Is Hyperdermium Congeneric with Ascopolyergus? Phylogenetic Relationships of Ascopolyergus spp. (Cordycipitaceae, Hypocreales) and a New Genus Neohyperdermium on Scale Insects in Thailand

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Abstract: During surveys of insect pathogenic fungi (IPF) in Thailand, fungi associated with scale insects and plants were found to represent five new species of the genus Ascopolyergus in Cordycipitaceae. Their macroscopic features resembled both Hyperdermium and Ascopolyergus. Morphological comparisons with the type and known Ascopolyergus and Hyperdermium species and phylogenetic evidence from a multigene dataset support the appointment of a new species of Ascopolyergus. Moreover, the data also revealed that the type species of Hyperdermium, H. caulium, is nested within Ascopolyergus, suggesting that Hyperdermium is congeneric with Ascopolyergus. The specimens investigated here differ from other Ascopolyergus species by phenotypic characters including size and color of stromata. Phylogenetic analyses of combined LSU, TEF1, RPB1 and RPB2 sequences strongly support the notion that these strains are distinct from known species of Ascopolyergus, and are proposed as Ascopolyergus albus, A. gallocatechiei, A. griseoperitheciatus, A. khaoyaiensis and A. purpuratus. Neohyperdermium gen. nov. is introduced for other species originally assigned to Hyperdermium and Cordycipitae occurring on scale insects and host plants as epitheca, accommodating two new combinations of Hyperdermium pulvinatum and Cordycipitae pipers.

Keywords: Cordycipitaceae; epiphyte; insect pathogenic fungi; multigene phylogeny; scale insects

1. Introduction

Scale insects are a diverse group of sap-sucking insects in the superfamily Coccoidea of the order Hemiptera, associated with aphids (Aphidoidea) and whiteflies (Aleurodoidae) [1,2]. These insects cause damage by sucking fluids from leaves, stems and other parts of host plants and excrete honeydew that favors sooty mold growth, which consequently decreases photosynthetic rates. They belong to seven families: Antennariellaceae, Capnodiaceae, Chaetothyriaceae, Coccodiniaceae, Euantennariaceae, Metacapnodiaceae and Trichomeriaceae [3–5]. In addition, many groups of fungi are known to grow on various scale insects by covering the whole surface of the insect body and can be found in the phyla (a) Basidiomycota: Septobasidiales (Septobasidium and Uredinella), (b) Chytridiomycota: Blastocladiales (Myiophagus) and (c) Ascomycota: Myriangiales (Myriangium), Pleosporales (Podonectria), and especially in a large group of entomopathogens in the Hypocreales [6–9].
Hypocrealean fungi associated with armored (Diaspididae) and soft-scale insects (Coccidae) can be found in various genera within five families: (1) Bionectriaceae viz. Clonostachys Corda; (2) Nectriaceae viz. Microcera Desm. and Fusarium Link; (3) Cordycipitaceae viz. Ascopolyergus Möller, Cordyceps Fr. and Hyperdermium J.F. White, R.F. Sullivan, Bills and Hywel-Jones; (4) Ophiocordycipitaceae viz. Ophiocordyceps Petch; and (5) Clavicipitaceae viz. Aschersonia Mont., Conioideocrella D. Johnson, G.H. Sung, Hywel-Jones and Spatafora, Dussiella Pat., Helicocollum Luangsa-ard, Mongkols., Noisrip. and Thanakitp., Hypocrella Sacc., Regiocrella P. Chaverri and K.T. Hodge, Orbiocrella D. Johnson, G.H. Sung, Hywel-Jones and Spatafora and Samuelsia P. Chaverri and K.T. Hodge [9–21]. Among them, the most abundant and widespread members are found in Clavicipitaceae and Cordycipitaceae.

The macromorphological characters of these genera in nature can be easily distinguished in each family. Scale insect pathogenic genera in Clavicipitaceae, such as Conioideocrella, Hypocrella, Moelleriella and Orbiocrella, possess diverse morphological characters, such as the formation of hard stromata, pulvinate, subglobose or hemispherical and ring-like stromata, as well as the presence of only superficial, cone-shaped perithecia, while in Cordycipitaceae, most have pulvinate, subglobose, hemispherical, soft stromata with crowded perithecia. Two different colors are found the upper and lower surface of stromata in some species of Ascopolyergus and Hyperdermium [11,12,14,16].

Ascopolyergus is an epiphytic fungal genus in Cordycipitaceae that produces stromata on the stems of living plants as biotrophs and infects scale insects as necrotrophs comprising only seven species [22]. Ascopolyergus species are commonly found in tropical forests where bamboo is present [23]. The type species of Ascopolyergus, A. polychrous, is a pathogen of bamboo scale insects that produces up to 4 cm large subglobose to polypore-like, bright rusty-red or white to yellow perithecial stromata, which are usually fertile only on the underside of the stroma [12,13,24]. In 2005, a new species of Ascopolyergus, A. philodendrus, was described by Bischoff et al. [14] on bamboo scale insects, and a new description for A. villosus was made. They considered that the morphology of perithecial stromata and the conidial states of Ascopolyergus resemble the scale insect pathogenic genus Hyperdermium, especially its type species, H. caulium [11,14]. Both of these species in the two genera share similar morphological characters, having large stromata, immersed perithecia, filiform ascospores and phialidic conidiogenous cells. The anamorph state is referred to as cylindrocarpon-like phialides, characterized by producing multisepate conidia, a unique character in the Cordycipitaceae. Moreover, a species of Cordyceps, C. piperis, is also capable of parasitizing scale insects but differ by producing verticillium-like anamorph with aseptate conidia [11,12].

During our continuous survey of insect pathogenic fungi (IPF) in national parks and community forests in Thailand, we encountered hyperdermium-like specimens with differences in phenotypic characters including colors and sizes of stromata. These morphologically diverse specimens were preliminarily identified as members of the genus Hyperdermium and Ascopolyergus. The aims of this study are thus (1) to determine the phylogenetic relationship of these two genera and (2) to identify and describe new species of hyperdermium-like fungi on scale insects from Thailand by combining morphological characteristics and reconstructing their phylogeny based on sequence data of LSU, TEF1, RPB1 and RPB2 loci.

2. Materials and Methods

2.1. Collection and Isolation

The 63 epiphytic isolates in this study were collected from various localities in Thailand since June 1992, representing the first recorded collection from Khao Yai National Park, Nakhon Ratchasima Province. Thereafter, these specimens have been found throughout every region in Thailand, albeit not frequently, including the Ban Hua Thung community forest in Chiang Mai Province; Chiang Dao, Khao Soi Dao and Khlong Nakha wildlife sanctuaries; Kaeng Krachan and Khlong Lan national parks; and the Khao Chong wildlife development and conservation promotion station. Specimens were examined for fungal
colonization from the stems and leaves of monocotyledonous and dicotyledonous plants. The specimens were collected and stored in plastic boxes before returning to the laboratory for isolation. Pure cultures were made from the isolation of the sexual morph following Luangsarad et al. [25]. The cultures and the voucher specimens were deposited in Thailand Bioresource Research Center (TBRC) and BIOTEC Bangkok Herbarium (BBH), Thailand, respectively.

2.2. Morphological Study

For obtaining morphological descriptions, all isolates were cultured on oatmeal agar (OA: oatmeal 60 g, agar 12.5 g, in 1 L distilled water, Difco) and potato dextrose agar (PDA: potato 200 g, dextrose 20 g, agar 15 g, in 1 L distilled water) for 14–20 days. Colony morphology was examined for color, size, shape and appearance. Fungal structures of teleomorph and anamorph states were mounted in lactophenol cotton blue solution, and their characters were investigated by light microscopy, as described by Mongkolsamrit et al. [26] and Khonsanit et al. [27]. Sections of the stroma on stems were prepared by using a freezing microtome (Slee Cryostat MEV, Mainz, Germany), and mounted in distilled water and in lactophenol cotton blue solution [28]. The Sixth Royal Horticultural Society (RHS) color chart was used to characterize the colors of fresh specimens and cultures [29]. Twenty to fifty individual length and width measurements were taken, and the amount of variability is provided as average ± standard deviation with absolute minima and maxima in parentheses.

2.3. DNA Extraction, PCR and Sequencing

The mycelial mass of fungi was obtained from cultures grown on PDA for 7 days at 25 °C. A modified CTAB protocol used for DNA extraction using polyvinylpyrrolidone instead of β-mercaptoethanol in CTAB buffer and increasing temperature in the incubation process from 60 °C to 65 °C was previously described by Thanakitpipattana et al. [30]. PCR was used to amplify the nuclear ribosomal large subunits (LSU), the region of the elongation factor 1-α (TEF1), and the largest and second-largest subunits of RNA polymerase II (RPB1 and RPB2). The reaction mix was prepared in 25 μL volumes containing 1× Dream Taq Buffer (with included 20 mM MgCl2), 0.4 M betaine, 200 μM dNTP mix, 0.5 μM of each primer, 1 Unit Dream Taq DNA polymerase (Thermo Scientific, Waltham, MA, USA), 50 ng of DNA template and Milli-Q water. PCR amplifications of four loci were carried out with the following primers: LROR and LR5 for LSU [31,32], 983F and 2218R for TEF1 [33], CRPB1 and RPB1-Cr for RPB1 [34], and RPB2-5F2 and RPB2-7Cr for RPB2 [35,36]. The PCR conditions were performed as follows: 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at a suitable temperature for 1 min, extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. The annealing temperature of each gene was 50 °C for RPB1 and RPB2, and 55 °C for TEF1 and LSU. PCR products were purified and subsequently sequenced with PCR amplification primers.

2.4. Sequence Alignment and Phylogenetic Analyses

The newly generated sequences from the twelve strains in this study were assembled using BioEdit v. 7.2.5 [37] and then deposited in the GenBank database under the accession numbers of TEF1 (OL322029-OL322040), LSU (OL322041-OL322052), RPB1 (OL322053-OL322059) and RPB2 (OL322060-OL322070) (Table 1). Sequences of each locus were aligned using MUSCLE 3.6 [38] together with other sequences of related taxa from previous studies for phylogenetic analyses (see Table 1), and manually refined to minimize gaps. The concatenated (LSU + TEF1 + RPB1 + RPB2) sequences were analyzed by maximum likelihood (ML) and Bayesian inference (BI), both on the CIPRES Science Gateway portal [39]. Maximum likelihood analysis was performed with RAxML-HPC2 on XSEDE v8.2.12 with default parameters [40] using the GTRCAT substitution model with 1000 rapid bootstrap replicates. The program MrModeltest v.2.2 [41] was used to determine the model of evolution under the Akaike Information Criterion (AIC) implemented in PAUP v.4.0a169 [42],
which selected SYM + G as the best nucleotide substitution model. The BI analysis was performed using MrBayes on XSEDE v.3.2.7a with default parameters [43]. The Markov Chain Monte Carlo (MCMC) searches were run for 5,000,000 generations with sampling every 1000 generations and a burn-in value of 10%. Nodes were considered as strongly supported with bootstrap and posterior probability values greater than 70% and 0.7, respectively.

**Table 1.** List of species and GenBank accession numbers of sequences used in this study. Bold accession numbers were generated for this study. The symbol “–” denotes no available data.

| Species | Strain | LSU | TEF1 | RPB1 | RPB2 | References |
|---------|--------|-----|------|------|------|------------|
| Akanthomyces aculeatus | HUA 186145 | MF416520 | MF416465 | – | – | [44] |
| Akanthomyces attenuatus | CBS 402.78 | AF339565 | EF468782 | EF468888 | EF468935 | [45] |
| Akanthomyces lecanii | CBS 101247 | AF339555 | DQ522359 | DQ522407 | DQ522466 | [45] |
| Akanthomyces neoaraneogenus | GZU1032 | KX845704 | KX845698 | KX845700 | KX845702 | [46] |
| Akanthomyces sabinense | ANDES-F 1024 | KC875225 | KC633266 | – | KC633249 | [47] |
| Akanthomyces tuberculatus | OSC 111002 | DQ518767 | DQ522338 | DQ522405 | DQ522493 | [45] |
| Ascopolyporus albus | BCC48975 | OL322048 | OL322035 | OL322056 | – | This study |
| Ascopolyporus albus | BCC48976 | OL322049 | OL322036 | OL322057 | – | This study |
| Ascopolyporus galloides | BCC22358 | OL322050 | – | OL322037 | – | This study |
| Ascopolyporus griseoperitheciatus | BCC22359 | OL322051 | OL322038 | OL322058 | OL322068 | This study |
| Ascopolyporus khaoyaiensis | BCC43314 | OL322052 | OL322039 | – | OL322069 | This study |
| Ascopolyporus polychrous | BCC 111024 | MF416534 | MF416479 | – | MF416341 | [47] |
| Ascopolyporus purpuratus | BCC88388 | OL322046 | OL322033 | – | OL322064 | This study |
| Ascopolyporus purpuratus | BCC88389 | OL322047 | OL322034 | – | OL322065 | This study |
| Akanthomyces foliatus | ARSEF 8043 | DQ118737 | DQ118745 | DQ127230 | DQ127236 | [15] |
| Beauveria bassiana | ARSEF 300 | – | – | – | – | [14,15] |
| Beauveria kipukae | ARSEF 7032 | – | – | – | – | [45] |
| Beauveria staphylinidicola | ARSEF 5718 | EF468836 | EF468776 | EF468881 | – | [45] |
| Blackwellomyces cardinalis | OSC 93609 | AY896525 | AY896525 | AY896525 | AY896525 | – | [17,49] |
| Blackwellomyces pseudomilitaris | BCC 1919 | MF416534 | MF416479 | – | MF416440 | [44] |
| Blackwellomyces pseudomilitaris | BCC 2091 | MF416535 | MF416479 | – | MF416441 | [44] |
| Cordyceps bisifusispora | EFCC 5690 | EF468806 | EF468746 | EF468854 | EF468909 | [45] |
| Cordyceps lepideroporum | TBRC 7263 | MF140699 | MF140819 | MF140768 | MF140792 | [51] |
| Cordyceps piperis | CBS 116719 | AY466442 | DQ118749 | DQ127240 | EU369083 | [15,17] |
| Cordyceps takaomontana | BCC 28612 | FJ765252 | FJ765252 | FJ765252 | FJ765252 | [52] |
| Cordyceps tenuipes | ARSEF 5135 | FJ416203 | FJ416203 | FJ416203 | FJ416203 | [53] |
| Engedodium araneorum | CBS 309.85 | AF339526 | DQ522341 | DQ522387 | DQ522439 | [49] |
| Gibellula leonopus | BBC 16025 | MF416548 | MF416492 | MF416492 | – | [44] |
| Gibellula pulchra | NHJ 10808 | EU369035 | EU369035 | EU369035 | EU369035 | [17] |
| Gibellula sp. | NHJ 10788 | EU369036 | EU369019 | EU369019 | EU369019 | [17] |
| Gibellula sp. | NHJ 13158 | EU369037 | EU369020 | EU369020 | EU369020 | [17] |
| Hevansia arachnophila | NHJ 10469 | EU369031 | EU369008 | EU369008 | EU369008 | [17] |
| Hevansia cinera | NHJ 3510 | EU369032 | EU369008 | EU369008 | EU369008 | [17] |
| Hevansia nelumboides | NHJ 4314 | EU369033 | EU369008 | EU369008 | EU369008 | [17] |
| Hyperdermium caulium | AF242354 | MF416530 | MF416473 | MF416473 | MF416437 | [44] |
| Hyperdermium pulvinatum | P.C. 602 | DQ118738 | DQ118746 | DQ127237 | DQ127237 | [15] |
| Lecanicillium antillanum | CBS 350.85 | AF339565 | DQ522350 | DQ522396 | DQ522450 | [45] |
| Lecanicillium psalliota | CBS 363.86 | AF339559 | EF468784 | EF468890 | – | [45] |
Table 1. Cont.

| Species                     | Strain      | GenBank Accession No.          | References |
|-----------------------------|-------------|--------------------------------|------------|
|                             |             | LSU                | TEF1  | RPB1 | RPB2 |          |
| Lecanicillium psalliottae   | CBS 532.81  | AF339560 EF469067  | EF469096 | EF469112 | [45] |
| Neotorrubiella chinghridicola | BCC 39684  | MK632096 MK632148  | MK632071 | MK632181 | [30] |
| Neotorrubiella chinghridicola | BCC 80733  | MK632097 MK632149  | MK632072 | MK632176 | [30] |
| Samsoniella aurantia        | TBRC 7271   | MF140728 MF140846  | MF140791 | –          | [51] |
| Samsoniella aurantia        | TBRC 7273   | MF140726 MF140844  | –        | MF140815 | [51] |
| Samsoniella inthanonensis   | TBRC 7915   | MF140725 MF140849  | MF140790 | MF140815 | [51] |
| Samsoniella inthanonensis   | TBRC 7916   | MF140724 MF140848  | MF140789 | MF140814 | [51] |
| Outgroup                    |             |                   |            |          |      |          |
| Flavocillium bifurcatum     | YFCC 6101   | MN576781 MN576951  | MN576841 | MN576897 | [54] |
| Lecanicillium sp.           | CBS 639.85  | KM283801 KM283824  | KM283843 | KM283865 | [54] |

3. Results

3.1. Molecular Phylogeny

The combined four-gene dataset of 54 taxa consisted of 3404 bp (LSU 861 bp, TEF1 954 bp, RPB1 730 bp, RPB2 859 bp). Flavocillium bifurcatum and Flavocillium sp. in Cordycipitaceae were used as an outgroups. Phylogenetic tree topology obtained from ML was similar to the BI analysis. Therefore, only the ML tree is shown (Figure 1). Multigene phylogenetic analyses revealed that the sequenced strains comprise five novel species and are nested with the type and other species of Ascopolyporus, A. polychrous and A. villosus, as well as type species of Hyperdermium, H. caulium, within the Ascopolyporus clade, with strong support (81% ML bootstrap (MLBS) and 0.99 BI posterior probability (BIPP)), as shown in Figure 1. The type species H. caulium is clustered within this clade, suggesting that Hyperdermium is congeneric with Ascopolyporus, although with low internal bootstrap support because only LSU sequence data are available (<50 MLBS and <0.5 BIPP, data not shown).

Three of our new species are found in the pulvinate subclade showing irregularly subglobose to globose stromata, namely, Ascopolyporus albus, A. galloides and A. griseoperithecatus, with 93% MLBS and 0.97 BIPP. Another subclade comprises both flattened and pulvinate stromata of two new and known species, including Ascopolyporus khaoyaiensis, A. purpuratus, A. polychrous, A. villosus and H. caulium (Figure 1). The Ascopolyporus clade is sister to the Blackwollomyces clade, which produces similar types of phialides and conidial arrangement as well as acrémone-like or lecanicillium-like anamorphs.

The position of Hyperdermium pulvinatum and Cordyceps piperis, on the other hand, is clearly distant from the Ascopolyporus clade, and these two species always clustered together separate from the type species of Hyperdermium, H. caulium. These two species form a basal clade to Akanthomyces, Samsoniella, Beauveria and Cordyceps. Based on their multigene phylogenetic position presented in this study, we propose to transfer these two species to the genus Neohyperdermium.

3.2. Taxonomy

**Ascopolyporus** Möller emend. Thanakitpippattana and Luangsa-ard.

Stromatal mass exceeding scale insect host. *Stroma* bulbous (lumpy or tuberous) or ungelate, flattened or pulvinate, fleshy or gall-like, polypore-like, white, yellowish white, purple to orange; sterile surface and fertile underneath the stroma. *Perithecia* semi-immersed to immersed, ovate to obclavate or cone-shaped. *Asci* hyaline, filiform. *Ascospores* hyaline, whole with septation or aseptate. Conidiogenous cells phialidic, solitary, slightly curved. *Conidia* hyaline, fusiform to subcylindrical, acerose, aseptate or 1–5 septate when mature, in chains or in sticky heads.

*Typification:* Ascopolyporus polychrous.

**Habit and type host:** On dead culms of bamboo, stems or leaf midrib of monocotyledonous and dicotyledonous plants.
Distribution: Argentina, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, Peru, Thailand [23]. *Ascopolyporus albus* Mongkolsamrit, Thanakitpipattana and Luangsa-ard sp. nov.

**Figure 1.** Phylogenetic reconstruction of *Ascopolyporus* and related genera in the *Cordycipitaceae* obtained from the combined LSU, *TEF1*, *RPB1* and *RPB2* sequence dataset based on maximum likelihood (RAxML) and Bayesian inference. Numbers on the nodes are ML bootstrap and Bayesian posterior probability values above 70% (MLBS) or 0.7 (BIPP). Thickened lines mean support for the two analyses was 100% (MLBS) or 1.0 (BIPP). □ represents species with pulvinate stromata while □ represents species with flattened stromata.
Figure 2. *Ascopolyporus albus*. (a) Stromata on living stem of bamboo (*Bambusae*); (b) cross-section through stroma showing perithecia (BBH30734); (c) perithecia; (d,e) asci; (f) ascospores; (g) colony obverse on OA; (h) colony reverse on OA; (i,j) phialide apex with conidial head on OA; (k) conidia on OA; (l) colony obverse on PDA; (m) colony reverse on PDA; (n,o) phialide and conidia on PDA; (p) conidia on PDA. Scale bars: (g,h,l,m) = 10 mm; (a) = 5 mm; (b) = 200 µm; (c) = 100 µm; (d,f,j,n,o) = 20 µm; (e,k,p) = 10 µm.

**Mycobank**: MB 841855.

**Etymology**: From the Latin “albus”, referring to the white color of the fresh stromata.

**Typification**: Thailand, Chiang Mai Province, Chiang Dao Wildlife Sanctuary, Doi Chiang Dao Wildlife Research Station; 19°23'10.70" N, 98°50'28.50" E, on scale insects (*Coccidae*: Hemiptera), on living stem of bamboo (*Bambusae*), 17 August 2011, K. Tasanathai (K.T.), P. Srikitikulchai (P.S.), S. Mongkolsamrit (S.M.), A. Khonsanit (A.K.) (holotype BBH30734, ex-holotype culture BCC48975). GenBank: ITS = OL331502, LSU = OL322048, TEF1 = OL322035, RPB1 = OL322056, RPB2 = OL322065.

**Description**: Stromata epibiotic, pulvinate, subglobose, globose, white (NN155C) to pinkish white (N155B), becoming dark brown when old, 3–6 mm wide, 2–3 mm thick. Perithecia semi-immersed, with slightly protruding orifices, obpyriform, 250–320 × 100–120 µm. Ascii cylindrical up to 250 µm long and 3–4 µm wide, Asci caps 2–3 × 3–4 µm. Ascospores hyaline, whole, filiform, multisepertate, 95–135 × 1 µm.

**Culture characteristics**: Colonies on OA attaining a diameter of 3.5–4 cm in 14 days, slightly convex to the agar surface, white, reverse moderate orange yellow (164C). Phialides arising from aerial hyphae, solitary, cylindrical, slightly curved, up to 80 µm long, 1–2 µm wide. Conidia hyaline, enteroblastic, fusiform to acerose, early in development asceptate, becoming 1–4 septa, aggregated at the apex of the phialides, (8–)10–23(–28) × (2–)2.5–3 µm.
Colonies on PDA attaining a diameter of 3.5–4 cm in 14 days, slightly convex to the agar surface, white, reverse light yellow (162C). Phialides arising from aerial hyphae, solitary, cylindrical, slightly curved, up to 120 µm long, 1–2 µm wide. Conidia hyaline, enteroblastic, fusiform to acerose, early in development aseptate, developing 1–4 septa, aggregated at the apex of the phialides, (8–)10.5–21(–30) × (2–)2.5–3(–3.5) µm.

Habitat: On scale insects (Coccidae; Hemiptera), found on living stems of bamboo (Bambuseae).

Additional specimen examined: Thailand, Chiang Mai Province, Chiang Dao Wildlife Sanctuary, Doi Chiang Dao Wildlife Research Station; 19°23′10.70″N, 98°50′28.50″E, on scale insects (Coccidae; Hemiptera), on the living stems, 17 August 2011, K.T., P.S., S.M., A.K. (BBH30734, BCC48976). GenBank: ITS = OL331503, LSU = OL322049, TEF1 = OL322036, RPB1 = OL322057, RPB2 = OL322066.

Notes: Ascopolyporus albus significantly differs from other species in Ascopolyporus herein. The difference is in the color of stromata. Ascopolyporus albus produces white to pinkish white stromata (Figure 2), whereas other species produce very pale violet (91D) to yellowish white (158) with strong orange (25A) stromata. Based on Ascopolyporus species from Thailand, the perithecia of A. albus are semi-immersed, similar to those in A. galloides, A. griseoperitheciatus and A. purpuratus. The perithecial shape of A. albus differs from A. galloides, A. griseoperitheciatus and A. purpuratus by having an obpyriform shape, whereas perithecia in A. galloides, A. griseoperitheciatus and A. purpuratus are obclavate, obovoid and ovoid, respectively.

Ascopolyporus caulium (Berk. and M.A. Curtis) Thanakitpipattana and Luangsa-ard, comb. nov. MycoBank: MB 842779.

≡ Corticium caulium Berk. and M.A. Curtis, J. Acad. nat. Sci. Philad. 2: 279. 1854.
≡ Hypocrella caulium (Berk. and M.A. Curtis) Pat., Bull. Soc. Mycol. France 30: 346. 1915.
≡ Hyperdermium caulium (Berk. and M.A. Curtis) P. Chaverri and K.T. Hodge, 2008.
= Hypocrella camerunensis (Henn.) Mains, Mycopath. Myc. Appl. 11: 311. 1959.
≡ Stigmatae brasiliana Henn., Hedwigia 36: 230. 1897.
≡ Hypocrella camerunensis var. brasiliana Henn., Hedwigia 43: 85. 1904.
= Hyperdermium bertonii J.F. White, R.F. Sullivan, Bills and Hywel-Jones, Mycologia 92: 910. 2000.
≡ Epichloë bertonii Speg., An. Mus. Nac. Hist. Nat. Buenos Aires 31: 416. 1922.

Ascopolyporus galloides Khonsanit, Thanakitpipattana and Luangsa-ard sp. nov. Figure 3.

MycoBank: MB 841853.

Etymology: Refers to the character of the stromata, which look similar to plant galls.

Typification: Thailand, Nakhon Ratchasima Province, Khao Yai National Park, Mo Singto Nature Trail; 14°26′21.46″N, 101°22′20.20″E, on scale insects (Coccidae; Hemiptera), on the living stems of dicotyledonous plant, 5 July 2011, A.K., K.T., K. Sansatchanon (K.S.), P.S., S.M., W. Noisripoom (W.N.) (holotype BBH30629, ex-holotype culture BCC48704). GenBank: ITS = OL331509, LSU = OL322044, TEF1 = OL322031, RPB1 = OL322055, RPB2 = OL322062.

Description: Stromata epibiotic, pulvinate, subglobose, hemispherical, upper surface white (NN155B) to yellowish white (158); lower surface strong orange (N25C), 1–7 mm wide. Perithecia semi-immersed, crowded, obclavate, (129–)133–153.5(–175) × (3–)3.5–5(–6) µm. Asci cylindrical, (129–)133–153.5(–175) × (3–)3.5–5(–6) µm. Asci caps 1–2 × 2.5–4 µm. Ascospores hyaline, whole, filiform, aseptate, (131–)154.5–211.5(–216) × 0.5 µm.

Culture characteristics: Colony on OA attaining a diameter of 3.5–4 cm in 20 days, flat, slightly convex to the agar surface, white (158), reverse pale greenish yellow (2D). Phialides arising from aerial hyphae, solitary, cylindrical, slightly curved, 35–161 × 1–2 µm. Conidia hyaline, enteroblastic, fusiform to acerose, early in development aseptate, becoming 1–3 septa, aggregated at the apex of the phialides, (4–)6–22(–34) × (1.5–)2–2.5(–3) µm.
**Figure 3.** *Ascopolyergus gallaoides.* (a,b) Stromata on living stem of dicotyledonous plant (BBH48704); (c) cross-section through stroma showing perithecia; (d) perithecia; (e) asci; (f) asci-caps; (g) ascospores; (h) colony obverse on OA; (i) colony reverse on OA; (j–l) phialide apex with conidial head on OA; (m) conidia on OA; (n) colony obverse on PDA; (o) colony reverse on PDA; (p–r) phialide apex with conidial head on PDA; (s) conidia on PDA. Scale bars: (h,i,n,o) = 10 mm; (a) = 5 mm; (b) = 1 mm; (c) = 200 μm; (d,g) = 100 μm; (j,k,l,q,r) = 20 μm; (e,f,m,p,s) = 10 μm.

Colony on PDA attaining a diameter of 4 cm in 20 days, fluffy in the middle, flat to umbonate, white in the middle, pale orange yellow (23D), light yellow (11B), brilliant yellow (11A), reverse moderate orange (173C) in the middle, strong orange yellow (N163D), light yellow (14D). *Phialides* arising from aerial hyphae, solitary, cylindrical, slightly curved, 30–294 × 1–2 μm. *Conidia* hyaline, enteroblastic, fusiform to acerose, cylindrical, early in development aseptate, becoming 1–4 septa, aggregated at the apex of the phialides, (5–)8–16(–27) × (2–)2.5–3.5(–4) μm.

*Habitat:* On scale insects (*Coccidae*, *Hemiptera*), found on living stems of dicotyledonous plant.
Additional specimens examined: Thailand, Ranong Province, Khlong Nakha Wildlife Sanctuary, Khlong Nakha Nature Trail; 9°27′33″ N, 98°30′16″ E, on scale insects (Coccidae; Hemiptera), on living stems of dicotyledonous plant, 5 October 2004, B. Thongnuch (B.T.), D. Johnson (D.J.), K.T., S.M., W. Chaygate (W.C.) (BBH10163, BCC16408; BBH10176, BCC16419; BBH10177, BCC16420; BBH10178, BCC16421; BBH10179, BCC16422; BBH10180, BCC16423); Nakhon Nayok Province, Khao Yai National Park, Tat Ta Phu Waterfall Nature Trail; 14°26′21.46″ N, 101°22′20.00″ E, on scale insects (Coccidae; Hemiptera), on living stems of dicotyledonous plant, 24 August 2005, K.T. (BBH14835, BCC18980); Phetchaburi Province, Kaeng Krachan National Park, Ban Krang Camp Nature Trail; 12°54′05″ N, 99°37′48″ E, on scale insects (Coccidae; Hemiptera), on living stems of dicotyledonous plant, 14 November 2005, B.T., K.T., R. Ridkaew (R.R.), W.C. (BBH15034, BCC19720); Ranong Province, Khlong Nakha Wildlife Sanctuary, Khlong Nakha Nature Trail; 9°27′33″ N, 98°30′16″ E, on scale insects (Coccidae; Hemiptera), on living stems of dicotyledonous plant, 10 January 2006, B.T., K.T., L.N. Yen (L.N.Y.), L.T. Huyen (L.T.H.), PS, SM, WC (BBH16500, BCC20115); 12 January 2006, B.T., K.T., L.N.Y., L.T.H., P.S., S.M., W.C. (BBH16554, BCC21023); Nakhon Ratchasima Province, Khao Yai National Park, Bueng Phai Nature Trail; 14°26′21.46″ N, 101°22′20.00″ E, on scale insects (Coccidae; Hemiptera), on living stems of dicotyledonous plant, 5 July 2006, B.T., J. Luangs-ard (J.J.L.), K.T., S.M., W.C. (BBH18631, BCC22237; BBH18632, BCC22238); Chanthaburi Province, Khao Soi Dao Wildlife Sanctuary, Withiphrai Nature Trail; 13°06′13″ N, 102°11′39″ E, on scale insects (Coccidae; Hemiptera), on living stems of dicotyledonous plant, 1 May 2007, B.T., K.T., R.R., S.M., W.C. (BBH19873, BCC25446; BCC25447; BCC25448); Nakhon Ratchasima Province, Khao Yai National Park, km. 33 Nature Trail; 14°26′21.46″ N, 101°22′20.00″ E, on scale insects (Coccidae; Hemiptera), on living stems of dicotyledonous plant, 8 August 2007, B.T., P. Puyngain (P.P.), W.C. (BBH22627, BCC26680), Trang Province, Khao Chong Wildlife Development and Conservation Promotion Station, 1.8 km. Nature Trail; 7°32′57″ N, 99°47′11″ E, on scale insects (Coccidae; Hemiptera), on living stems of dicotyledonous plant, 18 September 2007, B.T., K.T. (BBH23089, BCC27812); Nakhon Ratchasima Province, Khao Yai National Park, Mo Singto Nature Trail; 14°26′21.46″ N, 101°22′20.00″ E, on scale insects (Coccidae; Hemiptera), on living stems of dicotyledonous plant, 16 August 2009, K.T., P.S., R.R., S.M., T. Chohmee (T.C.) (BBH30139, BCC36656); 20 July 2009, K.T., P.S., R.R., S.M., T.C. (BBH27634, BCC37668); 25 July 2009, K.T., P.S., R.R., S.M. (BCC37879); Khao Yai National Park, km. 29 Nature Trail; 14°26′21.46″ N, 101°22′20.00″ E, on scale insects (Coccidae; Hemiptera), on living stems of dicotyledonous plant, 2 June 2011, A.K., K.T., K.S., P.S., S.M., W.N. (BBH30577, BCC47981); GenBank: ITS = OL331511, LSU = OL322043, TEFl = OL322030, RPB1 = OL322054, RPB2 = OL322061; Khao Yai National Park, Mo Singto Nature Trail; 14°26′21.46″ N, 101°22′20.00″ E, on scale insects (Coccidae; Hemiptera), on living stems of dicotyledonous plant, 3 August 2011, A.K., K.T., K.S., P.S., S.M., W.N. (BBH30683, BCC48951).

Notes: Based on the macromorphologies of the natural samples, the lower surface of stromata of A. galoides and A. griseoperitheciatus are orange and their perithecial layers are white and pale violet to light purplish gray, respectively. The perithecia in these two species are semi-immersed, but perithecia in A. galoides are obclavate, whereas those of A. griseoperetheciatus are obovoid. The colony color of A. galoides is pale orange, yellow, light yellow and brilliant yellow, whereas A. griseoperetheciatus is white and produces a pale purplish pink pigment diffusing in the medium.

Ascopolyporus griseoperitheciatus Khonsanit, Thanakitpipattana and Luangs-ard sp. nov. Figure 4.
Figure 4. Ascopolyporus griseoperitheciatus. (a,b) Stromata on living stem of dicotyledonous plants (a BBH18679, b BBH30155); (c,d) perithecia; (e) asci; (f) asci-caps; (g) colony obverse on OA; (h) colony reverse on OA; (i) microsclerotium-like structure on OA; (j) phialide apex with conidial head; (k) conidium germination; (l) phialide and conidia on OA; (m) conidia on OA; (n) colony obverse on PDA; (o) colony reverse on PDA; (p) conidia germination; (q–s) phialide and conidia on PDA; (t) conidia on PDA. Scale bars: (a,g,h,n,o) = 10 mm; (b) = 5 mm; (c) = 100 µm; (d,e,l) = 50 µm; (p,q,r,s) = 20 µm; (i,j,k,m,t) = 10 µm; (f) = 5 µm.

**MycoBank**: MB 841854.

**Etymology**: From the Latin “griseo”, referring to the gray color of the fresh stromata.

**Typification**: Thailand, Kamphaeng Phet Province, Khlong Lan National Park, Khlong Lan Waterfall; 16°07′50.20″ N, 99°16′36.30″ E, on scale insects (Coccidae; Hemiptera), on the living stems of dicotyledonous plant, 19 June 2006, B.T., J.J.L., K.T., P.S., R.R., S.M., W.C. (holotype BBH18679, ex-holotype culture BCC22358). GenBank: ITS = OL331507, LSU = OL322050, TEF1 = OL322037, RPB1 = RPB2 = OL322067.

**Description**: Stromata epibiotic, irregularly pulvinate or subglobose, upper surface very pale violet (91D) to light purplish gray (N187D); lower surface vivid yellow (14C) to strong orange (25A), 3–7 mm wide. Perithecia semi-immersed, crowded, obovoid, 150–320 × 80–140 µm. Asci cylindrical, (150–)154–320(–193) × 80–140 µm. Asci caps 150–320 × 80–140 µm. Ascospores hyaline, whole, filiform, aseptate, extending the length of ascus.

**Culture characteristics**: Colony on OA attaining a diameter of 4 cm in 20 days, flat, slightly convex to the agar surface, white with light yellow green (150D), reverse pale orange yellow (23D). Phialides arising from aerial hyphae, solitary, cylindrical or aceronomium-like, slightly curved, 50–250 × 1–2 µm. Conidia hyaline, enteroblastic, fusiform to acerose, early in development aseptate, becoming 1–2 septa, aggregated at the apex of the phialides, (6–)7–14(–19) × (1.5–)2–3 µm.
Colony on PDA attaining a diameter of 3.5–4 cm in 20 days, compact mycelium, slightly convex to the agar surface, white (158), pale purplish pink (56A) pigment diffusing in medium, reverse strong yellowish pink (31C). Phialides arising from aerial hyphae, solitary, cylindrical or acremonium-like, slightly curved, 43–265 × 1–2 µm. Conidia hyaline, enteroblastic, fusiform to acerose, early in development aseptate, becoming 1–2 septa, aggregated at the apex of the phialides, (4–)6–11–(17) × 2–3.5–(4) µm.

Habitat: On scale insects (Coccidae; Hemiptera), found on living stems of dicotyledonous plants.

Additional specimens examined: Thailand, Chanthaburi Province, Khao Soi Dao Wildlife Sanctuary, Withiphrai Nature Trail; 13°06′13″ N, 102°11′39″ E, on scale insects (Coccidae; Hemiptera), on living stems of dicotyledonous plant, 1 May 2007, B.T., K.T., R.R., S.M., W.C. (BBH19872, BCC25788); Nakhon Ratchasima Province, Khao Yai National Park, Mo Singto Nature Trail; 14°26′21.46″ N, 101°22′20.20″ E, on scale insects (Coccidae; Hemiptera), on living stems of dicotyledonous plant, 30 June 2010, A.K., K.T., K.S., P.S., R. Somnuk (R.S.), S.M. (BBH30155, BCC43315).

Notes: Our molecular phylogenetic study has shown that A. griseoperitheciatus is closely related to A. albus. However, A. griseoperitheciatus significantly differs from A. albus in having a perithecial layer on the upper surface of stromata that is very pale violet to light purplish gray, the lower surface of stromata is vivid yellow to strong orange, while in A. albus, the stromata are only white. Additionally, A. griseoperitheciatus produces a pale purplish pink pigment diffusing in PDA plates, whereas A. albus does not produce any pigment.

Ascopolyporus gollmerianus Henn., Hedwigia 41: 8. 1902.  
Ascopolyporus khaoyaiensis Mongkolsamrit, Thanakitpipattana and Luangsa-ard sp. nov. Figure 5. MycoBank: MB 841856.  
Etymology: Named after Khao Yai National Park, where the type specimen was found.  
Typification: Thailand, Nakhon Ratchasima Province, Khao Yai National Park, Mo Singto Nature Trail; 14°26′21.46″ N, 101°22′20.20″ E, on scale insects (Coccidae; Hemiptera), on the living stems of dicotyledonous plant, 5 August 2010, K.T., P.S., S.M., A.K., K.S. (holotype BBH30157, ex-holotype culture BCC43741). GenBank: ITS = OL331513, LSU = OL322041, TEF1 = OL322040, RPB2 = OL322070.

Description: Stromata epibiotic, flattened to convex, cylindrical to irregularly shaped, upper surface very pale violet (91D) to dark purple (59A); lower surface white to pale orange (20A), 3–25 mm wide, 1–3 mm thick. Perithecia semi-immersed, slightly protruding apices, narrow flask shaped, slightly protruding, obclavate, 300–360 × 100–120 µm. Ascii cylindrical, up to 215 µm long, 3–4 µm wide, Ascii caps 2–4 × 3–4 µm. Ascospores hyaline, whole, filiform, aseptate, 175–200 × 1 µm.

Culture characteristics: Colonies on OA attaining a diameter of 3.5 cm in 14 days, cottony, white, reverse moderate brown (165A). Phialides arising from aerial hyphae, solitary, cylindrical or acremonium-like, slightly curved, up to 60 µm long, 1–2 µm wide. Conidia hyaline, enteroblastic, fusiform to acerose, early in development aseptate, mostly becoming 1 septum, occasionally 2–3 septa, aggregated at the apex of the phialides, (5–)8–16–(20) × (1.5–)2–3 µm.

Colonies on PDA attaining a diameter of 3–4 cm in 14 days, cottony, white, pale orange in the middle of colony, reverse dark red (59A) in the middle, bright strong purplish red (60D) pigment diffusing in medium. Phialides arising from aerial hyphae, solitary, cylindrical or acremonium-like, up to 50 µm long, 1–2 µm wide. Conidia hyaline, enteroblastic, fusiform to acerose, early in development aseptate, mostly becoming 1 septum, occasionally 2–3 septa, aggregated at the apex of the phialides, (7–)9–16.5–(22) × (1.5–)2–3 µm.

Habitat: On scale insects (Coccidae; Hemiptera), found on living stems of dicotyledonous plant.
Figure 5. *Ascopolyporus khaoyaiensis*. (a,b) Stromata on living stem of dicotyledonous plants (a BBH30157, b BBH30154); (c) perithecia; (d) asci; (e) asci-caps; (f) ascospores; (g) colony obverse on OA; (h) colony reverse on OA; (i,j) phialide apex with conidial head on OA; (k) conidia on OA; (l) colony obverse on PDA; (m) colony reverse on PDA; (n–p) phialide apex with conidial head on PDA; (q) conidia on PDA. Scale bars: (b,g,h,l,m) = 10 mm; (a) = 5 mm; (c) = 100 µm; (i) = 50 µm; (d,f) = 20 µm; (g,j,k,n,o,p,q) = 10 µm; (e) = 5 µm.

Additional specimen examined: Thailand, Nakhon Ratchasima Province, Khao Yai National Park, Mo Singto Nature Trail; 14°26′21.46″ N, 101°22′20.20″ E, on scale insects (Coccidae; Hemiptera), on living stems of dicotyledonous plant, 30 June 2010, K.T., P.S., S.M., A.K., R.S., K.S. (BBH30154, BCC43314). GenBank: ITS = OL331512, LSU = OL322052, TEF1 = OL322039, RPB2 = OL322069.

Notes: The stromatal color of the natural samples of *A. khaoyaiensis* is similar to the purple stromata of *A. purpuratus*. However, perithecia in *A. khaoyaiensis* are immersed and obclavate, whereas perithecia in *A. purpuratus* are semi-immersed and ovoid. Asci of *A. khaoyaiensis* are shorter than those of *A. purpuratus* (up to 215 × 3–4 vs. 200–240 × 4–5 µm). Additionally, *A. khaoyaiensis* and *A. purpuratus* produce bright strong purplish red pigment diffusing in PDA plates.

*Ascopolyporus möllerianus* (Henn.) Möller, Phycomyc. Ascomyc. Bras.: 301. 1901. *Ascopolyporus philodendri* J.F. Bisch. (as “philodendrus”), Mycologia 97(3): 711. 2005.
Ascopolyporus polychrous Möller, Bot. Mitt. Trop. 9: 300. 1901.

Ascopolyporus polyphoroides Möller, Bot. Mitt. Trop. 9: 301. 1901.

Ascopolyporus purpuratus MongkolSarnrit, Thanakitpipattana, Himaman and Luangsaard sp. nov. Figure 6.

Figure 6. Ascopolyporus purpuratus. (a,b) Stromata on living midrib of leaves of dicotyledonous plant (BBH44511); (c) cross-section through stroma showing perithecia; (d) ovoid perithecia; (e) asci; (f) asci-caps; (g) ascospores; (h) colony obverse on OA; (i) colony reverse on OA; (j,k) phialide apex with conidial head on OA; (l) conidia on OA; (m) colony obverse on PDA; (n) colony reverse on PDA; (o,p) phialide apex with conidial head on PDA; (q) conidia on PDA. Scale bars: (h,i,m,n) = 10 mm; (a,b,c) = 1 mm; (d,e) = 100 µm; (g,j,o) = 20 µm; (f,k,l,p,q) = 10 µm.

MycoBank: MB 841857.

Etymology: Referring to the purple color of the fresh stroma.

Typification: Thailand, Nakhon Ratchasima Province, Khao Yai National Park, Pong Chang Chomrom Phoen (Nong Phakchi); 14°27’04.0” N, 101°22’03.60” E, on scale insects (Coccidae; Hemiptera), on the living stems and midrib of dicotyledonous leaves, 19 September 2018, J.J.L., K.T., D. Thanakitpipattana (D.T.), B. Sakolrak (B.S.), R.S., S.M., W.N., W. Himaman (W.H.), P.S. (holotype BBH44511, ex-holotype culture BCC88430). GenBank: ITS = OL331506, LSU = OL322045, TEF1 = OL322032, RPB1 = OL322059.

Description: Stromata epibiotic, flattened to convex, consisting of dense white mycelial mat, upper surface yellow (18D) to very pale purple-violet (75D), 5–12 mm long, 3–8 mm wide. Perithecia semi-immersed, crowded, ovoid, (300–)335–414(–420) × (100–)110–142(–150) µm.
Asci cylindrical, up to 240 × 2–4 µm. Asci caps 2–4 × 3–4 µm. Ascospores hyaline, whole, filiform, aseptate, (100–)131–190(–220) × 1–1.5 µm.

**Culture characteristics**: Colonies on OA attaining a diameter of 3–4 cm in 14 days, flat, white, reverse white. Phialides arising from aerial hyphae, solitary, cylindrical or aceremonium-like, slightly curved, up to 40 µm long, 1–2 µm wide. Conidia hyaline, enteroblastic, fusiform to acerose, aseptate, aggregated at the apex of the phialides, (5–)6.5–18(–25) × (1.5–)2–2.5(–3) µm.

Colonies on PDA attaining a diameter of 2.5–3.5 cm in 14 days, slightly convex to the agar surface, vivid orange (28B) with white in the middle of the colony, reverse strong reddish orange (34C), bright moderate reddish orange (N34D) pigment diffusing in medium. Phialides arising from aerial hyphae, solitary, cylindrical or aceremonium-like, slightly curved, up to 55 µm long, 1–2 µm wide. Conidia hyaline, enteroblastic, fusiform to acerose, aseptate, aggregated at the apex of the phialides, (5–)7–13.5(–18) × 1.5–2.5(–3) µm.

**Habitat**: On scale insects (Coccoidea; Hemiptera), found on living stem and midrib of leaves of dicotyledonous plants.

**Additional specimen examined**: Thailand, Phetchaburi Province, Kaeng Krachan National Park, Ban Krang Camp Nature Trail; 12°54′05″ N, 99°37′48″ E, on scale insects (Coccoidea; Hemiptera), on the midrib of leaves, 14 November 2005, K.T., W.C., R.R., B.T. (BBH15035, BCC19721); Nakhon Ratchasima Province, Khao Yai National Park, Mo Singto Nature Trail; 14°26′21.46″ N, 101°22′20.20″ E, on scale insects (Coccoidea; Hemiptera), on the twigs of tree, 23 July 2009, K.T., P.S., R.R., S.M. (BBH26373, BCC37880); Nakhon Ratchasima Province, Khao Yai National Park, Bueng Phai Nature Trail; 14°26′21.46″ N, 101°22′20.20″ E, on scale insects (Coccoidea; Hemiptera), on the midrib of leaves and twigs of tree, 18 September 2018, J.J.L., K.T., D.T., B.S., R.S., W.N., W.H., P.S. (BBH44547, BCC88388); GenBank: ITS = OL331505, LSU = OL322033, RPB2 = OL322064, (BBH44551, BCC88389); GenBank: ITS = OL331504, LSU = OL322047, TEF1 = OL322034.

**Notes**: Ascopolyporus purpuratus can be found on the midrib of leaves and living stems of dicotyledonous plants. In natural samples, the perithecia are pale yellow and/or very pale purple-violet. Additionally, A. purpuratus produces red pigment diffusing in PDA plates the same as A. griseoperitheciatus and A. khaoyaiensis. However, the mycelia of A. purpuratus are white in the middle of the colony with orange edges, whereas A. griseoperitheciatus produces only white mycelia and A. khaoyaiensis produces white mycelia that turn pale orange in the center of colony.

**Ascopolyporus villosus** Möller, Bot. Mitt. Trop. 9: 301. 1901.

**Neohyperdermium** Thanakitpipattana and Luangsa-ard, gen. nov.

**MycoBank**: MB 842780.

**Etymology**: Referring to the phenotypic similarity of the stromatal formation to Hyperdermium.

**Typification**: Neohyperdermium piperis (J.F. Bisch. and J.F. White) Thanakitpipattana and Luangsa-ard.

**Description**: Stromata epibiotic, flattened to pulvinate, white to yellow. Hosts are scale insects (Coccoidea, Hemiptera). Perithecia immersed, obpyriform, cymbiform to cone-shaped. Asci cylindrical, linear with enlarged refractive tip. Asexual morph verticillium-like.

**Notes**: This genus is a phylogenetically separate lineage from other scale insect pathogens in Cordycipitaceae, as shown in Figure 1. Two species are recognized in this genus that produce white to yellow stromata, immersed perithecia and a verticillium-like anamorph.

**Neohyperdermium piperis** (J.F. Bisch. and J.F. White) Thanakitpipattana and Luangsa-ard, comb. nov.

**MycoBank** MB 842782.

≡ Torrubieila piperis J.F. Bisch. and J.F. White, Studies in Mycology 50: 89–94. 2004.

≡ Cordyceps piperis (J.F. Bisch. and J.F. White) D. Johnson, G.H. Sung, J.F. Bisch. and Spatafora, Mycol. Res. 113(3): 284. 2009.

**Description and Illustration**: See J.F. Bisch. and J.F. White (2004).

**Typification**: Panama, Barro Colorado Island, Lutz Creek, scale insect (Coccoidea, Hemiptera) on Piper carrilloanum (Piperaceae) August 2003, J.F. Bischoff and J.F. White, Jr., New York Botanical Garden (NY), culture ex-type CBS 116719.
**Habitat:** Scale insects.

**Known distribution:** Panama.

**Note:** *Neohyperdermium piperis* is closely related to *N. pulvinatum* and can be distinguished from *N. pulvinatum* in producing part-ascospores, whereas in *N. piperis*, the ascospores are whole with multiple septations and the conidia are aseptate.

*Neohyperdermium pulvinatum* (J.F. White et al.) Thanakitpipattana and Luangsa-ard, comb. nov.

_MycoBank_: MB 842783.

≡ *Hyperdermium pulvinatum* J.F. White et al., _Mycologia_ 92(5): 908–918. 2000.

**Description and illustration:** See J.F. White et al. (2000).

**Typification:** Costa Rica, Guanacaste, Parque Nacional Guanacaste, Sector El Hacha, Puesto Los Almendros, on _Asteraceae_, 6 October 1998, J.F. White, G. Bills and S. Salas, RUTPP, culture ex-type ATCC MYA-69.

**Habitat:** Scale insects.

**Known distribution:** Costa Rica.

**Note:** *Neohyperdermium pulvinatum* is closely related to *N. piperis*, which can be distinguished by the type of ascospores and the presence of multiseptate conidia.

### 4. Discussion

The results of our multigene phylogenetic analyses show that our specimens were closely related to *Ascopolyporus polychorus*, *A. villosus* and *Hyperdermium caulium* (Figure 1). Importantly, the specimens in this study are clearly distinct species in *Ascopolyporus* because of the differences in the sizes, color, perithecial position and features of the stromata, which also overlap with morphological characters of some species previously treated as belonging to the genera *Hyperdermium* (H. caulium, H. bertonii, H. pulvinatum) and *Cordyceps* (C. piperis) in _Cordycipitaceae_, by producing flattened to pulvinate stromata and producing unique cylindrocarpon-like anamorph with multiseptate conidia [11,12,14,16]. The two genera, *Ascopolyporus* and *Hyperdermium*, differ only in the sizes and characters of ascomata [11,13,14]; *Hyperdermium* stromata are either flattened or pulvinate, whereas in *Ascopolyporus* sensu Möller, the stromata are subglobose to polypore-like. Based on these results, since the type species of *Hyperdermium*, *H. caulium*, is nested within *Ascopolyporus*, *Hyperdermium* is synonymized with *Ascopolyporus* and a new combination is proposed for *H. caulium*. The generic description of *Ascopolyporus* is therefore emended to include flattened to pulvinate stromata.

Our new species in *Ascopolyporus* are characterized by possessing flattened and pulvinate stromata, two groups that are supported as separate clades in phylogenetic analyses (Figure 1). The three pulvinate species (*Ascopolyporus albus*, *A. galloides* and *A. grisoperitheciatus*) have smaller stromata than previously described by Möller [24] and Bischoff et al. [14]. The two new species of *Ascopolyporus khaoyaiensis* and *A. purpuratus* have flattened stromata, and their sizes are in the same range as *A. caulium* (Table 2). All new *Ascopolyporus* species in this study possess semi-immersed perithecia with osti-oles slightly protruding on the surface of the fertile cushion, whereas *A. polychrous* and *A. philodendrus* have completely immersed perithecia; *A. villosus* does not produce perithecia on stromata [14].
Table 2. Morphological comparisons of *Ascopolyporus* and related species. NA, not applicable.

| Name                  | Host                      | Stromata (mm)                  | Perithecia (µm)          | Asci (µm)                  | Ascospores (µm)         | Conidiogenous Cell (µm) | Conidia (µm)          | References                  |
|-----------------------|---------------------------|-------------------------------|--------------------------|----------------------------|-------------------------|-------------------------|--------------------------|---------------------------|
| *Ascopolyporus albus* | Scale insect, Epiphyte    | pulvinate,                    | semi-immersed,           | hyaline, cylindrical,     | hyaline, filiform,      | solitary,               | enteroblastic,            | This study                 |
|                       |                           | subglobose to globose         | obpyriform,              | up to 250 × 3–4          | whole, multiseptate,    | slightly curved,         | fusiform to acerose,      |                           |
|                       |                           | white to pinkish white 3–6    | 250–320 × 100–120        |                            | 95–135 × 1              | cylindrical, up to 120 × 1–2 |                          |                           |
| *A. caulium*          | Scale insect, Epiphyte    | crustose, subcircular yellow to orange, 5–100 | cylindrical, 200–250 × 65–80 | cylindrical to slightly fusiform, 100–160 × 8–9 | filiform, multiseptate, extending to the length of ascus × 1 wide | phialidic, sparse layer | enteroblastic, cylindrical to fusiform, slightly truncate at end, aseptate: 5–7 × 1–1.5, 1–5 septate: 15–30 × 1.5–3 | Sullivan et al., 2000 |
| *A. galloides*        | Scale insect, Epiphyte    | pulvinate, hemispherical, upper: white-yellowish white 1–7, lower: strong orange 170–340 × 60–110 | semi-immersed, obclavate, | hyaline, cylindrical, 129–175 × 3–6 | hyaline, filiform, whole, aseptate, | solitary, mildly curved, cylindrical, 30–294 × 1–2 | aseptate to 1–4 septate, 5–27 × 2–4 | This study                 |
| *A. griseoperitheciatus* | Scale insect, Epiphyte    | pulvinate, irregular pulvinate to subglobose, 3–7 upper: very pale violet to light purplish gray lower: vivid yellow to orange 150–320 × 80–140 | semi-immersed, obovoid, 150–193 × 4–5 | hyaline, cylindrical, 150–265 × 8–9 | hyaline, filiform, whole, aseptate, extending to the length of ascus × 1 wide | solitary, mildly curved, cylindrical, 43–265 × 1–2 | fusiform to acerose, aseptate to 1–2 septate, 4–17 × 2–4 | This study                 |
| *A. khaoyaiensis*     | Scale insect, Epiphyte    | flattened to convex, cylindrical to irregular shaped, very pale violet to dark purple 3–25 | semi-immersed, obclavate, 300–360 × 100–120 | hyaline, cylindrical, up to 215 × 3–4 | hyaline, filiform, whole, aseptate, | solitary, mildly curved, cylindrical, 7–22 × 1.5–3 | fusiform to acerose, aseptate to 1–3 septate, 7–22 × 1.5–3 | This study                 |
| Name                  | Host                      | Stromata (mm)                  | Perithecia (µm)              | Asci (µm)            | Ascospores (µm)      | Conidiogenous Cell (µm) | Conidia (µm) | References                     |
|-----------------------|---------------------------|--------------------------------|-------------------------------|----------------------|----------------------|-------------------------|---------------|--------------------------------|
| **A. philodendrus**   | Scale insect, Epiphyte    | subglobe, upper: sterile, red-purple lower: fertile, white to tan 12–25 | immersed, obclavate, 200–300 × 40–80 | cylindrical, 90–140 × 3–5 | filiform, length of ascus | simple, phialidic,         | enteroblastic, subcylindrical, 30–60 × 1–3 aseptate to 1–4 septate, 7–25 × 3–4 | White et al., 2003 |
| **A. polychrous**     | Scale insect, Epiphyte    | polypore-like, bright-rusty red or white to yellow 40 | underside of stroma, immersed, narrow obclavate, up to 750 | hyaline, cylindrical, 500 × 4 | hyaline, filiform to spiroid, 300 × 1, disarticulate into part-spores, 6 × 1 | NA | enteroblastic, oval, 1-multiseptate, 7–12 × 4–6 | Möller, 1901; White et al., 2003 |
| **A. purpuratus**     | Scale insect, Epiphyte    | flattened to convex, yellow to very pale, purple-violet, 5–12 × 3–8 | semi-immersed, ovoid, 300–420 × 100–150 | hyaline, cylindrical, up to 240 × 2–4 | hyaline, filiform, whole, aseptate, 100–220 × 1–1.5 | solitary, slightly curved, cylindrical, aceremonium-like, up to 55 × 1–2 | enteroblastic, fusiform to acerose, aseptate, 5–18 × 1.5–3 | This study |
| **A. villosus**       | Scale insect, Epiphyte    | white to pale yellow, 12–25 | not produced | NA | NA | phialidic | enteroblastic, subcylindrical, guttulate, aseptate to 1–4 septate, 10–22 × 2–5 | White et al., 2003; Bischoff et al., 2005 |
| **Neohyperdermium piperis** | Scale insect, Epiphyte | pulvinate, subglobe, to cylindrical, white to yellow, 3–10 × 3–6 | immersed, obpyriform to cymbiform, 175–290 × 40–80 | cylindrical, 120–170 × 3–5 | filiform, disarticulating into part-spores, 4–9 × 1–2 | upright, verticillate, rarely subglobe, | hyaline, subcylindrical, aseptate, 3–5 × 1–2 | Bischoff and White, 2004 |
| **N. pulcinatum**     | Scale insect, Epiphyte    | pulvinate, white to tan, 3–6 | cone-shaped, linear, 150–250 × 100–130 | filiform, multiseptate, 130–225 × 1 | filiform, hyaline, 1 to several septa, 40–100 × 1–3 | phialidic, hyaline, enteroblastic, subcylindrical, 1–5 septate: 14–16 × 2.5–3 1–5 septate: 22–30 × 2.5–4 | Sullivan et al., 2000 |
Another species in *Hyperdermium* found in the family *Cordycipitaceae*, *H. pulvinatum*, did not cluster with type species *H. caulium*, which was congruent with Sung et al. [45], Kepler et al. [44] and Wang et al. [54], and is grouped together with *Cordyceps piperis* possessing pulvinate stromata that are white to yellow, producing aseptate, subcylindrical conidia on cultures (Table 2). These two species are proposed as new combinations in a new genus *Neohyperdermium*, as *Neohyperdermium pulvinatum* and *N. piperis*, which were described as epiphytes on scale insect pathogens in *Cordycipitaceae*.

The evolution and ecology of insect pathogenic fungi using insects and plants as the main source of nutrients remain not fully understood. Humber [55] suggested that the interaction between higher fungi and plants range from virulent pathogens to decomposer to mutualistic symbiosis. In *Hypocreales*, the genera *Aschersonia*, *Ascopolyporus*, *Conoidocrella*, *Dussiella*, *Hyperdermium*, *Hypocrella*, *Moelleriella*, *Regiocrella* and *Samuelsia* also utilize nutrients from the phloem of host plants through scale insects and white flies (*Coccidae* and *Aleyrodidae*) to continue their growth on plants [11,13–15]. Our new *Ascopolyporus* species are also found in this position, in which the scale insect attached to host plants was parasitized until it was consumed, but the fungus continues to utilize the nutrients that are being released through the stylet apparatus. The interactions occurred on the underside of fungal stroma, which is where the bridge for the exchange of nutrients between the fungus and plant exists (Figure 7).

![Image of fungal stroma](image_url)

**Figure 7.** Photographs showing the interaction between fungus, plant and scale insect. (a) Fungal stroma on the stem of a dicotyledonous plant. (b) Underside of fungal stroma removed from the stem. (c,d) Underside of fungal stromata with ventage (arrow) from which the scale insect stylet entered the plant host. Scale bars = (a,b,c) = 1 mm; (d) = 0.05 mm.

**Residual Species of *Ascopolyporus***.
The remaining taxon could not be accommodated in the genus *Ascopolyporus* because its morphological description resembles other genera in *Clavicipitaceae* by producing paraphyses, which are not found in *Ascopolyporus* (*Cordycipitaceae; Hypocreales*), and molecular phylogenetic data are not available.

*Ascopolyporus puttemansii* Henn., Hedwigia 48: 6. 1908.

**Key to *Ascopolyporus* species:**

1a. Conidia aseptate .................................................. 2
1b. Conidia aseptate to multiseptate .................................. 3
2a. Conidia oval .......................................................... *A. möllerianus*
2b. Conidia fusiform to acerose ........................................ *A. purpuratus*
3a. Conidia 1–5 septate, cylindrical to fusiform, 5–30 × 1–3 µm ...... *A. cauliun*
3b. Conidia 1–4 septate .................................................. 4
4a. Conidia subcylindrical ............................................... 5
4b. Conidia fusiform to acerose ........................................ 6
5a. Conidia subcylindrical, 7–25 × 3–4 µm .......................... *A. philodendron*
5b. Conidia subcylindrical, guttulate, 10–22 × 2–5 µm .......... *A. villosus*
6a. Ascospores hyaline, disarticulate into part-spores ............... 7
6b. Ascospores hyaline, whole .......................................... 8
7a. Ascospores filiform to spiroid, 6 × 1 µm ....................... *A. polychoirus*
7b. Ascospores filiform, 8–15 µm .................................... *A. polyporoides*
8a. Ascospores multiseptate, 95–135 × 1 µm ....................... *A. albus*
8b. Ascospores aseptate ................................................ 9
9a. Perithecia semi-immersed, obovoid .......................... *A. griseoperithciatus*
9b. Perithecia semi-immersed, obclavate ........................ 10
10a. Stromata pulvinate, hemispherical, 1–7 mm ........................ *A. galloides*
10b. Stromata flattened to convex, cylindrical to irregular shaped, 3–25 mm . *A. khaoyaiensis*

**Key to *Neohyperdermium* species**

1a. Ascospores filiform, multiseptate, whole .......................... *N. pulvinatum*
1b. Ascospores filiform, disarticulating into part-spores ............. *N. piperis*

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