RESEARCH ARTICLE

First report of the lichen *Ochrolechia akagiensis* (Ochrolechiaceae, Ascomycota) in Korea

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ABSTRACT

The genus *Ochrolechia* is a widespread, lichen genus in Korea. Despite being common, little is known about the species diversity and geographical distribution of *Ochrolechia*. In this study, we detailed the identification procedure of the genus *Ochrolechia* in a Korean collection and provided the description of each species. Using 104 specimens collected from 2003 to 2017, we identified four species of the genus *Ochrolechia* via morphological and/or molecular phylogenetic analysis: *O. parellula*, *O. trochophora*, *O. yasudae* and *O. akagiensis*. Among them, *O. akagiensis* had not been previously reported in Korea. Moreover, the species identified as *O. frigida* and *O. tartarea* in past studies were corrected as *O. yasudae* and *O. parellula*, respectively, based on morphological and/or molecular evidence. Phylogenetic analysis using the internal transcribed spacer regions including 5.8S rRNA gene showed that the four species separated clearly, indicating that the morphological identification corresponds to the phylogenetic identification. We provide a taxonomic key for the four species of the genus *Ochrolechia*.

Keywords: ITS region, lichen, new record, *Ochrolechia akagiensis*

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INTRODUCTION

The genus *Ochrolechia* is comprised of a common crustose lichen which can be found easily on rocks and/or bark which are exposed to sufficient sunlight in mountainous areas. The genus *Ochrolechia* is distributed worldwide ranging from the polar to tropical regions, with a total of 60 species reported worldwide [1-8].

The typical morphology of the genus is a grayish thallus with circular, disc-shaped, and pink apothecia [8]. Regarding the morphology, apothecia and ascospores of the genus *Ochrolechia* resemble those of the genus *Pertusaria*, with a thin layer of asci and ellipsoid ascospores [9]. In chemistry, however, the genus *Ochrolechia* is known to produce a depside, gyrophoric acid, as a secondary metabolite [9].

Ochrolechia spp. are identified by morphology, anatomy and chemistry [10]. Based on morphology, Verseghy [11] divided the genus into four groups: namely *tatareae*, *parellae*, *harmandii*, and *upsaliensis*.

Later, Brodo renamed the groups as *tartarea*, *parella*, *africana*, and *upsaliensis* [3].

More recently, phylogenetic studies have been applied in this genus using the ribosomal internal transcribed spacer (ITS) regions. Wei [12] has reported that the genus *Ochrolechia* is present in the same clade as the genus *Circinaria* with very low bootstrap support values and is a sister-clade to the genera *Varicellaria* and *Lepra*, which are more closely related to one another.

In Korea, Moon [13, 14] reported three *Ochrolechia* species in Mt. Seorak: *O. trochophora*, *O. parellula*, and *O. yasudae*. In addition, *O. tartarea* and *O. frigida* were also reported by Zhang [15] and Kondratyuk [16] through a survey of the coastal region of Korea, respectively. Based on these studies, most of the *Ochrolechia* specimens collected in Korea belong to the tartarea-group, which meet the following criteria: gyrophoric acid in the cortex, disc presence or absence in the margin, epruinose apothecia, and rarely having variolaric acid [10].

In this study, combining newly collected specimens with existing specimens in Korea, we identified all species belonging to the genus *Ochrolechia* at species level by a morphological, chemical and molecular examination. We also provide a taxonomic key and descriptions in detail.

MATERIALS AND METHODS

Collection and Morphological characteristics.

The specimens were collected and deposited in the Korea Lichen Research Institute (KoLRI). The morphological and anatomical characteristics of the specimens were observed using a dissecting microscope (Nikon SMZ645; Nikon, Tokyo, Japan) and a compound microscope (Olympus BX 50, Olympus, Tokyo, Japan). Zeiss Scope, A1 compound microscope (Carl Zeiss, Oberkochen, Germany) was used to take the microphotographs. Sections of the fruiting bodies were mounted in distilled water to measure the ascomata structure and spore size. A spot test was carried out on the thallus cortex, apothecial disc and apothecial margin, and the color reaction was observed under a compound microscope. Secondary compounds were determined by thin layer chromatography (TLC) in solvent system C (toluene: acetic acid = 85: 15) [17].

DNA extraction and amplification of ITS regions.

For molecular analysis, fresh lichen materials were ground with a mini bead-beater-16 (3450 RPM, 115V, 10A, Biospec products) and the grounded lichen materials were applied to extract DNA using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The extracted DNA was used to amplify the internal transcribed spacer (ITS) regions. Polymerase chain reaction (PCR) was performed using an AccuPower® PCR Premix (Bioneer, Daejeon, Korea) with initial denaturation for 5 min at 94°C, 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C, 1 min extension at 72°C, followed by a final extension for 5 min at 72°C. The PCR products were confirmed by gel electrophoresis purified using an AccuPrep® PCR purification kit (Bioneer, Daejeon, Korea) and bi-directionally sequenced on both strands with the

same primers used for PCR amplification. The ITS regions (ITS1-5.8S-ITS2) were amplified with ITS1F (5'- CTTGGTCATTTACAGGAAGTAA-3') [18] and ITS4 (5'- ATTTGAGCTCTTCCCGCTTCA-3') [19]. The sequence alignments were obtained using BIOEDIT 7.0.9 [20], in addition with the reference sequences downloaded from GenBank. The sequences were initially aligned using Clustal W ver. 1.83 [21].

Phylogenetic analysis.

Eight representative specimens were used for phylogenetic analysis. *Trapelia coarctata* (KR017098) was selected as an outgroup. Evolutionary history was inferred using the maximum likelihood (ML) method based on the general time reversible model [22]. The tree was drawn to scale, with branch lengths measured as the number of substitutions per site. A total of 26 nucleotide sequences were used in phylogenetic analysis. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7 [23]. One thousand bootstrap replications were tested for the reliability of the inferred tree.

RESULTS AND DISCUSSION

Morphological characteristics and dominant species in Korea.

During 2003-2017, a total of 104 specimens were collected. Applying the classification keys used in China and North America, we identified the specimens by morphological and chemical analysis [3, 8]. Based on thin layer chromatography (TLC) analysis, all 104 specimens produced gyrophoric acid as a secondary metabolite, which is a characteristic of the genus *Ochrolechia*.

Four species were identified from our specimens: *O. trochophora*, *O. parellula*, *O. yasudae,* and *O. akagiensis* (Fig 1). Among the four species, *O. trochophora* (40 specimens, 38.5%) and *O. parellula* (39 specimens, 37.5%) were the predominant species in Korea, followed by *O. akagiensis* (23 specimens, 22.1%) and *O. yasudae* (2 specimens, 1.9%). With the exception of *O. akagiensis,* the other three species identified, equating to 81 specimens (77.9%), have been reported in Korea [13-16].

Interestingly, all *O. trochophora* specimens were collected from mountainous regions, whereas all *O. parellula* were collected in coastal areas. In China and Poland, *O. trochophora* has been collected only from various trees in mountainous areas, but not from stones [8, 24]. In addition, *O. parellula* has been reported only in Japan and Korea until now [13]. In previous reports, these species have been found at altitudes below 500 m and existed in a saxicolous habit on acidic rocks [13]. Our findings are consistent with previous studies [13, 14]. This result implies that *O. trochophora* is likely to have adapted to higher altitudes, whereas *O. parellula* preferentially adapted to coastal areas in Korea.

In this study, we report the presence of *O. akagiensis* in Korea for the first time. *O. akagiensis* forms round granule-shaped isidia ranging from 0.2 to 0.3 mm in diameter, only on trees (Fig. 1A), and is very similar to *O. yasudae* in morphological characteristics. Unlike *O. akagiensis, O. yasudae* forms cylinder-shaped isidia ranging from 0.1 to 0.3 mm in diameter on rocks [8]. In a previous study, Moon [13] reported that *O. yasudae* are present on rocks with mosses or on tree bark. In this study, however, we found that

O. yasudae existed only on rocks and was rarely distributed in Korea. In addition, the specimens formerly identified as *O. yasudae* have been re-identified as *O. akagiensis* on account of the presence of granular isidia. Because these two species are very similar in the morphology, the probability of misidentification is very high. Molecular biological evidences should help clearly distinguish the two species.

Phylogenetic analysis by maximum likelihood method based on ITS rDNA sequences

We generated ITS region sequences from two representative specimens each of the four *Ochrolechia* species (*O. akagiensis*, *O. parellula*, *O. trochophora*, and *O. yasudae*). The length of the ITS1, 5.8S, and ITS2 regions ranges from 432 bp to 498 bp. The alignment of the ITS included 500 unambiguously aligned nucleotide positions, 301 of which were conserved and 187 were variable. All the generated ITS region sequences were deposited in GenBank.

Using the generated sequences, phylogenetic analysis was performed using the maximum likelihood (ML) method. The ML tree is shown in Figure 3. The clustering of *O. parellula* with *O. trochophora* is well-supported by a higher bootstrap value (Fig. 3). *O. yasudae* and *O. akagiensis* formed a monophyletic group and was a sister clade to the clade composed of *O. parellula* and *O. trochophora* with high bootstrap values.

In the morphological observation, *O. yasudae* and *O. akagiensis* are quite similar because both species have isidia. In this phylogenetic analysis, two species were clustered and placed in the same clade (Fig. 3). In a previous study, these two species were divided by the substrate [8], but phylogenetic analysis revealed clade division corresponding to the difference in the isidia type than that of the substrates.

In a previous study, specimen KU933682 was identified as *O. tartarea*, following morphological and chemical identification [15]. In this study, however, phylogenetic analysis showed that putative *O. parerulla* specimens, including specimen KU933682, is clearly clustered in the clade of *O. parellula* rather than with *O. tartarea* [15].

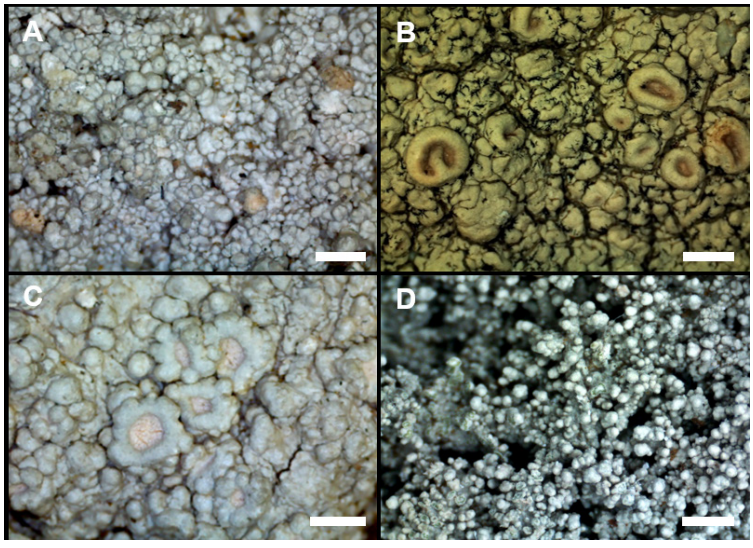

Fig. 1. Species of the genus *Ochrolechia* in Korea. A, *Ochrolechia akagiensis*; B, *O. parellula*; C, *O. trochophora*; D, *O. yasudae* (Scale bars: A-D=1mm).

Taxonomy

1) *Ochrolechia akagiensis* Yasuda & Vain., Bot. Mag., Tokyo 35: 54 (1921) (Figs. 1A, 2A).

Morphology: Thallus gray, thick, continuous, isidiate, granular, isidia covers thallus, 0.2~0.5 mm tall, 0.1~0.2 mm wide. Apothecia abundant or indistinct, round, sessile, 0.8~1.5 mm diam., apothecial disc pinkish, concave, without pruina; margin concolorous with thallus, prominent, smooth becoming rugose to verrucose; well-developed, epihymenium brownish, 40~44.5 μm, hymenium hyaline, 294~338 μm, hypothecium hyaline, 75.6~100 μm, algal layer mainly confined to margin, with spotty algal below hypothecium. Ascospores, simple, hyaline, ellipsoid. 50~77 × 28~36 μm.

Chemistry: Thallus and isidia: K-, C+ red, P-, UV-; apothecial disc and margin C+ red; gyrophoric acid.

Note: *Ochrolechia akagiensis* is confused with *O. yasudae*, which differs by having cylindrical isidia 0.1~0.3 mm in diam, and grows directly on rocks or moss-covered rocks, while *O. akagiensis* has granular isidia and grows on bark to moss-covered bark [3]. *O. frigida* is also similar to *O. akagiensis*, but differs by having a yellowish apothecial disc and granular thallus with spine-like extensions.

Representative specimen: Korea, Gyeongsangnam-do, Hadong-gun, Mt. Jiri, 35˚19'06.79"N, 127˚39'05.22"E, alt. 1346 m, on bark, Y. Joshi, X. Y. Wang, J.-S. Hur 091390 (KoLRI-011307); Gangwon-

Table 1. Specimens used in the phylogenetic analysis with voucher and GenBank numbers

Species	Voucher	GenBank Accession No. ITS
Ochrolechia akagiensis*	**Korea, Hur 091076 (KoLRI)**	**KU883364**
Ochrolechia akagiensis*	**Korea, Hur 170089 (KoLRI)**	**MG212681**
Ochrolechia androgyna	United Kingdom, E:DNA:EDNA09-01528	FR799238
Ochrolechia androgyna	United Kingdom, E:DNA:EDNA09-01547	FR799239
Ochrolechia androgyna	United Kingdom, E:DNA:EDNA09-01547	FR799240
Ochrolechia androgyna	Germany, FR:DNA28	JN943616
Ochrolechia balcanica	Greece (ESS-20968)	AF329172
Ochrolechia frigida	USA, AFTOL-ID 291	HQ650675
Ochrolechia frigida	Republic of Korea, Hur ANT050888	DQ534474
Ochrolechia microstictoides	United Kingdom, E:DNA:EDNA09-01548	FR799241
Ochrolechia microstictoides	United Kingdom, E:DNA:EDNA09-01589	FR799242
Ochrolechia microstictoides	United Kingdom, E:DNA:EDNA09-01590	FR799243
Ochrolechia parella	France (ESS-20864)	AF329174
Ochrolechia parella	Australia, Hafellner 47608 (GZU)	AF332123
Ochrolechia parellula*	**Korea, Hur 110733 (KoLRI)**	**KU933682**
Ochrolechia parellula*	**Korea, Hur 120666 (KoLRI)**	**KU883360**
Ochrolechia parellula*	**Korea, Hur 130353 (KoLRI)**	**KU883361**
Ochrolechia szatalaensis	United Kingdom, E:DNA:EDNA09-02104	FR799244
Ochrolechia szatalaensis	United Kingdom, E:DNA:EDNA10-00041	FR799245
Ochrolechia szatalaensis	United Kingdom, E:DNA:EDNA10-00042	FR799246
Ochrolechia tartarea	USA, FR:DNA7	JN943620
Ochrolechia trochophora*	**Korea, Hur 120983 (KoLRI)**	**KU883362**
Ochrolechia trochophora*	**Korea, Hur 121137 (KoLRI)**	**KU883363**
Ochrolechia upsaliensis	Australia, Hafellner 40763 (GZU)	AF332124
Ochrolechia yasudae*	**Korea, Hur 152863 (KoLRI)**	**MG212682**

* Bold letters indicate newly published sequences of *Ochrolechia* species in this study

do, Gangneung-si, Mt. Seokbyeong, 37°34'40.66"N, 128°51'36.11"E, alt. 807 m, on bark, J. S. Park, 170089 (KoLRI-044213).

2) *Ochrolechia parellula* (Müll. Arg.) Zahlbr., Cat. Lich. Univers. 5: 693 (1928) (Figs. 1B, 2B).

Morphology: Thallus gray, thick, 1.2~1.5 mm, continuous, cracked, verrucose, verrucae hemispheric, crowded, 0.1~0.3 mm in diam., prothallus indistinct to zonated. Apothecia abundant, scattered, sessile, round, 0.5~2.5 mm; disc white, pink to yellowish-pink, flat to concave, apothecial margin smooth, thick, concolorous with thallus, prominent above the disc level; epihymenium pale brown, 12.5~15 μm thick; hymenium hyaline, 270~290 μm tall; hypothecium pale brown to pale yellow, 40~70 μm thick, amphithecium fairly lax, algal layer continuous in margin, with spotty to continuous below the hypothecium. Ascospores eight per ascus, simple, hyaline, ellipsoid, 40~52.5 × 20~22.5 μm.

Chemistry: Thallus K-, C+ red, P-, UV-; apothecia disc K-, C+ red; apothecial margin cortex C+ red, medulla of thallus and margins K-, C-, P-; gyrophoric acid.

Note: *O. parellula* is a saxicolous species and grows on rock. In Moon's description, this species showed a C positive reaction, without soredia or isidia and apothecia with subdivided disc and grows on exposed rock, such as granite [25]. This species is similar to *O. trochophora* in the early stage, but the former has an increasingly thick thallus with a rimose-areolate form. Moreover, *O. trochophora* has a verrucose

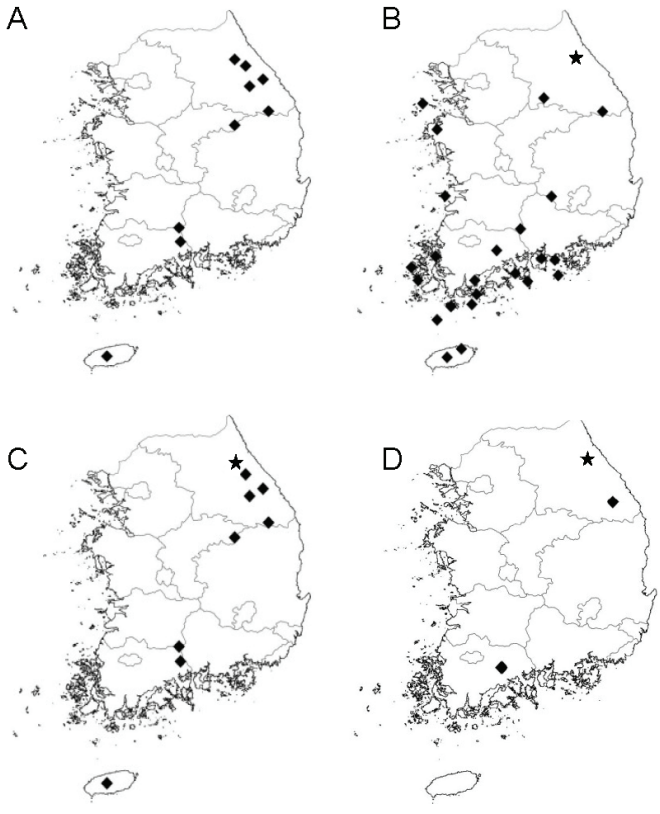

Fig. 2. Distribution of *Ochrolechia* species in Korea. Stars indicate species that were found in previous studies. Diamond indicates samples collected in this study. A, *O. akagiensis*; B, *O. trochophora*; C, *O. parellula*; D, *O. yasudae*.

apothecial margin when well-developed, while *O. parellula* has a smooth margin. This species has been reported in India and Japan [25, 26]. *O. parellula* specimens have been reported as *O. tartarea* because of the verruculose thallus [15], but this was corrected through a re-examination.

Representative specimen: Korea, Jeollanam-do, Wando-gun, Cheongsan Island, 34˚09'01.87"N, 126˚52'08.21"E, alt. 2 m, on a rock, X. Y. Wang, J. A. Ryu, 110733 (KoLRI-013758; Gyeongsangbuk-do, Ulleung-gun, Ulleung Island, 37˚28'59.09"N, 130˚54'40.07"E, alt. 20 m, on rock, J. S. Park, 162244 (KoLRI-040482).

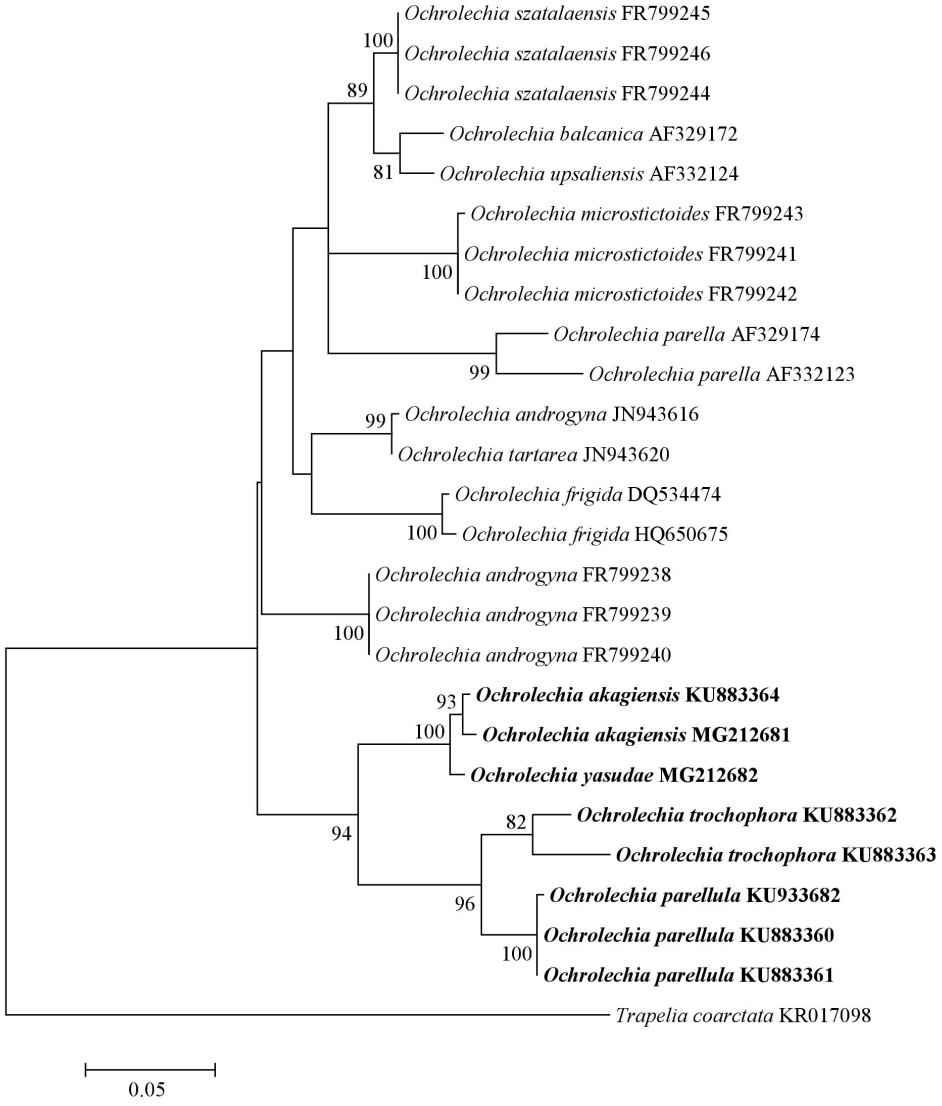

Fig. 3. The ML tree of *Ochrolechia* generated from the internal transcribed spacer (ITS) of rDNA sequences. The sequences newly generated in this study are indicated in bold. The numbers at nodes represent the percentage of their occurrence in 1,000 bootstrap replicates.

3) *Ochrolechia trochophora* (Vain.) Oshio, J. Sci. Hiroshima Univ., Ser. B, Div. 2, 12: 145 (1968) (Figs. 1C, 2C).

Morphology: Thallus gray to pale gray, thin when young, becoming verrucose to verruculose, thick, with dispersed pustulate areole, thick, prothallus indistinct. Apothecia round to subround, 1.1~1.3 mm in diam., apothecial disc pinkish, concave becoming flat, occasionally developing radiation bands of sterile tissue. Apothecial margin smooth becoming verrucose, epihymenium brownish, 25~37.5 μm, hymenium hyaline, 232~268 μm, hypothecium pale brownish 70~103 μm, amphithecium medulla lax, algal layer absent or spotty, bellow on hypothecium. Ascospores eight per ascus, simple, hyaline, ovoid to ellipsoid, 38.3~62.2.7 × 21~22.5 μm.

Chemistry: Thallus K-, C+ red, P-, Medulla K-, C-, P-, UV-; apothecial disc C+ red; apothecial margin cortex C+ red; apothecial margin medulla C-; gyrophoric acid.

Note: *Ochrolechia trochophora* is a common species in Korea. This species is characterized by having verrucose apothecia margin, the algal layer below the hypothecium is absent or spotty and grows on bark. This species has highly variable apothecial discs and margins. First, apothecial margin at the immature stage is very smooth, and the disc is flat. After the mature stage, the apothecial margin becomes verrucose and disc concave. In the final stage, the apothecial disc becomes wheel-like or rosulate. According to Kukwa, *O. trochophora* have two types: 1) *O. trochophora* s. str. from Europe with weakly developed thallus; 2) *O. trochophora* s. str. from U.S.A with pustulate areoles on the apothecial margin and over the thallus. Korean *O. trochophora* has both types. This species resembles *O. subpallescens* and *O. laevigata* but the former has a continuous algal layer below the hypothecium while the latter has a thin thallus and apothecial margin almost devoid of the algal layer, and lacking algae below the hypothecium.

Representative specimen examined: Korea, Jeju-do, Jeju-si, Mt. Halla, 33˚24'43.03"N, 126˚32'52.04"E, alt. 713 m, on bark, S.-O. Oh, U. Jayalal, J. S. Park, J.-S. Hur, 120983 (KoLRI-016013); Gangwan-do, Jeongseon-gun, Gohan-eup, 37˚11'31.26"N, 127˚31'77.1"E, alt. 957 m, on bark, J. S. Park, 163583 (KoLRI-041828); Jeollanam-do, Suncheon-si, Songgwang-myeon, Temple Songgwang, 35˚00'10.07"N, 127˚16'07.00"E, alt. 80 m, on bark, J. S. Park, J.-J. Woo, 164045 (KoLRI-042313).

4) *Ochrolechia yasudae* Vain., Bot. Mag., Tokyo 32: 155 (1918) (Figs. 1D, 2D).

Morphology: Thallus grows directly on rock or moss-covered rock, ashy-gray, thick, continuous, with a distinct, often zoned border, isidiate, isidia cylindrical, knobby, persistent, isidia covers the whole thallus, 0.2~0.5 mm tall, 0.1~0.2 mm wide. Apothecia not seen.

Chemistry: Thallus and isidia: K-, C+ red, P-; Medulla: K-, C-, P-; UV-; gyrophoric acid.

Note: *Ochrolechia yasudae* has been reported in East Asia and North America [8]. The main morphological characters include isidia covering the thallus and are coralloid or cylindrical in shape. *Ochrolechia frigida* is similar to *O. yasudae* but differs by having a yellowish apothecial disc and granular thallus with spine-like extensions.

Representative specimen examined: Korea, Gangwon-do, Yangyang-gun, Seo-myeon, Mt. Jobong, 37˚56'01.07"N, 128˚33'07.47"E, alt. 980 m, on rock covered by moss, Y. Joshi, X. Y. Wang, J. A. Ryu, J.-S.

Hur, 090283 (KoLRI-010027); Gangwon-do, Chuncheon-si, Mt. Maebong, 37°54'92.4"N, 127°58'48.6"E, alt. 576 m, on rock, J. S. Park, J.-J. Woo, B. G. Lee, 152863 (KoLRI-37172).

Key to the lichen genus *Ochrolechia* in South Korea

1. Thallus isidiate or with a finger-like extension 2
- Thallus lacking isidia and soredia 4
2. Thallus with a spine-like extension ..... *O. frigida*
- Thallus isidiate, isidia cylindrical or granular 3
3. Thallus isidia cylindrical, knobby; saxicolous ..... *O. yasudae*
- Thallus isidia granular; muscicolous or occasionally corticolous ..... *O. akagiensis*
4. Thallus corticolous, apothecial disc epruinose to haze-like pruina present, apothecial margin well-developed to verrucose ..... *O. trochophora*
- Thallus saxicolous 5
5. Thallus white and tartareous (strongly granular), disc yellowish ..... *O. tartarea*
- Thallus not tartareous, whitish-gray, continuous, rimose-areolate, verrucose, apothecial disc C+ red ..... *O. parellula*

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REFERENCES

1. Thomson JW. Lichens of the Alaskan arctic slope. 1st ed: University of Toronto Press; 1979.
2. Thomson JW. American Arctic Lichens: The Microlichens: University of Wisconsin Press; 1984.
3. Brodo IM. Studies in the lichen genus *Ochrolechia*. 2. Corticolous species of North America. Can J Bot 1991;69(4):733-72.
4. Brodo IM, Sharnoff SD, Sharnoff S. Lichens of north America: Yale University Press; 2001.
5. Øvstedal DO, Smith RL. Lichens of Antarctica and South Georgia: a guide to their identification and ecology: Cambridge University Press; 2001.
6. Galloway DJ. Flora of New Zealand: Lichens, including lichen-forming and lichenicolous fungi: Manaaki Whenua Press, Landcare Research; 2007.
7. Smith CW. Lichens of Great Britain and Ireland: British Lichen Society; 2009.
8. Ren QA. Revision of the lichen genus *Ochrolechia* in China. Lichenologist 2017;49(1):67-84.
9. Schmitt I, Lumbsch HT. Molecular phylogeny of the Pertusariaceae supports secondary chemistry as an important systematic character set in lichen-forming ascomycetes. Mol Phylogenet Evol 2004;33(1):43-55.
10. Jabłońska A, Kukwa M. The lichen genus *Ochrolechia* in Poland 2. Herzogia 2008;21:5-24.

11. Verseghy K. Die Gattung *Ochrolechia*. Nova Hedwigia 1962;1:1-146.
12. Wei X, Schmitt I, Hodkinson B, Flakus A, Kukwa M, Divakar PK., et al. Circumscription of the genus *Lepra*, a recently resurrected genus to accommodate the "*Variolaria*"-group of *Pertusaria* sensu lato (Pertusariales, Ascomycota). PLOS One 2017;12(7):1-14.
13. Moon KH. Lichen of Sorak in Korea: Sookmyung Women's University; 1997.
14. Moon KH. Lichens of Mt. Sorak in Korea. J Hattori Bot Lab 1999;(86):187-220.
15. Zhang LL, Wang XY, Zhao ZT, Hur JS. Lichens newly recorded from the South Korean coast. Mycotaxon 2012;122:421-32.
16. Kondratyuk S, Lőkös L, Tschabanenko S, Haji Moniri M, Farkas E, Wang XY, et al. New and noteworthy lichen-forming and lichenicolous fungi. Acta Bot Hung 2013;55(3-4):275-349.
17. Orange A, James PW, White FJ. Microchemical methods for the identification of lichens: British Lichen Society; 2001. p. 101.
18. Gardes M, Bruns TD. ITS primers with enhanced specificity for basidiomycetesapplication to the identification of mycorrhizae and rusts. Mol Ecol 1993;2(2):113-8.
19. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, editors. PCR protocols: a guide to methods and applications. San Diego: Academic Press; 1990. p. 315-22.
20. Hall T, Biosciences I, Carlsbad C. BioEdit: an important software for molecular biology. GERF Bull Biosci 2011;2(1):60-1.
21. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994;22(22):4673-80.
22. Nei M, Kumar S. Molecular evolution and phylogenetics: Oxford university press; 2000.
23. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 2016;33(7):1870-4.
24. Kukwa M. The lichen genus *Ochrolechia* in Poland III with a key and notes on some taxa. Herzogia 2009;22:43-66.
25. Moon KH. Lichens of Mt. Sorak in Korea. J Hattori Bot Lab 1999;86:187-220.
26. Awasthi DD. A key to the microlichens of India, Nepal and Sri Lanka. 1991.