First report of *Plasmopara sphagneticolae* on the native Hawaiian plant *Lipochaeta integrifolia*

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Abstract

Downy mildew disease was observed for the first time from *Lipochaeta integrifolia*, a G3-vulnerable plant native to Hawai'i. The oomycete infecting the plants was identified as *Plasmopara sphagneticolae* by morphological and molecular means. This marks the first report of *P. sphagneticolae* outside of Australia on a new host. This disease may have significant implications for conservation efforts of *L. integrifolia* and other endangered native Asteraceae on the islands.

Keywords *Melanthera* · Nehe · Peronosporales · *Sphagneticola trilobata* · Vulnerable plants

Lipochaeta integrifolia (= *Melanthera integrifolia*, Heliantheae, Asteraceae; common name = nehe) is a G3-vulnerable perennial coastal plant endemic to the main Hawaiian Islands including Kure Atoll and Laysan Island (Wagner et al. 1999). Flowers have been used for lei making by native Hawaiians (Elliot and Tamashiro 2009). In recent years, it has been used as a salt and wind tolerance native ground cover for landscaping in Hawai'i (Bornhorst and Rauch 2003; Benzona et al. 2009). During a recent (January 2019) visit to Dr. Orville Baldos's germplasm collection of *L. integrifolia* in his Hawaiian Native Plants for Floriculture and Landscape Use project at the University of Hawaii at Manoa, a Hawai'i Department of Agriculture (HDOA) staff member noticed that some of the live stands of *L. integrifolia* collected from Koko Head, Oahu and Southpoint, Hawai'i had symptoms of downy mildew infection. In these

greenhouse/nursery settings, the disease was prevalent, and symptoms were moderate to severe (Fig. 1a). In the field, the disease occurred sporadically, and symptoms were moderate to severe. Specifically, plants had brown to black leaves (Fig. 1a). The discoloration started at the tip of the leaves (Fig. 1b–c) and moved toward the stem. The abaxial leaf surface was colonized with a downy mass of sporangiophores and sporangia (Fig. 1d), which were subsequently determined at HDOA with light microscopy to be very similar to those of the oomycete genus *Plasmopara*. Affected plants were dried and shipped to the USDA-ARS Lab at Beltsville, Maryland for further identification to the level of species.

Sporangiophores and sporangia from the infected tissues were mounted in 85% w/v lactic acid and viewed under differential interference contrast (DIC) with a Zeiss Axio Imager.M2 compound microscope and imaged with an Axiocam 503 color camera and ZenPro 2 blue edition software (Carl Zeiss Microscopy, Cambridge, United Kingdom). Measurements did not differ between the two specimens and were therefore combined and are reported as minimum-average (standard deviation)-maximum. Sporangiophores were monopodial with a slightly bulbous base. Primary branches branched 1–3 times and terminated in 2–3 sporangium-bearing denticles (Fig. 1e). Sporangia ($n = 30$ per specimen, i.e. 60 sporangia total) were globose to ellipsoid and measured 12.69–15.35 (1.32)–19.95 μm by 15.58–19.81 (1.97)–24.72 μm (Fig. 1f). Microscope slides were dried overnight at 65 C, sealed with several layers of clear nail polish, and deposited along with the pressed leaf tissue in the U. S. National Fungus Collections Herbarium (BPI) as specimens BPI 919165 (Koko Head) and BPI 919166 (Southpoint).

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Fig. 1 (a) *Lipochaeta integrifolia* plant stands affected with downy mildew showing discolored leaves. (b) An individual *L. integrifolia* stem with affected leaves showing discoloration at the tip. (c) Close up of leaves with discoloration, top view. (d) Abaxial view of discolored

leaves with prominent white downy masses. (e) Monopodial sporangiophore, scale bar = 50 µm. (f) A range of subglobose to ellipsoid detached sporangia, scale bar = 25 µm. Images were edited in Gimp 2.10

Since the specimens were morphologically identical and grew in the same environment, DNA was extracted from BPI 919166 (Southpoint) using the HotSHOT method modified for microorganisms (Ishida et al. 2015). *Cox2* was amplified with the *cox2*-F/*cox2*-RC4 primer pair (Choi et al. 2015) in 10 µL reactions using MangoMix (BioLine Thomas Scientific, Swedesboro, New Jersey, USA) as previously described (Rivera et al. 2016). Amplicons were purified using Illustra ExoStar (GE Healthcare Life Sciences, Piscataway, New Jersey, USA), and then sequenced bidirectionally on an ABI 3739x Genetic Analyzer (Applied Biosystems). Sequences were trimmed to remove low quality bases and assembled into a consensus sequence in CLC Genomics Workbench 7 with default settings. The consensus sequence of BPI 919166 was deposited in NCBI GenBank (MT292790). BLASTn searches of the *cox2* sequence against the NCBI database revealed 99% nucleotide identity (618/620 with positions 539 and 609 in the BLAST alignment differing)

between BPI 919166 (MT292790) and *Plasmopara sphagneticolae* BRIP 61010 (KM085175), which is sister species to *P. halstedii* (McTaggart et al. 2015). *Cox2* sequences representing *P. halstedii*, *P. sphagneticolae*, and the outgroup taxa were downloaded from GenBank and aligned with BPI 919166 using the online version of MAFFT v7 (Katoh et al. 2002, 2017). The greedy algorithm (Lanfear et al. 2012) of PartitionFinder 2.1.1 (Lanfear et al. 2016) determined codon position as the best partition scheme and GTR + G as the best model of sequence evolution. A maximum likelihood (ML) phylogeny was inferred in RAxML 8.0.0 (Stamatakis 2014), and bootstrap values were generated from 1000 pseudoreplicates. In the ML tree, BPI 919166 formed a well-supported (100% bootstrap support) clade with BRIP 61010, the holotype of *P. sphagneticolae*, that is sister *P. halstedii* complex (Fig. 2).

Based off morphology and *cox2*, the oomycete species infecting *L. integrifolia* is *P. sphagneticolae*. *Plasmopara*

Fig. 2 Maximum likelihood phylogeny inferred from a *cox2* alignment partitioned by codon position under the GTR + G model of sequence evolution. Scale bar = 0.08 substitutions

sphagneticolae was originally described from *Sphagneticola trilobata* (Heliantheae, Asteraceae) in northern Queensland, Australia in 2015 (McTaggart et al. 2015), but there have been no subsequent published reports of the pathogen since its description (Farr and Rossman 2020). McTaggart et al. (2015) note that *S. trilobata* is native to tropical regions of central and South America and is an introduced weed in Australia. *Sphagneticola trilobata* is considered one of the 100 worst global invasive species (Lowe et al. 2000) and is found in Hawai'i (USDA, NRCS 2020). Thus, it is possible *P. sphagneticolae* was spread with *S. trilobata* to both Australia and Hawai'i, but further studies are needed to test that hypothesis. Understanding the origin and dispersal of *P. sphagneticolae* will be critical for conservation of *L. integrifolia*; and, further investigations into pathogenicity and host range are needed to determine the threat of *P. sphagneticola* to other endangered Hawaiian Asteraceae—e.g. *Melanthera fauriei*, *M. kamolensis*, *M. micrantha*, *M. tenuifolia*, *M. venosa*, and *M. waimeensis*.

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