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Intraspecific variations and phylogenetic relationships of threatened endemic Cameroonian freshwater crab species assigned to *Louisea* Cumberlidge, 1994 (Crustacea, Brachyura, Potamonautidae), with recommendations for conservation status

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Running title: Intraspecific variation and phylogenetic relationships of *Louisea* species

Abstract

The Cameroonian freshwater crab genus *Louisea* Cumberlidge, 1994 currently includes four threatened species: *L. edeaensis* (Bott, 1969) from Lake Ossa Island, *L. balssi* (Bott, 1959) from Mt. Manengouba, *L. yabassi* Mvogo Ndongo, von Rintelen & Cumberlidge, 2019 from the Ebo Forest zone, and *L. nkongsamba* Mvogo Ndongo, von Rintelen & Cumberlidge, 2019 from the Nlonako Ecological Reserve. We report here on collections of specimens of *L. yabassi* (from two new localities) and *L. nkongsamba* (from six new localities). The phylogenetic relationships of the four species of *Louisea* are currently unresolved. Here, we describe morphological variation within populations of both *L. nkongsamba* and *L. yabassi* that are supported by differences in characters of the carapace, mandible, thoracic sternum, and male second gonopods. In addition, three genetic lineages were found within *L. nkongsamba* that are supported by uncorrected *p*-distance and the haplotype network. No correlation, however, was found between the morphotypes within the species and the genetic lineages grouping populations of both of these species. Phylogenetic analyses based on three mtDNA loci (COI, 12S rRNA, and 16S rRNA) revealed divergence times of 5.6 million years ago (Ma) for when *L. edeaensis* diverged from *L. balssi*, 4.1 MA for when *L. yabassi* diverged from *L. nkongsamba* and *L.*

edeaensis, and 2.48 MA for when *L. yabassi* diverged from *L. nkongsamba*. Immediate threats to these rare and threatened species of freshwater crabs and to the rainforest ecosystem upon which they depend include deforestation, habitat fragmentation, agricultural encroachment, pollution and firewood gathering. Our results indicate that IUCN Red List reassessments of extinction risk for these four species would likely result in changes of category for *L. balssi* and *L. edeaensis* (both might be upgraded from EN to critically endangered (CR)), and first-time assessments for *L. yabassi* and *L. nkongsamba* would likely place them both in the endangered (EN) category. Recognition of species boundaries and of subpopulations of species will prove useful when making informed conservation decisions as part of the development of species action plans.

Key words: critically endangered, intraspecific variation, morphotypes, phylogenetic analysis

Introduction

The freshwater crab genus *Louisea* is endemic to remote forested ecosystems in Cameroon and was established by Cumberlidge (1994) and revised by Mvogo Ndongo et al. (2019) in order to accommodate four species: *L balssi* (Bott, 1959), *L. edeaensis* (Bott, 1969), *L. nkongsamba* Mvogo Ndongo, von Rintelen & Cumberlidge, 2019, and *L. yabassi* Mvogo Ndongo, von Rintelen & Cumberlidge, 2019. Both *L balssi*, *L. edeaensis* have been revised recently based on new material collected in Cameroon (Mvogo Ndongo et al. 2017a, b, 2018, 2019). *Louisea nkongsamba* was described from specimens collected from Mt. Nlonako in 2018, while *L. yabassi* was described only from two specimens collected in 1908 at Yabassi by German researchers during colonial period and deposited in the Museum für Naturkunde (ZMB), Berlin, Germany (Mvogo Ndongo et al. 2019). Recently, Mvogo Ndongo et al. (2021a), discovered new and threatened populations of *L. yabassi* living in streams in the Ebo Forest near Yabassi, Cameroon, and provided recommendations for their conservation. Other works on Cameroon fauna have focused on taxonomy, phylogenetic relationships, and conservation, but none has included all four species of the genus *Louisea* (e.g., Daniels et al. 2015, Mvogo Ndongo et al. 2017a, b, c, 2018, 2019, 2020, 2021b).

The genus *Louisea* includes some of the smallest freshwater crab species found in Africa (adult at CWs between 20 and 24 mm) (Cumberlidge 1994, 1999, Mvogo Ndongo et al. 2017b, 2017c, 2018, 2019). The present work is based on new collections of *L. yabassi* from two sites in the Ebo Forest and of *L. nkongsamba* from six localities in the Nlonako Ecological Reserve (Mvogo Ndongo et al. 2019, 2021a). These populations are compared with similar data from *L. balssi* from populations at Kumba and Mt. Manengouba (Cumberlidge 1994, 1999, Mvogo Ndongo et al. 2017, 2018, 2019) and *L. edeaensis* from populations at Yaounde, Edea, and Lake Ossa (Cumberlidge 1994, 1999, Mvogo Ndongo et al. 2017a, 2017c, 2019). This study is the first to include morphological and molecular data from all four species of *Louisea*.

The four species of *Louisea* are rare and threatened freshwater crabs that are all endemic to Cameroon and all are of great conservation importance because they are restricted-range endemics that are subjected to immediate threats from human activities (Cumberlidge 1994, 1999, Mvogo Ndongo et al. 2017b, 2017c, 2018, 2019, 2021a). These species are an important part of the fauna of a key biodiversity hotspot in southwestern Cameroon that is home to a number of other endemic and charismatic species of invertebrates and vertebrates found in both terrestrial and aquatic habitats (Cumberlidge 1994, 1999, Mvogo Ndongo et al. 2017, 2018, 2019, 2021a). For example, the aquatic ecosystems of this biodiversity hotspot are important spawning grounds for fish and invertebrates, and the forests are refuges for charismatic wildlife including monkeys, chimpanzees, gorillas, manatees, elephants, birds, bats, turtles, snakes, and amphibians (Gonwouo et al. 2006, Herrmann et al. 2005, Morgan and Abwe 2006, Morgan et al. 2013, Mvogo Ndongo et al. 2017, 2018, 2019, 2021a).

Louisea yabassi is endemic to Yabassi and the Ebo Forest, *L. nkongsamba* is endemic to Mount Nlonako, *L. balssi* is endemic to the area between Kumba and Mount Manengouba, and *L. edeaensis* is found between Yaounde, Edea, and Lake Ossa. The habitats in all four of these locations are threatened by encroachment from foresters, fishermen, and farmers, and any environmental changes arising from anthropogenic sources may threaten these endangered freshwater crabs. Changes in these populations can be detected by morphological and molecular studies, and these are likely to be of importance when considering long term conservation interventions (Stearns 1989, Griffiths et al. 2000).

The aim of the present work is to study intraspecific variation of morphological characters as a means of identifying species boundaries within *Louisea*, and to evaluate the genetic distance between the four species using molecular data. Accurate species delimitation is necessary for understanding levels of biodiversity and for adopting effective conservation and sustainable management strategies (Cornetti et al. 2015). The results of this study will enable the development of species action plans aimed at the conservation of these rare threatened species of endemic Cameroonian freshwater crabs.

Material and methods

Sample collection

Four species of *Louisea* were collected from four different locations in southwestern Cameroon between 2015 and 2021 and were each identified by reference to the identification keys provided by Cumberlidge (1994, 1999) and Mvogo Ndongo et al. (2019). The four species are from Mt. Nlonako (50 specimens of *L. nkongsamba*), Ebo Forest near Yabassi (35 specimens of *L. yabassi*), Bedimet Island in Lake Ossa (30 specimens of *L. edeaensis*), and Mt. Manengouba (8 specimens of *L. balssi*). All specimens of the two species collected in the present study (*L. nkongsamba* from Mt. Nlonako and *L. yabassi* from Ebo Forest) were measured, their gender and life stage (juvenile, sub adult, adult) recorded, the precise GIS location noted, and the details of their habitat noted. Descriptive

morphometrics of specimens of *L. edeaensis* (from Lake Ossa, 90 m asl) and *L. balssi* (from Mount Manengouba, 1,958 m asl) are given in Mvogo Ndongo et al. (2019: Tables 2 and 3 respectively) (Mvogo Ndongo et al. 2017, 2018, 2019). Most collected specimens were returned alive and unharmed to the place where they were collected after recording all relevant morphological data. A few adult specimens (males and females) were preserved in ethanol for further morphological descriptions, and single walking legs of selected specimens were removed and preserved in ethanol for further molecular analysis. The newly collected specimens were deposited either in the Museum Für Naturkunde, Berlin, Germany (ZMB) or the Unity of Taxonomy, Production and Sustainable Management of Aquatic Animals, in the Department of Management of Aquatic Ecosystems, Institut des Sciences Halieutiques, University of Douala, Cameroon (LABO-PASMAT). The terminology used follows Cumberlidge (1999) and the classification follows Cumberlidge and Daniels (2022). 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, and the software LAS V4 and Helicon Focus 6.7.1. Post processing was undertaken using Adobe Photoshop CC5.

Morphological analysis

Measurements of the carapace and gonopods of all specimens collected were made with digital calipers and the results recorded in millimetres (mm). Specimens were sorted according to their stage of development into juveniles, subadults, and adults. at which maturity is reached (signified by identifying specimens that had undergone the pubertal moult from subadult to adult). The pubertal moult was determined by examining the degree of development of the pleon of a series of juvenile, subadult and adult females. The pleon of juvenile females is undeveloped and resembles the slim pleon of juvenile males; the pleon of subadults is significantly widened and partially covers the thoracic sternum, while the pleon of adult females is dramatically enlarged and rounded such that its lateral margins overlap the coxae of the pereiopods and the telson covers S1–2. The lower limit of the range for the pubertal moult was judged as the CW of the largest non-adult female, while the upper limit of the pubertal moult was judged to be the CW of the smallest adult female specimen.

Molecular analysis

Genomic DNA was extracted from a tissue sample of up to 25 mg cut from the pereiopod muscle of 70% ethanol-preserved specimens using the Qiagen DNeasy Blood & Tissue kit following the manufacturer's instructions. Polymerase chain reaction (PCR) was used to amplify three mitochondrial gene fragments. A ~ 638 bp region of the 16S ribosomal RNA gene (16S) using primers 16L29 and 16HLeu (Schubart 2009), a ~ 594 bp region of the 12S ribosomal RNA gene (12S) using primers 12L4 and 12H2 (Schubart 2009), and a 648 bp region of the protein-coding mitochondrial

gene, Cytochrome Oxidase subunit I gene (COI) using primers LCOI-1490 and HCOI-2198 (Folmer et al. 1994). PCR was performed in 25 µl volumes containing 1x Taq buffer, 1.5 mM $MgCl_2$, 200 µM each dNTP, 1 U Taq polymerase, ca. 50–100 mg DNA and ddH_2O 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, 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 for purification and cycle sequencing of both strands of each gene. The sequences obtained were proofread manually using Chromas Lite (v. 2.1.1) (Technelysium Pty Ltd, Queensland, Australia) and aligned with ClustalW (Thompson et al. 1994) implemented in BioEdit 7.0.5 (Hall 1999). New sequences were submitted to the National Center for Biotechnology Information (NCBI) and are available from GenBank under the accession numbers in Table 1 (pending). Results from these genes were concatenated into a single alignment that was then converted into a Nexus file with FaBox (Villesen 2007).

Phylogeographic investigations

The COX1 mitochondrial gene was used here that is relatively variable and is commonly used for population genetics, and more recently also for animal species identification within the barcoding approach (Hebert et al. 2003). This was useful to examine the population genetic structure of *L. nkongsamba*, providing evidence for genetic substructure among the sampling sites in Nlonako Ecological Reserve. That was critical to investigate historical connectivity among populations of *Louisea* species and to implement the future management of genetic diversity.

In order to graphically depict the genetic distance between the mitochondrial genotypes, a maximum parsimony genotype networks (Templeton et al. 1992) were built with the software PopArt (Leigh and Bryant 2015). Haplotype and nucleotide diversities were used to compare genetic diversities among the sampling sites in terms of number of haplotypes and genetic distances of these haplotypes. Phylogeographic investigations have been successfully used by several authors to determine connectivity among populations of endemic freshwater crab species (see Stemmer and Schubart (2016) for populations of *Sesarma fossarum* in the Cockpit Country of Jamaica).

Phylogenetic investigations

The mitochondrial genes COX1, 12SrRNA, 16SrRNA were useful for identification of species boundaries and examination of the evolutionary origins and relationships of the endemic Cameroonian *Louisea* freshwater crabs and to determine whether morphological and ecological similarities between species are based on convergence or common ancestry.

Here two methods of phylogenetic inference were applied to our data set: maximum likelihood (ML) using the software PAUP*, and Bayesian inference (BI) as implemented in MrBayes (v.3.3; Huelsenbeck & Ronquist, 2001) (Mvogo Ndongo et al. 2017a, b, Fratini et al. 2005). The best

evolutionary model was determined with jModeltest v.2.1.7 (Darriba et al. 2012) based on the Akaike information criterion (Posada and Buckley 2004) and resulted in the GTR+I+G (COI), GTR+G (16S) and HKY+G (12S) models. ML tree was obtained for each alignment with 1000 bootstrap pseudoreplicates. Bayesian Inference (BI) was performed to infer phylogeny by using MrBayes v. 3.2.2 (Huelsenbeck and Ronquist 2001). The MCMC was run with four independent chains for 10,000,000 generations, samplefreq = 500, and burnin = 10,001. Analyses were conducted separately to test for topology congruence.

To estimate clade divergence times based on the COX1 gene, we conducted a Bayesian analysis with the software BEAST 2.6.2 (Bouckaert et al. 2019), using a strict clock model (Yule Model) with a rate of evolution for the COX1 of 2.33% per million years (my) (10% SD) (following Schubart et al. 1998). We ran Markov chains for 10 million generations, sampling every 1000th iteration and discarding the first 25% as burn-in. Overall, 7500 trees were obtained, and these trees were used to calculate the maximum clade credibility tree in TreeAnnotator v.1.6.1 (part of the BEAST package).

Results

Morphological analyses

Morphometric measurements of populations of *L. yabassi* and *L. nkongsamba* are provided in Table 2. The adult size range of *L. yabassi*, based on male and female specimens from the two populations, was between CW 16.5 and CW 24.0 mm. Subadults ranged from CW 11.0 to CW 15.5 mm, and juveniles were CW 10.0 mm or less. No significant differences were found between the carapace proportions (CW/FW, CL/FW, CH/FW) of these populations (Table 2). The adult size range of *L. nkongsamba* based on male and female specimens from four of the six sites, was between CW 15.8 and CW 20.0 mm. Subadults ranged from CW 11.5 to CW 14.4 mm (two populations, PAMN 02.12.19 and PAMN 10.12.19, consisted entirely of subadults), and juveniles of this species measured CW 10.0 mm or less. No significant differences were found between the carapace proportions (CW/FW, CL/FW, CH/FW) of any of the populations of these two species, and these proportions were virtually identical in all cases (Table 2). There was a slight difference in the adult size range of *L. yabassi* and *L. nkongsamba*, with the former species growing to CW 24 mm and the latter species reaching only CW 20 mm.

A number of morphological differences were found between selected characters of specimens of *L. yabassi* from populations living in the two localities in Ebo Forest (Table 3). Similarly, several morphological character differences were found between specimens from 6 sites of *L. nkongsamba* that were organised into two groups, refer here as two morphotypes (Table 4). Illustrations of the

character differences between the two populations of *L. yabassi* (based on adult males) are provided in Figs. 1-5, and the two morphotypes of *L. nkongsamba* (based on adult males) are provided in Figs. 6-9. Despite these morphological differences between populations of *L. nkongsamba* and *L. yabassi*, there was no genetic support for recognising these differences as indicating different genetic lineages that would warrant formal taxonomic recognition (Figs. 10-12).

The pubertal moult estimates indicate that the largest species of *Louisea* is *L. yabassi* (CW 24 mm), the smallest species is *L. balssi* (CW 16.2 mm), while the size ranges of *L. edeaensis* and *L. nkongsamba* are similar and fall in between these values (CW 20.00 mm) (Table 3). *Louisea balssi* is a high-altitude species found at 1989 m asl, *L. nkongsamba* is a submontane species (found between 938 and 1462 m asl), while both *L. yabassi* (300 m asl) and *L. edeaensis* (90 m asl) are low-altitude species (see Mvogo Ndongo et al. 2017, 2019, 2021a) (Table 3).

Molecular analyses

For the molecular analyses, a total of 138 sequences were obtained that included 46 sequences of CO1, 46 sequences of 16S RNA, and 46 sequences of 12S RNA (Tables 1). ML and BI trees were constructed for each individual gene. The tree presented here for ML topology (Fig. 10) has been reconstructed from the concatenation of the three partial loci (CO1, 16S RNA and 12S RNA) into a single alignment that was then converted into a Nexus file with FaBox. This tree (Fig. 10) includes the four species of *Louisea* (*L. balssi*, *L. edeaensis*, *L. nkongsamba* and *L. yabassi*) as in-groups, and *Potamonemus man* Mvogo Ndongo et al., 2021, and *Buea mundemba* Mvogo Ndongo et al., 2020 as out-group species. Statistical values at the nodes on Figure 10 indicate posterior probabilities or bootstrap values from ML (left) and Bayesian inference (BI) (right). The molecular evidence presented here subdivides *L. nkongsamba* into three lineages (populations 1, 2, and 3), but these groups do not correlate with the two main groups of morphotypes (represented by Nlonako Nlonako Enguegue n°1_1462 m and Nlonako Eyimba, Ngaltongue, Enguegue n°2_1382 m, Nguegue) whose characters are listed in Table 4. For example, population 1 of *L. nkongsamba* comprised specimens that were collected in all six of the localities on Mt. Nlonako (Table 1, 5, Fig. 10); population 2 of *L. nkongsamba* comprised specimens that were collected in five out of the six localities on Mt. Nlonako (Table 1, 5, Fig. 10); and population 3 of *L. nkongsamba* comprised specimens that were collected in four out of the six localities on Mt. Nlonako (Table 1, 5, Fig. 10). The two morphotypes are represented in all three populations of *L. nkongsamba* (Table 1).

The results of the uncorrected *p*-distance between species pairs of *Louisea* are given in Table 6, and the uncorrected *p*-distances between genetic populations of *L. nkongsamba* are given in Table 7. Divergence time calculations used to investigate the divergence time of *Louisea* species (Fig. 11)

showed that *L. edeaensis* diverged from the well-established *L. balssi* about 5.6 Ma, *L. yabassi* and *L. nkongsamba* diverged from *L. edeaensis* about 4.1 Ma, and *Louisea yabassi* separated from *L. nkongsamba* about 2.48 Ma. The haplotype network also distinguishes between the populations of *Louisea* species (Fig. 12).

Discussion

Intraspecific variation

The two populations of *L. yabassi* from localities 3 kilometres apart in the Ebo Forest genetically-form a single clade with little lineage differentiation (Fig. 10: Populations 1 and 2) and these individuals show relatively low levels of morphological variation (Table 4), but nevertheless two populations with a distinct morphotype can be identified (Table 1). Similarly, the 6 sampled localities around Mt. Nlonako 4-10 kilometres apart (Tables 1, 5) where *L. nkongsamba* is found host individuals who fall into three genetically-recognisable populations (Fig. 10: Populations 1, 2 and 3), which in turn have two distinct morphotypes (Table 5). Populations 1 and 3 consisted of individuals who all conformed to morphotype 1, while Population 2 included individuals of both morphotypes (Table 1).

Phylogenetic relationships

The four *Louisea* species were recovered here as a monophyletic clade (Fig. 10) with strong topological statistical support, and high pairwise uncorrected *p*-distance values between species pairs of *Louisea* (Table 6). This study, therefore, supports the continued recognition of four species of *Louisea* that are all endemic to the rainforests of Southwest Cameroon. Even the two morphologically variable species, *L. nkongsamba* and *L. yabassi*, were found in the molecular analyses to have low uncorrected *p*-distance values between population pairs, which does not support the recognition of any of these populations as distinct species in their own right (Mvogo Ndongo et al. 2019). The four *Louisea* species are endemic to different habitats within the rainforest zone: montane forest streams (*L. balssi*), submontane forest streams (*L. nkongsamba*), islands in a freshwater lake (*L. edeaensis*), and lowland forest streams (*L. yabassi*).

The results of the phylogenetic analysis indicate that all four species of *Louisea* form a single clade with *L. balssi* from Mt. Manengouba as the ancestral species, while *L. edeaensis* from Lake Ossa is the sister species of the clade that includes *L. yabassi* and *L. nkongsamba* (Fig. 10). The divergence time estimates for these species are provided in Fig. 11.

Mount Manengouba where *Louisea* first evolved consists mainly of a shield volcano overlain by a stratovolcano whose highest peaks are at 2,411 m asl, and which covers an area of 500 km^2. This mountain was formed 1.55 Ma in three stages (Kagou Dongmo 2006, Kagou Dongmo et al. 2001). The first stage, (from 1.55 to 0.7 Ma), corresponds to the formation of a basaltic shield volcano that evolved to a stratovolcano (named Elengoum) which was capped by trachyte domes and flows (Pouclet 2014).

The second stage (between 0.70 and 0.56 Ma), was when the summit if Elengoum collapsed and created a large caldera open to the west. The third stage (from 0.56 Ma to recent times), includes the formation of a new stratovolcano named Eboga inside the caldera (see Pouclet 2014).

Specimens of *L. nkongsamba* found in the cool mountain streams that drain the higher altitudes of the submontane zone of Mount Nlonako (938 to 1462 m asl) are small-bodied (adult males measure CW: 12.00 – 20.00 mm), while males of *L. balssi* from the high-altitude streams (1,958 m asl) draining into the caldera of Mount Manengouba are also remarkably small-bodied (CW: 13.00 - 16.2 mm). Genetic differentiation tends to be somewhat limited in small-bodied montane species of freshwater crabs (Daniels et al. 2016). On the other hand, limited genetic variation was found in populations of *L. edeaensis* (a lowland species living on an island in a lake). The moist tropical rainforests that surround Mt. Manengouba receive high annual rainfall amounts that have provided a stable climate that has consistently supported tropical rainforests even during periods of fluctuating drier periods (Brown and Ab'Saber 1979, Diamond and Hamilton 1980, Mayr and O'Hara 1986, Grubb 1992, Zimkus 2009). Species of freshwater crabs (such as *L. balssi*) found in such places would be sheltered from the harsher effects of rainforest disruption arising from prolonged dry periods in the past, making the Cameroon Highlands a Pleistocene forest refuge for forest species. Over time, *Louisea* dispersed from its original location around Mt. Manengouba and spread out into the surrounding forests in Southwest Cameroon, including Mount Nlonako where species divergence gave rise to *L. nkongsamba*, and the forested lowlands around Yabassi and Lake Ossa, where *L. yabassi* and *L. edeaensis* evolved.

Habitat, threats, and conservation measures

Louisea species are semi-terrestrial and prefer temporary water bodies such as puddles near small permanent streams as well as damp environments under small stones or fallen leaves on land adjacent to streams (Mvogo Ndongo et al. 2017, 2018, 2019, 2021). These freshwater crabs have limited dispersal abilities due to reproduction by direct development that restricts dispersal to the movements of adults, and to their relative isolation (dues the complicated topography and the fragmentary nature of their wetland habitats). These factors are probably responsible for much of the diversity and endemism of these freshwater crabs (Cumberlidge et al. 2009). Ongoing threats exist to the rainforest habitat of these crabs driven by human population increases, deforestation, rapid urbanization, infrastructure development and increased agriculture in southwestern Cameroon (Dudgeon et al. 2006). Other anthropogenic threats include increased noise pollution and altered terrestrial and aquatic landscapes (Kight and Swaddle 2011, Shannon et al. 2015, Buxton et al. 2017). Our surveys suggest that the original populations of both *L. edeaensis* (from Edea and Yaounde) and *L. balssi* (from near Kumba) could prove to be extirpated because the type localities are now in urban areas or in disturbed forest, and there have been no conservation measures in place for these species. Although several zoological expeditions have surveyed this region over the years, *L. edeaensis* and *L.*

balssi have still not been recorded from their original localities (but admittedly these expeditions were not focused on collecting freshwater crabs at these localities).

Although some of the recently collected *Louisea* specimens from Mount Manengouba, Ebo Forest, Nlonako Ecological Reserve, and Lake Ossa were found in protected areas, almost all of their original habitats are now threatened by anthropogenic sources. For example, although Lake Ossa is a protected area, the lake's fishes, manatees, molluscs, crabs and shrimps receive little actual protection, and Bedimet Island in Lake Ossa is under threat from deforestation and from intensive and encroaching agricultural practices. Although preliminary conservation actions have been carried out, more support is still needed.

So far, all four species of *Louisea* have only limited distributional data and little is known about other aspects of their lifecycle. What is clear, however, is that *L. edeaensis* and *L. balssi* should be upgraded to Critical Endangered (CR) and *L. yabassi* and *L. nkongsamba* needs to be assessed as Endangered (EN) once their conservation assessment will be carried out.

The results of the present study indicate a strong need for further research aimed at developing a database on the distributions, population biology, and ecology of these species of *Louisea*, as well as a need to develop and implement conservation measures to mitigate declines in freshwater crab diversity in Southwest Cameroon in general. Besides *Louisea*, the biodiversity-rich rainforests of southwestern Cameroon are home to 7 other endemic species of semi-terrestrial freshwater crabs: *Buea asylos* (Cumberlidge, 1993), *B. bangem* Mvogo Ndongo, von Rintelen & Cumberlidge, 2020, *B. mundemba* Mvogo Ndongo, von Rintelen & Cumberlidge, 2020, *B. nlonako* Mvogo Ndongo, von Rintelen & Cumberlidge, 2020, *Potamonemus man* Mvogo Ndongo, von Rintelen & Cumberlidge, 2021, *P. sachsi* Cumberlidge & Clark, 1993 and *Sudanonautes tiko* Mvogo Ndongo, Schubart & Cumberlidge, 2017 (Cumberlidge and Daniels 2022, Cumberlidge et al. 2019, Mvogo Ndongo et al. 2017a–c, 2018, 2019, 2020, 2021a, b). These species are of great conservation importance and also the most threatened by anthropogenic activities in these forests (Mvogo Ndongo et al. 2021b). This unique freshwater crab diversity is not yet fully appreciated by the relevant government agencies or indigenous communities. Therefore, capacity building through community outreach and collaboration with local communities and research institutes based in southwest Cameroon is recommended to raise awareness and facilitate monitoring of local endemic semi-terrestrial freshwater crab species. This initiative incudes training local field guides to aid with species identification, provision of resource guides for fisheries managers and park officials that highlight the role of threatened crabs in freshwater ecosystems, and the benefits that conservation development can bring in local areas (Brooks et al. 2008).

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References

Amiet J-L (1975) Ecologie et distribution des amphibiens anoures de la région de Nkongsamba (Cameroun). – Ann. Fac. Sci. Yaoundé, Cameroun 20: 33–107.

Bolnick DI, Amarasekare P, Araújo MS, Bürger R, Levine JM, Novak M, Rudolf VHW, Schreiber SJ, Urban MC, Vasseur DA (2011) Why intraspecific trait variation matters in community ecology. Trends in Ecology & Evolution 26 (4): 183–192 DOI 10.1016/j.tree.2011.01.009.

Brooks EGE, Allen DJ, Darwall WRT (2008) The status and distribution of freshwater biodiversity in Central Africa. IUCN, Cambridge, UK and Gland, Switzerland

Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, Heled J, Jones G, Kühnert D, De Maio N (2019) BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. PLOS Computational Biology 15:e1006650. doi:10.1371/journal. pcbi.1006650

Buxton RT, McKenna MF, Mennitt D, Fristrup K, Crooks K, Angeloni L, Wittemyer G (2017) Noise pollution is pervasive in U.S. protected areas. Science. 356: 531–533.

Cornetti L, Ficetola GF, Hoban S, Vernesi C (2015) Genetic and ecological data reveal species boundaries between viviparous and oviparous lizard lineages. Heredity (2015): 115, 517–526

Cumberlidge N (1994) *Louisea*, a new genus of fresh-water crab (Brachyura, Potamoidea, Potamonautidae) *Globonautes macropus edeaensis* Bott, 1969 from Cameroon. Proceedings of the Biological Society of Washington 107: 122–131.

Cumberlidge N (1999) *The Freshwater Crabs of West Africa, Family Potamonautidae*. Faune et Flore tropicales 35, IRD, Paris, pp. 1–382.

Cumberlidge N, Daniels S (2022) A new multilocus phylogeny reveals overlooked diversity in African freshwater crabs (Brachyura: Potamoidea): a major revision with new higher taxa and genera. Zoological Journal of the Linnean Society 2022, **XX**: 1–44

Daniels SD, Cumberlidge N, Pérez-Losada M, Marijnissen SAE, Crandall KA (2006) Evolution of Afrotropical freshwater crab lineages obscured by morphological convergence. Molecular Phylogenetics and Evolution 40: 225–235.

Daniels SR, Phiri EE, Klaus S, Albrecht C & Cumberlidge N (2015) Multi-locus phylogeny of the Afrotropical freshwater crab fauna reveals historical drainage connectivity and transoceanic dispersal since the Eocene. Systematic Biology 64: 549–567.

Daniels SR, McCleod C, Carveth C, Mexim KK, Cumberlidge N (2016) Using mtDNA sequence data to explore evolutionary relationships among three West African species of freshwater crabs in the genus *Liberonautes* (Brachyura: Potamoidea: Potamonautidae), with a discussion of conservation implications. Journal of Crustacean Biology 36: 731–739.

Darriba D, Taboada G L, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772–772.

Diamond AW, Hamilton AC (1980) The distribution of forest passerine birds and Quaternary climatic change in Africa. Journal of Zoology 191: 379–402.

Dudgeon D, Arthington AH, Gessner MO, Kawabata Z-I, Knowler DJ, Lévêque C (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81, 163–182.

Forsman A (2014) Effects of genotypic and phenotypic variation on establishment are important for conservation, invasion, and infection biology. Proceedings of the National Academy of Sciences of the United States of America 111(1): 302–307 DOI 10.1073/pnas.1317745111.

Fratini S, Vannini M, Cannicci S, Schubart CD (2005) Tree-climbing crabs: a case of convergent evolution. Evolutionary Ecology Research 7: 219–233.

Griffiths AJF, Miller JH, Suzuki DT, Lewontin RC, Gelbart WM (2000) An Introduction to Genetic Analysis. Seventh Edition. New York: W. H. Freeman

Gonwouo L N, Lebreton M, Wild, Chirio, Ngassam P, Tchamba M N (2006) Geographic and ecological distribution of the endemic montane chameleons along the Cameroon mountain range. ISSN 0036-3375. 42 (4): 213–230.

Grubb P (1992) Refuges and dispersal in the speciation of African forest mammals. In: Prance GT, ed. *Biological diversification in the tropics*. New York: Columbia University Press 537–543

Hall TA. 1999. Bioedit: a user–friendly biological sequence alignment editor and analysis program for windows 95/ 98/NT. Nucleic Acids Symposium Series 41: 95–98.

Herrmann H-W, Bohme W, Herrmann PA, Plath M, Schmitz A, Solbach M (2005) African biodiversity hotspots: the amphibians of Mt. Nlonako, Cameroon. Salamandra 41: 61–81.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755.

Kagou Dongmo A (2006) Le mont Manengouba: évolution volcanologique, caractères magmatologiques et risques naturels; comparaison avec les monts Bambouto et Bamenda (Ligne du Cameroun). Thèse d'Etat, Univ. Yaoundé I, Cameroon, p 239.

Kagou Dongmo A, Wandji P, Pouclet A, Vicat J-P, Cheilletz A, Nkouathio DG, Alexandrov P, Tchoua F (2001) Evolution volcanologique du mont Manengouba (Ligne du Cameroun), nouvelles données pétrographiques, géochimiques et géochronologiques. C. R. Acad. Sci. Paris, Sér. IIa 333, 155–162.

Kight CR, Swaddle JP (2011) How and why environmental noise impacts animals: an integrative, mechanistic review. Ecological Letter 14:1052–1061.

Lawson DP (1993) The reptiles and amphibians of the Korup National Park Project, Cameroon. Herpetological Natural History 1: 27–90.

Leigh JW, Bryant D (2015) PopART: Full-feature software for haplotype network construction. Methods in Ecology and Evolution. 6:1110–1116. doi:10.1111/2041-210X.12410

Mace G M (2004) The role of taxonomy in species conservation. Phil. Trans. R. Soc. Lond. B (2004) 359, 711–719. DOI 10.1098/rstb.2003.1454

Morgan BJ, Abwe EE (2006) Chimpanzees used stone hammers in Cameroon. Current Biology 16 (16): R632-R633.

Morgan BJ, Abwe EE, Dixson AF, Astaras C (2013) The distribution, status, and conservation outlook of the drill (*Mandrillus leucophaeus*) in Cameroon. International Journal of Primatology 34(2): 281–302.

Mvogo Ndongo PA (2018) *Diversity, systematics and geographical distribution of freshwater crabs from coastal of Cameroon*. Ph.D. dissertation, in Zoology, Dept. Animal Biology and Physiology, Fac. Science, University of Yaounde 1. p 138.

Mvogo Ndongo PA, von Rintelen T, Schubart CD, Albrecht C, Tamesse JL, Cumberlidge N (2017a) New data on the taxonomy, ecology, and conservation of the rediscovered *Louisea edeaensis* (Bott, 1969) (Brachyura: Potamoidea: Potamonautidae), an endangered freshwater crab from Cameroon. Zootaxa 4231 (2): 273–280.

Mvogo Ndongo PA, Schubart CD, von Rintelen T, Tamesse JL, Cumberlidge N (2017b) Morphological and molecular evidence for a new species of freshwater crab of the genus *Sudanonautes* Bott, 1955 (Brachyura: Potamoidea: Potamonautidae) from Cameroon, with notes on its ecology. Zootaxa 4242 (1): 161–173.

Mvogo Ndongo PA, Cumberlidge N, Poettinger T S, von Rintelen T, Tamesse JL, Schubart CD (2017c) Molecular evidence for the assignment of the Cameroonian freshwater crab genus *Louisea* Cumberlidge, 1994, to the Afrotropical subfamily Potamonautinae Bott, 1970 (Crustacea: Potamoidea: Potamonautidae). Zootaxa 4286 (3): 439–444.

Mvogo Ndongo PA, von Rintelen T, Albrecht C, Tamesse JL, Cumberlidge N (2018) Lost species in Cameroon: rediscovery of the endangered freshwater crab, *Louisea balssi* (Bott, 1959) (Brachyura: Potamonautidae), with notes on its ecology and conservation. *Zootaxa*, 4231 (2), 273–280.

Mvogo Ndongo PA, von Rintelen T, Cumberlidge N (2019) Taxonomic revision of the endemic Cameroonian freshwater crab genus *Louisea* Cumberlidge, 1994 (Crustacea, Decapoda, Brachyura, Potamonautidae), with descriptions of two new species from Nkongsamba and Yabassi. ZooKeys 881: 135–164.

Mvogo Ndongo PA, von Rintelen T, Tomedi-Tabi Eyango M, Cumberlidge N (2020) 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. Journal of Crustacean Biology 40: 288–300.

Mvogo Ndongo PA, von Rintelen T, Schubart CD, Clark PF, von Rintelen K, Missoup AD, Albrecht C, Rabone M, Ewoukem E, Tamesse JL, Cumberlidge N (2021a) Discovery of two new populations of the rare endemic freshwater crab *Louisea yabassi* Mvogo Ndongo, von Rintelen & Cumberlidge, 2019 (Brachyura: Potamonautidae) from the Ebo Forest near Yabassi in Cameroon, Central Africa, with recommendations for conservation action. Journal of Threatened Taxa. 13(6): 18551–18558. doi:10.11609/jott.6724.13.6.18551-18558.

Mvogo Ndongo PA, von Rintelen T, Cumberlidge N (2021b) A new species of the freshwater crab genus Potamonemus Cumberlidge & Clark, 1992 (Crustacea, Potamonautidae) endemic to the forested highlands of southwestern Cameroon, Central Africa. ZooKeys 1017: 111–125.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853–858.

Mayr E, O'Hara RJ (1986) The biogeographic evidence supporting the Pleistocene forest refuge hypothesis. Evolution 40: 55–67.

Ng PKL, Guinot D, Davie PJF (2008) Systema Brachyuorum: Part I. An annotated checklist of extant Brachyuran crabs of the world. Raffles Bulletin of Zoology Supplement 17: 1–286.

Ng PKL (1988) *The freshwater crabs of Peninsular Malaysia and Singapore*. Singapore: Department of Zoology, National University of Singapore, Shinglee Press.

Ng PKL, Rodriguez G (1995) Freshwater crabs as poor zoogeographical indicators: a critique of Banarescu (1990). Crustaceana 68: 636–645.

Pouclet A, Kagou Dongmo A, Bardintzeff J-M, Wandji P, Chakam Tagheu P, Nkouathio D, Bellon H, Ruffet G (2014) The Mount Manengouba, a complex volcano of the Cameroon Line: Volcanic history, petrological and geochemical features. Journal of African Earth Sciences (97) 297–321.

Palumbi S R (1996) Nucleic Acids II: The Polymerase Chain Reaction. In: Hillis D. M., Moritz C. & B. K. Mable (eds.): Molecular Systematics. 2nd Ed. Sinauer, Sunderland, USA. pp. 205–247.

Reuschel S, Schubart CD (2006) Geographic differentiation of two Atlanto-Mediterranean species of the genus *Xantho* (Crustacea: Brachyura: Xanthidae) based on genetic and morphometric analyses. Marine Biology 48: 853– 866.

Schubart CD (2009) Mitochondrial DNA and decapod phylogenies; the importance of pseudogenes and primer optimization. *In:* Martin, J.W., Crandall, K.A. & Felder, D. L. (eds.), Crustacean Issues 18: Decapod Crustacean Phylogenetics pp. 47-65. Boca Raton, Florida: Taylor & Francis/CRC Press.

Schubart CD, Diesel R, Hedges S (1998) Rapid evolution to terrestrial life in Jamaican crabs. Nature. 393:363–365. doi:10. 1038/30724

Shannon G, Mckenna MF, Angeloni LM, Crooks KR, Fristrup KM, Brown E, Warner KA, Nelson MD, White C, Briggs J (2015) A synthesis of two decades of research documenting the effects of noise on wildlife. Biological Reviews 91: 982–1005.

Scheiner SM Holt RD (2012) The genetics of phenotypic plasticity. X. variation versus uncertainty. Ecology and Evolution 2 (4): 751–767 DOI 10.1002/ece3.217

Stamatakis A (2006) RAxML-VI-HPC: maximum likehood – based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690.

Stearns SC (1989) The evolutionary significance of phenotypic plasticity. BioScience 39 (7): 436–445 DOI 10.2307/1311135.

Templeton AR, Crandall KA, Sing CF. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132: 619–633. doi:10.1093/genetics/132.2.619

Thompson JD, Higgins DG, Gibson TJ (1994) Clustal W: improving the sensitivity of progressive multiples sequence alignments through sequence weighting, position– specific gap penalties and weight matrix choice. Nucleic Acids Research. 22: 4673–4680. doi:10.1093/nar/ 22.22.4673

Stemmer M, Schubart CD (2016) Genetic analyses determine connectivity among cave and surface populations of the Jamaican endemic freshwater crab *Sesarma fossarum* in the Cockpit Country. International Journal of Speleology 45 (1): 35–41.

Villesen P (2007) FaBox: an online toolbox for Fasta sequences. Molecular Ecology Notes 7: 965–968.

Zimkus Breda M (2009) Biogeographical analysis of Cameroonian puddle frogs and description of a new species of *Phrynobatrachus* (Anura: Phrynobatrachidae) endemic to Mount Oku, Cameroon. Zoological Journal of the Linnean Society 157: 795–813.

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Figure 7. Morphotypes of *Louisea nkongsamba* endemic to Nlonako, Littoral Cameroon. A: dorsal view of adult male (CW 11.70 mm) from Nlonako_ Enguegue (site n°1). B: frontal view of adult male (CW 11.70 mm) from Nlonako_Enguegue (site n°1). C: Dorsal view of adult male (CW 18.20 mm) from Eyimba. D: Frontal view of adult male (CW 18.20 mm) from Eyimba. Scale bars: A, B = 3 mm, C, D = 4 mm.

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Figure 9. Morphotypes of *Louisea nkongsamba* endemic to Nlonako, Littoral Cameroon. A, right G1 ventral view of adult male (CW 12.00 mm) from Nlonako_ Enguegue (site n°1). D: right G1 ventral view of adult male (CW 18.20 mm) from Eyimba. B: left G1 dorsal view of adult male (CW 12.00 mm) from Nlonako_ Enguegue (site n°1). E: left G1 dorsal view of adult male (CW 18.20 mm) from Eyimba. C: G2 of adult male (CW 12.00 mm) from Nlonako_ Enguegue (site n°1). F: G2 of adult male (CW 18.20 mm) from Eyimba. A, B, C, D, E, F: 1mm.

Figure 10. ML tree topology for the four freshwater crab *Louisea* species from Cameroon included in this study derived from mtDNA sequences corresponding to three loci (partial 16S rRNA, COI and 12S rRNA genes). BI and ML statistical values (%) on the nodes indicate posterior probabilities and bootstrap support, respectively.

Figure 11. BI tree topology for the four freshwater crab *Louisea* species from Cameroon included in this study derived from COI mtDNA sequences constructed with BEAST 2.6.2. Statistical values on the nodes indicate age in million year.

Figure 12. Maximum parsimony genotype networks of COI, constructed with PopArt. Hatch marks stand for mutation steps.

Table 1: GenBank numbers of the three mtDNA markers used in this study for the four species of *Louisea* and the outgroups. Nl = Nlonako; Here = sequence available in the present study; * = Mvogo Ngongo et al. 2019; ** = Mvogo Ndongo et al. 2017c.

Species and Sample No. on Fig. 10	**Locality in Cameroon**	**Population No. (Fig. 10)**	**Morphotypes (Tables 3, 4)**	**Extraction/museum number**	**GenBank Accession Number (pending)**		
					CO1	**12S rRNA**	**16S rRNA**
L. nkongsamba (1)	Nlonako, Engugue1382	Population 1	Nl Morphotype 1	ZMB-X21	Here	Here	Here
L. nkongsamba (2)	Nlonako, NgaltongueS1	Population 1	Nl Morphotype 1	ZMB-X26	Here	Here	Here
L. nkongsamba (3)	Nlonako, NgaltongueS1	Population 1	Nl Morphotype 1	ZMB-X27	Here	Here	Here
L. nkongsamba (4)	Nlonako, NgaltongueS1	Population 1	Nl Morphotype 1	ZMB-X28	Here	Here	Here
L. nkongsamba (5)	Nlonako, NgaltongueS1	Population 1	Nl Morphotype 1	ZMB-X29	Here	Here	Here
L. nkongsamba (6)	Nlonako Engugue1462	Population 1	Nl Morphotype 1	ZMB-X31	Here	Here	Here
L. nkongsamba (7)	Nlonako, NgaltongueS2	Population 1	Nl Morphotype 1	ZMB-X36	Here	Here	Here
L. nkongsamba (8)	Nlonako, NgaltongueS2	Population 1	Nl Morphotype 1	ZMB-X37	Here	Here	Here
L. nkongsamba (9)	Nlonako, NgaltongueS2	Population 1	Nl Morphotype 1	ZMB-X38	Here	Here	Here
L. nkongsamba (10)	Nlonako, NgaltongueS2	Population 1	Nl Morphotype 1	ZMB-X39	Here	Here	Here
L. nkongsamba (11)	Nlonako, Eyimba	Population 1	Nl Morphotype 1	ZMB-X41	Here	Here	Here
L. nkongsamba (12)	Nlonako, Nguengue	Population 1	Nl Morphotype 1	ZMB-X46	Here	Here	Here
L. nkongsamba (13)	Nlonako, Nguengue	Population 1	Nl Morphotype 1	ZMB-X47	Here	Here	Here
L. nkongsamba (14)	Nlonako, Nguengue	Population 1	Nl Morphotype 1	ZMB-X48	Here	Here	Here
L. nkongsamba (15)	Nlonako, Engugue1382	Population 2	Nl Morphotype 1	ZMB-X22	Here	Here	Here
L. nkongsamba (16)	Nlonako, Engugue1382	Population 2	Nl Morphotype 1	ZMB-X23	Here	Here	Here
L. nkongsamba (17)	Nlonako, Engugue1382	Population 2	Nl Morphotype 1	ZMB-X24	Here	Here	Here
L. nkongsamba (18)	Nlonako, NgaltongueS1	Population 2	Nl Morphotype 1	ZMB-X30	Here	Here	Here
L. nkongsamba (19)	Nlonako Engugue1462	Population 2	Nl Morphotype 2	ZMB-X32	Here	Here	Here
L. nkongsamba (20)	Nlonako Engugue1462	Population 2	Nl Morphotype 2	ZMB-X33	Here	Here	Here
L. nkongsamba (21)	Nlonako Engugue1462	Population 2	Nl Morphotype 2	ZMB-X34	Here	Here	Here
L. nkongsamba (22)	Nlonako, Eyimba	Population 2	Nl Morphotype 1	ZMB-X42	Here	Here	Here
L. nkongsamba (23)	Nlonako, Eyimba	Population 2	Nl Morphotype 1	ZMB-X43	Here	Here	Here
L. nkongsamba (24)	Nlonako, Eyimba	Population 2	Nl Morphotype 1	ZMB-X44	Here	Here	Here
L. nkongsamba (25)	Nlonako, Nguengue	Population 2	Nl Morphotype 1	ZMB-X49	Here	Here	Here
L. nkongsamba (26)	Nlonako, Nguengue	Population 2	Nl Morphotype 1	ZMB-X50	Here	Here	Here
L. nkongsamba (27)	Nlonako, Engugue1382	Population 3	Nlon Morphotype 1	ZMB-X25	Here	Here	Here
L. nkongsamba (28)	Nlonako Engugue1462	Population 3	Nlon Morphotype 1	ZMB-X35	Here	Here	Here
L. nkongsamba (29)	Nlonako, NgaltongueS2	Population 3	Nlon Morphotype 1	ZMB-X40	Here	Here	Here
L. nkongsamba (30)	Nlonako, Eyimba	Population 3	Nlon Morphotype 1	ZMB-X45	Here	Here	Here
L. yabassi (31)	Eboforest Stream N°1	Population 1	Ebo Morphotype 1	ZMB-X11	Here	Here	Here
L. yabassi (32)	Eboforest Stream N°1	Population 1	Ebo Morphotype 1	ZMB-X12	Here	Here	Here
L. yabassi (33)	Eboforest Stream N°1	Population 1	Ebo Morphotype 1	ZMB-X13	Here	Here	Here
L. yabassi (34)	Eboforest Stream N°1	Population 1	Ebo Morphotype 1	ZMB-X14	Here	Here	Here
L. yabassi (35)	Eboforest Stream N°1	Population 1	Ebo Morphotype 1	ZMB-X15	Here	Here	Here

L. yabassi (36)	Eboforest Stream N°2	Population 2	Ebo Morphotype 2	ZMB-X16	Here	Here	Here
L. yabassi (37)	Eboforest Stream N°2	Population 2	Ebo Morphotype 2	ZMB-X17	Here	Here	Here
L. yabassi (38)	Eboforest Stream N°2	Population 2	Ebo Morphotype 2	ZMB-X18	Here	Here	Here
L. yabassi (39)	Eboforest Stream N°2	Population 2	Ebo Morphotype 2	ZMB-X19	Here	Here	Here
L. yabassi (40)	Eboforest Stream N°2	Population 2	Ebo Morphotype 2	ZMB-X20	Here	Here	Here
L. edeaensis	Lake Ossa, Bedimet Island	Population 1	--	ZMB Crust 30335	MN188068.1*	---	MN217395*
L. edeaensis	Lake Ossa, Bedimet Island	Population 1	--	T351-30	KY964474.1**	KY964479**	KY964472**
L. edeaensis	Lake Ossa, Bedimet Island	Population 1	--	ZMB_Crust 26930	KY964473.1**	KY964478**	-----
L. balssi	Manengouba, stream	Population 1	--	ZMB Crust 30319	MN188071.1*	MN217385*	MN217392*
L. balssi	Manengouba, stream	Population 1	--	ZMB Crust.29628	MN188070.1*	MN217384*	MN217391*
Potamonemus man	Bakossi National Park	Population 1	--	ZMB Crust 30327	MN188067.1*	MN217390*	MN217398*
Buea mundemba	Korup National Park	Population 1	--	ZMB_Crust 30321	MN188069.1*	MN217388*	MN217396*

Table 2. Morphometric and collection data of specimens of *Louisea yabassi* from Ebo Forest Cameroon and *L. nkongsamba* from 6 localities in Nlonako ecological reserve, Cameroon. PAMN: Pierre A. Mvogo Ndongo; Ad: adult; Sa: subadult, M: male; F: female.

Species	CW/FW Mean (n)	CL/FW Mean (n)	CH/FW Mean (n)	Size Range (CW in mm)	Collector & Date	Museum No. for few specimens	Locality	Latitude, Longitude	ASL (m)
L. yabassi	2.9 (19)	2.1 (19)	1.3 (19)	Ad M 16.4 to 20.2	PAMN, 10.12.19	LaboPasmat X100	Ebo Forest, Stream N°1	4.417150, 10.200210	162
L. yabassi				Ad F 12.0 to 24.1	PAMN, 10.12.19	ZMB Crust.(process)	Ebo Forest, Stream N°1	4.417150, 10.200210	162
L. yabassi	2.9 (16)	2.1 (16)	1.3 (16)	Ad M 16.6 to 21.3	PAMN, 13.12.19	LaboPasmat X101	Ebo Forest, Stream N°2	4.416460, 10.202130	254
L. yabassi				Ad F 17.4 to 22.5	PAMN, 13.12.19	ZMB Crust.(process)	Ebo Forest, Stream N°2	4.416460, 10.202130	254
L. nkongsamba	2.9 (8)	2.1 (8)	1.3 (8)	Ad M 15.8 to 20.0	PAMN, 05.12.19	LaboPasmat X102	Nlonako, Nguengue	4.912444, 9.980556	1176
L. nkongsamba	2.9 (5)	2.1 (5)	1.3 (5)	Ad M 12.8 to 18.5	PAMN, 11.11.16	ZMB Crust.(process)	Nlonako, NgaltongueS2	4.922320, 9.958620	1180
L. nkongsamba	2.9 (12)	2.1 (12)	1.3 (12)	Ad M 13.8 to 17.4	PAMN, 15.01.16	LaboPasmat X103	Nlonako, NgaltongueS1	4.922320, 9.961820	1180
L. nkongsamba	2.9 (10)	2.1 (10)	1.3 (10)	Sa M 11.7 to 11.8	PAMN, 02.12.19	ZMB Crust.(process)	Nlonako, Engugue1382	4.906010, 9.972400	1382
L. nkongsamba	2.9 (11)	2.1 (11)	1.3 (11)	Sa M 11.5 to 14.4	PAMN, 10.12.19	LaboPasmat X104	Nlonako, Eyimba	4.891870, 9.984760	1194
L. nkongsamba	2.9 (6)	2.1 (6)	1.3 (6)	Ad 12	PAMN 02.12.19	ZMB Crust.(process)	Nlonako, Engugue1462	4.906070, 9.972880	1462
L. nkongsamba				Ad F 14 to 15	PAMN 02.12.19	LaboPasmat X105	Nlonako, Engugue1462	4.906070, 9.972880	1462
L. nkongsamba				Sa 6.60	PAMN 02.12.19	ZMB Crust.(process)	Nlonako, Engugue1462	4.906070, 9.972880	1462
L. edeaensis	3.0 (21)	2.5 (21)	1.4 (21)	Ad M 14.1 to 17.5	Mvogo Ndongo et al. 2019	See Mvogo Ndongo et al. 2019, ('table 2, P. 143)	Lake Ossa	3.815583, 10.055139	90
L. edeaensis				Ad F 13.0 to 19.9	Mvogo Ndongo et al. 2019	See Mvogo Ndongo et al. 2019, ('table 2, P. 143)	Lake Ossa	3.815583, 10.055139	90
L. balssi	2.9 (8)	2.1 (8)	1.2 (8)	Ad M 13.3 to 16.2	Mvogo Ndongo et al. 2019	See Mvogo Ndongo et al. 2019, ('table 3, P. 147)	Manengouba	5.032472, 9.827167	1958
L. balssi				Ad F 13.3 to 14.8	Mvogo Ndongo et al. 2019	See Mvogo Ndongo et al. 2019, ('table 3, P. 147)	Manengouba	5.032472, 9.827167	1958

Table 3. Comparison of morphological characters between two populations of *Louisea yabassi* from Ebo Forest, Cameroon

Character	**Ebo Forest Stream Population 1 (morphotype 1)**	**Ebo Forest Stream Population 2 (morphotype 2)**
Epibranchial tooth	Reduced to granule (Fig. 2A)	Small tooth (Fig. 2C)
Intermediate tooth between exorbital & epibranchial teeth	Clearly distinct, but small (Fig. 2A)	Large and triangular (Fig.2-C)
Major cheliped dactylus	Slim and slightly arched (Fig. 4-A)	Slim and straight (Fig. 4-E)
Cheliped carpus inner margin teeth	Both distal & proximal teeth large & clearly separate (Fig. 4G)	Distal tooth larger than proximal tooth, positioned close to each other (Fig. 4C)
Mandible inferior lateral corner of coxa (biting edge)	Lacking pointed tip (Fig. 4I)	With pointed tip (Fig. 4J)
Margin of sternal sulcus S3	With long setae (Fig. 3-A)	Lacking setae (Fig. 3B)
Sternal sulcus S3/4	Reduced to two deep side notches (Fig. 3A)	Not visible (Fig. 3B)

Table 4. Comparison of morphological characters between two populations of *Louisea nkongsamba* from Mt. Nlonako, Cameroon

Characters	**Morphotype 1** **Nlonako Engugue1462**	**Morphotype 2** **Nlonako Eyimba, Ngaltongue, Engugue1382, Nguegue and type specimens (see Mvogo Ndongo et al. 2019)**
Exorbital tooth	Large (Fig. 7A)	Small (Fig. 7C)
Epibranchial tooth	Small tooth (Figs. 7A, B)	Reduced to granule (Figs. 7C, D)
Lateral margin posterior to epibranchial tooth	Lined by small granules (Figs. 7A, B)	Smooth (Figs. 7C, D)
Intermediate tooth between exorbital & epibranchial teeth	Large (Fig. 7A)	Small (Fig. 7C)
Postfrontal crest	Poorly defined, completely traversing carapace, meeting anterolateral margins at intermediate tooth (Figs. 7A, B)	Clearly defined, completely traversing carapace, not meet anterolateral margins (Figs. 7C, D)
Major cheliped dactylus	Slim and straight (Fig. 8-A)	Slim and slightly arched (Fig. 8G)
Cheliped carpus inner margin teeth	Distal tooth larger than proximal tooth, both slender, clearly separate positioned close to each other (Fig. 8C)	Distal tooth larger than proximal tooth, both robust, positioned close to each other (Fig. 8I)
Medial inferior margin of cheliped merus	With small but distinct jagged distal tooth angled outward at 60°, followed by numerous granules & small teeth (Fig. 8D)	With large jagged distal tooth angled outward at 90°, followed by numerous granules & small teeth decreasing in size proximally (Fig. 8J)
Mandible inferior lateral corner of coxa (biting edge)	Lacking pointed tip (Fig. 8E)	With pointed tip (Fig. 8K)
Distance from exopod and Ischium of third maxilliped	Large (Fig. 8F)	Small (Fig. 8L)
Sternal segment s3	Absent except for two deep side notches (Fig. 6B)	Absent, lacking side notches (Fig. 6D)
G2 SA length	Relatively short 2.1 mm (Fig. 9C)	Relatively long 2.8 mm (Fig. 9F)

Table 5. The number of individuals per site grouped in each of the three populations of *Louisea nkongsamba* included in the present study.

Sites	Altitude (m asl)	No. Individuals in Population 1	No. Individuals in Population 2	No. Individuals in Population 3
Enguegue n°2	**1382**	**1**	**3**	**1**
Ngaltongue n°1	**1176**	**4**	**1**	**0**
Ngaltongue n°2	**1256**	**4**	**0**	**1**
Enguegue n°1	**1462**	**1**	**3**	**1**
Nguegue	**1211**	**3**	**2**	**0**
Eyimba	**938**	**1**	**3**	**1**
Total		**14**	**12**	**4**

Table 6. The pairwise uncorrected *p*-distances of the COI, 16S rRNA, 12S rRNA partial sequences between species of *Louisea*.

***Louisea* species**	**Uncorrected *p*-distance**		
	CO1	**16S rRNA**	**12S rRNA**
L. nkongsamba and *L. yabassi*	3.97%	2.15%	3.77%
L. nkongsamba and *L. edeaensis*	8.61%	4.33%	4.92%
L. nkongsamba and *L. balssi*	7.98%	5.04%	12.42%
L. edeaensis and *L. yabassi*	8.88%	4.35%	4.27%
L. edeaensis and *L. balssi*	10.15%	7.77%	11.04%
L. yabassi and *L. balssi*	7.32%	5.36%	12.94%

Table 7. The pairwise uncorrected *p*-distances of the COI, 16S rRNA, 12S rRNA partial sequences of populations of *Louisea nkongsamba*

***Louisea* species**	**Uncorrected *p*-distance**		
	CO1	**16S rRNA**	**12S rRNA**
Population 2 and Population 3	0.48%	0.87%	0.95%
Population 2 and Population 1	0.70%	0.20%	0.71%
Population 3 and Population 1	0.52%	0.61%	1.18%

Figure 1. Whole specimen of *Louisea yabassi* endemic to Eboforest. A: Dorsal view of largest adult male (CW 20.18 mm) of site n°1 (population n°1). B: Ventral view of largest adult male (CW 20.18 mm) of site n°1 (population n°1). C: Dorsal view of largest adult male (CW 21.30 mm) of site n°2 (population n°2). D: Ventral view of largest adult male (CW 21.30 mm) of site n°2 (population n°2). Scale bars: A, B = 15 mm, C, D = 17 mm.

Figure 2. *Louisea yabassi* endemic to Eboforest. A: Dorsal view of largest adult male (CW 20.18 mm) of site n°1 (population n°1). B: Frontal view of largest adult male (CW 20.18 mm) of site n°1 (population n°1). C: Dorsal view of largest adult male (CW 21.30 mm) of site n°2 (population n°2). D: Frontal view of largest adult male (CW 21.30 mm) of site n°2 (population n°2). Scale bars: A, B = 8 mm, C, D = 9 mm.

Figure 3. *Louisea yabassi* endemic to Eboforest, thoracic sternites (s1–s8) and pleonal segments (a4–a7). A: largest adult male (CW 20.18 mm) of site n°1 (population n°1). B: largest adult male (CW 21.30 mm) of site n°2 (population n°2). Scale bars: A = 8 mm, B = 9 mm.

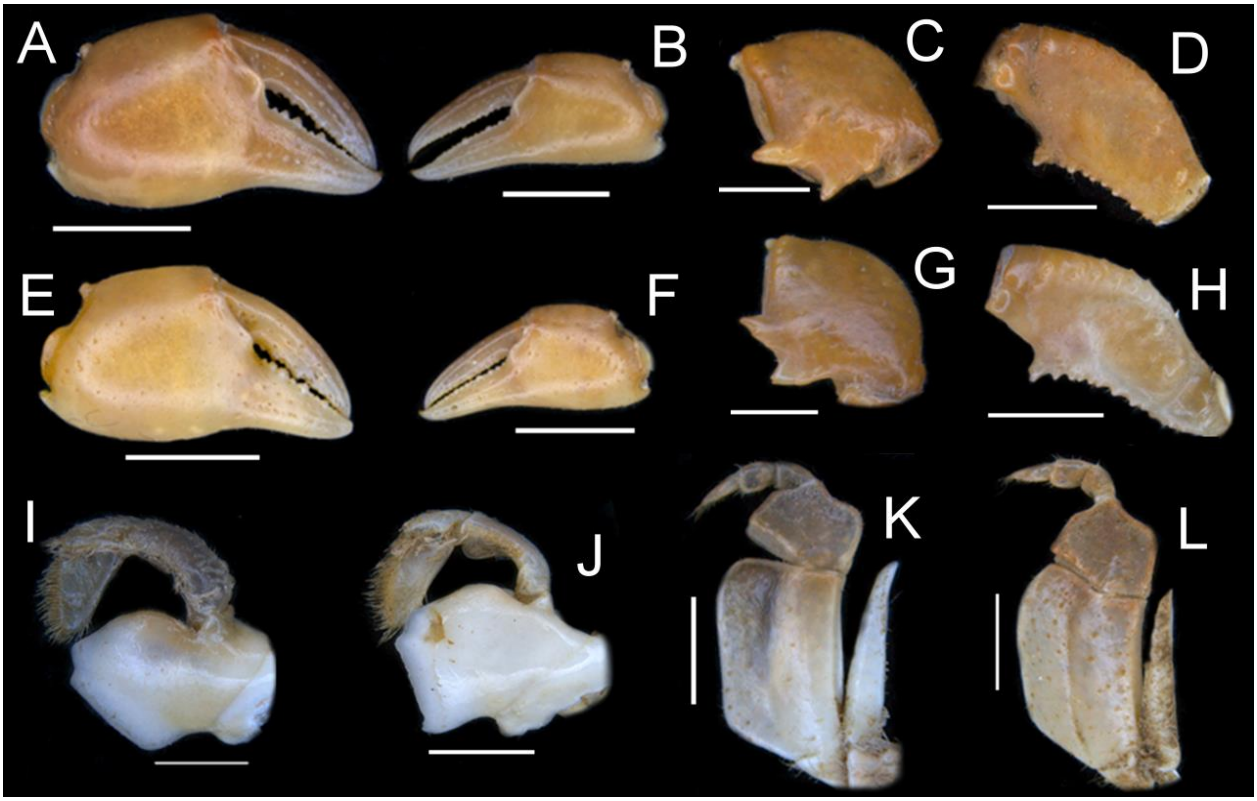

Figure 4. *Louisea yabassi* endemic to Eboforest, Cameroon. A, B: frontal view of right (A) and left (B) chela of largest adult male (CW 20.18 mm) of site n°1 (population n°1). E, F: frontal view of right (E) and left (F) chela of largest adult male (CW 21.30 mm) of site n°2 (population n°2). H: right cheliped merus of largest adult male (CW 20.18 mm) of site n°1 (population n°1). D: right cheliped merus of largest adult male (CW 21.30 mm) of site n°2 (population n°2). G: right cheliped carpus of largest adult male (CW 20.18 mm) of site n°1 (population n°1). C. right cheliped carpus of largest adult male (CW 21.30 mm) of site n°2 (population n°2). K. left third maxilliped of largest adult male (CW 20.18 mm) of site n°1 (population n°1). L. left third maxilliped of largest adult male (CW 21.30 mm) of site n°2 (population n°2). I: Ventral view of right mandible of largest adult male (CW 20.18 mm) of site n°1 (population n°1). J: Ventral view of right mandible of largest adult male (CW 21.30 mm) of site n°2 (population n°2). Scale bars: D, H, A, B, E, F, C, G: 5 mm; I, J: 1 mm; K, L: 2 mm

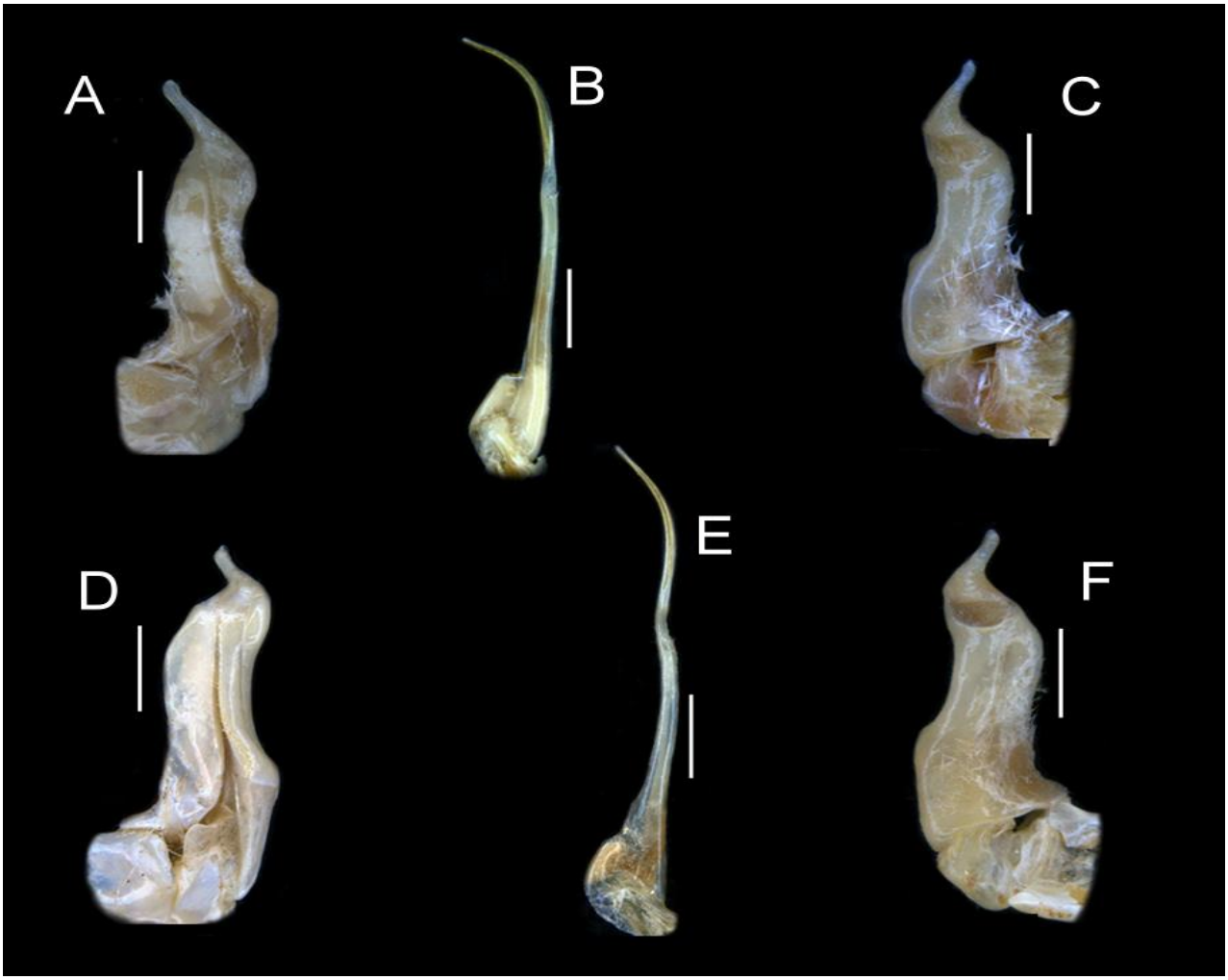

Figure 5. *Louisea yabassi* endemic to Eboforest, Cameroon. A, left G1 ventral view of largest adult male (CW 20.18 mm) of site n°1 (population n°1). D: left G1 ventral view of largest adult male (CW 21.30 mm) of site n°2 (population n°2). C: left G1 dorsal view of largest adult male (CW 20.18 mm) of site n°1 (population n°1). F: left G1 dorsal view of largest adult male (CW 21.30 mm) of site n°2 (population n°2). B: G2 of largest adult male (CW 20.18 mm) of site n°1 (population n°1). E: G2 of largest adult male (CW 21.30 mm) of site n°2 (population n°2). Scale bars: A, B, D, E, C, F: 1 mm.

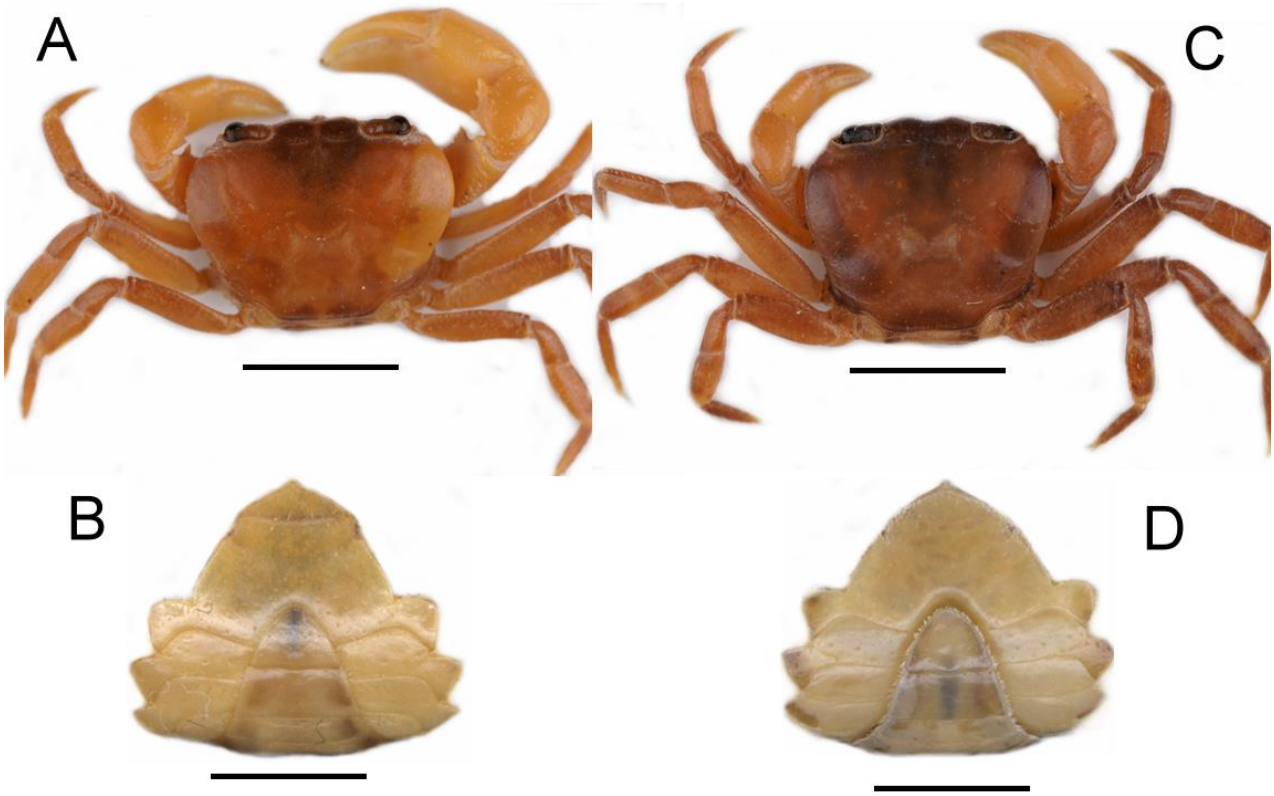

Figure 6. Morphotypes of *Louisea nkongsamba* endemic to Nlonako. A: Dorsal view of adult male (CW 18.20 mm) from Eyimba. B: thoracic sternites (s1–s8) and pleonal segments (a4–a7) of adult male (CW 18.20 mm) from Eyimba. C: dorsal view of adult male (CW 11.70 mm) from Nlonako_ Enguegue (site n°1). D: thoracic sternites (s1–s8) and pleonal segments (a4–a7) of adult male (CW 11.70 mm) from Nlonako_ Enguegue (site n°1). Scale bars: A, B: 12 mm; C, D: 8 mm.

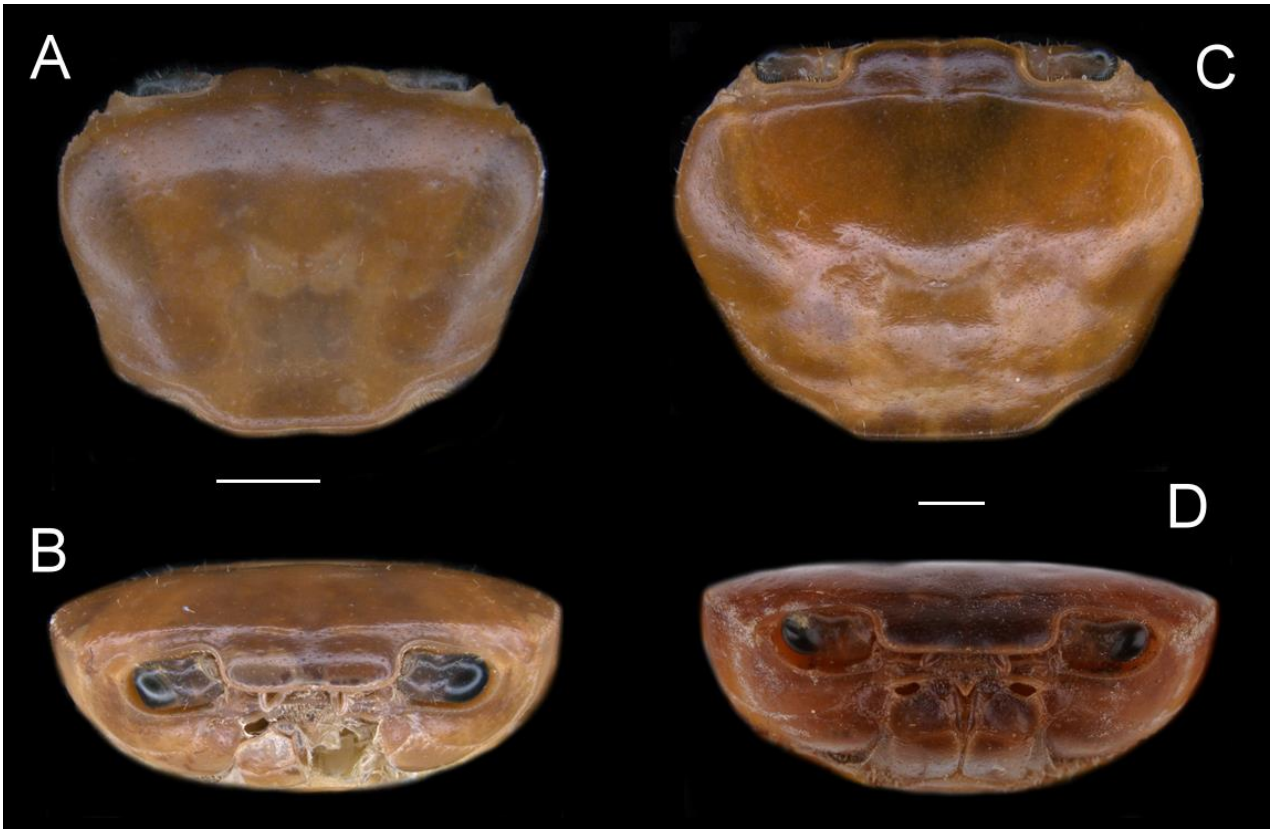

Figure 7. Morphotypes of *Louisea nkongsamba* endemic to Nlonako, Littoral Cameroon. A: dorsal view of adult male (CW 12.00 mm) from Nlonako_ Enguegue (site n°1). B: frontal view of adult male (CW 12.00 mm) from Nlonako_Enguegue (site n°1). C: Dorsal view of adult male (CW 18.20 mm) from Eyimba. D: Frontal view of adult male (CW 18.20 mm) from Eyimba. Scale bars: A, B = 3 mm, C, D = 4 mm.

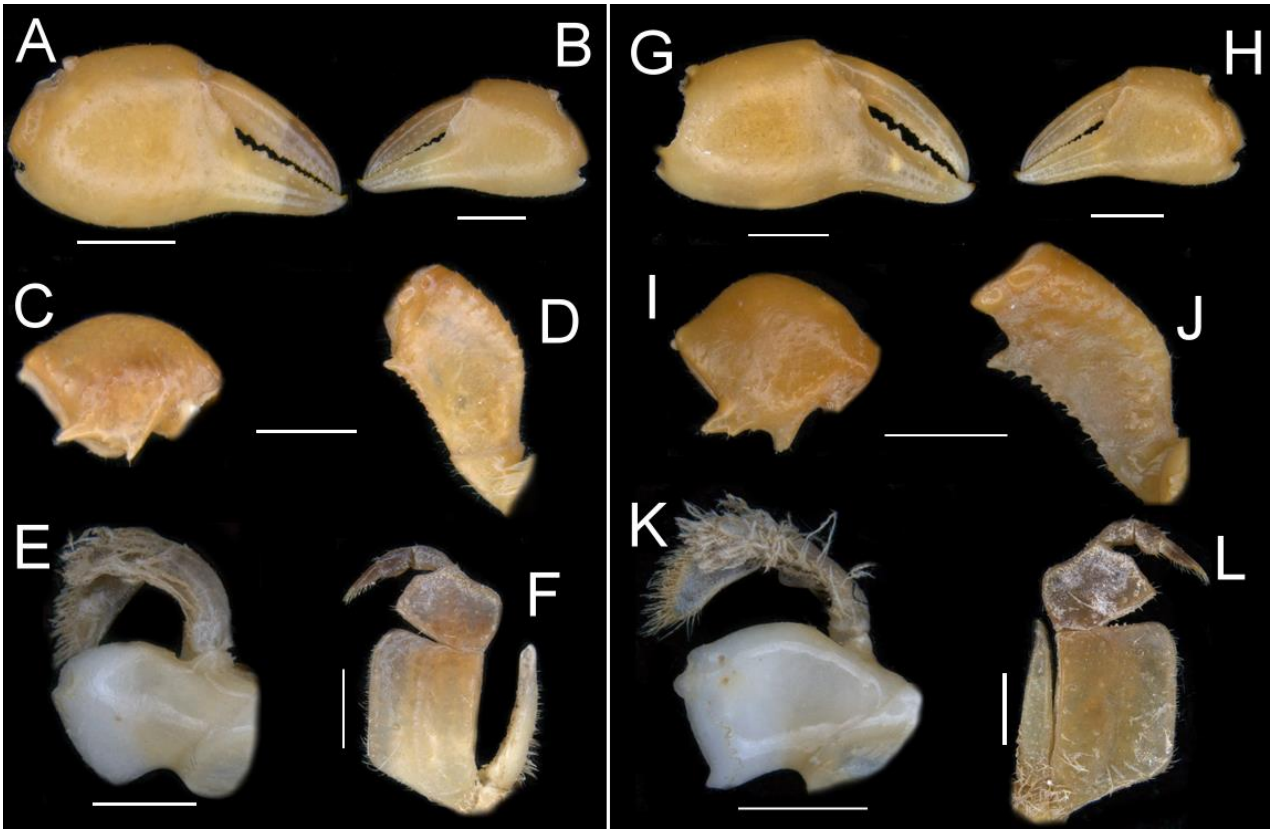

Figure 8. Morphotypes of *Louisea nkongsamba* endemic to Nlonako, Littoral Cameroon. A, B: frontal view of right (A) and left (B) chela of adult male (CW 12.00 mm) from Nlonako_ Enguegue (site n°1). G, H: frontal view of right (G) and left (H) chela of adult male (CW 18.20 mm) from Eyimba. D: right cheliped merus of adult male (CW 12.00 mm) from Nlonako_Enguegue (site n°1). J: right cheliped merus of adult male (CW 18.20 mm) from Eyimba. C: right cheliped carpus of adult male (CW 12.00 mm) from Nlonako_ Enguegue (site n°1). I: right cheliped carpus of adult male (CW 18.20 mm) from Eyimba. F: left third maxilliped of adult male (CW 12.00 mm) from Nlonako_ Enguegue (site n°1). L: right third maxilliped of adult male (CW 18.20 mm) from Eyimba. E: ventral view of right mandible of adult male (CW 12.00 mm) from Nlonako_ Enguegue (site n°1). K: Ventral view of right mandible of adult male (CW 18.20 mm) from Eyimba. Scale bars: A, B, C, D: 2 mm, G, H, I, J: 5 mm; E: 500 µm; K: 1 mm; F, L: 1 mm.

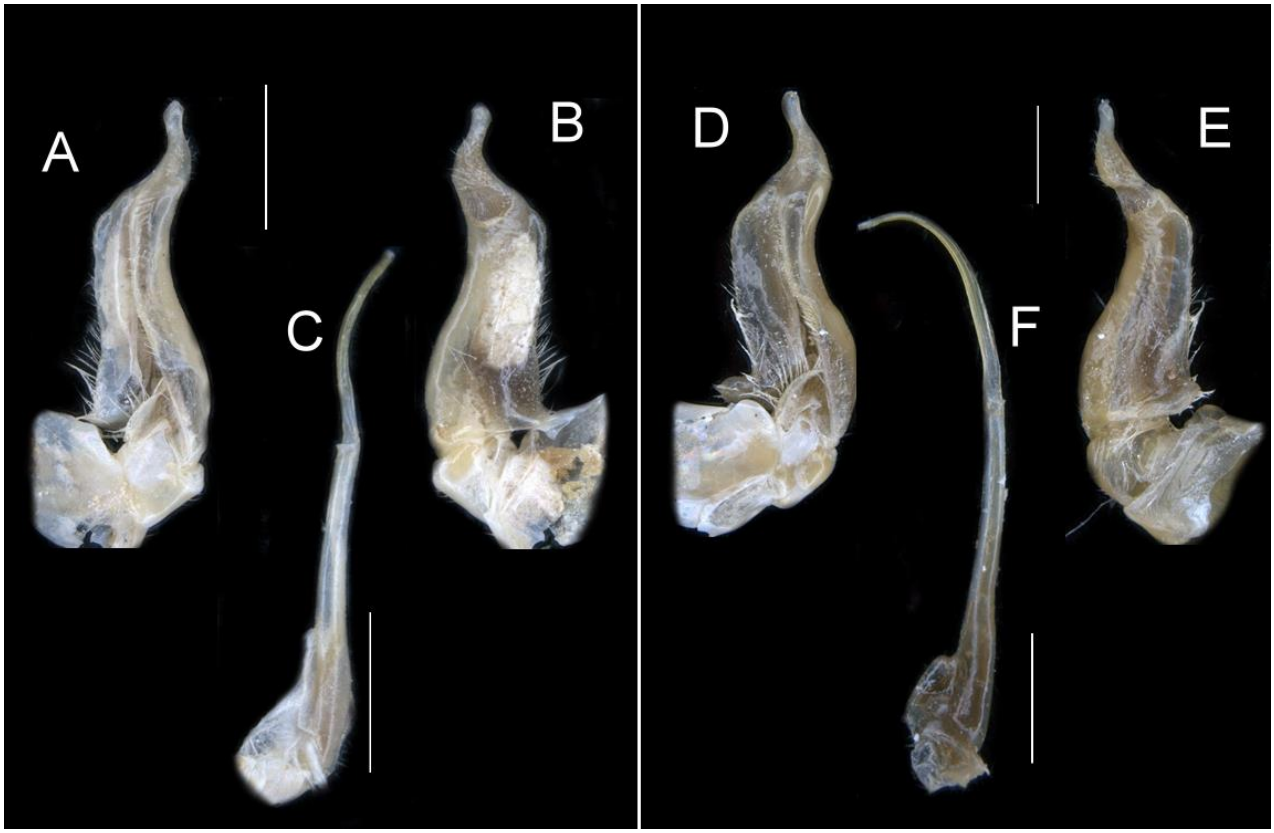

Figure 9. Morphotypes of *Louisea nkongsamba* endemic to Nlonako, Littoral Cameroon. A, right G1 ventral view of adult male (CW 12.00 mm) from Nlonako_ Enguegue (site n°1). D: right G1 ventral view of adult male (CW 18.20 mm) from Eyimba. B: left G1 dorsal view of adult male (CW 12.00 mm) from Nlonako_ Enguegue (site n°1). E: left G1 dorsal view of adult male (CW 18.20 mm) from Eyimba. C: G2 of adult male (CW 12.00 mm) from Nlonako_ Enguegue (site n°1). F: G2 of adult male (CW 18.20 mm) from Eyimba. A, B, C, D, E, F: 1mm.

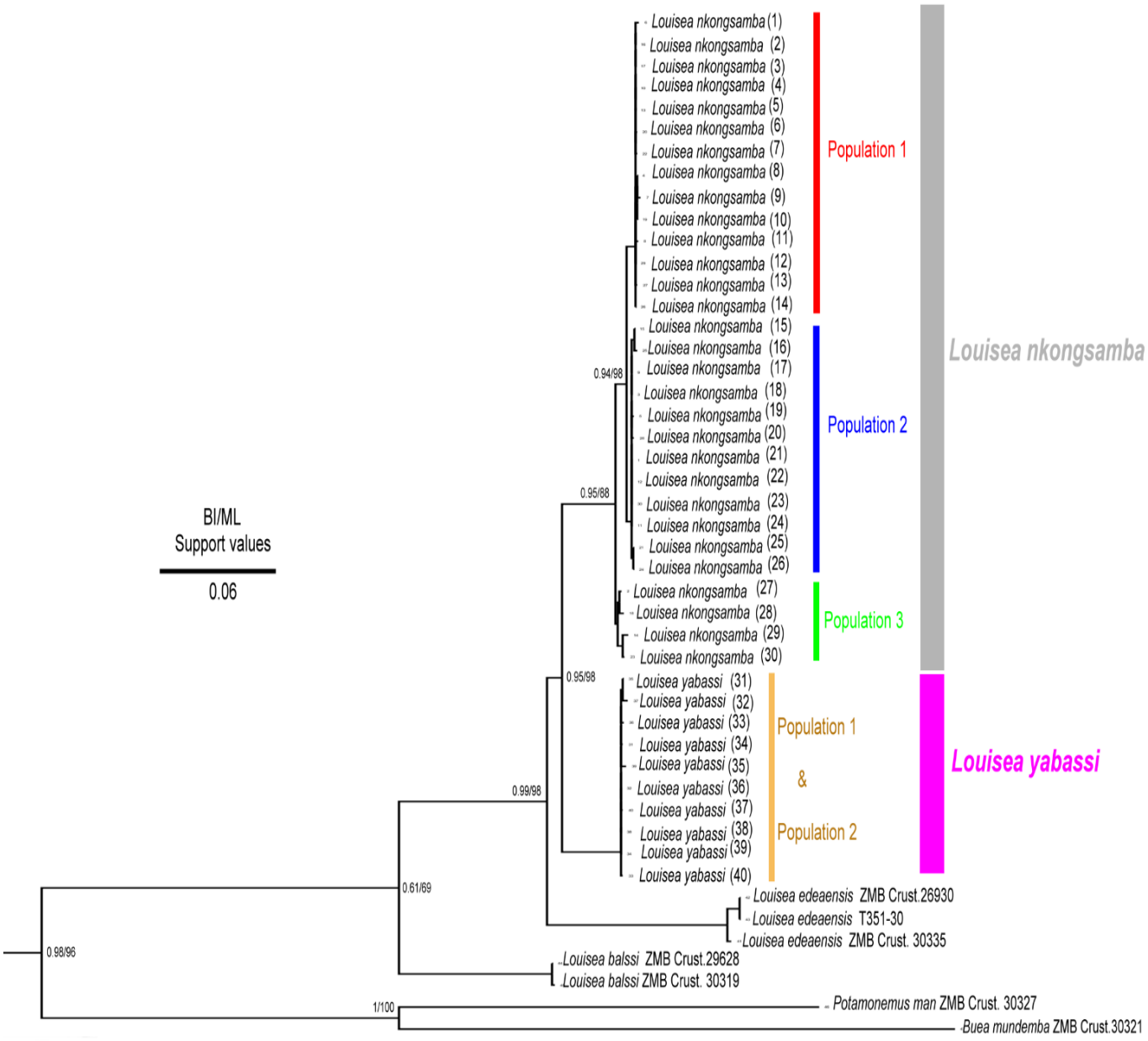

Figure 10. ML tree topology for the four freshwater crab *Louisea* species from Cameroon included in this study derived from mtDNA sequences corresponding to three loci (partial 16S rRNA, COI and 12S rRNA genes). BI and ML statistical values (%) on the nodes indicate posterior probabilities and bootstrap support, respectively.

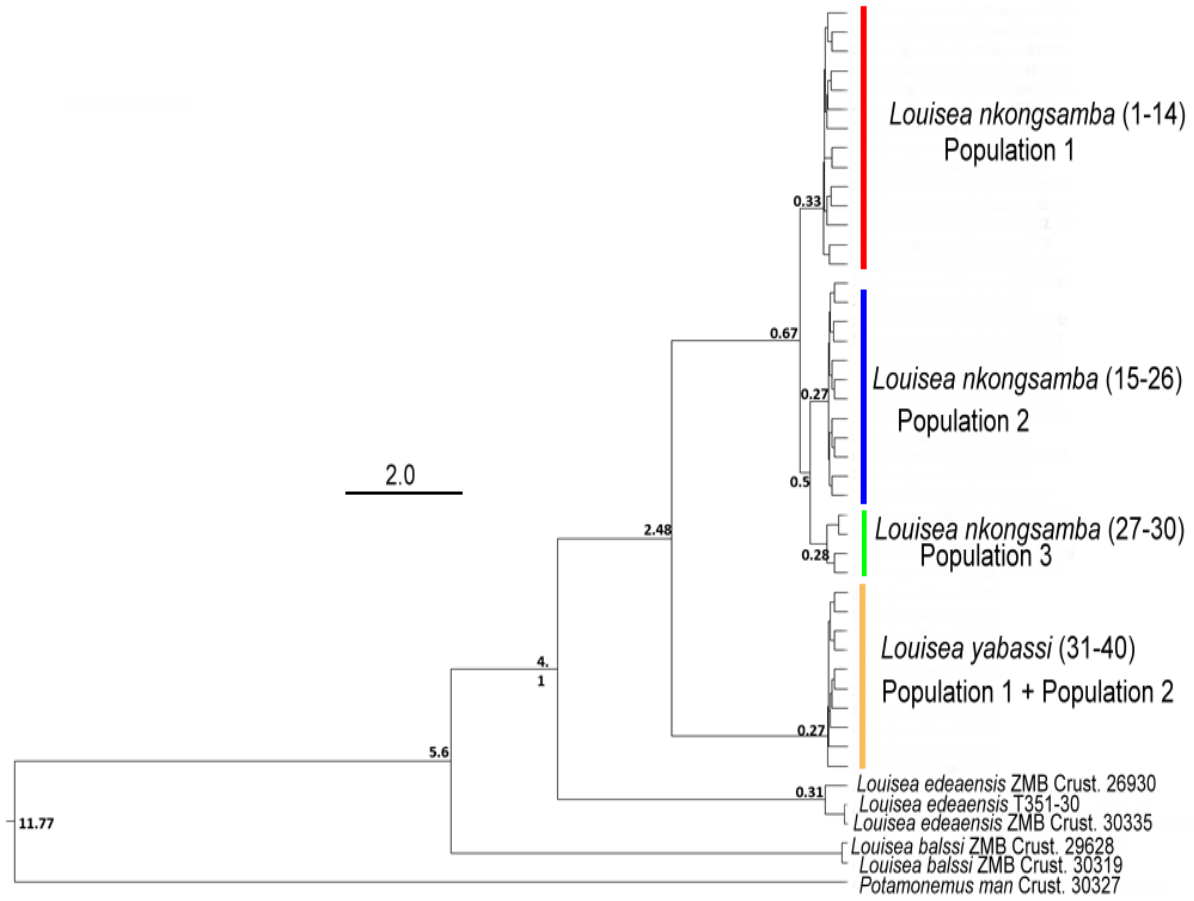

Figure 11. BI tree topology for the four freshwater crab *Louisea* species from Cameroon included in this study derived from COI mtDNA sequences constructed with BEAST 2.6.2. Statistical values on the nodes indicate age in million year.

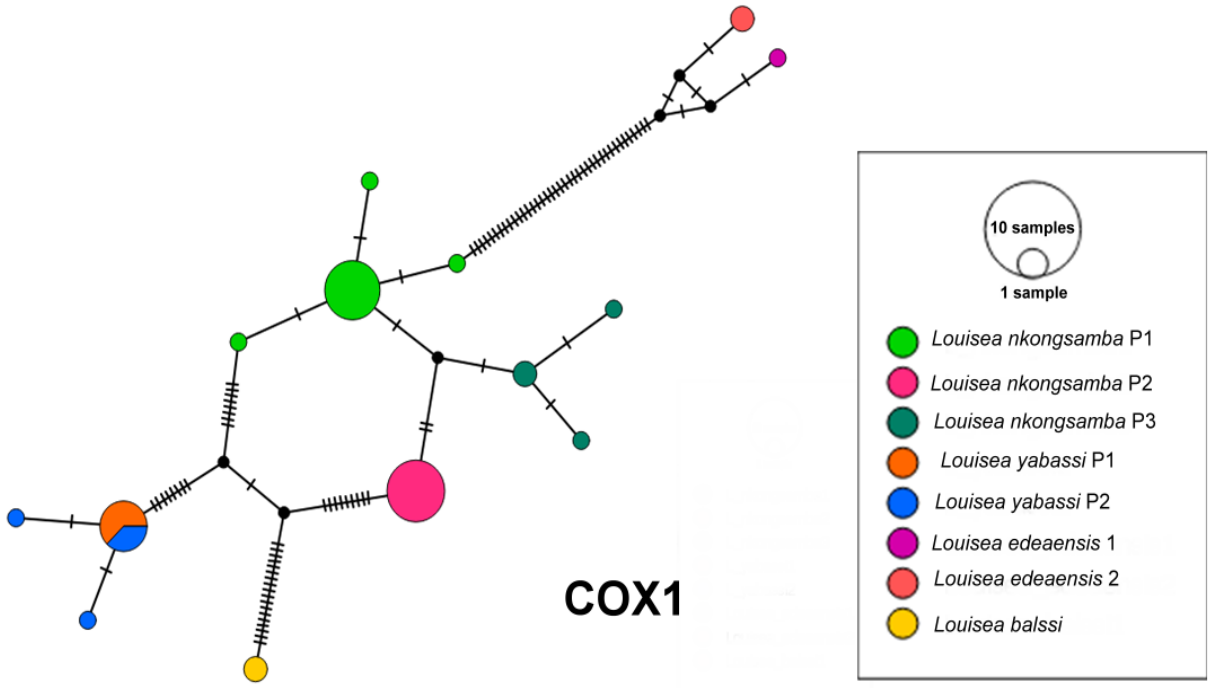

Figure 12. Maximum parsimony genotype networks of COI, constructed with PopArt. Hatch marks stand for mutation steps.