The First Record of *Rosellinia aquila* in Kanagawa Prefecture and the Analysis of Morphological Variation among the Collections

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Abstract. Ten stroma collections were obtained from 8 localities in Kanagawa Prefecture, and identified as *Rosellinia aquila* based on their morphological characteristics. This is the first record of *R. aquila* in Kanagawa Prefecture. The size of stromata ranged (0.76 –) 0.88 – 1.83 × 0.78 – 1.50 mm (mean 1.26 × 1.09 mm), and ascospores 17.2 – 30.2 (– 31.1) × (5.9 –) 6.3 – 11.6 (– 12.7) µm (mean 23.0 × 8.2 µm). A straight germ slit run across almost entire length on the ascospores. The ascospores generally had cellular appendage at one end, and cap-like slimy sheaths at both ends, except in old materials. Ascospore length varied mostly among collections. In one case, ascospore length greatly varied even between 2 collections in a single locality. The collections neither formed clusters nor showed geographic cline in principal component analysis based on morphological traits. The present study disclosed the morphological diversity of *R. aquila* in Kanagawa Prefecture.

Key words: analysis of variance (ANOVA), new record, principal component analysis (PCA), Xylariaceae

Introduction

Rosellinia aquila (Fr.) Ces. & de Not. is the type species of the genus *Rosellinia.* The species is basically a wood decomposing fungus (Abe, 1989), and can be found on dead and fallen logs and branches of broad-leaf trees, mainly *Quercus* spp. (Hennings 1902; Matsumura 1904; Shirai & Miyake 1917; Asahina *et al*., 1939; Abe, 1986; Abe & Doi, 2000; all listed in Kobayashi, 2007), though it was recently reported that *R. aquila* can persist in a stressed but still living twigs of *Quercus robur* (Kwaśna and Łakomy, 2006). Morphological studies on the teleomorph of *R. aquila* is quite limited in Japan, and as few as 10 specimens were observed so far for Japanese mycoflora, e.g., Abe (1986) and Nakamura *et al*. (2000). In Kanagawa Prefecture, there has been no record of distribution. In the present study, a wide area in Kanagawa Prefecture was searched for *R. aquila*. Ten stroma collections were obtained from 8 localities, and identified as *R. aquila* based on their morphological characteristics. Diversity in morphological characteristics, especially ascospore size, was statistically examined among collections.

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Materials and methods

Field search. Stromata tentatively identified as *R. aquila* were collected from a wide area in Kanagawa Prefecture, mainly in forest. Identification in the field was based on the habitat and appearance of stromata described by Petrini (1992) and Nakamura *et al*. (2000), i.e., almost globose, carbonaceous, dark brown to reddish brown, ca. 1 mm diam., papillate with an ostiole at the center top, often accompanying dark brown to brown subiculum, but never with synnemata, superficial and gregarious on decomposing wood. Information on the collections and isolates established from them are listed in Table 1.

Morphological observation. After stromata were gently

Table 1. Information on the collections of *Rosellinia aquila* in this study

Collection# (KPM-NC#[a])	Date	Locality[b]	Substrate	Collector	Isolate[d] (MAFF#)
1 (15966)	2007.11.08	Maruyama, Iryuda, Odawara City	Fallen branches, unknown broad-leaf tree	Y. Degawa & volunteer members[c]	
2 (15964)	2007.11.11	Ashigara Shinrin-kouen Forest Park, Hiromachi, Minamiashigara City	Piled logs, *Acer palmatum*	S. Takemoto	KnMn2-1 (625103) KnMn2-2 (625104) KnMn2-3 (625105)
3 (15973)	2007.11.18	Minoge Shizenkansatsu-no-mori Nature-watching Forest, Minoge, Hadano City	Piled branches, *Acer* sp.	S. Takemoto	KnHd1-1 (625106)
4 (15974)	2007.11.18	Nanasawa Forest Park, Morinosato, Atsugi City	Piled branches, unknown broad-leaf tree	S. Takemoto	KnAt1-1 (625107)
5 (15976)	2007.11.18	Susugaya, Kiyokawa Village	Piled branches, unknown broad-leaf tree	S. Takemoto	KnKy1-1 (625108)
6 (15979)	2007.11.23	Kannonzaki Point, Kamoi, Yokosuka City	Fallen branches, unknown broad-leaf tree	S. Takemoto	KnYs1-1 (625109)
7 (15980)	2007.11.24	Chuuou-kouen Park, Chigasaki, Chigasaki City	Fallen branches, unknown broad-leaf tree	S. Takemoto	
8 (15981)	2007.11.24	Chuuou-kouen Park, Chigasaki, Chigasaki City	Fallen branches, unknown broad-leaf tree	S. Takemoto	KnCh2-1 (625110)
9 (15982)	2007.11.24	Chuuou-kouen Park, Chigasaki, Chigasaki City	Fallen branches, unknown broad-leaf tree	S. Takemoto	KnCh3-1 (625111)
10 (15985)	2007.11.25	Houdaiyama Mt., Nagasawa, Yokosuka City	Fallen stems, *Aucuba japonica*	S. Takemoto	KnYs2-1 (625112)

a: Numbers under which collections are vouchered in Kanagawa Prefectural Museum of Natural History

b: All collections were from Kanagawa Prefecture, Japan

c: Volunteer members of Kanagawa Prefectural Museum of Natural History

d: Single-ascospore isolates derived from each collection. All of the isolates were deposited in the National Institute of Agrobiological Sciences Gene Bank, Tsukuba

detached with forceps from substrates, their height and diameter were measured with calipers. Average diameter was calculated from measurements along two axes crossing at right angles for each stroma. Ascospores were obtained separately from three stromata per collection. The ascospores were mounted on glass slides in lactophenol mounting solution (20% (w/w) phenol, 40% (w/w) glycerol, 20% (w/w) lactic acid, 20% (w/w) water), and the length and width of the ascospores were individually measured. Asci and paraphyses, if any, were similarly mounted, and ascus apical rings were observed after coloring with Meltzer's
reagent. Anamorphic state found in the subiculum also was observed.

Statistical analyses. Two-level nested ANOVA (Sokal & Rohlf, 1995) was applied to ascospore length to estimate variance components for three levels, i.e. among individual ascospores within single stromata, among stromata within single collections and among collections. The principal component analysis (PCA) was conducted to ordinate the collections and evaluate the contribution of each morphological trait to the ordination. PCA was run on the R software package version 2.5.1 (R Development Core Team, 2007) after data were standardized. Morphological data set used in PCA was composed by stroma height, stroma diameter, ascospore length and ascospore width.

Results

Field search. Ten stroma collections were obtained from 8 localities (Fig. 1). All of them were found on logs or branches of broad-leaf trees detached, fallen down or piled up on the ground, not on fresh substrates.

Morphological observation. The collections commonly had stromata almost globose or reversed cup-like, carbonaceous, dark brown to reddish brown, with a papillate ostiole at the center top, never accompanying synnemata, superficial and gregarious on the substrates (Fig. 2a, b, d), except one from Hadano City (Fig. 2c, e; collection No. 3). The size of stromata was (0.76 –) 0.88 – 1.83 × 0.78 – 1.50 mm with average 1.26 × 1.09 mm among collections. Stromata of the collection No. 3 were silvery to fuliginous with a faint reddish tone, and concentric winkles often appeared on the top surface around the ostiole, though other morphological characters were similar to the other collections. Brown to reddish brown subiculum was

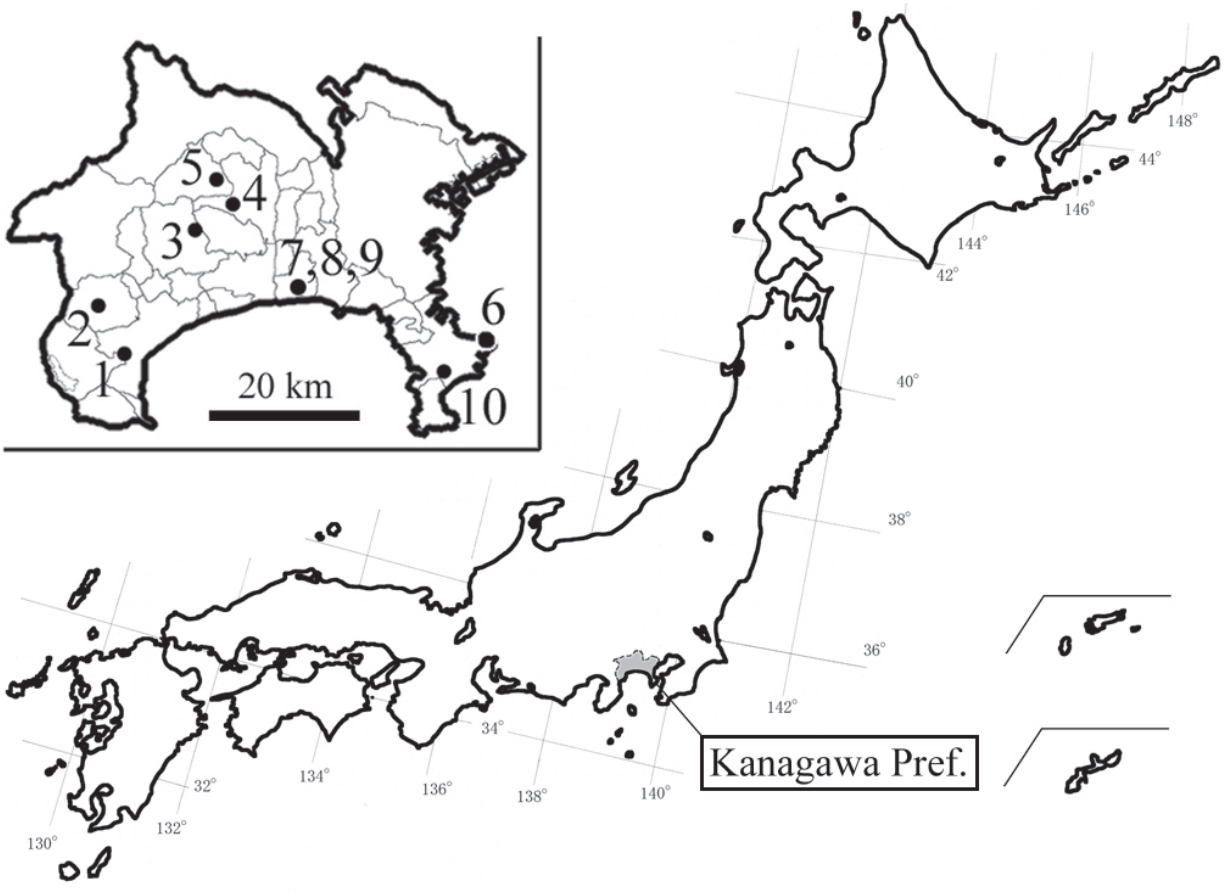

Fig.1 Sampling localities. The localities are indicated by closed circles labeled with collection numbers.

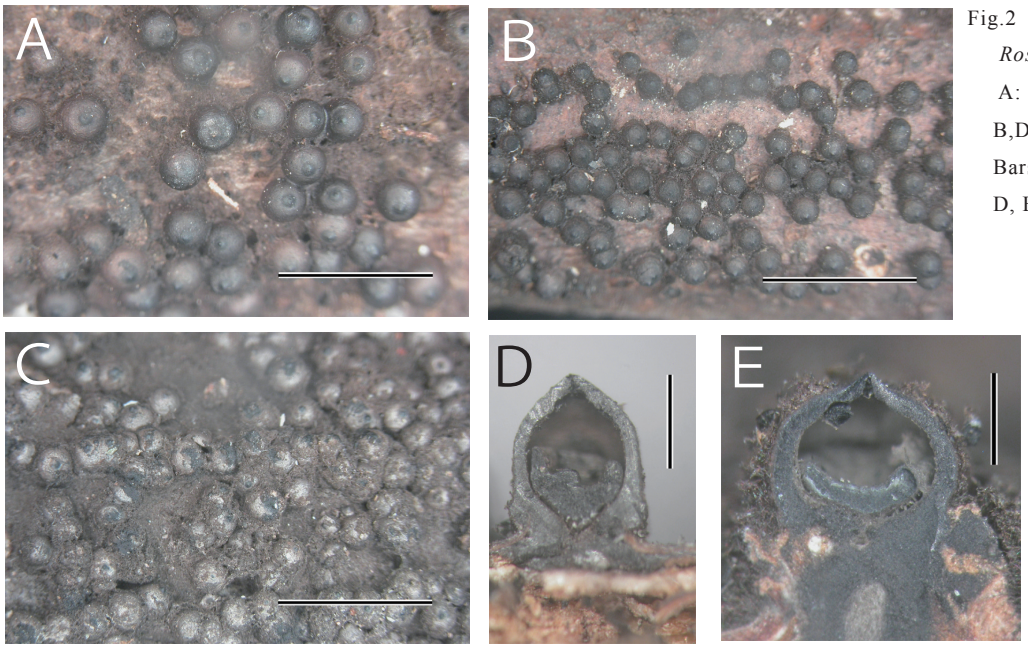

Fig.2 Stromata of *Rosellinia aquila*.
A: Collection No.4;
B,D: No.8; C, E: No.3.
Bars: A, C = 5 mm;
D, E = 500μm

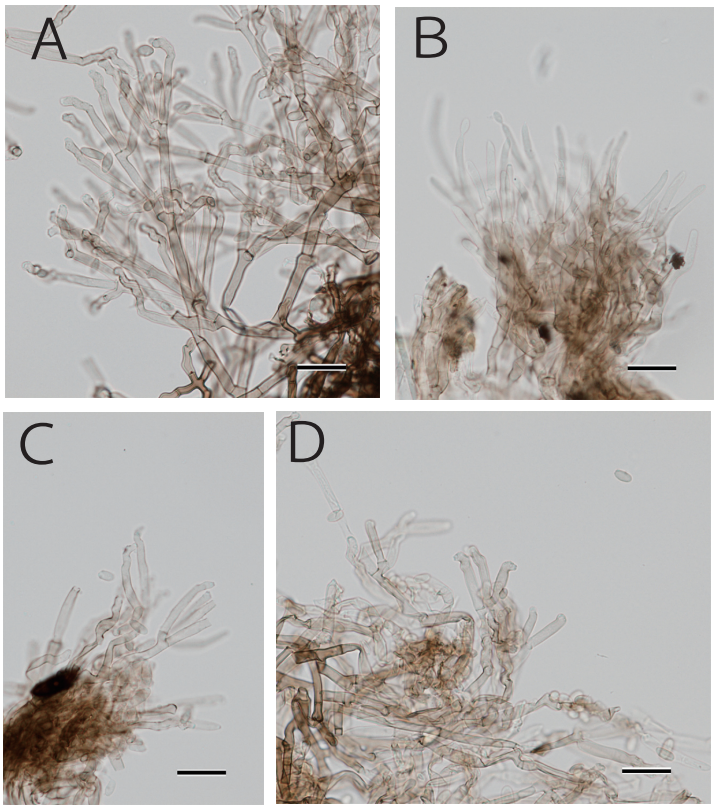

Fig.3 Anamorphic states found on the collections of *Rosellinia aquila*.
A: Collection No.4; B: No.8; C: No.3;
D: No.10. Bars = 20μm

generally accompanied on the collections, but almost lost from some old specimens (e.g., Fig. 2b). Geniculate conidiophores (*Geniculosporium* type), hyaline to light brown and noticeably cicatrized (Fig. 3) were present among subiculum or sometimes on the stromata. Conidia were oblong to elliptic, hyaline to light brown and truncate at the base. Structures inside stromata were as follows: Asci clavate, 8-spored (Fig. 4); paraphyses numerous, filiform, tapering toward the round apex (Fig. 4); ascus apical rings amyloid, variably vase-shaped, 5.4 – 11.2 μm high (mean 7.4 μm), 3.1– 4.8 μm diam. at superior part (mean 4.2 μm), 3.3 – 6.3 μm diam. at inferior part (mean 4.7 μm) (Fig.5); ascospores lemon-shaped to ellipsoid, slightly asymmetric, brown to dark brown, 17.2 – 30.2 (– 31.1) × (5.9 –) 6.3 –11.6 (– 12.7) μm with average 23.0 × 8.2 μm (Fig. 6), with a straight germ slit running almost from one end to the other (Fig. 6A), with cellular appendage at one end or rarely both ends (Fig.

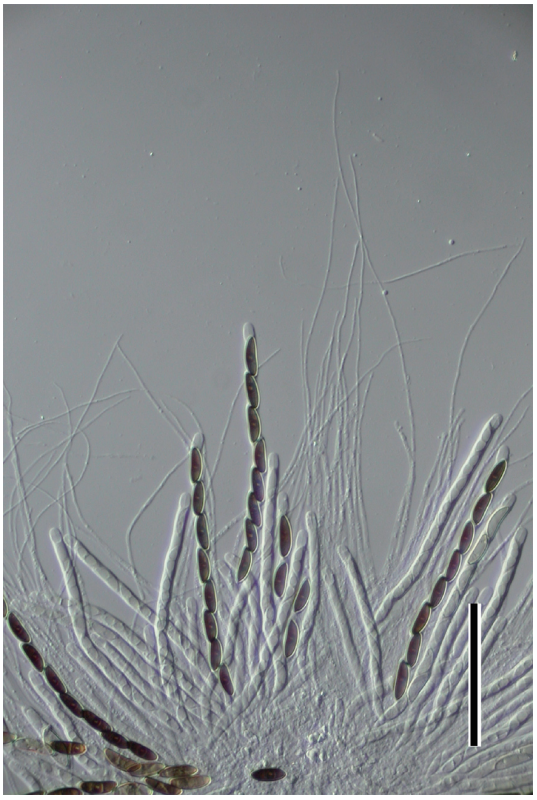

Fig.4 Asci and paraphyses of *Rosellinia aquila*. Bar = 10μm

6B, C), covered by cap-like slimy sheaths at both ends (Fig. 6B, C). The structures of soft tissues were lost from the old materials previously mentioned. Ascospores in most stromata were vigorous, and single-ascospore isolates were obtained from 8 of the 10 collections (Table 1). All the measurements and notes on morphological characters are summarized in Table 2.

Statistical analyses. A two-level nested ANOVA for ascospore length (Table 3) revealed that the variance (i.e., diversity) existed mainly among collections (58.7%) followed by among ascospores within stromata (34.7%). The variance component among stromata within single collections was small (6.6%), though it was significant as well as those at the other 2 levels.

Collections did not form clusters in the plane of PCA ordination, rather scattered (Fig. 7). The collection No.3, which distinctively had silvery stromata, was not located at distant from the others, rather intermingled together (Fig .7). The first axis of PCA (PC1) explained 78% of the total morphological variance and indicated graduation from small to large stroma and from large to small ascospore.

Discussion

Five of the collections in this study (Nos.1, 6, 7, 8 and 10) had ascospores and/or ascus apical rings larger than those of *R. aquila* reported in Petrini (1992), Nakamura *et al*. (2000) and

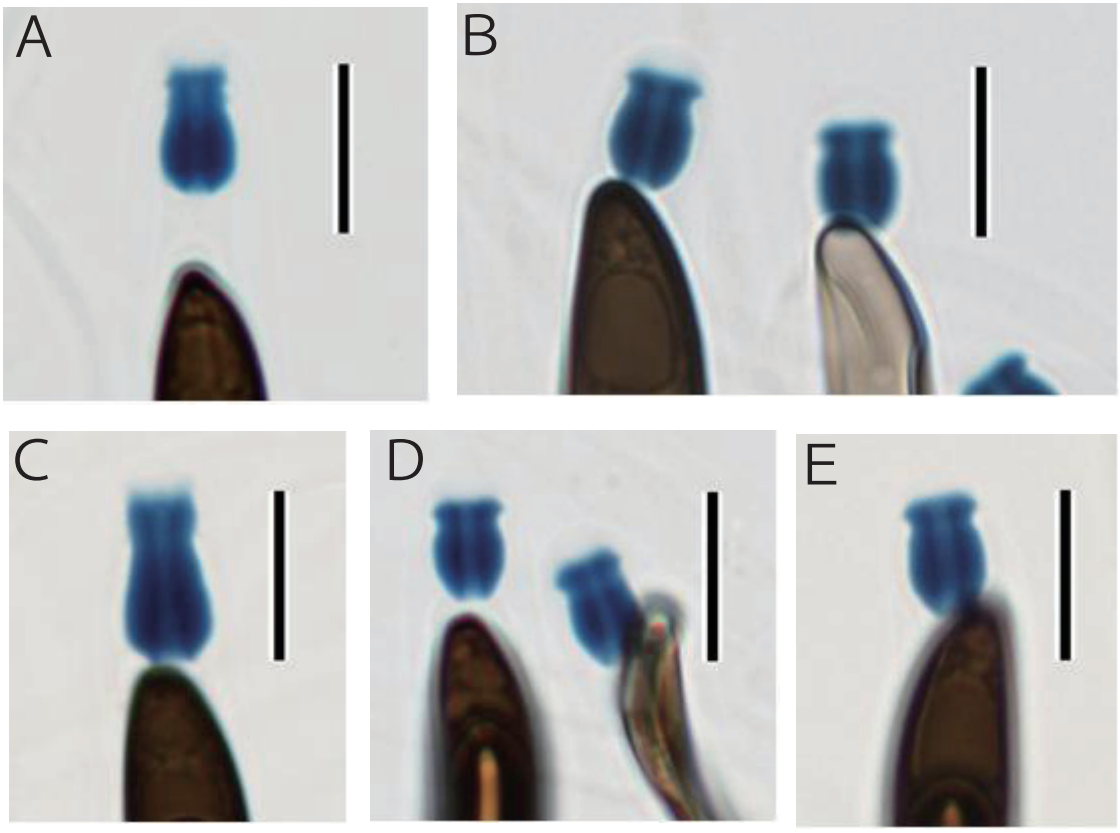

Fig.5 Ascus apical rings of *Rosellinia aquila*. A: Collection No.3; B: No.4; C: No.6; D: No.9; E: No.10. Bars = 10μm

Table 2. Characteristics of 10 *Rosellinia aquila* collections

No.	Stroma		Ascospore				Ascus apical ring						Anamorphic state[c]
							Height		Upper width		Lower width		
	Range [mm]	Average	Range [μm]	Average	Cellular appendage[a]	Slimy sheath[b]	Range [μm]	Average	Range [μm]	Average	Range [μm]	Average	
1	1.02–1.56 × 0.85–1.27	1.05 × 1.03	22.8–29.2 × 8.0–11.6(–12.7)	25.5 × 9.7	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	1.05–1.59 × 0.95–1.50	1.31 × 1.15	17.7–25.7 × 6.3–8.0	22.0 × 7.4	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	1.06–1.43× 0.97–1.38	1.27 × 1.14	(19.9–)21.8–25.8 (–26.5)×(5.9–)6.8–9.5	23.4 × 7.8	1+	C2	5.7–7.1	6.4	4.1–4.8	4.5	4.1–5.0	4.6	G
4	1.03–1.83 × 1.03–1.47	1.46 × 1.31	17.3–23.2(–25.7) × (6.6–)6.7–8.9	20.2 × 7.5	1	C2	5.4–8.4	6.7	3.1–3.9	3.5	3.3–4.7 (–5.5)	4.0	G
5	1.15–1.74 × 1.05–1.40	1.36 × 1.25	17.2–24.4 × 5.8–8.3	20.5 × 7.2	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	(0.76–)1.01–1.41 × 0.95–1.12	1.14 × 1.05	23.3–30.2(–31.1) × 7.3–10.9	25.9 × 9.2	1	BC2	7.5–10.8	8.7	3.8–4.6	4.2	4.5–5.7	5.0	G
7	0.88–1.22(–1.50) × 0.78–1.19	1.10 × 0.99	(18.6–)19.9–26.0 × 7.5–10.3(–10.5)	22.6 × 8.9	ND	ND	ND	ND	ND	ND	ND	ND	ND
8	0.88–1.26 × 0.82–1.22	1.08 × 0.98	21.2–28.3 × 7.2–9.7	23.8 × 8.3	1 or 0	C2	7.8–11.2	9.7	3.7–4.6 (–5.7)	4.3	5.2–6.3	5.8	G
9	1.00–1.68× 0.99 –1.21(–1.33)	1.35 × 1.09	18.5–23.0 × (6.3–)6.8–9.4	20.5 × 7.7	1	C2	5.5–6.5	6.1	3.8–4.5	4.0	3.8–4.5 (–5.1)	4.3	G
10	1.02–1.43 × 0.87–1.07	1.22 × 0.95	21.7–28.5 × 7.2–10.2	25.2 × 8.7	1	C2	6.5–6.9 (–7.5)	6.9	4.2–4.8	4.6	(4.0–)4.4 –4.8	4.5	G
Average		1.26 × 1.09		23.0 × 8.2				7.4		4.2		4.7	

ND: stands for no data

a: Ascospores had cellular appendage(s) at one end or none (1 or 0), at one end only (1) or at one end and rarely both ends (1+)

b: Ascospores had cap-like (C2) or broad cap-like slimy sheaths at both ends (BC2)

c: Anamorphic state was *Geniculosporium* type (G)

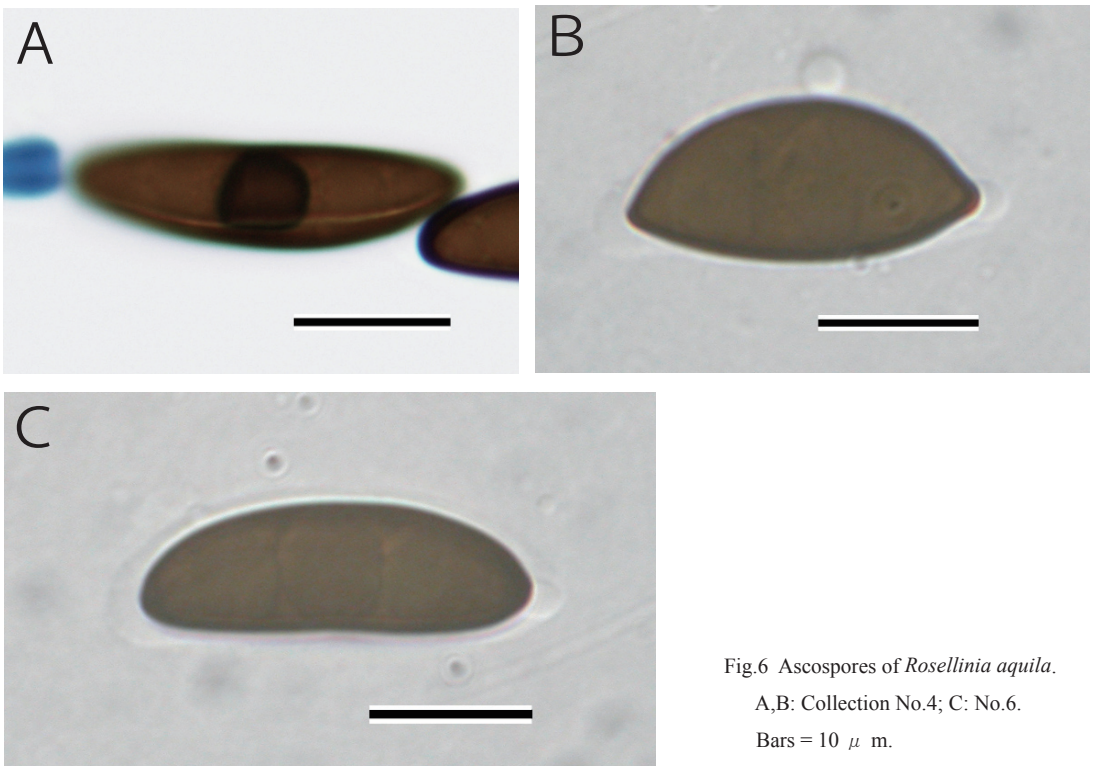

Fig.6 Ascospores of *Rosellinia aquila*.
A,B: Collection No.4; C: No.6.
Bars = 10 μ m.

Table 3. Two-level nested ANOVA for ascospore length

(a) ANOVA table

Source of variation	*df*	*SS*	*MS*	F_s	*p*
Among collections	9	1469.9	163.32	19.53	4.82×10^{-8}
Among stromata	20	167.3	8.36	3.10	1.44×10^{-5}
Within stromata	300	809.3	2.70		
Total	329	2446.4			

(b) Estimates of variance component at 3 levels.

Variance components	Estimation	Relative magnitude
s^2_C (among collections)	4.56	58.7%
$s^2_{S\subset C}$ (stromata within collections)	0.52	6.6%
s^2 (ascospores within stromata)	2.70	34.7%

Kwaśna and Łakomy (2006). Ascospore size of *R. aquila* ranged 18 – 22 × 6 – 8 μm in Kwaśna and Łakomy (2006), and 17.5 – 25.0 × 6.3 – 8.8 μm with average 22.0 × 7.5 μm in Nakamura *et al*. (2000). Petrini (1992) described as: ascospores 14.5 – 27.0 × 5.5 – 10.0 μm with average 19.5 × 7.5 μm; ascus apical rings 4.5 – 9.0 μm high, upper width 3.5 – 5.5 μm, lower width 3.5 – 6.0 μm. On the other hand, the five collections in this study were as follows: Ascospores (18.6 –) 19.7 – 30.2 (–31.1) × 7.2 – 11.6 (– 12.7) μm with average 24.6 × 9.0 μm; ascus apical rings 7.5 – 11.2 μm high, upper width 3.7 – 4.8 (– 5.7) μm, lower width 4.5 – 6.3 μm, which were more comparable to those of *R. corticium* in Petrini (1992): ascospores 19.0 – 33.5 × 6.5–14.0 μm with average 24.0 × 9.0 μm; ascus apical rings 5.5 –12.5 μm high, upper width 3.5 – 8.0 μm, lower width 3.5 – 8.0 μm. However, measurements on *R. aquila* ascospores ranged 20–35 × 7.5 – 12.0 μm in Abe (1986), and 20 – 38 × 7.5 – 12.8 μm in Dargan and Thind (1979), almost over-rapped to ours. The five collections as well as the others in the present study had slimy sheaths, if any, commonly reduced to cap-like structures at both ends of ascospores as *R. aquila* (Petrini, 1992); ascospores of *R. corticium* should be entirely covered with slimy sheath. Dargan and Thind (1979) used cellular appendages (hyaline apiculi) as main discriminative key between *R. aquila* and *R. corticium*, and described that the latter had no such structures. According

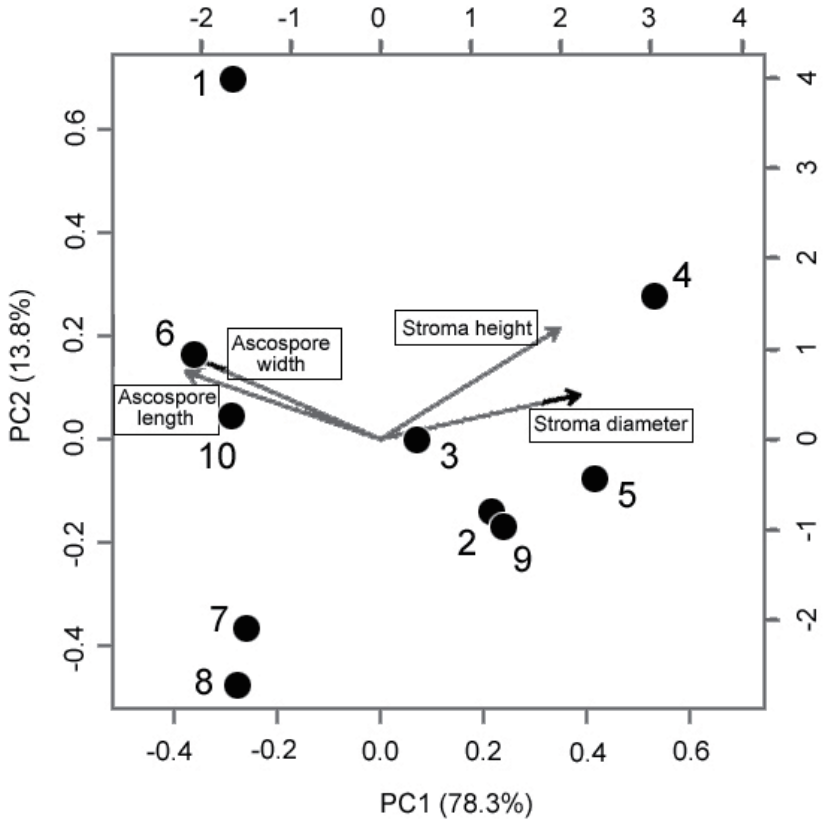

Fig.7 Ordination of the *Rosellinia aquila* collections by the principal component analysis based on the dimensions of stromata and ascospores. The data set was standardized before analysis. Collections are indicated by closed circles labeled with collection numbers. Vectors labeled with morphological traits (shown in boxes) represent the contribution of the traits to the first and second principal components (PC1 and PC2).

to Dargan and Thind (1979), the collections in the previous study were, thus, categorized clearly into *R. aquila*, since they generally had cellular appendage at one end of the ascospores, though Petrini (1992) pointed out that cellular appendages were sometimes hardly observed under light microscope when covered by slimy caps, and mentioned that *R. corticium* had a cellular appendage at one or seldom both ends of the ascospores, and *R. aquila* at one or both ends. The wide species concept of *R. aquila* includes *R. corticium* as its variety *byssiseda* (Traverso, 1906), and specimens of *R. corticium* has often been described as *R. aquila* among European mycologists (Petrini, 1992). *Rosellinia subsimilis* also resembles to our collections and *R. aquila* in the present studies, but differs by felty, woolly or web-like scarcely developed subiculum that completely disappears from fully mature or old material (Petrini, 1992). Since then, we identified all the collections as *R. aquila*. This is the first record of *R. aquila* in Kanagawa Prefecture. *Acer* spp. and *Aucuba japonica* are new substrate records in Japan.

Rosellinia aquila in this study were diverse in ascospore length noticeably among collections (58.7% of total variance, Table 3), but little among stromata in single collections (6.6%). Since variance component was estimated 4.56 at among-collection level against the allover average of ascospore length 22.7 μm, coefficient of variation was calculated as 9.4%. On the other hand, an allied plant-pathogenic species, *R. necatrix*, had size diversity mostly (70.2%) among individual ascospores within single stromata (Takemoto et al., 2009).

Two each collections from Chigasaki City (Nos. 7 and 8) and Yokosuka City (Nos.6 and 10) were located closely in the plane of PCA ordination (Fig. 7); however, one collection from Chigasaki City (No. 9) was distributed at a distance from 2 others collected from the same locality. In general, the collections formed no outstanding clusters, and no geographical cline was detected. *Rosellinia aquila* in Kanagawa Prefecture does not seem to differentiate geographically.

These facts suggest that *R. aquila* in Kanagawa Prefecture is composed by populations morphologically diverse, and maybe, also genetically diverse even in a single locality. Another hypothesis is that *R. aquila* includes one or more unidentified species to be recovered. Molecular study combined with further morphological examination is needed to disclose the diversity of *Rosellinia* fungi in Japan.

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摘　要

S. Takemoto, H. Nakamura & Y. Degawa, 2009. The first record of *Rosellinia aquila* in Kanagawa Prefecture and the analysis of morphological variation among the stroma collections. *Bull. Kanagawa prefect. Mus*. (*Nat. Sci*.), (38): 21-29.（竹本周平・中村仁・出川洋介 , 2008. カタツブタケ *Rosellinia aquila* の神奈川県における新産報告および形態的多様性の解析 . 神奈川県立博物館研究報告（自然科学）, (38): 21-29.）

神奈川県下の 8 地点において採集された計 10 点の子座標本を形態観察した結果、カタツブタケ *Rosellinia aquila* と同定した。本報は、*R. aquila* の神奈川県からの新産報告である。子座の大きさは (0.76–)0.88–1.83 × 0.78–1.50 mm (平均 1.26 × 1.09 mm) であった。子嚢胞子の大きさは 17.2–30.2(–31.1) × (5.9–)6.3–11.6(–12.7) μm (平均 23.0 × 8.2 μ m) であった。子嚢胞子は、ほぼ全長にわたる直線状の発芽スリットをひとつ有していた。また、古くなった標本以外では、子嚢胞子はおしなべて cellular appendage を一端に、キャップ状の slimy sheath を両端に有していた。子嚢胞子の長さの分散は標本間において最も大きく、一地点からの２標本間でも長さが大きく異なる場合があった。形態形質に基づく主成分分析では、標本はクラスターを形成せず、また、地理的な勾配もみとめられなかった。本研究の結果から、神奈川県における *R. aquila* の形態的多様性が示された。

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