Molecular data confirm the mitosporic state of *Hyphodermella rosae* (Phanerochaetaceae) as the pathogen of rosaceous fruits in northern Iran

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Hyphodermella is a genus of corticioid white rot fungi from the Phanerochaetaceae. Molecular data (partial SSU, LSU and complete ITS nrDNA) confirm the mitosporic state of *Hyphodermella rosae* as the causal agent of dry fruit rot of plum (*Prunus domestica*) and peach (*Prunus persica*) from Mazandaran, Iran. The asexual state of *H. rosae* is characterized by the presence of unicellular, spherical mitospores, which are terminal or intercalary in position. Both basidia and mitospores were observed in the matured cultures of *H. rosae*, although we did not observe any basidiospores. The phylogeny of Phanerochaetaceae confirms *Hyphodermella* as a monophyletic lineage within the family and sister group to *Phanerochaete* with considerable bootstrap support. Both the isolates of mitosporic *H. rosae* nest within a clade, which includes its sexual counterpart. Hence, we conclude that the lifecycle of *H. rosae* includes at least two reproductive states, i.e. sexual and asexual. Mitosporic *H. rosae* is capable of infecting plants and produce similar disease symptoms as its sexual state.

Keywords: Basidiomycota, dry fruit rot, mitospore, multi-gene phylogeny.

Corticioid species are a phylogenetically heterogeneous group of fungi within Agaricomycotina, Basidiomycetes. These fungi are characterized by resupinate, effused and crust like basidiomata that usually develop on the underside of decaying woody debris and logs (Hjortstam et al. 1988). Parmasto (1997) recognized 1733 species of corticioid Homobasidiomycetes, which include the majority of resupinate forms. Based on Parmasto's information, Hibbett & Binder (2002) estimated that approximately 13–15 % of described Homobasidiomycetes are resupinate.

Corticioid Basidiomycetes exhibit a broad range of habits as saprotrophs, pathogens of plants and lichens and also lichenization (Hibbett & Thorn 2001). The most common habits of corticioid Agaricomycetes are wood-decomposers and ectomycorrhizal symbionts, their role as saprotrophs is crucial in the nutrient recycling system (Rayner & Boddy 1988, Smith & Read 1997).

The genus *Hyphodermella* was erected by Eriksson & Ryvarden (1976) to accommodate *Hyphodermella corrugata* (Fr.) J. Eriksson & Ryvarden and was classified in the family Phanerochaetaceae (Larsson 2007). Based on morphological characters *Hyphodermella* comprises six species: *H. corrugata* (Eriksson & Ryvarden 1976), *H. maunakeaensis* (Gilbertson et al. 2001), *H. densa* (Melo & Hjortstam 2003), *H. rosae* (Nakasone 2008), *H. ochracea* (Duhem 2010) and *H. brunneocontexta* (Duhem & Buyck 2011). Telleria et al. (2010) while studying the morphology of three *Hyphodermella* species concluded that the morphological differences between *H. rosae* and *H. densa* are insignificant, which was also supported by molecular data. They concluded that *H. densa* and *H. rosae* are synonymous and that *H. corrugata* is distinct from *H. rosae*. *Hyphodermella rosae* as well as *H. corrugata* are widely distributed in the western Mediterranean area: France, Italy, Spain and Portugal (Nakasone 2008, Telleria et al. 2010). In Iran, *H. rosae* has been reported as a saprotroph species from East Azerbaijan (Ghobad-Nejhad & Hallenberg 2012) and as a pathogen causing dry fruit rot of peach (*Prunus persica*) and plum (*Prunus domestica*) from Mazandaran (Sayari et al. 2012, Babaeizad et al. 2012). *Hyphodermella corru-*

gata has also been reported causing white rot on apricot, sweet and sour cherry trees (Ogawa et al. 2003, Uyemoto et al. 2012).

There are no previous reports on the mitosporic state of *Hyphodermella*, although Ibáñez (1999) in his studies of non-poroid Aphyllophorales observed vacuolated, intensely staining generative hyphae of *H. corrugata* bearing spherical chlamydospores.

Here we report first the mitosporic state of *H. rosae* causing dry fruit rot of plum and peach from Mazandaran, Iran, which is confirmed using a multi-loci dataset (SSU, ITS and LSU nrDNA).

Materials and methods

Fungal specimens

Hyphodermella rosae was isolated from symptomatic fruits of plum and peach from Mazandaran, Iran. The fungi were initially isolated on Potato Dextrose Agar (PDA) medium and later sub-cultured onto modified Vogels medium N. All the plates were incubated for three weeks at 28 °C in order to sporulate (Gold & Cheng 1979). Both the isolates were deposited at the International Collection of Microorganisms from Plants (ICMP), under the accession no. 20104 and 20105, respectively. *Hyphodermella corrugata* isolate 16963 was retrieved from ICMP.

Light microscopy

Slides were prepared using 21 days old fungal cultures. Mean values of 50–100 measurements are indicated with the range within parentheses. Freehand drawings were made at a fixed scale, and photographs were taken with Nikon DS-Fi1 camera attached to the Nikon Eclipse 50i microscope.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from mycelia using CTAB method (Winnepenninckx *et al.* 1993). The nuclear LSU, ITS and SSU rDNA regions were amplified using fungal specific primer pairs LR0R/LR7 (Vilgalys & Hester 1990), ITS5/ITS4 and NS1/NS4 (White *et al.* 1990), respectively. Individual PCR reactions were carried out using 10 ng of DNA, 0.1 mM dNTPs, 1 U Smart-Taq DNA polymerase (CinnaGen Co.), 4 mM $MgCl_2$ and 0.2 µM of primers in a final volume of 30 µl. The PCR conditions were adjusted to initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 45 °C (SSU), 60 °C (ITS) and 58 °C (LSU) for 1 min, 72 °C for 1 min and final extension at 72 °C for 7 min using Bio-Rad MJ Mini™ PTC-1148 thermal cycler. Bioneer Corporation (South Korea) sequenced the amplicons. Sequences were deposited in GenBank (see Tab. 1).

Taxon selection and alignments

Sequences for each gene region for various taxa under Phanerochaetaceae were retrieved from GenBank (see Tab. 1). For consistency in the phylogenetic analysis, sequences were selected from the same isolate for each taxon where possible. Each gene region was aligned separately along with our *Hyphodermella* sequences using MAFFT (Katoh *et al.* 2005) through the EMBL-EBI server (http://www.ebi.ac.uk/Tools/msa/mafft/). Then the alignments were adjusted manually using Mesquite 2.75 (Maddison & Maddison 2011). We used RAxML 7.4.2 (Stamatakis 2006) to run several preliminary phylogenetic analyses using single gene dataset to check for incongruences in the phylogenetic position of the taxon (results not shown). Thereafter the concatenated dataset (LSU + SSU + ITS) was generated using Mesquite. Based on the phylogenies provided by Kim & Jung (2000), Binder et al. (2005) and Jia et al. (2014) the outgroups were selected from the *Antrodia* clade (brown-rot fungi).

The final alignment included 29 taxa with 1330 characters from LSU, 1589 from SSU and 676 from ITS. Alignment of the concatenated dataset is available at TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S16312).

Phylogenetic analysis

Phylogenetic analysis was performed using RAxML 7.4.2 with the concatenated dataset. The general time reversible (GTR) model along with a gamma distribution was selected using jModelTest 2.1 (Guindon & Gascuel 2003, Darriba et al. 2012). We performed 50 replicated likelihood searches followed by 1000 bootstrap replicates. The tree was rooted and modified using FigTree 1.4 (available at http://tree.bio.ed.ac.uk/software/figtree/). Clades with ≥70 % bootstrap support were considered as moderate, while ≥90 % as strong. Clades with <70 % support were considered unreliable.

We also performed several preliminary phylogenetic analyses using the concatenated dataset. After preliminary analyses (results not shown), *Ceriporiopsis* species indicated a polyphyletic distribution, as previously shown by Tomšovský et al. (2010). Hence, we excluded *Ceriporiopsis* from our analysis, due to its inconsistency with the objectives of this study. Among all three available *H. corrugata*

Tab. 1. Species selected for phylogenetic study. Species names in bold indicate new sequences generated for this study.

Species	Voucher number	GenBank accession number		
		LSU	SSU	ITS
Ingroup: Phanerochaetaceae				
Bjerkandera adusta	TNMF1405	GQ470629		
	DAOM 215869		DQ060084	DQ060097
Ceriporia purpurea	Dai6366	JX644047		JX623952
	DAOM 21316		U59065	
Ceriporia reticulata	KHL 11981 (GB)	EU118614		
	ATCC 24995		AB084587	
	Li1316			JX623947
Ceriporia spissa	Dai8168	KC182785		KC182768
	ATCC 62024		AB084588	
Ceriporia viridans	Dai5183	KC182786		
	USDA HHB-9594-Sp		AB084592	
	Yuan2747			KC182778
Ceriporia tarda	TNMF03650	GQ470632		
	USDA KY-214		AB084591	
Ceriporia tarda	Dai10226			JX623945
Gloeoporus pannocinctus	BRNM 709972	FJ496708		
	USDA L-15836-Sp		AB084590	
	DLL2010-123			JQ673106
Gloeoporus taxicola	GB98	AY586656		
	KEW213		AF334913	
	GL52			AM231907
Hyphodermella corrugata	**ICMP 16963**	**KM103083**	**KM103086**	**KM244653**
	MA-Fungi 61395	JN939584	JN940192	FN600380
	MA-Fungi 61382	JN939596	JN940188	FN600373
	MA-Fungi 5527	JN939597	JN940187	FN600372
Hyphodermella rosae	**H16**	**KM103081**	**KM103084**	
	AB1			**JN593086**
	P1	**KM103082**	**KM103085**	
	10			**JQ920375**
	MA-Fungi 38071	JN939588	JN940191	FN600389
	MA-Fungi 24292	JN939589	JN940190	FN600388
	MA-Fungi 75541	JN939595	JN940189	FN600383
Phanerochaete chrysosporium	ATCC MYA4764	JN874484		
	CBS 153.67		GU733344	
	ATCC MYA4764			JN882305
Phanerochaete sordida	KUC8037	AY858368		
	SFFPS YK624		AB084593	
	xsd08107			FJ481018
Phanerochaete stereoides	Wu9708-118	GQ470661		
	TMI TMIC33983		AB084598	
	VPCI 2073/12			KF291012
Phanerochaete velutina	FCUG1815	DQ679917		
	USDA RLG-11272-Sp		AB084599	
	FP-102157-sp			AY219351
Phanerochaete sanguinea	TNMF15264	GQ470655		
	USAD FP-105385-Sp		AB084597	
	FP-100391-sp			AY219353
Phlebia brevispora	BAFC-633	JX863667	JX863666	HM208154
Phlebia chrysocreas	KHL10216	AY586695		
	TMI TMIC31891		AB084601	AB084617
Phlebia radiata	P.CH-11	KF562010		
	ATCC 64658		AY946267	DQ056859
Phlebia tremellosa	KUC9161	JF416676		
	JHG-344		AY219402	
	CFMR:DLL2011-058			KJ140579
Outgroup: Fomitopsidaceae				
Amylocystis lapponica	HHB-13400-Sp	KC585059	AF518570	KC585237
Antrodia xantha	TFRI 879	EU232284		EU232210
	FCUG 100		AY336775	
Melanoporia nigra	FP-90888	KC585186		
	CBS 341.63		AF082674	DQ491420

SSU sequences in GenBank, JN940187 is missing a considerable part of the conserved region. However, due to unavailability of SSU sequences for *H. corrugata,* we included JN940187 in our final analysis along with other *H. corrugata* sequences.

Results

Phylogenetic analysis

Isolates of mitosporic *H. rosae* nest within a clade that includes its sexual counterpart, with 97 % bootstrap support from the concatenated dataset (Fig. 1). The isolate of *H. corrugata* from ICMP forms a monophyletic clade with sequences of the same taxon retrieved from GenBank. The *Phanerochaete* species *P. velutina, P. chrysospori-*

um, P. sanguinea, P. stereoides and *P. sordida* form a monophyletic clade within Phanerochaetaceae and a sister group to *Hyphodermella* with 99 % and 100 % bootstrap supports, respectively. *Ceriporia* forms a monophyletic clade with *C. tarda* as the earliest diverging taxon within *Ceriporia. Gloeoporus* species come out as the early diverging taxa in a clade that also includes *Ceriporia* species.

The mitosporic state of *Hyphodermella rosae* (Bresadola) Nakasone – Figs. 2–18.

D e s c r i p t i o n . – Colonies appressed, farinaceous to floccose, white to pale cream (Figs. 3, 9). Hyphae hyaline, multi-nucleate, (30)38–84(97) × 2–5 µm (n = 100), with few septa, clamp connection absent or limited to wide advancing hyphae. Mito-
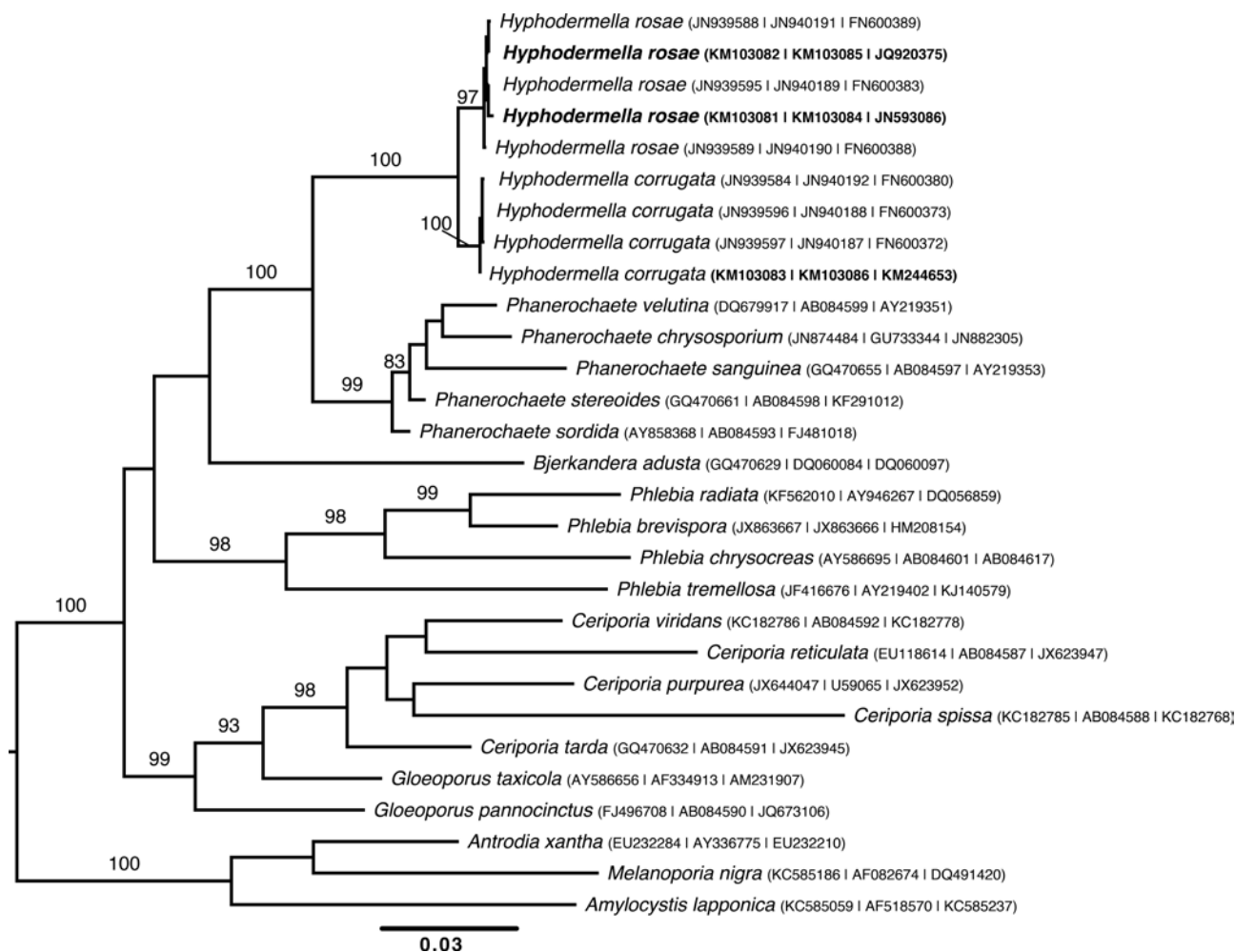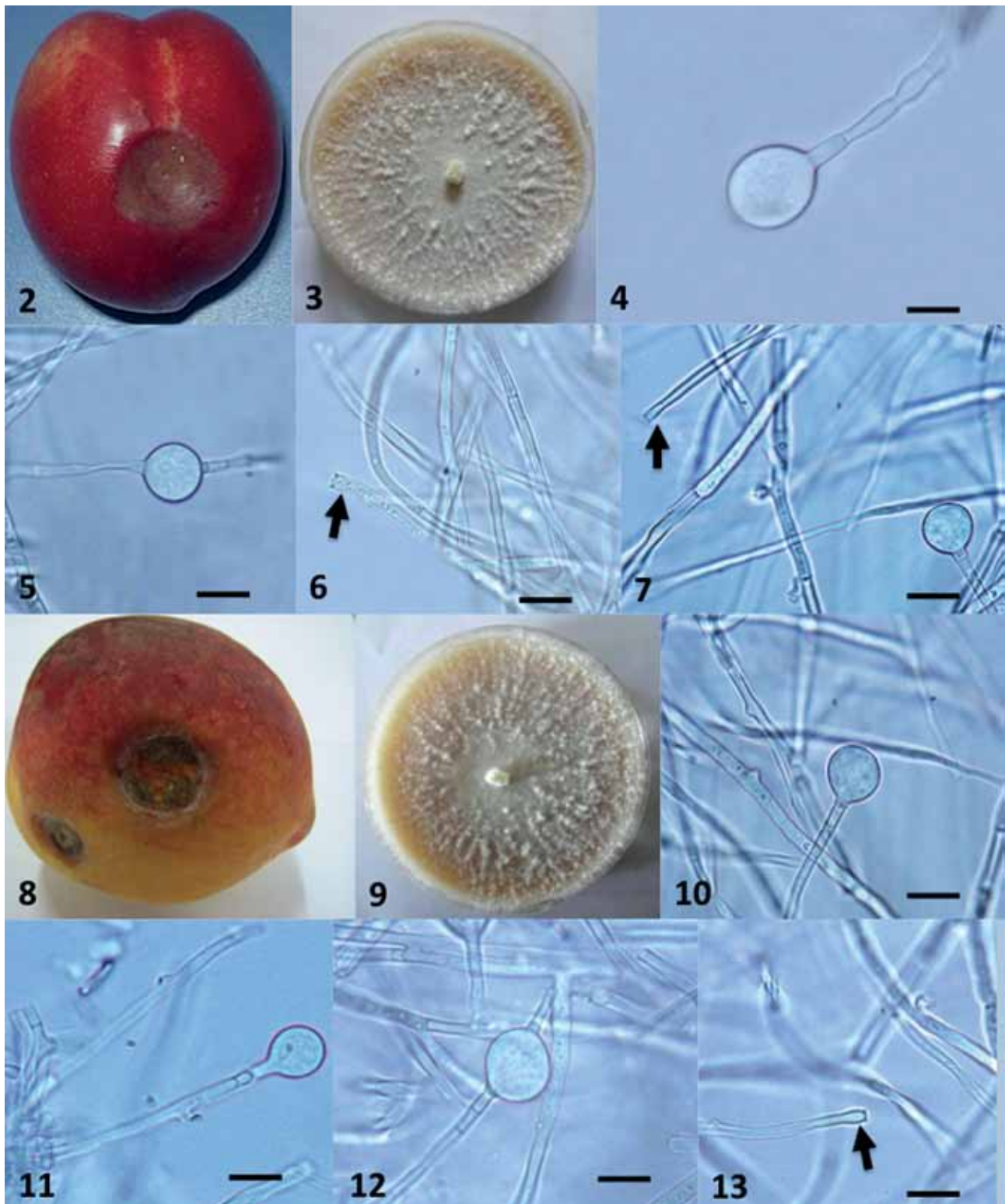

Fig. 1. *Hyphodermella rosae* plum and peach isolates in boldface represent mitosporic states. Accession numbers in boldface represent new sequences generated in this study. Maximum likelihood tree generated with RAxML for LSU and SSU as well as ITS. Bootstrap values from 1000 replicates. GenBank accession no. LSU/ SSU/ ITS. Bar = no. of nucleotide substitutions/site.

Figs. 2–13. *Hyphodermella rosae.* **Figs. 2–7.** Plum isolate. **2.** Disease symptom on fruit. **3.** Colony morphology on modified Vogels medium N. **4.** Terminal mitospore. **5.** Intercalary mitospore. **6.** Bisterigmate basidium (arrow). **7.** Terminal mitospore and basidium (arrow). **Figs. 8–13.** Peach isolate. **8.** Disease symptom on fruit. **9.** Colony morphology on modified Vogels medium N. **10, 11.** Terminal mitospore. **12.** Intercalary mitospore. **13.** Bisterigmate basidium (arrow). Bars 5 μm.
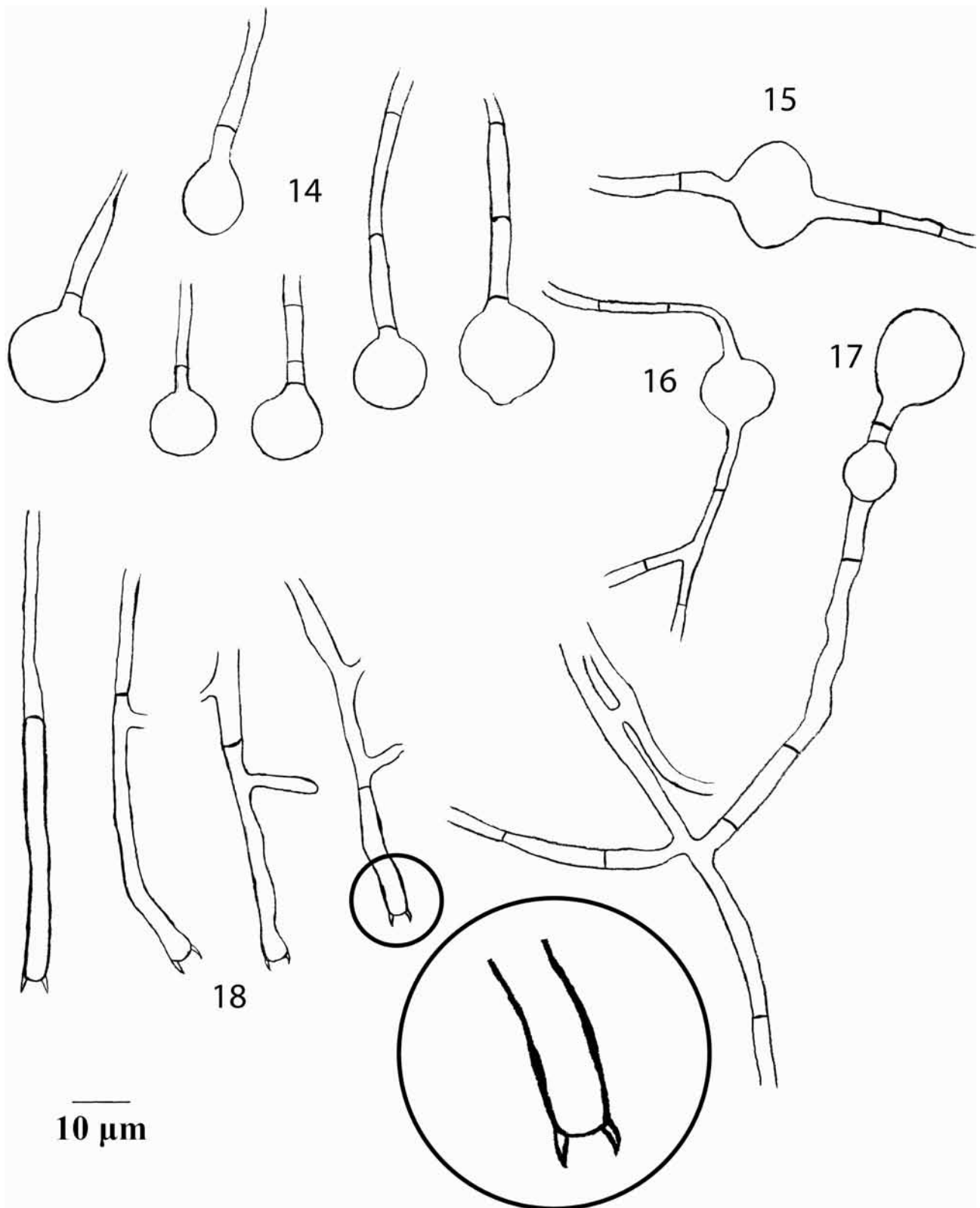
Figs. 14–18. *Hyphodermella rosae.* **14.** Terminal mitospores. **15, 16.** Intercalary mitospores. **17.** Branched mycelium with terminal mitospore. **18.** Bisterigmate basidia.

spores hyaline, (sub-)globose to ellipsoidal rarely pyriform, apical or intercalary, (4)5.5–8(10) × (3)4–7(7.5) µm (n = 100) and (3.5)4–8(8.5) × (3)4–7(7.5) µm (n = 100) for plum and peach isolates, respectively (Figs. 4, 5, 10, 11 and 12). In case of intercalary mitospores, probably the attached hyphae dissolving gradually to liberate the spores (Fig. 5). Along with mitospores, *H. rosae* also producing bi- rarely quadristerigmate basidia with size range (29)33–70(72) × 3–5 µm (n = 50) (Figs. 6, 7, 13 and 18); basidiospores not observed in culture. Mitospores acting as the secondary inoculum, confirmed by using *in vivo* pathogenicity trials.

Materials examined.– *Hyphodermella rosae* (Bresadola) Nakasone: IRAN, Sari, on fruits of *Prunus persica* (L.) Stokes, 12 August 2010, *leg.* M. Sayari, *det.* V. Babaeizad (ICMP 20105); Sari, on fruits of *Prunus domestica* L., 12 August 2010, *leg.* M. Sayari, *det.* M. Sayari (ICMP 20104).

Discussion

Phanerochaetaceous fungi are mostly lignicolous using cellulose and lignin as carbon source. In case of *Hyphodermella rosae* we notice a shift in nutritional mode, from saprotrophic to pathogenic (Sayari et al. 2012, Babaeizad et al. 2012), although we are unsure regarding the driving force that led to this change in habit.

Initially we isolated *H. rosae* from infected fruits onto Potato Dextrose Agar (PDA) medium. Even after 21 days of incubation the isolates of *H. rosae* as well as *H. corrugata* (ICMP 16963) failed to sporulate. Hence, we tried growing the isolates on three different media: Malt Extract Agar (MEA), Czapek Dox Agar (CZ) and modified Vogels medium N (1/3X concentration) (VM). Sporulation was only observed on VM after three weeks of incubation. This observation supports Gold & Cheng (1979) where they inferred in case of lignicolous fungi, that cellulose is the best carbon source for inducing sporulation *in vitro* whereas glucose suppresses sporulation.

In literature we found no reports of a mitosporic stage in the life cycle of *H. rosae*. Although we found evidence of chlamydospores in *H. corrugata* by Ibáñez (1999); the chlamydospores in this taxon were spherical and measured 5.3–8.6 × 5–8.2 µm. The shape and the size of the mitospores in *H. rosae* isolates were close to those observed and illustrated by Ibáñez (1999). Hence, we think the spherical spore-like structures in our *H. rosae* isolates are chlamydospores.

Our phylogeny of Phanerochaetaceae was mostly congruent with the trees published by Hibbett (2004) and Larsson (2007), where taxon sampling overlapped. In the phylogeny of Homobasidiomycetes by Binder et al. (2005) *Phanerochaete sanguinea* nested within the 'residual' polypore clade. Whereas, in our analysis *P. sanguinea* nests within *Phanerochaete s. str.*, making it a monophyletic lineage within Phanerochaetaceae. A similar trend was noticed in the tree published by De Koker et al. (2003) and Wu et al. (2010) where *P. sanguinea* was a part of the *Phanerochaete* core group. Moreover, Floudas & Hibbett (2015) identified three cryptic species: *P. citrinosanguinea*, *P. pseudosanguinea* and *P. sanguineocarnosa* under the name of *P. sanguinea*. These species as well as *P. sanguinea s. str.* are now placed in the *Phanerochaete* core group.

Mitosporic and phytopathogenic isolates of *H. rosae* from peach and plum nest within a group including their sexual counterpart. Hence, we confirm that the lifecycle of *H. rosae* comprises at least two reproductive states, i.e. sexual and asexual. Our molecular analysis also reconfirms *H. corrugata* as a species distinct from *H. rosa*, as inferred by Telleria et al. (2010).

Acknowledgements

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