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Phytophthora species isolated from alpine and sub-alpine regions of Australia, including the description of two new species; *Phytophthora cacuminis* sp. nov and *Phytophthora oreophila* sp. nov

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ABSTRACT

Plant deaths had been observed in the sub-alpine and alpine areas of Australia. Although no detailed aetiology was established, patches of dying vegetation and progressive thinning of canopy suggested the involvement of root pathogens. Baiting of roots and associated rhizosphere soil from surveys conducted in mountainous regions New South Wales and Tasmania resulted in the isolation of eight *Phytophthora* species; *Phytophthora cactorum*, *Phytophthora cryptogea*, *Phytophthora fallax*, *Phytophthora gonapodyides*, *Phytophthora gregata*, *Phytophthora pseudocryptogea*, and two new species, *Phytophthora cacuminis* sp. nov and *Phytophthora oreophila* sp. nov, described here. *P. cacuminis* sp. nov is closely related to *P. fallax*, and was isolated from asymptomatic *Eucalyptus coccifera* and species from the family *Proteaceae* in Mount Field NP in Tasmania. *P. oreophila* sp. nov, was isolated from a disturbed alpine herbfield in Kosciuzsko National Park. The low cardinal temperature for growth of the new species suggest they are well adapted to survive under these conditions, and should be regarded as potential threats to the diverse flora of sub-alpine/alpine ecosystems. *P. gregata* and *P. cryptogea* have already been implicated in poor plant health. Tests on a range of alpine/subalpine plant species are now needed to determine their pathogenicity, host range and invasive potential.

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1. Introduction

Mountains have been recognised as one of the world's richest biodiversity hotspots. Although mountains occupy about 12 % of the land surface, their complex mosaic of microenvironments and ecoclines support almost one quarter of its biodiversity (Körner et al., 2011). The sub-alpine zone alone occupies only 3 % of the global area, yet supports around 10,000 vascular plant species, most of which are endemic to mountains (Körner, 2004). In Australia, Kosciuszko National Park (KNP) alone contains about 1100 vascular native plant species, which represents one quarter of the New South Wales (NSW) flora in only 10 % of its land area (Doherty et al., 2015). Due to the steep environmental gradients over small spatial scales, mountainous regions are useful model systems for understanding ecological and evolutionary processes associated with biological invasions (Pauchard et al., 2016; Petitpierre et al., 2016). Any stress, biotic or abiotic, can have devastating and irreversible consequences on the distribution of species due to its very restricted climatic envelope.

Plants deaths had been observed in sub-alpine areas of Australia leading to concerns among land managers. Although no comprehensive aetiology had been established due to the assumption that lower temperatures in sub-alpine areas restrict the growth of *Phytophthora* species, such as *Phytophthora cinnamomi* (Podger et al., 1990), the progressive thinning of canopy and patches of dying vegetation in Barrington Tops National Park of KNP in the 1990's suggested the involvement of root pathogens. This assumption was confirmed when *P. cinnamomi* was isolated from dying *Oxylobium arborescens* and associated rhizosphere soil at Barrrington Tops National Park at an elevation of 1560 m above sea level (asl) (McDougall et al., 2003; Mills, 1999). Barrington Tops is a sub-alpine area with annual mean temperature 9.5 °C (extracted from downscaled 30 arc second resolution Worldclim layers) (Hijmans et al., 2005), with mean maximum and minimum temperatures of 16 °C and 3 °C, respectively at the highest altitude (Zoete, 2000).

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In 2013, surveys were conducted in sub-alpine areas of Tasmania (TAS), KNP, and Mt Toolbranup in Western Australia (WA) (Burgess et al., 2017b). Using high throughput sequencing (HTS), 33 *Phytophthora* species were detected in KNP, including *P. cinnamomi* that was detected at an elevation of 2100 m asl (almost at the highest point of mainland Australia) in asymptomatic vegetation, and in lower elevation ecosystems thought to be non-conducive, such as tall forests with deep loam soil. The detection of such a diverse *Phytophthora* community at such higher elevations was unexpected. The annual mean temperature in the sub-alpine area (Charlottes Pass; elevation 1757 m asl) of KNP is approximately 3.4 °C (Barrows et al., 2001; Edmonds et al., 2006). At Kiandra in KNP (1395 m asl) it is recorded as 6.8 °C, and for Kosciuszko Hotel (1530 m asl) it is 6.1 °C (Costin, 1954). Temperature at higher elevations would be even lower. These temperatures (except for Barrington Tops) are much lower than the mean annual temperature limit of 7.5 °C predicted for disease expression by *P. cinnamomi* (Podger et al., 1990).

A recent CLIMEX model predicted increased climate suitability for the growth of *P. cinnamomi* in most sub-alpine areas under present environmental conditions and increased suitability for the growth and survival of the pathogen under changing climatic variables (higher mean winter temperatures, seasonal precipitation shifts from summer into winter, and global warming) (Burgess et al., 2017a). This model supersedes the previous models that had only mapped the presence of the pathogen based on disease symptoms on susceptible plants, not on its survival, growth and lifecycle. The presence of a pathogen does not automatically lead to infection and disease rather the following conditions must be satisfied (i) a virulent pathogen, (ii) susceptible host(s), (iii) favourable environmental conditions, and (iv) favourable conditions for long enough for a pathogen to cause disease, and a host(s) to express symptoms.

Although many *Phytophthora* species were detected in alpine and sub-alpine areas through HTS in 2013, they were not proof of living organisms, as HTS can detect DNA from dead organisms. The current study was conducted to systematically survey sub-alpine and alpine areas to isolate living *Phytophthora* species to determine baseline *Phytophthora* species in these areas.

2. Materials and methods

2.1. Samples collection and isolation

Rhizosphere soil and associated roots were collected from asymptomatic vegetation within 5 m of road and track edges in the sub-alpine and alpine areas of NSW (KNP) in spring 2015/16, and asymptomatic vegetation in sub-alpine areas in TAS adjacent to walking tracks in May 2016. Special emphasis was placed on collecting rhizosphere soil including roots. The soil samples were placed into zip-lock plastic bags and kept in an insulated box to protect samples from high temperature and direct sunlight. In the laboratory, about 300 g of each soil sample was baited with juvenile leaves of *Quercus ilex*, *Q. suber*, *Pimelea ferruginea*, *Poplar* sp., *Scholtzia involucrata*, *Hedera helix* (Ivy), and *Hibbertia scandens*. The baited leaves were observed daily for a week. Leaves with brownish lesions were blotted dry on paper towelling, cut into 3 × 3 mm pieces, and plated onto modified NARH (Simamora et al., 2017). Plates were observed microscopically and any *Phytophthora*-like cultures (Erwin and Ribeiro, 1996) were transferred to vegetable juice agar V8A plates [100 ml/L filtered vegetable juice (Campbells V8 vegetable juice; Campbell Grocery products Ltd., Norfolk, UK), 900 ml/L distilled water, 0.1 g/L $CaCo_3$, pH adjusted to 7, and 17 g Grade A Agar Becton, Dickenson and Company, Sparks, MD, USA]. After a week, the soil was allowed to air dry and re-baited (double baiting) to increase isolation (Jeffers and Aldwinckle, 1987).

2.2. DNA isolation, amplification and sequencing

All the isolates were grown on half strength potato dextrose agar (PDA; Becton, Dickinson and Company, Sparks, USA, 19.5 g PDA, 7.5 g of agar and 1 L distilled water) for 7 d. Mycelia was harvested by scraping the agar surface with a sterile blade and placed it in a 1.5 ml sterile eppendorf® tubes. The mycelium was ground to a fine powder and genomic DNA was extracted using ZR Fungal/Bacterial DNA Miniprep™ (Zymo Research, Irvine, California). The region spanning the internal transcribed spacer (ITS) region of the ribosomal DNA was amplified using the primers DC6 (Cooke et al., 2000) and ITS-4 (White et al., 1990). The mitochondrial gene cox1 (COX) was amplified with primers FM84 and FM83 (Martin and Tooley, 2003). Heat shock protein 90 (HSP) was amplified with primers HSP 90-Fint and HSP-90 R1 (Blair et al., 2008). β-tubulin (TUB) was amplified with primers BTF1A and BTR1 (Kroon et al., 2004). NADH dehydrogenase subunit 1 was amplified with NADH-F1 and NADH-R1 primers according to Kroon et al. (2004).

Templates were sequenced in both directions with primers used in amplification for all gene regions. The clean up of PCR products and sequencing were performed as described by Sakalidis et al. (2011). All sequences derived in this study were deposited in GenBank and their accession numbers are given in Table 1. Cultures were maintained under long-term storage in water at CPSM (Centre for *Phytophthora* Science & Management), Murdoch University following identity confirmation through sequencing.

2.3. Phylogenetic analysis

The data set comprised of sequences of the new species *P.* 'cacuminis', *P.* 'oreophila', and those of closely related species in (Fig. 2), which were manually edited and compiled in Geneious v. R10 (http://www.geneious.com/). Parsimony analysis was performed in PAUP (Phylogenetic Analysis Using Parsimony) (Swofford, 2003), and Bayesian analysis with MrBayes (Ronquist et al., 2012) as plugins within Geneious software. Bayesian analyses were performed with applying a general time reversible (GTR) substitution model with inverse gamma (I). Alignment files and trees can be viewed on TreeBase (https://treebase.org/).

2.4. Cultural characteristics

Isolates were grown on V8A for seven days in the dark at 20 °C. Circular inoculum plugs were cut with a sterile cork borer (5 mm in diameter) from the colony edges and placed centrally in 90 mm Petri dishes of the test media. Colony growth patterns were described from 7-day-old cultures grown at 20 °C in the dark on V8A, 2 % malt extract agar (MEA; 20 g malt extract, 17 g agar and 1 L distilled water), carrot agar (CA; 0.1 L filtered carrot juice, 17 g agar and 0.9 L distilled water), and half-strength PDA for all species except *Phytophthora fallax* and *P.* 'cacuminis'. Colony growth patterns were described after 18 d for *P.* 'cacuminis' and *P. fallax* due to their very slow growth. Colony growth patterns were described according to Erwin and Ribeiro (1996). For temperature-growth relationship, 5 mm diameter agar plugs of all isolates were placed centrally onto V8A and incubated at 20 °C for 20 h to stimulate growth. The margins were marked and the isolates were then moved to incubators set at temperatures of 4, 10, 15, 20, 25, 30, 32.5, 35, and 37.5 °C. Plates were observed daily to make sure colonies did not reach the edges; radial growth rate was measured after 7 d for *P.* 'oreophila' and after 24 d for *P.* 'cacuminis' and *P. fallax*. Plates showing no growth at higher temperatures were returned to 20 °C to determine their viability.

Table 1
Identity, date and location of isolation, host information and GenBank accession numbers for isolates of *Phytophthora* species considered in this study. Shaded rows represent isolates that were recovered in this study. Additional information for isolates can be found in Table S1.

Isolate	Organism	Location	Vegetation	Date	GenBank Accession number				
					ITS	TUB	HSP	Cox	NADH
QLD13E	*Phytophthora* sp.	Australia, QLD[a], Koombooloomba	Tropical rain forest	2013	MG542958	MG543047	MG543034	MG543012	MG543024
U40	*P. cacuminis*	Australia, TAS[a], Mt Field NP[b]	*Eucalyptus coccifera*	2016	MG542997	MG543045	MG543032	MG543010	MG543019
U41	*P. cacuminis*	Australia, TAS, Mt Field NP	*Eucalyptus coccifera*	2016	MG542998	MG543046	MG543033	MG543011	MG543020
U11	*P. oreophila*	Australia, NSW[a], Merritts Creek	Disturbed alpine herbfield	2016	MG542976	MG543037	MG543025	MG543002	MG543013
VHS26182	*Phytophthora* sp.	Australia, WA, Fitzgerald River NP	Kwongan heathland	2006	MG543000				
TAS34	*P. cactorum*	Australia, TAS, Pine Lake	*Athrotaxis selaginoides*	2013	MG542959				
U1	*P. cactorum*	Australia, NSW, Merritts Creek	Disturbed alpine herbfield	2016	MG542966				
U2	*P. cactorum*	Australia, NSW, Merritts Creek	Disturbed alpine herbfield	2016	MG542967				
U3	*P. cactorum*	Australia, NSW, Merritts Creek	Disturbed alpine herbfield	2016	MG542968				
U4	*P. cactorum*	Australia, NSW, Charlottes Pass	*Eucalyptus niphophila*	2016	MG542969				
U5	*P. cactorum*	Australia, NSW, Charlottes Pass	*Eucalyptus niphophila*	2016	MG542970				
U6	*P. cactorum*	Australia, NSW, Merritts Creek	Disturbed alpine herbfield	2016	MG542971				
U7	*P. cactorum*	Australia, NSW, Charlottes Pass	*Eucalyptus niphophila*	2016	MG542972				
U8	*P. cactorum*	Australia, NSW, Charlottes Pass	*Eucalyptus niphophila*	2016	MG542973				
W1846	*P. cambivora*	Australia, NSW, Charlottes Pass	*Nematolepis ovatifolia*	2014	MG543001				
TAS188	*P. cinnamomi*	Australia, TAS, Condominium Creek	Riparian rain forest	2013	MG542963				
VHS16127	*P. constricta*	Australia, WA, Fitzgerald River NP	Kwongan heathland	2006	HQ013224				
VHS16130	*P. constricta*	Australia, WA, Fitzgerald River NP	Kwongan heathland	2006	HQ01327				
U21	*P. cryptogea*	Australia, NSW, Mt Kosciuszko	Walking track edge in alpine heath	2016	MG542983				
U22	*P. cryptogea*	Australia, NSW, Mt Kosciuszko	Walking track edge in alpine heath	2016	MG542984				
TAS126	*P. elongata*	Australia, TAS, Mt Field NP	Riparian rain forest	2013	MG542960				
U34	*P. fallax*	Australia, TAS, Hartz Mountain NP	Melaleuca	2016	MG542991	MG543043	MG543030	MG543008	MG543017
U35	*P. fallax*	Australia, TAS, Hartz Mountain NP	Alpine heath	2016	MG542992	MG543044	MG543031	MG543009	MG543018
U36	*P. fallax*	Australia, TAS, Hartz Mountain NP	*Melaleuca* sp.	2016	MG542993				
U37	*P. fallax*	Australia, TAS, Hartz Mountain NP	*Melaleuca* sp.	2016	MG542994				
U14	*P. gonapodyides*	Australia, NSW, Smiggins Hole	Road edge in subalpine heath	2016	MG542979	MG543038	MG543026	MG543003	MG543014
U15	*P. gonapodyides*	Australia, NSW, Kosciuszko Road	Disturbed alpine herbfield	2016	MG542980	MG543039	MG543027	MG543004	MG543015
TAS206	*P. gregata*	Australia, TAS, Pine Lake	Moorland	2013	MG542964				
TAS207	*P. gregata*	Australia, TAS, Pine Lake	Moorland	2013	MG542965				
U9	*P. gregata*	Australia, NSW, Pipers Gap	Road edge in subalpine heath	2016	MG542974				
U10	*P. gregata*	Australia, NSW, Pipers Gap	Road edge in subalpine heath	2016	MG542975				
U12	*P. gregata*	Australia, NSW, Perisher	Disturbed subalpine wetland	2016	MG542977				
U13	*P. gregata*	Australia, NSW, Perisher	Disturbed subalpine wetland	2016	MG542978				
U18	*P. gregata*	Australia, NSW, Pipers Gap	Road edge in subalpine heath	2016	MG542981				
U32	*P. gregata*	Australia, NSW, Perisher	Disturbed subalpine wetland	2016	MG542989				
U38	*P. gregata*	Australia, TAS, Hartz Mountain NP	*Melaleuca* sp.	2016	MG542995				
U39	*P. gregata*	Australia, TAS, Hartz Mountain NP	*Melaleuca* sp.	2016	MG542996				
U42	*P. gregata*	Australia, TAS, Hartz Mountain NP	*Melaleuca* sp.	2016	MG542999				
CBS139749	*P. pseudocryptogea*	Australia, WA, Fitzgerald River NP	*Isopogon buxifolius*	2006	KP288376	KP288392	KP288426	KP288342	KP288360
VHS5380	*P. pseudocryptogea*	Australia, WA, Fitzgerald River NP	*Xanthorrhoea preissii*	1992	KP288374	KP288390	KP288424	KP288340	KP288358
TAS143	*P. pseudocryptogea*	Australia, TAS, Steppes	Woodland	2013	MG542962				
U20	*P. pseudocryptogea*	Australia, NSW, Island Bend	Highly modified montane forest	2016	MG542982				
U23	*P. pseudocryptogea*	Australia, NSW, Island Bend	Highly modified montane forest	2016	MG542985				
U24	*P. pseudocryptogea*	Australia, NSW, Island Bend	Highly modified montane forest	2016	MG542986				
U30	*P. pseudocryptogea*	Australia, NSW, Island Bend	Highly modified montane forest	2016	MG542987				
U31	*P. pseudocryptogea*	Australia, NSW, Island Bend	Highly modified montane forest	2016	MG542988				
U33	*P. pseudocryptogea*	Australia, NSW, Island Bend	Highly modified montane forest	2016	MG542990				
CBS119107	*P. captiosa*	New Zealand, Rotoehu Forest	*Eucalyptus saligna*	1995	DQ297402				
NZFS310.35	*P. captiosa*	New Zealand, Rotoehu Forest	*Eucalyptus saligna*	1998	DQ297405				

(*continued on next page*)

Table 1 (*continued*)

Isolate	Organism	Location	Vegetation	Date	GenBank Accession number				
					ITS	TUB	HSP	Cox	NADH
MUCC761	*P. gonapodyides*	Australia, VIC[a], Toolangi North	*Eucalyptus oblique* forest	2008	HQ012937	JN547598	HQ012896	HQ012850	JN547686
CBS127954	*P. thermophila*	Australia, WA, Dwelling up	*Eucalyptus marginata*	2004	EU301155	JN547613	HQ012916	HQ012872	JN547700
TP13.29	*P. versiformis*	Australia, WA, Naturaliste	*Corymbia calophylla*	2013	KX011277	KX011318	KX011254	KX011220	KX011299
CBS 142005	*P. versiformis*	Australia, WA, Williams	*Corymbia calophylla*	2013	KX011279	KX011321	KX011256	KX011222	KX011302
HAS2313	*P. cooljarloo*	Australia, WA, Cooljarloo	Swamp native vegetation	1996	HQ012961	MF326817	HQ012929	HQ012885	MF326911
VHS24266	*P. pseudorosacearum*	Australia, WA, Albany	*Xanthorrhoea platyphylla*	2010	JN547637	MF326826	MF326877	MF326857	MF326909
OSU55	*P. rosacearum*	USA, Maryland	*Prunus armeniaca*		KJ372271	MF326833	MF326882	MF326854	MF326902
VHS29592	*P. pseudorosacearum*	Australia, WA, Jarrahdale	*Persoonia longifolia*	2013	KJ372267	MF326827	MF326878	MF326858	MF326907
VHS23298	*P. kwongonina*	Australia, WA, Bunbury	*Banksia grandis*	2010	JN547636	MF326824	MF326876	MF326847	MF326914
TAS35	*P. gonapodyides*	Australia, Tas, Houn River	Native vegetation	2009	JN547620	JN547642	MG543031	JN547581	JN547669
IMI389735	*P.* taxon walnut	USA, California, Merced County	*Juglans hindsii*	1988	AF541910				
CLJ0100	*P. cooljarloo*	Australia, WA, Cooljarloo	Hibbertia sp	2008	HQ012957	MF326816	HQ012925	HQ012881	MF326910
CBS124696	*P. rosacearum*	USA, California			EU925376				
P10725	*P. fallax*	New Zealand	*Eucalyptus fastigata*	2004	HQ261557				
CBS125801	*P. constricta*	Australia, WA, Fitzgerald River NP	Kwongon heathland	2006	HQ013225				
NZFS310.25	*P. captiosa*	New Zealand, Rotoehu Forest	*Eucalyptus saligna*	1998					

[a] QLD = Queensland, TAS = Tasmania, VIC = Victoria, NSW = New South Wales.
[b] NP = National Park.

2.5. Morphology of sexual and asexual structures

Isolates were grown on V8A for seven days and 3–4 agar plugs (5 mm diameter) were taken from the edges and placed in sterile empty Petri dishes. Each Petri dish was flooded with 10 % clarified V8 broth (Erwin and Ribeiro, 1996) until the broth was just above the surface of the agar plugs, and was kept in an incubator set at 20 °C to stimulate mycelial growth overnight. The following day, plates were flooded with deionized water. This water was decanted and replaced twice (after 4 and 6 h). In the final change, 7–10 drops of non-sterile pine (*Pinus radiata*) bark extract were added to the water in each plate. The pine bark extract was made by suspending 100 g of pine bark potting mixture in 1 L distilled water, and incubated overnight (Aghighi et al., 2012). After 18–22 h, dimensions and characteristic features of 50 mature sporangia, selected at random, were measured at 40x in a BX51 Olympus microscope for each isolate.

Phytophthora 'oreophila' was homothallic. *Phytophthora* 'cacuminis' was crossed with A1 and A2 mating types of two different species (*P. nicotianae* and *Phytophthora cryptogea*), but no oospore formation was observed. After four weeks, dimensions and characteristics of 50 randomly selected mature oogonia and oospores were measured at 40x for *P.* 'oreophila'. The oospore wall index was calculated as described by Dick (1990).

3. Results

3.1. Phytophthora *species isolated from sub-alpine and alpine areas*

Eight *Phytophthora* species were recovered from 11 (46 %) of the 24 soil samples tested. Thirty-two isolates corresponding to three species (*Phytophthora* 'cacuminis', *P. fallax* and *Phytophthora gregata*) were recovered from baiting the six samples collected from asymptomatic vegetation adjacent to walking tracks in TAS, and 57 isolates corresponding to six species (*Phytophthora cactorum*, *Phytophthora gonapodyides*, *Phytophthora pseudocryptogea*, *P. cryptogea*, *P. gregata* and *Phytophthora* 'oreophila') were recovered from baiting 18 samples collected from asymptomatic vegetation within 5 m of roads and track edges in NSW (Table 2). Isolates of all *Phytophthora* species with their closest relatives, considered in this study, and locations of isolation and altitude are listed in (Table 1, Supplementary Table 1). Of the six *Phytophthora* species recovered from KNP, four species i.e. *P. cactorum*, *P. gonapodyides*, *P. pseudocryptogea*, and *P.* 'oreophila' were isolated from this region for the first time through baiting. The most frequently isolated species in KNP from all sites were *P. cactorum* and *P. gregata*. *P. cryptogea* was isolated from the alpine area at the summit of Mt Kosciuszko (2228 m asl). This is also the first record on the recovery of living isolates of *P. fallax* and *P.* 'cacuminis' in TAS. The most frequently isolated species in TAS was *P. gregata*, and it was the only species isolated in both states. It has also been implicated in *Pimelea bracteatea* dieback in Rocky Plains in KNP (Fig. 1).

3.2. Phylogenetic analysis

The alignments for TUB, HSP, ITS, COX and NADH consisted of 1178, 936, 846, 1236 and 837 characters, respectively. Trees for the individual datasets produced similar topology (TreeBASE 22955) and the nuclear and mitochondrial gene regions were combined separately for the analyses presented here.

Support for terminal clades and their clustering was equivalent in both analyses and the Bayesian analysis is presented here (Fig. 2). All species reside in highly supported terminal clusters.

Fig. 1. Dieback disease symptoms on *Pimelea bracteatea* caused by *Phytophthora gregata* in Kosciuszko National Park. (A) healthy plants; (B) severe dieback and thinning leading to loss of aerial canopy giving the plants 'sticks' like appearance; and (C) root collar showing necrotic lesions resulting in the death of aerial stem.

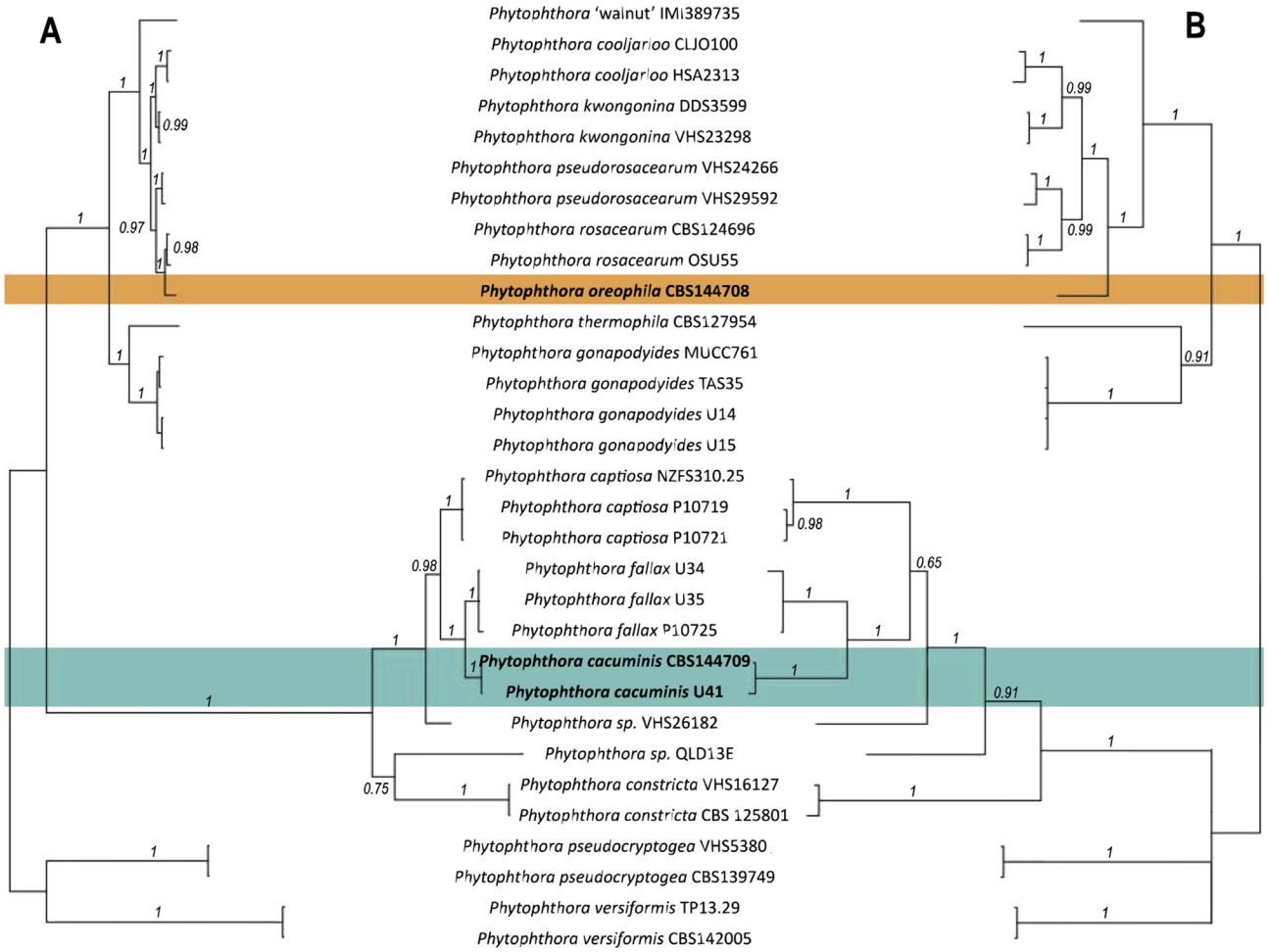

Fig. 2. Bayesian trees of (A) concatenated nuclear regions and (B) concatenated mitochondrial regions showing the phylogenetic position of *P. oreophila* (orange) and *P. cacuminis* (blue) in relation to related species. Bayesian posterior probabilities are listed above the branches. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Phytophthora 'oreophila' resides in clade 6a and is related to *P. rosacearum* and *P. pseudorosacearum*, but differs from them across the gene regions sequenced here by 39 and 64 polymorphisms, respectively. *Phytophthora* 'cacuminis' resides in clade 9 and is closely related to the known eucalypt pathogens *P. fallax and Phytophthora captiosa*, although it differs from them by 75 and 116 fixed polymorphisms, respectively.

4. Taxonomy

Phytophthora oreophila I. Khaliq and T.I Burgess, **sp. nov.**
Mycobank MB825232
(Figs. 3 and 5)

Etymology: '*oreos*' refers to a Greek word for a mountain and '*phila*' means loving. The name is given to the species due to its

Table 2
Number of isolates of each *Phytophthora* species recovered through baiting in Kosciuszko National Park and Tasmania.

Phytophthora species	Clade	Number of isolates recovered	
		NSW (KNP)	TAS
P. cactorum	1	24	
P. gregata	6	15	16
P. pseudocryptogea	8	8	
P. cryptogea	8	5	
P. fallax	9		14
P. gonapodyides	6	4	
P. cacuminis	9		2
P. oreophila	6a	1	
Total		57	32

mountainous origin, and its ability to grow at extremely low temperatures (less than 4 °C).

Type: **Australia**: New South Wales, Merritts Creek; by baiting rhizosphere soil and associated roots collected from a disturbed alpine herbfield, Jan 2016. Collected by *Keith McDougall* (Office of Environment and Heritage, PO Box 733, Queanbeyan NSW 2620). Holotype MURU 483 (dried culture on V8A, Herbarium of Murdoch University, Western Australia), cultures ex-type U11. ITS, cox1, NADH, HSP90 and β tubulin sequence GenBank numbers are MG542976, MG543002, MG543013, MG543025, and MG543037, respectively.

Original Description: Sporangia were exclusively non-papillate, persistent and frequently produced in non-sterile pine bark extract. They were borne terminally on simple sporangiophores rarely with globose swellings (2 %) produced near the base of sporangia (Fig. 3G). Although predominantly ovoid (80 %, Fig. 3A, B, F, H, I), a few ellipsoid (12 %, Fig. 3C–E, G), and distorted shaped (8 %, Fig. 3J) sporangia were also observed. Sporangia averaged 40.9 × 26.7 μm, ranged 19.9 × 59.9–13.4 × 38.5 μm, exit pores 12.8 μm in diameter, and length: breadth ratio was 1.5 (Table 3). Sporangia proliferated internally in both an extended (Fig. 3H) and a nested way (Fig. 3I, K). Intercalary hyphal swellings with radiating hyphae formed occasionally in non-sterile pine bark extract (Fig. 3 L, M). Zoospore cysts were spherical with average diameter 9.7 μm (Table 3).

P. oreophila is homothallic, readily produces oogonia, oospores and antheridia in single culture on CA, MEA and V8A. Time to oospore maturity was between 25 and 30 d. Oogonia averaged 36.8 μm in diameter ranging from 29.3 to 48.1 μm (Table 3). Plerotic oospores containing ooplasts when semi-mature to mature (Fig. 3O–R). Oospores averaged 33.8 μm in diameter ranging from 26.8 to 42 μm. Oospore walls were relatively thick (2.2 μm) (Fig. 3N–V), and oospore wall index was 0.34 μm (Table 3). Paragynous antheridia (Fig. 3 N-R, U, V) averaged 10.9 × 10.6 μm in diameter often (16 %) with multiple antheridium (Fig. 3 O, P, Q, V). Most of the oospores (90 %) observed aborted after wall formation (Fig. 3S–V).

Cultures: P. oreophila produced a slightly petaloid growth pattern on CA, petaloid growth pattern on V8A and MEA, and rosaceous growth pattern on PDA (Fig. 5). The colony morphology of *P. oreophila* was clearly distinguishable from *P. rosacearum* as the latter produced uniform colonies on V8A and MEA compared to the petaloid growth pattern of *P. oreophila*. Optimum temperature for the growth on V8A was 20 °C, and the average growth rate was 4.92 mm day^{-1} at this temperature. The maximum temperature for growth was 32.5 °C, and the lethal temperature for growth was recorded as 35 °C (Table 3).

Diagnosis: P. oreophila is closely related to *P. rosacearum* and *P. pseudorosacearum* but there are several differences; (1) *P. oreophila* has lower minimum, optimal and maximum temperatures for growth (Table 3, Fig. 6); (2) *P. oreophila* grows faster than related species at temperatures less than 20 °C (Fig. 6); (3) colony morphologies also differ on V8A and MEA, as *P. oreophila* produced a petaloid growth pattern compared to uniform colony growth by *P. rosacearum*, and *P. pseudorosacearum* (Fig. 5); and (4) *P. oreophila* has smaller sporangia and slightly larger oogonia and oospores (Table 3).

Phytophthora cacuminis I. Khaliq and T.I Burgess, **sp. nov**
MycoBank MB825231.
(Figs. 4–5)

Etymology: The species name *cacuminis* is derived from a Latin word '*cacumen*' for a 'peak'. The name is given to the species based on its isolation from a peak in Tasmania.

Type: **Australia:** south Australia: Tasmania, Mount Field NP, from asymptomatic vegetation (*Eucalyptus coccifera* and species in *Proteaceae*), May 2016, collected by *Treena Burgess*, holotype MURU 482 (dried culture on V8A, Herbarium of Murdoch University, Western Australia), cultures ex-type U40. ITS, cox1, NADH, HSP90 and β tubulin sequence GenBank numbers are MG542998, MG543011, MG543020, MG543033, and MG543046, respectively.

Original Description: Exclusively non-papillate, terminal, persistent and predominately ovoid sporangia (90 %, Fig. 4A–D, F, G, I, J), but a few globose sporangia (6 %, Fig. 4H) and lemoniform (4 %, Fig. 4E) sporangium were also observed. Sporangia averaged 27.4 × 22.4 μm in diameter ranging from 14.5 × 40.2 to 10.8 × 37.3 μm (Table 3). Internal proliferations both in a nested (Fig. 4F, H, I, J) and extended (Fig. 4G) way were observed. Exit pores diameter averaged 9.9 μm in diameter. Sporangiophores were mostly slightly twisted and narrowed (Fig. 4 A-C, E-I). Zoospore cysts were spherical with average diameter 10.7 μm. Chlamydospores were present (Fig. 4K and L), with average diameter 30.2 μm (Table 3). No hyphal swellings were observed.

Phytophthora cacuminis isolates were sterile in culture; no oogonia or oospores were formed when isolates were crossed with A1 and A2 mating type isolates of two different species (*P. nicotianae* and *P. cryptogea)*.

Cultures: All isolates produced colonies with distinctive growth patterns on different media (Fig. 5). Colonies had a halo of submerged hyphae on CA, plumose growth pattern on V8A, uniform growth on MEA and dense growth on half PDA. The colony morphology of *P. cacuminis* was clearly distinguishable from *P. fallax* on V8A as the former produced plumose growth pattern on V8A compared to uniform growth pattern of *P. fallax*. The optimum temperature for growth on V8A was 20 °C with a growth rate of 1.2 mm/day. The maximum temperature for growth was recorded as 25 °C (Table 3). No growth occurred at 30 °C, and this temperature was found to be lethal as isolates did not resume growth when subsequently incubated at 20 °C. *P. fallax* was markedly slower growing than *P. cacuminis* (Table 3, Fig. 6).

Diagnosis: P. cacuminis is closely related to *P. fallax* but it distinguishable from *P. fallax* in many ways; (1) *P. cacuminis* is sterile in culture compared to the homothallic nature of *P. fallax*; (2) *P. cacuminis* produces on average smaller sporangia; (3) sporangiophores are frequently slightly twisted and narrowed, similar to *P. constricta*, a closely related species in the same clade. It had been suggested by Jung et al. (2011) that *P. constricta* was in the process of becoming caducous because of this feature; (4) colony morphologies also differ, as *P. cacuminis* produces a plumose growth pattern on V8A, while *P. fallax* has a uniform growth pattern on the same medium; and (5) maximum temperature for growth for *P. cacuminis* is 25 °C, and for *P. fallax* is 30 °C. The later has also markedly slower growth rate than *P. cacuminis* (Fig. 6): 30 °C was found to be lethal for *P. cacuminis*, but not for *P. fallax*.

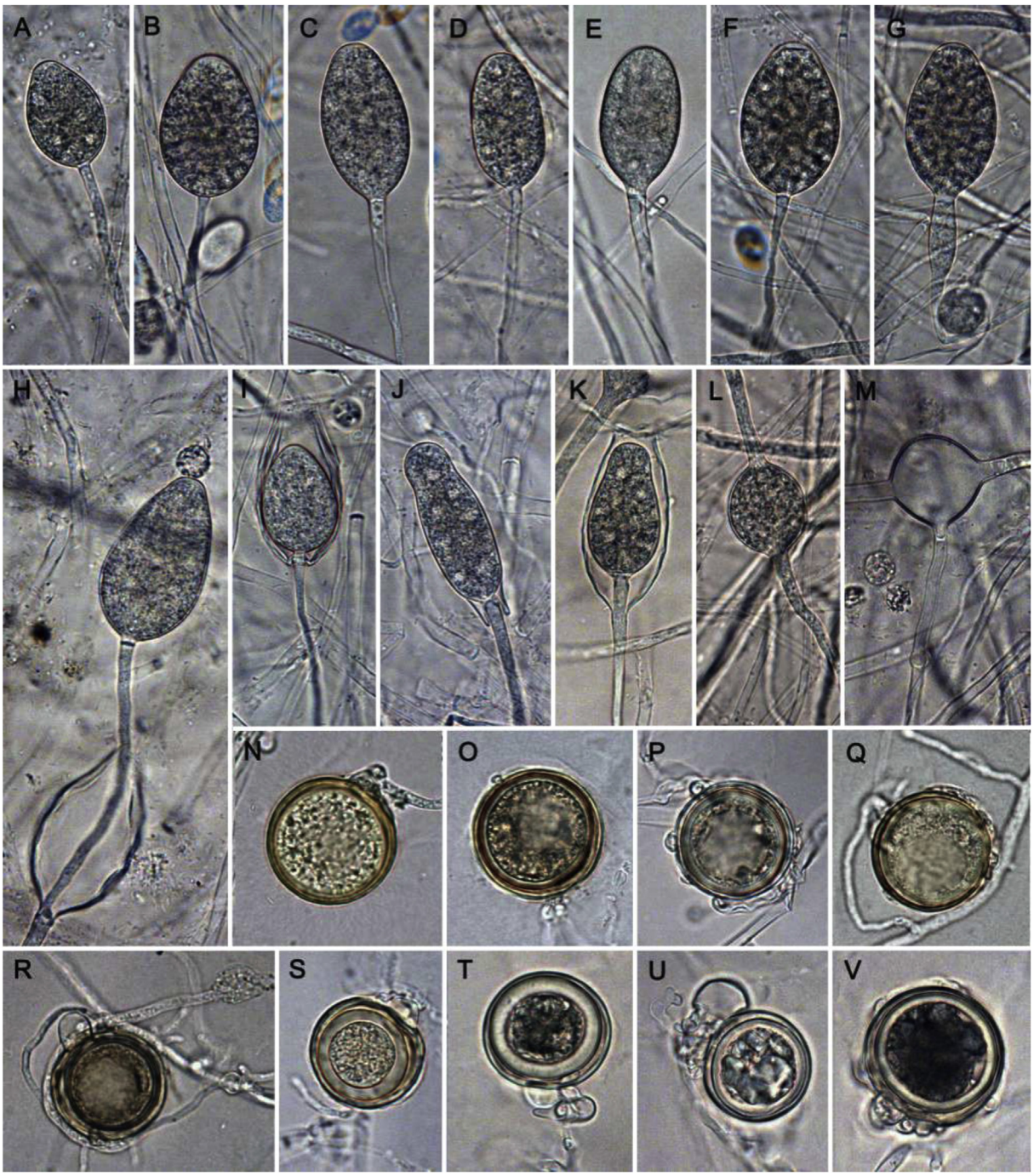

Fig. 3. Persistent non-papillate sporangia of *P. oreophila* formed on V8A flooded with pine bark extract; ovoid (A, B, F, H, I), ellipsoid (C–E, G), and distorted (J). Internal proliferations in a nested (I, K) and an extended way (H) were observed. Intercalary hyphal swellings (L, M). Swollen sporangiophore (G) rarely observed. Sporangiophores were occasionally twisted (F, I). Oogonia formed on solid media; globose oogonia with smooth margins that turned pale brown (N–R) on maturity, with plerotic oospores (N–R) and paragynous antheridia (N–R, U, V). Oospore with more than one antheridium was occasionally observed (O, P, Q, V). Oospores often abort after wall formation (S–V). Oospores rarely surrounded by hyphal coil (R). Scale bar = 25 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

5. Discussion

Eight *Phytophthora* species from phylogenetic clades 1, 6, 8 and 9 were recovered from alpine and sub-alpine areas of NSW and TAS by baiting rhizosphere soil and associated roots; two of these were new species. This is the first record on the recovery *P. cactorum*, *P. gonapodyides*, *P. pseudocryptogea*, and *P. oreophila* in KNP, and for *P. fallax*, and *P. cacuminis* in TAS. The other species have been recorded before, but this is the first time that any species, in this case *P. cryptogea*, has been recovered from the summit of Mt Kosciuszko-the highest point on mainland Australia (2228 m asl).

P. gregata was one of the most frequently isolated species. *P. gregata* belongs to phylogenetic clade 6 and has been previously isolated from natural vegetation in WA, and formally described by Jung et al. (2011). It had been referred to as *P.* taxon raspberry previously (Brasier et al., 2003; Jung et al., 2011). This species was also recovered from soil and water samples (Dunstan et al., 2016) and raspberry roots (Brasier et al., 2003) in Victoria, Pine Lake in

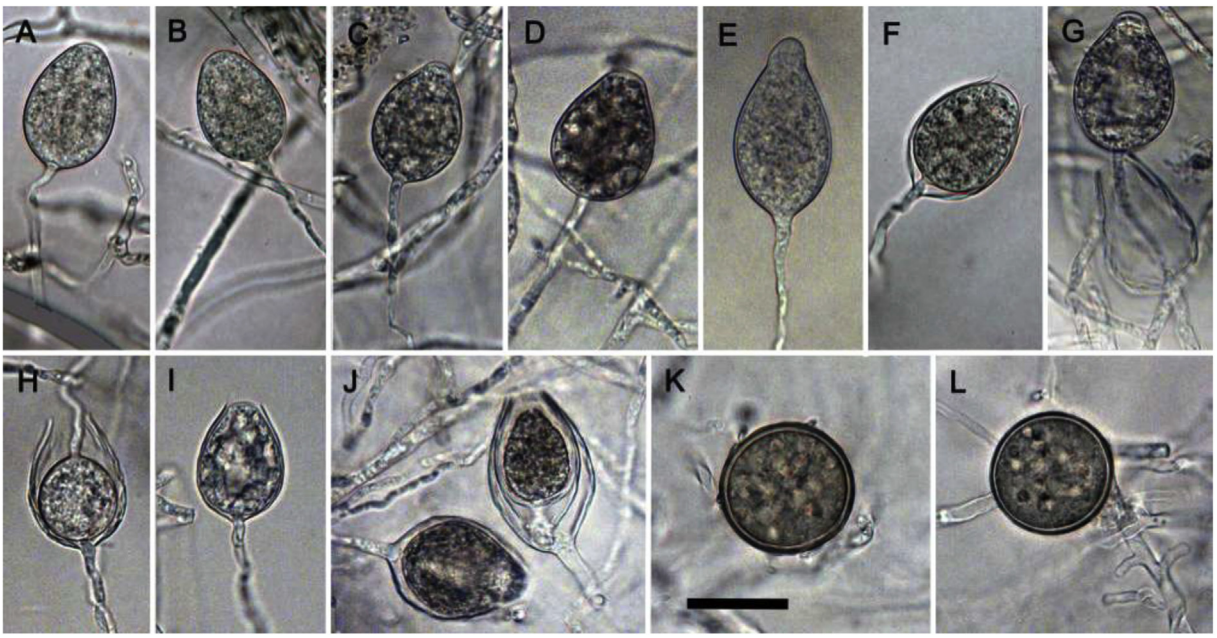

Fig. 4. Persistent non-papillate sporangia of *P. cacuminis* formed on V8A flooded with pine bark extract; predominantly ovoid (A–D, F, G, I, J) with internal proliferations in a nested (F, H, I, J) or an extended way (G). Lemoniform (E) and globose (H) sporangia were also observed. Chlamydospores were frequently observed (K, L). Sporangiophores frequently slightly twisted and/or narrowed (A–C, E–I). Scale bar = 25 μm.

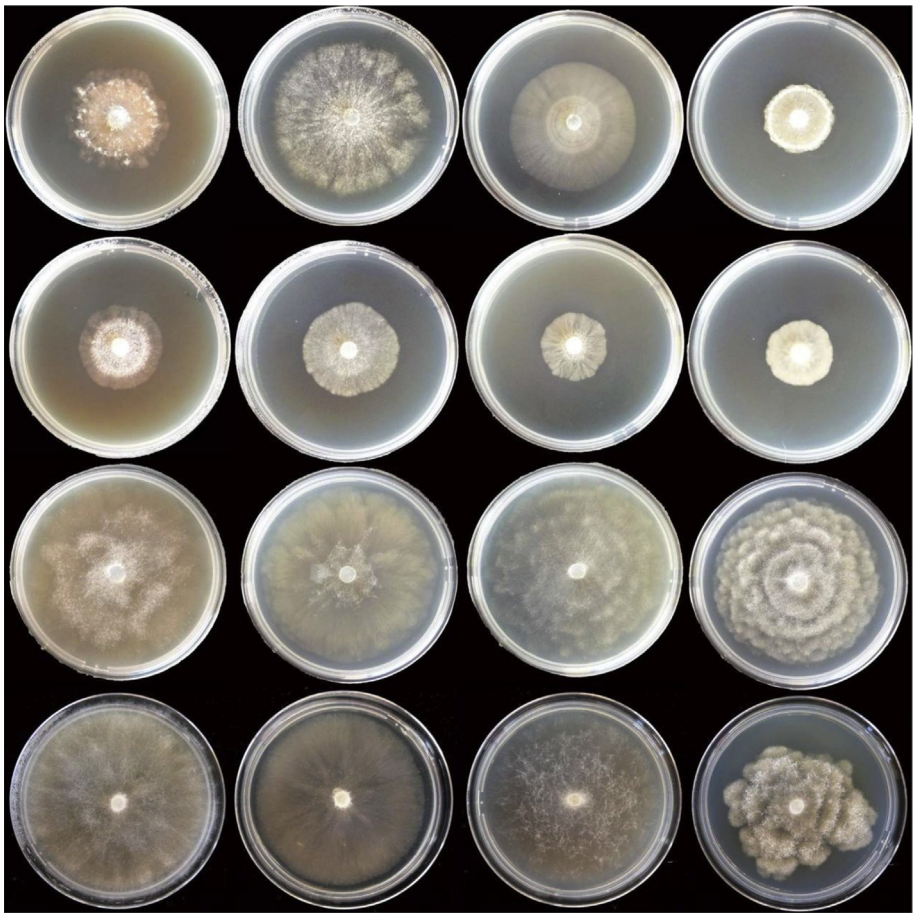

Fig. 5. Colony morphology (top to bottom) of isolates *P. cacuminis*, *P. fallax*, *P. oreophila*, and *P. rosacearum* on CA, V8A, MEA and half strength PDA (left to right).

Table 3
Comparison of morphological characters and dimensions, and temperature-growth relations of *P. rosacearum*, *P. pseudorosacearum*, *P. oreophila*, *P. fallax* and *P. cacuminis*. All measurements are in μm.

Species	*P. rosacearum*	*P. pseudorosacearum*	*P. oreophila*	*P. fallax*	*P. cacuminis*
no of isolates	6	3	1	9	2
sporangia					
LxB mean ± SD	46.4 ± 8.7 × 28.7 ± 4.8	52.7 ± 10.0 × 34.1 ± 5.6	40.9 ± 10.3 x ± 26.7 ± 6.08	55.5 ± 5 × 32 ± 3	27.4 ± 5.9 × 22.4 ± 4.42
Total Range	22.5–73.4 × 16.7–40.1	32.7–59.3 × 19.4–38.3	19.98–59.99 × 13.4–38.5	50.5–61.5 × 28 - 34	14.5–40.2 × 10.8–37.3
Range of isolates means	43.7–47.9 × 23.7–31.9	49.4–56.0 × 30.7–37.8	na[a]	nd[b]	27.4–22.4 × 27.4–22.4
L/B ratio (range)	1.63 ± 0.25 (1.05–2.36)	1.57 ± 0.31 (1.02–2.48)	1.54 ± 0.2 (0.79–2.35)	1.7	1.22 ± 0.13 (0.76–1.57)
Features	Terminal, persistent non papillate	Terminal, persistent non papillate	Terminal, persistent non papillate	Terminal, persistent non papillate	Terminal, persistent non papillate
Sporangiophores	Simple	Simple	Simple	Simple	Simple, but frequently slightly twisted and narrowed
Shapes	ovoid 56 % ellipsoid ovoid 29 % broad ovoid 2 % ellipsoid 10 % limonoform 2 %	ovoid 55 % elongated ovoid 30 % lemoniform 5 % ellipsoid 5 % broad ovoid 5 %	ovoid 80 % ellipsoid 12 % distorted 8 %	Obpyriform to distorted, often with a distinctive elongated neck and conspicuous basal plugs, hyphal projections at apex	ovoid 90 % globose 6 % lemoniform 4 %
Proliferation	Internal, both nested and extended	Internal, both nested and extended	Internal, both nested and extended	Internal, both nested and extended	Internal, both nested and extended
Exit pores					
Width (range)	12.6 ± 2.8 (5.7–18.6)	14.9 ± 2.7 (8.8–20.2)	12.84 ± 4.13 (5.88–19.83)	nd	9.88 ± 1.2 (7.4–11.6)
Zoospore cysts	11.9 ± 1.2 (9.6–16.0)	11.6 ± 1.8 (8.0–19.9)	9.70 ± 2.87 (6.11–13.13)	nd	10.68 ± 2.69 (8–15.69)
Chlamydospores	Absent	present	present	present	present
Diameter (range)		28.4 ± 5.3 (20.1–42.7)		12–26	30.2 ± 2.6
Hyphal swellings	present	present	present	absent	absent
Features	predominantly spherical and intercalary with radiating hyphae	predominantly spherical and intercalary with radiating hyphae	predominantly spherical and intercalary with radiating hyphae	nd	absent
Mean diam	17.6 ± 5.7 (9.0–27.8)	17.8 ± 6.0 (6.1–30.9)	20.7 ± 5.6 (10.9–31.2)		18.13
Breeding system	Homothallic	Homothallic	Homothallic	Homothallic	Sterile in culture
Oogonia					
Features	slightly wavy walls	wavy walls, sometimes with a slightly tapering base	spherical, pale brown on maturity	spherical, pale brown on maturity	
Mean diam	36.1 ± 3.9 (23.8–47.3)	35.8 ± 4.9 (23.8–49.0)	36.8 ± 4.8 (29.3–48.1)	33.5 ± 3	
Range of isolates means	32.6–38.8	33.1–37.4	na	30–39	
Oospores					
Features	Slightly aplerotic, pale on maturity	aplerotic, slightly golden on maturity and often slightly eccentric	plerotic, pale on maturity	Initially plerotic, become aplerotic with age	
Abortion	59 %	20 %	90 %	nd	
Mean diam	31.2 ± 3.4 (20.3–41.0)	30.8 ± 3.3 (22.3–38.1)	33.8 ± 4.49 (26.8–42)	31.5 ± 2.5	
Range of isolates means	28.4–35.4	29.5–31.8	na	29–35	
Wall diameter	2.05 ± 0.47	2.46 ± 0.47	2.23 ± 0.86	2 ± 0.5	
Oospore wall index	0.34 ± 0.06	0.41 ± 0.06	0.34 ± 0.11	nd	
Antheridia					
Features	Paragynous round-club shaped, predominantly adjacent to oogonial stalk, very few amphigynous in some isolates	Paragynous round-club shaped, predominantly adjacent to oogonial stalk	Paragynous round-club shaped, often with multiple antheridium	Paragynous (globose), amphigynous (cylindrical and single celled), attached near stalk	
LxB mean	12.9 ± 2.5 × 9.4 ± 2.1	13.8 ± 3.9 × 11.4 ± 3.2	10.9 ± 2.8 × 10.6 ± 14.7	18.5 ± 4 × 14 ± 1	
LxB range	7.5–19.2 × 4.7–13.9	6.1–26.6 × 5.5–22.1	5.65–16.9 × 3.7–11.9	nd	
Growth Characteristics					
Max temp (°C)	37.5	37.5	32.5	30	25
Opt temp (°C)	25–30	30	20	20	20
Min temp (°C)	4	4	<4	2	4
Lethal temp (°C)	>37.5	>37.5	35	> 30 <32.5	>25 < 30
Growth rate on V8A at optimum (mm day^{-1})	5.94 ± 0.1	5.2 ± 0.40	4.9 ± 0.06	0.83	1.24 ± 0.02

[a] na = not available.
[b] nd = not determined or reported in original species description.

TAS (Brasier et al., 2003; Jung et al., 2011), China (Huai et al., 2013), the US (Aram, 2017), and Sweden and France (Brasier et al., 2003; Redondo et al., 2018). It was also detected in KNP and TAS in 2013 using HTS (Burgess et al., 2017b). *P. gregata*, along with *P. cryptogea*, is implicated in the widespread death of the endemic shrub *Pimelea bracteata* in wetlands and riparian vegetation in northern KNP and surrounding areas. Plants at all growth stages are affected and there is very little or no regeneration (McDougall et al.

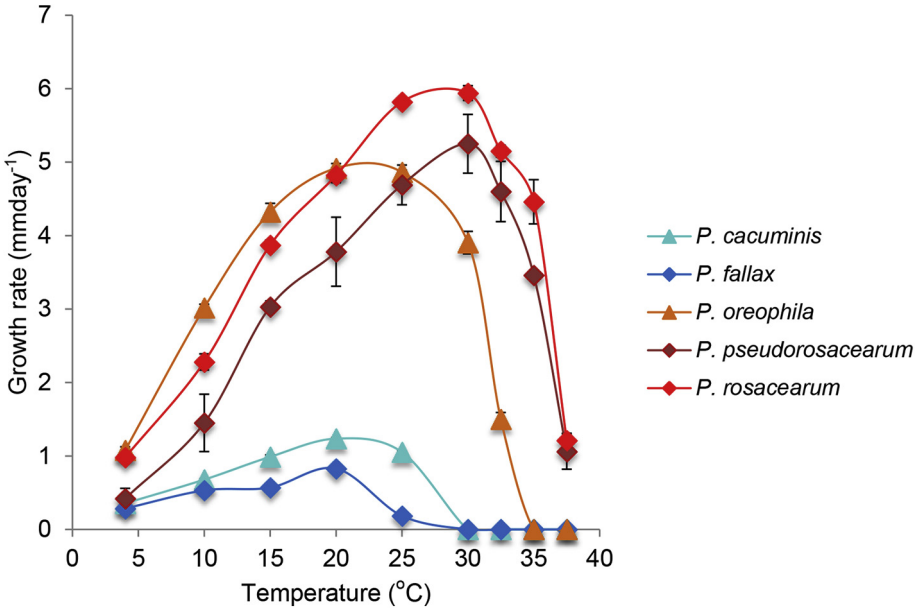

Fig. 6. Average growth rate (mm day^{-1}) of *P. cacuminis*, *P. fallax*, *P. oreophila*, *P. pseudorosacearum* and *P. rosacearum* on V8A across the temperature range from 4 to 37.5 °C.

2018). *P. gregata* has been shown to significantly reduce shoot/and or root growth of *Eucalpytus marginata*, *Corymbia calophylla*, *Banksia occidentalis*, *B. litoralis*, and *Lambertia infermis* in recent pathogenicity trials, although it did not kill them (Belhaj et al., 2018). It is not known if this species is native or introduced to Australia, however its widespread occurence in Europe in cooler climates suggests it is probably introduced.

P. cactorum was the other most frequently isolated species; it is a species in phylogenetic clade 1, has a broad host range affecting 150 plant species (Nienhaus, 1960), and causes diseases from topical to temperate climates (Liu et al., 2018; Rytkönen et al., 2008). It has been isolated in agricultural systems in much of temperate eastern Australia, and was also detected in KNP and TAS using HTS (Burgess et al., 2017b). Although it has not been associated with a disease in the sub-alpine areas in Australia so far, it should be regarded as a serious threat due to its capability to cause disease at relatively low temperatures, and in many hosts. Liu et al. (2018) studied the effect of temperature on infection and development of fruit rot caused by *P. cactorum* and observed that young apple fruits inoculated with zoospores of *P. cactorum* developed visible lesions from 10 to 30 °C, with optimum temperature being 23.5 °C. Similarly, incidence and severity of *P. cactorum* increased with increased wetness duration (1–12 h) over a temperature range of 10–30 °C on pears, and 7–10 °C on apples under controlled environmental conditions (Grove and Boal, 1991). *P. cactorum* has been reported to readily form chlamydospores in V8A juice broth and mycelial mats buried in pasteurized soil at 4 °C after 20 d of incubation (Darmono and Parke, 1990). Chlamydospores had high (60–80 %) germination rates even after incubating at −23 °C for 24 h (Darmono and Parke, 1990). It is considered to have been introduced to lowlands in Australia and then spread to mountain ecosystems by human activities (Burgess et al., 2017b).

P. fallax belongs to phylogenetic clade 9, and was first described on *Eucalyptus* causing leaf spots, petiole, twigs and small branches infection in New Zealand (Dick et al., 2006). It has previously been recovered in *Eucalyptus regnans* forests in Victoria (Cunnington et al., 2010; Dunstan et al., 2016), and in sub-alpine areas using HTS (Burgess et al., 2017b). Dick et al. (2006) evaluated its temperature-growth relationships when describing this species and suggested that it was adapted to colder conditions due to its low cardinal temperatures. To date, no disease has been associated with this species in Australia. The reason could be the nature of the lesions they cause, which usually have no distinctive patterns (Dick et al., 2006). Another reason could be its mode of infection. It has been observed to cause disease only in the inaccessible crown of trees, 6–20 m high, with an unknown mode of dispersal (Dick et al., 2006). Therefore, it is difficult to observe symptoms from the ground. Finally, due to its affinity to lower temperatures, it is also possible that *P. fallax* may have been living in the alpine and sub-alpine regions for a long time, and may have co-evolved in these ecosystems attaining an equilibrium and is, therefore, not causing any observable symptoms. This species appears to be native to Australia's mountain ecosystems due to its adaptability to these colder ecosystems and its growth and survival at relatively lower temperatures.

P. gonapodyides belongs to phylogenetic clade 6 and was first described from submerged fruits and twigs in Denmark by Petersen (1909). It has a worldwide distribution in all climates except the tropics (Zeng et al., 2009), and is ubiquitous in aquatic ecosystems in northwestern USA and Europe (Brasier et al., 2003; Jung et al., 1996; Reeser et al., 2011). It has been found in Denmark, New Zealand, Chile, Australia, UK, USA, France, and Czechoslovakia infecting minor roots and small seedlings of a limited number of hosts, such as *Tsuga*, *Pseudotsuga*, *Rhododendron* and *Hebe* (Brasier et al., 1993, 2003; Erwin and Ribeiro, 1996). In Australia, it has been isolated from native vegetation in Pine Lake in TAS (Brasier et al., 2003), and through soil baiting in Victoria (Dunstan et al., 2016). It has also been detected in KNP through HTS (Burgess et al., 2017b). *P. gonapodyides* has higher maximum (up to 38 °C) and lower minimum temperatures (3 °C) for growth (Brasier et al., 2003; Nechwatal et al., 2013). Its isolation from colder environments or even arctic alpine environments and its relatively higher optimum (up to 33 °C) and maximum temperature (up to 38 °C) for growth is rather contrasting/surprising. A possible explanation for still remaining as a 'high temperature taxon' could be its physiological adaptation to certain aspects of its ecology, such as litter breakdown, rather than climatic adaption (Brasier et al., 2003). Further research is recommended to investigate its survival strategies at these higher altitudes considering it does not produce chlamydospores. The origin of this species is uncertain due to its

wide distribution due to anthropogenic activities (Jung et al., 2011). It has perhaps been introduced to Australia's lowland ecosystems, and from these to mountain ecosystems.

P. cryptogea belongs to phylogenetic clade 8, and was first described from foot root of tomato in 1919 (Pethybridge and Lafferty, 1919). This species has been associated with *Rubus anglocandicans* decline in WA (Aghighi et al., 2016), and had also been isolated from aquatic ecosystems in WA (Hüberli et al., 2013). *P. cryptogea* has been reported to cause a collar rot in *P. bracteata* in south-eastern NSW (Bago State Forest; McDougall et al. 2018). *P. pseudocryptogea* also belongs to phylogenetic clade 8 and was formally described in 2015; it was given the name *pseudocryptogea* based on its physiological and morphological resemblance to *P. cryptogea* (Safaiefarahani et al., 2015). Recent pathogenicity trials have shown that this species is not pathogenic on *C. calophylla* (Croeser et al., 2018), and it has not been associated with a disease in KNP. This is the first record on the recovery of living isolates of *P. pseudocryptogea* in KNP, although it had been detected in TAS through HTS (Burgess et al., 2017b).

In vitro, both *P. cryptogea* and *P. pseudocrytogea* have high optimum (25 °C) and maximum temperatures (33 °C for *P. cryptogea* and 35 °C for *P. pseudocryptogea*) for growth (Safaiefarahani et al., 2015). On the other hand, their minimum temperature for growth is also very low (3 °C) (Safaiefarahani et al., 2015). This shows that these pathogens have the ability to grow at both low and high temperatures. Therefore, they have the capability to cause disease both in tropical and temperate climates. Their centre of origin is unknown, but it is believed that anthropogenic activities associated with nurseries, horticulture, and agriculture dispersed these pathogens globally (Brasier, 2008; Stukenbrock and McDonald, 2008). These invasive species were probably introduced to Australia's lowland ecosystems and then spread to mountains. It is interesting that *P. cryptogea* was isolated at these higher altitudes, and not *P. pseudocryptogea*, because the most frequently isolated species in the 'cryptogea' complex in lowlands in Australia is *P. pseudocryptogea* (CPSM unpublished data).

The two new *Phytophthora* species, *P. cacuminis* and *P. oreophila* described here have not been isolated anywhere in the world before. Therefore, it is not clear whether they are native to Australia or introduced. Nonetheless, their temperature growth-relationships suggests that these species are well adapted to these colder conditions. *P. oreophila* has a higher growth rate at temperatures less than 20 °C, and has a much lower optimal temperature than related species, *P. rosacearum*, *pseudorosacearum*, *P. kwongonina*, and *P. cooljarloo* and is the only species to have been isolated from cold environments. Additionally, *P. oreophila* exhibit prolific growth at temperatures less than 4 °C. *P. cacuminis* is closely related to *P. fallax* and *P. constricta*, and all of them have low optimum and minimum temperature for growth. *P. fallax* and *P. cacuminis* have been isolated from mountains, but *P. constricta* has only been isolated from lowlands.

Although *P. cacuminis* failed to produce oospores, its low cardinal temperatures and the ability to produce chlamydospores suggest that this species is well adapted to colder environments. The establishment of *Phytophthora* species in colder environments is mainly determined by low cardinal temperatures for growth and asexual structures rather than the ability to produce sexual structures (Redondo et al., 2018). Further research is recommended to understand their distribution, ecology, host-pathogen interactions, and to determine centres of origin of these new species.

Whilst the incidence of *Phytophthora* diseases is well documented in horticultural environments, ornamental plants grown in nurseries, and other lowland ecosytems (Català et al., 2015; Henricot et al., 2014; Jung et al., 2016), sub-alpine regions have received little attention. In our literature searches, there appear to be a very few records on the occurrence of *Phytophthora* species in the sub-alpine ecosystems (Green, 2016; Newby, 2014; Scarlett et al., 2015). This is due to the presumed assumption that lower temperatures restrict the growth and sporulation of *Phytophthora* and are not conducive to disease expression (Chee and Newhook, 1965; Phillips and Weste, 1985; Podger et al., 1990; Rafiei et al., 2018; Shearer et al., 1987; Shelley et al., 2017; Shepherd and Pratt, 1974). As such, the recovery of such a diverse *Phytophthora* community in sub-alpine and alpine areas previously thought to be pathogen-free leads to many concerns, and raises questions about their introduction, survival and subsequent dispersal, as they could potentially have a devastating effect on the rare and threatened species in these ecosytems.

Anthropogenic activities are known to have distributed *Phytophthora* species widely since European colonization, for example through contaminated mud on vehicles, road building and mining, replanting using infected seedlings because of poor hygiene, bushwalkers, feral horses and apiarists (Brasier, 2008; Cahill et al., 2008; Callaghan and Guest, 2015). Once introduced, *Phytophthora* species are dispersed by root to root contact between adjacent plants (Shearer et al., 2010), and movement of infested soil attached to visitor's boots, bicycles, management vehicles tires and feral horses (McDougall et al., 2003). Self sexual reproduction and parasexuality (Desprez-Loustau et al., 2007), phenotypic plasticity (Mariette et al., 2016), evolutionary potential (McDonald and Linde, 2002), survival in host tissues in deep soil layers (Marçais et al., 1996), and the ability to form asexual survival structures and low cardinal temperature for growth can then assist *Phytophthora* species to survive/invade extreme environments (Crone et al., 2013b; Redondo et al., 2018).

Thee recovery of apparently introduced (invasive) species *P. cactorum*, *P. gonapodyides*, *P. cryptogea* and *P. pseudocryptogea* surviving in these sub-alpine environments is of great concern. The involvement of *P. cryptogea* and the *P. gregata* in the decline of *P. bracteata* in Bago State Forest at an elevation of 1160 m asl, and Kellys Plains in KNP at an elevation of 1270 m asl, respectively shows that *Phytophthora* species present in the sub-alpine ecosystems have the potential to cause disease. While the other species have not been associated with a disease, it does not necessarily mean they are not causing disease because no studies or surveys have been conducted on these pathogens in relation to diseases/hosts in these areas. Also, *Phytophthora* species have been known to live in plants as biotrophs without causing observable disease symptoms (Crone et al., 2013a, 2013b). Therefore, asymptomatic areas where *Phytophthora* species have been detected/isolated should be explored more for soil suppression, host resistance and asymptomatic presence of *Phytophthora* species. Further work is required to determine the susceptibility of sub-alpine flora to a range of *Phytophthora* species. Glasshouse trials have recently tested the susceptibility of nine KNP sub-alpine shrub species to *P. cinnamomi* and *P. cambivora*, and found that one species *Phebalium squamulosum* was specially susceptible to both pathogens (Rigg et al., 2018). This is particularly important when temperature is rising globally, which will shift the climatic range of *Phytophthora* species and other pathogens, and render some host species more susceptible to disease.

In conclusion, the occurrence of such a diverse *Phytophthora* community in alpine and sub-alpine ecosystems, previously considered not suitable for *Phytophthora* indicate that alpine and sub-alpine areas of KNP are at risk. It is now important to restrict its further spread to protect the diverse and unique flora in alpine/sub-alpine ecosystem. Road closure is the best management strategy to reduce the spread of invasive pathogens. In areas where road closure is not possible, roads should be engineered in a way to stop the flow of water from roads to adjacent areas and/or reduce the

chances of infested soil uptake by vehicle from wet areas (Colquhoun and Hardy, 2000; Hansen et al., 2000). Besides landscape features, the inclusion of non-host and resistant vegetation can also reduce the dispersal of the pathogens (Holdenrieder et al., 2004). Visitors and staff need to be educated on hygiene and the potential spread of these pathogens via vehicles, cycling and walking. Overall, strict measures need to be taken to decrease introduction pathways, human land use, and habitat disturbance to reduce the spread of pathogens into colder environments.

Conflicts of interest

We confirm that we have no conflict of interest to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2018.10.006.

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