



Two new terrestrial species of *Hydraena* Kugelann from the island of Bioko, Gulf of Guinea (Coleoptera: Hydraenidae)

CARLES HERNANDO¹ & IGNACIO RIBERA^{2,3}

¹P.O. box 118, 08911 Badalona, Catalonia, Spain. E-mail: leptotyphlus@gmail.com

²Institut de Biologia Evolutiva (CSIC-Universitat Pompeu Fabra), Passeig Maritim de la Barceloneta 37-49, 08003 Barcelona, Spain. E-mail: ignacio.ribera@ibe.upf-csic.es

³Corresponding author

The knowledge of the Hydraenidae of sub-Saharan Africa, and in particular of the genus *Hydraena* Kugelann, 1794, is most incomplete. As an example, in southern Africa only three species were described before the revision of Perkins (2014), which raised the number to 31. Only 15 species are currently known from Central Africa, between the Sahel and South Africa, all belonging to subgenus *Hydraenopsis* Janssens, 1972 (Trizzino *et al.* 2013), but many more remain to be described and discovered. The islands of the Gulf of Guinea are no exception to this lack of knowledge (Jones 1994), and only one species of the family was previously known, *H. (Hydraenopsis) pagaluensis* Hernando & Ribera, 2001 from Annobón (Hernando & Ribera 2001; Trizzino *et al.* 2013). In this paper we describe the first known species of Hydraenidae from Bioko, the largest and closest to the continent of these islands. The species were collected by our friend and colleague Vasily Grebennikov during a survey of the forest litter fauna of Annobón and Bioko. Despite considerable efforts no Hydraenidae were found in Annobón, and only these two species, in a single locality, in Bioko.

Materials and methods

Specimens were collected by sifting forest leaf litter with the subsequent extraction of organisms using Winkler's funnels (see e.g. Grebennikov 2014). Genitalia were dissected and mounted in DMHF (dimethyl hydantoin formaldehyde) on transparent cards and pinned with the specimens. Aedeagi were figured from outlines traced from photographs obtained with a compound microscope. Exact label data are cited for specimens. A double slash (//) indicates separate label lines.

The DNA of the two holotypes and two female paratypes was extracted non-destructively with commercial kits ("DNeasy Tissue Kit"; Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions. DNA extractions are kept in the collections of the Institut de Biología Evolutiva, Barcelona (IBE). Two fragments of the cytochrome C oxidase subunit (COI) were sequenced, the 5' end (the barcode fragment, primers LCO1490 and HCO2198, Folmer *et al.* 1994) and the 3' end (primers Jerry-M202 and Pat-M70, Simon *et al.* 1994), and submitted to the EMBL database with accession numbers LT707761-LT707766.

The genitalia of the extracted male specimens were dissected before DNA extraction and mounted on transparent cards as described above; after extraction all four specimens were mounted on cards and pinned.

Abbreviations

CHB	Coll. Carles Hernando, Badalona, Barcelona, Spain
IBE	Institute of Evolutionary Biology, Barcelona, Spain
NMW	Naturhistorisches Museum Wien, Austria
MCZ	Museum of Comparative Zoology, Harvard University, Cambridge, MA, US

Taxonomy

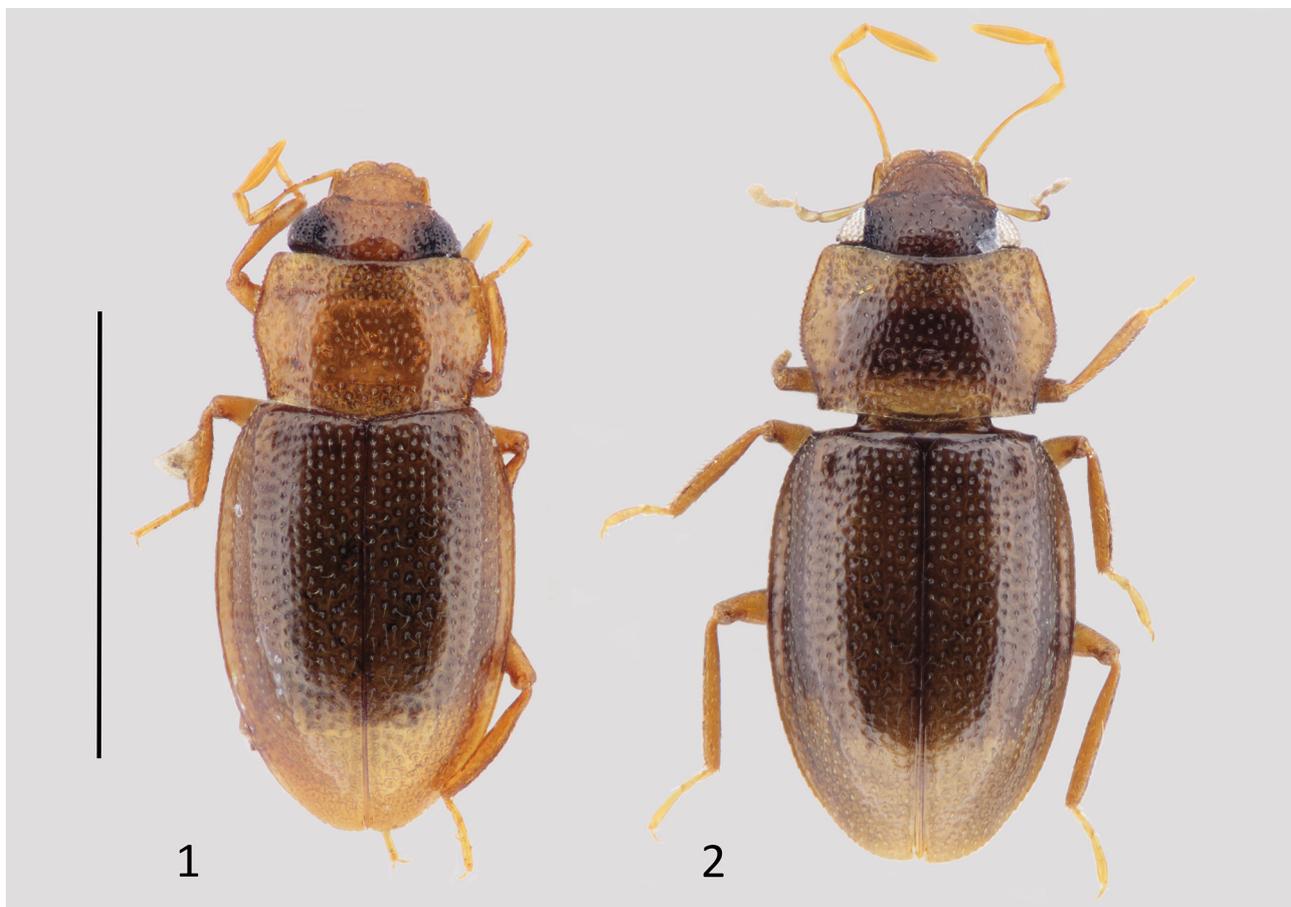
Hydraena (Hydraenopsis) bubu sp.n. (Figs 1, 3–4)

Type locality. Forest in Pico Biao, Province of Bioko Sur, island of Bioko, Equatorial Guinea.

Type material. Holotype male (NMW): "Eq. GUINEA, Bioko, // 03.3001, 008.6482, // 938m, 23.xii.2015, sift. // for. lit., V.Grebennikov"; "DNA voucher // IBE-AN370"; aedeagus dissected and mounted on a transparent card pinned

with the specimen, plus red holotype label. DNA extraction stored in the IBE with the same voucher number; COI with accession numbers LT707765 (COI-5', "barcode") and LT707761 (COI-3').

Paratypes. 8 males (CHB, IBE, MCZ) 1 female (NMW): same data as holotype, with red paratype labels. Female paratype used for DNA extraction with voucher number IBE-AN526, mounted after extraction on a card (COI-3' accession number LT707762). Four male paratypes stored in absolute ethanol in the IBE.



FIGURES 1&2. Habitus of 1) *Hydraena (Hydraenopsis) bubi* sp.n., male paratype and 2) *H. (Hydraenopsis) grebennikovi* sp.n., female paratype (voucher No. IBE-AN577, mounted after DNA extraction). Scale bar: 1 mm.

Description. length 1.34–1.50 mm (males), 1.54 mm (female paratype); width 0.60–0.70 mm (males), 0.70 mm (female paratype). Body form elongate, compact, maximum width of pronotum slightly smaller than maximum width of elytra (Fig. 1). Body and appendages uniformly pale brown.

Head: Labrum strongly excised anteriorly, with lobes rounded. Surface smooth and shiny, margins finely reticulated, with fine and sparse punctures. Puncturation on clypeus slightly coarser than on labrum. Frontoclypeal suture slightly arcuate. Frons with a coarser and denser puncturation than on clypeus, slightly less dense on disk; distance between punctures approximately equal to their diameter; surface between punctures smooth and shiny. Eyes large, prominent.

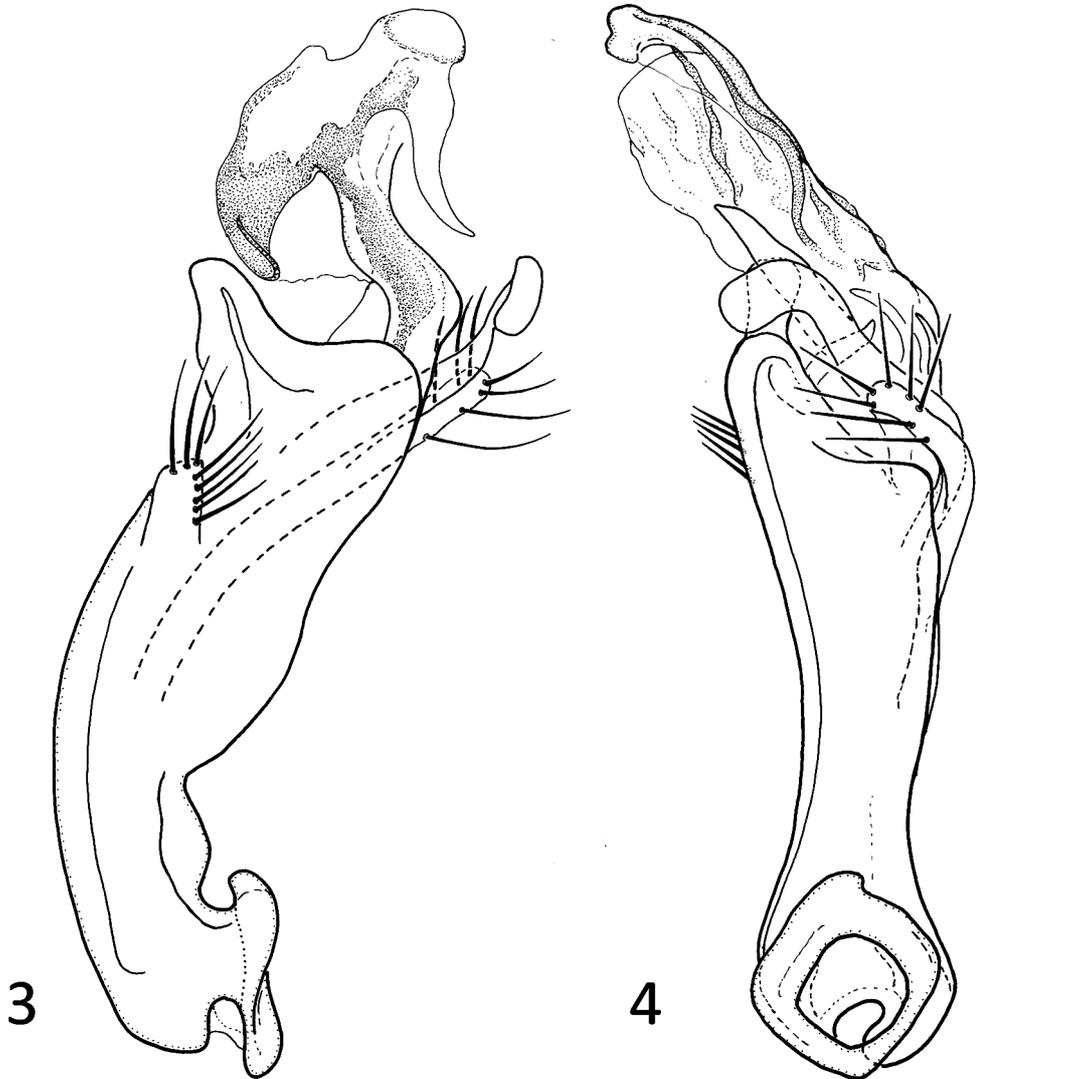
Pronotum: Transverse, lateral margins regularly curved, maximum width in middle part; clearly denticulate. Anterior and posterior margins slightly sinuated. Surface with coarse punctures, denser on the medial part, with two lateral bands almost without punctures; surface between punctures smooth and shiny.

Elytra: Approximately twice longer than pronotum; apex with margins finely serrated; with a broad explanate margin ending slightly before the apex, narrow at base, uniformly broadening towards apex; apex round. Ten very regular striae between suture and shoulder, with large and uniform punctures; punctures of apex of the elytra slightly less strong, with wider intervals. Interstriae smooth and shiny, slightly rugose at apex. Membranous wings well developed, longer than elytra.

Venter: Prosternum with distinct medial keel. Mesoventrite laterally bordered, apex truncated. Metaventral plaques densely and finely punctured, with a granulated appearance, covered by long hydrofuge pubescence; with two parallel glabrous areas on middle part, with smooth and shiny surface, without punctures. Abdominal sternites covered with fine punctures, space between punctures with fine transverse striolae; surface of abdominal sternites covered by short,

recumbent pubescence, with exception of last three sternites, which are glabrous (other than marginal setae) and with a transverse polygonal reticulum, giving a dull appearance.

Sexual variation: Aedeagus (Figs 3–4) with median lobe asymmetrical, straight; apex with digitiform expansions and a long membranous apical piece, somewhat resembling a hammer in lateral view; with two dorsal setae behind left paramere, not clearly seen in lateral view (not represented in Fig. 3). Left paramere very short and stout; right paramere longer, with apex recurved over the median lobe. Middle and hind tibia of most males slightly expanded, with external side curved (Fig. 1).



FIGURES 3&4. Aedeagus of *Hydraena (Hydraenopsis) bubi* sp.n. (holotype) 3) lateral view, 4) ventral view. Scale bar = 0.1 mm.

Females larger and more robust than males. Glabrous areas of the metaventrite smaller, less elongated. Tibia with external side straight, not expanded. Elytral margins with a wider explanation. Spermatheca very large and elongate; basal apophyses of gonocoxites long and curved inwards. Although there seem to be some differences in the gonocoxites between the two species (in e.g. the general shape and the size of the pseudostyli), they do not seem to be significant enough to allow unambiguous identification without DNA data. Therefore we do not designate females for which there is no molecular data as paratypes (11 specimens in total).

Etymology. Named after the Bubi, a Bantu ethnic group native to Bioko. Name is a noun in apposition.

Distribution. So far only known from the type locality.

Habitat and ecology. All specimens were found by sifting litter in a mid-altitude primary forest, without large trees, during the dry season and with no free water in the vicinity. The single sample was taken over two days along a single trail through the forest. Actual sifting, however, was done in four places along the trail, with intervals of some 300 meters between them (thus extreme sampling spots might be about 1 km apart). The forest was somewhat similar along the trail,

although some 150 meters altitudinal difference existed between extreme sampling spots (V. Grebennikov, personal communication, 2016). Some specimens have unidentified epibionts or ectoparasites on legs and palpi (Fig. 1).

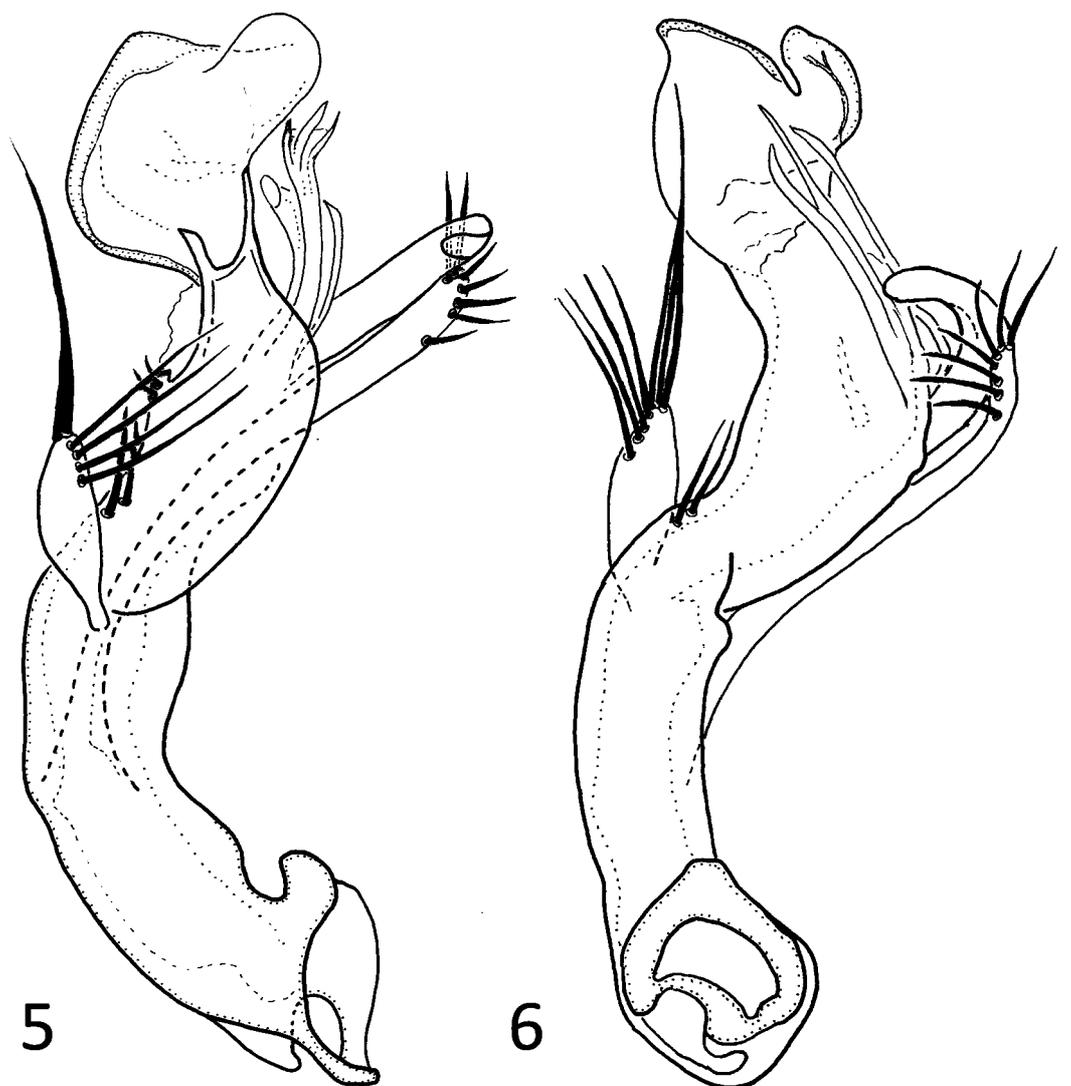
***Hydraena (Hydraenopsis) grebennikovi* sp.n. (Figs 2, 5–6)**

Type locality. Forest in Pico Biao, Province of Bioko Sur, island of Bioko, Equatorial Guinea.

Type material. **Holotype** male (NMW): "Eq. GUINEA, Bioko, // 03.3001, 008.6482, // 938m, 23.xii.2015, sift. // for. lit., V.Grebennikov"; "DNA voucher // IBE-AN525"; aedeagus dissected and mounted on a transparent card pinned with the specimen, plus red holotype label. DNA extraction stored in the IBE; COI with accession numbers LT707766 (COI-5', "barcode") and LT707763 (COI-3').

Paratypes. 2 males (IBE, NMW) 1 female (IBE): same data as holotype, with red paratype labels. Female paratype used for DNA extraction with voucher number IBE-AN577, mounted after extraction on a card (COI-3' accession number LT707764). One paratype male stored in absolute ethanol in the IBE.

Description. Length 1.35 mm (males), 1.54 mm (female paratype); width 0.60 mm (males), 0.70 mm (female paratype). External morphology very similar to *H. bubu* sp.n., with no clear consistent differences. In general males are slightly smaller, with straight middle and hind tibiae (similar to that of females) and a more griseous body colouration (more reddish in *H. bubu* sp.n.) (Figs 1–2). Differences in surface sculpture, frontoclypeal suture or pronotum shape do not seem to be diagnostic and are difficult to systematise.



FIGURES 5&6. Aedeagus of *Hydraena (Hydraenopsis) grebennikovi* sp.n. (holotype) 5) lateral view, 6) ventral view. Scale bar = 0.1 mm.

Sexual variation: Aedeagus (Figs 5–6) with median lobe asymmetrical, with a double sinuation in ventral view; apex with digitiform expansions and a short, globular membranous apical piece; with two clearly visible dorsal setae. Left paramere short and stout (but longer than in *H. bubi* sp.n., and with longer and more robust setae); right paramere longer, with apex recurved over the median lobe. Middle and hind tibia of males straight, similar to that of females. Females with spermatheca and last abdominal segments similar to those of *H. bubi* sp.n. (see above).

Etymology. Named after our friend and colleague Vasily V. Grebennikov, who collected the specimens as part of a systematic research on the forest litter beetle fauna of Central African mountain systems.

Distribution. So far only known from the type locality.

Habitat and ecology. All specimens were in the same sample as the specimens of *H. bubi* sp.n. (see above).

Discussion

The finding of two species of *Hydraena* with very similar external morphology in the same forest litter sample was unexpected. In aquatic habitats it is common to find multiple species in the same locality (e.g. Millán & Aguilera 2000), and even if some of them occupy different microhabitats (e.g. under stones in areas with stronger current, in coarse detritus, at the shore amongst gravel or sand, etc.) there may be more than one in each of them. There is, however, very little knowledge on the detailed ecology and feeding habits of most of the species of *Hydraena*, and in any case resources may be abundant enough to allow the coexistence of several species. The two species from Bioko may have different microhabitats within the litter, but they could also share what is likely to be an abundant resource. A similar situation may happen in tropical forest litter from other geographic areas, in which the presence of Hydraenidae, even if well known (e.g. Jäch & Balke 2008), may be more common than assumed. Whether these species are exclusive of forest litter or able to occupy a wider range of habitats, including streams of small water bodies within the forest, is generally not known.

Despite their very similar external morphology there are strong differences in the aedeagi of the two species, which share the same general structure (short and stout left paramere, right paramere recurved over the median lobe, apex of median lobe with digitiform expansions and a membranous distal piece) but have a very different general appearance. At present it is not known if the two species are the result of a single colonisation of Bioko, or if they have independently reached the island and when (meaning that they may not be sisters, one of them perhaps being closer to other species from the continent). There seems to be some resemblance between the general habitus of the two new species from Bioko and that of *H. pagaluensis*, all of them having explanate margins of the elytra, but differences in the shape of the aedeagus are substantial and do not suggest any close relationship between them. The collecting data of *H. pagaluensis* (in moss, in trickle on rock by stream, Hernando & Ribera 2001) also suggest an aquatic habit typical of other species of *Hydraena*, contrary to *H. bubi* sp.n. and *H. grebennikovi* sp.n., both found in forest litter in the dry season and far from any stream, suggesting a terrestrial habit.

Although the representation of African species was very poor, among the species included in the phylogeny of Trizzino *et al.* (2013) *H. bubi* sp.n. and *H. grebennikovi* sp.n. are most closely related to another undescribed forest litter species from Tanzania (voucher IBE-RA323), occupying a relatively isolated position within the subgenus *Hydraenopsis*. Unpublished molecular data show that this clade includes other central and eastern African species from Cameroon and Tanzania, but further studies are needed to establish the detailed phylogenetic relationships of the Central African species of *Hydraena*. Bioko was connected to mainland Africa during the Pleistocene, and in consequence its level of endemism is lower than that of the true oceanic islands of the Gulf of Guinea, Annobón, Sao Tomé and Príncipe (Jones 1994). This, together with the fact that both species have well developed membranous wings, does not allow us to discard the possibility that they may occur on nearby islands or the continent.

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