



Short communication

Gymnosporangium mori comb. nov. (*Pucciniales*) for *Caeoma mori* (\equiv *Aecidium mori*) inferred from phylogenetic evidence

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ABSTRACT

Caeoma mori (\equiv *Aecidium mori*), known as the mulberry rust which is an anamorphic rust fungus forming only aecidioid uredinia, were found on *Morus alba* in Ibaraki and Saitama Prefectures, Japan. Molecular phylogenetic analyses using the combined dataset of sequences from 28S and 18S of the nuclear ribosomal RNA gene and Cytochrome-c-oxidase subunit 3 of the mitochondrial DNA revealed that this anamorphic rust fungus was a member of the clade composed of the genus *Gymnosporangium*. Therefore, a new combination, *Gymnosporangium mori* is proposed for this species. Additionally, a new combination, *G. brucense* for *Roestelia brucensis* is proposed by phylogenetic evidence.

Keywords: *Gymnosporangiaceae*, *Moraceae*, nomenclature, phylogeny, taxonomy

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Mulberry (*Morus*, *Moraceae*) is shrubs or trees consisting of about 10 species, and mainly distributed in temperate regions of Asia and North America (Nepal & Ferguson, 2012). The leaves of these plants are used as foods for silkworm larvae. Some species are also cultivated for productions of edible fruits. Specimens of a rust fungus occurred on shoots and leaves of *Morus alba* L. (common mulberry or silkworm mulberry) were collected in the fields of Ibaraki and Saitama Prefectures, Japan in early summer of 2021 and 2023 (Fig. 1A). Morphological observations were made for the identification of this rust fungus using light microscopy and scanning electron microscopy by the same methods reported by Uzuhashi et al. (2022). Spermogonia were not found on specimens. Sori surrounded with fragile and short peridia (Fig. 1B, D, E) were amphigenous, densely formed, rounded to elliptical and cupulate. Peridial cells were loosely conjunct and their inner walls were verrucose. Spores (Fig. 1C, F) were catenulate, angularly globose to ellipsoid and 11.5–20 × 8–15.5 μm (avg. 16 × 12 μm; n = 50) in size. Their walls were hyaline, verrucose and 1–1.5 μm thick.

Five species of rust fungi, *Phakopsora fici-erectae* S. Ito & Y. Otani ex S. Ito & Muray., *Cerotelium fici* (Castagne) Arthur, *Aecidium mori* (Barclay) Barclay (\equiv *Caeoma mori* Barclay), *Uredo moricola* Henn. and *U. morifolia* Sawada have been reported on species of

Morus in Japan (Hiratsuka et al., 1992; Ito, 1950). Among them sorus structures of *P. fici-erectae*, *C. fici*, *U. moricola* and *U. morifolia* are different from the rust fungus collected on *M. alba* in Ibaraki and Saitama Prefectures. Namely, *P. fici-erectae* and *C. fici* has peripheral paraphyses in sori and two *Uredo* species have no peridium in their sori. Sorus structures of this rust fungus having fragile peridia is identical with descriptions of *A. mori* (Hiratsuka et al., 1992; Ito, 1950; Mordue, 1991). The morphology and size of spores are also similar to those of its descriptions. Therefore, the present rust fungus on *M. alba* is identified as *A. mori*. Specimens used in this observation were deposited in the Mycological Herbarium of the Department of Botany, National Museum of Nature and Science, Tsukuba, Japan (TNS).

Caeoma mori was originally described as a rust fungus on mulberry in 1890 by Barclay. Although this species was recorded as *Uredo mori* (Barclay) Sacc. in 1891 by Saccardo, Barclay (1891) treated this species as same species as *A. mori*, described by himself, because of the presence of peridia in the sori. *Aecidium mori* has been widely recorded on many species of *Morus* and *Broussonetia* (*Moraceae*) in Asia (Hiratsuka et al., 1992; Ito, 1950; Mordue, 1991; Tai, 1979). Sori and spores of this species are morphologically as same as *Aecidium*-type by Cummins and Hiratsuka (1983) because of catenulate spores and presence of peridia. Therefore, spores are morphologically categorized as aeciospores which are usually produced after spermogonial formation and produce uredinia after their infections to plants. However, no spermogonium is formed and same type of sori (*Aecidium*-type) is produced repeat-

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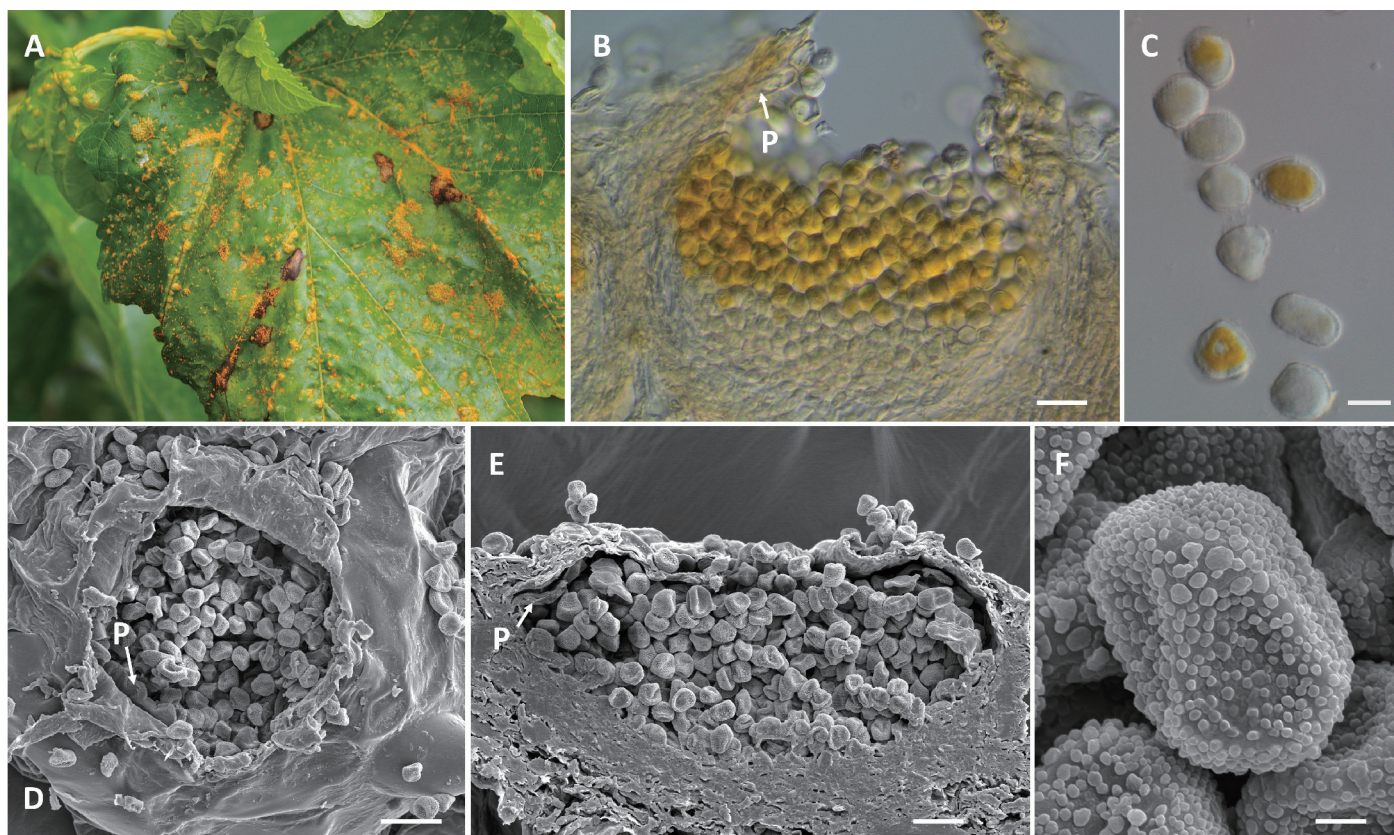


Fig. 1 – *Gymnosporangium mori* on *Morus alba* (TNS-F-99251). A: Sori on upper leaf surface. B: Vertical section of sorus. Peridium at margin of sori (P). C: Spores with hyaline walls. D: Sorus on leaf surface observed by scanning electron microscopy (SEM). Peridium at margin of sori (P). E: Vertical section of sorus observed by SEM. Peridium at margin of sori (P). F: Spores with verrucae on the surface observed by SEM. Bars: B, E 20 μ m; C 10 μ m; D 30 μ m; F 2.5 μ m.

edly after infections with these spores (Kaneko, 1973). This type of sori is called as aecidioid uredinia or uredinial aecia (Kaneko, 1973; Kasuya et al., 2020; Sato & Sato, 1981). Therefore, spores of this species are functionally as same as urediniospores of rust fungi.

Sathe (1969) described a new anamorphic genus, *Peridiopsis* Kamat & Sathe for rust fungi producing urediniospores in *Aecidium*-type sori, and *A. mori* was transferred to this genus as *P. mori* (Barclay) K.V. Prasad, B.R.D. Yadav & Sullia by Prasad et al. (1993). However, *A. mori* has been commonly used as species name of mulberry rust in the world. Because only anamorphic stage of this rust fungus has been known, its taxonomic position among rust fungi has been unknown for long time. Recently, Aime and McTaggart (2021) suggested that this species was phylogenetically close to *Gymnotelium* Syd. described for group of the genus *Gymnosporangium* R. Hedw. ex DC. having *Aecidium*-type of aecia, but their analyses was insufficient.

In the present study, to clarify the taxonomic position of *A. mori*, the phylogenetic analyses reported by Aime and McTaggart (2021) was applied. We obtained sequence data of the large subunit (28S) and the small subunit (18S) of the nuclear ribosomal RNA gene and Cytochrome-c-oxidase subunit 3 (CO3) of the mitochondrial DNA from specimens of *A. mori* which were collected from Ibaraki and Saitama Prefectures and used for morphological observations. Procedures of DNA extraction, PCR and sequencing followed the method reported by Virtudazo et al. (2001), Kasuya et al. (2012) and Aime and McTaggart (2021). 28S ribosomal RNA was amplified with Rust21NV (Aime, 2006)/LR6 or LR7 (Vilgalys & Hester, 1990) and, for weak products, nested with Rust28SF (Aime et al., 2018)/LR5 or LR6 (Vilgalys & Hester, 1990). 18S ribosomal RNA was amplified with NS1 (White et al., 1990)/Rust 18S-R (Aime, 2006) and

nested with RustNS2-F (Aime et al., 2018)/NS6 (White et al., 1990). The mitochondrial CO3 was amplified with CO3_F1/CO3_R1 (Vialle et al., 2009). DNA extraction, PCR and sequencing were mainly performed by TechnoSuruga Laboratory Co. Ltd. (Shizuoka, Japan).

A total of eight 28S, seven 18S and six CO3 sequences from eight specimens of *A. mori* were newly generated and used for the phylogenetic analyses. These sequences were deposited to the International Nucleotide Sequence Databases (INSD; Table 1). Phylogenetic analyses were conducted for the combined dataset of 28S, 18S and CO3 sequences under maximum likelihood (ML) and Bayesian inference (BI). The combined dataset of the three loci (Table 1) included 57 taxa with *Puccinia boroniae* Henn. used as the outgroup according to the result of phylogenetic analyses of *Urediniineae* Engl. by Aime and McTaggart (2021) since *Pucciniaceae* Chevall. has phylogenetically close relationship to *Gymnosporangiaceae* Chevall. A total of 48 28S, 40 18S and 10 CO3 sequences of ingroups obtained from the NCBI GenBank databases (<https://www.ncbi.nlm.nih.gov/>) were chosen from *Gymnosporangiaceae* and *Sphaerophragmiaceae* Cummins & Y. Hirats. based on the analyses by Zhao et al. (2020) and Aime and McTaggart (2021). The combined dataset was aligned using Muscle v.3.6 (Edgar, 2004a, 2004b), followed by manual alignment in the data editor of BioEdit ver. 7.0.1 (Hall, 1999). Hypervariable, indel-rich and ambiguously aligned regions were removed from the analyses, and gaps were scored as missing data. The final alignments were deposited in TreeBASE (<https://treebase.org>) under the accession number S30671.

ML analysis was performed using IQ-TREE v.1.6.12 (Nguyen et al., 2015). According to determine the lowest Bayesian information

Table 1. Specimen data used for the present phylogenetic analyses.

Species ^a	Voucher specimen numbers	Locality	INSD accession numbers ^b		
			28S	18S	CO3
<i>Austropuccinia psidii</i>	BRIP 57793	Australia, Queensland, Brisbane	KF318449	KF318457	KT199419
<i>Dasyscypha guianensis</i>	ZT Myc 3413	French Guiana	JF263479	JF263503	JF263519
<i>D. nitidae</i>	ZT Myc 3409	French Guiana	JF263484	JF263505	JF263521
<i>D. segregaria</i>	PMA MP4941	Panama	JF263488	JF263507	JF263523
<i>Gymnosporangium asiaticum</i>	IBAR 5704	Japan	KJ720161	KJ720161	n/a ^c
<i>G. asiaticum</i>	TNM F0027942	Taiwan, Taichung, Dongshi	KP308393	KP308393	n/a
<i>G. brucense</i>	DAOM 127906	Canada, Ontario, Ottawa	KJ720188	KJ720188	n/a
<i>G. brucense</i>	RSP 74-313	Mexico	KJ720189	n/a	n/a
<i>G. clavariiforme</i>	RSP 05-32	USA, New Mexico	KJ720164	KJ720164	n/a
<i>G. clavariiforme</i>	BRIP 59471	Australia	MW049261	MW049296	MW036499
<i>G. clavipes</i>	BPI 871102	USA	DQ354545	DQ354546	n/a
<i>G. clavipes</i>	NYBG 461394	USA	MN605691	MN604977	n/a
<i>G. clavipes</i>	CUP A-18207	USA, New York	MN605692	MN604978	n/a
<i>G. confusum</i>	DAOM 220748	Canada	KJ720165	KJ720165	n/a
<i>G. cupressi</i>	RSP 99-98	USA, Arizona	KJ720169	KJ720169	n/a
<i>G. ellisii</i>	YPM RN23	USA, North Carolina	KJ720156	KJ720156	n/a
<i>G. exiguum</i>	RSP 04-86	USA, California	KJ720170	KJ720170	n/a
<i>G. globosum</i>	CUP 1553	USA, New York	MN605698	MN604983	n/a
<i>G. globosum</i>	NYBG 237038	USA,	KU342738	MN604982	n/a
<i>G. juniperi-virginianae</i>	RSP 98-137	USA, Oklahoma	KJ720176	KJ720176	n/a
<i>G. juniperi-virginianae</i>	MCA 3585	USA	MG907217	MG917687	MG907268
<i>G. kernianum</i>	RSP 05-37	USA, Texas	KJ720177	KJ720177	n/a
<i>G. libocedri</i>	TDB 1519	USA	AF522168	AY123290	n/a
<i>G. libocedri</i>	PUR N10018	USA	MG907218	MG907206	MG907269
<i>G. libocedri</i>	HMAS 49246	USA, California	MN605717	MN605009	n/a
<i>G. libocedri</i>	HMAS 45643	USA	MN605718	MN605010	n/a
<i>G. libocedri</i>	GL3_3	Canada	OR567878	n/a	n/a
<i>G. libocedri</i>	GL4_6	Canada	OR568568	n/a	n/a
<i>G. libocedri</i>	GL2_1	Canada	OR654105	n/a	n/a
<i>G. mori</i>	PUR N11676	Taiwan, Taipei	MW147025	n/a	MW166323
<i>G. mori</i>	TNS-F-99251 (Epitype)	Japan, Ibaraki, Joso, Tategeta	OR415605	OR415613	OR423360
<i>G. mori</i>	TNS-F-99252	Japan, Ibaraki, Joso, Moto-toyoda	OR415606	n/a	n/a
<i>G. mori</i>	TNS-F-108304	Japan, Ibaraki, Tsukuba	OR415607	OR415614	n/a
<i>G. mori</i>	TNS-F-99265	Japan, Ibaraki, Joso, Tategeta	OR415608	OR415615	OR423361
<i>G. mori</i>	TNS-F-99266	Japan, Ibaraki, Shimotsuma	OR415609	OR415616	OR423362
<i>G. mori</i>	TNS-F-99267	Japan, Saitama, Kitamoto	OR415610	OR415617	OR423363
<i>G. mori</i>	TNS-F-99268	Japan, Saitama, Kawajima, Demaru-nakago	OR415611	OR415618	OR423364
<i>G. mori</i>	TNS-F-99269	Japan, Saitama, Kawajima, Demaru-shimogo	OR415612	OR415619	OR423365
<i>G. multiporum</i>	RSP 05-31	USA, New Mexico	KJ720179	KJ720179	n/a
<i>G. nidus-avis</i>	RSP 05-29	USA, New Mexico	KJ720181	KJ720181	n/a
<i>G. nidus-avis</i>	NYBG 237080	USA	KU342757	MN605019	n/a
<i>G. nidus-avis</i>	NYBG 461234	USA	KU342758	MN605015	n/a
<i>G. niitakayamense</i>	TNM F0027945	Taiwan, Nantou, Ren'ai	KP308396	KP308396	n/a
<i>G. niitakayamense</i>	TNM F0027946	Taiwan, Hualien, Sioulin	KP308397	KP308397	n/a
<i>G. nootkatense</i>	PUR 63656	Canada	KJ720159	KJ720159	n/a
<i>G. raphiolepidis</i>	TNS-F-79706	Japan, Chiba, Choshi	MT419964	n/a	n/a
<i>G. raphiolepidis</i>	TNS-F-70764	Japan, Chiba, Choshi	MT419965	n/a	n/a
<i>G. raphiolepidis</i>	TNS-F-70765	Japan, Ibaraki, Kamisu	MT419966	n/a	n/a
<i>G. sabinae</i>	TUB RB2066	Germany	AY512845	n/a	n/a
<i>G. sabinae</i>	TNM F0030477	Bulgaria, Sofia	KY964764	KY964764	n/a
<i>G. sabinae</i>	CUP 0477	USA	MN605721	MN605022	n/a
<i>G. speciosum</i>	RSP 99-96	USA, Arizona	KJ720160	KJ720160	n/a
<i>G. tsingchenense</i>	HMAS 133735	China	n/a	MN605032	n/a
<i>G. vauqueliniae</i>	RSP 05-87	USA, Arizona	KJ720186	KJ720186	n/a
<i>Puccinia boroniae</i>	BPI 57810	Australia	MW147045	MW147074	MW139655
<i>Puccorchidium polyalthiae</i>	ZT HeRB 251	n/a	JF263493	JF263509	JF263525
<i>Sphaerophragmium acaciae</i>	BRIP 56910	Australia, Western Australia, Kununurra	KJ862350	KJ862429	KJ862462
<i>S. longicorne</i>	PUR N16513	Nigeria, Abor, Enugu	MW147053	MW147077	n/a

^a Sequences newly generated in the present study are shown in bold.

^b The International Nucleotide Sequence Databases (INSD) accession numbers for the large subunit (28S) and the small subunit (18S) of the nuclear ribosomal RNA gene and Cytochrome-c-oxidase subunit 3 (CO3) of the mitochondrial DNA sequences. Identical accession numbers for 28S and 18S indicate a single rDNA sequence containing both regions.

^c "n/a" means unavailable information.

criterion scores (25315.04) by IQ-TREE, the GTR+F+I+G4 model was chosen as the best-fit evolutionary model for the analysis of the combined 28S, 18S and CO3 dataset. For the ML analysis, clade robustness was assessed using the Shimodaira-Hasegawa-like approximate likelihood ratio test (SH-aLRT; Guindon et al., 2010) and ultrafast bootstraps (UFBoot; Hoang et al., 2018) with 10000 replicates, respectively. BI analysis was performed using MrBayes version 3.2.7 (Ronquist et al., 2012) based on the same method of Kasuya and Ono (2018). The GTR+I+G model was selected as the best evolutionary model for the combined dataset by the hierarchical likelihood-ratio test using MrModeltest2 (Nylander, 2004). The support of nodes was tested by posterior probabilities (BPP), obtained from a 50% majority rule consensus after deleting the trees in the burn-in period.

The combined dataset of 283, 18S and CO3 had an aligned in length of 3371 characters including gaps, of which 2090 constant and phylogenetically uninformative, and 1281 phylogenetically informative. The highest log likelihood of the resulting ML tree of the combined dataset of the three loci was -12124.92. By BI, after 850,000 generations of Markov chain Monte Carlo runs, the analysis reached stationarity: the average standard deviation of split frequencies dropped below 0.01 after 405,000 generations. After discarding the burn-in phase, the trees had a likelihood score (harmonic mean) of -12304.44 with the potential scale reduction factor of 1.000 for all parameters, indicating that the analyses were run for a sufficient number of generations. The ML and BI analyses resulted in trees that were almost identical in topology. Hence, only the ML tree topology of the combined 28S, 18S and CO3 dataset is shown in Fig. 2.

By ML and BI analyses, 28S, 18S and CO3 sequences generated from specimens of *A. mori* were placed within a strongly supported clade [SH-aLRT(%) / UFBoot (%) / BPP = 100/100/1.00]. It was included into the major clade comprising *Gymnosporangium*, but clearly distinct from the other species (Fig. 2). This clade was phylogenetically close to *G. libocedri* (Henn.) F. Kern [= *Gymnotelium blasdaleanum* (Dietel & Holw.) Arthur] and *G. ellisii* (Berk.) Berk. [= *Gymnotelium myricatum* (Schwein.) Arthur] (Fig. 2). These two species have *Aecidium*-type of aecia, and species of *Gymnosporangium* producing this type of aecia were taxonomically separated as *Gymnotelium* because aecial structures were different from the other species of *Gymnosporangium* (*Roestelia*-type of Cummins & Hiratsuka, 1983) (Aime & McTaggart, 2021). Although Cummins and Hiratsuka (1983) treated *Gymnotelium* as a synonym of *Gymnosporangium*, Aime and McTaggart (2021) suggested *Gymnotelium* as an independent genus from *Gymnosporangium* based on its morphology, phylogeny and host plants. They also indicated that morphological similarities of *A. mori* (\equiv *P. mori*) with *Gymnotelium*. However, species of *Gymnosporangium* having *Aecidium*-type and *Roestelia*-type of aecia are scattered in our phylogenetic trees and species having each type do not make monophyletic group (Fig. 2). Results of the present analyses are also supported by phylograms of *Gymnosporangium* on *Malus* reported by Zhao et al. (2020). Moreover, results of our phylogenetic analyses (Fig. 2) strongly support the monophyly of *Gymnosporangium* including *G. sabinae* (Dicks.) G. Winter (= *G. fuscum* DC.), the type species of the genus [SH-aLRT(%) / UFBoot (%) / BPP = 87/95/1.00]. Simultaneously the present phylogram shows species hitherto recognized as *Gymnotelium* [*G. nootkatense* (Trel.) Arthur (\equiv *Gymnotelium nootkatense* (Trel.) Syd., the type species of *Gymnotelium*), *G. speciosum* Peck (\equiv *Gymnotelium speciosum* (Peck) Aime & McTaggart), *G. libocedri* and *G. ellisii*] are paraphyletic and do not form independent clade (Fig. 2). Therefore, we consider that *Gymnotelium* is included into *Gymnosporangium*.

From above phylogenetic analyses it is concluded that *A. mori* should be treated as a new member of *Gymnosporangium* although it produces only one stage of life cycle (aecidioid uredinia or uredinoid aecia). It is suspected that this species may be differentiated from aecial stage of *Gymnosporangium* having heteroecious life cycles and has changed function of its aeciospores to urediniospores for its survival (Cummins & Hiratsuka, 1983; Leppik, 1972), similar to *G. raphiolepidis* reported by Kasuya et al. (2020).

Caeoma mori is a legitimate earliest name for *A. mori* under ICN Shenzhen Code (Art. F.8, Turland et al., 2018). However, the application of an anamorphic genus name, *Caeoma* Link or *Aecidium* Pers. to a teleomorphic genus name, *Gymnosporangium* which is morphologically and taxonomically different from *Caeoma* or *Aecidium*, causes great confusion in the taxonomy of rust fungi because these anamorphic genera are connected with many teleomorphic genera (Ji et al., 2017, 2019; Kakishima et al., 2018; Kasuya et al., 2020; Ono, 2016). Therefore, we propose a new combination in *Gymnosporangium* for *C. mori* (\equiv *A. mori*). Uredinial anamorphic name, *P. mori* is also treated as its obligate synonym. In addition, the present phylogenetic analyses strongly suggest that *Roestelia bruceensis* Parmelee as a member of the genus *Gymnosporangium* (Fig. 2). Two sequences of *R. bruceensis* (KJ720189 is deposited as *Uredo apachea* R.S. Peterson in the NCBI GenBank databases but it was reassessed as *R. bruceensis* by Zhao et al., 2020) were placed within a strongly supported clade [SH-aLRT(%) / UFBoot (%) / BPP = 98/100/1.00] in the major clade comprising *Gymnosporangium* species. Although *Gymnosporangium* has been conserved against the older name *Roestelia* Rebert. (Aime & McTaggart, 2021), *R. bruceensis* has not been transferred to *Gymnosporangium*. Therefore, we here propose a new combination of *R. bruceensis* in the genus *Gymnosporangium*.

Taxonomy

Gymnosporangium mori (Barclay) T. Kasuya, K. Hosaka, Jing X. Ji & Kakish., **comb. nov.**

MycoBank no.: MB 849771.

Basionym: *Caeoma mori* Barclay, J. Asiat. Soc. Bengal, Pt. 2, Nat. Sci. 59: 97, 1890.

Obligate synonyms: *Aecidium mori* (Barclay) Barclay, J. Asiat. Soc. Bengal, Pt. 2, Nat. Sci. 60: 225, 1891; *Uredo mori* (Barclay) Sacc., Syll. Fung. (Abellini) 9: 334, 1891; *Peridiopsora mori* (Barclay) K.V. Prasad, B.R.D. Yadav & Sullia, Curr. Sci. 65: 426, 1993.

Typus: Pl. IV, Fig. 6 in Barclay (1890) (**Holotype**, cited from Index Fungorum); JAPAN, Ibaraki Prefecture, Joso, Tategata (approx. 36°7'56.17 N, 139°59'51.59 E, alt. 15.8 m), on *Morus alba* L., 16 Jun 2021, T. Kasuya, TNS-F-99251 (**Epitype**, designated here). Because a figure by Barclay (1890) is designated as the type of this species (Index Fungorum) we have selected an epitype specimen to specify the morphological characteristics and phylogenetic position of this species.

DNA sequence ex-Epitype: INSD accession no. OR415605 for 28S, OR415613 for 18S and OR423360 for CO3.

Additional specimens examined: JAPAN, Ibaraki Prefecture: Joso, Tategata, on *Morus alba*, 30 May 2023, M. Kakishima, TNS-F-99265; Joso, Moto-toyoda, on *M. alba*, 16 Jun 2021, T. Kasuya, TNS-F-99252; Tsukuba, Amakubo, Tsukuba Botanical Garden, on *M. alba*, 24 May 2023, M. Kakishima, TNS-F-108304; Shimotsuma, Shimoda, on *M. alba*, 19 Jun 2023, T. Kasuya, TNS-F-99266. JAPAN, Saitama Prefecture: Kitamoto, Ishitoshuku, on *M. alba*, 1 Jun 2023, T. Kasuya, TNS-F-99267; Kawajima, Demaru-nakago, on *M. alba*, 1 Jun 2023, T. Kasuya, TNS-F-99268; Kawajima, Demaru-shimogo, on *M. alba*, 1 Jun 2023, TNS-F-99269.

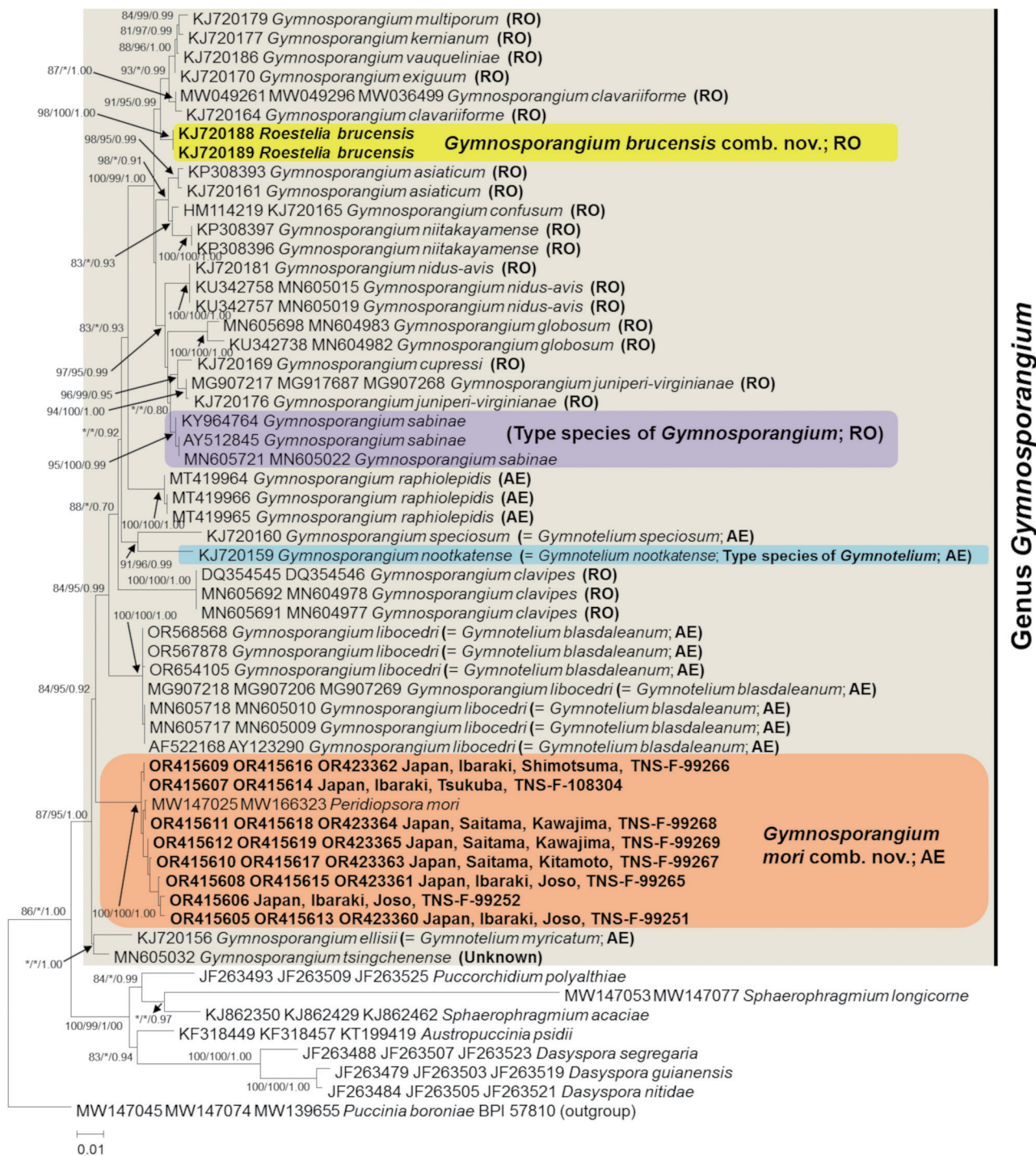


Fig. 2 – A phylogenetic tree generated from maximum likelihood (ML) analysis based on the combined dataset of sequences from the large subunit and the small subunit of the nuclear ribosomal RNA gene and Cytochrome-c-oxidase subunit 3 of the mitochondrial DNA of selected rust species. Taxon indicated by bold are newly generated sequences from the present study. “AE” and “RO” indicate that taxon produce *Aecidium*-type or *Roestelia*-type of aecia, respectively. “Unknown” means species which is not known the aecial stage. Numbers along branches are nodal supports by the Shimodaira-Hasegawa-like approximate likelihood ratio test (SH-aLRT; left), ultrafast bootstraps (UFBoot; middle) and Bayesian posterior probabilities (BPP; right). SH-aLRT, UFBoot and BPP less than 80%, 95% or 0.70 are indicated by an asterisk (*), respectively. Scale bar indicates the number of substitutions per site.

Distribution and host plants hitherto recorded: Asia (Afghanistan, Burma, China, India, Indonesia, Japan, Korea, Pakistan, Philippines, Taiwan and Thailand). On *Moraceae*: *Broussonetia kazinoki* Sieb., *B. papyrifera* (L.) L’Hér. ex Vent., *Morus acidosa* Griff., *M. alba* L. [= *M. atropurpurea* Roxb., *M. chinensis* Lodd. ex Loudon, *M. intermedia* Perr., *M. latifolia* Poir., *M. multicaulis* (Perr.) Perr., *M. tatarica* L.], *M. australis* Poir. (= *M. bombycis* Koidz.), *M. cathayana*

Hemsl., *M. indica* L., *M. kagayamae* Koidz., *M. mongolica* (Bureau) C.K. Schneid., *M. serrata* Roxb. (Ahmad et al., 1997; Boedijn, 1959; Cho & Shin, 2004; Dizon & Kakishima, 1995; Giatgong, 1980; Hiratsuka & Chen, 1991; Hiratsuka et al., 1992; Iqbal & Khalid, 1996; Ito, 1950; Kobayashi, 2007; Mordue, 1991; Prasad et al., 1993; Spaulding, 1961; Tai, 1979; Teng, 1996).

Gymnosporangium brucense (Parmelee) T. Kasuya, K. Hosaka, Jing X. Ji & Kakish., **comb. nov.**

Mycobank no.: MB 849772.

Basionym: *Roestelia brucensis* Parmelee, Can. J. Bot. 43: 259, 1965.

Distribution and host plants hitherto recorded: North America (Canada, Mexico and USA). On *Juniperaceae*: *Juniperus horizontalis* Moench. (Parmelee, 1965; Parmelee & Corlett, 1973).

Disclosure

The authors declare no conflict of interest. All the experiments undertaken in this study comply with the current laws of Japan.

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References

- Ahmad, S., Iqbal, S. H., & Khalid, A. N. (1997). *Fungi of Pakistan*. Mycological Society of Pakistan.
- Aime, M. C. (2006). Toward resolving family-level relationships in rust fungi (Uredinales). *Mycoscience*, 47, 112–122. <https://doi.org/10.1007/S10267-006-0281-0>
- Aime, M. C., & McTaggart, A. R. (2021). A higher-rank classification for rust fungi, with notes on genera. *Fungal Systematics and Evolution*, 7, 21–47. <https://doi.org/10.3114/fuse.2021.07.02>
- Aime, M. C., Bell, C. D., & Wilson, A. W. (2018). Deconstructing the evolutionary complexity between rust fungi (*Pucciniales*) and their plant hosts. *Studies in Mycology*, 89, 143–152. <https://doi.org/10.1016/j.simyco.2018.02.002>
- Barclay, A. (1890). A descriptive list of the Uredineae occurring in the neighbourhood of Simla (Western Himalya). Pt. III. *Journal of the Asiatic Society of Bengal*, 59, 75–112. <https://www.biodiversitylibrary.org/page/42643553>
- Barclay, A. (1891). Additional Uredineae from the neighbourhood of Simla. *Journal of the Asiatic Society of Bengal*, 60, 211–230. <https://www.biodiversitylibrary.org/page/32617848>
- Boedijn, K. B. (1959). The Uredinales of Indonesia. *Nova Hedwigia*, 1, 463–496.
- Cho, W. D., & Shin, H. D. (2004). *List of plant diseases in Korea. Fourth edition*. Korean Society of Plant Pathology.
- Cummins, G. B., & Hiratsuka, Y. (1983). *Illustrated genera of rust fungi. 2nd edition*. American Phytopathological Press.
- Dizon, T. O., & Kakishima, M. (1995). Symptomatology of mulberry rust and the morphology of its pathogen, *Aecidium mori* Barclay. *Philippine Phytopathology*, 31, 27–31.
- Edgar, R. C. (2004a). Muscle: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32, 1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Edgar, R. C. (2004b). Muscle: A multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics*, 5, 113. <https://doi.org/10.1186/1471-2105-5-113>
- Giatgong, P. (1980). *Host Index of Plant Diseases in Thailand. Second Edition*. Mycology Branch, Plant Pathology and Microbiology Division, Department of Agriculture and Cooperatives.
- Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML3.0. *Systematic Biology*, 59, 307–321. <https://doi.org/10.1093/sysbio/syq010>
- Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.
- Hiratsuka, N., & Chen, Z.C. (1991). A list of Uredinales collected from Taiwan. *Transactions of the Mycological Society of Japan*, 32, 3–22.
- Hiratsuka, N., Sato, S., Katsuya, K., Kakishima, M., Hiratsuka, Y., Kaneko, S., Ono, Y., Sato, T., Harada, Y., Hiratsuka, T., & Nakayama, K. (1992). *The rust flora of Japan*. Tsukuba-shuppankai.
- Hoang, D. T., Chernomor, O., von Haeseler, A., Minh, B. Q., & Vinh, L. S. (2018). UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, 35, 518–522. <https://doi.org/10.1093/molbev/msx281>
- Ito, S. (1950). *Mycological flora of Japan, vol. 2, no. 3* [in Japanese]. Yokendo.
- Iqbal, S. H., & Khalid, A. N. (1996). Material for the fungus flora of Pakistan. II. An updated check list of rust fungi (Uredinales) of Pakistan. *Sultania*, 1, 39–67.
- Ji, J.-X., Li, Z., Wang, Q., Li Y., & Kakishima, M. (2017). Life cycle of *Aecidium klugkistianum* on *Ligustrum* and its new combination, *Puccinia klugkistianiana*. *Mycoscience*, 58, 307–311. <https://doi.org/10.1016/j.myc.2017.01.004>
- Ji, J.-X., Li, Z., Li, Y., & Kakishima, M. (2019). Notes on rust fungi in China 7. *Aecidium caulophylli* life cycle inferred from phylogenetic evidence and renamed as *Puccinia caulophylli* comb. nov. *Mycotaxon*, 134, 719–730. <https://doi.org/10.5248/134.719>
- Kakishima, M., Ji J.-X., & Kasuya, T. (2018). *Puccinia neovelutina* nom. nov., a replaced name for *Aecidium elaeagni* and its new aecial host from Japan. *Phytotaxa*, 336, 197–200. <https://doi.org/10.11646/phytotaxa.336.2.7>
- Kaneko, S. (1973). Life cycle and behavior of nuclei of *Aecidium mori* Barclay, the causal fungus of mulberry rust. *Transactions of the Mycological Society of Japan*, 14, 294–301.
- Kasuya, T., & Ono, Y. (2018). *Herpobasidium filicinum* (Eocronartiaceae, Platygliales) occurs on *Dennstaedtia wilfordii* (Dennstaedtiaceae) in Japan. *Mycoscience*, 59, 443–448. <https://doi.org/10.1016/j.myc.2018.03.001>
- Kasuya, T., Hosaka, K., & Kakishima, M. (2020). *Gymnosporangium raphiolepidis* comb. nov. (*Pucciniales*) for *Aecidium raphiolepidis* inferred from phylogenetic evidence. *Phytotaxa*, 460, 110–114. <https://doi.org/10.11646/phytotaxa.460.1.7>
- Kasuya, T., Hosaka, K., Uno, K., & Kakishima, M. (2012). Phylogenetic placement of *Geastrum melanocephalum* and polyphyly of *Geastrum triplex*. *Mycoscience*, 53, 411–426. <https://doi.org/10.1007/s10267-012-0186-z>
- Kobayashi, T. (2007). *Index of fungi inhabiting woody plants in Japan. Host, Distribution and Literature* [in Japanese]. Zenkoku-Noson-Kyoiku Kyokai Publishing Co.
- Leppik, E. E. (1972). Evolutionary specialization of rust fungi (Uredinales) on the Leguminosae. *Annales Botanici Fennici*, 9, 135–148.
- Mordue, J. E. M. (1991). IMI descriptions of fungi and bacteria no. 1031. *Aecidium mori*. *Mycopathologia*, 114, 45–46. <https://doi.org/10.1007/BF00436693>
- Nepal, M. P., & Ferguson, C. (2012). Phylogenetics of *Morus* (Moraceae) inferred from ITS and *trnL-trnF* sequence data. *Systematic Botany*, 37, 442–450. <https://doi.org/10.1600/036364412X635485>
- Nguyen, L.-T., Schmidt, H. A., von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, 32, 268–274. <https://doi.org/10.1093/molbev/msu300>
- Nylander, J. A. A. (2004). *MrModeltest v2. Distributed by the author*. Evolutionary Biology Centre, Uppsala University.
- Ono, Y. (2016). *Phakopsora hornotina*, an additional autoecious rust species on *Meliosma* in the Philippines and the Ryukyu Islands, Japan. *Mycoscience*, 57, 71–78. <https://doi.org/10.1016/j.myc.2015.09.003>
- Parmelee, J. A. (1965). The genus *Gymnosporangium* in Eastern Canada. *Canadian Journal of Botany*, 43, 239–267. <https://doi.org/10.1139/b65-028>
- Parmelee, J. A., & Corlett, M. (1973). Development of the aecium and the nuclear condition of *Roestelia brucensis*. *Reports of the Tottori Mycological Institute*, 10, 189–201.
- Prasad, K. V., Yadav, B. R. D., & Sullia, S. V. (1993). Taxonomic status of rust on mulberry in India. *Current Science*, 65, 424–426. <https://www.currentscience.ac.in/Volumes/65/05/0424.pdf>
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61, 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Saccardo, P. A. (1891). Supplementum Universale, Pars I. Agaricaceae-Laboulbeniaceae. *Sylloge Fungorum*, 9, 1–1141.
- Sathe, A. V. (1969). *Peridiospora*, a new form-genus of Uredinales. *Transactions of the British Mycological Society*, 53, 143–145. [https://doi.org/10.1016/S0007-1536\(69\)80020-3](https://doi.org/10.1016/S0007-1536(69)80020-3)
- Sato, T., & Sato, S. (1981). *Aecidium raphiolepidis* and *A. pourthiaeeae*: two imperfect rust fungi with aecidioid uredinia. *Transactions of the Mycological Society of Japan*, 22, 173–179.
- Spaulding, P. (1961). Foreign diseases of forest trees of the world. *Agriculture Handbook, United States Department of Agriculture*, 197, 1–361. <https://naldc.nal.usda.gov/download/CAT10309136/pdf>

- Tai, F. L. (1979). *Sylloge fungorum Sinicorum*. Science Press.
- Teng, S. C. (1996). *Fungi of China*. Mycotaxon, Ltd.
- Turland, N. J., Wiersema, J. H., Barrie, F. R., Greuter, W., Hawksworth, D. L., Herendeen, P. S., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T. W., McNeill, J., Monro, A. M., Prado, J., Price, M. J., & Smith, G. F. (2018). *International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159*. Koeltz Botanical Books. <https://doi.org/10.12705/Code.2018>
- Uzuhashi, S., Horie, H., & Kakishima, M. (2022). First report of rust disease on *Potentilla indica* caused by *Phragmidium duchesneae* in Japan. *Journal of General Plant Pathology*, 88, 278–281. <https://doi.org/10.1007/s10327-022-01068-3>
- Vialle, A., Feau, N., Allaire, M., Didukh, M., Martin, F., Moncalvo, J., & Hamelin, R. (2009). Evaluation of mitochondrial genes as DNA barcode for Basidiomycota. *Molecular Ecology Resources*, 9, 99–113. <https://doi.org/10.1111/j.1755-0998.2009.02637.x>
- Vilgalys, R., & Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified DNA from several *Cryptococcus* species. *Journal of Bacteriology*, 172, 4238–4246. <https://doi.org/10.1128/jb.172.8.4238-4246.1990>
- Virtudazo, E. V., Nakamura, H., & Kakishima, M. (2001). Phylogenetic analysis of sugarcane rusts based on sequences of ITS, 5.8 S rDNA and D1/D2 regions of LSU rDNA. *Journal of General Plant Pathology*, 67, 28–36. <https://doi.org/10.1007/PL00012983>
- White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR Protocols: a guide to methods and applications* (pp. 315–322). Academic Press.
- Zhao, P., Qi, X. H., Crous, P. W., Duan, W. J., & Cai, L. (2020). *Gymnosporangium* species delineation, diversity and host alternation. *Persoonia*, 45, 68–100. <https://doi.org/10.3767/persoonia.2020.45.03>