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A new species and new records of *Lecanora* (Lecanoraceae, lichenized Ascomycota) with usnic acid from the Antarctic region

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Abstract

A new species of *Lecanora*, *L. flavocrassa*, is described from the Antarctic region. Additionally, *L. stenotropa* is registered for the first time from Antarctica and the distribution range of *L. intricata* is extended to the Antarctic Peninsula. A key to the species of *Lecanora* from Antarctica that contain usnic acid as secondary metabolite is presented.

Key words: Antarctica, artificial substrates, diversity, Lecanorales, lichens, saxicolous

Introduction

As a part of the Argentinean summer survey to the Antarctic territories carried out by A.P. and J.M.R. during February 2014, some interesting lichens belonging to the genus *Lecanora* were collected. All these species are characterized by a yellow-green thallus and contain usnic acid as mayor substance.

Previous information about lichen-forming fungi in Antarctica has been provided by Hale (1987), Jacobsen & Kappen (1988), Castello & Nimis (1995, 2000), Øvstedal & Lewis Smith (2001), Śliwa & Olech (2002), Olech (2004), Ruprecht *et al.* (2012). Regarding the genus *Lecanora*, 24 species were reported for the Antarctic region (Śliwa & Olech 2002). Notwithstanding, as occurs in other large genera of crustose lichens (*e.g.* *Caloplaca*, *Candelariella*, *Lecidea*) a reliable identification of the *Lecanora* species in Antarctica is extremely difficult, leading to an incomplete assessment of the number of lichens present in this region (Hertel 1988). The morphological modifications caused by the extreme environmental conditions under which these organisms have to grow (*e.g.* darker color, pulvinate surface, endolithic thallus) is one of the most important troubles encountered in the correct identification of Antarctic crustose lichens.

Approximately 12 of the *Lecanora* species present in Antarctica contain usnic acid, and in two of them this substance is the only or the main secondary metabolite (*L. intricata* and *L. polytropa*). The presence of this compound as major substance has been traditionally considered as an important diagnostic character in the delimitation of the *L. varia* group, as well as in the *L. polytropa* and *L. symmicta* groups (Śliwa & Wetmore 2000; Printzen 2001; Martínez & Aragón 2004). The *L. polytropa* group includes saxicolous species, while the other two groups are represented by corticolous or lignicolous lichens. These subgeneric groups are also distinguished by apothecial morphology (lecanorine *vs.* biatorine), and/or the presence of other lichens products, in addition to usnic acid. Some morphological studies on the genus *Lecanora* have indicated that usnic acid alone is not a strong enough taxonomic character for its segregation in natural groups (Brodo & Elix 1993; Lumbsch *et al.* 1995). Despite these conclusions, the division of *Lecanora* species in these subgeneric groups is still used for practical reasons (*e.g.* identification keys) and chemical characters are routinely studied for circumscription of species or species groups within the genus (Pérez-Ortega *et al.* 2010; LaGreca & Lumbsch 2013).

In this article, a new *Lecanora* species, *L. flavocrassa*, is described and illustrated. *Lecanora stenotropa* is registered for the first time in Antarctica. Additionally, the presence of *L. intricata* in the Antarctic region is confirmed

and its distribution range is extended to the Antarctic Peninsula. The new species belongs to the *L. varia* group and the other two are included in the *L. polytropa* group. Illustrations and comments of the new records are presented and a key to all currently known species of Antarctic *Lecanora* with usnic acid is provided.

Materials and Methods

Study area

The Antarctic territory is defined as all lands and adjoining ice shelves south of latitude 60°S (Fig. 1). Biogeographically, this region may be divided into two zones corresponding to distinct climatic regions: Continental Antarctica and Maritime Antarctica (Lewis Smith 2000; Øvstedal & Lewis Smith 2001; Olech 2004). Continental Antarctica comprises the main part of the continent, but excluding the western part of the Antarctic Peninsula (Ruprecht *et al.* 2012). Maritime Antarctica includes the South Sandwich, South Orkney, South Shetland Islands, and the west coast of the Antarctic Peninsula with its offshore islands north of 70°S (in southern Marguerite Bay).

The specimens examined in this study were collected during the 2014 Argentinean Antarctic survey. Two localities were explored: Carlini Station (ex Jubany Station) at King George Island (South Shetland Islands) and Primavera Station at Punta Cierva (Cierva Cove) on the Danco Coast (Antarctic Peninsula) (Fig. 1), both located in the Maritime Antarctic area.

Morphology, anatomy and chemistry

All material was examined using standard light microscope techniques. An Olympus SZ-STU1 dissecting microscope was used to prepare water-mounted sections of apothecia. Anatomical observations and measurements of ascomatal structures were made under an Olympus BX-50 microscope. The presence and solubility of granules and crystals were observed with polarized light; pol+ means a light reaction caused by crystals, while pol− means that no lighting can be observed (de la Rosa *et al.* 2012). The amyloid reaction of the ascus apex with Lugol's iodine (I) after pretreatment with dilute KOH 10% (K) was examined. Color reactions (spot tests) were made using standard methods (Orange *et al.* 2001). The ultra-violet fluorescence (UV) reaction was observed under UV-light of 254 nm and 360 nm wavelength. Routine chemical analyses were studied using standard methods of High Performance Thin Layer Chromatography (HPTLC) (Arup *et al.* 1993; Lumbsch 2002).

Results

The new species

Lecanora flavocrassa de la Rosa & Messuti *sp. nov.*

Mycobank No.: MB816991

Thallus crustose, lignicolous, yellowish, thick areolate, verrucose; with usnic acid, zeorin and thiophanic acid as secondary metabolites.

Apothecia yellowish, lecanorine, relatively large, 0.8–3.0 mm diam. Margin persistent, well-developed, flexuous. Asci clavate 8-spored. Ascospores hyaline, simple, narrowly ellipsoid, 10–14(–16) × 4–6 µm.

Type:—ANTARCTIC PENINSULA. Danco Coast: Primavera Station, Punta Cierva (Cierva Cove), on a small wooden building at the base near sea shore, north-west facing, on painted wood, elev. 13 m, 64°09'15.9"S, 60°57'17.4"W, 11 February 2014, *A. Passo & J.M. Rodríguez* (holotype BCRU 5388).

Figs. 1, 2

Thallus crustose, yellowish to pale yellowish-greenish, areolate, warted-verrucose, continuous to ±disperse, surface smooth to wrinkled, ±shiny, sometimes pruinose, areoles irregular, strongly convex, rough, scabrid. *Prothallus* not visible.

Apothecia lecanorine, disciform to more frequently irregular, immersed to adnate when young, widely sessile, strongly constricted at base to subpedicelate in mature apothecia, numerous, usually clustered in groups but not fused, 0.8–3.0 mm diam. Disc concave or flat-concave, sometimes slightly pruinose, yellowish, creamy-yellowish, pale

yellowish orange. Margin persistent, prominent, rough, rarely smooth, entire to more frequently strongly flexuous, epruinose, concolorous with thallus, parathecial ring well-developed, paler than disc. Amphithecium well-developed, without calcium oxalate crystals, with small granules (pol+), K-soluble; algal layer ±continuous, irregular; amphithecial cortex poorly developed, pale brownish, thin, uniform in thickness, 10–20 µm wide, with small crystals (pol+), soluble in K. Parathecium hyaline, without crystals (pol–), most developed at the upper part. Epihymenium brown, granular (pol+), ca. 16.5 µm high, granules and pigment soluble in K. Hymenium hyaline, with oil droplets, 50–65 µm high. Hypothecium well-developed, hyaline, prosoplectenchymatic, with oil droplets, hyphae thick walled, gelatinous, 50–260 µm wide. *Paraphyses* thick, up to 1.6 µm wide, branched and anastomosed, with dilated tips 3.2 µm wide. *Asci* *Lecanora*-type, 8-spored, clavate, 38–47 × 10–14 µm. Ascospores hyaline, simple, narrowly ellipsoid to bacilliform, 10–14(–16) × 4–6 µm. Pycnidia not seen.

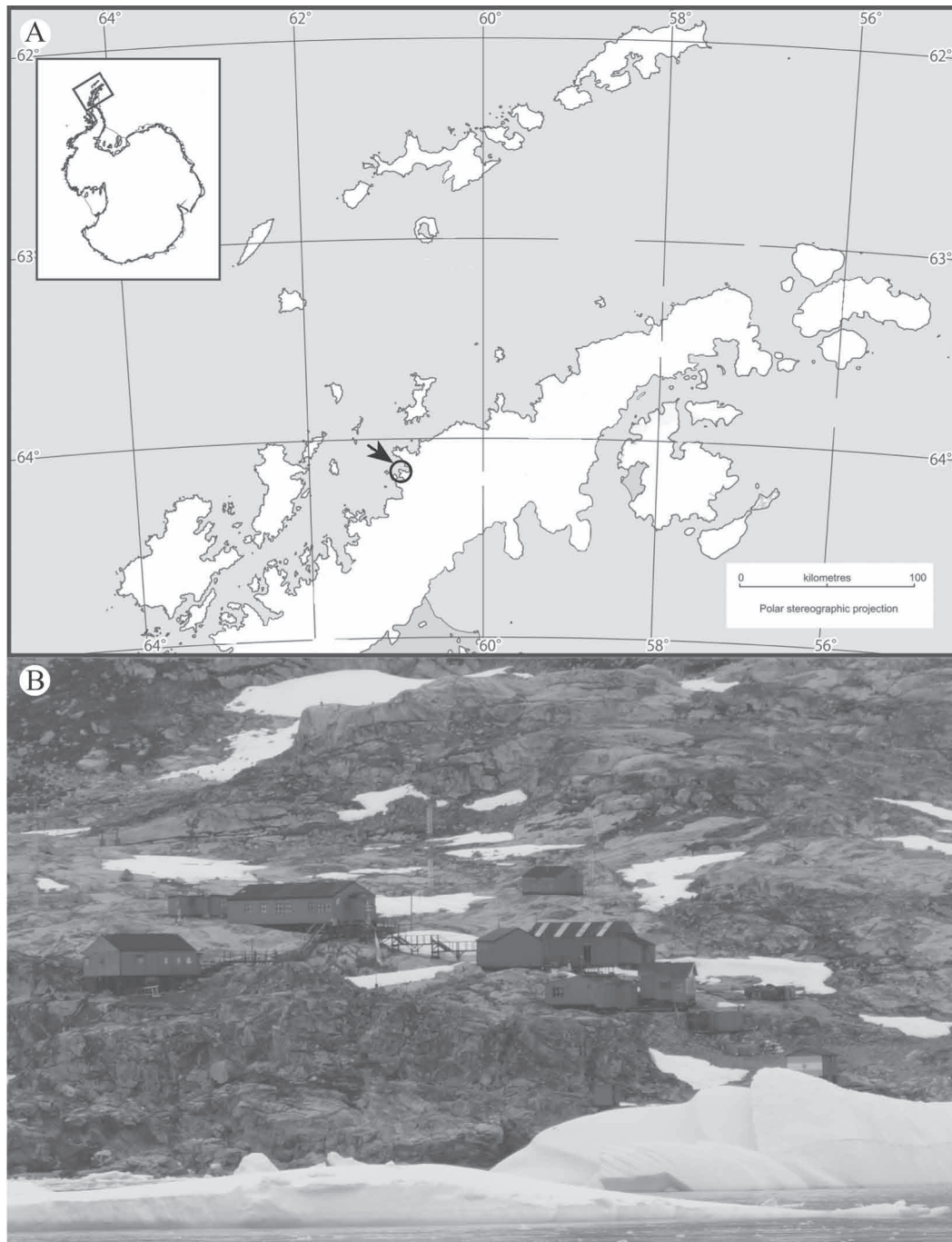


FIGURE 1. A. The Antarctic region, black arrow point to the area of the Primavera Station where the specimen of *L. flavocrassa* was found. B. A view of the Primavera Station.

Chemistry:—Thallus and apothecial margin K+ yellow-orange, C–, KC+ yellow, P–; UV–. Secondary metabolites detected by HPTLC: Usnic acid, zeorin, thiophanic acid and two unknown substances ([1] spot grey to pale greyish, UV+ orange after heating, R_{fA} 83 (class 8), R_{fC} 78 (class 8–9); 2) spot colorless, water-repellent, R_f classes 3, R_{fA} 36, R_{fC} 35.]).

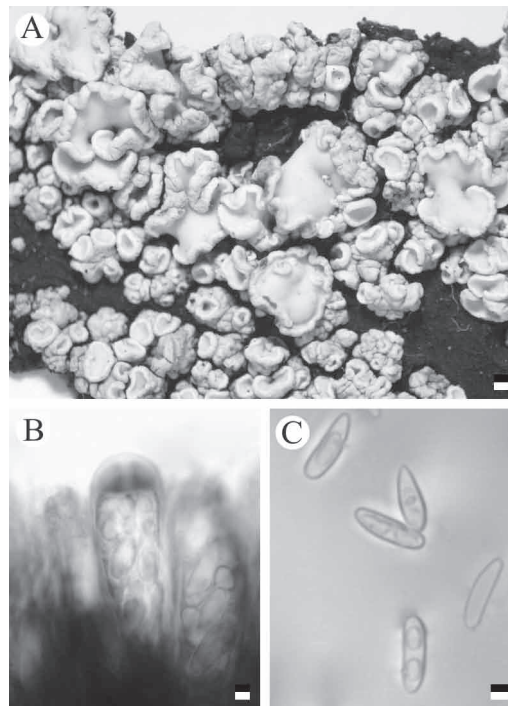


FIGURE 2. *Lecanora flavocrassa* de la Rosa & Messuti *sp. nov.* (holotype BCRU 5388). A. habitus. B. detail of ascus apex *Lecanora*-type. C. ascospores. Scales: A = 1 mm; B, C = 10 μ m.

Ecology and distribution:—The new species, *L. flavocrassa*, was found on anthropogenic substrate (worked wood), and it is known only from the type locality in the Primavera Station, Antarctic Peninsula. The possibility that this species has been introduced by man cannot be excluded. This lichen could have been established on imported timber, but additional material is required to clarify the substrate preference.

Etymology:—The specific epithet *flavocrassa* refers to the yellowish color of the thallus and the thick, strongly convex areoles.

Discussion:—*Lecanora flavocrassa* is characterized by a thick yellowish thallus, with relatively large apothecia and narrowly ellipsoid to bacilliform ascospores. The peculiar combination of morphological, anatomical and chemical characters, not observed in other species of the same genus, allows to put it apart as a new species. *Lecanora frustulosa* (Dicks.) Ach. is a saxicolous species, previously registered for South Shetland Islands (Livingston Island, South Bay) that is most likely to be confused with *L. flavocrassa* (Table 1). They are distinguished by the color of apothecial discs, being darker in *L. frustulosa* and by the absence of fatty acids, stictic, norstictic and cryptostictic acids in *L. flavocrassa*. Moreover, according to Vänškä (1984), the areoles in *L. frustulosa* have medullary hyphae which form a relatively broad but low central umbilicus-like structure, a character not observed in the new species. *Lecanora albellula* Nyl. a corticolous species distributed in Europe and North America, has also a corticated amphithecium, with a distinct cortex \pm uniform in thickness, ascospores 3.5–6.2 μ m in width and hymenium 40–60 μ m high (Table 1). However, this species has an inconspicuous thallus, smaller apothecia, beige to orange-brown discs, thalline margin often reduced or absent and isousnic acid as major compound (Printzen 2001; Ryan *et al.* 2004; Edwards *et al.* 2009). *Lecanora varia* (Hoffm.) Ach., another corticolous and lignicolous species present in Europe and North America, also resembles *L. flavocrassa* by its yellowish to greenish thallus, apothecia up to 2.0 mm diam., with persisting lecanorine margin and amphithecium with granules dissolving in KOH (Śliwa & Wetmore 2000; Printzen 2001; Ryan *et al.* 2004; Edwards *et al.* 2009). The thallus is clearly more developed in the new species, showing a thin amphithecial cortex of uniform thickness, longer ascospores and zeorin in addition to usnic acid (Table 1).

Lecanora flavocrassa can be regarded as a member of the *L. varia* group by its lignicolous, well-developed yellowish thallus, lecanorine apothecia, corticated amphithecium lacking oxalate crystals and usnic acid as main substance. Several members of the *L. varia* group also have large apothecia, with corticated amphithecia and

containing usnic acid, zeorin and thiophanic acid, but in those species the thallus is poorly developed and they show an inconspicuous parathecium and a more developed amphithecial cortex. In *L. flavocrassa* the thallus is clearly thicker and at the same time discs are pale yellowish. Additional collections are needed in order to register the phenotypic variation of this new species.

TABLE 1. Comparison of selected features between *L. flavocrassa* and the most similar species.

Character	<i>L. flavocrassa</i>	<i>L. albellula</i> ¹	<i>L. frustulosa</i> ²	<i>L. varia</i> ³
Thallus morphology	superficial, areolate, warty- verrucose	endosubstratal or sometimes of dispersed warts, areoles rounded or irregularly incised up to 0.2 mm diam.	superficial, areolate, areoles scattered to contiguous, strongly convex, 1–1.5 mm diam.	superficial, of dispersed to contiguous rounded granules, sometimes thickening into an areolate crust
Thallus color	yellowish to pale yellowish green	yellowish green to ochre or greyish beige	pale yellow or yellow-green	yellow-grey to dark grey green
Apothecial size	0.8–3.0 mm diam.	0.2–0.8(–0.9) mm diam.	0.4–2.0(–3.0) mm diam.	0.4–1.0(–1.5) mm diam.
Apothecial disc	yellowish, creamy-yellowish, pale yellowish orange	light ochre to reddish brown, orange-brown,	brown to red-brown	variable in color, cream to pink, pale orange or brown
Thalline margin	persistent prominent, frequently strongly flexuous	persistent to often excluded in old apothecia, ± level with disc	at first developed and entire, becoming crenate, flexuous or irregular, often excluded with age	poorly developed, entire, soon excluded
Amphithecial cortex	present, poorly developed, thin, uniform in thickness 10–20 µm wide	present, 10–20 µm wide	containing colorless crystals, 4–5 µm (?)	thick, 35–65 µm wide laterally and up to 105 µm high basally
Ascospores size	10–14 × 4–6 µm	(7–)9–11(–12) × (3–)4–5 µm	(9–)12–17 × 4–5(–7) µm	9–15(–16) × 4–5(–6) µm
Chemistry	usnic acid, zeorin, thiophanic acid and two unknown substances	isousnic acid, ± usnic acid	usnic acid, ± unidentified fatty acids, ± stictic, ± norstictic and ± cryptostictic acids, ± atranorin, ± epanorin, zeorin	usnic acid, psoromic acid
Substrate	on worked wood	on bark or wood	on siliceous rocks	mostly on wood but also on bark

1. Printzen (2001); 2. Vänskä (1984); 3. Šliwa & Wetmore (2000).

New records

Lecanora intricata (Ach.) Ach.

Fig. 3A

For a detailed description of this species see Ryan *et al.* (2004). The semi-immersed to sessile apothecia with dark discs and the presence of usnic acid and zeorin in the thallus are important diagnostic characters in this saxicolous species. The occurrence of intermediate morphotypes with *L. polytropa* (Hoffm.) Rabenh. make identification difficult. The presence of a more continuous yellowish thallus, areoles with crenulate margin and mainly immersed apothecia with

greenish brown to almost black discs that do not become strongly convex in *L. intricata*, differentiate both species (Messuti *et al.* 2003; Lumbsch & Elix 2004; Ryan *et al.* 2004; Edwards *et al.* 2009).

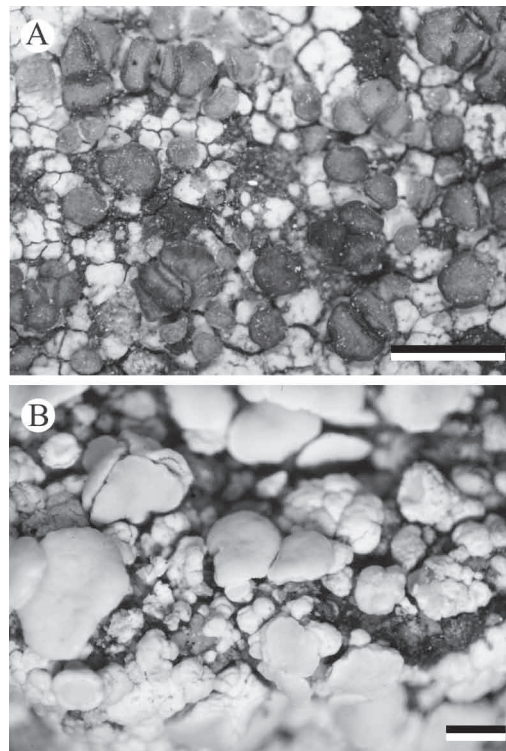


FIGURE 3. Habitus of the new records of *Lecanora* in the Antarctica. A. *L. intricata* (BCRU 5385). B. *L. stenotropa* (BCRU 5380). Scale = 1 mm.

The Antarctic material of *L. intricata* examined in this survey shows an extremely variable thallus, well-developed to almost absent (or endolithic), formed by areoles which are contiguous to disperse and scarce, thin to thick, sometimes growing on other lichens [*e.g. Rhizocarpon geographicum* (L.) DC.]. The apothecia are variable in shape and color (yellowish, blackish, bluish, greyish, greenish), adnate, sub-immersed to immersed, lecanorine to most frequently biatorine and the disc plane, convex to strongly convex.

Lecanora intricata is a cosmopolitan species of the *L. polytropa* group that grows on siliceous rocks. It occurs in holarctic-montane, boreal-arctic, arctic and high montane areas of Europe (*e.g.* British Isles), North America (United States) and South America (Argentina) (Messuti *et al.* 2003; Ryan *et al.* 2004; Edwards *et al.* 2009). It was reported from the South Shetland Islands (King George Island) in the Antarctic region (Øvstedal & Lewis Smith 2001; Śliwa & Olech 2002; Olech 2004). In this survey the distribution range of *L. intricata* is extended to the Antarctic Peninsula.

Specimens examined:—ANTARCTIC PENINSULA. Danco Coast: Primavera Station, Punta Cierva, near the base, 10° west-facing slopes close sea shore, between *Deschampsia antarctica* É. Desv. and bryophytes, on rock, elev. 17 m, 64°09'17.9"S, 60°57'23.0"W, 11 February 2014, A. Passo & J.M. Rodríguez (BCRU 5382); *ibid.*, 20° north-facing slope, elev. 131 m, 64°09'35.4"S, 60°57'05.2"W, 11 February 2014, J.M. Rodríguez & A. Passo (BCRU 5390).—SOUTH SHETLAND ISLANDS. King George Island: Potter Peninsula, 400 m south of Carlini Station, elev. 41 m, 62°14'28.9"S, 58°39'43.9"W, 16 February 2014, A. Passo & J.M. Rodríguez (BCRU 5384, 5392); *ibid.*, 1000 m south-west of Carlini Station, on mature community, on rock, 62°15'06.9"S, 58°39'33.7"W, 19 February 2014, A. Passo & J.M. Rodríguez (BCRU 5391).

Lecanora stenotropa Nyl.

Fig. 3B

This species has a yellowish, pale yellow-green thallus, plane to slightly convex areoles, strongly convex apothecia, with yellowish to brownish discs. It is easily confusable with *L. polytropa*, a species widely distributed in Antarctica. Although both species have been studied by many authors, its circumscription is still unresolved (LaGreca & Lumbsch 2001; Śliwa & Olech 2002; Ryan *et al.* 2004; Śliwa *et al.* 2012). The extreme variability of *L. polytropa* difficult the clarification of morphological differences between two species, since it may develop intermediate morphotypes

overlapping with some thallus of *L. stenotropa*. Formerly, they also were separated by the containing of rangiformic acid (in *L. polytropa*) or isorangiformic acid (in *L. stenotropa*). Nevertheless, HPTLC analysis have demonstrated that these species are chemically indistinguishable; both producing usnic, rangiformic, isorangiformic acids and sometimes zeorin. The most reliable character to separate these species is the ascospore shape, being narrowly ellipsoid in *L. stenotropa* (8–13 × 4–5 µm) and broadly ellipsoid in *L. polytropa* (8–13 × 4–8 µm). It is likely that *L. stenotropa* has been overlooked in the Antarctic collections, previously identified as *L. polytropa*.

After a detailed observation of the *L. stenotropa* specimens dealt with in the present paper, some rare morphological variations caused by extreme environmental conditions in Antarctica have been registered. In this habitat the mentioned species develops scanty, with areoles only around the apothecia, to well-developed thalli, superficial and extended, dispersed to ±continuous, verrucose, often forming rosette-like structures, with plane to slightly convex and emarginated apothecia, yellowish orange, pale orange, pale yellow, generally crowded in clusters.

Lecanora stenotropa was reported for Europe (e.g. Spain, Italy, British Isles), North America (Canada), South America (Bolivia) and New Zealand (LaGreca & Lumbsch 2001; Crespo *et al.* 2003; Ryan *et al.* 2004; Edwards *et al.* 2009; Śliwa *et al.* 2012). The present is the first record of the species for the Antarctic region.

Along with *L. intricata* and *L. polytropa*, *L. stenotropa* is considered as a member of the *L. polytropa* group, one of the least studied subgeneric groups within *Lecanora* that lacks taxonomic treatment (Śliwa & Flakus 2011). Due to notorious variability of this species group, a thorough survey of it and related species is required.

Specimens examined:—ANTARCTIC PENINSULA. Danco Coast: Primavera Station, Punta Cierva, near big penguin nests, on rock, elev. 167 m, 64°09'39.7"S, 60°57'15.3"W, 10 February 2014, A. Passo & J.M. Rodríguez (BCRU 5380); *ibid.*, near the base, 10° west-facing slopes close sea shore, between *Deschampsia antarctica* É. Desv. and bryophytes, on rock, elev. 17 m, 64°09'17.9"S, 60°57'23.0"W, 11 February 2014, A. Passo & J.M. Rodríguez (BCRU 5389).—SOUTH SHETLAND ISLANDS. King George Island: Potter Peninsula, 300 m west of Carlini Station, mature community, 62°14'25.5"S, 58°40'30.7"W, 16 February 2014, A. Passo & J.M. Rodríguez (BCRU 5383).

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Key to the Antarctic *Lecanora* species with usnic acid

1. Thallus growing on rocks4
- Thallus growing on bryophytes, soil or worked wood (lignicolous).....2

2. Thallus muscicolous or terricolous..... *L. geophila*
- Thallus lignicolous3

3. Thallus areolate, warted-verrucose; apothecia lecanorine with a well-developed thalline margin.....*L. flavocrassa*
- Thallus effuse, rimose to smooth; apothecia biatorine or lecideine, without thalline margin..... *L. symmicta*

4. Thallus with soredia5
- Thallus without soredia6

5. Thallus without atranorin; soralia always concolorous with thallus *L. handelii*
 - Thallus with atranorin; soralia sometimes somewhat blackened *L. orosthea*
6. Thallus without zeorin 7
 - Thallus with zeorin 9
7. Apothecia sessile, constricted at base *L. fuscobrunnea*
 - Apothecia slightly immersed in thallus 8
8. Thallus with stictic acid *L. atromarginata*
 - Thallus without stictic acid *L. physciella*
9. Areoles strongly convex; P± orange-yellow *L. frustulosa*
 - Areoles flat to slightly convex; P- 10
10. Thallus areolate, areoles plane; apothecia small, up to 0.5 mm diam., generally immersed in areoles, disc blackish *L. intricata*
 - Thallus areolate, areoles slightly convex; apothecia larger, up to 2.5 mm diam., sessile, constrict at base, disc yellowish 11
11. Ascospores broadly ellipsoid, 4–8 µm wide *L. polytropa*
 - Ascospores narrowly ellipsoid, 3–5 µm wide *L. stenotropa*