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First report of *Zeatylenchus pittosporum* on *Pittosporum crassifolium*

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Abstract *Zeatylenchus pittosporum* is reported for the first time from *Pittosporum crassifolium* at Auckland, New Zealand. Nematodes were isolated from the leaves with brown or yellow chlorotic spots and identified as *Z. pittosporum* based on morphology, SSU and LSU of rDNA sequences analysis.

Keywords *Pittosporum crassifolium* · *Zeatylenchus pittosporum* · New host · Nematode · Molecular · SSU · LSU · Morphology

Zeatylenchus pittosporum (Tylenchida: Anguinidae) causes brown or yellow chlorotic spots was first recorded in *Pittosporum tenuifolium* from Hahei, on the North Island of New Zealand in 2012 (Zhao et al. 2013). This was the first report of anguinids as parasites in the Pittosporaceae plants (Zhao et al. 2013). The anguinid host range includes both grasses and broad-leaved plants, and many gall formers are known to be host-specific. The family Anguinidae includes nematodes that are fungal feeders and parasites of the aerial parts of plants (Siddiqi 2000). Most genera and species of Anguinidae induce galls on aerial parts of plants (Siddiqi 2000).

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In June 2013, leaves of *P. crassifolium* with brown or yellow spots symptom were also observed and collected from the Auckland airport and sent to the Plant Health and Environment Laboratory, Ministry for Primary Industries (MPI) for identification. Nematodes were isolated from the leaves and identified as *Z. pittosporum*. Subsequently, permanent nematode slides with collection number 28185 and 30037 were received from the AureQuality, New Zealand. All the specimens on the slides were isolated from the leaves of *P. crassifolium*, collected from Bay of Islands in May 2006 and from Auckland in July 2007, respectively. The nematodes from both locations were identified as *Z. pittosporum*. In fact *Z. pittosporum* from Bay of Islands, collected by AureQuality, was the earliest records for *Z. pittosporum* from *Pittosporum* trees in New Zealand.

Zeatylenchus pittosporum causes brown or yellow chlorotic spots on both *P. tenuifolium* leaves (Zhao et al. 2013) and *P. crassifolium* (Fig. 1). Symptoms of infestation were non-specific, brown or yellow chlorotic spots and discoloured areas expanded along the vines on leaves (Fig. 1). The symptoms were patchy on the trees surveyed. No cavities, swellings or abnormal growths and no development of nutritive tissue were observed in the leaves. Although leaf spots on *Pittosporum* caused by fungi have been reported (Chase and Simone 2001), fungal hyphae were not observed in the *Pittosporum* leaves. The infested *P. crassifolium* leaves were examined under the dissecting microscope. All life stages of *Z. pittosporum* were found in the mesophyll, indicating that the life cycle is completed within the leaves, and the robust form of the stylet of the nematode is consistent with it being a plant feeder (Fig. 2). Therefore, the results indicated that the brown or yellow spots and discolouration of the leaves were caused by the nematodes, *Z. pittosporum*.

In general, the isolates of *Z. pittosporum* from *P. crassifolium* correspond well to the descriptions of

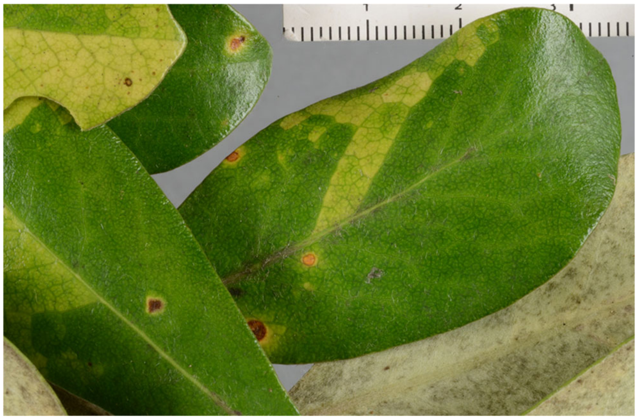

Fig. 1 Photograph of symptoms of yellow chlorotic spot on leaves of *Pittosporum crassifolium* caused by *Zeatylenchus pittosporum*

Zeatylenchus pittosporum given by Zhao et al. (2013) (Table 1). *Z. pittosporum* is morphologically characterised by having slender males and females with head offset and distinctly narrower than body diam (Fig. 2a and b). In some specimens, a small stylet, pharynx with a small non-muscular median bulb and glands in two parts with ventral and subventral glands overlapping the intestine, secretory/excretory pore opening at level of the retracted stylet knobs (Fig. 2b), anterior to the median bulb, females with a quadricolumella and post-uterine sac, males with arcuate spicules and an adanal bursa that extends to 30% of distance to the tail tip, and a conoid tail that is dorsally off-set with a spicate tip (Fig. 2d). It has both males and females free-living in leaves of *P. crassifolium* and its feeding does not induce galls (Fig. 1). Permanent slides of the *Z. pittosporum* specimens were stored in the nematode collection of AureQuality, New Zealand, with the collection numbers of 28185 and 30037.

To confirm the morphological identification, the individual nematode was used for DNA extraction. Individual live nematodes were lysed in a buffer of 20 μl 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 the nearly full length of small subunit (SSU) rDNA gene with primers, 1096 F and 1912R for the first fragment and 1813 F and 2646R for the second fragment (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 LSU and SSU genes, and sequencing were conducted as per Zhao et al. (2013). BLAST search analysis showed that the obtained DNA sequences of the LSU and SSU shared 99–100% maximal identity with those of the GenBank-derived *Z. pittosporum* isolates (JQ586255 SSU, JQ586256 LSU). Identical sequence for the SSU gene while 5 SNPs out of 729 bp were observed in the D2/D3 region of the LSU sequences for *Z. pittosporum*

Fig. 2 Light microscope photographs of *Zeatylenchus pittosporum* isolated from infected leaf of *Pittosporum crassifolium*. **a**: male anterior part and stylet; **b**: female anterior part, stylet and excretory pore; **c**: male lateral lines; **d**: male spicules and spicate tail tip. **e**: female tail and spike tail tip. **f**: female vulva (*Scale bars*: all = 10 μm)

Table 1 Comparative morphometrics of *Zeatylenchus pittosporum* from *Pittosporum crassifolium* and *P. tenuifolium*. All measurements given as mean (μm) ± s.d. (range)

Character	Female				Male	
	P. crassifolium (this study)	*P. tenuifolium* (Zhao et al. 2013)	*P. crassifolium* (this study)	*P. tenuifolium* (Zhao et al. 2013)	*P. crassifolium* (this study)	*P. tenuifolium* (Zhao et al. 2013)
n	13 (slender)	10 (slender)	2 (obese)	16 (obese)	4	15
L	462 ± 50 (352–558)	533 ± 31 (450–561)	592 ± 14 (583–602)	579 ± 23 (543–623)	514 ± 94 (391–618)	553 ± 35 (509–659)
a	53.1 ± 7.0 (36.8–63.7)	59.2 ± 4.9 (47.2–64.7)	36.0 ± 1.8 (34.7–37.3)	39.3 ± 3.2 (34.7–44.1)	43.5 ± 5.4 (35.9–48.5)	54.7 ± 5.9 (46.7–70.0)
c	20.3 ± 1.9 (17.2–22.7)	20.3 ± 1.1 (19.7–23.3)	23.5 ± 2.2 (21.9–25.0)	21.2 ± 1.7 (18.8–25.0)	16.8 ± 0.9 (16.3–17.9)	19.1 ± 1.5 (15.5–21.2)
c’	3.2 ± 0.4 (2.5–3.9)	3.5 ± 0.2 (3.1–3.7)	2.9 ± 0.3 (2.7–3.1)	3.4 ± 0.3 (2.7–3.9)	3.0 ± 0.5 (2.4–3.3)	3.6 ± 0.4 (3.0–4.3)
V/T	77.1 ± 1.2 (74.3–78.4)	77.1 ± 1.0 (76.0–78.9)	79.4 ± 0.6 (78.9–79.4)	78.3 ± 1.0 (75.5–79.9)	–	94.9 ± 0.4 (93.8–95.5)
Max. body diam.	8.8 ± 0.8 (7.7–9.7)	9.0 ± 0.4 (8.5–9.6)	16.5 ± 0.5 (16.1–16.8)	14.8 ± 1.3 (12.3–16.8)	11.7 ± 0.8 (10.9–12.8)	10.2 ± 0.7 (9.0–11.5)
Stylet length	7.3 ± 0.3 (6.9–8.0)	9.5 ± 0.9 (7.3–10.1)	10.4 ± 0.4 (10.1–10.7)	10.3 ± 0.4 (9.3–10.8)	8.3 ± 0.9 (7.0–9.2)	10.3 ± 0.3 (9.7–10.8)
Excretory pore from anterior end	5.9 ± 0.4 (5.6–6.2)	7.2 ± 0.7 (6.4–8.3)	8.5 ± 0.7 (8.0–9.0)	8.2 ± 05 (7.3–9.5)		7.9 ± 0.8 (7.2–9.4)
Spicule length		–	–	–	9.9 ± 1.5 (8.6–11.6)	11.8 ± 1.6 (9.2–14.3)
Post–uterine sac length	10.5 ± 1.2 (9.4–12.2)	12.5 ± 1.9 (8.4–15.8)	20.4 ± 0.8 (19.9–21.0)	20.1 ± 3.2 (15.7–26.4)		–
Tail length	23.0 ± 2.2 (18.7–26.1)	26.7 ± 1.6 (22.7–28.0)	25.3 ± 1.8 (24.0–26.6)	27.4 ± 1.7 (24.1–29.8)	29.5 ± 5.5 (23.9–34.6)	29.1 ± 2.6 (25.3–35.8)
Anal/cloacal body diam.	7.2 ± 0.6 (6.5–8.2)	7.7 ± 0.2 (7.3–8.1)	8.8 ± 0.3 (8.6–9.0)	8.2 ± 0.5 (7.3–8.6)	10.0 ± 0.6 (9.4–10.5)	8.0 ± 0.7 (6.9–9.2)

from *P. crassifolium* and *P. tenuifolium*, indicating there is slightly variation among *Z. pittosporum* species from different hosts. The sequences were submitted to GenBank under the accession numbers KY067422 for SSU and KY067423 for LSU.

To further resolve the phylogenetic relationship, the D2/D3 region of the 28S rRNA sequences derived from *P. crassifolium* was used to construct phylogenetic tree. The related LSU sequences were aligned in ClustalX2 (Larkin et al. 2007) with the default parameter values. ModelTest 3.04 (Posada and Crandall

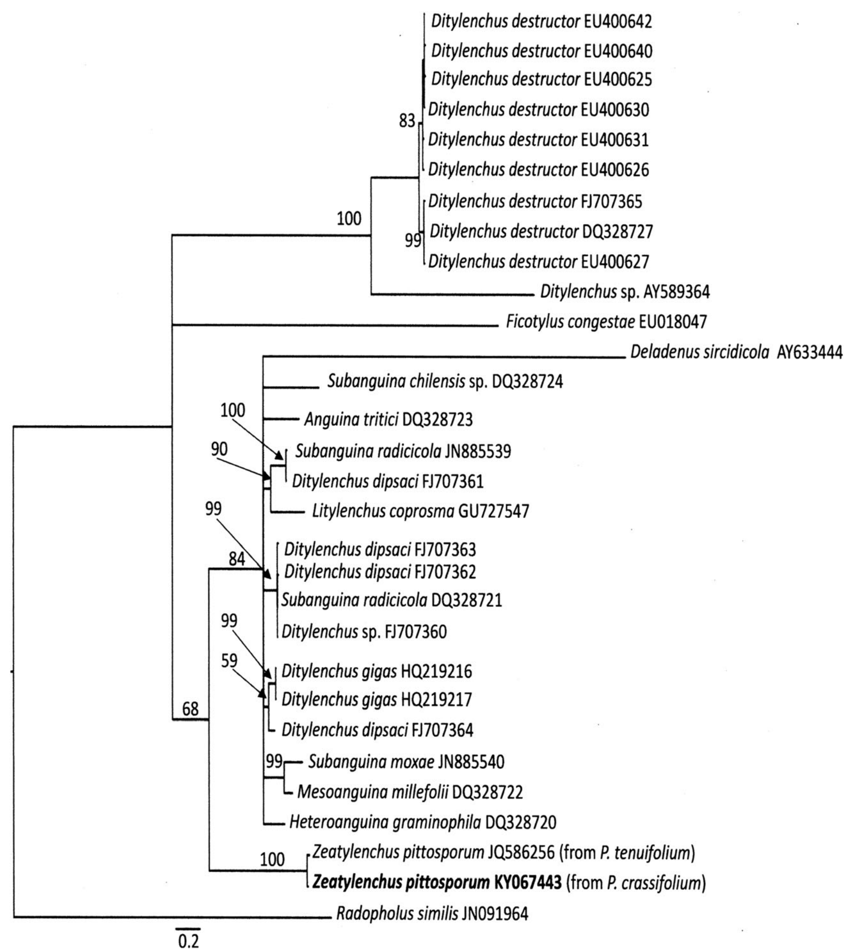

Fig. 3 Bayesian phylogenetic tree inferred from LSU gene DNA sequences. Posterior probabilities greater than 50% are given on appropriate clades. Nematode species and GenBank Accession numbers are listed for each taxon

1998) and PAUP*4.0b10 (Swofford 2002) were used to select the best AIC model. A Bayesian tree was constructed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) and rooted using *Radopholus similis* (Cobb 1893) Thorne, 1949 as outgroup. The phylogenetic tree showed that *Z. pittosporum* derived from *P. crassifolium* formed a clade with *Z. pittosporum* of *P. tenuifolium* with 100 *pp* values support, and well distant from the other genera (Fig. 3).

Although root-knot nematodes, *Meloidogyne* and spiral nematodes, *Helicotylenchus*, have been detected in the rhizosphere of *Pittosporum* trees (Knight et al. 1997), no *Z. pittosporum* were found in soil collected from under infested *P. crassifolium* and *P. tenuifolium* trees. *Pittosporum* Banks ex Sol. contains about 200 species across Asia, Australia, New Zealand, the Pacific Islands and South Africa (Poole and Lane 1990). *P. crassifolium* and *P. tenuifolium* are both native to New Zealand and widely grown as ornamental plants in subtropical areas. This nematode is probably endemic to New Zealand as it was collected from *P. tenuifolium* and *P. crassifolium* endemic New Zealand plants.

In conclusion, this is the first report of new host *P. crassifolium* for *Z. pittosporum*. The brown or yellow spots, caused by *Z. pittosporum* were observed only in the leaves of two *Pittosporum* species, *P. crassifolium* and *P. tenuifolium*. No other hosts have been reported so far. Whether *Z. pittosporum* occurs in other species of *Pittosporum* species needs to be further surveyed.

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