First report of *Litylenchus coprosma* on *Coprosma robusta*

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Abstract *Litylenchus coprosma* is reported for the first time from *Coprosma robusta* at two locations in Wellington, New Zealand. Nematodes were isolated from the leaves with angular yellow patches limited by veins and identified as *L. coprosma* based on morphology and sequence analysis of the SSU and LSU regions of rDNA.

Keywords Yellow patch · Nematode · New Zealand · Disease · New host · SSU · LSU

Litylenchus coprosma was first described as a new species from the leaves of *Coprosma repens* (Maori common name: taupata), from an amenity planting in Wellington, New Zealand (Zhao et al. 2011). On 10 Oct. 2012, angular yellow patches limited by veins were observed on the leaves of another plant, *Coprosma robusta* (Maori common name: karamu; Fig. 1) from Taylor Park, Tawa, Wellington, New Zealand (E: 1,753,272; N: 5,439,995). Subsequently, the same symptoms were also observed on *C. robusta* from Cambridge Terrace, Naenae, Lower Hutt, Wellington (E: 1,762,670; N: 5,437,032) in 2015. Additional symptomatic leaves were collected from both sites in Tawa and Lower Hutt in 2016. All the leaf samples collected were dissected and observed under

microscope, nematodes were extracted from the symptomatic leaves, but not from the healthy leaves. In one leaf, all life stages of *L. coprosma* were found in the mesophyll, indicating that the life cycle is completed within the leaves. The robust form of the stylet of the nematode suggests that it is a plant feeder (Fig. 2). No cavities, swellings or abnormal growths and no development of nutritive tissue were observed in the leaves (Fig. 1). No fungal hyphae were observed in the leaves of *C. robusta* and no plant pathogenic fungi and bacteria were isolated from the leaves. This indicates that the nematode was likely the primary cause of the discoloration of the leaves. This nematode was identified as *L. coprosma* by morphology and DNA sequence analysis.

The morphology of this nematode from *C. robusta* matched the descriptions of *L. coprosma* from *C. repens* given by Zhao et al. (2011) (Table 1). However, the main differences between the two populations are the body length, the post-uterine sac length and the de Man a ratio (Table 1). The differences could be caused by the host variation and environmental factors. *L. coprosma* is morphologically characterised by having slender males and females with head offset and distinctly narrower than body diameter (Fig. 2a, e). It has a short robust stylet with rounded knobs (Fig. 2a,e), pharynx with a weak non-muscular median bulb and glands in a terminal bulb which adjoins the intestine (Fig. 2b), secretory/excretory pore opening posterior to nerve ring (Fig. 2c), females with a monoprodelfic and post-uterine sac (Fig. 2d), males with arcuate spicules and an adanal bursa that extends nearly to the tail tip (Fig. 2g). Both males and females are living inside the leaves of *C. robusta* and they do not induce galls (Fig. 1). Permanent slides of the *L. coprosma* specimens were stored in the National Nematode Collection New Zealand, with the collection numbers of NNCNZ3255.

To confirm the morphological identification, the nematodes collected from Tawa were used for DNA extraction. Individual

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Fig. 1 Yellow patches on leaves of *Coprosma robusta* infected with *Litylenchus coprosma*

live nematodes were lysed in a buffer of 20 μ l containing proteinase K (Williams et al. 1992) and DNA was extracted using the method of Zheng et al. (2002). Extracted DNA was used for PCR amplification of nearly full length of small subunit (SSU) rDNA gene with two sets of primers, 1096F/1912R and 1813F/2646R (Holterman et al. 2006). Primers, D2A and D3B (Nunn 1992) were used to amplify the D2/D3 expansion segments of the large subunit (LSU) rDNA gene. The PCR compositions and cycling conditions for the amplification of the SSU and LSU genes, and sequencing were conducted as per Zhao et al. (2011, 2013). The sequences from SSU and LSU regions were 99% similar to sequences of *L. coprosma* in the GenBank (GU727546 SSU, GU727547 LSU). There are 6 SNPs out of 1537 bp for SSU and 2 SNPs out of 713 bp for D2/D3 fragment of the LSU between *L. coprosma* from *C. repens* and *C. robusta*, indicating there are slight differences between *L. coprosma* species from these two hosts. Furthermore, *L. coprosma* shared 99% similarity with *Subanguina radiculicola* at the SSU (AF202164, 10/1537 and EU682392, 13/1537), but only 93% similarity at LSU (DQ328721), suggesting that LSU gene is a better marker to separate these two species. The sequences were submitted to GenBank under the accession numbers KY679563 for SSU and KY679564 for LSU.

Fig. 2 Light microscope photographs of *Litylenchus coprosma* isolated from leaves of *Coprosma robusta*. **a**: Female anterior part and stylet; **b**: Female oesophagus and intestine junction; **c**: Female anterior part and excretory pore; **d**: Female vulva and genital track; **e**: Male anterior part and stylet; **f**: Female tail; **g**: Male spicules and bursa. (All scale bars = 10 μ m)

Table 1 Comparative morphometrics of *Litylenchus coprosma* from *Coprosma robusta* and *C. repens*. All measurements given as mean (μm) $\pm$ s.d. (range)

Character	Female				Male	
	<i>C. robusta</i> (this study)	<i>C. repens</i> (Zhao et al. 2011)	<i>C. robusta</i> (this study)	<i>C. repens</i> (Zhao et al. 2011)	<i>C. robusta</i> (this study)	<i>C. repens</i> (Zhao et al. 2011)
n	7 (slender)	13 (slender)	5 (obese)	15 (obese)	5	15
L	602 $\pm$ 44 (547–661)	743 $\pm$ 50 (649–816)	653 $\pm$ 62 (586–721)	856 $\pm$ 72 (710–940)	647 $\pm$ 25 (617–669)	899 $\pm$ 66 (768–994)
a	49.9 $\pm$ 5.6 (41.8–58.4)	55.2 $\pm$ 4.0 (51.5–63.3)	35.7 $\pm$ 5.1 (31.1–43.5)	32.8 $\pm$ 3.7 (24.9–37.7)	46.5 $\pm$ 7.6 (37.7–56.3)	52.0 $\pm$ 4.4 (44.5–60.2)
b	4.1 $\pm$ 0.6 (3.4–5.3)	4.4 $\pm$ 0.6 (3.9–5.8)	4.1 $\pm$ 0.8 (3.4–5.0)	5.1 $\pm$ 0.6 (4.2–6.8)	5.1 $\pm$ 0.6 (4.3–5.6)	5.4 $\pm$ 0.4 (4.8–6.2)
c	15.6 $\pm$ 1.4 (13.3–17.1)	18.7 $\pm$ 1.3 (16.3–21.3)	14.9 $\pm$ 1.6 (12.6–16.5)	19.4 $\pm$ 2.5 (15.4–25.0)	16.4 $\pm$ 1.8 (14.6–18.9)	21.1 $\pm$ 1.9 (18.2–24.1)
c'	4.7 $\pm$ 0.2 (4.3–5.0)	4.0 $\pm$ 0.4 (4.0–5.4)	4.6 $\pm$ 0.3 (4.3–5.0)	3.8 $\pm$ 0.4 (2.9–4.7)	3.5 $\pm$ 0.1 (3.4–3.6)	3.5 $\pm$ 0.4 (3.1–4.4)
V/T	80.8 $\pm$ 1.0 (79.0–82.3)	81.5 $\pm$ 2.4 (76.5–85.3)	81.0 $\pm$ 1.6 (78.8–82.8)	82.2 $\pm$ 1.6 (78.8–84.7)	55.8 $\pm$ 7.2 (49.3–63.5)	73.0 $\pm$ 6.6 (60.7–82.1)
Max. body diam.	12.2 $\pm$ 1.3 (10.9–14.5)	13.5 $\pm$ 0.6 (12.6–14.6)	18.4 $\pm$ 2.0 (16.2–21.1)	26.3 $\pm$ 3.1 (22.5–33.9)	14.3 $\pm$ 1.9 (11.9–16.9)	17.3 $\pm$ 1.3 (15.8–20.3)
Stylet length	10.2 $\pm$ 0.7 (9.2–10.9)	10.8 $\pm$ 0.9 (8.9–11.7)	10.0 $\pm$ 0.5 (9.6–10.6)	10.9 $\pm$ 0.3 (10.2–11.4)	10.2 $\pm$ 0.9 (9.5–11.5)	10.5 $\pm$ 0.5 (9.7–11.3)
Excretory pore from anterior end	90.3 $\pm$ 2.2 (86.4–93.0)	113 $\pm$ 6.0 (101–115)	91.3 $\pm$ 13.4 (79.6–106)	99 $\pm$ 13 (81–104)	88.1 $\pm$ 4.7 (82.6–91.1)	111 $\pm$ 9.0 (96–121)
Spicule length	–	–	–	–	14.3 $\pm$ 1.3 (13.0–15.9)	16.2 $\pm$ 0.7 (14.9–17.0)
Post-uterine sac length	18.8 $\pm$ 2.4 (14.8–21.3)	22.4 $\pm$ 6.8 (11.4–35.0)	17.7 $\pm$ 1.9 (15.6–20.0)	34.9 $\pm$ 9.5 (24.1–48.5)	–	–
Tail length	38.7 $\pm$ 1.5 (36.4–41.1)	40.1 $\pm$ 4.0 (32.2–46.3)	45.1 $\pm$ 1.7 (42.9–46.5)	45.1 $\pm$ 6.6 (31.2–55.1)	39.8 $\pm$ 3.2 (35.3–43.7)	42.8 $\pm$ 3.6 (37.1–50.7)
Anal/Cloacal body diam.	8.3 $\pm$ 0.4 (7.9–9.1)	8.7 $\pm$ 0.6 (7.6–9.6)	9.9 $\pm$ 0.7 (9.2–10.9)	11.7 $\pm$ 0.9 (10.4–12.8)	11.4 $\pm$ 1.1 (9.9–12.7)	12.3 $\pm$ 0.5 (11.4–12.9)

Fig. 3 Bayesian phylogenetic tree inferred from sequences of the D2D3 fragment of LSU gene. Posterior probabilities greater than 50% are given on appropriate clades. Nematode species and GenBank Accession numbers are listed for each taxon

To further resolve the phylogenetic relationship, the D2/D3 region of the 28S rRNA sequences derived from *L. coprosma* from *C. robusta* was used to construct phylogenetic tree. The related LSU sequences were aligned with ClustalX in Geneious 10.0.6 (<http://www.geneious.com>, Kearse et al. 2012) using the default parameter values. A Bayesian tree was constructed with MrBayes in Geneious using substitution model GTR and rate variation gamma. The tree was rooted using *Aphelenchoides fragariae* as the out group. The phylogenetic tree showed that *L. coprosma* derived from *C. robusta* formed a clade with *L. coprosma* of *C. repens* with 100 *pp.* value support, and well distant from the other genera (Fig. 3).

There are around 90 species of *Coprosma* (family: Rubiaceae) distributed mainly in the Pacific and Australasia. Among these, 58 species are endemic to New Zealand (New Zealand conservation network 2013). Both *C. repens* and *C. robusta* are native to New Zealand, common in the forest and coastal region of both North and South islands. They are also common ornamental plants. *Coprosma* are generally pest free, occasionally infected by root rot and scale insects (Polizzi et al. 2009). However, a number of fungal diseases have been reported: *Rhizoctonia solani* AG-4 strain causing root rot in *C. repens* and *C. lucida* in nurseries in Italy (Polizzi et al. 2009); *Puccinia coprosmae* causing leaf spot in several *Coprosma* species, including *C. repens* and *C. robusta* (Hood 2010); and *Cercospora* and *Coniothyrium* causing leaf spotting and blight, respectively (Ridley et al. 2000). The symptoms caused by *L. coprosma* are different from those caused by fungi, and no mycelia were observed in yellow patches leaves of *C. robusta*.

There were four species of nematodes, *Blandicephalanema pilatum*, *Helicotylenchus erythrinae*, *H. labiatus*, and *H. minzi* associated with *Coprosma* trees in the rhizosphere (Knight et al. 1997), but these nematodes have not been reported to cause disease symptoms on the trees. *L. coprosma* was not found in the soil collected under the infested *C. repens* and *C. robusta* trees.

In conclusion, this is the first report of *L. coprosma* causing yellow patches on *C. robusta* following the initial description of this nematode on *C. repens*. The yellow patches caused by *L. coprosma* were observed in the leaves of *C. repens* and *C. robusta*. Further sampling of other *Coprosma* species are needed to investigate the host distribution of *L. coprosma* in New Zealand.

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