Additions to the Inventory of the Genus *Alternaria* Section *Alternaria* (Pleosporaceae, Pleosporales) in Italy

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**Abstract:** The genus *Alternaria* is comprised of well-known plant pathogens causing various important diseases in plants, as well as being common allergens in animals and humans. Species of *Alternaria* can be found as saprobes associated with various dead plant materials. This research aims to enhance the taxonomy of saprobic species in the genus *Alternaria* found on grasses and herbaceous plants from Italy, based on multi-locus phylogenetic analyses of a concatenated ITS, LSU, SSU, *tefl-a*, *rpb2*, *gapdh* and *Alt-a1* DNA sequence dataset combined with morphological characteristics. Multi-locus phylogenetic analyses demonstrated six novel species belonging to the genus *Alternaria* sect. *Alternaria* as: *A. muriformispora* sp. nov., *A. obpyriconidia* sp. nov., *A. ozoidea* sp. nov., *A. pseudoorientaria* sp. nov., *A. rostroconidia* sp. nov. and *A. torilis* sp. nov. Detailed morphological descriptions, illustrations and an updated phylogenetic relationship of taxa in the genus *Alternaria* sect. *Alternaria* are provided herein.

**Keywords:** Dothideomycetes; Italian dematiaceous hyphomycetes; multi-locus phylogeny; saprobic fungi; taxonomy

1. Introduction

The genus *Alternaria* is classified in the family Pleosporaceae, order Pleosporales, class Dothideomycetes [1–3]. The genus contains over 700 species epithets [4], but approximately 378 species are accepted within 28 sections, of which less than 100 species have molecular data to clarify their phylogenetic affinities [1–3,5–7]. Species of *Alternaria* are well-known as serious plant pathogens and post-harvest pathogens, causing major crop losses, or can be the causative agents of animal and human pathogens, causing phaeohyphomycosis or acting as airborne allergens [8–11].

*Alternaria* is well-known as dematiaceous hyphomycetes which can be found everywhere. The genus is characterized by mononematous, macro- or micronematous, un-
branching or branched conidiophores, integrated to discrete, mono- to polytretic conidigenous cells, solitary or catenate, straight or curved, phragmo- or dictyoseptate, smooth or verrucose, and median brown to dark brown conidia with rounded or narrowly-beaked tip. *Alternaria* occupies diverse ecological niches through its life modes, which range from endophytes to pathogens to saprobes on a wide range of host substrates (e.g., agricultural products, animals, plants, seeds, soil as well as the atmosphere) [2,8,10–12]. The genus has a cosmopolitan distribution, and is widely distributed in Asia, Australia, Europe, and North America [13].

Lawrence et al. [14] introduced *Alternaria* sect. *Alternaria* to accommodate *Alternaria* species, commonly referred to as small-spored *Alternaria* groups. The members of *Alternaria* sect. *Alternaria* can be morphologically distinguished from other sections in having small conidia produced in short chains (frequently less than 60 μm in length *in vitro*) [8,14,15]. However, this small-spored criterion is not significant to distinguish species in *Alternaria* sect. *Alternaria* from other *Alternaria* sections, when multi-locus phylogeny has become an essential tool to discriminate species in *Alternaria* [2]. According to Li et al. [2], some species in *Alternaria* sect. *Alternaria* have conidia larger than 60 μm, but these species were affiliated with *Alternaria* sect. *Alternaria* based on multi-locus phylogenetic evidence. The holomorph of sect. *Alternaria* is known for *A. alternata*, the generic type of the section, and the sexual morph is described as typically erumpent, small-sized, smooth, globose to ovoid, dark brown; with papillate ascomata; cylindrical to cylindric-clavate asci and muriform, ellipsoid to fusoid, brown, eguttulate, smooth-walled ascospores [2,10,16]. Woudenberg et al. [8] estimated 60 species accommodating in sect. *Alternaria* based on ITS gene analysis. Consequently, Woudenberg et al. [17] accepted only 11 species and one species complex in this section based on polyphasic taxonomic approaches, while 35 morphospecies were treated as synonyms of *A. alternata*. Later, Li et al. [2] showed that these 35 synonymized species can be divided into 5 main subclades in their analyses of *A. alternata*, pending questions on their conspecific status. Gannibal [15] re-circumscribed and amended the section based on morphological assessments by Simmons [18], and included the other 37 morphospecies and accepted 59 species in sect. *Alternaria*. Subsequently, the other four species (i.e., *A. calystegiae*, *A. diversispora*, *A. guaranitica* and *A. macalpinei*) were included in this section by Gannibal and Lawrence [19]. *Alternaria doliconidium* and *A. italica* were also included in this section by Wanasinge et al. [20] and Jayawardena et al. [21] respectively. Nishikawa and Nakashima [22] also included *A. iridicola* in this section. Recently, Li et al. [2] introduced another 14 species in sect. *Alternaria*. Therefore, 83 species are currently accommodated in this section.

Recent molecular phylogenetic studies have shown that the identification of species in *Alternaria* and its close relative genera challenged their morphological basis [8,14,17,23–28]. In general, the molecular data tends to support the recent morphologically distinct sub-generic species groups [8,10,14,29]. However, the phylogenetic relationships of the *Alternaria* sections are normally variable, with the morphological characteristics used to identify morphospecies. On the other hand, Woudenberg et al. [8] delineate species in *Alternaria* sect. *Alternaria* based on ITS. The whole-genome sequencing has become an essential tool to delineate ambiguous species in *Alternaria* and other complex species by Woudenberg et al. [17]. Thus, Woudenberg et al. [17] used multi-locus phylogeny based on ITS, gapdh, rpb2, tef1-a, Alt-a1, endoPG and OPA10-2 gene loci coupled with whole-genome and transcriptome comparisons to discriminate species in sect. *Alternaria*, and accepted only 11 phylogenetic species and one species complex in *Alternaria* sect. *Alternaria*. Furthermore, Woudenberg et al. [17] synonymized 35 morphospecies under *A. alternata*. In addition, the lack of phylogenetic effective coding genes led to confusion in the identification of *Alternaria* species [8,10,17]; therefore, re-defining and expanding the generic concept of *Alternaria* sect. *Alternaria* and other *Alternaria* sections is necessary. These studies suggest that morphological characteristics typically used to delineate species (e.g., conidium length, width and septation; chain structure; and beak shape) may not reflect evolutionary relationships between taxa.
Alternaria species are major plant pathogens that infect a vast array of plant hosts [2,8,10,11,15,30]. Members in Alternaria sect. Alternaria are still confused in their delineation of species which are largely based on morphology and the clarity of their host species. The present study aims to introduce six novel species in Alternaria sect. Alternaria on different specific plant hosts based on a morpho-molecular approach.

2. Materials and Methods

2.1. Collection, Examination, Isolation, and Conservation

Samples were collected from dead branches, stems, and twigs of several plant hosts in Italy. The samples were dried and preserved in paper bags for further observation and examination under an Olympus SZ61 series stereo microscope. Micro-morphological features were mounted in sterilized distilled water on a clean slide for examination, and captured by a Nikon DS-Ri2 camera under a Nikon ECLIPSE Ni compound microscope. The size of micro-morphological features was measured by using Tarosoft (R) Image FrameWork version 0.9.7. Photographic plates were edited and combined in Adobe Photoshop CS6 software (Adobe Systems Inc., San Jose, CA, USA). The type specimens were deposited at the herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU).

Axenic cultures were obtained from single spore isolation using a spore suspension technique described by Senanayake et al. [31]. Germinated conidia were aseptically cultivated on potato dextrose agar (PDA) or malt extract agar (MEA) media under day/night lighting at room temperature (25–30 °C). The growth of fungal colonies and sporulation in cultures were observed after two weeks and eight weeks of incubation, respectively. The ex-type living cultures were deposited in the Mae Fah Luang University Culture Collection (MFLUCC). The novel species were registered in Index Fungorum (http://www.indexfungorum.org/names/IndexFungorumRegister.htm, accessed on 15 July 2022).

2.2. DNA Extraction, PCR Amplification, and Sequencing

Fungal genomic DNA were extracted from fresh mycelia growing on PDA/MEA for one month using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, Hangzhou, China). The duplicated strain of each species was extracted DNA from fungal fruiting bodies using Forensic DNA Kit (Omega®, Norcross, GA, USA).

DNA fragments were amplified by polymerase chain reaction (PCR) with seven gene loci, including the internal transcribed spacers (ITS: ITS1-5.8S-ITS2) using primers ITS5 and ITS4 [32], the 28S large subunit rDNA (LSU) using primers LR0R and LR5 [33], the 18S small subunit rDNA (SSU) using primers NS1 and NS4 [32], the partial RNA polymerase second largest subunit (rpb2) using primers fRPB2-5F and fRPB2-7cR [34], the translation elongation factor 1-alpha (tef1-α) using primers EF1-728F and EF1-986R [35], Alternaria major allergen (Alt-a1) using primers ALT-F and ALT-R [25] and Glyceraldehyde 3-phosphate Dehydrogenase (gapdh) using primers GDP-1 and GDP-2 [36]. The polymerase chain reaction (PCR) was performed in a Veriti™ 96-Well Fast Thermal Cycler (Applied Biosystem, California, USA) following the protocol described in Li et al. [2]. All PCR products were sent to TsingKe Biological Technology (Beijing) Co., Ltd., China for purification and sequencing. The quality of the sanger DNA sequences and sequence consensus from forward and reward directions was checked and assembled manually in BioEdit v. 7.2.3 [37], and the newly nucleotide sequences were deposited in GenBank (Table 1).

| Species Name          | Strains/Voucher No. | GenBank Accession Numbers |
|-----------------------|---------------------|---------------------------|
| Alternaria alstroemeriae | CBS 118808         | KP124917, KP124447, KP124764, KP124296, KP124153, KP125071, KP123845 |
| Species Name | Strains/Voucher No. | GenBank Accession Numbers | SSU | LSU | rpb2 | ITS | gapdh | tefl-a | Alt-a1 |
|--------------|--------------------|--------------------------|-----|-----|------|-----|-------|--------|--------|
| Alternaria alstroemeriae | CBS 118809 | NG063029 NG069882 | KP124765 NR163686 KP121454 KP125072 | MH084526 |
| Alternaria alternandilerae | CBS 124392 | KC584506 KC584251 KC584374 KC584179 KC584096 KC584633 | KP123846 |
| Alternaria alternata | CBS 102596 | KP124950 | MH74392 KP124796 MH62796 KP124183 KP125104 KP123877 |
| Alternaria alternata | CBS 102599 | KP124952 | MH74395 KP124798 MH62799 KP124185 KP125106 KP123879 |
| Alternaria alternata | CBS 102600 | KP124953 | MH74396 KP124799 MH62800 KP124186 KP125107 KP123880 |
| Alternaria alternata | CBS 102602 | KP124954 | MH77754 KP124800 KP124332 KP124187 KP125108 KP123881 |
| Alternaria alternata | CBS 102603 | KP124955 | KP124801 KP124333 KP124188 KP125109 KP123882 |
| Alternaria alternata | CBS 102604 | KP124956 | MH74399 KP124802 MH62803 - KP125110 - |
| Alternaria alternata | CBS 113013 | KP124963 | KP124493 KP124809 KP124341 KP124195 KP125117 KP123889 |
| Alternaria alternata | CBS 113014 | KP124964 | KP124494 KP124810 KP124342 KP124196 KP125118 KP123890 |
| Alternaria alternata | CBS 113015 | KP124965 | KP124495 KP124811 KP124343 KP124197 KP125119 KP123891 |
| Alternaria alternata | CBS 113016 | KP124966 | KP124496 KP124812 KP124344 KP124198 KP125120 KP123901 |
| Alternaria alternata | CBS 113017 | KP124967 | KP124497 KP124813 KP124345 KP124199 KP125121 KP123902 |
| Alternaria alternata | CBS 113018 | KP124968 | KP124498 KP124814 KP124346 KP124217 KP125122 KP123913 |
| Alternaria alternata | CBS 113019 | KP124969 | KP124499 KP124815 KP124347 KP124218 KP125123 KP123914 |
| Alternaria alternata | CBS 113020 | KP124970 | KP124500 KP124816 KP124348 KP124219 KP125124 KP123915 |
| Alternaria alternata | CBS 113021 | KP124971 | KP124501 KP124817 KP124349 KP124220 KP125125 KP123916 |
| Alternaria alternata | CBS 113022 | KP124972 | KP124502 KP124818 KP124350 KP124221 KP125126 KP123917 |
| Alternaria alternata | CBS 113023 | KP124973 | KP124503 KP124819 KP124351 KP124222 KP125127 KP123918 |
| Alternaria alternata | CBS 113024 | KP124974 | KP124504 KP124820 KP124352 KP124223 KP125128 KP123919 |
| Alternaria alternata | CBS 113025 | KP124975 | KP124505 KP124821 KP124353 KP124224 KP125129 KP123920 |
| Alternaria alternata | CBS 113026 | KP124976 | KP124506 KP124822 KP124354 KP124225 KP125130 KP123921 |
| Alternaria alternata | CBS 113027 | KP124977 | KP124507 KP124823 KP124355 KP124226 KP125131 KP123922 |
| Alternaria alternata | CBS 916.96 | KC584507 | DQ678082 KC584375 AF347031 AY278808 KC584634 - |
| Alternaria arborescens | CBS 101.13 | KP125016 | KP124546 KP124862 KP124392 KP125170 KP124244 KP123940 |
| Alternaria arborescens | CBS 105.24 | KP125017 | KP124547 KP124863 KP124393 KP125171 KP124245 KP123941 |
| Alternaria arborescens | CBS 105.49 | KP125020 | KP124550 KP124866 KP124396 KP125174 KP124248 KP123944 |
| Alternaria arborescens | CBS 108.41 | KP125018 | KP124548 KP124864 KP124394 KP125172 KP124246 KP123942 |
| Alternaria arborescens | CBS 113.41 | KP125019 | KP124549 KP124865 KP124395 KP125173 KP124247 KP123943 |
| Alternaria arborescens | CBS 750.68 | KP125021 | KP124551 KP124868 KP124398 KP125176 KP124250 KP123945 |
| Alternaria arborescens | CBS 102605 | KC584509 | KC584253 KC584377 AF347033 AY278808 KC584634 - |
| Alternaria arborescens | CBS 109730 | KP125022 | KP124552 KP124869 KP124399 KP125177 KP124251 KP123946 |
| Alternaria arborescens | CBS 119544 | NG063030 NG069254 | KP124878 MH63062 KP125186 JQ646321 KP123955 |
| Alternaria arborescens | CBS 119545 | KP125032 | KP124562 KY392798 KP124409 KP125187 KP124260 KP123956 |
| Alternaria arborescens | CBS 123267 | KP125035 | KP124565 KP124882 KP124412 KP125190 KP124263 KP123959 |
| Alternaria arctoseptata | MFLUCC 21-0139 | MZ621874 MZ621948 | OK236655 - 0K236608 OK236702 OK236755 |
| Alternaria betae-kenyensis | CBS 118810 | NG063032 NG069256 JQ905180 | NR136118 JQ905161 KP125197 JQ905104 |
| Alternaria baoshanensis | MFLUCC 21-0124 | MZ621878 MZ621952 | OK236659 OK236613 OK236670 OK236760 |
Table 1. Cont.

| Species Name          | Strains/Voucher No. | GenBank Accession Numbers |
|-----------------------|---------------------|--------------------------|
|                       | SSU | LSU | rpb2 | ITS | gapdh | tef1-α | Alt-a1 |
| Alternaria breviconiophora | MFLUCC 22-0075 T | MZ621870 | MZ621944 | OK236651 | MZ621997 | OK236604 | OK236698 | OK236751 |
| Alternaria breviconiophora | MFLU 21-0317 | MZ621871 | MZ621945 | OK236652 | MZ621998 | OK236605 | OK236699 | OK236752 |
| Alternaria burnsii     | CBS 107.38 T      | NG63033 | N669257 | JQ646457 | NR136119 | JQ646305 | KP125198 | JQ646388 |
| Alternaria burnsii     | CBS 110.50 T      | KP125044 | KP124574 | KP124890 | KP124421 | KP124271 | KP125199 | KP123968 |
| Alternaria burnsii     | CBS 118816        | KP125046 | KP124576 | KP124892 | KP124423 | KP124273 | KP125201 | KP123970 |
| Alternaria burnsii     | CBS 118817        | KP125047 | KP124577 | KP124893 | KP124424 | KP124274 | KP125202 | KP123971 |
| Alternaria burnsii     | CBS 130264        | KP125048 | KP124578 | KP124894 | KP124425 | KP124275 | KP125203 | KP123972 |
| Alternaria doliconidium| KUN-HKAS 100840 T | NG065142 | - | - | - | - | - |
| Alternaria doliconidium| KUMCC 17-0263 T   | MG829094 | MG828980 | - | MG828664 | - | - | - |
| Alternaria eichhorniae | CBS 119778       | KP125050 | KP124580 | KP124896 | KP124426 | KP124277 | KP125205 | - |
| Alternaria eichhorniae | CBS 489.92       | KP125051 | KP124581 | KP124897 | KP124427 | KP124278 | KP125206 | KP123975 |
| Alternaria ellipsoidalis| MFLUCC 21-0132 T | MZ621862 | MZ621936 | OK236643 | MZ621989 | OK236596 | OK236690 | OK236743 |
| Alternaria eupatoriicola| MFLUCC 21-0122 T | MZ621855 | MZ621929 | OK236636 | MZ621982 | OK236599 | OK236693 | OK236746 |
| Alternaria falcata     | MFLUCC 21-0123 T | MZ621865 | MZ62139 | OK236649 | MZ621992 | OK236599 | OK236693 | OK236746 |
| Alternaria gaisen      | CBS 118488 T      | KP125051 | KP124581 | KP124897 | KP124427 | KP124278 | KP125206 | KP123975 |
| Alternaria gaisen      | CBS 632.93       | KC584531 | KC584275 | KC584399 | KC584197 | KC584116 | KC584658 | KP123974 |
| Alternaria gaisen      | CPC 25268        | KP125052 | KP124582 | KP124898 | KP124428 | KP124279 | KP125207 | KP123976 |
| Alternaria gossypina   | CBS 100.23       | KP125053 | KP124583 | KP124899 | KP124429 | KP124280 | KP125208 | KP123977 |
| Alternaria gossypina   | CBS 104.32 T     | KP125054 | KP124584 | KP124900 | KP124430 | JQ646312 | KP125209 | JQ646395 |
| Alternaria gossypina   | CBS 107.36       | KP125055 | KP124585 | KP124901 | KP124431 | - | KP125210 | - |
| Alternaria gossypina   | CBS 102597       | KP125056 | MH674393 | KP124902 | MH622979 | KP124281 | KP125211 | KP123978 |
| Alternaria gossypina   | CBS 102601       | KP125057 | MH674397 | KP124903 | MH628011 | KP124282 | KP125212 | KP123979 |
| Alternaria iridianastralis | CBS 118486 T | NG_063035 | NG_069258 | KP124905 | NR_136120 | KP124284 | KP125214 | KP123981 |
| Alternaria iridianastralis | CBS 118407 | KP125060 | KP124590 | KP124906 | KP124436 | KP124285 | KP125215 | KP123982 |
| Alternaria iridianastralis | CBS 118404 | KP125058 | KP124588 | KP124904 | KP124434 | KP124283 | KP125213 | KP123980 |
| Alternaria italicata   | KUMCC 17-0090      | - | - | - | MG764018 | - | - | - |
| Alternaria italicata   | MFLUC 14-0421 T   | - | MG818319 | MG859737 | MG764017 | - | - | - |
| Alternaria jacinthicola| CBS 133751 T      | KP125062 | KP124592 | KP124908 | KP124438 | KP124287 | KP125217 | KP123984 |
| Alternaria jacinthicola| CBS 878.95       | KP125061 | KP124591 | KP124907 | KP124437 | KP124286 | KP125216 | KP123983 |
| Alternaria longipes    | CBS 113.35       | KP125064 | KP124594 | KP124910 | KP124440 | KP124289 | KP125219 | KP123986 |
| Alternaria longipes    | CBS 121332       | KP125067 | KP124597 | KP124913 | KP124443 | KP124292 | KP125222 | KP123989 |
Table 1. Cont.

| Species Name                | Strains/Voucher No. | GenBank Accession Numbers |
|-----------------------------|---------------------|---------------------------|
|                             | SSU                 | LSU | rpb2 | ITS | gapdh | tef1-α | Alt-a1 |
| Alternaria longipes         | CBS 121333          |     | KP125068 | KP12498 | KP124914 | KP124444 | KP124293 | KP125223 | KP123990 |
| Alternaria longipes         | CBS 539.94          |     | KP125065 | KP124995 | KP124911 | KP124441 | KP124290 | KP125220 | KP123987 |
| Alternaria longipes         | CBS 540.94          |     | KC584541 | KC584285 | KC584409 | -       | -       | KC584667 | -       |
| Alternaria longipes         | CBS 917.96          |     | KP125066 | KP124596 | KP124912 | KP124442 | KP124291 | KP125221 | KP123988 |
| Alternaria macilenta        | MFLUCC 21-0138      |     | MZ621845 | MZ621919 | OK236626 | MZ621972 | OK236579 | OK236673 | OK236726 |
| Alternaria macroconidia     | MFLUCC 21-0134      |     | MZ621876 | MZ621950 | OK236657 | MZ622001 | OK236610 | OK236704 | OK236757 |
| Alternaria minimispora      | MFLUCC 21-0127      |     | MZ621833 | MZ621927 | OK236634 | MZ621980 | OK236587 | OK236681 | OK236734 |
| Alternaria murriformispora  | MFLU 21-0309        |     | MZ621850 | MZ621924 | OK236630 | MZ621976 | OK236583 | OK236677 | OK236730 |
| Alternaria oblongoellipsoidea | MFLUCC 22-0074      |     | MZ621840 | MZ621914 | OK236621 | MZ621967 | OK236574 | OK236668 | OK236721 |
| Alternaria obpyriconidia    | MFLUCC 21-0121      |     | MZ621851 | MZ621925 | OK236633 | MZ621978 | OK236585 | OK236680 | OK236732 |
| Alternaria obpyriconidia    | MFLU 21-0300        |     | MZ621852 | MZ621926 | OK236632 | MZ621979 | OK236586 | OK236679 | OK236733 |
| Alternaria obpyriconidia    | MFLUCC 21-0137      |     | MZ621882 | MZ621956 | -       | MZ22007  | -       | OK236710 | OK236763 |
| Alternaria ovoidea          | MFLUCC 14-0427      |     | MZ621880 | MZ621954 | OK236661 | MZ622005 | OK236614 | OK236708 | OK236761 |
| Alternaria phragmictica     | MFLUCC 21-0125      |     | MZ621867 | MZ621941 | OK236649 | MZ621994 | OK236602 | OK236696 | OK236749 |
| Alternaria phragmictica     | MFLU 21-0316        |     | MZ621868 | MZ621942 | OK236650 | MZ621995 | OK236603 | OK236697 | OK236750 |
| Alternaria pseudoinfectoria | MFLUCC 21-0126      |     | MZ621857 | MZ621931 | OK236638 | MZ621984 | OK236591 | OK236685 | OK236738 |
| Alternaria pseudoinfectoria | MFLU 21-0311        |     | MZ621858 | MZ621932 | OK236639 | MZ621985 | OK236592 | OK236686 | OK236739 |
| Alternaria rostroconidia    | MFLUCC 21-0136      |     | MZ621842 | MZ621916 | OK236623 | MZ621969 | OK236576 | OK236670 | OK236723 |
| Alternaria rostroconidia    | MFLU 21-0318        |     | MZ621843 | MZ621917 | OK236624 | MZ621970 | OK236577 | OK236671 | OK236724 |
| Alternaria salicola         | MFLUCC 22-0072      |     | MZ621872 | MZ621946 | OK236653 | MZ621999 | OK236606 | OK236700 | OK236753 |
| Alternaria sp.              | CBS 108.27          |     | KC584601 | KC584343 | KC584468 | KC584236 | KC584162 | KC584727 | -       |
| Alternaria tomento          | CBS 103.30          |     | KP125069 | KP124599 | KP124915 | KP124445 | KP124294 | KP125224 | KP123991 |
| Alternaria tomento          | CBS 114.35          |     | KP125070 | KP124600 | KP124916 | KP124446 | KP124295 | KP125225 | KP123992 |
| Alternaria torilis          | MFLUCC 21-0133      |     | MZ621859 | MZ621933 | OK236640 | MZ621986 | OK236593 | OK236687 | OK236740 |
| Alternaria torilis          | MFLU 21-0299        |     | MZ621860 | MZ621934 | OK236642 | MZ621987 | OK236595 | OK236689 | OK236742 |
| Alternaria vitis            | MFLUCC 14-0433      |     | MZ621861 | MZ621935 | OK236641 | MZ621988 | OK236594 | OK236688 | OK236741 |

Abbreviations: CBS: the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CPC: Culture Collection of Pedro Crous, Netherlands; KUMCC: Kunming Institute of Botany Culture Collection, Yunnan, China; KUN-HKAS: Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica, Yunnan, China; MFLU: the Herbarium of Mae Fah Luang University Chiang Rai, Thailand; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand.

2.3. Sequence Alignment and Phylogenetic Analyses

The newly generated ITS, LSU, SSU, tef1-α, rpb2, gapdh and Alt-a1 sequences were subjected to the nucleotide BLAST search engine via the NCBI (https://www.ncbi.nlm.nih.gov).
nih.gov/, accessed on 10 April 2022) for checking potential contaminants or erroneous sequences as well as delineating the closely related taxa. All reference sequences were downloaded from GenBank. The multiple sequence matrixes were automatically aligned by MAFFT v. 7.452 (https://mafft.cbrc.jp/alignment/software/, accessed on 20 May 2022) [38]. Manual improvements were made where necessary in BioEdit v. 7.2.3 [37]. Individual gene alignments were separately analyzed by maximum likelihood (ML) in order to check the congruence of tree topology, and, thus, the combined multi-locus phylogenetic trees were inferred based on Bayesian inference (BI) and maximum likelihood (ML) analyses.

Maximum likelihood (ML) analyses were performed by Randomized Accelerated Maximum Likelihood (RAxML) [39,40] implemented in raxmlGUI 1.3 [41] using the default setting, but adjusted with 1000 bootstrap replicates and a GAMMAI model of nucleotide substitution. MrModeltest v. 2.3 [42] was used to determine the best-fit model of nucleotide substitution for each locus and incorporated into the analyses. GTR+I+G was the best-fit model for ITS, LSU and Alt-a1 loci under the Akaike Information Criterion (AIC), while TIM2+I+G was the best-fit model for SSU and rpb2, SYM+I+G was the best-fit model for tef1-a. Bayesian inference (BI) analyses were performed by MrBayes v.3.1.2 [43]. Markov Chain Monte Carlo (MCMC) of six simultaneous Markov chains was run with one million generations to determine posterior probabilities (PP) [44,45], and started from a random tree topology. Trees were frequently sampled at 100th generation and the temperature value of heated chain was set to 0.15. The extra runs were required when the average standard deviation of split frequencies did not lower than 0.01 after one million generation. The first 25% trees represented the burn-in phase of the analyses and were discarded. The remaining trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree. The phylogram were visualized in FigTree v. 1.4.0 [46] and edited in Microsoft Office PowerPoint 2016 (Microsoft Inc., Redmond, WA, USA).

3. Results

3.1. Phylogeny

Six new species collected from dead herbaceous and monocotyledonous plants in Italy were analyzed with other representative Alternaria species in sect. Alternaria including Alternaria muriformispora (strain MFLUCC 22-0073; on Plantago sp.), A. obpyriconidia (strains MFLUCC 21-0121 and MFLUCC 14-0435; on Vicia faba), A. ovoidea (MFLUCC 14-0427; on Dactylis glomerata), A. pseudoinfectoria (MFLUCC 21-0126; on Chenopodium sp.), A. rostroconidia (MFLUCC 21-0136; on Arabis sp.) and A. torilis (MFLUCC 14-0433 and MFLUCC 21-0133; on Torilis arvensis). The analyses represented phylogenetic relationships of taxa in Alternaria sect. Alternaria as well as the placement of six new species. Phylogenetic construction of sect. Alternaria based on a combined ITS, LSU, SSU, tef1-α, rpb2, gapdh and Alt-a1 DNA sequence dataset comprises 96 sequences of 34 representative species in sect. Alternaria, and Alternaria alternantherae (CBS 124392) was selected as the outgroup taxon. The best scoring RAxML tree is shown in Figure 1 with the final ML optimization likelihood value of -11313.33238 (ln). The dataset consists of 4377 total characters, including gaps (ITS: 1–514 bp, LSU: 515–1368 bp, SSU: 1369–2295 bp, tef1-α: 2296–2540 bp, rpb2: 2541–3311 bp, gapdh: 3312–3897 bp, Alt-a1: 3898–4377 bp). RAxML analysis yielded 511 distinct alignment patterns and 8.3% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.246733, C = 0.254032, G = 0.258489, T = 0.240746, with substitution rates AC = 0.896323, AG = 2.073824, AT = 1.043150, CG = 0.820017, CT = 4.179461 and GT = 1.000000. The gamma distribution shape parameter alpha = 0.020013 and the Tree-Length = 0.230674. The gamma distribution shape parameter alpha = 0.020013 and the Tree-Length = 0.230674. Bayesian posterior probabilities (PP) from MCMC were evaluated with a final average standard deviation of split frequencies = 0.008527.

Multi-locus phylogenetic analyses based on ML and BI criteria showed overall similarity in tree topologies. Alternaria muriformispora (MFLUCC 22-0073, MFLU 21-0309) has a close phylogenetic relationship with A. pseudoinfectoria (MFLUCC 21-0126, MFLU 21-0311) (76% ML, 0.98 PP; Figure 1) and also clustered with A. lathyrus (MFLUCC 21-0140,
MFLU 21-0297) and A. breviconidiophora (MFLUCC 22-0075, MFLU 21-0317). These four species formed a well-resolved subclade in sect. *Alternaria* with 97% ML and 0.98 PP support. *Alternaria obpyriconidia* (MFLUCC 21-0121, MFLU 21-0300) formed a clade with *A. macroconidia* (MFLUCC 21-0134), *A. arctoseptata* (MFLUCC 21-0139), *A. baoshanensis* (MFLUCC 21-0124) and *A. falcata* (MFLUCC 21-0123) with 93% ML and 1.00 PP support (Figure 1). While *A. ovoidea* (MFLUCC 14-0427) is sister to *A. baoshanensis* (MFLUCC 21-0124) with significant support (70% ML, 0.95 PP), and is also constituted in this clade. *Alternaria rostroconidia* (MFLUCC 21-0136, MFLU 21-0318) formed a separated branch with *A. minimispora* (MFLUCC 21-0127) with significant support in BI analysis (0.96 PP; Figure 1). *Alternaria torilis* (MFLUCC 14-0433, MFLUCC 21-0133, MFLU 21-0299) formed an independent subclade, related to *A. ellipsoidalis* (MFLUCC 21-0132) and *A. eupatoriicola* (MFLUCC 21-0122).

Figure 1. Cont.
Figure 1. Phylogenetic tree of *Alternaria* sect. *Alternaria* generated by RAxML-based analysis of a combined ITS, LSU, SSU, tef1-α, rpb2, gapdh and Alt-a1 DNA sequence dataset. Bootstrap support values for maximum likelihood (ML, black) equal to or greater than 60% and Bayesian posterior probabilities (PP, red) equal to or greater than 0.95 PP are shown above the nodes. The tree is rooted to *Alternaria alternantherae* (CBS 124392). Newly species and generated strains are in blue, and the type strains are indicated in bold. Strains obtained from ex-type living culture are indicated by (T) and strains obtained from holotype specimen are indicated by (H).

3.2. Taxonomy

*Alternaria muriformispora* J.F. Li, Camporesi, Phookamsak & Bhat, sp. nov. Figure 2

Index Fungorum number: IF 559795

Etymology: Named after its muriform conidia.

Holotype: MFLU 21-0309
Figure 2. *Alternaria muriformispora* (MFLU 21-0309, holotype). (a) Colonies on dead aerial stem of *Plantago* sp. (*Plantaginaceae*); (b–f) Conidiophores bearing conidiogenous cells; (g–p) Conidia. Scale bars: (a) = 100 µm, (b–f) = 50 µm, (g–p) = 30 µm, (h, i) = 20 µm.

Saprobic on dead aerial stems of *Plantago* sp. (*Plantaginaceae*). Sexual morph: Undetermined. Asexual morph: Mycelium superficial on the substrate, composed of septate, branched, smooth, thin-walled, brown hyphae. Conidiophores 185–201 × 12–13 µm (\(\bar{x} = 192 \times 12 \, \mu m, n = 30\)), macronematous, straight or flexuous, cylindrical, with swollen at the basal cell, slightly narrower towards the apex, dark brown, paler at the apex, smooth, septate, unbranched, thick-walled. Conidiogenous cells 4–5 × 5–7 µm (\(\bar{x} = 4.5 \times 6.2 \, \mu m, n = 20\)), polytretic, integrated, terminal, determinate or percurrent, cylindrical, subhyaline to light brown, smooth, thin-walled, apically doliiform with one conidiogenous locus. Conidia 75–88 × 23–35 µm (\(\bar{x} = 83 \times 29 \, \mu m, n = 30\)), acrogenous, solitary, dry, simple, straight, curved, ellipsoidal to ovoid, or obpyriform with short, narrow, paler brown, aseptate, unbranched, obtuse beak, copper brown to dark brown, four to seven transverse eusepta, with 1–2 longitudinal or oblique or Y-shaped septa in all middle transverse divisions, without oblique or longitudinal septa at both end cells, slightly thickened and constricted at middle septa, borne in chain, verruculose to verrucose, thin-walled. Conidial secession schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 h and germ tubes produced from lateral cells. Colonies hairy or cottony, brown to dark brown, reaching 5 cm in 7 days at 25 °C, mycelium superficial, effuse, radially striate, with irregular edge, brown to dark brown hyphae; conidia not formed *in vitro* within 60 days.

Material examined: Italy, Province of Forlì-Cesena, Meldola, on dead aerial stems of *Plantago* sp. (*Plantaginaceae*), 8 September 2014, E. Camporesi, IT2101 (MFLU 21-0309, holotype), ex-type living culture = MFLUCC 22-0073.
Notes: Multi-locus phylogeny showed that two strains of *Alternaria muriformispora* formed a robust clade (100% ML, 1.00 PP; Figure 1) sister to *A. pseudoinfectoria* with moderate support (76% ML, 0.98 PP; Figure 1). *Alternaria muriformispora* differs from *A. pseudoinfectoria* in having larger (83 × 29 µm vs. 33 × 19 µm), ovoid to ellipsoidal, or obpyriform, short beak and copper brown to dark brown conidia, with 4–7 transverse eu-septa and 1–2 longitudinal or oblique or Y-shaped septa in all middle transverse divisions. *Alternaria pseudoinfectoria* has subglobose to obclavate, or obpyriform, light brown conidia, with 3–4 transverse eusepta and 1–2 longitudinal or oblique or Y-shaped septa and conidia that form long, cylindrical, septate, unbranched secondary conidiophores with one apical conidiogenous locus. A nucleotide pairwise comparison of *rpb2* sequences showed that *A. muriformispora* differs from *A. pseudoinfectoria* in 10/559 bp (1.8% difference, no gap).

In *All-a1*, *A. muriformispora* differs from *A. pseudoinfectoria* in 9/474 bp (1.9% difference, no gap).

**Alternaria obpyriconidia** J.F. Li, Camporesi, Phookamsak & Bhat, sp. nov. Figure 3

Index Fungorum number: IF 559797

Etymology: Named after its obpyriform conidia.

Holotype: MFLU 21-0300

*Saprobic on dead stems of Vicia faba (Fabaceae).* Sexual morph: Undetermined. Asex-
ual morph: Mycelium superficial on the substrate, composed of septate, branched, smooth, thin-walled, subhyaline to pale white hyphae. Conidiophores (130–)139.5–155 × 11.5–13 µm
(\(\bar{x} = 145.8 \times 12.6 \, \mu m, n = 100\)), macronematous, mononematous, straight or flexuous, cylindrical, slightly swollen at the apical cell, copper brown to dark brown, septate, unbranched, smooth and thick-walled. Conidiogenous cells 19–23 × 9–12.5 \(\mu m\) (\(\bar{x} = 19.7 \times 10.8 \, \mu m, n = 100\)), polytretic, sympodial, integrated, terminal, determinate or percurrent, cylindrical to doliform, subhyaline, smooth, thick-walled, apically rounded or doliform, with 2–4 conidiogenous loci.

Conidia (58–)62.5–68(–69) × (12.5–)22.5–28(–30) \(\mu m\) (\(\bar{x} = 64 \times 25.4 \, \mu m, n = 100\)) acrogenous, solitary, dry, simple, straight or curved, ellipsoidal to obclavate or obpyriform, with 2–4 transverse eusepta, with 1–2 longitudinal to oblique or Y-shaped septa in the middle cells, constricted at the central septum, borne in chain, verruculose or verrucose and thin-walled. Conidial secession schizolytic.

Culture characteristics: Conidia germinating on PDA within 12 h and germ tubes produced from all cells. Colonies hairy or cottony, pale to dark brown, reaching 5 cm in 7 days at 25 °C, mycelium superficial, effuse, radially striate, with irregular edge, subhyaline to brown hyphae; conidia not formed \(in vitro\) within 60 days.

Material examined: Italy, Province of Forlì-Cesena, Bagno di Romagna, Valgianna, on dead aerial stems of \textit{Vicia faba} (Fabaceae), 29 January 2014, E. Camporesi, IT1688 (MFLUCC 21-0300, holotype), ex-type living culture = MFLUCC 21-0121; \textit{ibid.}, MFLUCC 14-0435.

Notes: In the multi-locus phylogenetic analyses, two strains of \textit{Alternaria obpyriconidia} formed a separate branch basal to \textit{A. macroconidia} (MFLUCC 21-0134), \textit{A. arctoseptata} (MFLUCC 21-0139), \textit{A. ovoidea} (MFLUCC 14-0427) and \textit{A. baoshanensis} (MFLUCC 21-0124), and also clustered with \textit{A. falcata} (MFLUCC 21-0123). \textit{Alternaria obpyriconidia} differs from \textit{A. macroconidia} in having smaller (58–69 × 12.5–30 \(\mu m\) vs. 68.5–95.5 × 20–30.5), pale brown to greyish brown conidia, with 3–4 transverse eusepta, while \textit{A. macroconidia} has olivaceous brown to golden brown or brown conidia, with 3–5 transverse disto- or eusepta and conidia that are not constricted in \textit{A. macroconidia} [2]. \textit{Alternaria arctoseptata} is distinct from \textit{A. obpyriconidia} in having larger (15–75 × 10–35 \(\mu m\)), yellowish-brown to dark brown, sectored conidia, varied in shape, with 2–3(–6) transverse septa. Conidiophores of \textit{A. arctoseptata} are shorter (50–100 × 8–12 \(\mu m\) vs. (130–)139.5–155 × 11.5–13 \(\mu m\)) and pale brown to light brown, arising from a stomatic base [2], while \textit{A. obpyriconidia} has copper brown to dark brown conidiophores. \textit{Alternaria ovoidea} can be distinguished from \textit{A. obpyriconidia} in having slightly smaller (48–65 × 15.5–30 \(\mu m\)), ovoid, orangish brown to copper brown, sectored, non-beak conidia with 1–3 indistinct transverse septa, whereas \textit{A. obpyriconidia} has short, narrow, pale brown, aseptate, rostrate beak conidia. \textit{Alternaria baoshanensis} can be distinguished from \textit{A. obpyriconidia} in having versicolorous, light brown to dark brown conidiophores, which sometimes branch with several aggregated at the base, and light brown to yellowish brown 3–6 transverse septa conidia [2], whereas \textit{A. obpyriconidia} has unbranched conidiophores. \textit{Alternaria falcata} differs from \textit{A. obpyriconidia} in having smaller (20–50 × 12–23 \(\mu m\)), olivaceous-brown to brown conidia, with 2–5 transverse disto- or eusepta [2]. A nucleotide base comparison of these species is shown in Table 2.

Table 2. A nucleotide base comparison of \textit{Alternaria obpyriconidia} with other phylogenetically related species.

| Species          | Alt-a1 | gapdh | ITS    | rpb2  | tefl-a |
|------------------|--------|-------|--------|-------|--------|
| \textit{A. arctoseptata} | 11/476 bp (2.3%) | 15/570 bp (2.6%) | -      | 39/560 bp (7.0%) | 4/240 bp (1.7%) |
| \textit{A. baoshanensis}   | 8/474 bp (1.7%)    | 15/568 bp (2.6%) | 5/515 bp (1%) | 40/559 bp (7.2%) | 3/240 bp (1.3%) |
| \textit{A. falcata}         | 10/474 bp (2.1%)   | 12/568 bp (2.1%) | 5/515 bp (1%) | 37/559 bp (6.6%) | 4/240 bp (1.7%) |
| \textit{A. macroconidia}    | 11/474 bp (2.3%)   | 11/567 bp (1.9%) | 4/515 bp (0.8%) | 54/560 bp (9.6%) | 4/240 bp (1.7%) |
| \textit{A. ovoidea}         | 16/470 bp (3.4%)   | 14/568 bp (2.5%) | 4/515 bp (0.8%) | 42/559 bp (7.5%) | 3/240 bp (1.3%) |
*Alternaria ovoidea* J.F. Li, Camporesi, Bhat & Phookamsak, sp. nov. Figure 4

Index Fungorum number: IF 559798

Etymology: Referring to its ovoid (droplets-like) conidia.

Holotype: MFLU 21-0298

Saprobic on stems of *Dactylis glomerata* (Poaceae). Sexual morph: Undetermined.

Asexual morph: Mycelium partly superficial on host substrate, composed of septate, branched, smooth, thin-walled, pale brown hyphae. Conidiophores 270–300 × 6.5–11 µm ($\bar{x} = 280 \times 8$ µm, $n = 100$), macronematous, mononematous, copper brown to dark brown, erect, flexuous or sigmoid, cylindrical, septate, branched, smooth to verrucose, thick-walled.

Conidiogenous cells 9–13 × 8.5–15 µm ($\bar{x} = 9.7 \times 11.4$ µm, $n = 100$), mono- to polytretic, integrated, terminal, determinate or percurrent, subcylindrical, pale brown to light brown,
smooth, thick-walled, apically doliiform with conidiogenous loci cicatrized on conidial secession. Conidia 48–65 × 15.5–30 µm (\(\bar{x} = 55.4 \times 27.2 \mu m, n = 100\)) acrogenous, solitary, ovoid, orangish brown to copper brown, sectored, with 1–3 indistinct transverse septa, and one longitudinal or oblique or Y-shaped septum in transverse divisions, verruculose, thick-walled. Conidial secession schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 h and germ tubes produced from all cells. Colonies cottony, brown to dark brown, reaching 5 cm in 7 days at 25 °C, mycelium superficial, effuse, radially striated, with irregular edge; conidia not formed in vitro within 60 days.

Material examined: Italy, Province of Forlì-Cesena, Fiumicello di Premilcuore, on dead aerial stems of Dactylis glomerata (Poaceae), 19 January 2014, E. Camporesi, IT1656 (MFLU 21-0298, holotype), ex-type living culture = MFLUCC 14-0427.

Notes: Multi-locus phylogenetic analyses showed that Alternaria ovoidea is sister to A. baoshanensis with significant support (70% ML, 0.95 PP; Figure 1). Alternaria ovoidea differs from A. baoshanensis in having solitary, flexuous or sigmoid, copper brown to dark brown conidiophores with a non-stomatic base, while the conidiophores are versicolorous, light brown to dark brown, arising from a stomatic base in A. baoshanensis. Conidia of A. ovoidea are slightly larger (48–65 × 15.5–30 µm vs. 25–60 × 12–22 µm), orangish brown to copper brown, sectored, with 1–3 indistinct transverse septa, while A. baoshanensis has light brown to yellowish brown, sometimes with a short beak, varied in shape, usually subglobose to ellipsoidal, or subcylindrical to obpyriform, 3–6 transverse septa conidia [2]. A nucleotide base comparison of A. ovoidea with A. baoshanensis showed that they are different in 4/515 bp (0.8%) of ITS, 11/474 bp (2.3%) of Alt-a1, 11/567 bp (1.9%) of gapdh, 37/559 bp (6.6%) of rpb2 and 3/238 bp (1.3%) of tef1-α.

**Alternaria pseudoinfectoria** J.F. Li, Camporesi, Bhat & Phookamsak, sp. nov. Figure 5

Index Fungorum number: IF 559799

Etymology: Referring to the conidial structures resemble Alternaria section infectoriae.

Holotype: MFLU 21-0311

Saprobiic on stems of Chenopodium sp. (Chenopodiaceae). Sexual morph: Undetermined. Asexual morph: Mycelium superficial on host substrate, composed of septate, branched, smooth, thin-walled, brown hyphae. Conidiophores 55–68 × 12–14 µm (\(\bar{x} = 62 \times 13 \mu m, n = 30\)), macronematous, mononematous, straight or flexuous, cylindrical, light brown to brown, septate, branched, smooth, thick-walled. Conidiogenous cells 11–12 × 10–14 µm (\(\bar{x} = 11.5 \times 12 \mu m, n = 20\)), monontretic, integrated, terminal, determinate or percurrent, cylindrical, subhyaline to light brown, smooth, thin-walled, apically doliiform with one conidiogenous locus. Conidia 25–40 × 13–25 µm (\(\bar{x} = 33 \times 19 \mu m, n = 30\)) acrogenous, holoblastic, solitary, straight, subglobose to obclavate, or obpyriform, sometimes with short, narrow, rostrate, paler brown, septate beak, light brown 3–4 transverse eusepta, with one longitudinal or oblique or Y-shaped septum in some transverse divisions, borne in chain, smooth to minutely verrucose, thin-walled, formed apically secondary conidiophores, with one conidiogenous locus. Conidiial secession schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 h and germ tubes produced from all cells. Colonies immersed in PDA, cottony, white to grey, reaching 5 cm in 7 days at 25 °C, mycelium superficial, effuse, radially striate, with irregular edge, white hyphae; conidia not sporulated in vitro within 60 days.

Material examined: Italy, Province of Forlì-Cesena, Forlì, Via Nenni, on dead aerial stems of Chenopodium sp. (Chenopodiaceae), 17 October 2014, E. Camporesi, IT2181 (MFLU 21-0311, holotype), ex-type living culture = MFLUCC 21-0126.
Holotype: MFLU 21-0311

Figure 5. Alternaria pseudoinfectoria (MFLU 21-0311, holotype). (a) Colonies on dead stem of Chenopodium sp.; (b,c,j) Conidia formed apical secondary conidiophores; (d–i) Conidiophores; (k–m) Conidia; (n) Germinated conidium; (o,p) Colonies on PDA. Scale bars: (o,p) = 2 cm, (a) = 300 µm, (b–f,h,n) = 20 µm, (g,i) = 15 µm, (k–m) = 10 µm.

Notes: Alternaria pseudoinfectoria resembles species in sect. Infectoriae due to its conidia often developing long secondary conidiophores. Although species in section Panax also formed long secondary conidiophores, conidiogenous loci on secondary conidiophores are rather monotretic in A. pseudoinfectoria, which more resemble structures of species in sect. Infectoriae [14,47]. However, A. pseudoinfectoria corresponds with sect. Alternaria in having straight or curved primary conidiophores, simple to branched, with one apical
conidiogenous locus, and conidia born in chain [8]. In phylogenetic analyses, two strains of *A. pseudoinfectoria* formed a well-resolved subclade (82% ML, 0.99 PP) and is sister to *A. muriformispora* with 76% ML and 0.98 PP support (Figure 1). The morphological comparison of these two species is detailed in notes of *A. muriformispora*.

**Alternaria rostroconidia** J.F. Li, Camporesi, Bhat & Phookamsak, sp. nov. Figure 6

Index Fungorum number: IF 559800

Etymology: Referring to the rostrate conidia.

Holotype: MFLU 21-0318

Saprobic on dead stems of *Arabis* sp. (*Brassicaceae*). Sexual morph: Undetermined. Asexual morph: Mycelium superficial on host substrate, with dark hyphae. Conidiophores 105–120 × 11–15 µm (x = 112 × 13 µm, n = 30), macronematous, solitary or 2–5 aggregated at the base, straight or flexuous, cylindrical, light brown to dark brown, septate, geniculate, smooth or sometimes semi-verrucose, thick-walled. Conidiogenous cells 12–18 × 5–8 µm (x = 15 × 6 µm, n = 20), mono- to polytretic, normally sympodial proliferations, integrated,
terminal, determinate or percurrent, cylindrical, subhyaline or semi-colored, smooth, thin-walled, apically doliiform, with 1–2 conidiogenous loci and swollen knots near conidiogenous loci. Conidia 50–80 × 25–30 µm (x = 66 × 22 µm, n = 30) acrogenous, solitary, straight or curved, ellipsoidial or ovoid to obpyriform, with short, narrow, rostrate, paler brown, aseptate beak, with distinct hilum at the apex, dark brown, 3–4 transverse eusepta, with one longitudinal or oblique or Y-shaped septum in some transverse divisions, sometimes sectored, slightly constricted at the septa, borne in chain, smooth, thick-walled. Conidial secession schizolytic.

Culture characteristics: Conidia germinating on PDA within 12 h and germ tubes produced from lateral cells. Colonies cottony, brown to dark brown, reaching 5 cm in 10 days at 25 °C, mycelium superficial, effuse, radially striate, with irregular edge, white to grey hyphae; conidia not sporulated in vitro within 60 days.

Material examined: Italy, Province of Forli-Cesena, Premilcuore, on dead aerial stems of Arabis sp. (Brassicaceae), 8 October 2017, E. Camporesi, IT3515 (MFLU 21-0318, holotype), ex-type living culture, MFLUCC 21-0136.

Notes: Alternaria rostroconidia corresponds with species in sect. Alternaria in having obpyriform, born in chain conidia with several transverse and longitudinal septa [8]. In multi-locus phylogenetic analyses, A. rostroconidia has a close relationship with A. minimispora with significant support in BI analyses (0.96 PP; Figure 1). A rp2 nucleotide pairwise comparison showed that A. rostroconidia differs from A. minimispora in 19/505 bp (3.8% difference, no gap). In gapdh, A. rostroconidia differs from A. minimispora in 10/545 bp (1.8% difference, no gap). The Alt-a1 nucleotide pairwise comparison shows that A. rostroconidia differs from A. minimispora in 8/474 bp (1.7% difference, no gap). Morphologically, A. rostroconidia can be distinguished from A. minimispora in having larger (50–80 × 25–30 µm vs. 13–25 × 8–11 µm), ellipsoidal or ovoid to obpyriform conidia, with 3–4 transverse eusepta and short, narrow, rostrate and distinct hilum at the apex. Alternaria minimispora has subglobose to ovoid, sometimes obpyriform or obturbinate, beakless, two to four transversely euseptate conidia [2].

Alternaria torilis J.F. Li, Camporesi, Bhat & Phookamsak, sp. nov. Figure 7

Index Fungorum number: IF 559801

Etymology: Named after the host genus “Torilis”.

Holotype: MFLU 21-0299

Saprobic on dead aerial stems of Torilis arvensis (Apiaceae). Sexual morph: Undetermined. Asexual morph: Mycelium superficial on host substrate, composed of septate, branched, smooth, thin-walled, brown to light brown hyphae. Conidiophores (155–)177–185(–191) × (7.5–)8–10(–11) µm (x = 175.2 × 8.8 µm, n = 100), macronematous, mononematous, straight or flexuous, cylindrical, dark brown, unbranched, septate, sometimes branched, smooth, thick-walled. Conidiogenous cells 7–9(–10) × (6.5–)7.5–10 µm (x = 8.2 × 8.9 µm, n = 100), mono- to polytretic, integrated, terminal, determinate or percurrent, cylindrical, subhyaline, smooth, thin-walled, apically doliiform, with 2 conidiogenous loci cicatrized on conidial secession. Conidia (55–)60–75(–82) × (23–)25–31.5(–32) µm (x = 68.5 × 28.5 µm, n = 100) acrogenous, solitary, dry, straight, fusiform to ovoid, or obturbinate to obpyriform, sometimes with short, narrow, pale brown to light brown, aseptate beak, brown to dark brown, 2–4 transverse eusepta, with one longitudinal or oblique or Y-shaped distoseptum in some transverse divisions, borne in chain, minutely verrucose, thin-walled, formed apically secondary conidiophores with one conidiogenous locus. Conidial secession schizolytic.

Culture characteristics: Conidia germinating on PDA within 14 h and germ tubes produced from lateral cells. Colonies growing on PDA, hairy or cottony, light brown to brown, reaching 5 cm in 14 days at 25 °C, mycelium superficial, effuse, radially striate, with irregular edge, colorless hyphae. Conidia sporulated on OA within 15 days, phragmosporous to muriform, oblong to ovoid, brown to dark brown, with short, doliiform, apical beak, formed apically or laterally, short, branched or unbranched secondary conidiophores
with one to two conidiogenous loci at apex and 1–3 transverse septa, with 1–2 longitudinal or Y-shape septa in transverse division, smooth to minutely verrucose and thin-walled.

Figure 7. *Alternaria torilis* (MFLU 21-0299, holotype). (a) Colonies on stems of *Torilis arvensis*; (b–h) Conidiophores bearing conidiogenous cells; (i) Secondary conidiophores arising from conidium; (j–q) Conidia; (r,s) Germinated conidia. Scale bars: (a) = 200 µm, (c,g) = 30 µm, (b,d–f,h–s) = 20 µm.

Material examined: Italy, Province of Forlì-Cesena, Forlì, San Lorenzo in Noceto, on dead aerial stems of *Torilis arvensis* (*Apiaceae*), 23 January 2014, E. Camporesi, IT1667 (MFLU 21-0299, holotype), ex-type living culture = MFLUCC 14-0433, *ibid.*, MFLUCC 21-0133.

Notes: *Alternaria torilis* resembles *A. alternata* in having a brown to dark brown short beak, 2–4 transverse septa conidia and forming secondary conidiophores. The conidial body can narrow gradually into a tapered beak or secondary conidiophore, with curved primary conidiophores and solitary conidiophores with mono- to polytretic conidiophores with conidiogenous loci at the apex. *Alternaria torilis* differs from *A. alternata* by its darker, ovoid to obturbinate or obpyriform, which is rather ovoid to chiefly obclavate or obpyriform in *A. alternata*. Conidiophores of *A. torilis* normally have 2 conidiogenous loci and are rostrate at the apex. In the phylogenetic analyses, three strains of *A. torilis* formed a well-resolved subclade (85% ML, 1.00 PP; Figure 1), independently constituted within sect. *Alternaria,*
and have a close relationship with A. ellipsoidalis and A. eupatoriicola distancing from A. alternata. Alternaria torilis can be distinguished from A. ellipsoidalis in having larger (55–82 × 23–32 μm vs. 35–60 × 18–25 μm), fusiform to ovoid, or obpyriform conidia, brown to dark brown conidia, with 2–4 transverse eusepta. Alternaria ellipsoidalis has oblong to ellipsoidal, ovoid, pale brown to brown, sectored, 4–7 transverse eusepta conidia [2]. Alternaria eupatoriicola is different from A. torilis in having smaller (40–65 × 15–30 μm vs. 55–82 × 23–32), ovoid to obpyriform, reddish brown to brown, 3–5 transverse septa conidia. In addition, conidia of A. torilis formed apically secondary conidiophores with one conidiogenous locus, whereas it was absent in A. eupatoriicola [2].

The nucleotide pairwise comparison of the ITS showed that Alternaria torilis differs from A. alternata (CBS 916.96, ex-type) in 9/485 bp (1.9% difference, no gap), differs from A. ellipsoidalis in 10/485 bp (2.1% difference, no gap) and differs from A. eupatoriicola in 9/480 bp (1.9% difference, no gap). A rpb2 nucleotide pairwise comparison showed that A. torilis differs from A. alternata (CBS 916.96, ex-type) in 42/558 bp (7.5% difference, no gap), differs from A. ellipsoidalis in 9/480 bp (1.9% difference, no gap) and differs from A. eupatoriicola in 40/558 bp (7.2% difference, no gap). A gapdh nucleotide pairwise comparison showed that A. torilis differs from A. alternata (CBS 916.96, ex-type) in 31/590 bp (5.3% difference, no gap), differs from A. ellipsoidalis in 18/560 bp (3.2% difference, no gap) and differs from A. eupatoriicola in 25/590 bp (4.2% difference, no gap). The nucleotide pairwise comparison of the Alt-a1 showed that A. torilis differs from A. alternata (CBS 916.96, ex-type) in 25/465 bp (5.4% difference, no gap), differs from A. ellipsoidalis in 20/465 bp (4.3% difference, no gap) and differs from A. eupatoriicola in 15/470 bp (3.2% difference, no gap).

4. Discussion and Conclusions

The aim of the present study was to introduce six novel Alternaria species in sect. Alternaria based on a morpho-molecular approach. These six saprobic species occurred on a variety of host plants in families Apiaceae, Brassicaceae, Chenopodiaceae, Fabaceae, Plantaginaceae, and Poaceae in Italy and could not be ascribed to any known taxon within sect. Alternaria. According to a recent classification provided by Woudenberg et al. [17] and Gannibal [15], we also note the morphological differences among extant species in this section. Hence, six new species: A. muriiformispora, A. obpyriconidia, A. ovoides, A. pseudoinfectoria, A. rostroconidia and A. torilis are introduced, described and illustrated herein.

Multi-locus phylogeny, based on a concatenated ITS, LSU, SSU, tef1-a, rpb2, gapdh and Alt-a1 DNA sequence matrix, revealed that these novel species formed well-resolved subclades within the sect. Alternaria, except for A. obpyriconidia that formed a distinct branch with other closely related species with low support in ML, but well-resolved species in BI analysis (1.00 PP; Figure 1). Based on the phylogenetic analyses and morphological characteristics, coupled with host preferences and nucleotide polymorphisms, A. obpyriconidia is justified as a new species following Jeewon and Hyde [48]. Furthermore, these six new species are distant from A. arborescens species complex (AASC) and A. alternata as well as other species in this section, which provided further evidence to support their phylogenetic affinities within the sect. Alternaria.

In the present analyses, Alternaria doliconidium and A. italica formed subclades, constituted within A. alternata, and that concurred with Li et al. [2]. Even though Woudenberg et al. [17] accepted only 11 phylogenetic species and one species complex in sect. Alternaria, and also treated 35 morphospecies as synonyms of A. alternata, Li et al. [2] re-analyzed the isolates of A. alternata with their new collections and mentioned that A. alternata could be separated to be at least five distinct species. However, more evidence is needed to support this conclusion. Similarly, A. doliconidium and A. italica lack informative cording genes such as Alt-a1, gapdh, rpb2 and tef1-a to justify their heterospecific status, with A. alternata pending further studies.

Woudenberg et al. [17] indicated that Alternaria species, including Alternaria sect. Alternaria, should be delineated by using phylogenomics due to a lack of effective gene sequences; however, the multi-locus phylogenetic analyses could well delineate species
in sect. Alternaria (Figure 1) in studies of Wanasinghe et al. [20], Jayawardena et al. [21], Nishikawa and Nakashima [22] and Li et al. [2]. In the present study, phylogenetically analyzed taxa in sect. Alternaria, based on combined the intervening ITS regions, nuclear ribosomal DNA SSU, LSU and protein-coding genes Alt-a1, tef1-α, gapdh and rpb2, demonstrated that the recent taxa in this section formed distinct clades and were well supported in the phylogenetic tree. Nucleotide polymorphic comparisons also show the differences between our new taxa, which support the justifications of the new species described herein. It is interesting to note that in the nucleotide polymorphic comparisons of gene sequences among the species in Alternaria sect. Alternaria, rpb2 contains the most nucleotide differences among the species (up to 3.5%), which implies that this protein-coding gene may be a potentially effective gene region to delineate species in sect. Alternaria.

Nevertheless, species of Alternaria in sect. Alternaria are similar in morphological characteristics, and it is difficult to distinguish these species based solely on morphology. However, the conidial characteristics (e.g., conidial septation and rostrate or non-beak conidia) of our six novel species are significant to distinguish them from other species. Multi-locus phylogenetic analyses also provided further evidence, confirming that these six species are novel. These six species clearly formed a separate branch with significant support values (≥70% ML and 0.95 PP; Figure 1) in the present study, and this concurs with the findings of Li et al. [2]. Jeewon and Hyde [48] suggested that the nucleotide polymorphic comparisons of reliable genes should be more than 1.5% different for justifying the novel species. Even though the ITS, LSU, SSU and tef1-α could not be used to delineate some species in sect. Alternaria, the remaining gene regions (i.e., Alt-a1, gapdh and rpb2) proved sufficient for distinguishing these new species. Therefore, the novel species introduced herein were justified based on the multi-locus phylogeny coupled with morphological characteristics and nucleotide polymorphic comparisons of reliable genes.

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