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FULL PAPER

Shigeki Inaba · Seiji Tokumasu

The genus *Brevilegnia* (Saprolegniales, Oomycetes) in Japan

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Abstract Three species of the genus *Brevilegnia* (Saprolegniales, Oomycetes) are described and illustrated from Japan: *B. megasperma* var. *brevicaulis*, *B. unisperma* var. *litoralis*, and *B. variabilis*. The Japanese *B. variabilis* agrees with the original description in demonstrating achlyoid- and dictyoid-type zoospore discharge patterns in addition to the thraustothecoid-type, which is the typical pattern in the genus, androgynous antheridial branch origin, and antheridial branches wrapping about the oogonia, but it produces larger oogonia and aplerotic oospores. All species are new records from Japan.

Key words *Brevilegnia* · New to Japan · Peronosporomycetes · Saprolegniaceae · Taxonomy.

Introduction

The genus *Brevilegnia* was established by Coker and Couch (Coker 1927), and 13 species are recognized in the genus (Dick 1973). The genus is characterized by forming depauperate mycelia, producing monosporous oogonia, and displaying thraustothecoid (as in the genus *Thraustotheca* Humphrey) spore release (Langsam 1987). Some species demonstrate achlyoid- or dictyoid-type spore discharge patterns besides the thraustothecoid-type, and their reassignment to the genus *Achlya* Nees or *Dictyuchus* Leitg. has been proposed by some authors (Langsam 1986; Chiou and Chang 1976). All the species and varieties of this genus have been mainly isolated from soil. In Japan, only one unidentified *Brevilegnia* species has been reported, from the mud of an eel culture pond in Shizuoka Prefecture (Hoshina et al. 1960).

In this article, we describe and illustrate three species of *Brevilegnia* isolated from soil samples collected in Japan and compare the three species.

S. Inaba (✉) · S. Tokumasu
Sugadaira Montane Research Center, University of Tsukuba, Sugadaira, Sanada, Chiisagata, Nagano 386-2201, Japan
Tel. +81-268-74-2002; Fax +81-268-74-2016
e-mail: s975116@icho.ipe.tsukuba.ac.jp

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Materials and methods

All isolates were collected from soil samples by the hemp seed baiting method (Seymour 1970), and established pure cultures by the single-spore isolation technique (Seymour and Fuller 1987). Stock cultures were maintained on cornmeal agar (CMA; Nissui, Tokyo, Japan) plates. For water culture, sterilized hemp seed halves were placed, cut-surface down, at the edge of the growing colonies on CMA plates, and incubated for about 48 h at 20°C. The infected hemp seed halves were then transferred to 60 × 15 mm petri dishes containing 15 ml of sterilized distilled water and incubated at 20° and 25°C.

Some isolates were also propagated on CMA plate cultures for inducing sexual reproductive organs. An agar block (5 × 5 mm) supporting actively growing hyphae was cut from the edge of colony growing on stock culture plates, inoculated into a new plate, and incubated at 20°C for 7 days. Then, sterilized hemp seed halves were placed on the colonies, and incubated until the reproductive organs formed around them.

Taxonomy

Brevilegnia megasperma J.V. Harv. var. ***brevicaulis*** Rossy-Vald., J. Elisha Mitchell Sci. Soc. **72**:133, figs. 23–29, 1956. Figs. 1, 4–6.

Mycelium dense. Hyphae slender, delicate, 15–60 μm in diameter at the base, frequently branched. Gemmae always present, very abundant, terminal or intercalary, short clavate, doliform in intercalary ones, often formed catenulately. Zoosporangia abundant, terminal or lateral, clavate or fusi-

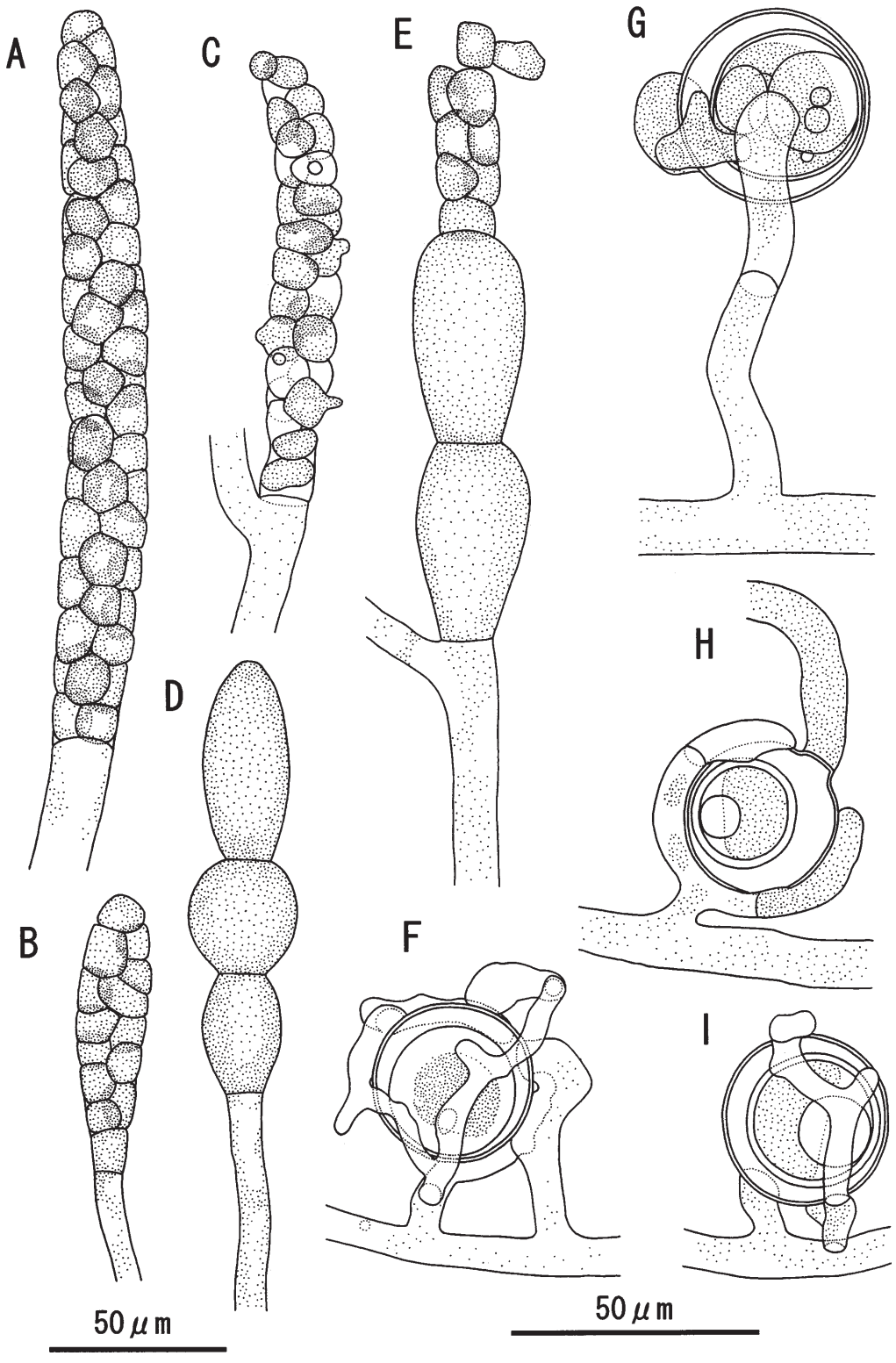


Fig. 1. *Brevilegnia megasperma* var. *brevicaulis* (IA1714). **A, B** Mature zoosporangia. **C** Zoosporangium with dictyoid spore release. **D** Terminal gemmae formed in chain. **E** Zoosporangium showing liberation of spores and gemmae. **F–I** Oogonia with various types of antheridia. *Bars* **A–E**, 50 μm; **E–I**, 50 μm

form, straight or curved, often bent at tip, (80–)120–250(–490) × 18–35(–40)μm, renewed by sympodial or cymose branching. Zoospore discharge rarely dictyoid in primary ones, later thraustothecoid, aplanoid in old culture, usually with two or three rows of spores. Spores more or less angular, mostly 12–19 μm in diameter, rarely cylindrical. Oogonia rare on mycelium colonizing on hemp seed in sterile distilled water, abundant in CMA plate with hemp seeds, lateral or intercalary, spherical or subspherical, doliform or cylindrical in intercalary ones, rarely irregular, (25–)30–42 μm in diameter, cylindrical ones reached 73 μm. Oogonial wall unpitted, smooth. Oogonial stalks variable in length,

stout, straight, curved, or contorted, often as long as to twice the diameter of the oogonium in length, sometimes rather long, usually not branched. Oospheres usually maturing. Oospores eccentric, spherical, rarely subspherical, single, rarely two in intercalary oogonia, not filling the oogonia, (19–)24–33(–49)μm in diameter; those in intercalary oogonia is usually long, cylindrical, reaching 55 μm. Antheridial branches generally monoclinous, infrequently androgynous, very rarely diclinous, sometimes lacking, often branched. Antheridial cells simple or tubular, laterally or apically appressed, or with projections.

Distribution: Puerto Rico. This species is a new record from Japan.

Isolates examined: IA 1711 isolated from soil of the shore of the Senbaru pond on the campus of University of Ryukyu, Nishihara, Nakagami, Okinawa Prefecture, collected and isolated by S. Inaba, 6 March 2000. Living culture is deposited in the Centraalbureau voor Schimmelcultures (CBS 109573) in the Netherlands. IA 1714 (CBS 109569) isolated from soil in a roadside ditch on the campus of University of Ryukyu, Nishihara, Nakagami, Okinawa Prefecture, collected and isolated by S. Inaba, 6 March 2000.

Notes. In our isolates, sexual reproductive organs were rarely formed on mycelia growing on hemp seeds in sterile distilled water, although zoosporangia and gemmae were abundantly observed. By contrast, sexual organs were abundantly produced in the agar around the hemp seeds placed on CMA plates. Consequently, we described asexual organs formed in water cultures and sexual organs formed on the CMA plates, respectively. In the original description of this species, the sexual reproductive organs were observed in 30 ml of charcoal-filtered distilled water at 23°–25°C (Rossy-Valderrama 1956). The cause of the poor sexual reproduction of our isolates in water culture is uncertain.

Brevilegnia megasperma J.V. Harv. can easily be distinguished from the other species by relatively larger oogonia and oospores and the production of gemmae (Rossy-Valderrama 1956). *Brevilegnia megasperma* resembles *B. globosa* Ziegler in the presence of dictyoid spore discharge, short oogonial stalks, and having monoclinous antheridial branches. Langsam (1987) suggested that the taxonomic status of *B. globosa* was questionable because of Ziegler's improper and inadequate description of the species. However, *B. megasperma* is easily distinguishable from *B. globosa* by the absence of pits in the oogonial wall.

Brevilegnia megasperma includes two varieties, var. *brevicaulis* and var. *megasperma* J.V. Harv. (Harvey 1930). The former can be distinguished from the latter by relatively short and stout oogonial stalks, smaller oogonia and oospores, and by commonly producing intercalary oogonia. Furthermore, two varieties differ in appearance of the antheridial branches, and those of the variety *brevicaulis* are almost monoclinous, whereas the variety *megasperma* produces only androgynous and hypogynous ones.

Brevilegnia unisperma (Coker & Braxton) Coker var. ***litoralis*** (Coker & Braxton) Coker, J. Elisha Mitchell Sci. Soc. **42**:213, 1927.

≡*Thraustotheca unisperma* Coker & Braxton var. *litoralis* Coker & Braxton, J. Elisha Mitchell Sci. Soc. **42**:141, pl. 11, figs. 8–13, 1926. Figs. 2, 7–9

Mycelium rather dense. Hyphae slender, delicate, opaque, 12–32(–51)μm in diameter at the base, frequently branched, profusely branched into secondary hyphae near their tips. Gemmae, when present, sparse, short clavate, terminal, usually formed single. Zoosporangia abundant, terminal, long, clavate, fusiform or cylindrical, occasionally tapering to the tip, (112–)120–350(–530) × 16–26(–33)μm, renewed by sympodial, occasionally cymose branching. Zoospore discharge thraustothecoid, many with only one row of spores, occasionally with two or three rows. Spores more or less angular, mostly 12–14 μm in diameter, rarely cylindrical, reaching 20 × 9 μm. Oogonia abundant, lateral or intercalary, spherical, subspherical or obovate, doliform in intercalary ones, rarely irregular, 14–26(–38)μm in diameter, often with hyphal swelling. Oogonial wall unpitted, more or less irregular, with or without apical projection. Oogonial stalks variable in length, often long, slender, straight, curved or contorted, occasionally branched, bearing oogonia cymosely. Oospheres usually maturing. Oospores eccentric, spherical, rarely subspherical, one or very rarely two in number, nearly filling the oogonia, 13–22(–25)μm in diameter. Antheridial branches and antheridia absent.

Distribution: United States, Germany. This species is a new record from Japan.

Isolates examined: IA1000 (CBS 109570) isolated from wet soil of a paddy field, Tamagusuku, Shimajiri, Okinawa Prefecture, collected by S. Tokumasu and isolated by S. Inaba, 25 March 1998. IA1712 isolated from soil of the shore of the Senbaru pond on the campus of University of Ryukyu, Nishihara, Nakagami, Okinawa Prefecture, collected and isolated by S. Inaba, 6 March 2000. IA1768 (CBS 109571) isolated from soil of the shore of a pond on the campus of Kinki University, Nara, Nara Prefecture, collected and isolated by S. Inaba, 21 May 2000. IA1800 isolated from boggy soil of the bank of the Nishi-kawa River, Sato, Mikura Island, Tokyo, collected and isolated by S. Inaba, 29 June 2000.

Notes. This species abundantly formed asexual and sexual reproductive organs on mycelia growing on hemp seeds in sterile distilled water and also on CMA plates with hemp seeds. The description was based on water cultures.

This variety was originally described as *Thraustotheca unisperma* var. *litoralis* when Coker and Braxton (1926) described the species. Subsequently, *T. unisperma* was transferred to the genus *Brevilegnia* erected by Coker and Couch (Coker 1927), and this variety was subsequently transferred to the present genus.

Our isolates are characterized by the entirely absence of antheridial branches. Three taxa lack antheridial branches in *Brevilegnia*: *B. unisperma* var. *litoralis*, *B. unisperma* var. *montana* Coker & Braxton, and *B. unisperma* var. *delica* Coker & Alexander. Johnson (1977) raised the variety *montana* to the species rank, and erected *B. montana* (Coker & Braxton) T.W. Johnson based this on his Scandinavian isolates. He also treated the variety *delica* as a synonym of this species. Our isolates were identified as the

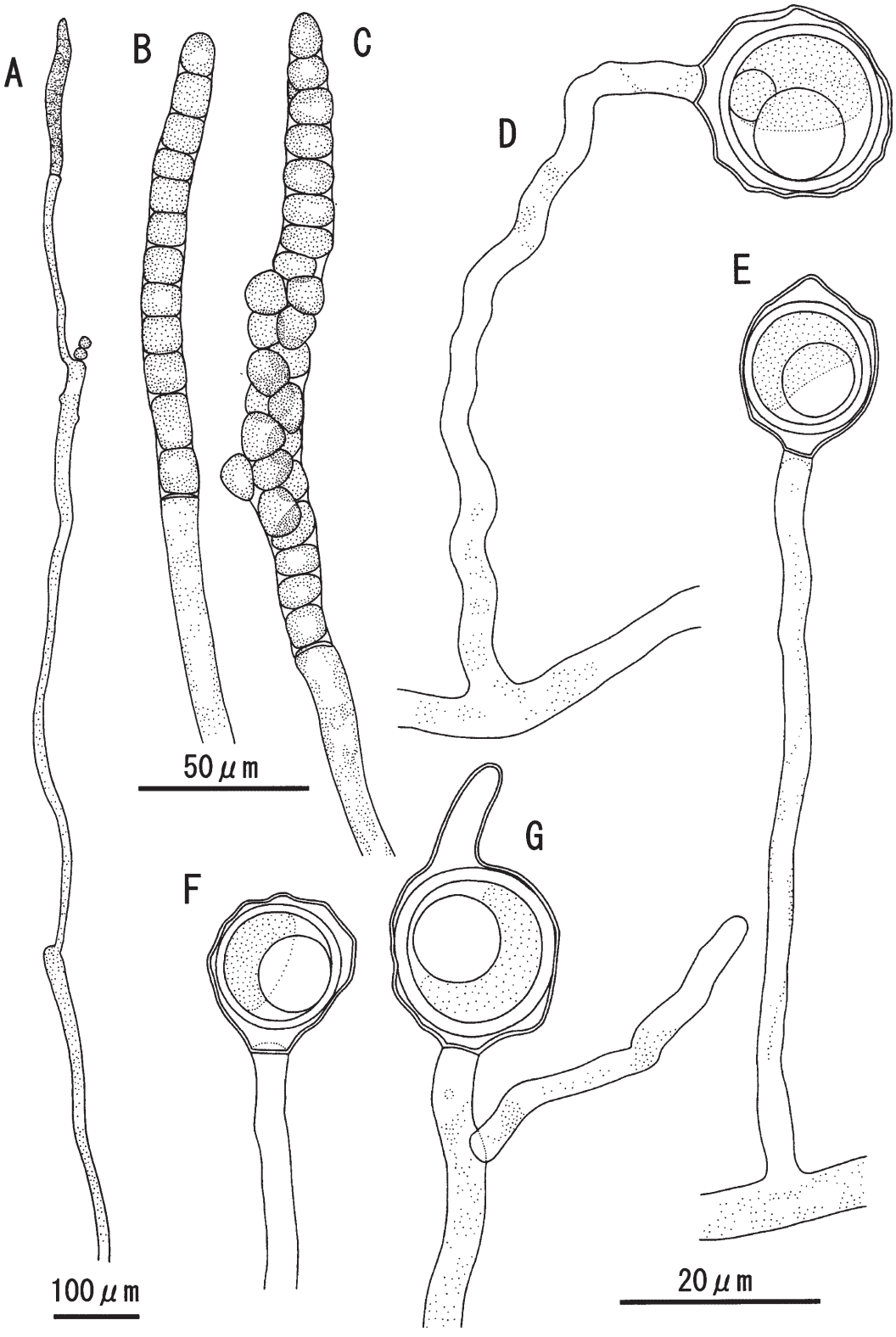


Fig. 2. *Brevilegnia unisperma* var. *litoralis* (IA1000). **A** Zoosporangia renewed by sympodial branching. **B** Mature zoosporangium with spores in a single row. **C** Zoosporangium showing disintegration of wall. **D–F** Oogonia with irregular wall. **G** Oogonia with apical projection. *Bars* **A**, 100 μm; **B–C**, 50 μm; **D–G**, 20 μm

variety *litoralis* by entirely thraustothecoid-type spore release, small oogonia, and an irregular and warted oogonial wall. The warted or waved oogonial wall is not observed in the other varieties.

In our isolates, secondary zoospore release, which was described as the feature of the variety *litoralis* in the key of the genus by Coker (1927), was not observed on water cultures. Milanez and Beneke (1968), however, also reported the lack of secondary zoospore release at *B. unisperma* var. *unisperma* (Coker & Braxton) Coker isolated from the United States. Salvin (1942) suggested that the presence or absence of secondary zoosporogenesis is

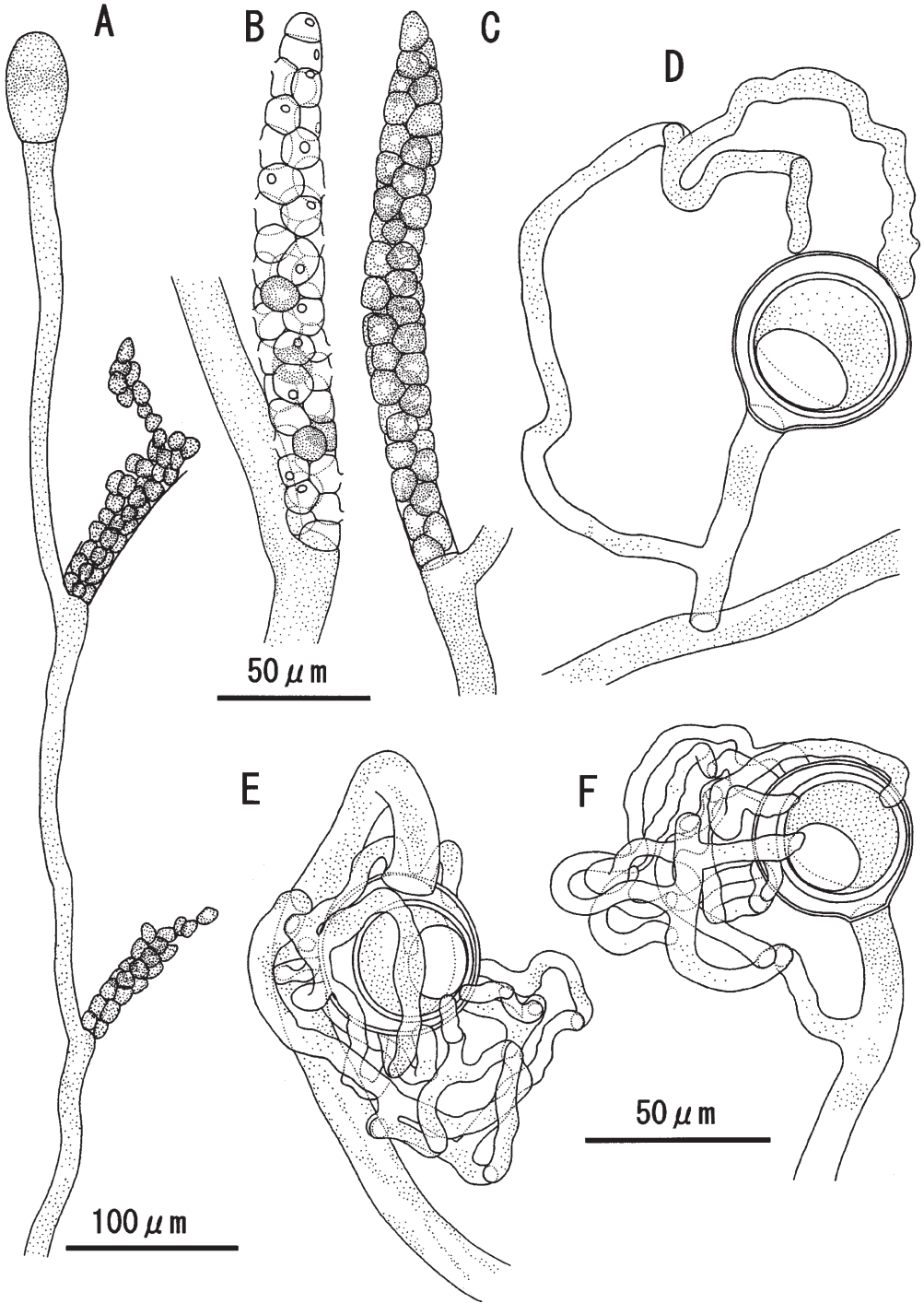


Fig. 3. *Brevilegnia variabilis* (IA1782). **A** Terminal gemma and zoosporangia with thraustothecoid spore release renewed by sympodial branching. **B** Zoosporangium with dictyoid spore release. **C** Mature zoosporangium. **D–F** Oogonia with androgynous antheridial branches. *Bars* **A**, 100 μm; **B, C**, 50 μm; **D–F**, 50 μm

greatly influenced by the environmental conditions. Therefore, we have no doubt that we can identify the isolates as *B. unisperma* var. *litoralis*.

Brevilegnia variabilis Indoh, Hakubutsugaku-zasshi, Tokyo (Mag. Nat. Hist.) **38**:87, figs. 1–2, 1941.

Figs. 3, 10–12

Mycelium dense. Hyphae slender, delicate, 12–32(–47)μm in diameter at the base, sparsely branched. Gemmae, when present, sparse, short, fusiform, clavate, pyriform or spherical, terminal or internal, single or catenulate. Zoosporangia abundant, terminal or lateral, long, cylindrical, fusiform or clavate, (95–)140–380(–440) × (18–)20–25(–28)μm, renewed by sympodial or cymose branching. Zoospore discharge dictyoid or very rarely achlyoid in primary zoosporangia, mainly thraustothecoid in older ones, single, or two or three rows of spores. Spores spherical in achlyoid- and dictyoid-type ones, more or less angular in thraustothecoid-type ones, mostly 11–14(–15.5)μm in diameter. Oogonia abundant, lateral or terminal, spherical or subspherical, rarely inflated, (31–)35–54(–97)μm in diameter. Oogonial wall unpitted, smooth. Oogonial stalks 1/2–3(–5) times diameter of oogonia, stout, straight or curved, usually not branched. Oospheres sometimes maturing. Oospores

eccentric, spherical, rarely subspherical, single, filling or not filling the oogonia, (26–)28–41(–48)μm in diameter. Antheridial branches abundant, monoclinous, androgynous, frequently branched, sometimes wrapping about the oogonia. Antheridial cells tubular or clavate, apically appressed, fertilization tubes not observed.

Distribution: Micronesia, Taiwan. This species is a new record from Japan.

Isolates examined: IA1782 (CBS 109572) isolated form soil of the shore of a pond in the ruins of Kohriyama Castle, Yamato-Kohriyama, Nara Prefecture, collected and isolated by S. Inaba, 22 May 2000. IA1728 isolated from the shore of a pond in the Mizutori Park, Yonago, Tottori Prefecture, collected by S. Iwamoto and isolated by S. Inaba, 13 April 2000.

Notes. This species easily formed asexual and sexual reproductive organs on mycelia growing on hemp seeds in sterile distilled water and also on CMA plates with hemp seeds. The description was based on water cultures.

This species was originally isolated from Micronesia by Indoh (1941). He characterized *B. variabilis* by possessing achlyoid- and true-net-type dictyoid zoosporangia in addition to thraustothecoid-type ones. Our isolates agreed with his description. Because the species possesses *Dictyuchus*-type sporangia, Johnson (1974) suspected that *B. variabilis* was a species of *Dictyuchus*. Chiou and Chang (1976) transferred this species to the genus *Dictyuchus* based on their Taiwanian isolate and treated it as *D. variabilis* (Indoh) T.S. Chiou & H.S. Chang. In their isolate, the majority of the zoosporangia were true-net dictyoid type, whereas those of thraustothecoid (as brevilegnoid) type were very rare. Indoh (1941), however, did not mention the proportion of individual types of zoosporangia, so the major discharge pattern of his original isolate is not clear. Because the majority of zoosporangia demonstrated thraustothecoid-type zoospore discharge pattern in our isolates, we consider that the species should be retained in *Brevilegnia*.

The origin of antheridial branches and the wrapping nature of antheridia branches around the oogonia of our isolates correspond to those of *B. variabilis* as described and illustrated in the original description (Indoh 1941). By contrast, the oospores of our isolates sometimes do not fill the oogonium (aplerotic), differing from the plerotic oogonia reported by Indoh (1941) and Chiou and Chang (1976). Furthermore, the oospores and oogonia of our isolates are larger than those of their isolates. Johnson (1950) noted that the general morphology of the oogonium and the size of oospores were relatively stable features in the genus. Although our isolates should be treated as a new variety of the species, we reserve any taxonomic treatment until we understand the bases of morphological variation in the species. Additional strains should be examined to determine the range of characteristics of the species.

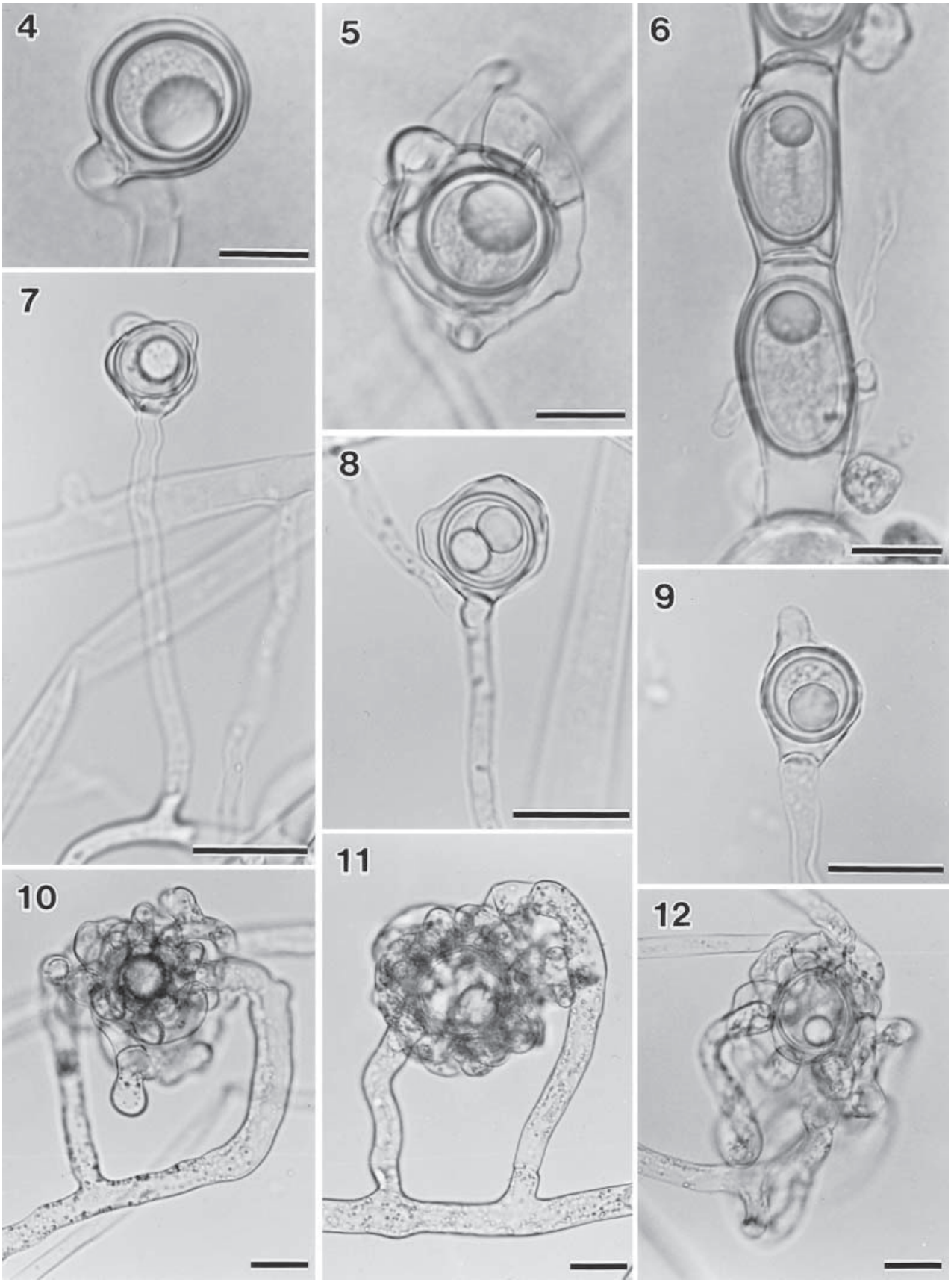
Discussion

In the genus *Brevilegnia*, there are some species that demonstrate the achlyoid- or dictyoid-type spore discharge patterns, although the typical discharge mode of the genus is thraustothecoid. *Brevilegnia megasperma* var. *brevicaulis* and *B. variabilis* reported in this article are examples of those species. Some authors suggested that those species should be transferred to the genus *Achlya* or *Dictyuchus*. Chiou and Chang (1976) suggested that *B. variabilis* should be transferred to the genus *Dictyuchus* because the species produces the true-net-type dictyoid zoosporangia. Because the achlyoid zoospore discharge occurs in the primary zoosporangia, Langsam (1986) also suggested that *B. bispora* Coker should be transferred to the genus *Achlya*. However, in the phylogenetic analysis of Saprolegniomycetidae based on 28S rDNA partial sequences by Riethmüller et al. (1999), *B. bispora* clustered together with *B. megasperma* and *Dictyuchus* spp. in a subcluster of the Saprolegniales, and the subcluster that consisted of *Brevilegnia* spp. and *Dictyuchus* spp. was supported by a bootstrap value of 100%. Based on the results, Riethmüller et al. (1999) suggested that this species should be assigned to the genus *Brevilegnia*. This opinion was supported by another analysis of 28S rDNA sequences (Petersen and Rosendahl 2000). From these results, we consider that the genus *Brevilegnia* is separable from *Achlya*, but the phylogenetic relationship between the genus *Brevilegnia* and *Dictyuchus* is still not certain.

By contrast, in the studies of 28S rDNA by Leclerc et al. (2000), *B. bispora* was not included in the cluster consisting of the species of Saprolegniales examined, and appeared as an outgroup species and adjoined the species of the order Pythiales (*Pythium aquatile* Höhnk) and Leptomitales (*Apodachlya brachynema* (Hildebr.) Pringsh.). From this result, Leclerc et al. (2000) suggested that the genus *Brevilegnia* should not be retained in the order Saprolegniales. Surprisingly, the strain of *B. bispora* (CBS 568.67) sequenced by Leclerc et al. (2000) was the same as examined by Riethmüller et al. (1999), and the DNA regions that they sequenced were almost common. It is uncertain why these two molecular studies showed quite different results.

Based on morphological characteristics, we consider that the genus should not be excluded from the order Saprolegniales, because the species of *Brevilegnia* possess the morphological characteristics of Saprolegniales, such as the hyphae increasing in diameter with age, zoosporogenesis by internal cleavage, sequential zoosporangium renewal, and the oogonia possessing thickened walls (Dick 2001).

In the genus *Brevilegnia*, the identification of species is generally difficult (Johnson et al. 1973; Johnson 1974, 1977). This difficulty mainly arises from the variability or instability of some taxonomic characters, such as the size and shape of zoosporangium, the presence or absence of antheridia, the motility of zoospores, and the presence or absence of gemmae, which tend to be changed by culture conditions (Salvin 1942; Johnson 1950). Furthermore, these features are also known to vary from isolate to isolate in a species (Johnson et al. 1973; Johnson 1974, 1977). In contrast to those characters, the characteristics such as the type of antheridia, general morphology of the oogonia, the size of



Figs. 4–6. *Brevilegnia megasperma* var. *brevicaulis* (IA1714). **4** Oogonium without antheridium. **5** Oogonium with antheridium. **6** Intercalary oogonia

Figs. 7–9. *Brevilegnia unisperma* var. *litoralis* (IA1000). **7** Oogonium on lateral oogonial stalk. **8** Oogonium with irregular oogonial wall. **9** Oogonium with apical projection

Figs. 10–12. *Brevilegnia variabilis* (IA1782). **10** Oogonium with androgynous antheridial branch. **11** Oogonium with monoclinous antheridial branch. **12** Oogonium with androgynous antheridial branch and aplerotic oospore. *Bars* 20 μm

Table 1. Comparison of morphological characters of four Japanese *Brevilegnia* species

Character	*B. megasperma* var. *brevicaulis*	*B. unisperma* var. *litoralis*	*B. variabilis*	*Brevilegnia* sp.[a]
Types of zoospore dicharge	Mainly thraustothecoid /dictyoid in primary zoosporangia	Thraustothecoid	Mainly thraustothecoid /dictyoid or achlyoid in primary zoosporangia	Thraustothecoid
Zoosporangial renewal	Sympodial or cymose	Sympodial, occasionally cymose	Sympodial or cymose	–
Gemmae	Always present, usually formed in chain	Usually absent	Occasionally present	–
Position of oogonia	Lateral or intercalary	Lateral or intercalary	Lateral or terminal	Terminal or lateral
Size of oogonia (μm)	(25–)30–42(–73)	14–26(–38)	(31–)35–54(–97)	25–30
Number of oospores	One, rarely two	One, very rarely two	One	One or two
Size of oospores (μm)	(19–)24–33(–49)	13–22(–25)	(26–)28–41(–48)	20–30
Origin of antheridial branches	Monoclinous, infrequently androgynous, very rarely diclinous	Absent	Androgynous or monoclinous	Androgynous

[a] Data from Hoshina et al. (1960)

oospores, and the manner of sporangial proliferation are regarded to be relatively stable (Johnson 1950).

Three species described in this article and one unidentified *Brevilegnia* species reported by Hoshina et al. (1960) can easily be distinguished from one another by the combination of characters such as the origin of antheridial branches, the shape of oogonia, and the size of oospores. The comparison of morphological characteristics of four Japanese *Brevilegnia* is shown in Table 1. We have observed little morphological variability among our isolates of the same species in the culture conditions examined. From these facts, we consider that the species identification of *Brevilegnia* is simple among Japanese isolates.

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