

The rise of *Ramularia* from the *Mycosphaerella* labyrinth

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Highlights

- *Ramularia* is the correct name for species in *Mycosphaerella* s. str.
- Most species named in *Mycosphaerella* represent other genera.
- Very few *Ramularia* spp. have confirmed *Mycosphaerella* sexual morphs.
- *Ramularia* contains both host-specific, and wide host range species.

Abstract

In this study we aimed to resolve the *Ramularia endophylla* species-complex by applying a polyphasic approach involving morphology and multi-gene phylogeny. Eleven partial genes were targeted for amplification and sequencing for a total of 81 isolates representing *R. endophylla* s. lat. and 32 isolates representing 11 *Ramularia* species that were previously linked to a *Mycosphaerella* sexual morph in literature. A Bayesian phylogenetic analysis, as well as a parsimony analysis, was performed on a combined five-locus dataset and the resulting trees showed significant support for three species within the complex, including the previously described *R. endophylla* and *R. vizellae*, and one novel species, *Ramularia unterseheri*. A parsimony analysis was also separately performed with mating-type gene sequences (MAT1-1-1 and MAT1-2-1) and the resulting tree topologies were in accordance with that of the multigene analysis. A bibliographic review of the proposed links between *Ramularia* spp. and their purported *Mycosphaerella* sexual morphs is also presented, confirming six connections in *Ramularia*. In spite of more than 10 000 species having been described in *Mycosphaerella*, the majority is shown to belong to other genera, suggesting that the taxa identified as *Mycosphaerella* in much of the plant pathology literature needs to be revisited.

Keywords : Cercosporoid; Cryptic species; Multigene; Nomenclature; Phylogeny

Introduction

Mycosphaerella s. lat. (Johanson 1884) is one of the largest genera of *Ascomycetes* and comprises numerous economically important crop pathogens. Over the years more than 10 000 species were described in this genus mainly based on host association or simply because their fruiting bodies were spherical (Aptroot, 2006 and Crous et al., 2009a; Koike et al. 2011). Although the morphology of the sexual morph is relatively uniform, the genus has been associated with more than 40 asexual genera (Crous et al. 2009a) including both coelomycetes and hyphomycetes. Early molecular work based on ITS DNA sequencing indicated that *Mycosphaerella* was monophyletic, although the subsequent introduction of additional loci and more taxa showed it was polyphyletic (Crous et al., 2009a and Crous et al., 2009c). As a consequence, members of this genus were allocated to different families such as *Schizothyriaceae* (Batzer et al. 2008), *Cladosporiaceae* (Schubert et al., 2007, Dugan et al., 2008, Bensch et al., 2010 and Bensch et al., 2012), *Dissoconiaceae*, *Mycosphaerellaceae* and *Teratosphaeriaceae* (Crous et al., 2009b and Li et al., 2012). From these results it became evident that the mycosphaerella-like morphology had evolved multiple times and a new circumscription of *Mycosphaerella* was urgently required.

The type species *Mycosphaerella punctiformis* was epitypified from freshly collected material and its asexual morph described as *Ramularia endophylla* (Verkley et al. 2004). Phylogenetic analyses based on DNA sequence data of the SSU and ITS regions grouped *Mycosphaerella* species with *Ramularia* asexual morphs in a monophyletic group with high bootstrap support (Verkley et al., 2004 and Crous et al., 2007). This partly led to the proposal by Crous et al. (2009a) that *Mycosphaerella s. str.* should be limited to species with *Ramularia* asexual morphs, and that the remaining mycosphaerella-like species should be allocated to other genera. In order to halt the unnecessary proliferation of generic names, it was proposed at the time that it would be preferable to not continue using the traditional dual nomenclature system, and that a single generic name should be attributed to each unambiguous phylogenetic lineage such as in the case of the *Botriosphaeriaceae* (Crous et al. 2006).

The widespread use of phylogenetic analyses, based on DNA sequence comparisons, has fuelled the idea that dual nomenclature in fungi is superfluous (Taylor 2012). A number of far reaching proposals were accepted at the eighteenth International Botanical Congress in Melbourne, which led to a revised and renamed International Code of Nomenclature for Algae, Fungi, and Plants (ICN), signalling the end of dual nomenclature (Hawksworth et al., 2011 and Wingfield et al., 2012). In pleomorphic fungi priority should be given to the oldest name, regardless of its sexuality. However, for widely used names, particularly where the asexual morph names replace sexual morph names, additional considerations are needed as specified in ICN Art. 57.2. The name *Ramularia* (Unger 1833) is older than *Mycosphaerella* (Johanson 1884) and, while *Mycosphaerella sensu lato* represents numerous genera distributed over different families, *Mycosphaerella sensu strictu* has *Ramularia* asexual morphs. Choosing *Ramularia* over *Mycosphaerella* requires less name changes since most established connections already have species names in *Ramularia*. Therefore, the name *Ramularia* has been selected for this genus and included in a list of protected names (Wijayawardene et al. 2014).

Ramularia includes species that are usually defined as hyphomycetes with hyaline conidiophores and conidia with thickened, darkened, and refractive conidial hila and conidiogenous loci (scars on conidiogenous cells). The structure and colour of conidiogenous

loci were considered important characters to define the genus and distinguish it from closely allied genera (Braun, 1995 and Braun, 1998). Recent molecular studies indicate that these characters were not always phylogenetically informative, and that the generic concept of some asexual genera warranted revision (Verkley et al., 2004 and Kirschner, 2009). For example, *Cercospora* is usually distinguished from *Ramularia* by bulging, hyaline conidiogenous loci. These characters are variable and difficult to distinguish with light microscopy (Kirshner 2009). A DNA phylogeny based on sequences obtained from the large nuclear ribosomal subunit (LSU) places the type species of *Cercospora* (*Cercospora virgaureae*) in a sister clade to *Ramularia*, but *Cercospora centaureicola*, for example, clustered within *Ramularia sensu stricto*. The ultrastructure of conidiogenous loci differed between these genera, with *Ramularia* having a raised rim with a central dome that is cladospore-like, while *Cercospora* has flat scars in the shape of a truncated cone (Kirschner 2009).

The genus *Ramularia* includes around 1000 species that vary in lifestyle from phytopathogenic to saprobic, endophytic and even hyperparasitic. Phytopathogenic species cause leaf spots, necrosis or chlorosis that lead to early defoliation and disease symptoms that usually develop under conditions of high air humidity and low temperatures. Endophytic species usually grow symptomless within the leaves and mature in overwintering leaves on the soil, releasing ascospores in spring that can re-infect young leaves in spring. *R. endophylla* (syn. *M. punctiformis*) is an endophyte often associated with broad-leaved trees, and has a worldwide distribution (Verkley et al. 2004). Recent DNA sequence comparisons based on sequence obtained from the intergenic nuclear ribosomal spacer region (ITS) has shown that a number of *R. endophylla* strains collected from several hosts appear to be heterogeneous, indicating the presence of cryptic species (Verkley et al., 2004 and Minnis et al., 2011). This applies to *Ramularia nyssicola*, which is morphologically indistinguishable from *R. endophylla*, but based on DNA sequence comparisons and host specificity represents a distinct species on *Nyssa* (Minnis et al., 2011 and Videira et al., 2015).

Identification of closely related species based on morphology is often difficult and the ITS barcode of fungi alone (Schoch et al. 2012) is unreliable for species identification among several cercosporoid genera (e.g. Groenewald et al., 2013, Crous et al., 2013 and Bakhshi et al., 2015). Several studies in recent years have highlighted the need to use additional phylogenetic markers to achieve accurate species identification (e.g. Bensch et al., 2012, Damm et al., 2012a, Damm et al., 2012b, Quaedvlieg et al., 2012, Phillips et al., 2013 and Wikee et al., 2013). In general, protein-coding genes have higher species resolution power due to their variable intron sequences. In addition, partial sequences from the mating-type ideomorphs (MAT1-1 and MAT1-2), specifically the alpha box (MAT1-1-1) and the high mobility group (MAT1-2-1), have also been found valuable due to their high interspecific variability and low intraspecific variability (Du et al., 2005 and Paoletti et al., 2005). Species delimitation is challenging and guided by several concepts but no strict rule applies. The use of concordance of multiple gene genealogies has been frequently used in mycology to determine species boundaries. This is known as the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) principle and is an adaptation of the Phylogenetic Species Concept (PSC) (Taylor et al. 2000). With the addition of ecological and morphological data to support the multiple gene phylogenies in a polyphasic approach, mycologists have been increasingly relying on the Consolidated Species Concept (CSC) for fungal species delimitation (Quaedvlieg et al. 2014).

The aims of this study were to: (i) resolve the variation in the *R. endophylla* species complex by applying morphology, ecology and multi-gene phylogeny based on five partial genes and partial mating-type locus DNA sequences; (ii) to investigate all purported links between *Ramularia* and *Mycosphaerella* in literature, and (iii) provide a platform that will enable a revision of this generic complex.

Materials and methods

Isolates

The isolates included in this study were obtained from the culture collection of the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, the Netherlands, from the working collection of Pedro Crous (CPC), housed at CBS-KNAW, or were freshly isolated from a range of different plant hosts (Table 1). Single-conidia and single-ascospore cultures were obtained using the techniques described for species of *Mycosphaerella* and its asexual morphs (Crous et al., 1991 and Crous, 1998). Representative cultures of the new species delineated in this study were deposited in the CBS culture collection.

Table 1. Collection details and GenBank accession numbers of strains included in this study.

| Species | Culture collection accession number(s) ^a | Host/isolation source | Location | Collector | GenBank Accession numbers ^b | | | | | | | | | | |
|----------------------------|---|--|------------------------|---------------|--|-----------|-----------|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| | | | | | LSU | ITS | ACT | TEF 1- α | GAP DH | RPB 2 | HIS3 | CAL | bTU B | MAT 1 | MAT 2 |
| <i>Ramularia aplospora</i> | CBS 109014 | <i>Alchemilla vulgaris</i> , leaf spot | Austria, Ötztal | G. Verkley | KP89 4107 | KP89 4216 | KP89 4322 | KP89 4432 | KP89 4542 | KP89 4653 | KP89 4764 | KP89 4875 | – | KP89 5037 | – |
| | CBS 109120 | <i>Alchemilla vulgaris</i> , leaf spot | Austria, Tirol | G. Verkley | KP89 4108 | KP89 4217 | KP89 4323 | KP89 4433 | KP89 4543 | KP89 4654 | KP89 4765 | KP89 4876 | – | – | KP89 5066 |
| | CBS 114118; UPSC 2679 | <i>Alchemilla vulgaris</i> | Sweden, Uppland | E. Gunnerbeck | KP89 4109 | KP89 4218 | KP89 4324 | KP89 4434 | KP89 4544 | KP89 4655 | KP89 4766 | KP89 4877 | – | – | KP89 5067 |
| | CBS 545.82 | mildew on <i>Alchemilla vulgaris</i> | Germany, Gössweinstein | T. Hijwegen | KP89 4110 | EU40 2328 | KP89 4325 | KP89 4435 | KP89 4545 | KP89 4656 | KP89 4767 | KP89 4878 | KP89 4965 | KP89 5038 | – |
| <i>Ramularia calcea</i> | CBS 101612 | <i>Symphytum</i> sp., leaf spot | Germany, Thüringen | G. Arnold | KP89 4111 | KP89 4219 | KP89 4326 | KP89 4436 | KP89 4546 | KP89 4657 | KP89 4768 | KP89 4879 | KP89 4966 | KP89 5039 | – |
| | CBS 10161 | <i>Symphytum</i> sp., leaf | Germany, Thüring | G. Arnold | KP89 4112 | KP89 4220 | KP89 4327 | KP89 4437 | KP89 4547 | KP89 4658 | KP89 4769 | – | KP89 4967 | KP89 5040 | – |

| Species | Culture collection accession number(s) ^a | Host/isolation source | Location | Collector | GenBank Accession numbers ^b | | | | | | | | | | | |
|-----------------------------|---|------------------------------------|----------------------|------------|--|-----------|-----------|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|--|
| | | | | | LSU | ITS | ACT | TEF 1- α | GAP DH | RPB 2 | HIS3 | CAL | bTU B | MAT 1 | MAT 2 | |
| | 3 | spot | en | d | | | | | | | | | | | | |
| <i>Ramularia endophylla</i> | CBS 101680 | <i>Castanea sativa</i> , dead leaf | Netherlands, Utrecht | A. Aptroot | KP89 4126 | KP89 4233 | KP89 4341 | KP89 4451 | KP89 4561 | KP89 4672 | KP89 4783 | KP89 4887 | KP89 4974 | KP89 5044 | | |
| | CBS 113265 DeepTy | <i>Quercus robur</i> , dead leaf | Netherlands, Utrecht | G. Verkley | AY49 0776 | AY49 0763 | KF90 3461 | KF25 3276 | KP89 4562 | KP89 4673 | KP20 7603 | KF25 3981 | KP89 4975 | KP89 5045 | | |
| | CBS 113868 | <i>Quercus robur</i> , living leaf | Netherlands, Utrecht | G. Verkley | KP89 4127 | KP89 4234 | KP89 4342 | KP89 4452 | KP89 4563 | KP89 4674 | KP89 4784 | KP89 4888 | | KP89 5046 | | |
| | CBS 113869 | <i>Quercus robur</i> , living leaf | Netherlands, Utrecht | G. Verkley | KP89 4128 | KP89 4235 | KP89 4343 | KP89 4453 | KP89 4564 | KP89 4675 | KP89 4785 | KP89 4889 | | KP89 5047 | | |
| | CBS 113870 | <i>Quercus robur</i> , living leaf | Netherlands, Utrecht | G. Verkley | KP89 4129 | KP89 4236 | KP89 4344 | KP89 4454 | KP89 4565 | KP89 4676 | KP89 4786 | KP89 4890 | KP89 4976 | | | |
| | CBS 113871 | <i>Quercus robur</i> , fallen leaf | Netherlands, Utrecht | G. Verkley | KP89 4130 | KP89 4237 | KP89 4345 | KP89 4455 | KP89 4566 | KP89 4677 | KP89 4787 | KP89 4891 | KP89 4977 | KP89 5048 | | |
| | CBS 115299 | <i>Quercus robur</i> , living leaf | Netherlands, Utrecht | G. Verkley | KP89 4131 | KP89 4238 | KP89 4346 | KP89 4456 | KP89 4567 | KP89 4678 | KP89 4788 | KP89 4892 | | KP89 5049 | | |
| | CBS 115302 | <i>Quercus robur</i> , living leaf | Netherlands, Utrecht | | KP89 4132 | KP89 4239 | KP89 4347 | KP89 4457 | KP89 4568 | KP89 4679 | KP89 4789 | KP89 4893 | KP89 4978 | | KP89 5073 | |
| | CBS 115303 | <i>Quercus robur</i> , living leaf | Netherlands, Utrecht | | KP89 4133 | KP89 4240 | KP89 4348 | KP89 4458 | KP89 4569 | KP89 4680 | KP89 4790 | KP89 4894 | | KP89 5050 | | |
| | CBS 115304 | <i>Quercus robur</i> , living leaf | Netherlands, Utrecht | | KP89 4134 | KP89 4241 | KP89 4349 | KP89 4459 | KP89 4570 | KP89 4681 | KP89 4791 | KP89 4895 | | | KP89 5074 | |
| | CBS 115310 | <i>Quercus robur</i> , dead leaf | Netherlands, Utrecht | | KP89 4135 | KP89 4242 | KP89 4350 | KP89 4460 | KP89 4571 | KP89 4682 | KP89 4792 | | KP89 4979 | KP89 5051 | | |
| | CBS 115311 | <i>Quercus robur</i> , | Netherlands, | | KP89 4136 | KP89 4243 | KP89 4351 | KP89 4461 | KP89 4572 | KP89 4683 | KP89 4793 | KP89 4896 | KP89 4980 | KP89 5052 | | |

| Species | Culture collection accession number(s) ^a | Host/isolation source | Location | Collector | GenBank Accession numbers ^b | | | | | | | | | | | |
|------------------------------|---|---|-----------------------|------------------------|--|-----------|-----------|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|--|
| | | | | | LSU | ITS | ACT | TEF 1- α | GAP DH | RPB 2 | HIS3 | CAL | bTUB | MAT 1 | MAT 2 | |
| | 1 | dead leaf | Utrecht | | | | | | | | | | | | | |
| | CBS 117876; CPC 11203 | <i>Quercus robur</i> | Netherlands, Utrecht | G. Verkleey | KP89 4137 | KP89 4244 | KP89 4352 | KP89 4462 | KP89 4573 | KP89 4684 | KP89 4794 | KP89 4897 | KP89 4981 | KP89 5053 | | |
| | CBS 117877; CPC 11204 | <i>Quercus robur</i> | Netherlands, Utrecht | G. Verkleey | KP89 4138 | KP89 4245 | KP89 4353 | KP89 4463 | KP89 4574 | KP89 4685 | KP89 4795 | KP89 4898 | KP89 4982 | | KP89 5075 | |
| | CBS 942.97 | <i>Quercus</i> sp., leaves | Belgium, Namur | A. Aptroot | KP89 4139 | KP89 4246 | KP89 4354 | KP89 4464 | KP89 4575 | EU87 4860 | KP89 4796 | KP89 4899 | KP89 4983 | | | |
| | CPC 11503 | – | South Korea | H.D. Shin | KP89 4140 | KP89 4247 | KP89 4355 | KP89 4465 | KP89 4576 | KP89 4686 | KP89 4797 | KP89 4900 | KP89 4984 | KP89 5054 | | |
| <i>Ramularia grevilleana</i> | CBS 114732; UPSC 3244 | <i>Fragaria ananassa</i> | Sweden, Uppland | E. Gunnerbeck | KP89 4113 | KP89 4221 | KP89 4328 | KP89 4438 | KP89 4548 | KP89 4659 | KP89 4770 | | KP89 4968 | | | |
| | CBS 259.36 | – | Netherlands | – | KP89 4114 | KP89 4222 | KP89 4329 | KP89 4439 | KP89 4549 | KP89 4660 | KP89 4771 | | | | KP89 5068 | |
| | CBS 298.34 | – | Netherlands | – | KP89 4115 | KP89 4223 | KP89 4330 | KP89 4440 | KP89 4550 | KP89 4661 | KP89 4772 | KP89 4880 | KP89 4969 | | | |
| | CBS 719.84 | <i>Fragaria</i> × <i>ananassa</i> 'Tioga' | New Zealand, Auckland | – | KP89 4116 | EU16 7605 | KP89 4331 | KP89 4441 | KP89 4551 | KP89 4662 | KP89 4773 | KP89 4881 | | | | |
| <i>Ramularia inaequalis</i> | CBS 250.96 | <i>Taraxacum officinale</i> | Canada, Nova Scotia | S. Green | KP89 4117 | KP89 4224 | KP89 4332 | KP89 4442 | KP89 4552 | KP89 4663 | KP89 4774 | KP89 4882 | KP89 4970 | | KP89 5069 | |
| | CPC 15752 | <i>Taraxacum</i> sp. | Mexico, Montecillo | M. de J. Yanez-Morales | KP89 4118 | KP89 4225 | KP89 4333 | KP89 4443 | KP89 4553 | KP89 4664 | KP89 4775 | | | KP89 5041 | | |

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|----------------------------------|---|------------------------------------|--------------------------|--------------------------|--|-----------|-----------|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| | | | | | LSU | ITS | ACT | TEF 1- α | GAP DH | RPB 2 | HIS3 | CAL | bTUB | MAT 1 | MAT 2 |
| | CPC 15753 | <i>Taraxacum</i> sp. | Mexico, Montecillo | M. de J. Yanez - Morales | KP89 4119 | KP89 4226 | KP89 4334 | KP89 4444 | KP89 4554 | KP89 4665 | KP89 4776 | KP89 4883 | KP89 4971 | - | - |
| | CPC 25741; X39 | <i>Taraxacum officinale</i> | Netherlands, Utrecht | U. Damm | KP89 4120 | KP89 4227 | KP89 4335 | KP89 4445 | KP89 4555 | KP89 4666 | KP89 4777 | - | - | - | KP89 5070 |
| | CPC 25742; X40 | <i>Taraxacum officinale</i> | Netherlands, Utrecht | U. Damm | KP89 4121 | KP89 4228 | KP89 4336 | KP89 4446 | KP89 4556 | KP89 4667 | KP89 4778 | - | - | - | KP89 5071 |
| <i>Ramularia lactea</i> | CBS 114442; UPSC 2727 | <i>Viola hirta</i> | Sweden, Uppland | E. Gunnerbeck | KP89 4122 | KP89 4229 | KP89 4337 | KP89 4447 | KP89 4557 | KP89 4668 | KP89 4779 | KP89 4884 | KP89 4972 | KP89 5042 | - |
| | CBS 135.23 | <i>Viola odorata</i> | - | - | KP89 4123 | KP89 4230 | KP89 4338 | KP89 4448 | KP89 4558 | KP89 4669 | KP89 4780 | - | KP89 4973 | - | - |
| <i>Ramularia nyssicola</i> | CBS 127664; AR 4629 | <i>Nyssageche×sylvatica</i> hybrid | USA, Maryland | R. Olsen | KP89 4124 | KP89 4231 | KP89 4339 | KP89 4449 | KP89 4559 | KP89 4670 | KP89 4781 | KP89 4885 | - | - | - |
| | CBS 127665 eepTy; AR 4656; DM 2 | <i>Nyssageche×sylvatica</i> hybrid | USA, Maryland | R. Olsen | KJ50 4724 | KJ50 4765 | KJ50 4429 | KJ50 4680 | KJ50 4548 | KJ50 4636 | KJ50 4592 | KJ50 4496 | KJ50 4473 | KP89 5043 | - |
| <i>Ramularia phacae-frigidae</i> | CBS 234.55 eTy | <i>Phaca frigida</i> | Switzerland, Corvegliana | E. Müller | KP89 4125 | KP89 4232 | KP89 4340 | KP89 4450 | KP89 4560 | KP89 4671 | KP89 4782 | KP89 4886 | - | - | KP89 5072 |
| <i>Ramularia pusilla</i> | CBS 124973; RoKi 3143 | <i>Poa annua</i> , leaves | Germany, Frankfurt | R. Kirshner | KP89 4141 | KP89 4248 | KP89 4356 | KP89 4466 | - | KP89 4687 | KP89 4798 | KP89 4901 | - | - | - |

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|------------------------------|---|--|----------------------|------------|--|----------|----------|-----------------|----------|----------|----------|----------|----------|----------|----------|
| | | | | | LSU | ITS | ACT | TEF 1- α | GAP DH | RPB 2 | HIS3 | CAL | bTUB | MAT 1 | MAT 2 |
| <i>Ramularia tricherae</i> | CBS 108973 | <i>Knautia arvensis</i> , leaf spot | Netherlands, Limburg | G. Verkley | KP894142 | KP894249 | KP894357 | KP894467 | KP894577 | KP894688 | KP894799 | KP894902 | KP894985 | KP895055 | – |
| | CBS 108989 | <i>Knautia dipsacifolia</i> , leaf spot | Austria, Ötztal | G. Verkley | KP894143 | KP894250 | KP894358 | KP894468 | KP894578 | KP894689 | KP894800 | KP894903 | KP894986 | – | KP895076 |
| | CBS 108990 | <i>Knautia dipsacifolia</i> , leaf spot | Austria, Ötztal | G. Verkley | KP894144 | KP894251 | KP894359 | KP894469 | KP894579 | KP894690 | KP894801 | KP894904 | – | – | – |
| | CBS 108994 | <i>Knautia arvensis</i> , leaf spot | Netherlands, Limburg | G. Verkley | KP894145 | KP894252 | KP894360 | KP894470 | KP894580 | KP894691 | KP894802 | KP894905 | KP894987 | KP895056 | – |
| | CBS 236.73; CCM F-369 | <i>Knautia drymeia</i> | Czechoslovakia | – | KP894146 | KP894253 | KP894361 | KP894471 | KP894581 | KP894692 | KP894803 | KP894906 | – | KP895057 | – |
| <i>Ramularia unterseheri</i> | CBS 117801; CPC 12091 | <i>Fagus sylvatica</i> , dead leaves | Netherlands, Utrecht | G. Verkley | KP894147 | KP894254 | KP894362 | KP894472 | KP894582 | KP894693 | KP894804 | KP894907 | KP894988 | – | – |
| | CBS 117807; CPC 12095 | <i>Fagus sylvatica</i> , dead leaves | Netherlands, Utrecht | G. Verkley | KP894148 | KP894255 | KP894363 | KP894473 | KP894583 | KP894694 | KP894805 | KP894908 | KP894989 | – | KP895077 |
| | CBS 117878; CPC 11206 | <i>Acer pseudoplatanus</i> , decaying leaves | Netherlands, Utrecht | G. Verkley | KP894149 | KP894256 | KP894364 | KP894474 | KP894584 | KP894695 | KP894806 | KP894909 | KP894990 | – | – |
| | CBS 117879; CPC 11207 | <i>Acer pseudoplatanus</i> , decaying leaves | Netherlands, Utrecht | G. Verkley | KP894150 | KP894257 | KP894365 | KP894475 | KP894585 | KP894696 | KP894807 | KP894910 | – | – | – |
| | CBS 117880; | <i>Tilia</i> sp. | Netherlands, | G. Verkley | KP894151 | KP894258 | KP894366 | KP894476 | KP894586 | KP894697 | KP894808 | KP894911 | KP894991 | – | – |

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|---------|---|--|----------------------|--------------|--|-----------|-----------|-----------------|-----------|-----------|-----------|-----------|-----------|-------|-----------|--|
| | | | | | LSU | ITS | ACT | TEF 1- α | GAP DH | RPB 2 | HIS3 | CAL | bTUB | MAT 1 | MAT 2 | |
| | CPC 11209 | | Utrecht | y | | | | | | | | | | | | |
| | CBS 117881; CPC 11211 | <i>Tilia</i> sp. | Netherlands, Utrecht | G. Verkleij | KP89 4152 | KP89 4259 | KP89 4367 | KP89 4477 | KP89 4587 | KP89 4698 | KP89 4809 | KP89 4912 | KP89 4992 | – | KP89 5078 | |
| | CBS 124827 | <i>Fagus sylvatica</i> , living leaves | Germany, Greifswald | M. Untereher | KP89 4153 | KP89 4260 | KP89 4368 | KP89 4478 | KP89 4588 | KP89 4699 | KP89 4810 | KP89 4913 | KP89 4993 | – | – | |
| | CBS 124830 | <i>Fagus sylvatica</i> , living leaves | Germany, Greifswald | M. Untereher | KP89 4154 | KP89 4261 | KP89 4369 | KP89 4479 | KP89 4589 | KP89 4700 | KP89 4811 | KP89 4914 | KP89 4994 | – | – | |
| | CBS 124831 | <i>Fagus sylvatica</i> , living leaves | Germany, Greifswald | M. Untereher | KP89 4155 | KP89 4262 | KP89 4370 | KP89 4480 | KP89 4590 | KP89 4701 | KP89 4812 | KP89 4915 | KP89 4995 | – | – | |
| | CBS 124834 | <i>Fagus sylvatica</i> , living leaves | Germany, Greifswald | M. Untereher | KP89 4156 | KP89 4263 | KP89 4371 | KP89 4481 | KP89 4591 | KP89 4702 | KP89 4813 | KP89 4916 | KP89 4996 | – | KP89 5079 | |
| | CBS 124836 | <i>Fagus sylvatica</i> , living leaves | Germany, Greifswald | M. Untereher | KP89 4157 | KP89 4264 | KP89 4372 | KP89 4482 | KP89 4592 | KP89 4703 | KP89 4814 | KP89 4917 | KP89 4997 | – | – | |
| | CBS 124838 | <i>Fagus sylvatica</i> , living leaves | Germany, Greifswald | M. Untereher | KP89 4158 | KP89 4265 | KP89 4373 | KP89 4483 | KP89 4593 | KP89 4704 | KP89 4815 | KP89 4918 | – | – | – | |
| | CBS 124844 | <i>Fagus sylvatica</i> , leaf litter | Germany, Greifswald | M. Untereher | KP89 4159 | KP89 4266 | KP89 4374 | KP89 4484 | KP89 4594 | KP89 4705 | KP89 4816 | KP89 4919 | KP89 4998 | – | – | |
| | CBS 124846 | <i>Fagus sylvatica</i> , leaf litter | Germany, Greifswald | M. Untereher | KP89 4160 | KP89 4267 | KP89 4375 | KP89 4485 | KP89 4595 | KP89 4706 | KP89 4817 | KP89 4920 | KP89 4999 | – | – | |
| | CBS 12485 | <i>Fagus sylvatica</i> , | Germany, Greifsw | M. Untereher | KP89 4161 | KP89 4268 | KP89 4376 | KP89 4486 | KP89 4596 | KP89 4707 | KP89 4818 | KP89 4921 | KP89 5000 | – | – | |

| Species | Culture collection accession number(s) ^a | Host/isolation source | Location | Collector | GenBank Accession numbers ^b | | | | | | | | | | | |
|-----------------------------|---|--|------------------------------|-----------------|--|-----------|-----------|-----------------|-----------|-----------|-----------|-----------|-----------|-------|-----------|--|
| | | | | | LSU | ITS | ACT | TEF 1- α | GAP DH | RPB 2 | HIS3 | CAL | bTUB | MAT 1 | MAT 2 | |
| | 2 | leaf litter | ald | eher | | | | | | | | | | | | |
| | CBS 124867 | <i>Fagus sylvatica</i> , leaf litter | Germany, Greifswald | M. Untereher | KP89 4162 | KP89 4269 | KP89 4377 | KP89 4487 | KP89 4597 | KP89 4708 | KP89 4819 | KP89 4922 | KP89 5001 | - | KP89 5080 | |
| | CBS 124884 eTy | <i>Fagus sylvatica</i> , leaf litter | Germany, Greifswald | M. Untereher | KP89 4163 | KP89 4270 | KP89 4378 | KP89 4488 | KP89 4598 | KP89 4709 | KP89 4820 | KP89 4923 | KP89 5002 | - | - | |
| | CBS 130721; DTO 162-C2 | Room inside castle (probably air sample) | Germany, Munich | - | KP89 4164 | KP89 4271 | KP89 4379 | KP89 4489 | KP89 4599 | KP89 4710 | KP89 4821 | KP89 4924 | - | - | - | |
| | CBS 355.90 | <i>Fagus sylvatica</i> , seed | Germany, former west Germany | U. Delfs-Siemer | KP89 4165 | KP89 4272 | KP89 4380 | KP89 4490 | KP89 4600 | KP89 4711 | KP89 4822 | - | KP89 5003 | - | - | |
| | CPC 25739; W6 | <i>Alnus</i> sp., leaf | Germany, Hesse | W. Quaedvlieg | KP89 4166 | KP89 4273 | KP89 4381 | KP89 4491 | KP89 4601 | KP89 4712 | KP89 4823 | - | - | - | - | |
| | CPC 25740; X2 | <i>Fagus sylvatica</i> , decaying leaves | Netherlands, Utrecht | S.I.R. Videira | KP89 4167 | KP89 4274 | KP89 4382 | KP89 4492 | KP89 4602 | KP89 4713 | KP89 4824 | KP89 4925 | KP89 5004 | - | KP89 5081 | |
| <i>Ramularia urticae</i> | CBS 105.26 | - | - | - | KP89 4169 | KP89 4276 | KP89 4384 | KP89 4494 | KP89 4604 | KP89 4715 | KP89 4826 | - | - | - | - | |
| | CBS 113974; UPSC 2359 | <i>Urtica dioica</i> | Sweden, Uppland | E. Gunnerbeck | KP89 4168 | KP89 4275 | KP89 4383 | KP89 4493 | KP89 4603 | KP89 4714 | KP89 4825 | KP89 4926 | KP89 5005 | - | - | |
| | CBS 162.91 | <i>Urtica dioica</i> , leaf spot | Germany, Weimar | G. Arnold | KP89 4170 | KP89 4277 | KP89 4385 | KP89 4495 | KP89 4605 | KP89 4716 | KP89 4827 | - | KP89 5006 | - | - | |
| <i>Ramularia variabilis</i> | CPC 16865 | <i>Verbascum</i> sp. | Canada, Ontario | K. Seifert | KP89 4171 | KP89 4278 | KP89 4386 | KP89 4496 | KP89 4606 | KP89 4717 | KP89 4828 | - | KP89 5007 | - | - | |

| Species | Culture collection accession number(s) ^a | Host/isolation source | Location | Collector | GenBank Accession numbers ^b | | | | | | | | | | | |
|---------------------------|---|---|-------------------------|------------|--|-----------|-----------|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|--|
| | | | | | LSU | ITS | ACT | TEF 1- α | GAP DH | RPB 2 | HIS3 | CAL | bTUB | MAT 1 | MAT 2 | |
| <i>lis</i> | | | | | | | | | | | | | | | | |
| | CPC 16866 | <i>Verbascum</i> sp. | Canada, Ontario | K. Seifert | KP89 4172 | KP89 4279 | KP89 4387 | KP89 4497 | KP89 4607 | KP89 4718 | KP89 4829 | - | KP89 5008 | - | - | |
| | CPC 25967 | <i>Verbascum</i> sp. | Austria, Graz | C. Scheuer | KP89 4173 | KP89 4280 | KP89 4388 | KP89 4498 | KP89 4608 | KP89 4719 | KP89 4830 | - | - | - | - | |
| <i>Ramularia vizellae</i> | CBS 113267 | <i>Quercus robur</i> , dead fallen leaves | Netherlands, Utrecht | G. Verkley | KP89 4174 | KP89 4281 | KP89 4389 | KP89 4499 | KP89 4609 | KP89 4720 | KP89 4831 | KP89 4927 | KP89 5009 | - | - | |
| | CBS 115980 | <i>Malus</i> sp., dead leaf litter | Netherlands, Gelderland | - | KP89 4175 | KP89 4282 | KP89 4390 | KP89 4500 | KP89 4610 | KP89 4721 | KP89 4832 | - | - | KP89 5058 | - | |
| | CBS 115981 | <i>Malus</i> sp., dead leaf litter | Netherlands, Gelderland | - | KP89 4176 | KP89 4283 | KP89 4391 | KP89 4501 | KP89 4611 | KP89 4722 | KP89 4833 | KP89 4928 | KP89 5010 | - | - | |
| | CBS 115982 | <i>Malus</i> sp., dead leaf litter | Netherlands, Gelderland | - | KP89 4177 | KP89 4284 | KP89 4392 | KP89 4502 | KP89 4612 | KP89 4723 | KP89 4834 | KP89 4929 | KP89 5011 | - | - | |
| | CBS 115983 | <i>Malus</i> sp., dead leaf litter | Netherlands, Gelderland | - | KP89 4178 | KP89 4285 | KP89 4393 | KP89 4503 | KP89 4613 | KP89 4724 | KP89 4835 | KP89 4930 | KP89 5012 | - | KP89 5082 | |
| | CBS 115984 | <i>Malus</i> sp., dead leaf litter | Netherlands, Gelderland | - | KP89 4179 | KP89 4286 | KP89 4394 | KP89 4504 | KP89 4614 | KP89 4725 | KP89 4836 | KP89 4931 | KP89 5013 | - | KP89 5083 | |
| | CBS 116015 | <i>Malus</i> sp., dead leaf litter | Netherlands, Gelderland | - | KP89 4180 | KP89 4287 | KP89 4395 | KP89 4505 | KP89 4615 | KP89 4726 | KP89 4837 | KP89 4932 | KP89 5014 | - | KP89 5084 | |
| | CBS 116069 | <i>Malus</i> sp., dead leaf litter | Netherlands, Gelderland | - | KP89 4181 | KP89 4288 | KP89 4396 | KP89 4506 | KP89 4616 | KP89 4727 | KP89 4838 | KP89 4933 | KP89 5015 | - | KP89 5085 | |
| | CBS | <i>Carpinus</i> | Netherlands | G. | KP89 | KP89 | KP89 | KP89 | KP89 | KP89 | KP89 | - | - | - | KP89 | |

| Species | Culture collection accession number(s) ^a | Host/isolation source | Location | Collector | GenBank Accession numbers ^b | | | | | | | | | | |
|---------|---|---|----------------------|------------|--|-----------|-----------|-----------------|-----------|-----------|-----------|-----------|-----------|-------|-----------|
| | | | | | LSU | ITS | ACT | TEF 1- α | GAP DH | RPB 2 | HIS3 | CAL | bTUB | MAT 1 | MAT 2 |
| | 117798; CPC 12088 | <i>Betulus</i> , fruit scales | Netherlands, Utrecht | Verkley | 4182 | 4289 | 4397 | 4507 | 4617 | 4728 | 4839 | | | | 5086 |
| | CBS 117799; CPC 12089 | <i>Acer pseudoplatanus</i> , dead leaves | Netherlands, Utrecht | G. Verkley | KP89 4183 | KP89 4290 | KP89 4398 | KP89 4508 | KP89 4618 | KP89 4729 | KP89 4840 | KP89 4934 | KP89 5016 | - | - |
| | CBS 117802; CPC 12092 | <i>Carpinus betulus</i> , dead leaves | Netherlands, Utrecht | G. Verkley | KP89 4184 | KP89 4291 | KP89 4399 | KP89 4509 | KP89 4619 | KP89 4730 | KP89 4841 | KP89 4935 | KP89 5017 | - | - |
| | CBS 117805; CPC 12094 | <i>Aesculus hippocastanum</i> , dead leaves | Netherlands, Utrecht | G. Verkley | KP89 4185 | KP89 4292 | KP89 4400 | KP89 4510 | KP89 4620 | KP89 4731 | KP89 4842 | KP89 4936 | KP89 5018 | - | KP89 5087 |
| | CBS 117806; CPC 12096 | <i>Tilia</i> sp., dead leaves | Netherlands, Utrecht | G. Verkley | KP89 4186 | KP89 4293 | KP89 4401 | KP89 4511 | KP89 4621 | KP89 4732 | KP89 4843 | KP89 4937 | KP89 5019 | - | KP89 5088 |
| | CBS 117870; CPC 11193 | <i>Quercus rubra</i> , decaying leaves | Netherlands, Utrecht | G. Verkley | KP89 4187 | KP89 4294 | KP89 4402 | KP89 4512 | KP89 4622 | KP89 4733 | KP89 4844 | KP89 4938 | KP89 5020 | - | KP89 5089 |
| | CBS 117871; CPC 11194 | <i>Quercus rubra</i> , decaying leaves | Netherlands, Utrecht | G. Verkley | KP89 4188 | KP89 4295 | KP89 4403 | KP89 4513 | KP89 4623 | KP89 4734 | KP89 4845 | KP89 4939 | KP89 5021 | - | - |
| | CBS 117872; CPC 11197 | <i>Amelanchier lamarckii</i> | Netherlands, Utrecht | G. Verkley | KP89 4189 | KP89 4296 | KP89 4404 | KP89 4514 | KP89 4624 | KP89 4735 | KP89 4846 | KP89 4940 | KP89 5022 | - | KP89 5090 |
| | CBS 117873; CPC 11198 | <i>Amelanchier lamarckii</i> | Netherlands, Utrecht | G. Verkley | KP89 4190 | KP89 4297 | KP89 4405 | KP89 4515 | KP89 4625 | KP89 4736 | KP89 4847 | KP89 4941 | KP89 5023 | - | - |

| Species | Culture collection accession number(s) ^a | Host/isolation source | Location | Collector | GenBank Accession numbers ^b | | | | | | | | | | |
|---------|---|--|----------------------|-----------------|--|----------|----------|-----------------|----------|----------|----------|----------|----------|----------|----------|
| | | | | | LSU | ITS | ACT | TEF 1- α | GAP DH | RPB 2 | HIS3 | CAL | bTUB | MAT 1 | MAT 2 |
| | CBS 117874; CPC 11200 | <i>Aesculus hippocastanum</i> | Netherlands, Utrecht | G. Verkleij | KP894191 | KP894298 | KP894406 | KP894516 | KP894626 | KP894737 | KP894848 | KP894942 | KP895024 | – | KP895091 |
| | CBS 117875; CPC 11201 | <i>Aesculus hippocastanum</i> | Netherlands, Utrecht | G. Verkleij | KP894192 | KP894299 | KP894407 | KP894517 | KP894627 | KP894738 | KP894849 | KP894943 | KP895025 | – | – |
| | CBS 117882; CPC 11212 | <i>Sorbus aucuparia</i> , decaying leaves | Netherlands, Utrecht | G. Verkleij | KP894193 | KP894300 | KP894408 | KP894518 | KP894628 | KP894739 | KP894850 | KP894944 | KP895026 | KP895059 | – |
| | CBS 117883; CPC 11213 | <i>Sorbus aucuparia</i> , decaying leaves | Netherlands, Utrecht | G. Verkleij | KP894194 | KP894301 | KP894409 | KP894519 | KP894629 | KP894740 | KP894851 | KP894945 | KP895027 | KP895060 | – |
| | CBS 124861 | <i>Fagus sylvatica</i> , leaf litter from 2007 | Germany, Greifswald | M. Unterstueher | KP894195 | KP894302 | KP894410 | KP894520 | KP894630 | KP894741 | KP894852 | KP894946 | – | – | KP895092 |
| | CBS 130601 eTy ; CPC 18283 | <i>Protea</i> sp., leaves | South Africa | P.W. Crous | JN712567 | KJ504808 | KJ504472 | KJ504723 | KJ504591 | KJ504679 | KJ504635 | – | KJ504495 | KP895061 | – |
| | CBS 184.97 | <i>Acer pseudoplatanus</i> , dead leaves | Netherlands, Utrecht | H.A. van der Aa | KP894196 | KP894303 | KP894411 | KP894521 | KP894631 | KP894742 | KP894853 | KP894947 | KP895028 | KP895062 | – |
| | CBS 185.97 | <i>Acer pseudoplatanus</i> , dead leaves | Netherlands, Utrecht | H.A. van der Aa | KP894197 | KP894304 | KP894412 | KP894522 | KP894632 | KP894743 | KP894854 | KP894948 | KP895029 | KP895063 | – |
| | CBS 324.87 | <i>Brassica</i> sp., in leaf spot | Netherlands | – | GU214581 | GU214581 | KP894413 | KP894523 | KP894633 | KP894744 | KP894855 | KP894949 | KP895030 | – | KP895093 |

| Species | Culture collection accession number(s) ^a | Host/isolation source | Location | Collector | GenBank Accession numbers ^b | | | | | | | | | | |
|---------|---|--|--------------------------------|-----------------|--|-----------|-----------|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| | | | | | LSU | ITS | ACT | TEF 1- α | GAP DH | RPB 2 | HIS3 | CAL | bTUB | MAT 1 | MAT 2 |
| | CBS 367.64 | <i>Malus sylvestris</i> , fruit | France | C. Moreau | KP89 4198 | KP89 4305 | KP89 4414 | KP89 4524 | KP89 4634 | KP89 4745 | KP89 4856 | KP89 4950 | KP89 5031 | – | KP89 5094 |
| | CBS 369.67 | <i>Lotus uliginosus</i> , young leaves | Netherlands, Utrecht | H.A. van der Aa | KP89 4199 | KP89 4306 | KP89 4415 | KP89 4525 | KP89 4635 | KP89 4746 | KP89 4857 | KP89 4951 | – | – | KP89 5095 |
| | CBS 428.74; IHEM 3995 | <i>Phaseolus</i> sp. | Switzerland | – | KP89 4200 | KP89 4307 | KP89 4416 | KP89 4526 | KP89 4636 | KP89 4747 | KP89 4858 | KP89 4952 | KP89 5032 | – | – |
| | CBS 515.69 | <i>Acer pseudoplatanus</i> | Netherlands, Utrecht | H.A. van der Aa | KP89 4201 | AY49 0759 | KP89 4417 | KP89 4527 | KP89 4637 | KP89 4748 | KP89 4859 | KP89 4953 | KP89 5033 | – | KP89 5096 |
| | CBS 724.79 | <i>Tilia</i> sp., overwintering leaf on the ground | Germany, München | A. John | KP89 4202 | KP89 4308 | KP89 4418 | KP89 4528 | KP89 4638 | KP89 4749 | KP89 4860 | KP89 4954 | KP89 5034 | – | KP89 5097 |
| | CBS 943.97 | <i>Quercus</i> sp., leaves | Netherlands | A. Aptroot | KP89 4203 | KP89 4309 | KP89 4419 | KP89 4529 | KP89 4639 | KP89 4750 | KP89 4861 | KP89 4955 | KP89 5035 | – | – |
| | CPC 15541 | <i>Acer campestre</i> | Ukraine, Seversky Donets river | A. Akulov | KP89 4204 | KP89 4310 | KP89 4420 | KP89 4530 | KP89 4640 | KP89 4751 | KP89 4862 | – | – | – | – |
| | CPC 25728; MP19 | <i>Corylus</i> sp. | Netherlands, Utrecht | S.I.R. Videira | KP89 4205 | KP89 4311 | KP89 4421 | KP89 4531 | KP89 4641 | KP89 4752 | KP89 4863 | KP89 4956 | – | – | – |
| | CPC 25729; MP20 | <i>Quercus</i> sp. | Netherlands, Utrecht | S.I.R. Videira | KP89 4206 | KP89 4312 | KP89 4422 | KP89 4532 | KP89 4642 | KP89 4753 | KP89 4864 | KP89 4957 | – | KP89 5064 | – |
| | CPC 25730; MP21 | <i>Carpinus</i> sp. | Netherlands, Utrecht | S.I.R. Videira | KP89 4207 | KP89 4313 | KP89 4423 | KP89 4533 | KP89 4643 | KP89 4754 | KP89 4865 | KP89 4958 | – | – | KP89 5098 |
| | CPC 25731; | <i>Quercus</i> sp. | Netherlands, | S.I.R. Videira | KP89 4208 | KP89 4314 | KP89 4424 | KP89 4534 | KP89 4644 | KP89 4755 | KP89 4866 | KP89 4959 | – | – | KP89 5099 |

| Species | Culture collection accession number(s) ^a | Host/isolation source | Location | Collector | GenBank Accession numbers ^b | | | | | | | | | | | |
|-------------------------------|---|---|----------------------|------------------|--|-----------|-----------|----------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|--|
| | | | | | LSU | ITS | ACT | TEF1- α | GAPDH | RPB2 | HIS3 | CAL | bTUB | MAT1 | MAT2 | |
| | MP23 | | Utrecht | a | | | | | | | | | | | | |
| | CPC 25732 ; MP24 | <i>Fagus</i> sp., decaying leaves | Netherlands, Utrecht | S.I.R. Videira | KP89 4209 | KP89 4315 | KP89 4425 | KP89 4535 | KP89 4645 | KP89 4756 | KP89 4867 | KP89 4960 | – | KP89 5065 | – | |
| | CPC 25733 ; W7 | Leaf of unidentified plant | Germany, Hesse | W. Quaeden-vlieg | KP89 4210 | KP89 4316 | KP89 4426 | KP89 4536 | KP89 4646 | KP89 4757 | KP89 4868 | – | – | – | – | |
| | CPC 25734 ; X1 | <i>Fagus</i> sp., decaying leaves | Netherlands, Utrecht | S.I.R. Videira | KP89 4211 | KP89 4317 | KP89 4427 | KP89 4537 | KP89 4647 | KP89 4758 | KP89 4869 | KP89 4961 | KP89 5036 | – | KP89 5100 | |
| | CPC 25735 ; X3 | <i>Fagus</i> sp., decaying leaves | Netherlands, Utrecht | S.I.R. Videira | KP89 4212 | KP89 4318 | KP89 4428 | KP89 4538 | KP89 4648 | KP89 4759 | KP89 4870 | KP89 4962 | – | – | – | |
| | CPC 25738 ; X31 | <i>Sambucus nigra</i> | Austria, Graz | C. Scheuer | KP89 4215 | KP89 4321 | KP89 4431 | KP89 4541 | KP89 4651 | KP89 4762 | KP89 4873 | – | – | – | – | |
| | CPC 25736 ; X4 | <i>Corylus</i> sp., decaying leaves | Netherlands, Utrecht | S.I.R. Videira | KP89 4213 | KP89 4319 | KP89 4429 | KP89 4539 | KP89 4649 | KP89 4760 | KP89 4871 | KP89 4963 | – | – | – | |
| | CPC 25737 ; X5 | <i>Aesculus hippocastanum</i> , decaying leaves | Netherlands, Utrecht | S.I.R. Videira | KP89 4214 | KP89 4320 | KP89 4430 | KP89 4540 | KP89 4650 | KP89 4761 | KP89 4872 | KP89 4964 | – | – | KP89 5101 | |
| <i>Zyloseptoria passerini</i> | CBS 12038 2 eepTy | <i>Hordeum vulgare</i> | USA, North Dakota | S. Goodwin | JQ73 9843 | JF700 877 | JF70 1046 | JQ73 9787 | KP89 4652 | KP89 4763 | KP89 4874 | JF70 1114 | JF70 0978 | – | – | |

^a AR: Personal culture collection of Amy Rossman; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CCM: Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic; CPC: Personal culture collection of Pedro Crous, housed at CBS; DTO: Personal culture collection of the Applied and Industrial Mycology, housed at CBS; IHM: Collection of the Laboratorium voor Microbiologie en Microbiële Genetica, Gent, Belgium; IPO: Research Institute for Plant Protection, Wageningen; RoKI: Personal culture collection of Roland Kirschner; UPSC: Uppsala University Culture Collection of Fungi, Botanical Museum University of Uppsala, Uppsala, Sweden.

^b LSU: large subunit (28S) of the nrRNA gene operon; ITS: internal transcribed spacers and intervening 5.8S nrDNA; ACT: partial actin gene; TEF1- α : partial translation elongation factor 1-alpha gene; GAPDH: partial glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene; RPB2: partial RNA polymerase II second largest subunit gene; HIS3: partial histone H3 gene; CAL: partial calmodulin gene; bTUB: partial beta-tubulin gene; MAT1: partial MAT1-1-1 mating type gene; MAT2: partial MAT1-2 mating type gene. eTy: ex-type; eepTy: ex-epitype; 'X' represents a DNA sequence that will be submitted to Genbank and '–' a DNA sequence that was not generated.

DNA extraction, amplification and sequencing

Fungal mycelium of strains (Table 1) was harvested with a sterile scalpel and the genomic DNA was isolated using the UltraClean™ Microbial DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA) following the manufacturers' protocols. Eleven partial nuclear genes were initially targeted for PCR amplification and sequencing: 28S nrRNA gene (LSU), internal transcribed spacer regions and intervening 5.8S nrRNA gene (ITS) of the nrDNA operon, actin (ACT), translation elongation factor 1- α (TEF1- α), histone H3 (HIS3), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), RNA polymerase II second largest subunit (RPB2), calmodulin (CAL), β -tubulin (bTUB), mating-type gene 1 (MAT1-1-1) and mating-type gene 2 (MAT1-2-1). The primers employed are listed in Table 2. The PCR amplifications were performed on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The PCR mixtures consisted of 1 μ L genomic DNA, 1 \times GoTaq® Flexi buffer (Promega, Madison, WI, USA), 2 μ M MgCl₂, 40 μ M of each dNTP, 0.2 μ M of each primer and 0.5 Unit GoTaq® Flexi DNA polymerase (Promega) in a total volume of 12.5 μ L. The PCR mixtures for HIS3, GAPDH, RPB2, CAL and bTUB contained 2 μ L genomic DNA. The PCR conditions were: initial denaturation (94 °C, 3 min); 35 cycles amplification (94 °C, 30 s; annealing temperature listed in Table 2, 30 s; 72 °C, 45 s), and final extension (72 °C, 5 min). For GAPDH and HIS3, 40 amplification cycles were used. To obtain the partial RPB2, a touchdown PCR protocol was used: initial denaturation (94 °C, 3 min), 5 amplification cycles (94 °C, 45 s; 60 °C, 45 s; 72 °C, 2 min), 5 amplification cycles (94 °C, 45 s; 58 °C, 45 s; 72 °C, 2 min), 30 amplification cycles (94 °C, 45 s; 54 °C, 45 s; 72 °C, 2 min) and a final extension (72 °C, 8 min). The resulting fragments were sequenced in both directions using the PCR primers and the BigDye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems Life Technologies, Carlsbad, CA, USA). DNA sequencing amplicons were purified through Sephadex G-50 Superfine columns (Sigma–Aldrich, St. Louis, MO) in MultiScreen HV plates (Millipore, Billerica, MA). Purified sequence reactions were analysed on an Applied Biosystems 3730xl DNA Analyzer (Life Technologies, Carlsbad, CA, USA). The DNA sequences generated were analysed and consensus sequences were computed using the BioNumerics v. 4.61 software package (Applied Maths, St-Martens-Latem, Belgium).

Table 2. Details of primers used for amplification and sequencing in this study.

| Locus ^a | Primer name | Primer sequence (5' → 3') | Annealing temperature (°C) | Orientation | Reference |
|--------------------|------------------|--------------------------------|----------------------------|-------------|----------------------------------|
| ACT | ACT-512F | ATG TGC AAG GCC GGT TTC GC | 55 | Forward | Carbone & Kohn (1999) |
| | ACT-783 R | TAC GAG TCC TTC TGG CCC AT | 55 | Reverse | Carbone & Kohn (1999) |
| | ACT-2Rd | ARR TCR CGD CCR GCC ATG TC | 55 | Reverse | Groenewald <i>et al.</i> (2013) |
| bTUB | T1 | AAC ATG CGT GAG ATT GTA AGT | 52 | Forward | O'Donnell & Cigelnik, 1997 |
| | β -Sandy-R | GCR CGN GGV ACR TAC TTG TT | 52 | Reverse | Stukenbrock <i>et al.</i> (2012) |

| Locus ^a | Primer name | Primer sequence (5' → 3') | Annealing temperature (°C) | Orientation | Reference |
|--------------------|----------------|------------------------------------|----------------------------|-------------|---------------------------------------|
| | Bt2a | GGT AAC CAA ATC GGT GCT GCT TTC | 52 | Forward | Glass & Donaldson (1995) |
| | Bt2b | ACC CTC AGT GTA GTG ACC CTT GGC | 52 | Reverse | Glass & Donaldson (1995) |
| CAL | CAL-228F | GAG TTC AAG GAG GCC TTC TCC C | 58 | Forward | Carbone & Kohn (1999) |
| | CAL-737R | CAT CTT TCT GGC CAT CAT GG | 58 | Reverse | Carbone & Kohn (1999) |
| | CAL2Rd | TGR TCN GCC TCD CGG ATC ATC TC | 58 | Reverse | Groenewald <i>et al.</i> (2013) |
| GAPDH | gpd1 | CAA CGG CTT CGG TCG CAT TG | 55 | Forward | Berbee <i>et al.</i> (1999) |
| | gpd2 | GCC AAG CAG TTG GTT GTG C | 55 | Reverse | Berbee <i>et al.</i> (1999) |
| HIS3 | CylH3F | AGG TCC ACT GGT GGC AAG | 52 | Forward | Crous <i>et al.</i> (2004b) |
| | CylH3R | AGC TGG ATG TCC TTG GAC TG | 52 | Reverse | Crous <i>et al.</i> (2004b) |
| ITS | V9G | TTA CGT CCC TGC CCT TTG TA | 52 | Forward | de Hoog & Gerrits van den Ende (1998) |
| | ITS4 | TCC TCC GCT TAT TGA TAT GC | 52 | Reverse | White <i>et al.</i> (1990) |
| LSU | LSU1Fd | GRA TCA GGT AGG RAT ACC CG | 52 | Forward | Crous <i>et al.</i> (2009a) |
| | LR5 | TCC TGA GGG AAA CTT CG | 52 | Reverse | Vilgalys & Hester (1990) |
| MAT1-1-1 | MgMfSpMat1-1f1 | CATTNGCNCATCCCTTTG | 54 | Forward | Groenewald <i>et al.</i> (2006) |
| | MgMfSpMat1-1r2 | GGCTTNGANACCATGGTGAG | 54 | Reverse | Groenewald <i>et al.</i> (2006) |
| MAT1-2-1 | MgMfSpMat1-2f2 | CAAAGAANGCNTTCNTGATCT | 54 | Forward | Groenewald <i>et al.</i> (2006) |
| | MgMfSpMat1-2r1 | TTCTTCTCNGATGGCTTGC | 54 | Reverse | Groenewald <i>et al.</i> (2006) |
| RPB2 | RPB2-5F | GAY GAY MGW GAT CAY TTY GG | 60 → 58 → 54 | Forward | Liu <i>et al.</i> (1999) |
| | RPB2-7cR | CCC ATR GCT TGY TTR CCC AT | 60 → 58 → 54 | Reverse | Liu <i>et al.</i> (1999) |
| | Rpb2-F1 | GGTGTGAGTCARGTGYTGAA | 60 → 58 → 54 | Forward | Videira <i>et al.</i> (2015) |
| | Rpb2-R1 | TCC TCN GGV GTC ATG ATR ATC | 60 → 58 → 54 | Reverse | Videira <i>et al.</i> (2015) |

| Locus ^a | Primer name | Primer sequence (5' → 3') | Annealing temperature (°C) | Orientation | Reference |
|--------------------|-------------|--------------------------------|----------------------------|-------------|--------------------------------|
| | | AT | | | |
| TEF1- α | EF1-728F | CAT CGA GAA GTT CGA GAA GG | 54 | Forward | Carbone & Kohn (1999) |
| | EF-2 | GGA RGT ACC AGT SAT CAT GTT | 54 | Reverse | O'Donnell <i>et al.</i> (1998) |
| | TEF-1R | CTT GAT GAA ATC ACG GTG ACC | 54 | Reverse | Videira <i>et al.</i> (2015) |

^a ACT: partial actin gene; bTUB: partial beta-tubulin gene; CAL: partial calmodulin gene; GAPDH: partial glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene; HIS3: partial histone H3 gene; ITS: internal transcribed spacers and intervening 5.8S nrDNA; LSU: large subunit (28S) of the nrRNA gene operon; MAT1: partial MAT1-1-1 mating type gene; MAT2: partial MAT1-2 mating type gene; RPB2: partial RNA polymerase II second largest subunit gene; TEF1- α : partial translation elongation factor 1-alpha gene.

Phylogenetic analysis

The generated sequences for each gene were aligned with MAFFT v. 7 (Katoh & Standley 2013) and the alignments were manually checked and improved where necessary using MEGA v. 5 (Tamura *et al.* 2011). From the strains listed in Table 1, only those with the complete dataset of genes were used in the phylogenetic analyses, with the exception of *Ramularia pusilla*, which was missing the sequence of GAPDH and was considered as missing data in the alignment. Phylogenetic analyses of sequence data consisted of both Neighbour-Joining analysis and parsimony analysis performed with PAUP v. 4.0b10 (Swofford 2003) and also a Bayesian analysis performed with MrBayes v. 3.2.1 (Ronquist *et al.* 2011).

The Neighbour-Joining analysis using the HKY85 substitution model was applied to each gene partition individually. The single gene trees were manually compared in order to check the stability of each species clade and exclude incongruent genes from the multigene analysis (data not shown, individual gene trees deposited on TreeBASE). Alignment gaps were treated as missing data and all characters were unordered and of equal weight. Any ties were broken randomly when encountered. The selected genes for the multigene parsimony and Bayesian analysis were concatenated with Mesquite v. 2.75 (Maddison & Maddison 2011).

The parsimony analysis was performed on three datasets, namely the concatenated alignment of five genes and the individual alignments of the mating-type sequences (MAT1-1-1 and MAT1-2-1). The analysis used a heuristic search with 100 random taxa additions and the branch-swapping algorithm for tree bisection and reconstruction. Alignment gaps were treated as fifth base and all characters were unordered and of equal weight. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the trees obtained was evaluated by 1000 bootstrap replications (Hillis & Bull 1993). Other measures calculated included tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC). The resulting trees were printed with Geneious v. 7.0.6 (Kearse *et al.* 2012).

The Bayesian analysis was performed on the combined multigene alignment only. MrModeltest v. 2.2 (Nylander 2004) was used to determine the best nucleotide substitution model settings for each data partition in order to perform a model-optimized Bayesian

phylogenetic reconstruction using MrBayes v. 3.2.1 (Ronquist *et al.* 2011). The heating chain was set to 0.15 and the Markov Chain Monte Carlo (MCMC) analysis of four chains was started in parallel from a random tree topology and lasted until the average standard deviation of split frequencies reached 0.01. Burn-in was set to 25 % after which the likelihood values were stationary. Trees were saved each 250 generations and the resulting phylogenetic tree was printed with Geneious v. 7.0.6 (Kearse *et al.* 2012). All new sequences generated in this study were deposited in NCBI's GenBank nucleotide database (www.ncbi.nlm.nih.gov) and the accession numbers of the sequences used for the phylogenetic analyses are listed in Table 1. The alignments and respective phylogenetic trees were deposited in TreeBASE (www.treeBASE.org).

Taxonomy

Isolates were cultivated for 7 d at 21 °C. Microscopic observations of the conidiogenous structures were performed using a Nikon Eclipse 80i light microscope with differential interference contrast (DIC) illumination. Slides were prepared using the inclined coverslip method (Kawato & Shinobu 1959, revised in Nugent *et al.* 2006) and also transparent adhesive tape (Titan Ultra Clear Tape, Conglom Inc., Toronto, Canada) (Bensch *et al.* 2012). Clear lactic acid was used as mounting medium for the measurements. The morphological structure terminology followed those used for *Ramularia* species by Crous *et al.* (2011). The recorded measurements represent the minimum value followed by the 95 % confidence interval of 30 individual measurements and the maximum value, for both length and width. For culture characterization the isolates were inoculated on 2 % potato dextrose agar (PDA), oatmeal agar (OA) and 2 % malt extract agar (MEA) (recipes according to Crous *et al.* 2009d), and incubated in the dark at 25 °C. After 14 d, the colony diameter was measured and the colony colour described according to the mycological colour charts of Rayner (1970). Nomenclatural novelties and descriptions were deposited in MycoBank (Crous *et al.* 2004a).

Results

DNA amplification and phylogenetic analysis

Of the 11 loci tested in this study, seven were successfully amplified for most strains (LSU, ITS, ACT, TEF1- α , HIS3, GAPDH, RPB2). The amplification of CAL and bTUB often resulted in multiple bands, despite the attempts of protocol optimization and were not used in the multigene analysis. The amplification of the mating-type loci was not successful for all the strains (Table 1) and was particularly challenging for the MAT1-2-1 with the use of the degenerate primers (Table 2) that were reported successful for other *Mycosphaerellaceae* (Groenewald *et al.*, 2006 and Groenewald *et al.*, 2007). Due to the observed variation of the position of these loci in other species, an attempt was made to amplify the loci using the forward primer for MAT1-1-1 and the reverse primer of MAT1-2-1 and vice-versa. A sequence of approximately 670 bp was obtained for several strains with the combination of the primers MgMfSpMat1-1f1 (MAT1-1-1 forward) and MgMfSpMat1-2r1 (MAT1-2-1 reverse) for which the last portion of approximately 200 bp corresponded to the MAT1-2-1 conserved high mobility group. When sequences of the MAT1-2-1 obtained with the regular primer combination were compared with the ones obtained with the described uncommon combination, they matched exactly. The mating-type genes were not used in the combined analysis since all isolates with a successful sequence had either the MAT1-1-1 or MAT1-2-1 amplicon. In addition, no sequences of MAT1-1-1 were obtained for any of the *Ramularia*

unterseheri strains available in this study. All the obtained sequences were deposited in GenBank (Table 1).

The Neighbour-joining analysis using the HKY85 substitution model used to check the stability and robustness of clades for the individual loci (data not shown) revealed that the both the LSU and ITS locus separated *Ramularia endophylla* strains in a unique clade but were not able to separate *Ramularia vizellae* from *R. unterseheri* (newly described). The single gene trees for ACT, HIS3, RPB2 and GAPDH could separate three species within the complex, namely *R. endophylla*, *R. vizellae*, and *R. unterseheri*. The partial sequences of TEF1- α were very heterogeneous and the resulting phylogenetic tree was not congruent with the other genes. The TEF1- α sequences were, therefore, not used in the multigene analysis.

The multigene analysis was based on a concatenated alignment of five loci (ITS, ACT, RPB2, GAPDH and HIS3) and contained 114 taxa, of which 81 belonged to the *R. endophylla* species complex, 32 represented other *Ramularia* species and the outgroup sequence of *Zymoseptoria passerini*. The final alignment contained a total of 2618 characters divided in five partitions containing 515 (ITS), 236 (ACT), 897 (RPB2), 575 (GAPDH) and 375 (HIS3) characters respectively, including alignment gaps. From the total alignment, 81 characters were excluded from the phylogenetic analysis: 20 characters that were artificially introduced as spacers between the genes; 10 characters (ITS) and 17 characters (GAPDH) that represented a longer sequence in the outgroup compared to the ingroup sequences; 20 characters (GAPDH) representing a longer intron that only existed for *Ramularia nyssicola*; 14 characters (ACT) representing a repetition in an intron on the strains in *Ramularia grevilleana* (see alignment in TreeBASE).

The results of the MrModelTest analyses for the multigene dataset indicated that the ITS partition had fixed (equal) base frequencies, whereas all the other partitions had dirichlet base frequencies. The optimised models for this alignment were SYM + I + G for ITS and GTR + I + G for all the other data partitions. The Bayesian analysis of the concatenated five-locus alignment generated 104 082 trees from which 26 020 trees were discarded (25 % burnin). The 50 % majority rule consensus tree (Fig 1) and posterior probabilities (values ≤ 1) were calculated from the remaining 78 062 trees. The alignment contained a total of 959 unique site patterns: 100 (ITS), 123 (ACT), 413 (RPB2), 209 (GAPDH), 114 (HIS3).

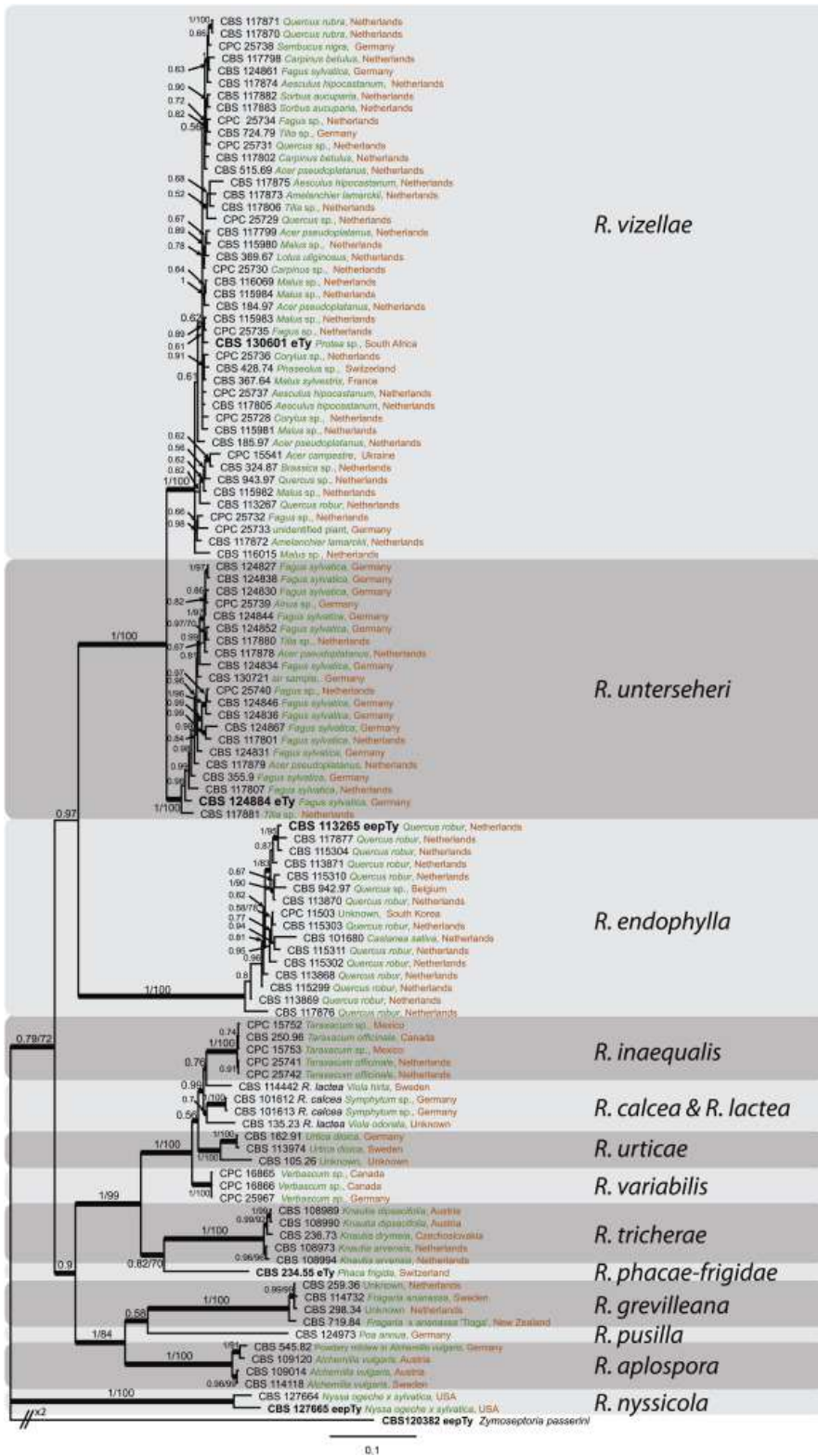


Fig 1. Phylogenetic tree resulting from a Bayesian analysis of the combined 5-gene sequence alignment. Both Bayesian posterior probabilities (left number) and parsimony bootstrap support values > 70 % (right number) are indicated at the nodes; the scale bar represents the expected number of changes per site. Branches depicted in a thicker represent the branches present in the strict consensus parsimony tree. Strains in bold and marked as ‘eTy’ are ex-types and those marked as ‘eepTy’ are ex-epitypes. The tree was rooted to *Zymoseptoria passerinii* (CBS 120382).

The parsimony analysis on the multigene dataset generated 1000 equally most parsimonious trees. From the analysed characters, 1559 were constant, 205 were variable and parsimony-uninformative and 772 were parsimony-informative. A parsimony consensus tree was calculated from the equally most parsimonious trees and the branches were mapped with a thicker stroke on the Bayesian tree (Fig 1; bootstrap support values >75). The overall parsimony phylogeny supported the same species clades as those presented in the Bayesian phylogeny (Fig 1). Phylogenetic trees based on the combined dataset (Fig 1) and generated with both parsimony and Bayesian analyses, separated strains into three well supported species within the original complex: *R. endophylla*, *R. vizellae*, and *R. unterseheri*. The other *Ramularia* strains represent species that, in literature, have been associated with a *Mycosphaerella* sexual morph (Table 3).

Table 3. Relations between *Ramularia* asexual morphs and their *Mycosphaerella* sexual morphs reported in literature. References in bold represent the reference where a link was experimentally proven.

| Asexual morph ^a | Sexual morph ^a | Current name | CA ^b | References | Sexual link ^c | Notes |
|---|--|--|-----------------|--|--------------------------|---|
| <i>R. endophylla</i> Verkley & U. Braun (2004) | <i>M. punctiformis</i> (Pers.) Starbäck (1889) [bas. <i>Sphaeria punctiformis</i> Pers. (1794)] | <i>R. endophylla</i> Verkley & U. Braun (2004) | Y | Verkley et al. (2004) | EP | Verkley et al. (2004) epitypified <i>M. punctiformis</i> and linked it with <i>R. endophylla</i> morphologically and genetically. |
| <i>R. grevilleana</i> (Oudem.) Jørst. (1945) [bas. <i>Cylindrosporium grevilleanum</i> Oudem. (1873)] | <i>M. fragariae</i> (Tul.) Lindau (1897) [bas. <i>Sphaeria fragariae</i> Tul. & C. Tul. (1856)] | <i>R. grevilleana</i> (Oudem.) Jørst. (1945) | Y | Dudley, 1889, Potebnja, 1908, Schellenberger, 1917, Plakidas, 1941, Klebahn, 1918, Tomilin, 1979, Sivanesan, 1984, Braun, 1998 and Braun and Pennycook, 2003 | EP | Dudley (1889) used conidia from pure cultures to infect strawberry leaves and observed perithecia formed in these lesions and that the ascospores germinated within the ascus, shot out through the ostiole and gave rise to conidia. |
| <i>R. inaequalis</i> (Preuss) U. Braun (1998) [bas. <i>Fusoma inaequale</i> Preuss (1855)] | <i>M. hieracii</i> (Sacc. & Briard) Jaap (1908) [bas. <i>Sphaerella nebulosa</i> var. <i>hieracii</i> Sacc.&Briard (1885)] | <i>R. inaequalis</i> (Preuss) U. Braun (1998) | Y | Jaap, 1908, Klebahn, 1918 and Sivanesan, 1984 | EP | Klebahn (1918) isolated ascospores and obtained a culture in which the conidiogenous stage was observed. |
| <i>R. variabilis</i> Fuckel (1870) | <i>M. mariae</i> (Sacc. & E. Bommer) Lindau (1903) [bas. <i>Sphaerella mariae</i> Sacc.&E.Bommer (1886)] | <i>R. variabilis</i> Fuckel (1870) | Y | Arx von, 1949, Tomilin, 1979, Sivanesan, 1984, Braun, 1998 and Aptroot, 2006 | EP | Ascospores were isolated from perithecia in overwintered leaves into pure cultures from which the conidial forms developed. |
| <i>R. nyssicola</i> (Cooke) Videira & Crous (2014) | <i>M. nyssicola</i> (Cooke) F.A. Wolf (1940) | <i>R. nyssicola</i> (Cooke) Videira & Crous (2014) | Y | Minnis et al., 2011 and Videira et al., 2015 | EP | The asexual morph has not observed but the available strains obtained from the sexual morph are genetically placed within the <i>Ramularia</i> clade. |
| <i>Ramularia</i> | <i>M. phacae-frigidiae</i> E. Müll. & Wehm. | <i>R. phacae-frigidiae</i> (E. Müll. & | Y | Müller & Wehmeyer (1954) , Aptroot (2006); | EP | Müller & Wehmeyer (1954) observed ramularia-like spores when he described the |

| Asexual morph ^a | Sexual morph ^a | Current name | CA ^b | References | Sexual link ^c | Notes |
|---|--|---|-----------------|--|--------------------------|---|
| | (1954) | Wehm.) Videira & Crous (2015), this study | | Present study | | species, but did not name the conidial form. |
| <i>R. atropae</i> Allesch. (1892) | ? <i>M. montellica</i> (Sacc.) Guyot (1946) | – | N | Tomilin, 1979 and Braun, 1998 | DB | Aptroot (2006) states the type belongs to <i>Davidiella</i> . |
| <i>R. chamerionis</i> Rostr. [as ' <i>chamaenerii</i> '] (1885) | <i>M. chamaenerii</i> Savile (1962) | – | N | Savile, 1962, Sivanesan, 1984 and Aptroot, 2006 | DB | Aptroot (2006) stated this belongs to <i>Davidiella</i> . |
| <i>R. evanida</i> (J. G. Kühn) Sacc. (1886) | <i>M. gentianae</i> (Niessl) Lindau (1897) [syn. <i>M. galatea</i> (Sacc.) Jacz. (1917)] | – | N | Petrak, 1940a, Tomilin, 1979 and Braun, 1998 | DB | Aptroot (2006) states the type and additional material studied belong to <i>Davidiella</i> . |
| <i>R. pteridiicola</i> Petr. (1927) | ? <i>M. aquilina</i> (Fr.) J. Schröt (1894) | – | N | Petrak, 1927, Eriksson, 1992, Braun, 1998 and Aptroot, 2006 | DB | Aptroot (2006) studied material from India (IMI 152515) and states it belongs to <i>M. punctiformis</i> . |
| <i>R. trifolii</i> Jaap (1910) | <i>M. carinthiaca</i> Jaap (1908) | – | N | Jaap, 1910, Tomilin, 1979, Braun, 1998 and Aptroot, 2006 | DB | Aptroot (2006) studied authentic material, states it is a parasitic species of <i>Davidiella</i> and proposed a new combination <i>Davidiella carinthiaca</i> (Jaap) Aptroot. |
| <i>Ramularia</i> sp. | <i>M. nawae</i> Hiura & Ikata (1929) | – | N | Kwon and Park, 2004 and Berbegal et al., 2013 | DB | Asexual ramularia-like morph observed but ITS closely related to <i>Phaeoipleospora</i> (<i>Mycosphaerellaceae</i>). LSU not available. |
| <i>R. aplospora</i> Speg. (1879) | <i>M. alchemillicola</i> Vassiljevsky 1925 | – | Y | Vasil'evskij and Karakulin, 1937, Tomilin, 1979 and Braun, 1998 | NEP | |
| <i>R. brunnea</i> Peck (1878) | <i>M. tussilaginis</i> (Rehm) Lindau (1903) | – | N | Wolf, 1912, Vasil'evskij and Karakulin, 1937, Tomilin, 1979, Braun, 1998 and Aptroot, 2006 | NEP | |
| <i>R. lactea</i> (Desm.) Sacc. (1882) | <i>M. violae</i> Potebnia 1910 | – | Y | Tomilin, 1979 and Braun, 1998 | NEP | |
| <i>R. obducens</i> Thüm. (1881) | <i>M. pedicularis</i> (P. Karst.) Lind (1913) | – | N | Savile, 1968 and Braun, 1998 | NEP | Aptroot (2006) could not locate the type but after observing other Scandinavian material states that it belongs to section |

| Asexual morph ^a | Sexual morph ^a | Current name | CA ^b | References | Sexual link ^c | Notes |
|--|--|--------------|-----------------|--|--------------------------|--|
| | | | | | | Caterva. |
| <i>R. onobrychidis</i> Allesch. (1892) | ? <i>M. onobrychidis</i> (Hollós) Tomilin (1968) | – | N | Švareman et al., 1973 and Braun, 1998 | NEP | Aptroot (2006) states the type may have been destroyed during the war. |
| <i>R. sambucina</i> Sacc. (1882) | <i>M. ebulina</i> Petr. (1915) | – | N | Petrak, 1915, Tomilin, 1979 and Aptroot, 2006 | NEP | Aptroot (2006) states the isotype in L belongs to section Caterva. |
| <i>R. tricherae</i> Lindr. (1902) | ? <i>Sphaerella sylvatica</i> Sacc. & Speg. (1878) [syn. <i>M. scabiosae</i> Tomilin (1971)] | – | Y | Laibach, 1921, Braun, 1998 and Aptroot, 2006 | NEP | Aptroot (2006) states the type belongs to section Caterva. |
| <i>R. ulmariae</i> Cooke (1876) | <i>M. filipendulae-denudatae</i> Kamilov (1973) | – | N | Tomilin, 1979 and Braun, 1998 | NEP | |
| <i>R. urticae</i> Ces. (1863) | <i>M. superflua</i> (Fuckel) Petr. (1940) | – | Y | Tomilin, 1979, Sivanesan, 1984 and Petrak, 1940b | NEP | |

^a bas.: basionym; syn.: synonym.

^bCA: Cultures of the *Ramularia* morph available; Y – Yes, N – No.

^cSexual link; EP – Experimentally Proven; DB – Doubtfull; NEP – Not Experimentally Proven.

The MAT1-1-1 alignment contained 30 taxa, including the outgroup *Cercospora beticola* (GenBank DQ192581), and 570 characters, including alignment gaps, from which 175 were constant, 74 were variable and parsimony-uninformative, and 321 were parsimony-informative. The MAT1-2-1 alignment contained 37 taxa, including the outgroup *Cercospora beticola* (GenBank DQ192582), and 233 characters, including alignment gaps. Of these characters, 67 were constant, 31 were variable and parsimony-uninformative, and 135 were parsimony-informative. Similar trees were obtained with both neighbour-joining and parsimony methods. Two most parsimonious trees were obtained from the MAT-1-1-1 sequence alignment and nine most parsimonious trees were obtained from the MAT1-2-1 sequence alignment. The most parsimonious trees differed slightly in the arrangement of the taxa within the clades of *R. vizellae* (Fig 2 and Fig 3) and of *R. unterseheri* (Fig 3) but the global tree topology was identical. The trees obtained for both MAT1-1-1 (Fig 2) and MAT1-2-1 (Fig 3) datasets showed that *R. endophylla* and *R. vizellae* cluster in separate clades with bootstrap support values of 100 % (MAT1-1-1) and 100 % and 97 % (MAT1-2-1), respectively. In the tree obtained for MAT1-2-1 the clade of *R. unterseheri* is supported with 98 % bootstrap. Strict consensus trees were calculated for each locus and the branches present were depicted in thicker lines (Fig 2 and Fig 3). The phylogenetic trees obtained from the mating-type sequences are in agreement with the parsimony and Bayesian analyses of the multigene dataset.

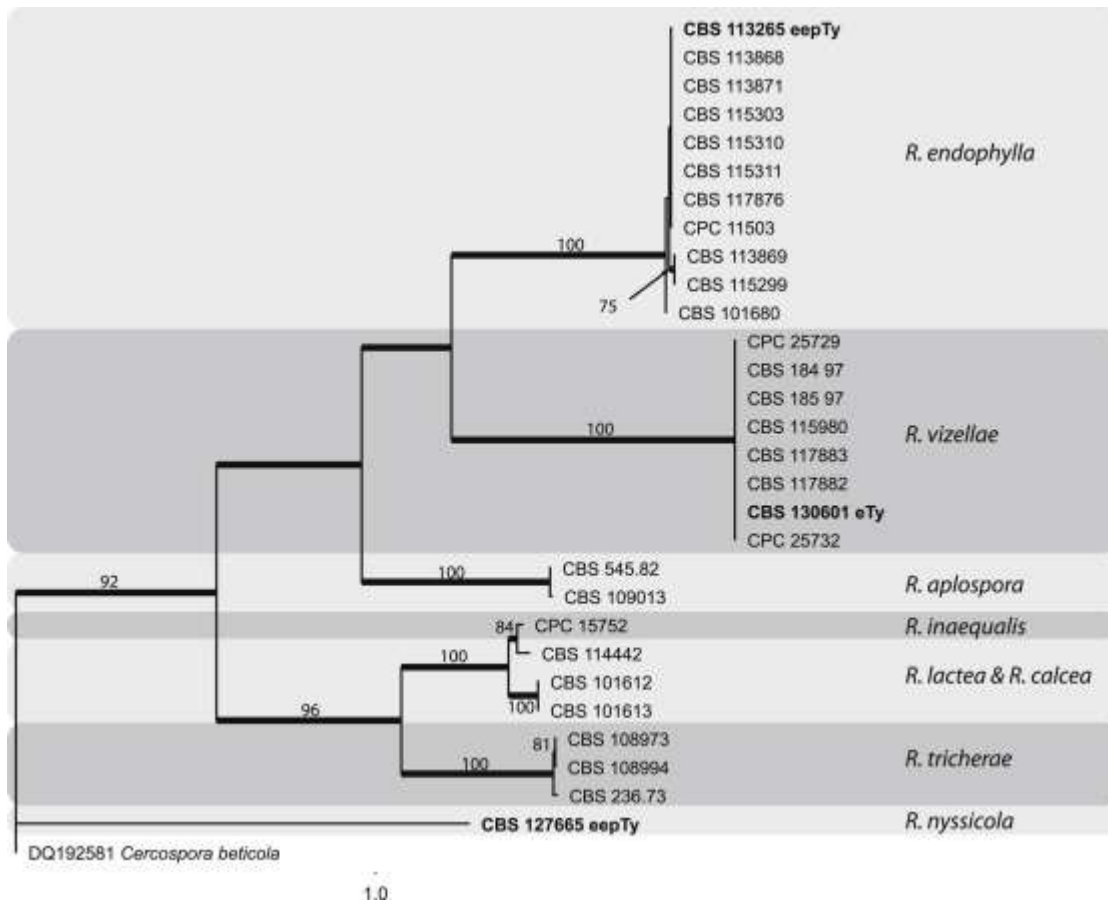


Fig 2. The first of two equally most parsimonious trees obtained from the MAT1-1-1 sequence alignment. Bootstrap support values from 1000 replicates are shown at the nodes. The tree was rooted to *Cercospora beticola* (GenBank DQ192581). TL = 912 steps, CI = 0.760, RI = 0.919, RC = 0.698, HI = 0.240. Strains in bold and marked as 'eTy' are ex-types and those marked as 'eepTy' are ex-epitypes.

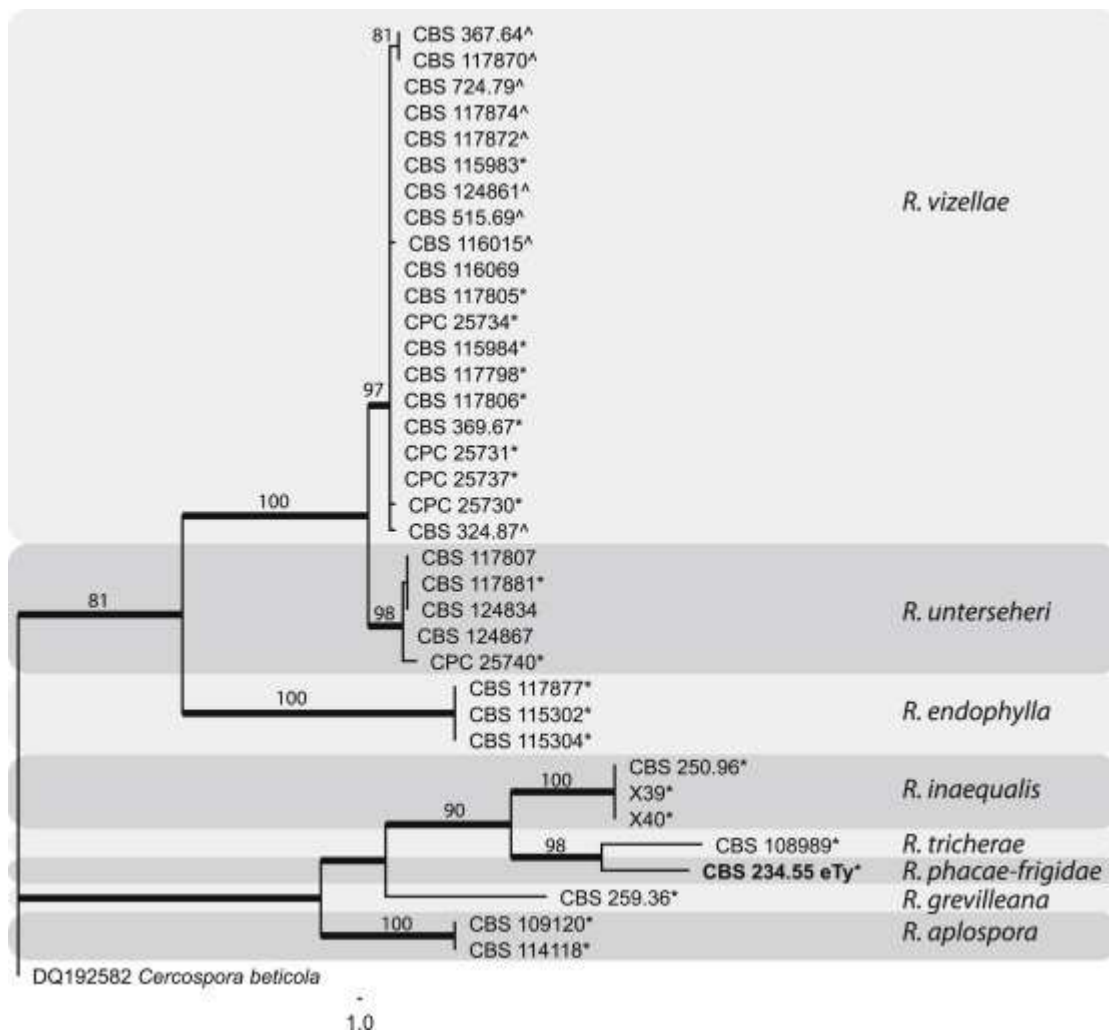


Fig 3. The first of nine equally most parsimonious trees obtained from the MAT1-2-1 sequence alignment. Bootstrap support values from 1000 replicates are shown at the nodes. The tree was rooted to *Cercospora beticola* (GenBank DQ192582). TL = 379 steps, CI = 0.736, RI = 0.873, RC = 0.643, HI = 0.264. Strains in bold and marked as ‘eTy’ are ex-types and those marked as ‘eepTy’ are ex-epitypes. The asterisk (*) represents sequences obtained with the primer combination MAT1-1-1 forward and MAT1-2-1 reverse (Table 2) and the caret (^) represents strains obtained with both the usual primer combination MAT1-2-1 forward and reverse and the unusual primer combination MAT1-1-1 forward and MAT1-2-1 reverse (Table 2). Strains without these symbols represent sequences obtained only with the regular primer combination MAT1-2-1 forward and reverse (Table 2).

Taxonomy

Ramularia endophylla Verkley & U. Braun, *Mycol. Res.* **108**: 1276 (2004)

Synonyms: *Sphaeria punctiformis* Pers., *Ann. Bot. (Usteri)* **11**: 26 (1794), non *Ramularia punctiformis* Sacc. (Saccardo, 1904).

Astoma punctiforme (Pers.) Gray, *Nat. Arr. Brit. Pl.* (London) **1**: 524 (1821)

Sphaerella punctiformis (Pers.) Rabenh., Klotzschii Herb. Viv. Mycol., ed. nov.: no. 264 (1856)

Mycosphaerella punctiformis (Pers.) Starbäck, *Bih. Kongl. Svenska Vetensk.-Akad. Handl.* **15** (2): 9 (1889)

Diatrype punctiformis (Pers.) Zahlbr., *Cat. Lich. Univers.* **7**: 780 (1931)

For further synonyms, see Tomilin (1979) and Aptroot (2006).

Specimens examined. **Belgium:** Namur, on leaves of *Quercus* sp., 7 May 1997, A. Aptroot (CBS 942.97). **d Netherlands:** Utrecht, on dead leaves of *Castanea sativa*, 23 Feb. 1999, A. Aptroot (CBS 101680); on dead leaves of *Quercus robur*, Apr. 2003, G. Verkley (CBS H-7949, epitype; ex-epitype culture CBS 113265); on living leaf of *Quercus robur*, G. Verkley (CBS 113868; CBS 113869; CBS 113870); on fallen leaf of *Quercus robur*, G. Verkley (CBS 113871); on living leaves of *Quercus robur*, Sep. 2008, G. Verkley (CBS 115299; CBS 115302); on living leaf of *Quercus robur*, May 2013 (CBS 115303; CBS 115304); on dead leaf of *Quercus robur*, Aug. 2002, (CBS 115310; CBS 115311); on *Quercus robur*, G. Verkley (CBS 117876); on *Quercus robur*, 18 Jun. 2009, G. Verkley (CBS 117877). **d South Korea:** unknown host, 1 Jan. 2004, H.D. Shin (CPC 11503).

Notes: The link between *Ramularia endophylla* (Verkley *et al.* 2004) and *Mycosphaerella punctiformis* (Starbäck 1889), initially described as *Sphaeria punctiformis* (Persoon 1794), was experimentally proven by Verkley *et al.* (2004) with morphological and phylogenetic data. Although the oldest epithet among these two names is ‘*punctiformis*’ (Persoon 1794), the name *Ramularia punctiformis* Sacc. (Saccardo, 1904) is already in use. Several possible epithets can be found among the synonyms proposed by Tomilin (1979) and Aptroot (2006). However, these specimens are often in poor state, contain immature perithecia or represent species never collected or observed after their first description (Aptroot 2006). In addition, several type specimens of other *Mycosphaerella* species have been found to be indistinguishable from *M. punctiformis* (Aptroot 2006) and the names represent valid epithets in case this material is recollected and prove to be the same species. With the objective to clarify and stabilize the taxonomy of this species, we propose that the epithet ‘*endophylla*’ is conserved since it represents an unequivocally proven link between sexual and asexual stage of this species, both biologically and phylogenetically.

Ramularia grevilleana (Oudem.) Jørst., *Meld. Stat. Plantepatol. Inst.* **1**: 17 (1945)

Basionym: *Cylindrosporium grevilleanum* Oudem., *Arch. Néerl. Sci. Exact. Nat.* **8**: 392 (1873), asexual morph [*Cylindrosporium* sp., in Tulasne & Tulasne (1863: 288)].

Synonyms: *Sphaeria fragariae* Tul. & C. Tul., *Ann. Sci. Nat., Bot.* **5**: 112 (1856), nom. illeg., non Schwein. 1832.

Stigmatea fragariae Tul. & C. Tul., *Select. Fung. Carpol.* **2**: 288 (1863).

Ramularia fragariae Peck, *Ann. Rep. N.Y. State Mus. Nat. Hist.* **32**: 43 (1880).

Sphaerella fragariae (Tul. & C. Tul.) Sacc., *Syll. Fung.* **1**: 505 (1882).

Mycosphaerella fragariae (Tul. & C. Tul.) Lindau, Nat. Pflanzenfam., Teil 1, 1(1): 424 (1897).

Ramularia punctiformis Sacc., Alaska Exp. Crypt.: 16 (1904).

For further synonyms based on asexual morphs, see Braun (1995: 248).

Specimens examined. **Sweden:** Uppland, Alsike, on *Fragaria ananassa*, 4 Oct. 1989, E. Gunnerbeck (CBS 114732 = UPSC 3244). **d Netherlands:** unknown district, host, collector and date (CBS 259.36; CBS 298.34). **d New Zealand:** Auckland, on *Fragaria*×*ananassa* ‘Tioga’ (CBS 719.84).

Notes: This pathogen is known for causing leaf spot disease in strawberry, both cultivated and wild species, and has a worldwide distribution (Braun 1998). The link between *Ramularia grevilleana* (Jørstad 1945) and *Mycosphaerella fragariae* (Lindau 1897) was experimentally proven by Dudley (1889) who observed the ascospores germinating within the ascus inside the perithecium, developing into mycelium that grew out through the perithecium wall and ostium, and produced conidia. Since then, most authors have treated this link as reliable and both names have appeared together in several publications (Maas, 1984, Crous et al., 2000, Braun and Pennycook, 2003 and Kirschner, 2009). *Ramularia grevilleana* (Jørstad 1945) was initially described as *Cylindrosporium grevilleanum* by Oudemans (1873). The confused nomenclatural history of this asexual morph name, previously attributed to Tulasne & Tulasne (1863), was discussed and clarified by Braun & Pennycook (2003). *Mycosphaerella fragariae* (Lindau 1897) was first described as *Sphaeria fragariae* (Tulasne 1856), which is a nom. illeg. (homonym). *Stigmatea fragariae* Tul. & Tul. (Tulasne & Tulasne 1863) is the first valid name for this species but a reallocation of this name to *Ramularia* is not possible because *R. fragariae* already exists. *Cylindrosporium grevilleanum* (Oudemans 1873) is the oldest available epithet among the synonyms and the basionym of the current name *Ramularia grevilleana* (Jørstad 1945), which is the nomenclaturally correct denomination for this species.

Ramularia phacae-frigidae (E. Müll. & Wehm.) Videira & Crous, **comb. nov.**

MycoBank No.: MB812600

Basionym: *Mycosphaerella phacae-frigidae* E. Müll. & Wehm., *Sydowia*8: 190 (1954).

Specimens examined. **Switzerland:** Corveglieria, above St. Moritz, from *Phaca frigida*, 20 Jul. 1953, E. Müller (ex-type culture CBS 234.55).

Notes: When *Mycosphaerella phacae-frigidae* was originally described (Müller & Wehmeyer 1954), the ascospores were isolated, producing a *Ramularia* state that was not named at that time. Based on morphological and molecular evidence we propose a new combination for this name in *Ramularia*. Culture CBS 234.55 was deposited by E. Müller in the CBS culture collection in May 1955 and is from the same host, locality and date as the original material used for the description of *M. phacae-frigidae*, which indicates that it is an ex-type strain.

Ramularia unterseheri Videira & Crous, **sp. nov.**

MycoBank No.: MB812599

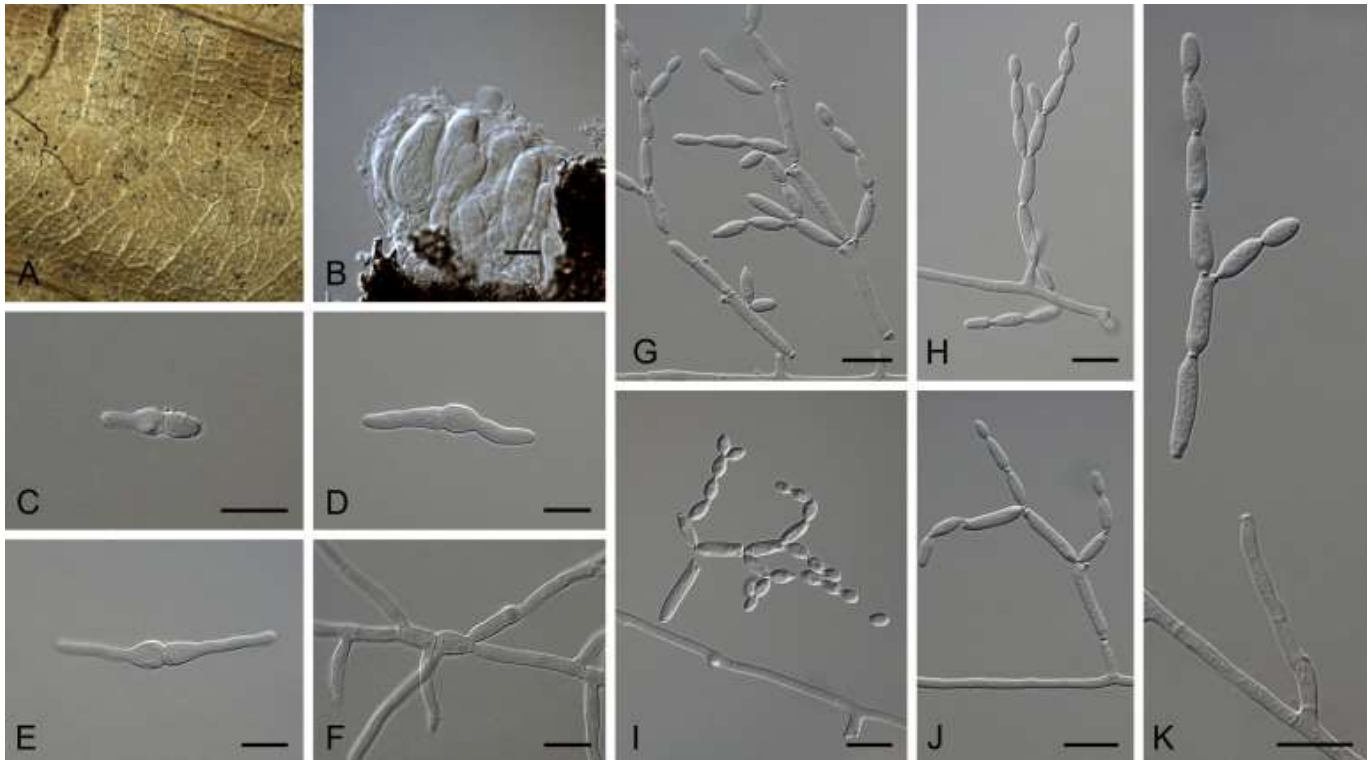


Fig 4. *Ramularia unterseheri*. (A). leaf of *Fagus sylvatica* (CPC 25740); (B). Broken ascoma bearing asci with ascospores (CPC 25740); (C–F). germinating ascospores (CPC 25740); (G–K). hypha, conidiophores and conidia (CBS 124884). — Scale bars = 10 µm.

Etymology. Named after Martin Unterseher, whose research focus on biodiversity and ecology of endophytic fungi, and the person who collected most of the strains of this species currently deposited at the CBS-KNAW collection.

Description: *Mycelium* consisting of septate, branched, smooth, hyaline hyphae, (1–)1.5–2 µm diam. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* smooth, hyaline, arising from hyphae, terminal and lateral, (5.5–)11–14(–20) × (1.5–)2 µm, sympodially proliferating with 1–3 apical loci, flattened or protuberant, cylindrical; scars thickened, darkened, refractive, 0.5–1 µm diam. *Ramoconidia* subcylindrical to oval or ovoid, 0–1-septate, hyaline, smooth, (8–)10–12(–18) × (1.5–)2–2.5(–3) µm. *Intercalary conidia* hyaline, smooth, aseptate, oval to ovoid, (6–)8–9(–13) × (2–)2.5–3 µm, in branched chains of up to six conidia. *Terminal conidia* hyaline, smooth, aseptate, obovoid to oval (3.5–)5–6(–7) × (1.5–)2–2.5(–3) µm; hila thickened, darkened, refractive, 0.5–1 µm diam.

Culture characteristics: On MEA surface raised, radially striated, with smooth mycelium, rosy vinaceous, with undulate margins and reverse cinnamon, reaching 10 mm after 2 wk at 25 °C. On OA surface flat, smooth mycelium, with undulate edge, reaching 15 mm after 2 wk at 25 °C. On PDA radially striated, smooth mycelium, rosy buff with undulate margins, reverse cinnamon, reaching 12 mm after 2 wk at 25 °C.

Specimens examined. Germany: Mecklenburg-Vorpommern, Greifswald, Elisenhain, on leaf litter of *Fagus sylvatica*, 4 Jan. 2008, M. Unterseher (holotype CBS H-22285, ex-type culture CBS 124884), additional collections with same details (CBS 124844, 124846, 124852, CBS 124867); on living leaves from understorey of *Fagus sylvatica*, 8 Jan. 2008, M. Unterseher (CBS 124826, CBS 124827, CBS 124830, CBS 124831, CBS 124834, CBS 124836, CBS 124838); without locality, on seed of *Fagus sylvatica*, date unknown, U. Delfs-Siemer (CBS

355.90); Hessen, Schlangenbad, on leaf of *Alnus* sp., 2012, W. Quaedvlieg (CPC 25739 = W6); Mecklenburg-Vorpommern, Bornhof, on *Lupinus* sp., U. Feiler 1993 (CBS 588.93); Bavaria, Munich, room inside a castle, May 2011, unknown collector (CBS 130721). **Netherlands:** Utrecht, Amelisweerd, on dead leaves of *Fagus sylvatica*, 25 Apr. 2005, G. Verkley (CBS 117801 = CPC 12091); Amersfoort, dead leaves of *Fagus sylvatica*, 25 Jul. 2005, G. Verkley (CBS 117807 = CPC 12095); Baarn, Baarnsche Bos, on *Tilia* sp., 26 Apr. 2004, G. Verkley (CBS 117880 = CPC 11209, CBS 117881 = CPC 11211); Baarn, decaying leaves of *Acer pseudoplatanus*, 26 Apr. 2004, G. Verkley (CBS 117878 = CPC 11206, CBS 117879 = CPC 11207); Rhijnauwen forest, decaying leaves of *Fagus sylvatica*, 17 May 2012, S.I.R. Videira (CPC 25740 = X2).

Notes – *Ramularia unterseheri* is a plurivorous species that is often found in *Fagus sylvatica* leaves in Germany and the Netherlands. It differs from *R. vizellae* by shorter and narrower ramoconidia [(8–) 10–12 (–18) × (1.5–) 2–2.5 (–3) μm versus (8–) 10–12 (–23) × (2.5–) 3–3.5 (–5) μm] and longer and narrower terminal conidia [(3.5–) 5–6 (–7) × (1.5–) 2–2.5 (–3) versus 4–5 (–5.5) × (2–) 3 (–3.5) μm]. It has been previously isolated from dead overwintered leaves as well as from living leaves (Verkley *et al.* 2004). In this study, a mycosphaerella-like sexual morph was observed (Fig 4, a–f) in newly collected samples of overwintered leaves in the Netherlands, but the available material was too scarce to provide a description.

Ramularia vizellae Crous, *Persoonia* 27: 37 (2011)

Mycobank No.: MB560566

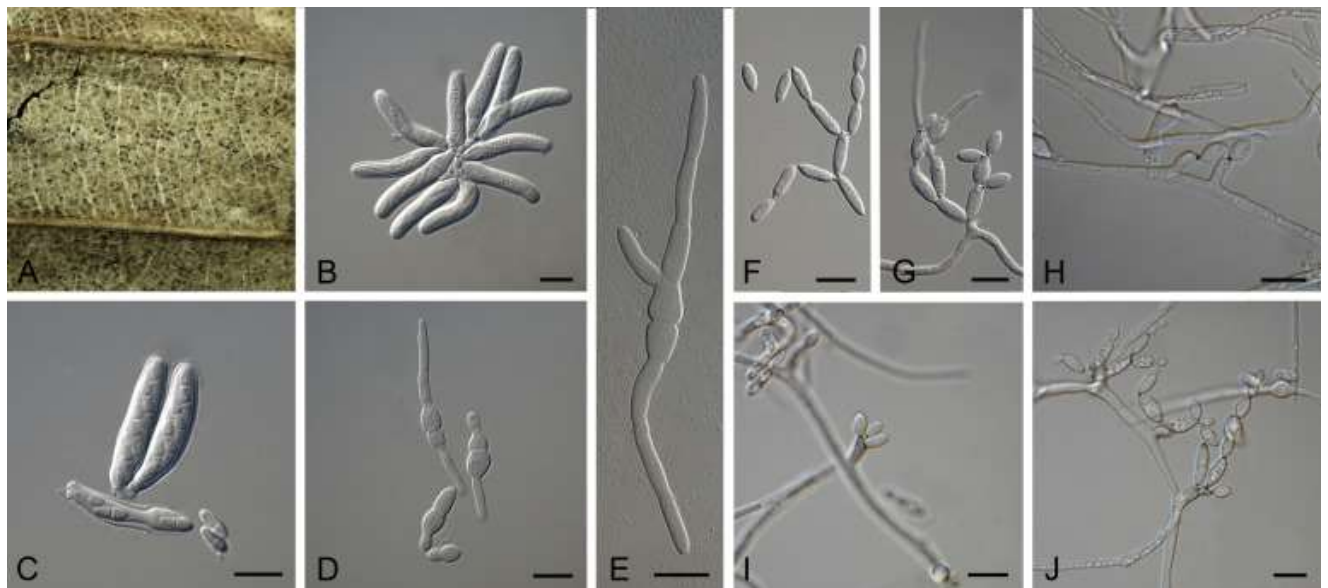


Fig 5. *Ramularia vizellae*. (A). leaf of *Carpinus* sp. (CPC 25730); (B), (C). Asci bearing ascospores (CPC 25730). (D, E). Germinating ascospores (CPC 25730); (F–J). hypha, conidiophores and conidia (F, G: CPC 25729; H: CBS 117871; I, J: CBS 117799). — Scale bars = 10 μm.

Specimens examined. **France:** on fruit of *Malus sylvestris*, C. Moreau (CBS 367.64) Austria: Graz, Innere Ragnitz, on *Sambucus nigra*, 12 Oct. 2012, C. Scheuer (CPC 25738 = X31). **d Germany:** Mecklenburg-Vorpommern, Greifswald, Elisenhain, on leaf litter from *Fagus sylvatica*, 4 Jan. 2008, M. Unterseher (CBS 124861); Hessen, Schlangenbad, on leaf of unidentified plant, W. Quaedvlieg (CPC 25733 = W7); Bavaria, München, on overwintering leaves of *Tilia* sp., Jul. 1979, A. John (CBS 724.79) **d Netherlands:** in leaf spot caused by

Mycosphaerella sp. on *Brassica* sp. (CBS 324.87); Gelderland: Randwijk, on dead leaf litter of *Malus* sp. (CBS 115980); Randwijk, on dead leaf litter of *Malus* sp. (CBS 115981, CBS 115982, CBS 115983, CBS 115984, CBS 116015, CBS 116069). Utrecht: Amelisweerd, on dead leaves of *Acer pseudoplatanus*, 25 Apr. 2005, G. Verkley (CBS 117799 = CPC 12089); on dead leaves of *Carpinus betulus*, 25 Apr. 2005, G. Verkley (CBS 117802 = CPC 12092); Amersfoort, on dead leaves of *Tilia* sp., 25 Apr. 2005, G. Verkley (CBS 117806 = CPC 12096); Baarn, Park Kasteel Groeneveld, on decaying leaves of *Quercus rubra*, 26 Apr. 2004, G. Verkley (CBS 117870 = CPC 11193), on decaying leaves of *Quercus rubra*, G. Verkley (CBS 117871 = CPC 11194), on *Amelanchier lamarckii*, 26 Apr. 2004, G. Verkley (CBS 117872 = CPC 11197, CBS 117873 = CPC 11198); Lage Vuursche, on *Aesculus hippocastanum*, G. Verkley (CBS 117874 = CPC 11200, CBS 117875 = CPC 11201), on decaying leaves of *Sorbus aucuparia*, 26 Apr. 2004, G. Verkley (CBS 117882 = CPC 11212, CBS 117883 = CPC 11213); garden, Eemnesserweg 90, on dead leaves of *Acer pseudoplatanus*, 7 May 1996, H.A. van der Aa (CBS 184.97, CBS 185.97), on *Acer pseudoplatanus*, 15 Oct. 1968, H.A. van der Aa (CBS 515.69); Baarn, ruderal terrain at Drakenburgerweg, on young leaves of *Lotus uliginosus*, 18 Jun. 1967, H.A. van der Aa (CBS 369.67); Utrecht Botanical Garden, on overwintered leaves of *Corylus* sp., 21 Apr. 2012, S.I.R. Videira (CPC 25728 = MP19), on overwintered leaves of *Quercus* sp., 21 Apr. 2012, S.I.R. Videira (CPC 25729 = MP20), on overwintered leaves of *Carpinus* sp., 21 Apr. 2012, S.I.R. Videira (CPC 25730 = MP21), on overwintered leaves of *Quercus* sp., 21 Apr. 2012, S.I.R. Videira (CPC 25731 = MP23), on overwintered leaves of *Fagus* sp., 21 Apr. 2012, S.I.R. Videira (CPC 25732 = MP24); Utrecht Rhijnauwen park, on *Aesculus hippocastanum*, 25 Apr. 2005, G. Verkley (CBS 117805 = CPC 12094), on overwintered leaves of *Fagus* sp., 17 May 2012, S.I.R. Videira (CPC 25734 = X1, CPC 25735 = X3), on fruit scales of *Carpinus betulus*, 25 Apr 2005, G. Verkley (CBS 117798 = CPC 12088), on overwintered leaves of *Corylus* sp., 17 May 2012, S.I.R. Videira (CPC 25736 = X4), on overwintered leaves of *Aesculus hippocastanum*, 17 May 2012, S.I.R. Videira (CPC 25737 = X5); Soesterberg 'De Stompert', on dead fallen leaves of *Quercus robur*, G. Verkley (CBS 113267). **d South Africa:** on leaves of *Protea* sp. in association with *Vizella interrupta*, 2 May 2010, P.W. Crous (ex-type culture CBS 130601 = CPC 18283). **d Switzerland:** on *Phaseolus* sp. (CBS 428.74 = IHEM 3995). **Ukraine:** Seversky Donets river, NNP Svjatje Gory, on *Acer campestre*, 21 Jul. 2008, A. Akulov (CPC 15539, CPC 15541).

Notes: This species was recently described from leaves of *Protea* sp. from South Africa (Crous *et al.* 2011) in association with lesions caused by *Vizella interrupta* in what was deemed as either a chance encounter, as sporulation was not observed in the leaf itself, or an indication that the species was a secondary invader of the diseased leaf tissue. *Ramularia vizellae* is now known from numerous hosts in many European countries (Fig 1, Table 1) as well as in South Africa. A mycosphaerella-like sexual morph was observed in freshly collected samples in the Netherlands (Fig 5, a–e), but a description is not provided due to the scarcity of material examined.

Discussion

Based on the epitypification of *Mycosphaerella punctiformis* (now *Ramularia endophylla*) (Verkley *et al.* 2004), and the molecular characterization of *Ramularia pusilla* (type species of *Ramularia*) by Kirschner (2009), the names *Mycosphaerella* and *Ramularia* are confirmed as congeneric. This means it is now possible to separate species closely allied to *R. endophylla*, such as *R. nyssicola* (Minnis *et al.* 2011). Based on the multigene phylogeny generated in this study (Fig 1), the host range and distribution of *R. endophylla* has been narrowed, since

most of the strains were isolated from *Quercus* leaves collected in the Netherlands, with the exception of one strain collected from *Castanea sativa* and another strain collected from Korea.

The heterogeneity observed in the ITS sequences in the past was further accentuated when protein coding genes were added to the analysis and both the Bayesian and parsimony analyses based on five genes in the present study split this species complex into three species: *R. endophylla*, *Ramularia vizellae* and the newly described species, *Ramularia unterseheri*. The identification of these closely related species based on morphology alone is difficult, and the ITS barcode alone is insufficient for species level identification. Based on the individual gene trees, each of the partial gene sequences of ACT, RPB2 and GAPDH are good phylogenetic markers to use in addition to the ITS barcode since they successfully separate the three species.

The new species described in this study, *R. unterseheri* (Fig 4), is only known from the Netherlands and Germany, but with a rather broad host range, namely *Acer* (*Sapindaceae*), *Alnus* (*Betulaceae*), *Fagus* (*Fagaceae*) and *Tilia* (*Malvaceae*). The intraspecific variation observed in each clade (Fig 1) is a result of the variation observed in the gene sequences among the strains. The internal structure of this variation was not consistent between different loci and cryptic speciation is unlikely to account for these genetic differences.

In this study, a mycosphaerella-like sexual morph was observed for both *R. unterseheri* (Fig 4) and *R. vizellae* (Fig 5) in overwintered leaves collected in the Netherlands. In addition, several of the strains of *R. unterseheri* were isolated from living material as endophytes in the previous work of Verkley *et al.* (2004). These observations indicate that these species most likely have a life cycle similar to that of *R. endophylla* but more work needs to be done in order to fully understand these fungal life-cycles. Even though the life-cycle of *R. endophylla* is well known (Verkley *et al.* 2004), some questions still remain unanswered, e.g. the role played by the *Asteromella* spermatial state in the development of the species.

Sexual reproduction plays an important role in the dynamics and fitness of a species by introducing variability through genetic recombination and the mating type genes are essential for the sexual cycle to occur. The similarity of homologous mating-type genes is usually very low except for the high mobility group and the alpha domains (Turgeon 1998). These conserved domains were successfully used to clarify the phylogenetic relationships among closely related species (Du *et al.*, 2005 and Paoletti *et al.*, 2005) but were not effective in resolving the *Cercospora apii* complex (Groenewald *et al.* 2006). The MAT1-2-1 tree showed that *R. vizellae*, *R. unterseheri* and *R. endophylla* strains cluster in separate well supported clades and the same can be observed in the MAT1-1-1 tree for the strains of *R. endophylla* and *R. vizellae*. In this study, the mating-type loci were effective in the separation of this complex. The complete characterization of the mating-type genes in *Ramularia* species has not been performed before and the evidence indicates these species are heterothallic since the strains with a MAT1-1-1 sequence did not amplify the MAT1-2-1 locus and vice-versa. However, transitions between heterothallic (self-sterile) and homothallic (self-fertile) sexual cycles are common among fungi and which represents the ancestral state is unknown.

Experimentally proven links in literature between *Ramularia* and *Mycosphaerella* are limited (Table 3). There are six cases where the authors reported they observed the complete life

cycle of the fungus from ascospore to conidia. Experimentally confirmed links include *R. endophylla*/*M. punctiformis* (= *R. endophylla*) (Verkley *et al.* 2004), *Ramularia grevilleana*/*Mycosphaerella fragariae* (= *R. grevilleana*) (Oudemans, 1873 and Braun and Pennycook, 2003), *Ramularia variabilis*/*Mycosphaerella mariae* (= *R. variabilis*) (von Arx 1949), and *Ramularia inaequalis*/*Mycosphaerella hieracii* (= *R. inaequalis*) (Klebahn 1918). In the case of *Mycosphaerella nyssicola*, no *Ramularia* morph has been observed, but based on molecular evidence the species belongs in *Ramularia*, and a new combination (= *R. nyssicola*) was made for this species (Videira *et al.* 2015). When *Mycosphaerella phacae-frigidae* was described (Müller & Wehmeyer 1954), the ascospores that were isolated produced a *Ramularia* state in culture that was not named at the time, and hence a new combination is introduced for this name in *Ramularia* (= *Ramularia phacae-frigidae*).

Sivanesan (1984) reported the links *Ramularia gossypii*/*M. areola*, *Ramularia nigromaculans*/*Mycosphaerella nigromaculans*, and *Ramularia urticae*/*Mycosphaerella superflua*, among others. *Ramularia gossypii* has been reassigned to the genus *Ramulariopsis* (Braun & Pennycook 1993) and *R. nigromaculans* has been excluded from *Ramularia* based on its pigmented conidia (Braun 1998). *Ramularia urticae*/*M. superflua*, and at least eight other links (Table 3), have not been experimentally proven, and await further collections and study.

Other existing links have been considered doubtful since Aptroot (2006) examined the herbarium type specimens of some *Mycosphaerella* that he considered belonged to *Davidiella* (Table 3). An interesting case is that of *Mycosphaerella nawae*, a pathogen causing circular leaf spot of persimmon that was originally reported from Japan (Ikata & Hitomi 1929) but has now spread worldwide (Berbegal *et al.* 2013). In Korea, a ramularia-like morph was observed (Kwon & Park 2004) but its importance during the infection processes was not established (Berbegal *et al.* 2013). Despite its importance as a plant pathogen, no cultures of this species are available in public culture collections. A recent study by Berbegal *et al.* (2013) generated two ITS sequences (GenBank GQ465767 & GQ465768) of *M. nawae* that, when compared with other dothideomyceteous ITS sequences in NCBI's GenBank, places the species near or in *Phaeophleospora* within the *Mycosphaerellaceae* (Quaedvlieg *et al.* 2014). This link is considered doubtful, awaiting further collections of fresh material.

In conclusion, we have shown that the *R. endophylla* species complex consists of three species, namely *R. endophylla*, *R. vizellae*, and a novel species described in this paper, *R. unterseheri*. We show that *R. vizellae* has a much wider host range and geographical distribution than originally assumed and observed its sexual stage. In spite of close to 1000 species names in *Ramularia*, and more than 10 000 species that have been described in *Mycosphaerella s. lat.*, the present study could only confirm six connections in *Ramularia*, and one new combination was proposed to accommodate *R. phacae-frigidae*. Additional collections of other names in *Mycosphaerella* may reveal more species that are true members of *Ramularia*, but presently the majority appears to belong to other genera (Quaedvlieg *et al.* 2014). In much of the plant pathology literature the name *Mycosphaerella* has been applied in a broad morphological and non-phylogenetic sense. For these fungi, the term mycosphaerella-like sexual morph is more appropriate. In accordance with the newly revised ICN code, the generic name *Ramularia* has been protected over that of *Mycosphaerella* and will be applied to this genus in the future.

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