



Revision of the morphology and biogeography of *Thysanorea papuana*

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Abstract

The monotypic genus *Thysanorea* (Chaetothyriomycetes) is characterized by *Periconiella*-like penicillate conidiophores. The morphology and geographic distribution of *Thysanorea papuana* are revised based on fresh collections from Taiwan, literature research, and type studies. It is revealed that the original morphological generic characterization is based on cultivation artifacts. On the natural substrate and in fresh isolates, conidiophore heads appear less complex and are shed off and replaced by repeated percurrent regeneration. The species was previously known from Papua New Guinea and India, while this is the first record from Taiwan. *Ramichloridium lignicola*, which was recorded from Hong Kong and Thailand is recognized as synonymous and *Alysidiopsis lignicola* recorded from Mexico is a possible synonym. The distribution of this species appears to be paleotropical or even pantropical.

Key words – dematiaceous hyphomycetes – ITS sequences – new record

Introduction

The genus *Thysanorea* with the single species, *Th. papuana* based on *Periconiella papuana* Aptroot, was proposed because of its phylogenetic placement among *Rhinocladiella* species in the Chaetothyriomycetes, whereas all other *Periconiella* species nest within a clade of Mycosphaerellaceae of Dothideomycetes, including species of *Ramichloridium* and *Zasmidium* (Arzanlou et al. 2007). As the species name indicates, the fungus was first discovered in Papua New Guinea, but was recently also found in India (Pratibha & Prabhugaonkar 2015). Since many dematiaceous hyphomycetes on leaf and branch litter show a distinct species-specific morphology, sufficient for species identification, many new species and records are presently being described based on morphology alone (Moro et al. 2015, Kodsueb et al. 2016, Ma et al. 2016a, b, Mena-Portales et al. 2016, Silva et al. 2016, Xia et al. 2016), although a high proportion could be cultivated and subjected to molecular analysis (Pratibha & Prabhugaonkar 2015, Hashimoto et al. 2016, Heidarian et al. 2016, Pratibha et al. 2016). DNA sequence data are helpful particularly when the generic delimitations are quite artificial, so that the same species is likely to be described independently by different researchers in different genera. Research of new collections of *Thysanorea* specimens in Taiwan and in recent literature, also indicated a widespread distribution, not only geographically, but also taxonomically, in different genera. Detailed comparison of the conidiophore morphologies in nature and in culture revealed a feature which might be useful for morphological characterization of the genus.

Materials & Methods

Isolates and morphology

Two specimens were collected on dead woody substrates in northern and southern Taiwan, respectively, and deposited in the Museum of Natural Science, Taichung, Taiwan (TNM). Isolation of pure culture and morphological investigation were conducted as described in Kirschner (2016). Air-dried specimens from field collections were mounted on stubs, sputter-coated with gold and investigated with scanning electron microscopy (SEM) using a low vacuum scanning electron microscope at National Central University. Loans of type specimens were requested from the herbaria of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS-H), Instituto de Ecología y Sistemática, Havana, Cuba (HAC), and the International Fungal Research & Development Centre, Kunming, China (IFRD). A number of Facesoffungi is included as explained in Jayasiri et al. (2015).

DNA techniques

Nuclear DNA extraction from a living culture (BCRC FU30287), PCR amplification of the internal transcribed spacer region (ITS) of the ribosomal RNA gene, sequencing and editing were performed as in Yeh & Kirschner (2014). The obtained sequence was deposited in GenBank (KX894451) and subjected to BLAST search.

Results

The original descriptions and illustrations of *Alysidiopsis lignicola* and *Ramichloridium lignicola* are almost identical to those of the specimens collected in Taiwan and identified with *Th. papuana*. Since the type of *A. lignicola* could not be traced in HAC (J. Mena, pers. comm.) and no conidiophore heads could be found in the type of *R. lignicola*, comparison between these species and *Th. papuana* was based only on the original publications. Light microscopic observations were shown in Figs 1–13. SEM photographs from the holotype of *Thysanorea papuana* (CBS-H) and specimen R. Kirschner & C.-J. Chen 1356 from Taiwan showed that conidia and conidiogenous cells were smooth and conidiogenous loci were conical and had plane secession scars (Figs 14–16).

BLAST search at GenBank with a 646 b long sequence from a Taiwanese cultured specimen (GenBank KX894451) revealed 97% (562/579 b) identity with the sequence of the ex-type culture of *Th. papuana* (NR111276) and 98% (587/596 b) identity with that of a strain from India (KR259881). The next similar sequences were from species of *Minimelanolocus* R.F. Castañeda & Heredia with unbranched conidiophores (Liu et al. 2015) with ITS sequence similarities of 93%.

Taxonomy

Thysanorea papuana (Aptroot) Arzanlou, W. Gams & Crous, Stud. Mycol. 58: 80 (2007) Figs 1–16
≡ *Periconiella papuana* Aptroot, Nova Hedwigia 67(3-4): 491 (1998)
= *Ramichloridium lignicola* K.M. Tsui, Goh, K.D. Hyde & Hodgkiss, Cryptog. Mycol. 22(2): 141 (2001), **syn. nov.**

? = *Alysidiopsis lignicola* Mercado, Figueras & J. Mena, Mycotaxon 60: 444 (1996)

Facesoffungi Number: FoF02731

Morphology on the natural substrate:

Specimens from Taiwan:

Sexual morph: Undetermined. **Asexual morph:** Hyphomycetous. *Conidiophores* single, micro- to macronematous, erect, straight, smooth, dark brown, becoming paler towards the apex, macronematous conidiophores 132–458 µm high, composed of unbranched stipe and branched head, stipe often with a percurrent proliferation close below the branched head, 6–14 µm wide at the base, 3–6 µm wide at the apex, head appr. 20–50 µm long and 15–55 µm wide, head and

branches easily breaking off, branches irregular in number, branching pattern and cellular composition, but always composed of basal sterile cells and conidiogenous cells and often constricted at the septa, smooth-walled, paler than the stipe. *Conidiogenous cells* intercalary and terminal, basal half often swollen and distinct from apical half bearing several conidiogenous scars and sometimes being bent, $(3-5-10.5(-13) \times (2-)3-4(-5) \times (2-)3-4(-5) \mu\text{m}$ ($n = 30$, R. Kirschner 4019). *Conidiogenous loci* short-cylindrical, with thickened and darkened wall at the truncate apex, $1 \times 1 \mu\text{m}$. *Conidia* solitary, clavate or short-cylindrical, sometimes slightly constricted, 0-3-septate, but 3-septate conidium ($12 \times 3 \mu\text{m}$) only found once, 2-septate conidia being rare, aseptate conidia rare in R. Kirschner 4019, pale brown, smooth, $(5-)6-8(-9) \times (1.5-)2-3 \mu\text{m}$ ($n = 15$, R. Kirschner & C.-J. Chen 1356) or $(6-)7-9(-9.5) \times 2.5-3$ ($n = 30$, R. Kirschner 4019), basal hilum conspicuous, thickened and darkened, 1-1.5 μm wide. *Micro- and semimacronematous conidiophores* inconspicuous, arising from superficial hyphae close to the macronematous conidiophores, branched or unbranched, with conidiogenous cells and conidia basically identical to those from macronematous conidiophores.

Holotype (from Papua New Guinea):

Conidiophores appr. 205 μm high, stipe 5-8 μm wide, dark brown, cell wall smooth, 1-1.5 μm thick, with percurrent proliferations below the conidiogenous head, head similar to that described above, with 3 levels of branching, *conidiogenous cells* terminal and intercalary, of similar shape as described above, pale brown, smooth, $8-12 \times 3-4 \mu\text{m}$, *conidiogenous loci* blackened, $0.5-1 \times 0.5-1 \mu\text{m}$. *Conidia* clavate or short-cylindrical, sometimes slightly constricted, 1-3-septate, but 1-septate conidia most frequent, pale brown, smooth, $8-11 \times 3-3.5 \mu\text{m}$, basal hilum conspicuous, darkened, $1 \times 1 \mu\text{m}$.

Morphology in culture on corn meal agar:

Colony dark brown, velutinous, hyphae pale brown, smooth, 1-3 μm wide. *Conidiophores* micronematous to macronematous, micronematous ones pale brown, reduced to a conidiogenous cell or few branches, semimacronematous ones dark brown, appr. 50-70 μm long, macronematous ones 125-230 μm long, with dark brown, smooth stipe, $100-200 \times 4-10 \mu\text{m}$, without percurrent proliferations, basal cell often subglobose and strongly swollen, up to 15 μm wide, conidiophore base sometimes rhizoidal, apex formed of sparse, irregularly clustered branches similar to those on the natural substrate, branches easily breaking off from the stipe, of the same color as the stipe or paler, paler towards the apex, smooth, mostly 2-4-celled, 3-5 μm wide at the swollen basal base that becomes delimited from the conidiogenous cell by the formation of a septum. Mature *conidiogenous cells* intercalary and terminal on the branches, cylindrical, geniculate, pale brown, smooth, terminal ones $(6-)7-10(-11) \times 2-3 \mu\text{m}$ ($n = 30$), covered by numerous blackened conidiogenous loci, $0.5-1 \times 0.5-1 \mu\text{m}$. *Conidia* solitary, clavate or short-cylindrical, sometimes slightly curved towards the base, 0-2-septate, but 1-septate conidia most frequent, pale brown, smooth, $(6-)7-10(-11) \times 2-3 \mu\text{m}$ ($n = 30$), basal hilum conspicuous, darkened, $1 \times 1 \mu\text{m}$.

Known habitats and distribution – on terrestrial and submerged branches, Hong Kong (Tsui et al. 2001), India (Pratibha & Prabhugaonkar 2015), ?Mexico (Mercado et al. 1996), Papua New Guinea (Aptroot & van Iperen 1998), Taiwan (new record), Thailand (Kodsueb et al. 2016).

Material examined – Hong Kong, Tai Po, Lam Tsuen River, on dead submerged branch, April 1997, K.M. Tsui KM 171 [formerly HKU(M), now IFRD, holotype of *Ramichloridium lignicola*, in poor condition, without conidiogenous heads and conidia], Papua New Guinea, Madang Province, foothill of Finisterre range, 40.8 km along road Madang-Lae, alt. 200 m, on stipe of unknown plant, 2 November 1995, A. Aptroot 36647 (CBS-H 6351, holotype of *Thysanorea papuana*), Taiwan, Kaohsiung County, between Baolai and Jiaxian, 400-600 m, on branch on ground, 6 July 2002, R. Kirschner & C.-J. Chen 1356 (TNM), Taoyuan City, Shihmen Dam area,

ca. 250 m, on rotting wood on ground, 16 April 2014, R. Kirschner 4019 (TNM), – living culture: BCRC FU30287, – ITS sequence: GenBank KX894451.

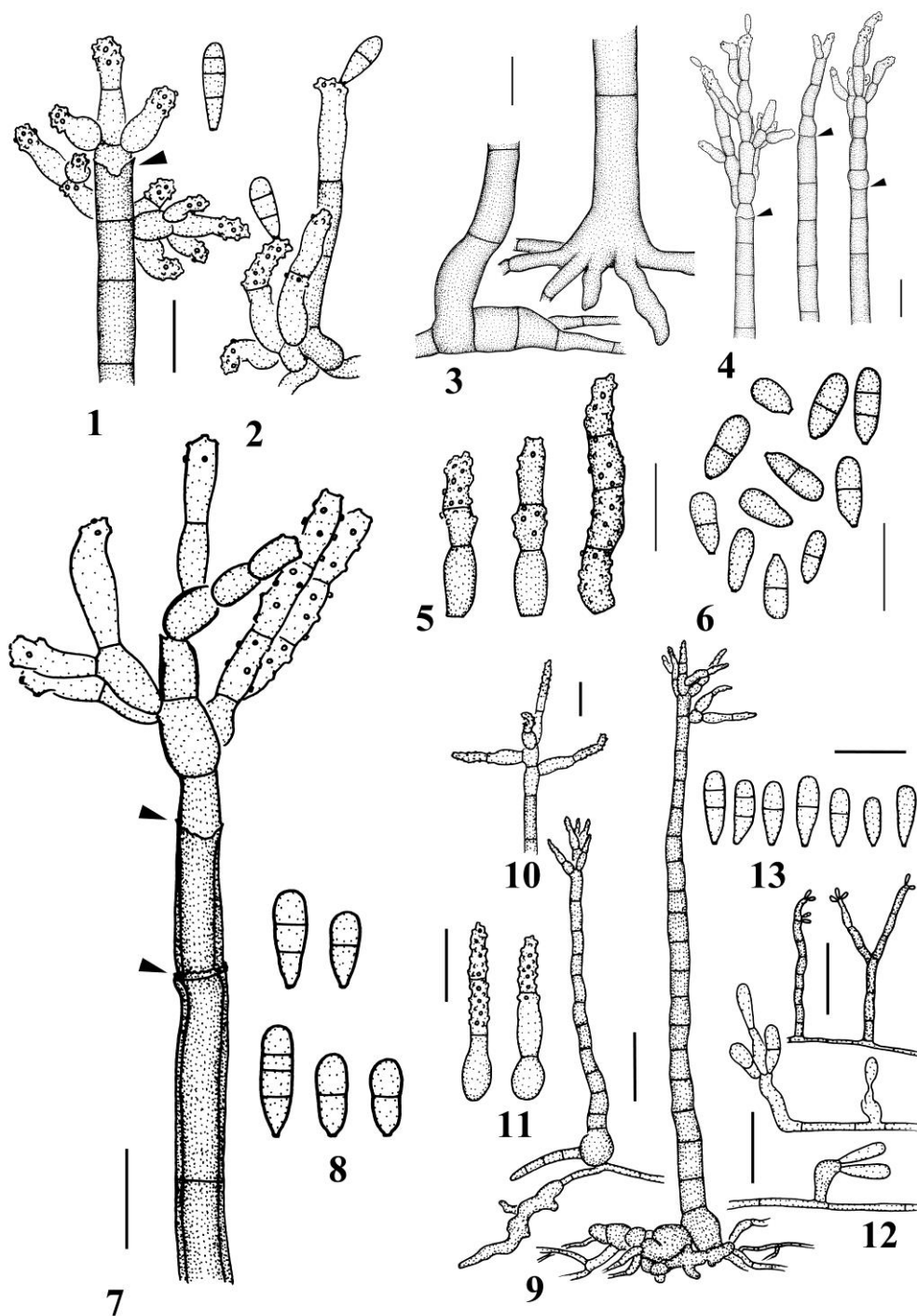
Discussion

Conspicificity

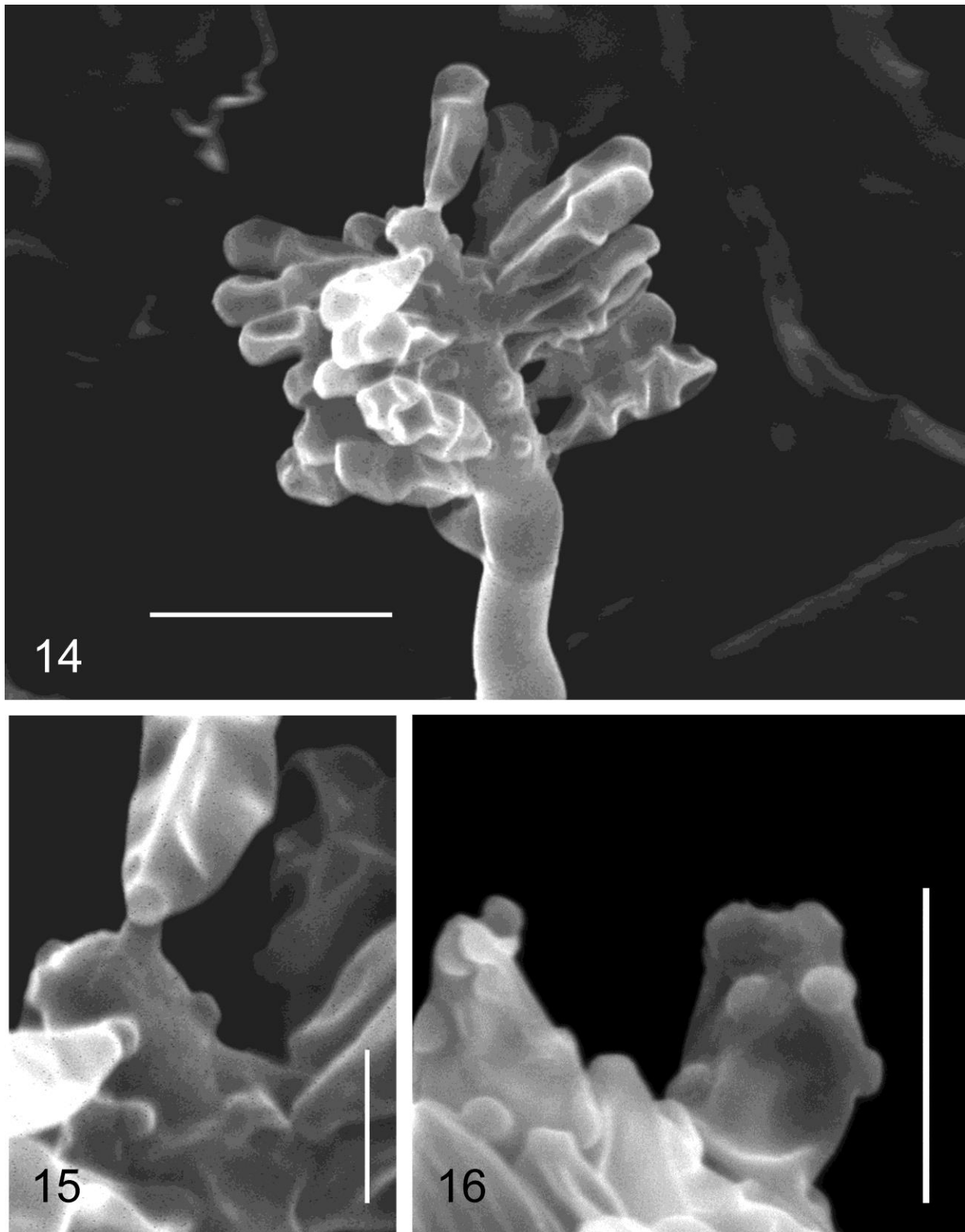
The morphological similarity between *Alysiidiopsis lignicola*, *Ramichloridium lignicola*, and *Thysanorea papuana* indicates conspecificity. The presence of short conidial chains in addition to solitary conidia mentioned for *A. lignicola* was indicated as the single difference to *R. lignicola* by Tsui et al. (2001). Catenate conidia cannot be deduced from the illustration of *A. lignicola* showing only solitary conidia with a basal hilum without a further scar at the apex (Mercado et al. 1996). Because no material of *A. lignicola* was available, a new synonymy with and combination into *Thysanorea* is not yet proposed. Conidia from field collections were 0–2-septate in the specimens from Taiwan and Hong Kong (Tsui et al. 2001), whereas 3-septate conidia were found in the field collection from Papua New Guinea (up to 2-septate in Aptroot & van Iperen 1998) and a single 3-septate conidium in a Taiwanese specimen, but only 0–1-septate ones in culture of *Th. papuana* (Arzanlou et al. 2007) and in the field collection of *A. lignicola* (Mercado et al. 1996) as well as in the specimen from India (Pratibha & Prabhugaonkar 2015). The number of septa found in the conidia, therefore, depends from environmental factors and the investigator, but in all cases, 1-septate conidia are predominant. Conidiogenous heads and conidia could not be found in the holotype of *R. lignicola*. The photographs of the type and the original description (Tsui et al. 2001), however, agree generally with the morphology of the Taiwanese specimens. Although the conidiophores exceed the length of that described by Tsui et al. (2001), the measurements of the specimen from Taiwan generally fit to the ranges given for the specimen described from Hong Kong (Tsui et al. 2001) and those from India (Pratibha & Prabhugaonkar 2015). The ITS divergence of 2–3% between the strains from India, Papua New Guinea and Taiwan is within the generally accepted 3% threshold for considering different strains as conspecific (Blaalid et al. 2013).

Generic delimitation

In order to avoid confusion by different morphologies in nature and in culture, both have to be studied and connected to each other in documentation of the same species (Gams 2002). Since this approach was not applied in *Thysanorea* previously, the species and genus concept has become confused, particularly since the type material has been practically lost in *Alysiidiopsis lignicola* and *Ramichloridium lignicola* so that the published illustrations serve as reference for taxonomic conclusions. Mercado et al. (1996) felt that *Alysiidiopsis* appeared artificial and suggested detailed comparison with *Cladosporium* and related genera. The ultrastructure of conidiogenous loci, though emphasized as important for generic delimitations of *Cladosporium*-like fungi by Arzanlou et al. (2007), was not investigated by the authors. The SEM photographs showing conical conidiogenous loci with smooth secession scar in the holotype of *Thysanorea papuana* and a specimen from Taiwan (Figs 14–16) were identical to those of *Alysiidiopsis lignicola* (Mercado et al. 1996). This ultrastructure is hardly distinguishable from that of *Periconiella* species (Kirschner & Piepenbring 2008, Kirschner & Chen 2010). The genus and species concept of *Th. papuana* with a complex conidiogenous head and 0–1-septate conidia was based only on culture characteristics (Arzanlou et al. 2007). Morphological separation of the genus from *Periconiella* was not accomplished, but based only on a gradually more (up to six times) branched head and presence of “micronematous” conidiophores in culture of *Th. papuana*. Less complex conidiophores (i.e. not composed of a long stipe and a branched head), however, also occur in true (phylogenetically supported) *Periconiella* species, e.g. *P. machilicola*, as well as complex heads with up to four levels of branches (Kirschner & Chen 2010). *Ramichloridium*-like conidiophores were also recorded for a species tentatively identified as *P. geonomae* (Kirschner et al. 2014). On the natural substrate and in fresh culture, conidiogenous heads of *Th. papuana* are not as complex as after



Figs 1–13 – Drawings of *Thysanorea papuana*. 1–8 Specimens from natural substrate (1, 2 R. Kirschner 4019, Taiwan; 3–6 R. Kirschner & C.-J. Chen 1356, Taiwan; 7, 8 holotype, Papua New Guinea). 1 Apex of penicillate conidiophore showing a percurrent extension (arrowhead) and a 3-septate conidium. 2 Semimacronematous conidiophores not differentiated into stipe and head. 3 Bases of penicillate conidiophores, the right one with aseptate rhizoids, the left one with few several-celled supporting hyphae. 4 Conidiophore apices, note percurrent proliferations of the stipe (arrowheads). 5 Detached conidiophore branches with intercalary and terminal conidiogenous cells. 6 Conidia. 7 Conidiophore from holotype showing percurrent extensions (arrowheads). 8 1–3-septate conidia from holotype. 9–13 Specimen from culture (R. Kirschner 4019). 9 Conidiophores associated with rhizoidal structures at the base. 10 Conidiophore head. 11 Branches detached from conidiophore head. 12 Non-penicillate conidiophores. 13 Conidia.
 – Scale bars = 10 μ m, except 9, 12 = 25 μ m



Figs 14–16 – SEM photographs of conidiogenous cells and conidiogenous loci from macronematous conidiophores of *Thysanorea papuana* (14, 15 from holotype, 16 from R. Kirschner & C.-J. Chen 1356). – Scale bars: 14 = 10 μm , 15 = 2.5 μm , 16 = 5 μm .

prolonged cultivation, but show up to 4 levels of branching (up to 3 levels in the specimen from India, Pratibha & Prabhugaonkar 2015) so that more complex branching appears to be an artifact of a degenerated culture.

In the material from field collections from Taiwan, the conidiophore heads and branches break off easily from the stipe and seem to be replaced by regeneration after percurrent extension of the stipe apex (Figs 1, 4, 5). The same characteristic was also found in the holotype of *Th. papuana* (Fig. 7). This phenomenon was described as “axial proliferation of conidiogenous cells” as particular feature of *R. lignicola* and illustrated in Tsui et al. (2001, Fig. 10). This feature was also clearly shown for the specimen from India (Pratibha & Prabhugaonkar 2015, Fig. 2). The type of *A. lignicola* was not available in HAC. A photograph of a conidiophore of *A. lignicola* in Mercado et al. (1996, Fig. 2) shows an abrupt change from the dark brown stipe to a pale head also indicating a break off of the stipe apex and percurrent extension by paler cells. The constrictions of septa and percurrent proliferation at the distal part of the conidiophores indicating a repeated break-off of the conidiogenous head or parts of it with subsequent regeneration appear to be different from other species retained in *Periconiella*. This easy break off and regeneration of the conidiogenous head by percurrent proliferation of the stipe apex seems to be a good morphological marker not only on the species level (Tsui et al. 2001), but also on the genus level. This kind of regeneration was not found for the conidiophores in culture by Arzanlou et al. (2007) nor in our study, probably due to the undisturbed growth in culture.

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