Four new freshwater fungi associated with submerged wood from Southwest Asia

D.M. Hu[1, 2], L. Cai[3]*, H. Chen[1], A.H. Bahkali[4] & K. D. Hyde[1,4,5]*

[1] International Fungal Research & Development Centre, The Research Institute of Resource Insects, Chinese Academy of Forestry, Bailongsi, Kunming 650224, P.R. China
[2] School of Chemistry and Life Science, Gannan Normal University, Ganzhou 341000, P.R. China
[3] Key Laboratory of Systematic Mycology & Lichenology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, P.R. China
[4] Botany and Microbiology Department, College of Science, King Saud University, Riyadh, Saudi Arabia
[5] School of Science, Mae Fah Luang University, Chiang Rai, Thailand

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One new teleomorphic and three new anamorphic ascomycetes from fresh water are introduced in this paper based on morphological characters. The new teleomorphic ascomycete *Ascominuta ovalispora* sp. nov. was collected from the north of Thailand. The three new anamorphic ascomycetes *Acrogenospora ellipsoidea* sp. nov., *Dictyosporium biseriale* sp. nov. and *Vanakripa menglensis* sp. nov. were collected from Yunnan, China. Keys to species of *Acrogenospora* and *Vanakripa* are provided.

Keywords: *Acrogenospora*, *Ascominuta*, ascomycetes, *Dictyosporium*, *Vanakripa*.

Fungi play an important role in decomposing woody debris in freshwater habitats (Wong *et al.* 1998, Cai *et al.* 2006, Simonis *et al.* 2008). Studies on freshwater fungi are, however, still limited and it is still fairly common to discover new species (e.g., Raja *et al.* 2009a). Knowledge concerning the geographic distribution of freshwater fungal species is also limited (Raja *et al.* 2009b). Investigations of lignicolous fungi in freshwater habitats have been conducted by our group (Luo *et al.* 2004, Cai *et al.* 2006, Hu *et al.* 2007, Kodsueb *et al.* 2008, Pinruan *et al.* 2008) to increase knowledge of freshwater fungi in China and the Asian region. During our present investigation of lignicolous fungi in Southeast Asia (Thailand & Yunnan Province, China), four species were discovered that appeared to be new to science.

* Corresponding authors: L. Cai: mrcailei@gmail.com; K.D. Hyde: kdhyde2@gmail.com

The first species is an ascomycete belonging to the monotypic genus *Ascominuta* V.M. Ranghoo & K.D. Hyde, but differs from the type species. It was collected in Thailand. Three new anamorphic fungi were collected in Yunnan Province, China. After checking the morphological characters of the three taxa, they should be classified in *Acrogenospora* M.B. Ellis, *Dictyosporium* Corda and *Vanakripa* Bhat, W.B. Kendr. & Nag Raj, respectively. The four taxa could be distinguished from other species within the four genera; and thus, we describe and illustrate them here as new species.

Materials and Methods

Visits were made to Chiang Mai Province, Thailand and Yunnan Province, China. Samples were collected from streams and lakes; Caution was taken to ensure that the wood samples were in a similar state of decay. During the visits, about 400 samples were collected. The dimensions of these samples ranged from ca. 2 cm to 6 cm diam. and were ca. 30 cm long. The samples were incubated following the method described by Cai *et al.* (2006).

Samples were examined for fungal fruiting bodies every week during the incubation period (usually 3 months). The fruiting bodies were picked off with a sterilized needle. Identification was made based on materials mounted in distilled water. The dry specimens are deposited in Mae Fah Luang University herbarium (MFLU) and International Fungal Research and Development Centre (IFRDC).

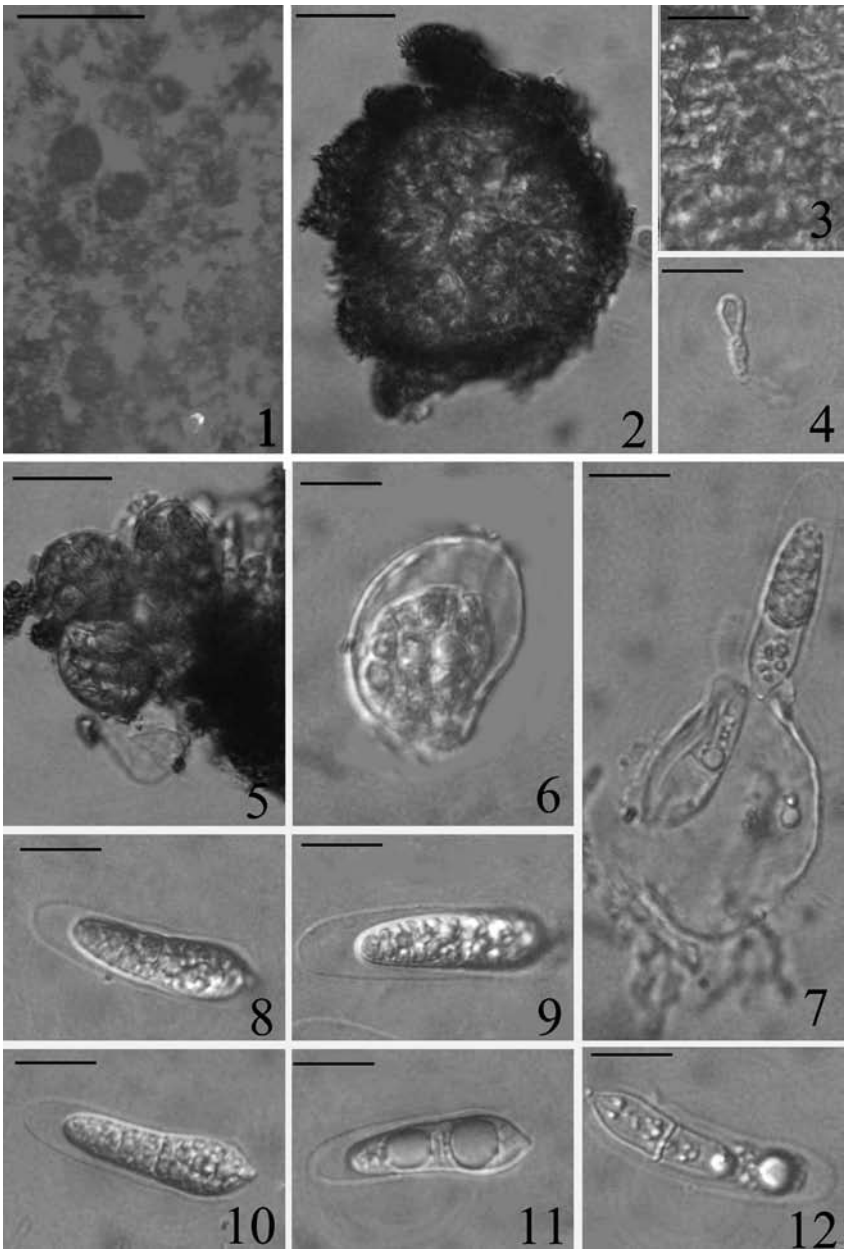
Taxonomy

Ascominuta ovalispora D.M. Hu & K.D. Hyde, **sp. nov.** – Figs. 1–12.
MycoBank no.: MB 518631

Ascomata 90–120 µm alta, 80–90 µm diam., superficialia vel partim immersa, globosa, atrobrunnea vel nigra, solitaria vel aggregata. Peridium perithecii 8–13 µm latum, 3–5-stratosum, ex cellulis hyalinis, extrinsecus textura angularis fuscis compositum. Paraphyses septatae, 8–20 µm longae, a septo constrictae. Asci 35–38 × 25–30 µm, 4-spori, globosi, pedicellati, bitunicati, sine apparatu apicali. Ascosporae 28–30 × 9–10 µm, multiseriatae, ovoides, 1-septatae, constrictae, hyalinae, muris laevigatae, tunica gelatinosa praeditae.

Holotypus. – Thailandia. D. M. Hu (MFUL08 1176).

Ascomata 90–120 µm high, 80–90 µm in diam., ostiolate, necks not visible, superficial to partly immersed in the host substrate, globose to subglobose, dark brown to black, solitary or aggregated. Ostiole periphysate. Peridium 8–13 µm wide, comprising three to five layers of hyaline compressed cells, with an outer layer of dark brown *textura angularis*. Pseudoparaphyses 8–20 µm long, sparse, comprising short chains of globose cells, apical cell swollen. Asci 35–38 × 25–30 µm, 4-spored, globose, non-pedicellate, bitunicate. Following



Figs. 1–12. – *Ascominuta ovalispora*. **1.** Appearance of ascomata on submerged wood. **2.** Section of ascomata. **3.** Surface of peridium. **4.** Pseudoparaphyses. **5–7.** Asci. **8–12.** Ascospores. Scale bars: 1 = 300 µm; 2 = 50 µm; 3–4 = 20 µm, 5 = 50 µm, 6–12 = 20 µm.

the dehiscence of the ectotunica, the endotunica extends and finally the ascospores are released. Ascospores 28–30 × 9–10 µm ($\bar{x}$ = 29 × 9 µm, n = 30), multiseriate, ovoid, 1-septate, slightly constricted at the septum, with a basal papilla-like swelling, hyaline, surrounded by a sac-like persistent sheath, usually with large globule in each cell when young, germinated from both ends of the ascospores.

Etymology. – *ovalispora*, in reference to the ovoid ascospores.
Habitat. – Saprobic on submerged wood.
Distribution. – Thailand.

Materials examined. – Holotype. – ***Ascominuta ovalispora*** D.M. Hu, H. Chen & K.D. Hyde: Thailand, Chiang Mai, Mushroom Research Center stream, on submerged wood, 24 Aug 2008, *leg*. D. M. Hu (MFUL08 1176).

Additional material examined: *ibid* MFUL08-1177; *ibid* MFLU10-0023; *ibid* MFLU10-0024.

Discussion. – The genus *Ascominuta* was established by Ranghoo & Hyde (2000) to accommodate the type species *A. lignicola* Ranghoo & K. D. Hyde. The genus was placed within the Ascomycetes *incertae sedis* by Ranghoo & Hyde (2000) and subsequently placed within the Dothideomycetes *incertae sedis* by Lumbsch & Huhndorf (2007).

This fungus should be placed in *Ascominuta* because it produces 4-spored, globose asci, 1-septate ascospores surrounded by an irregular mucilaginous sheath, sparse pseudoparaphyses comprising short chains of globose cells and relatively small ascomata. The new species differs from the type species in having larger ascomata, apically swollen pseudoparaphyses and ovoid ascospores with a sac-like persistent sheath. The ascospores of both species in the genus *Ascominuta* are compared in Tab. 1.

Tab. 1. – Comparison of the ascospores of *Ascominuta* species.

Ascospores	Picture	Size (µm)	Shape	Sheath
A. lignicola		22–25 × 8–10	broadly fusiform	irregular, mucilaginous sheath
A. ovalispora		28-30 × 9–10	ovoid	sac-like, persistent sheath

Acrogenospora ellipsoidea D.M. Hu, L. Cai & K.D. Hyde, **sp. nov.** – Figs. 13–19.
MycoBank no.: MB 518632

Coloniae sparsae, disseminatae, atrae, nitidae. Mycelium plerumque in substrato immersum, ex hyphis septatis, laevibus, subhyalinis vel pallide brunneis. Co-

nidiophora macronemata, mononemata, solitaria, erecta, laevia, pallide aurantiobrunnea vel modice brunnea, apicem versus pallidiora, septata, cylindrical, 87.5–162.5 µm longa, 6.5–7.5 µm lata, proliferations conidiogenas percurrentes elongescentia. Cellulae conidiogenae monoblasticae, integratae, terminals, cylindrical, pallide brunneis. Conidia acrogena, sicca, ellipsoidea, atro-brunnea, non-septata, 32–41 × 17–24 µm, ad fundamentum truncata et 4.5–5.5 µm lata. Conidiis secedentibus schizolyticibus.

Holotypus. – China. D. M. Hu (IFRDC 8883).

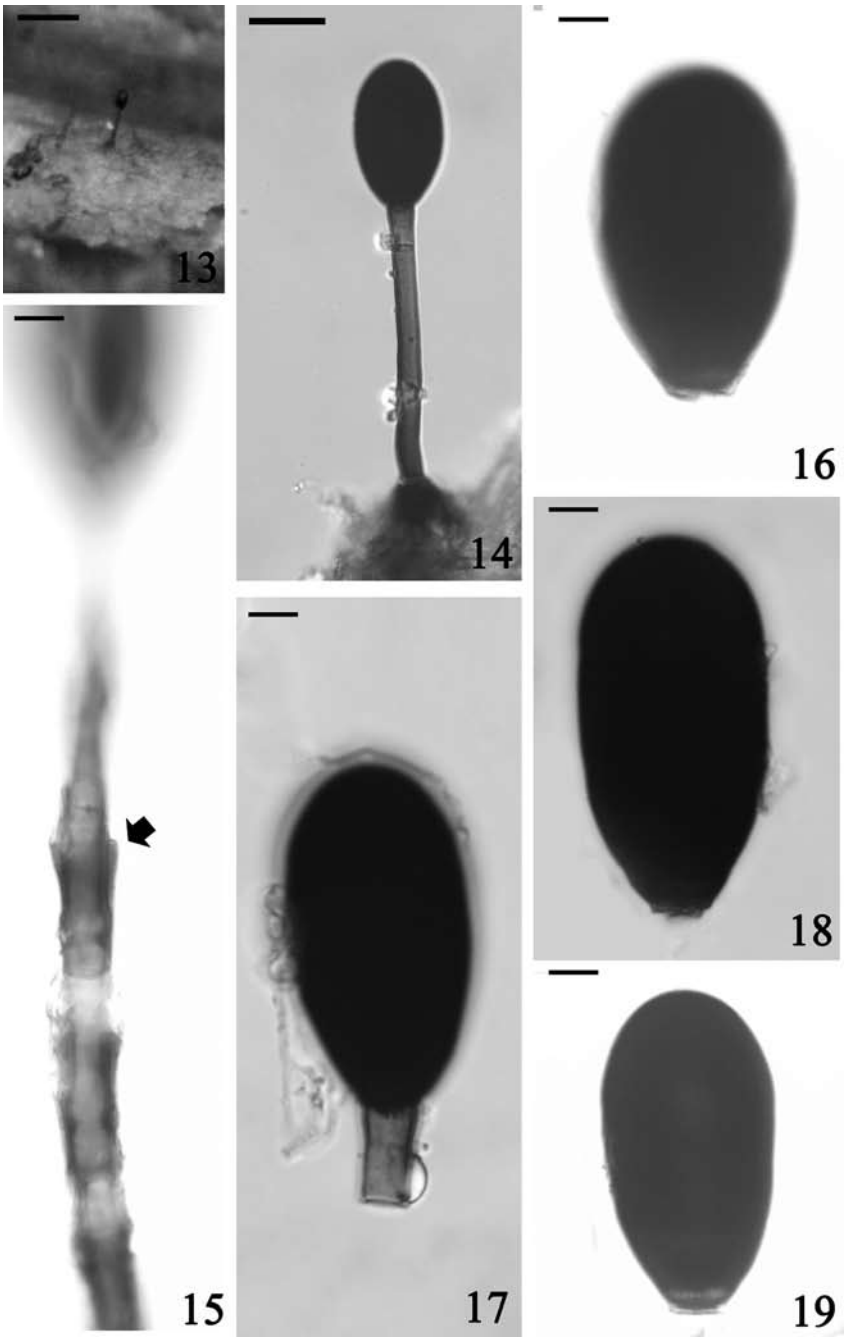
Colonies sparse, scattered, glistening. Mycelium mostly immersed in the substratum, consisting of septate, smooth, yellowish brown, 2–4 µm wide hyphae. Conidiophores macronematous, mononematous, solitary, erect, smooth, pale orange brown to mid brown, paler towards the apex, rarely septate, 1-2-septate, 87.5–162.5 µm long, 6.5–7.5 µm wide, with multiple percurrent proliferations. Conidiogenous cells monoblastic, integrated, terminal, cylindrical, pale brown. Conidia acrogenous, dry, ellipsoidal, dark brown, smooth, non-septate, 32–41 × 17–24 µm ($\bar{x}$ = 36.1 × 20.3 µm, n = 30), with a truncate base 4.5–5.5 µm wide; conidial secession schizolytic.

Etymology. – *ellipsoidea*, referring to the ellipsoidal conidia.
Habitat. – Saprobic on submerged wood.
Distribution. – China, Yunnan Province.

Materials examined. – Holotype. – ***Acrogenospora ellipsoidea*** D.M. Hu, L. Cai & K.D. Hyde: China, Yunnan Province, Xishuangbanna, Mengla stream, on submerged wood, 2 Apr 2009, *leg.* D. M. Hu (IFRDC 8883).

Discussion. – *Acrogenospora* was established by Ellis (1971) to accommodate *Acrogenospora sphaerocephala* (Berk. & Broome) M.B. Ellis (≡ *Monotosporella sphaerocephala* Berk. & Broome). *Monotosporella* S. Hughes is similar to *Acrogenospora* in many respects but differs in having septate conidia (Ellis, 1971). *Brachysporiella* resemble *Acrogenospora* in conidial shape, however, *Brachysporiella* have branched conidiophores. Nine species were accepted in *Acrogenospora* (Hughes 1978, Goh *et al.* 1998, Zhu *et al.* 2005).

Acrogenospora ellipsoidea is characterized by dark brown, ellipsoidal conidia, the size of conidia; and pale orange brown to mid brown, erect conidiophores. *Acrogenospora ellipsoidea* is similar to *Acrogenospora ovalia* described by Goh *et al.* (1998) in shape of conidia. However, *Ascogenospora ellipsoidea* produces larger dark brown conidia (32–41 × 17–24 µm vs. 24–33 × 18–22 µm), that are rarely septate, 1-2-septate, shorter and wider conidiophores (87.5–162.5 × 6.5–7.5 µm vs. 240 × 4–4.5 µm).



Figs. 13–19. – *Acrogenospora ellipsoidea*. **13.** Colony on submerged wood. **14.** Conidiophore. **15.** Upper part of a conidiophore (Arrow show the proliferation). **16–19.** Conidia. Scale bars: 13 = 100 µm, 14 = 20 µm, 15–19 = 5 µm.

Key to the species of *Acrogenospora*

1. Conidia verrucose, spherical or subspherical, 19–21.5 µm in diam. ..... *A. verrucospora*
1*. Conidia smooth 2
2. Conidia mostly less than 33 µm long 3
2*. Conidia mostly exceeding 35 µm long 6
3. Conidiophores pale brown to dark brown with clearly visible septa; conidia mostly subspherical or oval 4
3*. Conidiophores dark blackish brown to opaque, septa obscured or barely visible; conidia mostly broadly ellipsoidal to obovoid 5
4. Conidia subspherical, 17–30 × 15.5–30 µm ..... *A. sphaerocephala*
4*. Conidia oval to oblong, 24–33 × 18–22 µm ..... *A. ovalia*
5. Conidia 19–32 × 13–23.5 µm, obovoid, truncate base 5–8 µm wide ..... *A. megalospora*
5*. Conidia 14.5–24 × 10.5–19 µm, broadly ellipsoidal, truncate base 4–5 µm wide ..... *A. setiformis*
6. Conidia blackish brown or opaque 7
6*. Conidia paler 9
7. Conidia truncate base 4.5–5.5 µm wide, ellipsoidal, 32–41 × 17–24 µm ..... *A. ellipsoidea*
7*. Conidia truncate base 5.8–10 µm wide 8
8. Conidia blackish brown to opaque, broadly obovoid to spherical, 31–47 × 28–40 µm, truncate base 5.8–7.2 µm wide ..... *A. gigantospora*
8*. Conidia dark brown to blackish brown, broadly ellipsoidal, 40–60 × 30–36 µm, truncate base 8–10 µm wide ..... *A. altissima*
9. Conidiophores black (opaque), septa only visible in the upper part; conidia mostly oblong, medium brown to dark brown, 26-54 × 21.5-30.5 µm..... *A. novae-zelandiae*
9*. Conidiophores pale brown to mid brown, septa clearly visible; conidia mostly subprolate, pale orange-brown to olivaceous brown, 39–46 × 30–39 µm ..... *A. subprolata*

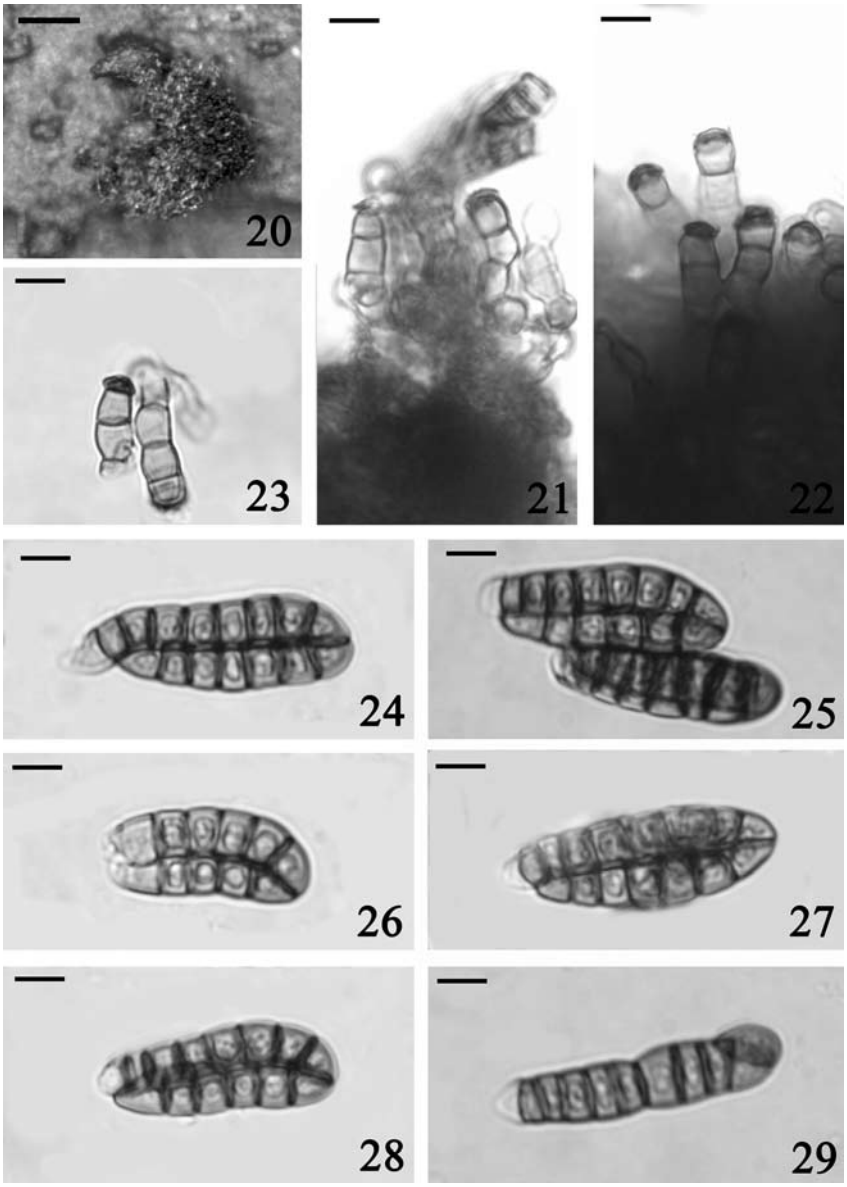
Dictyosporium biseriale D.M. Hu, L. Cai & K.D. Hyde, **sp. nov.** – Figs. 20–29.
MycoBank no.: MB 518633

Sporodochia in substrato naturali punctiformia, dispersa, nigra, granulate. Mycelium plerumque in substrato immersum, ex hyphis ramosis, septatis, laevibus compositum. Conidiophora micronemata, mononemata, tenuiparietales, septatis, pallide brunnea, 12–20 × 3–5 µm. Cellulae conidiogenae monoblasticae, integratae, terminals, cylindrical, pallide brunneis. Conidia 22–30 × 9–10 µm ($\bar{x}$ = 26 × 10 µm, n = 25), complanata, cheiroidea, laevia, pallide brunnea vel brunnea, in 10–17 cellulis, 2-serialibus composita, cellula basali cuneiformia. Conidiis secedentibus rhexolyticibus.

Holotypus. – China. D. M. Hu (IFRDC 8884)

Sporodochia on natural substratum punctiform, scattered, black, granular. Mycelium mostly immersed in the substratum, composed of branched, septate, smooth hyphae. Conidiophores micronematous, mononematous, thin-walled, septate, sparsely branched, pale brown, 12–20 × 3–5 µm. Conidiogenous cells monoblastic, integrated, terminal, determinate, pale brown, cylindrical. Conidia

22–30 × 9–10 µm ($\bar{x}$ = 26 × 10 µm, n = 25), complanate, cheiroid, smooth-walled, pale brown to brown, consisting of 10–17 cells arranged in 2 rows, with a cuneiform hyaline cell attached at the base of one of the arms, conidial secession rhexolytic.



Figs. 20–29. – *Dictyosporium biseriale*. **20.** Sporodochia on submerged wood. **21–23.** Conidiophores. **24–28.** Conidia. 29. An arm of a conidium. Scale bars: 20 = 100 µm, 21–29 = 5 µm.

Etymology. – *biseriale*, referring to the 2-armed conidia.
Habitat. – Saprobic on submerged wood.
Distribution. – China, Yunnan Province.

Materials examined. – Holotype. – ***Dictyosporium biseriale*** D.M. Hu, L. Cai & K.D. Hyde: China, Yunnan Province, Xishuangbanna, Mengla stream, on submerged wood, 2 Apr 2009, *leg.* D. M. Hu (IFRDC 8884).

Discussion. – Thirty-three species are presently accepted in the genus *Dictyosporium*. A key to the *Dictyosporium* species was provided by Crous *et al.* (2009). Nineteen species in the genus were recorded from freshwater habitats. Tsui *et al.* (2006) discussed the phylogenetic relationship among mitosporic fungi with cheiroid conidia in the genera *Cheiromoniliophora, Dictyosporium, Kamatia* and *Pseudodictyosporium*. *Dictyosporium* resembles the genera *Digitodesmium, Cheiromyces, Canalisporium* and *Berkleasmium* in having blackish sporodochia, dark brown to pale brown, multicelled conidia. The phylogenetic relationship among these five genera has not been investigated. The morphological differences among the five genera are listed in Tab. 2.

Dictyosporium biseriale fits the generic concepts of *Dictyosporium* (Ellis 1971) well in having sporodochial colonies, micronematous conidiophores, and cheiroid, euseptate, muriform conidia. *Dictyosporium biseriale* differs to other accepted species in *Dictyosporium* in having conidia with 2-arms.

Tab. 2. – Synopsis of *Dictyosporium* and closely related genera.

Genera	Conidiophore	Conidia			
		Cell arrangements	Septa	Arms	Base cells
Berkleasmium	macronematous	irregular, in different planes	euseptate		none
Canalisporium	macro-, micro- or semi-macronematous	complanate, cell lumen connected by septal canals	euseptate	not separate at the apex	1 or 3
Cheiromyces	micronematous to semimacronematous	cheiroid, in different planes	distoseptate	separate at the apex	1
Dictyosporium	micronematous	cheiroid, mostly complanate	euseptate	not separate at the apex	1
Digitodesmium	semi-macronematous	cheiroid	euseptate	separate at the apex	1

Vanakripa menglensis D.M. Hu, L. Cai and K.D. Hyde, **sp. nov.** – Figs. 30–42.
MycoBank no.: MB 518634

Coloniae in substrato naturali sporodochiales 60–210 µm in diam., dispersa, nigrae. Mycelium immersum, ex hyphis septatis, ramosis, incoloratis. Conidiophora

micronemata, cylindrical, aseptata, simplicia vel sparse ramose, laevia, hyaline, ca. 7.5 × 2.5 µm. Cellulae conidiogenae incorporatae, terminals, determinatae. Cellulae separabiles 20–40 × 4–6 µm, vermiformes, clavatae vel hyalinae. Conidia (8–)17–23 × 8–13 µm ($\bar{x}$ = 20.4 × 11.3 µm, n = 30), acrogena, solitaria, aseptata, clavata vel obpyriformia, laevia, brunnea vel atra. Conidiis secedentibus rhexolyticibus.

Holotypus. – China. D. M. Hu (IFRDC 8882).

Colonies on submerged wood sporodochial, 60–210 µm in diam., scattered, black. Mycelium mostly immersed in substratum, composed of septate, branched, hyaline hyphae. Conidiophores micronematous, ca. 7.5 × 2.5 µm, hypha-like, short cylindrical, aseptate, simple or sparsely branched, smooth, hyaline. Conidiogenous cells holoblastic integrated, terminal, determinate. Separating cells 20–40 × 4–6 µm, hyaline, clavate to vermiform. Conidia (8–)17–23 × 8–13 µm ($\bar{x}$ = 20.4 × 11.3 µm, n = 30), acrogenous, solitary, clavate to obpyriform, smooth, brown to dark brown, aseptate. Conidial secession rhexolytic.

Etymology. – *Menglensis*, referring to the location where this fungus was collected.

Habitat. – Saprobic on submerged wood.

Distribution. – China, Yunnan Province.

Materials examined. – Holotype. – ***Vanakripa menglensis*** D.M. Hu, L. Cai and K.D. Hyde: China, Yunnan Province, Xishuangbanna, Mengla stream, on submerged wood, 2 Apr 2009, *leg.* D. M. Hu (IFRDC 8882).

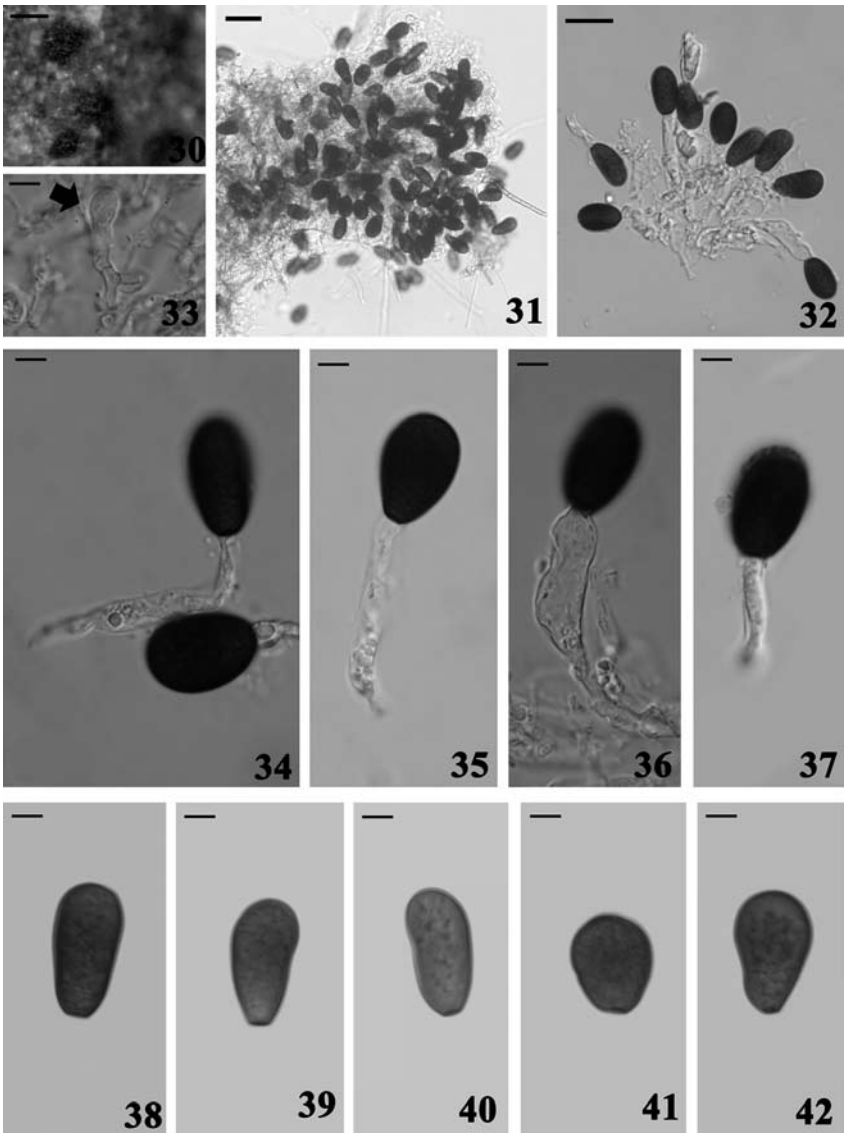
Discussion. – *Vanakripa* Bhat, W.B. Kendr. and Nag Raj was established by Bhat & Kendrick (1993) to accommodate *V. gigaspora* Bhat, W.B. Kendr. & Nag Raj and *V. parva* Bhat, W.B. Kendr. & Nag Raj. This genus can be easily distinguished by its conidial morphology and a separating cell attached at the base of conidia.

There were five described species within *Vanakripa* (Pinnoi *et al.* 2003, Tsui *et al.* 2003, Ruiz *et al.* 2005), *Vanakripa menglensis* differs in the clavate to obpyriform conidia and its unique size [(8–) 17–23 × 8–13 µm].

Key to the species of *Vanakripa*

1. Conidia with a submedian septum, 32–43 × 18–22 µm ..... *V. gigaspora*
1*. Conidia one-celled 2
2. Conidia less than 24 µm long 3
2*. Conidia longer than 24 µm 4
3. Conidia 7.5–10.5 × 4–6 µm ..... *V. parva*
3*. Conidia (8–) 17–23 × 8–13 µm ..... *V. menglensis*
4. Separating cells less than 9 µm wide, conidia 24–34 × 16–23 µm ..... *V. fasciata*

4*. Separating cells wider than 10 µm 5
5. Conidia 25–33 × 16–23 µm ..... *V. minutiellipsoidea*
5*. Conidia 33–42 × 20–25 µm .....*V. ellipsoidea*



Figs. 30–42. – *Vanakripa menglensis*. **30.** Colonies on submerged wood. **31.** Squash mount of a sporodochium with conidia. **32.** Conidiophores bearing conidia. **33.** Conidiogenous cell. **34–37.** Conidia bearing separating cells. **38–42.** Conidia. Scale bars: 30 = 100 µm, 31 = 30 µm, 32 = 20 µm, 33–42 = 5 µm.

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