ISSN (print) 0093-4666 ISSN (online) 2154-8889

MYCOTAXON

DOI: 10.5248/114.271

Volume 114, pp. 271–279 *October–December 2010*

Studies of *Exobasidium* new to China: *E. rhododendri-siderophylli* sp. nov. and *E. splendidum*

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Abstract—A new species, *Exobasidium rhododendri-siderophylli* causing leaf hypertrophy on *Rhododendron siderophyllum,* is described and a new Chinese record, *Exobasidium splendidum* on *Vaccinium fragile*, are reported from Yunnan Province, China. The new species is characterized by symptoms, number of sterigmata, and short germ tubes. Molecular sequence analyses of 22 *Exobasidium* species reveal that phylogenetic relationships within *Exobasidium* correspond to the host plants and symptoms.

Key words—*Exobasidiomycetes*, molecular analysis, taxonomy

A new species of *Exobasidium* on *Rhododendron siderophyllum* was collected from Yunnan Province. The host plant belongs to the subfamily *Rhododendroideae* of *Ericaceae*. The *Exobasidium* species is parasitic on young leaves and fruit, causing hypertrophy. The diseased leaf is almost wholly hypertrophied, pale yellow, and 2–3.3 cm long, 0.5–1.8 cm wide, and 2.5 mm thick; when mature, the underside is covered with a white hymenium. A transverse section of a diseased leaf shows a differentiation between the palisade and mesophyll cells, but it is not clear. The diseased fruit is entirely hypertrophied, 1.8 × 1.3 cm, and also covered with white hymenium when mature. The new species — characterized by the described symptoms, possession of 3–7 sterigmata, and short germ tubes — is described as:

Exobasidium rhododendri-siderophylli ZhenYing Li & L. Guo, **sp. nov.**

MycoBank MB 518411 Figs. 1–4

Hymenium hypophyllum. Basidia hyalina, cylindrica vel clavata, 5–9 µm lata, terminaliter 3–7 sterigmatibus 5–6(–7) × 1–1.5(–1.8) µm praedita. Basidiosporae ellipsoideae vel

*corresponding author

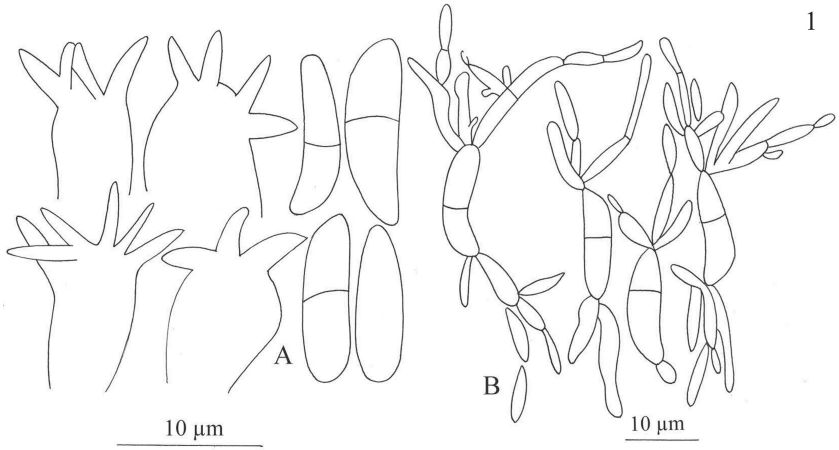


Fig. 1. Line drawings of *Exobasidium rhododendri-siderophylli* on *Rhododendron siderophyllum* (HMAS 183424, holotype). A. Basidia, sterigmata and basidiospores. B. Germinating basidiospores.

clavatae, interdum curvae, (12–)13–15(–18.5) × 3–4 µm, hyalinae, leves, primo continuae, dein 1-septatae.

Type: On *Rhododendron siderophyllum* Franch. (*Ericaceae*), Yunnan: Luquan, alt. 2520 m, 1.VII.2006, Z.Y. Li & L. Guo 339, HMAS 183424 (**holotype**).

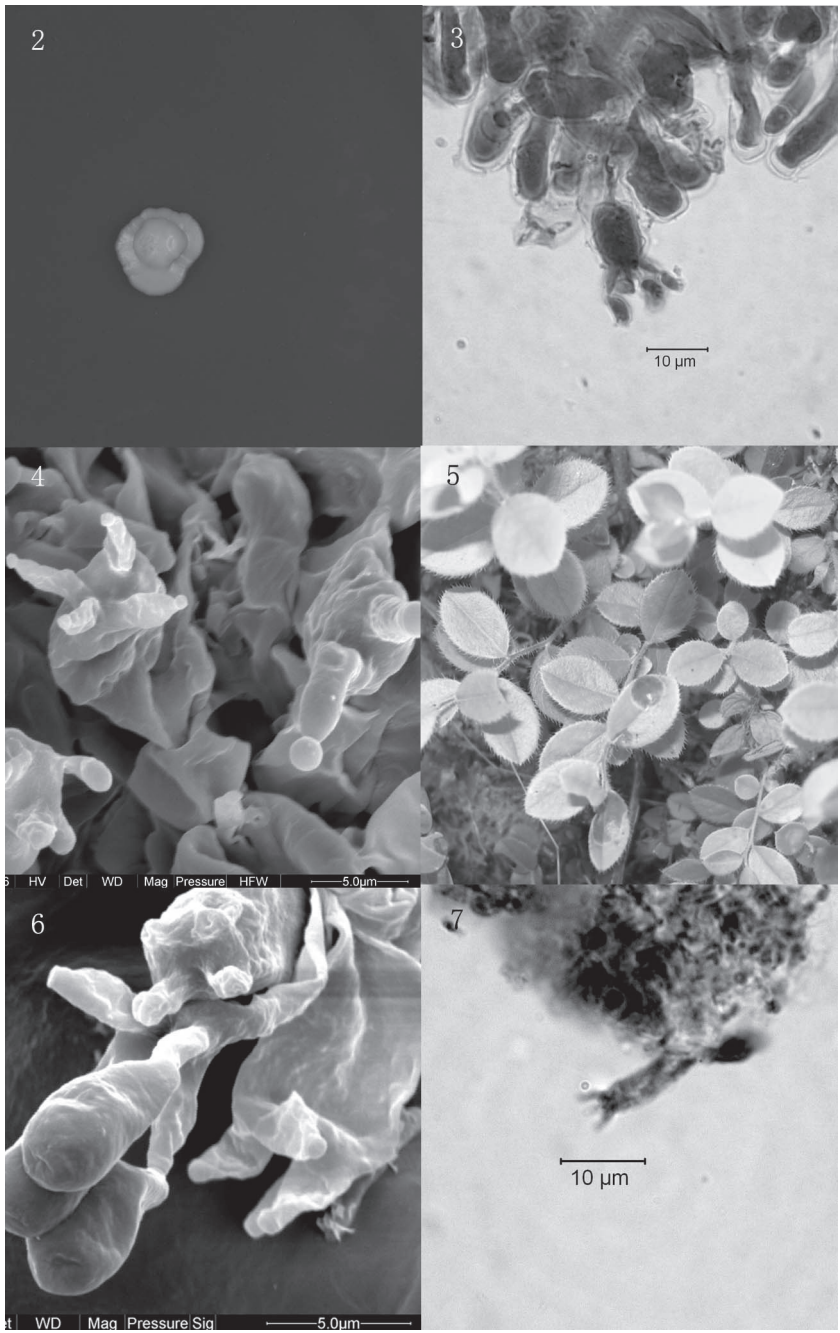
Hymenium hypophyllous. Basidia hyaline, cylindrical or clavate, 5–9 µm diam., with 3–7 sterigmata. Sterigmata conical, 5–6(–7) × 1–1.5(–1.8) µm. Basidiospores ellipsoidal or clavate, occasionally curved, (12–)13–15(–18.5) × 3–4 µm, hyaline, smooth, at first continuous, then 1-septate.

Colonies on potato dextrose agar (PDA) grew slowly, to a maximum 8 mm diameter after 21 days incubation at 25°C. The colony was pale yellow, composed of conidia. Conidia bacilliform, 5–7.5 × 1–2 µm.

Additional specimens examined: On *Rhododendron siderophyllum* Franch. (*Ericaceae*), Yunnan: Luquan, alt. 2520 m, 1.VII.2006, Z.Y. Li & L. Guo 338, HMAS 183429 (**paratype**); Z.Y. Li & L. Guo 336, HMAS 183428 (**paratype**). On *Rhododendron tatsienense* Franch. (*Ericaceae*), Yunnan: Luquan, alt. 2530 m, 1.VII.2006, Z.Y. Li & L. Guo 329 HMAS 183437 (**paratype**).

Remarks: Morphologically, *Exobasidium rhododendri* (Fuckel) C.E. Cramer (Nannfeldt 1981) on *Rhododendron ferrugineum* L. has similarly sized

Figs. 2–4. *Exobasidium rhododendri-siderophylli* on *Rhododendron siderophyllum* (HMAS 183424, holotype). 2. Colony on PDA. 3. Basidium, sterigmata and basidiospores as seen by LM. 4. Basidia and sterigmata as seen by SEM. Figs. 5–7. *Exobasidium splendidum* on *Vaccinium fragile* (HMAS 183436). 5. Symptoms. 6. Basidia, sterigmata and basidiospores as seen by SEM. 7. Basidium and sterigmata as seen by LM.


2
3
10 µm
4
HV Det WD Mag Pressure HFW 5.0µm
5
6
WD Mag Pressure Sig 5.0µm
7
10 µm

basidiospores (12–15 × 2.5–4 μm) but differs from *E. rhododendri-siderophylli* in that it causes galls.

Exobasidium splendidum, discovered in Yunnan Province, is a new Chinese record. It is parasitic on *Vaccinium fragile*, causing leaf spots, usually 1(–2) on each leaf. The upper side of the diseased parts is slightly concave and red to pale red, and the underside becomes covered with white hymenium during maturation. The leaf spots can be 3.5–5.5 mm in diam. Transverse sections of the diseased leaf show clear differentiation of the palisade and mesophyll cells. There is no hypertrophy and hyperplasia of plant cells.

Exobasidium splendidum Nannf., Symb. Bot. Upsal. 23(2): 58, 1981. Figs. 5–8

Specimen examined—On *Vaccinium fragile* Franch. (*Ericaceae*), Yunnan: Yangbi, Shangjie, Mopandi, alt. 2350 m, 14.IX.2005, Z.Y. Li, L. Guo & N. Liu 117, HMAS 183436.

Hymenium hypophyllous, white. Basidia hyaline, cylindrical, 4–8 μm, with 2–4 sterigmata. Sterigmata conical, 3–5 × 1–2 μm. Basidiospores cylindrical, clavate or obovoid, often curved, (7–)9–14(–16) × 3–4.2(–5) μm, hyaline, smooth, at first continuous, then 1–3-septate.

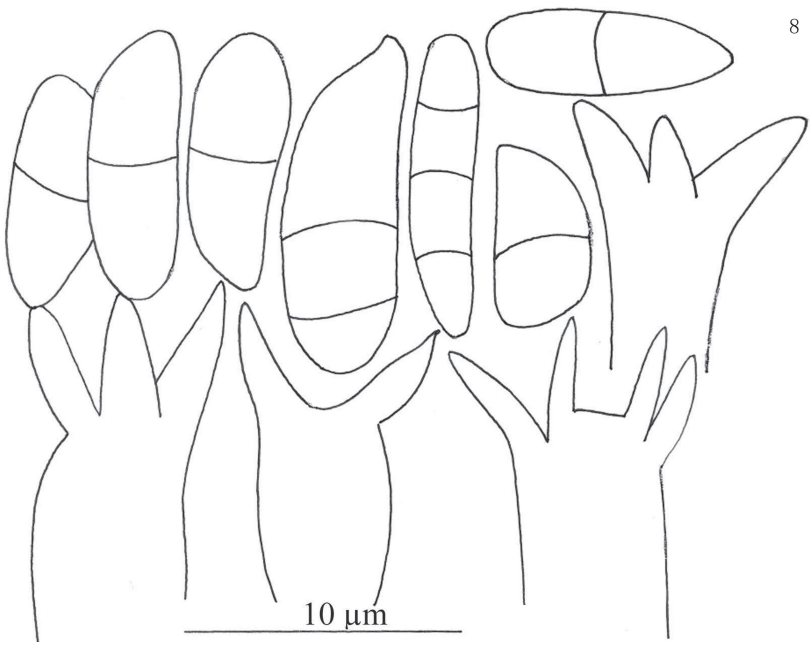


Fig. 8. Line drawings of *Exobasidium splendidum* on *Vaccinium fragile* (HMAS 183436). A. Basidia, sterigmata and basidiospores. B. Germinating basidiospores.

Thirty-three species of *Exobasidium* have been reported in China (Sawada 1922, Teng 1963, Tai 1979, Guo et al. 1991, Zang 1996, Li & Guo 2006a,b, 2008a,b, 2009a,b), including the two species recorded in this paper.

For phylogenetic analyses, the partial nrDNA-LSU (LSU) and ITS1-5.8S-ITS2 (ITS) genes were sequenced (White et al. 1990). Thirty-one sequences of 43 isolates (22 species) (Table 1), including 14 sequences (11 species) downloaded from Genbank, were used. Seventeen isolates (11 species) were collected by the authors. All strains collected by the authors were deposited in China General Microbiological Collection Center (CGMCC) (Table 1), and all sequences generated in this study were submitted to GenBank. Two *Entyloma* species were used as outgroup.

Table 1. Materials used in analysis of the nrDNA-LSU and nrDNA-ITS rDNA sequences

Taxon	Symptom	Host	Collection	Genbank no.	
				LSU	ITS
E. bisporum	leaf spots	*Eu. grayana* var. *glabra*	IFO9942	AB177598	AB180364
E. camelliae	fruit & leaf hypertrophy	*C. japonica*	MAFF238578	AB176712	AB180317
*E. canadense**	leaf spots	*R. mariesii*	HMAS 173409	EU692791	EU692771
E. cylindrosporum	leaf spots	*R.* sp.	MAFF238608	AB178245	
E. cylindrosporum	leaf spots	*R. pulchrum*	MAFF238579		AB180318
*E .cylindrosporum**	leaf spots	*R.* sp.	HMAS 183415	EU692795	EU692776
*E. euryae**	galls	*C. oleifera*	HMAS 97947	EU692779	EU692759
*E. formosanum**	galls	*R. delavayi*	HMAS 183418	EU692781	EU692775
*E. formosanum**	galls	*R.* sp.	HMAS 183445	EU692796	EU692777
*E. gracile**	leaf hypertrophy	*C. oleifera*	HMAS 140210	EU692780	
*E. gracile**	leaf hypertrophy	*C. oleifera*	HMAS 140502		EU692761
E. gracile	leaf hypertrophy	*C. sasanqua*	TUK-E30	AB177592	
E. gracile	leaf hypertrophy	*C. sasanqua*	MAFF238586		AB180322
E. inconspicuum	leaf spots	*V. hirtum* var. *pubescens*	MAFF238616	AB177556	
E. inconspicuum	leaf spots	*V. hirtum* var. *pubescens*	MAFF238619		AB180350
*E. japonicum**	leaf deform & hypertrophy	*R. pulchrum*	HMAS 172284	EU692788	EU692773
*E. japonicum**	leaf hypertrophy	*R. simsii*	HMAS 175467	EU692790	EU692766
*E. japonicum**	leaf deform & hypertrophy	*R.* sp.	HMAS 175457	EU692792	EU692772
*E. japonicum**	leaf deform & hypertrophy	*R.* sp.	HMAS 175455	EU692793	EU692768

Table 1, concluded.

Taxon	Symptom	Host	Collection	Genbank no.	
				LSU	ITS
*E. japonicum**	leaf deform & hypertrophy	*R.* sp.	HMAS 175454	EU692794	EU692769
E. japonicum	leaf deform & hypertrophy	*R. obtusum* var. *kaempferi*	MAFF238826	AB178253	
E. japonicum	leaf deform & hypertrophy	*R. lateritium*	IFO30756		AB180370
*E. kunmingense**	leaf spots	*L. ovalifolia*	HMAS 173147	EU692784	EU692763
*E. lushanense**	leaf spots	*R. simsii*	HMAS 173148	EU692789	EU692767
E. miyabei	leaf spots	*R. dauricum*	MAFF238583	AB177550	
E. miyabei	leaf spots	*R. dauricum*	MAFF238595		AB180330
E. nobeyamense	witches' broom	*R. wadanum*	MAFF238583	AB180378	
E. nobeyamense	witches' broom	*R. wadanum*	MAFF238598		AB180332
E. otanianum	leaf spots		IFO9960	AB177600	
E. otanianum	leaf spots	*R. hyugaense*	MAFF238612		AB180344
E. pentasporium	witches' broom & leaf spots	*R. obtusum* var. *kaempferi*	MAFF238601	AB177567	
E. pentasporium	witches' broom & leaf spots	*R. obtusum* var. *kaempferi*	MAFF238179		AB180316
E. pieridis-ovalifoliae	leaf spots	*L. neziki*	IFO9961	AB177601	AB180367
E. rhododendri	galls	*R. ferrugineum*	R.B.2050	AF009856	
E. rhododendri	galls	*R.* sp.	CBS101457		DQ667153
*E. rhododendri-russati**	galls	*R. russatum*	HMAS 183433	EU692797	EU692778
*E. rhododendri-siderophylli**	leaf hypertrophy	*R. tatsienense*	HMAS 183437	EU692782	EU692762
*E. rhododendri-siderophylli**	leaf hypertrophy	*R. siderophyllum*	HMAS 183428	EU692786	EU692765
*E. rhododendri-siderophylli**	leaf hypertrophy	*R. siderophyllum*	HMAS 183429	EU692786	EU692764
E. woronichinii	leaf spots	*R. brachycarpum*	MAFF238825	AB178252	
E. woronichinii	leaf spots	*R. brachycarpum*	MAFF238617		AB180348
E. yoshinagae	leaf spots	*R. wadanum*	MAFF238606	AB177551	
E. yoshinagae	leaf spots	*R. reticulatum*	IFO9959		AB180365
Entyloma ficariae		*Ra. ficaria*		AY081013	
Entyloma ficariae		*Ra. ficaria*			AY081035
Entyloma linariae		*Linaria vulgaris*		AY860054	
Entyloma linariae		*Linaria vulgaris*			AY081041

* = collected and sequenced by the authors.
C.= Camellia, E .= Exobasidium, Eu. = Eubotryoides, L. = Lyonia, R .= Rhododendron, Ra. = Ranunculus, V. = Vaccinium.

Two sequence sets, both independently and combined, were analyzed following the Minimum Evolution method (ME) (Rzhetsky & Nei 1992). As

all the ME trees derived from the independent and combined ITS and LSU sequence analyses share similar topologies structure and main clades, only the ME tree based on the combined ITS and LSU analysis is shown (Fig. 9).

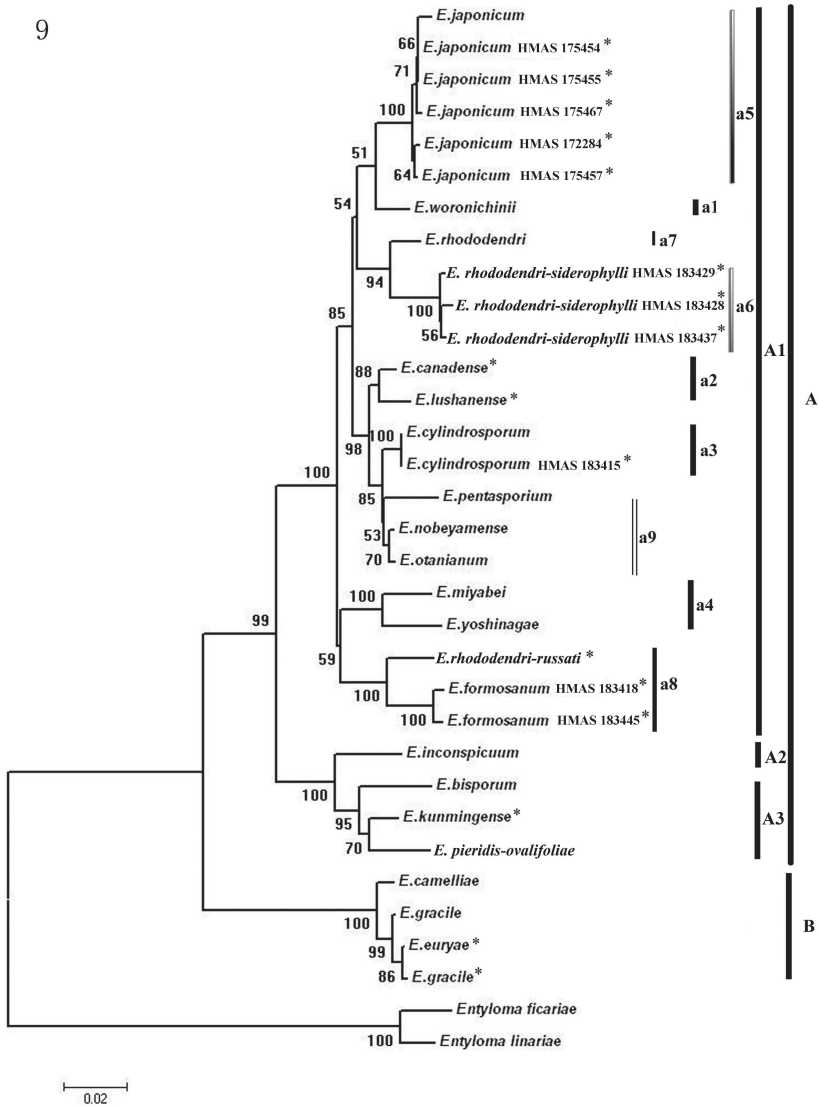


Fig. 9. ME tree based on analysis of nrDNA-ITS/nrDNA-LSU sequences. The numbers on the branches indicate bootstrap values, following the 50% majority rule. * = collected and sequenced by the authors; *E.*= *Exobasidium.* Bar types correspond to the different symptoms, i.e. leaf spots (a1–a4), leaf hypertrophy (a–a6), galls (a7–a8), and witches' broom (a9).

The combined tree is the most parsimonious following the 50% bootstrap majority-rule.

Two major clades (A–B) are identified in the ME tree: clade A consists of only the species parasitic on *Ericaceae,* while clade B contains species on *Theaceae*. Clade A includes three subclades: A1 on *Rhododendroideae* (*Rhododendron*), A2 on *Vaccinioideae,* and A3 on *Andromedoideae*. A1 encompasses nine small clades, including species causing different symptoms — a1–a4 causing leaf spots, a5–a6 leaf hypertrophy, a7–a8 galls, and a9 witches' broom.

Results of the molecular analyses indicate that the phylogenetic relationships within *Exobasidium* correspond to the host plants and symptoms. Host associations and symptoms should be regarded as important characteristics for morphological identification.

Acknowledgements

The authors would like to express their deep thanks to Drs Eric H.C. McKenzie (Auckland, New Zealand) and D. Begerow (Bochum, Germany) for serving as pre-submission reviewers, to Dr. Shaun Pennycook (Auckland, New Zealand) for nomenclatural review, to Mr. Ziyu Cao (Institute of Botany, Chinese Academy of Sciences) for identifying the host plants, to Mrs. Jiayi Xie and Jingnan Liang for assistance with SEM photographs, and to Mrs. Xiangfei Zhu for inking in line drawings. This study was supported by the National Natural Science Foundation of China (No. 30499340 and No. 30670005).

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