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Elaphomyces section *Elaphomyces* (*Eurotiales, Ascomycota*) — taxonomy and phylogeny of North European taxa, with the introduction of three new species

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Abstract: The North European species of *Elaphomyces* section *Elaphomyces* (*Eurotiales, Pezizomycotina*) are studied. Three new species, *E. citrinopapillatus*, *E. pusillus*, and *E. roseoviolaceus* are introduced and verified by morphology and sequence data from ITS, nuclear LSU, mitochondrial SSU, and β-tubulin. A lectotype for *Elaphomyces granulatus* is selected. *Elaphomyces granulatus* and *E. muricatus* are epitypified with sequenced material from the Femsjö region in South Sweden. *Elaphomyces striatosporus* is epitypified with sequenced material from the vicinity of the type locality in Norway. A key to all species of *Elaphomyces* occurring in Denmark, Norway, and Sweden is provided.

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INTRODUCTION

The genus *Elaphomyces* (Nees von Esenbeck & Nees von Esenbeck 1820) comprises hypogeous species forming ectomycorrhiza with a variety of forest trees. The genus was described based on a single species, *Scleroderma cervinum*, ascribed to Persoon. The genus name was later sanctioned by Fries (1829), who listed two species, *E. granulatus* (= *Scleroderma cervinum*) and *E. muricatus*. Index Fungorum (2018) counts nearly 100 described names but recent studies have estimated the number of valid species to be around 55 on a global scale (Castellano *et al.* 2012a). Early mycologists working on the European species of *Elaphomyces* were Vittadini (1831, 1842) and Tulasne & Tulasne (1841, 1851) and in their works several of the common and widespread species were described. In a comprehensive study of the genus Ławrynowicz (1988, 1989) recognized 20 species occurring in Europe. More recently Paz *et al*. (2017) combined morphology and molecular data in a revision of the European taxa and accepted 26 species. The genus has a global distribution and is recorded from all continents, except the Antarctic (Zhang & Minter 1989, Zhang 1991, Castellano *et al.* 2011, 2012a–c, 2016, Buyck *et al.* 2016, Castellano & Stephens 2017). Molecular phylogenetic studies indicate that *Elaphomyces*, as it is presently understood, covers a considerable genetic variation and its monophyly has been questioned (Reynolds 2011, Buyck *et al.* 2016, Paz *et al.* 2017).

Vittadini (1831) divided *Elaphomyces* in two major sections, *Malacodermei* and *Sclerodermei*. Within the latter section he distinguished two species groups: species with a more or less smooth peridium (*Cortice laevi*) and those with a verrucose peridium (*Cortice exasperato*). Fontana (1909), when dealing with Vittadini´s group "*cortice exasperato*", distinguished two lineages: species with a non-homogeneous peridium (the *E. variegatus*-group = *E. muricatus* and its allies) and those with a homogenous peridium (the *E. granulatus*-group). Phylogenetic analyses based on molecular data show that Fontana's two groups both belong to a well-supported clade that corresponds to *Elaphomyces sensu stricto* (= section *Elaphomyces*) (Buyck *et al.* 2016, Castellano *et al*. 2016, Paz *et al.* 2017). This section covers species with a mostly more or less brown peridial surface. In accordance with Fontana (1909) the molecular data confirm a division of sect. *Elaphomyces* in two subsections, *Muricati* and *Elaphomyces*, respectively. In addition, a third subsection, sect. *Papillati* has been recognized (Paz *et al.* 2017).

The present study extends on the work presented in Paz *et al.* (2017) and focuses on section *Elaphomyces* in North Europe (Denmark, Norway, Sweden). Truffle inventories using trained dogs have been undertaken in a variety of ecosystems. From newly collected ascomata a four-gene (ITS, nLSU, mtSSU and β-tubulin) sequence data set was generated and analysed by phylogenetic methods. The aims were to survey the diversity of *Elaphomyces* in the countries mentioned, relate all specimen sequences to existing names, describe new species when necessary, and infer phylogenetic relationships among the species.

MATERIALS AND METHODS

Specimen sampling

Our sampling has an emphasis on *Elaphomyces* in Europe and its northern part in particular, with the aim of finding and characterising all known or putative species of *Elaphomyces* subgenus *Elaphomyces* occurring in the Nordic countries. Representatives of *Pseudotulostoma* and *Aspergillus* were selected as outgroup taxa in the analyses. Sequence data of these were retrieved from GenBank and added to the dataset (FJ358278, AB161194). Two ITS sequences of *E. muricatus* and *E. granulatus* were retrieved from GenBank and included in the dataset (EU784198, EU784197). In addition, sequence data from southern Europe and type specimens in the study by Paz *et al*. (2017) were included for comparison of species concepts and genetic variation (Table 1). For the epitypification of species originally described by Fries from Sweden, new specimens from Femsjö, Småland, the place where Fries was collecting, were sampled and sequenced.

DNA extraction, amplification (PCR), and sequencing

Sequences from four regions were generated: the complete ITS region and about 1 200 base pairs (bp) of the 5´end of the LSU nuclear ribosomal DNA, the mitochondrial small subunit ribosomal DNA (mtSSU) and about 500 bp of the β-tubulin (*β-tub*) gene. DNA extractions, PCR reactions, and sequencing were performed as described in Larsson & Örstadius (2008). Primers used to amplify the complete ITS region and the 5´end of the LSU region were ITS1F (Gardes & Bruns 1993) and LR21, LR0R, and LR7 (Hopple & Vilgalys 1999); for mtSSU we used MS1 and MS2 (White *et al.* 1990); for *β-tub* Bt1a and Bt2b (Glass & Donaldson 1995). Primers used for sequencing were ITS1, ITS4, MS1, MS2 (White *et al.* 1990), Ctb6 (https://nature.berkeley.edu/brunslab/) and Lr5 (Hopple & Vilgalys, 1999), Bt2a and Bt2b. Some of the DNA barcode data were generated in collaboration with the Norwegian Barcode of Life project (NorBOL). Sequenced specimens are marked with an asterisk (*) in specimen lists.

Phylogenetic analyses

Sequences were edited and assembled using Sequencher v. 5.1 (Gene Codes, Ann Arbor). Alignment of individual genes was performed using the L-INS-i strategy as implemented in MAFFT v. 7.017 (Katoh & Standley 2013). The alignment was adjusted manually using the data editor in PAUP v. 4.0b10 (Swofford 2003). The sequences generated for this study have been deposited in GenBank with the following accession numbers: ITS-LSU KR029730–KR029767 and MF614923, mtSSU KR064762–KR064785, *β-tub* KR363193–363215 (Table 1). Some of these sequences were also used in the analyses carried out by Paz *et al.* (2017).

Sequences were concatenated and subjected to phylogenetic analyses through maximum parsimony and Bayesian inference. Variable regions with ambiguous alignment were excluded and gaps were treated as missing data. Heuristic searches for the most parsimonious trees were performed using PAUP (Swofford 2003). All transformations were considered unordered and equally weighted. Heuristic searches with 1 000 random-addition sequence replicates and TBR branch swapping were performed, saving at most 25 trees in each replicate. Relative robustness of clades was assessed by the bootstrap method using 1 000 heuristic search replicates with 100 random taxon addition sequence replicates and TBR branch swapping, the latter saving at most 25 trees in each replicate.

Bayesian analysis was carried out in MrBayes v. 3.2.6 (Ronquist *et al.* 2012), with a best-fit model of nucleotide evolution supplied separately for each genetic marker by MrModeltest v. 2.2 (Nylander 2004). The protein-coding β-tubulin gene was not subjected to partitioning of the third base in each codon. Eight default-setting Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) chains were run for 10 M generations with trees sampled every 5 000 generations and an initial burn-in of 1 000 trees. After discarding the trees prior to the burn-in threshold a 50 % majority-rule consensus phylogram was computed from the remaining trees.

Morphology

Ascomata of *Elaphomyces* were detected in the field, typically with the help of trained dogs. The samples were photographed *in situ* and later studied in the laboratory. The features of the peridial surface were studied after the ascomata had been carefully cleansed from soil, using a tooth brush. Ascomata were measured from fresh material, using a caliper. The measurements of the peridial thickness refer to fresh material and always include the outermost peridial layer (cortex). If fresh material was not available, dried samples were soaked in water for at least 30 minutes before measuring. Spore measurements are given inclusive of their ornamentation and were made at 1 000×, using a Leica DM LS microscope. Twenty mature (or putatively mature) spores with well-developed ornamentation were measured for each sample. All microscopic studies were conducted in Hoyer's medium (http://www.coloss.org/beebook/II/varroa/2/3/1/2). Micrographs were taken in a Zeiss AXIO Imager M2, using the software Zeiss ZEN 2 pro. Collections have been deposited at herbarium O, if not otherwise stated.

RESULTS

Phylogenetic analyses

The complete concatenated and aligned four-gene dataset consisted of 63 taxa and 3 165 nucleotide positions. After exclusion of ambiguous regions 2 871 characters remained for the analyses. Of these 2 510 were constant, 99 were variable and parsimony uninformative, and 262 were parsimony informative. The Maximum parsimony analysis yielded 23 225 equally most parsimonious trees (length = 479, CI = 0.8601, RI = 0.9712) one of which is presented in Fig. 1. Bootstrap values above 50 % are indicated above branches. A bootstrap value greater than 70 % is considered strong.

As suggested by MrModeltest (Nylander 2004), the following nucleotide evolution models were used in the partitioned Bayesian analysis: GTR+I for ITS1, JC+I for 5.8S, HKY+G for ITS2, GTR+I+G for LSU, GTR+I for mtSSU, and K80+G for β-tubulin. The MCMC analysis converged well in advance of the burn-in threshold and chain mixing was found to be satisfactory, as assessed by Tracer v. 1.5 (Drummond *et al*. 2012). Also in the Bayesian analysis, section *Elaphomyces* was recovered as monophyletic with strong support (BPP 1.00). The Bayesian tree topology is identical to the maximum

Table 1. Specimens of *Elaphomyces* spp. used for phylogenetic analyses, and their corresponding GenBank accession numbers.

Species	Status	Voucher ID	Country, year	Herbarium	ITS	LSU	mtSSU	β-tubulin
Elaphomyces asperulus	**Epitype**	IC13051208	Spain, 2012	LIP-0001131	KX238833	KX238877		
		A. Molia, AM-35-2014	Sweden	GB-0150464	KR029753	KR029753	KR064772	KR363209
		A. Molia s.n.	Denmark, 2014	O-F22178	KR029755	KR029755	KR064773	KR363210
		A. Molia, AM-179-2013	Norway, 2013	O-F21354	KR029754	KR029754	–	–
		G.F. Medardi	Italy	MCVE-00160	KR064762	KR064762	–	–
Elaphomyces barrioi	**Holotype**	IC16121209	Spain, 2012	LIP-0001133	KX238848	–	–	–
		A. Molia, AM-270a-2014	Norway, 2014	O-F22181	KR029744	KR029744	KR064767	–
		A. Molia, AM-184-2014	Norway, 2014	O-F22180	KR029746	KR029746	KR064768	–
		A. Molia, AM-347-2011	Norway, 2011	O-F21187	KR029745	KR029745	–	–
		A. Molia, AM-32-2014	Norway, 2014	O-F22301	KR029747	KR029747	KR064769	KR363206
Elaphomyces cf. barrioi		A. Molia, AM 153	Norway, 2011	A Molia pers. herb.	KR076543	–	–	–
Elaphomyces citrinopapillatus	**Holotype**	A. Molia, AM-23-2014	Norway, 2014	O-F21559	KR029765	KR029765	–	–
		A. Molia, AM-69-2014	Norway, 2014	O-F22184	KR029762	KR029762	KR064778	KR363213
		A. Molia, AM121a-2013	Norway, 2013	O-F21344	KR029763	KR029763	KR064779	KR363214
		A. Molia, AM-19-2014	Norway, 2014	O-F21561	KR029766	KR029766	–	–
		A. Molia, s.n.	Norway, 2013	O-F21556	KR029764	KR029764	–	–
Elaphomyces decipiens	**Neotype**	IC28011203	Spain, 2012	LIP-0001134	KX238832	KX238876	–	–
		IC27111118	Spain, 2011	A. Paz pers. herb.	KX238842	–	–	–
Elaphomyces cf. decipiens		IC12051208	Spain, 2012	A. Paz pers. herb.	KX238831	–	–	–
		A. Molia *et al*. s.n.	Norway, 2013	O-F21513	KR029743	KR029743	KR064766	KR363205
		M. Jeppson, MJ10151	Sweden	GB	MF614923	–	–	–
		A. Molia, AM-351-2013	Norway, 2013	O-F21484	KR029742	KR029742	KR064765	KR363204
Elaphomyces granulatus	**Epitype**	A. Molia, AM-44-2014	Sweden, 2014	GB-0147063	KR029767	KR029767	–	–
		M.C. Clark	Scotland, 1972	K(M)47712	EU784197	–	–	–
		A. Molia, AM-20-2012	Norway, 2012	O-F245217	KR029768	KR029768	–	KR363215
Elaphomyces granulatus f. pallidosporus	**Holotype**	IC21071103	Italy, 2011	LIP-0001132	KX238846	–	–	–
Elaphomyces muricatus	**Epitype**	A. Molia, AM-43-2014	Sweden, 2014	GB-0147062	KR029730	KR029730	–	KR363200
		A. Molia, AM-37-2014	Sweden, 2014	GB	KR029731	KR029731	–	KR363201
		A. Molia, AM-42-2014	Sweden, 2014	GB	KR029732	KR029732	KR064763	KR363202
		A. Molia, AM-157-2012	Norway, 2012	O-F245291	KR029733		–	–
		A. Molia, AM-264-2014	Norway, 2014	O-F22182	KR029734	KR029734	–	–
		A. Molia, s.n.	Norway, 2014	O-F22183	KR029735	KR029735	–	–
		IC01041301	Spain, 2013	A. Paz pers. herb.	KX238849	–	–	–
		M. Kelly	England, 2004	K(M)121442	EU784198			

Table 1. (Continued)

Species	Status	Voucher ID	Country, year	Herbarium	ITS	LSU	mtSSU	β-tubulin
		A. Molia, AM-212-2013	Norway, 2013	O-F21312	KR029741	KR029741	KR064764	KR363203
Elaphomyces muricatus var. *reticulatus*	**Epitype**	IC14011206	Spain, 2012	LIP-0001153	KX238851	–	–	–
Elaphomyces muricatus var. *variegatus*	**Epitype**	IC05011307	Spain, 2013	LIP-0001154	KX238850	–	–	–
		A. Molia, AM-351-2011	Norway, 2011	O-F21190	KR029737	KR029737	–	–
		A. Molia, AM-239-2012	Norway, 2012	O-F245312	KR029736	–	–	–
Elaphomyces cf. muricatus		R. Kristiansen s.n.	Norway, 2011	O-F245437	KR029738	–	–	–
		A. Molia, AM-352-2011	Norway, 2011	O-F21009	KR029739	KR029739	–	–
		A. Molia, AM-151-2014	Sweden, 2014	GB	KR029740	KR029740	–	–
Elaphomyces papillatus var. *papillatus*	**Epitype**	IC12051202	Spain, 2012	LIP-0001136	KX238819	KX238872	–	–
		IC26051201	Spain, 2012	A. Paz pers. herb.	KX238820	–	–	–
Elaphomyces papillatus var. *suphureopallidus*	**Holotype**	IC13051212	Spain, 2012	LIP-0001156	KX238830	–	–	–
Elaphomyces pusillus	**Holotype**	A. Molia, AM 121	Norway, 2014	O-F22174	KR029761	KR029761	KR064777	KR363212
		A. Molia, AM 132-2014	Sweden, 2014	GB-0179907	KR029758	KR029758	KR064774	–
		A. Molia, AM-123-2014	Norway, 2014	O-F22175	KR029760	KR029760	KR064776	KR363211
		S. Sivertsen, B.K.P. Sveum 79-205	Norway, 1979	TRH-1273	KR029759	–	KR064775	–
Elaphomyces cf. pusillus		A. Molia, AM-146-2012	Norway, 2012	O-F245285	KR029756	KR029756	–	–
		K. Killingmo, AKW-421	Norway, 2012	O-F21005	KR029757	KR029757	–	–
Elaphomyces roseoviolaceus	**Holotype**	A. Molia, AM-135-2013	Norway, 2013	O-F21376	KR029752	KR029752	KR064770	KR363207
		A. Molia, AM-271-2013	Norway, 2013	O-F21429	KR029751	KR029751	KR064771	KR363208
		A. Molia, AM-271-2013	Norway, 2013	O-F21429	KR029750	KR029750	–	–
Elaphomyces quercicola	**Holotype**	IC23071107	Spain, 2011	LIP-0001155	KX238837	KX238879	–	–
		IC23071104	Spain, 2011	A. Paz pers. herb.	KX238838	–	–	–
Elaphomyces striatosporus	**Epitype**	A. Molia, AM-269-2012	Norway, 2012	O-F245330	KR029748	–	–	–
		A. Molia, AM-273-2012	Norway, 2012	O-F245333	KX238861	–	–	–
		A. Molia, AM-280-2012	Norway, 2012	O-F245337	KR029749	–	–	–
Elaphomyces violaceoniger	**Holotype**	IC22011401	Spain, 2011	LIP-0001135	KX238857	–	–	–
		IC15031401	Norway, 2014	A. Paz pers. herb.	KX238858	–	–	–

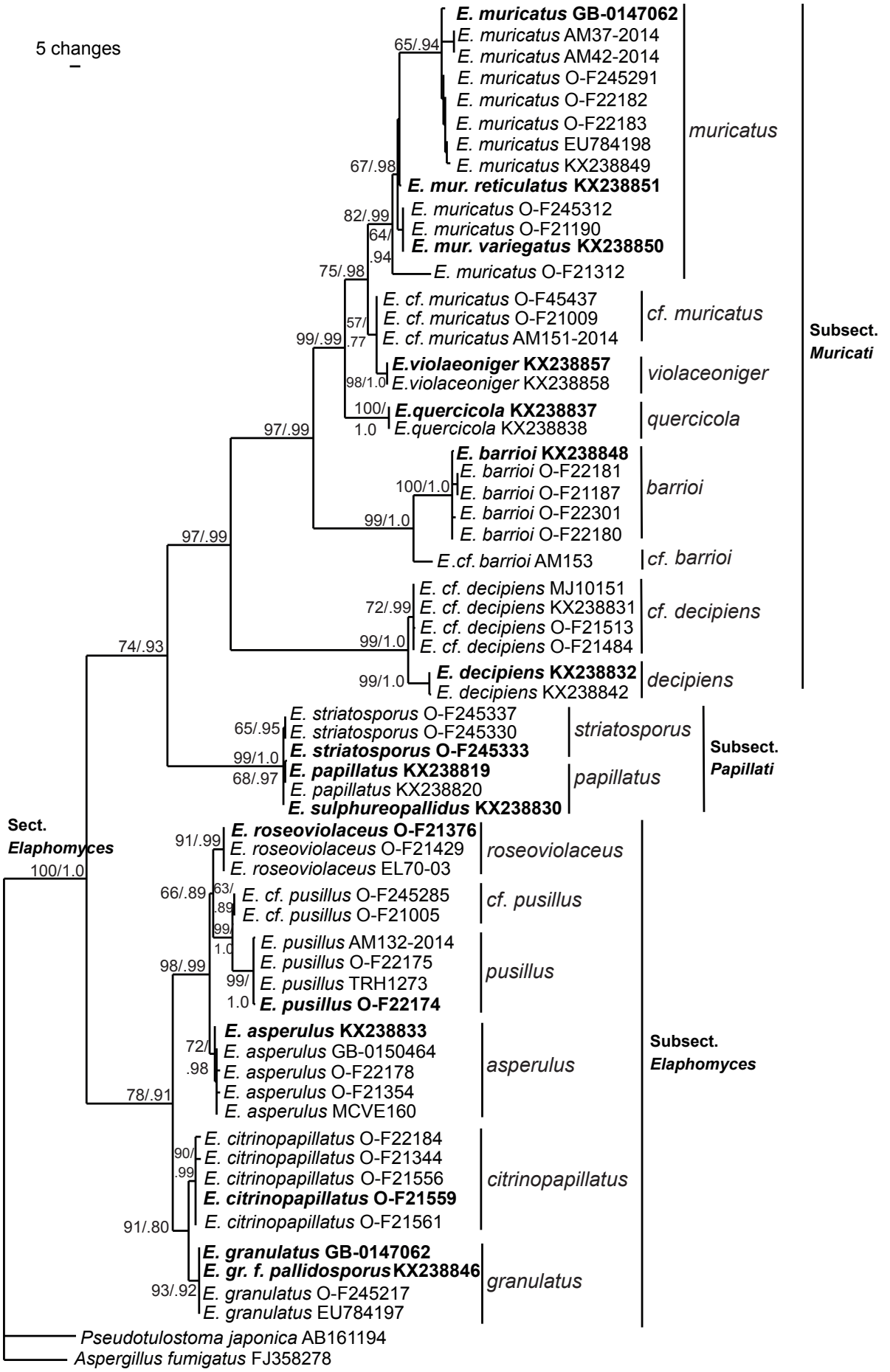

Fig. 1. One of the equally most parsimonious trees from the phylogenetic analysis based on nuclear rDNA ITS and partial LSU, mtSSU, and β-tubulin sequences. Parsimony bootstrap values and Bayesian posterior probabilities are indicated on branches. Clades discussed in the text are indicated with bars and species epithets. Sequences originating from type specimens are marked in bold.

parsimony tree. The same subclades and terminal clades supported in the bootstrap analysis were also recovered and supported in the Bayesian analysis. BPP values are indicated on the corresponding branches in Fig. 1. A BPP value above 0.95 is considered significant.

The analyses recovered section *Elaphomyces* (100/1.0) as monophyletic and divided in three supported subclades corresponding to subsection *Elaphomyces* (78/0.91), subsection *Papillati* (99 /1.0), and subsection Muricati (97/0.99). Within section *Elaphomyces* 15 terminal clades and one single

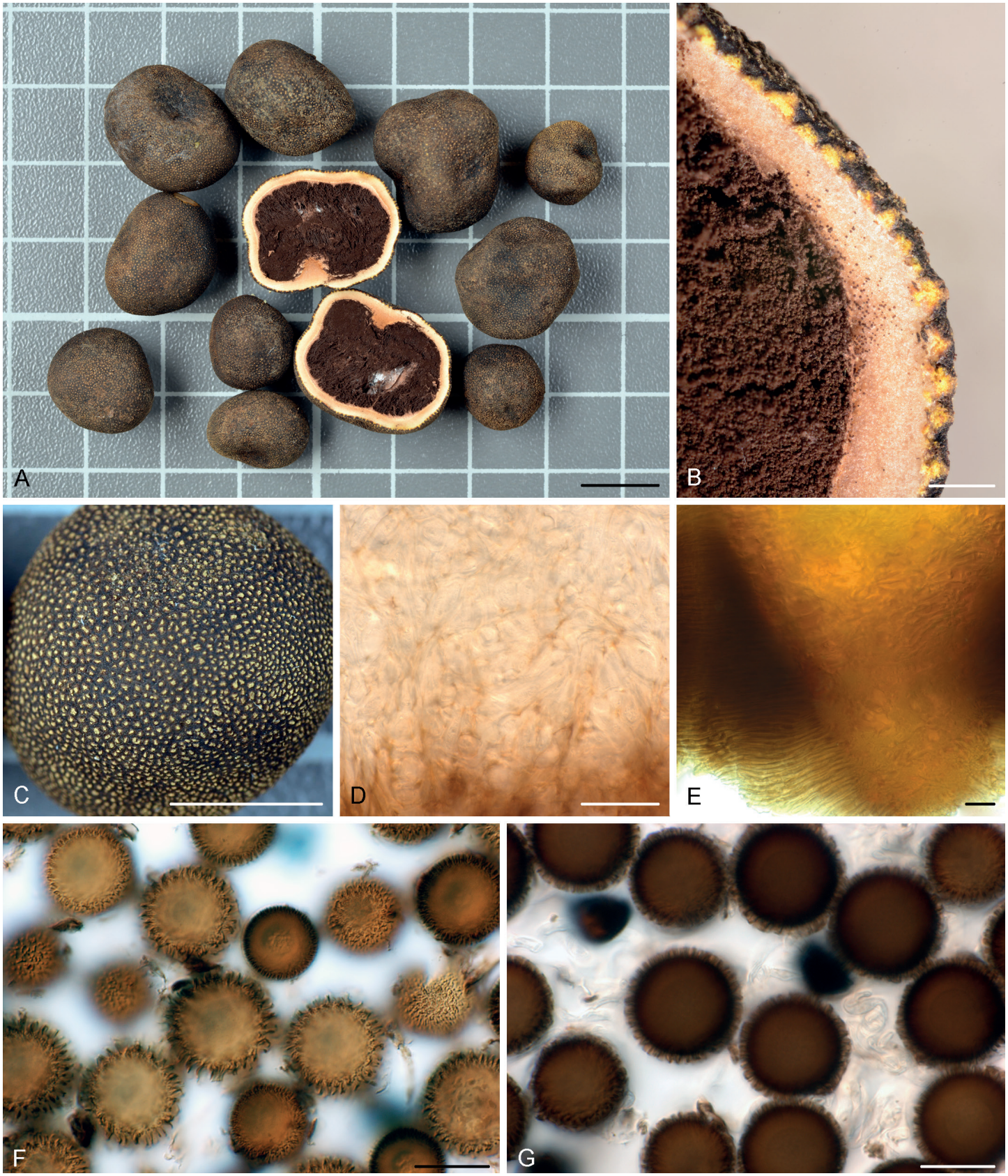

Fig. 2. *Elaphomyces citrinopapillatus*. Holotype (O-F21559). **A.** Mature ascomata. **B.** Section of peridium. **C.** Detail of cortex. **D.** Peridial structure. **E.** Section of cortex. **F, G.** Ascospores. Scale bars: A, C = 10 mm; B = 1 mm; D–G = 20 µm.

branch, weakly to strongly supported, were recovered. These correspond to *E. muricatus* (82/0.99), *E. cf. muricatus* (57/.77), *E. violaceoniger* (98/1.0), *E. quercicola* (100/1.0), *E. barrioi* (100/1.0), *E. cf. barrioi* (99/1.0), *E. decipiens* (99/1.0), *E. cf. decipiens* (72/0.99), *E. papillatus* (68/0.97), *E. striatosporus* (65/0.95), *E. roseoviolaceus* (91/0.99), *E. pusillus* (99/1.0), *E. cf. pusillus* (63/0.89), *E. asperulus* (72/0.98), *E. citrinopapillatus* (90/0.99), and *E. granulatus* (93/0.92) (Fig. 1).

The terminal clades corresponding to new species are described and discussed in the Taxonomy section. Also, typification measures are presented here. An overview of all species in section *Elaphomyces* is given in the Discussion section. For morphological descriptions of already described species in section *Elaphomyces* we refer to Paz *et al.* (2017).

Taxonomy

Elaphomyces citrinopapillatus A. Molia, A. Paz & Lavoise, ***sp. nov.*** Figs 2–3. MycoBank MB833573.

Etymology: *citrinus* (lemon yellow) and *papillatus* (warty), referring to the more or less enclosed, yellow warts of the peridium.

Typus: **Norway**, Akershus, Nittedal, Slattum (WGS84: N60.00808 E10.91350), in moist old-growth *Picea* stand on rich calcareous soil with moss cover, 10 Jan. 2014, *Lello* (dog) & *A. Molia*, **holotype** in herb. O, O-F21559*, barcode sequence GenBank KR029765.

Ascomata globose to depressed globose, 0.6–2.0(–2.6) cm (av. 1.3 cm, n = 310), black when fresh, dull brownish black when dry, with a brown, thin and ephemeral hyphal envelope. *Odour* strong and unpleasant, metallic. *Peridium* in section (0.65–)0.7–1.4 mm thick, white to cream, towards the gleba "café au lait"-colour, with enclosed, lemon yellow, rounded to elongated or ± conical warts, about 250 µm high, breaking through the cortex. After cleansing of the ascomata these warts are easily detected on the brownish black peridial surface. *Cortex* 350–500 µm thick, sharply delimited from the underlying peridial layer. The inner part of the peridium is constructed of loosely interwoven, thick-walled (up to 2 µm), angularly rounded to elongated cells. Near the gleba these cells are brownish and sometimes confluent and more or less shapeless. The exterior peridial layer, the cortex, is blackish brown, constructed of rounded and thick-walled cells on the surface, turning pyramidal to elongated inwards. *Gleba* black upon maturity, pulverulent. Young and immature ascomata have a continuous fleshy texture with coloured hyphae surrounding lumps of developing spore mass (Fig. 2). *Asci* 8-spored. *Ascospores* globose, (21–)23–32(–33) µm (av. 26 µm, n=240), brown, echinulate, spines 1.5–2.5 µm high, well separated and not coalescing.

Fig. 3. Type locality of *Elaphomyces citrinopapillatus*. Norway, Akershus, Nittedal, Slattum, 10 Jan. 2014.

Ecology and distribution: Develops gregariously in the upper soil layer (black to dark brown soil) in semi-rich to rich woodlands. It occurs in pure *Picea abies* stands, but also in mixed forests with *Picea abies, Pinus sylvestris, Betula* spp. and *Corylus avellana.* It is to date known from five counties in southern Norway. All sites were located by trained dogs.

Additional materials examined: **Norway**, Akershus, Nittedal, Slattum, in old, rich *Picea abies* forest, 10 Jan. 2014, *Lello* (dog) *& A. Molia*, O-F21560, O-F21561*, O-F21392; Akershus, Oppegård, Svartskog, by Svartskog kindergarten, in semi-rich mixed forest, under *Picea abies*, 9 Aug. 2014, *Lello* (dog), *P.-A. Moreau & A. Molia*, O-F22129; Buskerud, Flesberg, Lyngdal, Molia, Tritjenna, in semi-rich *Picea abies* forest with *Betula,* near forest road, 27 Dec. 2013, *Lello* (dog) *& A. Molia*, O-F21556*; *ibid.* in semi-rich *Picea abies* forest, 17 Apr. 2014, *Lello* (dog) *& A. Molia*, O-F22184*; *ibid.*, in young, semi-rich *Picea abies* forest near forest road, *A. Molia* 26-2015 (GB); *ibid.*, Botnan, in rich *Picea abies* forest, 3 Nov. 2013, *Lello* (dog) *& A. Molia*, O-F21458; Flesberg, Sølset, towards Hommelisetera, in semi-rich forest with *Picea abies, Salix* sp. and *Betula* sp., 18 Apr. 2016, *Lello* (dog) *& A. Molia*, O-F22515; Sigdal, Hagavollesetra, in semi-rich *Picea abies* forest, 2 Nov. 2013, *Lello* (dog), *Å. Borge & A. Molia*, O-F21457; Oslo, Sognsvann, in semi-rich *Picea abies* forest, 13 Sep. 2013, *Lello* (dog) *& A. Molia*, O-F21303, F21304; *ibid*., in semi-rich *Picea abies* forest, 25 Aug. 2013, *Lello* (dog)*, T. Læssøe & A. Molia*, O-F21344*; Oslo, Ekebergsåsen, in semi-rich conifer forest under *Picea abies*, 12 Jul. 2012, *Kokkos & Viktoria* (dogs), *K. Killingmo, M. Jeppson & A. Molia*, O- F245249, F245254; *ibid.* 18 Jul. 2012, O-F245258, O-F245258; Østfold, Marker, S of Ørje, in semi-rich *Picea abies* forest, 13 Apr. 2014, *Louise* (dog), *K. Killingmo & A. Molia*, O-F22128; Telemark, Bamble, Røsskleiva, rich mixed forest, under *Picea abies*, 10 Oct. 2011, *Lello* (dog), *A.K. Wollan & A. Molia*, (O-F21189). *ibid.*, 10 Oct. 2011, *Kokkos, Viktoria* (dogs), *K. Killingmo & T. Andersen* (O-F21194); Skien, Luksefjell-Ulfskollen, semi-rich forest with *Populus tremula, Corylus avellana, Quercus* sp. and *Picea abies*, 27 Sep. 2015, *Lello* (dog), *A. Molia & B.F. Høifødt,* (O- F260438); Porsgrunn, Frierflauane, rich mixed forest, under *Picea abies*, 28 Sep. 2013, *A. Paz*, O-F21277; *ibid.*, Frierstien, in mixed rich forest, under *Picea abies*, 2 Oct. 2015, *Lello* (dog) *& A. Molia*, O-F260439; Tinn Austbygd, Mæl, in rich mixed forest, under *Picea abies*, 18 Jun. 2015, *Lello* (dog) *& A. Molia*, O-F22483.

Remarks: *Elaphomyces citrinopapillatus* is usually easy to recognize already in the field due to its unique features of the peridium. The closely related species *E. asperulus, E. granulatus, E. pusillus* and *E. roseoviolaceus* also have yellowish peridial warts but with less contrasting colours. A detailed study of the literature did not reveal a suitable name for this species. Our molecular data confirm it as a distinct species, sister to *E. granulatus*, from which it differs by generally smaller ascomata, the strongly contrasting yellow warts and the production of gregarious ascomata.

Elaphomyces granulatus Fr., *Systema mycologicum* (Lundae) **3**: 58. 1829. Figs 4, 9A–B.
Synonyms: *Lycoperdon cervinum* L., *Species plantarum* **2**: 1183. 1753.
Elaphomyces leucocarpus Vittad., *Monographia Tuberacearum* (Milano): 72. 1831.

Lectotype for *Elaphomyces granulatus* Fr. (designated here): Mougeot JB, Nestler CG, Schimper WP (1812). *Stirpes cryptogamae vogeso-rhenanae; quas in Rheni superioris inferiorisque, nec non Vogesorum praefecturis, collegerunt J.B. Mougeot et C. Nestler*. Fasc 3, No. 282, third basidioma from left (UPS F-708238). MBT389802.

Epitype (designated here): **Sweden**, Småland, Femsjö, mixed coniferous forest, 16 Mar. 2014 *Lello* (dog) & *A. Molia*, in herb. GB, GB-0147063*, barcode sequence KR029767, MBT389803.

Remarks: When Fries (1829) described *Elaphomyces granulatus* he referred to *Lycoperdon cervinum* of Linnaeus (1753) and cited several early illustrations, among them Lobelius (1581: 276; *Tubera cervina*), Bauhin (1651: 835; *cervi boletus*), Micheli (1729: tab. 99, fig. 4; *Lycoperdastrum tuberosum*), Nees von Esenbeck (1817: fig. 147; *Tuber cervinum*). There is no original type material available of *E. granulatus* and none of the illustrations referred to by Fries are accurate enough to enable us to clearly distinguish *E. granulatus* from related species. Fries also cited number 282 from the series of exsiccate published by Mougeot & Nestler (Mougeot & Nestler 1812; as *Scleroderma cervinum*). This exsiccate was distributed to a number of museums and other institutions throughout Europe, among them the herbaria in Lund, Stockholm, and Uppsala. The exsiccate copy in Uppsala contains a mixture of *E. granulatus* and *E. hassiacus* and the one in Lund consists of *E. asperulus* or a closely related species. The specimen of interest from the exsiccate copy in Stockholm could not be located. According to the nomenclatural code all cited specimens are syntypes. In this case it means that all exsiccate

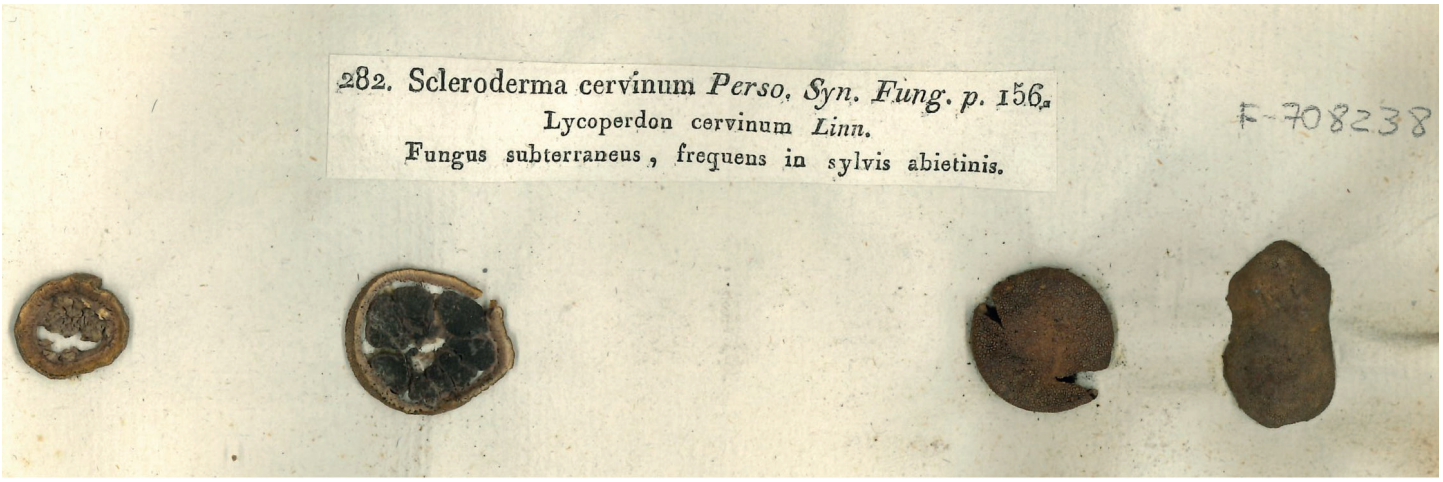

Fig. 4. *Elaphomyces granulatus*. Lectotype, third basidioma from left (UPS, F-708238).

copies are syntypes and that a lectotype has to be chosen among them. Here we select one basidioma from the exsiccate copy in the Uppsala herbarium (UPS) that corresponds to the current interpretation of *E. granulatus* (Fig. 4). However, with the introduction here of the new species *E. citrinopapillatus*, which in our analyses is recovered as a sister taxon to *E. granulatus*, a unanimous morphological identification of old, single basidiomata cannot be guaranteed. Thus, the selected lectotype needs to be supplemented by an epitype. The epitype is newly collected in Femsjö, Fries' mycological hunting-ground in southern Sweden (Fig. 9A–B).

Elaphomyces pusillus A. Molia & Sivertsen, ***sp. nov.*** Figs 5, 6. MycoBank MB833577.

Etymology: *pusillus* (small), referring to the size of the ascomata.

Typus: **Norway**, Nord-Trøndelag, Verdal, Inndalen, Elgstien at river Inna (WGS84: N63.70009 E11.89353), alt. 180 m, mesic, mossy *Picea abies* forest with some deciduous trees, with ferns and *Vaccinium myrtillus* in the field layer, 8 Sep. 2014, *Lello* (dog) *& A. Molia,* **holotype** in herb. O, O-F22174*, barcode sequence GenBank KR029761, **isotype** GB.

Ascomata globose to subglobose, 0.4–1.5(–1.8) cm, bright yellow (young) to dull brown (old; thus recalling *E. asperulus*). Peridial surface with angular and in relation to ascoma size rather big papillae, cream white to pale yellow. *Odour* weak, reminding of silver polish and reminiscent of the smell of *E.*

Fig. 5. *Elaphomyces pusillus*. Holotype (O-F22174). **A.** Mature ascomata **B.** Detail of cortex. **C.** Ascocarp in section. **D.** Peridial structure. **E.** Section of peridium showing peridial warts (yellowish). **F.** Peridial structure between warts. **G, H.** Ascospores. Scale bars: A–C = 10 mm; D = 10 µm; E–H = 20 µm.

Fig. 6. Type locality of *Elaphomyces pusillus*. Norway, Nord-Trøndelag, Verdalen, Inndalen, 8 Sep. 2014.

asperulus and *E. granulatus*. Ascomata are often incrusted in a thick hyphal mat of cream-yellow to yellow hyphae with interwoven roots and debris. *Peridium* in section 0.7–1.3 mm thick, pink to slightly violaceous, darkening towards the gleba, homogeneous, composed of elongated, pseudoparenchymatic, thick-walled cells (1.5–2 µm in diam.), with red-brown pigments. Old and presumably overwintered specimens have a blueish halo close to the cortex. Outer peridial layer (cortex) with prominent, cubic to prismatic or conical, often cog-wheel-like papillae, yellowish in section with a cellular structure, with cells of various shapes. *Gleba* initially greyish black, cottony, with age violaceous to brownish black, pulverulent. Pink tramal plates extend from the peridium into the gleba in young ascomata. *Asci* 8-spored. *Ascospores* globose, (20–)23–30(–33) µm (av. 26 µm, n = 100), when mature dark brown with an ornamentation of coalescing spines, forming a cracking pattern similar to that of *E. asperulus*. Spines 0.5–1(–1.5) µm high. Young spores are lighter brown and appear more regularly verrucose.

Ecology and distribution: Found with *Picea abies* and possibly *Betula* sp. on calcareous soil. The ascomata, which sometimes occur gregariously, develop in the upper soil, in a rather thick, cream-white hyphal mat. It is a rarely recorded species found on a few occasions in the boreal vegetation zone of Norway and Sweden.

Additional materials examined: **Norway**, Nordland, Rana, Jordbru, in *Betula* forest, 23 Sep. 1979, *S. Sivertsen & B.K.P. Sveum*, TRH 1273*; Nord-Trøndelag, Verdal, Inndalen, Elgstien at river Inna, in mesic, mossy *Picea abies* forest with some deciduous trees, ferns and *Vaccinium myrtillus*, 8 Sep. 2014, *Lello* (dog) *& A. Molia,* O-F22175*; Buskerud, Flesberg, Lyngdal, SE of Molia, in semi-rich *Picea abies* forest, 15 Aug. 2015, *A. Molia,* O-F22494, O-F22495. **Sweden**, Medelpad, Tuna, Runsvik, in rich *Picea abies* forest, 9 Sep. 2014, *A. Molia*, O-F22496; *ibid.*, *A. Molia*, GB-0179907*.

Remarks: *Elaphomyces pusillus* is in many ways similar to *E. asperulus*, from which it differs in having extremely small-sized ascomata imbedded in a thick hyphal mat, and smaller ascospores. *Elaphomyces pusillus* has been found growing gregariously with more than 10 ascomata on the same spot. It was first collected in Rana (Nordland) in northern Norway by Sigmund Sivertsen and Bodil K. Sveum in 1979. The ascomata were on that occasion found loose on the ground, having been dug up by animals (likely by reindeer, *Rangifer tarandus*). Later records were exclusively made by trained dogs. Our molecular data confirm it as a distinct species, related to *E. asperulus* and *E. roseoviolaceus*, described below.

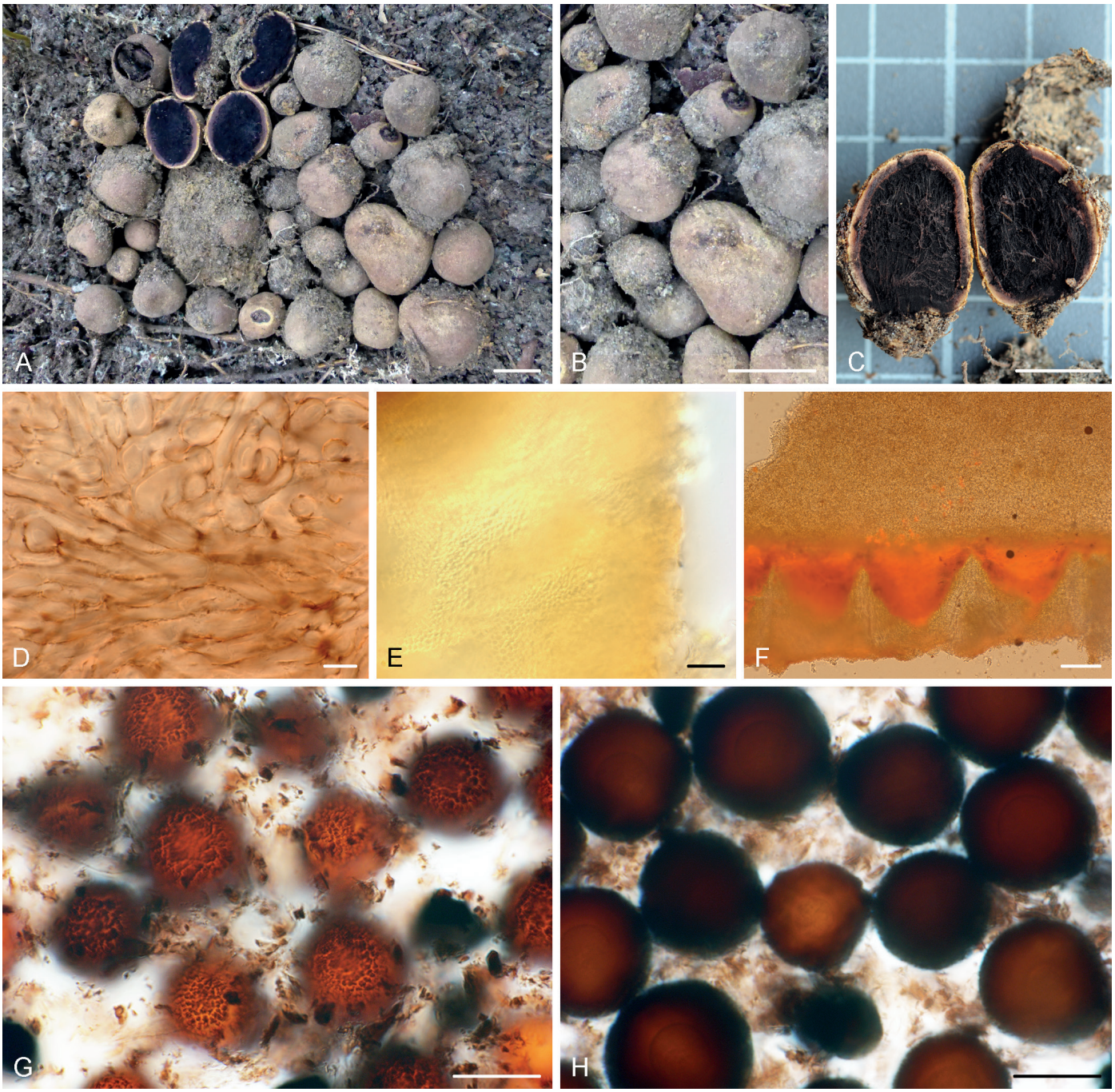

Fig. 7. *Elaphomyces roseoviolaceus.* Holotype (O-F21376). **A.** Mature ascomata. **B.** Detail of cortex. **C.** Ascoma in section. **D.** Peridial structure. **E.** Peridial structure between warts. **F.** Section of peridium showing warts (orange). **G, H.** Ascospores. Scale bars: A–C = 10 mm; D = 10 µm; E–H = 20 µm.

Elaphomyces roseoviolaceus A. Molia & E. Larss., ***sp. nov.*** Figs 7–8. MycoBank MB833578.

Etymology: from Latin *roseus* (pink/rose) and *violaceus* (violet), referring to the colour of the peridium in section.

Typus: **Norway**, Akershus, Frogn, Knardal NR (WGS84: N59.73711 E10.72019), in rich mixed forest with *Pinus, Picea, Tilia, Corylus*; under *Pinus sylvestris*, 3 Sep. 2013, *Louise, Lello* (dogs), *K. Killingmo & A. Molia*, **holotype** in herb. O, O-F21376*, barcode sequence KR029752.

Ascomata globose to subglobose, 1.3–2.5 cm (av. 1.6 cm, n = 23), pale sand brown to greyish brown, darker in old, overwintered specimens, with small flat and angular papillae. Ascomata are normally encrusted with soil particles and covered with a cream to dull yellow hyphal mat. *Odour* indistinct in the field, later (when enclosed in box) like freshly ground pepper. *Peridium* in section 1.4–2.1 mm thick, dark pink to bluish violaceous, most colourful towards the gleba; in periphery cream yellow. Peridium homogeneous in section with thick-walled (up to 2 µm), curved to elongated, sometimes rounded or angular cells, 8–40 µm. The cells contain pink to wine red pigments, particularly towards the cell wall. The cortex is 250–350 µm thick, with conical papillae unevenly distributed over the ascomatal surface. The papillae are yellow in section. Between the papillae there are pale yellow to colourless cells of two types: curved elongated thin-walled cells and thick-walled, irregularly arranged cells. The cortex is easily separated from underlying part of the peridium. *Gleba* black to

Fig. 8. Type locality of *Elaphomyces roseoviolaceus*. Norway, Akershus, Frogn, Knardal Nature reserve, 3 Sep. 2013.

violaceous when mature. *Asci* not seen. *Ascospores* (26–)27–33(–34) µm (av. 29.5 µm, n = 80), chestnut brown with darker ornamentation. Young spores have verrucose ornamentation whereas older spores have short (< 1 µm), coalescing spines, clustering in a patchy pattern.

Ecology and distribution: Occurs gregariously in the upper soil layer in calcareous, herb-rich woodlands with *Picea abies* and *Pinus sylvestris*. Hitherto known from four localities in southern Norway.

Additional materials examined: **Norway**, Telemark, Bamble, Langøya, in rich mixed coniferous forest, under *Picea abies*, 17 Oct. 2013, *Lello* (dog), *T. Læssøe & A. Molia*, O-F21324, O-F21429*; *ibid*., NE of Gjømle, rich mixed forest, under *Picea abies, Lello* (dog) *& A. Molia*, O-F22514; *ibid*., Røsskleiva, in rich mixed forest, under *Picea abies,* 20 May 2017, *Lello* (dog), *I. Rokseth & A. Molia,* O-F24144.

Remarks: *Elaphomyces roseoviolaceus* differs from *E. asperulus* by the more colourful, dark pink to violaceous peridium (in section), smaller ascomata and smaller spores. In the field, a thick cream or dull yellow mycelial mat is seen in the soil around the ascomata. In old and overwintered specimens, the peridium turns bluish-violaceous and the spores are slightly larger and have a more prominent ornamentation.

Elaphomyces muricatus Fr., *Systema mycologicum* (Lundiae) **3**: 59. 1829. Fig. 9C–E.

Lectotype (designated here): Willdenow, C.L. (1787), Florae Berolinensis Prodromus, plate VII, fig. 19. MBT389804.

Epitype (designated here): **Sweden**, Småland, Femsjö, SW of Femsjö church (WGS84: N56.89087 E13.33069), in mixed woodland with *Fagus sylvatica, Betula pendula* and *Pinus sylvestris*, quite open area on a ridge, 15 Mar. 2014, *Lello* (dog) *& A. Molia*, in herb. GB, GB-0147062*, barcode sequence GenBank KR029730. MBT389807.

Remarks: In the Friesian herbarium at UPS there is a specimen (F-127359) that was collected at Femsjö (Sweden) and labelled *Elaphomyces muricatus* in Fries' handwriting. The fruiting bodies show acute, pyramidal warts on the cortex surface and the peridium is clearly marbled in cross-section. However, only the peridium remains and no spores can be traced. Since the specimen is not dated it cannot be established if this material was available to Fries when describing the species and is thus not original material. In the protologue Fries refers to one illustration (in C.L. Willdenow Florae Berolinensis Prodromus, 1787) and this has to be selected as a lectotype. This type is here supplied with an epitype, based on newly collected and sequenced material from Femsjö.

Fig. 9. *Elaphomyces granulatus* and *E. muricatus* type specimens. **A, B.** *Elaphomyces granulatus*, epitype (GB-0147062). **C, D.** *Elaphomyces muricatus*, epitype (GB-0147063). **E.** *Elaphomyces muricatus*, holotype (UPS, F-127359) from Elias Fries' herbarium. **F.** Type locality for epitype of *Elaphomyces muricatus* at Femsjö, Sweden, 15 Mar. 2014. Scale bars: 10 mm.

Elaphomyces striatosporus Kers, *Botaniska Notiser* **133**(2): 149. 1980. Fig 10.
Synonym: *Elaphomyces papillatus* var. *striatosporus* (Kers) A. Paz & Lavoise, *Persoonia* **38**: 216. 2017.

Holotype: **Norway**, Oslo, ved Gausta, under *Corylus*, 22 Sep. 1952, F-E Eckblad, O-F72594.

Epitype (designated here): **Norway**, Oslo, Bygdøy, S. of Rodeløkken Café, under large *Tilia* sp. in a grazed field on calcareous soil, 21 Sep. 2012, *Kokkos, Lello, Viktoria* (dogs), *K. Killingmo, M. Mowinkel-Amundsen & A. Molia*, in herb. O, O-F245330*, barcode sequence GenBank KR029748. MBT389811.

Additional materials examined: **Norway**, Møre og Romsdal, Nesset, Eikesdal, under Rangåfjellet, in deciduous forest with *Corylus avellana* on semi-rich soil, 17 Sep. 2011, *Kokkos, Viktoria* (dogs), *M. Jeppson, K. Killingmo & A. Molia*, O-F21185*; Nord-Trøndelag, Inderøy, Råvika, in

Fig. 10. *Elaphomyces striatosporus* Kers. **A, C.** Epitype (O-F238861). **B, D.** A rich collection with overwintered ascocarps. (O-F21368). **E, F.** Holotype (O-F72594). Scale bars: A, B, D, F = 10 mm; C = 20 µm.

deciduous forest under Corylus avellana on rich soil, 21 Oct. 2011, *Lello* (dog) & *A. Molia*, O-F21184*; Oslo, Bygdøy, Klausåsen, S of Rodeløkken Café, in mixed deciduous forest under *Corylus* on calcareous ground, 21 Sep. 2012, *Kokkos* (dog), *K. Killingmo & A. Molia*, O-F245333*; Oslo, Hovedøya, deciduous forest with *e.g. Corylus, Tilia, Quercus, Fraxinus, Betula* on calcareous ground, 23 Sep. 2012, *Kokkos, Lello* (dogs), *K. Killingmo & A. Molia*, O-F245337*; Østfold, Jeløy, Albyskogen, in mixed forest with *Corylus*, *Quercus*, *Populus*, *Betula,* and *Picea*, 17 Mar. 2014, *Lello* (dog), *A. Fæste & A. Molia*, O-F21368.

Remark: We made several attempts to generate an ITS sequence from the holotype but failed. For this reason, we consider it desirable to select an epitype using recently collected and sequenced material from the vicinity of the type locality.

DISCUSSION

The phylogeny and taxonomy of *Elaphomyces* in Europe was recently treated in detail by Paz *et al*. (2017). Twenty-

six species were identified and an intrageneric division was established, supported by analyses of ITS and LSU sequence data. Four major clades were identified and classified as sections, viz. sect. *Elaphomyces, Ascoscleroderma, Ceratogaster* and *Malacodermei*. Representatives of the sections *Elaphomyces, Ceratogaster* and *Malacodermei* are currently known to occur in North Europe but only section *Elaphomyces* is treated in this paper. Paz *et al*. (2017) divided section *Elaphomyces* in three subsections: *Elaphomyces, Muricati* and *Papillati.* The species recovered in our analyses (Fig. 1) are briefly discussed below.

Subsection *Elaphomyces*

The main morphological character of subsection *Elaphomyces* is the presence of a homogenous (not marbled) peridium in section. Three European species belong to this subsection according to Paz *et al*. (2017): *E. asperulus, E. granulatus* and *E. hassiacus*. The former two species are well known and abundant in North Europe, whereas the latter one has no current records.

During our fieldwork we noticed the presence of morphologically deviating collections that could not be referred to the above species, but which clearly belonged in this subsection. These observations were later supported by molecular data (Fig. 1). The three new species, *E. citrinopapillatus, E. pusillus* and *E. roseoviolaceus* form ectomycorrhiza with *Picea abies* and are characteristic elements in hemiboreal-boreal coniferous woodlands on more or less calcareous soil. A sister species of *E. pusillus* was also recovered in the phylogenetic analyses (Fig. 1). It has an almost identical morphology and similar habitat preferences and is here treated as *E. cf. pusillus*, since we need additional material for a formal description. The new species are currently known from Norway, and *E. pusillus* in addition from Sweden.

Subsection *Muricati*

Subsection *Muricati* includes species with a peridium that is marbled when observed in section. In the phylogeny (Fig. 1) sequence data of the epitype of *E. muricatus*, designated above, as well as the epitypes of *E. muricatus* var. *variegatus* and *E. muricatus* var. *reticulatus* are marked in bold-face type. Although there are only a few base pair differences in ITS sequences compared with the epitype of *E. muricatus*, the two varieties can be distinguished by morphology (Paz *et al*. 2017). A sister taxon, here provisionally treated as *E. cf. muricatus* (Fig. 1), was recovered in the phylogenetic analyses. It is represented by three molecularly identical collections from Norway. In morphology it is close to *E. muricatus* var. *muricatus* but more specimens must be studied before it can be formally described. Paz *et al*. (2017) described *Elaphomyces violaceoniger,* a species characterized by a dark purplish peridium, and reported a single record from Norway. We have not been able to confirm this record and currently we do not accept *E. violaceoniger* as a member of the Nordic funga. *Elaphomyces quercicola*, formerly *E. muricatus f. quercicola*, was elevated to species rank by Paz *et al.* (2017); so far, no records from North Europe have been confirmed.

Another species in the *muricatus*-group is *E. barrioi*, described by Paz *et al.* (2017) with a holotype from Spain. It is likely to be confused with *E. muricatus* but careful observation of peridial features should make it possible to separate the two (see identification key below). It forms ectomycorrhiza with deciduous trees and has several records from southern Europe and is also present in southern Norway (Fig. 1). A sister taxon, collected in Norway and clearly separated by molecular data, is provisionally treated as *E. cf. barrioi*. Since only one small collection is known a formal description will have to await additional gatherings.

Elaphomyces decipiens also belongs to subsection *Muricati.* The species was neotypified with Spanish material and sequence data of the neotype generated (Paz *et al.* 2017). In our analyses, collections from Norway, and Sweden determined as *E. decipiens* based on morphology, form a sister clade to South European specimens (Fig. 1). The clades differ in ITS sequence data by four substitutions and three insertion/deletion events and should likely be regarded as two separate species and are in need of further attention (*cf.* Jeppson & Molia 2015).

Subsection *Papillati*

Subsection *Papillati* includes species with striate spores. Paz *et al.* (2017) divided *Elaphomyces papillatus* into three varieties, var. *papillatus*, var. *striatosporus* and var. *sulphureopallidus*. Of these only var. *striatosporus* occurs in northern Europe. The ITS sequence of this variety differs from var. *papillatus* by 2–4 insertions. In morphology *E. striatosporus* is best distinguished from *E. papillatus* by a less warty peridial surface and a more pronounced striation of the spore wall. Var. *striatosporus* was originally described on species level based on material collected by Finn-Egil Eckblad from Oslo, Norway, 1952 (Kers 1980). As this name is in current use in Norway and Sweden, and as the fungus can be distinguished by both morphology and ITS sequence data, we currently prefer to treat it on species level, as a northern taxon, closely related to the South European *E. papillatus*. As we were not able to generate ITS sequence data from the holotype material of *E. striatosporus* (O-F72594; Fig. 10E–F), the species is in this paper epitypified with newly collected material from Oslo.

Kers (1980) classified *E. striatosporus* in subgenus *Malacoderma*. The molecular analyses (Paz *et al*. 2017, this study) confirm its position among the "brown species" in section *Elaphomyces* and its peridium is in fact brown, particularly apparent in older stages.

In this paper we show that nine species belonging to *Elaphomyces* section *Elaphomyces* are present in northern Europe. This is more than a doubling of the number keyed out by Eckblad, Lange & Kers in the flora Nordic Macromycetes (Hansen & Knudsen 2000). In addition, our phylogenetic analyses of DNA sequences revealed the presence of genetically divergent specimens that could represent another four taxa (Fig 1.) It is obvious that much fieldwork remains in order to give a realistic picture of the diversity and the true distributions of *Elaphomyces* species in northern Europe. This is especially true for Denmark, where almost no collecting taking the advantage of trained dogs has taken place so far. Although *Elaphomyces* specimens often are found somewhat deeper in the ground than other hypogeous fungi, trained dogs are very efficient in locating the ascomata. For the prospective truffle-hunter *Elaphomyces* has the advantage over many other truffles in being present and detectable all year around.

Elaphomyces spp. are important contributors to forest ecosystem functioning, forming ectomycorrhiza with forest trees

(Bird & McCleneghan 2005). Three species from subsection *Elaphomyces* (*viz*. *E. asperulus, E. granulatus* and *E. muricatus*) have abundant records from North European hemiboreal and boreal coniferous forests according to our studies and to herbarium data and records in national databases. However, species identifications of older records are ambiguous due to the presence of previously unknown and morphologically similar species. The herein proposed new species occur mainly in old-growth herb-rich *Pic*ea forests on calcareous ground and may turn out to be useful indicators for this type of habitat that is under a continuous threat by modern forestry.

Key to North European species of *Elaphomyces – all sections*

1. With black, brownish black to blueish black or greyish black cortex, or at least black peridial spines 2
1. With brown or brownish cortex, never with black peridial spines 11

2. Cortex warty, or partly warty, with acute or blunt warts 3
2. Cortex smooth or nearly smooth 7

3. Ascospores with striate-ridged ornamentation 4
3. Ascospores non-striate 5

4. Ascomata 10–35 mm; peridial surface minutely and densely warty, very dark brown to black; ascospores 16–22 µm, ornamentation with sharp ridges ..... ***E. virgatosporus***
4. Ascomata usually < 10 mm, when young often wrapped in a thick white to cream-coloured mycelial felt; peridium scurfy to finely papillate, in young ascomata blackish blue, with age bluish grey to chestnut brown; ascospores 11–18 µm, ornamentation with blunt ridges ..... ***E. striatosporus***

5. Peridium with blackish spines penetrating the brown peridial surface ..... ***E. aculeatus***
5. Peridium different 6

6. Ascospores with coalescent ornamentation of low and dense warts ..... ***E. moretti***
6. Ascospores with small spines/warts, forming a dotted pattern on the surface ..... ***E. leveillei***

7. Ascospores >25 µm; ascomata 20–50 mm, with patches of a blue green mycelial felt on the peridium; peridial surface black, almost smooth ..... ***E. maculatus***
7. Ascospores < 25 µm 8

8. Ascomata greyish; mature gleba whitish-pinkish to dirty grey; ascospores 32–36 µm, pale-coloured ..... ***E. septatus***
8. Ascomata blackish, ascospores darker, gleba different 9

9. Ascomata with yellow papillae, breaking through the blackish peridium ..... ***E. citrinopapillatus***
9. Ascomata smooth 10

10. Ascomata small, normally between 5–15 mm, globose to ± pyriform, some with prominent depressions or ± flattened; ascomata gregarious ..... ***E. anthracinus***
10. Ascomata normally > 20 mm; spores often angular; ascomata solitary ..... ***E. anthracinus f. talosporus***

11. Inner peridium marbled in section 12
11. Inner peridium uniform in section 14

12. Peridial surface with blunt and flat warts, pale brown to dirty brown; a thick dull yellow hyphal mat covers the ascomata; ascospores 19–28 µm with a dense cover of curved, rod-like spines ..... ***E. cf. decipiens***
12. Peridial surface with ± acute warts 13

13. Peridial surface with prominent, acute warts, 1(–2) per mm; ascospores 18–21 µm, with thin, curved and rod-like spines ..... ***E. muricatus***
13. Peridial surface with small, truncate warts, 2(–3) per mm; ascospores 19–24 µm, with thick, rod-like spines with confluent apices, forming irregular meshes ..... ***E. barrioi***

14. Cortex surface blackish brown with yellow warts; ascomata 6–20 mm; ascopores 23–28 µm, with long, easily separable spines (similar ornamentation to other species in the *granulatus*-group) ..... ***E. citrinopapillatus***
14. Cortex surface brownish 15

15. Ascomata small, normally 4–15(–18) mm, often encrusted by a thick hyphal mat of cream-coloured to clearly yellow hyphae with interwoven roots and debris; ascospores 23–30 µm ..... ***E. pusillus***

15. Ascomata larger, normally > 20 mm in diam. 16

16. Peridium with pink to violaceous tints; ascomata 13–25 mm; peridial surface light brown with small, flat papillae; peridium strongly coloured, dark pink to violaceous in section; ascospores 26–31 µm with low, spiny verrucae, with age coalescing and forming small isolated groups (cheetah pattern), ascomata gregarious ..... ***E. roseoviolaceus***
16. Peridium dull pink, cream coloured, rarely bluish; ascomata normally larger 17

17. Cortex ochre yellow-brown; peridium whitish to yellowish white in section; ascomata 15–40 mm; ascospores 20–29 µm, with an ornamentation of isolated spines ..... ***E. granulatus***
17. Cortex dull brow; peridium with a pinkish tint in section, sometimes with a blue halo (in old/overwintered specimens); ascomata normally 30–40 mm; ascospores 25–38 µm with low, dense verrucae, with age forming a patchy pattern with groups of coalescing spines divided by narrow paths (giraffe-pattern) ..... ***E. asperulus***

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