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Sarcogyne endopetrophila (*Acarosporaceae*, Lichenized Ascomycota), a New Species from Japan

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Sarcogyne endopetrophila Tokiz. & Y. Ohmura, an endolithic lichen, is described as a new species from Hiroshima, western Japan, where it grows on granodiorite rocks along a river. *Sarcogyne endopetrophila* resembles *S. clavus*, *S. gibberella*, and *S. hypophaea* in having endolithic thalli and lecideoid apothecia with black to dark-red discs lacking pruina. However, it is distinguished from them by having smaller apothecia with smooth margin, pale brown and thick hypothecium, and indistinct subhymenium. Pycnidia of *S. endopetrophila* were also found on the bare rock surfaces. The identity of pycnidia was confirmed by comparisons with ITS rDNA sequences of the pycnidia, apothecia and endolithic thalli.

Key words: Endolithic lichen, ITS rDNA, pycnidia, taxonomy.

The genus *Sarcogyne* Flot. (*Acarosporaceae*, lichenized *Ascomycota*) is characterized by polyspory, simple hyaline spores, carbonized apothecial margin, bitunicate but non-fissitunicate asci with a non-amyloid thallus, and rarely branched paraphyses (Magnusson 1935a, 1935b, Seppelt et al. 1998, Knudsen and Standley 2007). The genus has a world-wide distribution and more than 50 species are estimated at present (Knudsen and McCune 2013). In Japan, only *S. gibberella* (Nyl.) H. Magn., an endemic species, was reported from Nagasaki Prefecture (as *Lecanora gibberella* Nyl., Nylander 1890). During our studies on Japanese lichen flora, an undescribed species of *Sarcogyne* was collected on granodiorite rocks along riverbanks of the Ôta River in Hiroshima Prefecture, western Japan.

The aim of this paper is to describe the species based on the morphological and chemical features. We also examined the genetic identity of apothecia, endolithic thalli, and pycnidia based on ITS rDNA sequences because they grow separately on the surface and inside of rocks.

Materials and Methods

Twenty-one specimens were collected from granodiorite rocks along riverbanks of the Ôta River in Hiroshima Prefecture, western Japan

Fig. 1. Habitat of *Sarcogyne endopetrophila*. During the high-water periods of the river, these habitats (arrows) are submerged under the water.

(N34°43′, E132°12′) (Fig. 1). All specimens examined are deposited in the Herbarium of the National Museum of Nature and Science (TNS), Tsukuba, Japan.

Morphological observations were made using a dissecting microscope or a bright field microscope. Cross sections of apothecia and pycnidia were made by hand with a razor blade, and observed after mounting in GAW (glycerin: ethanol: water = 1:1:1). Iodine reactions were performed using 0.5% of Lugol's iodine solution (I) or the I solution with pretreatment of 10% KOH (K/I) (Smith et al. 2009). Polarization microscopy was also applied to observe hyphae of endolithic thallus in the rock using a thin section of lichen-colonized rock. The measurements of morphological characters were calculated statistically and shown as "minimum - average - maximum (SD = standard deviation of the samples, *n* = number of samples examined)".

Lichen substances were examined by means of thin layer chromatography (TLC) (Culberson and Kristinsson 1970). Solvent B system (hexane: methyl tert.-butyl ether: formic acid, 140: 72: 18) (Culberson and Johnson 1982) was only used for the TLC analyses.

Total DNA from apothecia, pycnidia, and endolithic thalli were extracted by FastDNA SPIN Kit (MP Biomedicals) following the method of Ohmura et al. (2006). PCR amplification of the nuclear ribosomal internal transcribed spacer (ITS rDNA) region was performed using ITS1F (Gardes and Bruns 1993) as the 5' primer and LR1 (Vilgalys and Hester 1990) as the 3' primer. PCR was performed following the method of Ohmura et al. (2006) using PuReTaq Ready-To-Go PCR beads (GE Healthcare) and a thermal cycler (Gene Amp PCR system 9700, Applied Biosystems). PCR products were purified with ExoSAP-IT (GE Healthcare). Sequencing was carried out on ABI Prism 3130x genetic analyzer (Applied Biosystems) with BigDye Terminator ver. 3.1 Cycle Sequencing Kit according to the manufacturer's instructions. Obtained sequences were assembled and manually corrected using ATGC ver. 6.03 (Genetyx).

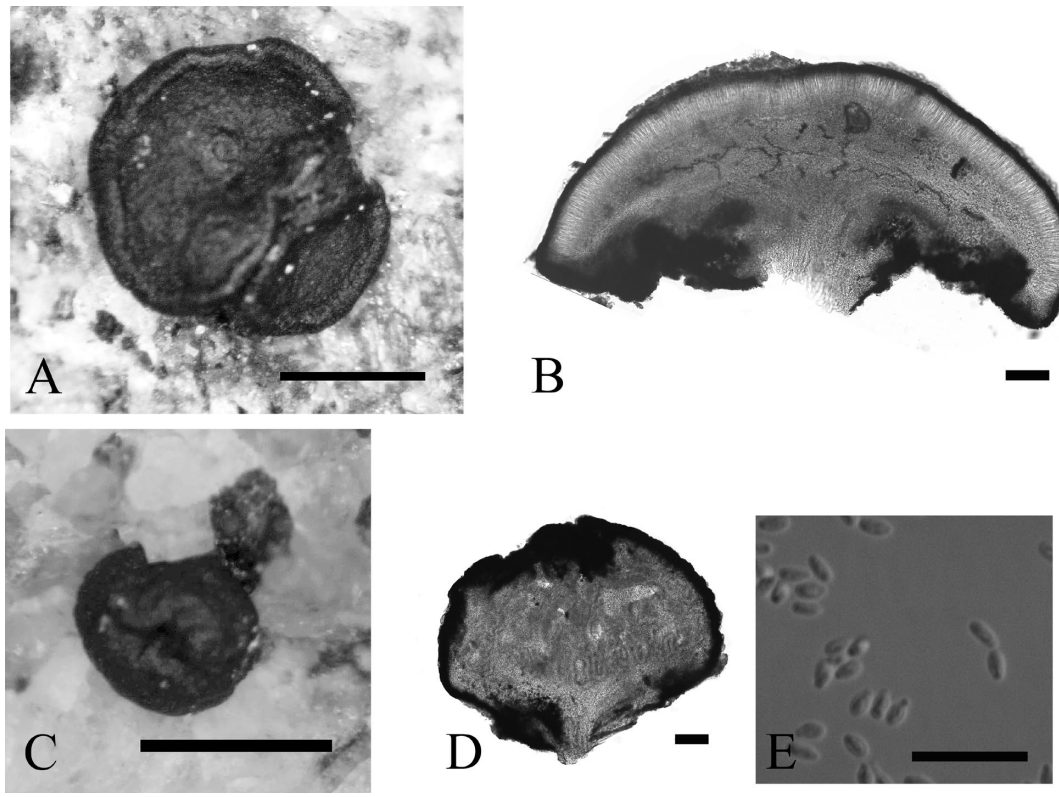

Fig. 2. *Sarcogyne endopetrophila* Tokiz. & Y. Ohmura (holotype, TNS). A. Apothecia. B. Cross section of an apothecium. C. Pycnidium. D. Cross section of a pycnidium. E. Conidia. Scales: 0.5 mm (A, C); 100 µm (B, D); 10 µm (E).

Results and Discussion

Sarcogyne endopetrophila Tokiz. & Y. Ohmura, sp. nov. [Figs. 2–3]

MycoBank No.: MB 808152.

Type: **JAPAN**. Honshu. Prov. Aki (Pref. Hiroshima): Akiôta-cho, Yamagata-gun, on/in granodiorite rocks along Ôta River, ca. 300 m elev., 25 July 2010, M. Tokizawa s.n. (TNS-L-130000–holotype).

Thallus crustose, immersed, inconspicuous, forming two layers with photobiont and mycobiont under 0.5–1.2–2.0 mm depth (SD = 0.5, *n* = 12) from the rock surface (Fig. 3A); hyphae across the rock crystals (Fig. 3B). Apothecia sessile, irregular to round in shape, 0.5–1.0–2.0 mm in diameter (SD = 0.4, *n* = 30); disc black to dark-red, lacking pruina; margin black, prominent, smooth and moderately undulate; proper exciple reddish brown, composed of compacted radiating hyphae that are more or less swollen at the surface; epihymenium yellow-brown, not carbonaceous, 10–15–30 µm thick (SD = 5.6, *n* = 26); hymenium hyaline, 50–73–150 µm thick (SD = 23, *n* = 26), I+ blue, paraphyses simple and unbranched (very rarely branched near the tip), 1.0–1.7–2.0 µm wide (SD = 0.4, *n* = 13); hypothecium prosoplechtenchymatous, pale brown, 90–110–150 µm thick (SD = 16, *n* = 14). Asci clavate, 55.0–64.0–77.5 × 7.5–10.0–12.5 µm (SD = 7.5, 1.5, *n* = 13), >100-spored, apical dome K/I−. Ascospores hyaline, simple, ellipsoid, 3.6–4.7–6.0 × 1.5–1.8–2.0 µm (SD = 0.9, 0.3, *n* = 9). Pycnidia black, sessile, verrucose to globose, 0.5–0.8–1.3 mm in diameter (SD = 0.2, *n* = 25). Conidia ellipsoid, 2.0–2.9–4.0 ×1.0–1.2–2.0 µm (SD = 0.4, 0.3, *n* = 25). Photobiont *Myrmecia* sp.

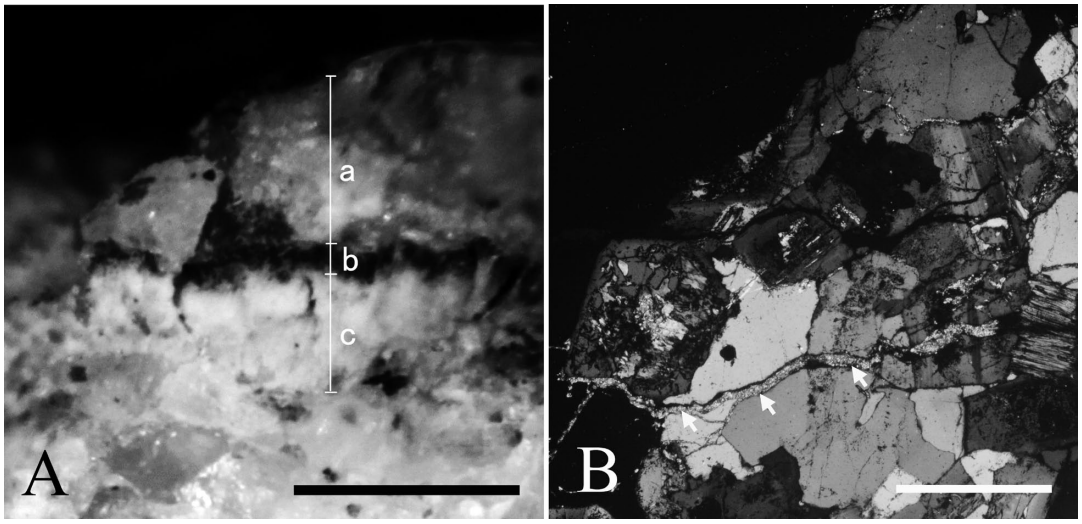

Fig. 3. Colonization of *Sarcogyne endopetrophila* Tokiz. & Y. Ohmura in a rock (M. Tokizawa 100315-3, TNS). A. Normal observation of endolithic thallus section. a. rock. b. photobiont layer. c. mycobiont layer. B. Polarized observation of endolithic thallus in a rock using a petrographic thin-section. arrows indicating lichen thallus across the rock crystals. Scales: 0.5 mm.

Chemistry. Fatty acids (Rf class 3, 3–4) minor or absent.

The diagnostic features of *S. endopetrophila* are (1) endolithic thallus (Fig. 3A), (2) small apothecia (0.5–1.2–2.0 mm in diam.) without pruina on the disc, (3) smooth margin of apothecium (Fig. 2A), (4) pale brown and thick hypothecium (90–110–150 µm) (Fig. 2B), (5) indistinct subhymenium, (6) verrucose to globose pycnidia (Fig. 2C, D), (7) ellipsoid conidia (Fig. 2E), (8) *Myrmecia* as the photobiont, and (9) the fatty acids when present.

Pycnidia growing on the bare rock surfaces were confirmed as being parts of *S. endopetrophila* based on the fungal ITS rDNA sequences of pycnidia, apothecia and endolithic thalli as well as on morphological features. Their sequences were 480 nt in length (including ITS1, 5.8S rDNA, and ITS2), and no differences were found among them (one representative sequence was registered to GenBank: AB915709). It was difficult to prove the morphological connection among apothecia, pycnidia, and endolithic thalli because they were scattered separately on/in the rock surface. However, the ITS rDNA sequences of the present study suggest their identity.

According to Golubic et al. (1981), three forms of endolithic lichen, cryptoendolithic, chasmoendolithic, and euendolithic forms, are recognized by the colonization in a rock. Cryptoendolithic lichens colonize the structural cavities within porous rocks; chasmoendolithic lichens colonize the fissures and cracks within rocks; and euendolithic lichens actively penetrate rocks. Polarization microscopy in our study suggests that the endolithic form of *S. endopetrophila* may belong to chasmoendolithic or euendolithic forms (Fig. 3B).

Sarcogyne endopetrophila resembles *S. clavus* (DC.) Kremp., *S. gibberella* (Nyl.) H. Magn., and *S. hypophaea* (Nyl.) Arnold [= *S. privigna* auct. non (Ach.) A. Massal., see Knudsen et al. 2013] which are also reported from Asia (Table 1) (Smith et al. 2009). These species have endolithic thalli and lecideoid apothecia with epruinose discs. Pycnidia of *S. endopetrophila* are also similar to those of *S. clavus* in having black colored, wart-like pycnidia and the same sized conidia. However, *S. endopetrophila* is distinguished

Table 1. Comparisons between *Sarcogyne endopetrophila* and its related species

	S. clavus	*S. endopetrophila*	*S. gibberella*	*S. hypophaea*
Apothecia (mm in diam.)	1.0–6.0	0.5–2.0	< 1.0	0.3–0.7
Disc margin	warty	smooth	warty	smooth
Hymenium (μm)	85–125	50–150	100–150	60–110
Subhymenium	indistinct	indistinct	indistinct	distinct, 20–50
Hypothecium (μm)	dark brown in the upper part, 60–100	pale brown, 90–150	dark brown, 50–100	yellow to very pale brown, 15–35
Pycnidia (mm in diam.)	0.1–0.3	0.5–1.3	not examined	0.1–0.2
Conidia (μm)	2.0–3.5×1.0–1.5	2.0–4.0 ×1.0–2.0	not examined	1.0–2.0 × 0.5–1.0
Secondary metabolites	nil	fatty acid (Rf class 3, 3–4) minor or ±	not examined	nil
Distribution	Europe, North America, Africa, Greenland and Asia	Japan	Japan	Europe, North America, Africa, Australia and Asia

Morphological values were obtained in this study and from references (Nylander 1890, Magnusson 1937, Knudsen and Kocourková 2011, Knudsen and Standley 2007). Specimens examined were *S. clavus* (Magnusson 24882, TNS), *S. endopetrophila* (holotype, TNS), and *S. hypophaea* (A. Vězda: Lichenes Selecti Exsiccati, no. 95, TNS). Morphological data of *S. gibberella* were obtained from the holotype (H-NYL 24516) by Dr. H. Kashiwadani (pers. comm.).

from *S. clavus* by the smooth apothecial margin, the pale brown hypothecium, and the larger pycnidia (0.5–1.3 mm in diameter); from *S. gibberella* by the smooth apothecia and the pale brown hypothecium; and from *S. hypophaea* by the indistinct subhymenium, the thicker hypothecium (90–150 μm thick) and the larger pycnidia (0.5–1.3 mm in diameter) (Table 1). Secondary metabolites in the related species of *S. endopetrophila* are nil or unknown (Table 1). Although fatty acids were sometimes detected in *S. endopetrophila*, their detection was unstable so that the taxonomic value of these substances appears to be low.

Sarcogyne endopetrophila is currently known only from Hiroshima Prefecture in western Japan, where it grows on/in the surface of granodiorite rocks along a river at ca. 300 m elevation (Fig. 1). This species might be overlooked easily in the field because it has an endolithic thallus and its outer appearance does not resemble that of lichen. Further careful investigations might enlarge the distribution in and outside of Japan. This is the second record of a *Sarcogyne* species from Japan.

Representative specimens examined. JAPAN. Honshu. Prov. Aki (Pref. Hiroshima): along Ôta River, Akiôta-cho, Yamagata-gun, on/in granodiorite rocks along river; ca. 300 m elev., 5 October 2009, S. Takeshita s.n. (herb. Y. Ohmura 6800); the same locality, 15 March 2010, M. Tokizawa 100315-3; Yoshiwago, Akiôta-cho, Yamagata-gun, on/in grandodiorite rocks, 310 m elev., 14 December 2010, Y. Ohmura, M. Tokizawa & S. Takeshita 7778, 7779.

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時澤味佳[a]，大村嘉人[a]，文　光喜[b]，竹下俊治[c]：**ホウネンゴケ科カクレホウネンゴケ属の 1 新種 *Sarcogyne endopetrophila***

広島県にてホウネンゴケ科カクレホウネンゴケ属（新称）の 1 新種 *Sarcogyne endopetrophila* Tokiz. & Y. Ohmura（イワカクレホウネンゴケ，新称）を記載した．日本で本属の報告は，1890 年に長崎県高島で発見された日本固有種の *S. gibberella*（カクレホウネンゴケ，新称）に続き 2 種目である．

本種は岩石内生地衣類 (endolithic lichen) であり，露岩表面には子器を露出させ，地衣体本体は岩石内部に存在している．同一露岩表面には，粉子器が多数確認された．粉子器がイワカクレホウネンゴケ由来であることは，粉子器，子器および地衣体の ITS rDNA 塩基配列が同一であることから確認された．粉子器は直径 0.5–1.3 mm，粉子は長円形で 2.0–4.0 × 1.0–2.0 μm であった．共生藻は *Myrmecia* sp.（緑藻類 トレボウクシア藻綱）であった．

本種は，岩石内生地衣類である *S. clavus* や *S. hypophaea*，*S. gibberella* と，子器盤が粉霜を欠くことや，果殻が炭化していることで似ているが，*S. clavus* とは，子器縁が瘤状にならないこと，子嚢下層が淡褐色，粉子器の大きさで区別され，*S. gibberella* とは，子器の縁が瘤状にならないことや，子嚢下層が淡褐色であることから区別される．さらに，*S. hypophaea* とは子嚢層下部 (subhymenium) が不明瞭であることや，子嚢下層が 90–150 μm と厚いことから区別される．

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