Article

Multigene Phylogeny Reveals *Haploanthostomella elaeidis* gen. et sp. nov. and Familial Replacement of *Endocalyx* (Xylariales, Sordariomycetes, Ascomycota)

Sirinapa Konta 1,2,3, Kevin D. Hyde 2, Prapassorn D. Eungwanchayapant 3, Samantha C. Karunarathna 1,4,5, Milan C. Samarakoon 2, Jianchu Xu 1,4,5, Lucas A. P. Dauner 1, Sasith Thanaranga Aluthwaththa 6,7, Saisamorn Lumyong 8,9 and Saowaluck Tibpromma 1,4,5,*

1 CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China; sirinapakonta@gmail.com (S.K.); samanthakarunarathna@gmail.com (S.C.K.); jxu@mail.kib.ac.cn (J.X.); luke.dauner1@gmail.com (L.A.P.D.)
2 Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand; kdhyde3@gmail.com (K.D.H.); milan.chameera@yahoo.com (M.C.S.)
3 School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand; prapassorn@mu.ac.th
4 World Agroforestry Centre, East and Central Asia, Kunming University, Xuedonglu 100, Nanning 530004, China; aluthwaththa@yahoo.com
5 State Key Laboratory of Conservation and Utilization of Subtropical Agro-Bioresources, College of Forestry, Guangxi University, Xuedonglu 100, Nanning 530004, China
6 Research Center of Microbial Diversity and Sustainable Utilization, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand; saisamorn.l@cmu.ac.th
7 Academy of Science, The Royal Society of Thailand, Bangkok 10300, Thailand
* Correspondence: saowaluckfai@gmail.com (S.T.)

**Abstract:** During our investigation of palm fungi in Thailand, two interesting taxa from *Elaeis guineensis* and *Metroxylon sagu* (Arecaeae) were collected. Based on phylogenetic analyses of a combined dataset of ITS, LSU, rpb2, and tub2 nucleotide sequences as well as unique morphological characteristics, we introduce the new genus *Haploanthostomella* within Xylariales, and a new species *Endocalyx metroxyli*. Additionally, in our study, the genus *Endocalyx* is transferred to the family Cai niaceae based on its brown conidia and molecular phylogenetic evidence.

**Keywords:** Apiosporaceae; Cai niaceae; fungi; palms; Thailand; Xylariales

1. Introduction

Palm trees represent a family of perennial lianas and consist of many diverse species worldwide, with the fossil record indicating around 65 million years of evolutionary history [1]. Microfungi on palms have been studied, but only a few have been analyzed using morphology and DNA sequence data. Several fungal species are currently unknown to science, with the total number estimated at somewhere between 2.2 and 3.8 million [2]. Thus, palms are a particularly interesting plant family for studying microfungi species unknown to science.

The subclass Xylariomycetidae has recently been updated to contain three orders (Amphisphaeriales, Delonicicolales, and Xylariales) and 35 families [3]. Recently, the family Induratiaceae was introduced in this subclass by Samarakoon et al. [4] with an updated phylogeny of Xylariales. Cai niaceae is a family of particular interest, as all members in this family tend to be found on monocotyledons, the majority of which are grasses [5]. In previous studies, Cai niaceae was accepted in the Xylariales [3,6]. Later, Hongsanan et al. [7], and Wijayawardene et al. [8] assigned Cai niaceae to the Xylariomycetidae as an incertae sedis family.
The Xylariales is one of the largest orders and includes 15 families, 160 genera, and 52 genera incertae sedis [3]. Family Cainiaceae was introduced by Krug [9] to include species of Cainia with unique apical rings in the ascus that consist of a series of rings and ascospores with longitudinal germ slits. An asexual morph of Cainiaceae was coelomycetous with black, scattered, immersed pycnidial conidiomata; hyaline, denticulate, sympodially proliferating conidiophores; hyaline, filiform, branched or simple, septate conidiogenous cells with one to three phialides; and hyaline, elongate fusiform, falcate to lunate, unicellular or septate conidia, with pointed ends [10]. At present, seven genera have been accepted into this family (Alishanica, Amphibambusa, Arecophila, Atrotorquata, Cainia, Longiappendispora, and Seynesia) [3,11].

Since 2014, fungal research in Thailand has revealed a high diversity of novel species [12–14]. In this study, we found fungal species unknown to science from Thailand. The phylogeny results show that Endocalyx grouped within Cainiaceae, and so we transferred Endocalyx from Apiosporaceae (Amphisphaeriales) to Cainiaceae (Xylariales) based on both morphology and multigene phylogeny. We also introduce the new species Endocalyx metroxyli, collected from the economically important oil palm host (Elaeis guineensis). Lastly, we introduce the new genus Haploanthostomella associated with true sago palm (Metroxylon sagu).

2. Materials and Methods

2.1. Collection, Isolation, and Identification

Saprobic fungi growing on dead leaves, petioles and rachis of Elaeis guineensis and Metroxylon sagu were collected in Krabi and Surat Thani Provinces of Thailand, placed in ziplock bags and brought to the mycology laboratory at the Center of Excellence in Fungal Research, and morphological characteristics were observed. Specimens were examined following the methods provided by Konta et al. [15]. Single spore isolates were obtained following the method of Senanayake et al. [16], using malt extract agar (MEA) and incubating at 25–28 °C overnight. Germinating conidia were transferred to new MEA media and pure cultures were kept at 25–28 °C. Specimens and cultures were deposited in the herbarium of Mae Fah Luang University (MFLU) and Mae Fah Luang University Culture Collection (MFLUCC), Chiang Rai, Thailand, respectively. Faces of Fungi and Index Fungorum numbers were registered as outlined in Jayasiri et al. [17] and Index Fungorum [18].

2.2. DNA Extraction and Amplification (PCR)

Genomic DNA was extracted from fruiting bodies of Haploanthostomella elaeidis and fungal mycelium of Endocalyx metroxyli. DNA extraction and amplification were followed Dissanayake et al. [19]. Konta et al.’s method [16] was followed for PCR amplification of ITS, LSU, SSU, tef1-α and rpb2, while O’Donnell and Cigelnik’s method [20] was followed for PCR amplification of the tub2 region. Amplification was done using the primers ITS5 and ITS4 for the internal transcribed spacer regions and intervening 5.8S rDNA (ITS), the primers LR5 and LR0R for the large subunit (LSU) rRNA gene, the primer pair rRPB2-5f and rRPB2-7cR for the RNA polymerase II second largest subunit (rpb2) gene, and the primers T1 and T22 for the partial gene β-tubulin (tub2). PCR amplifications were performed using 1× PCR buffer with 8.5 μL ddH2O, 12.5 μL 2× Easy Taq PCR SuperMix (mixture of Easy Taq TM DNA Polymerase, dNTPs and optimized buffer (Beijing Trans Gen Biotech Co., Beijing, China)), 2 μL of DNA template, and 1 μL each of forward and reverse primers (10 pM) in a final volume of 25 μL. The cycle conditions in the initiation step were started at 95 °C for 3 min, followed by 35 cycles at 95 °C for 30 s, 55 °C for 50 s, 72 °C for 30 s (for ITS, LSU); 95 °C for 5 min, followed by 35 cycles at 95 °C for 1 min, 54 °C for 2 min, 72 °C for 1:5 min (for rpb2); 95 °C for 5 min, followed by 35 cycles at 94 °C for 1 min, 52 °C for 1 min, 72 °C for 1:5 min (for tub2); a final elongation step at 72 °C for 10 min and a final hold at 4 °C were done as the last steps. Purification and sequencing were performed by
Table 1. Names, strain numbers and corresponding GenBank accession numbers of the taxa used in phylogenetic analyses, the ex-type strains are in bold.

| Order               | Family                  | Species                  | Strain No. | GenBank Accession No. | References |
|---------------------|-------------------------|--------------------------|------------|-----------------------|------------|
|                     |                         |                          |            |                       |            |
| Amphisphaeriales    | Apiosporaceae           | *Arthrinium balearicum*  | AP24118    | MK014869              | MK017946 [21] |
|                     |                         | *Arthrinium cariciola*   | CBS 145127 | MK014871              | MK017948 [21] |
|                     |                         | *Arthrinium hydei*       | CBS 114990 | KF144980              | KF144982 [22] |
|                     |                         | *Arthrinium phragmitis*  | CBS 135458 | KF144909              | KF144956 [22] |
|                     |                         | *Arthrinium pseudopagazzinii* | CBS 102052    | KF144911              | KF144958 [22] |
|                     |                         | *Nigrospora aurantia*    | CGMCC 3.18130 | NR_153477          | - [23] |
|                     |                         | *Nigrospora brasiliensis* | CBS 123.58 | -                      | KY806276 [24] |
|                     |                         | *Nigrospora zimmermannii* | CBS 290.62 | -                      | KY806276 [25] |

Xylariales

| Order               | Family                  | Species                  | Strain No. | GenBank Accession No. | References |
|---------------------|-------------------------|--------------------------|------------|-----------------------|------------|
|                     |                         | *Alishanica miscanthii*   | FLU31025   | MK503821              | MK503827 [3] |
|                     |                         | *Amphibambusa bambusicola* | MFLUCC 11-0617 | KP744433             | - [28] |
|                     |                         | *Atrotoxula lineata*     | HKUCC 3263 | AF009807              | - [29] |
|                     |                         | *Cainia anthoxanthis*     | MFLUCC 15-0539 | KR092787            | KL092777 [3] |
|                     |                         | *Cainia desmazieri*      | CAI         | KT949896              | KT949896 [29] |
|                     |                         | *Cainia globosa*         | MFLUCC 13-0663 | KX822127            | KX822123 [30] |
|                     |                         | *Cainia graminis*        | CBS 136.62  | KR092793              | AF431949 [5,31] |
|                     |                         | *Cainia globosa*         | MFLUCC 17-1485 | MT214370             | MT214464 [11] |
|                     |                         | *Endocalyx cinctus*      | JCM 7946   | LC228648              | LC228704 [32] |
|                     |                         | *Endocalyx metoxyphi*    | MFLUCC 15-0723A | MT292916           | MT292913 [32] |
|                     |                         | *Endocalyx metoxyphi*    | MFLUCC 15-0723B | MT292916           | MT292914 [32] |
|                     |                         | *Segesnia crampens*      | SMH 1291   | -                      | AF29410 [33] |
|                     |                         | *Chyphosphaeria numiliana* | CBS 140735    | KT949897            | KT949897 [29,34] |

Xylariales

| Order               | Family                  | Species                  | Strain No. | GenBank Accession No. | References |
|---------------------|-------------------------|--------------------------|------------|-----------------------|------------|
|                     |                         | *Contoecia anandra*      | Co108      | GU553338              | GU553349 [35] |
|                     |                         | *Contoecia cruciformis*  | Co116      | GU553336              | GU553347 [35] |

Sangon Biotech Co., Shanghai, China. Consensus sequences were computed using SeqMan software, and new sequences generated in this study were deposited in GenBank (Table 1).
| Order               | Family                  | Species                     | Strain No. | GenBank Accession No. | References |
|---------------------|-------------------------|-----------------------------|------------|-----------------------|------------|
| Xylariales          | Coniocessiaceae         | Coniocessia maxima          | Co117      | GU553332 GU553344    | [35]       |
| Xylariales          | Coniocessiaceae         | Coniocessia minima          | Co111      | GU553334 GU553345    | [35]       |
| Xylariales          | Coniocessiaceae         | Coniocessia nodulisporioides| CBS 281.77T| - AJ675224            | [36]       |
| Xylariales          | Coniocessiaceae         | Paracystara rosacearium     | TASM 6132  | MGS28941 MGS29050    | [37]       |
| Xylariales          | Diatrypaceae            | Allocryptovalsa polyspora   | MFLUCC 17-0364 | MF959500 MF959503 MG334556 | [38] |
| Xylariales          | Diatrypaceae            | Allodiutypa arengae         | MFLUCC 15-0713 | MN308411 MN308402 MN542886 MN340297 | [39] |
| Xylariales          | Diatrypaceae            | Cryptosalva rabenosoriitii  | Crel = CBS 125974 | KC774567 KC774567 - | [40]       |
| Xylariales          | Diatrypaceae            | Diutypa disciformis         | CBS 197.49 | - DQ470964 DQ470915  | [41]       |
| Xylariales          | Diatrypaceae            | Diutypella veruciformis     | UCROK1467  | JX144793 - - JX174093 | [42]       |
| Xylariales          | Diatrypaceae            | Eutypa lata                 | CBS 208.87 | DQ006927 MH873795 - | [43,44]    |
| Xylariales          | Diatrypaceae            | Entypella caricae           | ELC5       | AJ302460 - - -       | [45]       |
| Xylariales          | Diatrypaceae            | Halodiutypa salinicola      | MFLUCC 15-1277 | KX573915 - - KX573932 | [46]       |
| Xylariales          | Diatrypaceae            | Monosporascus cannobalus    | CMM3646    | JX971617 - - -       | Unpublished |
| Xylariales          | Diatrypaceae            | Neoentypella baoshanensis   | ELS1C, CBS 274.87 | AJ302460 - - -   | [45]       |
| Xylariales          | Diatrypaceae            | Pedantypella rhizophorae    | BCC44857   | KJ888853 KJ888850    | [47]       |
| Xylariales          | Diatrypaceae            | Peronutypa longiasca        | MFLUCC 17-0371 | MF959502 MF959505 MG334558 | [38] |
| Xylariales          | Fasciatisporaceae       | Fasciatispora arengae       | MFLUCC 15-0326a | MK120275 MK120300 MK890794 MK890793 | [48] |
| Xylariales          | Fasciatisporaceae       | Fasciatispora calami        | MFLUCC 15-0294 | - MF459055 - MF459056 | [49] |
| Xylariales          | Fasciatisporaceae       | Fasciatispora cocos         | MFLUCC 18-1445 | MN482680 MN482675 MN481517 MN350154 | [13] |
| Xylariales          | Fasciatisporaceae       | Fasciatispora nypae         | MFLUCC 11-0382 | - KP744484 - -       | [28]       |
| Xylariales          | Fasciatisporaceae       | Fasciatispora petrakii      | - AY083828 | - - Unpublished      |            |
| Xylariales          | Graphostromaceae        | Bicogniauxia numularia      | MUCL 51395 | KY610382 KY610427 KY624236 KY2271241 | [50] |
| Xylariales          | Graphostromaceae        | Camilla obularia            | ATCC 28093 | KY610384 KY610429 KY624238 KY2271243 | [50] |
| Xylariales          | Graphostromaceae        | Graphostroma platystemon   | CBS 270.87 | JX658535 DQ836906 KY624296 HK934108 | [50–53]    |
| Xylariales          | Graphostromaceae        | Obolarina dryophila         | MUCL 49882 | GQ428316 GQ428316 KY624284 GQ428322 | [50,54]    |
| Xylariales          | Hansfordiaceae          | Hansfordia pulvinata        | CBS 194.56 | MK442585 MH691922 KU684307 - | [24]       |
| Xylariales          | Hansfordiaceae          | Hansfordia pulvinata        | CBS 144422 | MK442587 MK442527 - - | [24]       |
| Xylariales          | Hypoxylaceae            | Anulidepoxylon truncatum    | CBS 140778 | KY610419 KY610419 KY624277 KO376382 | [50,55]    |
| Xylariales          | Hypoxylaceae            | Anthocanalis sparti         | MFLUCC 10-0010 | KP297394 KP340536 KP340522 KP340605 | [54]       |
| Xylariales          | Hypoxylaceae            | Anthostoma decipiens       | CD = CBS 133221 | KC774565 KC774565 - - | [40]       |
| Order                      | Family              | Species                | Strain No. | ITS       | LSU       | rpb2      | tub2      | References               |
|---------------------------|---------------------|------------------------|------------|-----------|-----------|-----------|-----------|--------------------------|
| Xylariales                | Hypoxylaceae        | Daldinia concentrica   | CBS 113277 | AY616683  | KY610434  | KY624243  | KY977274  | [50,56,57]               |
| Xylariales                | Hypoxylaceae        | Eustonema depressens   | ATCC 46302 | KY610389  | KY610443  | KY624253  | KY271248  | [50]                     |
| Xylariales                | Hypoxylaceae        | Hypomontagnella        | MUCL 54604 | KY610404  | KY610487  | KY624305  | KY271273  | [50]                     |
| Xylariales                | Hypoxylaceae        | Xyloxylin fragiforme   | MUCL 51264 | KC477229  | KM186295  | KM186296  | KY271282  | [50,59,60]               |
| Xylariales                | Hypoxylaceae        | Hypholoma              | CBS 119016 | KY610473  | KY624290  | KY271262  | [50,55,57]               |
| Xylariales                | Hypoxylaceae        | Pyrenomyxa             | CBS 116990T| AM749920  | -         | -         | -         | [61]                     |
| Xylariales                | Hypoxylaceae        | Pyrenomyxa pica        | ILLS 58257 | EF562506  | -         | -         | -         | [62]                     |
| Xylariales                | Pyrenopolyporus     | Pyrenopolyporus        | MUCL 52673 | KY610421  | KY610472  | KY624309  | KU159530  | [50,55]                  |
| Xylariales                | Xylariaceae         | Phylacia sagrana       | CBS 119992 | AM749919  | -         | -         | -         | [61]                     |
| Xylariales                | Xylariaceae         | Pyrenopolyporus        | TBRC:8873  | MH938529  | MH938538  | MK165428  | MK165419  | [64]                     |
| Xylariales                | Xylariaceae         | Creosphaeria           | STMA 14087 | KY610411  | KY610468  | KY624265  | KY271258  | [50]                     |
| Xylariales                | Xylariaceae         | Lobostoma              | CBS 133207 | KC774618  | KC774618  | KC774563  | MF489024  | [29,40]                  |
| Xylariales                | Xylariaceae         | Idriella lunata        | MFLU 18-0261| MFLU 18-0261| MFLU 18-0261| MFLU 18-0261| MFLU 18-0261| MFLU 18-0261| [66]                     |
| Xylariales                | Xylariaceae         | Microdochium           | CBS 423.78 | KP859012  | KP858948  | KP859121  | KP859076  | [67]                     |
| Xylariales                | Xylariaceae         | Polystigma folaeum     | MFLU 15-3091 | K594023  | M981079  | -         | -         | [68]                     |
| Xylariales                | Xylariaceae         | Polystigma rubrum      | MFLU 15-3091 | K594023  | M981079  | -         | -         | [68]                     |
| Xylariales                | Xylariaceae         | Acrocriella occulta    | RS9        | KT949893  | KT949893  | -         | -         | [29]                     |
| Xylariales                | Xylariaceae         | Acrocriella omanensis  | SQUCC 15091 | MG584568  | MG584570  | -         | -         | [69]                     |
| Xylariales                | Xylariaceae         | Requienella fraxini    | RS2        | KT949909  | KT949909  | -         | -         | [29]                     |
| Xylariales                | Xylariaceae         | Requienella foresti    | RS12 = CBS 140502 | KT949912  | KT949912  | MK523300  | -         | [29,64]                  |
| Xylariales                | Xylariaceae         | Abieticola koreana     | EML-F0010-1| JN977612  | JQ014618  | KP792128  | KP792126  | [70]                     |
| Xylariales                | Xylariaceae         | Amphimellinia nigrospora| HAST 91092308 | GU322457  | -         | GQ684340  | GQ495951  | [71]                     |
| Xylariales                | Xylariaceae         | Anthostomella fornsa   | MFLUCC 14-0170 | K97403  | K9340544  | K9340531  | K946614  | [59]                     |
Table 1. Cont.

| Order            | Family            | Species                        | Strain No.         | GenBank Accession No. | References |
|------------------|-------------------|--------------------------------|--------------------|-----------------------|------------|
|                  |                   |                                |                    |                       |            |
| Xylariales       | Xylariaceae       | Anthostomella helicofissa      | MFLUCC 14-0173     | KP297406              | [59]       |
| Xylariales       | Xylariaceae       | Anthostomella obesa            | MFLUCC 14-0171     | KP297405              | [59]       |
| Xylariales       | Xylariaceae       | Anthostomella pseudobambusica | MFLUCC 15-0192     | KU940153              | -          |
| Xylariales       | Xylariaceae       | Anthostomeloides braehetii    | CBS 110128         | EU355209              | [73]       |
| Xylariales       | Xylariaceae       | Anthostomeloides fortescensae| MFLUCC 14-0558     | KP297397              | [66]       |
| Xylariales       | Xylariaceae       | Anthostomeloides krabienis    | MFLUCC 15-0678     | KX305927              | -          |
| Xylariales       | Xylariaceae       | Anthostomeloides leucospermii | CBS 110126         | EU3552100             | [73]       |
| Xylariales       | Xylariaceae       | Anthostomeloides proteae      | CBS 110127         | EU3552101             | -          |
| Xylariales       | Xylariaceae       | Astrocystis mirabilis          | 94070803 HAST      | GU322448              | [71]       |
| Xylariales       | Xylariaceae       | Brunneiperidium gracilentum   | MFLUCC 14-0011 Ex-type | KP297400     | -          |
| Xylariales       | Xylariaceae       | Collodiocula japonica         | CBS 124266         | JF440974              | [50,74]    |
| Xylariales       | Xylariaceae       | Coniolariella ganssi          | Co27TRAN 842C, CBS114379 (T) | GU355325 | -          |
| Xylariales       | Xylariaceae       | Entalbostroma erumpens        | ICMP 21152         | KX258206              | [75]       |
| Xylariales       | Xylariaceae       | Entoleuca mammata             | J.D.R. 100         | GU300072              | [71]       |
| Xylariales       | Xylariaceae       | Entoleuca sphaeriostomum     | J.D.R. 261         | GU292821              | [71]       |
| Xylariales       | Xylariaceae       | Halorosellinia ocanica        | SGLA82             | EU715635              | -          |
| Xylariales       | Xylariaceae       | Hypoplospora rostrata         | NRRL 66178         | KM067909              | -          |
| Xylariales       | Xylariaceae       | Hypoploeostrum sanguineum     | J.D.R. 169         | GU322433              | [71]       |
| Xylariales       | Xylariaceae       | Kretzschmaria clavus          | YMJ 114            | EF026126              | [71,78]    |
| Xylariales       | Xylariaceae       | Linosporopsis notriochotica   | LIF1 = CBS 145761 | MN818952              | [79]       |
| Xylariales       | Xylariaceae       | Lunatiannulus irregularis     | MFLUCC 14-0014     | KP297398              | [57]       |
| Xylariales       | Xylariaceae       | Nemania serpens               | CBS 679.86         | KU683765              | [80]       |
| Xylariales       | Xylariaceae       | Neoclytrilla arengeae         | MFLUCC 15-0292     | MT496747              | -          |
| Xylariales       | Xylariaceae       | Podosordaria mexicana         | WSP 176            | GU324762              | [71]       |
| Xylariales       | Xylariaceae       | Poronia punctata              | CBS 656.78         | KT281904              | [5,50]     |
| Xylariales       | Xylariaceae       | Rosellinia aquila             | MUCL 51703         | KY610496              | [50]       |
| Xylariales       | Xylariaceae       | Rostrohypoxylon teretibranum | CBS 119137         | DQ631943              | [82,83]    |
| Xylariales       | Xylariaceae       | Ruwenzoria pseudoumatula      | MUCL 51394         | KY610494              | [50]       |
| Xylariales       | Xylariaceae       | Sarcoxylon compactum         | CBS 359.61         | KT281903              | [5,50]     |
| Order         | Family            | Species                  | Strain No. | GenBank Accession No. | References |
|--------------|-------------------|--------------------------|------------|-----------------------|------------|
| Xylariales   | Xylariaceae       | STILLHOHYPOXYLOXON       |            |                       |            |
|              |                   | claeicola                |            |                       |            |
|              |                   | Y.M.J. 173               | EF026148   | -                     | GQ844826   | EF025616   | [71]       |
| Xylariales   | Xylariaceae       | STILLHOHYPOXYLOXON       |            |                       |            |
|              |                   | claeidisi                |            |                       |            |
|              |                   | MFLUCC 15-0295a          | MT496745   | MT496755              | MT502416   | MT502420   | [81]       |
| Xylariales   | Xylariaceae       | STILLHOHYPOXYLOXON       |            |                       |            |
|              |                   | quisquiliarum            |            |                       |            |
|              |                   | Y.M.J. 172               | EF026119   | -                     | GQ853020   | EF025605   | [71]       |
| Xylariales   | Xylariaceae       | VAMSAPIRYA               |            |                       |            |
|              |                   | bambusiscola             |            |                       |            |
|              |                   | MFLUCC 11-0477           | KM462835   | KM462836              | KM462834   | KM462833   | [84]       |
| Xylariales   | Xylariaceae       | VAMSAPIRYA               |            |                       |            |
|              |                   | bre-viculiodiaphora      |            |                       |            |
|              |                   | MFLUCC 14-0436           | MF621584   | MF621588              | -          | -          | [39]       |
| Xylariales   | Xylariaceae       | VAMSAPIRYA               |            |                       |            |
|              |                   | indica                   |            |                       |            |
|              |                   | MFLUCC 12-0544           | KM462839   | KM462840              | KM462841   | KM462838   | [84]       |
| Xylariales   | Xylariaceae       | VAMSAPIRYA               |            |                       |            |
|              |                   | kluonkonensis            |            |                       |            |
|              |                   | MFLUCC 11-0475           | KM462630   | KM462631              | KM462629   | KM462628   | [84]       |
| Xylariales   | Xylariaceae       | VAMSAPIRYA               |            |                       |            |
|              |                   | yunnana                  |            |                       |            |
|              |                   | KUMCC 18-0008            | MG833874   | MG833873              | MG833875   | -          | [85]       |
| Xylariales   | Xylariaceae       | VIRGARIA                 |            |                       |            |
|              |                   | boninensis               |            |                       |            |
|              |                   | JCM 18624               | AB740956   | -                     | AB740960   | -          | [86]       |
| Xylariales   | Xylariaceae       | VIRGARIA                 |            | CBS 128006             |            | -          | [44]       |
| Xylariales   | Xylariaceae       | XYLARIA                   |            | CBS 122620             | KY610407   | KY610495   | KY624211   | KY272129   | [50,87]   |
| Sordariomyces| genera incertae sedis | XYLARIALES genera incertae sedis |            |                       |            |
|              |                   | Melanographium           |            |                       |            |
|              |                   | phoenicis                |            |                       |            |
|              |                   | MFLUCC 18-1481           | MN482677   | MN482678              | -          | -          | [13]       |
| Sordariomyces| genera incertae sedis | XYLARIALES genera incertae sedis |            |                       |            |
|              |                   | Ceratocladium            |            |                       |            |
|              |                   | micropernum              |            |                       |            |
|              |                   | CBS126092               | MH864077   | MH875534              | -          | -          | [44]       |
| Xylariales   | Xylariaceae       | ASCOTRICA                |            |                       |            |
|              |                   | chartarum                |            |                       |            |
|              |                   | CBS 234.97              | KF893284   | -                     | -          | KF893271   | [88]       |
| Xylariales   | Xylariaceae       | ASCOTRICA                |            |                       |            |
|              |                   | longipila                |            |                       |            |
|              |                   | OUCMB110118              | KC503896   | -                     | -          | KF893265   | [88]       |
| Xylariales   | Xylariaceae       | ASCOTRICA                |            |                       |            |
|              |                   | luzitanica               |            |                       |            |
|              |                   | CBS 462.70              | KF893289   | -                     | -          | KF893275   | [88]       |
| Xylariales   | Xylariaceae       | ASCOTRICA                |            |                       |            |
|              |                   | parciopora               |            |                       |            |
|              |                   | OUCMB1100003 (T)         | JX014298   | -                     | -          | KF893267   | [88]       |
| Xylariales   | Xylariaceae       | ASCOTRICA                |            |                       |            |
|              |                   | sinuosa                  |            |                       |            |
|              |                   | OUCMB1101190 (T)         | JX014299   | -                     | -          | KF893266   | [88]       |
| Xylariales   | Xylariaceae       | ALLOANTHOSTOMELLA        |            |                       |            |
|              |                   | rubicola                 |            |                       |            |
|              |                   | MFLUCC 14-0175           | KP297407   | KP340548              | KP340535   | KP406618   | [89]       |
| Xylariales   | Xylariaceae       | CIRCINOTRICHUM           |            |                       |            |
|              |                   | cypadis                  |            |                       |            |
|              |                   | CPC 17225               | KJ869121   | KJ869178              | -          | -          | [26]       |
| Xylariales   | Xylariaceae       | CIRCINOTRICHUM           |            |                       |            |
|              |                   | maccaliforme             |            |                       |            |
|              |                   | CPC 24566               | KR611874   | KR611895              | -          | -          | [90]       |
| Xylariales   | Xylariaceae       | CIRCINOTRICHUM           |            |                       |            |
|              |                   | papuense                 |            |                       |            |
|              |                   | CBS 101373              | KR611876   | KR611897              | -          | -          | [90]       |
| Xylariales   | Xylariaceae       | CIRCINOTRICHUM           |            |                       |            |
|              |                   | sinense                 |            |                       |            |
|              |                   | KY994106                | KY994107   | -                     | -          | [91]       |
| Xylariales   | Xylariaceae       | GYROTHRIX                |            |                       |            |
|              |                   | eucalypti                |            |                       |            |
|              |                   | CPC 36066               | MN562109   | MN567617              | -          | -          | [92]       |
| Xylariales   | Xylariaceae       | GYROTHRIX                |            |                       |            |
|              |                   | inops                    |            |                       |            |
|              |                   | BE108                    | KC775746   | KC775721              | -          | -          | [66]       |
| Xylariales   | Xylariaceae       | GYROTHRIX                |            |                       |            |
|              |                   | oleae                    |            |                       |            |
|              |                   | CPC 37069               | MN562136   | MN567643              | -          | -          | [92]       |
| Xylariales   | Xylariaceae       | GYROTHRIX                |            |                       |            |
|              |                   | ramosa                   |            |                       |            |
|              |                   | MUCUL54061              | KC775747   | KC775722              | -          | -          | [66]       |
| Xylariales   | Xylariaceae       | HAPLOANTHOSTOMELLA       |            |                       |            |
|              |                   | claeidisi               |            |                       |            |
|              |                   | MFLU 20-0522            | MT929161   | MT929312              | MT928154   | -          | This study |
Table 1. Cont.

| Order          | Family                   | Species                         | Strain No. | GenBank Accession No. | References |
|----------------|--------------------------|---------------------------------|------------|-----------------------|------------|
| Xylariales     | Xylaricales genera incertae sedis | Neanathostomella pseudostromaticica | MFLUCC 11-0610 | KU940158 | KU863146 | - | - | [72] |
| Xylariales     | Xylaricales genera incertae sedis | Neanathostomella viticola | MFLUCC 16-0243 | KX505957 | KX505958 | KX789496 | KX789495 | [89] |
| Xylariales     | Xylaricales genera incertae sedis | Pseudoanthostomella conorum | CBS 119333 | EU352099 | - | - | - | [73] |
| Xylariales     | Xylaricales genera incertae sedis | Pseudoanthostomella delitescens | MFLUCC 16-0477 | KX533451 | KX533452 | KX789491 | KX789490 | [89] |
| Xylariales     | Xylaricales genera incertae sedis | Pseudoanthostomella pulvigerae | MFLUCC 16-0478 | KX533453 | KX533454 | KX789492 | - | [89] |
| Xylariales     | Xylaricales genera incertae sedis | Pseudoanthostomella seepilitta | AY908989 | AY875645 | - | - | - | Unpublished |
| Xylariales     | Xylaricales genera incertae sedis | Xenoanthostomella chromolaenae | MFLUCC 17-1484 | MN638863 | MN638848 | - | - | [3] |
| Xylariales     | Zygosporiaceae            | Zygosporum oscheoides           | MFLUCC 14-0402 | MF621585 | MF621589 | - | - | [93] |
| Xylariales     | Zygosporiaceae            | Zygosporum minus                | HKAS99625 | MF621586 | MF621590 | - | - | [93] |

2.3. Phylogenetic Analyses

The consensus sequences were put through a BLAST search in the NCBI GenBank nucleotide database to search for the fungal sequences of closest relatives that have been deposited in the NCBI database. Dissanayake et al.’s study [19] was followed for the phylogenetic analyses. Voglmayr and Beenken’s study [79] was used as a reference of the dataset. Both individual and combined ITS, LSU, rpb2, and tub2 nucleotide sequences were analyzed. A total of 151 taxa were used for the phylogenetic analyses in order to find the taxonomic placement of each species. Three genera viz. *Delonicicola*, *Furfurella* (Delonicicolaceae), and *Leptosillia* (Leptosilliaceae) in Delonicicolales were used as the outgroup taxa.

The MAFFT online program was used to obtain initial alignments for each locus [94]. Alignments were manually edited and single gene sequence data sets were combined using MEGA7 [95]. The Alignment Transformation Environment online program was used to convert the file format [96]. MrModeltest [97] was used to find the best model for maximum likelihood (ML) and Bayesian analyses (BYPP). The six simultaneous Markov chains were run for 20,000,000 generations and trees were sampled every 1000th generation. Bayesian posterior probabilities from MCMC were evaluated with a final average standard deviation of the split frequency of <0.01. Bootstrap values for ML equal to or greater than 50% and BYPP equal to or greater than 0.90 are given at the nodes (Figure 1). Fig Tree v1.4.0 was used to configure the phylogenetic trees [98] and edited using Microsoft Office PowerPoint 2010 and Adobe Photoshop CS6 (Adobe Systems Incorporated, 345 Park Avenue, San Jose, CA, USA).
Figure 1. Maximum likelihood majority rule consensus tree for the analyses of selected Xylariomycetidae isolates based on a dataset of combined ITS, LSU, rpb2, and tub2 nucleotide sequence. Bootstrap support values for maximum likelihood (ML) equal to or higher than 50% are given above each branch. Bayesian posterior probabilities (BYPP) equal to or greater than 0.90 are given at the nodes. Novel taxa are in blue bold and ex-type strains are in black bold. The tree is rooted to Delonicicolaceae and Leptosilliaceae (Delonicicolales). The asterisks represent unstable species.
3. Results

3.1. Morphology and Phylogeny

The combined dataset comprised 151 taxa from selected taxa in Amphisphaeriales, Delonicicolales, and Xylariales (Table 1). The RAxML analyses of the combined dataset yielded the best-scoring tree (Figure 1) with a final ML optimization likelihood value of $-126584.196783$. The matrix had 4598 distinct alignment patterns, with 65.07% undetermined characters or gaps. Estimated base frequencies were: A = 0.243574, C = 0.257762, G = 0.258457, T = 0.240207; substitution rates AC = 1.296272, AG = 3.089851, AT = 1.400263, CG = 1.060328, CT = 9.900102, GT = 1.000000; gamma distribution shape parameter $\alpha$ = 0.443932. Tree-Length = 25.372161. Bayesian analysis resulted in a tree with similar topology and clades as the ML tree. Phylogenetic analyses of the combined ITS, LSU, rpb2, and tub2 loci show two novel taxa within the monospecific genus *Haploanthostomella* (type species *Haploanthostomella elaeidis*; Xylariales incertae sedis) and the novel taxa *Endocalyx metroxyli*, with the genus *Endocalyx* being placed in Cainiaceae.

3.1.1. *Haploanthostomella* Konta & K.D. Hyde. gen. nov.

Index Fungorum number: IF557876; Facesoffungi number: FoF09173

Etymology: “*haplos*” (απλός) in Greek means single; *Anthostomella* refers to its morphological similarity to *Anthostomella*.

Saprobic on dead leaves and rachis in terrestrial habitats. Sexual morph: *Ascomata* immersed in the host epidermis, beneath a clypeus, visible as slightly raised blackened areas, dark brown to black, coriaceous, solitary or aggregated into clusters, scattered, with an ostiolar canal. *Peridial wall* thick, comprised of several layers of cells, outwardly comprising dark brown cells of *textura prismatica* and inwardly comprising hyaline cells of *textura angularis*. *Paraphyses* septate, tapering hyphae-like, hyaline. *Asci* eight-spored, unitunicate, clavate to cylindric, short pedicellate, with J-, apical ring. *Ascospores* uni–biseriate into the asci, unicellular, obovoid, fusoid, hyaline or brown to dark brown, verrucose with a mucilaginous cap at apex. *Germ slit* straight, less than spore-length. Asexual morph: Not observed.

Type species: *Haploanthostomella elaeidis* Konta & K.D. Hyde.

Notes: *Anthostomella* species were proven to be polyphyletic, and it is of no surprise that a new genus with anthostomella-like characteristics was discovered in this study [99]. Phylogenetic analyses based on a single dataset of ITS (supporting information section) and combined sequence data indicated that *Haploanthostomella* belongs to Xylariales genera incertae sedis, separating well from other genera but with low bootstrap values (Figure 1). According to the phylogenetic tree (Figure 1), seven genera (*Ceratocladium*, *Circinotrichum*, *Gyrothrix*, *Idriella*, *Neoanthostomella*, *Virgaria* and *Xenoanthostomella*) are closely related to our new genus, but morphological characteristics of these genera are different. The genera *Neoanthostomella*, *Virgaria*, and *Xenoanthostomella* were compared morphologically since they are similar to our new taxon. *Haploanthostomella* differs from *Virgaria*, *Neoanthostomella*, and *Xenoanthostomella* in having a J- apical ring, fusoid-ovoid ascospores, and verrucose with a mucilaginous cap at the apex, while *Virgaria* has asci with a J+ apical ring and smooth-walled ellipsoidal ascospores lacking of a mucilaginous sheath; *Neoanthostomella* smooth-walled ellipsoidal ascospores surrounded by a thick mucilaginous sheath; *Xenoanthostomella* has unilocular ascoma, and ascospores lacking germ slits and mucilaginous sheaths [13,72,89]. Therefore, *Haploanthostomella* is described here as a new genus based on phylogeny coupled with morphology. In addition, we provide a key to genera with *Anthostomella*-like characteristics.

3.1.2. *Haploanthostomella elaeidis* Konta & K.D. Hyde., sp. nov.

Index Fungorum number: IF557877; Facesoffungi number: FoF09174 (Figure 2)
Figure 2. Haploanthostomella elaeidis (MFLU 20-0522, holotype). (A) Substrate. (B,C) Appearance of ascomata on the host surface. (D) Sections of ascomata. (E) Peridium. (F) Hamathecium. (G) Septa of paraphyses show in red arrows. (H–K) Asci. (L) J- apical ring in Melzer’s reagent. (M,N,P–R) Ascospores with mucilaginous cap (red arrows in M, Q, R) and germ slit (red arrows in P). (O) An ascospore with verrucose wall. Scale bars: B = 1000 μm, C = 200 μm, D = 500 μm, E, G, L = 20 μm, F, H–K = 50 μm, M–P = 10 μm, Q–R = 5 μm.

Etymology: Referring to the genus of palm trees Elaeis Jacq.
Holotype: MFLU 20-0522.

Saprobic on dead leaves and rachis of Elaeis guineensis. Sexual morph: Ascomata 160–280 × 130–350 μm (x̅ = 220 × 240 μm, n = 20), immersed in the host
epidermis, beneath a clypeus, visible as slightly raised blackened areas, dark brown to black, coriaceous, solitary or aggregated into clusters, scattered, with an ostiolar canal. *Peridial wall* 13–45 µm wide, thick, comprising several layers of cells, outwardly comprising dark brown cells of *textura irregularis* and inwardly comprising hyaline cells of *textura prismatica*, 7–20 µm wide. *Paraphyses* 1.5–4.5 µm wide, septate, hyphae-like, hyaline. *Asci* 50–90 × 10–15 µm (x⁻ = 70 × 12 µm, n = 40), 8-spored, unitunicate, clavate to cylindric, short pedicellate, with J- apical ring. *Ascospores* 10–18 × 5–8 µm (x⁻ = 14 × 6 µm, n = 100), uni–biseriate into the asci, unicellular, obovoid, fusoid, hyaline to light brown when immature and brown to dark brown when mature, mostly one, rarely two-guttulate, cell wall verrucose, with a mucilaginous cap at the apex. *Germslit* 3–6 µm length (x⁻ = 5 µm, n = 50), straight, less than spore-length. Asexual morph: Not observed.

Material examined: THAILAND, Surat Thani Province, on dead leaves and rachis of *Elaeis guineensis* Jacq. (Areaceae) on the ground, 21 July 2017, Sirinapa Konta, SRWD12 (MFLU 20-0522, holotype).

Notes: A BLAST search of *H. elaeidis* ITS sequence shows 83.87% similarity with *Gyrothrix oleae* (CPC 37069); LSU sequence shows 95.95% similarity with *Gyrothrix eucalypti* (CPC 36066); and *rpb2* sequence shows 80.95% similarity with *Lopadostoma meridionale* (LG). Only the sexual morph of *H. elaeidis* was found in nature, and we could not obtain a pure culture from fresh samples. Therefore, the morphological characteristics of *H. elaeidis* were not compared with *Ceratocladium*, *Circinotrichum*, *Gyrothrix*, and *Idriella*, as they only had asexual morphs found in nature. Hence, the morphological features of *H. elaeidis* were only compared with *Neoanthostomella*, *Virgaria*, and *Xenoanthostomella*, as they have sexual morphs.

### Key to genera related to Anthostomella-like genera

1. Hyaline ascospores
   1. Brown ascospores
   2. Asci with a J- apical ring
   3. Asci with or without J+ apical ring
   4. Ascospores with or without germ slit
   5. Ascospores with germ slit and the length less than spore length with a mucilaginous cap at the apex
   6. Ascospores with or without germ slit, with mucilaginous sheath
   7. Ellipsoid ascospores without mucilaginous sheath

2. Asci with or without J+ apical ring
   3. Asci with or without germ slit
   4. Asci with germ slit extending over full length with mucilaginous sheath
   5. Ellipsoid ascospores without mucilaginous sheath

3. Ascospores with or without germ slit, with mucilaginous sheath
   4. Asci with germ slit less than spore length, with or without mucilaginous sheath
   5. Ascospores with or without germ slit, with or without mucilaginous sheath
   6. Asci with germ slit extending over full length with mucilaginous sheath
   7. Inequilaterally oblong-ellipsoidal ascospores with mucilaginous sheath

3.1.3. **Endocalyx** Berk. & Broome, J. Linn. Soc., Bot. 15(1): 84 (1876) [1877]

*Saprobic* on various plants. *Colonies* on host plant, pustules nearly flat or raised, circular, discolored, dark brown to black, at last bursting, the conidiomata developing. Sexual morph: Undetermined. Asexual morph: *Conidiomata* scattered, erect, cupulate to cylindrical; peridial hyphae enclosing the inner conidial mass, nonsporiferous, brown to yellowish brown; some species consisting of two parts of conidiomata: (1) a basal cylinder covering a central column, rough-walled, carbonaceous, composed of black hyphae which are sometimes branched and are adherent to one another; (2) a slender central column, synnematous, expanding radially apically, high, enclosed by the peridial hyphae which are nonsporiferous, orange-yellow to lemon-yellow. *Peridial wall* thick, comprising dark brown, thick-walled cells of *textura angularis*. *Conidiophores* thread-like, septate, with or without short pegs bearing the conidia, meristematic at the base, colorless basally and gradually turning brown apically, 1–2 µm wide; *peridium* thick, comprising dark brown, thick-walled cells of *textura angularis*. *Conidiogenous cells* holoblastic, integrated, determinate. *Conidia*
solitary, unicellular, flattened, round, oval or slightly polygonal in face view, at first pale, dark brown to fuscous black at maturity, with or without guttules, often with a longitudinal hyaline straight germ slit extending the full-length (adapted from [99–101]).

Type species: *Endocalyx thwaitesii* Berk. & Broome

Notes: *Endocalyx* is a coelomycetous genus in Cainiaceae with *E. cinctus* collected from Japan *E. metroxyli* sp. nov. collected from Thailand. Phylogenetic analyses of a single dataset of ITS (supporting information section) and phylogenetic analyses of a combined dataset of ITS, LSU, rpb2, and tub2 regions (Figure 1) confirm the placement of *Endocalyx* within Cainiaceae. ITS analyses showed that *Endocalyx* is closely related to *Amphibambusa* and *Atrotorquata* (supporting information section), while Figure 1 shows that *Endocalyx* formed a basal clade to other cainiaceous genera (*Alishanica, Amphibambusa, Arecophila, Atrotorquata, Cainia, Longiappendispora*, and *Seynesia*) with high bootstrap support. Morphologically, *Endocalyx* has been revised and described only as an asexual morph of the genus [100,101], while all genera in Cainiaceae have been described in their sexual morphs, except the type genus *Cainia*, for which both asexual and sexual morphs have been described. We could not compare the morphology of *Endocalyx* to *Arecophila, Seynesia*, and *Amphibambusa* (sister species in Figure 1). Therefore, *Cainia* was used for morphological comparisons; *Endocalyx* differs from *Cainia* in having erect conidiomata and also the ostiole opening surrounded by yellow hyphae, ellipsoid-globose conidia, unicellular with brown to dark brown color, and a germ slit. *Cainia* has immersed conidiomata, conidiogenous cells with one to three phialides, and elongate fusiform conidia, unicellular or septate, hyaline, with pointed ends [100–102].

Table 2. Host and locality information of *Endocalyx* reported worldwide based on the records of Species Fungorum 2021.

| No. | Species | Host | Country | Reference |
|-----|---------|------|---------|-----------|
| 1   | *Endocalyx amarkantakensis* | *Shorea robusta* (Dipterocarpaceae) | India (Holotype) | [103] |
|     |         | *Livistona chinensis var. boninensis* (Arecaceae; solitary palm) | Japan | [104] |
|     |         | *Oncosperma fasciculatum* (Arecaceae; clustering, rarely solitary palm) | Japan | [101] |
|     | *E. cinctus* * | *Oncosperma* sp. (Arecaceae; clustering, rarely solitary palm) | Sri Lanka (Holotype) | [100] |
|     |         | *Phoenix canariensis* (Arecaceae; solitary palm) | Japan | [101] |
|     |         | *Phoenix hanceana* (Arecaceae; solitary palm) | Hong Kong | [105] |
|     |         | *Trachycarpus fortunei* (Arecaceae; solitary palm) | Japan | [101] |
| 3   | *E. collantensis* | Smilax sp. (Smilacaceae) | Cuba (Holotype) | [106] |
| 4   | *E. indicus* | twigs of woody | India (Holotype) | [107] |
| 5   | *E. indumentum* | *Livistona chinensis var. boninensis* (Arecaceae; solitary palm) | Japan (Holotype) | [101,104] |
|     |         | *Phoenix canariensis* (Arecaceae; solitary palm) | Japan | [104] |
| No. | Species                  | Host                      | Country      | Reference   |
|-----|--------------------------|---------------------------|--------------|-------------|
|     |                          | Eudicots                  | Monocots     |             |
| 6   | *Acrocomia mexicana*     | Mexico                     | [108]        |             |
|     | (Areaceae)               |                           |              |             |
|     | *Archontophoenix alexandrae* | Hong Kong (solitary palm) | [105,109]    |             |
|     | (Areaceae; solitary palm)| Malaysia                  | [109]        |             |
|     |                          | Singapore                 | [109]        |             |
|     | *Arenga engleri*         | Hong Kong                 | [105]        |             |
|     | (Areaceae; clustering palm)| Japan                    | [104]        |             |
|     | *Dypsis lutescens*       | Japan                     | [104]        |             |
|     | (=*Chrysalidocarpus lutescens*) | (Areaceae; clustering palm) | [100]        |             |
|     | *Caryota urens*          | Sri Lanka (Holotype)      | [100]        |             |
|     | (Areaceae; solitary palm)|                           |              |             |
|     |                          | Australia                 | [109]        |             |
|     |                          | Ghana                     | [110]        |             |
|     |                          | Hawaii                    | [111,112]    |             |
|     | *Cocos nucifera*         | Japanese                  | [104]        |             |
|     | (Areaceae; solitary palm)| Malaysia                  | [109,113]    |             |
|     |                          | Papua New Guinea          | [114]        |             |
|     | *Coffea arabica*         | Venezuela                 | [115]        |             |
|     | (Rubiaceae)              |                           |              |             |
| 6   | *Draecena fragrans*      | Cuba                      | [116]        |             |
|     | (Asparagaceae)           |                           |              |             |
|     | *Elaeis guineensis*      | Ghana                     | [110]        |             |
|     | (Areaceae; solitary palm)| Myanmar                   | [117]        |             |
|     |                          | Sierra Leone              | [113]        |             |
|     | *Elaeis sp.* (Areaceae; solitary palm) | Japan                  | [104]        |             |
|     | *Licuala longicalycata*  | Thailand                  | [118]        |             |
|     | (Areaceae; solitary palm)|                           |              |             |
|     | *Livistona chinensis*    | Hong Kong                 | [105]        |             |
|     | (Areaceae; solitary palm)|                           |              |             |
|     | *Livistona chinensis var. boninensis* (Areaceae; solitary palm) | Japan       | [104]        |             |
|     | *Livistona rotundifolia* | Taiwan                    | [119]        |             |
|     | (Areaceae; solitary palm)|                           |              |             |
|     | *Livistona speciosa*     | Myanmar                   | [117]        |             |
Table 2. Cont.

| No. | Species | Host                      | Country      | Reference |
|-----|---------|---------------------------|--------------|-----------|
|     |         | Eudicots                  | Monocots     |           |
|     |         | **Nannorrhops ritchiana** (Arecaceae; clustering palm) | Pakistan      | [120]    |
|     |         | **Phoenix canariensis** (Arecaceae; solitary palm) | Japan         | [104]    |
|     |         | **Phoenix hanceana** (Arecaceae; solitary palm) | Hong Kong    | [105,121] |
|     |         | **Phoenix reclinata** (Arecaceae; solitary palm) | Ghana         | [110]    |
|     |         | **Phoenix roebelenii** (Arecaceae; solitary palm) | Japan         | [104]    |
|     |         | **Phoenix roebelenii-senegalensis** (Arecaceae; solitary palm) | Japan         | [104]    |
|     |         | **Ravenala madagascariensis** (Strelitziaceae) | Japan         | [104]    |
|     |         | **Ripogonum scandens** (Ripogonaceae) | New Zealand  | [122]    |
|     |         | **Roystonea borinquena** (Arecaceae; solitary palm) | USA (Florida) | [123]    |
|     |         | **Roystonea regia** (Arecaceae; solitary palm) | Cuba         | [124–127] |
|     |         | **Sabal palmetto** (Arecaceae; solitary palm) | USA (Florida) | [128]    |
|     |         | **Serenoa serrulata** (Arecaceae; clustering and solitary palm) | USA (Florida) | [129]    |
|     |         | **Smilax sp.** (Smilacaceae) | USA (Florida) | [128]    |
|     |         | **Trachycarpus fortunei** (Arecaceae; solitary palm) | China         | [109]    |
|     |         | unknown, palm             | Australia    | [109]    |
|     |         |                           | China         | [109]    |
|     |         |                           | Hong Kong    | [109]    |
|     |         |                           | Malaysia     | [109]    |
|     |         |                           | Seychelles   | [109]    |
|     |         |                           | Singapore    | [109]    |
|     |         | **Wodyetia bifurcata** (Arecaceae; solitary palm) | Florida       | [123]    |
|     |         | **E. melannoxanthus** (=E. melannoxanthus var. grossus) |              |           |
|     |         | **Trachycarpus fortunei** (Arecaceae; solitary palm) | Japan         | [101]    |
### Table 2. Cont.

| No. | Species | Eudicots | Monocots | Country | Reference |
|-----|---------|----------|----------|---------|-----------|
| 6   | E. melanoxanthus (≡ E. melanoxanthus var. melanoxanthus) | | | | |
| 7   | E. thwaitesii (Type species) | | | | |

#### Eudicots

- **Acrocomia intumescens** (Arecaceae; solitary palm) - Brazil [102]
- **Butia yatay** (Arecaceae; solitary palm) - Argentina [130]
- **Cocos nucifera** (Arecaceae; solitary palm) - Ghana [101]
- **Euterpe edulis** (Arecaceae; solitary, or rarely clustering palm (growing in dense tufts or clumps) and then with few stems) - Argentina [130]
- **Euterpe oleracea** (Arecaceae; clustering palm) - Brazil [102]
- **Livistona chinensis var. boninensis** (Arecaceae; solitary palm) - Japan [101]
- **Livistona chinensis var. subglobosa** (Arecaceae; solitary palm) - Japan [101]
- **Phoenix canariensis** (Arecaceae; solitary palm) - Japan [101]
- **Phoenix roebelenii** (Arecaceae; solitary palm) - Japan [101]
- **Satakentia liukiuensis** (Arecaceae; solitary palm) - Japan [101]
- **Syagrus coronata** (Arecaceae; solitary palm) - Brazil [131]
- **Syagrus romanzoffiana** (Arecaceae; solitary palm) - Argentina [130]
- **Trachycarpus fortunei** (Arecaceae; solitary palm) - Japan [101]
- **Washingtonia robusta** (Arecaceae; solitary palm) - Japan [101]

#### Monocots

- **Cissus oreophila** (Vitaceae) - Ghana [132]
- **Cissus sp.** (Vitaceae) - Ghana [133]
- **Oncosperma sp.** (Arecaceae; clustering, rarely solitary palm) - Sri Lanka (Holotype) [133]

* Have molecular data.
Figure 3. *Endocalyx metroxyli* (MFLU 15-1454, holotype). (A) Forest in Krabi Province. (B) Palm samples. (C–E) Appearance of conidiomata on host. (F) Vertical cut of a conidioma. (G–H) Vertical section of a conidioma. (I) Section of peridium. (J) Group of conidia. (K) Conidiophores reduced to conidiogenous cell with conidium. (L–S) Conidia (P–R, Conidia with conidiogenous cells). (T) Germ slit (red arrow). (U) Germinated conidia. (V) Colonies on MEA media. Scale bars: B = 2 cm, C = 500 μm, D–H = 200 μm, I, J = 20 μm, L–T = 5 μm, U = 10 μm.
Recently, *Longiappendispora* was introduced under Cainiaceae, with seven genera in total included in the family by Mapook et al. [11]. In our study, detailed molecular analyses were done for *Endocalyx* and its placement in Cainiaceae (Xyaliales) was confirmed. Previously, *Endocalyx* was classified in Apiosporaceae (Xylariales, Sordariomycetes) based on morphological evidence. As the first detailed molecular data of *Endocalyx cinctus* have been made available from a Japan laboratory [32], their current placement is supported (Figure 1). However, there are no recent publications referring to the molecular data of this genus yet. Thus, in this study, we present the placement of *Endocalyx* based on multigene phylogenetic analyses with recent sequence data from the Japan collection as well as the Thailand collection. In addition, we accept eight genera in Cainiaceae (*Alishanica*, *Amphibambusa*, *Arecophila*, *Atrotorquata*, *Cainia*, *Endocalyx*, *Longiappendispora*, and *Seynesia*), and seven species by including our new species in the genus *Endocalyx* (Table 2). In addition, we provide a key for the members of Cainiaceae.

3.1.4. *Endocalyx metroxyli* Konta & K.D. Hyde. sp. nov.

**Index Fungorum number:** IF558116, **Facesoffungi number:** FoF09176 (Figure 3)

**Etymology:** Refers to the name of the host genus, *Metroxylon*.

**Holotype:** MFLU 15-1454.

*Saprobic* on dead petiole of *Metroxylon sagu*. **Colonies** on host plant, pustules. **Sexual morph:** Undetermined. **Asexual morph:** **Conidiomata** 340–660 µm wide, in vertical section 495–820 × 325–485 µm, acervulus, solitary, semi-immersed to immersed in the host epidermis, beneath a clypeus, visible as slightly raised and blackened, black, carbonaceous, fragile, with an ostiolar canal. **Ostiolar opening** surrounded by a yellow margin. **Peridial wall** 34–80 µm wide, thick, comprising dark brown cells of *textura angularis*. **Conidiomata** not observed with a basal cylinder covering a central column or a slender central column in our collection. **Conidiophores** reduced to conidiogenous cell, hyaline to pale-brown, unbranched, smooth. **Conidia** 13–16 × 7–10 µm (x = 13 × 10 µm, n = 30), unicellular, ellipsoid-globose, brown to dark brown, with short pegs bearing conidia, with germ slit, smooth-walled.

**Culture characteristics:** Colonies on MEA, at first white, raised, effuse, velvety to hairy, circular, smooth at the margin, white from above, pale-brown from below.

**Material examined:** Thailand, Krabi Province, on dead petiole of *Metroxylon sagu* Rottb. on the ground (Areaceae), 8 December 2014, Sirinapa Konta KBR04h2 (MFLU 15-1454, holotype); ex-type living culture, MFLUCC 15-0723A; *ibid*. MFLUCC 15-0723B, MFLUCC 15-0723C.

**Additional sequence data:** SSU: MT929310, MT929311, *tef1*-α: MT928152, MT928153.

**Notes:** *Endocalyx metroxyli* is phylogenetically well supported and is placed in Cainiaceae (Figure 1). *Endocalyx metroxyli* is closely related to *E. cinctus* with high bootstrap support but is distinct in morphological characteristics. A BLAST search of *E. metroxyli* ITS sequence shows 83.10% similarity with *Requienella seminuda* (CBS 140502) (CPC 37069), LSU sequence shows 96.14% similarity with *Entosordaria quercina* (RQ), *tub2* sequence shows 86.94% similarity with *Daldinia dennisi var. dennisi*, SSU sequence shows 97.92% similarity with *Xenoanthostomella chromolaenae* (MFLUCC 17-1484), and *tef1*-α sequence shows 89.39% similarity with *Barrmaelia macrospor* (BM).

*Endocalyx metroxyli* is morphologically similar to *E. melanoxanthus*. However, *Endocalyx metroxyli* does not have erect conidiomata developing from the pustules, as was mentioned by Petch [100], Okada and Tubaki [101], and Vitoria et al. [102,131]. In this study, we found only a black raised pustule structure with ostiole surrounded by a yellow hyphae ring, and hyaline conidiophore, unicellular, dark brown conidia with a longitudinal germ slit. *Endocalyx melanoxanthus* was collected and described from palm hosts (Areaceae), and a few collections were collected from other host plants (Table 2). According to Species Fungorum [134], *E. melanoxanthus var. Grossus* (G. Okada & Tubaki) and *E. melanoxanthus var. melanoxanthus* (Berk. & Broome) are considered as *E. melanoxanthus*, even though they have several different characteristics.
Endocalyx metroxyli is morphologically similar to *E. melanoxanthus var. melanoxanthus*, in having black raised pustules surrounded by yellow hyphae and smooth-walled conidia with no significant size differences [100–102]. However, our new taxon lacks cupulate or cylindrical conidiomata [101,102]. On the other hand, *E. metroxyli* differs from *E. melanoxanthus var. grossus* by lacking the production of ornamented conidia [100,101].

Keys to genera of Cainiaceae

1. Asexual morph
   1.1 Coelomycetous; 1–3 phialides conidiogenous cells, and elongate fusiform conidia with unicellular or septate, with pointed ends
   1.1 Coelomycetous; conidiomata with ostiolar opening surrounded by yellow, with unicellular conidia, ellipsoid-globose, pale to dark brown to black, with a straight germ slit extending the full-length

2. Sexual morph
   2.1 Cylindrical-clavate asci, ascospores with 1-septate
   2.1 Cylindrical, or cylindrical to elongate cylindrical asci, ascospores with 1-septate
   2.2 Ellipsoidal ascospores, with brown, and sheath
   2.2 Ellipsoidal to fusiform ascospores, with brown, and sheath
   2.3 Ellipsoid to broadly fusiform ascospores, longitudinal striations, bristle-like polar appendages from both ends, without a gelatinous sheath
   2.3 Fusiform to broad-fusiform ascospores with pointed at both ends, striation wall, and sheath
   2.3 Ellipsoidal or oblong ascospores
   2.4 Oblong ascospores with cap-like appendage, germ slits
   2.4 Ellipsoidal ascospores
   2.5 Ascospores with striation wall, brown, and sheath
   2.5 Ascospores with striate or verrucose wall, and subhyaline to brown

4. Discussion

Based on phylogeny and morphological characteristics, the new monotypic genus *Haploanthostomella* (type species: *Haploanthostomella elaeidis*) and the new species *Endocalyx metroxyli* have been established. The former new species was isolated from a dead rachis of *Elaeis guineensis*, and the latter from a dead petiole of *Metroxylon sagu* (Areaceae) in Thailand. Phylogenetic analyses of combined datasets together with morphological characteristics revealed that *Haploanthostomella* belongs to Xylariales incertae sedis, while *Endocalyx* belongs to the Cainiaceae (Xylariales).

Based on morphological features, *Endocalyx* was assigned to Apiosporaceae (Amphisphaeriales, Sordariomycetes), together with four other genera, viz. *Appendicospora*, *Arthrinium*, *Dictyoarthrinium*, and *Nigrospora* [3,8]. Later, *Dictyoarthrinium* was transferred to Didymosphaeriaceae (Pleosporales, Dothideomycetes) [135]. According to our phylogenetic analyses (Figure 1), *Arthrinium* and *Nigrospora* should be accepted under the Apiosporaceae, while *Appendicospora* did not clade to this family (supporting information section), and *Endocalyx* fits well within the Cainiaceae.

Interestingly, four out of seven species in the genus *Endocalyx* (*E. melanoxanthus*, *E. cinctus*, *E. indumentum*, and *E. thwaitesii*) were collected from palm hosts (Table 2).
Endocalyx metroxyli is similar to other species by having dark brown conidia with a full-length germ slit, but differs from other species by not having conidiomata produced from the pustulate and no thread-like structure of conidiophores. Morphological characteristics of species in the genus are mostly flat or raised pustules, capsule or slender conidiomata with or without branches at the apex, and brown to dark brown conidia with smooth walls (E. amarkantakensis, E. collantesis, E. indumentum, E. melanoxanthus, E. melanoxanthus var. melanoxanthus), while some species are verrucose-walled (E. cinctus, E. indumentum, E. melanoxanthus var. grossus, E. thwaitesii). We referred to previous publications for morphological comparisons to the taxa in this study, as we did not observe all holotype specimens [100–102].

According to the literature, there are also strains derived from another two species and two varieties. Excluding E. cinctus, no sequence data are available for generic types of Endocalyx and other species, and their morphology and host substrates are closely related to our novel taxon. Endocalyx species have been reported in several countries, especially in tropical and subtropical regions. Furthermore, palm trees (Areaceae) have most commonly been reported as the host, while several species have been presented from other hosts (Table 2).

The phylogenetic placement of many groups within the Xylariales remains unclear (e.g., Anthostomelloides, Calceomyces, Circinotrichum, Fasciatispora (only F. petrakii), Gyrothyrix, Melanographium, Neanthostomella, Pseudoanthostomella, and Xenoanthostomella, Figure 1). Thus, it is necessary to collect and analyze more fungal specimens from Xylariales using multigene phylogeny (with protein coding genes) and morphology to resolve their taxonomical placement and delimitation.

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