The *Ovulariopsis* anamorph of *Phyllactinia bauhiniae* var. *cassiae:* first record outside India and morphological characterization

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Based on an own collection from *Cassia fistula* in Taiwan and type specimens of *Oidium cassiae-siameae* and the *Ovulariopsis* anamorph of *Phyllactinia bauhiniae* var. *cassiae* (Ascomycota, Erysiphales), the latter is characterized in detail and recorded outside India for the first time. The conidiophores and conidia of *Oidium cassiae-siameae* have similar shapes, sizes and ornamentations as those of the anamorph of *Phyllactinia bauhiniae* var. *cassiae*, but both species differ by the presence of endophytic hyphae with haustoria formed in mesophyll cells in the latter species and their absence in the former. Different preparation techniques for light microscopy are compared.

Keywords: Fabaceae, Leguminosae, plant pathogenic fungi, powdery mildews

Powdery mildews (Ascomycota, Erysiphales) are easily recognizable as group of plant pathogenic fungi and can be quickly identified to species, when the host plant and details of ascomata are known. During a long period of the development of powdery mildews, however, a hyphomycetous anamorph precedes the production of ascomata in temperate regions, and this anamorphic stage is often predominant in tropical countries (Braun *et al.* 2002). Because morphological characteristics of anamorphic Erysiphales are variable within species and similar between species, species are often difficult to identify without the presence of the teleomorph and mainly based on assumed host specificity. In many species, anamorphic stages are not known or only superficially described. Anamorphs of species of Phyllactinioideae, however, can be separated from most of the other erysiphalean species with *Oidium* anamorphs by the production of endophytic hyphae with haustoria formed in mesophyll cells.

According to the present concepts of *Phyllactinia*, *Leveillula*, *Pleochaeta*, and their respective anamorphs, conspicuously dimorphic conidia do not occur in *Ovulariopsis* (anamorphic *Phyllactinia*), but in *Oidiopsis* (anamorphic *Leveillula*) and *Streptopodium* (anamorphic *Pleochaeta*) (Braun 1987, Liberato & Barreto 2005). An additional characteristic for the anamorphs of Phyllactinioideae is the reticulate-striate surface ornamentation of conidia visible with scanning electron microscopy (SEM) (Braun *et al*. 2002). Morphological differences of the anamorphs are more conspicuous between species of Phyllactinioideae than between those of other Erysiphales. By a combination of host data and morphological characteristics, species identification is possible in anamorphs of Phyllactinioideae even when the teleomorph is not known.

Cassia fistula L., a member of Fabaceae (Caesalpinioideae) native to India, has been pantropically distributed as ornamental tree because of its decorative yellow inflorescences (Bole 2004). An anamorphic powdery mildew forming copious colonies on leaves of *Cassia fistula* was collected on the campus of Cheng Kung University, Tainan, in tropical Southern Taiwan. A first microscopic examination revealed cylindrical to barrel-shaped conidia and short conidiophores typical for *Oidium* anamorphs (Fig. 1). The conidia showed a conspicuous reticulate-striate surface ornamentation rarely reported for *Oidium* species (Fig. 1 B, C). A closer examination of leaf-sections, however, proved the presence of endophytic hyphae with haustoria in mesophyll cells typical for *Ovulariopsis* anamorphs of *Phyllactinia* species. For the identification of the newly collected species, descriptions of *Ovulariopsis* and *Oidium* anamorphs on *Cassia* species and other Caesalpinioideae (Fabaceae) were compared. In descriptions and illustration of anamorphic Erysiphales from tropical and subtropical Asia by Yen (1966, 1967), only smooth or rugose surfaces of conidia were reported and no internal structures like endophytic hyphae or haustoria. A species described by Yen (1967) as *Oidium cassiae-siameae* did not only occur on a species of the same host genus in Taiwan, but also appeared morphological very similar to the newly collected specimen. For the anamorph of a taxon on *Cassia fistula* in India, recently described as *Phyllactinia bauhiniae* var. *cassiae* Y. S. Paul & V. Thakur, similar shapes and sizes were given (Paul & Thakur 2006), but without details of surface ornamentation. Type studies of this anamorph and of *Oidium cassiae-siameae* were, therefore, included in a comparison with the newly collected specimen from Taiwan. We aimed to clarify the previously undescribed localization of haustoria and surface ornamentation of conidiophores and conidia. In order to assess the influence of different preparation techniques on the appearance of the surface ornamentation, different techniques were applied in the specimens and the results compared.

Materials and Methods

Material was collected on fallen leaves of *Cassia fistula* in Taiwan, Tainan, Cheng Kung University, 30 Mar 2007. The same day, leaf sections made by hand with a razor blade and scratch preparations were mounted in 5–10 % aqueous KOH and observed with a light microscope. Measurements of microscopic structures are given as ranges from minimum to maximum values directly or as mean values $\pm$ 1 standard deviation with extreme values in brackets (n = 30). Photographs shown in Figs. 1 A, B and most of the free-hand drawings using scaled paper shown in Figs. 2 A, E were immediately made at the day of collection (30 Mar 2007). After drying of the specimen, portions were mounted in water without heat treatment, 5–10 % aqueous KOH without heat treatment, and in a mixture of 10 mL glycerol, 35 mL lactic acid, 10 g polyvinyl alcohol, 50 g chloral hydrate, and 60 mL distilled water (Schmutterer 1959, modified by M. Göker, personal communication) and gently heated with the flame of a lighter, respectively. The specimens were directly compared with a light microscope after treatment. The new specimen from Taiwan was deposited as R. Kirschner 3006 at the herbarium of National Museum of Natural Science, Taichung, Taiwan (TNM) and the Herbier Cryptogamique, Dépt. Systématique et Évolution, Muséum National d'Histoire Naturelle, Paris (PC). It was compared with type material of *Oidium cassiae-siameae* J. M. Yen (PC) and of *Phyllactinia bauhiniae* Y. S. Paul & V. Thakur var. *cassiae* Y. S. Paul & V. Thakur kindly provided by Y. S. Paul (Dept. Plant Pathology, College of Agriculture, CSKHPKV, Palampur, Himal Pradesh, India). Both specimens were treated with KOH and the lactic acid/cotton blue mixture as described above.

Results

Influence of mounting medium

The surface structures of conidiophores and conidia of the same specimen (R. Kirschner 3006) were identical after treatment with water and 5–10 % aqueous KOH, respectively (Fig. 1 B, C). Aqueous KOH appeared to penetrate into the cells and reduce air bubbles more effectively than water. In all three specimens, when material was mounted in 5–10 % aqueous KOH without heat treatment, conidiophores appeared finely verruculose and conidia reticulate-striate. Endophytic hyphae and haustoria could not be detected when using KOH. The ornamentation disappeared when using the lactic acid/cotton blue mixture with heat treatment and in many cases was replaced by large, flat wart-like wall appositions (Fig. 1 D). Endophytic hyphae, when present, and haustoria were detectable in heated lactic acid/cotton blue mountings.

Morphology

Ovulariopsis* anamorph of *Phyllactinia bauhiniae Y. S. Paul & V. Thakur **var. *cassiae*** Y. S. Paul & V. Thakur, Indian Erysiphaceae: 72, 2006. Figs. 1, 2 A-F.

Material on *Cassia fistula* from Taiwan, Figs. 1, 2 A-E.

Colonies hypophyllous, dense and white. Hyphae ectophytic and endophytic, hyaline, smooth, 3–4 µm wide, penetrating through stomata, forming haustoria of approx. 7–10 µm diam. within mesophyll cells. Ectophytic hyphae with slightly to strongly nipple-shaped or slightly lobed appressoria being 0.5–5 µm long and 2–5 µm broad, and with approx. 10–20 µm long, free ends covered by a cap formed of refractive material. Conidiophores arising from ectophytic hyphae, erect, straight or with a single bend at the base, hyaline, 1–4-septate, thin-walled, densely verruculose, especially in the upper part, ornamentation diminishing towards the base, 42–128 µm × 4–6 µm. Conidia mostly cylindrical, rarely oblong or ellipsoidal, hyaline, conspicuously reticulate-striate when seen with the light microscope, apex slightly apiculate in primary conidia or broadly rounded in secondary conidia, base truncate or broadly rounded, solitary on conidiophores or in chains of two, in some cases with hilum or amorphous material at the ends due to detachment, (33) 35–45 (60) µm × 11–14 (15) µm (n = 30), germinating at the ends.

Material examined. – *Ovulariopsis* anamorph of *Phyllactinia bauhiniae* var. *cassiae* on freshly fallen leaflets of *Cassia fistula* (Fabaceae), Taiwan, Tainan, Cheng Kung University, campus, ca. 50 m, 30 Mar 2007, R. Kirschner 3006 (PC, TNM).

Type of *Ovulariopsis anamorph* of *Phyllactinia bauhiniae* var. *cassiae* on *Cassia fistula* from India, Fig. 2 F.

The type specimen is morphologically identical to the specimen from Taiwan, except for slightly longer conidiophores, 75–140 µ × 5–6 µm, slightly broader conidia, (34) 38–45 (48) µm × (12) 14–17 (18) µm (n = 30), and the absence of refractive material covering free ends of ectophytic hyphae.

Material examined. – *Ovulariopsis* anamorph of *Phyllactinia bauhiniae* var. *cassiae* on leaves of *Cassia fistula* (Fabaceae), India, Panjgrain (Barmana), 12 Dec 2001, Y. S. Paul & V. K. Thakur DPDH-325, type.

Oidium cassiae-siameae J. M. Yen, Cahiers Pacif. 11: 96. 1967. Fig. 2 G.

Colonies mainly hypophyllous, but small epiphyllous colonies also present. Hyphae exclusively ectophytic, haustoria globose or

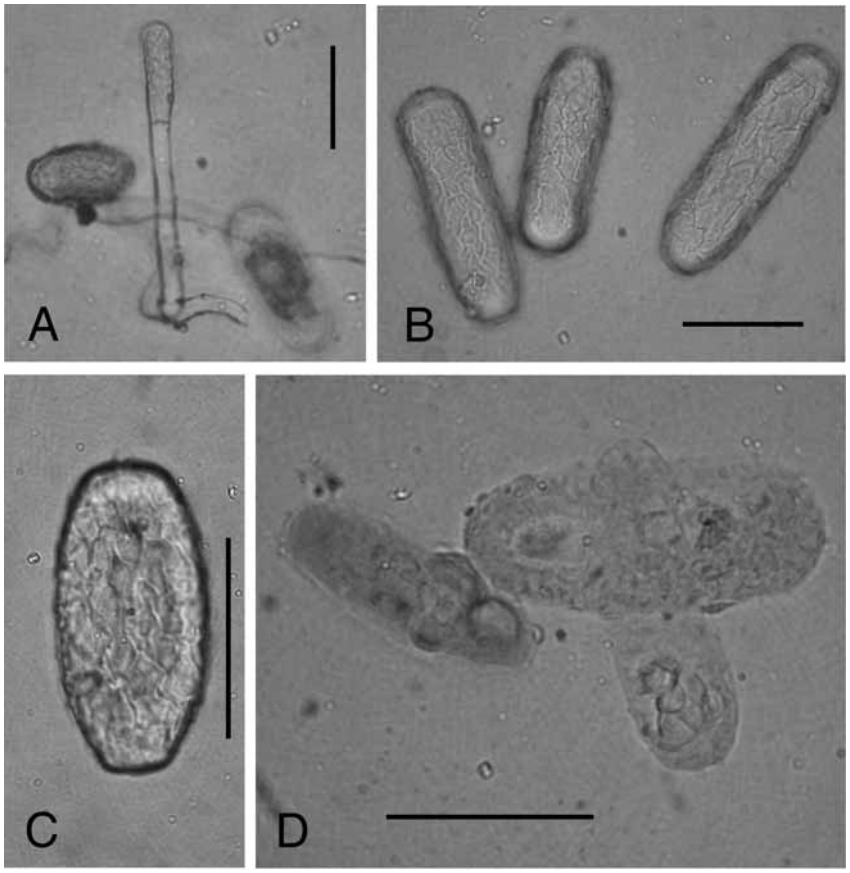

Fig. 1. Photographs of *Ovulariopsis* anamorph of *Phyllactinia bauhiniae* var. *cassiae* (R. Kirschner 3006, TNM) in different mounting media: in 5–10 % aqueous KOH (A, B), water (C), and medium containing lactic acid, heated (D). **A.** Conidiophore. **B, C.** Conidia showing characteristic reticulate-striate surface ornamentation in KOH and water, respectively, without heating. **D.** Conidia with smooth to irregularly rugose surface in lactic acid preparation after heating. Scale bars = 20 μm.

subglobose, exclusively in epidermis cells. Conidiophores hyaline, septate, finely verruculose in the apical part, approx. 65–70 μm × 7 μm. Conidia short to long cylindrical, oblong or ellipsoidal, hyaline, conspicuously reticulate-striate when seen with the light microscope, apex slightly apiculate in primary conidia or broadly rounded in secondary conidia, base truncate or broadly rounded, solitary on conidiophores, 23–34 μm × 10–15 μm.

Material examined. – *Oidium cassiae-siameae* J. M. Yen on leaves of *Cassia siamea* Lam. (Fabaceae), Taiwan, Taichung, Chung Hsing University, campus, 23 May 1964, M.-J. Chen No. 18 (PC: PC0092695, PC0092696, type).

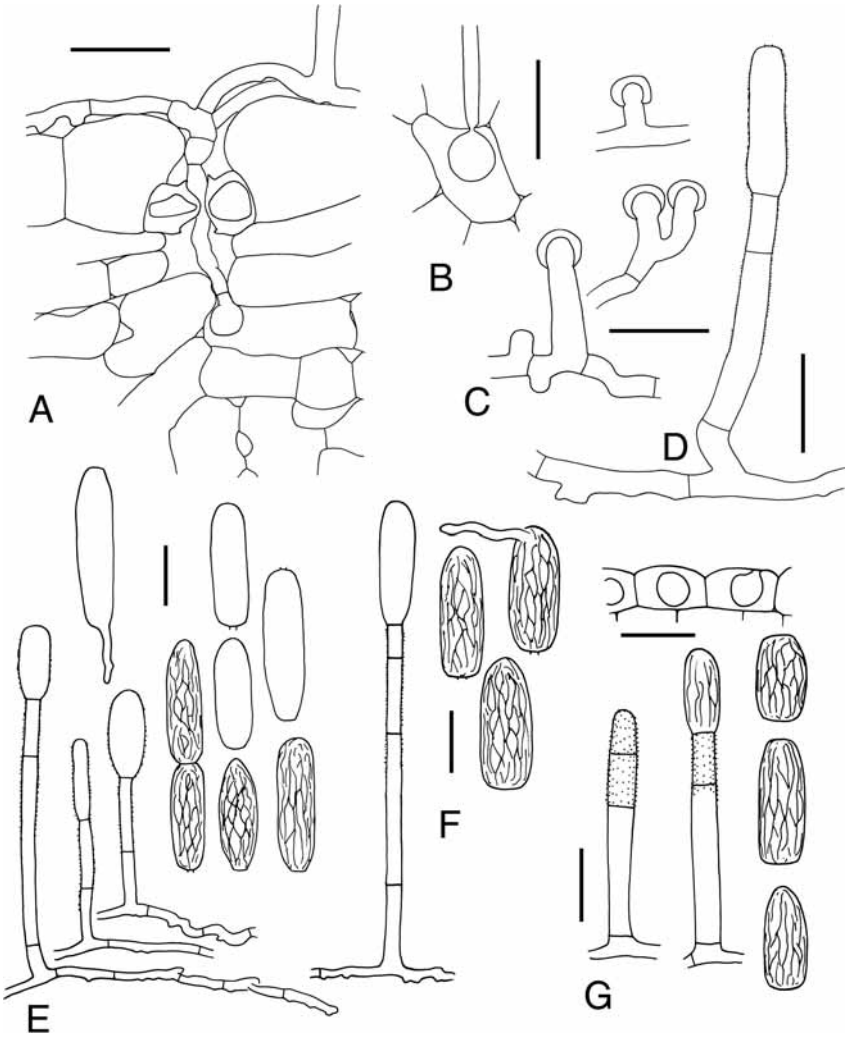

Fig. 2. Drawings of *Ovulariopsis* and *Oidium* species on *Cassia* species. **A–F.** *Ovulariopsis* anamorph of *Phyllactinia bauhiniae* var. *cassiae*. **A–E.** Collection on *Cassia fistula* from Taiwan (R. Kirschner 3006, PC, TNM). **A.** Leaf section showing external hyphae, a conidiophore base, a hypha penetrating through a stoma and forming a haustorium in a mesophyll cell. The constriction between hypha and haustorium lies behind the haustorium and is concealed by the haustorium. **B.** Endophytic hypha growing in an intercellular space and forming a globose haustorium in a mesophyll cell. **C.** Free ends of ectophytic hyphae covered by cap-like refractive material. **D, E.** Ectophytic hyphae with nipple-shaped appressoria and conidiophores with ornamentation seen in longitudinal optical section, and conidia with ornamentation seen in surface view (not shown in all conidia). **F.** Ectophytic hypha with conidiophore and conidia from type specimen from India. **G.** *Oidium cassiae-siameae*. Haustoria in epidermis cells (torn off from ectophytic hyphae during preparation), conidiophores and conidia with ornamentation seen in surface view (type, PC). Scale bars = 20 µm.

Discussion

Influence of mounting medium

Conidia developing singly with angular ornamentation were considered characteristic for *Oidium* subgen. *Pseudoidium* anamorphs of *Erysiphe* species in the modern sense and *Oidiopsis*, *Ovulariopsis*, and *Streptopodium* anamorphs of Phyllactinioideae by Braun *et al.* (2002). The ornamentation of conidia and conidiophores recognizable with the light microscope, however, depends on the mounting medium and treatment. The use of lactic acid preparations and heating leads to a significant change or disappearance of the characteristic ornamentation which is visible without this treatment in the light microscope (shown here) or scanning electron microscope (Braun *et al.* 2002). The smooth and rugose conidium surfaces illustrated and described for *Oidium* species by Yen (1966, 1967), for example in *Oidium cassiae-siameae*, can be explained as artifacts due to rigorous treatment of the specimens. In our study, conidia of *Oidium cassiae-siameae* revealed an angular, striate-reticulate ornamentation similar to that shown in SEM photographs of *Oidium bauhiniae* G. J. M. Gorter & Eicker (Gorter & Eicker 1985). Haustoria could, however, be observed only in lactic acid/cotton blue mounting. For a morphological characterization of anamorphic Erysiphales (see below), both kinds of preparations with and without lactic acid/cotton blue mounting are required.

Taxonomy

The own measurements of conidia and conidiophores overlapped with those given by Paul and Thakur (2006) and Yen (1967) and were combined in Tab. 1. The newly collected specimen on *Cassia fistula* from Taiwan was identified as *Ovulariopsis* anamorph of *Phyllactinia bauhiniae* var. *cassiae*, because it is morphologically almost identical and occurs on the same host species as the type. Both, the pathogen and its host, were first described in India, where the host occurs naturally. This new record for Taiwan indicates that the pathogen has followed its host outside India and might be expected in many other tropical countries.

The short conidiophores and cylindrical to barrel-shaped conidia of the *Ovulariopsis* anamorph are more similar to the anamorphic stages classified in *Oidium* than to typical anamorphs of Phyllactinioideae with long conidiophores and large, often clavate conidia. Since an attempt to sequence LSU rDNA from the new specimen from Taiwan yielded a *Cladosporium* contamination (data not shown), a decision about generic placement was possible with morphology only. Recently, a species previously accommodated in

Oidium because of barrel-shaped conidia on short conidiophores revealed to belong to *Ovulariopsis* when endophytic hyphae and haustoria were found and the connection with a *Phyllactinia* teleomorph was confirmed (Havrylenko *et al.* 2006). For these reasons, we compared the new specimen on *Cassia fistula* from Taiwan with *Oidium* species also described from *Cassia* species in tropical and subtropical Asia by Yen (1966, 1967). *Oidium cassiae-hirsutae* J. M. Yen could be excluded, because conidiophores are longer, 3–7-septate, and broader (exceeding 14 µm in width), and predominantly ellipsoidal conidia are formed in chains of more than three (Yen 1966). *Oidium cassiae-siameae* differs by its exclusively ectophytic hyphae, lobed appressoria, haustoria in epidermal cells, and slightly shorter conidia from the anamorph of *Phyllactinia bauhiniae* var. *cassiae* (Tab. 1). By detecting the haustoria in the epidermis, we confirm the correct assignment of *Oidium cassiae-siameae* to *Oidium*. Because of solitary conidium development (Yen 1967) and ornamentation we suggest a placement in the subgenus *Pseudoidium*.

Though in the new collection from Taiwan, conidiophores are not long and conidia not clavate as in typical species of *Ovulariopsis*, endophytic hyphae, verruculose conidiophores, and reticulate-striate conidia suggest a placement in *Ovulariopsis*. Endophytic hyphae with haustoria and verruculose conidiophores similar to the species shown here were illustrated for *Ovulariopsis* and *Phyllactinia* species in Taiwan by Sawada (1930), but without a species similar to the collection from *Cassia fistula*. A similar species described in South Africa on *Cajanus cajan* (L.) Millsp., *Ovulariopsis ellipsospora* G. J. M. Gorter, with predominantly ellipsoidal conidia was placed in *Ovulariopsis* mainly because of hypae penetrating stomata (Gorter 1989). It differs from the species on *Cassia fistula*, however, by broader conidiophores, 7.5–10 µm, broader primary conidia, (17.5) 22.5 (27.5) µm, and multilobed appressoria (Gorter 1989). Among *Phyllactinia* species on members of Fabaceae, anamorph stages are not known in three species, all occurring on members of Faboideae, and not available for comparison: *Phyllactinia desmodii* J. F. Tao, J. Z. Qin & Y. Z. Shen on *Desmodium sinuatum* Bl. (Braun 1987), *Phyllactinia phaseolina* N. Ahmad, D. K. Agarwal & A. K. Sarbhoy on *Phaseolus trilobus* Ait. (Ahmad *et al.* 1987), and *Phyllactinia verruculosa* D. Z. Xie on *Indigofera scabrida* Dunn. (Xie 1992). In contrast to those species, a typical *Ovulariopsis* anamorph with long conidiophores and clavate conidia is known in *Phyllactinia guttata* (Wallr.) Lév. s. l. with a broad host range including Fabaceae (Braun 1987).

Less typical anamorphs of *Phyllactinia* species with deviating characteristics on members of the Fabaceae are the anamorphs of

Phyllactinia dalbergiae Piroz. belonging to a group of species with spirally twisted conidiophore bases (Liberato & Barreto 2005), of *Phyllactinia adesmiae* Havryl. on *Adesmia campestris* (Rendle) Rowlee characterized by conidia with basal ring, 40–64 µm × 12–20 µm (Havrylenko 1995), and of *Phyllactinia cassiae* G. J. M. Gorter & Eicker with lanceolate conidia on *Cassia abbreviata* Oliv. (Gorter & Eicker 1987).

The hyphal endings covered by cap-like refractive material found in the *Ovulariopsis* specimen from Taiwan might be an artifact that was not found in the type specimen of *Phyllactinia bauhiniae* var. *cassiae*. They appear somewhat similar to the appendages typical of ascomata of *Bulbouncinula* (now accommodated in *Erysiphe*) or *Typhulochaeta* species (Braun 1987). Both genera are, however, characterized as exclusively ectophytic, and anamorphs are not known in *Typhulochaeta* species (Braun *et al*. 2002).

Though the host ranges of *Phyllactinia* species and their anamorphs need experimental investigation, members of this genus and their *Ovulariopsis* anamorph stages are considered specific to host families (Braun 1987). In a single species, *Phyllactinia guttata*, it is discussible whether it is a species with a broad host range (several plant families) or a complex of several species with narrower host ranges (Braun 1987). Paul & Thakur (2006) described several new species and varieties of *Phyllactinia* based on morphological differences and host genera, including *Phyllactinia bauhiniae* on *Bauhinia* sp. and *Phyllactinia bauhiniae* var. *cassiae* on *Cassia fistula*. The *Ovulariopsis* anamorph of the taxon proposed by Paul & Thakur (2006) for *Cassia fistula* as host is morphologically distinct from all other known *Ovulariopsis* anamorphs. Its distinct morphology and the new record from Taiwan on the same host species indicate that this parasite might be quite host specific, which supports the tendency to split *Phyllactinia* species into small taxa with narrow host ranges. If this will be supported by similar findings in other *Phyllactinia* species, the variety of *Phyllactinia bauhiniae* on *Cassia* species might even be raised to species rank, which would be morphologically supported by larger conidia (61–81 µm long) and ascomata of *Phyllactinia bauhiniae* on *Bauhinia* sp. (Paul & Thakur 2006). In this case, a nomen novum will be required in order to avoid homonymy with another parasite on *Cassia* species, *Phyllactinia cassiae* G. J. M. Gorter & Eicker (Gorter & Eicker 1987). Apparently, a considerable diversity of Erysiphales evolved on *Cassia* species. *Cassia* is also a host genus for a comparatively high number of species of *Cercospora* and *Pseudocercospora* (Nakashima 2004).

Tab. 1. – Characteristics of conidiophores, conidia, appressoria, and haustoria of *Oidium cassiae-siameae* and the *Ovulariopsis* anamorph of *Phyllactinia bauhiniae* var. *cassiae* in specimens from India and Taiwan. Measurements are combined from literature (Paul & Thakur 2006, Yen 1967) and own observations.

Specimen	*Phyllactinia bauhiniae* var. *cassiae*, type, India	*Phyllactinia bauhiniae* var. *cassiae*, Taiwan	*Oidium cassiae-siameae*, type, Taiwan
Conidiophore ornamentation	verruculose	verruculose	verruculose
Conidium ornamentation	reticulate-striate	reticulate-striate	reticulate-striate
Conidiophore size	44–140 µm × 5–8 µm	42–128 µm × 4–6 µm	63.6–103 µm × 7–12 µm
Conidium size	34–49 µm × 12–18 µm	(33–)35–45(–60) µm × 11–14(–15)	23–36 µm × 10–16.8 µm
Appressoria	nipple-shaped to slightly lobed	nipple-shaped to slightly lobed	slightly to strongly lobed (Yen 1967)
Haustorium localization	mesophyll	mesophyll	epidermis

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