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To cite this article: Adam Flakus, Javier Etayo, Sergio Pérez-Ortega, Martin Kukwa, Zdeněk Palice & Pamela Rodriguez-Flakus (2019) A new genus, *Zhurbenkoa*, and a novel nutritional mode revealed in the family Malmideaceae (Lecanoromycetes, Ascomycota), *Mycologia*, 111:4, 593-611, DOI: [10.1080/00275514.2019.1603500](https://doi.org/10.1080/00275514.2019.1603500)

To link to this article: <https://doi.org/10.1080/00275514.2019.1603500>



Published online: 28 May 2019.



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A new genus, *Zhurbenkoa*, and a novel nutritional mode revealed in the family Malmideaceae (Lecanoromycetes, Ascomycota)

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ABSTRACT

Lichen-inhabiting fungi are highly specialized mycoparasites, commensals or rarely saprotrophs, that are common components of almost every ecosystem, where they develop obligate associations with lichens. Their relevance, however, contrasts with the relatively small number of these fungi described so far. Recent estimates and ongoing studies indicate that a significant fraction of their diversity remains undiscovered and may be expected in tropical regions, in particular in hyperdiverse fog-exposed montane forests. Here, we introduce the new genus *Zhurbenkoa*, from South America and Europe, for three lichenicolous fungi growing on thalli of the widespread lichen genus *Cladonia* (Lecanorales). Phylogenetic analyses based on combined sequence data of mt and nuc rDNA obtained from Andean populations (Bolivia) placed *Zhurbenkoa* as a member of Malmideaceae, a recently introduced family of lichen-forming fungi in the class Lecanoromycetes. *Zhurbenkoa* is closely related to the genera *Savoronala* and *Sprucidea*. The new genus is characterized by the development of grayish brown to almost black apothecia lacking an evident margin, an epihymenium interspersed with crystals (often seen as pruina), a strongly conglutinated hymenium made of noncapitate and sparsely branched paraphyses, a colorless exciple composed of radially arranged hyphae, a *Lecanora*/*Micarea*-like ascus type, and aseptate or 1-septate ellipsoidal colorless ascospores. *Zhurbenkoa* includes two Neotropical (*Z. cladoniarum*, *Z. latispora*) and one widespread (*Z. epicladonia*) species. The lichenicolous trophic mode is documented for the first time in the Malmideaceae, which until now included only lichen-forming associations between fungi and green algae.

ARTICLE HISTORY

Received 2 December 2018
Accepted 2 April 2019

KEY WORDS

Arthonia epicladonia;
lichenicolous fungi;
Neotropics; Pezizomycotina;
phylogenetics; systematics; 4
new taxa

INTRODUCTION

About 2300 lichenicolous species are described worldwide, although recent estimates suggest that only a small part of their diversity is known (Hawksworth 2001; Lawrey and Diederich 2003; Diederich et al. 2018). Tropical ecosystems harbor a significant fraction of the known mycobiota (e.g., Hawksworth 1991, 1993, 2012), including the highest number of lichens—the hosts of lichenicolous fungi. This contrasts with the relatively small number of lichen-inhabiting species described from tropical regions (e.g., Diederich 1997; Etayo 2002, 2010, 2017). Therefore, one can hypothesize that the vast part of the missing diversity of lichen-inhabiting fungi is hidden in tropical lichen communities. Our recent observations in the Bolivian Andes, along with results of several other investigations in tropical regions, confirm that assumption (e.g., Hawksworth and Esslinger 1993; Diederich 1997; Etayo 2002, 2017; Flakus and Kukwa

2012; Diederich et al. 2014; Etayo et al. 2013, 2015, 2018; Flakus et al. 2014; Farkas and Flakus 2016; Suija et al. 2018). In particular, fog-exposed forests at high elevations are extremely diverse and rich in undescribed diversity of the lichen-inhabiting fungi (Diederich 1997; Flakus et al. 2016).

Most lichen-inhabiting fungi are considered obligate mycoparasites and commensals associated with lichens or to a lesser extent saprotrophs (Rambold and Triebel 1992; Hawksworth 2003; Lawrey and Diederich 2003). The trophic modes and the interactions with lichen symbionts vary among these fungi (de los Ríos et al. 2002). Although most lichenicolous species colonize the mycobiont, some species such as *Zwackhiomyces coepulonus* (Collembopsidiales, Dothideomycetes) or the recently described genus *Austrostigmidium* (Capnodiales, Dothideomycetes) associate with the photobiont (de los Ríos et al. 2002; Pérez-Ortega et al. 2015).

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Lichenicolous fungi are not monophyletic and are distributed across more than 50 different orders (Diederich et al. 2018). Most known lichenicolous fungi are Ascomycetes. Their phylogenetic relationships and evolutionary histories are only known for sparsely sampled groups, mainly of lichenicolous basidiomycetes (Sikaroodi et al. 2001; Lawrey et al. 2007, 2016; Millanes et al. 2011; Liu et al. 2016) or selected genera of Ascomycetes, including Abrothallales (Pérez-Ortega et al. 2014; Suija et al. 2015a), Asterinales (Ertz and Diederich 2015), Asterotexiales (Ertz et al. 2016), Arthoniales (Frisch et al. 2014), Capnodiales (Ruibal et al. 2011; Muggia et al. 2016), Chaetothyriales (Diederich et al. 2013; Muggia et al. 2015, 2016), Helotiales (Etayo et al. 2015; Suija et al. 2015b), Lecanoromycetes (Pino-Bodas et al. 2017), Lichenostigmatales (Ertz et al. 2014), Pleosporales (Lawrey et al. 2012; Trakunyingcharoen et al. 2014; Ertz et al. 2015; Muggia et al. 2016), and Trypetheliales (Ertz et al. 2015). This state of knowledge is mostly a consequence of the difficult access to fresh material from less-explored ecosystems and of rare lichenicolous species, as well as technical difficulties in obtaining axenic cultures, particularly when they grow biotrophically on their hosts.

Malmideaceae (Lecanoromycetes) was recently introduced to accommodate tropical, corticolous, lichen-forming fungi in the genus *Malmidea*, described for the former *Lecidea piperis* and *Lecanora granifera* groups (Kalb et al. 2011). Later studies added other tropical genera such as the peculiar genus *Savoronala* from Madagascar, which produces unique conidia that each include a single algal cell (Ertz et al. 2013), and the recently described genera *Kalbionora* from Thailand, Vietnam, and Australia (Sodamuk et al. 2017) and *Sprucidea* from Brazil (Cáceres et al. 2017). Lately, the temperate sporodochial genus *Cheiromycina* was shown to fall within Malmideaceae (Muggia et al. 2017). Additional members of the family include poorly known temperate-boreal taxa currently classified in *Lecidea* sensu lato (s.l.), which warrant recognitions as a separate genus. The placement in Malmideaceae was confirmed for two of them based on molecular data: the saxicolous '*Lecidea*' *cyrtidia* and the epixylic '*L.*' *plebeja* (Ertz et al. 2013). Several additional poorly known lecideoid lichens, mainly boreal taxa, which are morphologically close to '*L.*' *plebeja* (e.g., '*Lecidea*' *malmeana* complex or '*Lecidea*' *consimilis*) likely belong here as well (Printzen 1995; Holien et al. 2016; Palice et al. 2018). It was hypothesized that also an enigmatic sporodochial genus *Xyleborus*, an Appalachian endemic, is a member of this family (Muggia et al. 2017). Malmideaceae as currently understood contains approximately 60 species of lichen-forming fungi (Kalb et al. 2011; Ertz et al. 2013; Miadlikowska et al. 2014; Breuss and Lücking 2015;

Cáceres et al. 2017; Muggia et al. 2017; Sodamuk et al. 2017), and the number will probably considerably arise in the near future.

The goal of this study was to assess the systematic position of two enigmatic lichenicolous fungi growing on *Cladonia* species, *Lecidea epicladonia* and *Patellaria cladoniarum*, and a putatively closely related undescribed species from Bolivia and Ecuador. Using DNA sequence data from nuc and mt rDNA, we inferred their systematic position as members of the Malmideaceae in the Lecanorales. This is the first time that the lichenicolous nutritional mode has been observed in this family.

MATERIALS AND METHODS

Taxon sampling and morphological studies.—Our study was based on material freshly collected by the authors and on historical herbarium specimens deposited at G, H, KRAM, LPB, UGDA, MA, PRA, and the private herbarium of J. Etayo (Pamplona). Morphology and the anatomy were examined using standard stereo- and compound microscopes (Nikon SMZ 800, Nikon Eclipse 80i DIC; Tokyo, Japan). Sections were prepared manually using a razor blade or a Thermo Fisher Scientific Microm HM430 (Waltham, Massachusetts, USA) freezing microtome combined with BFS-MP freezing stage and BFS-3MP controller. Sections and squash mounts were examined in tap water, 10% KOH (K), or lactophenol cotton blue (LPCB; Sigma-Aldrich, catalog no. 61335-100ML; St. Louis, Missouri, USA). All photomicrographs showing anatomical characters were made using transmitted differential interference contrast (DIC) microscopy. Amyloid reactions of anatomical structures were tested using Lugol's solution (I) (Sigma-Aldrich, catalog no. 62650-1L-F), without (I) or with (K/I) pretreatment with 10% KOH solution. The solubility of crystals was tested using K and 50% nitric acid solution (N). All measurements were made in distilled water or LPCB. Ascospores measurements are presented as arithmetic mean (\bar{x}) and standard deviation (s) (in parentheses), flanked by the minimum and maximum observed values, and the length/breadth ratio (l/b) is presented in the same way, followed by the number of measurements (n). Host lichen substances were investigated by thin-layer chromatography (TLC) in solvents A, B', and C following the methods by Culberson and Kristinsson (1970) and Orange et al. (2001).

DNA extraction, PCR amplification, and DNA sequencing.—Ascomata, which were stored together with host pieces frozen at -20 C, were removed from

the host thallus and carefully cleaned in double-distilled water (dH₂O) on a microscope slide under sterile conditions to remove host tissues and other visible impurities using ultrathin tweezers and a razor blade. Genomic DNA was extracted from about five clean ascospores using the QIAamp DNA Investigator Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. We amplified both mtDNA small subunit DNA (mrSSU) using primers pair mrSSU1 and mrSSU3R (Zoller et al. 1999), and nuc rDNA large subunit (28S) with primers LROR, LR3, LR3R, and LR7 (Vilgalys and Hester 1990; Rehner and Samuels 1994). Polymerase chain reaction (PCR) reactions were performed in a volume of 25 µL comprising 1 µL of DNA template, 0.2 µL of AmpliTaq 360 DNA polymerase (Applied Biosystems, California, USA), 2.5 µL of 10× AmpliTaq 360 PCR Buffer, 2.5 µL 25mM MgCl₂, 1 µL of each primer (10 µM), 2 µL GeneAmp dNTPs (10 mM; Applied Biosystems, California, USA), 0.2 µL bovine serum albumin (BSA; New England Biolabs, Massachusetts, USA), and sterile distilled water was added to attain the final volume. PCR amplifications were performed using the thermocycling conditions of Rodriguez-Flakus and Printzen (2014). PCR products were visualized by running 3 µL of the PCR product on 1% agarose gels. Purified amplicons (using the ExoSAP method [EURx, Gdańsk, Poland]) were labeled with BigDye Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems, California, USA). The PCR products were sequenced using a 3100-Avant sequencer (Applied Biosystems) at the Laboratory of Molecular Analyses, W. Szafer Institute of Botany of Polish Academy of Sciences, Krakow. The newly generated mtSSU and nuLSU sequences were checked, assembled, and edited manually using Geneious Pro 8.0. (Biomatters, Auckland, New Zealand) and deposited in GenBank. Accession numbers are provided in TABLE 1.

Phylogenetic analyses and taxon selection.—All sequences generated were checked by BLAST (Altschul et al. 1990) to verify potential contaminations by an unrelated fungus. BLAST searches of both 28S and mrSSU sequences from species of the new genus *Zhurbenkoa* revealed the highest similarity with *Savoronala madagarcariensis* (Malmideaceae, Lecanoromycetes). Therefore, we aligned *Zhurbenkoa* sequences with all available members of Malmideaceae and representatives of all other families of Lecanorales following the taxonomy of Miadlikowska et al. (2014).

Alignments were generated for each region using MAFFT (Katoh et al. 2005) as implemented on the GUIDANCE2 Web server (Penn et al. 2010). GUIDANCE2 assigns a confidence score to each ambiguous nucleotide site in the alignment and later remove regions of uncertain columns. We used the default cutoff score of 0.93 in all single gene alignments. The following analyses were performed in the CIPRES Scientific Gateway (<http://www.phylo.org/portal2/>) (Miller et al. 2010). PartitionFinder 2 (Lanfear et al. 2016) was used to select the best partition for our data and substitution models. A single substitution model was selected for each region (TIM+G for mrSSU, GTR+I+G for 28S) under a greedy search algorithm and the Akaike information criterion (AIC) (Lanfear et al. 2012). Maximum likelihood (ML) analyses were carried out using a heuristic search as implemented in RAXML-HPC2 on XSEDE (Stamatakis 2006) applying the GTRGAMMAI model, and 100 bootstrap interactions on 1000 replicates to estimate branch support. Bayesian inference (BI) of the phylogenetic relationships was calculated using the Markov chain Monte Carlo (MCMC) approach as implemented in MrBayes 3.2.6 on XSEDE (Ronquist et al. 2012) using the partitions and substitution models obtained by PartitionFinder 2. Two independent parallel runs were started each with four incrementally heated (0.15) chains. This MCMC was allowed to run for 40 million generations, sampling every 1000th tree and discarding the first 50% of the samples tree as a burn-in factor. The analysis was stopped after 1 million generations when the standard deviation of split frequencies had dropped below 0.01. Resulting ML and BI phylogenetic trees were visualized in TreeView (Page 1996). The tree was rooted by using *Rusavskia elegans* as the outgroup. Alignments were submitted to TreeBASE (study no. TB2 523582).

Statistical analysis.—Statistical species-level differences among the studied taxa in nonparametric multivariate analysis of variance (MANOVA) using ascospore length ($n = 537$) and ascospore breadth ($n = 537$) as dependent variables were calculated. Homogeneity and quality of the variance-covariance matrix were assessed using Box's M and Levene's tests. The Wilks' lambda (Λ) multivariate test was used to analyze the variance, and multiple comparisons of each dependent variable was performed by Tukey's honest significant differences (HSD) post hoc tests on ascospore sizes among species. We considered statistically significant differences among groups when $P < 0.05$. All analyses were conducted using the Statistical

Table 1. Voucher data and GenBank accession numbers of sequences included in this study.

Taxon name	Source	GenBank accession no.	
		nLSU rDNA	mtSSU rDNA
<i>Bacidia rosella</i>	Sweden, Ekman 3117 (BG)	AY300829	AY300877
<i>Bacidia rubella</i>	Poland, Kukwa 4598 (DUKE)	DQ986793	DQ986808
<i>Biatora vernalis</i>	Norway, Klepsland JK09-L616 (O)	KF360446	KF360418
<i>Byssoloma leucoblepharum</i>	Portugal, Ekman 3502 (BG)	AY756317	AY567778
<i>Calopadia foliicola</i>	Costa Rica, Lücking 16011 (BG)	AY756318	AY567782
<i>Cheiromycina flabelliformis</i>	Czech Republic, Palice 18257 (PRA)	MF431804	MF431799
<i>Cheiromycina petri</i>	Czech Republic, Palice 17855 (PRA)	MF431805	MF431800
<i>Cheiromycina reimeri</i> 1	Poland, Kukwa 17422 (UGDA)	MF431807	MF431803
<i>Cheiromycina reimeri</i> 2	Poland, Kukwa 17681 (UGDA)	MF431806	MF431802
<i>Cladia moniliformis</i>	Australia, Lumbsch et al. 19991f (F)	GQ500971	GQ500934
<i>Cladia retipora</i>	Australia, Lumbsch et al. 19976a (F)	GQ500963	GQ500931
<i>Cladonia digitata</i>	Sweden, Ekman 3424 (BG)	AY756319	AY756366
<i>Evernia prunastri</i>	Belgium, Ertz 7596 (BR)	KJ766557	KJ766389
<i>Fellhanera bouteillei</i>	Sweden, Ekman 3417 (BG)	AY756348	AY567787
<i>Fellhanera subtilis</i>	Germany, Tønsberg 28199 (BG)	AY756321	AY567786
<i>Gypsoplaca macrophylla</i>	Russia, Zhurbenko 92104 (UPS)	DQ899298	DQ899299
<i>Gypsoplaca</i> sp.	USA, Spribille 38752 (herb. Spribille)	KP796393	KP822511
<i>Hypotrachyna caraccensis</i>	Costa Rica, Lutzoni et al. s.n. (DUKE 47519)	DQ912336	DQ912280
<i>Kalbionora palaeotropica</i> 1	Thailand, Sodamuk s.n. (RAMK 24533)	KY926786	N/A
<i>Kalbionora palaeotropica</i> 2	Viet Nam, Oh & Thanh 130046a (F)	KY926787	N/A
<i>Lecanora campestris</i>	Sweden, Arup L97370 (LD)	DQ787361	DQ787362
<i>Lecanora glabrata</i>	Sweden, Fritz s.n. (LD)/Arup L011003 (LD)	DQ787359	DQ787360
' <i>Lecidea</i> ' <i>cyrtidia</i>	USA, Lay 07-0076 (herb. Lay)	HQ660538	HQ660563
<i>Lecidea fuscoatra</i>	Germany, Feige 6236 (DUKE)	DQ912332	DQ912275
' <i>Lecidea</i> ' <i>nylanderii</i>	USA, Spribille 10020 (herb. Spribille)	HQ660545	HQ660572
' <i>Lecidea</i> ' <i>plebeja</i>	Lithuania, Motiejūnaitė 7695 (FH)	HQ660549	HQ660577
' <i>Lecidea</i> ' <i>roseincta</i>	Norway, Tønsberg 34577 (herb. Schmull)	HQ660547	HQ660575
<i>Lecidea silacea</i>	Finland, Stenroos 5682 (DUKE)	KJ766588	DQ986878
<i>Malmidea coralliformis</i>	Thailand, Kalb et al. s.n. (herb. Kalb 37082)	N/A	HM447597
<i>Malmidea eeuuae</i>	Thailand, Kalb 36993 (herb. Kalb)	N/A	HM447601
<i>Malmidea floridensis</i>	USA, May 3088 (herb. May)	HQ660540	HQ660565
<i>Micarea alabastrites</i>	Norway, Andersen 17 (BG)	AY756327	AY567764
<i>Miriquidica complanata</i>	Poland, Szczepańska 43 (herb. Szczepanska)	KP940386	KP940385
<i>Miriquidica garovaglii</i>	Norway, Ekman s.n. (BG)	AY756357	AY567711
<i>Mycoblastus affinis</i>	Canada, Scheidegger s.n. (Duke 47671); Sweden, Nordin 3216 (UPS)	KJ766601	DQ899307
<i>Parmotrema reticulatum</i>	USA, Lutzoni & Yahr s.n. (DUKE 5521)	DQ912339	DQ912283
<i>Peltigera degenii</i>	Russia, Zavarzin s.n. (DUKE 47504)	AY584657	AY584628
<i>Protoblastenia calva</i>	Croatia, Gueidan 659 (DUKE)	JQ301601	DQ986904
<i>Protoparmeliopsis achariana</i>	Finland, Stjernberg s.n. (H)	KJ766633	KJ766465
<i>Protoparmeliopsis muralis</i>	Germany, Schmull s.n. (herb. Schmull)	HQ660533	HQ660556
<i>Psilolechia leprosa</i>	Norway, Tønsberg & Botnen 27362 (BG)	AY756333	AY567730
<i>Psilolechia lucida</i>	Finland, Haikonen 24578 (H)	KJ766639	KJ766472
<i>Psora saviczii</i>	Spain, Burgaz s.n. (AFTOL 4858)	KJ766641	KJ766475
<i>Psora testacea</i>	Spain, Burgaz s.n. (AFTOL 4873)	KJ766642	KJ766476
<i>Ramalina complanata</i>	USA, Hillis s.n. (DUKE 47924)	DQ972938	DQ972986
<i>Ramalina fastigiata</i>	Hungary, Molnar & Varga s.n. (DUKE 47751)	KJ766647	KJ766481
<i>Ramboldia brunneocarpa</i>	Australia, Elix 36756 (F)	EU075520	EU075528
<i>Ramboldia elabens</i>	Austria, Türk 39749 (H)	KJ766648	KJ766482
<i>Rusavskia elegans</i>	Canada, Lutzoni & Miądlikowska s.n. (DUKE 47558)	DQ912352	DQ912304
<i>Savoronala madagascariensis</i> 1	Madagascar, Serusiaux DNA537 (LG)	KC020142	KC020144
<i>Savoronala madagascariensis</i> 2	Madagascar, Serusiaux DNA538 (LG)	KC020143	KC020145
<i>Scoliciosporum intrusum</i>	Norway, Ekman s.n. (BG)	AY756329	AY567767
<i>Scoliciosporum umbrinum</i>	Norway, Ekman 3005 (BG)	AY300861	AY567719
<i>Scutula epiblastematica</i>	Russia, Vershinina s.n. (LE 261003)	N/A	KY661688
<i>Scutula tuberculosa</i>	Sweden, Wedin 6356 (UPS)	N/A	AY567789
<i>Scutula miliaris</i>	Sweden, Wedin 6850 (UPS)	N/A	AY567790
<i>Solorina saccata</i>	Romania, Pócs s.n. (DUKE 47739)	KJ766661	KJ766494
<i>Sphaerophorus fragilis</i>	Canada, Lutzoni & Miądlikowska s.n. (DUKE 47564)	DQ986805	DQ986789
<i>Sphaerophorus globosus</i>	Canada, Scheidegger 45 (DUKE)	DQ986767	DQ986866
<i>Spruceidea gymnopiperis</i>	Brazil, Cáceres 28322 (ISE)	MF093874	MF093876
<i>Spruceidea rubropenicillata</i>	Brazil, Cáceres 28211 (ISE)	MF093875	MF093877
<i>Squamarina cartilaginea</i>	Belgium, Ertz 7600 (BR); Spain, Burgaz s.n. (AFTOL 4850)	DQ986763	KJ766496
<i>Squamarina lentigera</i>	Norway, Haugan & Timdal 4801 (O)	AY756363	AY756377
<i>Stereocaulon intermedium</i>	USA, Mc Cune 23483 (DUKE)	KJ766662	KJ766497
<i>Stereocaulon tomentosum</i>	Sweden, Wedin 5089 (UPS)	AY340569	AY340526
<i>Tephromela atra</i>	USA, Lutzoni s.n. (DUKE 47632)	DQ986764	DQ986894
<i>Toninia cinereovirens</i>	Norway, Haugan & Timdal 7953 (O)	AY756365	AY567724
<i>Xanthoparmelia conspersa</i>	USA, Lutzoni s.n. (DUKE 5525)	AY584641	AY584633
<i>Xanthoria parietina</i>	Sweden, Gaya et al. s.n. (BCN)	JQ301589	JQ301530
<i>Zhurbenkoa epiclادonia</i> 1	Bolivia, Flakus 25212 (KRAM)	MK491328	MK491331
<i>Zhurbenkoa epiclادonia</i> 2	Bolivia, Flakus 25624 (KRAM)	MK491329	MK491332
<i>Zhurbenkoa latispora</i>	Bolivia, Flakus 25452 (KRAM)	MK491330	MK491333

Note. Newly generated sequences are marked in bold.

Package for the Social Sciences (SPSS) 24.1 (IBM, Armonk, New York), and a scatter plot tool as implemented in R 2.12 (RStudio Team 2015; R Core Team 2016) using the package VEGAN (Oksanen et al. 2013) and SPSS software, respectively.

RESULTS

Phylogenetic placement of genus *Zhurbenkoa* and species delimitation.—The final concatenated alignment included 69 sequences of 1828 unambiguous nucleotide positions (28S with 1204 and mrSSU 624 sites), which belong to 65 operational taxonomic units (OTUs). The ML and BI analyses yielded similar topologies. FIG. 1 represents the topology recovered from the ML analysis. The tree is mostly congruent with the recent phylogeny of Lecanoromycetes presented by Miadlikowska et al. (2014). Fifteen well-supported clades were found, corresponding to the known monophyletic families of the Lecanorales, and separated from the outgroup, represented by Lecideales, Peltigerales, and Telochistales. Only the family Lecanoraceae is polyphyletic in our analyses.

Our phylogenetic tree is grouped within two main sister clades corresponding to the suborders Lecanorineae and Sphaerophorineae s.l. (including Psorineae), as delimited by Miadlikowska et al. (2014). Members of Lecanorineae form a strongly supported group (posterior probability [PP] = 0.99) including Malmideaceae together with seven additional families: Cladoniaceae, Gypsoplacaceae, Lecanoraceae, Parmeliaceae, Ramboldiaceae, Squamarinaceae, and Stereocaulaceae. In contrast to our results, three additional families, Mycoblastaceae, Ramalinaceae, and Tephromelataceae, were included in Lecanorineae by Miadlikowska et al. (2014). The second clade in our analyses represents the suborder Sphaerophorineae s.l. and combines Pilocarpaceae, Psilolechiaceae, Psoraceae, Ramalinaceae s.l., Scoliosporaceae, and Sphaerophoraceae—affirming the results obtained by Ekman et al. (2008)—with Mycoblastaceae and Tephromelataceae, two families belonging to the sister Sphaerophorineae s.l. clade according to Ekman et al. (2008) and Miadlikowska et al. (2014). This means that although the anatomical characters of *Zhurbenkoa* suggest its placement within Ramalinaceae s.l. (close to *Scutula* or *Toninia* sensu stricto), in our analyses both the families Malmideaceae (including *Zhurbenkoa*) and Ramalinaceae s.l. (including *Toninia cinerea* and two lichenicolous species, *Scutula tuberculosa* and *S. miliaris*) were resolved as distantly related clades, representing Lecanorineae and Sphaerophorineae s.l., respectively.

The lichenicolous fungi on *Cladonia* that are our main focus, included in the new genus *Zhurbenkoa*, were resolved within a strongly supported monophyletic clade including members of the Malmideaceae (Lecanoromycetes) (bootstrap [BS] 93%, PP 0.99), which includes genera such as *Cheiromycina*, *Kalbionora*, *Malmidea*, *Savoronala*, and *Sprucidea* and two poorly known, isolated species, '*Lecidea*' *cyrtidia* and '*L.*' *plebeja*. Such a circumscription of Malmideaceae agrees with previous studies (Kalb et al. 2011; Ertz et al. 2013; Miadlikowska et al. 2014; Cáceres et al. 2017; Muggia et al. 2017; Sodamuk et al. 2017). The two species of *Zhurbenkoa* included in our analyses formed a highly supported clade (BS 100%, PP 1) sister to a group including *Savoronala madagascariensis*, *Sprucidea gymnopiperis*, and *S. rubropenicillata*, members of two recently introduced genera from Madagascar and Brazil, respectively (Ertz et al. 2013; Cáceres et al. 2017).

The one-way MANOVA revealed a statistically significant difference among *Zhurbenkoa* species based on their ascospore sizes, $F(4, 1066) = 119.38$, $P < 0.0005$, Wilks' $\Lambda = 0.477$, partial $\eta^2 = 0.31$. A post hoc Tukey pairwise comparison revealed that the differences were highly significant ($P < 0.0005$ or 0.001) for all pairwise groups based on ascospore length or width. These differences are visualized as box plots (FIG. 2) and a scatter plot (FIG. 3). From the box plots, each *Zhurbenkoa* species can be recognized based on the width of the ascospores, which seems to be the more reliable character to delimit species in the genus. Additionally, the second graph (FIG. 3) shows an evident grouping between each species of *Zhurbenkoa*. Our results show a relatively clear difference between *Z. cladoniarum* and *Z. latispota* (the species with aseptate ascospores) in length/breadth ratio and width values of ascospores. The third species, *Z. epicladonia*, is characterized by the longest ascospores, which are 1-septate and narrowly ellipsoidal.

As a consequence of our analyses, we introduce the new genus *Zhurbenkoa* to accommodate the three species of lichenicolous fungi parasitic on *Cladonia* (*Z. cladoniarum*, *Z. epicladonia*, and *Z. latispota*) based on their strongly supported isolated position within the family Malmideaceae (BS 100%, PP 1) and the diagnostic morphological and anatomical characters discussed below.

TAXONOMY

Zhurbenkoa Flakus, Etayo, Pérez-Ortega and Rodr. Flakus, gen. nov.
MycoBank MB828469

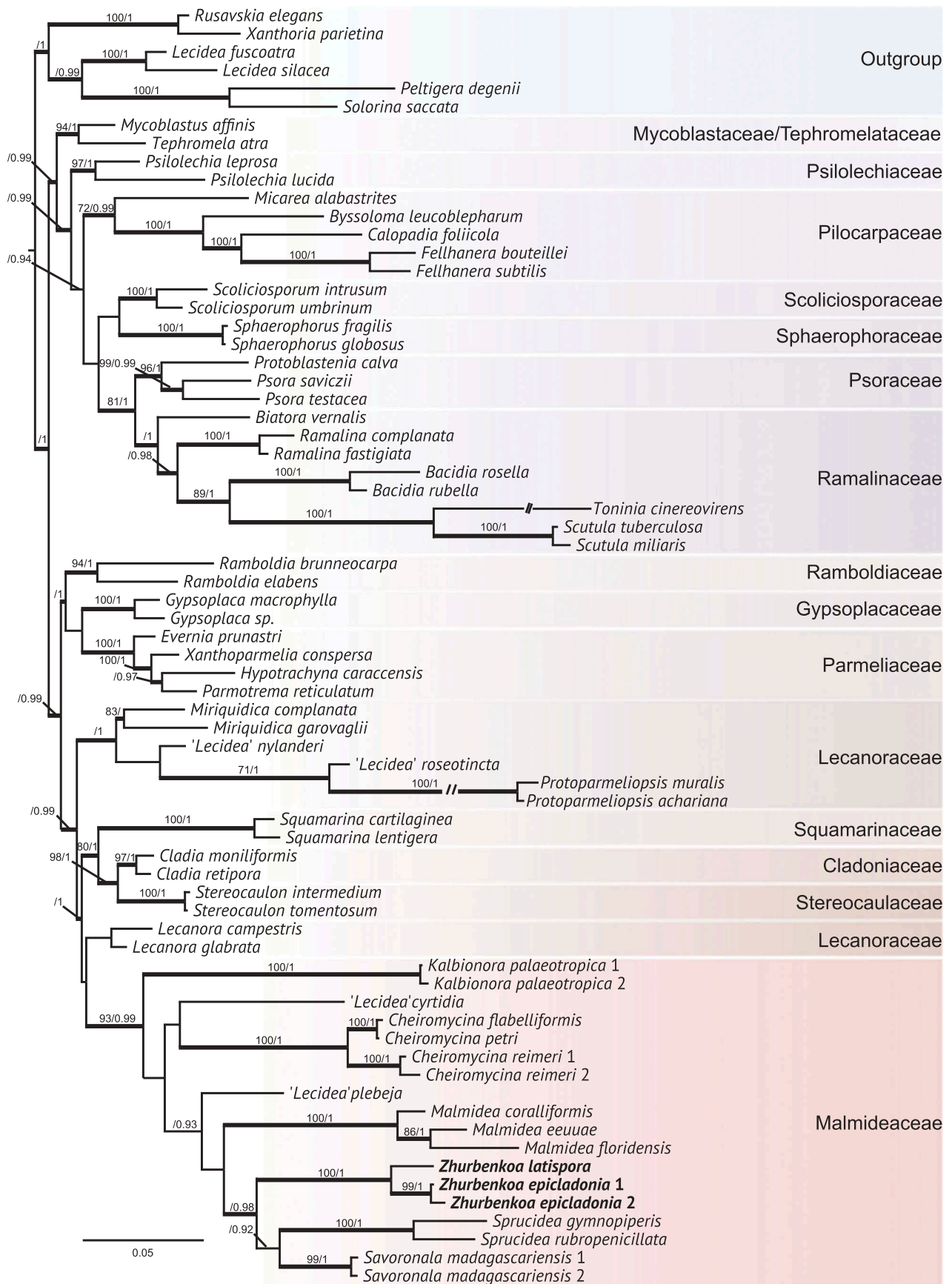


Figure 1. Phylogenetic placement of the genus *Zhurbenkoa* within Lecanorales inferred from ML analyses of combined mtSSU and 28S rDNA data set. Species from the Lecideales, Peltigerales, and Telochistales were used as the outgroup. Bold branches represent either bootstrap values ≥ 70 and/or Bayesian posterior probabilities ≥ 0.95 .

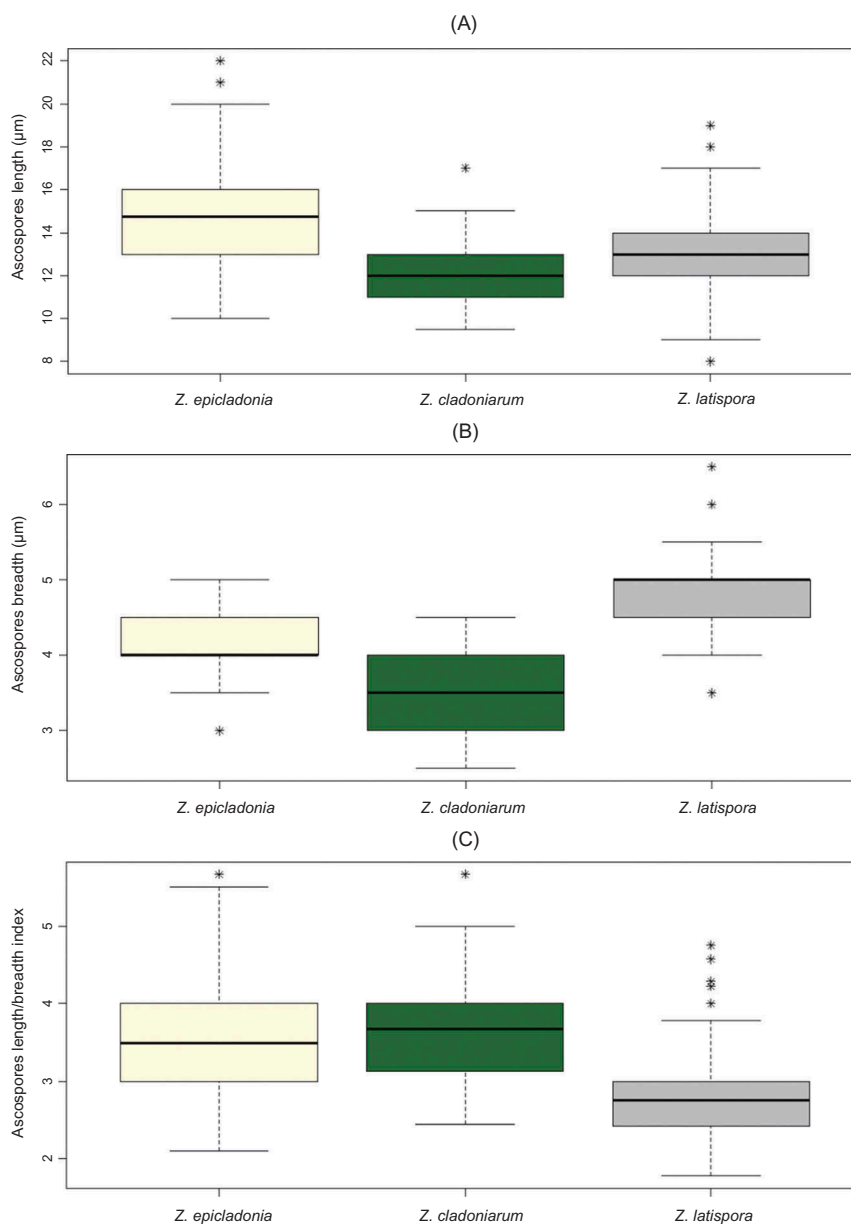


Figure 2. Box plots comparing ascospores measurements between *Zhurbenkoa epicladonia*, *Z. cladoniarum*, and *Z. latispora*. A. Ascospore length. B. Ascospore width. C. Ascospore l/b ratio. Bold vertical lines represent the median values, and asterisks represent outliers.

Typification: *Zhurbenkoa epicladonia* (Nyl.) Flakus, Etayo, Pérez-Ortega & Rodr. Flakus.

Etymology: Named in honor of our friend Dr. Mikhail P. Zhurbenko (Saint Petersburg), the prominent Russian lichenologist, for his magnificent contribution to knowledge on the biodiversity and systematics of lichenicolous fungi, including lichen parasites colonizing *Cladonia*.

Ascomata apothecioid, emarginate, mainly convex, numerous, dispersed on the upper surface of the healthy thallus of the host (*Cladonia*), almost round, sessile with a slightly constricted base. Disc dark brown to grayish

brown or almost black, matte or slightly shiny, pruinose to strongly pruinose, convex. Margin not evident from the beginning, concolorous with the disc. Exciple colorless inside, not interspersed with crystals or oil droplets, I–, composed of strongly gelatinized, radiately arranged hyphae slightly widened apically. Epithemium colorless, not evident, interspersed with small hyaline or yellowish crystals, K+ dissolving, N–. Hymenium colorless or gray to brownish in the lower part, not interspersed with oil droplets, strongly conglutinate, I+ blue, K/I+ blue. Paraphyses colorless, branched and anastomosed, composed of highly gelatinized hyphae, apically

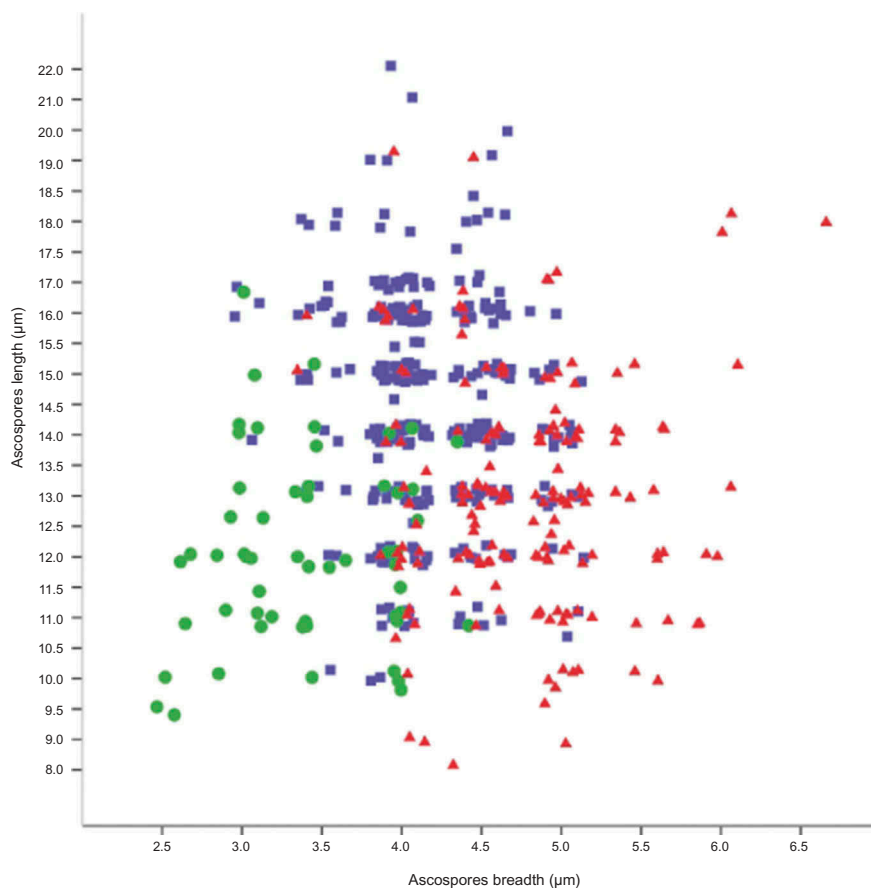


Figure 3. A scatter diagram showing 1074 measurements (in μm) of ascospore size distribution in three *Zhurbenkoa* species: *Z. cladoniarum* (circles), *Z. epicladoia* (squares), and *Z. latispora* (triangles). Each point may include values from more than one spore.

unthickened, unpigmented. Subhymenium colorless to grayish or pale brown. Hypothecium colorless to gray or brown pigmented (K⁻, N⁻), composed of highly gelatinized hyphae. Asci 8-spored, clavate, with K/I+ blue apical apparatus, developing in central part a broad hyaline vertical canal (nonamyloid apical cushion) with relatively wide opening at the top (similar to the *Lecanora*-type ascus apex described by Hafellner [1984] and Ekman et al. [2008]), and surrounded by a dark blue ‘tube’ (strongly amyloid, slightly darker boundary between nonamyloid apical cushion and moderate amyloid, pale blue apical dome), characteristic of the *Micarea*-type of ascus as described by Hafellner (1984) and Ekman et al. (2008), representing a transitional stage between *Lecanora*- and *Micarea*-type. Ascospores colorless, aseptate to 1-septate, ellipsoidal, sometimes slightly curved, with rounded ends, without an episporium. Conidiomata not seen.

Zhurbenkoa cladoniarum (Müll. Arg.) Flakus, Etayo, Pérez-Ortega & Rodr. Flakus, comb. nov.

FIG. 4

MycoBank MB828559

≡ *Patellaria cladoniarum* Müll. Arg., Hedwigia 30:232. 1891. (Basionym)

Typification: BRAZIL. SANTA CATARINA: Águas Mornas, Teresopolis (Teresópolis) in Serra dos Órgãos, on thallus of *Cladonia ceratophylla*, 1891, [*J.*] H. [R.] Schenck 4530 (holotype, G 00290673!). Thin-layer chromatography (TLC) of the host: fumarprotocetraric acid chemosyndrome.

Ascomata apothecioid, emarginate, mainly convex, numerous, dispersed on the upper surface of the healthy thallus of the host, almost round, sessile with a slightly constricted base, (0.15–)0.2–0.3 mm diam. Disc dark brown to grayish brown or rarely almost black, matte or slightly shiny, epruinose. Margin not evident from the beginning, concolorous with the disc. Exciple laterally 40–60(–80) μm wide, basally 40–60(–70) μm , colorless inside, not interspersed with crystals or oil droplets, I⁻, composed of strongly gelatinized, radiating hyphae with lumina 0.5–1.5 μm wide, sometimes apically slightly widened to 3 μm . Epithymenium colorless, not evident, ca. 5 μm thick, interspersed with small hyaline or yellowish crystals, K⁺ dissolving, N⁻. Hymenium colorless or grayish to brownish in the

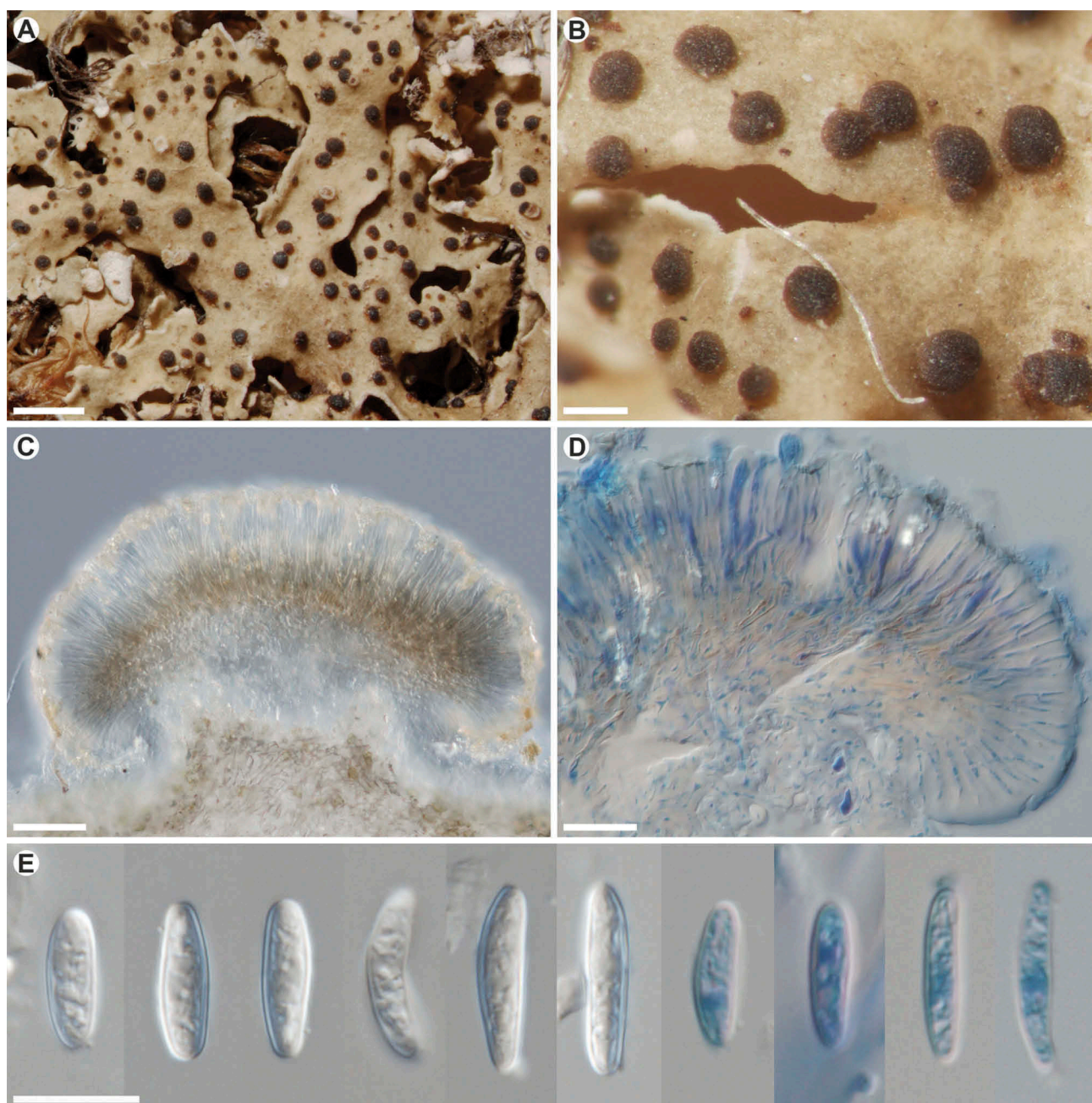


Figure 4. *Zhurbenkoa cladoniarum* (Schenck 4530, holotype). A, B. Habit of the apothecia dispersed on the upper side of the *Cladonia ceratophylla*. C. Section of ascoma mounted in tap water. D. Exciple of radiately arranged hyphae mounted in LPCB. E. Ascospores mounted in tap water (left) and LPCB (right). Bars: A = 1 mm; B = 250 μ m; C = 50 μ m; D = 25 μ m; E = 10 μ m.

lower part, not interspersed with oil droplets, 50–80 μ m high, strongly agglutinated, I+ blue, K/I+ blue. Paraphyses colorless, branched and anastomosed, composed of highly gelatinized hyphae with lumina 1–2 μ m wide, apically not thickened, not pigmented. Subhymenium colorless to grayish or pale brown, 10–20 μ m high. Hypothecium colorless to grayish or brown pigmented (K–, N–), composed of intricately arranged highly gelatinized hyphae with lumina 1–1.5 μ m wide, 30–60 μ m high. Asci 8-spored, clavate, with

K/I+ blue apical apparatus representing a transitional stage between *Lecanora*- and *Micarea*-type, 40–55 \times 8–14 μ m. Ascospores colorless, aseptate, narrowly ellipsoidal, sometimes slightly curved, with round ends, without an episporium, 9.5–(\bar{x} = 12.1, s = 1.5)–17 \times 2.5–(\bar{x} = 3.4, s = 0.5)–4.5 μ m, l/b ratio 2.4–(\bar{x} = 3.6, s = 0.7)–5.7 (n = 56). Conidiomata not seen.

Host: *Zhurbenkoa cladoniarum* inhabits the upper thallus surface of primary squamules of *Cladonia ceratophylla*, producing fumarprotocetraric acid

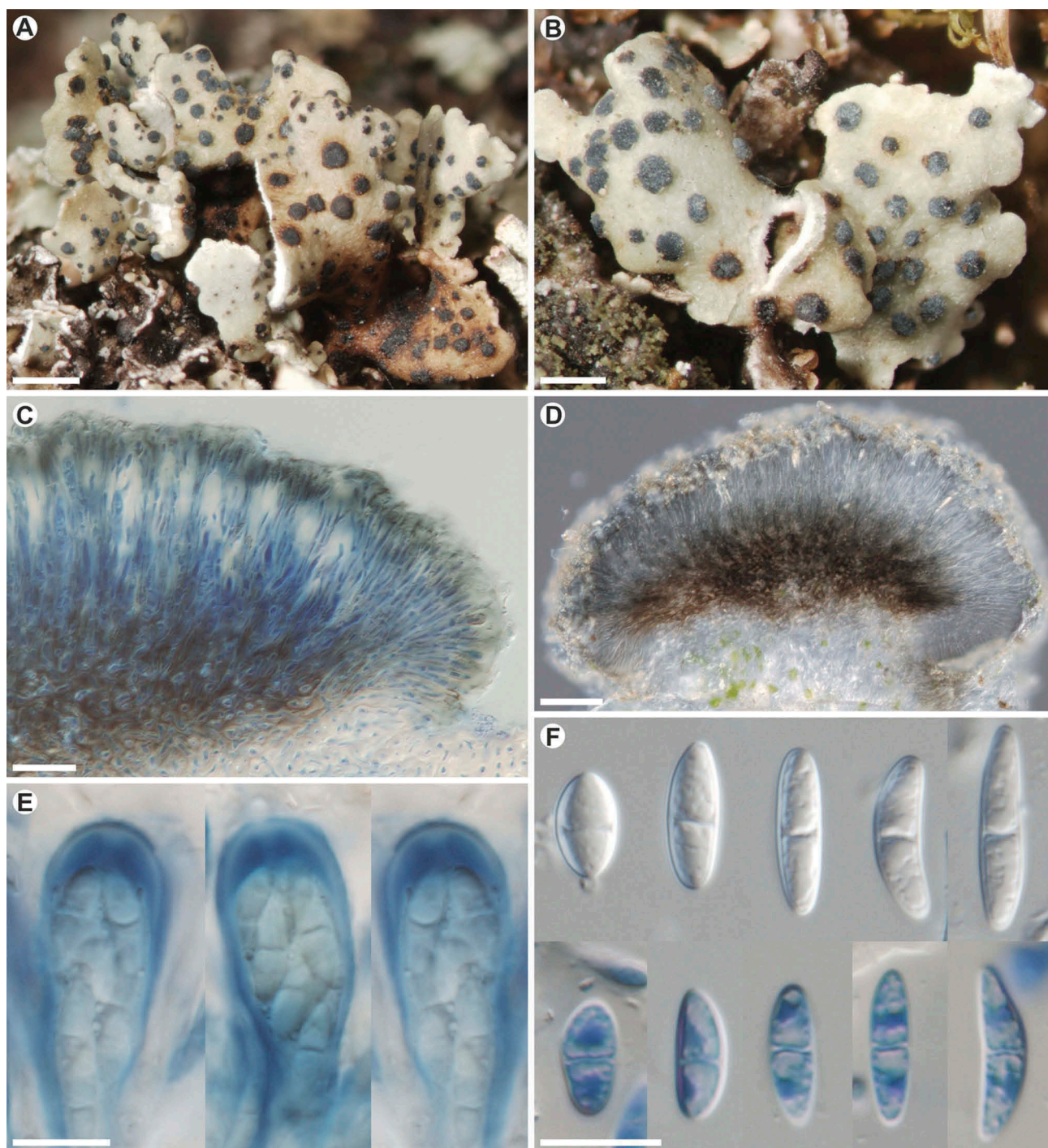


Figure 5. *Zhurbenkoa epicladonia* (Flakus 25624). A, B. Habit of the apothecia on the upper side of primary squamules of the *Cladonia* sp. C. Exciple of radiately arranged hyphae mounted in LPCB. D. Section of ascoma mounted in tap water. E. *Lecanora/Micarea*-type asci mounted in Lugol's iodine solution. F. Ascospores mounted in tap water (upper) and LPCB (lower). Bars: A = 1 mm; B = 500 μ m; C = 25 μ m; D = 50 μ m; E–F = 10 μ m.

chemosyndrome. The fungus appears on healthy parts of the thallus, and no damage to the host was observed.

Distribution and habitat. So far, the species is known only from the type locality in Serra dos Órgãos in Brazil (Müller 1891; Gumboski and Elisaro 2011).

Notes: *Zhurbenkoa cladoniarum* is characterized by dark brown to grayish brown, constantly epruinose ascomata up to 0.3 mm diam, a pale brown hypothecium, and narrowly ellipsoidal aseptate ascospores. The other two *Zhurbenkoa* species, *Z. epicladonia* and *Z.*

latispora, have apothecia that are usually pruinose and of slightly different color (grayish when pruinose or almost black) and size (up to 0.5 mm diam), with a darker hypothecium. They also can be separated by their larger or wider ascospores, respectively, which are 10–(\bar{x} = 14.5, s = 1.9)–22 × 3–(\bar{x} = 4.2, s = 0.4)–5 μ m for *Z. epicladoria* and 8–(\bar{x} = 13.1, s = 2)–19 × 3.5–(\bar{x} = 4.8, s = 0.6)–6.5 μ m for *Z. latispora*. Additionally, *Z. epicladoria* differs by the production of 1-septate ascospores.

Zhurbenkoa epicladoria (Nyl.) Flakus, Etayo, Pérez-Ortega & Rodr. Flakus, comb. nov. **FIG. 5**
Mycobank MB828560

≡ *Lecidea epicladoria* Nyl., Flora 70:132. 1887. (Basionym)

Typification: FRANCE. AVEYRON: Session de Millau, Cévennes Mts., on primary squamules of *Cladonia pocillum*, Jun 1886, F. [C.] Hy (holotype, H-Nyl 10962!). TLC of the host: not tested.

≡ *Arthonia epicladoria* (Nyl.) Alstrup & Zhurb., in Zhurbenko & Alstrup, Symb Bot Upsal 34:478. 2004.

≡ *Biatorina epicladoria* (Nyl.) Arnold, Öst Bot Zeitsch 45:106. 1895.

≡ *Catillaria epicladoria* (Nyl.) H. Olivier, Bull Acad Int Géobot 15:275. 1905.

≡ *Scutula epicladoria* (Nyl.) Zopf, Syll Fung 18:175. 1906.

Ascomata apothecioid, dispersed on the upper side of the healthy thallus of the host (occasionally causing slight hypertrophy), emarginate, immersed at first, soon sessile with a slightly constricted base, moderately to strongly convex, round, (0.1–)0.2–0.4(–0.5) mm diam. Disc pale to dark gray or almost black, matte, usually strongly grayish pruinose. Margin not evident from the beginning, concolorous with the disc. Exciple laterally 30–60 μ m wide, basally 40–50 μ m, colorless inside, not interspersed with crystals or oil droplets, I–, composed of strongly gelatinized, radiating hyphae with lumina 1–2.5 μ m wide, sometimes apically slightly widened to 3.5 μ m. Epithymenium pale to dark gray pigmented, K+ orange-brown, N–, 5–10 μ m thick, strongly interspersed with small hyaline or yellowish crystals (K+ dissolving, N–). Hymenium colorless to grayish blue (with less of the same pigment as in epithymenium), not interspersed with oil droplets, 50–70(–80) μ m high, strongly conglutinate, I+ blue, K/I+ blue. Paraphyses colorless, branched and anastomosed, composed of highly gelatinized hyphae with lumina 1–2 μ m wide, sometimes apically thickened (to 4.5 μ m wide) and with pigmented caps. Subhymenium grayish to dark brown, not clear, ca. 5–10 μ m high. Hypothecium dark brown to gray-brown pigmented, K+ yellow to orange-brown,

N+ reddish brown, composed of intricately arranged hyphae with lumina 2–3 μ m wide, 40–100 μ m high. Asci 8-spored, clavate, with K/I+ blue apical apparatus resembling the transitional stage between *Lecanora*- and *Micarea*-type, 50–70 × 10–15 μ m. Ascospores colorless, 1-septate to rarely aseptate, ellipsoidal, sometimes slightly curved, with round ends, without an epispore, 10–(\bar{x} = 14.5, s = 1.9)–22 × 3–(\bar{x} = 4.2, s = 0.4)–5 μ m, l/b ratio 2.1–(\bar{x} = 3.5, s = 0.6)–5.7 (n = 310). *Conidiomata* not seen.

Host: The species inhabits young terricolous and lignicolous thalli of several species of *Cladonia* (frequently composed of primary thallus only), producing fumarprotocetraric acid chemosyndrome. The fungus usually appears on healthy parts of the upper surface of primary squamules of the host thallus, but occasionally causes their slight hypertrophy.

Distribution and habitat: *Zhurbenkoa epicladoria* (as *Arthonia epicladoria*) is reported from British Isles, Greenland, Papua New Guinea, Russian Arctic, Sweden, Turkey, and USA (Alaska) (Alstrup and Hawksworth 1990; Zhurbenko and Santesson 1996; Aptroot et al. 1997; Zhurbenko 2001; Zhurbenko and Pospelova 2001; Hawksworth 2003; Zhurbenko and Alstrup 2004; Halıcı et al. 2010), but the majority of these records probably represent *Arthonia digitatae* (M. Zhurbenko, pers. comm.) or another similar species of *Arthonia* and should be revised. Although the species may be widespread, we suggest a conservative view of its distribution based only on specimens we studied. Thus, *Z. epicladoria* is known with certainty from the type locale in the Cévennes Mts. in France (Nylander 1887), the Eastern Carpathians (Ukraine; Kukwa and Zwolicki 2001), and the Andes (Bolivia, Ecuador, Peru).

Additional specimens examined: BOLIVIA. DEPT. CHUQUISACA: Prov. Zudañez, Área Natural de Manejo Integrado El Palmar, La Cascada bajo de El Palmar, 18°41'23"S, 64°54'26"W, 2740 m, Boliviano-Tucumano forest with *Podocarpus*, *Lauraceae*, and palms, 15 Jul 2015, P. Rodríguez-Flakus s.n. (LPB); Prov. Carrasco, Parque Nacional Carrasco, between Meruvia and Monte Punku, 17°34'43" S, 65°15'25"W, 3082 m, *Podocarpus* forest, 26 Nov 2014, A. Flakus 25624 (KRAM, LPB; TLC: fumarprotocetraric acid); Prov. Tiraque, Parque Nacional Carrasco, the crossroad below Cerro Juno, 17°19'50" S, 65°43'50"W, 4126 m, open high Andean vegetation, 29 Nov 2014, A. Flakus 25937 (KRAM, LPB; TLC: fumarprotocetraric acid); DEPT. LA PAZ: Prov. Franz Tamayo, Área Natural de Manejo Integrado Nacional Apolobamba, road Pelechuco-Keara near the turnoff to Puina, 14°41'23"S, 69°08'02"W, 4370 m, open high Andean vegetation, 17 Nov 2014, A. Flakus 25212 (KRAM, LPB; TLC: fumarprotocetraric acid); Aguas Blancas, close to Pelechuco-Sorta

road, 14°47'52"S, 69°08'51"W, 4396 m, open high Andean vegetation, 20 Nov 2014, *A. Flakus* 25474 (KRAM, LPB; TLC: fumarprotocetraric acid); DEPT. TARIJA: Prov. Aniceto Arce, Reserva Nacional de Flora y Fauna de Tariquía, between la Cumbre and campamento los Alisos, 22°00'41"S, 64°36'02"W, 2560 m, Boliviano-Tucumano forest with *Alnus acuminata* and *Polylepis*, 22 Jul 2016, on *Cladonia* squamules growing on epiphytic *Parmotrema*, *J. Etayo* 30348 (LPB, herb. Etayo). ECUADOR. PROV. COTOPAXI: N. P. Cotopaxi, NNE slope of the volcano Cotopaxi, a more than 100-y-old lava flow, 00°37'S, 78°24' W, 3840 m, on squamules of terricolous *Cladonia* sp., 27 Jun 1999, *Z. Palice* 2910 (PRA; TLC: fumarprotocetraric acid); 00°36'45"S, 78°24'15"W, 3800 m, on squamules (rarely podetia) of *Cladonia chlorophaea*, eroding slope by the brook, 27 Jun 1999, *Z. Palice* 2534 (PRA; TLC: fumarprotocetraric acid). PERU. DEPT. AREQUIPA: Prov. Caylloma, Cañon del Colca above Tapay village, open mountain area, 3705 m, 7 Jul 2006, 15°33'56"S, 71°55'32" W, *A. Flakus* 9708 (KRAM; TLC: fumarprotocetraric acid); DEPT. CUSCO: Prov. Anta, North of Zurite, a trail to queñua (*Polylepis* sp.) forest, 3794 m, 13°26.133'S, 72° 15.196'W, on squamules of *Cladonia* sp., 25 Oct 2010, *S. Pérez-Ortega* 2870 (MA-Lichen; TLC: fumarprotocetraric acid). UKRAINE. EASTERN CARPATHIANS: Chivchino-Grinyavskie Mts., below Budyjowska Wielka on squamules of *Cladonia* sp. growing on calcareous soil, 18 Jul 1935, *T. Sulma s.n.* (UGDA L-15357; TLC: fumarprotocetraric acid).

Notes: *Zhurbenkoa epiclادonia* was described as *Lecidea epiclادonia* and was found growing on *Cladonia pocillum* in France by Nylander (1887). For a long time, its systematic position was uncertain, and various taxonomists tried to resolve its relationships based on different anatomical characters. Consequently, the species was included in various genera, e.g., *Biatorina* (Arnold 1895), *Catillaria* (Olivier 1905), or *Scutula* (Saccardo and Saccardo 1906). Later, Alstrup and Hawksworth (1990) reported *Arthonia*-type asci in Greenland specimens (Christiansen 5574, as *Scutula epiclادonia*) and suggested that the generic position of the species needed further study. Zhurbenko and Alstrup (2004), based on specimens collected in Alaska and the drawings of the sample from Greenland, proposed to transfer the species into *Arthonia* (Arthoniomycetes). Recently, our study on the type collection of *Lecidea epiclادonia* revealed that the specimen has a well-developed exciple and asci of *Lecanora/Micarea*-type, critical characters that clearly exclude it from *Arthonia*.

Zhurbenkoa latispora Flakus, Etayo, Pérez-Ortega & Rodr. Flakus, sp. nov. FIG. 6
MycoBank MB828561

Typification: BOLIVIA. DEPT. LA PAZ: Prov. Franz Tamayo, Parque Nacional y Área Natural de Manejo Integrado Madidi, Chuñuna above Keara, 14°41'11"S, 69°05'30"W, 4053 m, *Polylepis pepeii* forest, on terricolous *Cladonia*, 19 Nov 2014, *A. Flakus* 25452 (**holotype** KRAM L-70178!). **Isotype** LPB! TLC of the host: sekikaik and homosekikaik acids chemosyndrome.

Etymology: *lata* (Latin), referring to the broadly ellipsoidal ascospores.

Diagnosis: Differs from other *Zhurbenkoa* species by having uniformly aseptate and broadly ellipsoidal ascospores, 8-(\bar{x} = 13.1, s = 2)–19 × 3.5-(\bar{x} = 4.8, s = 0.6)–6.5 μ m, l/b ratio 1.8-(\bar{x} = 2.8, s = 0.6)–4.8.

Ascomata apothecioid, dispersed on the upper surface of the healthy thallus of the host, emarginate, immersed at first, soon sessile with a slightly constricted base, moderately to strongly convex, round, (0.1–)0.2–0.4(–0.5) mm diam. Disc pale to dark gray or black, matte to shiny, strongly grayish pruinose or epruinose. Margin not evident from the beginning, concolorous with the disc. Exciple laterally 40–60 μ m wide, basally 30–50 μ m, colorless inside, not interspersed with crystals or oil droplets, I–, composed of strongly gelatinized, radiating hyphae with lumina 1–2 μ m wide, sometimes apically slightly widened to 3 μ m. Epithymenium pale to dark gray pigmented, K+ orange-brown, N–, 5–10 μ m thick, strongly interspersed with small hyaline or yellowish crystals (K+ dissolving, N–). Hymenium colorless to grayish blue, with a lesser amount of the same pigment as the epithymenium, not interspersed with oil droplets, 50–70(–80) μ m high, strongly conglutinate, I+ blue, K/I+ blue. Paraphyses colorless, branched and anastomosed, composed of highly gelatinized hyphae with lumina 1–2 μ m wide, sometimes apically thickened (to 5 μ m wide) and with pigmented caps. Subhymenium grayish to dark brown, not evident, ca. 5–10 μ m high. Hypothecium dark brown to gray-brown pigmented, K+ yellow to orange-brown, N+ reddish brown, composed of hyphae giving the appearance of paraplectenchymatous tissue, with lumina 2–3 μ m wide, sometimes strongly penetrating host thallus, 30–120 μ m high. Asci 8-spored, clavate, with K/I+ blue apical apparatus resembling the transitional form between *Lecanora*- and *Micarea*-type, 40–55 × 10–14 μ m. Ascospores colorless, aseptate, broadly ellipsoidal, sometimes slightly curved or narrowed at the top, without an epispore, 8-(\bar{x} = 13.1, s = 2)–19 × 3.5-(\bar{x} = 4.8, s = 0.6)–6.5 μ m, l/b ratio 1.8-(\bar{x} = 2.8, s = 0.6)–4.8 (n = 171). Conidiomata not seen.

Host: Upper surface of squamules of the primary and secondary thalli of various terricolous and lignicolous species of *Cladonia*, producing atranorin, sekikaik and homosekikaik acids, or fumarprotocetraric acid

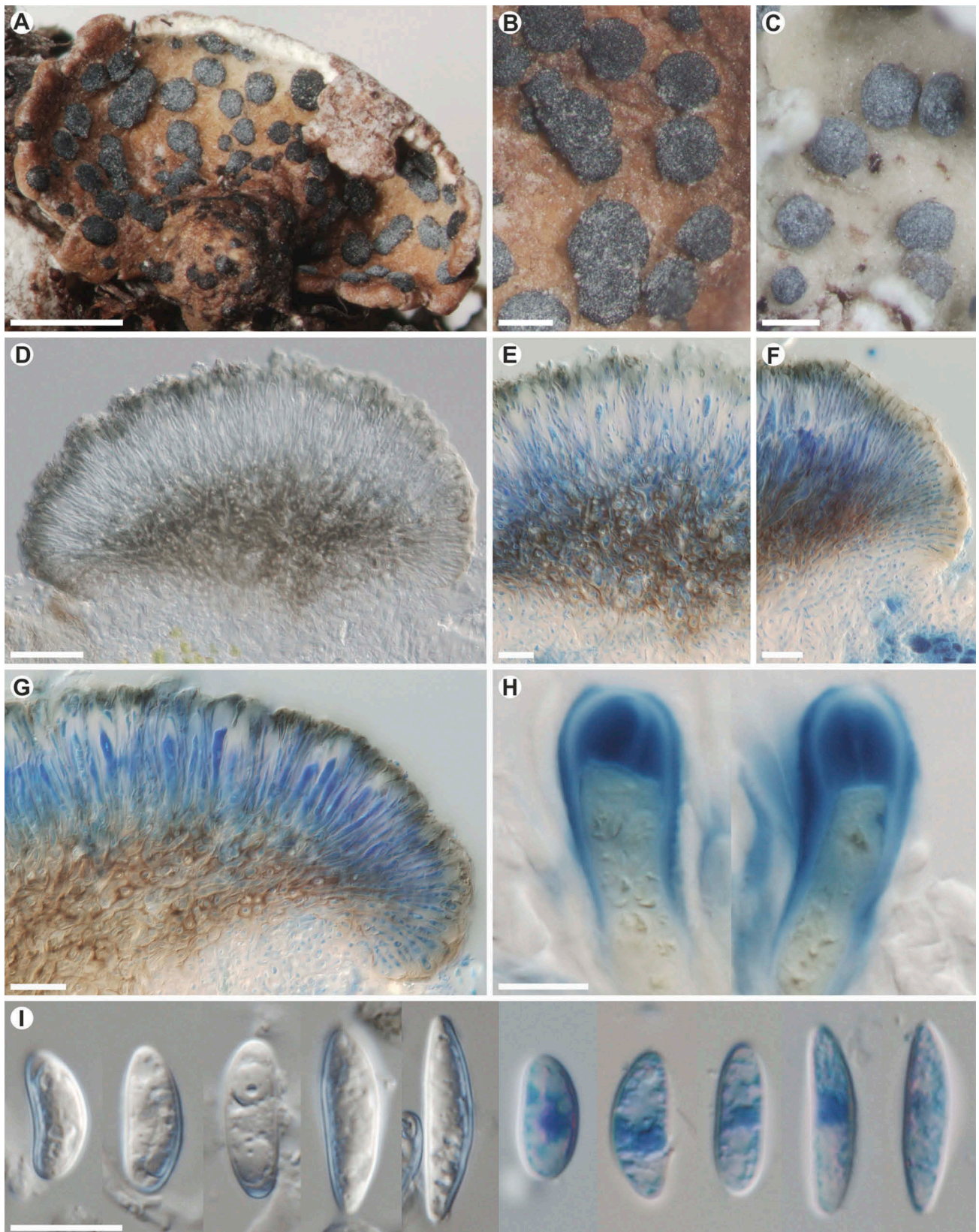


Figure 6. *Zhurbenkoa latispora* (all except C based on Flakus 25452, holotype). A, B. Habit of the apothecia on the upper side of primary squamules of the *Cladonia* sp. C. Apothecia on the podetial squamules of *C. rappii* (Flakus 4722/1). D. Section of ascoma mounted in tap water. E. Darkly pigmented hypothecium mounted in LPCB. F, G. Exciple of radiately arranged hyphae mounted in LPCB. H. *Lecanora/Micarea*-type asci mounted in Lugol's iodine solution. I. Ascospores mounted in tap water (left) and LPCB (right). Bars: A = 1 mm; B–C = 250 μ m; D = 50 μ m; E = 20 μ m; F–G = 25 μ m; H–I = 10 μ m.

chemosyndromes. The fungus appears on healthy parts of the host thallus.

Distribution and habitat: The species is known only from mountain areas in South America (Bolivia and Ecuador).

Additional specimens examined: BOLIVIA. DEPT. SANTA CRUZ: Prov. Manuel María Caballero, Siberia region near La Palma village, 17°49'12"S, 64°40'28"W, 2582 m, Yungas cloud forest, on podetia and squamules of *C. rappii*, 13 Dec 2004, A. Flakus 4722/1 (KRAM, LPBL; TLC: fumarprotocetraric acid). ECUADOR. PROV. AZUAY: Area Nacional de Recreación Cajas, Laguna Torreadora, 02°46'50"S, 79°13'40"W, on *Cladonia* sp., vertical side of a mossy boulder near the lake, 4100 m, 2 Aug 1999, J. Etayo & Z. Palice 3193 (PRA; TLC: sekikaic and homosekikaic acids); PROV. IMBABURA: Laguna Cuicocha, NE edge, ca. 9 km W of town Cotacachi, 00°18'43"N, 78°21'15"E, on squamules of *Cladonia* sp., trail cutting, 3300–3350 m, 8 Oct 2000, Z. Palice 4451 & Z. Soldán (PRA; TLC: atranorin and unidentified fatty acid).

Notes: Within *Zhurbenkoa*, this new species is characterized by the widest and broadly ellipsoidal ascospores. It further differs from *Z. epicladonia* by its aseptate ascospores.

KEY TO SPECIES OF *ZHURBENKOA*

1. Ascospores mostly 1-septate; widespread..... *Z. epicladonia*
- 1'. Ascospores aseptate; Neotropical..... 2
2. Ascospores narrowly ellipsoidal, 9.5–(\bar{x} = 12.1, s = 1.5)–17 × 2.5–(\bar{x} = 3.4, s = 0.5)–4.5 μ m, l/b ratio 2.4–(\bar{x} = 3.6, s = 0.7)–5.7; Brazil *Z. cladoniarum*
- 2'. Ascospores broadly ellipsoidal, 8–(\bar{x} = 13.1, s = 2)–19 × 3.5–(\bar{x} = 4.8, s = 0.6)–6.5 μ m, l/b ratio 1.8–(\bar{x} = 2.8, s = 0.6)–4.8; Bolivia, Ecuador *Z. latispora*

DISCUSSION

The newly described lichen genus *Zhurbenkoa* is recognized by following characters: (i) gray-brown to almost black emarginate apothecia; (ii) an epihymenium interspersed with crystals (often seen as pruina); (iii) a strongly conglutinated hymenium composed of noncapitate and sparsely branched and anastomosed paraphyses; (iv) a colorless exciple composed of radiately arranged hyphae; (v) a *Lecanora/Micarea*-like ascus type; (vi) aseptate or 1-septate ellipsoidal, colorless

ascospores without an epispore; and (vii) the lichenicolous lifestyle and specific host association (*Cladonia*).

Although the main morphological features of the type material of *Zhurbenkoa* and additional specimens studied here suggest that species of this genus are similar to those of *Scutula* and *Toninia* s.l., the phylogenetic analyses clearly indicate their affinities to Malmideaceae rather than Ramalinaceae s.l. (FIG. 1). Lichenicolous species of several fungal genera (Clauzade et al. 1989), such as *Arthonia*, *Carbonea*, *Catillaria*, *Micarea*, *Phacopsis* s.l., *Scutula*, *Tephromela*, and *Toninia* s.l., can be easily confused with *Zhurbenkoa* because of similar apothecial appearance, 8-spored asci, and the production of aseptate to transversely septate colorless ascospores. However, they substantially differ from *Zhurbenkoa* by their contrasting phylogenetic affinities, outside of the Malmideaceae, and a specific set of the following key characters: (i) *Arthonia* (Arthoniomycetes), absence of or with a strongly reduced exciple and *Arthonia*-type asci (Grube and Matzer 1997); (ii) *Carbonea* (Lecanoraceae), with a darkly pigmented inner exciple, the upper part of hymenium with aeruginose pigment, thin anastomosed paraphyses, and different host preferences, mainly saxicolous crustose lichens (Hertel 1983; Knoph et al. 2004; Pirogov et al. 2014); (iii) *Catillaria* (a polyphyletic assemblage in Lecanoromycetes), *Catillaria*-type asci, and strongly capitate simple paraphyses with pigmented cups (Kilias 1981; Hafellner 1982; Etayo 2000); (iv) *Micarea* (Pilocarpaceae), absence of or with a strongly reduced exciple, thin strongly anastomosing paraphyses, comparatively thinner ascospores, and in general presence of a lichenized thallus (Coppins 1983; Van den Boom and Ertz 2014; Brackel 2016; Etayo 2017); (v) *Phacopsis* s.l. (Parmeliaceae), ascomata immersed in host thallus, dark brown to black and shiny, often gall-inducing, reduced exciple, paraphyses apically swollen with dark brown-pigmented caps, inhabiting various genera of Parmeliaceae (Hafellner 1987; Triebel et al. 1995; Peršoh and Rambold 2002; Divakar et al. 2015); (vi) *Scutula* (Ramalinaceae s.l.), apothecia always black with a distinct margin, asci of the *Scutula*-type, exciple usually with greenish brown to red-brown pigments, hosts mainly from Peltigeraceae (Triebel et al. 1997; Wedin et al. 2007); (vii) *Tephromela* (Tephromelataceae; position of lichenicolous members unknown), apothecial sections with a violet pigment (N+ red), asci of the *Bacidia*-type, growing on *Dirinaria* and *Lecanora* (Rambold and Triebel 1992; Rambold 1993); and (viii) *Toninia* s.l. (Ramalinaceae), paraphyses strongly swelled apically, an apothecial section with different pigments (e.g., gray, green, reddish brown; frequently reacting N+ violet or K+ violet), asci of the *Bacidia*-type, and ascospores usually

more than 1-septate and/or larger (Timdal 1992; Etayo and Sancho 2008; Kistenich et al. 2018).

A revised classification of Lecanoromycetes, just published by Kraichak et al. (2018), confirmed the isolated position of Malmideaceae within Lecanoromycetes, strongly supported as a sister to Pilocarpaceae. As circumscribed previously, the family Malmideaceae was represented exclusively by lichen-forming Ascomycetes developing symbiotic associations with green chlorococoid algae of still unknown identity (Kalb et al. 2011; Ertz et al. 2013; Miadlikowska et al. 2014; Cáceres et al. 2017; Muggia et al. 2017; Sodamuk et al. 2017). This kind of mutualism plays essential role in evolution of the whole Lecanoromycetes, the most speciose class of lichen-forming fungi (Miadlikowska et al. 2014). Reversions to other nutritional strategies are rare in that class. Lichenicolous trophic modes, although very rare, repeatedly evolved through the Lecanoromycetes. Recent molecular evidence suggests that such events occurred in various families of the class, for example in Acarosporaceae (*Sarcogyne sphaeospora*), Lecanoraceae (*Carbonea supersparsa*, *C. vitellinaria*), Parmeliaceae (*Nesolchia oxyspora*, *Phacopsis vulpina*, *Raesaenenia huuskonenii*), Pilocarpaceae (*Epicladoia simplex*, *E. stenospora*, *Micarea usneae*), or Ramalinaceae (*Scutula epiblastematica*, *S. tuberculosa*, *S. miliaris*, *Toninia plumbina*) (Diederich et al. 2018 and papers cited therein). *Zhurbenkoa* species, however, are the first lichenicolous fungi discovered in Malmideaceae, and all other members of the family differing by an evident lichenized thallus. At the moment, the Malmideaceae contains (including *Zhurbenkoa*) 62 species spread across seven genera, including 2 species of unknown generic position that are temporarily placed in *Lecidea* s.l. (*L.* *cyrtidia* and *L.* *plebeja*).

Specimens of *Zhurbenkoa* are quite uniform in their apothecial anatomy. However, size and septation of ascospores are variable. Our preliminary analysis on a moderate number of ascospore measurements showed no evident patterns, and we at first thought that the genus included two species, one with aseptate and the other with septate ascospores. This hypothesis was supported by our phylogenetic analyses, which showed an obvious distinction between samples with septate and aseptate ascospores. When comparing only the length of ascospores specimens formed two groups (FIG. 2A), one with large and septate ascospores (*Z. epicladoia*) and the other one with short and aseptate ascospores (*Z. cladoniarum*, *Z. latispora*). But when the average values of ascospore width were compared, three groups were clear (FIG. 2B), characterized by medium (*Z. epicladoia*), narrowest (*Z. cladoniarum*), and widest (*Z. latispora*) ascospores. A comparison of l/b ratios consolidated specimens in two groups (FIG. 2C), one producing relatively narrow

ascospores (*Z. cladoniarum*, *Z. epicladoia*) and the second with broadly ellipsoidal ascospores (*Z. latispora*). A scatter diagram, for which 1074 measurements of the ascospores size were analyzed, showed three different clusters, representing *Z. epicladoia*, *Z. cladoniarum*, and *Z. latispora* (FIG. 3). Taking into consideration the results of the phylogenetic and statistical analyses together, we decided to distinguish three species within *Zhurbenkoa*: (i) *Z. epicladoia* for specimens with narrowly ellipsoidal, large and relatively wide, and septate ascospores; (ii) *Z. cladoniarum* for specimens with narrowly ellipsoidal, short and narrow, aseptate ascospores; and (iii) *Z. latispora* for specimens with broadly ellipsoidal, short and wide, aseptate ascospores.

Cladonia is a widespread lichen genus, the largest in the Cladoniaceae (Lecanoromycetes), with about 500 species (Stenroos et al. 2002), which frequently hosts lichenicolous fungi. Recent studies compiled up to 138 species of lichenicolous fungi inhabiting *Cladonia* (Zhurbenko and Pino-Bodas 2017); some of these could be mistaken for *Zhurbenkoa*. *Micarea kemmleri*, recently described from podetia of *Cladonia squamosa* from Germany, differs by having unpigmented, pale straw yellow, biatorine apothecia, a different kind of exciple, thinner and strongly branched paraphyses, an ascus apex with a narrower canal surrounded by K/I+ dark blue tube (reminiscent of the ascus type of Pilocarpaceae), and much smaller ascospores, 6–9 × 2.5–4 µm (Brackel 2016). The species presently is known from a single specimen, and further molecular studies are necessary to establish its phylogenetic position. *Bachmanniomyces punctum* (Trapeliales; Diederich et al. 2018) induces gall formation on the podetia and squamules of *Cladonia* and can be recognized by smaller black apothecia up to 0.2 mm diam), an ascus apex lacking evident structures, and small ascospores, 7–11 × 2.5–4 µm. *Brackelia lunkei* can be confused at first sight with very young specimens of *Zhurbenkoa*, but in contrast it has very small ascomata, only up to 0.12 mm diam, K/I– asci, sometimes forms capitate and brown-pigmented paraphyses, and produces smaller ascospores, 5.5–11 × 2.5–4 µm (Zhurbenko and Pino-Bodas 2017). Additionally, species of *Abrothallus*, *Sclerococcum* (syn. *Dactylospora*) and *Arthonia* species growing on *Cladonia* may have similar apothecia but produce darkly pigmented ascospores with more septa and/or a reduced exciple (for detailed information, see Zhurbenko and Pino-Bodas 2017). Additionally, *Lecanora lecanoricola*, described from Greenland, a species of unknown phylogenetic position having dark and pruinose lecideoid apothecia (Alstrup and Hawksworth 1990), might be confused with *Zhurbenkoa*, especially *Z. latispora*. It differs,

however, by the presence of a greenish pigment in apothecial sections, an exciple composed of apically swollen hyphae up to 6(–8) μm wide that may be indistinct when mature, apically swollen paraphyses up to 4 μm wide, and host selection, because the species is an obligate parasite of saxicolous *Lecanora cenisia* (Alstrup and Hawksworth 1990).







ACKNOWLEDGMENTS

We are greatly indebted to our colleagues and all staff of the Herbario Nacional de Bolivia, Instituto de Ecología, Universidad Mayor de San Andrés, La Paz, for their generous long-term cooperation. We would also like to thank the SERNAP (<http://sernap.gob.bo>), and all protected areas staff, for providing permits for scientific studies, as well as their assistance and logistical support during the field works. We thank especially Paul Diederich (Luxembourg) and an anonymous reviewer for their valuable comments on the manuscript, and also the curators of the cited herbaria for a loan of the specimens used in the study.

FUNDING

This research was financially supported by the National Science Centre (NCN) in Poland (DEC-2013/11/D/NZ8/03274). A.F. and P.R.F. received additional support under statutory funds from the W. Szafer Institute of Botany, Polish Academy of Sciences, Krakow, Poland. S.P.O. was supported by the grant RYC-2014-16784 from the Spanish Ministry of Economy, Industry, and Competitiveness. Z.P. acknowledges the support by the Academy of Science of the Czech Republic (AV0Z60050516, RVO 67985939).

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