Enumeration of Remarkable Japanese Discomycetes (9): Notes on Two *Lanzia* Species New to Japan

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Abstract Two *Lanzia* species newly documented for Japan are described and illustrated: *Lanzia longipes* and *L. pruni-serotinae* (Rutstroemiaceae, Helotiales), both characterized by apothecial structure and the presence of stroma. In the former species, occurrence of pigmentation of ascospores were observed after discharge.

Key words: Lanzia longipes, Lanzia pruni-serotinae, mycobiota, stroma, taxonomy.

Introduction

This is the ninth part of the series on remarkable Japanese discomycetes following Zhao and Hosoya (2014) to extend the knowledge of the Japanese mycobiota. The genus Lanzia (Rutstoemiaceae, Helotiales) is known to include more than 50 species (Kirk et al., 2008). It is thought to be a widespread genus, and local mycobiota have been studied in India (Sharma and Sharma, 1985), Australia (Spooner, 1987; Simpson and Grgurinovic, 2003), and China (Zhuang, 1996, 1999). In spite of its biodiversity, comprehensive studies have scarcely been conducted in Japan (Katumoto, 2010). Although the genus seems to be diverse, there have been no monographic studies (see Zhuang, 1996). Its segregation or similarity from allied genera such as Hymenoscyphus (Johnston and Park, 2013), Lambertella (Zhuang, 1996) and Poculum, Rutstoremia (Baral, 1994) have been discussed, but phylogenetic studies are limited (Holst-Jensen et al., 1997; Zhuang and Liu, 2007; Johnston and Park, 2013). Currently only two species have been recorded from Japan (Katumoto, 2010), but potentially more species await documentation. In the present paper, two species are documented for the first time from Japan, with attention to their cultural characteristics.

Materials and Methods

Observation procedures followed Hosoya and Otani (1997). Color codes followed the Pantone color code adopting CYMK system referring to a Pantone color bridge (Anonymous, 2005). To examine previously known distribution, the occurrence database of Global Biodiversity Information Facility (GBIF, http://www.gbif.org/ occurrence, as of June 20, 2015) was searched.

Single-spored isolates were obtained using Skerman's manipulator (Skerman, 1968), and deposited in the National Institute of Technology and Evaluation, Biological Resource Center (NBRC). To observe colony characteristics, potato dextrose agar (PDA, Nissui) plate or modified Weitzman-Silva-Hutner agar (WSH; 10g oatmeal, 1g KH₂PO₄, 1g MgSO₄·H₂O, 1g NaNO₃, 20g agar, 1,000 ml distilled water) plates were inoculated at the center with a 1 mm agar cube containing mycelium and incubated at 20 °C.

Descriptions

1. *Lanzia longipes* (Cooke & Peck) Dumont & Korf, Mycotaxon 7: 185. 1978.

[Figs. 1–3]

- *Peziza longipes* Cooke & Peck, Bull. Buffalo Soc. nat. Sci. 1: 295. 1875.
- *Phialea longipes* (Cooke & Peck) Sacc., Syll. fung. (Abellini) 8: 267. 1889.
- Hymenoscyphus longipes (Cooke & Peck) Kuntze, Revis. gen. pl. (Leipzig) 3: 485. 1898.
- *Rutstroemia longipes* (Cooke & Peck) W.L. White, Lloydia 4: 203. 1941.

Stroma substratal, visible as blackened zones on petioles of host. Apothecia long-stipitate, occurring on decaying petioles; disc flat to discoid when fresh, becoming concave when dry, 1.5-4 mm in diameter in dried specimen; hymenium cream white (1 PC = C2 M3 Y4 K5) when fresh, becoming buff (720 PC = C0 M20 Y32K2) to pale brown when dry; receptacle concolorous or slightly paler than the hymenium, furfuraceous; stipe subcylindric, concolorous with the receptacle, becoming much darker toward base, up to 30mm long when dry. Ectal excipulum three layered. Covering layer thin-walled, hyaline to pale brown, cells of irregular shaped, $2-4\,\mu m$ wide; outer ectal excipulum textura prismatica, subhyaline to pale brown, composed of slightly thick-walled, brick-shaped cells $8-25 \times$ $5-10\,\mu\text{m}$, becoming slightly globose in the margin; inner ectal excipulum hyaline, composed of $4-8\,\mu\text{m}$ wide hyphae. Hairs arising from outer covering layer, septate, hvaline to pale brown, smooth, usually with cylindrical or swollen apices. Medullary excipulum textura intricata, loosely interwoven, subhyaline to pale brown, smooth, composed of $5-10\,\mu\text{m}$ wide hyphae. Asci 70–95 \times 8–11 μ m, clavate, 8-spored, arising from crozier but obscure; apex $4-5\mu$ m thick; pore stained blue by Melzer's reagent with or without 3% KOH pretreatment. Ascospores $10-11 \times 3.5-5 \,\mu m$ ($10.6 \pm 0.5 \times 4.2 \pm 0.4 \,\mu m$ on average \pm SD, n = 23), uniseriate, ellipsoid to obovoid-reniform, usually inaequilateral, with one side much broader, smooth, some with 1–2 guttules; some discharged spores pale-brown, usually with one septum. When germinated on PDA, ascospores become unequally 1-septate near the bottom, and upper cell becomes thick and dark walled, much darker than the other cell. When germinated on 1% water agar, the whole spore becomes brown, thick-walled and 1–2 septate. Phialidic spermatia produced from the small tubes on the ascospore. **Paraphyses** filiform, septate, hyaline, slightly enlarged at the apex up to 2μ m wide.

Cultural characteristics. Colonies on PDA grows fast, covering the whole plate after 4 weeks incubation, circular, floccose, whitish to grayish. Aerial mycelium white, cottony, partially developed. Margin entire. Reverse pale brown, with the blackened areas diffusing from center. Rind delimiting irregular portions of the agar, composed of a single layer of cells with walls pigmented to various extent, epidermoid in face view.

Specimens examined. All on petiole of leaves unidentified tree. TNS-F-40082 (14 Oct. 2011), TNS-F-40097 (Culture FC-2832 = NBRC109877), 7 Nov. 2011, TNS-F-40177 (Culture FC-5098 = NBRC 110237), 15 Oct. 2012, Tsukuba Botanical Garden, Tsukuba City, Ibaraki Pref., Japan (140°6'44.0"N, 36°6'9.5"E, Alt. 44m), Col. Y.-J. Zhao. TNS-F-40148, Sugadaira Montane Research Center, University of Tsukuba, Ueda City, Nagano Pref. (138°20'51.2"N, 36°41'30"E, Alt. 1349m), 15 Sep. 2012, Col. Y.-J. Zhao. TNS-F-40160, Hanazono Shrine, Kitaibaraki-shi, Ibaraki Pref., 26 Sep. 2012, Col. Zhao. TNS-F-40167, Takayama-mura, Y.-J. Gunma Pref. (138°57'36.5"N, 36°35'19.3"E, Alt. 242 m), 2 Oct. 2012, Col. Y.-J. Zhao.

Known distribution. No GBIF data. Known from North America (US and Canada; White, 1941)

Japanese name: Naga-e-no-shiro-byou-take (newly proposed).

Notes. *Lanzia longipes* is a new record in Japan. It is easily distinguished by its long stipe



Fig. 1. Lanzia longipes (TNS-F-40082). A. Fresh apothecia on unknown petioles. B. Vertical section of an apothecium. C. Close up of ectal excipulum at the margin. D. Close up of ectal exipulum. E. Structure of medullay excipulum. F. Ascus. G. Reaction of ascal apex to MLZ. H. Paraphyses. I. Hairs. J. Ascospores (Hyaline ascospores, brown ascospores and germinating ascospores with spermatia). Bars, B, E, 40μm; C–D, F–J, 20μm.



Fig. 2. Camera lucida illustration of *Lanzia longipes* (TNS-F-40082). A. Asci. B. Reaction of ascal apex to MLZ. C. Paraphyses. D. Hairs. E. Vertical section of an apothecium through the margin showing the ectal excipulum. F. Ascospores.



Fig. 3. Cultural characters of *Lanzia longipes* (FC-2832, Culture of TNS-F-40097). A. Colony on PDA (20°C, 1 mo.). B. Colony on PDA (20°C, 3 mo.). C. Vertical view of rind. D. Surface view of rind showing the epidermoid to irregular cells. Bars, C, 40 μm; D, 20 μm.

of apothecia and remarkably large asci and ascospores.

Lanzia longipes was first described from New York under the name *Peziza longipes* Cooke & Peck in Cooke (1875) who emphasized the length of the stipe. The substratum was simply

indicated as "leaf petioles". White (1941) recognized that its simple structured apothecium differed from others. Korf and Gruff (1978) transferred it to *Lanzia* for its prismatic cells in ectal excipulum, but no previous authors recognized the pigmentation of ascospores and production of



Fig. 4. Lanzia pruni-serotinae (TNS-F-40119). A. Fresh apothecia on leaves of Prunus grayana. B. Fresh apothecia in a higher magnification. C. Vertical section of an apothecium. D. Close up of the vertical section. E. Close up of ectal excipulum at the margin. F. Close up of ectal excipulum at the flank. G. Structure of medullay excipulum. H. Ascus. I. Croziers at the base of asci. J. Reaction of ascal apex to MLZ. K. Paraphyses. L. Ascospores. Bars, A, 4mm; B, 1mm; C, 200µm; D, 40µm; E–L, 20µm.



Fig. 5. Camera lucida illustration of *Lanzia pruni-serotinae* (TNS-F-40119). A. Vertical section of an apothecium through the margin showing the ectal excipulum. B. Asci. C. Hairs. D. Reaction of ascal apex to MLZ. E. Croziers. F. Ascospores.

spermatia after dicharge.

2. *Lanzia pruni-serotinae* (Whetzel & W.L. White) M.P.Sharma & R.M.Sharma, Int. J. Mycol. Lichenol. 2: 109. 1985.

[Figs. 4–5] *Rutstroemia pruni-serotinae* Whetzel & W.L. White, Lloydia 4: 221. 1941.

Stroma substratal, visible as blackened zones

on leaf veins and petioles of the host. **Apothecia** stipitate, occurring on decaying leaves and petioles; disc flat to cupulate, 0.5-2.5 mm in diameter when fresh; hymenium beige to brown (137 PC = C0 M38 Y95 K0) when fresh, becoming dark brown (1615 PC = C11 M74 Y100 K50) to black when dry; receptacle beige when fresh, becoming brown when dry; stipe concolorous with the receptacle, 1–11 mm long when fresh, becoming dark brown towards the base, partially

encrusted by brown hyphal mass. Ectal excipulum three layered. Covering layer composed of 2-5 cells, thin-walled, hyaline to pale brown, slightly granulate; outer ectal excipulum textura prismatica, hyaline to pale brown, composed of slightly thick-walled, brick-shaped cells of $2.5-8\,\mu\text{m}$ wide; inner layer hyaline to pale brown, smooth, slightly thick-walled, hyphae of $5-10\,\mu\text{m}$ wide. Hairs protruded from outermost layer of the covering layer, hyaline to pale brown, smooth to roughened, septate, some expanded at the apices. Medullary excipulum textura intricata, hyaline, smooth, tightly interwoven, embedded in gelatinous matrices, composed of hypha of $3-6\mu m$ wide. Asci $60-75 \times$ $6-8\,\mu\text{m}$, clavate, 8-spored, arising from repeated croziers; apex rounded to truncate, $1.5-2\,\mu m$ thick; pore very faintly stained by Melzer's reagent with or without 3% KOH pretreatment. **Ascospores** $6-9 \times 3-4.5 \,\mu \text{m}$ $(8.0 \pm 1.0 \times 3.4 \pm$ $0.5\,\mu m$ on average \pm SD, n = 20, uniseriate or irregularly biseriate in upper region of the ascus, elongate ellipsoid, non-septate, smooth, 0-2 guttules. Paraphyses filiform, septate, hyaline, simple or branched near the base, usually expanded at the apex up to $2.5-3\,\mu\text{m}$ wide.

Specimens examined. On leaves and petioles of *Prunus grayana* Maxim.: TNS-F-40119, Tsu-kuba Botanical Garden, Tsukuba City, Ibaraki Pref. (140°6'44.0"N, 36°6'9.5"E, Alt. 44 m), 13 June 2012, Col. Y.-J. Zhao.

Known distribution. No GBIF data. Known from India (Sharma and Sharma, 1985) and North America (US, White, 1941)

Japanese name. Sakura-ba-chairo-byou-take (newly proposed)

Notes. The morphological features well agreed with the previous description of *L. pruni-sero-tinae* (Sharma and Sharma, 1985, as *Rutstroemia pruni-serotinae*). It is often confused with *Rut-stroemia renispora* (Ellis) W.L. White in apothecial structure and stromatic characters (White, 1941), but *L. pruni-serotinae* has the shorter asci, smaller non-curved ascospores and occurrence on *Prunus*. The discharged ascospores did not

germinate, hence the culture was not obtained. Although the name is after its host, White (1941) described it also occurs on *Crataegus*, *Ligustrum* and *Prunus domestica*.

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References

- Anonymous. 2005. Pantone color bridge/coated. Pantone Inc, New Jersey.
- Baral, H. O. 1994. Comments on "Outline of the asomycetes-1993". Systema Ascomycetum 1994: 113–128.
- Cooke, M. C. 1875. Synopsis of the discomycetous fungi of the United States. Bulletin of the Buffalo Society of Natural Sciences 2: 285–301.
- Hosoya, T. and Otani, Y. 1997. Hyaloscyphaceae in Japan (1): Non-glassy haired members of the tribe Hyaloscypheae. Mycoscience 38: 171–186.
- Holst-Jensen, A., Kohn, L. M. and Schumacher, T. 1997. Nuclear rDNA phylogeny of the Sclerotiniaceae. Mycologia 89: 885–899.
- Johnston, P. R. and Park, D. 2013. The phylogenetic position of *Lanzia berggrenii* and its sister species. Mycosystema 32: 366–385.
- Kirk, P. M., Cannon, P. F., David, J. C. and Stalpers, J. A. 2008. Anisworth & Bisby's Dictonary of the Fungi, 9th ed. International Mycological Institute, Egham, Surrey.
- Katumoto, K. 2010. List of fungi recorded in Japan. The Kanto Branch of the Mycological Society of Japan, Tokyo.
- Korf, P. R. and Gruff, S. C. 1978. Discomycetes exsiccate, Fasc. II & III. Mycotaxon 7: 185–203.
- Sharma, M. P. and Sharma, R. M. 1985. The genus *Lanzia* Sacc. in India. International Journal of Mycology and Lichenology 2: 95–118.
- Simpson, J. A. and Grgurinovic, C. A. 2003. A new species of *Lanzia* (Rutstroemiaceae) from Mt. Koschiuszko, Australia. Australasian Mycologist 22: 11–14.
- Skerman, V. B. D. 1968. A new type of micromanipulator and microforge. Journal of General Microbiology 54: 287–297.
- Spooner, B. M. 1987. Helotiales of Australasia: Geoglossaceae, Orbiliaceae, Sclerotiniaceae, Hyaloscyphaceae. Bibliotheca Mycologica 116: 1–711.
- White, W. L. 1941. A monograph of the genus Rutstro-

emia (Discomycetes). Lloydia 4: 153-240.

- Zhao, Y.-J. and Hosoya, T. 2014. Enumeration of remarkable Japanese discomycetes (8): Notes on two *Hymen*oscyphus species new to Japan. Bulletin of the National Museum of Nature and Science, Series B 40: 125–131.
- Zhuang, W. Y. 1996. The genera *Lambertella* and *Lanzia* (Sclerotiniaceae) in China. Mycosystema 8–9: 15–38.
- Zhuang, W. Y. 1999. Fungal flora of tropical Guangxi, China: Discomycetes of tropical China. IV. More fungi from Guangxi. Mycotaxon 72: 325–338.
- Zhuang, W. Y. and Liu, C. Y. 2007. Taxonomic reassessment of two taxa of helotialean fungi. Mycotaxon 99: 123–131.