



# Illuminating type collections of nectriaceous fungi in Saccardo's fungarium

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**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. tjibodensis* 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. tjibodensis* and new synonymies for *N. congesta*, *N. flageoletiana*, *N. phyllostachydis*, *N. sordescens* and *N. tjibodensis* var. *crebrior* are proposed. Furthermore, the current classification is confirmed for *Nectria coronata*, *N. cyanostoma*, *N. dolichospora*, *N. illudens*, *N. leucotricha*, *N. mantuana*, *N. raripila* 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 ascumatal 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 infra-generic 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 1 000 species were described and classified in *Nectria* s.lat. (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 *Bionectriaceae* and *Nectriaceae* (Samuels 1976, Brayford et al. 2004, Lechat & Fournier 2017). The *Bionectriaceae* 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) (Rossman et al. 1999). Members of the *Nectriaceae* typically have orange to red perithecial walls turning dark red or purple in KOH and yellow in LA (Rossman et al. 1999, Schroers 2001). Taxonomic studies based on DNA sequence data confirmed not only the separation between *Bionectriaceae* 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, Hirooka et al. 2010, 2012, Lombard et al. 2015).

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 *Lasionectria* (*Bionectriaceae*; Rossman et al. 1999) and *Dialonectria* (*Nectriaceae*; Gräfenhan 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.lat. is represented by over 111 different species comprising 434 specimens (Gola 1930). In addition, a further nine *Nectria* s.lat. species (15 specimens) were found under the genus *Polystigma* (Gola 1930). Among these, 38 *Nectria* s.lat. species, mainly from paleotropical areas and some represented by multiple specimens, were marked as *T!* or *cT!*, 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 and molecular description of new species. Others (seven) were described and named by contemporary

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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× and 1000× 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  $Q_{av}$  (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  $\mu$ L 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  $\mu$ L including 5  $\mu$ L of 5X Wonder Taq reaction buffer (5 mM dNTPs, 15 mM MgCl<sub>2</sub>; EuroClone), 0.5  $\mu$ L of bovine serum albumin (BSA, 10 mg/mL), 0.5  $\mu$ L each of two primers (10  $\mu$ M), 0.5  $\mu$ L of Wonder Taq (5 U/ $\mu$ L), 2  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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 × 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

**Table 1** List and details of *Bionectriaceae* and *Nectriaceae* specimens used in the ITS phylogenetic analyses.

Original identification <sup>1</sup> (GenBank/Herbarium)	Current name	Herbarium/ Voucher/Isolate	Origin	Reference(s)	GenBank accession numbers		
					ITS1	ITS2	ITS
<i>Bionectria apocyni</i>	<i>Clonostachys apocyni</i>	CBS 130.87	USA	Schroers (2001)	-	-	AF210688
<i>Bionectria aureofulvella</i>	<i>Clonostachys aureofulvella</i>	CBS 195.93	New Zealand	Schroers (2001)	-	-	AF358226
<i>Bionectria byssicola</i>	<i>Clonostachys farinosa</i>	CBS 914.97	Uganda	Schroers (2001)	-	-	AF358252
<i>Bionectria capitata</i>	<i>Clonostachys capitata</i>	CBS 218.93, isotype	Japan	Schroers (2001)	-	-	AF358240
<i>Bionectria compactiuscula</i>	<i>Clonostachys compactiuscula</i>	CBS 592.93	France	Schroers (2001)	-	-	AF358247
	<i>Clonostachys compactiuscula</i>	CBS 913.97, holotype	USA	Schroers (2001)	-	-	AF358245
<i>Bionectria coronata</i>	<i>Clonostachys buxi</i>	CBS 696.93	France	Schroers (2001)	-	-	AF210667
<i>Bionectria epichloe</i>	<i>Clonostachys epichloe</i>	CBS 101037, isotype	Japan	Schroers (2001)	-	-	AF210675
<i>Bionectria grammicospora</i>	<i>Clonostachys grammicospora</i>	CBS 209.93, holotype	French Guiana	Schroers (2001)	-	-	AF210678
<i>Bionectria grammicosporopsis</i>	<i>Clonostachys grammicosporopsis</i>	CBS 102834	Australia	Schroers (2001)	-	-	AF358256
<i>Bionectria levigata</i>	<i>Clonostachys levigata</i>	CBS 948.97	France	Schroers (2001)	-	-	AF210680
<i>Bionectria lucifer</i>	<i>Clonostachys lucifer</i>	CBS 100008, isotype	USA	Schroers (2001)	-	-	AF210683
<i>Bionectria oblongispora</i>	<i>Clonostachys oblongispora</i>	CBS 100285, isotype	Japan	Schroers (2001)	-	-	AF358248
<i>Bionectria ochroleuca</i>	<i>Clonostachys rosea</i>	CBS 193.94	Venezuela	Schroers (2001)	-	-	AF210686
	<i>Clonostachys rosea</i>	CBS 122171	Spain	Romón et al. (2008)	-	-	DQ674381
<i>Bionectria pityrodes</i>	<i>Clonostachys pityrodes</i>	CBS 102033, isotype	Mauritius	Schroers (2001)	-	-	AF210672
<i>Bionectria pseudochoroleuca</i>	<i>Clonostachys pseudochoroleuca</i>	CBS 192.94	French Guiana	Schroers (2001)	-	-	AF358238
<i>Bionectria pseudostriata</i>	<i>Clonostachys pseudostriata</i>	CBS 119.87	Indonesia	Schroers (2001)	-	-	AF358251
<i>Bionectria ralfsii</i>	<i>Clonostachys ralfsii</i>	CBS 129.87	New Zealand	Schroers (2001)	-	-	AF210676
<i>Bionectria rossmaniae</i>	<i>Clonostachys rossmaniae</i>	CBS 210.93	French Guiana	Schroers (2001)	-	-	AF358227
<i>Bionectria samuelsii</i>	<i>Clonostachys samuelsii</i>	CBS 699.97, isotype	Venezuela	Schroers (2001)	-	-	AF358236
<i>Bionectria sesquicillii</i>	<i>Clonostachys sesquicillii</i>	CBS 180.88, isotype	Venezuela	Schroers (2001)	-	-	AF210666
<i>Bionectria setosa</i>	<i>Clonostachys setosa</i>	CBS 834.91	Guyana	Schroers (2001)	-	-	AF210670
<i>Bionectria solani</i>	<i>Clonostachys solani</i>	CBS 101924	Cuba	Schroers (2001)	-	-	AF358232
<i>Bionectria sporodochialis</i>	<i>Clonostachys solani</i>	CBS 101921, isotype	Jamaica	Schroers (2001)	-	-	AF210685
<i>Bionectria vesiculosa</i>	<i>Clonostachys sporodochialis</i>	CBS 101921, isotype	USA	Schroers (2001)	-	-	AF210685
<i>Bionectria zelandiaenovae</i>	<i>Clonostachys vesiculosa</i>	HMAS 183151, holotype	China	Luo & Zhuang (2010)	-	-	NR_119828
	<i>Clonostachys zelandiaenovae</i>	CBS 100979, isotype	New Zealand	Schroers (2001)	-	-	AF358229
<i>Chaetopsina fulva</i>	<i>Chaetopsina fulva</i>	CBS 142.56, type	Italy	Lombard et al. (2015)	-	-	NR_145061
<i>Chaetopsina penicillata</i>	<i>Chaetopsina penicillata</i>	CBS 608.92, type	New Zealand	Gräfenhan et al. (2011)	-	-	NR_154780
<i>Chaetopsina pini</i>	<i>Chaetopsina pini</i>	CBS 136443, holotype	Thailand	Crous et al. (2013)	-	-	NR_137822
<i>Clonostachys pinicola</i>	<i>Chaetopsina pini</i>	CBS 136444, holotype	Thailand	Crous et al. (2013)	-	-	NR_137823
<i>Clonostachys agrawalii</i>	<i>Clonostachys agrawalii</i>	CBS 533.81, neotype of <i>Glodiadium agrawalii</i>	India	Schroers (2001)	-	-	AF358241
<i>Clonostachys buxi</i>	<i>Clonostachys buxi</i>	CBS 696.93	France	Lombard et al. (2015)	-	-	KM231840
<i>Clonostachys byssicola</i>	<i>Clonostachys farinosa</i>	CBS 364.78, isotype	Venezuela	Vu et al. (2019)	-	-	MH861151
	<i>Clonostachys sp.</i>	CML 2404	Brazil	Abreu et al. (2014)	-	-	KC806271
<i>Clonostachys byssicola</i>	<i>Clonostachys farinosa</i>	CML 2510	Venezuela	Abreu et al. (2014)	-	-	KJ499907
<i>Clonostachys candelabrum</i>	<i>Clonostachys farinosa</i>	CBS 504.67	Netherlands	Schroers (2001)	-	-	AF210668
<i>Clonostachys chlorina</i>	<i>Clonostachys candelabrum</i>	CBS 287.90, holotype	Brazil	Schroers (2001)	-	-	NR_137651
<i>Clonostachys compactiuscula</i>	<i>Clonostachys compactiuscula</i>	CBS 729.87	Germany	Schroers (2001)	-	-	AF358242
<i>Clonostachys divergens</i>	<i>Clonostachys divergens</i>	CBS 967.73, holotype	Germany	Schroers (2001)	-	-	NR_137532
<i>Clonostachys ericamporesiana</i>	<i>Clonostachys ericamporesiana</i>	MFLUCC 17-2620, holotype	Thailand	Hyde et al. (2020)	-	-	MN699132
<i>Clonostachys ericamporesii</i>	<i>Clonostachys ericamporesii</i>	MFLUCC 19-0486, holotype	Thailand	Hyde et al. (2020)	-	-	MN699133
<i>Clonostachys intermedia</i>	<i>Clonostachys intermedia</i>	CBS 508.82, holotype	Netherlands	Schroers (2001)	-	-	NR_137652
<i>Clonostachys kowhai</i>	<i>Clonostachys kowhai</i>	CBS 461.95, holotype	New Zealand	Schroers (2001)	-	-	NR_154748
<i>Clonostachys miodochialis</i>	<i>Clonostachys miodochialis</i>	CBS 997.69, holotype	Netherlands	Schroers (2001)	-	-	NR_137649
<i>Clonostachys phyllophila</i>	<i>Clonostachys phyllophila</i>	CBS 921.97, holotype	France	Schroers (2001)	-	-	NR_137531
<i>Clonostachys pityrodes</i>	<i>Clonostachys pityrodes</i>	CBS 126394	Sri Lanka	Vu et al. (2019)	-	-	MH864280

Table 1 (cont.)

Original identification <sup>1</sup> (GenBank/Herbarium)	Current name	Herbarium/ Voucher/Isolate	Reference(s)	GenBank accession numbers		
				ITS1	ITS2	ITS
<i>Clonostachys rhizophaga</i>	<i>Clonostachys rhizophaga</i>	CBS 202.37, holotype	Schroers (2001)	-	-	AF358225
<i>Clonostachys rosea</i>	<i>Clonostachys rosea</i>	CBS 127642	Vu et al. (2019)	-	-	MH864650
<i>Clonostachys rosea</i>	<i>Clonostachys rosea</i>	CBS 136.68	Vu et al. (2019)	-	-	MH859090
<i>Clonostachys setosa</i>	<i>Clonostachys setosa</i>	CBS 917.97, neotype of <i>Sesquicillium setosum</i>	Schroers (2001)	-	-	NR_154746
<i>Clonostachys subquaternata</i>	<i>Clonostachys subquaternata</i>	CBS 100003, isotype	<b>This study</b>	-	-	<b>MT537603</b>
<i>Clonostachys wenpingii</i>	<i>Clonostachys wenpingii</i>	HMAS 172156, holotype	Luo & Zhuang (2007)	-	-	NR_119651
<i>Cosmospora coccinea</i>	<i>Cosmospora coccinea</i>	CBS 341.70, type of <i>Verticillium olivaceum</i>	Gräfenhan et al. (2011)	-	-	HQ897827
<i>Cosmospora cymosa</i>	<i>Cosmospora cymosa</i>	CBS 762.69, isotype	Gräfenhan et al. (2011)	-	-	NR_111605
<i>Cyanonectria buxi</i>	<i>Cyanonectria buxi</i>	CBS 125551, epitype	Schroers et al. (2011)	-	-	NR_145049
<i>Dialonectria episphaeria</i>	<i>Dialonectria episphaeria</i>	CBS 125494	Vu et al. (2019)	-	-	MH863609
<i>Dialonectria ulivolea</i>	<i>Dialonectria ulivolea</i>	CBS 322.31	Vu et al. (2019)	-	-	MH855229
	<i>Dialonectria ulivolea</i>	CBS 125493	Gräfenhan et al. (2011)	-	-	KM231821
<i>Fusarium illudens</i>	<i>Necospora illudens</i>	CBS 119605	Lombard et al. (2015)	-	-	KM231806
<i>Fusarium merismoides</i>	<i>Fusicolla merismoides</i>	CBS 186.34	Vu et al. (2019)	-	-	MH855482
<i>Fusicolla acetilera</i>	<i>Fusicolla acetilera</i>	IMI 181488, ex-type of <i>Fusarium merismoides</i> var. <i>acetilereum</i>	Gräfenhan et al. (2011)	-	-	NR_111603
<i>Fusicolla aqueductuum</i>	<i>Fusicolla aqueductuum</i>	CBS 265.36	Vu et al. (2019)	-	-	MH855795
<i>Fusicolla matuoi</i>	<i>Fusicolla matuoi</i>	CBS 581.78	Vu et al. (2019)	-	-	MH861172
<i>Fusicolla melogrammae</i>	<i>Fusicolla melogrammae</i>	CBS 141092, holotype	Crous et al. (2016)	-	-	NR_155096
<i>Fusicolla ossicola</i>	<i>Fusicolla ossicola</i>	CBS 140161, holotype	Lechat & Rossman (2017)	-	-	MF628022
<i>Fusicolla septimanifimiscientiae</i>	<i>Fusicolla septimanifimiscientiae</i>	CBS 144935, ex-holotype		-	-	MK069422
<i>Fusicolla violacea</i>	<i>Fusicolla violacea</i>	CBS 634.76, ex-holotype	Lombard et al. (2015)	-	-	NR_137617
<i>Geejyessia cellidicola</i>	<i>Geejyessia cellidicola</i>	CBS 125502, holotype	Schroers et al. (2011)	-	-	NR_137580
<i>Geejyessia cicatricum</i>	<i>Geejyessia cicatricum</i>	CBS 125552	Schroers et al. (2011)	-	-	HQ728145
<i>Hydropisphaera bambusicola</i>	<i>Hydropisphaera fusigera</i>	CBS 124147, holotype	Schoch et al. (2014)	-	-	NR_119761
<i>Hydropisphaera erubescens</i>	<i>Hydropisphaera erubescens</i>	HMAS 91779	Luo & Zhuang (2007)	-	-	FJ969800
<i>Hydropisphaera fungicola</i>	<i>Hydropisphaera fungicola</i>	BPI 878275, holotype	Rossman et al. (2008)	-	-	NR_137701
<i>Hydropisphaera multiloculata</i>	<i>Hydropisphaera multiloculata</i>	CBS 339.77, type	Vu et al. (2019)	-	-	NR_160155
<i>Hydropisphaera peziza</i>	<i>Hydropisphaera peziza</i>	CBS 296.65	Vu et al. (2019)	-	-	MH858575
<i>Ijuhya chilensis</i>	<i>Ijuhya chilensis</i>	CBS 102803	Ashrafi et al. (2017)	-	-	KY607538
<i>Ijuhya favellana</i>	<i>Ijuhya favellana</i>	CBS 133850	Ashrafi et al. (2017)	-	-	KY607541
<i>Ijuhya parilis</i>	<i>Ijuhya parilis</i>	CBS 136677	Ashrafi et al. (2017)	-	-	KY607543
<i>Lanatonectria flocculenta</i>	<i>Sarcopodium tjobodense</i>	MAFF 2414.13	Hirooka et al. (2012)	-	-	JF-832657
<i>Lasionectria antillana</i>	<i>Lasionectria antillana</i>	CBS 122797	Ashrafi et al. (2017)	-	-	KY607537
<i>Lasionectria hihorstii</i>	<i>Lasionectria hihorstii</i>	CBS 144627, ex-holotype	Vu et al. (2019)	-	-	NR_161154
<i>Lasionectria krabiense</i>	<i>Lasionectria krabiense</i>	MFLUCC 15-0673		-	-	MH388352
<i>Lasionectria lecanodes</i>	<i>Lasionectria lecanodes</i>	NE322	Vu et al. (2019)	-	-	MH393445
<i>Lasionectria mantuana</i>	<i>Lasionectria sp.</i>	A.R. 4029	Chaverri et al. (2011)	-	-	HM484858
<i>Lasionectria marigotensis</i>	<i>Lasionectria marigotensis</i>	CBS 131606, ex-holotype	Lechat (2013)	-	-	KR105612
<i>Lasionectria martinicensis</i>	<i>Lasionectria martinicensis</i>	CBS 129746	Vu et al. (2019)	-	-	MH865378
<i>Lasionectria oenanthicola</i>	<i>Lasionectria boothii</i>	CBS 129747	Ashrafi et al. (2017)	-	-	KY607542
<i>Macroconia leptosphaeriae</i>	<i>Macroconia leptosphaeriae</i>	CBS 100001	Gräfenhan et al. (2011)	-	-	HQ897810
<i>Macroconia papilionacearum</i>	<i>Macroconia papilionacearum</i>	CBS 125495	Gräfenhan et al. (2011)	-	-	HQ897826
<i>Mariannaea camptospora</i>	<i>Mariannaea camptospora</i>	CBS 120801	Lombard et al. (2015)	-	-	KM231753
<i>Mariannaea catenulatae</i>	<i>Mariannaea catenulatae</i>	CBS 491.62, type of <i>Nectria chaetopsinae-catenulatae</i>	Lombard et al. (2015)	-	-	KM231752
<i>Mariannaea humicola</i>	<i>Mariannaea humicola</i>	CBS 102628	Lombard et al. (2015)	-	-	KM231756
<i>Mariannaea pinicola</i>	<i>Mariannaea pinicola</i>	CBS 745.88, holotype of <i>Nectria mariannaeae</i>	Lombard et al. (2015)	-	-	KM231754

Table 1 (cont.)

Original identification <sup>1</sup> (GenBank/Herbarium)	Current name	Herbarium/ Voucher/Isolate	Reference(s)	GenBank accession numbers		
				ITS1	ITS2	ITS
<i>Mariannaea samuelsii</i>	<i>Mariannaea samuelsii</i>	CBS 746.88	Lombard et al. (2015)	–	–	KM231757
<i>Microcera cocophila</i>	<i>Microcera cocophila</i>	CBS 310.34	Gräfenhan et al. (2011)	–	–	HQ897794
<i>Microcera larvarum</i>	<i>Microcera larvarum</i>	CBS 738.79	Lombard et al. (2015)	–	–	KM231825
<i>Microcera rubra</i>	<i>Microcera rubra</i>	CBS 638.76, isotype of <i>Fusarium larvarum</i> var. <i>rubrum</i>	Gräfenhan et al. (2011)	–	–	NR_111604
<b><i>Nectria albofimbriata</i></b>	<i>Lasionectria albofimbriata</i> (Penz. & Sacc.) Forin & Vizzini	PAD S00001: herbarium Saccardo, n. 436a, lectotype	<b>This study</b>	<b>MT554896</b>	<b>MT554874</b>	–
	<i>Lasionectria albofimbriata</i> (Penz. & Sacc.) Forin & Vizzini	PAD S00002: herbarium Saccardo, n. 172, syntype	<b>This study</b>	<b>MT554897</b>	<b>MT554875</b>	–
<b><i>Nectria ambigua</i></b>	<i>Clonostachys ambigua</i> (Penz. & Sacc.) Forin & Vizzini	PAD S00003: herbarium Saccardo, n. 119, holotype	<b>This study</b>	<b>MT554898</b>	–	–
	<i>Clonostachys pallens</i> (Penz. & Sacc.) Forin & Vizzini	PAD S00004: herbarium Saccardo, n. 452 ex p., lectotype	<b>This study</b>	<b>MT554899</b>	<b>MT554876</b>	–
	<i>Nectria asiatica</i>	MAFF 241439, ex-holotype	Hirooka et al. (2011)	–	–	NR_137760
	<i>Nectria balansae</i>	A.R. 4446	Hirooka et al. (2011)	–	–	HIM484552
	<i>Nectria berberidicola</i>	CBS 128669, holotype	Yu et al. (2019)	–	–	NR_160248
	<i>Nectria cinnabarina</i>	CBS 279.48	Lee et al. (2000)	–	–	AF163025
	<i>Nectria congesa</i>	PAD S00005: herbarium Saccardo, lectotype	<b>This study</b>	<b>MT554900</b>	<b>MT554877</b>	–
	<i>Nectria coronata</i>	PAD S00006: herbarium Saccardo, n. 452 ex p., holotype	<b>This study</b>	<b>MT554912</b>	<b>MT554889</b>	–
	<i>Cyanonectria cyanostoma</i>	PAD S00007: herbarium Saccardo, n. 32, syntype	<b>This study</b>	<b>MT554913</b>	<b>MT554890</b>	–
	<i>Cyanonectria cyanostoma</i>	CBS 101734	Samuels et al. (2009)	–	–	FJ474076
	<i>Nectria dematiosa</i>	CBS 126570, epitype	Vu et al. (2019)	–	–	NR_163554
	<i>Nectria dolichospora</i>	PAD S00008: herbarium Saccardo, n. 434, lectotype	<b>This study</b>	<b>MT554901</b>	<b>MT554878</b>	–
	<i>Hydropisphaera dolichospora</i>	PAD S00009: herbarium Saccardo, n. 442, syntype	<b>This study</b>	<b>MT554902</b>	<b>MT554879</b>	–
	<i>Nectria flageoletiana</i>	PAD S00010: herbarium Saccardo, holotype	<b>This study</b>	<b>MT554903</b>	<b>MT554880</b>	–
	<i>Nectria granuligera</i>	PAD S00011: herbarium Saccardo, n. 1082, lectotype	<b>This study</b>	<b>MT554904</b>	<b>MT554881</b>	–
	<i>Nectria illudens</i>	PAD S00012: herbarium Saccardo, neotype	<b>This study</b>	<b>MT554914</b>	<b>MT554891</b>	–
	<i>Nectria leucotricha</i>	G.J.S. 85-67	Hirooka et al. (2012)	–	–	JF-832660
	<i>Nectria mantuana</i>	PAD S00013: herbarium Saccardo, n. 150, lectotype	<b>This study</b>	<b>MT554905</b>	<b>MT554882</b>	–
	<i>Nectria nigrescens</i>	PAD S00014: herbarium Saccardo, holotype	<b>This study</b>	<b>MT554906</b>	<b>MT554883</b>	–
	<b><i>Nectria peziza</i> subsp. <i>reyesiana</i></b>	A.R. 4268	Hirooka et al. (2012)	–	–	JF-832634
	<i>Nectria phyllostachydis</i>	PAD S00015: herbarium Saccardo, n. 1609, holotype	<b>This study</b>	<b>MT554915</b>	<b>MT554892</b>	–
	<i>Nectria radians</i>	PAD S00016: herbarium Saccardo, isolectotype	<b>This study</b>	<b>MT554907</b>	<b>MT554884</b>	–
	<i>Nectria raripila</i>	PAD S00017: herbarium Saccardo, n. 86, holotype	<b>This study</b>	<b>MT554916</b>	–	–
	<i>Nectria sordescens</i>	PAD S00018: herbarium Saccardo, n. 923, holotype	<b>This study</b>	<b>MT554917</b>	<b>MT554893</b>	–
	<i>Nectria squamuligera</i>	PAD S00019: herbarium Saccardo, holotype	<b>This study</b>	<b>MT554918</b>	<b>MT554894</b>	–
	<i>Nectria squamuligera</i>	PAD S00020: herbarium Saccardo, lectotype	<b>This study</b>	<b>MT554908</b>	<b>MT554885</b>	–
	<i>Nectria tibodensis</i>	PAD S00021: herbarium Saccardo	<b>This study</b>	<b>MT554909</b>	<b>MT554886</b>	–
	<i>Nectria tibodensis</i>	PAD S00022: herbarium Saccardo, n. 318	<b>This study</b>	<b>MT554910</b>	<b>MT554887</b>	–
	<i>Nectria tibodensis</i>	PAD S00023: herbarium Saccardo, n. 166, lectotype	<b>This study</b>	<b>MT554919</b>	–	–
	<i>Nectria tibodensis</i> var. <i>crebrior</i>	PAD S00024: herbarium Saccardo, n. 123, holotype	<b>This study</b>	<b>MT554920</b>	<b>MT554895</b>	–

Table 1 (cont.)

Original identification <sup>1</sup> (GenBank/Herbarium)	Current name	Herbarium/ Voucher/Isolate	Origin	Reference(s)	GenBank accession numbers		
					ITS1	ITS2	ITS
<i>Nectriella noliniae</i>	<i>Nectriella noliniae</i>	CBS 110134	USA	Vu et al. (2019)	–	–	MH862853
<i>Nectriella pironii</i>	<i>Nectriella pironii</i>	CBS 171.75	USA	Vu et al. (2019)	–	–	MH860907
<b><i>Nectriella rufufusca</i></b>	<b><i>Hydrophisphaera rufufusca</i></b>	PAD 500025; herbarium Saccardo, n. 436, holotype	Indonesia, Java	<b>This study</b>	<b>MT554911</b>	<b>MT554888</b>	–
<i>Nectriopsis exigua</i>	<i>Nectriopsis rexiana</i>	CBS 305.70	England	Vu et al. (2019)	–	–	MH859679
<i>Nectriopsis fuliginicola</i>	<i>Nectriopsis fuliginicola</i>	CBS 400.82, ex-holotype	Russia	Zare & Gams (2016)	–	–	NR_154234
<i>Nectriopsis lindauiana</i>	<i>Nectriopsis lindauiana</i>	CBS 897.70, ex-neotype	Germany	Zare & Gams (2016)	–	–	NR_154235
<i>Nectriopsis rexiana</i>	<i>Nectriopsis rexiana</i>	CBS 248.70	Germany	Zare & Gams (2016)	–	–	KU382177
<i>Nectriopsis violacea</i>	<i>Nectriopsis violacea</i>	CBS 849.70	Germany	Vu et al. (2019)	–	–	MH859978
<i>Neocosmospora croci</i>	<i>Neocosmospora maritii</i>	CPC 27186, ex-holotype	Italy	Sandoval-Denis et al. (2017)	–	–	NR_163290
<i>Neocosmospora macrospora</i>	<i>Neocosmospora macrospora</i>	CPC 28191, ex-holotype	Italy	Sandoval-Denis et al. (2017)	–	–	NR_163291
<i>Neocosmospora ramosa</i>	<i>Neocosmospora lichenicola</i>	CBS 509.63	Brazil	Lombard et al. (2015)	–	–	KM231802
<i>Neocosmospora rubicola</i>	<i>Neocosmospora solani</i>	CBS 320.73	Sudan	Lombard et al. (2015)	–	–	KM231799
<i>Protocreopsis freycinetiae</i>	<i>Protocreopsis freycinetiae</i>	CBS 573.76, isotype	New Zealand	Vu et al. (2019)	–	–	MH861003
<i>Protocreopsis phormiicola</i>	<i>Protocreopsis phormiicola</i>	CBS 567.76, type	New Zealand	Vu et al. (2019)	–	–	MH861001
<i>Pseudocosmospora eutypae</i>	<i>Pseudocosmospora eutypae</i>	BPI 884164, holotype	France	Herrera et al. (2013)	–	–	NR_158889
<i>Pseudocosmospora eutypellae</i>	<i>Pseudocosmospora eutypellae</i>	CBS 133966, ex-holotype	USA	Herrera et al. (2013)	–	–	NR_158888
<i>Pseudocosmospora joca</i>	<i>Pseudocosmospora joca</i>	CBS 133967, ex-epitype	USA	Herrera et al. (2013)	–	–	NR_158891
<i>Pseudocosmospora rogersonii</i>	<i>Pseudocosmospora rogersonii</i>	BPI 1107121, ex-holotype	USA	Herrera et al. (2013)	–	–	NR_154295
<i>Pseudocosmospora vilfori</i>	<i>Pseudocosmospora rogersonii</i>	CBS 133971, ex-epitype	Argentina	Herrera et al. (2013)	–	–	NR_158890
<i>Pseudonectria buxi</i>	<i>Pseudonectria buxi</i>	CBS 125483	Spain	Gräfenhan et al. (2011)	–	–	HQ897800
<i>Pseudonectria foliicola</i>	<i>Pseudonectria foliicola</i>	CBS 123190, ex-holotype	New Zealand	Lombard et al. (2015)	–	–	KM231776
<i>Sarcopodium circinatatum</i>	<i>Sarcopodium circinatatum</i>	CBS 100998	Brazil	Lombard et al. (2015)	–	–	KM231786
<i>Sarcopodium circinatatum</i>	<i>Sarcopodium circinatatum</i>	CBS 587.92	Costa Rica	Lombard et al. (2015)	–	–	KM231787
<i>Sarcopodium circinosetiferum</i>	? <i>Sarcopodium tjobodense</i> (Penz. & Sacc.) Forin & Vizzini	CBS 100252	Argentina	Lombard et al. (2015)	–	–	KM231781
<i>Sarcopodium flavolanatum</i>	<i>Sarcopodium flavolanatum</i>	CBS 128370	China	Lombard et al. (2015)	–	–	KM231784
<i>Sarcopodium macalpiniei</i>	<i>Sarcopodium flavolanatum</i>	CBS 112283	Ecuador	Lombard et al. (2015)	–	–	KM231785
<i>Sarcopodium vanillae</i>	<i>Sarcopodium tjobodense</i> (Penz. & Sacc.) Forin & Vizzini	CBS 115296	Hong Kong	Lombard et al. (2015)	–	–	KM231783
<i>Sarcopodium vanillae</i>	<i>Sarcopodium vanillae</i>	CBS 100582	Ecuador	Lombard et al. (2015)	–	–	KM231780
<i>Sarcopodium vanillae</i>	<i>Sarcopodium vanillae</i>	MFLU 17-2595	Thailand	Chaiwan et al. (2019)	–	–	MK608516
<i>Sarcopodium vanillae</i>	<i>Sarcopodium vanillae</i>	MFLU 17-2597	Thailand	Chaiwan et al. (2019)	–	–	MK685870
<i>Selinia pulchra</i>	<i>Selinia pulchra</i>	CBS 126654	Argentina	Vu et al. (2019)	–	–	MH864186
<i>Stilbocrea walteri</i>	<i>Stilbocrea walteri</i>	CBS 144938, holotype	Portugal	Voglmayr & Jaklitsch (2018)	–	–	NR_160063
<i>Stylonectria norvegica</i>	<i>Stylonectria norvegica</i>	CBS 139239, type	Puerto Rico	Schroers (2001)	–	–	NR_154415
<i>Thelonectria cidaria</i>	<i>Thelonectria cidaria</i>	CBS 132323, holotype	Costa Rica	Vu et al. (2019)	–	–	NR_164437
<i>Thelonectria coronalis</i>	<i>Thelonectria coronalis</i>	CBS 132337, ex-holotype	Taiwan	Vu et al. (2019)	–	–	NR_160259
<i>Thelonectria coronata</i>	<i>Thelonectria coronata</i>	CBS 132335	Venezuela	Salgado-Salazar et al. (2012)	–	–	JQ403316
<i>Thelonectria coronata</i>	<i>Thelonectria coronata</i>	CBS 132334	Taiwan	Salgado-Salazar et al. (2012)	–	–	JQ403342
<i>Thelonectria nodosa</i>	<i>Thelonectria nodosa</i>	CBS 132327, ex-holotype	USA	Vu et al. (2019)	–	–	NR_160260
<i>Thelonectria stemmata</i>	<i>Thelonectria stemmata</i>	CBS 112468, ex-holotype	Jamaica	Salgado-Salazar et al. (2012)	–	–	JQ403312
<i>Thelonectria stemmata</i>	<i>Thelonectria stemmata</i>	CBS 132336	Jamaica	Salgado-Salazar et al. (2012)	–	–	JQ403313
<i>Varicosporella aquatica</i>	<i>Varicosporella aquatica</i>	CBS 126103, ex-holotype	France	Lechat & Fournier (2015)	–	–	KP192669
<i>Varicosporellopsis aquatilis</i>	<i>Varicosporellopsis aquatilis</i>	CBS 140158, ex-holotype	France	Lechat & Fournier (2016)	–	–	KU233187
<i>Volutella ciliata</i>	<i>Volutella ciliata</i>	CBS 483.61	Canada	Lombard et al. (2015)	–	–	KM231770
<i>Volutella consors</i>	<i>Volutella consors</i>	CBS 139.79	Netherlands	Lombard et al. (2015)	–	–	KM231768
<i>Volutella rosea</i>	<i>Volutella rosea</i>	CBS 128258	USA	Lombard et al. (2015)	–	–	KM231769

<sup>1</sup> 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 <sup>1</sup> (GenBank/Herbarium)	Current name	Herbarium/ Voucher/Isolate	Origin	Reference(s)	GenBank accession numbers			
					<i>TUB2</i>	ITS	ITS1	ITS2
<i>Bionectria apocyni</i>	<i>Clonostachys apocyni</i>	CBS 130.87	New York	Schroers (2001)	AF358168	AF210688	–	–
<i>Bionectria aureofulvella</i>	<i>Clonostachys aureofulvella</i>	CBS 200.93		Schroers (2001)	AF358181	AF358226	–	–
<i>Bionectria byssicola</i>	<i>Clonostachys farinosa</i>	CBS 914.97	Uganda	Schroers (2001)	AF358151	AF358252	–	–
<i>Bionectria capitata</i>	<i>Clonostachys capitata</i>	CBS 218.93, isotype	Japan	Schroers (2001)	AF358188	AF358240	–	–
<i>Bionectria oblongispora</i>	<i>Clonostachys oblongispora</i>	CBS 100285, isotype	Japan	Schroers (2001)	AF358169	AF358248	–	–
<i>Bionectria ochroleuca</i>	<i>Clonostachys rosea</i>	CBS 406.95		Schroers (2001)	AF358167	AF358249	–	–
	<i>Clonostachys rosea</i>	CBS 194.57	USA	Schroers (2001)	AF358165	AF358237	–	–
	<i>Clonostachys rosea</i>	CBS 193.94	Venezuela	Schroers (2001)	AF358159	AF210686	–	–
<i>Bionectria pseudocholeuca</i>	<i>Clonostachys rosea</i>	CBS 376.55	Massachusetts	Schroers (2001)	AF358162	AF358239	–	–
<i>Bionectria pseudostrigata</i>	<i>Clonostachys pseudocholeuca</i>	CBS 192.94	French Guiana	Schroers (2001)	AF358171	AF358238	–	–
<i>Bionectria solani</i>	<i>Clonostachys pseudostrigata</i>	CBS 119.87	Sulawesi	Schroers (2001)	AF358183	AF358251	–	–
	<i>Clonostachys solani</i>	CBS 702.97	France	Schroers (2001)	AF358177	AF210687	–	–
	<i>Clonostachys solani</i>	CBS 752.68, neotype	Germany	Schroers (2001), Vu et al. (2019)	AF358221	MH859224	–	–
<i>Bionectria sporodochialis</i>	<i>Clonostachys sporodochialis</i>	CBS 101921, isotype	Puerto Rico	Schroers (2001)	AF358149	AF210685	–	–
<i>Bionectria zelandiaenovae</i>	<i>Clonostachys zelandiaenovae</i>	CBS 232.80, isotype	New Zealand	Schroers (2001)	AF358185	AF210684	–	–
<i>Clonostachys agrawalii</i>	<i>Clonostachys agrawalii</i>	CBS 533.81, neotype of <i>Gliocladium agarwalii</i>	India	Schroers (2001)	AF358187	AF358241	–	–
<i>Clonostachys byssicola</i>	<i>Clonostachys farinosa</i>	CML 0422	Brazil	Abreu et al. (2014)	KF871150	KC806266	–	–
	<i>Clonostachys sp.</i>	CML 1942	Brazil	Abreu et al. (2014)	KF871148	KC806267	–	–
	<i>Clonostachys farinosa</i>	CML 2309	Brazil	Abreu et al. (2014)	KF871149	KC806269	–	–
	<i>Clonostachys sp.</i>	CML 2311	Brazil	Abreu et al. (2014)	KF871152	KC806270	–	–
	<i>Clonostachys sp.</i>	CML 2404	Brazil	Abreu et al. (2014)	KF871153	KC806271	–	–
	<i>Clonostachys farinosa</i>	CBS 364.78, isotype	Venezuela	Schroers (2001), Vu et al. (2019)	AF358153	MH861151	–	–
<i>Clonostachys divergens</i>	<i>Clonostachys divergens</i>	CBS 967.73, holotype	Germany	Schroers (2001)	AF358191	NR_137532	–	–
<i>Clonostachys eriocamporesiana</i>	<i>Clonostachys eriocamporesiana</i>	MFLUCC-17-2620, holotype	Thailand	Hyde et al. (2020)	MN699132	MN699132	–	–
<i>Clonostachys kowhai</i>	<i>Clonostachys kowhai</i>	CBS 461.95, holotype	New Zealand	Schroers (2001)	AF358170	NR_154748	–	–
<i>Clonostachys pseudocholeuca</i>	<i>Clonostachys pseudocholeuca</i>	CML 018	Brazil	Abreu et al. (2014)	KF871159	KC806258	–	–
	<i>Clonostachys pseudocholeuca</i>	CML 0520	Brazil	Abreu et al. (2014)	KF871160	KC806260	–	–
	<i>Clonostachys pseudocholeuca</i>	CML 0824	Brazil	Abreu et al. (2014)	KF871162	KC806259	–	–
	<i>Clonostachys pseudocholeuca</i>	CML 1940	Brazil	Abreu et al. (2014)	KF871161	KC806262	–	–
	<i>Clonostachys pseudocholeuca</i>	CML 1983	Brazil	Abreu et al. (2014)	KF871163	KC806264	–	–
<i>Clonostachys rhizophaga</i>	<i>Clonostachys rhizophaga</i>	CML 1210	Brazil	Abreu et al. (2014)	KF871156	KC806272	–	–
	<i>Clonostachys rhizophaga</i>	CML 1984	Brazil	Abreu et al. (2014)	KF871155	KC806274	–	–
	<i>Clonostachys rhizophaga</i>	CML 2312	Brazil	Abreu et al. (2014)	KF871157	KC806275	–	–
	<i>Clonostachys rhizophaga</i>	CBS 202.37, holotype	USA	Schroers (2001)	AF358156	AF358225	–	–
<i>Clonostachys rosea</i>	<i>Clonostachys rhizophaga</i>	CBS 361.77	Switzerland	Schroers (2001)	AF358158	AF358228	–	–
	<i>Clonostachys rosea</i>	CML 0817	Brazil	Abreu et al. (2014)	KF871147	KC806254	–	–
	<i>Clonostachys rosea</i>	CML 1820	Brazil	Abreu et al. (2014)	KF871145	KC806256	–	–
	<i>Clonostachys rosea</i>	CML 2310	Brazil	Abreu et al. (2014)	KF871146	KC806257	–	–
<i>Clonostachys rosea f. catenulata</i>	<i>Clonostachys rosea</i>	CBS 710.86	Netherlands	Schroers (2001), Vu et al. (2019)	AF358161	MH862010	–	–
	<i>Clonostachys rosea f. catenulata</i>	CBS 221.72b	Germany	Schroers (2001)	AF358203	AF358234	–	–
	<i>Clonostachys rosea f. catenulata</i>	CBS 154.27, type of <i>Gliocladium catenulatum</i>	Utah	Schroers (2001)	AF358160	MH862010	–	–
	<i>Clonostachys rosea f. catenulata</i>	CBS 443.65	Wyoming	Schroers (2001)	AF358166	MH858662	–	–
<i>Clonostachys wenpingii</i>	<i>Clonostachys wenpingii</i>	HMAS 172156, holotype	China	Schroers (2001), Vu et al. (2019)	AF358166	MH858662	–	–
<i>Nectria ambigua</i>	<i>Clonostachys ambigua</i> (Penz. & Sacc.) Forin & Vizzini	PAD S00003: herbarium Saccardo, n. 119, holotype	Indonesia, Java	This study	–	–	–	–
<i>Nectria congesta</i>	<i>Clonostachys rosea</i>	PAD S00005: herbarium Saccardo, lectotype	Italy, Padova	This study	–	–	–	–
<i>Nectria granulifera</i>	<i>Clonostachys farinosa</i>	PAD S00011: herbarium Saccardo, n. 1082, lectotype	Sweden, Uppsala	This study	–	–	–	–
<i>Nectria phyllostachidis</i>	<i>Clonostachys rosea</i>	PAD S00016: herbarium Saccardo, isoelectotype	Japan	This study	–	–	–	–
<i>Nectria squamifera</i>	<i>Clonostachys farinosa</i>	PAD S00020: herbarium Saccardo, lectotype	Portugal, Coimbra	This study	–	–	–	–
	<i>Clonostachys farinosa</i>	PAD S00021: herbarium Saccardo	Italy, Padova	This study	–	–	–	–
	<i>Clonostachys farinosa</i>	PAD S00022: herbarium Saccardo, n. 318	Italy, Padova	This study	–	–	–	–

<sup>1</sup> Newly obtained sequences are reported in bold.

**Table 3** List and details of specimens used in the combined ITS and LSU phylogenetic analysis.

Original identification <sup>1</sup> (GenBank/Herbarium)	Current name	Herbarium/ Voucher/Isolate	Origin	Reference(s)	GenBank accession numbers			
					ITS	ITS1	ITS2	LSU
<i>Clonostachys buxi</i>	<i>Clonostachys buxi</i>	CBS 696.93	France	Lombard et al. (2015)	KM231840	-	-	KM231721
<i>Cosmospora coccinea</i>	<i>Cosmospora coccinea</i>	CBS 341.70, type of <i>Verticillium olivaceum</i>	Germany	Gräfenhan et al. (2011)	HQ897827	-	-	KM231692
<i>Dialonectria ulivolea</i>	<i>Dialonectria ulivolea</i>	CBS 125493	USA	Gräfenhan et al. (2011)	KM231821	-	-	KM231696
<i>Fusarium merismoides</i>	<i>Fusicolla merismoides</i>	CBS 186.34	Germany	Vu et al. (2019)	MH855482	-	-	MH866963
<i>Fusicolla acetifera</i>	<i>Fusicolla acetifera</i>	IMI 181488, ex-type of <i>Fusarium merismoides</i> var. <i>acetiflerum</i>	Japan	Gräfenhan et al. (2011), Schoch et al. (2014)	NR_111603	-	-	-
<i>Fusicolla aquaeductuum</i>	<i>Fusicolla aquaeductuum</i>	CBS 265.36	Netherlands	Vu et al. (2019)	MH855795	-	-	MH867303
<i>Fusicolla bharatavarshae</i>	<i>Fusicolla bharatavarshae</i>	PUF D71	India	Jones et al. (2019)	MK152510	-	-	MK152511
<i>Fusicolla gigantispora</i>	<i>Fusicolla gigantispora</i>	HKAS 101990	Thailand	Dayaratne et al. (2020)	MN047106	-	-	MN017876
<i>Fusicolla gigantispora</i>	<i>Fusicolla gigantispora</i>	MFLU 16-1206, holotype	Thailand	Dayaratne et al. (2020)	MN047104	-	-	MN017869
<i>Fusicolla gigantispora</i>	<i>Fusicolla gigantispora</i>	MFLU 17-2620	Thailand	Dayaratne et al. (2020)	MN047105	-	-	MN017870
<i>Fusicolla matuoi</i>	<i>Fusicolla matuoi</i>	CBS 581.78	Japan	Vu et al. (2019)	MH861172	-	-	MH872940
<i>Fusicolla melogrammae</i>	<i>Fusicolla melogrammae</i>	CBS 141092, holotype	England	Lechat & Rossman (2017)	NR_155096	-	-	NG_058275
<i>Fusicolla ossicola</i>	<i>Fusicolla ossicola</i>	CBS 140161, holotype	Belgium	Lechat & Rossman (2017)	MF628022	-	-	MF628021
<i>Fusicolla septimanifiniscientiae</i>	<i>Fusicolla septimanifiniscientiae</i>	CBS 144935, ex-holotype	Netherlands	Lechat & Rossman (2017)	MK069422	-	-	MK069418
<i>Fusicolla violacea</i>	<i>Fusicolla violacea</i>	CBS 634.76, ex-holotype	Iran	Lombard et al. (2015)	NR_137617	-	-	MH872787
<i>Macroconia leptosphaeriae</i>	<i>Macroconia leptosphaeriae</i>	CBS 100001	Netherlands	Gräfenhan et al. (2011)	HQ897810	-	-	KM231705
<i>Macroconia papilionacearum</i>	<i>Macroconia papilionacearum</i>	CBS 125495	USA	Gräfenhan et al. (2011)	HQ897826	-	-	KM231704
<i>Microcera coccophila</i>	<i>Microcera coccophila</i>	CBS 310.34	Italy	Gräfenhan et al. (2011)	HQ897794	-	-	KM231703
<i>Microcera rubra</i>	<i>Microcera rubra</i>	CBS 638.76, isotype of <i>Fusarium larvarum</i> var. <i>rubrum</i>	Iran	Gräfenhan et al. (2011), Schoch et al. (2014)	NR_111604	-	-	KM231702
<i>Nectria peziza</i> subsp. <i>reyesiana</i>	<i>Fusicolla reyesiana</i> (Sacc.) Forin & Vizzini	PAD S00015: herbarium Saccardo, n. 1609, holotype	Philippines	This study	-	MT554915	MT554892	-

<sup>1</sup> Newly obtained sequence is reported in bold.

(Forin et al. 2018). VSEARCH v. 2.3.4 (Rognes et al. 2016) was used for the sequences dereplication (removing the singletons) and for a *de novo* chimera check. The ITS1 and ITS2 regions were extracted using ITSx (Bengtsson-Palme et al. 2013). The clustering into Operational Taxonomic Units (OTUs) was performed using VSEARCH with a 99 % similarity cut-off, according to Vu et al. (2019) which suggested a threshold of 99 % to discriminate among filamentous fungal species. OTUs represented by fewer than 10 sequences were discarded and the UNITE+INSD dataset v. 8.0 (<https://unite.ut.ee>) for QIIME was used as reference for the taxonomic assignment of the remaining OTUs. The OTUs were also checked comparing them with the sequences deposited in GenBank, excluding uncultured/environmental sample sequences, using a BLASTn search (Altschul et al. 1997). The final OTU abundance table was created with VSEARCH, considering a 99 % of identity.

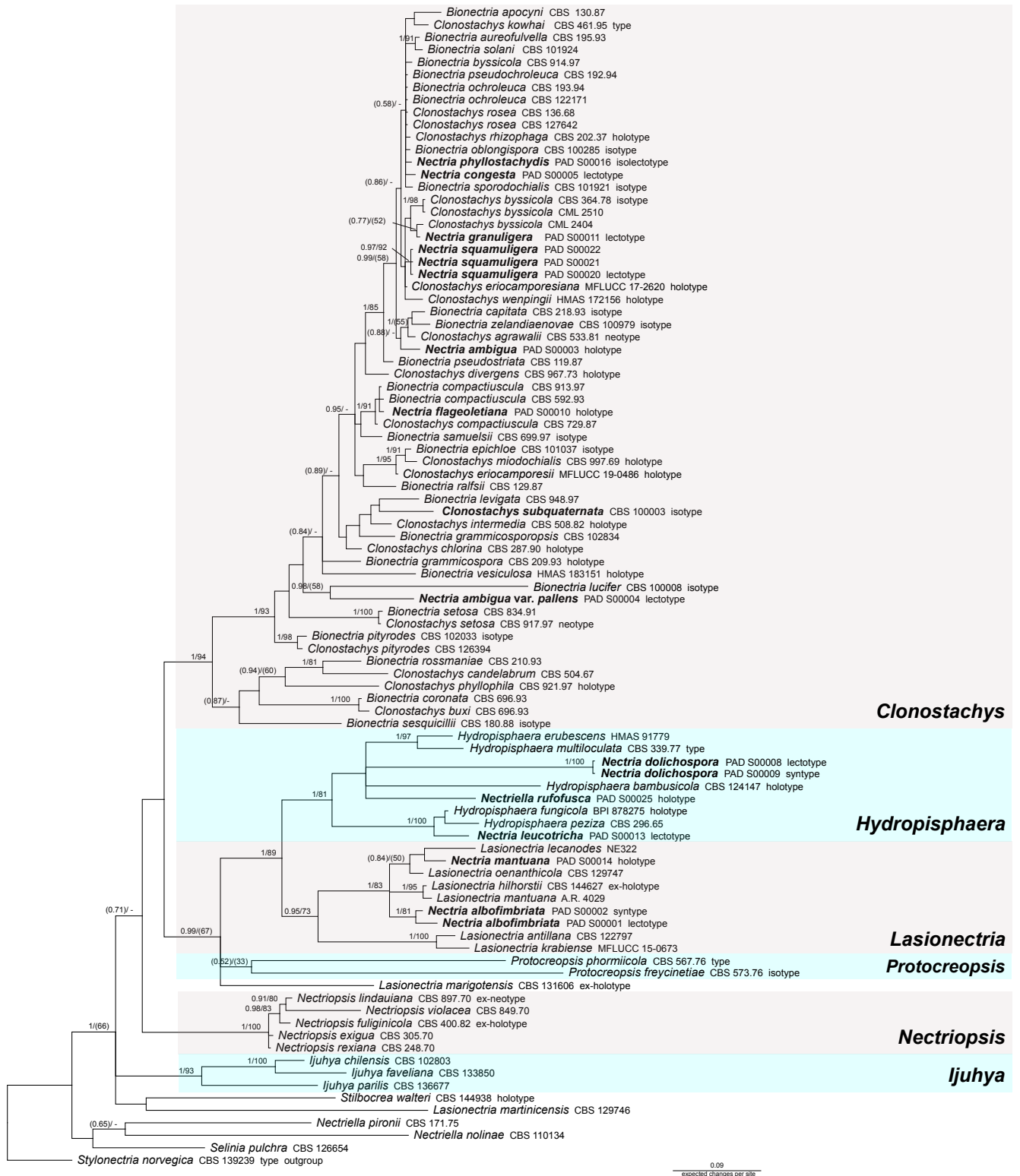
In order to assign the correct sequences to the analysed type specimens (discriminating between the target sequence and possible contaminations/coexisting species), the sequencing results were evaluated by taking into account: modern taxonomy of the specimens; information about asexual-sexual links reported in literature; notes reported on the sample labels; new morphological observations and previous morphological descriptions; number of sequences per OTUs and, in the case of specimens for which both ITS regions were amplified, comparing the taxonomic assignment obtained for ITS1 and ITS2.

### 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 *Bionectriaceae* and *Nectriaceae* (Schroers 2001, Chaverri et al. 2011, Gräfenhan et al. 2011, Hirooka et al. 2012, Lombard et al. 2015, Salgado-Salazar 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 one of the genus *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 (Katoch 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 was 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



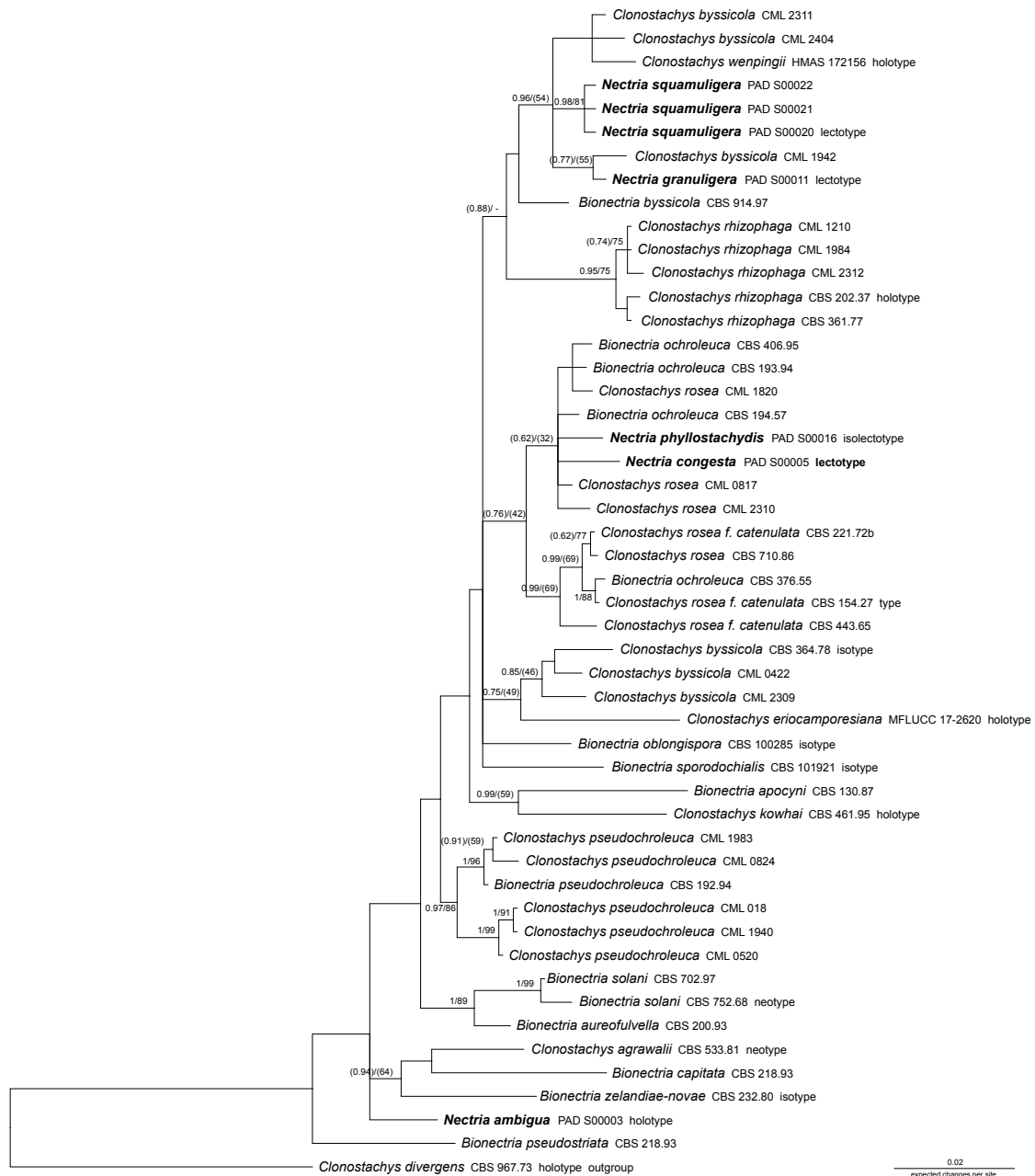
**Fig. 1** Phylogeny generated from Bayesian inference analysis based on ITS sequence data of species belonging to different *Bionectriaceae* genera. *Stylonectria norvegica* (*Nectriaceae*) is selected as the outgroup taxon. Bayesian posterior probability (BPP) values  $\geq 0.95$  (left) and maximum likelihood bootstrap (MLB) values  $\geq 70\%$  (right) are shown on the branches. Lower values are exceptionally represented inside parentheses. The scale bar indicates 0.09 changes. Newly obtained sequences are reported in **bold**.

novel rapid bootstrapping algorithm. Significance threshold was set  $\geq 0.95$  for posterior probability (BPP) and  $\geq 70\%$  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 *Hydropsisphaera* (*Bionectriaceae*), as *H. rufofusca* (Rossmann 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.



**Fig. 2** Phylogeny generated from Bayesian inference analysis based on combined *TUB2* and ITS sequence data of selected *Clonostachys* species. *Clonostachys divergens* is selected as the outgroup taxon. Bayesian posterior probability (BPP) values  $\geq 0.95$  (left) and maximum likelihood bootstrap (MLB) values  $\geq 70\%$  (right) are shown on the branches. Lower values are exceptionally represented inside parentheses. The scale bar indicates 0.02 changes. Newly obtained sequences are reported in **bold**.

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  $\mu\text{L}$  including 5  $\mu\text{L}$  of 5X Wonder Taq reaction buffer (5 mM dNTPs, 15 mM  $\text{MgCl}_2$ ; Euroclone), 0.5  $\mu\text{L}$  of bovine serum albumin (BSA, 10 mg/mL), 0.5  $\mu\text{L}$  each of two primers (10  $\mu\text{M}$ ), 0.5  $\mu\text{L}$  of Wonder Taq (5 U/ $\mu\text{L}$ ), 2  $\mu\text{L}$  of genomic DNA and ddH<sub>2</sub>O 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  $\mu\text{L}$  of the PCR products stained with Eurosafe DNA dye (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.

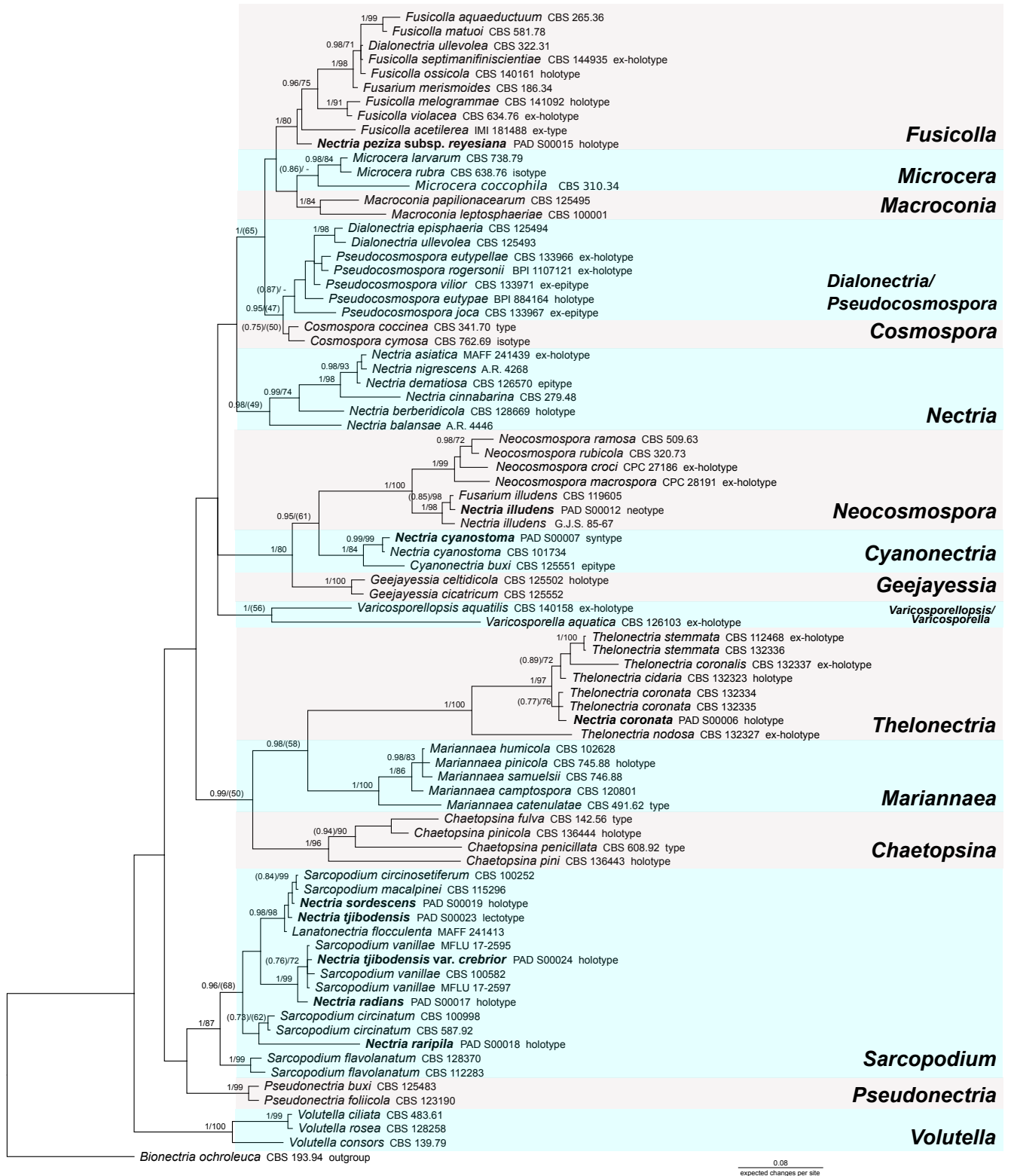
## RESULTS

### 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 for the *Clonostachys* subgroup comprises 50 ITS sequences (seven newly generated from the *Bionectriaceae* dataset and 43 from GenBank) and 43 *TUB2* sequences (43 from GenBank and corresponding to the same voucher of the ITS sequences) and 487 + 603 characters, respectively, including indels and missing data.

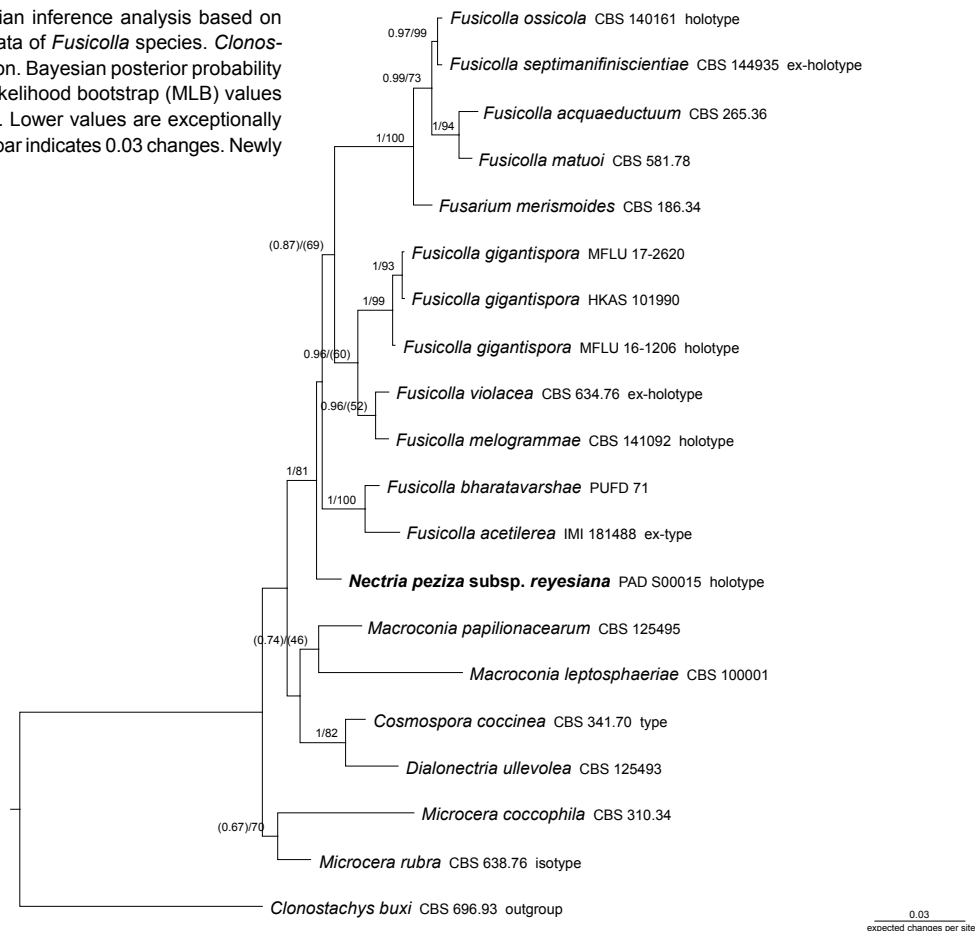


**Fig. 3** Phylogeny generated from Bayesian inference analysis based on ITS sequence data of species belonging to different *Nectriaceae* genera. *Bionectria ochroleuca* (*Bionectriaceae*) is selected as the outgroup taxon. Bayesian posterior probability (BPP) values  $\geq 0.95$  (left) and maximum likelihood bootstrap (MLB) values  $\geq 70\%$  (right) are shown on the branches. Lower values are exceptionally represented inside parentheses. The scale bar indicates 0.08 changes. Newly obtained sequences are reported in **bold**.

Most of the Saccardo specimens are distributed among three different genera of the *Bionectriaceae* (Fig. 1). *Nectria ambigua*, *N. ambigua* var. *pallens*, *N. congesta*, *N. flageoletiana*, *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 rufusca* 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 sequenced collections, 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 *Nectria ambigua*, *N. congesta*, *N. granuligera*, *N. phyllostachydis* and *N. squamuligera*.

**Fig. 4** Phylogeny generated from Bayesian inference analysis based on combined ITS and 28S rDNA sequence data of *Fusicolla* species. *Clonostachys buxi* is selected as the outgroup taxon. Bayesian posterior probability (BPP) values  $\geq 0.95$  (left) and maximum likelihood bootstrap (MLB) values  $\geq 70\%$  (right) are shown on the branches. Lower values are exceptionally represented inside parentheses. The scale bar indicates 0.03 changes. Newly obtained sequence is reported in **bold**.



## 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. raripila*, *N. sordescens*, *N. tjibodensis* and *N. tjibodensis* var. *crebrior* cluster with *Sarcopodium* species (BPP 1.0, MLB 87%), *N. cyanostoma* in the *Cyanonectria* clade (BPP 1.0, MLB 84%), *N. coronata* in the *Thelonectria* 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.* *Protocreopsis 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  $\mu\text{m}$  wide, usually fasciculate, smooth-walled hyphae, globose, non-papillate, yellow-orange, 200–280  $\mu\text{m}$  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)  $\times$  (7–)8–9–10(–10.3)  $\mu\text{m}$  ( $n = 10$ ), 8-spored, ascospores biseriolate. *Ascospores* fusoid, (15.1–)17–18.4–19.9(–22.6)  $\times$  (3.2–)3.6–4.1–4.6(–5.3)  $\mu\text{m}$ ,  $Q = (3.7–)4–4.5–5.1(–5.6)$ ,  $Q_{av} = 4.5$  ( $n = 35$ ), 1-septate, equally subdivided in two cells, not constricted or slightly constricted at the septum, hyaline, with many striations.

*Specimens examined.* INDONESIA, Java, Tjibodas, 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–)16.7–17.5–18.4(–18.8)  $\times$  4.4–4.9–5.4(–5.7)  $\mu\text{m}$  ( $n = 5$ ). 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 *Bionectriaceae* genus *Protocreopsis* 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 acremonium-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 *Protocreopsis* (Doi 1977, 1978). As



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 *Protocreopsis* 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 acromonium-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), *Protocreopsis freyinetiae* and *P. phormiicola* form an unsupported clade, and in Lechat et al. (2016), based on LSU sequences, *P. caricicola*, *P. korffii* 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 *Protocreopsis 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 *Protocreopsis pertusa*, reducing *N. scitula* to a later synonym. *Protocreopsis 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 *Nectria 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 *Protocreopsis caricicola* 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*, *Protocreopsis 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 perispore easily 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 ambigua*

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

*Basionym.* *Nectria ambigua* 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), *Q*<sub>av</sub> = 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. apocyni* (= *Bionectria apocyni*, Schroers 2001; *Clonostachys apocyni*, 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 ambigua* to the genus *Bionectria*. Presently, this species is considered a synonym of *Bionectria apocyni*, 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 ambigua* only the ITS1 sequence has been obtained and included in the phylogenetic analyses. The isolated position of *Nectria ambigua* in the phylograms (Fig. 1, 2) suggests that this is a distinct *Clonostachys* species, excluded from the doubtful synonymy with *Bionectria apocyni* 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 ambigua* and that of a *Bionectria apocyni* 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 ambigua* are shorter and narrower than those of *Bionectria apocyni* ((16–)20.6–22.6–24.6(–32) × (4.6–)6–6.8–7.6(–9.4) µm) (Schroers 2001). *Clonostachys agarwalii* (as 'agrawalii'), *C. capitata* and *C. zelandiae-novae* seem phylogenetically related to *C. ambigua*. *Clonostachys agarwalii*, 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 ambigua* var. *pallens*

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

*Basionym.* *Nectria ambigua* 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



**Fig. 6** a–e. *Nectria ambigua* (PAD S00003: herbarium Saccardo, n. 119, holotype). a. Original herbarium specimen; b. perithecia on natural substrate; c–e. ascus and ascospores in cotton blue. — f–j. *Nectria ambigua* var. *pallens* (PAD S00004: herbarium Saccardo, n. 452 ex p., lectotype). f. Original herbarium specimen; g. perithecia on natural substrate; h–j. asci and ascospores in cotton blue. — Scale bars: b, g = 500 µm; c–d = 20 µm; e, h = 10 µm; i–j = 5 µm. — Photos: a–e by S. Nigris; f–j by N. Forin.

diam ( $n = 5$ ); not changing colour in 3% KOH and 100% LA. Asci strictly clavate, (54–)54.9–59.7–64.5(–67.5) × (6.7–)7.5–8.6–9.7(–10) µm ( $n = 10$ ), 8-spored, ascospores biseriate above and uniseriate below. Ascospores ellipsoid to fusoid, (14.9–)16.2–17.2–18.2(–19) × (4.4–)4.7–5.1–5.5(–6.3) 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 (2) ex p., PAD S00004, lectotype designated here, MycoBank MBT393716.

**Notes** — This species co-occurs on the same substrate with *Nectria coronata* (Samuels et al. 1990). The specimen 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).

### *Nectria congesta*

***Clonostachys rosea*** (Link) Schroers et al., Mycologia 91: 369. 1999 — Fig. 7a–e

*Basionym.* *Penicillium roseum* Link, Mag. Ges. Naturf. Freunde, Berlin 3: 37. 1809.

*Synonyms.* *Sphaeria ochroleuca* Schwein., Trans. Amer. Philos. Soc., New Series 4: 204. 1832 '1834'.

*Cucurbitaria ochroleuca* (Schwein.) Kuntze, Revis. Gen. Pl. 3: 461. 1898.

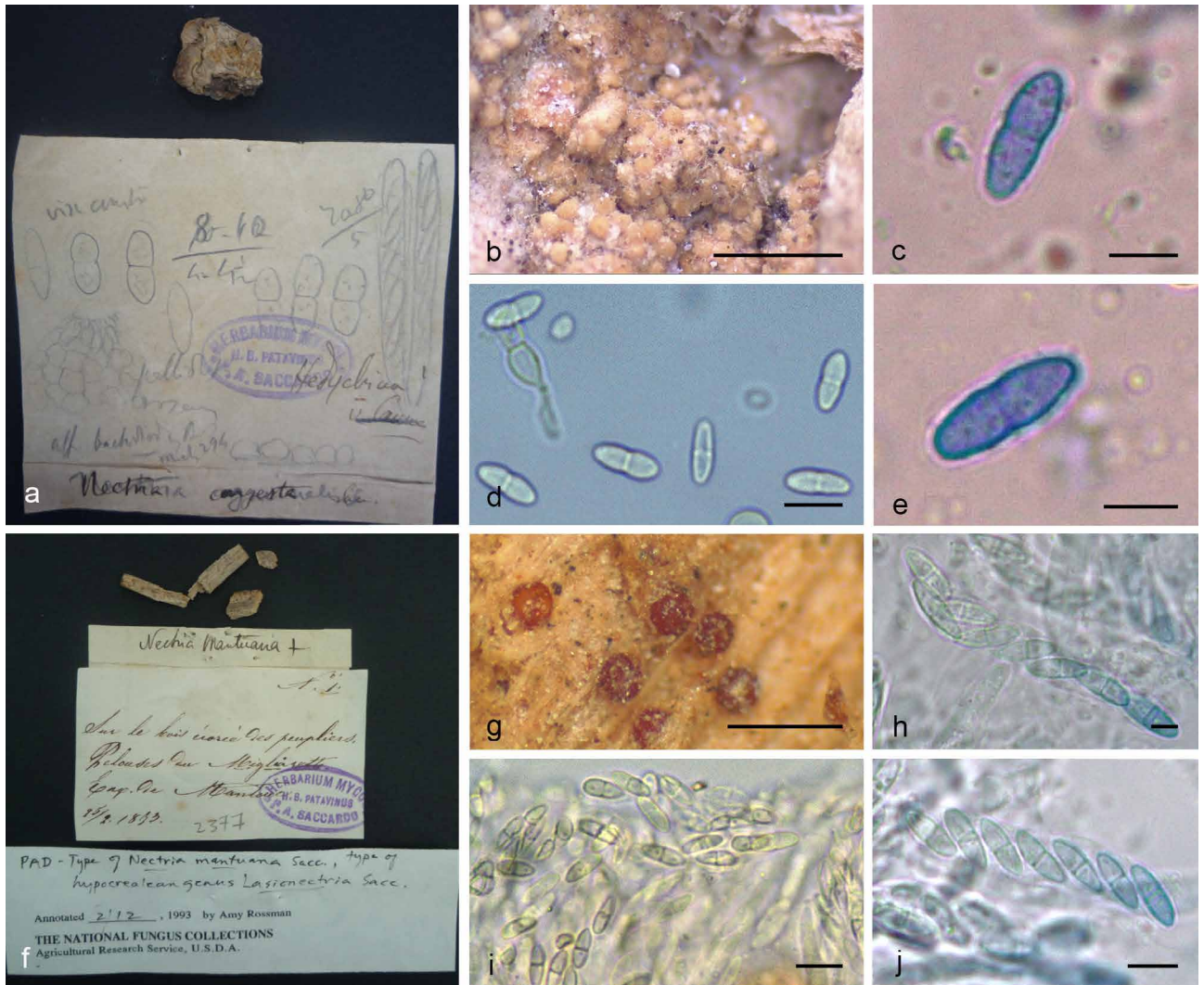
*Creonectria ochroleuca* (Schwein.) Seaver, Mycologia 1: 190. 1909.

*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.

**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



**Fig. 7** a–e. *Nectria congregata* (PAD S00005: herbarium Saccardo, lectotype). a. Original herbarium specimen; b. perithecia on natural substrate; c–e. ascospores in cotton blue and water. — f–j. *Nectria mantuana* (PAD S00014: herbarium Saccardo, holotype). f. Original herbarium specimen; g. perithecia on natural substrate; h–j. ascus and ascospores in cotton blue and water. — Scale bars: b, g = 500  $\mu$ m; c, e, h, j = 5  $\mu$ m; d, i = 10  $\mu$ m. — Photos: a–e by S. Nigris; f–j by N. Forin.

( $n = 10$ ); not changing colour in 3 % KOH and 100 % LA. Asci not observed. Ascospores ellipsoid, (9.4–)10.3–11–11.7(–12.5)  $\times$  (3.2–)3.5–3.8–4.1(–4.5)  $\mu$ m,  $Q = (2.6\text{--})2.7\text{--}2.9\text{--}3.1(3.6)$ ,  $Q_{av} = 2.9$  ( $n = 25$ ), 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 congregata* 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 congregata* and *N. phyllostachydis* ITS sequences ( $P\%iv = 99.4\%$ ) and between the morphologies of *N. congregata* and *B. ochroleuca* reported by Schroers (2001) suggest that *N. congregata* 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).

### *Nectria coronata*

***Thelonectria coronata*** (Penz. & Sacc.) P. Chaverri & C. Salgado, Stud. Mycol. 68: 76. 2011 — Fig. 8a–e

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

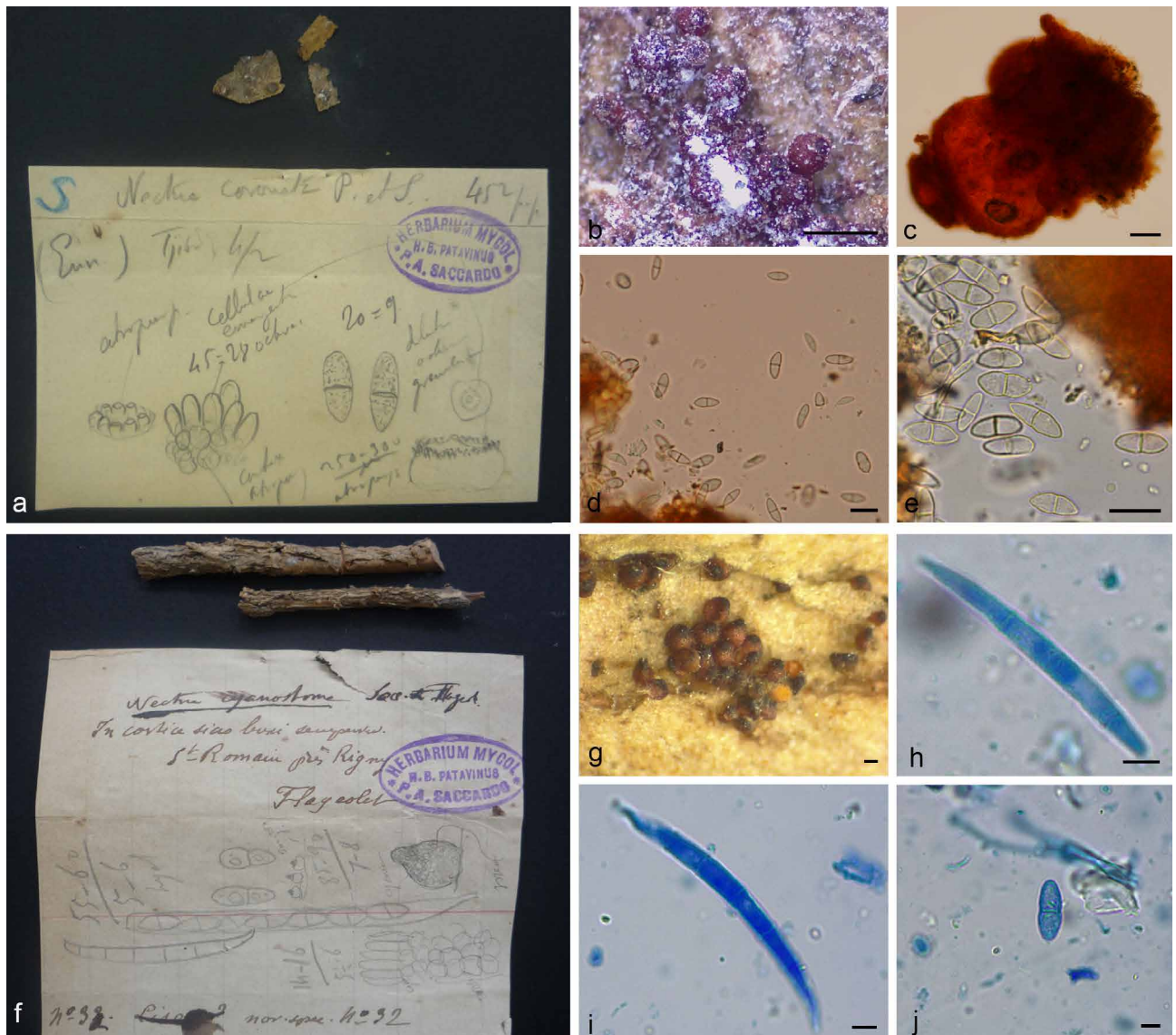
*Synonym.* *Neonectria coronata* (Penz. & Sacc.) Mantiri & Samuels, Canad. J. Bot. 79: 339. 2001.

*Cylindrocarpon coronatum* Brayford & Samuels, Sydowia 46: 91. 1993.

**Sexual morph.** *Perithecia* gregarious, superficial, globose to pyriform, brownish red with a darker ostiolar disc, 225–350  $\mu$ m 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)  $\times$  (5.5–)6.2–6.8–7.5(–8.4)  $\mu$ m,  $Q = (2.5\text{--})2.7\text{--}2.9\text{--}3.1(3.4)$ ,  $Q_{av} = 2.9$  ( $n = 25$ ), 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*)



**Fig. 8** a–e. *Nectria coronata* (PAD S00006: herbarium Saccardo, n. 452 ex p., holotype). a. Original herbarium specimen; b. perithecia on natural substrate; c. perithecium magnification with the typical crown which distinguishes the species; d–e. ascospores in water. — f–j. *Nectria cyanostoma* (PAD S00007: herbarium Saccardo, n. 32, syntype). f. Original herbarium specimen; g. perithecia on natural substrate; h–i. macroconidia in cotton blue; j. ascospore in cotton blue. — Scale bars: b = 500  $\mu\text{m}$ ; c, g = 100  $\mu\text{m}$ ; d–e = 20  $\mu\text{m}$ ; h–j = 5  $\mu\text{m}$ . — Photos: a–e by S. Nigris; f–j by N. Forin.

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 cylindrocarpon-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, Phytopathology 103: 404. 2013.

**Sexual morph.** *Perithecia* gregarious, superficial, red-brown, pyriform with darker apical region, 158–250  $\mu\text{m}$  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)  $\times$  (4.6–)4.9–5.3–5.7(–6.1)  $\mu\text{m}$ ,  $Q = (2\text{--})2.3\text{--}2.5\text{--}2.8(3)$ ,  $Q_{av} = 2.5$  ( $n = 16$ ), 1-septate, equally subdivided in two cells,

constricted at the septum, warty. *Macroconidia* 3–5-septate: 3-septate, 43  $\times$  4  $\mu\text{m}$  ( $n = 1$ ); 5-septate, 52–63  $\times$  5  $\mu\text{m}$  ( $n = 2$ ), 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 recombinated 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

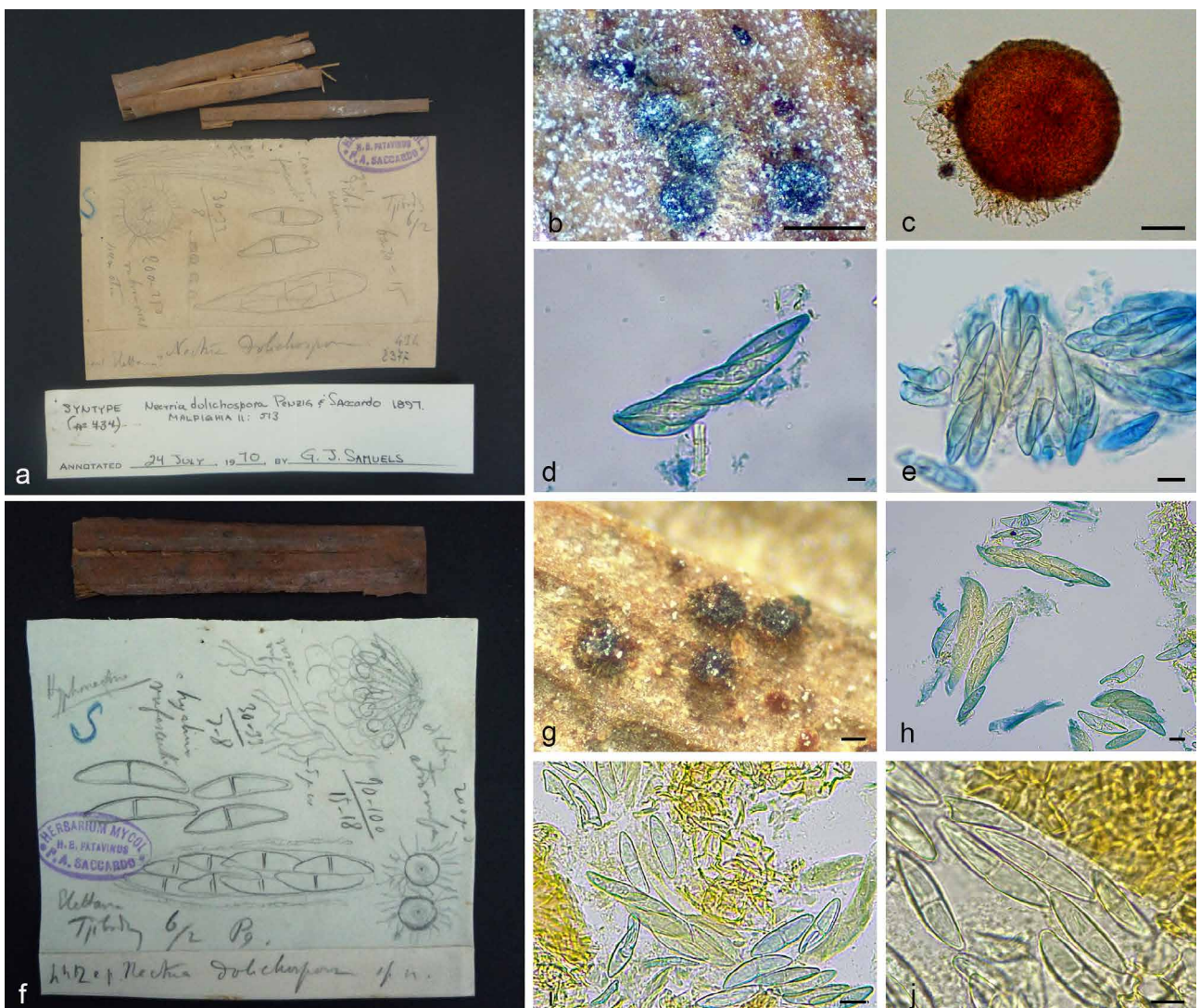
Basionym. *Nectria dolichospora* Penz. & Sacc., Malpighia 11: 513. 1897.

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 biseriata. *Ascospores* ellipsoidal to fusoid, (25.2–)28.3–30.7–33.1(–34.7) × (6–)7.1–7.8–8.6(–9.6) µm,  $Q = (3.1–)3.6–4–4.3(–5)$ ,  $Q_{av} = 3.9$  ( $n = 50$ ), 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 amber, 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 acronium-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** a–e. *Nectria dolichospora* (PAD S00008: herbarium Saccardo, n. 434, lectotype). a. Original herbarium specimen; b. perithecia on natural substrate; c. perithecium magnification; d–e. ascus and ascospores in cotton blue. — f–j. *Nectria dolichospora* (PAD S00009: herbarium Saccardo, n. 442, syntype). f. Original herbarium specimen; g. perithecia on natural substrate; h–j. asci and ascospores in cotton blue and water. — Scale bars: b = 500 µm; c = 100 µm; d, h–j = 5 µm; e = 10 µm; g = 50 µm. — Photos: a–e by S. Nigris; f–j by N. Forin.

*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 biserial 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,  $Q = (2.1–)2.5–2.8–3.2(–4.1)$ ,  $Q_{av} = 2.8$  ( $n = 38$ ), 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



**Fig. 10** a–e. *Nectria flageoletiana* (PAD S00010: herbarium Saccardo, holotype). a. Original herbarium specimen; b. perithecia on natural substrate; c–e. asci and ascospores in cotton blue. — f–j. *Nectria phyllostachydis* (PAD S00016: herbarium Saccardo, isolectotype). f. Original herbarium specimen; g. perithecia on natural substrate; h–j. ascospores in cotton blue and water. — k–n. *Nectria phyllostachydis* (TNS-F-210044: herbarium National Museum of Nature and Science, lectotype). k–l. Original herbarium specimen; m–n. perithecia on natural substrate. — Scale bars: b, g, m–n = 500 µm; c, j = 5 µm; d–e, i = 10 µm; h = 20 µm. — Photos: a–e by N. Forin; f–j by S. Nigris; k–n by Y. Tochihara.

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.* *Cucurbitaria granuligera* (Starbäck) Kuntze, Revis. Gen. Pl. 3: 461. 1898.

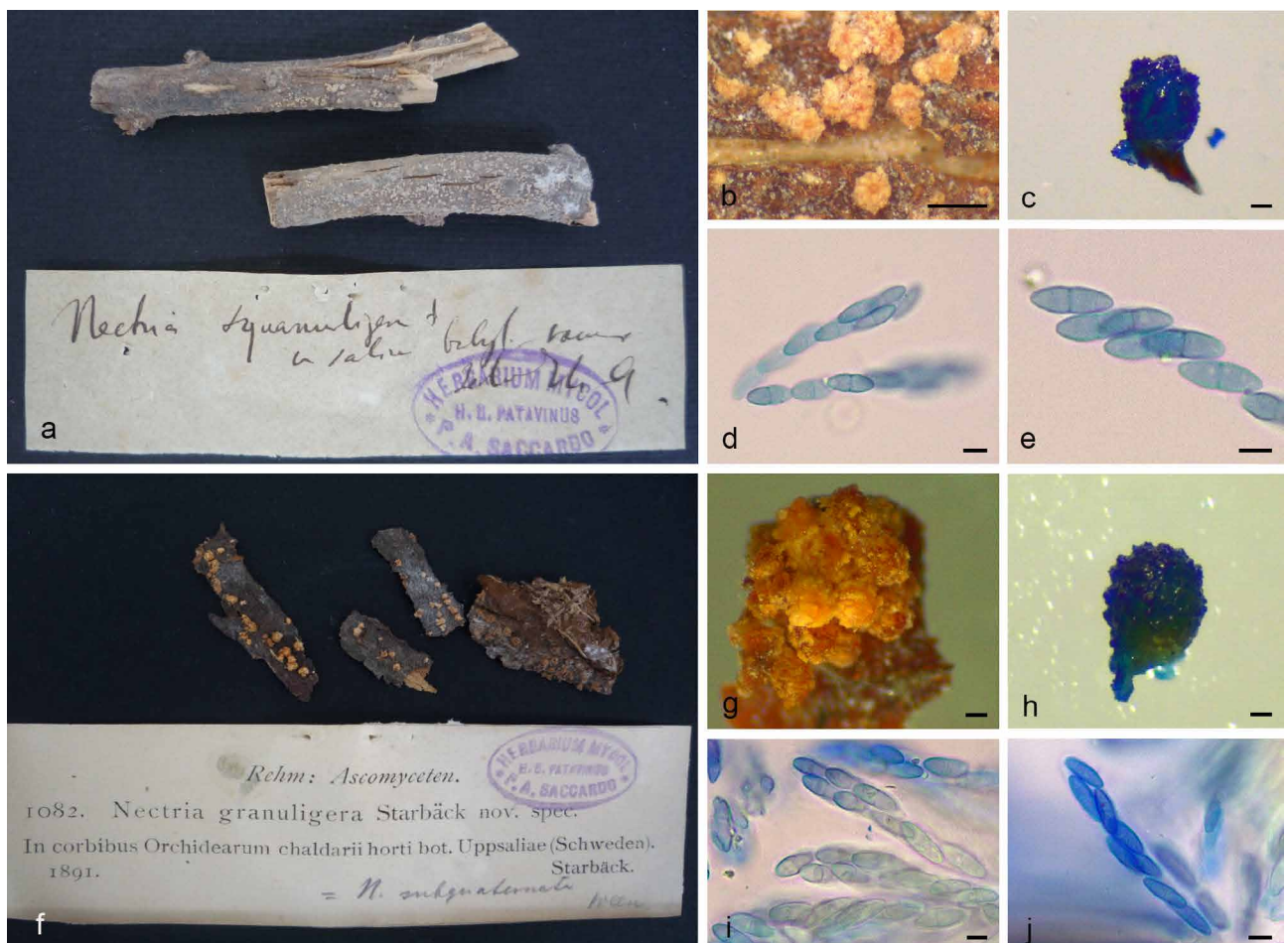
*Creonectria granuligera* (Starbäck) Seaver, Monogr. Univ. Puerto Rico, Ser. B 2: 130. 1934.

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) × (6–)6.1–6.5–7(–7.5) µm ( $n = 6$ ), 8-spored, ascospores biseriata above and uniseriate below. *Ascospores* ellipsoid, (8.9–)10.1–11.5–12.8(–15.1) × (3.4–)3.8–4.2–4.6(–4.9) µm,  $Q = (2.3–)2.5–2.8–3(–3.8)$ ,  $Q_{av} = 2.8$  ( $n = 50$ ), 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 *C. byssicola* has already been highlighted by Abreu et al. (2014).

*Clonostachys granuligera* is actually very similar morphologically to *C. 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 *C. eriocamporesiana* is difficult to differentiate from *C. byssicola* both on a morphological and molecular basis (Hyde et al. 2020) and, in our opinion, is probably synonymous with *C. byssicola*.

*Nectria squamuligera* is distinguished by solitary to gregarious (small groups), non-stromatic pale pink perithecia (see below). *Clonostachys wenpingii* differs from *C. 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.

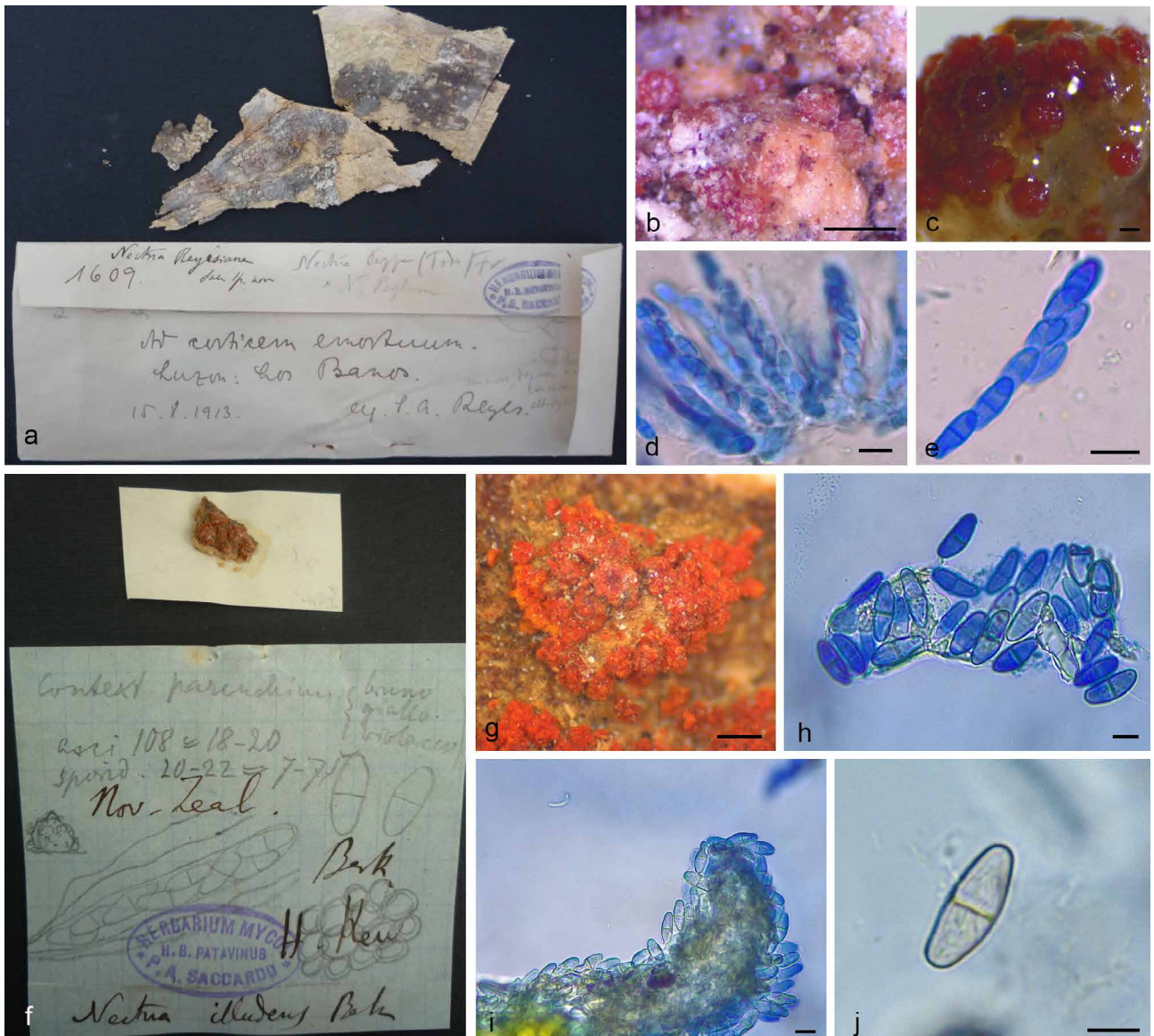
*Synonyms.* *Cucurbitaria illudens* (Berk.) Kuntze, Revis. Gen. Pl. 3: 461. 1898.

*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, warted, 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,  $Q = (2.5–)2.6–2.9–3.1(–3.6)$ ,  $Q_{av} = 2.9$  ( $n = 38$ ), 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. asci 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 fungarium 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).

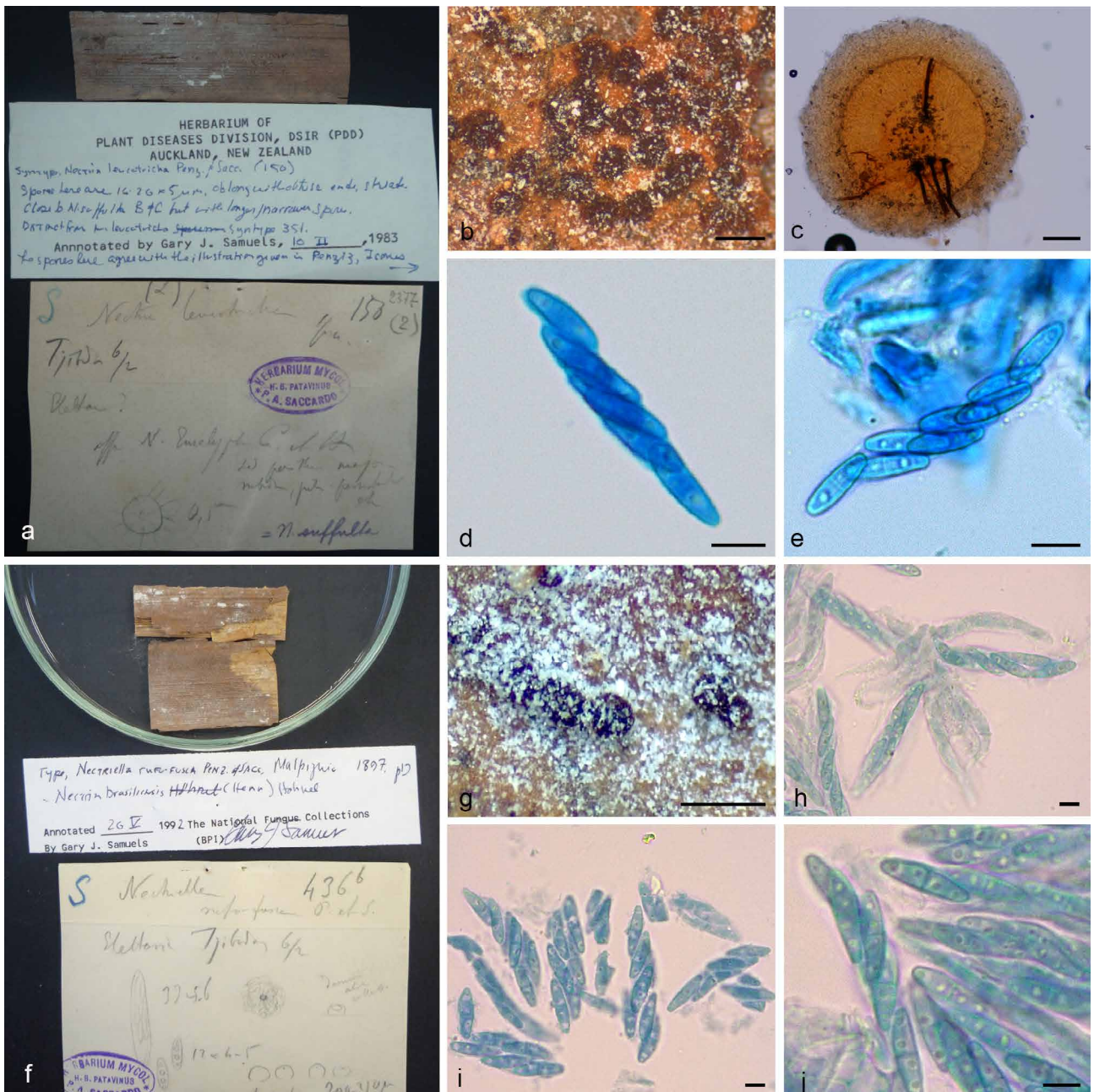
### *Nectria leucotricha*

***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 biserial. *Ascospores* ellipsoid, (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).



**Fig. 13** a–e. *Nectria leucotricha* (PAD S00013: herbarium Saccardo, n. 150, lectotype). a. Original herbarium specimen; b. perithecia on natural substrate; c. perithecium magnification with the visible trichomes on the surface; d–e. asci and ascospores in cotton blue. — f–j. *Nectriella rufofusca* (PAD S00025: herbarium Saccardo, n. 436, holotype). f. Original herbarium specimen; g. perithecia on natural substrate; h–j. asci and ascospores in cotton blue. — Scale bars: b, g = 500 µm; c = 100 µm; d–e = 10 µm; h–j = 5 µm. — Photos: a–e by S. Nigris; f–j by N. Forin.

Notes — The specimen was morphologically revised in 1983 by G.J. Samuels, as reported on the label associated with the sample (Fig. 13a). 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  $\pm$  200  $\mu$ m 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  $\mu$ m diam ( $n = 10$ ), sparsely covered with short hairs, 8–16  $\times$  2.4–3.8  $\mu$ m ( $n = 10$ ) 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)  $\times$  (5.8–)6.1–6.7–7.3(–7.6)  $\mu$ m ( $n = 7$ ), 8-spored, ascospores uniseriate. *Ascospores* ellipsoidal, (8.4–)9.5–10.3–11(–11.9)  $\times$  (2.9–)3–3.3–3.6(–4.1)  $\mu$ m,  $Q = (2.4–)2.8–3.1–3.4(–3.5)$ ,  $Q_{av} = 3.1$  ( $n = 32$ ), 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 mantuana* ITS sequence deposited in GenBank (P%iv = 92.4%; 25 nucleotide differences). The latter sequence (HM484858, voucher BPI 843540, on dead wood from Finland; Chaverri et al. 2011) does not belong to *L. mantuana*, but is closely related to *L. hilhorstii*, a species described based on its asexual morph (Crous et al. 2018). Among the morphologically closest species to *Lasionectria mantuana*, *L. vulpina* from North America and Europe differs in having slightly longer, striate ascospores, (7–)8–11(–13)  $\times$  3–4  $\mu$ m and up to 50  $\mu$ m long hairs on perithecia (Samuels 1976, as *Nectria vulpina*, Rossman et al. 1999). *Lasionectria marigotensis*, recently described from Guadeloupe on decaying leaves of *Cocos nucifera*, is distinguished by white to pale orange perithecia, smooth ascospores, (9–)10–12.5(–13.5)  $\times$  3–3.5  $\mu$ m, and 14–47  $\mu$ m long perithecial hairs (Lechat & Fournier 2012).

#### *Nectria peziza* subsp. *reyesiana*

***Fusicolla reyesiana*** (Sacc.) Forin & Vizzini, *comb. & stat. nov.* — MycoBank MB835771; Fig. 12a–e

*Basionym.* *Nectria peziza* subsp. *reyesiana* Sacc., *Ann. Mycol.* 12: 305. 1914.

Sexual morph. *Perithecia* gregarious, superficial or partially immersed in a pale-yellow sheet of hyphae, globose, non-papillate, red-orange, 234–342  $\mu$ m diam ( $n = 8$ ); not changing colour in 3% KOH but turning yellow in 100% LA. *Asci* narrowly clavate, (48.3–)51.2–58.8–66.4(–67.6)  $\times$  (7.1–)7.8–9.4–10.9(–11.3)  $\mu$ m ( $n = 5$ ), 8-spored, ascospores biseriate above and uniseriate below. *Ascospores* ellipsoid, (11.5–)12.4–13.5–14.5(–16.8)  $\times$  (4.2–)4.8–5.3–5.8(–6.2)  $\mu$ m,  $Q = (2.2–)2.3–2.6–2.8(–3.4)$ ,  $Q_{av} = 2.5$  ( $n = 40$ ), 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 the 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 *Fusicolla* (*Nectriaceae*) in the NCBI database. The phylogenetic analyses (Fig. 3, 4) confirm the taxonomic assignment by BLASTn, placing the analysed holotype within the well-supported clade formed by *Fusicolla* sequences (BPP 1.0, MLB 80/81%). The genus *Fusicolla*, typified by *Fusicolla betae*, includes 12 species (Jones et al. 2019, Index Fungorum 2020). *Fusicolla* species reported in Gräfenhan et al. (2011) and *F. septimanifiniscentiae* are only known from their asexual morphs (Crous et al. 2018); while for the recently introduced species, *F. bharatavarshae* (Jones et al. 2019), *F. gigantispora* (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 *Fusicolla*. *Fusicolla reyesiana* differs from *F. melogrammae* and *F. ossicola* in having shorter asci ((48.31–)51.16–66.44(–67.58)  $\times$  (7.09–)7.84–10.9(–11.32)  $\mu$ m vs (60–)70–80(–85)  $\times$  (9–)10–12(–14)  $\mu$ m and (70–)80–85(–90)  $\times$  8–11  $\mu$ m, respectively) (Crous et al. 2016, Lechat & Rossman 2017), and lacking spinulose, pale golden brown ascospores. The dimensions of asci in *Fusicolla reyesiana* are similar to those of *Fusicolla bharatavarshae*, but the ascospores are larger, ((11.51–)12.42–14.53(–16.76)  $\times$  (4.2–)4.84–5.8(–6.15)  $\mu$ m vs 7–12  $\times$  2–5  $\mu$ m). *Fusicolla gigantispora* is significantly different from all the other *Fusicolla* species and also from *F. reyesiana*, in having aseptate, larger ascospores (20–35  $\times$  20–28  $\mu$ m), that are dark brown when mature (Dayarathne et al. 2020), questioning its placement in *Fusicolla*.

#### *Nectria phyllostachydis*

***Clonostachys rosea*** (Link) Schroers et al., *Mycologia* 91: 369. 1999 — Fig. 10f–j

*Basionym.* *Penicillium roseum* Link, *Mag. Ges. Naturf. Freunde Berlin* 3: 37. 1809.

*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.

*Nectria ochroleuca* (Schwein.) Schroers & Samuels, *Z. Mykol.* 63: 15. 1997.

*Nectria congesta* Sacc., *Michelia* 2: 256. 1881.

*Nectria phyllostachydis* Hara (as *Nectoria 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 Kawauemura (Japan) in 1912.

Sexual morph. *Perithecia* gregarious, not immersed in a stroma, globose to subglobose-depressed, non-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,  $Q = (2.4–)2.8–3.1–3.5(–3.9)$ ,  $Q_{av} = 3.1$  ( $n = 25$ ), 1-septate, not equally subdivided in two cells, constricted at the septum, hyaline, warted.

*Specimen examined.* JAPAN, Mino prov., Kawauye-mura (currently Gifu pref., Nakatsugawa city), on *Phyllostachys bambusoides*. (= *P. reticulata*), Jan. 1912, K. Hara, TNS-F-210044 lectotype designated here (MBT392613); PAD S00016, isolectotype.

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 an isolectotype. 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 radians*

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

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

Original description — Peritheciis superficialibus, in soros ramoso-radiantes, 3,7 mm diam. congestis, lateritio-rubris, globoso-conoides, breve papillatis, 1/3 mm d., initio flavo-pruinosis; ascis fusoides utrinque acutulis, 50–60 × 9–12, aparaphysatis, (?), octosporidi; sporidiis 2–3-stichis, fusoides, 15–17 × 4–4.5, rectis, 1-septatis, non constrictis, hyalinis, intus nubilosus.

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.* INDONESIA, Java, Tjibodas, on bark (host not known), ? Penzig, n. 86, PAD S00017, holotype.

Notes — The original description of *Nectria radians* was based on a specimen with collector’s number 86. However, Samuels et al. (1990) considered the specimen *Nectria radians*

n. 86 as the holotype since it was the only specimen that they found with correlating details, and as a consequence, studied for a taxonomic revision of this species. Based on the morphological observations they synonymised this species under *Nectria flavolanata*, with some reservations (Samuels et al. 1990). *Nectria flavolanata* is now considered a member of the genus *Sarcopodium* (*Nectriaceae*) under the name *S. flavolanatum*. Species of this genus are characterised by red perithecia, not papillate or with a small papilla, solitary or in groups, subglobose to pyriform and with hyphal hairs; asci clavate to fusoid, 8-spored, and ascospores 1-septate and striate (Lombard et al. 2015). Unfortunately, we could not locate any asci and ascospores during the present study, and only the ITS1 sequence was obtained from this specimen. However, Samuels et al. (1990) reported a description of the spores from the holotype. The molecular analysis excludes the synonymy proposed by Samuels et al. (1990) suggesting that *Nectria radians* is a distinct species within the genus *Sarcopodium*, sister to the *S. vanillae*/*N. tjobodensis* var. *crebrior* (Fig. 3). The possible synonymy under *Sarcopodium vanillae* is rejected due to the difference in the ascospore size. *Sarcopodium vanillae* is characterised by smaller and narrower ascospores (8–12 × 3–4.5 µm; Chaiwan et al. 2019) than *Nectria radians* ((12–)12.4–14.8(–17) × (4–)4.5–5.3(–5.5) µm).

### *Nectria raripila*

*Sarcopodium raripilum* (Penz. & Sacc.) L. Lombard & Crous, *Stud. Mycol.* 80: 221. 2015 — Fig. 14a–e

*Basionym.* *Nectria raripila* Penz. & Sacc., *Malpighia* 15: 228. 1901.

*Synonym.* *Lanatonectria raripila* (Penz. & Sacc.) Samuels & Rossman, *Stud. Mycol.* 42: 140. 1999.

Sexual morph. *Perithecia* scattered, solitary or in small groups, superficial on the substratum, globose to pyriform, cupulate, yellow with hyphal hairs around the perithecium, 132–180 µm diam ( $n = 5$ ); orange in 3 % KOH and yellow in 100 % LA. *Asci* clavate, (66.6–)66.9–71.2–75.6(–78.8) × (10.3–)11.1–13–15(–15.3) µm ( $n = 5$ ), 8-spored, ascospores biseriolate. *Ascospores* fusoid, (24.2–)25.1–27.1–29(–31.5) × (4.6–)5–5.6–6.2(–6.7) µm,  $Q = (3.9–)4.4–4.9–5.4(–6.1)$ ,  $Q_{av} = 4.8$  ( $n = 44$ ), slightly curved, 1-septate, equally subdivided in two cells, constricted at the septum, hyaline, striate with wavy striae.

*Specimen examined.* INDONESIA, Java, ? Tjibodas, on *Elettaria* sp., ? 1898, ? M. Fleischer, n. 923, PAD S00018, holotype.

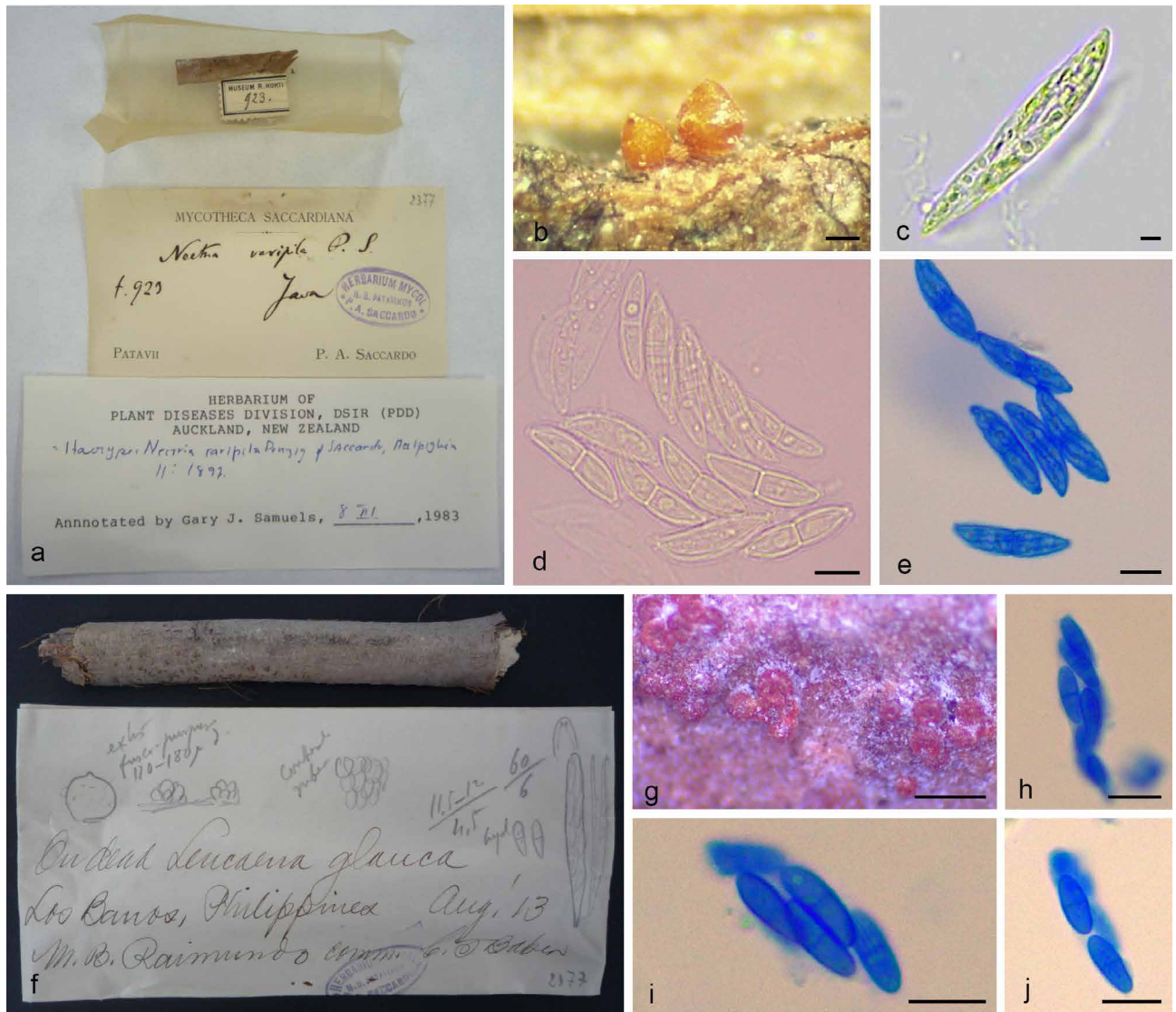
Notes — The holotype specimen was morphologically studied in 1983 by G.J. Samuels, as reported in the label associated with the sample (Fig. 14a). *Nectria raripila* is now considered a member of the genus *Sarcopodium* (*Nectriaceae*) as *S. raripilum*. The morphological observations of *Nectria raripila* 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 tjobodense* — Fig. 14f–j (see below)

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

Sexual morph. *Perithecia* 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



**Fig. 14** a–e. *Nectria rariipila* (PAD S00018: herbarium Saccardo, n. 923, holotype). a. Original herbarium specimen; b. perithecia on natural substrate; c–e. ascus and ascospores in cotton blue and water. — f–j. *Nectria sordescens* (PAD S00019: herbarium Saccardo, holotype). f. Original herbarium specimen; g. perithecia on natural substrate; h–j. ascospores in cotton blue. — Scale bars: b = 50  $\mu\text{m}$ ; c = 5  $\mu\text{m}$ ; d–e, h–j = 10  $\mu\text{m}$ ; g = 500  $\mu\text{m}$ . — Photos by N. Forin.

(–14)  $\times$  (2.7–)3.2–3.5–3.9(–4.4)  $\mu\text{m}$ ,  $Q = (2.7\text{--})3\text{--}3.3\text{--}3.6(\text{--}4)$ ,  $Q_{\text{av}} = 3.3$  ( $n = 30$ ), 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 *Lanatonectria flocculenta*, *Nectria tjobodensis*, *Sarcopodium circinosetiferum* and *S. macalpinei* (Fig. 3). The molecular analysis and the morphological comparison between *Nectria sordescens* and *N. tjobodensis* (see below) suggest that *N. sordescens* can be considered a synonym of *N. tjobodensis*.

### *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  $\mu\text{m}$  diam ( $n = 7$ ); not changing colour in 3% KOH and 100% LA. *Asci* clavate, (48.8–)49.3–53.9–58.6(–60)  $\times$  (5.7–)6–7–8.1(–8.2)  $\mu\text{m}$  ( $n = 4$ ), 8-spored, ascospores biseriata above and uniseriate below. *Ascospores* ellipsoid to fusoid, (10.2–)10.8–12.6–14.4(–17.4)  $\times$  (2.6–)3.3–3.7–4.1(–4.6)  $\mu\text{m}$ ,  $Q = (2.3\text{--})2.8\text{--}3.5\text{--}4.1(\text{--}5.1)$ ,  $Q_{\text{av}} = 3.4$  ( $n = 30$ ), 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 ( $P\%_{\text{iv}} = 99.8\%$ ), 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–)9.4–10–10.8(–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*

***Sarcopodium 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.

*Nectria flocculenta* (Henn. & E. Nyman) Höhn., *Sitzungsber. Kaiserl. Akad. Wiss., Wien. Math.-Naturwiss. Cl., Abt. 1*, 121: 360. 1912.

*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-Istria* 10: 69. 1917.

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

*Sarcopodium 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.

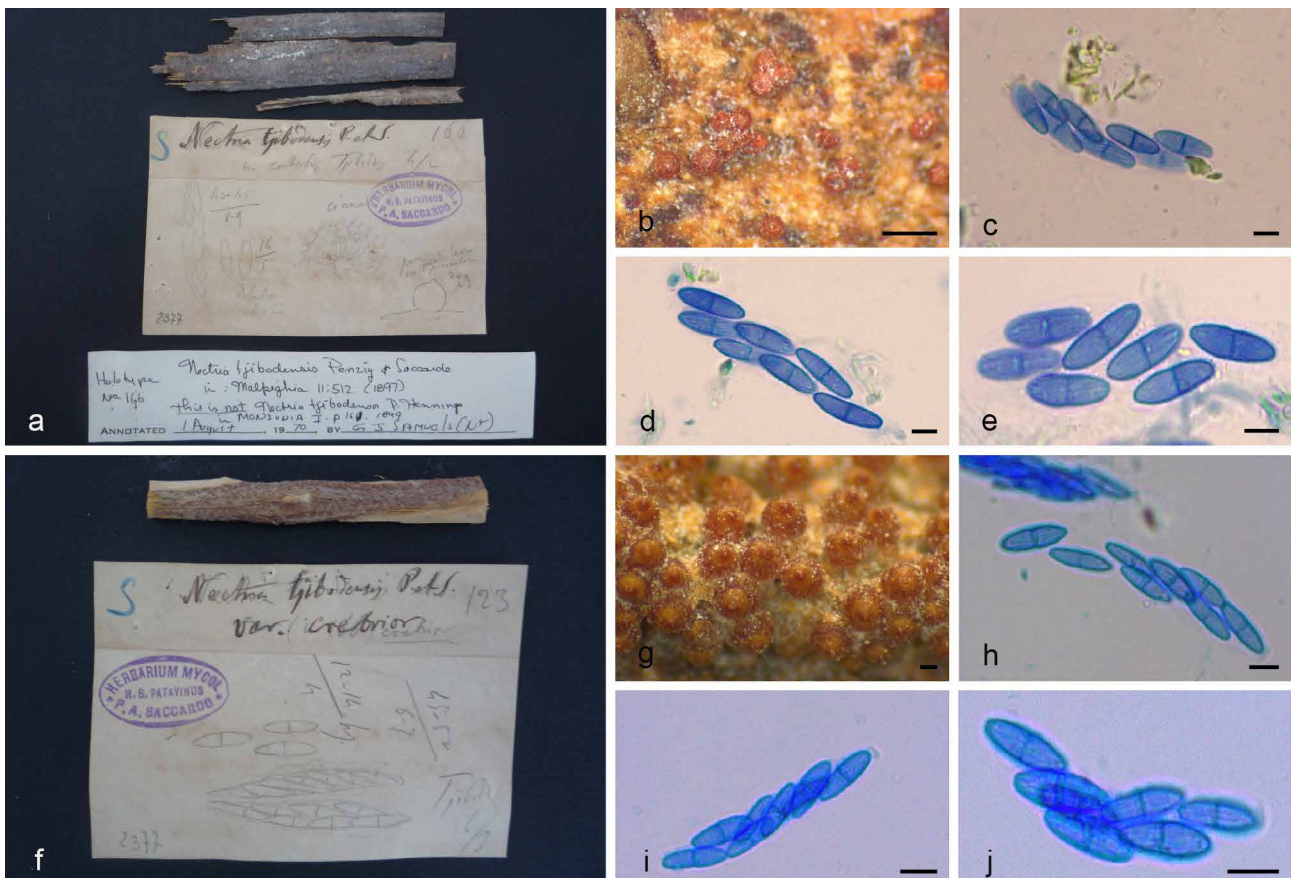
? = *Kutilakesa circinosetifera* Matsush., *Microfungi Solomon Isl. Papua New Guinea*: 34. 1971.

*Sarcopodium circinosetiferum* (Matsush.) Matsush., *Matsushima 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 biseriata. *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,  $Q = (2.8–)3.1–3.6–4.2(–4.8)$ ,  $Q_{av} = 3.6$  ( $n = 50$ ), 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* (Rossman 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*



**Fig. 15** a–e. *Nectria tjibodensis* (PAD S00023: herbarium Saccardo, n. 166, lectotype). a. Original herbarium specimen; b. perithecia on natural substrate; c–e. ascus and ascospores in cotton blue. — f–j. *Nectria tjibodensis* var. *crebrior* (PAD S00024: herbarium Saccardo, n. 123, holotype). f. Original herbarium specimen; g. perithecia on natural substrate; h–j. ascus and ascospores in cotton blue. — Scale bars: b = 500 µm; c–e, h–j = 5 µm; g = 200 µm. — Photos by N. Forin.

*flocculenta*, *Sarcopodium macalpinei* (= asexual morph of *L. flocculenta*), *S. circinosetiferum* and *Nectria sordescens* (see above) in a strongly supported clade (BPP 0.98, MLB 98 %), and distant from the ITS sequences of *S. flavolanatum* (Fig. 3). *Lanatonectria flocculenta* was synonymised under *Sarcopodium macalpinei*, but recently the name *Sarcopodium flocculentum* was proposed by Pennycook & Kirk (2019). The morphology of the lectotype of *Nectria tjibodensis* fits well with the detailed morphological description of *Lanatonectria flocculenta* (Rossman et al. 1999). Morphological and molecular analyses suggest that *Nectria tjibodensis* should be considered a synonym of *Sarcopodium flocculentum*, and not of *S. flavolanatum* as previously supposed. Based on the earliest available legitimate name, *Nectria tjibodensis* should be recombined as *Sarcopodium tjibodense* and *S. flocculentum* treated as a later synonym. *Sarcopodium circinosetiferum*, of which only the asexual morph is known, could be an additional synonym of *S. tjibodense* (Fig. 3).

*Nectria tjibodensis* var. *crebrior*

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

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

*Synonym.* *Nectria tjibodensis* 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. *Asci* 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 biserial 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,  $Q = (3.1–)3.3–3.6–3.9(–4.3)$ ,  $Q_{av} = 3.6$  ( $n = 41$ ), 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 tjibodensis* var. *crebrior* was synonymised under *Lanatonectria flocculenta* (= *S. macalpinei*) (Samuels et al. 1990). The ITS phylogenetic analysis placed *Nectria tjibodensis* 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 asci and ascospores of *Nectria tjibodensis* var. *crebrior* are very similar to *Sarcopodium vanillae* (asci 36–52 × 3–5 µm ( $x = 44 \times 4.5$  µm); ascospores 8–12 × 3–4.5 µm ( $x = 11 \times 3.9$  µm)). The only difference is the presence of striate ascospores in our sample (a feature shared by all the hitherto known species of *Sarcopodium*, Lombard et al. 2015), a character not observed for *Sarcopodium vanillae* (Chaiwan et al. 2019). Striae on the ascospore surface are very thin and difficult to observe if spores are not mounted in a high contrast medium-like cotton blue; Chaiwan et al. (2019) probably observed immature, not fully developed ascospores, or ascospores not mounted in cotton blue. Taking into account the morphological similarity and the high similarity among the sequences (ITS sequence, P%iv = 98.3 %) of *Sarcopodium vanillae* (CBS 100582, MFLU 17-2595, MFLU 17-2597) and *Nectria tjibodensis* var. *crebrior*, we conclude that *N. tjibodensis* var. *crebrior* is a synonym of *Sarcopodium vanillae*.

*Nectriella rufofusca*

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

*Basionym.* *Nectriella rufofusca* Penz. & Sacc., Malpighia 11: 507. 1897. *Synonyms.* *Neohenningsia stellatula* Koord., Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., ser.13: 164. 1907.

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

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

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

*Pseudonectria brasiliensis* (Henn.) Weese, Sitzungsber. 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. *Asci* clavate, (40.5–)41–42.6–44.3(–44.4) × (4.9–)5.2–6.1–7(–7.2) µm ( $n = 4$ ), 8-spored, ascospores biserial above and uniseriate below. *Ascospores* ellipsoid to fusoid, (10.8–)11.8–12.6–13.4(–14.7) × (2.6–)2.8–3–3.2(–3.3) µm,  $Q = (3.8–)3.9–4.2–4.5(–5)$ ,  $Q_{av} = 4.2$  ( $n = 31$ ), equally subdivided in two biguttulate cells, 1-septate, not constricted at the septum, hyaline, smooth.

*Specimen examined.* INDONESIA, Java, Tjibodas, on decaying leaf of *Elettaria* 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 *Hydropisphaera* (*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).

## 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. 2012). However, fungaria are an underused resource for this purpose due to the difficulty of obtaining high-quality DNA from historical biological material (Staats et al. 2011, Leavitt et al. 2019). 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., Liimatainen et al. 2014). In addition, the multicopy 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. ambigua*, *N. ambigua* var. *pallens*, *N. granuligera*, *N. peziza* subsp. *reyesiana*, *N. radians*,

*N. squamuligera* and *N. tjobodensis*) and five (*N. congesta*, *N. flageoletiana*, *N. phyllostachydis*, *N. sordescens* and *N. tjobodensis* 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 tjobodensis* 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 fungarium type specimens.

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