Persoonia 38, 2017: 136–155
www.ingentaconnect.com/content/nhn/pimj

RESEARCH ARTICLE

ISSN (Online) 1878-9080
https://doi.org/10.3767/003158517X694768

Juglanconis gen. nov. on *Juglandaceae*, and the new family *Juglanconidaceae* (*Diaporthales*)

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Key words

Ascomycota
Diaporthales
molecular phylogeny
new species
pathogen
systematics

Abstract Molecular phylogenetic analyses of ITS-LSU rDNA sequence data demonstrate that *Melanconis* species occurring on *Juglandaceae* are phylogenetically distinct from *Melanconis* s.str., and therefore the new genus *Juglanconis* is described. Morphologically, the genus *Juglanconis* differs from *Melanconis* by light to dark brown conidia with irregular verrucae on the inner surface of the conidial wall, while in *Melanconis* s.str. they are smooth. *Juglanconis* forms a separate clade not affiliated with a described family of *Diaporthales*, and the family *Juglanconidaceae* is introduced to accommodate it. Data of macro- and microscopic morphology and phylogenetic multilocus analyses of partial nuSSU-ITS-LSU rDNA, *cal*, *his*, *ms204*, *rpb1*, *rpb2*, *tef1* and *tub2* sequences revealed four distinct species of *Juglanconis*. Comparison of the markers revealed that *tef1* introns are the best performing markers for species delimitation, followed by *cal*, *ms204* and *tub2*. The ITS, which is the primary barcoding locus for fungi, is amongst the poorest performing markers analysed, due to the comparatively low number of informative characters. *Melanconium juglandinum* (= *Melanconis carthusiana*), *M. oblongum* (= *Melanconis juglandis*) and *M. pterocaryae* are formally combined into *Juglanconis*, and *J. appendiculata* is described as a new species. *Melanconium juglandinum* and *Melanconis carthusiana* are neotypified and *M. oblongum* and *Diaporthe juglandis* are lectotypified. A short description and illustrations of the holotype of *Melanconium ershadii* from *Pterocarya fraxinifolia* are given, but based on morphology it is not considered to belong to *Juglanconis*. A key to all treated species of *Juglanconis* is provided.

Article info Received: 22 November 2016; Accepted: 8 January 2017; Published: 19 January 2017.

INTRODUCTION

Melanconis is a well-known genus of *Diaporthales*, being the generic type of the family *Melanconidaceae*. However, its circumscription has substantially changed over the years. In his monograph of *Melanconis*, Wehmeyer (1941) used a wide generic concept. He included the genera *Macrodiaporthe*, *Melanconiella*, *Pseudovalsella* and even some species of *Prosthecium* and *Pseudovalsa*, making the genus very heterogeneous. This concept was largely accepted by Müller & Von Arx (1962). Subsequent researchers (e.g. Barr 1978) did not follow this wide concept, restricting the genus *Melanconis* mostly to Wehmeyer's (1941) subg. *Eumelanconis*. In this restricted sense, the genus *Melanconis* was defined by a distinct ectostromatic disc, more or less well-developed entostroma, two-celled hyaline to brown ascospores with or without appendages, in combination with melanconium- or discosporium-like asexual morphs (Barr 1978).

In the phylogenetic analyses of Castlebury et al. (2002), several species traditionally classified within the genus *Melanconis* were shown to be phylogenetically scattered throughout the *Diaporthales*, demonstrating the need of a critical taxonomic revision of the genus. It became evident that the genus *Melanconis*, based on the type species *M. stilbostoma*, has to be restricted to only few species (Castlebury et al. 2002, Rossman et al. 2007). All five *Melanconis* species currently accepted in the genus occur on *Alnus* and *Betula* (*Betulaceae*; Fan et al. 2016).

Following the results of phylogenetic analyses, several genera were recently segregated from *Melanconis*. Based on detailed molecular phylogenetic and morphological investigations, Voglmayr et al. (2012) re-established the genus *Melanconiella*, widened its circumscription and transferred several species of *Melanconis* to *Melanconiella*. These investigations also revealed an unexpectedly high species biodiversity. As a result, several previously synonymised taxa were recognised as distinct species, and several species were described as new. Another species placed in *Melanconis* by Wehmeyer (1941), *M. appendiculata*, has recently been shown to belong to the *Diaporthaceae* (Voglmayr & Jaklitsch 2014), and the genus *Phaeodiaporthe* described by Petrak (1919) was re-established. In the phylogenetic analyses of Castlebury et al. (2002), *Melanconis desmazieri* was also shown to be unrelated to *Melanconis* but formed an isolated lineage together with *Hercospora tiliae*. When describing *Melanconis desmazieri*, Petrak (1938) made the connection with its asexual morph, *Melanconium desmazieri*, for which Grove (1937) established the monotypic genus *Lamproconium*. Following the recent changes of the ICN, *Lamproconium desmazieri* is therefore the name to be used for *M. desmazieri*. Acknowledging their isolated phylogenetic position, Norphanphoun et al. (2016) placed *Lamproconium* and *Hercospora* in a new family *Lamproconiaceae*.

These results demonstrate the need of detailed investigations on the remaining *Melanconis* species for which no sequence data are yet available. In this respect, species on *Juglandaceae* are of particular interest. This group contains economically important pathogens of *Juglans* spp., causing black pustular dieback disease of walnut (Graves 1923, Belisario 1999). Two species are commonly known on *Juglans* spp., *Melanconis*

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Table 1 Strains and NCBI GenBank accessions used in the phylogenetic analyses of the combined multigene matrix of *Juglanconis*. All sequences were generated during the present study.

Taxon	Strain	Culture collection	Herbarium	Origin	Host	GenBank accession no.							
						ITS-LSU	*cal*	*his*	*ms204*	*rpb1*	*rpb2*	*tef1*	*tub2*
Juglanconis appendiculata	D140		WU 35956	Greece	*Juglans regia*	KY427138	–	–	KY427157	–	KY427188	KY427207	KY427226
	D96		WU 35954	Austria	*Juglans nigra*	KY427139	–	–	–	–	KY427189	KY427208	–
	D96A		WU 35954	Austria	*Juglans nigra*	KY427140	–	–	KY427158	–	KY427190	KY427209	–
	MC		WU 32010	Greece	*Juglans regia*	KY427141	KY427242	–	KY427159	KY427174	KY427191	KY427210	KY427227
	MC2		WU 35957	Spain	*Juglans regia*	KY427142	KY427243	–	KY427160	KY427175	KY427192	KY427211	KY427228
	MC4		WU 35958	Spain	*Juglans regia*	KY427143	KY427244	–	KY427161	KY427176	KY427193	KY427212	KY427229
	ME17, W.J.1665, A.R.3581	CBS 123194	WU 35951, BPI 840932	Austria	*Juglans regia*	KY427144	KY427245	–	KY427162	KY427177	KY427194	KY427213	KY427230
Juglanconis juglandina	D142		WU 35960	Austria	*Juglans regia*	KY427145	–	–	–	–	KY427195	KY427214	–
	MC1		WU 35967	Austria	*Juglans regia*	KY427146	KY427246	KY427128	KY427163	KY427178	KY427196	KY427215	KY427231
	MC3		WU 35968	Spain	*Juglans regia*	KY427147	KY427247	KY427129	KY427164	KY427179	KY427197	KY427216	KY427232
	ME16, W.J.1450, A.R.3420	CBS 121083	BPI 843622	Austria	*Juglans regia*	KY427148	KY427248	KY427130	KY427165	KY427180	KY427198	KY427217	KY427233
	ME22, W.J.1500, A.R.3860	CBS 133343	WU 35959	Austria	*Juglans regia*	KY427149	KY427249	KY427131	KY427166	KY427181	KY427199	KY427218	KY427234
	ME23		WU 35965	Austria	*Juglans nigra*	KY427150	KY427250	KY427132	KY427167	KY427182	KY427200	KY427219	KY427235
Juglanconis oblonga	ME14, A.R.4413	CBS 133344	–	USA	*Juglans cinerea*	KY427151	KY427251	KY427133	KY427168	KY427183	KY427201	KY427220	KY427236
	ME15, A.R.4529	CBS 133330	–	USA	*Juglans cinerea*	KY427152	KY427252	KY427134	KY427169	KY427184	KY427202	KY427221	KY427237
	ME18, M4-1	MAFF 410216	TFM FPH 2623	Japan	*Juglans ailanthifolia*	KY427153	KY427253	KY427135	KY427170	KY427185	KY427203	KY427222	KY427238
	ME19, M4-10	MAFF 410217	TFM FPH 3599, TFM FPH 3601	Japan	*Juglans ailanthifolia*	KY427154	KY427254	KY427136	KY427171	KY427186	KY427204	KY427223	KY427239
Juglanconis pterocaryae	ME20, LFP-M4-8	MAFF 410079	TFM FPH 3373	Japan	*Pterocarya rhoifolia*	KY427155	KY427255	KY427137	KY427172	KY427187	KY427205	KY427224	KY427240
Melanconis stilbostoma	D143		WU 35970	Poland	*Betula pendula*	KY427156	–	–	KY427173	–	KY427206	KY427225	KY427241

carthusiana, distributed from Europe to Central Asia, and *M. juglandis*, occurring in North America and Eastern Asia, with their asexual morphs described as *Melanconium juglandinum* and *M. oblongum*, respectively (Wehmeyer 1941).

Due to their economic importance, several studies dealing with *Melanconis* on *Juglandaceae* are available. Graves (1923) provided a detailed account on pathogenicity and taxonomy of *Melanconium oblongum*. He proved the connection of the sexual and asexual morphs by pure culture studies, provided detailed descriptions and combined the sexual morph, *Diaporthe juglandis*, in *Melanconis*, in analogy to the European *Melanconis carthusiana*. He also considered the North American *Melanconium oblongum* to be morphologically distinct from the European *Melanconium juglandinum*. In addition, he confirmed its pathogenicity on *Juglans cinerea* as a serious disease by inoculation experiments.

In his monograph on *Melanconis*, Wehmeyer (1941) recognised *M. carthusiana* and *M. juglandis* within his subg. *Eumelanconis* sect. *Chrysostromae*. He treated them as distinct species with some misgivings, as he considered the differences to be minor and probably insufficient for separation at the species level. Within *M. juglandis*, he described var. *caryae* from *Carya glabra* (Wehmeyer 1940), which differed in its host and the absence of a melanconium-like asexual morph, and var. *tiliae* from *Tilia americana* (Wehmeyer 1941), which he considered to be synonymous with the European *Melanconis desmazieri*, although he recognised the asexual morphs of American and European collections to be different.

Based on detailed morphological and pure culture studies, Kobayashi (1970) recorded, described and illustrated *M. juglandis* from Japan, stating that the Japanese collections agreed well with North American material. In addition, he reported the sexual morph of *Melanconium pterocaryae* and described it as *Melanconis pterocaryae*.

The lack of molecular phylogenetic investigations and of a taxonomic revision of *Melanconis* on *Juglandaceae* prompted us to initiate detailed molecular phylogenetic and morphological investigations, the results of which are presented here.

MATERIALS AND METHODS

Sample sources

The altogether 18 isolates of *Melanconis* from *Juglandaceae* included in this study either originated from ascospores or conidia of fresh specimens or from culture collections. Details of the 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 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 (2016). Freshly collected specimens have been deposited in the Fungarium of the Department of Botany and Biodiversity Research, University of Vienna (WU).

Morphology

Microscopic observations were made in tap water except where noted. Methods of microscopy included stereomicroscopy using a Nikon SMZ 1500 equipped with a Nikon DS-U2 digital camera, and Nomarski differential interference contrast (DIC) using a Zeiss Axio Imager.A1 compound microscope equipped with a Zeiss Axiocam 506 colour digital camera. Images and data were gathered using the NIS-Elements D v. 3.22.15 or Zeiss

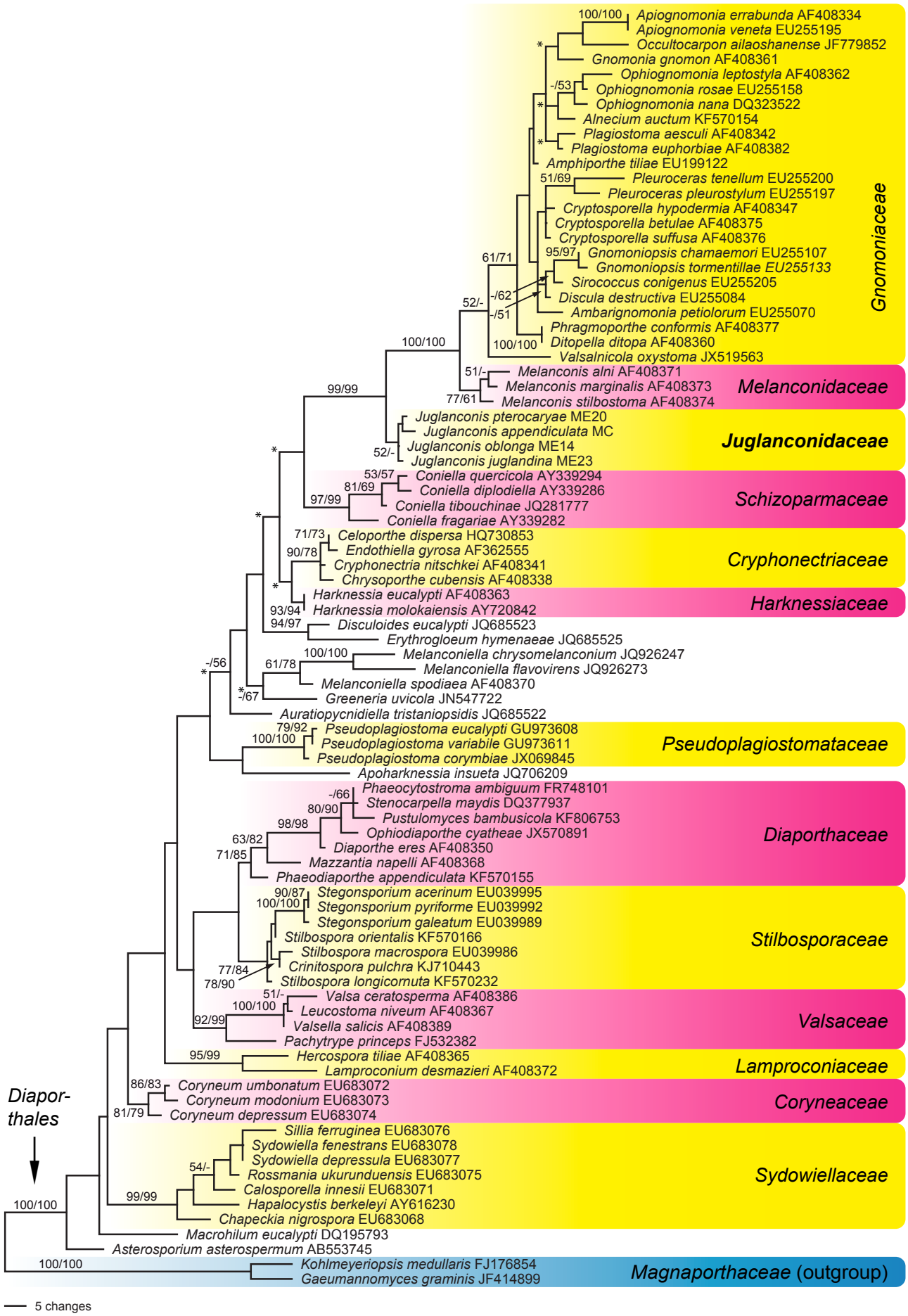

Fig. 1 Phylogram of one of 240 MP trees of 894 steps (CI = 0.41, RI = 0.81, RC = 0.33) revealed by PAUP from an analysis of the LSU matrix of selected *Diaporthales*, showing the phylogenetic position of *Juglanconis*. MP and ML bootstrap support above 50 % are given at the first and second position, respectively, above or below the branches. GenBank accession numbers are given following the taxon names; nodes marked by an asterisk (*) collapsed in the strict consensus of all MP trees.

ZEN Blue Edition softwares. For certain images of ascomata 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 range representing the mean plus and minus the standard deviation of a number of measurements given in parentheses. Due to poor or absent sporulation in pure culture, conidial and conidiophore morphology was only studied from natural substrates.

Culture preparation, DNA extraction, PCR and sequencing

Single ascospore or conidium isolates were prepared and grown on 2 % malt extract agar (MEA), or on 2 % corn meal agar plus 2 % w/v dextrose (CMD).

Growth of liquid culture and 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).

The following loci were amplified and sequenced: the complete internal transcribed spacer region (ITS1-5.8S-ITS2) and a c. 900 bp fragment of the large subunit nuclear ribosomal DNA (nuLSU rDNA), amplified and sequenced as a single fragment with primers V9G (De Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990); a 450–454 bp fragment of the calmodulin (*cal*) gene with primers CAL-228F and CAL-737R (Carbone & Kohn 1999); a 441–445 bp fragment of the histone H3 (*his*) gene with primers CYLH3F (Crous et al. 2004) and H3-1b (Glass & Donaldson 1995); a c. 1 kb fragment of the guanine nucleotide-binding protein subunit beta (*ms204*) gene with primers MS-E1F1 and MS-E5R1 (Walker et al. 2012); a 711 bp fragment of the RNA polymerase II subunit 1 (*rpb1*) gene with primers RPB1-Af and RPB1-Cr (Stiller & Hall 1997); a c. 1.2 kb fragment of the RNA polymerase II subunit 2 (*rpb2*) gene with primers fRPB2-5f and fRPB2-7cr (Liu et al. 1999) or dRPB2-5f and dRPB2-7cr (Voglmayr et al. 2016); a c. 1.3 kb fragment of the translation elongation factor 1-alpha (*tef1*) gene containing introns 4 and 5 and part of the exon with primers EF1-728F (Carbone & Kohn 1999) and TEF1LLErev (Jaklitsch et al. 2005); and a 441–445 bp fragment of the β-tubulin (*tub2*) gene with primers T1 (O'Donnell & Cigelnik 1997) and the newly designed BtHV2r (5' CATCATRCGRTCNGGGAACTC 3'). 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) and the PCR primers; in addition, primers ITS4 (White et al. 1990) and LR3 (Vilgalys & Hester 1990) were used as internal sequencing primers for the ITS-LSU rDNA region. Sequencing was performed on an automated DNA sequencer (ABI 3730xl Genetic Analyzer, Applied Biosystems).

Data analysis

To reveal the phylogenetic position of *Melanconis* species occurring on *Juglandaceae* within the *Diaporthales*, a phylogenetic analysis was performed with nuLSU rDNA sequences. Sequences of representative species were selected from Castlebury et al. (2002) and supplemented with sequences from GenBank. *Gaeumannomyces graminis* and *Kohlmeyeriopsis medullaris* (*Magnaporthaceae*) were included as outgroups. GenBank accession numbers of the sequences selected are given in the phylogenetic tree (Fig. 1). In addition, an ITS-LSU matrix was produced with a subset of taxa according to the results of the LSU analyses, including selected members of *Cryphonectriaceae*, *Gnomoniaceae*, *Harknessiaceae*, *Melanconidaceae* and *Schizoparmaceae*; for GenBank accession numbers see Table 1. For detailed investigations of species relationships and delimitation within *Melanconis* species from *Juglandaceae*, a combined matrix of eight loci (partial SSU-ITS-LSU rDNA, *cal*, *his*, *ms204*, *rpb1*, *rpb2*, *tef1* and *tub2*) was produced for phylogenetic analyses, with *Melanconis stilbostoma* as the outgroup. The GenBank accession numbers of sequences used in these analyses are given in Table 2.

Sequence alignments for phylogenetic analyses were produced with the server version of MAFFT (www.ebi.ac.uk/Tools/mafft or http://mafft.cbrc.jp/alignment/server/), checked and refined using BioEdit v. 7.0.9.0 (Hall 1999). After exclusion of a 355 bp insertion in the LSU of *Ditopella ditopa*, the LSU matrix contained 1 337 characters and the ITS-LSU matrix 1 591 characters. The combined data matrix contained 7 767 characters; viz. 1 600 nucleotides of SSU-ITS-LSU, 455 nucleotides of *cal*, 449 nucleotides of *his*, 1 037 nucleotides of *ms204*, 711 nucleotides of *rpb1*, 1 150 nucleotides of *rpb2*, 1 395 nucleotides of *tef1* and 970 nucleotides of *tub2*. Prior to phylogenetic analyses, the approach of Wiens (1998) was applied to test for significant levels of localised incongruence among the markers used for the combined analysis, using the level of bootstrap support (Sung et al. 2007) as described in Jaklitsch & Voglmayr (2014). For this, the 70 % maximum parsimony (MP) bootstrap consensus trees calculated for each individual partition, using the same parameters 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 eight loci (Wiens 1998).

Maximum parsimony (MP) analyses were performed with PAUP v. 4.0a150 (Swofford 2002). All molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data; the COLLAPSE command was set to MINBRLEN. For the LSU and the ITS-LSU matrices, first a parsimony ratchet approach was used. For this, nexus files were prepared using PRAP v. 2.0b3 (Müller 2004), implementing 1 000 ratchet replicates with 25 % of randomly chosen positions upweighted to 2, which were then run with PAUP. In a second step, the best trees obtained by the parsimony ratchet analyses were loaded in PAUP and subjected to heuristic search using TBR branch swapping (MULTREES option in effect, steepest descent option not in effect). MP analysis of the combined multilocus matrix was done 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). Bootstrap analyses with 1 000 replicates were performed in the same way, but using 5 rounds of random sequence addition and subsequent branch swapping during each bootstrap replicate; in addition, each replicate was limited to 1 million rearrangements in the LSU and ITS-LSU matrices.

Maximum likelihood (ML) analyses were performed with RAxML (Stamatakis 2006) as implemented in raxmlGUI v. 1.3 (Silvestro & Michalak 2012), using the ML + rapid bootstrap setting and the GTRGAMMAI substitution model with 1 000 bootstrap replicates. The matrix was partitioned for the different gene regions included in the combined multilocus analyses.

The sequence markers used for the multilocus analyses were also individually compared for their phylogenetic resolution within *Juglanconis*. Because several markers were not available for the outgroup taxon (*Melanconis stilbostoma*), only the accessions of *Juglanconis* were compared. The data matrices of the individual genes were subjected to MP bootstrap analyses with the same settings as in the analyses of the multilocus matrix and the resulting bootstrap support values of species and internal nodes were compared.

Table 2 Strains and NCBI GenBank accession numbers of the ITS and LSU sequences used in the phylogenetic analyses of the ITS-LSU matrix. Isolates/sequences in **bold** were isolated/sequenced in the present study.

Taxon	Isolate No.[1,2]	Herbarium no.[1]	Country	Host	GenBank accession numbers	
					ITS	LSU
Alnecium auctum	CBS 124263^{ET}	WU 30206	Austria	*Alnus glutinosa*	KF570154	KF570154
Ambarignomonia petiolorum	CBS 121227^{ET}	BPI 844274	USA	*Liquidambar styraciflua*	EU254748	EU255070
Amphiporthe tiliae	CBS 119289	BPI 843515	Austria	*Tilia platyphylla*	EU199178	EU199122
Apiognomonia hystrix	CBS 911.79	CBS-H 11343	Switzerland	*Acer pseudoplatanus*	DQ313549	EU255180
Apiognomonia veneta	CBS 897.79	NA	Switzerland	*Platanus orientalis*	DQ313532	EU255195
Celoporthe dispersa	CBS 118782^{T}	PREM 58896	South Africa	*Syzigium cordatum*	NR_119569	HQ730853
Coniella diplodiella	CBS 111858^{ET}	NA	France	*Vitis vinifera*	AY339323	KX833335
Coniella fragariae	CBS 172.49^{NT}	NA	Belgium	*Fragaria* sp.	AY339317	AY339282
Coniella quercicola	CBS 904.69^{NT}	NA	Netherlands	*Quercus robur*	KX833595	KX833414
Coniella tibouchinae	CBS 131594^{T}	NA	Brazil	*Tibouchina granulosa*	JQ281774	KX833418
Cryphonectria parasitica	ATCC 38755	NA	USA	*Castanea dentata*	AY141856	EU199123
Cryptosporella betulae	CBS 109763	BPI 748448	Austria	*Betula alba*	EU199180	AF408375
Cryptosporella hypodermia	CBS 171.69	NA	Netherlands	*Ulmus campestris*	EU199225	DQ862028
Cryptosporella suffusa	CBS 121077	BPI 871231	Austria	*Alnus incana*	EU199184	EU199124
Discula destructiva	CBS 109771	BPI 1107757	USA	*Cornus nuttallii*	EU199186	AF408359
Ditopella ditopa	CBS 109748	BPI 748439	Austria	*Alnus glutinosa*	DQ323526	AF408360
Gnomonia gnomon	CBS 199.53	NA	Italy	*Corylus avellana*	AY818956	AF408361
Gnomonia virginianae	CBS 121913^{T}	BPI 844264	USA	*Ostrya virginiana*	EU254801	EU255105
Gnomoniopsis chamaemori	CBS 803.79	NA	Finland	*Rubus chamaemorus*	EU254808	EU255107
Gnomoniopsis tormentillae	CBS 904.79	NA	Switzerland	*Potentilla erecta*	EU254856	EU255133
Harknessia eucalypti	CBS 342.97	NA	Australia	*Eucalyptus regnans*	AY720745	AF408363
Harknessia leucospermi	CBS 775.97^{T}	NA	South Africa	*Leucospermum* sp.	AY720727	AY720824
Harknessia molokaiensis	CBS 114877^{ET}	NA	USA	*Eucalyptus robusta*	AY720749	AY720842
Harknessia syzygii	CBS 111124^{ET}	NA	South Africa	*Syzygium cordatum*	AY720738	AY720834
Juglanconis appendiculata	**D96**^{T}	WU 35954	Austria	*Juglans nigra*	**KY427139**	**KY427139**
Juglanconis juglandina	**ME23**^{NT}	WU 35965	Austria	*Juglans nigra*	**KY427150**	**KY427150**
Juglanconis oblonga	**ME14**	NA	USA	*Juglans cinerea*	**KY427151**	**KY427151**
Juglanconis pterocaryae	MAFF 410079^{T} = ME20	TFM FPH 3373	Japan	*Pterocarya rhoifolia*	**KY427155**	**KY427155**
Melanconis alni	CBS 109773	BPI 748444	Austria	*Alnus viridis*	DQ323523	AF408371
Melanconis betulae	CFCC 50471	BJFC-S1319	China	*Betula albosinensis*	KT732952	KT732971
Melanconis itoana	CFCC 50474	BJFC-S1322	China	*Betula albosinensis*	KT732955	KT732974
Melanconis marginalis	CBS 109744	BPI 748446	Canada	*Alnus rubra*	EU199197	AF408373
Melanconis stilbostoma	CBS 121894	NA	Austria	*Betula alba*	JQ926229	JQ926229
Occultocarpon ailaoshanense	CBS 129146^{T}	BPI 879253	China	*Alnus nepalensis*	JF779849	JF779853
Ophiognomonia leptostyla	CBS 844.79	NA	Switzerland	*Juglans regia*	EU254910	EU255149
Ophiognomonia melanostyla	CBS 129144	BPI 879257	Germany	*Tilia cordata*	JF779850	JF779854
Ophiognomonia nana	CBS 883.79	NA	Finland	*Betula nana*	DQ323534	DQ323522
Ophiognomonia rosae	CBS 121267	BPI 877636	USA	*Rosa* sp.	EU254936	EU255158
Phragmoporthe conformis	CBS 109783	BPI 748450	Canada	*Alnus rubra*	DQ323527	AF408377
Plagiostoma aesculi	CBS 109765	BPI 748430	Austria	*Aesculus hippocastanum*	EU199179	AF408342
Plagiostoma apiculatum	CBS 109775^{ET}	BPI 747938	Austria	*Salix* sp.	DQ323529	AF408345
Plagiostoma euphorbiae	CBS 340.78	NA	Netherlands	*Euphorbia palustris*	EU199198	AF408382
Plagiostoma salicellum	CBS 121466^{E}	BPI 843527	Austria	*Salix alba*	EU254996	EU255166
Pleuroceras pleurostylum	CBS 906.79	NA	Switzerland	*Salix helvetica*	EU255061	EU255197
Pleuroceras tenellum	CBS 121082	BPI 871059	USA	*Acer rubrum*	EU199199	EU255202
Sirococcus conigenus	CBS 101225	BPI 871248	Austria	*Picea abies*	EU199201	EU199134
Sirococcus piceicola	CBS 119621	BPI 871166	Switzerland	*Picea abies*	EU199202	EU199135

[1] ATCC: American Type Culture Collection, Manassas, VA, USA; BJFC: Museum of the Beijing Forestry University, Beijing, China; BPI: U.S. National Fungus Collections USDA-ARS MD USA; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CFCC: China Forestry Culture Collection Center, Beijing, China; MAFF: MAFF Genebank, National Institute of Agrobiological Sciences, Ibaraki, Japan; NA: not applicable; PREM: South African National Collection of Fungi, Pretoria, South Africa; WU: Herbarium of the University of Vienna, Austria.

[2] T: ex-type strain; ET: ex-epitype strain; NT: ex-neotype strain.

RESULTS

Molecular phylogeny

Of the 1 337 characters of the LSU matrix, 219 were parsimony informative. MP analyses revealed 240 MP trees of score 894, one of which is shown in Fig. 1; tree topologies of all MP trees were identical except for some nodes within *Gnomoniaceae* and some deeper nodes in the tree (see nodes marked by an asterisk in Fig. 1). In both MP and ML analyses, the *Juglanconis-Gnomoniaceae-Melanconidaceae* clade was highly supported. The subclade containing *Melanconis* (*Melanconidaceae* s.str.) and *Gnomoniaceae* received maximum support, but *Melanconidaceae* and *Gnomoniaceae* received only low or insignificant support. The genus *Juglanconis* was revealed as sister clade to the *Gnomoniaceae-Melanconidaceae* s.str. clade and received low or insignificant support as well.

Of the 1 591 characters included in the ITS-LSU analyses, 291 were parsimony informative. MP analyses revealed two MP trees 1 156 steps long, one of which is shown in Fig. 2. Tree topologies of the two MP trees differed in an interchanged position of *Ophiognomonia rosae* and *O. nana*. The ML tree revealed by RAxML showed the same relationships between the families as the MP trees, but differed in some unsupported nodes within the *Gnomoniaceae* (not shown). The clade containing *Juglanconidaceae*, *Melanconidaceae* and *Gnomoniaceae* received maximum support in both analyses, and *Juglanconidaceae* were revealed as sister group to the highly supported clade containing *Melanconidaceae* and *Gnomoniaceae*. *Juglanconi-*

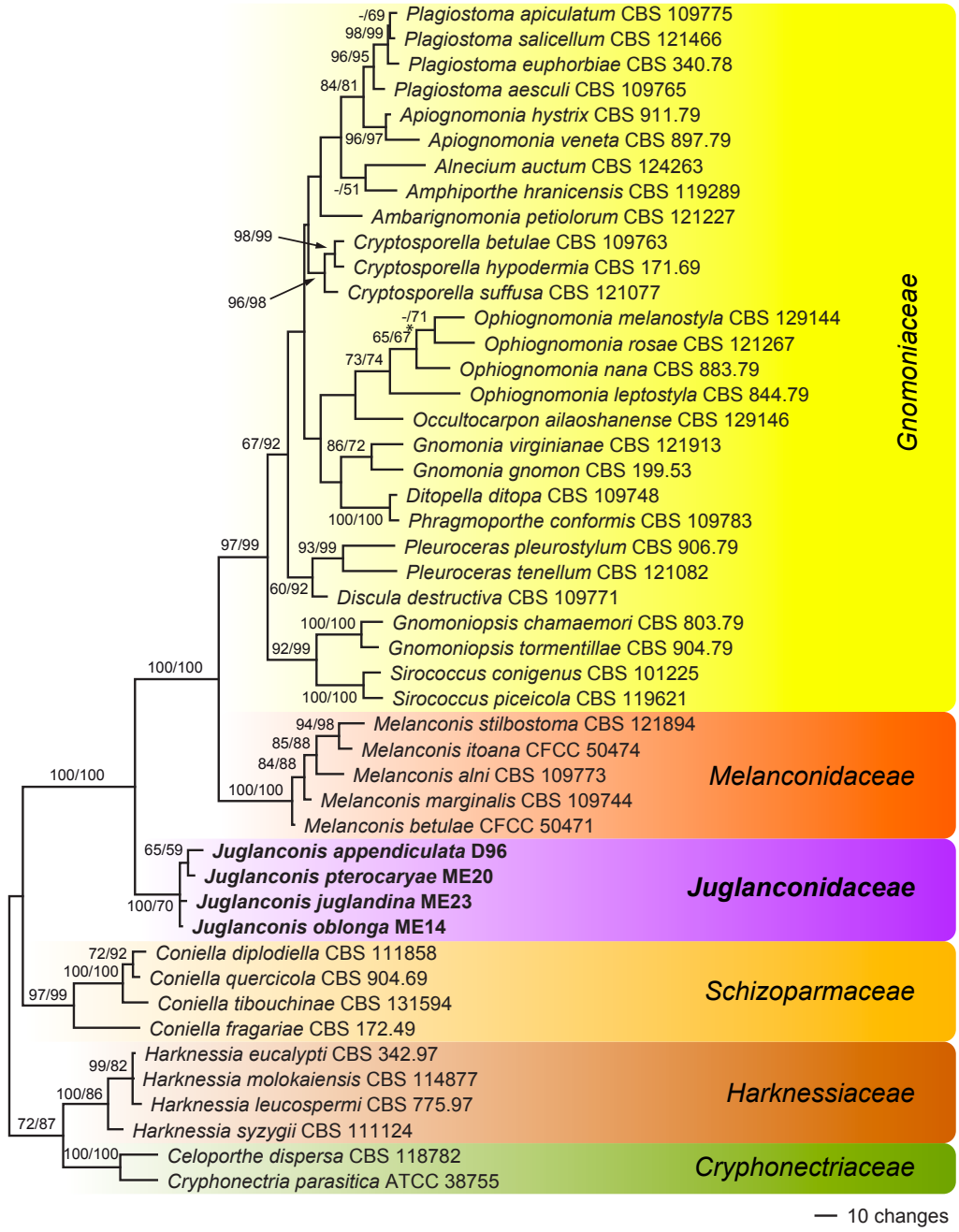

Fig. 2 Phylogram showing one of two MP trees of 1 156 steps (CI = 0.47, RI = 0.78, RC = 0.36) revealed by PAUP from an analysis of the ITS-LSU matrix of selected *Diaporthales*, showing the phylogenetic position of *Juglanconis*. MP and ML bootstrap support above 50 % are given at the first and second position, respectively, above or below the branches. Strain/culture numbers are given following the taxon names. The node marked by an asterisk (*) collapsed in the strict consensus of the two MP trees.

daceae received maximum and medium (70 %) support in MP and ML analyses, respectively.

Of the 7 767 characters included in the combined multilocus analyses, 315 were parsimony informative (16 from ITS-LSU, 32 from *cal*, 10 from *his*, 52 from *ms204*, 26 from *rpb1*, 45 from *rpb2*, 71 from *tef1* and 63 from *tub2*). The *his* gene consistently failed to amplify in *Juglanconis appendiculata* and is therefore missing for this species. The MP analysis revealed five MP trees 907 steps long, one of which is shown in Fig. 3. Tree topologies of all MP trees were identical except for minor differences within *J. appendiculata*. The ML tree revealed by RAxML was identical to the MP tree shown. All four species of *Juglanconis* received maximum support in both analyses, as well as the relationships between the species. *Juglanconis juglandina* and *J. oblonga* are revealed as closely related but distinct species, and conspecificity of Japanese and North American accessions of *J. oblonga* is confirmed.

The number of alignment characters, the number and percentage of parsimony informative characters of the different markers and the percentage of MP bootstrap support for species and internal nodes revealed in the phylogenetic analyses are shown in Table 3. A comparison of the markers focusing on bootstrap support shows that the *tef1* fragment containing introns 4 and 5 is the best resolving marker with 69 (5.1 %) parsimony informative characters (pic) and all nodes supported by 100 %, followed by *cal* with 32 (7 %) pic and support at all nodes above 98 %, except for *J. oblonga* with 94 %. Then followed *ms204* and *tub2* with 51 (5 %) and 62 (6.4 %) pic, respectively; all nodes were highly supported above 99 %, except for *J. oblonga* where support decreased to 87 % (*ms204*) and 86 % (*tub2*). In the *rpb2*, with 45 (3.9 %) pic, *J. oblonga* is supported by 95 %, whereas support of *J. juglandina* drops to 65 %. In the residual markers (*rpb1*, ITS and *his*) support of at least one node is absent.

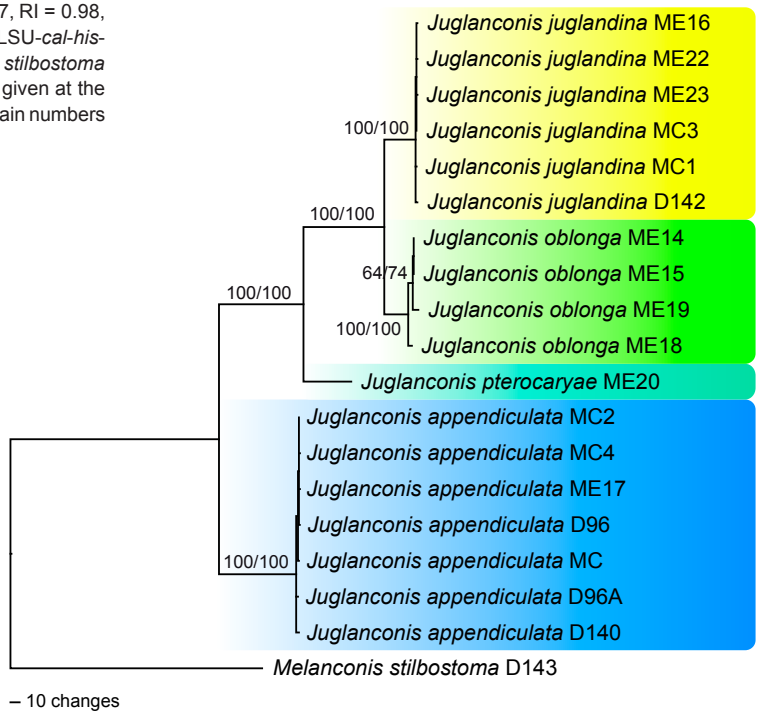

Fig. 3 Phylogram showing one of five MP trees of 907 steps (CI = 0.97, RI = 0.98, RC = 0.95) revealed by PAUP from an analysis of the combined ITS-LSU-*cal-his-ms204-rpb1-rpb2-tef1-tub2* matrix of *Juglanconis*, with *Melanconis stilbostoma* selected as outgroup. MP and ML bootstrap support above 50 % are given at the first and second position, respectively, above or below the branches. Strain numbers are given following the taxon names.

Table 3 Comparison of the phylogenetic markers used for the multigene analyses of *Juglanconis*. The markers were compared within *Juglanconis*. For the MP bootstrap support (% bts) of the respective clades, MP bootstrap analyses of the matrices of the respective markers were performed, applying the same rooting as in the multigene analyses (Fig. 3).

	ITS	LSU	*cal*	*his*	*ms204*	*rpb1*	*rpb2*	*tef1*	*tub2*
No. of alignment characters	518	910	455	449	1030	711	1144	1361	970
No. of variable characters	15	5	37	31	65	27	55	77	72
No. of parsimony-informative characters (pic)	13	2	32	10	51	26	45	69	62
% parsimony informative characters	2.5	0.2	7.0	2.2	5.0	3.7	3.9	5.1	6.4
% bts *oblonga*	–	–	94	71	87	95	95	100	86
% bts *juglandina*	69	63	99	100	99	–	65	100	99
% bts *oblonga+juglandina*	84	61	98	–	100	100	100	100	100
% bts *pterocaryae+oblonga+juglandina*	100	–	100	–	100	100	100	100	100

Taxonomy

Juglanconidaceae Voglmayr & Jaklitsch, *fam. nov.* — MycoBank MB819587

Etymology. Referring to the name of the type genus.

Type genus. *Juglanconis* Voglmayr & Jaklitsch.

Family of *Diaporthales*. *Pseudostromata* consisting of an inconspicuous ectostromatic disc causing a more or less pustulate bark surface. *Central column* beneath the disc more or less conical. *Stromatic zones* lacking. *Perithecia* surrounding the ectostromatic disc, with long lateral ostioles that emerge at the margin or within the ectostromatic disc. *Paraphyses* deliquescent at maturity. *Asci* octosporous, with an apical ring, becoming detached from their base. *Ascospores* hyaline, bicellular, with or without gelatinous appendages. *Asexual morph* melanconium-like. *Conidiomata* acervular, with ectostromatic disc and central column. *Conidiophores* aseptate or few-celled, smooth, hyaline to brownish. *Conidiogenous cells* annellidic. *Conidia* brown, with gelatinous sheath. *Conidial wall* smooth on the outer surface, with inconspicuous to distinct irregular verrucae on the inner surface.

Note — We describe this family, because the genus *Juglanconis* is consistently placed outside described families of *Diaporthales* in phylogenetic analyses.

Juglanconis Voglmayr & Jaklitsch, *gen. nov.* — MycoBank MB819582

Etymology. Referring to its occurrence on *Juglandaceae*.

Type species. *Juglanconis juglandina* (Kunze) Voglmayr & Jaklitsch.

Genus of *Diaporthales*. *Pseudostromata* consisting of an inconspicuous, erumpent, light to dark coloured ectostromatic disc causing a more or less pustulate bark surface. *Ectostromatic disc* convex, flat or concave, greyish to brownish, surrounded by bark flaps. *Central column* beneath the disc more or less conical. *Stromatic zones* lacking. *Perithecia* inconspicuous at the bark level, surrounding the ectostromatic disc, oblique or horizontal, usually more or less irregularly scattered, sometimes arranged in a circle around the central column, with long lateral ostioles that converge at the margin of the column and emerge at the margin or within the ectostromatic disc. *Ostioles* flat in the disc or slightly projecting, rarely distinctly projecting and cylindrical, often obscuring the disc, sometimes covered by a distinct white crust. *Paraphyses* deliquescent at maturity. *Asci* oblong or fusoid, octosporous, with a more or less distinct apical ring becoming inconspicuous in old herbarium specimens; asci becoming detached from their base. *Ascospores* hyaline, ellipsoid, symmetrical to asymmetrical, straight to curved, bicellular, with a central or slightly eccentric septum, constricted at the septum, smooth, with or without blunt or pointed appendages.

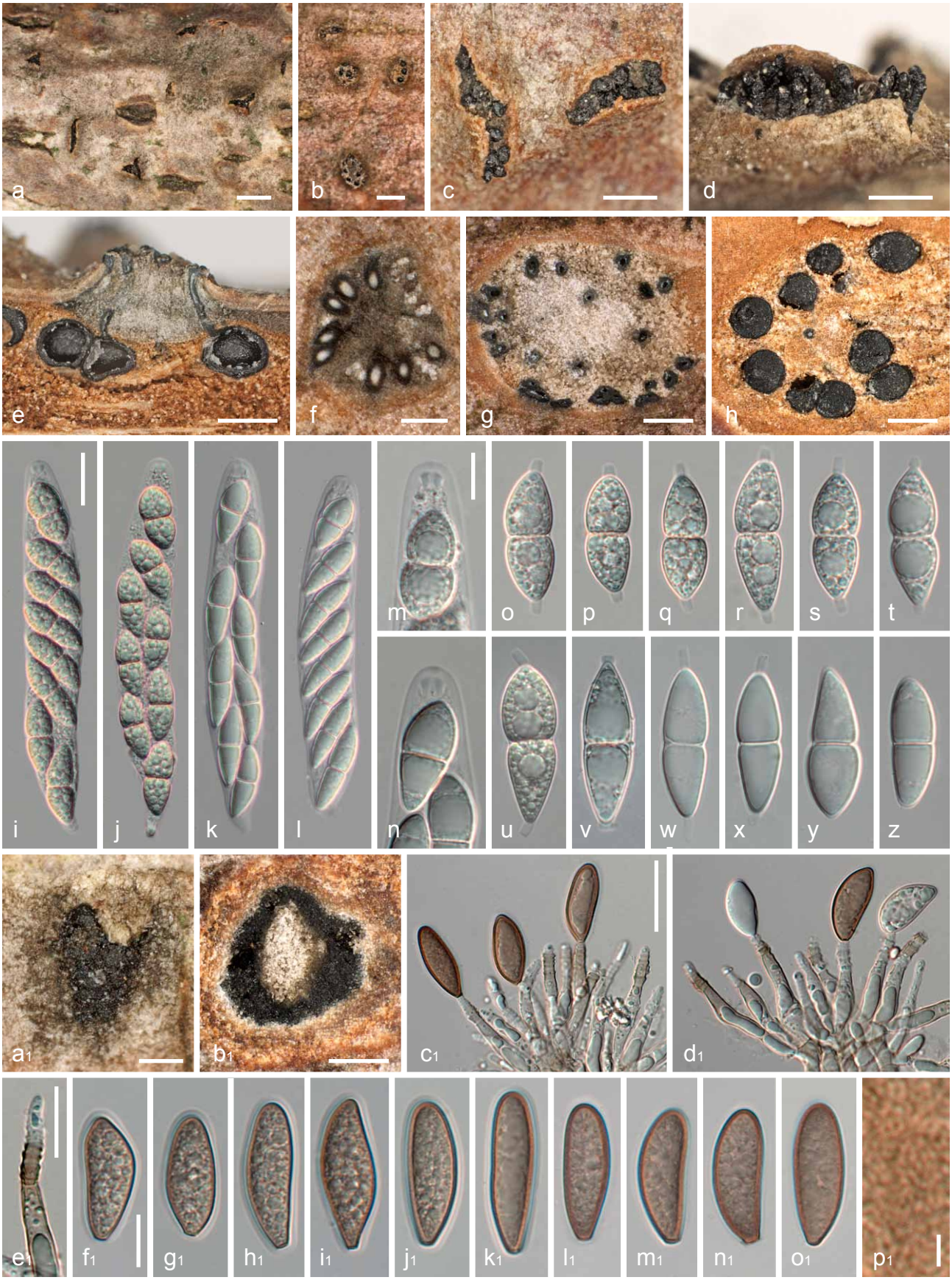

Fig. 4 *Juglanconis appendiculata*. a–c. Ectostromatic discs and ostioles in surface view; d. ectostromatic disc in side view showing protruding ostioles; e. pseudostroma in vertical section; f, g. transverse sections below ectostromatic disc; h. pseudostroma in transverse section, showing perithecia and indistinct whitish to light brown entostroma; i–l. mature asci with apical ascal ring (i, j vital, k, l dead); m, n. ascus apex with apical ring (m vital, n dead); o–u. vital ascospores with cylindrical gelatinous appendages; v–z. dead ascospores (v–x showing gelatinous appendages); a1. conidioma in surface view; b1. transverse section of conidioma, showing central column; c1–e1. conidiophores (annellides) with conidia (c1, d1); f1–o1. conidia (showing gelatinous sheath in f1–k1; f1–j1 vital, k1–o1 dead); p1. detail of verruculose inner conidial wall. All in water, except k, l, n, y, z, c1–e1, l1–p1 in 3 % KOH (a, c–e, h, u, a1, b1: WU 35955; b, g, k, l, n, w–z, c1–e1, j1–o1: WU 32010; f, i, j, o–s, f1–i1: WU 35954 (holotype); m, t: WU 35956; v: WU 35958, p1: WU 29730). — Scale bars: a = 1 mm; b, c, e, h = 0.5 mm; d, f, g, a1, b1 = 300 µm; i–l, c1–e1 = 20 µm; m–z, f1–o1 = 10 µm; p1 = 2 µm.

Asexual morph: melanconium-like. *Conidiomata* acervular, covered by the bark, erumpent at maturity; possessing the same type of ectostromatic disc and central column as the sexual morph, usually preceding it. *Conidiophores* branched only at the base, mostly aseptate, sometimes few-celled, smooth, hyaline when young, brownish with age. *Conidiogenous cells* distinctly annellidic, successively producing several generations of conidia. *Conidia* brown, variable in shape, subglobose, ellipsoid, elongate pyriform, pip-shaped to fusoid, with distinct gelatinous sheath when fresh. *Conidial wall* smooth on the outer surface, with inconspicuous to distinct, sometimes confluent irregular verrucae on the inner surface.

Juglanconis appendiculata Voglmayr & Jaklitsch, *sp. nov.* — MycoBank MB819583; Fig. 4

Etymology. Referring to its gelatinous ascospore appendages.

Holotype. Austria, Niederösterreich, Mühlleiten, Herrnau, on corticated branches of *Juglans nigra*, 28 Feb. 2015, *H. Voglmayr* (WU 35954; ex-epitype culture D96 (ex sexual morph) and culture D96A (ex asexual morph)).

Pseudostromata 1.5–3 mm diam, typically distinct, circular, projecting up to 0.3 mm beyond the host surface, without perithecial bumps. *Ectostromatic disc* distinct, circular or oblong, dark grey, brown or black, 0.3–2 mm diam, sometimes concealed by densely arranged ostioles, pulvinate. *Central column* yellowish, greenish to brownish grey. *Entostroma* indistinct. *Ostioles* 1–15 per disc, (80–)92–127(–154) µm diam (n = 34), plane or slightly papillate, black, sometimes covered by distinct white crust. *Perithecia* (380–)420–520(–560) µm diam (n = 20), arranged in various configurations. *Asci* (121–)131–147(–168) × (19.5–)20.5–24.2(–27.8) µm (n = 58), clavate to fusoid, containing 8 uni- to biseriate ascospores, with distinct funnel-shaped apical ring when fresh, 4.5–5.1 µm diam, 1.8–3.2 µm high, becoming faint in older herbarium specimens. *Ascospores* (23–)26–32(–38.5) × (7.7–)9.5–11.0(–13) µm, l/w = (2–)2.5–3.2(–4) (n = 141), hyaline, ellipsoid or broadly fusoid, symmetric to slightly asymmetric, distinctly constricted at the septum, with distinct appendages (1.6–)2.1–3.4(–4.2) µm long, (2.0–)2.2–2.6(–3.1) µm wide (n = 44); cells monomorphic to dimorphic with larger upper cell, with rounded to subacute ends, multiguttulate, often containing one large and numerous small guttules per cell; wall c. 0.5–0.7 µm thick, not swelling.

Asexual morph. *Conidiomata* acervular, 0.4–1 mm diam, dark brown to blackish, inconspicuous, scattered, with central or eccentric stromatic column; at maturity covered by brown to blackish discharged conidial masses. *Conidiophores* (25–)30–43(–52) × (3.0–)4.2–6.0(–7.5) µm (n = 48), narrowly cylindrical to lageniform, simple or branched at the base, smooth, subhyaline to pale brown. *Conidiogenous cells* annellidic with distinct annellations, integrated. *Conidia* (17.3–)21.3–26.2(–34.0) × (7–)8.6–10.2(–13) µm, l/w = (1.6–)2.2–2.9(–3.7) (n = 357), unicellular, hyaline when immature, brown when mature, variable in shape, pip-shaped, narrowly ellipsoid, elongate to suballantoid, truncate with distinct abscission scar at the base, densely multiguttulate, thin-walled; wall c. 0.5–0.7 µm, with indistinct ornamentation on the inside of the wall consisting of small irregular verrucae 0.2–0.5 µm diam, with 0.6–0.8 µm wide gelatinous sheath.

Habitat & Host range — Dead corticated twigs and branches of *Juglans* spp. attached to the tree.

Distribution — Europe; apparently common in Southern Europe.

Additional specimens examined (all on corticated branches of *Juglans regia* except where noted). Austria, Kärnten, St. Margareten im Rosental, Trieblach, 7 July 2013, *W. Jaklitsch* (WU 35950); St. Margareten im Rosental, Wograda, 27 Oct. 2000, *W. Jaklitsch W.J. 1665* (WU 35951, BPI 840932, culture CBS 123194); ibid., 7 July 2013, *W. Jaklitsch* (WU 35952); St. Margareten im Rosental, 20 June 2015, *H. Voglmayr* (WU 35953); Niederösterreich, Orth an der Donau, near Uferhaus, on corticated branches of *Juglans nigra*, soc. *J. juglandina*, 5 Mar. 2003, *H. Voglmayr* (WU 29730); ibid., soc. *J. juglandina*, 15 May 2016, *H. Voglmayr* (WU 35955). – France, Ariège, Rimont, Las Muros, 20 Sept. 2008, *J. Fournier* (J.F. 08180). – Greece, Crete, Pananiana, 4 June 2015, *H. Voglmayr & W. Jaklitsch* (WU 35956, culture D140); Crete, Vrysses, 26 Nov. 2011, *W. Jaklitsch* (WU 32010, culture MC). – Spain, Asturias, Planadera, near the crossing to Valbona and Borreras, 5 June 2013, *W. Jaklitsch & H. Voglmayr* (WU 35958, culture MC4); Asturias, Pineda, 27 Apr. 2013, *Enrique Rubio* (WU 35957, culture MC2).

Notes — *Juglanconis appendiculata* is easily distinguished from the other *Juglanconis* species by its conspicuous cylindrical ascospore appendages; the other species with appendaged ascospores, *J. pterocaryae*, has tapering appendages with rounded to subacute tips and also differs by the hosts, *Pterocarya* spp. Additional differences from the sympatric *J. juglandina* include distinct pseudostromata and light brown, distinctly narrower conidia (typically 8.6–10.2 µm wide, l/w = 2.2–2.9, vs 12.0–14.5 µm, l/w = 1.4–1.8). In contrast to *J. juglandina* its sexual morph is produced abundantly, whereas its asexual morph is inconspicuous. Remarkably, it has remained undetected until now, although it appears to be a common species in Southern Europe where it replaces *J. juglandina*; in eastern and southern Austria it is commonly co-occurring with *J. juglandina* on the same branches. Observational evidence suggests that it is currently expanding its range northwards which could be due to global warming.

Juglanconis juglandina (Kunze) Voglmayr & Jaklitsch, *comb. nov.* — MycoBank MB819584; Fig. 5, 6

Basionym. *Melanconium juglandinum* Kunze, in Schubert & Ficinus, Fl. Dresd., 2. Aufl.: 260. 1823.

Synonyms. *Melanconidium juglandinum* (Kunze) Kuntze, Revis. Gen. Pl. (Leipzig) 3, 2: 493. 1898.

Melanconium magnum (Grev.) Berk. subsp. *juglandinum* (Kunze) Sacc., Syll. Fung. (Abellini) 25: 580. 1931.

Melanconium juglandis Corda, Icon. Fungorum (Prague) 3: 21. 1839.

Melanconium juglandis Corda forma *diffusa* Corda, Icon. Fungorum (Prague) 3: 22. 1839.

Melanconium juglandinum Kunze forma *diffusa* (Corda) Sacc., Syll. Fung. (Abellini) 3: 753. 1884.

Melanconis carthusiana Tul. & C. Tul., Ann. Sci. Nat., Bot., sér. 4 5: 110. 1856.

Typification. Austria, Niederösterreich, Orth an der Donau, near Uferhaus, on corticated branches of *Juglans nigra*, 19 May 2013, *W. Jaklitsch & H. Voglmayr* (WU 35965, neotype of *Melanconium juglandinum* (MBT374383) and *Melanconis carthusiana* (MBT374385) here designated; culture ME23, culture lost).

Pseudostromata 0.8–2 mm diam, typically inconspicuous, sometimes distinct, circular, slightly projecting, without perithecial bumps. *Ectostromatic disc* indistinct, circular or oblong, dark grey, brown or black, 0.5–1.2(–2.3) mm diam, often concealed by densely arranged ostioles, often pulvinate. *Central column* yellowish, greenish to brownish grey. *Entostroma* indistinct. *Ostioles* 1–25 per disc, (94–)129–176(–208) µm diam (n = 30), plane or slightly papillate, black. *Perithecia* (375–)440–565 (–655) µm diam (n = 36), arranged in various configurations. *Asci* (126–)138–161(–184) × (14.2–)17.3–22.3(–25.0) µm (n = 75), clavate to fusoid, containing 8 uni- to irregularly biseriate ascospores, with indistinct apical ring when fresh 3.7–4.4 µm diam, 3.3–4.1 µm high, ring not visible in older herbarium specimens. *Ascospores* (20.5–)24.3–29.0(–36.5) × (6.7–)8.5–11.0(–12.7) µm, l/w = (2.0–)2.3–3.2(–4.7) µm (n = 359), hyaline, inequilaterally ellipsoid or broadly fusoid, asymmetric, usually slightly to distinctly curved, distinctly constricted at the septum, without appendages; cells usually distinctly dimorphic, upper cell mostly larger, with rounded to subacute end, lower cell subacute to narrowly rounded, multiguttulate, containing mostly one large and numerous small guttules per cell; wall c. 0.4–0.6 µm thick, not swelling.

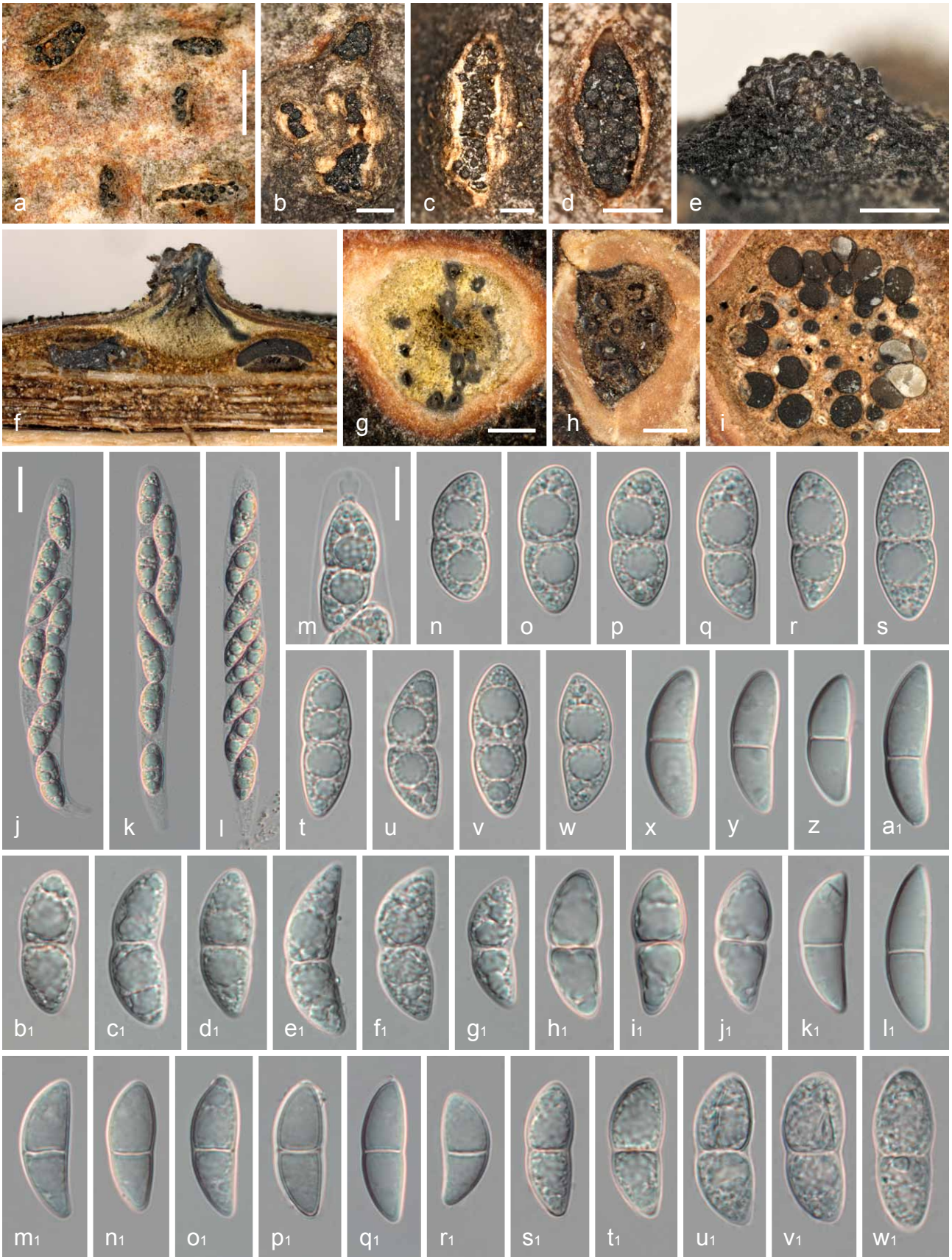

Fig. 5 *Juglanconis juglandina*, sexual morph. a–d. Ectostromatic discs and ostioles in surface view; e. ectostromatic disc in side view showing protruding ostioles; f. pseudostroma in vertical section; g, h. transverse sections below ectostromatic disc; i. pseudostroma in transverse section, showing perithecia and indistinct whitish to light brown entostroma; j–l. mature vital asci with apical ascal ring; m. vital ascus apex with apical ring; n–w. vital ascospores; x–w1. dead ascospores. All in water, except b1–j1, s1–w1 in 3 % KOH (a, f, i, h1–j1: WU 35965 (neotype); b–e, g, x–g1: WU 35961; h: PC 0723585; j–w: WU 35966; k1, l1: WU 35959; m1–t1: WU 35964; u1–w1: BPI 614906). — Scale bars: a = 1 mm; b–i = 0.5 mm; j–l = 20 µm; m–w1 = 10 µm.

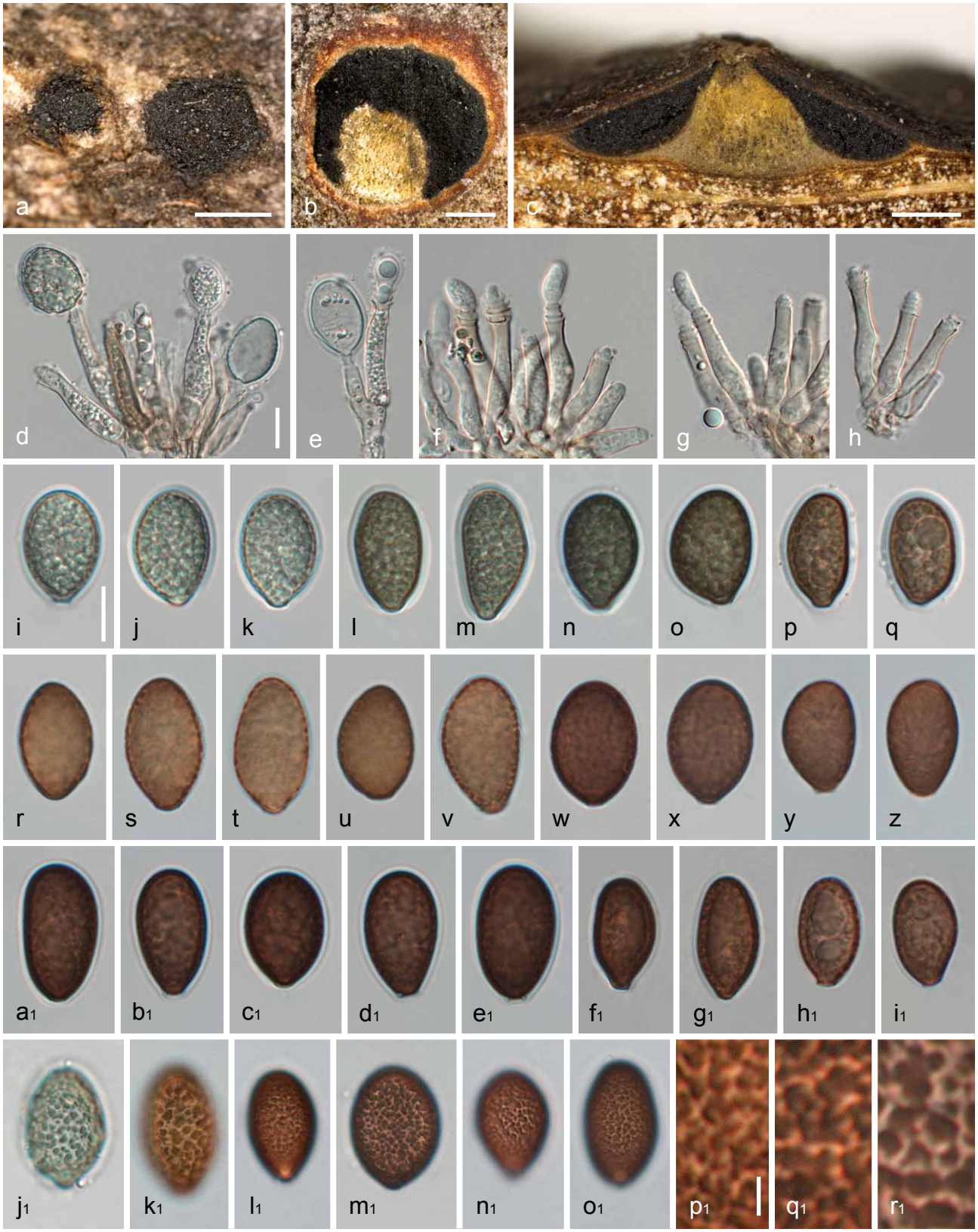

Fig. 6 *Juglanconis juglandina*, asexual morph. a. Conidiomata in surface view; b, c. transverse (b) and vertical (c) sections of conidiomata, showing central column; d–h. conidiophores (annellides) with conidia; i–o1. conidia (showing gelatinous sheath in i–q, a1–g1; i–q vital, r–o1 dead; in j1–o1 showing verruculose inner conidial wall); p1–r1. detail of inner conidial wall, showing confluent verrucae. All in 3 % KOH, except d, e, i–q, j1 in water (a–c, f–h, w, x, m1: WU 35961; d, e, i–o, j1: WU 35966; p, q: WU 35960; r–v, k1: PC 0723585; y, z: BPI 614909; a1–e1: WU 35968; f1–i1, q1: WU 35967; l1, p1: PC 0723587; n1: BPI 614907; o1: BPI 614910; r1: BPI 614908). — Scale bars: a = 1 mm; b, c = 0.5 mm; d–o1 = 10 µm; p1–r1 = 2 µm.

Asexual morph. *Conidiomata* acervular, 1–4 mm diam, blackish, scattered or occasionally confluent, with central or eccentric stromatic column; at maturity covered by black discharged conidial masses; usually conspicuous. *Conidiophores* (17–)26–37(–45) × (4.0–)4.8–6.5(–7.7) µm (n = 36), cylindrical to lageniform, simple, rarely branched at the base, smooth, subhyaline to pale brown. *Conidiogenous cells* annellidic with distinct annellations, integrated. *Conidia* (15–)19–23(–28.5) × (9.5–)12–14.5(–17.2) µm, l/w = (1.2–)1.4–1.8(–2.6) (n = 905), unicellular, hyaline when immature, brown to blackish when mature, broadly ellipsoid to broadly pip-shaped, truncate with distinct scar at the base, densely multiguttulate, thick-walled; wall c. 0.7–1.1 µm thick, with distinct ornamentation on the inside of the wall consisting of irregular confluent verrucae 0.5–1.5(–2.6) µm diam, with 0.8–1.1 µm wide gelatinous sheath.

Habitat & Host range — Dead corticated twigs and branches of *Juglans* spp. attached to the tree.

Distribution — Europe, Asia; common, particularly as asexual morph.

Additional specimens examined (all on corticated branches of *Juglans regia* except where noted). Austria, Kärnten, St. Margareten im Rosental, 20 June 2015, *H. Voglmayr* (WU 35960, culture D142); St. Margareten im Rosental, near Stariwald, 6 Dec. 1998, *W. Jaklitsch W.J. 1279* (WU 35961); ibid., 21 July 2000, *W. Jaklitsch W.J. 1500* (WU 35959); St. Margareten im Rosental, Wograda, 16 June 1995, *W. Jaklitsch W.J. 647* (WU 35962); ibid., 7 July 2013, *W. Jaklitsch* (WU 35963); St. Margareten im Rosental, Zugland, 21 Apr. 2000, *W. Jaklitsch W.J. 1450* (BPI 843622, culture CBS 121083); Niederösterreich, Orth an der Donau, near Uferhaus, on corticated branches of *Juglans nigra*, soc. *J. appendiculata*, 5 Mar. 2003, *H. Voglmayr* (WU 35964); Schönberg am Kamp, Olbersdorf, Dienbach-Tal, 25 Mar. 1984, *A. Hausknecht* (WU 16103); Oberösterreich, St. Willibald, Geitzedt, 6 Aug. 2016, *H. Voglmayr* (WU 35966); Wien, Floridsdorf, Neu-Stammersdorf, 14 Apr. 2013, *W. Jaklitsch* (WU 35967, culture MC1). – Czech Republic, Morava, Hranice, 23 July 1915, *F. Petrak*, *Flora Bohemiae et Moraviae exsiccata 761* (BPI 614906); ibid, Aug., *F. Petrak*, *Kryptogamae exsiccatae 2411* (BPI 614907). – France, Beaumont, on *Juglans*, Aug. 1852, Herb. *M.R. Tulasne* (PC 0723587); Chatenay, on *Juglans*, Feb. 1854, Herb. *M.R. Tulasne* (PC 0723583); ibid., Feb. 1858, Herb. *M.R. Tulasne* (PC 0723584, PC 0723585); Meudon (Clamart), on *Juglans*, Feb. 1854, Herb. *M.R. Tulasne* (PC 0723586); Gillancourt, on *Juglans*, Oct. 1852, Herb. *M.R. Tulasne* (PC 0723579); without place and date, on *Juglans* (PC 0723582). – Germany, Reichardtshausen, without date, *L. Fuckel*, *Fungi rhenani 595* (BPI 614908). – Russia, Circassia, Krasnaja Poljana, 6 July 1909, *J. Serebrianiko*, in *Tranzschel & Serebrianiko*, *Mycotheca Rossica 94* (BPI 614910). – Spain, Asturias, Saliencia, 3 June 2013, *W. Jaklitsch & H. Voglmayr* (WU 35968, culture MC3). – Ukraine, Odessa, Adrianovka, near Ovidiopolin, 28 Oct. 1898, *Kulikovski*, in *Jaczewski et al*., *Fungi Rossiae exsiccati 299* (BPI 614909).

Notes — *Melanconium juglandinum* is the first epithet unequivocally applicable to this taxon. No type specimen could be traced at B (R. Lücking, pers. comm.), and therefore specimen WU 35965, which contains the sexual and asexual morph and for which sequence data are available, is here designated as neotype.

In PC no type collection of *Melanconis carthusiana* is extant. In the original description Tulasne (1856) cited a single collection 'prope vicum S. Laurentii Carthusianorum' (i.e. Saint-Laurent-du-Pont north of Grenoble, France), without a collection date. In Tulasne & Tulasne (1863), 1855 is mentioned as collection year for this specimen, and they noted that after publication they found the sexual morph also on specimens from Bellemont, Isère (collected in August 1852) and Chatenay near Paris (collected in February 1858). In PC no collection from St Laurent is extant, but there are two collections from Chatenay and one from Beaumont corresponding to the data given in Tulasne & Tulasne (1863), which, however, are no types. As these collections are in poor condition, we do not select a neotype from them. To stabilise the nomenclatural connection of both names, we select the neotype specimen of *Melanconium juglandinum* also as neotype of *M. carthusiana*.

Corda (1839) nicely illustrated conidiomata, conidia and conidiophores under *Melanconium juglandis*, a younger synonym. The annellidic conidiogenesis was studied in detail by Belisario & Onofri (1995) by light and scanning electron microscopy. *Juglanconis juglandina* has been proven to be a virulent pathogen of *Juglans* spp. (Belisario 1999), being the causal agent of the European black pustular dieback of walnut. Compared to the asexual morph which is very common and conspicuous, the sexual morph has been infrequently found in fully developed condition.

Juglanconis oblonga (Berk.) Voglmayr & Jaklitsch, *comb. nov.* — MycoBank MB819585; Fig. 7, 8

Basionym. *Melanconium oblongum* Berk., Grevillea 2 (no. 22): 153. 1874.

= *Diaporthe juglandis* Ellis & Everh., Proc. Acad. Nat. Sci. Philadelphia 45: 448. 1893.

≡ *Melanconis juglandis* (Ellis & Everh.) A.H. Graves, Phytopathology 13: 311. 1923.

Typification. USA, Alabama, on corticated twigs of *Juglans cinerea*, without date, *Peters*, Herb. Berkeley no. 5250 (K(M) 200286, *lectotype* of *Melanconium oblongum* here designated; MBT374386); Massachusetts, on corticated twigs of *Juglans cinerea*, without date & collector, Herb. Berkeley no. 3380 (K(M) 200285, syntype of *Melanconium oblongum*); New York, Alcove, on corticated twigs of *Juglans cinerea*, 31 Aug. 1893, *C.L. Shear*, in *Ellis & Everhart*, *North American Fungi 3121* (BPI 616365, *lectotype* of *Diaporthe juglandis* here designated; MBT374387; BPI 616363, NY 00921841, NYS f4634 isotypes); same data, in *Shear*, *New York Fungi 340* (BPI 616364, isotype).

Pseudostromata 1–3 mm diam, usually distinct, circular, projecting up to 0.5 mm, without perithecial bumps. *Ectostromatic disc* indistinct, usually circular, greyish to brownish or black, 0.4–1.3(–2.7) mm diam, commonly concealed by densely arranged ostioles, often pulvinate. *Central column* yellowish, greenish to brownish grey. *Entostroma* indistinct. *Ostioles* 1–15(–25) per disc, (83–)103–163(–220) µm diam (n = 20), papillate, black, sometimes covered by distinctly white crust. *Perithecia* (490–)525–725(–780) µm diam (n = 31), arranged in various configurations. *Asci* (85–)100–132(–140) × (12.5–)14.5–18(–19) µm (n = 27), clavate to fusoid, containing 8 uni- to irregularly biseriate ascospores, ring cylindrical to funnel-shaped according to Kobayashi (1970), not seen in the herbarium specimens examined. *Ascospores* (17.5–)19.8–24(–28) × (6.7–)8.0–11.5(–17.5) µm, l/w = (1.5–)2–2.6(–3.3) (n = 322), hyaline, ellipsoid, broadly ellipsoid or broadly fusoid, symmetric to slightly asymmetric, straight, rarely slightly curved, constricted at the septum, without appendages; cells monomorphic to slightly dimorphic with larger upper cell, with broadly rounded to subacute ends, multiguttulate; wall c. 0.4–0.6 µm thick, not swelling.

Asexual morph. *Conidiomata* acervular, 1–4 mm diam, blackish, scattered or occasionally confluent, with central or eccentric stromatic column; at maturity covered by black discharged conidial masses. *Conidiophores* (21–)28–44.5(–55) × (4.0–)4.8–6.5(–7.5) µm (n = 30), cylindrical to lageniform, simple, rarely branched at the base, smooth, subhyaline to pale brown. *Conidiogenous cells* annellidic with distinct annellations, integrated. *Conidia* (13.7–)18–22.7(–27.7) × (7–)9.2–12(–15.5) µm, l/w = (1.3–)1.7–2.3(–3.2) (n = 1633), unicellular, hyaline when immature, brown to blackish when mature, ellipsoid, elongate to pip-shaped, sometimes slightly allantoid, truncate with distinct scar at the base, densely multiguttulate, thick-walled; wall c. 0.7–0.9 µm, with distinct ornamentation on the inner side of the wall consisting of irregular confluent verrucae 0.5–2 µm diam, with 0.6–0.8 µm wide gelatinous sheath.

Habitat & Host range — Dead corticated twigs, branches and trunks of *Juglans* spp.

Distribution — North America, Eastern Asia (Japan).

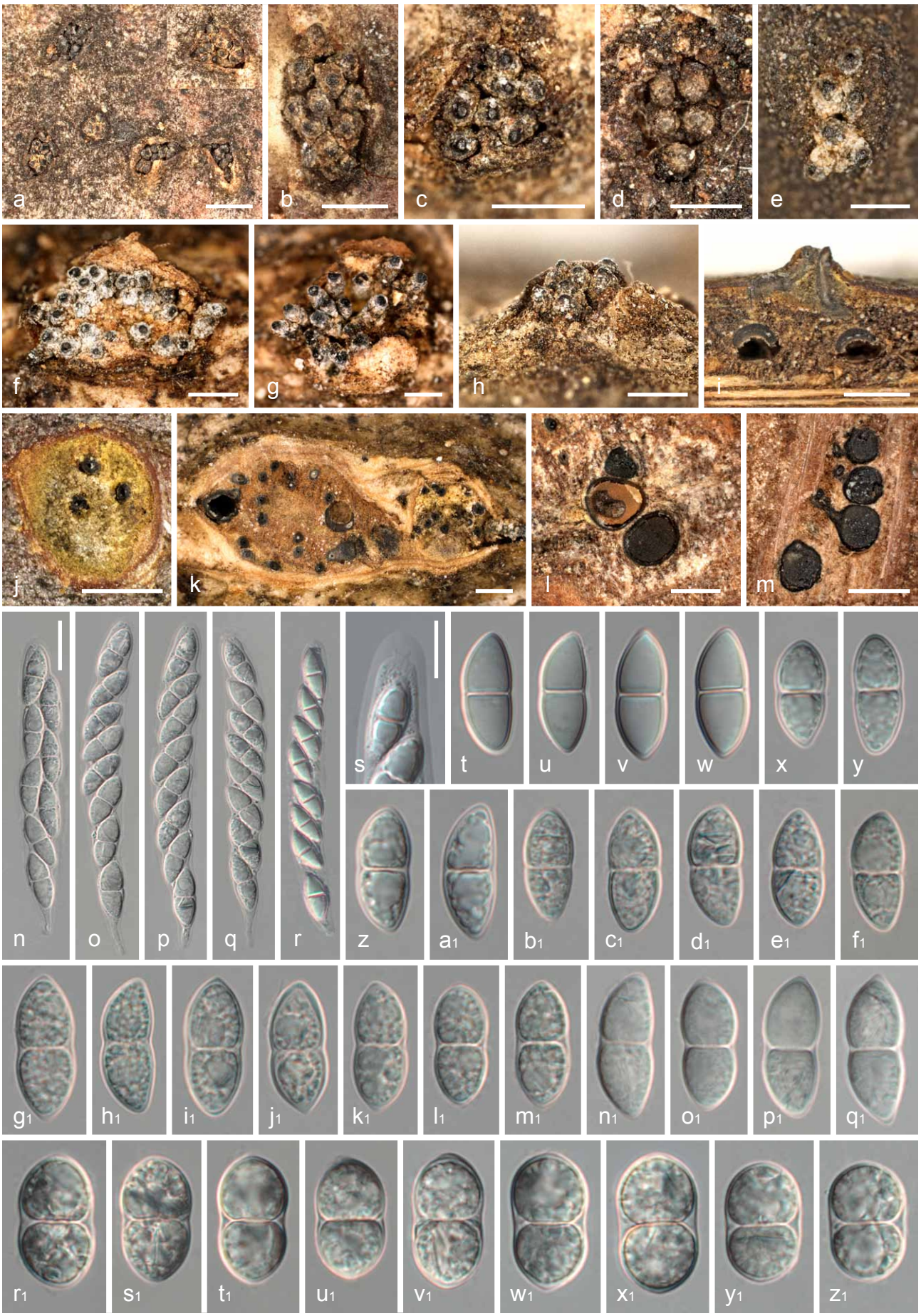

Fig. 7 *Juglanconis oblonga*, sexual morph. a–g. Ectostromatic discs and ostioles in surface view; h. ectostromatic disc in side view showing protruding ostioles; i. pseudostroma in vertical section; j, k. transverse sections below ectostromatic disc; l, m. pseudostromata in transverse section, showing perithecia and indistinct whitish to light brown entostroma; n–r. mature asci; s. ascus apex; t–z1. dead ascospores. All in 3 % KOH, except t–w in water (a, b: BPI 616364; c, h: BPI 616363; d, m–q, n1–q1: NY 01926933; e, r–a1: WU 35969; f, g, k, v1–z1: TFM FPH2623; i: BPI 614960; j, l, b1–f1: BPI 614954, g1–m1: BPI 616365 (lectotype of *Diaporthe juglandis*); r1–u1: TFM FPH3601). — Scale bars: a = 1 mm; b–m = 0.5 mm; n–r = 20 µm; s–z1 = 10 µm.

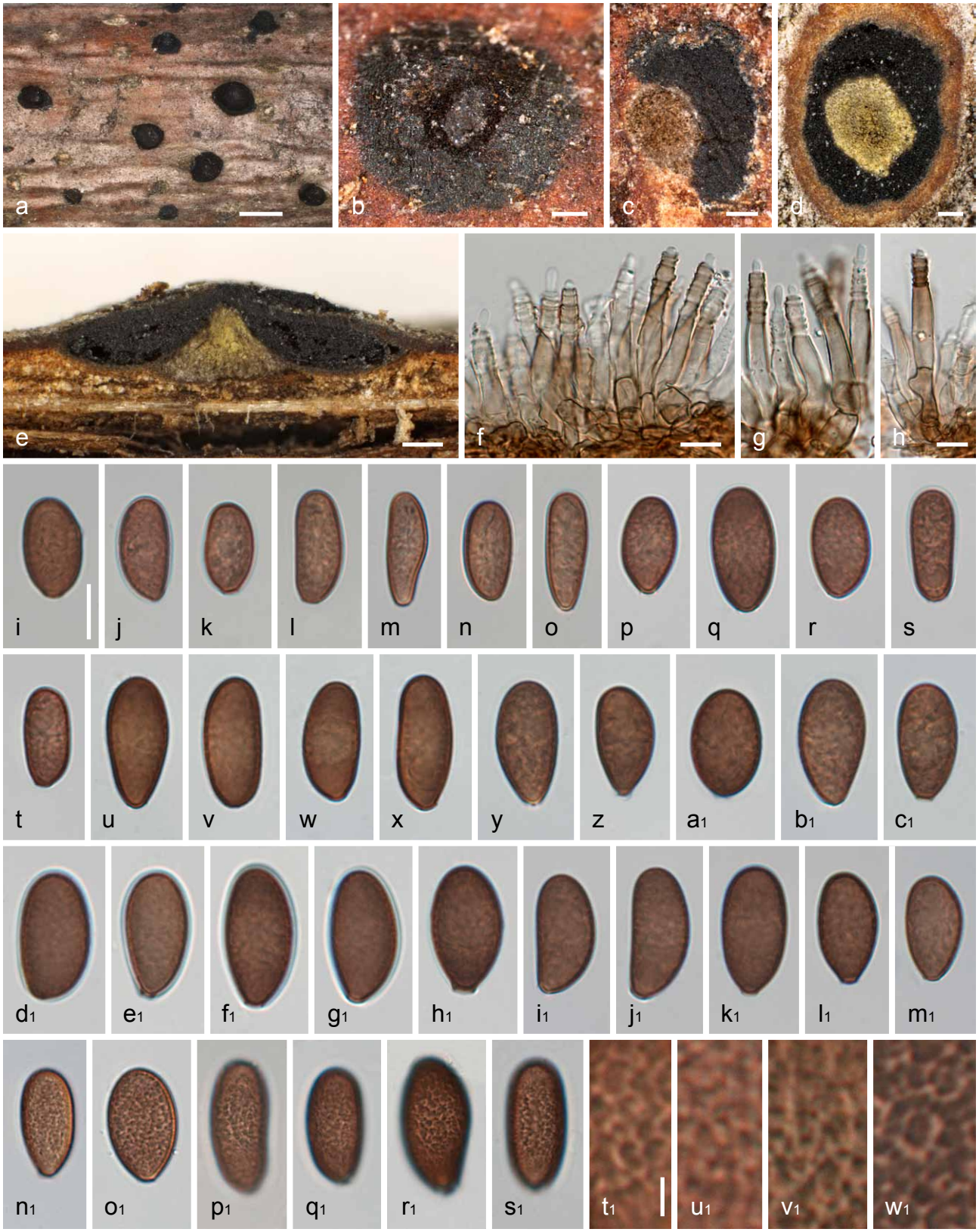

Fig. 8 *Juglanconis oblonga*, asexual morph. a, b. Conidiomata in surface view; c–e. transverse (c, d) and vertical (e) sections of conidiomata, showing central column; f–h. conidiophores (annellides); i–s1. dead conidia showing gelatinous sheath in j, k, s, d1–g1; in n1–s1 showing verruculose inner conidial wall; t1–w1. detail of inner conidial wall, showing confluent verrucae. All in 3 % KOH (a, f–h: NY 01926935; b, c, k–o, n1, t1: K(M) 200286 (lectotype); d: NY 01926937; e, d1–h1, r1: WU 35969; i, j: K(M) 200285; p–t, o1, u1: NY 00921841; u–x: TFM FPH3599; y–c1: TFM FPH2623; i1–m1, q1: BPI 614951; p1, v1: TFM FPH3601; s1, w1: BPI 614955). — Scale bars: a = 2 mm; b–e = 200 µm; f–s1 = 10 µm; t1–w1 = 2 µm.

Additional specimens examined (all on corticated twigs of *Juglans cinerea* except where noted). Canada, Ontario, Brant Co., E. of Harley, 27 June 1937, *R.F. Cain* (BPI 614954); Frontenac Co., Kingston, on corticated twigs of *Juglans nigra*, 8 June 1964, *E.D. Taylor* (BPI 614961); Lake Erie District, Aldsborough, 19 July 1962, *G.R. Irinnell* (BPI 614955); Renford Co., near Braeside, 31 Aug. 1960, *G.D. Darker* (BPI 614959); W of Richmond Hill, 12 Oct. 1935, *H.S. Jackson* (NY 01926938); Quebec, Plains d' Abraham, 27 July 2006, *H. Voglmayr* (WU 35969); Gatineau Park, Nature Trail, 9 Sept. 1958, *R. Arnold & J. Malvin* (BPI 615124). – Japan, Hokkaido, Bibai, Hokkaido Forest Research Institute Experimental Forest, on corticated twigs of *Juglans ailanthifolia*, 24 Sept. 1964, *T. Kobayashi* (TFM FPH3373); Iwate-gun, Iwate Pref., Takisawa, on corticated twigs of *Juglans ailanthifolia*, 5 Nov. 1970, *T. Kobayashi* (TFM FPH3599, TFM FPH3601, NY 01926932). – USA, Connecticut, Berlin, July 1924, *A.H. Graves* (BPI 615128); Hamden, 16 Sept. 1922, *A.H. Graves* (BPI 614960); Meriden, 16 Sept. 1922, *A.H. Graves* (BPI 614957); Maine, North Windham, 23 July 1923, *A.H. Graves* (BPI 614956); Maryland, Beltsville, North Farm walnut planting, on corticated twigs of *Juglans ailanthifolia*, 7 July 1953, *F.H. Berry* (BPI 614842); Massachusetts, Conway, Baptist Hill, 10 Feb. 1980, *M.E. Barr* (NY 01926937); New York, Highlands of Rockland Co., 16 June 1929, *A.H. Graves* (BPI 614951); Putnam Co., Carmel, S. of Nichols Rd. between Gypsy Trail and Horsepound Rds., 22 June 1998, *R.C. Harris* (NY 01926935); Walton, Mountain Home Farm, 1 June 1924, *A.E. Jenkins* (BPI 614958, NY 01926934); Pennsylvania, Lancaster, on corticated twigs of *Juglans nigra*, 12 June 1940, *J.D. Diller* (BPI 614843); Vermont, Lamoille Co., Stowe, Loomis Hill Rd., 10 July 1964, *H.E. Bigelow & M.E. Barr* (NY 01926933); Washington D.C., Ft. Kemble Park, on corticated twigs of *Juglans nigra*, 8 June 1943, *G.F. Gravatt* (BPI 614836).

Notes — Two authentic collections of *Melanconium oblongum* are extant at K, of which K(M) 200286 is here selected as lectotype. The type collection of *Diaporthe juglandis* has been distributed in two exsiccates (Ellis & Everhart, North American Fungi 3121 and in Shear, New York Fungi 340, the latter bearing the annotation 'These specc. are from the same collection as the type'), therefore numerous copies are present. A remaining part of the original collection has been deposited as BPI 616365; it does not bear an original label, but contains a note by Shear 'issued in N.Y.F., Cent. III'. This note also gives the exact date of the collection, whereas the labels of the exsiccata only give the month. Of all duplicates of the original collection we examined, BPI 616365 contains the most abundant and best preserved material; it is therefore here selected as lectotype of *Diaporthe juglandis*.

Juglanconis oblonga, previously also known as *Melanconis juglandis*, has been reported as the agent of walnut dieback in North America (Graves 1923) and Japan (Kobayashi 1968). It appears to be confined to North America and Eastern Asia where it replaces the similar, closely related *J. juglandina*. Wehmeyer (1941) expressed some doubts about its status as a separate species and gave slightly smaller stromata ('pustules'), shorter and less inequilateral or curved ascospores and narrower conidia as main distinctions from the European *J. juglandina*. However, investigation of representative collections from North America revealed a remarkable variability in conidial width, and while conidia in general are narrower in *J. oblonga* (typically 8.5–11 vs 12–14.5 µm in *J. juglandina*), there is some size overlap in certain collections. No fresh collections were available for morphological investigations, therefore it has not been possible to observe the apical ascus ring. Only few specimens containing the sexual morph were available for study, but our investigations confirmed shorter (typically 19.8–24 vs 24.3–29 µm in *J. juglandina*), mostly symmetric spores with monomorphic to slightly dimorphic cells. In addition, the asci are significantly shorter in *J. oblonga*. The Japanese collections available for study differed from North American collections by distinctly wider ascospores (11–13 µm) with rounded ends, but the sequence data demonstrated conspecificity with material from eastern North America.

Within *Melanconis juglandis*, Wehmeyer established var. *caryae* from *Carya glabra* (Wehmeyer 1940), and var. *tiliae* from *Tilia americana* (Wehmeyer 1941). The former differs in host and the absence of a melanconium-like asexual morph, and the latter was considered to be synonymous with the European *Melanconis* (now *Lamproconium*) *desmazieri*, although the American collections did not produce a lamproconium-like but a melanconium-like asexual morph. In absence of fresh collections and of DNA data, their status cannot be evaluated, but it is likely that at least the latter does not belong to *Juglanconis*. They are certainly not conspecific with *J. oblonga*.

Juglanconis pterocaryae (Kuschke) Voglmayr & Jaklitsch, *comb. nov.* — MycoBank MB819586; Fig. 9

Basionym. *Melanconium pterocaryae* Kuschke, Trudy Tiflissk. Bot. Sada 28: 25. 1913.

Synonym. *Melanconis pterocaryae* Tak. Kobay., Bull. Govt. Forest Exp. Stn Meguro 226: 24. 1970.

Typification. Japan, Shizuoka, Fuji, on corticated twigs of *Pterocarya rhoifolia*, 5 Aug. 1968, *T. Kobayashi* (TFM FPH2623, holotype of *Melanconis pterocaryae*).

Pseudostromata 1–2 mm diam, typically distinct, circular, projecting up to 0.3 mm, without perithecial bumps. *Ectostromatic disc* reduced to almost absent, circular, grey or brown, 0.15–0.5 mm diam, concealed by densely arranged ostioles, pulvinate. *Central column* poorly developed, grey to brownish grey, or absent. *Entostroma* indistinct. *Ostioles* 1–9 per disc, (79–)91–123(–135) µm diam (n = 31), plane or slightly papillate, black. *Perithecia* (410–)470–600(–640) µm diam (n = 14), arranged in various configurations. *Asci* (67.5–)79–96(–105) × (11.5–)12–14.2(–15.2) µm (n = 25), clavate to fusoid, containing 8 uni- to irregularly biseriate ascospores; ring cylindrical according to Kobayashi (1970), not seen in the herbarium specimen. *Ascospores* (16.5–)17.5–20(–21.5) × (5.3–)6–7(–7.5) µm, l/w = (2.5–)2.7–3.1(–3.5) (n = 51), hyaline, broadly fusoid to fusoid, symmetric to slightly asymmetric, straight or slightly curved, slightly constricted at the septum, with distinct tapering appendages having rounded to subacute tips, (1.6–)2–3.4(–4.6) µm long, (1.9–)2.2–2.5(–2.6) µm wide (n = 42); cells monomorphic to dimorphic with slightly larger upper cell, with narrowly rounded to subacute ends, multiguttulate; wall c. 0.5 µm thick, not swelling.

Asexual morph. *Conidiomata* acervular, 0.4–1.2 mm diam, dark brown to blackish, inconspicuous, scattered, with central or eccentric greenish yellow to grey stromatic column; at maturity covered by brown to blackish discharged conidial masses. *Conidiophores* (14–)17–28(–38) × (2.5–)3.5–4.7(–5.5) µm (n = 35), narrowly cylindrical to lageniform, simple or branched at the base, smooth, subhyaline to pale brown. *Conidiogenous cells* annellidic with distinct annellations, integrated. *Conidia* (10.8–)14.5–17.5(–20) × (5.2–)6.7–7.7(–8.7) µm, l/w = (1.5–)2–2.5(–3.1) (n = 100), unicellular, hyaline when immature, medium brown when mature, narrowly ellipsoid to elongate, rarely pip-shaped, often truncate with scar at the base, densely multiguttulate, thick-walled; wall c. 0.6–0.8 µm, with faint ornamentation on the inside of the wall consisting of small irregular confluent verrucae 0.5–0.8(–1.5) µm diam, with c. 0.5–0.9 µm wide gelatinous sheath.

Habitat & Host range — Dead corticated twigs and branches of *Pterocarya* spp. attached to the tree.

Distribution — Asia (Georgian Republic, Iran, Japan).

Notes — *Melanconium pterocaryae* was described from the Georgian Republic (Abkhazia) from *Pterocarya fraxinifolia*, but no type collection could be traced and no collections from the original host were available for morphological investigations and for DNA sequencing. Kobayashi (1970) described *Melanconis pterocaryae* from *Pterocarya rhoifolia* collected in Japan as sexual morph of *Melanconium pterocaryae*. However, the conidial sizes of the Japanese collection were slightly

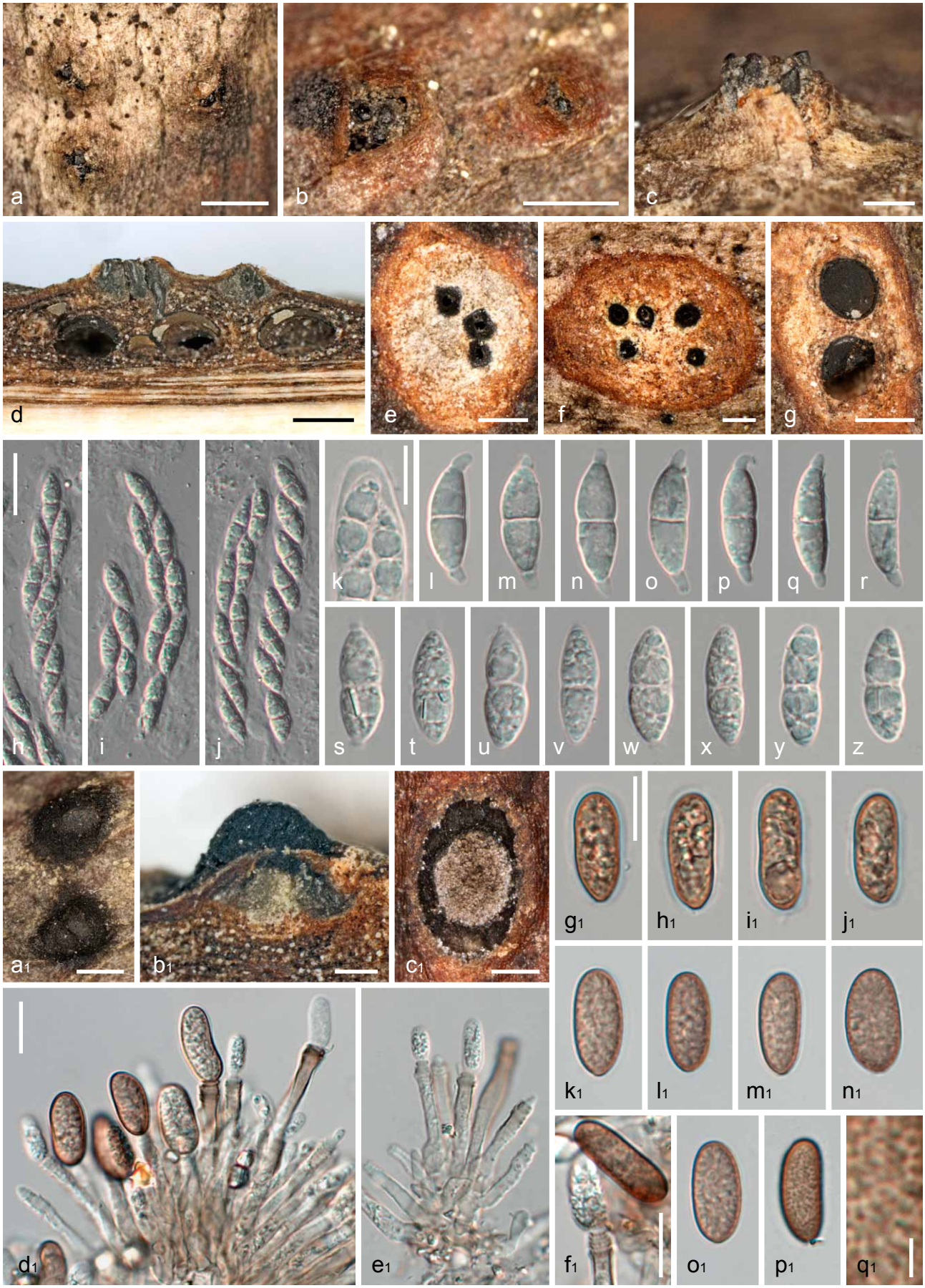

Fig. 9 *Juglanconis pterocaryae* (TFM FPH3373). a, b. Ectostromatic discs and ostioles in surface view; c. ectostromatic disc in side view showing slightly protruding ostioles; d. pseudostromata in vertical section; e, f. transverse sections below ectostromatic disc; g. pseudostroma in transverse section, showing perithecia and indistinct entostroma; h–j. mature asci; k. ascus apex (no apical ring visible); l–z. dead ascospores (l–r with tapering, rounded to subacute gelatinous appendages); a1. conidiomata in surface view; b1. vertical section of conidioma; c1. transverse section of conidioma, showing central column; d1, e1. conidiophores (annellides) with conidia; f1. annellations of conidiophore and young conidium; g1–p1. dead conidia (showing gelatinous sheath in g1–j1; in p1 showing verrucae on inner conidial wall); q1. detail of verruculose inner conidial wall. All in 3 % KOH, except l–r, g1–j1 in water. — Scale bars: a, b, d, g, a1 = 0.5 mm; c, e, f, b1, c1 = 200 µm; h–j = 20 µm; k–z, d1–p1 = 10 µm; q1 = 1 µm.

narrower than those given in the protologue of *Melanconium pterocaryae* (14–21 × 6–10 µm vs 14–19 × 8–12 µm), which was confirmed in the present study. Riedl & Ershad (1977) also reported narrower conidia (12–15.5 × 6.5–9.5 µm) from an Iranian collection on the original host, *Pterocarya fraxinifolia*, and we therefore consider these size differences to be within the range of intraspecific variability.

In the original description of *Melanconis pterocaryae*, Kobayashi (1970) mentioned absence of ascospore appendages. However, re-investigation of the type in water mounts revealed the presence of small tapering appendages with rounded to subacute tips, whereas in KOH the appendages were faint and disappearing quickly.

Melanconium ershadii Riedl, in Riedl & Ershad, Sydowia 29 (1–6): 163. 1977 (1976–77). — Fig. 10

Holotype. Iran, S Gorgan, Nahār Khorān, on corticated twigs of *Pterocarya rhoifolia*, 21 Apr. 1974 (given as 24 Apr. 1974 in Riedl & Ershad 1977), *H. Riedl & D. Ershad* (W 1978-03131).

Sexual morph unknown. *Conidiomata* acervular, 0.3–0.8 mm diam, dark brown to blackish, flat, inconspicuous, scattered, with small central or eccentric whitish to light grey stromatic column; at maturity sometimes covered by brown to blackish discharged conidial masses. *Conidiophores* (20–)24–39(–46) × (2.5–)3–4(–5) µm (n = 22), narrowly cylindrical to lageniform, simple or branched at the base, smooth, hyaline. *Conidiogenous cells* annellidic with few indistinct annellations, integrated. *Conidia* (7.5–)9.5–11.5(–13.3) × (4–)4.7–5.7(–6.8) µm, l/w = (1.2–)1.7–2.4(–3) (n = 100), unicellular, hyaline when immature, pale to medium brown when mature, very variable in shape from subglobose, narrowly ellipsoid, allantoid, pip-shaped to

Fig. 10 *Melanconium ershadii* (W 1978-03131, holotype). a, b. Conidiomata in surface view; c. transverse section of conidioma, showing reduced central column; d–g. conidiophores (annellides) with conidia; h–y. dead conidia surrounded by gelatinous sheath. All in 3 % KOH. — Scale bars: a = 1 mm; b = 400 µm; c = 200 µm; d–y = 10 µm.

elongate, often truncate with scar at the base, with few faint guttules, thin-walled; wall smooth, c. 0.4 µm, without ornamentation on the inside of the wall, with c. 0.5 µm wide gelatinous sheath.

Habitat & Host range — Dead corticated twigs of *Pterocarya fraxinifolia*.

Distribution — Only known from Iran.

Notes — *Melanconium ershadii* and *M. pterocaryae* share the same host, *Pterocarya fraxinifolia*, and the former was reported to differ from the latter in flatter conidiomata and shorter conidia (10.5–11.5 × 5.5–6.2 µm; Riedl & Ershad 1977). This was confirmed by re-investigation of the type. No sexual morph is known and no cultures and sequence data are available, which currently makes an appropriate phylogenetic placement impossible. Whereas the annellidic conidiation and the unicellular brown conidia are melanconium-like, we do not think that it belongs to *Juglanconis*. Important differences concern the consistently hyaline conidiophores with few indistinct annellations (vs at least partly light brown conidiophores with distinct annellations in *Juglanconis*), the entirely smooth inner conidial wall (vs at least finely verrucose in *Juglanconis*), and the highly variable, and commonly irregular, shape of the conidia. These characters indicate that *Melanconium ershadii* may rather belong to *Melanconis* s.str., which needs to be confirmed by sequence data.

Key to species of Juglanconis

NB: For observation of ascospore appendages the ascospores should be mounted in water, as in KOH the appendages usually disappear quickly.

1. Ascospores with hyaline terminal appendages; conidia light to medium brown at maturity, inner surface of conidial wall with finely verruculose ornamentation 2
1. Ascospores without appendages; conidia dark brown at maturity, inner surface of conidial wall with distinct verruculose ornamentation 3
2. Ascospore appendages cylindrical with truncate ends; most conidia distinctly longer than 20 µm, variable in shape from pip-shaped, narrowly ellipsoid, elongate to suballantoid; on *Juglans* in Europe . . . . . *J. appendiculata*
2. Ascospore appendages tapering, with rounded to subacute ends; most conidia distinctly shorter than 20 µm, narrowly ellipsoid to elongate; on *Pterocarya* spp. in Asia . . . . . *J. pterocaryae*
3. Ascospores (20.5–)24.3–29(–36.5) µm long, distinctly inequilateral, commonly curved, cells dimorphic; conidia in average usually wider than 12 µm; on *Juglans* spp. in Europe and Asia . . . . . *J. juglandina*
3. Ascospores (17.5–)19.8–24(–28) µm long, mostly symmetrical, only rarely curved, cells monomorphic to slightly dimorphic; conidia in average usually narrower than 12 µm; on *Juglans* spp. in North America and Eastern Asia (Japan) . . . . . *J. oblonga*

DISCUSSION

Molecular phylogeny, species delimitation and barcoding

The molecular phylogenetic analyses reveal that *Juglanconis* is a genus distinct from *Melanconis*, which cannot be classified within any existing family (Fig. 1–3). Therefore, we consider it justified to describe the family *Juglanconidaceae* for it. In the LSU tree (Fig. 1) the genus receives low (52 %) MP and no ML bootstrap support, whereas in the ITS-LSU tree (Fig. 2) bootstrap support rises to 100 % (MP) or 70 % (ML), which shows that the LSU alone does not contain sufficient information for providing a sound resolution of all phylogenetic relationships within *Diaporthales*. This has been also shown for other groups of *Diaporthales* like *Stilbospora* (Voglmayr & Jaklitsch 2014), which appeared as paraphyletic in the LSU tree but was resolved as a highly supported monophylum in trees obtained from other markers (ITS, *rpb2*, *tef1*). Similarly, also other families like *Gnomoniaceae* and *Melanconidaceae* receive only low to medium support in the LSU analyses (Fig. 1), but become highly supported in multilocus analyses (Sogonov et al. 2008). Unfortunately, for most representatives of *Diaporthales* no sequence data are available apart from ITS and LSU, therefore it has not been possible to perform a multilocus phylogenetic analyses which includes a representative taxon sampling. However, the genus becomes well supported upon addition of the ITS, and also the similar morphological and ecological traits provide good characters for generic and familial delimitation.

Wehmeyer (1941) questioned the status of *Juglanconis juglandinum* and *J. oblongum* as distinct species, but the molecular phylogenetic analyses of the multilocus matrix (Fig. 3) clearly reveal them as distinct species, which also differ morphologically by ascospore and conidial characters (see notes at the respective species).

The comparison of the different markers used for multilocus analyses (Table 3) showed that the *tef1* fragment containing introns 4 and 5 is the best suited marker for species resolution within *Juglanconis*, which is in line with other investigations on *Diaporthales* (e.g. Voglmayr et al. 2012, Voglmayr & Jaklitsch 2014, Wang et al. 2014, Udayanga et al. 2014, 2015), and it should be adopted as barcoding marker for the group. On the other hand, the ITS which is the primary barcoding locus for fungi (Schoch et al. 2012) is amongst the poorest performing markers, which is clearly due to the comparatively low number (13) of informative characters. This is in line with what is observed in other ascomycete lineages like *Hypocreales* (e.g. Jaklitsch et al. 2013, Jaklitsch & Voglmayr 2015). In phylogenetic multilocus analyses within closely related species of *Diaporthales*, *tef1* should be combined with other informative markers like *ms204*, *cal* and *tub2*, whereas for higher-level relationships the *rpb2* may be more suitable, as the sequenced fragment consists of a coding region, which facilitates a better alignment.

Host range, distribution and other species

The genus *Juglanconis* appears to be confined to hosts from *Juglandaceae* (*Fagales*). Three species (*J. appendiculata*, *J. juglandina* and *J. oblonga*) are so far known from the genus *Juglans* and one (*J. pterocariae*) from *Pterocarya*. In contrast to e.g. *Stegonsporium* (Voglmayr & Jaklitsch 2008, 2014), which is highly host specific and where European plantations of North American *Acer* hosts consistently also harbour the North American *S. acerinum*, the genus *Juglanconis* appears to be less host specific. In Europe, *J. appendiculata* and *J. juglandina* are occurring on the indigenous *Juglans regia* as well as on the introduced North American *J. nigra*, which has been planted and become widely naturalised in Central European alluvial forests. *Juglanconis oblonga* has been confirmed from *J. cinerea* and *J. nigra* in North America and from *J. ailanthifolia* in Japan, and it has been recorded from several additional *Juglans* species (see Farr & Rossman 2016), but these records need to be critically evaluated by morphological and DNA data.

Additional *Juglanconis* species may be hidden within the *Juglandaceae*, especially in America and Eastern Asia, the biodiversity centres of *Juglandaceae*, which are still largely understudied except for the few economically important *Juglans* species. It cannot be ruled out that *Melanconis juglandis* var. *caryae* also belongs to *Juglanconis*, but no specimens were available for study. It has been described from *Carya alba*, and differs from *J. oblonga* primarily in hyaline conidia sized

10.5–14 × 5–7 µm; in addition, hyaline beta-conidia of size 2–2.5 × 0.8–1 µm were recorded (Wehmeyer 1940). Already Wehmeyer (1940) assumed that *Melanconis juglandis* var. *caryae* may represent a distinct species, and considering the results of recent molecular phylogenetic investigations of corticolous *Diaporthales* with similar ecology (e.g. Mejía et al. 2008, 2011a, b, Voglmayr & Jaklitsch 2008, 2014, Voglmayr et al. 2012, Walker et al. 2014), which revealed a much higher species biodiversity than previously perceived, we are convinced that it represents a distinct species. However, DNA sequence data as well as detailed morphological investigations are necessary to evaluate its generic affiliation. Wehmeyer (1936) also transferred *Melanconiella pallida*, a species growing on *Carya* spp., to *Melanconis*. This species differs from all *Juglanconis* species in dark brown ascospores. We have not been able to investigate this species morphologically, and in the absence of DNA sequence data its generic affiliation cannot be evaluated, but its morphological features indicate that it may not belong to *Juglanconis*.

The current investigations once again show that the traditional generic classification in *Diaporthales* needs to be critically re-evaluated by detailed morphological and molecular phylogenetic analyses. Even supposedly well-studied areas and hosts still harbour undescribed, morphologically distinct species. Our results also confirm that the ITS-LSU rDNA region, which has mostly been used for phylogenetic analyses in *Diaporthales*, commonly does not contain sufficient information for satisfactory phylogenetic resolution, and should be supplemented by additional suitable single-copy markers like *tef1*, *cal*, *tub2*, *ms204* and *rpb2*.

Acknowledgements We thank Enrique Rubio for providing fresh material, Jack Fournier for providing his detailed documentation of a French collection of *J. appendiculata*, Robert Lücking from B for information on specimens of *Melanconium juglandinum*, and the fungarium curators of BPI, K, NY, NYS, PC, TFM, W, and Walter Till at WU for sending and managing collections. The financial support by the Austrian Science Fund (FWF; project P27645-B16) is gratefully acknowledged.

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