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Morphological differentiation and phylogenetic homogeneity in *Usnea aurantiaco-atra* reveal the complexity of lichen symbiosis

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Abstract: *Usnea aurantiaco-atra* is the dominant flora around King George Island, Antarctica, whose specimens exhibited various phenotypes, even for those with the same ITS sequences in both mycobiont and photobiont. A comprehensive analysis of morphological traits of *U. aurantiaco-atra* including the reproductive structures, growth forms and ornamentation, cross section of the branches, and the substratum was carried out. Four arbitrary groups were identified based on their reproductive characters, but these groups cannot be distinguished from molecular phylogenetic trees based on fungal or algal ITS sequences. Further, the complicated morphological diversity of the thalli with the same ITS haplotypes in both mycobiont and photobiont suggest that some other factors in addition to the symbionts could influence the morphology of lichens. This implies that lichen is indeed a complex-mini-ecosystem rather than a dual symbiotic association of fungus and alga. Also, a lichenous fungi *Phacopsis* sp. was identified based on its anatomical characters and ITS sequence, which was also responsible for the black burls-like structures on *U. aurantiaco-atra*.

Key words: Antarctic, lichens, symbiont, ITS, molecular phylogeny, morphology, anatomy.

Introduction

Lichen is a typical symbiotic partnership between a mycobiont (fungal partner) and a photosynthetic phycobiont partner, usually green alga or cyanobacteria. The association becomes successful as the fungus provides a suitable niche for its photobiont partner while the alga serves as a carbon source through the products of photosynthesis (Nash 2008). This symbiosis is characterized by poikilohydric lifestyle (Green and Lange 1995) that allows it to colonize almost all terrestrial environments, ranging from the tropical to polar climate zones (Osyczka and Węgrzyn 2008; Green *et al*. 2015). Further, lichen could grow on almost every substrate type, such as rocks, plant surfaces, bare soil, man-made material surfaces and marine intertidal zones (Nash 2008; Cao *et al*. 2015a).

Lichens are also known to adapt well to harsh environments (Kranner *et al*. 2008). In the Antarctic, these holobionts have been reported to not only grow on oligotrophic rocks or other substrates (Cao *et al*. 2015a), but could also actively photosynthesize below freezing point even as low as -15°C (Barták *et al*. 2007; Cao *et al*. 2015b). Normally, photobionts exhibit a higher diversity than mycobionts in high latitudes or polar regions, ensuring lichen adaptation to various environments (Printzen *et al*. 2012; Cao *et al*. 2015c).

Among the macrolichen flora found in the Antarctic region, *Usnea* Dill. ex Adans. species of the *Neuropogon* group are the most widespread and abundant. However, identification of this group has been challenging due to their morphological differentiation and innate atypical morphologies. For example, a specimen with apothecia was originally identified as *U. aurantiaco-atra* (Jacq.) based on the morphological characters, but identified to be *U. sphacelata* R. Br. using molecular and chemical marker (Seymour *et al*. 2007). In contrasts, classification of sterile or bearing apothecia *U. subantarctica* F.J. Walker specimens strongly supported a possible Antarctic Peninsula sub-clade (Seymour *et al*. 2007), whose apothecia was once recorded as infrequent (Øvstedal and Smith 2001).

Antarctica is an ideal system to study the composition of the associated microbes in lichens and their influences on their symbiotic partners. For *U. aurantiaco-atra*, which is the most dominant fruticose lichen species in King George Island (http://www.aari.aq/KGI/Vegetation/lst_lichens.html), various growth forms are present in the current study. The morphological variability in *U. aurantiaco-atra* was investigated to illustrate the complexity of lichen symbiosis, and understand other factors that could influence lichen morphology aside from their known symbionts. This could provide further evidence that lichen is more than a dual symbiotic association of fungus and alga but actually a microcosm environment.

Material and methods

Sample collection. — A total of 16 *U. aurantiaco-atra* lichen specimens with different morphologies were collected and investigated from Nelson Island (six specimens from sites a, b, c), Ardley Island (three specimens from site d) and Fildes Peninsula (seven specimens from sites e, f, g). These sampling sites spread across the three islands were influenced by varying environmental conditions. Sampling sites a and c located at the front of glacier in Nelson Island were mainly influenced by the ice sheet, while sampling site d located in Ardley Island was mainly inhabited by penguins. The sites b, f and g were found in a rocky environment near the shore where the winds would be expected to influence lichen activity, and the sampling site e in the middle of Fildes Peninsula could be characterized as a reference site which was not influenced by above factors dramatically. Among these sampling sites, the shortest distance was about 0.7 km between sites a and b, and the farthest was over 7.6 km between sites a and g (Fig. 1).

Fig. 1. Map of sampling sites around King George Island. This map was modified from Google Earth (screenshot was captured on 5/22/2017).

Based on the reproductive structure of lichen, which is usually used in the traditional classification of *Usnea* species, the 16 specimens were classified into four major groups. These include the groups that had common apothecia but rare or absent soredia (Group A), those with rare or absent apothecia but with common soredia (Group S), or those with both apothecia and soredia (Group B), and lastly, the specimens where reproductive structures were rare or not seen (Group N). The detailed information was summarized in Table 1.

Morphological and molecular characterization. — A compound microscope (Zeiss Axioskop 2 plus) and a dissecting microscope (Motic SMZ-168) were used to study the lichen's morphology and anatomy. For the light microscopy, 15–20 μm sections were cut from frozen specimens with a sliding microtome (Leica 151 SM 2000R) and were observed with the Zeiss Axioskop 2 plus microscope equipped with an Olympus SC100-10.6 camera.

Molecular investigation. — Total DNA of lichen specimens was extracted using a modified CTAB method (Cao *et al*. 2015c) and a 50× diluted DNA was used as template for PCR amplication. ITS regions of mycobiont and photobiont were separately amplified using the fungal specific primer pair ITS5 and ITS4 (White *et al*. 1990) and the algal specific primer pair ITS5 (White *et al*. 1990) and nrSSU-1780 (Piercey-Normore and Depriest 2001), respectively. PCR reactions were carried out in 50 μL mixture containing 5 μL amplification buffer with 25 mmol l^{-1} of $MgCl_2$, 1.25 units of Taq DNA polymerase (TaKaRa Biotechnology Co. Ltd.), 4 μL 2.5 mmol l^{-1} of each dNTP, 2 μL 10 μmol l^{-1} of each primer, 2μL of diluted template DNA, add ddH_2O up to 50 μL and run using the PCR conditions described by Cao *et al*. (2015c). The amplicons were verified using electrophoresis in 0.8% agarose gel and purified with Gel Extraction Mini Kit (Omega Bio-tek, Inc.).

DNA sequencing and data analysis. — Sequencing reactions were carried out in an ABI3730XL Sequencer. Both forward and reverse sequences for each amplicon were sequenced, which were assembled in the SEQMAN program (DNASTAR Inc.). The regions of small subunit and large subunit rDNA flanking the ITS region were trimmed off. Sequence alignments of the mycobiont and photobiont were performed in ClustalW within MEGA 5 (Tamura *et al*. 2011) and adjusted manually. Phylogenetic trees were constructed based on the alignments with Minimum Evolution (ME) method in MEGA 5 (Tamura *et al*. 2011) using default parameters. The reproducibility of the inferred trees was tested with bootstrap searches with 1000 resamplings. The ITS region of both *U. aurantiaco-atra* symbionts (mycobiont and photobiont) were sequenced and submitted to GenBank (Table 1).

Table 1

Summary of the information of the lichen specimens investigated in this study.

BIRDS ID[#]	Specimens ID[*]	Date	Location	Altitude	Substrates	GenBank Accession No.	
						Mycobiont	Photobiont
2131C0001ASBM100061	d-N1	02-14-2014	62°12'42.03"S, 58°55'42.07"W	36 m	Moss	KX147238	KX147254
2131C0001ASBM100062	e-A1	02-14-2014	62°12'39.39"S, 58°59'03.10"W	40 m	Stone	KX147239	KX147255
2131C0001ASBM100063	d-B1	02-14-2014	62°12'42.03"S, 58°55'42.07"W	44 m	Stone	KX147240	KX147256
2131C0001ASBM100064	d-N2	02-14-2014	62°12'42.03"S, 58°55'42.07"W	44 m	Stone	KX147241	KX147257
2131C0001ASBM100065	f-A2	03-01-2014	62°12'40.68"S, 59°00'28.25"W	94 m	Stone	KX147242	KX147258
2131C0001ASBM100066	f-N3	03-01-2014	62°12'40.68"S, 59°00'28.25"W	94 m	Stone	KX147243	KX147259
2131C0001ASBM100067	f-S1	03-01-2014	62°12'40.68"S, 59°00'28.25"W	94 m	Stone	KX147244	KX147260
2131C0001ASBM100068	f-S2	03-01-2014	62°12'40.68"S, 59°00'28.25"W	94 m	Stone	KX147245	KX147261
2131C0001ASBM100069	b-A3	03-01-2014	62°15'55.67"S, 58°52'36.29"W	12 m	Stone	KX147246	KX147262
2131C0001ASBM100070	b-N4	03-01-2014	62°15'55.67"S, 58°52'36.29"W	12 m	Stone	KX147247	KX147263
2131C0001ASBM100071	a-A4	03-01-2014	62°16'17.41"S, 58°52'27.34"W	75 m	Stone	KX147248	KX147264
2131C0001ASBM100072	a-N5	03-01-2014	62°16'17.41"S, 58°52'27.34"W	75 m	Moss	KX147249	KX147265
2131C0001ASBM100073	g-B2	03-01-2014	62°10'29.70"S, 58°58'18.97"W	-1 m	Stone	KX147250	KX147266
2131C0001ASBM100074	g-N6	03-01-2014	62°10'29.70"S, 58°58'18.97"W	-1 m	Stone	KX147251	KX147267
2131C0001ASBM100075	c-N7	03-01-2014	62°14'46.80"S, 59°00'34.65"W	96 m	Stone	KX147252	KX147268
2131C0001ASBM100076	c-A5	03-01-2014	62°14'46.80"S, 59°00'34.65"W	96 m	Stone	KX147253	KX147269

[#] The specimens were kept in the Resource-sharing Platform of Polar Samples (including samples of Biology, Ice-snow, Rock, Deep-space and Sediment)

[*] The lower-case letters "a" to "g" indicate sampling site (see Fig. 1); the capital letters "A, S, B, N" represent reproductive characters (A: apothecia common but soredia rare or not seen; S: apothecia rare or not seen but soredia common; B: both apothecia and soredia are common; N: neither apothecia nor soredia is common).

Haplotype networks of the mycobiont and photobiont were calculated by TCS version 2.1 (Clement *et al*. 2000) using the neat ITS sequence data with the parameters as "Calculate 97% connection limit" and "Gaps=missing data".

Results

Morphological characterization. — Four major groups were identified based on the reproductive structures. Five out of the 16 specimens were classified with group A (with apothecia and without soredia: (e-A1, f-A2, b-A3, a-A4, c-A5, Fig. 2a), two specimens in group S (with soredia but without apothecia: f-S1 and f-S2, Fig. 2b), two specimens in group B (with both apothecia and

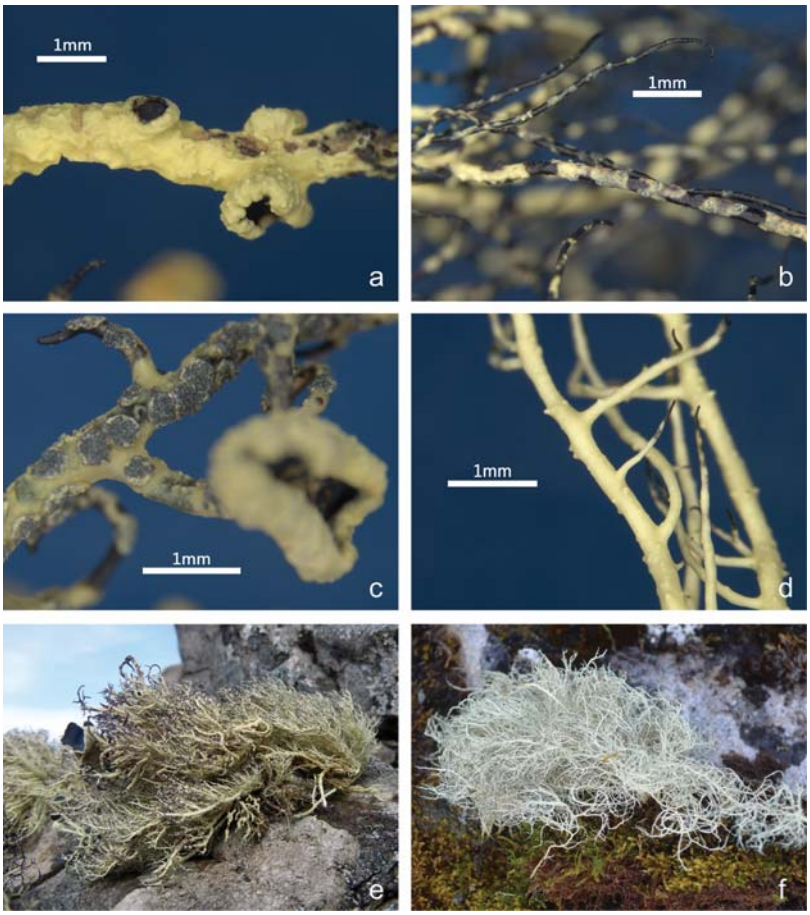

Fig. 2. Reproductive structure and habitats of *Usnea aurantiaco-atra*. a, thallus with apothecia (b-A3); b, thallus with soredia (f-S1); c, thallus with both apothecia and soredia (g-B2); d, thallus without apothecia or soredia (a-N5); e, thallus with apothecia on stone (f-A2); f, thallus without apothecia on moss (d-N1).

soredia: d-B1 and g-B2, Fig. 2c), and seven specimens in group N (without apothecia and soredia: d-N1, d-N2, f-N3, b-N4, a-N5, g-N6 and c-N7, Fig. 2d). Lastly, 14 of the investigated specimens were growing on stones, and only two (d-N1 and a-N5) were found growing with moss (Figs. 2e and 2f).

For the *U. aurantiaco-atra* specimens of group A, S, B and most in group N, their thalli were usually erect, and occasionally prostrates (d-N1 and a-N5). The branches were always ornamented with black rings and three specimens had black rings on the stem (g-N6 and those in group S) (Figs 3a, b), but sometimes these black rings were constricted making branches rhizoma nelumbinis-like (Fig. 3c). For the specimens in groups A, B, and N (except g-N6), the black rings were at the tip of the branches (Fig. 3d). The branch transversal sections

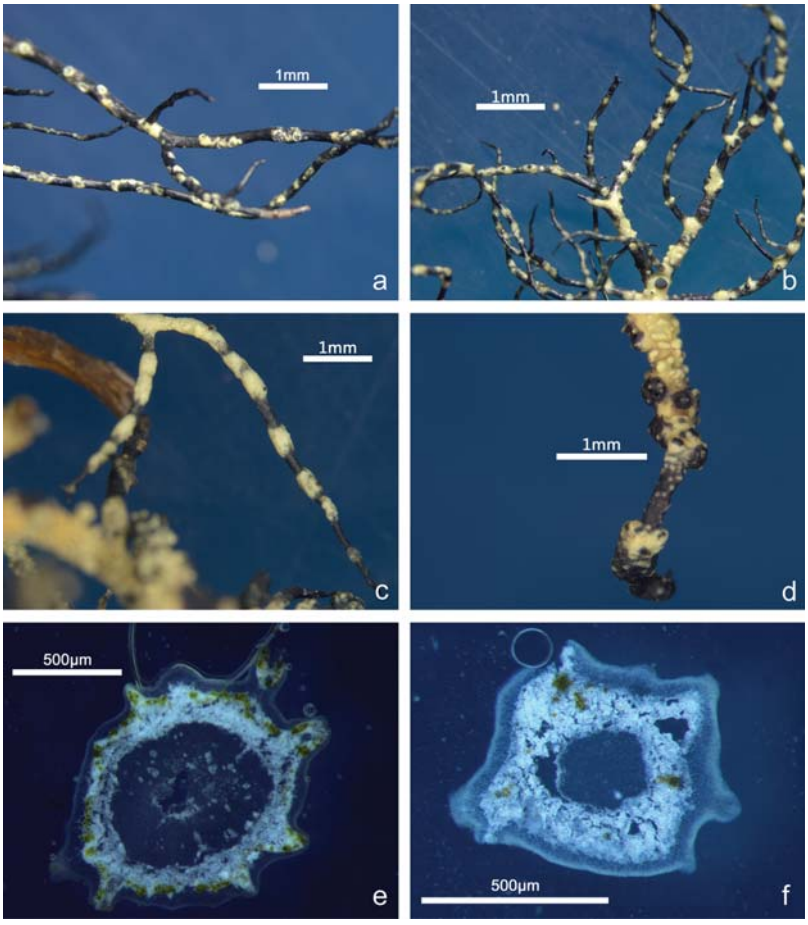

Fig. 3. Position of the black rings and shapes of the branches. a and b, black rings distributed on the branches (f-S1, c-N7); c, black rings constricted on the branches (b-N4); d, black rings located at the tip of the branches (f-N3); e, terete shaped branch (c-N7); f, angular shaped branch (c-A5).

Fig. 4. Branches decorated with warty papillae. a and b, densely crowded papillae (0.1–0.5 mm diam) (e-A1, c-A5); c and d, where papillae were loosely arranged (f-S1, a-N5).

were observed terete (Fig. 3e) or angular (Fig. 3f), and the central cord occupied more than 80% of the transversal sections in *U. aurantiaco-atra*.

All lichen thalli surfaces were papillate with warty, densely (Figs 4a, b) to loosely distributed (Figs 4c, d) papillae, 0.1–0.5 mm diam.

Five of the 16 investigated specimens had black burls on their branches (c-A5, f-S2, g-B2, b-N4, g-N6) (Fig. 5). The black burls looked like endokapylic fungus on the thallus of *U. aurantiaco-atra*, whose apothecia were circular within distinct immersed margin. Asci of this lichenicolous fungus were broadly clavate, with 8 aseptate, and ellipsoid to broadly ellipsoid colorless spores (Figs. 5e and 5f).

ITS Sequence alignment. — All mycobiont ITS sequences phylogenetically grouped as *U. aurantiaco-atra* with high bootstrap support of 0.984 (Fig. 6), while all of the photobionts were identified as the green alga *Trebouxia jamesii* (Hildreth *et* Ahmadjian) Gärtner (Fig. 7). Haplotype networks of the mycobiont and photobiont were calculated (Fig. 8). For the mycobiont, one genotype was shared with all the four groups, except that of d-N1, e-A1 and f-S1, which

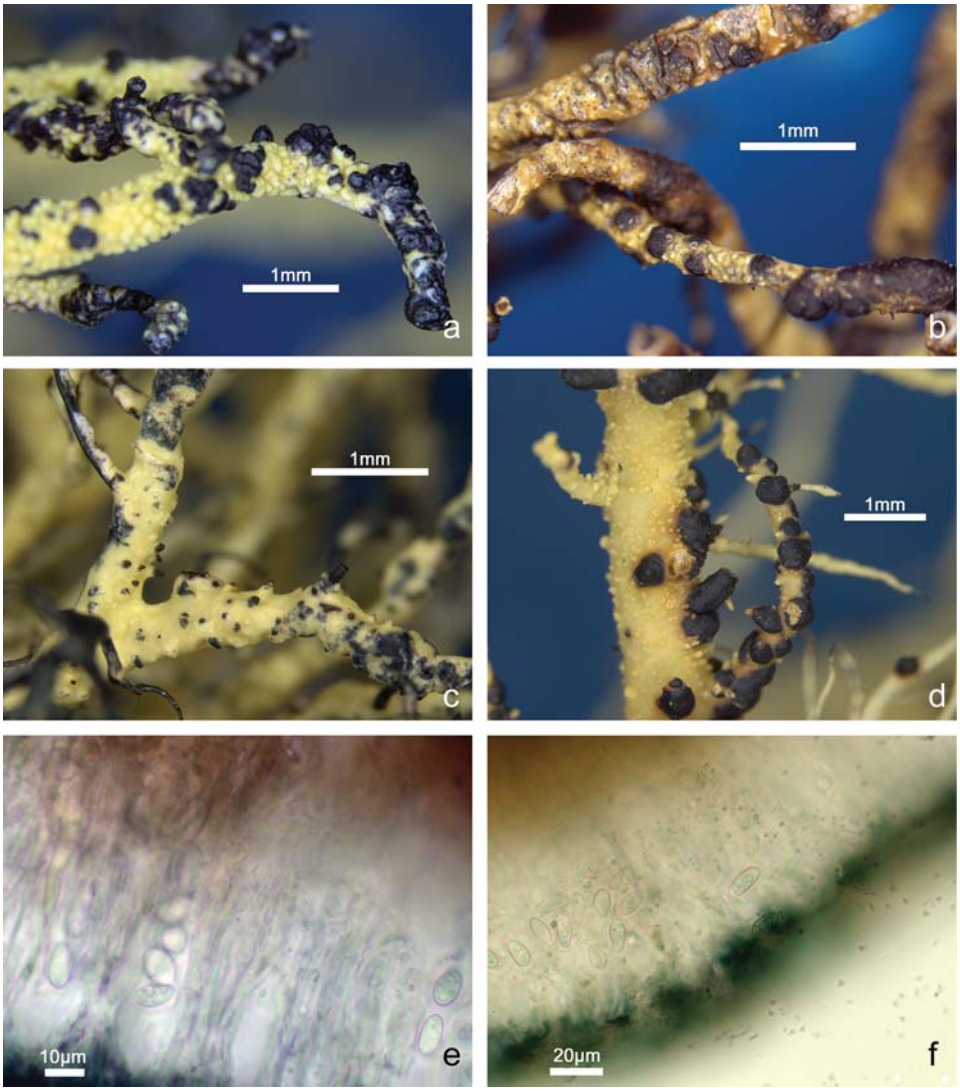

Fig. 5. Black burls-like forms and asci of the epiphytic fungi from *Usnea aurantiaco-atra*. a–d, black burls-like forms of the lichenicolous fungi (a: c-A5; b: f-S2; c: g-B2; d: b-N4); asci (e) and ascospores (f) of the lichenicolous fungus (from g-B2).

were unique compared to the others; for the photobiont, four genotypes were identified, three of which were shared by the different groups.

Phylogenetic analysis showed that the sequence of the black burls from g-B2 clustered closely with *Phacopsis huuskonenii* Räsänen (Fig. 9), which was strongly supported having a bootstrap value 0.99. The taxonomic position of this lichenicolous fungus will be reported in another study.

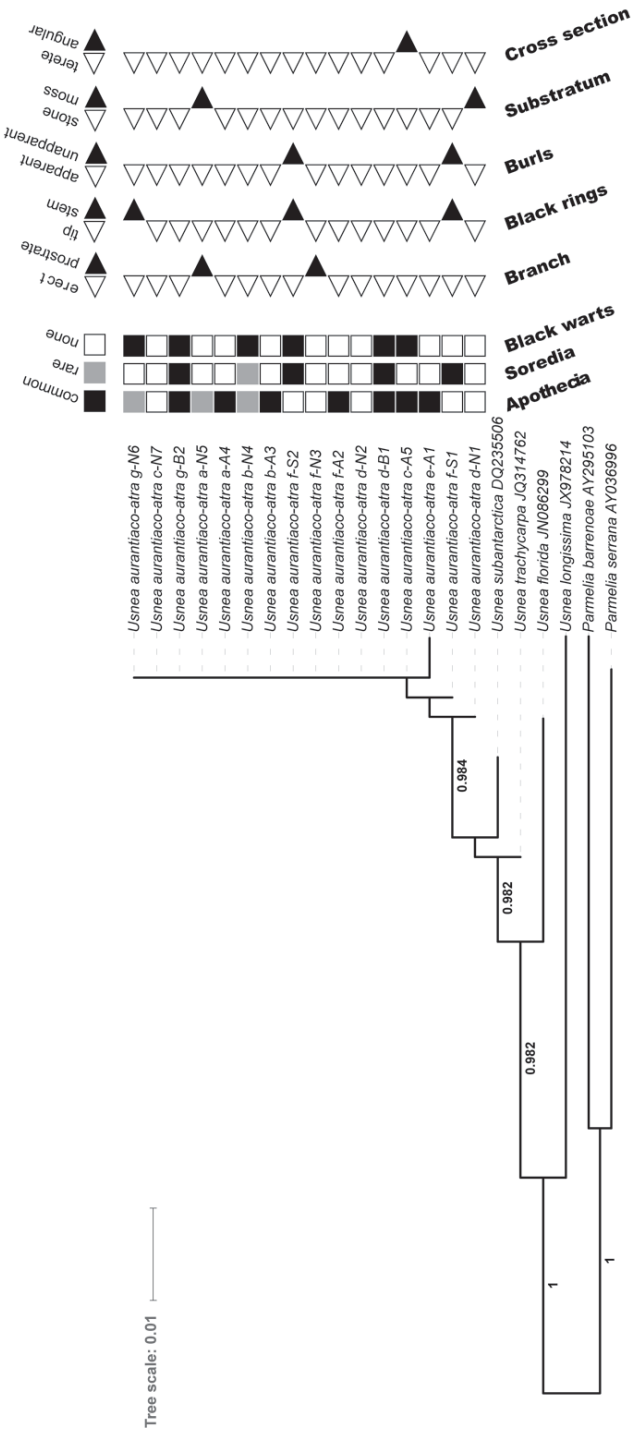

Fig. 6. The minimum evolution (ME) tree of mycobiont based on the ITS sequences. The reliability of the inferred tree was tested by 1000 bootstrap replications. Numbers at the nodes present the bootstrap support (<0.5 not shown).

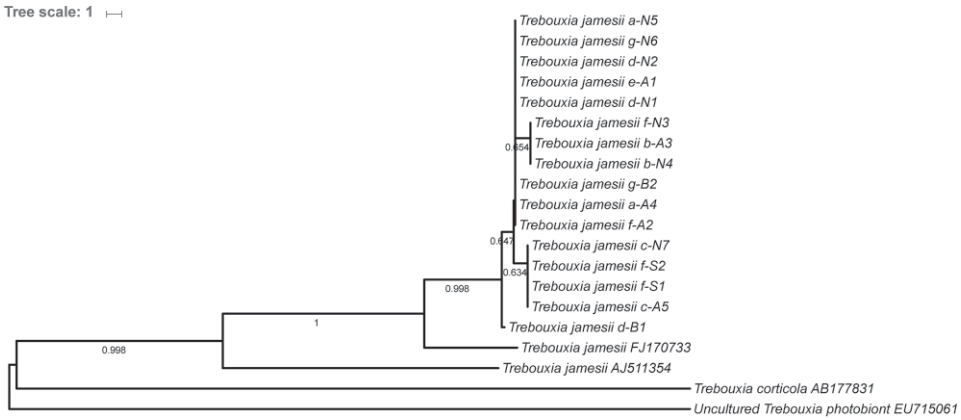

Fig. 7. The minimum evolution (ME) tree of the photobionts based on the ITS sequences. The reliability of the inferred tree was tested by 1000 bootstrap replications, and numbers at the nodes represent the bootstrap supports (<0.5 not shown).

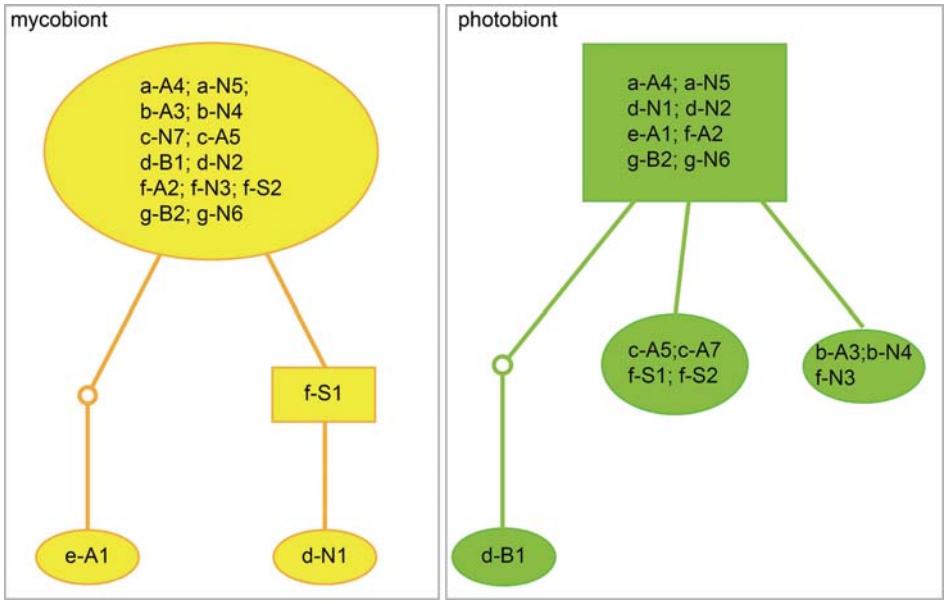

Fig. 8. Haplotype network of the mycobiont (left) and photobiont (right).

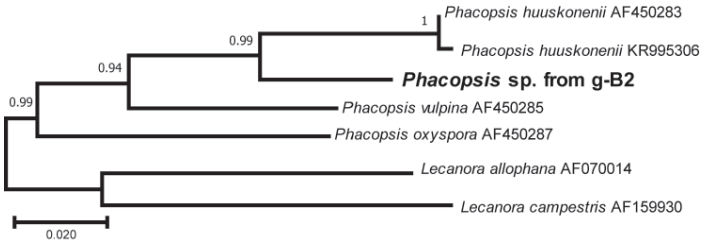

Fig. 9. The minimum evolution (ME) tree of the black burls-like fungi from *Usnea aurantiaco-atra* based on their ITS sequences. The reliability of the inferred tree was tested by 1000 bootstrap replications, and numbers at the nodes represent the bootstrap support (<0.5not shown).

Discussion. — The lichen thallus evolved as early as terrestrial plant life, and the first ancestors of lichens can be traced back to the Devonian era around 400 million years ago (Remy *et al*. 1994; Honegger *et al*. 2013). Lichens exhibit strong tolerance to harsh environments, with distributions ranging from the equator to the polar regions, and could be considered as pioneer organisms. *Usnea aurantiaco-atra* is the most dominant flora around King George Island, Antarctica. Interestingly, within the studied region (about 10 km long, 2–3 km wide), *U. aurantiaco-atra* showed high degree of morphological diversity. Notably, the reproductive structures apothecia and soredia, which were used to distinguish lichen species in traditional taxonomy, were observed in one specimen.

Although four morphological groups of *U. aurantiaco-atra* were identified based on their individual reproductive structures, this was not supported by the results of phylogenetic analysis (Figs. 6 and 7), indicating the absence of genetic marker for such morphological differentiation. For both mycobiont and photobiont, most genotypes were shared among groups (Fig. 8). For example, a-A4, f-A2 and g-B2; a-N5, d-N2, e-A1 and g-N6; c-A5, c-N7 and f-S2; b-A3, b-N4 and f-N3 had the same fungal and algal ITS haplotypes. Although three specimens (d-N1, e-A1 and f-S1) with unique genotypes were observed in the mycobionts, the genotypes of their symbionts were the same for all of the other specimens. Also, one unique mycobiont genotype was recovered from e-A1, but its photobiont genotype was shared with a-A4, a-N5, d-N1, d-N2, f-A2, g-B2, g-N6. On the other hand, a unique photobiont genotype was observed for d-B1, whose mycobiont genotype was shared with some specimens in group A, S, and N, such as e-A1, f-S1, d-N1.

Neither the morphological characters nor a set of these characters, proved to be a completely unambiguous marker for a monophyletic group of *U. aurantiaco-atra*. This strong similarity among strains was observed despite significant differences among the sites where they were collected. The specimens of group N were distributed at the sampling sites a–f and g, and the specimens of group A distributed at sites a, b, c, e and f. This suggests that the environment did not have enough influence on the morphological differentiation of *U. aurantiaco-atra*.

The presence or absence of apothecia/soredia instead could indicate different stages of ontogeny of a lichen species but not an informative character in classifying *Usnea* species. Suetina and Glotov (2010) proposed four periods and 11 ontogenetic states of the lichen *U. florida* (L.) Weber *ex* F.H. Wigg. thallus. They reported that the apothecia appears on branches at the eighth stage (Young generative) of period three (Generative) and absent at 11th stage (Subsenile) of period four (Postgenerative). Therefore, the morphological differences observed in *U. aurantiaco-atra* could represent spatiotemporal variation of the individual development of the lichen. Our early work also revealed that the two main growth forms of *U. aurantiaco-atra*, *i.e.* the erect form with apothecia on rock and the prostrate form without apothecia associated with mosses, appeared to reflect different stages of lichen–moss community succession (Cao *et al.* 2017).

Lichenicolous microbes may take effort on the morphological variety, for example, the black burls were formed by fungi *Phacopsis* sp. The importance of lichenicolous microbes had always been neglected in the past, and up to now, and there is still nearly no study about the influence of lichenicolous fungi, the endophytic fungi nor the bacteria on the morphology of lichen thallus. This could be attributed in part to the limitations of traditional taxonomic approaches in recognizing lichen symbionts, which in turn results in failure to reflect actual species diversity accurately (Leavitt *et al.* 2016). There is a high degree of cryptic diversity in both the myco- and photobionts in rock-posy lichens (Erlacher *et al.* 2015; Fleischhacker *et al.* 2015; Leavitt *et al.* 2016). Many lichens were infected by phenotypically distinct lichenicolous fungi of diverse lineages (Muggia *et al.* 2016). For example, the basidiomycete yeasts were found in the lichen cortex, and their abundance correlated with previously unexplained variations in phenotype (Spribille *et al.* 2016). The complicated morphological variety of *U. aurantiaco-atra* also reflects that the lichen is more than a dual-symbiont. The symbiotic structures of the lichen thalli appeared to be a shared habitat of phylogenetically diverse and stress-tolerant fungi, which potentially benefit from the lichen niche in an otherwise hostile habitat, such as the Antarctic.

Although only one epiphytic fungus was observed morphologically in the present study (Figs. 5 and 9), in reality, there were various eukaryotes that thrived in the lichen thalli. For example, more than 10 fungal and six algal cultures have been isolated from *U. aurantiaco-atra* using a tissues isolation method in this same study (Table 2), and at least three of the algal cultures have not yet been described.

Besides the eukaryotes, a large number of prokaryotes were also detected, which has been gaining more attention recently in the field of lichen studies. Bacterial microbiome could be involved in nutrient provision and degradation of older lichen thallus parts, biosynthesis of vitamins and hormones, detoxification processes, and the protection against biotic as well as abiotic stresses (Grube *et al.* 2015). Furthermore, volatile organic compounds (VOCs) profiles from bacterial isolates showed that lichen-associated bacteria emit a broad range

Table 2

List of fungal and algal cultures isolated from *Usnea aurantiaco-atra*.

Lichenicolous fungi or algae*	Number of isolates (sequenced)	Kinds of isolates
Fungi		
Cryptococcus magnus	2	1
Dothideales sp.	1	1
Epicoccum sp.	1	1
Helotiales sp.	2	1
Rhodotorula glacialis	1	1
Uncultured basidiomycetes	6	4
Uncultured fungi	3	2
Algae		
Coccomyxa spp.	4	2
Uncultured Chlorophyta	10	4

* The names of the isolates were referred to the first species in ITS rDNA BLAST results from NCBI.

of volatile substances. These molecules are most likely involved in various interactions (*e.g.*, communication between microorganisms and the host) and might also increase the overall resistance against various pathogens (Cernava *et al*. 2015). The presence of varying prokaryotes and eukaryotes could also have direct or indirect influences on the morphological characters of the lichen thallus.

In summary, the lichen should not be taken as a simple dual-symbiont system. In our study we showed wide morphological variations in lichens with the same mycobiont and photobiont genotype. Such differences were so strong that the specimens could be classified into different species using the traditional taxonomic method. For example, the specimens with apothecia (*e.g.*, e-A1, f-A2, b-A3, a-A4) were classified as *U. aurantiaco-atra*, but those with soredia (*e.g.*, f-S1 and f-S2) were thought to be *U. antarctica* (Seymour *et al*. 2007). However, the ITS sequences of both mycobiont and photobiont showed that the morphologically different strains were indeed the same. This could indicate that some factors not involving the lichenized fungus and alga could have played important roles in shaping the form of the lichens. This further suggests that the lichen should be regarded as a mini-ecosystem and that various lichenicolous microbes contribute to thallus flexibility.

Because specimens of *U. aurantiaco-atra* having the same ITS sequences for both mycobiont and photobiont exhibited various phenotypes, it is then an ideal species model to study morphological flexibility in lichens. The comprehensive analysis of morphological traits in *U. aurantiaco-atra* implies that phenotypic differentiation was caused by multiple factors, especially, it became clear that some black burls-like structures were formed by lichenicolous fungi such as *Phacopsis* Tul. species; and the

phenotypic differentiation can provide an effective way for studying the diversities of lichenicolous microbes. Our study further indicates that other organisms beyond lichenized fungus and alga may play important roles in shaping thalli morphology.

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Reference

BARTÁK M., VACZI P., HAJEK J. and SMYKLA J. 2007. Low–temperature limitation of primary photosynthetic processes in Antarctic lichens *Umbilicaria antarctica* and *Xanthoria elegans*. *Polar Biology* 31: 47–51.

CAO S.N., ZHENG H.Y., LIU C.P., TIAN H.M., ZHOU Q.M. and ZHANG F. 2015a. The various substrates of *Usnea aurantiaco-atra* and its algal sources in the Fildes Peninsula, Antarctica. *Advance in Polar Science* 26: 274–281.

CAO S.N., ZHANG J., ZHENG H.Y., LIU C.P. and ZHOU Q.M. 2015b. Photosynthetic performance in Antarctic lichens with different growth forms reflect the diversity of lichenized algal adaptation to microhabitats. *Polish Polar Research* 36: 175–188.

CAO S.N., ZHANG F., LIU C.P., HAO Z.H., TIAN Y., ZHU L.X. and ZHOU Q.M. 2015c. Distribution patterns of haplotypes for symbionts from *Umbilicaria esculenta* and *U. muehlenbergii* reflect the importance of reproductive strategy in shaping population genetic structure. *BMC Microbiology* 15: 212.

CAO S.N., PENG F., ZHENG H.Y., FENG W., LIU C.P. and ZHOU Q.M. 2017. Patterns of fungal-algal symbiont association in *Usnea aurantiaco-atra* reveal the succession of lichen-moss communities in Fildes Peninsula, Antarctica. *Polar Research* 36: 1374123.

CERNAVA T., ASCHENBRENNER I.A., GRUBE M., LIEBMINGER S. and BERG G. 2015. A novel assay for the detection of bioactive volatiles evaluated by screening of lichen-associated bacteria. *Frontiers in Microbiology* 6: 398.

CLEMENT M., POSADA D. and CRANDALL K.A. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657–1659.

ERLACHER A., CERNAVA T., CARDINALE M., SOH J., SENSEN C.W., GRUBE M. and BERG G. 2015. Rhizobiales as functional and endosymbiontic members in the lichen symbiosis of *Lobaria pulmonaria* L. *Frontiers in Microbiology* 6: 53.

FLEISCHHACKER A., GRUBE M., KOPUN T., HAFELLNER J. and MUGGIA L. 2015. Community analyses uncover high diversity of lichenicolous fungi in Alpine habitats. *Microbiology Ecology* 70: 348–360.

GREEN T.G.A. and LANGE O.L. 1995. Photosynthesis in poikilohydric plants: a comparison of lichens and bryophytes. *In*: E.D. Schulze and M.M. Caldwell (eds) *Ecophysiology of Photosynthesis*. Springer Study Edition, Springer, Berlin, Heidelberg. 100: 319–341.

GREEN T.G.A., SEPPELT R.D., BRABYN L.R., BEARD C., TURK R. and OTTO L.L. 2015. Flora and vegetation of Cape Hallett and vicinity, northern Victoria Land, Antarctica. *Polar Biology* 38: 1825–1845.

GRUBE M., CERNAVA T., SOH J., FUCHS S., ASCHENBRENNER I., LASSEK C., WEGNER U., BECHER D., RIEDEL K., SENSEN C.W. and BERG G. 2015. Exploring functional contexts of symbiotic sustain within lichen-associated bacteria by comparative omics. *Multidisciplinary Journal of Microbial Ecology* 9: 412–424.

HONEGGER R., EDWARDS D. and AXE L. 2013. The earliest records of internally stratified cyanobacterial and algal lichens from the Lower Devonian of the Welsh Border land. *New Phytologist* 197: 264–275.

KRANNER I., BECKETT R., HOCHMAN A. and NASH III T.H. 2008. Desiccation-tolerance in lichens: a review. *The Bryologist* 114: 576–593.

LEAVITT S.D., KRAICHAK E., VONDRAK J., NELSEN M.P., SOHRABI M., PEREZ-ORTEGA S., CLAIR ST., THORSTEN L.L. and LUMBSCH H. 2016. Cryptic diversity and symbiont interactions in rock-posy lichens. *Molecular Phylogenetics and Evolution* 9: 261–274.

MUGGIA L., FLEISCHHACKER A., KOPUN T. and GRUBE M. 2016. Extremotolerant fungi from alpine rock lichens and their phylogenetic relationships. *Fungal Diversity* 76: 119–142.

NASH T.H. III (2008) *Lichen biology*. 2nd Edition. Cambridge University Press, Cambridge: 486 pp.

OSYCZKA P. and WĘGRZYN M. 2008. Lichens on lignum in the coastal regions of western Spitsbergen (Svalbard). *Biologia* 63: 1069–1072.

ØVSTEDAL D.O. and SMITH R.I.L. 2001. *Lichens of Antarctica and South Georgia*. Cambridge University Press, Cambridge: 411 pp.

PIERCEY-NORMORE M.D. and DEPRIEST P.T. 2001. Algal switching among lichen symbioses. *American Journal of Botany* 88: 1490–1498.

PRINTZEN C., FERNÁNDEZ-MENDOZA F., MUGGIA L., BERG G. and GRUBE M. 2012. Alphaproteobacterial communities in geographically distant populations of the lichen *Cetraria aculeata*. *FEMS Microbiology Ecology* 82: 316–325.

REMY W., TAYLOR T.N., HASS H. and KERP H. 1994. Four hundred million year old vesicular arbuscular mycorrhizae. *Proceedings of the National Academy of Sciences, USA* 91: 11841–11843.

SEYMOUR F.A., CRITTENDEN P.D., WIRTZ N., ØVSTEDAL D.O., DYER P.S. and LUMBSCH H.T. 2007. Phylogenetic and morphological analysis of Antarctic lichen forming *Usnea* species in the group *Neuropogon*. *Antarctic Science* 19: 71–82.

SPRIBILLE T., TUOVINEN V., RESL P., VANDERPOOL D., WOLINSKI H., AIME M.C., SCHNEIDER K., STABENTHEINER E., TOOME-HELLER M., THOR G., MAYRHOFER H., JOHANNESSON H. and MCCUTCHEON J.P. 2016. Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science* 353: 488–492.

SUETINA Y.G. and GLOTOV N.V. 2010. Ontogeny and Morphogenesis of the Fruticose Lichen *Usnea florida* (L.) Weber *ex* F.H. Wigg. *Russian Journal of Developmental Biology* 411: 24–31.

TAMURA K., PETERSON D., PETERSON N., STECHER G., NEI M. and KUMAR S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731–2739.

WHITE T.J., BRUNS T., LEE S. and TAYLOR J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis M. and Gelfand D. (ed.) *PCR – Protocols and Applications – A Laboratory Manual*. Academic Press 38: 315–322.

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