P.R. Johnston, 26 March 2024

Helotiales on Elaeocarpus leaves

Elaeocarpus spp. are small trees widespread in New Zealand forests and several apparently host-specialised *Helotiales* species are common on their fallen leaves. Those that are typically associated with recently fallen leaves (e.g. "*Lanzia*" *ovispora*, *Poculum* sp. "hinau", and *Helotiaceae* sp. PJ-2024c) could have an endophytic stage to their life cycle, initially symptomlessly infecting the living leaves. This has been demonstrated experimentally for host-specialised *Helotiales* on native New Zealand hosts such as *Metrosideros*, but not yet for *Elaeocarpus*.

Here I discuss several species of glabrous, mostly pale-coloured *Helotiales* found on fallen *Elaeocarpus* leaves in New Zealand and Australia. Also common on *Elaeocarpus* leaves in both New Zealand and Australia is an unnamed *Hymenotorrendiella* sp., distinguished from the fungi treated here by the dark setae on its apothecia. Based on cultures grown from *Elaeocarpus* litter cited in Collado et al. (2007), there are several other *Leotiomycetes* species that inhabit *Elaeocarpus* leaves in New Zealand forests. These belong in taxa such as *Pezizellaceae*, *Dermateaceae*, basal *Helotiales*, *Arachnopeziza*, *Mollisiaceae* and *Cyathicula*, but none of these litter-inhabiting taxa have yet been linked to specimens with apothecia.

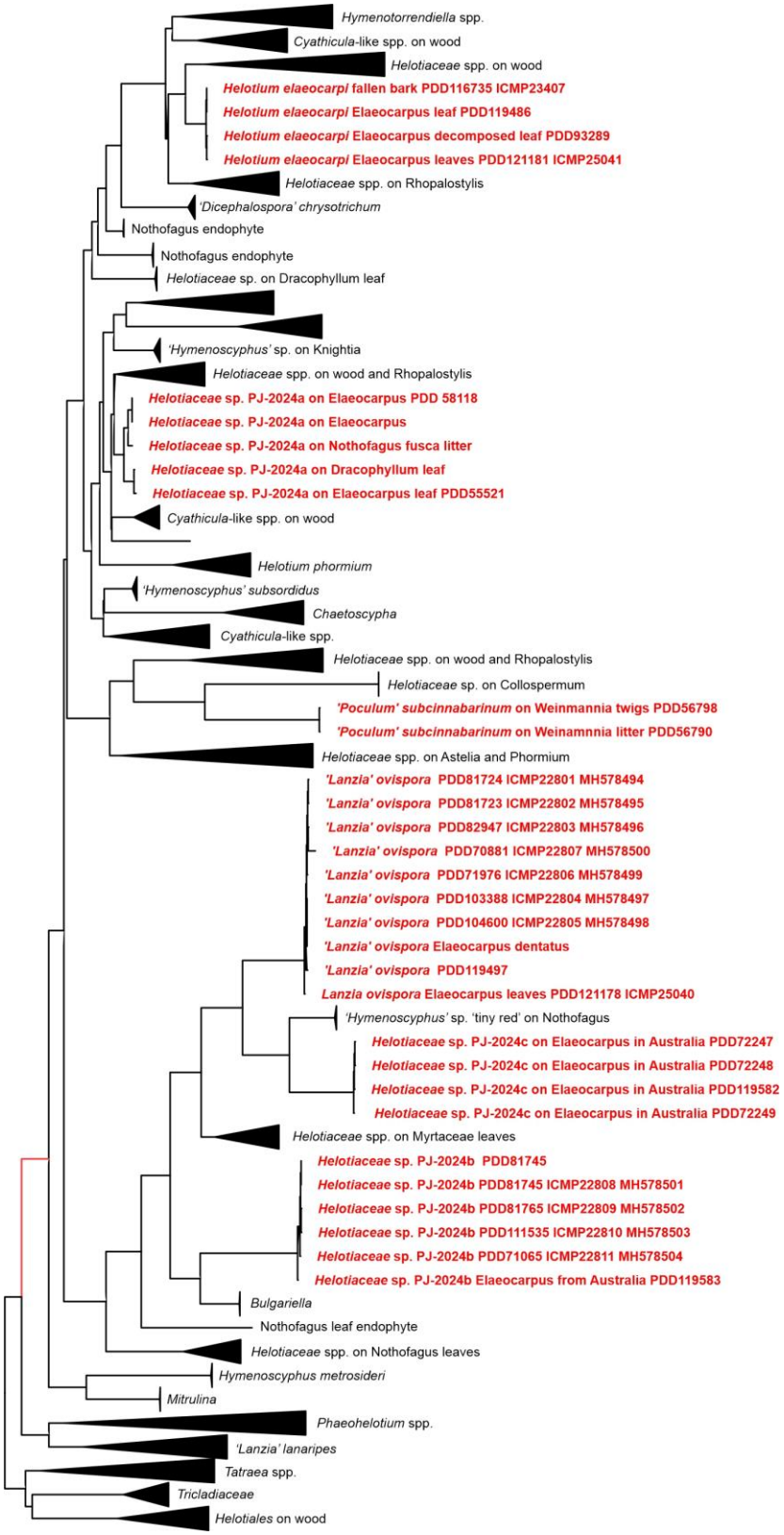
Three of the glabrous, pale-coloured species have names, "*Helotium*" *elaeocarpi*, "*Lanzia*" *ovispora* and "*Poculum*" *subcinnabarinum*. I have sequenced specimens that appear to match all three species and all are in *Helotiaceae* but without any clear generic position. Also in *Helotiaceae* are three additional unnamed species, one in New Zealand (genetically very similar fungi are also found on other hosts), one in Australia, and one in both New Zealand and Australia. Another morphologically distinctive, unnamed species (*Poculum* sp. "hinau") lacks DNA sequences but based on morphology it is likely to be either *Rutstroemiaceae* or *Helotiaceae*.

"*Lanzia*" *ovispora* and *Helotiaceae* sp. PJ-2024c form a sister relationship in an ITS phylogeny with an unnamed species commonly found on fallen *Nothofagus fusca* and *N. solandri* leaves in New Zealand, "*Hymenoscyphus*" sp. 'tiny red' (see https://biotanz.landcareresearch.co.nz/scientific-names/1f76cfa6-3e62-48eb-b3f3-fc1fd1a0d278). "*Hymenoscyphus*" sp. 'tiny red' shares with these other species broad, ovate ascospores are an outer network on the receptacle of encrusted hyphae with thin walls.

Morphologically most of these *Elaeocarpus*-inhabiting species have large, broad-elliptic to ovate ascospores mostly more than 6 µm wide. "*P*". *subcinnabarinum* and *Helotiaceae* sp. PJ-2024a differ in having ascospores less than 5 µm wide. Most species have ascospores 12–20 µm long, "*Poculum*" *ovispora* differs in having ascospores 11-12 µm long, and *Poculum* sp. "hinau" in having ascospores more than 20 µm long.

All of the species except "*Helotium*" *elaeocarpi* and "*P*". *subcinnabarinum* have an ectal excipulum comprising cylindric to short-cylindric cells with slightly thickened walls and an outer layer of thin-walled hyphae meandering across the receptacle surface, the end cells of these hyphal elements sometimes are swollen clavate or fusoid in some species, the walls sometimes encrusted with darkly pigmented material in some species, the cellular vacuoles sometimes packed with coloured material. "*H*". *elaeocarpi* and "*P*". *subcinnabarinum* lack

the outer layer, or it is very poorly developed, and the excipular cells are cylindric to long cylindric and with gelatinous walls or embedded in a gelatinous matrix.



ITS gene tree. The sequenced species from *Elaeocarpus* in red, other taxa represent *Helotiaceae* from New Zealand, many in unnamed genera. Metadata including PDD, ICMP and GenBank numbers in results below.

Key to the *Elaeocarpus*-inhabiting discomycetes treated here

1. Ascospores less than 5 µm wide 2
   Ascospores more than 5 µm wide.....3

2. Ascospores more than 12 µm long .....*Helotiaceae* sp. PJ-2024a
   Ascospores less than 12 µm long..... "*Poculum*" *subcinnabarinum*

3. Ascospores average less than 12 µm long, asci J- ..... "*Lanzia*" *ovispora*
   Ascospores average more than 12 µm long, asci J+ 4

4. Ascospores average more than 20 µm long .....*Poculum* sp. "hinau"
   Ascospores average less than 20 µm long 5

5. Excipular cells more or less square to short cylindric .....*Helotiaceae* sp. PJ-2024b
   Excipular cells brick-shaped to cylindric 6

6. Known only from New Zealand. Apothecia sessile to substipitate, excipulum of long-cylindric cells with thickened walls, lacking an outer layer on receptacle surface ....*Helotium elaeocarpi*
   Known only from Australia. Apothecia with well-developed stipe, excipulum of broad-cylindric to brick-shaped cells, outer surface of receptacle with layer of meandering hyphae with encrusted walls .....*Helotiaceae* sp. PJ-2024c

***Helotium elaeocarpi* Dennis**

On often partly decomposed leaves. Small, sessile to substipitate yellow to pale yellow apothecia. Excipular cells cylindric to long-cylindric in more or less parallel rows, surface of receptacle lacking an outer hyphal layer.

Description: Apothecia on fallen leaves, sessile to subsessile, concolorous pale yellow, glabrous. Ectal excipulum with cylindric to long-cylindric cells in more or less parallel rows, with gel matrix between the rows; surface of receptacle lacking a distinct layer of thin-walled hyphae, although the ends of ectal elements are sometimes somewhat free. Paraphyses 2–2.5 µm diam., undifferentiated at apex. Asci 130–175 × 10–12 µm, with faint J+ reaction. Ascospores (12.5-)16-18.5 x (6.5-)7-8.5 µm, broad-elliptic to ovate, usually somewhat flat on one side in side view, not curved, ends broadly rounded.

Notes: Morphologically specimens accepted as this species match the isotype specimen of *Helotium elaeocarpi* (PDD 19047) and the description of Dennis (1961). PDD 116735 is on fallen bark rather than *Elaocarpus* leaves, but both morphologically and phylogenetically it matches *H. elaeocarpi*.

Sequenced specimens: PDD 93289 (PRJ D2093, GenBank PP692168), PDD 119486 (TTT1135, GenBank PP692167), PDD 121181 (PRJ D2662, ICMP 25041, GenBank PP692165), PDD 116735 (PRJ D2560, ICMP 23407, GenBank PP692166), PDD 93289 (PRJ D2093, GenBank PP692168)
Other specimens examined: PDD 19047 (isotype), PDD 56330 (D441), PDD 50182, PDD 55511 (D356).

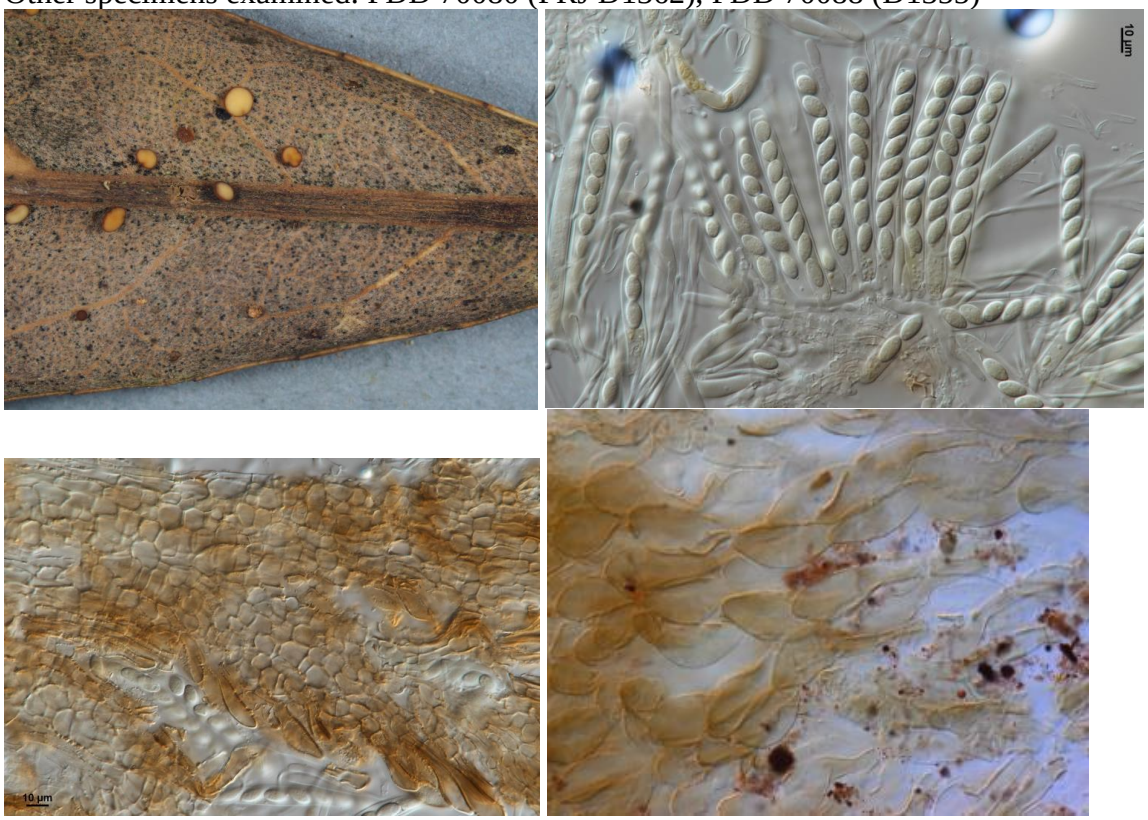
Fresh apothecia (up to 1.5 mm diam.); asci, ascospores and paraphyses; surface of receptacle; ectal excipulum (PDD 116735)

"*Lanzia*" *ovispora* Spooner

Common on recently fallen leaves, with apothecia short-stipitate, reddish to orange-brown, hymenium deep yellow when fresh, drying reddish. Receptacle with a well developed network of surface hyphae with encrusted walls and end cells swollen-clavate. Asci J-.

Description: Apothecia about 1.5 mm diam., short-stipitate, receptacle orange-brown with fine radiating network of darker cells, slightly furfuraceous when dry, hymenium paler, orange-yellow. Excipulum of more or less square or globose cells with thick, hyaline (agglutinated) walls; outer layer of meandering hyphae with walls encrusted with orange-brown pigment, end cells broad-fusoid. In vertical section excipular cells at high angle to surface of receptacle. Paraphyses swollen clavate to fusoid at apex. Asci 85–110 × 8–11.5 µm, cylindric, wall thickened at apex, J-. Ascospores 11–12 × 6–7.5 µm, ovate, symmetrical along long axis.

Sequenced specimens: PDD 70881 (PRJ D1425, ICMP 22807, GenBank MH578500, MH587172, MH578567), PDD 71976 (PRJ D1284, ICMP 22806, GenBank MH578499), PDD 81724 (PRJ D1870, ICMP 22801, GenBank MH578494), PDD 81723 (PRJ D1866, ICMP 22802, GenBank MH578495), PDD 119497 (TTT1138, GenBank PP692182), PDD 82947 (PRJ D1964, ICMP 22803, GenBank MH578496), PDD 103388 (PRJ D2348, ICMP 22804, GenBank MH578497), PDD 104600 (PRJ D2354, ICMP 22805, GenBank MH578498), PDD 121178 (PRJ D2661, ICMP 25040, GenBank PP692183).
Other specimens examined: PDD 70080 (PRJ D1362), PDD 70088 (D1355)



Fresh apothecia (about 1.5 mm diam.); asci, ascospores and paraphyses; ectal excipulum (PDD 121178). Detail of surface of receptacle (PDD 103388).

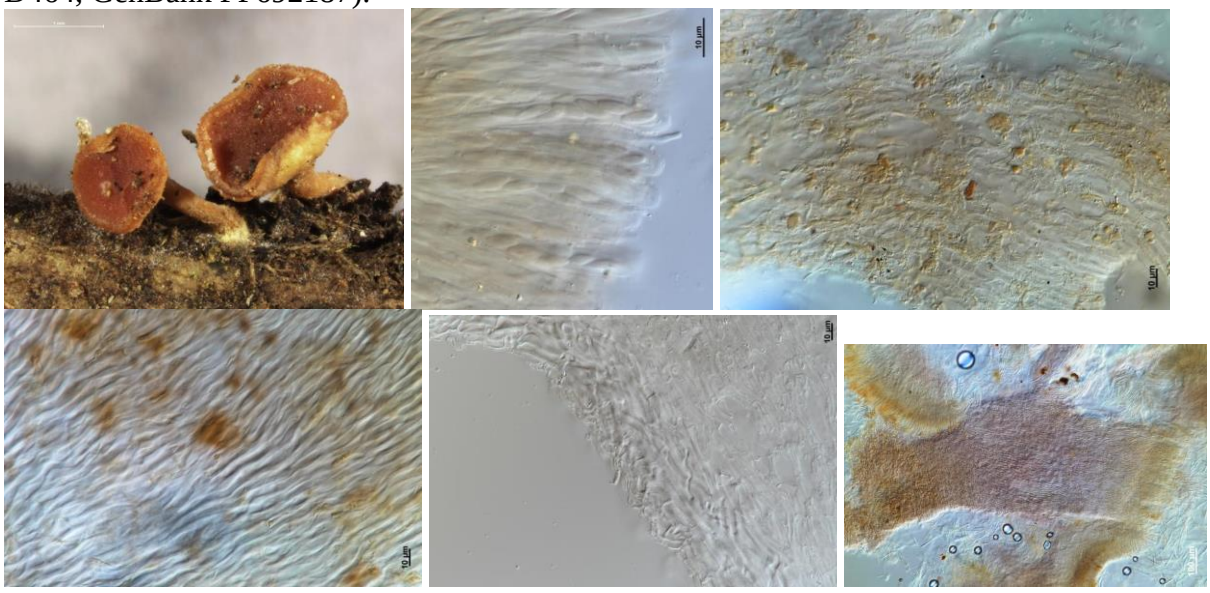
“*Poculum*” *subcinnabarinum* (Dennis) Dumont

On partly decomposed leaves and litter of several hosts. Robust, stipitate apothecia, glabrous with strong orange-brown pigments, hymenium often reddish. Excipular cells long-cylindric, partly tangled, embedded in gel.

Description: Apothecia stipitate, when fresh receptacle and stipe orange-brown in colour, base of stipe with tuft of whitish mycelium, hymenium pale creamy-brown, when dry receptacle orange-brown, hymenium reddish. Excipulum of long-cylindric cells somewhat tangled or in more or less parallel rows, embedded in gel; surface of receptacle with patchy layer of thin-walled hyphae with dense yellowish cell contents. Paraphyses 1.5–2 µm diam., undifferentiated at apex. Asci 80–95 × 6.5–8 µm, cylindric, taper slightly to broad subtruncate apex, wall thick with faint J+ pore. Ascospores 8–12 × 3–3.5 µm, oblong-elliptic, slightly wider to apex, flat one side, slightly curved, 0–septate.

Notes: When Dennis (1961) named *Helotium subcinnabarinum* he described the ectal excipulum as composed of interwoven hyphae with gelatinized walls and extending at a low angle to the surface of the apothecium, a structure he noted as somewhat intermediate between *Helotium* and *Phialea*. Dumont (1975) also described a gelatinous excipulum, with hyphae partly tangled that he regarded as *Calycella*-like. Although the type specimen of “*P*”. *subcinnabarinum* is on *Elaeocarpus* (on partly decomposed leaves and twigs), other specimens identified as this species by K.P. Dumont and with a very similar morphology, are on *Weinmannia* litter and *Nothofagus* bark. The sequenced species identified here as “*P*”. *subcinnabarinum* are on *Weinmannia*, collected from the same locality as the *Weinmannia*-inhabiting specimens placed in “*P*”. *subcinnabarinum* by Dumont (annotation slips in packets PDD 19402 and PDD 19403).

Sequenced specimens: PDD 56798 (PRJ D461, GenBank PP692188), PDD 56790 (PRJ D464, GenBank PP692187).



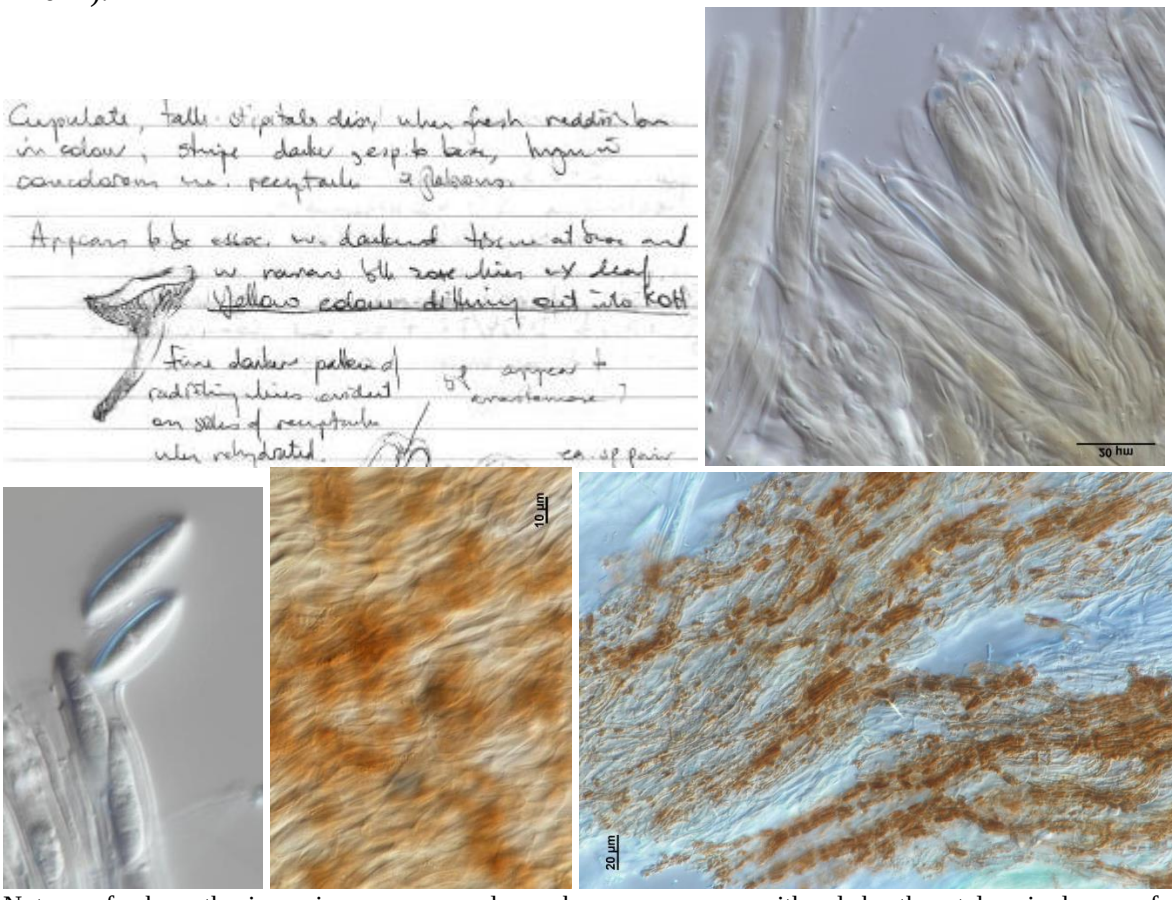
Dry apothecia; asci, ascospores and paraphyses; surface of receptacle; ectal excipulum; excipulum in vertical section; squash showing red reaction of excipulum in Melzers. (PDD 56798).

***Poculum* sp. "hinau"**

Apothecia developing on recently fallen leaves. Stipe well-developed, spores greater than 20 µm long and with a gelatinous sheath.

Apothecia stipitate, hymenium mustard yellow, receptacle similar but patterned with a loose network of darker cells. Excipular cells cylindric with walls slightly thickened, surface of receptacle with a well developed outer layer of thin-walled, meandering hyphae with pale brown walls, sometimes encrusted, undifferentiated at apex. Paraphyses 1.5–2 µm diam., swollen to 3–5 µm at the clavate apex. Asci 110–125 × 11–12 µm, with a well-developed J+ pore extending through the thickened apex. Ascospores 21–25 × 4–6 µm, elliptic-fusoid, flat one side, not curved, tapering to more or less acute ends, surrounded by a gelatinous sheath.

Specimens examined (no DNA sequences): PDD 55507 (PRJ D357), PDD 93710 (PRJ D1014).



Notes on fresh apothecia; asci, ascospores and paraphyses; ascospores with gel sheath; ectal excipulum; surface of receptacle (PDD 55507).

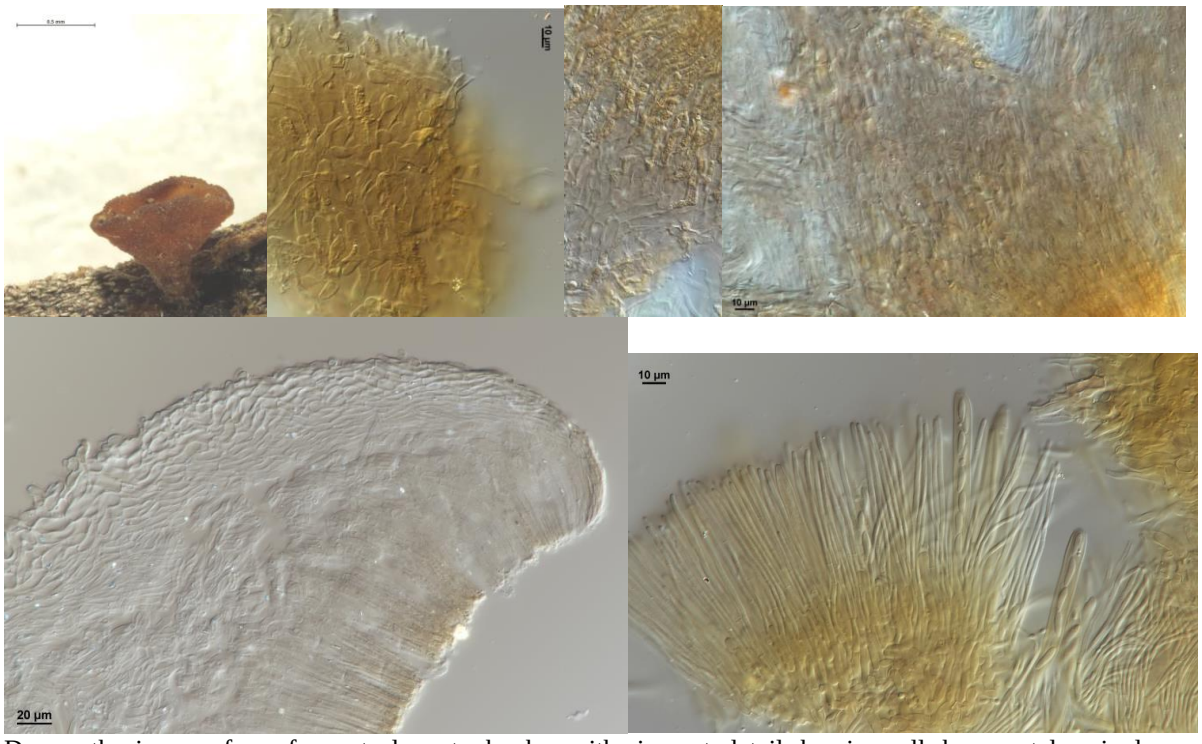
***Helotiaceae* sp. PJ-2024a**

Apothecia on partly decomposed leaves, white when fresh, sometimes staining rusty-brown with damage, orange-yellow when dry. Surface of receptacle with a layer of thin-walled hyphae with swollen clavate end cells, walls not encrusted. Usually on partly decomposed leaves.

Description: Apothecia short-stipitate, whitish when fresh (sometimes staining rusty-brown with damage), drying orange-yellow. Excipulum with brick-shaped to short cylindric cells, walls hyaline, slightly thickened; surface of receptacle with layer of hyaline, thin-walled hyphae, end cells of these hyphae often swollen, clavate. Paraphyses 1.5 µm diam., undifferentiated. Asci 85–100 × 7.5–10 µm, croziers at base, J+. Ascospores 14-17(-20) × 3-4 µm, flat one side, sometimes slightly curved, widest point towards one end, tapering to narrow rounded ends.

Notes: A species complex with specimens on partly decomposed, fallen leaves of *Elaeocarpus, Nothofagus* and *Dracophyllum* that are phylogenetically and morphologically similar. Pairwise similarities across the four sequenced specimens are between 97% and 99.4%, the most similar being one of the *Elaeocarpus* specimens and the specimen on *Dracophyllum.*

Sequenced specimens: PDD 58118 (PRJ D586, GenBank PP692178, on *Elaeocarpus*), PDD 55521 (PRJ D359, GenBank PP692179, on *Elaeocarpus*), PDD 122822 (PRJ cam128, GenBank PP692180, on *Dracophyllum*), PDD 82944 (PRJ D1954, GenBank PP692181, on *Nothofagus*)
Another possible specimen of this species with no DNA sequences is PDD 57545 (on *Nothofagus* leaves).



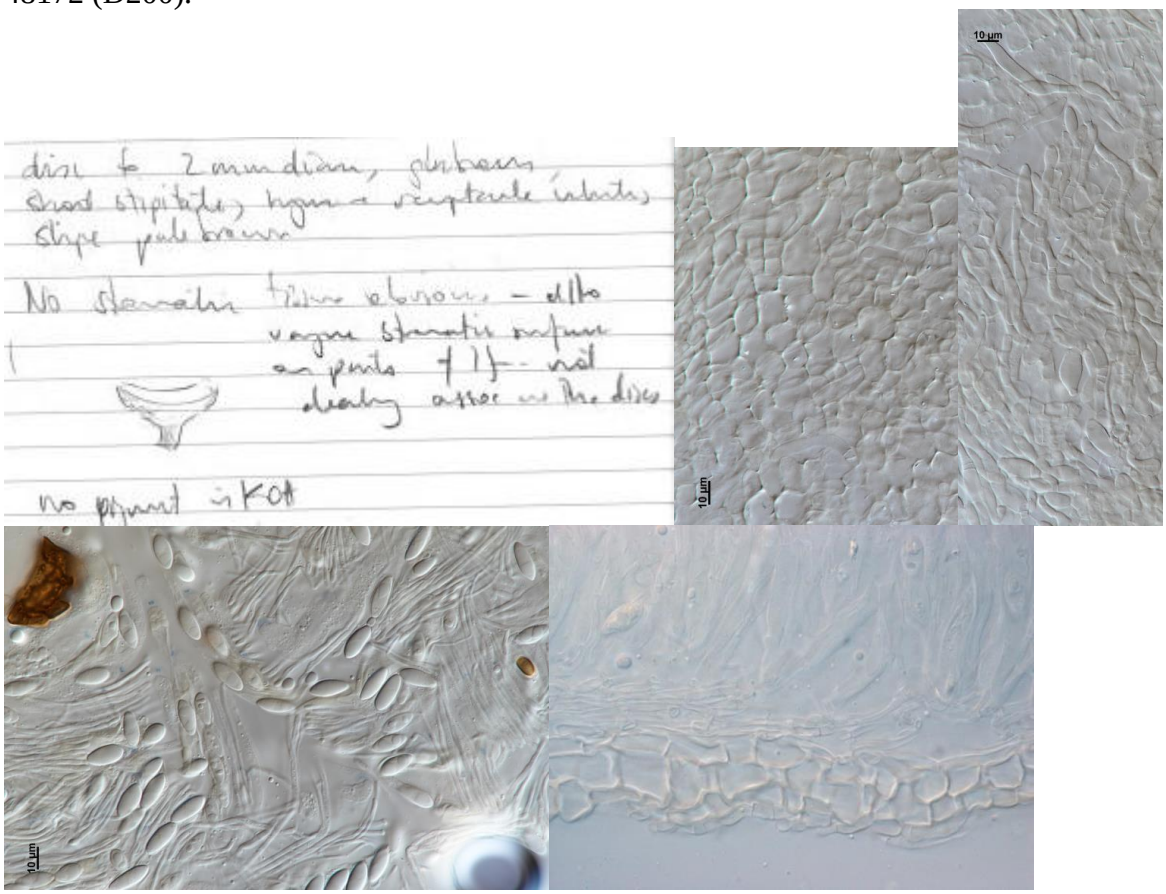
Dry apothecium; surface of receptacle, outer hyphae with pigment, detail showing cell shape; ectal excipulum; excipulum in vertical section; asci, ascospores and paraphyses (PDD122822).

***Helotiaceae* sp. PJ-2024b**

Apothecia on partly decomposed leaves, short-stipitate, white when fresh, drying pale yellowish. Excipular cells more or less square. Surface of receptacle with surface hyphae thin-walled, not encrusted, end cells more or less fusoid. Known from both New Zealand and Australia.

Description: Apothecia short-stipitate, more or less white when fresh, pale orange-yellow when dry. Excipulum of short-cylindric, square or globose cells with thick, hyaline (agglutinated) walls; outer layer poorly developed, of meandering hyphae with walls thin, not encrusted, end cells long and narrow-fusoid. Paraphyses 1.5–2 µm diam., undifferentiated to apex.
Asci 95–125 × 8–10 µm, cylindric, amyloid pore at thickened apex. Ascospores 14–20(–22) × 6–8 µm ovate, more or less symmetrical along the long axis, or one side slightly flattened in side view, tapering to subacute ends.

Sequenced specimens: PDD 81765 (PRJ D1498, ICMP 22809, GenBank MH578502), PDD 71065 (PRJ D1424, ICMP 22811, GenBank MH578504), PDD 81745 (PRJ D1898, ICMP 22808, GenBank MH578568), PDD 111535 (TTT1139, ICMP 22810, GenBank MH578503), PDD 119583 (PRJ AU09-83, ICMP 25239, GenBank PP701707, from Australia).
Other specimens examined: PDD 45316 (D85), PDD 81138 (TTT243), PDD 74280, PDD 81766 (D1894), PDD 81787 (D1899), PDD 98227 (D1271-2), PDD 55555 (D403), PDD 48172 (D200).



Sketch of apothecium (PDD 81765); ectal excipulum with square to subglobose cells; surface of receptacle, surface hyphae end cells fusoid (PDD 74280); asci, ascospores and paraphyses; excipulum in vertical section (PDD 98227).

***Helotiaceae* sp. PJ-2024c ex Australia**

Found on recently fallen leaves of several *Elaeocarpus* spp. Apothecia up to 5 mm diam., colour similar to *L. ovispora*, with orange-coloured discs, but with a more well-developed stipe. When dry the surface of the receptacle with a more or less leathery rather than the slightly furfuraceous appearance of *L. ovispora*. Excipular cells are brick-shaped, cylindric, longer than *L. ovispora*, wall slightly thickened, outer layer of narrow-cylindric hyphae encrusted with clumps of pigment. Asci J+, spores symmetrical, ovate, about 15-18 x 7.5-8 µm.

Sequenced specimens: PDD 72248 (PRJ AU96-123, ICMP 25306, GenBank PP701705), PDD 72247 (PRJ AU96-118, ICMP 25302, GenBank PP701704). PDD 72249 (PRJ AU96-218, ICMP 25324, GenBank PP701706), PDD 119582 (PRJ AU09-45, ICMP 25230, GenBank PP701703).

Fresh apothecia; excipulum in vertical section (PDD 72247).

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