



Three new *Pronectria* species in terricolous and saxicolous microlichen communities (Bionectriaceae, Ascomycota)

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With 2 figures

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Abstract: Three new species of Bionectriaceae are described; (1) *Pronectria algicola* grows within *Gloeocystis*-like algal crusts with *Steinia geophana* and *Sarcosagium campestre*; (2) *P. caloplacae* is lichenicolous on areoles of *Caloplaca austroclitrina*; (3) *P. diplococca* grows in squamules of *Collema* spp. The latter species has early dissolving asci and 1-septate ascospores disintegrating into simple globose mature ascospore fragments. Fragmentation of ascospores appears in three additional species of *Pronectria*. A key to the known *Pronectria* species is provided.

Key words: Ascospore fragmentation, Bionectriaceae, Hypocreales, lichenicolous fungi, microbial crust, Sordariomycetes.

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Introduction

Pronectria (Bionectriaceae) is characterized by non-stromatic perithecia occurring on host lichens; perithecial walls that do not react with KOH, and usually 1-septate (rarely 2–7-septate) ascospores, which are either smooth or ornamented. A recent monograph of Bionectriaceae (Rossman et al. 1999) increased the number of species in *Pronectria* to 18. However, many further species have been described afterwards (Brackel & Etayo 2010, Etayo 2001, 2002, Etayo & Sancho 2008, Etayo & Triebel 2010, Lopez de Silanes et al. 2009, Motiejūnaitė & Kukwa 2008, Zhurbenko et al. 2005, Zhurbenko 2007, Zhurbenko 2009); most of them are lichenicolous on conspicuous epiphytic and terricolous macrolichens. Only four species, *P. dealbans* (Etayo & Breuss 1996), *P. dillmaniae* (Zhurbenko et al. 2005), *P. lecideicola* (Zhurbenko 2009), *P. terrestris* (Lowen & Diederich 1990), and *Pronectria verrucariae* (Lowen 1990) are known from crustose lichens. Other lichenicolous *Pronectria* species may exist, but they are easily overlooked; a number of *Pronectria* spp. is only known from the type locality.

Two of the three species, which we describe here as new, are also known from one locality only. The new species are from three different substrata; (1) *Gloeocystis*-like algal crust with *Sarcosagium campestre* and *Steinia geophana* in the Czech Republic; (2) *Caloplaca austrocitrina* growing on concrete in the Ukraine; and (3) terricolous *Collema squamules* in Europe.

Materials and methods

The study is based on specimens collected by the authors and deposited in BILAS, CBFS, KHER, PRA, PRM and the private hb. J.Kocourková & K.Knudsen. The morphology was studied using light microscopy. Observations and measurements were made in water. The measurements are given as (min.–) $X \pm SD$ (–max.), where X = mean value and SD = standard deviation with the number of measurements in square parentheses. Colour reactions were examined in 10% KOH (K), 1% Lugol's iodine solution (I); asci were also observed in I after the K pre-treatment (KI). Type specimens of each described lichenicolous *Pronectria* species have been photographed and pictures are available on <http://botanika.bf.jcu.cz/lichenology/index.php?pg=5>.

The Species

***Pronectria algicola* Vondrák, Naumovich & Khodos. sp. nov.**

Figs 1A, B, C

Fungus lichenicola in algis typi *Gloeocystis*. A *Pronectria dillmaniae* ascosporisque maioribus (6.25–)8.17 \pm 1(–9.75) \times (4.25–)5.37 \pm 0.69(–6.75) μm , ostiolisque rubellis et tomentosis differt.

TYPE: Czech Republic. S Bohemia, České Budějovice, Mydlovary, the setting pit "MAPE", alt. 400 m, 49°5'58"N, 14°20'8"E, on microbial crust over iron-rich sediments, 30 Nov. 2009, coll. J.Vondrák (CBFS – 7424 holotype, CBFS, KHER – isotypes).

Mycelium immersed in the algal crust, consisting of simple or branched hyphae among gelatinous sheets of *Gloeocystis*-like algae. Ascomata perithecia, immersed in soil crust, dispersed, subglobose in vertical section, (105–)135 \pm 22(–170) μm in width and (120–)155 \pm 22(–190) μm in height [$n = 10$]; Perithecial apex visible on host surface, reddish-orange, (30–)45 \pm 8(–60) μm diam. [$n = 10$]. The apex surrounded by delicate colourless upright papillae, (4–)6 \pm 1.75(–10) \times (2–)2.7 \pm 0.45(–3.5) μm

[n = 15]. Perithecial wall (12–)17 ± 3.5(–25) µm thick [n = 10], of homogeneous tissue, but outer part orange-brown and inner part colourless, of narrowly ellipsoid cells, (3.5–)5.6 ± 1.5(–7.5) × (1.0–)1.5 ± 0.45(–2.25) µm [n = 10] in 4–7 layers. Perithecial centrum with numerous orange oil drops. Paraphyses absent. Periphyses distinct in the upper "neck-like" perithecial part, (10.5–)13.03 ± 1.52(–16.0) µm long [n = 17] and c. 1 µm wide. Asci 8-spored, clavate, (37–)48 ± 8(–63) × (9–)11 ± 2(–14) µm, [n = 25]. Ascospores colourless, biseriata, fusiform (11.25–)16.25 ± 2.0(–21.0) × (4.0–)5.25 ± 0.75(–6.75) µm, [n = 50], 2-celled with 1–2 oil drops in each cell, indistinctly constricted at the septum; upper cell (6.25–)8.25 ± 1(–9.75) × (4.25–)5.37 ± 0.69(–6.75) µm wide; lower cell narrower, (5.5–)7.75 ± 1.0(–9.5) × (–3.5) 4.3 ± 0.72(–6.0) µm wide [n = 30], thin and smooth-walled. The ratio of ascospore length/width (2.04–)3.11 ± 0.48(–3.94). Anamorph unknown.

REACTIONS: Perithecial wall K–, I–; perithecial centrum I–; asci I–, KI–.

ETYMOLOGY: The epithet refers to the occurrence in algal crusts.

ECOLOGY AND DISTRIBUTION: The new species grows in terricolous microbial crusts with an abundance of *Gloeocystis*-like cells. These algae may be in partnership with *Sarcosagium campestre* and *Steinia geophana*, which co-occur with the *Pronectria*. Although ascomata of both lichens grow closely to perithecia of *Pronectria*, we have no clear evidence for *Pronectria* being lichenicolous on them. In the type locality, other microlichens were found in the microbial crust: *Absoconditella trivialis*, *Agonimia vouauxii*, *Epigloea soleiformis*, *Lemmopsis pelodes*, *Thelocarpon lichenicola*, *Verrucaria bryoctona* and *Veizdaea leprosa*. It is only known from the type locality in the Czech Republic, where it was repeatedly collected.

DIAGNOSTIC CHARACTERS: Medium-sized perithecia (100–170 µm in width); reddish-orange perithecial apex with colourless papillae; ascospores smooth-walled, 1-septate, c. 14–18 × 4.5–6 µm with ± narrower lower cell.

NOTES: The smooth-walled 1-septate ascospores are known in several *Pronectria* species. The ascospore size in *Pronectria tenacis* and *P. terrestris* are similar to the new species, but those have much larger perithecia. *Pronectria collematis* and *P. occulta* differ by their narrower ascospores and *P. dillmaniae*, *P. leptogii*, *P. microspora* and *P. sticticola* have smaller ascospores. *Pronectria dealbans* and *P. oligospora* are distinguished by the absence of colourless papillae in the ostiolar area. The papillae in the ostiolar area of *P. anisospora* and *P. pilosa* are much longer. *Pronectria tenacis* and *P. tenuispora* have larger perithecia without papillae. *P. minuta* has smaller ascomata and ascospores.

PARATYPES: The type locality, 19 Oct. 2007, coll. J.Vondrák (CBFS 5852, 6051).

***Pronectria caloplacae* Khodos., Vondrák & Naumovich sp. nov.** Figs 1D, E, F

Fungus lichenicola in thallis *Caloplacae austrocitrinae*. A *Pronectria echinulata* ascosporis angustioribus (12.5–)15.2 ± 1.6(–20) × (5–)6.4 ± 0.8(–8) µm et verruculosus differt.

TYPE: Ukraine. Kherson region, Chaplynsky district, 5 km W from the village Zaozerne, bank of the water channel "Kachovskiy kanal", alt. 30 m, on concrete, lichenicolous on *Caloplaca austrocitrina*, 15 Nov. 2006, coll. A.Khodosovtsev (KHER 6248 – holotype).

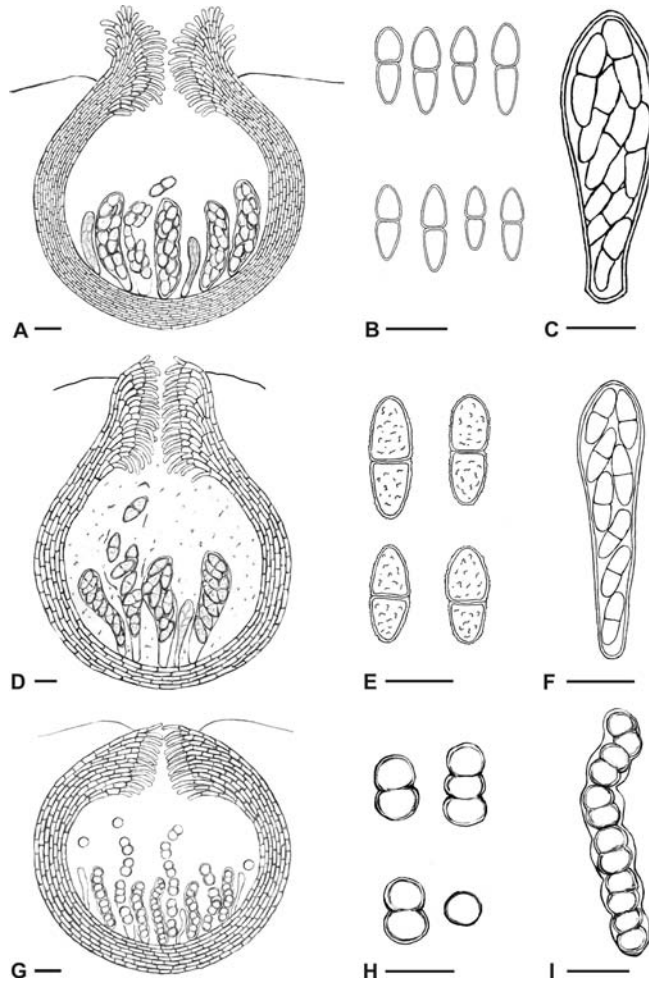


Fig. 1. Ascomatal characters of new *Pronectria* spp. A–C *P. algicola*: A, perithecium; B, ascospores; C, ascus. D–F *P. caloplacae*: D, perithecium; E, ascospores; F, ascus. G–I *P. diplococca*: G, perithecium; H, ascospores; I, ascus. Bars = 10 μ m.

Ascomata perithecia, numerous, immersed in discoloured host thallus, in group of 10–30 per one areole, subglobose to pyriform, (110–)130 \pm 17(–160) μ m in width and (140–)170 \pm 24(–220) μ m in height [n = 21], orange. Perithecial apex visible on host surface, orange, (30–)50 \pm 12(–75) μ m diam. [n = 20]. Perithecial wall (13–)16 \pm 2.6 (–22) μ m wide [n = 15] in its medium height, but up to 25–35 μ m in its upper part, light orange, formed of one homogeneous layer, composed typically of 5–6 rows of cells; the cells \pm fusiform, thin-walled, rectangular, (–4)6.8 \pm 2.2(–10) \times (1.5–)2.3 \pm 0.45 (–3) μ m [n = 15]. Perithecial centrum orange, with numerous oil drops. Paraphyses absent. Paraphyses (9–)12 \pm 2(–15) long [n = 10] and c. 1 μ m wide. Asci unitunicate,

8-spored, cylindrical to subclavate, $(40-)\text{60} \pm 11(-75) \times (8-)\text{10} \pm 2(-13) \mu\text{m}$ [$n = 12$], with apical ring. Ascospores biseriate, colourless, narrowly ellipsoid to ellipsoid, oblong to fusiform, with rounded (rarely somewhat acute) apices, 1-septate, the cells usually equal in size, not constricted at the septum, $(12.5-)\text{15.3} \pm 1.5(-20.0) \times (5.0-)\text{6.4} \pm 0.7(-8.0) \mu\text{m}$ [$n = 45$], markedly verrucose, with one or more oil drops. The ratio of ascospore length/width $(1.73-)\text{2.4} \pm 0.38(-3.2)$. Anamorph unknown.

REACTIONS: Perithecial wall K-, I-; perithecial centrum I-; asci I-, KI-.

ETYMOLOGY: The epithet refers to the host lichen genus *Caloplaca*.

ECOLOGY AND DISTRIBUTION: This species is lichenicolous on areoles of *Caloplaca austroctrina*. Perithecia of *Pronectria caloplacae* are abundant on the host in the type locality, which is the only known occurrence of the new species.

DIAGNOSTIC CHARACTERS: Ornamented, 1-septate ascospores, c. $12-16 \times 5-7 \mu\text{m}$; small perithecia $110-160 \mu\text{m}$ in width.

NOTES: Ornamented, 1-septate ascospores are known in several species. *Pronectria sticticola* and *P. invisibilis* differ by smaller ascospores. *Pronectria echinulata* has wider ascospores. *Pronectria lecideicola* differs by taller perithecia and thicker perithecial walls. *Pronectria xanthoriae* has wider perithecia and longer and narrower ascospores. *Nectria epicallopisma* differs in having 4 ascospores per ascus and a K+ purple perithecial wall.

Pronectria diplococca Kocourk., Khodos., Naumovich, Vondrák & Motiej. **sp. nov.**

Figs 1G, H, I, 2

Fungus lichenicola in thallis speciei Collema. A *Pronectria fissuriprodiente* ascosporis maioribus $(8.75-)\text{10.95} \pm 1.39(-13.5) \times (4.75-)\text{6.66} \pm 0.9(-8.75) \mu\text{m}$ et fatiscentibus differt.

TYPE: Ukraine. Kherson region, Belozersky district, village Nikolskoye, left bank of the river Ingulets, alt. 12 m, $46^{\circ}43'02.7''\text{N}$, $32^{\circ}48'51.2''\text{E}$, on young squamules of *Collema* cf. *crispum*, 13 Feb. 2011, coll. A.Khodosovtsev, Yu.Khodosovtseva (KHER 6243 – holotype, CBFS, KHER 6244 – isotypes).

Ascomata perithecia, fully immersed in the host thallus tissue, c. 1–14 per each *Collema* squamule, globose to pyriform, $(100-)\text{140} \pm 20(-200) \mu\text{m}$ in width and $(120-)\text{150} \pm 20(-170) \mu\text{m}$ in height [$n = 20$], orange-brown to reddish-brown. Perithecial apex visible on host surface, \pm reddish-brown, urceolate to flat, $(40-)\text{70} \pm 20(-110) \mu\text{m}$ diam. [$n = 50$]. Perithecial wall $(12-)\text{18.3} \pm 3.5(-25) \mu\text{m}$ wide [$n = 11$] in its medium height, but up to $30-35 \mu\text{m}$ in the upper part, rose to light orange to almost colourless, homogeneous, composed typically of 5–6 rows of cells. The cells fusiform, thin-walled, rectangular, $(-4)\text{6.75} \pm 1.74(-10) \times (1.75-)\text{2.4} \pm 0.58(-3.5) \mu\text{m}$ [$n = 15$]. Perithecial centrum orange, with oil drops, with numerous free ascospore fragments (globose cells) and 1-septate ascospores in mature ascoma. Paraphyses absent. Periphyses $(7-)\text{11} \pm 2(-14) \times (0.75-)\text{0.93} \pm 0.21(-1.25) \mu\text{m}$ [$n = 20$]. Asci unitunicate, 4–6-spored, narrowly cylindrical, $(30-)\text{34} \pm 2(-37) \times (4-)\text{6} \pm 1.6(-9) \mu\text{m}$ [$n = 15$], disappearing early. Ascospores uniseriate, colourless, diplococcoid strongly constricted at septa, 1-septate, \pm halonate, $(8.75-)\text{10.95} \pm 1.39(-13.5) \times (4.75-)\text{6.66} \pm 0.9(-8.75) \mu\text{m}$ [$n = 30$], but both cells soon disintegrating into simple globose cells, c. $5-8 \mu\text{m}$ diam., which predominate in the old perithecia. Simple "spore fragments" may secondarily

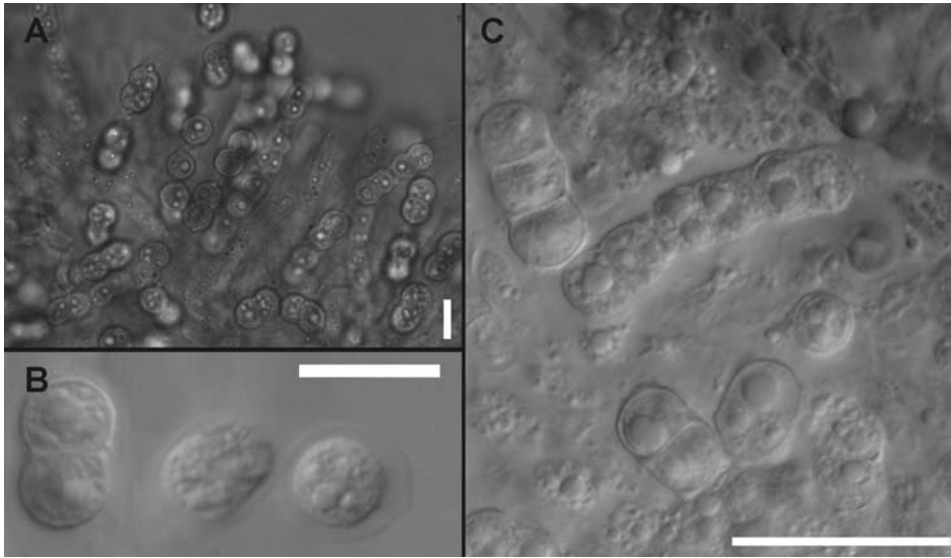


Fig. 2. *Pronectria diplococca*; A, dissolving asci with ascospores; B, halonate ascospores; C, ascospores and ascospore fragments. Bars = 10 μm (A, B), 20 μm (C).

form 2-cell aggregates. The ratio of the original ascospore length/width (1.29–)1.67 \pm 0.29(–2.5). Anamorph unknown.

REACTIONS: Perithecial wall K–, I–; Perithecial centrum I–; Asci I–, KI–.

ECOLOGY: The Ukrainian specimens grew in \pm young squamules of terricolous *Collema* cf. *crispum* on bare soil, usually in disturbed landscapes. Accompanying species were *Athelium imperceptum*, *Polyblastia agraria*, *Thelidium zwackhii*, *Verrucaria bryoctona* and mosses. All Czech collections were made on abandoned terraces in limestone quarries on *Collema coccophorum* and *C. crispum*. The species was accompanied by *Collema tenax*, *Lempholemma chalazanum*, *L. polyanthes*, and *Verrucaria bryoctona*.

DIAGNOSTIC CHARACTERS: Perithecia fully immersed; asci with uniseriate ascospores, the asci disappearing early; 1-septate young ascospores, strongly constricted at septa, soon disintegrating to simple globose ascospore fragments; simple ascospore fragments 5–8 μm diam. occurring free in the perithecial centrum.

NOTES: Some *Pronectria* species and allied species from *Haematonectria* have 1-septate ascospores with strongly constricted septa or with disintegrating ascospores. *Pronectria subimperspicua* is similar in having uniseriate asci, but differs in having smaller ascospores slightly constricted at septa and in having a K+ (red) reaction of the outer ascomatal wall. *Pronectria fissuriprodiens* differs in having smaller ascospores. Both species have non-disintegrating ascospores. The saprotrophic *Haematonectria monilifera* and *H. termitum* with disintegrating ascospores differ by having large sessile perithecia, longer asci and smaller ascospores. None of the known lichenicolous

Nectria, *Nectriopsis*, *Trichonectria* on *Collemataceae* (Brackel & Etayo 2010; Lopez de Silanes et al. 2009; Diederich & Puntillo 1995; Etayo 2001) have disintegrating ascospores.

NOTES: We have placed the new species in *Pronectria* on the basis of immersed perithecia, generally 1-septate ascospores and the lichenicolous substrate. However, the shape of ascospores and their fragmentation is a rare character in *Pronectria*; Rossman et al. (1999) do not provide any examples. Fragmentation of ascospores was also found in *Pronectria oligospora* (Etayo 1998), *P. fragmospora* (Etayo & Sancho 2008) and *Pronectria parmotrematis* (Etayo 2001). Ascospore fragmentation is otherwise typical for stromatic Hypocreaceae and is known in two *Haematonectria* species in Nectriaceae (Rossman et al. 1999).

PARATYPES: UKRAINE. Kherson region, Belozersky distr., Nikolskoye, A.Khodosovtsev (KHER 6245); Sofievka, J.Vondrák, A.Khodosovtsev (KHER 6246); A. & Y.Khodosovtsev (KHER 6247). CZECH REPUBLIC. Central Bohemia: distr. Praha-west, N of Kosoř village, J.Kocourková 7002 (PRM 915043); Loděnice, Branžovy quarry, J.Kocourková (hb. Kocourková & Knudsen 7868); Loděnice, Branžovy quarry, J.Kocourková (hb. Kocourková & Knudsen 7869, 7877); S Moravia, distr. Brno Co., Mokrý-Horákov, J.Kocourková (hb. Kocourková & Knudsen 7867). LITHUANIA. Pakruojis distr., Petrašiūnai, J. Motejūnaitė (BILAS 9770).

Key to lichenicolous *Pronectria* species

1	Ascospores 1-septate.....	2
1*	Ascospores 2- or more septate	41
2	Ascospores disintegrating or strongly constricted at the septa	3
2*	Ascospores not disintegrating or slightly constricted at the septa	9
3	Ascospores disintegrating	4
3*	Ascospores not disintegrating	7
4	Ascospore fragments of conical cells	5
4*	Ascospore fragments of globose or ellipsoid cells	6
5	Ascospores 20–25 × 5–6 µm, on <i>Usnea</i>	<i>P. fragmospora</i>
5*	Ascospores 13–16 × 4.5–6 µm, on <i>Parmotrema</i>	<i>P. parmotrematis</i>
6	Ascospores 9–13 × 5–8.5 µm, uniseriate, strongly disintegrating, ascospore fragments globose, on <i>Collema</i>	<i>P. diplococca</i>
6*	Ascospores 12.5–17 × 5–6 µm, biseriate, rarely disintegrating, ascospore fragments ellipsoid, on <i>Punctelia</i>	<i>P. oligospora</i>
7	Ascospores < 10 µm long	8
7*	Ascospores 12.5–17 × 5–6 µm, on <i>Punctelia</i>	<i>P. oligospora</i>
8	Ascospores smooth, 6.5–10 × 3–5 µm, on <i>Lobaria</i>	<i>P. fissuriproidens</i>
8*	Ascospores verrucose, 6.5–8 × 5–6 µm, on <i>Punctelia</i>	<i>P. subimperspicua</i>
9	Mature ascospores ornamented.....	10
9*	Mature ascospores smooth.....	27
10	Perithecia < 200 µm wide	11
10*	Perithecia > 200 µm wide	16
11	Ascospores < 12 µm long.....	12
11*	Ascospores > 12 µm long.....	13

12	Ascospores 7–11 × 4–5 µm, perithecial wall 25–30 µm wide, on <i>Nephroma</i>	<i>P. invisibilis</i>
12*	Ascospores 8–11.5 × 2.5–3.5 µm, perithecial wall 10–15 µm wide, on <i>Sticta</i>	<i>P. sticticola</i>
13	Ascospores > 6 µm wide	14
13*	Ascospores 17–22 × 4–5.5 µm, on <i>Anaptychia</i>	<i>P. tincta</i>
14	Ascospores with short spines, 12–18 × 5.5–10 µm, on <i>Physcia</i>	<i>P. echinulata</i>
14*	Ascospores verrucose, on other hosts	15
15	Ascospores wall < 20 µm wide, ascospores 12.5–20 × 5–8 µm, on saxicolous <i>Caloplaca</i> spp.	<i>P. caloplacae</i>
15*	Ascospores wall > 20 µm wide, ascospores 9–21 × 4–9 µm, on terricolous <i>Lecidea</i>	<i>P. lecideicola</i>
16	Mature ascospores < 16 µm long	17
16*	Mature ascospores > 16 µm long	21
17	Ascospores < 6 µm wide	18
17*	Ascospores > 6 µm wide	19
18	Perithecial wall 20–30 µm wide, ascospores 8–16 × 3–5 µm, on <i>Cladonia</i>	<i>P. tibellii</i>
18*	Perithecial wall 17–20 µm width, ascospores 9–12 × 4.5–5 µm, on <i>Pertusaria</i>	<i>P. pertusariicola</i>
19	Ascospores with spines	20
19*	Ascospores with papillae, 9.5–15 × 6.5–9.5 µm, on <i>Protopannaria</i>	<i>P. protopannariae</i>
20	Lichenicolous on <i>Peltigera</i>	<i>P. robergei</i>
20*	Lichenicolous on <i>Anaptychia</i>	<i>P. santessonii</i>
21	Ascospores < 6 µm wide	22
21*	Ascospores > 6 µm wide	23
22	Perithecia 170–220 × 170–230 µm, ascospores 17–22 × 4–5.5 µm with cells of unequal size, on <i>Anaptychia</i>	<i>P. tincta</i>
22*	Perithecia 150–300(–400) × 140–250(–400) µm, ascospores 17–24 × 4–5 µm with cells of same size, on <i>Xanthoria</i>	<i>P. xanthoriae</i>
23	Ascospores 21–26 × 10–13 µm, on <i>Solorina</i>	<i>P. rolfiana</i>
23*	Ascospores < 10 µm wide	24
24	Perithecial wall 30–50 µm wide, ascospores 9–21 × 4–9 µm, on terricolous <i>Lecidea</i>	<i>P. lecideicola</i>
24*	Perithecial wall < 30 µm thick, on various hosts	25
25	Ascospores 17–30 × 5.5–8 µm, on <i>Peltigera</i>	<i>P. erythrella</i>
25*	Ascospores shorter	26
26	Perithecia 180–320 × 150–280 µm, perithecial wall 16–20 µm thick, ascospores 16–21 × 5–7 µm, on <i>Verrucaria</i>	<i>P. verrucariae</i>
26*	Perithecia 250–350 × 250–400 µm, perithecial wall 20–30 µm thick, ascospores 8–20 × 4–10 µm, on <i>Ochrolechia</i>	<i>P. walkerorum</i>
27	Perithecia > 200 µm wide	28
27*	Perithecia < 200 µm wide	30
28	Ascospores 22–28(–33) × 3.5–5 µm, ascospore length/width > 3, on <i>Peltigera</i>	<i>P. tenuispora</i>
28*	Ascospores < 20 µm length, ascospore length/width < 3	29
29	Perithecia orange, ascospores 10–16 × 5–6 µm, on <i>Collema</i>	<i>P. tenacis</i>
29*	Perithecia pale red to yellowish, ascospores 12–17 × 5.5–7 µm, on <i>Thrombium</i> ...	<i>P. terrestris</i>
30	Ostiole with tomentum or hairs.....	31
30*	Ostiole smooth	33

31	Hairs at ostiole 10–30 µm long.....	32
31*	Hairs at ostiole 4–10 µm long, forming white tomentum, ascospores 12–21.0 × 4.5–6.5, on <i>Gloeocystis</i> -like algae.....	<i>P. algicola</i>
32	Perithecia small, 50–80 µm wide, ascospores 10–13.5(–15) × 3–3.5 µm, ascospore length/width ratio 3.3–3.9, on <i>Collema</i>	<i>P. pilosa</i>
32*	Perithecia lager, 150–200 µm width, yellow to bright orange, ascospores 14–17 × 4–6.5 µm, ascospore length/width ratio 2.8–3, on <i>Hypogymnia</i>	<i>P. anisopora</i>
33	Ascospores < 12 µm long.....	34
33*	Ascospores > 12 µm long.....	37
34	Ascospores < 5 µm wide.....	35
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