**Palicella lueckingii** (Lecanorales, Ascomycota), a new lichen species inhabiting **Araucaria** from the extratropical South America

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**Abstract**

*Palicella lueckingii* is a newly described corticolous lecideoid lichen from the Malalcahuelo National Reserve growing on the bark of *Araucaria araucana* in Chile. Detailed morphological studies and inference from molecular phylogenetic analyses, based on maximum likelihood and Bayesian analyses of single gene locus (ITS), clearly indicate that the new species is a member of the recently introduced genus *Palicella*. *Palicella lueckingii* is most closely related to *P. glaucopa*, but clearly differs in having smaller ascospores, comparatively thicker thallus, epruinose apothecia, lack of oil droplets inside of exciple and presence of thiophanic acid as a major metabolite.

**Keywords:** biodiversity, Chile, ITS, Lecanoromycetes, *Lecidea* s. lat., new species, phylogeny

**Introduction**

The recently introduced lichen-forming fungal genus *Palicella* Rodriguez Flakus & Printzen (2014a: 540) contains three species occurring in Europe, North and South America. The genus is characterized by a highly variable concentration of pigments in the apothecia, varying from very pale to completely black, hymenium composed of branched and sparsely anastomozed paraphyses, a specific ascus apex (intermediate between *Lecanora-* and *Lecidella*-type), and production of atranorin as a main secondary metabolite (Rodriguez Flakus & Printzen 2014a).

In the most recent molecular studies, *Palicella* species form monophyletic lineage nested inside the Lecanoraceae Körber (1855: 104) (Zhao et al. 2016, Printzen et al. 2017). Many efforts to adjust the placement of genera and species within Lecanoraceae based on large dataset phylogenies and several gene markers have been done in the past decades (e.g., Ekman & Wedin 2000, Papong et al. 2012, Miądlikowska et al. 2014, Zhao et al. 2016). However, further research is still necessary to elucidate relationships and species boundaries among most of lecanoroid and lecideoid species.

During recent lichenological research on non-saxicolous species of *Lecidea* Acharius s.l. (1803: 32) conducted in extratropical South America (Argentina, Chile) unknown diversity of this group was revealed, and some of the new species/records have already been published (Rodriguez Flakus & Printzen 2014b, Printzen et al. 2016). Recently, while studying lichens collected in the Valdivian temperate forest in Chile (IX Región, de la Araucania, the Malalcahuelo National Reserve) another new species was found growing on the bark of *Araucaria araucana*. Here it is formally described as new species of *Palicella* and its phylogenetic position is resolved. The paper provides also a comparative table including key characters for determination of *Palicella* species.

**Material and Methods**

**Morphological study**

Microscopical examinations were carried out on thin handmade sections mounted in distillate water using standard microscopy techniques and reagents according to Rodriguez Flakus & Printzen (2014a). Spore measurements are given as follow: (arithmetic mean—standard deviation) *arithmetic mean* (arithmetic mean + standard deviation), flanked by the minimal and maximal measurements in parentheses, and followed by the number of measurements (n).
Secondary metabolites were identified by thin-layer chromatography according to Culberson & Kristinsson (1970), and Orange et al. (2001). Nomenclature for apothecial pigments follows Meyer & Printzen (2000).

Molecular techniques
The DNA was extracted using DNeasy™ Plant Mini Kit (QIAGEN) following the manufacturer’s protocol. The Polymerase chain reaction (PCR) contained 5 µl of the extracted DNA in a 25 µl final reaction volume using PCR PuReTaq™ Ready-To-Go™ beads (GE Healthcare, USA). I amplified and sequenced, the fungal barcode locus, internal transcribed spacer (ITS) using primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). Thermal cycling parameters were performed according to Rodríguez Flakus & Printzen (2014a).

PCR products were visualized on a 1% agarose gel in Tris-Acetate EDTA (TAE) buffer with a 1000-bp ladder size standard (Invitrogen Corp.), later bands were cut out and purified using the QIAquick Gel Extraction Kit (QIAGEN). Purified bands were labeled with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequences were determined on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems). All sequences were assembled and manually edited in Geneious Pro, version 5.0.4 (Biomatters Ltd). GenBank accession numbers for used sequences are listed in Figure 1.

![Figure 1](image-url)

**FIGURE 1.** Bayesian inference phylogenetic tree of *Palicella lueckingii* sp. nov. and related species within Lecanoraceae. Nodal support values are indicated by bold branches, including ML bootstrap values ≥ 75% and MCMC posterior probability ≥ 0.9. *Protoparmelia badia* was used as outgroup.

Phylogenetic analyses and taxon selection
Taxa, including members of the *Lecidella* Körber (1855: 233), *Lecanora* Acharius (1810: 77), and *Palicella*, were selected for the phylogenetic analyses based on previously published studies by Rodríguez Flakus & Printzen (2014a). Due to the small scale of the current study, the complete dataset includes 22 specimens. The closely related species were selected as outgroup based on large phylogeny provided by Zhao et al. (2016). Originally obtained and acquired from GenBank sequences were aligned using MAFFT (Katoh et al. 2002) under default settings.

In final alignment, ambiguously aligned regions were removed using Gblocks version 0.91b (Castresana 2000), with default settings but allowing gaps in 50% of the sequences. Substitution model selection was performed to
find the best-fitting model of evolution for the dataset including Bayesian Information Criterion (BIC) and greedy algorithm to search for good partitioning scheme were selected as implemented in PartitionFinder version 1.0.1 (Lanfear et al. 2012). Maximum likelihood (ML) and Bayesian analyses were performed using the CIPRES Scientific gateway portal (http://www.phylo.org/portal2/) (Miller et al. 2010). Maximum likelihood bootstrapping analysis was performed with RAxML-HPC v.8 (Stamatakis 2014), using the default parameters as implemented on the CIPRES, NSF XSEDE resource with bootstrap statistics calculated from 1000 bootstrap replicates. Bayesian phylogenetic inference was performed using the best fitting model as inferred by PartitionFinder in MrBayes v. 3.2.2 (Ronquist et al. 2011) on CIPRES platform, NSF XSEDE resource with the follow parameters Nst=6, rates=gamma (GTR+G) with 3 independent runs, 4 chains per run that were incrementally heated using a factor of 0.15. Each run searching for 1000000 generations sampling every 500th generation, discarding the first 50% of the sampled trees as burn-in. Generated phylogenetic trees were visualized under Figtree (Rambaut 2009).

Results & Discussion

Phylogenetic analyses

A total of 22 sequences were included in this study. The final alignment contained 8 OTUs and 467 unambiguously aligned nucleotide positions. The Bayesian phylogenetic tree is presented in Figure 1 including ML bootstrap values (BP) and Posterior probabilities (PP). The MCMC analysis and maximum likelihood inference resulted show *Palicella* as a well-supported monophyletic clade (BP = 100% and PP = 1) separate from Parmeliaceae Eschweiler (1824: 19). The molecular analyses revealed *Palicella lueckingii* as a sister clade to *P. glaucopa* (Hook. f. & Taylor) Rodriguez Flakus & Printzen (2014: 543) (BP = 100%, PP = 1), a species previously reported from southern South America. The other two holarctic species of *Palicella* [*P. filamentosa* (Stirt.) Rodr. Flakus & Printzen (2014: 540), and *P. schizochromatica* (Pérez -Ortega et al.) Rodriguez Flakus & Printzen (2014: 547)] form a separate clade.

According to Pérez-Ortega et al. (2010) the clade containing *Lecanora symmicta* (Acharius) Acharius (1814: 340) was closely related and sister to *Palicella* clade (formely named “*Lecanora filamentosa* group”). Despite similar morphology of *Lecanora symmicta* group and *Palicella* members, both clades can be clearly segregated by ascus types, and absence or presence of atranorin respectively. This suggests that composition of secondary metabolites in *Palicella* is not only stable at species level, being important for their taxonomy, but also can be linked to species phylogeography. For example, clade represented by two species known from the Southern Hemisphere (*P. glaucopa* and *P. lueckingii*) is characterized by presence of pannarin and xanthones (Rodriguez Flakus & Printzen 2014a), instead of species from the Holarctic region (*P. filamentosa* and *P. schizochromatica*) which are producing usnic acid (Printzen & May 2002, Palice et al. 2011, Pérez-Ortega et al. 2010).

Although several phylogenetic studies based on large sampling dataset and at least five loci (e.g. Miądlikowska et al. 2006, 2014, Papong et al. 2012, Printzen et al. 2017, Zhao et al. 2016) have been provided in the past decade, the final generic position of many taxa within Lecanoraceae remain still not resolved. In this study, species clades inside *Palicella* are statistical supported and the phylogenetic position of the new species is resolved. The relationship of *Palicella* to other genera in Lecanoraceae was not tested here, but obtained topology is similar to previously published studies (Pérez-Ortega et al. 2010, Rodriguez Flakus & Printzen 2014a), and confirms that *Palicella* is sister to *Lecanora symmicta* clade. Here, I describe *Palicella lueckingii* Rodr. Flakus as the fourth member of the genus, based on morphological, anatomical and chemical characteristics (discussed below) and confirm its placement within the *Palicella* clade (BP = 100, PP = 1). It is the second species of *Palicella* discovered in South America at the moment.

Taxonomic treatment

**Palicella lueckingii** Rodr. Flakus, sp. nov. (Fig. 2)

MycoBank no. 824257

**Diagnosis:**—Diffs from its relative *P. glaucopa* (Hook. f. & Taylor) Rodriguez Flakus & Printzen in having smaller ascospores, comparatively thicker thallus, epruinose apothecia, the inner part of exciple not inspersed by oil droplets, and presence of thiophanic acid as a major metabolite.

**Type:**—CHILE. IX Región, de la Araucanía, Malleco, Reserva Nacional Malalcahuelo, very close to Lonquimay Vulcan, 38°25′14.5″S, 71°32′38.9″W, 1431 m, Valdivian temperate forest, on bark of Araucaria araucana, 22 November 2011, *P. Rodriguez Flakus 2124* & R. Vargas (CONC—holotype, FR—isoype).
FIGURE 2. Palicella lueckingii (Rodríguez Flakus 2124). A habit: A, thick thallus with black marginate apothecia, B, section through apothecium showing hyaline exciple; C, asci showing amyloid Lecanora/Lecidella-like ascus apex (mounted in K/I); D, ascospores. Scales: A = 1000 µm, B = 50 µm, C–D = 10 µm.

**Etymology:**—The epithet is named in honor of Dr. Robert Lücking, prominent German lichenologist, who has made an outstanding contribution to the knowledge of Neotropical lichens.

**Description:**—Thallus greyish to pale yellow, crustose, very thick, (0.3–)0.6–1.3 mm high, cracked-areolate, split into elevated areoles of different size, 0.4–1.5(–3.0) mm in diam., surface smooth to rough, isidia and soredia absent. Photobiont chlorococcoid, cells 8–11 µm diam. Apothecia usually numerous, rounded to irregular in shape, sessile with constricted base, 0.2–0.6(–0.8) mm in diam., when well-developed sometimes growing in groups. Disc black, matte or shiny, epruinose, flat to moderately convex (in old apothecia), usually P+ orange. Margin concolorous with disc, persistent, shiny. Exciple laterally 15–20 µm, basally 50–60 µm wide, colourless inside, not inspersed by crystals or oil droplets, I–, outer layer dark pigmented by Cinereorufa-green (Meyer & Printzen 2000), and additional undetermined brown pigment, K+ green, N+ purple-red, composed of highly conglutinate radiate hyphae, 1–2 µm wide, in which apically widened to 5 µm. Hypothecium colourless, 45–50 µm high. Subhymenium colourless, ca. 15 µm high. Hymenium colourless, inspersed by oil droplets, 45–55 µm high, strongly agglutinated. Epihymenium greenish black, mainly because of Cinereorufa-green pigment (the same as in the exciple), 8–10 µm thick. Paraphyses colourless, branched and anastomosed, 1–2 µm wide, strongly apically thickened, pigmented caps not evident, apical cells 3–6 µm wide. Asci 8-spored, clavate, with K/I+ blue tholus resembling a transitional form between Lecanora- and Lecidella-type, usually with a small region at the top with non-amyloid reaction, 35–45 × 10–15 µm. Ascospores colourless, simple to 1-septate, broadly ellipsoid, without epispore, (8–)10.4–12.9–15.6(–17) × (4–)4.7–5.3–5.9(–6.5) µm, length-width ratio (1.8–)2.0–2.4–2.8(–3.0) µm (n=26). Pycnidia not seen.

**Chemistry:**—Atranorin (minor to trace), pannarin (minor; on disc), thiophanic acid (major). Thallus K–, P–, C+ orange, KC+ orange, UV+ pale yellowish; apothecial disc K–, P+ rust orange to yellowish, C–, KC–.

**Ecology and distribution:**—So far, Palicella lueckingii is known only from the type locality in Chile, where it grows exclusively on the bark of Araucaria araucana in the Malalcahuelo National Reserve in humid Valdivian temperate forest.

**Remarks:**—The new species is characterized by small ascospores, thick thallus, epruinose apothecia, not inspersed...
inner part of exciple, and production of atranorin, pannarin and thiophanic acid as secondary metabolites. *Palicella lueckingii* is rather similar to the epruinose forms of *P. glaucopa*, a common species growing on *Nothofagus* spp. bark in the southern part of Argentina and Chile. Both species have similar black and marginate lecideoid apothecia reacting P+ rust-orange. *Palicella glaucopa*, however, can be easily distinguished by its larger ascospores, (9.0–15.9–19.5–23.0(–32.0) × (4.8–)5.5–6.5–7.4(–9.5) μm, inner part of exciple composed of loosely arranged narrow hyphae and strongly inspersed by oil droplets, much thinner thallus (0.04–0.15 mm), larger apothecia, (0.3–)0.5–0.7–1.0(–1.4) mm, and different chemistry (Rodriguez Flakus & Printzen 2014a). The key characters to separate *Palicella lueckingii* from other known species of *Palicella* are presented in Table 1.

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<tr>
<th>TABLE 1. Key characters for determination of <em>Palicella</em> species.</th>
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<td><strong>P. lueckingii</strong></td>
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<td>Thallus thickness</td>
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<td>Disc</td>
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<td>Distribution</td>
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References


Acharius, E. (1814) *Synopsis methodica lichenum, sistens omnes hujus ordinis detectas plantas, quas, secundum genera, species et varietates disposit, characteribus et differentiis emendatis definitiv, nec non synonymis et observationibus selectis illustravit auctor*. Lund.


http://dx.doi.org/10.1093/oxfordjournals.molbev.a026334


http://dx.doi.org/10.1016/S0021-9673(00)83967-9

Eschweiler, F.G. (1824) *Systema lichenum, genera exhibens rite distincta, pluribus novis adacta.* Norimberga.


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