Taxonomic Revision of the Lichen Genera Pertusaria, Varicellaria, and Variolaria (Pertusariales, Ascomycota) in South Korea

Jung Shin Park¹, Sook-Young Park², Chan-Ho Park¹, Sergii Y. Kondratyuk³, Soon-Ok Oh⁴ and Jae-Seoun Hur^{1,*}

¹Korean Lichen Research Institute, Sunchon National University, Suncheon 57922, Korea ²Department of Plant Medicine, Sunchon National University, Suncheon 57922, Korea ³M. H. Kholodny Institute of Botany, 01004 Kiev, Ukraine ⁴Korea National Arboretum, Pocheon 11186, Korea

Abstract The crustose lichen genus *Pertusaria* comprises over ca. 800 species worldwide. In total, 20 *Pertusaria* species were localized to the Mt. Sorak and Jeju-do in Korea. To date, information regarding the distribution of *Pertusaria* species in the South Korean peninsula is scarce. In this study, we collected *Pertusaria* species across South Korea and identified them based on morphological, chemical, and molecular characteristics. Of the 387 samples collected, we identified 24 taxa and 1 variety, of which 17 were previously recorded, and 6 taxa were newly found in South Korea (*P. leioplaca, P. leucosora var. violascens, P. texana, P. thiospoda, P. thwaitesii*, and *P. xanthodes*), 2 known species were transferred to *Varicellaria* (*Varicellaria lactea* and *V. velata*), one species was transferred to *Variolaria* as a new record (*Variolaria multipunctoides*) and one was a new species (*P. jogyeensis* J. S. Park & J.-S. Hur, sp. nov.). Characteristics of the newly discovered species, *P. jogyeensis*, are as follows: smooth to bumpy thallus, scattered to crowded poriform apothecia, blackish ostioles, definitely sunken, thin yellowish green rims around ostioles, 8-spored ascus, and the presence of perlatolic acid and thiophaninic acid (chlorinated xanthone). Phylogenetic studies on *P. jogyeensis* based on the mitochondrial small subunit sequence revealed proximity to *P. flavicans* and *P. texana*, and supported its classification as a new species within the genus *Pertusaria*. Additionally, we describe the chemical composition and morphology of all listed species in detail and provide an artificial key for identification.

Keywords Internal transcribed spacer, mtSSU, Pertusaria, Phylogenetic analysis, Taxonomy

The lichen genus *Pertusaria* is distributed worldwide from the poles to the tropics. Although over 800 species belong to the genus *Pertusaria* [1], several undiscovered species likely exist. *Pertusaria* species are differentiated by several morphological characteristics, including the presence of isidia or soredia, shape of apothecia (poriform or disciform), the number of ascospores within an ascus, and the presence

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*Corresponding author E-mail: jshur1@sunchon.ac.kr

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of single or double-walled ascospores with smooth or rough inner wall. In addition, secondary metabolite profiles are also crucial in determining *Pertusaria* species [2].

The genus Pertusaria is divided into three subgenera according to Archer's classification [2-4], namely, Pertusaria (chloroxanthones and C_3-C_7 orcinol depsides), Pionospora (chloroxanthones, 6-O-methylnorlichexanthones with variolaric or gyrophoric acid), and Monomurata (C₁ orcinol depsides, C_3-C_7 depondence or β -orcinol depoidones) that are differentiated based on lichen compounds and different forms of apothecia (disciform in Monomurata, poriform in Pertusaria, and both forms in Pionospora) [2]. Based on Archer's classification, phylogenetic analysis performed by Lumbsch and Schmitt [5] using the nuclear large subunit DNA (nuLSU) revealed that the genus Pertusaria is polyphyletic, and are separated into two clades, while Monomurata is separated from Pertusaria and Pionospora. In addition, the results also indicate agreement between phylogenetic classification, and classification based on some morphological characters (apothecia type, number of ascospore walls, and ascus type), and chemical production profile (chlorinated xanthone) [5].

Schmitt and Lumbsch [6] conducted further molecular

phylogenetic analysis of Pertusariaceae based on the nuLSU, mitochondrial small subunit (mtSSU), and mitochondrial large subunit (mtLSU) sequences. They suggested that the genus Pertusaria can be re-divided into three subgenera: Pertusaria s. str. (subgenera Pertusaria and Pionospora), Varicellaria (composed of Varicellaria rhodocarpa and three members of the Pertusaria subgenus Monomurata: Pertusaria lactea, P. hemisphaerica, and P. velata), and Variolaria (subgenus Monomurata) [6]. Interestingly, these phylogenetic divisions are in accordance with differences in chemical compositions. The subgenus Pertusaria s. str. group produces chlorinated xanthones, and gyrophoric and planaic acids; the subgenus Varicellaria group produces lecanoric acid, but not chlorinated xanthones; the subgenus Variolaria group produces picrolichenic and thamnolic acids, but not chlorinated xanthones and orcinol depsides. Besides the chemical differences, the three subgenera show morphological differences. The Pertusaria s. str. group has poriform apothecia, while the Variolaria and Varicellaria groups have disciform apothecia. The Variolaria group has a thin ascospore wall, while the Varicellaria group has a thick ascospore wall [6].

Recently, several molecular markers such as nuLSU [5], mtSSU [6, 7] and mtLSU [6] are being used for phylogenetic analysis of the genus *Pertusaria*. However, mtSSU is commonly used to identify genera below the family level, and ample mtSSU reference sequences are available than any other type of molecular marker used for *Pertusaria* [7-11]. RNA polymerase II subunit (RPB2), DNA replication licensing factor (MCM7) [12], and more recently, internal transcribed spacer (ITS) region sequences have also been used for the identification of the genus *Pertusaria* [13].

The genus *Pertusaria* has been renamed *Variolaria* Ach. [6, 14], *Marfloraea* S. Y. Kondr., L. Lőkös & Hur [13], and *Lepra* Scop. [15, 16] based on their morphology, chemicals produced and molecular data. A subgenus *Monomurata* within *Pertusaria* has been proposed *Variolaria* [6]. However, the name *Variolaria* has been proposed as the new name for *Marfloraea* [13]. More recently, a new name *Lepra* has been proposed for the genus because the name was already used and published by Lendemer and Harris [16].

Pertusaria species have been reported in Korea by many lichenologists since 1950 [17-27]. Extensive studies on Pertusaria were conducted in 1999 and 2002 by Moon et al. [21, 22]. In these studies, 20 species (P. amara, P. astomoides, P. composita, P. glauca, P. lactea, P. laeviganda, P. leucosora, P. multipuncta, P. nakamurae, P. pertusa, P. pseudamara, P. pustulata, P. quartans, P. radiata, P. stenostoma, P. subfallens, P. sublaeviganda, P. submultipuncta, P. subobductans, and P. velata) were identified through morphological analysis. By 2016, a total of 30 Pertusaria species has been reported in Korea [17-27].

However, using DNA sequence analysis, a number of genera and species have been re-established [6]. For example, *P. lactea*, *P. velata*, and *P. multipunctoides* have been re-assigned to the genus *Varicellaria* (*V. lactea* and *V. velata*)

and Variolaria (V. multipunctoides) [12, 14]. P. coreana has been also suggested to be synonymous with P. astomoides based on its morphological and chemical characteristics [22].

In this study, lichen specimens belonging to the genus *Pertusaria* were collected throughout the South Korean peninsula, including various mountains and islands. The collected specimens were identified by morphological, chemical, and molecular analyses. Based on the recent identification, we divided them into three subgenera, *Pertusaria* s. str., *Variolaria*, and *Varicellaria*. We identified six species previously unknown in Korea and one new species, *P. jogyeensis* J. S. Park & J.-S. Hur, sp. nov. Here, we describe an artificial key of Korean *Pertusaria* and allied species based on previous and current studies, and include detailed descriptions.

MATERIALS AND METHODS

Morphological examination. The specimens have been deposited at the Korea National Arboretum (KH). The morphological and anatomical characteristics of the specimens were observed using a stereo microscope (SMZ-645; Nikon, Tokyo, Japan) and compound microscope (BX-50; Olympus, Tokyo, Japan). All specimens were examined to characterize the thallus shape, ascomata structure, color, and size. A spot test was performed on the thallus, medulla, and soredia, and the color reaction was observed under a compound microscope. Thin layer chromatography (TLC) was performed in solvent system A (toluene : dioxane : acetic acid = 180 : 45:5), B (hexane : methyl *tert*-butyl ether : formic acid = 140:72:18), and C (toluene : acetic acid = 85:15) [28].

DNA extraction and amplification of ITS region and mtSSU. ITS region and mtSSU were sequenced to identify the specimens. Lichen materials were ground briefly using a Mini Beadbeater-16 (3450 RPM, 115 V, 10 A; Biospec Products, Bartlesville, OK, USA). DNA was extracted from the ground samples using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The amplified DNA was concentrated and purified using a PCR Quick-spin PCR Product Purification Kit (INTRON Biotechnology, Inc., Seongnam, Korea). PCR was performed in a total volume of 20 mL using a PCR premix (Bioneer, Daejeon, Korea). The ITS regions (ITS1-5.8S-ITS2) and mtSSU were amplified using primers ITS1F (5'-CTTGGTCATTTACAGGAAGTAA-3') [29] and ITS4 (5'-ATTTGAGCTCTTCCCGCTTCA-3') [30], and mtSSU1 (5'-AGCAGTGAGGAATATTGGTC-3') and mtSSU3R (5'-ATGTGGCACGTCTATAGCCC-3') [31]. For amplification of the mtSSU sequence, we designed two primer pairs: mtSSU_PC1_F (5'-GGTCAATAGCCTAACGGC-3') and mtSSU_PC1_R (5'-CCCACA ATATTCAGGCCATG-3') for the Pertusaria s. str. group, and mtSSU_PC2_F (5'-GCCTAACGGCTGAACC-3') and mtSSU_PC2_R (5'-GTCCCTCTTATCACATTCCAA-3') for the Variolaria group.

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Phylogenetic analysis. The sequences obtained from each sample were aligned to selected sequences of *Pertusaria* species obtained from GenBank, and the sequences obtained from *Trapelia coarctata* (AY212874) were used as the outgroup. Aligned sequences were assembled separately in BIOEDIT 7.0.9 [32] and the sequences were aligned using Clustal W ver. 1.83 [33]. Phylogenetic analysis was performed using the maximum parsimony (MP) method on MEGA ver. 6.0 [34]. The reliability of the inferred tree was tested by 1,000 bootstrap replications.

RESULTS AND DISCUSSION

Taxonomy and distribution of the genus Pertusaria and allied taxa in Korea. From 2003 to 2016, 387 specimens were collected in South Korea, including the western and southern coast and Ulleung Island. Morphological and chemical identification of Pertusaria species in Korea was carried out using Moon et al's Korean [22], Oshio's Japanese [35], and the Chinese identification keys developed by Zhao et al. [36]. Pertusaria species were identified based on the presence or the absence of four lichen compound classes such as xanthone, orcinol depside, depsone, and βorcinol depsidone. In the spot test and TLC analysis, we found seven different chemicals from the 387 specimens: two xanthones (4,5-dichlorolichexanthone and thiophaninic acid), one orcinol depside (perlatolic acid), one depsone (picrolichenic acid), and three β -orcinol depsidones (stictic acid, norstictic acid, and protocetraric acid).

Among the 25 species identified in this study, 17 taxa (367 out of 387 specimens, 94.8%) were previously recorded in Korea: *P. amara (Marfloraea amara)*, *P. astomoides*, *P. commutata*, *P. composita*, *P. flavicans*, *P. laeviganda*, *P. leucosora*, *P. multipuncta*, *P. pertusa*, *P. quartans*, *P. stenostoma*, *P. subfallens*, *P. sublaeviganda*, *P. submultipuncta*, and *P. subobductans*, *Varicellaria lactea*, and *V. velata*. Six species and one allied (17 of 387 specimens, 4.4%) specimens were newly found in South Korea: *P. leioplaca*, *P. leucosora* var. *violascens*, *P. texana*, *P. thiospoda*, *P. thwaitesii*, *P. xanthodes*, and *Variolaria multipunctoides*. One new species (3 of 387 specimens, 0.8%) was discovered and named as *P. jogyeensis* J. S. Park & J.-S. Hur, sp. nov.

Within the genus *Pertusaria* and allied taxa, *P. multipuncta* was the most common species in Korea with 23.5% occurrence (91 specimens) (Fig. 1A). This species was distributed in various altitudes from the Mt. Odae (1,400 m) to coastal areas. *P. multipuncta* is also one of the most common species in Japan [35]. Fifty-four specimens (14%) of *P. subobductans* were collected (Fig. 1B). This species is abundantly distributed from the sea-level to high mountains in Japan and Taiwan [35]. Forty-three specimens of *P. amara* (11.1%) (Fig. 1C), 39 specimens (10.1%) of *P. flavicans* (Fig. 1D), 30 specimens (7.8%) of *P. astomoides* (Fig. 1E), and 30 specimens (7.8%) of *P. quartans* (Fig. 1F) were collected. *P. flavicans* and *P. astomoides* were mainly distributed on the islands and in the coastal areas. Twenty-seven specimens

(7%) of *P. subfallens* (Fig. 1G) and 21 specimens (5.4%) of *P. laeviganda* were collected (Fig. 1H).

Thirteen species appeared to be rare in Korea. Seven specimens of P. submultipuncta (1.8%) (Fig. 1I), 6 specimens of P. thiospoda (1.6%) (Fig. 1J), 5 specimens of P. stenostoma (1.3%) (Fig. 1K), 3 specimens of P. commutata (0.8%) (Fig. 1L), 3 specimens of P. leucosora (0.8%) (Fig. 1M), 3 specimens of P. sublaeviganda (0.8%) (Fig. 1N), 3 specimens of P. texana (0.8%) (Fig. 1O), 3 specimens of P. xanthodes (0.8%) (Fig. 1P), 2 specimens of P. thwaitesii (0.5%) (Fig. 1Q), 1 specimen of P. composita (0.3%) (Fig. 1R), 1 specimen of P. leioplaca (0.3%) (Fig. 1S), 1 specimen of P. leucosora var. violascens (0.3%) (Fig. 1T), 1 specimen of P. pertusa (0.3%) (Fig. 1U) were collected. Three specimens (0.87%) of P. jogyeensis (Fig. 1V) were classified as new species, and were collected only from Mt. Jogye. Among the Varicellaria group, 8 specimens of V. velata (Fig. 1W) and one specimen of V. lactea (Fig. 1X) were collected. Among the Variolaria group, only one specimen of V. multipunctoides (Fig. 1Y) was collected in Jeju-do.

Phylogenetic analysis of the MP tree based on mtSSU.

To clarify the phylogenetic status of the putative new species of *P. jogyeensis*, we analyzed the mtSSU and ITS sequences. To date, phylogenetic studies using ITS sequences have been rarely conducted for the genus *Pertusaria*. We obtained ITS sequences from 12 *Pertusaria* species, including *P. jogyeensis*, and deposited them in GenBank. The combined length of the entire ITS1, 5.8S, and ITS2 regions range from 443 to 595 bp. All the deposited ITS sequences are listed in Table 1. BLAST search revealed that the ITS sequence from *P. jogyeensis* did not match with any of the ITS sequences previously deposited for other members of the genus *Pertusaria* in GenBank, supporting the idea that *P. jogyeensis* could be a new species.

We also deposited mtSSU sequences from 11 Pertusaria species (P. amara, P. astomoides, P. flavicans, P. jogyeensis, P. laeviganda, P. multipuncta, P. quartans, P. subobductans, P. texana, P. thiospoda, and P. xanthodes). All the mtSSU sequences obtained in this study are listed in Table 1. Unfortunately, we were unable to obtain representative sequences for all 25 Pertusaria species identified. Several factors may have affected PCR amplification: (1) use of non-specific primers, (2) presence of lichen polysaccharides, and (3) low quantity of template DNA. Indeed, when we used primer pairs designed by us based on specific sequences, mtSSU_PC1_F & mtSSU_PC1_R, and mtSSU_PC2_F & mtSSU_PC2_R, we successfully obtained mtSSU DNA sequences from 6 species (P. astomoides, P. flavicans, P. laeviganda, P. quartans, P. subobductans, and P. jogyeensis) using mtSSU_PC1_F & mtSSU_PC1_R, and two more species (P. amara and P. multipuncta) using mtSSU_PC2_F & mtSSU_PC2_R primers.

The alignment of the mtSSU included 846 unambiguously aligned nucleotide positions, 381 of which were conserved and 456 were variable. The MP tree is shown in Fig. 2. In

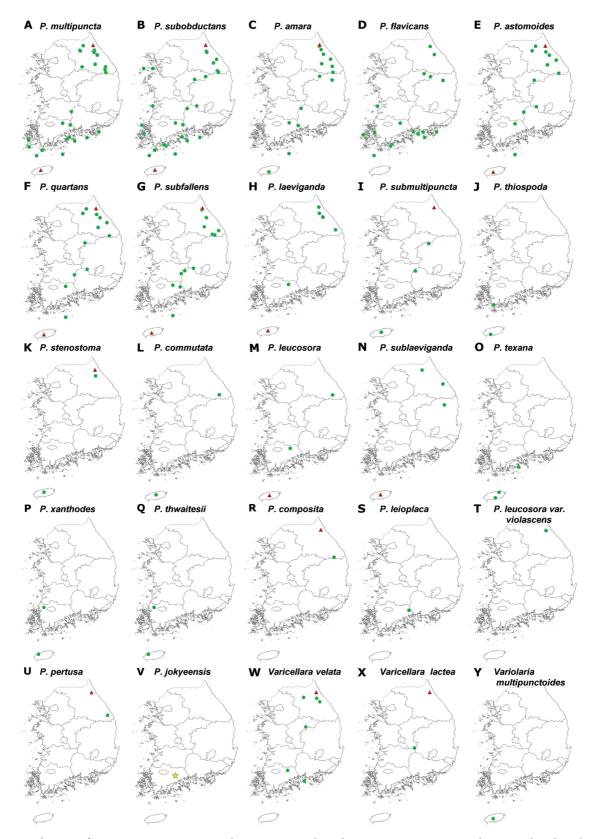


Fig. 1. Distribution of *Pertusaria* species in South Korea. Triangle indicates *Pertusaria* species that was also described in a previous study. Pentagon indicates samples collected in this study. A, *Pertusaria multipuncta*; B, *P. subobductans*; C, *P. amara*; D, *P. flavicans*; E, *P. astomoides*; F, *P. quartans*; G, *P. subfallens*; H, *P. laeviganda*; I, *P. submultipuncta*; J, *P. thiospoda*; K, *P. stenostoma*; L, *P. commutata*; M, *P. leucosora*; N, *P. sublaeviganda*; O, *P. texana*; P, *P. xanthodes*; Q, *P. thwaitesii*; R, *P. composita*; S, *P. leioplaca*; T, *P. leucosora* var. *violascens*; U, *P. pertusa*; V, *P. jogyeensis*; W, Varicellaria velata; X, V. lactea; Y, Variolaria multipunctoides.

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

Species	Source	GenBank accession No.	
		mtSSU	ITS
Ochrolechia androgyna	Germany, Schmitt (ESS 21066)	AY300897	_
Ochrolechia frigida	Alaska, Spribille 39444 (GZU)	KR017339	KR017062
Ochrolechia pallescens	Spain, Lumbsch, 9 Aug 2004 (F)	DQ780277	_
Ochrolechia parella	Culture 1114M (AKITA)	DQ780278	_
Ochrolechia tartarea	Scotland, Coppins (ESS 21493)	AY300899	_
Ochrolechia trochophora	Japan, Yamamoto 10052703 (AKITA)	DQ780280	
Ochrolechia trochophora	Culture 0162M (AKITA)	-	_
		DQ780281	-
Ochrolechia yasudae	Culture 0217M (AKITA)	DQ780282	-
Ochrolechia yasudae	Isolate AFTOL-ID 882	DQ986902	-
Pertusaria albescens	Czech Republic, Bohemia, Schmitt (ESS 20967)	AF329175	-
Pertusaria amara	Korea, Kondratyuk 121116 (KoLRI 016152)	MF095418	-
Pertusaria amara	Germany, Schmitt (ESS 21067)	AY300900	-
Pertusaria amara	Isolate AFTOL-ID 379	AY584713	-
Pertusaria cf. amara	Canada, Lumbsch, Schmitt, Wirlz, 20 Aug 2003 (F)	DQ780283	-
Pertusaria aspergilla	France, I. Schmitt (ESS 21505)	AY567986	_
Pertusaria astomoides	Korea, Lőkös 140384-1 (KoLRI 022752)	MF095419	KU894624
Pertusaria astomoides	Korea, Lőkös 140753-2 (KoLRI 023226)	MF095420	KU894626
Pertusaria cineraria	Antarctica, R. S. Poulsen 1127 (HB R. S. Poulsen)	AY567983	
Pertusaria coccodes	Germany, O. Duerhammer D-1403 (HB O. Duerhammer)	AY567984	
Pertusaria corallina	Culture 1118M (AKITA)		_
		DQ780286	-
Pertusaria corallophora	Antarctica, Lumbsch 19026e (F)	DQ780285	-
Pertusaria coronata	Czech Republic, Schmitt (ESS 21494)	AY300902	-
Pertusaria flavicans	Korea, Joshi & So 140182-1 (KoLRI 022552)	MF095421	KU894629
Pertusaria flavicans	Korea, Oh, Park & Hur 140219 (KoLRI 022585)	MF095422	KU894630
Pertusaria flavicans	Yamamoto & Tanaka 14112710 (AKITA)	DQ780287	-
Pertusaria flavicunda	-	AF381562	-
Pertusaria dactylina	Sweden, B. Kanz & C. Printzen 5435 (HB C. Printzen)	AY567985	_
Pertusaria erythrella	Australia, Archer (ESS 20866)	AF431958	_
Pertusaria excludens	France, N. Stapper (ESS 21497)	AY567987	_
Pertusaria gibberosa	Australia, Archer, Brown & Sureeporn, Mar 2003 (NSW)	DQ780288	_
Pertusaria graphica	New Zealand, Wright 756! (WELT)	DQ78O290	
	New Zealand, Wright 750: (WEET)		—
Pertusaria hemisphaerica	-	AF381563	_
Pertusaria hermaka	Australia, Mangold, 22 Mar 2005 (F)	DQ780299	
Pertusaria jogyeensis	Korea, Park & Woo 163707 (KoLRI 041956)	MF095423	MF176877
Pertusaria jogyeensis	Korea, Park & Woo 164063 (KoLRI 042331)	MF095424	MF176878
Pertusaria kalelae	Argentina, M.I. Messuti 2006 (F)	AY567989	-
Pertusaria lactea	-	AF381564	-
Pertusaria laeviganda	Korea, Hur 111028 (KoLRI 014103)	MF095425	KU894632
Pertusaria laeviganda	Korea, Hur 121704 (KoLRI 017019)	MF095426	KU894633
Pertusaria laeviganda	Japan, Yamamoto 11081304 (AKITA)	DQ780291	_
Pertusaria lecanina	USA, S. Tucker, 2000 (HB Santa Barbara Botanical Garden)	AY567991	_
Pertusaria leioplaca	Germany, Schmitt (ESS 21502)	AY300903	_
Pertusaria mammosa	Greece, Sipman & Raus 47130 (B)	AY212854	_
Pertusaria multipuncta	Korea, Joshi & So 140738-1 (KoLRI 023196)	MF095428	KU894635
Pertusaria multipuncta	Korea, Joshi & So 141053 (KoLRI 023608)	MF095429	KU894636
Pertusaria multipuncta	Korea, Jayalal, Park & Woo 141380 (KoLRI 023834)	MF095430	KU894637
Pertusaria ophthalmiza	Scotland, B. Coppins (ESS 21498)	AY567993	-
Pertusaria panyrga	Canada, C. Printzen 5718 (HB C. Printzen)	AY567994	-
Pertusaria pertusa	-	AF381565	-
Pertusaria plittiana	USA, Lumbsch & Kautz, 6 Jun 2004 (F)	DQ780294	-
Pertusaria pustulata	Japan, Yamamoto & Takahagi 14122626 (AJCJTA)	DQ780295	_
Pertusaria quartans	Korea, Hur 121645 (KoLRI 017099)	MF095431	KU894638
Pertusaria quartans	Korea, Jayalal, Park & Woo 141386 (KoLRI 023840)	MF095432	KU894639
Pertusaria scaberula	Australia, Archer (ESS-20867)	AF431959	
Pertusaria signyae	Antarctica, U. Søchting 7807	AY567997	-
0,	5		- VI 100 47 45
Pertusaria subobductans	Korea, Joshi & So 141051 (KoLRI 023606)	MF095433	KU894645
Pertusaria subobductans	Korea, Jayalal, Park & Woo 141285 (KoLRI 023739)	MF095434	KU894646

Species	Source	GenBank accession No.	
		mtSSU	ITS
Pertusaria subobductans	Korea, Jayalal, Park & Woo 141324 (KoLRI 023740)	MF095435	KU894647
Pertusaria subobductans	Japan, Yamamoto 10092230 (AKITA)	DQ780300	_
Pertusaria subobductans	Culture 1108M (AKITA)	DQ780301	-
Pertusaria subventosa	-	AY300905	_
Pertusaria subfallens	Korea, Hur 091211 (KoLRI 011210)	-	KU883367
Pertusaria subfallens	Korea, Hur 100543 (KoLRI 012134)	-	KU883368.
Pertusaria subfallens	Korea, Hur 121134 (KoLRI 016170)	-	KU883369
Pertusaria texana	Korea, Kondratyuk 140616-1 (KoLRI 023011)	KX165269	KU894634
Pertusaria texana	Korea, Park, Woo & Lee 152872 (KoLRI 037181)	KX165270	-
Pertusaria thiospoda	Korea, Park, Woo & Lee 152891 (KoLRI 037792)	KX165271	-
Pertusaria thwaitesii	Korea, Park, Woo & Liu 141461 (KoLRI 032134)	-	KU894648
Pertusaria werneriana	Greece, Sipman 47336 (B)	AY300907	-
Pertusaria xanthodes	Korea, Park, Lee & Woo 152908 (KoLRI 037881)	KX165272	MF176876
Trapelia coarctata	Czech Republic, Palice, Schmitt (ESS 20966)	AY212874	_
Varicellaria velata	Australia, Archer (ESS 21500)	AY300906	-
Varicellaria velata	Korea, Hur 080533 (KoLRI 008756)	-	KU883370
Varicellaria velata	Korea, Hur 090615 (KoLRI 010288)	-	KU883371

Table 1. Continued

The bold letter indicates newly published sequences from Pertusaria species described in this study.

mtSSU, mitochondrial small subunit; ITS, internal transcribed spacer.

the phylogenetic tree, we observed that the putative new species, *P. jogyeensis*, belongs to the *Pertusaria* s. str. group, and is closely related to *P. flavicans* and *P. texana. Pertusaria flavicans*, *P. texana*, and *P. jogyeensis* have eight-spored ascus with thiophaninic acid. We further describe the chemistry and morphology of all species in detail, and provide an artificial key to all the species listed, including the putative new species.

New species.

Pertusaria jogyeensis J. S. Park & J.-S. Hur, sp. nov. (Fig. 3). Mycobank: MB 821302.

Similar to *P. injuneana* A. W. Archer & Elix, but differs by having confluent poriform apothecia and contains thiophaninic acid as a major compound.

Type: Korea, Jeollanam-do, Suncheon-si, Temple Songgwang, 35°00'09.6" N, 127°16'18.11" E, alt. 220 m, on bark (*Zelkova*), 16 Dec 2016, J. S. Park & J.-J. Woo, 164063 (holotype: KoLRI 042331); same locality with holotype, alt. 218 m, on bark (*Zelkova*), 20 Nov 2016, J. S. Park & J.-J. Woo, 163707 (isotype: KoLRI 041956).

Morphology: Thallus crustose, developed on bark, continuous, thin, greenish gray, margin not definite, unzonated; surface smooth to bumpy, locally cracked deeply, areoles forming between apothecia, generally matt, lacking soredia and isidia. Apothecia poriform, numerous, crowded, solitary to grouped in 2–3 confluent verrucae, scattered, flattened-hemispherical, occasionally elongate, 1.2–2.1 mm diameter, concolorous with thallus, not constricted at the base. Ostioles 1–3 per verruca, blackish, conspicuous, slightly sunken, having thin yellowish green rims around ostioles, somewhat inconspicuous, 0.1–0.2 mm. Ascospores 8 per ascus, uniseriate, rarely irregular, hyaline, ellipsoid, $60-70 \times$

27.5–30.0 μ m in size with smooth inner walls, 3.5–5.0 μ m. **Chemistry:** Thallus K–, C–, P–, KC+ orange-yellow, UV+ dark orange; medulla K–, C–, P– or P+ orange. TLC: thiophaninic acid (major), perlatolic acid (major) (e and i in Fig. 4).

Substrate and ecology: This new species is known only from the type collection at Mt. Jogye. It grows on *Zelkova* at an altitude of 220 m.

Etymology: The species epithet refers to the place of origin, Mt. Jogye.

Note: *P. jogyeensis* is characterized by thick and bumpy thallus; convex verrucae with black ostioles; having thin yellowish green rims around ostioles; ascospores 8 per ascus, uniseriate. Chemical analysis revealed that *P. jogyeensis* contains thiophaninic acid and perlatolic acid. Although over 47 *Pertusaria* species have eight-spored ascus, the combination of uniseriate ascospores and the production of thiophaninic acid and perlatolic acid has been found only in *P. injuneana* to date [1].

However, *P. injuneana* and *P. jogyeensis* differ greatly in their morphology. For example, (1) thallus form: bumpy type in *P. jogyeensis*; areolate and minutely tuberculate in *P. injuneana*; (2) poriform: confluent verrucae in *P. jogyeensis*; solitary verrucae in *P. injuneana*; (3) ostioles: flattened, blackish, and conspicuous ostioles in *P. jogyeensis*; mammiform in *P. injuneana*; (4) chemicals: both thiophaninic and perlatolic acids produced as major compounds in *P. jogyeensis*; only perlatolic acid produced as a major compound and thiophaninic acids as a minor compound in *P. injuneana*. *Pertusaria lignicola* Malme, a similar species from South America has greenish gray smooth thallus and produces thiophaninic, stictic, and protocetraric acid. *Pertusaria jogyeensis* is closely related to *P. flavicans* and *P. texana*

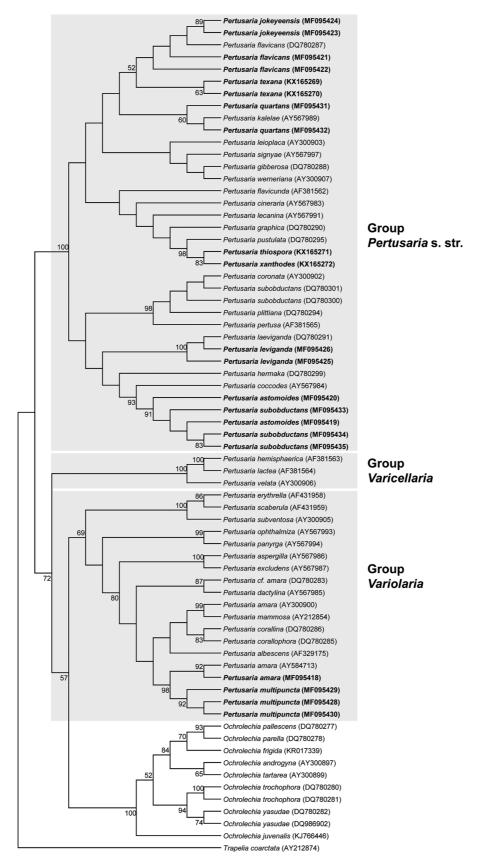


Fig. 2. Phylogenetic tree generated from mitochondrial small subunit sequences (mtSSU). Bold letter with asterisk indicates new mtSSU sequences identified in this study. Bootstrap values are shown near the corresponding branch. Only nodes supported by 50 or more bootstraps are shown.

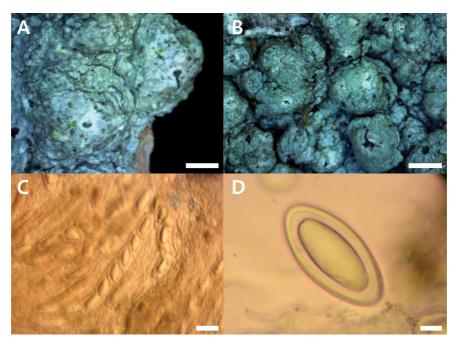


Fig. 3. The features of the putative new species, *Pertusaria jogyeensis.* A, Poriform and ostioles; B, Ostioles; C, Ascospore number; D, form of a spore (scale bars: A, B = 1 mm, $C = 50 \text{ \mum}$, $D = 10 \text{ \mum}$).

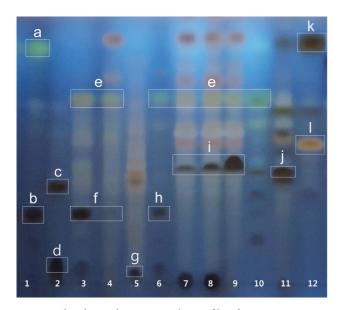


Fig. 4. Thin layer chromatography profile of *Pertusaria* species in solvent A under UV light: Line 1: *P. leioplaca* with 4,5-dichlorolichexanthone (a), stictic acid (b); Line 2: *P. leucosora var. violascens* with unknown 1 (c), unknown 2 (d); Line 3: *P. texana* with thiophaninic acid (e), stictic acid (f); Line 4: *P. thiospoda* with thiophaninic acid (e), stictic acid (f); Line 5: *P. thwaitesii* with protocetraric acid (g); Line 6: *P. xanthodes* with thiophaninic acid (e), stictic acid (h); Line 7, 8, 9: *P. jogyeensis* (163707; 164063; 164064) with thiophaninic acid (e), perlatolic acid (i); Line 10: control [*P. flavicans* Lamy] with thiophaninic acid (e); Line 11: control [*Dirinaria applanata* (Fée) D. D. Awasthi] with divaricatic acid (j); Line 12: Control [*Heterodermia leucomelos* (L.) Poelt] with atranorin (k), and zeorine (l).

because the two species produce thiophaninic acid. *Pertusaria flavicans* has sorediate 8-spored thallus [37]. However, *P. jogyeensis* does not have sorediate thallus and contains perlatolic acid. *P. texana* is also similar but differs in the presence of blackish ostioles and convex verrucae, and the production of perlatolic acid instead of stictic acid. **Additional specimen examined:** Same locality with holotype, 35°00′07.3″ N, 127°17′29.03″ E, alt. 220 m, on bark, J. S. Park & J.-J. Woo, 164064 (isotype: KoLRI 042332).

New records.

Pertusaria leioplaca DC., in de Candolle & Lamarck, Fl. Franç., Ed. 3 (Paris) 5/6: 173 (1815) (Fig. 5A).

Morphology: Thallus determinate, thin; surface greenish gray, smooth, even to somewhat roughened, weakly cracked, thallus margin definite, little zonated, yellowish brown. Apothecia poriform, concolorous with thallus, not constricted at the base, convex, hemispherical, 0.5-0.6 mm diameter, 0.3-0.5 mm high, small, often flat-topped, solitary, fertile verrucae with 1(-2) apothecia. Ostioles gray to black. Ascospores 4 per ascus, uniseriate, hyaline, ellipsoid, 77.5– $105 \times 33.8-37.5$ µm, and inner wall smooth.

Chemistry: Thallus K– or K+ yellowish, C–, P–, UV+ pale orange; medulla K–, C–, KC–, P– or P+ brownish. TLC: 4,5-dichlorolichexanthone, stictic acid (a and b in Fig. 4).

Note: *P. leioplaca* is characterized by solitary apothecia with 4-spored ascus. *P. pertusa* also has the same chemical reaction but differs in having verrucae with a constricted base and 2-spored ascus.

Specimen examined: Korea, Jeollanam-do, Mt. Beakwoon, 35°06′48.08″ N, 127°36′45.03″ E, alt. 1,051 m, on bark, 27 Jun 2006, J.-S. Hur, 060435 (KoLRI 004803).

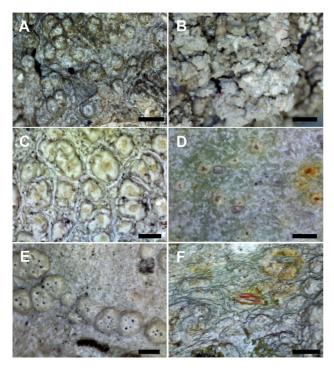


Fig. 5. New records of *Pertusaria* species in South Korea. A, *Pertusaria leioplaca*; B, *P. leucosora* var. *violascens*; C, *P. texana*; D, *P. thiospoda*; E, *P. thwaitesii*; F, *P. xanthodes*.

Pertusaria leucosora var. violascens (Räsänen) Oshio, J. Sci. Hiroshima Univ., Ser., B, Div. 2 12: 98 (1968) (Fig. 5B). **Morphology:** Thallus thick; surface whitish gray, sorediate, verrucose to rugose, largely cracked, thallus margin conspicuous, flat, slightly zonated, brownish to grayish. Sterile, sorediate at apices, white to grayish white and somewhat pinkish, 0.5–1.2 mm diameter, irregular to globose, convex, round, minute granular to powdery, abundant, confluent.

Chemistry: Thallus K–, C–, P–, UV–; medulla K+ purple, C–, P+ yellow; soredia K+ purple, KC+ purple, C–. TLC: unknown 1 (dark green), unknown 2 (purple-brown) (c and d in Fig. 4).

Note: This species is a variety of *P. leucosora*. *P. leucosora* var. *violascens* is characterized by the presence of a more verrucose thallus and purple color with K positive chemical reaction in the medulla. The species has been found only in three localities in Japan, and grows on rocks [35].

Representative specimen examined: Korea, Gyeongsangnamdo, Mt. Cheontea, 36°09′26.6″ N, 127°36′22.7″ E, alt. 542 m, on bark, 3 Jun 2006, J.-S. Hur, 060089 (KoLRI 005577).

Pertusaria texana Müll. Arg., Flora, Regensburg 67: 399 (1884) (Fig. 5C).

Morphology: Thallus determinate, thin; surface yellowish green to whitish gray, flat, smooth to somewhat roughened, weakly cracked, thallus margin distinct, blackish. Apothecia poriform, concolorous with thallus, not constricted at the base, hemispherical, convex, 0.5–1.0 mm diam, small,

somewhat rugose to irregular, solitary to confluent in 2–3 verrucae. Ostioles greenish yellow, distinct, even to slightly concave. Ascospores 8 per ascus, biseriate, hyaline, ellipsoid, $70-75 \times 25-30 \mu m$, and inner wall smooth.

Chemistry: Thallus K–, C–, P–, UV+ orange; medulla K+ yellow, C–, P+ orange red; ostioles K–, KC+ orange, C–. TLC: thiophaninic acid, stictic acid, constictic acid (e and f in Fig. 4).

Note: *P. texana* is characterized by the presence of thiophaninic acid and yellowish ostioles. This species is similar to *P. leioplacella* Nyl., but the latter has a uniseriate ascospore form. *P. xanthothelia* Müll. Arg. has the same characteristic for ostioles, but has been recorded only from East Africa [1].

Specimen examined: Korea, Jeju-do, Seogwipo-si, Donnaeko Valley, 33°18′04.3″ N, 126°34′53.07″ E, alt. 330 m, on bark, 19 Jun 2014, S. Y. Kondratyuk, 140616-1 (KoLRI 023011); Jeju-do, Jeju-si, 33°30′35.6″ N, 126°43′00.01″ E, alt. 114 m, on bark, 19 Aug 2015, J. S. Park, J.-J. Woo and B. G. Lee, 152872 (KoLRI 037181).

Pertusaria thiospoda C. Knight, Trans. Linn. Soc. Lond., Bot. 2: 47 (1882) (Fig. 5D).

Morphology: Thallus thin; surface continuous, smooth, slightly cracked, greenish gray to yellowish gray, prothallus inconspicuous. Apothecia poriform, not constricted at the base, concolorous with thallus, convex, hemispherical, 0.4–0.6 mm diam. wide, surrounded by a yellowish border. Ostioles conspicuous, brownish to colorless. Ascospores 2 per ascus, 95–100 × 25–30 μ m, ellipsoid, hyaline, double-walled, inner wall smooth, 5–7.5 μ m thick.

Chemistry: Thallus K–, C+ yellow P–, KC+ orange, UV+ orange; medulla K+ yellow, C–, P+ orange. TLC: thiophaninic acid, stictic acid (e and f in Fig. 4).

Note: *Pertusaria thiospoda* is distinguished from *P. pustulata* by the presence of thiophaninic acid instead of 2-chloro-6-O-methylnorlichexanthone, having pale ostioles and smooth inner wall.

Representative specimen examined: Korea, Jeollanam-do, Haenam-gun, 34°23'39.89" N, 126°33'54.03" E, alt. 58 m, on bark, 31 May 2015, J. S. Park, D. Liu, B. G. Lee, 152891 (KoLRI 037792).

Pertusaria thwaitesii Müll. Arg., Flora, Regensburg 67: 470 (1884) (Fig. 5E).

Morphology: Thallus thin; surface continuous, flat, smooth to slightly verrucose, whitish gray, soredia and isidia absent, prothallus conspicuous, black, and rarely zonated. Apothecia poriform, concolorous with thallus, conspicuous, numerous, convex, hemispherical, flattened above, 1-2 mm wide solitary to grouped. Ostioles black, 3-5(-6) per verrucae. Ascospores 2 per ascus, $105-125 \times 35-42.5 \mu m$, ellipsoid, hyaline, double-walled, inner wall rough, $3-5 \mu m$, having many lateral ribs.

Chemistry: Thallus K–, C–, P+ orange-red, UV+ orange; medulla K–, C–, P+ orange red. TLC: protocetraric acid,

lichexanthone (trace) (g in Fig. 4).

Note: *P. thwaitesii* resembles *P. pustulata*, which also has a rough inner wall, but contains protocetraric acid instead of stictic acid. *P. thwaitesii* is also similar to *P. pertusa* but the latter has ascospores with smooth inner wall and prominent black ostioles.

Specimen examined: Korea, Jeju-do, Mt. Halla, 33°25'18.04" N, 126°32'58.09" E, alt. 577 m, on bark, 1 Aug 2012, S.-O. Oh, U. Jayalal, J. S. Park, J.-S. Hur 120946 (KoLRI 015976); Jeju-do, Mt. Halla, 33°21'30.08" N, 126°30'13.09" E, alt. 1,626 m, on bark, Nov 2014, J. S. Park, J.-J. Woo, D. Liu, 141461 (KoLRI 032134).

Pertusaria xanthodes Müll. Arg., Flora, Regensburg 67: 286 (1884) (Fig. 5F).

Morphology: Thallus determinate, thin; surface smooth to slightly cracked, somewhat rugose, grayish green to yellowish gray, prothallus indistinct. Apothecia poriform, abundant, concolorous with thallus, convex, globose to sub-globose, flattened, 0.5–0.9 mm diam., solitary to confluent. Ostioles distinct, pale black to brownish, 0.1–0.2 mm diam., 1–2 ostioles in verruca. Ascospores 2 per ascus, 50– 95×20 – 28μ m, ellipsoid, hyaline, double-walled, inner wall rough, 5.0–7.5 μ m, having many lateral ribs.

Chemistry: Thallus K–, C+ yellow, P–, KC+ bright yellow to orange, UV+ orange; medulla K+ bright yellow, C–, P–. TLC: thiophaninic acid, stictic acid, constictic acid (e and h in Fig. 4).

Note: *P. xanthodes* is confused with *P. pustulata*; however, it contains a different chemical, 2-O-methynorlichexanthon. It has pale ostioles but *P. pustulata* has cracked black ostioles. It is difficult to distinguish this species from *P. pustulata* without chemical tests.

Representative specimen examined: Korea, Jeju-do, Geumsan Park, subtropical forest, 33°26′03.06″ N, 126°19′50.09″ E, alt. 100 m, on bark, Aug 2015, J. S. Park, B. G. Lee, J.-J. Woo, 152908 (KoLRI 037881).

Variolaria multipunctoides (Dibben) Lendemer, B. P. Hodk. & R. C., Harris, Mem. N. Y. Bot. Gard. 104: 88 (2013).

Lepra multipunctoides (Dibben) Lendemer & R. C. Harris, Bryologist 120: 187 (2017).

Pertusaria multipunctoides Dibben, Publications in Biology and Geology, Milwaukee Public Museum 5: 59 (1980).

Morphology: Thallus definite, thick; surface verrucose, cracked, somewhat shiny, white, thallus margin smooth, grayish green, zonated. Apothecia disciform, 0.5-1.1 mm diam. wide, flattened-hemispherical, whitish brown to pale brown, not constricted at the base, soredia on the disc, margin slightly rolled, solitary to grouped with 2–3 verrucae, abundant, confluent. Ascospores one per ascus, $147.5 \times 55 \mu$ m, one-celled, single walled.

Chemistry: Thallus K+ brownish, C-, P+ orange red, UV-; medulla K+ brownish, C-, P+ orange red; soredia KC-, P+ orange red. TLC: protocetraric acid, furmarprotocetraric acid.

Note: This species has been recorded in North America [38]. It is similar to *P. subfallens* but differs by having a shiny white thallus, and no papillae. *P. sphaerophora* is distinguished from *V. multipunctoides* by medulla K+ yellow turning to a red-violet reaction.

Representative specimen examined: Korea, Jeju-do, Mt. Halla (Youngsil Trail), 33°21'30.91" N, 126°30'13.94" E, alt. 1,615 m, on bark, 7 Jul 2012, S. Y. Kondratyuk, L. Lőkös, S.-O. Oh, S. Joshi, 121793 (KoLRI 016727).

Description of already published species.

Pertusaria astomoides Nyl., Lich. Jpn.: 51 (1890).

Morphology: Thallus definite, 0.1–0.5 mm thick, whitish gray; surface continuous, moderately cracked-areolate, irregular to square-like, 1–1.2 mm thick, thallus margin distinct, unzonated. Apothecia poriform, rare, scattered, concolorous with thallus, 0.5–1.5 mm diam., 2–4 ostioles in each verruca. Ostioles black, same level with surface of verruca. Ascospores 2 per ascus, ellipsoid, $115-150 \times 40-50 \mu$ m, double-walled, inner wall smooth, pycnidia present, with black ostioles, conspicuous, bacillar pycnoconidia, 5–9 µm long.

Chemistry: Thallus and medulla K+ yellow turning red, C-, P+ yellow to orange; UV-. TLC: norstictic acid and connorstictic acid.

Note: *P. coreana* is synonymous with *P. astomoides* which was reported by Räsänen (1940) [17]. Pycnoconidia are produced in pycnidia and are 7–8 μ m in length, similar to that in *P. astomoides*. In addition, production of norstictic acid was confirmed by Moon *et al.* [22]. *P. astomoides* is similar to *P. subobductans*, but differs by the absence of pycnidia with black ostioles.

Representative specimens examined: Korea, Jeollanamdo, Shin-ui Island, 34°32′27.02″ N, 126°02′10.00″ E, alt. 1 m, on rock, 28 Jun 2013, S.-O. Oh, J. S. Park, J.-J. Woo, 130607 (KoLRI 018952); Jeju-do, 33°20′31.6″ N, 126°10′12.08″ E, alt. 1 m, 18 Jun 2014, Y. Joshi, J.-E. So, 140179 (KoLRI 022548).

Pertusaria commutata Müll. Arg., Flora, Regensburg 67: 269 (1884).

Lepra commutata (Müll. Arg.) Lendemer & R. C. Harris, Bryologist 120: 186 (2017).

Morphology: Thallus thick, dark green to grayish green; surface somewhat shiny, smooth, cracked, margin indefinite, thickly covered with white soralia, appearing as small spots, and soon becoming flat or convex. Apothecia disciform, 0.3–0.9 mm diam., pulverulent disc and irregular disc margin, grayish white, pale gray inside the disc, most verrucae solitary or 2–3 are aggregated. Ascospores 1 per ascus, $127.5-135 \times 30-37.5 \mu m$, ellipsoid, hyaline, single-walled.

Chemistry: Thallus K–, C–, P–, UV–; medulla K–, C–, KC+ violet-red, P–. TLC: no result.

Note: *P. commutata* is similar to other pulverulent disciform species such as *P. amara*, *P. multipuncta*, and *P. subfallens*.

This species is identical to *P. amara* in morphology. In detail, soredia is thickly covered with pulverulent disc, similar to that in *P. commutata*, and the soredia is not granulate. *P. amara* contains picrolichenic acid and protocetraric acid through medulla KC+ violet chemical reaction. *P. commutata* has been described differently by researchers. Following Archer's concept [39], this species has K+ yellow, C–, and P+ yellow reactions with hemathamnolic acid (major), lichexanthone (variable), and thamnolic acid (trace). According to Oshio [35], this species is synonymous with *P. amara*, but it was changed to *P. commutata* due to different verrucae size and chemistry (P+ orange red). However, the chemical reactions described between Nylander's specimen and Oshio's do not match (P–). In the present study, we followed Oshio's concept [35].

Representative specimens examined: Korea, Jeju-do, Temple Gwanum, 33°25'21.50" N, 126°33'34.80" E, alt. 615 m, on bark, 7 Jul 2012, S. Y. Kondratyuk, L. Lőkös, 121931 (KoLRI 016959); Gangwon-do, Mt. Hambeak, 37°11'50.07" N, 128°54'41.94" E, alt. 1,413 m, on bark, 20 Sep 2014, J. S. Park, J.-J. Woo, 141606 (KoLRI 037787).

Pertusaria composita Zahlbr., Ann. Mycol. 14: 58 (1916). **Morphology:** Thallus determinate, corticolous, 0.3–0.9 mm thick, gray; surface strongly cracked-areolate. Apothecia disciform, 1.0–1.5 mm diam., 0.3–0.5 mm height, prominent, constricted at the base, concolorous with thallus, pulverulent disc with exposed inner part, inside brownish, irregular margin, 2 ascospores per ascus, $97.5-100 \times 20-30 \,\mu\text{m}$, hyaline, ellipsoid, double-walled, 5.0–7.5 μm , inner wall smooth.

Chemistry: Thallus K+ brownish, C–, P–, UV–; medulla K+ brownish, C–, P+ yellow or yellowish orange. TLC: protocetraric acid.

Note: *P. composita* presented 2 ascospores per ascus unlike other species that contain one ascospore per ascus. This species is similar to *P. subcomposita*, which also has thick thallus (up to 1.2 mm) and contains thamnolic acid.

Representative specimen examined: Korea, Gangwondo, Mt. Taebeak, 37°05′30.08″ N, 128°56′44.08″ E, alt. 1,481 m, on bark, 18 Jun 2007, J.-S. Hur, 070590 (KoLRI 007431).

Pertusaria flavicans Lamy, Bull. Soc. Bot. Fr. 25: 427 (1878).

Morphology: Thallus determinate or effuse, growing on rock, greenish yellow to yellow, rather thick and rough, up to 1 mm thick; surface slightly rimose to rimose-areolate, areoles irregular, flat to slightly verrucose, up to 1 mm diam., farinose soredia on the thallus, flattened-hemispherical, whitish-yellow, aggregated, rarely solitary, 0.2–1.1 mm diameter, rarely definite margin, gray. Apothecia not seen, unknown.

Chemistry: Thallus K–, C+ orange, P–, KC+ orange, UV+ orange; medulla P–; soredia K–, P+ orange. TLC: thiophaninic acid, norstictic acid.

Note: The species has been reported from the British Isles, Europe, and Asia [37]. It usually grows on siliceous

rock and is commonly observed in coastal rocks. This species produces a yellowish soredia and yellow thallus.

Representative specimen examined: Korea, Gyeongsangnamdo, Mt. Mangeun, 34°51′01.02″ N, 127°49′06.37″ E, alt. 176 m, on rock, 11 Oct 2011, J.-S. Hur, 110124 (KoLRI 013340).

Pertusaria laeviganda Nyl., Lich. Jpn.: 53 (1890).

Morphology: Thallus definite, thick, 0.5–0.8 mm thick; surface smooth, waxy, cracked, grayish green to dark green, cortex well developed. Apothecia poriform, scattered, irregularly round to sub-round, large and prominent, 1.5–2.2 mm wide, 2–5 ostioles in each verruca. Ostioles minute, conspicuous, pale gray, protruding. Ascospores 8 per ascus, 2-seriate, 107.5–175 μ m × 35–50 μ m, hyaline, ellipsoid to oblong-ellipsoid, double-walled, inner wall smooth, 6.25–7.5 μ m.

Chemistry: Thallus K–, C–, P–, UV–; medulla K–, C–, P+ orange red. TLC: 4,5-dichlorolichexanthone, stictic acid, methyl stictic acid, and connorstictic acid.

Note: This species is characterized by the protruding verrucae with minute ostioles, large verrucae size (up to 3.5 mm) and waxy thallus. This species is distributed at high altitudes in the Korean Mountains (Fig. 1H). Eighteen species of *P. laeviganda* (18 out of 21) occurred at elevations higher than 700 m. According to Moon [21], this species appears over 1,000 m on Mt. Sorak and at elevations higher than 900 m in Jeju-do [22].

Representative specimen examined: Korea, Jeju-do, Mt. Halla, 33°25′04.08″ N, 126°32′52.02″ E, alt. 619 m, on bark, 1 Jun 2012, S.-O. Oh, U. Jayalal, J. S. Park, J.-S. Hur, 120965 (KoLRI 015995).

Pertusaria leucosora Nyl., Flora, Regensburg 60: 223 (1877).

Morphology: Thallus definite, developed on rock, 0.5 mm thick; surface pale to dark gray, continuous, rimose-areolate, irregular, areole 0.1–1.0 mm wide, papillate and soralia, thallus margin blackish. Sterile, sorediate, erumpent on the thallus, soredia 0.1–0.2 mm diam. wide, abundant, conspicuous, globose to irregular, crowded soredia spreading over the entire thallus.

Chemistry: Thallus and medulla K+ brownish, C-, P+ orange-red; UV-; soralia KC-. TLC: protocetraric acid, furmarprotocetraric acid.

Note: This species is difficult to distinguish from the other sterile species. This species is morphologically similar to *P. subfallens*, but differs by the absence of fertile vertucae and contains protocetraric acid.

 Representative
 specimens
 examined:
 Korea,

 Gyeongsangnam-do,
 Mt.
 Ungseokbong,
 35°22′63.6″
 N,

 127°52′28.3″
 E,
 alt.
 360 m,
 on rock,
 11
 Oct
 2010,
 X.
 Y.

 Wang,
 J.
 A.
 Ryu,
 101327
 (KoLRI
 012680);
 Jeollanam-do,

 Hacho
 Island,
 34°16′40.5″
 N,
 126°03′30.7″
 E,
 alt.
 1 m,
 on

 rock,
 23
 Oct
 2011,
 X.
 Y.
 Wang,
 J.
 A.
 Ryu,
 110921
 (KoLRI

 013909).
 E
 A.
 Ryu,
 110921
 (KoLRI
 013909).

Pertusaria multipuncta (Turner) Nyl., Lich. Scand. (Helsinki): 179 (1861).

Variolaria multipuncta Turner, Trans. Linn. Soc. Lond. 9: 137 (1806).

Morphology: Thallus definite, thin to thick; surface smooth, cracked, somewhat shiny, greenish gray. Apothecia disciform, soredia on the tip of verrucae, erumpent, white, farinose, 0.1–0.5 mm diam. wide, with a wide blackish disc and irregular margin. Ascospore one per ascus, colorless, ellipsoid, $75-112.5 \times 20-27.5 \mu$ m, wall thin.

Chemistry: All chemical reactions negative, thallus and medulla K-, C-, KC-, P-; UV-. TLC: fatty acid.

Note: This species is most common and widely distributed in Korea (Fig. 1A). This species is very closely related to *Pertusaria ophthalmiza* (Nyl.). Both species have the same chemical reaction profile. *Pertusaria ophthalmiza* also has a negative chemical reaction on thallus, medulla, and soredia, but contains fatty acid. *P. multipuncta* is a variable species, which has a different chemical reaction depending on the country: *P. multipuncta* of Japan [35], Korea [22], and India [40] have no chemical reaction in thallus, medulla, and soredia, while *P. multipuncta* of China [36] and England [37] have a yellow color with K positive and orange color with P positive reactions of the medulla.

Representative specimens examined: Korea, Gyeonggido, Soheul Town, 37°44′57.05″ N, 127°09′05.06″ E, alt. 242 m, on bark, 9 Jul 2014, R. U. G. Jayalal, Y. Joshi, S.-O. Oh, J. S. Park, 141191 (KoLRI 022327); Jeju-do, Chuja Island, 33°57′09.09′ N, 126°20′13.08″ E, alt. 56 m, on rock, 21 Jun 2014, Y. Joshi, J.-E. So, 141053 (KoLRI 023608).

Pertusaria pertusa (L.) Tuck., Enum. N. Am. Lich.: 56 (1845).

Morphology: Thallus thick; surface warted in the center, rest of the parts flat and smooth, slightly cracked, greenish gray, thallus margin definite, conspicuous, pale to whitish. Apothecia poriform, scattered, abundant, convex, irregularly globe to sub-globose, 0.5-1.1 mm thick, 0.3-0.5 mm height, constricted at the base. Ostioles conspicuous, black, round, slightly immersed, 1-5 per ostioles in each verruca. Ascospores 2 per ascus, $117.5-148 \times 45.8-52.5 \ \mu m$, ellipsoid, hyaline, double-walled, inner wall smooth, $5.0-7.5 \ \mu m$.

Chemistry: Thallus K–, C–, P–, UV+ brick red; medulla K+ yellow, C–, P+ orange red. TLC: stictic acid, ± norstictic acid, ± constictic acid.

Note: This species is found on the smooth to rough barks of tree, rarely on rock [36]. This species has a typical pertusaroid type of verrucae that are well-developed, irregularly globed with conspicuous black ostioles. This species is widely distributed from Japan [35] to England [37], but few specimens have been collected in Korea to date.

Specimen examined: Korea, Gangwon-do, Mt. Duta, 37°26′27.3″ N, 128°59′17.14″ E, alt. 855 m, on bark, 11 May 2008, J.-S. Hur, 080124 (KoLRI 008368).

Pertusaria quartans Nyl., Lich. Jpn.: 54 (1890).

Morphology: Thallus muscicolous, definite, thin; surface yellowish gray to whitish gray, mostly smooth to a part of thallus verrucose, thallus margin yellowish brown, irregular. Apothecia poriform, abundant, conspicuous, concolorous with thallus, 0.5–1.0 mm diam. wide, sub-globose to globe, round, constricted at the base becoming sessile, margin usually smooth to slightly verrucose. Ostioles in the center of verrucae, black to dark brown, 0.1–0.2 mm diam., Ascospores 4 per ascus, uniseriate, colorless, ellipsoid, 95–132.5 × 37.5–40 μ m, double-walled, inner wall smooth, 7–10 μ m thick.

Chemistry: Thallus K+ yellow, C–, KC–, P–, UV+ brick red; medulla P– to P+ weak orange. TLC: 4,5- dichlorolichexanthone, and stictic acid.

Note: This species is similar to *P. nakamurae*, but the latter has a poriform apothecia with granules.

Representative specimens examined: Korea, Gangwondo, Mt. Baekseokbong, 37°28'739" N, 128°39'760" E, alt. 494 m, 16 May 2009, Y. Joshi, X. Y. Wang, J. A. Ryu, J.-S. Hur, 090445 (KoLRI 010150); Jeju-do, Mt. Halla (Seongpanak Trail), 33°22'48.44" N, 126°35'26.70" E, alt. 1,025 m, on bark, 6 Jul 2012, S. Y. Kondratyuk, L. Lőkös, S.-O. Oh, S. Joshi, 121883 (KoLRI 016862).

Pertusaria stenostoma Vain., Bot. Mag., Tokyo 35: 55 (1921). **Morphology:** Thallus determinate, thin; surface flat, smooth, yellowish gray, thallus margin irregular. Apothecia poriform, scattered, convex, globe to sub-globose, small, 0.7–1.0 mm diam. wide, 0.2–0.5 mm height, solitary. Ostioles inconspicuous, the tip of verruca irregularly cracked and exposed inner part, black to brownish. Ascospores 8 per ascus, $72.5-85 \times 22.5-30 \mu$ m, hyaline, ellipsoid, single walled, smooth, 2.5–5 µm.

Chemistry: Thallus K–, C–, P–, UV–; medulla K+ yellow turning orange-yellow, KC–, C–, P–. TLC: stictic acid.

Note: This species resembles *P. texana*, but is distinguished by cracked verrucae and the presence of 8 biseriate ascospores per ascus.

Representative specimen examined: Korea, Gangwondo, Mt. Galjeongokbong, 37°52′91.9″ N, 128°30′18.8″ E, alt. 1,084 m, on bark, 22 May 2009, Y. Joshi, X. Y. Wang, J. A. Ryu, J.-S. Hur, 090552 (KoLRI 010240).

Pertusaria subfallens Vain., Bot. Mag., Tokyo 35: 55 (1921).

Morphology: Thallus definite; surface continuous, smooth, greenish gray, papillose, isidia-like, spread whole thallus, 0.1–0.5 wide, 0.1–0.5 mm long, the tip of papillae white, thallus margin conspicuous, zonated, surrounded by thin black line. Apothecia disciform, pulverulent disc and irregular disc margin, granular to powdery soredia on the blackish disc, 0.6–1.2 mm diam., one per ascus, ellipsoid, $(75–)120–156 \times 20–30 \mu$ m, hyaline, single walled, 5.0–7.5 µm. **Chemistry:** Thallus K–, C–, P–, UV–; medulla K– brownish, C–, P+ orange red. TLC: protocetraric acid,

± furmarprotocetraric acid.

Note: This species is characterized by the pulverulent disc and papillae on thallus, which is large than the other disciform verrucae species.

Representative specimens examined: Korea, Jeollanamdo, Mt. Jiri, 35°18′40.2″ N, 127°35′55.3″ E, alt. 1,502 m, on bark, 8 Sep 2006, J.-S. Hur, 060833 (KoLRI 005215); Gangwon-do, Mt. Gariwang, 37°24′05.2″ N, 128°32′10.6″ E, alt. 993 m, on bark, 10 May 2008, J.-S. Hur, 080066 (KoLRI 008309).

Pertusaria sublaeviganda Vain., Bot. Mag., Tokyo 35: 58 (1921).

Morphology: Thallus definite, thin; surface continuous to somewhat discontinuous, smooth, cracked-areolate, greenish gray, thallus margin inconspicuous. Apothecia poriform, scattered, 1.9–2.5 mm diam., constricted at the base, prominent, convex and irregularly sub-globose. Ostioles inconspicuous, minute, opening in the depressions. Ascospores 8 per ascus, biseriate, $65-90 \times 25-33 \mu m$, double-walled, inner wall smooth.

Chemistry: Thallus K–, C–, P–, UV–; medulla K– or K+ brownish, C–, P–. TLC: confluentic acid.

Note: According to Oshio [35], *P. sublaeviganda* is differentiated from *P. laeviganda* by the fertile verrucae as apothecia with irregular, uneven, depressed ostioles and spores. *P. glauca* is also closely related to this species but is distinguished by the 4 ascospores and rough inner wall.

Representative specimen examined: Korea, Gyeongsangbukdo, Mt. Cheongryeang, $36^{\circ}47'21.5''$ N $128^{\circ}54'49.0''$ E, alt. 490 m, on bark, 28 Feb 2004, J.-S. Hur, 040087 (KoLRI 000850).

Pertusaria submultipuncta Nyl., Lich. Jpn.: 55 (1890).

Morphology: Thallus thin, 0.1 mm thick; surface smooth to cracked, whitish gray to yellowish gray, without a definite margin, irregular. Apothecia disciform, concolorous with thallus, 0.6–0.9 mm diam. wide, round, heavy soredia on blackish disc, surrounded by a thick margin, 0.1–0.3 mm thick. Ascospore 1 per ascus, ellipsoid, $(77–)100–130 \times (14–)22–55 \mu$ m, double-walled, 4–5 µm thick.

Chemistry: Thallus K+ yellow turning orange-red, UV-; C-, P+ yellow or yellowish orange, medulla K+ red, C-, P+ yellow. TLC: norstictic acid.

Note: This species is characterized by abundant soredia on disciform apothecia and contains norstictic acid. This species is also difficult to separate from other disciform species because of similarities in the disciform shape and size.

Representative specimens examined: Korea, Gyeongsangbukdo, Mt. Gongduk, 36°45'13.0″ N, 128°16'07.1″ E, alt. 814 m, on bark, 28 Sep 2006, J.-S. Hur, 070809-2 (KoLRI 007647); Jeju-do, Mt. Halla (Gwaneumsa Trail), 33°21'58.06″ N, 126°32'03.03″ E, alt. 1,800 m, on bark, 19 Jun 2012, S.-O. Oh, U. Jayalal, S. Joshi, J. S. Park, F. H. Tian, J.-S. Hur, 121177 (KoLRI 016213).

Pertusaria subobductans Nyl., Lich. Jpn.: 52 (1890).

Morphology: Thallus determinate, thick; surface continuous, smooth to granulate, dark gray to whitish gray, somewhat shiny. Apothecia poriform, scattered, constricted at the base, round to irregular. 2–3 small verruca united, one large verruca, 0.5–2 mm diam. wide, variable, convex, upper part of verruca flat to slightly bumpy, verruca with 2–4 ostioles. Ostioles light gray, slightly depressed. Ascospores 2 per ascus, 125–190 μ m × 40–55 μ m, ellipsoid, hyaline, double- walled, inner wall rough.

Chemistry: Thallus K+ yellowish turning red, C–, P–, UV–; medulla K–, C–, P–. TLC: norstictic acid, and perlatolic acid.

Note: *P. subobductans* has a typical scattered poriform, and it is easily found on the mountains and coastal region in Korea. The species grows on bark or rocks. The species is very closely related to *P. mendax*, but the latter contains perlatolic acid and grows only on the bark.

Representative specimens examined: Korea, Gangwondo, Mt. Taebeak, 37°05′05.4′ N 128°57′13.3″ E, alt. 1,184 m, on bark, 18 Jun 2007, J.-S. Hur, 070575 (KoLRI 007421); Jeju-do, Mt. Halla (Youngsil Trail), 33°21′57.0″ N, 126°30′9.16″ E, alt. 1,670 m, on rock, 4 Jul 2012, S. Y. Kondratyuk, L. Lőkös, S.-O. Oh, S. Joshi, J.-S. Hur, 121488 (KoLRI 016395).

Pertusaria amara (Ach.) Nyl., Bull. Soc. Linn. Normandie, Sér. 2, 6: 288 (1872).

Lepra amara (Ach.) Hafellner, Stapfia 104: 171 (2016).

Marfloraea amara (Ach.) S. Y. Kondr., L. Lőkös & J.-S. Hur. Studia Botanica Hungarica 46: 95-110.

Variolaria amara Ach., K. Vetensk Acad. Nya Handl. 30: 163 (1809).

Morphology: Thallus definite, corticolous, grayish green to gray; surface smooth, rather shiny, small white dot-like spread onto thallus, prothallus distinct, zonated, lacking isidia. Apothecia disciform, constricted at the base, round, sorediate, white pulverulent disc and irregular margin, becoming erumpent, scattered over the thallus. Ascospores 1 per ascus, colorless, oblong-ellipsoid, $62.5-162.4 \times 20-70$ µm, thin walled, up to 2.5 µm.

Chemistry: Thallus K–, C–, P–, UV–; medulla K–, KC+ violet, C–, P–; soredia KC+ violet. TLC: picrolichenic acid, protocetraric acid (trace).

Note: *P. amara* is hardly confused with other sorediate types of species in *Pertusaria*. The species has picrolichenic acid and thus has a bitter taste. Above all, the chemistry of soredia and medulla yielding KC+ violet is the most remarkable character of this species.

Representative specimen examined: Korea, Jeju-do, Mt. Halla (Temple Gwanum trail), 33°23'32.2" N, 126°32'16.0" E, alt. 1,128 m, on bark (Quercus and Acer), 8 Aug 2012, S. Y. Kondratyuk, S.-O. Oh, Y. Kusama, 121620 (KoLRI 017074).

Varicellaria lactea (L.) I. Schmitt & Lumbsch, in Schmitt, Otte, Parnmen, Sadowska-Deś, Lücking & Lumbsch, MycoKeys 4: 31 (2012).

Pertusaria lactea (L.) Arnold, Verh. Zool.-Bot. Ges. Wien 22: 283 (1872).

Morphology: Thallus determinate, somewhat radiated, thin, 0.2–0.4 mm thick; surface gray, smooth, rimose-areolate, areole 0.5–0.9 mm wide, thallus margin conspicuous, zonated, whitish gray, 0.8–0.9 mm wide, irregular to flat. Sterile, soredia on the thallus, abundant, confluent, convex, hemispherical, whitish gray, 0.2–0.5 mm diam. wide.

Chemistry: Thallus K–, C+ red or C–, P–, UV–; medulla K+ pale yellow, C+ red, KC+ red, P–. TLC: lecanoric acid.

Note: This species resembles *P. leucosora*, but the latter has a medulla C negative chemical reaction. *V. velata* is also similar to this species but is distinguished by the sterile with white soredia on the thallus.

Specimen examined: Korea, Chungcheongbuk-do, Mt. Samdobong, 36°01′15.1″ N, 127°52′34.3″ E, alt. 1,115 m, on rock, 1 Jul 2015, J.-J. Woo, G. S. Park & S.-O. Oh, 150491 (KoLRI 035810).

Varicellaria velata (Turner) I. Schmitt & Lumbsch, in Schmitt, Otte, Parnmen, Sadowska-Deś, Lücking & Lumbsch, MycoKeys 4: 31 (2012).

Pertusaria velata (Turner) Nyl., Lich. Scand. (Helsinki): 179 (1861).

Morphology: Thallus determinate, corticolous, waxy, smooth or rugose, becoming verrucose at maturity, gray to white, with distinct margin, brownish gray, soredia and isidia absent. Apothecia disciform, concolorous with thallus, immersed, becoming sessile, round, 0.5–0.7 mm diameter, abundant. Ascospore 1 per ascus, colorless, oblong-ellipsoid, simple, 137.5–200 × 37.5–80 μ m, 5–12- μ m-thick wall when it matures.

Chemistry: Thallus K–, C–, P–, UV+ yellowish white; medulla C+ red. TLC: lecanoric acid.

Note: This species belonged to genus *Pertusaria* but has been segregated by different characteristics such as disciform ascomata, non-amyloid hymenial gel, 1–2 spored asci, and the presence of lecanoric acid [12].

Representative specimens examined: Korea, Gangwondo, Mt. Odae, 37°46'17.02" N, 128°36'04.01" E, alt. 1,454 m, on bark (Quercus), 15 Aug 2008, J.-S. Hur, 080533 (KoLRI 008756); Gangwon-do, Mt. Galjeongokbong, 37°52'08.80" N, 128°26'08.49" E, alt. 1,141 m, on bark (Quercus), 22 May 2009, J.-S. Hur, 090615 (KoLRI 010288).

Species not found in this study. In the present study, we could not confirm six species reported in the literature [19, 21, 22, 25]; they are *P. corallina*, *P. glauca*, *P. nakamurae*, *P. radiata*, *P. pseudamara*, and *P. pustulata*. *P. sphaerosphora* and *P. subcomposita*, which were transferred to *Variolaria multipunctoides* and *P. submultipuncta*, respectively. *P. commutans* Vain. is synonymous with *V. velata* [41] and has been segregated to *Varicellaria*. Consequently, a short description of the following six species is provided according to the previously available literature.

P. corallina (L.) Arnold [Flora, Regensburg 44: 658 (1861)] was reported by Park [19]. This species is characterized by an effuse thallus with crowded coralloid papillate, the isidia simple or branched. It has a strong chemical reaction, with medulla K and KC+ yellow, then yellowish brown or red; C+ yellowish; P+ reddish brown [35].

P. glauca Zahlbr. [Annals of Mycology 14: 57 (1916)] is characterized by the presence of 4 ascospores in each ascus with a double wall and inner wall having numerous lateral ribs [35]. It has been reported from the Jeju-do by Moon [22].

P. nakamurae (Yasuda ex Räsänen) Dibben [Biology and Geology, Milwaukee Public Museum 5: 19 (1980)] is characterized by the presence of a poriform apothecia, scattered with numerous large irregular granules [35]. It is very similar to *P. quartans* in having 4 ascospores in each ascus, but no granule. It has been reported from the Jejudo by Moon *et al.* [22].

P. radiata Oshio [Journal of Science of the Hiroshima University, Series B, Division 2 (Botany) 12 (1968)] is characterized by the presence of 4 ascospores in each ascus with xanthone, small spores and the inner wall of ascospore. It was a new species in Japan, has a rough thallus with fine cracks all over [35]. It has been reported from Jeju-do by Moon *et al.* [22].

P. pseudamara K. H. Moon & Shibuichi [Journal of the Hattori Botanical Laboratory 86: 207 (1999)] has been reported as a new species from the Korean Mt. Sorak. *P. pseudamara* resembles *P. leucosora*. However, it can be distinguished from the latter by the absence of soredia, and the presence of picrolichenic acid [21].

P. pustulata (Ach.) Duby [Bot. Gall., Ed. 2 (Paris) 2: 673 (1830)] is characterized by the presence of a thin thallus; 2 ascospores in each ascus, the inner wall having numerous lateral ribs [35]. This species has been reported from Europe, South Africa, North America, India, Thailand, and Japan [39]. This species also has been reported from the Korean Mt. Sorak and Jeju-do by Moon *et al.* [21, 22].

An updated taxonomic key to the lichen genus *Pertusaria* and allied taxa in South Korea

- 1. Apothecia sterile or not seen, thallus isidiate or sorediate
- ------ 2

- 4. Medulla and soredia C+ yellow, thallus yellow and
- sulfur*P. flavicans*Medulla and soredia C- or C+ red, thallus various color
 5
- 5. Thallus saxicolous, C+ red, containing lecanoric acid *Varicellaria lactea*
- Thallus saxicolous, C–, containing picrolichenic acid

6. Thallus P+ orange red, containing protocetraric acid P. leucosora Thallus P+ yellow, medulla KC+ purple P. leucosora var. violascens Apothecia poriform 17 8. Thallus not sorediate, C+ red, containing lecanoric acid Varicellaria velata 9. Ascospore 2 per ascus P. composita Ascospore 1 per ascus 10 10. Medulla and soredia KC+ violet, containing picrolichenic acid P. amara Medulla and soredia KC-, not containing picrolichenic acid 11 11. Medulla and soredia P- or P+ yellow 12 Medulla and soredia P+ red 13 12. Medulla and soredia P- or P+ yellow, C-, KC+ rose red P. commutata Medulla and soredia P-, KC-, no chemical reaction P. multipuncta 13. Thallus and soredia K+ red P. submultipuncta Thallus and soredia K- or K+ yellow, then purple 14 14. Medulla and soredia K+ yellow, then purple (reddish violet) 15 Medulla and soredia K- 16 15. Ascospore single P. sphaerophora Ascospore two P. subcomposita 16. Thallus papillate P. subfallens Thallus not papillate Variolaria multipunctoides 17. Ascospore 2 or 4 per ascus 18 Ascospore 8 per ascus 29 18. Ascospore 2 per ascus 19 Ascospore 4 per ascus ----- 25 19. Thallus KC+ yellowish orange, containing thiophaninic acid 20 Thallus KC-, not containing thiophaninic acid 21 20. Ascospore wall smooth P. thiospoda Ascospore wall having numerous lateral ribs P. xanthodes 21. Thallus containing 2-chloro-6-O-methylnorlichexanthone P. pustulata Thallus not containing 2-chloro-6-O-methylnorlichexanthone 22. Ascospore inner wall rough, thallus containing protocetraric acid or perlatolic acid 23 Ascospore inner wall smooth ----- 24 23. Thallus containing protocetraric acid, ostioles black, conspicuous, scattered P. thwaitesii Thallus containing norstictic and perlatolic acid P. subobductans 24. Thallus saxicolous, pycnidia common P. astomoides Thallus corticolous, pycnidia not common, ostioles black P. pertusa 25. Thallus muscicolous, UV+ brick red P. quartans

26. Thallus and apothecia having granules P. nakamurae
- Thallus and apothecia lacking granules
27. Ascospore wall smooth, ostioles located at the apices of
verrucae P. leioplaca
- Ascospore wall rough
28. Ostioles located at the apices of verrucae, cracked
P. radiata
- Ostioles not located at the apices of verrucae, scattered
P. glauca
29. Thallus not containing thiophaninic acid or 4,5-
dichlorolichexanthone, ostioles cracked P. stenostoma
- Thallus containing thiophaninic acid or 4,5-
dichlorolichexanthone
30. Thallus containing 4,5-dichlorolichexanthone, stictic
acid
- Thallus containing thiophaninic acid
31. Ostioles with protruding margins, ascospore > 100 μ m
long P. laeviganda
- Ostioles with depressed, ascospore < 100 µm long
P. sublaeviganda
32. Ascospores uniseriate in the asci P. jogyeensis
- Ascospores biseriate in the asci P. texana

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