



Royal Irish Academy Grants Report

Title:	Dr
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Discipline:	Sciences
Year of Award:	2018
Project Title	Describing new species of fungus-like oomycete from Irish streams belonging to the new genus <i>Nothophytophthora</i>

1. Research background:

The fungus-like genus *Nothophytophthora* (oomycota) was erected in 2017 and contains 6 described species, with limited evidence on their role as plant pathogens. Little is known about oomycete diversity on the island of Ireland, just 14 *Phytophthora* species have been recorded in Northern Ireland and 28 in Ireland. Recent global surveys in aquatic habitats are revealing the large diversity of oomycetes found in the wild. During surveys in 2014 – 2018, a number of *Phytophthora*-like cultures were isolated. These were different from other described species, therefore may constitute new species. The aim of this work was to examine the morphology and distribution of the novel taxa in comparison to other described species. To the best of the authors knowledge, no new species of fungus or fungus like organisms have been published from Ireland since 1995.

2. Please outline the findings of your research and/or milestones achieved (did you achieve the primary objectives - if not, what did you learn from the process)?

The primary objective was achieved. Almost 4000 microscopic measurements on 21 isolates were made during the research exchange. Preliminary analysis indicates that the new taxa are different enough to be designated as a species new to science. Further collaborations with the PRC are planned in the near future, as a result of the networking provided by this research exchange.

Describing two new species of *Nothophytophthora* (oomycota) from Ireland and Northern Ireland

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Introduction

The fungus-like genus *Nothophytophthora* (oomycota) was erected in 2017 and contains 6 described species, with limited evidence on their role as plant pathogens. Little is known about oomycete diversity on the island of Ireland, just 14 *Phytophthora* species have been recorded in Northern Ireland and 28 in Ireland (O Hanlon et al. 2016, *Biology and Environment*). Recent global surveys in aquatic habitats are revealing the large diversity of oomycetes found in the wild. During surveys in 2014 – 2018, a number of *Phytophthora*-like cultures were isolated. These were different from other described species, therefore may constitute new species. The aim of this work was to examine the morphology and distribution of the novel taxa in comparison to other described species

Methods

- *Rhododendron* leaves were collected from in and around streams at several locations in Ireland and Northern Ireland between 2014 - 2017.
- The leaves were plated onto selective agar (PARP).
- Plates were incubated at 18-22°C and oomycetes hyphal tips transferred to carrot piece agar for further growth.
- Sporulation was induced by flooding plates with non-sterile water.
- Visible sexual and asexual structures were measured and compared to morphological keys. 40 spores and 40 chlamydospores were measured per isolate.
- The 18s ITS region of several isolates was barcoded to further establish identity.



Figure 1 (rhododendron leaves from where the *Nothophytophthora* taxa was isolated; figure 2 the location where several of the isolates have been collected; figure 3 chlamydospore of isolate Pr12-475; figure 4 empty sporangia Pr13-27b showing basal plug characteristic of *Nothophytophthora*; figures 5, 6, 7 time lapse of zoospore release from isolate Pr13-109

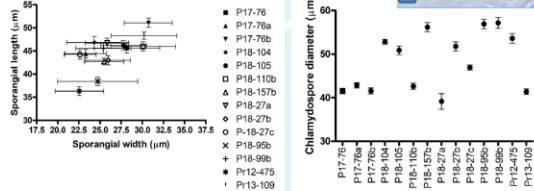


Figure 8 (above left): Mean and standard deviations of sporangia length and breadth measurements on 40 sporangia per isolate; Figure 9 (above right): Mean and standard deviation of chlamydospore diameter measurements on 40 chlamydospore per isolate.

Table 1 (below) ITS sequencing and BLAST results from Genbank for 5 sequenced isolates

Isolate	Location	BLAST match (% similarity)
Pr12-475	Co. Waterford	<i>Nothophytophthora chlamydospora</i> (98%)
Pr13-109	Co. Waterford	<i>Nothophytophthora chlamydospora</i> (99%)
Pr18-27a	Co. Down	<i>Nothophytophthora chlamydospora</i> (98%)
Pr13-662	Co. Tipperary	<i>Nothophytophthora chlamydospora</i> (97%)
Pr17-76b	Co. Waterford	<i>Nothophytophthora chlamydospora</i> (98%)

Acknowledgements

Thanks to AFBI and DAFM staff for their support and to the Royal Irish Academy for support to visit and collaborate with the Phytophthora Research Centre, Czech Republic through a Charlemont grant. The collaboration of the team at the Phytophthora Research Centre is gratefully acknowledged

Results and Discussion

- 15 isolates fitting the description of *Nothophytophthora* genus were collected from 2 counties in Ireland and 1 county in Northern Ireland.
- 14 of these isolates were examined morphologically
- The isolates could be separated into 2 taxonomic groups, based on a combination of their chlamydospore size and their sporangial dimensions (Fig. 3 and 5).
- The species *Phytophthora gonapodyides*, *P. chlamydospora* and *P. ramorum* were also detected during surveys in Ireland and Northern Ireland.
- The isolates collected differ from other described species of *Nothophytophthora* based on their morphology (Table 1) and ITS phylogeny (Fig. 5)
- The *Nothophytophthora* taxa were only isolated from rhododendron leaves collected in or directly around rivers.

Table 2: *Nothophytophthora* morphology data

Taxa	no. isolates examined	Mean chlamydospore diameter (SD)	Mean sporangial width (SD)	Mean sporangial length (SD)
<i>Nothophytophthora</i> sp.1 (Pr13-109)	7	42.3 (5.1)	27.3 (3.1)	46.6 (6.2)
<i>Nothophytophthora</i> sp.2 (Pr12-475)	8	54.2 (6.4)	25.2 (3.1)	42.7 (6.5)
<i>Nothophytophthora</i> amphigynosa	8	N/A	24.6 (1.8)	47 (5.6)
<i>N. caduca</i>	14	N/A	25.7 (3.1)	37.9 (4.6)
<i>N. chlamydospora</i>	5	43.7 (7)	22.1 (2.5)	37.6 (4.9)
<i>N. intricata</i>	6	N/A	24.8 (1.5)	38.5 (2.8)
<i>N. valdiviana</i>	5	N/A	28 (3.5)	42.7 (4.6)
<i>N. vietnamensis</i>	8	N/A	29.3 (8.1)	36.4 (12.7)

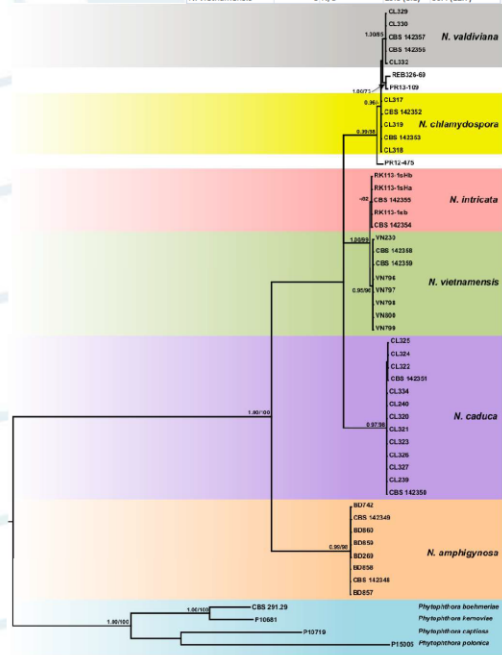


Figure 10 (above): ITS Phylogeny of the described *Nothophytophthora* species, including placement of two of the isolates collected in Ireland (Pr12-475, Pr13-109). Taken from Jung et al. 2017. Permission

Conclusions

- The isolates studied here appear to be different enough from described *Nothophytophthora* species to warrant publication of formal species descriptions. This will be the first new species of an oomycete described from the island of Ireland for over 50 years.

Further work

- To further compare these isolates against the described *Nothophytophthora* species, the following work will be carried out
- Temperature growth rate studies
- Mating type studies
- Sequence multiple gene regions