Taxonomic aspects of Peronosporaceae inferred from Bayesian molecular phylogenetics

M. Göker, H. Voglmayr, A. Riethmüller, M. Weiß, and F. Oberwinkler

Abstract: We present the results of a Bayesian phylogenetic analysis of parts of the nuclear 28S rDNA of a representative sample of the Peronosporales. *Peronospora* s.l. is shown to be paraphyletic. Based on molecular and morphological evidence, several species of the genus *Peronospora* are transferred to *Hyaloperonospora*. *Plasmopara oplismeni* appears to be related only distantly to the other *Plasmopara* species, and is transferred to the new genus *Viennotia* based on molecular, morphological, and ecological evidence. The remaining *Plasmopara* species are likely to be paraphyletic with respect to *Bremia*, *Paraperonospora*, and *Basidiophora*. *Phytophthora* is shown to be paraphyletic with respect to the obligatory biotrophic genera. Evidence for the assumption that obligatory biotrophism arose independently at least twice in Peronosporales is demonstrated.

Key words: LSU rDNA, Straminipila, Peronosporomycetes, Peronosporales, downy mildews, Bayesian phylogenetic analysis.

Résumé : Les auteurs présentent les résultats d'une analyse phylogénétique bayésienne effectuée sur une partie du rADN 28S nucléaire, provenant d'un échantillonnage représentatif des Péronosporales. On montre que le *Peronospora* s.l. est paraphylétique. En se basant sur la preuve moléculaire et morphologique, on transfère plusieurs espèces du genre *Peronospora* au genre *Hyaloperonospora*. Le *Plasmopara oplismeni* ne semble être relié que de façon éloignée avec les autres espèces de *Plasmopara*, et est transféré au nouveau genre *Viennotia*, en se basant sur la preuve moléculaire, morphologique et écologique. Les autres espèces de *Plasmopara* semblent être paraphylétiques par rapport aux *Bremia*, *Paraperonospora* et *Basidiophora*. On montre que le genre *Phytophthora* est paraphylétique relativement aux genres biotrophes obligatoires. On démontre que l'hypothèse suggérant que le biotrophisme obligatoire soit apparu au moins à deux reprises chez les Péronosporales est bien fondée.

Mots clés : rDNA LSU, Straminipila, Pénonosporomycètes, Péronosporales, mildious, analyse phylogénétique bayésienne.

[Traduit par la Rédaction]

Introduction

Like many other groups of plant parasitic fungi, the downy mildews and their allies (Oomycetes, Peronosporomycetes) present many difficulties for a natural classification. This is due to the fact that, as a rule, taxonomically useful morphological or ecological characters are few, so as to make the identification of synapomorphic states impossible. To cope with these problems, a combination of classical systematics and modern molecular methods is required. Recent publications (Dick et al. 1999; Matsumoto et al. 1999; Riethmüller et al. 1999; Cooke et al. 2000; Förster et al. 2000; Leclerc et al. 2000; Petersen and Rosendahl 2000; Hudspeth et al. 2000) have indeed used DNA sequence information for phylogenetic inference in Oomycetes. These studies dealt mainly with marine or non-obligatory biotrophic genera like members of the Saprolegniales, or *Pythium*, or *Phytophthora*. The first comprehensive molecular study to cover the majority of genera within the Peronosporaceae was Riethmüller et al. (2002). Although some new insights into the evolution of Peronosporaceae were gained and greater certainty about several taxonomic questions achieved, a general lack of resolution in the backbone of the Peronosporales part of the phylogenetic trees made it difficult to clear up evolutionary relationships between *Phytophthora* and the obligatory biotrophic genera. Recently, Constantinescu and Fatehi (2002) divided *Peronospora* into three genera based on the morphology of haustoria, conidia, and conidiosporangiophores. They also presented molecular evidence for their taxonomic changes, but the phylogenetic tree included in their publication concentrated on *Peronospora* and contained few other taxa.

Therefore, the relationships among genera of the Peronosporaceae have remained largely unclear. The goal of the present publication is to improve knowledge of their evolu-

Received 19 November 2002. Published on the NRC Research Press Web site at http://canjbot.nrc.ca on 12 August 2003.

M. Göker,[1] M. Weiß, and F. Oberwinkler. Lehrstuhl für Spezielle Botanik und Mykologie, Botanisches Institut, Universität Tübingen, Auf der Morgenstelle 1, D-72076 Tübingen, Germany.
H. Voglmayr. Institut für Botanik, Universität Wien, Rennweg 14, A-1030 Wien, Austria.
A. Riethmüller. Fachgebiet Ökologie, Fachbereich 18 Naturwissenschaften, Universität Kassel Heinrich-Plett-Straße, 40 D-34132 Kassel, Germany.

[1]Corresponding author (e-mail: markus.goeker@uni-tuebingen.de).

 doi: 10.1139/B03-066

tion. We concentrated on a representative sample of Peronosporaceae and Pythiaceae with *Albugo candida* as outgroup. Species for phylogenetic analyses were chosen such that each of the major clusters recognized by Riethmüller et al. (2002) was represented. Secondly, a larger section of the large ribosomal unit (LSU rDNa) was sequenced, now including not only the variable regions D1 and D2, but also D3, D7, and D8 (Hopple and Vilgalys 1999). The use of more characters instead of more taxa is in accordance with the results of recent simulation studies (Rosenberg and Kumar 2001; Whelan et al. 2001). Thirdly, Bayesian phylogenetic inference (Larget and Simon 1999; Huelsenbeck and Ronquist 2001) was used to cope with the systematic problems mentioned above. This method has already been used successfully by, e.g., Murphy et al. (2001) for mammalian phylogeny, and by Maier et al. (2003), Garnica et al. (2003), and Wubet et al. (2003) for several fungal groups. If compared with maximum parsimony or distance analyses, the new approach seems to yield better resolution, especially of higher order clusters.

Materials and methods

Sample sources and DNA extraction

The organisms included in this study are listed in Table 1. The classification system used, the DNA extraction, PCR, and cycle sequencing procedures have been described in Riethmüller et al. (2002). In the present study, the following additional primers were used: LR3R (5′-GTCTTGAAACACGGACC-3′; Hopple and Vilgalys 1999), LR16–0 (5′-TTGCACGTCAGAATCG-3′), LR7 (5′-TACTACCACCAAGATCT-3′; Hopple and Vilgalys 1999), LR7R (5′-GCAGATCTTGGTGGTAG-3′; Hopple and Vilgalys 1999), LR7R-O (5′-GAAGCTCGTGGCGTGAG-3′), and LR9 (5′-AGAGCACTGGGCAGAAA-3′; Hopple and Vilgalys 1999).

Unfortunately, the primer pair LR7R and LR9 often resulted in amplification of both host and parasite gene and made separation with gel electrophoresis necessary. Using the modified LR7R-O instead of LR7R solved this problem. LR16-O is the corresponding modification of LR16 (Hopple and Vilgalys 1999).

Data analysis

The MEGALIGN module of the Lasergene System (DNASTAR, Inc.) was used to align the segments between NL1 and LR16-O and between LR7R and LR9 separately. Both regions were assembled, checked, and edited with SeAl version 2.0 (Rambaut 1996). The corresponding nexus file was edited in PAUP* version 4b8 (Swofford 2001). The computer program MrBayes (version 3.0b3; Huelsenbeck and Ronquist 2001) was used to perform Metropolis-coupled Markov chain Monte Carlo analyses (Mau et al. 1999; Larget and Simon 1999) based on the general time reversible model including a proportion of invariant sites with gamma-distributed substitution rates of the remaining sites (GTR + I + G; see Swofford et al. 1996). Four incrementally heated simultaneous Markov chains were run over 1 000 000 generations from which every 100th tree was sampled. From these, the first 1000 trees were discarded. MrBayes was used to compute a 50% majority rule consensus of the remaining trees to obtain estimates for the a posteriori probabilities of groups of species. Branch lengths were computed as mean values over the sampled trees. This analysis was repeated five times on Macintosh G4 computers, always starting with random trees and default parameter values to test whether the results were reproducible.

Additionally, the data were first analysed with Modeltest version 3.04 (Posada and Crandall 1998) to find the most appropriate models of DNA substitution, which were then used for heuristic maximum likelihood analysis (five random addition replicates with TBR branch swapping, MULTREES option in effect, STEEPEST option not in effect) with PAUP*, version 4b10 (Swofford 2001). The support for the internal nodes of the trees was calculated with bootstrap analysis (Felsenstein 1985) using 100 replicates. Every bootstrap replicate performed heuristic maximum likelihood analysis with SPR branch swapping and starting trees obtained with neighbor joining (Saitou and Nei 1987) in the BIONJ version of Gascuel (1997).

Justified by the results of Cooke et al. (2000), Petersen and Rosendahl (2000), and Riethmüller et al. (2002), the phylogenetic trees were rooted with the two *Pythium* species included in our sample.

Additional microscopical studies

All organisms listed in Table 1 were thoroughly examined with respect to the morphology of haustoria, conidiosporangia, and conidiosporangiophores. Whole mounts of infected leaf pieces were prepared using the method of McNicol and Williamson (1989). Single conidiosporangiophores and freehand sections of infected plant tissue were mounted in Hoyer's Fluid (Cunningham 1972). A Zeiss Photomikroskop 3 was used for Nomarski contrast micrographs.

Results

Sequence alignment

The final length of the alignment was 1036 bp for the first segment from NL1 to LR16-O containing the D1, D2, and D3 regions, and 856 bp for the second segment between LR7R and LR9 including D7 and D8. After exclusion of alignment regions containing a large amount of gaps (because of differences in sequence length), 1659 bp remained for phylogenetic analysis. The final alignment and the trees obtained are deposited in TreeBase (http://www.treebase.org/) as SN1409.

Main characteristics of the resulting phylogenetic trees

A consensus *Pythium*-rooted tree with mean branch lengths achieved through Bayesian analysis and the denotations of the clusters found are shown in Fig. 1. No significant deviations in other trees were observed among the results of different runs of Bayesian analysis; minor ones are mentioned below.

Using *Pythium undulatum* and *Pythium monospermum* as outgroups, the remaining species representing the Peronosporaceae were highly supported by an a posteriori probability of 100% (Fig. 1). Within the Peronosporaceae, the *Phytophthora arecae* cluster (61% support) separates basally, the cluster containing the remaining groups characterized by a probability value of 91%. The latter divide into a group con-

Table 1. Collection data and GenBank accession number of the taxa studied.

Taxon	Collection data: Isolated from (or host)	Origin or source	GenBank accession No.: D1–D2–D3	GenBank accession No.: D7–D8
Peronosporales, Peronosporaceae				
Basidiophora entospora Roze & Cornu*	*Conyza canadensis* (L.) Cronquist	Austria, Lower Austria, Langenlois; leg. HV (WU)	AY035513	AY273990
Bremia lactucae Regel*	*Cirsium oleraceum* (L.) Scop.	Austria, Upper Austria, St. Willibald; leg. HV (WU)	AY035507	AY273984
Paraperonospora leptosperma (De Bary) O. Const.*	*Tripleurospermum perforatum* (Mérat) M. Laínz	Austria, Upper Austria, St. Willibald; leg. HV (WU)	AY035515	AY273989
Peronophythora litchii Chen ex Ko et al.*	*Litchi sinensis* Sonn. (fruits)	CBS 100.81	AY035531	AY273993
Peronospora aestivalis H. Sydow in Gäum.	*Medicago sativa* L.	Austria, Lower Austria, Pfaffstätten; leg. HV (WU)	AY035482	AY273948
Peronospora alpicola Gäum.	*Ranunculus aconitifolius* L.	Germany, Baden-Württemberg, Titisee; leg. MG (TUB)	AY271990	AY273953
Peronospora alta Fuckel	*Plantago major* L.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035493	AY273962
Peronospora aparines (De Bary) Gäum.	*Galium aparine* L.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035484	AY273955
Peronospora aquatica Gäum.	*Veronica anagallis-aquatica* L.	Germany, Bavaria, Birkenried near Günzburg; leg. MG (TUB)	AY271991	AY273956
Peronospora arvensis Gäum.	*Veronica hederifolia* L.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035491	AY273957
Peronospora boni-henrici Gäum.	*Chenopodium bonus-henricus* L.	Germany, Bavaria, Oberjoch; leg. MP (TUB)	AY035475	AY273952
Peronospora brassicae Gäum.	*Sinapis alba* L.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035503	AY273974
Peronospora calotheca De Bary	*Galium odoratum* (L.) Scop.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035483	AY273960
Peronospora conglomerata Fuckel	*Geranium pyrenaicum* L.	Germany, Baden-Württemberg, Heidelberg; leg. MG (TUB)	AY271993	AY273961
Peronospora dentariae Rabenh.(1)	*Cardamine hirsuta* L.	Germany, Nordrhein-Westfalen, Wuppertal; leg. MG (TUB)	AY035505	AY273975
Peronospora dentariae Rabenh.(2)	*Cardamine impatiens* L.	Germany, Baden-Württemberg, Bebenhausen; leg. MG (TUB)	AY272000	AY273976
Peronospora erophilae Gäum.	*Erophila verna* (L.) Chev.	Germany, Baden Württemberg, Criesbach; leg. MG (TUB)	AY271998	AY273972
Peronospora hiemalis Gäum.	*Ranunculus acris* L.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY271992	AY273958
Peronospora lamii A. Braun	*Lamium pupureum* L.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035494	AY273968
Peronospora lunariae Gäum.	*Lunaria rediviva* L.	Germany, Bavaria, Munich; leg. MG (TUB)	AY271997	AY273970
Peronospora niessleana Berlese	*Alliaria petiolata* (M. Bieb.) Cavara & Grande	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035498	AY273971
Peronospora parasitica (Pers.:Fr.) Fr.	*Capsella bursa-pastoris* (L.) Medik.	Germany, Baden-Württemberg, Criesbach; leg. MG (TUB)	AY271996	AY273969
Peronospora potentillae-sterilis Gäum.	*Potentilla sterilis* (L.) Garcke	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035486	AY273967
Peronospora pulveracea Fuckel	*Helleborus niger* L.	Austria, Styria, Mariazell; leg. WM (TUB)	AY035470	AY273959
Peronospora rumicis Corda*	*Rumex acetosa* L.	Austria, Upper Austria, Kopfing; leg. HV (WU)	AY035476	AY273951
Peronospora sanguisorbae Gäum.	*Sanguisorba minor* Scop.	Austria, Tyrol, Schattwald; leg. MG (TUB)	AY035487	AY273954
Peronospora sordida Berk. & Br.	*Scrophularia nodosa* L.	Germany, Baden-Württemberg, Heidelberg; leg. MG (TUB)	AY271995	AY273964
Peronospora thlaspeos-perfoliati Gäum.	*Thlaspi perfoliatum* L.	Germany, Baden-Württemberg, Niedernhall; leg. MG (TUB)	AY271999	AY273973

Table 1 (*concluded*).

Taxon	Collection data: Isolated from (or host)	Origin or source	GenBank accession No.: D1–D2–D3	GenBank accession No.: D7–D8
Peronospora trifolii-alpestris Gäum.	*Trifolium alpestre* L.	France, Le Bout du Monde; leg. MG (TUB)	AY271989	AY273946
Peronospora trifolii-repentis Sydow	*Trifolium repens* L.	Austria, Tyrol, Tannheim; leg. AR (TUB)	AY271988	AY273945
Peronospora trifoliorum De Bary	*Trifolium medium* L.	France, Mont Blanc; leg MG (TUB)	AY035478	AY273947
Peronospora trivialis Gäum.	*Cerastium fontanum* Baumg.	Germany, Baden-Württemberg, Niedernhall; leg. MG (TUB)	AY035471	AY273950
Peronospora variabilis Gäum.	*Chenopodium album* L.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035477	AY273949
Peronospora verna Gäum.	*Veronica arvensis* L.	Germany, Baden Württemberg, Niedernhall; leg. MG (TUB).	AY271994	AY273963
Phytophthora arecae (Coleman) Pethybridge	*Cocos nucifera* L.	IMI 348342	AY035530	AY273992
Phytophthora infestans (Montagne) De Bary*	*Solanum tuberosum* L.	CBS 560.95	AF119602	AY273991
Plasmopara baudysii Scalicky	*Berula erecta* (Huds.) Coville	Austria, Lower Austria, Gramatneusiedl; leg. HV (WU)	AY035517	AY273985
Plasmopara densa (Rab.) Schroet.	*Rhinanthus alectorolophus* (Scop.) Poll.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035525	AY273983
Plasmopara megasperma (Berlese) Berlese	*Viola rafinesquii* Greene	U.S.A., Tennessee, Knoxville; leg. HV (WU)	AY035516	AY273981
Plasmopara obducens (Schroet.) Schroet.	*Impatiens capensis* Meerb.	U.S.A., Tennessee, Knoxville; leg. HV (WU)	AY035522	AY273980
Plasmopara oplismeni Viennot-Bourgin	*Oplismenus hirtellus* (L.) Beauv.	Africa, Guinea, Kindia; leg. JK (GZU)	AY035527	AY273977
Plasmopara pimpinellae O. Savul.	*Pimpinella major* (L.) Huds.	Austria, Tyrol, Obertilliach; leg. HV (WU)	AY035519	AY273988
Plasmopara pusilla (De Bary) Schroet.	*Geranium pratense* L.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035521	AY273979
Plasmopara pygmaea (Ung.) Schroet.*	*Anemone ranunculoides* L.	Germany, Baden-Württemberg, Tübingen-Bebenhausen; leg. AR (TUB)	AF119605	AY273986
Plasmopara umbelliferarum (Casp.) Schroet.	*Aegopodium podagraria* L.	Germany, Baden-Württemberg, Tübingen-Bebenhausen; leg. AR (TUB)	AF119604[†]	AY273982
Plasmopara viticola (Berk. & M. A. Curtis) Berlese & De Toni	*Vitis vinifera* L.	Germany, Baden-Württemberg, Tübingen; leg. MG (TUB)	AY035524	AY273978
Pseudoperonospora humuli (Miyabe & Takah.) G. W. Wilson	*Humulus lupulus* L.	Austria, Lower Austria, Langenlois; leg. HV (WU)	AY035496	AY273965
Pseudoperonospora urticae (Libert ex Berk.) E. Salmon & Ware	*Urtica dioica* L.	Austria, Upper Austria, St. Willibald; leg. HV (WU)	AY035497	AY273966
Sclerospora graminicola (Sacc.) Schroet.*	*Setaria viridis* (L.) P. Beauv.	Austria, Lower Austria, Theresienfeld; leg. HV (WU)	AY035514	AY273987
Pythiales				
Pythium monospermum Pringsheim*		Culture collection Reading, U.K., strain no. 4114a	AY035535	AY273995
Pythium undulatum Petersen		Germany, Baden-Württemberg, Blinder See; leg. AR (TUB)	AF119603[‡]	AY273994

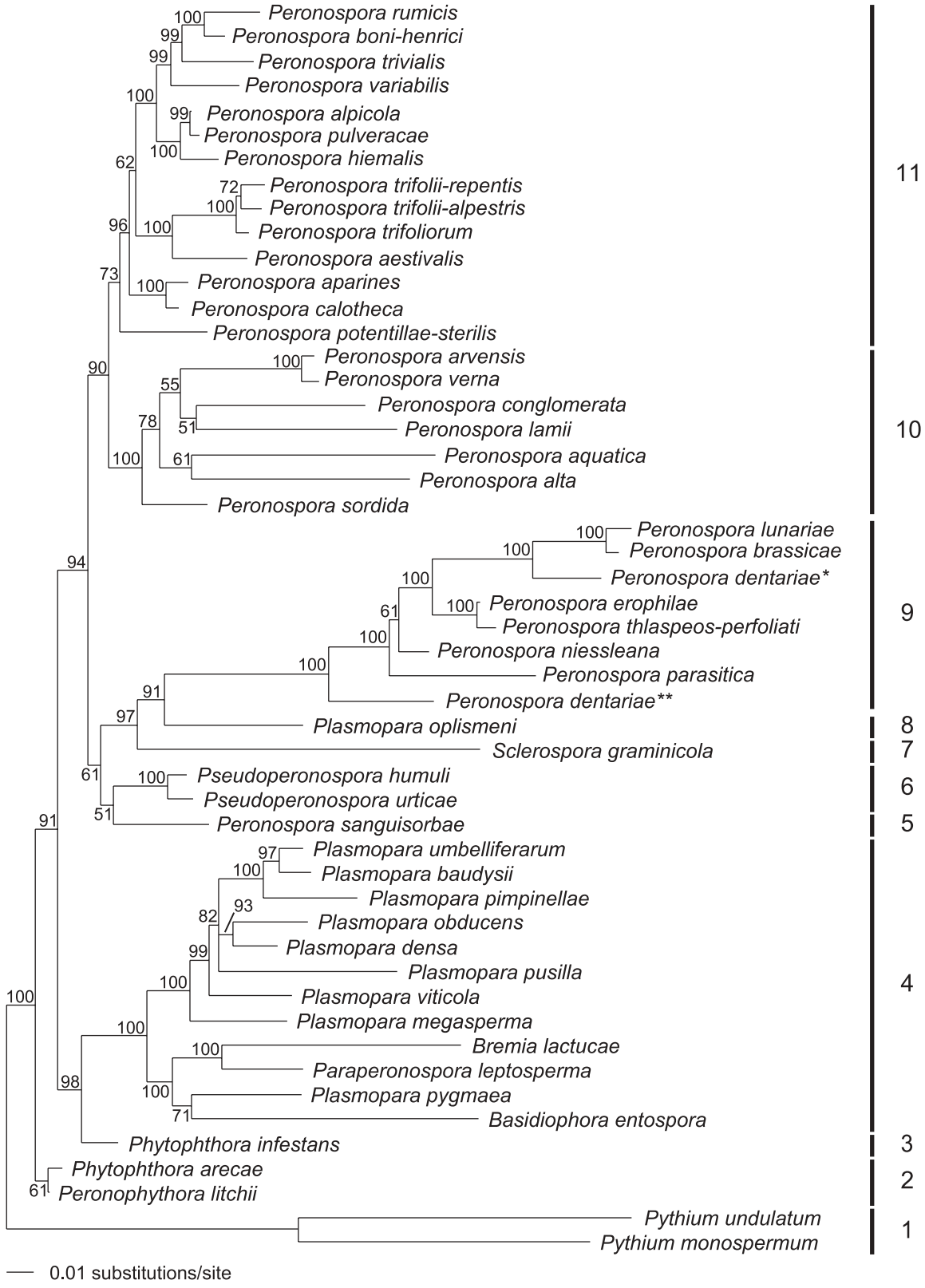
Note: The taxa were grouped taxonomically; the classification follows Hawksworth et al. (1995) and Dick (2001), respectively, including some changes proposed in Riethmüller et al. (2002). Collectors: AR, A. Riethmüller; HV, H. Voglmayr; JK, J. Kranz; MG, M. Göker; MP, M. Piepenbring; WM, W. Maier. Vouchers: TUB, University of Tübingen; WU, University of Vienna; GZU, University of Graz. Sources: CBS, Centraalbureau voor Schimmelcultures, AG Baarn, Netherlands; IMI, CABI Bioscience, Egham, Surrey, U.K.

*Type species.

[†]Published in GenBank as *Plasmopara aegopodii*.

[‡]Published in GenBank as *Phytophthora undulata*.

Fig. 1. 50% majority rule consensus tree with mean branch lengths from a representative Bayesian analysis of nuclear LSU rDNA data. Numbers on branches represent their respective a posteriori probabilities. The following clusters or isolated species were recognized: 1, *Pythium* cluster; 2, *Phytophthora arecae* cluster; 3, *Phytophthora infestans*; 4, *Plasmopara* cluster; 5, *Peronospora sanguisorbae*; 6, *Pseudoperonospora* cluster; 7, *Sclerospora graminicola*; 8, *Plasmopara oplismeni*; 9, *Hyaloperonospora* cluster; 10, *Peronospora lamii* cluster; 11, *Peronospora rumicis* cluster. **Peronospora dentariae* on *Cardamine hirsuta*. ***Peronospora dentariae* on *Cardamine impatiens*.



taining the *Plasmopara* cluster together with *Phytophthora infestans*, which is highly supported by a probability of 98% and indicates that the genus *Phytophthora* is paraphyletic. The *Plasmopara* cluster itself, containing members of the genera *Plasmopara* (except for *Plasmopara oplismeni*), *Bremia*, *Paraperonospora*, and *Basidiophora*, is also highly supported (100%). Within this cluster, *Plasmopara* is clearly paraphyletic because of the position of *Plasmopara pygmaea*.

Another group (supported by 87%) harbours *Plasmopara oplismeni*, *Sclerospora graminicola,* and all members of the genera *Peronospora* s.l., and *Pseudoperonospora* included in the present analysis. The cluster containing all *Peronospora* species that belong to *Hyaloperonospora*, according to Constantinescu and Fatehi (2002), is the sister group of *Plasmopara oplismeni* (91% support), indicating that both *Plasmopara* and *Peronospora* s.l. are not monophyletic. A closer relationship of *Sclerospora graminicola*, *Plasmopara oplismeni* and the *Hyaloperonospora* cluster is supported by 97%. These taxa appear as sister groups of a cluster containing *Peronospora sanguisorbae* and *Pseudoperonospora*. The latter relationships were only weakly supported (61% and 51%, respectively) and not present in all Bayesian analyses. However, the close relationship of the two *Pseudoperonospora* species included in the analyses is indicated by 100% support. The remaining *Peronospora* species are distributed over the moderately supported (73%) *Peronospora rumicis* cluster and the highly supported *Peronospora lamii* cluster (100%); the close relationship of these two groups is supported by 90%.

The application of Modeltest version 3.04 proposed the model TrN + I + G or GTR + I + G (see Swofford et al. 1996 for a survey of these DNA substitution models) using likelihood ratio tests or the Akaike information criterion, respectively. The maximum likelihood tree based on GTR + I + G with bootstrap values is shown in Fig. 2. Topologically identical with the results of the Bayesian analysis, it was generally characterized by a lack of bootstrap resolution in the backbone. However, the branches with significant bootstrap support were in agreement with the groups supported by high a posteriori probability in the Bayesian analysis. The *Hyaloperonospora*, *Plasmopara*, and *Pseudoperonospora* clusters were highly to moderately supported (100, 90, or 82% bootstrap values, respectively).

Morphology of haustoria in *Plasmopara* and conidiosporangiophores in *Plasmopara oplismeni*

With the exception of *Plasmopara oplismeni*, all *Plasmopara* species examined showed small to medium-sized, elliptic to pyriform haustoria. This is illustrated for the type species, *Plasmopara pygmaea*, in Fig. 7. The same shape of haustoria was found in *Bremia*, *Paraperonospora*, and *Basidiophora*. Our results confirm the results of the comprehensive treatise of haustoria in Peronosporales by Fraymouth (1956). On the other hand, haustoria in *Plasmopara oplismeni* were hyphoid, long and slender, and tightly coiled (Figs. 5, 6, 8). Conidiosporangiophores of *Plasmopara oplismeni* were monopodially branched (Fig. 3) and showed typical swellings on the terminal branches, which were straight to only slightly bent (Fig. 4). This is in accordance with the descriptions given by Viennot-Bourgin (1959) and Kenneth and Krantz (1973).

Discussion

Delimitation of Peronosporaceae

In contrast to most of the recent classifications of Peronosporomycetidae (e.g., Dick 1999; Dick 2001), the molecular data of Cooke et al. (2000), Petersen and Rosendahl (2000), and Riethmüller et al. (2002) showed that *Pythium* should be regarded as the sister group of a cluster comprising *Phytophthora* and the former Peronosporaceae. The transfer of *Phytophthora* to this family (Riethmüller et al. 2002) is clearly supported by the present study. *Sclerospora*, formerly ascribed to Saprolegniomycetidae (Dick 2001), had been demonstrated to be also a member of Peronosporaceae. The present study confirms this view, since *Sclerospora* is nested within Peronosporaceae sensu Riethmüller et al. (2002) with high support.

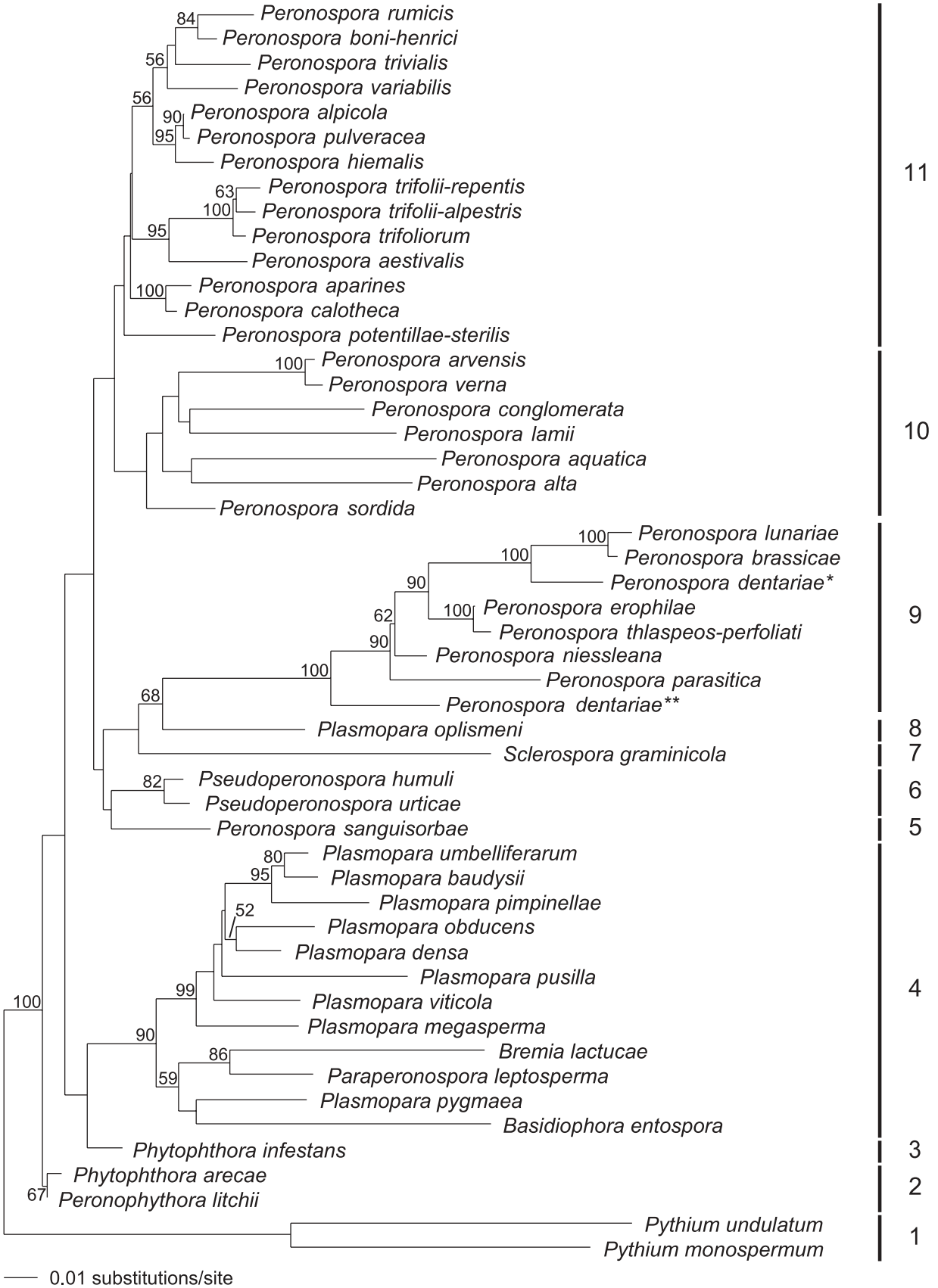
Phytophthora

Another phylogenetic result of Riethmüller et al. (2002) was the close relationship of *Peronophythora litchii* and *Phytophthora arecae*. This led to the suggestion that the former genus should be dismissed, and *Peronophythora litchii* transferred to the latter, a conclusion that could be confirmed by the present study. According to our results *Phytophthora* is paraphyletic as indicated by Cooke et al. (2000). This could have been expected on the basis of a consequently Hennigian approach (Hennig and Hennig 1982; Hennig 1965) to classical Oomycete phylogeny, since the genus *Phytophthora* seems to be defined mostly, or even completely, by plesiomorphic character states when compared with the other taxa of Peronosporaceae. For example, the ability of *Phytophthora* species to grow on synthetic media (Erwin and Ribeiro 1996) is usually regarded as a primitive trait. Furthermore, the present study reveals that the *Plasmopara* cluster is more closely related to *Phytophthora infestans* than to the other biotrophic genera *Peronospora*, *Sclerospora*, *Pseudoperonospora*, and *Plasmopara oplismeni*. If we assume that the saprotrophic type is in all cases plesiomorphic, it follows that the obligate plant parasitism within Peronosporaceae arose at least twice independently. For determining the taxonomic consequences, future molecular research in Peronosporaceae should include a sufficiently large sample of both *Phytophthora* and the biotrophic genera.

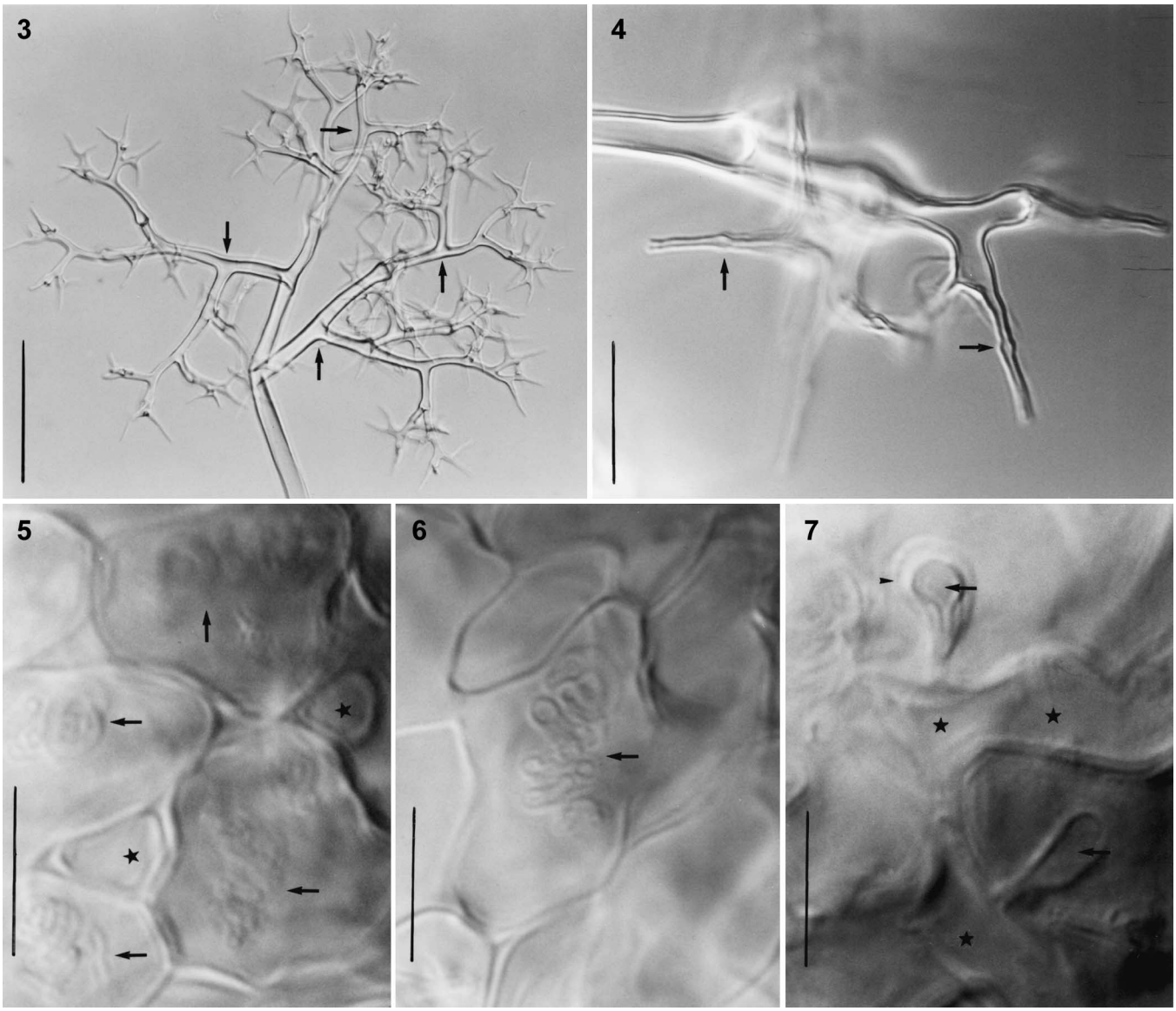
Plasmopara cluster

Riethmüller et al. (2002) proposed a closer relationship of *Plasmopara* (except for *Plasmopara oplismeni*), *Paraperonospora*, *Basidiophora*, *Sclerospora*, and *Bremia*, although without bootstrap support. With the exception of *Sclerospora*, a morphological interpretation could be based on the similar type of haustoria developed in these genera. The present results are even more in accordance with morphology, since *Sclerospora graminicola* is not included in the *Plasmopara* cluster, an assemblage supported by an a posteriori probability of 100%. Therefore, the typical ellipsoid or pyriform haustoria (Fraymouth 1956; personal observations; Fig 7) may be regarded as an autapomorphy of this group,

Fig. 2. Tree topology found by heuristic maximum likelihood analysis. Numbers on branches represent bootstrap values from 100 replicates. For denotations of the clusters see legend to Fig. 1.



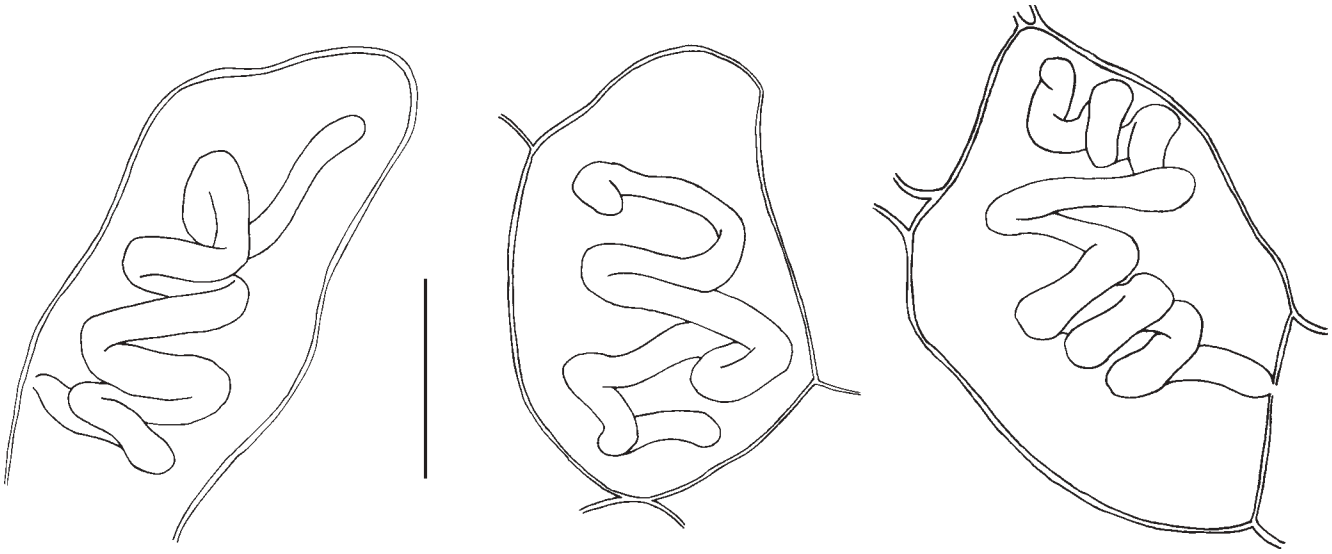
Figs. 3–7. Morphology of *Plasmopara oplismeni*. Nomarski contrast. Fig. 3. Apical part of a conidiosporangiophore of *Plasmopara oplismeni*. Monopodial branching is obvious (arrows). Scale bar = 100 µm. Fig. 4. Straight to slightly curved terminal branches of a conidiosporangiophore of *Plasmopara oplismeni* with typical swellings (arrows). Scale bar = 20 µm. Figs. 5–6. Hyphoid, coiled haustoria (arrows), and intercellular hyphae (stars) of *Plasmopara oplismeni* in the leaf tissue of *Oplismenus hirtellus*. Scale bars = 10 µm. Fig. 7. Ellipsoid to pyriform haustoria (arrows) of *Plasmopara pygmaea* in leaf tissue of *Anemone ranunculoides*. Intercellular hyphae (stars) and a haustorial sheath (arrowhead) surrounding a probably senescent haustorium are also visible. Scale bar = 10 µm.



derived from the slender, hyphoid haustoria found in *Phytophthora infestans* (Erwin and Ribeiro 1996) and some other *Phytophthora* species. This change in haustorial morphology seems to have accompanied the shift to obligatory biotrophism in the *Plasmopara* cluster.

Riethmüller et al. (2002) also suggested eliminating the genus *Bremiella* and transferring *Bremiella baudysii* and *Bremiella megasperma* to *Plasmopara*, a change in nomenclature already followed in the present publication. The Bayesian analyses distinctly show that *Plasmopara baudysii* is not at all closely related to *Plasmopara megasperma*, but belongs to a cluster comprised of solely Apiaceae-infecting *Plasmopara* species that is supported by an a posteriori probability of 100%. Other parallelisms of host and parasite phylogeny are rarely seen in the tree topology of the *Plasmopara* species belonging to the *Plasmopara* cluster. *Plasmopara* (even if *Plasmopara oplismeni* is ignored) appears to be paraphyletic, as *Plasmopara pygmaea*, the type species, clusters together with the genera *Bremia*, *Paraperonospora* and *Basidiophora*. The latter share hosts belonging to the Asteraceae, but represent rather different shapes of conidiosporangiophores. However, we do not believe this represents a real contradiction between morphological and molecular data, since it seems possible to regard

Fig. 8. Line drawings of haustoria of *Plasmopara oplismeni* in leaf cells of *Oplismenus hirtellus*. Scale bar = 5 μm.



the conidiosporangiophore type found in *Plasmopara* as plesiomorphic with respect to the types developed in *Bremia*, *Paraperonospora*, and *Basidiophora*.

Based on the present study, it would be premature to divide *Plasmopara* (except for *Plasmopara oplismeni*) into two genera. Additional microscopical studies and sequencing of more species is required to investigate the interrelationships of *Bremia*, *Paraperonospora*, and *Basidiophora*.

Plasmopara oplismeni and *Sclerospora*

Like the other *Plasmopara* species (Skalicky 1966), *Plasmopara oplismeni* develops monopodially branched conidiosporangiophores (Fig. 3). It deviates from the other members of the genus by its graminaceous host and the shape of the terminal branches of the conidiosporangiophores (Viennot-Bourgin 1959, Fig. 4). A closer relationship between *Plasmopara oplismeni* and the Brassicaceae-infecting *Peronospora* species, now included in *Hyaloperonospora* (Constantinescu and Fatehi 2002), is consistent with the phylogenetic trees in Riethmüller et al. (2002), but no significant bootstrap support for this was present. The present analysis reveals *Plasmopara oplismeni* as the sister group of the *Hyaloperonospora* cluster with a probability value of 91% and thus shows that *Plasmopara* as traditionally circumscribed is polyphyletic.

In his original description of *Plasmopara oplismeni*, Viennot-Bourgin (1959) did not make any statements about the shape of the haustoria. Our examinations revealed that this species develops hyphoid haustoria that are often tightly coiled (Figs. 5, 6, 8). This kind of haustorial morphology resembles that found in *Sclerospora*, *Peronospora* s. str., and *Pseudoperonospora*; all of these genera share slender, hypha-like haustoria with an irregular outline (Fraymouth 1956; Skalicky 1966, own observations). The same morphology is also found in some species of *Phytophthora* (Erwin and Ribeiro 1996), and may represent a plesiomorphic character state. The probably apomorphic shape of haustoria found in the whole *Plasmopara* cluster, including the type species, *Plasmopara pygmaea* (see above; Fig 7), is absent in *Plasmopara oplismeni*. Thus, the molecular result that *Plasmopara* is polyphyletic is confirmed by morphology, indicating that *Plasmopara oplismeni* should be transferred to a new genus. In addition, *Sclerospora*, its probable closest relative *Peronosclerospora* (Dick 2001), and *Plasmopara oplismeni* have graminaceous hosts in common. The new molecular results indicate that this ecological feature may have had evolutionary significance with respect to these taxa and should be considered in their taxonomy.

Further morphological evidence for a closer relationship of *Sclerospora*, *Peronospora* s. str., *Pseudoperonospora*, the *Hyaloperonospora* cluster, and *Plasmopara oplismeni* as indicated by the molecular results is hard to find. These taxa show quite diverse types of conidiosporangiophore morphology.

Hyaloperonospora cluster

The results presented here support the suggestion of Constantinescu and Fatehi (2002) to divide the former *Peronospora* into *Peronospora* s. str., and *Hyaloperonospora*. As mentioned above, our analyses reveal that the cluster containing the species of this new genus is the sister group of *Plasmopara oplismeni*. Most of these species (*Peronospora brassicae*, *Peronospora dentariae*, *Peronospora erophilae*, and *Peronospora thlaspeos-perfoliati*, also *Peronospora camelinae*, which was contained in cluster 5 in Riethmüller et al. 2002) were not mentioned in Constantinescu and Fatehi (2002), but show the generic characteristics of *Hyaloperonospora*, such as mainly globose to lobate haustoria, colourless conidial walls, and conidiosporangiophores with slightly curved to recurved ultimate branchlets (own observations). Furthermore, these taxa are well characterized ecologically by their parasitism of Brassicaceae, a family that apparently is not infected by any member of *Peronospora* s. str. (Constantinescu and Fatehi 2002). Our molecular data do not disprove the opinion that *Peronospora* s. str. is paraphyletic with respect to *Pseudoperonospora*, *Plasmopara oplismeni*, *Hyaloperonospora*, or *Sclerospora*. The tree topology within the *Hyaloperonospora* cluster resembles that presented by Riethmüller et al. (2002; cluster 5), but the Bayesian approach, together with the lon-

ger alignment, results in a better resolution for the branches of higher order. The *Peronospora dentariae* specimen from *Cardamine impatiens* is again shown not to be closely related to the sample from *Cardamine hirsuta*. The cluster containing *Peronospora erophilae* and *Peronospora thlaspeos- perfoliati* may be an example of an ecological rather than taxonomic influence of the host plant, since the annual *Erophila verna* and *Thlaspi perfoliatum* both appear early in spring (at least in Central Europe).

The comparatively large genetic distances within this cluster are in contrast to the opinion of Yerkes and Shaw (1959) that all these species should be merged with *Peronospora parasitica*. Likewise, it would not be appropriate to include them in the broad concept of *Hyaloperonospora parasitica* applied by Constantinescu and Fatehi (2002). Instead, new combinations are necessary, which are listed below.

Remaining *Peronospora* species and *Pseudoperonospora*

With the exception of *Peronospora conglomerata*, a parasite of *Geranium* species, the *Peronospora lamii* cluster contains species infecting Lamiales (sensu Angiosperm Phylogeny Group 1998). The relatively large branch lengths within the *Peronospora lamii* cluster may indicate an early radiation and a long period of independent evolution of the included species, even in cases where they share the same host genus, like *Peronospora aquatica* on the one hand and *Peronospora arvensis* and *Peronospora verna* on the other hand, all infecting members of the genus *Veronica*.

The *Peronospora rumicis* cluster, named after the type species of *Peronospora*, contains species that each infect different host families. Compared with our earlier results (Riethmüller et al. 2002; cluster 10) the present analysis better resolves some higher-level relationships between detected clusters. This is especially important for the relationships between the different clusters characterized by host taxonomy that could not be resolved by the analyses in Riethmüller et al. (2002). For instance, the group of those *Peronospora* species that infect Caryophyllales or Polygonaceae (*Peronospora variabilis*, *Peronospora trivialis*, *Peronospora. rumicis*, and *Peronospora boni-henrici*) and the cluster consisting of parasites of Ranunculaceae (*Peronospora alpicola, Peronospora pulveracea*, and *Peronospora hiemalis*), both strongly supported, cluster together with an a posteriori probability of 100%. Other ecologically interpretable groups are the cluster of Fabacean parasites (*Peronospora aestivalis*, *Peronospora trifoliorum*, *Peronospora trifolii-alpestris*, *Peronospora trifolii-repentis*, the latter three species infecting *Trifolium* species) and the cluster containing the two *Peronospora* species found on *Galium* (*Peronospora aparines* and *Peronospora calotheca*) included in this analysis. Within the *Peronospora rumicis* cluster, *Peronospora potentillae-sterilis* takes the basal position. For a discussion of the fact that infrageneric taxonomy of *Peronospora* based on oospore characters is not in accordance with molecular phylogeny, see Riethmüller et al. (2002).

Peronospora sanguisorbae is characterized by an isolated position in our phylogenetic tree. Like *Peronospora potentillae-sterilis,* it infects Rosaceae. As Riethmüller et al. (2002) have shown, *Peronospora sanguisorbae* is closely related to *Peronospora sparsa*, another parasite of Rosaceae that was formerly assigned to the genus *Pseudoperonospora* by Jaczewski (Skalicky 1966). Indeed, Skalicky (1966) dismissed the genus *Pseudoperonospora*, partly because some *Peronospora* species, *e.g.*, *Peronospora sparsa,* show a *Pseudoperonospora*-like germination behaviour of conidiosporangia. However, molecular data support the view of Waterhouse and Brothers (1981) and Constantinescu (2000) who maintained the genus *Pseudoperonospora*. The members of *Pseudoperonospora* included in both the analyses of Riethmüller et al. (2002) and the present study appeared as a well supported monophyletic group.

Nevertheless, a close relationship of *Pseudoperonospora*, *Peronospora sanguisorbae*, and the *Peronospora* species contained in the *Peronospora rumicis* and the *Peronospora lamii* clusters seems plausible because of the fact that these species are characterized by brownish to brown coloured conidiosporangial walls (Skalicky 1966, own observations). Constantinescu and Fatehi (2002) used this feature to separate the species now included in *Hyaloperonospora* from *Peronospora*. Since brownish or even darker coloured conidiosporangial walls are present in neither *Phytophthora* nor any other members of the Peronosporaceae, and since the obligatory biotrophic species are likely to be derived from *Phytophthora* species (see the discussion above), coloured conidiosporangial walls should be regarded as apomorphic. Therefore, this character is an indicator that *Pseudoperonospora*, *Peronospora sanguisorbae*, and the *Peronospora* species contained in the *Peronospora rumicis* and the *Peronospora lamii* cluster might form a monophyletic group. This hypothesis is contradictory to our molecular results at first glance, but the relevant branches are only poorly supported in both Bayesian and maximum likelihood analysis.

Taxonomic implications of the current study

The following new combinations are proposed:

Hyaloperonospora brassicae (Gäumann) Göker, Voglmayr, Riethmüller, Weiß et Oberwinkler, comb. nov.

BASIONYM: *Peronospora brassicae* Gäumann, Beih. Bot. Zentbl. 35(1) (1918): 521.

Hyaloperonospora camelinae (Gäumann) Göker, Voglmayr, Riethmüller, Weiß et Oberwinkler, comb. nov.

BASIONYM: *Peronospora camelinae* Gäumann, Beih. Bot. Zentbl. 35(1) (1918): 522.

Hyaloperonospora erophilae (Gäumann) Göker, Voglmayr, Riethmüller, Weiß et Oberwinkler, comb. nov.

BASIONYM: *Peronospora erophilae* Gäumann, Beih. Bot. Zentbl. 35(1) (1918): 525.

Hyaloperonospora thlaspeos-perfoliati (Gäumann) Göker, Voglmayr, Riethmüller, Weiß et Oberwinkler, comb. nov.

BASIONYM: *Peronospora thlaspeos-perfoliati* Gäumann, Beih. Bot. Zentbl. 35(1) (1918): 530–531.

Viennotia Göker, Voglmayr, Riethmüller, Weiß, and Oberwinkler, gen. nov.

ETYMOLOGY: Named after George Viennot-Bourgin, the French mycologist who described *Plasmopara oplismeni*.

Fungi Peronosporacearum sensu Riethmüller et al. (2002). Hyphae intercellulares. Haustoria intracellularia, hyphoidea, tenuia, longa, saepe arcte helica. Conidiosporangiophora incolorata, monopodialiter ramosa, ramunculis rectis vel parum curvis. Conidiosporangia parietibus incoloratis. Hospes ad familiam Poacearum pertinent.

TYPUS GENERIS: ***Viennotia oplismeni*** (Viennot-Bourgin) Göker, Voglmayr, Riethmüller, Weiß et Oberwinkler, comb. nov.

BASIONYM: *Plasmopara oplismeni* Viennot-Bourgin, Bull. Soc. Mycol. Fr. 75 (1959): 33–37.

Members of the Peronosporaceae sensu Riethmüller et al. (2002). Hyphae intercellular. Haustoria intracellular, hyphoid, slender, long and often tightly coiled. Conidiosporangiophores colourless, monopodially branched. Ultimate branches straight to slightly curved. Conidiosporangia with colourless walls. Hosts belong to the grass family (Poaceae).

Acknowledgements

The authors are grateful to O. Constantinescu for sending us a preprint of his recent publication, to the curators of the International Mycological Institute (IMI) and the Herbarium of the Institute of Botany, University of Graz (GZU), for the loan of herbarium specimens, and to I. Kottke for help with Nomarski contrast optics. We thank F. Albrecht for technical assistance with photographic work, G. Pasteur for critically reading the manuscript, and R. Bauer and D. Begerow for many helpful comments. The present paper is part of the GLOPP (Global Information System for the Biodiversity of Plant Pathogenic Fungi) project financed by the German Bundesministerium für Bildung und Forschung (BMBF), which is gratefully acknowledged.

References

Angiosperm Phylogeny Group. 1998. An ordinal classification of the families of flowering plants. Ann. Mo. Bot. Gard. **85**: 531–553.

Constantinescu, O. 2000. The fine structure of the sporangium in *Pseudoperonospora humuli* (Chromista, Oomycota, Peronosporales). Cryptogam. Mycol. **21**: 93–101.

Constantinescu, O., and Fatehi, J. 2002. *Peronospora*-like fungi (Chromista, Peronosporales) parasitic on Brassicaceae and related hosts. Nova Hedwigia, **74**: 291–338.

Cooke, D.E.L, Drenth, A., Duncan, J.M., Wagels, G., and Brasier, C.M. 2000. A molecular phylogeny of *Phytophthora* and related Oomycetes. Fungal Genet. Biol. **30**: 17–32.

Cunningham, J.L. 1972. A miracle mounting fluid for permanent whole-mounts of microfungi. Mycologia, **64**: 906–911.

Dick, M.W. 1999. Classification of the Peronosporomycetes. *In* Encyclopaedia of food microbiology. Vol. 2. *Edited by* R. Robinson, C. Batt, and P. Patel. Acacemic Press, London. pp. 871–882.

Dick, M.W. 2001. The Peronosporomycetes. *In* The Mycota. Vol. VIIA. *Edited by* D.J. McLaughlin, E.G. McLaughlin, and P.A. Lemke. Springer, Berlin. pp. 39–72.

Dick, M.W., Vick, M.C., Gibbings, J.G., Hedderson, T.A., and Lopez Lastra, C.C. 1999. 18S rDNA for species of *Leptolegnia* and other Peronosporomycetes: justification of the subclass taxa Saprolegniomycetidae and Peronosporomycetidae and division of the Saprolegniaceae sensu lato into the Leptolegniaceae and Saprolegniaceae. Mycol. Res. **103**: 1119–1125.

Erwin, D.C., and Ribeiro, O.K. 1996. *Phytophthora* diseases worldwide. APS Press, St. Paul, Minn.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, **39**: 783–791.

Förster, H., Cummings, M.P., and Coffey, M.D. 2000. Phylogenetic relationship of *Phytophthora* species based on ribosomal ITS I DNA sequence analysis with emphasis on Waterhouse groups V and VI. Mycol. Res. **104**: 1055–1061.

Fraymouth, J. 1956. Haustoria of the Peronosporales. Trans. Br. Mycol. Soc. **39**: 79–107.

Garnica, S., Weiß, M., Oertel, B., and Oberwinkler, F. 2003. Phylogenetic relationships of European *Phlegmacium* species (*Cortinarius*, Agaricales). Mycologia, In press.

Gascuel, O. 1997. BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. Mol. Biol. Evol. **14**: 685–695.

Gäumann, E. 1918. Über die Formen der *Peronospora parasitica* (Pers.) Fries. Beih. Biol. Zentbl. **35**: 395–533.

Hawksworth, D.L., Kirk, P.M., Sutton, B.C., and Pegler, D.N. 1995. Ainsworth & Bisby's dictionary of the Fungi. 8th ed. CAB International, Wallingford, U.K.

Hennig, W. 1965. Phylogenetic systematics. Annu. Rev. Entomol. **10**: 97–116.

Hennig, W., and Hennig, W. 1982. Phylogenetische Systematik. Parey, Berlin.

Hopple, J.S., and Vilgalys, R. 1999. Phylogenetic relationships in the mushroom genus *Coprinus* and dark-spored allies based on sequence data from the nuclear gene coding for the large ribosomal subunit RNA: divergent domains, outgroups, and monophyly. Mol. Phylogenet. Evol. **13**: 1–19.

Hudspeth, D.S.S., Nadler, S.A., and Hudspeth, M.E.S. 2000. A COX2 molecular phylogeny of the Peronosporomycetes. Mycologia, **92**: 674–684.

Huelsenbeck, J.P., and Ronquist, F.R. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics, **17**: 754–755.

Kenneth, R., and Kranz, J. 1973. *Plasmopara penniseti* sp. nov., a downy mildew of pearl millet in Ethiopia. Trans. Br. Mycol. Soc. **60**: 590–593.

Larget, B., and Simon, D.L. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Mol. Biol. Evol. **16**: 750–759.

Leclerc, M.C., Guillot, J., and Deville, M. 2000. Taxonomic and phylogenetic analysis of Saprolegniaceae (Oomycetes) inferred from LSU rDNA and ITS sequence comparisons. Antonie Leeuwenhoek, **77**: 369–377.

Maier, W., Begerow, D., Weiß, M., and Oberwinkler, F. 2003. Phylogeny of the rust fungi: an approach using nuclear large subunit ribosomal DNA sequences. Can. J. Bot. **81**: 12–23.

Matsumoto, C., Kageyama, K., Suga, H., and Hyakumachi, M. 1999. Phylogenetic relationships of *Pythium* species based on ITS and 5.8S sequences of the ribosomal DNA. Mycoscience, **40**: 321–331.

Mau, B., Newton, M.A., and Larget, B. 1999. Bayesian phylogenetic inference via Markov chain Monte Carlo methods. Biometrics, **55**: 1–12.

McNicol, R.J., and Williamson, B. 1989. Systemic infection of black currant flowers by *Botrytis cinerea* and its possible in-

volvement in premature abscission of fruits. Ann. Appl. Biol. **114**: 243–254.

Murphy, W.J., Eizirik, E., O'Brien, S.J., Madsen, O., Scally, M., Douady, C.J., Teeling, E., Ryder, O.A., Stanhope, M.J., De Jong, W.W., and Springer, M.S. 2001. Resolution of the early placental mammal radiation using Bayesian phylogenetics. Science (Washington, D.C.), **294**: 2348–2356.

Petersen, A.B., and Rosendahl, S. 2000. Phylogeny of the Peronosporomycetes (Oomycota) based on partial sequences of the large ribosomal subunit (LSU rDNA). Mycol. Res. **104**: 1295–1303.

Posada, D., and Crandall, K.A. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics, **14**: 817–818.

Rambaut, A. 1996. Se-Al. Sequence Alignment Editor v2.0. Available from http://evolve.zoo.ox.ac.uk/software/ [updated 22 January 2002; cited 17 October 2002].

Riethmüller, A., Weiß, M., and Oberwinkler, F. 1999. Phylogenetic studies of Saprolegniomycetidae and related groups based on nuclear large subunit DNA sequences. Can. J. Bot. **77**: 1790–1800.

Riethmüller, A., Voglmayr, H., Göker, M., Weiß, M., and Oberwinkler, F. 2002. Phylogenetic relationships of the downy mildews (Peronosporales) and related groups based on nuclear large subunit ribosomal DNA sequences. Mycologia, **94**: 834–849.

Rosenberg, M.S., and Kumar, S. 2001. Incomplete taxon sampling is not a problem for phylogenetic inference. Proc. Nat. Acad. Sci. U.S.A. **98**: 10 751–10 756.

Saitou, N., and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**: 406–425.

Skalicky, V. 1966. Taxonomie der Gattungen der Familie Peronosporaceae. Preslia, **38**: 117–129.

Swofford, D.L. 2001. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4b10. Sinauer Associates, Sunderland, Mass.

Swofford, D.L, Olsen, G.J., Waddell, P.J., and Hillis, D.M. 1996. Phylogenetic inference. *In* Molecular systematics. *Edited by* D.M. Hillis, C. Moritz, and B.K. Mable. Sinauer Associates, Inc., Sunderland, Mass. pp. 407–514.

Viennot-Bourgin, G. 1959. Champignons nouveaux de la Guinée. Bull. Soc. Mycol. Fr. **75**: 33–37.

Waterhouse, G.M., and Brothers, M.P. 1981. The taxonomy of *Pseudoperonospora*. Mycol. Pap. **148**: 1–28.

Whelan, S., Lio, P., and Goldman, N. 2001. Molecular phylogenetics: state-of-the-art methods for looking into the past. Trends Genet. **17**: 262–272.

Wubet, T., Weiß, M., Kottke, I., and Oberwinkler, F. 2003. Morphology and molecular diversity of arbuscular mycorrhizal fungi in wild and cultivated yew (*Taxus baccata* L.). Can. J. Bot. **81**: 255–266.

Yerkes, D.R. Jr., and Shaw, C.G. 1959. Taxonomy of the *Peronospora* species on Cruciferae and Chenopodiaceae. Phytopathology, **49**: 499–507.