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Standard Paper

Placopsis craterifera (*Trapeliaceae, Lecanoromycetes*), a new lichen species from alpine habitats on Mount Meru, Tanzania

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Abstract

During a field trip to the highlands of Mount Meru in Tanzania, two *Placopsis* specimens were collected. Morphological analyses showed a unique combination of characters not observed in any other published taxa within the genus. The specimens are characterized by their circular soralia, not confluent, crater-shaped, with a prominent white margin and coarse granular pinkish central soredia. Considering the morphological, geographical and genetic data, we propose the designation of a new species, *Placopsis craterifera* Boluda sp. nov.

Keywords: Africa; Afroalpine; conservation; lichens; new species

(Accepted 2 November 2023; first published online 8 February 2024)

Introduction

The lichenized fungal genus *Placopsis* (Nyl.) Linds., with *c.* 64 accepted species (Index Fungorum Partnership 2023; www.indexfungorum.org), is mainly distributed in highly oceanic temperate or alpine habitats of the Southern Hemisphere, with only a small number of taxa reaching the Northern Hemisphere (Galloway 2013). *Placopsis* species typically grow on compacted alpine soils or siliceous rocks, often in periglacial areas. It is a remarkable crustose genus with frequent conspicuous cephalodia, structures that enclose cyanobacteria as secondary photobionts that can fix carbon and nitrogen from the atmosphere (Hitch & Stewart 1973; Haselkorn 1986; Rai 1990). This allows them to be pioneer colonizers in oligotrophic habitats such as rocks exposed after glacier melting, where they are sometimes a major component of the lichen flora.

With at least 22 species reported in South America (Galloway 2010) and a minimum of 39 in Oceania (Lumbsch *et al.* 1992; Galloway 2013), it might be expected that Africa, with nearly half of the continent situated in the Southern Hemisphere, could harbour a significant proportion of *Placopsis* species. However, only three species have been reported so far from Africa (*Placopsis gelida* (L.) Linds., *P. lambii* Hertel & V. Wirth, *P. parellina* (Nyl.) I. M. Lamb) and all three are also present in other continents (Lambinon & Sérusiaux 1983; Moberg & Carlin 1999). This reduced number may be partly due to the cold and humid environment preferred by *Placopsis*, which is not common in Africa. However, such conditions do occur in some regions in the east of the continent, especially around the highlands of Ethiopia, Kenya, the Democratic Republic of Congo and Tanzania. Since the number of collections and knowledge of African lichen diversity is lower than for South America and Oceania, the small number of *Placopsis* species known from Africa can be explained by sparse sampling, suggesting that the collection of African *Placopsis* may result in new species for science.

During a field trip conducted in February 2016 to Mount Kilimanjaro and Mount Meru in Tanzania, some lichen specimens were collected. Examination showed new records for the African continent, as well as new lichen species. Among them, two collections of *Placopsis* could not be identified as any known taxa, and we propose their description here as a new species based on morphological and genetic data.

Materials and Methods

Sampling, morphology and chemistry

Two specimens growing 2 m apart were collected on Mount Meru, Tanzania, at the base of the Little Meru peak (3°12′58.1″S, 36°46′27.5″E), 3608 m above sea level (*Boluda* 17841 and *Boluda* 17842). They were growing on a compacted siliceous soil among small mosses and other crustose lichens in a sparse alpine scrubland. Specimens were examined morphologically using a Leica MZ75 stereomicroscope (up to ×50), and hand-cut sections were studied with a Leica DM2000 microscope. The morphology of these two specimens was compared to all published species and subspecific taxa of the genus *Placopsis*, using either species descriptions (Lamb 1947; Lambinon & Sérusiaux 1983; Lumbsch *et al.* 1992; Moberg & Carlin 1999;

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Galloway *et al.* 2005; Galloway 2010, 2013; Awasthi & Agarwal 2011) or herbarium specimens deposited in G, the general collection of the Geneva herbarium (*Placopsis bicolor* (Tuck.) B. de Lesd., *P. chilena* I. M. Lamb, *P. contortuplicata* I. M. Lamb, *P. gelida*, *P. parellina*, *P. perrugosa* (Nyl.) Nyl., *P. rhodocarpa* (Nyl.) Nyl., *P. rhodophthalma* (Müll. Arg.) Räsänen, *P. subcribellans* (I. M. Lamb) D. J. Galloway, and *Placopsis* sp.).

Spot tests reactions were observed using solutions of 10% potassium hydroxide (K), 8% sodium hypochlorite (C) and para-phenylendiamine (P) according to Orange *et al.* (2010). Thin-layer chromatography (TLC) was carried out following Orange *et al.* (2010). Solvent systems A (toluene:1,4-dioxane: acetic acid, 180:45:5), B (hexane:methyl tert-butylether:formic acid, 140:72:18) and C (toluene:acetic acid, 170:30) were used according to Culberson & Ammann (1979), with solvent B modified according to Culberson & Johnson (1982).

Phylogenetic reconstruction

DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Barcelona, Spain) with a slight modification to the manufacturer's instructions (Crespo *et al.* 2001; Divakar *et al.* 2012). The ITS locus (internal transcribed spacers of the nuclear ribosomal DNA including the 5.8S region and partial sequences of the 18S and 28S) was amplified using the primers ITS1FKYO2 (5′-TAG AGG AAG TAA AAG TCG TAA-3′) and ITS4KYO2 (5′-RBT TTC TTT TCC TCC GCT-3′; Toju *et al.* 2012). For PCR amplification, a reaction mixture of 25 μl was used containing 18 μl sterile water, 2.5 μl 10× buffer with 2 mM MgCl2, 0.5 μl dNTPs (10 mM of each base), 1.25 μl of each primer at 10 μM, 0.625 μl of DNA polymerase (1U μl^{-1}), and 1 μl DNA template. Amplifications were run in a thermocycler (XP Cycler, Bioer, Hangzhou, China) using the following parameters: initial denaturation of 5 min at 95 °C, then 35 cycles of 1 min at 95 °C, 1 min at 56 °C, 1 min 30 s at 72 °C, and a final extension of 10 min at 72 °C. Polymerase chain reaction (PCR) products were cleaned using Illustra^{TM} ExoProStar (GE Healthcare, Little Chalfont, UK), according to the manufacturer's instructions. Sequencing was performed by the Unidad de Genómica (Parque Científico de Madrid).

BLAST (Altschul *et al.* 1990) was used to search for the most similar ITS sequences available on GenBank. Those sequences, as well as others representing all the known *Placopsis* species available in the database, were downloaded (Fig. 1). Sequence alignment was performed using MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/; Katoh & Standley 2013) with the G-INS-i alignment algorithm, a '200PAM/k = 2' scoring matrix, with an offset value of 0.1, and the remaining parameters set to default values. Gblocks v. 0.91b (Talavera & Castresana 2007) was used to remove ambiguously aligned positions. The final DNA alignment contained 486 base pairs. PartitionFinder (Lanfear *et al.* 2012) was used to detect possible intra-locus substitution model variability, resulting in the splitting of the ITS region into ITS1, 5.8S and ITS2. DNA substitution models for each locus partition were selected with jModelTest v. 2.0 (Darriba *et al.* 2012), using the Akaike information criterion (AIC; Akaike 1974). The best-fit models of evolution obtained were: TVM + G for ITS1, TrNef + I for 5.8S, and TIM3 + I + G for ITS2.

Datasets were analyzed using maximum likelihood (ML) and Bayesian (B/MCMCMC) approaches. For ML tree reconstruction, we used RAxML v. 8.2.10 (Stamatakis 2006) implemented on the CIPRES Science Gateway (https://www.phylo.org/; Miller *et al.* 2010) with the GTRGAMMA model (Stamatakis 2006, 2014; Stamatakis *et al.* 2008). Support values were assessed using the 'rapid bootstrapping' option with 1000 replicates. For the Bayesian reconstruction, MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2003) was used. Two simultaneous runs with 2 million generations each, starting with a random tree and employing 12 simultaneous chains, were executed. Every 100th tree was saved to a file. To correct for putative overestimation of branch lengths, we used the uniform compound Dirichlet prior 'brlenspr = unconstrained:gammadir (1, 1, 1, 1)' (Zamora *et al.* 2015). We plotted the log-likelihood scores of sample points against generations using Tracer v. 1.5 (Rambaut *et al.* 2014) and determined that stationarity had been achieved when the log-likelihood values of the sample points reached an equilibrium and effective sampling size (ESS) values exceeded 200 (Huelsenbeck & Ronquist 2001). Posterior probabilities (PPs) were obtained from the 50% majority-rule consensus of sampled trees after excluding the initial 25% as burn-in. The phylogenetic tree was drawn with FigTree v. 1.4 (Rambaut 2009) and edited with CorelDRAW v. 11.

Results

The morphological comparison of the specimens *Boluda* 17841 and *Boluda* 17842 against all other published *Placopsis* taxa (101 taxa including species and subspecific categories; www.indexfungorum.org) showed a combination of characters absent in any other taxon. These two *Placopsis* collections are characterized by their circular not confluent soralia, with a protruding white margin and coarse granular pinkish central soredia that do not form pits after soredia dispersion.

The maximum likelihood and Bayesian phylogenetic trees (Fig. 1) are congruent except for a small number of unsupported branches indicated with the symbol '-'. However, they show some unsupported backbone nodes. This may be expected when a single locus is used. With few exceptions, the different species appear highly supported. The species *Aspiciliopsis macrophthalma* (Hook. f. & Taylor) B. de Lesd. appeared nested within *Placopsis*, but lacked support. The collections *Boluda* 17841 and *Boluda* 17842 appear clustered together with 100/1 bootstrap/posterior probability values, with a branch length similar to other *Placopsis* species. However, the relationships between the Tanzanian collections and the other species are not supported.

Discussion

The specimens *Boluda* 17841 and 17842 appear genetically distinct from all sequenced *Placopsis* species and differ from all the described species in their soralia characteristics. Soralia are not frequent in the genus *Placopsis*, with 51 of the 69 taxa published at species-rank being esorediate. Following Galloway (2013), the soralia of *Placopsis* can be classified into two distinct types: capitate, lacking a characteristic margin (as observed in *P. rhodocarpa* and *P. lateritioides* I. M. Lamb), and eroding, with a well-defined and often sharp margin (as seen in most sorediate species, such as *P. argillacea* (C. Knight) Malcolm & Vězda, *P. lambii*, *P. erosa* D. J. Galloway, *P. fusciduloides* D. J. Galloway or *P. microphylla* (I. M. Lamb) D. J. Galloway). Occasionally, both types of soralia may be found in the same species, as in *Placopsis gelida*. Additionally, some taxa may produce soralia that do not align well with these two types, as seen in *Placopsis antarctica* D. J. Galloway, R. I. L. Sm. & Quilhot, where they form on eroding dactyls (Galloway *et al.* 2005).
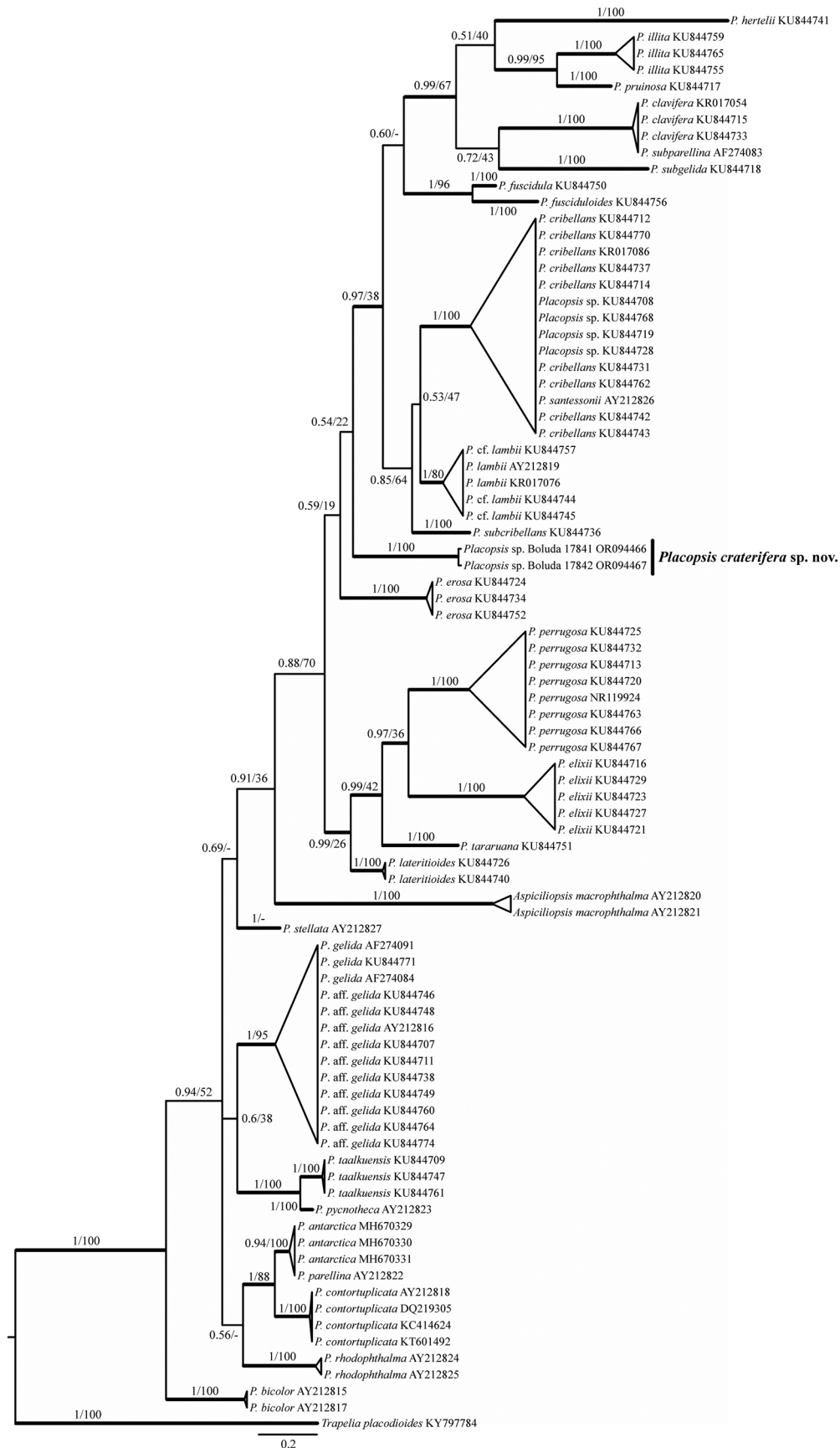
Figure 1. Phylogenetic reconstruction of 85 *Placopsis* specimens representing at least 26 species, based on the nuITS matrix with 486 base pairs. Tree topology depicts the results of the Bayesian Markov chain Monte Carlo (B/MCMC). Posterior probabilities and maximum likelihood bootstrap values, when congruent with the Bayesian tree, are given on each branch. The nuITS GenBank Accession numbers are provided for each specimen.
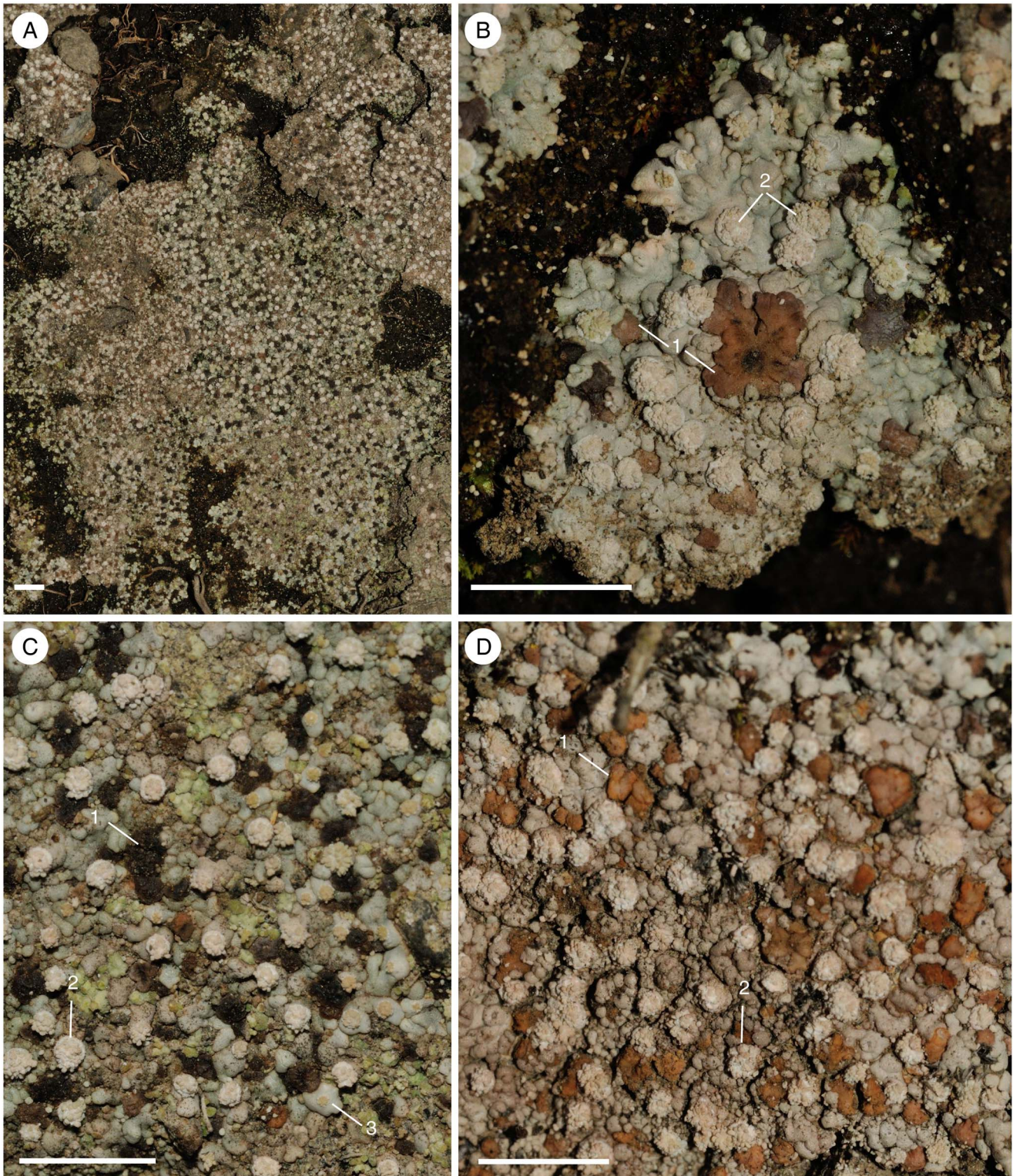
Figure 2. A–D, Field views of four non-collected thalli from the type locality of *Placopsis craterifera* sp. nov.; 1 = cephalodia, 2 = soralia, 3 = thallus squamules. Scales: A, C & D = 1 cm; B = 0.5 cm. Images courtesy of Christoph Scheidegger.

The Mount Meru collections produce soralia of the eroding type, yet these are never confluent as commonly seen in most sorediate species. Furthermore, the soralia margin is noticeably thicker, and more developed and raised compared to any described species. The soredia are pinkish to cream, different from the greenish, greyish, olive or blackened colouring found in other taxa, at least when fresh (Galloway 2010, 2013). *Placopsis erosa* forms discrete, coarsely pustular, crater-like soralia that generate coarsely granular soredia, which are the most similar to those in the Tanzanian collections. However, after soredia

dispersion they form pits, whereas the soralia in the Tanzanian samples remain protruding.

The species *Aspiciliopsis macrophthalma* was found to be nested within *Placopsis* in the present study. However, its inclusion in this genus lacks support, since the phylogenetic tree's backbone is unsupported. *Aspiciliopsis*, along with *Ducatina* and *Orceolina*, formed a clade sister to *Placopsis* in previous phylogenetic studies (Schmitt *et al.* 2003; Ertz *et al.* 2017), including reconstructions that utilized only nuITS. These studies were conducted with fewer specimens than were used in the present study, and including more specimens could potentially reduce the support of the tree's backbone due to the presence of missing data. This could result in long branch attraction phenomena that might misplace *Aspiciliopsis* within *Placopsis*, especially if *Ducatina* and *Orceolina* are not included.

The combination of morphological, genetic and geographical data indicate that the specimens *Boluda* 17841 and *Boluda* 17842 belong to an undescribed species of the genus *Placopsis*. Consequently, we propose its description below as *Placopsis craterifera* Boluda sp. nov.

The Species

Placopsis craterifera Boluda sp. nov.

MycoBank No.: MB 849792

Vaguely resembling the Australasian *Placopsis erosa* in its soralia, but with a bullate-squamulose thallus instead of angular areolated, and soralia that are pustular to crateriform, 0.7–1.1 mm diam., not forming pits after soredia spreading, versus 0.2–0.4 mm diam. and soon eroding and spreading to form pits.

Type: Tanzania, Reg. Arusha, Mount Meru, base of the Little Meru peak, on compacted siliceous soil in an alpine sparse scrubland, 3608 m, 3°12′58.1″S, 36°46′27.5″E, 20 February 2016, *Boluda* 17841 (G 00576113—holotype) (GenBank Accession no.: OR094466).

(Fig. 2)

Thallus closely attached to the substratum, forming irregular rosettes when young, squamulose when mature, coalescing to form larger colonies, 1–8 cm diam., 0.3–0.8 mm thick, margins shortly lobed or squamulose, without any marginal prothallus. *Marginal lobes* shallowly to strongly convex, contiguous to imbricate, 0.6–4.2 × 0.4–2.1 mm. *Squamules* 0.5–1.3(–2.3) mm diam., isodiametric or frequently elongated or lobed, slightly to strongly convex, ±contiguous, sometimes brownish at the edges. *Upper surface* pale greyish green to greyish brown, matt, sometimes faintly rough, sorediate; *isidia*, *maculae* and *pseudocyphellae* absent. *Soralia* scattered, solitary, never confluent, at the beginning slightly prominent, circular, brownish, *c.* 0.3 mm diam., soon becoming very prominent, pustular to crateriform, circular, 0.7–1.1(–1.5) mm diam.; with up to 40 (sometimes more) coarse granular soredia longer than wide, 90–210 × 60–120 μm, more or less guttiform, inserted by the thinnest end, pale pink to pale brown, easily eroding, becoming an apothecium-like structure with a whitish wrinkled-uneven disc with scars originating from the soredia insertions; margin of soralia permanent, 120–300 μm wide, 90–210 μm in height, whitish. *Medulla* white. *Primary photobiont* a green chlorococcoid alga, cells rounded, 6–10 μm diam. *Cephalodia* sessile, spreading slightly or strongly over the thallus surface, orbicular at first, 0.4–0.9 mm diam.,

smooth or shallowly wrinkled, becoming deeply plicate at maturity, furrowed, with the margin lobulated and sometimes splitting and separating, 1–5 mm diam., from pinkish brown to orange-brown, blackened when old or in older parts, epruinose. *Cyanobiont* in chains, cells rounded, 3–6 μm diam., somewhat compressed.

Apothecia and *conidiomata* unknown.

Chemistry. Gyrophoric acid.

Etymology. Named after its crateriform soralia, as well as its habitat in the volcanic crater of Mount Meru.

Distribution and ecology. So far known only from the type locality on the Little Meru peak, a secondary summit of Mount Meru, the African continent's fifth highest mountain, in Arusha, Tanzania. It grows on compacted siliceous soil of volcanic origin on subvertical to horizontal substrata, in exposed areas in sparse alpine scrubland with frequent fogs and warm days with cold nights.

Conservation status. We are reluctant to perform a conservation assessment based only on two collections, which would end in a Critically Endangered status according to the IUCN (2012) criteria. These two collections were situated in a protected area; however, the type locality is affected by frequent fires and human activities, especially damage resulting from trampling by mountain climbers.

Additional specimen examined (paratype). **Tanzania:** *Reg. Arusha*: Mount Meru, base of the Little Meru peak, on compacted siliceous soil in an alpine sparse scrubland, 3608 m, 3°12′58.1″S, 36°46′27.5″E, 2016, *Boluda* 17842 (G 00576112) (GenBank Accession no.: OR094467).

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