Contrasting variation patterns in *Austroplaca hookeri* and *Rusavskia elegans* (*Teloschistaceae*, lichenized *Ascomycota*) in maritime Antarctica

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Abstract

*Teloschistaceae* is one of the largest lichen-forming fungal lineages, with more than one thousand species worldwide distributed, including areas with extreme environmental conditions, such as Antarctica. Two species of this family, *Austroplaca hookeri* and *Rusavskia elegans* were investigated with molecular, morphological, and anatomical data to understand their diversity patterns in maritime Antarctica. These species can be confounded in a superficial identification due to their apparent similarities in color, shape, size, and dispersal mode (spores). Sampling area included the King George Island (South Shetland Islands), James Ross Island, and the Antarctica Peninsula. New nuITS sequences revealed low divergence in *A. hookeri* (haplotypes with a maximum divergence of 0.8%) and high phylogenetic diversity in *R. elegans* (haplotypes with up to 5.5% of divergence distributed in three different lineages). The same pattern was found examining the morphological and anatomical features, with phenotypic uniformity in *A. hookeri* and several variations among the *R. elegans* specimens (such as the presence and location of the isidioid structures, the margin of the hymenial disc, and the parahimenial tissue thickness). The phenotypic variability found in *R. elegans* is not linked to the different nuITS lineages or to the geographic origin of the specimens analyzed. These patterns probably reflect the unique evolutionary history of each species and their different pathways in the colonization of Antarctica.

Keywords DNA barcoding · Lichens · nuITS sequences · Species complex

Introduction

Lichens are complex structures composed of multiple symbiotic partners, including mycobionts, photobionts, and associated microbiota (Sørbø et al. 2016; Mark et al. 2020). They are key elements of the Antarctic flora, occupying various environments and substrates, and exhibiting an extraordinary diversity of shapes and colors (Øvstedal and Lewis-Smith 2001; Spielmann and Pereira 2012), like the bright yellow–orange members of the *Teloschistaceae* (Kraichak et al. 2015). This highly diverse group of lichenized *Ascomycota* is composed mainly of saxicolous thalline lineages but also includes crustose and fruticose growth forms (Gaya et al. 2012; Lücking et al. 2017). With exceptions (e.g., Aptroot and Cáceres 2016), the species of this family have anthraquinone pigments in their cortex, which confers UV light protection and enable them to adapt to the harsh environmental conditions of extreme habitats (Gaya et al. 2015).

*Austroplaca hookeri* (C.W. Dodge) Søchting, Frödén & Arup is a rosette-forming species of the *Xanthoriioidae* subfamily that is found on rocky seashores of the Antarctic Peninsula, Falkland Islands, and South Shetland Islands in maritime Antarctica (Olech 2004; Arup et al. 2013; Fryday et al. 2019). Another member of this subfamily, *Rusavskia elegans* (Link) S.Y. Kondr. & Kärnefelt (syn. *Xanthoria elegans* (Link) Th. Fr.) is a widely distributed species that is reported from Africa, Antarctica, Asia, Europe, North America, Oceania, and South America (Øvstedal and Lewis-Smith 2001; Arup et al. 2013; Joshi et al. 2019; Lücking
et al. 2021; and many others). In Antarctica, this species is reported from nitrogen-rich environments (Øvstedal and Lewis-Smith 2001). Previous molecular and morphological analyses revealed substantial variability among specimens from different regions, indicating that R. elegans possibly comprises a species complex (Lindblom 1997; Murtagh et al. 2002). Interestingly, this foliose lichen has also been investigated in experiments that involves the exposure of specimens to space conditions (extreme temperatures, UV irradiation, and vacuum), revealing remarkable viability (Brandt et al. 2015).

The increase of available molecular data combined with powerful analytical methods discloses a broader view of fungal diversity (Lücking et al. 2020). Comprehensive studies with phenotypic and genetic traits reveal species with extraordinary phenotypic variability and low genetic divergence (Pérez-Ortega et al. 2012) or even the disclosure of cryptic lineages (Leavitt et al. 2016). However, there are significant sampling gaps in lichens’ integrative taxonomy, including several megadiverse tropical regions and particularly remote areas (Kitaura et al. 2018; Wilk et al. 2021).

This study details two species of Teloschistaceae commonly registered in maritime Antarctica, A. hookeri and R. elegans. We described the extent of variation of phenotypic characters and the genetic variation of the nuITS region, discussing the divergence levels and the geographical distribution of their lineages.

Materials and methods

Taxon sampling

Sampling collections were made during four Brazilian summer expeditions (OPERANTARs), between 2014 and 2017. Twenty-eight specimens of A. hookeri were collected in King George Island (South Shetland Islands) and the Antarctic Peninsula. Eight specimens of R. elegans were found in Clearwater “mesas” in James Ross Island and one in the Antarctic Peninsula (Table 1).

DNA isolation, PCR, and sequencing

DNA was extracted using 0.015–0.025 g of the thallus with Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). The nuclear ribosomal internal transcribed spacer region (nuITS), considered the universal DNA barcode for fungi (Schoch et al. 2012), was amplified using the primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). The 25 μL PCR contained 1 x PCR Buffer (Promega), 0.2 μM of each primer, 0.2 μM of dNTPs, 2 μM of MgCl₂, 1 unit of DNA polymerase (Promega), and 5–20 ng of genomic DNA. The PCR were carried out in a Veriti Thermal Cycler (Applied Biosystems), using the following program: initial denaturation at 94 °C for 5 min, 30 cycles of 95 °C for 30 s, 54.8 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. The sequencing was performed in Macrogen (Republic of Korea), and the generated sequences were deposited in GenBank (Table 1).

Phylogenetic analyses


The alignments were constructed in Geneious v9.1.2 (Kearse et al. 2012) using the plugin MAFFT v7.308 (Katoh et al. 2002) with posterior manual inspection. The settings used were the G-INS-i algorithm, 1PAM/k = 2 scoring matrix, and the remaining parameters as default. The best-fit models of nucleotide substitution were selected using the Bayesian Information Criterion (BIC) implemented in jModelTest 2 (Darriba et al. 2012).

Phylogenetic relationships were estimated using both Bayesian and maximum likelihood approaches on the
Table 1  Information about the specimens of *Austroplaca hookeri* and *Rusavskia elegans* selected for the present study

<table>
<thead>
<tr>
<th>Species</th>
<th>Collection Sites</th>
<th>Voucher</th>
<th>nuITS haplotype(^b)</th>
<th>ITS GenBank Accession code</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Austroplaca hookeri</em></td>
<td></td>
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</tr>
<tr>
<td>(1) Ardley Island, South Shetland Islands</td>
<td>62°12'57.5&quot; S, 58°56'07.7&quot; W</td>
<td>A.A. Spielmann 11674</td>
<td>H2a</td>
<td>MN819087</td>
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<tr>
<td></td>
<td></td>
<td>D.C. Santos 211</td>
<td>H2a</td>
<td>MN819075</td>
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<tr>
<td></td>
<td></td>
<td>D.C. Santos 213</td>
<td>H2a</td>
<td>MN819078</td>
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<tr>
<td></td>
<td></td>
<td>D.C. Santos 215</td>
<td>H2a</td>
<td>MN819092</td>
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<tr>
<td></td>
<td></td>
<td>D.C. Santos 221</td>
<td>H2a</td>
<td>MN819077</td>
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<tr>
<td></td>
<td></td>
<td>A.A. Spielmann 11753</td>
<td>H1a</td>
<td>MN819071</td>
</tr>
<tr>
<td>(2) Esperanza Base, Hope Bay, Antarctic Peninsula</td>
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<tr>
<td>(3) Chabrier Point, King George Island, South Shetland Islands</td>
<td>62°10'59.6&quot; S, 58°16'56.3&quot; W</td>
<td>A.P. Lorenz 732</td>
<td>H2a</td>
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<tr>
<td></td>
<td></td>
<td>S.C. Feuerstein 1675</td>
<td>H2a</td>
<td>MN819088</td>
</tr>
<tr>
<td>(4) Turret Point, King George Island, South Shetland Islands</td>
<td>62°05'14.3&quot; S, 57°57'07.1&quot; W</td>
<td>A.P. Lorenz 419</td>
<td>H2a</td>
<td>MN819083</td>
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<tr>
<td></td>
<td></td>
<td>S.C. Feuerstein 1676</td>
<td>H2a</td>
<td>MN819091</td>
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<tr>
<td>(5) Barton Peninsula, King George Island, South Shetland Islands</td>
<td>62°13'26.3&quot; S, 58°47'29.6&quot; W</td>
<td>S.C. Feuerstein 1650</td>
<td>H4a</td>
<td>MN819096</td>
</tr>
<tr>
<td>(6) Base Almirante Brown, Paradise Bay, Antarctic Peninsula</td>
<td></td>
<td>M.J. Kitaura 2396</td>
<td>H2a</td>
<td>MN819082</td>
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<tr>
<td></td>
<td></td>
<td>A.P. Lorenz 117</td>
<td>H2a</td>
<td>MN819080</td>
</tr>
<tr>
<td>(7) Ullmann Point, King George Island, South Shetland Islands</td>
<td>62°04'43.8&quot; S, 58°21'11.1&quot; W</td>
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<td>H2a</td>
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<td></td>
<td></td>
<td>S.C. Feuerstein 1669</td>
<td>H5a</td>
<td>MN819097</td>
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<tr>
<td><em>Rusavskia elegans</em></td>
<td></td>
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<tr>
<td>(8) James Ross Island</td>
<td>64°01'31.9&quot; S, 57°43'19.6&quot; W</td>
<td>M.J. Kitaura 3066</td>
<td>H3r</td>
<td>MN628618</td>
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<td>H2r</td>
<td>MN628619</td>
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<td>M.J. Kitaura 3143</td>
<td>H4r</td>
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<td>M.J. Kitaura 3192</td>
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<td></td>
<td></td>
<td>M.J. Kitaura 2861</td>
<td>H7r</td>
<td>MN628616</td>
</tr>
<tr>
<td>(2) Esperanza Base, Hope Bay, Antarctic Peninsula</td>
<td></td>
<td>A.P. Lorenz 522</td>
<td>H1r</td>
<td>MN820449</td>
</tr>
</tbody>
</table>

\(^a\)Numbers in parentheses are indicated in the map of Fig. 2

\(^b\)The codes correspond to the haplotypes presented in Fig. 2
CIPRES Science gateway (Miller et al. 2010). The Bayesian analyses were carried out using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), with GTR + I + G as nucleotide substitution and site heterogeneity models. The posterior probabilities were approximated by sampling trees using Markov Chain Monte Carlo (MCMC). Two simultaneous runs with 10,000,000 generations, each starting with a random tree and employing four simultaneous chains were executed, sampling every 10,000 steps. The first 25% of the generated trees were discarded as burn-in and the consensus tree was built. Tracer v. 1.7 (Rambaut et al. 2018) was used to verify if the chains reached the stationary stage. Maximum likelihood analyses were implemented with RAxML 8.2.12 (Stamatakis 2014), assuming the GTRGAMMA model and the remaining settings as default. Support for each branch was considered significant when ML bootstrap ≥ 75 and Bayesian posterior probabilities ≥ 0.95.

Morphological descriptions

All voucher material were deposited in the CGMS Herbarium. The specimens were described in detail, including all the characters usually examined in the literature (Link 1791; Fries 1860; Lindblom 1997; Øvstedal and Lewis-Smith 2001). The isoneotype of *R. elegans*, designed by Filson in 1984, gently sent from the Helsinki herbarium as a loan, was also examined. Unfortunately, due to the time since the collection, it was not possible to obtain genetic sequences from this material.

Results

Phylogenetic relationships

Thirty-eight new nuITS sequences were generated and all the materials were identified at species level: 29 sequences of *A. hookeri* and nine sequences of *R. elegans*. The specimens of *A. hookeri* were sampled in six collection sites on King George Island (South Shetland Islands) and two sites of the Antarctic Peninsula, while almost all *R. elegans* specimens (eight out of nine) were found on James Ross Island and the remaining in the Antarctic Peninsula (Table 1). The results showed that the nuITS region, widely used for molecular recognition of fungi (Schoch et al. 2012), effectively identified these species. The complete nuITS alignments comprised 529 bp in the *Austroplaca* dataset and 548 bp in the *Rusavskia* dataset.

Bayesian and ML analyses resulted in similar phylogenetic relationships, recovering highly supported clades (posterior probabilities > 0.95 and bootstrap > 75%); therefore, only the ML analyses are shown. The sequences generated from specimens identified as *A. hookeri* grouped with the two other *A. hookeri* sequences retrieved in GenBank (KC179085 and KJ133447) that were also generated from Antarctic specimens. Besides, there are two sequences reported as *Caloplaca hookeri* (C.W. Dodge) Sochting, Øvstedal & Sancho in GenBank (JQ074201 and JQ074202) that are closely related to *A. ambitiosa* and to *A. everta* (Fig. 1), respectively. Both specimens (JQ074201 and JQ074202) were collected in the extreme southern South America (Tierra del Fuego, Chile), and probably were erroneously determined. This relationship was already shown in Sochting and Castello (2012), in which *C. hookeri* sequences were close to *A. millegrana* (classified at the time as *Caloplaca millegrana* (Müll. Arg.) Zahlbr.).

Among the nuITS sequences of *A. hookeri*, six haplotypes were detected (maximum 0.8% divergence). The nuITS sequences distribution revealed a widespread and frequent haplotype (H2a) that occurs on King George Island and the Antarctic Peninsula. Haplotypes H3a and H6a are exclusive from the Antarctic Peninsula populations, H1a and H5a from King George Island, and H4a occurs in both locations (Fig. 2).

In a contrasting pattern, *R. elegans* had a greater availability of nuITS sequences in GenBank. Fifty-five nuITS sequences named *R. elegans* or *X. elegans* were selected, covering a wide geographical range (Online Resource 1). Bayesian and ML reconstructions resulted in similar branching patterns with several polytomies and moderate to weak statistical support (Fig. 3). The nuITS phylogenetic analyses did not recover reciprocal monophyly among *Rusavskia* species and grouped *R. dasanensis* and *R. sorediata* with *R. elegans* sequences from the northern hemisphere. The single-locus analyses also lacked phylogenetic resolution to separate the closely related genera *Zeroviella*. This was already shown by Vondrák et al. (2019), with nuITS analyses that included a wide variety of *R. elegans* sequences and did not recovered the *Zeroviella* genus as monophyletic. *Z. coreana*, *Z. mandschurica*, and *Z. papillifera* (the *Rusavskia papillifera*-group) grouped with two undescribed species of *Rusavskia* from the Altai-Sayan region (Central Asia), while *Z. esfahanensis* is closer to a *R. elegans* sequence from Switzerland.

The results revealed high diversity among the nuITS sequences obtained from the nine specimens of *R. elegans* examined. Seven haplotypes with up to 5.5% divergence were detected (Fig. 2), and there was no correspondence between them and the morphological variations. Sequences of *R. elegans* from James Ross Island and the Antarctic Peninsula did not cluster with other Antarctic specimens (AF279772 from Signy Island and AF281306 from Vestfold Hills). Instead, they were positioned in different lineages that
included specimens from China, Iceland, Norway, Russia, Svalbard, Switzerland, and the USA. These results indicated that much more remains to be investigated about the relationships among *Rusavskia* species, especially regarding *R. elegans*.

**Species characterization**

*Austroplaca hookeri* (C.W. Dodge) Søchting, Frödén & Arup, Nordic Journal of Botany 31(1): 37 (2013). Figure 4a, b.


Description: Thallus crustose, 1.5–6.0 cm broad, yellowish orange to reddish under fluorescent light, opaque, matt, orange to reddish under stereomicroscope, macules absent. Branches laciniform at the distal end and probably verruciform or areolate at the proximal end, 0.2–1.5 mm wide, up to 6.0 mm long, continuous to agglomerated, adnate, adpressed, upper surface smooth to the naked eye, rugulose at ×10 magnification; apices of the branches rotund.
smooth to involute, without ornamentation; lateral margin of the branches smooth, plane, or rarely involute, irregular; lower side attached on the rock or with substrate. Soredia, isidia, and lobules absent. Vegetative ornaments spherical (verrucae), 0.2–0.7 mm diam., simple, concolorous with the thallus, frequent to abundant. Thallus attached by the medullar hyphae; hapters and rhizines absent. Apothecia usually present, up to 1.5 mm diam., laminal, adnate to subpedicellate, on the verruciform or placodioid part of the thallus; disc plane with granular appearance; margin of the apothecia concolorous with the thallus, smooth, without ornaments; lateral of apothecia covered by the thallus, concolorous with the thallus, without ornaments, photobiont rarely present on the lateral of apothecia; pedicel absent or short, smooth, ca. 0.5 mm, without ornaments. Anatomy: Thallus 350–800 µm thick; upper cortex with collo- or prosoplectenchymatous tissues, 20.0–37.5 µm (5–7(–11) cells) thick; photobiont frequent to abundant, green, spherical cells with 10.0–12.5 µm diam.; medullar hyphae usually abundant, without intercellular space; lower cortex absent, but can be found on the apices forming colloplectenchymatous tissue. Apothecia with hymenium 75–125 µm thick, subhymenium 37.5–100 µm thick, uncolored; hypothecium 37.5–50.0(–200) µm thick, when extend to the pedicel, colloplectenchymatous cells; parahymenial tissue continuous with the hypothecium, prosoplectenchymatous cells, 12.5–37.5 µm thick at the base, 25–37.5 µm thick at the apices; photobiont layer usually covered 1/3 of the lateral apothecia (proper margin), cortex of amphithecia 12.5–25.0 µm (ca. 5 cells) thick. Ascospores polaricircular, 12.5–17.5 × 5.0–7.5 µm, apices acute to obtuse, septae ca. 2.0 µm long. Pycnidia not observed.

Examined material: Antarctica, the Antarctic Peninsula, Base Esperança, 63°24′32.5″ S, 57°00′49.0″ W, 18 Jan 2017, Leg. A.P. Lorenz 700; IDEM, Paradise Bay, near Almirante Brown Base, 64°53′41.2″ S, 62°52′24.6″ W, 07 Jan. 2016, Leg. A.P. Lorenz 104, 117; IDEM, South Shetland Islands, Ardley Island, 62°12′57.6″ S, 58°55′06.4″ W, 11 m elev., 19 Feb 2015, Leg. D.C. Santos 211; IDEM, Barton Peninsula, near the King Sejong Station, 62°13′50.6″ S, 58°47′05.4″ W, 34 m elev., 11 Feb. 2015, Leg. M.J. Kitaura 2396; IDEM, Turret Point, 62°05′14.3″ S, 57°57′07.1″ W, 08 Dec 2014, Leg. S.C. Feuerstein 1626; IDEM, Ullmann Point, 62°04′43.8″ S, 58°21′11.1″ W, 13 Dec 2014, Leg. S.C. Feuerstein 1669; and IDEM, Vauréal Cabe, 62°10′59.6″ S, 58°17′23.6″ W, 21 Jan. 2016, Leg. A.P. Lorenz 419.

**Austroplaca hookeri** is characterized by a crustose thallus, strongly attached and usually collected with the substrate. The thallus has laciniform branches on the distal end and the proximal end is verruciform or areolate.

**Rusavskia elegans** (Link) S.Y. Kondr. & Kärnefelt, Ukrayins’kyi Botanicnyi Zhurnal. Kiev. (Ukrayins’kyi Bot. Zhurn.). 60: 434. 2003. Figure 4c, d.


Description: Thallus foliose, 2.0–6.0 cm broad, orange under fluorescent light, opaque, matt to bright, orange...
Fig. 3  Phylogenetic relationships among *Rusavskia* species based on maximum likelihood analysis of the nuITS region. Sequences generated in this study are indicated in bold and their respective nuITS haplotypes are color coded. Bootstrap values > 75% are given above branches.
to reddish under stereomicroscope, discreet to salient maculae, originated when the cortex is degraded, subtle. Lacinia 0.4–1.5 mm wide, 4–15 mm long., overlapping to agglomerate, adpressed to attached at points, adnate to ascending, upper surface smooth under all magnifications; apices rounded, plane, without ornamentation, smooth to inflated; lateral margin plane, sinuous to irregular, without ornamentation; lower cortex yellow to whitish, smooth. Soredia absent. Isidioid structure granular (verrucae) without constricted base, 0.2–0.3 × 0.2–0.4(–0.6) mm diam., simple, firm, erect, concolorous with the thallus to slightly orange, laminal, casual or grouped, rare to abundant, usually on older parts. Thallus attached by rhizines, frequent. Apothecia 0.5–2.5 mm diam., laminal to marginal, subpedicellate, disc plane; margin of apothecia orange, smooth, without ornamentation, only the older apothecia discreet; amphithecia concolorous with the thallus, without ornamentation, usually covering the margin. Anatomy: Thallus 350–750 µm; upper cortex proso- to slightly colloplectenchymatous, 12.5–50.0 µm (3–6 or undefined cells); lower cortex colloplectenchymatous, 15.0–25.0 µm (3–6 cells). Medullar layer irregularly distributed, inflated (with intercellular space); photobiont abundant, frequent, near the cortices, ca. 10 µm thick of spherical cells. Apothecia with hymenium 50–62.5 µm high, subhymenium 12.5–37.5 µm thick, colorless, hypothecium 12.5–37.5 µm thick, colorless, prosoplectenchymatous; paraphymenium tissue continuous with the hypothecium, prosoplectenchymatous of irregular cells, 12.5–25.0 µm thick at the base and apex; cortex of thalline exciple until 1/3 high of hymenia; 10.0–12.5 µm (ca. 2 cells) thick at the apex; 25–37.5 µm (4–6 cells) thick at the base, colloplectenchymatous. Ascospores fusiform, polaricicular, 10–17.5 × 5.0–7.5 µm, apices rounded to obtuse, septum less than 2.5 µm. Pycnidia absent.

Examined specimens: Antarctica, James Ross Island, Clearwater mesa, 64°01’50.1’’ S, 57°42’24.5’’ W, 253 m elev. 17 Jan 2016, Leg. M.J. Kitaura 2861; 64°01’36.9’’ S, 57°41’13.7’’ W, 177 m elev., 26 Jan 2016, Leg. M.J. Kitaura 3062, 3066; 30 Jan 2016, 64°00’56.9’’ S, 57°41’16.6’’ W,
184 m elev., Leg. M.J. Kitaura 3141, 3143; 64°01′47.1″ S, 57°39′10.7″ W, 100 m elev., 02 Feb 2016, Leg. M.J. Kitaura 3170, MJK 3171; 64°01′31.9″ S, 57°43′19.6″ W, 182 m elev., 07 Feb 2016, Leg. M.J. Kitaura 3192; and Antarctica, the Antarctic Peninsula, near Esperanza Base, on rocks, 63°23′55.8″ S, 56°59′34.0″ W, 13 Jan 2017, Leg. A.P. Lorenz 522.

*Rusavskia elegans* is characterized by a foliaceous thallus and adnate or attached by rhizines at points on the substrate. The laciniae have lower cortex that is not restricted to the margin.

Both species, *A. hookeri* and *R. elegans*, are ornamented by verrucae. *Austroplaca hookeri* has verrucae on the thallus, which is laciniform at the distal end and verruculose or areolate in the proximal region (Fig. 4b), whereas *R. elegans* has verrucae on the laciniate thallus in both regions (Fig. 4d). The verrucose appearance has generated erroneous determinations in the austral region, as reported by Sochting et al. (2004); then the earlier reports must be revised.

In our study, the specimens of *A. hookeri* occurred in rocky outcrops near the seashore in nitrogen-rich environments of bird-nesting areas of King George Island, Ardley Island, and the Antarctic Peninsula (Fig. 4a). *Rusavskia elegans* specimens were found on rocky substrates within cracks, in James Ross Island Clearwater mesa (100 to 253 m of elevation), usually far from the coast and not, at least recently, bird-influenced areas (Fig. 4c).

### Discussion

Analyses of several specimens of *A. hookeri* revealed low divergence of the nuITS sequences and morphological variations. Most specimens shared the same haplotype, widely distributed in the sampling points (distant for up to 380 km). It was also possible to identify exclusive haplotypes, two from King George Island and two from the Antarctic Peninsula. This pattern may indicate a recent origin or colonization as found in the Antarctic populations of *Cetraria aculeata* (Schreb.) Fr. (Domaschke et al. 2012), *Placopsis* (Brock et al. 2019), *Pseudephebe minuscula* (Nyl. ex Arnold) Brodo & D. Hawksw (Garrido-Benavent et al. 2021), and *Usnea aurantiacoatra* (Jacq.) Bory (Lagostina et al. 2021).

In contrast, the phylogenetic analyses using a comprehensive nuITS dataset of *R. elegans* revealed several genetic lineages, with no relation with the sequences geographical origins. Previous studies already show that *R. elegans* comprises a species complex (Lindblom 1997; Dyer and Murtagh 2001; Murtagh et al. 2002). Dyer and Murtagh (2001) generated nuITS sequences of specimens identified as *R. elegans* from Vestfold Hills and Mawson station in Eastern Antarctica. However, only the Mawson sequence (GenBank accession number AF281306) clustered within the *R. elegans* complex, with a sequence from Signy Island (AF279772) and Russia (MG954149). Here, the surprise was the placement of the specimens from James Ross Island (JRI) in different lineages of the *R. elegans* phylogeny. JRI is a volcanic island located in the Antarctic Peninsula east coast, a region with vegetation mainly composed of lichens, due to the limited availability of liquid water (Láska et al. 2011). The sequences from JRI are closely related to sequences from very distant regions, a pattern that suggests multiple long-distance dispersal events. These events are generally related to reproductive strategy and dispersal vectors, such as wind (Muñoz et al. 2004) and migratory birds (Garrido-Benavent et al. 2017). The specimens of *A. hookeri* and *R. elegans* examined in this study demonstrate sexual reproduction structures (apothecia). These structures produce ascospores that can be dispersed for long distances compared to vegetative propagules, which can restrict distribution capacity at the regional dispersion scale (Werth et al. 2006; Buschbom 2007; Lättman et al. 2009; Jones et al. 2015).

The morphological variation among *R. elegans* thalli must also be highlighted. This wide variation is also reported for populations of *R. elegans* from North America, that were distinguished in three morphotypes based on color, shape, thallus size, medulla type, and apothecium density (Poelt 1969; Lindblom 1997). The variation in the thallus size is also reported for *R. elegans* specimens from the Altai-Sayan region in Central Asia, with no correspondence between the morphological diversity of *R. elegans* specimens and their phylogenetic relationships (Vondrak et al. 2019). The morphological analysis of the JRI specimens revealed variation in several characters, such as the presence and location of the isidioid structures, the margin of the hymenial disc, the parahymenial tissue thickness, and others, with a considerable overlap of characteristics. However, the morphological variation was not structured in the lineages; therefore, it was not possible to delimitate morphotypes. Diagnostic characteristics used to delimitate *Teloschistaceae* species, like the color of the thallus and density of apothecia, are already reported as variable in several groups (Scherrer and Honegger 2003; Lindblom and Ekman 2007, 2012; Arup et al. 2013). Different lineages that show similar morphology (or overlap of characteristics) are common in lichens and combined approaches should be used to delimitate species (Lücking et al. 2020). Therefore, it is evident that *R. elegans* comprises a species complex with much to be investigated about its phenotypic variation, environmental preferences, and evolutionary history throughout its wide distribution.

Due to their apparent similarities in color and size, specimens of *A. hookeri* and *R. elegans* can be hard to be distinguished during a superficial identification in the field. The morphological features detailed in this study, as the thallus surface and how they are attached to the substrate, can be easily used to differentiate both species. In addition, the literature reported that *R. elegans* has preferences for...
nitrogen-rich environments and that the species is widespread in South Shetland Islands (Övstedal and Lewis-Smith 2001). However, in our study, we found R. elegans in areas without birds, and collections in the South Shetland Islands were confirmed as other species after detailed morphological and anatomical examinations. Therefore, a review of the Antarctic collections identified as R. elegans is necessary. There is the possibility that this taxon has a more restricted distribution in Antarctica than previously thought, and may not have an ecological preference for nitrogen-rich sites.

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**Author contributions** AAS designed and coordinated the project. MCS, JBP and MJK conducted experiments. MSC, MKJ, and APL analyzed data. MCS, MKJ and APL wrote the manuscript. All authors read and approved the manuscript.

**Declarations**

**Conflict of interest** The authors declare that there are no conflicts of interest at any level for the publication of this study.

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Fries TM (1860) Lichenes Arctoi Europae Groenlandiaeque Hacte- nus cogniti. Upsalia


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