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Phylogenetic relationships of the downy mildews (Peronosporales) and related groups based on nuclear large subunit ribosomal DNA sequences

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Abstract: In order to investigate phylogenetic relationships of the Peronosporomycetes (Oomycetes), nuclear large subunit ribosomal DNA sequences containing the D1 and D2 region were analyzed of 92 species belonging to the orders Peronosporales, Pythiales, Leptomitales, Rhipidiales, Saprolegniales and Sclerosporales. The data were analyzed applying methods of neighbor-joining as well as maximum parsimony, both statistically supported using the bootstrap method. The results confirm the major division between the Pythiales and Peronosporales on the one hand and the Saprolegniales, Leptomitales, and Rhipidiales on the other. The Sclerosporales were shown to be polyphyletic; while Sclerosporaceae are nested within the Peronosporaceae, the Verrucalvaceae are merged within the Saprolegniales. Within the Peronosporomycetidae, Pythiales as well as Peronosporales as currently defined are polyphyletic. The well supported *Albugo* clade appears to be the most basal lineage, followed by a *Pythium-Lagenidium* clade. The third, highly supported clade comprises the Peronosporaceae together with *Sclerospora*, *Phytophthora*, and *Peronophythora*. *Peronophythora* is placed within *Phytophthora*, indicating that both genera should be merged. *Bremiella* seems to be polyphyletic within the genus *Plasmopara*, suggesting a transfer to *Plasmopara*. The species of *Peronospora* do not appear as a monophyletic group. *Peronospora* species growing on Brassicaceae form a highly supported clade.

Key Words: LSU rDNA, molecular evolution, Oomycota, Peronosporomycetidae, plant pathogen

INTRODUCTION

Recently, various phylogenetic studies of ribosomal DNA sequences have been published to resolve phylogenetic relationships within the Peronosporomycetes (Dick et al 1999, Matsumoto et al 1999, Riethmüller et al 1999, Cooke et al 2000, Förster et al 2000, Leclerc et al 2000 and Petersen and Rosendahl 2000). Hudspeth et al (2000) used mitochondrial COX2 sequence data to estimate phylogenetic hypotheses and obtained results compatible with the rDNA studies.

The 28S rDNA (LSU) has proved to be particularly useful in phylogenetic analyses; it has been used to test phylogenetic assumptions within the Saprolegniaceae (Leclerc et al 2000) and to analyse the relationships of the major lineages of Peronosporomycetes (Riethmüller et al 1999, Petersen and Rosendahl 2000). A particular emphasis of Riethmüller et al (1999) was to construct hypotheses about phylogenetic relationships within the Saprolegniomycetidae. However, a detailed study of the Peronosporomycetidae is still lacking, demonstrating the urgent need for further studies.

A new taxonomic system for the Peronosporomycetes has been proposed by Dick (1995), in which he divided them into three subclasses, the Saprolegniomycetidae, Rhipidiomycetidae, and Peronosporomycetidae. His ordinal classification was based on morphological and ultrastructural characters, e.g., oosporogenesis, oospore wall, and protoplasmic structure of the oospore. This subdivision could be largely confirmed by subsequent phylogenetic studies based on sequence data (Dick et al 1999, Riethmüller et al 1999, Hudspeth et al 2000, Petersen and Rosendahl 2000). However, phylogenetic relationships within these clades are much less clear, which is especially true for the Peronosporomycetidae.

The evolutionarily advanced Peronosporomycetidae are recognized as some of the most important plant pathogens commercially; they constitute an interesting study group with respect to evolutionary

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processes. Their evolutionary success is reflected by rapid radiation that is probably the result of increasingly specialized parasitism on angiosperms and from the transition to more effective wind dispersal with the formation of elaborate conidiosporangiophores. These evolutionary trends have greatly influenced phylogenetic hypotheses of the whole group. It has generally been accepted that the highly evolved downy mildews and white rusts originated from ancestors of a more primitive *Pythium-Phytophthora* group (e.g., Gäumann 1964, Shaw 1978, 1981, Barr 1983, Dick et al 1989). As a result, the suprageneric classification of the Peronosporomycetidae has largely remained stable over the years.

In current classifications (Hawksworth et al 1995, Dick 2001a), the Peronosporomycetidae contain two orders. The Pythiales are considered to represent a more primitive lineage and consist of two families: the little known Pythiogetonaceae and the Pythiaceae with the genera *Pythium* and *Phytophthora*, both important facultative plant parasites, and seven less prominent genera. The second order, Peronosporales, obligatory parasites on aerial parts of angiosperms, contains two families: the monotypic Albuginaceae (white rusts), and the Peronosporaceae (downy mildews), with currently eight genera (*Basidiophora*, *Benua*, *Bremia*, *Bremiella*, *Paraperonospora*, *Peronospora*, *Plasmopara*, and *Pseudoperonospora*). With about 600 species altogether (Dick 2001a), the Peronosporaceae is the largest family of Peronosporomycetes.

This classification was mainly based on the interpretation of obligate parasitism and elaborate, determinate conidiosporangiophores as apomorphic characters. On the other hand, recent molecular data (Riethmüller et al 1999, Cooke et al 2000, Petersen and Rosendahl 2000) have cast some doubts on this interpretation. However, comparatively few representatives of the Peronosporomycetidae have been included in these studies, concentrating on the genera *Phytophthora* and *Pythium*. Data on the Peronosporaceae and Albuginaceae are almost entirely wanting. Therefore, the present publication was intended to elucidate phylogenetic relationships in the Peronosporomycetidae by analyzing new sequence data, and to stimulate further research on this fascinating group of organisms.

MATERIALS AND METHODS

Sample sources, DNA-extraction, PCR and sequencing.—The organisms included in the study are listed in TABLE 1. For *Peronospora*, the narrow species concept of Gäumann (1918, 1923) was used due to the lack of a modern, comprehensive classification; the nomenclature followed Constantinescu (1991). The other downy mildews were determined and classified mainly using Brandenburger (1985). The sequences of *Lagenidium chthamalophilum*, *Phytophthora megasperma*, *Pythium aphanidermatum*, and *Py. aquatile* were obtained from GenBank. Genomic DNA from pure cultures was isolated using a modified version of the SDS method (Riethmüller et al 1999). From the obligate plant parasites, DNA was extracted either using herbarium specimens, host tissue dried in silica-gel, or spore suspensions frozen in 1.5 mL Eppendorf tubes and stored at −20 C. Material from herbarium specimens was extracted either with the SDS method (Riethmüller et al 1999) or with the DNeasy Plant Kit (Quiagen Inc.) according to the manufacturer's instructions. In most cases, tissue was disrupted using a Retsch MM 300 Mixer Mill and tungsten carbide balls of 3 mm diam. From silica-gel dried host tissue and frozen spore suspensions, DNA was extracted using a modified CTAB-protocol (Doyle and Doyle 1987). For disruption of the cells, the material dried in silica-gel was placed in 2 mL Eppendorf tubes containing 5–7 glass beads (3 mm diam) and ground to powder with a homogenizing mill. The frozen spore suspensions were ground with glass-beads (100 μm diam) and a conical homogenizing pestle. Towards the end of grinding, 300–500 μL 2× CTAB extraction buffer (100 mM Tris pH 8, 1.4 M sodium chloride, 20 mM EDTA, 2% CTAB, 0.5% mercaptoethanol) was added, and the tubes were thoroughly vortexed and incubated at 65 C for 60 min. Then an equal volume of chloroform-isoamyl alcohol (24:1) was added, the tubes vortexed and spun at 14 000 × *g* for 5 min. The resulting supernatant was transferred into a 1.5 mL Eppendorf tube, an equal volume of isopropanol was added, and the tube was vortexed and placed on ice for at least 30 min to precipitate the DNA, followed by centrifugation at 14 000 × *g* for 10 min. The DNA pellet was rinsed with 500 mL ice cold 70% ethanol, dried in a thermoblock at 50 C, dissolved in 30–50 μL dd H_2O and stored at −20 C.

The 5′ terminal domain of the nuclear DNA coding for the large ribosomal subunit (LSU rDNA) was amplified using the polymerase chain reaction PCR (Mullis and Faloona 1987; White et al 1990) with NL1, NL4 (O'Donnell 1993), LR0R (Moncalvo et al 1995), LR6 (Vilgalys and Hester 1990) and LR6-O (5′-CGC CAG ACG AGC TTA CC-3′) as primers. LR6-O is a modification of LR6 to fit the Oomycete LSU gene and was used in most cases. PCR products were purified with the QIAquick Kit (QIAGEN) according to the manufacturer's instructions. DNA was sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Kit (Perkin Elmer) with NL1 or NL4 as primers and an automated DNA sequencer (ABI 373, Perkin Elmer).

Data analysis.—The sequence alignment was initially produced with the aid of the MEGALIGN Module of the LASERGENE System (DNASTAR, Inc.) and visually checked and refined with Se-Al version 2.0 (A. Rambaut, University of Oxford, U.K.). Phylogenetic analyses were performed according to the neighbor-joining method (Saitou and Nei 1987), as well as the maximum parsimony method (e.g., Fitch 1971) using PAUP* version 4b8 (Swofford 2001). For neighbor-joining analysis, the data were first analyzed with

TABLE I. Collection data and GenBank accession numbers of the taxa studied. The taxa were grouped taxonomically; the familial and ordinal classification follows Hawksworth et al (1995) and Dick (2001), respectively. Collectors: AR A. Riethmüller; FB V. Faust-Berndt, FO F. Oberwinkler, HV H. Voglmayr, KW K. Wurm, MG M. Göker, MP M. Piepenbring, RB R. Berndt, RBa R. Bauer, RK R. Kirschner, WM W. Maier. Vouchers: TUB University of Tübingen, WU University of Vienna, GZU University of Graz

Taxon	Collection data		GenBank accession No.
	Isolated from/host	Origin/source (voucher)[a]	
Peronosporales/Albuginaceae			
Albugo achyranthis (Henn.) Miyabe	*Achyranthes aspera* L.	Costa Rica, Prov. Limón, Bribri; leg. MP (TUB)	AY035545
Al. bliti (Steud.) Kuntze	*Amaranthus* sp.	Austria, Upper Austria, St. Willibald; leg. HV (WU)	AY035543
Al. candida (Pers.) Kuntze	*Arabis alpina* L.	Germany, Bavaria, Oberjoch; leg. MP (TUB)	AY035539
Al. candida	*Capsella bursa-pastoris* (L.) Med.	Germany, Mecklenburg-Vorpommern, Greifswald; leg. MG (TUB)	AY035538
Al. candida	*Dentaria* sp.	USA, Tennessee, Knoxville; leg. HV (WU)	AY035540
Al. portulacae (DC. ex Duby) Kuntze	*Portulaca oleracea* L.	Austria, Vienna; leg. HV (WU)	AY035544
Al. tragopogonis (DC.) Gray	*Centaurea scabiosa* L.	Switzerland, Appenzell, Schwendenbachtal; leg. RB & FB (TUB)	AY035542
Al. tragopogonis	*Cirsium oleraceum* L.	Austria, Styria, Mariazell; leg. WM (TUB)	AY035541
Peronosporales/Peronosporaceae			
Basidiophora entospora Roze & Cornu	*Conyza canadensis* (L.) Cronquist	Austria, Lower Austria, Langenlois; leg. HV (WU)	AY035513
Bremia lactucae Regel	*Cirsium oleraceum* (L.) Scop.	Austria, Upper Austria, St. Willibald; leg. HV (WU)	AY035507
B. lactucae	*Lactuca sativa* L.	Germany, Baden-Württemberg; leg. MP 2768 (TUB)	AY035511
B. lactucae	*Lapsana communis* L.	Germany, Baden-Württemberg, Tübingen; leg. HV (TUB)	AY035509
B. lactucae	*Picris hieracioides* L.	Austria, Tyrol, Prägraten; leg. HV (WU)	AY035512
B. lactucae	*Senecio vulgaris* L.	Austria, Upper Austria, St. Willibald; leg. HV (WU)	AY035508
B. lactucae	*Sonchus asper* (L.) Hill	Austria, Upper Austria, St. Willibald; leg. HV (WU)	AY035510
Bremiella baudysii (Scalický) Constant. & Negrean	*Berula erecta* (Huds.) Coville	Austria, Lower Austria, Gramatneusiedl; leg. HV (WU)	AY035517
Br. megasperma (A. Berl.) G. W. Wilson	*Viola rafinesquii* Greene	USA, Tennessee, Knoxville, leg. HV (WU)	AY035516
Paraperonospora leptosperma (de Bary) Constant.	*Tripleurospermum perforatum* (Mérat) M. Laínz	Austria, Upper Austria, St. Willibald; leg. HV (WU)	AY035515
Peronospora aestivalis Syd.	*Medicago sativa* L.	Austria, Lower Austria, Pfaffstätten; leg. HV (WU)	AY035482
Pe. alsinearum Casp.	*Stellaria media* (L.) Vill.	Austria, Vienna; leg. HV (WU)	AY035472
Pe. alta Fuckel	*Plantago major* L.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035493
Pe. aparines (de Bary) Gäum.	*Galium aparine* L.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035484
Pe. arvensis Gäum.	*Veronica hederifolia* L.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035491
Pe. barbareae Gäum.	*Barbarea vulgaris* R. Br.	Austria, Tyrol, Schattwald; leg. MG (TUB)	AY035499
Pe. boni-henrici Gäum.	*Chenopodium bonus-henricus* L.	Germany, Bavaria, Oberjoch; leg. MP (TUB)	AY035475
Pe. brassicae Gäum.	*Raphanus raphanistrum* L.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035503
Pe. bulbocapni Beck	*Corydalis cava* (L.) Schweigg. & Körte	Germany, Baden-Württemberg, Tübingen; leg. FO (TUB)	AF119599
Pe. calotheca de Bary	*Galium odoratum* (L.) Scop.	Austria, Lower Austria, Gießhübl; leg. HV (WU)	AY035483

TABLE I. Continued

Taxon	Collection data		GenBank accession No.
	Isolated from/host	Origin/source (voucher)[a]	
Pe. camelinae Gäum.	*Camelina sativa* (L.) Crantz	Austria, Lower Austria, Hainburg; leg. HV (WU)	AY035506
Pe. conglomerata Fuckel	*Geranium molle* L.	Austria, Lower Austria, Hainburg; leg. HV (WU)	AY035489
Pe. coronillae Gäum.	*Coronilla varia* L.	Austria, Lower Austria, Pfaffstätten; leg. HV (WU)	AY035474
Pe. dentariae Rabenh.	*Cardamine hirsuta* L.	Germany, Nordrhein-Westfalen, Wuppertal; leg. MG (TUB)	AY035505
Pe. dentariae	*Cardamine impatiens* L.	Austria, Tyrol, Schattwald; leg. MG (TUB)	AY035500
Pe. dentariae	*Cardamine pratensis* L.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035504
Pe. ficariae Tul. ex de Bary	*Ranunculus ficaria* L.	Germany, Baden-Württemberg, Tübingen; leg. FO (TUB)	AF119600
Pe. grisea (Unger) Unger	*Veronica beccabunga* L.	Germany, Bavaria, Unterjoch; leg. HV (TUB, WU)	AY035492
Pe. hiemalis Gäum.	*Ranunculus acris* L.	Austria, Vienna; leg. HV (WU)	AY035469
Pe. lamii A. Braun	*Lamium pupureum* L.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035494
Pe. myosotidis de Bary	*Myosotis* sp.	Germany, Bavaria, Hinterstein; leg. MG (TUB)	AY035473
Pe. niessleana Berl.	*Alliaria petiolata* (M. Bieb.) Cavara & Grande	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035498
Pe. parasitica (Pers.:Fr.) Fr.	*Capsella bursa-pastoris* (L.) Medik.	Germany, Baden-Württemberg, Tübingen-Unterjesingen; leg. RB (TUB)	AY035501
Pe. potentillae-sterilis Gäum.	*Potentilla sterilis* (L.) Garcke	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035486
Pe. pulveracea Fuckel	*Helleborus niger* L.	Austria, Styria, Mariazell; leg. WM (TUB)	AY035470
Pe. rumicis Corda	*Rumex acetosa* L.	Austria, Upper Austria, Kopfing; leg. HV (WU)	AY035476
Pe. sanguisorbae Gäum.	*Sanguisorba minor* Scop.	Austria, Tyrol, Schattwald; leg. MG (TUB)	AY035487
Pe. silvestris Gäum.	*Veronica urticifolia* Jacq.	Germany, Bavaria, Oberjoch; leg, HV (TUB)	AY035490
Pe. sparsa Berk.	*Prunus laurocerasus* L. cv. Etna	South of Germany, tree nursery (TUB)	AY035488
Pe. thlaspeos-arvensis Gäum.	*Thlaspi arvense* L.	Germany, Baden-Württemberg, Niedernhall; leg. MG (TUB)	AY035502
Pe. trifolii-alpestris Gäum.	*Trifolium alpestre* L.	Germany, Baden-Württemberg, Tübingen; leg. FO (TUB)	AY035481
Pe. trifolii-hybridi Gäum.	*Trifolium hybridum* L.	Austria, Tyrol, Schattwald; leg. MG (TUB)	AY035480
Pe. trifolii-minoris Gäum.	*Trifolium badium* Schreb.	Germany, Bavaria, Oberjoch; leg. MG (TUB)	AY035479
Pe. trifoliorum de Bary	*Trifolium medium* L.	France, Mont Blanc; leg. MG (TUB)	AY035478
Pe. trivialis Gäum.	*Cerastium fontanum* Baumg.	Germany, Baden-Württemberg, Niedernhall; leg. MG (TUB)	AY035471
Pe. variabilis Gäum.	*Chenopodium album* L.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035477
Pe. violacea Berk.	*Knautia dipsacifolia* Kreutzer	Germany, Bavaria, Oberjoch; leg. HV (TUB, WU)	AY035485
Plasmopara densa (Rabenh.) J. Schroet.	*Rhinanthus alectorolophus* (Scop.) Poll.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035525
Pl. geranii (Peck) Berl. & De Toni	*Geranium maculatum* L.	USA, Tennessee, Knoxville; leg. HV (WU)	AY035520
Pl. helianthi Novot.	*Helianthus annuus* L.	Germany, Baden-Württemberg, Tübingen-Unterjesingen; leg. AR (TUB)	AY035523
Pl. isopyri-thalictroides (Wartenw.) Săvul.	*Isopyrum thalictroides* L.	Austria, Lower Austria, Mannersdorf/Leitha; leg. HV (WU)	AY035526
Pl. obducens (Schroet.) Schroet.	*Impatiens capensis* Meerb.	USA, Tennessee, Knoxville; leg. HV (WU)	AY035522
Pl. oplismeni Vienn.-Bourg.	*Oplismenus hirtellus* (L.) Beauv.	Africa, Guinea, Kindia (GZU)	AY035527
Pl. pimpinellae Săvul.	*Pimpinella major* (L.) Huds.	Austria, Tyrol. Obertilliach; leg. HV (WU)	AY035519
Pl. pusilla (de Bary) Schroet.	*Geranium pratense* L.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035521

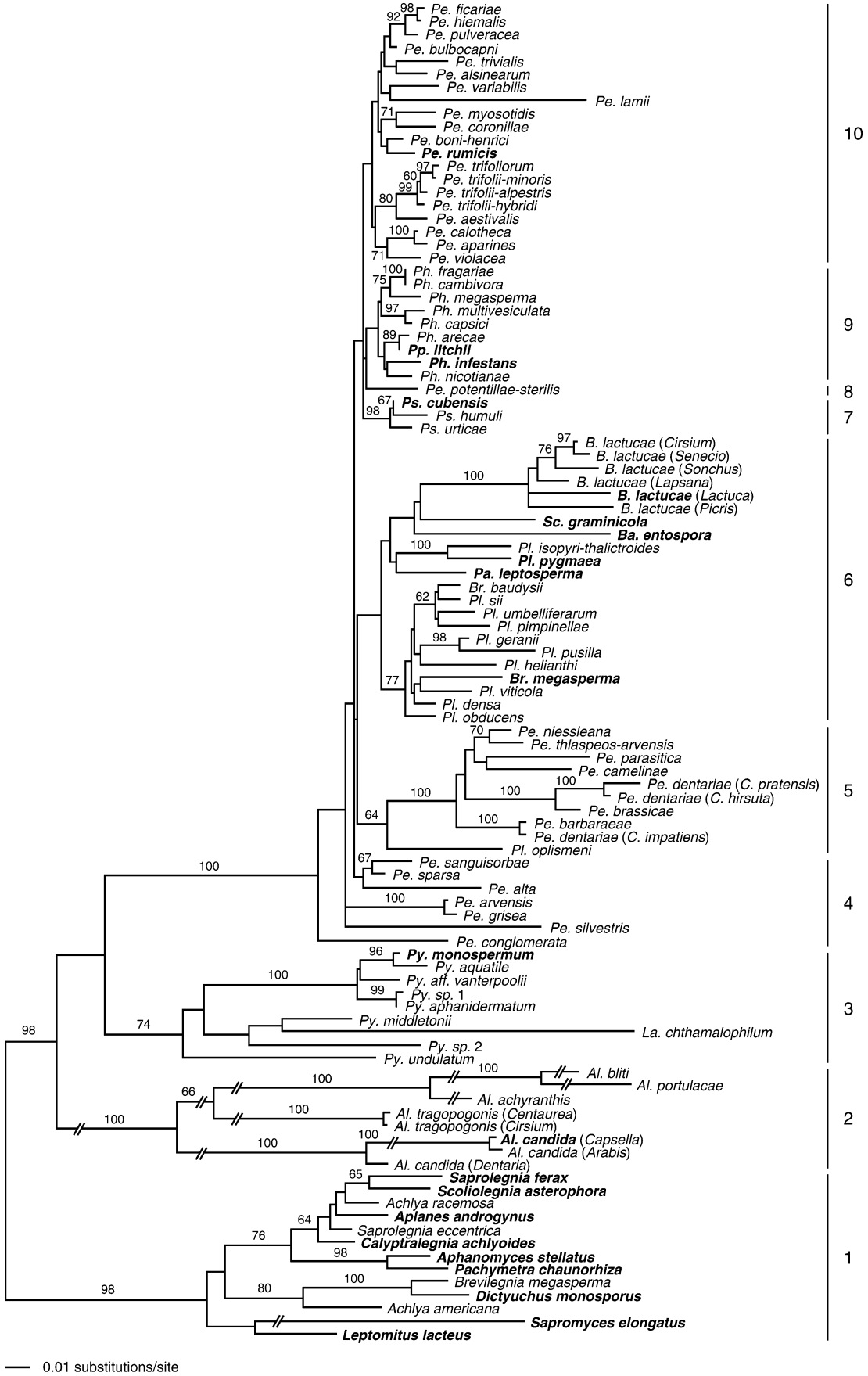
TABLE I. Continued

Taxon	Collection data: Isolated from/host	Collection data: Origin/source (voucher)[a]	GenBank accession No.
Pl. pygmaea (Unger) Schroet.	*Anemone ranunculoides* L.	Germany, Baden-Wüttemberg, Tübingen-Bebenhausen; leg. AR (TUB)	AF119605
Pl. sii Gaponenko	*Sium latifolium* L.	Austria, Lower Austria, Marchegg; leg. HV (WU)	AY035518
Plasmopara umbelliferarum (Casp.) Schroet.	*Aegopodium podagraria* L.	Germany, Baden-Württemberg, Tübingen-Bebenhausen; leg. AR (TUB)	AF119604[c]
Plasmopara viticola Berk. & M. A. Curtis	*Vitis vinifera* L.	Germany, Baden-Württemberg, Tübingen; leg. RK (TUB)	AY035524
Pseudoperonospora cubensis (Berk. & M. A. Curtis) Rostovzev	*Cucumis sativus* L.	Austria, Upper Austria, St. Willibald; leg. HV (WU)	AY035496
Ps. humuli (Miyabe & Takah.) G. W. Wilson	*Humulus lupulus* L.	Austria. Lower Austria, Langenlois; leg. HV (WU)	AY035497
Ps. urticae (Libert ex Berk.) E. S. Salmon & Ware	*Urtica dioica* L.	Austria, Upper Austria, St. Willibald; leg. HV (WU)	AY035495
Pythiales			
Lagenidium chthamalophilum T. W. Johnson		Sequence from GenBank	AF235946
Peronophythora litchii Chen ex W. H. Ko, H. S. Chang, H. J. Su, C. C. Chen & L. S. Leu	*Litchi sinensis* Sonn. (fruits)	CBS 100.81	AY035531
Phytophthora arecae (L. C. Coleman) Pethybr.	*Cocos nucifera* L.	IMI 348342	AY035530
Ph. cambivora (Petri) Buisman	*Prunus cerasus* L.	IMI 340630	AY035533
Ph. capsici Leonian	*Piper nigrum* L. (stem)	IMI 352321	AY035532
Ph. fragariae Hickman	*Fragaria* sp. (fruits)	CBS 309.62	AF119601
Ph. infestans (Mont.) de Bary	*Solanum tuberosum* L.	CBS 560.95	AF119602
Ph. megasperma Drechsler		Sequence from GenBank	X75631
Ph. multivesiculata Ilieva, Man in 't Veld, Veenbaas-Rijks & Pieters	*Cymbidium* sp.	CBS 545.96	AY035529
Ph. nicotianae Breda de Haan	*Nicotiana tabacum* L.	CBS 305.29	AY035528
Pythium aphanidermatum (Edson) Fitzp.		Sequence from GenBank	AF235956
Py. aquatile Höhnk		Sequence from GenBank	AF218200
Py. middletonii Sparrow	Soil	CBS 528.74	AF119608
Py. monospermum Pringsh.		Culture collection Reading, UK, strain no. 4114a	AY035535
Py. undulatum H. E. Petersen	Water	Germany, Baden-Württemberg, Kanzach; leg. AR	AF119603[d]
Py. aff *vanterpoolii* V. Kouyeas & H. Kouyeas	Water	Germany, Baden-Württemberg, Kanzach; leg. AR	AY035534
Py. sp. 1	Soil from field with *Zizania latifolia* (Griseb.) Stapf	Taiwan, Taipei; leg. RK	AY035536
Py. sp. 2	*Juncus articulatus* L. (roots)	Germany, Baden-Württemberg, Crailsheim; leg. RBa	AY035537

TABLE I. Continued

Taxon	Collection data		GenBank accession No.
	Isolated from/host	Origin/source (voucher)[a]	
Rhipidiales			
Sapromyces elongatus (Cornu) Coker	*Pinus* sp. (needles)	Germany, Baden-Württemberg, Kanzach; leg. AR	AF119618
Leptomitales			
Leptomitus lacteus (Roth) C. Agardh	Water	Germany, Baden-Württemberg, Pfullendorf; leg. KW	AF119597
Saprolegniales			
Achlya americana Humphrey	Water	Germany, Baden-Württemberg, Bad Buchau; leg. AR	AF119574
Ac. racemosa Hildebr.	Water	Germany, Baden-Württemberg, Kanzach; leg. AR	AF119581
Aphanomyces stellatus de Bary	Water	Germany, Baden-Württemberg, Kanzach; leg. AR	AF119587
Aplanes androgynus (Archer) Humphrey	Water	Germany, Baden-Württemberg, Kanzach; leg. AR	AF119588
Brevilegnia megasperma J. V. Harv.	Water	Germany, Baden-Württemberg, Nagold; leg. AR	AF119592
Calyptralegnia achlyoides (Coker & Couch) Coker	Water	Germany, Baden-Württemberg, Bad Buchau; leg. AR	AF119593
Dictyuchus monosporus Leitg.	Water	CBS 467.81	AF119595
Saprolegnia eccentrica (Coker) R. L. Seym.		CBS 551.67	AF119611
S. ferax (Gruith.) Thur.	Water	Germany, Baden-Württemberg, Bad Buchau; leg. AR	AF119612
Scoliolegnia asterophora (de Bary) M. W. Dick	Water	Germany, Baden-Württemberg, Kanzach; leg. AR	AF119619
Sclerosporales/Sclerosporaceae			
Sclerospora graminicola (Sacc.) Schroet.	*Setaria viridis* (L.) P. Beauv.	Austria, Lower Austria, Theresienfeld; leg. HV (WU)	AY035514
Sclerosporales/Verrucalvaceae			
Pachymetra chaunorhiza B. J. Croft & M. W. Dick	*Saccharum officinarum* L.	CBS 960.87[b]	AF119598

[a] Source acronyms: *CBS* Centraalbureau voor Schimmelcultures, AG Baarn, The Netherlands; *IMI* CABI Bioscience, Egham, Surrey, UK.
[b] CBS, Isotype.
[c] Published in GenBank as *Plasmopara aegopodii.*
[d] Published in GenBank as *Phytophthora undulata.*


Pe. ficariae
Pe. hiemalis
Pe. pulveracea
Pe. bulbocapni
Pe. trivialis
Pe. alsinearum
Pe. variabilis
Pe. lamii
Pe. myosotidis
Pe. coronillae
Pe. boni-henrici
Pe. rumicis
Pe. trifoliorum
Pe. trifolii-minoris
Pe. trifolii-alpestris
Pe. trifolii-hybridi
Pe. aestivalis
Pe. calotheca
Pe. aparines
Pe. violacea
Ph. fragariae
Ph. cambivora
Ph. megasperma
Ph. multivesiculata
Ph. capsici
Ph. arecae
Pp. litchii
Ph. infestans
Ph. nicotianae
Pe. potentillae-sterilis
Ps. cubensis
Ps. humuli
Ps. urticae
B. lactucae (Cirsium)
B. lactucae (Senecio)
B. lactucae (Sonchus)
B. lactucae (Lapsana)
B. lactucae (Lactuca)
B. lactucae (Picris)
Sc. graminicola
Ba. entospora
Pl. isopyri-thalictroides
Pl. pygmaea
Pa. leptosperma
Br. baudysii
Pl. sii
Pl. umbelliferarum
Pl. pimpinellae
Pl. geranii
Pl. pusilla
Pl. helianthi
Br. megasperma
Pl. viticola
Pl. densa
Pl. obducens
Pe. niessleana
Pe. thlaspeos-arvensis
Pe. parasitica
Pe. camelinae
Pe. dentariae (C. pratensis)
Pe. dentariae (C. hirsuta)
Pe. brassicae
Pe. barbaraeae
Pe. dentariae (C. impatiens)
Pl. oplismeni
Pe. sanguisorbae
Pe. sparsa
Pe. alta
Pe. arvensis
Pe. grisea
Pe. silvestris
Pe. conglomerata
Py. monospermum
Py. aquatile
Py. aff. vanterpoolii
Py. sp. 1
Py. aphanidermatum
Py. middletonii
La. chthamalophilum
Py. sp. 2
Py. undulatum
Al. bliti
Al. portulacae
Al. achyranthis
Al. tragopogonis (Centaurea)
Al. tragopogonis (Cirsium)
Al. candida (Capsella)
Al. candida (Arabis)
Al. candida (Dentaria)
Saprolegnia ferax
Scoliolegnia asterophora
Achlya racemosa
Aplanes androgynus
Saprolegnia eccentrica
Calyptralegnia achlyoides
Aphanomyces stellatus
Pachymetra chaunorhiza
Brevilegnia megasperma
Dictyuchus monosporus
Achlya americana
Sapromyces elongatus
Leptomitus lacteus
10
9
8
7
6
5
4
3
2
1
0.01 substitutions/site

Modeltest version 3.04 by D. Posada (Posada and Crandall 1998) to find the most appropriate model of DNA substitution, which was then used for calculation of the neighbor-joining tree. Support for internal nodes of the trees was obtained using bootstrap analysis (Felsenstein 1985) from 1000 replicates.

Heuristic maximum parsimony analysis was performed with 10 000 rounds of branch swapping (TBR; MULTREES option in effect) on starting trees obtained by stepwise addition of the DNA sequences in random order. Gaps were treated as missing data. In order to prevent PAUP* crashing because of the large amount of suboptimal trees of length 2347 detected in previous analyses, the options CHUCK = 1 and CHUCKSCORE = 2345 were used. Thus, no more than one tree of length greater than or equal to 2345 was saved in each replicate. Bootstrap analysis was done with 1000 resamplings, each with 10 rounds of random addition without branch swapping.

Leptomitus lacteus (Leptomitales), *Sapromyces elongatus* (Rhipidiales) and 11 taxa of the Saprolegniales were chosen as outgroups on the basis of the results of Riethmüller et al (1999) and Petersen and Rosendahl (2000).

RESULTS

Sequence alignment.—The total alignment was 774 bp long; most of the sequences could be accurately aligned over the whole range. However, in some *Albugo* species (*A. tragopogonis*, *A. bliti*, *A. portulacae*, *A. achyranthis*) parts of the sequences were excluded as they could not be aligned; these regions have been recoded in the matrix as ''missing data''. The final alignment and the trees obtained are deposited in TreeBase (http://www.treebase.org/study accession number S766).

Phylogenetic analysis.—Performing hierarchical likelihood ratio tests, the model of Tamura and Nei (1993) was chosen for the neighbor joining (NJ) analysis, with the following settings: base frequencies A = 0.2430, C = 0.1479, G = 0.2669, T = 0.3421; rate matrix [A-C] = 1.0, [A-G] = 4.8878, [A-T] = 1.0, [C-G] = 1.0, [C-T] = 7.0636, [G-T] = 1.0; additionally assuming a proportion I = 0.3079 of invariant nucleotide sites and a gamma-distributed substitution rate for the remaining sites (Gu et al 1995) with gamma distribution shape parameter α = 1.0309. The dendrogram obtained by the NJ analysis is shown in FIG. 1. Heuristic maximum parsimony (MP) analysis yielded 426 trees of length 2344 from six different islands; the strict consensus tree of these is shown in FIG. 2. These trees were already detected after 2138 of 10 000 rounds of heuristic search. Tree topologies of both distance and parsimony analysis are broadly similar, with the exception of some minor differences. The division between the Saprolegniales, Leptomitales, and Rhipidiales (cluster 1) on the one hand and the Peronosporales and Pythiales (cluster 2–10) on the other hand is well supported (bootstrap values of 98% in FIG. 1 and 88% in FIG. 2, respectively).

Leptomitus lacteus (Leptomitales) is sister to the Saprolegniales according to MP analysis (FIG. 2), whereas in NJ analysis, *Leptomitus lacteus* and *Sapromyces elongatus* are joined (FIG. 1), though without significant bootstrap support.

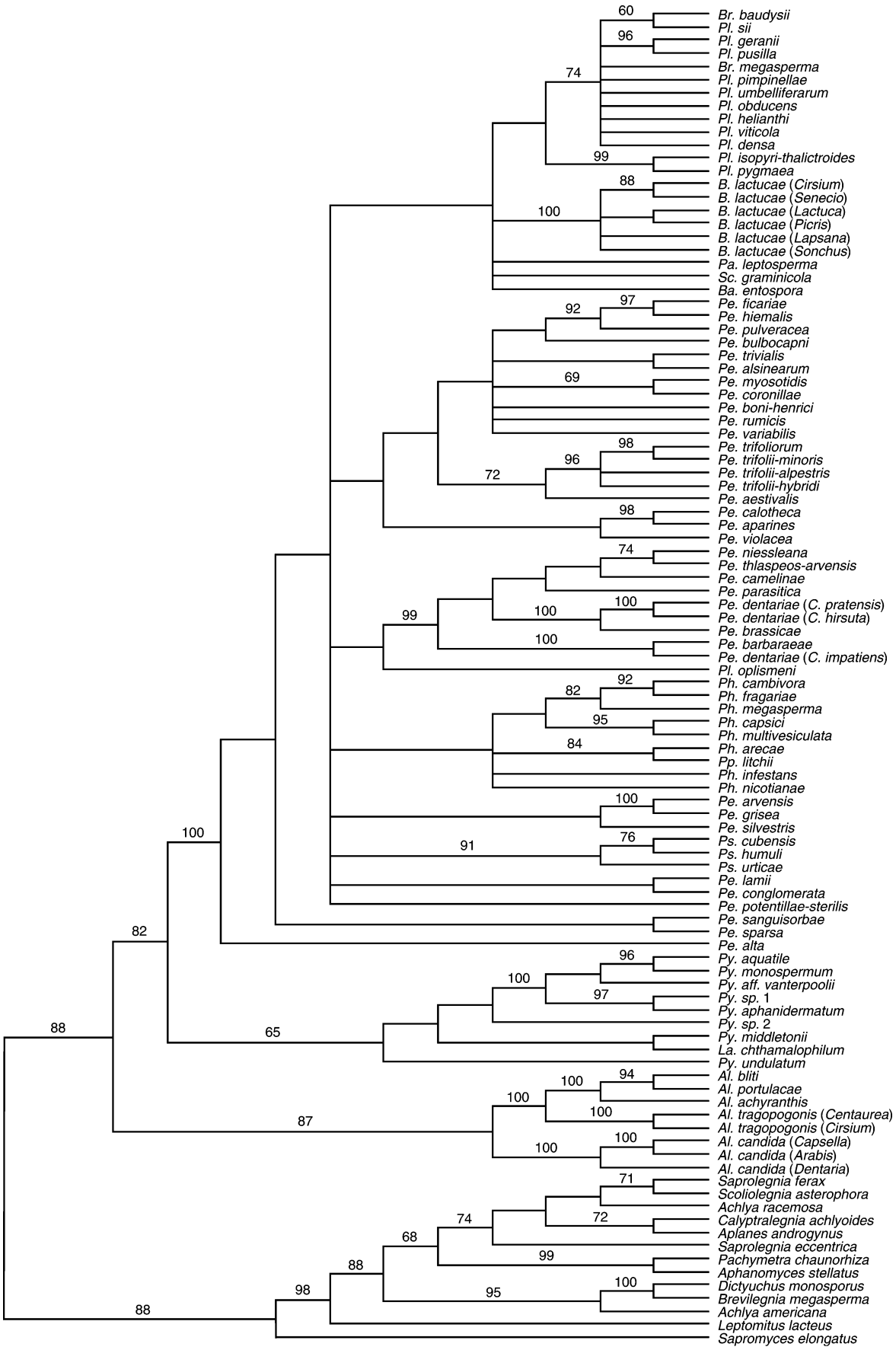
Except for *Calyptralegnia achlyoides*, the tree topologies within the Saprolegniales are identical in both analyses; *Pachymetra chaunorhiza*, classified in the Sclerosporales, clusters with *Aphanomyces* of the Saprolegniales with high bootstrap support (FIGS. 1, 2).

Within the Peronosporomycetidae, three major groups are present (cluster 2, cluster 3, and cluster 4–10). A well supported *Albugo* clade (bootstrap values of 100% in FIG. 1 and 87% in FIG. 2) is most basal; however, its basal position is only well supported in parsimony analysis (82%), indicated by the common ancestry of the remaining species of Peronosporomycetidae (clusters 3–10). Within *Albugo*, topologies are identical in both trees and supported by high bootstrap values. In the NJ tree, branch lengths within the *Albugo* clade are very long, reflecting higher substitution rates in this clade.

Next to *Albugo* comes a *Pythium-Lagenidium* clade (cluster 3) which is moderately supported in both analyses with bootstrap values of 74% (FIG. 1) and 65% (FIG. 2), respectively. However, after removal of *Lagenidium*, bootstrap support for the *Pythium* clade became highly significant in NJ analysis (97%; data not shown). Except for *Py.* sp. 2, topologies within the clade are identical in both trees, but the topologies of the basal taxa receive no bootstrap support, notably the position of *Lagenidium* within a paraphyletic *Pythium*.

←

FIG. 1. Neighbor-joining analysis of an alignment of nuclear DNA coding for the 5′ terminal domain of the 28S ribosomal large subunit. Genetic distances were computed according to the model of Tamura and Nei (1993), additionally assuming a proportion of invariant nucleotide sites and a gamma-distributed substitution rate for the remaining sites. Interrupted branches were scaled to half of the length. Branch lengths of *Al. bliti*, *Al. candida* (*Capsella*), *Pe. dentariae* (*C. hirsuta*) and *Pe. trifolii-minoris* were negative. Tree topology was rooted with the group of Saprolegniales, Leptomitales and Rhipidiales (cluster 1). Numbers on branches are bootstrap values (1000 replicates, values smaller than 60% not shown). Bold taxa are the type species of the respective genera.


Br. baudysii
Pl. sii
Pl. geranii
Pl. pusilla
Br. megasperma
Pl. pimpinellae
Pl. umbelliferarum
Pl. obducens
Pl. helianthi
Pl. viticola
Pl. densa
Pl. isopyri-thalictroides
Pl. pygmaea
B. lactucae (Cirsium)
B. lactucae (Senecio)
B. lactucae (Lactuca)
B. lactucae (Picris)
B. lactucae (Lapsana)
B. lactucae (Sonchus)
Pa. leptosperma
Sc. graminicola
Ba. entospora
Pe. ficariae
Pe. hiemalis
Pe. pulveracea
Pe. bulbocapni
Pe. trivialis
Pe. alsinearum
Pe. myosotidis
Pe. coronillae
Pe. boni-henrici
Pe. rumicis
Pe. variabilis
Pe. trifoliorum
Pe. trifolii-minoris
Pe. trifolii-alpestris
Pe. trifolii-hybridi
Pe. aestivalis
Pe. calotheca
Pe. aparines
Pe. violacea
Pe. niessleana
Pe. thlaspeos-arvensis
Pe. camelinae
Pe. parasitica
Pe. dentariae (C. pratensis)
Pe. dentariae (C. hirsuta)
Pe. brassicae
Pe. barbaraeae
Pe. dentariae (C. impatiens)
Pl. oplismeni
Ph. cambivora
Ph. fragariae
Ph. megasperma
Ph. capsici
Ph. multivesiculata
Ph. arecae
Pp. litchii
Ph. infestans
Ph. nicotianae
Pe. arvensis
Pe. grisea
Pe. silvestris
Ps. cubensis
Ps. humuli
Ps. urticae
Pe. lamii
Pe. conglomerata
Pe. potentillae-sterilis
Pe. sanguisorbae
Pe. sparsa
Pe. alta
Py. aquatile
Py. monospermum
Py. aff. vanterpoolii
Py. sp. 1
Py. aphanidermatum
Py. sp. 2
Py. middletonii
La. chthamalophilum
Py. undulatum
Al. bliti
Al. portulacae
Al. achyranthis
Al. tragopogonis (Centaurea)
Al. tragopogonis (Cirsium)
Al. candida (Capsella)
Al. candida (Arabis)
Al. candida (Dentaria)
Saprolegnia ferax
Scoliolegnia asterophora
Achlya racemosa
Calyptralegnia achlyoides
Aplanes androgynus
Saprolegnia eccentrica
Pachymetra chaunorhiza
Aphanomyces stellatus
Dictyuchus monosporus
Brevilegnia megasperma
Achlya americana
Leptomitus lacteus
Sapromyces elongatus

The largest, well supported clade (bootstrap support of 100% in both trees) comprises the Peronosporaceae together with the genera *Sclerospora*, *Phytophthora* and *Peronophythora* (cluster 4–10). Topologies within this clade while not identical in the two trees are generally similar, particularly in the case of those groupings that are well supported by bootstrap analyses.

The genera *Plasmopara* (except *Pl. oplismeni*), *Bremiella*, *Bremia*, *Paraperonospora*, *Basidiophora* and, remarkably, *Sclerospora* form one group (cluster 6), without, however, significant bootstrap support. Remarkably, the Verrucalvaceae (*Pachymetra*) appear within the Saprolegniales, and the Sclerosporaceae (*Sclerospora*) within the Peronosporaceae.

Another group common to both trees is the *Phytophthora-Peronophythora* clade (cluster 9), which, however, lacks bootstrap support in both analyses. Internal branching is compatible in both trees. *Pe. litchii* is sister to *Ph. arecae*, which is supported by bootstrap values of 89% (FIG. 1) and 84% (FIG. 2).

Plasmopara (exclusive of *Pl. oplismeni*) is monophyletic in the parsimony analysis but paraphyletic in the NJ tree; *Bremiella* is consistently nested within the *Plasmopara* clade and polyphyletic. The *Bremia lactucae* complex forms an evolutionarily distinct group within clade 6 with high bootstrap support (100%) in both analyses. The three species of *Pseudoperonospora* form another small, well supported clade (98% in FIG. 1 and 91% in FIG. 2). The largest genus of the Peronosporaceae, *Peronospora*, is polyphyletic with several distantly related groups (cluster 4, 5, 8, 10). The species on Brassicaceae (*Pe. parasitica* group) form a highly supported clade (cluster 5, 100% in FIG. 1 and 99% in FIG. 2). Another large group of *Peronospora* species (cluster 10, with poor bootstrap support) includes species infecting Ranunculales, Caryophyllales, Fabales, Boraginaceae, Dipsacaceae, and Rubiaceae.

DISCUSSION

The phylogenetic trees are compatible with the basal division of Peronosporomycetes into Peronosporomycetidae and Saprolegniomycetidae as proposed by Dick et al (1984), which is consistent with the results of previous investigations based on LSU (Petersen and Rosendahl 2000), SSU (Dick et al 1999) and COX2 (Hudspeth et al 2000). Dick (1995) proposed a third subclass, Rhipidiomycetidae, which is represented by *Sapromyces* in our analysis. Its phylogenetic position remains unclear. This is consistent with the other analyses where it sometimes seems to be more closely related to the Saprolegniomycetidae (Petersen and Rosendahl 2000), or to the Peronosporomycetidae (Hudspeth et al 2000). Therefore, as already indicated (Riethmüller et al 1999), more sequence data are necessary to resolve the evolutionary position of the Rhipidiales.

Within the Saprolegniales, tree topologies of the present analyses differ from those in Riethmüller et al (1999). Nevertheless, the polyphyly of centric and eccentric *Achlya* species was confirmed, and polyphyly of the centric and eccentric *Saprolegnia* species could be assumed.

Within the Peronosporomycetidae, our phylogenetic trees are not fully compatible with the hierarchical classification in current use (Hawksworth et al 1995, Dick 2001a), as both Pythiales and Peronosporales appear to be polyphyletic. The genus *Phytophthora*, assumed to be a member of the Pythiales, is more closely related to Peronosporaceae than to *Pythium*, which occupies a basal position within the Peronosporomycetes. This is in accordance with the molecular phylogenies of Riethmüller et al (1999), Cooke et al (2000) and Petersen and Rosendahl (2000). The Peronosporales as currently defined are also polyphyletic, as the genus *Albugo* appears to be basal to the rest of the Peronosporomycetidae.

Peronosporaceae.—As the common ancestry of the Peronosporaceae and allied genera (*Phytophthora*, *Peronophythora*, and *Sclerospora*) is strongly supported in the analyses, there can be little doubt that this family represents a monophyletic group. However, this strong support contrasts with a lack of resolution of the basal branches within the clade. Many of the internal nodes are not supported by bootstrap analysis, presenting an unclear picture of the overall phylogeny of the group. Nevertheless, this study may offer some interesting insights into the evolutionary processes. The lack of resolution of these basal branches may be evidence for a rapid radiation of the whole clade, which is also supported by the short

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FIG. 2. Maximum parsimony analysis of an alignment of nuclear DNA coding for the 5′ terminal domain of the 28S ribosomal large subunit. A strict consensus tree of the 426 trees of length 2344 found in heuristic search (10 000 rounds of random addition of sequences and subsequent TBR branch swapping) is shown. Numbers on branches are bootstrap values from 1000 replicates (each with 10 rounds of random addition of sequences without branch swapping), values smaller than 60% not shown. Tree topology was rooted with the group of Saprolegniales, Leptomitales and Rhipidiales.

branch lengths between many nodes in relation to the comparatively long terminal branches (FIG. 1). The main trigger responsible for this rapid radiation of the downy mildews may have been the transition to parasitism of aerial parts of angiosperms, in combination with effective wind dissemination. It is not surprising that also *Phytophthora* is contained within this clade, supporting the theories of Brasier and Hansen (1992) who, on the basis of sexual mechanisms and conidiosporangial dispersal, suggested that *Phytophthora* might be more closely related to *Peronospora* and *Bremia* than to *Pythium*. The ecology of the species of the genus *Phytophthora* is very diverse, they either occur in soil and water or are aerially dispersed. While most species of *Phytophthora* are also parasites on higher land plants, they differ from the downy mildews in their ability to grow on agar.

The phylogenetic position of the monotypic genus *Peronophythora* has been extensively debated (Chen 1961, Ko et al 1978, Chi et al 1982, Ho et al 1984, Hall 1989). Considering the combination of determinate conidiosporangiophores and *Phytophthora*-like characters, Ko et al (1978) placed *Peronophythora* in a family of its own, Peronophythoraceae, which was expected to occupy a phylogenetic position between *Phytophthora* and Peronosporaceae. On the other hand, Chi et al (1982) noted indeterminate growth of the conidiosporangiophores and proposed a transfer to *Phytophthora*. Ho et al (1984) confirmed the presence of indeterminate growth but argued that the conidiosporangiophores are usually determinate, which should justify the retention of a separate genus. However, many *Phytophthora* taxa demonstrate determinate sporangiophore growth as well. Hence, the presence of determinate or indeterminate growth does not appear to be a suitable character for distinguishing between the genera.

Our analyses do not support a position of *Peronophythora* intermediate between *Phytophthora* and Peronosporaceae, and there is strong evidence for a transfer of *Peronophythora* to *Phytophthora*. The position of *Pe. litchii* as sister to *Ph. arecae* receives high bootstrap support in both analyses (FIGS. 1, 2). The caducous but water-dispersed and shortly pedicellate sporangia, the partly amphigynous oogonia, the lack of oogonial periplasm and, perhaps most significantly, its ability to grow on synthetic media are clear evidence for a close relationship of *Peronophythora* and *Phytophthora*.

Interestingly, the genera *Plasmopara*, *Bremiella*, *Paraperonospora*, *Bremia*, *Basidiophora*, and *Sclerospora*, despite their diverse conidiosporangiophore morphology, form a monophyletic group in both NJ and MP analyses. Although this clade is not supported by significant bootstrap values, its members share features probably indicating a closer relationship: ellipsoid to pyriform haustoria (except for *Sclerospora* with peglike haustoria) and hyaline to pale yellowish conidia or sporangia (our observations). This may indicate that conidiosporangiophore morphology has been overemphasized in previous evolutionary hypotheses (see diagrams in Dick et al 1984, Dick 1988) and also demonstrate that unequivocal interpretation of condiosporangiophore morphology is often difficult.

The genus *Bremiella* may be an example for a challenging interpretation of conidiosporangiophore morphology. Its sequenced species clustering among species of *Plasmopara*, *Bremiella* appears to be polyphyletic. A re-examination of the conidiosporangiophores showed that branching in *Bremiella* is more similar to *Plasmopara* (own observations) than strictly dichotomous (Constantinescu 1979), which is evidence for reincorporating the genus *Bremiella* into *Plasmopara* (the two sequenced species of *Bremiella* have already been assigned to the genus *Plasmopara*). It should also be mentioned that the second feature assumed to be typical of *Bremiella*, the terminal knobs on the sterigmata, does not seem to be of high taxonomic value as it is also seen in some species of the genus *Paraperonospora* (Constantinescu 1989).

Currently, the genus *Bremia* includes two species, *Br. lactucae* on Asteraceae and the little-known *Br. graminicola* on Poaceae. Besides *Br. lactucae*, several additional species of *Bremia* have been described from Asteraceae, which are listed in Skidmore and Ingram (1985). However, extensive investigations made by these authors demonstrated that morphological characters were not useful for taxonomic differentiation within the genus. Cross-infection studies with various isolates from different hosts showed the presence of several host-specific groups (Skidmore and Ingram 1985). Based on the lack of morphological differentiation, these authors accepted only one species, *Br. lactucae*, but proposed several *formae speciales* in relation to the groups of host species presented in their studies.

Our analyses support the presence of distinct entities within *Br. lactucae*, which may correspond to the groups proposed by Skidmore and Ingram (1985). The isolates from the Cichorioideae are consistently basal to those on the Asteroideae (*Cirsium oleraceum* and *Senecio vulgaris*) hosts (FIGS. 1, 2), possibly indicative of an early radiation of *Br. lactucae* on Cichorioideae and subsequent parasitism of the Asteroideae.

Both generic circumscription and infrageneric classification of *Plasmopara* have changed greatly over the years (Fischer 1892, Berlese 1902, Wilson 1907, Skalický (1966). Wilson (1907) transferred the

species releasing zoospores from the sporangium to the new genus *Rhysotheca*; however, this has not been widely accepted. The present study neither supports the genus *Rhysotheca* nor the sectional and subgeneric classifications proposed by Fischer (1892) and Skalický (1966), because neither of these entities appear to be monophyletic groups (FIGS. 1, 2). On the other hand, the inclusion of *Bremiella* into *Plasmopara* as proposed by Berlese (1902) is corroborated. At present, a monophyletic *Plasmopara* genus is not supported in our analyses; however, morphologically the genus can be well defined by the monopodial sporangiophores and the hyaline sporangia. *Plasmopara oplismeni*, which is only distantly related to the majority of *Plasmopara* taxa (FIGS. 1, 2), is distinguished from the latter by unique conidiosporangiophore features (long, tortuous, and bloated sterigmata) and the graminaceous host (Kenneth and Kranz 1973), possibly indicating an independent origin.

Within the genus *Plasmopara* the relatedness of the studied species does not parallel that of their angiosperm host species (APG 1998), which may be evidence for frequent host jumping. On the other hand, also radiation on related hosts may be observed, as the species on the same host families are closely allied; the species on Ranunculaceae (*Pl. pygmaea* and *Pl. isopyri-thalictroides*) and Geraniaceae (*Pl. geranii* and *Pl. pusilla*) form highly supported clades. In addition, in the NJ tree the species parasitizing Apiaceae (*Pl. sii*, *Pl. umbelliferarum*, *Pl. pimpinellae*) form a cluster together with *Bremiella baudysii*, which is also parasitic on a host belonging to the Apiaceae. This is additional evidence for polyphyly of *Bremiella* and for reincorporation of the genus into *Plasmopara*.

For a long time, the genus *Pseudoperonospora* represented a heterogeneous assemblage, and numerous species were excluded after critical re-examination (Waterhouse and Brothers 1981). As a consequence, the status of the genus has been questioned by Skalický (1966) and by Kochman and Majewski (1970), and suggestions have been made that it should be merged with *Peronospora* and/or *Plasmopara* (Waterhouse and Brothers 1981). However, Shaw (1978) maintained the genus, because the asexual reproductive structures in *Pseudoperonospora* are sporangia with apical opercula (poroid according to Shaw 1978), in contrast to the conidia of *Peronospora*, which lack modifications in the apical region (nonporoid according to Shaw 1978). Based on sporangial ultrastructure and the phenetic characters shared by the species of the genus, Constantinescu (2000) suggested that the genus *Pseudoperonospora* is justified.

Our analyses confirm monophyly of the genus *Pseudoperonospora* (FIGS. 1, 2). Relationships to other genera remain unclear; however, NJ analysis suggests that *Pseudoperonospora* may be affiliated with *Peronospora* (FIG. 1), which is supported by similar conidiosporangiophore morphology, haustoria and conidiosporangium color (our observations).

The large genus *Peronospora* obtained its present circumscription (some later changes have been mentioned above) mainly with the work of Schroeter (1889), who limited it to De Bary's section *Pleuroblastae* (Fischer 1892). So far, subgeneric classification of *Peronospora* has been primarily based on oospore morphology (Fischer 1892, Gäumann 1923, Savulescu 1948, Skalický 1966). However, our data do not support any of the sections (*Calothecae* and *Leiothecae*) or subsections (*Verrucosae*, *Reticulatae*, *Effusae*, and *Parasiticae*) of the genus defined by this feature. Only the members of the *Parasiticae*, except for *Pe. bulbocapni*, can be found in a single clade (cluster 5).

In the present analyses, the genus *Peronospora* appears to be polyphyletic (FIGS. 1, 2); two major (cluster 5, 10) and two minor (cluster 4, 8) groups are present in the NJ tree (FIG. 1). Most of the *Peronospora* species included in the present analysis belong to cluster 10. Although not supported by the bootstrap, except for *Pe. lamii* this clade comprises the same species in both trees (FIGS. 1, 2) and appears to be closest to the *Pseudoperonospora* and *Phytophthora-Peronophythora* clades in the NJ tree. This arrangement receives support from morphological characters because the *Peronospora* species of cluster 10 show the same type of conidiophores as *Pseudoperonospora* and the same type of haustoria as both *Pseudoperonospora* and *Phytophthora*, in cases where haustoria are developed in the latter genus (Fraymouth 1956, our observations). Therefore, cluster 10 possibly represents a valid phylogenetic hypothesis.

Within cluster 10, some well supported subgroups relate well with the systematics of their respective hosts. For example, most species parasitizing Fabaceae (*Pe. aestivalis*, *Pe. trifolii-alpestris*, *Pe. trifolii-hybridi*, *Pe. trifolii-minoris*, *Pe. trifoliorum*) included in the present analysis belong to a single clade supported by bootstrap values of 80 or 72%, respectively (FIGS. 1, 2). In addition, within this Fabaceae clade the species growing on *Trifolium* form a highly supported subclade. Similarly, a clade with bootstrap values of 92% (FIGS. 1, 2) consists of the three species of *Peronospora* present in our analysis that infect Ranunculaceae (*Pe. ficariae*, *Pe. hiemalis*, *Pe. pulveracea*). Thus, the present molecular study results in a preliminary confirmation of Gäumann's concept of ''Formenkreise'' in *Peronospora* (Gäumann 1923) adopted

by Gustavsson (1959), but clearly many more taxa must be considered to draw definitive conclusions.

A number of *Peronospora* species (*Pe. alta*, *Pe. arvensis*, *Pe. conglomerata*, *Pe. grisea*, *Pe. potentillae-sterilis*, *Pe. sanguisorbae*, *Pe. silvestris*, *Pe. sparsa*) can be found in more or less isolated positions in both trees, none of them supported by significant bootstrap values and with no topological accordance between the NJ and the MP tree in detail. Thus, our molecular data give no consistent results concerning the phylogenetic position of these taxa, and their position in the trees presented here gives no evidence for considering *Peronospora* to be polyphyletic. Equally, no microscopic differences between them and those species belonging to cluster 10 were evident.

Within cluster 5, the same group of *Peronospora* species is present in both trees and in each case supported by high bootstrap values of 100 and 99%, respectively (FIGS. 1, 2). Therefore, this subclade may be considered as monophyletic. In perfect accordance between molecular and pathogenicity data, it comprises all *Peronospora* species infecting Brassicaceae (*Pe. parasitica* group). Both analyses show *Plasmopara oplismeni* to be basal to this clade. This grouping is poorly supported, but is perhaps an indication that the genus *Peronospora* is polyphyletic. The species of *Peronospora* parasitizing Brassicaceae show morphological differences in comparison with the remaining species concerning haustoria (Fraymouth 1956, our observations) and other microscopic characters (unpubl data). The comparatively long genetic distances within cluster 5 (FIG. 1) agree with the results of Rehmany et al (2000), who compared ITS and AFLP data from *P. parasitica* s. l. infecting *Brassica oleracea* and *Arabidopsis thaliana*, respectively. This confirms Gäumann's concept of splitting the former *Pe. parasitica* into a lot of distinct species (Gäumann 1918), which was rejected by Yerkes and Shaw (1959). On the other hand, not all of the species mentioned in Gäumann (1918) appear to be natural groups. For example, according to our analysis, *Pe. dentariae* seems to be polyphyletic.

Sclerosporales.—Dick et al (1984) moved the graminicolous downy mildew genera *Sclerospora* and *Peronosclerospora* from the Peronosporales to the new order Sclerosporales on the basis of their parasitism on graminaceous hosts, a thickened, sclerified oogonial wall, and more or less plerotic oospores. They suggested two families, the Sclerosporaceae with the genera *Peronosclerospora* and *Sclerospora*, and the Verrucalvaceae with the genera *Sclerophthora* and *Verrucalvus*. With some delay, they considered the Sclerosporales to be part of the subclass Peronosporomycetidae. Later, a third genus, *Pachymetra*, was added to the Verrucalvaceae (Dick et al 1989) and the Sclerosporales were removed to the subclass Saprolegniomycetidae. This classification has remained unchanged in the most recent classifications of Peronosporomycetes (e.g., Dick 1999, 2001a, Hawksworth et al 1995). However, our data indicate that the Sclerosporales in the current circumscription are polyphyletic, as *Pachymetra chaunorhiza* is more closely related to members of the Saprolegniomycetidae whereas the type species of the genus *Sclerospora*, *Sc. graminicola*, clusters within the downy mildew clade, which is well supported with bootstrap values of 100% (FIGS. 1, 2). Therefore, the separation of an order Sclerosporales is not justified, and the Sclerosporaceae should again be classified as Peronosporales, whereas at least part of the Verrucalvaceae should be grouped within the Saprolegniaceae. The morphological data support such a separation; the Verrucalvaceae lack conidiosporangiophores and no haustoria were observed (Dick 1999). In addition, in *Verrucalvus* zoosporangial dehiscence closely resembles that of *Aphanomyces* (Dick et al 1984), which is remarkable as *Pachymetra*, considered to be its closest relative (Dick et al 1989), clusters with *Aphanomyces* in our analysis (FIGS. 1, 2). However, it is necessary to collect data for all genera of the Sclerosporales before the status of the Verrucalvaceae can be finally resolved. It seems that parasitism on grasses as a phylogenetic character has been overemphasized by Dick et al (1984, 1989) and has evolved independently in *Pachymetra chaunorhiza*, *Plasmopara oplismeni*, and *Sclerospora graminicola*.

Albuginaceae.—The basal position of the Albuginaceae in the trees was unexpected as they have consistently been considered to be close relatives of the Peronosporaceae within the Peronosporales (see e.g., Hawksworth et al 1995, Dick 2001a). However, the position of *Albugo* as the most basal taxon of the Peronosporomycetidae is consistent with previous molecular phylogenetic analyses of Cooke et al (2000) and Petersen and Rosendahl (2000). In addition, according to Shaw (1978), the asexual state of *Albugo* is unique for the order and should have segregated from a presumed *Pythium-Phytophthora* line of development relatively early. A basal position is also supported by the phylogenetic position of host families that are reported to be ancient lineages, e.g., the Piperales (Dick 2001b), and by oosporogenesis as, according to Gäumann (1964: 73–75), some species of *Albugo* show primitive traits. On the other hand, the highly derived sporangiophore morphology, the obligate parasitism on aerial parts of dicots and the adaptation to dry habitats indicate that the *Albugo* clade is highly evolved. As many morphological features are

either autapomorphies for *Albugo* (e.g., percurrent conidiosporangiophore) or synapomorphies for the whole Peronosporomycetidae clade (e.g., a single oospore per oogonium, thin hyphal diameter), morphology neither supports nor falsifies a basal position of *Albugo* within the Peronosporomycetidae, and further thorough molecular and ultrastructural investigations are needed to confirm its phylogenetic position.

Despite the obviously high DNA substitution rate within the genus (FIG. 1), sequences of *Albugo candida* and *Al. tragopogonis*, respectively, from different host genera showed little variation. Contrary to this, the species on Amaranthaceae (*Al. achyranthis* and *Al. bliti*), although related, exhibit a high sequence divergence and do not form a monophyletic group (FIGS. 1, 2).

Taxonomic implications of the current study.—The present study confirms the results of recent studies (Cooke et al 2000) that the current classification of the Peronosporomycetidae (Hawksworth et al 1995, Dick 2001a) is in part unsatisfactory, since it contains polyphyletic groups. In addition, further polyphyletic assemblages (e.g., Sclerosporales, *Bremiella*) have been detected. However, it would be premature to make major rearrangements as data both from other regions of the genome and from more taxa should be collected first. For suprageneric classification, we suggest that a slightly modified classification of Waterhouse (1973) should be used meanwhile. Within Peronosporomycetidae, three families (which may be elevated to orders) are accepted; these are identical in circumscription to Waterhouse (1973) except for the following changes: *Lagenidium* is placed within the Pythiaceae; *Phytophthora* and *Peronophythora* are transferred from the Pythiaceae to the Peronosporaceae.

Peronophythora litchii should be transferred to *Phytophthora*, and the species of the genus *Bremiella* to *Plasmopara*. No formal nomenclatural changes have to be proposed as the respective binomials have previously been published.

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