Illuminating type collections of nectriaceous fungi in Saccardo’s fungarium

N. Forin1, A. Vizzini1,2,3,* S. Nigris1,4, E. Ercole2, S. Voyron2,3, M. Girlanda2,3, B. Baldan1,4,*

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Ascomycota
Hypocreales
Illmina
ribosomal sequences
Sordariomycetes

Abstract Specimens of Nectria spp. and Nectriella rufofusca were obtained from the fungarium of Pier Andrea Saccardo, and investigated via a morphological and molecular approach based on MiSeq technology. ITS1 and ITS2 sequences were successfully obtained from 24 specimens identified as ‘Nectria’ sensu Saccardo (including 20 types) and from the type specimen of Nectriella rufofusca. For Nectria ambigua, N. radians and N. tibiodensis only the ITS1 sequence was recovered. On the basis of morphological and molecular analyses new nomenclatural combinations for Nectria albofimbriata, N. ambigua, N. ambigua var. pallens, N. granuligera, N. peziza subsp. reyesiana, N. radians, N. squamuligera, N. tibiodensis and new synonyms for N. congesta, N. flageoleliana, N. phyllostachydia, N. sordecents and N. tibiodensis var. crebror are proposed. Furthermore, the current classification is confirmed for Nectria coronata, N. cyanostoma, N. dolichospora, N. iludens, N. leuconicha, N. mantuana, N. ranipila and Nectriella rufofusca. This is the first time that these more than 100-yr-old specimens are subjected to molecular analysis, thereby providing important new DNA sequence data authentic for these names.

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INTRODUCTION

Nectria, typified with N. cinnabarina, is an ascomycete genus of the family Nectriaceae comprising filamentous fungal species with a Tubercularia asexual morph and a sexual morph producing ascomatal perithecia. Normally perithecia are fleshy, uniloculate, red to bay, subglobose to globose with a smooth or warted surface; they are superficially localized on a well-developed stroma and produce cylindrical to clavate asci with ellipsoidal to fusoid, hyaline, smooth to spinulose ascospores (Rossman et al. 1999, Hirooka et al. 2012, Lombard et al. 2015). Members of this genus, as well as most nectriaceous fungi, are typically parasites of woody plants, occurring on hardwood trees and shrubs in tropical, subtropical, or temperate regions worldwide (Rossman et al. 1999, Hirooka et al. 2012).

The name Nectria was proposed by Fries in 1825 as an infrageneric section of the fungal genus Hypocrea. Subsequently, in 1849, Fries raised Nectria to generic rank (Booth 1959, Schroers 2001). For many years the taxonomic concept of this genus was broadly defined, and more than 1000 species were described and classified in Nectria s.l. (Hirooka et al. 2012). Rossman (1989) restricted the genus to species morphologically similar to the type species of the genus, Nectria cinnabarina. As a consequence, species excluded from Nectria s.str. were placed in different or old-resurrected genera of the hypocrealean families Biorectiaceae and Nectriaceae (Samuels 1976, Brayford et al. 2004, Lechat & Fournier 2017). The Biorectiaceae includes nectria-like species that have white to orange or brown perithecia which do not change colour in 3 % potassium hydroxide (KOH) or 100 % lactic acid (LA) (Rossmen et al. 1999). Members of the Nectriaceae typically have orange to red perithelial walls turning dark red or purple in KOH and yellow in LA (Rossmen et al. 1999, Schroers 2001). Taxonomic studies based on DNA sequence data confirmed not only the separation between Biorectiaceae and Nectriaceae, but also the relationships among the genera within the two families where nectria-like species were segregated (Rossman et al. 1999, 2001, Schroers 2001).

Saccardo (1878, 1883) restricted the genus Nectria to species with 1-septate ascospores, and described new species following this generic concept. He rearranged the genus into 10 different subgenera according to the presence or absence and nature of a stroma, perithecial surface characteristics, and ascospore morphology (Booth 1959, Samuels 1976, Schroers 2001). Many of the subgenera were raised to generic rank, but today only Lasonectria (Biorectiaceae; Rossman et al. 1999) and Dialonectria (Nectriaceae; Graevenhan et al. 2011), originally introduced by Saccardo, are accepted genera in Hypocreales.

In the Saccardo fungarium (1874–1916), stored in the Herbarium of the Botanical Garden of Padova (PAD), the genus Nectria s.l. is represented by over 111 different species comprising 434 specimens (Gola 1930). In addition, a further nine Nectria s.l. species (15 specimens) were found under the genus Polystigma (Gola 1930). Among these, 38 Nectria s.l. species, mainly from paleotropical areas and some represented by multiple specimens, were marked as T1 or cT1, indicating the presence of type or co-type material. In total, considering the presence of more than one specimen per species, 45 type specimens (e.g., holotype, lectotype, neotype or isotype) were identified. Many of these specimens (38) were used directly by Saccardo, or in collaboration with mycological colleagues (e.g., Penzig), for the first morphological description of new species. Others (seven) were described and named by contemporary...
mycologists (e.g., Berkeley or Traverso), sent to Saccardo, and deposited in his fungarium. All these specimens were deposited prior to the year 1920 and they are stored on the substrates on which they were originally found (bark, dead wood and plant stems). Over time, many of these types were morphologically revised and placed in synonymy with other existing species or reclassified as members of new genera within the families Bionectriaceae and Nectriaceae (e.g., Rossman et al. 1999, Schroers 2001, Chaverri et al. 2011, Lombard et al. 2015) while others were not considered in subsequent morphological revisions. However, none of these specimens has ever been subjected to molecular analysis.

The task of recovering molecular data from old types preserved in the Saccardo collection is highly relevant since many of them represent the only known record for a specific taxon. In addition, molecular information may help to clarify the current systematic status of these species. In the present study we report the molecular results obtained from a selection of specimens (24 Nectria sensu Saccardo specimens, including 20 types, and one nectria-like sensu Saccardo type). Based on these data, the taxonomy of these fungal specimens was re-evaluated by combining the molecular data provided by the internal transcribed spacer region (ITS) analysis with new morphological observations. Furthermore, the ITS sequence data and morphology were also compared to other known species belonging to the Bionectriaceae or Nectriaceae.

**MATERIALS AND METHODS**

**Sampling and morphological observations**

Fungal specimens were collected from the Saccardo fungarium and observed under a dissecting microscope (Leica EZ4W) to identify and sample the fungi on their natural substrates. Considering the inestimable value of the specimens, the sampling was done with the permission of the Botanical Garden of Padova, owner of the Saccardo’s collection, and under the supervision of the PAD herbarium curator. Particular attention was given to preserve the overall integrity of each specimen. The specimens were sampled by removing a small number of dried perithecia from the substrate (plant material or bark), without damaging them, using sterilized tweezers. The material was used both for new morphological observations and for molecular analyses.

The morphological observations were focused on features linked to the visible sexual morphs such as shape, dimension and colour of the perithecia, asci and ascospores. The specimens were placed under a dissecting microscope (Leica EZ4W) to observe the perithecial distribution on the host material, and macroscopic features such as shape and colour. Digital images were captured using the integrated camera system on the dissecting microscope. One or two perithecia were placed on a glass slide, and rehydrated with water. Slides were then flooded with 3 % KOH and subsequently with 100 % LA to observe the colour reactions.

The internal microscopic characters such as asci and ascospores were observed after the colour test with KOH and LA by making squash preparations of perithecia. To observe spore surface ornamentation, 3 % LA solution (plus cotton blue) was used as mounting medium. Microscopic structures were examined using a Leica DM500 light microscope with 400 x and 1000 x magnifications and photographed with a Leica ICC50W camera. After capturing digital images, the diameter of perithecia, and the length and width of asci and ascospores were measured using Fiji software (Schindelin et al. 2012). Measurements of asci and ascospores are indicated as: (minimum–) average minus standard deviation – average – average plus standard deviation (–maximum) of length × (minimum–) average minus standard deviation – average – average plus standard deviation (–maximum) of width. In addition, Q (spore quotient, length/width ratio) = (minimum–) average minus standard deviation – average – average plus standard deviation (–maximum), and Qw (average spore quotient) are indicated.

**DNA extraction, ITS1/ITS2 amplification and sequencing**

DNA was extracted with the CTAB method described in Forin et al. (2018). The success of the DNA extraction was verified by running 3 μL of the extracted DNA stained with Eurosafe DNA loading dye (Euroclone) for each sample in 0.8 % agarose gel in TRIS acetate-EDTA buffer and visualised under UV light. The extracted DNAs were then purified using OneStep™ PCR Inhibitor Removal Kit (Zymo research) in order to remove potential contaminants that might inhibit downstream PCR reactions.

For the preparation of the Illumina sequencing libraries, the nuclear ribosomal ITS1 and ITS2 regions were amplified using a two-step PCR process. The first PCR (PCR1) was carried out using the universal primers ITS1F/ITS2 (White et al. 1990, Gardes & Bruns 1993) for the ITS1 amplification and the universal primers ITS3/ITS4 (White et al. 1990) for the ITS2 amplification. In the second PCR (PCR2) the products of the first amplification of the ITS1 and ITS2 regions were amplified using the same couple of primers tagged with different 5 bp identifier tags to distinguish sequences from each specimen. The second PCR was done in four replicates for each couple of tagged primers.

PCR1 was carried out in a total volume of 25 μL including 5 μL of SX Wonder Taq reaction buffer (5 mM dNTPs, 15 mM MgCl2, EuroClone), 0.5 μL of bovine serum albumin (BSA, 10 mg/mL), 0.5 μL each of two primers (10 μM), 0.5 μL of Wonder Taq (5 U/μL), 2 μL of genomic DNA and water to reach the final volume.

The PCR conditions used for the ITS1 were: 95 °C for 3 min; 35 cycles of 95 °C for 30 s, 53 °C for 40 s and 72 °C for 45 s; 72 °C for 5 min. The PCR conditions used for the ITS2 were: 95 °C for 3 min; 35 cycles of 95 °C for 30 s, 52 °C for 40 s and 72 °C for 45 s; 72 °C for 5 min. PCR2 was performed similarly to the PCR1 except for the absence of the BSA, the use of 2 μL of the first PCR amplicons as template and the use of the tagged primers. The success of the amplifications (PCR1 and PCR2) was checked in 1.2 % agarose gel in TRIS acetate-EDTA buffer using 5 μL of the PCR products stained with Eurosafe DNA loading dye (EuroClone) under UV light.

The four replicates of each sample were pooled and purified using the PureLink PCR Purification Kit (Invitrogen). After the quantification with a Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific), the purified amplicons were mixed in an equimolar amount to prepare different ITS1 and ITS2 libraries, according to the specifications provided by the DNA sequencing services (IGATech, Italy; Fasteris, Switzerland), for a paired-end sequencing using the Illumina MiSeq technology 2 x 300 bp.

**Data analyses**

Forward and reverse fastq files from each library were merged using PEAR v. 0.9.10 (Zhang et al. 2014) with the quality score threshold set at 28, the minimum length of reads after trimming set at 150 bp and the minimum overlap size set at 100 bp. QIIME v. 1.9.1 (Caporaso et al. 2010) was used for the demultiplexing and the quality filtering of the merged reads considering: no errors in the tag sequence, no ambiguous bases in the sequences, a minimum sequence length cut-off of 150 bp, a minimum quality score of 28, a sliding window test of quality score of 50, a maximum length of homopolymers of 13, a maximum number of ambiguous bases of 0 and a maximum number of mismatches in forward and reverse primers of 3.

**R**
| Original identification | Current name | Voucher/Isolate | Origin | Reference(s) | GenBank accession numbers |
|------------------------|--------------|----------------|--------|--------------|--------------------------|
| Nectria apocyni         | Clonostachys apocyni | CBS 130.87 | USA     | Schroers (2001) | – – AF210688 |
| Nectria aureofulvella   | Clonostachys aureofulvella | CBS 195.93 | New Zealand | Schroers (2001) | – – AF358226 |
| Nectria byssicola       | Clonostachys byssicola | CBS 914.97 | Uganda | Schroers (2001) | – – AF358202 |
| Nectria canadensis      | Clonostachys canadensis | CBS 218.93, isotype | Japan | Schroers (2001) | AF358246 |
| Nectria compactiiculata | Clonostachys compactiiculata | CBS 692.93 | France | Schroers (2001) | – – AF358245 |
| Nectria capitis          | Clonostachys capitis | CBS 913.97, holotype | USA | Schroers (2001) | – – AF358245 |
| Nectria corinna         | Clonostachys corinna | CBS 696.93 | France | Schroers (2001) | – – AF210667 |
| Nectria epichloe        | Clonostachys epichloe | CBS 101037, isotype | Japan | Schroers (2001) | – – AF210675 |
| Nectria grammicospora   | Clonostachys grammicospora | CBS 209.93, holotype | French Guiana | Schroers (2001) | – – AF210678 |
| Nectria grammicosporopsis | Clonostachys grammicosporopsis | CBS 102834 | Australia | Schroers (2001) | AF210656 |
| Nectria levisi           | Clonostachys levisi | CBS 948.97 | France | Schroers (2001) | – – AF210680 |
| Nectria lucifer          | Clonostachys lucifer | CBS 100008, isolate | USA | Schroers (2001) | – – AF210683 |
| Nectria oblongispore     | Clonostachys oblongispore | CBS 100285, isolate | Japan | Schroers (2001) | AF358248 |
| Nectria ochroleuca       | Clonostachys ochroleuca | CBS 193.94 | Venezuela | Schroers (2001) | – – AF210686 |
| Nectria ophiopsora       | Clonostachys ophiopsora | CBS 122171 | Spain | Romón et al. (2008) | – – DQ674381 |
| Nectria pityrodes        | Clonostachys pityrodes | CBS 102033, isotype | Mauritius | Schroers (2001) | – – AF358238 |
| Nectria pseudochroleuca  | Clonostachys pseudochroleuca | CBS 192.94 | France | Schroers (2001) | – – AF210666 |
| Nectria pseudodisistria  | Clonostachys pseudodisistria | CBS 119.87 | Indonesia | Schroers (2001) | – – AF358251 |
| Nectria ralfsi           | Clonostachys ralfsi | CBS 129.87 | New Zealand | Schroers (2001) | – – AF210676 |
| Nectria rosmaniae        | Clonostachys rosmaniae | CBS 210.93 | France | Schroers (2001) | – – AF358227 |
| Nectria samuelisi        | Clonostachys samuelisi | CBS 699.97, isotype | Venezuela | Schroers (2001) | – – AF358236 |
| Nectria sequoiae         | Clonostachys sequoiae | CBS 180.88, isolate | Guyana | Schroers (2001) | – – AF210666 |
| Nectria setosa           | Clonostachys setosa | CBS 834.91 | Cuba | Schroers (2001) | – – AF210670 |
| Nectria solani           | Clonostachys solani | CBS 101924 | Jamaica | Schroers (2001) | – – AF210672 |
| Nectria sporodochialensis | Clonostachys sporodochialensis | CBS 101921, isolate | USA | Schroers (2001) | – – AF358232 |
| Nectria vesiculosa       | Clonostachys vesiculosa | HMAS 183151, holotype | China | Luo & Zhuang (2010) | – – NR_119828 |
| Nectria zelandiaevariae  | Clonostachys zelandiaevariae | CBS 100979, isolate | New Zealand | Schroers (2001) | – – AF358229 |
| Chaetopsis fulva         | Chaetopsis fulva | CBS 142.56, type | Italy | Lombard et al. (2015) | – – NR_145061 |
| Chaetopsis penicillata   | Chaetopsis penicillata | CBS 608.92, type | New Zealand | Gräfenhan et al. (2011) | – – NR_154780 |
| Chaetopsis pini          | Chaetopsis pini | CBS 136443, holotype | Thailand | Crous et al. (2013) | – – NR_137822 |
| Chaetopsis pinicola      | Chaetopsis pinicola | CBS 136444, holotype | Thailand | Crous et al. (2013) | – – NR_137823 |
| Clonostachys agerwallii  | Clonostachys agerwallii | CBS 533.81, neotype of Gliocladium agerwallii | India | Schroers (2001) | – – AF358241 |
| Clonostachys buxi        | Clonostachys buxi | CBS 696.93 | France | Lombard et al. (2015) | – – KF218440 |
| Clonostachys byssicola   | Clonostachys byssicola | CBS 364.78, isolate | Venezuela | Vu et al. (2019) | – – MH661151 |
| Clonostachys farinosa    | Clonostachys farinosa | CML 2404 | Brazil | Aube et al. (2014) | – – KC806271 |
| Clonostachys farinosa    | Clonostachys farinosa | CML 2510 | Venezuela | Aube et al. (2014) | – – KJ499907 |
| Clonostachys candidubrum | Clonostachys candidubrum | CBS 504.67 | Netherlands | Schroers (2001) | – – AF210668 |
| Clonostachys chlorina    | Clonostachys chlorina | CBS 287.90, holotype | Brazil | Schroers (2001) | – – NR_137651 |
| Clonostachys compactiiculata | Clonostachys compactiiculata | CBS 729.87 | Germany | Schroers (2001) | – – AF360423 |
| Clonostachys divergens   | Clonostachys divergens | CBS 967.73, holotype | Germany | Schroers (2001) | – – NR_137532 |
| Clonostachys ericamoresiana | Clonostachys ericamoresiana | MFLUCC 17-2620, holotype | Thailand | Hyde et al. (2020) | – – MN991132 |
| Clonostachys ericamoresii | Clonostachys ericamoresii | MFLUCC 19-0486, holotype | Thailand | Hyde et al. (2020) | – – MN991133 |
| Clonostachys intermedia  | Clonostachys intermedia | CBS 508.82, holotype | Netherlands | Schroers (2001) | – – NR_137652 |
| Clonostachys kowhai      | Clonostachys kowhai | CBS 461.95, holotype | New Zealand | Schroers (2001) | – – NR_134748 |
| Clonostachys miodochialis | Clonostachys miodochialis | CBS 997.99, holotype | Netherlands | Schroers (2001) | – – NR_137649 |
| Clonostachys phyllophila | Clonostachys phyllophila | CBS 921.97, holotype | France | Schroers (2001) | – – NR_137531 |
| Clonostachys pityrodes   | Clonostachys pityrodes | CBS 126394 | Sri Lanka | Vu et al. (2019) | – – MH864280 |
| Original identification | Current name | Herbarium/GenBank accession numbers | Origin | Reference(s) | GenBank accession numbers |
|-------------------------|--------------|------------------------------------|--------|--------------|--------------------------|
| Clonostachys rhizophaga | Clonostachys rhizophaga | CBS 202.37, holotype | USA | Schroers (2001) | AF358225 |
| Clonostachys rosea | Clonostachys rosea | CBS 127642, USA | Vu et al. (2019) | -- | -- |
| Clonostachys setosa | Clonostachys setosa | CBS 917.97, neotype Sesquicillium setosum | USA | Schroers (2001) | NR_154746 |
| Clonostachys subquaterna | Clonostachys subquaterna | CBS 100003, isotype | Puerto Rico | This study | -- |
| Clonostachys wenpingii | Clonostachys wenpingii | HMAS 172156, holotype | China | Luo & Zhuang (2007) | -- |
| Cosmospora coccinea | Cosmospora coccinea | CBS 341.70, type of Verticillium olivaceum | Germany | Gräfenhan et al. (2011) | HQ97927 |
| Cosmospora cymosa | Cosmospora cymosa | CBS 762.69, isotype | Germany | Gräfenhan et al. (2011) | NR_11605 |
| Cyanonectria buxi | Cyanonectria buxi | CBS 125581, epitype | Slovenia | Schroers et al. (2011) | -- |
| Dialonectria episphaeria | Dialonectria episphaeria | CBS 125494, Canada | Vu et al. (2019) | -- | MH855409 |
| Dialonectria ullevolea sp | Dialonectria ullevolea | CBS 125493, USA | Gräfenhan et al. (2011) | KM231821 |
| Fusarium illudens | Neocosmospora illudens | CBS 119605, New Zealand | Lombard et al. (2015) | -- | KM231806 |
| Fusarium metsmoids | Fusicola metsmoids | CBS 186.34, Germany | Vu et al. (2019) | -- | MH855482 |
| Fusicola acsseria | Fusicola acsseria | CBS 184148, ex-type of Fusarium metsmoids var. acsseria | Japan | Gräfenhan et al. (2011) | NR_11603 |
| Hydropisphaera bimucilata | Hydropisphaera fungicola | CBS 124147, holotype | Martinique | Schoch et al. (2014) | NR_11976 |
| Hydropisphaera buxica | Hydropisphaera erubescens | CBS 120801, Germany | Lombard et al. (2015) | -- | -- |
| Ijuhya chilensis | Ijuhya faveliana | CBS 136677, Spain | Ashrafi et al. (2017) | -- | -- |
| Ijuhya faveliana | Ijuhya faveliana | CBS 133850, France | Ashley et al. (2017) | -- | -- |
| Ijuhya parilis | Ijuhya parilis | CBS 136677, Spain | Ashrafi et al. (2017) | -- | -- |
| Lasionectria antillana | Lasionectria multiloculata | CBS 129746, holotype | Netherlands | Schroes et al. (2011) | -- |
| Lasionectria hiliortii | Lasionectria hiliortii | CBS 144627, ex-holotype | Netherlands | Schroes et al. (2011) | -- |
| Lasionectria lecanodes | Lasionectria lecanodes | CBS 127797, holotype | Martinique | Schroes et al. (2011) | -- |
| Lasionectria maritizensis | Lasionectria maritizensis | CBS 131606, ex-holotype | Guadeloupe | Schroes & Stork (2011) | -- |
| Lasionectria maritimensis | Lasionectria maritimensis | CBS 129746, holotype | Martinique | Schroes et al. (2011) | -- |
| Lasionectria oenanthica | Lasionectria oenanthica | CBS 129747, holotype | France | Ashrafi et al. (2017) | -- |
| Macroconia leptosphaeria | Macroconia leptosphaeria | CBS 100001, Netherlands | Schroes et al. (2011) | -- | -- |
| Macroconia papilloneura | Macroconia papilloneura | CBS 125495, USA | Schroes et al. (2011) | -- | HQ897826 |
| Marraniacea cattaneae | Marraniacea cattaneae | CBS 120801, Germany | Lombard et al. (2015) | -- | KM231753 |
| Marraniacea cattaneae | Marraniacea cattaneae | CBS 491.62, type of Nectria chaetopsinae-catenatae | Venezuela | Lombard et al. (2015) | KM231753 |
| Marraniacea humicola | Marraniacea humicola | CBS 102601, Spain | Lombard et al. (2015) | -- | KM231756 |
| Marraniacea pinicola | Marraniacea pinicola | CBS 745.88, holotype of Nectria marianae | Venezuela | Lombard et al. (2015) | KM231764 |
| Original identification | Current name | Herbarium/ Voucher/Isolate | Origin | Reference(s) | GenBank accession numbers |
|-------------------------|--------------|---------------------------|--------|--------------|--------------------------|
| Marsarnaeae samuelisi   | Marsarnaeae samuelisi | CBS 746.88 | Venezuela | Lombard et al. (2015) | – | – | KM231757 |
| Microcera coccophila    | Microcera coccophila | CBS 310.34 | Italy | Gallenhan et al. (2011) | – | – | HQ87794 |
| Microcera larvarum      | Microcera larvarum | CBS 738.79 | Iran | Lombard et al. (2015) | – | – | KMS1825 |
| Nectria albofimbriata   | Lasconectria albofimbriata (Penz. & Sacc.) Forin & Vizzini n. 436a | Pad S00001: herbarium Saacoardo, lectotype | Indonesia, Java | This study | MT554896 | MT554874 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 452 ex p. | Pad S00006: herbarium Saacoardo, lectotype | Indonesia, Java | This study | MT554901 | MT554878 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 119, holotype | Pad S00003: herbarium Saacoardo, holotype | Indonesia, Java | This study | MT554902 | MT554879 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 172, syntype | Pad S00004: herbarium Saacoardo, holotype | Indonesia, Java | This study | MT554903 | MT554880 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 452 ex p., holotype | Pad S00005: herbarium Saacoardo, holotype | Indonesia, Java | This study | MT554904 | MT554881 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 32, syntype | Pad S00007: herbarium Saacoardo, holotype | Indonesia, Java | This study | MT554905 | MT554882 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 150, lectotype | Pad S00008: herbarium Saacoardo, lectotype | Indonesia, Java | This study | MT554906 | MT554883 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 86, holotype | Pad S00009: herbarium Saacoardo, holotype | Indonesia, Java | This study | MT554907 | MT554884 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 923, holotype | Pad S00010: herbarium Saacoardo, holotype | Indonesia, Java | This study | MT554908 | MT554885 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 166, lectotype | Pad S00011: herbarium Saacoardo, lectotype | Indonesia, Java | This study | MT554909 | MT554886 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 168, lectotype | Pad S00012: herbarium Saacoardo, lectotype | Indonesia, Java | This study | MT554910 | MT554887 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 225 | Pad S00013: herbarium Saacoardo, holotype | Indonesia, Java | This study | MT554911 | MT554888 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 318 | Pad S00014: herbarium Saacoardo, holotype | Indonesia, Java | This study | MT554912 | MT554889 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 452 ex p., holotype | Pad S00015: herbarium Saacoardo, holotype | Indonesia, Java | This study | MT554913 | MT554890 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 452 ex p., holotype | Pad S00016: herbarium Saacoardo, holotype | Indonesia, Java | This study | MT554914 | MT554891 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 119, holotype | Pad S00017: herbarium Saacoardo, holotype | Indonesia, Java | This study | MT554915 | MT554892 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 172, syntype | Pad S00018: herbarium Saacoardo, holotype | Indonesia, Java | This study | MT554916 | MT554893 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 452 ex p., holotype | Pad S00019: herbarium Saacoardo, holotype | Indonesia, Java | This study | MT554920 | MT554895 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 32, syntype | Pad S00020: herbarium Saacoardo, holotype | Indonesia, Java | This study | MT554921 | MT554896 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 150, lectotype | Pad S00021: herbarium Saacoardo, holotype | Indonesia, Java | This study | MT554922 | MT554897 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 86, holotype | Pad S00022: herbarium Saacoardo, holotype | Indonesia, Java | This study | MT554923 | MT554898 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 923, holotype | Pad S00023: herbarium Saacoardo, holotype | Indonesia, Java | This study | MT554924 | MT554899 |
| Nectria ambigua var. pallens | Clonostachys ambigua (Penz. & Sacc.) Forin & Vizzini n. 166, lectotype | Pad S00024: herbarium Saacoardo, holotype | Indonesia, Java | This study | MT554925 | MT554900 |
| Original identification¹ | Current name | Herbarium/Voucher/Isolate | Origin | Reference(s) | ITS1 | ITS2 | ITS3 |
|--------------------------|-------------|--------------------------|--------|--------------|------|------|------|
| *Nectriella nolinae*     | *Nectriella nolinae* | CBS 110134 | USA  | Vu et al. (2019) | – | – | MH862853 |
| *Nectriella pirionii*    | *Nectriella pirionii* | CBS 171.75  | USA  | Vu et al. (2019) | – | – | MH860907 |
| *Hydropisphaera rufofusca* | *Nectriella nolinae* | CBS 305.70  | Indonesia | This study | MT554911 | MT554888 |
| *Nectriella rufofusca*   | *Hydropisphaera rufofusca* | CBS 987.70  | ex-nedotype | Germany | Zare & Gams (2016) | – | – | NR_154234 |
| *Nectriella rexiana*     | *Nectriella rexiana* | CBS 248.70  | Germany | Zare & Gams (2016) | – | – | NR_154235 |
| *Nectriopsis exigua*     | *Nectriopsis exigua* | CBS 248.70  | Germany | Zare & Gams (2016) | – | – | KU382177 |
| *Nectriopsis pironii*    | *Nectriopsis pironii* | CBS 400.82  | ex-holotype | Russia | Zare & Gams (2016) | – | – | NR_154234 |
| *Nectriopsis lindauiana* | *Nectriopsis lindauiana* | CBS 509.63  | ex-neotype | Germany | Zare & Gams (2016) | – | – | NR_154235 |
| *Nectriopsis rexiana*    | *Nectriopsis rexiana* | CBS 320.73  | ex-holotype | England | Vu et al. (2019) | – | – | NR_154297 |
| *Nectriopsis violacea*   | *Nectriopsis violacea* | CBS 849.70  | – | Germany | Vu et al. (2019) | – | – | MH869978 |
| *Neocosmospora croci*    | *Neocosmospora croci* | CPC 27186   | ex-holotype | Italy | Sandoval-Denis et al. (2017) | – | – | NR_163290 |
| *Neocosmospora macrospora* | *Neocosmospora macrospora* | CPC 28191   | ex-holotype | Italy | Sandoval-Denis et al. (2017) | – | – | NR_163291 |
| *Neocosmospora ramosa*   | *Neocosmospora ramosa* | CBS 599.63  | ex-neotype | Brazil | Lombard et al. (2015) | – | – | KM231802 |
| *Neocosmospora rubicola* | *Neocosmospora rubicola* | CBS 305.70  | ex-neotype | Sudan | Lombard et al. (2015) | – | – | KM231798 |
| *Protocreopsis freycinetiae* | *Protocreopsis freycinetiae* | CBS 573.76  | ex-isotype | New Zealand | Vu et al. (2019) | – | – | MH861003 |
| *Protocreopsis phormiicola* | *Protocreopsis phormiicola* | CBS 567.76  | type | New Zealand | Vu et al. (2019) | – | – | MH861001 |
| *Pseudocosmospora eutypae* | *Pseudocosmospora eutypae* | BPI 884164  | holotype | France | Herrera et al. (2013) | – | – | NR_158889 |
| *Pseudocosmospora eutypellae* | *Pseudocosmospora eutypellae* | BPI 1107121 | ex-holotype | USA | Herrera et al. (2013) | – | – | NR_158888 |
| *Pseudocosmospora rugerisonii* | *Pseudocosmospora rugerisonii* | CBS 133967  | ex-epitype | Argentina | Herrera et al. (2013) | – | – | NR_158889 |
| *Pseudocosmospora vilior* | *Pseudocosmospora vilior* | CBS 125493  | ex-neotype | Spain | Gürkenhan et al. (2011) | – | – | KU897100 |
| *Sarcopodium circinatum* | *Sarcopodium circinatum* | CBS 100998  | ex-holotype | Brazil | Lombard et al. (2015) | – | – | KM231776 |
| *Sarcopodium cirratus*   | *Sarcopodium cirratus* | CBS 857.92  | ex-holotype | Costa Rica | Lombard et al. (2015) | – | – | KM231786 |
| *Sarcopodium cirrosetiferum* | *Sarcopodium cirrosetiferum* | CBS 112283  | ex-holotype | Ecuador | Lombard et al. (2015) | – | – | KM231785 |
| *Sarcopodium flavolanatum* | *Sarcopodium flavolanatum* | CBS 128370  | ex-neotype | China | Lombard et al. (2015) | – | – | KM231784 |
| *Sarcopodium flavolanatum* | *Sarcopodium flavolanatum* | CBS 112283  | ex-neotype | Ecuador | Lombard et al. (2015) | – | – | KM231785 |
| *Sarcopodium macalpinei* | *Sarcopodium macalpinei* | CBS 115296  | ex-epitype | Hong Kong | Lombard et al. (2015) | – | – | KM231783 |
| *Sarcopodium vanillae*   | *Sarcopodium vanillae* | CBS 100582  | ex-epitype | Ecuador | Lombard et al. (2015) | – | – | KM231780 |
| *Selinia pulchra*        | *Selinia pulchra* | CBS 126654  | holotype | Argentina | Vu et al. (2019) | – | – | MH864186 |
| *Stilbocrea walteri*     | *Stilbocrea walteri* | CBS 144938  | holotype | Portugal | Voglmayr & Jaklitsch (2018) | – | – | NR_160063 |
| *Stylonectria norvegica* | *Stylonectria norvegica* | CBS 139239  | type | Puerto Rico | Schroes (2001) | – | – | NR_154415 |
| *Thelonectria cidaria*   | *Thelonectria cidaria* | CBS 132323  | holotype | Costa Rica | Vu et al. (2019) | – | – | NR_164437 |
| *Thelonectria coronalis* | *Thelonectria coronalis* | CBS 132337  | ex-holotype | Taiwan | Vu et al. (2019) | – | – | NR_160299 |
| *Thelonectria coronata*  | *Thelonectria coronata* | CBS 132335  | ex-holotype | Venezuela | Salgado-Salazar et al. (2012) | – | – | QJ033316 |
| *Thelonectria coronata*  | *Thelonectria coronata* | CBS 132334  | ex-holotype | Taiwan | Salgado-Salazar et al. (2012) | – | – | QJ033342 |
| *Thelonectria nodosa*    | *Thelonectria nodosa* | CBS 132327  | ex-holotype | USA | Vu et al. (2019) | – | – | NR_160290 |
| *Thelonectria stemmata*  | *Thelonectria stemmata* | CBS 112468  | ex-holotype | Jamaica | Salgado-Salazar et al. (2012) | – | – | QJ033312 |
| *Varicospora aquatica*   | *Varicospora aquatica* | CBS 126103  | ex-holotype | France | Lechat & Fournier (2015) | – | – | KP126969 |
| *Varicosporopsis aquatilis* | *Varicosporopsis aquatilis* | CBS 140158  | ex-holotype | France | Lechat & Fournier (2016) | – | – | KU233187 |
| *Volutella ciliata*      | *Volutella ciliata* | CBS 483.61  | – | Canada | Lombard et al. (2015) | – | – | KM231770 |
| *Volutella consors*      | *Volutella consors* | CBS 139.79  | – | Netherlands | Lombard et al. (2015) | – | – | KM231768 |
| *Volutella rosea*        | *Volutella rosea* | CBS 128258  | – | USA | Lombard et al. (2015) | – | – | KM231769 |

1 Newly obtained sequences are reported in **bold**.
Table 2  List and details of specimens used in the combined TUB2 and ITS phylogenetic analysis.

| Original identification | Current name | Herbarium/ Voucher/Isolate | Origin | Reference(s) | GenBank accession numbers |
|-------------------------|--------------|-----------------------------|--------|--------------|--------------------------|
| **TUB2**                | **ITS**      | **ITS1**                    | **ITS2** |              |                           |
| Bionectria apocyni      | Clonostachys apocyni | CBS 130.87               | New York | Schroers (2001) | AF358186 AF210688 – – |
| Bionectria aureofulvella | Clonostachys aureofulvella | CBS 200.93               | Uganda | Schroers (2001) | AF358181 AF358226 – – |
| Bionectria capitata     | Clonostachys capitata | CBS 218.93, isotype     | Japan | Schroers (2001) | AF358188 AF358240 – – |
| Bionectria ochroleuca   | Clonostachys ochroleuca | CBS 406.95               | USA    | Schroers (2001) | AF358167 AF358249 – – |
| Bionectria pseudochroleuca | Clonostachys pseudochroleuca | CBS 192.94            | Venezuela | Schroers (2001) | AF358185 AF358237 – – |
| Bionectria rosea        | Clonostachys rosea | CBS 193.94, isotype    | Massachusetts | Schroers (2001) | AF358182 AF358239 – – |
| Bionectria solani       | Clonostachys solani | CBS 702.97               | France  | Schroers (2001) | AF358177 AF210687 – – |
| Bionectria sporadichialis | Clonostachys sporadichialis | CBS 101921, isotype | Puerto Rico | Schroers (2001) | AF358149 AF210685 – – |
| Bionectria zelandiaeoviae | Clonostachys zelandiaeoviae | CBS 232.80, isotype | New Zealand | Schroers (2001) | AF358185 AF210684 – – |

1 Newly obtained sequences are reported in **bold**.
List and details of specimens used in the combined ITS and LSU phylogenetic analysis.

GenBank accession numbers

| GenBank accession numbers | ITS | ITS1 | ITS2 | LSU |
|---------------------------|-----|------|------|-----|
| GenBank/Herbarium         |     |      |      |     |
| Current name              |     |      |      |     |
| Origin                    |     |      |      |     |
| Reference(s)              |     |      |      |     |

| Specimen                    | Current name | GenBank accession numbers | ITStr | ITStr1 | ITStr2 | LSUstr |
|-----------------------------|--------------|----------------------------|-------|--------|--------|--------|
| Clonostachys buxi            | Clonostachys buxi | HJ921597                   | –     | –      | –      | –      |
| Cosmospora coccinea          | Cosmospora coccinea | HJ921601                   | –     | –      | –      | –      |
| Fusicolla acetileerea        | Fusicolla acetileerea | HJ921599                   | –     | –      | –      | –      |
| Fusicolla aquaeductuum        | Fusicolla aquaeductuum | HJ921602                   | –     | –      | –      | –      |
| Fusicolla bharatavarshae     | Fusicolla bharatavarshae | HJ921600                   | –     | –      | –      | –      |
| Fusicolla gigantispora       | Fusicolla gigantispora | HJ921603                   | –     | –      | –      | –      |
| Fusicolla matuoi             | Fusicolla matuoi | HJ921604                   | –     | –      | –      | –      |
| Fusicolla melogrammae        | Fusicolla melogrammae | HJ921660                   | –     | –      | –      | –      |
| Macroconia leptosphaeriae    | Macroconia leptosphaeriae | HJ921661                   | –     | –      | –      | –      |
| Microcera rubra              | Microcera rubra | HJ921662                   | –     | –      | –      | –      |
| Nectria peziza               | Nectria peziza | HJ921663                   | –     | –      | –      | –      |
| Fusicolla reyesiana          | Fusicolla reyesiana | HJ921664                   | –     | –      | –      | –      |

Phylogenetic analyses

The sequences used for the phylogenetic analyses were chosen on the basis of BLAST results, selecting taxonomically close, well-annotated and published sequences in accordance with recent phylogenetic studies regarding the families Biocorticaceae and Nectriaceae (Schroers 2001, Chaverri et al. 2011, Gräfenhan et al. 2011, Hirooka et al. 2012, Lombard et al. 2015, Salgado-Sahazar et al. 2017; Table 1). Two different ITS datasets were generated and analysed separately: one for taxa belonging to the family Bionectriaceae and the other for those of the family Nectriaceae. ITS1 and ITS2 sequences, when both identified, of the Saccardo type specimens were combined and used in the phylogenetic analyses. Two additional analyses were done to better elucidate the systematic position of several types: one of a Clonostachys subgroup encompassing our specimens combining partial beta-tubulin (TUB2) gene and ITS sequences; the other of one of the genera Fusicolla combining ITS sequences and 28S rDNA gene partial sequences (Table 2, 3). TUB2 and 28S rDNA gene sequences are not available for the Saccardo specimens.

The sequences were aligned using the online version of MAFFT v. 7 (Katoh et al. 2019) using the algorithm L-INS-I. The alignments were manually refined with Geneious R11 (https://www.geneious.com) where necessary. ITS alignments were partitioned into ITS1, 5.8S and ITS2 regions.

Phylogenetic analyses were performed using the Bayesian Inference (BI) and Maximum likelihood (ML) approaches. BI analyses were performed using MrBayes v. 3.2.6 (Ronquist et al. 2012). Two independent Monte Carlo Markov Chains (MCMC) runs were performed, each with four chains of 10 M generations, under GTR+G evolutionary model. Trees were sampled every 1000 generations and the first 25 % of the trees were discarded as burn-in. A majority rule consensus tree of the remaining 10001 trees was calculated to obtain estimates for Bayesian posterior probabilities (BPP). ML analyses were performed using RAxML v. 8 (Stamatakis 2014) with 1000 replicates and a general time reversible (GTR) model of nucleotide substitution with a GAMMA distribution rate variation across sites. Maximum likelihood trees were generated using the ‘-f a’ option and ‘-x 12345’ as a random seed to invoke the
novel rapid bootstrapping algorithm. Significance threshold was set ≥ 0.95 for posterior probability (BPP) and ≥ 70 % for ML bootstrap (MLB) values. Non-significant support values are presented inside parentheses.

Pairwise % identity values of ITS sequences (P%iv) were calculated using Geneious R11 (https://www.geneious.com). Alignments were submitted to TreeBASE (https://www.treebase.org, submission number 26427).

Additional specimens involved in the study

The type specimen of Nectriella rufofusca, stored in the Saccardo collection, was also sampled. This species was transferred to Hydropisphaera (Bionectriaceae), as H. rufofusca (Rossman et al. 1999), where other Nectria sensu Saccardo types were accommodated (e.g., Nectria dolichospora and N. leucotricha). ITS1 and ITS2 regions were amplified with the two-step PCR process previously described, and then included in the Illumina sequencing libraries.
The ITS sequence of the Clonostachys subquaternata isotype collection CBS 100003 was amplified using the universal primers ITS1/ITS4 (White et al. 1990) and used in the phylogenetic analyses to obtain a better taxonomic identification of some of Saccardo’s type specimens. The PCR reaction was carried out in a total volume of 25 μL including 5 μL of 5X Wonder Taq reaction buffer (5 mM dNTPs, 15 mM MgCl₂; Euroclone), 0.5 μL of bovine serum albumin (BSA, 10 mg/mL), 0.5 μL each of two primers (10 μM), 0.5 μL of ddH₂O to reach the final volume. The PCR conditions used were: 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 55 °C for 45 s and 72 °C for 70 s; 72 °C for 7 min. The success of the amplifications was evaluated in 1.2 % agarose gel in TRIS acetate-EDTA buffer using 5 μL of the PCR products stained with Eurosafe DNA dying (Euroclone). The PCR products were quantified with Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific) and sent to Eurofins Genomics (Germany) service for the sequencing.

RESULTS

Phylogenetic analyses

Bayesian Inference and ML analyses produced trees with congruent topologies. Consequently, only Bayesian consensus trees with BPP and MLB values are reported (Fig. 1–4).

Bionectriaceae

The Bionectriaceae dataset comprises 93 ITS sequences (17 newly generated and 76 obtained from GenBank) and 639 characters including indels and missing data. The combined dataset comprises 50 ITS sequences (seven newly generated from the Bionectriaceae dataset and 43 from GenBank) and 639 characters, respectively, including indels and missing data.
Most of the Saccardo specimens are distributed among three different genera of the Nectriaceae (Fig. 1). *Nectria ambiguа*, *N. ambigua var. pallens*, *N. congestа*, *N. flagoletiana*, *N. granuligera*, *N. phyllostachydis* and *N. squamuligera* cluster in a main clade that includes representative species of the genus *Clonostachys*. This clade is highly supported by BI analysis with a 1.0 BPP and by ML analysis with a 94 % MLB. *Nectria dolichospora*, *N. leucotricha* and *Nectriella rufofusca* belong to the genus *Hydropisphaera* (BPP 1.0, MLB 81 %), while *N. albofimbriata* and *N. mantuana* cluster in the Lasionectria clade (BPP 0.95, MLB 73 %). A deeper placement of several newly collected sequences, affiliated to the genus *Clonostachys* based on ITS sequences, is further analysed in the combined TUB2/ITS tree focused on a subset of *Clonostachys* species (Fig. 2) comprising *Clonostachys*, *Neocosmospora*, *Cosmospora*, *Nectria*, *Cyanonectria*, *Thelonectria*, *Marianneae*, *Chaetopsina*, *Sarcopodium* and *Volutella*. Newly obtained sequences are reported in bold.

**Fig. 3** Phylogeny generated from Bayesian inference analysis based on ITS sequence data of species belonging to different genera of the *Bionectriaceae* (Fig. 1). *Nectria ambiguа*, *N. ambigua var. pallens*, *N. congestа*, *N. flagoletiana*, *N. granuligera*, *N. phyllostachydis* and *N. squamuligera* cluster in a main clade that includes representative species of the genus *Clonostachys*. This clade is highly supported by BI analysis with a 1.0 BPP and by ML analysis with a 94 % MLB. *Nectria dolichospora*, *N. leucotricha* and *Nectriella rufofusca* belong to the genus *Hydropisphaera* (BPP 1.0, MLB 81 %), while *N. albofimbriata* and *N. mantuana* cluster in the Lasionectria clade (BPP 0.95, MLB 73 %). A deeper placement of several newly collected sequences, affiliated to the genus *Clonostachys* based on ITS sequences, is further analysed in the combined TUB2/ITS tree focused on a subset of *Clonostachys* species (Fig. 2) comprising *Clonostachys*, *Neocosmospora*, *Cosmospora*, *Nectria*, *Cyanonectria*, *Thelonectria*, *Marianneae*, *Chaetopsina*, *Sarcopodium* and *Volutella*. Newly obtained sequences are reported in bold.

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Nectriaceae

The Nectriaceae dataset comprises 82 ITS sequences (nine newly generated and 73 from GenBank) and 630 characters including indels and missing data. The combined dataset for the genus Fusicolla comprises 20 ITS sequences (one newly generated from the Nectriaceae dataset and 19 from GenBank) and 18 28S rDNA sequences (18 from GenBank and corresponding to the same voucher of the ITS sequences) and 566 + 805 characters, respectively, including indels and missing data. Within the phylogram comprising different representative Nectriaceae taxa, Saccardo’s specimens were included in five different genera (Fig. 3). Nectria radians, N. rarepilis, N. sordescens, N. tibodensis and N. tibodensis var. crebrilobus cluster with Sarcosporidium species (BPP 1.0, MLB 87 %), N. cyanostoma in the Cylaneckoria clade (BPP 1.0, MLB 84 %), N. corallina in the Thelenectria clade (BPP 1.0, MLB 100 %) and N. illudens in the Neocosmospora clade (BPP 1.0, MLB 100 %) (Fig. 3). Nectria peziza subsp. reyesiana is included in the Fusicolla clade supported by BI analysis with a 1.0 BPP (Fig. 3, 4).

TAXONOMY

(taxa presented in alphabetical order based on species epithets; current names are in bold)

Nectria albofimbriata

Lasionectria albofimbriata (Penz. & Sacc.) Forin & Vizzini, comb. nov. — MycoBank MB835768; Fig. 5

Basionym. Nectria albofimbriata Penz. & Sacc. (as 'albo-fimbriata'), Malpighia 11: 513. 1897.
Synonym. Proctocreopsis albofimbriata (Penz. & Sacc.) Yoshim. Doi (as 'albo-fimbriata'), Bull. Natl. Sci. Mus., Tokyo, B 4: 117. 1978.

Sexual morph. Perithecia gregarious, surrounded by white-yellow, 3–4 µm wide, usually fasciculate, smooth-walled hyphae, globose, non-papillate, yellow-orange, 200–280 µm diam (n = 5); not changing colour in 3 % KOH and 100 % LA. Asci clavate to fusiform, (46.6–)47.9–52.2–56.5 (–58) × (7–)8–9–10–10.3 µm (n = 10), 8-spored, ascospores biseriate. Ascospores fusoid, (15.1–)17–18.4–19.9–(22.6) × (3.2–)3.6–4.1–4.6–5.3 µm, Q = (3.7–)4–4.5–5.1 (–6.5), Qav = 4.5 (n = 35), 1-septate, equally subdivided in two cells, not constricted or slightly constricted at the septum, hyaline, with many striations.

Specimens examined. Indonesia, Java, Tijobas, on dead stems of Elettaria sp., 6 Feb. 1897; ? Penzig, n. 436a, PAD S00001, lectotype designated by Samuels (1976); ? Penzig, n. 172, PAD S00002, syntype.

Notes — The morphological observation of Nectria albofimbriata n. 436a (PAD S00001, Fig. 5a–e) agrees with the description reported by Doi (1978), Samuels et al. (1990) and Rossman et al. (1999). Specimen number 172 (PAD S00002, Fig. 5f–j) has slightly larger asci, (16.6–)17–18.4–18.8) × (4.4–4.9–5.4–(5.7) µm (n = 5). However, despite these morphological differences, the phylogeny in Fig. 1 confirms that these two specimens belong to the same species. This species was considered a member of the Bieneckriaceae genus Proctocreopsis as P. albofimbriata (Doi 1978, Rossman et al. 1999). Species of this genus generally grow on decaying monocotyledonous leaves (Arecaceae or Musaceae) in tropical regions and are characterised by pale perithecia surrounded by a hyphal stroma, striate ascospores and acerominium-like asexual morphs (Doi 1977, 1978, Rossman et al. 1999). Nectria albofimbriata and other morphologically similar species were placed in the Nectria subfalcata group by Samuels (1976), and then moved to the genus Proctacreopsis (Doi 1977, 1978). As
Fig. 5  a–e. *Nectria albofimbriata* (PAD S00001: herbarium Saccardo, n. 436a, lectotype). a. Original herbarium specimen; b. perithecia on natural substrate; c–e. asci and ascospores in cotton blue. — f–j. *Nectria albofimbriata* (PAD S00002: herbarium Saccardo, n. 172, syntype). f. Original herbarium specimen; g. perithecia on natural substrate; h–j. asci and ascospores in cotton blue. — k–o. *Nectria albofimbriata* (PAD S00028: herbarium Saccardo, n. 430). k. Original herbarium specimen; l. perithecia on natural substrate; m–o. asci and ascospores in cotton blue. — Scale bars: b, g, l = 500 μm; c–d, m–o = 5 μm; e, h–j = 10 μm. — Photos: a–e, k–o by N. Forin; f–j by S. Nigris.
reported in Fig. 1, the ITS sequences of the two specimens cluster with sequences of species belonging to the Bionectriaceae genus Lasionectria (typified by L. mantuana, see below). From a morphological point of view, the species of Lasionectria and those of Protocereopsis are similar. Lasionectria species are characterised by yellow to dark brown perithecia surrounded by solitary hairs or often triangular fascicles of densely packed hyphae; 1-septate ascospores that can be striate and acremonium-like asexual morphs (Rossman et al. 1999, Lechat & Fournier 2012, Tibpromma et al. 2018). Therefore, key morphological differences are difficult to distinguish between the two genera. In addition, it is important to take into account that two former Nectria species previously included in the Nectria subfalcata group (N. sylvana and N. vulpina) are now considered members of the genus Lasionectria (Rossman et al. 1999).

In our ITS phylogenetic analysis (Fig. 1), Protocereopsis freycinetiae and P. phormicola form an unsupported clade, and in Lechat et al. (2016), based on LSU sequences, P. caricola, P. korfii and P. pertusa cluster in a monophyletic group, but the latter study suffers from a very poor taxon sampling of Bionectriaceae. These considerations and our results show that N. albofimbriata should be considered a member of the genus Lasionectria. A third specimen of Nectria albofimbriata (n. 430) is present in Saccardo’s fungarium (Fig. 5k–o). Although we were unable to obtain molecular data from this sample, Doi (1978) revised and reclassified it as Protocereopsis scitula. The same specimen was morphologically revised in 1983 by Samuels (see label, Fig. 5k) who identified it as Nectria pertusa, a taxon later recombined by Samuels & Rossman (in Rossman et al. 1999) as Protocereopsis pertusa, reducing N. scitula to a later synonym. Protocereopsis pertusa differs from Lasionectria albofimbriata mainly by the smaller ascospores, hardly reaching 17 μm in length with only 1–3 striations visible in one plane of view (Samuels 1976 as N. pertusa, Samuels et al. 1990 as N. cf. pertusa and Rossman et al. 1999). Our morphological analysis of Lasionectria albofimbriata (n. 430, PAD S00026) (Fig. 5m–o) has highlighted that its spore measurements, (13.7–14.7–15.8–16.9(–17.6) × (4.1–)4.4–4.8–5.1(–5.4) μm (n = 20), are perfectly corresponding with those reported by Rossman et al. (1999), as well as the fact that the ascospores are practically smooth or with very few striations. The recently described Protocereopsis caricola from Germany differs from P. pertusa by smooth and smaller ascospores, (11.5–)12–13.5(–14.5) × 3–3.5 μm (Lechat et al. 2016).

Among the morphologically closest species to Lasionectria albofimbriata, Protocereopsis javanica (= P. palmicola, = Cryptothecium javanicum, fide Rossman et al. 1999) is distinguished by hyphae enveloping perithecia that are typically roughened/warted (Penzig & Saccardo 1897 as Cryptothecium javanicum, Doi 1977 as P. palmicola, Rossman et al. 1999). Lasionectria martinicensis, on dead stems of Passiflora in Martinique, differs mainly by perithecia without a hyphal coating and narrower ascospores (3–3.7 μm wide) with a conspicuously striate perispor evesely loosening from the epispore (Lechat & Fournier 2012). It falls outside Lasionectria based on our phylogenetic inference (Fig. 1). Lasionectria krabiense on dead leaf of Pandanus sp. in Thailand, has similar ascospores but it is distinguished by papillate, orange to brownish orange perithecia, which collapse and become cupulate when dry, and are not surrounded by a hyphal coating (Tibpromma et al. 2018).

**Nectria ambigu**

**Clonostachys ambigu** (Penz. & Sacc.) Forin & Vizzini, comb. nov. — MycoBank MB835769; Fig. 6a–e

Basionym. Nectria ambigu Penz. & Sacc., Malpighia 11: 511. 1897.

Sexual morph. Perithecia solitary or in groups of a few, superficial on bark, yellow-orange, globose, not papillate, warted, about 450 μm diam (n = 1); not changing colour in 3% KOH and in 100% LA. Asci narrowly clavate, 73.8 × 10.6 μm (n = 1), 8-spored, ascospores biseriate above and uniseriate below. Ascospores ellipsoidal to fusoid, (16.5–)17–18.2–19.4(–21) × (4.6–)5.1–5.6–6.2(–6.6) μm, Q = (2.7–)2.9–3.3–3.6(–4), Qₙ = 3.2 (n = 35), 1-septate, equally subdivided in two cells, not constricted at the septum, hyaline, warted.

*Specimen examined. Indonesia. Java, Tjibodas, on bark (host not known),? Penzig, n. 119, PAD S00003, holotype.*

Notes — The specimen was morphologically revised in 1983 and in 1997, as shown on the labels associated with the sample. In the first revision it was hypothesized to be linked to *Nectria aureofulva* (= Bionectria aureofulva, Clonostachys rosea, Schroers 2001) and *N. apocygni* (= Bionectria apocygni, Schroers 2001; Clonostachys apocygni, Lombard et al. 2015), having an affinity to the *Nectria ochroleuca* complex (Samuels et al. 1990). In the second revision it was suggested to transfer *Nectria ambigu* to the genus *Bionectria*. Presently, this species is considered a synonym of *Bionectria apocygni*, although with some doubt (Schroers 2001). Recently, Rossman et al. (2013) proposed generic names for acceptance or rejection in the families Bionectriaceae, Hypocreaceae and Nectriaceae. In this treatment, Clonostachys was recommended over Bionectria in the Bionectriaceae. Accordingly, Lombard et al. (2015) proposed new combinations in *Clonostachys* for several bionectrioid taxa.

For *Nectria ambigu* only the ITS1 sequence has been obtained and included in the phylogenetic analyses. The isolated position of *Nectria ambigu* in the phylograms (Fig. 1, 2) suggests that this is a distinct Clonostachys species, excluded from the doubtful synonymy with *Bionectria apocygni* as proposed by Schroers (2001) in his monograph of *Bionectria*. This result is supported by the low identity (P%iv = 92.9 %; 11 nucleotide differences) between the ITS1 of *Nectria ambigu* and that of a *Bionectria apocygni* collection deposited in GenBank (AF210688, CBS 130.87). From a morphological point of view the two species are very similar (the reason why they were placed in synonymy), but they differ in ascospore dimensions: the ascospores of *Nectria ambigu* are shorter and narrower than those of *Bionectria apocygni* ([16–20.6–22.6–24.6(–32) × (4.6–)6–6.8–7.6–(9.4) μm) (Schroers 2001). *Clonostachys agarwali* (as ‘agarwali’), *C. capitata* and *C. zelandiae-novae* seem phylogenetically related to *C. ambigu*. *Clonostachys agarwali*, first isolated in India from decomposing buffalo horn pieces from animal house floor sweepings, is known only based on its asexual morph (Schroers 2001). *Clonostachys capitata* and *C. zelandiae-novae* have ascospores that are less than 15 μm long on average (Schroers 2001).

**Nectria ambigu var. pallens**

**Clonostachys pallens** (Penz. & Sacc.) Forin & Vizzini, comb. & stat. nov. — MycoBank MB835770; Fig. 6f–j

Basionym. Nectria ambigu var. pallens Penz. & Sacc., Malpighia 11: 511. 1897.

Sexual morph. Perithecia solitary or aggregated in groups, not immersed in a stroma, globose to subglobose-depressed, non-papillate, superficial on bark, pale yellow, 240–375 μm...
Asci strictly clavate, (54.9–)54.9–64.5(–67.5) × (6.7–)7.5–8.6(–9) µm (n = 10), 8-spored, ascospores biseriate above and uniseriate below. Ascospores ellipsoid to fusoid, (14.9–)16.2–17.2(–18.2) × (4.4–)4.7–5.1(–5.5) µm, Q = (2.8–)3.1–3.4–3.7(–4.1), Q av = 3.4 (n = 50), 1-septate, equally subdivided in two cells, not constricted at the septum, hyaline, striate.

Specimen examined. INDONESIA, Java, Tjibodas, on bark (host unknown), ? Penzig, n. 452 (ex p., PAD S00004, lectotype designated here, MycoBank MBT393716.

Notes — This species co-occurs on the same substrate with Nectria corona (Samuels et al. 1990). The species has been revised from a morphological point of view more than once, suggesting a synonymy with two different Bionectria (Clonostachys) species: B. subquaternata (note on the label, Fig. 6f) and B. grammicospora (Samuels et al. 1990). The molecular analysis excludes these possible synonymies suggesting that Nectria ambigua var. pallens is a distinct species within the genus Clonostachys, sister to Bionectria (Clonostachys) lucifer (Fig. 1) which differs in length and width of asci and ascospores. Nectria ambigua var. pallens has asci and ascospores shorter and narrower than those of Clonostachys lucifer (asci (85–)100–115–124(–160) × (18–)19–20.5–21.5(–23.5); ascospores (21.4–)27–28.8–30.6(–37) × (6–)8.8–9.4–10(–13.8)) (Samuels 1988, Schroers 2001).

Sexual morph. Perithecia aggregated into dense groups, partially immersed in a stroma superficial on the substrate, globose, smooth to rough, non-papillate, yellow, 190–240 µm diam and narrower than those of Clonostachys lucifer (asci (85–)100–115–124(–160) × (18–)19–20.5–21.5(–23.5); ascospores (21.4–)27–28.8–30.6(–37) × (6–)8.8–9.4–10(–13.8)) (Samuels 1988, Schroers 2001).
Asci not observed. Ascospores ellipsoid, (9.4–)10.3–11–11.7(–12.5) × (3.2–)3.5–3.8–4.1(–4.5) µm, Q = (2.6–)2.7–2.9–3.1(–3.6), Qav = 2.9 (n = 25), equally subdivided in two cells, 1-septate, not constricted or strongly constricted at the septum, warted, hyaline.

Specimen examined. Italy, Padova, Botanical Garden, on dead rhizome of Hedychium coronarium, Saccardo, PAD S00005, lectotype designated here, MycoBank MBT392609.

Notes — The specimen of Nectria congesta has never been taxonomically re-evaluated. In the ITS phylogram, the sequence of the lectotype clusters with different Bionectria/Clonostachys species without any statistical support (Fig. 1). In the combined phylogram the type sequence clusters with Bionectria ochroleuca, Clonostachys rosea and our type of Nectria phyllostachydis with low statistical support (Fig. 2). The high similarity among B. ochroleuca (CBS 193.94, CBS 194.57, CBS 406.95), Nectria congesta and N. phyllostachydis ITS sequences (P%iv = 99.4 %) and between the morphologies of N. congesta and B. ochroleuca reported by Schroers (2001) suggest that N. congesta can be considered a synonym of Clonostachys rosea, a taxon which probably represents a species complex that will be difficult to untangle (Abreu et al. 2014).

Sexual morph. Perithecia gregarious, superficial, globose to pyriform, brownish red with a darker ostiolar disc, 225–350 µm diam (n = 5); darker in 3 % KOH and yellow in 100 % LA; ostiolar disc with saccate cells which forms a fringe giving the perithecium a coronate aspect. Asci not found. Ascospores ellipsoid to fusiform, (17.1–)18–19.5–21.1(–22.7) × (5.5–)6.8–7.5(–8.4) µm, Q = (2.5–)2.7–2.9–3.1(–3.4), Qav = 2.9 (n = 25), 2-celled, symmetrical or eccentric, sometimes with one side curved and one side flattened, 1-septate, constricted or not constricted at the septum, hyaline, striate.

Specimen examined. Indonesia, Java, Tjibodas, on bark (host unknown), ? Penzig, n. 452 ex p., PAD S00006, holotype.

Notes — Morphological observations of Nectria coronata agree with the description provided by Samuels et al. (1990). The species co-occurs on the same substrate with Nectria ambigua var. pallens (Samuels et al. 1990). Our results confirm the transfer of this species to the genus Thelonectria (Nectriaceae)
reported by Chaverri et al. (2011) (Fig. 3). The genus encompasses species characterised by bright red-brown perithecia with a prominent sometimes darker papilla (main feature of *Thelonectria* species); asci cylindrical to clavate and 8-spored; ascospores 1-septate, spinulose or striate; and a cylindroconidium-like asexual morph (Chaverri et al. 2011, Salgado-Salazar et al. 2016).

*Nectria cyanostoma*

*Cyanonectria cyanostoma* (Sacc. & Flageolet) Samuels & Chaverri, *Mycol. Progr.* 8: 56. 2009 — Fig. 8f–j

*Basionym.* *Nectria cyanostoma* Sacc. & Flageolet, *Rendiconti Congr. Bot. Palermo:* 53. 1902.

*Synonym.* *Fusarium cyanostomum* (Sacc. & Flageolet) O’Donnell & Geiser, *Phytotaxonomy* 103: 404. 2013.

Sexual morph. *Perithecia* gregarious, superficial, red-brown, pyriform with darker apical region, 158–250 µm diam (*n* = 10); dark red in 3 % KOH and yellow in 100 % LA. *Asci* not found. *Ascospores* ellipsoidal to ovoidal, (11.9–)12.3–13.3–14.4–(15.8) × (4.6–)4.9–5.3–5.7–(6.1) µm, *Q* = (2–)2.3–2.5–2.8–(3), *Q*<sub>av</sub> = 2.5 (*n* = 16), 1-septate, equally subdivided in two cells, constricted at the septum, warded. *Macroconidia* 3–5-septate: 3-septate, 43 × 4 µm (*n* = 1); 5-septate, 52–63 × 5 µm (*n* = 2), curved.

*Specimen examined.* France, St. Romain near Rigny, on bark of *Buxus sempervirens*, Flageolet, n. 32, PAD S00007, syntype; lectotype in BPI as BPI 551652.

Notes — The genus *Cyanonectria* (*Nectriaceae*) was proposed for *N. cyanostoma* and, as a consequence, the name was recombined as *Cyanonectria cyanostoma* (Samuels et al. 2009). The two species of this genus (*Cyanonectria cyanostoma* and *C. buxi*) are distinguished by red perithecia with a bluish purple papilla and a fusarium-like asexual morph (Samuels et al. 2009). The ITS of the syntype specimen clusters with an ITS sequence of *Cyanonectria cyanostoma* (CBS 101734) in a highly-supported clade (BPP 0.99, MLB 99 %) in the *Nectriaceae* (Fig. 3). The morphological observation of *Nectria cyanostoma* fits with the description reported by Samuels et al. (2009). The molecular and morphological analyses confirm that this species belongs to the genus *Cyanonectria* as *C. cyanostoma*, together with *C. buxi* (Schroers et al. 2011). Geiser et al. (2013) proposed expanding the concept of the genus *Fusarium* as the sole name for a group that includes virtually all *Fusarium* species of...
importance in plant pathology, mycotoxicology, medicine, and basic research. A number of genera have fusarium-like asexual morphs, and Lombard et al. (2015) argued to retain the sexual morph generic names Albonectria, Cyanonectria, Geejayessia and Neocosmospora as proposed by Gräfenhan et al. (2011), Schroers et al. (2011) and Nalim et al. (2011) for these genera. Fusarium should be restricted to the monophyletic clade of species associated with a Gibberella sexual morph (the clade that includes the lectotype of the genus, F. sambucinum).

**Nectria dolichospora**

*Hydropisphaera dolichospora* (Penz. & Sacc.) Rossman & Samuels, Stud. Mycol. 42: 30. 1999 — Fig. 9

**Basionym.** *Nectria dolichospora* Penz. & Sacc., Malpighia 11: 513. 1897.

Sexual morph. *Perithecia* solitary or gregarious, superficial, brown, globose with hyphae around the perithecium base, non-papillate, 187–257 μm diam (n = 10); not changing colour in 3 % KOH and 100 % LA. *Asci* clavate, (74.6–)75.6–81–86.5(–87.9) × (6–)7.1–7.8–8.6(–9.6) μm (n = 5), 8-spored, ascospores biseriate. *Ascospores* ellipsoidal to fusoid, (25.2–)28.3–33.1(–34.7) × (6–)7.1–7.8–8.6(–9.6) μm, Q = (3.1–)3.6–4.3(–5), Q_av = 3.9 (n = 50), straight or with one flat side and one side curved, 1-septate, equally subdivided in two cells, not constricted at the septum, hyaline, smooth-walled to slightly striate.

**Specimens examined.** Indonesia, Java, Tjibodas, on dead stem of *Elettaria* sp., 6 Feb. 1897, Penzig, n. 434, PAD S00008, lectotype designated by Samuels et al. (1990); Penzig, n. 442, PAD S00009, syntype.

Notes — The two types were morphologically revised in 1970 by G.J. Samuels, as reported on the labels associated with the samples (Fig. 9a). Presently, this species belongs to the genus *Hydropisphaera* (*Bionectriaceae*) (Rossman et al. 1999). The genus is characterised by species with superficial, non-stromatic perithecia, pale yellow to umber, globose to subglobose and a perithecial wall more than 25 μm thick. *Asci* are clavate and 8-spored. *Ascospores* ellipsoid, 1- to multi-septate, hyaline, generally striate, rarely smooth-walled or spinulose. The asexual morph of *Hydropisphaera* is considered to be acremonium-like (Rossman et al. 1999). However, a *Hydropisphaera* species (*H. bambusicola*) was found producing an asexual morph identified as *Gliomastix fusigera* (Lechat et al. 2010). The morphological observations of *Nectria dolichospora* fit with the detailed description reported by Samuels et al. (1990). The molecular analysis of the two types (Fig. 1) confirms the taxonomic reclassification proposed by Rossman et al. (1999).

![Fig. 9](image_url)
Nectria flageoletiana

Clonostachys compactiuscula (Sacc.) D. Hawksw. & W. Gams, Trans. Brit. Mycol. Soc. 64: 90. 1975 — Fig. 10a–e

Basionym. Verticillium compactiusculum Sacc., Fungi Italica: 17–28: t. 724. 1881.
Synonyms. Bionectria compactiuscula Schroers, Stud. Mycol. 46: 104. 2001.
Nectria flageoletiana Sacc., Atti Mem. R. Accad. Sci. Lett. Arti, Padova 33: 161. 1917.

Sexual morph. Perithecia solitary or in small groups, erumpent through bark, globose and slightly sunken when dry, pale yellow, 190–255 µm diam (n = 10); not changing colour in 3 % KOH and 100 % LA; ostiolar openings slightly papillate. Asci narrowly clavate, (37.6–)41.2–48.9–56.6–(60.2) × (5.2–)5.4–6.1–6.8–(7.2) µm (n = 10), 8-spored, ascospores biseriate above and uniseriate below. Ascospores ellipsoid to oblong-ellipsoidal, (7.8–)8.6–10–11.3–(13.4) × (2.9–)3.2–3.5–3.9–(4.3) µm, Q = (2.1–)2.5–2.8–3.2–(4.1), Qav = 2.8 (n = 38), equally subdivided in two cells, aseptate or 1-septate, slightly constricted at the septum, hyaline, finely roughened.

Specimen examined. FRANCE, Rigny, on bark of Prunus laurocerasus, 1916, Flageolet, PAD S00010, holotype.

Notes — The ITS sequence of *N. flageoletiana* clusters with ITS sequences of *Clonostachys compactiuscula* (CBS 729.87, CBS 913.97, CBS 592.93) (BPP 1.0, MLB 91 %) (Fig. 1). P%iv of the ITS sequences in this clade resulted in 99.4 %. Combining this result with the high similarity between the morphological characteristics of *Nectria flageoletiana* and those reported by Schroers (2001) for *Bionectria compactiuscula*, it is plausible to suppose that these two species are synonymous. This is
also supported by the geographical distribution of *Bionectria compactiuscula*, that includes France (Schroers 2001), and the place of origin of *Nectria flageoletiana* (Rigny, France). As a consequence, *Nectria flageoletiana* is reduced here to synonymy with *Clonostachys compactiuscula*.

**Nectria granuligera**

*Clonostachys granuligera* (Starbäck) Forin & Vizzini, comb. nov. — MycoBank MB836934; Fig. 11f–j

_Basionym._ *Nectria granuligera* Starbäck, Hedwigia 31: 308. 1892.

_Synonyms._ *Creonectria granuligera* (Starbäck) Seaver, Monogr. Univ. Puerto Rico, Ser. B 2: 130. 1934.

**Sexual morph.** _Perithecia_ gregarious in large clusters, on stroma erumpent from bark, globose to subglobose, warted, not papillate, yellow-orange, 230–300 µm diam (n = 8); not changing colour in 3 % KOH and 100 % LA. _Asci_ clavate (36.9–)37.4–43.3–49.2–(53.1) x (6–)6.1–6.5–7–(7.5) µm (n = 6), 8-spored, ascospores biseriate above and uniseriate below. _Ascospores_ ellipsoid, (8.9–)10.1–11.5–12.8–(15.1) x (3.4–)3.8–4.2–4.6–(4.9) µm, Q = (2.3–)2.5–2.8–3–(3.8), Qav = 2.8 (n = 50), equally subdivided in two biguttulate cells, 1-septate, slightly constricted at the septum, warted, hyaline.

_Specimen examined._ SWEDEN, Uppsala, Botanical Garden, on orchid bark, 1891, Starbäck, Rehm, Ascomyc. nr. 1082, PAD S00011, lectotype designated here, MycoBank MBT393970.

Notes — The specimen *Nectria granuligera* stored in the Saccardo collection is part of a series from the same original exsiccate (Rehm, Ascomyc. nr. 1082). An identical specimen of the series, stored in the New York Botanical Garden, was defined as isotype and morphologically revised by Samuels in 1982 which proposed a possible synonymy with *Nectria byssicola* (http://sweetgum.nybg.org/science/vh/specimen-details/?irn=1052023). This synonymy, however, has not been formally published. Another exsiccate marked as type is deposited at Swedish Museum of Natural History under the name *Stilbocrea gracilipes* (S-F10151, Rehm, Ascomyc., nr. 1082, http://herbarium.nrm.se/specimens/F10151). Neither MycoBank nor Index Fungorum provides a link between these names and we could not locate any record in literature.

Our ITS molecular analysis (Fig. 1) placed _N. granuligera_ in the genus _Clonostachys_ within an unsupported clade comprising specimens of _Clonostachys byssicola_ (CML 2404; CML 2510; CBS 364.78 isotype), _N. squamuligera_ (PAD S00020 lectotype, PAD S00021, PAD S00022), _C. eriocamporesiana_ (MFLUCC 17-2620 holotype) and _C. wenpingii_ (HMAS 172156 holotype). In the combined analysis (Fig. 2), _N. granuligera_, sister to _C. byssicola_ (CML 1942), forms a partly supported clade (BPP 0.96, MLB 54 %) together with two other _C. byssicola_ collections (CML 2311 and CML 2404), _N. squamuligera_ (PAD S00020 lectotype, PAD S00021, PAD S00022) and _C. wenpingii_ (HMAS 172156 holotype), whereas the isotype of _C. byssicola_ (CBS 364.78) clusters with two other _C. byssicola_ collections (CML 0422 and CML 2309) and _C. eriocamporesiana_ (MFLUCC 17-2620 holotype). Another _C. byssicola_ collection (CBS 914.97)

_Fig. 11_ a–e. *Nectria squamuligera* (PAD S00020: herbarium Saccardo, lectotype). a. Original herbarium specimen; b. perithecia on natural substrate; c. perithecium magnification with details of the surface in cotton blue; d–e. asci and ascospores in cotton blue. — f–j. *Nectria granuligera* (PAD S00011: herbarium Saccardo, n. 1082, lectotype). f. Original herbarium specimen; g. perithecia on natural substrate; h. perithecium magnification with details of the surface in cotton blue; i–j. asci and ascospores in cotton blue. — Scale bars: b = 500 µm; c, g–h = 100 µm; d–e, i–j = 5 µm. — Photos by N. Forin.
occupies an uncertain position. The polyphyly of the strains assigned to Clonostachys byssicola has already been highlighted by Abreu et al. (2014).

Clonostachys granuligera is actually very similar morphologically to Clonostachys byssicola (now Clonostachys farinosa, fide Rossman 2014) as circumscribed by Samuels (1976, as Nectria byssicola), Samuels et al. (1990, as N. byssicola), Schroers & Samuels (1997, as Bionectria byssicola) and Schroers (2001, as B. byssicola), but the latter has larger asci, (44–)55–60–65(–90) × (5.5–)7.5–8.5–9(–12.5) μm (Schroers 2001). The recently described species Clonostachys eriocamporesiana is difficult to differentiate from Clonostachys byssicola both on a morphological and molecular basis (Hyde et al. 2020) and, in our opinion, is probably synonymous with Clonostachys byssicola.

Nectria squamuligera is distinguished by solitary to gregarious (small groups), non-stromatic pale pink perithecia (see below). Clonostachys wenpingii differs from Clonostachys granuligera by smaller perithecia (175–210 μm diam) which are smooth (non-warted), solitary, non-stromatic and pale yellow, and narrower ascospores, × 2.7–4 μm (Luo & Zhuang 2007).

Nectria illudens

**Neocosmospora illudens** (Berk.) L. Lombard & Crous, Stud. Mycol. 80: 227. 2015 — Fig. 12f–j

Basionym. Nectria illudens Berk., in Hooker, Bot. Antarc. Voy. II (Fl. Nov. Zel.): 203. 1855.

**Synonyms.** Cucurbitaria illudens (Berk.) Kuntze, Revis. Gen. Pl. 3: 461. 1898.

_Haematonectria illudens_ (Berk.) Samuels & Nirenberg, Stud. Mycol. 42: 136. 1999.

_Fusarium illudens_ C. Booth, The genus Fusarium: 54. 1971.

Sexual morph. **Perithecia** gregarious, superficial, red-orange, wartner, globose, papillate with a darker papilla, 224–349 μm diam (n = 6); dark red in 3 % KOH and yellow in 100 % LA. Asci clavate 125–138 × 16.8–18.7 μm (n = 2), 8-spored, ascospores biseriate above and uniseriate below. **Ascospores** ellipsoidal to fusoid, (19–)21.5–23.5–25.5(–27.7) × (5.8–)7.2–8.3–9.3(–10) μm, Q = (2.5–)2.6–2.9–3.1(–3.6), Qav = 2.9 (n = 38), straight to curved, 1-septate, equally subdivided in two cells, not constricted at the septum, finely striate.

**Specimen examined.** NEW ZEALAND, on bark (host unknown), Berkeley, PAD S00012, neotype designated here, MycoBank MBT392611.

**Fig. 12** a–e. Nectria peziza subsp. reyesiana (PAD S00015: herbarium Saccardo, n. 1609, holotype). a. Original herbarium specimen; b–c. perithecia on natural substrate; d–e. ascii and ascospores in cotton blue. — f–j. Nectria illudens (PAD S00012: herbarium Saccardo, neotype). f. Original herbarium specimen; g. perithecia on natural substrate; h–j. ascospores in cotton blue. — Scale bars: b, g = 500 μm; c = 150 μm; d–e, h–j = 10 μm. — Photos: a–e by N. Forin; f–j by S. Nigris.
Notes — *Nectria illudens* was recombined in *Neocosmospora* based on morphological and molecular data (Lombard et al. 2015, Sandoval-Denis et al. 2019). The specimen analysed here has the same information as the *Nectria illudens* specimen deposited at Kew, and marked as possible type. According to Samuels & Brayford (1994) a holotype should exist (collector name J. Hooker, based on the protologue), but they were unable to locate it. As Sandoval-Denis et al. (2019), we could not find information about the location of a type collection, and therefore designate a specimen stored in the Saccardo fungalarium as neotype. The ITS sequence of the neotype clusters with sequences of *Neocosmospora illudens* (CBS 119605 and G.J.S. 85-67) in a highly supported clade (BPP 1.0, MLB 98 %) within the genus *Neocosmospora* (*Nectriaceae*) (Fig. 3), as previously highlighted by Lombard et al. (2015).

*Nectria leucotricha*  
*Hydropisphaera leucotricha* (Penz. & Sacc.) Rossman & Samuels, Stud. Mycol. 42: 31. 1999 — Fig. 13a–e

Basionym: *Nectria leucotricha* Penz. & Sacc., Malpighia 11: 512. 1897.

Sexual morph. *Perithecia* characterised by the presence of hyphal trichomes on the surface, solitary or gregarious, brown, superficial, globose, with hyphae around the perithecial base, non-papillate, 280–385 µm diam ($n = 10$); not changing colour in 3 % KOH and 100 % LA. *Asci* clavate, (51.3–)51.4–56–60.5(–62.1) × (8.3–)8.4–8.6–8.8(–8.9) µm ($n = 4$), 8-spored, ascospores biseriate. *Ascospores* ellipsoidal, (14.4–)15.9–16.7–17.6(–18.8) × (3.9–)4.3–4.7–5.1(–5.4) µm, $Q = (2.5–)2.6–2.9–3.1(–3.6)$, $Q_{av} = 3.6$ ($n = 38$), 1-septate, equally subdivided in two cells, not constricted at the septum, hyaline, striate.

Specimen examined. INDONESIA, Java, Tjibodas, on decaying leaf of *Elettaria* sp., 6 Feb. 1897, ? Penzig, n. 150, PAD S00013, lectotype designated by Samuels et al. (1990).
Notes — The specimen was morphologically revised in 1983 by G.J. Samuels, as reported on the label associated with the sample (Fig. 1a). This species is now considered a member of the genus *Hydropisphaera* (*Bionectriaceae*). The morphological observations of *Nectria leucotricha* fit with the detailed description provided by Samuels et al. (1990). The species is well-characterised by the presence of hyphae that form ≥ 200 μm long triangular hairs on the surface of perithecia (Samuels et al. 1990). The molecular analysis of the lectotype (Fig. 1) confirms the taxonomic reclassification proposed by Rossman et al. (1999).

*Nectria mantuana*

*Lasionectria mantuana* (Sacc.) Cooke, Grevillea 12: 112. 1884 — Fig. 7f–j  
Basionym. *Nectria mantuana* Sacc., Michelia 1: 52. 1877.  
Synonym. *Cucurbitaria mantuana* (Sacc.) Kuntze, Revis. Gen. Pl. 3: 461. 1898.

Sexual morph. *Perithecia* solitary, superficial, brown, globose-depressed, non-papillate, 164–280 μm diam (n = 10), sparsely covered with short hairs, 8–16 × 2.4–3.8 μm (n = 10) which sometimes are fasciculate; not changing colour in 3 % KOH and 100 % LA. *Asci* clavate, (41.4–)42.6–47.2–51.9–(52.6) × (5.8–)6.1–7.3–(7.6) μm (n = 7), 8-spored, ascosporae uniseriate. *Ascosporae* ellipsoid, (8.4–)9.5–10.3–11–(11.9) × (2.9–)3–3.3–3.6–(4.1) μm, Q = (2.4–)2.6–3.1–3.4–(3.5), Qav = 3.1 (n = 32), 1-septate, equally subdivided in two cells, not constricted at the septum, hyaline, smooth to slightly striate.

Specimen examined. **ITALY**, Mantova, Migliaretto, on decorticated poplar wood, Feb. 1873, A. Magnaguti-Rondinini, PAD S00014, holotype.

Notes — The specimen was morphologically revised in 1993 by A.Y. Rossman, as reported on the label associated with the sample (Fig. 7f). This taxon is the type species of the genus *Lasionectria* (*Bionectriaceae*) (Rossman et al. 1999). The morphological observations of *Nectria mantuana* fit the description provided by Rossman et al. (1999). The molecular analysis of the holotype shows that *Nectria mantuana* is a distinct species of the genus *Lasionectria* (Fig. 1), sister to *L. lecanodes*, a lichenicolous species with minutely warted spores (Petch 1938, Sérusiaux et al. 1999, Lechat & Fournier 2019). In addition, the recovered ITS sequence from the holotype *Nectria mantuana* differs compared to the single *Lasionectria* species described based on its asexual morph (Crous et al. 2018; while for the recently introduced species, *F. bharatavarshae* (Dayarathne et al. 2020), *F. melogrammae* (Crous et al. 2016) and *F. ossicola* (Lechat & Rossman 2017), a detailed description of the sexual morphs is presented. Species of this genus are characterised by superficial, yellow to pale orange perithecia that do not change colour in KOH but become yellow-orange in LA and a fusarium-like asexual morph (Lechat & Rossman 2017). These characters have also been observed in the *Nectria peziza* subsp. *reyesiana* specimen. These observations suggest that this species should be considered as a species of *Fusicola*. *Fusicola reyesiana* differs from *F. melogrammae* and *F. ossicola* in having shorter ascii ((48.3–)51.1–66.4–(67.6) × (7.1–)7.8–9.4–10.9–(11.3) μm (n = 5), 8-spored, ascosporae biseriate above and uniseriate below. *Ascosporae* ellipsoid, (11.5–)12.4–13.5–14.5–(16.8) × (4.2–)4.8–5.3–5.8–(6.2) μm, Q = (2.2–)2.3–2.6–2.8–(3.4), Qav = 2.5 (n = 40), 1-septate, equally subdivided in two cells, not constricted at the septum, hyaline, verrucose.

Specimen examined. **PHILIPPINES**, Luzon, Los Banos, on bark (host not known), 15 Aug. 1913, S.A. Reyes, n. 1609, PAD S00015, holotype.

Notes — *Nectria peziza* subsp. *reyesiana* was synonymized with *N. peziza* (*Bionectriaceae*) and, as a consequence, with *Hydropisphaera peziza* (Rossman et al. 1999). However, this original material has never been examined since its original description. The target ITS1 and ITS2 sequences identified after the sequencing data analyses clearly indicate a different scenario with respect to the current status of this species. In fact, the BLASTn analysis showed that the ITS1/ITS2 sequences have a high similarity value with ITS sequences belonging to the genus *Fusicola* (*Bionectriaceae*) in the NCBI database.

*Fusicola reyesiana* (Link) Schroers et al., Mycologia 91: 369. 1999 — Fig. 10f–j  
Basionym. *Nectria peziza* Sacc., Ann. Mycol. 12: 305. 1914.

Sexual morph. *Perithecia* gregarious, superficial or partially imersed in a pale-yellow sheet of hyphae, globose, non-papillate, red-orange, 234–342 μm diam (n = 8), not changing colour in 3 % KOH but turning yellow in 100 % LA. *Asci* narrowly clavate, (48.3–)51.2–66.4–(67.6) × (7.1–)7.8–9.4–10.9–(11.3) μm (n = 5), 8-spored, ascosporae biseriate above and uniseriate below. *Ascosporae* ellipsoid, (11.5–)12.4–13.5–14.5–(16.8) × (4.2–)4.8–5.3–5.8–(6.2) μm, Q = (2.2–)2.3–2.6–2.8–(3.4), Qav = 2.5 (n = 40), 1-septate, equally subdivided in two cells, not constricted at the septum, hyaline, verrucose.

*Clonostachys rosea* (Link) Schroers et al., Mycologia 91: 369. 1999 — Fig. 10f–j  
Basionym. *Nectria peziza* Sacc., Ann. Mycol. 12: 305. 1914.

SYNONYMS

*Sphaeria ochroleuca* Schwein., Trans. Amer. Philos. Soc., New Series 4: 204. 1832 *1834*  
*Cucurbitaria ochroleuca* (Schwein.) Kuntze, Revis. Gen. Pl. 3: 461. 1898  
*Creonectria ochroleuca* (Schwein.) Seaver, Mycologia 1: 190. 1909  
*Bionectria ochroleuca* (Schwein.) Schroers & Samuels, Z. Mykol. 63: 15. 1997.
Nectria congesta Sacc., Michelia 2: 256. 1881.
Nectria phyllostachydis Hara (as Nectria phyllostachydis), Bot. Mag. (Tokyo) 27: 247. 1913.

Original description (translated from Japanese) — Perithecia orange-red, solitary or in groups of 3–7 on a small protruding stroma, opening by an oval ostiole, fleshy. 250–300 µm diam. Ascus clavate, 8-spored. Spores transparent, 1-septate, fusoid, slightly constricted at the septum, 10–14 × 2–3 µm. It grows on young trunk of Phyllostachys reticulata. Collected at Mino Kawaiumura (Japan) in 1912.

Sexual morph. Perithecium gregarious, not immersed in a stroma, globose to subglobose-depressed, not papillate, superficial on bark, pale yellow, 175–300 µm diam (n = 5); not changing colour in 3 % KOH and 100 % LA. Asci not observed. Ascospores ellipsoid to fusoid, (8.1–)9.1–10.3–11.4(–12.6) × (2.8–)3–3.3–3.6(–4.1) µm, Q = (2.4–)2.8–3.1–3.5(–3.9), Qav = 3.1 (n = 25), 1-septate, not equally subdivided in two cells, constricted at the septum, hyaline, warped.

Specimen examined. Japan, Mino prov., Kawaiyume-mura (currently Gifu pref.), on Phyllostachys bambusoides. (=P. reticulata), Jan. 1912, K. Hara, TNS-F-210044 lectotype designated here (MBT392613); PAD S00016, isolecotype.

Notes — The type specimen of Nectria phyllostachydis has never been morphologically revised or systematically re-evaluated. Another specimen of Nectria phyllostachydis is deposited at the National Museum of Nature and Science (TNS) in Japan (Tsukuba) with the number 210044 (Fig. 10k–n) and has the same information found in the specimen stored in the Saccardo fungarium (JAPAN, Gifu pref., on Phyllostachys bambusoides, Jan. 1912, K. Hara). Based on the protologue, we designate the specimen TNS-F-210044 as lectotype (MycoBank MBT392613) and our specimen, which is a duplicate of the lectotype, as the holotype. The morphological observations of Nectria phyllostachydis fit the original description. The ITS sequence of Nectria phyllostachydis clusters with different Clonostachys species, including N. congesta, without statistical support in the ITS tree (Fig. 1) and with Bionectria ochroleuca, Clonostachys rosea, C. rosea f. catenulata and Nectria congesta with low statistical support in the combined phylogram (Fig. 2). However, the high morphological similarity between this type, Bionectria ochroleuca (Schroers 2001) and Nectria congesta suggests that N. phyllostachydis is a synonym of Clonostachys rosea.

Nectria radicans

Sarcopodium radians (Penz. & Sacc.) Forin & Vizzini, comb. nov. — MycoBank MB835772

Basionym. Nectria radicans Penz. & Sacc., Malpighia 11: 510. 1897.

Original description — Perithecium superficialis, in soros ramoso-radiantes, 3.7 mm diam. congestis, latericito-rubris, globoso-conoides, breve papillatis, 1/3 mm d., initio flavo-pruinosis; ascis fusoidibus utrinque acutulis, 50–60 × 9–12, a paraphysatis, (?), octosporidio; sporidios 2–3-stichis, fusoidis, 15–17 × 4–4.5, rectis, 1-septatis, non constrictis, hyalinis, intus nubilosis.

From Samuels et al. (1990) — “Ascospores in this collection measure (12–)12.4–14.8(–17) × (4–)4.5–5.3(–5.5) µm. Because these are somewhat larger than ascospores of N. flocculenta, we consider this name to be synonymous with N. flavo-lanata”.

Specimen examined. IndoneIs, Java, ? Tjibodas, on bark (host not known). 1898, M. Fleischer, n. 923, PAD S00018, holotype.

Notes — The holotype specimen was morphologically studied in 1983 by G.J. Samuels, as reported in the label associated with the sample (Fig. 14a). Nectria radicans is now considered a member of the genus Sarcopodium (Nectriaceae) as S. rapi- lum. The morphological observations of Nectria radicans fit with the detailed description reported by Samuels et al. (1990). It is distinguished by its large, non-spinulose ascospores, and smooth hyphal hairs as in other Sarcopodium species (Samuels et al. 1990, Rossman et al. 1999, Lombard et al. 2015). The molecular analysis of the holotype (Fig. 3) confirms the taxonomic placement made by Lombard et al. (2015).

Nectria sordescens

Sarcopodium tjiobodense — Fig. 14f–j (see below)

Synonym. Nectria sordescens Sacc., Atti Accad. Sci. Veneto-Trentino-Istriana 10: 69. 1917.

Sexual morph. Perithecium gregarious, superficial, globose, non-papillate or with a small darker papilla, red, 182–232 µm diam (n = 6); dark red in 3 % KOH and yellow in 100 % LA. Asci not observed. Ascospores ellipsoid, (10.1–)10.6–11.5–12.5
Nectria squamuligera

**Clonostachys squamuligera** (Sacc.) Forin & Vizzini, comb. nov. — MycoBank MB836924; Fig. 11a–e

Basionym. *Nectria squamuligera* Sacc., Atti Soc. Veneto-Trentino Sci. Nat. Padova, sér. 4: 122. 1875.

Synonyms. *Dialonectria squamuligera* (Sacc.) Cooke, Grevillea 12: 110. 1884.

*Cucurbitaria squamuligera* (Sacc.) Kuntze, Revis. Gen. Pl. 3: 461. 1898.

(-14) × (2.7–)3.2–3.5–3.9(–4.4) μm, Q = (2.7–)3–3.3–3.6(–4), Qav = 3.3 (n = 30), 1-septate, equally subdivided in two cells, not constricted, hyaline, striate.

Specimen examined. PHILIPPINES, Los Baños, on bark of *Leucaena glauca*, Aug. 1913, M.B. Raimundo, comm. Baker, PAD S00019, holotype.

Notes — The holotype specimen *Nectria sordescens* was never morphologically re-evaluated since its description. The ITS sequence of the type clusters with sequences of *Lanatonec- tria flocculenta*, *Nectria tjibodensis*, *Sarcopodium circinosetiferum* and *S. macalpinei* (Fig. 3). The molecular analysis and the morphological comparison between *Nectria sordescens* and *N. tjibodensis* (see below) suggest that *N. sordescens* can be considered a synonym of *N. tjibodensis*.

*Nectria squamuligera*

**Clonostachys squamuligera** (Sacc.) Forin & Vizzini, comb. nov. — MycoBank MB836924; Fig. 11a–e

Basionym. *Nectria squamuligera* Sacc., Atti Soc. Veneto-Trentino Sci. Nat. Padova, sér. 4: 122. 1875.

Synonyms. *Dialonectria squamuligera* (Sacc.) Cooke, Grevillea 12: 110. 1884.

*Cucurbitaria squamuligera* (Sacc.) Kuntze, Revis. Gen. Pl. 3: 461. 1898.

Sexual morph. *Perithecia* solitary to gregarious in small groups, superficial to erumpent through bark, non-stromatic, globose to subglobose, squamulose, warted, non-papillate, yellow with a darker ostiolar region, 230–320 μm diam (n = 7); not changing colour in 3 % KOH and 100 % LA. *Asci* clavate, (48.8–)49.3–53.9–58.6(–60) × (5.7–)6–7–8.1(–8.2) μm (n = 4), 8-spored, ascospores biseriate above and uniseriate below. *Ascospores* ellipsoid to fusoid, (10.2–)10.8–12.6–14.4(–17.4) × (2.6–)3.3–3.7–4.1(–4.6) μm, Q = (2.3–)2.8–3.5–4.1(–5.1), Qav = 3.4 (n = 30), 1-septate, equally subdivided in two cells, not constricted or slightly constricted at the septum, warted, hyaline.

Specimens examined. ITALY, on branch bark of *Salix babylonica*, PAD S00020, lectotype designated here, MycoBank MBT392615; Padova, Botanical Garden, on bark of *Glycine sinensis*, D. Saccardo, Dec. 1898, n. 318, PAD S00022, (Nectria squamuligera f. glycines). – PORTUGAL, Coimbra, Botanical Garden, on *Hardenbergia violacea*, Nov. 1891, A. Moller, PAD S00021.

Notes — Based on morphological and molecular data (P%iv = 99.8 %), the three *Nectria squamuligera* specimens examined in this study are conspecific and belong to *Clonostachys* (Fig. 1, 2). The only information found about this species is reported in Samuels (1976) where he placed *N. squamuligera* in synonymy with *Nectria ochroleuca* (= *Clonostachys rosea*). The sequences of the three *Nectria squamuligera* specimens form a well-supported clade (BPP 0.98, MLB 81 %) phylogenetically...
close to those of three collections misidentified as Clonostachys byssicola (CML 1942, CML 2311, CML 2404), N. granuligera (PAD S00011 isotype) and C. wenpingii (HMAS 172156 holotype) (Fig. 2), excluding the synonymy proposed by Samuels (1976). Clonostachys rosea, as delimited by Samuels (1976, as Nectria ochroleuca), Schroers & Samuels (1997, as Bionectria ochroleuca), Schroers et al. (1999, as B. ochroleuca) and Schroers (2001, as B. ochroleuca), is really morphologically very close to C. squamuligera but mainly differs in having stromatic, yellowish orange, light orange to brown orange perithecia (vs non-stromatic perithecia which are pale pink in fresh condition, Saccardo 1875) and smaller ascospores, (7.4–)9.4–10.8(–14.4) × (2.2–)3–3.4–3.6(–4.8) µm. Clonostachys wenpingii has smaller (175–210 µm diam), pale yellow, smooth perithecia and shorter asci, 33–44 × 5.5–8.0 µm (Luo & Zhuang 2007).

**Nectria tjibodensis**

*Sarcocordium tjibodense* (Penz. & Sacc.) Forin & Vizzini, *comb. nov.* — MycoBank MB835773; Fig. 15a–e

*Basionym.* *Nectria tjibodensis* Penz. & Sacc., Malpighia 11: 512. 1897.

*Synonyms.* *Nectriella flocculenta* Henn. & E. Nyman, Monsunia 1: 160. 1899.

*Lanatonectria flocculenta* (Henn. & E. Nyman) Samuels & Rossman, Stud. Mycol. 42: 138. 1999.

*Actinostilbe flocculenta* (Henn. & E. Nyman) Rossman et al., IMA Fungus 4: 46. 2013.

*Sarcopodium flocculentum* (Henn. & E. Nyman) Pennycook & P.M. Kirk, Index Fungorum 418: 1. 2019.

*Nectria sordescens* Sacc., Atti Accad. Sci. Veneto-Trentino-Istriana 10: 69. 1917.

*Kutilakesisraphiopina* Agnihothr. & G.C.S. Barua, J. Indian Bot. Soc. 36: 309. 1957.

*Sarcocordium macalpinei* (Agnihothr. & G.C.S. Barua) B. Sutton, Trans. Brit. Mycol. Soc. 76: 99. 1981.

*Actinostilbe macalpinei* (Agnihothr. & G.C.S. Barua) Seifert & Samuels, Stud. Mycol. 42: 138. 1999.

* = *Kutilakesis cirinosetifera* Matsu, Microfungi Solomon Isl. Papua New Guinea: 34. 1971.

*Sarcocordium cirinosetifera* (Matsus.) Matsush., Micropalma Mycol. Mem. 9: 24.1996. [Nom. inval., Art. 41.4 (Melbourne)].

Sexual morph. *Perithecia* solitary or gregarious in groups, superficial on bark, globose, papillate, cupulate, not collapsing when dry, red, 225–298 µm diam (n = 10); dark red in 3 % KOH and yellow in 100 % LA. *Asci* clavate, (41.3–)41.4–43–45(–45.4) × (6.2–)6.3–7.4–8.5(–8.7) µm (n = 5), 8-spored, ascospores biseriate. *Ascospores* ellipsoid to fusoid, (11.6–)12.1–13.3–14.6(–17.6) × (2.7–)3.3–3.7–4.1(–4.6) µm, Q = (2.8–3.1–3.6–4.2(–4.8), Qav = 3.6 (n = 50), 1-septate, equally subdivided in two cells, constricted at the septum, hyaline, striate.

*Specimen examined.* INDONESIA, Java, Tjibodas, on bark (host not known), 4 Feb. 1897, ? Penzig, n. 166, PAD S00023, lectotype designated by Samuels et al. (1990).

Notes — *Nectria tjibodensis* was placed in synonymy with *Lanatonectria flavolanata* (Rosman et al. 1999), but presently the sexual genus *Lanatonectria* is considered a synonym of the asexual genus *Sarcopodium* (Lombard et al. 2015). *Lanatonectria flavolanata* was recombined as *Sarcopodium flavolanatum* and, as a consequence, *Nectria tjibodensis* a synonym of *S. flavolanatum*. However, the present ITS phylogenetic analysis has placed *Nectria tjibodensis* close to *Lanatonectria*...
floucculenta, Sarcopodium macalpinei (= asexual morph of L. floucculenta), S. circinostetiferum and Nectria sordeens (see above) in a strongly supported clade (BPP = 0.98, MLB 98 %), and distant from the ITS sequences of S. flavolanaunt (Fig. 3). Lanatoneectria floucculenta was synonymised under Sarcopodium macalpinei, but recently the name Sarcopodium flocculentum was proposed by Pennycook & Kirk (2019). The morphology of the lectotype of Nectria tijbodensis fits well with the detailed morphological description of Lanatoneectria floucculenta (Rossman et al. 1999). Morphological and molecular analyses suggest that Nectria tijbodensis should be considered a synonym of Sarcopodium flocculentum, and not of S. flavolanaunt as previously supposed. Based on the earliest available legitimate name, Nectria tijbodensis should be recombined as Sarcopodium tijbodense and S. flocculentum treated as a later synonym. Sarcopodium circinoisetiferum, of which only the asexual morph is known, could be an additional synonym of S. tijbodense (Fig. 3).

Nectria tijbodensis var. crebrior

Sarcopodium vanillae (Petch) B. Sutton, Trans. Brit. Mycol. Soc. 76: 99. 1981 — Fig. 15f–j

Basionym: Actinostitibie vanillae Petch, Ann. Roy. Bot. Gard. (Peradeniya) 9: 327. 1925.

Synonym. Nectria tijbodensis var. crebrior Sacc., Syll. Fung. 14: 636. 1899.

Sexual morph. Perithecia solitary or gregarious, partially immersed in an erumpent stroma, globose to subglobose with a papilla in the middle of the perithecial apex, red-orange, 260–310 µm diam (n = 10); dark red in 3 % KOH and yellow in 100 % LA. Ascii clavate (38.5–39.2–42–44.8–(44.9) × (6.2–)6.3–6.9–7.5(–7.6) µm (n = 4), 8-spored, ascospores biseriate above and uniseriate below. Ascospores ellipsoid to fusoid, (9.5–)10.4–11.2–11.9–(12.8) × (2.5–)2.8–3.1–3.4–(3.8) µm, Q = (3.1–)3.3–3.6–3.9(–4.3), Qav = 3.6 (n = 41), 1-septate, equally subdivided in two cells, not or slightly constricted at the septum, hyaline, striate.

Specimen examined. Indonesia, Java, Tjibodas, on bark (host not known), 6 Mar. 1897, ? Penzig, n. 123, PAD S00024, holotype.

Notes — Nectria tijbodensis var. crebrior was synonymised under Lanatoneectria flocculentum (= S. macalpinei) (Samuels et al. 1990). The ITS phylogenetic analysis placed Nectria tijbodensis var. crebrior in a clade with Sarcopodium vanillae (Fig. 3). Sutton (1981) described Sarcopodium vanillae based on characters linked only to an asexual morph; however, recently the sexual morph of this species has been observed for the first time (Chaiwan et al. 2019). The perithecial features and the dimensions of ascii and ascospores of Nectria tijbodensis var. crebrior are very similar to Sarcopodium macalpinei in the detailed description provided by Samuels et al. (1990). The molecular analysis of the holotype (Fig. 1) confirms the taxonomic reclassification proposed by Rossman et al. (1999).

DISCUSSION

Type specimens preserved in fungaria have an extraordinary scientific value as they represent the only link between a taxonomic hypothesis and a scientific name. Therefore, the recovery of DNA barcodes from these old specimens and their inclusion in phylogenetic analyses may greatly contribute to the taxonomy of complex genera, such as Nectria, as they enable species names to be applied with absolute certainty (Puillandre et al. 2011, Leavitt et al. 2011). The ITS region, the universal barcode marker for fungi (Schoch et al. 2012), is the most used DNA barcode to obtain molecular information from old mycological material as only short DNA regions can be successfully obtained from degraded DNA (e.g., Lilimatainen et al. 2014). In addition, the multiplicity nature of the ITS region and the possibility to get ITS1 and ITS2 sequences separately increase the amplification/sequencing success (Larsson & Jacobsson 2004).

In this study, high-throughput sequencing was applied to obtain ITS sequences from 22 Nectria types (plus two non-types) and one nectria-like type, stored in Saccardo’s fungarium, to overcome the problems of the DNA fragmentation of the fungal samples and the presence of exogenous DNA contaminants (Forin et al. 2018). ITS1 and/or ITS2 sequences from 21 different types have been obtained using the MiSeq approach, confirming for eight specimens the current species name; while for eight (Nectria albofimbriata, N. ambiguа, N. ambiguа var. pallens, N. granuligera, N. peziza subsp. reyesiana, N. radians, Nectriella rufofusca

Hydropischaea rufofusca (Penz. & Sacc.) Rossman & Samuels, Mycologia 85: 702. 1993 — Fig. 13f–j

Basionym. Nectriella rufofusca Penz. & Sacc., Malagisia 11: 507. 1897.

Synonyms. Neohenningsia stellatula Koord., Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurw., ser.13: 164. 1907.

Nectria stellatula (Koord.) Höhn., Sitzungsb. Kaiserl. Akad. Wiss. Wien. Math.-Naturwiss. Cl., Abt. 1, 118: 819. 1909.

Neohenningsia brasiliensis Henn., Hedwigia 48: 102. 1908.

Nectria brasiliensis (Henn.) Höhn., Sitzungsb. Kaiserl. Akad. Wiss. Wien. Math.-Naturwiss. Cl., Abt. 1, 118: 1186. 1909.

Pseudoneohenningsia brasiliensis (Henn.) Weese, Sitzungsb. Kaiserl. Akad. Wiss. Wien. Math.-Naturwiss. Cl., Abt. 1, 125: 518. 1916.

Sexual morph. Perithecia solitary to gregarious, superficial, non-stromatic, globose, red brownish, 184–237 µm diam (n = 5); not changing colour in 3 % KOH and 100 % LA. Ascii clavate, (40.5–)41–42.6–44.3–(44.4) × (4.9–)5.2–6.1–7.2(–7.2) µm (n = 4), 8-spored, ascospores biseriate above and uniseriate below. Ascospores ellipsoid to fusoid, (10.8–)11.8–12.6–13.4(–14.7) × (2.6–)2.8–3.2–3.3(–3.3) µm, Q = (3.8–)3.9–4.2–4.5(–5), Qav = 4.2 (n = 31), equally subdivided in two biguttulate cells, 1-septate, not constricted at the septum, hyaline, smooth.

Specimen examined. Indonesia, Java, Tjibodas, on decaying leaf of Elletaria sp., 6 Feb. 1897, ? Penzig, n. 436, PAD S00025, holotype.

Notes — The holotype specimen was morphologically studied in 1992 by G.J. Samuels, as noted on the label associated with the sample (Fig. 13f). Samuels suggested a subsequent synonymy with Nectria brasiliensis. Nectriella rufofusca was described in Samuels et al. (1990) as Nectria brasiliensis (Rossman et al. 1999). It is now considered a member of the genus Hydropischaea (Bionectriaceae) with the name H. rufofusca. The morphology of Nectriella rufofusca fits with the detailed description provided by Samuels et al. (1990). The molecular analysis of the holotype (Fig. 1) confirms the taxonomic reclassification proposed by Rossman et al. (1999).
N. squamuligena and N. tjibodensis) and five (N. congesta, N. flageolitiana, N. phyllostachydis, N. sordescens and N. tjibodensis var. crebrior) new nomenclature combinations and synonymies have been proposed here. The importance of obtaining DNA sequences from type material is demonstrated here for those species previously placed in synonymy with other existing species or reclassified as member of other genera on the basis of morphological similarities, for which new nomenclature combinations are newly proposed. The presence of morphologically indistinguishable species is common in many fungal groups but, integrating morphological studies with molecular information, cryptic species are continuously discovered within already described morphological species (e.g., Salgado-Salazar et al. 2017). However, the opposite is also true. Taxa previously described and classified as distinct morphological species can actually be considered conspecific. For instance, here we have demonstrated the synonymy between species that were considered as distinct taxa (e.g., Nectria tjibodensis and Nectria sordescens). Therefore, the importance of combining molecular and morphological approaches is clearly demonstrated in fungal systematic studies.

For nectriaceous fungi alternative barcode markers to ITS have been proposed for a rapid and accurate species identification such as TUB2 or TEF3 (translation elongation factor 3) genes (Zhao et al. 2011, Zeng et al. 2012). Unfortunately, the amplification of more informative barcodes (e.g., 28S rDNA gene domains D1 and D2 or D3 and D4) from the specimens studied here was impossible due to the high level of DNA degradation. Despite the lack of additional useful molecular markers, our study demonstrates that the information obtained from the ITS region (also from a part of this region), combined with morphological observations, might be sufficient for a correct species identification.

These results highlight the possibility to obtain molecular information from fungarium specimens collected more than 100 years ago, which have not been maintained at optimal storage conditions for DNA preservation, and are exposed to possible exogenous DNA contaminants. The relevance of DNA data obtained from old type specimens to fungal taxonomy is clearly demonstrated. In addition, this study provides additional evidence of the scientific value of mycological collections as treasure troves of valuable genetic information, showing that the application of a high-throughput sequencing approach can be applied to historical collections with the aim to generate molecular data from taxonomically important fungal type specimens.

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