

Journal of Crustacean Biology 40(3), 288-300, 2020. doi:10.1093/jcbiol/ruaa019

# Morphological and molecular analyses reveal three new endemic species of the freshwater crab genus *Buea* Cumberlidge, Mvogo Ndongo, Clark & Daniels, 2019 (Crustacea: Brachyura: Potamonautidae) from a rainforest biodiversity hotspot in Cameroon, Central Africa

Pierre A. Mvogo Ndongo<sup>1,2,4</sup>, Thomas von Rintelen<sup>2,•</sup>, Minette Tomedi-Tabi Eyango<sup>1,3</sup> and Neil Cumberlidge<sup>4,•</sup>

<sup>1</sup>Département de Gestion des Écosystèmes Aquatiques, Institut des Sciences Halieutiques, Université de Douala à Yabassi, PO. Box. 7236 Douala-Bassa, Cameroon; <sup>2</sup>Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, Invalidenstrasse 43, 10115 Berlin, Germany; <sup>3</sup>Département d'Aquaculture, Institut des Sciences Halieutiques, Université de Douala à Yabassi, PO. Box. 7236 Douala-Bassa, Cameroon; and <sup>4</sup>Department of Biology, Northern Michigan University, Marquette, MI, 49855-5376, USA

Correspondence: P.A. Mvogo Ndongo; e-mail: mpierrearmand@yahoo.fr

(Received 13 November 2019; accepted 19 March 2020)

### ABSTRACT

Three new species of the Cameroonian endemic freshwater crab genus *Buea* Cumberlidge, Mvogo Ndongo, Clark & Daniels, 2019 are described. The new species were collected from protected areas in the Southwest region of Cameroon, the Korup and Bakossi National Parks and the Mt. Nlonako Ecological Reserve. These species are distinguished from each other and from the type species of the genus, *Buea asylos* (Cumberlidge, 1993), by a combination of morphological characters (carapace, thoracic sternum, chelipeds, and male first gonopod) and by partial sequences of three mitochondrial DNA genes (COI, 12S rRNA, and 16S rRNA). A phylogenetic analysis that included representatives of all other freshwater crab genera found in Cameroon recovered each of the new species as a well-supported distinct lineage. An identification key is also provided for the four species of *Buea* and the conservation status of the new species is discussed.

Key Words: molecular phylogeny, phylogenetic trees, Potamonautinae, taxonomy

#### INTRODUCTION

The freshwater crab genus *Buea* Cumberlidge, Mvogo Ndongo, Clark & Daniels, 2019 was recently described from the Southwest region of Cameroon from specimens that had previously been included in *Potamonemus* Cumberlidge & Clark, 1992 (as *P. asylos* Cumberlidge, 1993) (Cumberlidge, 1993, 1999; Cumberlidge *et al.*, 2019). Daniels *et al.* (2015) recognized *P. asylos* as a separate genetic lineage from the other species assigned to *Potamonemus*, and this resulted in the subsequent transfer of *P. asylos* to the newly erected genus *Buea* (Cumberlidge *et al.*, 2019). The remaining species of *Potamonemus* are found in Southwest Cameroon and eastern Nigeria, whereas *Buea* is endemic to Southwest Cameroon. *Buea* is recognised by the following characters (from Cumberlidge *et al.*, 2019). 2019): the third maxilliped exopod either lacks, or has an extremely reduced, flagellum; the lower inner margin of the cheliped merus has a large pointed distal tooth; the G1 terminal article (TA) is elongated (almost as long as the male first gonopod (G1)); the G1 subterminal segment (SS)) is broad, and curves outward distally before tapering to a pointed tip; the lateral margins of the G1 TA and SS are lined by very long setae; and the TA of the male second gonopod (G2) is extremely short. *Buea asylos* (Cumberlidge, 1993) is endemic to the rainforest zone of Southwest Cameroon in an area that lies between the towns of Kumba, Buea, and Limbe (Cumberlidge, 1993, 1999; Cumberlidge *et al.*, 2019).

Southwest Cameroon (Fig. 1) includes biodiversity hotspots for a number of freshwater groups but this area was not been well surveyed for freshwater decapods until recently (Mvogo Ndongo *et al.*,



Figure 1. Collection localities of the four species of *Buea* in Southwest Cameroon region. Area shaded grey represents the biodiversity hotspot. This figure is available in color at *journal of Crustacean Biology* online.

2017a, b, c, 2018, 2019). Extensive surveys of the lowland and upland forested zones of two National Parks (Bakossi and Korup) in the Southwest region, and the Mt. Nlonako Ecological Reserve in the Littoral region by PAMN in 2017 and 2018 resulted in the discovery of the three new species of *Buea* described herein. The present work raises the number of the species of *Buea* from one to four.

## MATERIAL AND METHODS

## Sampling

Specimens from Bakossi National Park (Bangem locality; Fig. 1) were collected in March 2017 (at the end of the dry season and the beginning of the rainy season) and were hand-caught from their burrows under stones in small streams. It was very challenging to collect specimens during the height of the rainy season (June to October) due to high water levels and difficulties in accessing the collecting sites. Specimens from Korup National Park (Mundemba locality) were collected in April 2017 (at the end of the dry season and the beginning of the rainy season) from medium-size rivers and small streams, either by hand or with a dip net. The specimens from Mt. Nlonako Ecological Reserve (Nkongsamba locality) were hand-caught in May 2018 (at the beginning of the rainy season) from puddles near small permanent streams, under fallen leaves, and stones, and from their burrows close to water bodies. Specimens collected from the Mt. Nlonako Ecological Reserve were found in a similar habitat to that of L. nkongsamba Myogo Ndongo, von Rintelen & Cumberlidge, 2019, but the two species were not found in the same locality within the reserve.

## Morphological analyses

Morphological analyses were conducted at the Museum für Naturkunde, Berlin, Germany (ZMB). Characters of the gonopods, carapace, thoracic sternum, chelipeds, third maxillipeds, and mandibles were examined in detail, and photographs were taken using a Leica microscope (model Z16A POA; Leica Biosystems, Wetzlar, Germany), and LAS V4 and Helicon Focus 6.7.1 software (Helicon Focus, Kharkiv, Ukraine). All measurements (in mm) were taken with digital calipers. The terminology used follows Cumberlidge (1999), and the classification follows Ng *et al.* (2008). Post-processing of the images was undertaken using Adobe Photoshop CC5. The holotypes of the three new species are deposited in the ZMB, while the paratypes and other specimens are deposited in the Institute of Fisheries and Aquatic Sciences, University of Douala at Yabassi, Cameroon (IFAS).

## Abbreviations used

The following abbreviations are used: A, pleonal (abdominal) segment or pleomere; asl, above sea level; CW, carapace width measured at widest point; CL, carapace length measured along medial line from anterior to posterior margin; CH, carapace height measured at maximum height of cephalothorax; E, episternite; FW, front width measured along anterior frontal margin between inner angles of orbits; G1, male first gonopod; G2, male second gonopod; P2–P5, pereiopods 2–5 or walking legs 1–4; SS, subterminal segment of G1 or G2; S4/E4, S5/E5, S6/E6, S7/E7, episternal sulci between adjacent thoracic sternites and episternites; S, thoracic sternite; S1/S2, S2/S3, S4/DS5, S5/S6, S6/S7, sternal sulci between adjacent thoracic sternites; TA, terminal article of G1 or G2; TS, terminal segment of mandibular palp.

## PCR, DNA sequencing, and molecular phylogenetic analyses

Genomic DNA was extracted from a 25 mg tissue sample cut from the pereiopod muscle of individuals preserved in 70% ethanol using a Qiagen DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) was used to amplify three mitochondrial gene fragments, a  $\sim$  638 bp region of the 16S ribosomal RNA gene (16S) using primers 16L29 and 16HLeu or 16H10 (Schubart, 2009), a ~ 594 bp region of the 12S ribosomal RNA gene (12S) using primers 12L4 and 12H2 (Schubart, 2009), and a 648 bp fragment of the Cytochrome Oxidase subunit I gene (COI) using primers COL6a (Schubart, 2009) and COH1b (Schubart, 2009), COH6 (Schubart & Huber, 2006), or CO1a (Palumbi *et al.*, 1991). PCR was performed in 25  $\mu$ l volumes containing 1× Taq buffer, 1.5 mM MgCl<sub>2</sub>, 200  $\mu$ M each dNTP, 1 U Taq polymerase, ca. 50–100 ng DNA, and ddH<sub>2</sub>O up to volume. After an initial denaturation step of 4 min at 94 °C, cycling conditions were 35 cycles at 94 °C for 30 s, 45 °C for 60 s, and 72 °C for 90 s, with a final elongation step of 5 min at 72 °C. The same primers were used in PCR and sequencing.

PCR products were sent to Macrogen Europe (Amsterdam, The Netherlands) for purification and cycle sequencing of both strands of each gene. The sequences obtained were proofread manually using Chromas and aligned with Bioedit (Thompson et al., 1994). Results from these genes were concatenated into a single alignment that was then converted into a Nexus file with FaBox (Villesen, 2007). The best evolutionary model was determined with jModeltest v.2.1.7 (Darriba et al., 2012) based on the Akaike information criterion (Posada & Buckley, 2004) and resulted in the GTR+I+G (COI), GTR+G (16S) and HKY+G (12S) models. The phylogenetic reconstruction was conducted with maximum likelihood (ML) using the software RAxML (Stamatakis, 2006) under the GTR + (I) + G model of sequence evolution. Bayesian Inference (BI) was performed to infer phylogeny by using MrBayes v. 3.2.2 (Huelsenbeck & Ronquist, 2001). The MCMC was run with four independent chains for 10,000,000 generations, samplefreq = 500, and burni $\mathcal{N}$  = 10,001. Analyses were conducted separately to test for topology congruence. The trees were drawn to scale, with branch lengths measured as the number of substitutions. Sequences of two specimens of B. bangem n. sp. and B. mundemba n. sp. were published in Mvogo Ndongo et al. (2019), and sequences of three other specimens were generated in the present study. All sequences used here are from species of freshwater crabs that represent the five genera known to occur in Cameroon, and all of these belong to the subfamily Potamonautinae Bott, 1970. The outgroup taxa all belong to the subfamily Deckeniinae Ortmann, 1897, and include Afrithelphusa monodosa (Bott, 1959) and Globonautes macropus (Rathbun, 1904) from West Africa, and Deckenia imitatrix (Hilgendorf, 1868) from East Africa. Afrithelphusa monodosa and G. macropus (both endemic to the Upper Guinea forests) share some taxonomically important characters with Louisea Cumberlidge, 1994, which is endemic to the Lower Guinea forests in Cameroon (Mvogo Ndongo et al., 2019), whereas D. imitatrix (from coastal Kenya and Somalia) share very few taxonomic characters with any species from Cameroon. All sequences used in this study are given in Table 1.

We calculated the uncorrected p-distances (%) in MEGA 7 (Kumar *et al.*, 2016) and compared these with those based on representative COI sequence data from morphologically well-defined Afrotropical genera from Daniels *et al.* (2015). The uncorrected p-distances based on COI sequence data were used as a guide (along with morphological data) to distinguish existing and novel freshwater crab species.

## SYSTEMATICS

Infraorder Brachyura Latreille, 1802

Superfamily Potamoidea Ortmann, 1896

Family Potamonautidae Bott, 1970

Subfamily Potamonautinae Bott, 1970

Genus Buea Cumberlidge, Mvogo Ndongo, Clark & Daniels, 2019

**Buea bangem** Mvogo Ndongo, von Rintelen, Tomedi-Tabi & Cumberlidge **n. sp.** 

(Figs. 2A, B, 3C, D, 4A, 5A, E, I, 6G-I, 7A-C, 8A, E)

*Type material:* Holotype adult male (CW 26.54 mm, CL 18.07 mm, CH 10.16 mm, FW 7.12 mm), Cameroon, Southwest region, Bangem, Bakossi National Park (05°01′25.5″N, 09°42′17.6″E), 1,253 m asl, 16 March 2017, coll. P. A. Mvogo Ndongo (ZMB Crust. 30325).

*Paratypes:* 5 adult males (CW 24.56 mm, CL 16.73 mm, CH 8.92 mm, FW 6.81 mm; CW 24.46 mm, CL 16.24 mm, CH 8.87 mm, FW 7.51 mm; CW 21.20 mm, CL 14.34 mm, CH 8.65 mm, FW 7.17 mm; CW 20.72 mm, CL 14.55 mm, CH 7.99, FW 6.14 mm; CW 20.05 mm, CL 13.82 mm, CH 7.78 mm, FW 6.00 mm) (IFAS-010); 2 subadult males (CW 18.60 mm, CL 13.27 mm, CH 7.23 mm, FW 5.42 mm; CW 16.74 mm, CL 12.43 mm, CH 6.51 mm, FW 5.09 mm), Cameroon, Southwest region, Bangem, Bakossi National Park (05°02′05.5″N, 09°41′57.9″E), 1,248 m asl, 15 March 2017, coll. P. A. Mvogo Ndongo (IFAS-011).

Diagnosis: Carapace height greatly exceeding FW (CH/FW 1.43,  $\mathcal{N} = 8$ ), anterior carapace surface with fields of granules, carinae; cervical, urogastric, cardiac, branchial grooves all distinct, shallow (Figs. 2A, 5E); carapace branchiostegal wall with distinct vertical (pleural) groove (Figs. 2B, 5A). Thoracic sternal sulcus S2/S3 slightly arched (2B, 5I). Major cheliped dactylus stout, slightly arched, lined by small teeth, with large tooth in middle; major cheliped propodus inferior margin distinctly concave; pollex with 3 evenly-spaced large teeth (Fig. 3C). Lower margin of cheliped merus lined by pointed teeth, distal tooth jagged, largest (Figs. 2B, 6G, H). Terminal segment (TS) of mandibular palp bilobed, with large distinct anterior lobe (0.4× TS length) (Fig. 8A). G1 TA very long, equal to SS length (TA/SS 1.00) (Figs. 7A, B); G2 TA extremely short (TA/SS 0.12) (Fig. 7C).

Description: Carapace ovoid, height greatly exceeding FW (CH/ FW 1.43,  $\mathcal{N} = 8$ ), medium width (CW/FW 3.73,  $\mathcal{N} = 8$ ), surface texture granular; anterior corners of carapace each with field of carinae; cervical, urogastric, cardiac, branchial grooves all distinct. shallow (Figs. 2A, 5E). Postfrontal crest clearly defined, lateral ends meeting anterolateral margins at epibranchial teeth (Figs. 2A, 5A, E). Exorbital tooth small, low; intermediate, epibranchial teeth small, reduced to granule (Figs. 2A, 5A). Anterolateral margin behind epibranchial tooth granular (Figs. 2A, 5A). Carapace branchiostegite with longitudinal, vertical sutures dividing wall into suborbital, subhepatic, ptervgostomial regions; vertical suture distinct, beginning behind epibranchial tooth (Figs. 2A, 5A). Mandibular palp 2-segmented; terminal segment (TS) bilobed, with large, distinct a nterior lobe  $(0.4 \times \text{ TS length})$  (Fig. 8A). Third maxillipeds filling entire buccal cavern, except for transversely oval efferent respiratory openings in superior lateral corners, exopod lacking flagellum, ischium with distinct vertical sulcus (Fig. 8E). Sternal sulcus S2/S3, slightly arched, completely crossing sternum; S3/S4 incomplete, reduced to 2 small side notches (Figs. 2B, 5I); episternal sulci S4/E4, S5/E5, S6/E6 complete, S7/E7 missing (Figs. 2B, 5I).

Chelipeds of adult male greatly unequal (Figs. 2A, B, 3C, D). Fingers slim, elongated, major cheliped dactylus, stout, straight, with large pointed tooth in middle, propodus with large rectangular tooth on proximal part, large tooth distally, fingers enclosing oval interspace when closed (Fig. 3C). Minor cheliped with occluding margins of fingers lined by row of small teeth enclosing narrow oval interspace when closed (Fig. 3D). Cheliped carpus with 2 large pointed, unequal teeth, distal tooth broad, proximal tooth smaller (Fig. 6I). Lower margin of merus of major, minor chelipeds with large jagged blunt rectangular tooth followed by row of small stout teeth (Figs. 2B, 6G, H). Walking legs (pereiopods P2–P5) slender, posterior margins of propodi serrated with small pointed teeth, dactyli tapering, each bearing rows of downward-pointing large sharp spines Table 1. Specimens of species of *Louisea, Buea, Potamonemus, Sudanonautes*, and *Potamonautes* and the outgroup taxa included in the molecular analysis. All measurements in mm. LZUY, Zoological Collection of the Laboratory of Zoology, University of Yaounde 1, Cameroon; NHM, Natural History Museum, London, UK; NMU, Northern Michigan University Museum, USA; ZMB, Museum für Naturkunde, Berlin, Germany.

| Species                                      | Locality           | Catalog number      | Reference                    | GenBank accession number |          |          |
|--|--------------------|---------------------|------------------------------|--------------------------|----------|----------|
|  |                    |                     |                              | CO1                      | 12S rRNA | 16S rRNA |
| L. nkongsamba (CW 20.0)                      | Mt. Nlonako        | ZMB Crust. 31618    | Mvogo Ndongo et al., 2019    | MN188072                 | MN217386 | MN217393 |
| L. nkongsamba CW 18.4)                       | Mt. Nlonako        | ZMB Crust. 31620    | Mvogo Ndongo et al., 2019    | MN188065                 | MN217387 | MN217394 |
| L. balssi (CW 16.2)                          | Manengouba         | ZMB Crust. 30319    | Mvogo Ndongo et al., 2019    | MN188071                 | MN217385 | MN217392 |
| L. balssi (CW 14.8)                          | Manengouba         | ZMB Crust.29628     | Mvogo Ndongo et al., 2019    | MN188070                 | MN217384 | MN217391 |
| L. edeaensis (CW 17.2)                       | Lake Ossa          | LZUY 15-3 (T351-30) | Mvogo Ndongo et al.,2017c    | KY964474                 | KY964479 | KY964472 |
| L. edeaensis (CW 16.2)                       | Lake Ossa          | ZMB Crust. 30335    | Mvogo Ndongo et al., 2019    | MN188068                 |          | MN217395 |
| <i>B. mundemba</i> . <b>n. sp.</b> (CW 26.2) | Korup N. P.        | ZMB Crust. 30321    | Mvogo Ndongo et al., 2019    | MN188069                 | MN217388 | MN217396 |
| <i>B. mundemba</i> <b>n. sp.</b> (CW 30.0)   | Korup N. P.        | ZMB Crust. 30322    | this study                   | MT019690                 |          | MT021450 |
| <i>B. mundemba</i> <b>n. sp.</b> (CW 25.0)   | Korup N. P.        | ZMB Crust. 30322(1) | this study                   | MT019689                 |          | MT021449 |
| <i>B. bangem</i> <b>n. sp.</b> (CW 26.5)     | Bakossi N. P.      | ZMB Crust. 30325    | Mvogo Ndongo et al., 2019    | MN188066                 | MN217389 | MN217397 |
| <i>B. bangem</i> <b>n. sp.</b> (CW 24.6)     | Bakossi N. P.      | IFAS-010            | This study                   | MT019691                 | MT021447 |          |
| <i>B. nlonako</i> <b>n. sp.</b> (CW 25.8)    | Mt. Nlonako        | ZMB Crust. 31786    | This study                   | MT019692                 |          | MT021451 |
| B. nlonako (CW 30.1)                         | Mt. Nlonako        | ZMB Crust. 31786    | This study                   | MT019693                 | MT021448 |          |
| B. asylos                                    | Buea & Kumba       | NHM 1994.588-591    | Daniels <i>et al.</i> , 2015 | KP640489                 | KP640410 | KP640453 |
| Potamonemus sp.                              | Bakossi N. P.      | ZMB Crust. 30327    | Mvogo Ndongo et al., 2019    | MN188067                 | MN217390 | MN217398 |
| P. mambilorum                                | Southwest Cameroon | NHM 1991.183        | Daniels et al., 2015         |                          | KP640409 | KP640452 |
| P. sachsi                                    | Southwest Cameroon | NMU09.04.1983       | Daniels <i>et al.</i> , 2015 |                          | AY803490 | AY803530 |
| Potamonautes idjiwiensis                     | D. R. Congo        | SAM A78437          | Daniels et al., 2015         | KP640481                 | KP640402 | KP640446 |
| Potamonautes obesus                          | Tanzania           | Unaccessioned       | Daniels et al., 2015         | AY803647                 | AY803497 | AY803537 |
| Afrithelphusa monodosa                       | Guinea             | NMU 25.IV.2005.C    | Daniels <i>et al.</i> , 2015 | KP640469                 | KP640386 | KP640430 |
| Globonautes macropus                         | Guinea             | NMU VII. 1988       | Daniels <i>et al.</i> , 2015 |                          | KP640391 | KP640435 |
| Deckenia imitatrix                           | Kenya              | NMU 1998.1          | Daniels <i>et al.</i> , 2015 | AY803576                 | AY803503 | AY803544 |
| Sudanonautes aubryi                          | Cameroon           | LZUY-06             | Mvogo Ndongo et al., 2017c   | KY069938                 | KY964475 | KY069950 |
| Sudanonautes floweri                         | Cameroon           | LZUY-10 (T262-03)   | Mvogo Ndongo et al., 2017c   | KY069939                 | KY964477 | KY069952 |
| Sudanonautes tiko                            | Cameroon           | ZMB Crust.29628     | Mvogo Ndongo et al., 2017c   | KY069941                 | KY964476 | KY069954 |

(Figs. 2A, B). Male pleon large, triangular, sides not indented, bordered by row of setae; telson (A7) large, with rounded distal margin (Figs. 2B, 5I). G1 TA very long, equal to SS length (TA/SS 1.0), proximal half straight, distal half directed outward ending in short tip, with long setae along ventral margins (Figs. 7A, B); G1 SS with long setae along proximal margins (Fig. 7A, B). G2 SS base broadened tapering to long thin process that supports G2; G2 TA extremely short (TA/SS 0.12), broadened, tip small (Fig. 7C).

Size: A small-size species, adult size range CW 20.1-26.5 mm.

*Color in life:* Dorsal carapace and appendages P2–P5 all dark brown; chelipeds (P1) red except for white tips (Fig. 4A).

*Etymology:* The species is named for Bangem, the closest town to the type locality in Bakossi National Park. The species epithet is a noun in apposition.

*Habitat: Buea bangem* **n. sp.** is found in rivers and streams in the Bakossi National Park (NP), Southwest Cameroon (Fig. 4B). This area covers a surface area of approximately 29,320 ha (293 km<sup>2</sup>) that includes submontane and montane zones (between 900 m and 1,800 m asl).

*Nomenclatural statement:* A life science identifier (LSID) number was obtained for the new species: urn:lsid:zoobank. org:pub:399269F4-4FA2-4858-ADE0-C281CBF79278.

# **Buea mundemba** Mvogo Ndongo, von Rintelen & Cumberlidge **n. sp.**

# (Figs. 2C, D, 3E, F, 4C, 5B, F, J, 6C, D, K, 7D–F, 8B, F)

*Type material:* Holotype, adult male (CW 26.15 mm, CL 17.60 mm, CH 10.26 mm, FW 7.04 mm), Cameroon, Southwest region, Korup National Park (05°0136.2"N, 08°52′18.5″E), 300 m asl, 12 April 2017, coll. P. A. Mvogo Ndongo (ZMB Crust. 30321).

Paratypes: 6 adult males (CW 30 mm, CL 20.16 mm, CH 11.42 mm, FW 8.31 mm; CW 25 mm, CL 17.52 mm, CH 9.83 mm, FW 6.72 mm; CW 23.41 mm, CL 16.54 mm, CH 9.00 mm, FW 6.62 mm; CW 23.52 mm, CL 16.68 mm, CH 8.04 mm, FW 6.66 mm; CW 23.53 mm, CL 16.65 mm, CH 9.10 mm, FW 6.73 mm; CW 23.91 mm, CL 16.86 mm, CH 9.20 mm, FW 6.57 mm; CW 24.39 mm, CL 16.95 mm, CH 9.68 mm, FW 7.35 mm), same data as holotype (ZMB Crust. 30322); 3 subadult males (CW 20.77, CL 14.72, CH 8.47, FW 5.70 mm; CW 21.04 mm, CL 14.77 mm, CH 8.80 mm, FW 6.41 mm; CW 18.26 mm, CL 13.23 mm, CH 6.91 mm, FW 5.60 mm), Southwest region, Korup National Park (05°01'36.2"N, 08°52'18.5"E; 05°02'17.6"N, 08°52'36.3"E), 300 m asl, 12 April 2017, coll. P. A. Mvogo Ndongo (IFAS-007); 2 juvenile males (CW 13.31 mm, CL 9.44 mm, CH 5.10 mm, FW 4.51 mm; CW 10.90 mm, CL 11.11 mm, CH 5.91 mm, FW 3.80 mm); 3 adult females (CW 28.24 mm, CL 19.34 mm, CH 10.34 mm, FW 8.07 mm; CW 24.03 mm, CL 16.38 mm, CH 9.59 mm, FW 7.77 mm; CW 23.49 mm, CL 16.71 mm, CH 9.28 mm, FW 6.95 mm) Southwest region, Korup National Park (05°01'36.2"N,



Figure 2. Buea bangem n. sp.: dorsal (A), ventral (B) views of adult male (CW 26.54 mm; ZMB Crust. 30325), Bakossi National Park, Southwest Region, Cameroon. Buea mundemba n. sp.: dorsal (C), ventral (D) views of adult male (CW 26.15 mm; ZMB Crust. 30321), Korup National Park, Southwest Region, Cameroon. Buea nlonak n. sp.: dorsal (E), ventral (F) views of adult male (CW 25.80 mm; ZMB Crust. 31786), Mt. Nlonako Ecological Reserve, Littoral region, Cameroon. This figure is available in color at *Journal of Crustacean Biology* online.

 $08^{\circ}52'18.5''E;\,05^{\circ}02'17.6''N,\,08^{\circ}52'36.3''E),\,300$  m asl, 12 April. 2017, coll. P. A. Mvogo Ndongo (IFAS-008); 4 subadult females (CW 17.03 mm, CL 12.24 mm, CH 6.56 mm, FW 5.57 mm; CW 15.81 mm, CL 11.67 mm, CH 5.96 mm, FW 4.86 mm; CW 15.58 mm, CL 11.59 mm, CH 5.95 mm, FW 4.86 mm; CW 18.38 mm, CL 12.70 mm, CH 7.06 mm, FW 5.50 mm), Southwest region, Korup National Park (05^01'36.2''N, 08^52'18.5''E; 05^02'17.6''N, 08^52'36.3''E), 300 m asl, 12 April 2017, coll. P. A. Mvogo Ndongo (IFAS-009).

*Diagnosis:* Carapace highly arched (CH/FW 1.45, N = 20), anterior carapace surface with fields of granules and carinae; cervical, urogastric, cardiac, branchial grooves all distinct, but shallow (Figs. 2C, 5F); carapace branchiostegal wall with distinct vertical (pleural) groove (Figs. 2C, 5B). Sternal sulcus S2/S3 completely crossing sternum, slightly arched (Figs. 2D, 5J). Major cheliped dactylus stout, arched, lined by small teeth, largest pointed tooth in middle, propodus with large (molar) tricuspid tooth proximally, followed by smaller teeth interspersed by 4 larger teeth (Fig. 3E). Lower margin of cheliped merus lined

by blunt small teeth, distal tooth largest (sharp spine) (Figs. 2D, 6C, D). Terminal segment (TS) of mandibular palp bilobed, with small distinct anterior lobe (0.25× TS length) (Fig. 8B). G1 TA extremely long, almost as long as SS (TA/SS 0.98) (Figs.7D, E); G2 TA extremely short (TA/SS 0.20) (Fig. 7F). Major cheliped propodus inferior margin straight or only slightly indented; pollex with large molar proximally, followed by 4 distinct teeth interspersed by smaller teeth (Fig. 3E).

Description: Carapace ovoid, high (CH/FW 1.45,  $\mathcal{N} = 20$ ), very wide (CW/FW 3.72,  $\mathcal{N} = 20$ ), surface slightly granular, anterior corners of carapace each with field of carinae; cervical, urogastric, cardiac, branchial grooves all distinct, shallow, intestinal groove faint (Figs. 2C, 5F); postfrontal crest distinct, complete, lateral ends meeting anterolateral margins (Figs. 2C, 5B, F); exorbital angle low, blunt, intermediate, epibranchial teeth each reduced to small granule (Figs. 5B); anterolateral margin behind epibranchial tooth granular (Figs. 2C, 5B). Carapace branchiostegite with distinct vertical (pleural) groove with longitudinal, vertical suture dividing wall into suborbital, subhepatic, pterygostomial regions,



Figure 3. Buea asylos (Cumberlidge, 1993), holotype: dorsal (A) ventral (B) views of adult male (CW 27.6 mm; NHM 1994.587), between Buea and Kumba, Cameroon (photographs by Phillip Crabb, NHM, London (Cumberlidge et al., 2019; fig. 1a, b). Buea bangem n. sp.: frontal view of the major (C) and minor (D) chelipeds of adult male (CW 26.54 mm; ZMB Crust. 30325), Bakossi National Park, Southwest Cameroon. Buea mundemba n. sp.: frontal view of the major (E) and minor (F) chelipeds of adult male of (CW 26.15 mm; ZMB Crust. 30321), Korup National Park, Southwest Cameroon. Buea nlonako n. sp.: frontal view of the major (G) and minor (H) chelipeds of adult male of (CW 25.80 mm; ZMB Crust. 31786), Mt. Nlonako Ecological Reserve, Littoral Region, Cameroon. Buea asylos: frontal view of the major (I) and minor (J) chelipeds of adult male (CW 27.6 mm; NHM 1994.587) between Buea and Kumba, Southwest Cameroon region (Cumberlidge et al., 2019; figs. 1a, b, 5 a, b). This figure is available in color at *Journal of Crustacean Biology* online.

vertical suture beginning on posterior margin of epibranchial tooth (Figs. 2D, 5B). Sternal sulcus S2/S3 completely crossing sternum, slightly arched; S3/S4 incomplete, reduced to 2 small side notches (Figs. 2D, 5J). Episternal sulci S4/E4, S5/E5, S6/E6 complete, S7/E7 missing (Figs. 2D, 5J).

Mandibular palp 2-segmented, terminal segment (TS) bilobed, with small distinct anterior lobe  $(0.25 \times \text{TS length})$  (Fig. 8B). Third maxillipeds filling entire buccal cavern, except for transversely oval efferent respiratory openings in superior lateral corners (Fig. 8F); exopod lacking flagellum; ischium with distinct vertical sulcus (Fig. 8F).

Chelipeds of adult male greatly unequal (Figs. 2C, D, 3E, F). Fingers slim, elongated; movable finger (dactylus) arched, with large tooth, enclosing oval interspace when closed (Fig. 3E); pollex of fixed finger (propodus) with 3 jagged, large teeth proximally, 2 distinct teeth distally interspersed by row of small teeth (Fig. 3E). Minor cheliped with occluding margins of each finger lined by row of small teeth, enclosing oval interspace when closed (Fig. 3F). Cheliped carpus with 2 large, unequal teeth, distal tooth bigger than proximal tooth (Figs. 2C, 6K). Medial inferior margin of merus of right, left chelipeds identical, both with large tooth onethird from distal margin followed by row of small teeth (Figs. 2D, 6C, D). Walking legs (pereiopods P2–P5) moderately slender, posterior margins of propodi serrated with small, blunt teeth, dactyli tapering, each bearing rows of downward-pointing large sharp spines (Figs. 2C, D). Male pleon triangular, sides not indented, telson (A7) rounded at distal margin (Figs. 2D, 5J). G1 TA extremely long, almost as long as SS (TA/SS 0.98), tip long (Figs. 7D, E), G1 TA proximal half straight, distal half directed outward ending in long tip with long setae along ventral margin (Figs. 7D, E). G1 SS slightly longer than TA with long marginal setae (Figs. 7D, E). G2 SS robust at base, longer than G1 SS, reaching middle of G1 TA; G2 TA extremely short (TA/SS 0.20), broadened, tip short (Fig. 7F).

Size: A small-size species, adult size range CW 23.4-30 mm.

Color in life: Dorsal carapace and perciopods P1–P5 all dark purple/brown (Fig. 4C).

*Etymology:* The species is named for Mundemba, the closest town to the type locality in Korup National Park, Cameroon. The species epithet is a noun in apposition.

*Habitat: Buea mundemba* **n. sp**. is found in rivers and streams in Korup National Park in Southwest Cameroon (8°42'N to 9°16'E) that comprises more than 1,260 km<sup>2</sup> of closed canopy lowland primary forest (Fig. 4D).

*Nomenclatural statement:* A life science identifier (LSID) number was obtained for the new species: urn:lsid:zoobank. org:pub:399269F4-4FA2-4858-ADE0-C281CBF79278.



**Figure 4.** Dorsal view of freshly caught (**A**) and habitat (**B**) of adult male of *Buea bangem* **n. sp.** (CW 26.54 mm; ZMB Crust. 30325), Bakossi National Park, Southwest Region, Cameroon. Dorsal view of freshly caught (**C**) and habitat (**D**) of adult male of *B. mundemba* **n. sp.** (ZMB Crust. 30321), Korup National Park, Southwest Region, Cameroon. Dorsal view of freshly caught (**E**) and habitat (**F**) of adult male of *B. nlonako* **n. sp.** (ZMB Crust. 31786), Nlonako Ecological Reserve, Littoral region, Cameroon. This figure is available in color at *Journal of Crustacean Biology* online.

# **Buea nlonako** Mvogo Ndongo, von Rintelen & Cumberlidge **n. sp.**

## (Figs. 2E, F, 3G, H, 4E, 5C, G, K, 6E, F, J, 7G–I, 8C, G)

*Type material:* Holotype adult male (CW 25.80 mm, CL 17.80 mm, CH 10.55 mm, FW 7.41 mm), Cameroon, Littoral region, Mt. Nlonako Ecological Reserve (04°54′14.7″N, 09°58′16.4″E), 1,500 m asl, 25 May 2018, coll. P. A. Mvogo Ndongo (ZMB Crust. 31786).

*Paratypes:* 3 adult males (CW 22.17 mm, CL 15.93 mm, CH 9.09 mm, FW 7.17 mm; CW 22.21 mm, CL 15.82 mm, CH 8.18 mm, FW 6.76 mm; CW 20.91 mm, CL 14.67 mm, CH 7.74 mm, FW 6.29 mm); 2 adult females (CW 30.10 mm, CL 21.78 mm, CH 11.04 mm, FW 8.43 mm; CW 25.12 mm, CL 17.13 mm, CH 9.58 mm, FW 7.17 mm), Littoral region, Mt. Nlonako Ecological Reserve (04°53′33.04″N, 09°59′14.0″E), 900 m asl, 26 May 2018, coll. P. A. Mvogo Ndongo (ZMB Crust. 31787).

*Other material examined:* 2 adult males (CW 23.31 mm, CL 16.44 mm, CH 8.59 mm, FW 7.60 mm; CW 24.00 mm, CL 17.24 mm, CH 9.30 mm, FW 7.30 mm); 3 adult females (CW 27.47 mm, CL 19.21 mm, CH 10.88 mm, FW 8.24 mm; CW 25.67 mm, CL 18.22 mm, CH 9.80 mm, FW 7.87 mm; CW

26.06 mm, CL 18.56 mm, CH 10.12 mm, FW 7.38 mm); sub-adult male (CW 16.76 mm, CL 12.60 mm, CH 6.39 mm, FW 5.82 mm), Littoral region, Mt. Nlonako Ecological Reserve (04°53′33.4″N, 09°59′14.0″E), 900 m asl, 26 May 2018, coll. P. A. Mvogo Ndongo (IFAS-012).

Diagnosis: Carapace medium height (CH/FW 1.28, N=12), anterior carapace surface completely smooth; cervical, urogastric, cardiac, branchial grooves distinct, very deep (Figs. 2E, 5G); postfrontal crest complete, meeting anterolateral margins (Fig. 5C); vertical (pleural) groove on carapace branchiostegite wall faint or absent; carapace branchiostegite lacking vertical (pleural groove) (Fig. 2F, 5C). Sternal sulcus S2/S3 horizontal, completely crossing sternum (Figs. 2F, 5K). Major cheliped dactylus stout, relatively straight, lined by small teeth, medium size tooth in middle, pollex of propodus with 3 large teeth proximally, followed by smaller teeth interspersed by 2 distinct teeth (Fig. 3G). Lower margin of cheliped merus lined by pointed teeth, distal tooth largest (sharp spine) (Figs. 2F, 6E, F). Terminal segment (TS) of mandibular palp bilobed, with small but distinct setae-fringed anterior lobe (0.1× TS length) (Fig. 8C). G1 TA broad, long, but not equal to SS length (TA/SS 0.70) (Figs. 7G, H); G2 TA extremely short (TA/SS 0.18) (Fig. 7I).

*Description:* Carapace ovoid, medium height (CH/FW 1.28,  $\mathcal{N} = 12$ ), width more than 3× FW (CW/FW 3.31,  $\mathcal{N} = 12$ ); carapace surface smooth, anterior corners of carapace each with



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Figure 5. Frontal view showing details of the carapace of adult males of species of *Buea: B. bangem* **n. sp.** (CW 26.54 mm; ZMB Crust. 30325) (**A**); *B. mundemba* **n. sp.** (CW 26.15 mm; ZMB Crust. 30321) (**B**); *B. nlonako* **n. sp** (CW 25.80 mm; ZMB Crust. 31786) (**C**); *B. asylos* (CW 27.6 mm; NHM 1994.587) (Cumberlidge *et al.*, 2019: fig. 4a) (**D**). Dorsal view showing details of the carapace of adult males of species of *Buea: B. bangem* **n. sp.** (CW 26.54 mm; ZMB Crust. 30325) (**E**); *B. mundemba* **n. sp.** (CW 26.15 mm; ZMB Crust. 30321) (**F**); *B. nlonako* **n. sp.** (CW 25.80 mm; ZMB Crust. 31786) (**G**); *B. asylos* (CW 27.6 mm; NHM 1994.587) (Cumberlidge *et al.*, 2019: fig. 1a) (**H**). Ventral view showing details of the anterior thoracic sternum and distal part of the pleon of adult males of species of *Buea: B. bangem* **n. sp.** (CW 26.54 mm; ZMB Crust. 30325) (**I**); *B. mundemba* **n. sp.** (CW 25.80 mm; ZMB Crust. 30321) (**J**); *B. nlonako* **n. sp.** (CW 25.80 mm; ZMB Crust. 31786) (**K**); *B. asylos* (CW 27.6 mm; NHM 1994.587) (Cumberlidge *et al.*, 2019: fig. 1b) (**L**). This figure is available in color at *Journal of Crustacean Biology* online.



Figure 6. Dorsal view of the cheliped merus of adult males of species of *Buea*: *B. asylos* (CW 27.6 mm; NHM 1994.587) (Cumberlidge *et al.*, 2019: fig. 6a, b) (**A**, **B**); *B. mundemba* **n. sp.** (CW 26.15 mm; ZMB Crust. 30321) (**C**, **D**); *B. nlonako* **n. sp** (CW 25.80 mm; ZMB Crust. 31786) (**E**, **F**); *B. bangem* **n. sp.** (CW 26.54 mm; ZMB Crust. 30325) (**G**, **H**). Dorsal view of the cheliped carpus of adult males of species of *Buea*: *B. bangem* **n. sp.** (CW 26.54 mm; ZMB Crust. 30325) (**J**); *B. nlonako* **n. sp.** (CW 26.15 mm; ZMB Crust. 30325) (**J**); *B. nlonako* **n. sp.** (CW 26.15 mm; ZMB Crust. 30321) (**K**); *B. asylos* (CW 27.6 mm; NHM 1994.587) (Cumberlidge *et al.*, 2019: fig. 5g) (**L**).



Figure 7. Dorsal view of left (**D**, **G**, **J**) and right (**A**) and ventral view of left (**E**, **H**, **K**) and right (**B**) G1 of adult males of species of *Buea*: *B. bangem* **n. sp.** (CW 26.54 mm; ZMB Crust. 30325) (**A**, **B**); *B. mundemba* **n. sp.** (CW 26.15 mm; ZMB Crust. 30321) (**D**, **E**); *B. nlonako* **n. sp.** (CW 25.80 mm; ZMB Crust. 31786)) (**G**, **H**); *B. asylos* (CW 27.6 mm; NHM 1994.587) (Cumberlidge *et al.*, 2019: fig. 7a, d) (**J**, **K**). G 2 of adult males of species of *Buea*: *B. bangem* **n. sp.** (CW 26.54 mm; ZMB Crust. 30325) (**C**); *B. mundemba* **n. sp.** (CW 26.15 mm; (ZMB Crust. 30321) (**F**); *B. nlonako* **n. sp.** (CW 25.80 mm; ZMB Crust. 31786) (**I**); *B. asylos* (CW 27.6 mm; NHM 1994.587) (Cumberlidge *et al.*, 2019: fig. 7a, d) (**J**, **K**). G 2 of adult males of species of *Buea*: *B. bangem* **n. sp.** (CW 26.54 mm; ZMB Crust. 30325) (**C**); *B. mundemba* **n. sp.** (CW 26.15 mm; (ZMB Crust. 30321) (**F**); *B. nlonako* **n. sp.** (CW 25.80 mm; ZMB Crust. 31786) (**I**); *B. asylos* (CW 27.6 mm; NHM 1994.587) (Cumberlidge *et al.*, 2019: fig. 8a) (**L**).

field of carinae; cervical, urogastric, cardiac, branchial grooves distinct, very deep (Figs. 2E, 5G); postfrontal crest distinct, complete, lateral ends meeting anterolateral margins (Figs. 2E, 5C, G); exorbital angle low, clearly distinct, intermediate, epibranchial teeth each reduced to small granule (Fig. 5C); anterolateral margin behind epibranchial tooth with small granules (Fig. 5C). Carapace branchiostegite with longitudinal suture dividing wall into 2 regions, lacking vertical (pleural) groove (Figs. 2F, 5C). Margin of sternum with setae; sternal sulcus S2/S3 horizontal, completely crossing sternum; S3/S4 incomplete, reduced to 2 small side notches (Figs. 2F, 5K); episternal sulci S4/E4, S5/E5, S6/E6 complete, S7/E7 missing (Figs. 2F, 5K).

Chelipeds of adult male greatly unequal (Figs. 2E, F, 3G, H). Fingers slim, elongated; movable finger (dactylus) stout, straight, lined by small teeth, medium size tooth in middle, pollex of propodus with 3 large teeth proximally, followed by smaller teeth interspersed by 2 distinct teeth (Fig. 3G). Minor cheliped dentition similar to major cheliped but smaller, enclosing oval interspace



Figure 8. Frontal view of the left mandible of adult males of species of *Buea: B. bangem* **n. sp.** (CW 26.54 mm; ZMB Crust. 30325) (**A**); *B. mundemba* **n. sp.** (CW 26.15 mm; ZMB Crust. 30321) (**B**); *B. nlonako* **n. sp.** (CW 25.80 mm; ZMB Crust. 31786) (**C**); *B. asylos* (CW 27.6 mm; NHM 1994.587) (Cumberlidge et al., 2019: fig. 8g) (**D**). Frontal view of the left third maxilliped of adult males of species of *Buea: B. bangem* **n. sp.** (CW 26.54 mm; ZMB Crust. 30325) (**E**); *B. mundemba* **n. sp.** (CW 26.15 mm; ZMB Crust. 30321) (**F**); *B. nlonako* **n. sp.** (CW 25.80 mm; ZMB Crust. 31786) (**G**); *B. asylos* (CW 27.6 mm; NHM 1994.587) (Cumberlidge et al., 2019: fig. 8d) (**H**).

when closed (Figs. 3H). Cheliped carpus with 2 large, unequal teeth, distal tooth largest, proximal tooth smallest (Figs. 6J). Medial inferior margin of cheliped merus with large distal tooth, lined by small pointed teeth proximally (Figs. 2F, 6E, F). Walking legs (pereiopods P2–P5) moderate length (not unusually elong-ated), posterior margins of propodi lined by small pointed teeth, dactyli tapering, each bearing rows of downward-pointing large sharp spines, tip with long spine (Figs. 2E, F).

Mandibular palp 2-segmented, terminal segment (TS) bilobed, with short but distinct setae-fringed anterior lobe  $(0.1 \times \text{TS length})$ (Fig. 8C). Third maxillipeds filling entire buccal cavern, except for transversely oval efferent respiratory openings in superior lateral corners (Fig. 8G); ischium with distinct vertical sulcus; third maxilliped exopod either completely lacking flagellum, or with extremely reduced short process (Fig. 8G).

Male pleon triangular, sides not indented, telson (A7) rounded at distal margin (Figs. 2F, 5K). G1 TA long (TA/SS 0.70), proximal half straight, robust, distal half directed outward tapering evenly to pointed tip, G1 TA with long setae lining ventral margin (Figs. 7G, H). G2 SS robust at base, tapering to long thin rod, reaching middle of G1 TA. G2 TA extremely short (TA/SS 0.18), slender (Fig. 7I).

Size: A medium-size species, adult size range CW 20.9-30.1 mm.

*Color in life*: Dorsal carapace and appendages all dark brown; P1, chelipeds dark brown with white fingertips (Fig. 4E).

*Type locality:* Nlonako Wildlife Reserve, Nkongsamba, Littoral region, Cameroon.

*Etymology:* The new species is named for the Nlonako Wildlife Reserve. The species epithet is a noun in apposition.

*Habitat: Buea nlonako* **n. sp**. is known only from the Nlonako Wildlife Reserve, which includes Mt. Nlonako, which rises from 400 m asl on its southern side to 1,825 m at its peak (Fig. 4G). The reserve lies in the Littoral region of Cameroon, which is one of the most threatened tropical rainforest habitats in this part of Africa.

*Nomenclatural statement:* A life science identifier (LSID) number was obtained for the new species: urn:lsid:zoobank. org:pub:399269F4-4FA2-4858-ADE0-C281CBF79278.

#### DISCUSSION

The three new species described in this study are consistent with the diagnostic characters of *Buea* (Cumberlidge *et al.*, 2019). The four species can be distinguished from each other by comparing a number of characters (Table 2), including the texture of the anterior corners of the carapace surface; the cervical, urogastric, cardiac, branchial carapace grooves; the position of the vertical (pleural) groove on the carapace wall; the shape of the major cheliped dactylus; the dentition of the pollex of the major cheliped propodus; and the length and aspect of the TA of both G1 and G2.

A phylogenetic tree (Fig. 9) based on 1,873 base pairs representing the combined partial sequences of three mtDNA markers (COI, 16S RNA, 12S RNA) recovered all four species of *Buea* as a single clade with strong BI and ML confidence values (1/100 at this node). Three of the species of *Buea* (*B. bangem* **n. sp.**, *B. nlonako* **n. sp.** and *B. asylos* **n. sp.**) form well-supported independent lineages, whereas *B. mundemba* **n. sp.** is basal and unresolved with respect to *B. nlonako* **n. sp.** Incomplete lineage sorting might be invoked as an explanation and this hypothesis should be tested in the future using nuclear markers.

#### Table 2. Differences between the four species of Buea

| Characters   | <b>Buea asylos (</b> Cumberlidge, 1993)   | <i>Buea nlonako</i> n. sp.   | Buea bangem n. sp.   | Buea mudemba n. sp.  |
|--|---|--|--|--|
| Carapace height  | Medium (CH/FW 1.10)<br>(Figs. 3A, 5H)   | Medium (CH/FW 1.28)<br>(Figs. 2E, 5G)  | High (CH/FW 1.43)<br>(Figs. 2A, 5E)  | High (CH/FW 1.45) (Figs. 2C, 5F)   |
| Texture of anterior<br>corners of cara-<br>pace surface            | Smooth (Figs. 3A, 5H)   | Smooth (Figs. 2E, 5G)  | Highly granulated (Figs. 2A, 5E)   | Slightly granulated (Figs. 2C, 5F)   |
| Cervical,<br>urogastric, cardiac,<br>branchial carapace<br>grooves | Faint or absent (Figs. 3A,<br>5H)   | Distinct and very deep<br>(Figs. 2E, 5G)   | Distinct and shallow<br>(Figs. 2A, 5E)   | Distinct and shallow (Figs. 2C, 5F)  |
| Postfrontal crest  | Incomplete, not meeting<br>anterolateral margins<br>(Figs. 3A, 5D)  | Complete, lateral ends<br>meeting anterolateral mar-<br>gins (Figs. 2E, 5C, G)   | Complete, clearly defined,<br>meeting anterolateral mar-<br>gins at epibranchial teeth<br>(Figs. 2A, 5A, E)  | Complete, lateral ends meeting<br>anterolateral margins (Figs. 2C,<br>5B, 5F)  |
| Vertical (pleural)<br>groove on cara-<br>pace wall                 | Faint (Figs. 3A, 5D)  | Absent (Fig. 2F, 5C)   | Distinct (Figs. 2B, 5A)  | Distinct (Figs. 2D, 5B)  |
| Sternal sulcus<br>S2/S3  | Straight, deep, ends not<br>meeting side margins of<br>sternum (Figs. 3A, 5L)                                   | Straight, ends meeting<br>side margins of sternum<br>(Fig. 2F, 5K)   | Slightly arched, ends<br>meeting side margins of<br>sternum (Figs. 2B, 5I)   | Slightly arched, ends meeting<br>side margins of sternum (Figs. 2D,<br>5J)   |
| Major cheliped<br>dactylus   | Broad, not arched, with blunt<br>tooth (Fig. 3I)  | Stout, relatively straight,<br>lined by small teeth, me-<br>dium size tooth in middle<br>(Fig. 3G).  | Stout, slender and slightly<br>arched, lined by small<br>teeth, with one large tooth<br>in middle (Fig. 3E).   | Stout, arched, lined by small teeth, one large pointed tooth in middle (Fig. 3C)   |
| Major cheliped<br>propodus   | Ventral margin of the palm<br>slightly concave, 3 large<br>teeth proximally, one tooth<br>medially (Fig. 3I)    | Ventral margin of the palm<br>slightly concave, one large<br>(molar) tricuspid tooth<br>proximally, followed by 4<br>distinct teeth interspersed<br>by smaller teeth (Fig. 3G) | Ventral margin of the palm<br>strongly concave, one<br>large (molar) bicuspid<br>tooth proximally, and one<br>large pointed tooth in<br>middle (Fig. 3E) | Ventral margin of the palm slightly<br>concave, one large (molar)<br>tricuspid tooth proximally, followed<br>by 4 distinct teeth interspersed by<br>smaller teeth (Fig. 3C). |
| G1 TA and G1 SS  | G1 TA long (TA/SS 0.86),<br>broadened in mid-section,<br>with long marginal setae and<br>long tip (Figs. 7J, K) | G1 TA long (TA/SS 0.70),<br>broadened in mid-section,<br>with long marginal setae<br>and long tip (Fig. 7G, H)   | G1 TA extremely long (TA/<br>SS 1.00), broadened in<br>mid-section, with long<br>marginal setae and short<br>tip (Figs. 7A, B)                           | G1 TA extremely long (TA/SS<br>0.98), broadened in mid-section,<br>with long marginal setae and long<br>tip (Fig. 7D, E)   |
| G2 TA  | Short (TA/SS 0.23) (Fig. 7L)  | Extremely short (TA/SS 0.18), slender (Fig. 7I).   | Extremely short (TA/SS 0.12), medium width, with short tip (Fig. 7C).  | Short (TA/SS 0.20), broad, with short tip (Fig. 7F).   |

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Uncorrected p-distances based on COI sequence data ranged from 1.8% to 13.2% among species of *Buea*, and 0% to 2.5% within species (Table 3). The genetic relationship between *B. bangem* **n. sp.**, *B. mundemba* **n. sp.**, and *B. nlonako* **n. sp.** as described above may reflect adaptations to differences in their habitats: *B. bangem* **n. sp.** and *B. mundemba* **n. sp.** prefer rivers and streams, whereas *B. nlonako* **n. sp.** is more semi-terrestrial, and is found in puddles near small permanent streams, under fallen leaves, under stones, and in burrows sited close to water bodies (a habitat preference that is similar to that of *Louisea nkongsamba* Mvogo Ndongo, von Rintelen & Cumberlidge, 2019).

*Conservation status and threats:* Recent discoveries of new species and genera of freshwater crabs in Southwest Cameroon (Cumberlidge, 1999; Mvogo Ndongo *et al.*, 2017a, c, 2018, 2019) have established that this is one of the most species-rich areas on the African continent. The conservation status of the newly discovered species have yet to be determined, but we do know that *B. asylos* (as *Potamonemus asylos*) was judged to be Data Deficient

(DD) according to the International Union for the Conservation of Nature (IUCN) Red List due to a lack of basic biological information required to carry out the protocols (Cumberlidge, 2008; Cumberlidge et al., 2009). Similarly, it is likely that the extinction risk of the three new species (B. bangem n. sp., B. mundemba n. sp., and B. nlonako n. sp.) will also prove to be DD. Our surveys of the freshwater decapod fauna throughout Southwest Cameroon, however, indicate that there are numerous immediate threats to freshwater species including habitat encroachment, habitat destruction from deforestation and agriculture, as well as water pollution from pesticides and herbicides (Mvogo Ndongo et al., 2017a, c, 2018). There is, therefore, an urgent need for more research aimed at assessing the conservation status of these potentially threatened species of freshwater crabs. New surveys of this biodiversity hotspot in Cameroon would be expected to discover new species, refine species distributions, define specific habitat requirements, describe population levels and trends, assess status, identify specific threats, and suggest target areas for conservation actions.



Figure 9. Maximum likelihood (ML) tree topology for the freshwater crab taxa from Cameroon included in this study derived from mtDNA sequences corresponding to three loci (partial 16S rRNA, COI, and 12S rRNA genes). Bayesian Inference (BI) and ML statistical values (%) on the nodes indicate bootstrap support and posterior probabilities, respectively.

Table 3. The pairwise uncorrected p-distance (%) of the COI partial sequences of species of Buea.

| Species                   | Maximum distance<br>within species (%) | Distance range<br>between species (%) |                         |                          |                    |
|---------------------------|--|---------------------------------------|-------------------------|--------------------------|--------------------|
|                           |  | Buea asylos                           | B. bangem <b>n. sp.</b> | B. nlonako <b>n. sp.</b> | B. mundemba n. sp. |
| Buea asylos               | _                                      | -                                     |                         |                          |                    |
| B. bangem <b>n. sp.</b>   | 2.5                                    | 10.5–13.2                             | -                       |                          |                    |
| B. nlonako <b>n. sp.</b>  | 0.0                                    | 12.3–12.4                             | 6.4-8.8                 | -                        |                    |
| B. mundemba <b>n. sp.</b> | 0.6                                    | 7.7–10.3                              | 2.6-8.8                 | 1.8–3.9                  | -                  |

# KEY TO THE SPECIES OF BUEA

- Carapace highly arched (CH/FW 1.4–1.5), anterior carapace surface with fields of granules and carinae; distinct vertical (pleural) groove on carapace branchiostegite wall; thoracic sternal sulcus S2/S3 slightly arched; G1 TA extremely long, equal to SS length (TA/SS 1.0)
- Major cheliped propodus inferior margin distinctly concave; pollex with three evenly-spaced large teeth (Fig. 3C) ...... B. bangem n. sp.
- Major cheliped propodus inferior margin straight or only slightly indented; pollex with large molar proximally, followed by 4 distinct teeth interspersed by smaller teeth (Fig. 3E)
  B. mundemba n. sp.
- **3.** Cervical, urogastric, cardiac, branchial carapace grooves either faint or absent (fig. 3A); postfrontal crest incomplete, not meeting anterolateral margins (Figs. 3A, 5D, H); major cheliped dactylus with 3 large teeth proximally, one tooth medially (3I); pollex of propodus with molar consisting of 3

## ACKNOWLEDGEMENTS

We thank the Rufford Small Grant Foundation (24245-B, 20242-2, 17672-1) for funding the field work in south and Southwest Cameroon, and the Museum für Naturkunde, Berlin (MfN) for funding the first author during a 2018 research visit to Germany. We are also grateful to Bernhard Schurian, Lukas Kirschey, and Robert Schreiber (MfN) for their important collaboration during the first author's visit. We are also grateful to Drs. Charles Fransen (Naturalis Biodiversity Center, Leiden, The Netherlands), Tomoyuki Komai (Natural History Museum and Institute, Chiba, Japan), Kristina von Rintelen (MfN), Christian Albrecht (University of Giessen, Germany), Christoph D. Schubart (University of

Regensburg, Germany), and Joseph L. Tamesse (University of Yaounde 1, Cameroon) who provided constructive advice to the first author during the course of the project from 2015 to 2018. The field surveys were conducted under permits 0265/L/MINFOF/ SG/DFAP/SDAP/SPN/BSVE issued 18 January 18 2017 and 1698/L/MINFOF/SETAT/SG/DFAP/SDAP/SPN/BSVE issued April 12, 2018 by the Ministry of Forestry and Wildlife of Cameroon. We thank Dr. Andrew Polaszek for kindly granting us permission to reproduce the figures of *Buea asylos* originally published in *Journal of Natural History*.

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