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Highlighted Student Research

Racoleus japonicus sp. nov. (Teratosphaeriaceae, Ascomycota), a new sterile filamentous lichen collected from Japan

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ABSTRACT: *Racoleus japonicus* is described as a new species. It is characterized by a blackish brown minutely filamentous thallus with lateral spines, vertical arrangement of hyphae with uneven and undulate to corrugated hyphal walls, and a *Trentepohlia* photobiont. In this genus, only *R. trichophorus* was known before the present study. *Racoleus japonicus* is distinguished from that species by having broader filaments (10–15 μm wide), larger hyphal cells (10–20 × 3–7 μm), and lateral sinuous spines. It was collected on shady rocks from northern to southern Japan at elevations between 290 and 1700 m. A molecular phylogeny based on nuSSU, nuLSU and mtSSU inferred that *R. japonicus* has a close relationship with unidentified fungi of Capnodiales, and that is not related to *Cystocoleus* and *Racodium* which have similar filamentous thalli. Based on the molecular phylogenetic analyses, *R. japonicus* is classified within Teratosphaeriaceae.

KEY WORDS: Capnodiales, *Cystocoleus*, lichenized fungi, mtSSU, nuLSU, nuSSU, *Racodium, Trentepohlia*.

INTRODUCTION

The genera *Cystocoleus* Thwaites, *Racodium* Fr. and *Racoleus* R. Sant. & D. Hawksw. are known as sterile filamentous lichens that are characterized by blackish brown fungal hyphae surrounding a filamentous green alga of the genus *Trentepohlia*. According to Hawksworth *et al*. (2011), each genus is characterized by the following morphology: *Cystocoleus* has a twisted arrangement of hyphae with a corrugated hyphal wall; *Racodium* has a vertical arrangement of hyphae with a straight hyphal wall; and *Racoleus* has a vertical arrangement of hyphae with a corrugated wall. Furthermore, by scanning electronic microscopy, a warted ornamentation on the surface of hyphal wall is observed only in *Cystocoleus* (vs. smooth surface in *Racodium* and *Racoleus*). Emphasizing the lateral spines as a feature of *Racoleus* by Hawksworth *et al*. (2011) will be discussed later in this paper. These genera were considered to belong to Capnodiales because of the similar appearance in morphology (Hawksworth *et al*., 2011). Abdollahzadeh *et al*. (2020) delimited Capnodiales *s. str.* and transferred *Cystocoleus* to Mycosphaerellales and *Racodium* to Racodiales, but those authors did not mention *Racoleus*.

Cystocoleus and *Racodium*, but not *Racoleus*, were analyzed with other related fungi based on nuSSU, nuLSU and mtSSU sequence data (Muggia *et al*., 2008) or by using nuLSU, ITS rDNA, *TEF-1α*, and *RPB2* sequences (Abdollahzadeh *et al*., 2020). The molecular phylogenetic analyses of those authors suggested that the relationship between *Cystocoleus* and *Racodium* is not sister and that the lichenization occurred independently in each group within Capnodiales *s. lat.*, a group of fungi in which other taxa are non-lichenized (i.e., saprophytes, parasites, ectophytes, or epiphytes; Abdollahzadeh *et al*., 2020). While the taxonomic position of *Cystocoleus* and *Racodium* has been discussed based on molecular phylogeny and morphological data, the position of *Racoleus* remains unclear due to a lack of DNA sequences.

While examining the Japanese sterile filamentous lichens housed in the herbarium of the National Museum of Nature and Science (TNS), Tsukuba, Japan, several specimens were recognized as an undescribed species of the genus *Racoleus*. The aim of this study is to describe and illustrate the new species, *Racoleus japonicus*, and to infer its phylogenetic position based on molecular data.

MATERIALS AND METHODS

Morphology and chemistry

All voucher specimens examined in this study are housed in the herbarium of the National Museum of Nature and Science (TNS), Tsukuba, Japan.

Morphological observations and photography were performed using a dissecting microscope (SZX16; Olympus) and a differential interference contrast microscope (BX53; Olympus) with a digital camera (EOS Kiss X10i; Canon). Anatomical examinations were performed using hand-cut sections mounted in GAW (glycerin: ethanol: water = 1: 1: 1) solution (Asahina, 1936).

Color spot tests for K, C, KC, and Pd were performed according to Orange *et al*. (2001). Secondary substances were examined using high-performance thin layer

Table 1. Collections/strains and their GenBank accession numbers. New sequences obtained in this study are in bold.

Taxa	Collection/strain No.	nuSSU	nuLSU	mtSSU	Reference
Capnodiales sp.	A557	KT263481	KT263447	KT263516	Muggia *et al*., 2016
	A571	KT263492	KT263457	KT263527	Muggia *et al*., 2016
	A577	KT263494	KT263459	KT263529	Muggia *et al*., 2016
	A863	KT263485	KT263451	KT263520	Muggia *et al*., 2016
	A886	KT263488	KT263453	KT263523	Muggia *et al*., 2016
	A913	KT263484	KT263450	KT263519	Muggia *et al*., 2016
	A951	KT263482	KT263448	KT263517	Muggia *et al*., 2016
	A959	KT263487	KT263460	KT263522	Muggia *et al*., 2016
	A960	KT263486	KT263452	KT263521	Muggia *et al*., 2016
	A1043	KT263480	KT263446	KT263515	Muggia *et al*., 2016
Capnodium coffeae	CBS 147.52	DQ247808	DQ247800	FJ190609	Schoch *et al*., 2006a, 2009
Cladosporium cladosporioides	CBS 170.54	DQ678004	DQ678057	FJ190628	Schoch *et al*., 2006b, 2009
Cystocoleus ebeneus	L161	EU048571	EU048578	EU048584	Muggia *et al*., 2008
	L348	EU048573	EU048580	EU048586	Muggia *et al*., 2008
Friedmanniomyces endolithicus	CCFEE 522 & 524	DQ066715	GU250364	GU250409	Selbmann *et al*., 2005
Mycosphaerella fijiensis	OSC 100622	DQ767652	DQ678098	FJ190656	Schoch *et al*., 2006a, 2009
Myriangium duriaei	CBS 260.36	AY016347	AY016365	AY571389	Lumbsch and Lindemuth, 2001; Lumbsch *et al*., 2005
Neodevriesia strelitziae	CBS 122379	GU296146	GU301810	GU561845	Ruibal *et al*., 2009; Schoch *et al*., 2009
Petrophila incerta	TRN 62	GU323991	GU323961	GU324022	Ruibal *et al*., 2009
	TRN 77	GU323993	GU323963	GU324024	Ruibal *et al*., 2009
Racodium rupestre	L346	EU048575	EU048583	EU048588	Muggia *et al*., 2016
	L424	EU048577	EU048582	EU048589	Muggia *et al*., 2016
Racoleus japonicus	YO9473	**LC779815**	**LC779821**		**This study**
	YO9651 (Holotype)	**LC779816**	**LC779822**	**LC779827**	**This study**
	KeM1561	**LC779817**	**LC779825**		**This study**
	KeM1700	**LC779818**	**LC779823**		**This study**
	KeM1701	**LC779819**	**LC779824**	**LC779828**	**This study**
	KeM1702	**LC779820**	**LC779826**	**LC779829**	**This study**
Ramularia punctiformis	CBS 113265	DQ471017	DQ470968	FJ190611	Spatafora *et al*., 2006; Schoch *et al*., 2009
Recurvomyces mirabilis	CCFEE 5475	KC315865	KC315876	KC315887	Unpublished
Scorias spongiosa	CBS 325.33	DQ678024	DQ678075	FJ190643	Schoch *et al*., 2006a, 2009

chromatography (HPTLC) following Schumm and Elix (2015). Solvent system B′ (*n*-hexane: methyl *tert*-butyl ether: formic acid, 140: 72: 18) (Culberson and Johnson, 1982) was used for HPTLC. The spot color was checked under 254 and 366 nm UV light and visible light, before and after spraying with 10% sulfuric acid on the HPTLC plate and charring at 90°C for 20 minutes.

DNA extraction, PCR amplification and sequencing

DNA was extracted from fresh material collected within one year according to a modified method of Izumitsu *et al.* (2012) (see also Miyazawa *et al*., 2022) or a modified CTAB protocol (Hosaka, 2009). The voucher specimens for DNA extractions are housed in TNS.

For PCR amplification, 10 μL of PCR mix contained 1 μL genomic DNA extraction, 0.25 μL of each primer (10 pmol/μL) and 5 μL EmeraldAmp® MAX PCR Master Mix (TaKaRa Bio Inc.). The partial sequences of the small subunit and the large subunit of the nuclear ribosomal RNA gene (nuSSU and nuLSU) and of the small subunit of the mitochondrial ribosomal RNA gene (mtSSU) were amplified with the primer sets nuSSU0021 (Gargas and DePriest, 1996) and nuSSU0852 (Gargas and Taylor, 1992) for nuSSU, LIC24R (Miadlikowska and Lutzoni, 2000) and LR7 or LR3 (Vilgalys and Hester, 1990) for nuLSU, and mrSSU1 and mrSSU3R (Zoller *et al*., 1999) for mtSSU, according to the modified PCR conditions of Frisch *et al*. (2014) (45 cycles to 35 cycles) using an Applied Biosystems Veriti® 96-Well Thermal Cycler (Thermo Fisher Scientific). The PCR products were purified with illustra™ ExoProStar™ (GE HealthCare). 1.2 μL of PCR products were incubated with 0.2 μL ExoProStar™ and 0.5 μL ddH_2Oat 37°C for 30 minutes and then at 80°C for 15 minutes.

DNA sequencing was performed on an Applied Biosystems™ 3500xL Genetic Analyzer (Thermo Fisher Scientific) using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) following the manufacturer's instructions. The taxon name and the GenBank accession numbers for the obtained sequences are shown in Table 1.

Molecular phylogenetic analyses

The newly obtained nuSSU, nuLSU and mtSSU sequences of *Racoleus japonicus* from the Japanese material were aligned with sequences of selected taxa in

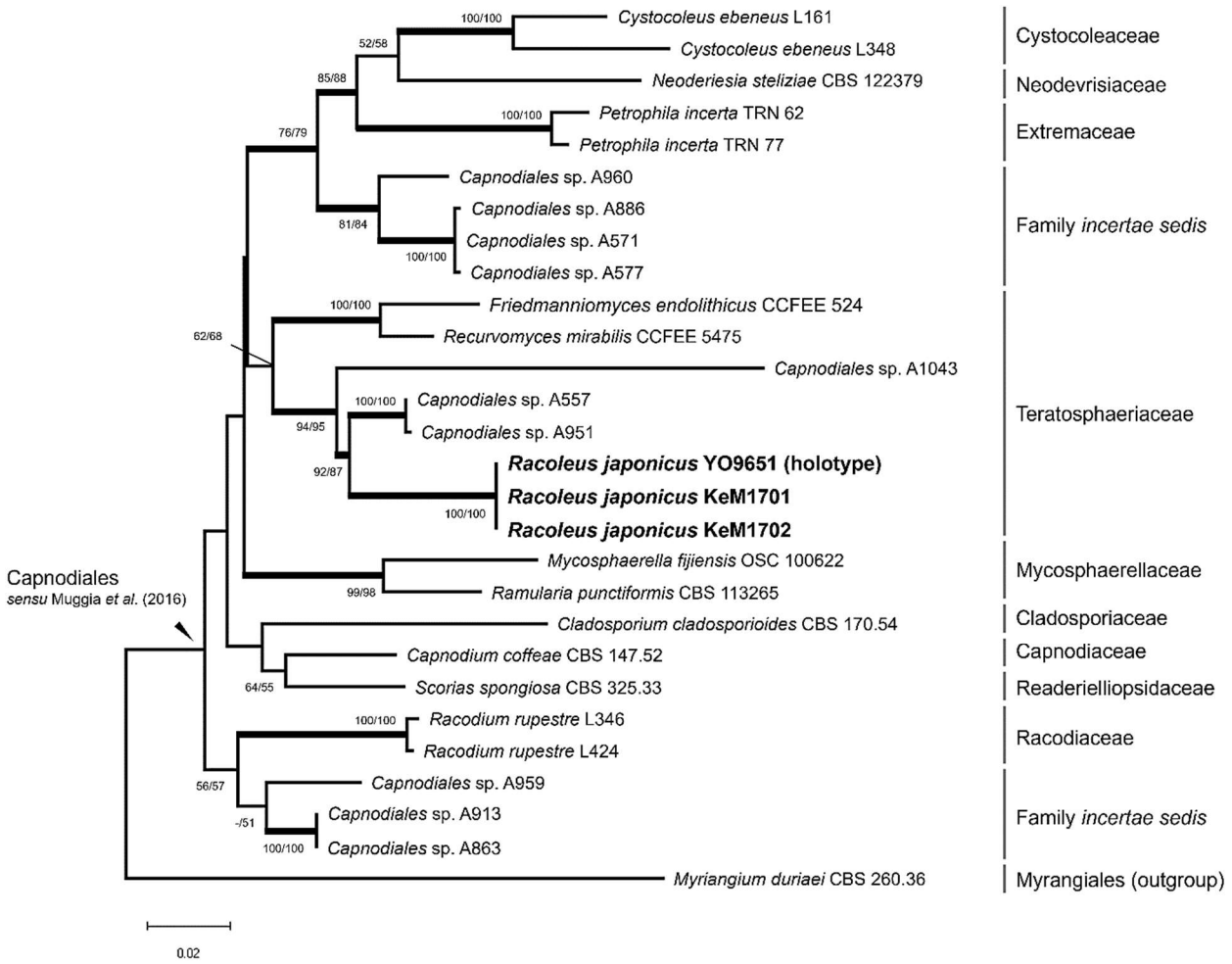

Fig. 1. Maximum likelihood tree of selected taxa in **Capnodiales** *s. lat.* showing the phylogenetic position of ***Racoleus japonicus*** collected from Japan (in bold). ***Myriangium duriei*** is used as the outgroup. NJ and ML support values are presented for each node. Branches highly supported (≥70) by both analyses are indicated with bold black lines.

Capnodiales *s. lat.* from GenBank (Table 1) in MAFFT ver. 7 (Katoh *et al*., 2019) using the default settings. Taxa included in the alignment, including *Myriangium duriaei* (Myrangiales) as the outgroup (see Table 1), were selected according to the BLAST results in GenBank and previous phylogenetic studies (Muggia *et al*., 2008, 2016; Abdollahzadeh *et al*., 2020). Each single locus data set (nuSSU, nuLSU and mtSSU) was separately aligned. After removing sites with gaps, missing or ambiguous data, the initial molecular phylogenetic trees were reconstructed based on each single locus, then the data sets were concatenated. The final alignment of 1153 sites was used for the molecular phylogenetic analyses.

Neighbor-joining (NJ) and maximum likelihood (ML) analyses were performed using the Tamura-Nei model (Tamura and Nei, 1993) plus gamma distribution with invariant sites (G + I), which was selected as the best fitting model based on the lowest Bayesian information criterion score. The bootstrap values (≥50%) of 1,000 replicates for NJ and ML are shown on each branch. All calculations were conducted in MEGA X (Kumar *et al*., 2018).

RESULTS AND DISCUSSION

Molecular analysis

Among the aligned sites of each locus within the Japanese material of *Racoleus japonicus*, those of nuSSU (857 sites) had ten variable sites including singleton and/or parsimony-informative sites (98.9–100% identity among six specimens), those of nuLSU (1285 sites) had twelve variable sites and one gap site (99.4–99.9% identity among six specimens), and those of mtSSU (826 sites) had no variable sites or gaps (100% identity among three specimens).

Before conducting the phylogenetic analysis with combined sequences of three loci, each locus was separately analyzed. The phylogenetic tree based on nuSSU could not resolve the relationships among the families within Capnodiales *s. lat*. However, each tree based on nuLSU or mtSSU shows supportable bootstrap values on some branches in the trees.

The concatenated phylogenetic tree based on three loci for *Racoleus japonicus* within Capnodiales *s. lat.* is

shown in Fig. 1. The topology of our phylogenetic ML tree fundamentally shows no conflict with those of Muggia *et al*. (2008, 2016) and Abdollahzadeh *et al*. (2020), and the bootstrap values were higher than those in the separate analysis of each locus.

The monophyly of *R. japonicus* is confirmed with high support values (NJ/ML = 100/100). This new species shows a close phylogenetic relationship with unidentified fungi of Capnodiales *s. lat.* (A557 and A951 in Fig. 1) that were collected from alpine rock lichen communities (Muggia *et al*. 2016) which is supported by relatively high support values (NJ/ML = 92/87, Fig. 1). These lichen-associated fungi of Capnodiales *s. lat.* were treated under Teratosphaeriaceae (Cometto *et al*., 2023). This clade and other fungi of Teratosphaeriaceae including *Friedmanniomyces endolithicus* and *Recurvomyces mirabilis* form a common clade that is not well-supported in our analyses (NJ/ML = 62/68, Fig. 1). Regardless, based on the currently available sequence data and phylogenetic analyses, we consider it is appropriate to place *R. japonicus* in Teratosphaeriaceae. Furthermore, our phylogenetic analyses revealed that *Racoleus* is not closely related to *Cystocoleus* (Cystocoleaceae) and *Racodium* (Racodiaceae) despite having extremely similar appearance in morphology with their unusual, sterile, filamentous thalli. Although they all belong to a broadly defined Capnodiales *s. lat.*, each genus appears to represent an independent lichenization event.

Teratosphaeriaceae has been delimited based on phylogenetic analysis of nuLSU sequence data (Crous *et al*., 2007), and presently does not have well defined phenotypic synapomorphies. However, within this family, there are taxa with similar traits to *R. japonicus* such as the brown fungal filaments (Crous *et al*., 2007; Si *et al*., 2023) and living in harsh environments like rock surfaces (Ruibal *et al*., 2008, 2009).

Because DNA sequences of the type species of *Racoleus*, *R. trichophorus* R. Santt. & D. Hawksw., have not been obtained, further study is needed to confirm the monophyly including both *R. trichophorus* and *R. japonicus*. However, based on the morphological features described and discussed below, we consider the new species belongs to *Racoleus*.

TAXONOMIC TREATMENT

Racoleus japonicus K. Miyaz. & Y. Ohmura, ***sp. nov.***
Figs. 2 & 3C,F

MycoBank No.: MB 850089

Japanese common name: *Iwa-goke*.

Similar to *Racoleus trichophorus* but differs by wider filaments (10–15 μm vs. 7–9 μm wide in *R. trichophorus*), larger hyphal cells (3–7 μm vs. 2–3 μm wide in *R. trichophorus*), and irregularly sinuous lateral spines.

***Type*: JAPAN.** Honshu. Shinano Prov. (Nagano Pref.): Azusayama, Kawakami-mura, Minamisaku-gun (N35°57′, E138°40′), 1370 m elev., on rock, 26 May 2013, *Y. Ohmura 9651, K. Yoshida & A. Frisch* [TNS-L-132529, holotype: TNS, isotypes: B, BG, BM, BRY, C, CANB, CANL, DUKE, F, FH, G, GZU, H, KRAM, LIV, M, MIN, MVM, NY, O, S, TAI, TSB, TUR, UPS, US, W, and hb. Kalb, distributed to each herbarium as an exsiccata specimen of Y. Ohmura: Lich. Minus Cogn. Exs. 494 as "*Racodium rurestre* Dill." in Ohmura (2014)].

THALLUS minutely filamentous, blackish brown, forming a dense fluffy colony reaching up to ca. 30 mm diam. Filaments suberect to decumbent or spreading on the surface of substrate, occasionally sympodially branched, 10–15(–20) μm wide, with lateral spines; outer wall undulating and irregularly corrugated, reflecting the morphology of the fungal hyphae. HYPHAE surrounding the algal filaments in a single layer, orientated vertically along and parallel to the axis of the filament, brown, 3–7 μm wide, septate; the septa generally 10–20 μm apart, thick-walled; walls uneven and undulate to corrugated, corrugations tending to interlink with those to adjacent hyphae. SPINES arising at broadly acute to almost right angles from the vertical axis, brown, septate, stiff, irregularly sinuous, up to 150 μm in length and 2–3 μm wide, the base expanded into a foot like cell (12–15 × 4–5 μm). CONIDIOGENOUS CELLS and CONIDIA not observed. PHOTOBIONT *Trentepohlia* sp., in single filaments surrounded by fungal hyphae; cells rounded rectangular, 25–50 × 8–10 μm.

Chemistry: C–, K–, KC–, Pd–. No secondary substances were detected by HPTLC.

Etymology: The epithet '*japonicus*' refers to Japan where the new species was collected.

Habitat and distribution: This species grows on shady rocks or rock walls (Fig. 2A) in subboreal to temperate regions of Japan (i.e., from Hokkaido to Kyushu) at elevations between 290 and 1700 m.

Remarks: *Racoleus japonicus* closely resembles *R. trichophorus* by its dense, minutely filamentous, blackish brown fluffy colonies (Fig. 2B), that are formed by sympodially branching filaments (Fig. 2C) with corrugated hyphal walls (Fig. 2C). However, *R. japonicus* differs from *R. trichophorus* in wider filaments (10–15 μm vs. 7–9 μm wide in *R. trichophorus*), larger hyphal cells (3–7 μm vs. 2–3 μm wide in *R. trichophorus*), and irregularly sinuous lateral spines (Figs. 2D, 3F; vs. straight in *R. trichophorus*). Furthermore, *R. japonicus* is distributed in temperate to subboreal regions, whereas *R. trichophorus* is found in tropical regions of Africa (Ivory Coast), Asia (southern China), and South America (Peru) (Hawksworth *et al*., 2011).

The lateral spines of *Racoleus* were considered as an important feature for the genus distinguishing it from *Cystocoleus* and *Racodium* (Hawksworth *et al*., 2011). Indeed, there is a difference that the spines are straight in *R. trichophorus* (Hawksworth *et al*., 2011), sinuous in *R. japonicus* (Figs. 2D, 3F), *Cystocoleus* (Sukuja and Ore,

Fig. 2. *Racoleus japonicas*. **A**. Habitat of type locality. **B**. Thallus (holotype, TNS). **C**. Sympodially branched filamentous thallus with corrugated hyphal walls (*K. Miyazawa 1700*, TNS). **D**. Filamentous thallus with spines (lateral hyphae) (holotype, TNS). Scale bars: B = 0.5 mm; C = 50 µm; D = 10 µm.

1934; Fig. 3D) and *Racodium* (Fig. 3E), but the following morphological features are common in these genera. The spines are composed of a single hypha extending outside the lichenized part of the thallus. The hyphae are 1.5–4 µm wide and up to 150 µm in length with some septa [the presence of septa in *R. trichophorus* was confirmed by the photo in Hawksworth *et al*., (2011, Fig. 1D)]. There is no large morphological difference except being straight or sinuous in the lateral spine among these taxa, and this difference serves as taxonomic character at species level rather than for genus level. The arrangement of the hyphae and the presence/absence of corrugated walls in the filamentous thallus should be an important taxonomic character to recognize each genus.

Racoleus japonicus may be confused with *Cystocoleus ebeneus* (Dillwyn) Thwaites and *Racodium rupestre* Pers. because of the similar blackish brown filamentous thalli in which the fungal hyphae surround *Trentepohlia* filaments. However, *Racoleus japonicus* differs in the vertical arrangement of hyphae with a

Fig. 3. Hyphal arrangements of ***Cystocoleus***, ***Racodium*** and ***Racoleus***. (**A**–**C**) and their lateral spines (hyphae) (**D**–**F**). **A**. ***Cystocoleus ebeneus***. The hyphae with corrugated walls are arranged twisted to the algal filament (*Follmann s.n.* [Follmann & Werner: Lich. Exs. Sel. Colon. 505], TNS). **B**. ***Racodium rupestre***. The hyphae with straight walls are arranged parallel to the algal filament (*R. Santesson 22567* [Moberg: Lich. Sel. Exs. Upsal. 45], TNS). **C**. ***Racoleus japonicus***. The hyphae with corrugated walls are arranged parallel to the algal filament (*K. Miyazawa 1700*, TNS). **D**. A lateral hypha of ***Cystocoleus ebeneus*** (*W. A. Weber s.n.* [Weber: Lich. Exs. 491], TNS)]. **E**. A lateral hypha of ***Racodium rupestre*** (*R. Santesson 22567* [Moberg: Lich. Sel. Exs. Upsal. 45], TNS). **F**. A lateral hypha of ***Racoleus japonicus*** (*K. Miyazawa 1700*, TNS). Scale bars: A–F = 10 μm.

corrugated wall (Fig. 3C). On the other hand, *Cystocoleus* has a weakly twisted arrangement of hyphae with a corrugated wall (Fig. 3A), and *Racodium* has a vertical arrangement of hyphae without a corrugated wall (Fig. 3B). *Racoleus japonicus* might be confused with taxa that belong to *Spilonema* Bornet (Coccocarpiaceae) and *Thermutis* Fr. (Lichinaceae), which have blackish filamentous thalli. However, *Spilonema* and *Thermutis* have stiffer thalli composed of wider filaments (at least 20 μm vs. up to 20 μm wide in *R. japonicus*), blackish apothecia, and cyanobacteria photobionts (observed materials in TNS are cited below).

Some Japanese specimens previously identified as '*Racodium rupestre*' housed in TNS were found to be *R. japonicus* by our study, while *R. rupestre* was confirmed to occur in Japan (at about 2480 m elevation in Mt. Kinpu). The habitat of *R. rupestre* in Japan seems to be alpine to subalpine areas. The collections of '*Racodium rupestre*' reported from Mt. Ryokami by Yoshimura (1964) were confirmed as *Racoleus japonicus* in the present study by examining materials collected from the same locality. The taxon reported by Kato and Harada (2011) from Mt. Haruna, central Honshu in Japan, should be *Racoleus japonicus* according to their illustration and description.

Additional specimens examined. **JAPAN. Hokkaido.** Kitami Prov.: Ikutahara-Kiyosato, Engaru-cho, Monbetsu-gun (N43°51′,

E143°29′), 290 m elev., on shady rock wall in riverine forest, 29 May 2012, *A. Frisch 12/Jp152 & Y. Ohmura* (TNS). **Honshu.** Musashi Prov. (Saitama Pref.): mountain path between Hinataooyaguchi and the summit of the Mt. Ryokami, Ogano-machi, Chichibu-gun (N36°01′, E138°50′), about 1500 m elev., on shady rock, 17 August 2023, *K. Miyazawa 1700* (TNS); ditto, about 1700 m elev., *K. Miyazawa 1701* (TNS), *K. Miyazawa 1702* (TNS). Aki Prov. (Hiroshima Pref.): Mt. Misen, Miyajima Island, Hatsukaichi-city (N34°16′, E132°19′), 340 m elev., on rock, 8 November 2012, *Y. Ohmura 9473, A. Frisch & K. Fedrowitz* (TNS). **Kyushu.** Higo Prov. (Kumamoto Pref.): Hakusuishizenshinrin Park, Mizukami-mura, Kuma-gun (N32°22′, E131°02′), 780 m elev., on rock, 29 May 2023, *K. Miyazawa* 1561 (TNS).

Exsiccati of other species examined. [***Cystocoleus niger*** (Huds.) Har.]: **AUSTRIA**. Westösterreich, Stubaier Alpen: flockenrasig an schattig-trockenen, aber luftfeuchten überhängenden Urgesteinswänden im *Cystocoleo-Racodietum rupestris* SCHADE, 1900 m elev., NW, pH 6.2, Osthang des Lisenser Tales bei Jufenau unter dem Windegg, October 1975, *G. Follmann s.n.* (Follmann: Lich. Exs. Sel. Cassel. 167, TNS). [***Cystocoleus ebeneus*** (Dillwyn) Thwaites]: **GERMANY**: Rheinlasnd-Pfalz, southern Eifel Mountains, German-Luxemburgian Nature Park, Gutland District, on vertical or slightly overhanging faces of red sandstone cliffs in open woodland not far from the Prümerburg north of Irrel, occasionally growing intermixed with *Racodium rupestre* Pers. which is, nevertheless, always of secondary importance in comparison with the above species, about 300 m elev., W-S exposition, forming extensive mats, October 1989, *Follmann s.n.* (Follmann & Werner: Lich. Exs. Sel. Colon. 505, TNS). **COLOMBIA**: Depart. Cundinamarca, Municipio Supatá, Alto El Tablazo, along trail from radar station to Suptatá, subparamo scrub, 3400 m elev., on perpendicular sandstone rockface, 11 September 1984. *J. Aguirre C. & H. Sipman 5228* (Sipman: Lichenoth. Latinoamer. 17, TNS). **U.S.A.** Colorado. Rocky Mountain National Park: Odessa Gorge, on trail from Odessa to Fern Lake; vertical faces of granite cliffs, locally humid site, 3000–3100 m elev., 28 September 1975, *W. A. Weber s.n.* (Weber: Lich. Exs. 491, TNS). [***Racodium rupestre*** Pers.]: **SWEDEN**, Härjedalen Prov.: Tännäs par., southern slope of Mt. Gruvvålen (SW of Mt. Mittåkläppen) (N62°43′, E12°25′), about 900 m elev., on exposed rock in the upper part of the subalpine (birch) region, 1 September 1970, *R. Santesson 22567* (Moberg: Lich. Sel. Exs. Upsal. 45, TNS). **Czech Republic**, Sudeti occident., Teplice nad Metují: loco dicto 'Skalní město', 620 m elev., ad parietes altos umbrosos rupium arenacearum, 18 June 1962, *A. Vězda s.n.* (Vězda: Lich. Sel. Exs. 450, TNS). [***Spilonema paradoxum*** Bornet]: **U. S. A.**, Alabama, Lee Co., Waverly, auf zeitweise überrieseltem Glimmerscheifer am Bachufer, 23 February 1963, *A. Henssen & H. McCullough 15166* (Henssen: Lich. Cyanoph. 15, TNS). [***Spilonema revertens*** Nyl.] **U. S. A.**, California, Humboldt Co., Coast Range, Pine Ridge Summit bei Arcata, in Sickerwasserstreifen auf vulkanischem Gestein, ca. 1000 m elev., 31 October 1961, *A. Henssen 13627a* (Henssen: Lich. Cyanoph. 14, TNS). [***Thermutis velutina*** (Ach.) Flot.]: **SWEDEN**, Bohuslän: Ödsmål, Kolhättan, on irrigated, sunny rocks near the see, fertile, 13 August 1932, *A. H. Magnusson s. n.* (Magnusson: Lich. Sel. Scand. Exs. 296, TNS). **CROATIA**, Dalmatia: Duba prope vicum Drvenik, 30 m elev., ad saxa calcarean in litore maris, *A. Vězda s.n.* (Vězda: Lich. Sel. Exs. 509, TNS).

Specimens examined of other species. [***Racodium rupestre*** Pres.: Japanese common name "*Iwa-goke-modoki*"]: **JAPAN**. Honshu. Kai Prov. (Yamanashi Pref.): Mt. Kinpu, Hokuto-city (N35°52′ E138°37′), about 2480 m elev., on shady rock-wall in forest, 4 July 2012, *A. Frisch 12/Jp464, Y. Ohmura & G. Thor* (TNS).

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