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Assessment of the amphibians of Batéké Plateau National Park, Gabon, including results of chytrid pathogen tests

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Abstract. We report on the amphibians of five sites within the Batéké Plateau National Park in southeastern Gabon. During our survey we recorded 11 genera and at least 18 frog species, including four new country records (*Kassina maculosa*, *Phrynobatrachus ruthbeateae, Ptychadena uzungwensis* and *Xenopus pygmaeus*). Most of the recorded frogs were typical savanna or farmbush species; some preferred forested habitats and were collected in gallery forest or small forest patches within the savanna. Larvae were also collected, and the identifications of tadpoles and select adults were confirmed using mtDNA. The presence of *Batrachochytrium dendrobatidis* (*Bd*), the fungus that causes the amphibian disease chytridiomycosis, was tested using Taqman PCR, including community-level sampling. All samples were *Bd*-negative. This amphibian survey represents the first within Batéké Plateau National Park and is valuable because the fauna of this expansive, true savanna habitat differs from other protected areas that have isolated savanna pockets. The results of this survey also assist in predicting which species may exist in the contiguous savanna habitat in the Republic of the Congo.

Key words. Amphibia, Anura, *Batrachochytrium dendrobatidis*, chytridiomycosis, conservation, Gabon.

Résumé. Nous rapportons sur les amphibiens de cinq sites dans le Parc National des Plateaux Batéké dans le sud du Gabon. Au cours de notre enquête, nous avons enregistré 11 genres et au moins 18 espèces de grenouilles, dont quatre nouveaux records nationaux (*Kassina maculosa*, *Phrynobatrachus ruthbeateae*, *Ptychadena uzungwensis*, et *Xenopus pygmaeus*). La plupart des grenouilles enregistrés étaient des espèces typiques de savane ou «farmbush», tandis que d'autres étaient des espèces qui préfèrent des habitats forestiers et ont été recueillies dans la forêt-galerie. Les têtards ont aussi été recueillis, et des identifications de certains adultes et les têtards ont été confirmées par l'ADNmt. La présence de *Batrachochytrium dendrobatidis* (*Bd*), le mycète qui provoque la chytridiomycose, une maladie, a été testée en utilisant Taqman PCR, y compris au niveau communautaire d'échantillonnage. Tous les échantillons étaient *Bd* négatifs. Cette enquête constitue la première des amphibiens dans le Parc National de Plateaux de Batéké et est précieuse parce que la faune de cet habitat de savane vrai et expansif diffère quelque peu des autres zones protégées qui ont des poches de savane isolées. Les résultats de cette enquête aussi aideront à prédire quelles espèces peuvent exister dans l'habitat de savane contigus dans la République du Congo.

Introduction

The Batéké Plateau extends from southeastern Gabon through the central parts of the Republic of the Congo and the southern Democratic Republic of the Congo to northern Angola (Christy 2001). The plateau consists of ancient Kalahari sands, which date from the Eocene period, approximately 50 million years ago (Walters et al. 2006). A significant portion of the plateau has been eroded away, leaving a mosaic of smaller plateaus that are separated by sandstone escarpments. Batéké Plateau National Park is situated in the northwestern portion of the plateau and lies within the southeastern corner of Gabon. The eastern boundary of the park follows the national border between Gabon and the Republic of the Congo. Due to its purported universal cultural and natural significance, it was added to the tentative list of UNESCO World Heritage sites in 2005. The park includes a series of plateaus and rolling hills incised by valleys and intermittent streams. The landscape is dominated by savanna grassland and interspersed with watercourses bordered by gallery forest, at low to medium altitudes (400–830 m a.s.l.).

Pauwels & Rödel (2007) synthesized all known amphibian records from the national parks of Gabon. They concluded that 76 of the 88 species known to occur in Gabon (86%), all ten near-endemics (100%), and three of the six Gabonese endemic species (50%) are currently represented in these parks. However, the addition of more than 15 new country records within the last five years indicates that the batrachofauna of Gabon is perhaps far from fully inventoried. The amphibian fauna of Batéké Plateau National Park is completely unknown, and field surveys of

this region are especially important because this represents the only national park with expansive, true savanna habitat. It is likely that the fauna differs from those national parks that have a savanna-forest mosaic, such as Lopé National Park.

In addition to learning about the diversity of amphibians present in Batéké Plateau National Park, it is essential that amphibians are tested for *Batrachochytrium dendrobatidis* (*Bd*), the fungus that causes the amphibian disease chytridiomycosis. Recent studies indicate that *Bd* is prevalent within several of the national parks of Gabon. Bell et al. (2011) reported the presence of *Bd* from two national parks, Monts de Cristal and Ivindo, which were surveyed in 2009. Within these two national parks, *Bd* was detected in 20 of the 42 species from four of the seven families (Arthroleptidae, Hyperoliidae, Ranidae, Rhacophoridae). Daversa et al. (2011) did not detect *Bd* in any samples collected in the lowlands of Lastoursville, Lebamba and Lopé National Park in 2008. In addition, Gratwicke et al. (2011) did not detect *Bd* in two lowland areas surveyed on the west coast (Gamba and Libreville) from samples collected in 2009. In light of the confirmed existence of *Bd*, surveys are warranted to identify the prevalence of this fungal pathogen within Gabon.

Materials and methods

Study sites and sampling

Batéké Plateau National Park (BPNP) is located in southeastern Gabon and covers approximately 2,044 km². The eastern boundary of the park follows the national border between Gabon and the Republic of the Congo, and this border also represents the boundary between two major watersheds: watercourses that flow eastwards to feed the tributaries of the Congo River and those that flow northwest to feed the Ogooué River. The park is characterized by savanna at medium altitudes (400–830 m). Most of the forest is concentrated within the western portion of the park, while the eastern side is characterized by savanna with gallery forests along watercourses. Much of the plateau has been eroded away, leaving a number of smaller plateaus separated by dramatic sandstone escarpments and watercourses. The rainy season lasts from early October to mid-June with an annual precipitation of approximately 2,500 mm (Pearson et al. 2007). Maximum daily temperatures during the rainy season rarely exceed 33°C, and minimum temperatures are rarely lower than 18°C, with an average temperature of approximately 23–24°C. Humidity is generally in the mid to high 90s, but drops to approximately 90% during the dry season.

Fieldwork was carried out at the end of the rainy season between 23 May and 6 June 2011, by BZ and JL. The park was accessed by a 4×4 utility vehicle from the city of Franceville via Léconi (Lekoni), located 60 km to the north, across the savanna to the park's northern border. Transport within the park was mainly facilitated by boat along the Mpassa River, which runs from south to north and almost bisects the park. Study sites were selected along the Mpassa River for ease of access and due to limited time in the field (Fig. 1). Most amphibian specimens were collected during visual encounter surveys, while some species were located via acoustic encounter surveys at night. Larvae were collected by dip-netting. Surveys were undertaken during both day and night by up to three people; surveys after dusk were not possible at some sites because of the presence of forest elephants (*Loxodonta cyclotis*). Searching techniques included visual scanning of the terrain and investigation of potential hiding places (see also Heyer et al. 1994, Rödel & Ernst 2004). Geographical and habitat details of the five study sites follow: 1) Projet Protection des Gorilles (PPG) "Cambodge City" Camp; 02°07.304' S, 014°03.064' E; 463 m (Fig. 1, Site 1; Fig. 2A). This area included forest patches with small streams, as well as a spring-fed, permanent savanna pond partially edged with trees. The savanna in which this pond was located was bounded on three sides by tall, steep hills and a sizeable forest with streams (Fig. 2B) on the fourth side. This locality was surveyed both during the day and at night for two days. 2) PPG "Camp Mbié"; 02°11.500' S, 014°01.771' E; 435 m (Fig. 1, Site 2). This camp on the banks of the Mpassa River included a nearby savanna pond that was partially surrounded by forest. Surveys were conduct-

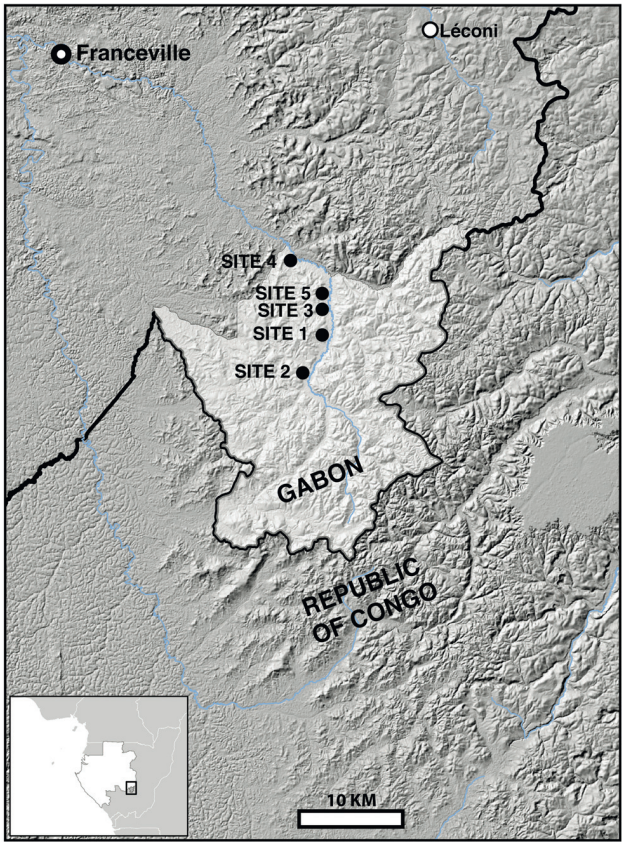

Figure 1. Map of Batéké Plateau National Park in southeast Gabon illustrating the five study sites along the Mpassa River.

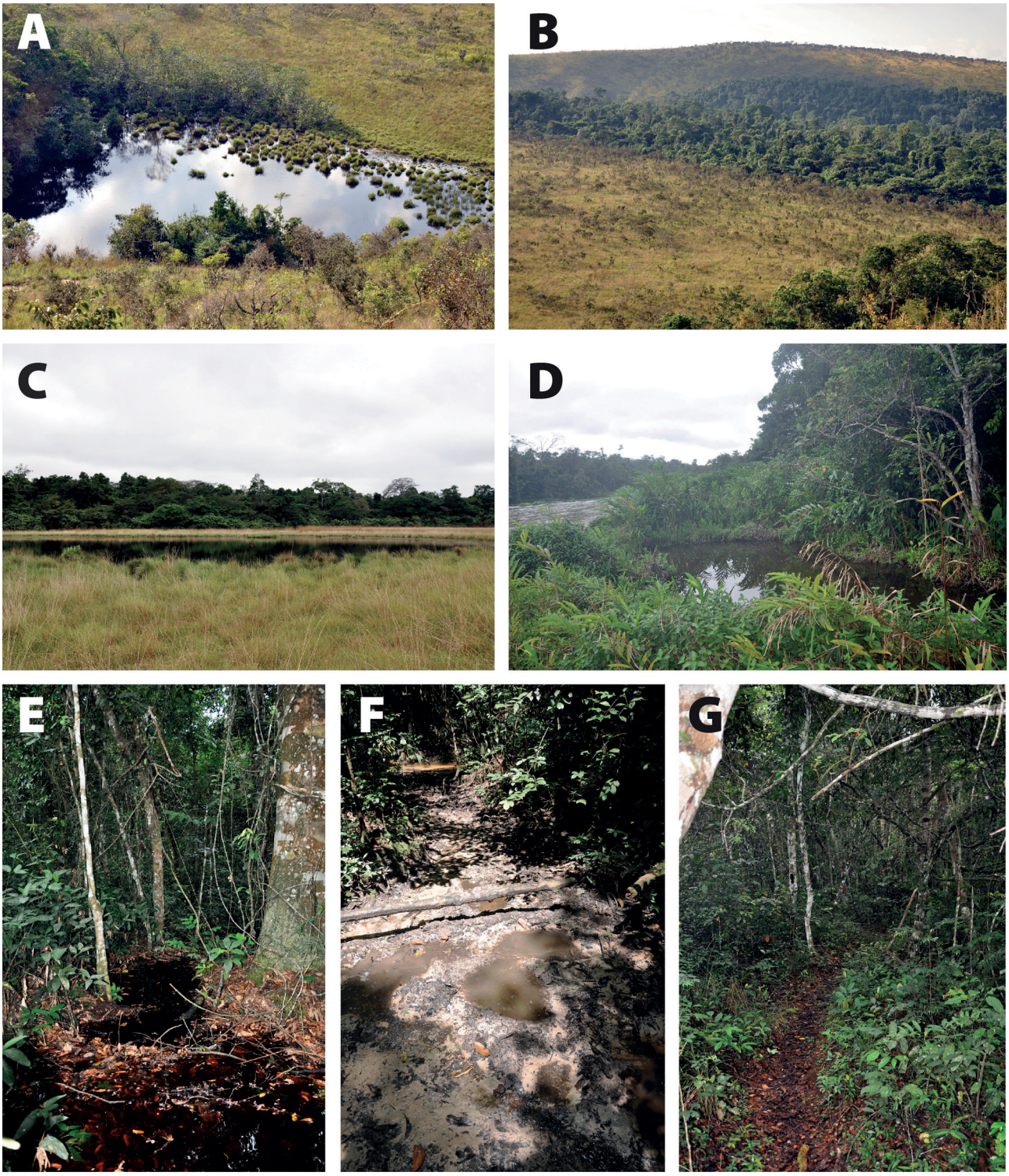

Figure 2. Surveyed habitats of Batéké Plateau National Park; a) Site 1: spring-fed, permanent savanna pond, partially edged with forest, in a basin of larger hills; b) Site 1: photograph of savanna in which the pond pictured in a) is located; c) Site 3: one of several small, savanna lakes at Lac Osséré; d) Site 5: small inlet surrounded by vegetation on the banks of the Mpassa River; e) Site 3: small stream in tunnel forest adjacent to Lac Osséré; f) Site 4; water accumulated in footprints made by elephants in gallery forest outside of Camp Ntsa; g) Site 5; gallery forest surrounding the Mpassa River at Camp Dallas.

ed during the day and night for two days. 3) Lac Osséré; 02°04.478' S, 014°03.855' E; 418 m (Fig. 1, Site 3; Fig. 2C). This area included forest adjacent to the Mpassa River and a group of small savanna lakes and associated puddles that were a very short distance from the river. This region was surveyed both during the day and at night for two days. 4) Former Wildlife Conservation Society "Camp Ntsa"; 01°58.896' S, 014°00.068' E; 395-400 m (Fig. 1, Site 4). We surveyed the gallery forest on the banks of the Mpassa River in close proximity to the northern border of the park for 3 days. We surveyed the gallery forest directly adjacent to the Mpassa River, inland forest patches, and a small savanna pond frequented by elephants. 5) PPG "Camp Dallas; " 02°06.888' S, 014°04.169' E; 433 m (Fig. 1, Site 5). This area included gallery forest and vegetation on the banks of the Mpassa River, which was surveyed for two days and three nights.

Collection methods

Voucher specimens were anaesthetized using Orajel™, fixed in formalin, and subsequently preserved in 70% ethanol. Tissue samples for DNA analysis were taken from every specimen that was collected; liver and/or skeletal muscle tissue was preserved in either 99% ethanol or RNA*later*®. Photographs of live animals were taken, and skin swabbing was completed prior to anaesthetization to test for the presence of *Bd*. Geographical positions were recorded with a hand-held GPS receiver (Garmin GPSMAP® 76CSX). Vouchers and tissue samples are currently deposited at the Museum of Comparative Zoology (MCZ), Harvard University, Cambridge, Massachusetts, USA.

DNA barcoding and species identification

DNA barcoding was conducted on larvae, metamorphs and select adult specimens for species verification purposes, using 16S rRNA, which has been suggested as a universal marker to barcode amphibians (Vences et al. 2005). DNA was extracted from liver or skeletal muscle tissue using Qiagen DNeasy tissue kits (Qiagen). A polymerase chain reaction (PCR) was used to amplify approximately 550 bp of the 16S rRNA gene, using the following primers of Palumbi et al. (1991): 16SA (5'-CGCCTGTTTATCAAAAACAT-3') and 16SB (5'-CCGGTCTGAACTCAGATCACGT-3'). Amplification followed the standard PCR conditions (Palumbi 1996) with the following thermal cycle profile: 2 min at 94°C, followed by 35 cycles of 94°C for 30 s, 46°C for 30 s, and 72°C for 60 s, and a final extension phase at 72°C for 7 min. All amplified PCR products were verified using electrophoresis on a 1.0% agarose gel stained with SYBR Safe DNA gel stain (Invitrogen Corporation). PCR products were purified using the Qiagen DNeasy DNA Purification System (Qiagen), and DNA sequences were sequenced using an automated DNA sequencer (ABI PRISM 3730xl). Sequences were compared with others collected during this survey and preliminarily identified by standard nucleotide-nucleotide sequence BLAST searches in GenBank (Benson et al. 2004). DNA sequences with the highest similarity in GenBank were aligned with the novel sequence using ClustalX v.1.83.1 (Thompson et al. 1997) and further improved by eye with MacClade 4.08 (Maddison & Maddison 2005). Percent pairwise uncorrected molecular distances were calculated using PAUP* 4.0b10 (Swofford 2002). Identification was considered to be unequivocal when the novel sequence was 98–100% identical to the reference sequence. If the novel sequence was greater than 3% divergent from all reference sequences, we assigned a genus but left the species unspecified. Future analyses will determine whether these unidentified specimens belong to unconfirmed candidate species or deep conspecific lineages (sensu Vieites et al. 2009). Sequences have been deposited in GenBank (KF178889-KF178894); accession numbers for particular specimens are listed in the results.

The various families, subfamilies, genera and species discussed in the species accounts are presented alphabetically and do not reflect a phylogenetic arrangement. Our nomenclature follows the taxonomy of Frost (2013) with additional information adopted from Pauwels & Rödel (2007). Identification of some species to genus level does not necessarily imply that such specimens represent undescribed taxa, even though this may be found to be true in some cases. For example, species identification in the genus *Hyperolius* is notoriously difficult because of the large numbers of valid species and synonyms, as well as lack of a molecular phylogeny for the genus (Frost 2013).

Chytrid testing

Frogs were handled with nitrile gloves, and individuals were collected in plastic bags. Adults were kept individually in bags, and bags were not reused. We used fine-tipped rayon swabs (Dryswabs™ MW113, Medical Wire & Equipment) to collect samples by rubbing the ventral skin. Using a single swab, the ventral surfaces were swabbed approximately 25 times; target areas included the pelvic patch (5 passes), ventral thighs (5 passes each side with the swab) and toe webbing (5 passes on each foot). Swab samples were air-dried for approximately 5 min, clipped approximately 2–3 cm from the tip and placed individually in 2 ml plastic vials. Samples were kept at ambient temperature for approximately 10 days in the field before being frozen. Samples were then shipped overnight on dry ice to the Amphibian Disease Laboratory at the San Diego Zoo's Institute for Conservation Research (San Diego, California, USA) for analysis. The methods of Boyle et al. (2004) with minor modifications (see Kinney et al. 2011) were used for *Bd* DNA extraction and qPCR detection of *Bd*. Template DNA was prepared by treatment of air-dried swabs with Prepman Ultra (Applied Biosystems). PCR assays were run on an ABI/Applied Biosystems 7900HT thermocycler, using 384 well plates with Applied Biosystems exogenous in-

ternal positive control reagents labelled with Vic placed in separate wells to test for the presence of PCR inhibitors. For each sample, 5 µl of a 1:10 solution (10 µl Prepman Ultra DNA extract and 90 µl water) swab DNA was added to each well for a final total volume of 20 µl. Standard curves were generated with 10-fold serial dilutions (range: 10,000 to 0.001 zoospores) of laboratory-cultivated *B. dendrobatidis* zoospores. With Taqman PCR, fluorescent reporter probes were used to detect *Bd* spores. Internal controls were run to detect the presence of PCR inhibitors. Samples were run in triplicate. Intensity of infection from Taqman PCR results was expressed as zoospore equivalents/swab.

Results

During ten days in BPNP, we collected 64 adult amphibians, representing ten genera and at least 14 species. We also collected 84 tadpoles, which included one additional genus (*Kassina*) and four additional species (*Kassina maculosa*, *Ptychadena perreti*, *Ptychadena taenioscelis, Ptychadena uzungwensis*) of which we did not collect representative adults. Identifications were verified using DNA barcoding. Summaries of amphibian species recorded during the study are presented in Table 1. *Bd* was not detected in any of the nine genera and 14 species of adults tested; see Appendix for a list of samples tested. Tadpoles were not tested for the presence of *Bd*.

Species accounts

Arthroleptidae: *Arthroleptis*

The genus currently includes 47 species, almost 30% of which have been described in the past five years (Frost 2013). Species of this genus are notable because they exhibit direct development with miniature froglets hatching from terrestrial eggs. Five species of *Arthroleptis* are currently known from Gabon (Frost 2013, IUCN 2012). We recorded two of them in BPNP.

Arthroleptis cf. *poecilonotus* Peters, 1863. MCZ A-147851, 147855, 147876, 147877, 147878. 147894. Sites 1, 2, 4, 5. We collected individuals identified as *A.* cf. *poecilonotus* from all sites, except Lac Osséré, from within both savanna ponds and leaf litter within gallery forests (Fig. 3A). Specimens were collected during the day, at dusk and after dark. Numerous populations are currently referred to as *A. poecilonotus*, and these likely represent a species complex with taxa being restricted to either Central or West Africa (Rödel 2000, Rödel & Agyei, 2003, Rödel & Bangoura 2004, Rödel et al. 2005, Blackburn 2008, 2010). We follow Pauwels & Rödel (2007) who suggested that Gabonese populations of *A. poecilonotus* may be distinct from the typical West African populations and refer to these species as "cf." until their status is clarified.

Arthroleptis sylvaticus (Laurent, 1954). MCZ A-147868, 147880, 147881, 147906. Sites 3, 4, 5. We collected individuals identified as *A. sylvaticus* at three different sites (Fig. 3B). All were collected from the leaf litter in gallery forests during the day. This species is known from rainforests within Central Africa from sea level up to 1,300 m (Burger et al. 2004).

Dicroglossidae: *Hoplobatrachus*

There are currently five species of *Hoplobatrachus* described, but only a single species, *Hoplobatrachus occipitalis*, is distributed in sub-Saharan Africa (Frost 2013).

Hoplobatrachus occipitalis (Günther, 1858). MCZ A-147852. Site 1. This species is widespread and common over much of its range, and it is found mainly in savanna habitats (Rödel 2000). We collected a single groove-crowned bullfrog during the night at a permanent savanna lake at the first collection site. Numerous other adults were heard calling at the same locality, but they could not be caught.

Hyperoliidae

There are currently 17 genera and 210 described species within the family Hyperoliidae, which is distributed across Africa and is also known from Madagascar and the Seychelles Islands (Frost 2013). There are nine genera currently known from Gabon: *Acanthixalus, Afrixalus, Alexteroon, Chlorolius, Cryptothylax, Hyperolius, Kassina, Opistothylax* and *Phlyctimantis* (Frost 2013, Frétey et al. 2011). We recorded four of them within BPNP.

Afrixalus

This genus includes 31 species of which four are known from Gabon: *A. dorsalis*, *A. laevis*, *A. paradorsalis* and *A. quadrivittatus* (Frost 2013, Frétey et al. 2011). We collected a single species in BPNP.

Afrixalus quadrivittatus (Werner, 1908) MCZ A-140498, MCZ A-147864. Sites 1, 3. Pauwels & Rödel (2007) referred to Gabonese populations of this species as *A.* cf. *fulvovittatus*. Pickersgill (2007) restricted application of *A. fulvovittatus* to West Africa within Côte d'Ivoire, Guinea, Liberia and Sierra Leone, referring records from all other countries to *A. quadrivittatus*. We collected a single adult from the savanna lakes at Site 3 during the day (Fig. 3 C). We also collected a single tadpole of this species from shallow, standing water at Site 1, for which the identification was confirmed using 16S rRNA (GenBank No. KF178889).

Cryptothylax

The genus includes two species, *Cryptothylax minutus*, which is known from the Democratic Republic of the Congo, and a more widespread species, *C. greshoffii*, which is distributed across Central Africa, including Angola, Cameroon, Central African Republic, Democratic Republic of the Congo, Equatorial Guinea, Guinea, Republic of the Congo and Gabon (Frost 2013).

Table. 1. List of amphibian species recorded in Batéké Plateau National Park with site of record (1 = Cambodge City; 2 = Projet Protection des Gorilles camp; 3 = Osséré; 4 = Camp Ntsa; 5 = Camp Dallas), microhabitat, habitat preference, and geographic distribution. Microhabitat in which specimens were collected: (1) savanna pond; (2) Mpassa River edge (vegetation); (3) gallery forest, floor; (4) gallery forest, stream; (5) gallery forest, temporary puddle; (6) forest vegetation. Habitat preference: (S) = savanna; (FB) = farmbush (degraded forest and farmland); (GF) = gallery forest; (F) = forest. Geographic distribution: (C) = Central Africa (east of the Dahomey Gap, including the Lower Guinean Forest Zone and lowland forests of Congo River Basin); (W) = Western Africa (west of, but not including, Nigeria); (E) = Eastern Africa; (S) Southern Africa (countries south of Malawi and excluding northern Mozambique). [1)] = first country record; [2)] = first record from within a Gabonese national park; [*)] = tadpole only, identification supported by mtDNA sequence data.

Species	Site	1	2	3	4	5	6	S	FB	GF	F	W	C	E	S
Arthroleptis cf. *poecilonotus* (Arthroleptidae)	1, 2, 4, 5	x		x					x	x	x	x	x		
Arthroleptis sylvaticus (Arthroleptidae)	3, 4, 5			x						x	x		x		
Hoplobatrachus occipitalis (Dicroglossidae)	1	x						x	x	x	x	x	x	x	x
Afrixalus quadrivittatus (Hyperoliidae)	1, 3	x							x				x	x	
Cryptothylax greshoffii (Hyperoliidae)	5		x				x		x	x	x		x		
Hyperolius adspersus (Hyperoliidae)	3	x						x	x				x		
Hyperolius bolifambae (Hyperoliidae)	5						x		x				x		
Hyperolius sp. 1 (Hyperoliidae)	1	x											x		
Hyperolius sp. 2 (Hyperoliidae)	2, 5		x										x		
Hyperolius sp. 3 (Hyperoliidae)	5		x										x		
Kassina maculosa (Hyperoliidae)	3	x						x	x	x	x		x		
Phrynobatrachus ruthbeateae [1] (Phrynobatrachidae)	3, 4			x				x	x	x	x		x		
Hymenochirus boettgeri (Pipidae)	4					x				x	x		x		
Xenopus pygmaeus [1] (Pipidae)	4					x			x	x	x		x		
Ptychadena perreti [*] (Ptychadenidae)	3	x						x	x				x	x	x
Ptychadena taenioscelis [2*] (Ptychadenidae)	1	x						x	x				x	x	x
Ptychadena uzungwensis [1*] (Ptychadenidae)	1	x						x					x	x	x
Hylarana albolabris (Ranidae)	2, 3, 4, 5			x	x				x	x	x	x	x	x	

Cryptothylax greshoffii (Schilthuis, 1889). MCZ A-147903–147905. Site 5. We collected a number of *C. greshoffii*, a large orange-red treefrog with a golden-green eye and diamond-shaped pupil. All specimens were either collected in vegetation on the banks of the Mpassa River or in the adjacent gallery forest within the camp during the night (Fig. 2D, G; Fig. 3D).

Hyperolius

Hyperolius contains more than 130 species in sub-Saharan Africa, making it the most speciose African anuran genus (Frost 2013). Frétey et al. (2011) reported there were 11 confirmed species of this genus in Gabon, not including *Chlorolius koehleri*, which was identified as *Hyperolius* by Frost (2013). According to Frost (2013), 11 species are currently known from Gabon. The single species that is not concordant between Frétey et al. (2011) and Frost (2013) is *H. marmoratus*, and according to the latter, Pauwels & Rödel (2007) recorded this species in error from Gabon due to their subscription to an alternative taxonomic arrangement. One of the more recent phylogenetic studies of African hyperoliids of the Congo Basin by Schick et al. (2010) emphasized the taxonomic difficulties of this lineage, highlighting the lack of external diagnostic characters and substantial intraspecific polymorphism in both colour and pattern. In addition, inadequate original descriptions have resulted in considerable difficulty in identifying species of this genus. We recorded five species in BPNP.

Hyperolius bolifambae Mertens, 1938. MCZ A-147895. Site 5. This species ranges from southeastern Nigeria through Cameroon to the southwestern Central African Republic, and it is presumably found in the Republic of the Congo and Equatorial Guinea as well. A single adult was collected in the gallery forest adjacent to the Mpassa River (Fig. 2G; Fig. 3E). The specimen exhibits a black ventral side with large white spots (Schiøtz 1999; phase F). This represents the second record of the Bolifamba reed frog in Gabon, which was first reported by Bell et al. (2011) from the vicinity of Ipassa Station in Ivindo National Park.

Hyperolius adspersus Peters, 1877. MCZ A-147858, 147863, 147872. Site 3. *Hyperolius adspersus* is part of the *H. nasutus* group and was once considered a subspecies of *H. nasutus* (Laurent 1957). Amiet (2005) revived it from synonymy and provided several characters to distinguish it from other species within the complex. A recent taxonomic revision of the *H. nasutus* group was provided by Channing et al. (2013), confirming the presence of *Hyperolius adspersus* in Gabon. Specimens were collected in vegetation surrounding the savanna lakes both during the day and at night (Fig. 2C; Fig. 3F; GenBank No. KF178890). This

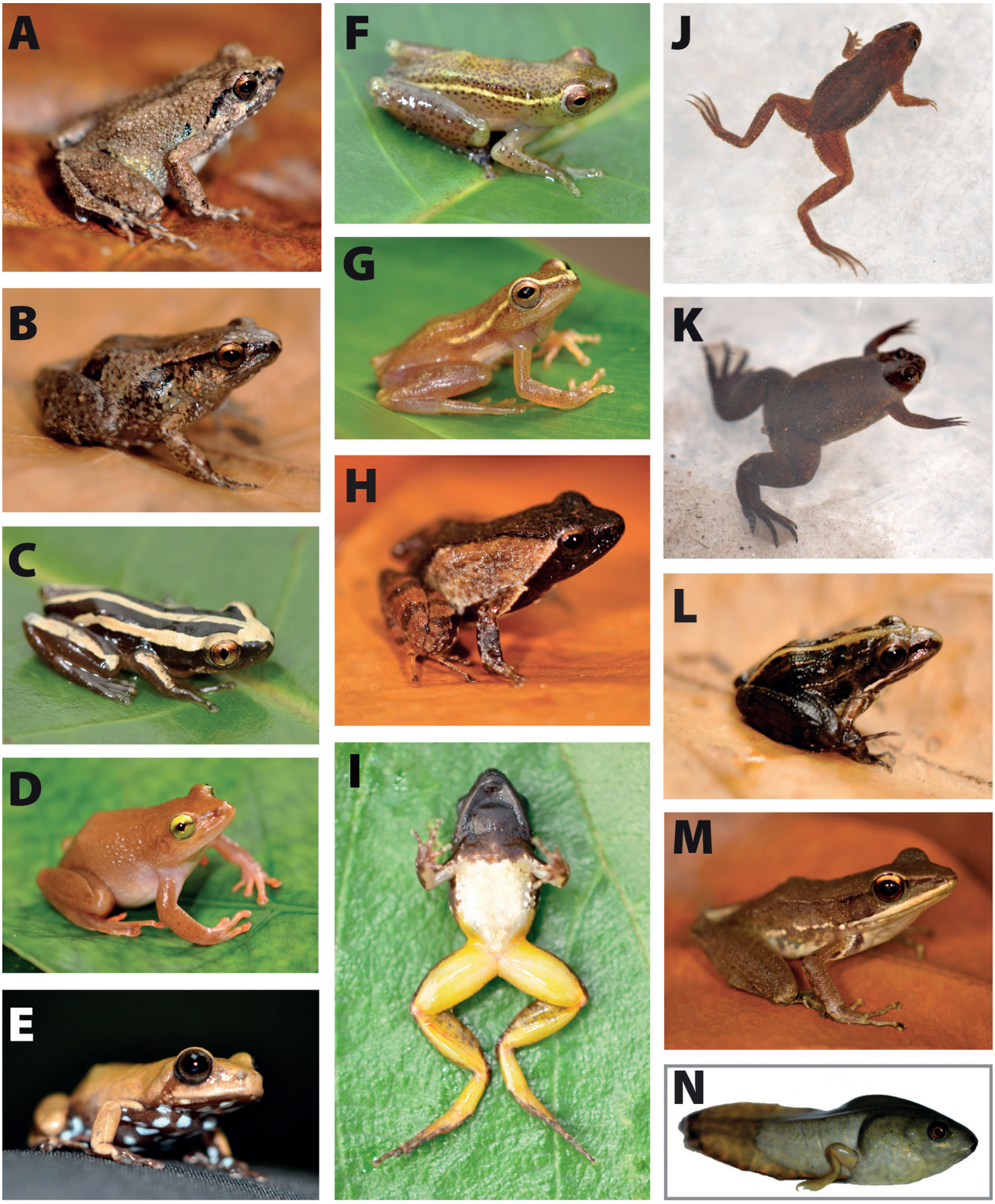

Figure 3. Photographs of amphibians collected in Batéké Plateau National Park; a) *Arthroleptis* cf. *poecilonotus* in lateral view; b) *Arthroleptis sylvaticus* in lateral view; c) *Afrixalus quadrivittatus* in lateral view; d) *Cryptothylax greshoffii* in lateral view; e) *Hyperolius bolifambae* in lateral view; f) *Hyperolius adspersus* in lateral view; g) *Hyperolius* sp. 2 in lateral view; h) *Phrynobatrachus ruthbeateae* in lateral view; i) male *Phrynobatrachus ruthbeateae* in ventral view; j) *Hymenochirus boettgeri* in dorsolateral view in water; k) *Xenopus pygmaeus* in dorsolateral view in water; l) *Ptychadena* sp. juvenile in lateral view; m) *Hylarana albolabris* in lateral view; n) *Kassina maculosa* tadpole in lateral view.

species is also listed as occurring in the Moukalaba-Doudou National Park (as *H. nasutus;* Pauwels & Rödel 2007).

Hyperolius sp. 1. MCZ A-147848–147850. Site 1. Three males, which were heard calling at dusk, were collected from trees surrounding a savanna lake (Fig. 2A, B). All individuals had light dorsolateral lines extending to the sacroiliac joint. The dorsum was either green or brown with small, dark spots. Fingers and toes were yellow. The throat was yellow and tuberculated. These individuals displayed morphological features, such as a large snout–vent length (SVL: 24.3–26.8 mm), that suggested they were not conspecific with any *Hyperolius* species collected during this expedition.

Hyperolius sp. 2. MCZ A-147853–147854, 147897–1478902. Sites 2, 5. Numerous males of this unidentified species were collected in vegetation on the edge or overhanging the Mpassa River (Fig. 3G). Specimens were smaller (SVL: 20.3–23.0 mm) when compared to *H.* sp. 1. All specimens had pale dorsolateral lines and approximately half of them displayed pale dorsal lines in the caudal region. Dorsum colour ranged from brown to pale yellowish-green; brown speckling was present in some specimens. Throats were yellow and tuberculated with an opaque white gular flap. The anterior half of the ventrum was opaque white, while the lower half was usually translucent and reddish.

Hyperolius sp. 3. MCZ A-147896. Site 5. A single male specimen was collected in vegetation along the Mpassa River during the day (Fig. 2D). Other males of this species were heard calling high up in trees along the edge of the river at Site 5. Similar in size to *H.* sp. 2 (SVL: 21.0 mm), this individual had a pale dorsolateral line edged with dark brown running over the eyelid, and a dorsum with light brown and dark speckling. The iris was pale yellow. The throat and gular flap were bright yellow, and the ventral side was opaque white.

Kassina

This genus, which includes 16 described species, has thus far only been recorded from a single, unnamed species collected in Moukalaba-Doudou National Park in southwestern Gabon (Burger et al. 2004, Frost 2013, IUCN 2012). Here we present the first record of *Kassina maculosa* from Gabon.

Kassina maculosa (Sternfeld, 1917). MCZ A-147846. Site 3. A single tadpole collected in the savanna lakes at Osséré during the day (Fig. 2C; Fig. 3N). The specimen was identified as *K. maculosa* by its oral morphology (Channing et al. 2012) and verified using DNA barcoding data (GenBank No. KF178891). Photographs of an adult *Kassina maculosa* taken at Osséré weeks before this expedition support the identification of this species (G. Jongsma pers. comm.).

Phrynobatrachidae: *Phrynobatrachus*

Phrynobatrachus is one of the most speciose sub-Saharan genera with more than 85 species currently recognized (Zimkus et al. 2010, IUCN 2012, Frost 2013). Both Frétey & Blanc (2000) and IUCN (2012) reported that five species are currently known from Gabon: *P. africanus, P. auritus, P. batesii, P. cornutus* and the endemic *P. ogoensis*. Frétey (2011) and Frost (2013) both also include *P. hylaios*, and Frost (2013) additionally includes *P. ruthbeateae*, which was first recorded from Gabon by Zimkus & Larson (2013) from specimens collected on this expedition.

Phrynobatrachus ruthbeateae Rödel, Doherty-Bone, Kouete, Janzen, Garrett, Browne, Gonwouo, Barez, & Sandberger, 2012. MCZ A-147865–147867, 147870–147871, 147873, 147882–147886, 14788–147891. Sites 3, 4. This collection represents the first confirmed records for this species from Gabon, extending the known range significantly from its type locality in southern Cameroon (Rödel et al. 2012, Zimkus & Larson 2013). This species is believed to be more widespread within lowland forests of Cameroon and Gabon, including Moukalaba-Doudou National Park in southwestern Gabon (*Phrynobatrachus* sp. 2; Burger et al. 2004). It has not yet been recorded from the Republic of the Congo, but its presence in BPNP makes it highly likely that it is also distributed across the border. Specimens were collected in leaf litter at two sites during the day and at night (Fig. 3H, I).

Pipidae

The family Pipidae includes two genera in Gabon: *Hymenochirus* and *Xenopus* (Frost 2013, Frétey et al. 2011). Frogs of this family are exclusively aquatic with completely webbed feet and a lateral line system.

Hymenochirus

Of the four species of *Hymenochirus* described, two are currently known to be distributed in Gabon: *H. boettgeri* and *H. feae* (Frost 2013, IUCN 2012). We recorded one species in BPNP.

Hymenochirus boettgeri (Tornier, 1896). MCZ A-147874. Site 4. A single specimen of *H. boettgeri* was collected in the forest adjacent to the savanna at Camp Ntsa during the night (Fig. 2F; Fig. 3I). It was found in the same small pool as the single specimen of *Xenopus pygmaeus*.

Xenopus

IUCN (2012) and Frost (2013) included five species occuring within Gabon: *X. andrei*, *X. epitropicalis* (as *Silurana epitropicalis* by IUCN), *X. fraseri*, *X. laevis* and *X. petersii*. *Xenopus pygmaeus* has only recently been recorded in Gabon from a single specimen collected on this expedition (Zimkus & Larson 2012).

Xenopus pygmaeus Loumont, 1986. MCZ A-147875. Site 4. A single specimen of *X. pygmaeus* was collected in the forest adjacent to Camp Ntsa in the same large puddle where *Hymenochirus boettgeri* was collected during the night (Fig. 2F; Fig. 3J). The identification of *X. pygmaeus* was supported by mitochondrial data from 16S ribosomal DNA (GenBank No. JQ302191). This represents the first confirmed country record of this species in Gabon, extending the known range by more than 750 km SW from the

type locality in Bouchia, Central African Republic (Zimkus & Larson 2012).

Ptychadenidae: *Ptychadena*

The genus *Ptychadena* currently includes 49 species, although preliminary molecular analyses suggest that there are many cryptic species (Frost 2013, B. M. Zimkus unpublished). Frost (2013) recorded four species from Gabon (*P. aequiplicata*, *P. mascareniensis*, *P. perreti* and *P. pumilio*) and noted that *P. taenioscelis* is also possibly found in Gabon. IUCN (2012) did not report *P. pumilio* from Gabon, provisionally assigning records from coastal Gabon and southern Congo to *P. taenioscelis*, although they mention that these could refer to *P. pumilio*. We record three species in BPNP based on collected tadpoles, two of which are not currently known from Gabon and the third we provisionally identify as *P. taenioscelis*.

Ptychadena perreti Guibé & Lamotte, 1958. MCZ A-147845 (larvae), 147847 (larva), 147907 (juvenile). Site 3. Tadpoles of *P. perreti* were collected from a savanna lake during the day. Our species identification was confirmed using DNA barcode methods (GenBank No. KF178892). Genetic data suggests that *P. perreti* may be synonymous with *P. christyi* from the northeastern Democratic Republic of the Congo and western Uganda, but additional investigation is needed. A single tadpole was reared through metamorphosis (Fig. 2C; Fig. 3L).

Ptychadena taenioscelis Laurent, 1954. MCZ A-140499 (larvae). Site 1. Tadpoles of *P. taenioscelis* were collected from small pools on the edge of a savanna pond at the first site. Our species identification was confirmed using DNA barcode methods (GenBank No. KF178893). Pauwels & Rödel (2007) tentatively regarded *P. taenioscelis* as synonymous with *P. pumilio*. Pickersgill (2007) provided an account and rejected the idea of any close relationship between *Ptychadena taenioscelis* and *Ptychadena pumilio*.

Ptychadena uzungwensis (Loveridge, 1932). MCZ A-140497 (larvae). Site 1. Tadpoles were collected from small pools on the edge of a savanna pond at the first site. Our species identification was confirmed using DNA barcode methods (GenBank No. KF178894). This species is currently known from medium to high altitude grassland in Eastern and Southern Africa, including Rwanda, Burundi, southeastern Democratic Republic of the Congo, Tanzania, Malawi, Zambia, and Zimbabwe to the Mozambican uplands and northern Angola. This represents the first record of this species from Gabon. It is likely that it is distributed more widely in Central Africa, including central and western portions of the Democratic Republic of the Congo and Republic of the Congo.

Ranidae: *Hylarana*

The genus *Hylarana* is a speciose clade with 84 species and a disjunct distribution in sub-Saharan Africa and tropical Asia, through the Indo-Australian Archipelago (Frost 2013). IUCN (2012) and Frost (2013) recorded 11 species from sub-Saharan Africa with three species being listed from Gabon: *H. albolabris*, *H. amnicola* and *H. lepus*. A single species was found in BPNP.

Hylarana albolabris (Hallowell, 1856). MCZ A-140482 (larva), 140483 (larva), 140484 (metamorph), 140495 (juvenile), 140496, 147856, 147857, 147869, 147879, 147892, 147893. Sites 2, 3, 4, 5. This large-bodied species is currently distributed widely across West and Central Africa to Uganda, western Kenya and northwestern Tanzania in the east (Frost, 2013). Tadpoles are notable for their bright orange colour and prominent poison glands (Lamotte et al. 1957). We collected numerous specimens at various sites both during the day and at night with the majority of specimens being found in gallery forest (Fig. 2E, F, G; Fig. 3M).

Discussion

This field survey resulted in a number of additions to the known Gabonese herpetofauna and supports the importance of the conservation of the savanna habitat in southeast Gabon. BPNP is the only national park within Gabon with extensive, true savanna habitat, and the fauna differs from those areas that have savanna-forest mosaic, such as the Lopé National Park. We assume that we were not able to assemble a complete picture of the amphibian community of BPNP due to the short survey period and our arrival at the end of the rainy season. However, the fact that we succeeded in detecting at least 18 amphibian species despite these obstacles suggests that this region has high species richness. Photographs of *Aubria subsigillata* (at the Boat Launch located at the northern border of BPNP), *Chiromantis rufenscens* (Osséré; Fig. 1, Site 3; Fig. 2C), and a juvenile *Cardioglossa* sp. (PPG "Camp Dallas;" Fig. 1, Site 5) taken between 24 and 30 March 2011 also indicate that these taxa are present (G. Jongsma pers. comm.). In addition, although no bufonids were encountered during this survey, photographs suggest that at least one species is present in BPNP (Pearson et al. 2007). For these reasons, further amphibian surveys in this region are highly recommended, and it is suggested that collections be made during times of heaviest rains to maximize the diversity of species collected. Exploration should also target the southern region of the park on the Congolese border, an area that is especially hard to access.

Several specimens collected as part of this expedition represent first records for these species from Gabon, but perhaps the most notable are those that are reported via tadpoles whose identifications are confirmed by means of DNA sequence data (*Kassina maculosa* and *Ptychadena uzungwensis*). A short fragment of the 16S rRNA gene (approximately 550 bp) is a highly conserved mitochondrial marker that has been found to be an ideal gene for amphibian barcoding. DNA barcoding is a taxonomic method that uses short DNA sequences (400–800 bp) for species identification, comparing sequence data against a reference li-

brary (Hebert et al. 2003, 2004, Hebert & Gregory 2005, Kress & Erickson 2008). It is an additional tool that may assist in species identification if morphological characteristics are inconclusive. This tool is ideal for assisting with the identification at various amphibian life stages, such as eggs, tadpoles and newly metamorphosed juveniles, because there may be few identifying morphological features at these points in development (Parmalee et al. 2002). During certain periods of the year, adults may be difficult to find and other life stages may be the only evidence that a species is present at a given site. In addition, time may not allow researchers to rear these life stages to adulthood. The use of DNA barcoding in the identification of tadpoles or other sub-adults may allow those doing rapid biodiversity assessments to truncate their time in the field. In addition, DNA barcoding gives researchers flexibility with regard to the time of year that they travel, allowing them to access a site at times that may not be ideal for finding breeding adults (i.e., dry season), but when other life stages may be present. It must be noted that there are a number of caveats associated with DNA barcoding. The first is that the ability to identify barcode sequences is highly dependent on the representation of the particular species in publicly available genetic sequences databases, such as GenBank (Benson et al. 2004). The second is that users must assume that the sequences in the database are correctly identified to species level, and ideally, these sequences are associated with a vouchered specimen. Lastly, there is a question of how similar the variant sequence must be to the reference sequence to be assigned to a specific taxon. For these reasons, DNA barcoding is a technique that currently must be used alongside morphological examination for taxonomic identification.

A number of species recorded in BPNP are not currently known from other national parks in Gabon (see Christy et al. 2008), including *Kassina maculosa*, *Phrynobatrachus ruthbeateae, Ptychadena taenioscelis*, *Ptychadena uzungwensis* and *Xenopus pygmaeus*. It is also possible that additional phylogenetic and taxonomic study on the genus *Hyperolius* will reveal unique species not found in other protected areas within Gabon. The species distributed in Batéké Plateau National Park are likely also distributed across the border in the Republic of the Congo, as the Batéké Plateau savanna is a direct continuation of the Congolese savanna; however, due to safety issues, no herpetological fieldwork has been conducted on the Congolese side. The government of the Republic of the Congo has proposed this area to become the Ogooué-Lékéti National Park (State of the Forests 2008); however, the protection of this area has not yet been formalized. The creation of this park, as well as trans-boundary management agreements between the Congolese and Gabonese governments, would be beneficial to both sides in that it may curb illegal activities and promote the conservation of the unique savanna fauna and flora found in this region.

The difficulty in accessing this region has kept the human population density low around BPNP, with the nearest villages at least 10 km from the park boundaries. However, poaching by commercial bushmeat hunters and associated arson does threaten wildlife populations in this area (Bout 2006). Most poachers originate from the Republic of the Congo, crossing into the park on foot along the eastern border. Projet Protection des Gorilles, a project funded by The Aspinall Foundation that operates a lowland gorilla sanctuary within BPNP, now helps to fund the anti-poaching and biological monitoring missions conducted throughout the park. Regular patrols are conducted in association with park authorities to stop poaching and associated arson.

Bd was not detected in any samples collected from BNPN. Similarly, *Bd* was not detected in samples from the lowlands of Gamba, Lastoursville, Lebamba, Libreville or Lopé National Park (Daversa et al. 2011, Gratwicke et al. 2011). In contrast, *Bd*-positive species were found in Monts de Cristal and Ivindo National Parks at all altitudes (75–565 m) and across all habitat types (pristine forest, bais, disturbed forest) by Bell et al. (2011). Infection was reported from 20 species representing four families (Arthroleptidae, Hyperoliidae, Ranidae and Rhacophoridae), but no dead or dying frogs showing symptoms of chytridiomycosis were found. *Bd* was also recently reported from amphibians from the southeastern lowlands of Cameroon and eastern lowlands of Nigeria (Reeder et al. 2011, Baláž et al. 2012). Continued sampling for *Bd* in Gabon and across Africa is needed to understand the prevalence of this fungal pathogen, determine if specific species are more susceptible to infection, and ascertain which environmental characteristics make communities most vulnerable to the disease.

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Appendix

Adult amphibian specimens that tested negative for *Batrachochytrium dendrobatidis* (*Bd*), the fungus that causes the disease chytridiomycosis.

MCZ Catalogue Number	Species
MCZ A-147848	*Hyperolius* sp. 1
MCZ A-147849	*Hyperolius* sp. 1
MCZ A-147851	*Arthroleptis* cf. *poecilonotus*
MCZ A-147852	*Hoplobatrachus occipitalis*
MCZ A-147853	*Hyperolius* sp. 2
MCZ A-147854	*Hyperolius* sp. 2
MCZ A-147855	*Arthroleptis* cf. *poecilonotus*
MCZ A-147856	*Hylarana albolabris*
MCZ A-147857	*Hylarana albolabris*
MCZ A-147858	*Hyperolius adspersus*
MCZ A-147863	*Hyperolius adspersus*
MCZ A-147864	*Afrixalus quadrivittatus*
MCZ A-147865	*Phrynobatrachus ruthbeateae*
MCZ A-147866	*Phrynobatrachus ruthbeateae*
MCZ A-147867	*Phrynobatrachus ruthbeateae*
MCZ A-147868	*Arthroleptis sylvaticus*
MCZ A-147869	*Hylarana albolabris*
MCZ A-147870	*Phrynobatrachus ruthbeateae*
MCZ A-147871	*Phrynobatrachus ruthbeateae*
MCZ A-147872	*Hyperolius adspersus*
MCZ A-147873	*Phrynobatrachus ruthbeateae*
MCZ A-147874	*Hymenochirus boettgeri*
MCZ A-147875	*Xenopus pygmaeus*
MCZ A-147876	*Arthroleptis* cf. *poecilonotus*
MCZ A-147877	*Arthroleptis* cf. *poecilonotus*
MCZ A-147878	*Arthroleptis* cf. *poecilonotus*
MCZ A-147879	*Hylarana albolabris*
MCZ A-147880	*Arthroleptis sylvaticus*
MCZ A-147881	*Arthroleptis sylvaticus*
MCZ A-147882	*Phrynobatrachus ruthbeateae*
MCZ A-147883	*Phrynobatrachus ruthbeateae*
MCZ A-147888	*Phrynobatrachus ruthbeateae*
MCZ A-147891	*Phrynobatrachus ruthbeateae*
MCZ A-147892	*Hylarana albolabris*
MCZ A-147893	*Hylarana albolabris*
MCZ A-147894	*Arthroleptis* cf. *poecilonotus*
MCZ A-147895	*Hyperolius bolifambae*
MCZ A-147896	*Hyperolius* sp. 3
MCZ A-147897	*Hyperolius* sp. 2
MCZ A-147898	*Hyperolius* sp. 2
MCZ A-147899	*Hyperolius* sp. 2
MCZ A-147903	*Cryptothylax greshoffii*
MCZ A-147904	*Cryptothylax greshoffii*
MCZ A-147905	*Cryptothylax greshoffii*
MCZ A-147906	*Arthroleptis sylvaticus*