



Nothophytophthora gen. nov., a new sister genus of *Phytophthora* from natural and semi-natural ecosystems

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Abstract During various surveys of *Phytophthora* diversity in Europe, Chile and Vietnam slow growing oomycete isolates were obtained from rhizosphere soil samples and small streams in natural and planted forest stands. Phylogenetic analyses of sequences from the nuclear ITS, LSU, β -tubulin and *HSP90* loci and the mitochondrial *cox1* and *NADH1* genes revealed they belong to six new species of a new genus, officially described here as *Nothophytophthora* gen. nov., which clustered as sister group to *Phytophthora*. *Nothophytophthora* species share numerous morphological characters with *Phytophthora*: persistent (all *Nothophytophthora* spp.) and caducous (*N. caduca*, *N. chlamydospora*, *N. valdiviana*, *N. vietnamensis*) sporangia with variable shapes, internal differentiation of zoospores and internal, nested and extended (*N. caduca*, *N. chlamydospora*) and external (all *Nothophytophthora* spp.) sporangial proliferation; smooth-walled oogonia with amphigynous (*N. amphigynosa*) and paragynous (*N. amphigynosa*, *N. intricata*, *N. vietnamensis*) attachment of the antheridia; chlamydospores (*N. chlamydospora*) and hyphal swellings. Main differing features of the new genus are the presence of a conspicuous, opaque plug inside the sporangiophore close to the base of most mature sporangia in all known *Nothophytophthora* species and intraspecific co-occurrence of caducity and non-papillate sporangia with internal nested and extended proliferation in several *Nothophytophthora* species. Comparisons of morphological structures of both genera allow hypotheses about the morphology and ecology of their common ancestor which are discussed. Production of caducous sporangia by *N. caduca*, *N. chlamydospora* and *N. valdiviana* from Valdivian rainforests and *N. vietnamensis* from a mountain forest in Vietnam suggests a partially aerial lifestyle as adaptation to these humid habitats. Presence of tree dieback in all forests from which *Nothophytophthora* spp. were recovered and partial sporangial caducity of several *Nothophytophthora* species indicate a pathogenic rather than a saprophytic lifestyle. Isolation tests from symptomatic plant tissues in these forests and pathogenicity tests are urgently required to clarify the lifestyle of the six *Nothophytophthora* species.

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INTRODUCTION

The *Peronosporaceae*, a sister family of the *Pythiaceae*, belongs to the *Peronosporales*, class *Peronosporomycetes*, kingdom *Stramenipila*, and currently comprises 22 genera, i.e., *Phytophthora*, *Halophytophthora*, *Phytopythium* and 19 genera of downy mildews (Dick 2001, Hulvey et al. 2010, Beakes et

al. 2014, Thines & Choi 2016). While *Halophytophthora* and *Phytopythium* species are mostly saprophytes and/or necrotrophic facultative plant pathogens most *Phytophthora* species have a hemibiotrophic or necrotrophic lifestyle as primary plant pathogens although for mostly aquatic *Phytophthora* species a partially saprophytic lifestyle seems likely (Erwin & Ribeiro 1996, Brasier et al. 2003, Jung et al. 2011). In contrast, all c. 600 species of downy mildews are highly specialized, obligate biotrophic plant pathogens (Göker et al. 2007, Runge et al. 2011, Beakes et al. 2012, Thines & Choi 2016). However, the production of RxLR-type effectors, which play a crucial role for pathogenesis, by both *Phytophthora* and the downy mildews indicates a close relationship between the two groups (Baxter et al. 2010, Thines & Kamoun 2010). Several phylogenetic studies demonstrated that the genus *Phytophthora* is monophyletic and that all downy mildews reside within *Phytophthora* (Cooke et al. 2000, Kroon et al. 2004, Göker et al. 2007, Runge et al. 2011, Martin et al. 2014, Thines & Choi 2016). However, due to the description of the obligate biotrophic downy mildews as 19 distinct genera, mainly before the advent of molecular phylogenetic analyses, *Phytophthora* exhibits a high degree of paraphyly (Cooke et al. 2000, Göker et al. 2007, Runge et al. 2011, Thines & Choi 2016). The molecular results confirmed the hypothesis of Gäumann (1952) who, based on morphological and pathogenic data, postulated an evolutionary development

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from saprophytic *Pythium* species via hemibiotrophic or necrotrophic *Phytophthora* species to the obligate biotrophic downy mildews. Unlike *Phytophthora*, the genus *Pythium* was in DNA sequence-based phylogenetic analyses shown to be polyphyletic (Briard et al. 1995, Cooke et al. 2000, De Cock & Lévesque 2004, Kroon et al. 2004, Villa et al. 2006). Consequently, the genus was recently divided in *Pythium* s.str. and four new genera, i.e., *Phytopythium* (syn. *Ovatisporangium*; previously *Pythium* Clade K), *Elongisporangium*, *Globisporangium* and *Pilasporangium* (Bala et al. 2010, Uzuhashi et al. 2010, De Cock et al. 2015). While *Phytopythium* together with the other four genera was originally assigned to the *Pythiaceae* (De Cock et al. 2015), Thines & Choi (2016) considered *Phytopythium* belonging to the *Peronosporaceae* due to both phylogenetic relatedness and morphological similarity to *Phytophthora*.

Stimulated by the increasing number of epidemics caused by exotic invasive *Phytophthora* species including *P. austrocedri*, *P. cinnamomi*, *P. lateralis*, *P. plurivora*, *P. ramorum*, *P. xalni* or *P. xcambivora* in both managed and natural ecosystems (Brasier et al. 1993, Erwin & Ribeiro 1996, Jung et al. 1996, 2000, 2013, 2016, Hansen et al. 2000, 2012, Rizzo et al. 2002, Vettraino et al. 2002, 2005, Balci & Halmschlager 2003a, b, 2007, Jung & Blaschke 2004, Hardham 2005, Greslebin et al. 2007, Jung 2009, Jung & Burgess 2009, Brasier & Webber 2010, Green et al. 2013, 2015, Ginetti et al. 2014, Henricot et al. 2014, Scanu et al. 2015) numerous *Phytophthora* surveys have been performed during the past two decades in forests and river systems in most continents. Using classical isolation methods and, more recently, also metagenomic approaches, these surveys have uncovered an astonishing diversity of described and previously unknown *Phytophthora* taxa (Jung et al. 2000, 2011, 2013, 2016, 2017a, b, Balci & Halmschlager 2003a, b, 2007, Jung 2009, Zeng et al. 2009, Rea et al. 2011, Reeser et al. 2011, Hansen et al. 2012, Huai et al. 2013, Hüberli et al. 2013, Oh et al. 2013, Shrestha et al. 2013, Burgess 2015, Català et al. 2015, Burgess et al. 2017).

During surveys of *Phytophthora* diversity in Europe, Chile and Vietnam slow growing isolates which morphologically resemble *Phytophthora* species were obtained from rhizosphere soil samples and small streams in natural and planted forest stands. A preliminary phylogenetic analysis of ITS rDNA sequences resulted in six distinct clades belonging to a potentially new genus in sister position with *Phytophthora*. In this study, morphological and physiological characteristics were used in combination with DNA sequence data from four nuclear gene regions, i.e., ITS, part of the 28S large subunit (LSU), heat shock protein 90 (*HSP90*) and β -tubulin (*Btub*), and the two mitochondrial *cox1* and *NADH1* genes to characterise and officially describe the new oomycete genus as *Nothophytophthora* gen. nov., and the six new taxa as *N. amphigynosa* sp. nov., *N. caduca* sp. nov., *N. chlamydospora* sp. nov., *N. intricata* sp. nov., *N. valdiviana* sp. nov. and *N. vietnamensis* sp. nov.

MATERIAL AND METHODS

Isolate collection and maintenance

Details of all isolates used in the phylogenetic, morphological and temperature-growth studies are given in Table 1. Sampling and isolation methods from forest soil and streams were according to Jung et al. (1996, 2017a). For baiting of soils young leaves of *Lithocarpus bacgiangensis* (Vietnam), and *Fagus sylvatica* and *Quercus robur* (Germany) were used as baits. Stream baiting was performed using young leaves of *Castanea sativa*, *F. sylvatica*, *Nothofagus obliqua* and *Q. robur* (Chile), and *Citrus sinensis* and *Quercus suber* (Portugal). For all isolates, single hyphal tip cultures were produced under the stereomicroscope

from the margins of fresh cultures on V8-juice agar (V8A; 16 g agar, 3 g CaCO₃, 100 mL Campbell's V8 juice, 900 mL distilled water). Stock cultures were maintained on grated carrot agar (CA; 16 g agar, 3 g CaCO₃, 200 g carrots, 1 000 mL distilled water; Brasier 1967, Scanu et al. 2015) at 10 °C in the dark. All isolates of the six new *Nothophytophthora* spp. are preserved in the culture collections maintained at the University of Algarve, the University of Sassari and the Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences. Ex-type and isotype cultures were deposited at the Westerdijk Fungal Biodiversity Institute (previously Centraalbureau voor Schimmelcultures CBS; Utrecht, The Netherlands) (Table 1).

DNA isolation, amplification and sequencing

For all *Nothophytophthora* isolates obtained in this study mycelial DNA was extracted from pure cultures grown in pea-broth medium (Erwin & Ribeiro 1996). Pea-broth cultures were kept for 7–10 d at 25 °C without shaking. Mycelium was harvested by filtration through filter paper, washed with sterile deionized water, freeze-dried and ground to a fine powder in liquid nitrogen. Total DNA was extracted using the E.Z.N.A.® Fungal DNA Mini Kit (OMEGA Bio-tek, Norcross, GA) following the manufacturer's instructions and checked for quality and quantity by spectrophotometry. DNA was stored at –20 °C until further use to amplify and sequence four nuclear and two mitochondrial loci (Table 1). The internal transcribed spacer (ITS1-5.8S-ITS2) region (ITS) and the 5' terminal domain of the large subunit (LSU) of the nuclear ribosomal RNA gene (nrDNA) were amplified separately using the primer-pairs ITS1/ITS4 (White et al. 1990) and LR0R/LR6-O (Moncalvo et al. 1995, Riethmüller et al. 2002), respectively, using the PCR reaction mixture and cycling conditions described by Nagy et al. (2003) with an annealing temperature of 57 °C (ITS) or 53 °C (LSU) for 30 s. Partial heat shock protein 90 (*HSP90*) gene was amplified with the primers HSP90F1int and HSP90R1 as described previously (Blair et al. 2008). Segments of the β -tubulin (*Btub*) and the mitochondrial genes cytochrome c oxidase subunit 1 (*cox1*) and NADH dehydrogenase subunit 1 (*NADH1*) were amplified with primers TUBUF2 and TUBUR1, FM80RC (the reverse complement of FM80) and FM85, and NADHF1 and NADHR1, respectively, using the PCR reaction mixture and cycling conditions as described earlier (Martin & Tooley 2003, Kroon et al. 2004). Products of Thermo Fisher Scientific Inc. (Waltham, MA, USA) and Bio-Rad C1000™ or Applied Biosystems® 2720 Thermal Cyclers were used for the PCR reactions. Amplicons were purified and sequenced in both directions using the primers of the PCR reactions by LGC Genomics GmbH (Berlin, Germany). Electrophoregrams were quality checked and forward and reverse reads were compiled using Pregap4 v. 1.5 and Gap v. 4.10 of the Staden software package (Staden et al. 2000). Clearly visible pronounced double peaks were considered as heterozygous positions and labelled according to the IUPAC coding system. All sequences derived in this study were deposited in GenBank and accession numbers are given in Table 1.

Phylogenetic analysis

The sequences obtained in this work were complemented with sequences deposited in GenBank. Four datasets were established to analyse different phylogenetic questions. The sequences of the loci used in the analyses were aligned using the online version of MAFFT v. 7 (Katoh & Standley 2013) by the E-INS-I strategy (ITS) or the auto option (all other loci). When indel positions of ITS sequences were used to increase robustness of phylogenetic analyses (Nagy et al. 2012), the program GapCoder was used (Young & Healy 2003).

Table 1 (cont.)

Species	Isolate numbers ^a		Origin Location; Year	Collector; reference	GenBank accession numbers						
	International collections	Local collections			ITS	LSU	<i>Btub</i>	<i>HSP90</i>	<i>Cox1</i>	<i>NADH1</i>	
<i>Haliophytophthora avicenniae</i> ^b	CBS 188.85 ATCC 64709	DAR 50187	Australia; –	S. Wilkens; Robideau et al. 2011	HQ643147	HQ665146	–	–	HQ708219	–	
<i>H. batemanensis</i> ^b	CBS 679.84 IMI 327602	DAR 41559	Australia; 1982	J. Simpson; Robideau et al. 2011	HQ643148	HQ665286	–	–	HQ708220	–	
<i>H. epistomia</i> ^b	CBS 590.85 ATCC 28293 IMI 330183	–	Florida; –	I.M. Master & J.W. Feli; Robideau et al. 2011	HQ643220	HQ665279	–	–	HQ708285	–	
<i>H. exoprolifera</i> ^b	CBS 252.93 ATCC 76607	IFO 32420	Japan (Okinawa island); 1988	–; Robideau et al. 2011	HQ643132	HQ665174	–	–	HQ708205	–	
<i>H. operculata</i> ^b	CBS 241.83	–	Australia; –	–; De Cock et al. 2015	KJ128038	KJ128038	–	–	KF853238	–	
<i>H. polymorphica</i> ^b	ATCC 44952 CBS 680.84	DAR 41562	Australia; 1982	J. Simpson; Robideau et al. 2011, De Cock et al. 2015	HQ643313	HQ665288	–	–	HQ708363	–	
<i>Hyaloperonospora sisymbrii-sophiae</i> ^b	HV276	–	Austria; 2000	H. Voglmayr; Voglmayr 2003	AY198253	EU054910	–	–	HM033186	–	
<i>Peronospora tumidis</i> ^b	HV312	–	Austria; 2000	H. Voglmayr; Voglmayr 2003	AY198287	KC495032	–	–	KC494952	–	
<i>Phytophthora asparagi</i> ^b	WPC P10690 ICMP 9495	–	New Zealand; 1986	P.G. Falloon; Robideau et al. 2011	HQ261683	EU080569	–	–	HQ261430	–	
<i>P. boehmeriae</i> ^b	CBS 291.29	–	Japan; –	K. Sawada; Robideau et al. 2011	HQ643149	HQ665190	EU080162	EU080165	HQ708221	AY563992	
<i>P. cactorum</i> ^b	IMI 180614	–	The Netherlands; 1930	W.L. White; Robideau et al. 2011	HQ261514	EU080282	–	–	HQ261261	–	
<i>P. capitosa</i> ^b	WPC P10719 ICMP 15576	–	New Zealand; 1992	M.A. Dick & C.W. Barr; Robideau et al. 2011	HQ261522	EU079663	–	–	HQ261269	–	
<i>P. castaneae</i> ^b	CBS 587.85 ATCC 36818	–	Taiwan; –	H.S. Chang; Robideau et al. 2011	HQ643255	HQ665278	–	–	HQ708315	–	
<i>P. coloccasiae</i> ^b	IMI 328914	–	Indonesia; 1989	M.D. Coffey; Robideau et al. 2011	HQ261539	EU079911	–	–	HQ261286	–	
<i>P. foliorum</i> ^b	WPC P6317	–	California; 2005	C. Blomquist; Robideau et al. 2011	HQ261561	EU079684	–	–	HQ261308	–	
<i>P. heveae</i> ^b	WPC P10989 CBS 296.29	1307997-MI	Malaysia; 1929	A. Thompson; Robideau et al. 2011	HQ643238	HQ665194	–	–	HQ708301	–	
<i>P. humicola</i> ^b	IMI 180616 CBS 200.81 ATCC 52179	–	Taiwan; –	P.J. Ann & W.H. Ko; Robideau et al. 2011	HQ643243	HQ665148	AY564069	EU080172	HQ708305	AY564011	
<i>P. ilicis</i> ^b	WPC P3939 ATCC 56615	–	British Columbia, Canada; 1988	H. Ho; Robideau et al. 2011	HQ261583	EU079864	–	–	HQ261330	–	
<i>P. infestans</i> ^b	CBS 366.51	–	The Netherlands; –	–; Robideau et al. 2011	HQ643247	HQ665217	–	–	HQ708309	–	
<i>P. kernoviae</i> ^b	WPC P10681	–	New Zealand; 2002	C.F. Hill; Robideau et al. 2011	HQ261603	EU079650	–	–	HQ261350	–	
<i>P. litchii</i> ^b	ICMP 14761	–	Taiwan; –	C.W. Kao; Voglmayr 2003	AY198308	AF235949	–	–	HQ708323	–	
<i>P. megalakarya</i> ^b	CBS 100.81	–	Sao Tome and Principe; –	–; Robideau et al. 2011	HQ261609	EU079974	–	–	HQ261356	–	
<i>P. niederhauserii</i> ^b	WPC P8516	–	North Carolina, USA; 2001	G. Abad; Robideau et al. 2011	HQ261702	EU080233	–	–	HQ261449	–	
<i>P. polonica</i> ^b	WPC P10616	–	Poland; –	T. Oszak; Robideau et al. 2011	HQ261646	EU080261	–	–	HQ261393	–	
<i>P. quercina</i> ^b	WPC P10334 CBS 782.95	–	Germany; 1995	T. Jung; Robideau et al. 2011	HQ261659	EU080494	–	–	HQ261406	–	
<i>P. rubi</i> ^b	CBS 967.95 ATCC 90442 IMI 355974	–	Scotland; 1985	J.M. Duncan & D.M. Kennedy; Robideau et al. 2011	HQ643340	HQ665306	KU899234	KU899391	HQ708388	KU899476	
<i>Phytophthora boreale</i> ^b	CBS 551.88	–	China; –	Y. Yang-nian; Robideau et al. 2011	HQ643372	HQ665261	–	–	HQ708419	–	
<i>Ph. helioides</i> ^b	CBS 286.31	–	USA; –	C. Drechsler; Robideau et al. 2011	HQ643383	HQ665186	–	–	HQ708430	–	
<i>Ph. oedocheilum</i> ^b	CBS 292.37	–	USA; –	C. Drechsler; Robideau et al. 2011	HQ643392	HQ665191	–	–	HQ708439	–	

Table 1 (cont.)

Species	Isolate numbers ^a		Origin Location; year	Collector; reference	GenBank accession numbers						
	International collections	Local collections			ITS	LSU	Btub	HSP90	Cox1	NADH1	
<i>Ph. ostracodes</i> ^b	CBS 768.73	–	Spain (Ibiza); 1972	A.J. van der Plaats-Niterink; Robideau et al. 2011	HQ643395	HQ665295	–	–	–	HQ708442	–
<i>Pythium attrantheridium</i> ^b	–	DAOM 230383	Canada; –	N. Alain-Boulé; Robideau et al. 2011	HQ643477	HQ665308	–	–	–	HQ708524	–
<i>Py. caudatum</i> ^{bc}	CBS 584.85	–	Pennsylvania, USA; 1984	B.A. Jaffee; Robideau et al. 2011	HQ643136	HQ665277	–	–	–	HQ708209	–
<i>Py. insidiosum</i> ^b	ATCC 58393	–	Costa Rica; –	–; Robideau et al. 2011	HQ643570	HQ665273	–	–	–	HQ708614	–
<i>Py. oligandrum</i> ^b	ATCC 58643	–	UK; –	n.a.; Robideau et al. 2011	HQ643715	HQ665223	–	–	–	HQ708759	–
<i>Py. rostratum</i> ^b	CBS 382.34	–	The Netherlands; 1971	A.J. van der Plaats-Niterink; Robideau et al. 2011	HQ643767	HQ665252	–	–	–	HQ708808	–
<i>Py. ultimum</i> var. <i>ultimum</i> ^b	CBS 122650	–	France; 2012	T. Rintoul; Robideau et al. 2011	HQ643864	HQ665103	–	–	–	HQ708905	–
<i>Py. venterpoolii</i> ^b	CBS 295.37	–	UK; 1936	T.C. Venterpool; Robideau et al. 2011	HQ643952	HQ665193	–	–	–	HQ708993	–
<i>Salisapillia tartarea</i> ^b	CBS 208.95	IFO 32606	Florida, USA; 1991	S.Y. Newell; Robideau et al. 2011	HQ643135	HQ232464	–	–	–	HQ708208	–

– = not available; authentic strains, ex-types, isotypes, neotypes and paratypes are printed in **bold italics**-type.

^a Abbreviations of isolates and culture collections: ATCC = American Type Culture Collection, Manassas, USA; CBS = CBS collection at the Westerdijk Fungal Biodiversity Institute (previously Centraalbureau voor Schimmelcultures), Utrecht, Netherlands; DAOM = Canadian National Mycological Herbarium, Agriculture and Agri-Food Canada, Ottawa, Canada; DAR = New South Wales Plant Pathology Herbarium, Orange Agricultural Institute, Orange, Australia; ICMP = International Collection of Micro-organisms from Plants, Auckland, New Zealand; IFO = Institute for Fermentation, Osaka, Japan; WPC = World Phytophthora Collection, University of California Riverside, USA; other isolate names and numbers are as given by the collectors and on GenBank, respectively.

^b Isolates used in the phylogenetic studies.

^c Submitted to GenBank as *Phytophthora* sp. PR12-475.

^d Submitted to GenBank as *Phytophthora* sp. PR13-109.

^e Submitted to GenBank as *Phytophthora* sp. PR13-109.

^f Submitted to GenBank as *Phytophthora* sp. PR13-109.

^g Submitted to GenBank as *Phytophthora* sp. PR13-109.

^h Submitted to GenBank as *Phytophthora* sp. PR13-109.

ⁱ Submitted to GenBank as *Phytophthora* sp. PR13-109.

^j Submitted to GenBank as *Phytophthora* sp. PR13-109.

^k Submitted to GenBank as *Phytophthora* sp. PR13-109.

To study the (i) phylogenetic position of the potentially new genus among other oomycete genera, a 3-locus dataset (ITS-LSU-*cox1*) of representative species from all genera of the *Peronosporales* together with the representatives of all species from the potentially new genus were analysed with *Salisapillia tartarea* (CBS 208.95), *Salisapillia* spp., *Peronosporales*, *Halophytophthora epistomium* (CBS 590.85), *Peronosporales*, and *Aphanomyces euteiches* (CBS 156.73), *Leptolegniaceae*, *Saprolegniales*, as outgroups (dataset: 48 isolates and 3 020 characters). To analyse the (ii) intrageneric phylogeny of the potential new genus a 6-partition dataset (6 loci: ITS-LSU-*Btub*-*HSP90*-*cox1*-*NADH1*) complemented with the indel motifs of the ITS region) was analysed with *Phytophthora boehmeriae* (CBS 291.29), *P. humicola* (CBS 200.81) and *P. rubi* (CBS 967.95) as outgroup taxa (dataset: 42 isolates and 5 366 characters). A GenBank blast search revealed ITS sequences of three isolates from Ireland and New Zealand which possibly represent congeneric taxa. To analyse their relation to the six new taxa, a (iii) full ITS dataset (complemented with the indel motifs) of all isolates from the six new taxa together with three GenBank entries (dataset: 51 isolates and 1 244 characters) and (iv) a partial ITS dataset (complemented with the indel motifs) of all isolates from the six new taxa together with those three and one partial ITS sequence originating from an environmental sample (MOTU 33 from Català et al. 2015) (dataset: 51 isolates and 1 phylotype; 504 characters) were used. In the ITS datasets *P. boehmeriae* (CBS 291.29), *P. captiosa* (P10719), *P. kernoviae* (P10681) and *P. polonica* (P15005) were used as outgroup taxa.

A Maximum likelihood (ML) and a Bayesian (BI) analysis were carried out with all datasets except the partial ITS dataset with which only an ML analysis was run (data not shown). Bayesian analyses were performed with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) into partitions with GTR+G model for nucleotide partitions and a two-parameter Markov (Mk2 Lewis) model for the indel partitions. Four Markov chains were run for 10 M generations, sampling every 1 000 steps, and with a burn in at 4 000 trees. ML analyses were carried out with the raxmlGUI v. 1.3 (Silvestro & Michalak 2012) implementation of the RAXML (Stamatakis 2014). A GTR+G nucleotide substitution model was used for the nucleotide partitions and indel data were treated as binary data. There were 10 runs of the ML and bootstrap ('thorough bootstrap') analyses with 1 000 replicates used to test the support of the branches. Phylogenetic trees were visualized in MEGA6 (Tamura et al. 2013) and edited in figure editor programs. Datasets presented and trees deriving from Maximum likelihood and Bayesian analyses are available from TreeBASE (20801; <http://purl.org/phylo/treebase/phyloids/study/TB2:S20801>).

Morphology of asexual and sexual structures

Morphological features of sporangia, oogonia, oospores, antheridia, chlamydospores, hyphal swellings and aggregations of the six new species (Table 1, 12) were compared with each other.

Formation of sporangia was induced by submersing two 12–15 mm square discs cut from the growing edge of a 3–7-d-old V8A colony in a 90 mm diam Petri dish in non-sterile soil extract (50 g of filtered oak forest soil in 1 000 mL of distilled water, filtered after 24 h) (Jung et al. 1996). The Petri dishes were incubated at 20 °C in natural light and the soil extract was changed after c. 6 h (Jung et al. 2017b). Shape, type of apex, caducity and special features of sporangia and the formation of hyphal swellings and aggregations were recorded after 24–48 h. For each isolate 40 sporangia were measured at ×400 using a compound microscope (Zeiss Imager.Z2), a digital camera (Zeiss AxioCam ICc3) and a biometric software (Zeiss AxioVision).

The formation of chlamydospores and hyphal swellings was examined on V8A after 21–30 d growth at 20 °C in the dark. If present, for each isolate each 40 chlamydospores and hyphal swellings chosen at random were measured under a compound microscope at $\times 400$.

The formation of gametangia (oogonia and antheridia) and their characteristic features were examined after 21–30 d growth at 20 °C in the dark on CA which for oogonia production proved to be superior to V8A in a preliminary study. For each isolate each 40 oogonia, oospores and antheridia chosen at random were measured under a compound microscope at $\times 400$. The oospore wall index was calculated according to Dick (1990). Self-sterile isolates were paired with isolates from the same and from other self-sterile *Nothophytophthora* species according to Jung et al. (2017b). In addition, isolates from all self-sterile and homothallic *Nothophytophthora* species were paired with A1 and A2 tester strains of *P. cinnamomi* using a modified membrane method (Ko et al. 1978, Gallegly & Hong 2008) with nitrocellulose instead of polycarbonate membranes (pore size 0.22 μm ; Millipore, Merck, Germany) to test whether they are able to stimulate oogonia production in *P. cinnamomi* and, hence, share the A1/A2 compatibility system of *Phytophthora*.

Colony morphology, growth rates and cardinal temperatures

Colony growth patterns of all six *Nothophytophthora* species were described from 10-d-old cultures grown at 20 °C in the dark in 90 mm plates on CA, V8A, malt-extract agar (MEA; Oxoid Ltd., UK) and potato dextrose agar (PDA; Oxoid Ltd., UK) according to Jung & Burgess (2009), Jung et al. (2017b) and Erwin & Ribeiro (1996).

For temperature-growth relationships, representative isolates of the six *Nothophytophthora* species (Table 1) were subcultured onto 90 mm V8A plates and incubated for 24 h at 20 °C to stimulate onset of growth (Jung et al. 1999). Then three replicate plates per isolate were transferred to 5, 10, 15, 20, 25, 26, 27, 28, 29 and 30 °C. Radial growth was recorded after 6 d, along two lines intersecting the centre of the inoculum at right angles and the mean growth rates (mm/d) were calculated. To determine the lethal temperature, plates showing no growth at 26, 27, 28, 29 or 30 °C were re-incubated at 20 °C.

RESULTS

Phylogenetic analysis

Compared to the ML analyses the BI analyses provided with all three datasets more support for terminal clades and with the 3-loci dataset also for the deeper branches. Since the topology of all trees resulting from BI and ML analyses was similar the Bayesian trees are presented here with both Bayesian Posterior Probability values and Maximum Likelihood bootstrap values included (Fig. 1–3, TreeBASE: 20801). When the phylogenetic position of the new genus *Nothophytophthora* among other oomycete genera was studied with the help of the 3-loci dataset (ITS-LSU-*cox1*), both BI and ML analyses resulted in a fully supported distinct clade of the isolates of the new genus which formed a monophyletic group with the genus *Phytophthora*. The clade of the two genera was supported by a 0.98 PP in BI analysis (Fig. 1) and 61 % bootstrap value in ML analysis (not shown). The phylogeny of the other oomycete genera included in the analyses was in accordance with results from previous studies (Hulvey et al. 2010, Marano et al. 2014, Martin et al. 2014, De Cock et al. 2015). The downy mildews, represented by *Peronospora rumicis* and *Hyaloperonospora sisymbrii-sophiae*, resided within the paraphyletic genus *Phytophthora*. The genus *Halophytophthora* proved to be polyphyletic with *Halophytoph-*

thora s.str., represented by *H. avicenniae*, *H. batemanensis* and *H. polymorphica*, clustering in a basal position to the monophyletic *Phytophthora-Nothophytophthora* clade and *H. exoprolifera* clustering basal to the previous three genera. *Halophytophthora operculata* resided in a basal position to the genus *Phytophythium* whereas *H. epistomium* clearly belongs to an unknown genus outside of the *Peronosporaceae* and *Pythiaceae* (Fig. 1). *Phytophythium* constituted the basal genus within the *Peronosporaceae* sensu Dick (2001) and Hulvey et al. (2010) which also comprised *Halophytophthora*, *Phytophthora* inclusive the downy mildew genera, and *Nothophytophthora*.

When the intrageneric phylogeny of *Nothophytophthora* was analysed with the 6-partition dataset (ITS-LSU-*Btub*-*HSP90-cox1*-*NADH1*), the isolates formed six fully supported distinct clades (Fig. 2). In both BI and ML analyses the two populations of *N. caduca* from two different forest streams formed two separate clusters within the *N. caduca* clade which resided in a basal position to the well-supported clade formed by the other five *Nothophytophthora* species (Fig. 2). Within that clade, *N. vietnamensis* with *N. intricata* and *N. valdiviana* with *N. chlamydospora* clustered in sister position to each other with both subclades having high support in both analyses. However, the relative position of these two clades and the position of the fifth lineage, *N. amphigynosa*, have been fully resolved only in the BI analysis (Fig. 2). Across a 4 136 character alignment of the five coding genes, LSU, *Btub*, *HSP90*, *cox1* and *NADH1*, *N. amphigynosa*, *N. intricata*, *N. vietnamensis*, *N. caduca*, *N. chlamydospora* and *N. valdiviana* had 31, 7, 9, 53, 29 and 31 unique polymorphisms, respectively, and differed from each other at 19–116 positions corresponding to sequence similarities of 97.2–99.5 % (Table 8, 9). The six *Nothophytophthora* species differed from *Phytophthora* spp. (*P. boehmeriae*, *P. humicola* and *P. rubi*), *Halophytophthora avicenniae* and *Phytophythium helicoides* at 328–379, 370–382 and 472–491 positions corresponding to sequence similarities of 90.8–92.1 %, 90.8–91.0 % and 88.1–88.6 % (Table 8, 9). Due to the presence of heterozygous positions *N. amphigynosa* and *N. chlamydospora* had four and two LSU haplotypes, respectively (Table 3). Also, the LSU sequence of all isolates of *N. vietnamensis* contained one heterozygous position (Table 3). Heterozygous sites were also present in the ITS sequences of *N. amphigynosa*, *N. chlamydospora* and *N. vietnamensis* (Table 2) and in the *HSP90* sequences of *N. caduca* and *N. chlamydospora* (Table 4). The *Btub* sequence of all isolates of *N. valdiviana* contained nine heterozygous positions (Table 5). No heterozygous positions were found in the mitochondrial *cox1* and *NADH1* sequences of any *Nothophytophthora* isolate (Table 6, 7).

When the ITS sequences of the six new *Nothophytophthora* species together with three (partial) ITS sequences from GenBank were analysed the phylogeny gained was less supported in both BI and ML analyses (Fig. 3). *Nothophytophthora amphigynosa* clustered in a basal position to the other five *Nothophytophthora* species of which the relative positions could not be fully resolved (Fig. 3). The ITS sequences of the three congeneric isolates from streams in Ireland and New Zealand grouped into the clade formed by *N. valdiviana* and *N. chlamydospora* with the Irish isolate PR12-475 being basal to this clade. Isolates PR13-109 from Ireland and REB326-69 from New Zealand showed only 4–6 characters differences to *N. chlamydospora* and *N. valdiviana* (Table 10) but their phylogenetic position was vague and they could not unambiguously be assigned to any of the two new species (Fig. 3). In a separate analysis of a shorter ITS sequence alignment (data not shown), a similar situation was found. The partial ITS sequence representing the 'Uncultured *Phytophthora* clone R2 MOTU33' from a metagenomic stream survey in Spain (Català et al. 2015) grouped within the clade formed by the sister spe-

cies *N. vietnamensis* and *N. intricata*. Unfortunately, the short ITS sequence, even complemented with the indels, could not resolve the clade of these two new species (data not shown) and the species assignment of this environmental sequence was ambiguous (NB: the sequence of 'MOTU 33' was not included in the analyses presented in Català et al. 2015).

In the ITS region with its non-coding parts, both intrageneric variability and differences between *Nothophytophthora* and related genera were considerably higher than in the coding genes. In the 1 140 bp ITS alignment used for the intrageneric comparison the sequences of the six *Nothophytophthora* species contained in total 417 polymorphic sites (36.6 %; Table 2) and showed pairwise differences at 5–356 positions, equivalent

to sequence similarities of 68.8–99.7 % (Table 10, 11). The large differences of *N. amphigynosa* and *N. caduca* to other *Nothophytophthora* spp. were caused by the high numbers of 189 and 179 unique polymorphisms, respectively, which mainly comprised indels (Table 2). Including the three congeneric isolates from Ireland and New Zealand increased the number of polymorphic sites to 427 (data not shown). In the 1 230 characters ITS alignment used for the intergeneric comparison the six *Nothophytophthora* species differed from *Phytophthora* spp. (*P. boehmeriae*, *P. humicola* and *P. rubi*), *H. avicenniae* and *Ph. helicoides* at 392–531, 446–567 and 510–654 positions corresponding to sequence similarities of 56.8–68.1 %, 53.9–63.7 % and 46.8–58.5 % (Table 10, 11).

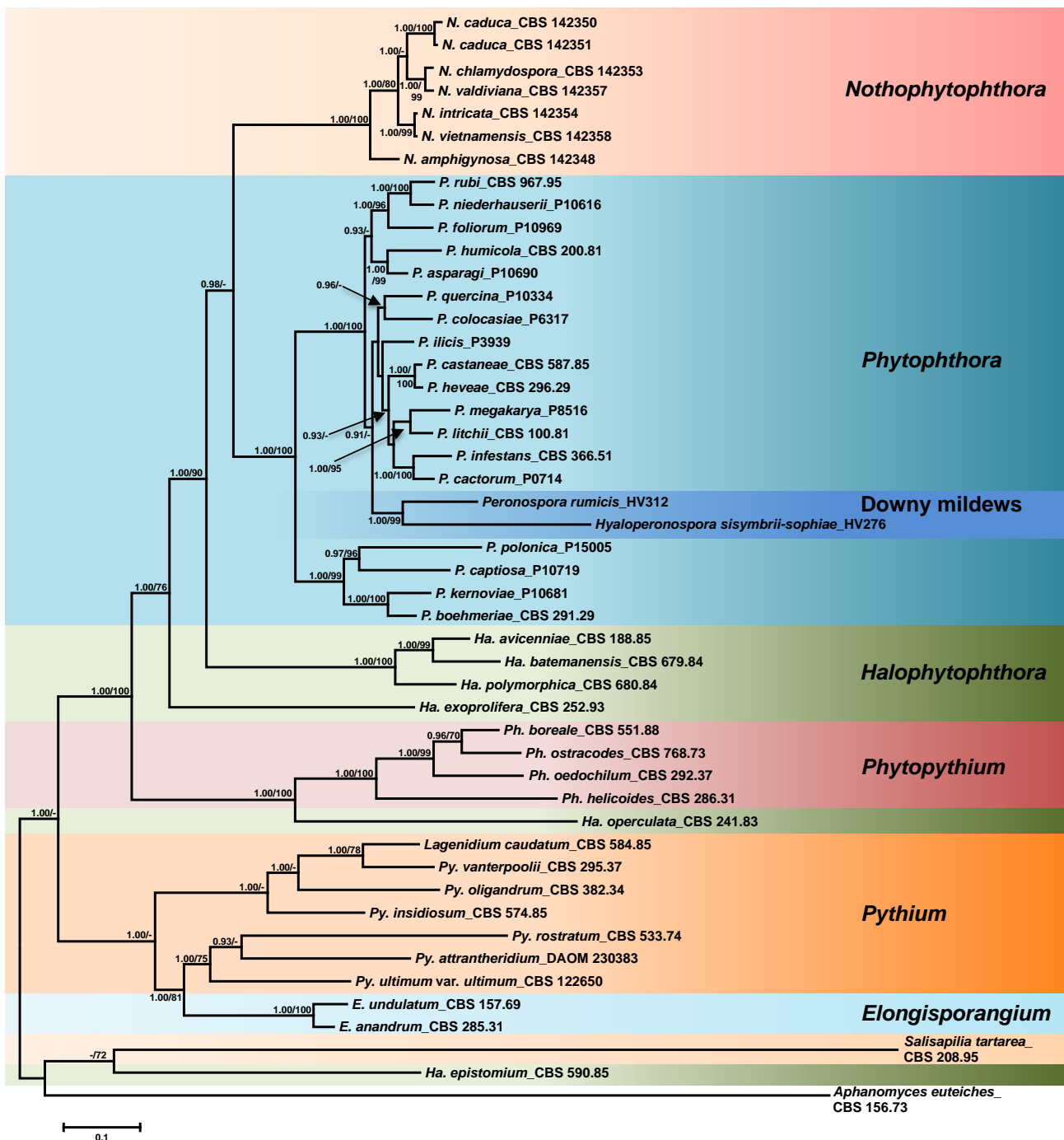


Fig. 1 Fifty percent majority rule consensus phylogram derived from Bayesian phylogenetic analysis of three-loci (LSU, ITS, *cox1*) dataset of *Nothophytophthora* gen. nov. and representative species from other genera of the *Peronosporales*. Bayesian posterior probabilities and Maximum Likelihood bootstrap values (in %) are indicated, but not shown below 0.9 and 70 %, respectively. *Salisapillia tartarea*, *Halophytophthora epistomium* and *Aphanomyces euteiches* were used as outgroup taxa. Scale bar indicates 0.1 expected changes per site per branch.

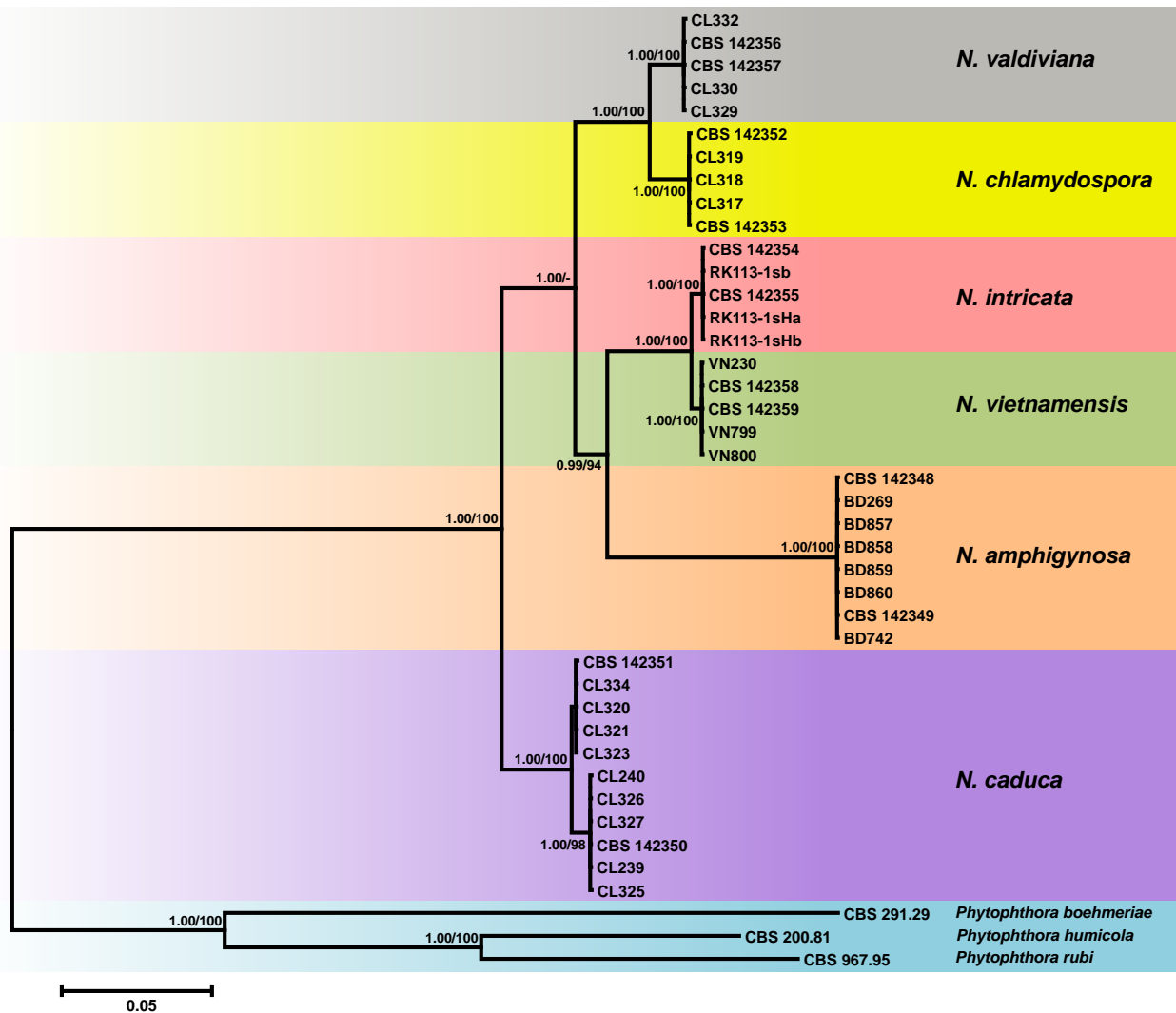


Fig. 2 Fifty percent majority rule consensus phylogram derived from Bayesian phylogenetic analysis of six-loci (LSU, ITS, *Btub*, *HSP90*, *cox1*, *NADH1*) dataset of *Nothophytophthora* gen. nov. to examine intrageneric variability and phylogenetic structure. Bayesian posterior probabilities and Maximum Likelihood bootstrap values (in %) are indicated, but not shown below 0.9 and 70 %, respectively. *Phytophthora boehmeriae*, *P. humicola* and *P. rubi* were used as outgroup taxa. Scale bar indicates 0.05 expected changes per site per branch.

TAXONOMY

Nothophytophthora T. Jung, Scanu, Bakonyi & M. Horta Jung, gen. nov. — MycoBank MB820530

Etymology. Name refers to the morphological and ecological similarity to *Phytophthora* (Nothus Lat = false).

Type species. *Nothophytophthora amphigynosa*.

Sporangia mostly ovoid, limoniform, ellipsoid or obpyriform, and usually non-papillate. Sporangial proliferation is in all known species external, leading in some species to dense sympodia, and in some species also internal in both a nested and extended way. In all known species, a conspicuous opaque plug separates most sporangia from the sporangiophores. In some species, varying proportions of the sporangia are caducous, breaking off at the base of this plug which is synonymous with the pedicel of airborne *Phytophthora* species. As in *Phytophthora*, the sporangial cytoplasm differentiates inside the sporangia into biflagellate zoospores which are released without discharge tube. *Chlamydospores* are formed in some species and are absent in others. Some species are homothallic, forming smooth-walled oogonia, containing thick-walled oospores with a large ooplast, and amphigynous and/or paragynous antheridia. Several species are sterile both in single culture and when mated with isolates from the same or

from other self-sterile *Nothophytophthora* species, and also when mated with A1 and A2 tester strains of *Phytophthora cinnamomi*. *Nothophytophthora* is morphologically similar to *Phytophthora* and phylogenetically constitutes a monophyletic sister genus of *Phytophthora*.

Notes — Morphological and physiological characters and morphometric data of the six new *Nothophytophthora* species are given in the comprehensive Table 12.

Nothophytophthora amphigynosa T. Jung, Scanu, Bakonyi & M. Horta Jung, sp. nov. — MycoBank MB820532; Fig. 4

Etymology. Name refers to the predominantly amphigynous antheridia.

Typus. PORTUGAL, Sintra, isolated from a stream in a temperate Atlantic forest, T. Jung, 13 Mar. 2015 (CBS H-23007 holotype, dried culture on CA, Herbarium Westerdijk Fungal Biodiversity Institute, CBS 142348 = BD268, ex-type culture). ITS and *cox1* sequences GenBank KY788382 and KY788473, respectively.

Sporangia, hyphal swellings and chlamydospores (Fig. 4a–j) — Sporangia were not observed in solid agar but were produced abundantly in non-sterile soil extract. Sporangia of *N. amphigynosa* were typically borne terminally on unbranched sporangiophores (Fig. 4a–e) or less frequently in lax sympodia

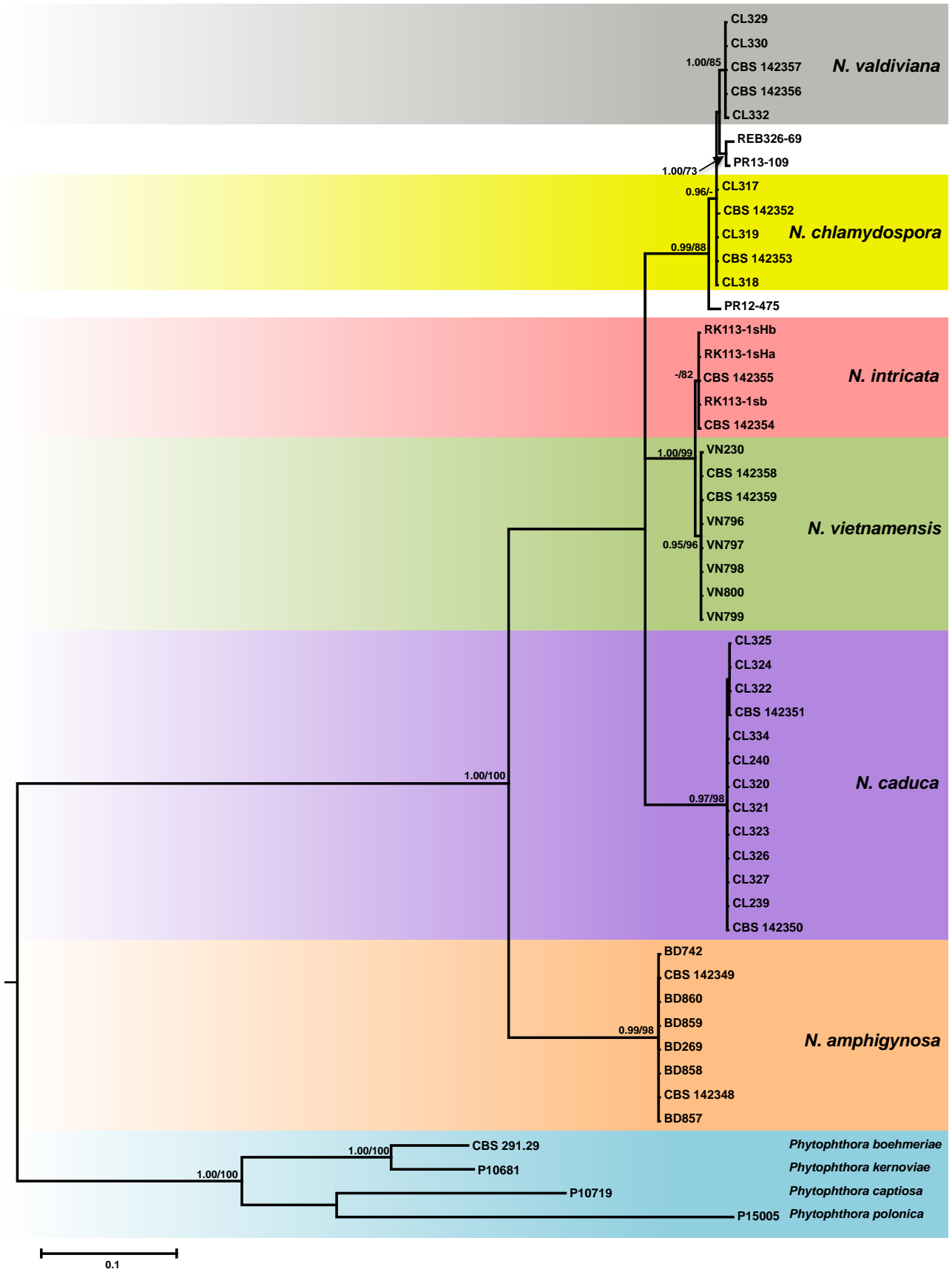


Fig. 3 Fifty percent majority rule consensus phylogram derived from Bayesian inference analysis of a full ITS dataset (complemented with the indel motifs) of the six new *Nothophytophthora* species and three GenBank entries from Ireland and New Zealand. Bayesian posterior probabilities and Maximum Likelihood bootstrap values (in %) are indicated, but not shown below 0.9 and 70 %, respectively. *Phytophthora boehmeriae*, *P. captiosa*, *P. kernoviae* and *P. polonica* were used as outgroup taxa. Scale bar indicates 0.1 expected changes per site per branch.

Table 5 Polymorphic sites from a 897-character long partial β -tubulin sequence alignment showing inter- and intraspecific variation of six new *Nothophytophthora* species represented by 39 isolates. Polymorphisms unique to a species are highlighted in **bold**.

<i>Nothophytophthora</i> species	No. of isolates	Haplotype	0	0	0	0	0	0	0	0	0	1	1	2	3	3	4	5	6	6	6	7	7	7	7	8	No. of unique polymorphic sites
			0	2	3	5	6	6	7	7	8	1	6	6	4	5	5	6	1	3	7	1	3	6	8	3	
			8	0	5	9	2	5	4	7	9	0	7	9	4	4	8	9	7	5	2	0	7	2	8	0	
<i>N. amphigynosa</i>	8 ^a	H1	C	T	T	C	C	C	A	T	G	C	T	T	C	C	G	G	C	C	C	G	C	C	T	A	6
<i>N. intricata</i>	5 ^b	H2	C	C	C	T	T	C	A	T	A	T	T	C	C	C	G	C	C	C	T	G	C	C	T	G	1
<i>N. vietnamensis</i>	5 ^c	H3	C	C	C	T	T	T	A	T	G	T	T	C	C	C	G	C	C	C	T	G	C	C	T	G	1
<i>N. caduca</i>	11 ^d	H4	C	C	C	C	T	C	G	C	G	T	T	C	G	C	A	C	C	T	C	A	T	C	T	G	7
<i>N. chlamydospora</i>	5 ^e	H5	T	C	C	C	T	C	A	T	G	T	T	T	C	T	G	C	T	C	C	G	C	T	A	G	5
<i>N. valdiviana</i>	5 ^f	H6	Y	C	C	C	Y	C	A	T	G	T	Y	Y	S	Y	G	C	Y	C	C	G	C	Y	W	G	9

^a CBS 142348, CBS 142349, BD269, BD742, BD857, BD858, BD859, BD860.
^b CBS 142354, CBS 142355, RK113-1sb, RK113-1sHa, RK113-1sHb.
^c CBS 142358, CBS 142359, VN230, VN799, VN800.
^d CBS 142350, CBS 142351, CL239, CL240, CL320, CL321, CL323, CL325, CL326, CL327, CL334.
^e CBS 142352, CBS 142353, CL317, CL318, CL319.
^f CBS 142356, CBS 142357, CL329, CL330, CL332.

Table 6 Polymorphic sites from a 643-character long partial *cox1* sequence alignment showing inter- and intraspecific variation of six new *Nothophytophthora* species represented by 39 isolates. Polymorphisms unique to a species are highlighted in **bold**.

<i>Nothophytophthora</i> species	No. of isolates	Haplotype	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	No. of unique polymorphic sites				
			1	3	4	5	6	9	0	0	1	1	2	5	5	7	7	8	8	0	0	2	2	3	5	6	7	7	8	9	0	1	
			1	5	5	9	5	8	7	8	3	6	2	5	8	6	9	5	8	3	9	1	4	9	7	1	0	8	1	9	5	1	
<i>N. amphigynosa</i>	8 ^a	H1	A	T	C	T	A	A	T	G	T	T	T	T	T	C	T	T	T	T	T	C	T	G	T	A	A	A	A	C	5		
<i>N. intricata</i>	5 ^b	H2	T	T	C	A	T	A	A	A	T	T	T	T	T	T	T	T	T	A	C	T	T	A	T	T	A	A	T	T	1		
<i>N. vietnamensis</i>	5 ^c	H3	T	T	T	T	A	C	T	A	T	T	T	T	T	T	T	T	T	A	C	T	T	A	T	T	A	A	A	T	T	1	
<i>N. caduca</i>	5 ^d	H4	T	T	C	A	A	A	A	G	T	T	C	T	T	T	T	T	T	A	T	T	C	T	T	T	A	A	A	T	T	13	
<i>N. caduca</i>	6 ^e	H5	A	T	A	A	A	G	T	G	T	C	C	T	T	A	C	C	T	T	T	T	T	T	C	T	A	G	C	T	T	13	
<i>N. chlamydospora</i>	5 ^f	H6	A	T	C	A	T	A	T	G	A	A	T	A	G	A	C	T	A	T	A	C	A	T	C	G	T	A	A	A	T	T	9
<i>N. valdiviana</i>	5 ^g	H7	T	A	A	A	A	A	G	T	A	T	T	T	C	C	T	A	T	T	T	C	T	T	C	T	A	A	A	T	T	8	

^a CBS 142348, CBS 142349, BD269, BD742, BD857, BD858, BD859, BD860.
^b CBS 142354, CBS 142355, RK113-1sb, RK113-1sHa, RK113-1sHb.
^c CBS 142358, CBS 142359, VN230, VN799, VN800.
^d CBS 142351, CL320, CL321, CL323, CL334.
^e CBS 142350, CL239, CL240, CL325, CL326, CL327.
^f CBS 142352, CBS 142353, CL317, CL318, CL319.
^g CBS 142356, CBS 142357, CL329, CL330, CL332.

Table 7 Polymorphic sites from a 812-character long partial *NADH1* sequence alignment showing inter- and intraspecific variation of six new *Nothophytophthora* species represented by 39 isolates. Polymorphisms unique to a species are highlighted in **bold**.

<i>Nothophytophthora</i> species	No. of isolates	Haplotype	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	3	No. of unique polymorphic sites			
			0	1	2	3	3	4	5	7	8	8	9	9	0	1	5	5	5	0	2	2	2	2	4	5	8	8	0	0	3		
			1	0	2	7	8	3	0	3	2	8	1	7	3	5	2	4	5	3	0	3	6	9	1	0	0	3	4	7	4		
<i>N. amphigynosa</i>	8 ^a	H1	A	T	C	C	T	T	C	A	T	T	T	T	G	T	A	A	G	A	A	T	T	A	C	T	T	A	A	T	C	8	
<i>N. intricata</i>	5 ^b	H2	A	T	T	T	T	T	T	T	T	C	A	A	T	A	A	G	A	T	T	A	G	C	T	C	A	A	T	C	2		
<i>N. vietnamensis</i>	5 ^c	H3	A	T	T	T	T	T	T	G	C	C	A	A	G	A	A	G	A	T	T	A	A	C	T	C	A	A	G	C	6		
<i>N. caduca</i>	11 ^d	H5	A	C	T	C	T	T	C	C	T	T	T	A	A	T	A	A	G	G	A	T	T	A	T	A	T	A	T	G	T	C	12
<i>N. chlamydospora</i>	5 ^e	H6	G	T	T	T	C	C	C	C	A	T	A	A	A	T	A	A	G	A	A	A	T	A	C	T	T	G	T	T	C	12	
<i>N. valdiviana</i>	5 ^f	H7	A	T	T	T	T	C	A	A	T	C	A	A	C	G	T	A	A	A	T	A	T	T	T	T	T	T	T	T	T	14	

^a CBS 142348, CBS 142349, BD269, BD742, BD857, BD858, BD859, BD860.
^b CBS 142354, CBS 142355, RK113-1sb, RK113-1sHa, RK113-1sHb.
^c CBS 142358, CBS 142359, VN230, VN799, VN800.
^d CBS 142350, CBS 142351, CL239, CL240, CL320, CL321, CL323, CL325, CL326, CL327, CL334.
^e CBS 142352, CBS 142353, CL317, CL318, CL319.
^f CBS 142356, CBS 142357, CL329, CL330, CL332.

of 1–3 sporangia (Fig. 4f–j), and some were formed intercalary (0.3 %). Small subglobose to limoniform hyphal swellings (11.1 ± 2.8 µm) were sometimes observed on sporangiophores. Sporangia were non-caducous and non-papillate (Fig. 4a–j). In almost all mature sporangia (98.5 %) a conspicuous opaque plug was formed inside the sporangiophore close to the sporangial base which averaged 2.9 ± 0.6 µm (Fig. 4a–j). Sporangia were mostly ovoid to elongated-ovoid (over all isolates 81.5 %; Fig. 4a–c, f, h–j), ellipsoid (11.6 %; Fig. 4d, j), obpyriform (5.1 %; Fig. 4g) or infrequently limoniform (0.9 %; Fig. 4e), mouse- or club-shaped (0.9 %). Sporangia with special features such as slightly lateral attachment of the sporangiophore (over all isolates 14.1 %; Fig. 4e, g), slightly curved apex (3.1 %; Fig. 4c) or the presence of a vacuole (5.9 %; Fig. 4f) were common in all isolates. Sporangial proliferation was exclusively external (28.8 % of sporangia; Fig. 4f–j). Sporangial dimensions of eight isolates of *N. amphigynosa* averaged 47.0 ± 5.6 × 26.4 ± 1.8 µm (overall range 33.6–60.6 × 21.3–32.4 µm) with a range of isolate means of 41.5–52.0 × 25.4–27.3 µm and a length/breadth ratio of 1.78 ± 0.17 (range of isolate means 1.62–1.91) (Table 12). Zoospores of *N. amphigynosa* were differentiated inside

the sporangia and discharged through an exit pore 5.2–16.3 µm wide (av. 8.9 ± 1.4 µm) (Fig. 4h–j). They were limoniform to reniform whilst motile, becoming spherical (av. diam = 9.0 ± 1.1 µm) on encystment. Direct germination of sporangia was not observed. In solid agar, hyphal swellings or chlamydozoospores were not observed.

Oogonia, oospores and antheridia (Fig. 4k–t) — Gametangia were produced in single culture by all isolates of *N. amphigynosa* in CA within 10–14 d. Gametangia formation was usually starting at and was sometimes restricted to the areas of the colonies close to the walls of the Petri dishes. Oogonia were borne terminally, had smooth walls, short thin stalks and were globose to slightly subglobose with non-tapering bases (on av. 87.5 %; Fig. 4k–p, t) or less frequently elongated pyriform to ellipsoid (12.5 %) sometimes with tapering bases (2.9 %) (Fig. 4q–s). Mean diameter of oogonia was 25.3 ± 1.7 µm (overall range 18.4–29.7 µm and range of isolate means 24.3–25.5 µm). They were almost exclusively plerotic (99.2 %). Oospores of *N. amphigynosa* were usually globose (Fig. 4k–q) but could be slightly elongated in elongated oogonia (Fig. 4r–s). Oospores contained large ooplasts (Fig. 4k–s) and had

Table 8 Pairwise numbers of different positions along a 4 136-character long multigene alignment (LSU, *Btub*, *HSP90*, *cox1*, *NADH1*) among the six *Nothophytophthora* species and between the *Nothophytophthora* species and representative species of the related genera *Phytophthora*, *Halophytophthora* and *Phytophythium*.

<i>Nothophytophthora</i> species	<i>N. amphigynosa</i>	<i>N. intricata</i>	<i>N. vietnamensis</i>	<i>N. caduca</i>	<i>N. chlamydozoospora</i>	<i>N. valdiviana</i>	<i>Phytophthora boehmeriae</i> (Clade 10)	<i>Phytophthora humicola</i> (Clade 6)	<i>Phytophthora rubi</i> (Clade 7)	<i>Halophytophthora avicenniae</i>	<i>Phytophythium helicoides</i>	Total no. of unique polymorphisms in <i>Nothophytophthora</i> spp.
<i>N. amphigynosa</i>	0/2	88/90	86/88	101/107	100/101	103/104	358/359	328/329	339/340	370/371	472/473	31
<i>N. intricata</i>	88/90	0	19	103/115	96	98	366	338	335	375	478	7
<i>N. vietnamensis</i>	86/88	19	0	104/116	99	99	367	338	338	378	481	9
<i>N. caduca</i>	101/107	103/115	104/116	0/28	103/107	101/104	365/376	339/344	337/348	374/380	484/491	53
<i>N. chlamydozoospora</i>	100/101	96	99	103/107	0/1	73	379	341	345/346	382	476	29
<i>N. valdiviana</i>	103/104	98	99	101/104	73	0	371	334	344	381	484	31

Nucleotides missing from the terminal part(s) of partial sequences and undetermined bases (N) were not considered as polymorphisms. Due to intraspecific variation at individual loci in several *Nothophytophthora* species pairwise differences between species also showed slight variations which is indicated by two numbers separated by a slash.

Table 9 Pairwise sequence similarities (%) along a 4 136-character long multigene alignment (LSU, *Btub*, *HSP90*, *cox1*, *NADH1*) among the six *Nothophytophthora* species and between the *Nothophytophthora* species and representative species of the related genera *Phytophthora*, *Halophytophthora* and *Phytophythium*.

<i>Nothophytophthora</i> species	<i>N. amphigynosa</i>	<i>N. intricata</i>	<i>N. vietnamensis</i>	<i>N. caduca</i>	<i>N. chlamydozoospora</i>	<i>N. valdiviana</i>	<i>Phytophthora boehmeriae</i> (Clade 10)	<i>Phytophthora humicola</i> (Clade 6)	<i>Phytophthora rubi</i> (Clade 7)	<i>Halophytophthora avicenniae</i>	<i>Phytophythium helicoides</i>
<i>N. amphigynosa</i>	99.5/100	97.8/97.9	97.9	97.4/97.6	97.6	97.5	91.3	92.0/92.1	91.8	91.0	88.6
<i>N. intricata</i>	97.8/97.9	100	99.5	97.2/97.5	97.7	97.6	91.1	91.8	91.9	90.9	88.4
<i>N. vietnamensis</i>	97.9	99.5	100	97.2/97.5	97.6	97.6	91.1	91.8	91.8	90.9	88.4
<i>N. caduca</i>	97.4/97.6	97.2/97.5	97.2/97.5	99.3/100	97.4/97.5	97.5/97.6	90.9/91.2	91.7/91.8	91.6/91.8	90.8/91.0	88.1/88.3
<i>N. chlamydozoospora</i>	97.6	97.7	97.6	97.4/97.5	99.8/100	98.2	90.8	91.8	91.6/91.7	90.8	88.5
<i>N. valdiviana</i>	97.5	97.6	97.6	97.5/97.6	98.2	100	91.0	91.9	91.7	90.8	88.3

Due to intraspecific variation at individual loci in several *Nothophytophthora* species pairwise sequence similarities among species also showed slight variations which is indicated by two numbers separated by a slash.

Table 10 Pairwise numbers of different positions along ITS rDNA sequence alignments among the six *Nothophytophthora* species and between the *Nothophytophthora* species, the congeneric three isolates PR13-109, PR12-475 and REB326-69 and representative species of the related genera *Phytophthora*, *Halophytophthora* and *Phytophythium*.

<i>Nothophytophthora</i> species	<i>N. amphigynosa</i>	<i>N. intricata</i>	<i>N. vietnamensis</i>	<i>N. caduca</i>	<i>N. chlamydospora</i>	<i>N. valdiviana</i>	PR13-109	PR12-475	REB326-69	<i>Phytophthora boehmeriae</i> (Clade 10)	<i>Phytophthora humicola</i> (Clade 6)	<i>Phytophthora rubi</i> (Clade 7)	<i>Halophytophthora avicenniae</i>	<i>Phytophythium helicoides</i>	Total no. of unique polymorphisms in <i>Nothophytophthora</i> spp.
<i>N. amphigynosa</i>	0/2	236	236	356	235/237	239	213	233	173	431/432	452/453	461	481	510	189
<i>N. intricata</i>	236	0	5	239	83/85	87	72	99	61	409	406	409	461	519	1
<i>N. vietnamensis</i>	236	5	0	242	82/84	86	71	98	61	407	406	409	459	518	2
<i>N. caduca</i>	356	239	242	0	234/236	236	219	244	199	531	524	530	567	654	179
<i>N. chlamydospora</i>	235/237	83/85	82/84	234/236	0/1	4/5	4/6	27/28	4/6	393/394	392/393	397/398	446/447	510/511	0/2
<i>N. valdiviana</i>	239	87	86	236	4/5	0	6	29	5	395	393	400	446	513	3

Pairwise differences between the six *Nothophytophthora* species and between them and the three congeneric isolates PR13-109, PR12-475 and REB326-69 were calculated using a 1 140 bp alignment. Pairwise differences between the six *Nothophytophthora* species and representative species of related genera were calculated using a 1 230 bp alignment. Nucleotides missing from the terminal part(s) of partial sequences and undetermined bases (N) were not considered as polymorphisms. Due to intraspecific variation in several *Nothophytophthora* species pairwise differences between species also showed slight variations which is indicated by two numbers separated by a slash.

Table 11 Pairwise sequence similarities along ITS rDNA alignments among the six *Nothophytophthora* species and between the *Nothophytophthora* species, the three congeneric isolates PR13-109, PR12-475 and REB326-69 and representative species of the related genera *Phytophthora*, *Halophytophthora* and *Phytophythium*.

<i>Nothophytophthora</i> species	<i>N. amphigynosa</i>	<i>N. intricata</i>	<i>N. vietnamensis</i>	<i>N. caduca</i>	<i>N. chlamydospora</i>	<i>N. valdiviana</i>	PR13-109	PR12-475	REB326-69	<i>Phytophthora boehmeriae</i> (Clade 10)	<i>Phytophthora humicola</i> (Clade 6)	<i>Phytophthora rubi</i> (Clade 7)	<i>Halophytophthora avicenniae</i>	<i>Phytophythium helicoides</i>
<i>N. amphigynosa</i>	99.8/100	79.3	79.3	68.8	79.2/79.4	79.0	81.3	79.6	84.8	64.9/65.0	63.2/63.3	62.5	60.9	58.5
<i>N. intricata</i>	79.3	100	99.6	79.0	92.5/92.7	92.4	93.7	91.3	94.7	66.8	67.0	66.8	62.5	57.8
<i>N. vietnamensis</i>	79.3	99.6	100	78.8	82/84	92.5	93.8	91.4	94.7	66.9	67.0	66.8	62.7	57.9
<i>N. caduca</i>	68.8	79.0	78.8	100	79.3/79.5	79.3	80.8	78.6	82.5	56.8	57.4	56.9	53.9	46.8
<i>N. chlamydospora</i>	79.2/79.4	92.5/92.7	82/84	79.3/79.5	99.9/100	99.6/99.7	99.5/99.7	97.5/97.6	99.5/99.7	68.0/68.1	68.1	67.6/67.7	63.7	58.5
<i>N. valdiviana</i>	79.0	92.4	92.5	79.3	99.6/99.7	100	99.5	97.5	99.6	67.9	68.1	67.5	63.7	58.3

Pairwise sequence similarities among the six *Nothophytophthora* species and between them and the three congeneric isolates PR13-109, PR12-475 and REB326-69 were calculated using a 1 140 bp alignment. Pairwise sequence similarities between the six *Nothophytophthora* species and representative species of related genera were calculated using a 1 230 bp alignment. Due to intraspecific variation in several *Nothophytophthora* species pairwise sequence similarities among species also showed slight variations which is indicated by two numbers separated by a slash.

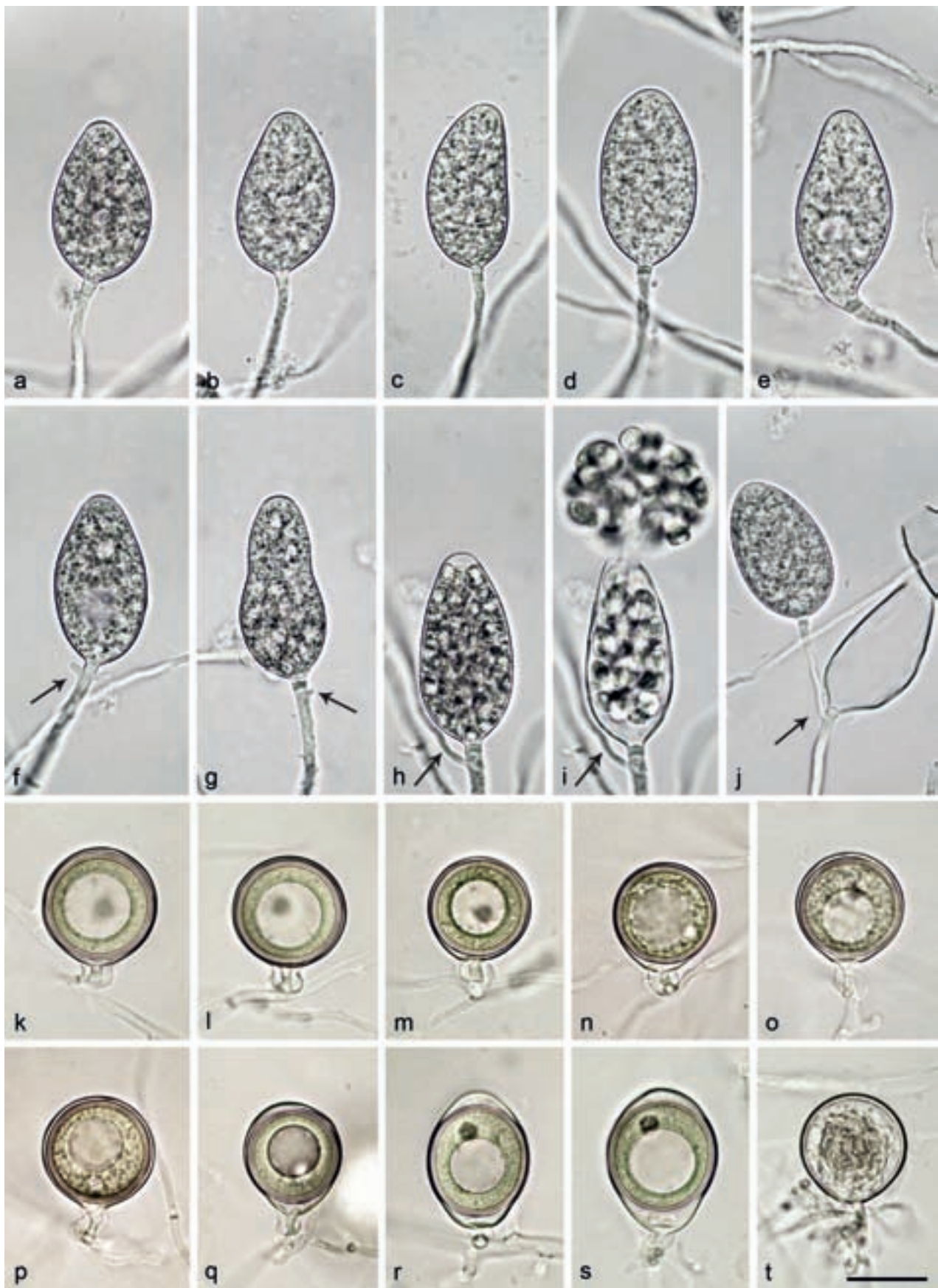


Fig. 4 Morphological structures of *Nothophytophthora amphigynosa*. — a–j. Non-papillate sporangia with a conspicuous basal plug formed on V8 agar flooded with soil extract. a–e. Sporangia borne terminally on unbranched sporangiophores; a–c. ovoid; c. with a slightly curved apex; d. ellipsoid; e. limoniform with slightly lateral attachment of the sporangiophore; f–j. sporangia with external proliferation immediately below sporangial base (arrows); f. ovoid with vacuole; g. elongated obpyriform with slightly lateral attachment of the sporangiophore; h. elongated-ovoid with already differentiated zoospores; i. same sporangium as in h releasing zoospores; j. lax sympodium of two ovoid sporangia. — k–p. Smooth-walled mature oogonia with non-tapering bases and short, thin stalks, containing plerotic, medium thick-walled oospores with each one large ooplast and one nucleus, formed in single culture in V8A; k–o. globose to subglobose with amphigynous antheridia; p. globose with paragynous antheridium behind oogonial stalk; q. slightly elongated with tapering base and amphigynous antheridium; r–s. elongated-ellipsoid with tapering bases, slightly elongated almost plerotic oospores and amphigynous antheridia; t. smooth-walled oogonium aborted before forming an oospore. — Scale bar = 25 μ m, applies to a–t.

a diameter of $23.4 \pm 1.7 \mu\text{m}$ (overall range 17.2–28.0 μm), a wall diam of $1.7 \pm 0.3 \mu\text{m}$ (range 1.0–2.5 μm) and an oospore wall index of 0.38 ± 0.05 (Table 12). Oospore abortion was low (4.2 % after 4 wk; Fig. 4t). The antheridia often had twisted intricate stalks (28.8 %) and were club-shaped to subglobose, mostly amphigynous (87.2 %; Fig. 4k–o, q–s) or less frequently paragynous (12.8 %; Fig. 4p) and averaged $8.5 \pm 1.8 \times 6.5 \pm 0.9 \mu\text{m}$. In the nitrocellulose membrane test all isolates tested stimulated abundant oogonia production in the A2 tester strain of *P. cinnamomi*.

Colony morphology, growth rates and cardinal temperatures (Fig. 10, 11) — Colonies on V8A, CA and MEA were largely submerged with limited aerial mycelium around the inoculum plug. They had a chrysanthemum pattern on V8A and CA and were uniform on MEA. On PDA colonies were dense felty with a rosaceous pattern (Fig. 10). Temperature-growth relations are shown in Fig. 11. All four isolates included in the growth test had similar growth rates and cardinal temperatures. The maximum growth temperature was 27 °C. The isolates did not resume growth when plates incubated for 5 d at 28 °C were transferred to 20 °C. The average radial growth rates at the optimum temperature of 20 °C and at 25 °C were 3.1 ± 0.05 and 3.0 ± 0.06 mm/d, respectively (Fig. 11).

Additional specimens. PORTUGAL, Sintra, isolated from a stream in a temperate Atlantic forest, T. Jung, 13 Mar. 2015; CBS 142349 = BD741; BD269; BD742; BD857; BD858; BD859; BD860.

Nothophytophthora caduca T. Jung, Scanu, Bakonyi, A. Durán & M. Horta Jung, *sp. nov.* — MycoBank MB820534; Fig. 5

Etymology. Name refers to the caducity of the sporangia (*caduca* Lat = caducous, shedding).

Typus. CHILE, isolated from a stream in a temperate Valdivian rainforest, T. Jung, 25 Nov. 2014 (CBS H-23011 holotype, dried culture on CA, Herbarium Westerdijk Fungal Biodiversity Institute, CBS 142350 = CL328, ex-type culture). ITS and *cox1* sequences GenBank KY788401 and KY788489, respectively.

Sporangia, hyphal swellings and chlamydospores (Fig. 5a–r) — Sporangia of *N. caduca* were not observed in solid agar but were produced abundantly in non-sterile soil extract. Sporangia were borne terminally on unbranched sporangiophores or less frequently in lax sympodia of 1–3 sporangia (Fig. 5r). A conspicuous, pedicel-like opaque plug ($2.6 \pm 0.7 \mu\text{m}$) formed inside the sporangiophore close to the base of most sporangia (over all isolates 87.0 %; Fig. 5b–n, r). In all isolates, sporangia were partially caducous (10–53 %, on av. 32.1 %; Fig. 5i–n) breaking off at the base of the basal plug. Sporangial shapes ranged from broadly ovoid, ovoid or elongated ovoid (83.4 %; Fig. 5a–e, i–j, l, o–r) to ellipsoid (7.4 %; Fig. 5k, m–n), limoniform (4.1 %; Fig. 5g–h), mouse-shaped (3.0 %), obpyriform (15.3 %; Fig. 5f), subglobose (0.7 %) or pyriform (0.7 %). Sporangia with laterally attached sporangiophores (44.6 %; Fig. 5e–f, h, j–k, m–n) and undulating sporangiophores (74.1 %; Fig. 5d–g, i, r–s) were commonly observed. Sporangial proliferation was external (Fig. 5r) and internal in both a nested and extended way (Fig. 5o–r) often with the sporangiophore showing multiple branching and undulating growth inside the empty sporangium (Fig. 5o–q). Sporangial dimensions of 14 isolates of *N. caduca* averaged $37.9 \pm 4.6 \times 25.7 \pm 3.0 \mu\text{m}$ (overall range 24.1–54.4 \times 18.1–35.9 μm) with a range of isolate means of 34.7–43.1 \times 23.3–28.2 μm . The length/breadth ratio averaged 1.48 \pm 0.15 with a range of isolate means of 1.38–1.66 (Table 12). Germination was indirect with zoospores (Fig. 5n) discharged through an exit pore 4.3–16.9 μm wide (av. 10.4 \pm 2.2 μm ; Fig. 5n–r). They were limoniform to reniform whilst motile, becoming spherical (av. diam = 7.4 \pm 0.6 μm) on encystment. Subglobose to limoniform swellings were infrequently formed on sporangiophores. Chlamydospores were not observed.

Oogonia, oospores and antheridia — All 14 isolates of *N. caduca* were self-sterile and did not form gametangia when paired against each other or with isolates of *N. chlamydospora*, *N. valdiviana* and with A1 and A2 tester strains of *P. cinnamomi*. Since in the nitrocellulose membrane test all isolates tested stimulated abundant oogonia production in the A2 tester strain of *P. cinnamomi*, their breeding system was considered as silent A1 mating type.

Colony morphology, growth rates and cardinal temperatures (Fig. 10, 11) — All isolates of *N. caduca* formed similar colonies on the same agar medium. Colonies on V8A, CA and PDA had a rosaceous to chrysanthemum pattern, largely submerged with limited felty aerial mycelium around the inoculum on V8A and CA and more woolly on PDA. On MEA irregular to dendroid, dense-felty colonies were formed (Fig. 10). The temperature-growth relations on V8A are shown in Fig. 11. The two populations from different streams had slightly different optimum and maximum temperatures for growth of 25 and 26 °C in one population and 20 and 28 °C in the other population (Fig. 11). Lethal temperatures were 28 and 30 °C, respectively. All isolates showed slow growth with average radial growth rates of 3.1 ± 0.2 mm/d at 20 °C and 3.6 ± 0.08 mm/d at 25 °C (Fig. 11).

Additional specimens. CHILE, isolated from streams in a temperate Valdivian rainforest, T. Jung, 25 Nov. 2014; CBS 142351 = CL333; CL235b; CL239; CL240; CL320; CL321; CL322; CL323; CL324; CL325; CL326; CL327; CL334.

Nothophytophthora chlamydospora T. Jung, Scanu, Bakonyi, A. Durán & M. Horta Jung, *sp. nov.* — MycoBank MB820536; Fig. 6

Etymology. Name refers to the production of chlamydospores by all known isolates.

Typus. CHILE, isolated from a stream in a temperate Valdivian rainforest, T. Jung, 25 Nov. 2014 (CBS H-23008 holotype, dried culture on CA, Herbarium Westerdijk Fungal Biodiversity Institute, CBS 142353 = CL316, ex-type culture). ITS and *cox1* sequences GenBank KY788405 and KY788493, respectively.

Sporangia, hyphal swellings and chlamydospores (Fig. 6a–v) — Sporangia of *N. chlamydospora* were not observed on solid agar but were produced abundantly after 24 hr in non-sterile soil extract. Sporangia were borne terminally (Fig. 6a–e, g) or infrequently intercalary (Fig. 6f) on unbranched sporangiophores or in dense sympodia (Fig. 6k). Up to 6–8 sporangia per sympodium were observed although there were usually fewer. Sporangia were non-papillate or sometimes shallow semi-papillate (Fig. 6k) and partially caducous (over all isolates 11–41 %, on av. 25.2 %; Fig. 6h–k) breaking off below a pedicel-like opaque plug formed inside the sporangiophore close to the base of 77.5 % of all sporangia (Fig. 6b–e, g–k, m–n). Sporangial shapes ranged from ovoid or elongated ovoid (44 %; Fig. 6c–d, f–g, j–k, m), ellipsoid (27.5 %; Fig. 6a–b, h) and limoniform (22.5 %; Fig. 6i, k) to obpyriform (2.5 %), mouse-shaped (1.5 %; Fig. 6e) or pyriform (1.5 %). Sporangia with special features like lateral attachment of the sporangiophore (14.5 %; Fig. 6d–e, j), curved apex (2.0 %; Fig. 6n), hyphal extensions (1.5 %; Fig. 6l), a vacuole (13.0 %; Fig. 6g–i) or undulating sporangiophores (2.0 %) occurred in all isolates. Sporangia proliferated exclusively externally, usually immediately below the old sporangium (Fig. 6g, k, m–n). Sporangial dimensions of five isolates averaged $37.6 \pm 4.9 \times 22.1 \pm 2.5 \mu\text{m}$ (overall range 27.4–57.2 \times 17.0–30.8 μm and range of isolate means 35.6–38.9 \times 20.4–23.2). The length/breadth ratio averaged 1.71 \pm 0.17 with a range of isolate means of 1.64–1.75 (Table 12). In all isolates, a few sporangia failed to form a basal septum and continued to grow at the apex, functionally becoming hyphal swellings (Fig. 6l). Zoospores were discharged through an exit pore 4.8–13.1 μm wide (av. 8.2 \pm 1.7 μm ; Fig. 6j, m–n). They were limoniform to

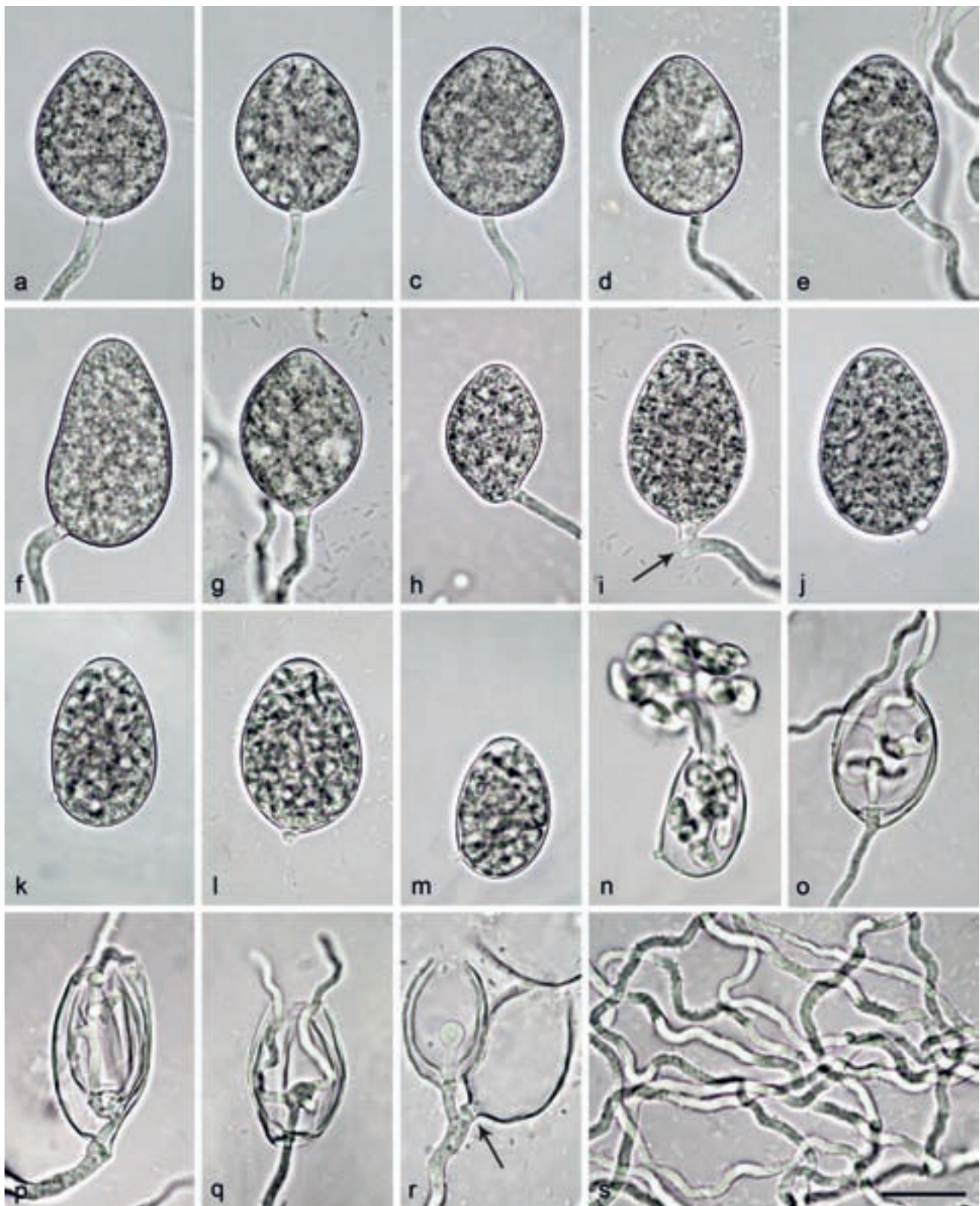


Fig. 5 Morphological structures of *Nothophytophthora caduca* formed on V8 agar flooded with soil extract. — a–j. Mature non-papillate sporangia; a. ovoid without basal plug; b–n. with conspicuous basal plug; b. ovoid; c–e. ovoid with undulating sporangiophores; f. obpyriform with undulating sporangiophore; g. limoniform with undulating sporangiophore; h. limoniform with laterally attached sporangiophore; i. ovoid, just being shed (arrow); j–n. caducous ovoid sporangia with short pedicel-like basal plug; k–m. with differentiated zoospores and swollen semipapillate apex; n. same sporangium as in m releasing zoospores; o–q. empty sporangia with internal nested and extended proliferation and multiple branching and undulating growth of hyphae inside the sporangium; r. small sympodium of two sporangia resulting from external proliferation; one sporangium showing nested proliferation and the other one breaking off from the sporangiophore (arrow); s. undulating hyphae. — Scale bar = 25 μ m in a–r and 40 μ m in s.

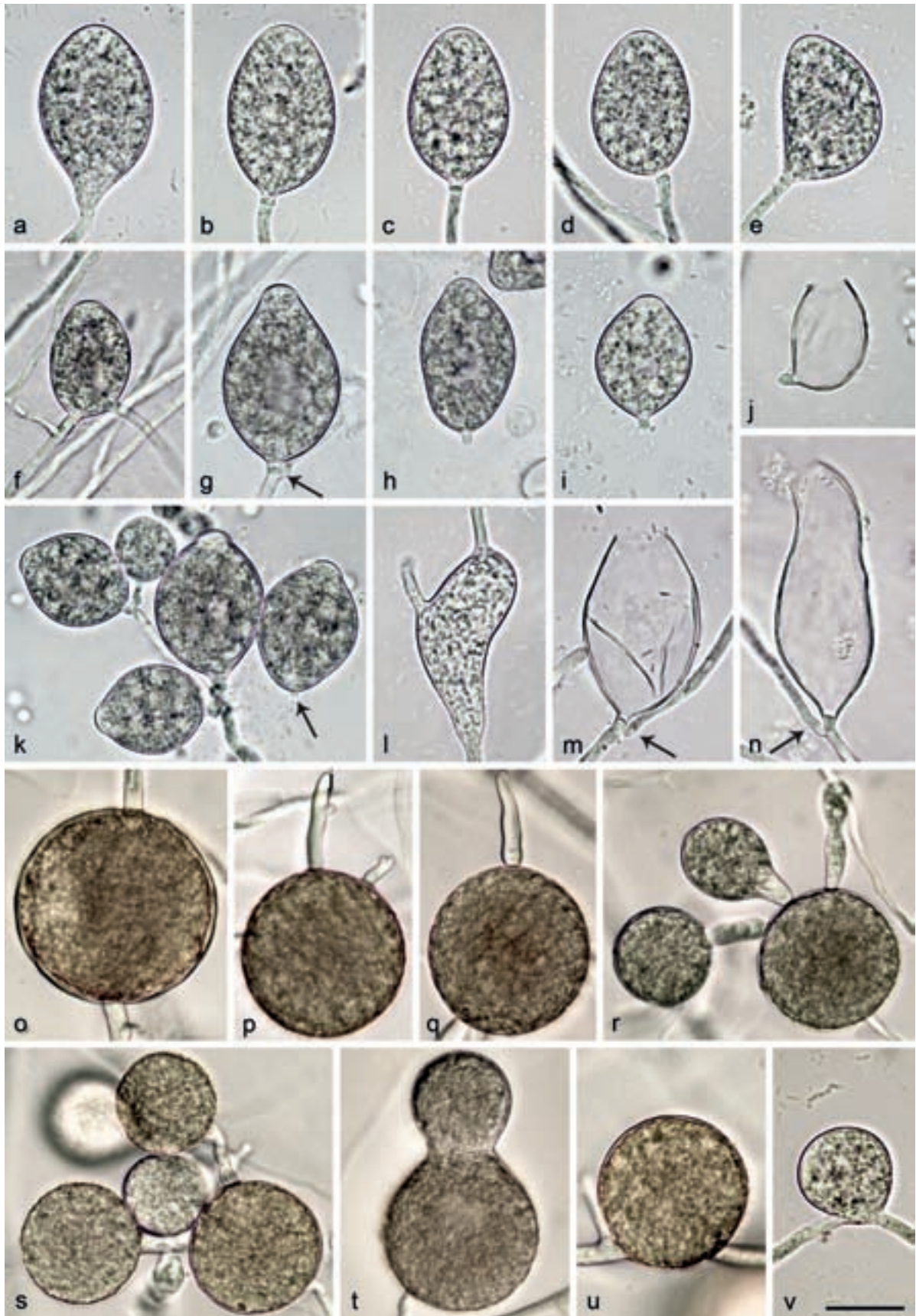


Fig 6 Morphological structures of *Nothophytophthora chlamydospora*. — a–n. Structures formed on V8 agar flooded with soil extract; a–i. mature non-papillate sporangia; a–e. borne terminally on unbranched sporangiophores; a. ellipsoid with tapering base; b–e. with conspicuous basal plugs; b. ellipsoid; c. ovoid; d. ovoid with slight lateral attachment of sporangiophore; e. mouse-shaped with laterally attached sporangiophore; f. ovoid, intercalary inserted; g. ovoid with vacuole, basal plug and beginning external proliferation (arrow); h–i. caducous with short pedicel-like basal plugs and small vacuoles; h. ellipsoid; i. limoniform; j. ovoid caducous sporangium with short pedicel-like basal plug, after release of zoospores; k. dense symposium of ovoid to limoniform sporangia with shallow semipapillate apices; one sporangium caducous with short pedicel-like basal plug (arrow); l. sporangium which failed to form a basal septum and continued to grow at the apex, functionally becoming a hyphal swelling; m–n. empty sporangia after release of zoospores, with conspicuous basal plugs and external proliferation close to the base; m. ovoid; n. elongated-obpyriform with curved apex; o–v. structures formed in solid V8 agar; o–u. chlamydospores; o. globose, intercalary inserted; p–q. globose, terminally inserted with hyphal outgrowths; r–s. globose with radiating hyphae forming hyphal swellings or secondary chlamydospores; t. ampulliform, terminally inserted; u. globose, laterally sessile; v. intercalary globose hyphal swelling. — Scale bar = 25 µm, applies to a–v.

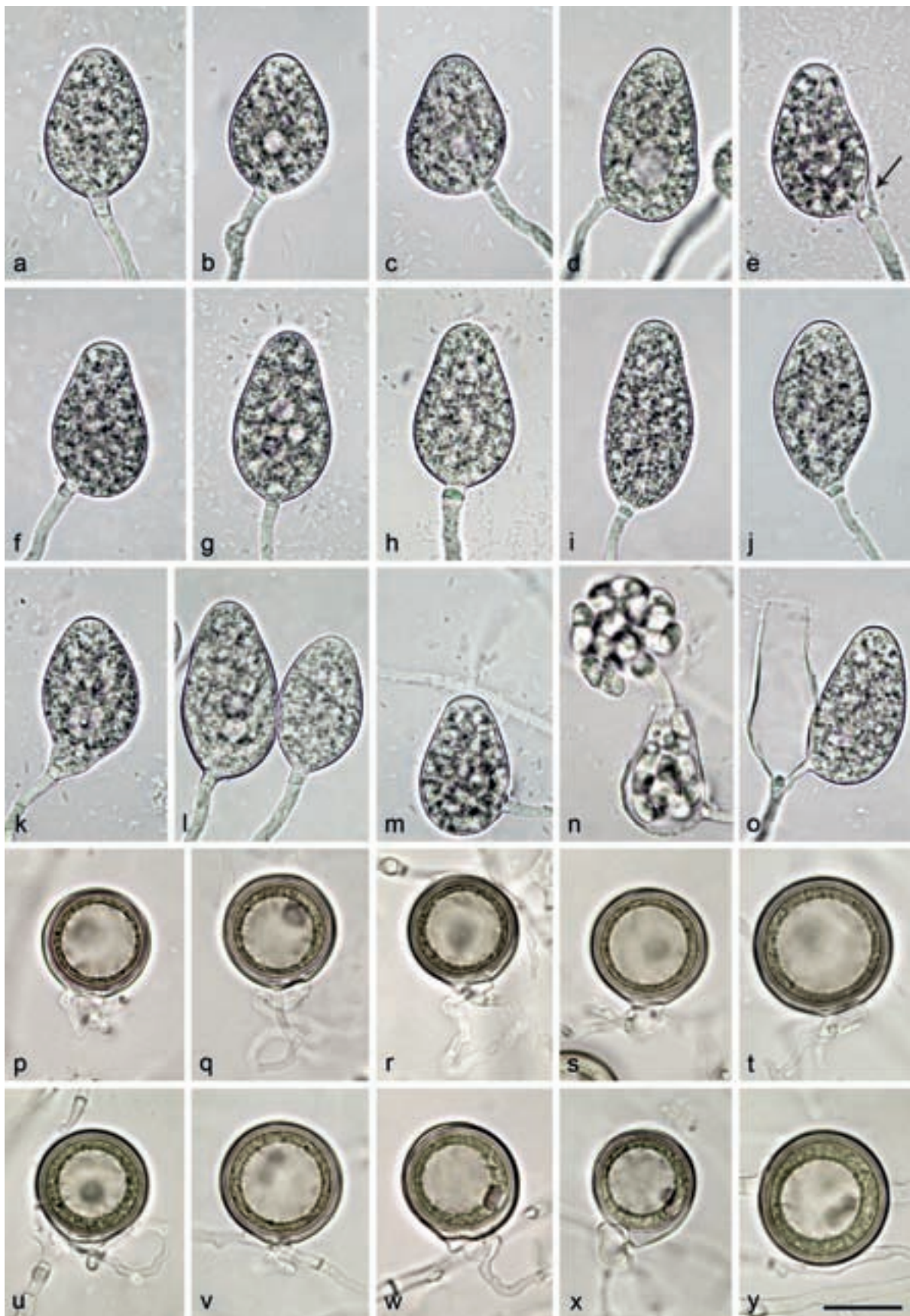


Fig. 7 Morphological structures of *Nothophytophthora intricata*. — a–o. Non-papillate sporangia with conspicuous basal plugs formed on V8 agar flooded with soil extract; a–n. borne terminally on unbranched sporangiophores; a–d. ovoid to elongated-ovoid; b–d. with small vacuoles; c–d. with laterally attached sporangiophores; e–h. obpyriform; e–f. with laterally attached sporangiophores and swollen apices before release of zoospores; i. elongated-obpyriform; j. ellipsoid with tapering slightly curved base; k. pyriform with vacuole and curved base; l. elongated-ovoid, one with vacuoles and laterally attached sporangiophore; m. ovoid with laterally attached sporangiophore and swollen apex, before release of zoospores; n. same sporangium as in m releasing zoospores; o. small sympodium of two sporangia resulting from external proliferation, empty sporangium elongated obpyriform and the other ovoid with laterally attached sporangiophore; p–y. mature, smooth-walled globose oogonia formed in single culture in CA, containing thick-walled plerotic oospores with particularly big ooplasts; p–x. with paragynous antheridia; p–w. with non-tapering bases; p–r. terminally inserted on thin stalks; q–r. with twisting intricate antheridial stalks; u–x. laterally inserted, sessile or on very short stalks; u. with undulating antheridial stalk; w. with twisting intricate antheridial stalk; x. with tapering base; y. intercalary inserted. — Scale bar = 25 μ m, applies to a–y.

reniform whilst motile, becoming spherical (av. diam = 8.6 ± 0.8 μm) on encystment. Cysts germinated directly. Intercalary or terminal, globose or limoniform, sometimes catenulate hyphal swellings, measuring 29.2 ± 6.1 μm , were formed by all isolates (Fig. 6v). Globose (98.1 %) or less frequently pyriform to irregular (1.9 %) chlamydospores (Fig. 6o–u) were produced intercalary or terminally and measured 43.7 ± 7.0 μm (Table 12). They often had radiating hyphae bearing hyphal swellings or secondary chlamydospores, thus, forming small clusters of chlamydospores and swellings (Fig. 6p–s).

Oogonia, oospores and antheridia — All five isolates of *N. chlamydospora* were self-sterile and did not form gametangia when paired with each other or with isolates of *N. chlamydospora*, *N. valdiviana* and with A1 and A2 tester strains of *P. cinnamomi*. Since in the nitrocellulose membrane test all isolates stimulated abundant oogonia production in the A2 tester strain of *P. cinnamomi*, their breeding system was considered as silent A1 mating type.

Colony morphology, growth rates and cardinal temperatures (Fig. 10, 11) — Colonies on V8A had a striate to chrysanthemum pattern and were largely submerged with very limited aerial mycelium. On CA and MEA colonies with limited aerial mycelium were produced, petaloid on CA and uniform to faintly petaloid on MEA. Colonies on PDA were rosaceous with dense-felt to woolly aerial mycelium (Fig. 10). Temperature-growth relations are shown in Fig. 11. All four isolates included in the growth test had similar growth rates and cardinal temperatures. The maximum and lethal growth temperatures were 25 and 26 °C, respectively. The average radial growth rate at the optimum temperature of 20 °C was 3.2 ± 0.05 mm/d (Fig. 11).

Additional specimens. CHILE, isolated from a stream in a temperate Valdivian rainforest, T. Jung, 25 Nov. 2014; CBS 142352 = CL195; CL317; CL318; CL319.

Nothophytophthora intricata T. Jung, Scanu, Bakonyi & M. Horta Jung, *sp. nov.* — MycoBank MB820538; Fig. 7

Etymology. Name refers to the intricate, intertwining antheridial stalks (*intricata* Lat = intricate or intertwining).

Typus. GERMANY, Wiesbaden, rhizosphere of a declining mature *Aesculus hippocastanum* tree in the floodplain of the river Main, T. Jung, 5 Aug. 2011 (CBS H-23009 holotype, dried culture on CA, Herbarium Westerdijk Fungal Biodiversity Institute, CBS 142354 = RK113-1s, ex-type culture). ITS and *cox1* sequences GenBank KY788413 and KY788501, respectively.

Sporangia, hyphal swellings and chlamydospores (Fig. 7a–o) — Sporangia were not observed in solid agar but were produced abundantly in non-sterile soil extract. Sporangia of *N. intricata* were typically borne terminally on unbranched sporangiophores (Fig. 7a–n) or less frequently forming lax sympodia of 1–3 sporangia (5.8 %; Fig. 7e, o). Subglobose to limoniform hyphal swellings (9.8 ± 1.5 μm) were sometimes formed on sporangiophores. Sporangia were non-papillate and non-caducous (Fig. 7a–o). Mature sporangia were usually delimited by a conspicuous opaque plug (91.1 %; 2.9 ± 0.7 μm) formed inside the sporangiophore close to the sporangial base (Fig. 7a–o) which sometimes protruded into the empty sporangium (Fig. 7o). Sporangia with special features such as lateral attachment of the sporangiophore (40.0 %; Fig. 7c–f, m–o), curved base (4.6 %; Fig. 7j–k), curved apex (2.1 %) or the presence of a vacuole (20.0 %; Fig. 7b–e, k, l) and undulating sporangiophores (10.4 %) were common in all isolates. Sporangia were mostly ovoid to elongated-ovoid (70.5 %; Fig. 7a–d, k–o), obpyriform (15.4 %; Fig. 7e–i, o), limoniform (6.3 %), ellipsoid (5.0 %; Fig. 7j) and less frequently pyriform (1.3 %), ampulliform (0.8 %) or mouse-shaped (0.7 %). Sporangial proliferation was exclusively external (Fig. 7e, o). Sporangial dimensions of six isolates of *N. intricata* averaged $38.5 \pm 2.8 \times 24.8 \pm 1.5$ μm (overall range 27.8–49.2 \times 18.6–30.2 μm) with a range of isolate means of

37.6–40.5 \times 23.4–26.3 μm and a length/breadth ratio of 1.55 \pm 0.18 (range of isolate means 1.47–1.65) (Table 12). Zoospores of *N. intricata* were discharged through an exit pore 4.8–13.8 μm wide (av. 9.0 ± 1.6 μm) (Fig. 7n, o). They were limoniform to reniform whilst motile, becoming spherical (8.1 ± 1.1 μm) on encystment. Direct germination of sporangia was not observed. In solid agar, hyphal swellings or chlamydospores were not formed.

Oogonia, oospores and antheridia (Fig. 7p–y) — Gametangia were readily produced in single culture on CA by all isolates of *N. intricata* within 10–14 d. Gametangia formation was usually starting at and was sometimes restricted to the edges of the colonies close to the walls of the Petri dishes. Oogonia had smooth walls and were borne terminally on thin, often undulating stalks (Fig. 7p–t) or were sessile (Fig. 7u–x) or less frequently intercalary inserted (Fig. 7y). They were usually globose to slightly subglobose (94.4 %) with mostly non-tapering bases (Fig. 7p–w, y) or less frequently slightly elongated (5.6 %) with tapering bases (Fig. 7x) and almost exclusively plerotic (96.9 %; Fig. 7p–y). Mean diameter of oogonia was 30.1 ± 3.9 μm with an overall range of 16.7–41.8 μm and a range of isolate means of 28.1–31.8 μm (Table 12). Oospores of *N. intricata* were globose and contained particularly large ooplasts (Fig. 7p–y). Oospore dimensions averaged 28.3 ± 3.5 μm (overall range 15.7–38.4 μm) with a wall diam of 2.1 ± 0.4 μm (range 1.0–3.2 μm) and an oospore wall index of 0.38 ± 0.05 (Table 12). Oospore abortion was low (4.5 % after 4 wk at 20 °C increasing to 10.8 % after 12 mo storage at 8 °C). The antheridia were club-shaped to subglobose and exclusively paragnous (Fig. 7p–y). Antheridial stalks were often intricate and undulating (63.3 %; Fig. 7q–r, u, w). Antheridial dimensions averaged $10.0 \pm 1.9 \times 6.9 \pm 1.2$ μm . In the nitrocellulose membrane test all isolates stimulated abundant oogonia production in the A2 tester strain of *P. cinnamomi*.

Colony morphology, growth rates and cardinal temperatures (Fig. 10, 11) — All isolates of *N. intricata* formed similar colonies on the same agar medium. Colonies on all media were round with regular margins (Fig. 10). On V8A faintly stellate colonies with limited aerial mycelium were formed. On CA and MEA colonies had stellate patterns and were dense-felt on CA and largely submerged with limited aerial mycelium on MEA. Colonies on PDA were faintly striate with moderate aerial mycelium (Fig. 10). Temperature-growth relations are shown in Fig. 11. All five isolates included in the growth test had similar growth rates and cardinal temperatures. The maximum and lethal temperatures were 27 and 28 °C, respectively. The average radial growth rates at 20 °C and at the optimum temperature of 25 °C were 2.2 ± 0.06 and 2.5 ± 0.07 mm/d, respectively (Fig. 11).

Additional specimens. GERMANY, Wiesbaden, rhizosphere of declining mature *Aesculus hippocastanum* trees in the floodplain of the river Main, T. Jung, 5 Aug. 2011; CBS 142355 = RK113-1sH; RK113-1sa; RK113-1sb; RK113-1sHa; RK113-1sHb.

Nothophytophthora valdiviana T. Jung, Scanu, Bakonyi, A. Durán & M. Horta Jung, *sp. nov.* — MycoBank MB820539; Fig. 8

Etymology. Name refers to the origin of all known isolates in Valdivian rainforests.

Typus. CHILE, isolated from a stream in a temperate Valdivian rainforest, T. Jung, 25 Nov. 2014 (CBS H-23010 holotype, dried culture on CA, Herbarium Westerdijk Fungal Biodiversity Institute, CBS 142357 = CL331, ex-type culture). ITS and *cox1* sequences GenBank KY788417 and KY788505, respectively.

Sporangia, hyphal swellings and chlamydospores (Fig. 8a–q) — Sporangia of *N. valdiviana* were not formed on solid agar but were produced abundantly in non-sterile soil extract. Sporangia

were non-papillate to shallow semi-papillate (Fig. 8a–o). In all isolates, a low proportion of sporangia were caducous (4–10 %, on av. 6.8 %) breaking off below a pedicel-like opaque plug (2.4 ± 0.5 µm; Fig. 8m–o) which is formed inside the sporangiophore close to the base of 78 % of all sporangia (Fig. 8a–p). Sporangia were ovoid to elongated ovoid (51.0 %; Fig. 8a–f, k–q), limoniiform (40.5 %; Fig. 8i–l) and less frequently ellipsoid (6.0 %; Fig. 8g, q) or obpyriform (1.5 %; Fig. 8h). Sporangiohores were sometimes undulating (15.0 %) and infrequently laterally

attached to the sporangia (5.5 %). A vacuole was observed in 28.1 % of sporangia (Fig. 8j–l, n, o). Mean sporangial dimensions of five isolates were 42.7 ± 4.6 × 28.0 ± 3.5 µm (overall range 30.2–55.7 × 18.6–47.5 µm) with a range of isolate means of 40.4–44.7 × 25.6–29.5 µm (Table 12). The length/breadth ratio averaged 1.53 ± 0.14 with a range of isolate means of 1.48–1.62. In all isolates, sporangia proliferated internally in both a nested and extended way (Fig. 8q). In addition, sporangiophores often branched externally close to the sporangial

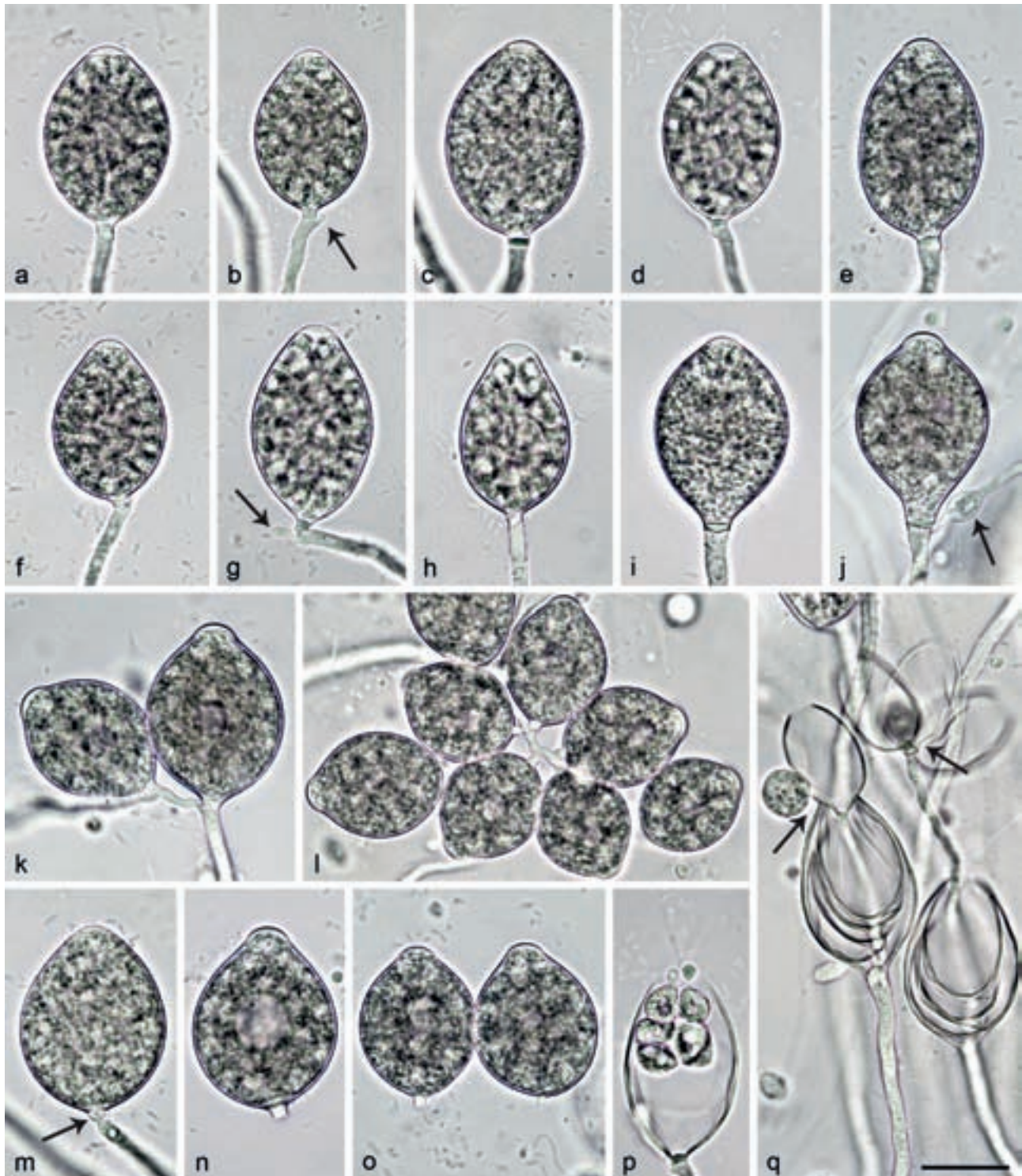


Fig. 8 Sporangia of *Nothophytophthora valdiviana* formed on V8 agar flooded with soil extract. — a–p. Mature non-papillate to shallow semipapillate sporangia with conspicuous basal plugs; a–f. ovoid; a–b, d. with differentiated zoospores to be released soon; b. with beginning external proliferation close to the sporangial base (arrows); g. ellipsoid with beginning external proliferation, just breaking off at base of pedicel-like basal plug; h. obpyriform, just before zoospore release; i. limoniiform; j. limoniiform with vacuole, external proliferation and swelling on the sporangiophore (arrow); k. small symposium of two ovoid to limoniiform sporangia with vacuoles, resulting from external proliferation; l. dense symposium of ovoid and mostly limoniiform sporangia, some with small vacuoles; m. ovoid sporangium breaking off at base of pedicel-like basal plug (arrow); n–o. ovoid, caducous sporangia with vacuoles and short pedicel-like basal plugs; p. same sporangium as in d releasing zoospores; q. empty sporangia showing internal nested and extended proliferation, and external proliferation (arrows). — Scale bar = 25 µm, applies to a–q.

base (Fig. 8b, g, j–l, q) forming lax or dense sympodia of up to 8–10 sporangia (Fig. 8k–l). Subglobose to limoniform swellings, averaging $14.0 \pm 2.7 \mu\text{m}$, were infrequently produced on sporangiophores (Fig. 8j) by all isolates. Zoospores of *N. valdiviana* were discharged through exit pores $9.4 \pm 1.8 \mu\text{m}$ (5.5 – $13.0 \mu\text{m}$) wide (Fig. 8p–q). They were limoniform to reniform whilst motile (Fig. 8p), becoming spherical (av. diam = $8.6 \pm 1.1 \mu\text{m}$) on encystment. Cysts germinated directly. Chlamydospores were not observed.

Oogonia, oospores and antheridia — All five isolates of *N. valdiviana* were self-sterile and did not form gametangia when paired with each other or with isolates of *N. caduca*, *N. chlamydospora* and A1 and A2 tester strains of *P. cinnamomi*. In the nitrocellulose membrane test all isolates stimulated abundant oogonia production in the A2 tester strain of *P. cinnamomi*. Therefore, their breeding system was considered as silent A1 mating type.

Colony morphology, growth rates and cardinal temperatures (Fig. 10, 11) — All *N. valdiviana* isolates formed similar colonies on the same agar medium. Colonies on CA and V8A were largely submerged with limited aerial mycelium, with a chrysanthemum pattern on V8A and a stellate to chrysanthemum pattern on CA. On PDA and MEA colonies were appressed with dense felty aerial mycelium and rosaceous patterns, respectively (Fig. 10). The temperature-growth relations on V8A are shown in Fig. 11. All isolates showed slow growth and had similar growth rates at the same temperature. Optimum and maximum growth temperatures were 25 and 28 °C, respectively. Isolates did not resume growth when plates incubated for 5 d at 30 °C were transferred to 20 °C. The average radial growth rates at 20 and 25 °C were $2.9 \pm 0.05 \text{ mm/d}$ and $3.1 \pm 0.1 \text{ mm/d}$, respectively (Fig. 11).

Additional specimens. CHILE, isolated from a stream in a temperate Valdivian rainforest, T. Jung, 25 Nov. 2014; CBS 142356 = CL242; CL329; CL330; CL332.

Nothophytophthora vietnamensis T. Jung, Scanu, Bakonyi, P.Q. Thu & M. Horta Jung, *sp. nov.* — MycoBank MB820541; Fig. 9

Etymology. Name refers to the origin of all known isolates in Vietnam.

Typus. VIETNAM, Fansipan, rhizosphere soil of *Castanopsis* sp. and *Acer campbellii*, T. Jung, 27 Mar. 2016 (CBS H-23012 holotype, dried culture on CA, Herbarium Westerdijk Fungal Biodiversity Institute, CBS 142358 = VN794, ex-type culture). ITS and *cox1* sequences GenBank KY788420 and KY788508, respectively.

Sporangia, hyphal swellings and chlamydospores (Fig. 9a–m) — Sporangia were not observed in solid agar but were produced abundantly in non-sterile soil extract. Sporangia of *N. vietnamensis* were borne terminally on unbranched sporangiophores (Fig. 9c–g) or in lax or dense sympodia of 2–7 sporangia (Fig. 9h). Small subglobose to limoniform hyphal swellings were only rarely observed on sporangiophores. Mature sporangia were non-papillate and usually delimited by a pedicel-like, conspicuous opaque plug ($2.7 \pm 0.7 \mu\text{m}$) formed inside the sporangiophore close to the sporangial base (over all isolates 93.5 %; Fig. 9a–m). Sporangia were partially caducous (4–36 %, on av. 15.8 %) breaking off just below the basal plug (Fig. 9h, j–l). Sporangial shapes were mostly ovoid to elongated-ovoid (90.5 %; Fig. 9a–e, h, j–m) or infrequently ellipsoid (6.0 %; Fig. 9h), limoniform (3.4 %; Fig. 9f) or pyriform (0.1 %; Fig. 9g, i). Special features such as the presence of a vacuole (55.0 %; Fig. 9b, d–l), slightly lateral attachment of the sporangiophore (14.0 %; Fig. 9c–d, j–l), a curved apex (0.4 %; Fig. 9e) or undulating sporangiophores (3.1 %) were common in all isolates. Sporangial dimensions of eight isolates of *N. vietnamensis* averaged $36.4 \pm 12.7 \times 29.3 \pm 8.1 \mu\text{m}$ with an overall

range of 28.4 – 42.1×20.6 – $28.1 \mu\text{m}$, a range of isolate means of 34.1 – 37.9×24.1 – $25.8 \mu\text{m}$ and a length/breadth ratio of 1.47 ± 0.08 (range of isolate means 1.42 – 1.52) (Table 12). Sporangial proliferation was exclusively external (20.5 % of sporangia; Fig. 9a–b, h–j, m). Zoospores of *N. vietnamensis* were discharged through an exit pore 4.1 – $11.7 \mu\text{m}$ wide ($7.6 \pm 1.5 \mu\text{m}$) (Fig. 9m). They were limoniform to reniform whilst motile, becoming spherical ($8.4 \pm 0.7 \mu\text{m}$) on encystment. Direct germination of sporangia was not observed. In solid agar, no hyphal swellings or chlamydospores were produced.

Oogonia, oospores and antheridia (Fig. 9n–w) — Gametangia were readily produced in single culture by all isolates of *N. vietnamensis* on CA within 10–14 d. Gametangia formation was usually starting at and was sometimes restricted to the edges of the colonies close to the walls of the Petri dishes. Oogonia were borne terminally, had smooth walls and due to their mostly long tapering (75.4 %) and curved (24.4 %) bases were mostly elongated pyriform to ellipsoid (70.6 %; Fig. 9n–v) or less frequently globose to slightly subglobose (49.4 %; Fig. 9w). Only 3.1 % of oogonia had particularly thin stalks. Oogonia were small with a mean diameter of $23.9 \pm 3.0 \mu\text{m}$ (overall range 18.6 – $33.0 \mu\text{m}$ and range of isolate means 22.3 – $27.3 \mu\text{m}$) (Table 12). They were almost exclusively pleurotic (96.9 %). Oospores of *N. vietnamensis* contained large ooplasts and were usually globose (Fig. 9n, p–s, u, w) or less frequently slightly elongated (10.6 %; Fig. 9o, t, v). Oospores had diameters of $22.5 \pm 2.4 \mu\text{m}$ (overall range 17.6 – $29.5 \mu\text{m}$) with walls averaging $1.8 \pm 0.3 \mu\text{m}$ (range 1.1 – $2.5 \mu\text{m}$) and an oospore wall index of 0.42 ± 0.05 (Table 12). Oospore abortion was low (1.0 % after 4 wk). The antheridia were club-shaped to subglobose, exclusively paragynous (Fig. 9n–w) and small with dimensions averaging $7.2 \pm 1.2 \times 4.6 \pm 0.9 \mu\text{m}$. Antheridial stalks were often twisted and undulating (46.7 %; Fig. 9n, p, r, v). In the nitrocellulose membrane test none of the isolates tested stimulated oogonia production in the A1 and A2 tester strains of *P. cinnamomi*.

Colony morphology, growth rates and cardinal temperatures (Fig. 10, 11) — All isolates produced similar colonies on the same agar medium. Colonies on V8A and MEA were radiate to slightly radiate with limited aerial mycelium around the inoculum plug and a submerged edge on V8A (Fig. 10). On CA, appressed radiate colonies with limited aerial mycelium were formed whereas colonies on PDA had a chrysanthemum pattern with dense-felty aerial mycelium (Fig. 10). Temperature-growth relations are shown in Fig. 11. All eight isolates included in the growth test had similar growth rates and cardinal temperatures. The maximum growth temperature was 27 °C. The isolates did not resume growth when plates incubated for 5 d at 29 °C were transferred to 20 °C. The average radial growth rates at 20 °C and at the optimum temperature of 25 °C were 2.5 ± 0.04 and $2.9 \pm 0.05 \text{ mm/d}$, respectively (Fig. 11).

Additional specimens. VIETNAM, Fansipan, rhizosphere soil of *Castanopsis* sp. and *Acer campbellii*, 27 Mar. 2016, T. Jung; CBS 142359 = VN795; VN230; VN796; VN797; VN798; VN799; VN800.

NOTES

The genera *Nothophytophthora* and *Phytophthora* share numerous morphological characters like persistent and caducous sporangia with variable shapes, internal differentiation of zoospores, and external and internal nested and extended sporangial proliferation; smooth-walled oogonia with amphigynous and/or paragynous attachment of the antheridia; chlamydospores and hyphal swellings. Several of these characters are also common to *Halophytophthora* but general absence of caducity and internal proliferation of sporangia and of amphigynous antheridia, and the release of zoospores through



Fig. 9 Morphological structures of *Nothophytophthora vietnamensis*. — a–m. Mature non-papillate sporangia with conspicuous basal plugs formed on V8 agar flooded with soil extract; a–e. ovoid; a–b. with beginning external proliferation close to sporangial base (arrows); b. with vacuole; d. with slightly lateral attachment of sporangiophore; e. with vacuole and curved apex; c–g. borne terminally on unbranched sporangiophores; f. limoniform with vacuole; g. pyriform with vacuole; h. dense symposium of ellipsoid and ovoid sporangia with vacuoles, one sporangium caducous with pedicel-like basal plug (arrow); i. elongated-pyriform with vacuole and external proliferation; j. ovoid sporangium with vacuole and external proliferation, breaking off at base of pedicel-like basal plug (arrow); k–l. ovoid, caducous sporangia with vacuoles and short pedicel-like basal plugs; m. ovoid sporangium with external proliferation, releasing 40 zoospores; n–w. mature, smooth-walled oogonia formed in single culture in CA, with thick-walled plerotic oospores containing big ooplasts and with paragynous antheridia; n–v. elongated-pyriform with tapering curved bases; o, t. with elongated oospores; n, p, r, v. with undulating antheridial stalks; w. subglobose with short tapering base. — Scale bar = 25 μ m, applies to a–w.

Table 12 Morphological characters and dimensions (μm), cardinal temperatures ($^{\circ}\text{C}$) and temperature-growth relations (mm/d) on V8-juice agar^a of *Nothophytophthora* species. Most discriminating characters are highlighted in bold. Percentages in brackets are ranges of isolate means.

	<i>N. amphigynosa</i>	<i>N. caduca</i>	<i>N. chlamydospora</i>	<i>N. valdiviana</i>	<i>N. intricata</i>	<i>N. vietnamensis</i>
No. of isolates	8 ^b	14 ^b	5 ^b	5 ^b	6 ^b	8 ^b
Sporangia	82 % ovoid, 12 % ellipsoid, 5 % obpyriform (limoniform, mouse-shaped)	83 % ovoid , 7 % ellipsoid, 4 % limoniform (obpyriform, pyriform, mouse-shaped)	44 % ovoid, 27.5 % ellipsoid , 22.5 % limoniform (obpyriform, pyriform, mouse-shaped)	50.5 % ovoid, 40.5 % limoniform , 6 % ellipsoid , (obpyriform, pyriform, mouse-shaped)	71 % ovoid, 15 % obpyriform , 7 % limoniform, 5 % ellipsoid (pyriform, mouse-shaped)	91 % ovoid , 6 % ellipsoid, 3 % limoniform
l × b mean	47.0 ± 5.6 × 26.4 ± 1.8	37.9 ± 4.6 × 25.7 ± 3.0	37.6 ± 4.9 × 22.1 ± 2.5	42.7 ± 4.6 × 28.0 ± 3.5	38.5 ± 2.8 × 24.8 ± 1.5	36.4 ± 12.7 × 29.3 ± 8.1
range of isolate means	41.5–52.0 × 25.4–27.3	34.7–43.1 × 23.3–28.2	35.6–38.9 × 20.4–23.2	40.4–44.7 × 25.6–29.5	37.6–40.5 × 23.4–26.3	34.1–37.9 × 24.1–25.8
total range	33.6–60.6 × 21.3–32.4	24.1–54.4 × 18.1–35.9	27.4–57.2 × 17.0–30.8	30.2–55.7 × 18.6–47.5	27.8–42.1 × 20.6–28.1	28.4–42.1 × 20.6–28.1
l/b ratio	1.78 ± 0.17	1.48 ± 0.15	1.71 ± 0.17	1.53 ± 0.14	1.55 ± 0.18	1.47 ± 0.08
caducity	2.9 ± 0.6	32.1 % (10–53 %)	25.2 % (11–41 %)	6.8 % (4–10 %)	–	15.8 % (4–36 %)
pedicel-like basal plug	–	2.6 ± 0.7	2.8 ± 1.6	2.4 ± 0.5	2.9 ± 0.7	2.7 ± 0.7
internal proliferation	–	nested and extended	–	nested and extended	–	–
exitpores	8.9 ± 1.4	10.4 ± 2.2	8.2 ± 1.7	9.4 ± 1.8	9.0 ± 1.6	7.6 ± 1.5
sympodia	infrequent, lax	frequent, lax	frequent, lax or dense	frequent, lax or dense	infrequent, lax	frequent, lax or dense
zoospore cysts	9.0 ± 1.1	7.4 ± 0.6	8.6 ± 0.8	8.6 ± 1.1	8.1 ± 1.1	8.4 ± 0.7
sporangiospore swellings	11.1 ± 2.8; rare	10.2 ± 2.0; rare	15.2 ± 6.3; rare	14.0 ± 2.7; rare	9.8 ± 1.5; rare	n/a; rare
Breeding system	homothallic	self-sterile	self-sterile	self-sterile	homothallic	homothallic
Oogonia						
mean diam	25.3 ± 1.7	–	–	–	30.1 ± 3.9	23.9 ± 3.0
range of isolate means	24.3–25.5	–	–	–	28.1–31.8	22.3–27.3
total range	18.4–29.7	–	–	–	16.7–41.8	18.6–33.0
tapering base	2.9 % (0–7.5 %)	–	–	–	7.5 % (0–30 %)	75.4 % (42–95 %)
thin stalks	58.3 % (10–100 %)	–	–	–	29.4 % (2.5–45 %)	3.1 % (0–12.5 %)
curved base	–	–	–	–	1.3 % (0–5 %)	24.4 % (7.5–32.5 %)
elongated	12.5 % (5–20 %)	–	–	–	5.6 % (0–17.5 %)	70.6 % (60–85 %)
Oospores						
plerotic oospores	99.2 %	–	–	–	96.9 % (92.5–100 %)	96.9 % (87.5–100 %)
mean diam	23.4 ± 1.7	–	–	–	28.3 ± 3.5	22.5 ± 2.4
Total range	17.2–28.0	–	–	–	15.7–38.4	17.6–29.5
wall diam	1.7 ± 0.3	–	–	–	2.1 ± 0.4	1.8 ± 0.3
oospore wall index	0.38 ± 0.05	–	–	–	0.38 ± 0.06	0.42 ± 0.05
Abortion rate	4.2 % (1–25 %)	–	–	–	10.8 % (1–18 %)	1.0 % (0–4 %)
Antheridia						
size	87.2 % amphigynous	–	–	–	100 % paragnynous	100 % paragnynous
intricate stalks	8.5 ± 1.8 × 6.5 ± 0.9	–	–	–	10.0 ± 1.9 × 6.9 ± 1.2	7.2 ± 1.2 × 4.6 ± 0.9
	28.8 % (22.5–35 %)	–	–	–	63.3 % (50–72.5 %)	46.7 % (42.5–52.5 %)
Chlamydospores	–	–	98.1 % globose, 1.9 % pyriform; radiating, forming clusters	–	–	–
	–	–	43.7 ± 7.0	–	–	–
	–	–	globose, (pyriform, limoniform)	–	–	–
	–	–	29.2 ± 6.1	–	–	–
Hyphe swellings	–	–	–	–	–	–
Lethal temperature	28	28 or 30	26	30	28	29
Maximum temperature	27	26 or 28	25	28	27	27
Optimum temperature	20	20 or 25	20	25	25	25
Growth rate at 20 °C	3.1 ± 0.05	3.1 ± 0.21	3.2 ± 0.05	2.9 ± 0.05	2.2 ± 0.06	2.5 ± 0.04
Growth rate at 25 °C	3.0 ± 0.06	3.6 ± 0.08	0.5 ± 0	3.1 ± 0.1	2.5 ± 0.07	2.9 ± 0.05

^a Oogonia and oospores were studied and measured on carrot agar.^b Numbers of isolates included in the growth tests: *N. amphigynosa* = 4; *N. caduca* = 10; *N. chlamydospora* = 4; *N. valdiviana* = 4; *N. intricata* = 5; *N. vietnamensis* = 8.

– = character not observed.

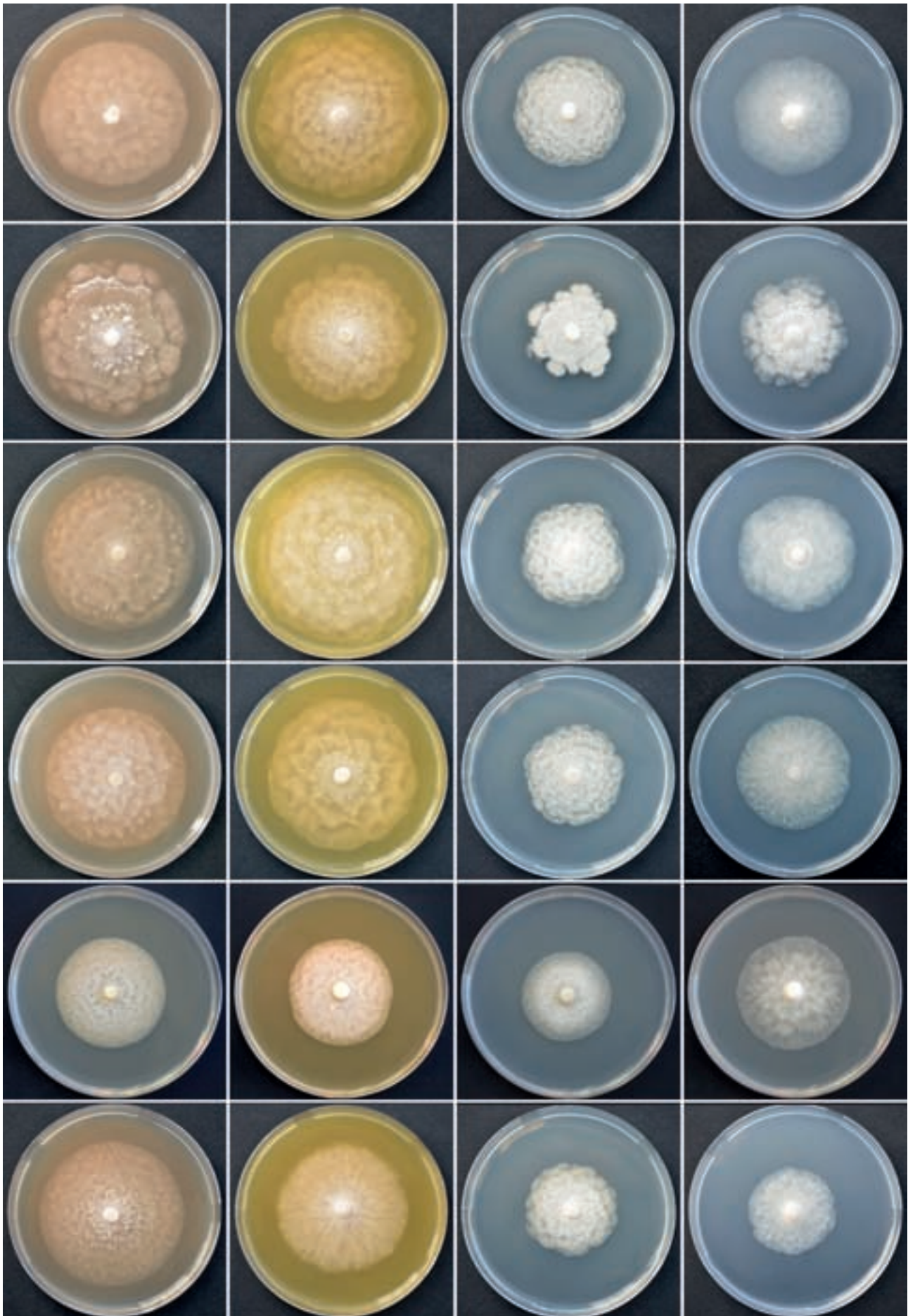


Fig. 10 Colony morphology of *Nothophytophthora amphigynosa*, *N. caduca*, *N. chlamydospora*, *N. valdiviana*, *N. intricata* and *N. vietnamensis* (from top to bottom) after 10 d growth at 20 °C on V8 agar, carrot agar, potato-dextrose agar and malt extract agar (from left to right).

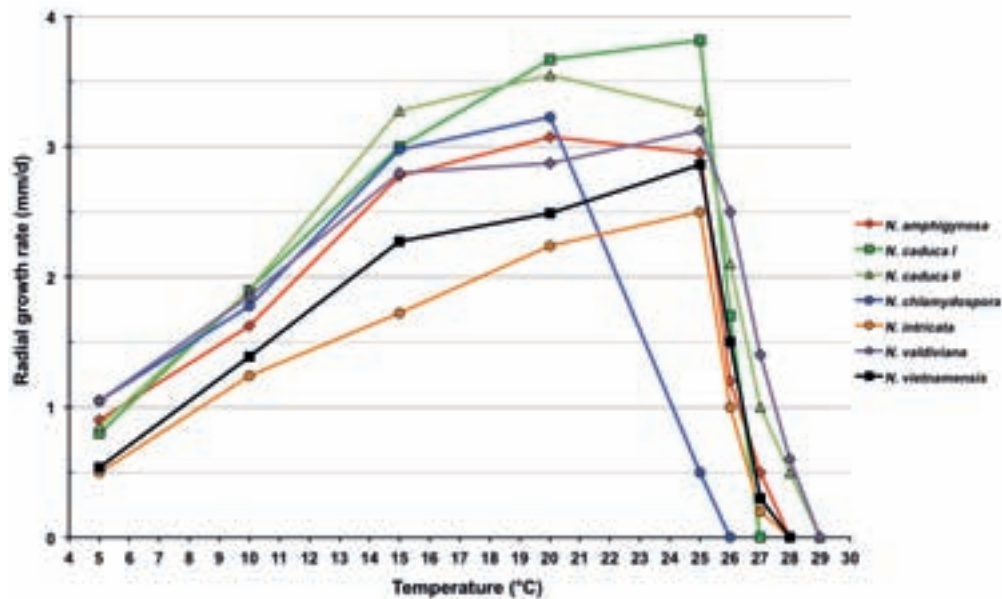


Fig. 11 Mean radial growth rates of *Nothophytophthora amphigynosa* (4 isolates), *N. caduca* (6 isolates from population *N. caduca* I; 4 isolates from population *N. caduca* II), *N. chlamydospora* (4 isolates), *N. intricata* (5 isolates), *N. valdiviana* (4 isolates) and *N. vietnamensis* (8 isolates) on V8 agar at different temperatures.

dehiscence tubes or into semi-persistent or persistent vesicles clearly differentiate *Halophytophthora* from the other two genera (Ho & Jong 1990, Nakagiri et al. 2001, Yang & Hong 2014). In *Nothophytophthora*, morphological structures are on average smaller than in most *Phytophthora* and *Halophytophthora* species but the ranges overlap widely. A significant difference between *Nothophytophthora* and *Phytophthora* is the presence of a conspicuous, opaque plug inside the sporangiophore close to the base of most mature sporangia in all known *Nothophytophthora* species which enables partial caducity in several species including *N. caduca*, *N. chlamydospora*, *N. valdiviana* and *N. vietnamensis*. A similar plug is also found in many *Halophytophthora* species (Ho & Jong 1990, Nakagiri et al. 2001). Also, intraspecific co-occurrence of caducity and non-papillate sporangia with internal nested and extended proliferation as in *N. caduca* and *N. valdiviana* is not known from any *Phytophthora* species except the recently described hybrid *P. xhetero hybrida* from subtropical monsoon forests in Taiwan (Jung et al. 2017b).

The LSU, *Btub*, *HSP90*, *cox1* and *NADH1* sequences of *N. amphigynosa*, *N. caduca*, *N. chlamydospora*, *N. intricata*, *N. valdiviana* and *N. vietnamensis* contain 31, 53, 29, 7, 31 and 9 unique polymorphisms, respectively, and differ from each other in 19–116 positions (Table 3–8). In the ITS rDNA region the six *Nothophytophthora* species had 0–189 unique polymorphisms and differed from each other at 5–356 positions (Table 2, 10). In addition, they can be easily separated from each other by a combination of morphological and physiological characters of which the most discriminating are highlighted in bold in Table 12. *Nothophytophthora amphigynosa* differs from the other two homothallic species *N. intricata* and *N. vietnamensis* by its predominant production of amphigynous antheridia and by having on average considerably larger sporangia (Table 12; Fig. 4, 7, 9). In addition, *N. vietnamensis* is distinguished from *N. amphigynosa* by its partial caducity of sporangia, the predominance of elongated oogonia with tapering often curved bases, different colony morphologies on CA, MEA and V8A, markedly slower growth at 15 and 20 °C, and a higher optimum temperature for growth (Table 12; Fig. 4, 9–11). *Nothophytophthora intricata* differs from *N. amphigynosa* by its lower sporangial l/b ratio, larger oogonial dimensions, the frequent occurrence of intricate, intertwining antheridial stalks, different colony morphologies on all four agar media tested, slower growth at

all temperatures, and higher optimum temperature for growth (Table 12; Fig. 4, 7, 10–11). *Nothophytophthora vietnamensis* can be separated from its sister species *N. intricata* by having sporangia which are partially caducous and often formed in dense sympodia, considerably smaller and mostly elongated oogonia with tapering bases, different colony morphologies on all four agar media tested, and faster growth between 10 and 25 °C (Table 12; Fig. 7, 9–11). *Nothophytophthora chlamydospora* differs from all other five *Nothophytophthora* species by the production of chlamydospores. It can also be distinguished from its closest relative *N. valdiviana* by a markedly higher proportion of caducous sporangia, higher sporangial l/b ratio, absence of internal sporangial proliferation, different colony growth patterns on CA and MEA, considerably slower growth at 25 °C, and lower optimum and maximum temperature for growth (Table 12; Fig. 6, 8, 10–11); and from *N. caduca* by its higher sporangial l/b ratio, absence of internal sporangial proliferation, production of sporangia in dense sympodia, different colony growth patterns on all four agar media, considerably slower growth at 25 °C, and lower maximum temperature for growth (Table 12; Fig. 5–6, 10–11). *Nothophytophthora caduca* and *N. valdiviana* can easily be separated by the predominance of ovoid sporangial shapes, considerably higher level of caducity, absence of dense sympodia and faster growth at 20 °C in *N. caduca*, and by different colony growth patterns on all four agar media (Table 12; Fig. 5, 8, 10–11). Although the two populations of *N. caduca* from two different Valdivian rainforest streams had slightly different optimum and maximum temperatures for growth (Fig. 11) and differed in *cox1* at 25 positions (Table 6) their morphology was indistinguishable and, hence, they were considered to belong to the same species.

HOSTS AND GEOGRAPHIC DISTRIBUTION

Nothophytophthora amphigynosa was exclusively isolated from a small forest stream running through a planted mature forest of *Eucalyptus globulus*, *Cupressus sempervirens* and *Quercus* spp. close to Sintra, Portugal (N38°47'28.0" W9°25'28.7", 163 m above sea level (a.s.l.)) with a humid Atlantic climate. It co-occurred with two aquatic sterile *Phytophthora* species, *P. amnicola* and *P. chlamydospora*. *Nothophytophthora intricata* was isolated alongside *P. xcambivora*, *P. megasperma*

and *P. plurivora* from the rhizosphere of declining mature *Aesculus hippocastanum* trees in the floodplain of the river Main in Wiesbaden, Germany (N49°59'48.8" E8°18'3.2", 84 m a.s.l.). *Nothophytophthora vietnamensis* was recovered from rhizosphere soil of *Castanopsis* sp. and *Acer campbellii* trees with dieback symptoms on the banks of a small stream in a humid, montane monsoon forest at the Fansipan in Vietnam (N22°19'40.2" E103°46'53.1", 2 242 m a.s.l.) where it co-occurred with *P. attenuata*, *P. castaneae* and *P. cinnamomi*. *Nothophytophthora caduca* (S39°58'9.2" W73°34'13.4", 198 m a.s.l.; S39°58'17.6" W73°34'5.3", 193 m a.s.l.), *N. chlamydospora* (S39°57'47.2" W73°37'9.3", 9 m a.s.l.) and *N. valdiviana* (S39°58'3.7" W73°33'41.9", 175 m a.s.l.) were detected in four forest streams in the Reserva Costera Valdiviana in Chile with the catchments covered by natural Valdivian rainforests and plantations of *E. globulus*. All three species co-occurred in these streams with *P. chlamydospora* and *P. kernoviae*.

DISCUSSION

During various surveys of oomycete diversity in Europe, Chile and Vietnam slow growing cryptic isolates were detected alongside a diverse community of *Phytophthora*, *Pythium* and *Phytopythium* species from rhizosphere soil and streams in natural and semi-natural ecosystems. Phylogenetic analyses of sequences from the nuclear ITS, LSU, *Btub* and *HSP90* genes and the mitochondrial *cox1* and *NADH1* genes placed them into six distinct previously unknown species belonging to a new genus described here as *Nothophytophthora* gen. nov. Phylogenetic analyses together with detailed morphological and physiological studies allowed the taxonomic description of these six taxa as *N. amphigynosa*, *N. caduca*, *N. chlamydospora*, *N. intricata*, *N. valdiviana* and *N. vietnamensis*.

Sparrow (1960, 1976) accepted only four orders in the oomycetes, i.e., *Leptomitales*, *Saprolegniales*, *Lagenidiales* and *Peronosporales*. Dick et al. (1984) and Dick (2001) proposed the order *Pythiales*. In the latter publications the number of orders in the oomycota was increased to 12 and in early phylogenies (Riethmüller et al. 1999, Cooke et al. 2000) the *Pythiales* were accepted and *Pythium* and the *Pythiaceae* were assigned to this order. However, *Pythium* and the *Pythiaceae* were originally assigned to the *Peronosporales* (Fischer 1892, Schröter 1893). More recently, refined multigene phylogenies have abandoned the order *Pythiales* and returned to the four orders accepted by Sparrows (1960, 1976). We followed this trend since in the multigene phylogeny of the present study *Pythium* s.lat. grouped within a larger group that included the *Salisapiliaceae* and the *Peronosporaceae*, both considered belonging to the *Peronosporales* (Hulvey et al. 2010, Thines & Choi 2016). The multigene phylogeny of this work demonstrated that *Nothophytophthora* and *Phytophthora* including the downy mildews form a monophyletic clade with *Halophytophthora* s.str. clustering basal to this clade and *Phytopythium* residing in a basal position of this group which forms the *Peronosporaceae*, order *Peronosporales*, sensu Baxter et al. (2010), Hulvey et al. (2010) and Thines & Choi (2016). *Halophytophthora operculata* resided in a basal position to the genus *Phytopythium* supporting earlier suggestions that this species should be transferred to *Phytopythium* (Marano et al. 2014, De Cock et al. 2015). In contrast, *H. epistomium* clearly belongs to a new genus outside of both the *Peronosporaceae* and *Pythiaceae*. The six *Nothophytophthora* species showed nucleotide sequence similarities to the related genera *Phytophthora* (*P. boehmeriae*, *P. humicola* and *P. rubi*), *Halophytophthora* (*H. avicenniae*) and *Phytopythium* (*Ph. helicoides*) of 90.8–92.1 %, 90.8–91.0 % and 88.1–88.6 % across the five coding genes LSU, *Btub*,

HSP90, *cox1* and *NADH1*, and of 56.8–68.1 %, 53.9–63.7 % and 46.8–58.5 % in the ITS region.

Despite the high number of oomycete surveys performed during the previous two decades in both managed and natural ecosystems, only four GenBank entries match *Nothophytophthora*. Isolates PR13-109 and PR12-475 (GenBank accessions KT633938 and KT633937) obtained in 2014 and 2015 from streams in Ireland and isolate REB326-69 (JX122744) from a stream in New Zealand, all originally designated as *Phytophthora* sp. (Table 1; Than et al. 2013, O'Hanlon et al. 2016). Unfortunately, only ITS sequences were available for these *Nothophytophthora* isolates preventing their inclusion in the multigene phylogenetic analyses of the present study. However, in a phylogenetic analysis of ITS sequences the three isolates resided in a clade formed by *N. chlamydospora* and *N. valdiviana* from Chile with the Irish isolate PR12-475 being basal to the clade and isolates PR13-109 and REB326-69 clustering in a non-supported sister position to *N. valdiviana*. The relatedness of these congeneric isolates to *N. chlamydospora* is also demonstrated by the production of chlamydospores by both Irish isolates (Richard O'Hanlon, pers. comm.). In a metagenomic stream survey in the Spanish Pyrenees, Català et al. (2015) found a phylotype ('MOTU 33') which resided in *Nothophytophthora* in a separate phylogenetic analysis of shorter ITS sequences (data not shown). In a yet unpublished metagenomic stream survey in Scotland another *Nothophytophthora* phylotype was found (David E.L. Cooke, pers. comm.). The findings of *Nothophytophthora* isolates and phylotypes in Germany, Portugal, Ireland, Scotland, Spain, Chile, New Zealand and Vietnam indicate a wide distribution of this new genus. The scarcity of records in GenBank is most likely caused by the slow growth of *Nothophytophthora* species which hampers their isolation in presence of faster growing oomycetes like *Pythium* s.lat., *Phytopythium* and *Phytophthora*.

Multiple heterozygous positions in the ITS and LSU sequences of *N. amphigynosa*, *N. chlamydospora* and *N. vietnamensis*, in the *HSP90* sequences of *N. caduca* and *N. chlamydospora*, and in particular in the *Btub* sequence of *N. valdiviana* might indicate reticulation events. In the genus *Phytophthora*, several studies have demonstrated that interspecific hybridisations play a major evolutionary role by facilitating adaptation to new environments and expansion of host ranges (Brasier et al. 2004, Man in' t Veld et al. 2012, Bertier et al. 2013, Burgess 2015, Husson et al. 2015, Jung et al. 2017a, b). Also autopolyploidisations and whole genome duplications are suggested having contributed to the evolutionary and pathogenic success of *Phytophthora* species (Sansome & Brasier 1974, Sansome 1977, Martens & Van de Peer 2010). Determination of nuclear genome sizes and ploidy levels using flow cytometry or genotyping-by-sequencing, and cloning and sequence analyses of single-copy nuclear genes are required to clarify whether and which of the six *Nothophytophthora* species originate from interspecific hybridisations or autopolyploidisations (Martens & Van de Peer 2010, Bertier et al. 2013, Burgess 2015, Jung et al. 2017b). Interestingly, the two populations of *N. caduca* have identical *NADH1* sequences but differ in their *cox1* sequences at 25 positions. This is similar to what has been observed in the three allopolyploid hybrid species *P. xcambivora*, *P. xinocrassata* and *P. xheterohybrida* and might be explained by paternal leakage occurring during an interspecific hybridisation event (Jung et al. 2017b) which has been demonstrated as a feasible pathway by which mtDNA might become non-clonal (Eyre-Walker & Awadalla 2001).

Despite being either homothallic or self-sterile, five of the six *Nothophytophthora* species were able to stimulate oogonia production in an A2 tester strain of *P. cinnamomi*, a phenomenon also common in homothallic and self-sterile *Phytophthora*

species (Erwin & Ribeiro 1996, Brasier et al. 2003, Jung et al. 2011). Consequently, both genera most likely share the same A1/A2 compatibility system. Traits shared between closely related taxa or groups of taxa were most likely already present in their common ancestor (Yokohama 2002, Carroll 2009, Baum & Smith 2012). Therefore, comparisons of morphological structures of *Nothophytophthora* and *Phytophthora* allow clues about the potential morphology and ecology of their common ancestor for which the provisional name '*ProtoPhytophthora*' is suggested. '*ProtoPhytophthora*' most likely had a heterothallic A1/A2 breeding system, smooth-walled oogonia with both amphigynous and in the case of selfing also paragynous antheridia, non-papillate sporangia which released already differentiated zoospores without dehiscence tube and proliferated externally and internally in a nested and extended way. Presence of a plug of wall material above the septum where sporangia are being shed and uniform pedicel length are considered as main criteria for true caducity of sporangia in *Phytophthora* (Al-Hedaiithy & Tsao 1979a, b, Erwin & Ribeiro 1996). Since sporangia of *N. caduca*, *N. chlamydospora*, *N. valdiviana* and *N. vietnamensis* exclusively break off at the base of particularly big opaque plugs of uniform size formed inside the sporangiophore close to the sporangial base, these species should be contemplated as truly caducous and partially airborne species. However, in contrast to airborne species from *Phytophthora* clades 1–4, 8 and 10, no separate pedicel is formed between the plug and the sporangial base. It can, therefore, be inferred that caducity of sporangia in both genera most likely evolved separately in a convergent way and that their common ancestor '*ProtoPhytophthora*' most likely had persistent sporangia indicating a soil- and/or waterborne lifestyle.

Four of the six *Nothophytophthora* species, *N. amphigynosa*, *N. caduca*, *N. chlamydospora* and *N. valdiviana*, and the *Nothophytophthora* isolates from Ireland (O'Hanlon et al. 2016) and New Zealand (Than et al. 2013) originated from river systems while *N. intricata* and *N. vietnamensis* were isolated from rhizosphere soil of riparian forests. Even though this might indicate an aquatic lifestyle, major morphological and physiological features of these *Nothophytophthora* species are differing from typical aquatic oomycetes, in particular from the genus *Phytophthora*. The majority of predominantly aquatic *Phytophthora* species is characterised by persistent sporangia with abundant nested and extended, internal proliferation, a sterile or disrupted breeding system, fast growth and high optimum and maximum temperatures for growth, all considered specific adaptations to an aquatic lifestyle as saprophytes and opportunistic pathogens (Brasier et al. 2003, Jung et al. 2011, 2017a, Yang & Hong 2013, Yang et al. 2014). In contrast, all known *Nothophytophthora* species show very slow growth and low maximum temperatures for growth disqualifying them as competitive colonisers of leaf litter. In addition, *N. amphigynosa*, *N. intricata* and *N. vietnamensis* are homothallic and together with *N. chlamydospora* lack internal proliferation. Moreover, caducity of sporangia as in *N. caduca*, *N. chlamydospora*, *N. valdiviana* and *N. vietnamensis* has never been observed in predominantly aquatic or in any saprophytic oomycete species. Therefore, it seems possible that their inoculum in the forest streams resulted from canopy drip and surface water flows rather than indicating an aquatic lifestyle. This was also recently suggested for the occurrence of *P. xheterohybrida*, a hybrid species with functional heterothallic breeding system and partially caducous sporangia, in Taiwanese forest streams (Jung et al. 2017b).

Besides *Nothophytophthora* and *Phytophthora*, the only oomycetes with caducous sporangia are the white rusts from the genera *Albugo*, *Pustula* and *Wilsoniana* (*Albuginaceae*) and the 19 downy mildew genera including *Bremia*, *Graminivora*, *Hyaloperonospora*, *Peronospora*, *Plasmopara*, *Sclerophthora*

and *Viennotia* (*Peronosporaceae*) (Thines & Choi 2016). White rusts and downy mildews are exclusively obligate biotrophic whereas all airborne and partially airborne *Phytophthora* species from Clades 1–4, 8 and 10 are facultative, hemibiotrophic or necrotrophic pathogens (Erwin & Ribeiro 1996, Thines & Choi 2016). Since to date no airborne saprophytic oomycetes are known the partial caducity of sporangia in *N. caduca*, *N. chlamydospora*, *N. valdiviana* and *N. vietnamensis* suggests that these four partially airborne species are most likely facultative, hemibiotrophic or necrotrophic pathogens. Partial caducity of sporangia with varying proportions of caducity between different isolates of the same species also occurs in several *Phytophthora* species, including *P. pseudosyringae* and *P. psychrophila* (Jung et al. 2002, 2003) providing these pathogens with flexible pathogenic opportunities. Both *Phytophthora* species cause fine root losses in several tree species (Jung et al. 2002, 2003, Jung 2009, Pérez-Sierra et al. 2013). In addition, *P. pseudosyringae* causes leaf necroses and shoot dieback in *Hedera helix* and *Vaccinium myrtillus*, collar rot in *Alnus glutinosa*, *Castanea sativa* and *Fagus sylvatica* and aerial bark cankers on *F. sylvatica*, *Nothofagus alpina*, *Nothofagus obliqua* and *Notholithocarpus densiflora* (Jung et al. 2003, 2013, Jung & Blaschke 2004, Beales et al. 2009, Jung 2009, Scanu et al. 2010, 2014b, Hansen et al. 2012, Scanu & Webber 2016). In order to clarify whether a similarly flexible pathogenic lifestyle also applies to the four partially caducous *Nothophytophthora* species, surveys of both root symptoms and above-ground symptoms like wilting of leaves and shoots, leaf and fruit blights and aerial bark cankers for presence of *Nothophytophthora* species in Valdivian rainforests and Vietnamese mountain forests are needed. Presence of tree dieback in all forests from which the six *Nothophytophthora* spp. were recovered also suggests that they may have a pathogenic rather than a saprophytic lifestyle. However, pathogenicity tests are urgently required to clarify this. In addition, molecular studies are currently underway to examine whether the six *Nothophytophthora* species produce elicitors like many *Phytophthora* species. Since elicitors constitute a group of small proteins involved as effectors in pathogenesis of *Phytophthora* spp. (Derevnina et al. 2016), their presence in *Nothophytophthora* would strongly indicate a pathogenic lifestyle for members of this new genus.

Three *Nothophytophthora* species, *N. caduca*, *N. chlamydospora* and *N. valdiviana*, were recovered from four streams within a few kilometres range in natural Valdivian rainforests in the Reserva Costera Valdiviana. In both the 3-genes (LSU-ITS-*cox1*) and the 6-genes (LSU-ITS-*Btub*-*HSP90*-*cox1*-*NADH1*) phylogenetic analyses *N. chlamydospora* and *N. valdiviana* constituted sister species. In the 3-gene-analysis they formed a Chilean cluster with *N. caduca* whereas in the 6-gene-analysis the latter species resided in a basal position of the genus. The two populations of *N. caduca* from different streams clustered in both analyses separately due to differences of 25 bp in their *cox1* sequences. These results strongly indicate that these three *Nothophytophthora* species are endemic resulting from a sympatric species radiation in the Valdivian rainforests. However, it cannot be excluded that they were introduced with infested nursery stock used in the *Eucalyptus globulus* and *Pinus radiata* plantations established in the area of the Reserva Costera Valdiviana in previous decades before the park obtained protection status. *Nothophytophthora vietnamensis* appears to be native to the mountain forests at the Fansipan due to their remote location and absence of any planting activities. The production of caducous sporangia by *N. caduca*, *N. chlamydospora*, *N. valdiviana* and *N. vietnamensis* as adaptation to a partially aerial lifestyle in these humid habitats with long term average annual precipitations of approximately 2 500 mm in Valdivia and 2 763 mm in Hoàng Liên Nationalpark, respec-

tively (<https://en.wikipedia.org/wiki/Valdivia#Climate>; https://en.wikipedia.org/wiki/Sa_Pa#Climate), supports the endemism hypothesis. Interestingly, the two *Nothophytophthora* species with exclusively persistent sporangia, *N. amphigynosa* and *N. intricata* which is the closest relative of *N. vietnamensis*, were isolated from regions with considerably drier climates less suitable for aerial sporangial production, in Sintra, Portugal (849 mm average annual precipitation) and Wiesbaden, Germany (562 mm) (<https://en.climate-data.org>).

Some of the most important questions in current oomycete research relate to the evolutionary history and the divergence times of different genera and phylogenetic clades, and how these relate to biogeographical data and the potential centres of origin of invasive pathogens. Using three distinct Bayesian molecular clock models on complete genome sequences of a representative range of oomycete genera, diatoms and a brown algae species, Matari & Blair (2014) estimated the divergence time of the major phylogenetic clades of *Phytophthora* at 19.8–39.0 million years (Myr) ago. However, such an evolutionary young age does not correspond to recent insights into the natural biogeography of the genus *Phytophthora*. An accumulating body of indirect evidence resulting from numerous *Phytophthora* surveys in natural, semi-natural and managed ecosystems across different continents, host range studies, various whole-genus phylogenetic studies and population genetic studies of several globally distributed *Phytophthora* species is suggesting for the genus *Phytophthora* two main centres of origin in Southeast Asia and South- and Central America and secondary centres in Western Australia, Europe and North America (Goodwin 1997, Hansen et al. 2000, 2012, 2017, Jung et al. 2002, 2003, 2011, 2016, 2017a, b, Zeng et al. 2009, Rea et al. 2011, Reeser et al. 2011, Vettraino et al. 2011, Blair et al. 2012, Brasier et al. 2012, Kroon et al. 2012, Huai et al. 2013, Oh et al. 2013, Martin et al. 2014, Scanu et al. 2014a, b, Burgess et al. 2017, Arentz 2017). This biogeographical pattern suggests that the genus *Phytophthora* was already existing before the separation of Gondwana and Laurasia c. 210–175 Myr ago. The considerable discrepancy between an age of 19.8–39 Myr estimated by the molecular clocks and the minimum age suggested by biogeography and plate tectonics might be explained by problems arising from the necessity of calibrating molecular clocks using fossil records. The low preservation potential of oomycetes in combination with the similarity of structures of primitive oomycetes to those from various fungal groups including *Zygomycetes*, *Chytridiomycetes* and other zoosporic true fungi make a reliably identified fossil record extremely difficult (Krings et al. 2011). Due to the generally poor microfossil record it is likely that much older oomycete fossils are still awaiting their detection which would change the average evolutionary rates and, hence, the divergence times considerably. Including *Nothophytophthora* in future coalescence analyses might help to date the divergence time between *Phytophthora* and *Nothophytophthora* and give new insights into the evolutionary history of *Phytophthora*.

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