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Morphology and DNA barcoding reveal a new species of *Eudicella* from East Africa (Coleoptera: Scarabaeidae: Cetoniinae)

MATTHIAS SEIDEL[1,2,3]

[1]*Department of Zoology, Faculty of Science, Charles University, Viničná 7, 123 83 Praha 2, Czech Republic*
[2]*Allgemeine Zoologie, Martin-Luther-Universität Halle-Wittenberg, Hoher Weg 8, 06120 Halle (Saale), Germany*
[3]*Zentralmagazin Naturwissenschaftlicher Sammlungen, Martin-Luther-Universität Halle-Wittenberg, Domplatz 4, 06108 Halle (Saale), Germany. E-mail: seidelma@natur.cuni.cz*

Abstract

A new species of *Eudicella* White, 1839 (Coleoptera: Scarabaeidae: Cetoniinae), is described from Uganda and Kenya: *E. nana* **new species**. Morphological and genetic analyses of the new taxon and phenotypically allied species are given. *Eudicella nana* is compared with its hypothesized sister species, *E. darwiniana* Kraatz, 1880, and diagnostic characters that distinguish it from other species occurring in the same region are provided.

Key words: beetle, COI, Uganda, Kenya, integrative taxonomy

Introduction

The genus *Eudicella* White, 1839 (Coleoptera: Scarabaeidae: Cetoniinae: Goliathini: Coryphocerina) is a diverse group of large fruit chafers broadly distributed in sub-Saharan Africa. Holm (1993) included *Cyprolais* Thomson, 1880 as a subgenus in *Eudicella*. *Eudicella* (*Eudicella*) was recently revised (De Palma 2009), whereas the subgenus *Cyprolais* is still in need of revision. In the past, multiple local variants have been described as new species or subspecies. As the local variants are often uniform and discrete in their appearance, they were mistakenly described as separate species. With modern techniques such as DNA barcoding available in taxonomy, we can identify and resolve cases where the spectrum of intraspecific variation is unclear. In this paper, a new species of *Eudicella* is described based on morphology and cytochrome oxidase I (COI) DNA sequence distances. The new species, *Eudicella nana*, is also compared with its hypothesized sister species, *Eudicella darwiniana* Kraatz, 1880, and other phenotypically similar species occurring in its range.

Material and methods

Specimens and collections. The material examined in the present study is kept in the following collections:

BMNH—Natural History Museum, London, United Kingdom (M. Barclay)
GBPC—Gerhard Beinhundner Personal Collection, Euerbach, Germany
MSPC—Matthias Seidel Personal Collection, Prague, Czech Republic
PLPC—Philippe Leonard Personal Collection, Embourg, Belgium
SDEI—Senckenberg Deutsches Entomologisches Institut, Müncheberg, Germany (S. Blank)
SMNS—Staatliches Museum für Naturkunde, Stuttgart, Germany (W. Schawaller)

Illustrations. Photographs were taken with a digital camera and processed with Helicon focus 6 stacking software. To photograph the labia the heads of the specimens were incubated at 60 °C in a 1% Pepsin-HCl solution (pH = 2) for 24 hours. The mouthparts were removed and then dry mounted.

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DNA extraction, amplification, and sequencing. Genomic DNA was extracted from thorax muscle tissue of dried museum specimens. DNA extractions were carried out according to the protocol of Paxton *et al*. (1996), but all centrifugation steps were performed at 11800 g and DNA was precipitated with one volume of 2-propanol instead of ethanol. Polymerase chain reactions were performed using standard protocols and standard barcoding primers (Table 1). An internal primer pair for COI (Table 1) was designed to amplify smaller fragments of COI in *Eudicella* when DNA did not amplify with the original primers. Polymerase chain reaction products were purified using EXOSAP-it (Affymetrix, Santa Clara, California, United States of America) following the manufacturer's instructions. The forward and reverse Sanger sequencing was performed by GATC-Biotech (Konstanz, Germany) on a capillary DNA sequencer.

TABLE 1. Polymerase chain reaction primers used to amplify the COI barcode region in *Eudicella* (*Eudicella*).

Name	Sequence (5'->3')	Reference
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al.* 1994
LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer *et al.* 1994
nanaF	YGGAACAGGATGAACRGTCT	This study
nanaR	AATATCGCCCATAGAGGTGCT	This study

Taxon sampling. For genetic analysis, 29 specimens belonging to the new species *E. nana*, its hypothesized sister species, *E. darwiniana*, and three similar species: *E. morgani* (White, 1839), *E. pauperata* Kolbe, 1884, and *E. grallii* (Buquet, 1836), were sequenced (Table 2).

Molecular phylogenetic analyses. The consensus of forward and reverse sequences was generated with BioEdit (Hall 2011) for each individual. The resulting sequences were BLASTed against the Genbank nucleotide collection (http://www.ncbi.nlm.nih.gov) to confirm gene and taxon identity. Furthermore, the Barcode of Life Data Systems (BOLD) was used for genus level confirmation (http://www.boldsystems.org). Sequence data on *Eudicella* deposited in BOLD are not publicly accessible. The sequences were aligned using the MUSCLE algorithm implemented in MEGA6 (Tamura *et al.* 2013). The alignment was tested for the best nucleotide substitution model using jModelTest (Darriba *et al.* 2012). A phylogenetic analysis using a maximum likelihood approach and 1000 bootstrap replicates was performed in MEGA6.

Results and discussion

Eudicella (Eudicella) nana Seidel, new species

Type material. Holotype male at BMNH with label data: a) "Uganda, Budongo Forest, 6.1.1995" (handwritten), b) "No.: GB 222" (typeset and handwritten), c) "ex. Gerhard Beinhundner Collection" (handwritten), d) male genitalia card mounted, e) wing card mounted, f) "no suitable thorax tissue for DNA extraction" (typeset), g) red holotype label. Allotype female at GBPC with label data: a) "Uganda, Budongo Forest, 6.1.1995" (handwritten), b) "DNA extract No.: GB 220" (typeset and handwritten), c) "Gerhard Beinhundner Collection" (handwritten), d) mouthparts card mounted, e) wing card mounted, f) red allotype label. Paratype male at GBPC with label data: a) "Uganda, Budongo Forest, 6.1.1995" (handwritten), b) "No.: GB 223" (typeset and handwritten), c) "Gerhard Beinhundner Collection" (handwritten), d) male genitalia card mounted, e) wing card mounted, f) "no suitable thorax tissue for DNA extraction" (typeset), g) red paratype label. Paratype male at GBPC with label data: a) "Uganda, Budongo Forest, 6.1.1995" (handwritten), b) "DNA extract No.: GB 215" (typeset and handwritten), c) "Gerhard Beinhundner Collection" (handwritten), d) male genitalia card mounted, e) wing card mounted, f) red paratype label. Paratype male at GBPC with label data: a) "Uganda, Budongo Forest, 6.1.1995" (handwritten), b) "No.: GB 216" (typeset and handwritten), c) "Gerhard Beinhundner Collection" (handwritten), d) male genitalia card mounted, e) wing card mounted, f) "no suitable thorax tissue for DNA extraction" (typeset), g) red paratype label. Paratype male at MSPC with label data: a) "Uganda, Budongo Forest, 6.1.1995" (handwritten), b) "DNA extract No.: GB 35" (typeset and handwritten), c) "ex. Gerhard Beinhundner Collection" (handwritten), d) "Coll. Matthias Seidel 2015" (typeset), e) male genitalia card mounted, f) mouthparts card mounted, g) wing card

mounted, h) red paratype label. Paratype female at BMNH with label data: a) "Uganda, Budongo Forest, 6.1.1995" (handwritten), b) "No.: GB 219" (typeset and handwritten), c) "ex. Gerhard Beinhundner Collection" (handwritten), d) mouthparts card mounted, e) wing card mounted, f) red paratype label. Paratype female at MSPC with label data: a) "Uganda, Budongo Forest, 6.1.1995" (handwritten), b) "No.: GB 221" (typeset and handwritten), c) "ex. Gerhard Beinhundner Collection" (handwritten), d) "Coll. Matthias Seidel 2015" (typeset), e) eggs card mounted, f) wing card mounted, g) red paratype label. Paratype male at GBPC with label data: a) "Uganda, Semuliki Forest, 10.1994" (handwritten), b) "DNA extract No.: GB 218" (typeset and handwritten), c) "Gerhard Beinhundner Collection" (handwritten), d) male genitalia card mounted, e) mouthparts card mounted, f) wing card mounted, g) red paratype label. Paratype male at GBPC with label data: a) "Uganda, near Kampala, V.1994" (typeset), b) "DNA extract No.: GB 34" (typeset and handwritten), c) "Gerhard Beinhundner Collection" (handwritten), d) male genitalia card mounted, e) mouthparts card mounted, f) wing card mounted, g) red paratype label. Paratype male at GBPC with label data: a) "Kenya, Teita hills, 10-15.10.1995, coll. G. Bentz" (typeset), b) "DNA extract No.: GB 217" (typeset and handwritten), c) "Gerhard Beinhundner Collection" (handwritten), d) male genitalia card mounted, e) mouthparts card mounted, f) wing card mounted, g) red paratype label. Paratype male at SMNS with label data: a) "Uganda – Kisogo, Kiala, VI-06, P. Stobbia" (handwritten), b) "Eudicella woermanni ugandensis (Allard, 1985), R. Giannatelli det. 2008" (typeset), c) "DNA extract No.: E 272" (typeset and handwritten), d) male genitalia card mounted, e) red paratype label.

Description. Holotype male (Figs. 1, 3, 4, 6, 7, 17)**.** Total length 32 mm; width across humeri 14.5 mm. **Colour:** Head (except clypeus), pronotum, scutellum, pygidium, legs, and venter green with weak red reflections. Elytra yellow with dark green band at the disc ending in black humeral and apical maculae; humeral and apical maculae elongate to midpoint of elytral band, not abutting; elytral suture black with green border reduced from scutellum towards elytral apices; elytral margin black. Clypeus tawny and green iridescent at base. **Head:** Frons posteriorly smooth with fine, sparse punctures becoming densely punctate near lateral and anterior margins; small and large punctures mixed; lateral margin with short, tawny setae. Interocular width equals 5.5 transverse eye diameters. Clypeus ending in a narrowly forked horn with black tips (Fig. 17); width of clypeal fork 2.9 mm; external tubercles bilateral of the clypeal horn ending in transversely blunt apices (Fig. 17). Labium moderately deeply punctate, setigerous; setae long, tawny (Fig. 4). **Pronotum:** Surface evenly convex in lateral view; sparsely punctate at disc and densely punctate towards margins. **Scutellum:** Surface sparsely punctate, punctures small. **Elytron:** Surface punctate; punctures small and dense; 1 row of moderately large punctures adjacent to suture. **Pygidium:** Disc regularly convex in lateral view; surface rugose with sparse punctation (Fig. 7); setigerous; setae short, tawny. **Venter:** Mesosternal apex produced; abdominal ventrites 1 to 5 with longitudinal impression. **Legs:** Colour of the body; protibia denticulate at interior margin; metatibia half green, apically tawny; metatarsi reddish brown, others black (Fig. 1). **Parameres:** Form symmetrical (Fig. 6). **Wings:** transparent, tawny with a dark brown bar close to the tip (Fig. 3).

Allotype. Female (Figs. 2, 5, 8). Total length 29.5 mm; width across humeri 14 mm. **Colour:** Same as in the holotype. Elytra with elongate humeral and rounded, apical maculae; Clypeus tawny with green iridescence. **Head:** Frons posteriorly with fine to medium, sparse punctures becoming densely, confluently rugopunctate at disc and margins; small and large punctures mixed; lateral margin with short, tawny setae. Interocular width equals 5.0 transverse eye diameters. Clypeus unarmed; Labium rugose (wavy ridges) with both small and deep, large punctures, setigerous; setae long, tawny (Fig. 5). **Pronotum:** Surface evenly convex in lateral view; sparsely punctate. **Scutellum:** Surface with small punctures evenly distributed and moderately large punctures sparsely distributed. Large deeper punctures at the anterior margin. **Elytron:** Surface punctate; punctures small, dense; three parallel rows of large punctures between suture and discal green band. **Pygidium:** Disc evenly convex in lateral view; surface highly rugose-reticulate without punctation (Fig. 8); setigerous; setae short, tawny. **Venter:** Mesosternal apex produced; abdominal ventrites without impressions. **Legs:** Colour of the body; metatibia half green, apically tawny; metatarsi reddish brown, others black. **Wing:** Same as in holotype.

Paratypes. Male (n = 8). Total length 24–34 mm. Width across humeri 11.0–14.8 mm. The paratypes from Budongo (Uganda), Semuliki Forest (Uganda), Kisogo (Uganda), and the Taita Hills (Kenya) possess the same appearance as the holotype. Humeral and apical maculae vary in length. Width of clypeal fork: 1.9–2.9 mm. The paratype from Kampala (Uganda) differs in colour; the discal band on the elytron is brighter, the maculae are brown. All legs are reddish brown. **Female** (n = 2). Total length 30.5–31.0 mm. Width across humeri 14 mm. The paratypes possess the same appearance as the allotype. In one specimen all tarsi are reddish brown.

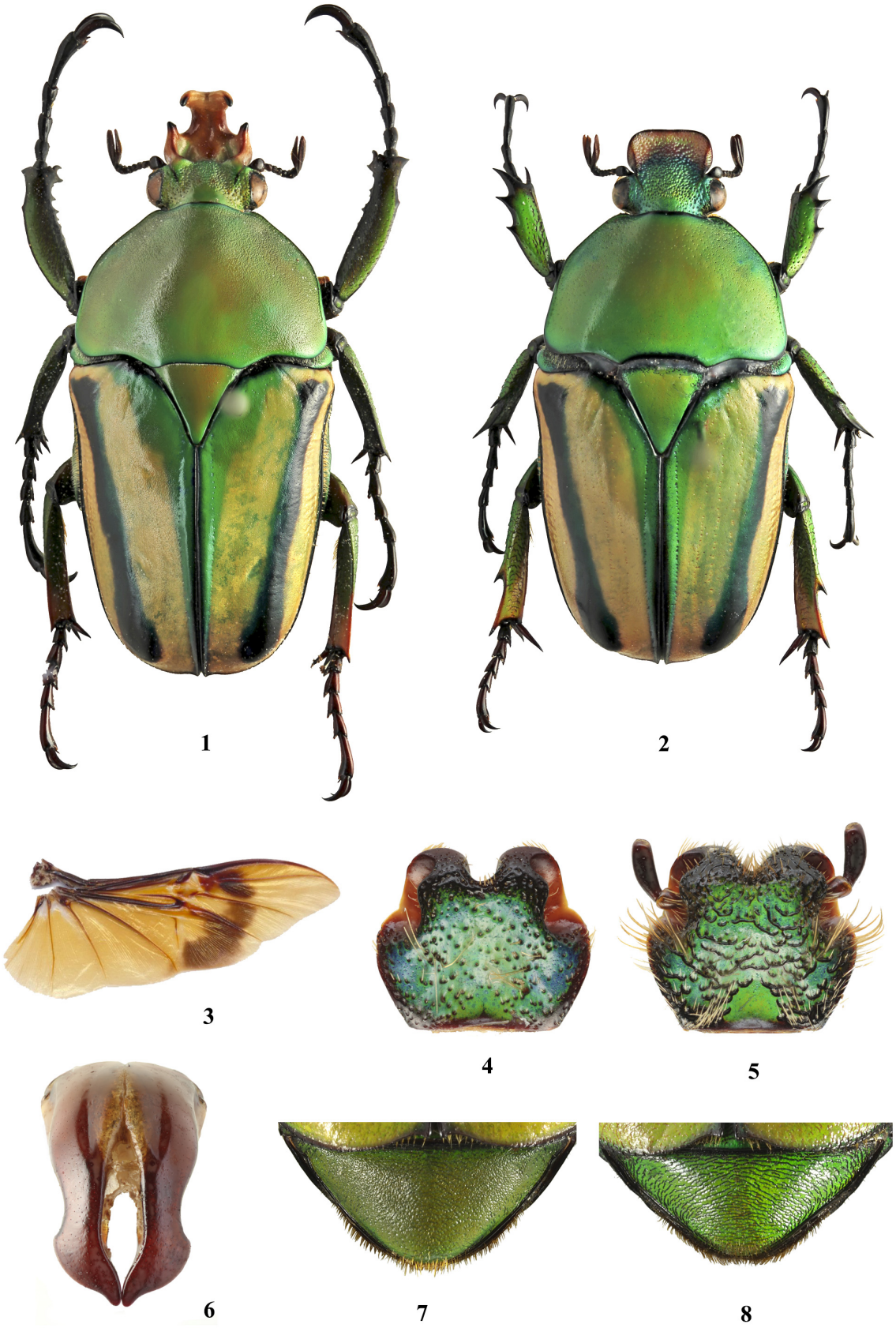

FIGURE 1–8. *Eudicella nana* **new species**. **1.** Male holotype, dorsal view. **2.** Female allotype, dorsal view. **3.** Male wing, holotype. **4.** Male labium, paratype, ventral view. **5.** Female labium, paratype, ventral view. **6.** Male parameres, holotype, frontal view. **7.** Male pygidium, holotype, frontal view. **8.** Female pygidium, allotype, frontal view.

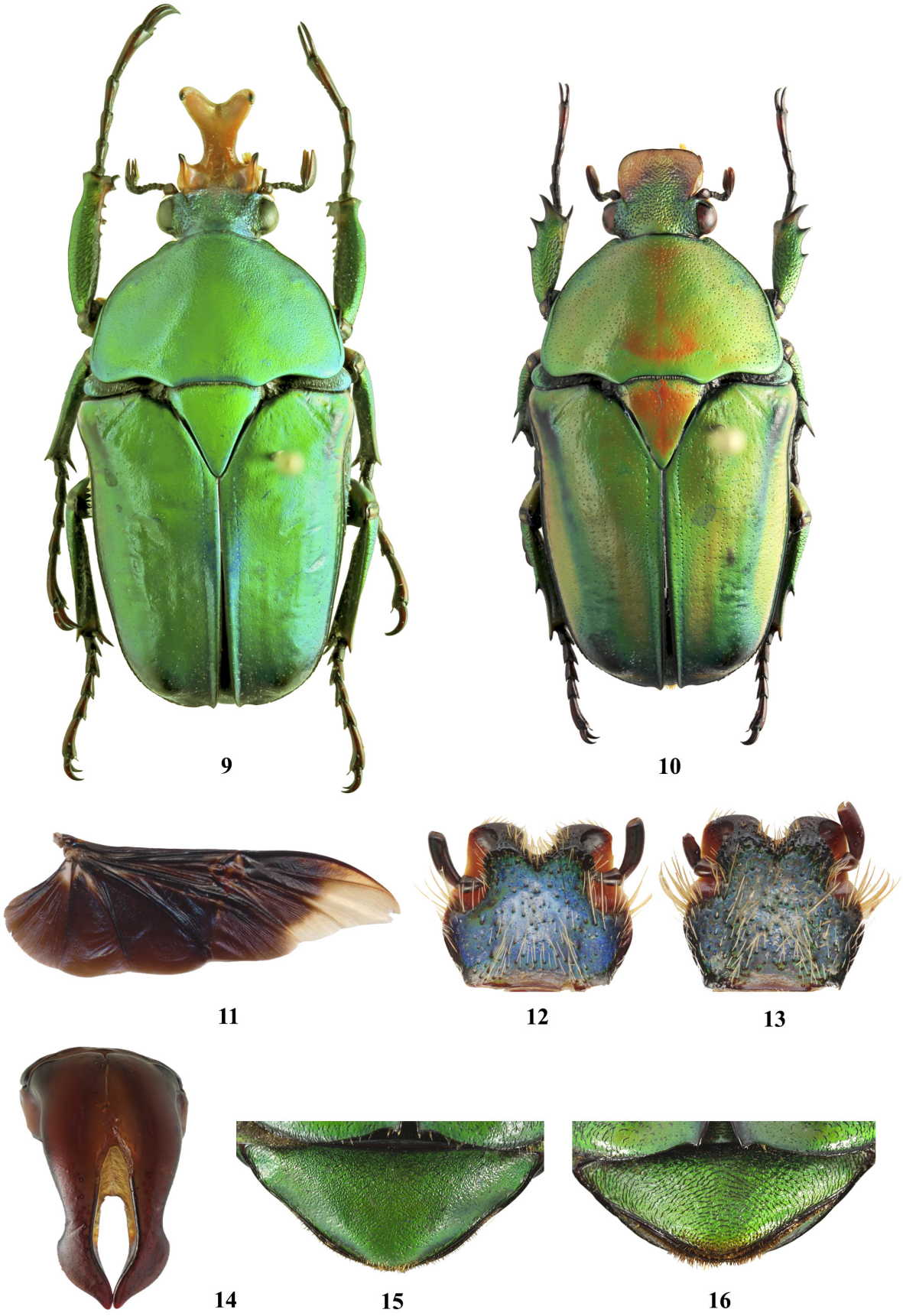

FIGURE 9–16. *Eudicella darwiniana* Kraatz, 1880. **9.** Male, dorsal view. **10.** Female, dorsal view. **11.** Male wing. **12.** Male labium, ventral view. **13.** Female labium, ventral view. **14.** Male paramers, frontal view. **15.** Male pygidium, frontal view. **16.** Female pygidium, frontal view.

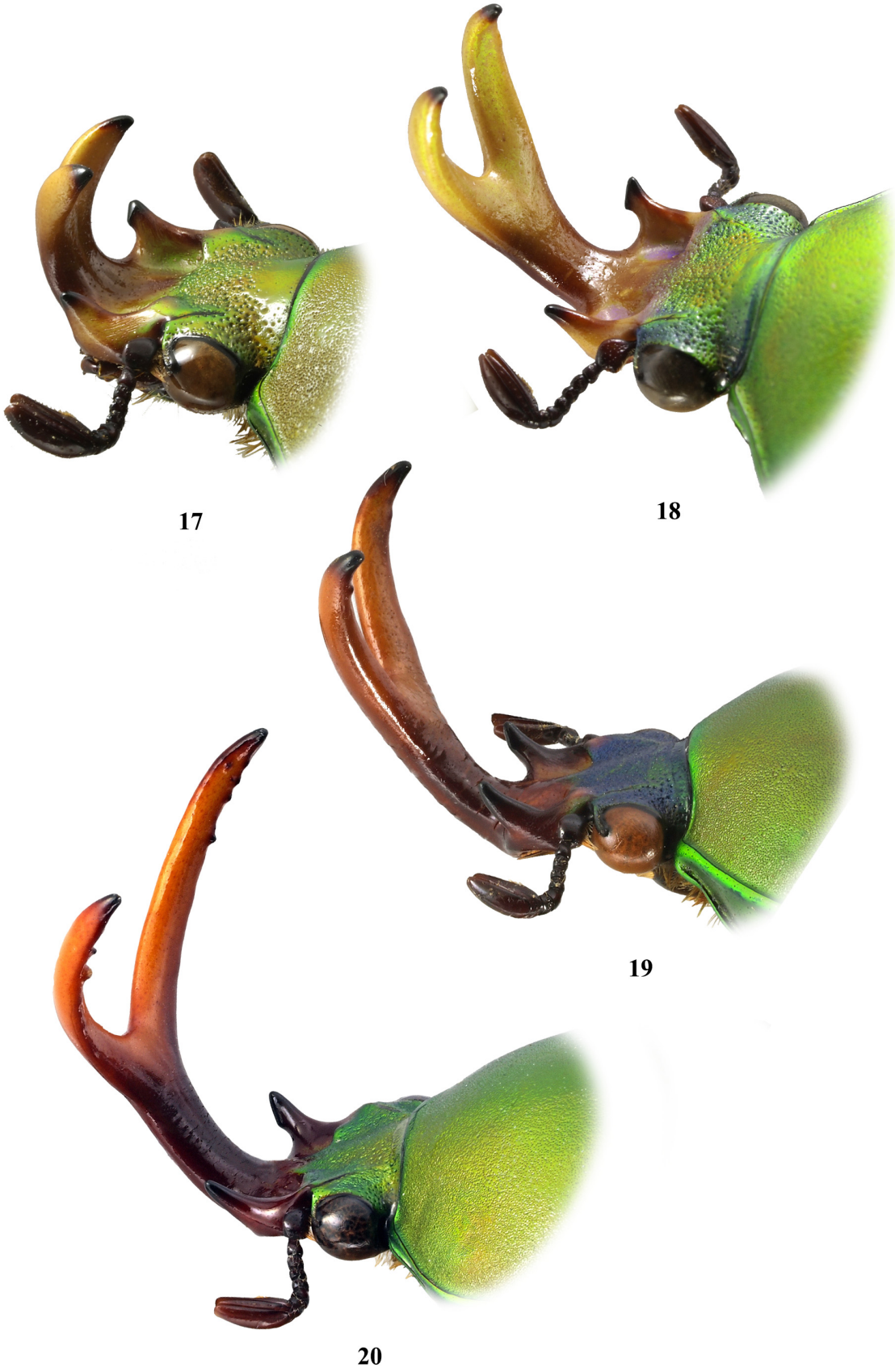

FIGURE 17–20. Clypeal horns of *Eudicella* (*Eudicella*). **17.** *Eudicella nana* **new species**. **18.** *Eudicella darwiniana* Kraatz, 1880. **19.** *Eudicella pauperata* Kolbe, 1884. **20.** *Eudicella grallii* (Buquet, 1836).

Diagnosis. This species can be distinguished from other *Eudicella* (*Eudicella*) species based on the following combination of characters: clypeus tawny and green iridescent at its base; clypeal fork short and narrow (Fig. 17); pygidium rugosely sculptured (Figs. 7–8); labium deeply punctate in males (Fig. 4) and rugose in females (Fig. 5); wings transparent, tawny with a dark brown bar close to the tip (Fig. 3). Furthermore, the form of the parameres is unique for *E. nana* and *E. darwiniana* and can be distinguished from the other species in the genus (Fig. 6).

Etymology. The species name is derived from the Latin word for dwarf (*nanus*) as it is the smallest species in the subgenus *Eudicella*.

Comparison of *Eudicella nana* with *E. darwiniana*

Material examined. *Eudicella darwiniana*: 1 ♂ holotype, Ghana, Ashanti, 1880; 3 ♂, 4 ♀, Togo, Forêt d´Imoussa, X.2013; 4 ♂, Togo, Kloto, Forêt de Missahoe, XI.2011; 1 ♂, 1 ♀, Ghana, Kibi, RCI Border, XII.2003. *Eudicella nana*: see above for details of the type series.

Eudicella darwiniana resembles *E. nana* morphologically and is regarded as its hypothesized sister species, pending a phylogenetic analysis. Both species possess a narrowly forked clypeal horn with partial green iridescence and black tips. The horn of *E. nana* is less elongate than in *E. darwiniana*. The horn of *E. nana* elevates with a steeper angle than in *E. darwiniana* (Figs. 17–18). The elytra of *E. nana* are yellow with a marked dark-green discal band (Figs. 1–2). In *E. darwiniana,* the elytra are either entirely green (except for the humeral and apical calli, which are dark-green or black) or yellowish-green with a more-or-less developed, green discal band (Figs. 9–10). The humeral maculae are usually more elongate in *E. nana*. The male labiae are very similar, deeply punctate in males (Figs. 4, 12) but distinct in females. The labial surface in female *E. nana* is rugose with deep punctures (Fig. 5), in contrast to female *E. darwiniana*, which do not possess the wavy ridges (Fig. 13). In both species, the pygidia are similar within sexes (Figs. 7–8, 15–16). The membranous wings in *E. nana* are tawny with a narrow black bar near its tips (Fig. 3). In *E. darwiniana*, the wings are black with tawny tips (Fig. 11). The parameres are very similar in the two species (Figs. 6, 14). The two species are allopatric.

Comparison of *Eudicella nana* with similar species

Material examined: *Eudicella morgani*: 1 Cameroon, Obout, V.2013; 1 ♂, 1 ♀, Cameroon, Banguem, XII.2007; 2 ♀, Togo, Forêt de Bala, X.2011; 1 ♂, 1 ♀, Gabon, Kinguele, XI.2011; 2 ♀, Togo, 4 ♂, Togo, Forêt de Missahoe, X.2014; 5 ♀, Togo, Forêt de Missahoe, X.2014. *Eudicella grallii*: 2 ♂, 1 ♀, Uganda, Lamwo, XII.2012; 1 ♂, Uganda, Budongo, 06.I.1996; 1 ♂, 2 ♀, Kenya, Aberdares Mountains, X.2011; 2 ♂, 1 ♀, Burundi, Kigwena, 01.VII.2009; 1 ♂, Burundi, Kigwena, VI.1994; 1 ♂, Democratic Republic of Congo, Sud Kivu, 28.IV.–10.V.2010; 1 ♀, Democratic Republic of Congo, Kyankwale, VI.2004. *E. pauperata*: 1 ♂, 1 ♀, Uganda, Lamwo, XII.2012; 1 ♂, Democratic Republic of Congo, Likasi, II.2011; 1 ♂, 1 ♀, Democratic Republic of Congo, Kolwezi, XI–XII.2009; 1 ♀, Uganda/Democratic Republic of Congo, Goma, XI.2010; 1 ♂, Democratic Republic of Congo, Nord Kivu, XII.2006; 2 ♂, Democratic Republic of Congo, Katanga, II–III.2008; 2 ♂, 1 ♀, Uganda, Mpigi, V.2010; 1 ♂, 1 ♀, Central African Republic, Mbaiki, 2013.

Eucidella nana can be confused with species with green body colour and yellow elytra with green discal bands. *Eucidella morgani* (*sensu* De Palma 2009) resembles *E. nana* but is only known to occur in West Africa (Ivory Coast, Togo, Ghana, Cameroon, Gabon). *Eudicella pauperata* and *E. grallii* are sympatric with *E. nana*. Large to small-sized male specimens of the former species can be easily distinguished from *E. nana* by the shape and development of the clypeal horn. The horns of the similar taxa are often more elongate and do not possess green iridescence at the clypeal base (Figs. 19–20). Furthermore, the horns of large *E. grallii* specimens can possess denticulate branches (Fig. 20). In contrast to its resembling species, *E. nana* females have strongly rugose labia. In female *E. pauperata* the labiae are deeply puncture, sometimes slightly rugose. *Eucidella grallii* females possess deeply punctate labiae.

Molecular divergence and phylogeny

COI sequences of the 29 *Eudicella* sampled resulted in a 553 base pair alignment. JModelTest (Darriba *et al.* 2012) determined the GTR+I+G model as best nucleotide substitution model. The resulting maximum-likelihood tree revealed five clades that could be assigned to taxonomic units (species) (Fig. 21). The statistical support for most clades was high (bootstrap > 98%), whereas the backbone of the phylogeny was poorly supported. Indeed the phylogeny is not a species-level phylogeny as just the morphological similar taxa were included. The interspecific divergence was lowest (5.6%) between *E. nana* and its hypothesized sister species, *E. darwiniana*, and highest (9.5%) between *E. nana* and *E. morgani* (Table 2). The intraspecific variation was generally low (0.12–0.89%) for all species (Table 3).

TABLE 2. Details of the specimens used in the genetic analysis. Type specimens are in bold.

Sample ID	Identification	Depository	GenBank#	Sex	Date	Locality
MSPC_E203	*E. morgani*	MSPC	KT240062	M	V.2013	Cameroon, Obout
MSPC_E63	*E. morgani*	MSPC	KT240063	F	XII.2007	Cameroon, Banguem
MSPC_E65	*E. morgani*	MSPC	KT240064	F	X.2011	Togo, Bala
MSPC_E118	*E. morgani*	MSPC	KT240065	F	XI.2011	Gabon, Kinguele
MSPC_E64	*E. morgani*	MSPC	KT240066	F	X.2011	Togo, Bala
MSPC_E62	*E. morgani*	MSPC	KT240067	M	XII.2007	Cameroon, Banguem
MSPC_E70	*E. morgani*	MSPC	KT240068	M	XI.2011	Gabon, Kinguele
MSPC_E67	*E. grallii*	MSPC	KT240069	F	XII.2012	Uganda, Lamwo
GBPC_GB51	*E. grallii*	GBPC	KT240070	M	I.1994	Uganda, Budongo
MSPC_E66	*E. grallii*	MSPC	KT240071	M	XII.2012	Uganda, Lamwo
MSPC_E8	*E. grallii*	MSPC	KT240072	F	X.2011	Kenya, Gatamayu
PLPC_PL14	*E. grallii*	PLPC	KT240073	M	VII.2009	Burundi, Kigwena
GBPC_GB13	*E. grallii*	GBPC	KT240074	M	VI.1996	Burundi, Kigwena
MSPC_E110	*E. grallii*	MSPC	KT240075	M	XII.2012	Uganda, Lamwo
MSPC_E93	*E. grallii*	MSPC	KT240076	M	V.2010	Democratic Republic of Congo, South Kivu
PLPC_PL10	*E. grallii*	PLPC	KT240077	F	VI.2004	Democratic Republic of Congo, Kyankwale
MSPC_E60	*E. pauperata*	MSPC	KT240078	F	XII.2012	Uganda, Lamwo
MSPC_E21	*E. pauperata*	MSPC	KT240079	M	II.2011	Democratic Republic of Congo, Likasi
MSPC_PL112	*E. pauperata*	MSPC	KT240080	M	VII.2009	Democratic Republic of Congo, Kolwezi
MSPC_E6	*E. pauperata*	MSPC	KT240081	F	XI.2010	Democratic Republic of Congo, Goma
GBPC_GB48	*E. pauperata*	GBPC	KT240082	M	XII.2006	Democratic Republic of Congo, North Kivu
MSPC_E61	*E. pauperata*	MSPC	KT240083	M	XII.2012	Uganda, Lamwo
MSPC_E73	*E. darwiniana*	MSPC	KT240084	M	X.2013	Togo, Fôret d'Imoussa
PLPC_PL24	*E. darwiniana*	PLPC	KT240085	F	XII.2003	Ghana, Kibi
MSPC_E111	*E. darwiniana*	MSPC	KT240086	M	X.2013	Togo, Fôret d'Imoussa
GBPC_GB217	***E. nana***	**GBPC**	**KT240087**	**M**	**X.1995**	**Kenya, Teita Hills**
GBPC_GB34	***E. nana***	**GBPC**	**KT240088**	**M**	**V.1994**	**Uganda, Kampala**
GBPC_GB215	***E. nana***	**GBPC**	**KT240089**	**M**	**I.1995**	**Uganda, Budongo**
GB220	***E. nana***	**GBPC**	**KT240090**	**F**	**I.1995**	**Uganda, Budongo**

TABLE 3. Distances (%) within (shaded cells) and between species were calculated using the maximum composite likelihood model (Tamura *et al.* 2004) based on analysis of the complete DNA barcode dataset. Standard error estimates (in brackets) obtained by bootstraping (1000 replicates) implemented in MEGA6.

	E. morgani	*E. pauperata*	*E. grallii*	*E. darwiniana*	*E. nana*
E. morgani	0.89 [0.25]				
E. pauperata	7.5 [1.5]	0.31 [0.15]			
E. grallii	6.5 [1.2]	5.8 [1.3]	0.40 [0.13]		
E. darwiniana	9.2 [1.6]	8.6 [1.6]	8.5 [1.7]	0.12 [0.11]	
E. nana	9.5 [1.2]	9.2 [1.6]	8.4 [1.8]	5.6 [1.8]	0.18 [0.12]

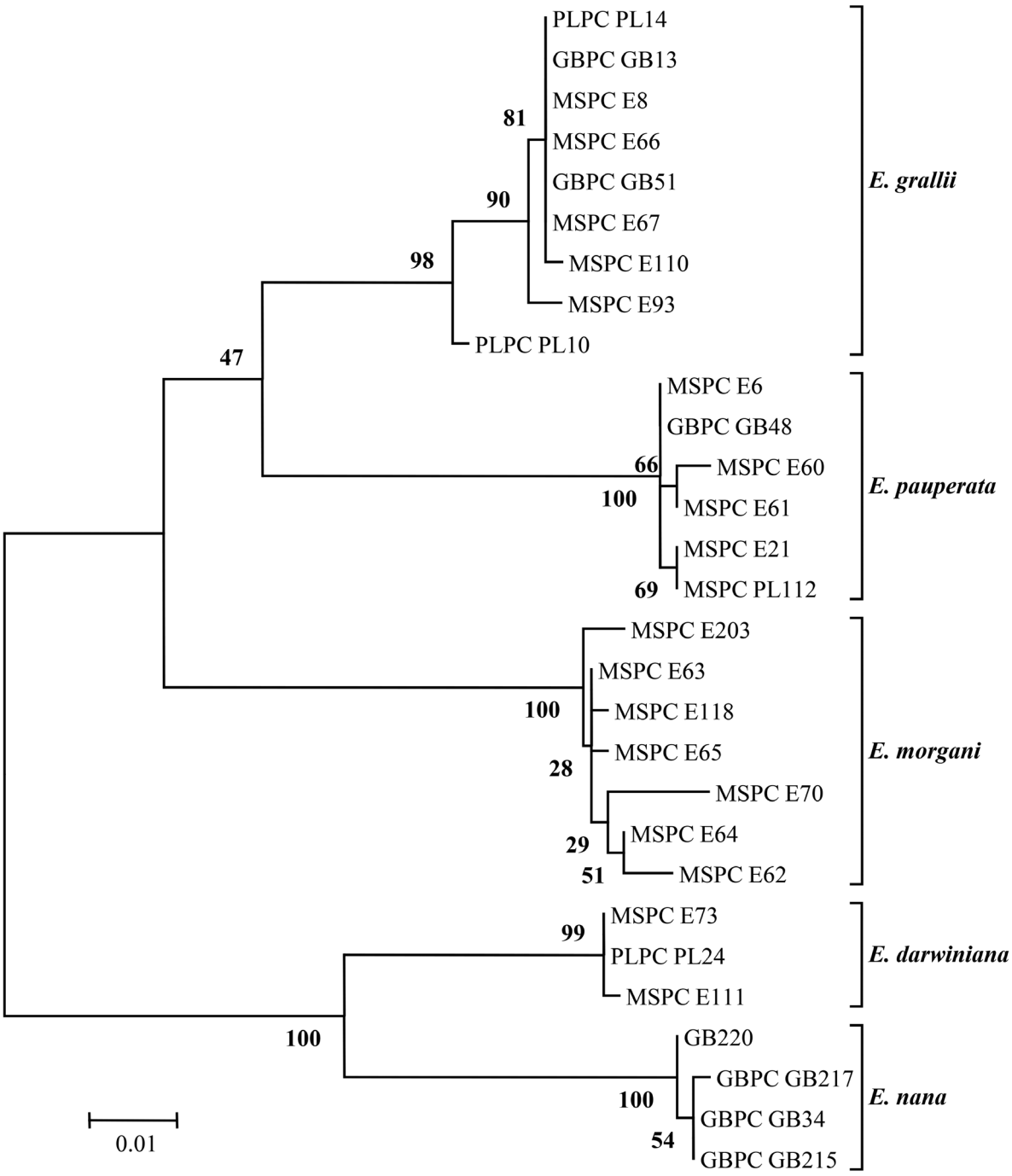

FIGURE 21. Maximum likelihood tree (1000 bootstrap replicates) resulting from the phylogenetic analysis of the complete dataset of DNA barcode sequences for 29 specimens. Each specimen is identified by its SampleID code (see Table 2).

Concluding remarks

Eudicella nana is a new species from East Africa, which can be easily distinguished from other taxa by several morphological characters. Additionally, COI DNA sequences indicate that it represents a distinct genetic lineage. The species distribution probably extends over the western borders of Uganda as the Budongo and Semuliki Forest are very close to the Democratic Republic of Congo. The occurrence of the species in Kenya should be regarded with caution as only one specimen was so far reported from that country. The male specimens of *E. nana* from the four localities studied here are smaller than the other known *Eudicella* (*Eudicella*) (De Palma 2009) species. It is possible that the description of this new species is based only on minor males. Besides finding of the type specimens in January, May, June, and October, the natural history of *E.nana* is unknown.

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