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Updates to the list of Estonian lichenized, lichenicolous and allied fungi

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Herewith, we continue to upgrade Estonian checklist of lichenized, lichenicolous and allied fungi and report twelve fungal species as new for Estonia, of them eleven are lichenized and one is lichenicolous fungus.

The specimens were determined by using standard techniques of microscopy commonly used for determination of lichens (Randlane & Saag, 2004; Smith et al., 2009). In addition, DNA was extracted, amplified and sequenced from selected specimens to confirm identifications. For extraction, High Pure PCR Template Preparation Kit (Roche Applied Science®) or alkaline lysis method described in for example Voitk et al. (2020) was used. The fungal internal transcribed spacer (ITS) region was amplified using the primer pair ITS0F / LA-W (Tedersoo et al., 2008), and for *Scytinium* also mitochondrial small subunit (mtSSU) DNA region with primers mrSSU1 / mrSSU3r (Zoller et al., 1999). The extracted DNA samples are deposited in the DNA and Environmental Sample Collection of the Natural History Museum in University of Tartu (TUE). The new DNA sequences are publicly available under UDB-codes in PlutoF workbench (https://plutof.ut.ee) and in eElurikkus data portal (https://elurikkus.ee). A blast search (Altschul et al., 1990) was used to compare new sequences with those deposited in NCBI (https://www.ncbi.nlm.nih.gov) and UNITE nucleotide repository (https://unite.ut.ee). To ascertain determinations, the most similar sequences were downloaded, aligned using MUSCLE (Edgar, 2004) and clustered using PhyML (Guindon et al., 2010) in SeaView ver. 4.7. (Gouy et al., 2010).

The abbreviations of the country regions and frequency classes follow Randlane & Saag (1999): (1) the country regions: NE – northeastern part, NW – northwestern part, SE – southeastern part, SW – southwestern part, WIs – Western islands; (2) frequency classes (Freq.): rr – very rare, 1–2 localities, r – rare, 3–5 localities, st fq – rather frequent, 11–20 localities. The cited specimens are kept in the fungarium of the Natural History Museum and Botanic Garden, University of Tartu (TUF), in the lichen collection of the Tallinn Botanic Garden (TALL), in the herbarium ICEB (deposited in TUF), National Museum Wales (NMW) or in the private herbarium of Alan Orange (Hb. A. Orange). The following abbreviations are used for persons: AS – Ave Suija, IJ – Inga Jüriado, LM-O – Liis Marmor-Ohtla, TR – Tiina Randlane; for spot tests: Pd – paraphenylenediamine (in ethanol), C – calcium hypochlorite (in water).

Biatora fallax Hepp – NW: Rapla county (Co.), Kehtna community (comm.), Kastna (58.87164°N, 25.08149°E), *Carex* type pine forest, on dried *Betula* sp., leg. T. Sepp 3 July 2020, det. AS 8 Oct. 2022 (TUF091314; UDB0781140). Freq.: rr.

The thallus of Estonian specimen is granulose to small-squamulose, esorediate, reacts Pd+ orange, ascomata are beige to light brown, strongly convex; all microstructures are hyaline, ascospores 1-celled, a few 2-celled, 8–16 × 3.5–4.5 µm (n=15). The fungal ITS sequence corresponds to sequences of *B. fallax* (AJ247548, KX389593, etc., percent identity 99–100%). In Europe, *Biatora fallax* grows in old-growth coniferous forest preferring old, somewhat decaying trees (Holien, 1994; Printzen & Palice, 1999).

Biatora fallax differs from other *Biatora* species occurring in Estonia and having beige ascomata with non-septate ascospores as follows: *B. helvola* Körb. contains gyrophoric and lecanoric acids (thallus and ascomata C+ red) while *B. fallax* contains additionally argopsin (thallus

Pd+ orange); *B. efflorescens* (Hedl.) Räsänen and *B. chrysantha* (Zahlbr.) Printzen are obligately sorediate while soredia occur only secondarily in well-developed small-squamulose thalli of *B. fallax*; *B. vernalis* (L.) Fr. grows primarily on ground-layer mosses and contains no lichen substances (all spot tests negative) in contrast to other *Biatora* species which are epiphytic and contain lichen substances. There are numerous *Biatora* species described in recent years (e.g., Printzen, 2014; Printzen et al., 2016), and it is possible that at least some of these new species occur also in Estonia.

Buelliella minimula (Tuck.) Fink – SW: Pärnu Co., Saarde comm., Lodja (58.16277°N, 24.88274°E), on *Pertusaria leioplaca* growing on *Acer platanoides*, leg. P. Lõhmus 23 May 2020, det. AS 20 Oct. 2021 (TUF091771; UDB01004662). Freq.: rr.

The species is scatterly recorded, but globally known from North and South America, Australia and Europe (Ertz & Diederich, 2015). Before this record, only one *Buelliella* species – *B. lecanorae* Suija & Alstrup was known from Estonia (Suija & Alstrup, 2004). There are no ITS sequences available for this genus in NCBI sequence repository, but the closest matches belong to environmental samples representing taxa of Dothideomycetes (e.g., KU550122 percentage of identity 91.26%, JF519200 – 90.58%). Taxonomically, the obligately lichenicolous genus *Buelliella* belongs to Asterotexiales, Dothideomycetes within which it has been shown to be polyphyletic (Ertz et al., 2016).

Calogaya arnoldii (Wedd.) Arup, Frödén & Søchting – NE: Ida-Viru Co., Toila comm., Saka village, Värava Cottage (59.42910°N, 27.22778°E), on granite stone in wall of farm house, leg. IJ 21 July 2021, det. IJ & AS 16 May 2022 (TUF092325.a; UDB07672062); Harju Co., Kuusalu comm., Kuusalu village, Kuusalu Cemetery (59.44472°N, 25.44722°E), calcareous stone in stone wall around the cemetery, leg. TR 17 Aug. 2020, det. AS 2021 (TUF090751; UDB01004471). Freq.: rr.

Within the well-known, widely distributed and species-rich genus *Caloplaca* s. lato, this saxicolous lobed-effigurate taxon belongs to the taxonomically most controversial *C. saxicola* group (Gaya et al., 2011). Moreover, *Calogaya arnoldii* represents the *C. arnoldii* complex (part of the *C. saxicola* group) with especially complicated taxonomy, where intermediate phenotypes are frequently observed within a population (Gaya et al., 2011). The *C. arnoldii* complex includes four subspecies that are differentiated based on morphology (Gaya, 2009); however, their circumscription is unclear and phylogenetic relationships are not established even when combined morphological and ITS data were analyzed (Gaya et al., 2011). Two specimens are known from Estonia, the specimen TUF092325.a is morphologically in accordance with the description of British authors as *Caloplaca arnoldii* in a strict sense (Smith et al., 2009) which corresponds to *C. arnoldii* ssp. *obliterata* (Pers.) Gaya (Gaya, 2009). Moreover, in comparison of fungal ITS sequences of this sample, 100% similarity with *C. arnoldii* ssp. *obliterata* (e.g. HM800856 – 100%, JQ301657 – 99.84%) occurred. This specimen was found in coastal region on vertical wall of siliceous stones, which has been considered characteristic for this subspecies and has good distinction value from other subspecies taxa, which prefer calcareous rocks (e.g. *C. arnoldii* ssp. *arnoldii*). The other specimen found in Estonia (TUF090751) was growing on calcareous stone and represents another infraspecific taxon (closest match HM800861 – 98.63% similarity). However, further taxonomic studies on the *C. arnoldii* subgroup are required as none of the infraspecific taxa included in the *C. arnoldii* species complex is phylogenetically well circumscribed (Gaya et al., 2011).

Flavoplaca flavocitrina (Nyl.) Arup, Frödén & Søchting – Distr.: NW, NE, SE, WIs. NW: Harju Co., Tallinn, Lasnamäe (59.4529°N, 24.85431°E), on limestone, leg. LM-O 21 Oct. 2021, det. IJ & AS Nov. 2021 (TALL L009165; UDB01004675; TUF092685) and (59.45362°N, 24.81709°E; 59.45286°N, 24.81388°E), on limestone, leg. IJ 10–17 May 2022, det. IJ Aug. 2022; Aegna (59.57284°N, 24.75841°E), on mortar, leg. IJ 1 July 2021, det. IJ 2021 (TUF092370); Lääne Co., Dirhami (59.20964°N, 23.49211°E), on limestone, leg. IJ 17 Aug. 2021, det. IJ 2021 (TUF092484.a); Rapla Co., Haimre manor (58.87222°N, 24.46694°E), on limestone wall, leg. TR 5 Aug. 2020, det. IJ Dec. 2021 (TUF092493.b); NE: Lääne-Viru Co., Kunda (59.48530°N, 26.53168°E), on mortar, leg. IJ 9 June 2021, det. IJ 2021 (TUF092468; TUF092243.a); Porkuni manor

(59.18643°N, 26.19662°E), on limestone, leg. IJ 10 June 2021, det. IJ 2021 (TUF092473.a); Vainupea (59.58232°N, 26.27135°E), on limestone wall, leg. IJ 9 June 2021, det. IJ & TR 2021 (TUF092360.a, TUF092252.a); SE: Tartu Co., Alatskivi castle (58.60185°N, 27.13696°E), on mortar, leg. IJ 4 Aug. 2021, det. IJ 2021 (TUF092482.a); WIs: Saare Co., Karja (58.52220°N, 22.69988°E), on limestone, leg. IJ 28 July 2020, det. IJ 2021 (TUF092461.g); Orissaare (58.56259°N, 23.09162°E), on limestone, leg. IJ 28 July 2020, det. IJ Dec. 2021 (TUF092460.a). Freq.: st fq.

The species is a quite overlooked taxon of the *Caloplaca citrina* group and was previously considered as a variety of *C. citrina* (Smith et al., 2009). However, distinct morphology and ITS ribosomal DNA gene analyses prove the consideration of the taxa at species level (Arup, 2006), with new combination within the genus *Flavoplaca* established according to modern classification (Arup et al., 2013). Thallus consists of contiguous areoles, soralia are marginal but may eventually cover most of the thallus surface. Characteristically, color of soredia is contrasting with the rest of the thallus, being usually paler than the thallus (pale yellow to pale orange). Apothecia occur quite frequently (Arup, 2006; Smith et al., 2009). The species can be both saxicolous and corticolous. It occurs on calcareous rock, and on man-made calcareous substrata such as mortar and concrete. When corticolous, it prefers bark with subneutral pH, growing frequently on *Ulmus*, *Fraxinus*, and *Acer* (Arup, 2006). In Estonia the specimens of *F. flavocitrina* have been collected only recently and so far, found only on calcareous stones and mortar. The determination of the specimen from Lasnamäe (TALL L009165) was confirmed using comparison of fungal ITS sequence (percent identity 99–100%) and phylogenetic clustering. The species is widely distributed; it is common in Europe and North America, and known also from Macaronesia, Asia, Africa and Australia (https://www.gbif.org/species/8591446; Smith et al., 2009).

Flavoplaca oasis (A. Massal.) Arup, Frödén & Søchting – WIs: Saare Co., Saaremaa Island, Saaremaa comm., Liigalaskma village (58.577116°N, 23.00795°E), alvar grassland (no. 474), on calcareous stone and on *Verrucaria* thallus, together with *Acarospora cervina*, *Circinaria calcarea*, *C. hoffmanniana*, *Xanthocarpia lactea* and *Verrucaria nigrescens*, leg. IJ & AS 11 Sept. 2009, det. IJ & TR 5 Apr. 2022 & 4 Aug. 2022 (TUF092688.a, TUF092689.a); Atla village (58.30046°N, 23.93086°E), alvar grassland (no. 27, Atla_3), on calcareous stone and on *Verrucaria* thallus, together with *Circinaria calcarea*, *C. hoffmanniana*, *Clauzadea monticola*, *Sarcogyne regularis*, *Thelidium incavatum*, *Xanthocarpia lactea*, *Verrucaria fuscella* and *V. nigrescens*, leg. E. Oja & K. Kolnes 18 Sept. 2009, det. IJ & TR 4 Aug. 2022 (TUF092687.a); Muhu Island, Muhu comm., Kallaste village (58.67113°N, 23.23955°E), alvar grassland on calcareous cliff (LC_85), on calcareous stone and on *Verrucaria* thallus, together with *Gyalecta jenensis*, *Hymenelia heteromorpha, Verrucaria* sp. and *Xanthocarpia crenulatella*, leg. IJ 9 July 2020, det. IJ & TR 4 Aug. 2022 (TUF092690.b). Freq.: r.

The species belonged to the so-called *Caloplaca holocarpa* group (Arup, 2009) but is now accepted in a separate genus *Flavoplaca* (Arup et al., 2013). It grows on limestone, mortar and concrete but may also be parasitic on thalli of *Verrucaria* s. lat. All our samples were parasitic on *Verrucaria*, at least partly. The characteristic features of the species are related to the measures of ascospores, including the thickness of septum: spores are ellipsoid, (8.0–)9.5–13.0(–14.0) × (4.0–)4.5–6.5(–7.0) µm, septum is pretty wide, (2.5–)2.8–4.0(–4.6) µm, >1/4 of spore length (Arup, 2009). Spore measures of our samples were: 12.8–14.4 × 4.8–6.4 µm, while septum width was 2.5–3.2 µm. The species is distributed in Europe, mainly in Great Britain and Central and Southern Europe (https://www.gbif.org/species/2610000); in Fennoscandia it is recorded in the southern parts of Sweden and Norway, but not in Finland (Westberg et al., 2021).

Polyblastia baltica Savić & Tibell – WIs: Saare Co., Saaremaa, north-west of Tagamõisa village, south of Undva nina (58.507500°N, 21.911833°E), on limestone stones at edge of *Pinus sylvestris* forest by sea, leg. & det. A. Orange 18095 12 May 2009 (NMW, Hb. A. Orange). Freq.: rr.

This is a rare species found from a few localities in Sweden and Finland (Westberg et al., 2021). The detailed description of this species is given in Tibell & Tibell (2017: 38).

Scytinium biatorinum (Nyl.) Otálora, P.M. Jørg. & Wedin [lubja-tardsamblik] – NE: Harju Co., Tallinn, Lasnamäe (59.45245°N, 24.85685°E), on calcareous rock, leg. LM-O 2021, det. IJ, AS & TR 6 Oct. 2022 (TUF092686; TALL L009166, UDB01004674). Freq.: rr.

Scytinium biatorinum has granular-crustose to small-squamulose thallus, usually forming a blackish thin crust. Squamules are 0.3–0.5 mm diam., with crenulate margins, some are reduced to granules. Apothecia are common, to 0.5 mm diam., concave to flat, with thick proper margin (Jørgensen, 2007; Smith et al., 2009). Spores are colourless, submuriform, ellipsoid, 25–30 × 10–14 µm. The species grows on calcareous soil, rocks and walls (Jørgensen, 2007). A variable species, specimens from dry habitats (calcareous rock and soil) tend to be granulose, while those from mossy soil are squamulose (Jørgensen, 2007; Smith et al., 2009). The Estonian specimen is crust-like, with minute lobes and granules; apothecia are abundant, spores are colourless, ellipsoid, submuriform, 20–26 × 9–11 µm (n=13), and it was found on calcareous rock from old, abandoned quarry. The closest hit for the ITS sequence was *Scytinium lichenoides* (MK101210) with 94.2% of identity. The closest matches (99%) for the additionally sequenced mtSSU DNA region were JX992940 and JX992941, both representing *S. biatorinum*.

The ascospores of Estonian specimen are smaller than presented by Jørgensen (2007), but closer to the measurements presented by Nimis (2022): (23–)25–30 × (8–)11–14 µm. *Scytinium biatorinum* often occurs in ephemeral or disturbed habitats. The species is widely distributed in warm-temperate parts of the Northern Hemisphere (Jørgensen, 2007; Smith et al., 2009) and has been recorded also in the neighbouring countries of Estonia – Finland, Latvia, Lithuania, Russia and Sweden (Stepanchikova et al., 2013).

Strigula glabra (A. Massal.) V. Wirth – NE: Harju Co., Tallinn, Pirita (59.45324°N, 24.82618°E; 59.45325°N, 24.83031°E; 59.45241°N, 24.83912°E; 59.45486°N, 24.86042°E), on bark of deciduous trees (*Acer platanoides, Corylus avellana, Fraxinus excelsior* and *Sorbus aucuparia*) in a broadleaved slope forest by limestone outcrop, leg. LM-O & IJ May 2022, det. LM-O & AS 2022 (TALL L010461, UDB07672549; L010463; L010464; L010465; L010466). Freq.: rr.

Strigula glabra is a crustose epiphytic lichen with thin whitish or light grey thallus, black flattened perithecia and black pycnidia (Roux & Sérusiaux, 2004; Wirth et al., 2013). Compared to *S. stigmatella* (Ach.) R. C. Harris (the only previously known *Strigula* species from Estonia), *S. glabra* is characterised by flattened perithecia with involucrellum reaching to the base of the perithecium (Roux & Sérusiaux, 2004). The spores of collected samples were 4–7-septate, hyaline, fusiform, (19–)21–27(–31) × 5–5.5(–7) µm (n=16); conidia were 5–7-septate, hyaline, cylindrical with rounded apices, (23–)25–28(–30) × 4–5 µm (n=16). Pycnidia were present on all samples whereas perithecia occurred only occasionally (TALL L010461, L010465 and L010466). The species was recorded on 17 trees; the actual abundance is likely to be higher as only a small proportion of trees was examined in the habitat. There are no ITS sequences available neither for *S. stigmatella* nor for *S. glabra* in NCBI nucleotide repository. For the moment, we have only one ITS sequence available from the Estonian material. The closest matches for this sequence were KX710253 (*Preussia* sp., Pleosporales, Dothideomyces) and KP335515, KP335515, etc. (Fungal endophyte; UNITE annotation *Preussia* sp.) with 85.53% and 85.36% of identity respectively. According to Hongsanan et al. (2020), genus *Strigula* is restricted to foliicolous members of this genus, and non-foliicolous species are transferred to a new genus *Swinscowia*. In the moment, we retain the older name because of lack of phylogenetic data supporting this distinction.

According to GBIF (https://www.gbif.org/species/5259739), *S. glabra* is a rare species that is previously not known from the Baltic and Fennoscandian region; in Europe it occurs mainly in the Alpes, some localities are also known outside of Europe.

Verrucaria ahtii Pykälä, Launis & Myllys – WIs: Saare Co., Saaremaa, south-west of Tagamõisa village, near Kõruse (58.472000°N, 21.947717°E), on limestone stone on unshaded ground amongst *Pinus sylvestris-Juniperus communis* scrub, leg. & det. A. Orange 18106 12 May 2009. (Hb. A. Orange). Freq.: rr.

The species occurs in Finland, Lithuania, Russia (Pykälä et al., 2017) and Switzerland (Oïhénart et al., 2018). The detailed description of this species is given in Pykälä et al. (2017).

Verrucaria fusconigrescens Nyl. – NE: Harju Co., Viimsi comm., Rohuneeme, Muuga Bay (59.562789°N, 24.812083°E), on granite boulder in water ca 3 m from the coastline, leg. J. Martin 9 July 2006, det. L. Martin 12 July 2006 (ICEB # 13279). Freq.: rr.

The species is distinguished by the well-developed brown thallus on a dark brown to nearly black prothallus, absence of thalline layer over perithecia and medium-sized ascospores [(17–)19.5–23.5(–26) × (6.5–)8–9.5(–11) µm]; the habitat on siliceous rocks on or near the coast is also considered characteristic (Smith et al., 2009). Such treatment has been widely accepted and the taxon is recorded from Europe (Germany, Greece, France, Ireland, Netherlands, Norway, Sweden, UK, Ukraine), Asia and Australia (https://www.gbif.org/species/5259502). The closest record to ours is from Leningrad Region, Russia (Himelbrant et al., 2013). However, Pykälä (2010) pointed out that the infraspecific variation of this taxon was poorly known and it remained uncertain if *V. fusconigrescens* was a species on calcareous or siliceous seashore rocks.

Verrucaria pallidomurina Orange – WIs: Saare Co., Lümanda comm., Himmiste village (58.26493°N, 21.98072000°E), on limestone, leg. V. Liiv 19 June 2014, det. AS 8 Oct. 2022 (TUF073085.g, sub *Verrucaria nigrescens*; UDB022934). Freq.: rr.

Verrucaria pallidomurina is a species that has been reported only from the United Kingdom and Ireland (Orange, 2020). The Estonian specimen was identified primarily based on the 100% similarity to corresponding ITS sequences (MT127198, MT127221, etc.). As noted by Orange (2020), there are number of species with small perithecia, medium-sized ascospores and discontinuous thalli that are difficult to distinguish morphologically from each other. The Estonian specimen has epilithic thallus, greyish to dirty brown in color, cracked; perithecia are half-immersed, with apical involucrellum, that spreads downwards, exciple dark, periphyses c. 20 µm in length, ascospores 20–22 × 9–12 µm (n=3). The accompanying species are *Verrucaria nigrescens*, *Protoblastenia rupestris*, *Clauzadea monticola*, *Bagliettoa calciseda* and *Polyblastia albida*.

Verrucaria raesaenenii Pykälä & Myllys – NW: Rapla Co., Märjamaa comm., Iganõmme landscape reserve, Iganõmme Pakamägi (58.929205°N, 24.186474°E), limestone cliff, on limestone, leg. AS & M. Suija, 27 July 2021, det. AS 8 Oct. 2022 (TUF091674.a; UDB01004455). Freq.: rr.

Verrucaria raesanenii is a few years ago described species that is known from Finland and Norway (Pykälä et al., 2019). We identified this specimen based on 100% similarity of ITS sequence to MK138816 (sequence from the holotype). The thallus of the Estonian specimen is epilithic, but discontinuous, perithecia are one-third to half-immersed, black, c. 200 µm in diameter, involucrellum is well-developed, reaching almost to the base level, exciple dark, ascospores 15–19 × 6–8 µm (n=10) and periphyses are c. 15 µm in length. The stone is covered with white limestone dust, and therefore the characteristic fimbriate prothallus (Pykälä et al., 2019) is only partly distinguishable. The accompanying species are *Sagiolechia protuberans* and *Verrucaria nigrescens*.

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