The taxonomic status of *Nephroma parile* chemotype 2

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Chemotype 2 of *Nephroma parile*, occurring in southern South America, is confirmed as a distinct chemotype. However, it is genetically (nrITS and mtSSU) not differentiated from chemotype 1 in the Northern Hemisphere and does not merit status as a separate taxon. *Nephroma parile* is hence regarded as a monotypic, bipolar species.

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Introduction

Based on hopane triterpene chemistry, James & White (1987) recognized three chemotypes ('races') in *Nephroma parile* (Ach.) Ach. (Nephromataceae, Lecanorales). Chemotypes 1 and 2 contain compounds denoted T2 (15α-acetoxyhopane-22-ol; dolichorrhizin), T3 (hopane-6α,22-diol; zeorin), and T5 (hopane-15α,22-diol), and chemotype 3 contains compounds T1 (7β-acetoxyhopane-22-ol; peltidactylin), T3, T4 (hopane-7β,22-diol), and T6 (hopane-6α,7β,22-triol). The distinction between chemotype 1 and 2 was based on additional minor compounds. Chemotype 1 is the widely distributed chemotype in the Northern Hemisphere and was found to be present in the lectotype (H-ACH 1468B); chemotype 2 occurs in southern South America (see also White & James 1988); and chemotype 3 was known from a few collections from Europe and North America.

Based on the nrITS marker, Fedrowitz et al. (2012) recognized eight genotypes in *N. parile* in the Northern Hemisphere, denoted NP1–NP8. Timdal et al. (2020) recognized two major clades within *N. parile*, one consisting of genotypes NP1–NP6 and one of NP7. Genotype NP8 was excluded from the analysis as it appeared to be a possible contamination. The 14 sequenced specimens of chemotype 1 and the 12 specimens of chemotype 3 fell within the two clades, respectively, and the clades were hence regarded as two distinct species. The latter clade (NP7, chemotype 3) was described as the new species *Nephroma orvoi* Timdal et al. (Timdal et al. 2020).

No recent material of chemotype 2 was known to Timdal et al. (2020), hence its taxonomic status remained unresolved. Later, I have examined three specimens from Chile in TROM collected by Arve Elvebakk and Jarle Werner Bjerke in 1998, and I here treat its taxonomy.

Material and Methods

Five specimens of *N. parile* from Chile in TROM were initially studied (L-564711, L-564714–17), but one (L-564715) was soon identified by TLC as *N. pseudoparile* (Räsänen) Zahlbr. and one (L-564716) was regarded as too scanty for further study.

Thin-layer chromatography (TLC) was performed in accordance with the methods of Culberson (1972), Menlove (1974), and Culberson et al. (1981) using solvent systems C and G, and aluminium plates.

The specimens were sequenced for the nrITS and mtSSU markers at the Natural History Museum, University of Oslo, using the primer pairs ITS1F/ITS4 (Gardes & Bruns 1993, White et al. 1990) and mtSSU1/mtSSU3r (Zoller et al. 1999), respectively. The two newly obtained nrITS sequences and the single mtSSU sequence were added to the 41 sequence data set of *N. parile* by Timdal et al. (2020). See *Specimens examined* for the new sequences in this paper and Table 1 in Timdal et al. (2020) for sequence number, voucher information, genotype, chemotype, and GenBank ID's for the 41 other sequences.

The obtained sequences were subjected to BLASTn searches against the GenBank nucleotide database. For the mtSSU sequence, no further analysis was performed. For the nrITS sequences, a phylogenetic reconstruction under the maximum likelihood criterion was inferred by SATé-II ver. 2.2.7 (Liu et al. 2012), using MAFFT (Katoh et al. 2005, Katoh & Toh 2008) as aligner, MUSCLE (Edgar 2004) as merger, FastTree (Price et al. 2010) as tree evaluator, and with the default settings in the GUI except that the number of iterations after last improvement in the maximum likelihood score was set to 10. A haplotype network was constructed by PopART ver. 1.7 (Leigh and Bryant 2015) using the alignment provided by MAFFT, the Median-joining algorithm (Bandelt et al. 1999), and with geographical region as displayed trait.

Results

Chemistry: The three specimens of chemotype 2 contained the three major triterpenes T2 (dolichorrhizin), T3 (zeorin), and T5 (hopane-15α,22-diol), and several minor, unknown compounds that were different from those of chemotype 1 (Fig. 1).

DNA: One nrITS sequence (558 bp long) was obtained from TROM-L-564714, and both nrITS and mtSSU sequences (501 bp and 913 bp long, respectively) were obtained from TROM-L-564717. The two nrITS sequences were identical (except for length).

The BLASTn search of the mtSSU marker showed 100% identity with GenBank sequences AY584625 and KJ150442 (alignment lengths 782 and 780 bp, mtSSU sequences from specimens 55 and 32 in Timdal et al. 2020, respectively).

The phylogenetic reconstruction (not shown) recovered the two nrITS sequences within the clade containing genotypes NP1–NP4 in Fig. 2 in Timdal et al. (2020). In the haplotype network (Fig. 2), the two sequences constitute a genotype that is only a single substitution (T→C in the ITS1 region) from genotype NP1. The other genotypes of *N. parile* (NP2–NP6, plus an unnamed genotype) differ from NP1 in 1–3 substitutions and from the Chilean sequences in 2–4 substitutions (Fig. 2).

Discussion

The nrITS and mtSSU sequences indicate that the South American population of *N. parile* is conspecific with the Northern Hemisphere population, as they differ only in a single nucleotide substitution in the nrITS marker and are identical in the mtSSU marker. *Nephroma parile* should, from current evidence, be regarded as a monotypic, bipolar species.

Still, this study corroborates the observation of James & White (1987) that the Northern and Southern Hemisphere populations of *N. parile* differ in the additional minor compounds. It should be noticed that the sequenced specimens of chemotype 1 analyzed by Timdal et al. (2020) all belong in genotype NP1. No other chemotype of *N. parile* (excluding *N. orvoi*) has been reported from the

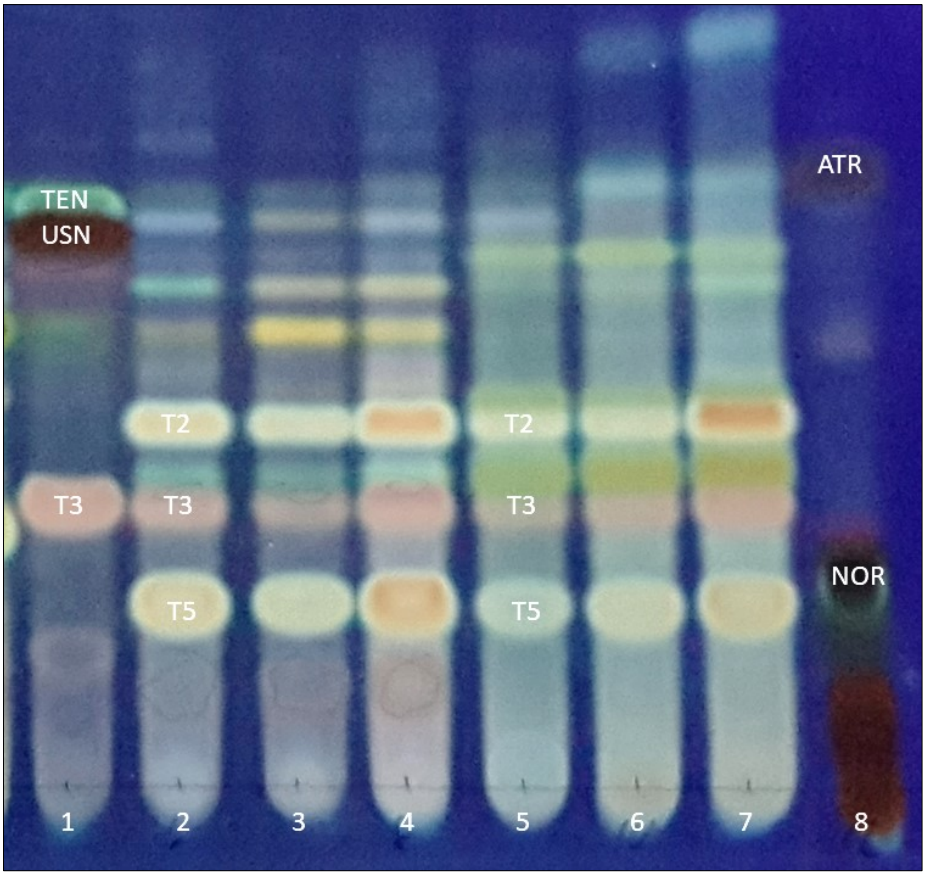

Figure 1. Chromatogram in solvent system C, photographed in UV 366 nm after treatment with sulphuric acid and heat. Lane 1: *Nephroma pseudoparile*, TROM-L-564715 (Chile); lanes 2–4: *N. parile* chemotype 2, TROM-L-564711 (Chile), TROM-L-564714 (Chile), TROM-L-564717 (Chile), respectively; lanes 5–7: *N. parile* chemotype 1, O-L-131146 (Finland), O-L-131276 (USA, Alaska), O-L-131158 (Sweden), respectively. ATR: atranorin, NOR: norstictic acid, TEN: tenuiorin, USN: usnic acid, T2: dolichorrhizin, T3: zeorin, T5: hopane-15α,22-diol.

Northern Hemisphere by James & White (1987) and Timdal et al. (2020), but there is actually no proof that the other genotypes (NP2–NP6) also belong in chemotype 1. The genetic basis for intraspecific chemotypes is unknown and may not be reflected in the two markers used in this taxonomy.

Specimens examined: **Chile**. *Magallanes y de la Antarctica Chilena*: Parque Nacional Torres del Paine, 0.5 km S of Hostería Pehoe, 51.1083°S, 73.0000°W, alt. 90 m, on *Berberis* sp. in scrub vegetation, 1998-03-12, A. Elvebakk 98:347 (TROM L-564711); Parque Nacional Torres del Paine, S slope of Cerro Paine, 50.9667°S, 72.8833°W, alt. 650 m, on a rotten branch of *Nothofagus pumilio* near ground, 1998-03-13, J.W. Bjerke 166/98 (TROM L-564717) [nrITS: OQ076287, mtSSU: OQ091693]; Parque Nacional Torres del Paine, W side of the little lake c. 1.5 km WSW of Refugio Pehoe, 51.0750°S, 73.1500°W, alt. 170 m, on dead twigs of *Nothofagus* sp. on the forest floor, 1998-03-18, A. Elvebakk 98:289 (TROM L-564714) [nrITS: OQ076288].

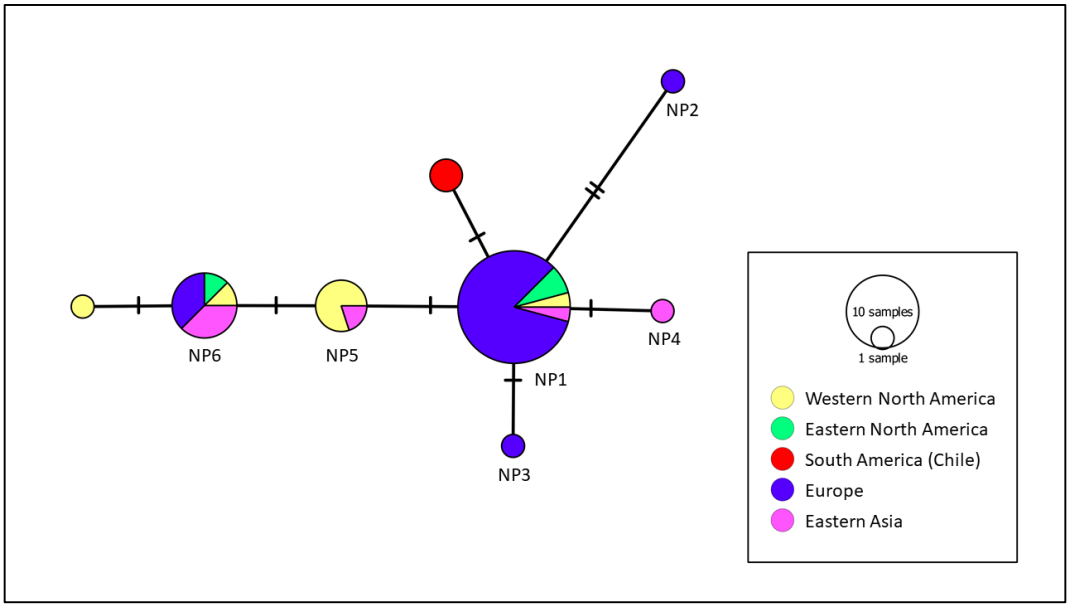

Figure 2. Median-joining haplotype network of *Nephroma parile* based on the nrITS marker. Genotypes are represented by circles whose sizes are proportional to the number of individuals. Different colors represent geographical regions. Each hatchmark represents one nucleotide substitution. NP1–NP6: Genotype codes according to Fedrowitz et al. (2012). NP1 consists of specimens: 18, 20, 23, 24, 25, 28, 29, 30, 35, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 51, and 52; NP2: 26; NP3: 27; NP4: 31; NP5: 16, 17, 21, 33, and 54; NP6: 15, 19, 22, 32, 34, 36, 50, and 53; unnamed, yellow: 55 (see Table 1 in Timdal et al. 2020 for specimen data); unnamed, red: the two newly obtained sequences from Chile.

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