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BOECKELLA

Genetic variability of New Caledonian *Boeckella* De Guerne & Richard, 1889 (Copepoda: Calanoida), with description of a new species

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ABSTRACT

Thirteen new freshwater populations of the copepod genus *Boeckella* De Guerne & Richard, 1889 were found during three expeditions to New Caledonia ('La Planète Revisitée,' 2016-2018). The 12 populations from the Plaine des Lacs, which show remarkable genetic diversity among themselves, were identified as *B. spinogibba* Defaye, 1998, the only species of its genus known from New Caledonia until now. The sole exception, the population from Mont-Dore 22 km further east, appeared genetically and morphologically distinct from the others and is described herein as a new species. The two species are distinguished from each other by the shapes of the male and female P5, female Th5, and body colour. A previously published key is amended to separate the species. Our concatenated COI+28S phylogeny places the two New Caledonia species as a new branch within *Boeckella*, distinct from the branches consisting of South American, Antarctic and Australasian species. All 13 *Boeckella* populations inhabit the extreme south of New Caledonia, an area known for endemism and high heavy metal concentrations in the soil. Extensive mining activity in this metal-rich area, begun in 1873, is now leading to conflict with conservation goals. By using aerial

photographs to trace the fate of the pond that is the type locality of *B. spinogibba*, we confirmed that it disappeared between 2007 and 2014 as a result of the expansion of the Goro nickel mine.

KEY WORDS: *Boeckella spinogibba*, conservation, Crustacea, micro-endemism, freshwater zooplankton, mining perturbation, morphology, phylogeny, population genetics

INTRODUCTION

The main island of New Caledonia (Grande Terre) separated from Australia around 80 mya. It later became submerged until approximately 37 mya, when uplift caused the re-emersion of Grande Terre along with rocks from the upper mantle. Laterization of these mantellic rocks formed ultramafic massifs abundant in heavy metals across the island (Dubois *et al.*, 1974; Pelletier, 2007; Grandcolas *et al.*, 2008). Ultramafic massifs are mainly located in the southeast of New Caledonia (Fig. 1), a site of high endemism (Nattier *et al.*, 2013; Isnard *et al.*, 2016). Intensive mining of, e.g., nickel and cobalt, constitutes a major threat to the area's biodiversity (L'Huillier & Jaffré, 2010; Pitoiset, 2016).

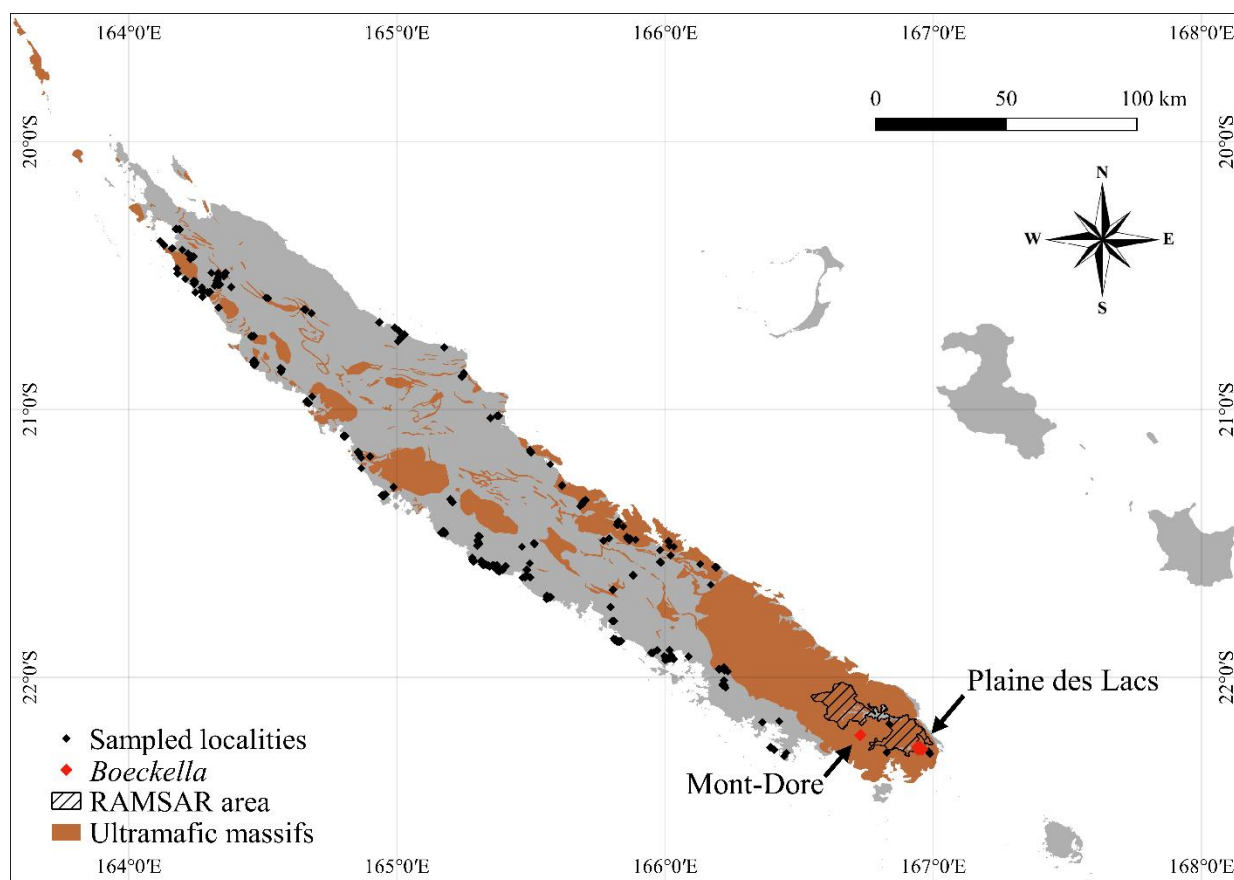


Figure 1. Localities sampled in New Caledonia during the three ‘La Planète Revisitée’ expeditions (2016–2018). Red represents localities where *Boeckella* was found.

New Caledonia is globally acknowledged to be a major biodiversity hotspot despite its relatively small surface area (18,576 km²). The fauna of its lentic freshwater bodies is, however, relatively little-known. Freshwater ponds are diverse environments; the turbidity of the water, size of the pool, flora outside and inside, and average water temperature are some of the characteristics that vary over time and space and condition the fauna inhabiting them. The fauna of New Caledonia freshwater ponds includes such invertebrates as the coleopteran *Phalilus oberthuri* Guignot, 1935, the ostracod *Cyprinotus cingalensis* Brady, 1886, and the branchiopod *Lynceus insularis* Olesen, Pöllabauer, Sigvardt & Rogers, 2016 [Olesen *et al.* 2016] (Inventaire National du Patrimoine Naturel, 2023). When such bodies are temporary, their cyclic drying-out prevents most members of the aquatic fauna from colonizing them permanently so that most fishes and some macro-crustaceans are excluded. As planktonic crustaceans in temporary freshwater can produce diapausing eggs, they often represent a prominent fraction of biodiversity in these environments (Brock *et al.*, 2003).

The diversity of freshwater zooplankton remains inadequately studied globally, even more so in New Caledonia. To fill in knowledge gaps there, many temporary ponds and sinkholes all across Grande Terre were sampled during three ‘La Planète Revisitée’ expeditions between 2016 and 2018 (Fig. 1). Preliminary examinations of these samples identified many previously unknown populations of freshwater crustaceans. More specifically, the copepod genus *Boeckella* De Guerne & Richard, 1889 was exclusively found on ultramafic massifs known for hosting high endemism. Only one species of *Boeckella* had previously been reported in New Caledonian temporary freshwater ponds: *B. spinogibba* Defaye, 1998. It was described and reported from a single locality on the ultramafic massifs of the south of Grande Terre (Defaye, 1998) and has not been further studied or sought for since.

We aimed to describe part of the taxonomic and genetic diversity of *Boeckella* in New Caledonia. This genus is currently composed of 42 valid species, including 23 from Australasia and 19 from South America, various sub-Antarctic islands, and Antarctica (Supplementary material Fig. S1; Maturana *et al.*, 2021; Walter & Boxshall, 2021). Extensive sampling conducted between 2016 and 2018 resulted in the identification of new populations of *Boeckella* in temporary ponds, one of which is geographically isolated in Mont-Dore, approximately 22 km away from the others, which inhabit ponds in the Plaine des Lacs. The morphological and genetic features of the *Boeckella* populations from both locations were investigated, including a population genetics analysis based on the cytochrome C oxidase I (COI) gene in these populations. The left antennule (A1) of *B. spinogibba* is redescribed.

Some of these populations are in ponds located in the RAMSAR area defined by the Convention on Wetlands (Fig. 1) and very close to the Goro mine, the largest nickel mine in New Caledonia. This facility is an open-pit complex where nickel and cobalt minerals are extracted. It has notably expanded since the description of *B. spinogibba* in 1998, and the effect of such expansion on the fate of the type locality of *B. spinogibba* is here tracked through time.

MATERIALS AND METHODS

Study sites and sampling

The survey of 469 ponds on Grande Terre was conducted between 2016 and 2018. Ponds filled with water were sampled using a plankton net (NHBS, Bonn, Germany) with a frame diameter of 25 cm and a 55 cm-long bag of 200 μ m nylon mesh with a filter at the tip. The net was either tossed and pulled from the edge of the pond or towed while swimming in the pond. Samples were preserved in 80–90% ethanol at 4 °C as soon as possible to avoid alteration of genetic material. Individuals of *Boeckella* were found in 13 temporary ponds. Twelve of these were in the Plaine des Lacs (Figs. 1, 2A–E) in a small area of 2.5 km² at around 290 m elevation situated between 22°16.2'S and 22°15'S latitude and between 166°56.4'E and 166°57.6E longitude (sites HYNC918, HYNC920, HYNC925, HYNC927, HYNC931, HYNC4102, HYNC4103, HYNC4104, HYNC4105, HYNC4109, HYNC4110 and HYNC4111) (Table 1). The remaining pond was in Mont-Dore (site HYNC928, 22°12.9'S, 166°43.6'E, 117 m elevation; Figs 1, 2F–G).

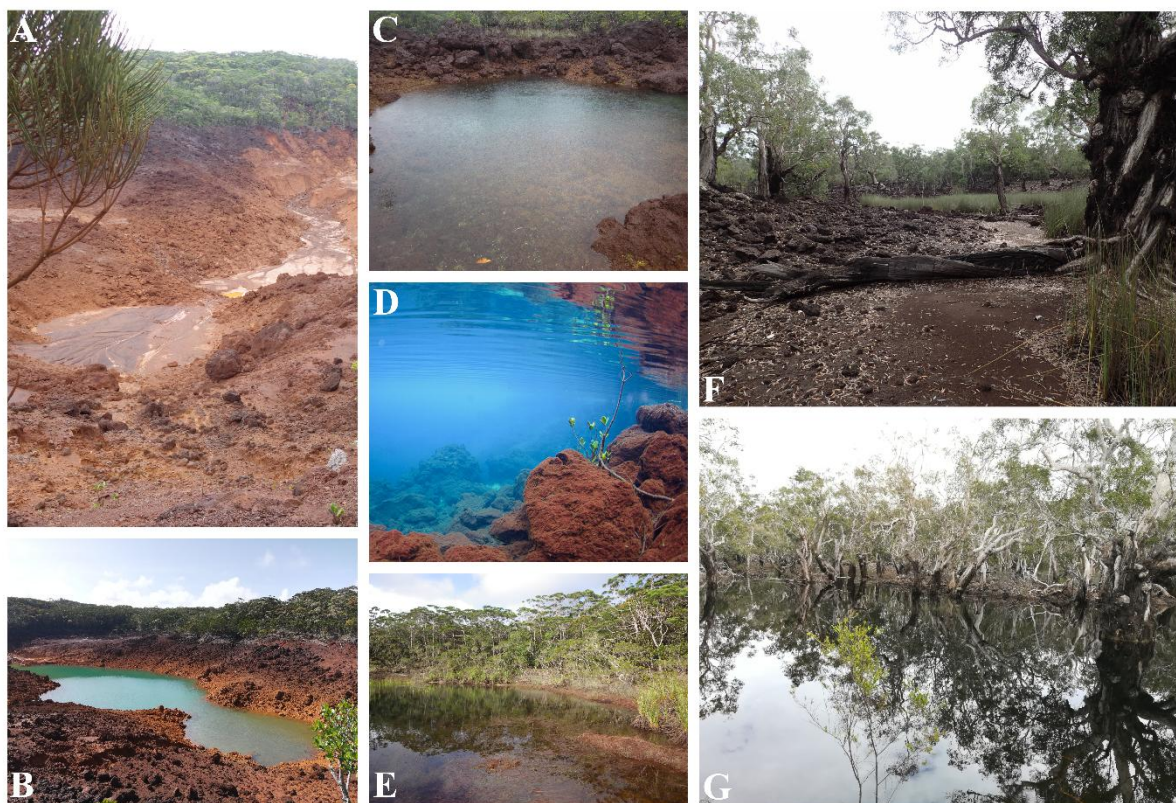


Figure 2. Sampled ponds in the Plaine des Lacs where species of *Boeckella* were found. Pond HYNC918 dry in November 2016 (A); filled in June 2018 (B). Pond HYNC920 in November 2016 (C); and in June 2018 (D). Pond HYNC931 in June 2018 (E). Sampled pond (HYNC928) in Mont-Dore where *Boeckella sibleti* sp. nov. was found (type locality). Dry in November 2016 (F); filled in June 2018 (G).

Table 1. *Boeckella* sampling sites, coordinates and aliases.

| Locality (pond) single identifier | Area in New Caledonia | Latitude | Longitude | Other names given to the locality |
|--------------------------------------|--------------------------|-------------|--------------|---|
| HYNC918 | Plaine des Lacs | 22°16.1982S | 166°57.5412E | HYNC4101 |
| HYNC920 | Plaine des Lacs | 22°15.7692S | 166°57.126E | HYNC4005 |
| HYNC925 | Plaine des Lacs | 22°15.6642S | 166°57.1554E | HYNC4107 ; Lac en long |
| HYNC927 | Plaine des Lacs | 22°15.726S | 166°57.1938E | HYNC4108 |
| HYNC931 | Plaine des Lacs | 22°15.5502S | 166°56.901E | HYNC4106 ; DT53 |
| HYNC4102 | Plaine des Lacs | 22°15.5556S | 166°56.4846E | |
| HYNC4103 | Plaine des Lacs | 22°16.125S | 166°56.4996E | |
| HYNC4104 | Plaine des Lacs | 22°16.101S | 166°56.8638E | |
| HYNC4105 | Plaine des Lacs | 22°15.7542S | 166°56.865E | |
| HYNC4109 | Plaine des Lacs | 22°15.7182S | 166°57.201E | |
| HYNC4110 | Plaine des Lacs | 22°15.7686S | 166°57.207E | |
| HYNC4111 | Plaine des Lacs | 22°15.7176S | 166°57.2382E | Doline croissante |
| HYNC928 | Mont-Dore | 22°12.93S | 166°43.662E | HYNC4004 |

Morphological analysis

Specimens were dissected under a binocular dissecting microscope (SZX7, Olympus, Tokyo, Japan) and microscopically observed in glycerol or 96% ethanol. Photographs serving as the basis for drawings and measurements were taken with a digital camera (MC170 HD, Leica, Wetzlar, Germany) attached to an optical compound microscope (BX51, Olympus); the drawings and measurements were made using Inkscape 1.2.2 (The Inkscape Project; <https://inkscape.org/>) and Gimp 2.10 (GNOME Project; <https://www.gimp.org/>).

Some specimens from the Plaine des Lacs and Mont-Dore were prepared for SEM examination and underwent sequential dehydration in 96% and 100% ethanol followed by critical point-drying in liquid CO₂ (Emitech K850, Quorum Technologies, Lewes, UK). After mounting on flat aluminium stubs (12.7 mm diameter) with double-sided conductive carbon adhesive tape, specimens were sputter-coated with platinum (EM ACE600, Leica) and examined at 15 kV accelerating voltage and at 90–5500× magnification in a scanning electron microscope (SU3500, Hitachi, Tokyo, Japan).

Abbreviations used in the text: Th (1–5), thoracic segment; A1, antennules; Ur (1–5), urosomites; P (1–5), legs; B1, coxa; B2, basis; Re (1–3), exopod; Ri (1–3), endopod.

All the sampled material except for specimens used destructively for SEM or sequencing is preserved in ethanol and deposited in the Muséum national d'Histoire naturelle, Paris (see Supplementary material Fig. S2 for registration numbers of samples).

DNA extraction and PCR

Apart from site HYNC4105, where only a few individuals of *Boeckella* were found, DNA was extracted from 5–10 individuals from each pond where this genus was detected (90 individuals in total). Lysis was performed on whole bodies, and DNA was extracted from tissues by the NucleoSpin® 96 robot using the NucleoSpin® 96 Tissue Core Kit (Macherey-Nagel, Düren, Germany).

In order to amplify the mitochondrial gene COI (cytochrome C oxidase I), Folmer primers LCO1490 and HCO2198 (Folmer *et al.*, 1994) were used with the following PCR (polymerase chain reaction) cycle: 5 min at 94 °C; 40 cycles of 30 sec at 94 °C, 30 sec at 50 °C, 1 min at 72 °C, and 8 min at 72 °C. To ensure proper amplification of the sequences, PCR products were subjected to gel electrophoresis. The expected size of amplified DNA strands was 658 bp without primers. Detailed protocols are available on protocols.io (DNA

extraction: <https://dx.doi.org/10.17504/protocols.io.j8nlk4x7wg5r/v2>; PCR: <https://dx.doi.org/10.17504/protocols.io.261ge4y4jv47/v3>).

Sequencing and phylogenetic analysis

Properly amplified DNA sequences were sent to Eurofins Scientific (Luxembourg City, Luxembourg) for Sanger sequencing (Sanger *et al.*, 1977). The obtained sequences were cleaned, complementary strands were aligned, and consensus sequences were determined using Galaxy (<https://ecology.usegalaxy.eu>; The Galaxy Community, 2022).

To obtain more genomic information on individuals from New Caledonia, library preps from DNA amplified from one individual from the Plaine des Lacs (Site HYNC931; DNA sample identifier M156) and 50 individuals from Mont-Dore (DNA sample identifier M223) were performed with a Nextera XT kit (Illumina; <https://www.illumina.com/>) using fragmentation and Illumina index adapter ligation. Equimolar pools of each library were established. Qualification and quantification of the final library was established before sequencing on Illumina Miseq at Plateforme iGenSeq, Institut du Cerveau (ICM) (Hôpital Pitié Salpêtrière, Paris) with 2×25 million reads cartridge of 300 bases each (30–45 libraries per run).

All raw sequences were assembled and sequencing errors corrected using Geneious (Geneious® 11.1.4, Dotmatics; <https://www.geneious.com/>). The consensus sequences are available in GenBank (see Supplementary material Fig. S3 for NCBI accession numbers). In addition, gene concatenations were performed by adding the assembled genes from the present study to the concatenation (ribosomal RNA large subunit 28S+COI) published by Maturana *et al.* (2021) and retrieved in Genbank. In total, the phylogeny was computed on 40 individual sequence concatenations (Supplementary material Fig. S4). Apart from the two species from New Caledonia, 16 other species of *Boeckella* were included in the phylogeny. *Calamoecia ampulla* (Searle, 1911) and *Parabroteas sarsi* (Daday, 1901) were used as an external group, as Adamowicz *et al.* (2010) demonstrated *Calamoecia* Brady, 1906 and *Parabroteas* Mrázek, 1901 formed a monophyletic group closest to *Boeckella* in the family Centropagidae. Genes were independently aligned with Geneious alignment in Geneious 11.1.4 (Dotmatics). The 28S was delimited by hand. The COI sequences were aligned and verified for the absence of a stop codon in the reading phase. The COI alignment was then subjected to a Gblock performed with the default options on the LIRMM web platform (Dereeper *et al.*, 2008). The concatenation was realized on PhyloSuite v1.2.3 (Zhang *et al.*, 2020; Xiang *et al.*, 2023). Evolution models were estimated with distinct codon positions for

COI using ‘model finder’ in PhyloSuite 1.2.3 (Kalyaanamoorthy *et al.*, 2017; Zhang *et al.*, 2020; Xiang *et al.*, 2023).

The concatenated 28S+COI phylogeny was constructed from a Bayesian analysis performed with MrBayes 3.2.7 (Ronquist *et al.*, 2012) using the models TIM+I+G (28S), TIM+G, TRN+I, and F81+I for each codon position (COI). Maximum likelihood analysis was performed with IQTree 2.2.0 (Minh *et al.*, 2020) using the models TIM+I+G (28S), TIM+G, TRN+I, and F81+I for each codon positions (COI), always with 1,000 bootstrap resamplings. The multilocus phylogenetic tree was formatted using FigTree 1.4.4 (<https://tree.bio.ed.ac.uk/software/figtree>) and Inkscape 1.2.2 (The Inkscape Project).

Two species-delimitation models were performed on the bPTP server (Bayesian Poisson Tree Processes; <https://species.h-its.org/>), one on the concatenated 28S+COI phylogenetic tree and one on a COI phylogenetic tree (Supplementary material Fig. S5). Both analyses were calibrated with 100,000 MCMC generations and exclusion of the outgroup.

Population structure of *Boeckella spinogibba*

To assess the genetic structure of the populations of *Boeckella spinogibba* found in the Plaine des Lacs, an exact test of population differentiation and an analysis of molecular variance (AMOVA) were performed using R 4.1.2 (CRAN) packages `mmod` 1.3.3 (Winter, 2012) and `ade4` 1.7-18 (Dray & Dufour, 2007), respectively. Significance of the AMOVA was computed through randomised matrix permutations. An alignment was also produced using Galaxy (mafft method, tool align seqs 1.9.1.0; Katoh *et al.*, 2002) in order to compute a median-joining haplotype network (Bandelt *et al.*, 1999) using R package `pegas` 1.2 (Paradis, 2010). Median-joining haplotype networks represent genetic links between haplotypes. The median-joining algorithm occasionally computes hypothetical haplotypes (referred to as ‘median vectors’), that could potentially be unsampled sequences, to resolve the network. The network was computed using several tolerance (epsilon) parametrisations in order to obtain several possible representations of the links between haplotypes. The tolerance parameter set in a median-joining network affects the search for median vectors. The augmentation of the tolerance parameter beyond 2 had little impact on the organisation of the network.

All sequence cleaning and analyses conducted in Galaxy have been implemented as reusable workflows at: https://usegalaxy.fr/u/coline_royaux/w/sanger1--from-ab1-to-aligned-fasta-imported-from-uploaded-file; https://usegalaxy.eu/u/coline_royaux/w/sanger-5--from-hand-corrected-haplotypes-table-to-population-haplotypes-map-and-euclidean-distance-matrix.

History of type locality of *Boeckella spinogibba*

The coordinates of the type locality of *B. spinogibba* provided by Defaye (1998) are precise within about 2 km². Aerial photographs of the region in question were downloaded from the Observatoire de l'environnement Nouvelle-Calédonie (www.oeil.nc), and satellite images were obtained from Google Earth Pro 7.3.2. The description of the surrounding environment by Defaye (1998) and these aerial and satellite images allowed us to find the pond and monitor its condition from 1976 to 2014.

TAXONOMY

Order Calanoida G.O. Sars, 1903

Family Centropagidae Giesbrecht, 1892

Boeckella De Guerne & Richard, 1889

Boeckella spinogibba Defaye, 1998

(Fig. 3)

Material examined: 2 males examined in SEM from *B. spinogibba* in pond HYNC931 (MNHN-UI-2017-2310, MNHN-UI-2017-2311); 5 males examined in photonic microscope from *B. spinogibba* in pond HYNC931 (4 individuals; MNHN-UI-2017-2310 and MNHN-UI-2017-2311) and in pond HYNC920 (1 individual; MNHN-UI-2017-2303).

Morphological remarks: On the right A1 of males, Defaye (1998) described five types of sensory structure: aesthetascs (Fig. 3A–C, G); thick setae (Fig. 3B, C); thin setae (Fig. 3C, G); setae with hairs (Fig. 3F); and pectinate setae (Fig. 3D, E). Detailed examination with SEM of specimens of *B. spinogibba* in pond HYNC931 of Plaine des Lacs (Table 2) showed that the distribution of these sensory structures on the right antennule of males is different from the original description by Defaye (1998). Namely, the original description made no mention of the thin seta on segments 3, 12, 17, and 19; the supplementary pectinate seta and the seta with hairs on segment 19; and the two thin setae on segment 20. No hairs were observed on the thin setae of segments 3, 5, 9, and 14, contrary to the original description, which mentioned such structures (Defaye, 1998).

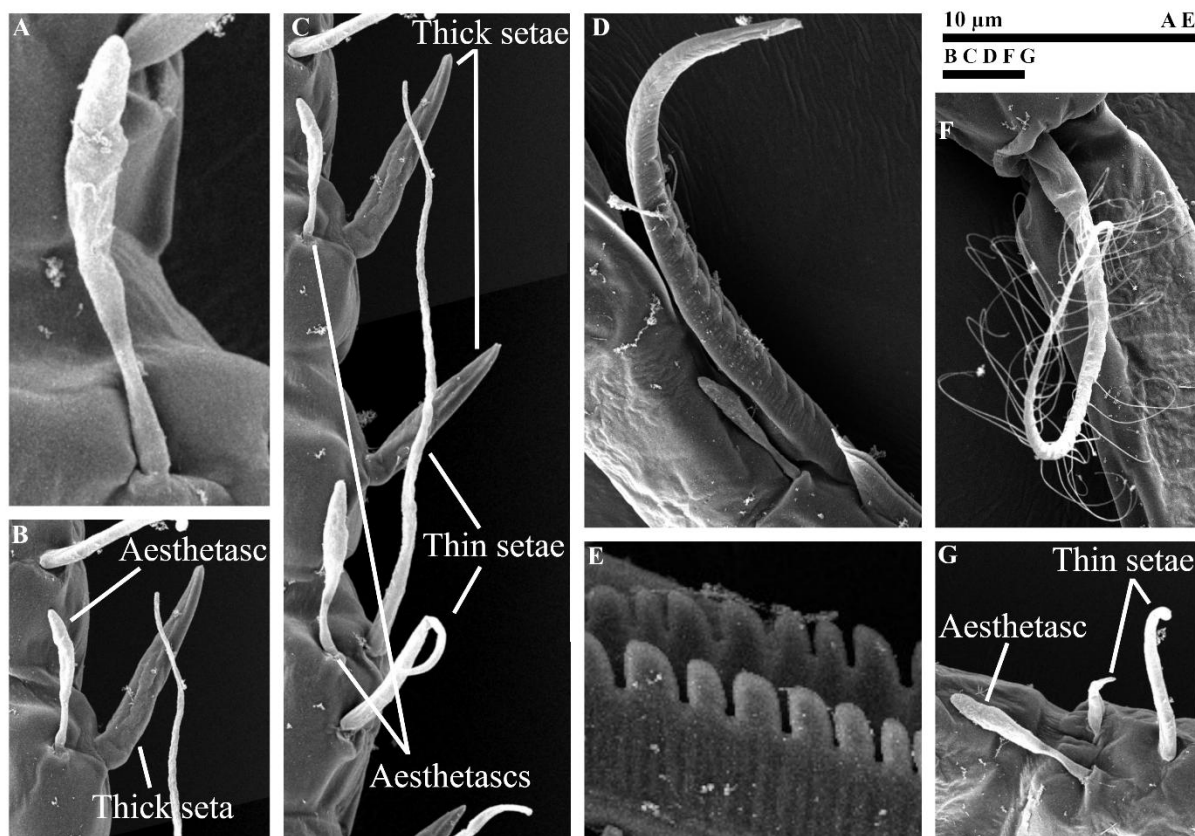


Figure 3. Types of sensory structures on male right antennule of *Boeckella spinogibba*. Lanceolate aesthetasc on segment 7 (A). Thick seta next to aesthetasc on segment 11 (B); long and short thin setae with two aesthetascs and two thick setae on segments 9–11 (C); pectinate seta on segment 19 (D); pectinate edge of pectinate seta on segment 18 (E); long seta on segment 19 with thin setules on distal third (F); short seta and tiny thin seta next to aesthetasc on segment 12 (G).

Table 2. Sensory armature of each segment (seg.) of the right antennules of male *Boeckella spinogibba* and *Boeckella sibleti sp. nov.*

| Seg. | Structures | Seg. | Structures | Seg. | Structures | Seg. | Structures |
|------|------------------------------|------|------------------------------|------|---------------------------------|------|---|
| 1 | 1 aesthetasc 1 thin seta | 7 | 2 thin setae 1 aesthetasc | 13 | 1 thin seta 1 aesthetasc | 19 | 2 pectinate setae 1 aesthetasc 1 thin seta 1 long, setulose seta |
| 2 | 3 thin setae 1 aesthetasc | 8 | 1 thin seta 1 thick seta | 14 | 2 thin setae 1 aesthetasc | 20 | 2 thin setae 2 long, setulose setae |
| 3 | 2 thin setae 1 aesthetasc | 9 | 1 aesthetasc 2 thin setae | 15 | 2 thin setae 1 aesthetasc | 21 | 2 long, setulose setae |
| 4 | 2 thin setae | 10 | 1 thick seta | 16 | 2 thin setae 1 aesthetasc | 22 | 3 long, setulose setae 2 thin setae 1 aesthetasc |
| 5 | 2 thin setae 1 aesthetasc | 11 | 1 thick seta 1 aesthetasc | 17 | 1 pectinate seta 1 thin seta | | |
| 6 | 1 thin seta | 12 | 2 thin setae 1 aesthetasc | 18 | 1 pectinate seta 1 thin seta | | |

Boeckella sibleti sp. nov.

(Figs. 4–6)

Material examined: Holotype, MNHN-UI-2017-2299, male. Allotype, MNHN-UI-2017-2300, female. Paratypes, MNHN-UI-2017-2298, 25 males; MNHN-UI-2017-2297, 20 females. Topotypes, MNHN-UI-2017-2301, MNHN-UI-2017-2302, >1,000 males (length, without furcal setae, 1.00–1.14 mm) and females (length, without furcal setae, 1.07–1.28 mm).

Type locality: HYNC928, a pond near the Pirogues River in Mont-Dore, Southern Province, New Caledonia, 22°12.9'S, 166°43.68'E, elevation 117 m, collected 5 June 2018. The pond occupies a pseudo-karstic formation called a 'doline' or 'sinkhole' (Fig. 2F-G). Its water is rather heavily tannic and the surrounding extensive area of shrubby vegetation includes a large number of niaouli trees (bark tea tree, *Melaleuca quinquenervia*, Blake).

Etymology: Named in honour of Jean-Philippe Sibley, a fervent defender of nature conservation.

Colouration: Body red in both sexes.

Male: Holotype, MNHN-UI-2017–2299, length without furcal setae 1.13 mm. Body elongated as a tapered droplet. Cephalothorax 6-segmented with cephalosome longest, 5 free thoracic segments (Th1, closest to cephalosome, to Th5, closest to urosome) successively shorter towards urosome (Fig. 4A).

Antennules (A1) asymmetrical as in *B. spinogibba* (Defaye, 1998: figs. 1F, 4C); left one 25-segmented, reaching tips of caudal rami; right one 22-segmented, slightly longer than left, with geniculations between 18, 19th and 19, 20th segments (Fig. 4B). Mouthparts as in *B. spinogibba* (Defaye, 1998: figs. 2, 3A, 5A).

Th5 (Fig. 5A) symmetrical, with each 'wing' ending in pointed lobe nearly reaching posterior end of first urosomite. Wing ending with small terminal spine and bearing second small spine towards posterior margin.

Natatory legs P1 to P4 identical to those of *B. spinogibba* (Defaye, 1998: fig. 5B–E).

Urosome with 5 urosomites successively decreasing in length towards furca from Ur1 to Ur4 but with Ur5 longer than Ur4 (Fig. 4A, 5A).

P5 (Fig. 5B–D) asymmetrical, with left limb's coxa (B1) twice as long as that of right limb. Left P5: Basis (B2) with medially swollen, finely denticular protrusion on inner margin, overlapping proximal margin of exopod (Re1), latter with distally denticulate terminal lateral spine. Endopod (Ri) atrophied but bearing fine hairs, inserted beneath B2's protrusion before Re1. Re2 with proximolateral distally denticulate spine. Re3 elongated, spine-like with slight medial curve, denticulate, pointed at apex. Right P5: B2 without medial protrusion, Ri

elongated, digitiform, swollen at midlength, medially curved, rounded terminally. Re1 approximately half as long as Re2, bearing distal spine armed with approximately uniformly-sized denticles. Re2 with small medial bulge on inner margin and large, distally denticulate distolateral spine of almost equal length to segment. Re3 elongated, medially curved at midlength with slight medial bulge, ending in pointed apex.

Female: Allotype, MNHN-UI-2017-2300. Larger than male, length without furcal setae 1.23 mm.

Body shape slender, elongated. Cephalothoracic tagmosis as in male (Fig. 6A). Mouthparts as in *B. spinogibba* (Defaye, 1998: figs. 2, 3A).

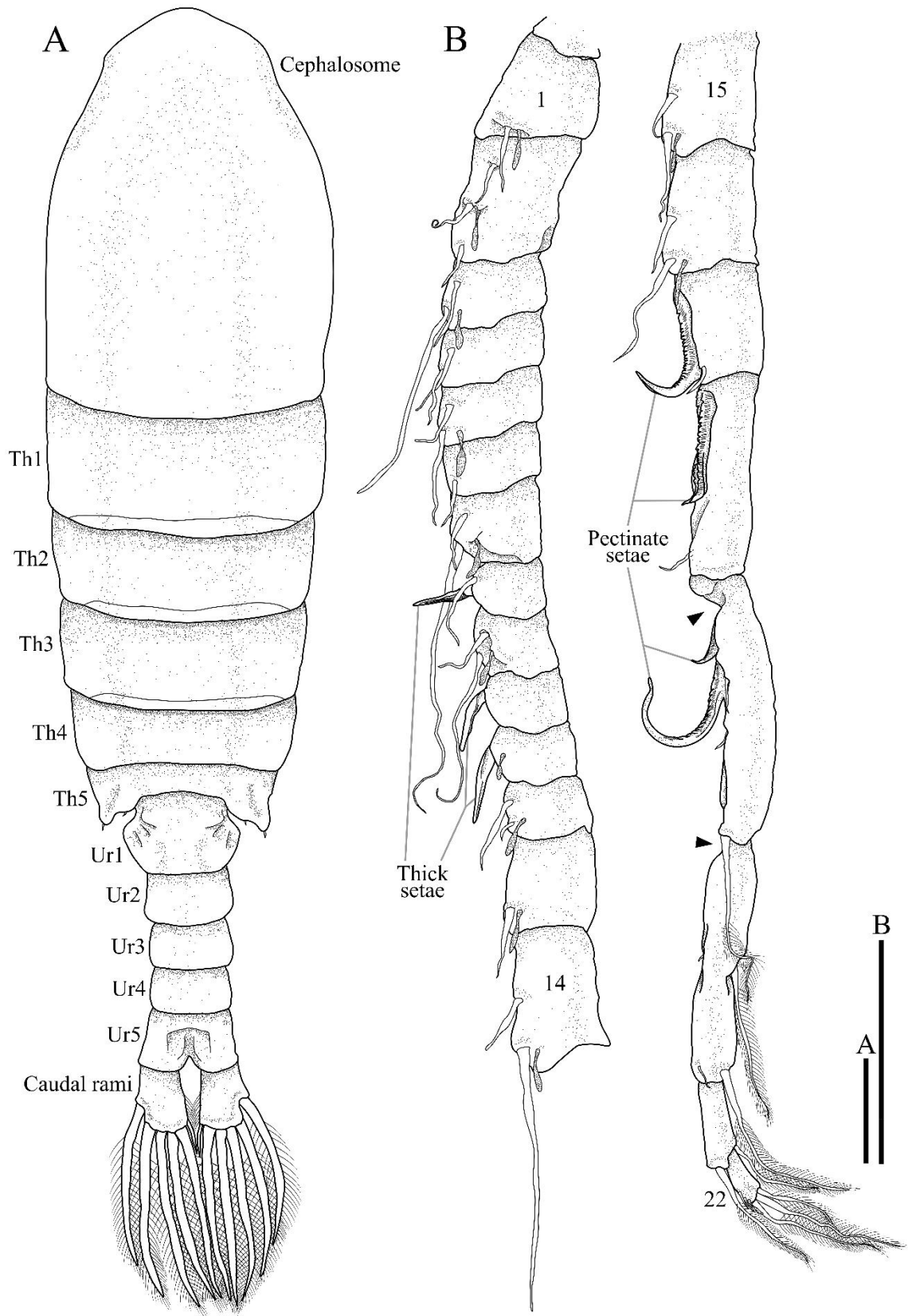


Figure 4. *Boeckella sibleti* sp. nov., male. Whole body without antennules, posterior view (A); right antennule, with thick setae and pectinate setae labelled, aesthetascs stippled, and long, setulose setae drawn with hairs, all others are thin setae; geniculation points indicated by black arrows (B). Scale bars = 100 μm.

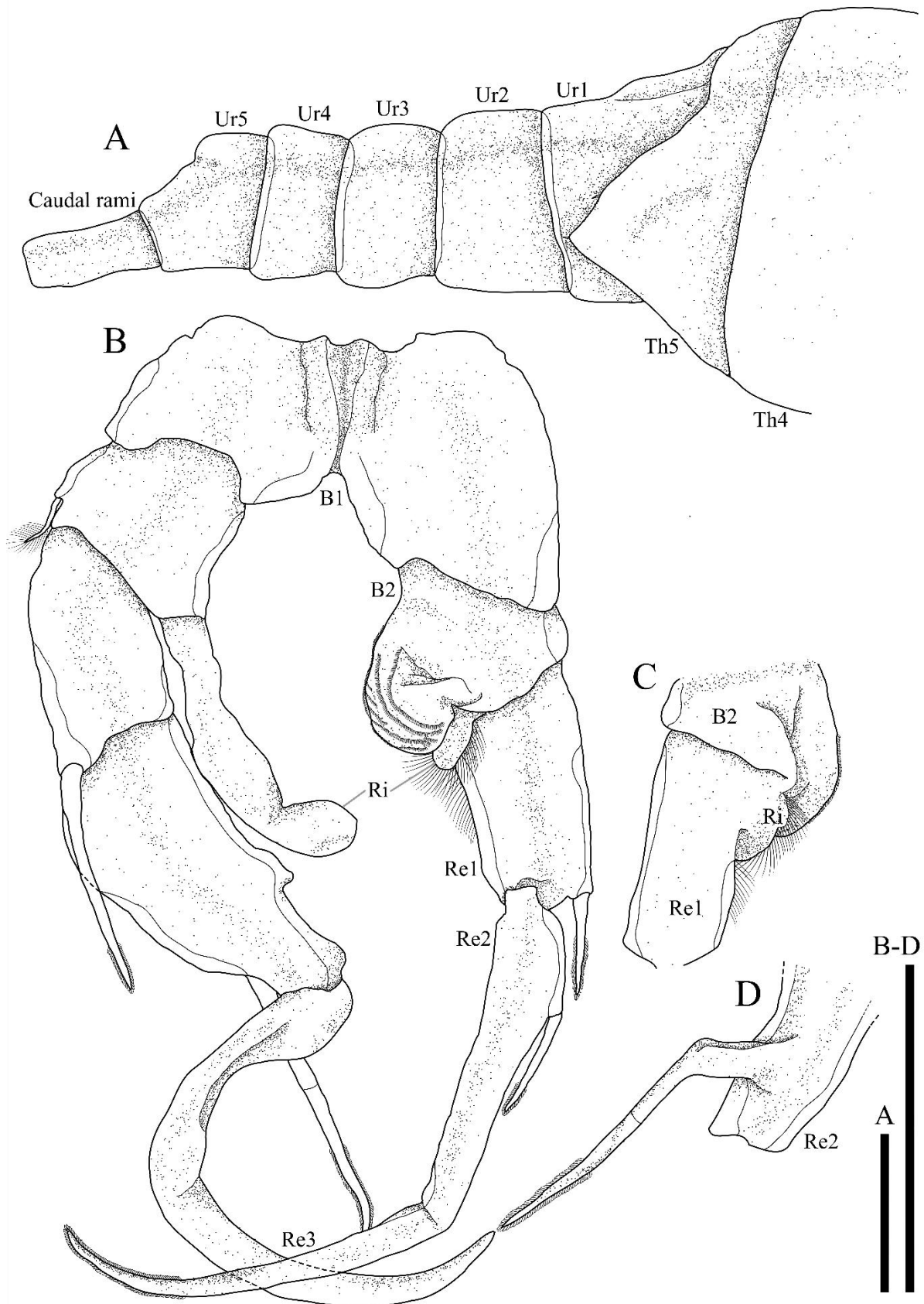


Figure 5. *Boeckella sibleti* sp. nov., male. Th4, Th5, and urosome, viewed from right side (A); P5, anterior view (B); left B2, Ri, and Re1, posterior view (C); distal part of left Re2, posterior view (D).

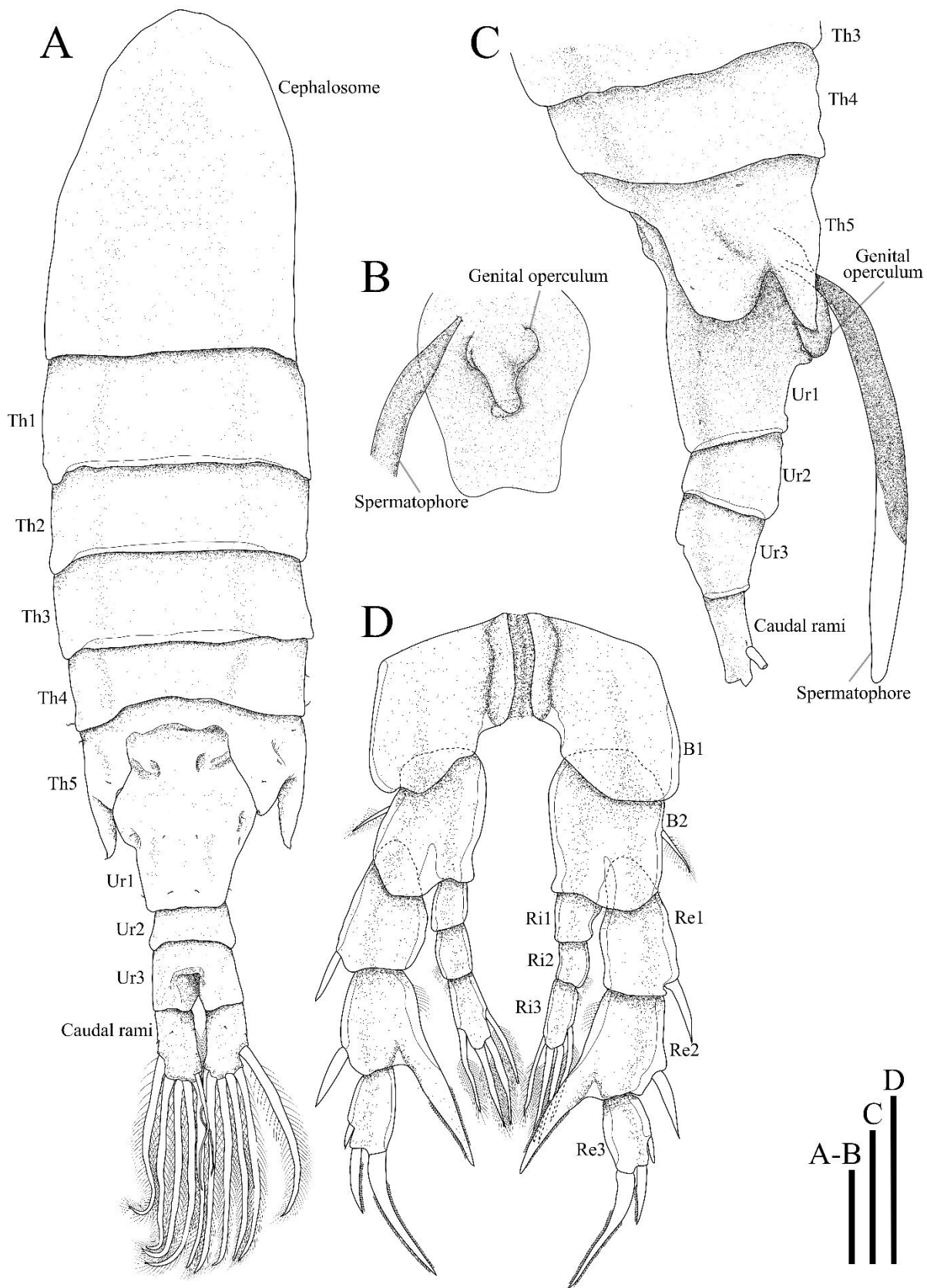


Figure 6. *Boeckella sibleti* sp. nov., female. Whole body without antennules, posterior view (A); genital operculum with part of spermatophore, anterior view (B); Th3, Th4, Th5, and urosome with spermatophore and genital operculum, viewed from right side (C); P5, anterior view (D). Scale bars = 100 μ m.

Antennules 25-segmented, extending beyond caudal rami, identical to those of *B. spinogibba* (Defaye, 1998: fig. 1F).

Left, right lateral ‘wings’ of Th5 ending in 2 lobes (Fig. 6A, C), and each lobe bearing small spine on outer margin.

Natatory legs P1–P4 identical to those of *B. spinogibba* (Defaye, 1998: fig. 5B–E).

Urosome 3-segmented. Urosomite 1 (Ur1) longest, widest on medial transversal axis, narrowing towards anterior and posterior margins from posterior view (Fig. 6A), asymmetrical anterior genital operculum, latter with wide anterior part, thinner posterior part in anterior view (Fig. 6B). Ur1 also bearing spermatophore (Fig. 6C).

P5 (Fig. 6D) symmetrical, distolateral spines of Re1-2 equal in length. Distomedial spiniform process of Re2 extending beyond Re3, Re3 armed with small distolateral spine and 2 longer terminal spines, medial one of which longest and medially curved. Ri 3-segmented; Ri3 bearing 4 spines with fine hairs.

Remarks: The body of *B. sibleti* **sp. nov.** is red, whereas that of *B. spinogibba* is blue. The body shape of males is similar in both species, and the mouthparts and swimming legs appear identical between the species. The main differences are found in P5 of both sexes (Fig. 7), and, for the female, Th5 (see Table 3).



Figure 7. SEM images of male copulatory organ (P5) of *Boeckella sibleti* **sp. nov.** (A) and of *B. spinogibba* (B). Major differences listed in Table 3 are indicated with a white arrow and a numeric identifier.

Table 3. Morphology-based differential diagnosis of *Boeckella spinogibba* and *B. sibleti* sp. nov. See Scheihing *et al.* (2010: 47, fig. 2) for abbreviations of parts of legs. Numbers (no.) given to differences shown in Figure 8 of text are provided for male P5.

| | | <i>Boeckella spinogibba</i> | | <i>Boeckella sibleti</i> | Numbers in Figure 8 |
|--------|-----|-----------------------------|--|---|---------------------|
| Female | Th5 | Right | Ending in two lobes of same length | Ending in two lobes with ventral lobe slightly longer | |
| | | Left | Ending in one lobe with a slight swelling bearing a small spine on the dorsal side | Ending in two lobes | |
| | P5 | Endopodite 3 | 3 spines | 4 spines | |
| Male | P5 | Right | Short spines on Re1 and Re2 | Longer spines on Re1 and Re2 | no. 1, 2 |
| | | | Ri thin and long | Ri thick and long | no. 3 |
| | | | Re3 slightly curved | Re3 highly curved like a hook | no. 4 |
| | | | No slit | Inner margin of Re2 with a small slit | no. 5 |
| | | Left | Round process on B2 rather flat and bonded to B2 | Round process on B2 rather plump and detached from B2 | no. 6 |

Both *B. spinogibba* and *B. sibleti* **sp. nov.** bear three spines on the Re3 of the female P5 (according to Defaye, 1998: 697), this is ‘the usual setation pattern of [this exopodite in] *Boeckella*.’ By contrast, most of the Australian species of *Boeckella* (*B. pilkililli* Quinlan & Bayly, 2017, *B. montana* Bayly, 1964, *B. opaqua* Fairbridge, 1945, and *B. triarticulata* Thomson, 1883) bear five to seven spines on this segment (Bayly, 1964; Quinlan & Bayly, 2017).

The female P5 in *B. spinogibba* has three spines on the Ri3, whereas the females of *B. sibleti* **sp. nov.** have four spines. By contrast, Australasian species usually bear four to six spines on the Ri3 in females (Bayly, 1964; cf. *B. pilkililli* Quinlan & Bayly, 2017 and *B. shieli* Bayly, 1985; Quinlan & Bayly, 2017: fig. 4D; Bayly, 1985: fig. 1E).

Phylogenetic analysis

Among the 90 DNA samples submitted to PCR, 84 were successfully amplified, sequenced, and cleaned. The haplotypes and NCBI accession numbers are provided in Supplementary material Fig. S3 (OQ117983–OQ118066). Pairwise genetic distances between each of the B_spi_COI01–B_spi_COI13 haplotypes and the single Mont-Dore haplotype (B_sib_COI01) ranged 8.51%–9.73%. Pairwise genetic distances among the B_spi_COI01–B_spi_COI13 haplotypes of 74 individuals were 2.9% or less. A unique haplotype was found at Mont-Dore in 10 individuals (Fig. 8, Supplementary material Fig. S2).

| | | <i>B. spinogibba</i> haplotypes from the Plaine des Lacs | | | | | | | | | | | | |
|--|---|--|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| | | B_spi_COI01 | B_spi_COI02 | B_spi_COI03 | B_spi_COI04 | B_spi_COI05 | B_spi_COI06 | B_spi_COI07 | B_spi_COI08 | B_spi_COI09 | B_spi_COI10 | B_spi_COI11 | B_spi_COI12 | B_spi_COI13 |
| <i>B. spinogibba</i> haplotypes from the Plaine des Lacs | B_spi_COI02 | 2.13 | | | | | | | | | | | | |
| | B_spi_COI03 | 2.89 | 1.67 | | | | | | | | | | | |
| | B_spi_COI04 | 2.43 | 1.22 | 1.98 | | | | | | | | | | |
| | B_spi_COI05 | 2.28 | 0.76 | 1.82 | 1.06 | | | | | | | | | |
| | B_spi_COI06 | 2.74 | 2.13 | 1.37 | 2.43 | 2.28 | | | | | | | | |
| | B_spi_COI07 | 2.43 | 0.91 | 1.98 | 1.22 | 0.15 | 2.43 | | | | | | | |
| | B_spi_COI08 | 2.58 | 1.37 | 2.13 | 0.15 | 1.22 | 2.58 | 1.37 | | | | | | |
| | B_spi_COI09 | 1.98 | 0.15 | 1.82 | 1.37 | 0.91 | 2.28 | 1.06 | 1.52 | | | | | |
| | B_spi_COI10 | 0.61 | 2.13 | 2.89 | 2.43 | 2.28 | 2.43 | 2.43 | 2.58 | 1.98 | | | | |
| | B_spi_COI11 | 0.46 | 1.98 | 2.74 | 2.28 | 2.13 | 2.58 | 2.28 | 2.43 | 1.82 | 0.15 | | | |
| | B_spi_COI12 | 2.28 | 0.15 | 1.82 | 1.37 | 0.76 | 2.28 | 0.91 | 1.52 | 0.30 | 2.28 | 2.13 | | |
| | B_spi_COI13 | 2.28 | 0.15 | 1.82 | 1.37 | 0.91 | 2.28 | 1.06 | 1.52 | 0.30 | 2.28 | 2.13 | 0.30 | |
| | <i>B. sibleti</i> sp. nov. haplotype from Mont-Dore | | 8.51 | 8.97 | 8.66 | 9.57 | 9.12 | 8.66 | 8.97 | 9.73 | 9.12 | 8.51 | 8.66 | 9.12 |

Figure 8. Matrix of cytochrome C oxydase I (COI) haplotype uncorrected genetic distance (%). All haplotypes of *Boeckella spinogibba* (Plaine des Lacs) and *B. sibleti* sp. nov. (Mont-Dore) are represented.

The consensus sequences for 28S were established with 2,087 reads for *B. sibleti* sp. nov. (M223; NCBI accession: OR612784) and 545 reads for *B. spinogibba* (M156; NCBI accession: OR612785).

The concatenated tree (28S+COI) based on the concatenation used in Maturana *et al.* (2021) has been reproduced with the additional concatenated sequences from New Caledonia (Fig. 9, Supplementary material Fig. S4). One concatenated sequence, identified as ‘Hap_19’ in Maturana *et al.* (2021), was excluded from the original data because, after verification, this sequence appeared likely to be a chimeric concatenation of two distinct species. Originally identified as *B. brevicaudata* (Brady, 1875), this sequence has been corrected in NCBI (accession MZ757027.3) since the publication of Maturana *et al.* (2021) and is now identified as *B. vallentini* (Scott, 1914). The final alignment obtained includes 1045 bp (673 for 28S, 372 for COI). The results are very similar to those obtained by Maturana *et al.* (2021) except that, in addition to the Australia/New Zealand group and the group of *Boeckella* from South America, Antarctica, and various sub-Antarctic Islands, the two New Caledonian

species together form a third robust monophyletic group within this genus.

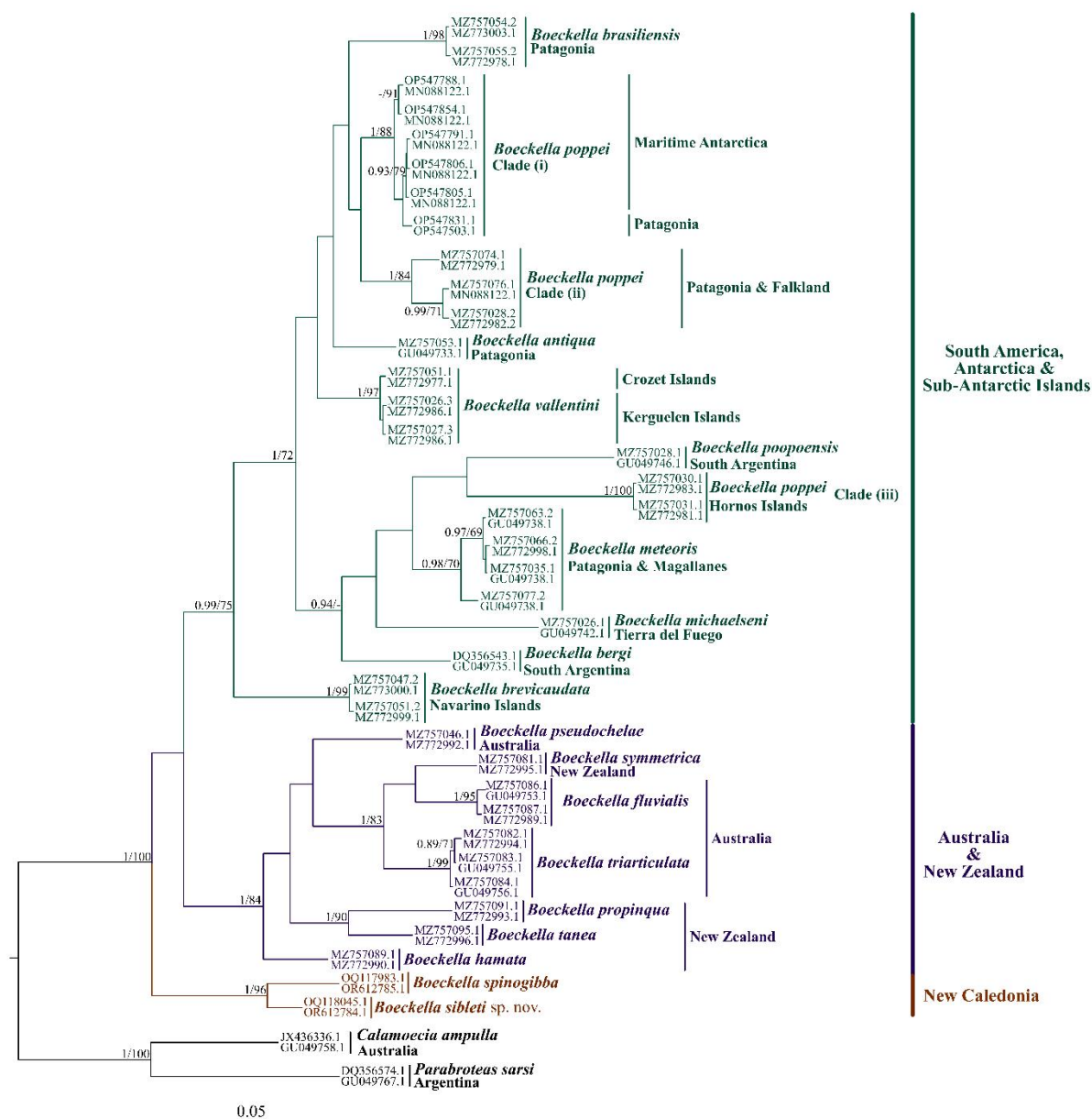


Figure 9. Concatenated phylogeny (28S+COI) of *Boeckella* in New Caledonia, other *Boeckella* species from South America, Antarctica, Australia, and New Zealand, and the outgroup *Calamoecia ampulla* - *Parabroteas sarsi* (nodes: probability support values according to Bayesian/bootstrap value for Maximum Likelihood).

The raw results from the two bPTP analyses are available in Supplementary material Figure S6), and the COI phylogenetic tree used for one of the analyses is also available there (Supplementary material Fig. S5). *Boeckella spinogibba* and *B. sibleti* **sp. nov.** are distinct in the two models, with good support.

Population structure

The AMOVA test performed on populations of *B. spinogibba* from the Plaine des Lacs showed these populations had a genetic variance rate of 17.24% within ponds and 82.76% among ponds ($P = 0.001$). Exact tests of population differentiation showed that the *Boeckella* populations were generally significantly genetically differentiated among themselves.

The haplotype network of *B. spinogibba* (Fig. 8, 10) shows the mutations, distribution, and abundance of haplotypes in the sampled localities. The network presented in Figure 10 has a tolerance parameter of 2; this network was chosen for its closeness to the distance matrix (Fig. 8). For the sake of clarity, the network is presented without alternative links, and six median vectors have been removed as they do not directly interact with several of the sampled haplotypes (see Supplementary material Fig. S7 for the network with all alternative links and median vectors). The network on Supplementary material Fig. S7 has a tolerance parameter of 1. Its main difference with Figure 10 concerns the link between haplotypes B_spi_COI03 and B_spi_COI06. The median-joining network was unable to resolve the links between these two haplotypes and the rest of the network as regards the real number of mutations between the sequences (e.g., 18 mutations between B_spi_COI01 and B_spi_COI06 versus 22 mutations displayed on Fig. 10 and 30 displayed in Supplementary material Fig. S7).

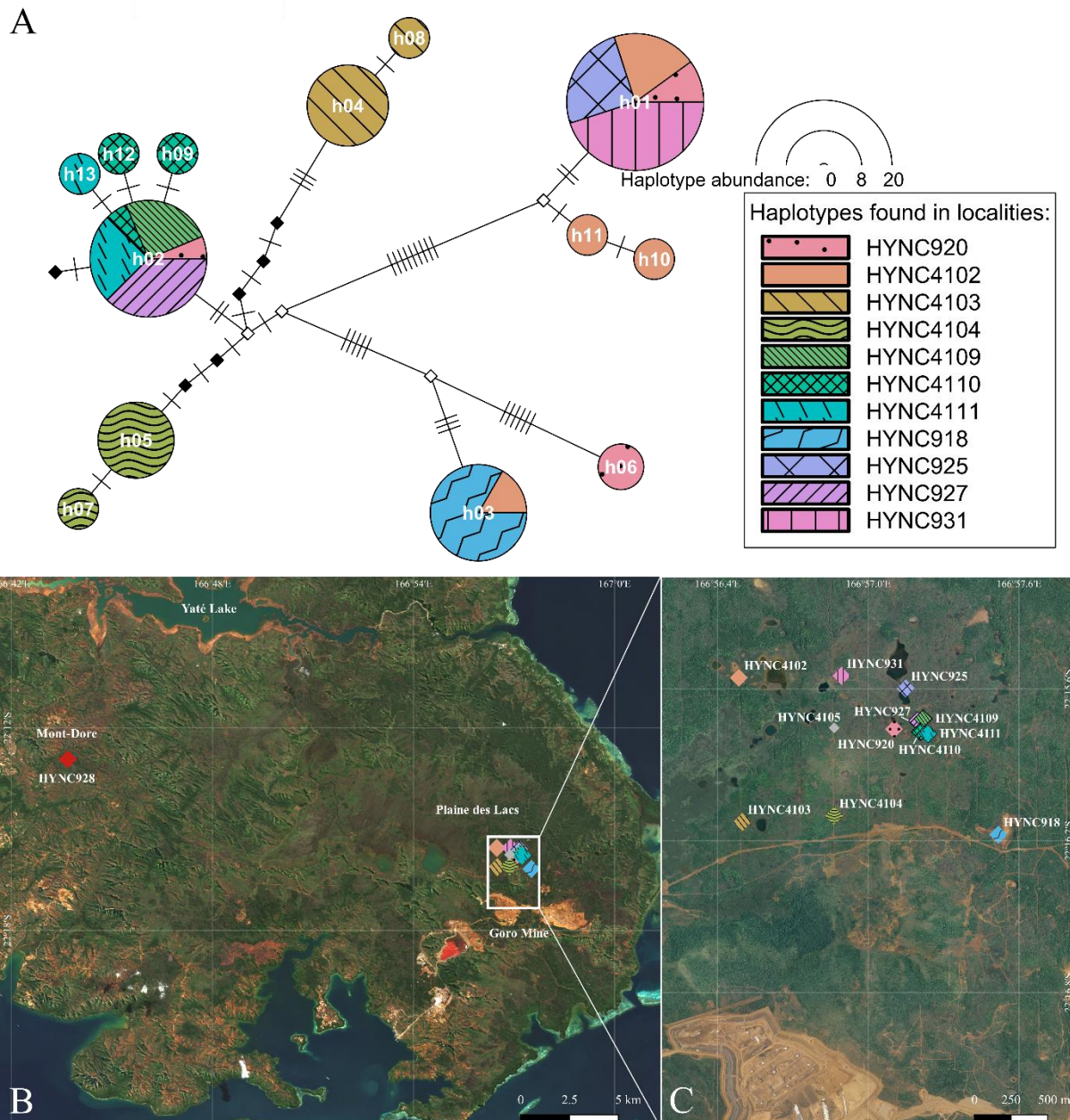


Figure 10. Haplotype network of 74 individuals of *Boeckella spinogibba* from 11 ponds in the Plaine des Lac; haplotype names, originally of the form B_spi_COIXX, are abbreviated to hXX for the purpose of this representation (A). Satellite images and locations of sampled localities where species of *Boeckella* were found (B). Closer view of satellite images of locations where *B. spinogibba* was found in the Plaine des Lac (outlined by a white rectangle in Fig. 10B). Localities are represented with colours and patterns corresponding to those in the haplotype network (Fig. 10A), except locality HYNC4105, which is represented in grey as no sequences were obtained from copepods from this pond (C).

The pond at site HYNC4102 displayed the highest haplotype richness (four haplotypes), whereas six other ponds contained only one haplotype each (sites HYNC918, HYNC925, HYNC927, HYNC928, HYNC931, and HYNC4109; Supplementary material Fig.

S2). The median-joining model inferred four main haplotypes, missed in the sampling (white diamonds in Fig. 10), being needed to articulate the structure of the network: one between haplotypes B_spi_COI01 and B_spi_COI11; one between B_spi_COI02, B_spi_COI04, and B_spi_COI05; one between B_spi_COI03 and B_spi_COI06; and one at the centre of the network between the B_spi_COI03–B_spi_COI06 and the B_spi_COI01–B_spi_COI11–B_spi_COI10 haplotype complexes. The other missing haplotypes inferred by the model support alternative links (black diamonds in Fig. 10, Supplementary material Fig. S7).

History of the type locality of *Boeckella spinogibba*

Defaye (1998: 687) described the type locality of *B. spinogibba* at the time of collection in 1996 as ‘a small pond in the Plaine des Lacs [...] on the route between Grand Lac and the fork to Parc Boisé, the pond nearest to the fork.’ As the area has been damaged by the Goro mine, roads on recent satellite images (Fig. 11C) are very different from those in 1996. Aerial photographs nonetheless allowed the pond to be located at 22°17.652’S, 166°58.044’E, while also showing that it was filled in by the mine between 2007 and 2014 (filled with water: Fig. 11A, B; filled with tailings from the mine: Fig. 11C).

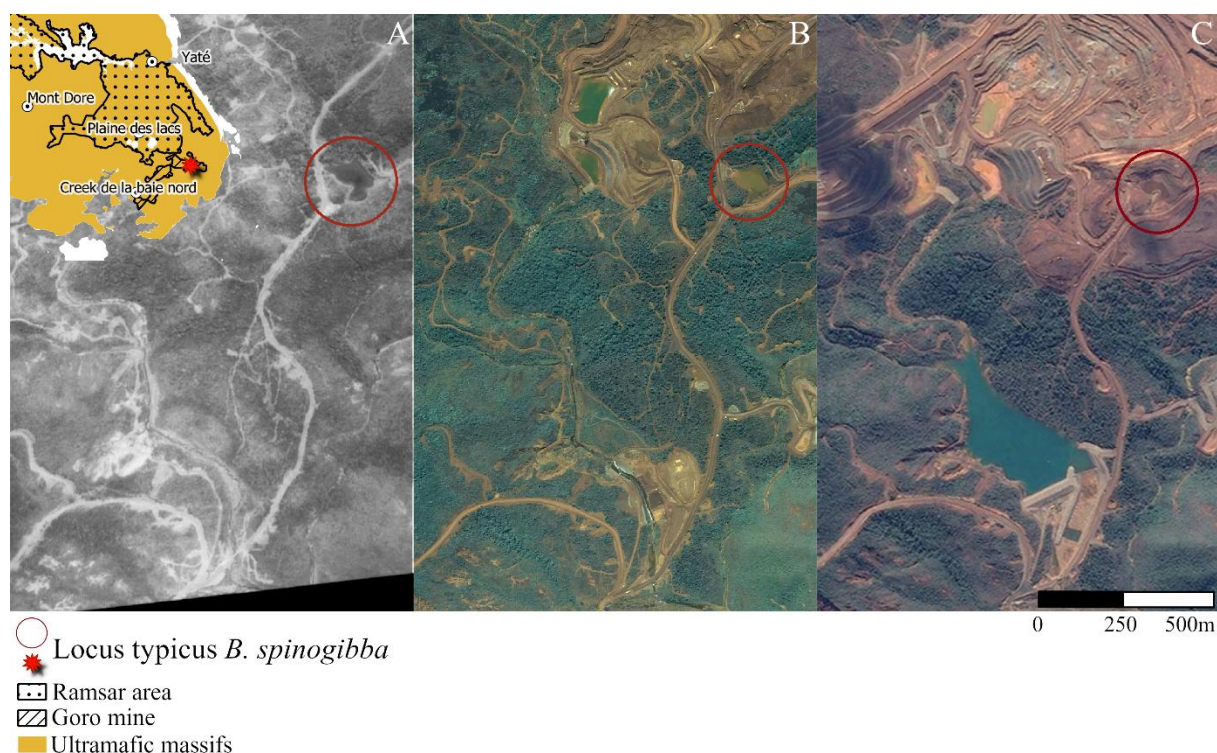


Figure 11. Aerial photographs and satellite images of the type locality of *Boeckella spinogibba* in the Goro mine area, New Caledonia in 1992 (A), 2007 (B), and 2014 (C).

DISCUSSION

Boeckella in New Caledonia

The genus *Boeckella* is generally considered a Gondwanan taxon (Bayly & Morton, 1978; Bayly, 1992; Maturana *et al.*, 2021) with three centres of distribution. It occurs in South America from the Andes Mountains in Colombia to the plains of southern Chile and Argentina, on sub-Antarctic islands and in coastal Antarctica; and in Australasia, especially in South Australia and New Zealand (Maturana *et al.*, 2019). *Boeckella triarticulata* Thomson, 1883 in Mongolia (Bayly, 1992) and Italy (Alfonso & Belmonte, 2008) is the only species present outside the Gondwanan area. Our concatenated COI+28S phylogeny places the two New Caledonian species as a monophyletic branch in the phylogeny of the genus, distinct from other such groups composed of South American and Australian species (Fig. 9). This suggests that the evolutionary history of the genus is more complex than previously suspected and merits further investigation.

No further information has been published on *Boeckella* in New Caledonia since the original observation of *B. spinogibba* from a single pond by Defaye (1998). We, however, found several populations of the species in the Plaine des Lacs which permitted the redescription of the left male antennule (A1) (Fig. 3) and the acquisition of genomic information on the species. We also found a population of *Boeckella* at Mont-Dore, 22.5 km from the nearest originally sampled population of *B. spinogibba*, that showed 8-9.6% genetic divergence from the latter in the COI gene and clear morphological differentiation, particularly in the male fifth leg, the copulatory organ (Fig. 7, Table 3) and its different body colour. According to the principles of integrative taxonomy (Padial *et al.*, 2010), several criteria, including evidence of genetic, morphological, and geographical differentiation, must be fulfilled to confirm that the species are distinct. Geographically, although the present two species are separated from each other, they are yet close enough to possibly indicate micro-endemism, something that is common in New Caledonia among, for example, plants and insects (Caesar *et al.*, 2017) and freshwater invertebrates such as gastropods (Haase & Bouchet, 1998). Such phenomenon seems to be particularly prominent on ultramafic soils (Pillon *et al.*, 2021), which is consistent with the unusual environmental conditions in New Caledonia, notably a high pH and high concentrations of heavy metals in the surface waters (Marques *et al.*, 2008). In New Caledonia, *Boeckella* has been found only in temporary freshwater ponds on ultramafic soils despite a fairly large amount of sampling in other types of ponds. This is a peculiar habitat, with water that is toxic for most plant and animal species and particularly poor in nutrients (Kierczak *et al.*, 2021). We still lack enough data on the

distribution of *Boeckella* in New Caledonia to confirm the extent of its micro-endemism.

The body colouration of copepods is mainly due to carotenoids, and freshwater copepods tend to have higher carotenoid concentrations than marine copepods. As highlighted in the review by Vilgrain *et al.* (2023), body colouration can vary widely in individuals depending on biotic and abiotic factors, making its study challenging. *Boeckella spinogibba* is blue, which is typical for copepods occurring at the water surface where this colour may be useful for camouflage, especially so for *B. spinogibba*, which lives in ponds with bright blue water (Fig. 2D). Red colouration like that of *B. sibleti* **sp. nov.** occurs in copepods from all ecosystems (marine and freshwater, shallow and deep) and can provide many physiological and ecological advantages, including, protection from UV light, antioxidant activity, and higher reproduction rates (Powers *et al.*, 2019; Vilgrain *et al.*, 2023).

Genetic divergence can occur over time when connectivity and the genetic flows that depend on it are no longer maintained, (Rundle & Nosil, 2005). Indeed, poor connectivity between different populations can lead to speciation (Schluter, 2001). The small size and weight of freshwater zooplankton permits their transportation, either as eggs, larvae, or adult individuals, by other animals, mostly wading birds, which may get eggs stuck in their feathers or ingest them accidentally (Incagnone *et al.*, 2015; Fontaneto, 2019; Parry *et al.*, 2023). In New Caledonia, *Boeckella* has only been found in ultramafic ponds, which are mostly oligotrophic if not dystrophic, with low prey density. Wading birds occur where there is a high density of prey (Bryant, 1979; Herteux *et al.*, 2019), which greatly limits the potential for dispersion of zooplanktonic species from these ultramafic ponds by birds. Moreover, *B. spinogibba* showed high genetic variability within a very small, 2.5 km² area, with up to 2.9% COI differentiation between individuals from different ponds and with highly structured populations in terms of haplotype distribution (Fig. 8, 10). AMOVA revealed that 83% of the observed genetic variance is attributed to variance among ponds, with only 17% being within ponds. The exact test also identified significant genetic divergence among ponds. As it has also been observed in *B. poppei* populations from Signy Islands, South Orkney Islands (Maturana *et al.*, 2020), these results tend to confirm weak connectivity for this genus in New Caledonia, which can support the hypothesis of an allopatric speciation between *B. spinogibba* and *B. sibleti* **sp. nov.**

Updated key by Quinlan & Bayly (2017)

Quinlan & Bayly (2017) updated the key of Australasian *Boeckella* by including *B. spinogibba* at couplet 18, using characters that are similar in *B. sibleti* **sp. nov.** as well. To

incorporate the new species in the key, couplet 18 is left unchanged but it now points towards a newly created couplet 24 for the two New Caledonian species, not just to *B. spinogibba*.

‘18. Left Ri small, reduced to a triangular plate and hidden by a rounded process covered in thick spiniform hairs; left B1 noticeably longer than right B1’.... 24

24. Right Re3 with acute claw-like, rounded shape with bulge at mid-length disto-lateral spine of Re1 approximately two-thirds as long as Re2 *B. sibleti* **sp. nov.**

Right Re3 slightly curved; disto-lateral spine of Re1 less than one-third as long as Re2 *B. spinogibba* Defaye, 1998

Impact of Goro mine

The pond that was the type locality of *B. spinogibba* was destroyed and filled in between 2007 and 2014 as a result of expansion of the Goro mine (Fig. 11). This event exemplifies the threat that mines pose for ponds and other freshwaters inside and outside their enclosures. Along with the destruction of the environment to build roads and other infrastructure and to extract minerals, mines in New Caledonia produce clouds of fine tailings that fill ponds outside the exploited perimeter. The open-pit complex of the Goro mine is very close to the present populations of *B. spinogibba* and the southern border of the RAMSAR area. According to Jeanpert *et al.* (2016), about 8,500 ponds occupied the 148 km² area around the future mining site in 2006 before the construction of the mine. Supplementary studies are needed in such areas, in particular in the RAMSAR area, where ponds and many other habitats are threatened by mining activities (Pascal *et al.*, 2008).

Perspectives and limitations

New Caledonia has been extensively sampled, but ultramafic soils are often less accessible. Our discovery of a new species in Mont-Dore suggests that other still unknown species may be found in unexplored parts of the Plaine des Lacs and elsewhere in New Caledonia.

The concatenated phylogeny computed in this study was mainly constructed from sequences concatenated by other workers, and the sources did not specify which individual copepods provided the sequences. Hence, there is a possibility that COI and 28S sequences of different individuals were concatenated for some portion of the Australian, New Zealand, Antarctic, and South American species. Because the mitochondrial marker (COI) we used only provides information about the maternal lineage and only two nuclear (28S) sequences were retrieved from shotgun sequencing, further exploration of the genetic structure of *Boeckella* populations in New Caledonia, notably using other genes, is desirable.

Microsatellite sequences of populations and species could also be used to test the hypothesis of isolation by distance and to help assess the dispersal potential of *Boeckella* in New Caledonia (Teske *et al.*, 2018).

SUPPLEMENTARY MATERIAL

S1 Figure. Table of present *Boeckella* species and associated regions.

S2 Figure. Distribution of amplified *Boeckella sibleti* **sp. nov.** and *B. spinogibba*.

S3 Figure. Accession numbers for sequences obtained for *Boeckella sibleti* **sp. nov.** and *B. spinogibba*.

S4 Figure. Accession numbers for sequences used in producing the 28S+COI phylogeny after Maturana *et al.* (2021).

S5 Figure. COI phylogeny of *Boeckella* in New Caledonia, other *Boeckella* species, and the outgroup *Calamoecia ampulla*.

S6 Figure. Raw results from bPTP analyses.

S7 Figure. Haplotype network with all alternative links and median vectors of 74 individuals of *B. spinogibba*.

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Data availability statement

ABI files from sequencing and datasets generated during and/or analysed during the current study are available in the Data.InDoRes repository [<https://doi.org/10.48579/PRO/DGVWPW>].

REFERENCES

- Adamowicz, S.J., Menu-Marque, S., Halse, S.A., Topan, J.C., Zemplak, T.S., Hebert, P.D. N. & Witt, J.D.S. 2010. The evolutionary diversification of the Centropagidae (Crustacea, Calanoida): A history of habitat shifts. *Molecular Phylogenetics and Evolution*, **55**: 418–430.
- Alfonso, G. & Belmonte, G. 2008. Expanding distribution of *Boeckella triarticulata* (Thomson, 1883) (Copepoda: Calanoida: Centropagidae) in Southern Italy. *Aquatic Invasions*, **3**: 247–251.
- Bandelt, H.J., Forster, P. & Rohlf, A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**: 37–48.
- Bayly, I.A.E. 1964. A revision of the Australasian species of the freshwater genera *Boeckella* and *Hemiboeckella* (Copepoda: Calanoida). *Australasian Journal of Marine and Freshwater Research*, **15**: 180–238.
- Bayly, I.A.E. 1985. A new species of *Boeckella* (Copepoda: Calanoida) from Western Australia, and comments on two other congeners. *Records of the West Australian Museum*, **12**: 79–84
- Bayly, I.A.E. 1992. *The non-marine Centropagidae (Copepoda, Calanoida) of the world*. SPB Academic Publishing, The Hague, The Netherlands.
- Bayly, I.A.E. & Morton, D. W. 1978. Aspects of the zoogeography of Australian microcrustaceans. *SIL Proceedings*, **20**: 2537–2540.
- Brady, G.S. 1875. Note on Entomostraca from Kerguelen’s Land and the south Indian Ocean. *Annals and Magazine of Natural History*, **16**: 162.

- Brady, G.S. 1886. Notes on freshwater Entomostraca from South Australia. *Proceedings of the Zoological Society of London*, **1886**: 82–93.
- Brady, G.S. 1906. On the Entomostracan Fauna of the New Zealand Lakes. *Proceedings of the Zoological Society of London*, **1906**: 696–697.
- Brock, M.A., Nielsen, D.L., Shiel, R.J., Green, J.D. & Langley, J.D. 2003. Drought and aquatic community resilience: the role of eggs and seeds in sediments of temporary wetlands. *Freshwater Biology*, **48**: 1207–1218.
- Bryant, D.M. 1979. Effects of prey density and site character on estuary usage by overwintering waders (Charadrii). *Estuarine and Coastal Marine Science*, **9**: 369–384.
- Caesar, M., Grandcolas, P. & Pellens, R. 2017. Outstanding micro-endemism in New Caledonia: More than one out of ten animal species have a very restricted distribution range. *PLoS ONE*, **12**: [<https://doi.org/10.1371/journal.pone.0181437>].
- Daday, E 1901. Diagnoses praecursoriae Copepodorum novorum e Patagonia. *Természetrájsz Füzetek*, **24**: 345–350.
- Defaye, D. 1998. Description of the first *Boeckella* (Copepoda, Calanoida, Centropagidae) from New Caledonia. *Crustaceana*, **71**: 686–699.
- De Guerne, J. & Richard, J. 1889. Révision des Calanides d'eau douce. *Mémoires de la Société Zoologique de France*. **2**: 1–129.
- Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.F., Guindon, S., Lefort, V., Lescot, M., Claverie, J.M. & Gascuel, O. 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research*, **36** (supplement 2): W465–W469.
- Dray, S. & Dufour, A.-B. 2007. The ade4 package: Implementing the duality diagram for ecologists. *Journal of Statistical Software*, **22**: 1–20.
- Dubois, J., Launay, J. & Recy, J. 1974. Uplift movements in New Caledonia-Loyalty Islands area and their plate tectonics interpretation. *Tectonophysics*, **24**: 133–150.
- Fairbridge, W.S. 1945. West Australian fresh-water calanoids (Copepoda). Three new species of *Boeckella*, with an account of the developmental stages of *B. opaqua* n. sp. and a key to the genus. *Journal and Proceedings of the Royal Society of Western Australia*. **29**: 25–65.
- Fontaneto, D. 2019. Long-distance passive dispersal in microscopic aquatic animals. *Movement Ecology*, **7**: [<https://doi.org/10.1186/s40462-019-0155-7>].
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan

- invertebrates. *Molecular Marine Biology and Biotechnology*, **3**: 294–299.
- Grandcolas, P., Murienne, J., Robillard, T., Desutter-Grandcolas, L., Jourdan, H., Guilbert, E. & Deharveng, L. 2008. Review. New Caledonia: A very old Darwinian island? *Philosophical Transactions of the Royal Society B: Biological Sciences*, **363**: 3309–3317.
- Haase, M. & Bouchet, P. 1998. Radiation of crenobiontic gastropods on an ancient continental island: The *Hemistomia*-clade in New Caledonia (Gastropoda: Hydrobiidae). *Hydrobiologia*, **367**: 43–129.
- Herteux, C.E., Gawlik, D.E. & Smith, L.L. 2019. Habitat characteristics affecting wading bird use of geographically isolated wetlands in the U.S. Southeastern Coastal Plain. *Wetlands*, **40**: 1149–1159.
- Incagnone, G., Marrone, F., Barone, R., Robba, L. & Naselli-Flores, L. 2015. How do freshwater organisms cross the “dry ocean”? A review on passive dispersal and colonization processes with a special focus on temporary ponds. *Trends in Aquatic Ecology*, **750**: 103–123.
- Inventaire National du Patrimoine Naturel. 2023. Inventaire des collectivités d'outre-mer et de la métropole (icom_16) issu des données biogéographiques de TAXREF V16 [<https://inpn.mnhn.fr/espece/jeudonnees/72419>].
- Isnard, S., L’Huillier, L., Rigault, F. & Jaffré, T. 2016. How did the ultramafic soils shape the flora of the New Caledonian hotspot? *Plant and Soil*, **403**: 53–76.
- Jeanpert, J., Genthon, P., Maurizot, P., Folio, J.-L., Vendé-Leclerc, M., Sérino, J., Join, J.-L. & Iseppi, M. 2016. Morphology and distribution of dolines on ultramafic rocks from airborne LiDAR data: the case of southern Grande Terre in New Caledonia (SW Pacific). *Earth Surface Processes and Landforms*, **41**: 1854–1868.
- Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., Von Haeseler, A. & Jermini, L.S. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* **14**: 587–589.
- Katoh, K., Misawa, K., Kuma, K. & Miyata, T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, **30**: 3059–3066.
- Kierczak, J., Pietranik, A. & Pędziwiatr, A. 2021. Ultramafic geoecosystems as a natural source of Ni, Cr, and Co to the environment: A review. *Science of the Total Environment*, **755**: [<https://doi.org/10.1016/J.SCITOTENV.2020.142620>].
- L’Huillier, L. & Jaffré, T. 2010. L’exploitation des minerais de nickel en Nouvelle-Calédonie, pp. 21–31. In: *Mines et environnement en Nouvelle-Calédonie : les milieux sur substrats*

- ultramafiques et leur restauration* (L. L’Huillier, T. Jaffré & A. Wulff, eds.). Éditions IAC, Païta, New Caledonia.
- Marques, J.M., Carreira, P.M., Carvalho, M.R., Matias, M.J., Goff, F.E., Basto, M.J., Graça, R.C., Aires-Barros, L. & Rocha, L. 2008. Origins of high pH mineral waters from ultramafic rocks, Central Portugal. *Applied Geochemistry*, **23**: 3278–3289.
- Maturana, C.S., Rosenfeld, S., Biersma, E.M., Segovia, N.I., González-Wevar, C.A., Díaz A., Naretto, J., Duggan, I.C., Hogg, I.D., Poulin, E., Convey, P. & Jackson, J. A. 2021. Historical biogeography of the Gondwanan freshwater genus *Boeckella* (Crustacea). *Diversity and Distributions*, **27**: 2330–2343.
- Maturana, C.S., Rosenfeld, S., Naretto, J., Convey, P., Poulin, E., Lopez, Z., Gerard, K., Contador, T., Rendoll, J., Jackson, J.A., Hogg, I.D., Duggan, I.C. & Bahamonde, F. 2019. Occurrences of *Boeckella* at high latitudes in the southern hemisphere. *GBIF*: [<https://doi.org/10.15468/ZC6Y59>].
- Maturana, C.S., Segovia, N.I., González-Wevar, C.A., Díaz, A., Rosenfeld, S., Poulin, E., Jackson, J.A. & Convey P. 2020. Evidence of strong small-scale population structure in the Antarctic freshwater copepod *Boeckella poppei* in lakes on Signy Island, South Orkney Islands. *Limnology and Oceanography*, **65**: 2024–2040.
- Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A. & Lanfear R. 2020. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, **37**: 1530–1534.
- Mrázek, A. 1901. Süßwasser-Copepoden. *Ergebnisse der Hamburger Magalhaensischen Sammelreise 1892/93, Hamburg Natural History Museum*, **2**: 1–29.
- Nattier, R., Grandcolas, P., Pellens, R., Jourdan, H., Couloux, A., Poulain, S. & Robillard, T. 2013. Climate and soil type together explain the distribution of microendemic species in a biodiversity hotspot. *PLoS ONE*, **8**: e80811 [<https://doi.org/10.1371/journal.pone.0080811>].
- Olesen, J., Pöllabauer, C., Sigvardt, Z.M.S. & Rogers, D.C. 2016. A new species of *Lynceus* Müller, 1776 from New Caledonia (Crustacea: Branchiopoda: Laevicaudata) from dolines, with remarks on zoogeography. *European Journal of Taxonomy*, **224**: 1–18.
- Padial, J.M., Miralles, A., De la Riva, I. & Vences M. 2010. The integrative future of taxonomy. *Frontiers in Zoology*, **7**: 16 [<https://doi.org/10.1186/1742-9994-7-16>].
- Paradis, E. 2010. pegas: an R package for population genetics with an integrated-modular approach. *Bioinformatics*, **26**: 419–420.
- Parry, V., Kiemel, K., Pawlak, J., Eccard, J., Tiedemann, R. & Guntram, W. 2023. Drivers of

- zooplankton dispersal in a pond metacommunity. *Hydrobiologia*: [https://doi.org/10.1007/s10750-023-05232-4]
- Pascal, M., Richer de Forges, B., Le Guyader, H. & Simberloff, D. 2008. Mining and other threats to the New Caledonia biodiversity hotspot. *Conservation Biology*, **22**: 498–499.
- Pelletier, B. 2007. Geology of the New Caledonia region and its implications for the study of the New Caledonian biodiversity. *Compendium of Marine Species of New Caledonia*, **2**: 17–30.
- Pillon, Y., Jaffré, T., Birnbaum, P., Bruy, D., Cluzel, D., Ducouso, M., Fogliani, B., Ibanez, T., Jourdan, H., Lagarde, L., Léopold, A., Munzinger, J., Pouteau, R., Read, J. & Isnard, S. 2021. Infertile landscapes on an old oceanic island: the biodiversity hotspot of New Caledonia. *Biological Journal of the Linnean Society*, **133**: 317–341.
- Pitoiset, A. 2016. *Le nickel en Nouvelle Calédonie*. Maison de la Nouvelle-Calédonie, Paris.
- Powers, M.J. Hill, G.E., and Weaver, R.J. 2019. An experimental test of mate choice for red carotenoid coloration in the marine copepod *Tigriopus californicus*. *Ethology*, **126**: 344–52.
- Quinlan, K. & Bayly, I.A.E. 2017. A new species of *Boeckella* (Copepoda: Calanoida) from arid Western Australia, an updated key, and aspects of claypan ecology. *Records of the Western Australian Museum*, **32**: 191–206.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**: 539–542.
- Rundle, H.D. & Nosil, P. 2005. Ecological speciation. *Ecology Letters*, **8**: 336–352.
- Sanger, F., Nicklen, S. & Coulson, A.R. 1977. DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America*, **74**: 5463–5467.
- Scheihing, R., Cardenas, L., Nespolo, R.F., Krall, P., Walz, K., Kohshima, S. & Labarca, P. 2010. Morphological and molecular analysis of centropagids from the high Andean plateau (Copepoda: Calanoidea). *Hydrobiologia*, **637**: 45–52.
- Schluter, D. 2001. Ecology and the origin of species. *Trends in Ecology & Evolution*, **16**: 372–380.
- Scott, T. 1914. Remarks on some Copepoda from the Falkland Islands collected by Mr. Rupert Vallentin, F. L.S. *Annals and Magazine of Natural History*, **13**: 1–11.
- Searle, J. 1911. Some Victorian Copepoda new to science. *Victorian Naturalist*, **27**: 174–178.

- Teske, P.R., Golla, T.R., Sandoval-Castillo, J., Emami-Khoyi, A., van der Lingen, C.D., von der Heyden, S., Chiazzari, B., Jansen van Vuuren, B. & Beheregaray, L.B. 2018. Mitochondrial DNA is unsuitable to test for isolation by distance. *Scientific Reports*, **8**: [https://doi.org/10.1038/s41598-018-25138-9].
- The Galaxy Community. 2022. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2022 update. *Nucleic Acids Research*, **50**: W345–W351.
- Thomson, G.M. 1883. On the New Zealand Copepoda. *Transactions and Proceedings of the New Zealand Institute*, **15**: 93–116.
- Vilgrain, L., Maps, F., Basedow, S., Trudnowska, E., Madoui, M.A., Niehoff, B. & Ayata, S.D. 2023. Copepods' true colors: astaxanthin pigmentation as an indicator of fitness. *Ecosphere*, **14**: e4489 [https://doi.org/10.1002/ecs2.4489].
- Walter, T.C. & Boxshall, G. 2021. World of Copepods database. *Boeckella* Guerne & Richard, 1889 [https://www.marinespecies.org/copepoda/aphia.php?p=taxdetails&id=346853].
- Winter, D.J. 2012. mmod: an R library for the calculation of population differentiation statistics. *Molecular Ecology Resources*, **12**: 1158–1160.
- Xiang, C.Y., Gao, F., Jakovlić, I., Lei, H.P., Hu, Y., Zhang, H., Zou, H., Wang, G.T. & Zhang, D. 2023. Using PhyloSuite for molecular phylogeny and tree-based analyses. *iMeta*, **2**: e87 [https://doi.org/10.1002/imt2.87].
- Zhang, D., Gao, F., Jakovlić, I., Zou, H., Zhang, J., Li, W.X. & Wang G.T. 2020. PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources*, **20**: 348–355.