Protothelenella sphinctrinoides (Protothelenellaceae) new to Japan and new chemical features for several species in the genus

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Abstract: Ohmura, Y. & Mayrhofer, H. 2016. *Protothelenella sphinctrinoides* (Protothelenellaceae) new to Japan and new chemical features for several species in the genus. – Herzogia **29**: 137–142.

Protothelenella sphinctrinoides is newly reported for Japan (Hokkaido and central Honshu). It is a bryophilous lichen in arctic-alpine to boreal-montane and circumpolar regions of the Northern Hemisphere. Japanese collections were found on the bryophytes *Andreaea rupestris* var. *fauriei*, *Cephalozia otaruensis*, *Dicranum viride* var. *hakkodense* and *Jungermannia* sp. growing on soil or rock in alpine areas. Although no chemical substance has been reported for this species previously, an unidentified substance that can react C+ red was detected in all specimens collected in Europe and Japan. Other species of *Protothelenella* which also give a C+ red reaction are discussed.

Zusammenfassung: Ohmura, Y. & Mayrhofer, H. 2016. *Protothelenella sphinctrinoides* (Protothelenellaceae) neu für Japan und neue chemische Merkmale für einige Arten der Gattung. – Herzogia **29**: 137–142.

Protothelenella sphinctrinoides wird neu für Japan von Hokkaido und Zentral-Honshu gemeldet. Die bryophile Flechte ist in boreal montanen bis arktisch alpinen Regionen der Nordhalbkugel zirkumpolar verbreitet. Die japanischen Aufsammlungen kommen auf den Bryophyten *Andreaea rupestris* var. *fauriei*, *Cephalozia otaruensis*, *Dicranum viride* var. *hakkodense* and *Jungermannia* sp. auf Erde oder Felsen in alpinen Lagen vor. Obwohl bisher keine Flechtenstoffe für diese Art angegeben gewesen sind, wurde eine nicht identifizierte Substanz in allen überprüften Belegen aus Europa und Japan nachgewiesen, die mit C eine Rotreaktion hervorrufen kann. Die C+ rot reagierenden Arten der Gattung werden diskutiert.

Key words: Lichenised Ascomycota, biodiversity, alpine belt, Nothern Hemisphere, secondary chemistry.

Introduction

A large number of crustose lichens favor decaying bryophytes as a substrate and such species are generally termed bryophilous lichens (Honegger 2008). Because many bryophilous lichens are extremely small and inconspicuous, they are often overlooked in the field.

As part of our biodiversity studies on Japanese lichens, the interesting bryophilous lichen, *Protothelenella sphinctrinoides* (Nyl.) H.Mayrhofer & Poelt, was collected from alpine areas of Hokkaido and central Honshu. The genus *Protothelenella* Räsänen is characterized by the non-lichenized or lichenized crustose thallus with globose to pear-shaped, dark brown to blackish perithecia, bitunicate asci with an amyloid tholus, and by the colourless, multiseptate or muriform ascospores (Mayrhofer & Poelt 1985, Mayrhofer 1987, 2002, Schmitt et al. 2005, Orange et al. 2009). Species of *Protothelenella* occur on a variety of acidic substrata including rocks, soil, bryophytes, plant detritus or rotten wood, or they can be lichenicolous

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on species of *Solorina*, *Peltigera*, *Pseudocyphellaria* or *Cladonia* (Mayrhofer 2002, Etayo & Sancho 2008). In some species of the genus, an unidentified substance that can give a red color reaction with sodium hypochlorite solution (C+ red) has been observed in some species (Orange et al. 2009). However, because some species have a very thin thallus covering bryophytes or are lichenicolous, the detection of chemical characters can be difficult.

The purpose of this study was to examine the features of *P. sphinctrinoides* based on Japanese and European collections, particularly focusing on the chemistry and comparisons between the C+ red species of the genus.

Materials and methods

Field investigations in Japan were carried out by the first author in 1995 and 2012. The voucher specimens and additional samples of *Protothelenella* housed in TNS and GZU were also examined.

Morphological observations were made using a dissecting microscope (Olympus SZX16) and a differential interference contrast microscope (Olympus BX51). Anatomical examinations were undertaken using hand-cut sections mounted in GAW (glycerine : ethanol : water, 1:1:1). Ascospore size is presented as (minimum value–)$\bar{x} \pm$ SD(–maximum value) ($\bar{x}$ = mean; SD = standard deviation; *n* = number of measurements).

Secondary substances present in *Protothelenella* were examined using thin-layer chromatography (TLC) with solvent B' (hexane : methyl *tert*.-butyl ether : formic acid, 140 : 72 : 18) and solvent C (toluene : acetic acid, 170 : 30) (Culberson & Kristinsson 1970, Culberson & Johnson 1982).

The ascus apex was examined using 0.5 % aqueous iodine solution (I), with pretreatment with 10 % aqueous potassium hydroxide (KI). The color reactions of the thallus were observed in response to 5 % aqueous potassium hydroxide (K), sodium hypochlorite as applied in 1/2 diluted common household bleach (C), and *para*-phenylendiamine (PD) according to Orange et al. (2001).

Results and discussion

New record for Japan

Protothelenella sphinctrinoides (Nyl.) H.Mayrhofer & Poelt, Herzogia **7**: 53 (1985).

Holotype: Finland, Lapponia inarensis, Inari, ad Padasjoki ("Enari, ad Patsjoki"), 1856, E. Nylander (H-NYL 1619!; isotype: H!).

Basionym: *Verrucaria sphinctrinoides* Nyl., Not. Sällsk. Fauna et Fl. Fenn. Förh., Ny Ser. **1**: 6 (1858).

See synonyms in Mayrhofer & Poelt (1985).

Specimens examined: JAPAN, Hokkaido, Mt. Me-akan, 1025 m, 7.9.1995, Y. Ohmura 1712 & H. Kashiwadani (TNS). Honshu, Pref. Nagano, Mt. Senjogatake, 2750 m, 4.9.2012, Y. Ohmura 9300 & A. Frisch (TNS). – AUSTRIA, Styria, Schladminger Tauern, Kleinsölktal: Innere Neualm, Aufstieg zum Hüttkar (Predigtstuhl), ca. 1900 m, 9.8.1985, H. Mayrhofer 5054 & T. H. Nash (GZU); Fleischkögel über der Lafenbergalm, ca. 1900 m, 3.8.1984, H. Mayrhofer 4304 (GZU); NW-exponierte Abhänge der Großen Kesselspitze über der Sacherseealm, ca. 2200 m, 8.7.1985, M. & H. Mayrhofer 4942 (GZU); Großsölktal S Gröbming, Sölkpaß über St. Nikolai, ca. 1700 m, 22.7.1984, H. Mayrhofer 4257 & J. W. Sheard (GZU); Sattental S Gröbming, Kochofen, 1900–1915 m, 8.7.1984, H. Mayrhofer 4116 & C. Scheuer (GZU); Seerieszinken SE von Schladming, NW-Hänge ober der Lärnachscharte, ca. 2150 m, 13.8.2001, R.

Türk 32511 (GZU). Seetaler Alpen: W-exponierte Abhänge des Schlosserkogels über dem Großen Winterleitensee, ca. 1900 m, 1.11.1989, H. Mayrhofer 8412 (GZU); SW-Abhänge der Wenzel Alpe NE von Neumarkt, Oberberg Alpe, ca. 1900 m, 12.6.1984, H. Mayrhofer 4062 (GZU). Wölzer Tauern: Hänge am Grat zwischen Karlspitze und Schreinl östlich über Donnersbachwald, ca. 2000 m, 7.1972, J. Poelt 11342 (GZU). – SWEDEN, Jämtland, Handöl, 1868, S. Almquist (TNS-L-11754); Torne Lappmark, Jukkasjärvi, Vassijaure, 20.7.1906, E. P. Vrang (TNS-L-125466).

The morphological features of the Japanese material (Fig. 1) agree well with the descriptions provided by MAYHROFER & POELT (1985) and ORANGE et al. (2009). The statistical values of morphological features for the Japanese material are consistent with the range of the known sizes as follows: perithecia up to 0.65 mm diameter; ascospores (36.7−)42.9±4.2(−50.1) × (10.9−)12.8±2.0(−16.7) µm (n = 15).

Chemical characters were also identical between Japanese and European material although these differed from previous reports (see section below): K−, PD−, C+ red (thallus); and the presence of an unidentified substance [P1] as a major substance detected by TLC (Fig. 2).

The Japanese collections of *P. sphinctrinoides* were found on *Andreaea rupestris* Hedw. var. *fauriei* (Besch.) Takaki and *Dicranum viride* (Sull. & Lesq.) Lindb. var. *hakkodense* (Cardot) Takaki on rock at 2750 m elev. in Nagano Prefecture on Honshu and on *Cephalozia otaruensis* Steph. and *Jungermannia* sp. on soil at 1025 m elev. in Hokkaido. Both localities have an alpine climate. *Protothelenella sphinctrinoides* was previously known from Europe, Greenland, North America and North Asia (Russia) (ORANGE et al. 2009). It is reported here for the first time from Japan.

C+ red species in the genus *Protothelenella*

This study revealed that the chemical features of *P. sphinctrinoides* differed from previous reports. The gelatinous thallus reacted C+ red (as checked under a stereo-microscope with a small amount of C solution). TLC analyses consistently detected an unidentified substance [P1] located in R*f* class 4, slightly higher than norstictic acid in solvent B', and R*f* class 4 – 5 in solvent C (Fig. 2). This spot develops a pale brown colour after spraying with 10 % H_2SO_4 and heating and is visible at UV_{254nm} but quenches at UV_{366nm}.

A secondary substance with a C+ red reaction had previously been reported for *P. leucothelia* (Nyl.) H.Mayrhofer & Poelt as an unknown *para*-depside of the orcinol-type (R*f* classes in solvents A/B'/C = 4/5/5-6) (MAYRHOFER & POELT 1985) with absorption maxima in chromatogram-spectrophotometry at 215 nm, 274 nm and 309 nm following the protocol of LEUCKERT (1984). This substance [P2] was also detected by TLC in the present study with the R*f* classes in solvent B'/C = 5/5 (Fig. 2). Interestingly, the chemical substance P1 of *P. sphinctrinoides* was also detected in *P. corrosa* (Körb.) H.Mayrhofer & Poelt and *P. croceae* (Bagl. & Carestia) Hafellner & H.Mayrhofer. The latter species is a lichenicolous parasite of *Solorina crocea* (L.) Ach. The C+ red reaction was confirmed on the thallus of *P. corrosa* and on the hymenium of perithecia of *P. croceae*. By contrast, *P. sphinctrinoidella* (Nyl.) H.Mayrhofer & Poelt, which has smaller ascospores (22–33 × 7–10 µm) than *P. sphinctrinoides*, was C-negative and no spot was detected by TLC.

Specimens examined for chemistry: ***Protothelenella corrosa***. AUSTRIA, Carinthia, Saualpe, Geierkogel südlich vom Klippitztörl, 1850 m, 17.6.1984, H. Mayrhofer 4099 (GZU). Salzburg, Radstädter Tauern, Weg vom Zauchensee auf dem Strimskogel über die Strimshütte, 2110 m, 23.5.1986, H. Wittmann (GZU). Styria, Schladminger Tauern: Kleinsölk-Obertal, unterste Abhänge der Kesselspitze über der Kohlungbrücke, 1050 m, 7.7.1998, H. Mayrhofer 13718 & P. Bilovitz (GZU); Wanderweg Richtung Unterer Zwieflersee, 1539 m, 10.11.2011, M. Sebernegg & H. Mayrhofer (GZU); südexponierte Abhänge der Handalpe östlich der Weinebene, 1760 m, 25.9.2011, H. Mayrhofer 18159 (GZU). – CZECH REPUBLIC, Vězda, Lich. Sel. Exs. 51 (TNS). – ITALY, Arnold, Lich. Exs. 201 (TNS). –

Fig. 1: *Protothelenella sphinctrinoides* collected from Japan (Y. Ohmura 9300 & A. Frisch, TNS). Scale = 0.5 mm.

POLAND, Nowak, Lich. Polon. Merid. Exs. 1 (TNS). – SLOVAKIA, Vězda, Lich. Sel. Exs. 505 (TNS). – SWEDEN, Närke, St. Mellösa, 1868, P. J. Hellbom (TNS); Värmland, Munkfors, 6.6.1953, S. W. Sundell (TNS).

Protothelenella croceae. AUSTRIA, Santesson, Fungi Lichenicoli Exs. 194, 281 (TNS). – SWEDEN, Santesson, Fungi Lichenicoli Exs. 118 (TNS).

Protothelenella leucothelia. AUSTRIA, Arnold, Lich. Exs. 613b (GZU).

Protothelenella sphinctrinoidella. AUSTRIA, Vorarlberg: Rätikon, WSW Schruns, Gipfelbereich des Golmer Joches, 2000 – 2124 m, 27.7.1986, M. & H. Mayrhofer 7060 (GZU). Kärnten: Nationalpark Hohe Tauern, Ankogel Gruppe, am Südgrat des Greilkopf E der Hagener Hütte, 2480 m, 27.8.1994, J. Hafellner (GZU). Steiermark, Niedere Tauern: Wölzer Tauern, Schreinl östlich über Donnersbachwald, 2130 – 2150 m, 7.1972, J. Poelt 11763 (GZU). Ende des Triebentales SO Trieben, unweit der Griesmoarhube, 1200 – 1300 m, 8.1973, J. Poelt 12417 (GZU). Schladminger Tauern: Katschtal, 1430 m, 19.5.2011, M. Sebernegg & H. Mayrhofer (GZU). Sattental S Gröbming, Kochofen, 1900 – 1915 m, 8.7.1984, H. Mayrhofer 4117 & C. Scheuer (GZU). – ITALY, Poelt, Pl. Graec. Lich. 425 (TNS). South Tyrol, High Tauern, Venediger Gruppe, Rieserferner-Ahrn Nature Park, SE of Kasern, Röttal, 2466 m, 13.8.2013, P. Bilovitz et al. 4644 (GZU). – RUSSIA, Kola Peninsula, Khibiny Mts., Kukisvum valley, 400 m, 10.8.2000, M. Kukwa (GZU). – SWEDEN, Magnusson, Lich. Sel. Scand. Exs. 201 (TNS); Rabenhorst, Lich. Europaei 852 (TNS). – GREENLAND, Disko, Umgebung von Godhavn, N der Arktischen Station Godhavn, um 20 m, 13.8.1982, J. Poelt & H. Ullrich (GZU). – USA, Colorado, Rocky Mountains, Summit Co., N-facing slope of North Star Mountain S of Quandary Peak, near Blue Lakes Reservoir, 3580 – 3750 m, 6.9.1977, J. Poelt (GZU).

Protothelenella sphinctrinoides. See chapter above.

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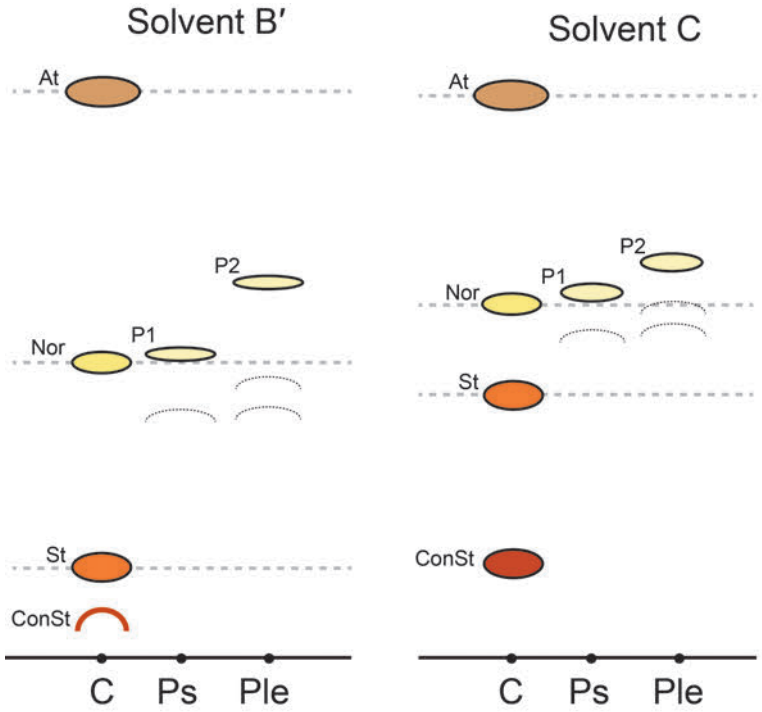

Fig. 2: Diagnostic TLC spots of lichen substances detected in C+red species in the genus *Protothelenella* in Solvents B' and C. Control (C) using *Stereocaulon japonicum* which contains atranorin (At) for R*f* class 7, norstictic acid (Nor) for R*f* class 4, stictic acid (St) for R*f* class 2, and constictic acid (ConSt) for R*f* class 1. Ps = *P. sphinctrinoides*; Ple = *P. leucothelia*; P1 and P2 = unidentified substances.

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References

Culberson, C. F. & Johnson, A. 1982. Substitution of methyl *tert*.-butyl ether for diethyl ether in the standardized thin-layer chromatographic method for lichen products. – J. Chromatogr. **238**: 483–487.

Culberson, C. F. & Kristinsson, H. D. 1970. A standardized method for the identification of lichen products. – J. Chromatogr. **46**: 85–93.

Etayo, J. & Sancho, L. G. 2008. Hongos liquenícolas del Sur de Sudamérica, especialmente de Isla Navarino (Chile). – Biblioth. Lichenol. **98**: 1–302.

Honegger, R. 2008. Mycobionts. – In: Nash T. H. III (ed.) Lichen Biology. Second Edition. Pp. 27–39. – Cambridge: Cambridge University Press.

Leuckert, C. 1984. Die Identifizierung von Flechtenstoffen im Rahmen chemotaxonomischer Routineanalysen. – Beih. Nova Hedwigia **79**: 839–869.

Mayrhofer, H. 1987. Ergänzende Studien zur Taxonomie der Gattung *Protothelenella*. – Herzogia **7**: 313–342.

Mayrhofer, H. 2002. *Protothelenella*. – In: Nash, T. H. III, Ryan, B. D., Gries, C. & Bungartz, F. (eds.) Lichen Flora of the Greater Sonoran Desert Region. Vol. I. Pp. 408–409. – Tempe, Arizona: Lichens Unlimited, Arizona State University.

Mayrhofer, H. & Poelt, J. 1985. Die Flechtengattung *Microglaena* sensu Zahlbruckner in Europa. – Herzogia **7**: 13–79.

Orange, A., James, P. W. & White, F. J. 2001. Microchemical methods for the identification of lichens. – London: British Lichen Society.

Orange, A., Purvis, O. W. & James, P. W. 2009. *Protothelenella* Räsänen (1943). – In: Smith, C. W., Aptroot, A., Coppins, B. J., Fletcher, A., Gilbert, O. L., James, P. W. & Wolseley, P. A. (eds). The Lichens of Great Britain and Ireland. Pp. 755–757. – London: The British Lichen Society.

Schmitt, I., Mueller, G. & Lumbsch, H. T. 2005. Ascoma morphology is homoplaseous and phylogenetically misleading in some pyrenocarpous lichens. – Mycologia **97**: 362–374.

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