Didymella corylicola sp. nov., a new fungus associated with hazelnut fruit development in Italy

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Abstract

A new fungal species constantly associated with hazelnut (Corylus avellana) fructification starting from its primordia is described. The fungus is associated with hazelnut fruit during all their developmental stages, being consistently more present in spring (March–June). A 4-year survey has been conducted, from young fruit formation to full kernel maturity including also the post-harvest phase, to collect fungi associated with damaged/discoloured kernels. A collection of 60 isolates of a new species has been obtained in this study, which is here described as Didymella corylicola sp. nov. Multi-locus phylogenies based on four genomic loci (nuITS and LSU rDNA, RPB2 and TUB2) in combination with morphological data confirmed the fungus to represent a new species of Didymella (Didymellaceae). The occurrence of D. corylicola sp. nov. might have an impact on the quality of hazelnut production by contributing to kernel defects.

Keywords Corylus avellana · Kernel defects · Nut disease · Phoma · Pleosporales · 1 new species

Introduction

Hazelnut (Corylus avellana L.) is native to Europe and Western Asia where it is widely distributed (Olsen 2013; Enescu et al. 2016). The main hazelnut producing countries are Turkey, Italy and the USA. Turkey produces over 60% of the world total, followed by Italy, Azerbaijan and the United States (FAOSTAT; data for 2017 retrieved from http://www.fao.org/faostat/en/#data/QC). Commercial hazelnut orchards have expanded significantly in the last years due to an increasing demand for direct consumption of fruit as well as often in combination with chocolate. Nearly 90% of the harvested yield is destined to processing companies, whereas fresh consumption represents the residual 10%. Hazelnut cultivation is dispersed all over Italy, from north to south, with main production located in Piedmont (26%), Latium (32%), Campania (28%) and Sicily (9%) regions (ISTAT, http://dati.istat.it/). These percentages just give an indication since production may vary from year to year. Hazelnut is characterized by good rusticity which makes it an adaptable fruit tree species compared to other nut species such as almond, walnut and pistachio. Nevertheless, it is affected by several diseases and fungal pathogens which can be particularly harmful in altering the kernel and consequently reducing harvest quality and yield. Hazelnut represents a high value product suffering kernel defects which might be climate dependent, inducing production fluctuations. In Piedmont and in Campania, gleosporiosis (purple necrotic spots on female flowers, petioles, nut bracts and husk), caused by Elsinoe coryli (syn. Sphaceloma coryli), has been reported (Minutolo et al. 2016), while in central Italy (Latium, Viterbo province) and in Campania, Alternaria spp., Colletotrichum spp., Fusarium spp. and Diaporthe spp. have
also been isolated (Librandi et al. 2006). From the year 2000 onwards, special attention has been given to identify the causal agent of hazelnut fruit drop, known as nut grey necrosis (NGN) disease, which was identified as *Fusarium lateritium* (Vitale et al. 2011). In this extensive survey, an unknown fungus with typical deeply red-pigmented colonies was isolated in every phenological phase, particularly in the early stages of hazelnut fruit formation. This fungus was also isolated in the surveys from 2016 onwards, which were addressed to identify the fungi associated with kernel damage/discoloration and the potential causal agents of kernel defects.

The aims of this study were to provide morphological, taxonomic and phylogenetic data for the red-pigmented fungus which proved to be an undescribed species of the genus *Didymella*. Experimental evidence on its relation with hazelnut kernel defects is given.

### Materials and methods

#### Field surveys and isolations

In past years, numerous surveys were carried out, mainly in Latium region, on the causal agent of the nut grey necrosis (NGN) disease, which caused heavy losses for about 10 years, starting from its outbreak in 2000 (Vitale et al. 2011). More recently, surveys of a total of 9 hazelnut orchards were conducted from 2016 to 2019 in Campania, as well as in 7 hazelnut orchards located in Piedmont. Except for 2016, in which the survey started at fruit yield, sampling started from the end of March onwards, considering several phenological stages, namely, bud break/initial fruit formation (March–April), nut development (May–June), embryo and kernel development (late June–July), ripening nuts-harvest phase (August–September), and post-harvest sampling (September–October). About 300 fruits were sampled per orchard. Samples were subjected to surface disinfection in 10% sodium hypochlorite for 60 s, rinsed in sterile water for 60 s, and dried on sterile filter paper in a laminar flow. Tissue fragments were placed onto potato dextrose agar (PDA) Petri dishes at 25 °C in the dark, and daily observed for mycelial growth.

Numerous red-pigmented cultures were obtained, and single-spore isolations were performed with conidia collected from pycnidia produced on those cultures within 1 month of incubation at room temperature under daylight. More than 60 single-spore isolates were obtained from symptomatic tissue isolation. Amongst these, 10 isolates representative of the three main colony morphologies were characterized by molecular data and phylogenetic analyses (Table 1), and the three isolates CREADC-F2281, CREADC-F2402 and CREADC-F2403 were subjected to detailed morphological, taxonomic, phylogenetic and pathogenic studies.

#### Morphological characterization

For culture characteristics, colonies were grown on 2% (w/v) malt extract agar (MEA, VWR), potato dextrose agar (PDA, Sigma-Aldrich), corn meal agar (CMA, Sigma-Aldrich) supplemented with 2% w/v dextrose (CMD), oatmeal agar (OA, Sigma-Aldrich) and hazelnut extract agar (HEA; 25 g/l fresh chopped hazel twigs, autoclaved in 2% agar). Colony diameters were measured after 7 days, and colony morphologies were determined after 14 days of incubation at room temperature (22 °C) and daylight. To promote pycnidial formation, cultures were grown on HEA and hazel twig agar (HTA; autoclaved split hazel twigs placed on 2% agar plates, shortly before solidification). The isolates used in this study are maintained in the culture collection of the CREA-DC (ex CREA-PAV. The ex-holotype isolate (CREADC-F2403) of the new hazelnut pathogen was deposited at the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands, and the holotype specimen in the fungarium of the Department of Botany and Biodiversity Research, University of Vienna (WU).

Microscopic observations were made in tap water except where noted in the figure legend. Sections of 8 μm thickness of pycnidia were prepared with a Leica FrigoCut 2700 freezing microtome. Methods of microscopy included stereomicroscopy using a Nikon SMZ 1500 equipped with a Nikon DS-U2 digital camera, and Nomarski differential interference contrast (DIC) using a Zeiss Axio Imager.A1 compound microscope equipped with a Zeiss Axiocam 506 colour digital camera. Images and data were gathered using the NIS-Elements D v. 3.2.22.15 or Zeiss ZEN Blue Edition software packages. Measurements are reported as maxima and minima in parentheses and the range representing the mean plus and minus the standard deviation of a number of measurements given in parentheses.

#### Temperature-growth relationships

For investigations of temperature-growth relationships of the new hazelnut fungus, the ex-holotype isolate CREADC-F2403, and the isolates CREADC-F2281 and CREADC-F2402 were used. Agar plugs (5 mm diameter) were taken from the edge of actively growing cultures on PDA and transferred onto the centre of 9-cm PDA Petri dishes. Three replicate plates were incubated at 5, 10, 15, 20, 25, 30 and 35 °C in the dark and measurements were taken after 7 days at right angles along two lines intersecting the centre of the inoculum and the mean growth rates plus and minus the standard deviation (± SD) were calculated.
Table 1 Isolates and accession numbers of sequences used in the phylogenetic analyses. Isolates and sequences in bold were obtained in the present study.

| Species                  | Strain number1 | GenBank accession numbers2 |
|--------------------------|---------------|---------------------------|
|                          |               | LSU | ITS | RPB2 | TUB2 |
| Didymella acetosellae    | CBS 179.97    | GU238034 | GU237793 | KP330415 | GU237575 |
| D. aeria                 | LC 8120       | KY742052 | KY742206 | KY742138 | KY742294 |
| D. aliena                | CGMCC 3.18353 | KY742051 | KY742205 | KY742137 | KY742293 |
|                          | CBS 379.93; PD 82/945 | GU238037 | GU237851 | KP330416 | GU237578 |
| D. americana             | CBS 185.85; PD 80/1191 | GU237990 | FJ426972 | KT389594 | FJ427088 |
|                          | CBS 568.97; ATCC 44494; PD 94/1544 | GU237991 | FJ426974 | – | FJ427090 |
| D. anserina              | CBS 253.80    | KT389715 | KT389498 | KT389595 | KT389795 |
|                          | CBS 285.29    | KT389716 | KT389499 | – | KT389796 |
|                          | CBS 360.84    | GU237993 | GU237839 | KT389596 | GU237551 |
|                          | CBS 397.65    | KT389717 | KT389500 | KT389597 | KT389797 |
| D. aquatica              | CGMCC 3.18349 | KY742055 | KY742209 | KY742140 | KY742297 |
|                          | LC 5555       | KY742056 | KY742210 | KY742141 | KY742298 |
| D. arachidicola          | CBS 333.75; ATCC 28333; IMI 386092; PREM 44889 | GU237996 | GU237833 | KT389598 | GU237554 |
| D. aurea                 | CBS 269.93; PD 78/1087 | GU237999 | GU237818 | KT389599 | GU237557 |
| D. bellidis              | CBS 714.85; PD 74/265 | GU238046 | GU237904 | KP330417 | GU237586 |
|                          | PD 94/886     | GU238047 | GU237923 | – | GU237587 |
| D. boeremae              | CBS 109942; PD 84/402 | GU238048 | FJ426982 | KT389600 | FJ427097 |
| D. brunneospora          | CBS 115.58; DSM 62044 | KT389723 | KT389505 | KT389625 | KT389802 |
| D. calidophila           | CBS 448.83    | GU238052 | FJ427059 | – | FJ427168 |
|                          | PD 84/109     | GU238053 | FJ427069 | – | FJ427169 |
| D. chenopodi             | CBS 128.93; PD 79/140 | GU238055 | GU237775 | KT389602 | GU237591 |
| D. chloroguttulata       | CGMCC 3.18351 | KY742057 | KY742211 | KY742142 | KY742299 |
|                          | LC 8122       | KY742058 | KY742212 | KY742143 | KY742300 |
| D. coffea-arabicae       | CBS 123380; PD 84/1013 | GU238005 | FJ426993 | KT389603 | FJ427104 |
| D. corylica              | CREADC-F2281  | MN954299 | MN954288 | MN958321 | MN958331 |
|                          | CREADC-F2402  | MN954300 | MN954289 | MN958322 | MN958332 |
|                          | CREADC-F2403  | MN954301 | MN954290 | MN958323 | MN958333 |
|                          | CREADC-F2404  | MN954302 | MN954291 | – | – |
|                          | CREADC-F2405  | MN954303 | MN954292 | MN958324 | MN958334 |
|                          | CREADC-F2406  | MN954304 | MN954293 | MN958325 | MN958335 |
|                          | CREADC-F2407  | MN954305 | MN954294 | MN958326 | MN958336 |
|                          | CREADC-F2408  | MN954306 | MN954295 | MN958327 | MN958337 |
|                          | CREADC-F2409  | MN954307 | MN954296 | MN958328 | MN958338 |
|                          | CREADC-F2410  | MN954308 | MN954297 | MN958329 | MN958339 |
|                          | CREADC-F2411  | MN954309 | MN954298 | MN958330 | MN958340 |
| D. curtisii              | CBS 251.92; PD 86/1145 | GU238013 | FJ427038 | – | FJ427148 |
|                          | PD 92/1460    | GU238012 | FJ427041 | KT389604 | FJ427151 |
| D. dactylidis            | CBS 124513; PD 73/1414 | GU238061 | GU237766 | – | GU237599 |
| D. dimorpha              | CBS 346.82    | GU238068 | GU237835 | – | GU237606 |
| D. ellipsoidea           | CGMCC 3.18350 | KY742060 | KY742214 | KY742145 | KY742302 |
|                          | LC 8123       | KY742061 | KY742215 | KY742146 | KY742303 |
| D. eucalyptica           | CBS 377.91; PD 79/210 | GU238007 | GU237846 | KT389605 | GU237562 |
| D. exigua                | CBS 183.55    | EU754155 | GU237794 | EU874850 | GU237525 |
| D. gardeniae             | CBS 626.68; IMI 108771 | GQ387595 | FJ427003 | KT389606 | FJ427114 |
| D. glomerata             | CBS 133.72    | KT389718 | FJ427004 | – | FJ427115 |
|                          | CBS 528.66; PD 63/590 | EU754184 | FJ427013 | GU371781 | FJ427124 |
Table 1 (continued)

| Species                      | Strain number1          | GenBank accession numbers2 |
|------------------------------|-------------------------|---------------------------|
|                              |                         | LSU | ITS | RPB2 | TUB2 |
| *D. heteroderae*             | CBS 109.92; PD 73/1405  | GU238002 | FJ426983 | KT389601 | FJ427098 |
| *D. illicicola*              | CGMCC 3.18355           | KY742065 | KY742219 | KY742150 | KY742307 |
|                             | LC 8127                 | KY742066 | KY742220 | KY742151 | KY742308 |
| *D. infuscatispora*          | CGMCC 3.18356           | KY742067 | KY742221 | KY742152 | KY742309 |
|                             | LC 8129                 | KY742068 | KY742222 | –        | KY742310 |
| *D. keratinophila*           | CBS 143032; UTHSC:DI16–200; FMR 13690 | LT592901 | L907343 | LT593039 | LT592970 |
|                             | UTHSC:DI16–228; FMR 13718 | LT592915 | L907371 | LT593053 | LT592984 |
|                             | UTHSC:DI16–282; FMR 13774 | LT592938 | L907425 | LT593077 | LT593007 |
| *D. lethalis*                | CBS 103.25              | GU238010 | GU237729 | KT389607 | GU237564 |
| *D. longicolla*              | CBS 124514; PD 80/1189  | GU238095 | GU237767 | –        | GU237622 |
| *D. macrophilla*             | CGMCC 3.18357           | KY742070 | KY742224 | KY742154 | KY742312 |
|                             | LC 8132                 | KY742071 | KY742225 | KY742155 | KY742313 |
| *D. macrostoma*              | CBS 482.95              | GU238099 | GU237869 | KT389609 | GU237626 |
|                             | CBS 529.66; PD 66/521   | GU238098 | GU237885 | –        | GU237625 |
|                             | CBS 223.69              | GU238096 | GU237801 | KT389608 | GU237623 |
|                             | CBS 247.38              | KT389719 | KT389501 | –        | KT389798 |
| *D. maydis*                  | CBS 588.69              | EU754192 | FJ427086 | GU371782 | FJ427190 |
| *D. microchlamydospora*      | CBS 105.95              | GU238104 | FJ427028 | KP330424 | FJ427138 |
| *D. molleriana*              | CBS 229.79; LEV 7660    | GU238067 | GU237802 | KP330418 | GU237605 |
|                             | CBS 109179; PD 90/835–1 | GU238066 | GU237744 | –        | GU237604 |
| *D. musae*                   | CBS 463.69              | GU238011 | FJ427026 | LT623248 | FJ427136 |
| *D. negriana*                | CBS 358.71              | GU238116 | GU237838 | KT389610 | GU237635 |
| *D. nigricans*               | CBS 444.81; PDDCC 6546  | GU238000 | GU237867 | –        | GU237558 |
|                             | PD 77/919               | GU238001 | GU237915 | KT389611 | GU237559 |
| *D. oecimicola*              | CGMCC 3.18358           | KY742078 | KY742232 | –        | KY742320 |
|                             | LC 8138                 | KY742079 | KY742233 | –        | KY742321 |
| *D. pedeiae*                 | CBS 124517; PD 92/612A  | GU238127 | GU237770 | KT389612 | GU237642 |
| *D. pinodella*               | CBS 318.90; PD 81/729   | GU238016 | FJ427051 | –        | FJ427161 |
| *D. pinodes*                 | CBS 531.66              | GU238017 | FJ427052 | KT389613 | FJ427162 |
| *D. pomorum*                 | CBS 285.76; ATCC 26241; IMI 176742; VKM F-1843 | GU238025 | FJ427053 | KT389615 | FJ427163 |
|                             | CBS 388.80              | GU238027 | FJ427055 | KT389617 | FJ427165 |
|                             | CBS 539.66; ATCC 16791; IMI 122266; PD 64/914 | GU238028 | FJ427056 | KT389618 | FJ427166 |
|                             | CBS 354.52              | KT389720 | KT389502 | KT389616 | KT389799 |
| *D. protuberans*             | CBS 132.96; PD 93/853   | GU237989 | GU237778 | –        | GU237550 |
|                             | CBS 377.93; PD 80/976   | GU238014 | GU237847 | KT389619 | GU237565 |
|                             | CBS 391.93; PD 80/87    | GU238015 | GU237858 | KT389621 | GU237566 |
|                             | CBS 381.96; PD 71/706   | GU238029 | GU237853 | KT389620 | GU237574 |
| *D. pteridis*                | CBS 379.96              | KT389722 | KT389504 | KT389624 | KT389801 |
| *D. rhei*                    | CBS 109177; LEV 15165; PD 2000/9941 | GU238139 | GU237743 | KP330428 | GU237653 |
| *D. rosea*                   | BRIP 50788              | KT287003 | KT338640 | –        | KT286945 |
| *D. rumicicola*              | CBS 683.79; LEV 15094   | KT389721 | KT389503 | KT389622 | KT389800 |
| *D. sancta*                  | CBS 281.83              | KT389603 | FJ427063 | KT389623 | FJ427170 |
| *D. segeticola*              | CGMCC 3.17489           | KP330443 | KP330455 | KP330414 | KP330399 |
|                             | CGMCC 3.17498           | KP330442 | KP330454 | KP330413 | KP330398 |
| *D. senecionica*             | CBS 160.78; LEV 11451   | GU238143 | GU237787 | –        | GU237657 |
| *D. sinensis*                | LC 8142                 | KY742087 | KY742241 | KY742166 | KY742329 |
DNA extraction, PCR amplification and sequencing

The extraction of genomic DNA from pure cultures was performed as reported in previous studies (Voglmayr and Jaklitsch 2008; Adekambi et al. 2009). The PCR product was purified using an enzymatic PCR cleanup (Welte et al. 1994) as described in Voglmayr and Jaklitsch (2008). DNA was cycle-sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Warrington, UK) with the same primers as in PCR; in addition, primers ITS4, LR2-R-A (Voglmayr et al. 2012) and LR3 (Vilgalys and Hester 1990) were used for the ITS-LSU fragment. Sequencing was performed on an automated DNA sequencer (3730xl Genetic Analyser, Applied Biosystems).

Phylogenetic analyses

The newly generated sequences were aligned with selected sequences of Chen et al. (2017) and complemented with a few recent additions from GenBank. The GenBank accession numbers of sequences used in the analyses are given in Table 1. A combined matrix of the three loci (partial nuITS-LSU rDNA, RPB2 and TUB2) was produced for phylogenetic analyses, with two species of Macroventuria (M. anomochaeta, M. wentii) and three species of Paraboeremia (P. adianticola, P. putaminum and P. selaginellae) added as the outgroup taxa according to the results of the phylogenetic analyses of Chen et al. (2017). Sequence alignments were produced with the server
version of MAFFT (http://mafft.cbrc.jp/alignment/server/) , checked and refined using BioEdit v. 7.2.6 (Hall 1999). The combined data matrix contained 3010 characters; viz. 493 nucleotides of ITS, 1327 nucleotides of the LSU, 846 nucleotides of RPB2 and 344 nucleotides of TUB2.

Maximum parsimony (MP) analyses were performed with PAUP v. 4.0a166 (Swofford 2002). All molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data; the COLLAPSE command was set to MINBRLEN. MP analysis of the combined multi-locus matrix was done using 1000 replicates of heuristic search with random addition of sequences and subsequent TBR branch swapping (MULTREES option in effect, steepest descent option not in effect). Bootstrap analyses with 1000 replicates were performed in the same way, but using 5 rounds of random sequence addition and subsequent branch swapping during each bootstrap replicate.

Maximum likelihood (ML) analyses were performed with RAxML (Stamatakis 2006) as implemented in raxmlGUI 1.3 (Silvestro and Michalak 2012), using the ML + rapid bootstrap setting and the GTRGAMMA substitution model with 1000 bootstrap replicates. The matrix was partitioned for the different gene regions. In the “Results” and “Discussion” sections, bootstrap values below 70% are considered low, between 70 and 90% medium and above 90% high.

Pathogenicity

Pathogenicity tests with the three fungal strains of the undescribed hazelnut pathogen were performed to fulfil Koch’s postulates. For this, conidial suspensions were prepared by washing the conidia from actively sporulating agar cultures with sterile distilled water; the conidial concentration was determined using a haemocytometer, and the concentration adjusted with sterile distilled water to 1 × 10⁶ conidia/ml. The isolates were tested either by inoculating the apex of fresh and mature hazelnuts after making a little hole with a sterile needle and introducing 25 μl of conidial suspension into each kernel cavity (Scarpari et al. 2018) or a drop (10 μl) of conidial suspension was adjusted at the centre of halved hazelnut kernels. The inoculated nuts (twenty replicates for each isolate) and negative controls (ten replicates) treated with sterile water only were all incubated in the dark at 25 °C in a humid growth chamber for the first 72 h. After 2 weeks, the results of inoculations were checked by halving the whole nuts or directly on the halved kernels.

Results

Isolations and morphological characterization

In the recent surveys carried out from 2016 to 2019, the undescribed fungal species associated with hazelnut fructifications was only isolated in Campania, during the whole vegetative season (from March to September) and occasionally from post-harvest nuts. Until now, the novel Corylus fungus has never been isolated in Piedmont. In the previous investigations of 2000–2010, colonies of this fungus were obtained in surveys conducted on hazelnut orchards in Viterbo province (Latium Region).

Culture images of three strains (CREADC-F2281, CREADC-F2402, CREADC-F2403) grown on OA, PDA, CMD and HEA for 2 weeks at room temperature are shown in Fig. 1. Detailed descriptions of morphological traits are given in the “Taxonomy” section below.

Temperature-growth relationships

The growth rate experiments (Fig. 2) revealed 20 °C as optimal temperature for all the three isolates with an evidently better growth of culture CREADC-F2281 (10.54 ± 0.25 mm/day), originating from Latium region, compared to the ex-holotype culture CREADC-F2403 with 7.50 ± 1.07 mm/day. Conversely, the ex-holotype culture showed a faster growth at 25 °C in comparison with the other two isolates. No growth was recorded at 35 °C, while all three isolates were able to grow at 5 °C, at which the ex-holotype culture performed best (1.55 ± 0.1 mm/day).

Phylogenetic analyses

Of the 3010 characters included in the phylogenetic analyses, 449 were parsimony informative (41 from the ITS, 20 from the LSU, 256 from RPB2, 132 from TUB2). MP analyses revealed 114 MP trees 2199 steps long, one of which is shown as Fig. 3. The tree backbone was identical in all MP trees, except for topologies of several deeper nodes of the Peyronellaea clade marked by an asterisk in Fig. 3. The best ML tree (lnL = −14.674.1908) revealed by RAxML was largely compatible with the MP tree shown in Fig. 3, except for differences in some deeper unsupported nodes (not shown). In the MP and ML analyses, the novel Corylus fungus was placed within a clade containing D. pedeiae, D. ilicicola and D. subherbarum with maximum support (group G of Aveskamp et al. 2010), but it remained unresolved whether D. ilicicola or D. subherbarum is its closest relative (Fig. 3).

Pathogenicity

Necrotic lesions were evident on the halved kernels while rather limited on the whole nuts. Re-isolations from the margin of lesions cultured on PDA gave colonies with the same morphological characters as those used for inoculation, thus confirming Koch’s postulates.
Taxonomy

*Didymella corylicola* Voglmayr, Scarpari, Di Giambattista, Vitale and Luongo sp. nov. Figs 1 and 4.

*Mycobank*: MB 833929

*Etymology*: *corylicola*, referring to its occurrence on *Corylus* fructifications.

*Diagnosis*: *Didymella corylicola* is recognized by colonies producing bright red diffusible pigments in pure culture.

*Description*: Pycnidia (46–55–82(–110) μm diameter (*n* = 100), solitary, produced within the agar, on the agar surface or on the aerial mycelium, (sub-)globose to broadly pyriform, glabrous, brown, with 1 (rarely 2) conspicuous, non-papillate ostioles (8–)11–17(–22) μm wide (*n* = 32). Pycnidal wall (4–)4.5–6.5(–7.5) μm thick (*n* = 31), brown, pseudoparenchymatous, composed of 1–3 layers of isodiametric to oblong cells (4–)6–10(–16) × (3–)4–8(–11) μm (*n* = 66). *Conidiogenous cells* (4.3–)4.5–7.8(–10.0) × (2.8–)3.5–5.0(–5.8) μm (*n* = 18), phialidic, hyaline, simple, smooth, globose to flask-shaped. *Conidia* (3.2–)3.8–4.5(–5.3) × (1.4–)1.6–1.9(–2.1) μm, l/w = (1.7–)2.1–2.6(–3.1) (*n* = 221), ellipsoidal to oblong, commonly slightly allantoid, thin-walled, smooth, hyaline, aseptate, with 0–2 tiny guttules. *Conidial matrix* white.
Culture characteristics: Colonies on PDA 47–62 mm diameter after 7 days at 22 °C, margin regular or irregular, covered by floccose aerial mycelium, grey brown to dark vinaceous brown, at least in the centre becoming dark vinaceous brown with age, sometimes with concentric rings; reverse blackish with bright red brown margins. Colonies on MEA 53–58 mm diameter after 7 days at 22 °C, with culture characteristics similar to those on PDA. Colonies on CMD 67–72 mm diameter after 7 days at 22 °C, with culture characteristics similar to those on PDA. Colonies on OA with regular or irregular margin, covered by floccose aerial mycelium, olivaceous brown, grey brown to dark vinaceous brown, at least in the centre becoming dark vinaceous brown with age, sometimes with concentric rings; reverse buff to olivaceous brown with a dark vinaceous brown centre. Colonies on HEA with regular or irregular margin, covered by floccose aerial mycelium, olivaceous brown, at least in the centre becoming dark vinaceous brown with age, sometimes with concentric rings; reverse buff to olivaceous brown with a dark vinaceous brown centre. Colonies on OA with regular or irregular margin, covered by floccose aerial mycelium, grey brown to dark vinaceous brown, at least in the centre becoming dark vinaceous brown with age, sometimes with concentric rings; reverse buff to olivaceous brown with a dark vinaceous brown centre.

Holotype: Italy, Campania region, Caserta province, Teano, from kernel of Corylus avellana (Betulaceae) at harvest phase, August 2017 (WU 40039; ex-holotype culture CBS 146357 = CREADC-F2403).

Other specimens examined (all from kernels of Corylus avellana): Italy, Campania, Caserta province, Teano, August 2017 (WU 40040, culture CREADC-F2402); Latium, Viterbo province, Ronciglione, August 2006 (WU 40041, culture CREADC-F2281).

Notes: Didymella corylicola is phylogenetically closely related to D. pedeiae, D. ilicicola and D. subherbarum (Fig. 3), from which it differs in 1, 1 and 2 nucleotides, respectively, in the ITS; in 25, 3 and ? (no sequence available for D. subherbarum) nucleotides, respectively, in RPB2; and in 14–15, 6–7 and 11–12 nucleotides, respectively, in TUB2. However, D. corylicola differs significantly from D. pedeiae, D. ilicicola and D. subherbarum in its bright red pigment produced in agar cultures and by its specific host, Corylus avellana. Morphologically, D. corylicola has conidial sizes similar to those of D. ilicicola, D. pedeiae and D. subherbarum (D. corylicola: 3.2–5.3 × 1.4–2.1 μm; D. ilicicola: 3–4 × 1.5–2.5 μm (Chen et al. 2017); D. pedeiae 3–4.5 × 1.5–2.5 μm (Aveskamp et al. 2010); D. subherbarum: 4–6.4 × 1.6–2.2 μm (de Gruyter et al. 1993)). However, in contrast to its closest relatives which all have straight ellipsoid to oblong conidia, the conidia of D. corylicola are commonly slightly allantoid.

Discussion

The Didymellaceae in general and the genus Didymella in particular are a species-rich lineage within Pleosporales, containing numerous plant pathogens (Chen et al. 2015, 2017). The taxonomy of the group is complex and challenging, as it contains, amongst others, highly speciose sexual (Didymella, Ascochyta) as well as asexual (e.g., Epicoccum, Phoma) morph genera. Until recently, the simple morphology of both sexual and asexual morphs was a serious obstacle for reliable genus and species circumscriptions as well as for species identification. However, with the application of multigene phylogenies, substantial progress in genus and species delimitation...
Fig. 4 Didymella corylicola. a–c Pycnidia in face view. d Substrate hyphae. e, f Torulose hyphae. g Amorphous red pigments in agar. h, i Pycnidia in side (h) and face (i) view. j, k Pycnidia in section. l–q Conidiogenous cells (phialides). r, s Conidia (s ejected and swollen, with amorphous red pigments in-between). All in water, except h, i in 3% KOH. Media: a HEA; b–d, g–s HTA; e, f MEA. Sources: a–c, e, f, h–o, r ex-holotype strain CBS 146357 = CREADC-F2403; d, g, p, q, s CREADC-F2402. Scale bars a, b 200 μm; c 100 μm; d–k 10 μm; l–s 5 μm.
within Didymellaceae has been achieved in recent years (Aveskamp et al. 2010; Chen et al. 2015, 2017), in particular for the large genera *Ascochyta*, *Didymella* and *Phoma*. Several new genera were established, the genus *Phoma* was restricted to the type species, *P. herbarum*, and numerous species of *Phoma* were transferred to the genera *Allophoma*, *Ascochyta*, *Boeremia*, *Calophoma*, *Didymella*, *Epicoccum*, *Heterophoma*, *Neascochyta*, *Neodidymellioptis*, *Nothophoma*, *Paraboeremia*, *Stagonosporopsis* and *Xenodidymella* (Chen et al. 2015). In addition, new species of *Didymella* have been described (e.g. Chen et al. 2015, 2017; Valenzuela-Lopez et al. 2018), highlighting that the species diversity of *Didymella* is still insufficiently known.

In the molecular phylogenetic analyses, *D. corylicola* forms a highly supported clade with *D. pedeiae*, *D. ilicicola* and *D. subherbarum*, which corresponds to *Phoma* group G of Aveskamp et al. (2010). In contrast to *D. corylicola*, which is, as far known, host specific to *Corylus*, *D. pedeiae* and *D. subherbarum* are plurivorous (Aveskamp et al. 2010), while *D. ilicicola* is so far only known from seedlings of *flex chinensis* (Chen et al. 2017). All species of the clade share a negative NaOH reaction of the colonies, and while conidial sizes are overlapping with its closest relatives, *D. corylicola* is well characterised by the bright red pigment produced in agar cultures and its specific host. In addition, the genetic differences of *D. corylicola* justify its status as a distinct species.

*Didymella corylicola* represents a fungal species associated with hazelnut fructification from the primordial to post-harvest phase. Its abundance in the early stages of fruit development may be facilitated by the psychrophilic nature of this fungus which is able to grow at 5 °C. In addition, it seems to be associated only with hazelnut, since it has never been isolated from other common nut species such as walnut and pistachio, which were intensely surveyed in the past years by the authors (Belisario et al. 2002; Vitale et al. 2007, 2018; Scotton et al. 2015), and it has never been reported from almond. Although weak pathogenicity was revealed in the inoculation experiments, the impact of *D. corylicola* on the development of hazelnut fruit is yet unclear. Ongoing studies might show whether this fungus plays a direct role in the emergence of kernel defects, or whether it has an indirect effect by an interaction with known hazelnut fruit pathogens such as *Colletotrichum* spp., *Diaporthe* spp. or *Fusarium* spp.

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