

Full Length Research Paper

Ultramicro-morphological observation of *Usnea longissima* Ach.

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The *Usnea longissima* Ach. grew as an epiphyte on *Abies georgei* and was collected at an altitude of 3640 m above sea level from the Pudacuo National Park in the Shangri-La County of the Diqing Tibetan Autonomous Prefecture in the Yunnan Province of China. Scanning electron microscopy revealed the ultramicro-organisation of the *U. longissima*. We observed that the interior of the specimen contained a large amount of cubic and needle-like secretions; images of the cross-section of the central axis show many densely distributed holes, and the longitudinal section of the central axis revealed many vessel-like structures, similar to vessels in plants; some hyphae specialised into ring-shaped. These structures have not been reported even in other *Usnea*. This study gives us more comprehensive understanding of the structure and function of lichens.

Key words: *Usnea longissima* Ach., ultramicro-morphological observation, scanning electron microscopy.

INTRODUCTION

Lichens are composite organisms formed by the symbiotic association of fungi and algae. This unique mutualistic symbiotic relationship endows lichens with novel biological characteristics that are different from those of regular fungi and algae (Blasco et al., 2011). Lichens contain large amounts of usnic acid, licheterinic acid, and other substances that possess potent antiseptic and antibacterial activities (Asahina, 1967; Cocchietto et al., 2002). Therefore, they are a biological resource with great potential for development.

Usnea longissima refers to species in the genus *Usnea*, the family *Parmeliaceae*, the order *Lecanorales*, and the class *Lecanoromycetes* (Tavares, 1997; Halonen et al., 1998). It is edible and is utilized in the preparation of traditional foods and medicines in both Eastern and Western countries. It is pale green or silvery-yellowish-green in color, fruticose, and pendulous. Its main branches are cylindrical and can reach up to 3 m or more in length. The main branches rarely divide but they have

numerous short perpendicular side branches and fibrils of approximately equal length (3 to 40 mm). Papillae are lacking but soredia or isidia occasionally form on the side branches. Apothecia are extremely rare. When present, they are disc-shaped, 1 to 3 (5) mm across, and terminate on the ends of the side branches, with numerous fibrils extending from the thalline margin (Halonen et al., 1998; Keon, 2002).

In recent years, molecular biology techniques have been used to study the species diversity and thallus composition of *Usnea* (Ohmura, 2002; DePriest, 2004; Fabian et al., 2007; Arnold et al., 2009; Jana et al., 2010; Ana and Lumbsch., 2010); but morphological observations of the thallus have been limited to traditional optical microstructural observations of paraffin-embedded sections (Su et al., 2007), and observations of hand-sectioned or partially dissected dried herbs and fixed samples (Fabian et al., 2007; Suetina and Glotov., 2010; Wirtz et al., 2008). Little information is currently available on the morphological characteristics of the *U. longissima* at the ultra-microscopic level. In this study, we utilised scanning electron microscopy to characterise the ultra-microscopic morphological structure of the *U. longissima*, to discover some unknown or uncertain structure.

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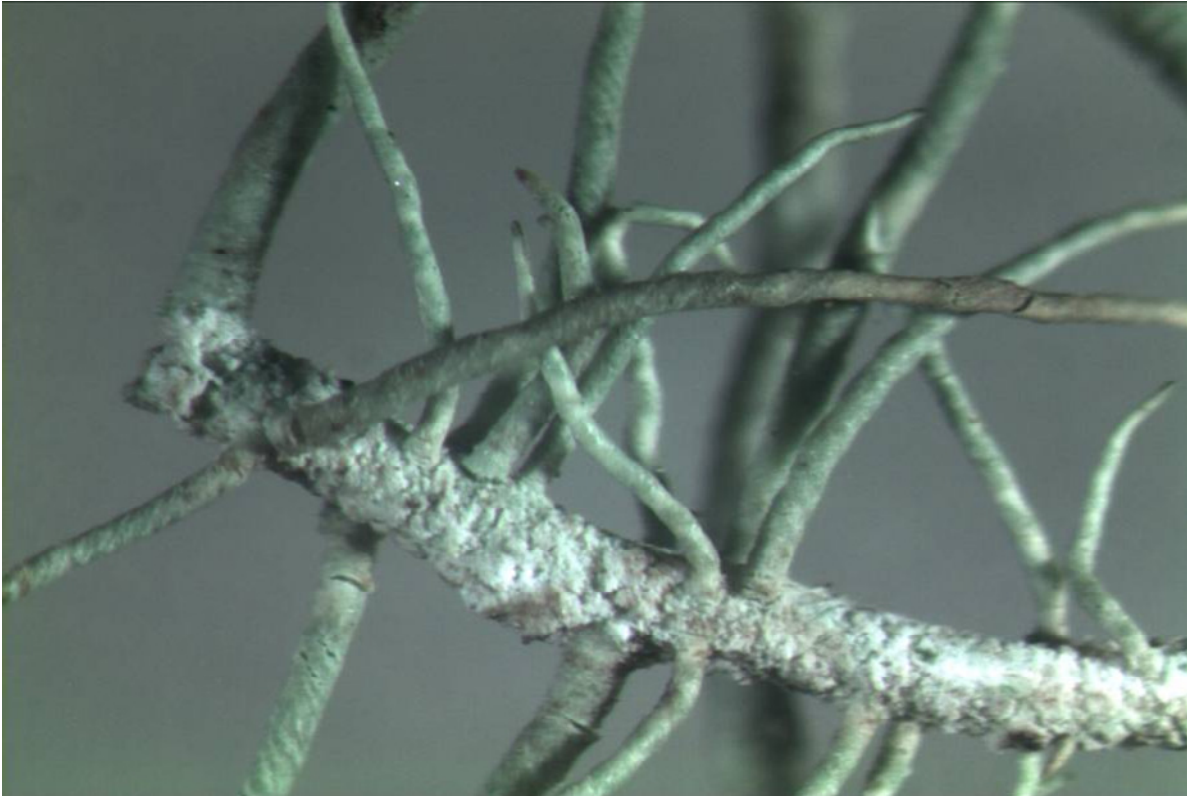


Figure 1. The morphology of the *Usnea longissima* thallus and the features of the main stems and side branches.

MATERIALS AND METHODS

Specimen collection

Fresh thalli of *U. longissima* were collected from Pudacuo National Park, Shangri-la County, Yunnan Province, China (27°49'N, 99°59'E). The mean annual temperature is 5.4°C, and the mean annual precipitation is approximately 580 mm. The lichens were located at an altitude of ca. 3640 m above sea level. The collected samples from *Abies georgei* were identified by their morphological characteristics.

Morphological and anatomical methods

Whole specimen and specimen sections were coated with platinum using a JFC-1600 auto fine coater at a current of 10 mA for 2 min. The platinum-coated samples were stored in a dryer before use. A JSM-7500F scanning electron microscope was used to observe the external morphology and the internal structure of the lichen.

RESULTS AND DISCUSSION

Dissection and observation under a stereoscopic biological microscope

The *U. longissima* is filamentous and cylindrical, with a length of approximately 50 to 130 cm and a diameter of approximately 0.6 to 1.5 mm. The main stems are long,

with branches, some parts of which have dense growths of short side branches. The surface is gray-green or yellow-green, slightly smooth with white powder in certain areas, and covered with fine white rings (Figure 1). The specimen is supple, slightly flexible, and can be easily broken by pulling. The exposed section is greenish-white, with a faint smell and weak taste. The original *U. longissima* thallus from which the specimen was obtained grows as an epiphyte hanging on the surface of the branches of *A. georgei*. The base of its attachment with the tree branch is enlarged, with a width of approximately 5 mm.

Microscopic images of the cross-sections (Figure 2) demonstrated that the *U. longissima* thallus is composed of three concentric rings of tissue, each with a radial structure. The outermost cortex layer, which has a thickness of 20 to 30 μm , is a tissue layer composed of 4 to 5 columns of hyphae woven together and has annular-pseudocyphellae which are characteristic of the subgenus *Dolichousnea* (Ohmura, 2001). The gonidial layer is immediately inside the cortex and is composed of 2 to 3 staggered layers of Tsengia-like algal cells. The algal cells are either oval or semi-round in shape, with a diameter of 8 to 13 μm . The chloroplasts are the source of the green colour. In the middle of the thallus is the centre axis, which has a diameter of approximately 200 to 250 μm . The centre axis accounts for about one-half to

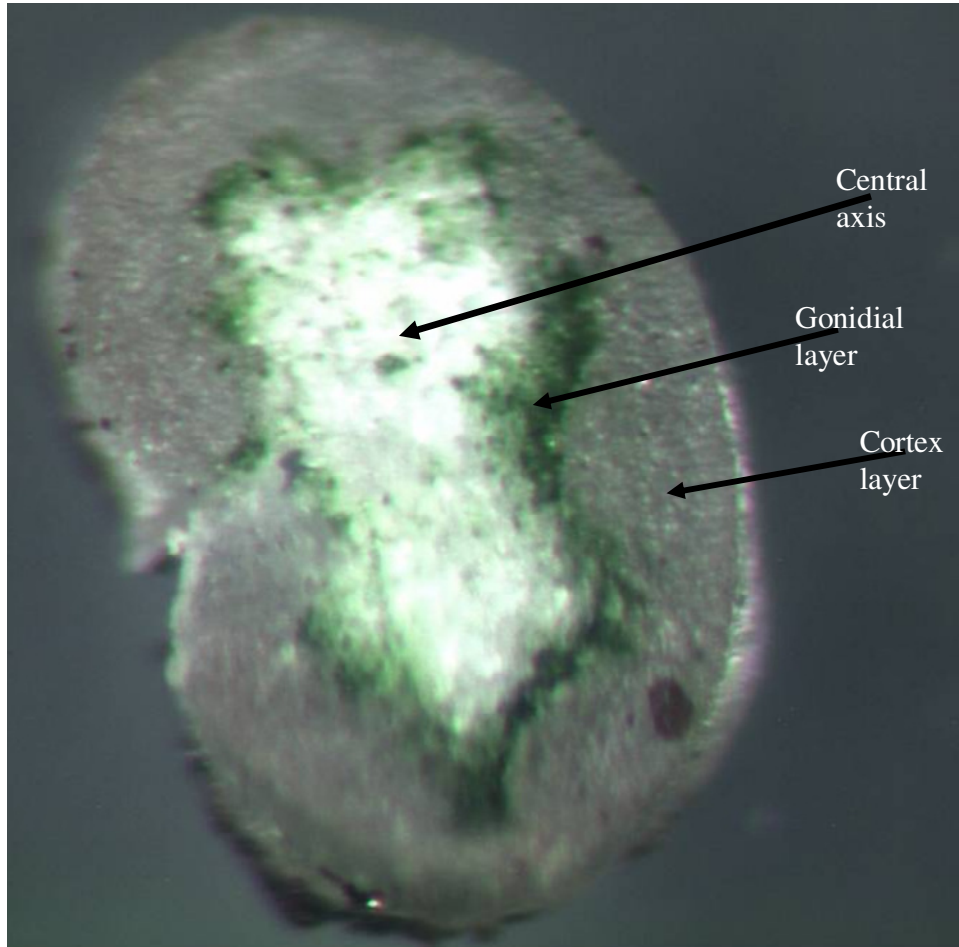


Figure 2. Tissue cross-sections of the *Usnea longissima* thallus, 50× magnification

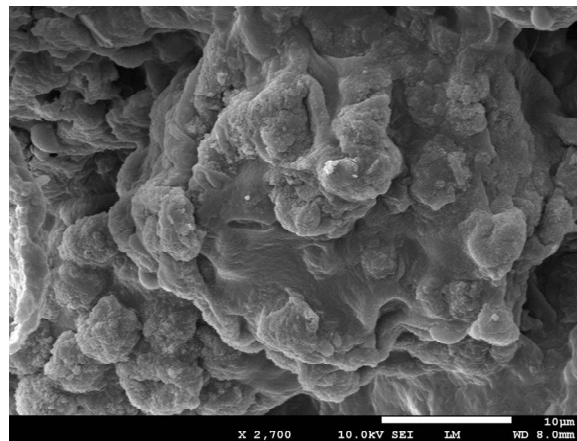
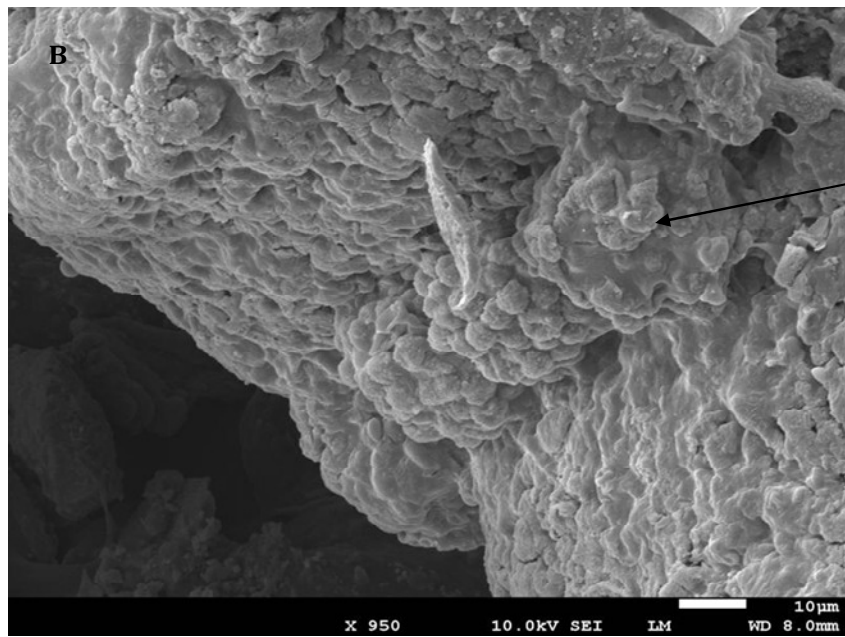
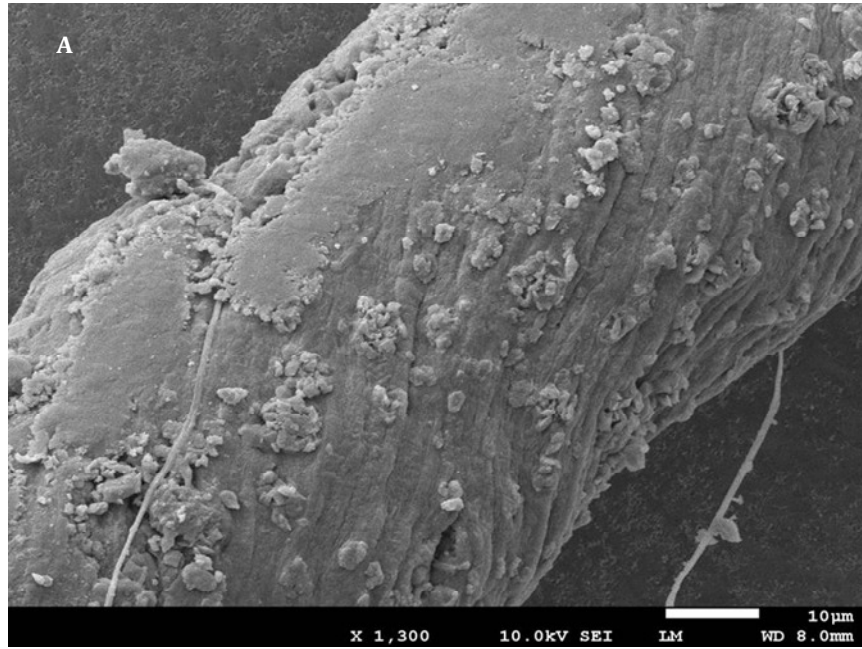
two-thirds of the entire cross-section and is composed of tightly packed specialised hyphae.

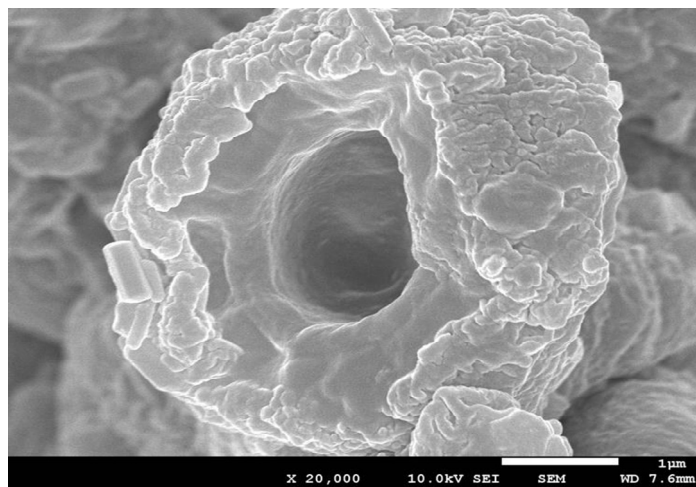
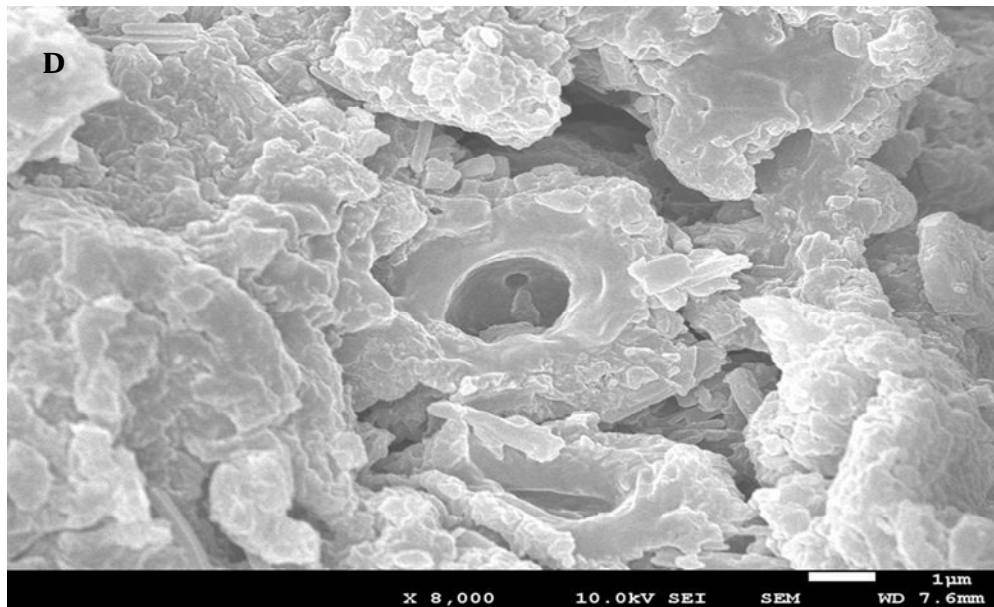
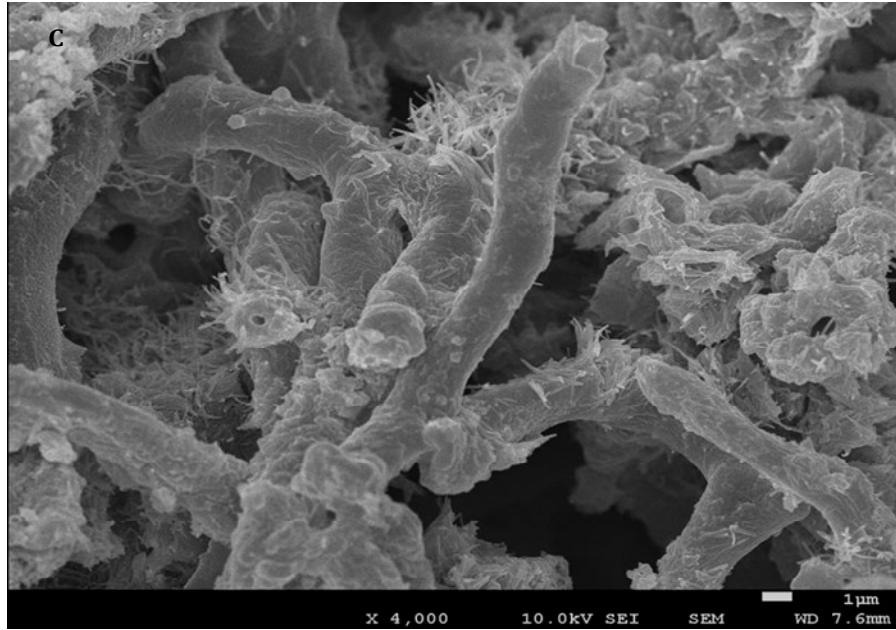
Scanning electron microscopy

Scanning electron microscopy was used to observe the surface and anatomical structures of the specimen (Figure 3). On the surface of the thallus, the dense cortex is formed by left-handed vertical helical arrangements of hyphae (Figure 3a). Regular square- or diamond-shaped crystals and spherical or irregular granular secretions are also located on the surface (Figure 3a). These structures are also found on the exposed surfaces of the cortex. These secretions might be deposits of secondary metabolites, including multiple lichen acids such as usnic acid and lichen polysaccharide, which are produced by the biological processes of the lichen. Ring fractures in the cortex are present on the surface of the thallus or in the branch bases. Vertical cracks are also present. We can observe pink buds growing in the branch bases, spherical spores growing on top of the buds, and scars on the surface of the buds resulting from the loss of the

spores (Figure 3b).

The hyphal surface is covered with needle-like or granular secretions of varying thickness (Figure 3c), scattered with small amounts of algae in groups or as single algal cells. The connections among the hyphae are loose, with many large gaps, and some specialised hyphae are ring-shaped (Figure 3d) which may form more space and be helpful for gas exchange. The central axis is composed of tightly packed specialised mycelium, oriented longitudinally, appearing like an elastic cord or cylindrical rubber band in the center of the lichen thallus, which is surrounded by gonidial layer and cortex, coincides with other *Usnea* species (Tavares, 1997; Clerc, 1998; Halonen et al., 1998; 2000). However, a cross-section of the central axis shows many densely distributed holes (Figure 3e), and images of the longitudinal section of the central axis reveal many vessel-like structures (Figure 3f), similar to vessels in plants. These ultramicro-morphological structures have not been reported by others (Ohmura, 2001, 2002, Ohmura and Kanda, 2004; Fabian et al., 2007). We assume that the structure probably has two roles; one is that it can increase the space, so as to facilitate the





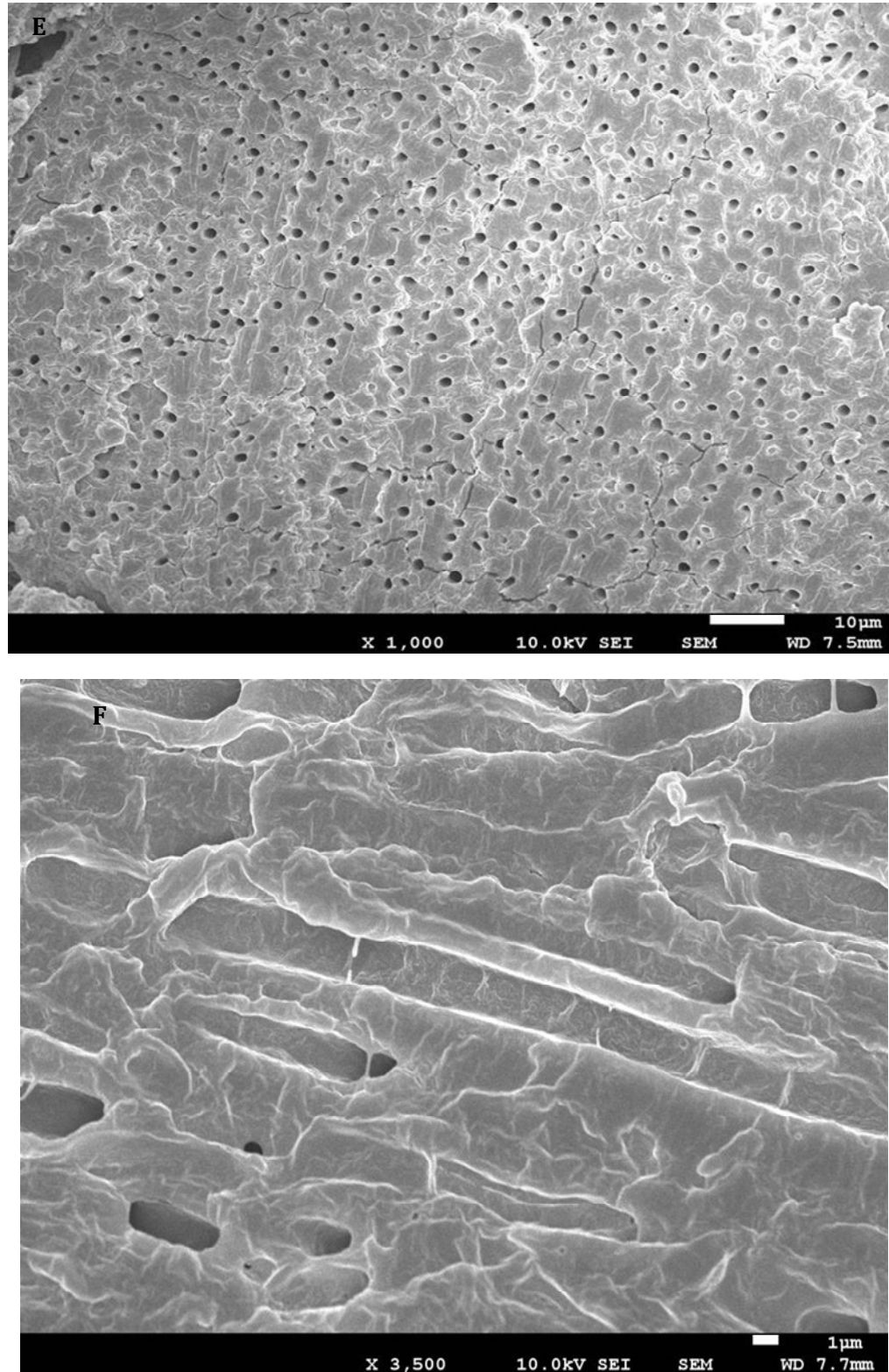


Figure 3. The surface and anatomical structures of *Usnea longissima* specimen observed by scanning electron microscopy; a) left-handed vertical helical arrangement of mycelium and granular secretions located on the surface, 1,300 \times magnification; b) pink buds formed in the base of a side branch with spherical spores on top, 950 \times magnification, and 2,700 \times magnification; c) the hyphal surface is covered with needle-like or granular secretions, 4,000 \times magnification; d) ring-shaped structure composed of specialised mycelia, 8,000 \times magnification, and 20,000 \times magnification; e) cross-section of the central axis; f) longitudinal section of the central axis.

exchange of gases, while the other is support, the *U. longissima* is longer, hollow, can improve toughness, ensure uniform stress, and is not easily broken.

Conclusions

The *U. longissima* Ach. grew as an epiphyte on *A. georgei* and was collected at an altitude of 3640 m above sea level from the Pudacuo National Park in the Shangri-La County of the Diqing Tibetan Autonomous Prefecture in the Yunnan Province of China. After treatment with 5% potassium hydroxide and 1% iodine solution, the *Usnea* specimen showed characteristics of *U. longissima* Ach. and the typical morphological features of a *Usnea* lichen symbiont. Scanning electron microscopy demonstrated that the tissue organisation of the thallus includes three basic parts: cortex layer, gonidial layer, and central axis. We observed that the central axis is a specialised structure of hyphae that resembles plant vessels. The surface is covered with an off-white powder, whereas the interior of the specimen contains a large amount of cubic and needle-like secretions. Some hyphae also specialised into ring-shaped.

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REFERENCES

- Ana C, Lumbsch HT (2010). Cryptic species in lichen-forming fungi. *IMA Fungus*, 1(2): 167-170.
- Arnold AE, Jolanta M, Higgins KL, Sarvate SD (2009). A phylogenetic estimation of trophic transition networks for ascomycetous fungi: are lichens cradles of symbiotrophic fungal diversification? *Syst. Biol.* 58(3): 283-297.
- Asahina Y (1967). Lichenologische Notizen. *J. Jpn. Bot.* 42: 289-294.
- Blasco M, Domeño C, López P, Nerín C (2011). Behaviour of different lichen species as biomonitors of air pollution by PAHs in natural ecosystems. *J. Environ. Monit.* 13(9): 2588-2596.
- Clerc P (1998). Species concepts in the genus *Usnea* (lichenized Ascomycetes). *Lichenologist*. 30: 321-340.
- Cocchietto M, Skert N, Nimis PL, Sava G (2002). A review on usnic acid, an interesting natural compound. *Naturwissenschaften*. 89(4): 137-46.
- DePriest PT (2004). Early molecular investigations of lichen-forming symbionts: 1986-2001. *Annu. Rev. Microbiol.* 58: 273-301.
- Fabian AS, Peter DC, Nora W, Paul SD (2007). Phylogenetic and morphological analysis of Antarctic lichen-forming *Usnea* species in the group *Neuropogon*. *Antarctic Sci.* 19(1): 71-82.
- Jana MU, François L, Jolanta M, Arnold AE (2010). Community analysis reveals close affinities between endophytic and endolichenic fungi in mosses and lichens. *Microb. Ecol.* 60: 340-353.
- Halonen P, Clerc P, Goward T, Brodo IM, Wulff K (1998). Synopsis of the genus *Usnea* (Lichenized Ascomycetes) in British Columbia, Canada. *Bryologist*. 101: 36-60.
- Halonen P (2000). *Usnea pacificana* sp. nov. and *U. wasmuthii* (Lichenized Ascomycetes) in Pacific North America. *Bryologist*. 103: 38-43.
- Keon DB (2002). Fertile *Usnea longissima* in the Oregon Coast Range. *Lichenologist*. 34: 13-17.
- Ohmura Y (2001). Taxonomic study of the genus *Usnea* (lichenized Ascomycetes) in Japan and Taiwan. *J. Hattori Botanical Lab.* 90: 1-96.
- Ohmura Y (2002). Phylogenetic evaluation of infrageneric groups of the genus *Usnea* based on ITS regions in rDNA. *J. Hattori Botanical Lab.* 92: 231-243.
- Ohmura Y, Kanda H (2004). Taxonomic status of section *Neuropogon* in the genus *Usnea* elucidated by morphological comparisons and ITS rDNA sequences. *The Lichenologist*. 36(3&4): 217-225.
- Suetina IG, Glotov NV (2010). Ontogeny and morphogenesis of the fruticose lichen *Usnea florida* (L.) Weber ex F.H. Wigg. *Russian J. Dev. Biol.* 41(1): 32-40.
- Su YQ, Peng F, Li B (2007). Improvement of paraffin section methods and morphological studies on *Usnea betulina*. *Acta Botanica Boreali-Occidentalia Sinica*. 27(5): 859-863.
- Tavares II (1997). A preliminary key to *Usnea* in California. *Bull. California Lichen Soc.* 4: 19-23.
- Wirtz N, Printzen C, Lumbsch HT (2008). The delimitation of Antarctic and bipolar species of neuropogonoid *Usnea* (Ascomycota, Lecanorales): a cohesion approach of species recognition for the *Usnea perpusilla* complex. *Mycol. Res.* 112(4): 472-484.