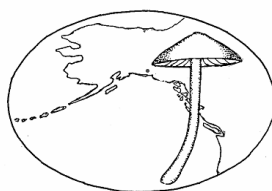


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First Report of *Erysiphe (Uncinuliella) flexuosa* in western North America

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Abstract: *Erysiphe flexuosa*, a powdery mildew parasite of *Aesculus* species, is believed to have originated in North America where distribution records were confined to regions east of the Rocky Mountains. The fungus recently was found in eastern Washington State and northern Idaho. The fungus can be distinguished readily from other powdery mildews attacking *Aesculus* species by the presence of two distinctive kinds of chasmothecial appendages. Consistent with previous reports on similar species, observations determined that subulate appendages formed first, on dorsal sides of chasmothecia. The longer, uncinuate appendages formed equatorially during later chasmothecial maturation. There are no *Aesculus* species native to northwest North America that could have hosted an undetected population of the fungus. It appears likely that *E. flexuosa* became established relatively recently in the inland Pacific Northwest, possibly being introduced on infected host material, similar to the situation in Europe where several reports documented the presence of the fungus beginning in 2000.

Key words: *Uncinula*, *Uncinuliella*, *Erysiphe*, *Aesculus*, powdery mildew, biogeography, fungal morphology, ascocarp appendage, chasmothecia, exotic plant pathogen.

Introduction: The powdery mildew fungus *Erysiphe flexuosa* (Peck) U. Braun & S. Takam. originally was described as *Uncinula flexuosa* Peck (Braun and Takamatsu, 2000). Although chasmothecia (ascocarps termed perithecia or cleistothecia by some authors) of the fungus bear uncinuate appendages resembling those in species traditionally classified in *Uncinula*, it also produces shorter, subulate appendages (Braun, 1987). Demonstration that these two kinds of appendages are distinctly different, and not merely developmental stages of the same kind of appendage, led Braun (1981) to make the new combination *Uncinuliella flexuosa* (Peck) U. Braun for this fungus. Subsequent analysis of rDNA sequence data suggested that teleomorph characters such as chasmothecial appendages are not reliable indicators of phylogenetic relationships (Braun et al., 2002), leading Braun and Takamatsu (2000) to make the combination *Erysiphe flexuosa* which is used herein.

Erysiphe flexuosa is relatively easy to distinguish from other powdery mildew fungi because of the distinctive chasmothecial morphology and the host range that is restricted to species of *Aesculus* (Hippocastanaceae). Other species of Erysiphales recorded from Hippocastanaceae include *Phyllactinia guttata* (Wallr.:Fr.) Lév., distinguished on the basis of acicular chasmothecial appendages and fusiform-clavate conidia, and species of *Sawadaea*, distinguished on the basis of dichotomously or trichotomously branched chasmothecial appendages and synanamorphs producing both microconidia and macroconidia with fibrosin bodies (Braun, 1987).

Although the fungus has remained unreported from the western USA (regions

west of the Rocky Mountains), *Erysiphe flexuosa* is thought to be native to North America (Braun, 1987; Zimmermannová-Pastirčáková et al., 2002). Ing and Spooner (2002) also noted another possibility—that the fungus is native to Europe but until recently was undetected. European reports of the fungus began appearing in 2000 and it is now known to occur in Croatia, France, Germany, Poland, Slovakia, Switzerland, United Kingdom (Zimmermannová-Pastirčáková et al., 2002 and references therein), Hungary (Kiss et al., 2003), Slovenia (Milevoj, 2004), Ukraine (Heluta and Vojtjuk, 2004), Lithuania (Grigaliūnaitė et al., 2005) as well as Serbia and Montenegro (Ranković and Mišić, 2006). Recently the authors examined collections of the fungus from Moscow, Idaho and Pullman, Washington. This paper documents for the first time the occurrence of this fungus in North America west of the Rocky Mountains. In addition, information is presented on chasmothecial development, particularly on relative timing of development of the two kinds of chasmothecial appendages characteristic of this fungus.

Materials and Methods: Samples of *Aesculus* sp. leaves collected from trees growing in urban landscapes, and bearing *E. flexuosa* were examined and photographed using a Leica DMR compound microscope and a Leica MZ95 stereo microscope, both equipped with Leica DC300 digital cameras. The following material was characterized in this study: Glawe 03-383, from *Aesculus* sp., University of Idaho campus, Moscow, Latah Co., Idaho, coll. September 4, 2002 by Jennifer Fallacy; Glawe 02-90, from *Aesculus* sp., Pullman, Whitman Co. Washington, coll. Autumn, 2005 by Wayne Shull; Glawe 06-609, from *Aesculus* sp., University of Idaho Arboretum, Latah Co.,

Idaho, coll. September 13, 2006 by F. M. Dugan; Glawe 06-610 from *Aesculus* sp., Pullman, Whitman Co., Washington, collected September 4, 2006 by F. M. Dugan. Voucher material was deposited with the Mycology Herbarium in the Department of Plant Pathology, Washington State University (WSP).

Results: Morphological features of the fungus were as follows: mycelia on leaves amphigenous, sparse to whitish. Chasmothecia initially whitish to pale yellow to amber-colored (Figs. 1-3), becoming black at maturity (Figs. 4-5), (135-)140-160(-180) μm , dehiscence appearing to result from separation of basal hyphae from vegetative mycelium (Figs. 6-8). Appressoria on vegetative hyphae were lobed (Fig. 9). Chasmothecia with two forms of appendages: subulate appendages (Figs. 10, 11) formed primarily on dorsal surfaces of chasmothecia, tapering toward apices, walls displaying encrustation-like ornamentations, (20-)22.5-39(-41) \times (3-)3.5-7(-8) μm ; uncinat appendages (Fig. 10) primarily equatorial, rather narrow at base and widening toward middle, distal portions geniculate to helically-twisted, strongly recurved at apices, (110-)135-165(-225) \times 7-10(-10.5) μm . Asci (Fig. 12) clavate, short-stipitate, (52.5-)54-66.5(-67) \times (24-)36.5-40 μm , containing 5-8 ascospores. Ascospores (Fig. 13) hyaline, ellipsoid-ovoid, (16-)17.5-24.5(-27.5) \times (7-)9-11(-13) μm . Anamorph scarce, conidia short-cylindrical to ovoid (Fig. 14), (22-)22.5-27.5 \times (10.5-)11-14.5 μm , germ tube originating at site near end of conidium (Fig. 15).

Discussion: Based on morphological features of chasmothecia and conidia, the powdery mildew fungus on *Aesculus* sp. was determined to be *E. flexuosa*. Previous reports of the fungus, on several species of *Aesculus*, included Georgia, Indiana, Iowa, Mississippi, New York, North Carolina, Pennsylvania, and Vermont (Farr et al., n. d.). The only powdery mildew fungus reports on *Aesculus* from a western state were *Phyllactinia guttata* (Wallr.:Fr.) Lév.

and *Phyllactinia coryli* (Pers.) P. Karst. (regarded by Braun [1987] as synonymous with *P. guttata*), both of which were from California (Farr et al., n. d.). *Phyllactinia guttata* is easily differentiable from *E. flexuosa* on the basis of its acicular chasmothecial appendages (Braun, 1987).

The current study appears to be the first to document formally the presence of *E. flexuosa* in North American west of the Rocky Mountains. Collections made by students and retained in the teaching herbarium of Lori Carris at the Department of Plant Pathology, Washington State University, indicate that the fungus also was collected by students in 2002 and 2004 (L. Carris, personal communication). A photograph of a chasmothecium from the former collection was published (Callan and Carris 2004) but without reference to geographic origin of the specimen or geographic distribution of the species. Interestingly, the fungus has not been found in the Pacific Northwest west of the cascade mountains. The fungus is not listed in records of the Oregon State University Plant Disease Diagnostic Clinic covering the last 50 years of samples submitted to the clinic (J. Pscheidt, personal communication). Nor was the fungus encountered in western Washington despite several years of active collecting of Erysiphales in the region (Glawe, 2004). Preston (1976) included information on geographical distributions for seven species of *Aesculus* native to the USA, of which only *A. californica* (Spach) Nutt. was native to the western USA. On the basis of existing information it is reasonable to hypothesize that *E. flexuosa* arose on one of the native North American *Aesculus* species somewhere in the eastern USA. The fungus most likely was introduced on infected plant material into the inland Pacific Northwest. Perhaps the use of appropriate molecular markers would allow testing this hypothesis by assessing genetic variation of populations of this fungus on native *Aesculus* species in the eastern USA and comparing them with newly found populations in Europe and the

northwestern USA. Mycologists and plant pathologists in coastal Washington and Oregon might anticipate the eventual arrival of *E. flexuosa*, given its geographic proximity in eastern Washington and Idaho.

Observations on chasmothecia in differing states of maturity revealed developmental patterns consistent with previous work by Takamatsu et al. (2003) on the similar species *Erysiphe wadae* S. Takam. & Y. Sato. They (Takamatsu et al., 2003) observed two kinds of appendages arising from different parts of chasmothecia and produced at different stages of chasmothecial development. Subulate appendages were produced early in chasmothecial development from peridial cells on the dorsal surface of chasmothecia. Uncinate appendages were produced later and were equatorial. In the present study chasmothecia formed dorsal, subulate appendages early in development. They were distinguishable from hyphae on ventral surfaces that appeared to connect developing chasmothecia to vegetative mycelia. Uncinate appendages were produced later from chasmothecia that were larger in size and with peridial cells that were becoming melanized. Ultimately, chasmothecia bore both subulate and uncinete appendages. Chasmothecia eventually dehiscid from vegetative mycelia and on the ventral surface exhibited a concave depression with remnants of basal hyphae.

The chasmothecial morphology and uncinete appendages of *E. flexuosa* are reminiscent of features of species traditionally included in *Uncinula* but more recently included in *Erysiphe*, such as *Erysiphe necator* Schwein (Braun and Takamatsu, 2000). Observations reported herein for *E. flexuosa* resemble those reported by Gadoury and Pearson (1988) for development of *E. necator* chasmothecia. In that fungus, chasmothecia also produced equatorial, uncinete appendages, basal anchoring hyphae, and became ventrally concave as

they matured. They (Gadoury and Pearson, 1988) reported that the appendages appeared effective in attaching dispersed chasmothecia to host bark, suggesting that uncinete appendages in *E. flexuosa* might function similarly in attaching chasmothecia to substrates following dispersal.

The function or functions of subulate appendages is unclear. The position on the dorsal surface of the chasmothecium is reminiscent of the site of origin of digitate cells of *P. guttata*, which also produces a different kind of equatorially-arranged appendages, the acicular appendages (Braun, 1987). In *P. guttata*, the acicular, equatorial appendages dislodge mature chasmothecial from host leaves (Cullum and Webster, 1977). The digitate cells form a mucilaginous mass that attaches dispersed chasmothecia to substrates prior to ascospore discharge (Cullum and Webster, 1977; Webster, 1979). In contrast to the mucilaginous material formed by deliquescing digitate cells in *P. guttata* (Hartney et al., 2005), subulate appendages in *E. flexuosa* observed in the present study did not undergo deliquescence but retained their integrity even after uncinete appendages matured.

While determining a clear role or roles for subulate appendages will require further research, it seems noteworthy that they form early and develop heavily encrusted, durable cell walls. Perhaps subulate appendages could play a role in discouraging small arthropods from consuming chasmothecia. Regardless of the functional attributes of subulate appendages, it appears likely that their evolution has been favored by strong selection pressures because Takamatsu et al. (2003) reported that analysis of rDNA ITS regions suggested that subulate appendages evolved independently in several clades of Erysiphales. The putative multiple origins of subulate appendages is reminiscent of suggestions based on molecular data that both uncinete and dichotomously branched chasmothecial

appendages also evolved independently in different lineages (Takamatsu, 2004). Such findings suggest that investigating the role of chasmothecial appendages in *E. flexuosa* could provide significant insight into the biology and ecology of this fungus.

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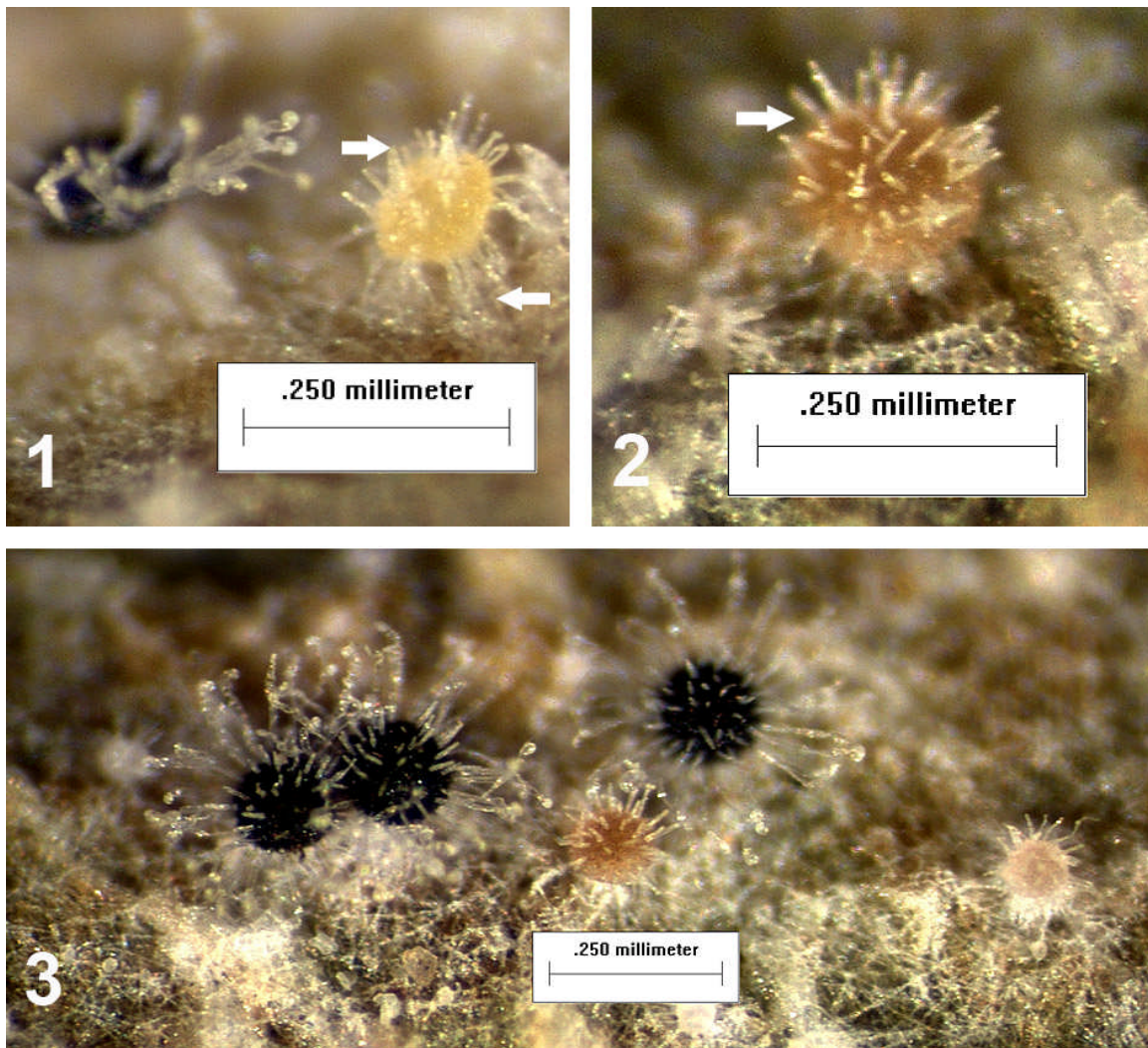
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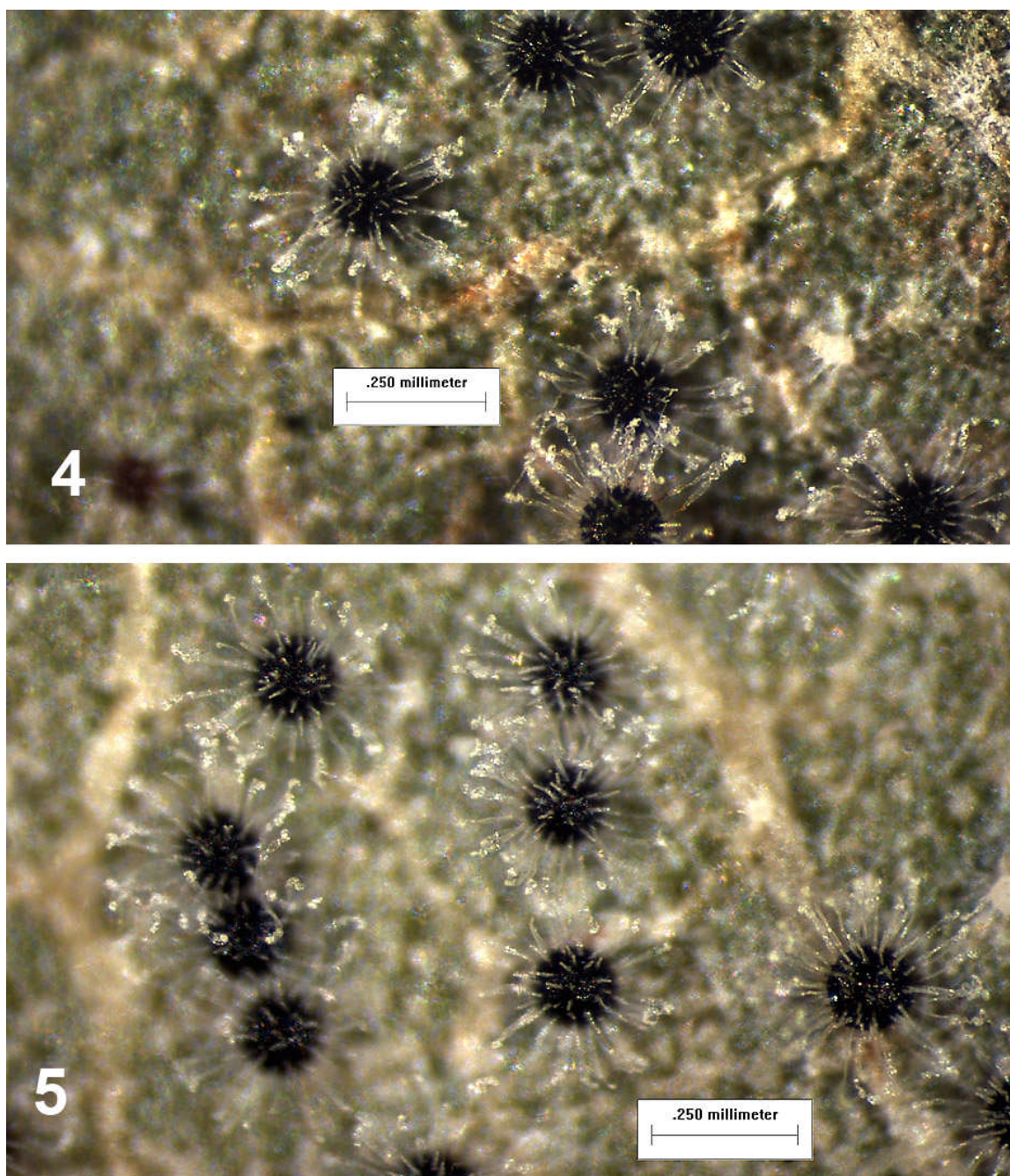
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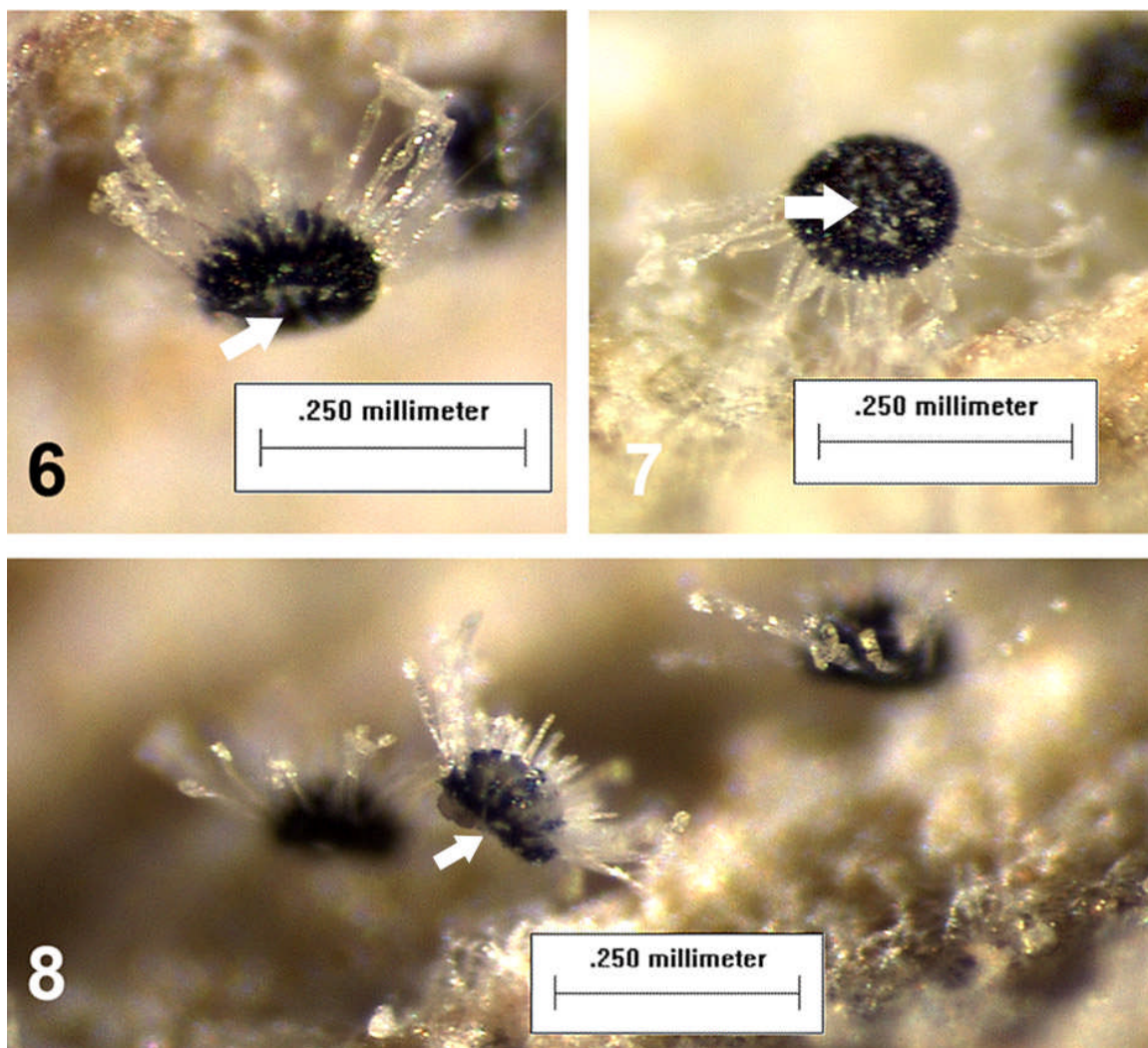
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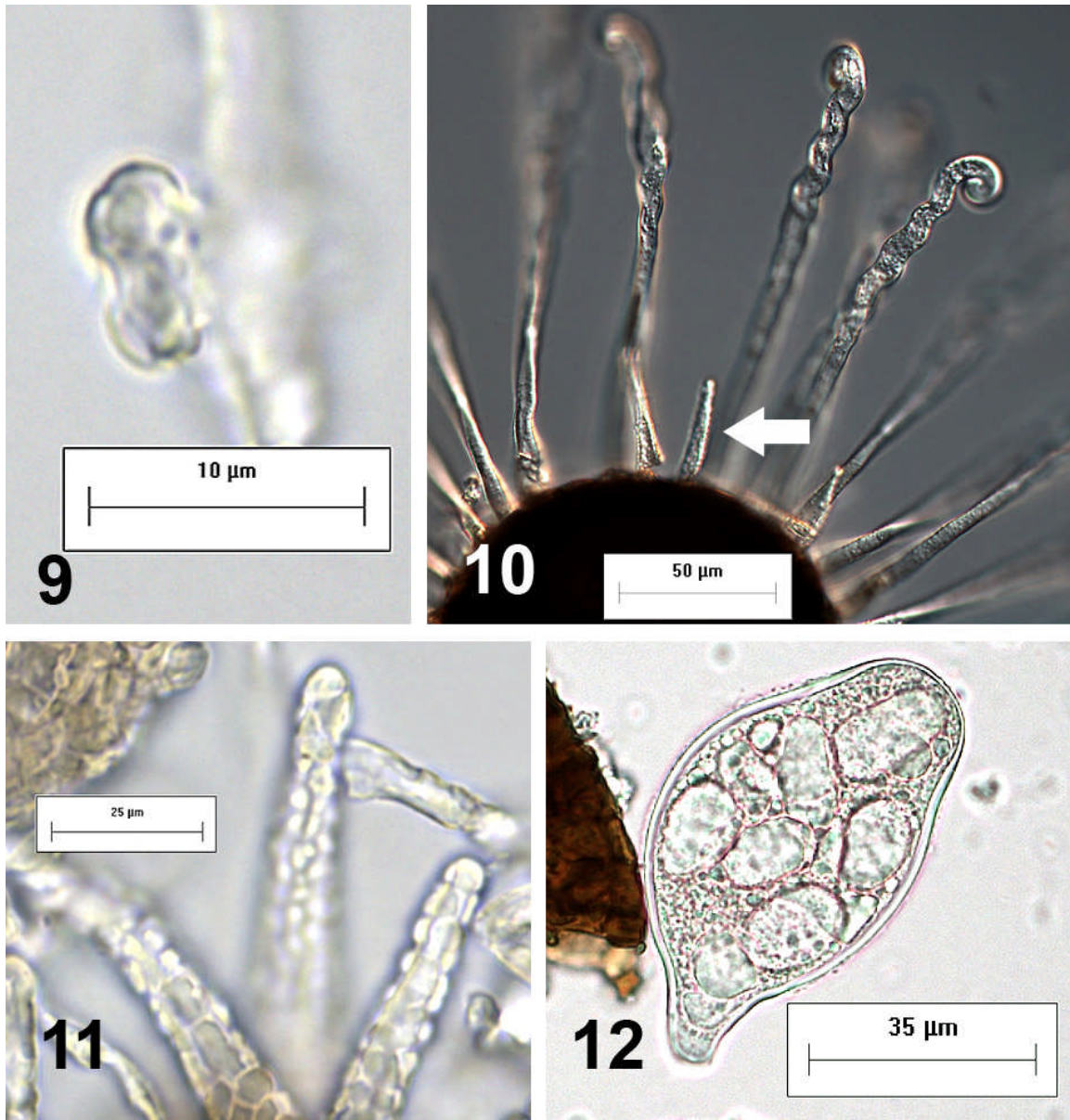
Figs. 1-3. *Erysiphe flexuosa*. 1. Immature chasmothecium; upper arrow denotes developing subulate appendage, lower arrow denotes hyphae connecting chasmothecium to thallus. Note black chasmothecium on left that has produced uncinuate, more-or-less equatorial appendages. Scale bar = 0.25 mm. 2. Immature chasmothecium bearing subulate appendages (arrow). Scale bar = 0.25 mm. 3. Chasmothecia of different developmental stages, ranging from immature (pale yellow to amber chasmothecia lacking long, uncinuate appendages) to mature (black chasmothecia with long, uncinuate appendages). Scale bar = 0.25 mm.



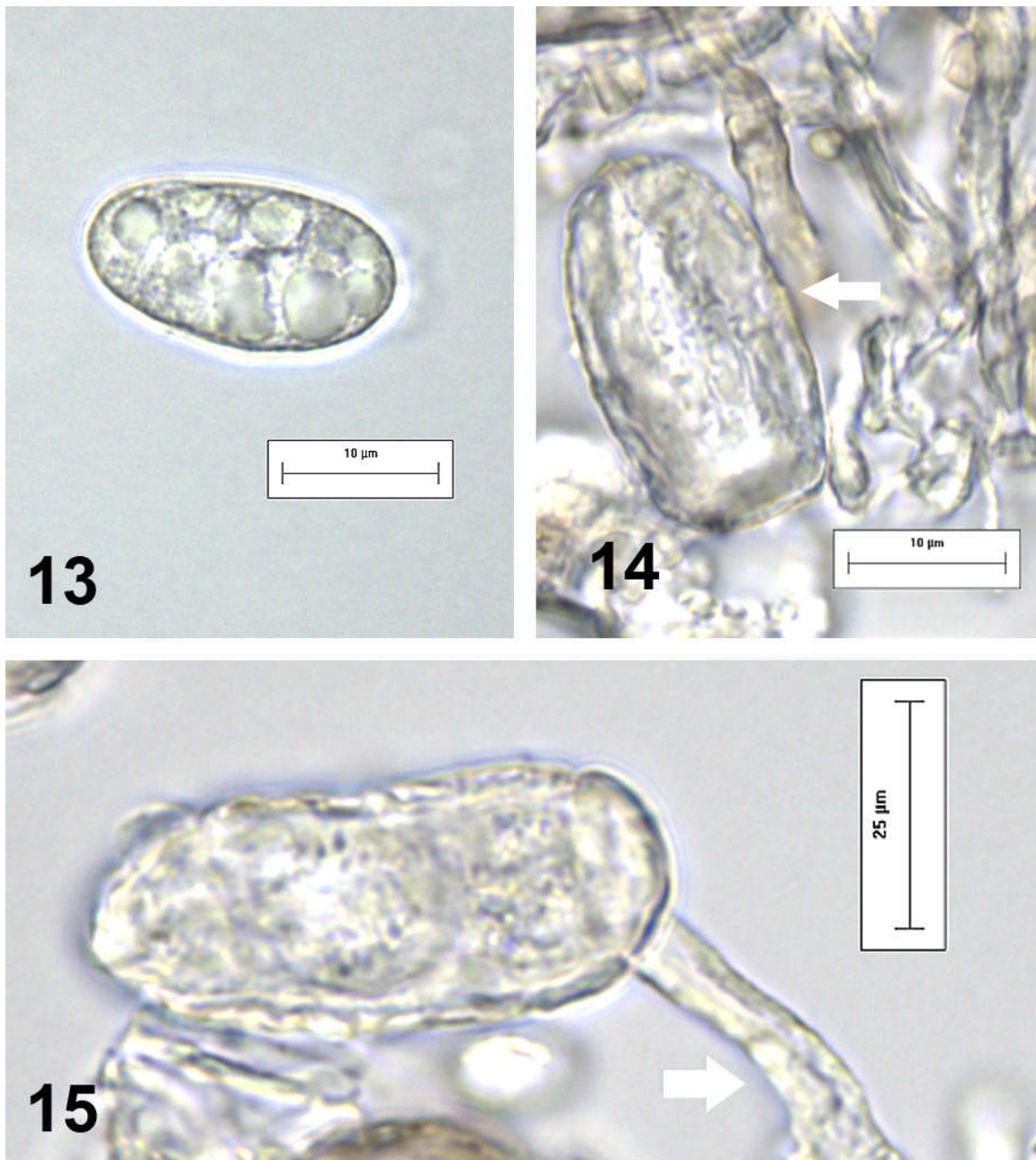
Figs. 4, 5. *Erysiphe flexuosa*. Chasmothecia exhibiting both uncinata and subulate appendages. Scale bars = 0.25 mm.



Figs. 6-8. *Erysiphe flexuosa*. Dehiscent chasmothecia. 6. Chasmothecium exhibiting concave ventral surface (arrow). 7. Ventral surface of chasmothecium exhibiting remnants of anchoring hyphae. 8. Chasmothecium exhibiting concave ventral surface, subulate appendages on dorsal surface, and equatorially-arranged uncinuate appendages. Scale bars = 0.25 mm.



Figs. 9-12. *Erysiphe flexuosa*. 9. Lobed appressorium. Scale bar = 10 µm. 10. Chasmothecium exhibiting short, subulate appendages (one designated by arrow) and longer, helically coiled uncinuate appendages. Scale bar = 50 µm. 11. Subulate appendages exhibiting wall ornamentation. Scale bar = 25 µm. 12. Ascus. Scale bar = 35 µm.



Figs. 13-15. *Erysiphe flexuosa*. 13. Ascospore. Scale bar = 10 µm. 14. Conidium (designated by arrow). Scale bar = 10 µm. 15. Conidium with germ tube (arrow). Scale bar = 25 µm.