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## Identity of the plasmodial slime mold *Phytoceratiomyxa osmundae* and the lectotypification of *Taphrina osmundae*, the basionym of *Mixia osmundae*

Received: September 21, 2007 / Accepted: January 16, 2008

**Abstract** *Phytoceratiomyxa osmundae*, parasitic on fronds of *Osmunda japonica* var. *sublancea* in Taiwan, was described as a new genus and species in the Myxomycetes by Sawada in 1929. Our investigations on the type specimen, and related descriptions and illustrations of *P. osmundae*, revealed that this microorganism, originally identified as a myxomycete, was phenotypically identical with *Mixia osmundae*, which was transferred from the Ascomycota to the Basidiomycota based on the integrated analysis of molecules and morphology by Nishida et al. in 1995. In addition, a lectotype for *Taphrina osmundae*, the basionym of *Mixia osmundae*, is also designated. A nomenclatural proposal regarding the generic names *Phytoceratiomyxa* and *Mixia* related to this study will be published elsewhere.

**Key words** Lectotypification · *Mixia* · Phylogeny · *Phytoceratiomyxa* · *Taphrina*

### Introduction

Recent advances in molecular research and the study of subcellular morphology have produced several dramatic changes in the taxonomic positions of fungi and fungus-like organisms. Representative examples are the Amoebidiales and the Eccrinales (reclassified from the Trichomycetes to the Mesomycetozoea: Benny and O'Donnell 2000; Benny et al. 2001; Tanabe et al. 2005), *Pneumocystis* (moved from the Protozoa to the Ascomycota: Edman et al. 1988; Sugiyama et al. 2007), and *Mixia* (transferred from the Ascomycota to the Basidiomycota: Nishida et al. 1995).

In 1929, Sawada described, as a myxomycete, a new genus and species *Phytoceratiomyxa osmundae* Sawada, parasitic on fronds of the fern *Osmunda japonica* Thunb. var. *sublancea* (H. Christ) Nakai, based on only a single specimen (Fig. 1) that was collected at about 2600 m altitude in Meishang, Taichung Pref., Taiwan, on August 4, 1928. Subsequently, Sawada (1931) redescribed this genus and species with several illustrations based on the same specimen. To date, this myxomycete genus has not been examined since Sawada's proposal and descriptions (1929, 1931).

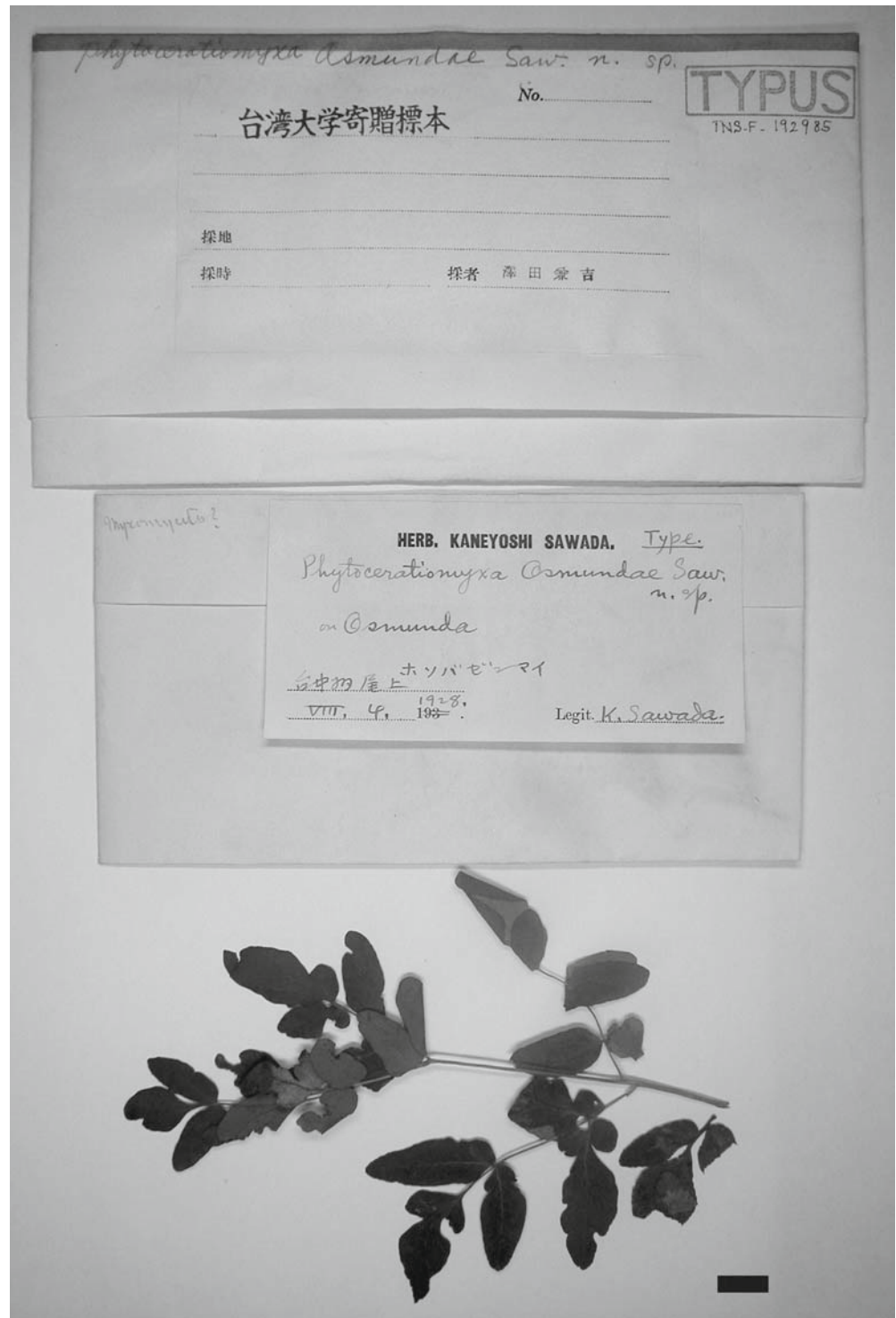
Last year we encountered a possible type specimen of *Phytoceratiomyxa osmundae* in the mycological herbarium of the National Science Museum (now National Museum of Native and Science), Tsukuba (TNS-F). The designation "Type" was written on the original label. This specimen was presented to TNS from the Herbarium of National Taiwan University as one of Sawada's collections in 1955 (for details, see Sawada 1959). Greuter et al. (1993) listed *Phytoceratiomyxa* as a current generic name in the Myxomycetes, whereas Kirk et al. (2001) treated it as "nom. dub., ? Myxomycetes." We carefully examined this type specimen and compared it with data on *Mixia osmundae* (Nishida) C.L. Kramer (1959 [1958]) Kramer [and its basionym *Taphrina osmundae* Nishida (1911)]. *Mixia* is the type genus for the Mixiaceae Kramer (1987). Additional information on *M. osmundae*, especially genotypic (molecular) and phenotypic (mainly morphological and ultrastructural) characters reported by Nishida et al. (1995), were utilized. In the course of the previous report (Nishida et al. 1995) and this study, one of the authors (J.S.) has investigated the whereabouts of the two voucher specimens of *Taphrina osmundae* cited by Nishida (1911) and could not locate original material in several Japanese herbaria. As a result, it had been concluded that his two specimens were missing, but recently we located one of the original collections presumably cited in Nishida's description (1911) at the New York Botanical Garden Herbarium (NY), as is explained below.

The aim of this article is to describe in detail the type of *Phytoceratiomyxa osmundae* and to lectotypify *Taphrina osmundae*, the basionym of *Mixia osmundae*.

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**Fig. 1.** Type specimen of *Phytocerasomyxa osmundae* Sawada (1929), with the original label (middle), the cover of Mycological Herbarium of National University of Taiwan (top), and fronds of *Osmunda japonica* var. *sublancea* (bottom)



## Materials and methods

Macroscopic and microscopic observations were made based on the dried specimens. Parts of the infected spots on fronds of *Osmunda japonica* var. *sublancea* were mounted

in Shear's mounting fluid (Bills and Foster 2004) and examined with an Olympus DX 51 light microscope fitted with Olympus UPlanF1 100 $\times$ /1.30 oil immersion and 40 $\times$ /0.75 objectives (Olympus, Tokyo, Japan) and photographed with a Nikon Coolpix 5000 digital camera (Nikon, Tokyo, Japan). See specimens examined for more details.

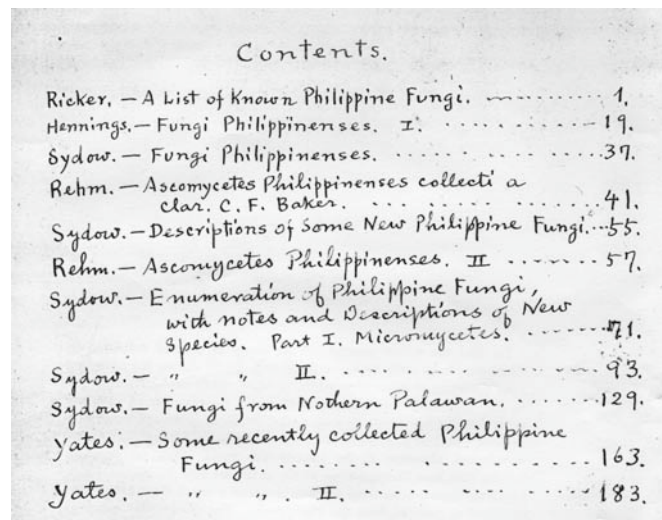
**Table 1.** Dimensions of sporogenous cells and spores of *Phytoceratiomyxa osmundae* and *Mixia osmundae*

Reference	<i>P. osmundae</i> Sawada (1929/1931)	<i>M. osmundae</i>	Nishida (1911)	Mix (1947)	Kramer (1959 ["1958"])	Ito (1964)	Nishida et al. (1995)
Sporogenous cell (µm)	50–70 × 20–26		32–44 × 17–25	26–43 × 17–23	40–80 × 13–23	26–63 × 17–27	24–60 × 9–25.5
Spore form	Ellipsoidal – ovoid		No mention	No mention	Ovate – obovate	No mention	Obovoid
Spore size (µm)	4–6.5 × 2–4		No mention	3–4 × 2–3.5	2–5 × 1.5–4	3–4 × 2–3.5 (3.6 × 3 on average)	3–4 × 1.5–2.5

Illustrations of sporogenous cell

a, reprinted from Sawada (1931; pl. 1, figs. 1–4); b, reprinted from Nishida (1911; pl. 15, figs. 5, 6); c, reprinted from Mix (1947; fig. 1B,G,H) with kind permission from *Mycologia* (©The Mycological Society of America); d, reprinted from Kramer (1959 ["1958"]; figs. 4, 8, 14, 15) with kind permission from *Mycologia* (©The Mycological Society of America); e, LM (left) was taken by K. Ando, reprinted from Nishida et al. (1995; fig. 3c) with kind permission from NRC Research Press. Scanning electron micrograph (SEM) (middle) and transmission electron micrograph (TEM) (right) were taken by K. Ando and A. Hirata, respectively, in the course of the *Mixia* study by Nishida et al. (1995). For the scale of the respective illustrations, see the previously published descriptions

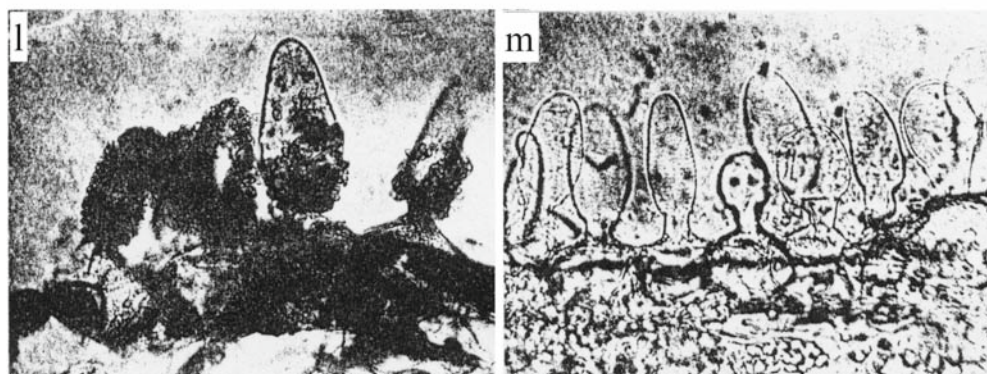
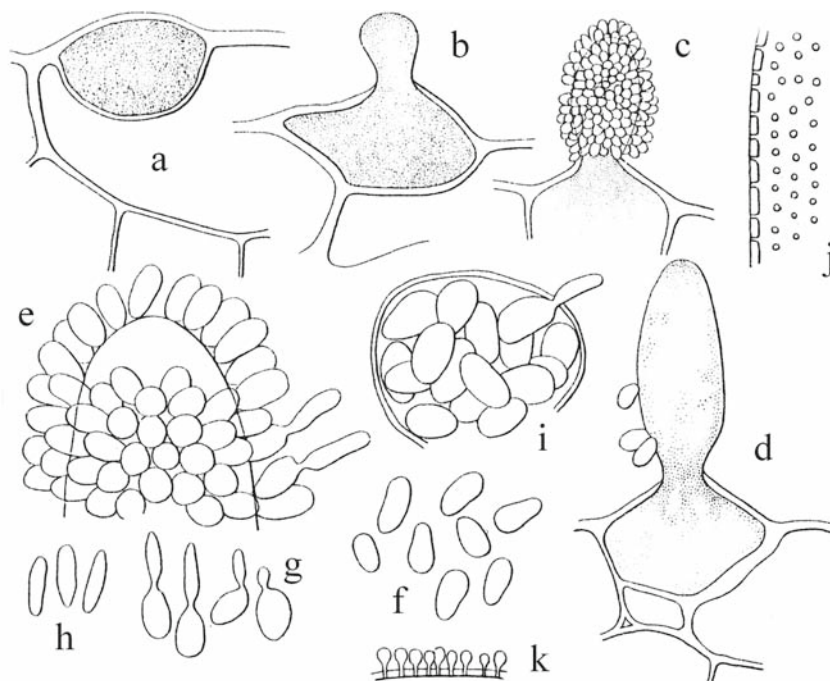
**Fig. 2.** Sawada's authentic calligraphy written on a compilation of reprints relating to the Philippine fungi

## Results and discussion

### The identity of *Phytoceratiomyxa osmundae* Sawada

As mentioned in the Introduction, Sawada's proposal for a myxomycete *Phytoceratiomyxa osmundae*, parasitic on fronds of *Osmunda japonica* var. *sublancea* (the fern family Osmundaceae) in Taiwan, was based on only a single specimen (now in TNS-F, as no. 192985; cf. Fig. 1). The designation "Type" was written on its original label. Sawada's calligraphy on the original label of the specimen (see Fig. 1) was identical with his authentic writing on a compilation of reprints relating to the Philippine fungi (Fig. 2). Although Sawada's protologue lacked a type designation and both a Latin diagnosis and description, which are today required for new names by the International Code of Botanical Nomenclature (ICBN) (McNeill et al. 2006: Arts. 9, 36), both the generic name and the species binomial are valid and legitimate. Latin diagnoses or descriptions were not required before 1935 (ICBN Art. 36.1), and a type designation was not required until 1958 (ICBN Art. 37.1). Additionally, a single collection was cited by Sawada, as already stated, and the type designation was made on his specimen label. Therefore, we conclude that the single specimen is the holotype (ICBN Art. 9.1, Note 1). Our investigations on the type specimen, and related descriptions and illustrations of *P. osmundae* (Sawada 1929, 1931; Fig. 3), revealed that this microorganism originally identified as a myxomycete was phenotypically identical with those of *Mixia osmundae*. The latter genus was transferred from the Ascomycota to the Basidiomycota based on the integrated analysis of DNA sequences and morphology by Nishida et al. (1995). Both diagnostic characters such as the disease symptoms on the host fern, *Osmunda*, and the shape and size of sporangia and spores were closely matched to the previously published descriptions (Nishida 1911; Sawada 1929, 1931; Mix 1947, 1949; Ito 1964; Nishida et al. 1995;

**Fig. 3.** *Phytoceratiomyxa osmundae* illustrated by Sawada (1931). **a** Immature fructification (fruiting body). **b** Early stage of fructification formation. **c** Formation of endogenous spores. **d** Fructification after endogenous spore abscission. **e** Formation of endogenous spores. **f** Endogenous spores. **g** Microspore germination from endogenous spores. **h** Microspores. **i** Formation of endogenous spores and microspores. **j** Sporogenous cells after endogenous spore abscission. **k** Early stage of endogenous spore formation. **l** Formation of endogenous spores (photograph). **m** Fructification after spore abscission (photograph). [Reprinted from drawings and photographs from Sawada (1931), from Reports on Formosan Fungi, Part 5, Rep Central Res Inst Formosan Gov, No. 51, pp. 11–12, pl. 1 (figs. 1–11), pl. 5 (Photos I–II)]



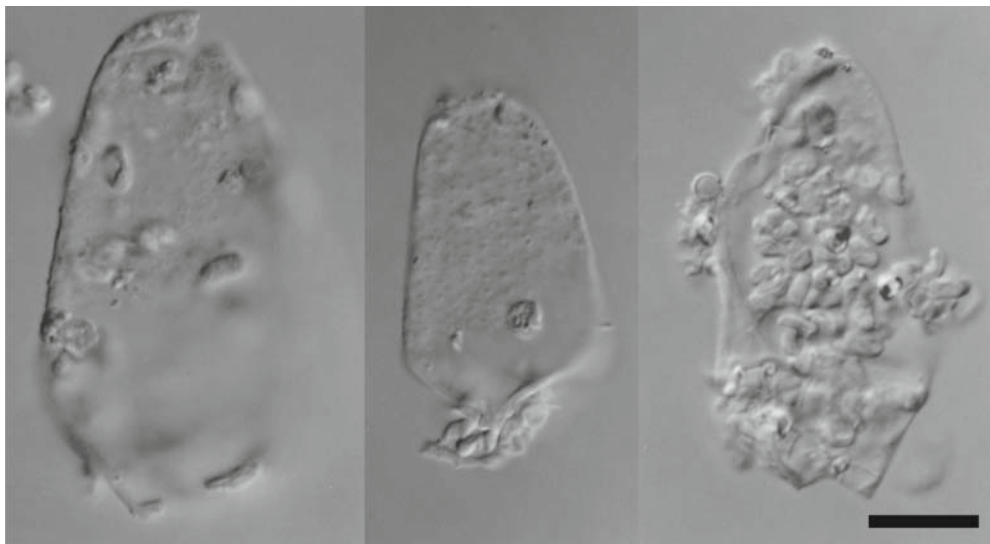
cf. Table 1). In addition to these, our investigations of slide preparations from the type specimen of *P. osmundae* (Fig. 4) proved that the shape and size of sporangia and spores agreed well with those of *M. osmundae* in Nishida et al. (1995).

In conclusion, the genus *Phytoceratiomyxa* Sawada (1929) is a member of the Mixiomycetes in the subphylum Pucciniomycotina (Bauer et al. 2006; Aime et al. 2007; Hibbett et al. 2007), and is not a member of the Myxomycetes. *Phytoceratiomyxa osmundae* Sawada (1929) is congeneric and conspecific with *Mixia osmundae* (Nishida) C.L. Kramer (1959) and its basionym, *Taphrina osmundae* (Nishida 1911). In the light of the current ICBN Rule IV and Art. 11 (McNeill et al. 2006), *Phytoceratiomyxa* has priority as the correct generic name. Therefore, we propose to conserve the name *Mixia* C.L. Kramer (1959) against the name *Phytoceratiomyxa* Sawada (1929) as allowed by ICBN Art. 14. The nomenclatural proposal will be officially published in *Taxon*, as is required.

#### Lectotypification of *Taphrina osmundae* Nishida

The name *Taphrina osmundae*, for a fungus parasitic on fronds of the fern *Osmunda regalis* L. var. *japonica* Willd., was proposed by Nishida (1911) based on two collections: these were from Prov. Higo (now Kumamoto Pref.) collected by T. Nishida and from Prov. Echigo (now Niigata Pref.) collected by K. Yoshino. Nishida's description for *T. osmundae* had neither a Latin diagnosis nor a Latin description and it was not typified, but, similar to the name *Phytoceratiomyxa osmundae*, as already mentioned, these were not required in 1911. Since June 1994, one of the authors (J.S.), in relation to the *Mixia* paper (Nishida et al. 1995), searched for the whereabouts of the two original collections but could not find Nishida's specimens in the major herbaria such as the mycological herbaria of the Hokkaido University Museum in Sapporo and the National Science Museum in Tsukuba. Initially, we concluded that both specimens are missing from Japanese mycological herbaria. Correspond-

**Fig. 4.** Sporogenous cells and spores from the type specimen (TNS-F-192985) of *Phytocercariomyxa osmundae*. Bar 10  $\mu\text{m}$

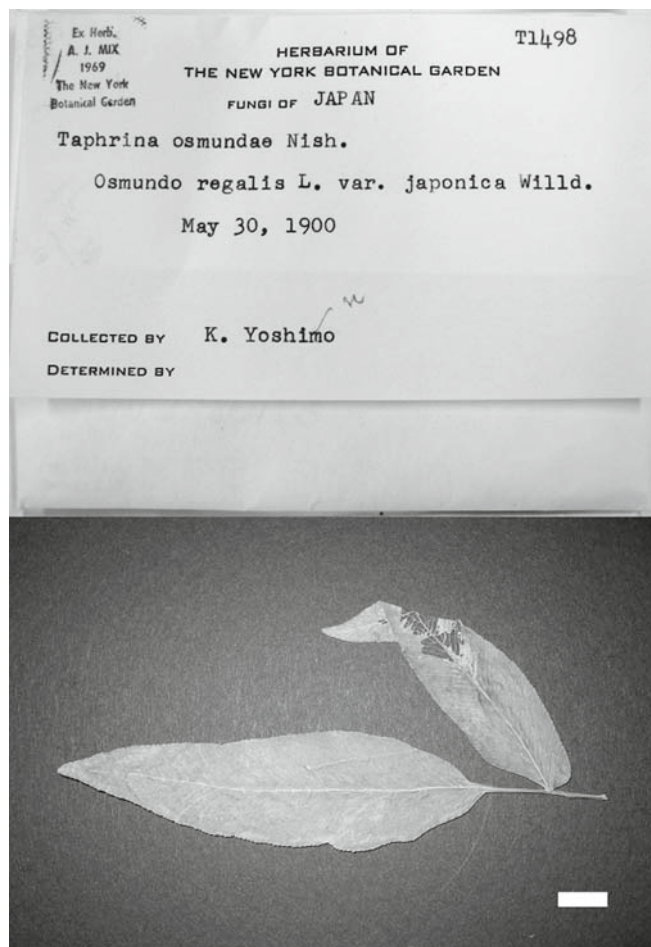


dence with Dr. C. Kramer (personal communication, 2006) revealed that mycological collections originally deposited in the Kansas State University herbarium (KSC) had been transferred to the New York Botanical Garden (NY) and that the collections may have included materials he cited earlier (Kramer 1959). Among the collections cited by Kramer (1959) was a May 30, 1900, collection by K. Yoshino. Materials transferred to NY from KSC were borrowed, and we discovered that one of the original materials of *Taphrina osmundae*, i.e., a collection from “Prov. Echigo (K. Yoshino)” (sic), presumably part of the collection cited in Nishida’s description (1911), is being maintained in the New York Botanical Garden Herbarium (NY). The specimen in question (on *Osmunda regalis* L. var. *japonica* Willd., May 30, 1900, collected by K. Yoshino; T1498 in NY; Ex Herb. A.J. Mix 1969; see Fig. 5) agrees well with data from Nishida’s description (l. c.) and dates. There is no reason to believe the collection is not original material. Original materials, as defined by ICBN Art. 9, Note 2, include illustrations and specimens, and therefore a lectotype must be chosen from original materials if such exist (ICBN Art. 9.2 and 9.10), which would include his illustrations and this specimen. Therefore, we hereby designate the specimen (T1498 in NY) to serve as the lectotype of the name *Taphrina osmundae*.

*Mixia osmundae* (Nishida) C.L. Kramer, *Mycologia* 50: 924. 1959 [“1958”].

Basionym: *Taphrina osmundae* Nishida, *Miyabe Festschrift* p. 167, 206. 1911.

Specimens examined: Japan: (1) locality unknown, on *Osmunda regalis* var. *japonica*, 30 May 1900, leg. K. Yoshino, T1498 in NY – LECTOTYPE of *Taphrina osmundae* designated here (see also Fig. 5); (2) locality unknown, on the



**Fig. 5.** Label for the lectotype specimen (T1498 in NY) designated here for *Taphrina osmundae* Nishida (1911) (upper) and part of an infected frond of *Osmunda regalis* var. *japonica* (sic) in that collection (lower)



**Fig. 6.** Symptoms of *Osmunda japonica* fronds infected with *Mixia osmundae*; part of a voucher specimen IAM-F 0148. Pencil indicates 9-cm. [Photo taken by J. Sugiyama on May 31, 1993; reprinted from Nishida et al. (1995) with kind permission from NRC Research Press, Canada]

same host plant, July 1912, leg. K. Yoshino, T1495 in NY; (3) Shizuoka-ken, Haibara-gun, Nakakawane-mura, on fronds of *Osmunda japonica*, 12 May 1963, leg. A. Ezuka, NIAES 10529; (4) Shizuoka-ken, Haibara-gun, Nakakawane-cho; Mitsuma, alt. about 200 m, *ibid.*, 9 May 1991, leg. A. Ezuka, NIAES 10557; (5) Shizuoka-ken, Haibara-gun, Nakakawane-cho, Sezawa, alt. about 220 m, on fronds of *Osmunda japonica* (Osmundaceae), 31 May 1993, leg. J. Sugiyama, H. Nishida & Y. Ando, IAM-F 0148 (TNS-F-13368 – an authentic voucher specimen of *Taphrina osmundae* cited by Nishida et al. in 1995; see Fig. 6). A representative living isolate derived from the authentic voucher collection (IAM-F 0148) is maintained in Japan Collection of Microorganisms, RIKEN BioResource Center in Wako as JCM 22182 (= IAM 14324) and in the Centraalbureau voor Schimmelcultures in Utrecht as CBS 9802 (= IAM 14324), respectively; that derived from the specimen NIAES 10557 is also maintained in JCM as 22200 (= IAM 14511, = IFO 32408) and in CBS as 6302 (= IAM 14511, = NBRC 32408, = IFO 32408, = MAFF 306200).

**Acknowledgments** We are grateful to Dr. Tsuyoshi Hosoya, Department of Botany, National Museum of Nature and Science in Tsukuba, for the loan of a specimen of *Phytoceratiomyxa osmundae* Sawada; Dr. Toyozo Sato, Microorganisms Section of the NIAS Genebank, National Institute of Agrobiological Sciences, Japan, in Tsukuba for the loan of NIAES specimens of *Taphrina osmundae*; Prof. Emeritus Hideaki Ohba, The University Museum, The University of Tokyo, and Prof. Nitaro Maekawa, Tottori University, for providing Sawada's references; Dr. Takahito Kobayashi, The Hokkaido University Museum in Sapporo, for searching Nishida's collections; Dr. C. Kramer, retired from Kansas State University, for advice on collections; Drs. B. Thiers and R. Halling and Ms. Ellen Bloch for the loan of specimens from the New York Botanical Garden (NY); and Mr. Tomohiko Kiyuna, TechnoSuruga Laboratory Co., Ltd., in Shizuoka for assistance with light microscopic observations. The Mycological Society of America (*Mycologia*) and NRC Research Press (*Canadian Journal of Botany*) granted us permission to use copyrighted materials. Finally we are pleased to acknowledge the exacting criticism, stimulating discussion (by e-mails), and helpful cooperation of Dr. Scott Redhead (DAOM), without which this study could not have been completed.

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