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Herpetological Survey of the Proposed Bambama-Zanaga Mine Site, Department of Lékoumou, Republic of Congo

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***Abstract.*—We sampled amphibians and reptiles using rapid assessment techniques in primary forest and forest-savannah mosaic habitat in Lékoumou Department, Republic of Congo, near the Batéké Plateau, as part of an environmental impact assessment for a mining project. We detected 38 amphibian and 36 reptile species. Amphibian species represented 14 genera and 10 families of frogs. Reptile species included 23 snakes (representing 18 genera and six families), 11 lizards (representing 8 genera and five families) and two turtles (representing two genera and two families). Five frogs (Foulassi Screeching Frog, *Arthroleptis adelphus,* Rio Benito Long-fingered Frog, *Cardioglossa gracilis,* Ongot Long-fingered Frog, *Cardioglossa gratiosa,* Bates' River Frog, *Phrynobatrachus batesii,* and Uzungwe Grassland/Ridged Frog, *Ptychadena uzungwensis*), two snakes (Steinhaus' Worm Snake, *Afrotyphlops steinhausi*, and Underwood's Tree Snake, *Dipsadoboa underwoodi*), and two lizards (Kamdem Toham's Half-toed Gecko, *Hemidactylus kamdemtohami*, and Reichenow's Skink, *Lacertaspis reichenowi*) represents the first confirmed records for the Republic of Congo. The snake *Bothrolycus ater* (Loreal-pitted Snake) was previously known from only a few specimens; we present here the first photograph of this species found alive. This study represents the herpetological component of a broader biodiversity survey carried out as an early step toward the goal of establishing Ogooué-Leketi National Park as a protected area.**

Key Words.—Amphibia; Batéké Plateau; Central Africa; Ogooué-Leketi National Park; Reptilia

***Resumé.*—Nous avons échantillonné les amphibiens et les reptiles dans un format d'évaluation rapide, en forêt primaire et en mosaïque forêt-savane dans le département Lékoumou de la République du Congo, près du plateau Batéké, dans le cadre d'une étude d'impact environnemental pour un projet minier. Nous avons trouvé 38 espèces d'amphibiens et 36 espèces de reptiles. Les espèces d'amphibiens comprenaient des représentants de 14 genres et de 10 familles. Les espèces de reptiles comprenaient 23 serpents (représentant 18 genres et six familles), 11 lézards (représentant huit genres et cinq familles) et deux chéloniens (représentant deux familles). Dix espèces représentent les premiers rapports confirmés pour la République du Congo, dont cinq grenouilles (*Arthroleptis adelphus*, *Cardioglossa gracilis*, *Cardioglossa gratiosa*, *Phrynobatrachus batesii* et *Ptychadena uzungwensis*), deux serpents (*Afrotyphlops steinhausi* et *Dipsadoboa underwoodi*) et deux lézards (*Hemidactylus kamdemtohami* et *Lacertaspis reichenowi*). Le serpent *Bothrolycus ater*, bien que précédemment connu de la République du Congo n'est connu que de quelques spécimens de musée et nous présentons ici la première photo de cette espèce vivante. Cette étude herpétologique fait partie d'une plus grande enquête sur la biodiversité réalisée comme première étape vers l'établissement du parc national Ogooué-Leketi comme aire protégée.**

Mots Clés.—Afrique centrale; Amphibiens; Batéké Plateau; Ogooué-Leketi Parc National; Reptilia

Introduction

The amphibians and reptiles of central Africa remain poorly known compared with those of other regions of the continent (Tolley et al. 2016). Within central Africa, the herpetofauna of the Republic of Congo has been less studied than those of neighboring Cameroon (e.g., Diffo 2001; Lawson and Klemens 2001; Foruekem 2002; Gonwouo et al. 2005; Herrmann et al. 2005) and Gabon (e.g., Burger et al. 2004; Chirio and Lebreton 2007; Pauwels and Vande Weghe 2008). The earliest herpetological studies from the Congo date back more than a century (Mocquard 1887; 1889). Efforts to study the herpetofauna of the region intensified during the 1960s (Guibé 1946; Ravisse 1960; De Witte 1967). Studies carried out since the colonial period have improved knowledge of Congolese herpetofauna. These primarily include studies carried out in the savannahs

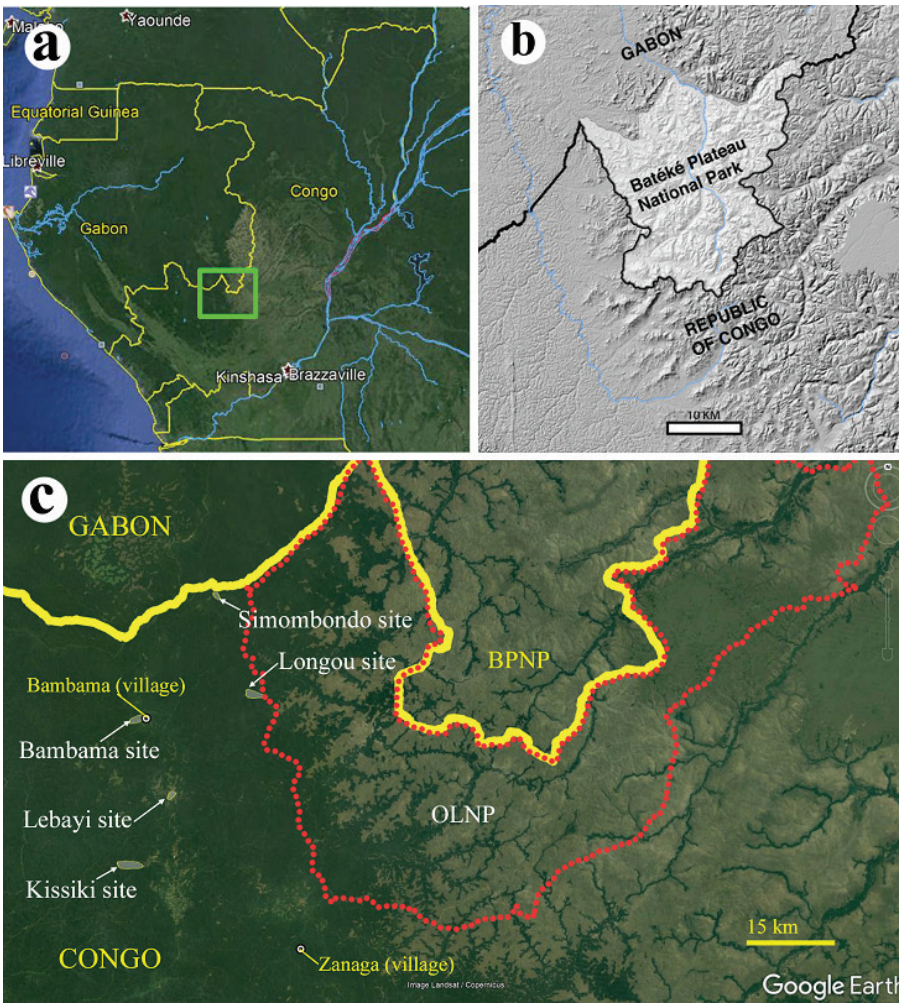

Figure 1. Study zone in Lékoumou Department, southwestern Congo, Africa: (a) Area of interest on the border between Congo and Gabon; (b) Location of (existing) Batéké Plateau National Park on the Gabon side of the border (adapted from Zimkus and Larson 2013); (c) Study sites in the area to be impacted by the Zanaga mine project. Yellow line indicates border between Congo and Gabon. Dotted red line indicates the boundaries of the proposed Ogooué-Lékéti National Park on the Congo side of the border adjacent to the (existing) Batéké Plateau National Park on the Gabon side of the border. (Map from Google Earth™ 2018).

and forests of southern Congo in the 1980s (Trape and Carme 1982; Trape 1985), in the 1990s (Trape and Roux-Estève 1990; Largen 1991; Largen and Dowsett-Lemaire 1991; Rasmussen 1991), and in the 21st Century (Jackson and Blackburn 2010; Zassi-Boulou et al. 2010, 2011; Boudzoumou et al. 2013; Hirschfeld et al. 2015). A smaller number of studies have been carried out in the forests of northern Congo (Jackson and Blackburn 2007; Jackson et al. 2007).

The purpose of this survey was to establish the baseline herpetofaunal diversity in an area surrounding the village of Zanaga, Lékoumou Department, southwestern Congo, near the border with Gabon. This is an area of dense primary forest and the site of a proposed iron ore mine (Mabiala et al. 2014). Mitigation of the proposed mining activity includes the possibility of creating the Ogooué-Lékéti National Park (OLNP), planned for an area adjacent to the Gabonese Batéké Plateau National Park (BPNP; Fig. 1; Mabiala et al. 2014). This is a valuable location for a park because large areas of protected habitat extending across international borders are more useful than many separate fragments of protected habitat (Telfer et al. 2008) and especially desirable because they allow megafauna such as Forest Elephants (*Loxodonta cyclotis*) to roam freely (Telfer et al. 2008; Mabiala et al. 2014).

The survey was commissioned by a mining company: Mining Project Development Congo SA (MPD Congo), and was carried out by Wildlife Conservation Society Congo (WCS Congo) as part of a larger effort undertaken by WCS Congo to survey several taxonomic groups (including mammals, birds, insects, and plants) in a rapid assessment format at several sites within the zone potentially impacted by the Zanaga-Bambama

iron ore mining project (Zanaga Project). Resources for basic scientific surveys necessary to document the herpetological biodiversity of remote areas of the Congo are scarce, and surveys commissioned by the extractive industries as part of required environmental impact assessments represent an important source of funding for biodiversity fieldwork. This survey provided a unique opportunity to better understand the amphibian and reptile biodiversity in an area never previously surveyed by herpetologists.

No previous herpetological surveys had been carried out in the Lékoumou Department, Congo; however, a large collection of snakes was accumulated over several years in the Mayombe Forest, in the Niari Department of southwestern Congo (Trape 1985). Additionally, several surveys have been conducted in the Kouilou River basin, in the Kouilou Department of southern Congo (snakes: Rasmussen 1991; lizards, turtles, and tortoises: Largen 1991; amphibians: Largen and Dowsett-Lemaire 1991). A more complete picture of the amphibian diversity in the neighboring Haut-Ogooué Province of Gabon, including BPNP, is known based on recent surveys (Zimkus and Larson 2013; Jongsma et al. 2017; Larson and Zimkus 2018). Jackson et al. (2007) and Jackson and Blackburn (2007) surveyed amphibians and reptiles at sites in northern Congo (Lac Télé Community Reserve in Likouala Department, and Nouabalé-Ndoki National Park in Sangha Department). Additionally, Jackson and Blackburn (2010) surveyed in degraded habitat near the city of Pointe-Noire (Pointe Noire Department), and Ange-Ghislain Zassi-Boulou et al. (unpubl. data) surveyed an urban population of snakes in Brazzaville (Brazzaville Department), in southern Congo.

Materials and Methods

Study sites.—We conducted sampling between 22 May and 16 June 2010. We surveyed four sites within the study area (Bambama, Simombondo, Kissiki and Lebayi) for a few days each as part of the rapid assessment mission (Fig. 1). Our herpetological work was carried out as part of a broader rapid assessment survey in which a team of specialists in taxonomic groups (including mammals, birds, and invertebrates, as well as amphibians and reptiles) surveyed a series of sites for a few days at each. A fifth site, Longou, was not surveyed by the herpetology team, but specimens were collected opportunistically by mammal and insect teams.

We surveyed the Bambama Site, near the village of Bambama, on 22–26 May 2010. This site (between 2°31’48”S and 2°32’24”S and between 13°30’36”E and 13°32’24”E, 540–574 m elevation), was located in dense forest. We surveyed the Simombondo Site and an area just outside the border of the proposed OLNP from 28 May–4 June 2010. This site (between 2°18’36”S and 2°19’48”S and between 13°39’00”E and 13°39’36”E longitude, 511–531 m elevation) was densely forested and located on the Ogooué River. We surveyed the Kissiki Site (between 2°18’36”S and 2°19’48”S and between 13°39’00”E and 13°39’36”E, 604–651 m elevation) from 6–11 June 2010. This site was forested and included several streams, freshwater springs, and ponds. We surveyed the Lebayi Site (between 2°39’36”S and 2°40’12”S and between 13°34’48”E and 13°35’24”E, 593–630 m elevation), located in wooded savannah, from 13–16 June 2010. Longou Site was located between 2°46’48”S and 2°47’24”S and between 13°30’00”E and 13°32’24”E longitude, 515–550 m elevation (Fig. 2).

Collecting methods.—We used a combination of passive and active survey techniques. Our passive collection methods included pitfall traps with drift fences on land and gill nets placed in waterways primarily to catch aquatic snakes. Drift fences, comprised of long (20 m), tall (1.5 m) lengths of plastic sheeting, were designed to channel small leaf-litter species into pitfall traps. We set traps at 5 m intervals along the drift fence. These consisted of 20 L buckets, sunk into the ground with the rim at ground level. We typically placed gill nets across flowing streams. We checked pitfall traps and gill nets on a daily basis.

Our team conducted active searches and visual observations during the day and at night using headlamps on land and in wetlands. On land, we carefully turned over logs, rocks, and other potential refuges. Whenever possible, we photographed captured specimens that were not collected.

We collected voucher specimens for all species except tortoises, chameleons, and varanids. We euthanized amphibians by application of a drop of benzocaine gel (OrajelTM brand, Church and Dwight, Inc., Ewing, New Jersey, USA) to the skin and euthanized reptiles by intracardiac injection of xylocaine. For all voucher specimens, we preserved tissue samples of 1–3 mm^3 of liver or muscle in 95% ethanol to allow DNA extraction prior to fixation of the whole animal in 10% formalin. Voucher specimens were fixed overnight in 10% formalin on flat trays and stored, wrapped in formalin-saturated cheesecloth, in plastic bags. We deposited voucher specimens and tissue samples in the Division of Amphibians and Reptiles, National Museum of Natural History, Smithsonian Institution, USA.

DNA barcoding.—Some amphibian specimens reported here were ultimately identified by genetic barcoding using a number of methods, including the Barcode Index Number (BIN) System generated in BOLD with COI sequences, as well as 16S sequence

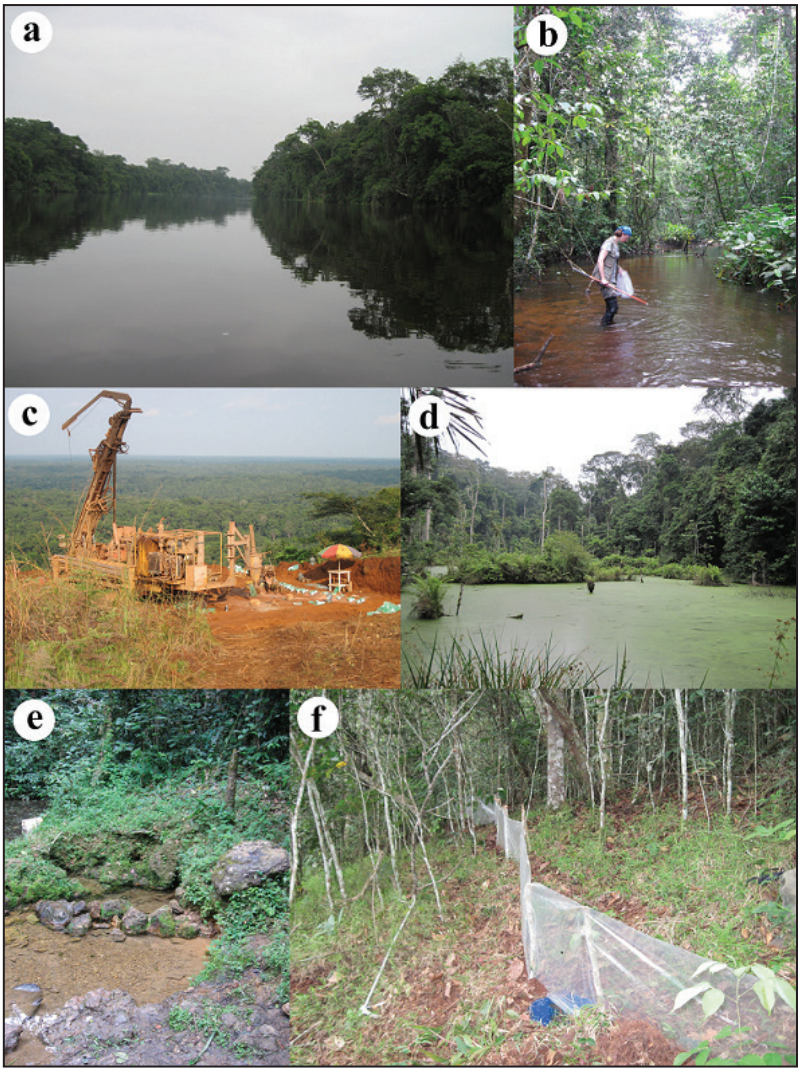

Figure 2. Habitat photos from the Lékoumou Department, southwestern Congo: (a) Ogooué River at the Simombondo site; (b) A forest stream at the Simombondo site; (c) Machinery left by mining company overlooking forest near Lebayi camp; (d) Forest pond at the Kissiki site; (e) Freshwater spring in the forest near Lebayi site; (f) Pitfall trapline at the Lebayi site. (a, c, d, e, and f photographed by Sylvestre Boudzoumou: b photographed by Ange-Ghislain Zassi-Boulou).

data compared with GenBank material (Deichmann et al. 2017). Specimens placed in the same COI BIN were considered the same species. We report relevant GenBank numbers in the description of each species in the Supplemental Information file; BIN numbers are reported in Table 2 of Deichmann et al. (2017). As part of this study, we extracted DNA for a single unidentified amphibian species that was not included in the previous study. We used the polymerase chain reaction (PCR) to amplify approximately 780 base pairs of the 16S rRNA genes using the 16SC and 16SD primers (Darst and Cannatella, 2004). We followed standard PCR conditions for amplification (Palumbi 1996) with the following thermal cycle profile: 2 min at 94° C, followed by 35 cycles of 94° C for 30 sec, 46° C for 30 sec, and 72° C for 60 sec and a final extension phase at 72° C for 7 min. We verified all amplified PCR products using electrophoresis on a 1.0% agarose gel stained with SYBR Safe DNA gel stain (Invitrogen Corporation, Carlsbad, California, USA). We purified PCR products using the Qiagen DNeasy DNA Purification System according to the manufacturer's recommendations. DNA sequences were obtained on an automated DNA sequencer (ABI PRISM 3730xl Foster City, California, USA). We completed editing and assembly of contigs in SEQUENCHER 5.0 (Gene Codes Corp., Ann Arbor, Michigan, USA). We confirmed identification using BLAST and deposited the sequence data in GenBank. The family-level classification used here for amphibians is consistent with that proposed by Frost et al. (2018. Amphibian Species of the World: an Online Reference. Version 6.0 [January 2, 2018]. Available from: http://

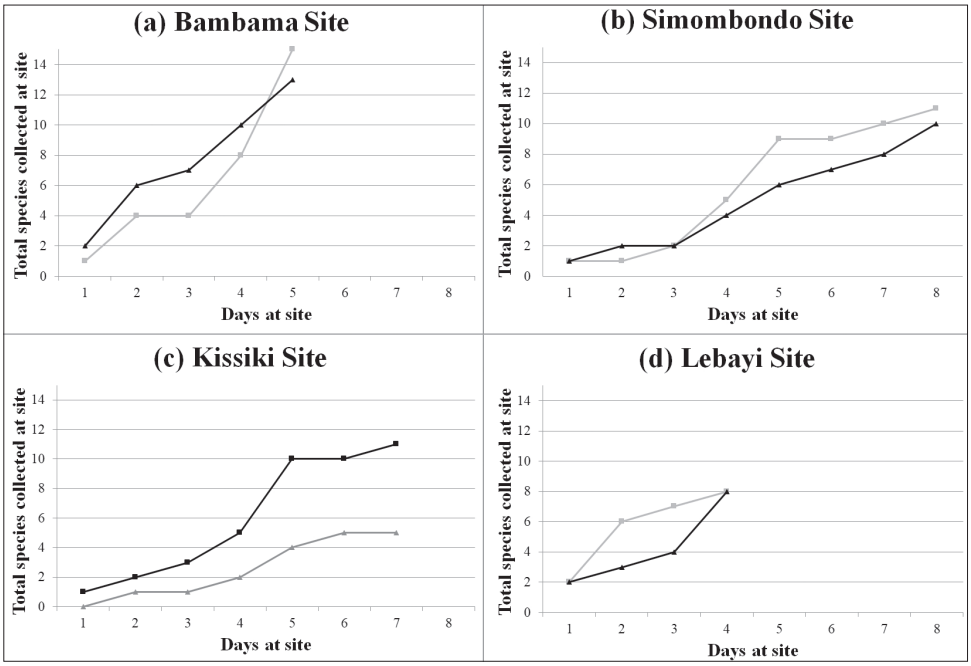

Figure 3. Species accumulation curves for amphibian (light gray) and reptile (black) species collected at: (a) Bamabama; (b) Simombondo; (c) Kissiki; and (d) Lebayi sites, Lékoumou Department, southwestern Congo. The x-axis shows number of days of sampling effort and the y-axis shows number of species collected.

research.amnh.org/herpetology/amphibia/index.html. [Accessed 6 December 2018]).

Results

We documented the presence of 38 amphibian and 36 reptile species in the course of this study. The amphibian species included 14 genera and 10 families of anurans. The reptile species included 23 snakes (representing 18 genera and six families), 11 lizards (eight genera and five families) and two chelonians (two genera and two families). See the Supplemental Information file for a detailed checklist with information for each species.

At Bambama, we documented 15 amphibian and 13 reptile species over 5 d. At Simombondo, we documented 11 amphibian and 10 reptile species over 8 d. We documented 11 amphibian and five reptile species at Kissiki over 6 d and eight amphibian and eight reptile species at Lebayi over 4 d (Fig. 3). Using opportunistic collections at Longou, we documented eight amphibian and five reptile species.

The rapid assessment of amphibians of this region identified 38 frog species in approximately 23 d, which included the first confirmed records of five species for the country (Fig. 4). These were *Arthroleptis adelphus* (Foulassi Screeching Frog), *Cardioglossa gracilis* (Rio Benito Long-fingered Frog), *Cardioglossa gratiosa* (Ongot Long-fingered Frog), *Phrynobatrachus batesii* (Bates' River Frog), and *Ptychadena uzungwensis* (Uzungwe Grassland/Ridged Frog). The most commonly observed species was *Sclerophrys camerunensis* (Oban Toad) with 42 individuals documented at three sites. In contrast, we documented over half of the species (22 of 38), representing 11 of the 14 genera, with only one or two individuals at one or two sites. The greatest number of species (15) we documented was at Bambama with the observation of 69 individuals in approximately 5 d. Although more time (8 d) was spent at Simombondo and we observed more individuals (73) there compared to Bambama, we documented fewer total species (11) at Simombondo. We documented the same number of species (11) from Kissiki and Simombondo, but we observed fewer individuals (32) in fewer days (seven) at Kissiki. The fewest number of species (eight) we documented was from Lebayi and Longou with 36 individuals observed from the former and only 10 at the latter; however, it should be noted that we only surveyed Lebayi for 4 d and that the survey work conducted at Longou was only completed opportunistically during rapid assessments focused on other taxonomic groups. The majority of individuals (165 of 220) we collected was from the forest floor with far fewer (43) from other habitats, including in or near water (28), near human habitation (nine), in savannah (four), or from arboreal habitats (two). Numbers of individuals of each amphibian species sampled by site and by habitat are listed in Table 1 in the Supplemental Information file.

The rapid assessment of reptiles of this region

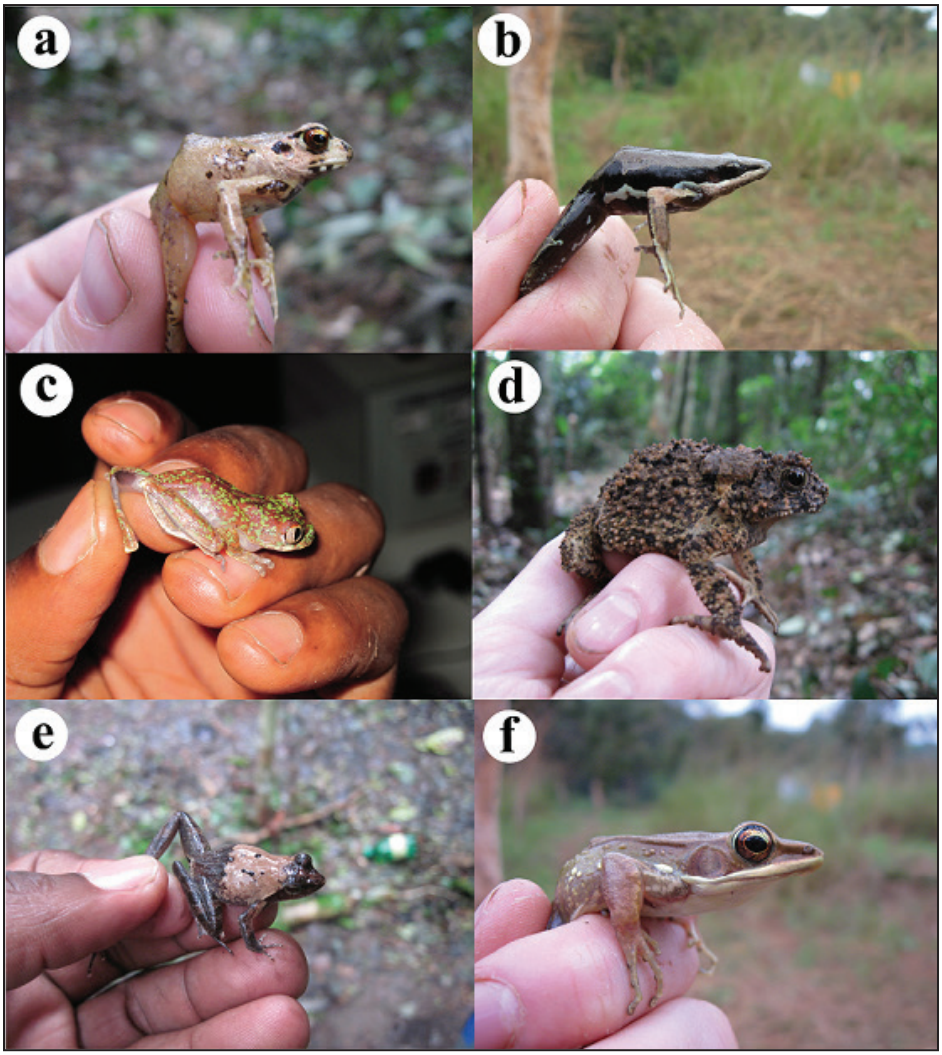

Figure 4. Select amphibians found during surveys in the Lékoumou Department, southwestern Congo: (a) *Arthroleptis variabilis* (Variable Screeching Frog); (b) *Cardioglossa gracilis* (Rio Benito Long-fingered Frog); (c) *Leptopelis aubryoides* (Kala Forest Treefrog); (d) *Sclerophrys tuberosus* (Warty/Fernando Po Toad); (e) *Phrynobatrachus auritus* (Eared River/Golden Puddle Frog); (f) *Amnirana albolabris* (White-Lipped Frog). (a, b, d, e, and f photographed by Kate Jackson; c photographed by Sylvestre Boudzoumou).

identified 36 reptile species in approximately 23 days (Fig. 5). These included 23 snakes (representing 18 genera and six families), 11 lizards (representing 8 genera and five families) and two turtles (representing two genera and two families). Among the reptiles documented are first confirmed records of two lizards: *Hemidactylus kamdemtohami* (Kamdem Toham's Half-toed Gecko) and *Lacertaspis reichenowi* (Reichenow's Skink). We also report first records for two snakes, including *Afrotyphlops steinhausi* (Steinhaus' Worm Snake) and *Dipsadoboa underwoodi* (Underwood's Tree Snake), and present the first photograph of *Bothrolycus ater* (Loreal-pitted Snake) found alive (Fig. 5c). The greatest number of species (15) was documented at Bambama during a period of approximately 5 d. Although more time (8 d) was spent at Simombondo, we documented fewer total species (11). We documented nine reptile species in just 4 d at Lebayi. The fewest number of species (five) we documented was from Kissiki and Longou; however, it should be noted that the survey work conducted at Longou was only completed opportunistically during rapid assessments focused on other taxonomic groups. Numbers of individuals of each reptile species sampled by site and by habitat are listed in Table 2 in the Supplemental Information file.

Discussion

Our survey sought to document amphibian and reptile species present in the area of the Zanaga-Bambama mine site, using a rapid assessment format. Our herpetological work was part of a broader rapid assessment survey (including mammals, birds, invertebrates) aimed at ultimately protecting an area of habitat as a national park

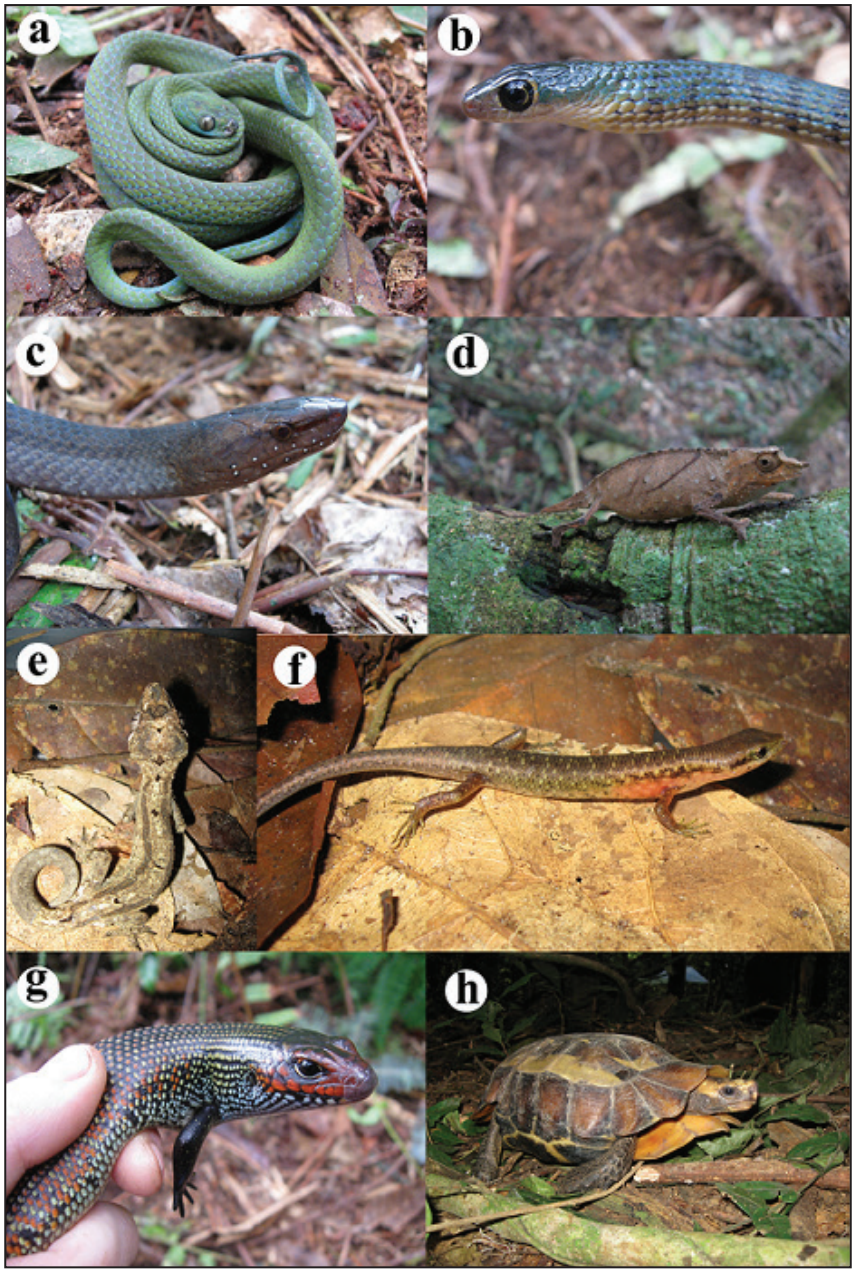

Figure 5. Reptiles from the Lékoumou Department, southwestern Congo: (a) *Dipsadoboa viridis* (Green Tree Snake) in defensive posture with body rolled into a ball to protect its head; (b) *Philothamnus carinatus* (Thirteen-scaled Bush Snake); (c) *Bothrolycus ater* (Loreal-pitted Snake); (d) *Rhampholeon spectrum* (Cameroon Pygmy Chameleon); (e) *Hemidactylus kamdemtohami* (Kamdem Toham's Half-toed Gecko) (f) *Lacertaspis reichenowi* (Reichenow's Skink); (g) *Lepidothyris striatus* (Central African Red-flanked Skink); (h) *Kinixys erosa* (Forest Hinged Tortoise). (Photographed by Kate Jackson).

as a mitigation measure for the impacts of surface mining. Of the 38 amphibian and 36 reptile species documented during this survey, several species represent the first confirmed records for the Republic of Congo. These include five frogs (*Arthroleptis adelphus, Cardioglossa gracilis, Cardioglossa gratiosa, Phrynobatrachus batesii,* and *Ptychadena uzungwensis*), two snakes (*Afrotyphlops steinhausi* and *Dipsadoboa underwoodi*), and two lizards (*Hemidactylus kamdemtohami* and *Lacertaspis reichenowi*). Additionally, there are records of *Scotobleps gabonicus* **(**Gaboon Forest Frog) from southern and northwestern Congo according to the International Union for the Conservation of Nature (IUCN; 2018), but this species was not reported by Frost et al. (*op. cit*.) and the record reported herein represents the first occurrence in western Congo.

We used a rapid assessment format as a practical way to quickly survey a large area. This technique is valuable because so many locations and habitats can be included in a short time period. In this approach, researchers sample for a few days at several locations instead of spending an extended time at a single location. This approach, however, can be logistically demanding because of the cost of moving and re-establishing camp several times with support staff (such as cooks, porters, and guides). Additionally, species lists derived from rapid assessment sampling are not expected to be exhaustive. Consistent with this expectation, species accumulation curves for sites did not asymptote during sampling. This suggests that a more comprehensive sampling protocol would have resulted in the identification of additional amphibian and reptile species.

Although we did not observe *Python sebae* (African Rock Python), credible accounts by locals and the presence of suitable habitat suggest that it is present at Lebayi, Simombondo, and possibly some other sites. *Python sebae* is broadly distributed in sub-Saharan Africa, with its range extending from Senegal to Somalia and as far south as Zimbabwe (Chippaux and Jackson 2019). Although it occupies almost all habitat types, it spends a substantial amount of time in the water (Chippaux and Jackson 2019), and subadult specimens are often caught in gill nets set by fishermen (Kate Jackson, pers. obs.). This species is also hunted locally for food, although it is listed as Convention on International Trade in Endangered Species (CITES) Appendix II (Convention on International Trade in Endangered Species of Wild Fauna and Flora. 2018. Appendices I, II and III. Available from https://www.cites.org/eng/app/appendices.php [Accessed 5 December 2018]). Similarly, although we did not observe *Mecistops cataphractus* (Slender-snouted Crocodile), fishermen on the Ogooué River at Simonbomdo provided convincing descriptions of this species, which is listed as CITES Appendix I (CITES. 2018. *op. cit.*) and as Critically Endangered (CR) by the IUCN (IUCN 2018).

In 2004, the Congolese government proposed the establishment of the Ogooué-Leketi National Park (Telfer et al. 2008). The area proposed, a 5,300 km^2 protected area that borders BPNP, was chosen based on the findings of the Zanaga-Bambama rapid assessment survey, and the goal of creating a trans-border protected area by protecting an area in Congo adjacent to BPNP in Gabon (Mabiala et al. 2014). Fieldwork for our surveys was conducted in 2010 as part of an environmental assessment in preparation for surface mining for iron ore of the Zanaga-Bambama area; however, global economic factors have prevented the start of the planned surface mining. Ogooué-Leketi National Park was finally established in November 2018. OLNP spans 3,500 km^2 and borders BPNP in Gabon. Together, the two national parks form a transboundary protected area covering more than 5,500 km^2 (https://news.mongabay.com/2018/11/republic-of-congo-names-new-national-park-home-to-gorillas-elephants/).

Larson and Zimkus (2018) found that the frog assemblages in the savannah dominated BPNP are distinct from other Gabonese protected regions. The identification of many previously unreported species for Congo in the Bambama-Zanaga mine site suggests that protection of the lowland forest surrounding the Batéké Plateau may be just as important as prioritizing the unique savannah habitat (Larson and Zimkus 2018). There is an increased urgency to more completely understand the biodiversity of regions before habitats are altered, and this survey begins documentation of herpetofaunal assemblages in the region before alteration of the landscape through mining; however, other threats are also present. Although bushmeat hunting and ivory poaching remain serious threats to wildlife, arguably the most serious problem in the region is deforestation by industrial logging (https://news.mongabay.com/2014/10/the-zanaga-iron-ore-mine-a-test-of-best-laid-plans-for-preserving-wildlife/). Currently, there are three Asian logging companies operating in the Zanaga-Bambama mine concession. Baseline data of community assemblages will facilitate long-term species monitoring projects to address these threats.

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