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Taxonomic rearrangement of *Anthostomella* (Xylariaceae) based on a multigene phylogeny and morphology

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Abstract – The genus *Anthostomella* is heterogeneous and recent DNA based studies have shown that species are polyphyletic across Xylariaceae. In this study, we present a morphology based, taxonomic treatment, coupled with a molecular phylogenetic reassessment of relationships within *Anthostomella*. This has resulted in the establishment of two new genera, eight new combinations and three new species among anthostomella-like taxa. Seventeen strains from 16 anthostomella-like species have been revisited. A re-description of morphological characters among these taxa suggests that *Anthostomella* can be circumscribed based on immersed ascomata, cylindrical asci with short pedicels and pigmented, equilateral ascospores with germ slits, while *Anthostomella brabeji*, *A. forlicesenica*, *A. leucospermi* and *A. proteae* are transferred to *Anthostomelloides*. *Pseudoanthostomella* gen. nov., possesses solitary ascomata, as well as asci with apical rings and is closely related to *Neoanthostomella*,

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but can be distinguished based on a combination of characters, such as multiple ascomata and asci completely lacking apical rings. *Anthostomella conorum, A. delitescens* and *A. sepelibilis* are reallocated to *Pseudoanthostomella* with two new species, *P. pini-nigrae* and *P. senecionicola*. *Neoanthostomella viticola* sp. nov. is added to the previously monotypic genus *Neoanthostomella*. *Alloanthostomella* gen. nov is introduced to accommodate *Anthostomella rubicola*. The only anthostomella-like genus diagnosed with hyaline ascospores so far, is *Alloanthostomella* gen. nov. This study represents a comprehensive molecular phylogenetic study based on four independent molecular markers (ITS, 28S rDNA, RPB2 and β -tubulin) with a broad taxon sampling across *Anthostomella*.

Character evolution / Classification / Pyrenomycetes / Sordariomycetes / Taxonomy / Xylariales

INTRODUCTION

The family Xylariaceae comprises 87 genera (Maharachchikumbura *et al.* 2015; 2016) and over 1300 known species (Stadler *et al.* 2013). Although many species are characterized by large, conspicuous stromata, others have rudimentary stromata (Daranagama *et al.* 2016a). Species delimitation and generic recognition criteria in Xylariaceae have shifted from morphology (Dennis 1956; Miller 1961; Rogers 1977; Læssøe & Spooner 1994; Ju & Rogers 1996; Daranagama *et al.* 2016b) to DNA based phylogenetic schemes (Sánchez-Ballesteros *et al.* 2000; Hsieh *et al.* 2005; Bitzer *et al.* 2008; Pelaez *et al.* 2008; Tang *et al.* 2009; Daranagama *et al.* 2015a; b; 2016c; d; Senanayake *et al.* 2015). Most recently chemotaxonomic data has been used in identification of species and genera (Stadler & Hellwig 2005; Stadler *et al.* 2008; 2010; 2014a; Daranagama *et al.* 2014).

Anthostomella Sacc. is a complex genus with approximately 95 species (Lu & Hyde 2000; Daranagama et al. 2015a). The morphological circumscription of Anthostomella is based on the type species Anthostomella tomicoides Sacc. with immersed, dark, clypeate ascomata with periphysate, ostiolar canals, 8-spored, cylindrical, unitunicate asci and mostly dark, unicellular ascospores sometimes with small cells or appendages at the ends. Daranagama et al. (2015a) confirmed that Anthostomella is polyphyletic and several anthostomella-like species can be accommodated into several genera across Xylariaceae. Apart from Anthostomella, there are other genera in Xylariaceae, such as Nemania, Rosellinia, Stilbohypoxylon and Xylaria (Hsieh et al. 2010; Stadler et al. 2013; Daranagama et al. 2015a; b) which may not be monophyletic. *Anthostomella* species within Xylariaceae are polyphyletic (Stadler et al. 2013; Daranagama et al. 2015a; Tibpromma et al. 2016). There have been a number of contentious issues in connection with the taxonomy of species that are polyphyletic. In some cases, polyphyletic genera have been treated as orphans (i. e. Shenoy et al. 2006; Wang et al. 2007). In this study a practical taxonomic treatment for the polyphyletic genus Anthostomella is proposed, which is in concordance with phylogenies recovered from multigene sequence analyses. To provide a more robust justification on our taxonomic rearrangement, it has been necessary to redefine generic concepts based on reexamination of type species and micro-morphological variations, which is common taxonomic practice nowadays (Senanayake et al. 2015; Thambugala et al. 2015; Maharachchikumbura et al. 2016). For many years, large number of species has been allocated to *Anthostomella*, making it more complex and difficult to resolve species relationships. Around 90% of these species had been described only from a morphological

perspective and never been reported with a living culture hence no DNA sequence data were available to propose a more natural classification. For a meaningful phylogenetic and morphological reappraisal of *Anthostomella*, it is essential to obtain fresh collections of *Anthostomella* species and get axenic cultures to acquire DNA sequence data. In this study we obtained cultures from fresh specimens allowing for additional DNA sequence data to reevaluate the evolutionary relationships of anthostomella-like species.

It is important to emphasize the need of reference isolates with accessible molecular data, particularly for species identified and described several decades ago. Based on the preliminary phylogenetic analysis on *Anthostomella* (Daranagama *et al.*, 2015a) and previous morphological studies (Lu & Hyde 2000; Lee & Crous 2003), it is essential to reorganize the taxonomy of *Anthostomella* species to reflect a more natural classification.

The objectives of this study are as follows: 1) to reassess morphological species concepts to define species in *Anthostomella* and anthostomella-like genera based on multi-gene phylogenies; 2) to investigate phylogenetic relationships of *Anthostomella* and describe new genera for anthostomella-like species where necessary; 3) and evaluate resolving power of various phylogenetic markers for anthostomella-like species.

MATERIAL AND METHODS

Sampling, morphological observation and isolation. – Fresh specimens were obtained from various host plants collected in different provinces of Italy. Morphological examination and microphotography was carried out as described by Daranagama *et al.* (2014; 2015). Axenic cultures were initiated and maintained in oat agar (OA) medium for observation of asexual morph as described by Daranagama *et al.* (2015). Dried materials are deposited in Mae Fah Luang University herbarium (MFLU), Chiang Rai, Thailand and Kunming Institute of Botany (HKAS), China and cultures are deposited at Mae Fah Luang University Culture Collection (MFLUCC), Thailand and the Kunming Institute of Botany Culture Collection (KIBCC), China. Faces of Fungi numbers and Index Fungorum numbers were obtained as explained in Jayasiri *et al.* (2015) and Index Fungorum (2016).

DNA extraction, PCR and sequencing. – DNA was extracted and the ITS, LSU, β -TUB and RPB2 genes were amplified following the protocols outlined by Daranagama *et al.* (2014; 2015a; b) and Thambugala *et al.* (2015). PCR products were visualized in 1 % agarose gel electrophoresis, stained with Goldview (Geneshun Biotech, China) with D2000 DNA ladder (Realtimes Biotech, Beijing, China). All PCR products were purified using the kits according to the manufacture protocols and DNA sequencing was performed using the same primers as used for PCR in an Applied Biosystem 3730 DNA analyzer at Sinogenomax Company, Beijing, China.

Sequence alignment and phylogenetic analyses. – Raw sequences were assembled with Contig Express 2003 (Invitrogen, Carlsbad, CA). The assembled consensus sequences were implemented automatically by Fast Fourier Transformation (MAFFT v7.017) using the E-INS-i algorithm (Katoh & Standley 2013) (http://mafft.cbrc.jp/alignment/server/) and adjusted manually where necessary. The alignments were edited with an online version of the Gblocks program v. 0.91b9, using parametrical settings for a very low stringency to avoid poorly aligned regions.

Maximum Likelihood (ML) phylogenetic trees were reconstructed using RAxML 7.4.2 Black Box (Stamatakis 2006; Stamatakis *et al.* 2008) available in the CIPRES Science Gateway platform (Miller *et al.* 2010). A combined dataset was included with all default modal parameters in RAxML with ML estimate of 25 per site rate categories. The RAxML software accommodated the GTR model of nucleotide substitution with the additional options of modeling rate heterogeneity (Γ) and proportion invariable sites (I) Gaps were treated as missing data.

Phylogenetic trees and data files were viewed in MEGA 5 (Tamura *et al.* 2011) and Fig tree v1.4 (Rambaut & Drummond 2008). Maximum Likelihood bootstrap values (equal to or above 50%) are indicated above or below nodes and considered significant if equal or above 70%. The sequences generated in this study are indicated in bold and deposited in GenBank (Table 1). The alignments and trees were submitted to TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S19489).

RESULTS

Phylogenetic analyses

Four different alignments corresponding to each individual gene of ITS, LSU, RPB2 and β -tubulin and a combined alignment of all four genes were analyzed. The ITS alignment consisted of 15 flanks with 314 characters (20% of the original 1556 positions). The LSU alignment consisted of 33 flanks with 558 characters (52% of the original 1063 positions). The RPB2 alignment consisted of 22 flanks with 927 characters (70% of the original 1316 positions). The β -tubulin alignment consisted of 40 flanks with 858 characters (32% of the original 2631 positions). The phylogram inferred from the combined analysis of four genes for all isolates is presented in Fig. 1. Sequence data from 58 isolates, including 17 isolates of *Anthostomella* species were used. *Sordaria fimicola* was used as the outgroup taxon. GenBank accession numbers of the isolates are provided in Table 1.

The Hypoxyloideae (HY) clade is well-supported (90% BS) in the combined phylogenetic analysis and the analyzed *Anthostomella* species do not form a monophyletic clade in Xylarioideae (XY). The data show species previously identified as *Anthostomella sensu lato* to be split across the two subfamilies of Xylariaceae, forming five separate clades (A-E). *Anthostomella formosa* (similar in morphology to the type species) is in clade A together with *A. obesa, A. pinea* and *A. helicofissa*, which is named herein as *Anthostomella sensu stricto*. *Anthostomella formosa* has close affinities with *A. helicofissa* and both cluster together with 90% bootstrap support while *Anthostomella obesa* and *A. pinea* cluster together with 100% bootstrap support (Figs 1 & 2).

Alloanthostomella, Neoanthostomella and Pseudoanthostomella cluster into a subgroup outside of the two subfamilies Hypoxyloideae and Xylarioideae. Biscogniauxia is suggested without support as the sister clade to Alloanthostomella, Neoanthostomella and Pseudoanthostomella.

A group of anthostomella-like species clusters in a monophyletic clade (Clade B, 73% BS) within Xylarioideae and is introduced in this study as the new genus *Anthostomelloides*. *Anthostomelloides* consists of five species including the type species, *Anthostomelloides krabiensis*. *Anthostomelloides leucospermi* clusters with *An. krabiensis* with 100% bootstrap support, while our analyses place *An. brabeji* basal to the other *Anthostomelloides* species.

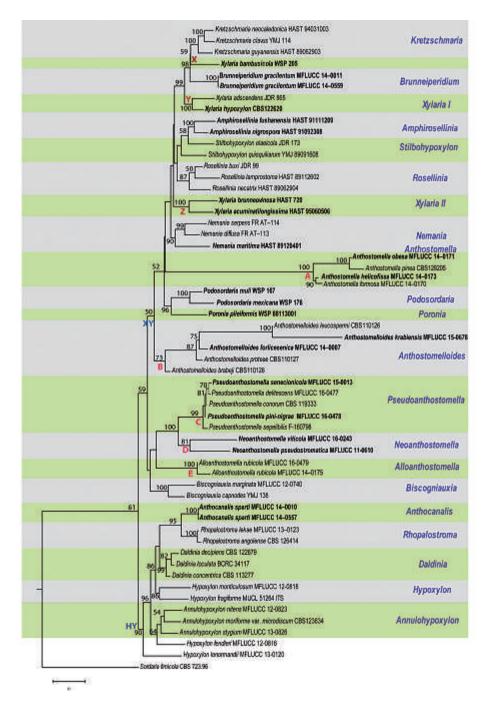


Fig. 1. The best scoring phylogenetic tree based on a combined dataset of ITS, LSU, RPB2 and β -tubulin sequence data produced by maximum likelihood analysis. Bootstrap support values for maximum likelihood greater than 50% are given. The type strains/specimens are in bold letters and indicated by *. The tree is rooted to *Sordaria fimicola*. Ex-type strains are in bold.

	Culture collection/	E		0	GenBank accession numbers	ssion number	S
species	specimen number	type status	Kejerence	STI	RPB2	β -tubulin	TSU
Amphirosellinia fushanensis	HAST Isolate 91111209	Ex-type	Hsieh et al., 2010	GU339496	GQ848339	GQ495950	
A. nigrospora	HAST Isolate 91092308	Ex-type	Hsieh et al., 2010	GU322457	GQ848340	GQ495951	Ι
Anthocanalis sparti	MFLUCC 14-0010	Ex-type	Daranagama <i>et al.</i> , 2015a	KP297394	KP340522	KP406605	KP340536
A. sparti	MFLUCC 14-0557	Ex-paratype	Daranagama <i>et al.</i> , 2015a	KP297395	KP340523	KP406606	KP340537
Annulohypoxylon moriforme var. microdiscum	CBS123834	Authentic	Tang et al., 2009	DQ631935	DQ631960	DQ840095	DQ840061
A. nitens	MFLUCC 12-0823	Authentic	Daranagama <i>et al.</i> , 2015a	KJ934991	KJ934994	KJ934993	KJ934992
A. stygium	MFLUCC 13-0826	Authentic	Daranagama et al., 2015a	KJ940870	KJ940868	KJ940867	KJ940869
Anthostomella formosa	MFLUCC 14-0170	Authentic	Daranagama <i>et al.</i> , 2015a	KP297403	KP340531	KP406614	KP340544
A. helicofissa	MFLUCC 14-0173	Ex-type	Daranagama <i>et al.</i> , 2015a	KP297406	KP340534	KP406617	KP340547
A. obesa	MFLUCC 14-0171	Ex-type	Daranagama <i>et al.</i> , 2015a	KP297405	KP340533	KP406616	KP340546
A. pinea	CBS 128205	I	Crous & Groenewald 2010	HQ599578	I	Ι	Ι
Anthostomelloides brabeji	CBS 110128	I	Jaklitsch and Voglmayr 2011	EU552098	I	I	EU552098
An. forlicesenica	MFLUCC 14-0007	Ex-type	Daranagama <i>et al.</i> , 2015a	KP297396	KP340524	KP406607	KP340538
An. krabiensis	MFLUCC 15-0678	Ex-type	Tibpromma et al., 2016	KX305927	KX305929	Ι	KX305928
An. leucospermi	CBS 110126	I	Marincowitz et al., 2008	EU552100	I	Ι	Ι
An. proteae	CBS 110127	I	Marincowitz et al., 2008	EU552101	I	I	Ι
Alloanthostomella rubicola	MFLUCC 14-0175	Authentic	Daranagama <i>et al.</i> , 2015a	KP297407	KP340535	KP406618	KP340548
Al.rubicola	MFLUCC 16-0479	Authentic	This study	KX533455	KX789493	KX789494	KX533456
Biscogniauxia capnodes	YMJ 138	Authentic	Hsieh et al., 2010	EF026131	JX507779	AY951675	Ι
B. marginata	MFLUCC 12-0740	Authentic	Daranagama et al., 2015a	KJ958407	KJ958409	KJ958406	KJ958408
Brunneiperidium gracilentum	MFLUCC 14-0011	Ex-type	Daranagama <i>et al.</i> , 2015a	KP297400	KP340528	KP406611	KP340542
B. gracilentum	MFLUCC 14-0559	Ex-paratype	Daranagama <i>et al.</i> , 2015a	KP297401	KP340529	KP406612	KP340549
Daldinia concentrica	CBS 113277	Authentic	Kuhnert et al., 2013	AY616683	I	KC977274	Ι
D. decipiens	CBS 122879	Authentic	Hsieh et al., 2005	JX658441	I	AY951694	I
D. loculata	BCRC 34117	Authentic	Hsieh et al., 2005	EF026145	I	AY951698	I
Hypoxylon fendleri	MFLUCC 12-0816	Authentic	Daranagama <i>et al.</i> , 2015a	KM017563	KM017566	KM017564	KM017565
H. fragiforme	MUCL 51264	Authentic	Triebel <i>et al.</i> , 2005/ Daranagama <i>et al.</i> , 2015a	KM186294	KM186296	KM186301	KM186295
H. lenormandii	MFLUCC 13-0120	Authentic	Daranagama <i>et al.</i> , 2015a	KM039135	KM039135 KM039137 KM039138 KM039136	KM039138	KM039136
H. monticulosum	MFLUCC 12-0818	Authentic	Daranagama <i>et al.</i> , 2015a	KM052716	KM052719	KM052718	KM052717

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Table 1. GB accession numbers of the isolates used in this study

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Kretzschmaria clavus	YMJ 114	Authentic	Hsieh et al., 2010	EF026126	GQ844789	EF025611	I
K. guyanensis	HAST 89062903	Authentic	Hsieh et al., 2010	GU300079	GQ844792	GQ478214	I
K. neocaledonica	HAST 94031003	Authentic	Hsieh et al., 2010	GU300078	GQ844788	GQ478213	I
Nemania diffusa	FR AT-113	I	Tang et al., 2009	DQ658238	DQ631947	DQ840088	DQ840073
N. maritima	HAST 89120401	Ex-type	Hsieh et al., 2010	GU292822	GQ844775	GQ470225	I
N. serpens	FR AT-114	I	Tang et al., 2009	DQ631942	DQ631948	DQ840086	DQ840075
Neoanthostomella pseudostromatica	MFLUCC 11-0610	Ex-type	Dai et al., 2016	KU940158	I	Ι	KU863146
N. viticola	MFLUCC 16-0243	Ex-type	This study	KX505957	KX789496	KX789495	KX505958
Podosordaria mexicana	WSP 176	Authentic	Hsieh et al., 2010	GU324762	GQ853039	GQ844840	I
P. muli	WSP 167	Ex-type	Hsieh et al., 2010	GU324761	GQ853038	GQ844839	Ι
Poronia pileiformis	WSP 88113001	Ex-epitype	Hsieh et al., 2010	GU324760	GQ853037	GQ502720	Ι
Pseudoanthostomella conorum	AR173	I	Jaklitsch and Voglmayr 2011	FN435813	Ι	Ι	FN435813
P. delitescens	MFLUCC 16-0477	Authentic	This study	KX533451	KX789491	KX789490	KX533452
P. pini-nigrae	MFLUCC 16-0478	Ex-type	This study	KX533453	KX789492	ī	KX533454
P. senecionicola	MFLUCC 15-0013	Ex-type	This study	KX505960	KX789489		KX505959
P. sepelibilis	F-160	I	Pelaez et al., 2008	AY908989	I	Ι	Ι
Rhopalostroma angolense	CBS 126414	Authentic	Stadler et al., 2010/	FN821965	KM186297	KM186297 KM186299	KM186298
			Daranagama <i>et al.</i> , 2015a				
R. lekae	MFLUCC 13-0123	Authentic	Daranagama <i>et al.</i> , 2014	KJ472428	KJ472427	I	KJ472429
Rosellinia buxi	JDR 99	Authentic	Hsieh et al., 2010	GU300070	GQ844780	GQ470228	I
R. lamprostoma	HAST 89112602	Authentic	Hsieh et al., 2010	EF026118	GQ844778	EF025604	Ι
R. necatrix	HAST 89062904	Authentic	Hsieh et al., 2010	EF026117	GQ844779	EF025603	Ι
Sordaria fimicola	CBS 723.96	I	Tang et al., 2009	AY 681188	DQ368647	DQ840087	AF132330
Stilbohypoxylon elaeicola	JDR 173	I	Hsieh et al., 2010	EF026148	GQ844826	EF025616	Ι
S. quisquiliarum	YMJ 89091608	I	Hsieh et al., 2010	EF026120	GQ853021	EF025606	Ι
Xylaria acuminatilongissima	HAST Isolate 95060506	Ex-type	Hsieh et al., 2010	EU178738	GQ853028	GQ502711	Ι
X. adscendens	JDR 865	Authentic	Hsieh et al., 2010	GU322432	GQ844818	GQ487709	I
X. bambusicola	WSP 205	Ex-type	Hsieh et al., 2010	EF026123	GQ844802	AY951762	I
X. brunneovinosa	HAST 720	Ex-type	Hsieh et al., 2010	EU179862	GQ853023	GQ502706	I
X. hypoxylon	CBS122620	Ex-epitype	Stadler et al., 2014b/	AM993141	KM186302	KM186300 KM186301	KM186301
			Uaranagama <i>et al.,</i> 2012				

Ex-types strains and newly generated sequences are highlighted in **bold**. Abbreviations: **AT**: Taxa collected and identified by Alvin M. C. Tang, **CB**S: Centraalbureau voor Schimmeleultures, Utrecht, The Netherlands, **BCRC**: Bioressource Collection and Research Centre, Taiwan; **HAST**: Herbarium, Research Centre for Biodiversity, Academia Sinica, Taipei; **JDR**. Herbarium of Jack D. Rogers; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **MUCL**: Mycothèque de l'Université catholique de Louvain, Germany; **YMJ**: Herbarium of Yu Ming Ju; **WSP**: Washington State University, USA.

Taxonomic rearrangement of Anthostomella

Another group of anthostomella-like species (Clade C, 99% BS) is here introduced as the new genus *Pseudoanthostomella* with five genetically very close species, which were previously treated as *Anthostomella*. *Pseudoanthostomella senecionicola*, *P. delitescenes* and *P. conorum* cluster in a terminal clade with 81% and 70% bootstrap support. *Pseudoanthostomella pini-nigrae* branches off from them and nested in between *P. sepelibilis* and the above species. ML analysis places

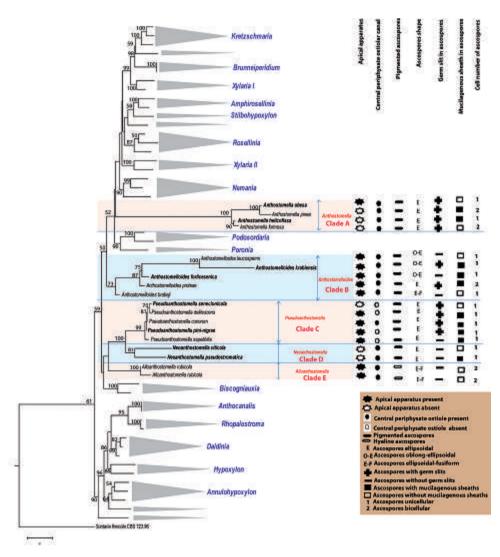


Fig. 2. The best scoring phylogenetic tree based on a combined dataset of ITS, LSU, RPB2 and β -tubulin sequence data produced by Maximum Likelihood analysis. Groups labeled are the same as in Fig. 1. Clades labeled as A-E indicate the different monophyletic subclades of anthostomella-like species. Morphological characters distinguishing each group pertaining to each clade are shown on the right of the cladogram with the legend below. Bootstrap support values for maximum likelihood greater than 50% are given. The tree is rooted to *Sordaria fimicola*. Ex-types strains are in bold.

Pseudoanthostomella sepelibilis basal to other species of *Pseudoanthostomella*. *Neoanthostomella pseudostromatica* has close affinities with *N. viticola* (81% bootstrap support) and together form a separate clade (Clade D), which appears as a sister clade to *Pseudoanthostomella* with 100% bootstrap support. *Alloanthostomella* clade (Clade E) is also a well-defined and basal to both *Neoanthostomella* and *Pseudoanthostomella* (although poorly supported in the combined analysis). However, the single gene phylogenetic tree of RPB2 (data not shown) supports the separation of *Alloanthostomella* with 64% bootstrap support from *Neoanthostomella* and *Pseudoanthostomella*. The morphological characters linked to species in each of these clades are displayed in Fig. 2.

The genera such as, *Amphirosellinia, Anthocanalis, Annulohypoxylon, Biscogniauxia, Brunneiperidium, Daldinia, Kretzschmaria, Nemania, Podosordaria, Poronia, Rhopalostroma* and *Rosellinia* are resolved as distinct groups in the combined phylogenetic analysis (Fig. 1). However *Xylaria* species grouped into three clades (X, Y, Z) and intermingled with other taxa. *Hypoxylon fendleri* and *H. lenormandii* appeared as sister clades while *H. fragiforme* and *H. monticulosum* clustered together (Fig. 1).

The phylogenetic trees of single gene analyses obtained from the ML analysis were studied and compared in terms of position of each species, topology of the tree and clade stability (results not shown). ITS based phylogeny reveals a very limited resolution within the family, often resulting in an inconclusive branching order and lack of bootstrap support at the internodes. Anthocanalis, Biscogniauxia, Brunneiperidium. Daldinia and Rhopalostroma were resolved as monophyletic clades but with low bootstrap support values. There was weak resolution and lack of bootstrap support as well for our ingroup under study. LSU based phylogeny revealed that the isolates of *Pseudoanthostomella* formed in a monophyletic clade with 56% bootstrap support while other anthostomella-like species intermingle with other genera. RPB2 based phylogenies display better resolution than both ITS and LSU with considerable bootstrap support and the separation of Neoanthostomella and *Pseudoanthostomella* receives high bootstrap support. B-tubulin gene tree resolves the Anthostomella species into a monophyletic clade with 78% bootstrap support. When the β -tubulin gene sequences were analyzed independently, better resolution and clade stability were obtained as compared to the other single gene phylogenies.

TAXONOMY

Key to genera mentioned in this study

1.	a. Asci with apical ring	2
	b. Asci without apical ring, sometimes with apical thickenings only	
	Neoanthoston	
	2. a. Pigmented ascospores	3
	b. Hyaline ascospores Alloanthoston	mella
3.	a. Ascomata with a central periphysate ostiolar canal	4
	b. Ascomata lack a central periphysate ostiolar canal Pseudoanthoston	
	4. a. Ascospores oblong-ellipsoidal, usually lack germ slits Anthostomell	oides
	b. Ascospores equilaterally-ellipsoidal, usually with germ slits	
	Anthostor	nella

Alloanthostomella Daranagama, Camporesi & K. D. Hyde, gen. nov.

Index Fungorum number: IF552371; *Facesoffungi number:* FoF 02526 *Etymology:* referring to different; yet anthostomella-like species.

Saprobic on living branch of Cornus sanguinea L. and on Rubus. Sexual morph: Ascomata immersed, black, slightly raised conical areas, solitary, sometimes aggregated into clusters, scattered, in cross section globose, with a disc-like, central, black ostiolar dot. Clypeus carbonaceous, comprising intracellular fungal hyphae and host tissues. Peridium few cell layers, compressed, outwardly comprising thick-walled, light to dark brown cells of textura irregularis, inwardly comprising thinwalled, hyaline cells of textura angularis. Paraphyses few, filamentous, septate. Asci 8-spored, unitunicate, cylindrical, short-pedicellate, apically rounded, with a wedge-shaped-cylindrical, J+, apical ring. Ascospores overlapping uniseriate-biseriate, elongate-ellipsoidal, one end tapered and pointed, remain hyaline in asci, bicellular with rostrate, hyaline, dwarf cell, larger cell hyaline, rarely dark brown, smooth-walled. Asexual morph: Undetermined.

Type species: Alloanthostomella rubicola (Speg. [ex Sacc. & Trotter) Daranagama, Camporesi & K. D. Hyde.

Notes: The monotypic genus Alloanthostomella is introduced to accommodate Anthostomella rubicola, which is morphologically and phylogenetically different from other Anthostomella species. Another morphologically reminiscent genus to Alloanthostomella is Emarcea Duong et al. which shares certain characters, such as ascomata developing beneath a blackened clypeus, papillate ostioles, unitunicate asci with a J+, subapical ring and bicellular, hyaline ascospores lacking germ slits (Duong *et al.* 2004). However, the other morphological differences between these two genera (e.g long fusiform ascospores in Emarcea with an obclavate apical cell and a cylindrical basal cell, while Alloanthostomella have dwarf cell and an elongate ellipsoidal, larger cell and further discussed in the taxonomy section) can be used to differentiate them. In this study, we have been selective with the ingroup taxa and excluded *Emarcea* in our analysis since most of the available data cannot be authenticated. Along the same line, several xylariaceous species had to be excluded from the phylogenetic analyses because of the generation of ambiguous sequence alignment, lack of sequence data from protein gene and high degree of polytomies encountered in phylograms coupled with low bootstrap support. Previous rDNA based phylogenetic analyses by Duong et al. (2003) indicate that Emarcea castanopsidicola is closely related to Muscodor species which is now treated as genera *incertae sedis* (Maharachchikumbura *et al.* 2016). The phylogenetic position of *Emarcea* with more taxa, such as *Emarcea eucalyptigena*, E. rostrispora and Anthostomella eucalyptorum warrant further investigations.

Alloanthostomella rubicola (Speg.) Daranagama, Camporesi & K. D. Hyde, *comb. nov*.

Index Fungorum number: IF552372; Facesoffungi number: FoF 00320

Basionym: Entosordaria rubicola Speg., Revta Fac. Agron. Vet. Univ. nac. La Plata, Ser. 2, 6(1): 40 (1910)

 \equiv Anthostomella rubicola Speg. ex Sacc. & Trotter, Syll. fung. (Abellini) 22: 100 (1913)

Saprobic on branch of Cornus sanguinea Sexual morph: Pseudostromata black, carbonaceous, comprising fungal tissues and epidermal host cells. Ascomata $250-300 \times 280-320 \ \mu m \ (\bar{x} = 275 \times 310 \ \mu m, n = 10)$, immersed, visible as blackened,

Fig. 3

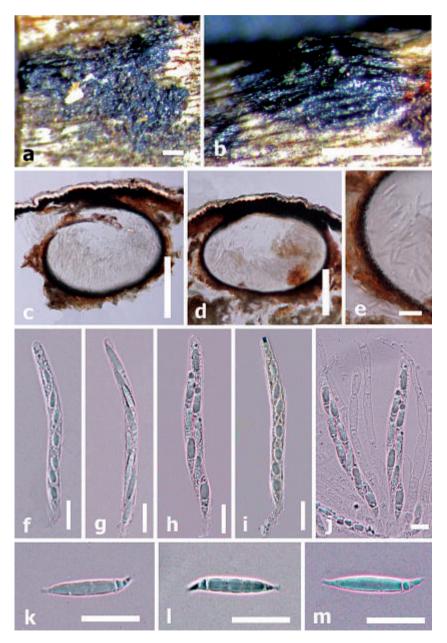


Fig. 3. *Alloanthostomella rubicola* (MFLU 15-0661). **a**, **b**. Pseudostromata on host **c**, **d**. Cross section of ascoma **e**. Peridium **f-i**. Asci **j**. Paraphyses **k-m**. Ascospores. Scale bars: a, b = 500 μ m, c, d = 100 μ m, e = 30 μ m, f-j = 10 μ m, k-m = 10 μ m.

slightly raised, conical, stromata-like areas, solitary or aggregated into clusters, scattered, in cross section globose, with a central black ostiolar dot, disc-like, black. *Clypeus* 60-83 μ m diam. at base, 75-80 μ m high ($\bar{x} = 68 \times 77 \ \mu$ m, n = 10), comprising host cells and intracellular fungal hyphae, surrounded by carbonaceous tissues. *Peridium* 20-30 μ m diam. ($\bar{x} = 25.6 \ \mu$ m, n = 10), with a few compressed cell layers, outwardly comprising thick-walled, light-dark brown cells of *textura irregularis* and inwardly comprising thin-walled, hyaline cells of *textura angularis*. *Paraphyses* 5-6 μ m diam. ($\bar{x} = 5.8 \ \mu$ m, n = 20), few, filamentous, septate. *Asci* 120-165 × 6-9 μ m ($\bar{x} = 157 \times 8.4 \ \mu$ m, n = 30), 8-spored, unitunicate, cylindrical, short-pedicellate, apically rounded, with a wedge-shaped to cylindrical, J+ apical ring, 3-5 × 4-5 μ m ($\bar{x} = 4 \times 4.5 \ \mu$ m, n = 30). *Ascospores* 22-35 × 5.5-8.7 μ m ($\bar{x} = 27 \times 6.5 \ \mu$ m, n = 40), overlapping uniseriate to biseriate, ellipsoidal, one end tapered and pointed, in asci always remain hyaline, unicellular with rostrate, hyaline dwarf cell, 4-6 μ m long ($\bar{x} = 5.5 \ \mu$ m, n = 40), larger cell rarely dark brown or mostly hyaline, smoothwalled. **Asexual morph:** Undetermined.

Culture characteristics: Colonies on Difco OA at 25-27°C reaching 9 cm in 4-5 weeks, at first whitish, felty, azonate, with diffuse grey colour margins, reverse turning citrine (13). Asexual morph was not produced in cultures after 3 months.

Material examined: ITALY, Province of Forlì-Cesena, Trivella di Predappio, on dead branch of *Cornus sanguinea* (Cornaceae), 26 December 2014, Erio Camporesi, IT 2316 (MFLU 15-0661, **reference specimen designated here**), living cultures MFLUCC 16-0479, KUMCC.

Notes: Ascospores of *Al. rubicola* remain hyaline in the asci. However, rarely mature, brown ascospores occur with a hyaline dwarf cell which is rostrate (Lu & Hyde 2000; Daranagama *et al.* 2015a). This species has morphological similarities to *Anthostomella appendiculosa* (Berk. & Broome) Sacc. However, the ascospores in *A. appendiculosa* are larger and become brown in the asci with a cordate dwarf cell and a straight germ slit, while those in *Al. rubicola* lack a germ slit. Attempts to obtain the asexual morph in culture were not successful even after 3 months observation. The holotype of *A. rubicola* collected by Spegazzini, deposited at LPS is from Chile and on *Rubus fruticosus* L. Due to the differences in the host and the geographical differences between our collections and the holotype, we refrained from epityification and only designated the fresh collections as reference specimens.

Anthostomella Sacc., Atti Soc. Veneto-Trent. Sci. Nat., Padova, Sér. 4 4: 84 (1875) Facesoffungi number: FoF 00316

Generic description and illustration: See Lu & Hyde (2000) and Daranagama *et al.* (2015a).

Type species: Anthostomella tomicoides Sacc., *Atti Soc. Veneto-Trent. Sci. Nat., Padova, Sér.* 4 4: 101 (1875).

Synonymy: Entosordaria tomicoides (Sacc.) Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 129: 166 (1920)

= Anthostomella tomicoides var. crassispora Bat., Fischman & Da Matta, in Batista et al. Atas Inst. Micol. Univ. Recife 4: 224 (1967)

Notes: The typification of the genus *Anthostomella* is long confused as Saccardo never designated any type material for *Anthostomella*. Eriksson (1966) designated *Anthostomella limitata* Sacc. as the lectotype material, the only one with the original three species with non-appendiculate ascospores. Francis (1975) disagreed to this typification because *A. limitata* has no true clypeus, which is the

key taxonomic feature of the genus and ascospores have diagonal or spiral germ slit that is not common to many *Anthostomella* species. Hence Francis (1975) selected *Anthostomella tomicoides* Sacc. as the type of the genus but she was unable to locate the material and selected *Anthostomella italica* subsp. *affinis* Sacc. as the neotype. Lu and Hyde (2000) located the original material of *A. tomicoides* and lectotypified it. Since no living culture of *A. tomicoides* is available for now, we used *A. formosa*, which is morphologically similar to the type for stabilization of *Anthostomella sensu stricto. Anthostomella formosa* is similar to *A. tomicoides* by having darkened, coriaceous ascomata immersed beneath the clypeus, with central, periphysate ostiolar canal and appendage bearing ascospores with thin mucilaginous sheaths and straight germ slits (Lu and Hyde 2000).

Anthostomella in a broad sense is currently included in the Xylariaceae with 85 species (Lu & Hyde 2000; Daranagama et al. 2015a) but this placement still needs molecular support and is also not supported in our phylogeny. Furthermore, in our phylogeny the genus sits on a very long branch in between Nemania, Poronia and *Podosordaria* and its true affinities may be with other genera A world monograph of Anthostomella was published by Lu & Hyde (2000). The placement of Anthostomella sensu stricto was established in Daranagama et al. (2015a). Based on the molecular data the genus is clearly heterogeneous and true affinities of many species are poorly understood. The genus is characterized by immersed, dark, clypeate ascomata with periphysate ostiolar canals, 8-spored, cylindrical, unitunicate asci and mostly dark, unicellular ascospores, sometimes with small cells or appendages at the ends. The asexual morph of *Anthostomella* is characterized by conidiophores that are simple, light brown, branched or single and having a few, small, disc-like conidiogenous cells occurring along the denticles of conidiophores and hyaline, easily dehiscent, ellipsoidal, unicellular conidia (Daranagama et al. 2015a). The genus is saprobic and cosmopolitan, distributed in tropics, subtropics and even temperate regions. The species are distributed on a wide range of hosts and some species may be host-specific (e. g. A. eucalyptorum).

Anthostomelloides Tibpromma & K.D. Hyde, Turkish Journal of Botany. (2016) 40: 2.

Index Fungorum number: IF552117, Facesoffungi number: FoF 02190

Saprobic on dead leaves, branches and twigs. Sexual morph: Ascomata immersed, black, globose, visible as conical, blackened dots, with central periphysate ostiolar canal. Clypeus short, margins indistinct, sometimes reduced, comprising dark, thick-walled, fungal and host tissues, black, globose. Peridium coriaceous, composed of several layers, outwardly comprising brown cells and inwardly comprising hyaline cells. Hamathecium comprising numerous, hyaline, filamentous, septate, tapering paraphyses. Asci 8-spored, rarely 6-spored, unitunicate, cylindrical, short pedicellate, with a wedge-shaped, J+ apical ring. Ascospores mostly uniseriate, inequilaterally oblong-ellipsoidal, dark brown at maturity, guttulate, with or without a conspicuous mucilaginous sheath, with or without germ slit, if present germ slit straight, less than the spore length. Asexual morph: undetermined.

Type species: Anthostomelloides krabiensis Tibpromma & K.D. Hyde, *Turkish Journal of Botany.* (2016) 40: 2.

Notes: This genus was introduced recently to accommodate *Anthostomelloides krabiensis* isolated from dead leaves of *Pandanus odorifer* (Forssk.) Kuntze (Tibpromma *et al.* 2016). The morphological similarity of *Anthostomelloides* to other genera such as *Anthostomella, Brunneiperidium, Fasciatispora* and *Nipicola* is discussed in Tibpromma *et al.* (2016). Immersed, globose ascomata with a central,

papillate, periphysate ostiolar canal, short or reduced, black clypeus, asci with wedged-shaped, J+ apical ring and oblong-inequilaterally ellipsoidal ascospores are considered as characteristics of this genus. In this study four previously described *Anthostomella* species are transferred to *Anthostomelloides*. Most of the *Anthostomelloides* species lack germ slits and even when present they are indistinct as in *An. krabiensis* and *An. proteae*. The separation of this genus from others is morphologically and phylogenetically well-supported.

Key to Anthostomelloides species

1.	a. Ascospores unicellular	2
	b. Ascospores bicellular	A. proteae
	2. a. Ascospores with mucilaginous sheath	
	b. Ascospores without mucilaginous sheath	4
3.	a. Ascospores with germ slitsb. Ascospores without germ slits	A. krabiensis A. forlìcesenica
	4. a. Ascomata with an intact clypeusb. Ascomata with a reduced clypeus	A. leucospermi

Anthostomelloides brabeji (S.J. Lee & Crous) Daranagama & K. D. Hyde, comb. nov.

Index Fungorum number: IF552373, Facesoffungi number: FoF 02527 Basionym: Anthostomella brabeji S.J. Lee & Crous, Mycol. Res. 107(3): 361 (2003)

Illustration and description: See Lee & Crous (2003)

Notes: According to the description provided (Lee & Crous 2003) *An. brabeji* is characterized by perithecioid, immersed ascomata in the host tissue. These ascomata developed under a clypeus visible as blackened, shiny, slightly raised dots with a central papillate, periphysate ostiolar canal and globose in cross section. The clypeus of *An. brabeji* is short and reduced, when compared to other species, but comprises both fungal and host tissues. Asci of *An. brabeji* contain wedged-shaped, amyloid apical rings and differ from other species in *Anthostomelloides* by having elongate ellipsoidal-fusiform, pale brown ascospores (Lee & Crous 2003).

Anthostomelloides forlicesenica (Daranagama, Camporesi & K.D. Hyde) Daranagama & K. D. Hyde, *comb. nov.*

Index Fungorum number: IF552374, Facesoffungi number: FoF 02528

Basionym: Anthostomella forlicesenica Daranagama, Camporesi & K.D. Hyde, Fungal Diversity: 73: 214 (2015)

Illustration and description: See Daranagama et al. (2015a), Fungal Diversity: 73: 214, Fig. 5.

Notes: Anthostomelloides forlicesenica was reported from Italy on *Spartium junceum* L. Due to the morphological similarity of this species to *An. krabiensis* in having immersed, globose ascomata, a central periphysate ostiolar canal and asci containing wedged-shaped apical rings (Daranagama *et al.* 2015a), this species is transferred to *Anthostomelloides*, a move also supported by the phylogenetic analysis. In this study *An. forlicesenica* clustered with the type species *An. krabiensis*. Similar

to *An. brabeji*, *An. forlicesenica* also has a reduced clypeus. *Anthostomelloides forlicesenica* lacks a visible germ slit, while a conspicuous mucilaginous sheath is present in *An. brabeji* (Daranagama *et al.* 2015a).

Anthostomelloides leucospermi (S.J. Lee & Crous) Daranagama & K. D. Hyde, comb. nov.

Index Fungorum number: IF552375, Facesoffungi number: FoF 02529

Basionym: Anthostomella leucospermi S.J. Lee & Crous, Mycol. Res. 107(3): 364 (2003)

Illustration and description: See Lee & Crous (2003) *Mycol. Res.* 107(3): 364. Fig. 2.

Notes: This species also exhibits a range of similar characters to the previously described species of *Anthostomelloides*, such as immersed, globose ascomata with a central periphysate, papillate ostiolar canal, asci with wedged-shaped apical ring and oblong-ellipsoidal ascospores. *Anthostomelloides leucospermi* is similar to *An. brabeji* as both lack ascospore germ-slits and gelatinous sheaths, but differs as the latter has more fusiform ascospores (Lee & Crous 2003). *Anthostomelloides leucospermi* has a more distinct clypeus than in *An. brabeji*, where the clypeus is reduced.

Anthostomelloides proteae (S.J. Lee & Crous) Daranagama & K. D. Hyde, comb. nov.

Index Fungorum number: IF552376, Facesoffungi number: FoF 02530 Basionym: Anthostomella proteae S.J. Lee & Crous, Mycol. Res. 107(3): 366 (2003)

Illustration and description: See Lee & Crous (2003), Mycol. Res. 107(3): 366, Fig. 3.

Notes: Anthostomelloides proteae was reported from South Africa on dead leaves of *Protea nitida* Mill. (Lee & Crous 2003). *Anthostomelloides proteae* also possess immersed and globose ascomata with a central periphysate ostiolar canal but it lacks a clypeus. According to the illustrations provided by Lee & Crous (2003) the clypeus is reduced and visible as slightly darkened area around the ostiole comprising subepidermal cells and intracellular fungal hyphae. The ascospores of *An. proteae* are inequilaterally ellipsoidal and bicellular with a small, hyaline, dwarf cell at one end (Lee & Crous 2003). Although Lee & Crous (2003) mentioned the presence of an indistinct germ slit in ascospore, it is not visible in the illustrations provided. However, *An. proteae* has a conspicuous mucilaginous sheath similar to *An. forlicesenica* and *An. krabiensis*.

Neoanthostomella D.Q. Dai & K.D. Hyde, *Fungal Divers*. 10.1007/s13225-016-0367-8 (2016)

Index Fungorum number: IF552041; Facesoffungi number: FoF 02004

Saprobic on dead bamboo culms and branches of Vitis vinifera. L. Sexual morph: Pseudostromata forming blackened, elliptical-irregular-shaped, raised areas, sometimes visible as pustules on the host surface. Ascomata immersed, gregarious, 2-5 growing together in a single pseudostroma, in cross section globose-subglobose, dark brown, coriaceous, with a central, periphysate, ostiolate neck. Clypeus black, sometimes elongate, margin indistinct, mixed with dark, thick-walled hyphae in epidermal and subepidermal cells. Peridium comprising several layers of compressed, brown to hyaline cells of textura angularis. Hamathecium comprising dense, long, septate paraphyses intermixed with asci. Asci 8-spored, unitunicate, cylindrical,

short pedicellate, without an apical ring. *Ascospores* uniseriate-overlapping uniseriate, equilaterally ellipsoidal, with pointed ends, unicellular, brown-olivaceous, guttulate, smooth-walled, with or without mucilaginous sheath, lacking a germ slit. **Asexual morph:** Hyphomycetous. *Conidiophores* macronematous, septate, densely branched in the upper part, hyaline, smooth, similar to nodulisporium-like branching pattern. *Conidiogenous cells* with an apical collarette, cylindrical, hyaline, smooth. *Conidia* hyaline, globose-ellipsoidal, slightly verruculose.

Type species: Neoanthostomella pseudostromatica D.Q. Dai & K.D. Hyde, *Fungal Divers.* 10.1007/s13225-016-0367-8 (2016)

Notes: Neoanthostomella was introduced by Dai *et al.* (2016) with a single species *N. pseudostromatica*, which has morphological affinities with *Anthostomella* by having cylindrical, 8-spored asci containing brown ascospores with mucilaginous sheath. However, in *N. pseudostromatica* the presence of multiple ascomata, which cluster together in groups of 2-5 within single pseudostroma differ it from *Anthostomella* (Lu & Hyde 2000; Daranagama *et al.* 2015a). According to the description provided in Dai *et al.* (2016) there is a straight germ slit in ascospores although this is not shown in the illustration. We have observed the holotype material (MFLU 15-1190) and noted that the ascospores lack a germ slit. Hence we amended the generic description and provided details of the asexual morph.

It is noteworthy to mention herein that our analyses of molecular phylogeny do reveal a close relationship between *Pseudoanthostomella* and *Neoanthostomella* (Clade C & D) and hence one may argue that these phenotypically similar genera could be treated as congeneric. Despite our limited taxon sampling here, we consider *Neoanthostomella* as evolutionary significant and maintain our new genus given that species are differentiated by strictly specific morphological characters (e.g absence of apical apparatus and presence of central periphysate ostiole) as compared to *Pseudoanthostomella*. Should future studies with an increasing diversity of *Neoanthostomella* species reveal an overlap of characters between those two genera, then the possibility of treating those two as congeneric cannot be excluded.

Neoanthostomella viticola Daranagama, Camporesi & K. D. Hyde, sp. nov. Figs 4, 5 Index Fungorum number: IF552248; Facesoffungi number: FoF 02392 Etymology: Species epithet refers to the host genus Vitis. Holotype: MFLU 15-0691

Saprobic on dead branch of Vitis vinifera. L. Sexual morph: Ascomata $160-203 \times 180-225 \ \mu m \ (\overline{x} = 186 \ \mu m \times 205 \ \mu m, n = 10)$, immersed, visible as black, raised, conical-irregular-shaped areas, coriaceous, clustered, rarely solitary, in cross section globose, with wide ostiolar neck. Ostiole 82-110 μ m diam. at the base \times 50-66 µm high ($\overline{x} = 102 \text{ µm} \times 64 \text{ µm}$, n = 10), grey, papillate, with a central periphysate ostiolar canal. Clypeus black, thick-walled, margin indistinct, mixed with dark, fungal hyphae in host cell layers. *Peridium* 34-53 μm wide ($\overline{x} = 41 \ \mu m$, n = 10), with two cell layers, outwardly comprising thick-walled, compressed, light brown cells of *textura irregularis* and inwardly comprising thick-walled, several layers of hyaline cells of *textura angularis*. Paraphyses 2.5-3.4 μm wide at base ($\overline{x} = 3.1 \ \mu m$, n = 30), slightly longer than the asci, numerous, filamentous, septate. Asci $85-117 \times$ 5-7 μm ($\bar{x} = 91.5 \times 6.7 \mu m$, n = 20), 8-spored, unitunicate, cylindrical, long pedicellate, lacking a visible apical ring, sometimes with apical thickenings. Ascospores 5.7-11 \times 3.4-4.8 μm ($\overline{x} = 9.2 \times 4.1 \ \mu m$, n = 20) uniseriate-overlapping uniseriate, ellipsoidal, with broad ends, light brown, smooth-walled, lacking a germ slit. Asexual morph: Hyphomycetous. Conidiophores $60-80 \times 5-8 \ \mu m$ ($\overline{x} = 76 \ \mu m$ \times 5.7 µm, n = 40), macronematous, septate, densely branched in the upper part,

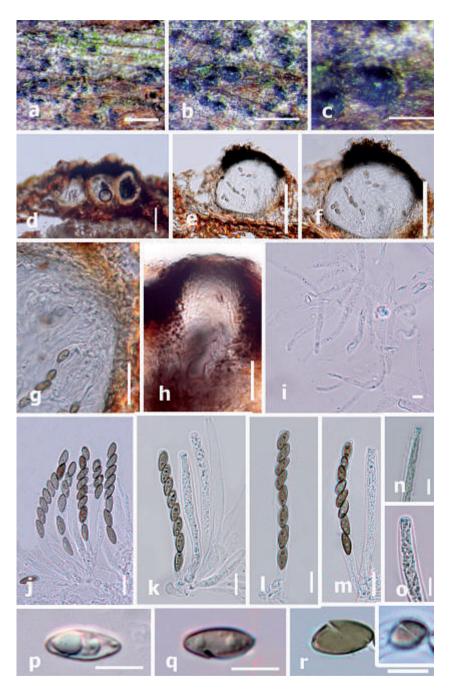


Fig. 4. *Neoanthostomella viticola* (MFLU 15-0691). **a**, **b**, **c**. Appearance of ascomata in host **d-f**. Cross section of ascomata **g**. Peridium **h**. Clypeus **i**. Paraphyses **j-m**. Asci **n**, **o**. Ascal apical apex in Melzer's reagent (Note: lacks an apical ring, apical thickenings visible) **p-r**. Ascospores. Scale bars: a, $c = 500 \mu m$, $b = 1000 \mu m$, d, $e = 100 \mu m$, $f = 30 \mu m$, $h-l = 10 \mu m$.

hyaline, smooth. *Conidiogenous cells* 9-12 × 3-6 μ m (\bar{x} = 10.5 μ m × 4.5 μ m, n = 40), phialidic, with an apical collarette, cylindrical, slightly wider at the base, hyaline, smooth. *Conidia* 5-8 × 4-6 μ m (\bar{x} = 6.5 μ m × 4.3 μ m, n = 40), hyaline, globose to ellipsoidal, slightly vertuculose.

Culture characteristics: Colonies on Difco OA at 25-27°C reaching the edge of 9 cm Petri-dish in 5 weeks, at first whitish, felty, azonate, with fluffy margins; reverse turning light yellow. Production of conidiophores sparse, after 3-4 weeks, occurring in the center of the colony.

Material examined: ITALY, Province of Forlì-Cesena, Trivella di Predappio, on dead branch of *Vitis vinifera* L. (Vitaceae), 31 December 2014, Erio Camporesi, IT 2326 (MFLU 15-0691, **holotype**), *ibid* (HKAS 95066, **isotype**), ex-type living cultures MFLUCC 16-0243, KUMCC.

Notes: Neoanthostomella viticola is similar to *Anthostomella variabilis* B.S. Lu & K.D. Hyde in characters such as its smooth-walled, light brown, ellipsoidal ascospores and cylindrical, slender asci. However, *A. variabilis* has deeply immersed

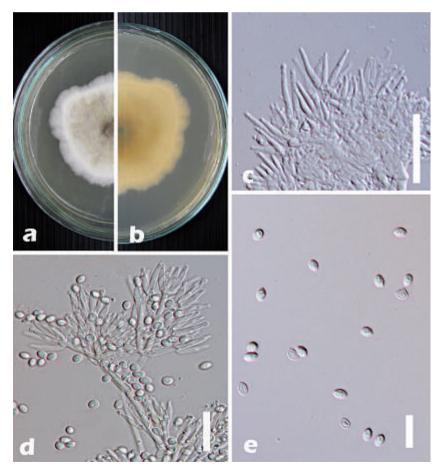


Fig. 5. Asexual morph of *Neoanthostomella viticola* (MFLUCC 16-0243). Culture on OA; **a.** Upper view **b.** Lower view **c.** Appearance of conidiophores on 3 weeks **d.** Conidiophores and conidiogenous cells **e.** Conidia. Scale bars: $c = 50 \mu m$, $d = 20 \mu m$, $e = 10 \mu m$.

ascomata in the host surface which results in a long, periphysate ostiolar canal (240-400 μm) as observed by Lu & Hyde (2000), which is unique to the species. In contrast, *Neoanthostomella viticola* has an ostiolar canal which is wider than its length. *Neoanthostomella viticola* is similar to *A. limitata* Sacc., *A. okatina* Whitton *et al.* and *A. helicofissa* Daranagama *et al.* However *N. viticola* differs because *A. limitata* has ascospores with a thin mucilaginous sheath and *A. okatina* has asci that lack an apical ring and circular papilla in the ascomata, asci without apical structures and rather small ascospores with a thin mucilaginous sheath (Daranagama *et al.* 2015a). *Neoanthostomella viticola* differs from *N. pseudostromatica* by having smaller, pale brown ascospores without a mucilaginous sheath. In addition, *N. viticola* produced a hyphomycete asexual morph in culture, while an asexual morph has not been determined in *N. pseudostromatica*.

Pseudoanthostomella Daranagama, Camporesi & K. D. Hyde, gen. nov.

Index Fungorum number: I IF552377; *Facesoffungi number:* FoF 02531 *Etymology:* Refers to the morphological similarity to *Anthostomella.*

Saprobic on dead leaves, branches, twigs and cones of Pinus. Sexual morph: Ascomata immersed to semi-immersed, developed beneath the clypeus, blackened, raised, conical-dome-shaped areas, coriaceous or carbonaceous, solitary, rarely aggregated, in cross section mostly subglobose, with reduced clypeus, usually lacking a periphysate ostiolar canal. *Clypeus* black, thick-walled, short, comprising dark fungal hyphae and host epidermal cells. *Peridium* with two cell layers; outwardly comprising thick-walled, carbonaceous, compressed, dark brown cells of *textura* irregularis and inwardly thin-walled, hyaline cells of *textura angularis*. Paraphyses less than 5 μm wide at base, shorter than the asci, numerous, filamentous, septate. Asci 8-spored, unitunicate, broadly cylindrical-clavate, short-pedicellate, apically rounded, with discoid-wedged shaped, J+ apical ring. Ascospores overlapping uniseriate, ellipsoidal, dark brown, smooth-walled, mostly with conspicuous mucilaginous sheath, germ slit on ventral side, straight, extending over the full length, rarely absent. Asexual morph: Hyphomycetous; Conidiophores erect, arising from hyphae, complex, septate, dichotomously branched, smooth, hyaline-light brown. Conidiogenous cells monoblastic, discrete, terminal on the branches, arising in clusters of 3-4, denticulate. Conidia hyaline, elongated ellipsoidal-fusiform, sometimes curved at apical end, aseptate, unicellular, smooth.

Type species: Pseudoanthostomella pini-nigrae Daranagama, Camporesi & K. D. Hyde.

Notes: Pseudoanthostomella is erected to accommodate five anthostomellalike species clustered together in a strongly supported monophyletic clade in the phylogenetic analysis. These species share common characters such as blackened, conical to dome shaped, semi-immersed to immersed ascomata, which mostly occur as solitary and rarely aggregated into small groups. The clypeus is usually reduced, but they form thick-walled, dark and short clypei without ostiolar necks. All *Pseudoanthostomella* species have a peridium comprising two layers of *textura irregularis* and *textura angularis*. All the species except *P. sepelibilis* possess ascospores with straight germ slits running the full length of the spore. *Pseudoanthostomella* has a strong morphological resemblance to the genus *Anthostomella* but the former does not have a central, periphysate ostiolar canal. The asci are more clavate than those of *Anthostomella* and many *Anthostomella* species have ascospores with sigmoid and curved germ slits rather than straight germ slits as found in *Pseudoanthostomella* species. *Pseudoanthostomella* differs from *Neoanthostomella* by having asci with J+ apical ring and ascospores with germ slits. It differs from *Alloanthostomella* by having unicellular, pigmented ascospores with germ slits. *Anthostomelloides* species always have immersed ascomata with a central periphysate ostiolar canal with more oblong-ellipsoidal ascospores which lack germ slits or sometimes have a mucilaginous sheath.

Key to Pseudoanthostomella species

1.	a. Ascomata immersed	2
	b. Ascomata semi-immersed	
	 a. Asci with J+ apical ring b. Asci without apical ring 	P. conorum P. senecionicola
3.	a. Ascospores with germ slitsb. Ascospores without germ slits	4 P. sepelibilis
	4. a. Germ slits less than the spore lengthb. Germ slits, full length of spore	

Pseudoanthostomella conorum (Fuckel) Daranagama, Camporesi & K. D. Hyde, comb. nov.

Index Fungorum number: IF552378; Facesoffungi number: FoF 02532

Basionym: Amphisphaeria conorum Fuckel, Jb. nassau. Ver. Naturk. 29-30: 20 (1875) [1877]

 \equiv Anthostomella conorum (Fuckel) Sacc., Syll. fung. (Abellini) 1: 283 (1882)

Illustration and Descriptions: See Lu & Hyde (2000), Lee & Crous (2003).

Notes: Similar to other *Pseudoanthostomella* species, *P. conorum* has black, conical ascomata, reduced, short clypeus, cylindrical-slightly clavate asci with J+ discoid apical ring and ellipsoidal, brown ascospores with straight, full length germ slit and surrounded by an even mucilaginous sheath (Lee & Crous 2003). However, *P. conorum* possesses a central periphysate ostiolar canal though it is not elongate to form an ostiolar neck (Lu & Hyde 2000). According to the description by Lu & Hyde (2000) ascomata in cross section are obpyriform and the other *Pseudoanthostomella* species have subglobose ascomata.

Pseudoanthostomella delitescens (De Not.) Daranagama, Camporesi & K. D. Hyde, comb. nov. Fig. 6

Index Fungorum number: IF552379; Facesoffungi number: FoF 02389

Basionym: Sphaeria delitescens De Not., Monogr. Tuberac. (Milano) 8: no. 9 (1846)

≡ Anthostomella delitescens (De Not.) Sacc., Michelia 1(no. 3): 328 (1878)

Saprobic on dead and land cones of Pinus nigra J.F. Arnold. Sexual morph: Ascomata 340-360 × 285-320 μ m ($\bar{x} = 355 \times 302 \mu$ m, n = 10), semi-immersed, visible as blackened, raised, conical areas, carbonaceous, solitary, rarely clustered, in cross section conical-subglobose, with reduced clypeus. Clypeus 44-55 × 55-73 μ m near to the neck ($\bar{x} = 51 \times 68 \mu$ m, n = 10), dark brown, comprising host cells and intracellular light brown fungal tissues. Peridium 18-30 μ m wide ($\bar{x} = 25 \mu$ m,

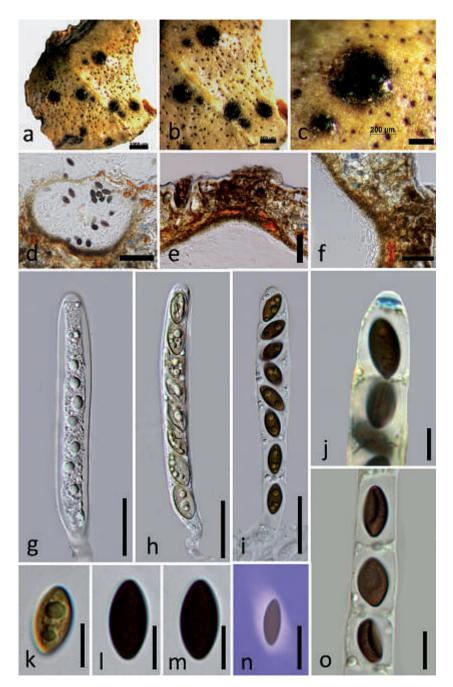


Fig 6. *Pseudoanthostomella delitescens* (MFLU 15-2660). **a, b.** Appearance of ascomata on host surface **c.** Single ascoma **d.** Cross section of ascoma **e.** Reduced clypeus **f.** Peridium **g, h.** Immature asci **i.** Mature ascus **j.** Discoid, J+ apical ring **k.** Immature ascospore **l, m.** Mature ascospores **n.** Sheath around ascospore in Indian ink **o.** Germ slit. Scale bars: $a = 1000 \ \mu\text{m}$, $b = 500 \ \mu\text{m}$, $c = 200 \ \mu\text{m}$, $d = 100 \ \mu\text{m}$, $e, f = 50 \ \mu\text{m}$, $g-i = 20 \ \mu\text{m}$, $j = 5 \ \mu\text{m}$, $k-o = 10 \ \mu\text{m}$.

n = 10), with two cell layers, outwardly comprising thick-walled, dark brown cells of *textura irregularis* and inwardly comprising thin-walled, loosely arranged, hyaline cells of *textura angularis*. Paraphyses 4-5 μm wide at base ($\bar{x} = 4.4 \ \mu m$, n = 30), shorter than the asci, numerous, filamentous, septate. Asci 120-145 × 10-15 μm ($\bar{x} = 132 \times 13.8 \ \mu m$, n = 20), 8-spored, unitunicate, cylindrical-clavate, short pedicellate, apically rounded, with a discoid, J+ apical ring, 1.5-2 μm high × 4-6 μm wide ($\bar{x} = 1.8 \times 5.4 \ \mu m$, n = 20), ascospores appears as if in compartments due to the presence of mucilaginous sheaths. Ascospores 14-17 × 8-10 μm ($\bar{x} = 15.6 \times 9.5 \ \mu m$, n = 20), uniseriate, broadly ellipsoidal, with pointed ends, dark brown, smooth-walled, with mucilaginous sheath visible in Indian ink, 2.9-3.1 μm thick ($\bar{x} = 3 \ \mu m$, n = 10), germ slit straight, slightly less than spore length. Asexual morph: Undetermined.

Material examined: ITALY, Province of Forli-Cesena, near Passo delle Forche – Galeata, on dead and land cone of *Pinus nigra* (Pinaceae), 16 June 2014, Erio Camporesi, IT 1937 (MFLU 15-2660), *ibid* (MFLU 15-3277, **reference specimen designated here**, HKAS 95062), living cultures MFLUCC 16-0477, KUMCC.

Culture characteristics: Colonies on Difco OA plates at 25-27°C reaching the edge of 9 cm Petri dish in 4 weeks, at first whitish, felty, azonate, with diffuse margins; reverse turning light yellow after 2-3 weeks.

Notes: Our fresh collection is morphologically similar to the holotype material of *P. delitescens* as described in Lu & Hyde (2000). The holotype was also collected from Italy in 1842 on *Erica umbellata* L. Morphological characters of *P. delitescens* described in Lu & Hyde (2000) such as semi-immersed, subglobose to conical ascomata, appearing as blackened dots, asci with a J+, discoid apical ring and brown ascospores, surrounded by a thin mucilaginous sheath are similar to those in our collection. However, the colour of ascomata in the holotype is recorded as yellowish brown and our collection has more dark brown to black ascomata which may be due to the host differences. *Pseudoanthostomella delitescens* is different from other *Pseudoanthostomella* species as it has ascospores with germ slits less than the spore length while others have germ slits running the full length of the ascospores.

Pseudoanthostomella pini-nigrae Daranagama, Camporesi & K. D. Hyde, sp. nov. Figs 7, 8

Index Fungorum number: IF552250; *Facesoffungi number:* FoF 02390 *Etymology:* Species epithet refers to the host plant *pinus nigra. Holotype:* MFLU 15-0652

Saprobic on dead and land cones of Pinus nigra J.F.Arnold. Sexual morph: Ascomata 250-275 × 330-350 µm ($\bar{x} = 263.5 µm × 342 µm$, n = 10), semi-immersed, visible as blackened, raised, dome-shaped areas, black, carbonaceous, solitary, in cross section globose-subglobose, with flattened top. Clypeus black, thick-walled, short, comprising dark fungal hyphae and host epidermal cells. Peridium 17-25 µm wide ($\bar{x} = 20.5 µm$, n = 10), with two cell layers, outwardly comprising thick-walled, carbonaceous, compressed, dark brown cells of textura irregularis and inwardly comprising thin-walled, hyaline cells of textura angularis. Paraphyses less than 5 µm wide at base ($\bar{x} = 4.5 µm$, n = 30), slightly shorter than the asci, numerous, filamentous, septate. Asci 90-120 × 11-13.5 µm ($\bar{x} = 106.4 × 12.3 µm$, n = 20), 8-spored, unitunicate, cylindrical-clavate, short pedicellate, sometimes reduced or absent, apically rounded, with a discoid-inverted hat-shaped, J+ apical ring, with thin lower ring, 1.5-2.5 µm high × 4.2-5 µm wide ($\bar{x} = 1.7 × 4.7 µm$, n = 20).

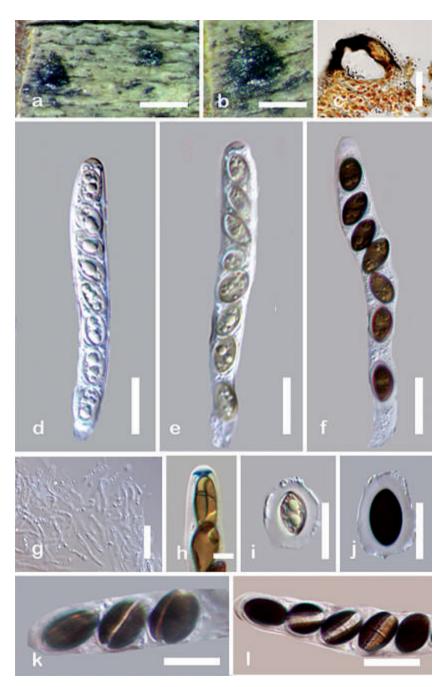


Fig. 7. *Pseudoanthostomella pini-nigrae* (MFLU 15-3274). **a**, **b**. Appearance of ascomata on host surface **c**. Cross section of ascoma **d-f**. Asci g. Paraphyses **h**. Apical ring in Melzer's reagent **i**, **j**. Ascospores **k**. Ascospore showing the germ slit **l**. Ascospores with a wide band. Scale bars: $a-b = 200 \ \mu m$, $c = 100 \ \mu m$, $g = 30 \ \mu m$, $h = 5 \ \mu m$, $d-f \ \& i-l = 20 \ \mu m$.

Ascospores 10-15 × 8.5-12 μm ($\bar{x} = 14.4 \times 10.3 \mu m$, n = 20), overlapping uniseriate, broadly ellipsoidal, dark brown-black, smooth-walled, with conspicuous mucilaginous sheath, 3-3.4 μm thick, germ slit on ventral side of the ascospore, straight, extending over the full length, sometimes visible as wide horizontal pallid band. Asexual **morph:** Hyphomycetous; *Conidiophores* more than 80 μm long and 2.5-3 μm wide ($\bar{x} = > 80 \times 2.7 \mu m$, n = 40), erect, arising from horizontal hyphae, complex, septate,

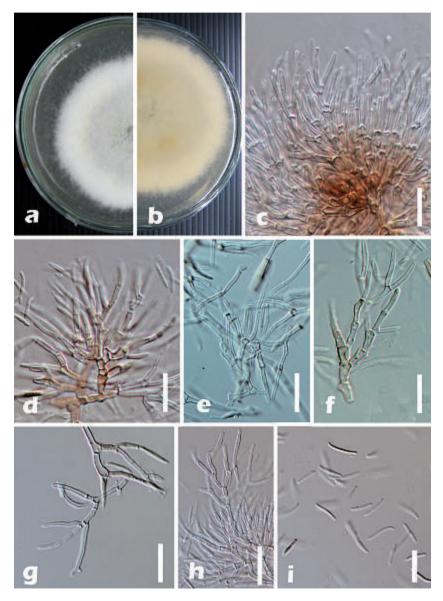


Fig. 8. Asexual morph of *Pseudoanthostomella pini-nigrae* (MFLUCC 16-0478). Culture on OA; a. Upper view b. Lower view c-g. Conidiophores h. Conidiogeneous cells i. Conidia. Scale bars: $c-h = 20 \mu m$, $i = 10 \mu m$.

dichotomously branched, smooth, upper region hyaline, lower region light brown. *Conidiogenous cells* 10-15 μm long and 2-3 μm diam ($\bar{x} = 13.5 \times 2.6 \ \mu m$, n = 40), monoblastic, discrete, terminal on the branches, arising in clusters of 3-4, denticulate. *Conidia* 6-8.5 × 2-3.5 μm ($\bar{x} = 7.3 \times 2.5 \ \mu m$, n = 40), hyaline, elongated ellipsoidalfusiform, sometimes curved at apical end, aseptate, smooth.

Culture characteristics: Colonies on Difco OA plates at 25-27°C reaching the edge of 9 cm Petri dish in 4 weeks, at first whitish, puffy, azonate, with diffuse margins, developing greyish oily spots in the center of the culture; reverse turning citrine after 2 weeks. Production of conidiophores rare, starting as greyish spots as the mycelium becomes melanized.

Material examined: ITALY, Province of Forli-Cesena, Montecoronaro – Verghereto, on dead and land cones of *Pinus nigra* (Pinaceae), 30 July 2014, Erio Camporesi, IT 2027 (MFLU 15-0652, **holotype**), *ibid* (MFLU 15-3274, HKAS 95065, **isotypes**), ex-type living cultures MFLUCC 16-0478, KUMCC.

Notes: Ascospores of *P. pini-nigrae* are similar to those of *A. nigroannulata* (Berk. & M.A. Curtis) Sacc. but in *P. pini-nigrae* they are smaller, ellipsoidal and surrounded with a wide mucilaginous sheath. In addition, the asci of *P. pini-nigrae* have a smaller, discoid apical ring with a thin lower ring, while in *A. nigroannulata* the apical ring is wedge-shaped (Lu & Hyde 2000) which also makes it different from other species of *Pseudoanthostomella*. Ascomata in *A. nigroannulata* are immersed, whereas *P. pini-nigrae* has semi-immersed ascomata appearing as conical areas on the host surface. *Anthostomella nigroannulata* has been recorded from *Daemonorops* (Arecaceae) and *Yucca* (Asparagaceae). *Pseudoanthostomella pini-nigrae* has only been recorded from *Pinus nigra* (Pinaceae).

Pseudoanthostomella senecionicola Daranagama, Camporesi & K. D. Hyde, sp. nov. Fig. 9

Index Fungorum number: IF552249; *Facesoffungi number:* FoF 02391 *Etymology:* Species epithet refers to the host plant *Senecio. Holotype:* MFLU 15-3276

Saprobic on dead stem of Senecio sp. Sexual morph: Ascomata 160-215 \times 206-240 µm (\overline{x} = 204 µm \times 232 µm, n = 10), immersed, visible as blackened, raised, dome-shaped areas, coriaceous, solitary, in cross section subglobose, with ostiole. Ostiole central, black, enclosed with carbonaceous tissues, lacking a periphysate ostiolar canal. Clypeus 40-48 \times 95-103 μm near the neck ($\overline{x} = 45 \times$ 98 μm , n = 10), black, comprising host cells and intracellular dark brown fungal tissues, carbonaceous tissues around. Peridium 18-20 μm wide ($\overline{x} = 20 \ \mu m$, n = 10), with two cell layers, outwardly comprising thick-walled, light brown cells of *textura* angularis and inwardly comprising thin-walled, hyaline cells of *textura irregularis*. *Paraphyses* 3-4 μm wide at base ($\overline{x} = 3.2 \ \mu m$, n = 30), shorter than the asci, numerous, filamentous, septate. Asci 70-98 × 14-17 μm ($\overline{x} = 88.2 \times 15 \mu m$, n = 20), 8-spored, unitunicate, cylindrical-clavate, short pedicellate, apically rounded, without apical ring. Ascospores 10-16 × 8-10 μm ($\bar{x} = 15 \times 9 \mu m$, n = 20), uniseriate, broadly ellipsoidal- ovoid, with one broad ends and one narrow end, dark brown, smoothwalled, germ slit straight, extending over the full length. Asexual morph: Undetermined.

Culture characteristics: Colonies on Difco OA plates at 25-27°C reaching the edge of 9 cm Petri dish in 4 weeks, at first whitish, puffy, azonate, with diffuse margins, developing greyish oily spots in the middle of the culture; reverse turning citrine (13) after 2 weeks. Production of conidiophores rare, starting as greyish spots as the mycelium becomes melanized.

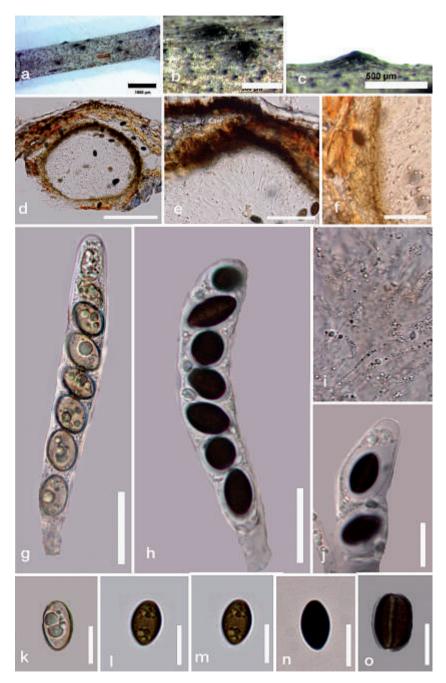


Fig. 9. *Pseudoanthostomella senecionicola* (MFLU 15-3276). **a.** Habitat **b.** Appearance of ascomata on host **c.** Appearance of ascomata on host in side view **d.** Cross section of an ascoma **e.** Reduced clypeus **f.** Peridium **g, h.** Asci in water **i.** Paraphyses **j.** Asci in Melzer's reagent lack of an apical ring **k-n.** Ascospores in water **o.** Ascospore with straight germ slit. Scale bars: a, b = 2000 μ m, c = 500 μ m, d = 100 μ m, e, f = 50 μ m, g-o = 10 μ m.

Material examined: ITALY, Province of Forli-Cesena, near Passo delle Forche – Galeata, on dead stem of *Senecio* sp. (Asteraceae), 25 June 2014, Erio Camporesi, IT 1959 (MFLU 15-3276, **holotype**), *ibid* (HKAS 95063, **isotype**) ex type living cultures MFLUCC 15-0013, KUMCC.

Notes: Pseudoanthostomella senecionicola is distinct from other *Pseudoanthostomella* species as the asci lack any visible apical ring. However, species such as *Anthostomella sphaeroidea* Speg. and *A. maderensis* Petr. also lack apical ring, but they are thought to be conspecific (Francis 1975, Lu & Hyde 2000). The lack of an apical ring is also characteristic for many species in the stromatic Xylariaceae, i.e *Hypoxylon notatum* Berk. & M.A. Curtis, *H. intermedium* (Schwein.: Fr.) Y.-M. Ju & J. D. Rogers (Ju and Rogers 1996). *Pseudoanthostomella senecionicola* has larger ascospores and unlike *A. sphaeroidea*, they lack a periphysate ostiolar canal. The latter has a very prominent central ostiolar canal (Lu & Hyde 2000). Considering the ascospore characters and shape, *P. senecionicola* is reminiscent to *A. vestita* Speg. Ascospores of *A. vestita* however, lack germ slits and have 2-3 μ m wide mucilaginous sheaths. In addition *A. vestita* has globose, larger ascomata with a central periphysate ostiolar canal and longer and narrower asci, with J+ apical ring (Lu & Hyde 2000).

Pseudoanthostomella sepelibilis [(Berk. & M.A. Curtis) Sacc.] Daranagama, Camporesi & K. D. Hyde, *comb. nov.*

Index Fungorum number: IF552380; Facesoffungi number: FoF 02533

Basionym: Sphaeria sepelibilis Berk. & M.A. Curtis, Grevillea 4(no. 32): 146 (1876)

 \equiv Anthostomella sepelibilis (Berk. & M.A. Curtis) Sacc., Syll. fung. (Abellini) 1: 281 (1882)

Illustration and Descriptions: See Lu & Hyde (2000)

Notes: Pseudoanthostomella sepelibilis has semi-immersed, brown, domeshaped and solitary ascomata, cylindrical-clavate asci with J+ apical ring and ellipsoidal ascospores with mucilaginous sheath (Lu & Hyde 2000), which justifies its position in the genus *Pseudoanthostomella*. The ascospores of *Pseudoanthostomella* lack germ slits and they are bicellular, with a large, brown cell and a small, hyaline dwarf cell and a mucilaginous sheath which is slightly thicker at both ends (Lu & Hyde 2000).

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