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Phytophthora niederhauserii sp. nov., a polyphagous species associated with ornamentals, fruit trees and native plants in 13 countries

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Abstract: A non-papillate, heterothallic *Phytophthora* species first isolated in 2001 and subsequently from symptomatic roots, crowns and stems of 33 plant species in 25 unrelated botanical families from 13 countries is formally described here as a new species. Symptoms on various hosts included crown and stem rot, chlorosis, wilting, leaf blight, cankers and gumming. This species was isolated from Australia, Hungary, Israel, Italy, Japan, the Netherlands, Norway, South Africa, Spain, Taiwan, Turkey, the United Kingdom and United States in association with shrubs and herbaceous ornamentals grown mainly in greenhouses. The most prevalent hosts are English ivy (*Hedera helix*) and Cistus (*Cistus salvifolius*). The association of the species with acorn banksia (*Banksia prionotes*) plants in natural ecosystems in Australia, in affected vineyards (*Vitis vinifera*) in South Africa and almond (*Prunus dulcis*) trees in Spain and Turkey in addition to infection of shrubs and herbaceous ornamentals in a broad range of unrelated families are a sign of a wide ecological adaptation of the species and its potential threat to agricultural and natural ecosystems. The morphology of the persistent non-papillate ellipsoid sporangia, unique toruloid lobate hyphal swellings and amphigynous antheridia does not match any of the described species. Phylogenetic analysis based on sequences of the ITS rDNA, *EF*-1α, and β-*tub* supported that this organism is a hitherto unknown species. It is closely related to species in ITS clade 7b with the most closely related species being *P. sojae*. The name *Phytophthora niederhauserii* has been used in previous studies without the formal description of the holotype. This name is validated in this manuscript with the formal description of *Phytophthora niederhauserii* Z.G. Abad et J.A. Abad, sp. nov. The name is coined to honor Dr John S. Niederhauser, a notable plant pathologist and the 1990 World Food Prize laureate.

Key words: β-*tub*, *EF*-1α, ITS, Oomycetes, plant pathogen, Straminipila, taxonomy

INTRODUCTION

During the past two decades an increased emphasis has been placed on the problems caused by *Phytophthora* species in natural ecosystems and in ornamental nursery production. During this time an increasing number of damaging species have been described in various ecosystems. For example, *P. ramorum*, *P. pinifolia* and *P. kernoviae* are notable for the economic and environmental damage they have caused in natural ecosystems and nursery production (Brasier et al. 2005, Brasier and Webber 2010, Duran et al. 2008, Hansen et al. 2012, Martin et al. 2012). Several new species also have been found during surveys targeting the quarantine pathogens above (Érsek and Ribeiro 2010, Kroon et al. 2012). Among them is a non-papillate, heterothallic species morphologically distinct from other species, which originally was isolated from the necrotic collars, stems and roots of arborvitae (*Thuja occidentalis* L.) and English ivy (*Hedera helix* L.) in North Carolina USA in 2001 (Abad and Abad 2003). The authors submitted the ITS sequences on GenBank-NCBI (AY550915, AY550916) before publication of the species. This placed the authors in the USA in contact with other scientists who conducted sequence similarity searches of GenBank and found identical ITS sequences and indicated that several groups were working on the same species independently. To date the species has been found in 13 countries. Among of the most interesting findings are those from Italy and Spain. In Italy, during a survey of nurseries of ornamentals that started in 2001, the species was isolated from rotted roots of potted plants of sageleaf rockrose and crimson bottlebrush in Sicily (Cacciola et al. 2009a) (FIG. 1). Moreover, during this survey a decline associated with root and basal stem rot of 2–3 y old plants of showy Banksia, Baxter's Banksia and acorn Banksia grown in soil was observed in a commercial nursery in Liguria (Pane et al. 2005; Martini et al. 2007; Benson and Magnano di San Lio 2008; Cacciola et al. 2008, 2009b) (FIG. 2). In 2007 the species was found at the end of the summer associated with severe decline of 2 y old almond trees in a nursery in Valencia province (eastern-central Spain), according to Pérez-Sierra et al. (2010). The symptoms observed were leaf chlorosis, leaf drop, wilting, cankers and gum exudation (FIGS. 3, 4). Up to 40% of the affected trees died in the orchard within a few weeks. The symptoms in spring were failure to leaf out, death of the scion and sprouting from the rootstock. The name *Phytophthora niederhauserii* is a nomen nudum due to the publication of the species in previous manuscripts using this name before the official description of the species, according to Kroon et al. (2012) (Martini et al. 2007, Moralejo et al. 2009, Luongo et al. 2010, Spies et al. 2011). To remedy this situation, we herein validate the name of this species as *Phytophthora niederhauserii* Z.G. Abad and J.A. Abad. This paper represents the united efforts of scientists working with Phytophthora diseases in different areas of the world to describe this organism as a new *Phytophthora* species.

MATERIALS AND METHODS

Isolates.—We included information on associated hosts, plant families, location, year and collectors of samples including isolate codes and accession numbers for different

FIG. 1. Wilting and dieback of potted crimson bottlebrush (*Callistemon citrinus*) plants with root rot infection incited by *Phytophthora niederhauserii* in an ornamental nursery in Sicily. Photo by S.O. Cacciola.

FIG. 2. Wilting and dieback of showy banksia (*Banksia speciosa*) caused by *Phytophthora niederhauserii*; these symptomatic banksia plants were obtained from seeds imported from Australia and planted in the ground of a commercial ornamental nursery in Liguria. Irrigation was provided through a drip system (see the black plastic pipe on the ground). Photo by S.O. Cacciola.

FIGS. 3, 4. Two-year-old almond (*Prunus dulcis*) trees affected by *Phytophthora niederhauserii* showing severe wilting and profuse stem gum exudation. Photos by A. Pérez-Sierra.

loci for *P. niederhauserii* sp nov. as well as information for the *Phytophthora* species in Clade 7b used for the molecular evaluation presented in this study (TABLE I).

Isolation and morphological characterization.—At the Plant Disease and Insect Clinic (PDIC), Department of Plant Pathology, North Carolina State University (NCSU) at Raleigh, potted plants of English ivy, arborvitae, iris (*Iris* sp.), coral bell (*Heuchera* sp.), Leyland cypress (x *Cupressocyparis leylandii*) and juniper (*Juniperus* sp.) with symptoms of collar, stem or root rot were processed for isolation. Affected necrotic tissues were thoroughly washed with tap water to remove soil and plant debris. Small sections from roots or from the edge of rotted and healthy tissue of collars and stems were selected and blotted on sterile paper towel. Tissue pieces were placed on cornmeal agar (CMA) P_{10}ARP (Kanwischer and Mitchell 1978). The plates were maintained at 20 C in the dark 2–4 d. Hyphal tips of coralloid mycelia were transferred into new CMA P_{10}ARP, then subcultured into CMA and incubated 4 d at 20 C.

Ten *Phytophthora* isolates from hosts were selected for further analysis. Isolates were transferred into baby lima bean agar (B-LBA, 50 g fresh lima bean/500 mL water, autoclaved 5 min, filtered, for total 1 L, plus 17 g agar), potato dextrose agar 30 (PDA, Difco 30 gL^{-1}), and clarified V8 (cV8A) (100 mL V8 juice, 2 g calcium carbonate, 18 g agar, distilled water for 1 L total) for morphological characterization. Production of sporangia was induced by placing small mycelial plugs from the edge of growing colonies in B-LBA (4–7 d) into 10% soil solution and incubating under continuous fluorescent light at room temperature (22–25 C) 2–3 d. Fifty sporangia were measured for each isolate. Production of the sexual structures (oogonium, antheridium, oospore) was stimulated by pairing the isolates with *P. cryptogea* A1 (BBA65909) and A2 (BBA62660) testers in B-LBA + oil (400 mL B-LBA supplemented with 200 µL 3.6.9 omega fatty acids [alpha-linolenic, linoleic, gamma linoleic, oleic] in UDO oil [Flora Inc., Lynden, Washington]) and incubated in darkness at 20 C. For each isolate, 50 oogonia, oospores and antheridia, chosen at random, were measured from 4 wk old cultures. Isolates were morphologically evaluated with published keys for identification of *Phytophthora* species (Waterhouse 1963, Stamps et al. 1990, Gallegly and Hong 2008) and the ''Online identification tools to *Phytophthora*: lucid key, tabular key and sequencing analysis'' based on the types and putative epitypes and neotypes (Abad ZG, Bienapfl J, Balci Y, Burgess T, Coffey M, Martin F, Kang S; USDA-APHIS-PPQ-CPHST, Center of Plant Health Science and Technology). Colony morphology and analysis of the minimum, optimum and maximum growth temperatures were performed in cV8A and PDA growth media. Cultures were maintained on slants of B-LBA, CMA and in glass tubes with 15 mL distilled sterilized water at room temperature (ca. 22 C) during this study.

Isolates from California and countries including Australia, Hungary, Israel, Italy, Japan, Norway, South Africa, Spain and the United Kingdom were processed with similar isolation methods. Various other isolation and identification techniques, including different growth media, were used in Hungary, UK, Norway and Italy. In Hungary isolates were obtained on carrot agar P_{10}ARPH, and pure cultures were maintained on carrot agar (CA) slants (Brasier 1972) at 10 C. Compatibility types of two Hungarian isolates were determined by pairing with *P. cambivora* A1 (WPC P1432) and A2 (Bu KN 4, courtesy of J. Nechwatal, Institute for Plant Protection, Bavarian State Research Center for Agriculture (LfL), Freising, Germany) testers in CA in the dark at 25 C. In the UK, the leading edge of symptomatic stems and roots and soil from the rhizosphere around the roots were wetted and placed in apple (cv. Granny Smith) baits (Erwin and Ribeiro 1996). Apple baits were kept in the dark at 20°C, until a firm rot was seen. Sections from the leading edge of the rot were placed onto CMA P_{10}ARPH plates to culture. In Norway, to induce the formation of oogonia and antheridia, the cultures were paired on CPA (50 g carrot pieces, 22 g agar, 1000 mL dH_2O) (Werres et al. 2001) with tester strains of *P. cryptogea* (BBA 65909 A1, BBA62660 A2). In Italy original isolations were made in potato dextrose agar PDA-BNPRAH selective medium (20 mg benomyl, 25 mg nystatin, 25 mg PCNB, 10 mg rifampicin, 500 mg ampicillin, 25 mg hymexazol) (Masago et al. 1977).

Molecular characterization.—Ten isolates of the putative new *Phytophthora* species obtained from the NCSU-PDIC

TABLE I. Isolates, location, hosts, families, year and collectors of samples associated with *Phytophthora niederhauserii* sp. nov. and related species in Clade 7b evaluated in this study

Isolate code(s)	Location	Host-family	Year-collector(s)	Tests in paper[a]	GenBank accession nos.[b]		
					ITS	β-*tub*	EF-1α
P. niederhauserii WPC P10616 (TYPE)	USA: NC	English Ivy (*Hedera helix*)-Araliaceae	2001-G. Abad	1, 2	AY550915	EU080230	EU080231
WPC P10617	USA: NC	Arborvitae (*Thuja occidentalis*)-Cupressaceae	2001-G. Abad	1	AY550916	EU080243	EU080244
PH287 (01.5429)	USA: NC	Arborvitae-Cupressaceae	2001-G. Abad				
PH525 (05_1705)	USA: NC	Iris (*Iris* sp.)-Iridaceae	2005-G. Abad				
PH526 (05_1708)	USA: NC	Coral Bell (*Heuchera* sp.)-Saxifragaceae	2005-G. Abad				
PH540, PH541, PH542	USA: NC	Leyland Cypress (× *Cupressocyparis leylandii*)-Cupressaceae	2005-G. Abad				
PH656 (06-407)	USA: NC	Juniper (*Juniperus* sp.)-Cupressaceae	2006-G. Abad				
PH2464	USA: NC	Catawba rhododendron (*Rhododendron catawbiense*)- Ericaceae	2009-S. Spencer/G. Abad				
1281334A&B	USA: CA	Cistus (*Cistus salvifolius*)-Cistaceae	2003-C. Blomquist				
1281654E	USA: CA	Ceanothus, CA Wild Lilac (*Ceanothus* sp.)-Rhamnaceae	2007-C. Blomquist				
WPC P10976	USA: CA	Iron-Tree (*Metrosideros villosa*)-Myrtaceae	2005-M. Coffey	2	FJ801538		
WPC P16237 (MN 084)	USA: CA	Schefflera (*Schefflera hoi* var fantsipanensis)-Araliaceae	2008-M. Coffey	2	GU259119		
PhHe1	Italy	English Ivy-Araliaceae	2001-S.O. Cacciola, D. Cooke				
IMI 391712 (Cistus1)	Italy	Cistus or Sageleaf Rockrose-Cistaceae	2001-S.O. Cacciola, A. Pane	2, 3	JF900372		
IMI 391713 (Cistus2)	Italy	Cistus or Sageleaf Rockrose-Cistaceae	2003-S.O. Cacciola, A. Pane	2	JF900373		
IMI 391708 (PhCa12)	Italy	Crimson Bottlebrush (*Callistemon citrinus*)-Myrtaceae	2003-S. Cacciola, A. Chimento	2, 3	JX494411		
IMI 393960 (465/03-SCRP978)	Italy (Liguria)	Showy Banksia (*Banksia speciosa*)-Proteaceae	2003-S.O. Cacciola, P. Martini, D. Cooke	1, 2, 3	FJ648808		
466/03 (SCRP979)	Italy (Liguria)	Baxter's Banksia (*Banksia baxteri*)-Proteaceae	2003-S.O. Cacciola, P. Martini, D. Cooke	1, 2, 3	FJ648809		
AB154	Italy (Marche)	English Ivy, collar rot-Araliaceae	2009-Belisario et al.	2	FN252857		
IMI 500394 (465/10)	Italy	Silver Wattle, Mimosa tree (*Acacia dealbata*)-Fabaceae	2010-S.O. Cacciola	2	JF900371		
LEN1	Italy	Lentisk (*Pistacia lentiscus*)-Anacardiaceae	2010-B. Scanu	2	GU119914		
P731	Spain (Mallorca)	Montpelier cistus (*Cistus monspeliensis*)-Cistaceae	2001-E. Moralejo	2	AY943297		
P831	Spain (Mallorca)	Cistus or Rockrose-Cistaceae	2001-E. Moralejo	2	EF050511		
PS-54, PS-55	Spain	English Ivy-Iridaceae	2004-L. Alvarez				
PS-56	Spain	English Ivy-Iridaceae	2005-L. Alvarez	2	EU244850		

TABLE I. Continued

Isolate code(s)	Location	Host-family	Year-collector(s)	Tests in paper[a]	GenBank accession nos.[b]		
					ITS	β-*tub*	EF-1α
PS-57	Spain	English Ivy-Iridaceae	2005-L. Alvarez	2	EU194436	EU195073	EU195072
PS-58	Spain	English Ivy-Iridaceae	2005-L. Alvarez				
PS-180	Spain	Almond (*Prunus dulcis*)-Rosaceae	2007-A Ma. Perez-Sierra				
PS-183	Spain	Almond-Rosaceae	2007-A Ma. Perez-Sierra	2	GQ385965	GQ418174	GQ418177
PS-184, PS-186	Spain	Almond-Rosaceae	2007-A Ma. Perez-Sierra				
PS-673	Spain	Almond-Rosaceae	2008-A Ma. Perez-Sierra				
PS-690	Spain	Plumbago and leadwort (*Plumbago* sp.)- Plumbaginaceae	2009-A Ma. Perez-Sierra	2	GQ385966	GQ418175	GQ418178
PS-722, PS-729, PS-730, PS-731, PS-732, PS-733, PS-734	Spain	Almond-Rosaceae	2009-A Ma. Perez-Sierra				
PS-737	Spain	Pomegranate (*Punica granatum*)-Lythraceae	2009-A Ma. Perez-Sierra	2	GQ385967	GQ418176	GQ418179
PS-757, PS-758, PS762	Spain	Almond-Rosaceae	2009-A Ma. Perez-Sierra				
CBS 125247 (10045)	Spain	English Ivy-Iridaceae	2006-M.L. Herrero	2, 3	GQ925808		
B0606	Norway	Begonia-hybrid (*Begonia* × *hiemalis*)-Begoniaceae	2006-B. Toppe	3			
10154	Norway	Begonia-hybrid (*Begonia* × *cheimantha*)-Begoniaceae	2006-M.L. Herrero	3			
CBS 125246 (10041)	Norway	Gloxinia (*Sinninga speciosa*)-Gesneriaceae	2006-M.L. Herrero	2, 3	GQ925807		
10170	Norway	kalanchoë (*Kalanchoë blossfeldiana*)-Crassulaceae	2006-M.L. Herrero	3			
10448	Norway	Peperomia (*Peperomia clusiifolia* cv Isabella)- Piperaceae	2007-M.L. Herrero				
CBS 124086 (P214)	Hungary (Nova)	Lawson Cypress (*Chamaecyparis lawsoniana* cv. White Spot)-Cupressaceae	2007-A. Józsa	1, 2, 3	GU230789	GU477613	GU477615
CBS 123840 (P218)	Hungary (Nova)	Nordmann Fir (*Abies nordmanniana*)-Pinaceae	2007-A. Józsa	1, 2, 3	GU230790	GU477614	GU477616
WPC P10279 (IMI 390920)	Hungary (Budapest)	Common Box (*Buxus sempervirens*)-Buxaceae	2002-J. Bakonyi	2	FJ801357		
VPRI 32022	Australia (Melbourne)	*Grass-tree* (*Xanthorrhoea australis*)-Xanthorrhoeaceae	2004-P. symes				
VPRI 32023	Australia (Melbourne)	Grass-tree, soil around dead plant-Xanthorrhoeaceae	2004-P. Symes	3			
VPRI 32086	Australia (Western)	Ord River Irrigation Area	2004-unknown	3			
VHS17577	Australia (Western)	Acorn Banksia (*Banksia prionotes*)-Proteaceae	2009-T.I. Burgess	2	JX113307		
WPC P2264 (IMI 66288)	Australia	Cassava, yuca (*Manihot esculenta*)-Euphorbiaceae	M. Coffey				

TABLE I. Continued

Isolate code(s)	Location	Host-family	Year-collector(s)	Tests in paper[a]	GenBank accession nos.[b]		
					ITS	β-*tub*	EF-1α
STE-U6970 (OW1611)	South Africa (Ashton)	Grapevine (*Vitis vinifera*)-Vitaceae	2005-C.F.J. Spies	2	GQ911576		
STE-U6971 (OW1474)	South Africa (Ashton)	Grapevine-Vitaceae	2005-C.F.J. Spies	2	GQ911577		
STE-U6972 (OW1466)	South Africa (Ashton)	Grapevine-Vitaceae	2005-C.F.J. Spies	2	GQ911578		
STE-U6973 (OW2289)	South Africa (Wellington)	Grapevine-Vitaceae	2005-C.F.J. Spies	2	GQ911574		
STE-U6974 (OW2061)	South Africa (Hermanus)	Grapevine-Vitaceae	2005-C.F.J. Spies	2	GQ911575		
IK-KD/Kayseri	Turkey (Kayseri)	Almond-Rosaceae	2009-İlker Kurbetli	2	HQ681251		
WPC P7377 (PD90/418)	The Netherlands	Spathiphyllum (*Spathiphyllum* sp.)-Araceae	M. Coffey	2	FJ802128		
P9000.06	United Kingdom	Olive Grevillea (*Grevillea olivacea*)-Proteaceae	2006-B. Henricot, G. Denton	2	GQ848201		
CH96HE1: *P. drechsleri*	Japan (Chiba)	English Ivy-Araliaceae	2007-S. Uematsu	2	AB367382		
CH96HE2	Japan (Chiba)	English Ivy-Araliaceae	2007-S. Uematsu				
MYA-4163: *P. drechsleri*	Israel	Ornamental plant	Gallegly and Hong/Igo et al.	2	FJ746649	EU595777	
TARI 26231: *P. melonis*	Taiwan (Changhua)	Fig (*Ficus carica*)-Moraceae	2006-Ann et al.	2	GU111617		
P. cajani WPC P3105 (ATCC4438)	India	Pigeon pea (*Cajanus cajani*)			FJ801827[1]	EU080101[2]	EU080102[2]
P. cinnamomi WPC P2110 (TYPE)	Indonesia	Indonesian cinnamon (*Cinnamomum burmannii*)			FJ801806[1]		
WPC P2428	USA	Avocado (*Persea americana*)			AY302170[4]	EU079770[2]	EU079771[2]
P. cinnamomi var. parvispora WPC P8494 (TYPE)	Germany	Ponytail palm (*Beaucarnea recurvata*)			FJ802006[1]		
WPC P7154	Israel	Avocado			FJ801964[1]	EU080454[2]	EU080455[2]
P. melonis MYA-4079 (TYPE)	Japan	Cucumber (*Cucumis sativus*)			EU088256[3]		
WPC P10994	India	Pointed Gourd (*Trichosanthes dioica*)			FJ801540[1]	EU079712[2]	EU079713[2]
P. pistaciae MYA-4082 (TYPE)	Iran	Pistachio (*Pistacea vera*)			FJ746648[5]		
WPC P6196	Iran	Pistachio			FJ801903[1]	EU080320[2]	EU080321[2]
P. sojae WPC P3114	USA	Soybean (*Glycine max*)			FJ801828[1]	EU079790[2]	EU079791[2]
P. vignae WPC P3019 (CBS241.73)	Australia (Q)	Cowpea (*Vigna unguiculata*)			FJ801824[1]	AY564090[6]	AY564146[6]

[a] Tests in paper: 1 = morphological, 2 = molecular, 3 = pathogenicity.

[b] References from sequences in GenBank: [1] Coffey et al. direct submission, [2] Blair et al. 2008, [3] Ho et al. 2007, [4] Garbeloto et al. direct submission, [5] Igo et al. direct submission, [6] Kroon et al. 2004.

were grown 14 d in potato dextrose broth (Difco, USA) at the USDA-APHIS-PPQ Center for Plant Health Science and Technology (CPHST, formerly Molecular Diagnostics Laboratory in Beltsville, Maryland). DNA was extracted with the DNeasy Plant Mini Kit (QIAGEN) according to manufacturer's protocols. Concentration and quality of total DNA were estimated by agarose gel electrophoresis. Two oligonucleotide universal primers ITS5 and ITS4 (White et al. 1990) were used to amplify the internal transcribed spacer rDNA region (ITS rDNA) (Cooke and Duncan 1997). Oligonucleotide primers ELONGF1/ELONGR1, and TUBUF2/TUBUR1 were used to amplify the translation elongation factor 1 alpha gene (*EF-1*α), and the β-tubulin gene (*B-tub*) respectively (Kroon et al. 2004). PCR amplifications were performed following protocols of Cooke and Duncan (1997) and Kroon et al. (2004). Amplicon purification and sequencing was performed at the McLab Sequencing facility (South San Francisco, California, http://www.mclab.com/home.php). In other countries DNA extraction, PCR and sequencing of the ITS, *EF-1*α, *B-tub*, *Cox*-I & II, NADH1 were performed with slight modifications as at USDA-APHIS-PPQ-CPHST. Accession numbers for sequences generated and used in the present study are included (TABLE I). Data for sequences from the *Cox*-I & II, NADH1 are not shown. Assembly of sequences and consensus sequences including those generated in different countries for different molecular markers were processed at the USDA-APHIS-PPQ-CPHST with the Geneious bioinformatics software platform (Drummond et al. 2011). Sequences of the putative new *Phytophthora* sp. and the closest species in clade 7b (including the exholotypes, if available in GenBank) were selected for final phylogenetic analysis (TABLE I, FIG. 5). Alignments and consensus trees of the selected sequences were generated with same software using Geneious Alignment and Geneious Tree Builder. Each of the three phylogenetic trees was estimated with a neighbor joining (NJ) algorithm, and bootstrap support values were derived from 1000 replicates (Drummond et al. 2011).

Pathogenicity tests.—Pathogenicity of novel *Phytophthora* isolates, described below as *P. niederhauserii* sp. nov., was investigated separately in multiple countries on various plant species.

Italy.—Pathogenicity tests were performed on potted plants of showy banksia (*Banksia speciosa* R. Br.) as well as on apples by with isolates IMI 393960 from showy banksia and 466/03 from Baxter's banksia (*B. baxteri* R. Br.). One year old plants of Baxter's banksia (20 plants for each isolate) were transplanted into pots (12 cm diam) filled with infested soil prepared by mixing steam-sterilized sandy loam with 1% inoculum produced on autoclaved wheat kernels. Twenty control plants were transplanted in pots containing non-infested soil. Plants were kept in greenhouse at 25 ± 2 C and watered to field capacity once a week. PDA-BNPRAH selective medium was used for re-isolation. In addition mature apples (cv. Golden Delicious) were wound-inoculated by inserting into the pulp a mycelial plug of 7 d old colonies grown on PDA. Control fruits were inoculated with sterile agar plugs. Wounds were sealed with Parafilm® and incubated at 25 ± 2 C. All tests were performed twice. Pathogenicity also was tested with isolates IMI 391708 from crimson bottlebrush (*Callistemon citrinus* [Curtis] Skeels) and IMI 391712 from Cistus (*Cistus salvifolius* L.) on their respective hosts. For these experiments, 6 mo old potted plants of crimson bottlebrush and sageleaf rockrose (10 plants of each species for each isolate) were transplanted into 12 cm diam pots containing a mixture of 1 : 1 steam-sterilized, sandy loam (vol/vol) with 4% inoculum produced on autoclaved wheat kernel seeds. Plants were maintained at 25–28 C and watered once a week. Ten control plants were transplanted into pots containing non-infested soil.

Hungary.—Pathogenicity tests were performed with isolates P214 and P218, which represented cultures isolated from Lawson cypress (*Chamaecyparis lawsoniana* [A. Murray] Parl.) and Nordmann fir (*Abies nordmanniana* [Steven] Spach) respectively. For this purpose, lower stems of 2 y old potted plants of both hosts were inoculated at 3–5 cm above the ground. A 4 mm diam wound was made in the bark on each of three replicate plants with a cork borer. Agar plugs (4 mm diam) were cut from the margins of the actively growing colonies on CA and inserted into the wounds, covered with moist cotton and sealed with Parafilm® and aluminium foil. Equivalent control inoculations were made with sterile agar plugs. Koch's postulates were completed with successful re-isolations from necrotic inner bark tissues of the infested plants after 4 wk incubation at 18–26 C. Isolation was attempted from necrotic tissue after the outer bark around the wound was removed as described previously.

Norway.—Koch's postulates were completed in greenhouse assays with potted plants including English ivy, begonia hybrids including Hiemalis begonia or Rieger begonia (*Begonia* × *hiemalis*) and Christmas begonia (*Begonia* × *cheimantha*), kalanchoë (*Kalanchoë blossfeldiana* Poelln.) and gloxinia (*Sinninga speciosa* Baill.). Isolates from the different host plants were selected and grown on V8 agar. The cultures were incubated on the laboratory bench at room temperature approximately 2 wk, then the agar with mycelial growth in each Petri dish was divided into eight parts and each of these parts was used to inoculate one pot. For inoculation each plant was carefully removed from its pot and a small amount of growing media was added to the bottom of the empty pot. The eighth part of the colonized agar was placed onto the media surface and the plant was returned to the pot. In some cases it was necessary to transplant plants into larger pots.

RESULTS

Phylogenetic analysis.—All isolates designated *P. niederhauserii* (TABLE I) had identical 826 bp ITS rDNA, 1154 bp β-*tub*, 961 bp *EF*-1α sequences. Representative ITSrDNA, β-*tub* and *EF*-1α sequence data from this study have been submitted to GenBank (TABLE I). Sequence alignments of the ITS rDNA region against the collection in the NCBI database

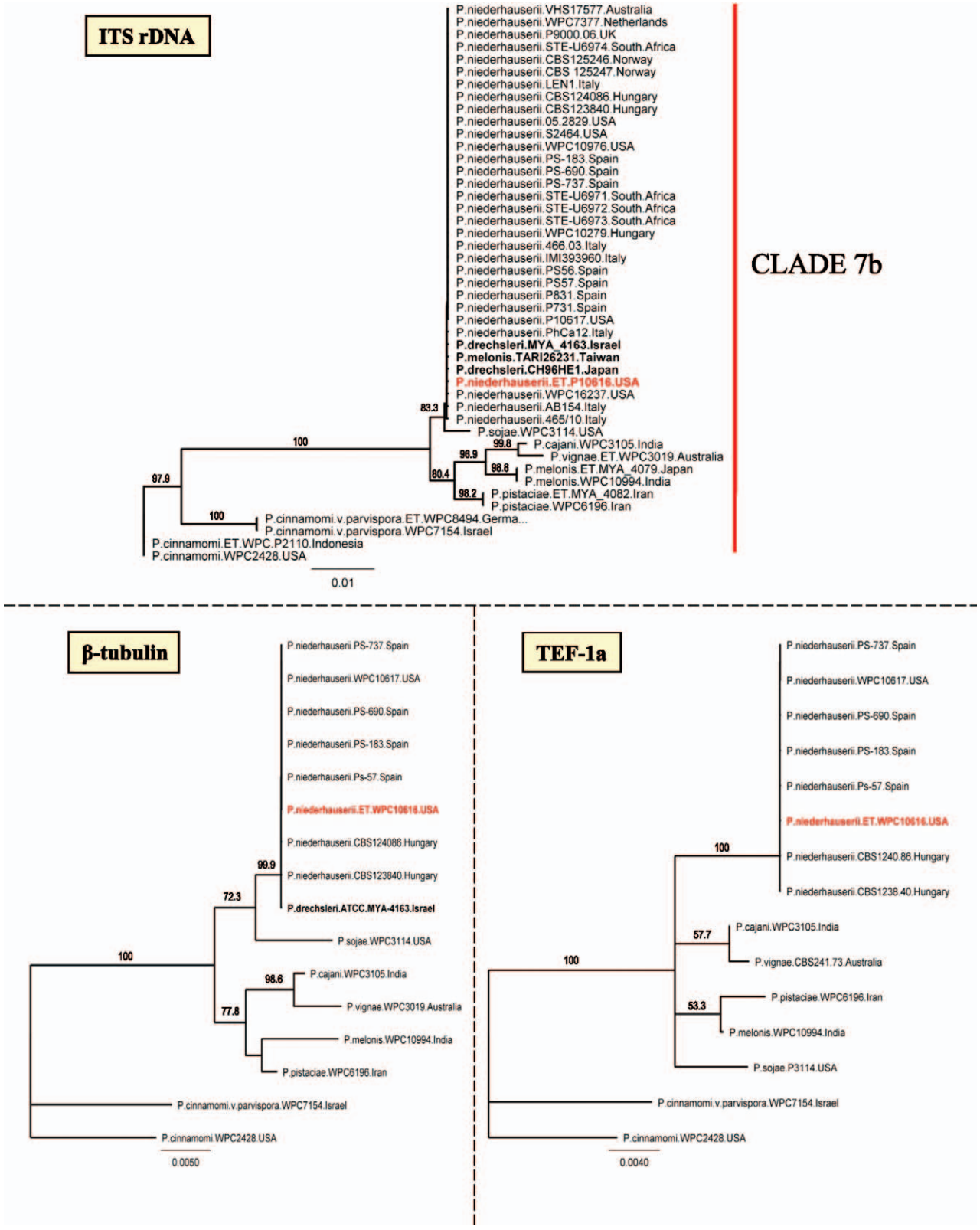


FIG. 5. Neighbor joining phylogenetic trees with 1000 bootstraps based on the internal transcribed spacer ribosomal DNA, translation elongation factor 1α, and β-tubulin regions of *Phytophthora* species in subclade 7b showing the position of representative isolates of *P. niederhauserii* in relation to other known taxa in the subclade. *P. cinnamomi* and *P. parvispora* in

revealed that *P. niederhauserii* is phylogenetically distinct from all described species. The closest relative of *P. niederhauserii* is *P. sojae*, as shown in phylogenetic trees of the ITS rDNA and β-*tub* (FIG. 5). Isolates of *P. niederhauserii* were readily distinguished from related species by ITS rDNA, β-*tub* and *EF*-1α sequences (FIG. 5). Based on phylogenetic analyses of the ITS rDNA region *P. niederhauserii* falls in subclade 7b sensu Cooke et al. (2000), which includes host-specific pathogens of crops such as *P. cajani* Amin, Baldev & Williams, *P. vignae* Purss, *P. melonis* Katsura K., *P. pistaciae* Mirabolfathy and *P. sojae* Kaufm. & Gerd. (FIG. 5). Phylogenetic analysis of *cox*-I spacer sequences supported this placement (data not shown).

Isolate variation and characteristics.—In Italy isolates IMI 393960 from showy Banksia and 466/03 from Baxter's Banksia grew more slowly on PDA than on V8A; colonies were stoloniform with irregular margins. Isolates grew at all tested temperatures except 5 and 40 C. Optimum growth temperature was 30 C on both PDA and V8A media; minimum temperatures were 5–10 C and maximum temperatures were 38–40 C. Growth resumed when isolates were returned to 25 C after they have been maintained at 40 C for 9 d. Mean growth at 30 C of the two representative isolates, IMI 393960 and 466/03, was 9.3 ± 0.3 and 10.2 ± 0.5 mm d^{-1} respectively on PDA and 14.2 ± 0.1 mm d^{-1} for both isolates on V8A. Sporangia produced in the saline solution of Chen and Zentmeyer (1970), as well as in soil extract, were ellipsoid, non-papillate and persistent. The mean sporangial dimensions of 10 isolates examined were 55.3 ± 3.8 × 36.2 ± 3.1 μm (range 47–70 × 30–44 μm) with a mean length/width ratio of 1.5 ± 0.1 μm (range 1.25–1.75 μm) in saline solution. In soil extract mean dimensions were 31.6 ± 8.3 × 21.4 ± 5.7 μm with a mean length/width ratio of 1.5 ± 0.2 μm (range 1.2–2.0 μm). All isolates produced catenulate hyphal swellings in liquid media. Gametangia were observed in dual cultures with A2 mating type of *P. drechsleri*, indicating that all isolates from *Banksia* species, bottlebrush and sageleaf rockrose are A1 mating type. Oogonia were spherical with a smooth wall and amphigynous antheridia.

In Hungary colonies of isolates P214 and P218 on CA were stellate-radiate with low aerial mycelium, on PDA patternless, submerged at the edge and with sparse aerial mycelium in the center, on cV8A stellate-radiate, submerged at the edge and little aerial mycelium in the center. Each isolate developed slightly irregular colony margin and few lobate or rounded hyphal swelling in each medium. Optimum temperature for growth was ca. 30 C, maximum 37 C. At this temperature radial growth was 0.5–1 mm for 3 d and the isolates continued to grow at 25 C. At 39–40 C no growth occurred and isolates did not grow when returned to 25 C. Radial growth at 30 C on CA was 9.4 mm d^{-1} (isolate P214) and 9.2 mm d^{-1} (isolate P218). Sporangia were absent in each tested medium but abundantly produced in non-sterile stream water. They were terminal, mostly single, or in small groups, persistent, ellipsoid-ovoid, non-papillate. The mean sporangial dimensions were 73.5 ± 7.3 × 33.7 ± 3.6 μm (isolate P214) and 71.4 ± 5.9 × 31.8 ± 3.1 μm (isolate P218). Nested and extended proliferation of sporangia was seen. Sporangiophores were single or simple sympodial. No chlamydospores were observed. The sexual stage was determined for isolates P214 and P218. Gametangia were not produced in single cultures in pairings of the two isolates of *P. niederhauserii* and *P. cambivora* A1 tester but were abundantly produced in pairings with *P. cambivora* A2 tester, indicating the presence of A1 mating type for isolates P214 and P218.

Pathogenicity tests.—In Italy, on wound-inoculated apples, the two isolates tested induced brown lesions; no significant differences in pathogenicity were observed. All *Banksia* plants transplanted into pots filled with infested soil developed symptoms of wilt, leaf chlorosis and basal stem rot within 2–3 wk and collapsed within 40 d after transplanting. Similarly plants of sageleaf rockrose and crimson bottlebrush transplanted into pots containing infested soil developed symptoms of wilt and root rot 2–3 wk after transplanting. Non-inoculated plants remained healthy. *P. niederhauserii* was re-isolated from all symptomatic plants. In Hungary both tested isolates, P214 and P218, were pathogenic to Lawson cypress and Nordmann fir. Necrosis and expanding stem lesions were observed in both hosts 3 wk after inoculation. *P. niederhauserii* was re-isolated from the necrotic bark tissue. Control plants remained healthy. In Norway after 2–3 wk the inoculated plants presented symptoms of stunted growth, necrotic stem

←

Clade 7 are outgroups. Scale bars unit: number of nucleotide substitutions per site. Note the position of sequences of *P. melonis* TARI26231 from Taiwan, *P. drechsleri* CH96HE1 from Japan and *P. drechsleri* MYA_4163 from Israel that have perfect alignment with the type of *P. niederhauserii* in the ITS rDNA and β-tubulin phylogenetic trees.

lesions or wilting. Re-isolations were made from aerial lesion of infected plants or from roots.

TAXONOMY

Phytophthora niederhauserii Z.G. Abad & J.A. Abad, sp. nov. FIGS. 6–22

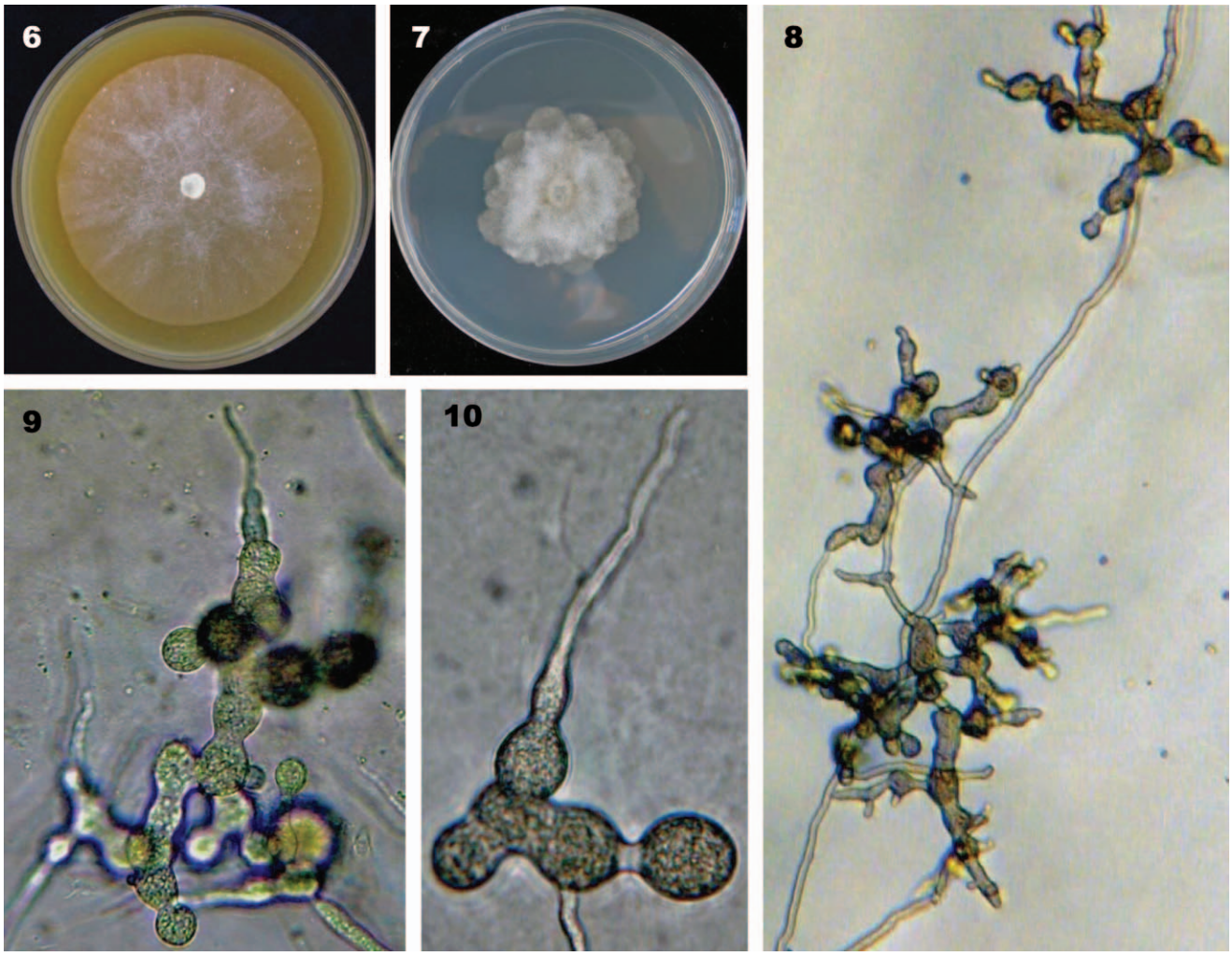
MycoBank MB515114

Colonies on cV8A radiate with regular margins and moderate aerial mycelia (FIG. 6), on B-LBA chrysanthemum with irregular margins (FIG. 7). On both cV8A and B-LBA minimum temperature for growth was 10 C, optimum 30 C and maximum 37 C. Colonies on cV8A were fast growing at 14.2 ± 0.1 mm d^{-1}. Mycelium was typical coralloid, main hyphae 4–8 µm wide. Chlamydospores were absent. Hyphal swellings were frequently produced; toruloid to lobate and irregularly branched (FIGS. 8–10). Sporangiophores were mainly single (FIG. 11) or simple sympodial or rarely branched. Zoospores were actively produced in liquid cultures (FIG. 12). Sporangia were non-papillate, persistent, ellipsoid, obpyriform, ovoid or irregular. They were 56–112 µm long (av. 89 µm) and 32–52 µm wide (av. 44 µm) (FIGS. 13–18). They proliferated internally with both nested (FIG. 19) and extended proliferation (FIGS. 20, 21). The sexual stage was heterothallic, oospores were produced in pairings with *P. cryptogea* A2 (FIGS. 22–27), oogonia was spherical and 24–40 µm (av. 36 µm) diam. Oospores were plerotic or nearly so, 25–38 µm (av. 33 µm) diam and oospore wall was 4–6 µm (av. 5 µm) thick. Antheridia were amphigynous, some with lobes that are frequently asymmetrical around the oogonia stalk (giving the appearance of being paragynous when observed at low magnification or at a different angle). Some unusual shapes of antheridia and oogonia occurred occasionally. These included oogonia with a neck (FIG. 25) or with funnel-shaped base (FIG. 26) and large elongate antheridia (FIG. 27).

Holotype: UNITED STATES OF AMERICA, North Carolina, from English ivy (*Hedera helix* L.) affected with collar and root rot from a greenhouse in Henderson, 10 Oct 2001, collector Z. Gloria Abad, WPC_P10616 ''preserved in a permanent inactive state'' cryopreserved in liquid nitrogen at the World Oomycetes/Phytophthora Genetic Resource Collection, Univ. of California. *Ex-holotype:* P10616. Other codes: Ph289 and 01.6056. GenBank accession numbers: ITS = AY550915, β-*tub* = EU080230, *EF*-1α = EU080231. Alignments and phylogenetic trees of the ITS, β-*tub* and *EF*-1α were submitted to TreeBASE and are available at http://purl.org/phylo/treebase/phylows/study/TB2:S14140

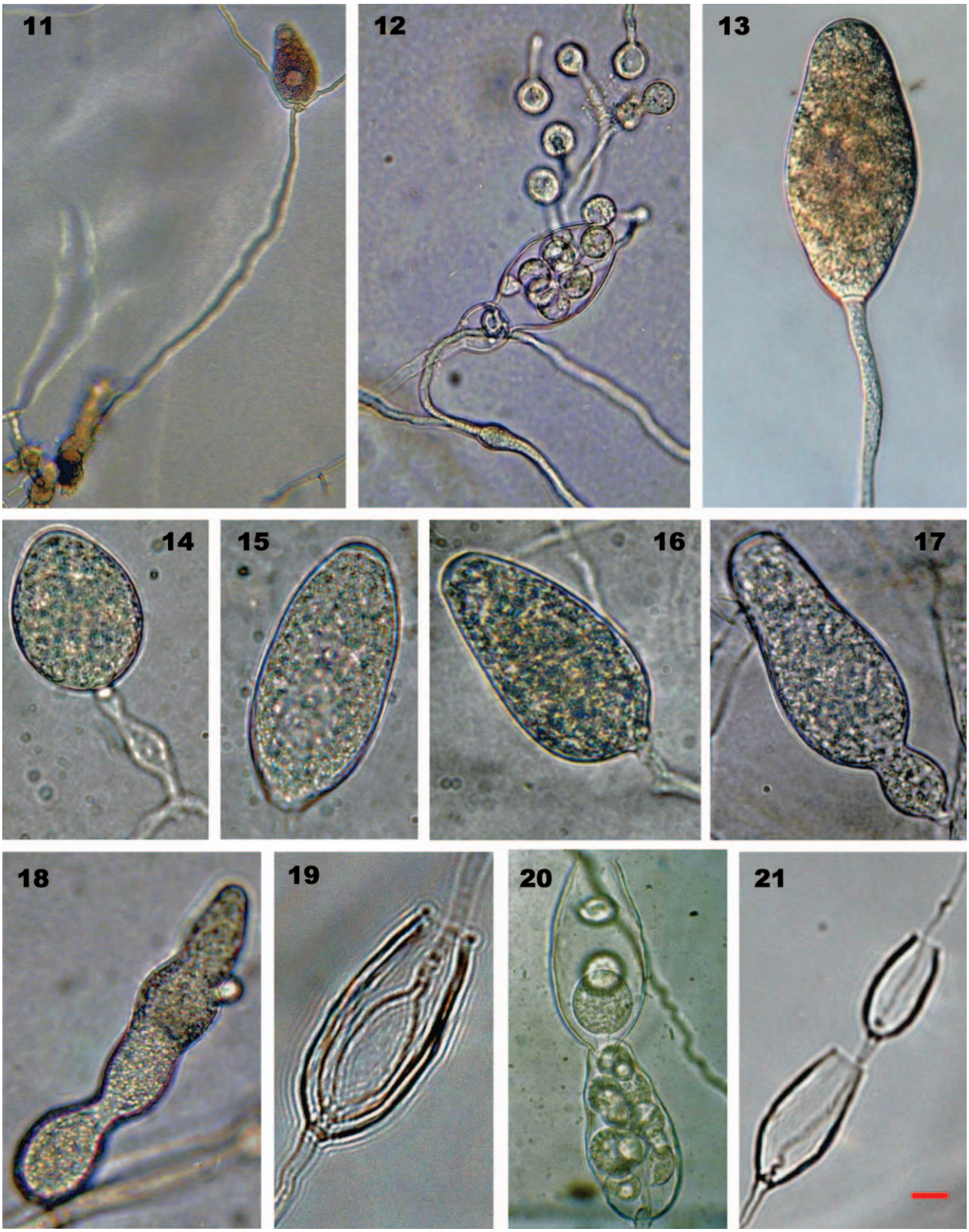
Etymology: Named to honor Dr John Niederhauser, winner of the 1990 World Food Prize and best known in the scientific community for his research to control the potato late-blight pathogen *Phytophthora infestans* (Mont.) de Bary.

Additional important isolates for the species: AUSTRALIA: Isolate VPRI 32023 from soil around Grasstree or Black Boy (*Xanthorrhoea australis* R. Br.), pear baited from soil around dead plant in Royal Botanic Gardens, South Yarra, Melbourne, Victoria; collector P. Symes, 27 Jul 2004. Isolate VPRI 32086 from mango baited from water in Ord River Irrigation Area, Western Australia; collector unknown, Nov 2004. Isolate VHS17577 from Acorn Banksia (*Banksia prionotes* Lindl.), Western Australia; collector Treena I. Burgess, GenBank accession numbers: ITS = JX113307. HUNGARY: Isolate H-3/02 (WPC P10279, IMI 390920) from necrotic roots of common box, European box or boxwood (*Buxus sempervirens* L.) in a public garden, Budapest; collector József Bakonyi, 1 Oct 2002. GenBank accession number: ITS = FJ801357. Isolate P214 (CBS 124086) from necrotic bark of Lawson Cypress or Port Orford cedar (*Chamaecyparis lawsoniana* cv. white spot) in a nursery, Nova, Hungary; collector András Józsa, 10 Nov 2007. Compatibility type A1. GenBank accessions: ITS = GU230789, β-*tub* GU477613, *EF*-1α = GU477615, *Cox*-1 GU477617, nad1 GU477619. Isolate P218 (CBS 123840) from necrotic bark of Normann fir (*Abies nordmanniana*) growing in a nursery, Nova, Hungary; collector András Józsa, 10 Nov 2007. Compatibility type A1. GenBank accession numbers: ITS = GU230790, β-*tub* GU477614, *EF*-1α = GU477616, *Cox*-1 GU477618, nad1 GU477620. ITALY: Isolate 465/03 (SCRP978, IMI393960) from Showy Banksia (*B. speciosa*) roots in Liguria, northern Italy, collector Santa Olga Cacciola, 2003, GenBank accession numbes: ITS = FJ648808. Isolate 466/03 (SCRP979) from Baxter's Banksia (*B. baxteri*) roots in Liguria, northern Italy, collector Santa Olga Cacciola, 2003, GenBank accession number: ITS = FJ648809. Isolate PhCa12 (IMI391708) from crimson bottlebrush (*Callistemon citrinus*) roots Sicily, southern Italy, collector Santa Olga Cacciola, 2003, GenBank accession number: ITS = JX494411. Isolates Cistus1 (IMI 391712) and Cistus2 (IMI 391713) from sageleaf rockrose (*Cistus salvifolius*) roots in Sicily, southern Italy, collector Santa Olga Cacciola, 2003, GenBank accession numbers: ITS = JF900372 and JF900373 respectively. Isolate 465/10 from silver wattle or mimosa tree (*Acacia dealbata* Link.) roots and collar in Liguria, northern Italy, collector Patrizia Martini, 2010, GenBank accession number: ITS = JF900371. SPAIN: Isolate Ps-57 from *Hedera helix*, Jun–Jul 2005. GenBank accession numbers: ITS = EU194436, *EF*-1α = EU195072, β-*tub* = EU195073, *Cox*-II = EU195074. Isolate PS-183 from almond (*Prunus dulcis*) stem, 2007. GenBank accession numbers: ITS = GQ385965,

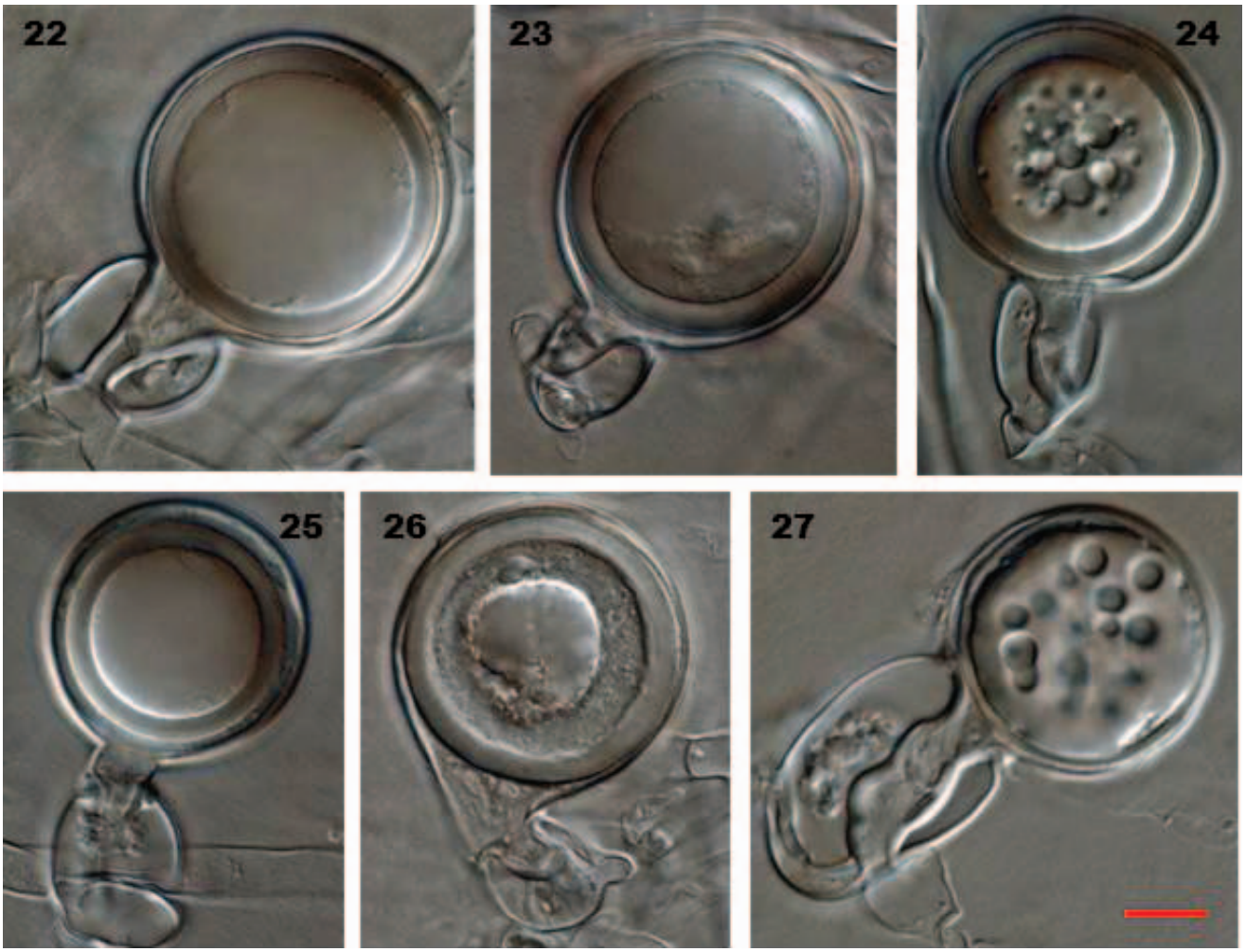


FIGS. 6–10. *Phytophthora niederhauserii*. 6. Colony morphology on cV8 agar. 7. Colony morphology on B-LBA showing a chrysanthemum pattern. 8–10. Toruloid lobate branching hyphal swellings.

EF-1α = GQ418177, β-*tub* = GQ418174, *Cox*-I = GQ418180. Isolate PS-690 from *Plumbago s*p. roots, 2009. GenBank accession numbers: ITS = GQ385966, *EF*-1α = GQ418178, β-*tub* = GQ418175, *Cox*-I = GQ418181. Isolate PS-737 from Pomegranate (*Punica granatum* L.) roots, 2009. GenBank accession numbers: ITS = GQ385967, *EF*-1α = GQ418179, β-*tub* = GQ418176, *Cox*-I = GQ418182. All isolates from Valencia; collector Ana Pérez-Sierra. SOUTH AFRICA: Isolates STE-U 6970, STE-U 6971 STE-U 6972 from grapevine (*Vitis vinifera* L.) roots collected from an established vineyard in Ashton, Western Cape; collector C.F.J. Spies, 11 Jul 2005. GenBank accession numbers: ITS = GQ911576 (STE-U 6970), GQ911577 (STE-U 6971) and GQ911578 (STE-U 6972). Isolate STE-U 6973 from grapevine (*V. vinifera*) roots collected from an established vineyard in Hermanus, Western Cape; collector C.F.J. Spies, 10 Oct 2005. GenBank accession number: ITS = GQ911574. Isolate STE-U 6974 from the crown of a rooted grapevine (*V. vinifera*) cutting collected from a nursery in Wellington, Western Cape; collector C.F.J. Spies, 7 Nov 2005. GenBank accession number: ITS = GQ911575. All isolates maintained in the STE-U collection (Department of Plant Pathology, University of Stellenbosch, Stellenbosch, South Africa). TURKEY: Isolate IK-KD/Kayseri from root, crown and stem of almonds (*Prunus dulcis*) in the orchard, Kayseri, Turkey; collector İlker Kurbetli, 17 Aug 2009. GenBank accession number: ITS = HQ681251. UNITED KINGDOM: Isolate P9000/06 from olive grevillea (*Grevillea olivacea* A.S. George) stem lesion and soil from the rhizosphere of the roots, in Surrey, collector G Denton, Nov 2006. GenBank accession number: ITS= GQ848201. UNITED STATES: Isolate WPC P10617 (PH281 01.5212.-2) from arborvitae (*Thuja occidentalis*), in the family Cupressaceae, affected with root rot in a nursery in Henderson, North Carolina,



FIGS. 11–21. *Phytophthora niederhauserii*. Sporangia (asexual stage), sporangiophores and internal proliferation of the sporangia. 11. Single or rarely branched sporangiophore. 12. Zoospores production. 13–18. Non-papillate, persistent, ellipsoid, obpyriform, ovoid or irregular sporangia on baby lima bean agar flooded with 10% soil extract after 24–36 h flooding. 19. Nested proliferation. 20 and 21. Extended proliferation. Bar = 10 μm.



FIGS. 22–27. *Phytophthora niederhauserii*. Gametangia and oospores (sexual stage). 22–24. Spherical, non-ornamented oogonia; thick-walled, plerotic oospores; amphigynous antheridia. 25–27. Unusual shapes of antheridia and oogonia occasionally detected. 25. Oogonia with neck-shaped base. 26. Oogonia with funnel base. 27. Elongated. Bar = 10 μm.

Sep 2001; collector Z. Gloria Abad. GenBank accession numbers: ITS = AY550916, *EF*-1α = EU080244, β-*tub* = EU080243.

DISCUSSION

A new *Phytophthora* species isolated from 33 host species in 25 families from Australia, Hungary, Israel, Italy, Japan, the Netherlands, Norway, South Africa, Spain, Taiwan, Turkey, the United Kingdom and United States is newly described as *Phytophthora niederhauserii*. *P. niederhauserii* is a distinct species based on morphological comparisons of isolates of this species with descriptions published for 134 *Phytophthora* taxa, including recently described species such as *P. cichorii* Bertier, H. Brouwer, de Cock & D.E.L. Cooke, *P. dauci* Bertier, H. Brouwer & de Cock, and *P. lactucae* Bertier, H. Brouwer & de Cock (Bertier et al. 2013), *P. lacustris* Brasier, Cacciola, Nechwatal, Jung & Bakonyi (Nechwatal et al. 2013), *P. mississippiae* X. Yang, W. E. Copes, and C. X. Hong (Yang et al. 2013), *P. parvispora* Scanu and Denman (Scanu et al. 2014), *P. pisi* F. Heyman (Heyman et al. 2013) and *P. pluvialis* Reeser, Sutton and E Hansen (2013), as well as other putative novel species. *Phytophthora niederhauserii* is self-sterile (heterothallic), fast growing and a high temperature-adapted species that produces non-papillate, mostly ellipsoid, persistent, proliferating sporangia, and has unique toruloid lobate irregular branched hyphal swellings. Phylogenetic analysis of ITS, β-*tub* and *EF-1α* sequences of *P. niederhauserii* and those from other reported *Phytophthora* species, including undescribed putative new species, strongly validates its novel status. *Phytophthora sojae* is phylogenetically the closest species of *P. niederhauserii* in the ITS rDNA and

β-*tub* trees. The main morphological differences between the two species are that *P. niederhauserii* is heterothallic while *P. sojae* is homothallic; *P. niederhauserii* presents amphigynous antheridia, while *P. sojae* produces antheridia that are predominantly paragynous. Distinguishing characters for *P. niederhauserii* are the production of the lobate toruloid hyphal swellings, the shape of the ellipsoid sporangia and the amphigynous antheridia with lobes that are frequently asymmetrical around the oogonia stalk (FIGS. 22–27). *P. sojae* and *P. niederhauserii* have similar temperature requirements; growth optimum for both is close to 30 C.

Phytophthora niederhauserii originally was isolated from arborvitae and English ivy in North Carolina USA in 2001. The most prevalent hosts for the species were English ivy in Italy, Japan, Norway, Spain and the United States and sageleaf rockrose in Italy, Spain and the United States. In USA the species also has been found associated with catawba rhododendron (*Rhododendron catawbiense* Michx.), juniper and Leyland cypress and in ornamentals including iris and coral bell and in an unspecified ornamental in South Carolina (Robayo-Camacho et al. 2009), in California on sageleaf rockrose and *Ceanothus* sp. in Santa Barbara County (Anonymous 2007).

The species also has been isolated consistently from hosts in Italy, Spain, Norway, Hungary and Australia (TABLE I). In Italy, during a survey in the 2001, *P. niederhauserii* was isolated from rotten roots of potted plants of sageleaf rockrose and crimson bottlebrush in Sicily (Cacciola et al. 2009a) and from root and basal stem rot of 2–3 y old plants of showy Banksia, Baxter's Banksia and acorn Banksia in a commercial nursery in Liguria (Pane et al. 2005; Martini et al. 2007; Benson and Magnano di San Lio 2008; Cacciola et al. 2008, 2009b). Other Banksia species, including coast Banksia, *B. marginata* Cav., *B. media* R. Br. and *B. ericifolia* L.f. grown in distinct plots of the same field, were unaffected (S.O. Cacciola unpubl). The species also has been found in ornamental plant nurseries in Sicily associated with infected roots of potted *Banksia* sp. (Martini et al. 2007). In Liguria *P. niederhauserii* recently has been isolated from infected roots and bark of the basal stem of silver wattle seedlings grown in the ground in an ornamental nursery. Affected seedlings had symptoms of decline, root and basal stem rot and gum exudates oozing from the basal stem (S. O. Cacciola unpubl data). The species also has been isolated in central Italy from rotten roots of English ivy (Luongo et al. 2010) and in a forest nursery in Sardinia from Lentisk (*Pistacia lentiscus* L.) (Scanu et al. 2011). On some hosts in Italy, such as *Banksia* spp., crimson bottlebrush and Cistus, *P. niederhauserii*, has been found associated with other *Phytophthora* species including *P. nicotianae*, *P. cryptogea* and *P. drechsleri*. Banksia plants in some nurseries were produced from seeds imported from Australia. In recent years the cultivation of *Banksia* species whose ornamental flowers are commercially used in cut-flower arrangements is continuously increasing in Italy as well as in other European countries. Silver wattle (*A. dealbata*) recently was found to be a host of *P. niederhauserii*, and Australian blackwood (*A. melanoxylon*) has been found susceptible to the pathogen in artificial inoculations (S.O. Cacciola unpubl data).

In Spain the new species was found during a 2001–2006 survey in garden centers and nurseries of the Balearic Islands and eastern Spain (Moralejo et al. 2009). During the surveys it was detected in 2001 on Montpelier cistus (*Cistus monspeliensis* L.) and sageleaf rockrose and in 2005 on English ivy. *P. niederhauserii* has been isolated in fruit nurseries in Spain from 2 y old almond trees with symptoms of chlorosis, wilting, cankers and profuse gumming (FIGS. 26, 27) and from ornamental pomegranate (Pérez-Sierra et al. 2010). In Norway the new species was isolated consistently from diseased English ivy, begonia hybrids (*Begonia* × *hiemalis* and *Begonais* × *cheimantha*), gloxinia and kalanchoë originated from seven greenhouses during a survey of root diseases in greenhouse pot plants started in 2006 (Herrero et al. 2008). Symptoms on begonia, gloxinia and English ivy included necrotic roots and stems with the necrosis advancing to the leaves via the petioles. In the case of English ivy and gloxinia, wilting of the whole plant was observed. In kalanchoë discoloration of roots and reduced plant growth was seen only at first (Herrero et al. 2008). Later necrosis of stems and wilting of kalanchoë and peperomia (*Peperomia clusiifolia* [Jacq.] Hook. cv Isabella) plants also was observed (M.L. Herrero unpubl data).

In Hungary *P. niederhauserii* has been reported causing collar and root necrosis associated with severe wilting and desiccation of foliage on boxwood in a public garden in Budapest (2002) and on Nordmann fir and Lawson cypress produced in a 12 ha ornamental nursery of western Hungary (2007) (Józsa et al. 2010). The disease has been sporadic on Nordmann fir but was abundant on box and Port Orford cedar, resulting in 25–30% mortality.

In Australia *P. niederhauserii* was found in interceptions associated with imported potting mix taken from consignments of nursery-grown plants from the Northern Territory (NT) to Western Australia (WT) during a small survey conducted 2001–2002 (Davidson et al. 2006). The species also has been isolated from soil around dead plants of Austral grass tree (*X. australis*) in natural ecosystems in Melbourne. In the

present study two of these isolates aligned 100% with the type of *P. niederhauserii* (data not shown). More recently the new *Phytophthora* was found established in natural ecosystems in southwestern Western Australia (WA) on *Banksia prionotes* Lindl. (Burgess et al. 2009). *P. niederhauserii* also has was isolated from *Manihot esculenta* in Australia.

In other countries including South Africa, the United Kingdom, the Netherlands and Turkey the pathogen has been detected in one or two hosts and the pathogen has been considered of concern. In South Africa the new species was isolated from grapevine nurseries and established vineyards and was shown to cause stunting of grapevines in greenhouse pathogenicity trials (Spies et al. 2011). The isolation of *P. niederhauserii* from the crowns of nursery vines in that study indicated the potential of the species to cause crown rot in grapevines (Spies et al. 2011). *P. niederhauserii* also was found in 2009 on almonds in Turkey. Out of 600 2 y old almond saplings in an orchard containing cvs. Nonpareil, Ferraduel and Ferragnes in Kayseri province, 30 were affected by the pathogen (Kurbetli and Değirmenci 2011). Symptoms included small chlorotic leaves, wilting, root and crown rot, cankers on the basal stems and gum exudation just above grafting. In the United Kingdom the new species was isolated and identified from stem and soil samples of Olive Grevillea in Nov 2006 through a survey of *Phytophthora* species in UK garden and greenhouse plants (Denton et al. 2008). It is assumed that *Grevillea* plants purchased in Italy by the Royal Horticultural Society as visibly healthy were subsequently found to be infected with *P. niederhauserii*, according to Brasier (2008). This author said that such observations have profound implications for the effectiveness of visual inspection in international trade programs. In addition, exporting nurseries often make extensive use of pesticides and synthetic chemical feeds with the aim of controlling pests and pathogens and providing healthy-looking plants (Brasier 2008). However the use of pesticides may simply suppress the pathogen and thus mask its presence within an infected plant.

There are some misidentifications of *P. niederhauserii*, including the report of *P. drechsleri* associated the rot of English ivy (accession number AB367382) in Japan (Uematsu 1998), a culture of *P. drechsleri* from an unknown ornamental in Israel (ATCC MIYA-4163 [P57, 23J6, Israel2]) listed as a key isolate of the species in Gallegly and Hong (2008) and which sequence accession number FJ746649 corresponds to *P. niederhauserii*. The sequence of a specimen obtained from fig (*Ficus carica* L.) in Taiwan deposited as *P. melonis* at the GenBank (accession number GU111617) also corresponds to the pathogen described in the present study.

The wide host range exhibited by *P. niederhauserii*, which includes at the present 33 plant species in 25 families, strongly suggests that the species is a polyphagous pathogen emerging from nurseries of ornamental plants in Australia, Europe, the USA and possibly other parts of the world. The distribution that includes five continents indicates that the intercontinental ornamental plant trade might have contributed to the introductions of the species globally. The recent discovery of *P. niederhauserii* in fruit nurseries, including almond in Spain (Pérez-Sierra et al. 2010) and Turkey (Kurbetli and Değirmenci 2011), is significant because it suggests that the species also could represent a risk for fruit orchards around the world. As mentioned above, it is possible that the plant trade is accidentally accelerating the worldwide spread of well known or undescribed *Phytophthora* species and creating novel niches for emerging pathogens (Moralejo et al. 2009). Mediterranean shrubs introduced as ornamentals, such as sageleaf rockrose and Montpelier cistus, were fatally affected by *P. niederhauserii* to the extent that a leading producer abandoned production in Mallorca, Spain (Moralejo et al. 2009). Although it is not clear where the species originated, its isolation from acorn Banksia in native forests of midwestern Australia is interesting. In recent years, indeed, several *Banksia* species and other plants in the *Proteaceae* native to the Western Australia, one of the richest regions of floristic biodiversity in the world, are being increasingly cultivated as ornamental and cut flower plants. Several *Phytophthora* species have been isolated from declining Banksia plants in native ecosystems in Australia, which is the center of origin of most *Banksia* species (Scott et al. 2009). A genetic comparison of isolates from Australia and those from the other continents using neutral genetic markers in the way that has been done for *P. ramorum* (Grünwald et al. 2009) will be important in elucidating aspects of the origin and migrations pathways of this important pathogen.

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