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NOTE

Noteworthy foliicolous lichens collected from Iriomote Island, southern Japan

Kento MIYAZAWA[1,*], Yoshihito OHMURA[2], Yuichi YAMAOKA[3]

1. *Degree Program in Life and Earth Sciences, Graduate School of Science and Technology, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, 305-8572, Japan.* ***2.*** *Department of Botany, National Museum of Nature and Science, 4-1-1 Amakubo, Tsukuba, Ibaraki, 305-0005, Japan.* ***3.*** *Faculty of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, 305-8572, Japan. [*]Corresponding author's email: tentomushi.3838@gmail.com*

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ABSTRACT: The following noteworthy species were identified based on foliicolous lichen material collected in 2011 and 2019 on Iriomote Island: *Byssoloma chlorinum*, *B. vanderystii* and *Gallaicolichen pacificus*, reported as new to Japan, and *B. minutissimum* and *Fellhanera rhapidophylli*, reported as new to Iriomote Island. Hyphophores and diahyphae of *Tricharia kashiwadanii* were described for the first time, based on the original material and a collection from Iriomote Island. The mtSSU sequences of *B. chlorinum*, *Porina kamerunensis* and *T. kashiwadanii* were obtained for the first time as well as additional sequences of *B. annuum*, *B. vanderystii*, *Eugeniella micromata*. The mtSSU sequence was obtained from the specimens were compared in terms of homology with those registered in GenBank.

KEY WORDS: Ascomycota, lichenized fungi, mtSSU, mycota, red-listed taxa, taxonomy, the Tropics, threatened species.

INTRODUCTION

There are more than 800 species of foliicolous lichens worldwide, distributed mainly in tropical regions (e.g., Santesson, 1952; Lücking, 2008). In Japan, 85 taxa of foliicolous lichens have been reported (Thor *et al*., 2000; Suto and Ohtani, 2015; Miyazawa *et al*., 2020). Among them, 14 taxa are categorized as "Near Threatened" (NT) and 14 taxa as "Data Deficient" (DD) in the Red List for Japanese species (Ministry of the Environment, 2020).

Iriomote Island, one of the Ryukyu Islands, is located near the southernmost part of Japan (N24°17′33″, E123°51′43″) and about 200 km east of Taiwan (Matsuzawa, 2019). Approximately 90% of the island is covered with subtropical rainforest, forming a semi-closed ecosystem with little influence from anthropogenic activities (Matsuzawa, 2019), and a large part of island is well-preserved old-growth forest located within the national park. Sixty-five taxa of foliicolous lichens were reported from Iriomote Island before the present study (Thor *et al*., 2000; Ohmura, 2012; Miyazawa *et al*., 2020). Although this represents the highest number of taxa among the foliicolous lichens reported in Japan, further investigations are expected to find additional taxa (Thor *et al*., 2000).

The aim of this study was to conduct taxonomic examinations of the foliicolous lichens collected from Iriomote Island and to report additional information on the foliicolous lichen mycota of Japan.

MATERIALS AND METHODS

Collections

The field investigations were performed by Y. Ohmura and G. Tanaka on 8–11 March 2011 and by K. Miyazawa and Y. Ohmura on 18–21 November 2019 on Iriomote Island, and approximately 370 specimens of foliicolous taxa ware collected. All voucher specimens are housed in the herbarium of the National Museum of Nature and Science (TNS), Tsukuba, Japan.

Morphology and chemistry

Morphological observations were performed using a dissecting microscope (MZ-8 [Leica, Wetzlar, Germany] or SZ-PT [Olympus, Tokyo, Japan]) and a differential interference contrast microscope (BX51; Olympus). Anatomical examination was performed using hand-cut sections mounted in a glycerin/ethanol/water (1:1:1) solution (Asahina, 1936). Photographs were taken using a digital camera (COOLPIX P6000 [Nikon, Melville, NY, USA] or NEX-5 [Sony, New York, NY, USA]), and the images obtained were subjected to depth stacking using CombineZP (https://combinezp.software.informer.com/) (Fig. 2A). Spore size is expressed as follows: (minimum–) range of mean ± standard deviation (–maximum) (n = number of measurements).

Ascus amyloidity was assessed using with 5–10% KOH solution and Lugol's solution. Crystals was evaluated by polarization under differential microscopy. Secondary substances from the thallus were examined by high-performance thin-layer chromatography (HPTLC)

Table 1. BLAST results of mtSSU sequences obtained in this study. If the homology of the top-hit sequence was greater than 95%, three sequences were presented, but if it was less than 95%, only the top-hit sequence was presented.

Species/ Voucher	GenBank No.[a]	BLAST Result[b]
Byssoloma chlorinum		
K. Miyazawa 372 & Y. Ohmura (TNS)	LC648410	737/795 (93%), 1136, 0.0 (MN105612, *Byssoloma leucoblepharum*)
K. Miyazawa 549 & Y. Ohmura (TNS)	LC648417	731/787 (93%), 1133, 0.0 (MN043721, *Byssoloma leucoblepharum*)
K. Miyazawa 566 & Y. Ohmura (TNS)	LC648419	770/832 (93%), 1182, 0.0 (MN105612, *Byssoloma leucoblepharum*)
K. Miyazawa 567 & Y. Ohmura (TNS)	LC648420	777/839 (93%), 1195, 0.0 (MN105612, *Byssoloma leucoblepharum*)
K. Miyazawa 569 & Y. Ohmura (TNS)	LC648421	793/862 (92%), 1201, 0.0 (MN043695, *Byssoloma* cf. *leucoblepharum*)
K. Miyazawa 570 & Y. Ohmura (TNS)	LC648422	763/822 (93%), 1181, 0.0 (MN105612, *Byssoloma leucoblepharum*)
K. Miyazawa 575 & Y. Ohmura (TNS)	LC648423	792/861 (92%), 1199, 0.0 (MN043695, *Byssoloma* cf. *leucoblepharum*)
B. annuum		
K. Miyazawa 451 & Y. Ohmura (TNS)	LC648414	819/824 (99%), 1496, 0.0 (MN043716, *Byssoloma annuum*) 816/821 (99%), 1491, 0.0 (MN043716, *Byssoloma annuum*) 817/843 (97%), 1496, 0.0 (MN105600, *Byssoloma brunneodiscum*)
K. Miyazawa 501 & Y. Ohmura (TNS)	LC648415	806/807 (99%), 1496, 0.0 (MN043726, *Byssoloma annuum*) 788/789 (99%), 1452, 0.0 (MN043716, *Byssoloma annuum*) 786/808 (97%), 1367, 0.0 (MN105603, *Byssoloma brunneodiscum*)
K. Miyazawa 558 & Y. Ohmura (TNS)	LC648418	806/809 (99%), 1476, 0.0 (MN043726, *Byssoloma annuum*) 788/791 (99%), 1443, 0.0 (MN105603, *Byssoloma annuum*) 787/809 (97%), 1367, 0.0 (MN105603, *Byssoloma brunneodiscum*)
K. Miyazawa 583 & Y. Ohmura (TNS)	LC648424	759/762 (99%), 1389, 0.0 (MN043726, *Byssoloma annuum*) 759/762 (99%), 1389, 0.0 (MN043716, *Byssoloma annuum)* 741/762 (97%), 1286, 0.0 (MN105603, *Byssoloma brunneodiscum*)
B. vanderystii		
K. Miyazawa 400 & Y. Ohmura (TNS)	LC648411	795/796 (99%), 1465, 0.0 (MN043715, *Byssoloma vanderystii*) 795/796 (99%), 1465, 0.0 (MN043713, *Byssoloma vanderystii*) 794/796 (99%), 1459, 0.0 (MN043719, *Byssoloma vanderystii*)
Eugeniella micromata		
K. Miyazawa 437 pr.p. & Y. Ohmura (TNS)	LC648413	783/792 (99%), 1413, 0.0 (MK957161, *Eugeniella micrommata*)
Porina kamerunensis		
K. Miyazawa 504 & Y. Ohmura (TNS)	LC648416	611/660 (93%), 935, 0.0 (DQ168394, *Porina subnitidula*)
Tricharia kashiwadanii		
K. Miyazawa 405 & Y. Ohmura (TNS)	LC648412	663/741 (89%), 929, 0.0 (KF833340, *Echinoplaca* sp.)

a. GenBank accession number for mtSSU.
b. BLAST result with GenBank sequences. Identity; score; e-value accession number, species) of the top 3 sequences (maximum) of related taxa are shown.

following the method of Schumm and Elix (2015).

The detailed morphologies of peltidiangia and peltidia were observed by scanning electron microscopy (SEM) (S-4200; Hitachi, Tokyo, Japan). A fragment of thallus containing peltidiangia was cut and attached to a sample stand with double-sided tape. The sample was coated with platinum palladium by an ion sputtering device (E1030; Hitachi) for SEM.

DNA extraction, PCR amplification and sequencing

DNA extraction from selected samples was conducted following the method of Izumitsu *et al*. (2012). One to three apothecia were picked from a colony or a fragment of thallus, ground using sterile glass slides, and placed in a 1.5-mL tube containing 100 μL TE buffer. The tubes were heated in a microwave oven (600 W) for 1 min., kept at room temperature for 30 s, and heated again in a microwave oven (600 W) for 1 min. The heated microtubes were immediately stored in a freezer at −20°C for approximately 1 day, and 2 μL of solution from each microtube was used as a DNA template for PCR.

The partial sequence of the fungal mitochondrial small subunit (mtSSU) was amplified using the primer set mrSSU1 and mrSSU3R (Zoller *et al*., 1999) according to the following protocol. PCR was performed in a 25 μL reaction volume containing 2 μL DNA template, 12.5 μL GenRED PCR Mix Plus (Nippon Gene, Tokyo, Japan), 2.5 μL each primer (2 pmol/μL), and 5.5 μL distilled water. The PCR conditions were according to Wang *et al*. (2020) using a TaKaRa PCR Thermal Cycler Dice® Touch (TaKaRa, Tokyo, Japan). The PCR products were checked by electrophoresis on a 1.5 % agarose gel stained with Midori Green Direct DNA Stain (Nippon Genetics, Tokyo, Japan) and visualized using WSE-5200 Printgraph 2 M (ATTO Corporation, Tokyo, Japan). The PCR products were purified using a FastGene™ Gel/PCR extraction kit (Nippon Genetics) following the manufacturer's instructions. Sequences were obtained by a DNA sequencing service (Eurofins Genomics, Tokyo, Japan).

The obtained sequences were analyzed by the GenBank BLAST search (blastn) using the default settings. The voucher specimen data and GenBank accession numbers for the obtained mtSSU sequences are shown in Table 1.

RESULTS AND DISCUSSION

Taxonomic examination of 209 of the 370 specimens identified 51 taxa at the species level (Supplement, Table S1). Among them, 3 species were new to Japan and an additional 2 species were new to Iriomote Island but known from other localities in Japan; 13 species are those listed in the Red List for Japanese species (Ministry of the Environment, 2020) (Table S1).

For the species newly recorded in Japan, full descriptions are given based on the Iriomote Island specimens. For the species newly recorded on Iriomote Island and the red-listed species, including the DD species, newly obtained taxonomic and ecological information are noted. The mtSSU sequences obtained from the specimens were compared with those registered in GenBank in terms of homology.

Species new to Japan

Byssoloma chlorinum (Vain.) Zahlbr. Lich. Univ. 8: 233. 1932. **Fig. 1A, B**

Thallus continuous, 15–30 mm across, 10–50 µm thick, minutely farinose to granulose, light green, sometimes whitish green. Apothecia rounded, up to 0.6 mm diam., 100–250 µm tall; margin byssoid, reduced to well-developed, persistent or rarely evanescent, occasionally spreading laterally over thallus surface, 50–100 µm broad, white, composed of loosely woven colorless to pale brown hyphae (2–3 µm wide); disc plane to slightly convex, brownish black; epithecium indistinct; hymenium 40–100 µm tall, colorless to pale brown; hypothecium 40–70 µm tall, dark brown, K–; apothecial base brownish black to aeruginous, K–; paraphyses branched, 0.8–1.0 µm wide, not widened apically. Asci clavate, I+ blue, tholus amyloid (*Byssoloma*-type), 40–45 × 10–12 µm. Ascospores oblong-ellipsoid, 3-septate (rarely 4-septate), with or without slight constrictions at septa, colorless, (8.0–)9.7–12.7(–16.0) × (2.5–)2.8–3.8(–4.0) µm (n = 40). Pycnidia subglobose to barrel-shaped, 80–180 µm diam., grey to black with white margin, walls aeruginous. Conidia pyriform, non-septate, colorless, (3.0–)3.3–4.3(–5.0) × (1.0–)1.2–1.5(–1.5) µm (n = 20). Photobiont trebouxioid, globose to subglobose, (5.0–)5.1–7.5(–9.0) × (4.0–)5.0–6.8(–8.0) (n = 20).

Chemistry: no secondary substances were detected by HPTLC.

Byssoloma chlorinum is characterized by a light green farinose thallus (Fig. 1A), blackish apothecia with whitish developed byssoid margin (Fig. 1A), and 3-septate ascospores (Fig. 1B).

The ascospore size in the materials in the Iriomote Island collections (see above) (Fig. 1B) largely overlapped with the range (9.6–15 × 2.0–4.3 µm) of the protologue (Vainio, 1924) (10–12 × 2–2.5 µm), the description by Lücking (2008) (10–15 × 2.5–3.5 µm), and our measurements for an exsiccata (Lücking: Lich. Fol. Exs. 132, TNS) [(9.6–)10.0–11.9(–12.6) × (3.0–)3.3–4.1(–4.3) µm (n=14)].

Hypothecium of the Iriomote Island materials is well developed and much thicker (40–70 µm tall) than the measurement (15–25 µm) reported by Lücking (2008), causing the disc shape to be plane to slightly convex.

The byssoid excipulum varies from distinctly reduced to well-developed spreading laterally over the thallus surface. Such variations were observed even within the same thallus in some specimens.

The BLAST results for the mtSSU sequences of the Iriomote Island samples showed less than 93% identity with those of other *Byssoloma* species in GenBank (Table 1). It is because no sequence of *B. chlorinum* was registered in GenBank before this study.

Byssoloma chlorinum resembles *B. vanderystii* Sérus., but the latter is readily distinguished by chocolate-brown discs and 7-sptate ascospores. Among foliicolous taxa, *B. chlorinum* may be confused with *B. leucoblepharum* (Nyl.) Vain. However, *B. chlorinum* is distinguished from the latter by having a light green farinose thallus and dark apothecia with a whitish byssoid margin, while *B. leucoblepharum* has a dark smooth thallus and apothecia with a chamois-colored byssoid margin (Lücking, 2008). Further taxonomic research is needed for these species because the features are variable and sometimes overlapping (see Lücking, 2008).

Byssoloma chlorinum is from Asia (India [Gupta and Sinha, 2017], Indonesia [Santesson, 1952], Thailand [Buaruang *et al.*, 2017], and Vietnam [Aptroot and Sparrius, 2006]), Oceania (Australia [Lücking *et al.*, 2001], French Polynesia [type locality], and Papua New Guinea [Aptroot and Sipman, 1993]), North and South America (Bermuda [Berger and LaGreca, 2014], and other Neotropical regions [Lücking, 2008]), Europe (Azores [Breuss, 2018]), and Africa (Kenya [Lücking and Kalb, 2002] and Seychelles [Seaward and Aptroot, 2009]), showing a pantropical distribution (Lücking, 2008). This species is new to Japan.

Exsiccati examined. COSTA RICA. Puntarenas Province: Cocos Island National Park, ca. 540 km SW of Puntarenas in the NW Pacific Ocean (N05°33′, W87°04′), on leaves of *Calophyllum brasiliense*, 0 m elev., April 1992, Lücking 92-1020 (Lücking: Lich. Fol. Exs. 132, TNS); Limon Province: Cahuita National Park, "Kelly Creek" section, 140 km ESE of San José and 45 km SE of Limon at the Atlantic coast (N09°44', W82°50'), on leaves of undetermined dicotyledon, 0 m elev., October 1991, Lücking 91-2956 (Lücking: Lich. Fol. Exs. 133, TNS).

Specimens examined. JAPAN. RYUKYU ISLANDS (Okinawa Pref.): Haemida Beach, Iriomote Island, Yaeyama Islands (N24°16′24″, E123°49′54″) on *Arenga engleri*, 10 m elev., 21 November 2019, *K. Miyazawa 575 & Y. Ohmura* (TNS); along the stream at SE foot of Mt. Goza, Iriomote Island, Yaeyama Islands (N24°17′, E123°49′), on broad-leaf tree, 10 m elev., 11 March 2011, *Y. Ohmura 8037 & G. Tanaka* (TNS); ditto, on *Arenga engleri*, *Y. Ohmura 8058 & G. Tanaka* (TNS); along trail of Ohtomi-yuhodo, Iriomote Island, Yaeyama Islands (N24°17′58″, E123°51′45″), on *Arenga engleri*, 50 m elev., 18 November 2019, *K. Miyazawa 372 & Y. Ohmura* (TNS), *K. Miyazawa 549 & Y. Ohmura* (TNS), *K. Miyazawa 566 & Y. Ohmura* (TNS), *K. Miyazawa 567 & Y. Ohmura* (TNS), *K. Miyazawa 569 & Y. Ohmura* (TNS), *K. Miyazawa 570 & Y. Ohmura* (TNS), *K. Miyazawa 577 & Y. Ohmura* (TNS), *K.*

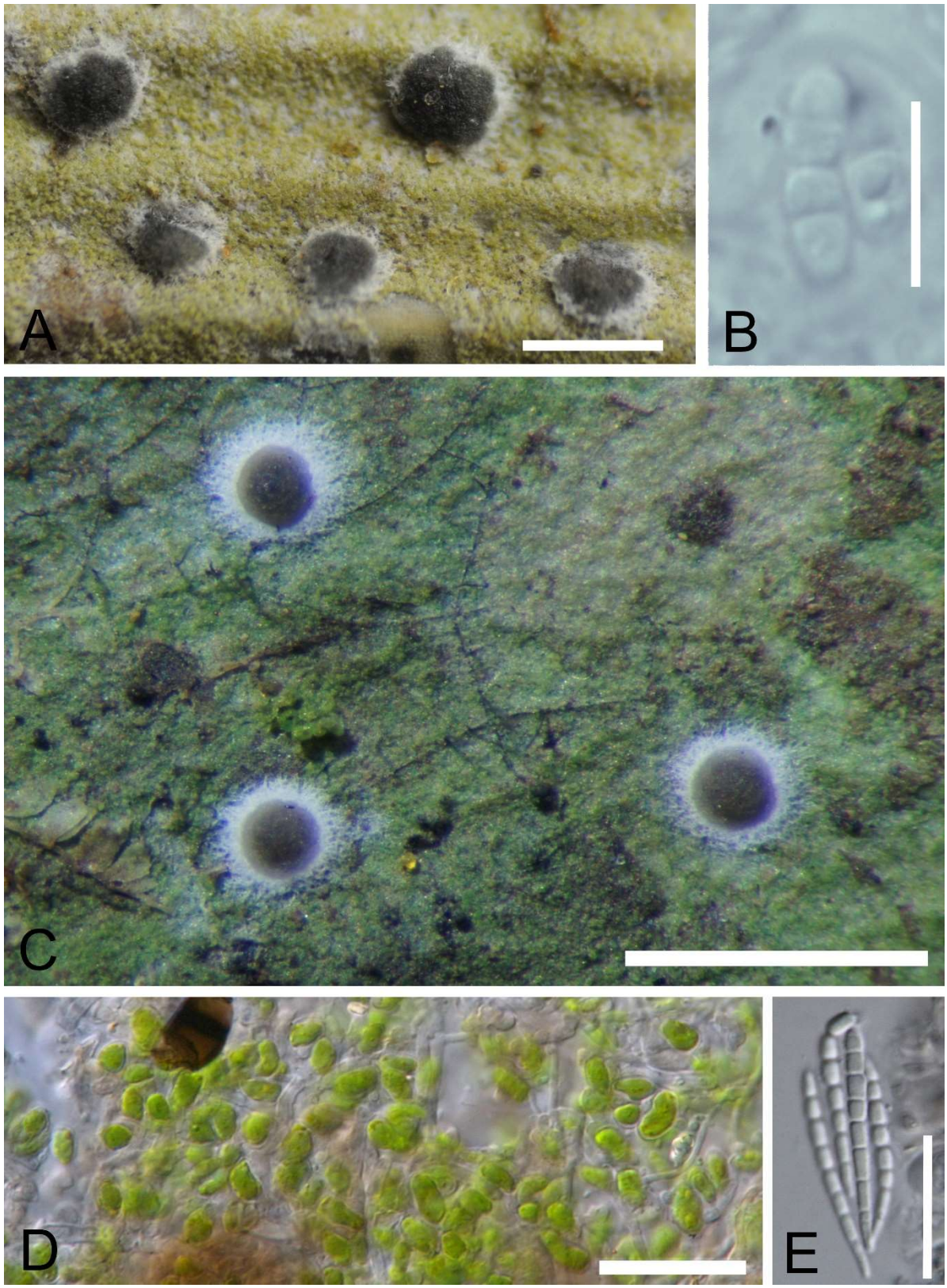

Fig. 1. ***Byssoloma chlorinum*** and ***B. vanderystii***. **A**. Thallus and apothecia of ***B. chlorinum*** (*K. Miyazawa 372 & Y. Ohmura*, TNS). **B**. Ascospore of ***B. chlorinum*** (*K. Miyazawa 372 & Y. Ohmura*, TNS). **C**. Thallus and apothecia of ***B. vanderystii*** (*K. Miyazawa 400 & Y. Ohmura*, TNS). **D**. Photobionts of ***B. vanderystii***. **E**. Ascospores of ***B. vanderystii***. Scales: A = 0.5 mm; B = 10 μm; C = 1 mm; D–E = 20 μm.

Miyazawa 580 & Y. Ohmura (TNS), *K. Miyazawa 581 & Y. Ohmura* (TNS); along the trail by Urauchi River, ca. 0.5 km NW of Maryudo Waterfall, Iriomote Island, Yaeyama Islands (N24°21′, E123°48′), on dead leaf of *Arenga engleri*, 80 m elev., 9 March 2011, *G. Tanaka 364 & Y. Ohmura* (TNS), *Y. Ohmura 7966 & G. Tanaka* (TNS).

Byssoloma vanderystii Sérus., Lichenologist 11: 181. 1979.

Fig. 1C–E

Thallus crustose, irregular in shape, continuous, ca. 25 mm across, 15–25 µm thick, smooth to minutely farinose, pale to dark greenish grey. Apothecia sessile to adnate, rounded, 0.2–0.6 mm diam., 150–200 µm tall; margin well-developed, densely byssoid, persistent, spreading laterally over thallus surface, 80–200 µm broad, white, composed of loosely woven colorless hyphae; disc slightly to strongly convex, chocolate-brown, with paler marginal zone; epithecium indistinct; hymenium 70–100 µm tall, colorless; hypothecium 30–40 µm tall, dark brown, K–; apothecial base dark brown, K–. Asci clavate, I+ blue, tholus amyloid (*Byssoloma*-type), 40–50 × 10–13 µm. Ascospores 8 per ascus, cylindrical, (6–)7-septate, with or without slight constrictions at septa, colorless, (22.0–)25.4–30.6(–31.0) × (2.0–)1.9–2.5(–3.0) µm (n = 20). Pycnidia subglobose, 60–140 µm diam., brownish black to black, covered by whitish loose tissue. Conidia bacillar, non-septate, colorless, (4.0–)4.1–4.7(–5.3) × (1.0–)1.1–1.3 µm (n = 30). Photobiont trebouxioid, ellipsoid, (4.6–)6.5–6.7(–8.1) × (2.2–)2.8–4.2(–5.3) µm (n = 30).

Chemistry: no secondary substances were detected by HPTLC.

Byssoloma vanderystii is characterized by convex apothecia with a well-developed byssoid margin spreading laterally over the thallus surface (Fig. 1C) and 7-septate ascospores (Fig. 1E).

The morphology of the Iriomote Island material was fundamentally consistent with the protologue and the description provided by Lücking (2008), except the size of the asci and photobiont. The ascus size of the Iriomote Island material (40–50 × 10–13 µm) is approximately half that (70–90×12–15 µm) reported by Lücking (2008). Although the ascus size is not mentioned in the protologue, it was estimated to be 50–60 × 14–18 µm based on the illustrations of the type specimen (Sérusiaux, 1979; Vězda, 1987). The ascus size seems to be highly variable in this species. However, whether this variability lies within the species or is attributed to *B. vandersytii* comprising different species is uncertain based on the current data. This study treats these differences as variation within a single species. The photobiont of Iriomote Island material is ellipsoid in shape (Fig. 1D), while that described in the protologue is spherical with a 5–10 µm diameter. The photobiont of Iriomote Island material could represent a different species from the algal taxon described in the protologue.

In addition to the identification based on morphology and chemistry, the BLAST results showed high identity (99%) to *B. vanderystii* sequences registered in GenBank (Table 1).

Within the genus *Byssoloma*, *B. braulioi* Lücking, *B. catarinense* L.I. Ferraro & Lücking, *B. octomerum* Malcolm & Vězda, *B. usambarense* Vězda, *B. syzygii* Vězda & Vivant, and *B. vanderystii* consistently have 7-septate ascospores. Among the six species, *B. vanderystii* is easily distinguished from others by a convex apothecia with a well-developed byssoid margin spreading laterally over the thallus surface.

Byssoloma vanderystii has been reported from Asia (Malaysia [Sipman, 1993], Thailand [Wang *et al.*, 2020], and Vietnam [Nguyen, 2010]), Oceania (Australia [Sipman, 1991]), North and South America (Argentina [Ferraro, 1997], Brazil, Costa Rica, French Guiana [Lücking, 2008]), and Africa (Democratic Republic of the Congo [type locality] [Sérusiaux, 1979; Van den Broeck *et al*., 2014]), showing a pantropical distribution (Lücking, 2008). This species is reported as new to Japan. At present, Iriomote Island is the northernmost point of the species.

Specimen examined. JAPAN. RYUKYU ISLANDS (Okinawa Pref.): along trail in the mountains along Urauchi river, Iriomote Island, Yaeyama Islands (N24°19′01″, E123°50′32″), on *Rhynchotechum discolor*?, 210 m elev., 19 November 2019, *K. Miyazawa 400 & Y. Ohmura* (TNS).

Gallaicolichen pacificus Sérus. & Lücking, Biblioth. Lichenol. 95: 510, 2007.

Figs. 2 & 3

Thallus crustose, epiphyllous, pale yellowish gray, rounded and usually coalescing with adjacent thalli to form irregular shape, 1.2–2.5 mm in diam., smooth or slightly uneven, up to 20 µm thick, composed of a loose network of interwoven hyphae and plates of photobiont cells, containing crystals (up to 50 × 30 µm); prothallus usually present, membranaceous and whitish. Peltidiangia, 1–6 per a patch of thallus, almost perfectly circular, 0.1–0.19 mm in diam. and 80–100 µm tall, formed by a rather thick erect margin with its inner part typically whitish and its outer part usually covered by the thallus. Peltidia numerous, usually filling up the peltidiangia cavity, disc-like, 20–30 µm in diam. and 10–15 µm thick, composed of fungal hyphae and algal cells of Trentepohliales, all linked to a central structure and coiled up inwards. Ascomata not seen. Conidiomata not seen. Photobiont Trentepohliales, greenish brown, cells 6–11 × 3–5 µm, regularly and radiately arranged rows in plates.

Chemistry: no secondary substances were detected by HPTLC.

Gallaicolichen pacificus is characterized by a pale yellowish gray rounded patched thallus with crystals (Fig. 2A, C), peltidiangia filled with peltidia (Figs. 2A, 3A, B), and photobiont of Trentepohliales (Fig. 2C).The peltidia size of the Iriomote Island materials is slightly smaller (20–30 µm in diam.) than that mentioned in the protologue

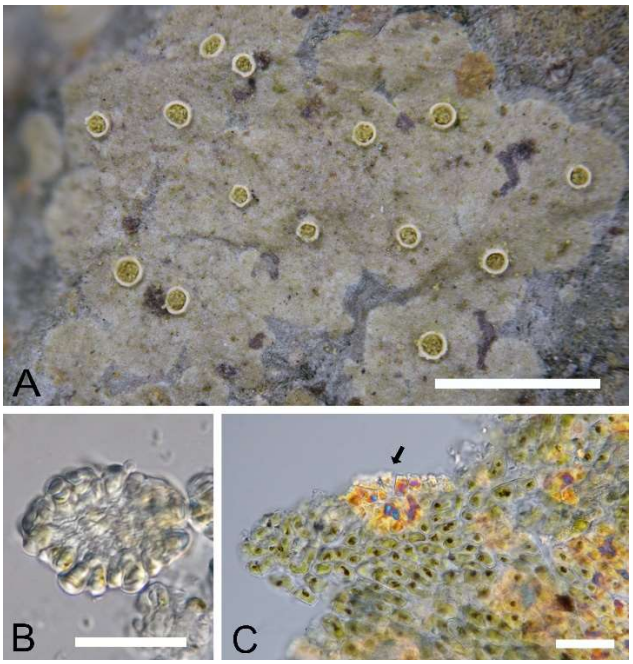

Fig. 2. ***Gallaicolichen pacificus*** collected from Iriomote Island. **A**. Thallus with peltidiangia (*Y. Ohmura 7952 & G. Tanaka*, TNS). **B**. Peltidium (*G. Tanaka 357 & Y. Ohmura*, TNS). **C**. Plate of photobionts and crystals (black arrow indicates) (*G. Tanaka 355 & Y. Ohmura*, TNS). Scales: A = 1 mm; B–C = 20 μm.

(25–55 μm) (Figs. 2B, 3B), but is considered within the range of variation because the ranges partially overlap. As the ascomata and conidiomata of this genus have not been found to date, the taxonomic position under the phylum Ascomycota is still unclear (Sérusiaux and Lücking, 2007; Wijayawardene *et al*., 2018). The mtSSU sequence from the Iriomote Island materials could not be obtained in this study.

Gallaicolichen pacificus is from the southeast Pacific region: Australia (Queensland), Vanuatu, New Caledonia, and Hawaii (Sérusiaux and Lücking, 2007). The distribution is now extended to the west side of the Pacific Ocean as the northern limit. This species is reported as new to Asia.

Specimens examined. JAPAN. RYUKYU ISLANDS (Okinawa Pref.): along mountain path between Ohtomi-guchi Entrance and Daiichi Yamagoya-ato, Iriomote Island, Yaeyama Islands (N24°19′49″, E123°51′04″), on *Lasianthus attenuatus*, 180 m elev., 19 November 2019, *K. Miyazawa 492C & Y. Ohmura*; along the trail by Urauchi River from the landing point to Maryudo Waterfall, Iriomote Island, Yaeyama Islands (N24°21′, E123°47′), on *Arenga engleri*, 60 m elev., 9 March 2011, *G. Tanaka 355 pr.p. & Y. Ohmura*, (in collection of *Porina kamerunensis*) (TNS); ditto, *G. Tanaka 356 pr.p. & Y. Ohmura*, (in collection of *Fellhanera bouteillei*) (TNS); ditto, *G. Tanaka 357 pr.p. & Y. Ohmura* (in collection of *Asterothyrium* cf. *decipenes*) (TNS); ditto, *Y. Ohmura 7950 pr.p. & G. Tanaka* (in collection of *Coenogonium subluteum*) (TNS); ditto, *Y. Ohmura 7952 & G. Tanaka* (TNS); along the stream at SE foot of Mt. Goza, Iriomote Island, Yaeyama Islands (N24°17′, E123°49′), on *Lasianthus cyanocarpus*, 10 m elev., 11 March 2011, *G. Tanaka 432 pr.p. & Y. Ohmura* (in collection of *Calopadia* sp.) (TNS); ditto, *Y. Ohmura 8053 pr.p. & G. Tanaka* (in collection of *Mazosia bambusae*) (TNS); ditto, on *Lasianthus cyanocarpus*, *Y. Ohmura 8080 pr.p. & G. Tanaka* (in collection of *Byssoloma leucoblepharum*) (TNS); ditto, on *Lasianthus cyanocarpus*, *Y. Ohmura 8081 pr.p. & G. Tanaka* (in collection of *Calopadia puiggarii*) (TNS).

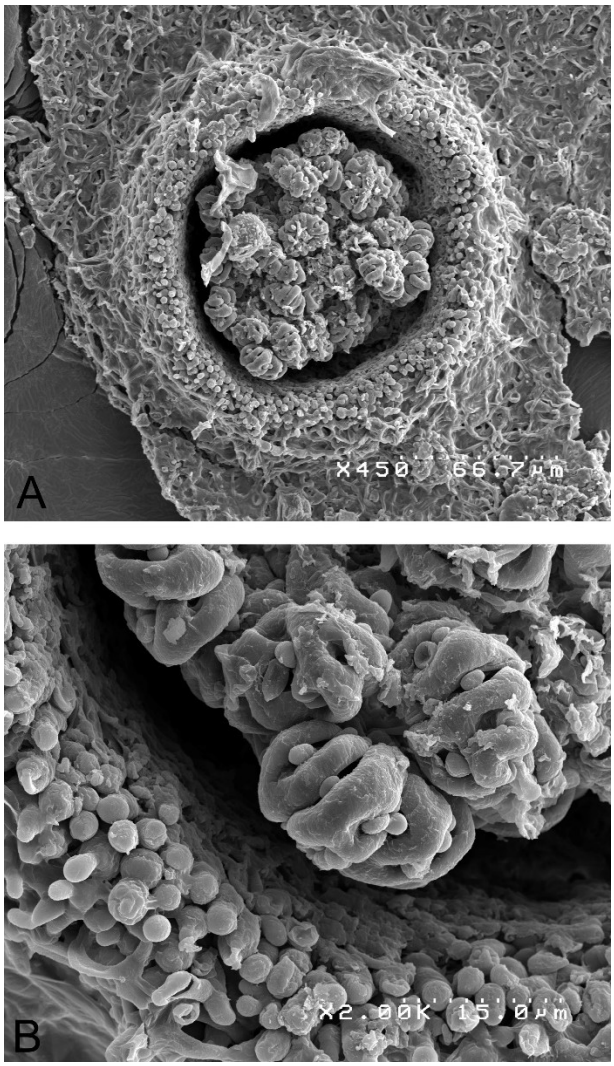

Fig. 3. SEM images of ***Gallaicolichen pacificus*** (*Y. Ohmura 7952 & G. Tanaka*, TNS). **A**. Peltidiangium with many peltidia. **B**. Margin of peltidiangium and peltidia.

Species new to Iriomote Island

Byssoloma minutissimum Kalb & Vězda, Nova Hedwigia 51: 445. 1990.

Thor *et al*. (2000) reported *B. minutissimum* from Ishigaki Island in Japan as new to the Paleotropics but showed that the ascospores size range is broader in width than usual (10–12 × 2.5–3.5 μm vs. 2–2.5 μm). The ascospore size range of the Iriomote Island material is even greater [(10–)10.8–13.2(–16) × (2.5–)2.7–3.9(–5.5) μm (n = 50)]. The wider size range of ascospores was treated as a variation within this species in Thor *et al*. (2000). Our study follows their treatment, and *B. minutissimum* is reported as new to Iriomote Island. However, further taxonomic study treating on this variation is needed after sufficient materials have been examined.

Specimen examined. JAPAN. RYUKYU ISLANDS (Okinawa Pref.): along the trail in the mountains along Urauchi river, Iriomote Island, Yaeyama Islands (N24°18′42″, E123°50′24″), on leaf

of *Cleyera japonica*, 220 m elev., 19 November 2019, *K. Miyazawa 387 & Y. Ohmura* (TNS).

Fellhanera rhapidophylli (Rehm) Vězda, Folia Geobot. Phytotax. 21: 214. 1986.

In Japan, *Fellhanera rhapidophylli* has been reported only once from Yakushima Island and the main island of Okinawa (Thor *et al*., 2000).

Specimen examined. JAPAN. RYUKYU ISLANDS (Okinawa Pref.): along the trail by Urauchi River from the landing point to Maryudo Waterfall, Iriomote Island, Yaeyama Islands (N24°21′, E123°47′), on *Cleyera japonica*, 60 m elev., 9 March 2011, *G. Tanaka 353 pr.p. & Y. Ohmura* (in collection of *Mazosia bambusae*) (TNS).

Additional data on the list of threatened species in Japan

Thirteen species in the Red List for Japanese species including DD (Ministry of the Environment, 2020) were identified among the materials collected in this study. Among these species, eight are treated as NT and five as DD (Appendix 1). The list was formerly evaluated based mainly on the rarity of the species. However, more quantitative evaluations are needed to clarify the rank of each species strictly according to the IUCN criteria. Our additional data in this study will contribute to future quantitative evaluation of red-listed species in Japan.

Byssoloma annuum (Vain.) G. Thor, Lücking & Tat. Matsumoto

In addition to the morphological similarities, the BLAST results showed high identity (99%) to *B. annuum* sequences in GenBank (Table 1).

Specimens examined. JAPAN. RYUKYU ISLANDS (Okinawa Pref.): along mountain path between Ohtomi-guchi Entrance and Daiichi Yamagoya-ato, Iriomote Island, Yaeyama Islands (N24°19′26″, E123°50′49″), on broad-leaf tree, 190 m elev., 19 November 2019, *K. Miyazawa 451 pr.p. & Y. Ohmura* (in collection of *Sporopodium flavescens*) (TNS); along trail in the mountains along Urauchi river, Iriomote Island, Yaeyama Islands (N24°20′26″, E123°49′58″), on *Arenga engleri*, 160 m elev., 19 November 2019, *K. Miyazawa 501 & Y. Ohmura* (TNS); ditto (N24°20′52″, E123°49′24″), on *Arenga engleri*, 100 m elev., 20 November 2019, *K. Miyazawa 558 & Y. Ohmura* (TNS), *K. Miyazawa 583 & Y. Ohmura* (TNS).

Eugeniella micrommata (Kremp.) Lücking, Sérus. & Kalb

In addition to the morphological similarities, the BLAST results showed high identity (99%) to an *Eugeniella micrommata* sequence in GenBank (Table 1).

Specimens examined. JAPAN. RYUKYU ISLANDS (Okinawa Pref.): along mountain path between Ohtomi-guchi Entrance and Daiichi Yamagoya-ato, Iriomote Island, Yaeyama Islands (N24°19′01″, E123°50′32″), on broad-leaf tree, 210 m elev., 19 November 2019, *K. Miyazawa 403 & Y. Ohmura* (TNS); ditto, (N24°19′26″, E123°50′49″), on broad-leaf tree, 190 m elev., 19 November 2019, *K. Miyazawa 437 pr.p. & Y. Ohmura* (in collection of *Sporopodium flavescens*) (TNS), *K. Miyazawa 450 pr.p. & Y. Ohmura* (in collection of *Sporopodium flavescens*) (TNS).

Porina kamerunensis F. Schill.

Although Thor *et al*. (2000) reported Japanese specimens collected from Ishigaki Island and Iriomote Island as *Porina chrysophora* (Stirt.) R. Sant., Lücking (2008) concluded that the specimens are *P. kamerunensis* (as *P. kameruniensis*). This study follows the same treatment.

The ascospore size of the Iriomote Island materials was (19.0–)19.7–21.8(–22.0) × (2.5–)2.8–3.5(–4.0) µm (n = 15). This is larger than that of the protologue (13–15.6 × 2–2.6 µm, Cameroon) and the description of Lücking (2008) (14–20 × 2.5–4 µm, the Neotropics) but is smaller than the description of Vězda (1997) (18–26 × 3.5–4.5 µm, Vietnam, reported as *P. chrysophora*). Thor *et al*. (2000), who examined Japanese materials of *Porina kamerunensis* (as *P. chrysophora*), reported a similar measurement to that in this study [(19–)20–23(–25) × (3–)3–4(–4) µm)]. Although the ascospore size differs by region, we tentatively consider these differences to be intraspecific variations because they are continuous.

Since the sequence of *P. kamerunensis* was not registered in GenBank, the BLAST result showed less than 93% identity to the sequences of other *Porina* species (Table 1).

Specimens examined. JAPAN. RYUKYU ISLANDS (Okinawa Pref.): along trail of Ohtomi-yuhodo, Iriomote Island, Yaeyama Islands (N24°18′08″, E123°51′31″), on *Asplenium setoi*, 30 m elev., 18 November 2019, *K. Miyazawa 376 pr.p. & Y. Ohmura* (in collection of *Coenogonium subluteum*) (TNS); along mountain path between Ohtomi-guchi Entrance and Daiichi Yamagoya-ato, Iriomote Island, Yaeyama Islands (N24°19′01", E123°50′32"), on broad-leaf tree, 210 m elev., 19 November 2019, *K. Miyazawa 400 pr.p. & Y. Ohmura* (in collection of *Byssoloma vanderystii*) (TNS); along mountain path between Daini Yamagoya-ato and Kambire-guchi Entrance, Iriomote Island, Yaeyama Islands (N24°20′52″, E123°48′59″), on broad-leaf tree; 100 m elev., 20 November 2019, *K. Miyazawa 504 & Y. Ohmura* (TNS), *K. Miyazawa 517 & Y. Ohmura* (TNS); along mountain path between Itachikigawa River Junction and Daini Yamagoya-ato, Iriomote Island, Yaeyama Islands (N24°20′52″, E123°49′18″), on broad-leaf tree, 100 m elev., 20 November 2019, *K. Miyazawa 511 pr.p. & Y. Ohmura* (in collection of *Porina cupreola* var. *nipponica*) (TNS); along the trail by Urauchi River from the landing point to Maryudo Waterfall, Iriomote Island, Yaeyama Islands (N24°21′, E123°47′), on *Arenga engleri*, 60 m elev., 9 March 2011, *G. Tanaka 355 & Y. Ohmura* (TNS); Haemida Beach, Iriomote Island, Yaeyama Islands (N24°16′, E123°49′), on *Ipomoea* sp., 10 m elev., 10 March 2011, *Y. Ohmura 8029 pr.p. & G. Tanaka* (in collection of *Coenogonium subluteum*) (TNS).

Semigyalecta paradoxa Vain.

Fig. 4A

The Iriomote Island material exhibited thalli composed of brown branched hyphae of the symbiotic fungus and plates of symbiotic algae (Fig. 4A). The plates of symbiotic algae were composed of irregular and usually elongate cells, approximately 3–10 × 2.5–3 µm (Fig. 4A). This observation is more or less similar to the description provided by Santesson (1952) (~3–5 × 2–4 µm). Although it is debated whether *Semigyalecta paradoxa* is lichenized fungus (Santesson, 1952; Kalb and Vězda, 1994; Thor *et al*., 2000; Wang and Wei, 2018), our observation implies that it is lichenized.

Specimen examined. JAPAN. RYUKYU ISLANDS (Okinawa Pref.): along trail in the mountains along Urauchi river,

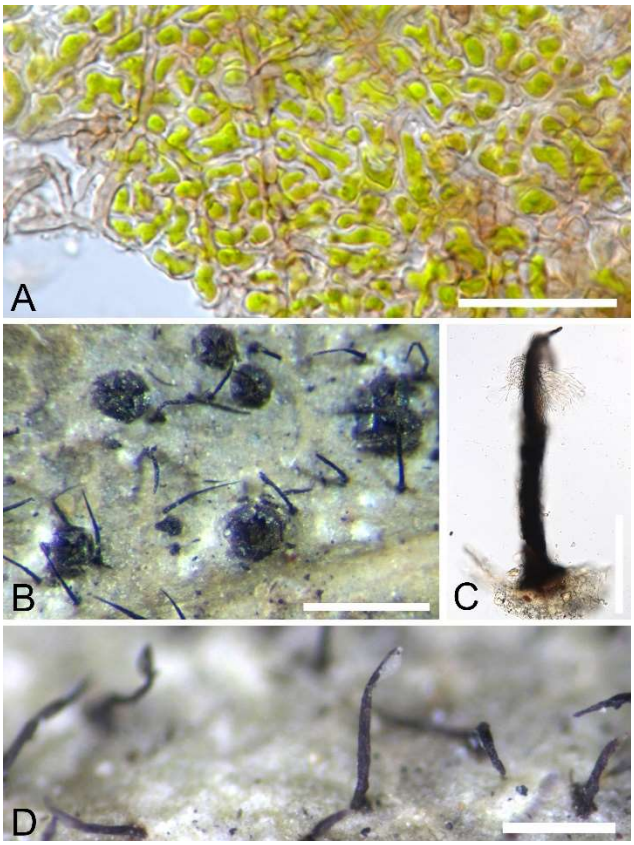

Fig. 4. ***Semigyalecta paradoxa*** and ***Tricharia kashiwadanii***. **A**. Thallus of ***S. paradoxa*** composed of brown branched hyphae of the mycobiont and plates of the photobiont (*K. Miyazawa 436 & Y. Ohmura*, TNS). **B**. Thallus with apothecia of ***T. kashiwadanii*** (holotype, TNS). **C**. Black hyphophore with diahyphae of ***T. kashiwadanii*** (holotype, TNS). **D**. Black hyphophores on the thallus of ***T. kashiwadanii*** (holotype, TNS). Scales: A = 20 μm; B = 0.5 mm; C = 100 μm; D = 0.2 mm.

Iriomote Island, Yaeyama Islands (N24°19′26″, E123°50′49″), on herbaceous plant, 190 m elev., 19 November 2019, *K. Miyazawa 436 & Y. Ohmura* (TNS).

Tricharia kashiwadanii G. Thor, Lücking & Tat. Matsumoto **Fig. 4B–D**

Hyphophores and diahyphae of *T. kashiwadanii* were found in the type specimen and in other material collected in this study, although they were not described in the protologue. Their features based on these specimens are described as follows. Hyphophores are abundant, black, setiform with an acute apex and up to 0.3 mm tall (Fig. 4C, D). Diahyphae are inserted apically, branched throughout, moniliform, fusiform to sausage-shaped, and 5–9 × 1–2 μm segments (Fig. 4C).

The BLAST results show less than 90% identity to *Gomphilloideae* (including *Tricharia*) sequences in GenBank (Table 1). This low homology is due to the absence of registered sequences for this species in GenBank.

Specimen examined. JAPAN. RYUKYU ISLANDS (Okinawa Pref.): along trail in the mountains along Urauchi river, Taketomi-cho, Iriomote Island, Yaeyama Islands (N24°19′01″, E123°50′32″), on broad-leaf tree, 210 m elev., 19 November 2019, *K. Miyazawa 405 & Y. Ohmura* (TNS).

Additional specimen examined. JAPAN. RYUKYU ISLANDS (Kagoshima Pref.): 7 km SW of Nishinakama village, 100–200 m N of Yakugachi River, near the road, Suniyo-cho, Oshima-gun, Amami Islands, (N28°13′, E129°21′), on *Quercus* sp., 60–80 m elev., 5 February 1995, *G. Thor 12987* (holotype, TNS).

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Supplementary materials are available from Journal Website.