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The genus *Placolecis* (Catillariaceae, Lichenized Ascomycota) in Russia

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Summary. A review of the lichen genus *Placolecis* in Russia is presented. Localities in the Primorye Territory of *Placolecis loekoesiana*, a new to Russia species, and *P. opaca*, a new to the Russian Far East species, are reported. For *P. opaca*, this is a second locality in Russia after Trans-Baikal Territory (South Siberia). *Placolecis loekoesiana* was previously known exclusively from the "locus classicus" in South Korea. New localities in South Korea are also reported. The description and localities of the new to Russia lichen species *Placolecis loekoesiana* in the Primorye Territory are reported and results of the phylogenetic analysis (nrITS/5.8S) of *Placolecis* species are presented, confirming the distinctness of *P. loekoesiana* and indicating conspecifity of Far Eastern specimens with specimens from South Korea. The studied specimens of *P. loekoesiana* from Russia and South Korea differ from the protologue by hyaline hymenium smaller in size, exclusively ellipsoid ascospores smaller in size and thallus bigger in size. The verified diagnostic traits of *P. loekoesiana* based on studied specimens from Russia and South Korea are given. Besides, the data on pycnidia and conidia for *P. loekoesiana* are presented for the first time. Thus, the species is characterized by its placodioid, yellow-brown or yellow-olivaceous thallus, lecideine apothecia with glossy permanent proper margin, yellow to orange-yellow medulla, hyaline hymenium, pale brownish to hyaline hypothecium, 8-spored *Catillaria*-type asci with simple, hyaline, ellipsoid ascospores. New data on ecology of the species are reported: so far *P. loekoesiana* was known occurring on calcareous rocks in habitats with periodically flowing water, while wherevers in the Russian Far East, it grows on open, dry surfaces of calcareous rocks at the elevation 290 to 480 m.

Род *Placolecis* (Catillariaceae, лихенизированные Ascomycota) в России

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Ключевые слова: биогеография, Восточная Азия, лишайники, Приморский край, Южная Корея, *Placolecis loekoesiana, Placolecis opaca*.

Аннотация. Представлен обзор рода *Placolecis* в России. Приводятся местонахождения нового для России вида лишайника *Placolecis loekoesiana* и нового для российского Дальнего Востока вида *P. opaca,* выявленных в Приморском крае. Первый вид ранее был известен только из "locus classicus" в Южной Корее. Новое место-

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нахождение в Южной Корее также указано. Для второго вида приводится второе местонахождение в России после даурского (Забайкальский край, Южная Сибирь). Представлены результаты филогенетического анализа видов *Placolecis*. На основании сопоставления молекулярных данных (nrITS/5.8S) подтверждена самостоятельность вида *P. loekoesiana* и показано полное сходство дальневосточных образцов с образцами из Южной Кореи. Изученные образцы из России и Южной Кореи отличаются от диагноза, помещенного в протологе, бесцветным гипотецием меньшего размера, более низким гимением, исключительно элипсоидными аскоспорами меньшего размера и бóльшими размерами таллома. По результатам детального анализа морфолого-анатомического строения изученных талломов приводятся уточненные диагностические признаки *P. loekoesiana*, составлено описание вида на русском языке. Кроме этого, впервые дается информация о пикнидах и конидиях этого вида. Таким образом, *P. loekoesiana* характеризуется плакодиоидным желто-коричневым, желто-оливковым талломом, лецидеиновыми апотециям с сохраняющимся блестящим собственным краем, сердцевиной желтой до оранжево-желтой, бесцветным гимением, светло-коричневатым до бесцветного гипотецием, восьмиспоровыми сумками *Catillaria*-типа с одноклеточными, бесцветными элипсоидными спорами. Представлены новые сведения по экологии *P. loekoesiana*, который ранее был известен только на карбонатных скалах в периодически заливаемых местообитаниях, тогда как на российском Дальнем Востоке вид произрастает на открытых, сухих поверхностях карбонатных скал.

Introduction

Placolecis Trevis. (Catillariaceae, Ascomycota) is a small genus included five species worldwide (Kousar et al., 2021). The representatives of *Placolecis* are characterized by crustose-placodioid to squamulose, dark brown to dark yellow, yellow-brown or yellow-olivaceous thallus with yellow to reddish orange medulla K+ red due to the presence of anthraquinones. It has lecideine apothecia, black, sessile, up to 1.3 mm in diam., with a plane to convex disc and a persistent proper margin. Hypothecium is colorless to dark reddish-brown; the apical cells of paraphyses are swollen, with a dark brown cap. Eight-spored, clavate, *Catillaria*-type asci include simple, hyaline, ellipsoid ascospores, (5.0–)10.0–14.0 × (3.0–)5.0–7.5 μm. Pycnidia are immersed to slightly protruding with hyaline, bacilliform, up to 6 μm long conidia. The representatives of *Placolecis* inhabit calcareous rocks in well insolate and exposed sites at low and height elevation.

The type species, *Placolecis opaca* (Dufour) Hafellner, is generally known from Mediterranean region of Europe, and has been infrequently reported from North Africa and Asia (Hertel, 1977; Nimis, Poelt, 1987; Roux, 1991; Kotlov, 2003; Makryi, 2003; Mies, Schultz, 2004; Czarnota et al., 2006; Bilovitz et al., 2008; Aptroot, Moon, 2014; Sinha et al., 2015; Amrani et al., 2018; Ravera et al., 2019). Four other species have been described recently and have a restricted distribution within East and Central Asia: *P. loekoesiana* (S. Y. Kondr. et al.) An. C. Yin has been described from South Korea (Kondratyuk et al., 2017), *P. kunmingensis* A. C. Yin et Li S. Wang and *P. sublaevis* A. C. Yin et Li S. Wang from China (Yin et al., 2019) and *P. kashmirensis* R. Kousar et al. from Pakistan (Kousar et al., 2021). In Russia, the only one species *P. opaca* has been previosly known from the southeastern Transbaikalia (Trans-Baikal Territory, South Siberia), where this species is considered by Makryi (2003) as a relic of Eurasian xerophilic-thermophilic flora and can be dated, presumably, to the Cretaceous period.

Several specimens belonging to *Placolecis* were collected in the southern part of the Sikhote-Alin Range (Primorye Territory, Russian Far East) from limestone outcrops that locally distributed in this area (Yakovchenko et al., 2020). When identifying the specimens, some questions arose that did not allow them to be unambiguously identified.

The aim of the study was to prepare a review of the genus *Plasolecis* in Russia basing on comprehensive analysis of materials, which included morphological and anatomical study of samples, identification of secondary metabolites and molecular phylogenetic analysis.

Materials and Methods

Sampling and phenotypic studies

The material for study comprises eleven specimens of *Placolecis* collected by authors in 2011, 2017 and 2022 on limestone outcrops in the lowlands of the Central and Southern Sikhote-Alin Range (ALTB, herbarium of Davydov and Yakovchenko), as well as specimens of *P. opaca* from Transbaikalia, collected by T. Makryi (NSK, LE). Moreover, our recent collections of presumably *P. loekoesiana* from South Korea, kept in NIBR, were additionally studied, as well as exsiccata of *P. opaca* from Europe deposited in LE.

The specimens were examined using a stereomicroscope (Zeiss Stemi 2000-C) and a compound microscope (Zeiss Axio Lab.A1). Anatomical ex-

amination was undertaken using hand-cut sections mounted in water with following reagents (R): 10 % KOH (K), 10 % HNO_3 (N), lactophenol cotton blue (LCB) and Lugol' s solution (I). Polarized light (pol) was used for locating crystals in the sections. Measurements of ascospores, apothecia, squamules and hymenium are presented as follows: (smallest value recorded–) ($\bar{x}$ – SE) – $\bar{x}$ – ($\bar{x}$ + SE) (–largest value recorded), where $\bar{x}$ is the (arithmetic) sample mean, and SE is the sample standard error. Other measurements are presented as: (extreme minimum) minimum – maximum (extreme maximum). The measurements of anatomical structures were made to the nearest 0.5 μm.

Lichen substances were studied using spot tests with potassium hydroxide solution (K), sodium hypochlorite solution (C) and 1,4-p-phenylendiamine (PD), and by high performance thin-layer chromatography (TLC) with solvent systems A (toluene: 1,4-dioxane: acetic acid, 180 : 45 : 5), B' (hexane: methyl tert-butyl ether: formic acid, 140 : 72 : 18) and C (toluene: acetic acid = 170 : 30) following Orange et al. (2001). We used Merk silicagel 60 UV 254 glass HPTLC plates 10 × 10 cm and made photo by SLR camera in transmitted UV light.

DNA extraction, amplification, and sequencing

Single thallus parts (100–200 mg) or 3–4 apothecia were carefully checked for fungal infections and thoroughly cleaned of extraneous matter. DNA extraction, amplification, and sequencing followed the methods of Davydov and Yakovchenko (2017). Cycling conditions included initial denaturation at 94 °C for 35 cycles of 95 °C for 20 s, 52 °C for 40 s, 72 °C for 60 s, and a final extension step at 72 °C for 7 min. The program Geneious 6.0 (Biomatters Ltd, New Zealand) was used for assembling partial and complementary sequences.

Sequences and phylogenetic reconstructions

To test the phylogenetic relationships of *Placolecis loekoesiana* collected in the Russian Far East and its conspecifity with the Korean material (GenBank Acc. No. MN052962), the ITS region of the nrDNA (ITS1, 5.8S, and ITS2), was sequenced (Table). These markers were used because they were used in phylogenetic analyses recently (Yin et al., 2019; Kousar et al., 2021), and sequences of all species are present in GenBank, whereas other loci are available only for *P. opaca*.

Table

Sample numbers and their GenBank accession numbers for the phylogenetic analyses in this study. Newly obtained sequence in bold

Species	Source: collection location, and collection number or reference	GenBank accession number
Placolecis loekoesiana	South Korea, 041238 (KoLRI)	MN052962
Placolecis loekoesiana	Russia, Primorye Territory, 2014, E. A. Davydov 18307 and L. S. Yakovchenko (ALTB)	**OR195131**
Placolecis sublaevis	China, Yunnan, 19-62675 (KUN)	MK995874
Placolecis kunmingensis	China, Yunnan, 18-58078 (KUN)	MK995884
Placolecis kunmingensis	China, Yunnan, 56795 (KUN)	MK995879
Placolecis opaca	Spain, Inv. Nr. 8764	MK995885
Placolecis kashmirensis	Pakistan, Azad Jammu and Kashmir (LAH36831) (MAK – 11 - holotype), (LAH36831),	MW586931
Solenopsora marina	–	KF689880
Solenopsora olivacea	–	KF689888
Catillaria scotinodes	–	ON380913

Newly generated sequence of *P. loekoesiana* supplemented with sequences obtained from GenBank (Table), representing all five known species of *Placolecis*. *Catillaria scotinodes* (Nyl.) Coppins, *Solenopsora marina* (Zahlbr.) Zahlbr., and *Solenopsora olivacea* (Fr.) H. Kilias were used as the outgroup. This selection is based on the recent studies, in which *Solenopsora* and *Catillaria* appeared more closely related to *Placolecis* (Yin et al., 2019; Kousar et al., 2021). GenBank Accession numbers are provided in Table. ITS\5.8S 556 bp matrix was aligned using the MAFFT algorithm (Katoh et al., 2005) as implemented on the GUIDANCE web server (Sela et al., 2015). The most likely tree and 1000 rapid bootstrap replicates were calculated using RAxML 8.0.26 (Stamatakis, 2014) by raxmlGUI software

version 1.3.1 (Silvestro, Michalak, 2012) applying the GTRGAMMA model of substitution to ITS1, 5.8S, and ITS2 subsets.

The tree topologies were taken from RAxML. Bootstrap support values and BMCMC posterior probability were noted onto the best scoring tree. To provide additional support for our phylogenetic reconstruction a heuristic search for the maximum likelihood (ML) bootstrap tree with simultaneous inference of the optimal partitioning scheme and substitution models for each data partition was performed using the online version of IQ-TREE (Nguyen et al., 2015; Trifinopoulos et al., 2016) with default settings.

Results

A few specimens from the Sikhote-Alin Range were identified as *P. opaca* (Fig. 1) basing on morphology and anatomy. The other part of the specimens morphologically resembled *Placolecis loekoesiana* (Fig. 1), which is characterized by its yellow-brown to yellow-olivaceous effigurate thallus with black lecideine apothecia and yellow medulla and is distributed in East Asia (Kondratyuk et al., 2017) but anatomically does not fit into the description well due to hyaline hymenium (vs. dark brown hymenium in the protologue). They also do not fit well to *Placolecis opaca* because of hyaline to pale-brownish hypothecium (in *P. opaca,* hypothecium is brown) and dissimilar habitus.

Morphological and anatomical study of these specimens and specimens from South Korea, showed their identity. In all specimens, hymenium is colorless and does not exceed 55.0 μm height (Fig. 2). It was also found that the size and shape of the ascospores in studied specimens differ from those described in the protologue (Fig. 3). Based on these facts, a verified diagnostic traits of the *P. loekoesiana* are presented. In addition, pycnidia and conidia are described for the first time for this species.

Both species found in the Sikhote-Alin Range are interesting geographical records. *Placolecis loekoesiana* is a new species for Russia, *P. opaca* is new for the Russian Far East. Its locality is the second in Russia. Both species were reported to South Korea (Aptroot, Moon, 2014; Kondratyuk et al., 2017); these localities are the nearest to those of the Russian Far East.

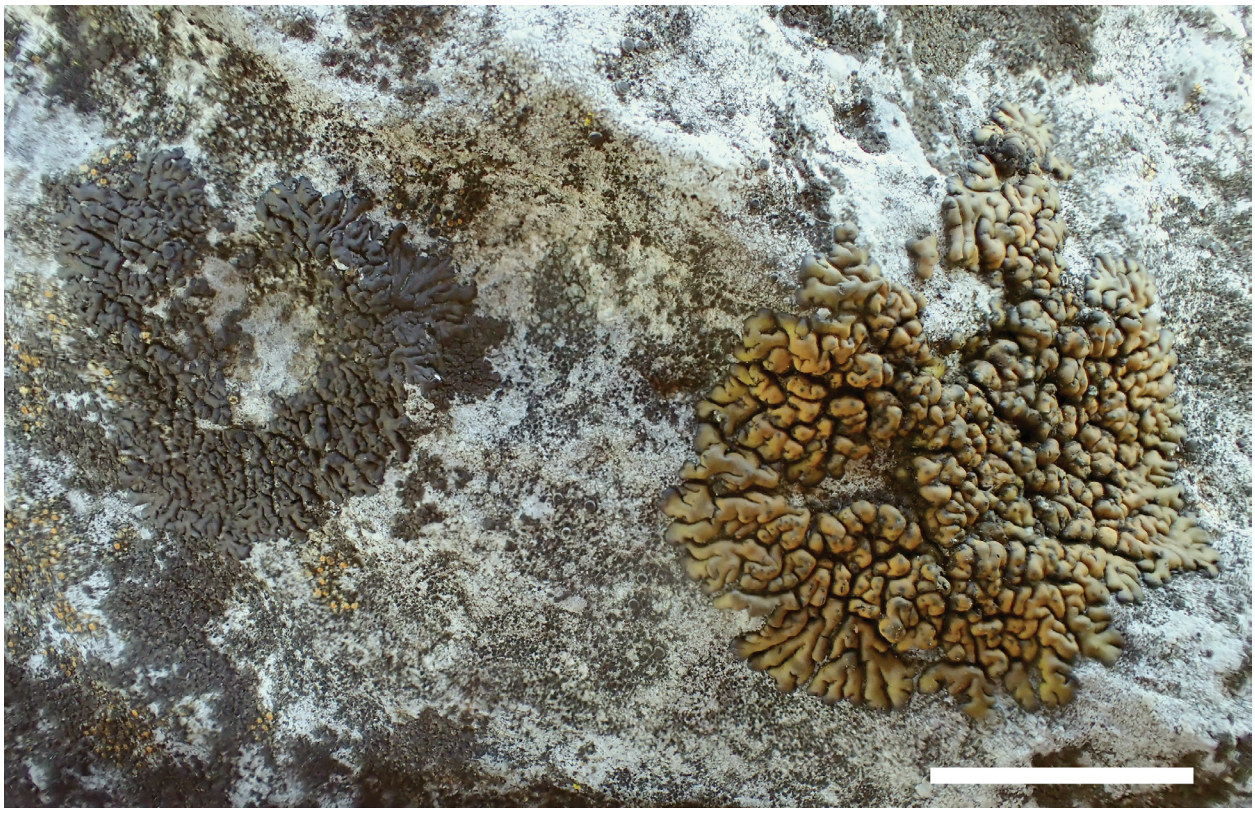

Fig. 1. *Placolecis opaca* (dark brown, left) and *Placolecis loekoesiana* (yellow-olivaceous, right) growing on one rock in the Primorye Territory (field photo). Scale = 1 cm.

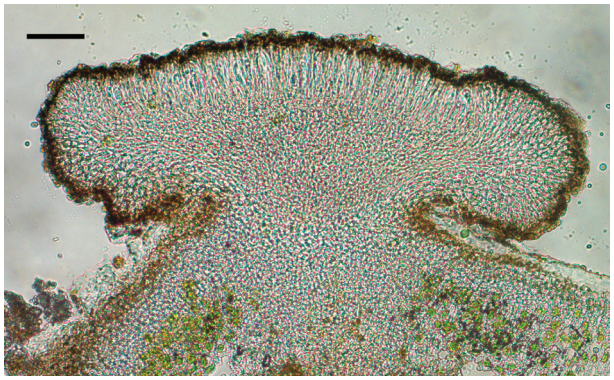

Fig. 2. Apothecium section of *Placolecis loekoesiana* with pale brownish hypothecium. Scale = 50 µm.

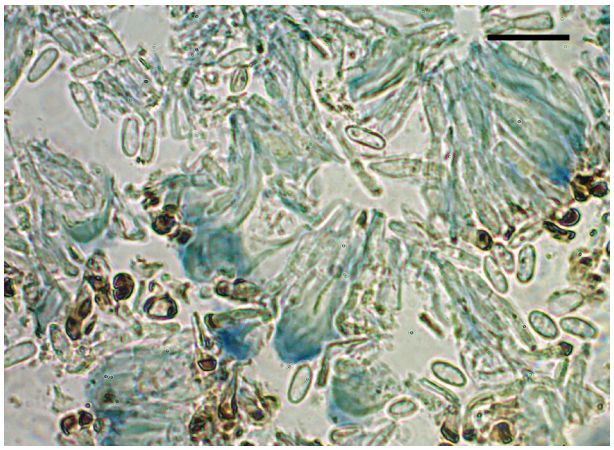

Fig. 3. Asci and ascospores of *Placolecis loekoesiana* in Iodine. Scale = 20 µm.

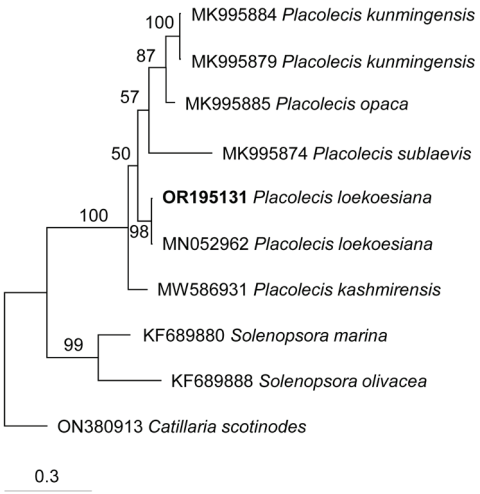

Fig. 4. Maximum likelihood (ML) phylogeny of selected *Placolecis* ITS sequences. Numbers at tree nodes indicate ML bootstrap percentages (right). Thicker branches indicate when the bootstrap value of ML is ≥ 70 %. Accession numbers are given to serve as operational taxonomic unit (OTU) names (see Table). Originally produced sequence is marked in bold. *Catillaria scotinodes, Solenopsora marina*, and *S. olivacea* were used as an outgroup. Branch lengths represent the estimated number of substitutions per site assuming the respective models of substitution.

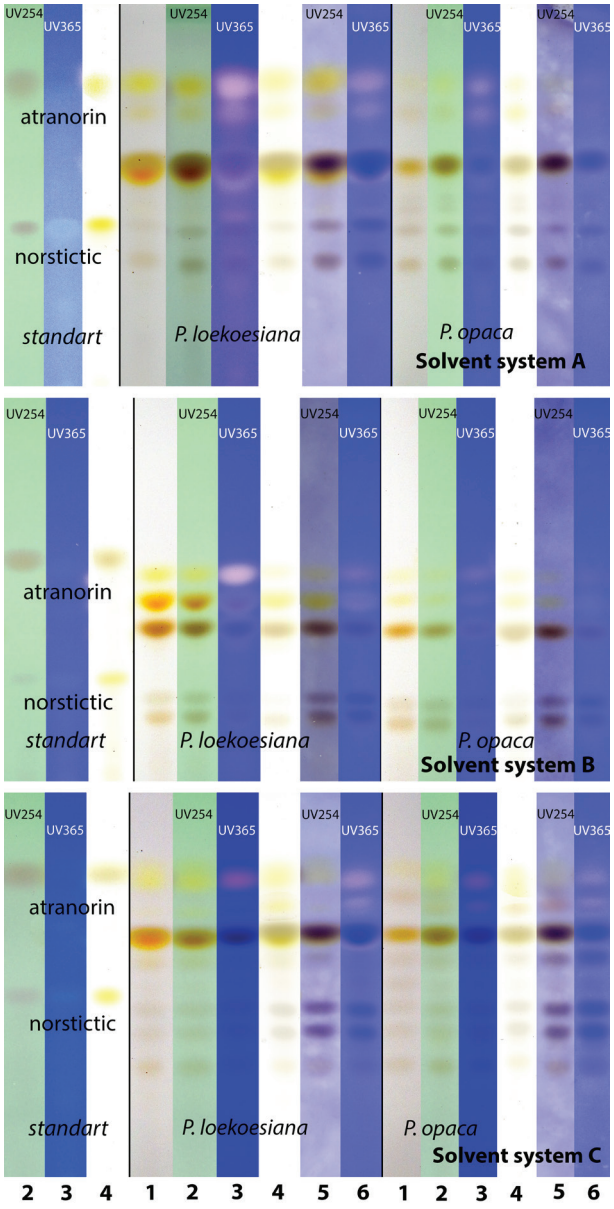

Fig. 5. Fragments of TLC plate with acetone extracts of standart, *Placolecis loekoesiana,* and *P. opaca* in solvent systems A (upper), B (middle), and C (lower): 1 – in day light before heating with sulfuric acid, after two days of exposure; 2 – in UV light 254 nm; 3 – in UV light 365 nm; 4 – in day light after heating with sulfuric acid after two days of exposure; 5 – in UV light 254 nm after heating with sulfuric acid; 6 – in UV light 365 nm after heating with sulfuric acid.

The phylogenetic study

A molecular-phylogenetic analysis of *Placolecis* species was carried out. According to the ITS\5.8S phylogram (Fig. 4), the sequences of *P. loekoesiana* from the Primorye Territory and the sequence of this species from South Korea clustered together (RAxML 98 % BS, IQ 97 % BS), which indicates their identity. This confirms the distinctness of *P. loekoesiana* and indicates a conspecificity of the

specimens from the Primorye Territory and South Korea. We therefore report *Placolecis loekoesiana* for the first time to Russia based on ITS data.

The secondary chemistry

All studied specimens were investigated by TLC. We compared secondary metabolites in *P. loekoesiana* and *P. opaca*. Both species showed the same spots pattern in TLC, however the concentrations of compounds were always higher in *P. loekoesiana* (Fig. 5). As Steiner et al. (1974) reported, fragilin, 2-chlor-1-hydroxy-3,8-dimethyloxy-6-methylanthrachinon, 7-chlor-emodin ('tentatively identified'), 2-chlor-1,2,3-trihydroxy-6-methyl-10-anthron were found in *P. opaca*, we assume that the same antraquinones are contained in *P. loekoesiana.* Secondary metabolites of *Placolecis* require further investigation, six, five, and nine compounds were revealed in solvent systems A, B, and C respectively (Fig. 5).

The species

Placolecis loekoesiana (S. Y. Kondr., Farkas, J. J. Woo et Hur) A. C. Yin, 2019, in A. C. Yin et al., Mycobiology 47(4): 406.

≡ *Astroplaca loekoesiana* S. Y. Kondr., Farkas, J. J. Woo et Hur, 2017, in Kondratyuk et al., Acta Bot. Hung. 59(1/2): 139.

Type: "Republic of Korea. Gangwon-do: Jeongseon-gun, Jeongseon-eup, Aesan-ri, limestone rocky wall along river, on calcareous rocks. 37°22'18.66"N, 128°40'27.76"E. Alt.: 325 m a. s. l. Coll.: S. Y. Kondratyuk and L. Lőkös (163000), 16 IX 2016" (holo – KoLRI 041238); the same locality, (163086) (iso – KoLRI 041327, BP).

Placolecis loekoesiana is characterized by its yellow-brown to yellow-oliveoceous, effigurate to iregular thallus, crustose centrally with areoles (0.3–) 0.7–1.0–1.4(–2,0) mm wide and with elongated marginal lobes expanded up to 4 mm long and up to 1.5 (–1.7) mm wide and lecideine apothecia up to 1.0 mm in diam. with a black, flat disc and black, glossy, permanent proper margin. Medulla yellow to orange-yellow, K+ red to violet due to antraquinones presence.

Apothecia lecideine, hymenium hyaline up to 55(–?75) μm tall; hypotecium pale (hyaline to yellowish, grayish or brownish); paraphyses with a brown terminal cells, up to 7.5 μm wide; asci 8-spored, *Catillaria*-type; ascospores simple, hyaline, ellipsoid, (7.5–)10.0–12.5(–14.0) × 4.0–6.0 μm. Pycnidia abundant, black, submerged to slightly projecting; conidia are colorless, rod-shaped, straight, 4.0–6.0 × 1.0 μm.

Since the description of *P. loekoesiana* has never been published in Russian, we give it here based on studied specimens from the Russian Far East and South Korea.

Description (in Russian)

Таллом накипной, плакодиоидный, округлый (розетки до 3,0 см в диам.) или бесформенный (Fig. 1). *Подслоевище* отсутствует. *Ареолы* выпуклые, 0,3–0,5–0,7 мм толщиной, в центре скученные, округлые и овальные до изогнуто-бесформенных, (0,3–)0,7–1,0–1,4(–2,0) мм шир. (*n* = 65), по краям удлиненные в виде лопастинок, (1,8–) 2,6–3,1–3,6(–4,0) мм дл. и (0,6–)0,7–1,0–1,3(–1,5) мм шир. (*n* = 55), веерообразно расширяющиеся, от перисто-надрезанных до рассеченных на вторичные доли, с округлыми краями. *Верхняя поверхность* темно-желтая, желто-коричневая, желто-оливковая, матовая до слегка блестящей, гладкая до складчатой, без налета. *Вегетативные пропагулы* отсутствуют. *Сердцевина* желтая до оранжево-желтой. Пигмент распределен неравномерно, ближе к верхней коре. *Кора таллома* 25,0–45,0–75,0 мкм толщ., бесцветная, параплектенхимная, сложена гифами с изодиаметрическими клетками, 4,5–6,0–7,5 мкм в диам., расположенных в 3–4(5) ряда, сверху покрыта коричневыми коровыми кристаллами и бесцветным эпинекральным слоем, 5,0–10,0–12,5 мкм высотой. *Водорослевый слой* сплошной, до 112,5 мкм толщ., водоросли хлорококковые, максимальный диаметр 14 мкм; *сердцевина* состоит из рыхло расположенных гиф до 5,0 мкм в диам. с бесформенными золотисто-коричневыми кристаллами, от K+ краснеют.

Апотеции лецидеевые, обычно присутствуют, в центре таллома, по 1–2 на ареоле, одиночные, реже скученные и попарно сливающиеся, округлые до бесформенных, сидячие, широко прикрепленные до чуть суженных у основания, (0,3–)0,4–0,6–0,7(–1,0) мм в диам. и до 2,5 мм выс. *Диск* черный, плоский до слабо выпуклого, матовый до чуть блестящего, гладкий, без налета. *Собственный край* постоянный, ровный, черный, блестящий, на одном уровне с диском до слабо возвышающегося, 0,03–0,08 мм толщ., иногда незаметный. *Гимений* (42,5–) 47,7–50,1–52,5(–55,0) мкм выс. (*n* = 20), бесцветный (даже на толстых срезах). *Эпигимений* (7,5–)8,6–10,5–12,4(–15,0) мкм толщ. (*n* = 15), коричневый. *Гипотеций* (87,5–)95,3–110,5–125,7 (–150,0) мкм толщ. (*n* = 15), бесцветный до чуть коричневатого, без капель масла, состоит из компактно расположенных, вытянутых, беспорядочно ориентированных гиф (Fig. 2). *Эксци-*

пул в базальной части сливается с гипотецием, в латеральной части расширяющийся, из радиально расходящихся гиф, (100,0–)111,3–122,5–133,8(–150,0) мкм толщ. (n = 15), параплектенхимный, из изодиаметрических клеток 5,0–12,5 мкм в диам., бесцветный до чуть коричневатого, а слой наружных клеток с коричневыми шапочками. *Парафизы* простые до разветвленных на концах, септированные, 2,0–3,5 мкм в средней части гимения, верхушки 5,0–7,5 мкм шириной, булавовидные до шаровидных, с коричневыми шапочками. *Сумки Catillaria*-типа, булавовидные, 8-споровые (35,0–)38,3–40,7–43,0(–45,0) × (11,8–)12,4–13,2–14,0(–15,0) мкм (n = 20). *Аскоспоры* (Fig. 3) простые, бесцветные, эллипсоидные до узко элипсоидных, (7,5–)9,6–10,8–11,9(–12,5) × (4,0–)4,8–5,0–5,2(–5,5) мкм (n = 65). *Пикниды* обильные, черные, погруженные до слегка выступающих; *конидии* бесцветные, палочковидные, прямые, 4–6 × 1 мкм.

Таллом K–, C–, KC–, P–; сердцевина K+ краснеет, C+ краснеет, KC+ краснеет, P–.

Substrate and ecology. In the Primorye Territory *P. loekoesiana* grows on open, dry, well-insolated surfaces of carbonate limestone rocks in the mountains at the elevation up to 480 m above the sea level (Fig. 6). It commonly grows together with representatives of the Verrucariaceae, Teloschistaceae, and Lichinaceae. Associated species include *Porpidinia brevispora* Yakovchenko et Davydov, *Endocarpon pusillum* Hedw., *Placynthium nigrum* (Huds.) Gray, *Protoblastenia rupestris* (Scop.) J. Steiner, *Vericaria nigrescens* Pers., and others.

Distribution. The species is known only in East Asia – in South Korea and in the southern part of the Russian Far East (Primorye Territory).

Specimens examined. **Russia**. ***Primorye Territory***: "vicinity of Nakhodka City, Sestra Mt. (Sister Mt.) – conical calcareous rocks, south slope, "42°49'39.7"N, 132°59'40.0"E, elev. 301 m. a. s. l., limestone cliffs, on rock. 21 IX 2011. Yakovchenko 1254" (VLA); ibid., "on S exposed calcareous rock, 16 VIII 2022, E. A. Davydov 19341 and P. Yu. Ryzhkova" (ALTB); ibid., "42°49' 39.8"N, 132°59'39.9"E, elev. 304 m a. s. l., on rocks. 21 IX 2011. Yakovchenko 1255" (herbarium Davydov and Yakovchenko); "Dalnegorskiy district, eastern macroslope of Sikhote-Alin Range: at 6.5 km NE from Dalnegorsk toward to Cheremshany settlement, the valley of Gorbusha River (Rudnaya River's basin), 44°37'01.4"N, 135°39'26.3"E, elev. 349 m a. s. l., limestone cave at the top of the mountain, on calcareous rocks. 16 IX 2011. L. S. Yakovchenko 1252" (herbarium Davydov and Yakovchenko); ibid., "at 2.5 km N from Dalnegorsk City, Partizanskaya Sopka, 44°35'17.7"N, 135°33'17.9"E, elev. 380 m a. s. l., lower part of the slope, calcareous rocks, on rocks. 15 IX 2011. Yakovchenko 1256" (herbarium Davydov and Yakovchenko); ibid., "at 3 km NW from Dalnegorsk, upstream the Barachnyi Stream, 44°34'55"N, 135°33'10"E, elev. 480 m a. s. l., polydominant mixed forest with a calcareous rock massif, on calcareous rocks. 2 IX 2017. E. A. Davydov 18308 and L. S. Yakovchenko" (ALTB); ibid., "at 3.5 km NW from Dalnegorsk, upstream the Barachnyi Stream, 44°35'21"N, 135°33'17"E, elev. 470 m a. s. l., polydominant broadleaf deciduous forest, calcareous rocks massif, on S exposed calcareous rock. 23 VIII 2022. E. A. Davydov 19347 and P. Yu. Ryzhkova"; "Dal'negorsky District, Sikhote-Alin' at 3.5 km NW from Dalnegorsk, upstream the Barachnyi Stream polydominant broadleaf deciduous forest, calcareous rocks massif, 44°35'21"N, 135°33'15"E, elev. 570 m a. s. l., on S exposed calcareous rock. 3 IX 2017. E. A. Davydov 18308 and L. S. Yakovchenko" (herbarium Davydov and Yakovchenko); Kavalerovsky District: "at S from the Kavalerovo, eastern macroslope of central Sikhote-Alin Range, at the right bank of the Zerkalnaya River, 44°14'50"N, 135°03'38.5"E, elev. 290 m a. s. l., near the summit of the calcareous rock cliff surrounded by polydominant broadleaf deciduous forest, on calcareous rocks. 1 IX 2017. E. A. Davydov 18307 and L. S. Yakovchenko" (herbarium Davydov and Yakovchenko); "at S from the Kavalerovo, at right bank of the Zerkal'naya River, near the top of the calcareous rock cliff surrounded by polydominant broadleaf deciduous forest, 44°14'50"N, 135°03'38.5"E, elev. 290 m a. s. l., on S exposed calcareous rock. 1 IX 2017. E. A. Davydov 18307 and L. S. Yakovchenko" (ALTB).

Additional specimens examined. **South Korea:** "Gangwon-do, Sokcho city, Seorak-dong, Mt. Seorak, Seoraksan National Park, on route from Gwengum Castle to Hwachae-bong, 38°09'36"N, 128°29'01"E, elev. 700–1200 m a. s. l., local summit on stone. 14 V 2015. E. A. Davydov 12163" (NIBR); "Samcheok city, Singi-myeoun, Shingi-ri, along the Ohship stream, around Shindong elementry School, 37°21'06"N, 129°04'55"E, elev. 100 m a. s. l., S exposed calcareous rocks, on soil over the rocks. 11 V 2015. E. A. Davydov 12098" (NIBR); ibid., "on S exposed calcareous rocks, E. A. Davydov 12091, 12097" (NIBR).

Placolecis opaca (Dufour) Hafellner, 1984, Beih. Nova Hedwigia 79: 317.

≡ *Lecidea opaca* Dufour, 1831, in Fries, Lich. eur. reform. (Lund): 289.

≡ *Lecidea adglutinata* var. *opaca* (Dufour) Nyl., 1855, Mém. Soc. Imp. Sci. Nat. Cherbourg 3: 183.

≡ *Astroplaca opaca* (Dufour) Bagl., 1857, Mém. R. Accad. Sci. Torino, Ser. 2 17: 415.

≡ *Biatora opaca* (Dufour) Jatta, 1911, Fl. Ital. Crypt. (Florence) 3: 254.

Type: France, "in rupibus alpinis Galliae", leg. Dufour. [in Herbarium Elias L. Fries in UPS, as in G, not a single authentic material was found (Hertel, 1977)].

Thallus crustose placodioid, forming up to 2.0 cm wide, regular rosettes to irregular in outline (Fig. 1). *Prothallus* absent. *Areoles* flat to slightly convex, rounded to elongated, up to 1.0 mm wide in the central part, marginally with distinctly elongated, fan-shaped expanding lobes, up to 4.0 mm long and 0.4–1.5(–2.0) mm wide and up to 0.35 mm hight. *Upper surface* olive to brown, matt, smooth, without pruine. *Vegetative* propagules absent. *Medulla* orange to yellow, darker in the upper part and paler in the lower part. *Cortex* paraplectenchymatous brown in upper part, colourless to pale brown in lower part, 20.0–25.0 µm high, of 3–4 rows of hyphae with isodiametric cells, up to 7.5 µm in diam., without hyaline epynecral layer. *Algae layer* even, up to 100 µm tall, chlorococcal algae, the maximum diameter is 15.5 µm. *Medulla* consists of loosely arranged hyphae up to 5.0 µm thick with irregular golden brown crystals that turn red from K.

Apothecia lecideine, usually present, in the centre of the thallus, single to rare crowded, rounded to irregular, 0.25–0.75 mm in diam., sessile, widely attached to somewhat constricted at the base. *Disc* black, flat, matt, smooth, without pruina. *Proper margin* black, persistent, glossy, even, at the same level as disc, up to 0.07 mm thick. *Hymenium* colourless up to 60.0 µm tall. *Epihymenium* brown, 7.0–10.0 µm high. *Hypothecium* brown, non inspersed by oil droplets. *Proper margin* brown in outer part, colourless within, up to 120.0 µm thick in uppermost part, paraplectenchymatous, consist of isodiametric cells 5.0–10.0 µm in diam. *Paraphyses* simple to branched near the tips, septate, 2.0–2.5 µm thick in mid hymenium, apically clavate to capitate, the apical cells with an internal pigment cap, up to 6.5 µm wide. *Asci* 8-spored, clavate, *Catillaria*-type, 32.0–45.0 × 12.0–15.0 µm. *Ascospores* simple to 1-septate, hyaline, ellipsoid, (8.0–)10.0–12.0 × (4.0–)5.0–6.5 µm. *Pycnidia* frequent, immersed or slightly projecting. *Conidia* hyaline, bacilliform, straight, 4.0–7.5 × ca. 1 µm. *Spot tests* thallus K–, C–, KC–, P–; medulla K+ red, C+ red, KC+ red, P–.

Chemistriy fragilin and other antraquinones detected by TLC (Fig. 5).

The morphological features of the Far Eastern specimen of *P. opaca* (Fig. 1) agree with the descriptions provided by Schneider (1979), Makryi (2002) and Kotlov (2003) as well as with herbarium specimens of the species from Europe (J. Schuler 55, LE!, C. Sbarbaro, LE!) and Asia (T. Makryi D-501, LE-L1332!, T. Makryi 6856, NSK!).

Substrate and ecology. In Primorye and Trans-Baikal Territories *P. opaca* grows on open, dry, well-insolated surfaces of limestone rocks in the mountains at the elevation up to 500 m a. s. l. (Fig. 6). It commonly grows together with representatives of the Verrucariaceae, Teloschistaceae, and Lichinaceae. In one locality in Primorye it was observed growing together with *P. loekoesiana* (Fig. 1).

Distribution. Mediterranean region of Europe, North Africa (Algeria) and Asia (India, Yemen, South Korea and Russia) (Hertel, 1977; Nimis, Poelt, 1987; Roux, 1991; Makryi, 2003; Mies, Schultz, 2004; Czarnota et al., 2006; Bilovitz et al., 2008; Aptroot, Moon, 2014; Sinha et al., 2015; Amrani et al., 2018; Ravera et al., 2019).

Specimens examined. **Russia. *Primorye Territory***: "Nakhodka City, at the vicinity of Nakhodka, the left bank of the Partizanskaya River near its mouth, the conical summit of the Sestra Mt. (318 m), calcareous rocks, 42°49'40"N, 132°59'39"E, elev. 310 m a. s. l., on S exposed calcareous rock. 16 VIII 2022. E. A. Davydov 19348 and P. Yu. Ryzhkova" (ALTB); "Partizansky District, at 1.5 km NE from Ekaterinovka Village, the right bank of the Partizanskaya River, 13.5 km upstream from its mouth, Prizhevalskogo Range, 42°56'09"N, 133°03'54"E, elev. 80 m a. s. l., oak forest (*Quercus mongolica*) with calcareous rocks, on S exposed calcareous rock. 17 VIII 2022. E. A. Davydov 19351 and P. Yu. Ryzhkova" (hb. Davydov and Yakovchenko); ibid., "E. A. Davydov 19342 and P. Yu. Ryzhkova" (ALTB). ***Trans-Baikal Territory***: "Nerchinsko-Zavodskiy District, vicinity of Nerchinskiy Zavod Village, [51°18'N, 119°37'E], elev. ca. 500 m a. s. l., upper part of the slope, meadow steppe with *Paeonia lactiflora* Pall. near the *Betula dahurica* Pallas forest. On big stone of crystalline calcite, calcareous rock. 1 VII 1985. T. V. Makryi" (NSK 4001000); ibid., "steppe slope, on stones of dolomitic limestone. 31 VII 2001. T. V. Makryi D-501" (LE-L1332; NSK 4001001; duplicate in ALTB).

Fig. 6. The sun exposed calcareous rock outcrop is a typical habit for *Placolecis loekoesiana* and *P. opaca* in the Primorye Territory (Sestra Mt.).

Additional specimens examined. **Italy**. Kryptogamae exsiccatae editae a Museo Palatino Vindobonensi № 55, "litorale austriacum: ad saxa calcarea in agro tergestino. Leg. J. Schuler" (LE); Italy. "*Liguria occidentalis*: Alassio. IV 1955. C. Sbarbaro" (LE).

Discussion

The genus *Placolecis* in Russia is represented by two species: *P. opaca* which is distributed in South Siberia and southern part of Far East and *P. loekoesiana* which is first discovered in Russia in southern part of the Russian Far East. The species are well identifiable, the worldwide key is given in Kousar et al. (2021).

Placolecis loekoesiana is a conspicuous epilithic calcicolous lichen characterized by its yellow-brown to yellow-oliveoceous, effigurate to iregular thallus, crustose centrally. The closest species, *P. opaca*, is mainly differ by its smaller and darker, brown thallus, with smaller areoles in the central part, up to 1.0 mm wide (vs. up to 1.0(–2.0) mm wide), also by brown to red-brown hypotecium (vs. hyaline to yellowish, grayish or brownish).

The specimens of *P. loekoesiana* from the Russian Far East, in general, fit into the description of the species, but mainly differ in the following: the hymenium is colorless (dark brown in the protologue) and lower, up to 55.0 μm tall (in the protologue up to 75.0 μm tall) (Fig. 2), the spores are ellipsoid to narrowly ellipsoid (the shape of the spores in the protologue was "round to ellipsoid", but according to the sizes "10.0–12.0(–14.0) × 5.0–6.0 μm" they cannot be round), the minimum and maximum spore length is less than given in the protologue (Fig. 3). In addition, we have studied the material of the species from South Korea. In all investigated specimens the hymenium was hyaline and up to 50.0 μm tall. Moreover, the maximum size of the thallus of studied specimens is twice as large as indicated in the protologue. Possibly, it depends on different method of measuring. Among the studied specimens, we observed variability in the morphology of the thalli: some specimens are characterized by a radial effigurate thallus with clearly elongated marginal lobes, in other specimens the thallus possesses forms of a compact crust but the marginal lobes are less distinct, specimens of the third group have irregular thallus and elongated lobes occur arranged randomly, and not only along the edge of the thallus. We measured only distinct placodioid rossetes, ignoring specimens from the last group or confluent thalli. The hyaline epinecral layer was found in all specimens of *P. loekoesiana* and lack in specimens of *P. opaca*. The characteristics of the pycnidia and conidia of *P. loekoesiana* reported here for the first time almost do not differ from those in *P. opaca.*

According to the protologue, "medulla yellow in the upper portion and white in the lower portion, K+ violet" and it is a one of the characters to split the species with *P. opaca*. According to our observations, the medulla is yellow to orange-yellow. It is remarkable that the color of the medulla is connected with the color of the upper surface of the thallus: under the darker upper surface, the medulla is darker, orange- yellow. The specimens with paler upper surface have a lighter, yellow medulla. The pigment is distributed unevenly: its concentration is high under the upper cortex, gradually decreasing to the lower cortex. When the medulla is yellow in the upper part its coloration is getting white close to the lower cortex. However, the yellow coloration is observed throughout the section in specimens with a brighter colored medulla. We could not observe K+ violet reaction in *P. loekoesiana*. The reaction after K was similar for *P. opaca* and *P. loekoesiana*, and it was K+ red. Moreover, we showed the same chemosindrome in both species (Fig. 5).

Both species of *Placolecis* are found in the same localities at altitudes of 290 to 480 m, sometimes grow together. Sun exposed surfaces of calcareous rocks are the typical habitats for *P. opaca.* However, *P. loekoesiana* grows in South Korea on calcareous rocks in habitats with periodically flowing water, while wherevers in the Russian Far East, it grows on open, dry surfaces of calcareous rocks. Thus, our observations from the Russian Far East expand data on its ecology. The species was found in five localities in the Primorye Territory at a latitude between 44° to 42° and at a maximum distance of about 300 km from each other. The localities of *P. loekoesiana* in the Primorye Territory are the northernmost known ones. The South Siberian locality of *P. opaca* is situated 1400 km west from the Far Eastern locality. The nearest localities in Juwang Mts, South Korea (Aptroot, Moon, 2014) are about 700 and 1000 km from newly reported localities in the Primorye Territory. The doubts expressed by Kondratyuk et al. (2017) about the reality of the presence of *P. opaca* in South Korea are most likely erroneous, since our research has confirmed the presence of both species in East Asia.

Thus, *P. loekoesiana*, previously known exclusively from *"locus classicus"*, is recorded as a new species for Russia. A new location of it in South Korea

is also reported. *Placolecis opaca* is reported for the first time for the Russian Far East, and this locality is the second in Russia after Transbaikal locality.

A verified diagnostic traits of *P. loekoesiana* are presented on the basis of the studied samples from Russia and South Korea.

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