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By Hamid Kheyrodin and Khosro Ghazvinian

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RESEARCH PAPER

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Study of some Plant Inhabiting Fungi

Hamid Kheyrodin^{*} and Khosro Ghazvinian

Faculty of Desert Science-Semnan University, Iran*. Faculty of Veterinary and Animal Science-Semnan University, Iran.

ABSTRACT

Plant pathogenic fungi are moved around the world on plant material and accidentally introduced into new regions where they can be very damaging. Different kinds of plant material pose higher or lower levels of risk as pathways for introducing harmful fungi. In this paper, four plant-inhabiting fungi newly collected from the Nansei Islands, Japan, are reported. Among them, Phaeodiscula tremae Sawada from Tokunoshima Island was transferred to the genus Capnodiastrum and given a new name C. sawadae, because in the genus Capnodiastrum, the specific epithet tremae has already been used for the different South American species. Nyssopsora formosana on Koelreuteria formosana and Pestalotiopsis fici on Ardisia quinquegona collected in Okinawa Main Island and Pseudocercospora acaciaeconfusae on Acacia confusa collected in Okinoerabu Island were newly recorded from Japan. In this paper we reported that Propagative plant material including woody nursery stock, herbaceous plant germplasm and seeds are considered the most risky pathways.

Keywords: Plant-inhabiting fungi and Pseudocercospora acaciae-confusae.

INTRODUCTION

Fungi that inhabit terrestrial plants present many challenges when evaluating their invasion pathways and the risk posed by these pathways. One means of obtaining insight into potential invasion pathways is by reviewing past introductions, particularly how these fungi were moved from country to country. Using examples primarily from the United States, the risk of specific invasion pathways is assessed. Propagative plants, including woody nursery stock, herbaceous plant germplasm and seeds, and non-propagative plants, especially wood and wood products, are high-risk invasion pathways for fungi. Because global trade is increasing at a rapid rate, the introduction of new disease-causing fungi poses a threat to their new environment. Modern research tools exist that can be used to increase our knowledge of fungi in order to predict more precisely the risk posed by individual fungi.

Nansei Island is a group of many of islands located further south of Japan than the Kyushu Main Islands, and most of them are in a subtropical climate. In our previous two papers (Kobayashi et al. 2003; Motohashi et al. 2010), seven fungi collected in Nansei Islands were recorded as new to Japan, and one was transferred to the genus Phyllosticta from Strasseria. In this paper, three fungi, Nyssopsora formosana (Sawada) Lu tjeh. on Koelreuteria formosana, Pestalotiopsis fici Steyaert on Ardisia quinquegona, and Pseudocercospora acaciae-confusae (Sawada) Goh & Hsieh on Acacia confusa were described as new to Japan. The other one, Phaeodiscula tremae Sawada (1944) on Trema orientalis has now been transferred to the genus Capnodiastrum and given a new name, C. sawadae Tak. Kobay. & Ky. Watan., because the specific epithet tremae has been shared by old species C. tremae (Syd.) Petr. (1952). Descriptions 9. Capnodiastrum sawadae Tak. Kobay. & Ky. Watan. nom. nov. Figs. 1, 2a-d Synonym: Phaeodiscula tremae Sawada 1944, Rept. Taiwan Agr. Exp. Sta. 87:61, Pl.4: 18–19. non *Capnodiastrum tremae* (Syd.) Petr., 1952. Capnodiastrum guaraniticum sensu Katumoto & Harada, Trans. Mycol. Soc. Jpn. 20: 423, 1979, non Spegazzini 1886. Symptom and morphology (see Figs. 1 and 2a–d): Many small, dark brown to black fungal colonies develop mainly on the lower leaf surface as sooty patches. Diseased leaves turn pale yellow on the upper surface. The surfaces of fungal colonies are rough and granular with many minute pycnidia. Pycnidia are formed on short branches from the creeping hyphae on leaves, which are brown to dark brown

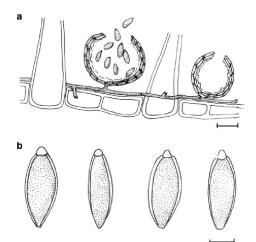


Fig. 1. *Capnodiastrum sawadae*. a Pycnidia and conidia on the lower leaf surface. b Brown matured conidia having small projection at the tip.

Barsa 50 Im; b 10 Im and 4–6 Im in diameter. Young pycnidia are globular at first, then widely open and cup- shaped, 100–125 Im in diameter, with a dark brown wall composed of irregularshaped cells and are 15–20 Im thick. Conidiogenous cells bear from the inner layer of the pycnidial wall and are hyaline to pale brown, small, conic, and produce conidia holoblastically. Conidia are elliptic to fusoid with a thick cell wall, truncate basal end, and rounded tip with a small, horn-like projection covered with a sticky cap, dark brown to sooty brown, often with a hyaline equatorial band, 27.5–35.9 13.8–17.5 Im (32.38.9 14.96 Im on average). Disease name: Komaru-susu-byo in Japanese (Sawada 1944), Sooty granular spot.

Specimen examined: On living leaves of Trema orientalis *Blume* (Urajiro-enoki in Japanese), Japan: Mt. Inokawa, Tokunoshima, Kagoshima Pref., Nov. 9, 1993, by T. Kobayashi (TK) and M. Muramoto (TFM: FPH-8057). FORMOSA (=Taiwan): Jen-tzou Hot Spring, Ilan Prefecture, Taiwan, October 13, 1982; by Amano, N. (TNS-F 176491). Taxonomic note: The present fungus was first described by Sawada (1944) from Formosa as Phaeodiscula tremae Sawada. The symptoms on the diseased leaves and the morphological characteristics of the fungus collected at Tokunoshima Island, Kagoshima Prefecture, were identical with those of Sawada. On the other hand, Katumoto and Harada (1979) reported quite similar fungus to Phaeodiscula tremae Sawada based on the diseased materials collected from Chichijima Island, Bonin Isles, Tokyo, as Capnodiastrum guaraniticum Speg. as shown in Table 1. Morphological characteristics of these three fungous materials in guestion was not identical to those of the monotypic species Phaeodiscula celottii Cub. (Saccardo 1892; Kirk et al. 2008). The latter do not develop a mycelial mat on the leaf surface, in which pycnidia are densely formed and have long filamentous conidiophores. On the other hand, these three fungous materials equally develop many pycnidia within the blackish mycelial mat mainly on the lower leaf surface and produce conidia holoblastically from short conidiogenous cells. Morphological characteristics of these 3 fungous specimens well correspond to those of the genus Capnodiastrum, as Katumoto and Harada (1979) identified their fungus as a member of the genus Capnodiastrum. The genus Capnodiastrum was established by Spegazzini in 1886 with 2 species. One is C. guaraniticum Speg., the type species and the other one is C. paraquaiensis Speg., which was treated as a synonym of C. guaraniticum by Sutton (1980). As 4 species were added in this genus by Petrak (1952), a total of 5 species has been recognized in the genus Capnodiastrum (Kirk et al. 2008). Among them, C. guaraniticum and C. tremae (Syd.) Petr. was known on ulmaceous plants, Celtis or Trema. In Table 1 and Fig. 3, the dimensions of the fruiting bodies of these two species and Phaeodiscula tremae are compared. As shown, Phaeodiscula tremae from Formosa and Tokunoshima Island and Capnodiastrum guaraniticum sensu Katumoto and Harada from Chichijima Island comprised one group according to the sizes of pycnidia and conidia and were clearly separated from C. guaraniticum Speg. from Paraguay and C. tremae from Venezuela. From these results, three East-Asian materials seem to be the same species in the genus Capnodiastrum, and a new specific epithet sawadae is given for them as C. sawadae Tak. Kobay. & Ky. Watan., because C. tremae (Syd.) Petr. 1952 has authentic priority in this genus. 10. Nyssopsora formosana (Sawada) Lu⁻⁻tjeh., Blumea Suupl. I (29), 6:160, and 1937: Ito, Mycol. Flora of Japan II (3): 50, 1950; Sawada, Spec. Bull. Coll. Agr., Natn. Taiwan Univ. 8: 84, 1959 Figs. 2e-g, 4a-d Synonym: Triphragmium formosana Sawada, Rept. Cent. Inst. Dept. Agr., Taiwan Gov. 51: 52, Pl. II: 38-40, 1931; Hiratsuka & Hashioka, Bot. Mag. (Tokyo) 48(568): 236, 1934; Hiratsuka, J. Jpn. Bot. 13(4): 250, Fig. 2, 1937. Symptom and morphology (see Figs. 2e–g, 4a– d): After the new leaves sprout, yellow and powdery uredinia are formed densely on the lower leaf surface. Upper leaf surface becomes yellowish due to the formation of numerous yellow dots. Then, uredinia gradually disappear and alter to a telial state, with black masses of teliospores.

Uredinia are mostly hypophyllous, immersed at first, then break through the epiderm, widely open like acervulus, and expose the masses of urediniospores, 80–160 Im in diameter.

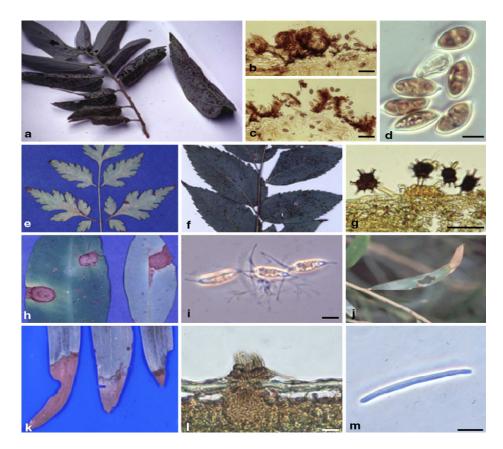


Fig. 2. a-d Capnodiastrum sawadae on Tremaorientalis.

A. Black sooty mycelial colonies developed on the lower leaf surface. B. Closed pycnidia and conidia. C. Matured disc-like pycnidia and conidia. D. Conidia (phase contrasted). Barsb, c 50 lm; d 15 lm. e-g Nyssopsora formosana on Koelreuteria formosana. E. Densely formed yellowish uredinia on the lower leaf surface. F. Densely formed black dots of telia on the lower leaf surface. G. Telium and teliospores. Bar g 100 lm. h, i Pestalotiopsis fici on Ardisia quinquegona. H. Brown spots surrounded by dark brown border. I. Conidia having knobbed appendages at the tip (phase contrasted). Bar i 10 lm j-m Pseudocercospora acaciaeconfusae on Acacia confuse. J. Diseased leaf showing both the dark brown spot and leaf blight lesion on the upper leaf surface. K. Leaf blight lesions on the lower leaf surface. L. Stroma and conidiophores. M. Conidium (phase contrasted). Bars I 10 lm; m 15 lm.

Species	$\begin{array}{llllllllllllllllllllllllllllllllllll$			Literature	Symbol and number in Fig. 3	
Capnodiastrum sp. from Tokunoshima Island	100-125 × 105-113	27.5-35 × 14-17.5 (av. 31.0 × 15.3)	<i>Trema orientalis</i> Kyushu, Japan	This paper	• 1	
Phaeodiscula tremae Sawada	57–104 × 57–104	21-32 × 13-18	T. orientalis Formosa	Sawada (1944)	• 2	
C. guaraniticum sensu Katum, & Y. Harada	65-100	22.5–32 × 12.5–17	<i>T. orientalis</i> Bonin Island, Japan	Katumoto and Harada (1979)	• 3	
C. guaraniticum Speg.	50-60	16-18 × 9-11	Celtis boliviensis Paraguay	Spegazzini (1886)	02	
C. paraguai ense Speg. ^a	70-80	14-16 × 7-8	Celtis sp. Paraguay	Spegazzini (1886)	01	
C. tremae Petr.	40-100	12-23 × 8-12.5	T. micranthae Venezuera	Sydow (1930)	• 4	

Table 1 Comparison of the taxonomic data among species of the genus Capnodiastrum parasitic to ulmaceous plants

^a Sutton (1980) treated this species as a synonym of C. guaraniticum

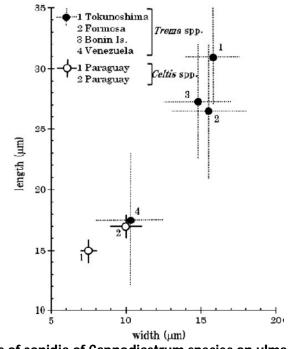


Fig. 3. Sizes of conidia of Capnodiastrum species on ulmaceousPlants

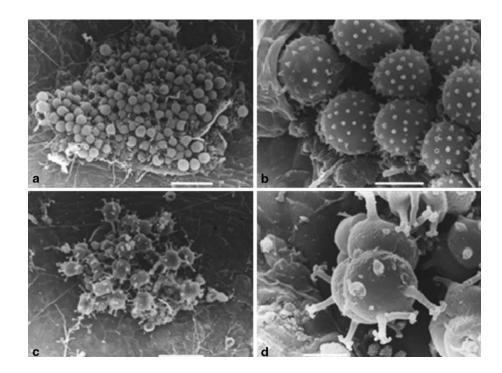


Fig. 4. *Nyssopsora* formosana on *Koelreuteria formosana*. a Uredinia dispersing urediniospores. b Urediniospores ornamented with many spicules. c Telia and teliospores. d Teliospores ornamented with anchor-like projections. (Scanning electron micrograph. Barsa 20 Im; b 10 Im, c 50 Im, d 15 Im)

Urediniospores are subglobular to broadly elliptic, hyaline to pale yellow, 14-18 lm in diameter, covered with a thin cell wall set by minute numerous thorns. Telia are mostly hypophyllous, immersed at first, then break through the epiderm, 75–150 lm in diameter Teliospores are composed of 3 cells, trigonal-subglobose, 25–30 9 25–32.5 lm (27.4 9 27.4 Im on average), dark brown to dark olive, with hyaline to pale pedicels 12-30 lm long, and ornamented with anchor-like projections, 9-15 per spore, 12-30 lm long, and mostly 2-4 branches at its tip. Disease name: Sabi-byo in Japanese (Sawada 1931), Rust. Specimen examined: On living leaves of Koelreuteria formosana Hayata (Taiwan-mokugenji in Japanese), Japan: Southeast Bot. Gard., Okinawa-city, Okinawa-Honto, Okinawa Prefecture, November 7, 1994, by TK (TFM: FPH-8056); November 11, 1995, by TK (TFM: FPH- 8081). Formosa: Taipei, Nov. 1, 1928, by Kaneyoshi Sawada (KS), as Triphragmium formosanum Sawada (Herb. Hiratsuka 101943); Taipei, December 9, 1933, by Yoshio Hashioka (YH), as Triphragmium formosanum Sawada (Herb. Hiratsuka 101945); Taipei, January 8, 1933, by YH, Triphragmium formosanum Sawada (Herb. Hiratsuka 101948). Taxonomic note: The rust fungus was observed and collected at the Southeast Botanical Gardens, Okinawa Main Island, in 1994 and 1995. On the lower surface of the diseased leaves, yellowish uredinia and blackish telia were densely formed. From the unique morphological characteristics of teliospores with anchor-like projections on their

Species	Urediniospore Length × width (µm)	Teliospore Length × width (µm)	Pedicel Length (µm)	Projection no. and Length	Host and locality	Literature
Nyssopsora sp. from Okinawa	14-18 (av. 15.5)	25-30 × 25-33 (av. 27 × 27)	12-30 (av. 18.5)	9–15 7.5–15	K. formosana Okinawa, Japan	This paper
Triphragmium formosanum Sawada	$17-21 \times 16-19$ (av. 19 × 17.5)	$26-28 \times 27-29$ (av. 27 × 28)	18-32 (av. 25)	11–15 9–16	K. formosana Formosa	Sawada (1931, 1959)
	$17-23 \times 16-20$ (av. 20 × 18)	$26-32 \times 27-31$ (av. 29 × 29)	-42	11-17	K. formosana Formosa	Hiratsuka (1937)
Nyssopsora formosanum (Sawada) Lütjeh.	16-23 × 14-20 (av. 19.5 × 17)	25-32 × 27-32 (av. 28.5 × 29)	-42	11–17 9–16	K. formosana Formosa	Ito (1950)
Nyssopsora koelreuteriae (Syd. & P. Syd.) Tranzsche	17-26 × 15-18 (av. 21.5 × 21.5)	26-34 × 24-28 (av. 30 × 26)	-	10-20 ~9	K. paniculata Honshu, Japan	Ito (1950)
	18-35 × 14-25 (26.5 × 19.5)	30-40 × 28-39 (35 × 33.5)	~ 52	18-30 ~9	K. paniculata Honshu, Japan	Hiratsuka et al. (1992)

Table 2 Comparison of taxonomic data among the species of Nyssopsora recorded on Koelreuteria

surfaces, this rust fungus belongs to the genus Nyssopsora. On Koelreuteria plants, two Nyssopsora species have been known. One is Nyssopsora koelreuteriae (Syd.) Tranzschel and the other is N. formosana (Sawada) Lu⁻tjeh. In Table 2, morphology of the rust collected from Okinawa is compared with those of two Nyssopsora species hitherto known. Sizes of urediniospore and teliospore, length of pedicel of teliospore, number and length of projection on teliospore in the rust from Okinawa were well accorded with those of N. formosana, but not with those of N. koelreuteriae. Urediniospores and teliospores of the rust fungus were smaller than those of N. koelreuteriae. Pedicels of teliospore in the former are shorter than those of the latter. Teliospores of the former have a smaller number of projections than those of the latter. Conversely, the projections in the former fungus are longer than those of the latter. From these facts, the fungus was identified as N. formosana. This is the first record of this fungus from Japan. 11. Pestalotiopsis fici Steyaert, Bull. Jard. Bot. Brux. 19(3): 324, 1949; Kobayashi et al. Forest Pests 58 (2): 59, 2009 Figs. 2h-i, 5 Synonym: Pestalotia fici (Steyaert) Guba, Monograph of Monochaetia and Pestalotia, 91, 1961. Symptom and morphology (see Figs. 2h-i. 5): Leaf spots are pale brown to brown, subcircular, 5–15 mm in diameter, surrounded by dark brown zone. Minute black dots (acervuli) scattered on the leaf spot. Acervuli are immersed within epidermal cell layer, and then break through it mature. Conidia are oblong to fusoid, 5-celled by 4 transverse septa, 20–25 9 5–7 lm, with both hyaline end cells. Three intermedial cells are pale brown and 14– 17.5 Im in length. Appendages are (2) 3 on the top of the upper end

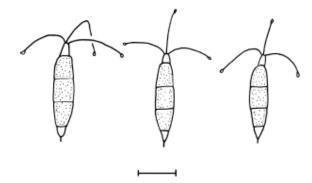


Fig. 5. Conidia of Pestalotiopsis fici having knobbed appendage. Bar 10 Im

cell, 17.5–22.5 Im in length, and swell as a small ball at their tip. One basal appendage is 1.3–7.5 Im in length.

Disease name: Pestalotia-byo in Japanese (Kobayashi et al. 2009), Pestalotia disease. Specimen examined: On living leaves of Ardisia guinguegona Blume (Shishiakuchi, in Japanese), Minami-meijiyama, Nago, Okinawa Prefecture (Okinawa Island), November 10, 1994, by TK & Choei Ogimi (TFM: FPH- 8047). Taxonomic note: This fungus has the unique feature that the conidia have appendages with a swollen ball-like structure at their tip. In the genus Pestalotiopsis (Pestalotia), 15 species have been known as having knobbed appendage (Guba 1961). Among them, 5 species are apparently different from the fungus on Ardisia, in their median colored cells composed of two upper brown to dark brown cells and one lower pale brown cell. Seven of 10 remaining species also differ from Pestalotiopsis in their large conidia and long apical appendages. Among the remaining 3 species, Pestalotiopsis javanica (Guba) Y. X. Chen (Guba 1961) is clearly distinguishable from this fungus by its small and strongly constricted conidia, long apical appendages, and quite long filamentous basal appendages, as shown in Table 3. Pestalotiopsis phoenicis (Vize) Y. X. Chen (Guba 1961) is also different from this fungus in its small conidia and darker colored intermediate cells (Table 3). Morphological characteristics of the fungus described here on Ardisia well accorded with those of Pestalotiopsis fici Steyaert (1949) originally described on Ficus sp. from Uganda and then on Cocos nucifera from Sierra Leone (Guba 1961). This is the first record of this fungus species in Japan, and Ardisia guinguegona is a new host of the fungus. In Japan, another Pestalotiopsis species, P. theae (Sawada) Steyaert on several broad-leaved trees (Kobayashi 2007) is well known as having similar unique appendages. 12. Pseudocercospora acaciae-confusae (Sawada) Goh & W. H. Hsieh, Trans. Mycol. Soc. R.O.C. 4(2): 41, 1989; Hsieh & Goh, Cercospora and similar fungi from Taiwan: 176, 1990; Guo & Hsieh, The genus Pseudocercospora in China: 36, 1995: Crous & Braun: Mycosphaerella and its anamorph I: 39, 2003; Kobayashi et al., Forest Pests 56(5): 24, 2007 Figs. 2j-m; 6 Basionym: Cercospora acaciae-confusae Sawada, Rep. Gov. Agr. Res. Inst. Taiwan 35: 105, 1928. Synonym: Pseudocercospora acaciae-confusae (Sawada) Y.L.Guo & X.J. Liu, Mycosystema 2: 226, 1989. Later homonym of Pseudocercospora acaciae-confusae (Sawada) Goh & Hsieh. Symptom and morphology (see Figs. 2j-m, 6): From the tip of leaves

brown blight lesions extend downward. Fruiting bodies are mainly produced on the upper surface of the lesions as minute black masses of stroma, which are first immersed within the epidermal layer then erumpent, 17–34 Im in diameter and pale brown to pale olive in color. Conidiophores are 5–10 in number, loosely fascicled on the surface of the stroma, usually 1-septate, pale olive brown, 12–30 9 2.5–3 Im, with thin conidial scars. Conidia are long, cylindric, straight or curved, obtuse at the apex, with truncate and thin basal end, pale

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brown to pale olivaceous, 2–5-septate, 36–61 9 3–4.5 Im (54.5 9 3.6 Im in average), smooth. Numerous conidia were produced when the pieces of mycelial colonies were placed on a potato dextrose agar (PDA) plate at 25_C after they were stored for 1 month in liquid nitrogen. These conidia were long, cylindric, straight or curved, hyaline to very pale olivaceous, 3–7-septated, 43–82 9 2.4–4.5 Im (59.1 9 3.4 Im on average), smooth. Disease name: Maruhoshibyo in Japanese (Sawada 1928), Circular leaf spot.

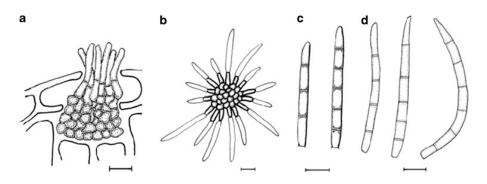


Fig. 6. *Pseudocercospora acaciae-confusae*. a Stroma and conidiophores. b Stroma, conidiophores and young conidia (overview). c Mature conidia on host. d Conidia produced on the colonies on a potato dextrose agar (PDA) plate at 25_C, after pieces of colony were stored for 1 month with liquid nitrogen. Bars, a, c, d 10 lm; b 15 lm.

Specimen examined: On living leaves of Acacia confuse Merr. (Soushiju in Japanese), Round road of Mt. Ohyama, China-cho, Ohshima-gun, Kagoshima Prefecture (Okinoerabu Is.), November 20, 2001, by TK and Yasunori Ono (TFM: FPH-8083; Isolates: MAFF 239180). Taxonomic note: Morphological characteristics of this fungus well accorded with those of Pseudocercospora acaciae-confusae (=Cercospora acaciae-confusae), which was described on the same host, Acacia confusa Merr., from Formosa and continental China (Sawada 1928; Hsieh and Goh 1990; Guo and Hsieh 1995). In Formosa and China, the fungus forms circular to somewhat angular leaf spots. However, in Okinoerabu Island of Japan, the fungus mainly causes leaf blight lesions from the tip of leaves, except for a small number of the typical spot. On this island, the host plant is exposed strong winds; those in Formosa and China may exist in a different environment. This is the first record of this fungus from Japan. According to Crous and Braun (2003), the species has been collected from Hong Kong and Libya on Acacia sp. Acknowledgements The authors express heartfelt thanks to Professor Makoto Kakishima, Institute of Agriculture and Forestry, University of Tsukuba, for his courtesy to loan the authentic specimens of Nyssopsora formosana and N. koelreuteriae, and for his useful advice in identifying the species of Nyssopsora. We are also grateful to Miss Machiko Sano, Graduate School of Agriculture, Tokyo University of Agriculture, for her support in preparing figures and tables.

As the periderm of woody plants represents a structurally heterogeneous substrate, it is more difficult to differentiate truly internal from external fungi. Of all fungal taxa isolated at least *Kabatina* sp. can be regarded as a true endophyte. The mode of infection of xylem specific fungi such as *Kabatina* sp. remains unclear but the presence of this slow-growing fungus in the bark cannot be excluded, since other fast-growing species could have concealed its presence. The use of other isolation methods which involve alternate moisture regimes and extensive histology studies are needed to further understand the mode of infection of xylotropic fungi.

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Corresponding author: Dr. Hamid Kheyrodin, Faculty of Desert Science-Semnan University, Iran. Email id: <u>hkhyrodin@yahoo.com</u>