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A new species of *Phrynobatrachus* (Amphibia: Anura: Phrynobatrachidae) from north-western Guinea, West Africa

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Abstract

A new small *Phrynobatrachus* species from a gallery forest in north-western Guinea is described. *Phrynobatrachus pintoi* sp. nov. exhibits a combination of unique morphological characters and a distinctive color pattern, including: compact, oval body, short snout, warty dorsum and eyelid (although no eyelid cornicle is present), three pairs of large symmetric black spots on throat and breast, black spots on belly, more than one black bar on thighs and lower leg, finger and toe tips not expanded, and rudimentary web on foot. Furthermore, analysis of mitochondrial DNA from 16S rRNA reveals that this new species differs from other West African species of the genus by a minimum distance of 7%. Genetically the new species is closest to *Phrynobatrachus fraterculus*, *P. cornutus*, and *P. gutturosus*.

Key words: *Phrynobatrachus pintoi* sp. nov., conservation, gallery forest, Upper Guinea Forest zone

Introduction

The genus *Phrynobatrachus* Günther, 1862, family Phrynobatrachidae Laurent, 1941, currently comprises 76 species and is endemic to savannas and forests in sub-Saharan Africa (Poynton 1999; IUCN *et al.* 2004; Frost 2007). Seventeen valid species are recognized to occur in West Africa west of the Dahomey Gap, the majority living in forest habitats (Schjøtz 1964; Perret 1988; Rödel & Ernst 2002b; Rödel *et al.* 2005a). Currently 13 *Phrynobatrachus* species are known to occur in Guinea (Guibé & Lamotte 1963; Rödel & Bangoura 2004; Rödel *et al.* 2004; Greenbaum & Carr 2005). During a recent survey in north-western Guinea (Hillers *et al.* 2006) we detected a small forest *Phrynobatrachus* that was not assignable to a described species. Subsequent morphological and genetic analyses revealed that it represents an unknown species that we describe herein.

Material and methods

Measurements were taken by one person (MOR) with a dial caliper (± 0.1 mm) or with an ocular micrometer in a dissecting microscope (± 0.1 mm, Zeiss Stemi SV 6). Additionally, we recorded the structure of the dorsal and ventral skin, and the color pattern. For comparative material investigated see Rödel and Ernst (2002b) and Appendix 1. Collection abbreviations: MCZ = Museum of Comparative Zoology at Harvard University, ZMB = Zoologisches Museum Berlin (Museum of Natural History, Humboldt University Berlin). The geographic position of the type locality was collected with a handheld GPS receiver (Garmin 12XL).

We analyzed approximately 560 base pairs (bp) of mitochondrial 16S ribosomal RNA from all available West African *Phrynobatrachus* species (Table 1, Appendix 1). DNA was extracted using either QIAamp or DNeasy tissue extraction kits (Qiagen) or High Pure PCR Template Preparation kits (Roche). We used the primers 16SA-L and 16SB-H of Palumbi *et al.* (1991) to amplify the 16S rRNA gene. Standard PCR protocols were used and PCR products were purified using QIAquick purification kits (Qiagen) or High Pure PCR Product Purification kits (Roche). Purified templates were directly sequenced using an automated sequencer (ABI 377 or ABI 3100). Sequences were validated using SEQUENCE NAVIGATOR (Applied Biosystems), aligned using the Clustal option in MEGA 3.1 and refined by eye. Uncorrected pairwise sequence divergence was calculated using PAUP* (version 4beta10).

TABLE 1. Genetic distances (uncorrected p) in the mitochondrial 16S ribosomal DNA of *Phrynobatrachus* species, compared to *P. pintoii* **sp. nov.** (GB: EU718711). * *P. calcaratus* from various West African countries were included as this name seems to comprise several cryptic species. For locality data of the tissue vouchers see Appendix 1.

Species	GenBank #	Genetic distances to <i>P. pintoii</i> sp. nov.
<i>P. accraensis</i>	EU718712	0.1375
<i>P. alleni</i>	EU718713	0.1527
<i>P. annulatus</i>	EU718714	0.1080
<i>P. batesii</i>	EU718715	0.1440
<i>P. calcaratus</i> (Ghana)*	EU718716	0.1058
<i>P. calcaratus</i> (Guinea)*	EU718717	0.1187
<i>P. calcaratus</i> (Togo)*	EU718718	0.1076
<i>P. cornutus</i>	EU718719	0.0875
<i>P. francisci</i>	EU718720	0.1592
<i>P. fraterculus</i>	EU718721	0.0744
<i>P. ghanensis</i>	EU718722	0.1450
<i>P. guineensis</i>	EU718723	0.1458
<i>P. gutturosus</i>	EU718724	0.0901
<i>P. liberiensis</i>	EU718725	0.1433
<i>P. natalensis</i>	EU718726	0.1624
<i>P. phyllophilus</i>	EU718727	0.1404
<i>P. plicatus</i>	EU718728	0.1413
<i>P. tokba</i>	EU718729	0.1447
<i>P. villiersi</i>	EU718730	0.0910
Min-Max		0.07-0.16
Mean \pm sd		0.1253 \pm 0.0275

Results

Phrynobatrachus pintoii **sp. nov.**

Figs 1–2

Holotype. ZMB 70689 (GB: EU718711, field and tissue #: MOR BO167), gallery forest, 11°06.377' N, 13°57.595' W, Kewewol / Boulléré, Boké Préfecture / Sangaredi sub-Préfecture, Guinea, 5 May 2005, collected by A. Hillers, N.S. Loua and M.A. Bangoura.

Diagnosis. The genetic results indicate that the new species belongs to the genus *Phrynobatrachus*. Morphologically this is visible by the body shape; presence of a tarsal tubercle; presence of rudimentary webbing, presence of dorsal warts in the neck region; lack of black spot in the tympanal region; lack of fine medial dorsal depression; lack of parallel dorsal ridges; neither finger nor toe tips heart shaped nor expanded to discs.

Small, compact *Phrynobatrachus*, characterized by combination of warty back; warty eyelids (eyelid cornicle absent); large dark spots on white throat, breast and belly; rudimentary webbing.



FIGURE 1. Dorsolateral and ventral view of *Phrynobatrachus pintoii* sp. nov. (ZMB 70689, holotype).

Description (measures in mm). Typical, semi-adult *Phrynobatrachus* with oval, compact body shape; snout-vent-length: 10.19; short, rounded snout; canthus rostralis indistinct; loreal region straight to slightly concave; head-width directly behind the eyes: 3.75; eye-diameter: 0.96; distance eye-nostril: 0.96; distance nostril-snout tip: 0.39; nostril closer to snout than to eye; tympanum indistinct, much smaller than diameter of

eye; femur: 5.62, slightly shorter than tibio-fibulare: 5.89; foot including longest toe: 8.41 (right foot missing); hand with large and oval palmar and thenar tubercle; fingers with small roundish subarticular tubercles, no additional tubercles on hands; relative finger length: $4=1=2<3$; palmar webbing absent; tarsal tubercle present but not conspicuous; larger internal and smaller external metatarsal tubercle; relative toe length: $1<2<5<3<4$; only rudiments of webbing on toe bases; neither toe nor finger tips expanded to discs.

Dorsal skin grainy; back and eyelids with warts; larger pointed wart in posterior corner of eyelid, resembles eyelid cornicle of various other small *Phrynobatrachus* species but is less distinct; small whitish warts between posterior corner of the eye and forearm bases; two symmetrical pairs of dorsal warts especially pronounced: smaller roundish warts on neck and comma shaped warts directly posterior to symmetrical pairs; various other, smaller warts on back and hind legs; skin of throat corrugated; ventral skin otherwise smooth.

Overall coloration of the dorsum clear brown; indistinct darker bars on upper lip; warts on back rimmed almost black: unpaired roundish spot between posterior part of eyelids, paired roundish and comma shaped pairs of black bordered spots posterior to it; further dark bordered spots irregularly spread on posterior part of back; large pair of symmetrical black bordered spots between base of hind legs; flanks with dark blotch originating dorsal to base of forelimbs, extending ventrally to the middle of body; blackish spots in groin area just extending to back; lower arm with one black bar; thighs and lower leg with three black bars each; vent bordered black; outer parts of thighs with clear longitudinal band, bordered black posterior ventrally; basic color of throat white; mandible with eight black spots; throat and breast with three symmetric pairs of large blackish spots, increasing in size posteriorly; belly with eight irregularly spread blackish spots; ventral parts of thighs white with blackish spots, posterior part near vent speckled brownish; ventral parts of lower legs whitish, border of dorsal black bars visible; color in preservation possibly a bit faded, otherwise not different to life.

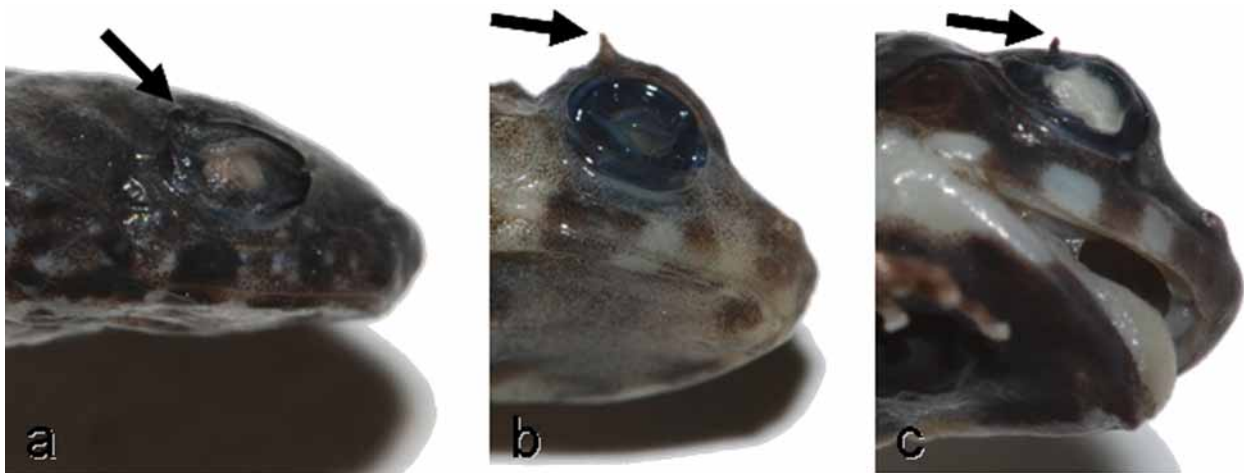


FIGURE 2. Eyelids of a) *Phrynobatrachus pintoi* sp. nov. (ZMB 70689, holotype), b) *P. cornutus* (ZMB 70745) and c) *P. villiersi* (ZMB 70746).

Genetics. *Phrynobatrachus pintoi* differed between 7-16% in the 560 investigated base pairs of the 16S rRNA gene from 17 West African and Central African species of the genus *Phrynobatrachus* (Table 1).

Natural History. The holotype was collected in a gallery forest in the Boulléré / Sangaredi area. This gallery forest was situated in a small depression surrounded by savanna habitat. In the area where *P. pintoi* was found, the forest was approximately 150 m wide. The edges were dominated by shrubs, while there were a small number of larger trees in the central section, with a more important canopy and open shrub stratum. *P. pintoi* was found in the leaf litter of this central area, close to the river. The soil was partly sandy with some stones and mainly covered with leaf litter. With the exception of the gallery forests, the general landscape in the Boulléré / Sangaradi area was dominated by savanna habitats on bauxite outcrops. These habitats were highly disturbed due to a number of settlements and agricultural encroachment (Wright *et al.* 2006). The

recorded anuran fauna was mainly dominated by savanna species but also included farmbush species, e.g. *Phrynobatrachus tokba*, and one forest species, *Astylosternus* cf. *occidentalis* (Hillers *et al.* 2006). No other data on the biology and ecology of *P. pintoi* are known. As all other morphologically similar *Phrynobatrachus* species are forest dwelling, we believe *P. pintoi* is forest dependant as well. Further data on habitat, flora and fauna of the region are provided by Wright *et al.* (2006).

Distribution. *Phrynobatrachus pintoi* is only known from the type locality.

Etymology. The species is named in honor of Mr. Sidy Mohamed Diawara, known as Pinto. With his outstanding personality and effort he contributed greatly to successful field research and nature conservation in Guinea. He was team-member of the NGO “Guinée Ecologie” and was involved in several Rapid Assessment Programs (RAP). The RAP in the Boké region was his last before he died in September 2006.

Discussion

Apart from *Phrynobatrachus brongersmai* Parker, 1936, *P. taiensis* Perret, 1988, and an undescribed species from mountainous south-eastern Guinea (Rödel & Bangoura 2004), we could compare the 16S rRNA of the new species to all other *Phrynobatrachus* known to occur in West Africa. *P. brongersmai* differs from the new species by having small but distinct discs on toes that are half webbed, a presumably larger size (SVL: 21–27 mm), four dorsal warts that form an X-shaped pattern and brown stippling on the gular and pectoral regions (Parker 1936); whereas *P. pintoi* has no discs, almost no webbing, differently shaped dorsal warts and distinct black blotches on the throat. The *Phrynobatrachus* sp. from the Simandou Range has a differently colored venter (less distinctly delimited or smaller spots), a more distinctly black lateral band, a more elongated body and a more pointed snout than *P. pintoi*.

Morphologically *P. pintoi* is most similar to a variety of other small West African *Phrynobatrachus* species, i.e. *P. annulatus* Perret, 1966, *P. calcaratus* (Peters, 1863), *P. cornutus* (Boulenger, 1906), *P. ghanensis* Schiøtz, 1964, *P. guineensis* Guibé and Lamotte, 1961, *P. phyllophilus* Rödel and Ernst, 2002, *P. taiensis* Perret, 1988 and *P. villiersi* Guibé, 1959. Within this group the new species is genetically closest to *P. cornutus* (8% difference) and *P. villiersi* (9% difference). These two differ by the presence of an eyelid cornicle. *P. villiersi* has a venter with a blackish, reticulated pattern on clear blue ground. *P. cornutus* usually has an almost uniform dark (males) or white throat with black bars on the mandible (females, for an exception see MHNG 1031.14 in Fig. 4, Perret 1988). From *P. calcaratus*, *P. annulatus*, and *P. taiensis* the new species likewise differs by the lack of an eyelid cornicle and a differing ventral coloration (see Perret 1988). *P. pintoi* differs from *P. guineensis* and *P. phyllophilus* by its almost absent webbing and the lack of expanded toe and finger tips. These are yellow in *P. guineensis*, but do not differ in color from the rest of the finger or toe in *P. pintoi*. *P. guineensis* has similar warts on the eyelids and on the back as *P. pintoi*, but always possess bright yellow markings in the groin area. This region is black in the new species. *P. phyllophilus* has almost always only one dark bar on thighs and lower leg, *P. pintoi* has three. *P. ghanensis* is similar to *P. pintoi* in body shape, warty eyelids and ventral coloration but differs genetically by almost 15% and morphologically by better developed webbing and very well developed discs on toes. Small differences in color pattern are black bars on the upper lip that are very distinct in *P. ghanensis* (Fig. 4 in Asseman *et al.* 2006). The black ventral spots of the latter species often have a clearer center or are less distinctly delimited (Schiøtz 1964; Perret 1988) than in *P. pintoi*.

Numerous morphological characteristics differentiate *P. pintoi* from other West African *Phrynobatrachus* species. *Phrynobatrachus alleni* Parker, 1936, *P. batesii* (Boulenger, 1906), *P. liberiensis* Barbour and Loveridge, 1927, *P. natalensis* (Smith, 1849), and *P. plicatus* (Günther, 1859 “1858”) are all much larger than *P. pintoi*, differ in body shape, have uniform colored white or yellow (adult *P. alleni* males) bellies and have extensive webbing. In *P. alleni* and *P. plicatus* the dorsal warts are short (*P. alleni*) or elongate (*P. plicatus*) X-shapes. *P. accraensis* (Ahl, 1925 “1923”) and *P. francisci* Boulenger, 1912 have white venters and distinct

webbing. *P. gutturosus* (Chabanaud, 1921) lacks webbing, but has a uniform white belly and a much more elongated body shape. *P. fraterculus* (Chabanaud, 1921), which was most similar to *P. pinto* genetically (7% difference), possess a very distinctly marked black lateral band, small blackish spots on the belly, has a more elongated body shape, completely smooth skin and presumably is larger (females known to reach 26 mm; see Fig. 2c in Rödel & Bangoura 2004).

From our knowledge with other, similar shaped *Phrynobatrachus* species, we believe that adults of *P. pinto* will not be much larger than 20 mm body length (for measurements of West African *Phrynobatrachus* see Guibé & Lamotte 1963; Perret 1988; Rödel 2000; Rödel & Ernst 2002b). *P. tokba* (Chabanaud, 1921) is also a very small species but differs from the new one by a differently shaped, more elongated body and converging dorsal warts (see Fig. 1A & B in Rödel & Ernst 2002a and Fig. 3 in Rödel *et al.* 2005a; second pair of neck warts comma shaped in *P. pinto*), as well as a uniform clear colored breast and belly (Rödel *et al.* 2005a).

The genus *Phrynobatrachus* is a very diverse and taxonomically difficult African frog genus (Poynton 1999), with very variable species (Stewart 1974; Rödel 2000). A description of a new species at first did not seem to be advisable because only a single sub-adult specimen was available for analysis. However, even with extensive examination of thousands of living and preserved specimens from almost all West African *Phrynobatrachus* species, we (AH, MOR) failed to assign this frog to a described species. As we found both morphological and genetic evidence for the specific distinctiveness of our new taxon, we regarded this description as justified. Giving the presumably restricted distribution of the new species in an area of intense human pressure, naming was a necessity also concerning conservation. Currently *P. pinto* has to be classified as Data Deficient. However, future work in the area may reveal that the conservation status of the new species, potentially having a very restricted distribution in a habitat type that is under huge human pressure, may be Critically Endangered. This discovery is a further hint that even very small and / or isolated West African forests still may hold herpetological novelties (Ernst *et al.* 2008) and facing the current rates of deforestation (FAO 2006) should be urgently assessed for their fauna.

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Appendix 1: Central and West African *Phrynobatrachus* tissue and voucher specimens examined for genetic and morphological comparison (MOR: field numbers M.-O. Rödel / A. Hillers; GB: GenBank accession numbers):

- P. accraensis*: ZMB 70691, MOR La5, male, Côte d'Ivoire, Lamto, 06°13.057' N; 05°01.493' W, 15 September 2004, P.J. Adeba and M.-O. Rödel; MOR C3 (C6_2), no voucher, GB: EU718712, female, Côte d'Ivoire, Comoé National Park, M.-O. Rödel.
- P. alleni*: ZMB 70692, MOR P5, GB: EU718713, female, Côte d'Ivoire, Mont Péko National Park, 06°53.259' N, 07°10.005' W, 21 May 2000, R. Ernst and M.-O. Rödel; ZMB 70693, MOR P4 and ZMB 70694, MOR P6, both males, both same collection details as ZMB 70692.
- P. annulatus*: ZMB 70690, MOR WOL92, GB: EU718714, female, Liberia, North Lorma National Forest, 08°02.023' N, 09°44.143' W, 22 November 2005, A. Hillers.
- P. batesii*: ZMB 70695, MOR K MK, male, Cameroon, Mount Kupe, W. Böhme; MCZ 136783, GB: EU718715, Cameroon, Southwest, E of Ntale Village, Banyang Mbo Forest Reserve, N/NW of Ntale Plateau, 05°15' N, 09°36' E.

- P. calcaratus*: ZMB 70698, MOR FD100, male, Guinea, Fouta Djallon/Foret de Nialama, 11°48.160' N, 12°44.593' W, 3 June 2006; N.-S. Loua and A. Hillers; ZMB 70697, MOR FD74, male, Guinea, Fouta Djallon/Foret de Nialama, 11°43.239' N, 12°43.404' W, 1 June 2006, N.-S. Loua and A. Hillers; MOR FD78, no voucher, GB: EU718717, male, Guinea, Fouta Djallon/Foret de Nialama, 11°44.221' N, 12°41.099' W, 2 June 2006, N.-S. Loua and A. Hillers; ZMB 70695, MOR AM30, GB: EU718716, female, Ghana, Amedzofe, 06°50.429' N, 00°25.582' E, 13 July 2005, A.C. Agyei and A. Hillers; ZMB 70699, MOR TOG47, GB: EU718718, male, Togo, Missahöhe/Kuma-Konda, 06°57.056' N, 00°36.018' E, G. Segniagbeto, C.O. Boateng and A. Hillers; ZMB 70700, MOR TOG75, female, Togo Missahöhe/Yo, 06°56.641' N; 00°35.619' E, 10 April 2007, G. Segniagbeto, C. Ofori Boateng and A. Hillers.
- P. cornutus*: ZMB 70745, MOR DS 29, GB: EU718719, female, Central African Republic, Dzanga-Sangha, Forest Reserve Bayanga, Bai Hokou, 02°51.576' N, 16°28.050' E, 22 June 2003, M.-O. Rödel.
- P. francisci*: ZMB 70701, MOR La10, male, Côte d'Ivoire, Lamto, 06°13.057' N; 05°01.493' W, 15 September 2004, P.J Adeba and M.-O. Rödel; MOR C8, no voucher, GB: EU718720, male, Côte d'Ivoire, Comoé National Park, M.-O. Rödel.
- P. fraterculus*: ZMB 70702, MOR GO44, female, Liberia, Gola National Forest, 07°27.376' N, 10°41.736' W, 1 December 2005, A. Hillers; GO31, GB: EU718721, no voucher, male, 29 November 2005, other data as ZMB 70702, A. Hillers; ZMB 70703, MOR WOL51, male, Liberia, North Lorma National Forest, 08°01.434' N, 09°44.414' W, 21 November 2005, A. Hillers; ZMB 70704, MOR WOL52, other data as ZMB 70703.
- P. ghanensis*: ZMB 70722, MOR ANK67, male, Ghana, Ankasa National Park, 05°15.714' N, 02°38.703' W, 8 August 2005, A.C. Agyei and A. Hillers; ZMB 70723, MOR ANK70, female, other data as ZMB 70722; ZMB 70721, MOR KAK24, female, Ghana, Kakum National Park, 05°26.916' N, 01°25.009' W, 9 August 2005; BTR0503, no voucher, GB: EU718722, Ghana, Boi Tano, 05°31' 55.58" N, 02°37' 07.26" W, 30 October 2003, A.C. Agyei and R. Ernst.
- P. guineensis*: ZMB 70706, MOR MTN269, male, Guinea, Nimba Mountains, 07°35.422' N, 08°27.105' W, 19 May 2006, N.-S. Loua and A. Hillers; ZMB 70705, MOR MTN263, female, Guinea, near Diécké forest, 07°31.087' N, 08°50.148' W, 16 May 2006, N.-S. Loua and A. Hillers; MOR T13, no voucher, GB: EU718723, Côte d'Ivoire, Taï National Park, 05°49.648" N, 07°19.862" W, M.-O. Rödel.
- P. gutturosus*: ZMB 70724, MOR MPCu1, female, Côte d'Ivoire, Marahoué National Park, 27 August 2000, R. Ernst and M.-O. Rödel; MOR C 9, GB: EU718724, no voucher, juvenile, Côte d'Ivoire, Comoé National Park, M.-O. Rödel.
- P. liberiensis*: ZMB 70725, MOR ANK68, juvenile, GB: EU718725, Ghana, Ankasa National Park, 05°15.714' N, 02°38.703' W, 8 August 2005, A.C. Agyei and A. Hillers; ZMB 70726, MOR ANK69, other data as ZMB 70725; ZMB 70727, MOR KAK77, female, Ghana, Kakum National Park, 05°26.819' N, 01°24.873' W, 10 August 2005, A.C. Agyei and A. Hillers; ZMB 70728, MOR KAK79, female and ZMB 70729, MOR KAK81, male, other data as ZMB 70727.
- P. natalensis*: ZMB 70730, MOR Gu110, GB: EU718726, Guinea, Diécké Classified Forest, 07°35'46.9" N, 08°52'18.8" W, 21-23 November 2003, M.A. Bangoura and M.-O. Rödel; ZMB 70731, MOR Gu111; ZMB 70732, MOR Gu113 ZMB 70733, MOR Gu114, other data as in ZMB 70730.
- P. phyllophilus*: ZMB 70736, MOR JP0177, female, Sierra Leone, Tingi Hills, 08°52.035' N, 10°47.536' W, 4 June 2007, J. Johnny and A. Hillers; ZMB 70734, MOR JP0178, male, other data same as ZMB 70736; ZMB 70735, MOR JP0179, male, other data same as ZMB 70736; MOR GOL35, no voucher, GB: EU718727, male, Sierra Leone, Gola North Forest Reserve, 07°34.869' N, 11°01.012' W, 15 September 2005, A. Barrie and A. Hillers.
- P. pintoii* sp. nov.: ZMB 70689, GB: EU718711, MOR BO167, holotype.
- P. plicatus*: ZMB 70737, MOR GRE30, female, Liberia, Grebo National Forest, 05°24.358' N, 07°44.106' W, 10 December 2005, A. Hillers; GRE19, no voucher, GB: EU718728, male, Liberia, Grebo National Forest, 05°24.285' N, 07°43.786' W, 9 December 2005, A. Hillers; ZMB 70738, MOR GO42, male, Liberia, Gola National Forest, 07°27.178' N, 10°41.522' W, 30 November 2005, A. Hillers; ZMB 70739, MOR WOL71, female, Liberia, North Lorma National Forest, 08°02.023' N, 09°44.143' W, 22 November 2005, A. Hillers.
- P. tokba*: ZMB 70742, MOR GRE63, juvenile, Liberia, Grebo National Forest, 05°24.127' N, 07°43.965' W, 9 December 2005, A. Hillers; GRE77, no voucher, GB: EU718729, juvenile, Liberia, Grebo National Forest, 05°23.827' N, 07°44.160' W, 11 December 2005, A. Hillers; ZMB 70740, MOR GO9, juvenile, Liberia, Gola National Forest, 07°27.178' N, 10°41.522' W, 28 November 2005, A. Hillers; ZMB 70741, MOR WOL53, juvenile, Liberia, North Lorma National Forest, 08°01.434' N, 09°44.414' W, 21 November 2005, A. Hillers.
- P. villiersi*: ZMB 70743, MOR T18, GB: EU718730, male, Côte d'Ivoire, Taï National Park, 05°49.648' N, 07°19.862' W, M.-O. Rödel; ZMB 70744, MOR T10, male, same collection details as ZMB 70743; ZMB 70746, MOR GRE32, female, Liberia, Grebo National Forest, 05°24.358' N, 07°44.106' W, 10 December 2005, A. Hillers.