The trouble with *Neophyllis pachyphylla* (lichenised Ascomycetes)

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Abstract: The genus *Neophyllis* F.Wilson in Australia is considered to comprise two morphologically and chemically distinct species, *N. pachyphylla* (Müll.Arg.) Gotth.Schneid. and *N. melacarpa* (F.Wilson) F.Wilson. The confusion surrounding the application of the former name is clarified and revised descriptions of both are presented.

Keywords: Australia, grayanic acid, lichens, melacarpic acid, New Zealand, Tasmania

Introduction

The genus Neophyllis F.Wilson (Sphaerophoraceae) is a distinctly Australasian taxon with two described species, N. melacarpa (F.Wilson) F.Wilson and N. pachyphylla (Müll.Arg.) Gotth.Schneid. The former is generally well-known to lichenologists, being widespread on the south-eastern Australian mainland, in Tasmania and in New Zealand. It is commonly seen on rotting wood where it forms extensive colonies of greenish or olive-coloured, often digitate squamules bearing black, globose apothecia. Neophyllis pachyphylla, on the other hand, is rarely seen, collected or mentioned in inventories. In his Flora of Australia account, Filson (1992) cited collections from Mt William (Victoria) and Pigeon House Mountain (N.S.W.) and little data have been added since that time. This paper addresses some of the complexities surrounding the application of this name and provides revised descriptions of both species.

Material and methods

The study is based on the extensive collections of *Neophyllis*, compiled mostly by the author and housed in the Tasmanian Herbarium (HO); these are chiefly from Tasmania and the Australian mainland, with a small number from New Zealand. Examination of thallus and apothecial anatomy was undertaken on hand-cut sections mounted in water, 10% KOH (K), 50% HNO₃ (N), and in Lugols Iodine after pre-treatment in K and rinsing with water (KI). Measurements of ascospores are based on at least 50 observations per taxon and are presented in the format 5th percentile–*average*–95th percentile, with outlying values in brackets. Routine chemical analysis was undertaken using standard methods of thin-layer chromatography (TLC), with solvent A as the preferred medium (Orange *et al.* 2010).

History

Neophyllis melacarpa was described by Wilson (1889), originally in the genus Phyllis. As this is a genus of the Angiosperm family Rubiaceae, he soon redescribed it within a new genus, Neophyllis (Wilson 1891). At the time of description, Wilson perceived affinities to the genus Cladonia, and the species remained classified in Cladoniaceae, even as it was transferred to Phyllopsora (Müller 1895), Gymnoderma (Yoshimura 1973) and eventually back to Neophyllis (Schneider 1979); see also Jahns (1970) and Wei & Ahti (2002). It was not until Döring et al. (1999) and Döring & Wedin (2000) investigated the ontogeny of the genus that it, together with another Australasian endemic genus, Austropeltum Henssen, Döring & Kantvilas, was transferred to Sphaerophoraceae. This classification was also confirmed by molecular methods (Wedin & Döring 1999).

The other species of the genus, N. pachyphylla, was originally described by Müller (1887) in the genus Psora, and transferred to Neophyllis by Schneider (1979). Schneider characterised N. melacarpa by its forked, digitate squamules with rounded apices and a corticolous habitat, and N. pachyphylla was distinguished from it by having sparsely branched, crowded squamules with flattened apices and a terricolous habitat. He also mentioned the medulla of the latter being brown, but this is almost certainly due to the single specimen that he saw (the type) being in very poor condition. Müller (1895) had also compared the two species, claiming, *inter alia*, that the ascospores of N. melacarpa were narrower. Subsequently Filson (1992) again distinguished the species in a similar way, but added the additional and critical observation of a chemical difference, namely that N. pachyphylla contained melacarpic acid only, whereas N. melacarpa contained grayanic acid plus fumarprotocetraric and melacarpic acids.

Present observations

Generic features

Critical features of the genus *Neophyllis* include: the squamulose to minutely coralloid-fruticose thallus with black, globose, immarginate apothecia (Fig. 1); the green photobiont with globose cells 7–11 μ m diam.; the particular, 8-spored asci ("*Neophyllis*-type") with an amyloid tholus penetrated by a darker-staining tube-structure and lacking an ocular chamber; the relatively robust, parallel paraphyses with few branches and anastomoses; and the simple, ellipsoid, non-halonate, hyaline ascospores (Fig. 2). The conidiomata are reported by Döring *et al.* (1999) as being laminal, subglobose pycnidia with bacilliform conidia, but none were observed in the present study.

Chemistry

Thallus chemistry in Neophyllis is dominated by the dibenzofuranes, grayanic and/or melacarpic acids, and is a critical taxonomic character. In the course of the present study, scores of specimens were examined by TLC, confirming that grayanic acid is always present in *N. melacarpa*. This substance appears about half-way up TLC plates as a UV+ pale blue spot before heating, and as a pale pinkish brown, UV+ purple spot after acid spray and heating. Additional melacarpic acid is commonly present as a major or minor compound, and fumarprotocetraric acid (a slow grey spot) may also be present in minor concentrations. In contrast, N. pachyphylla always contains melacarpic acid as the sole major compound. This substance appears on TLC plates as a UV+ pale blue spot before heating, and as a pale blue-grey, UV+ purple spot after heating; it is faster than grayanic acid in all standard solvents.

Morphology

Neophyllis melacarpa is a highly variable species. On wood, it forms loose aggregations of apically divided squamules, with the ultimate segments being terete and coralloid (Fig. 1A). With increasing exposure, the squamules become ever more densely packed together, more erect and form swards and cushions (Fig. 1B). In extreme cases, the thallus consists entirely of \pm erect, terete lobes (Fig. 1C). Whilst the two extreme forms look very different from each other, a continuum exists between the ascending squamulose and erect teretiform morphologies.

The morphological criteria by which Schneider (1979) and Filson (1992) delimited *N. pachyphylla* fall within the range of variation displayed by *N. melacarpa*. The two species also overlap ecologically, in that *N. melacarpa* can grow on soil as well as wood. Moreover, at every locality where *N. pachyphylla* has been observed during the present study, *N. melacarpa* is also present. However, at these locations, *N. melacarpa* is usually common, whereas *N. pachyphylla* is typically rare. Chemically, however, *N. pachyphylla* is distinct in never containing grayanic acid (which is always present in *N. melacarpa*) and always containing melacarpic acid (which is only sometimes present in *N. melacarpa*). Thus, to distinguish the two species ecologically is untenable, but to do so chemically is unequivocal, although this requires TLC.

In the course of the present work, it was found that the two species can be readily distinguished morphologically. The squamules of *Neophyllis pachyphylla* tend to be more dispersed (Fig. 1D) and, whereas terete segments are often developed, they do not dominate the thallus (Figs 1E–F). When forming extensive thalli, *N. pachyphylla* is particularly distinctive, with the squamules being rather tongue-like and having slightly thickened, rounded apices (Fig. 3). This observation was tested in the field when the author collected and chemically analysed multiple specimens of both taxa and was always able to identify them correctly on morphology alone.

The status of N. pachyphylla

An argument has been made in the past for considering N. pachyphylla as simply a chemical variant of N. melacarpa. This was suggested in the literature (Döring et al. 1999; Wei & Ahti 2002), and the type specimen of N. pachyphylla (in G) was annotated to that effect by the American lichenologist Paula de Priest in 1999. However, the nomenclatural situation is complicated by N. pachyphylla being the older name, as also noted by Döring et al. (1999) and Wei & Ahti (2002). Synonymisation would necessitate the adoption of a rarely used name of what had been regarded as an extremely rare taxon, for a species with a currently widely used name that is applied to an extremely common lichen. Alternatively, a case for conservation of N. melacarpa would need to be made. The conclusion of the present study, however, is that in addition to their chemical difference, the two taxa also differ morphologically, and should be maintained as separate.

Key to the species of Neophyllis

 Grayanic acid present, frequently with additional melacarpic and/or fumarprotocetraric acids; thallus forming dense swards or cushions, composed of apically divided squamules with the ultimate segments terete and coralloid, or with the thallus consisting entirely of terete lobes; common on wood, but also found on moist peaty or sandy soil. 	. melacarpa
1: Melacarpic acid present as the sole major compound; thallus composed of usually rather dispersed, ascending squamules with slightly thickened, rounded apices; terete projections often present but not dominating the thallus; rare on coarse, sandy or gravelly soil over granite, or directly on rock; not observed on wood	pachyphylla

Taxonomy

Neophyllis melacarpa (F.Wilson) F.Wilson

J. Linn. Soc., Bot. 28: 372 (1891). — Phyllis melacarpa F.Wilson, Victorian Naturalist 6: 68 (1891). — Gymnoderma melacarpum (F.Wilson) Yoshim., J. Jap. Bot. 48: 287 (1973). — **Type citation:** "Habitat supra truncos arborum permagnorum viventes atque putridos in regionibus montanis, Warburton et Mt Macedon." — **Lectotype:** Victoria: Black Spur, on dead bark of large eucalyptus, Feb, 1888, F.R.M. Wilson s.n. (NSW 423991!), fide Filson, Austral. Fl. Fauna Ser. 4: 231 (1986), as "Holotype". **Isolectotype:** G00292739 (ex Herb. Müll.Arg.). **Residual syntypes:** G00293378, G00292381 (see below).

Psora dactylophylla Müll.Arg., Bull. Herb. Boissier 1: 35 (1893). — Lecidea dactylophylla (Müll. Arg.) Zahlbr., Cat. Lich. Univ. 3: 867 (1925). — Phyllopsora melanocarpa Müll.Arg., Hedwigia 34: 28 (1895), nom. superfl. & illeg. (based on the same type). — **Type citation:** "Ad ligna emortua, Mt Macdon : Wilson, n. 150." — Lectotype (here designated): On decaying log, Mt Macedon, Victoria, F.R.M. Wilson 150 (G00292381, ex Herb. Müll.Arg., annotated "L.[ichenes] Wils.[onianae] n. 15."). Isolectotype: G00293378 (ex Herb. Müll.Arg., annotated "L.[ichenes] Exot.[ici] n. 109."). Mycobank typification number: MBT10005953.

Thallus squamulose to diminutively coralloid-fruticose, forming extensively spreading colonies or contiguous tufts, swards or cushions 5-40 mm wide. Squamules bright green to olive when fresh and moist, drying to a yellowish green to olive brownish, glossy, commonly dorsiventrally flattened at the base, with a pale lower surface, ascending or decumbent, to 15 mm long, 0.3-0.6 (-1) mm wide, pinnately or digitately branched, with the ultimate segments terete, very fragile and brittle, to c. 0.15 mm wide, sometimes segmented by slight constrictions, or with the terete segments arising directly from the upper surface of the squamules, less frequently with all parts entirely coralloid-terete, erect, 5-10 mm tall, to 0.3 (-0.5) mm wide, sparsely branched and entangled in mats or cushions. Apothecia to 1 (-1.4) mm wide, subsessile and nestling amongst the squamules or elevated above the thallus on terete stalks to c. 3 mm tall. Proper exciple in section hyaline within, at the outer edge dark brown, K± unchanged, N+ reddish, soon reflexed and ± excluded, composed of radiating, anastomosing hyphae in a gel matrix. Hypothecium massive, diffusely brown to hyaline. Hymenium hyaline, not inspersed, 50-70 µm thick, overlain by a dark chocolate-brown epithecium c. 10 µm thick, ± unchanged in K, N+ red. Asci 40-50 \times 10–15 $\mu m.$ Paraphyses 2–3 μm thick, with the apices sometimes capitate and brown, 3 (-5) μ m wide. Ascospores (8–) 9-10.9-12.5 (–13) × 4-4.8-6 µm. Conidiomata not seen. Chemistry: grayanic acid, melacarpic acid (±), fumarprotocetraric acid (±), plus biosynthetically related compounds such as congravanic acid or 4-O-demethylgrayanic acid in trace amounts

only; medulla K-, KC-, C-, P- or + yellowish to orange-red, UV+ white. **Figs 1A-C.**

Typification. Type material is present in both the Conservatoire et Jardin botaniques de la Ville de Genève (G), and the National Herbarium of New South Wales (NSW). Filson (1986) referred to the latter specimen as the holotype, although in doing so he inadvertently selected it as the lectotype, here corrected under Art. 9.10. A putative isolectotype cited by Filson (1992) as being in BM was not located.

Remarks. Neophyllis melacarpa is common and widespread in south-eastern Australia, Tasmania and New Zealand. Although it occurs mainly on wood, it can also colonise peaty or sandy soil. In Tasmania, it occurs in a wide range of rainforest, sclerophyll forest, woodland and heathland vegetation communities.

In shaded forest situations, this species is mostly found on wood, especially rotting logs, buttresses and stumps of old eucalypts. It also colonises mature trunks of other trees which produce a similarly soft, moistureretaining substratum, such as Nothofagus and Athrotaxis. In these situations, the thallus is usually bright green (when fresh), composed of ascending or decumbent, dorsiventral, ± pinnately branched squamules, and forms extensive, spreading swards, potentially covering up to several square metres, often intermixed with Cladia aggregata (Sw.) Nyl. and Cladonia rigida (Hook.f. & Taylor) Hampe. The coralloid extensions of the squamules may be scattered or very abundant to the extent of dominating the entire thallus, even within the same colony. In more exposed, sunny situations, the species is found mainly on soil. Here the thallus tends to comprise a dense cushion of erect, terete, coralloid lobes. This form is particularly common in high rainfall areas of the west and south-west of Tasmania, where it is found on soil in crevices of quartzitic boulders, or on the ground in buttongrass (Gymnoschoenus sphaerocephalus)-dominated moorland or in alpine or subalpine heathland. Such caespitose, terricolous forms are also seen in lower rainfall areas on coastal granite pinnacles in eastern Tasmania, on Triassic sandstone in south-eastern Tasmania, and on the coarse sandstones of the New South Wales Tablelands.

Selected specimens examined

AUSTRALIA. NEW SOUTH WALES. Braidwood district, S of Rossi, 19.xii.1967, *W.A. Weber & D. McVean (Lich. Exsicc. Colo. 246*) (HO); track to Wentworth Falls, 34°44'S 150°22'E, 820 m, 2.x.1999, *R.G. Coveney 18075 & M. Sherring* (HO, NSW); Pigeon House Mountain, 35°21'S 150°16'E, 700 m, 21.x.1999, *G. Kantvilas 348/99* (HO).

AUSTRALIAN CAPITAL TERRITORY. Tidbinbilla N.R., tributary of Hurdle Ck, 35°25'S 148°54'E, 1000 m, 26.i.1995, *N. Williams 7* (CANB, HO).

VICTORIA. Bonang Hwy near Tigaringa Track, 37°05'S 148°46'E, 890 m, 26.ix.1985, *J.A. Curnow 383* (CANB, HO); Bulga N.P., Lyrebird-Ash Tracks, 38°26'S 146°34'E, 500 m, 14.iv.1993, *J.A. Elix 29763* (CANB, HO).



Fig. 1. Morphological variation in *Neophyllis*. **A–C** *N. melacarpa*: **A** Growing on rotting wood: squamulose thallus developing coralloid, finger-like projections; **B** thallus dominated by coralloid projections (different section of same colony); **C** on soil: robust thallus consisting entirely of erect, terete, coralloid lobes. **D–F** *N. pachyphylla*: **D** Growing on soil: thallus of dispersed squamules with \pm rounded, intact apices; **E** on soil: thallus dominated by squamules with \pm rounded apices and occasional coralloid-terete projections; **F** thallus of ascending squamules. Scale = 2 mm. — A, B *G. Kantvilas 336/21*, C *GK 337/21*, D *GK 189/05*, E, F *GK 41/95*. Photos: J. Jarman.

TASMANIA. Flinders Island, Mt Strzelecki, 40°12'S 148°05'E, 710 m, 21.i.2006, G. Kantvilas 41/06 (HO); Guy Fawkes Rivulet, 42°54'S 147°17'E, x.1912, L. Rodway s.n. (HO); Mt Wellington, 42°53'S 147°15'E, iii.1963, P.W. James s.n. (BM, HO); St Columba Falls, 41°20'S 147°55'E, 300 m, 10.x.1968, G.C. Bratt 68/1307 (HO); Five Road, Florentine Valley, 42°43'S 146°26'E, 450 m, 1.v.1981, G. Kantvilas 259/81 (BG, BM, HO); Grasstree Hill, 42°47'S 147°21'E, 400 m, 14.viii.1981, G. Kantvilas 710/81 & P. James (BM, HO); Strathgordon Road, near Boyd River, 400 m, 29.ii.1984, G. Kantvilas 493/84 (A. Vézda: Lich. Sel. Exsicc. 2020) (HO); Perambulator Ridge, 42°31'S 146°11'E, 7.ii.1985, G. Kantvilas 50/85 (HO); Yarlington Tier, 42°32'S 147°18'E, 620 m, 30.xi.1988, G. Kantvilas 588/88 (HO); Denison Road, 43°00'S 146°50'E, 60 m, 24.vii.2002, G. Kantvilas 388/02 (HO); Alum Cliffs, 41°32'S 146°26'E, 350 m, 13.viii.2005, G. Kantvilas 234/05 (HO); Windsong Property, Callitris Gully, 42°21'S 147°55'E, 40 m, 26.x.2017, G. Kantvilas 354/17 (HO); Hungry Flats Road, 42°32'S 147°27'E, 230 m, 17.viii.2021, G. Kantvilas 336/21 (H, HO, NY, UPS); Hungry Flats, 540 m, 17.viii.2021, G. Kantvilas 337/21 (HO).

NEW ZEALAND. SOUTH ISLAND. Arthurs Pass, 42°55'S 171°30'E, 11.xi.1972, *G.C. Bratt 72/1814* (HO); Cobb Valley, track to Mt Mytton, 41°07'S 172°35'E, 1060 m, 21.ii.1989, *A.J. Fife 9184* (CHR, HO); Westport, Denniston Plateau, Mt Rochfort, 750 m, 21.iv.1997, *W. Malcolm & A. Vézda (A. Vézda: Lich. Rariores Exsicc.* 380) (HO).

Neophyllis pachyphylla (Müll.Arg.) Gotth.Schneid.

Biblioth. Lichenol. 13: 168 (1980) [1979]. — Psora pachyphylla Müll.Arg., Flora 70: 319 (1887). — Lecidea pachyphylla (Müll.Arg.) Zahlbr., Cat. Lich. Univers. 3: 888 (1925). — Holotype: Australia, Victoria, Mt William, 5000' [1500 m], Oct. 1882, Dr Sullivan 86 (G00292738!, ex Herb. Müll.Arg., annotated "L.[ichenologische] B.[eiträge] n. 1155").

Thallus small-squamulose, forming spreading colonies. Squamules olive-green to brownish, glossy, 1-4 mm long, 0.2–0.6 mm wide, dorsiventrally flattened, with a pale lower surface, ascending or, more rarely, decumbent, sparsely to occasionally branched, with the apices remaining flattened, a little thickened and tongue-like, or developing terete, \pm erect segments 0.2–0.3 (–0.5) mm wide, dispersed or crowded together in mats. Apothecia to 1.5 (-1.7) mm wide, subsessile and nestling amongst the squamules. Proper exciple in section hyaline within, at the outer edge dark brown, K± unchanged, N+ reddish, soon reflexed and ± excluded, composed of radiating, anastomosing hyphae in a gel matrix. Hypothecium massive, diffusely brown to hyaline, typically darkest and with additional dilute greenish, K+ intensifying, N+ red pigment in the upper part. Hymenium hyaline, not inspersed, 55–65 µm thick, overlain by a dark chocolatebrown epithecium c. 10 µm thick, ± unchanged in K, N+ reddish. Asci $35-55 \times 12-15 \,\mu\text{m}$. Paraphyses 2-3 µm thick, with apices sometimes capitate and brown, 3 (-5) μ m wide. Ascospores 9–11.2–13.5 (-14) × 4.5–5.2–6 (–6.5) μm. Conidiomata not seen. Chemistry: melacarpic acid; medulla K–, KC–, C–, P–, UV± whitish. Figs 1D-F, 2, 3.

Remarks. The description is based on Australian and Tasmanian specimens that contain melacarpic acid as the sole major metabolite. In general, Neophyllis pachyphylla is a more robust species, with thicker squamules that remain largely dispersed and do not form caespitose clumps (Fig. 3). With their slightly thickened, rounded apices, the ascending squamules sometimes resemble tiny tongues. Terete projections are sometimes formed on the squamules, but they do not dominate the thallus, nor are they as brittle as in N. melacarpa. After examining large numbers of herbarium specimens representing both species, and studying the species in the field, the conclusion is that one is highly unlikely to ever mistake N. melacarpa for *N. pachyphylla*. When the two species grow together, for example on soil, they are also unlikely to be confused, as the former is more delicate, brittle and often caespitose. However, it is strongly recommended that any inferred specimens of N. pachyphylla be checked chemically. The putative spore size difference (Müller 1895) was not supported in the present study. The additional, subhymenial greenish pigment observed in N. pachyphylla is likely to be environmentally induced and not of taxonomic significance.

On this basis, *N. pachyphylla* is known from the type locality in the Grampians, Victoria, from parts of eastern Tasmania, and from the southern tablelands of New South Wales. At the Tasmanian and N.S.W. localities, *N. melacarpa* is also present. Specimens in HO from Pigeon House Mountain contain grayanic acid and are identified as *N. melacarpa*, although *N. pachyphylla* has been recorded from there (Filson 1992). The species



Fig. 2. Neophyllis pachyphylla anatomy, showing the "Neophyllis-type" ascus (with amyloid parts stippled), paraphyses and ascospores. Scale = $10 \mu m$.



Fig. 3. *Neophyllis pachyphylla* habit on granite (Mt Stronach, Tasmania). Photo J. Jarman.

occurs directly on rock (Devonian granite in Tasmania) or on coarse sandy or gravelly soil over granite or sandstone (in N.S.W.) in open sclerophyll woodland. All specimens collected by the author were from shaded rocks subject to moisture seepage, or to accumulations of silt in drainage channels on large rock outcrops.

Specimens containing melacarpic acid only are also known from New Zealand although the detailed ecology of their provenance is unknown. Whilst these could be included under *N. pachyphylla* on chemical characters alone, they are likely to represent a further, undescribed species of the genus. They consist of erect well-separated terete lobes, up to 7 mm tall and 0.4–1 mm wide; no flattened squamules are evident in what are rather small, fragmented specimens.

Specimens examined

AUSTRALIA. NEW SOUTH WALES. Braidwood Road, c. 5 km NE of Nerriga, 35°05'S 150°08'E, 17.xi.2012, *G. Kantvilas 625/12* (HO); Bulee Gap, 8 km N of Nerriga, 35°05'S 150°08'E, 690 m, 18.iv.2014, *G. Kantvilas 382/14* (HO).

TASMANIA. Mt Cameron, 40°59'S 147°56'E, 550 m, 5.vii.1995, *G. Kantvilas 41/95* (HO); The Hazards, near the Wineglass Bay Lookout, 42°09'S 148°17'E, 180 m, 19.vii.2005, *G. Kantvilas 188/05, 189/05, 190/05 & J. Jarman* (HO); northern ridge of Mt Stronach, 41°10'S 147°34'E, 400 m, 11.ix.2021, *G. Kantvilas 360/21* (HO).

New Zealand specimens examined, currently identified as Neophyllis pachyphylla

NEW ZEALAND. SOUTH ISLAND. Stockton Plateau, above Granity, 41°38'S 171°54'E, 16.ii.1986, *A.M. Buchanan 13086* (HO); Westport, Denniston Plateau, 670 m, 12.iii.2009, *W. Malcolm s.n.* (HO); Denniston, near road to old Escarpment Mine, 645 m, 10.xii.2013, *D. Glenny 12028* (CHR, HO).

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