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A great leap – the first record of *Xenopus pygmaeus* (Anura: Pipidae) from south of the Congo Basin

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The genus Xenopus WAGLER, 1827 is endemic to sub-Saharan Africa and presently consists of 17 valid, highly aquatic species (Evans et al. 2011). Of those species, eleven belong to the so-called "X. fraseri group", which we define as a group of species closely related to X. fraseri (see e.g., EVANS et al. 2011) and distinct from the other species by possessing a fourth claw on the metatarsal tubercle, large eyelids and long tentacles. This group comprises X. amieti KOBEL, DU PASQUIER, FISCHBERG & GLOOR, 1980; X. andrei LOUMONT, 1983; X. boumbaensis LOUMONT, 1983; X. fraseri BOULEN-GER, 1905; X. itombwensis Evans, Carter, Tobias, Kel-LEY, HANNER & TINSLEY, 2008; X. lenduensis Evans, Green-BAUM, KUSAMBA, CARTER, TOBIAS, MENDEL & KELLEY, 2011; X. longipes LOUMONT & KOBEL, 1991; X. pygmaeus LOU-MONT, 1986; X. ruwenzoriensis TYMOWSKA & FISCHBERG, 1973; X. vestitus LAURENT, 1972; and X. wittei TINSLEY, KO-BEL & FISCHBERG, 1979.

Xenopus pygmaeus, the Bouchia clawed frog, was originally described as a diploid species of this group, distinct from others by its small size, very small eyes and a dark iris (LOUMONT 1986). However, there is some controversy about the ploidy level of several Xenopus taxa and EVANS et al. (2011) consider X. pygmaeus as tetraploid. Since its description, the species has become known only from Bagandou, Etoi and Bouchia in the southern Central African Republic, ranging eastwards to the northeastern Democratic Republic of the Congo and Semliki in western Uganda (TINSLEY et al. 1996). However, there have been unconfirmed records from northern Congo, and because of the difficulties associated with identifying cryptic Xenopus species, some supposed records of X. fraseri might in fact be referable to X. pygmaeus. These records include, e.g., localities from the Garamba and Virunga National Parks (TINSLEY & MEASEY 2004).

Herein we report on seven specimens that were recently collected in Zambia: Zoologisches Forschungsmuseum Alexander Koenig, Bonn, ZFMK 92758 (field no.: PW HF-093), ZFMK 92759 (PW HF-094), ZFMK 92760 (field tag lost), ZFMK 92761 (PW HF-097), ZFMK 92762 (PW HF-092); Museum für Naturkunde, Berlin, ZMB 77058 (PW HF-096); and Natural History Museum of Geneva, MHNG 2726.97 (PW HF-099). All specimens were collected within the Nchila Reserve (Sakeji Stream next to the "bush camp" of the reserve) of Hillwood Farm, near Ikelenge (Mwinilunga District) in northwestern Zambia by PHILIPP WAGNER in June 2008. These specimens possess a fourth claw and are therefore an addition to the Zambian herpetofauna, as only three-clawed *Xenopus* frogs (*X. laevis* [DAUDIN, 1802]; X. muelleri [PETERS, 1844]; X. petersii BOCAGE, 1895) have so far been known from this country (CHANNING 2001).

In order to identify the specimens we conducted direct morphological comparisons with the type series of *X. pyg-maeus*, produced molecular sequences for three Zambian vouchers, and then used a DNA barcoding approach, which has proven to be one of the most reliable identification tools in amphibian taxonomy (for the exact methods used see SCHMITZ et al. [2005] and WILDENHUES et al. [2011]). We used the mitochondrial 16S rRNA gene (using the primers 16sar-L and 16sbr-H of PALUMBI et al. [1991]) for an unambiguous genetic assessment of the three voucher specimens (ZFMK 92760–62; GenBank sequence accession numbers: KF738289–KF738291; all three produced identical 16S sequences). The resulting 16S fragments (551 bp) were compared with the published extensive datasets of various *Xenopus* species (EvANS et al. 2004, 2011).

This analysis revealed that the Zambian specimens represented an integral part of the *X. fraseri* species group (as

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Figure 1. Comparison of *Xenopus* specimens: Left = holotype of *Xenopus pygmaeus* (MHNG 2196.4); right = a specimen from Ikelenge (ZFMK 92758, field no. PW HF-099).

Table 1. Selected measurements of the specimens from Ikelenge, Zambia, and the type series of *X. pygmaeus*. SVL = Snout-vent length; HW = Head width; HL = Length of the left hind leg; BW = Body width just before hind legs; LA = Length from foreleg angle to hind leg angle; TW = Width of thigh. All measurements in mm.

-	Coll. no.	SVL	HW	HL	BW	LA	TW	SVL/HW	HW/HL	HL/BW	LA/TW
Ikelenge	ZMB 77058	23.0	5.9	29.1	8.8	9.4	4.5	3.898	0.203	3.307	2.088
	MHNG 2726.97	22.9	6.3	25.2	9.1	8.6	5.9	3.635	0.25	2.769	1.458
	ZFMK 92760	17.4	7.0	26.4	8.1	7.3	4.8	2.486	0.265	3.259	1.521
	ZFMK 92758	25.0	6.9	32.2	10.2	10.9	7.2	3.623	0.214	3.157	1.514
	ZFMK 92761	14.7	5.4	25.5	9.3	8.7	4.2	2.722	0.212	2.742	2.071
	ZFMK 92759	22.1	6.8	30.6	9.0	8.8	5.7	3.25	0.222	3.4	1.544
	ZFMK 92762	17.5	5.9	28.4	8.9	7.8	5.4	2.966	0.208	3.191	1.444
Туре	MHNG 2238.29	33.4	8.9	42.9	14.9	15.1	9.2	3.753	0.207	2.879	1.641
Series	MHNG 2238.28	27.9	8.0	40.6	9.6	10.3	8.2	3.488	0.197	4.229	1.256
	MHNG 2238.27	27.5	8.8	43.7	11.2	10.9	8.1	3.125	0.201	3.902	1.346
	MHNG 2196.4	31.5	8.1	35.7	13.9	13.3	8.8	3.888	0.227	2.568	1.511

defined above). A direct comparison with the species included in the latter group (*X. amieti* [GenBank#: AY581634], *X. andrei* [AY581627], *X. boumbaensis* [AY581633], *X. fraseri* "1" [AY581632], *X. fraseri* "2" [AY581631], *X. itombwensis* [EU594660], *X. longipes* [AY581625], *X. ruwenzoriensis* [AY581624], *X. pygmaeus* [AY581626, HQ225690], *X. wittei* [HQ225701, EU566831]) showed a very strong genetic similarity to *X. pygmaeus* from the DRC (uncorrected p-distances of 0.9%). The genetic distances to all other described species of this group, including a so-far undescribed species (*X.* "fraseri 2" sensu EVANS et al. 2011), are significantly higher by comparison (uncorrected p-distances of 2.18%–4.18%).

Moreover, the Zambian specimens all exhibit many typical morphological characters of X. pygmaeus in spite of them being slightly smaller (Tab. 1) and lacking the parallel or curved stripes behind the eyes that are often present in this species. However, the latter difference is only of minor importance, since LOUMONT (1986) already mentioned that specimens without this pattern were present in every population of X. pygmaeus. The smaller size could be due to the fact that the Zambian populations are on the southernmost distribution limit of the species and thus have to cope with comparatively less favourable environmental parameters (BÖHME 1978). Because of their genetic and morphological similarity, we tentatively recognize the Zambian specimens as conspecific with X. pygmaeus, but further research and more specimens of this species are needed to investigate if the noted minor differences are due to intraspecific variation within what now appears to be a large geographic distribution range, or if the Zambian specimens possibly represent an as yet undescribed taxon.

The specimens were collected within the Nchila Reserve of Hillwood Farm. The area comprises a heterogeneous landscape of mesic Miombo woodland with small but significant patches of tropical gallery forests and wetland within the Reserve. According to TINSLEY & MEASEY (2004), *X. pygmaeus* can be found in stagnant waters like small water holes, pools and shady swamps in lowland forest, but it was also found in swamps at the edge of villages. In Zambia, the habitat is different (see below) and one must probably



Figure 2. A) Specimen of *Xenopus pygmaeus* from the Nchila Reserve on Hillwood Farm near Ikelenge, northwestern Zambia. B) Sakeji stream within the Nchila Reserve, the natural habitat of the clawed frog population.



Figure 3. Known distribution of *Xenopus pygmaeus*: 1) Ikelenge (see text for further information); 2) Boende (Evans et al. 2004), Democratic Republic of the Congo (DRC); 3) Etoi (LOUMONT 1986), Central African Republic (CAR); 4) Bagandou (voucher unknown), CAR; 5) Bouchia (type locality, 40 km southeast of M'Baiki), CAR; 6) Buta (MCZ 21629–31), DRC; 7) Banalia (AMNH 9790), DRC; 8) Kisangani (JACKSON & TINSLEY 1995), DRC; 9) Ngayu (AMNH 9764–70), DRC; 10) Avakubi (AMNH 9793–9801), DRC; 11) Niapu (AMNH 9749–78), DRC; 12) Medje (AMNH 9736–65), DRC; 13) Isoro (LOUMONT 1986), DRC; 14) Poko (LOUMONT 1986), DRC; 15) Niangara (LOUMONT 1986), DRC; 16) Dungu (AMNH 9783–85), DRC; 17) Semliki (TINSLEY & MEASEY 2004), Uganda.

categorise the species here as an autochthonous rainforest species with a capability of dispersing to other habitats. The specimens were all found in gallery forests along the Sakeji stream (Fig. 2). Here, adult frogs were collected (tadpoles were only observed) on sandy ground in a small clearing on the bank in a slow-flowing section of the Sakeji. The individuals of various *Xenopus* species are known to migrate between habitats, e.g., between rivers in the dry and ponds in the rainy season (RÖDEL 2000) and this could explain the finds of specimens outside forests in the Congo (TINSLEY & MEASEY 2004). RÖDEL (2000) also recorded the rainforest species *Silurana tropicalis* GRAY, 1864 from rainforest-like gallery forests far out in the savannah zone of West Africa.

In summary, the specimens of *X. pygmaeus* are now known from 17 different localities (Fig. 3). The northernmost records lie between Bagandou and Niangara, but the Zambian record, as the southernmost locality, presently seems to be isolated, about 1,300 km south of the closest other known localities (Boende and Kisangani, see Fig. 3). Therefore, it is possible that *X. pygmaeus* has a disjunct circular distri-

bution pattern similar to Guineo-Congolian forest species like *Adolfus africanus* (Köhler et al. 2003) or *Feylinia currori* (WAGNER & SCHMITZ 2006), or possibly a so-far unrevealed continuous distribution, which remained undetected because of scientific undercollecting in the Congo Basin.

To date, *X. pygmaeus* is only known from lowland forests (TINSLEY et al. 1996, TINSLEY & MEASEY 2004), and only narrow stands of such forests persist across the southern parts of the basin for more than 250 km south of the main moist forest belt. The forests of Ikelenge are gallery or riparian in character, known locally as 'mushitu', and extend deep into the surrounding mesic savannah woodland landscape. Because of these forests and the region's mesic climate, several typical Guineo-Congolian forest species (e.g., *Adolfus africanus, Thelotornis kirtlandii, Causus lichtensteini;* see WAGNER et al. 2008, WAGNER 2010) occur in the area far south of the main forest blocks. The connection of gallery forests with the main forest blocks farther north, east and west is the most plausible explanation for the occurrence of Guineo-Congolian species within the Miombo savannah (COTTERILL 2002). However, while Ikelenge was not a stable forest refuge during the Pleistocene, the gallery forests today serve as retreat rainforest habitats within an extensive savannah area and seem to be remnants of an old continuous post-Pleistocene rainforest block that has only recently been fragmented by natural climate changes.

This new country record of *X. pygmaeus* from northwestern Zambia represents an enormous extension of its known range (about 1,300 km) and the first record from south of the Congo Basin.

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