



# Persistent hamathecial threads in the *Nectriaceae*, *Hypocreales*: *Thyronectria* revisited and re-instated

W.M. Jaklitsch<sup>1</sup>, H. Voglmayr<sup>1,2</sup>

## Key words

*act*  
*Ascomycota*  
*Hypocreales*  
*Mattiroliia*  
*Nectriaceae*  
new species  
*Pleonectria*  
pyrenomyces  
*rpb1*  
*rpb2*  
*tef1*  
*tub*  
*Thyridiaceae*  
*Thyridium*  
*Thyronectroidea*

**Abstract** Based on type studies and freshly collected material we here re-instate the genus *Thyronectria* (*Nectriaceae*, *Hypocreales*). Species of this genus were recently for the most part classified in the genera *Pleonectria* (*Nectriaceae*) or *Mattiroliia* (*Thyridiaceae*), because *Thyronectria* and other genera had been identified as members of the *Thyridiaceae* due to the presence of paraphyses. Molecular phylogenies based on several markers (*act*, ITS, LSU rDNA, *rpb1*, *rpb2*, *tef1*, *tub*) revealed that the *Nectriaceae* contain members whose ascospores are characterised by long, more or less persistent, apical paraphyses. All of these belong to a single genus, *Thyronectria*, which thus has representatives with hyaline, rosy, green or even dark brown and sometimes distoseptate ascospores. The type species of *Thyronectria*, *T. rhodochlora*, syn. *T. patavina*, syn. *T. pyrrochlora* is re-described and illustrated. Within the *Nectriaceae* persistent, apical paraphyses are common in *Thyronectria* and rarely also occur in *Nectria*. The genus *Mattiroliia* is revised and merged with *Thyronectria* and also *Thyronectroidea* is regarded as a synonym of *Thyronectria*. The three new species *T. asturiensis*, *T. caudata* and *T. obscura* are added to the genus. Species recently described in *Pleonectria* as well as some species of *Mattiroliia* are combined in the genus, and a key to *Thyronectria* is provided. Five species are epitypified. The type species of the genus *Thyridium* (*Thyridiaceae*), *T. vestitum*, is included in phylogenetic analyses to illustrate the phylogenetic distance of *Thyronectria* from the *Thyridiaceae*.

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## INTRODUCTION

The family *Nectriaceae* includes about 20 genera of which two major genera were monographed by Hirooka et al. (2012) in a comprehensive and voluminous work. One of the generic names revived in that work is *Pleonectria* Sacc. (1876). The genus *Thyronectria* Sacc. (1875), based on *T. patavina* Sacc., is older and has therefore priority over *Pleonectria*. As described by Saccardo (1875a: 21), the generic name refers to superficial similarities of the immersed stromata and muriform ascospores to the genus *Thyridium*, whereas the nectriaceous perithecial context and the hyaline ascospores being similar to *Nectria* or *Calonectria*. Seeler (1940) monographed this genus, which he characterised by light-coloured perithecia immersed in or superficial on erumpent stromata, often clothed with yellowish or greenish scales or powder, with eventually 'evanescent pseudo-paraphyses' and hyaline, yellowish, green or brown, muriform ascospores that may form conidia by budding in the ascus. He determined the asexual morphs to belong to *Gyrostroma*, *Dendrodochium* and *Stilbella*. Hamathecial elements in the *Hypocreales* have been determined to be restricted to paraphyses and apical paraphyses, while true paraphyses, i.e. sterile filaments emerging from the subhymenium between asci, are not known in the *Hypocreales*. Apical paraphyses develop in a cushion at the top of the perithecialium and grow downward to the bottom of the perithecial cavity, forming a compact palisade or appearing like a pseudoparenchyma. After growth of asci into this palisade, the apical paraphyses become disintegrated

and are not present between mature asci (Hanlin 1961, 1971). Zhang et al. (2006) used the absence of true paraphyses as a typical character of the subclass *Hypocreomycetidae* of the *Sordariomycetes*.

When Rossman et al. (1999) detected paraphyses in the holotype of *T. patavina*, they concluded that *Thyronectria* was not available for muriform-spored nectriaceous species, and they referred the genus *Thyronectria* to the *Thyridiaceae*. They placed other species regarded as *Thyronectria* by Seeler (1940) in the genus *Nectria*. Rossman et al. (1999) relegated also *Balzania* Speg., *Mattiroliia* Berl. & Bres. and *Thyronectroidea* Seaver to the *Thyridiaceae*. In a morphotaxonomic work, Checa et al. (2013) accepted the placement of the latter genera in the *Thyridiaceae* and recognised *Balzania* and *Thyronectroidea* as synonyms of *Mattiroliia*.

The species classified by Rossman et al. (1999) in *Nectria* were revised by Hirooka et al. (2012), who recognized three genera. The authors also determined that yellow scurf on ascospores or stromata is confined to *Allantonectria* Earle and *Pleonectria* and that two species with muriform ascospores included by Seeler (1940) in *Thyronectria* belong to *Nectria* s.str., namely *N. antarctica* and *N. pseudotrichia*. These latter species have sporodochial or synnematos asexual morphs, while Hirooka et al. (2012) characterised *Pleonectria* as having pycnidial asexual morphs, although this was not present in all species. *Pleonectria* now contains also species devoid of longitudinal septa in their ascospores, as had also been determined by Jaklitsch & Voglmayr (2011) using molecular phylogeny.

We have occasionally seen persistent filiform hamathecial threads in perithecial mounts of *Pleonectria coryli*, *P. lamyi* or *P. pyrrochlora*, just as had been described for *Thyronectria patavina*. This is why we started to investigate those genera that were relegated to the *Thyridiaceae* by Rossman et al.

<sup>1</sup> Division of Systematic and Evolutionary Botany, Department of Botany and Biodiversity Research, University of Vienna, Rennweg 14, A-1030 Vienna, Austria; corresponding author e-mail: walter.jaklitsch@univie.ac.at.

<sup>2</sup> Institute of Forest Entomology, Forest Pathology and Forest Protection, Department of Forest and Soil Sciences, BOKU-University of Natural Resources and Life Sciences, Peter Jordan-Straße 82, 1190 Vienna, Austria.

(1999) such as *Mattirolia*. An important species in this context is *Pleonectria pyrrochlora*, below recognised as *Thyronectria rhodochlora*, also considered an earlier name for *T. patavina*, which led us to conclude that *Thyronectria* is the correct genus for these species. Another important step in this work was the collection of *Mattirolia roseovirens* on its original host genus *Laburnum* in its original region. In conclusion we synonymise *Mattirolia*, *Pleonectria* and *Thyronectroidea* with *Thyronectria* below.

## MATERIALS AND METHODS

### Isolates and specimens

All isolates used in this study originated from ascospores or conidia of fresh specimens. Numbers of strains including NCBI GenBank accession numbers of gene sequences used to compute the phylogenetic trees are listed in Table 1. Strain acronyms other than those of official culture collections (ATCC, CBS, MAFF) are used here primarily as strain identifiers throughout the work. Representative isolates have been deposited at the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands (CBS). Details of the specimens used for morphological investigations are listed in the Taxonomy section under the respective descriptions. Herbarium acronyms are according to Thiers (2014). Freshly collected specimens have been deposited in the Herbarium of the Institute of Botany, University of Vienna (WU).

### Culture preparation, growth rate determination and phenotypic analysis

Cultures were prepared and maintained as described previously (Jaklitsch 2009) except that CMD (CMA: Sigma, St Louis, Missouri; supplemented with 2 % (w/v) D(+)-glucose-mono-hydrate) or 2 % malt extract agar (MEA; 2 % w/v malt extract, 2 % w/v agar-agar; Merck, Darmstadt, Germany) was used as the isolation medium. Cultures used for the study of asexual morph micro-morphology were grown on CMD or 2 % MEA (or potato dextrose agar (PDA, 39 g/L; Merck, Darmstadt, Germany) where noted) at room temperature (RT), defined here as 22 ± 3 °C, or at 25 °C under alternating 12 h cool daylight and 12 h darkness. Sectioning with a freezing microtome was carried out after short rehydration and treatment with 3 % KOH at 8–12 µm as described previously (Jaklitsch & Voglmayr 2011). Microscopic observations were generally made in de-ionised water or 3 % KOH, lactic acid or 50 % glycerol where noted. Morphological analyses of microscopic characters were carried out as described earlier (Jaklitsch 2009). Data were gathered using a Nikon Coolpix 4500 or a Nikon DS-U2 digital camera and measured by using the NIS-Elements D v. 3.0 software. Methods of microscopy included stereomicroscopy using a Nikon SMZ 1500 and Nomarski differential interference contrast (DIC) using the compound microscope Nikon Eclipse E600. For certain images of stromata the stacking software Zerene Stacker v. 1.04 (Zerene Systems LLC, Richland, WA, USA) was used. Measurements are reported as maxima and minima in parentheses and the mean plus and minus the standard deviation of a number of measurements given in parentheses. The colour term rosy is used for a certain range of pinkish colours as exemplified by ascospores of *T. rhodochlora* (Fig. 4) and conidiation structures of *T. roseovirens* (Fig. 12h, i).

### DNA extraction and sequencing methods

The extraction of genomic DNA was performed as reported previously (Voglmayr & Jaklitsch 2011, Jaklitsch et al. 2012) using the DNeasy Plant Mini Kit (QIAGEN GmbH, Hilden, Germany) or the modified CTAB method of Riethmüller et al. (2002). Seven loci were amplified and sequenced of which six correspond to

those included in Hirooka et al. (2012): a c. 700 bp fragment of alpha-actin (*act*) with primers Tact1 and Tact2 (Samuels et al. 2006); the complete internally transcribed spacer region (ITS1-5.8S-ITS2) and a c. 900 bp fragment of the large subunit nuclear ribosomal DNA (nLSU rDNA), amplified and sequenced as a single fragment with primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990); a c. 700 bp fragment of the RNA polymerase II subunit 1 (*rpb1*) with primers crpb1a and rpb1c (Castlebury et al. 2004); a c. 1.2 kb fragment of the RNA polymerase II subunit 2 (*rpb2*) with primers fRPB2-5f and fRPB2-7cr (Liu et al. 1999); a c. 1.3 kb fragment of the translation elongation factor 1-alpha (*tef1*) with primers EF1-728F (Carbone & Kohn 1999) and TEF1LLErev (Jaklitsch et al. 2005); and a c. 1 kb fragment of β-tubulin (*tub*) with primers Btub-T1 and Btub-T222 (O'Donnell & Cigelnik 1997). PCR products were purified using an enzymatic PCR cleanup (Werle et al. 1994) as described in Voglmayr & Jaklitsch (2008). DNA was cycle-sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Warrington, UK) with the same primers as in PCR and an automated DNA sequencer (3730xl Genetic Analyzer, Applied Biosystems); in addition, internal primers ITS4 (White et al. 1990) and LR3 (Vilgalys & Hester 1990) were used for sequencing the partial nuSSU-complete ITS-partial nuLSU rDNA region.

### Analysis of sequence data

All alignments were produced with the server version of MAFFT (www.ebi.ac.uk/Tools/msa/mafft), checked and refined using Bio-Edit v. 7.0.4.1 (Hall 1999). To investigate the phylogenetic relationships of '*Mattirolia*' and *Thyronectria* to *Thyridium* and *Nectriaceae*, a multigene matrix comprising LSU, *rpb1*, *rpb2* and *tef1* sequences was produced and analysed. In addition to sequences obtained in the current study, representative GenBank sequences were selected from Hirooka et al. (2012) and Spatafora et al. (2006). The resulting combined 4-gene sequence matrix contained 42 taxa and 4 178 alignment positions (825, 779, 1 129 and 1 445 characters from LSU, *rpb1*, *rpb2* and *tef1*, respectively). According to Spatafora et al. (2006), *Diatrype disciformis* and *Xylaria hypoxylon* (*Xylariales*, *Xylariomycetidae*) were selected as outgroup. Prior to phylogenetic analyses, the approach of Wiens (1998) was applied to test for significant levels of localised incongruence among the 4-gene partitions, using the level of bootstrap support (Sung et al. 2007). For this, the 70 % maximum parsimony (MP) bootstrap consensus trees calculated for each individual partition, using the same parameters as for the combined analysis given below, were compared. No topological conflicts were observed between these bootstrap trees of the various genes, indicating the absence of significant incongruence and combinability of the four loci (Wiens 1998).

For detailed investigation of phylogenetic relationships within *Thyronectria*, sequences of the six loci included in Hirooka et al. (2012) were downloaded from GenBank and combined with those generated during the present study; in addition, sequence data for the *rpb2* were added when available. *Nectria asiatica*, *N. cinnabarina*, *N. dematiosa* and *N. nigrescens* were selected as outgroup. The resulting combined sequence matrix contained 6 150 alignment positions from seven genes (630 from *act*, 512 from ITS, 807 from LSU, 695 from *rpb1*, 1 187 from *rpb2*, 1 278 from *tef1* and 1 041 from *tub*). Prior to phylogenetic analyses, the approach of Wiens (1998) was applied to test for significant levels of localised incongruence among the 7-gene partitions as described above. No topological conflicts were observed between the bootstrap trees of the various genes, indicating the absence of significant incongruence and combinability of the seven loci (Wiens 1998).

Table 1 Isolates and accession numbers used in the phylogenetic analyses. Isolates/sequences in bold were isolated/sequenced in the present study.

Species	Isolate No.	Herbarium No.	Substrate/Host	Country	act	ITS	LSU	rbp1	rbp2	tef1	tub
<i>Allantonecchia militina</i>	CBS 121121	BPI 878442	<i>Agave americana</i>	Italy	HM484514	HM484547	HM484572	HM484587	–	HM484524	HM484609
<i>Camaropus ustulinooides</i>	AFTOL-ID 72, D.E.H. 2164										
<i>Cosmospora coccinea</i>	CBS 114050	BPI 802729	<i>Inonotus nodulosus</i>	Germany			DQ470941	DQ471121	DQ470882	DQ471050	
<i>Cyanonectria cyanostoma</i>	CBS 101734	BPI 748307	<i>Buxus sempervirens</i>	France			GQ505990	GQ506020	–	HM484515	
<i>Diaporthe eres</i>	CBS 109767	BPI 748435	<i>Acer campestre</i>	Austria			FJ474081	GQ506017	–	HM484535	
<i>Diatrype disciformis</i>	CBS 197.49		<i>Alnus</i> sp.	Netherlands			AF408350	DQ471161	DQ470919	DQ479931	
<i>Gnomonia gnomon</i>	CBS 199.53		<i>Corylus avellana</i>	Italy			DQ470964	DQ471085	DQ470915	DQ471085	
<i>Nectria antarctica</i>	ATCC 204178, CBS 115033	BPI 746217	<i>Berberis aquifolium</i>	USA			AF408361	DQ471167	DQ470922	DQ471094	
<i>N. balsanae</i>	MAFF 241439	BPI 879972	unid. dead bark	USA			HM484560	HM484575	–	HM484516	
<i>N. cinnabarina</i>	CBS 123351	BPI 878477	<i>Coronilla</i> sp.	Japan			HM484563	–	JQ014140	–	HM484604
<i>N. dematiosa</i>	CBS 125165	BPI 879981	<i>Aesculus</i> sp.	France			GQ505996	GQ506026	–	HM484525	
<i>N. nigrescens</i>	CBS 126570	BPI 749337	unid. dead bark	France			HM484562	HM484577	JQ014125	HM484527	HM484606
<i>N. pseudotrachia</i>	CBS 125148	BPI 871083	unid. dead twigs	USA			HM484561	HM484576	JQ014144	HM484534	HM484603
<i>Ophiostoma piliferum</i>	CBS 652.83	PDD 7908	unid. dead bark	Venezuela			HM484720	HM484781	JQ014123	HM484672	HM484806
<i>Papulosa amerospora</i>	CBS 158.74		unid. wood	Chile			DQ470955	DQ471147	DQ470905	DQ471074	
<i>Pseudonectria pachysandricola</i>	AFTOL-ID 748, J.K. 5547F										
<i>Rodentomyces reticulatus</i>	CBS 128674	BPI 879936	<i>Pachysandra</i> sp.	USA			DQ470950	DQ471143	DQ470901	DQ471069	
<i>Rugonectria rugulosa</i>	CBS 128675	BPI 881070	Rodent dung	USA			JF832715	JF832791	–	JF832544	
<i>Thelonectria westlandica</i>	CBS 129158		unid. dead bark	Italy			JF832761	–	–	JF832543	
<i>Thyridium vestitum</i>	CBS 112464	BPI 842278	<i>Dacrydium cupressinum</i>	USA			GQ505987	–	–	HM484533	
<i>Thyronectria aquifolii</i>	CBS 113027	BPI 550125	<i>Acer pseudoplatanus</i>	New Zealand			QO506015	–	–	–	
	CBS 307.34	WU 30360	<i>flex aquifolium</i>	Austria			AY544671	DQ471129	DQ470890	DQ471058	JF832842
	<b>NAK, CBS 125027</b>	WU 32124	<i>flex aquifolium</i>	UK			JF832718	JF832792	–	JF832548	JF832842
<i>T. asturiensis</i>	CBS 109874	BPI 841465	<i>Fraxinus excelsior</i>	UK			HM534891	<b>KJ570715</b>	HM534881	HM534870	<b>KJ570638</b>
<i>T. aurigera</i>	CBS 125134	WU 32664	<i>Quercus ilex</i>	Spain			<b>KJ570690</b>	<b>KJ570716</b>	<b>KJ570741</b>	<b>KJ570760</b>	<b>KJ570639</b>
<i>T. austroamericana</i>	CBS 125135	BPI 746395, NCSU	<i>Gleditsia triacanthos</i>	France			HM484551	HM484573	–	HM484521	HM484600
	CBS 126114	BPI 746395	<i>Gleditsia triacanthos</i>	USA			HM484551	HM484573	–	HM484521	HM484600
	<b>GG</b>	WU 32664	<i>Gymnocladus dioica</i>	USA			JF832513	JF832759	–	JF832881	JF832882
	CBS 125132	BPI 746322	<i>Abies balsamea</i>	USA			JF832655	JF832760	–	JF832588	JF832882
	CBS 129159	BPI 881047	<i>Abies fraseri</i>	USA			JF832514	JF832655	–	–	–
	CBS 125136	NCSU	<i>Abies balsamea</i>	USA			GQ505960	HM484555	–	–	–
	CBS 129160	BPI 881050	<i>Abies balsamea</i>	USA			<b>KJ570665</b>	<b>KJ570691</b>	<b>KJ570717</b>	<b>KJ570742</b>	<b>KJ570640</b>
	CBS 129429	BPI 881048	<i>Abies balsamea</i>	USA			<b>KJ570665</b>	<b>KJ570691</b>	<b>KJ570717</b>	<b>KJ570742</b>	<b>KJ570640</b>
	CBS 125137	NCSU	<i>Abies fraseri</i>	Austria			JF832453	JF832719	JQ014122	JF832556	JF832846
	CBS 129428	BPI 881049	<i>Abies balsamea</i>	USA			JF832456	JF832721	–	JF832557	–
<i>T. berolinensis</i>	CBS 126112	BPI 746346	<i>Ribes rubrum</i>	USA			JF832456	JF832721	–	JF832557	–
	CBS 128980	HB7896A	<i>Ribes nigrum</i>	Austria			JF832479	JF832623	–	JF832559	JF832847
	<b>NB, CBS 127382</b>	WU 30361	<i>Ribes sanguineum</i>	Mongolia			<b>KJ570666</b>	HM534893	–	JF832559	JF832847
<i>T. boothii</i>	CBS 128977	BPI 881052	<i>Picea abies</i>	Austria			JF832475	JF832755	–	JF832552	JF832871
<i>T. caudata</i>	<b>NL2, CBS 136003</b>	WU 32130	<i>Berberis cretica</i>	Greece			<b>KJ570667</b>	<b>KJ570692</b>	<b>KJ570719</b>	<b>KJ570762</b>	<b>KJ570642</b>
	<b>NCA</b>	WU 33429	<i>Berberis hispanica</i>	Slovakia			<b>KM225674</b>	<b>KM225679</b>	<b>KM225690</b>	<b>KM225691</b>	<b>KM225698</b>
	<b>NCA1</b>	WU 33428	<i>Berberis hispanica</i>	Spain			<b>KM225675</b>	<b>KM225680</b>	<b>KM225686</b>	<b>KM225693</b>	<b>KM225698</b>
<i>T. coryli</i>	CBS 129358	BPI 881053, C.L.L. 651	<i>Corylus avellana</i>	France			JF832476	JF832740	–	JF832553	JF832872
	CBS 115619	BPI 746347	<i>Viburnum lantana</i>	Austria			JF832477	JF832618	–	JF832554	JF832873
	CBS 129156	BPI 880697	<i>Rhus copallinum</i>	USA			HM484509	HM484539	–	–	–
	CBS 129744	BPI 881054	<i>Celastrus orbiculatus</i>	USA			JF832478	JF832742	–	–	–
	<b>NCP, CBS 127384</b>	WU30362	<i>Pyrus communis</i>	Austria			<b>KJ570668</b>	HM534895	–	–	–
	<b>NeCo1, CBS 137264</b>	WU 32129	<i>Corylus avellana</i>	Austria			<b>KJ570669</b>	<b>KJ570693</b>	<b>KJ570721</b>	<b>KJ570744</b>	<b>KJ570643</b>
<i>T. cucurbitula</i>	CBS 301.75		<i>Pinus nigra</i>	France			JF832461	JF832621	–	JF832563	JF832854
	CBS 259.58		<i>Pinus sylvestris</i>	Netherlands			GQ505974	HM484541	–	–	–
	CBS 541.70		Dead twig in witch's broom	Netherlands			JF832463	JF832722	–	JQ014131	HM484550
	CBS 125130		<i>Pinus sylvestris</i>	Netherlands			JF832464	JF832723	–	–	–
	CBS 178.73		<i>Pinus sylvestris</i>	Austria			JF832462	JF832733	–	JQ014134	JF832855
	CBS 125147		<i>flex aquifolium</i>	UK			HM484506	HM484538	–	–	–
<i>T. illicicola</i>	CBS 125170	BPI 881055, C.L.L. 7159	<i>flex aquifolium</i>	France			JF832445	JF832625	–	–	–

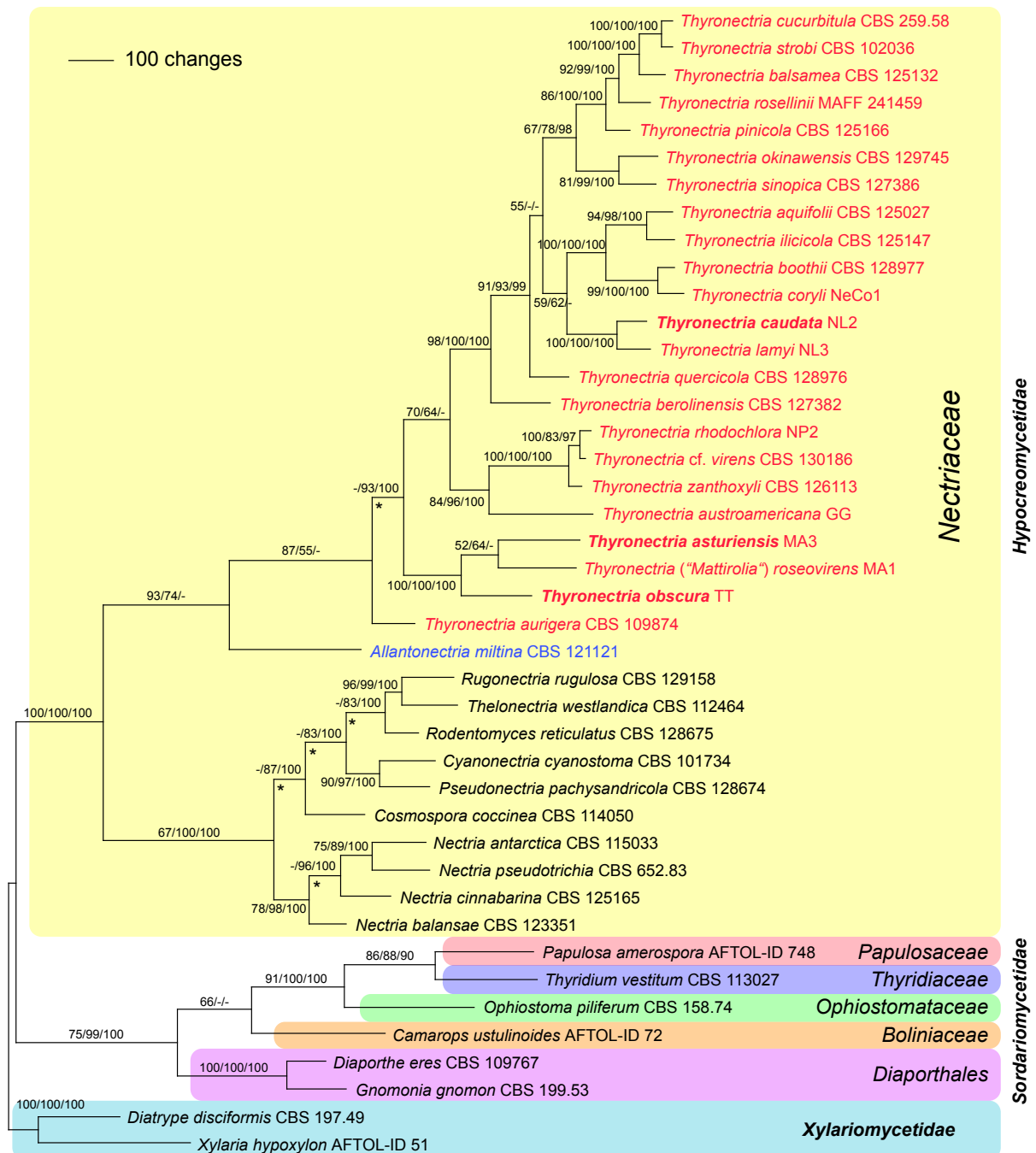




Maximum parsimony (MP) analyses were performed with PAUP v. 4.0 b10 (Swofford 2002), using 1 000 replicates of heuristic search with random addition of sequences and subsequent TBR branch swapping (MULTREES option in effect, steepest descent option not in effect). All molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data. The COLLAPSE command was set to NO for the combined 4-gene and to MINBLEN for the combined 7-gene matrix. Bootstrap analyses with 500 replicates were performed in the same way, but using five rounds of random sequence addition and subsequent branch swapping during each bootstrap replicate; in addition, each replicate was limited to 10 million rearrangements in the analyses of the combined 7-gene matrix.

In ML and Bayesian analyses, substitution model parameters were calculated separately for the different gene regions included in the combined analyses. For ML analyses, 500 fast bootstrap replicates were computed with RAxML (Stamatakis 2006a) as implemented in raxmlGUI v. 1.3 (Silvestro & Michalak 2012) using the GTRCAT1 substitution model, which efficiently approximates the well-known general time reversible model (GTR; Rodríguez et al. 1990) with gamma-distributed substitution rates, additionally assuming a proportion of invariant sites (GTR+I+G) (Stamatakis 2006b).

Bayesian analyses were performed with the computer program MrBayes (v. 3.2.2; Huelsenbeck & Ronquist 2001). The following substitution models were selected by Modeltest v. 3.6 (Posada & Crandall 1998) under the Akaike Information Criterion: in

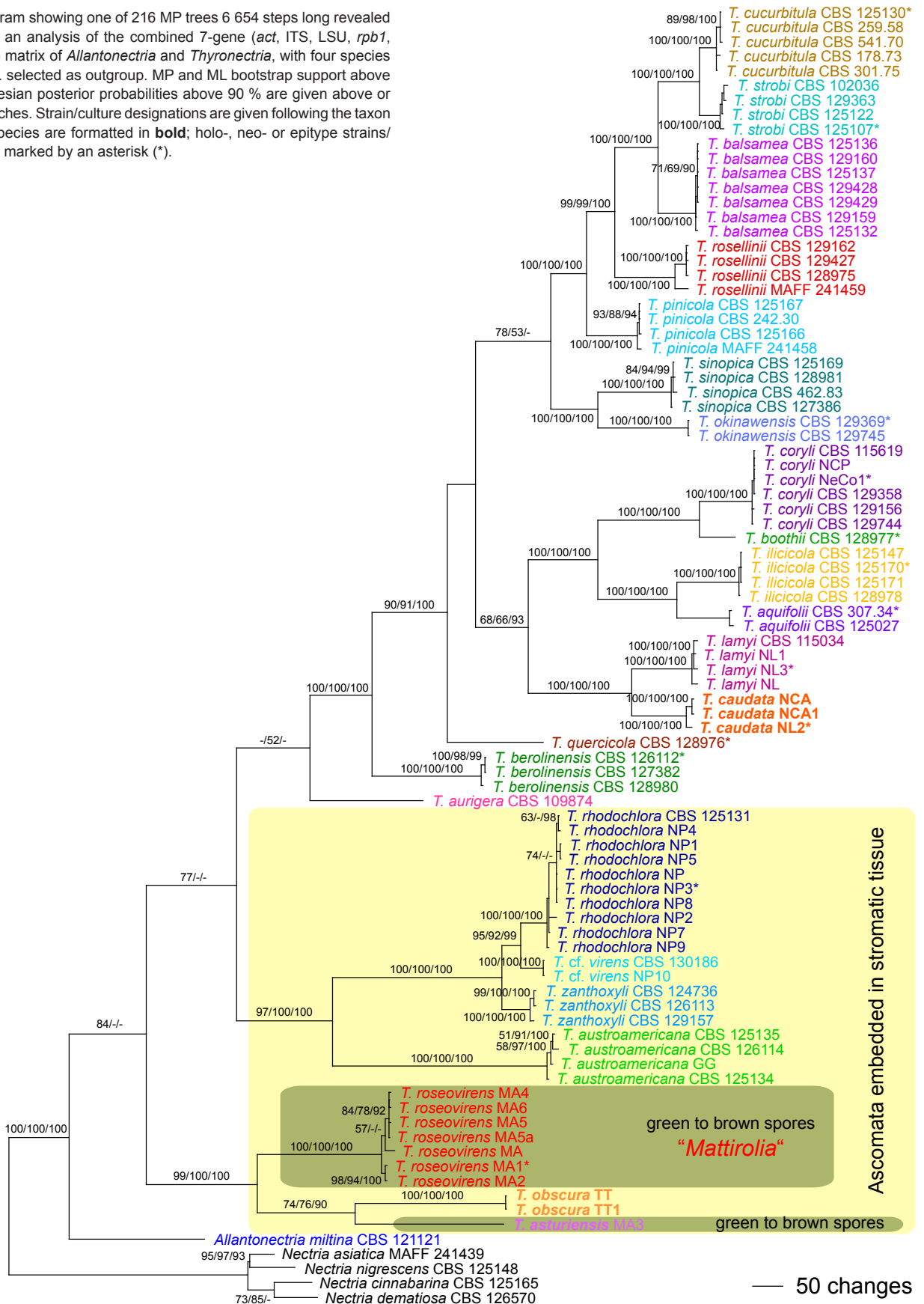


**Fig. 1** Phylogram showing one of four MP trees 9 282 steps long revealed by PAUP from an analysis of the combined 4-gene (LSU, *rpb1*, *rpb2*, *tef1*) matrix of selected *Xylariomycetidae*, *Sordariomycetidae* and *Nectriaceae*, showing the phylogenetic position of *Thyridium*, *Thyronectria* and *Mattiolia* (given as *Thyronectria roseovirens* in the tree). MP and ML bootstrap support above 50 % and Bayesian posterior probabilities above 90 % are given above or below the branches. Strain/culture designations are given following the taxon names; new species are marked in **bold italics**. Nodes marked by an asterisk (\*) collapsed in the strict consensus of the four MP trees.

the 4-gene matrix for all loci the GTR+I+G model; in the 7-gene matrix for *tub* TRN+G and for the remaining loci the GTR+I+G model. As the TRN+G model could not be implemented in Mr-Bayes, the GTR+I+G model was applied as most similar model for all partitions. Three parallel runs of four incrementally heated, simultaneous Markov chains were performed over 10 million

generations, of which every 1 000th tree was sampled in each run. The first 500 trees sampled were discarded, and a 90 % majority rule consensus of the remaining trees was computed to obtain posterior probabilities (PP). To test convergence of runs, the results were analysed using AWTY (Nylander et al. 2008); no indication of lack of convergence was detected.

**Fig. 2** Phylogram showing one of 216 MP trees 6 654 steps long revealed by PAUP from an analysis of the combined 7-gene (*act*, ITS, LSU, *rpb1*, *rpb2*, *tef1*, *tub*) matrix of *Allantonectria* and *Thyronectria*, with four species of *Nectria* s.str. selected as outgroup. MP and ML bootstrap support above 50 % and Bayesian posterior probabilities above 90 % are given above or below the branches. Strain/culture designations are given following the taxon names; new species are formatted in **bold**; holo-, neo- or epitype strains/specimens are marked by an asterisk (\*).



## RESULTS

### Molecular phylogeny

Of the 4 178 characters included in the combined 4-gene matrix, 1 573 were parsimony informative (161 in LSU, 403 in *rpb1*, 524 in *rpb2* and 485 in *tef1*). MP analyses revealed four MP trees with a score of 9 282, one of which is shown as Fig. 1. The four MP trees differed in the nodes lacking MP bootstrap support (Fig. 1); i.e. the position of *Thyronectria aurigera* which is either sister to all other *Thyronectria* species or sister to the *T. asturiensis*/*T. obscura*/*T. roseovirens* clade, and in some topological differences within the residual *Nectriaceae* clade. Tree topologies of the Bayesian analyses were fully congruent with the MP tree. The three Bayesian runs revealed almost identical posterior probabilities. MP and ML bootstrap support above 50 % and Bayesian posterior probabilities above 90 % are given in Fig. 1 in this order above or below the branches.

Of the 6 150 characters included in the combined 7-gene matrix, 1 794 were parsimony informative (159 in *act*, 102 in ITS, 78 in LSU, 280 in *rpb1*, 440 in *rpb2*, 419 in *tef1* and 316 in *tub*). MP analyses revealed 216 MP trees with a score of 6 654, one of which is shown as Fig. 2. All MP trees were identical except for minor topological differences within the same species (data not shown). Tree topologies of the Bayesian analyses were fully congruent with the MP tree. The three Bayesian runs revealed almost identical posterior probabilities. MP and ML bootstrap support above 50 % and Bayesian posterior probabilities above 90 % are given in Fig. 2 in this order above or below the branches.

As shown in Fig. 1, molecular data confirm the placement of *Thyronectria* and '*Mattirolia*' (*T. roseovirens*) within *Nectriaceae* (*Hypocreomycetidae*), whereas *Thyridium vestitum* is placed as sister to *Papulosaceae* within *Sordariomycetidae*. Within *Thyronectria*, tree topologies agree well with Hirooka et al. (2012) except for slightly different positions of *T. aurigera* in the 7-gene tree and of *T. lamyi* and *T. quercicola* in both trees (Fig. 1, 2).

*Thyronectria roseovirens*, the type species of *Mattirolia*, and the two newly described *T. asturiensis* and *T. obscura* form a highly supported clade at near basal (Fig. 1) or basal (Fig. 2) position within *Thyronectria* (Fig. 1, 2). The third new species, *T. caudata*, originally described as a variety of *T. lamyi*, is sister species of *T. lamyi* with maximum support in all analyses (Fig. 1, 2).

### Taxonomy

Available generic names for the monophylum treated here are *Aponectria* (Sacc.) Sacc., *Chilonectria* Sacc., *Mattirolia* Berl. & Bres., *Pleonectria* Sacc., *Scoleconectria* Seaver, *Thyronectria* Sacc. and *Thyronectroidea* Seaver. The oldest of these names is *Thyronectria*. The original species *Thyronectria patavina* Sacc. (1875b), on which Saccardo based his genus, was determined to be a synonym of *Sphaeria rhodochlora* Mont. (1834), which is older than *T. patavina*. Therefore the correct epithet of the type species of *Thyronectria* is *T. rhodochlora*.

***Thyronectria* Sacc., Grevillea 4: 21. 1875**

- = *Pleonectria* Sacc., Mycoth. Veneta no. 688. 1876.
- = *Chilonectria* Sacc., Michelia 1: 279. 1878.
- = *Nectria* subg. *Aponectria* Sacc., Michelia 1: 296. 1878.
- = *Aponectria* (Sacc.) Sacc., Syll. Fung. 2: 516. 1883.
- = *Mattirolia* Berl. & Bres., Micromycet. Trident.: 55. 1889.
- = *Scoleconectria* Seaver, Mycologia 1: 197. 1909.
- = *Thyronectroidea* Seaver, Mycologia 1: 206. 1909.

*Type species. Thyronectria rhodochlora* (Mont.) Seeler.

*Stromata* immersed-erumpent from bark, uni- to multiperitheciate, scattered, aggregated in groups or compact and pulvinate;

stromatic tissue soft, light coloured, surrounding individual ascomata or formed as a hypostroma, pseudoparenchymatous or prosenchymatous or both, KOH- or KOH+, typically upper surface covered by KOH- yellow-green amorphous scurf. *Ascomata* perithecial, immersed in a stroma or superficial and typically densely aggregated on a hypostroma immersed below the bark epidermis, variously shaped, mostly globose to flask-shaped, often collapsed cupulate when dry; apex (ostiole region) obtuse, often black when mature. *Peridium* pseudoparenchymatous, consisting of 2–4 layers, the pigmented outer layer(s) in section subhyaline, yellow, orange, red or light brown, when dry yellow-orange, shades of red to brown or black, often distinctly thickened around the ostiole; KOH- or KOH+. *Hamathecium* present as periphyses in the ostiole and as apical paraphyses, branched and anastomosing, descending from an apical cushion to the bases of asci, typically present at maturity, cylindrical or as submoniliform bands. *Asci* unitunicate, oblong or clavate, with undifferentiated apex, containing 8 uni- or biseriate ascospores. *Ascospores* ellipsoid, oblong, fusiform, globose, clavate or vermiform, hyaline, yellowish, rosy, green or brown, with eusepta and/or distosepta becoming indistinct in KOH, 1- to several-septate or muriform, smooth or striate, sometimes budding in the ascus to produce oblong to allantoid, 1-celled, hyaline, ascoconidia.

**Asexual morph on natural substrates** — When present, effuse, conidial masses forming on white mycelium and conidiophores or pycnidial or both. *Pycnidia* occurring together with perithecia on the same hypostroma or separately, solitary or aggregated in groups, erumpent-superficial, subglobose to irregularly discoid to cupulate or elongate and erect, rosy, orange, red, violaceous brown to nearly black, KOH+ or KOH-. Sterile hyphae sometimes present inside the pycnidium. *Conidiophores* densely packed, simple, irregularly or verticillately branched; conidia formed on minute pegs or cylindrical to subulate phialides, conidial formation enteroblastic. *Conidia* hyaline, oblong, ellipsoid or (sub)allantoid, 1-celled.

**Asexual morph in culture** — Conidia formed on minute pegs produced on hyphae in the colony above the agar surface or, particularly on CMD, submerged in agar; conidia also formed on pegs and lageniform to ampulliform phialides produced on simple, unbranched or branched, sometimes verticillate conidiophores or formed in pycnidia on mostly lageniform to subulate phialides on densely aggregated, simple, asymmetrically or verticillately branched, often shrub- or fan-shaped conidiophores. *Conidia* oblong, ellipsoid, cylindrical or allantoid, hyaline, 0- (rarely 1- or 2-)septate, smooth.

**Habitat** — On dead corticated twigs or branches of woody plants and/or fungi colonizing them.

**Distribution** — Mostly north and south temperate, sometimes also found in subtropical regions of Asia, the Caribbean, Central and South America.

***Thyronectria rhodochlora* (Mont.) Seeler, J. Arnold Arbor. 21: 455. 1940. — Fig. 3–5**

*Basionym. Sphaeria rhodochlora* Mont., Anns Sci. Nat., Bot., sér. 2, 1: 307. 1834.

= *Trichosphaeria rhodochlora* (Mont.) Sacc., Syll. Fung. (Abellini) 1: 454. 1882.

= *Mattirolia rhodochlora* (Mont.) Berl. (as '*rhodochlora*'), Atti Congr. Bot. Int. Genova: 574. 1892.

= *Pleosphaeria rhodochlora* (Mont.) Sacc., Syll. Fung. (Abellini) 2: 306. 1883.

= *Pleosphaeria mutabilis* Sacc., Syll. Fung. 2: 306. 1883.

= *Mattirolia mutabilis* (Sacc.) Checa, M.N. Blanco & G. Moreno, Mycotaxon 125: 153. 2013.

= *Strickeria mutabilis* (Sacc.) G. Winter, Rabenh. Krypt.-Fl., ed. 2, 1, 2: 288. 1885.

= *Thyronectria patavina* Sacc., Atti Soc. Veneto-Trentina Sci. Nat. 4: 123. 1875.



- ≡ *Nectria patavina* (Sacc.) Rossman, Mem. New York Bot. Gard. 49: 260. 1989.
- ≡ *Valsonectria patavina* (Sacc.) Cooke, Grevillea 12: 105. 1884.
- = *Nectria pyrrochlorata* Auersw. (as '*pyrrochlorata*'), in Rabenhorst, Hedwigia 8: 88. 1869.
- ≡ *Calonectria pyrrochlorata* (Auersw.) Sacc. (as '*pyrrochlorata*'), Michelia 1: 251. 1878.
- ≡ *Thyronectria pyrrochlorata* (Auersw.) Sacc., Michelia 2: 325. 1881.
- ≡ *Valsonectria pyrrochlorata* (Auersw.) Cooke, Grevillea 12: 105. 1884.
- ≡ *Pleonectria pyrrochlorata* (Auersw.) G. Winter, Rabenh. Krypt.-Fl., ed. 2, 1, 2, II. Abt.: Ascomyc.: Gymnoascen: 108. 1884.
- ≡ *Mattirolia pyrrochlorata* (Auersw.) Starbäck, Bih. Kongl. Svenska Vetensk.-Akad. Handl., Afd. 3 19 (no. 2): 43. 1894.

**Typification.** Lectotype of *Sphaeria rhodochlora*, here designated: FRANCE, Lyon, Chateau de Rocheardon, sub cortice alni (not *Alnus*, but *Acer campestre* or *A. platanoides* as determined by xylotomy), no date given, *Montagne* (PC0084652; MBT177536); isolectotype: FRANCE, Lyon, no date given, *Montagne* (K(M) 171594; as *Trichosphaeria rhodochlora* (Mont.) Sacc.). Lectotype of *Pleosphaeria mutabilis*, designated by Checa et al. (2013): FRANCE, Jura, La Bouloie, Saule marceau (*Salix*), soc. ?*Diplodia* sp., L. Quélet 406223 (UPS F-126406, as *Sphaeria mutabilis*). Holotype of *Thyronectria patavina*: ITALY, Padua, host given as *Jugans regia* (not supported by xylotomy), Dec. 1874, P. Saccardo (PAD). Isotype of *Pleonectria pyrrochlorata*: GERMANY, Arnstad, on *Acer campestre*, on/soc. *Diplodia* sp., *Fleischhack*, in Rabenhorst, *Fungi Europaei exsiccati* 1234 (WU). Epitype of *Sphaeria rhodochlora*, *Pleosphaeria mutabilis* and *Thyronectria patavina*, here designated: AUSTRIA, Niederösterreich, Gießhübl, on *Acer campestre*, on/soc. *Diplodia* sp., 18 Mar. 2012, H. Voglmayr (WU 31656; culture CBS 136006 = NP3; MBT177537). All mentioned type materials were studied.

**Stromata** immersed-erumpent from bark, at the sides usually surrounded by bark flaps, rarely superficial, stromatic tissue surrounding ascomata that are scattered or aggregated in groups of 2–40(–80) individually, sometimes uniting them into compound pulvinate stromata 0.7–5.3(–9.8) mm long (n = 29), (0.2–)0.3–0.6(–0.8) mm high (n = 25), often with rosy to light or reddish brown sides; tissue consisting of loosely or densely interwoven, (2.0–)3.0–5.5(–7.0) µm wide (n = 30) hyphae with walls to 1 µm thick, in places appearing more cellular, subhyaline to yellowish; covered by greenish yellow or light green scurf on the upper surface. **Scurf** amorphous, consisting of minute particles, turning brown in lactic acid. **Ascomata** subglobose, ellipsoid or cylindrical, (215–)300–500(–730) µm diam (n = 72) in surface view including stroma, in section (335–)435–620(–675) µm high, (175–)225–395(–505) µm diam (n = 21). **Peridium** orange-red, dark red, brown to nearly black when dry, (22–)30–47(–55) µm (n = 21) wide at the sides, consisting of a thin, up to 15 µm thick, inner layer of strongly compressed hyaline filiform cells and a pigmented outer layer of compressed, thick-walled cells (5.0–)5.5–12.5(–21.0) × (2.2–)3.2–5.5(–7.5) µm (n = 50), yellowish to dull orange-red in water, orange(-red) in KOH, lactic acid and 50 % glycerol, lighter and more yellow-brown at the top; cells around the ostiole small and more isodiametric; without a distinct pH-dependent colour change. **Ostiolar region** 65–190(–290) µm diam (n = 30), mostly obscure, usually concealed by the scurf, less commonly broad, black, smooth. **Ostioles** (120–)128–187(–213) µm long, at the apex (21–)48–91(–105) µm wide inside and (72–)105–180(–250) µm outside (n = 21), filled with periphyses. **Periphyses** narrow, 0.5–2.5 µm wide, pointed, short, 20–25 µm projecting into ostioles and slightly downwards. **Apical paraphyses** usually numerous, indistinct in KOH, embedded in a slime matrix when immature, descending from the top of the ascoma, richly branched and anastomosing, mostly 1.5–4.5 µm wide, free ends between ascus bases widened to 6–8 µm, sometimes becoming submoniliform, distinctly longer and wider, clearly differentiated from periphyses. **Asci** oblong or clavate, (92–)101–129(–137) × (16–)17–26(–31) µm (n = 30), with variable stipe and undifferentiated apex, containing 8 obliquely uniseriate or biseriate ascospores. **Ascospores** ellipsoid or oblong, straight or curved, (15–)18–25(–37) ×

(7–)9–12(–16) µm, l/w = (1.4–)1.8–2.4(–3.3) (n = 419), muriform, with (3–)5–7(–9–10) transverse and (1–)2(–3–4) longitudinal, less commonly oblique septa, hyaline and often more oblong when immature, turning yellowish to rosy or pale brownish at full maturity, smooth, sometimes budding when overmature; ascoconidia 1-celled, hyaline, oblong to mostly allantoid, (3.5–)4.2–5.5(–6.0) × (0.9–)1.1–1.4(–1.5) µm, l/w = (3.0–)3.5–4.3(–4.7) (n = 25).

**Asexual morph on natural substrates** — None seen.

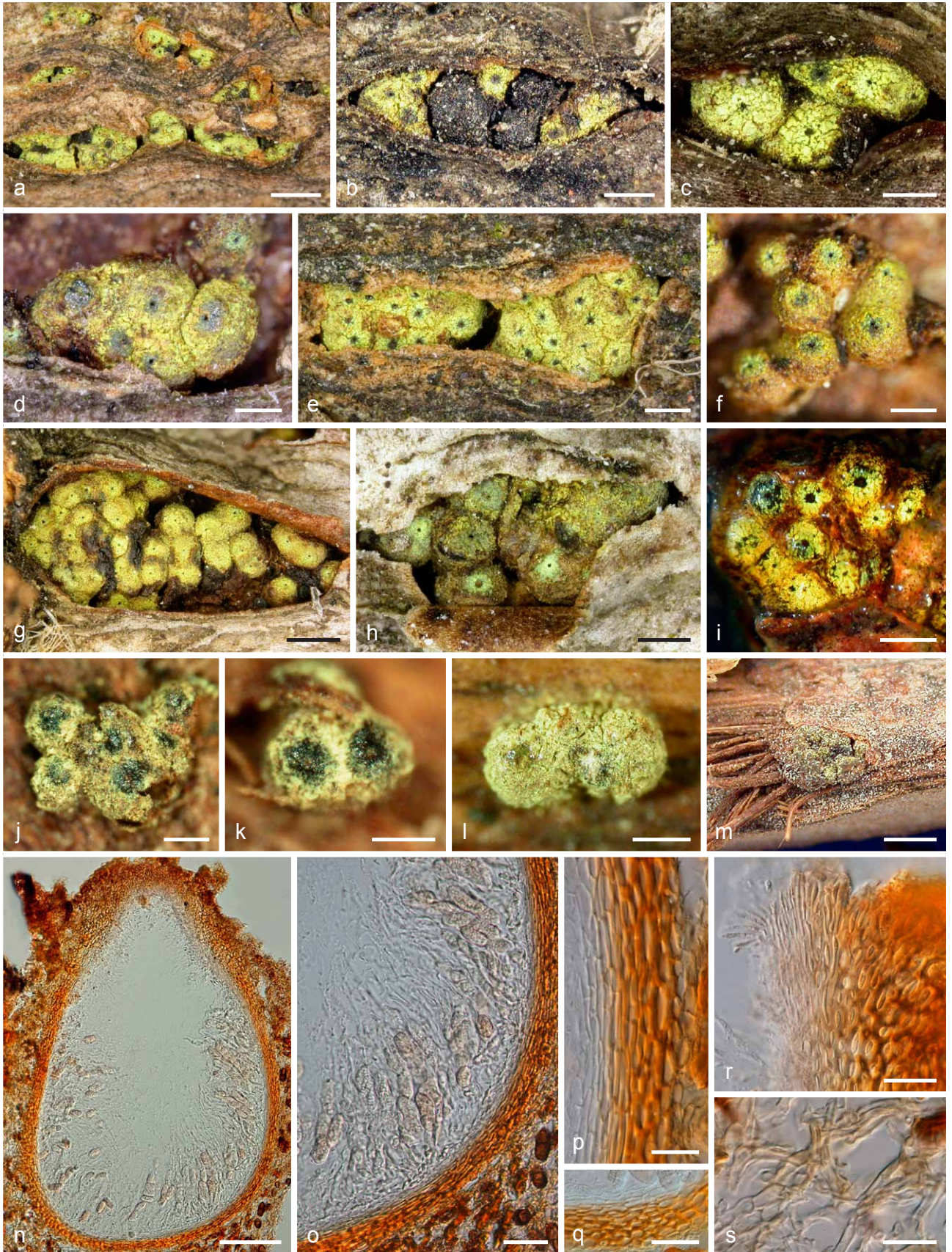
**Cultures and asexual morph** — Germination of ascospores with conidia and/or hyphae; growth slow, on MEA slightly better than on CMD and PDA, on CMD at 25 °C after 10 d colony radius e.g. 27 mm, on CMD and MEA centrally inoculated plate entirely covered after 2–4 wk at 20–25 °C, colony circular, dense, colourless to yellowish or dull brownish, surface turning rosy from the centre due to conidial masses, sometimes surface after c. 1 mo covered by yellow 'scurf' of aerial hyphae, odour indistinct to yeast-like. **Conidiation** effuse; conidia formed on CMD at 25 °C within 24 h on minute pegs on hyphae in the colony or on solitary, rarely paired phialides on short, more or less erect, simple or loosely branched, narrow conidiophores. **Phialides** lageniform to ampulliform, (4.3–)4.5–7.7(–11.2) × (2.3–)2.5–3.5(–4.2) µm, l/w = (1.5–)1.6–2.6(–3.3) (n = 22), more or less straight, mostly inequilateral. **Conidia** oblong to suballantoid, 1-celled, (3.8–)4.3–7.8(–14.2) × (1.0–)1.2–2.2(–4.0) µm, l/w = (2.4–)3.2–4.3(–5.0) (n = 163), when swollen sometimes with 1–2 thin septa, smooth. Pycnidia sometimes formed in culture, e.g. in NP7 on MEA after 1 mo at RT and a further 1 mo at 15 °C. **Pycnidia** 0.4–0.8(–1.4) mm diam, subglobose to globose, often on a short broad stipe, solitary or in dense clusters up to 2.5 mm diam, first white, turning pale to greenish yellow, contents hyaline, with a rosy shine. **Peridium** pseudo-parenchymatous, of cells (3.5–)4.0–7.5(–10) µm diam (n = 30) with walls to c. 1 µm thick, yellow, not changing in 3 % KOH. **Conidiophores** densely packed, parallel and simple or short shrub- or fan-shaped, often dichotomously branched, hyaline, filiform, 1.5–3 µm wide, cells sometimes thickened to 6 µm. **Phialides** solitary or in small clusters of 2–3, subulate, (9.0–)10.2–13.2(–14.3) × (1.5–)1.7–2.2(–2.5) µm, l/w = (4.4–)5.4–7.0(–7.5) (n = 30). **Conidia** oblong to mostly allantoid, (3.2–)4.0–5.2(–5.7) × (1.1–)1.2–1.5(–1.7) µm, l/w = (2.6–)2.8–4.0(–5.2) (n = 30), 1-celled, hyaline, smooth, eguttulate, oozing out from the pycnidial apex in mucous masses.

**Habitat** — On fungi, typically *Diplodia* spp. colonising dead corticated branches or twigs, mainly of *Acer campestre*, but also found on *Acer opalus*, *Corylus avellana*, *Koelreuteria paniculata*, *Prunus tenella*, *Robinia pseudoacacia*, *Salix caprea* and *Ulmus minor*; also recorded from *Cydonia oblonga* and *Vitis vinifera* (Seeler 1940).

**Distribution** — Europe (Austria, Czech Republic, France, Germany, Italy).

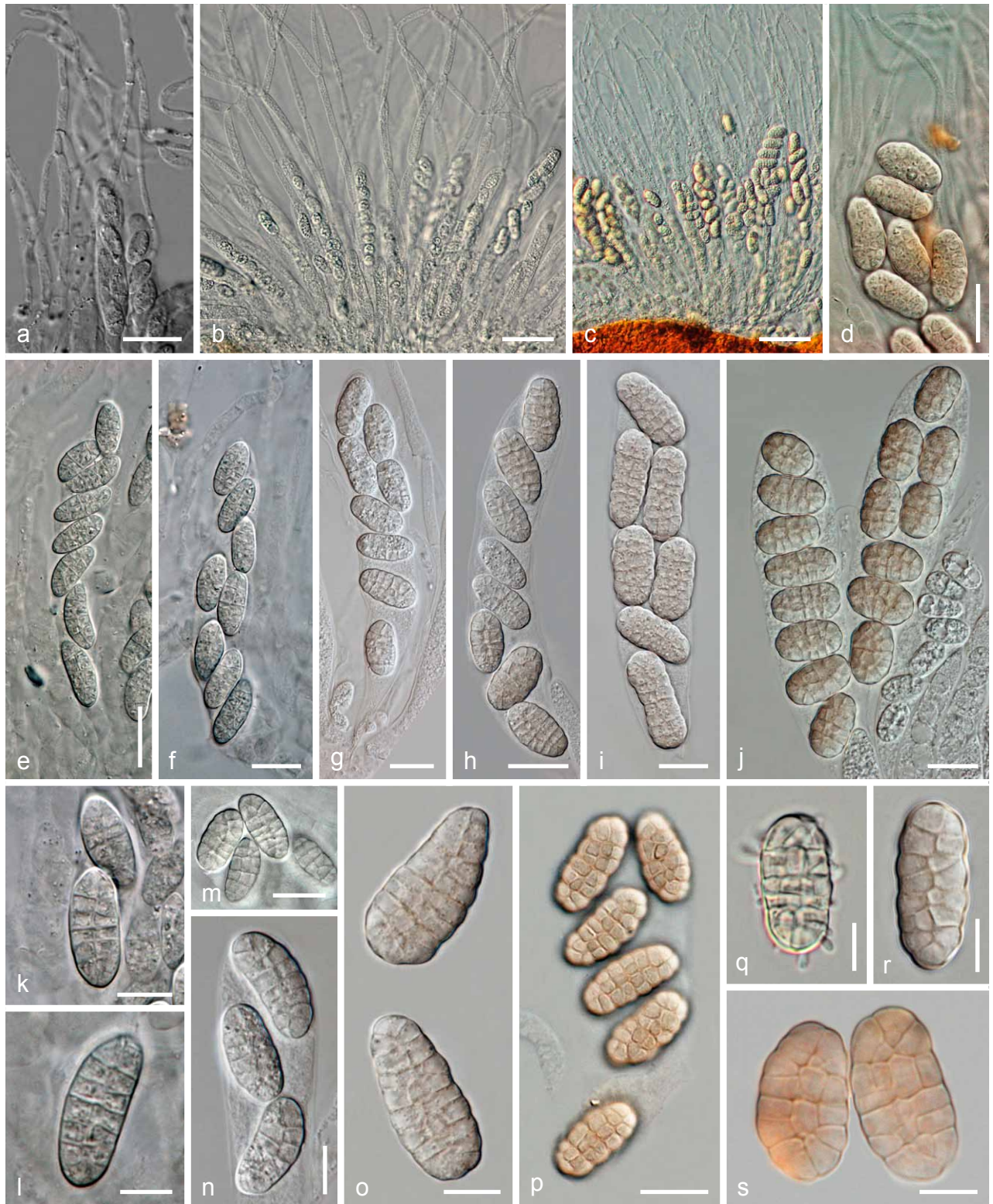
**Other material studied** (all on dead corticated twigs on the ground or attached to the trees): AUSTRIA, Burgenland, Breitenbrunn, Tenauriegel, on *Ulmus minor* (part a), *Corylus avellana* (part b) and *Acer campestre* (part c), on/soc. *Diplodia* sp., 22 Mar. 2014, H. Voglmayr & I. Greilhuber (WU 33425; culture NP8 from WU 33425a, culture NP9 from WU 33425b); Niederösterreich, Bad Fischau, on *Acer campestre*, on/soc. *Diplodia* sp., 13 Nov. 2011, H. Voglmayr (WU 31655; culture CBS 136005 = NP2); Mühlleiten, on *Acer campestre*, on/soc. *Diplodia* sp., *Fusarium* sp., *Valsa* sp. and *Valsaria insitivata*, 23 Mar. 2013, H. Voglmayr (WU 32149; culture NP4); Vienna, 3rd district, Botanical Garden, on *Prunus tenella*, on/soc. *Pyrenochaeta* sp. and *Diplodia* sp., 10 Sept. 2013, H. Voglmayr (WU 32152; culture NP7); *ibid.*, same host, on/soc. *Diplodia* sp., 18 Mar. 2009, H. Voglmayr (WU 32628); *ibid.*, on *Acer campestre*, on/soc. *Diplodia* sp., 11 Nov. 2013, W. Jaklitsch & H. Voglmayr (WU 32160); 19th district, Bellevuestraße, grid square 7763/2, on *Acer campestre*, on/soc. *Diplodia* sp. and *Pyrenochaeta* sp., 11 Mar. 1995, W. Jaklitsch W.J. 514 (WU 32145); between Himmelstraße and Unterer Reisenbergweg, grid square 7763/2, on *Acer campestre*, on/soc. *Diplodia* sp., *Dothidotthia ramulicola*, 24 Apr. 1999, W. Jaklitsch W.J. 1306 (WU 32146, BPI 746398);





**Fig. 3** *Thyronectria rhodochlora*, stromata and ascomata. a–h, j–m. Dry stromata in surface view (a. habit; b. with pycnidia of a *Diplodia* sp.); i. stromata in 3% KOH after rehydration; n. perithecium in vertical section; o. lower lateral portion of a perithecium showing asci and apical paraphyses in section; p–r. peridium in section (q. basal region; r. ostiolar region with paraphyses and scurf); s. stroma hyphae (n–p, r: in 50% glycerol; q, s: in lactic acid). a, e, i, n–s: epitype WU 31656 (NP3); b, c, g: WU 32152 (NP7); d, f, h: WU 31654 (NP1); j, k: lectotype (PC); l: isolectotype (K); m: holotype of *T. patavina* (PAD). — Scale bars: a, m = 1 mm; b, e, h, i = 0.4 mm; c, d, f, j–l = 0.3 mm; g = 0.7 mm; n = 100  $\mu$ m; o, q = 30  $\mu$ m; p, r, s = 15  $\mu$ m.





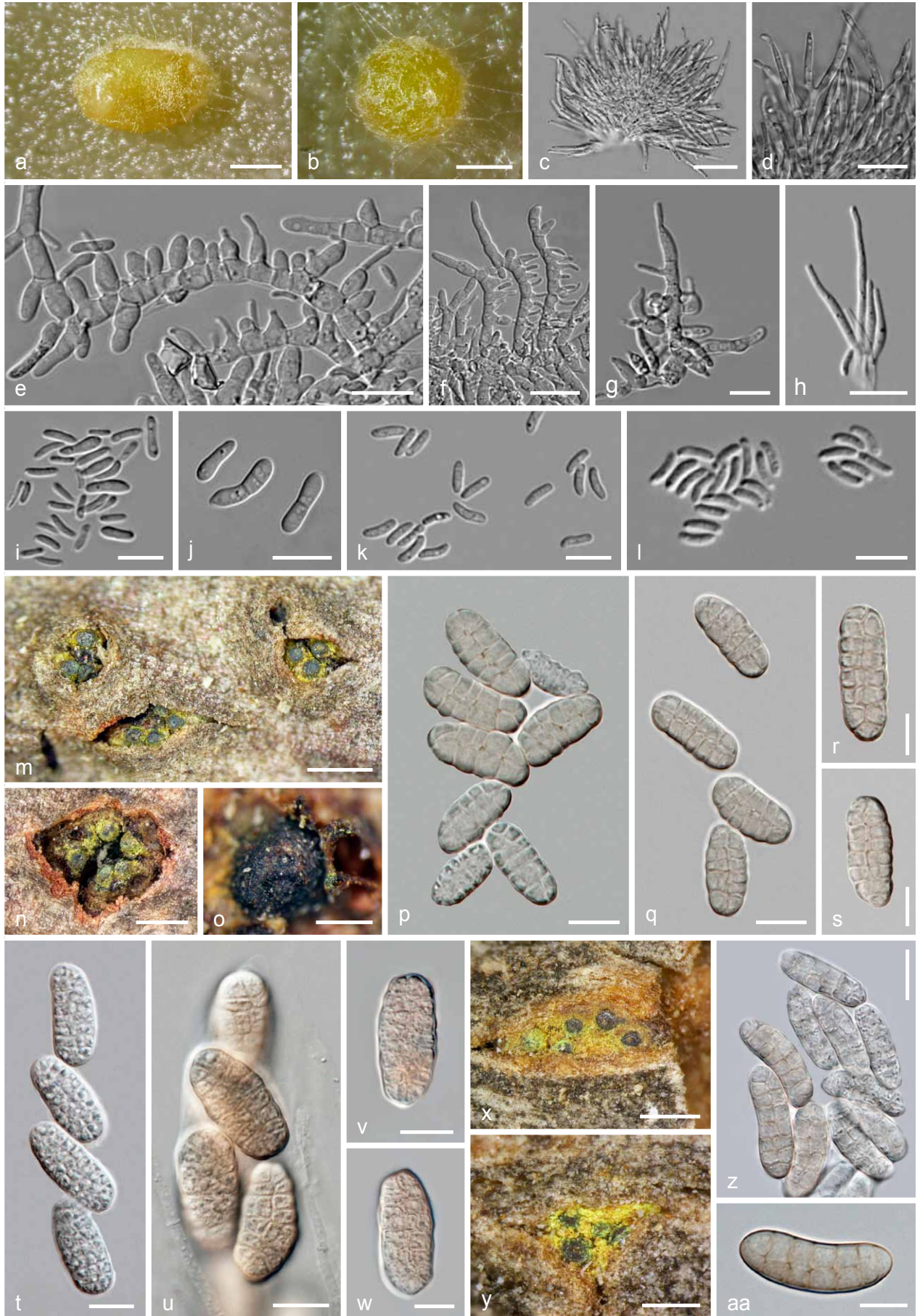
**Fig. 4** *Thyronectria rhodochlora*, centrum characteristics. a–d. Apical paraphyses among immature (a, b) and mature (c, d) asci in water; e–j. asci with ascospores; k–s. ascospores (q. overmature, producing conidia; all in water except p (in water after swelling in 3 % KOH) and s. (in lactic acid in a microtome section)). a, e, f, k, l: *T. patavina* holotype (PAD); b: WU 32150 (NP5); c, h, i, o, s: epitype WU 31656 (NP3); d, p: *T. rhodochlora* isolectotype (K); g, n, r: WU 31654 (NP1); j: WU 32152 (NP7); m: *T. rhodochlora* lectotype (PC); q: WU 33425a (NP8). — Scale bars: a, e, h = 20 µm; b = 30 µm; c = 50 µm; d, f, g, i, j, m, p = 15 µm; k, n, o = 10 µm; l, q–s = 7 µm.

culture CBS 125131); 21st district, Donauturmstraße, grid square 7764/3, on *Koelreuteria paniculata*, on/soc. *Camarosporium* sp., 8 Sept. 2002, W. Jaklitsch W.J. 1935 (WU 32147); *ibid.*, on *Koelreuteria paniculata*, on/soc. *Diplodia* sp., *Camarosporium* sp. and ?*Pyrenochaeta pycnidia*, 31 Aug. 2013, W. Jaklitsch & H. Voglmayr (WU 32150; culture NP5); *ibid.*, on *Acer campestre*, on/soc. *Diplodia* sp., 31 Aug. 2013, H. Voglmayr & W. Jaklitsch (WU 32151; culture NP6); 22nd district, Lobau, Panozzalacke, grid square 7865/1, on *Acer campestre*, on/soc. *Diplodia* sp., 29 Oct. 2002, W. Jaklitsch W.J. 2019 (WU 32148). — FRANCE, Rougon, Gorge du Verdon, on *Acer opalus*, soc. *Fene-*

*stella* sp., 29 July 2011, H. Voglmayr (WU 31653; culture CBS 136004 = NP). — ITALY, Veneto, Galzignano, at Turri, on *Robinia pseudoacacia* (part a) and *Acer campestre* (part b), on/soc. *Diplodia* sp., 23 Oct. 2011, W. Jaklitsch & H. Voglmayr (WU 31654a, b; culture NP1 from WU 31654a).

**Notes** — As already outlined by Hirooka et al. (2012), *T. rhodochlora* (as *Pleonectria pyrrochlora*) is similar to the closely related *T. virens* and *T. zanthoxyli* in that they have ascomata covered by bright yellowish green scurf. Additionally, ascomata





**Fig. 5** a–l: *Thyronectria rhodochlora*, asexual morph. a, b. Pycnidia (MEA, 15 °C, 65 d); c–h. conidiophores and phialides (c, d, h. from pycnidium on MEA, 15 °C, 65 d; c. shrub-like; e–g. from effuse conidiation, showing pegs and phialides; MEA, RT, 3 d); i–l. conidia (i, j: MEA, RT, 3 d; k, l: from pycnidium on MEA, 15 °C, 65 d) (all in water except h (in 3 % KOH)). a–d, h, k, l: NP7; e–g, i, j: NP. — m–s. *Thyronectria virens* (isolectotype BPI 631193). m–o. Ascomata in bark (o. old open ascoma attached to the side of a *Diplodia* conidioma); p–s. ascospores. — t–w. Ascospores of *Thyronectria* cf. *virens* (WU 33426; u. in ascus, note apical paraphyses). — x–aa. *Thyronectria zanthoxyli*. x, y. Stromata; z, aa. ascospores. x, y, aa. lectotype NYS 3611; z. isolectotype NYS 3438. — Scale bars: a, m, n, x = 0.5 mm; b, o, y = 0.3 mm; c = 20 µm; d, f = 15 µm; e, g–j, p, q, t, u, v, z = 10 µm; k, l, r, s, w, aa = 7 µm.



of these species are embedded in stromatic tissue. *Thyronectria rhodochlora* differs from the other species by the width of mature ascospores averaging > 9 µm and mostly two longitudinal septa and by the absence of pycnidia. Here we report the formation of pycnidia in cultures of this species for the first time, i.e. this character is shared among the three species. Initially, ascospores of *T. rhodochlora* are hyaline and tend to be oblong, often curved and mostly < 10 µm wide. When fully mature, they are rosy or yellowish with a rosy tint in water, KOH and lactic acid, and this colour is characteristic for *T. rhodochlora* and its closest relatives, *T. virens* and *T. zanthoxyli*. No greenish colour has been seen in ascospores of any of the numerous specimens examined. Ascospores have commonly 5–7 transverse septa, but, as also shown by Saccardo (1877), a small fraction may form up to 9, rarely 10 septa. We have seen this septation consistently in fresh material of several specimens, but also in type material of *P. mutabilis*, *T. rhodochlora* and *T. patavina*. *Thyronectria rhodochlora* (formerly *Pleonectria pyrrochlora*) has been thought to specifically occur on *Acer campestre*, but as can be seen from the list above, we have collected this species also on other trees, in association with or directly on *Diplodia* spp. or other fungi.

In the lecto- and isolectotype specimens of *T. rhodochlora* stromata are superficial on wood and partly covered by bark fibres; lower free sides of the perithecia are light to reddish brown, turning slightly more orange-red in 3% KOH; the ostiolar area is large, convex or flattened, black, or scarcely visible and diffusely delimited due to the scurf. The apical paraphyses are numerous, the asci mostly oblong, with ascospores that are hyaline, yellowish to pale rosy, ellipsoid or oblong, partly curved, with (4–)6–7(–9) transverse and (1–)2 longitudinal septa, smooth, and not budding. Based on xyotomy and bark structure, the host is clearly a species of *Acer*, either *A. campestre* or *A. platanoides*.

The holotype specimen of *T. patavina* in PAD consists of several, partly corticated twig fragments. As already reported by Rossman et al. (1999), only little material of the described fungus remains; on two small fragments there are some scattered perithecia and one small stroma, consisting of densely aggregated, minute perithecia with yellow-green scurf. We studied a perithecial section: The perithecial wall is dull orange-red to nearly black when dry; numerous branched, 2–4 µm wide apical paraphyses are present. The asci are oblong-fusoid, with a short stipe, without a differentiated apex, each containing 8 uni- to biseriolate ascospores. Ascospores are oblong to ellipsoid, also slightly inequilateral or curved, (14–)16–20(–22) × 6.5–8.5(–10.7) µm, hyaline, muriform, with 5–7(–9) transverse and 1(–3) longitudinal septa, and sometimes more irregularly septate, i.e. with some oblique instead of longitudinal septa. The host is not *Juglans regia*, because the twigs of the holotype do not have the characteristically chambered pith of *Juglans*. The overall appearance including the coarse cortical fibres below the epidermis of the bark suggests *Populus* sp. or *Salix* sp. No *Thyridaria incrustans* as reported by Saccardo is present, but among other fungi black pycnidia of a *Diplodia* sp. occur in the bark, just as usually found with *T. rhodochlora*. Rossman et al. (1999) studied another part of the type. They reported that the material was scant, that it contained black stromata covered by yellow-green powder, with yellowish ascomata aggregated in the stroma and included persistent paraphyses in a gelatinous matrix. The ascospores were described as hyaline, irregularly muriform, 16.5–30 × 6.8–8 µm with 5–11 and 1–3 vertical septa in clavate asci. In the material that we examined ascospores had 5–7, only rarely 9 transverse septa, as is typical for *T. rhodochlora*. In the original description Saccardo (1875b) gives ascospores as 25 × 9–11 µm, rarely 30 × 8 µm, with 7–9 transverse septa, which fits *T. rhodochlora*.

The species *T. patavina* was described in the same year as the genus *Thyronectria*, but in a different journal (see above under the synonyms). This has been cited wrongly in all available databases and papers. Only Seeler (1940) found the original, detailed species description and translated it to English. Even Saccardo (1877) himself gave the wrong page number (23 instead of 123) of his publication. The asexual morph that Saccardo (1875b) described as acervuli arranged in a valsoid manner and hyaline allantoid conidia 'mixed with asci' could not be detected in the residual material of the holotype of *T. patavina*. We, however, found ascoconidia in overmature perithecia of two recent collections of the species resembling the conidia depicted by Saccardo (1877).

The lectotype of *Pleospaeria mutabilis* (UPS) includes only scant material that does not permit a re-assessment of the host plant given as *Salix caprea*. The perithecia are scattered and grew directly on a black host fungus with large, 2-celled, dark brown conidia or possibly ascospores that are 18–21 × 8.5–12.5 µm. These most probably represent a *Diplodia* sp. All morphological features of the lectotype are fully in agreement with other specimens of *T. rhodochlora*. Its ascospores have a yellowish colour with a pale rosy tint as shown by illustrations in Checa et al. (2013).

## OTHER SPECIES OF THYRONECTRIA

Below we list species in alphabetical order, describe the three new species *T. asturiensis*, *T. caudata* and *T. obscura*, make 15 new combinations in *Thyronectria*, emend or enlarge descriptions and illustrations or epitypify several selected species that were originally described from Europe, and list some examined specimens that are available in the herbarium WU.

### *Thyronectria aquifolii* (Fr.) Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB808291

- Basionym.* *Sphaeria aquifolii* Fr., Elench. Fung. 2: 82. 1828.  
= *Nectria aquifolii* (Fr.) Berk., Outl. Brit. Fungol.: 393. 1860.  
= *Pleonectria aquifolii* (Fr.) Hirooka, Rossman & P. Chaverri, Stud. Mycol. 71: 106. 2012.  
= *Nectria inaurata* Berk. & Broome, Ann. Mag. Nat. Hist., ser. II, 8: 467. 1854.  
= *Aponectria inaurata* (Berk. & Broome) Sacc., Michelia 1: 296. 1878.  
= *Nectria aquifolii* (Fr.) Berk. var. *appendiculata* Feltgen, Vorstud. Pilzfl. Luxemb. 3: 305. 1903.

*Specimen examined.* UK, Surrey, Richmond, Royal Botanic Gardens, Kew, on dead twigs of *Ilex aquifolium* attached to the tree, 11 Nov. 2008, H. Voglmayr (WU 30360; culture NAK = CBS 125027).

*Notes* — This species seems to be confined to *Ilex aquifolium* in Western Europe. The strain cited above is included in our phylogenetic analyses.

### *Thyronectria asturiensis* Jaklitsch & Voglmayr, *sp. nov.* — MycoBank MB808293; Fig. 6

*Etymology.* Referring to its occurrence in Asturias, Spain.

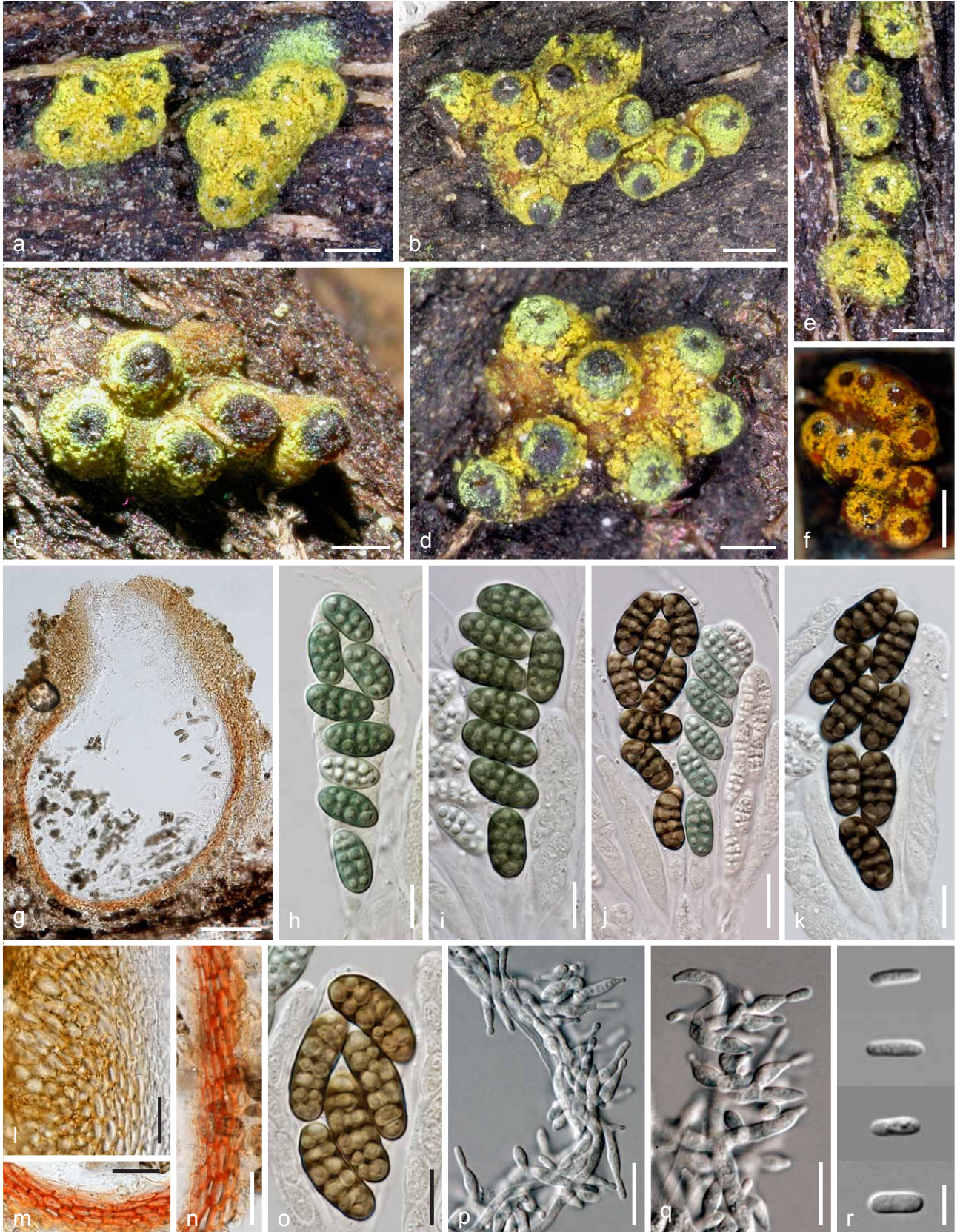
*Holotype.* SPAIN, Asturias, Santiago, on a black subiculum on wood of *Quercus ilex*, soc. *Thyridaria rubronotata*, 23 Mar. 2013, E. Rubio (WU 32124; culture CBS 136000 = MA3).

*Stromata* small, of variable outline, (0.5–)0.6–1.2(–1.4) mm long, (120–)210–420(–550) µm high (n = 20), superficial on wood or partly immersed in black hyphal subiculum of the host; stromatic tissue encasing ascomata that are scattered or aggregated in small numbers to 15, excluding the apex, individually or uniting them into compound stromata; tissue usually narrow, dull yellow to brownish, consisting of pale yellow-brown cells (5.0–)6.5–11.5(–14.0) × (3.0–)4.5–8.5(–10.5) µm (n = 20)



directly around the peridium, otherwise of subhyaline to yellowish, thick-walled, 2–8(–11)  $\mu\text{m}$  wide hyphae, at the base interwoven with host hyphae and wood; in the upper part, except for the perithecial apex, covered by yellow, sometimes partly rosy scurf. Scurf finely granulose, turning dark brown in KOH and lactic acid, releasing some yellow pigment in KOH. *Ascomata* pyriform, blunt-conical to subglobose, (208–)260–400(–470)

$\mu\text{m}$  diam (n = 20) including stromatic tissue when dry, in section (375–)405–500(–530)  $\mu\text{m}$  high, (225–)250–325(–355)  $\mu\text{m}$  diam (n = 18), highly variable in configuration; base orange to reddish or black. *Peridium* (16–)18–23(–25)  $\mu\text{m}$  wide at the base, (13–)17–25(–30)  $\mu\text{m}$  at the sides (n = 18), thickened up to 85  $\mu\text{m}$  around the ostiole, consisting of a narrow subhyaline inner layer of filiform cells, broader and distinct at the apex, and



**Fig. 6** *Thyronectria asturiensis* WU 32124 (MA3). a–f. Stromata/ascomata (f. in 3% KOH); g. perithecium in vertical section; h–k. asci with ascospores; l–n. peridium in section (l. ostiole region; m. base; n. lateral; l. in lactic acid; m, n. in 3% KOH); o. ascospores; p, q. conidiophores and phialides (20 °C, MEA, 6 d); r. conidia (20 °C, MEA, 6 d). — Scale bars: a, b, e = 0.3 mm; c, d = 0.2 mm; f = 0.5 mm; g = 100  $\mu\text{m}$ ; h, i, k, o, q = 10  $\mu\text{m}$ ; j, l, p = 15  $\mu\text{m}$ ; m, n = 20  $\mu\text{m}$ ; r = 5  $\mu\text{m}$ .



a pigmented outer layer of thick-walled (0.5–2 µm), compressed cells, orange to orange-red in lower regions, paler orange upward in water, only slightly more intense in 3% KOH, slightly paler to light brown in lactic acid, paler, more longish and walls yellow incrusting in the ostiole, outwardly tending to be isodiametric. Perithecial apex (ostiole region) (59–)62–186(–295) µm diam (n = 25) when dry, black, papillate, convex or flattened, with circular outline, centrally pierced by the minute umbilicate reddish ostiole, usually only partly covered by the yellow scurf. *Ostioles* (104–)107–158(–195) µm long, (39–)44–77(–108) µm wide inside at the apex, (95–)109–156(–180) µm wide outside (n = 18), with acute lanceolate periphyses partly directed downward. *Apical paraphyses* usually numerous, forming a reticulum, mostly 2.5–4 µm wide, descending to the bases of asci. *Asci* clavate or oblong, (59–)61–81(–96) × (15–)17–23(–26) µm (n = 27), with an indistinct apex and a short but variable stipe, containing 6–8 ascospores bi- to triseriate in the upper part. *Ascospores* oblong or inequilaterally ellipsoid, (14.0–)16.5–20.7(–22.0) × (6.3–)7.3–8.7(–9.3) µm, l/w = (1.6–)2.0–2.7(–3.0) (n = 40), straight or often curved, muriform, with (3–)5 transverse distosepta and 1 longitudinal or oblique distoseptum, first hyaline, turning green and finally dark brown when mature, with a large guttule per cell, ends broadly rounded, no sheath, not budding.

**Cultures** — Growth slow, on CMD colony radius < 5 mm after 7 d, 18 mm after 21 d, colony flat, lacking aerial hyphae, whitish to yellow, centre turning rosy due to conidial masses; colony radius on MEA at 20 °C, 2–3 mm after 6 d, 14 mm after 14 d; colony flat, dense, whitish to orange, margin whitish due to strands of aerial hyphae, central conidial masses mucous, hardening with time, yellow to carrot; odour unpleasant to fruity. *Conidiophores* emerging as fasciculate side branches on strands of parallel hyaline aerial hyphae, short, 1.8–5.2 µm wide, simple, scarcely branched, with few verticils of 2–3 branches. *Phialides* (4.5–)6.0–9.0(–10.8) × (2.0–)2.5–3.2(–3.5) µm, l/w = (1.8–)2.1–3.4(–4.1) (n = 40), solitary or in whorls of 2–4, lageniform, straight, curved or sigmoid, mostly inequilateral; conidia also forming on small pegs. *Conidia* (after 6–14 d at 20 °C on MEA and CMD) cylindrical, (3.0–)4.0–6.3(–8.2) × (1.3–)1.5–2.0(–2.7) µm, l/w = (1.9–)2.4–3.3(–4.4) (n = 120), sometimes swollen toward one end, 1-celled, smooth, straight, scarcely curved, mostly eguttulate; scar indistinct to truncate.

**Habitat** — On dark subicular hyphae of a fungus on dead blackened wood of *Quercus ilex*.

**Distribution** — Europe (Spain). Only known from the holotype.

**Notes** — In ascospore colour *T. asturiensis* is similar to its close relative *T. roseovirens*, but differs from that species in the host fungus and plant, by oblong, often curved, distoseptate ascospores that contain a single guttule per cell and the absence of pycnidia. The host is a subiculum of black hyphae, possibly belonging to the associated *Thyridaria rubronotata*. Mature ascospores resemble those of *Thyridium vestitum*. No difference was seen between conidia examined after 6 and 14 d on MEA and on CMD. They are longer and wider than those of *T. roseovirens*. In phylogenetic analyses (Fig. 1, 2), *T. asturiensis* forms a highly supported clade with *T. roseovirens* and *T. obscura*, which is remarkable, as *T. obscura* is morphologically quite distinct (see below).

***Thyronectria aurigera*** (Berk. & Ravenel) Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB808294

*Basionym.* *Nectria aurigera* Berk. & Ravenel, Grevillea 4: 46. 1875.

≡ *Calonectria aurigera* (Berk. & Ravenel) Sacc., Michelia 1: 308. 1878.

≡ *Pleonectria aurigera* (Berk. & Ravenel) Hirooka, Rossman & P. Chaverri, Stud. Mycol. 71: 110. 2012.

***Thyronectria austroamericana*** (Speg.) Seeler, J. Arnold Arbor. 21: 405. 1940. — Fig. 7a–d

*Basionym.* *Pleonectria austroamericana* Speg., Anales Mus. Nac. Hist. Nat. Buenos Aires 10: 22. 1880.

≡ *Nectria austroamericana* (Speg.) Rossman, Mem. New York Bot. Gard. 29: 257. 1989.

= *Pleonectria denigrata* G. Winter, Bull. Torrey Bot. Club 10: 49. 1883.

≡ *Thyronectria denigrata* (G. Winter) Seaver, Mycologia 1: 204. 1909.

= *Pleonectria guaranitica* Speg., Anales Mus. Nac. Hist. Nat. Buenos Aires 19: 44. 1885.

= *Pleonectria nigropapillata* Starbäck, Ark. Bot. 2: 13. 1904.

*Materials examined.* AUSTRIA, Vienna, 3rd district, Botanical Garden, in bark of *Gymnocladus dioica*, 9 Sept. 2010, H. Voglmayr (WU 32664; culture GG). — USA, Kentucky, Lexington, on dead twigs of *Gleditsia triacanthos*, June 1882, W.A. Kellermann, in: Winter, L. *Rabenhorstii fungi Europaei et extraeuropaei exsiccati* 2948 (WU s.n., isotype of *Pleonectria denigrata*).

**Notes** — This species has been only known from North and South America, but it also occurs in Europe as asexual morph: pycnidia purplish- to dark brown, densely aggregated in large numbers, forming a strongly tubercular to cerebriform structure; conidia ellipsoid to oblong, (2.5–)2.8–3.2(–3.5) × (1.3–)1.4–1.6(–1.8) µm, l/w = (1.6–)1.8–2.2(–2.5) (n = 30).

Microscopic investigation of an isotype of *Pleonectria denigrata* from WU revealed the presence of numerous branched, 2–4 µm wide, apical paraphyses that are persistent in fully mature ascomata.

***Thyronectria berolinensis*** (Sacc.) Seaver, Mycologia 1: 205. 1909

*Basionym.* *Pleonectria berolinensis* Sacc., Michelia 1: 123. 1878.

≡ *Nectria berolinensis* (Sacc.) Cooke, Grevillea 12: 107. 1884.

≡ *Nectria fenestrata* Berk. & M.A. Curtis, in Cooke, Grevillea 12: 81. 1884.

≡ *Pleonectria fenestrata* (Berk. & M.A. Curtis) Berl. & Voglino, Fung. Addit. 1–4: 216. 1886.

*Materials examined.* AUSTRIA, Kärnten, St. Margareten im Rosental, village area, grid square 9452/4, on *Ribes rubrum*, 31 July 1994, W. Jaklitsch W.J. 163 (WU 32125); *ibid.*, 25 Oct. 1998, W. Jaklitsch W.J. 1248 (WU 32126); Vienna, 21st district, Marchfeldkanalweg, on *Ribes sanguineum*, 13 Apr. 2009, W. Jaklitsch (WU 30361; culture CBS 127382 = NB).

***Thyronectria boothii*** (Hirooka, Rossman & P. Chaverri) Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB808295

*Basionym.* *Pleonectria boothii* Hirooka, Rossman & P. Chaverri, Stud. Mycol. 71: 124. 2012.

***Thyronectria caudata*** (Malençon) Jaklitsch & Voglmayr, *comb. & stat. nov.* — MycoBank MB808296; Fig. 8, 9

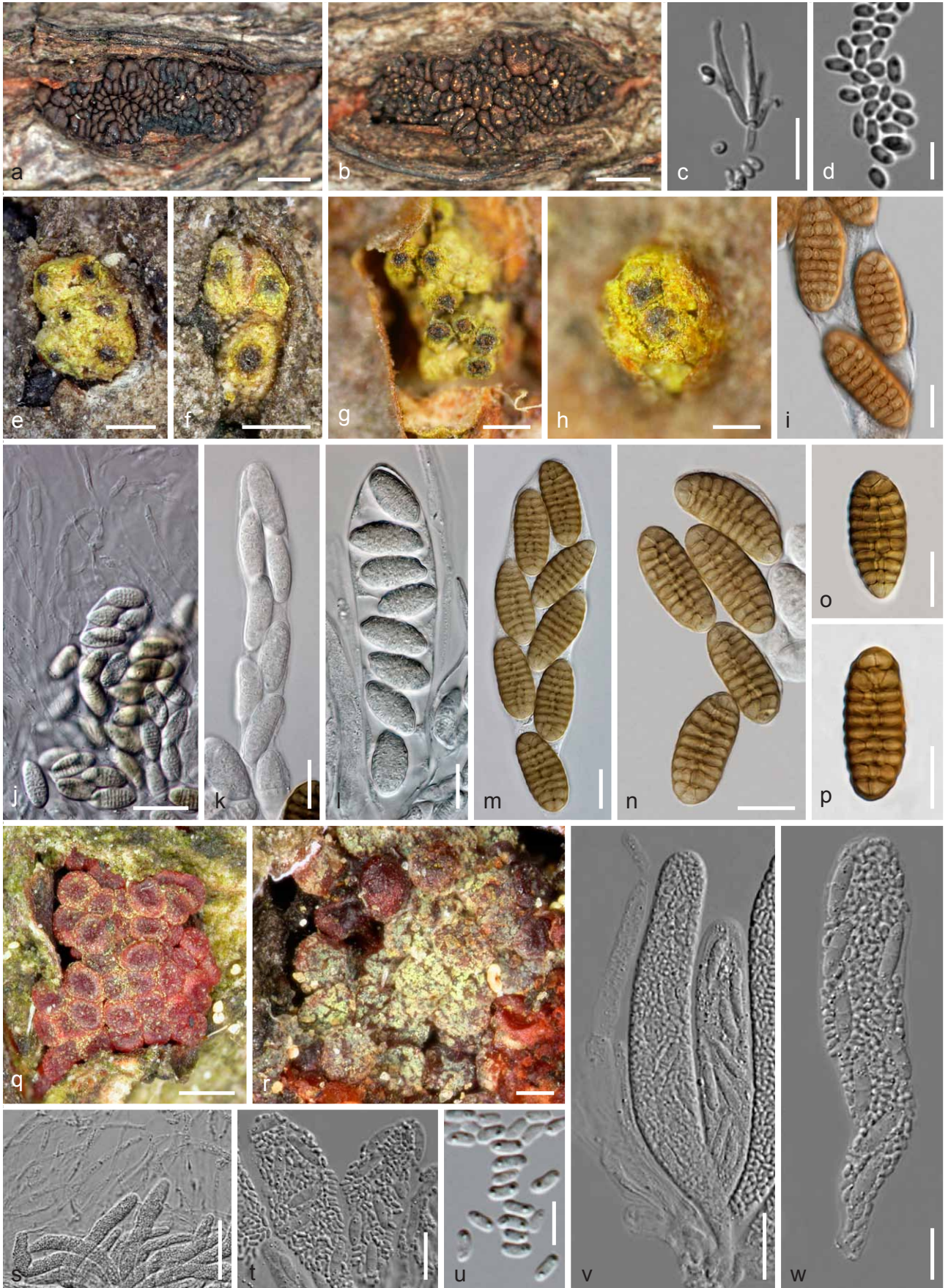
*Basionym.* *Thyronectria lamyi* var. *caudata* Malençon, Bull. Trimestriel Soc. Mycol. France 95: 99. 1979.

= *Thyronectria lamyi* var. *pakistani* E. Müll. & S. Ahmad, Biologia (Lahore) 8, 2: 155. 1962.

*Holotype.* MOROCCO, Middle Atlas, northern slope of Bou-Ighitten, above Aguelmam N' Sidi-Ali, elev. 2200 m, on *Berberis hispanica*, 30 May 1957, G. Malençon (MPU). Epitype, here designated: GREECE, Crete, path to a waste dump off the road to Omalos, on *Berberis cretica*, on/soc. *Cucurbitaria* cf. *berberidis*, *Thyridium* sp. and on inner bark, 28 Nov. 2011, W. Jaklitsch (WU 32130; culture CBS 136003 = NL2; MBT177538).

*Hypostromata* erumpent-superficial, crustose to pulvinate, tubercular, yellow, dark reddish to black when dry, inside yellow, 0.2–0.6 mm thick; pale yellow in water, KOH and lactic acid, pseudoparenchymatous, of isodiametric to oblong cells (4.5–)6.5–14.0(–18.5) × (3.8–)5.0–8.5(–10.0) µm (n = 30) with walls to 1 µm thick, extending as hyphae in the bark and mixed with hyphae of the host, the latter orange in lactic acid and 2.5–4.5 µm wide. *Ascomata* superficial on the hypostroma, typically not surrounded by bark flaps, generally aggregated in numbers of



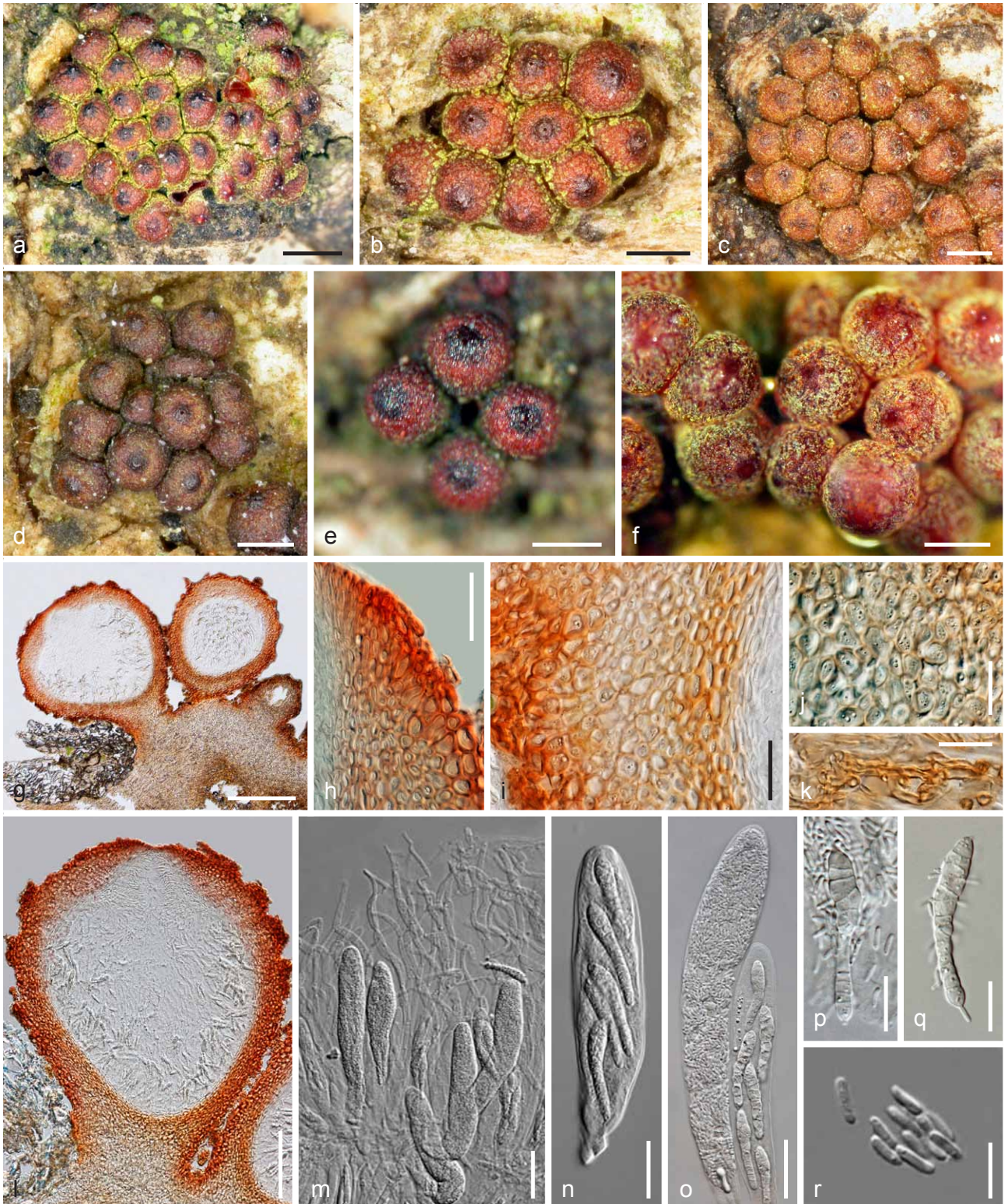


**Fig. 7** a–d: *Thyronectria austroamericana* WU 32664. a, b. Pycnidial aggregates on hypostromata; c. phialides; d. conidia. — e–p: *Thyronectria chrysogramma*. e–h. Stromata (e, f. holotype; g, h. paratype); i, n–p. ascospores; j. apical paraphyses and asci; k–m. asci (k, l. immature; k. showing oblong ascospores). — q–w: *Thyronectria coryli*. q, r. Perithecia with greenish yellow scurf; s. apical paraphyses and mature asci; t. ascospores and ascoconidia within asci; u. ascoconidia; v, w. asci with ascospores and ascoconidia (q: WU 32129; r: WU 30362 (NCP); s–w: WU 32127 (W.J. 1262)). — Scale bars: a, b = 1.5 mm; c, i, v, w = 10  $\mu$ m; d, u = 5  $\mu$ m; e–g, q = 0.4 mm; h, r = 0.2 mm; j = 30  $\mu$ m; k–p, t, v = 15  $\mu$ m; s = 75  $\mu$ m.



up to 42 in round or elongated clusters 1.0–2.5(–3.0) mm long ( $n = 12$ ), 0.5–1.0 mm high, rarely solitary, globose to obovoid, only rarely collapsing cupulate from above, sometimes formed in 2 layers, (260–)320–467(–496)  $\mu\text{m}$  diam in surface view ( $n = 20$ ) when dry, in section (340–)360–444(–457)  $\mu\text{m}$  high, (300–)350–437(–450)  $\mu\text{m}$  diam ( $n = 15$ ), dark reddish-, purplish brown to grey-brown, deep red in 3 % KOH, laterally often covered by greenish yellow scurf of minute particles. *Peridium* (29–)36–67(–78)  $\mu\text{m}$  thick at the base, (47–)60–79(–82)  $\mu\text{m}$  at the sides ( $n = 15$ ), up to 100  $\mu\text{m}$  around the ostiole, at the

base often paler and thinner and poorly delimited from the hypostroma, consisting of up to four layers laterally, the hyaline thin inner layer consisting of strongly compressed, elongate cells, the outer layers of thick-walled (1.5–2.5  $\mu\text{m}$ ), compressed cells (6.8–)7.0–14.8(–19.3)  $\mu\text{m}$  ( $n = 30$ ) diam, tending to be more isodiametric outward, pigmented from outside red/yellow-orange/rosy in 3 % KOH, the pigmented part in lactic acid and 50 % glycerol brightly yellow to orange. Peridial surface with warts consisting of outer peridial cells. Ostiolar region (78–)93–169(–204)  $\mu\text{m}$  diam ( $n = 20$ ), slightly papillate or flat-umbilicate,



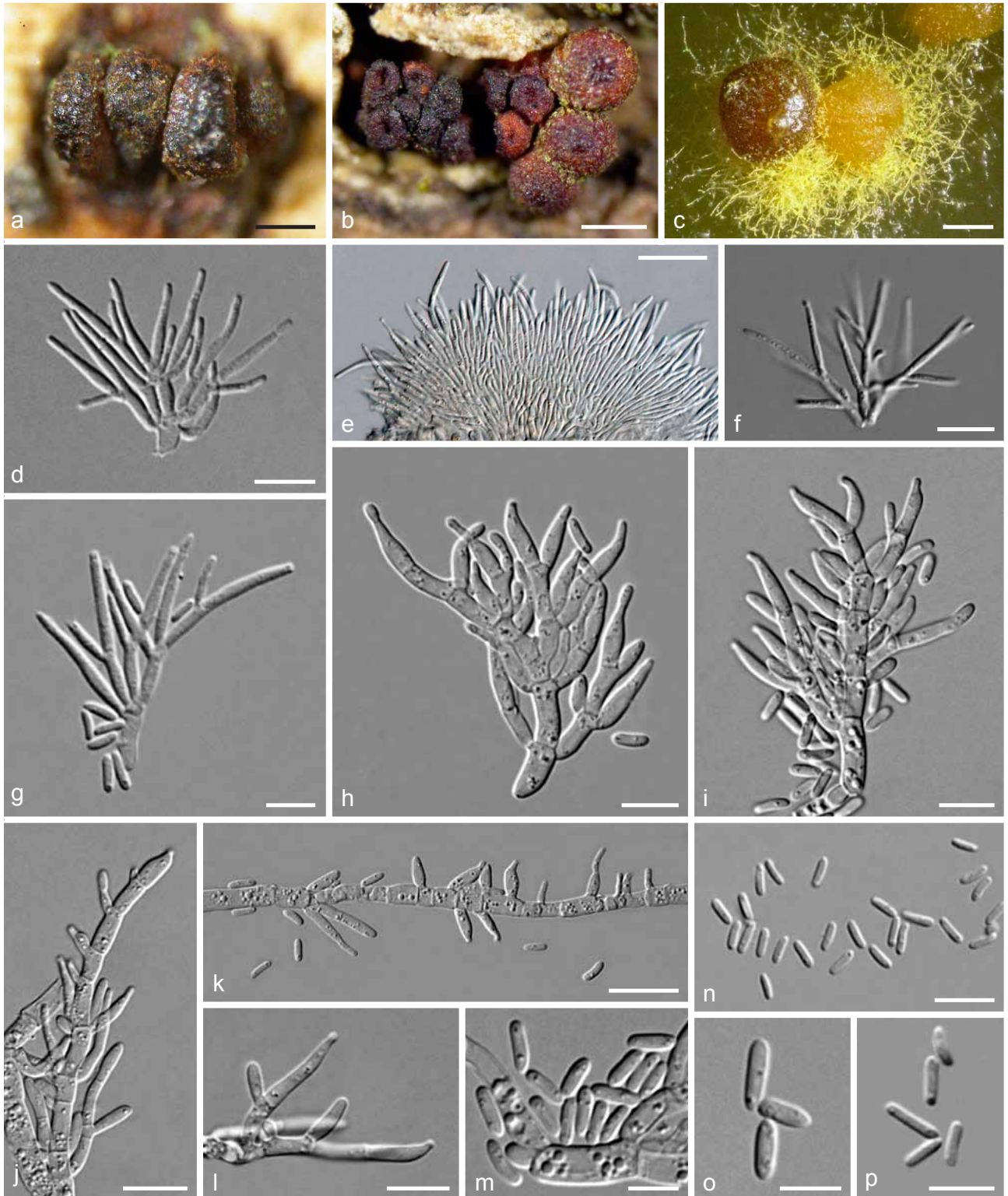
**Fig. 8** *Thyronectria caudata*, sexual morph. a–f. Perithecial aggregates (f. in 3 % KOH); g, l. perithecia and hypostroma in vertical section; h, i. peridium in section (3 % KOH; h. ostiolar region); j. hypostroma cells (3 % KOH); k. host hyphae (lactic acid); m. mature asci and apical paraphyses; n, o. asci with ascospores and ascoconidia; p, q. ascospores; r. ascoconidia. a, b, d–m, o, q, r: epitype WU 32130; c, p: holotype (MPU); n. BPI 552469. — Scale bars: a = 0.5 mm; b–f = 0.3 mm; g = 0.2 mm; h, m = 30  $\mu\text{m}$ ; i–k, o = 20  $\mu\text{m}$ ; l = 100  $\mu\text{m}$ ; n = 15  $\mu\text{m}$ ; p, q = 10  $\mu\text{m}$ ; r = 5  $\mu\text{m}$ .



shiny, darker than the main part of the perithecium, red, brown to black. *Ostioles* (87–)89–116(–135)  $\mu\text{m}$  long, apically (50–)55–92(–123)  $\mu\text{m}$  wide inside, (99–)113–185(–235)  $\mu\text{m}$  outside ( $n = 15$ ), periphysate. *Apical paraphyses* numerous, anastomosing, descending to the bases of asci, (1.5–)2–6(–8)  $\mu\text{m}$  wide. *Asci* cylindrical to clavate, (82–)104–143(–163)  $\times$  (16.3–)16.5–22.3(–26.8)  $\mu\text{m}$  ( $n = 30$ ), with 8 bi- to triseriate ascospores and mostly filled with ascoconidia when mature, with croziers and stipe of variable length; apex undifferentiated. *Ascospores* narrowly clavate, (21–)25–33(–39)  $\times$  (4.0–)5.0–6.2(–7.2)  $\mu\text{m}$ ,  $l/w = (3.5–)4.5–6.0(–7.4)$  ( $n = 103$ ), hyaline, at-

tenuated downward, with 5–11(–12) transverse septa and 1 longitudinal or oblique septum in 1 or few cells of the upper part, all cells budding to produce oblong, 1-celled, hyaline, mostly straight ascoconidia (2.6–)3.3–4.2(–4.7)  $\times$  (0.8–)1.0–1.2(–1.4)  $\mu\text{m}$ ,  $l/w = (2.5–)3.0–4.0(–5.0)$  ( $n = 127$ ).

Asexual morph on the natural host — *Pycnidia* either associated with perithecia on a common hypostroma or separate, solitary or in small groups, subglobose to somewhat vertically elongated, often laterally compressed in groups, collapsed-discoid when old, dull orange-red to dark reddish brown, 0.15–0.5 mm diam, surface slightly warted. *Peridium* in water



**Fig. 9** *Thyronectria caudata* WU 32130 (NL2), asexual morph. a, b. Pycnidia on the natural substrate (b. in association with perithecia); c. pycnidia on CMD; d–l. conidiophores and phialides (d, g. from natural host; e, f, h–l. from MEA, 20 °C, 7–9 d); m–p. conidia (m, o. from MEA, 20 °C, 7 d; n, p. from the natural host). — Scale bars: a, c = 0.2 mm; b = 0.3 mm; d, h, i, l, n = 7  $\mu\text{m}$ ; e, k = 15  $\mu\text{m}$ ; f, j = 10  $\mu\text{m}$ ; g, m, o, p = 5  $\mu\text{m}$ .



orange, of isodiametric cells 4–11(–13)  $\mu\text{m}$  diam with walls up to 1  $\mu\text{m}$  thick; the interior densely clothed with numerous fascicles of shrub-like or fan-shaped, 1–2  $\mu\text{m}$  wide, cylindrical conidiophores on a common, up to c. 3.5  $\mu\text{m}$  wide stipe. *Phialides* terminal, solitary, long cylindrical, 6–12  $\times$  1–2  $\mu\text{m}$ . *Conidia* oblong-cylindrical, (2.8–)3.5–4.5(–5.2)  $\times$  (0.9–)1.0–1.2(–1.4)  $\mu\text{m}$ , l/w = (2.8–)3.2–4.1(–4.8) (n = 40), hyaline, 1-celled, straight to scarcely curved, eguttulate, smooth. Sterile hyphae absent.

Cultures — On CMD conidiation sparse and mostly submerged in the agar, yellow pigment diffusing into the agar. On MEA colony radius 5 mm after 7 d at 20 °C; colony very dense, first whitish, turning yellow and finally orange by mucous, (rosy-)orange conidial masses, a yellow pigment diffusing into the agar. *Conidiophores* on the colony surface erect, white, (1.5–)2.0–3.5(–4.3)  $\mu\text{m}$  wide, comprising straight hyphae with conidia formed on short pegs, phialides scattered along the axis or brush-like to fan-shaped, consisting of a main axis with 1–3 verticils each of 2–5 steeply ascending 1- to few-celled side branches or phialides. *Phialides* solitary or in whorls of 2–3, lageniform to cylindrical, (5.3–)7.0–10.0(–12.2)  $\times$  (1.5–)2.0–2.7(–3.2)  $\mu\text{m}$ , l/w = (2.1–)2.9–4.7(–6.0) (n = 46), often slightly curved, inequilateral or sigmoid, with a narrow collarette. *Conidia* formed holoblastically, solitary, oblong to cylindrical, (3.8–)4.5–5.7(–6.5)  $\times$  (1.2–)1.4–1.8(–2.0)  $\mu\text{m}$ , l/w = (2.4–)2.7–3.7(–4.5) (n = 60), hyaline, 1-celled, straight or suballantoid, eguttulate or with few minute, often subterminal guttules, often mixed with pegs. *Pycnidia* observed on CMD after c. 1 mo at 15 °C subsequent to 1 wk pre-cultivation at 20 °C, forming a concentric ring on the agar surface, 0.1–0.5 mm diam, subglobose, first yellow, turning to violaceous to violaceous brown, KOH- or slightly more violaceous or purple in 3 % KOH; after 2 mo sterile; peridium a *textura porrecta* of short, thick-walled, pale orange, 2–4.5(–5)  $\mu\text{m}$  wide hyphae. *Pycnidia* also formed on a plug of PDA stored for 8 mo at 15 °C and placed on MEA. *Conidiophores* fan-shaped, phialides and conidia as described above for the effuse conidiation.

Habitat — On *Cucurbitaria* cf. *berberidis* on dead twigs of *Berberis cretica*, *B. hispanica* and *B. cf. lycium*.

Distribution — Mediterranean region (Southern Europe, North Africa), Asia (Pakistan).

*Other materials studied.* PAKISTAN, Kaghan Valley, Shogran, on *Berberis* cf. *lycium*, soc. *Cucurbitaria* cf. *berberidis*, 26 July 1956, S. Ahmad 14057 (BPI 552469, lectotype of *Thyronectria lamyi* var. *pakistani*, here designated; MBT198113); same place and host, 13 June 1967, S. Ahmad 20137 (BPI 552468); Naran, on *Berberis* cf. *lycium*, soc. *Cucurbitaria* cf. *berberidis*, 12 Aug. 1968, S. Ahmad 21198 (BPI 552470); Naran, Nathia Gali, on *Berberis* cf. *lycium*, 22 Aug. 1968, S. Ahmad 21223 (BPI 552471). — SPAIN, Andalucía, Granada, El Trevenque, above Jardín Botánico La Cortijuela, elev. c. 1700 m, on *Berberis hispanica*, soc. *Cucurbitaria* cf. *berberidis*, 14 May 2014, S. Tello & W. Jaklitsch (WU 33428; culture NCA1); Andalucía, Jaén, Jaén, La Panadera, N37°37'54" W3°46'34.4", elev. 1800 m, on *Berberis hispanica*, 12 May 2014, S. Tello, W. Jaklitsch, D. Extrada & D. Merino (WU 33429; culture NCA).

Notes — Material from Crete, Pakistan and Spain is in perfect agreement with the holotype. Characteristic of *T. caudata*, which is otherwise similar to *T. lamyi*, are the long, narrowly clavate ascospores, superficial ascomata that are usually not enclosed by bark flaps and particularly by narrow ascoconidia that have a l/w ratio of (2.5–)3.0–4.0(–5.0), similar to conidia of the asexual morph in nature and in culture, while ascoconidia of *T. lamyi* have a l/w ratio of (1.7–)2.1–2.6(–2.9). *Thyronectria caudata* is obviously drought-tolerant and in Europe confined to oromediterranean regions. The peridial colour of *T. caudata* is strongly pH-dependent, red to purple in KOH and bright yellow in lactic acid. The asexual morph of *T. caudata* on the natural host is virtually identical to that in culture, except for slightly narrower phialides and conidia. *Thyronectria clavatispora*, described from *Ribes* in North America (Hirooka et al. 2012, as *Pleonectria clavatispora*), has also clavate ascospores, but

these are distinctly wider than those of *T. caudata*; in addition *T. clavatispora* has red, collabent ascomata that resemble those of e.g. *T. berolinensis* or *T. coryli*.

***Thyronectria chrysogramma*** Ellis & Everh., Proc. Acad. Nat. Sci. Philadelphia 42: 245. 1890. — Fig. 7e–p

≡ *Mattiolia chrysogramma* (Ellis & Everh.) Sacc., Syll. Fung. (Abellini) 9: 993. 1891.

≡ *Nectria chrysogramma* (Ellis & Everh.) Rossman, Mem. New York Bot. Gard. 49: 259. 1989.

≡ *Thyronectroidea chrysogramma* (Ellis & Everh.) Seaver, Mycologia 1: 206. 1909.

*Holotype.* USA, Kansas, Manhattan, on *Ulmus americana*, Mar. 1889, Kellerman & Swingle 1421 (NY 00927545); *paratype.* USA, New York, Potsdam, on elm limbs (NY 00927565 = NY 1944).

*Ascomata* immersed and erumpent from bark, subglobose, 0.3–0.5 mm diam, scattered or aggregated, individually surrounded by yellowish stromatic tissue, sometimes immersed in soft, pulvinate, erumpent stromata to c. 1 mm diam, covered with yellow to yellow-green scurf. *Peridium* reddish, weakly reacting in 3 % KOH. Ostiolar area black, the interior of ostioles filled with numerous periphyses. *Apical paraphyses* numerous, richly branched, 1.5–5  $\mu\text{m}$  wide. *Asci* c. 100–150  $\times$  28–37  $\mu\text{m}$ , clavate, containing 8 biseriolate ascospores, apex undifferentiated. *Ascospores* (24–)27–33(–35.5)  $\times$  (10–)12–15(–17)  $\mu\text{m}$ , l/w = (1.6–)2.0–2.5(–3.2) (n = 58), first hyaline and oblong, turning yellowish and finally medium to reddish brown, often with an olivaceous tinge and oblong or ellipsoid, with 4–9(–11) transverse and (1–)2–3 longitudinal, densely disposed eu- and distosepta, smooth.

Distribution — North America, on *Ulmus americana*.

Notes — The above description is based on a study of the holo- and paratype. They represent the same fungus, but in the paratype many perithecia are solitary and scattered. This fungus is clearly a species of the genus *Thyronectria*, resembling, in its distoseptate ascospores, *T. asturiensis* or *T. roseovirens*. *Thyronectria chrysogramma* is obviously fungicolous like other species of *Thyronectria*, as some of the perithecial aggregates occur directly on an effete coelomycete (?*Diplodia* sp.). Superficially, stromata look much like those of *T. rhodochlora* or *T. roseovirens*. From the latter it differs mostly by ascospore size and septation and the absence of the green colour of immature ascospores, although nearly mature ascospores have some olivaceous tinge. For good additional descriptions see Rossman et al. (1999) as *Thyronectroidea chrysogramma* and Checa et al. (2013) as *Mattiolia chrysogramma* in which the distoseptate ascospores are clearly illustrated.

***Thyronectria clavatispora*** (Hirooka, Rossman & P. Chaverri) Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB808297

*Basionym.* *Pleonectria clavatispora* Hirooka, Rossman & P. Chaverri, Stud. Mycol. 71: 128. 2012.

***Thyronectria coryli*** (Fuckel) Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB808298; Fig. 7q–w

*Basionym.* *Nectria coryli* Fuckel, Fung. Rhenani Exsicc., suppl. 1, no. 1582. 1865.

≡ *Chilonectria coryli* (Fuckel) Ellis & Everh., N. Amer. Pyrenomyc.: 117. 1892.

≡ *Creonectria coryli* (Fuckel) Seaver, Mycologia 1: 186. 1909.

≡ *Pleonectria coryli* (Fuckel) Hirooka, Rossman & P. Chaverri, Stud. Mycol. 71: 128. 2012.

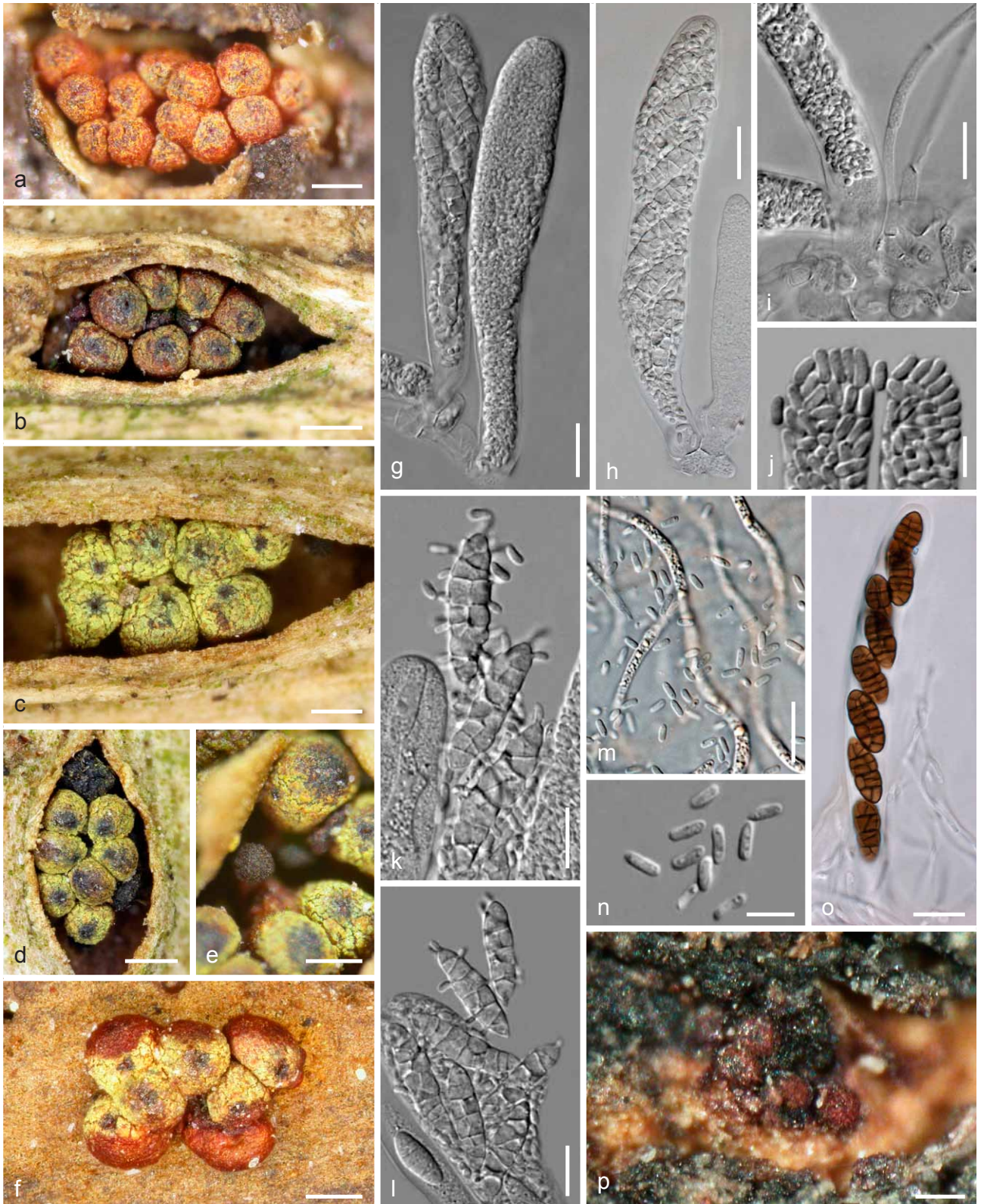
= *Coelospaeria acervata* P. Karst., Meddel. Soc. Fauna Fl. Fenn. 5: 56. 1879.

= *Nectria coryli* f. *salicis* Rehm, Ascomyceten Exsicc. No. 680. 1882.



**Typification.** Lectotype of *Nectria coryli*, designated by Hirooka et al. (2012): GERMANY, on twigs of *Corylus avellana*, *Fuckel, Fungi Rhenani Exsiccati 1582* (FH!). Epitype here designated: AUSTRIA, Kärnten, St. Margareten im Rosental, Gupf, Brici, grid square 9452/2, on *Corylus avellana*, 25 July 2009, W. Jaklitsch & H. Voglmayr (WU 32129; culture CBS 137264 = NeCo1; MBT177539).

*Ascomata* erumpent-superficial, usually densely aggregated in convex groups on a hypostroma, covered by yellowish green scurf when immature, globose and light red when fresh, covered by yellow-green scurf when young, collapsed cupulate and dark red when dry, usually glabrous when mature. *Hamathecium* of descending, branched and anastomosing apical paraphyses forming a reticulum, present among immature and mature asci,



**Fig. 10** a–n: *Thyronectria lamyi*. a–f. Perithecial aggregates (a. immature; d. with an ascoma of *Cucurbitaria berberidis* at the upper end; e. erect pycnidium surrounded by perithecia; f. superficial perithecia after bark removal); g, h. asci with ascospores and ascoconidia; i. apical paraphyses at ascus bases; j. ascoconidia; k, l. budding ascospores; m, n. sterile hyphae and conidia from pycnidium on natural host (a: epitype WU 32159 (NL3); b–n: WU 32141 (NL1)). — o, p: *Mattirolia macluriae* (holotype, LISE). o. ascus; p. ostioles in surface view. — Scale bars: a, c, e, f = 0.25 mm; b, d = 0.4 mm; g–i, m, o = 15 µm; j, n = 5 µm; k, l, p = 10 µm.



not basally attached, easily removed from asci. Asci narrowly clavate to oblong, (58–)66–110(–129) × (9–)11–16(–18) µm (n = 14), containing 8 biseriate ascospores budding in the ascus to produce numerous ascoconidia when mature. Ascospores oblong, (8.5–)9.5–12.2(–13.0) × (2.5–)2.8–3.4(–3.7) µm, l/w = (2.7–)3.0–4.0(–4.5) (n = 35), with a central, non-constricted septum, hyaline, straight. Ascoconidia suballantoid, (2.8–)3.0–4.0(–4.5) × (1.3–)1.4–1.6(–1.7) µm, l/w = (1.9–)2.1–2.5(–2.7) (n = 30), hyaline, smooth, with a subterminal guttule at each end.

Habitat — On dead bark or twigs of deciduous trees, in Europe recorded on *Corylus avellana*, from own observations also on *Betula pendula*, *Cornus sanguinea*, *Crataegus monogyna*, *Euonymus europaeus*, *Fraxinus ornus*, *Ligustrum vulgare*, *Pyrus communis*, *Viburnum lantana* and *V. opulus*, often on blackened inner bark, on *Corylus* e.g. associated with *Valsa* spp., *Otthia* cf. *spiraeae*; in mixed piles of cut twigs apparently spreading from one plant host to others, in one occasion e.g. found on six different hosts in a single pile.

Distribution — Europe, North America.

*Selected specimens examined.* AUSTRIA, Kärnten, St. Margareten im Rosental, Stariwald, grid square 9452/4, on *Viburnum lantana*, soc. *Diplodia* sp., 26 Oct. 1998, W. Jaklitsch W.J. 1262 (WU 32127); *ibid.*, on *Betula pendula*, 18 Mar. 2000, W. Jaklitsch (WU 32128); Oberösterreich, Schärding, St. Wilibald, Aichet, on *Pyrus communis*, 22 May 2009, H. Voglmayr (WU30362, culture CBS 127384 = NCP).

Notes — We provide a short description and illustration of *T. coryli* in addition to the detailed description by Hirooka et al. (2012), because the greenish yellow scurf on perithecia has been rarely reported; in addition, we epitypify this species with material grown on its original host for which a culture and *act*, ITS-LSU, *rpb1*, *rpb2*, *tef1* and *tub* sequences are available. In *T. coryli* the hamathecial threads between asci are clearly not basally attached, as they are easily removed in mounts from mature asci, more so than in other species we studied. In perithecia covered by greenish yellow scurf asci are usually immature. When collected late in the year, e.g. from the end of November in Austria, perithecia may occasionally be entirely yellow.

***Thyronectria cucurbitula* (Tode) Jaklitsch & Voglmayr, comb. nov. — MycoBank MB808299**

*Basionym.* *Sphaeria cucurbitula* Tode, Fungi Mecklenb. sel. 2: 38. 1791. ≡ *Pleonectria cucurbitula* (Tode: Fr.) Hirooka, Rossman & P. Chaverri, Stud. Mycol. 71: 132. 2012.

*Materials examined.* AUSTRIA, Kärnten, St. Margareten im Rosental, Stariwald, grid square 9452/4, on *Pinus sylvestris*, 18 Dec. 1994, W. Jaklitsch W.J. 388 (WU 32131); *ibid.*, 25 Mar. 1995, W. Jaklitsch W.J. 541 (WU 32132); same village, Wograda, grid square 9452/3, on *Pinus sylvestris*, 26 May 1995, W. Jaklitsch W.J. 614 (WU 32133).

***Thyronectria ilicicola* (Hirooka, Rossman & P. Chaverri) Jaklitsch & Voglmayr, comb. nov. — MycoBank MB808300**

*Basionym.* *Pleonectria ilicicola* Hirooka, Rossman & P. Chaverri, Stud. Mycol. 71: 136. 2012.

*Material examined.* UK, Slough, Burnham Beeches, Buckinghamshire, on *Ilex aquifolium*, 15 Sept. 2004, W. Jaklitsch W.J. 2720 (WU 32134).

***Thyronectria lamyi* (Desm.) Seeler, J. Arnold Arbor. 21: 449. 1940. — Fig. 10a–n**

*Basionym.* *Sphaeria lamyi* Desm., Ann. Sci. Nat., Bot., sér. 2, 6: 246. 1836.

≡ *Nectria lamyi* (Desm.) De Not., Sfer. Ital. 1: 13. 1863.

≡ *Pleonectria lamyi* (Desm.) Sacc. (as '*lamyiii*'), Michelia 1: 325. 1878.

*Typification.* Lectotype of *Sphaeria lamyi*, designated by Seeler (1940): FRANCE, Limoges, on dead branches of *Berberis vulgaris*, *Desmazières*, *Plantes Cryptogames France* No 839 (FH!). Epitype, here designated:

AUSTRIA, Vienna, 3rd district, Botanical Garden, on *Berberis vulgaris*, on soc. *Cucurbitaria berberidis*, 11 Nov. 2013, W. Jaklitsch & H. Voglmayr (WU 32159; ex-epitype culture CBS 137263 = NL3; MBT177540).

*Ascomata* superficial on hypostromata, usually surrounded by bark flaps, only rarely erumpent above bark, (200–)260–425 (–470) µm diam in surface view (n = 20), varying in colour from orange over red to brown, covered by greenish yellow scurf or not. Ostiole periphysate. *Apical paraphyses* numerous, apically attached, mostly 2–5 µm wide, hanging down, branched and anastomosing, forming a coarse-meshed reticulum, with free widened ends near ascus bases, without basal attachment. *Asci* clavate, (94–)108–143(–160) × (17–)18.7–26.5(–30) µm, with croziers, thickened but otherwise undifferentiated apex, with 8 biseriate or obliquely uniseriate ascospores, usually packed with numerous ascoconidia when mature. Ascospores oblong or fusoid, (13.5–)18.5–25.5(–30.0) × (4.8–)5.5–7.2(–8.2) µm, l/w = (1.7–)2.8–4.3(–5.4) (n = 60), muriform, with 5–9 transverse septa and 1(–2) longitudinal septum, variable, budding in the ascus to produce hyaline, thin-walled, suballantoid ascoconidia (3.0–)3.3–4.2(–5.0) × (1.3–)1.5–1.7(–2.0) µm, l/w = (1.7–)2.1–2.6(–2.9) (n = 35).

*Pycnidia* on the natural host solitary or scattered on the inner bark in bark fissures, lacking a hypostroma or aggregated singly or in small numbers in direct association with perithecia on a common hypostroma, variable in shape, from discoid or lenticular and sometimes convoluted to subglobose or stipitate-elongate to subcylindrical, 110–260 µm diam, orange-red, dark reddish- or purplish brown to nearly black, often disintegrating with orange cup-like bases remaining. Pycnidial interior with or without long, hyaline to pale yellowish or brownish, sterile, 2–4 µm wide, capillitium-like hyphae, densely packed, fasciculate conidiophores with cylindrical to subulate phialides and masses of oblong, straight or slightly curved, hyaline, smooth, 1-celled conidia, (3.4–)3.7–4.3(–4.6) × (1.3–)1.4–1.6 µm, l/w = (2.2–)2.4–3.0(–3.3) (n = 30).

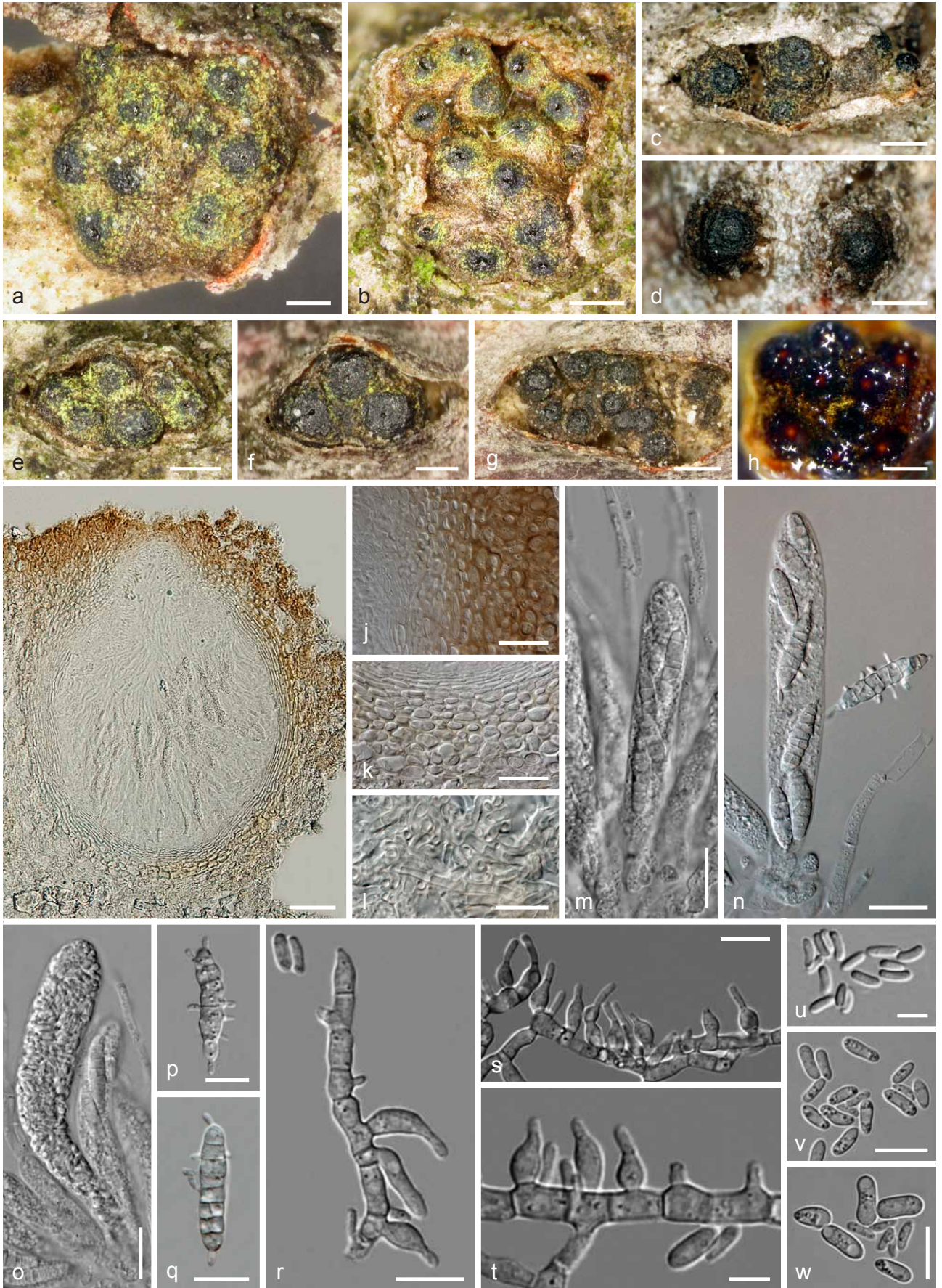
Cultures — Colony on CMD at 25 °C usually reaching a radius of only a few mm after one month, pale rosy conidial masses spreading from the centre; on PDA at 25 °C colony covering the laterally inoculated 90 mm diam plate entirely after 20–30 d, whitish to yellow, mucous rosy-orange conidial masses spreading from the centre. *Conidia* hyaline, rod-shaped, long cylindrical or fusoid.

Distribution — Asia, Europe, North America.

Habitat — Common in Central Europe on *Cucurbitaria berberidis* on the naturally occurring *Berberis vulgaris*, in urban areas on many different planted species of *Berberis*, including *B. aquifolium*, *B. candidula*, *B. gagnepainii* and *B. thunbergii*.

*Additional materials examined.* AUSTRIA, Kärnten, St. Margareten im Rosental, shrubs in village area, grid square 9452/4, on *Berberis vulgaris*, 2 Dec. 1995, W. Jaklitsch W.J. 811 (WU 32136); Stariwald, grid square 9452/4, on *Berberis vulgaris*, 26 Oct. 1998, W. Jaklitsch W.J. 1264 (WU 32138); Niederösterreich, Schwarzensee, at the cross-country ski track, grid square 7962/3, on *Berberis vulgaris*, 25 Feb. 1996, W. Jaklitsch W.J. 825 (WU 32137); *ibid.*, 6 Jan. 2000, W. Jaklitsch W.J. 1407 (WU 32139); Vienna, 19th district, Aslangasse, grid square 7763/2, on *Berberis gagnepainii*, 1 May 2001, W. Jaklitsch W.J. 1751 (WU 32140); Bellevuestraße, grid square 7763/2, on *Berberis vulgaris*, 25 Feb. 1995, W. Jaklitsch W.J. 505 (WU 32135); 21st district, Felix-Slavik-Straße, on *Berberis thunbergii*, 31 May 2009, W. Jaklitsch (WU 30363; culture CBS 127385 = NL); *ibid.*, on *Berberis candidula*, 20 Feb. 2011, W. Jaklitsch (WU 32141; culture NL1). — SPAIN, Andalucía, Jaén, Camino de los Bojes, Valdepeñas de Jaén, N37°34'42.43" W3°47'56.54", elev. 1275 m, on *Berberis hispanica*, 18 Dec. 2013, S. Tello JA-CUSSTA 7789 (WU 32165; culture NL4); Malaga, Sierra de las Nieves Natural Park, Parauta Pinsapar de la Escalereta, elev. 1150 m, on *Berberis hispanica*, 26 Dec. 2013, M. Becerra (WU 32166; culture NL5); Aragón, Huesca, Jaca, estuarine forest along the river Aragón, N42.55951 W0.59626, elev. 740 m, on *Berberis hispanica* ssp. *seroi*, 27 Dec. 2013, A. Lorenzo & J. Hernanz (WU 32169; culture NL7); Teruel, 3.5 km W from Cedrillas, N40°26'21" W0°53'43", 1502 m, on *Berberis hispanica* subsp. *seroi*, 12 Dec. 2013, R. Tena Lahoz RT13121201 (WU 32167; culture NL6).





**Fig. 11** *Thyronectria obscura*. a–h. Stromata/Perithecia (h. in 3 % KOH); i. perithecium in vertical section; j, k. peridium in section in 3 % KOH (j. ostiolar region; k. base); l. stroma hyphae in lactic acid; m–o. asci with ascospores and ascoconidia, and apical paraphyses; p, q. ascospores; r–t. conidiophores and phialides (MEA, RT, 2d); u. ascoconidia; v, w. conidia (MEA, RT, 2 d) (a–e, h–l, n, p, r–t, v, w: WU 32142 (TT); f, g, m, o, q, u: WU 32143 (TT1)). — Scale bars: a, c, d, f = 0.2 mm; b, e, g, h = 0.3 mm; i = 50  $\mu$ m; j–l = 25  $\mu$ m; m–o = 15  $\mu$ m; p–s, v, w = 10  $\mu$ m; t, u = 5  $\mu$ m.



**Notes** — We include here a short description and illustration of *T. lamyi* in addition to the detailed description by Hirooka et al. (2012), in order to facilitate comparison with *T. caudata*, to describe the apical paraphyses, to show some variation in the greenish yellow scurf on perithecia and pycnidia containing sterile hyphae. Sometimes apical paraphyses appear to be entrapped between ascus bases, but this may be only a consequence of mount preparation. The asexual morph in nature was found on the plant host *Berberis vulgaris* similar to that described by Hirooka et al. (2012), i.e. irregularly discoid to lenticular pycnidia lacking sterile internal hyphae, particularly when occurring separately from the sexual morph. Pycnidia in WU 32141 from *B. candidula*, especially when formed in association with perithecia on the same hypostroma, are typically subglobose to cylindrical and are darker, dark reddish brown to nearly black. Such pycnidia contain sterile hyphae. We here select a well-developed specimen for which a culture and *act*, ITS-LSU, *rpb1*, *rpb2*, *tef1* and *tub* sequences are available as epitype to ensure nomenclatural stability. For delimitation from *T. caudata*, see under notes to that species. Both *T. lamyi* and *T. caudata* at least partly share the same plant hosts but differ in their ecology, the former being mesophilic with wide distribution in the temperate zone, whereas the latter has mostly been found in dry oromediterranean areas.

***Thyronectria obscura*** Jaklitsch & Voglmayr, *sp. nov.* — MycoBank MB808301; Fig. 11

**Etymology.** Referring to its dark colour.

**Holotype.** AUSTRIA, Vienna, 3rd district, Botanical Garden, on dead twigs of *Tamarix tetrandra* attached to the tree, 1 Aug. 2013, H. Voglmayr (WU 32142; culture TT = CBS 136923).

**Stromata** completely immersed in linear groups or erumpent from inner bark, at the sides usually surrounded by bark flaps; tissue surrounding ascomata that are scattered or aggregated in clusters of 2–21 individually or less commonly uniting them into compound pulvinate, 0.4–1.2(–1.9) mm long ( $n = 42$ ) and 0.2–0.5(–0.7) mm high ( $n = 16$ ) stromata; consisting of hyaline, pale yellow to pale brown, (1.5–)2.5–5.8(–9.7)  $\mu\text{m}$  wide ( $n = 31$ ) hyphae with walls up to 1.5  $\mu\text{m}$ , on its surface covered by a layer of brown cells corresponding to outer peridial cells, on the upper surface also partly covered by usually scant, sometimes well-developed, dark yellow-green or brown scurf of yellowish brown amorphous matter and brown hyphae, turning entirely black in 3% KOH; below the ascomata continuing as a loose to compact hyphal network into the bark. **Ascomata** globose, subglobose to flask-shaped, (200–)225–325(–400)  $\mu\text{m}$  diam ( $n = 17$ ) in surface view, in vertical section (270–)300–410(–420)  $\mu\text{m}$  high, (200–)250–310(–347)  $\mu\text{m}$  diam ( $n = 14$ ), surrounded by stromatic tissue, becoming glabrous with age, black. **Peridium** (34–)40–52(–57)  $\mu\text{m}$  wide at the base, (36–)42–55(–60)  $\mu\text{m}$  at the sides ( $n = 14$ ), thickened to c. 110  $\mu\text{m}$  around the ostiole, consisting of a hyaline inner layer of thin-walled, strongly compressed, filiform cells and an outer layer of compressed cells (3.5–)6.5–15(–21)  $\mu\text{m}$  diam ( $n = 40$ ), in the upper part hyaline, yellowish, pale orange to brown in 3% KOH, lactic acid and water, tending to be larger and more pigmented out- and upward. Outer pale brown cells with incrustated, up to nearly 2  $\mu\text{m}$  thick walls, becoming loose in the upper part to form a cellular layer beyond the surrounding stroma. **Ostiolar region** (62–)90–170(–220)  $\mu\text{m}$  diam ( $n = 21$ ), broad, black, flat or convex, non-papillate, with shiny centre. **Ostioles** (85–)94–120(–129)  $\mu\text{m}$  long, (44–)54–84(–103)  $\mu\text{m}$  wide inside at the apex ( $n = 14$ ), filled with up to 2.5  $\mu\text{m}$  wide paraphyses sometimes continuing down beyond the ostiole on the inner wall surface. **Apical paraphyses** numerous, 1.5–4.5(–8)  $\mu\text{m}$  wide, descending from the top as a reticulum, distinctly

differentiated from the paraphyses, branching-anastomosing, with ends widened to 8  $\mu\text{m}$  between ascus bases. **Asci** clavate or oblong, (69–)75–99(–112)  $\times$  (10.3–)11.8–16.5(–18.2)  $\mu\text{m}$  ( $n = 18$ ), containing 8 biseriata ascospores, with short stipe and undifferentiated apex. **Ascospores** fusiform, oblong, vermiform or clavate, (12.5–)17.0–24.5(–28.5)  $\times$  (3.5–)4.0–5.2(–6.5)  $\mu\text{m}$ , l/w = (2.6–)3.7–5.4(–6.9) ( $n = 61$ ), hyaline, with 3–9(–10) transverse septa, with or without 1 longitudinal or oblique septum in 1–3 cells, straight or curved, smooth; all cells budding to produce cylindrical, hyaline, 1-celled, straight to slightly curved ascoconidia, (3.3–)3.7–4.7(–5.0)  $\times$  (1.0–)1.3–1.7(–2.0)  $\mu\text{m}$ , l/w = (2.4–)2.6–3.4(–4.1) ( $n = 50$ ).

**Asexual morph on the natural host** — None seen.

**Cultures and asexual morph** — Colony on CMD and MEA colourless, on CMD remaining hyaline, on MEA turning pale yellowish to rosy from the centre due to conidial masses; odour unpleasant, sourly. **Conidiation** effuse, formation of conidia commencing after short growth on minute pegs on hyphae in the colony or even before a distinct colony is formed, or on solitary, rarely paired phialides on short, more or less erect, simple or loosely branched conidiophores. **Phialides** lageniform to ampulliform, (4.3–)5.7–8.8(–11.5)  $\times$  (2.5–)3.0–3.5(–4.0)  $\mu\text{m}$ , l/w = (1.3–)1.7–2.7(–3.4) ( $n = 67$ ), straight to distinctly curved. **Conidia** (after 3 d on MEA at room temperature) oblong to suballantoid, (4.0–)5.0–8.0(–11.0)  $\times$  (1.1–)1.7–3.2(–4.3)  $\mu\text{m}$ , l/w = (1.5–)2.3–3.2(–4.2) ( $n = 150$ ).

**Habitat** — On dead twigs of *Tamarix* spp.

**Distribution** — Europe (Austria).

**Additional material examined.** AUSTRIA, Niederösterreich, Hagenbrunn, village entrance, on dead twigs of *Tamarix parviflora* attached to the tree, 18 Aug. 2013, W. Jaklitsch (WU 32143; culture TT1).

**Notes** — This species is difficult to interpret as a nectriaceous fungus at first sight, as the perithecia may be entirely black. As in other species of the genus, the apical paraphyses are distinctly differentiated from the paraphyses. The former are easily removed from the asci in microscopic mounts by pressure on the cover slip. Remarkably, the close phylogenetic relationship of *T. obscura* to *T. asturiensis* and *T. roseovirens* is highly supported (Fig. 1, 2), despite their morphological differences.

***Thyronectria okinawensis*** (Hirooka, Rossman & P. Chaverri) Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB808302

**Basionym.** *Pleonectria okinawensis* Hirooka, Rossman & P. Chaverri, Stud. Mycol. 71: 146. 2012.

***Thyronectria pinicola*** (Kirschst.) Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB808303

**Basionym.** *Pleonectria pinicola* Kirschst., Abh. Bot. Ver. Prov. Brandenburg 48: 59. 1906.

**Material examined.** AUSTRIA, Steiermark, Klösch, Steinriegelwald, 9261/2, on *Pinus sylvestris*, 17 Sept. 1996, W. Jaklitsch W.J. 947 (WU 32144).

***Thyronectria pseudomissouriensis*** (Hirooka, Rossman & P. Chaverri) Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB808304

**Basionym.** *Pleonectria pseudomissouriensis* Hirooka, Rossman & P. Chaverri, Stud. Mycol. 71: 153. 2012.

***Thyronectria quercicola*** (Hirooka, Checa, Arenal & P. Chaverri) Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB808305

**Basionym.** *Pleonectria quercicola* Hirooka, Checa, Arenal & P. Chaverri, Stud. Mycol. 71: 157. 2012.



***Thyronectria rosellinii*** (Carestia) Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB808306

*Basionym.* *Nectria rosellinii* Carestia, in Rabenh., Fung. Europ. Exs. No. 923. 1866.

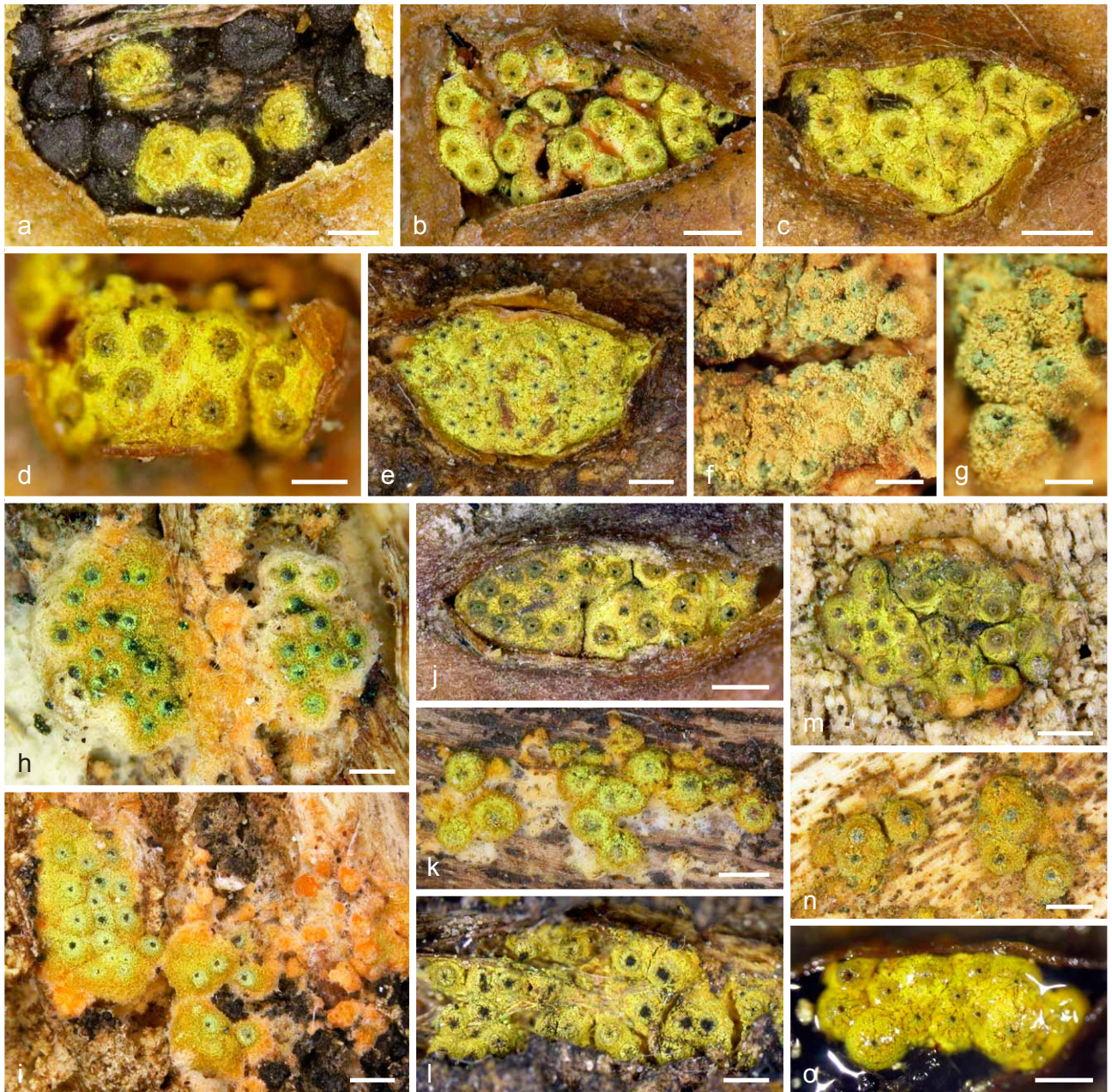
≡ *Pleonectria rosellinii* (Carestia) Hirooka, Rossman & P. Chaverri, Stud. Mycol. 71: 157. 2012.

***Thyronectria roseovirens*** (Berl. & Bres.) Seeler, J. Arnold Arbor. 21: 455. 1940. — Fig. 12, 13

*Basionym.* *Mattirolia roseovirens* Berl. & Bres., Micromycet. Trident.: 55. 1889.

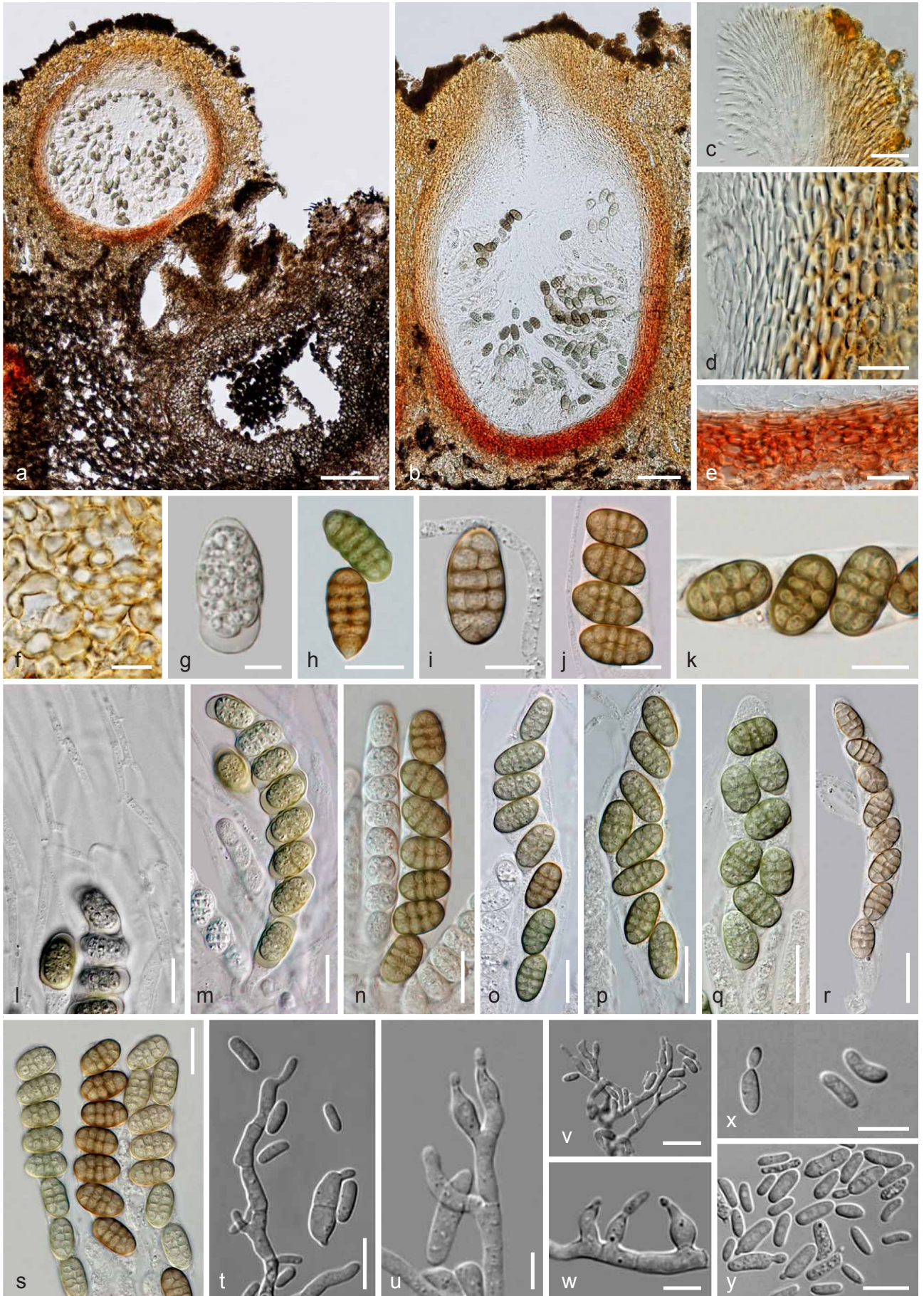
*Typification.* Lectotype of *Mattirolia roseovirens*, designated by Rossman et al. (1999): ITALY, Trentino, Trento, on branches of *Laburnum anagyroides* (or possibly *L. alpinum*; given as *Cytisus laburnum*), *S. Bresadola*, year not given (FH 00258826). Epitype here designated: ITALY, Trentino, Molveno, Lago di Molveno, Volta delle Assi, on *Laburnum alpinum*, on/soc. *Cucurbitaria laburni*, *Valsaria* sp., soc. asexual morph and setose pycnidia, 19 Oct. 2011, *W. Jaklitsch & H. Voglmayr* (WU 32154; ex-epitype culture CBS 135999 = MA1; MBT177541).

*Stromata* usually on black fungi immersed in bark, often only visible through fissures, typically surrounded by the epidermis of the plant host, less commonly superficial on wood; stromatic tissue thin, subhyaline to pale yellow, pseudoparenchymatous, of thick-walled (1–1.5  $\mu\text{m}$ ), rounded to angular cells, (3.8–) 4.5–9.0(–16.0)  $\mu\text{m}$  diam ( $n = 30$ ), variably containing some thick-walled, (2–)3–6(–8)  $\mu\text{m}$  wide ( $n = 35$ ) hyphae. Stromatic tissue below ascomata often mixed with subicular hyphae of the fungal host, surrounding ascomata that are scattered or densely aggregated in groups of 2–60 individually or uniting them into compact, pulvinate, more or less tubercular stromata, 0.3–2.8(–6.5) mm long ( $n = 58$ ), 0.3–0.7(–0.8) mm high ( $n = 34$ ); stroma sides often rosy to light honey-brown or surrounded by rosy spots or tufts; surface covered by bright yellow scurf, sometimes with a rosy tone, often becoming dull green to black due to spore deposits; in 3% KOH without a distinct macroscopic colour change; scurf typically present in coarse flakes, consisting of minute granules, brown in KOH, golden yellow in lactic acid. *Ascomata* globose to flask-shaped or conical, perithecia mostly 0.3–0.5 mm diam in surface view,



**Fig. 12** *Thyronectria roseovirens*, ascomata and stromata (h, i, k. showing rosy asexual morph). Note direct growth on the host fungus *Cucurbitaria laburni* in a (a–d, o: WU 32154 (MA1); o. in 3% KOH); e, k: WU 32153 (MA); f, g: lectotype (FH); h, i, m: WU 32156 (MA4); j: WU 32157 (MA5); l: WU 32155 (MA2); n: WU 32158 (MA6)). — Scale bars: a, d, g = 0.3 mm; b, c, e, f, h–o = 0.5 mm.





**Fig. 13** *Thyronectria roseovirens*, microscopic traits. a. Section of perithecium including natural host; b. perithecium in vertical section; c–e. peridium in section (c. ostiolar region with periphyses in water; d. lateral region in lactic acid; e. base in water); f. stroma cells in lactic acid; g–k. ascospores (g. immature, with projecting sheath; h. showing distosepta); l. apical paraphyses; m–s. asci, ascospores and apical paraphyses; t–w. conidiophores and phialides (MEA, RT, 3–4 d); x, y. conidia (MEA, RT, 3–4 d) (a–f, h: WU 32154 (MA1); g, i: WU 32158 (MA6); j, o, p, s: WU 32155 (MA2); k–n: lectotype (FH); q: WU 32156 (MA4); r: WU 32157 (MA5); t–y: WU 32153 (MA)). — Scale bars: a = 100  $\mu$ m; b = 50  $\mu$ m; c, e, p–s, v = 20  $\mu$ m; d, h, l–o = 15  $\mu$ m; f, i–k, t, x, y = 10  $\mu$ m; g, u, w = 5  $\mu$ m.



in section (360–)405–515(–580)  $\mu\text{m}$  high, (170–)240–350(–360)  $\mu\text{m}$  diam ( $n = 20$ ). *Peridium* (27–)30–43(–52)  $\mu\text{m}$  wide at the base and sides ( $n = 40$ ), distinctly thickened around the ostiole, consisting of a thin inner layer of hyaline, elongate-filiform cells, and a thick outer pigmented layer of thick-walled, elongate, compressed, out- and upward more isodiametric cells (3.2–)4.5–11(–16)  $\mu\text{m}$  diam ( $n = 60$ ), at the base often grading into the hypostroma, in the basal region orange in water, lactic acid and 50 % glycerol to red in 3 % KOH, in the upper region generally distinctly lighter, subhyaline, yellow to yellow-brown. Ostiolar region (78–)106–185(–235)  $\mu\text{m}$  diam ( $n = 51$ ), flat-convex, sometimes prominent, dull ochre or yellowish grey when young, turning grey-green to black with age. *Ostioles* (95–)115–160(–170)  $\mu\text{m}$  long, apically (51–)62–89(–103)  $\mu\text{m}$  wide inside, (113–)131–189(–206)  $\mu\text{m}$  outside ( $n = 20$ ), containing short, 1–2  $\mu\text{m}$  wide periphyses. *Apical paraphyses* numerous, formed in a mucous matrix, 2–5  $\mu\text{m}$  wide, branched and anastomosing. *Asci* oblong, clavate to subfusoid, (85–)96–129(–144)  $\times$  15–25(–36)  $\mu\text{m}$  ( $n = 30$ ), with short stipe and undifferentiated apex, containing 8 uni- to biseriolate ascospores. *Ascospores* ellipsoid to subglobose, (13–)15–20(–25.5)  $\times$  (7.5–)9–11(–13)  $\mu\text{m}$ ,  $l/w = (1.4–)1.6–2.0(–2.3)$  ( $n = 150$ ), muriform, with 3–5(–6) transverse and 1–2 longitudinal distosepta, first hyaline, turning green and finally pale to medium brown at maturity, sometimes with a sheath projecting to c. 3.5  $\mu\text{m}$  at the ends, cells eguttulate or finely multiguttulate when alive; rarely more fusiform and pale brownish without a preceding green stage, smooth.

**Asexual morph on natural substrates** — Either surrounding stromata partly as rosy margin, occurring separately in bark fissures or on the surface of the fungal host; first white mycelium formed, producing rosy to pale orange masses of convoluted hyaline hyphae, conidiophores to 6  $\mu\text{m}$  wide and numerous conidia, or sometimes flat, compound, rosy or pale orange, 0.1–0.6 mm long pycnidia with variable outline, not changing colour in 3 % KOH; peridium pseudoparenchymatous, of pale yellowish, isodiametric to elongated cells (3.5–)6–11(–15) ( $n = 40$ ) with walls to 1  $\mu\text{m}$  thick; interior whitish and rosy mottled, consisting of numerous short, parallel, simple, filiform conidiophores arranged in palisades, mostly 2–4.5  $\mu\text{m}$  wide. Conidia formed on short pegs and numerous phialides arranged solitarily or in whorls of 2–4. *Phialides* variable, lageniform to subulate or ampulliform, (4.2–)6.3–9.2(–11.2)  $\times$  (2.0–)2.2–3.5(–4.2)  $\mu\text{m}$ ,  $l/w = (1.4–)2.0–3.7(–5.3)$  ( $n = 40$ ), straight or curved, sometimes constricted in the middle. *Conidia* oblong, narrowly ellipsoid or allantoid, (2.8–)3.5–5.0(–6.3)  $\times$  (1.5–)1.7–2.2(–2.8)  $\mu\text{m}$ ,  $l/w = (1.8–)1.9–2.6(–3.2)$  ( $n = 40$ ), 1-celled, hyaline, smooth, eguttulate. Sometimes sulphur yellow mycelium or perithecia developing directly on the asexual morph.

**Cultures and asexual morph** — Ascospores germinating to produce conidia and hyphae, colony on CMD reaching a radius of c. 18 mm after 3 wk (strain MA3), conidia produced in masses within 24 h, colony hyaline, turning yellowish, centre rosy due to conidial masses; on PDA growth slower than on CMD, colony bright yellow with rosy conidial masses; on MEA growth usually faster than on CMD, colony yellow, centre turning rosy and eventually bright orange as conidial masses develop and extend, odour yeast-like. Culture from conidia predominantly hyphal, conidia formed after 2–3 d, colony turning yellow, crystals formed in the agar. Asexual morph of strain MA on MEA after 4–5 d at 25 °C: On CMD conidia formed on minute pegs along hyphae submerged in the agar. On MEA conidiophores formed on the agar surface, simple, acropleurogenous, sometimes densely aggregated and botryose, 2–5  $\mu\text{m}$  wide, mostly asymmetric, with few-celled branches or phialides typically only on one side. *Phialides* mostly solitary, lateral on main axes, mixed with small pegs, or terminal on branches, then solitary

or in pairs, lageniform, (4.5–)6.5–11.5(–16.5)  $\times$  (2.0–)2.5–3.2(–3.5)  $\mu\text{m}$ ,  $l/w = (1.8–)2.2–4.0(–5.7)$  ( $n = 43$ ), straight, hooked or sigmoid. *Conidia* oblong to allantoid, sometimes ellipsoid, (3.7–)5.8–9.0(–14.5)  $\times$  (1.3–)2.2–3.5(–5.2)  $\mu\text{m}$ ,  $l/w = (1.9–)2.3–3.0(–3.7)$  ( $n = 86$ ), hyaline, 1-celled, rarely with 1 or 2 non-constricted septa, smooth, eguttulate, scar indistinct.

**Habitat** — On species of *Cucurbitaria*, *Diplodia* and *Valsaria* occurring on dead corticated branches and twigs of *Fabaceae* (*Genista*, *Laburnum*, *Ononis*, *Retama*, *Ulex*).

**Distribution** — Southern Europe (Italy, Spain).

**Additional materials examined.** ITALY, Trentino, Volgaria, on *Laburnum alpinum*, soc. *Diatrype* sp., *Cyphellopsis* sp., 20 Oct. 2011, W. Jaklitsch & H. Voglmayr (WU 32155; culture MA2). — SPAIN, Andalucía, Granada, La Zubia, Cerro del Trevenque, N37°04'58" W3°30'26.3", elev. 1440 m, on *Cucurbitaria* sp. on *Ononis aragonensis*, 14 May 2014, S. Tello & W. Jaklitsch (WU 33431); Jaén, near N-432 exit to Castillo de Locubín, N37°31'31" W3°58'26.7", elev. 695 m, on *Cucurbitaria* sp. on *Retama sphaerocarpa*, 11 May 2014, W. Jaklitsch (WU 33430); near Villa Luenga, on *Retama sphaerocarpa*, on/soc. *Valsaria* cf. *insitiva* and *Cucurbitaria* sp., 23 Mar. 2011, W. Jaklitsch & H. Voglmayr (WU 32153; culture MA); Asturias, Santa Maria, Vega Cimera, on *Genista florida*, on wood, soc. asexual morph and *Cucurbitaria* sp., 4 June 2013, C. Lechat CLL 13027 (WU 32158; culture CBS 136002 = MA6); Soto de Los Infantes, near Viescas, on *Genista florida*, on ascumata and subiculum of *Cucurbitaria* sp., 4 June 2013, J. Linde (WU 32156; culture MA4); Villar de Vildas, on *Genista florida*, partly on *Cucurbitaria* sp., both morphs also on pycnidia of a *Diplodia* sp., soc. *Coniochaeta* sp., *Valsaria* sp., 6 June 2013, W. Jaklitsch & H. Voglmayr (WU 32157; culture from conidia CBS 136001 = MA5a; MA5: culture from ascospores); Navarra, Sorauren, on burnt, standing branches of *Ulex europaeus*, N42°52'14" W1°36'22", elev. 500 m, 11 Feb. 2014, J. Balda, comm. E. Rubio (WU 33424). — UNITED KINGDOM, England, South Hampshire, Southampton, Peartree Green, N50°54' W1°22", grid reference SU436115, on burnt branches of *Ulex europaeus*, 26 Apr. 2014, S. Rogerson, comm. P. Cannon (K(M) 191916).

**Notes** — Stromata of *T. roseovirens* are superficially similar to those of *T. chrysogramma* or other species, e.g., *T. rhodochlora*, but the scurf has a deep yellow tone. Sometimes they appear hypocrea-like (see Jaklitsch 2009, 2011) and are widely erumpent from bark. The apical paraphyses are numerous, and it is difficult to determine their points of attachment. A basal attachment could not be observed. In contrast to its close relative *T. asturiensis*, mature ascospores of *T. roseovirens* are generally medium brown and eguttulate or with many small guttules. Their distoseptation is evident in Checa et al. (2013: f. 5). These authors also pointed out that parts of the original material (see lectotype) was distributed to several other herbaria. The pycnidia in nature develop before the perithecia, which form directly on top with remnants of the asexual morph appearing as rosy margins of stromata.

***Thyonectria rubicarpa* (Cooke) Jaklitsch & Voglmayr, comb. nov.** — MycoBank MB808307

*Basionym.* *Nectria rubicarpa* Cooke, Grevillea 7: 50. 1878.

≡ *Pleonectria rubicarpa* (Cooke) Hirooka, Rossman & P. Chaverri, Stud. Mycol. 71: 162. 2012.

***Thyonectria sinopica* (Fr.) Jaklitsch & Voglmayr, comb. nov.** — MycoBank MB808308

*Basionym.* *Sphaeria sinopica* Fr., Elench. Fung. 2: 81. 1828.

≡ *Pleonectria sinopica* (Fr.) Hirooka, Rossman & P. Chaverri, Stud. Mycol. 71: 165. 2012.

**Materials examined.** AUSTRIA, Niederösterreich, Maierhöfen, asexual morph on *Hedera helix*, 20 June 2009, W. Jaklitsch & H. Voglmayr (WU 30364; culture from conidia NS = CBS 127386); Wöllersdorf, Dreistätten, Burgruine Starhemberg, on *Hedera helix*, 13 Mar. 2010, H. Voglmayr & I. Greilhuber (WU 32162).

***Thyronectria strobi*** (Hirooka, Rossman & P. Chaverri)  
Jaklitsch & Voglmayr, *comb. nov.* — MycoBank MB808309

*Basionym.* *Pleonectria strobi* Hirooka, Rossman & P. Chaverri, in Hirooka et al., *Stud. Mycol.* 71: 169. 2012.

***Thyronectria virens*** Harkn., in Ellis & Everhart, *North Amer. Pyrenomyc.*: 92. 1892. — Fig. 5m–w

≡ *Pleonectria virens* (Harkn.) Hirooka, Rossman & P. Chaverri, in Hirooka et al., *Stud. Mycol.* 71: 175. 2012.

*Materials examined.* FRANCE, Bourgogne, Dijon, Jardin de l'Arquebuse, on *Ostrya carpinifolia*, 13 Mar. 2014, A. Gardiennet AG14076 (WU 33426; culture NP10). — USA, California, Sausalito, on *Rhus diversiloba*, on/soc. *Diplodia* sp., H.W. Harkness (isoelectotype BPI 631193, as *Valsonectria virens*).

*Notes* — Ascospore colour is the same as in *T. rhodochlora*, i.e. ranging from hyaline over yellowish to rosy. Also the host is a *Diplodia* sp., as a perithecium was found directly on a pycnidium in the isoelectotype (Fig. 5o). French material differs from the isoelectotype by larger ascospores ((19.0–)19.8–24.5(–26.7) × (7.5–)9.0–11.3(–12.0) μm (n = 20) vs (14–)16.5–20(–22) × (7.0–)7.5–8.8(–9.2) μm (n = 30)) with mostly two vertical septa approaching those of *T. rhodochlora*, but differ by multiguttulate cells, more oblong shape and rather indistinct septa. Fresh material from North America is thus necessary to find out, whether European material labelled *T. virens* (Hirooka et al. (2012)) or *T. cf. virens* (this work) is conspecific with the American taxon or an undescribed species. See Fig. 5m–s for an illustration of the isoelectotype (BPI 631193) of *T. virens* and Fig. 5t–w for ascospores of WU 33426.

***Thyronectria zanthoxyli*** (Peck) Ellis & Everh. (as '*xanthoxyli*'),  
N. Amer. *Pyrenomyc.*: 92. 1892. — Fig. 5x–aa

*Basionym.* *Valsa zanthoxyli* Peck, *Ann. Rep. N.Y. State Mus.* 31: 49. 1879.

≡ *Fenestella zanthoxyli* (Peck) Sacc., *Syll. Fung.* 2: 332. 1883.

≡ *Nectria zanthoxyli* (Peck) Rossman, *Mem. New York Bot. Gard.* 49: 264. 1989.

≡ *Pleonectria zanthoxyli* (Peck) Hirooka, Rossman & P. Chaverri, *Stud. Mycol.* 71: 177. 2012.

≡ *Pseudovalsa zanthoxyli* (Peck) Sacc., *Syll. Fung.* 2: 137. 1883.

*Material examined.* USA, New York, West Troy, on *Zanthoxylum americanum*, Oct. 1878, C.H. Peck (lectotype NYS 3611 and isoelectotype NYS 3438 of *Valsa zanthoxyli*).

*Note* — This species is similar in many respects including ascospore colour to both *T. rhodochlora* and *T. virens* but has distinctly curved ascospores. See Fig. 5x–aa for an illustration of the type material.

### Residual species in *Thyronectria* and *Pleonectria*

Some additional names in *Thyronectria* were dealt with by Seeler (1940), which he recognised as synonyms of other fungi. Based on the description and in the absence of a useful type specimen, he regarded *T. sambucina* Ellis & Everh., *Bull. Torrey Bot. Club* 24, 10: 458 (1897), as doubtful. Although he did not see the type specimen, he combined *Pleonectria coffeicola* Zimm., *Zentbl. Bakt. Parasitkde, Abt. II* 8: 183 (1902) in *Thyronectria*, a fungus that occurs on living leaves of *Coffea* in Java and is thus likely not a species of this genus. Seeler (1940) did not mention *T. manihoticola* Sousa da Câmara, *Revista Agronomica* 17, 2: 8 (extr.) (1929), described from *Manihot* in Portugal. Four additional species of *Thyronectria* were described later: *T. hyperantarctica* (D. Hawksw.) D. Hawksw. & Spooner, *Kew Bull.* 35, 3: 519 (1980) and *T. inconspicua* Döbbeler, *Mitt. Bot. Staatssamml. München* 14: 116 (1978) occurring on bryophytes in Argentina and Austria, respectively, and *T. indica* A. Pandey & V.G. Rao, *Geobios, New Rep.* 7: 49

(1988) and *T. odinae* V.G. Rao & Varghese, *Sydowia* 32: 257 (1980) [1979] from India. No recent information about these species is available as well as for *Pleonectria affinis* Sacc., *Bol. Soc. Brot., Coimbra, sér. 2* 1: 139 (1922), which was not mentioned by Hirooka et al. (2012). *Pleonectria affinis* was described from an unidentified host in Africa and said to be similar to *T. berolinensis*.

### Residual species in *Mattirolia*

The remaining three species placed in *Mattirolia* are *M. macluræ* M.T. Lucas & Sousa da Câmara, *Agron. Lusit.* 15, 2: 159 (1953), *M. nivea* Speg., *Anales Mus. Nac. Hist. Nat. Buenos Aires* 6: 292 (1898) [1899] and *M. ohiensis* (Ellis & Everh.) Checa, M.N. Blanco & G. Moreno, *Mycotaxon* 125: 155. 2013.

Examination of the holotype of *M. macluræ* (Portugal, Coimbra, Botanical Garden, on a twig of *Maclura pomifera* (as *M. aurantiaca*), 16 Apr. 1952, *M.T. Lucas 1064* (LISE)) revealed a fungus immersed in bark, surrounded by reddish hyphae, with only the reddish cylindrical ostioles (80–110 μm diam) visible on the surface (Fig. 10p). The centrum of the ascomata consists of 1–2 μm wide trabeculae with indistinct septa, and narrowly clavate, fissitunicate asci with short stipe, measuring 103–115 × 13–15 μm, with 8 uni- to partly biseriolate ascospores. The ascospores are ellipsoid-oblong, dark brown, muriform, with 5–7 transverse and 1(–2) longitudinal septa, (16–)17–20 × (7.0–)7.5–8.7(–9.2) μm, l/w = 2.1–2.5(–2.7) (n = 20) (Fig. 10o). Based on this morphology, *M. macluræ* may be assigned to the genus *Karstenula*. The fungus is accompanied by *Valsaria* cf. *insitiva*, an effete *Diaporthe* sp. and a *Diplodia* sp.

*Mattirolia nivea* (syn. *Leucocrea nivea* (Speg.) Sacc. & P. Syd. ex Lindau, *Syll. Fung.* (Abellini) 16: 601 (1902)) was synonymised with *Balzanina platensis* Speg. by Rossman et al. (1999). Checa et al. (2013) included this species in *Mattirolia*; however, we have insufficient data to determine its placement. Likewise, as illustrated by Checa et al. (2013), perithecia of *Mattirolia ohiensis* lack yellow-green scurf and in the absence of molecular data its placement is unclear.

### KEY TO SPECIES OF THYRONECTRIA

(adapted from the *Pleonectria* key in Hirooka et al. 2012; conidia given below are mature conidia in the sense of these authors)

1. Ascospores hyaline when immature, becoming distinctly pigmented, green to brown, muriform, distoseptate, not budding ..... 2
1. Ascospores hyaline to yellowish or rosy, variously euseptate ..... 4
2. Ascospores brown; on *Ulmus americana*; in North America ..... *T. chrysogramma*
2. Ascospores green to brown ..... 3
3. On *Fabaceae* in Southern Europe; ascospores mostly ellipsoid, with finely multiguttulate cells when fresh ..... *T. roseovirens*
3. On *Quercus ilex* in Spain; ascospores mostly oblong, often curved, with one large guttule per cell when fresh ..... *T. asturiensis*
4. Ascospores in nature typically not budding ..... 5
4. Ascospores in nature budding in or outside asci ..... 16
5. Ascospores 1- to multiseptate but lacking longitudinal septa ..... 6
5. Ascospores muriform ..... 10
6. Ascospores (3–6–)7-septate, ellipsoid, oblong to allantoid, with broadly rounded ends, (15–)17–21(–25) × (4.5–)5.0–6.5(–7.3) μm; in culture conidia formed on pegs, 1-celled, long-cylindrical, (7.2–)8.7–11.3(–12.7) × (1.3–)1.5–



- 2.2(–3.0)  $\mu\text{m}$ ; on bark of dead deciduous trees, mainly *Oleaceae* . . . . . *T. aurigera*
6. Ascospores 1-septate, smooth or striate . . . . . 7
7. Ascospores striate, ellipsoid to fusiform, (13–)14–17(–18.5)  $\times$  (4.5–)5.3–6.7(–7.3)  $\mu\text{m}$ ; known from Argentina . . . . . *T. pseudomissouriensis*
7. Ascospores smooth . . . . . 8
8. Ascospores ellipsoid to fusiform, not constricted at the central septum, (9–)10–12(–13.5)  $\times$  (3.3–)4.0–5.0(–5.7)  $\mu\text{m}$ ; on *Citrus*, *Gelsemium* and *Ilex* . . . . . *T. rubicarpa*
8. Ascospores ellipsoid to fusiform, slightly constricted at the central septum; on *Hedera* or *Ilex* . . . . . 9
9. On *Ilex*; ascospores ellipsoid, (9–)11–13.5(–15.5)  $\times$  (4.0–)5.5–6.8(–7.5)  $\mu\text{m}$ ; conidia in culture (5.5–)6.5–9.5(–12.5)  $\times$  (2.0–)2.3–3.0(–3.3)  $\mu\text{m}$  . . . . . *T. illicicola*
9. On *Hedera*; ascospores ellipsoid to fusiform, (8–)10.5–13(–14.5)  $\times$  (3.7–)5.0–6.5(–8.0)  $\mu\text{m}$ ; conidia in culture (5.2–)6.0–11.0(–13.5)  $\times$  (1.0–)1.5–2.5(–3.0)  $\mu\text{m}$  . . . . . *T. sinopica*
10. Perithecia typically superficial on or partly immersed in a hypostroma . . . . . 11
10. Perithecia surrounded by stromatic tissue . . . . . 13
11. Perithecia partly immersed in a hypostroma; ascospores subglobose to ellipsoid, (9.7–)10–12.5(–15)  $\times$  (4.8–)6.0–7.5(–10.2)  $\mu\text{m}$ ; on *Fabaceae*; in nature pycnidia forming tubercular to cerebriform masses; conidia ellipsoid to oblong, (1.7–)2.3–3.0(–3.5)  $\times$  (1.0–)1.3–2.0(–2.5)  $\mu\text{m}$  . . . . . *T. austroamericana*
11. Perithecia superficial on a hypostroma . . . . . 12
12. Ascospores of two sizes: micro-ascospores allantoid to short-cylindrical, (21–)25–30(–32.5)  $\times$  (8.2–)9.5–12(–13)  $\mu\text{m}$ , macro-ascospores cylindrical, (37–)39–47(–49.5)  $\times$  (10–)10.5–12(–13)  $\mu\text{m}$ ; on *Carya*, in USA . . . . . *T. missouriensis*
12. Ascospores uniform, oblong to ellipsoid, 17–25  $\times$  6.5–8.5  $\mu\text{m}$ ; on *Ribes* . . . . . *T. berolinensis*
13. Saffron to sienna stroma surrounding perithecia immersed in bark, scarcely protruding, with red-orange apex, asci long/slender; on *Lonicera involucrata* and *Symphoricarpos*; USA . . . . . *T. loniceræ*
13. Perithecia immersed in yellowish stroma erumpent from bark; ascospores yellowish to rosy at maturity . . . . . 14
14. Ascospores ellipsoid or oblong, (15–)18–25(–37)  $\times$  (7–)9–12(–16)  $\mu\text{m}$ ; chiefly on *Acer campestre*, but also other trees in Europe . . . . . *T. rhodochlora*
14. Ascospores oblong and often curved, averaging < 9  $\mu\text{m}$  wide . . . . . 15
15. Ascospores (13–)16–21(–23)  $\mu\text{m}$  long; mean colony diameter 14 mm on PDA after 7 d at 25 °C; chiefly on *Rhus*, but also on other trees and shrubs . . . . . *T. virens*
15. Ascospores (18–)19–24(–26.5)  $\mu\text{m}$  long, distinctly curved; mean colony diameter > 67 mm on PDA after 7 d at 25 °C; chiefly on *Zanthoxylum* . . . . . *T. zanthoxyli*
16. Ascospores not budding or budding outside asci in perithecia . . . . . 17
16. Ascospores budding inside asci . . . . . 18
17. Ascospores 1-septate, (8.7–)10–12.5(–13.5)  $\times$  (3.7–)4.5–6.0(–6.8)  $\mu\text{m}$ ; in culture conidia 1-celled, ellipsoid, fusiform or allantoid, (5–)7–10(–11.5)  $\times$  (1.8–)2.0–2.8(–3.3)  $\mu\text{m}$ ; on *Castanopsis* in Japan . . . . . *T. okinawensis*
17. Ascospores muriform, mostly with 7 transversal septa and 1 longitudinal septum, 17–25  $\times$  6.5–8.5  $\mu\text{m}$ ; in culture conidia swollen, ellipsoid, oblong-allantoid, (0–)1(–2)-septate, (9–)10–14(–20)  $\times$  (2.2–)3.3–4.7(–5.5)  $\mu\text{m}$ ; on *Ribes* . . . . . *T. berolinensis*
18. Ascospores 1-septate . . . . . 19
18. Ascospores multiseptate or muriform . . . . . 20
19. Ascospores ellipsoid to fusiform, (8–)9–11(–13)  $\times$  (3.2–)4–5.5(–6.5)  $\mu\text{m}$ ; on *Ilex aquifolium*; in Europe *T. aquifolii*
19. Ascospores cylindrical or fusiform, (8.3–)10–13(–15)  $\times$  (2.2–)2.8–4(–5.3)  $\mu\text{m}$ ; on *Corylus avellana* and other deciduous trees and shrubs . . . . . *T. coryli*
20. Ascospores filiform, transversely multiseptate . . . . . 21
20. Ascospores muriform . . . . . 24
21. Ascospores 8–15-septate, hyaline, (26–)31–44(–49)  $\times$  (1.3–)2.3–4.0(–4.7)  $\mu\text{m}$ ; on *Quercus ilex* ssp. *rotundifolia* . . . . . *T. quercicola*
21. Ascospores 8–44-septate, long-filiform, 22–75  $\mu\text{m}$  long, hyaline; on conifers . . . . . 22
22. On *Abies*; ascomatal surface scaly; ascospores 8–31-septate, (22–)29–45(–60)  $\times$  (1.5–)2.0–3.2(–4.0)  $\mu\text{m}$  . . . . . *T. roselinii*
22. On *Pinus*; ascomatal surface generally scurfy . . . . . 23
23. On *Pinus* subg. *Pinus*; ascospores 15–39-septate, (33–)43–65(–75)  $\times$  (2.3–)2.7–3.5(–3.7)  $\mu\text{m}$  . . . . . *T. cucurbitula*
23. On *Pinus* subg. *Strobus*; ascospores 12–44-septate, (22–)33–52(–64)  $\times$  (2.0–)2.2–3.2(–4.0)  $\mu\text{m}$  . . . . . *T. strobi*
24. Ascospores disarticulating; part-ascospores subglobose to ellipsoid, (7.7–)8.7–12(–13.5)  $\times$  (5.0–)6.5–8.5(–9.0)  $\mu\text{m}$ ; on *Platanus occidentalis* and *Ulmus americana*; in USA . . . . . *T. chlorinella*
24. Ascospores not disarticulating . . . . . 25
25. Ascospores subglobose to ellipsoid, (5.0–)5.5–7.5(–9.5)  $\times$  (4.0–)4.5–6.5(–8.5)  $\mu\text{m}$ ; on *Fabaceae* . *T. sphaerospora*
25. Ascospores differently shaped . . . . . 26
26. Ascospores clavate . . . . . 27
26. Ascospores ellipsoid, oblong to fusiform . . . . . 28
27. Ascospores broadly clavate, (16–)18–23(–36.5)  $\times$  (4.3–)4.8–6.2(–7.0)  $\mu\text{m}$ ; on *Ribes*; in North America . . . . . *T. clavatispora*
27. Ascospores narrowly clavate, (21–)25–33(–38)  $\times$  (4.0–)5.0–6.2(–7.2)  $\mu\text{m}$ ; on *Berberis cretica*, *B. hispanica*, *B. cf. lycium*; in Greece, Morocco, Spain and Pakistan . . . . . *T. caudata*
28. Perithecia black; ascospores fusiform, oblong, vermiform or clavate; (12.5–)17.0–24.5(–28.5)  $\times$  (3.5–)4.0–5.2(–6.5)  $\mu\text{m}$ ; on *Tamarix* spp.; in Europe (Austria) . . . . . *T. obscura*
28. Perithecia with shades of red . . . . . 29
29. Ascospores fusoid or oblong, (13.5–)18.5–25.5(–30.0)  $\times$  (4.8–)5.5–7.2(–8.2)  $\mu\text{m}$ ; on *Berberis* spp. . . . . *T. lamyi*
29. Ascospores ellipsoid, fusiform, cylindrical to vermiform, averaging < 5  $\mu\text{m}$  wide; on conifers . . . . . 30
30. On *Picea*; perithecial apex of three regions; ascospores with 7–25 transverse septa, cylindrical to vermiform, (15.5–)20–30(–36)  $\times$  (2.8–)3.2–4.2(–4.5)  $\mu\text{m}$ ; in culture conidia long-cylindrical to allantoid, (7.5–)9–11(–12.3)  $\times$  (1.3–)1.5–2.0  $\mu\text{m}$  . . . . . *T. boothii*
30. On *Abies* or *Pinus*; perithecial apex of two regions; ascospores averaging > 4.5  $\mu\text{m}$  wide . . . . . 31
31. On *Abies*; ascospores ellipsoid to fusiform with 5–9 transverse septa, (16–)20–24(–28.5)  $\times$  (3–)4–5.5(–7)  $\mu\text{m}$ ; in culture conidiophores not abundant; conidia subglobose to ellipsoid, (6.0–)6.5–7.2(–9.0)  $\times$  (2.2–)2.5–3.3(–3.5)  $\mu\text{m}$  . . . . . *T. balsamea*
31. On *Pinus*; ascospores ellipsoid, fusiform to vermiform with 5–15 transverse septa, (14–)18–28(–46.5)  $\times$  (3.2–)4.3–5.3(–7)  $\mu\text{m}$ ; in culture conidiophores abundant, conidia oblong, slightly swollen at both ends, (5.5–)7–11(–13)  $\times$  (1.7–)2.0–2.7(–3.0)  $\mu\text{m}$  . . . . . *T. pinicola*

## DISCUSSION

Here we report a change in the phylogenetic placement of several fungi that until recently have been classified in the *Thyridiaceae*. We have been reluctant to accept the placement of *Thyronectria* and some other genera in the *Thyridiaceae*, because the genus *Thyridium* differs from nectriaceous fungi in the following fundamental traits: species of *Thyridium* s.str. (excluding *Sinosphaeria* J.Z. Yue & O.E. Erikss. and *Bivonella* (Sacc.) Sacc.) have a dark brown to black peridium, macro- and microscopically, that never shows a pH-dependent colour reaction, they have true paraphyses, asci that become easily detached in microscopic mounts and have sometimes a ring in the ascus apex. Also the more or less yellow entostroma surrounding the perithecia is KOH-negative. Stromatic tissues of *Sinosphaeria* and *Bivonella* yield a yellow pigment in KOH and ethanol, it is therefore questionable, whether they are synonyms of *Thyridium* as advocated by Eriksson & Yue (1989) and accepted by Checa et al. (2013). The yellow scurf of *Thyronectria* species also releases such pigment in 3 % KOH and to a lesser extent in ethanol. These morphological differences are also reflected by different phylogenetic positions, as *Thyridium* (*Thyridiaceae*) is a member of *Sordariomycetidae* (Spatafora et al. 2006), whereas *Thyronectria* is embedded within *Nectriaceae* (*Hypocreomycetidae*) (Fig. 1).

Here we also re-instate the genus *Thyronectria* for *Pleonectria* as recently monographed by Hirooka et al. (2012) on sound molecular and morphological evidence. One of the central themes that form the basis of our conclusions was the recollection of *Thyronectria roseovirens* in the area of its original collection site. This species is the generic type of *Mattiolia*, which has been regarded to possess true paraphyses and was therefore placed in the *Thyridiaceae*. Using molecular data, we determined that this fungus belongs to a genus of the *Nectriaceae* (*Hypocreales*). This raised the question whether the persistent hamathecial threads characterising this species are true paraphyses as interpreted earlier (Rossmann et al. 1999, Checa et al. 2013) or apical paraphyses that originate at the top of the perithecium and continue down to the ascus bases. We found these threads in all other species we studied, but could not find any evidence that these threads may be attached to or originate in the subhymenium between asci, i.e. they seem to be in fact apical paraphyses. A study of immature perithecia of *T. rhodochlora* revealed that a hyaline apical cushion was present at the top of the perithecium, from which a 3-dimensional reticulum of anastomosing threads continues down to the base of the perithecium. In the *Nectriaceae* these threads are usually evanescent, but in at least a number of *Thyronectria* spp., and apparently also in *Nectria himalayensis* (Hirooka et al. 2012: f. 36D, E), they are persistent until maturation of ascospores. These apical paraphyses can be often removed from the asci by pressure on the cover slip of microscopic mounts, but sometimes this is difficult. Sometimes threads remain and this is apparently because they may become entrapped between asci near their bases (see e.g. *T. lamyi*, Fig. 10i). The stability of the apical paraphyses during development depends on the species, as sometimes septa may become strongly constricted and cells inflated.

As we have seen that the genus '*Pleonectria*' contains long apical paraphyses that are clearly differentiated and much distinct from paraphyses, the next question was which generic name should be applied. In this context *Thyronectria* clearly has priority. We had then to clarify the correct epithet of its type species. The type species of *Thyronectria*, *T. patavina*, is said to occur on *Juglans regia* in association with *Thyridaria incurstantans*. After examination of thousands of branches and twigs of *Juglans regia* and *J. nigra* in several countries including Italy,

also in Padua, in vain, we re-examined the type specimen of *T. patavina* in PAD. The equipment in the herbarium unfortunately did not allow us taking better images of the ascospores than that given in Fig. 3m. However, gross morphology as well as a section prepared in PAD, enabled us to make measurements in our lab and to produce illustrations of the apical paraphyses and asci with ascospores (see Fig. 4a, e, f, k, l). Only hyaline ascospores were present in the section, which are in accordance with those of fresh specimens and type material of '*Pleonectria*' *pyrrhochlora*, but also with those of the type material of *T. rhodochlora* (see below).

In his original species description Saccardo (1875b) wrote that the perithecia are yellow-powdered outside, that the context is slightly reddening and that the ascospores are  $25 \times 9\text{--}11 \mu\text{m}$ , rarely  $30 \times 8 \mu\text{m}$ , first full of oil drops, later thinly and profusely 7–9-septate, muriform, hyaline. An ascospore size of  $25 \times 8\text{--}11 \mu\text{m}$  is given by Saccardo (1877) on his illustration of the fungus. These data fully agree with *T. rhodochlora*.

Subsequently we studied two parts of the original collection of *Thyronectria rhodochlora* and found that it is clearly conspecific with *T. patavina* and *T. pyrrhochlora* and that it was collected on a species of *Acer*, not *Alnus* as originally stated. As *T. rhodochlora* is older than both *T. patavina* and *T. pyrrhochlora*, it is the correct name for the type species of *Thyronectria*. *Thyronectria rhodochlora* is, however, not specific for *Acer campestre* as stated previously (Hirooka et al. 2012). The host of the *T. patavina* holotype specimen (PAD) is not *Juglans*, as noted above. A probable host of the type of *T. patavina* could be *Populus* sp. or *Salix* sp., the latter with somewhat higher probability, because '*Mattiolia*' *mutabilis*, another synonym of *T. rhodochlora*, was collected on *Salix caprea* in France.

All these data given above and also the fact that we found *T. rhodochlora* close to the original collection area of *T. patavina* south from Padua in the Colli Euganei on *Acer campestre* and *Robinia pseudoacacia*, are sound and convincing evidence of conspecificity of *T. patavina* with *T. rhodochlora*. We therefore epitypify *Thyronectria patavina* with the same epitype specimen designated for *Sphaeria rhodochlora* to stabilize this connection also nomenclaturally.

Except for the muriform-spored *Nectria antarctica* and *N. pseudotrachia* and the recently added species characterised by didymo- or phragmospores, Seeler (1940) was essentially correct with his generic concept of *Thyronectria* and synonymies of genera, although the types of *T. patavina* and *T. rhodochlora* were not available to him. In essence, three species that he included, *T. patavina*, *T. pyrrhochlora* and *T. rhodochlora*, are here merged into one. At first sight it may be surprising that the *Nectriaceae* contain fungi that have green to distinctly brown ascospores, but already Seeler (1940) concluded that ascospore colour is insignificant on the generic level. Surprising is also that fungi that were thought to contain true paraphyses belong to a genus of the *Nectriaceae*. This can be explained by the difficulty to determine the origin and to find the ends of the hamathecial threads, i.e. their interpretation as true paraphyses was erroneous.

Morphologically, two groups of species are recognisable in *Thyronectria*: the first, which contains the majority of species, is characterised by superficial perithecia usually aggregated on a hypostroma, a strong pH-dependent colour reaction of the peridium in most species, and budding ascospores. In this group the yellow-green scurf is situated directly on the perithecial surface. It may be scant on mature perithecia, but this often varies within species e.g. depending on the host. Usually the scurf is more abundant on young perithecia, especially in those species where the perithecium collapses upon drying. Repeated drying and rehydration in nature apparently results



in loss of the scurf particles because of mechanical stress. Apical paraphyses in this group are highly variable in abundance, can be generally easily removed from the asci in microscopic mounts, and they are often present as evanescent, sometimes submoniliform threads in mature perithecia. The species of this group we studied here, e.g. *T. coryli*, *T. caudata* and *T. lamyi*, all have such threads when asci are mature, i.e. when filled with ascoconidia (Fig. 7s, 9m, 10i).

The second group of species, contained within two highly supported clades at the base of *Thyronectria* (Fig. 1, 2), have scattered or aggregated perithecia, each of which is surrounded by a stroma that continues into the substrate. Here the scurf is situated on the stroma, not the perithecium. To this group, recognised by Hirooka et al. (2012) as containing *T. austroamericana*, *T. rhodochlora* (as *P. pyrrochlora*), *T. loniceræ*, *T. virens* and *T. zanthoxyli*, we add *T. asturiensis*, *T. obscura* and *T. roseovirens*. *Thyronectria chrysogramma* belongs also here, although that species has not been sequenced. In this group the apical paraphyses are much more persistent than in the other, which is clearly shown by the fact that other workers (Rossman et al. 1999, Checa et al. 2013) interpreted them as true paraphyses. Even in this group cells of the paraphyses may sometimes become inflated with age, as seen in some perithecia in *T. rhodochlora*.

As reported by Hirooka et al. (2012), colour reactions to KOH may be obscured by the yellow scurf, but also by stromatic tissues encasing perithecia. Most species have some orange to red peridium a priori, which may result in a weak macroscopic colour reaction to KOH, thus there is a need to check for the colour reaction in lactic acid in which the peridium may turn bright yellow. This is best done on a slide using diluted lactic acid on dry perithecia or undiluted lactic acid after rehydration of the perithecia.

Earlier workers did not mention the fungicolous habit of the genus. That a species of a genus is not always specific for its preferred plant species or genus obviously depends on the host specificity of the respective fungal host. As we did not study all species of *Thyronectria*, we cannot confirm that all species are fungicolous, but it seems that the situation is similar to *Trichoderma* (syn. *Hypocrea*) in the *Hypocreaceae*, as usually various different fungi accompany ascocata of *Thyronectria* spp., but they often do not grow directly on macroscopically visible parts of fungi, which may mean that they can also attack hyphae of the host fungus.

Of the 26 species recognised by Hirooka et al. (2012) in *Thyronectria* (as *Pleonectria*) 19 are characterised by molecular data, i.e. many need recollection and sequencing, particularly those that were originally described from North America. For *T. aurigera* and *T. virens* only accessions are available in GenBank that were obtained from material collected in Europe (France). Hirooka et al. (2012) described seven new species in *Thyronectria* (as *Pleonectria*). We add three new species, provide DNA data for them and some additional species.

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