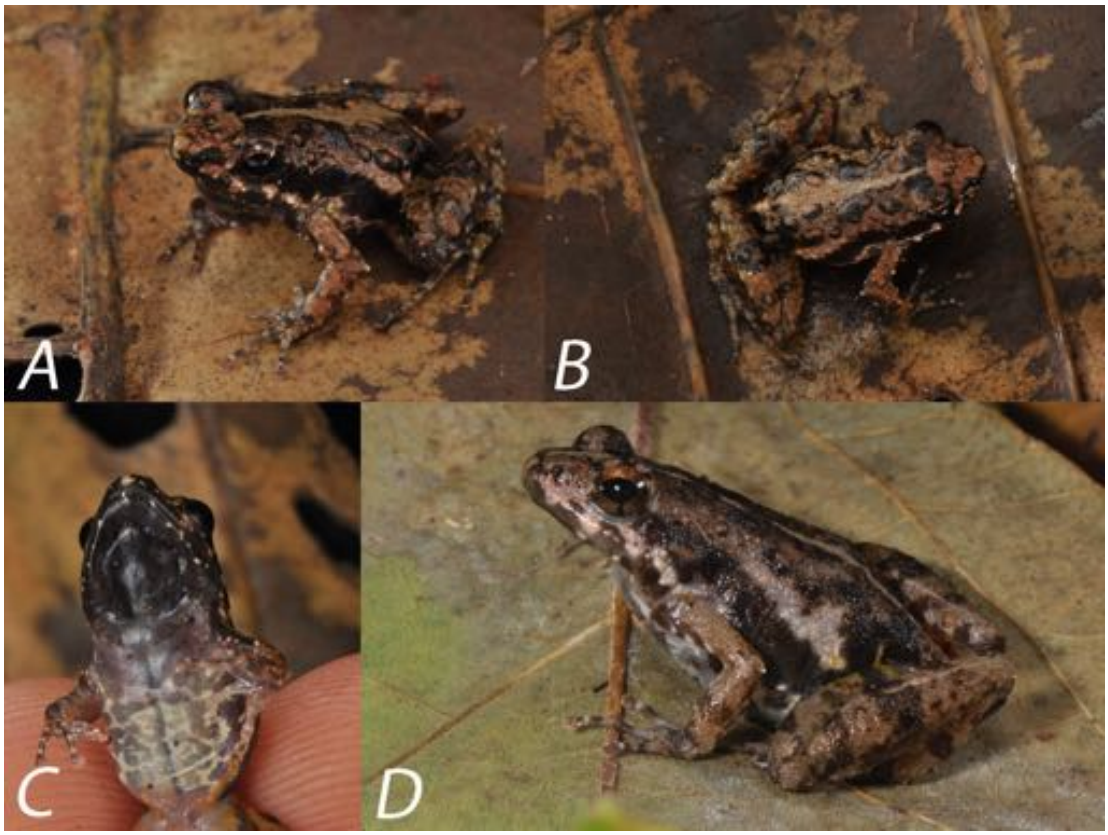

FIGURE 2. Ventral (A), dorsal (B), and dorsolateral (C–D) views of *Phrynobatrachus afiabirago* **sp. nov.** from the Atewa Range, Ghana (male holotype: A–C, UWBM 05925, and D: female paratype, UWBM 5924).

FIGURE 3. Variations in ventral pattern among males (A; from left to right UWBM 9044, 9045 and 9043) and females (B; from left to right UWBM 9042, 9041 and 5924) of *Phrynobatrachus afiabirago* **sp. nov.**

FIGURE 4. West African *Phrynobatrachus* species, similar to *Phrynobatrachus afiabirago* **sp. nov.** Dorsal (A) and ventral (B) view of a male *Phrynobatrachus gutturosus* from Taï National Park, Ivory Coast; dorsal (C) and ventral (D) view of a male *Phrynobatrachus fraterculus* from Gola Rainforest National Park, Sierra Leone; and dorsal (E) and ventral (F) view of a male *Phrynobatrachus maculiventris* from south-eastern Guinea.

Holotype description (UWBM 5925, all measures in mm; Fig. 2A–C). Typical, small, adult male *Phrynobatrachus* with short, slender-oval body shape; snout-vent length: 18.4 mm; short snout, rounded in dorsal and rounded to slightly protruding in lateral view; canthus rostralis rounded; loreal region slightly concave; head-width directly behind the eyes: 7.1; eye-diameter: 2.0; distance eye-nostril: 1.7; distance eye-snout tip: 2.2; nostril closer to snout than to eye; tympanum present but indistinct, tympanum diameter: ~ 0.9, nearly half the size of eye diameter; femur: 8.2, shorter than tibio-fibulare: 10.9; foot including longest toe: 14.8; hand with large oval palmar and thenar tubercles; fingers with small roundish subarticular tubercles, no additional tubercles on hands; finger tips very slightly expanded without forming discs; relative finger length: III>I<II=IV; palmar webbing rudimentary, webbing only between the most basal phalange of each toe; small and oval inner and outer metatarsal tubercles present, the inner one being larger approximately 0.25 of shortest toe length; relative toe length: I<II<III=V<IV; toes expanded forming slightly triangular to roundish discs; tarsal tubercle present, size almost equal to outer metatarsal tubercle.

Dorsal skin slightly warty, two converging—in almost x-shape—pairs of scapular warts, followed by three pairs of smaller oval to roundish black warts along posterior back; pair of very small warts on posterior part of snout, edges of back, flanks and upper surfaces of hind limbs with smaller round warts on granular skin; eyelids faintly granular without a spiny tubercle; ventral skin smooth.

Colour in life. Overall coloration of dorsum light to dark bronze brown with numerous black spots scattered particularly along edges of dorsal warts; light vertebral line extending from dark inter-orbital band to almost vent; anterior canthal region black; a conspicuous broad black band extends from behind eye across tympanum region along flanks to groin; narrow area between this band and back light brown; narrow area from tympanum to groin and between the lateral band and belly light brown; dorsal parts of hind limbs brownish with one broad and 2–3 narrow black cross-bars on either thighs and lower legs; anterior legs light brown with very faint black markings; brown upper lip with black bars, two white spots below anterior and posterior edge of eye; lower mandible almost completely black with few minute white spots; posterior part of thighs with light longitudinal lines; throat with black blotches on dark grey background; pectoral region grey; belly white with larger and smaller black spots and blotches, posterior part of belly slightly lighter than anterior one; groin area, posterior-most part of belly and lower part of thighs yellowish orange with few black spots; antero-ventral part of thighs with black longitudinal line. Throat covered by disc like vocal sac skin, slightly extending to pectoral region; throat skin slightly granular without spines; dorsal part of first finger with light nuptial pad; femoral glands absent.

Colour in preservation: Pattern as in life but faded; black may turn into darker brown; yellowish-orange colour turns into beige or white.

Paratype variation (measures in mm, summarized in Tab. 1; see Figs. 2D, 3). Body shape and dimensions and colouration, mostly as in holotype; male snout-vent length ranges from 17.2–20.3, females from 21.8–23.4 mm; canthal region with or without black line; back colour varies from bronze brown to greyish brown, median yellowish to orange vertebral line always present, broadest at mid of back, edges often indistinctly delimitated; two to three black, broad cross bars on thighs and lower legs; anterior part of thighs often with clear longitudinal band; narrow to broader yellowish line turning down from dorsal hind leg insertion to middle of posterior part of thighs, from there as longitudinal line with dark borders to knee, rarely interrupted; male throats range from entirely black to densely covered with black blotches on dark grey ground, tip sometimes with clear spot, sometimes longitudinal folds parallel to lower mandible; female throats smooth, from almost dark brown to black within irregular white median line and few white spots to very light with several dark blotches; dark lower lips of females partly interrupted by clearer spots; pectoral region, belly and lower side of hind legs from densely best with larger or smaller dark brown to black botches to almost white with few dark spots; some individuals with a fine light longitudinal line on posterior part of thighs and from mid lower leg to heel; females with slightly more expanded finger tips, compared to males; webbing may vary slightly, web between toe 4 and 5 often extending along 1.5 to 2 phalanges.

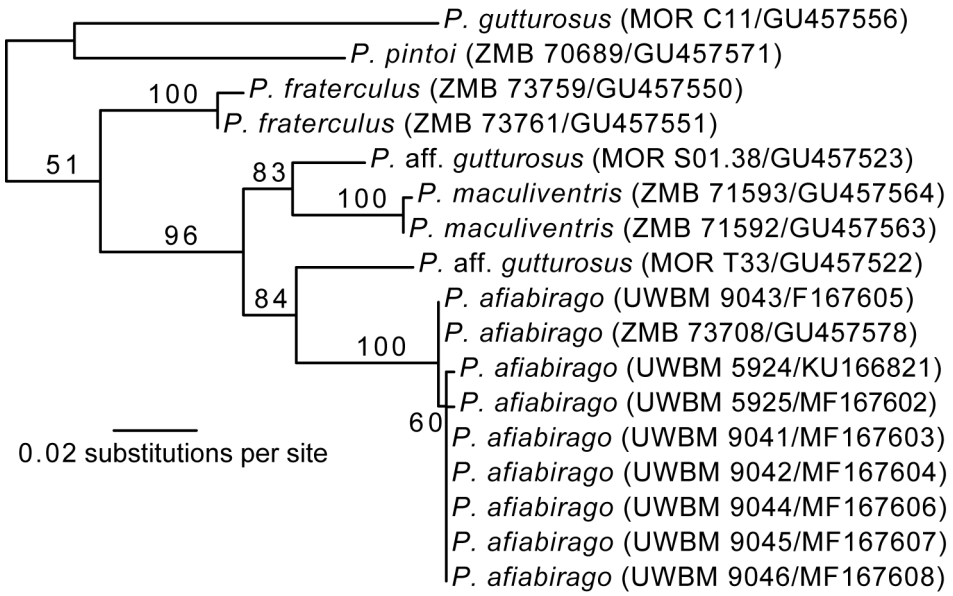

FIGURE 5. Phylogenetic relationship of *Phrynobatrachus afiabirago* **sp. nov.** with its closest congeners, based on mtDNA (520 bp fragment of the 16S gene; ML tree); nodes supported by bootstrap values ≥ 50% are indicated; in parentheses: collection or field number/GenBank accession number. The out-group species used to root the tree, *P. dispar,* is not shown.

In ZMB 73708 (male) and ZMB 73709 (female) from Jukwa, in life the yellowish-orange colouration of groin and ventral thighs was replaced by lemon green; posterior surfaces of thighs with lemon-green longitudinal bands; ventral spots on belly restricted to edges leaving 90% of belly clear white; in preservation colouration much faded, specimens partly shrunken due to preservation.

Genetics. The 16S alignment contained 538 bp and 111 variable sites (75 parsimony-informative). There is 100% bootstrap support for the monophyly of *P. afiabirago* **sp. nov.** (Fig. 5). *Phrynobatrachus afiabirago* **sp. nov.** together with two undescribed taxa, *P.* aff. *gutturosus* (bootstrap = 84% and 83%), and *P. maculiventris* (bootstrap = 100%) formed a highly supported clade (bootstrap = 96%). The genetic diversity within *P. afiabirago* **sp. nov.** samples was low (average pairwise distance = 0.0016; F81 substitution model). The alignment for *P. afiabirago* **sp. nov.** comprised 533 bp with 3 variable sites (1 parsimony-informative). We found four unique haplotypes among the nine samples, but there was no geographic structure in the distribution of these haplotypes. For example, ZMB 73708 from Jukwa in western Ghana shared the same haplotype as UWBM 9043 from the Atewa Range in south-eastern Ghana.

We finally compared the 16S sequence of the new species to all 20 West African *Phrynobatrachus* species for which genetic data have been published (Table 2, average pairwise distance: mean ± sd = 13.03 ± 3.65%, range = 5.00–18.63%). Concerning described taxa, *P. maculiventris* (5.00% difference) and *P. fraterculus* (6.45%) were the genetically most similar taxa.

FIGURE 6. Habitats of *Phrynobatrachus afiabirago* **sp. nov.** from the Atewa Range (type locality, site 1 = A; and site 2 = B; compare Fig. 1), south-eastern Ghana.

Habitat and natural history. *Phrynobatrachus afiabirago* **sp. nov.** always occurred around large forest ponds and forest swamps (Fig. 6). The frogs were usually collected from around the stilt-like root system of trees along the bank of swamps, including palms. The altitudinal range of the species extended from 300–800 m asl in the Atewa Range, but they occurred as low as 135–250 m in the south-western Jukwa forest. The Atewa Range, type locality, is located in south-eastern Ghana, west of Lake Volta (Eastern Region, see Fig. 1). It is one of the highest upland forests in Ghana, characterized by an interconnecting series of hills that runs from north to south (Swaine & Hall 1977). This unique area comprises mountain forest like vegetation, not occurring anywhere else in West Africa (McCullough *et al*. 2007).

Distribution. *Phrynobatrachus afiabirago* **sp. nov.** is so far known from only two localities in the Ghanaian forest zone; the Atewa Range in south-eastern Ghana and the small (0.5 ha) Jukwa forest, situated near the Kakum National Park in south-western Ghana (Fig. 1). We assume that it will occur in other forests of the area as well.

Etymology. The specific epithet honours Afia Birago, the mother of the first author for overcoming all odds as a widow, woman and a single parent to provide the highest level of education to all her eight children. Her tenacity and love for nature is a source of inspiration to the first author's professional career. The specific epithet is treated as a noun in apposition. We suggest Afia Birago's Puddle Frog as common name.

TABLE 2. Genetic similarity of *Phrynobatrachus afiabirago* **sp. nov.** with 20 other West African *Phrynobatrachus* species, based on mtDNA (520 bp fragment of the 16S gene).

species	museum or field numbers / GenBank accession numbers	Genetic distance to the new species
Phrynobatrachus alleni Parker, 1936	ZMB 73747 / GU457535	0.15141
Phrynobatrachus annulatus Perret, 1966	ZMB 73746 / GU457538	0.10581
Phrynobatrachus calcaratus (Peters, 1863)	MVZ 245140 / EU075282	0.10177
Phrynobatrachus dispar (Peters, 1870)	CAS 219386 / EU075278	0.08685
Phrynobatrachus francisci Boulenger, 1912	MVZ 249545 / GU457548	0.14781
Phrynobatrachus fraterculus (Chabanaud, 1921)	ZMB 73761 / GU457551	0.06405
Phrynobatrachus ghanensis Schiøtz, 1964	ZMB 70721 / GU457553	0.16203
Phrynobatrachus guineensis Guibé & Lamotte, 1962	MOR T13 / GU457555	0.15837
Phrynobatrachus gutturosus (Chabanaud, 1921)	MOR C11 / GU457556	0.11696
Phrynobatrachus intermedius Rödel, Boateng, Penner & Hillers, 2009	ZMB 71539 / GU457557	0.14615
Phrynobatrachus latifrons Ahl, 1924	ZMB 73753 / GU457559	0.18633
Phrynobatrachus liberiensis Barbour & Loveridge, 1927	ZMB 73768 / GU457562	0.15001
Phrynobatrachus maculiventris Guibé & Lamotte, 1958	ZMB 71592 / GU457563	0.05002
Phrynobatrachus natalensis (Smith, 1849)	ZMB 73717 / GU457566	0.16584
Phrynobatrachus phyllophilus Rödel & Ernst, 2002	ZMB 73763 / GU457570	0.15328
Phrynobatrachus pintoi Hillers, Zimkus & Rödel, 2008	ZMB 76883 / JN813916	0.09848
Phrynobatrachus plicatus (Günther, 1858)	ZMB 73742 / GU457575	0.15683
Phrynobatrachus rainerguentheri Rödel, Onadeko, Barej & Sandberger, 2012	ZMB 77742 / JQ954865	0.15515
Phrynobatrachus tokba (Chabanaud, 1921)	ZMB 73774 / GU457585	0.14155
Phrynobatrachus villiersi Guibé, 1959	ZMB 73740 / GU457589	0.10777

Discussion

The distinctness of *Phrynobatrachus afiabirago* **sp. nov.** from other congeners is supported by both morphological and molecular data. From other West African congeners, *P. afiabirago* can be readily distinguished by a combination of size, shape and colour of males' vocal sacs, amount of pedal webbing, head, dorsal and ventral colour pattern, absence of eyelid spines, as well as skin structure and size and shape of scapular ridges (see diagnosis and e.g. Guibé & Lamotte 1963; Rödel 2000; Rödel *et al*. 2009a). Morphologically and genetically, *P. afiabirago* is most similar to the *P. gutturosus*-complex, *P. fraterculus* and *P. maculiventris* (Fig. 6; Chabanaud 1921; Guibé & Lamotte 1958; Rödel 2000; Blackburn 2005; Rödel *et al*. 2009c).

Phrynobatrachus gutturosus, P. fraterculus and *P. maculiventris* have been recorded predominantly from secondary forests, forest edges (Rödel 2000; Rödel & Ernst 2005; Rödel *et al*. 2004, 2009c) and even savannah (*P. gutturosus*, this name comprising a complex of species ranging from primary rainforest to humid savannah; Rödel 2000; Nago *et al*. 2006; Zimkus *et al*. 2010), whereas *P. afiabirago* seems to be a real forest species. The new species is currently known from only three sites in Ghana, including two large swamp habitats in the Atewa Range, and a small swamp near the village of Jukwa, located approximately 10 km south of the Kakum National Park (Figs. 1, 6). In 2007, the Jukwa site contained only 0.5 ha of forest cover, and was surrounded by cocoa farms and a major road. Encroachment of cocoa farms into the forest decreased the size of the forest patch by half, and a

resurvey of the site in 2009 conducted by COB did not produce any new specimens. Without any formal protection status, the long-term survival of this species at Jukwa cannot be guaranteed. Additional survey work in swampy forest habitats across southern Ghana is needed to determine whether *P. afiabirago* has a geographic distribution that extends the known sites.

New species continue to be described at a frequent rate throughout West Africa. In the last decade various amphibian species have been described from West Africa and many more are awaiting formal description (e.g. Ernst *et al*. 2008; Hillers *et al*. 2008; Rödel *et al*. 2009a, b, 2010, 2012a, b). Seventy-eight amphibian species are currently known to occur in Ghana (Frost 2017 and IUCN Red List; sorting by country and deleting synonyms and erroneous records). This is much less species compared to Ivory Coast (95 species) and Guinea (94), the two countries with remaining Upper Guinea rainforest and a comparatively well-known amphibian fauna. It is however, comparable to the known number of amphibian species from Liberia (76), a country where only very few people surveyed amphibians after the 20th and 30th of last century. These species number comparisons, as well as the herein presented new description and other recent discoveries (compare Rödel & Agyei 2003; Leaché 2005; Leaché *et al*. 2005; Rödel *et al*. 2005; Hillers *et al*. 2009; Kouamé *et al*. 2007), let us assume that Ghana's amphibian fauna is only incompletely known. As more investments are made in field studies and data analyses (acoustic and molecular data), many more species are likely to be still discovered and described.

The type locality of this species, the Atewa Range, is one of the few montane forest habitats in West Africa (Hall & Swaine 1981; Abu-Juam *et al*. 2003). As a result of comparatively low temperatures, short dry season, and high precipitation, this ecosystem is unique within the eastern part of the Upper Guinea forest zone, harbouring exceptional species richness (Bakarr *et al*. 2004; McCullough *et al*. 2007), and presumably high number of endemic species (e.g. some more recent arthropod descriptions: Naskrecki 2008a–c). Although our knowledge of the amphibian diversity from the Atewa Range is based on just a few surveys (Kouamé *et al*. 2007 and unpublished data of the authors), the composition of the frog fauna seems to indicate a still healthy moist evergreen forest (e.g., *Conraua* aff. *derooi, Hyperolius bobirensis, Leptopelis occidentalis, L. macrotis, Amnirana occidentalis*). Unfortunately, the persistence of the original habitats of the Atewa Range are threatened by plans of bauxite mining, and by ongoing illegal logging, artisanal mining, poaching, and farming (Kusimi 2015). The discovery and description of *Phrynobatrachus afiabirago* adds to the ongoing arguments of the importance of the Atewa Range ecosystem and the need to urgently protect it.

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APPENDIX 1

Specimens examined

Phrynobatrachus afiabirago.—see type series.

Phrynobatrachus fraterculus.—LIBERIA: UWBM 09409-UWBM 09412, Welequah; MNHN 1998.1593–94, Mt. Tocadeh; MNHNP 1998.1193–94, New-camp road side, south-east Nimba, Nimba County; MNHNP 1998.1520, Mt. Nimba, Grassfield; ZMB 70702, Gola National Forest; ZMB 70703–70704, North Lorma National Forest; SIERRA LEONE: ZMUC R074944, R075020, 3 miles south of Joru; ZMUC R075825–29, Gola Forest Reserve; ZMB 73799, Tiwai Island; ZMB 73800, Loma Mountains; ZMB 73801, Tingi Hills; ZMB 73802, Nimini Forest Reserve; GUINEA: MNHNP 1921.153–57 (syntypes), Macenta; ZMB 73759–73760, Pic de Fon, Simandou Range; ZMB 73761, Diécké Forest Reserve; IVORY COAST: SMNS 9744.1–2, SRET transects, Taï National Park; SMNS 9743, same locality; ZMB 73750–73752, same locality; MNHNP 1958.348, MNHN A 360, Nimba Ziéla.

Phrynobatrachus gutturosus.—GHANA: COB 1056, Ajenjua Bepo; LIBERIA: MNHNP 1998.1501–12, Grassfield, Nimba county; MNHNP 1998.2581–2617, Mt. Nimba; MNHNP 1921.280, 280 A, 280D, 280 F, 280 H, 281 C, 282, 282 E, 282 F, 282 I, (syntypes), Sanikole´; IVORY COAST: MNHNP 1998.1561–70, Lamto; SMNS 9738, 9751.1–6, Guiroutou, Taï National Park; SMNS 9749.1–2, SRET station, Taï National Park; SMNS, 9739.1–2, same locality; SMNS 9752, south-western Comoé National Park; SMNS 9750, western park, Mt. Péko National Park; ZMB 70724, Marahoué National Park.

Phrynobatrachus maculiventris.—GUINEA: ZMB 71592–71594, Diécké Forest Reserve.

APPENDIX 2

Samples of *Phrynobatrachus* included in the phylogenetic analyses. GenBank accessions with asterisks denote new samples sequenced for this study.

Species	Voucher	GenBank	Country
P. dispar	CAS 218995	DQ283223	Sao Tome and Principe
P. gutturosus	MOR C11	GU457556	Ivory Coast
P. aff. *gutturosus* 1	MOR T33	GU457522	Ivory Coast
P. aff. *gutturosus* 2	MOR S01.38	GU457523	Ivory Coast
P. pintoi	ZMB 70689	GU457571	Guinea
P. fraterculus	ZMB 73759	GU457550	Guinea
P. fraterculus	ZMB 73761	GU457551	Guinea
P. maculiventris	ZMB 71592	GU457563	Guinea
P. maculiventris	ZMB 71593	GU457564	Guinea
P. afiabirago **sp. nov.**	ZMB 73708	GU457578	Ghana
P. afiabirago **sp. nov.**	UWBM 5924	KU166821	Ghana
P. afiabirago **sp. nov.**	UWBM 5925	MF167602*	Ghana
P. afiabirago **sp. nov.**	UWBM 9041	MF167603*	Ghana
P. afiabirago **sp. nov.**	UWBM 9042	MF167604*	Ghana
P. afiabirago **sp. nov.**	UWBM 9043	MF167605*	Ghana
P. afiabirago **sp. nov.**	UWBM 9044	MF167606*	Ghana
P. afiabirago **sp. nov.**	UWBM 9045	MF167607*	Ghana
P. afiabirago **sp. nov.**	UWBM 9046	MF167608*	Ghana