Mycoscience (2010) 51:75–80
DOI 10.1007/s10267-009-0006-2

SHORT COMMUNICATION

Asterostroma species (*Basidiomycota*) from mangrove forests in Japan

Hiroto Suhara · Nitaro Maekawa · Shuji Ushijima · Kazuhiko Kinjo · Yoshikazu Hoshi

Received: 6 August 2008 / Accepted: 2 July 2009 / Published online: 25 December 2009

Abstract A new homobasidiomycete, *Asterostroma macrosporum*, was found in mangrove forests of Iriomote Island, Japan. This species is morphologically characterized by having resupinate basidiomata, a monomitic (asterodimitic) hyphal system, simple septate generative hyphae, dextrinoid asterosetae, four sterigmate basidia and globose, tuberculate and amyloid basidiospores measuring 8.5–11 × 7.5–9 μm. It is similar to *A. muscicola*, but basidiospores in the latter are smaller (7–8 × 5.5–7 μm). Furthermore, phylogenetic analysis using internal transcribed spacer (ITS) region revealed that *A. macrosporum* is distinctly separated from *A. muscicola*. In Japan, *A. muscicola* is widely distributed in warm-temperate to subtropical regions, growing on a variety of broadleaved trees including mangroves, while *A. macrosporum* has been found only on mangroves.

H. Suhara (✉) · N. Maekawa
Fungus/Mushroom Resource and Research Center,
Faculty of Agriculture, Tottori University,
4-101 Koyama-Minami, Tottori 680-8553, Japan
e-mail: h_suhara@muses.tottori-u.ac.jp

S. Ushijima
The United Graduate School of Agricultural Science,
Tottori University, Koyama, Tottori, Japan

K. Kinjo
Laboratory of Applied Bioresource Science,
Department of Bioscience and Biotechnology,
Faculty of Agriculture, University of Ryukyus,
Senbaru, Nishihara-cho, Okinawa, Japan

Y. Hoshi
Department of Plant Science, School of Agriculture,
Tokai University, Kawayou, Minamiaso-mura,
Aso-gun, Kumamoto 869-1404, Japan

Keywords Internal transcribed spacer (ITS) region · *Lachnocladiaceae* · Taxonomy

The genus *Asterostroma* Massee belonging to the family *Lachnocladiaceae* (*Homobasidiomycetes*) is characterized by resupinate and felted-membranous basidiomata, and the presence of gloeocystidia, clampless generative hyphae and dextrinoid asterosetae (asterohyphidia). Based on the morphological characters of the basidiospores, the genus is divided into two subgenera, *Austroasterostroma* Parmasto and *Asterostroma*: the former produces smooth and inamyloid basidiospores whereas those of the latter are amyloid (Parmasto 1970). Furthermore, the subgenus *Asterostroma* is subdivided into two sections, *Laevispora* Parmasto (with smooth basidiospores) and *Asterostroma* (with ornamented basidiospores) (Boidin et al. 1997). According to the Aphyllophorales taxonomic database (http://www.cbs.knaw.nl/databases/aphyllo/database.aspx) provided by the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, twenty-five species have been reported for this genus, and most have been isolated in tropical areas. In Japan, only one species, *A. cervicolor* (Berk. & M.A. Curtis) Massee, was reported (Aoshima et al. 1963) from temperate areas.

Recently, we collected several *Asterostroma* specimens with tuberculate and globose basidiospores in mangrove forests of Iriomote Island, located in the subtropical southwest of Japan. These specimens were apparently distinct from *A. cervicolor*, and we carried out a detailed study on their morphology and phylogeny.

The materials examined in this study are listed in Table 1; the specimens were deposited in the Tottori University Mycological Herbarium (TUMH), Tottori or herbarium of the Tottori Mycological Institute (TMI),

Table 1 DDBJ accession numbers of *Asterostroma* specimens and isolates used in the present phylogenetic analysis

Species	Specimen	Isolate no.	DDBJ accession no.
Asterostroma macrosporum	TMI25696	TUFC10014	AB439544
	TMI25697	TUFC10013	AB439545
Asterostroma musicola	TMI25737	TMIC35030	AB439547
	TMI25744	TMIC35031	AB439546
	TMI25842	TMIC35077	AB439548
	TMI25738	–	AB439549
	TMI25698	TMIC35098	AB439556
	TUMH10017	–	AB439557
	TMI25191	TMIC34896	AB439555
	TMI25197	TMIC34903	AB439554
	TUMH10016	TMIC34316	AB439552
	TMI25769	TMIC35096	AB439550
	–	TMIC34317	AB439553
	TMI25860	TMIC35097	AB439551
Asterostroma cervicolor	TMI20621	TMIC33878	AB439559
	TMI21362	TMIC34374	AB439560
	TMI21361	TMIC34378	AB439558

Tottori, and the cultures in Tottori University Fungal Culture Collection (TUFC), or the Tottori Mycological Institute Culture Collection (TMIC). In the description, colour names in double quotation marks are based on Rayner (1970). For microscopic observations, pieces of dried specimens were mounted in 3% KOH, Melzer's reagent (Weresub 1953), sulphobenzaldehyde reagent (Boidin 1951) or distilled water. In general, twenty measurements were made per element for each specimen in Melzer's reagent. Polysporous isolates obtained from each specimen were grown on malt extract agar [MA; 1.5% (w/v) malt extract and 1.5% (w/v) bacto agar; Difco, Detroit, MI, USA] at 25°C. Cultural characters were described after Nakasone (1990). To determine the optimum growth temperature, isolates were grown on MA plates at ten different temperatures (4°C, 10°C, 15°C, 21°C, 24°C, 27°C, 30°C, 33°C, 36°C and 39°C).

Molecular phylogenetic analysis was carried out using a total of 17 isolates of *Asterostroma* species, including the newly described species, *A. muscicola* (Berk. & M.A. Curtis) Massee and *A. cervicolor* (Table 1). *Asterostroma muscicola* is closest to the newly described species in terms of basidiospore characteristics among the known species of this genus. *Asterostroma cervicolor* was selected as the control group for the phylogenetic analysis because its basidiospore characters are distinct from the other two species. DNA extraction from cultural mycelia and specimens was performed by a method described previously (Suhara et al. 2002, 2003). Polymerase chain reaction (PCR) amplification was carried out for the internal transcribed spacer (ITS) region [ITS1, 5.8S rDNA and ITS2], using the primers ITS1-F and ITS4-B (Gardes and Bruns 1993). Amplifications were performed as previously reported (Suhara et al. 2002). Sequencing was performed on an ABI 310 genetic analyzer (Applied Biosystems Japan, Tokyo, Japan) according to the manufacturer's protocols. Phylogenetic analyses were carried out according to our previous report using an ITS dataset (Maekawa et al. 2005). All sequences used in this study were deposited in the DNA Data Bank of Japan (DDBJ); their accession numbers are listed in Table 1. The alignments were deposited in TreeBASE (http://www.treebase.org/) under accession number SN4784.

Asterostroma macrosporum N. Maek. & Suhara, sp. nov. Figs. 1, 2

MycoBank no.: MB515683.

Systema hyphale monomiticum; hyphae generatoriae cum septis, sine fibulis, 2–4 μm diametro, leaves, tenui-vel parum crassitunicatae (usque 0.5 μm), simpliciseptatae; asterohyphidia numerosa, radii ad 70 μm longi. Basidiomata resupinata, adnata, effusa, mollia, 200–800 μm crassa; superficies hymenialis "Buff", "Ochreous" vel "Fulvous" (see Rayner 1970), laevis vel tuberculata; margo albus vel "Buff", tenuescens, interdum sub lente (20×) fibrillosus vel fimbriatus. Cystidia (gloeocystidia) parum numerosa, subcylindracea, ventricosa vel fusoidea, 40–80 × 10–17 μm. Basidia subcylindrica vel utriformia, 38–65 × 8–9.5 μm, four sterigmata gignentia. Basidiosporae globosae apiculo distincto armatae, 8.5–11 × 7.5–9 μm (praeter tuberclis), tubercula (tuberclis usque 2 μm longi), tenuitunicatae, amyloideae (Figs. 1, 2).

Fig. 1 Basidiomata of *Asterostroma macrosporum* (TMI25697, holotype). *Bar* 1 cm

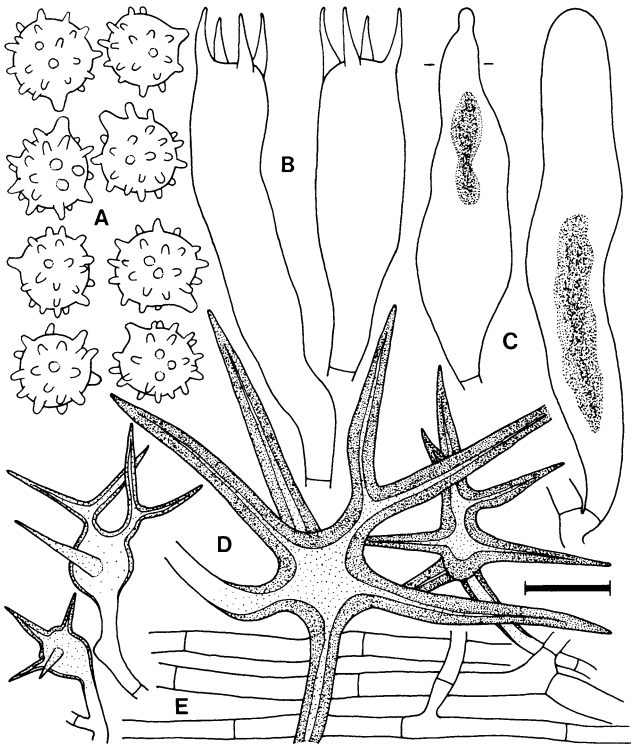

Fig. 2 *Asterostroma macrosporum* (TMI25697, holotype). *A* Basidiospores, *B* basidia, *C* cystidia (gloeocystidia). *Short horizontal lines* indicate the level of the hymenial surface. *D* Asterohyphidia (asterosetae). *E* Subicular hyphae. *Bar* 10 μm

Holotype: TMI25697 on dead branch of *Bruguiera gymnorrhiza* Lam., Komi (in mangrove forest of the mouth of Aira River, Iriomote Island), Taketomi-cho, Yaeyama-gun, Okinawa Pref., Japan, 7 November 2002, collected by N. Maekawa.

Etymology: macro + sporum (Latin), referring to the large basidiospores.

Basidiomata resupinate, adnate, effused, soft, felt-like, 200–800 μm thick; hymenial surface "Buff", "Ochreous" to "Fulvous", smooth to tuberculate, pruinose under the lens (20×), sometimes cracked when dried; margin "Buff" to white, thinning out, usually fimbriate under the lens (20×). Context in vertical section ocher, membranous, sometimes containing crystals in the subiculum. Hyphal system monomitic (asterodimitic); generative hyphae 2–4 μm in diameter, smooth, thin- to slightly thick-walled (up to 0.5 μm), clampless-septate, loosely intertwined in the subiculum; asterohyphidia (asterosetae) numerous in the subiculum and subhymenium, subhyaline to brownish coloured, diverging 2–9 branches, the branches acicular to subulate, up to 70 μm in length; cystidia (gloeocystidia) subcylindrical, ventricose to subfusiform, sometimes with schizopapillae, 40–80 × 10–17 μm, without a basal clamp, thin-walled, with pale yellowish oily contents, usually imbedded in the hymenium; basidia subcylindrical to utriform, 38–65 × 8–9.5 μm, thin-walled, without a basal clamp, producing four sterigmata; basidiospores globose, 8.5–11 × 7.5–9 μm (except for tubercles), with a distinct apiculus, tuberculate (tubercles up to 2 μm in length), thin-walled, amyloid. Associated with white rot.

Specimens examined: Japan: TMI25696 and TMI25697 (Holotype) on dead branch attached to a living tree of *Bruguiera gymnorrhiza*, Komi (in mangrove forest of the mouth of Aira River, Iriomote Island), Taketomi-cho, Yaeyama-gun, Okinawa Pref., 7 November 2002, collected by N. Maekawa; TMI25717 on dead branch of *B. gymnorrhiza*, Funaura (in mangrove forest, Iriomote Island), Taketomi-cho, Yaeyama-gun, Okinawa Pref., 8 November 2002, collected by N. Maekawa; TMI25750 on dead branch attached to a living tree of *Rhizophora mucronata* Lam., Komi (in mangrove forest of the mouth of Aira River, Iriomote Island), same locality, 14 July 2003, collected by N. Maekawa.

Culture characteristics: Optimal temperature for the two polysporous isolates (TUFC10013 and TUFC10014) examined was between 21°C and 25°C. These isolates could grow between 15°C and 30°C, but no visible growth was observed at 10°C and 33°C. Growth on MA 7.6–8.8 mm in radius at 24°C for 14 days. Mycelial mats white to pale buff, partly pale olivaceous or reddish in colour, downy, cottony to felty at 6 weeks; margin even, appressed, with irregularly fan-like extensions; odor slightly aromatic; colony reverse white to cream, pale ochraceous to pale olivaceous around inocula; no fruiting or discolouration, but occasionally changing to pale red by 6 weeks.

Marginal and aerial hyphae hyaline, 1.5–2.5 μm in diameter, sometimes with oily contents, smooth, thin-walled, clampless-septate, sparsely branched; submerged hyphae hyaline, 1.5–3 μm in diameter, usually with oily contents, thin-walled, clampless-septate, sometimes with

many short-branches, occasionally swelling; skeletal and binding hyphae lacking.

Species code (Nakasone 1990): 9. 13. 15. 16. 17. 25. 30. (31.) 36. (38.) 48. 52. (60.) (61.)

Asterostroma muscicola (Berk. & M.A. Curtis) Massee, J. Linn. Soc. Bot. 25: 155, 1889 Fig. 3

Basidiomata resupinate, loosely adnate, effused, soft, felt-like, 150–300 μm thick; hymenial surface "Saffron", "Buff", "Ochreous", "Fulvous" to "Cinnamon", smooth, pruinose under the lens (20×), sometimes slightly cracked when dried; margin concolourous with the hymenial surface, determinate, but sometimes thinning out, white, fimbriate, occasionally with thin hyphal strands under the lens (20×). Context in vertical section ocher, pellicular to submembranous, sometimes with thin mycelial strands in the subiculum, sometimes containing crystals in the subiculum. Hyphal system monomitic (asterodimitic); generative hyphae 1.5–4 μm in diameter, smooth, thin- to slightly thick-walled (up to 0.5 μm), clampless-septate, loosely intertwined in the subiculum; asterohyphidia (asterosetae) numerous in the subiculum and subhymenium, subhyaline to brownish, diverging 2–10 branches, the branches acicular to subulate, up to 70 μm in length; cystidia (gloeocystidia) subcylindrical, ventricose to fusiform, sometimes with schizopapillae, 35–55 × 7–15 μm, without a basal clamp, thin-walled, with pale yellowish oily contents, imbedded in the basidiomata, but sometimes projecting 10 μm beyond the hymenial surface; basidia subcylindrical to utriform, 28–40 × 5.5–6.5 μm, thin-walled, without a basal clamp, producing 2(–4) sterigmata; basidiospores globose, 7–8 × 5.5–7 μm (except for tubercles), with a distinct apiculus, tuberculate (tubercles up to 1.5 μm in length), thin-walled, amyloid (Fig. 3).

Newly reported for Japan.

Specimens examined: Japan: TMI25191 on dead blanch attached to a living tree of *B. gymnorrhiza*, Higashi-son, Kunigami-gun, Okinawa Pref., 8 July 2001, collected by N. Maekawa; TMI25197 on dead branch of a living tree of *Kandelia candel* Druce, Kin-cho (in mangrove forest of the mouth of Okkubi River, Okinawa Island), Kunigami-gun, Okinawa Pref., 8 July 2001, collected by N. Maekawa; TUMH10017 on dead bark of a living tree of *B. gymnorrhiza*, same location, same date, collected by N. Maekawa; TMI25698 on dead branch of *B. gymnorrhiza*, Funaura (in mangrove forest, Iriomote Island), Taketomi-cho, Yaeyama-gun, Okinawa Pref., 11 November 2002; TMI25240, TMI25737, TMI25738, TMI25739 and TMI25740 on dead branch of a living tree of *B. gymnorrhiza*, Kin-cho (in mangrove forest of the mouth of Okkubi River, Okinawa Island), Kunigami-gun, Okinawa Pref., 12 July 2003, collected by N. Maekawa.

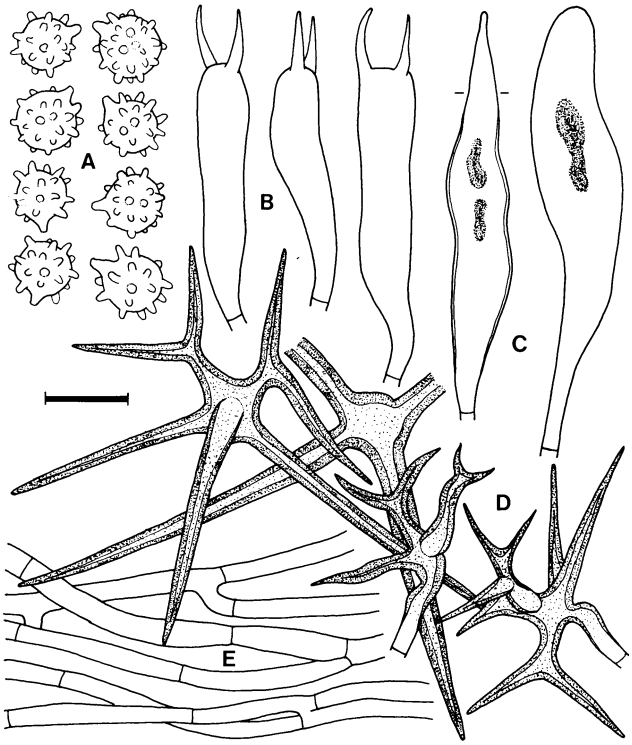

Fig. 3 *Asterostroma muscicola* (TMI25197). *A* Basidiospores, *B* basidia, *C* cystidia (gloeocystidia). *Short horizontal lines* indicate the level of the hymenial surface. *D* Asterohyphidia (asterosetae). *E* Subicular hyphae. *Bar* 10 μm

Additional specimens examined (substrates other than mangroves): Japan: TMI25769 on living trunk of *Cinnamomum camphora* (L.) T. Nees & C.H. Eberm., Minamitateishi, Beppu-shi, Oita Pref., 11 July 2004, collected by H. Suhara; TMI25860 on living trunk of *C. camphora*, Showa-cho, Omuta-shi, Fukuoka Pref., 29 July 2004, collected by H. Suhara; TMI25744 and TMI25842 on dead branch of a broadleaved tree, Mt. Onnadake, Kunigami-gun, Okinawa Pref., 12 July 2003, collected by N. Maekawa.

Figure 4 shows the phylogenetic tree obtained from neighbor-joining (NJ) analysis running 100 bootstrap replicates using overlapping datasets of the ITS region. The sequences were aligned in 546 positions, of which 80 (14.7%) were phylogenetically informative. *Asterostroma macrosporum*, *A. muscicola* and *A. cervicolor* formed a monophyletic clade with high bootstrap values (100%, respectively), and phylogenetic distance among *A. cervicolor*, *A. muscicola* and *A. macrosporum* was almost equivalent. These data suggest that *A. macrosporum* is distinguishable from *A. muscicola* and *A. cervicolor* molecular-phylogenetically.

Asterostroma macrosporum is characterized by the ochraceous, smooth to tuberculate basidiomata with pale ochraceous to white, fimbriate margins, globose and

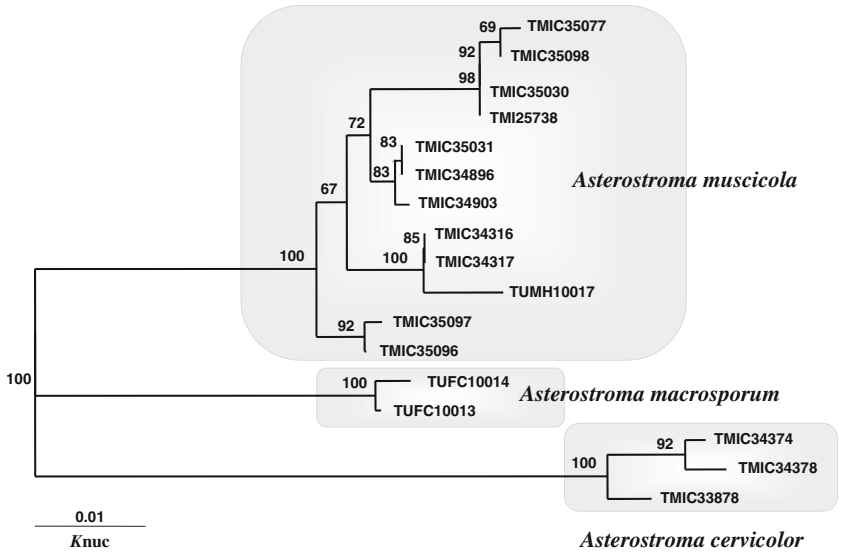

Fig. 4 Neighbor-joining tree derived from the ITS region sequence (ITS1, ITS2 and 5.8S). Phylogenetic tree shows unrooted tree. Confidence values from 100 bootstrap replications that are higher than 50% are given above branches. *Bar* indicates 0.01 *K*nuc in nucleotide sequences

Table 2 Basidiospores of *Asterostroma macrosporum* and its related species in the section *Asterostroma*

Species	Shape	Size (μm)
A. cervicolor	Ellipsoid	5–6 (–6.5) × 4–4.5
A. gaillardii[a]	Globose	6–7 × 6–6.5
A. macrosporum	Globose	8.5–11 × 7.5–9
A. muscicola	Globose	7–8 × 5.5–7
A. ochroleucum[a]	Globose	5.5–6.5 (–7.5)
A. persimile[a]	Globose	6 × 5

[a] According to Boidin et al. (1997)

tuberculate basidiospores measuring 8.5–11 × 7.5–9 μm (except for ornamentation) and consistently 4-sterigmate basidia. Because of the amyloid and ornamented basidiospores, this species belongs to the section *Asterostroma*, where nine species were reported (Boidin et al. 1997). Of these species, *A. echinosporum* Boidin, Lanq. & Gilles, *A. medium* Bres., *A. praeacutesporum* Boidin, Lanq. & Gilles and *A. spinososporum* Boidin, Lanq. & Gilles are different from *A. macrosporum* by possessing basidiospores with conical spiny ornamentation. The remaining five species, *A. cervicolor*, *A. gaillardii* Pat., *A. muscicola*, *A. persimile* Wakef. and *A. ochroleucum* Bres., have tuberculate basidiospores as in *A. macrosporum*, but *A. macrosporum* has larger basidiospores than them (Table 2). Among the five species, *A. muscicola* is closest to *A. macrosporum* in size and shape of the basidiospores, but the present study reveals that they are phylogenetically distinct. In Japan, substrates and geographical distribution of *A. muscicola* overlap with those of *A. macrosprum*. However, *A. macrosporum* has been found only on mangroves, namely *B. gymnorrhiza* and *R. mucronata*, while *A. muscicola* occurs on various broadleaved trees including mangroves and is widely distributed in warm-temperate to subtropical regions. So far there have been no reports of the members of the section *Asterostroma* from mangroves, therefore *A. macrosporum* and *A. muscicola* would represent the first members of the section occurring in mangrove forest.

Acknowledgments This study was partially supported by a Sasakawa Scientific Research Grant from the Japan Science Society (16-291), and a Grant-in-Aid for Scientific Research (KAKENHI 14540653) from the Japan Society for the Promotion of Science (JSPS).

References

Aoshima K, Hayashi Y, Furukawa H (1963) The genera *Asterodon* and *Asterostroma* of Hymenochaetaceae (in Japanese). Trans Mycol Soc Jpn 4:136–140

Boidin J (1951) Les réactifs sulfo-aldehydiques, leur intérêt pour la determination et la classification des Théléphoracées (Basidiomycètes). Bull Soc Nat Oyonnax 5:72–79

Boidin J, Lanquetin P, Gilles G (1997) Contribution a la connaissance du genre *Asterostroma* Massee 1889 (basidiomycotina). Bull Soc Mycol Fr 113:269–301

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetous—application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Maekawa N, Suhara H, Kinjo K, Kondo R, Hoshi Y (2005) *Haloaleurodiscus mangrovei* gen. sp. nov. (Basidiomycotina) from mangrove forests in Japan. Mycol Res 109:825–832

Nakasone KK (1990) Cultural studies and identification of wood-inhabiting Corticiaceae and selected Hymenomycetes from North America. Mycologia memoir no.15. Gebrüder Borntraeger, Berlin

Parmasto E (1970) The Lachnocladiacea of the Soviet Union. With a key to the boreal species. Institute of Zoology and Botany, Tartu, Estonia

Rayner RW (1970) A mycological colour chart. Commonwealth Mycological Institute and British Mycological Society, Kew, Surrey

Suhara H, Maekawa N, Kubayashi T, Sakai K, Kondo R (2002) Identification of the basidiomycetous fungus isolated from butt rot of the Japanese cypress. Mycoscience 43:477–481

Suhara H, Maekawa N, Kaneko S, Hattori T, Sakai K, Kondo R (2003) A new species, *Ceriporia lacerata*, isolated from white-rotted wood. Mycotaxon 86:335–347

Weresub LK (1953) Studies of Canadian Thelephoraceae. X. Some species of Peniophora, section Tubuliferae. Can J Bot 31:760–778