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Observations on two glassy-haired lichenicolous discomycetes

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Abstract: Two lichenicolous species are discussed: the recently described *Unguiculariopsis nephromae*, which occurs on *Nephroma* species in Canada, Fennoscandia, North America and Russia, is combined into *Protounguicularia*; and *Hyalopeziza rapax* sp. nov. is described from *Pseudocyphellaria crocata* in Alaska. In discussing generic placements, it is concluded that the species now known as *Olla transiens* is better accommodated in *Protounguicularia* and the species is combined into that genus. Attention is drawn to variability in general appearance of *P. nephromatis*, particularly the occurrence of both superficial and erumpent apothecia in the same species.

Key words: *Helotiales*, *Hyalopeziza*, *Hyaloscyphaceae*, lichens, *Nephroma*, *Olla*, *Protounguicularia*, *Pseudocyphellaria*, *Unguiculariopsis*

Introduction

Amongst old collections by Rolf Santesson, P. G. I. discovered an abundant, lichenicolous fungus with some characteristics indicating the material should be referred to the *Hyaloscyphaceae*. He then contacted S. H. and D. L. H., and also learned that T. Tønsberg had apparently collected similar material in the USA and Canada. In all, the large amount of material generously placed at our disposal revealed two glassy-haired species, both of which had not been formally described at that time. However, while we were in the process of describing them as new species, we learnt that one of them had also been discovered and described by Zhurbenko & Zavarzin (*in* Zurbenko 2007) as *Unguiculariopsis nephromae*. That species does, however, require transferring to a more appropriate genus. Here we make that new combination in the genus *Protounguicularia* and provide an emended description and discussion of the variability of the species and also comment on the generic placements of the glassy-haired, lichenicolous discomycetes. During this study, *Olla transiens* was found to be more appropriately referred to *Protounguicularia* and is also formally transferred here to that genus. The second undescribed glassy-haired species discovered in this study is described here as *Hyalopeziza rapax*.

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Material and Methods

The material was studied with an Olympus BX40 research microscope using bright field optics. All measurements and critical observations were made using a ×100 Olympus UPlanFl objective. Drawings were made to a ×2500 scale using a drawing tube. Media and staining procedures used were those given in Huhtinen (1990) and their abbreviations are: MLZ (Melzer's reagent), IKI (1% Lugol's solution), CR (ammoniacal Congo red), KOH (5% aqueous solution). Colour standards used follow Cailleux (1981). Means of measurement ranges are placed in italics, and atypical extremes in parenthesis; similarly for the Q value (= length : breadth ratio).

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Taxonomy

Protounguicularia nephromatis (Zhurb. & Zavarzin) Huhtinen, D. Hawksw. & Ihlen, comb.nov.

MycoBank no.: 512448.

Basionym: *Unguiculariopsis nephromae* Zhurb. & Zavarzin, *in* Zhurbenko, *Mikol. Fitopat.* **41:** 126 (2007); grammatical error, to be changed (Art. 32.7.)

Corticifraga nephromae R. Sant., *in* Santesson *et al.*, *Lich. Lichenic. Fungi Fennoscand.*: 107 (2004); nom. inval. (Art. 32.1).

(Figs 1 & 2)

Apothecia typically densely gregarious, sunken and erumpent on the upper side of the host lichen, in one collection only superficial when on the upper side, always superficial when growing on the lower side, discoid to cupulate, up to 330 µm diam. when dry, in most collections up to only 200 µm diam., disc faint yellowish to faint brownish (Cailleux K75, M77), margin whitish. *Ectal exciple* composed of *textura prismatica* to *textura angularis* depending on the growth habit, cells 9–16 × 2·5–4·0 µm to *c.* 4–5 × 5–6 µm, firm-walled, true wall hyaline, 0·5–1·0 µm thick, MLZ–, IKI–, CR–, except for those areas where the glassy, solidifying substance forms clear CR+ thickenings. *Marginal hairs* always present, very variable in size and abundance between populations, best developed in apothecia on the lower surface of the host thallus, from minute and aseptate up to 140 µm long and 2-septate, cylindrical to slightly tapering, to 4 µm wide, apex blunt, 1·5-4·0 µm wide, smooth, hyaline, often apically covered with a hyaline layer sometimes gluing the hairs together, this substance CR–, MLZ–; walls mostly slightly thickened, more rarely thin throughout, CR–, MLZ– but inside most of the hairs with apical glassy material MLZ– and CR+, occasionally such material is also present in the lower parts forming irregular thickenings; glassy material inert in 5 % KOH. *Paraphyses* cylindrical or slightly clavate, to 3 µm wide, simple to apically branched, devoid of pigments. *Asci* cylindrical-clavate, 37–*58·9*–74 × 8–*6·7*–9 µm in MLZ (*n*= 10, from three populations), 36–*44*–61 × 7–*8·3*–9·5 µm in CR (*n*= 10, from two populations), eight-spored, firm-walled in MLZ, apex rounded, wall of even thickness, MLZ–, IKI–; asci arising from croziers. *Ascospores* fusoid-subfusoid to hirudoid [leech-shaped], 8·5–*11·5*–16 (–17·5) × 2·5–*2·9*–3·2 µm, Q = 3·1–*4*·0–5·3 in MLZ (*n* = 50, from six populations), smooth, hyaline, aseptate, apically often beaked, with or without guttulae in MLZ, typically guttulate in CR.

Ecology. So far the species has been found on at least three species of *Nephroma*, *viz*. *N. bellum, N. laevigatum,* and *N. resupinatum,* as well as undetermined specimens of the genus. In all but two collections the apothecia are formed only on the upper surface. Zhurbenko (2007) found apothecia on both sides of the thallus, but with most growing on the lower side. Our material showed the opposite; one collection only (*Tønsberg* 15495) had apothecia on the lower side. In the majority of collections, the apothecia are sunken and erumpent through the upper cortex. They fruit typically in large swarms and do not seem to cause any damage to the host. In one abundant collection only (*Tønsberg* 37035) all the apothecia were superficial on the upper surface. When on the lower surface, the apothecia are sparser. Well-developed, separate, long hairs were seen only in these superficial apothecia, whereas the erumpent apothecia have a wide, furry margin where individual hairs do not stand out when viewed under a dissecting microscope. The overall difference between these two apothecial types is of such magnitude that without strong microscopical evidence and their co-occurrence it would be easy to conclude that they were different species.

Holien & Tønsberg (1996) suggested that *P. nephromatis* (as "*Corticifraga nephromae*") might be confined to boreal rain forest. Its presence in such 'better' habitats could, at least in Finland, be due to the association between *Nephroma* spp. and old forest stands (Kuusinen & Siitonen 1998). A study of 42 old-growth forests in Finland showed that *N. bellum* occurred in 41, and *N. resupinatum* in 38, showing they are excellent indicators of such forests (Kuusinen *et al.* 1995).

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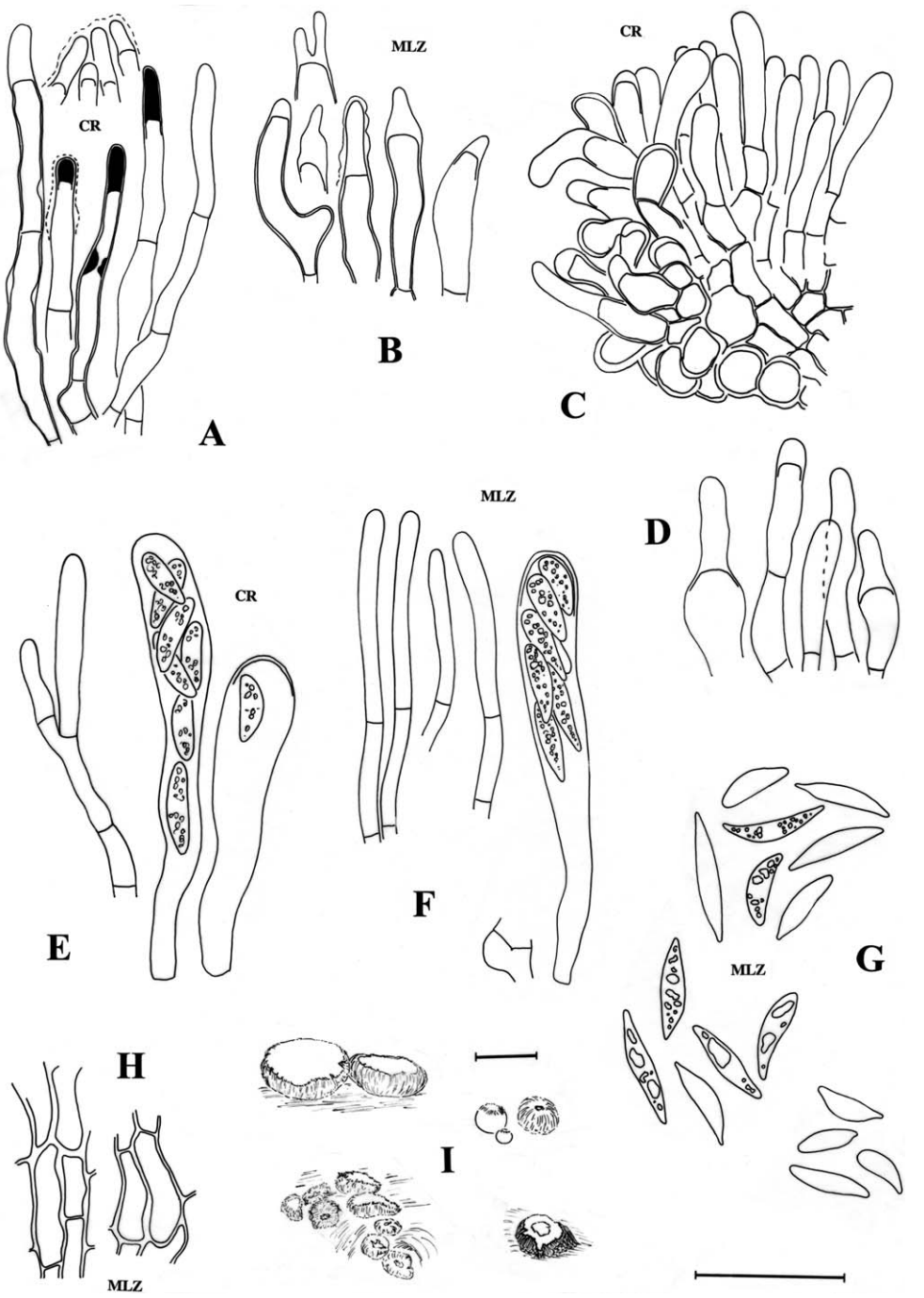


FIG. 1. *Protounguicularia nephromatis*. A, hairs (*Tønsberg* 15496b); B, hairs (*Santesson* 12516a); C, detail from exciple (*Tønsberg* 20731); D, hairs (*Santesson* 32676); E, asci and paraphyses (*M. Laurila*, s.n).; F, asci and paraphyses (*Tønsberg* 15495); G, ascospores [*Tønsberg* 15495 and *Tønsberg* 20730a (lowermost four s.n.)]; H, ectal exciple (*Tønsberg* 15495); I, variability in dry apothecia. Scales: A–H = 20 μm; I = 200 μm.

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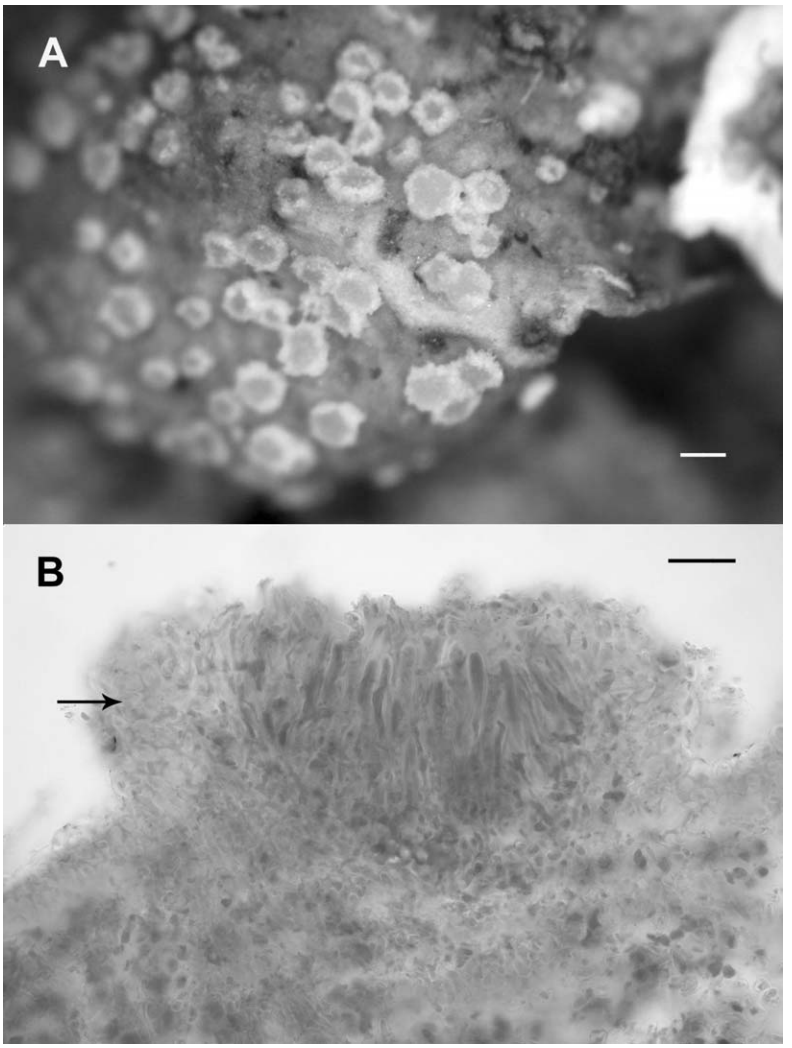


FIG. 2. *Protounguicularia nephromatis* (*Santesson* 12516a). A, dry apothecia; B, section of an apothecium (arrow points to exciple with only stunted hairs). Scales: A = 200 μm; B = 20 μm. Photo: P.G. Ihlen.

Distribution. The species seems to be widespread in boreal regions of the Northern Hemisphere, and is known from Canada, Finland, Norway, Russia, Sweden, UK and the USA. It might be common since additional material was found with ease in collections of *Nephroma* in TUR.

Notes. The overall agreement of our observations with the original description by Zhurbenko (2007) is good. The excipular hairs were reported as up to 35 μm long only and aseptate; the additional material examined here adds markedly to the range of variability of the hairs. The ascus size range and chemical reactions are similar. However, the ascospore mean width was given as 3·5 μm in the type. This could be due to measuring the spores in KOH. Especially noteworthy is the characteristic shape of the ascospores; Zhurbenko's illustration shows the same rare hirudoid shape so we are confident that our material is conspecific with that described by Zhurbenko & Zavarzin (*in* Zhurbenko 2007).

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The immersed to erumpent apothecia led Santesson (in Santesson *et al*. 2004) to propose placing this species in *Corticifraga* D. Hawksw. & R. Sant. 1990. However, the presence of the excipular hairs, the evenly thin-walled asci, simple ascospores, and the sometimes superficial apothecia indicate that *Corticifraga* is not an appropriate genus for this species. Indeed, that genus may be most closely related to the *Lecanoromycetes*, although its position remains obscure. The solidified, blunt, KOH-insoluble hairs indicate that the most logical genus in which to place this species is *Protounguicularia* Raitv. & R. Galán 1986 or *Olla* Vel. 1934. These hairs make a placement in *Unguiculariopsis* Rehm 1909 impossible as the principal diagnostic character in both the fungicolous and lichenicolous species of that genus is the aseptate ascomatal hairs with swollen bases, and narrow, curved glassy apices, with or without a lumen (Zhuang 2000). Zhuang did not comment on newly added lichenicolous species, all of which lack the typical hairs of *Unguiculariopsis*, and require re-examination, also using the stains employed here.

Raitviir & Galán (1986) established *Protounguicularia* for three species, all of which had long, cylindrical, blunt and septate hairs. Two of the species were soon transferred to *Arachnopeziza* Fuckel 1870 by Huhtinen (1987*c*) because of a lack of glassy material in the excipular hairs. The remaining species was characterized by septate, apically solidified hairs, inside which the glassy material was KOH-insoluble, dextrinoid, and congophilous. In a separate paper (Huhtinen 1987*a*) the type species, *Protounguicularia brevicapitata* Raitv. & R.Galán 1986, was placed in synonymy under an earlier name introduced by Velenovský (1934) and the new combination, *P. barbata* (Vel.) Huhtinen 1987 was made. However, that placement did not remain in use for long as Engel & Svrček (1983) had reported the same taxon under the name *Pezizellaster transiens* Höhn. 1918. Subsequently, Svrček (1987) hinted at the possible synonymy of *P. brevicapitata* and Höhnel's species, and this was later verified by Baral (1993) who combined Höhnel's name into the genus *Olla*.

Baral's (1993) transfer of *Pezizellaster transiens* to *Olla* was commented on a year later (Baral 1994). As had Svrček, he stressed the urn-like apothecial shape, and also the short, aseptate, totally glassy hairs of species belonging to *Olla*. The discoid apothecia and only shortly and apically glassy hairs of *Olla transiens* were considered satisfactory for the genus, but the solution was admittedly somewhat tentative (Baral 1994).

A third species was transferred to *Protounguicularia* by Svrček (1991), *Chrysothallus vandae* Vel. 1934 the lectotype of which was collected on a twig of *Quercus*; the same collection has also been studied by S. Huhtinen Svrček (*loc.cit*) illustrated typical *Protounguicularia* hairs with an apical, dextrinoid, solidifying substance. He considered the hairs to be smooth, but mentioned large crystals on the margin. Based on studies of the same specimen, Galán & Raitviir (1994) came to a different conclusion and combined this species name into *Graddonidiscus* Raitv. & R. Galán 1992, but failed to mention the solidifying substance in the hair apices.

These contrasting conclusions arose from the use of different methods. The surface crystals are lost in both heated Cotton blue mounts and in 10 % KOH. Crystals partly persist in MLZ mounts, which were apparently used by Svrček, but are dissipated by heavy tapping to reveal naked hair apices. Because of the short, aseptate and totally to heavily crystal-covered hairs, *Chrysothallus vandae* should not be accepted in *Protounguicularia*.

We consider that *Protounguicularia* is sufficiently different from *Olla* to merit reinstating its generic status. The long hairs are cylindrical and septate, in contrast to the short and aseptate hairs in *Olla*. The hairs also have a slightly thickened true proper wall, and are histochemically different in the nature of the solidifying material. They differ from the typical hairs of *Olla* in not having just a thin membrane surrounding the glassy hair material. In mounts of, for example, *O. scrupulosa* (P. Karst.) Svrček 1986 or *O. millepunctata* (Libert) Svrček 1986, the hairs are frequently fractured, holding their basic shape apparently by a very thin true wall

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covering the glassy part. The solidifying material does not stain in ammoniacal Congo red (contrary to the erroneous indication in Huhtinen 1987*b*: table 1).

The hairs of the present species differ in their MLZ reaction from both genera in being non-dextrinoid, but the hair morphology and the CR+ reaction of the solidifying substance seem to bring it closer to *Protounguicularia* than *Olla*. Although the totally sunken and then erumpent apothecia seem to be distinct from either genus, when much more material became available it was clear that the apothecia could vary from erumpent (mostly on the upper surface) to totally superficial (mostly on the lower surface). In the sunken apothecia, the hairs remain short and the margin becomes broad, while in superficial apothecia the hairs are longest and morphologically identical to those of *Protounguicularia*, possibly reflecting the more favourable growth environment. Although the appearance of the fruit bodies is totally different, all other microscopical features are identical and we have no doubt that they represent a single species. For comparison of the genera treated see Table 1.

In both *O. transiens* and *P. nephromatis,* well-developed hairs have solidifying CR+ matter located locally at the apex, or more rarely as infrequent thickenings in more basal parts. The hairs are tougher and keep their basic shape, and no fracturing usually occurs as the glassy substance is encapsulated by the CR– true wall. The apothecia are discoid when fully mature, whereas in *Olla* they are typically urceolate. We therefore also transfer *O. transiens* to *Protounguicularia* below.

Material of P. nephromatis examined. **Canada:** *British Columbia*: N of Hazelton, 10 km WNW of Kispiox, N of Date Creek, 55°23.4′N, 127°49.3′, alt 720 m, on *Nephroma* sp., 1994, *T. Tønsberg* 20730, 20731 (BG L-71898, 71900). —**Finland:** *Pohjois-Karjala*: Pielisjärvi, Louhivaara, Kirkisenvaara, on *N. bellum*, *M. Laurila* (TUR 178812). —**Norway:** *Hordaland*: Fjell, Sotra, Steinland, on *N. laevigatum*, 1967, *R. Santesson* 19286 (UPS). *Nordland*: Brønnøy, 3·5 km SE of Hommelstø, Holmarklia, alt. 150, on *N. bellum*, 1995, *T. Tønsberg* 23558 (BG L-43904); Brønnøy, 3·5 km SE of Hommelstø, Holmarklia, alt. 150 m, on *N. bellum* on base of trunk of *Sorbus aucuparia,* 1995, *T. Tønsberg* 23558 (BG); Rana, Olderlia, alt 120–160, on *N. bellum* on trunk of *Salix caprea*, 2006, *T. Tønsberg* 37034, 37035

TABLE 1. *Some typical distinguishing characteristics of the genera,* Protounguicularia, Unguiculariopsis, Olla, Hyalopeziza, Arachnopeziza *and* Corticifraga

	Protounguicularia	*Unguiculariopsis*	*Olla*	*Hyalopeziza*	*Arachnopeziza*	*Corticifraga*
Hairs						
walls/contents	apically solid	firm or apically solid	solid or with lumen	mostly firm-walled	no hairs	
shape	cylindric	tapering, curved	cylindric	tapering, straight	cylindric	no hairs
septa	present	lacking	lacking	present/lacking	present	no hairs
MLZ-reaction	red/lacking	lacking	red	lacking	lacking/blue	no hairs
Asci	single-layered	single-layered	single-layered	single-layered	single-layered	two-layered
Mostly on						
lichens	+/–	–	–	–	–	+
other fungi	–	+	–	–	–	–

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(BG). *Nord-Trøndelag*: Snåsa, *c.* 1 km N of Hammer, alt. 60–140 m, on *N. bellum* on trunk of *Salix* in brook ravine in spruce forest, 1993, *R. Santesson* 33586 (UPS); Grong, W of river Gartlandselva, alt. 100 m, on *N. bellum* on twigs of *Picea abies*, 1995, *H. Holien* 6863 (TRH). —**Russia:** *Republic of Karelia*: Korpiselkä, Hirvasvaara, on *N. bellum*, 10 ix 1901, *K. J. Ehnberg* (TUR 178813). —**Sweden:** *Härjedalen*: Tännäs parish, Hamrafjället, SW slope E of Hotel of Hamrafjället, alt. 800–850 m, on *N. bellum*, 1958, *R. Santesson* 12516a (UPS); Tännäs parish, valley of the river Ljusnan, Ramundberget, alt. 700 m, on *N.* bellum in the subalpine birch forest, 1962, *R. Santesson* 14873b (UPS); Tännäs parish, valley of the river Ljusnan, *c.* 2·5 km SSE of Ramundbergets Fjällgård, alt. 700 m, on *N. bellum*, 1987, *R. Santesson* 31910 (UPS); Tännäs parish, Hamrafjället, southwestern slope E of Hotel Hamrafjället, alt. 800–850 m, on *N. bellum* on a mossy boulder in the birch forest, 1958, *R. Santesson* 12516 (UPS); Tännäs parish, Ljusnan, slope of Mt. Gruvvålen, alt. 800 m, on *N. bellum*, 1989, *R. Santesson* 32676 (UPS). *Värmland*: Dalby parish, Norra Brattmoviken, on *N. bellum* on twigs of *Picea abies*, 1956, *R. Santesson* 11225d (UPS). *Lycksele Lappmark*: Tarna parish, Ume river, Bredviken, on *N. bellum*, 1960, *G. E. Du Rietz* 2211b (UPS L-121463). —**Great Britain:** *Scotland*: **V.C.96** Easterness: Inverfarigaig SSSI, Pass of Inverfarigaig, alt. 60m, on *N. laevigatum* on *Corylus*, 2008, *B. J. Coppins* 22629 (e). —**USA:** *Alaska*: Kodiak Island Borough, Kodiak Island E, S of Isthmus Bay, bank of Roslyn Creek, 57°37′N, 152°19.4′W, alt 0–10 m, on *Nephroma* sp., 1991, *T. Tønsberg* 15495, 15496b (BG L-66114, 66116).

Protounguicularia transiens (Höhn.) Huhtinen, comb. nov.

MycoBank no.: 572449.

Basionym: *Pezizellaster transiens* Höhn., *Sber. Akad. wiss. Wien, Math.-nat. Kl., Abt. 1*, **127:** 608 (1918). — *Olla transiens* (Höhn.) Baral, *Z. Mykol.* **59:** 9 (1993).

Protounguicularia barbata (Vel.) Huhtinen, *Beitr. Kenntn. Pilze Mitteleur.* **3:** 457 (1987).—*Hyaloscypha quercina* var. *barbata* Vel., *Monogr. Discom. Bohem.* **1:** 276 (1934).—*Hyaloscypha barbata* (Vel.) Svrček, *Česká Mykol.* **39:** 216 (1985).

Protounguicularia brevicapitata Raitv. & R. Galán, *Int. J. Mycol. Lichen.* **2:** 222 (1986).

Hyaloscypha quercina var. *resinacea* Dennis, *Kew Bull.* **30:** 353 (1975).—*Protounguicularia barbata* f. *resinacea* (Dennis) Huhtinen, *Beitr. Kenntn. Pilze Mitteleur.* **3:** 458 (1987).

Ecology. Saprobic on wood; not lichenicolous.

Distribution. Europe (Austria, Czech Republic, Denmark, Finland, France, Germany, Norway, Poland, Spain, Sweden, Switzerland, The Netherlands, and the UK) and South America (Argentina).

Notes. This species has identical excipular hairs to those of well-developed *Protounguicularia nephromatis,* but differs in that the solidifying apices are both CR+ and MLZ+ (i.e. dextrinoid) whereas those of *P. nephromatis* are only CR+.

Hyalopeziza rapax Huhtinen, sp. nov.

MycoBank no.: 512450.

Apothecia pallide grisea vel pallide ochracea, ad usque 0.2 mm diametro, cupulata, margine minute puberula. Excipulum ectale ex cellulis prismaticis compositur. Pili cylindraceo-angustati, aseptati, hyalini, in basi tenuiter tunicati, sursum crassotunicati vel valde crassotunicati sed lumen habentes, in iodo non colorati, leves. Paraphyses filiformes, 1·5–2·0 µm in diametro, sine guttulae. Asci 25–33 × 6–7·5 µm, octospori, non amylodei, in basi non uncinati. Ascosporae 7–11 × 1·5–2·0 µm, cylindraceo-ellipsoideae, saepe subfusoideae, hyalinae, aseptatae.

Typus: USA, Alaska, Kodiak Island Borough, Kodiak Island E, S of Isthmus Bay, bank of Roslyn Cr., 57°37′N, 152°19.4′W, alt 0–10 m, on the lower side of *Pseudocyphellaria crocata* on twigs of *Picea sitchensis* in rain forest, 16 May 1991 *T. Tønsberg* 15496a (BG L-66115—holotypus).

(Fig. 3)

Apothecia gregarious, discoid, minute, to 200 µm diam. when dry, broadly sessile; watery greyish (Cailleux L92) to straw-coloured (K79), margin hairy, whitish. *Ectal exciple* composed of *textura prismatica*, cells 6·5–8·5 × 3–6 µm, walls firm, 0·5–1·2 µm thick, IKI–, MLZ–, CR–; lower parts of the exciple with firm-walled *textura angularis*. Hairs numerous, at the margin 20–43(–57) × 2–4 µm, tapering, more rarely cylindrical, smooth, aseptate, very rarely 1-septate, typically apically glassy and without a lumen, more rarely with a discontinuous lumen or walls only slightly glassy and thus with a continuous lumen; hairs with irregular, glassy thickenings very rare; glassy material MLZ–, CR+. *Paraphyses* cylindrical, 1·5–2 µm wide, simple to apically branched, devoid of prominent guttulae in KOH and MLZ. *Asci* cylindrical-clavate, 25–33 × 6–7·5 µm, eight-spored, MLZ–, IKI–, arising from simple septa. *Ascospores* 7–*8·7*–11 × 1·5–2 µm, Q= 3·5–*4·6*–5·2, (*n*=27, in MLZ and CR) elongate-subfusoid, smooth, aseptate, with prominent guttulae in MLZ and CR.

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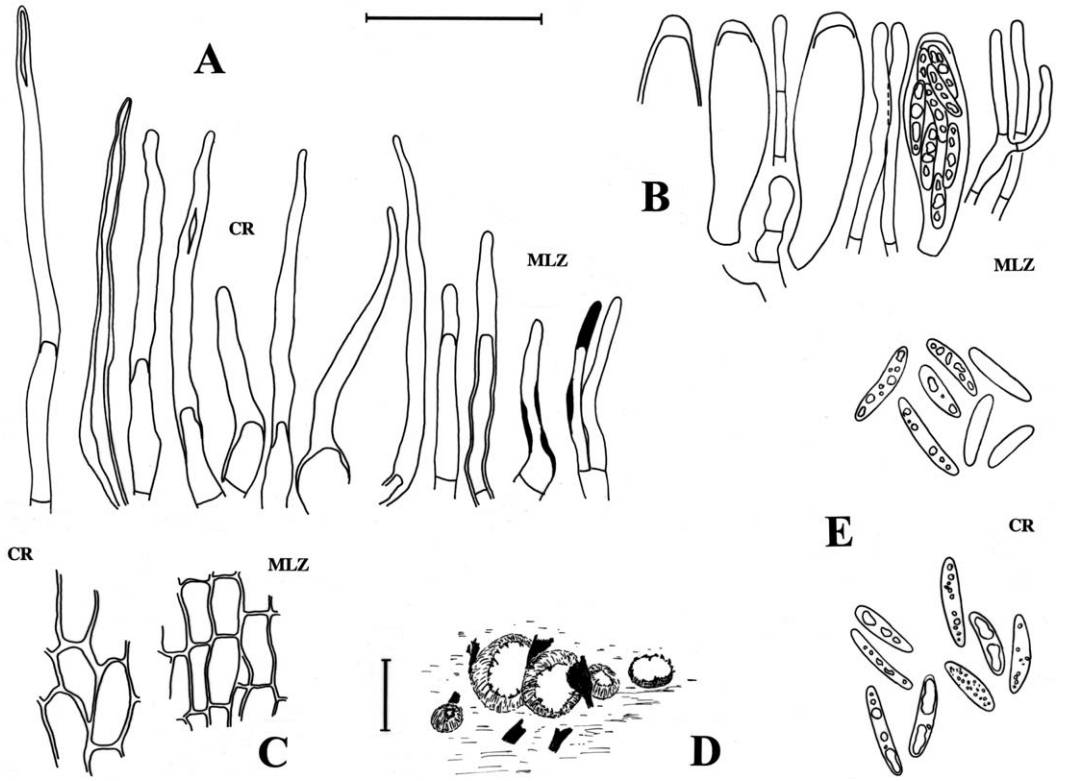


FIG. 3. *Hyalopeziza rapax* (holotype) A, marginal hairs; B, asci and paraphyses; C, ectal exciple; D, dry apothecia; E, ascospores. Scale A–C & E = 20 μm; D = 200 μm.

Etymology. *Rapax*, referring to the seizing or catching habit.

Ecology. On the thallus of *Pseudocyphellaria crocata*.

Distribution. North America (USA); known only from the holotype collection.

Notes. The KOH inert, tapering, and glassy hairs place this species in *Hyalopeziza* Fuckel 1870. The genus was recently treated by Raitviir (2004) who included 16 species. Of those only *H. latispora* Raitv. and *H. niveocincta* (Graddon) Raschle have pale apothecia, MLZ– asci and short, aseptate to basally septate hairs. The former differs clearly in having wide spores, the latter in having non-glassy hairs (Baral & Galán 1999). In addition to the shape of the hairs, the strong CR+ reaction is also indicative of a relationship to *Hyalopeziza*. In some of the hairs there is a tendency for a continuous lumen to also be seen, a character also present in the genus. The character combination and ecology clearly separate the present species from other species in the genus.

We thank T. Tønsberg for placing his many collections at our disposal and A. Puolasmaa for his efforts in going through Finnish material of *Nephroma* until he found the species as new to Finland. We also acknowledge the first observations of the new species on *Nephroma* by R. Santesson. This work was supported by the Ministry of the Environment, Finland, as a part of the research programme of deficiently known and threatened forest species (PUTTE).

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