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FULL PAPER

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Three ascomycetes on leaves of evergreen *Ilex* trees from Japan: *Rhytisma ilicis-integrae* sp. nov., *R. ilicis-latifoliae*, and *R. ilicis-pedunculosae* sp. nov.

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Abstract Three species belonging to the genus *Rhytisma* causing tar spot were collected on leaves in evergreen trees of *Ilex* species from Japan. *Rhytisma ilicis-latifoliae*, the known species, is found on *Ilex latifolia*, and *R. ilicis-integrae* sp. nov. and *R. ilicis-pedunculosae* sp. nov. are found on *I. integra* and *I. pedunculosa*, respectively. Ascomata are formed on the abaxial part of the stromata in all the *Rhytisma* species studied, and spermogonia are formed on the amphigenous parts in *R. ilicis-latifoliae* and on the adaxial part in *R. ilicis-integrae* and *R. ilicis-pedunculosae*. Shape and size of asci, ascospores, and spermatia are distinctly different among the three species. The morphology of germination tubes from ascospores and appressoria is unique for each *Rhytisma* species. Yellowish spots arise on the newly developing leaves in mid-May, then abundant spermatia are produced in spermogonia in the three *Rhytisma* species. In the next year, ascospores are produced in ascomata from early April to late May in *R. ilicis-integrae* and from early April to early June in *R. ilicis-latifoliae* and *R. ilicis-pedunculosae*, and they are considered to be the inocula of disease infection.

Key words Infection cycle · Morphology · Rhytismatales · Taxonomy

Introduction

In 1890, Tanaka reported *Rhytisma curtisii* Berk. & Ravenel on *Ilex integra* Thunb. collected from the Tokyo Metropolitan area, and this is the first record on *Rhytisma* on *Ilex* trees from Japan. No morphological characteristics of his fungus, however, were shown. In about 1900, some Japanese phytopathologists sent several specimens of the tar spot on *Ilex* trees collected from Japan to Dr. P. Hennings, at the Berlin Botanical Museum, asking him for identification of the causal fungus. Hennings (1899) reported a new species of *Rhytisma*, *R. ilicis-latifoliae* Henn., based on a specimen of *I. latifolia* Thunb. collected from Nagasaki Prefecture, although the description of the fungus was very brief (Saccardo 1902). Hennings (1900, 1901, 1903) also identified the *Rhytisma* specimens on *I. integra* collected from Chiba Prefecture and *I. pedunculosa* Miq. collected from Kochi Prefecture as *R. ilicis-latifoliae* and the fungus on *I. macropoda* Miq. collected from Tokyo Metropolitan and Kochi Prefecture as *R. prini* (Schwein.) Fr. From the identification by Hennings, Yoshinaga (1902) noted *R. ilicis-latifoliae* on *I. latifolia* and *I. pedunculosa* and *R. prini* on *I. macropoda* collected from Kochi Prefecture. Yoshinaga (1901), however, noted that the *Rhytisma* on *I. integra* collected from Kochi Prefecture was *R. ilicis-integrifoliae* Henn., but no original paper by Hennings has been found. Later, Yoshinaga (1904) identified *Rhytisma* on *I. integra* collected from Kanagawa Prefecture as *R. ilicis-latifoliae*.

Several *Ilex* tree species have been planted in gardens and parks throughout the western part of Japan, and the "tar spot" caused by *Rhytisma* species has been frequently reported as a serious disease of the ornamental *Ilex* trees (Kobayashi 1983; Horie et al. 2001). On the causal fungi, several Japanese reporters have noted *Rhytisma* on evergreen *Ilex* trees as *R. ilicis-latifoliae* and the fungus on deciduous *Ilex* trees as *R. prini* (Kobayashi 2007). The same treatment on the causal fungi was carried out in "Common names of plant diseases in Japan" (The Phytopathological Society of Japan 2000). Morphological characteristics of these species of *Rhytisma*, however, have not been described in detail.

The genus *Melasmia* has been known as an anamorph of the genus *Rhytisma* (Cannon and Minter 1986). Although no teleomorph was described, Yoshinaga (1911) and Yamamoto and Yasumori (1960) noted *Melasmia ilicis* Henn. on *Ilex geniculata* Maxim., but the original paper on the species by Hennings has not been found. The conidia of *Melasmia*, however, are minute, and their germination has never been observed, which has led to speculation that they are spermatia (Jones 1925; Cannon and Minter 1986).

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Several *Rhytisma* inhabiting *Ilex* trees were reported from other countries as follows: *R. bontocense* Syd. on *Ilex buergeri* Miq. var. *rolfei* (Elm.) Loesen. from the Philippines; *R. concavum* Ellis & Kellerm. on *I. verticillata* (L.) A. Gray from North America (Kellerman 1902; Saccardo 1906); *R. himalense* Syd., P. Syd. & E. J. Butler on *I. dipyrena* Wall., *I. fargesii* Franch., and *Ilex* sp. from India, Pakistan, and China (Sydow et al. 1911; Cannon and Minter 1986; Hou and Piepenbring 2005); *R. ilicis* Sawada on *I. micrococca* Maxon var. *longifolia* Hayata from China (Taiwan) (Sawada 1959); *R. ilicincolum* Schwein. on *I. prinoides* Sol. (= *I. decidua* Walter) from North America (Saccardo 1895); *R. loseneriana* Henn. on *I. dumosa* Reissek from Urguay (Saccardo 1889); *R. prini* on *I. verticillata* (L.) A. Gray from North America (Saccardo 1895); and *R. velatum* (Schwein.) Fr. on *I. prinoides* from North America (Saccardo 1895). For *Melasmia*, Sawada (1959) reported *M. ilicicola* Sawada on *I. asprella* Champ. ex Benth. from China (Taiwan). Fungi belonging to the *Rhytisma* and *Melasmia* on *Ilex* trees collected from Japan should be morphologically compared with those foreign fungi, but it is impossible to compare with those on *R. ilicincolum*, *R. prini*, and *R. velatum* because no dimensions of asci and ascospores were reported.

Under these circumstances, I investigated the *Rhytisma* species on three evergreen *Ilex* tree species, i.e., *I. integra*, *I. latifolia*, and *I. pedunculosa*, collected mainly from Shimane Prefecture, Japan. Morphology was distinctly different among these fungi with respect to the host, and they could be treated as three different fungi including two new species. The purpose of this article is to describe and illustrate the morphology of the *Rhytisma* on these hosts and discuss their taxonomy. To identify the infection cycle of the disease, seasonal developments of symptoms, signs, and fruiting bodies were examined and germination of ascospores and formation of appressoria were observed.

Materials and methods

From February 2002 to December 2007, I collected fresh materials of *Rhytisma* from three evergreen *Ilex* trees, *I. integra* (Japanese name: Mochinoki), *I. latifolia* (Tarayo), and *I. pedunculosa* (Soyogo), mostly at Matsue, Shimane Prefecture, Japan. The numbers of specimens were 19, 19, and 29 on *I. integra*, *I. latifolia*, and *I. pedunculosa*, respectively. These specimens were deposited in the Herbarium of Y. Suto (YSH). Five specimens of *Rhytisma* (3 and 2 specimens on *I. integra* and *I. pedunculosa*, respectively), deposited in the Herbarium in the Mountainous Region Research Center in Shimane Prefecture (SFH), were also examined. Two holotype specimens, on *I. integra* and *I. pedunculosa*, were deposited in the Herbarium of Forest Mycology and Pathology, Forestry and Forest Products Research Institute, Japan (TFM:FPM).

Macroscopic features of the fungi were observed under a hand lens and a stereoscope micrometer. Hand-sectioned pieces were mounted in Shear's fluid, and morphological characteristics of the fungi were observed and measured under a light microscope.

Because the minute conidia produced in conidiomata never germinated in repeated experiments, conidiomata and conidia of these fungi were, respectively, considered as spermogonia and spermatia in this article. Seasonal development of the stromata, ascomata and spermogonia, and production of ascospores and spermatia were recorded for each species.

A piece of stroma with maturing ascoma was attached onto the inner surface lid and was placed over 2% sucrose agar. Discharged ascospores were examined to check the germination and formation of appressoria.

Descriptions

Rhytisma ilicis-integrae Y. Suto, sp. nov.

Figs. 1–10, 31, 34

MycoBank no.: MB 513379.

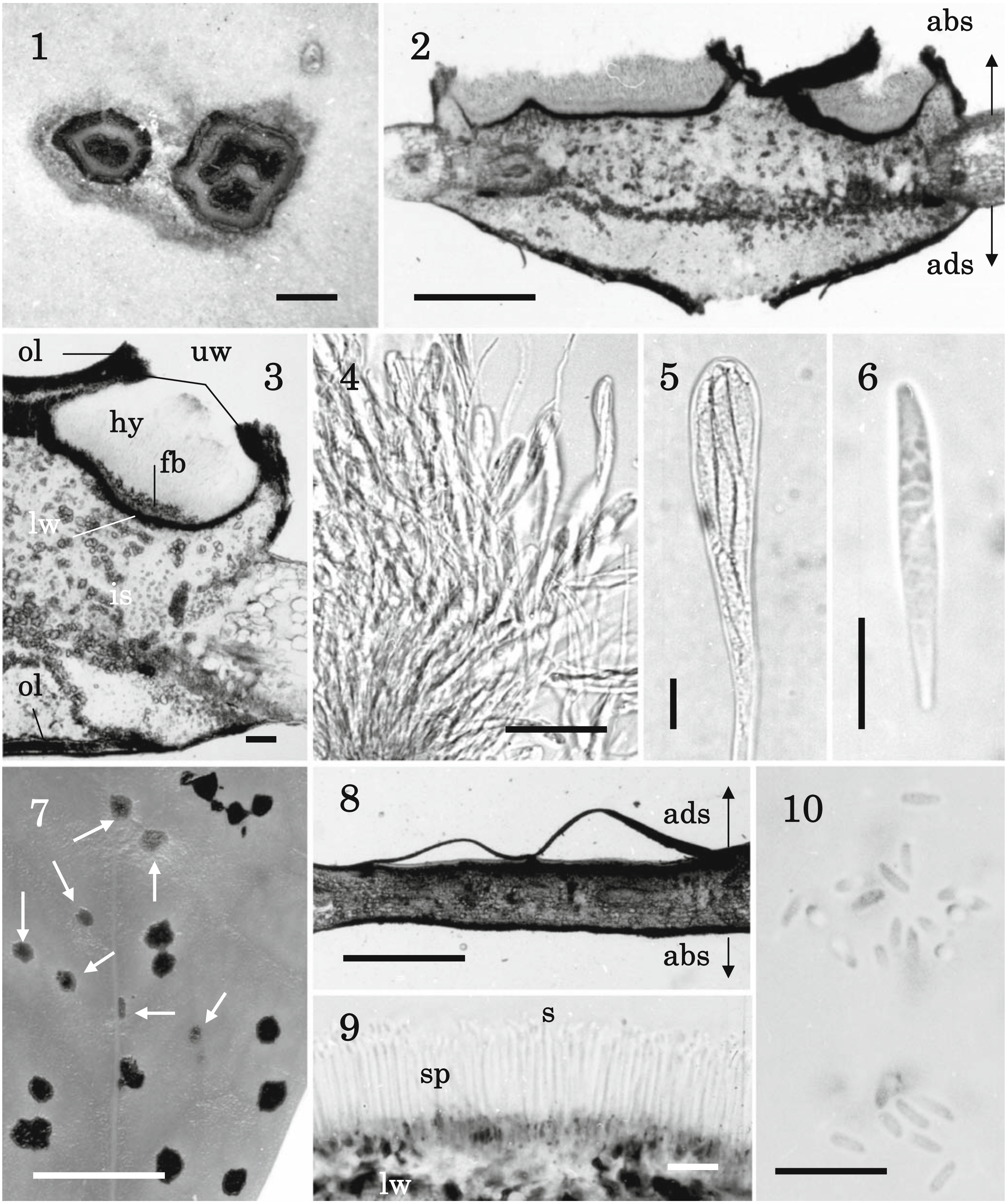
Stromatibus amphigenis, plus minusve circularibus, 0.8–3.7 mm diametris, nigris; ascomatibus abaxialibus, stromatibus innatis, 2–3 locularibus in aspectu medio-verticali, 290–430 μm profundis; paraphysibus filiformibus, simplicibus, 170–200 × 0.5 μm, asepatatis, apice non circinatis; ascis invicem maturantis, elongato-clavatis, longistipitatis, apice rotundatis, annulo apicali jodo non caerulescenti praeditis, 95–180 × 9.5–12 μm, octosporis; ascosporis uni- ad tristichis, elongato-fusiformibus, basem versus angustatis, 16–32 × 2.5–3.5 μm, hyalinis, aseptatis, in tunica gelatinosa non inclusis. Spermogoniis adaxialibus, immaturis strobatibus innatis, multilocaribus, 50–60 μm profundis; spermatiophoris cylindricis, apicem versus angustatis, 20–25 × 1 μm, hyalinis; spermatiis ellipticis ad allantoideis, 2–4 × 1 μm, hyalinis, aseptatis.

Etymology: Named after its host species name.

Holotype: On living leaves of *Ilex integra*, Tonomachi, Hamada, Shimane Pref., Japan, April 26, 2004, Y. Suto, TFM:FPH-7970.

Stromata developing on the amphigenous surfaces of living leaves, roughly circular but with irregular outlines, 0.8–3.7 mm in diameter, occasionally confluent, black, surrounded by yellow halos.

At the ascomatal stage, stromata rising significantly above the amphigenous leaf surfaces, 0.5–1.2 mm thick, developing through the host mesophyll; outer layer of stroma developing into 35–50 μm thick on the adaxial surface, 40–60 μm thick on the abaxial surface, consisting of strongly blackened tissue joined with dark brown textura angularis cells with blacking in the intercellular spaces; inner part of the stroma consisting of tightly packed hyphae and hyaline thick-walled textura intricata cells, containing degraded mesophyll cells of the host. At maturity of ascomata, lower outer layer of the stroma rupturing by an irregular circle in the outer portion.



Figs. 1–10. *Rhytisma ilicis-integrae*. **1** Opened ascostroma on abaxial surface. **2** General view of ascostroma in vertical section. *ads*, adaxial leaf surface; *abs*, abaxial leaf surface. **3** Enlarged ascoma formed in stroma. *hy*, hymenium; *fb*, fragments of blackened material; *ol*, outer layer of stroma; *is*, inner part of stroma; *uw*, upper wall of ascoma; *lw*, lower wall of ascoma. **4** Asci and paraphyses. **5** Upper portion of ascus. **6** Ascospore. **7** Yellowish spots (*arrows*) and blackened stromata on adaxial surface. **8** General view of spermogonia in vertical section. *ads*, adaxial leaf surface; *abs*, abaxial leaf surface. **9** Spermatiophores and spermatia. *s*, spermatia; *sp*, spermatiophores; *lw*, lower wall of spermogonia. **10** Spermatia. *Bars* **1, 2, 8** 1 mm; **3, 4** 100 µm; **5, 6, 9, 10** 10 µm; **7** 10 mm

Ascomata confined to the abaxial part of the stromata, toroidal or horseshoe shaped, exposing yellowish hymenium in surface view, formed 290–430 μm deep, with two to three loculi at median vertical section, hymenial space roughly circular; upper wall developing beneath the outer layer of stroma, 50–75 μm thick, consisting of brown textura globulosa cells, with dark browning in the intercellular spaces; lower wall 15–30 μm thick, consisting of dark brown textura intricata cells; subhymenium 20–80 μm thick, consisting of hyaline textura intricata cells, often with fragments of blackened material at the lower portion. Paraphyses filiform, simple, 170–190 × 0.5 μm, aseptate, not coiled at the apex. Asci ripening sequentially, elongated clavate, long-stalked, with rounded apex, without a pore bluing in Melzer's reagent, 95–180 × 9.5–12 μm, 8-spored. Ascospores 1- to 3-seriate, elongated fusiform, tapering toward the base, 16–32 × 2.5–3.5 μm, smooth-walled, hyaline, aseptate, without a gelatinous sheath.

At spermogonial stage developing before ascomatal stage, stromata black, shining, rugose, and rising slightly on the adaxial surface, not shining and flat on the abaxial surface; outer layer of stroma formed beneath the host cuticle, 15–45 μm thick, consisting of strongly blackened tissue with structure difficult to discern, on the adaxial surface, 15–25 μm thick, consisting of dark brown textura angularis cells with blacking in the intercellular spaces on the abaxial surface.

Spermogonia confined to the adaxial part of the stroma, formed at a depth of 50–60 μm; upper wall formed as the outer layer of stroma; lower wall 8–13 μm thick, consisting of heavily degraded epidermal and dark browned textura intricata cells; spermogonial lumen divided into several loculi by blackened rampart tissue, which arises from the lower wall; the lower layer of spermogonium 3–5 μm thick, hyaline tissue with structure difficult to discern. Spermatiophores arising directly from the lower layer cells, cylindrical, slightly tapering towards the apex, 20–25 × 1 μm, hyaline with dark browning at the basal portion, holoblastic, proliferating mostly percurrently but occasionally sympodially. Spermatia elliptical to allantoid, 2–4 × 1 μm, hyaline, aseptate.

Additional specimens examined: On living leaves of *Ilex integra*, Agenogi, Matsue, Shimane Pref., Japan, April 20, 1973 (SFH-221); Nishikawatsu, Matsue, Shimane Pref., Japan, June 1, 1972 (SFH-151*), April 30, 2002 (YSH-421); Ohba, Matsue, Shimane Pref., Japan, April 18, 2002 (YSH-401), May 25, 2002 (YSH-440*), May 17, 2003 (YSH-800), April 15, 2004 (YSH-1131), April 28, 2004 (YSH-1149), May 5, 2004 (YSH-1161), May 15, 2004 (YSH-1170), May 20, 2004 (YSH-1189), June 14, 2004 (YSH-1217*), April 27, 2005 (YSH-1423, 1425), April 6, 2006 (YSH-1632), May 15, 2006 (YSH-1682), April 12, 2007, (YSH-1813), May 2, 2007 (YSH-1823), May 16, 2007 (YSH-1833), May 30, 2007 (YSH-1839*); Shinji, Matsue, Shimane Pref., Japan, April 13, 1973 (SFH-284); Uehama, Tsu, Mie Pref., Japan, March 29, 2003 (YSH-754) (* indicates spermogonia- and spermatia-producing specimens).

Notes: *Rhytisma ilicis-integrae* is characterized by small stromata, rounded apices of asci, and long narrowly fusiform ascospores (Table 1; Figs. 1, 5–7, 31). The spermogonial stage of *R. ilicis-integrae* is characterized by spermogonia formed only on the adaxial leaf surface and elliptical to allantoid spermatia (Figs. 8, 10, 34). Ascospores of *R. ilicis-integrae* are similar to *R. bontocense* (Sydow 1932) in shape and size. Asci of *R. ilicis-integrae* are larger in length and smaller in width than those of *R. bontocense*.

Rhytisma curtisii (Tanaka 1890), *R. ilicis-integrifoliae* (Yoshinaga 1901), and *R. ilicis-latifoliae* (Hennings 1900; Yoshinaga 1904) were reported on *Ilex integra*, and taxonomic confusion has been introduced. It is a question how Tanaka (1890) identified his fungus as *R. curtisii*. Because *R. curtisii* did not agree with the circumscription of *Rhytisma*, Höhnel (1917) designated the fungus as the type of a new genus *Macroderma*, *M. curtisii* (Berk. & Ravenel) Höhn., and then Luttrell (1940) transferred the fungus to the genus *Phacidium* and designated *P. curtisii* (Berk. & Ravenel) Luttr.

The original paper on *R. ilicis-integrifoliae* described by Hennings has not been found, and the fungal name reported by Yoshinaga (1901) should be eliminated. Because the morphological characteristics of the *Rhytisma* on *I. integra* clearly differed from *R. ilicis-latifoliae*, the identification of the fungus by Hennings (1900) and Yoshinaga (1904) is considered to be incorrect.

Rhytisma ilicis-latifoliae Henn., in Warburg, Monsunia 1: 29, 1899. Figs. 11–20, 32, 35

Stromata developing on amphigenous surfaces of living leaves, roughly circular with irregular outlines, 1.7–4.4 mm in diameter, occasionally confluent, black, surrounded by yellow halos.

At ascomatal stage, stromata rising significantly above the amphigenous leaf surfaces, 1.2–1.8 mm thick, developing through the host mesophyll; outer layer of stroma developing to 50–150 μm thick, consisting of strongly blackened tissue joined with dark brown textura angularis cells with blacking in the intercellular spaces; inner part of the stromata consisting of tightly packed hyphae and hyaline thick-walled textura intricata cells, containing degraded mesophyll cells of the host. At maturity, lower outer layer of stroma peeled off from outer portion of the stroma as a circular disk.

Ascomata confined to the abaxial part of the stromata, developing below the spermogonia formed on the abaxial surface, daedaleoid, exposing yellowish hymenium in surface view, and formed 310–600 μm deep, with four to five loculi at median vertical section, hymenial space roughly circular; upper wall developing beneath the outer layer of the stroma, 40–75 μm thick, consisting of browned textura globulosa cells, with dark browning in the intercellular spaces; lower wall 25–50 μm thick, consisting of dark brown textura intricata cells; subhymenium 20–80 μm thick, consisting of hyaline textura intricata cells, occasionally with fragments of blackened material in the intercellular spaces at the lower portion. Paraphyses filiform, simple, 200–230 × 0.5 μm, aseptate, not coiled at the apex. Asci ripening sequentially, narrowly clavate, long-stalked, rostrate at

Table 1. Morphology of asci and ascospores of *Rhytisma* species on *Ilex*

Species	Specimen	Host	Ascus, µm (average)	Ascospore	
				Size, µm (average)	Shape
R. bontocense[a]		*I. buergeri* var. *rolfei*	60–100 × 13–16	25–32 × 3	Cylindrical to cylindrical fusoid
R. concavum[b]		*I. verticillata*	80–100 × 8–10	20–35 × 2–3	Elongated clavate (from figure)
R. himalense[c]		*I. dipyrena*, *I.* sp.	145–215 × 10.2–14	25–27.5 × 3–4	Elongated clavate
R. himalense[d]		*I. fargesii*	90–160 × 9–13	26–40 × 3–4.5	Clavate to cylindrical clavate
R. ilicis[e]		*I. micrococca* var. *longifolia*	67–73 × 10–13	20–31 × 3.5–4.5	Long-cylindrical clavate
R. ilicis-integrae[f]	YSH-401	*I. integra*	95–180 × 11–12 (130 × 11.5)	16–29 × 2.5–3 (25 × 3)	Elongated fusiform, tapering toward the base
	YSH-754	do.	95–170 × 9.5–11.5 (12.5 × 10.5)	17.5–30.5 × 2.5–3.5 (23 × 3)	do.
	TFM:FRH-7970	do.	105–155 × 10–12 (140 × 11)	21–32 × 2.5–3 (26.5 × 3)	do.
R. ilicis-latifoliae[g]		*I. latifolia*	90–150 × 9–12	15–17 × 4–5	Elongated fusoid
R. ilicis-latifoliae[f]	YSH-1151	*I. latifolia*	145–200 × 11–12.5 (170 × 12)	16–22.5 × 3–5 (20.5 × 3.5)	Clavate to fusoid
	YSH-1152	do.	145–180 × 10–11.5 (160 × 10.5)	17.5–24 × 3–4.5 (20.5 × 3.5)	do.
	YSH-1821	do.	153–235 × 9.5–12 (178 × 10)	10.5–21 × 3–3.5 (18.5 × 3)	do.
R. ilicis-pedunculosae[f]	YSH-400	*I. pedunculosa*	120–155 × 10.5–12 (130 × 12)	29–43 × 2.5–4 (35.5 × 3.5)	Elongated clavate, tumidiuscule at the base
	YSH-419	do.	95–110 × 9.5–12 (100 × 10.5)	29–43 × 2.5–4 (34 × 3.5)	do.
	TFM:FRH-7974	do.	110–170 × 9.5–11 (130 × 10.5)	24–38.5 × 3–3.5 (31 × 3)	do.
R. loeseneriana[h]		*I. dumosa*	70–87 × 18–21	21–24 × 4–6	Not described (1-septate)

Data are derived from Sydow (1932),[a] Kellerman (1902),[b] Cannon and Minter (1986),[c] Hou and Piepenbring (2005),[d] Sawada (1959),[e] this paper,[f] Hennings (1899),[g] Saccardo (1889)[h]

apex, without a pore bluing in Melzer's reagent, 145–235 × 9.5–12.5 µm, 8-spored. Ascospores 1- to 3-seriate, clavate to fusoid, 16–24 × 3–5 µm, hyaline, aseptate, covered by a gelatinous sheath.

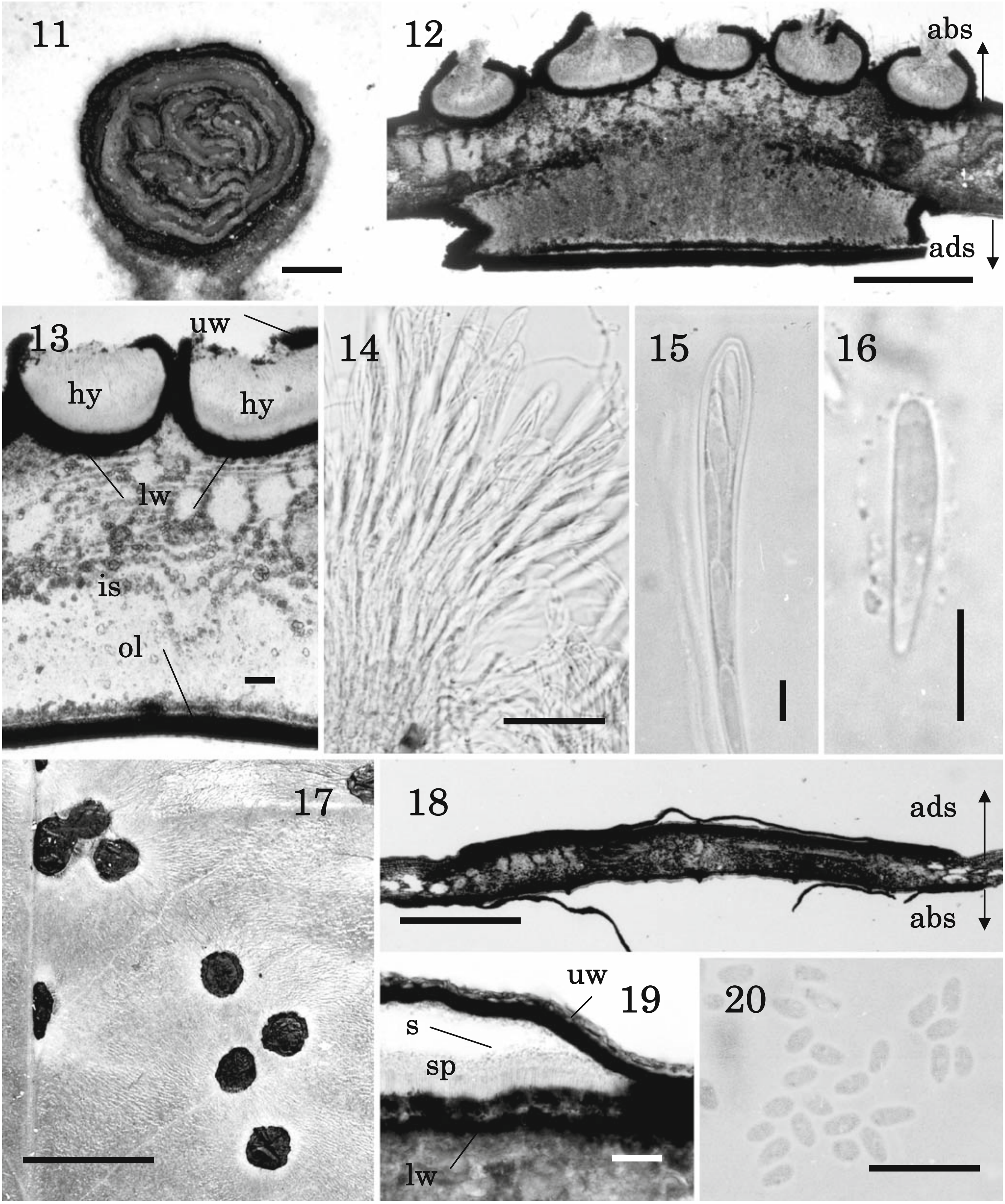
At spermogonial stage developing before ascomatal stage, stromata black, shining, rugose, and rising slightly on the amphigenous leaf surfaces; outer layer of stroma formed beneath the host cuticle, 15–30 µm thick, consisting of strongly blackened tissue with structure difficult to discern.

Spermogonia formed on both parts of the stromata, formed 50–60 µm deep; upper wall formed as the outer layer of stroma; lower wall 8–13 µm thick, consisting of heavily degraded epidermal cells and dark brown textura intricata cells; spermogonial lumen divided into several loculi by blackened rampart tissue, which arises from the lower wall; the lower layer of spermogonium 3–6 µm thick, consisting of hyaline tissue with structure difficult to discern. Spermatiophores arising directly from the lower layer cells, cylindrical, slightly tapering toward the apex, 20–25 × 1–1.5 µm, hyaline with dark browning at the basal portion, holoblastic, proliferating mostly percurrently but occasionally sympodially. Spermatia elliptical to ovoid, 2.5–3 × 1.5–2 µm, hyaline, aseptate.

Specimens examined: On living leaves of *Ilex latifolia* Thunb., Kumano, Yakumo, Matsue, Shimane Pref., Japan, April 8, 2004 (YSH-1125), April 15, 2004 (YSH-1128, 1129), April 28, 2004 (YSH-115l), May 5, 2004 (YSH-1152), May 15, 2004 (YSH-1168), May 26, 2004 (YSH-1184), June 14, 2004 (YSH-1208*), June 24, 2004 (YSH-1231*), April 28, 2005 (YSH-1431), May 12, 2005 (YSH-1439), April 19, 2006 (YSH-1648, 1651, 1652), April 11, 2007 (YSH-1805), May 2, 2007 (YSH-1821), May 16, 2007 (YSH-1832), June 1, 2007 (YSH-1843), June 21, 2007 (YSH-1857) (* indicates spermogonia- and spermatia-producing specimens).

Notes: The present fungus is characterized by large stromata, long asci with rostrate apices, and small clavate to fusoid ascospores covered by a gelatinous sheath (Table 1; Figs. 11, 14–17, 32). It is a unique feature that the lower layer of the stroma peels off as a circular disk at maturity of the ascomata. The spermogonial stage of this species is characterized by spermogonia formed on the amphigenous leaf surfaces and elliptical to ovoid spermatia (Figs. 18, 20, 35).

The present fungus is morphologically similar to *R. ilicis-latifoliae* described by Hennings (1899), except for slightly longer asci and ascospores. The present fungus were collected on the same host (*Ilex latifolia*) and in the same locality (Japan) as Hennings described. Ascospores of the present fungus are similar in shape to those of *R. concavum* (Kellerman 1902), *R. himalense* (Cannon and Minter 1986; Hou and Piepenbring 2005), and *R. ilicis* (Sawada 1959), but are smaller in size. The present fungus has larger asci than those of *R. concavum* and *R. ilicis*. *Rhytisma loseneriana* (Saccardo 1889) is different from all the three *Rhytisma* species studied for this report in having one-septate ascospores, although the shape of ascospores is not described (see Table 1). Therefore, the present fungus is identified as *R. ilicis-latifoliae*.



Figs. 11–20. *Rhytisma ilicis-latifoliae*. **11** Opened ascostroma on abaxial surface. **12** General view of ascostroma in vertical section. *ads*, adaxial leaf surface; *abs*, abaxial leaf surface. **13** Enlarged ascoma formed in stroma. *hy*, hymenium; *ol*, outer layer of stroma; *is*, inner part of stroma; *uw*, upper wall of ascoma; *lw*, lower wall of ascoma. **14** Asci and paraphyses. **15** Upper portion of ascus. **16** Ascospore. **17** Blackened stromata on adaxial surface. **18** General view of spermogonia in vertical section. *ads*, adaxial leaf surface; *abs*, abaxial leaf surface. **19** Enlarged spermogonium in stroma. *s*, spermatia; *sp*, spermatiophores; *uw*, upper wall; *lw*, lower wall. **20** Spermatia. *Bars* **11, 12, 18** 1 mm; **13, 14** 100 μm; **15, 16, 19, 20** 10 μm; **17** 10 mm

Rhytisma ilicis-pedunculosae Y. Suto, sp. nov.
Figs. 21–30, 33, 36
MycoBank no.: MB 513380.

Stromatibus amphigenis, plus minusve circularibus, 0.6–3.1 mm diametris, nigris; ascomatibus abaxialibus, stromatibus innatis, 1–2 locularibus in aspectu edio-verticali, 260–600 µm profundis; paraphysibus filiformibus, simplicibus, 170–200 × 0.5 µm, asepatatis, apice circinatis; ascis invicem maturantis, elongato-clavatis, longistipitatis, apice truncatis, annulo apicali jodo non caerulescenti praeditis, 95–170 × 9.5–12 µm, octosporis; ascosporis uni- ad tristichis, elongato-clavatis, basi tumidulis, 24–43 × 2.5–4 µm, hyalinis, aseptatis, in tunica gelatinosa inclusis. Spermogonis adaxialibus, immaturis strobatibus innatis, multilocaribus, 50–60 µm profundis; spermatiophoriis cylindricis, apicem versus angustatis, 20–25 × 1 µm hyalinis; spermatiis clavatis, 3–5 × 1 µm, hyalinis, aseptatis.

Etymology: Named after its host species name.

Holotype: On living leaves of *Ilex pedunculosa*, Ohba, Matsue, Shimane Pref., Japan, May 2, 2007, Y. Suto, TFM: FPH-7974.

Stromata developing on the amphigenous surfaces of living leaves, roughly circular but with irregular outlines, occasionally polygonal, 0.6–3.1 mm in diameter, occasionally confluent, black, surrounded by yellow halos.

At ascomatal stage, stromata rising significantly above the amphigenous leaf surfaces, 1–1.5 mm thick; outer layer of stroma developing 60–100 µm thick on the adaxial surface, 50–75 µm thick on the abaxial surface, consisting of strongly blackened tissue joined with dark brown textura angularis cells with blackening in the intercellular spaces; inner part of the stromata consisting of tightly packed hyphae and hyaline thick-walled textura intricata cells, containing degraded mesophyll cells of the host. At maturity of ascomata, lower outer layer of the stroma rupturing by an irregular circle in the outer portion.

Ascomata confined to the abaxial lower part of the stromata, toroidal or horseshoe shaped, exposing yellowish hymenium in surface view, 290–600 µm thick, with one to two loculi at median vertical section, hymenial space roughly circular; upper wall developing beneath the lower layer of the stroma 50–75 µm thick, consisting of browned textura globulosa cells, with dark browning in the intercellular spaces; lower wall 25–35 µm thick, consisting of dark brown textura intricata cells; subhymenium 20–100 µm thick, consisting of hyaline textura intricata cells, often with fragments of blackened material in the intercellular spaces at the lower portion. Paraphyses filiform, simple, 170–200 × 0.5 µm, aseptate, circinately coiled at the apex. Asci ripening sequentially, narrowly clavate, long-stalked, 95–170 × 9.5–12 µm, truncate at apex, without a pore bluing in Melzer's reagent, 8-spored. Ascospores 1- to 3-seriate, elongated clavate, tumescent at the base, 24–43 × 2.5–4 µm, hyaline, aseptate, occasionally covered by a thin gelatinous sheath.

At spermogonial stage developing before ascomatal stage, stromata black, shining, rugose and rising slightly on the adaxial surface, not shining and flat on the abaxial surface; outer layer of stroma formed beneath the host cuticle, 15–50 µm thick, consisting of strongly blackened tissue with structure difficult to discern, on the adaxial surface, 10–25 µm thick, consisting of dark brown textura angularis cells with blackening in the intercellular spaces on the abaxial surface.

Spermogonia confined to the adaxial part of the stroma, formed 50–60 µm thick; upper wall formed as the outer layer of stroma; lower wall 5.5–13 µm thick, consisting of heavily degraded epidermal cells and brown textura intricate cells; spermogonial lumen divided into several loculi by blacked rampart tissue, which arises from the lower wall; the lower layer of spermogonium 3–5 µm thick, hyaline tissue with structure difficult to discern. Spermatiophores arising directly from the lower layer cells, cylindrical, slightly tapering towards the apex, 20–25 × 1 µm, hyaline with dark brown at the basal portion, holoblastic, proliferating mostly percurrently but occasionally sympodially. Spermatia clavate, 3–5 × 1 µm, hyaline, aseptate.

Additional specimens examined: On living leaves of *I. pedunculosa*, Agenogi, Matsue, Shimane Pref., Japan, April 25, 2002 (YSH-419), May 11, 2004 (YSH-1162), April 21, 2006 (YSH-1655), May 30, 2006 (YSH-1701), April 6, 2007 (YSH-1797), April 20, 2007 (YSH-1814), May 11, 2007 (YSH-1831), June 17, 2007 (YSH-1856*); Ohba, Matsue, Shimane Pref., Japan, March 11, 2002 (YSH-367), April 19, 2002 (YSH-400), May 25, 2002 (YSH-448*), May 17, 2003 (YSH-803), April 28, 2004 (YSH-1148), May 5, 2004 (YSH-1157), May 15, 2004 (YSH-1169), May 26, 2004 (YSH-1187), June 14, 2004 (YSH-1214*), April 27, 2005 (YSH-1427), May 3, 2005 (YSH-1435), April 12, 2007 (YSH-1810), May 2, 2007 (YSH-1827), May 16, 2007 (YSH-1834, 1835), June 4, 2007 (YSH-1844–1, 1844–2*, 1845–1, 1845–2*, 1849–1, 1849–2*); Shinji, Matsue, Shimane Pref., Japan, May 19, 1987 (SFH-1033); Shimo-tadokoro, Ounan, Ouchi, Shimane Pref., Japan, April 12, 1990 (SFH-1108) (* indicates spermogonia- and spermatia-producing specimens).

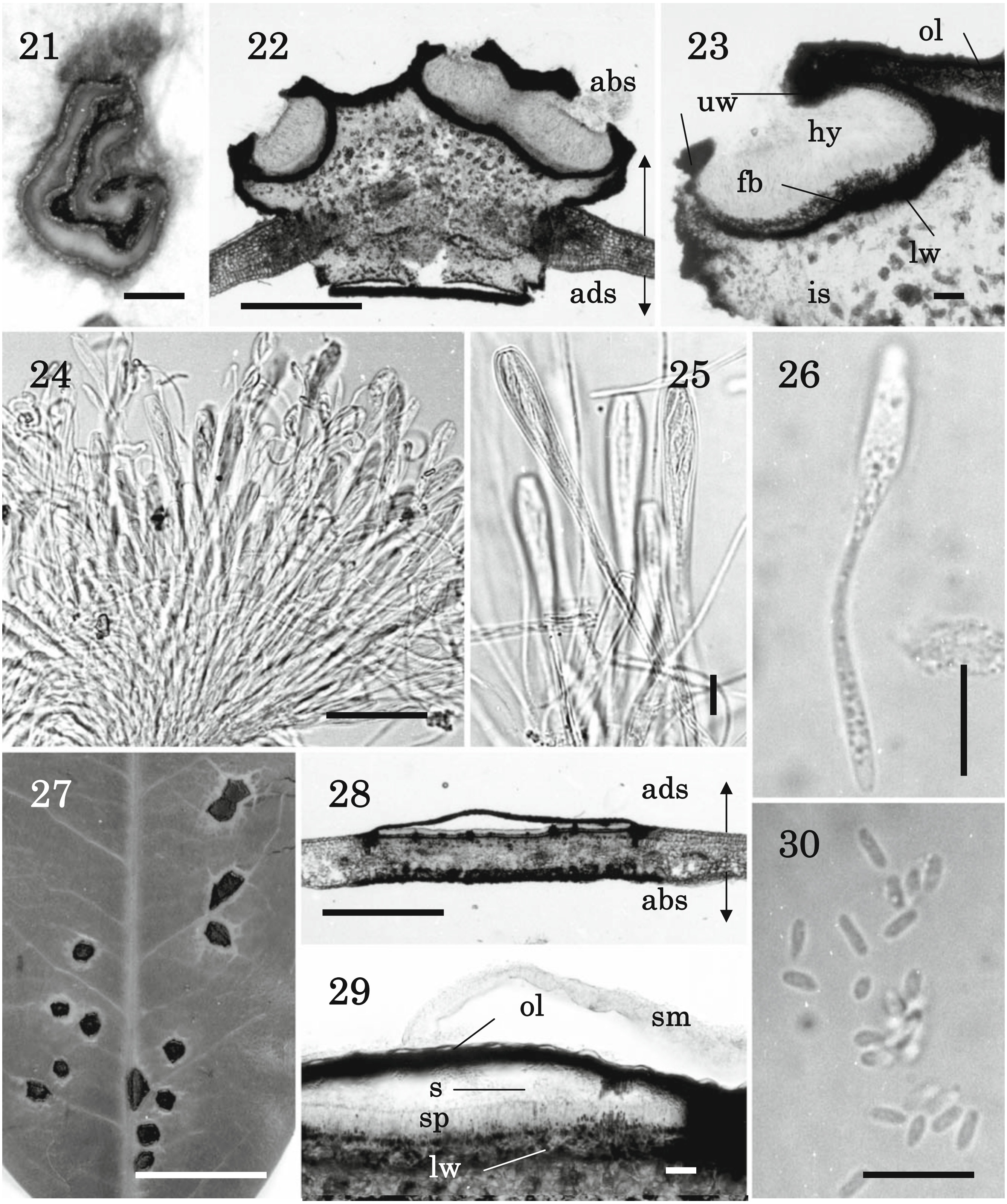
Notes: *Rhytisma ilicis-pedunculosae* is characterized by small stromata, truncate apices of asci, and long clavate ascospores tumidiuscule at the base (Table 1; Figs. 21, 25–27, 33). It is a unique feature that paraphyses are circinately coiled at the apex. The spermogonial stage of *R. ilicis-pedunculosae* is characterized by spermogonia formed only on the adaxial leaf surface and clavate spermatia (Figs. 28, 30, 36).

Yoshinaga (1902) and Hennings (1903) described *Rhytisma* on *I. pedunculosa* as *R. ilicis-latifoliae*. Because the morphology of *R. ilicis-pedunculosae* is clearly different from *R. ilicis-latifoliae*, their identification is considered to be incorrect.

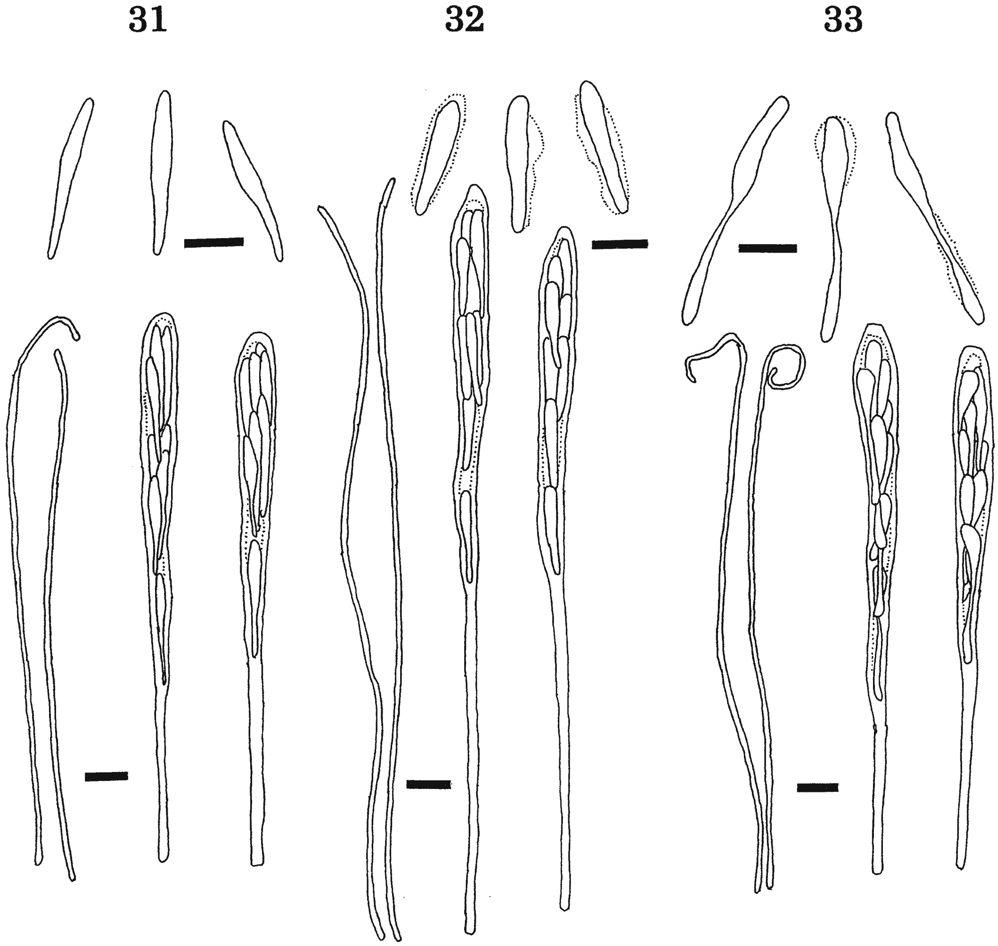
Results and discussion

Seasonal development of symptoms, signs, and fruiting bodies and infection cycle of the tar spot disease

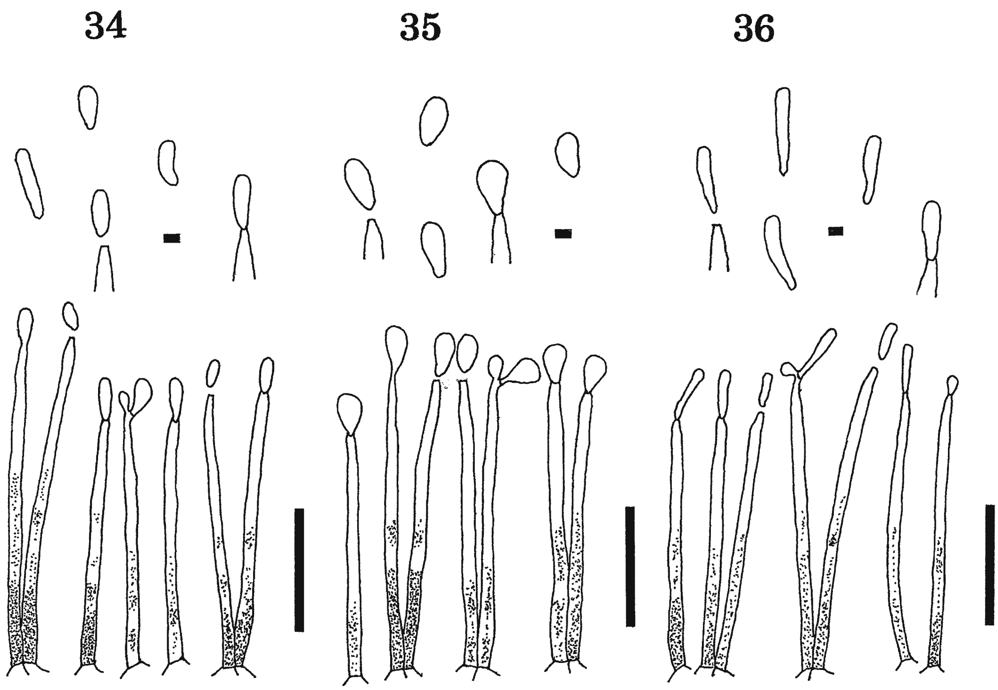
The seasonal development of symptoms and production of ascospores and spermatia is collectively shown in Fig. 37.



Figs. 21–30. *Rhytisma ilicis-pedunculosae*. **21** Opened ascostroma on abaxial surface. **22** General view of ascostroma in vertical section. *ad*s, adaxial leaf surface; *abs*, abaxial leaf surface. **23** Enlarged ascoma formed in stroma. *hy*, hymenium; *fb*, fragments of blackened material; *ol*, outer layer of stroma; *is*, inner portion of stroma; *uw*, upper wall of ascoma; *lw*, lower wall of ascoma. **24** Asci and paraphyses. **25** Upper part of asci. **26** Ascospore. **27** Blackened stromata on upper leaf surface. **28** General view of spermogonium in vertical section. *ads*, adaxial leaf surface; *abs*, abaxial leaf surface. **29** Enlarged spermogonium in stroma. *s*, spermatia; *sp*, spermatiophores; *sm*, spermatial mass; *ol*, outer layer of stroma; *lw*, lower wall of spermogonium. **30** Spermatia. *Bars* **21, 22, 28** 1 mm; **23, 24** 100 µm; **25, 26, 29, 30** 10 µm; **27** 10 mm



Figs. 31–33. Asci (*right*), ascospores (*upper*), and paraphyses (*left*) of *Rhytisma* spp. **31** *R. ilicis-integrae*. **32** *R. ilicis-latifoliae*. **33** *R. ilicis-pedunculosae*. *Bars* 10 μm



Figs. 34–36. Spermatiophores (*lower*) and spermatia (*upper*) of *Rhytisma* spp. **34** *R. ilicis-integrae*. **35** *R. ilicis-latifoliae*. **36** *R. ilicis-pedunculosae*. *Bars* 1 μm (*upper*), 10 μm (*lower*)

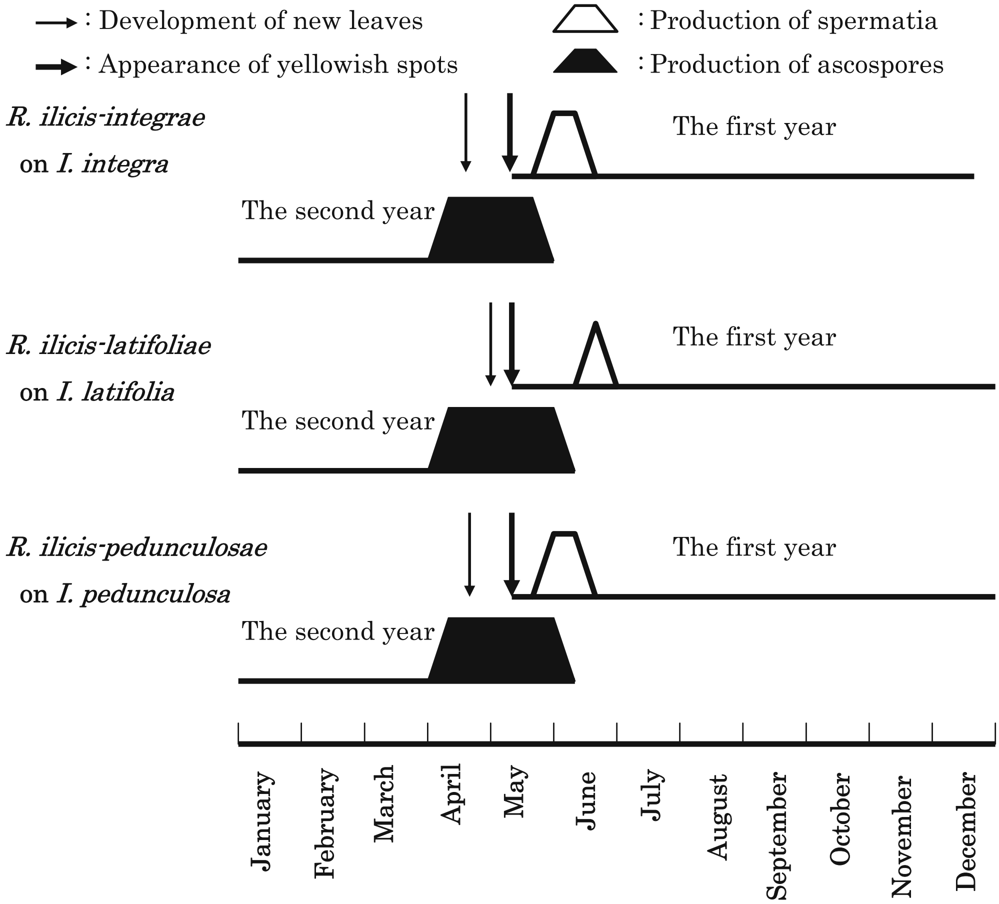
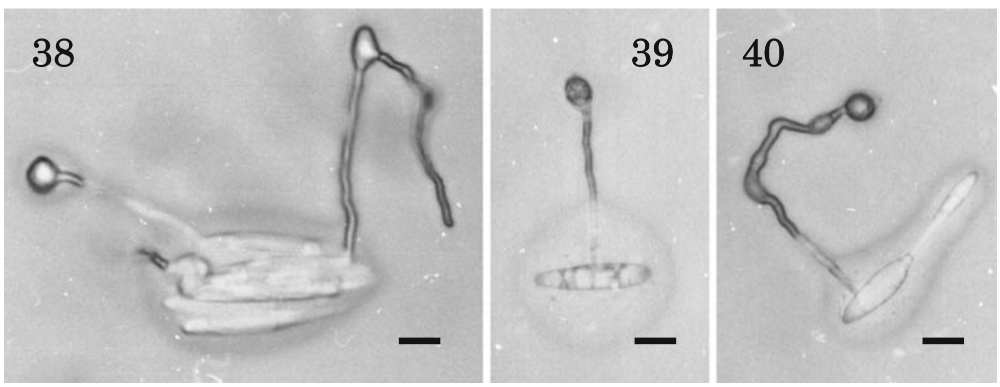


Fig. 37. Infection cycle of *Rhytisma (R.) ilicis-integrae*, *R. ilicis-latifoliae*, and *R. ilicis-pedunculosae* on leaves of *Ilex* (*I.*) trees



Figs. 38–40. Germination and forming appressorium of ascospore. **38** *Rhytisma ilicis-integrae*. **39** *R. ilicis-latifoliae*. **40** *R. ilicis-pedunculosae*. *Bars* 10 µm

New leaves of the current year develop from late April on *Ilex integra* and *I. pedunculosa* and from early May on *I. latifolia*. Yellowish spots, roughly circular and about 1–4 mm in diameter, arise in mid-May on newly developing leaves by the three *Rhytisma* species studied. After about 5–10 days of the appearance of symptoms, a black stromatal layer develops on the yellowish spots and spermogonia are formed. Abundant spermatia are produced from late May to mid-June in *R. ilicis-integrae* and *R. ilicis-pedunculosae* and from mid-June to late June in *R. ilicis-latifoliae*. Spermatia are frequently ejected from irregular cracks of the stromatal layer to the surfaces of the infected leaves as a gelatinous shiny fluid.

From early August, the black outer layer of the stroma gradually grows in thickness on amphigenous leaf surfaces and no loculi of spermogonia become detectable. Immature ascoma gradually develop in early September beneath the lower outer layer of the stroma.

In the following year, the inner part of the stromata increases in thickness, the stromata rise significantly above the amphigenous surfaces, and the ascomata develop beneath the stromatal layer of the abaxial surface. Asci are produced from early February in the ascomata. Asci are ripening sequentially and ascospores are produced from early April to late May in *R. ilicis-integrae* and from early April to early June in *R. ilicis-latifoliae* and *R. ilicis-pedunculosae*. After most of the ascospores are released from asci, the ascomata of these fungi become rotten or dried and decayed.

From the foregoing survey, the only inoculum of the tar spot disease in evergreen *Ilex* trees is considered to be ascospores produced on the previous-year leaves for about 1 month in early spring when the current-year leaves are developing. The tar spots of the disease appeared only in May, but then no disease development was observed. The infection source of tar spot diseases in evergreen *Ilex* trees in Japan, therefore, is living leaves infected in previous spring.

The host of *R. himalense* is also the evergreen *Ilex* tree, and ascomata are reported to be formed on living leaves in November (Cannon and Minter 1986); spermogonia and spermatia of the fungus were not observed in India (Cannon and Minter 1986; Hou and Piepenbring 2005). In contrast, the hosts of the following *Rhytisma* species are deciduous trees and ascomata develop in spring directly from the spermogonia (described as conidiomata), which are formed in the previous year on the fallen leaves: *Rhytisma acerinum* (Pers.) Fr., *R. americanum* Hudler & Banik, and *R. punctatum* (Pers.) Fr. on *Acer* spp. (Jones 1925; Woo and Partridge 1969; Cannon and Minter 1986; Hudler and Banik 1987; Hudler et al. 1998), *R. salicinum* (Pers.) Fr. and *R. umbonatum* Hoppe on *Salix* spp. (Cannon and Minter 1986), and *R. xylostei* Naumov on *Lonicera* spp. (Cannon and Minter 1986). It is noteworthy that the infection sources of these tar spot diseases are different between evergreen and deciduous trees of the host.

Germination of ascospores and formation of appressoria

Ascospores of the three *Rhytisma* species germinated on 2% glucose agar at 20°C after 12 h and ended forming of the appressoria. Germination tubes developed from mostly apical or basal sites in *R. ilicis-integrae* and mostly apical or lateral sites in *R. ilicis-latifoliae* and *R. ilicis-pedunculosae*.

Connecting hyphae between ascospores and appressoria of *R. ilicis-integrae, R. ilicis-latifoliae*, and *R. ilicis-pedunculosae* were 34–69 × 2–2.5 μm, 10–32(–49) × 1–2 μm, and 20–78 × 1–2 μm, respectively. The shape of appressoria was spherical, fusiform to elliptical in all the species. Appressoria of *R. ilicis-integrae*, *R. ilicis-latifoliae*, and *R. ilicis-pedunculosae* were 6–14.5 × 6–9.5 μm, 4.5–10 × 4.5–6.5 μm, and 4.5–7.5 × 4.5–7 μm, respectively (Figs. 38–40).

Osorio and Stephan (1989) reported that the morphology of the appressorium is unique for each species of the family Rhytismataceae and stated possibilities for use as additional taxonomic characteristics. My observation suggests that *R. ilicis-latifoliae* can be distinguished from *R. ilicis-integrae* and *R. ilicis-pedunculosae* by shorter connecting hyphae and that *R. ilicis-integrae* can be distinguished from *R. ilicis-latifoliae* and *R. ilicis-pedunculosae* by larger appressoria. Hudler et al. (1998) recognized differences in morphology of the germinating tube of *Rhytisma acerinum* and *R. americanum*. No cultures of these fungi, however, could be obtained from the germinating ascospores.

Slender hyphae developed from these appressoria but did not elongate more than 20 μm (Fig. 40). My repeated attempts to obtain cultures of these *Rhytisma* species by transferring the ascospores germinating and forming appressoria onto potato glucose agar media failed.

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