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Morphology, biology and phylogeny of African seed beetles belonging to the *Bruchidius ituriensis* species group (Coleoptera: Chrysomelidae: Bruchinae)

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Abstract. A small group of East African morphologically similar species is reviewed, four of which are assigned to a new species group. Three species are described as new: *Bruchidius arcuatus, B. pardellus,* and *B. snizeki*. The leguminous host plants of three species are identified: the larva of *Bruchidius ituriensis* develops in the seeds of *Indigofera tinctoria* L., *Ind. swaziensis* Bolus (tribe Indigofereae) and in an unidentified species of *Teramnus* (tribe Phaseoleae), while *B. pardellus* and *B. mulunguensis* were reared from *Neonotonia wightii* (Wight & Arn.) seeds (tribe Phaseoleae). The biology of other species remains unknown. Based on a comprehensive sample of 26 seed beetle species, the phylogenetic relationships of three of these species are investigated using molecular phylogenetics. The resulting phylogeny allows us to identify their likely sister-groups and to assess the usefulness of several taxonomically significant morphological characters in a phylogenetic context.

Key words: entomology, taxonomy, Afrotropical region, *Bruchidius*, Bruchinae, Coleoptera, host plant, *Indigofera*, *Neonotonia,* new species, phylogenetics, seed beetle.

INTRODUCTION

Several members of genus *Bruchidius* were recently collected in East Africa as larvae in the seeds of their host plants. Because of the high level of variability observed among adults thus obtained, a comprehensive study of related species was felt necessary. Four species described by Decelle were found to be closely related with reared

specimens: *B. djemensis* from Ethiopia, *B. ituriensis* from Western, Central and Eastern Africa, *B. lusingaensis* from Congo, and *B. mulunguensis* from Central and Eastern Africa. One reared series of specimens was found to belong to a new species, described here as *B. pardellus*. During the course of this study, two additional species belonging to the same group were identified and are described as *B. arcuatus* and *B. snizeki*.

We propose, together with the description of the three new species, a redescription of *B. ituriensis* and *B. mulunguensis*; male genitalia of the latter two are figured for the first time. In this study we also aim to provide a phylogenetic framework for the studied species, through the analysis of a six gene molecular dataset that encompasses representatives from 26 seed beetle species (including individuals from *B. ituriensis*, *B. mulunguensis*, *B. pardellus* and *B. snizeki*). The resulting phylogeny will also allow us to investigate several morphological characters in a phylogenetic context.

Abbreviations used

CBAD, first author's Collection, Fontenay-aux-Roses, France; CKWA, second author's Collection, Emmendingen, Germany; CBGP, Centre de Biologie pour la Gestion des Populations, Montpellier, France; MNHN, Muséum national d'Histoire naturelle, Paris, France; MRAC, Musée royal de l'Afrique Centrale, Tervuren, Belgique; NHRS, Naturhistoriska Riksmuseet, Stockholm; OÖLM, Oberösterreichisches Landesmuseum, Linz, Austria; ZFMK, Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany.

MATERIAL AND METHODS

Morphological studies

Measurement of body parts were obtained from digital photographs transferred to a graphics editing software; similarly, genitalia drawings were obtained from microphotographs transferred to a vector graphics editor. Numerous microscopic preparations (mostly of male genitalia) were made by J. Decelle (slides M.P.51 and Br.47 to Br.1100), K.-W. Anton (slides 21.05.95 I to 28.11.11 II), and A. Delobel (slides 06807 to 05011); additional dissections were performed, and the genitalia glued in a drop of DMHF (dimethyl hydantoin formaldehyde) on a cardboard pinned under the specimen, or fixed in water-soluble polyvinyl-pyrrolidon on specimen's card; these additional preparations are not mentioned in the text.

Molecular data

In total, eight specimens from the group of interest were processed for DNA extractions: three individuals of *B. ituriensis*, two of *B. mulunguensis*, two of *B. pardellus* and one of *B. snizeki*. To precise their placement, a comprehensive set of morphologically related seed beetle species was defined. To do so, we included representatives from species groups *Bruchidius afrasperae*, *B. centromaculatus*, *B. chloroticus, B. hinnulus* and *B. kiliwaensis*, which also possess an elongated median lobe, with a strongly chitinized ventral valve (e.g. see Anton & Delobel 2003; Delobel & Le Ru 2008). We also included specimens from species associated with the same host-plant

tribes, such as *Callosobruchus* spp., *Kingsolverius malaccanus* (on Phaseoleae) or *Conicobruchus* spp. (on Indigofereae). More distantly related outgroups were chosen based on the results of several molecular studies on seed beetles (Kergoat et al. 2005a, 2005b, 2011). The tree was rooted using a representative of the subfamily Criocerinae, *Crioceris duodecimpunctata*. In total, we used 41 individuals encompassing 28 species (see Fig. 31).

DNA was extracted following the non-invasive protocol of extraction of Gilbert et al. (2007). Four mitochondrial gene fragments were sequenced, namely 1,015 bp of the cytochrome oxidase I (COI), 782 bp of the cytochrome b (Cyt b), 415 bp of the ribosomal 12S RNA (12S), and 555 bp of the ribosomal 16S RNA (16S). Two nuclear gene regions were sequenced, namely 994 bp of the domain D2-D3 of the 28S ribosomal DNA (28SD2-D3), 715 bp of the domain D4-D5 of the 28S ribosomal DNA (28SD4-D5). All these genes were chosen because they are known to be informative in phylogenetic analyses of seed beetles (Kergoat et al. 2007a, 2007b, 2011). Polymerase chain reaction (PCR) amplifications were performed with standard settings for primer sequences and thermocycler procedures (see Kergoat et al. 2011 for additional information). Unfortunately, we did not manage to obtain suitable DNA material from the specimen of *B. snizeki*, which was collected in 1997. The PCR products were processed by the French sequencing centre Genoscope using a BigDye 3.1 sequencing kit and Applied 3730xl sequencers. The resulting sequences of complementary strands were further edited and reconciled using Geneious 5.1 (available at: www.geneious.com). For all protein-coding genes, we used Mesquite 2.75 (available at: www.mesquiteproject.org) to check the coding frame for possible errors or stop codons. The sequences of the four gene regions (12S, 16S, 28SD2-D3 and 28SD4-D5) contained some variations in length. Their alignment was accomplished using ClustalX 2.0 (Larkin et al. 2007) with default option settings. The combination of the six gene fragments resulted in a matrix of 40 individuals and 4,476 aligned characters.

Phylogenetic analyses

Phylogenetic analyses were carried out using Bayesian inference. We used partitioned analyses (Nylander et al. 2004) with a standard analytic scheme of one partition per gene fragment. For each partition, the best-fit model of sequence evolution was selected with jModelTest (Posada 2008) using the corrected Akaike information criterion. Bayesian analyses were carried out with MrBayes v3.2.1 (Ronquist et al. 2012). The following settings were used: two independent runs with four Markov Chains Monte Carlo (MCMC, one cold and three incrementally heated) that ran for $20x10^6$ generations, sampling the trees every 100^{th} cycle. A conservative burn-in of 25% was applied after checking for the PSRF (Potential Scale Reduction Factor) and the standard deviation of split-frequencies of the runs. All sampled trees prior to reaching these generations were discarded and remaining trees were used to generate a 50% majority rule consensus tree in which support of trees was estimated by posterior probabilities (PP).

RESULTS AND DISCUSSION

MORPHOLOGICAL STUDIES

Bruchidius arcuatus sp. nov.

Type material

Holotype: Male, "RWANDA, Rusumo / Ibanda Makera, X. / Th. Wagner leg. 93", "Genit ♂ / Br. 1100", dissected by J. Decelle. Paratypes, 2♂, 5♀, same data as holotype, one ♂ dissected (slide 04811). Paratype 1♂, CONGO, "Usumbura / 3.III.57 / Decelle" (slide 02.11.11 III). All type specimens deposited in MRAC.

Description

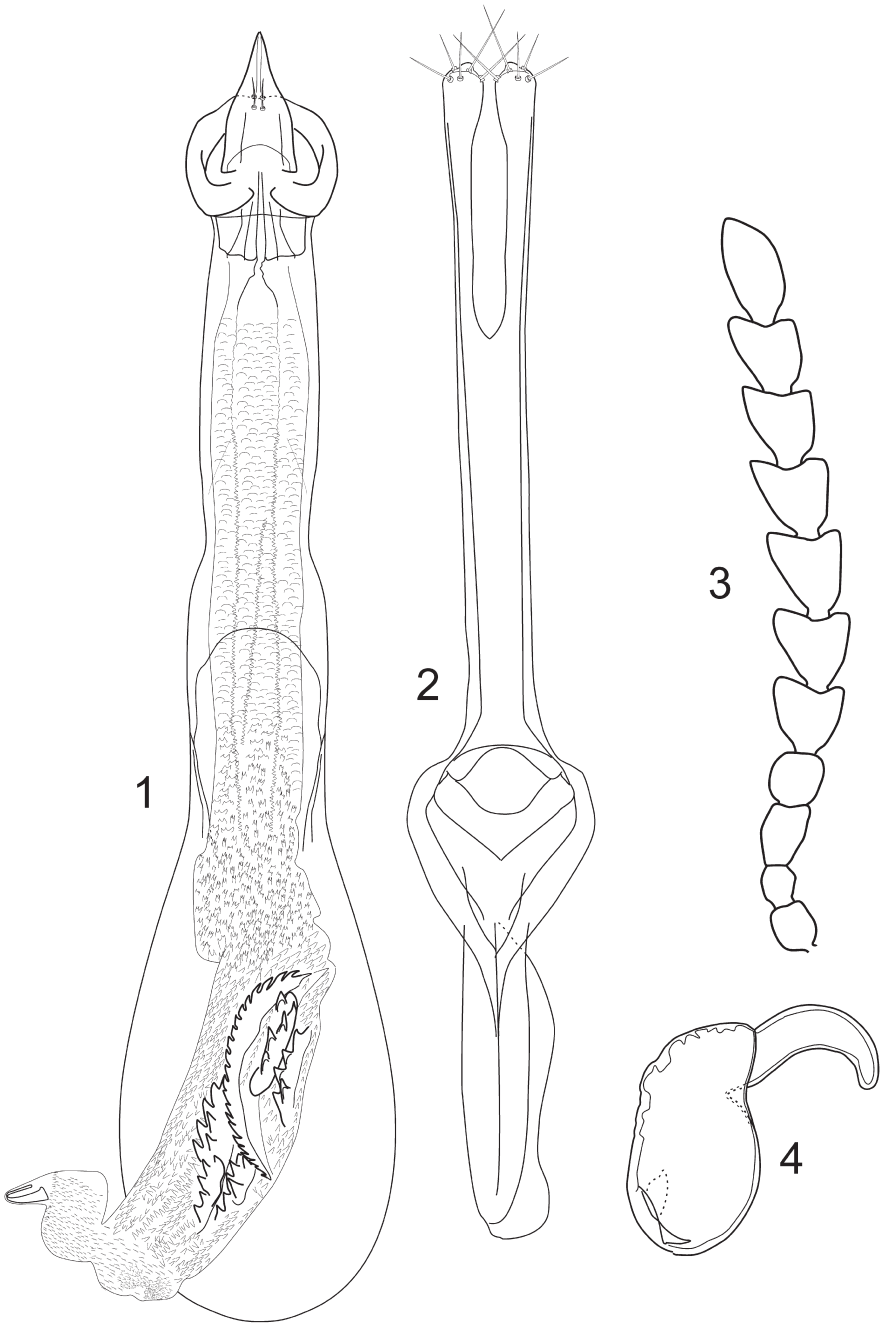
Length (pronotum-pygidium): 1.7-1.9 mm; width: 1.1-1.2 mm.

Body stout, rather thick, 1.7 times longer than deep, pygidium subvertical. Integument predominantly black, with four anterior legs testaceous, (except median femora blackened or brownish), posterior legs black with tarsi and small preapical area on tibia, reddish brown; last tarsal segments partly blackened; antennae testaceous, with four apical segments darkened (last one often less so). Vestiture dark, with well-defined white spots, and scattered light brown setae; on pronotum, two wide longitudinal black stripes on white background, a large patch of long white setae on basal lobes, a smaller one on middle of pronotal sides, scutellum and anterior part of suture white; odd intervals with small white squarish spots, the longest one being on middle of third interval; even intervals with mixed yellowish and whitish setation; last visible tergite with basal triangle of dense white scaly setae, prolonged in a thin longitudinal stripe.

Male. Head moderately elongated; eyes strongly bulging, maximum head width about 1.5 times width behind eyes; eyes separated by 0.27 times head width including eyes; distance between posterior rim of eyes and apex of clypeus / distance between eyes = 2.8; eye deeply cleft, width at bottom of sinus composed of 4-5 ommatidia; carina on frons well defined, interocular tubercule indistinct. Punctation of face strong, dense, confluent, becoming alutaceous on clypeus. Antenna long, measuring 0.7 times body length (Fig. 3); antennal segment 3 slightly bulging medially, 4 subtriangular, 5-10 widened apically, moderately eccentric, 11 oval (L/W = 2.1). Length ratio of antennomeres: 1.3; 1; 1.7; 1.4; 1.9; 2.0; 2.2; 2.0; 1.8; 1.8; 3.2.

Pronotum trapezoidal, with greatest width at base (W/L = 1.6), its sides almost straight, not expanded behind eyes; with shallow oblique impression on sides of basal lobe; disc shining, with dense strong, ocellate punctures, and smaller punctures between them. Elytra only 1.05 times longer than combined width, their sides convex, maximum width near middle; disc not flattened; a blunt swelling but no teeth at base of interstriae 3 and 4. Striae on disc deep and narrow, interstriae shining, with strong micropunctation.

Hind femora moderately incrassated, mesoventral margin with small acute preapical denticle; hind tibia stout, with dorsomesal and ventral carinae complete, lateral reaching base, ventrolateral reaching apex; apex of tibia with mucro slightly longer



1-4. *Bruchidius arcuatus*: 1 - median lobe; 2 - lateral lobes and tegminal strut; 3 - male antenna; 4 - spermatheca

than width of tarsomere 1 in middle; lateral and dorsal denticles much shorter. First tarsomere ventrally with blunt denticle.

Abdomen with ventrite 5 moderately emarginate, medially about as long as sternite 4; ventrite 1 basally with small patch of dense setae. Last visible abdominal tergite subtriangular, about as wide basally as long, almost flat, with apex not turned under.

Genitalia: Median lobe (Fig. 1) elongated, narrow (maximum width excluding basal hood / total length = 0.12), apically widened; basal hood narrowly ovate, not emarginate; ventral valve strongly concave, spear-shaped, moderately sclerotized, with apex acute, bearing two lateral groups of 2 setae; dorsal valve braced by a strongly sclerotized ring; no hinge sclerites; internal sac basally lined with weakly sclerotized tubercles, gradually transformed into multifid scales; saccus with a large crescentic dented sclerite and several variously shaped dented rods or plates; its wall lined with densely packed, weakly sclerotized spines. Basal strut with large and transparent keel; lateral lobes cleft to 40% their length, pubescent; apex of parameres with 6 long setae (Fig. 2).

Female. Similar to male, but first ventrite without patch of dense setae, ventrite 2 to 3 times longer than ventrite 4, so that last visible tergite is not vertical, but slanted. Antennae shorter than in male, less serrate. Spermatheca thick-walled, smooth, with subcylindrical body, diverticulum slightly curved.

Etymology

Specific name (adjective masculine) from Latin word for curved.

Host Plants

Biology unknown.

Discussion

Distinct from *ituriensis* by ornamentation of saccus, number and shape of dented sclerites; also color of posterior tibia, almost entirely black; parameres more deeply cleft than in *ituriensis*.

Distribution

Congo and Rwanda.

Bruchidius djemensis Decelle

Bruchidius djemensis Decelle, 1971: 251.

Type material examined

Paratype: Male, "Abyssinia, between Addis Allem and Djem-Djem, 7000-8000ft, 20.ix.1926, Dr. H. Scott" (slide 24.06.02 IV), MRAC

Other material: ETHIOPIA: 1♀, Bale Province, Sof Omar, 1200m, 25.xii.1971, R.O.S. Clarke, MRAC. 1♂, Ilubator Province, 60 km SSE Bedele, K. Werner (slide 08.02.01 V), CKWA.

The species was described in sufficient detail, and male median lobe drawn by Decelle. Additions to this description: mesoventral margin of posterior femur with inconspicuous preapical denticle; hind tibia stout, with dorsomesal and ventral carinae complete, lateral not reaching base, absent; mucro of hind tibia short, about as long as width of tarsomere 1 in middle, dorsal denticle as long as mucro. First tarsomere ventrally with blunt denticle. First ventrite without patch of dense setae. Median lobe moderately slender; ventral valve acutely triangular, with two groups of 4 setae; proximal part of internal sac densely lined with smooth then dented hyaline tubercles, becoming gradually smaller and transformed into spines; saccus densely lined with spines of different sizes, among which five much larger and more sclerotized teeth; distally four narrow-based teeth. Tegminal strut with wide keel, extending posteriorly well beyond apex of strut; lateral lobes cleft to 38% their length, pubescent; apex of parameres with about 10 setae; no sclerotized gonopore.

***Bruchidius ituriensis* Decelle**

Bruchus ituriensis Decelle, 1958: 80; *Bruchidius ituriensis*: Decelle, 1971: 251

Type material examined

Holotype: Male, CONGO, Haut-Uélé, Moto (later Kibali-Ituri), Abimva, iv.1925, L. Burgeon (slide Br.252). Allotype: 1♀, same data as holotype. Paratypes: 2♂, 2♀, same data as holotype, 1920, iv. 1925, ii.1927 (slide Br.47); 1♂, 6♀, same data as holotype but without "Moto", v., vi.-vii., vii., viii.1925; 1♀, Haut-Uélé, Madyu, xii.1918, L.Burgeon; 2♂, Haut-Uélé, Watsa, xi.1919, L.Burgeon (slide 28.11.11 I); 3♂, 1♀, Haut-Uélé, Yebo, i., ii., xi.1926, L.Burgeon (slide 28.11.11 II); 1♂, same data, but Yebo-Tora, ii.1926; 1♀, Kivu: Katana, xi.1932, L. Burgeon;1♀, Tanganika, Albertville (later Kalémié), xii.1918, R. Mayné; 4♂, 4♀, Urundi, Kihanga, 2, 12.iv.1957, sur Acacia, Decelle (slide Br.248, not seen); 3♂, 1♀, same data but without "sur Acacia", Ruzizi, 12.iv.1957 (according to original description exist only 3 type specimens); 1♀, Congo, Kisantu, 1931, R. P. Vanderyst. All type specimens deposited in MRAC.

Additional material

CAMEROON: 1♂, Adamaoua, 20km S Minim, 6°49N, 12°52E, 14.iii-6.iv.1979, Flacke, Müller, Nagel; 2♀, Adamaoua, 20km S Minim, 6°49N, 12°50E, 1200m, 24.ii-21.iii.1981, Flacke, Nagel; 1♂, Adamaoua, 30km NE Tignere, 7°34N, 12°50E, 1000m, 27.ii-17.iii.1982, Flacke, Klein, Konzmann; 3♂, same but 18.iii-14.iv.1982, ZFMK.

CONGO: 1♀, Haut-Uélé, Moto, N'Dare, i.1925, L.Burgeon, MRAC; 1♂, Kivu: Ibanda, 1952, M. Vandelannoite, MRAC; 3♂, 6♀, Kivu: Rutshuru, 1938, Hendrickx (slide Br.48); 1♂, Kivu, Rutshuru 1285m, 23-30.xi.1933, G.F. de Witte, *B. ituriensis* J. Decelle det. 1954 (slide 03.01.03 I), MRAC; 1♂, P.N.A., Kamande, 10.vi.1935, Mission H. Damas: 150, *B. ituriensis* J. Decelle det. 1954, MRAC.

ETHIOPIA: 1♂, Gorgora, 1933, Miss. del Tana di G. Dainelli, *B. ituriensis* det. J. Decelle 1989 (slide Br.1033); 1♂, Lago Bass Narok, ix.1896, Bottego, *B. ituriensis* det J. Decelle 1989 (slide Br.1032).

IVORY COAST: 2♂, 3♀, Lamto, 6°13N, 5°02W, 23.i-25.ii.1983, "ex sp. 144, n.sp. 961 = *ituriensis* Dec., cf lettre 5.2.85 et 5.2.93, *Teramnus* sp." (Decelle's hdw.), MRAC.

KENYA: 10♂, 12♀, Galana River Bridge / ex *Indigofera tinctoria*, S 03°01.657', E 38°28.727', 530m, 27.xii.2008, B. Le Ru, MNHN; 4♂, 2♀, Lake Bogoria, ex *Indigofera tinctoria*, N 00°22. 093', E 36°02.676', 1128m, 22.x.2007, B. Le Ru (slide 03208); 5♂, 2♀, Race Course Forest, ex *Ind. swaziensis swaziensis,* S 00°52.580', E 37°24.390', 1754m, 25 avril 2008, B. Le Ru (slide 12408), MNHN; 1 specimen, Tsavo East, Buchuma env., 28.xi.1997, M. Snizek; 3 specimens, Voi, 11-14.iv.1997, M. Snizek; 1 specimen, S.E. Kenya, Voi, 23.iii-4.iv.1997, Ma. Halada, OÖLM; 1♂, 1♀, Mombasa, Diani, 5-20.vii.1985, R. Mourglia (slide Br.967); 3♂, 1♀, Ukunda (Mombasa), vii.1979, W. Rossi (slide Br.841), MRAC; 1♀, Kitale, Uasin Gishu, 2100 m, Mission de l'Omo, C. Arambourg et al. 1932-33, *Bruchidius ituriensis* det. J. Decelle 1976, MRAC.

MAURITIUS: 1♂, Black River Canyon N.P., 21.xii.1997, Chamarel, 370m, Wiesner, CKWA; 3♂, 1♀, Grande Rivière Noire, 21.ii.2000, 40m, 20°22'19S, 57°24'08E, Wiesner (slide 03.01.03 IV), CKWA and CBAD.

RWANDA: 12♂, 16♀, Rusumo, Ibanda Makera, x.1993, Th. Wagner; 3♂, Kerengere, Cyamudongo, x.1993, Th. Wagner, MRAC.

TANZANIA: 1♀, Kijichame, S 04°59.022', E 39°48.189', *ex Indigofera tinctoria*, 7.v.2008, B. Le Ru, MNHN; 1♀, Ukewere, ex Coll. Oberthur, MNHN; 4♂, Tanzania bor., Mombo or., 9-11.i.1996, M. Snizek, (slide 14207) OÖLM; 1♂, Pinyini, S 02°17.967, E 35°56.540, ex *Indigofera tinctoria*, 01.vi.2008, B. Le Ru, MNHN; Ngare Sero 1200m, nr Usa river, 17.ii.-6.iii.1982, H.J. Bremer, *B. ituriensis* det. J. Decelle 1995 (slide 21.05.95 I), CKWA.

UGANDA: 1♂, 2♀, Kasese 600m, 13-19.ii.1994, M. Snizek (slide 12.05.96 III), CKWA.

Redescription

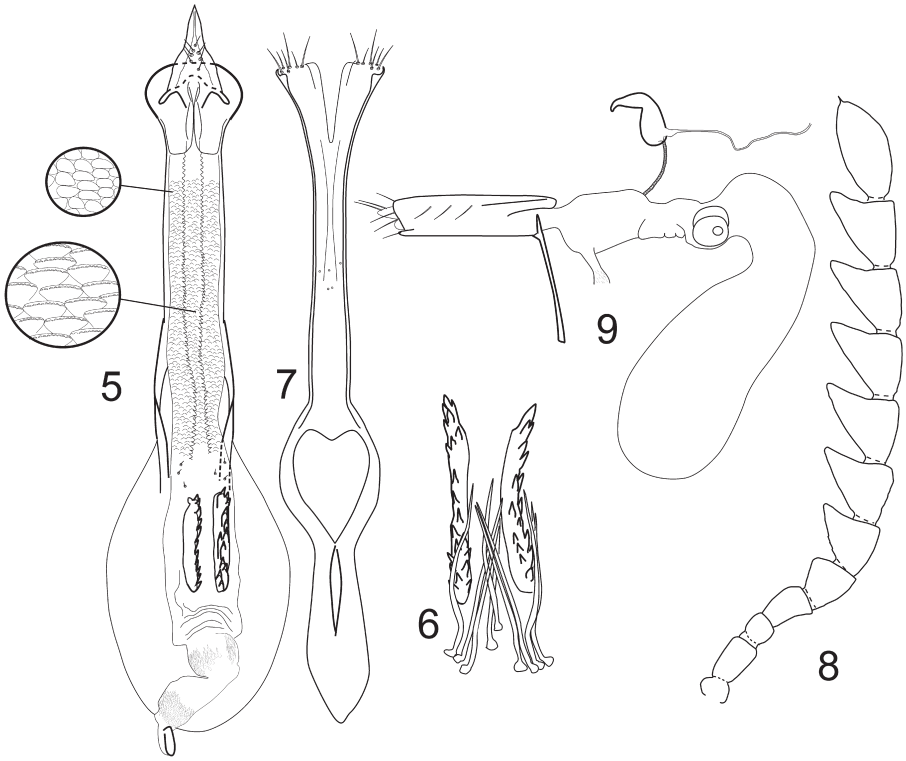
Length (pronotum-pygidium): 1.6-2.0 mm; width: 1.1-1.3 mm.

Body short, thick, pygidium vertical. Integument reddish brown, with head often entirely black, except mouthparts; antennae entirely testaceous or with segments 8-10 darkened to black; anterior and median legs testaceous, hind femora entirely reddish or with basal third to two-thirds blackened; base of pronotum and elytra more or less extensively darkened, sometimes elytral suture and external margins with black markings; thoracic sternites partly to entirely black; ventrites more or less extensively blackened medially, in some specimens underside entirely black; last visible tergite almost always entirely reddish, blackened medially in a few specimens. Vestiture made of white, yellowish and dark, thin setae, not covering integument; pronotum with two wide, longitudinal stripes of dark setae; elytra with odd intervals with alternating yellowish and white elongated spots; sometimes black spots on external intervals beyond middle of elytron; last visible tergite with dense white setation, denser on small basal triangle and along a thin longitudinal stripe; in males, base of first ventrite with a small patch of very dense white setae.

Male. Head short; eyes strongly bulging, maximum head width 1.7 times width behind eyes; eyes separated by 0.12 times head width including eyes; face long and very narrow, with distance between posterior rim of eyes and apex of clypeus / distance between eyes = 6.2; eye deeply cleft, width at bottom of sinus composed of 4-5 ommatidia; carina on frons well defined, sharp, shining, interocular tubercule distinct. Punctation of face shallow, dense, clypeus strongly alutaceous. Antenna measuring 0.80 times body length (Fig. 8); 8th antennomere wider than long (L/W = 0.92); antennal segments 1 to 4 submoniliform, 4 slightly widened apically on mesal side, 5-10 strongly serrate, 11 oval (L/W = 2.1). Length ratio of antennomeres: 2.5; 1.0; 1.7; 1.8; 2.0; 2.0; 2.2; 2.3; 2.2; 2.4; 3.9.

Pronotum subtrapezoidal, with greatest width at base (W/L = 1.4), its sides almost straight, slightly bulging medially, not expanded behind eyes; without oblique impression on sides of basal lobe; disc with very dense and regular punctation.

Elytra 1.15 times longer than combined width, their sides convex, maximum width before middle; disc slightly flattened; two strong teeth at base of striae 3 and 4. Striae with strong and wide punctures; interstriae shining, with strong microsculpture.



5-9. *Bruchidius ituriensis*: 5 - median lobe (specimen without hair-like sclerites); 6 - saccus of specimen with hair-like sclerites; 7 - lateral lobes and tegminal strut; 8 – male antenna; 9 – female genital tract, side view

Hind femur moderately incrassate; mesoventral margin with small preapical denticle before moderately deep notch; hind tibia apically strongly widened, its dorsal side with small tubercles, dorsomesal and ventral carinae complete, lateral not reaching base; apex of tibia with mucro about as long as width of tarsomere 1 at middle; lateral and dorsal denticles about one fourth mucro length. First tarsomere ventrally without basal denticle.

Abdomen with ventrite 5 emarginate; ventrite 1 basally with small patch of long setae. Last visible abdominal tergite shield-shaped (W/L = 1.0), almost flat, with apex not turned under.

Genitalia: Median lobe (Fig. 5) slender (maximum width excluding basal hood / total length = 0.11), widened before apex; ventral valve acutely triangular, strongly sclerotized, bearing two lateral groups of 3-4 setae; dorsal valve braced by a wide sclerotized ring; no hinge sclerites; internal sac basally lined with weakly sclerotized tubercles, gradually transformed into shortly dented tubercles; saccus with two moderately elongated rods, each rod with 6 to 15 teeth; in some specimens, one to eight strong hair-like sclerites with rounded base (Fig. 6). Basal strut with a small keel (Fig. 7); lateral lobes cleft to 22% their length, pubescent; apex of parameres with about 8 short setae and a small rounded bulge.

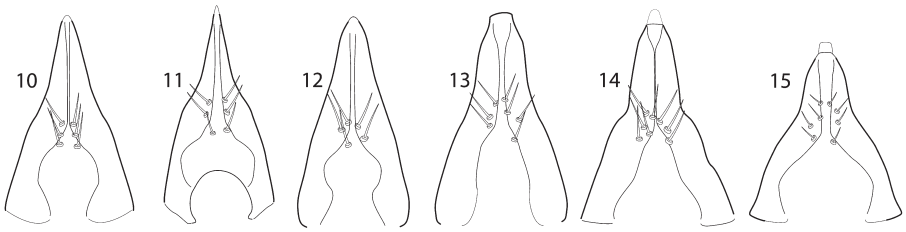
Female. Similar to male, but antennae shorter, not serrate, segments 5-10 less eccentric, almost as long as wide; first ventrite without patch of long white setae; last ventrite not emarginate, pygidium not different from male. Female genitalia without sclerite at opening of bursa copulatrix (Fig. 9); spermathecal body subcylindrical, diverticulum wide, almost at a right angle with body.

Host plants

Kenyan and Tanzanian material was reared from *Indigofera tinctoria* and *I. swaziensis swaziensis* pods; specimens from Ivory Coast were reared from an unidentified species of *Teramnus*.

Distribution

Cameroon, Congo, Ethiopia, Ivory Coast, Kenya, Mauritius, Rwanda, Tanzania, and Uganda.



10-15. Ventral valves of *B. ituriensis* males of different geographic origins: 10 - Holotype (slide Br.252); 11 - Rwanda (slide 04611); 12 - Rwanda (slide Br.1098); 13 - Ivory Coast (slide Br.961); 14 - Ethiopia (slide Br.1032); 15 - Mauritius (slide 03.01.03 IV)

Discussion

Bruchidius ituriensis as presently defined is a highly variable species. Samples of various geographical origins show differences in external and male genital morphology. In addition to usual trait variability found in seed beetles concerning integument and vestiture coloration, antenna length or pronotum shape, main identified variations concern face width, number of hair-like sclerites and dented rods in the saccus, shape of the ventral valve, level of sclerotization of ventral valve tip. Integument color varies from almost uniformly testaceous to almost entirely black; four anterior legs and antennae however remain testaceous, except sometimes segments 8-10 blackened. Specimens reared from pods of *I. swaziensis* (Kenya, Race Course Forest) differ from those from *I. tinctoria* in body size slightly larger, elytra with white spots stronger than usually seen in other specimens; male genitalia are however identical with these of other Kenyan populations. The number of hair-like sclerites in internal sac ranges from zero (all Kenyan specimens reared from *Indigofera*, specimens from Ivory Coast) to eight. The number of dented rods is usually two, but in several dissected specimens two to four additional sclerites were found (specimens from Cameroon, some specimens from Rwanda, one specimen from Ethiopia, some specimens from Congo, including seven paratypes). Lateral lobes cleft to 45% their length.

Ventral valve shape varies from narrowly triangular with more or less acute tip (Fig. 10, 11) to wide, with tip blunt or rounded (Fig. 12, 13). Mauritius specimens show a particularly wide valve (Fig. 15), with rounded tip, prolonged by a small transparent strip; this feature exists also in a specimen from southern Ethiopia (Fig. 14) and in some Kenyan specimens. It is very narrow, with a long and acute tip in specimens from Cameroon. The number of setae on the ventral valve ranges from three to eight.

We have chosen to consider as true species three series of specimens previously identified as *B. ituriensis*: *B. pardellus*, *B. snizeki* and *B. arcuatus* exhibit constant morphological traits that appear sufficient to differentiate them from all other *B. ituriensis* specimens.

***Bruchidius lusingaensis* Decelle, comb. nov.**

Bruchus lusingaensis Decelle, 1960: 140.

Type material examined

Holotype: Male, "Congo Belge, P.N.U. / Mukana Lusinga (1810m) / 16.iv.1947 (mousses) / Miss. G.F. de Witte. 265a", "J. Decelle det, 1954: *Bruchus lusingaensis* Decelle". Paratypes, 2♂, 4♀, same data as holotype, but different dates, 1♂ dissected (slide 04311). All type specimens deposited in MRAC.

Redescription

Length (pronotum-pygidium): 1.9-2.1 mm; width: 1.1-1.2 mm.

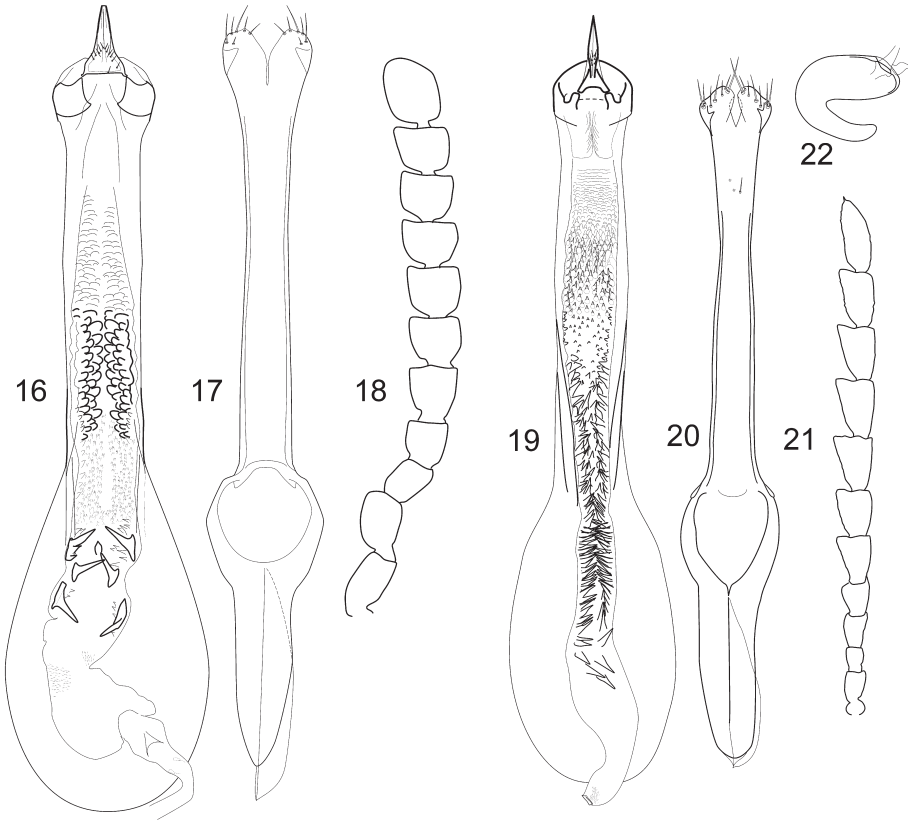
Body stout, rather thick, pygidium subvertical. Integument mainly black, antennae, legs testaceous (except femoral base black). Vestiture greyish-yellow, with a large

lighter area towards middle of outer side of elytron, and tawny shades on humerus and apical fourth of elytron.

Male. Head moderately elongated; eyes strongly bulging, maximum head width about 1.6 times width behind eyes; eyes separated by 0.24 times head width including eyes; face wide, with distance between posterior rim of eyes and apex of clypeus / distance between eyes = 2.9; eye deeply cleft, width at bottom of sinus composed of 3-4 ommatidia; carina on frons well defined, shining, interocular tubercule indistinct.

Antenna moderately long (Fig. 18), measuring about 60% body length; antennal segment 2 greatly enlarged, 5 as wide as long, 6-10 slightly wider than long, cup-shaped, 11 oval (L/W = 1.4). Length ratio of antennomeres: 1.1; 1.0; 0.7; 0.8; 0.8; 0.8; 0.9; 0.7; 0.8; 0.8; 1.2.

Pronotum campaniform, with greatest width at base (W/L = 1.6), its sides bulging medially, not expanded behind eyes. Elytra 1.15 times longer than combined width, their sides convex, maximum width near middle; disc flattened; no teeth at base of interstriae 3 and 4.



16-22. Genitalia and antennae: 16-18 – *Bruchidius lusingaensis*; 19-22 – *B. mulunguensis*; 16, 19 – median lobe; 17, 20 - lateral lobes and tegminal strut; 18, 21 – male antenna; 22 - spermatheca

Hind femora moderately incrassated, mesoventral margin with small preapical denticle; hind tibia apically strongly widened, with mucro about half as long as width of tarsomere 1 at base.

Abdomen with ventrite 5 strongly emarginate; ventrite 1 without particular arrangement of setae. Last visible abdominal tergite shield-shaped, about as wide at base as long, with apex not turned under.

Genitalia: Median lobe (Fig. 16) of moderate length (maximum width excluding basal hood / total length = 0.13), subcylindrical; basal hood ovate, not emarginate; ventral valve acutely triangular, with apex narrowly rounded, bearing two lateral groups of 5-7 setae; dorsal valve braced by a wide sclerotized ring; no hinge sclerites; internal sac basally with dense hyaline tubercles of increasing size, followed by a zone with large and strongly sclerotized tubercles, then multifid scales and spines; distally two lateral groups of three strong teeth with large base. Basal strut with large transparent keel (Fig. 17); lateral lobes cleft to 13% their length, pubescent; apex of parameres with 6 or 7 short setae.

Female. Similar to male, but ventrite 5 not emarginate, antennae shorter, with segment 2 not as enlarged as in male holotype; antennae more or less darkened beyond segment 5 (black in some specimens).

Host plants

Biology unknown.

Discussion

One male paratype has antennal segment 2 less enlarged than in holotype, and segments 5-10 not transverse, but distinctly longer than wide.

Distribution

Congo.

***Bruchidius mulunguensis* (Decelle)**

Bruchus (s.l.) *mulunguensis* Decelle, 1951: 183.
Bruchidius latescapularis Pic, 1952: Decelle, 1971: 251.
Bruchidius mulunguensis Decelle, 1971: 251.

Type material examined

Holotype: Male, "Coll. Mus. Congo / Kivu, Mulungu / Hendrickx, 1938", "R. Det. B.5710", "*Bruchus mulunguensis* n. sp. / Decelle det. 1951", "Genit ♂ / Br 669" "Holotypus / *mulunguensis*". Paratype: 1♂, Elisabethville, octobre 1949, Ch. Seydel, R Det. C.5710. All type specimens deposited in MRAC.

Additional Material

CONGO: 1♂, Elisabethville, à la lumière, 1957-1958, Ch. Seydel (slide 02.11.11 I), CKWA.

KENYA: 19♂, 16♀, Timau, ex *Neonotonia wightii,* N 00°03.963', E 37°14.903', 2286m, 25.xii.2006 (B. Le Ru); 13♂, 12♀, Naro Moru River Lodge, ex *Neonotonia wightii*, S 00°09.278', E 37°00.706', 1956m, 1.xi.2007 (B. Le Ru), CBAD, MNHN, CBGP; 1♂, Tsavo, Hundayi, 18-22.iii.1997, M. Snizek, OÖLM.

RWANDA: 2♂, 3♀, Karengera, Cyamudongo, x.1993, Th. Wagner (slide 02.11.11 II), MRAC; 1♀, Mahembe, terr. Nyanza, 1400 m, 13.-15.i.1953, P.Basilewsky, CKWA.

Redescription

Length (pronotum-pygidium): 1.7-2.0 mm; width: 1.1-1.3 mm.

Body stout, rather thick, pygidium vertical. Color of integument highly variable, from almost entirely pale reddish brown to almost entirely black; in light colored specimens, central part of thoracic sternites and basal abdominal sternites, humerus and a small area about mid-length of elytral side are almost always black; in darker specimens, only the following areas remain testaceous: whole antenna, legs (except femoral base), and last visible tergite; intermediate specimens show a particular elytral pattern, with basal half testaceous and apical half (except some testaceous squarish dots), black. Vestiture very variable, in some specimens almost uniformly pale yellowish, with two elongated spots of denser pale setae in third interstria; setation variegated in other specimens; pronotum often with a pair of longitudinal dark stripes; base of first ventrite with a very small patch of long whitish setae.

Male. Head elongated; eyes strongly bulging, maximum head width 1.5 times width behind eyes; eyes separated by 0.12 times head width including eyes; face long and very narrow, with distance between posterior rim of eyes and apex of clypeus / distance between eyes = 6.3; width at bottom of sinus composed of 7 ommatidia; carina on frons well defined, high, shining, interocular tubercule distinct. Punctation of face small, dense, irregular, clypeus alutaceous. Antenna measuring 0.93 times body length (Fig. 21); antennal segment 2 subspherical, 4 to 10 slightly widened apically but longer than wide, eccentric, eight 1.3 times longer than wide, 11 oval (L/W = 2.5). Length ratio of antennomeres: 2.0; 1.0; 2.3; 2.2; 2.9; 2.8; 3.3; 3.4; 3.5; 3.3; 4.8.

Pronotum narrowly conical, with greatest width at base (W/L = 1.3), its sides almost straight, not expanded behind eyes, with faint oblique impression on sides of basal lobe. Disc of pronotum with small, dense ocellate punctures. Elytra 1.2 times longer than combined width, their sides convex, maximum width near middle; disc flattened; a tubercle with two small teeth at base of interstriae 3 and 4. Striae on disc deep and narrow, with small punctures; interstriae shining, with strong micropunctation.

Hind femora moderately incrassated; mesoventral margin with well-defined preapical denticle; hind tibiae apically strongly widened, with dorsomesal, lateral and ventral carinae complete; apex of tibia with mucro wide, strongly produced outwardly; lateral and dorsal denticles minute.

Abdomen with ventrite 5 slightly emarginate, its length medially about two thirds of sternite 4; first ventrite without particular arrangement of setae. Last visible abdominal tergite subtriangular, 1.05 times longer than wide at base, with apex not turned under.

Genitalia: Median lobe (Fig. 19) elongate (maximum width excluding basal hood / total length = 0.10), apically widened; basal hood elongate oval; ventral valve acutely triangular, almost needle-like, bearing two lateral groups of three setae; no hinge sclerites; internal sac basally with hyaline tubercles progressively transformed into spines of increasing size, followed by two strands of slightly larger and more sclerotized spines of decreasing size, and oriented almost transversally. Basal strut with large and transparent keel; lateral lobes cleft to 10% their length; apex of parameres broad, with seven setae (Fig. 20).

Female. Similar to male, but antennae shorter, (8th antennomere slightly wider than long); 8 to 10 squarish, last ventrite not emarginate; last visible tergite 1.1 times longer than wide. Spermathecal body as in Fig. 22; entrance of bursa copulatrix without dorsal sclerite.

HOST PLANTS

The Kenyan material was reared from larvae developing in *Neonotonia wightii* (Leguminoseae, Fabaceae, Phaseoleae) seeds; the species is widespread in Africa South of the Sahara, and also present in the Arabic Peninsula and in India.

DISCUSSION

This extremely variable species is characterized by antennal shape and color; male genitalia differ from these of other species treated here in the lack of large sclerites in the internal sac; spermathecal body shape is also characteristic.

DISTRIBUTION

Congo, Ethiopia, Kenya, Rwanda, and Tanzania.

***Bruchidius pardellus* sp. nov.**

TYPE MATERIAL

Holotype: Male, KENYA, "Nairobi, ICIPE, ex *Neonotonia wightii* / S 01°13.230, E 36°53.636' / 1619m, 04.vi.2008, B. Le Ru", MNHN. Paratypes: KENYA, 14♂, 15♀, same data as holotype (slides 06109 and 05011); 2♂, 2♀, same data as holotype, but 12.xi.2007; 1 specimen, same data as holotype, but 11.i.2008; 5♂, 8♀, Suam, ex *Neonotonia wightii*, N 01°11.182', E 34°49.538' / 1951m, 12.xi.2008 (slide 06409), B. Le Ru; 2♀, Tambach, Kerio Valley, ex *Neonotonia wightii* /N 00°32.440', E 35°31.518' / 1611m, 17.v.2007, B. Le Ru (slide 06807), MNHN, CBDA, CBGP; 1♀, Mt Elgon, Kaptega (1980m), 3.ii.1979, T.E. Leiler, NHRS; 3♂, 2♀, Tsavo, Hundayi, 18-22.iii.1997, M. Snizek (slide 07409); 1♂, Taita Hills, Wundanyi, 5-10.iv.1997, Ma. Halada; 1♀, Voi, 22.xi-2.xii.1996, Mi. Halada, OÖLM.

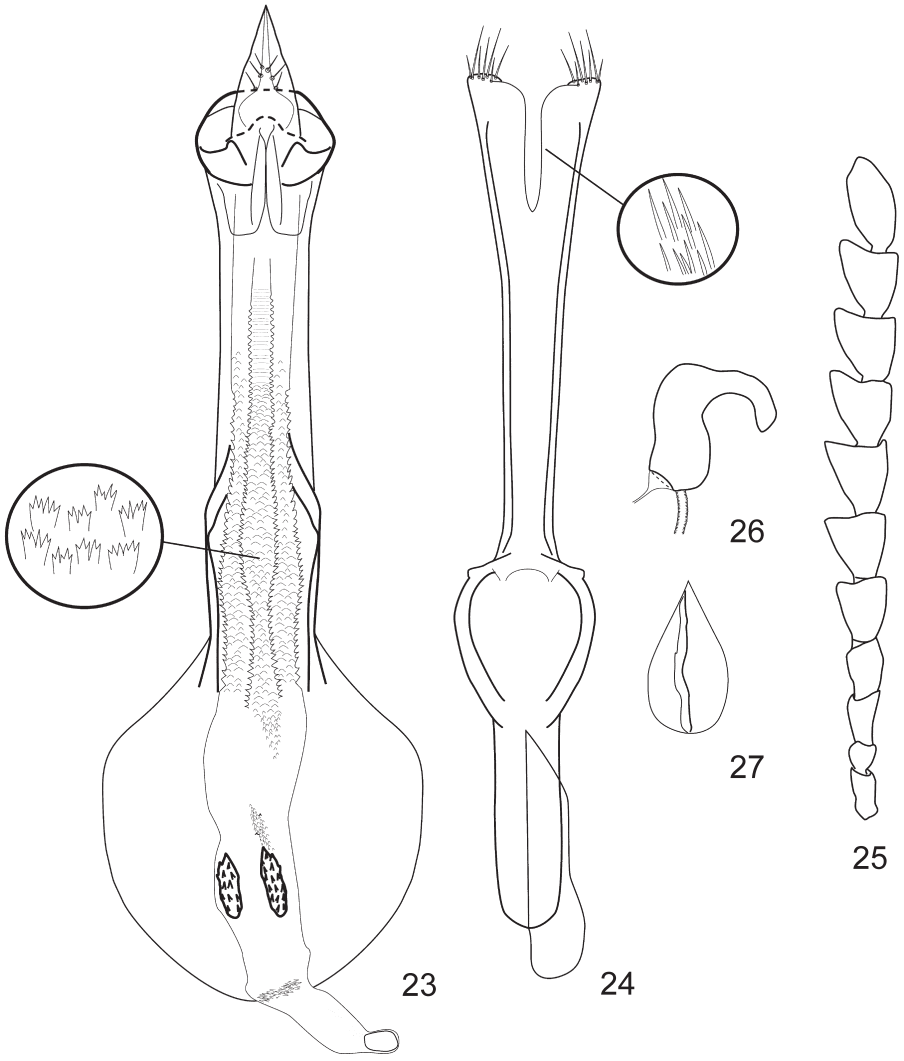
ADDITIONAL MATERIAL

ETHIOPIA, 1♂, Abyssinie, Schimper, 1850 (slide M.P.51), MNHN. YEMEN, 1♂, 18.iv.2001, nr Hammam Ali, A. van Harten (slide 06.02.02 I), CKWA.

Description

Length (pronotum-pygidium): 1.7-2.3 mm; width: 1.0-1.5 mm.

Body stout, rather thick, pygidium vertical. Integument reddish brown, with head black (apex of clypeus lighter), antennal base testaceous, segments 8-9 to 6-10 more or less blackened, anterior and median legs testaceous, hind legs reddish, with basal third to two-thirds of femora black; pronotum more or less extensively blackened; elytra with four transversal black areas: base, anterior third, beyond middle, before apex; thoracic sternites black, ventrites more or less extensively blackened medially,



23-27. *Bruchidius pardellus*: 23 - median lobe; 24 - lateral lobes and tegminal strut; 25 - male antenna; 26 - spermatheca; 27 - dorsal sclerite at entrance of bursa copulatrix

last visible tergite reddish, sometimes blackened on disc. Vestiture made of white, yellowish and dark setae, not hiding integument; pronotum with V- or O-shaped dark markings on disc, rest with whitish setae; elytral intervals 3, 5, 7, and 9 with alternating white and black spots; last visible tergite with dense white setation, denser on basal triangle and along a thin longitudinal stripe; in males, base of first ventrite with a patch of long white setae.

Male. Head short; eyes strongly bulging, maximum head width almost twice width behind eyes; eyes separated by 0.1 times head width including eyes; face long and very narrow, with distance between posterior rim of eyes and apex of clypeus / distance between eyes = 7.1; eye deeply cleft, width at bottom of sinus composed of 7 ommatidia; carina on frons well defined, shining, interocular tubercle distinct. Punctation of face dense, small, confluent. Antenna long, measuring 0.8 times body length (Fig. 25); antennal segments 1 to 4 submoniliform, 4 only slightly widened apically, 5-10 strongly eccentric, 8-10 slightly wider than long (L/W = 0.95), 11 oval (L/W = 2.16). Length ratio of antennomeres: 1.6; 1; 2.0; 2.0; 2.3; 2.3; 2.3; 2.2; 2.0; 2.1; 3.5.

Pronotum subtrapezoidal, with greatest width at base (W/L = 1.4), its sides almost straight, not expanded behind eyes; with shallow oblique impression on sides of basal lobe. Disc with dense, more or less coalescent, ocellate punctation. Elytra 1.13 times longer than combined width, their sides convex, maximum width before middle; disc flattened; two strong teeth at base of interstriae 3 and 4. Striae on disc deep, with strong punctures; interstriae shining, with strong microsculpture.

Hind femur moderately incrassate; mesoventral margin with small preapical denticle before moderately deep notch; hind tibia apically strongly widened, its dorsal side with small tubercles, dorsomesal and ventral carinae complete, lateral not reaching base; apex of tibia with mucro about as long as width of first tarsomere at middle; lateral and dorsal denticles about one fourth mucro length. First tarsomere ventrally without denticle.

Abdomen with ventrite 5 emarginate; ventrite 1 basally with small patch of long setae. Last visible abdominal tergite shield-shaped (W/L = 1.17), almost flat, with apex not turned under.

Genitalia: Median lobe (Fig. 23) moderately slender (maximum width excluding basal hood / total length = 0.14), widened before apex; ventral valve acutely triangular, strongly sclerotized, bearing two lateral groups of 3-4 setae; dorsal valve braced by a wide sclerotized ring; no hinge sclerites; internal sac basally with transverse wrinkles, followed by hyaline tubercles and strongly dented scales; saccus with two elongate-oval dented sclerites (Fig. 24). Basal strut with a large keel; lateral lobes cleft to 28% their length, pubescent; apex of parameres with 7 short setae.

Female. Similar to male, but antennae shorter, less serrate (8th antennomere 1.16 times wider than long); antennal segments less markedly eccentric, 8 to 10 squarish; first ventrite without patch of long setae, last ventrite not emarginate; last visible tergite 1.10 times wider than long. Spermathecal body as in Fig. 26; entrance of bursa copulatrix with a flat, pear-shaped sclerite in dorsal wall (Fig. 27).

ETYMOLOGY

Masculine Latin noun in apposition, a forged diminutive of *pardus*, "leopard". It refers to the color of elytral vestiture.

HOST PLANTS

Type material was reared from *Neonotonia wightii* pods.

DISCUSSION

Differs from typical *ituriensis* only in a few morphological details (body usually darker, elytra more strikingly checkered, 4th antennomere more slender, last visible tergite wider); male genitalia are also quite similar, but aedeagus less slender, ornamentation of internal sac different, dented sclerites smaller and shorter; spermathecal body of a different shape, and presence of a sclerite in dorsal wall of bursa copulatrix.

DISTRIBUTION

Ethiopia, Kenya and Yemen.

***Bruchidius snizeki* sp. nov.**

TYPE MATERIAL

Holotype: Male, KENYA, "SE Kenya / Voi (Tsavo) / 23.3.-4.4.1997 / leg. M. Snizek", dissected, genitalia on slide 13907, ÖOLM. Paratypes: 2♂, same data as holotype (slides 13507, 07509); 3♂, Voi, 22.xi.-2.xii.1996, Mi. Halada (slides 06509, 03911); 6♀, Voi, 11.14.iv.1997, leg. Ma. Halada (one dissected, slide 07909); 1♀, Taita Hills, Wundanyi, 5-10.iv.1997, Ma. Halada; 1♀, Voi, 22.xi.-2.xii.1996, M. Snizek, all in ÖOLM, except one pair in MNHN; 1♂, Taita, Mwatate, 9.xi.1999, Snizek (slide 04.11.01 I), CKWA. YEMEN: 5♂, 9♀, Al Lahima, 1.i.-9.iv.2001, A. van Harten (slide 07.02.02 I), CKWA and CBAD; 2♂, 1♀, same data but 9.iv.-5.vi.2001 (slide 05.02.02 II), CKWA; 1♀, same data but 5.vi.-24.vii.2001, CKWA; 2♂, 1♀, same data but 17.ix.-14.xi.2001 (slide 24.07.02 I), CKWA; 3♂, 3♀, same data but 14.xi.2001–5.iii.2002, CKWA and CBAD; 1♂, 3♀, Al Lahima, 16.x.-31.xii.2000, A. van Harten & A.M. Hager, CKWA.

DESCRIPTION

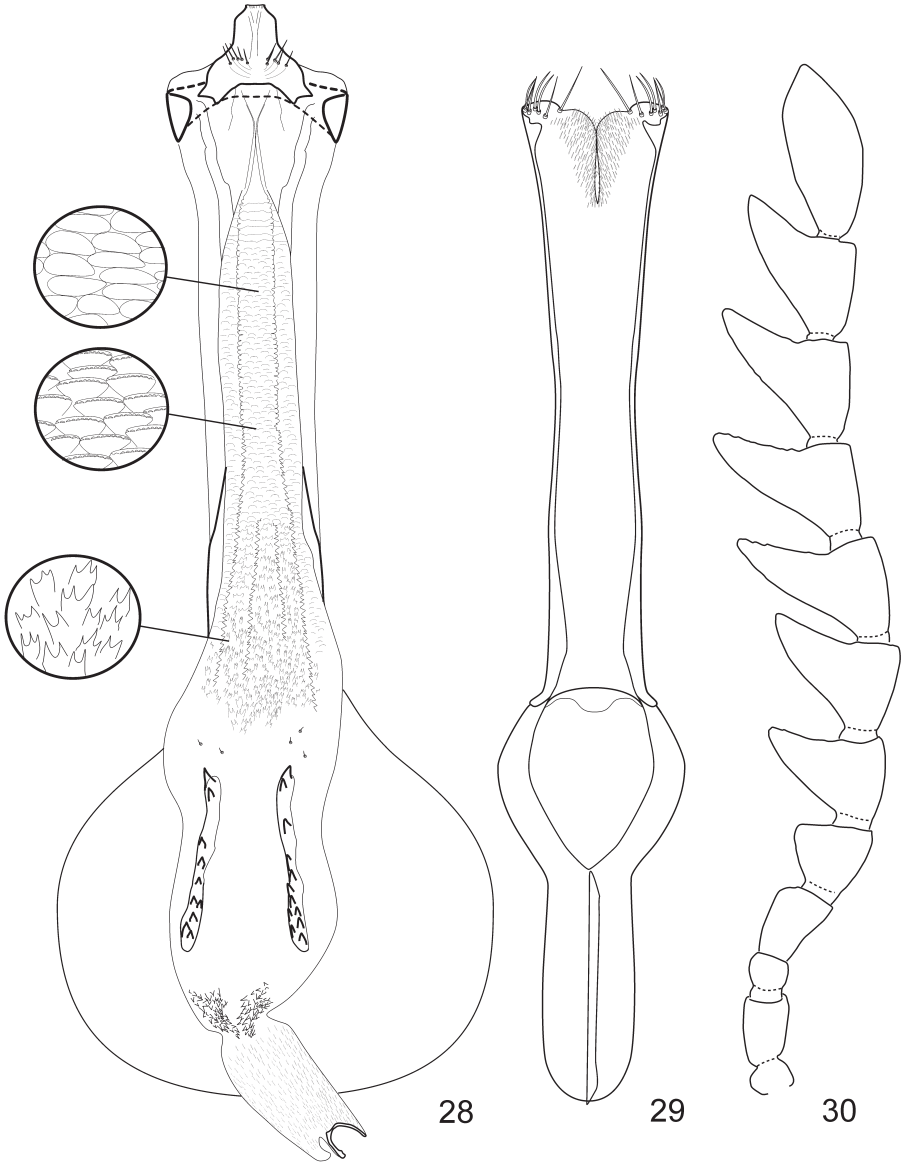
Length (pronotum-pygidium): 1.8-2.0 mm; width: 1.1-1.3 mm.

Body stout, rather thick, pygidium vertical. Integument mainly black, antennae and four anterior legs testaceous; posterior legs (except basal half of femora) and last visible tergite reddish; elytra with more or less extended reddish-brown elongated spots on disc and at apex. Some specimens of Yemen have antennomeres 8-10 darkened to blackish, in both sexes.

Vestiture made of whitish, yellowish, and brown to black scaly setae; diffuse brownish spots on pronotum, elytra with checkered black and yellowish pattern, showing three irregular transversal whitish stripes: at basal fourth, before middle and

at posterior 2/3; spots of whitish denser setae on striae 3, 5, 7, and 9; odd striae with thinner yellowish setation; last visible tergite with whitish setation, denser on small basal triangle and along a thin longitudinal stripe; in males, base of first ventrite with a patch of denser white setae.

Male. Head elongated; eyes strongly bulging, maximum head width about 1.5 times width behind eyes; eyes separated by 0.2 times head width including eyes; face



28-30. *Bruchidius snizeki*: 28 - median lobe; 29 - lateral lobes and tegminal strut; 30 - male antenna

long and very narrow, with distance between posterior rim of eyes and apex of clypeus / distance between eyes = 3.9; eye deeply cleft, width at bottom of sinus composed of 6 ommatidia; carina on frons well defined, shining, interocular tubercule not defined. Punctation of face small, dense, irregular, clypeus alutaceous. Antenna long, measuring 0.8 times body length (Fig. 30); 8th antennomere wider than long (L/W = 0.71); antennal segments 2 half as long as adjacent segments, 3 slightly widened apically, 4-10 strongly eccentric, much wider than long, 11 acutely oval (L/W = 2.3). Length ratio of antennomeres: 2.1; 1.0; 2.1; 2.0; 2.3; 2.7; 2.8; 2.8; 3.0; 3.0; 5.1.

Pronotum narrowly conical, with greatest width at base (W/L = 1.46), its sides straight slightly bulging before middle, not expanded behind eyes; disc rather strongly humped, with oblique impression on sides of basal lobe. Disc with punctures shallow, regular, coalescent, ocellate.

Elytra short, only 1.05 times longer than combined width, their sides convex, maximum width at middle; disc convex, depressed around scutellum; a well-defined tubercle at base of stria 4. Striae on disc deep and wide, diameter of punctures much larger than width of striae; interstriae with strong microsculpture.

Hind femora moderately incrassated; mesoventral margin with small preapical denticle; hind tibiae apically strongly widened, with dorsomesal and ventral carinae complete, lateral not reaching base; apex of tibia with mucro about as long as width of first tarsomere at base; lateral denticle about one third mucro length, and dorsal denticles minute.

Abdomen with ventrite 5 emarginate; first ventrite basally with patch of denser setation. Last visible abdominal tergite subtriangular, 1.1 times longer than wide at base, regularly convex.

Genitalia: Median lobe (Fig. 28) moderately slender (maximum width excluding basal hood / total length = 0.17), widened before apex; ventral valve subtriangular, strongly sclerotized, with apex truncated, bearing two lateral groups of 4-6 setae; dorsal valve braced by a wide sclerotized ring; no hinge sclerites; internal sac basally lined with weakly sclerotized tubercles, gradually transformed into shortly dented tubercles and ctenoid scales; saccus with two long dented rods, each rod with about 10 teeth, and 0 to 4 strong hair-like sclerites. Basal strut with minute keel; lateral lobes cleft to 17% their length, pubescent; apex of parameres broad, with about 6-7 short setae (Fig. 29).

Female. Similar to male, but antennae shorter, segments less eccentric, segments 8-10 darkened; first ventrite without patch of denser setation; last visible tergite similar to male.

ETYMOLOGY

Masculine Latin noun, genitive case of the holotype collector's name, M. Snizek, to whom this species is dedicated.

HOST PLANTS

Biology unknown.

DISCUSSION

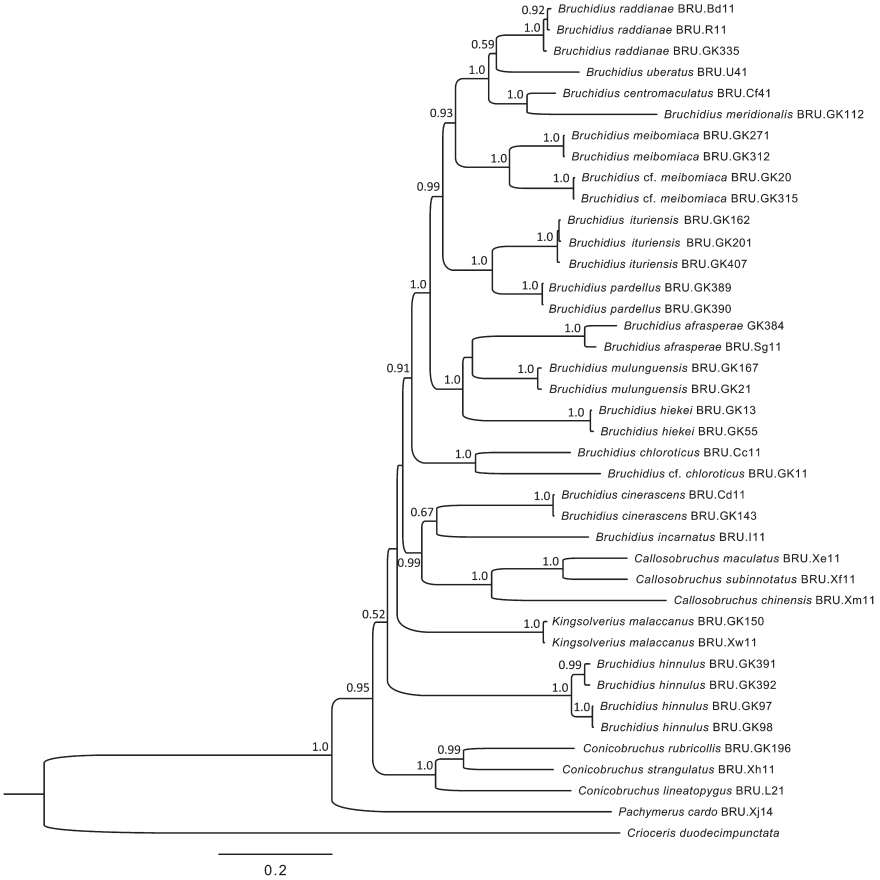
Bruchidius snizeki could easily be mistaken for a dark form of *B. pardellus*, but the short elytra with checkered pattern are typical, as are the shape of ventral valve and the long dented rods in saccus. *Bruchidius snizeki* has a distinctly wider face and shorter elytra than *ituriensis, mulunguensis* and *pardellus*.

DISTRIBUTION

Kenya and Yemen.

PHYLOGENETIC ANALYSES

For each gene of interest the best-fit model of sequence evolution was of the general time reversible family, with a gamma distribution of rates. The two runs of the BI analysis reach convergence well before the end of the burn-in period as indicated



31. Phylogenetic tree corresponding to the result of a Bayesian inference analyses under MrBayes 3.2.1. Clade posterior probability values are figured on nodes

by PSRF values of 1.00 for all parameters and standard deviation of split-frequencies below 1%. Overall, the resulting topology (Fig. 31) is well supported by the CPP as most nodes are supported by CPP ≥ 95% (29 nodes out of 38).

The five individuals from species group *ituriensis* are recovered in a sister group position (CPP of 0.99) to a clade encompassing members of the *centromaculatus* group (CPP of 1.00) and the four individuals of the *Bruchidius meibomiaca* complex (CPP of 1.00). All these species are sister to another clade (CPP of 1.00), which includes the two individuals of *B. mulunguensis* along with the specimens of *B. afrasperae* and *B. hiekei*. The respective position of the three species is not well resolved as indicated by the low CPP value (0.48) for node grouping *B. afrasperae* and *B. mulunguensis*. The sampled specimens of the *Bruchidius chloroticus* complex are sister to these two clades. More distantly related taxa belong to the genera *Callosobruchus* and *Kingsolverius* and to the *Bruchidius hinnulus* group. It is also the case for species in genus *Conicobruchus*, which are even more distantly related to *B. mulunguensis* and species in the *ituriensis* group.

CONCLUSION

Of the seven species treated here, four (*arcuatus, ituriensis, pardellus* and *snizeki*) clearly belong to a single species group, the *Bruchidius ituriensis* group. As we presently conceive it, that group represents a widely distributed complex which was so far treated as a single species, *B. ituriensis*. Among this complex, we recognize three new species (*arcuatus, pardellus* and *snizeki*), on the basis of small but constant differences in genital morphology, and (partly) of the nature of the larval host. At least in one case, there is evidence that the larval diet may have some bearing on genetic isolation of populations, possibly leading to speciation. There is some probability that in the future more morphological forms may be recognized as genetically isolated entities within the *B. ituriensis* species complex, especially when considering populations with a disjunct distribution.

The group is characterized by the following combination of characters: body short, stocky; integument essentially reddish-brown, blackened in some specimens; antenna with strong sexual dimorphism, subserrate in female, serrate in male; eyes bulging; pronotum trapezoidal to slightly campaniform, elytra with two basal teeth or tubercles; legs without sexual dimorphism, hind femur with minute preapical denticule; hind tibial apex with mucro almost as long as width of 1st tarsal segment, rest of corona much shorter; base of first male ventrite with a patch of denser hair, remaining segments much shortened, last one indented to receive the pointed apex of the last visible tergite (pygidium), which is subtriangular or shield-shaped, convex but not turned under apically, vertical; male genitalia exhibit a strong homogeneity: a slim, elongated median lobe, with a strongly chitinized, bow-shaped dorsal valve; ventral valve acute; internal sac bearing feebly sclerotized denticles, and sclerotized plates; lateral lobes fused well beyond middle; spermathecal wall without concentric ridges, appearing smooth at 400x magnification; spermathecal body subcylindrical, with apical diverticulum moderately long, evenly curved or straight basally and hooked at tip, dorsal sclerite at opening of

bursa copulatrix absent or small, undentate; bursa copulatrix without visible spines or needles; ovipositor elongated, tergite 9 much longer than wide, spiculum gastrale long, with very short basal arms.

In spite of its close morphological similarity, *B. mulunguensis* does not belong to the *ituriensis* group, as shown by the present phylogenetic reconstruction. In fact, a major distinctive character in genital morphology is found in *B. mulunguensis*: the saccus is lined with isolated spines instead of bearing dented rods as seen in the *ituriensis* group. Among other important characters, shape of the ventral valve, basal strut size, more or less complete fusion of parameres, or shape of antennomeres (see Fig. 1-9 and 23-30) are clearly variable within the group. Therefore, these characters cannot be considered as phylogenetically informative in the present particular situation. As saccus morphologies similar to *mulunguensis* exist in both *lusingaensis* and *djemensis*, it may be assumed that both species do not belong to the *ituriensis* group.

In the present case, we must partly disagree with Kingsolver's (2004) remark on the apparent lack of taxonomic significance of female genitalia. This observation is usually related with the lack of strongly sclerotized structures that could be easily compared, as is the case in male genitalia. Within the present group of species, a few characters of taxonomic value were identified: general shape of the spermatheca, which is comma-shaped in *B. mulunguensis*, but shows a subcylindrical body in *arcuatus, ituriensis*, *pardellus* and *snizeki*; shape of the apical spermathecal diverticulum, almost straight in *B. ituriensis*, curved at a right angle in *snizeki* and *pardellus,* moderately curved in *arcuatus*; presence of a dorsal sclerite at the entrance of the bursa copulatrix in *B. pardellus*, absent in the other species. It is worth mentioning that female genitalia in this group of species show much similarity with those described in *B. afrasperae* Delobel & Le Ru, a species feeding in seeds of Aeschynomeneae (Delobel & Le Ru 2008): ovipositor long, spiculum gastrale Y-shaped, with short basal arms, and spermathecal body smooth. Yet, as underlined by the phylogenetic reconstruction these taxonomically significant characters are not always phylogenetically informative because of their homoplasious nature. All these results and observations highlight the complexity of the revisional task at hand, and how it is important to consider in an integrative way ecological, morphological and molecular information.

The *B. ituriensis* group has obvious morphological similarities with the *Bruchidius chloroticus* group, which is composed of *B. chloroticus* Dalman, *B. garambaensis* Decelle and *B. sesbaniae* Decelle; these species mostly differ from members of the *ituriensis* group in the presence of a pair of large teeth at the anterior third of the internal sac. However both groups are quite distantly related in the phylogenetic reconstruction, thus highlighting the importance of considering every character of the internal sac. Instead a closer relationship is suggested with *Bruchidius meibomiaca* and species from *B. centromaculatus* group, despite the fact that they present less morphological similarities overall. For instance, contrary to what is observed in the *B. ituriensis* group, there is a quasi absence of fusion of parameres in *B. centromaculatus* group. As already noted by Decelle (1951) for *B. mulunguensis*, the *ituriensis* group also bears some similarities (ventral valve shape, strong sclerotization of the dorsal valve, and partial fusion of parameres) with the *Bruchidius hinnulus* species group,

which is made up of *B. hinnulus* Fahr. and an undescribed species from Kenya. Our phylogenetic reconstruction does not support this relationship, suggesting instead that *B. hinnulus* is distantly related to the *ituriensis* group or to *B. mulunguensis*. All these inconsistencies illustrate the fact that most of these morphological similarities are likely the product of evolutionary convergence.

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REFERENCES

Anton, K.-W., Delobel, A., 2003. African species of the *Bruchidius centromaculatus* group with "eyed" female pygidium (Coleoptera: Bruchidae: Bruchinae). Genus, **14**:159-190.

Decelle, J., 1951. Contribution à l'étude des Bruchidae du Congo belge. Rev. Zool. Bot. Afr., **45**: 172-191.

—, 1958. Contribution à l'étude des Bruchidae du Congo belge (Deuxième note). Rev. Zool. Bot. Afr., **58**: 75-84.

—, 1960. Bruchidae (Coleoptera Phytophagoidea). Parc National de la Garamba. Mission H. de Saeger, **18**: 45-73.

—, 1971. Bruchides (Coleoptera) récoltés en Abyssinie centrale par l'expédition H. Scott et J. Omer Cooper (IX.1926 - I.1927). Bull. Ann. Soc. Roy. Entomol. Belg., **107**: 243-259.

Delobel, A., Le Ru, B., 2008. Les *Bruchidius* consommateurs de graines d'*Aeschynomene*; définition du groupe d'espèces *Bruchidius kiliwaensis* (Decelle) (Coleoptera, Bruchidae). Bull. Soc. Entomol. Fr., **113**: 5-14.

Gilbert, M.T.P., Moore, W., Melchior, L., Worobey, M., 2007. DNA extraction from dry museum beetles without conferring external morphological damage. PLoS One, **2**: 272-275.

Kergoat, G.J., Alvarez, N., Hossaert-McKey, M., Faure, N., Silvain, J.-F., 2005a. Evidence for a parallel evolution in the two largest New and Old World seed-beetle genera (Coleoptera: Bruchidae). Mol. Ecol., **14**: 4003-4021.

Kergoat, G.J., Delobel, P., Delobel, A., 2007a. Phylogenetic relationships of a new species of seed-beetle infesting *Cercis siliquastrum* L. in China and in Europe (Coleoptera: Chrysomelidae: Bruchinae: Bruchini). Ann. Soc. Entomol. Fr., **43**: 265-271.

Kergoat, G.J., Delobel, A., Fédière, G., Le Ru, B., Silvain, J.-F., 2005b. Both host-plant phylogeny and chemistry have shaped the African seed-beetle radiation. Mol. Phylogenet. Evol., **35**: 602-611.

Kergoat, G.J., Le Ru, B.P., Genson, G., Cruaud, C., Couloux, A., Delobel, A., 2011. Phylogenetics, species boundaries and timing of resource tracking in a highly specialized group of seed beetles (Coleoptera: Chrysomelidae: Bruchinae). Mol. Phylogenet. Evol., **59**: 746-760.

Kergoat, G.J., Silvain, J.-F., Delobel, A., Tuda, M., Anton, K.-W., 2007b. Defining the limits of taxonomic conservatism in host-plant use for phytophagous insects: molecular systematics and evolution of host-plant associations in the seed-beetle genus *Bruchus* Linnaeus (Coleoptera: Chrysomelidae: Bruchinae). Mol. Phylogenet. Evol., **43**: 251-269.

Kingsolver, J.M., 2004. Handbook of the Bruchidae of the United States and Canada (Insecta, Coleoptera). Vol. 1. USDA, ARS, Techn. Bull., **1912**: 1-324.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics, **23**: 2947-2948.

Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P., Nieves-Aldrey, J.L., 2004. Bayesian phylogenetic analysis of combined data. Syst. Biol., **53**: 47-67.

Pic, M., 1952. Coléoptères du globe (suite). L'échange, 68ème année, **528**: 5-8.
Posada, D., 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol., **25**: 1253-1256.
Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol., **61**: 539-542.