Phylogenetic analyses of a combined DNA data matrix containing ITS, LSU, rpb2 and tub2 sequences of representative Xylariales revealed that the genus *Barrmaelia* is a well-defined monophylum, as based on four of its described species (*B. macrospora*, *B. moravica*, *B. oxyacanthae*, *B. rhamnicola*) and the new species *B. rappazii*. The generic type of *Entosordaria, E. perfidiosa*, is revealed as the closest relative of *Barrmaelia*, being phylogenetically distant from the generic type of *Clypeosphaeria, C. mamillana*, which belongs to Xylariaceae sensu stricto. *Entosordaria* and *Barrmaelia* are highly supported and form a distinct lineage, which is recognised as the new family Barrmaeliaceae. The new species *E. quercina* is described. *Barrmaelia macrospora, B. moravica* and *B. rhamnicola* are epitypified and *E. perfidiosa* is lecto- and epitypified. Published sequences of *Anthostomella* and several *Anthostomella*-like species from the genera *Alloanthostomella, Anthostomelloides, Neoanthostomella, Pseudoanthostomella* and *Pyridoformiascoma* are evaluated, demonstrating the necessity of critical inspection of published sequence data before inclusion in phylogenies. Verified isolates of several species from these genera should be re-sequenced to affirm their phylogenetic affinities. In addition, the generic type of *Anthostomella* should be sequenced before additional generic rearrangements are proposed.

**Keywords** *Anthostoma* · Ascomycota · *Clypeosphaeria* · Phylogenetic analysis · Pyrenomycetes · Sordariomycetes · *Stereosphaeria* · Xylariaceae

**Introduction**

Xylariaceae have long been treated in a conservative, morphology-based concept, and only informal subgroupings like Hypoxyloideae and Xylarioideae were accepted despite polyphyly of several genera. Recently, Wendt et al. (2017) subdivided Xylariaceae into three families based on multigene phylogeny of an ITS–LSU–rpb2–tub2 matrix. They resurrected and emended the family Hypoxylaceae, widened the Graphostromataceae to include the genera *Biscogniauxia, Camillea, Obolarina* and *Vivantia*, and restricted Xylariaceae mostly to genera with geniculosporium-like asexual morphs. This facilitates phylogenetic placement of other genera affiliated with Xylariaceae sensu lato. One example is the genus *Anthostomella*, which houses a number of species, whose morphological traits vary considerably and may, thus, be phylogenetically uninformative. Ascomata are usually immersed in the host tissue, covered by a clypeus or not, have amyloid or...
sometimes non-amyloid ascus apices and brown amerosporous ascospores with or without a hyaline appendage cell, with or without a gelatinous sheath. One major challenge to study them on hosts other than palms is the difficulty to spot them, as they cannot be collected regularly, and, often, they produce very limited material. Francis (1975) performed a study on the systematics of *Anthostomella* species on the stems and leaves of herbaceous plants and gymnosperms based on morphology alone. A similar but more voluminous study was carried out by Lu and Hyde (2000). Using a few newly collected specimens, Daranagama et al. (2015, 2016) determined that *Anthostomella* is polyphyletic within Xylariaceae and described several new genera.

There is some confusion in the literature about the generic type of *Anthostomella*. Eriksson (1966) pointed out that lectotypification of *Anthostomella* with *A. phaeoestica* by Clements and Shear (1931) was in error and that *A. limitata* is the true generic type of *Anthostomella*. According to the ICN, this lectotypification is valid and has to be followed unless conservation with a different type is formally approved, and *A. limitata* is correctly listed as the generic type in Index Fungorum. In arguing that *A. limitata* does not exhibit several morphological characters then considered typical for the genus, Francis (1975) proposed *A. tomicoides* as the generic type, but this change has never been formally proposed and approved to become in effect. However, in the subsequent publications cited above, Francis (1975) was followed and *A. tomicoides* was accepted as the generic type. Neither *A. limitata* nor *A. tomicoides* have yet been sequenced.

Several genera have been segregated from *Anthostomella* or newly described, or subgenera were elevated to the generic rank. One of the latter is *Lopadostoma* (Jaklitsch et al. 2014) and another *Entosordaria*. The generic type of *Entosordaria*, *E. perfidiosa*, is characterised by non-amyloid asci and ascospores, which have a unique apical germ apparatus consisting of radiating slits (Eriksson 1966; Eriksson and Hawksworth 1986). Nonetheless, the genus was subsumed by Barr (1989) under *Chypeosphaeria* (see also Jaklitsch et al. 2016).

A transition to and now a member of the Diatrypaceae is the genus *Anthostoma*, which currently encompasses the single lignicolous species *A. decipiens* (Rappaz 1992; Jaklitsch et al. 2014). In a study designed to assess *Anthostomella* on hardwoods, Rappaz (1995) described the genus *Barrmaelia*, whose species, in part, also resemble Diatrypaceae, particularly in ascospore features, but, in contrast, have short-pedicellate asci and non-amyloid ascus apices. Furthermore, *Barrmaelia* species are typically characterised by ascomata that are immersed in the wood or bark and stromata that tend to blacken the host surface, in combination with light to dark brown, one-celled, smooth, ellipsoid to allantoid ascospores without sheath or appendages and with or without a germ slit. Rappaz (1995) combined six species in *Barrmaelia* (*B. macrospera*, *B. moravica*, *B. oxycanthae*, *B. picacea*, *B. pseudobombarda* and *B. sustenta*) and described one new species, which he also selected as the generic type, *B. rhannicola*. No new taxa have been added to this genus since then.

Although Rappaz (1995) only had morphology at hand, his concept withstands molecular phylogenetic analyses, as we show below. We, therefore, describe the new species *B. rappazii* to honour him, present the molecular systematics of five species of *Barrmaelia* and two of *Entosordaria*, including the new species *E. quercina*. The genera *Barrmaelia* and *Entosordaria* form a distinct lineage, which we name as the new family *Barrmaeliaceae*.

### Materials and methods

#### Isolates and specimens

All newly prepared isolates used in this study originated from ascospores of fresh specimens. The numbers of strains including NCBI GenBank accession numbers of gene sequences used to compute the phylogenetic trees are listed in Table 1. Isolates have been deposited at the Westerdijk Fungal Biodiversity Institute (CBS-KNAW), Utrecht, the Netherlands. Details of the specimens used for morphological investigations are listed in the Taxonomy section under the respective descriptions. Herbarium acronyms are according to Thiers (2017). Specimens have been deposited in the Fungarium of the Institute of Botany, University of Vienna (WU).

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

Cultures were prepared and maintained as described previously (Jaklitsch 2009). Microscopic observations were made in tap water, except where noted. Morphological analyses of microscopic characters were carried out as described earlier (Jaklitsch 2009). Methods of microscopy included stereomicroscopy using Nikon SMZ1500, Olympus SZX10 and Euromex Novex RZ 65.560, light microscopy using Euromex XHR MIC 625, Olympus BX51 and Nomarski differential interference contrast (DIC) using the compound microscopes Nikon Eclipse E600 and Zeiss Axio Imager.A1. Images and data were gathered with Nikon Coolpix 4500, Nikon DS-U2, Nikon D90, Olympus DP72 and Zeiss Axiocam 506 colour digital cameras and measured directly with the microscope, or with Olympus cellSens Dimension, NIS-Elements D v.3.0 and Zeiss ZEN Blue Edition softwares. Amyloidity of ascii was assessed using Lugol or Melzer reagent. Measurements are reported as maximum and minimum in parentheses and the range representing the mean plus and minus the standard deviation of a number of measurements given in parentheses.
Table 1  Isolates and accession numbers used in the phylogenetic analyses. Isolates/sequences in **bold** were isolated/sequenced in the present study. For details about sequence accessions retrieved from GenBank, see Jaklitsch and Voglmayr (2012), Jaklitsch et al. (2014, 2016), Danmagama et al. (2016), Hernández-Restrepo et al. (2016) and Wendt et al. (2017)

| Species | Specimen or strain number | Origin | Status | GenBank accession numbers |
|---------|--------------------------|--------|--------|--------------------------|
|         |                          |        | ITS    | LSU          | rpb2   | tub2 | tef1 |
| **Amphirosellinia fushanensis** | HAST 9111209 | Taiwan | HT     | GU339496     | N/A    | GQ48339 | GQ495950 |
| **Amphirosellinia nigrospora** | HAST 91092308 | Taiwan | HT     | GU322457     | N/A    | GQ48340 | GQ495951 |
| **Annulohypoxylon annulatum** | CBS 140775 | Texas | ET     | KY610418     | KY610418 | KY624263 | KX376353 |
| **Annulohypoxylon atroroseum** | ATCC 76081 | Thailand | HT     | AJ390397     | KY610422 | KY624233 | DQ480083 |
| **Annulohypoxylon michelianum** | CBS 119993 | Spain | ET     | KX376320     | KY610423 | KY624234 | KX271239 |
| **Annulohypoxylon moriforme** | CBS 123579 | Martinique | HT     | KX376321     | KY610425 | KY624289 | KX271261 |
| **Annulohypoxylon nitens** | MFLUCC 12-0823 | Thailand | HT     | KJ934991     | KJ934992 | KJ934994 | KJ934993 |
| **Annulohypoxylon stygium** | MFLUCC 54601 | French Guiana | HT     | KY610409     | KY610475 | KY624292 | KX271263 |
| **Anthostomella formosa** | MFLUCC 14-0170 | Italy | ET     | KP297403     | KP340544 | KP340531 | N/A |
| **Anthostomella helicofissa** | MFLUCC 14-0173 | Italy | HT     | KP297406     | KP297406 | KP340534 | KP406617 |
| **Anthostomella obesa** | MFLUCC 14-0171 | Italy | HT     | KP297405     | KP340546 | KP340533 | N/A |
| **Anthostomelloides praefites** | MFLUCC 14-0007 | Italy | HT     | KX533455     | KX533456 | KX789493 | KX789494 |
| **Barrmaelia macrospora** | BM = CBS 142768 | Austria | ET     | KY610419     | KY610419 | KY624277 | KX376352 |
| **Barrmaelia moravica** | Cr1 = CBS 142769 | Austria | ET     | MF488987     | MF488987 | MF489006 | MF488976 |
| **Barrmaelia oxyacanthae** | BO = CBS 142770 | Austria | ET     | MF488988     | MF488988 | MF489006 | MF488997 |
| **Barrmaelia rappazii** | Cr 2 = CBS 142771 | Norway | HT     | MF488989     | MF488989 | MF489006 | MF488998 |
| **Barrmaelia rubicola** | BR = CBS 142772 | France | ET     | MF488990     | MF488990 | MF489006 | MF488999 |
| **Biscogniauxia arima** | WSP 122 | Mexico | FT     | EC026150     | N/A    | GQ480473 | AY951672 |
| **Biscogniauxia avrocutata** | YMJ. 128 | USA | ET     | JK570799     | N/A    | JK507778 | AY951673 |
| **Biscogniauxia armata** | MFLUCC 12-0740 | France | ET     | KY610382     | KY610427 | KY624236 | KX271241 |
| **Biscogniauxia nummularia** | MFLUCC 51395 | France | ET     | KY610383     | KY610428 | KY624237 | KX271242 |
| **Biscogniauxia repanda** | ATCC 6506 | USA | ET     | KY610384     | KY610427 | KY624237 | KX271241 |
| **Camillea obularia** | ATCC 28893 | Puerto Rico | HT     | KY610385     | KY610429 | KY624238 | KX271243 |
| **Camillea tinctoria** | YMJ. 136 | Martinique | ET     | KY610386     | KY610430 | KY624239 | KX271243 |
| **Chapeosphaeria ornithohippica** | CLM = CBS 140735 | France | ET     | KT949897     | KT949897 | MF488991 | MF488990 |
| **Collocladus bambusae** | GZUH 0102 | China | FT     | KJ945279     | KJ945280 | KP276675 | KP276674 |
| **Collocladus fangshangensis** | GZUH 0109 | China | FT     | KJ945279     | KJ945280 | KP276675 | KP276674 |
| **Collocladus japonicus** | CBS 124266 | China | FT     | KY610411     | KY610468 | KY624265 | KX271258 |
| **Collocladus sasquatch** | ST.MA. 14087 | Argentina | FT     | AM749918     | KY610430 | KY624239 | KX271258 |
| **Daldinia andina** | CBS 146071 | Ecuador | HT     | AM749918     | KY610430 | KY624239 | KX271258 |
| **Daldinia bambusicola** | CBS 122872 | Thailand | HT     | KY610385     | KY610431 | KY624241 | AY951684 |
| **Daldinia caldariorum** | MFLUCC 49211 | France | ET     | AM749934     | AM749934 | KY624243 | KX271258 |
| **Daldinia concentrica** | CBS 113277 | Germany | HT     | AM749934     | AM749934 | KY624243 | KX271258 |
| **Daldinia demissii** | CBS 114741 | Australia | HT     | KY658477     | KY610435 | KY624244 | KX271258 |
| **Daldinia escholtzii** | MFLUCC 45435 | Benin | FT     | KY658484     | KY610437 | KY624246 | KX271258 |
| **Daldinia luculata** | CBS 146071 | UK | ET     | KY610385     | KY610431 | KY624241 | AY951684 |
| **Daldinia macaronica** | CBS 121040 | Spain | PT     | KY610398     | KY610477 | KY624294 | KX271258 |
| Species                  | Specimen or strain number | Origin      | Status | GenBank accession numbers |
|-------------------------|---------------------------|-------------|--------|--------------------------|
|                         |                           |             |        | ITS LSU rpb2 tub2 tef1    |
| Daldinia petriniae      | MUCL 49214                | Austria     | ET     | AM749937 KY610439 KY624248 KC977261 |
| Daldinia placentiformis | MUCL 47603                | Mexico      | ET     | AM749921 KY610440 KY624249 KC977278 |
| Daldinia pyren aria     | MUCL 53969                | France      | ET     | KY610413 KY610413 KY624274 KY624312 |
| Daldinia steglichii     | MUC 43512                 | Papua New Guinea | PT     | KY610399 KY610479 KY624250 KX271269 |
| Daldinia thiesiennis    | CBS 113044                | Argentina    | PT     | KY610388 KY610441 KY624251 KX271247 |
| Daldinia vernicosa      | CBS 119316                | Germany     | ET     | KY610395 KY610442 KY624252 KC977260 |
| Diatrype disciformis    | CBS 197.49                | Netherlands | N/A    | N/A DQ470964 DQ470915 N/A |
| Entoleuca mammata       | J.D.R. 100                | France       | ET     | GU300072 N/A GQ484782 GQ470230 |
| Entosordaria liquescens | ATCC 46302               | USA          | PT     | KY610389 KY610443 KY624253 KX271248 |
| Euepixylon sphaeriostomum | J.D.R. 261              | USA          | ET     | GU292821 N/A GQ484774 GQ470224 |
| Eutypa lata             | UCR-EL1                   | USA          | GJ     | GJ GJ GJ GJ |
| Graphostroma platys stomum | CBS 270.87           | France       | HT     | JX658535 DQ36906 KY624296 HG934108 |
| Hypocreodendron sanguineum | J.D.R. 169              | Mexico       | HT     | GU322433 N/A GQ484819 GQ487710 |
| Hypoxylon carneum       | MUC 54177                 | France       | HT     | KY610400 KY610480 KY624297 KX271270 |
| Hypoxylon cercidicola   | CBS 119009                | France       | HT     | K968908 KY610444 KY624254 KC977263 |
| Hypoxylon crocephium    | CBS 119004                | France       | HT     | K968907 KY610445 KY624255 KC977268 |
| Hypoxylon fendleri      | MUC 54792                 | French Guiana| ET     | K234421 KY10481 KY624298 KF300547 |
| Hypoxylon fragiforme    | MUC 51264                 | Germany      | ET     | KC77229 KM186295 KM186296 KX271282 |
| Hypoxylon fragiforme    | CBS 113049                | France       | HT     | KY610401 KY610482 KY624254 KX271270 |
| Hypoxylon griseobrunneum| CBS 331.73                | India        | HT     | KY610402 KY610483 KY624300 KC977303 |
| Hypoxylon haematostroma | MUC 53301                 | Martinique   | ET     | K968911 KY610484 KY624301 KC977291 |
| Hypoxylon howarmianum   | MUC 47599                 | Germany      | ET     | KY610433 KY624258 KC977277 |
| Hypoxylon hypomithium   | MUC 51845                 | Guadeloupe   | HT     | KY610403 KY610449 KY624302 KX271249 |
| Hypoxylon investiens    | CBS 118183                | Malaysia     | HT     | K968925 KY610450 KY624259 KC977270 |
| Hypoxylon lateripigmentum | MUC 53504               | Martinique   | HT     | K968933 KY610486 KY624304 KC977290 |
| Hypoxylon lenorandii    | CBS 19003                 | Ecuador      | HT     | K968943 KY610452 KY624261 KC977273 |
| Hypoxylon monticulosum  | MUC 54604                 | French Guiana| ET     | KY610404 KY610487 KY624305 KX271273 |
| Hypoxylon mucemum       | MUC 53765                 | Guadeloupe   | HT     | K968926 KY610488 KY624306 KC977280 |
| Hypoxylon ochraceum     | MUC 54625                 | Martinique   | HT     | K968937 N/A KY624271 KC977307 |
| Hypoxylon papillatum    | ATCC 58729                | USA          | HT     | K968919 KY610454 KY624223 KC977258 |
| Hypoxylon perforatum    | CBS 115281                | France       | HT     | KY610391 KY610455 KY624224 KX271250 |
| Hypoxylon petriniiae    | CBS 114746                | France       | HT     | KY610405 KY610491 KY624279 KX271274 |
| Hypoxylon piletterum    | ST.MA. 13455              | Martinique   | HT     | KY610412 KY610412 KY624308 KX264315 |
| Hypoxylon porphyreum    | CBS 119022                | France       | HT     | KC968921 KY610456 KY624225 KC977264 |
| Hypoxylon pulcicidum    | CBS 122622                | Martinique   | HT     | JX183075 KY610492 KY624280 JX183072 |
| Hypoxylon rubiginosum    | MUC 53309                 | Martinique   | HT     | K968932 KY610416 KY624281 KC977288 |
| Hypoxylon samuelsii     | MUC 52887                 | Germany      | HT     | K477232 KY610469 KY624266 KX243111 |
| Hypoxylon submonticolum | CBS 115280                | France       | HT     | K968923 KY610457 KY624226 KC977267 |
| Hypoxylon ticenense     | CBS 115271                | France       | HT     | JQ009317 KY610471 KY624272 AY951757 |
| Hypoxylon trugodes      | MUC 54794                 | Sri Lanka    | ET     | K234422 KY610493 KY624282 KF300548 |
| Hypoxylon vogesiacum    | CBS 115273                | France       | HT     | K968920 KY610417 KY624283 KX271275 |
| Species                        | Specimen or strain number | Origin          | Status | GenBank accession numbers |
|-------------------------------|---------------------------|-----------------|--------|--------------------------|
| Jackrogersella cohaerens      | CBS 119126                | Germany         |        | KY610396 KY610407        |
| Jackrogersella minutella      | CBS 119015                | Portugal        |        | KY610381 KY610424        |
| Jackrogersella multiformis    | CBS 119016                | Germany         |        | ET KC477234 KY610473     |
| Kretzschmaria deusta          | CBS 163.93                | Germany         |        | KY610458 KY610497        |
| Lopadostoma dryophilum        | CBS 133213                | Austria          |        | ET JC4774570 KY610516    |
| Lopadostoma turgidum          | CBS 133207                | Austria          |        | ET JC4774618 KY610517    |
| Microdochium lycopodinum      | CBS 122885                | Austria          |        | ET JC4774654 KY610518    |
| Microdochium phragmitis       | CBS 285.71                | Poland           |        | ET KC477237 KY610519     |
| Microdochium seminicola       | CBS 139951                | Switzerland      |        | ET KC477240 KY610520     |
| Nemania abortiva              | BISH 467                  | USA              |        | GU292816 N/A            |
| Nemania beaumontii            | HAST 405                  | Martinique       |        | GU292819 N/A            |
| Nemania bipapillata           | HAST 9009610              | Taiwan           |        | N/A N/A                 |
| Nemania maritima              | HAST 8908610              | Taiwan           |        | N/A N/A                 |
| Nemania primolutea            | HAST 9102010              | Taiwan           |        | N/A N/A                 |
| Obolarina dryophila           | MUCL 49882                | France           |        | GQ428316 GQ428316        |
| Podosordaria mexicana         | WSP 176                   | Mexico           |        | GU324762 N/A            |
| Poronia pileiformis           | CBS 656.78                | Australia        |        | HT GU324760 N/A         |
| Poronia punctata              | CBS 1256.78               | Australia        |        | HT GU324760 N/A         |
| Pseudoanthostomella delitescens| MFLUCC 16-0477           | Italy            |        | KX533451 KX533452       |
| Pseudoanthostomella pini-nigrae | MFLUCC 16-0477           | Italy            |        | KX533453 KX533454       |
| Pseudoanthostomella senecionicola | MFLUCC 16-0477          | Italy            |        | KX533455 KX533456       |
| Pyrenopolyporus hunteri       | MUCL 52673                | Ivory Coast      |        | KY610421 KY610422       |
| Pyrenopolyporus laminosus     | CBS 117339                | Martinique       |        | KY610423 KY610424       |
| Pyrenopolyporus nicaraguensis | CBS 151983                | Brazil           |        | KX590659 KX590660       |
| Pyrenopolyporus wingrei       | CBS 151984                | Brazil           |        | KX590661 KX590662       |
| Pyrenopolyporus zonatus       | CBS 151985                | Brazil           |        | KX590663 KX590664       |
| Pyrenopolyporus zonatus       | CBS 151986                | Brazil           |        | KX590665 KX590666       |
| Rostrohypoxylon terebratum    | CBS 119137                | Thailand         |        | KY610420 KY610421       |
| Rosellinia aquila             | MUCL 51703                | France           |        | KY610392 KY610460       |
| Rosellinia buxi               | J.D.R. 99                 | France           |        | GU300070 N/A            |
| Rosellinia corticium          | MUCL 51693                | France           |        | KY610393 KY610461       |
| Rosellinia necatrix           | CBS 349.36                | Argentina        |        | AY909001 KY610425       |
| Ruwenzoria pseudoannulata     | MUCL 51394                | Thailand         |        | KY610406 KY610407       |
| Sarcoxylon compuchamaticum    | CBS 5367                  | South Africa     |        | KY610410 KY610411       |
| Stilbohypoxylon elaeicola     | Y.M.J. 173                | French Guiana    |        | EF025151 EF025152       |
| Xylaria acuminatilongissima   | HAST 95060506             | Taiwan           |        | KY610462 KY610463       |
| Xylaria bambusicola           | WSP 205                   | Taiwan           |        | KY610464 KY610465       |
| Xylaria brunneovinosa         | CBS 349.36                | Thailand         |        | KY610466 KY610467       |
| Xylaria curta                 | CBS 119137                | Taiwan           |        | HT EF025151 EF025152    |
| Xylaria curta                 | CBS 119137                | Taiwan           |        | HT EF025151 EF025152    |
| Xylaria curta                 | CBS 119137                | Taiwan           |        | HT EF025151 EF025152    |
| Xylaria curta                 | CBS 119137                | Taiwan           |        | HT EF025151 EF025152    |
DNA extraction and sequencing methods

The extraction of genomic DNA was performed as reported previously (Voglmayr and Jaklitsch 2011; Jaklitsch et al. 2012) using the DNeasy Plant Mini Kit (QIAgen GmbH, Hilden, Germany). The following loci were amplified and sequenced: the complete internally transcribed spacer region (ITS1–5.8S–ITS2) and a ca. 1.3-kb fragment of the large subunit nuclear ribosomal DNA (nLSU rDNA), amplified and sequenced as a single fragment with primers V9G (de Hoog and Gerrits van den Ende 1998) and LR5 (Vilgalys and Hester 1990); a ca. 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 dRPB2-7r (Voglmayr et al. 2016a); a ca. 1.3–1.5-kb fragment of the translation elongation factor 1-alpha (tef1) gene with primers EF1-728F (Carbone and Kohn 1999) and TEF1LLErev (Jaklitsch et al. 2005) or EF1-2218R (Rehner and Buckley 2005); and a ca. 1.6-kb fragment of the beta-tubulin (tub2) gene with primers T1 and T22 (O’Donnell and Cigelnik 1997). PCR products were purified using an enzymatic PCR cleanup (Werle et al. 1994) as described in Voglmayr and Jaklitsch (2008). DNA was cycle-sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit v.3.1 (Applied Biosystems, Warrington, UK) with the same primers as in PCR; in addition, primers ITS4 (White et al. 1990), LR2R-A (Voglmayr et al. 2012) and LR3 (Vilgalys and Hester 1990) were used for the ITS–LSU region, TEF1_INTF (Jaklitsch 2009) and TEF1D_iR (5′ GTCTGGCCATCCTTGGAGAT 3′) for tef1 and BtHV2r (Voglmayr et al. 2016b, 2017) for tub2. Sequencing was performed on an automated DNA sequencer (3730xl Genetic Analyser, Applied Biosystems).

Analysis of sequence data

Following the phylogenetic placement of *Barrmaelia macrospora* within the Xylariaceae sensu lato clade in earlier analyses (Jaklitsch et al. 2014, 2016), sequences of *Barrmaelia* and *Entosordaria* were analysed within the combined ITS, LSU rDNA, rpb2 and tub2 matrix of Wendt et al. (2017). As only a few tef1 sequences are available for Xylariales, this marker was not included in the matrix but the sequences were deposited at GenBank as a secondary barcode marker. To obtain a more representative taxon sampling, selected sequences were added to this matrix from Hernández-Restrepo et al. (2016) and from Daranagama et al. (2015, 2016). From the latter two publications dealing with *Anthostomella*-like representatives, only accessions for which at least three of the four loci are available were included; before addition, it proved necessary to check these sequences with NCBI nucleotide BLAST searches for their correct gene and lineage identity, and obviously erroneous sequences as well as regions of poor sequence...
quality were excluded. For *Eutypa lata*, sequences were retrieved from the genome of strain UCR-EL1 deposited at JGI-DOE (http://genome.jgi.doe.gov/). Following the analyses of Jaklitsch et al. (2016), sequences of *Microdochium* (Microdochiaeae) were selected as the outgroup to root the trees. Familial classification of Xylariaceae sensu lato follows Wendt et al. (2017). All alignments were produced with the server version of MAFFT (http://www.ebi.ac.uk/Tools/msa/mafft), checked and refined using BioEdit version 7.0.9.0 (Hall 1999). After exclusion of ambiguously aligned regions and long gaps, the final matrix contained 4668 nucleotide characters, i.e. 600 from the ITS, 1359 from the LSU, 1162 from *rpb2* and 1547 from *tub2*.

Maximum parsimony (MP) analysis of the combined matrix was performed using a parsimony ratchet approach. For this, a nexus file was prepared using PRAP v.2.0b3 (Müller 2004), implementing 1000 ratchet replicates with 25% of randomly chosen positions upweighted to 2, which was then run with PAUP v.4.0a151 (Swofford 2002). The resulting best trees were then loaded in PAUP and subjected to heuristic search with TBR branch swapping (MULTREES option in effect, steepest descent option not in effect). Bootstrap analysis with 1000 replicates was performed using five rounds of replicates of heuristic search with random addition of sequences and subsequent TBR branch swapping (MULTREES option in effect, steepest descent option not in effect) during each bootstrap replicate. In all MP analyses, molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data; the COLLAPSE command was set to minbrlen.

Maximum likelihood (ML) analyses were performed with RAxML (Stamatakis 2006) as implemented in raxmlGUI 1.3 (Silvestro and Michalak 2012), using the ML + rapid bootstrap setting and the GTRGAMMA substitution model with 1000 bootstrap replicates. The matrix was partitioned for the individual gene regions, and substitution model parameters were calculated separately for them.

**Results**

**Assessment of published sequences**

NCBI Nucleotide BLAST searches revealed serious problems for some sequences of Daranagama et al. (2015), which were, therefore, excluded from the analyses (Table 1). LSU sequences KP340547 (*Anthostomella helicofissa*) and KP340538 (*Anthostomelloides forlicesenica*) were not added to the matrix, as they did not correspond to the LSU part (ca. 540 bp) included in the ITS sequences KP297406 and KP297396 of the same accessions. Whereas LSU sequence KP340547 was revealed as xylarialean by BLAST searches but differed in 60 positions (3 gaps and 57 substitutions) from the LSU part of KP297406, a BLAST search of KP340538 revealed various Pleosporales (*Kalmusia, Coniothyrium, Dendrothyrium*) as the closest match (84% sequence similarity). Therefore, for these two accessions, only the LSU part of the ITS sequences was included in the LSU matrix. *rpb2* sequence KP340524 (*Anthostomelloides forlicesenica*) was excluded as well, as a BLAST search also revealed pleosporalean affinities (80% similarity to sequence LK936413 of *Leptosphaerulina chartarum*, 77% similarity to sequences DQ677970 of *Phaeodothis winterti* and DQ677956 of *Coniothyrium palmarum*). *tub2* sequences KP406614 (*Anthostomella formosa*) and KP406616 (*Anthostomella obesa*) were also excluded, as BLAST searches actually revealed them as *rpb2* sequences. This was also confirmed in an alignment containing the *rpb2* sequences included in the present study, where they were highly similar to *rpb2* sequences of various *Anthostomella* species (not shown); however, both were different from the *rpb2* sequences KP340531 and KP340533 published for the same isolates in the same publication.

**Molecular phylogeny**

Of the 4668 nucleotide characters of the combined matrix, 2210 are parsimony informative (338 of ITS, 422 of LSU, 638 of *rpb2* and 812 of *tub2*). Figure 1 shows a simplified phylogram of the best ML tree (lnL = −136212.706) obtained by RAxML. Maximum parsimony analyses revealed four MP trees 32,311 steps long, which were identical except for a polytomy within the three terminal taxa of *Anthostomella* and an unresolved position of *Hypoxylon ochraceum* and *H. pilgerianum* relative to each other; the strict consensus tree of the four MP trees is provided in the Supplementary Information. The backbone of the MP trees was similar to the ML tree, except for a sister group relationship of Lopadostomataceae and Diatrypaceae and a slightly different position of the *Calceomycyes*–*Neoanthostomella* clade; in addition, there were a few minor topological differences within the Xylariaceae and Graphostomataceae.

All families received high to maximum support in all analyses, as did the Xylariaceae sensu lato (Fig. 1). The genera *Barrmaelia* and *Entosordaria* were revealed as the closest relatives with maximum support but formed a separate lineage within Xylariaceae sensu lato, and are classified here within the new family Barrmaeliaceae. Within the Xylariaceae sensu lato, the basal position of *Hypoxylaceae* was highly supported, but the phylogenetic relationships between the other three families (*Barrmaeliaceae*, Graphostomataceae and Xylariaceae sensu stricto) remain uncertain due to the lack of significant backbone support. *Clypeosphaeria mamillana* is revealed as the closest relative of *Anthostomelloides krabiensis* with high (99% MP BS) to maximum (ML) support, and both are sister clade to the rest of the Xylariaceae sensu stricto with high support as well (Fig. 1). The second species of *Anthostomelloides*, *A. forlicesenica*, is not closely related to *A. krabiensis* but sister species of *Brunneiperidium*...
Fig. 1 Simplified phylogram of the best ML trees (lnL = −136212.706) revealed by RAxML from an analysis of the combined ITS–LSU–rpb2-tub2 matrix of selected Xylariales. Strains in bold were sequenced in the current study. The Hypoxylaceae clade, which is not treated in detail, is collapsed to provide sufficient space for the other clades of interest. ML and MP bootstrap support above 50% are given at the first and second positions, respectively, above or below the branches. The arrows denote topological conflict with previous phylogenies (Anthostomelloides forticesenica) or major incongruence with the morphology of the clade in which it is placed (Pyriormiascoma trilobatum).

gracilentum within Xylariaceae sensu stricto with high (98% MP BS) to maximum (ML) support. The genera Anthostomella and Pseudoanthostomella are placed outside Xylariaceae sensu lato and form a highly supported lineage; sister group relationship to the highly supported Calceomyces–Neoanthostomella clade is revealed with medium support only in the ML analyses. Pyriormiascoma trilobatum is placed within Microdochium with maximum support in both analyses.

**Taxonomy**

**Barrmaeliaceae** Voglmayr & Jaklitsch, fam. nov.
MycoBank MB 822042
Type genus: *Barrmaelia* Rappaz.
Other genus in the family: *Entosordaria* Höhn.
Saprobioc on wood or bark. Stroma if present mostly in wood and blackening the surface in wide areas or in elongate bands, sometimes darker around the ostioles; entostroma
Barrmaelia macrospora (Nitschke) Rappaz, Mycol. Helv. 7(1): 130 (1995). 

_Basionym_ Valsa macrospora Nitschke, Pyrenomyc. Germ. 1: 145 (1867).

For synonyms, see Rappaz (1995).

Stromata blackening the wood surface in areas of up to 5 × 1.5 cm. Wood usually unchanged among ascomata, sometimes slightly pale brown. Ascomata perithecial, 400–600 μm diam., 300–500 μm high (n = 10), usually gregarious but separate, rarely two in contact, immersed, depressed globose to ellipsoid. Ostiolar apices inconspicuous, sometimes slightly raised, circular. Peridium 20–35 μm thick (n = 10), pseudoparenchymatous at the outer side and consisting of moderately thick-walled cells encrusted with brown material, tending to be pseudoparenchymatous at the outer side and consisting of moderately thick-walled cells encrusted with brown material, tending to be prosenchymatous, lighter coloured and thinner-walled at the inner side, partly filled with oil drops. Paraphyses numerous, filled with oil drops, 2–4 μm wide, slightly tapering towards the apex, obtuse. Asci 108–143 × 9–11 μm, spore part 91–123 μm long, stipe 5–21 μm long (n = 20), cylindrical, containing eight biseriate or obliquely uniseriate ascospores, with short stipe and an inamyloid apical apparatus. Ascospores (18.2–)20.5–24.0(–26.0) × (4.0–)4.8–5.9(–6.5) μm, l/w = (3.1–)3.7–4.7(–5.4) μm (n = 60), one-celled, narrowly ellipsoid to fusoid, asymmetric, ends sometimes slightly pointed, brown, germ slit hard to observe, with a lighter coloured band at the concave side, apically also sometimes lighter coloured, filled with minute oil drops, smooth.

Colonies on CMD and MEA white; aerial hyphae abundant. No asexual morph observed.

_Habitat_: In wood of (partly) decorticated twigs and branches of _Populus_ spp., also on _Ligustrum_ (fide Rappaz 1995).

_Distribution_: Europe (Czech Republic, France, Germany, Netherlands, Norway, Sweden, Switzerland, United Kingdom), possibly also the USA (fide Rappaz 1995).

_Typification_. Germany, Nordrhein-Westfalen, Münsterland, [Münster-] Handorf, on _Sarothamnus scoparius_, without date, Th. Nitschke, (B 70 0009297; sub Valsa macrospora, holotype, labelled as “Lectotype”). Epitype of _Valsa macrospora_, here designated: France, Côte-d’Or (21), Marcilly-sur-Tille, les Creux, on branch of _Populus_ aff. _nigra_, 2 Sep. 2012, A. Gardiennet A.G. 12107 (WU 36920; ex-epitype culture CBS 142768 = BM; MBT377828).

_Other material examined_: Germany, Nordrhein-Westfalen, Münsterland, [Münster-] Nienberge; on wood of _Populus_ sp. (originally given as _Quercus_). Dec. 1865, Th. Nitschke (B 70 0009349).

_Notes_: For synonyms, see Rappaz (1995). Concerning typification, Nitschke (1867) only cited material from Handorf on _Sarothamnus_ in his protologue. In their list of type specimens of Nitschke deposited in B, Gerhardt and Hein (1979) mention two envelopes mounted on a sheet without a place or date on the envelopes. However, the holotype B 70 0009297 now only contains a single envelope with an asexual morph with hyaline conidia, i.e. no sexual morph is present. Therefore, epitypification became necessary. Rappaz (1995) selected B 70 0009349 as the lectotype, but that material was not cited in the protologue. It is, however, authentic material of _Valsa macrospora_ (collected by Nitschke before publication), as both Nitschke and Rappaz considered it to be the fungus described in the protologue.

_Barrmaelia macrospora_ is usually easy to identify due to its large and relatively narrow ascospores with one lighter coloured side. The inconspicuous germ slit was best visible...
in B 70 0009349 (Fig. 2t). Cannon (2015) provides a description of a slightly deviating British collection with larger, occasionally one-septate ascospores measuring (23.5–26–29 × 7–8.5 μm), which may represent a distinct species.

**Barrmaelia moravica** (Petr.) Rappaz, Mycol. Helv. 7(1): 134 (1995). Fig. 3.

Basionym: *Eutypa moravica* Petr., Ann. Mycol. 25(3/4): 224 (1927).

Stromata immersed in bark, covered by the periderm except for the ostiolar openings; in areas lacking periderm visible as black spots of up to 6 mm diam., not discolouring the periderm but sometimes blackening the bast around the perithecium. Ascomata perithecial, 300–700 μm (n = 15) diam., 200–500 mm high (n = 10), usually crowded to gregarious, rarely solitary, globose, ellipsoid to pyriform, contents whitish when immature, brown when mature. Ostioles conspicuous, papillate, often elongate, ostiole pore rounded. Peridium 15–25 μm thick (n = 10), pseudoparenchymatous at the outer side and consisting of thick-walled dark brown cells, tending to be prosenchymatous, lighter coloured and thinner-walled at the inner side, partly filled with oil drops. Paraphyses numerous, 2–3.5 μm wide in the middle, filled with oil drops, slightly tapering towards the apex, obtuse. Ascii 98–130 × 8–9 μm, spore part 73–100 μm long, stipe 15–34 μm long (n = 20), cylindrical, containing eight obliquely uniseriate ascospores, with an inamyloid apical apparatus. Ascospores (11.5–12.3–14.2(−16.2) × (4.6–)5.3–6.3(−7.5) μm, l/w = (1.9–)2.1–2.5(−3.2) (n = 151), one-celled, ellipsoidal, slightly inequilaterally, with a straight germ slit of spore-length (sometimes slightly shorter), brown to dark brown, filled with several small oil drops in the pores, smooth.

Colonies on CMD and MEA white; aerial hyphae abundant. No asexual morph observed.

**Habitat:** In wood of twigs and branches of various hardwoods.

**Distribution:** Widespread (Africa, Asia, Europe and North America); for details, see Cannon and Minter (2013).

**Holotype:** France, place and date unknown, in branches of *Crataegus oxyacantha*, s. *Sphaeria lata var. corticalis*, L. Castagne (PC 0706585 ex herb. C. Montagne).

**Other material examined:** Austria, Steiermark, Deutschlandsberg, Koralpe, near the parking place of the walking path to the Grünanger- and Bärentalhütte; 15°00’52”E 46°49’37”N, on dead attached branches of *Salix cf. caprea*, 6 May 2016, G. Friebes (WU 36925: culture CBS 142770 = BO); Schadminger Tauern: Kleinsölk-Obertal, Schwarzensee, 1163 m, on *Salix* sp., 18 Sep. 1991, Ch. Scheuer 2897 (GZU 000317705), Germany, Sachsen, Königstein, on dead branches of *Salix purpurea*, Oct. 1880.
**Fig. 3** Barrmaelia moravica (a-c, f-o, v-y: WU 36924; d, e, p-u: W 1970-0024077, lectotype). a, d Ostioles protruding through the peridium. b Stroma beneath the peridium. c, e Peridium in transverse section. f, g Perithecium in vertical section. h, i Vertical section of perithecial wall. j-u Ascospores. v, w Paraphyses apices. x, y Asci. All in water. Scale bars: a, b, d = 500 μm; c = 250 μm; e = 300 μm; f = 200 μm; g = 100 μm; h, i, x, y = 10 μm; j-w = 5 μm

and Apr. 1881, W. Krieger (GZU 000317701; as Anthostoma schmidii); Schkeuditz, on dead branches of Fraxinus excelsior, spring 1874, G. Winter (GZU 000317700; as Anthostoma schmidii). Italy, Venetia, Treviso, Selva, on decorticated dead branches of Castanea vesca, autumn 1873, P.A. Saccardo (GZU 000317702; as Anthostoma schmidii). USA, South Dakota, Mellette, in a glacial valley, on branches of Fraxinus sp., Aug. 1950, F. Petrak (GZU 000317704; as Anthostoma melanotes); same data, 9 Aug. 1950, F. Petrak (GZU 000317703; as Anthostoma melanotes).

**Notes:** For synonyms, see Rappaz (1995). He found a libertella-like asexual morph in pure culture. Rappaz (1995) recognised three groups based on ascospore size within his broad concept of *B. oxyacanthae*. The first group with the smallest ascospores (“mean length between 12.5 and 13”) contains the type of *B. oxyacanthae* and agrees very well with GZU 000317702, whose mean length of 12.6 μm (n = 30) corresponds exactly with our measurements of the type collection. The sequenced collection WU 36925 has a mean length of 13.3 μm (n = 31) and, thus, appears to be an intermediate between the first and second group, the latter of which has a “mean length between 13.5–14”. The group with the longest ascospores (“between 14.5–15”) is said to mostly contain material on Salix. Of the three collections studied on this substrate, WU 36925 belongs to either the first or second group (see above), whereas GZU 000317705 falls in the second group (mean length 13.6 μm, n = 30) and GZU 000317701 best fits in the third group (mean length 14.3 μm, n = 30). GZU 000317700 does not contain mature ascomata. GZU 000317704 and GZU 000317703 from South Dakota (USA) have mean lengths of 16.2 and 19.6 (n = 30), respectively; thus, they likely represent different, probably undescribed, species.

*Barrmaelia oxyacanthae* differs from other *Barrmaelia* species in its relatively dark brown, ellipsoid ascospores with a well-visible germ slit. It is most similar to *B. pseudobombarda*, which has narrower ascospores (Rappaz 1995; Mathiassen et al. 2015). Cannon and Minter (2013) give a morphological description and illustrations of *B. oxyacanthae* and details on its ecology and distribution.

**Barrmaelia rappazii** Jaklitsch, Friebes & Voglmayr, sp. nov. Fig. 5.

Mycobank no.: MB 822043

**Etymology:** In honour of F. Rappaz, who established the genus *Barrmaelia*.

Stromata discolouring the wood surface grey to black in areas extending up to 6 × 0.6 cm; wood internally either nearly white between ascomata or darkened in patches. Ascomata perithecial, (450–)560–795–(900) μm (n = 14) diam., (420–)480–635–(660) μm (n = 9) high, sparsely distributed within the stromata and distantly spaced, immersed, depressed globose to ellipsoid. Ostioles forming minute, shiny black, rounded papillae above the wood surface. Peridium 20–45 μm thick (n = 7), pseudo- to prosenchymatous, cells moderately thick-walled and encrusted with brown material. Paraphyses up to 3.2 μm wide in the lower part, tapering, ca. 1 μm wide at the apex, filled with numerous oil drops when vital. Ascii 117–158 × 5.8–8.5 μm, spore part 95–136 μm long, stipe 11.5–29.5 μm long (n = 11), cylindrical, containing eight uniseriate ascospores, with an inamyloid apical apparatus. Ascospores (12.8–15.5–18.0–(19.5) × (2.8–3.0–3.5–(3.8) μm, l/w = (3.8–)4.5–5.7(6.5) (n = 39), one-celled, allantoid, brown, without germ slit, filled with few small oil drops, smooth.

Colonies on CMD and MEA white; aerial hyphae abundant. No asexual morph observed.

**Habitat:** In wood of twigs and branches of *Populus tremula*.

**Distribution:** Europe, only known from the type location in Norway.

**Holotype:** Norway, Stange, Hedmark, Rotlia Naturereservat, 7.5 km S Stange Kirke, on decorticated wood of *Populus tremula*, soc. *Platystomum compressum*, 30 Nov. 2015, P. Vetlesen PV-R221 (WU 36926; ex-holotype culture CBS 142771 = Cr2).

**Other material examined:** USA, North Dakota, Nylands Grove, on *Populus deltoides*, 29 Mar. 1914, J.F. Brenchle (W 1978-0018347, as *Anthostoma flavoviride*).

**Notes:** *Barrmaelia rappazii* can be recognised by its allantoid, brown and relatively narrow ascospores without a germ slit, and its black stromata with sparsely distributed ascomata. Morphological differences to the most similar species, *B. moravica*, are given there. *Barrmaelia rhamicola* is another species with allantoid ascospores without a germ slit but they contain larger oil drops and are somewhat longer and wider.

Rappaz (1995) mentions a collection (W 1978-0018347) similar to *B. moravica* but growing on *Populus* and having larger and darker ascospores, thus apparently resembling *B. rappazii*. However, the examination of this collection revealed it to be a different species with shorter and wider ascospores measuring (12.0–14.0–16.0(16.8) × (3.3–)3.7–4.5–(5.0) μm, l/w = (2.5–)3.2–4.3(4.9) (n = 30), as was already indicated by the congruent measurements given in Rappaz (1995). In the absence of sequence data, we currently refrain from describing it as a new species.
Notes: Barrmaelia rhamnicola is distinguished from other species of the genus by the often slightly curved, relatively large ascospores, which are filled with conspicuous oil drops and lack a germ slit. For comparison with the other allantoid-spored species without germ slit, see notes under B. moravica and B. rappazii. Rappaz (1995) observed a libertella-like asexual morph in pure cultures.

Entosordaria (Sacc.) Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1129: 167 (1920), emend.

Type species: Entosordaria perfidiosa (De Not.) Höhn.

Ascobata perithecial, scattered, immersed to erumpent, depressed globose to ellipsoid, circular in transverse section. Peridium brown. Hamathecium of apically free, thin, sparsely branched paraphyses. Asci unistriate, cylindrical, with uniseriate ascospores; apex inamyloid without distinct ring or amyloid with a discoid ring. Ascospores two-celled with septum near one end, the small cell hyaline, the large cell dark brown and with an apical germ apparatus consisting of radial slits. Asexual morph unknown.

Notes: Entosordaria was first described as a subgenus of Anthostomella (Saccardo 1882) and raised to the generic rank by Hönnel (1920), with E. perfidiosa as the generic type. Eriksson (1966) outlined the fundamental morphological differences from Anthostomella, i.e. inamyloid asci and dorsiventrally flattened ascospores with an apical germ apparatus consisting of radiating slits. He confined Entosordaria to the generic type and removed the genus from the Xylariaceae. Later, he (in Eriksson and Hawksworth 1986) argued that Stereosphaeria is the valid generic name to be used, considering Entosordaria (Sacc.) Höhn. to be a younger heterotypic homonym of Entosordaria Spec. However, Entosordaria Spec. has not been validly described according to ICN Art. 38.1, as Specazzini (1910) neither provided a diagnosis nor a reference to a previous valid description. Therefore, Entosordaria (Sacc.) Höhn. remains available and, based on priority, is the generic name to be used.

Barr (1989) classified E. perfidiosa in Clypeosphaeria, based on similarities of their ascospores, apical ascus apparatus, ascomata, clypeu and peridium structure. However, molecular phylogenies do not support a close relationship, as the generic type, Clypeosphaeria mamillana, is placed in Xylariaceae s. str. with high support (Fig. 1).

With the addition of the closely related E. quercina, the genus Entosordaria also includes a species with an amyloid ascus ring, which shows that this character is not suitable for generic classification within Xylariales.

Entosordaria perfidiosa (De Not.) Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1129: 166 (1920). Fig. 7.
For synonyms, see Barr (1989).

Ascomata perithecial, scattered, solitary or in groups, partly immersed to erumpent, depressed globose to ellipsoid, circular in transverse section, 400–800 μm diam., with a distinct central apical papilla 120–200 μm wide. Peridium 20–40 μm wide, brown, pseudoparenchymatous, of dark brown isodiametric to elongate cells 2–12 μm diam. Paraphyses numerous, sparsely branched, 1–2 μm wide. Ascii in 3% KOH (185–)200–220(–225) × (11–)12–14(–15) μm (n = 27), uniseriate, cylindrical, with a short stipe, with eight (partly obliquely) uniseriate ascospores, with an amyloid apical apparatus, no apical ring seen. Ascospores (20.5–)21.8–25.8(–29.5) × (8.7–)10.0–11.2(–12.0) μm, l/w = (1.8–)2.1–2.5(–2.7) (n = 71), ellipsoid, with subacute to rounded ends, two-celled with septum near one end, the small cell hyaline, the large cell dark brown at maturity and with an apical germ apparatus consisting of radial slits, multiguttulate when young, at maturity often with a large and several small guttules.

Colonies on CMD and MEA white; aerial hyphae abundant. No asexual morph observed.

Habitat: In bark of old trunks of living Acer pseudoplatanus.

Distribution: Europe.

Typification. Lectotype of Sordaria perfidiosa, here designated: Italy, Riva, Corteccia dell’Acer pseudoplatanus, 30. Oct. 1863, Ab. Carestia, no. 413 (RO; MBT377831). Syntype: Riva, Sulla corteccia dell’Acer pseudoplatanus, 22 Apr. 1858, Ab. Carestia, no. 222 (RO). Epitype of Sordaria perfidiosa, here designated: Austria, Kärnten, St. Margareten im Rosental, at Brici (“Writze”), on bark of Acer pseudoplatanus, 10 Apr. 2016, H. Voglmayr & W. Jaklitsch (WU 35981; ex-epitype culture CBS 142773 = EPE; MBT377832).

Other material examined: Germany, Baden-Württemberg, Hornberg, on bark of Acer pseudoplatanus; Dec. 2015, B. Wergen (WU 35982; culture BW3). France, Hautes-Alpes (05), Vallouise-Pelvoux, Ailefroide, on bark of living trunk of Acer pseudoplatanus, soc. Decaisneilla mesascium, 28 Jul. 2017, A. Gardiennet A.G. 17056.

Notes: Entosordaria perfidiosa is well characterised by the ascospores with an apical germ apparatus consisting of radiating slits in combination with inamyloid asci and its growth on bark of old Acer pseudoplatanus trees. It has been classified in Cyphosphaeria by Barr (1989); however, it is only distantly related with C. mamillana, the generic type (see Fig. 1). For comparison with E. quercina, see below.

Two syntypes of Sphaeria perfidiosa are present at RO, which were studied in detail by O. Eriksson (see Eriksson and Hawksworth 1986), but he did not select a lectotype. Type specimens at RO are no longer sent out for study, but detailed photographic documentation of the two syntypes was generously provided by Mrs. A. Millozza (pers. comm.). Based on the abundance of ascomata, we select no. 413 as the lectotype. For nomenclatural stability, a recent Austrian collection for which a culture and DNA sequences are available is designated as the epitype.

Entosordaria quercina

Voglmayr & Jaklitsch, sp. nov. Fig. 8.

MycoBank no.: MB 822044

Etymology: Referring to the host genus Quercus.

Ascomata perithecial, scattered, solitary, immersed below bark and raising it, depressed globose to ellipsoid, circular in transverse section, 400–800 μm diam., without an apical papilla, ostiole not to slightly protruding above cortex. Peridium 20–30 μm wide, brown, pseudoparenchymatous, of dark brown isodiametric to elongate cells 3–11 μm diam. Paraphyses numerous, sparsely branched, 1.5–2.5 μm wide. Asci in 3% KOH (258–)270–293(–310) × (17–)18.5–21.5(–22) μm (n = 17), uniseriate, cylindrical, with a short stipe, with eight (partly obliquely) uniseriate ascospores, ascus apex containing a discoid amyloid apical ring 5.3–6.8 × 1.3–1.8 μm (n = 15; in 3% KOH + Lugol). Ascospores (31–)34–38(–40) × 12–13.5(–16) μm, l/w = (2.4–)2.7–3.0(–3.2) (n = 50), ellipsoid to allantoid, two-celled with septum near one end, the small cell hyaline, the large cell dark brown at maturity and with an apical germ apparatus consisting of radial slits, multiguttulate when young, at maturity often with a large and several small guttules.

Colonies on CMD white, on MEA a reddish and yellowish pigment developing; aerial hyphae abundant. No asexual morph observed.

Habitat: In bark of dead twigs of Quercus coccifera.

Distribution: Only known from the type locality in Crete (Greece).

Holotype: Greece, Crete, Chania, Omalos, 920 m s.m., 35.37° N, 23.897° E, in bark of Quercus coccifera, 5 June 2015, H. Voglmayr & W. Jaklitsch (WU 35983; ex-holotype culture CBS 142774 = RQ).

Notes: Ascospore morphology of Entosordaria quercina fits E. perfidiosa, from which it differs in an amyloid ascus ring, much larger ascospores and asci, immersed ascomata without a distinct apical papilla and the host. In addition, the ascospores of E. quercina are commonly allantoid. The radiating slits of the apical germ apparatus are shorter than in E. perfidiosa; thus, they are less distinct.
Fig. 6 Barrmaelia rhamnicola (a, c, e, g–l, s–u: WU 36927; d, m–o: WU 36928, epitype; b, f, p–r: F. Rappaz no 890611–2, LAU, holotype). a, b Ostioles protruding through the blackened wood surface. c Perithecia in transverse section. d–f Perithecia in vertical section. g Vertical section of perithecial wall. h Paraphyses apices. i–r Ascospores. s–u Asci (with paraphyses in u). All in water. Scale bars: a = 500 μm; b = 1 mm; c = 250 μm; d = 150 μm; e = 200 μm; f = 300 μm; g, h, s–u = 10 μm; i–r = 5 μm.
Fig. 7  Entosordaria perfidiosa (a–n, p–v: WU 35981, epitype; o, w–a1: WU 35982).  
a–c Erumpent ascomata with apical papilla in face view (two fused ascomata in c).  
d Two ascomata in transverse section.  
e Ascoma with apical papilla in side view.  
f Ascoma in vertical section.  
g Transverse section of perithecial wall (in 3% KOH).  
h–j Asci (in 3% KOH; with paraphyses in h).  
k, l Asci apices (in 3% KOH + Lugol).  
m–a1 Ascospores (m–p immature; v in 3% KOH); the arrows denote radial slits of the apical germ apparatus (v, a1).  
All in water, except where noted.  
Scale bars:  
a = 1 mm;  
b–f = 200 μm;  
g, k–a1 = 10 μm;  
h–j = 20 μm.
Fig. 8 Entosordaria quercina, holotype (WU 35983). a, b Ascomata immersed in bark in face view. c Ascoma in transverse section. d Ascoma in vertical section. e–h Asci (with paraphyses in e, f). i–l Ascus apices (j in Lugol; k, l in 3% KOH + Lugol; note the gelatinous sheath surrounding the ascospores, the arrows in k and l denote the radial slits of the apical germ apparatus of the ascospore). m Transverse section of perithecial wall. n–y Ascospores; the arrows denote the short radial slits of the apical germ apparatus. All in 3% KOH, except where noted. Scale bars: a = 500 μm; b–d = 200 μm; e–h = 20 μm; i–y = 10 μm.
Discussion

Phylogenetic relationships and familial classification within Xylariaceae sensu lato

Our phylogenetic analyses are fully concordant with Wendt et al. (2017) in revealing Hypoxylaceae, Graphostomataceae and Xylariaceae sensu stricto as highly supported distinct lineages within the former Xylariaceae sensu lato, with Hypoxylaceae being placed basal to the rest (Fig. 1). The highly supported Barrmaelia–Entosordaria clade is also contained within the highly supported Xylariaceae sensu lato, but not affiliated with any of these families; a sister group relationship to Xylariaceae sensu stricto receives only moderate support (71%) in ML analyses and is unsupported in the MP analyses. Therefore, to be consistent with the new familial classification of Wendt et al. (2017), the Barrmaelia–Entosordaria clade is classified here within the new family Barmaeliaceae.

Our data also demonstrate that the genus Entosordaria is phylogenetically distinct from Clypeosphaeria, disproving the generic concept of Barr (1989). In the phylogenetic analyses of Jaklitsch et al. (2016) based on ITS–LSU sequence data, the generic type Clypeosphaeria mamillana was contained within Xylariaceae sensu lato, but its closest relatives remained unclear due to the lack of internal backbone support. In our multigene analyses, the phylogenetic position of Clypeosphaeria mamillana is now resolved to belong to Xylariaceae sensu stricto, where it forms a highly supported basal clade together with Anthostomelloides krabiensis, the generic type of Anthostomelloides (Fig. 1). Although both species differ in their ascospore characters, they share a similar wedge-shaped amyloid apical apparatus (Jaklitsch et al. 2016; Tibpromma et al. 2017). The second species of Anthostomelloides included in our analyses, A. forlicesenica, is not revealed as being closely related to the generic type, but as a sister species to Brunneiperidium gracilentum with high to maximum support (Fig. 1), with which it shares a discoid amyloid apical apparatus. This discrepancy in phylogenetic placement compared to Daranagama et al. (2016) may be caused by their obviously erroneous LSU and rpb2 sequences (see above), which were excluded from our analyses. This has been confirmed by an MP analysis of the matrix including the erroneous (pleosporalean) rpb2 and LSU sequences of A. forlicesenica, which result in an unsupported phylogenetic position of the latter as sister to the A. krabiensis–Clypeosphaeria clade (not shown).

As, apart from the commonly sequenced ITS–LSU rDNA, few sequence data are available for most lineages of Xylariales, the phylogenetic position of many taxa of putative xylariaceous affinities remains unresolved (Wendt et al. 2017). Whereas the ITS–LSU sequences are useful for barcoding purposes, molecular phylogenies solely based on these markers commonly do not provide sufficient phylogenetic resolution, and backbone support of many deeper nodes is often low (e.g. Jaklitsch and Voglmayr 2012; Jaklitsch et al. 2014, 2016). Considering the substantial increase of phylogenetic resolution observed in the multigene analyses of Wendt et al. (2017) and the current study (Fig. 1), rpb2 and tub2 should be included as standard markers in future phylogenetic studies of Xylariales, in addition to the usually sequenced ITS–LSU rDNA.

Anthostomella and Anthostomella-like genera

Recently, several investigations were published on Anthostomella (Daranagama et al. 2015, 2016; Tibpromma et al. 2017). In these publications, the genus Anthostomella was recognised to be polyphyletic, and several new genera and species were established.

Due to the lack of sequence data for rpb2 and tub2, only a subset of these taxa could be incorporated in our analyses. However, for most new Anthostomella-like genera, at least the generic type could be included, and we believe that some of the results are conclusive and should encourage more detailed studies and a critical evaluation of the published data. There are some topological differences between our analyses and those of Daranagama et al. (2015, 2016), which may be caused by the inclusion of some obviously erroneous sequences in the latter (see the Results section above). In our analyses, Pseudoanthostomella and Anthostomella in the sense of Daranagama et al. (2016) were united in a highly supported clade clearly placed outside Xylariaceae sensu lato (Fig. 1), whereas in Daranagama et al. (2016), Pseudoanthostomella and Anthostomella formed separate clades (clades A and C in their fig. 2). However, they only included members of Xylariaceae sensu lato in their analyses, with a single distantly related sordariomycete, Sordaria fimicola, as the outgroup, and internal support of the tree backbone relevant for the topology of Anthostomella-like fungi was low or absent. In our ML analysis, the Pseudoanthostomella–Anthostomella clade is the sister group of a highly supported clade containing Neoanthostomella viticola and Calceomyces lacunosus, the latter representing a genus of uncertain affinities within Xylariales (Wendt et al. 2017). The monotypic genus Alloanthostomella, introduced by Daranagama et al. (2016) for Anthostomella rubicola, is not supported in our analyses, as it is placed within the highly supported Anthostomella clade (Fig. 1), a position which was also revealed in Daranagama et al. (2015).

Our phylogenies suggest that the Pseudoanthostomella–Anthostomella clade may represent a distinct family (Fig. 1). However, we refrain from formally establishing a new family because the generic type, Anthostomella limitata, has not been sequenced, and it is, as yet, unclear whether Anthostomella in the sense of Daranagama et al. (2015, 2016) phylogenetically includes the generic type. Therefore, the correct application and circumscription of Anthostomella remains uncertain until sequences of the generic type become available.
In our molecular phylogenetic analyses, the Anthostomella-like Pyriformiascoma trilobatum is placed within Microdochium with maximum support (Fig. 1). The sexual morphs of Microdochium have thin-walled, hyaline to pale brown, fusoid ascospores with commonly variable but more or less regular septation and asci with a distinct funnel-shaped amyloid apical ascus apparatus (Parkinson et al. 1981; Jaklitsch and Voglmayr 2012; Hernández-Restrepo et al. 2016). Pyriformiascoma trilobatum differs substantially from all known sexual morphs of Microdochium by two-celled, inequilateral, oblong-ellipsoid ascospores consisting of a large olivaceous-brown cell and a hyaline dwarf cell and by an indistinct inamyloid apical ascus apparatus (Daranagama et al. 2015). Considering that Microdochium is morphologically homogeneous, it is unlikely that Pyriformiascoma belongs there, and the sequences of the latter may, rather, originate from a Microdochium contaminant. The “conidia” illustrated for P. trilobatum in Daranagama et al. (2015) recall unicellular terminal chlamydomosporas which are known from Microdochium species (Hernández-Restrepo et al. 2016). Pyriformiascoma trilobatum should be re-sequenced from verified cultures to ascertain its phylogenetic position.

An evaluation of published sequences reveals that sequence data quality should be critically checked by BLAST searches and detailed inspection of alignments before inclusion into phylogenetic analyses. An indicator for problems in the sequence data used for phylogenetic analyses are exceptionally long branch lengths in phylogenograms like, for example, those seen there, and the sequences of the latter may, rather, originate from other sequences which have been identified and removed from our matrix. These errors may cast general doubts on the accuracy of the sequences published for these species, and all markers should be re-sequenced from verified material to corroborate their phylogenetic affinities.

Acknowledgements Open access funding provided by Austrian Science Fund (FWF). We thank Rosella Marcucci at PAD for access to the fungarium and support, Anna Millozza at RO for providing detailed documentation and photographs of the syntypes of Sphaeria perfidiosa, the herbarium curators of B, LAU, PC and W for sending specimens and Christian Scheurer at GZU for managing collections. Per Vetlesen, Jacques Foumier and Björn Wergen are gratefully acknowledged for providing fresh specimens. Financial support by the Austrian Science Fund (FWF; project P27645-B16) to HV is gratefully acknowledged.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

Barr ME (1989) Clypeosphaeria and the Clypeosphaeriaceae. Syst Ascomycet 8:1–8

Cannon PF (2015) Barrmaelia macrospora. Fungi of Great Britain and Ireland. Available online at: http://fungi.myspecies.info/taxonomy/term/6795/descriptions

Cannon PF, Minter DW (2013) Barrmaelia oxyacanthae. IMI Descriptions of Fungi and Bacteria. Set 195 No. 1941. 4 pp. CABI, Egham, Surrey, UK

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553–556

Clements FE, Shear CL (1931) The genera of fungi. H.W. Wilson, New York

Daranagama DA, Camporesi E, Tian Q, Liu XZ, Chamyuang S, Stadler M, Hyde KD (2015) Anthostomella is polyphyyletic comprising several genera in Xylariaceae. Fungal Divers 73:203–238

Daranagama DA, Camporesi E, Jeewon R, Liu ZX, Stadler M, Lumyong S, Hyde KD (2016) Taxonomic rearrangement of Anthostomella (Xylariaceae) based on a multigene phylogeny and morphology. Cryptogam Mycol 37:509–538

de Hoog GS, Gerrits van den Ende AHG (1998) Molecular diagnostics of clinical strains of filamentous basidiomycetes. Mycoses 41:183–189

Eriksson O (1966) On Anthostomella Sacc., Entosordaria (Sacc.) Höhn. and some related genera (Pyrenomycetes). Svensk Bot Tidskr 60: 315–324

Eriksson O, Hawksworth DL (1986) Notes on ascomycete systematics. Nos. 1–224. Syst Ascomycet 5:113–174

Francis SM (1975) Anthostomella Sacc. (part I). Mycol Pasp 139:1–97

Gerhardt E, Hein B (1979) Die nomenklatorischen Typen der von Th. Nitschke beschriebenen Arten im Pilzherbar des Botanischen Museums Berlin-Dahlem [De herbario berolinensi notulae 12]. Wildenowia 9:313–330

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Hernández-Restrepo M, Groenewald JZ, Crous PW (2016) Taxonomic and phylogenetic re-evaluation of Microdochium, Monographella and Idriella. Persoonia 36:57–82

Höhnel F (1920) Fragmente zur Mykologie XXIV . Sitzungsber Akad Wiss Wien. Math-naturw Kl, Abt 1 129:137–184

Jaklitsch WM, Gardiennet A, Voglmayr H (2016) Resolution of morphological clade in Hypocrea. Mycologia 97:1365–1378

Jaklitsch WM, Stadler M, Voglmayr H (2012) Blue pigment in Hypocrea caeruleus sens sp. nov. and two additional new species in sect. Trichoderma. Mycologia 104:925–941

Jaklitsch WM, Fournier J, Rogers JD, Voglmayr H (2014) Phylogenetic and taxonomic revision of Lopadostoma. Persoonia 32:52–82

Jaklitsch WM, Gardiennet A, Voglmayr H (2016) Resolution of morphology-based taxonomic delusions: Acrocoridiella, Basidiopistera, Blogiascospora, Clypeosphaeria, Hymenopalea, Lepteutypa, Pseudapispora, Requienella, Serigidium and Strickeria. Persoonia 37:82–105

Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Mol Biol Evol 16:1799–1808

Lu BS, Hyde KD (2000) A world monograph of Anthostomella. Fungal Diversity Series 4:1–376. Fungal Diversity Press, Hong Kong
