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Two new species of *Lecanora* sensu stricto (Lecanoraceae, Ascomycota) from east Africa

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Abstract

The new sorediate species *Lecanora kenyana* from Mount Kenya and *L. orientoafricana* from the Rift Valley in Kenya are described. *L. kenyana* has red-brown apothecia with a constricted base, a *melacarpella*–type amphithecium, *pulicaris*–type epihymenium, a hyaline hypothecium, and contains usnic acid as major constituent. *L. orientoafricana* is characterized by having a dark hypothecium, *pulicaris*-type amphithecium, *chlarotera*-epihymenium, and contains atranorin and gangaleoidin. A phylogenetic analysis using maximum likelihood and a Bayesian approach based on DNA sequence data of mtSSU and ITS rDNA support that both new species belong to *Lecanora* sensu stricto and cluster with species containing usnic acid or having a dark hypothecium, respectively.

Keywords

Kenya, Lecanorales, new species, taxonomy, tropical lichens

Introduction

Lecanora is the major genus of Lecanoraceae (Lumbsch and Huhndorf 2010) and includes crustose (incl. placodioid) lichens with hyaline, usually non-septate ascospores, *Lecanora*–type asci and mostly lecanorine apothecia. The morphological and chemical diversity is large in this heterogeneous genus and molecular data have supported that the genus as currently circumscribed is not monophyletic (Arup and Grube 1998; 2000; Grube et al. 2004; Lumbsch 2002). The core group of *Lecanora* sensu stricto is characterized by the presence of calcium oxalate crystals in the amphithecium, filiform conidia, and the presence of atranorin and/or usnic acid. This agrees with an extended circumscription of the *Lecanora subfusca* group to include taxa containing usnic acid and a dark hypothecium (Guderley 1999; Lumbsch 1995; Lumbsch et al. 1995; Lumbsch et al. 1996; Lumbsch et al. 2003; Papong et al. in press). African species of *Lecanora* sensu stricto are poorly known but our recent studies resulted in the description of a new species and new records of *Lecanora* species for Kenya (Kirika et al. in press; Lumbsch et al. 2011). Among the collections from the Mount Kenya area and the Rift Valley we found two corticolous species, one sorediate taxon with usnic acid and a morphologically somewhat similar species with a dark hypothecium. The two taxa do not agree with known species (Lumbsch 1995; Lumbsch et al. 1995; Lumbsch et al. 1996; Papong et al. 2011; Papong and Lumbsch 2011) and consequently are described as new. To confirm the placement of the new species in *Lecanora* sensu stricto, we also generated DNA sequence data of the internal transcribed spacer region (ITS) and partial sequences of the small subunit of the mitochondrial ribosomal DNA (mtSSU) and performed a phylogenetic analysis with sequences available in Genbank.

Materials and methods

Taxon sampling and molecular methods

The study is based on material deposited in EA and F and DNA sequences downloaded from Genbank. Sequences of five *Ramboldia* spp. were included as outgroup since the genus has been shown previously to be related to *Lecanora* (Kalb et al. 2008). Sequence data of the two new species were assembled with sequences of the mitochondrial small subunit (mtSSU) and nuclear ITS rDNA downloaded from Genbank (Table 1). Sample preparation, DNA isolation, PCR and direct sequencing were performed as described previously (Mangold et al. 2008; Wirtz et al. 2012). Primers for amplification were: mr SSU1 (Zoller et al. 1999) and MSU7 (Zhou and Stanosz 2001) for mtSSU and ITS1F and ITS4 (Gardes and Bruns 1993) for ITS rDNA. Sequence fragments obtained were assembled with SeqMan 4.03 (DNASTAR) and manually adjusted.

Table 1. Species and specimens used in the present study. Newly obtained sequenced in bold.

Species	Collection data	mtSSU acc. no.	ITS acc. no
Lecanora achroa		JQ782663	JN943714
Lecanora aff. *achroa*		JQ782662	JQ782708
Lecanora allophana		AY567710	AF070031
Lecanora argentata		JQ782664	JQ782704
Lecanora argopholis		DQ787358	JQ782705
Lecanora austrotropica		JQ782665	JQ782706
Lecanora californica		JQ782668	JQ782707
Lecanora campestris		DQ787362	AF159930
Lecanora elatinoides		JQ782669	JQ782709
*Lecanora flavopallida*1		JQ782673	JN943723

Species	Collection data	mtSSU acc. no.	ITS acc. no
*Lecanora flavopallida*2		JQ782674	JN943724
Lecanora flavoviridis		JQ782675	JQ782711
Lecanora gangaleoides		JQ782676	JQ782712
*Lecanora helva*1		JQ782679	JQ782716
*Lecanora helva*2		JQ782680	JQ782715
Lecanora aff. *helva*		JQ782677	JQ782713
Lecanora aff. *helva*		JQ782678	JQ782714
Lecanora hybocarpa		DQ912273	DQ782849
Lecanora kenyana	Kenya, *Kirika 1179* (F)	**JQ900616**	**JQ900618**
*Lecanora leproplaca*1		JQ782683	JQ782718
*Lecanora leproplaca*2		JQ782684	JQ782719
*Lecanora leprosa*1		JQ782682	JQ782721
*Lecanora leprosa*2		JQ782685	JQ782720
Lecanora orientoafricana	Kenya, *Kirika 2205* (F)	**JQ900617**	**JQ900619**
Lecanora pacifica		JQ782686	JQ782722
Lecanora paramerae		EF105418	EF105413
*Lecanora phaeocardia*1		JQ782687	JQ782724
*Lecanora phaeocardia*2		JQ782688	JQ782723
*Lecanora plumosa*1		JQ782689	JQ782725
*Lecanora plumosa*2		JQ782690	JQ782726
Lecanora pseudogangaleoides ssp. *verdonii*		JQ782691	JQ782727
Lecanora queenslandica		JQ782692	JQ782728
Lecanora sp.C		JQ782693	JQ782729
Lecanora sp.E (sorediate)		JQ782694	JQ782730
*Lecanora subimmergens*1		JQ782696	JQ782732
*Lecanora subimmergens*2		JQ782695	JQ782731
Lecanora subimmersa		JQ782697	JQ782733
Lecanora toroyensis		JQ782698	JQ782734
Lecanora tropica		JQ782699	JN943720
Lecanora ulrikii		JQ782700	-
*Lecanora vainioi*1		JQ782702	JN943716
*Lecanora vainioi*2		JQ782701	JN943717
Lecanora wilsonii		JQ782703	-
Ramboldia arandensis		EU075527	EU075541
Ramboldia brunneocarpa		EU075528	EU075542
Ramboldia laeta		EU075530	EU075544
Ramboldia petraeoides		EU075531	EU075545
Ramboldia stuartii		EU075535	EU075549

Sequence alignments and phylogenetic analysis

Alignments were done using Clustal W (Thompson et al. 1994). Ambiguously aligned regions were removed manually. The single locus and concatenated alignments were analyzed by maximum likelihood (ML) and a Bayesian approach (B/MCMC). To test for potential conflict, ML bootstrap analyses were performed on the individual data sets, and 75% bootstrap consensus trees were examined for conflict (Lutzoni et al. 2004). Maximum likelihood analyses were performed using the program GARLI (Zwickl 2006), employing the general time reversible model of nucleotide substitution (Rodriguez et al. 1990), including estimation of invariant sites, and assuming a discrete gamma distribution with six rate categories. Bootstrapping (Felsenstein 1985) was performed based on 2000 replicates. The B/MCMC analysis was conducted on the concatenated data set using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001), with the same substitution model as in the ML analysis. The dataset was portioned into the two parts (mtSSU, ITS) and each partition was allowed to have own parameters (Nylander et al. 2004). A run with 20,000,000 generations, starting with a random tree and employing 4 simultaneous chains, was executed. Every 100th tree was saved into a file. The first 500,000 generations (i.e. the first 5000 trees) were deleted as the “burn in” of the chain. We used AWTY (Nylander et al. 2007) to compare splits frequencies in the different runs and to plot cumulative split frequencies to ensure that equilibrium was reached. Of the remaining trees a majority rule consensus tree with average branch lengths was calculated using the sumt option of MrBayes. Posterior probabilities were obtained for each clade. Only clades that received bootstrap support equal or above 70% under ML and posterior probabilities ≥ 0.95 were considered as strongly supported. Phylogenetic trees were visualized using the program Treeview (Page 1996).

Anatomical and chemical studies

Anatomical studies were conducted using standard light microscopy on hand-cut sections mounted in water. Secondary lichen substances were identified by high performance thin-layer chromatography (HPTLC) according to the standard methods (Arup et al. 1993).

Data resources

The data underpinning the analyses reported in this paper are deposited in the Dryad Data Repository at doi: 10.10.5061/dryad.b1068.

Results and discussion

The Species

Lecanora kenyana **Kirika & Lumbsch, sp. nov.**
Mycobank no. MB 800051
http://species-id.net/wiki/Lecanora_kenyana
Figure 1

Type. Kenya, Eastern Prov., Mount Kenya National Park, Chogoria Track, close to Chogoria Gate, open *Juniperus-Podocarpus* woodland, 0°09’S, 37°26’E, 2960m alt., 27.01.2010, on *Juniperus*, *P. Kirika 1179, G. Mugambi & H.T. Lumbsch* (holotype EA, isotype F).

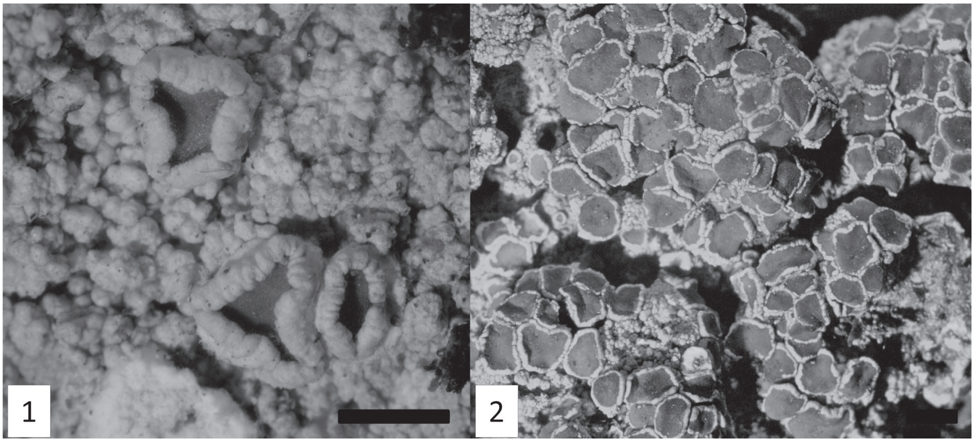

Figure 1, 2. Morphology of the new *Lecanora* species. **1** *L. kenyana*, isotype (F). **2** *L. orientoafricana*, isotype (F). Scale bars = 1mm

Description. Thallus crustose, verrucose to verruculose, thin to thick, glossy, whitish to greenish; margin indistinct; prothallus not visible; sorediate. Soralia roundish, concave, 0.5–1.2 mm diam., remaining distinct or coalescing, with granular soredia, yellowish green to yellowish gray. Apothecia sessile, strictly constricted at base, 0.6–2.0 mm diam., lecanorine; disc red-brown, shiny, plane, epruinose; margin concolourous with thallus, prominent, thick, smooth, entire to verruculose, flexuose. Amphithecial cortex uniform, gelatinous, inspersed with crystals, hyaline, 25–45 µm thick, with hyphae growing out basally. Amphithecium with small and large crystals (=*melacarpella*–type). Hypothecium hyaline, 25–30 µm high, parathecium hyaline, with yellowish crystals, 5–7 µm thick. Hymenium hyaline, 55–70 µm high, clear. Epihymenium red–brown, 10–12 µm thick, with numerous, small crystals; pigmentation and crystals dissolving in K (=*pulicaris*–type). Paraphyses sparingly branched, apically slightly swollen, hyaline. Asci clavate, 50–60 × 10–14 µm, 8–spored. Ascospores ellipsoid to narrowly ellipsoid, 12–17 × 4.5–6.5 µm. Pycnidia not seen.

Chemistry. Thallus and apothecial margin K+ yellow, C-, KC–, containing atranorin (minor), and usnic acid (major).

Etymology. The new species is named after the country Kenya where the new species has been found.

Notes. *Lecanora kenyana* is characterized by relatively large, red-brown apothecia with a constricted base, a *melacarpella*–type amphithecium, *pulicaris*–type epihymenium, the presence of usnic acid as major constituent, and the presence of soralia. There are only few sorediate *Lecanora* sensu stricto species with usnic acid, including *L. brodoana, L. elatinoides, L. floridula, L. jamesii, L. mobergiana,* and *L. transvaalensis* (Brodo and Elix 1993; Lumbsch and Elix 1998; Lumbsch et al. 1995; Lumbsch and Nash 1995). The saxicolous *L. brodoana* and *L. mobergiana* differ in having an egranulose epihymenium among other characters, whereas *L. elatinoides* (containing pannarin) and *L. jamesii* (containing 2-*O*-methylsulphurellin) are readily distinguished by their alternative chemistry and smaller ascospores. Similar species include *L. floridula* described from Florida (USA) and *L. transvaalensis* from South Africa. The former species can be distinguished by having smaller apothecia (up to 1 mm), thinner and poorly developed amphithecial cortex, a *chlarotera*-type epihymenium, smaller ascospores, and the presence of unidentified triterpenes (Lumbsch et al. 1995). *Lecanora transvaalensis* differs from *L. kenyana* in having smaller apothecia (up to 0.9 mm), a thinner and poorly developed amphithecial cortex, and smaller, broadly ellipsoid ascospores (9.5–11.5 × 7.0–8.5 µm). Further, it contains unidentified terpenoids (Lumbsch et al. 1995).

Ecology and distribution. At present this species is known from bark of juniper trees in open habitats at altitudes above 2800m in forests dominated by *Hagenia* and *Podocarpus.* Associated lichens included *Heterodermia leucomelos, Lecanora caesiorubella, Leptogium laceroides, Lobaria pulmonaria, Pannaria fulvescens, Physcia albata, Pseudocyphellaria aurata, P. crocata, Varicellaria velata,* and several *Usnea* spp.

Additional specimen examined. Kenya: Eastern Prov., Mt. Kenya National Park, Sirimon route, ca. 3km for KWS gate towards Old Moses Camp, 00˚00'N, 37˚15'E, mature montane forest with *Podocarpus, Olea, Hagenia* and *Arundinaria alpina*, 2870m, on bark, 7.10.2010, *P. Kirika 2051, G. Mugambi, G. Gatere and M. Mutembei* (EA).

***Lecanora orientoafricana* Kirika & Lumbsch, sp. nov.**

Mycobank no. MB 800052

http://species-id.net/wiki/Lecanora_orientoafricana

Figure 2

Type. Kenya, Rift Valley Prov., Cherangani Hills, Kerer forest, degraded montane forest, 3240m, on bark, 25.07.2011, *P. Kirika* 2205 (EA, holotype, F-isotype).

Description. Thallus crustose, verrucose to verruculose, thin to thick, glossy, whitish to greenish grey; margin indistinct; prothallus not visible; sorediate. Soralia roundish, 0.3–1.0 mm diam., with granulose soredia, light pale greenish white to grayish green. Apothecia sessile, constricted at base, 0.4–1.4 mm diam., lecanorine; disc light red-brown to brown, matt, plane or concave, sparsely grayish pruinose; margin concolourous with thallus, prominent, thick, smooth, verruculose. Amphithecial cortex uniform, gelatinous, inspersed with crystals, hyaline, 20–30 µm thick. Amphithecium with large crystals (=*pulicaris*–type). Hypothecium red-brown to yellowish brown, 30–40 µm high, parathecium hyaline, lacking crystals, 5–7 µm thick. Hymenium hyaline, 70–85 µm high, clear. Epihymenium red–brown, 10–12 µm thick, with coarse crystals; pigmentation and crystals dissolving in K (=*chlarotera*–type). Paraphyses sparingly branched, apically slightly swollen, hyaline. Asci clavate, 50–60 × 10–12 µm, 8–spored. Ascospores ellipsoid to broadly ellipsoid, 12.5–15.5 × 6.0–8.5 µm. Pycnidia not seen.

Chemistry. Thallus and apothecial margin K+ yellow, C-, KC–, containing atranorin and gangaleoidin.

Etymology. The new species is named after the area East Africa where it has been collected.

Notes. *Lecanora orientoafricana* is characterized by the presence of granular soredia, sparsely pruinose, brown apothecia, a *pulicaris*–type amphithecium, *chlarotera*–type epihymenium, dark hypothecium, broadly ellipsoid ascospores, and the presence of atranorin and gangaleoidin. Soredia are rare among *Lecanora* sensu stricto species with a dark hypothecium. Some specimens of *L. coronulans* are sorediate, but this species is readily distinguished by epruinose apothecial discs, an egranulose epihymenium, and the presence of protoconstipatic acid and zeorin and major constituents in addition to atranorin (Lumbsch et al. 1996). Similar esorediate species include *L. egranulosa* and *L. phaeocardia.* The latter differs from *L. orientoafricana* in having epruinose apothecial discs, a thinner amphithecial cortex, and alternative chemistry. *Lecanora egranulosa* is readily distinguished by darker, epruinose apothecial discs, an indistinct, thin amphithecial cortex, small crystals in the epihymenium, shorter ascospores, and the presence of zeorin (Lumbsch et al. 1996).

Ecology and distribution. This new species is currently only known from the type locality in the Rift Valley province of Kenya, where it was found growing on bark in a degraded montane forest dominated by *Podocarpus falcatus, Rapanea melanophloes* and *Faurea saligna* at an altitude of 3240m. Associated species included *Sphaerophorus melanocarpus*, *Pannaria* cf. *rubiginosa*, and *Ramalina* spp.

Phylogenetic study

Four new sequences were generated for this study and aligned with sequences downloaded from Genbank (Table 1). The single gene locus did not show any conflicts and hence the concatenated data set was analyzed. Our combined data set included 820 unambiguously aligned positions, 174 of which were constant. The ML tree had a likelihood value of –3718.083 and in the B/MCMC analysis of the combined data set, the likelihood parameters in the sample had the following mean (Variance): LnL = -3794.172 (0.21). The ML tree and the tree from the B/MCMC tree sampling were almost identical with no differences in well-supported clades. Thus, only the ML tree is shown here (Fig. 3). In our analysis, species of the genus *Lecanora* form a strongly supported monophyletic group as in a previously published study (Papong et al. in press). Since Papong et al. (in press) discussed the relationships of the different *Lecanora* groups in detail, these discussions are not reiterated here, but we focus only on the relationships of the two newly described species here. *Lecanora kenyana* clusters strongly supported with *L. ulrikii* and *L. wilsonii,* two species which also contain usnic acid (Lumbsch et al. 1995; Papong et al. 2011; Papong et al. in press; Papong and Lumbsch 2011). *Lecanora orientoafricana* is sister to *L. flavoviridis,* which also has a dark hypothecium (Lumbsch et al. 1996; Papong et al. in press). The molecular data support the placement of the new species in *Lecanora* sensu stricto. However, given the few sequences available from tropical *Lecanora* in Genbank, the molecular data cannot be used to confirm that the species have indeed not been described previously. We conclude that they are new based on our database of known *Lecanora* species and our examinations of type material of *Lecanora* spp. over more than 24 years.

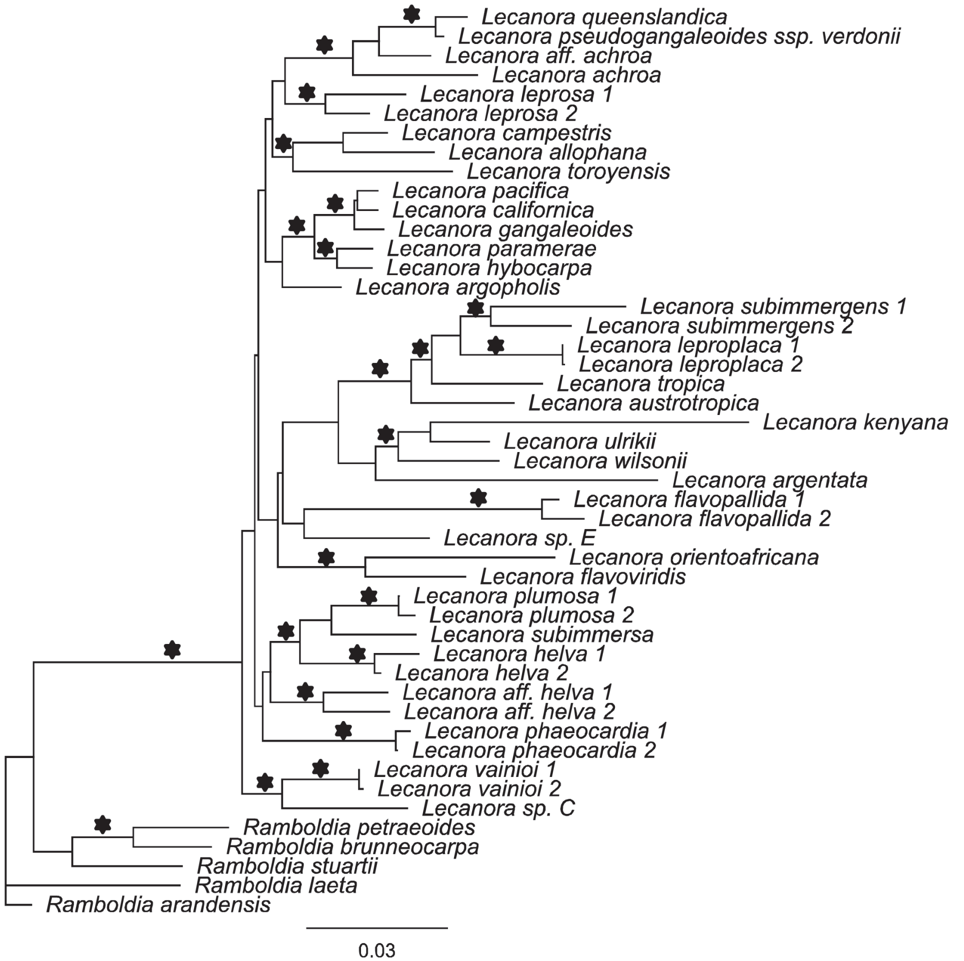

Figure 3. Phylogenetic placement of the two new *Lecanora* species as inferred from a concatenated alignment of mtSSU and ITS DNA sequences. This is the optimal tree under maximum likelihood. Branches in bold received likelihood bootstrap support values above 70%, and posterior probabilities equal or above 0.95

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