

Ultrastructure of the D-haustoria of *Coleosporium* spp. (rust fungi, Uredinales)*

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The ultrastructure of D-haustoria of seven *Coleosporium* species was investigated. The species were selected to represent host plants of different systematic positions. D-haustoria were similar in all species investigated. Haustoria each had a ± bulb-shaped haustorial body and were morphologically similar to D-haustoria of many other rust fungi. They differed, however, from typical D-haustoria by the ultrastructure of the haustorial neck. The neck was wrapped by a fold of the extra-haustorial membrane (velopedunculate type of D-haustorium). The importance of haustorial ultrastructure for the systematics of *Coleosporium* and Melampsoraceae *s. l.* is discussed.

Keywords: *Coleosporium*, D-haustoria, ultrastructure, systematics.

The haustoria of rust fungi which develop after infection by dikaryotic spores, i.e., aecio- or urediniospores, are called D-haustoria. Typical D-haustoria form a variably shaped haustorial body and a slender haustorial neck which are wrapped by the extrahaustorial membrane. By the ultrastructure of the neck region two groups of D-haustoria can be distinguished. In the melampsoraceous rust genera *Cronartium*, *Hyalopora*, *Milesina*, *Pucciniastrum* (*p.p.*), and *Uredinopsis* the haustorial necks are covered by a fold of the extrahaustorial membrane (Berndt, 1993; Gray & al., 1982; Khan & al., 1982), and they were thus designated as 'velopedunculate haustoria' by Berndt & al. (1994). The species of Pucciniaceae which have been investigated so far possess a haustorial neck which is not covered by a fold of the extrahaustorial membrane (Littlefield & Heath, 1979; Harder & Chong, 1984 and references therein) and is therefore called 'gymnopedunculate' (Berndt & Oberwinkler, 1995). *Coleosporium* belongs to the Melampsoraceae *s. l.* and is supposed to have a systematic position close to the genus *Cronartium* because of the blister-like aecia both genera produce on *Pinus* spp. (Gäumann, 1959). To obtain further evi-

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dence for the systematic position of the genus, the haustorial ultra-structure of *Coleosporium* was investigated and compared with that of *Cronartium* and other melampsoraceous genera.

Coleosporium is a large genus with mostly extratropical distribution. It is quite uniform morphologically without true teliospores and basidia produced in crusts beneath the host epidermis. *Coleosporium* species whose basidia are arranged in several layers or are truly catenulate sometimes were placed in a separate genus *Stichopsora*.

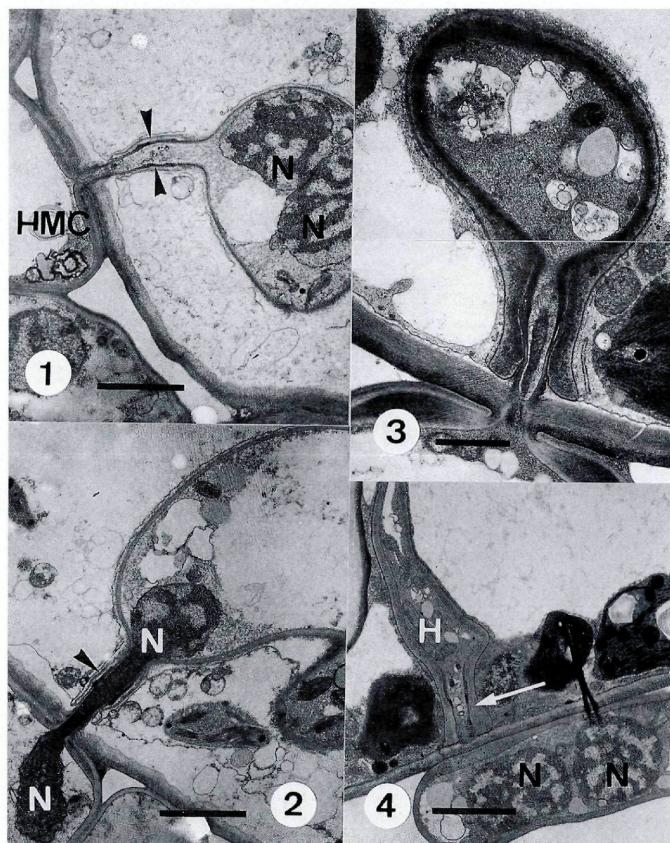
The author has knowledge of about 120 legitimately described *Coleosporium* species. As species delimitation is sometimes poor, however, several species may not prove to be separate ones. The host range of *Coleosporium* is rather wide. Most species occur on dicots in the dikaryophase, especially on members of families of the Lamiidae and Asteridae with a concentration on Asteraceae. Fewer species parasitize species of Ranunculaceae and rosoid and dilleniid families. *Coleosporium* apparently has not been found on members of Caryophyllidae and Hamamelididae. Two of the investigated *Coleosporium* species, *C. asterum* and *C. paraphysatum*, are on Asteraceae. *C. asterum* was selected to represent the *Stichopsora* segregate of *Coleosporium*. *C. clematidis* was chosen as a parasite of the Ranunculaceae, a more "primitive" host family. *C. paederiae* and *C. domingense* both occur on species of Gentianales (Cornidae), *C. ipomoeae* on the lamiid Convolvulaceae.

Material and methods

Infected plants were collected in the field. Small pieces of infected leaves were fixed in 2% glutaraldehyde in 0.1 M cacodylate-buffer for transmission electron microscopy. Specimens were postfixed for 1h in 1% OsO₄ dissolved in cacodylate-buffer, washed with distilled water (6 times) and stained with 1% aqueous uranyl acetate for 1h. The samples were dehydrated in a graded acetone series (10% – 100%) and embedded in Spurr's resin (Spurr, 1969). Blocks were sectioned with a diamond knife using a Reichert-Jung Ultratome-E. Sections, about 80 nm thick, were collected on formvar-coated one-hole grids, stained with lead citrate (Reynolds, 1963) and examined with a Zeiss EM 109.

Species investigated

Coleosporium asterum (Dietel) P. & H. Sydow. Taiwan, Prov. Taichung, Ku Kwan recreation area, on *Aster formosana* Hayata, 21. 4. 1990, leg. R. Berndt. – *Coleosporium clematidis* Barclay. Taiwan, Prov. Nantou, NE Puli, Hui Sun recreation area, on *Clematis* sp., 24. 6. 1990, leg. R. Berndt. – *Coleosporium domingense* (Berk.)



Figs. 1-4. - 1-2. *Coleosporium euphrasiae*. - 1. HMC and haustorium. The neck fold (arrowheads) is inconspicuous. The nuclei (N) lie in the haustorial body. Scale bar = 2 μ m. - 2. Immature haustorium. A nucleus (N) passes from the HMC through the haustorial neck into the haustorial body. The neck fold (arrowhead) is already fully developed. Scale bar = 2 μ m. - 3. *Coleosporium asterum*, HMC and haustorium. Scale bar = 1 μ m. - 4. *Coleosporium paraphysatum*, young haustorium (H). The nuclei (N) are still in the HMC. The haustorial neck is already wrapped by the neck fold (arrow). Scale bar = 2 μ m.

Arthur. Venezuela, Distr. Federal, Caracas, Botanical Garden, on *Plumeria* sp., 15. 11. 1993, leg. R. Berndt. – *Coleosporium euphrasiae* (Schum.) Winter. Germany, Pfullingen, slopes of the Ursulaberg, on *Rhinanthus aristatus* (Čelak) Hauskn., 25. 7. 1987, leg. R. Berndt. – *Coleosporium ipomoeae* (Schw.) Burr. Costa Rica, Prov. Cartago, near Paraiso, on *Ipomoea* sp., 28. 3. 1991, leg. R. Berndt. – *Coleosporium paederiae* Dietel ex Hirats. f. Taiwan, Prov. Taichung, Ku Kwan recreation area, on *Paederia* cf. *scandens* (Lour.) Merr., 15. 7. 1990, leg. R. Berndt. – *Coleosporium paraphysatum* Dietel & Holway. Costa Rica, Prov. Alajuela, San Ramón, at the trail to the Río S. Lorencito field station of the University of Costa Rica, on *Liabum* sp., 21. 3. 1991, leg. R. Berndt.

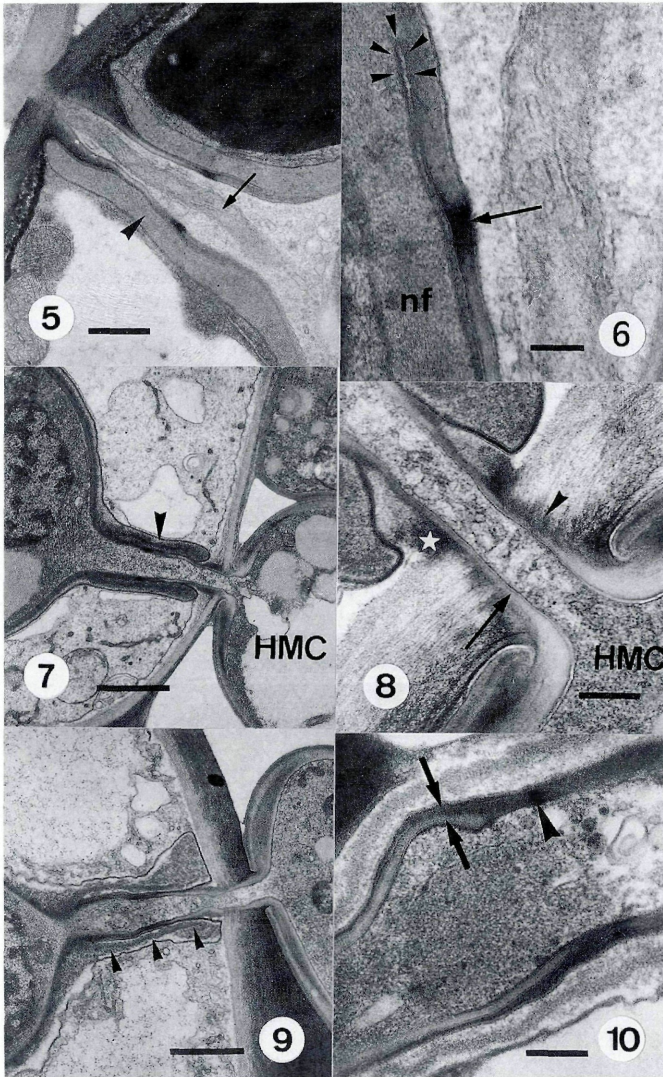
Results

The morphology and ultrastructure of the D-haustoria of all *Coleosporium* species investigated were similar. D-haustoria were delicately stalked and had a simple, bulb-shaped to oblong haustorial body (Figs. 1-3).

Extrahaustorial membrane

A more or less tubular fold (neck fold) of the haustorial membrane covered the haustorial neck along its entire length (Figs. 3-5, 7, 9, 11). The origin of the fold was marked by a sharp bend of the extrahaustorial membrane distal to the neckband (Figs. 5, 6). The bend was positioned at the distal part of the neck in front of the transition between neck and haustorial body. From the bend the membrane continued backwards along the haustorial neck and finally turned upwards

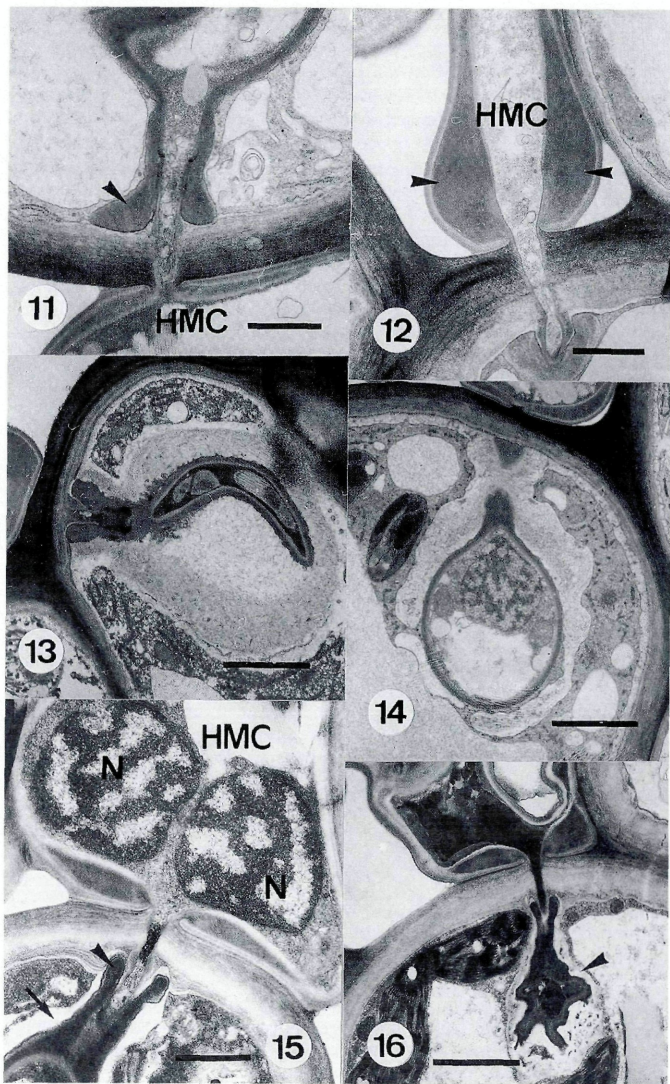
Figs. 5-10. – 5-6. *Coleosporium paraphysatum*. – 5. Haustorial neck. The content of the neck fold (arrowhead) is homogeneous. A mitochondrion can be seen (arrow) within the neck. Scale bar = 0.5 μm . – 6. Detail of Fig. 5. showing the distal part of the neck with neck fold (nf), neckband (arrow) and the sharp bend of the extrahaustorial membrane (arrowheads). Scale bar = 0.1 μm . – 7-8. *Coleosporium ipomoeae*. – 7. HMC and proximal part of the haustorium. The neck fold (arrowhead) covers the entire length of the neck. Scale bar = 1 μm . – 8. HMC and haustorial neck at the penetration site. The wall of the HMC reveals several differently stained layers at the penetration site. Two fungal wall layers can be discerned in the penetration peg. The outer "layer" forms a fuzzy halo (arrowhead) within the host cell wall and has an irregular outline. The inner layer (arrow) is thin, distinct and less electron-dense. Material of the outer "layer" seems to form the swelling (star) at the base of the neck. Scale bar = 0.2 μm . – 9. *Coleosporium clematidis*. Neck fold with indistinct band of aggregated electron-dense material (arrowheads). Scale bar = 1 μm . – 10. *Coleosporium euphrasiae*. Haustorial neck. Proximal to the neckband (arrowhead) the wall of the neck consists of an inner, moderately electron-dense layer and an outer layer that is more electron-dense (arrows). The inner layer wedges out towards the neckband. Scale bar = 0.2 μm .



again to enclose the haustorial body. The contents of the neck fold were granular. In most species investigated the granular material was distributed non-homogeneously and partly aggregated in an ill-defined layer in the middle of the neck fold (Figs. 2, 9). Sometimes the material formed a distinct band which extended through the length of the neck fold (Fig. 9). The layer of aggregated material in the neck fold seemed to be continuous with the dense granular or flocculent material which was accumulated in the extrahaustorial matrix adjacent to the fungal wall (Figs. 3, 7). In *C. domingense*, *C. paraphysatum* (Figs. 5, 11, 13), and the necrotic haustoria of *C. paederiae* (Figs. 15, 16) the contents of the neck fold were distributed almost homogeneously and moderately electron-dense. The formation of the neck fold took place early in the ontogeny of the haustoria. The fold was already present in young haustoria where nuclei had not passed from the haustorial mother cell (HMC) into the haustorial body (Fig. 4) or were about to stream into the body (Fig. 2). The neck fold was variously shaped in the different *Coleosporium* spp. (Figs. 2, 3, 5, 7, 11), but there was also some infraspecific variability. Therefore, in most *Coleosporium* spp. investigated, the extension and form of the neck fold could not be used as a feature to definitely distinguish the species. In *C. euphrasiae* the neck fold was consistently extremely thin and inconspicuous (Figs. 1, 2).

A collar formed by host wall material sometimes was present at the haustorial neck. In several haustoria material of the collar enveloped the entire haustorium (Figs. 13, 14). Some of the enveloped haustoria did not show obvious differences to non-enveloped haustoria and appeared to be vigorous (Fig. 14). Other haustoria were deformed and apparently necrotic (Figs. 13, 16). In *C. paederiae* haustoria became necrotic and compressed by the encapsulation before the nuclei passed into the haustorial body from the HMC (Fig. 15). Thus it can be concluded that in these haustoria encapsulation took place early in haustorial ontogeny.

Figs. 11–16. – 11–14. *Coleosporium domingense*. – 11. Penetration peg, neck and proximal haustorial body. The content of the neck fold (arrowhead) is more or less homogeneously moderately electron-dense. Scale bar = 1 μm . – 12. HMC and basal part of the neck. The lumen of the HMC is extremely narrow. The thickening of the wall of the HMC is due to a prominent electron dense layer (arrowheads). Scale bar = 1 μm . – 13. Encapsulated haustorium. The haustorial body is compressed, and the haustorium appears to be necrotic. Scale bar = 2 μm . – 14. Haustorium that is almost encapsulated. The haustorium is not deformed and seems to be vigorous. Scale bar = 2 μm . – 15–16. *Coleosporium paederiae*. – 15. HMC and haustorial neck. The haustorium is necrotic and surrounded by an electron-translucent layer (arrow) of host wall material. The nuclei (N) lie in the HMC in front of the penetration site. The neck fold (arrowhead) is already present. Scale bar = 1 μm . – 16. Necrotic D-haustorium with deformed haustorial body. The haustorium is encapsulated by host wall material (arrowhead). Scale bar = 2 μm .



Haustorial mother cell, penetration peg and haustorial neck

The wall of each HMC was thickened at the penetration site. The thickened wall consisted of several layers which could be distinguished by various electron-densities (Figs. 3, 8, 15). In a few HMCs the wall was extraordinarily thick around the penetration site (Fig. 12). This thickening was due to the extremely thick, electron-dense central wall layer of the HMC. In most haustoria two layers of the fungal wall could be distinguished within the penetration peg (Fig. 8). The inner layer was thin, rather distinct and moderately electron-dense. The outer layer was more electron-dense, indistinct, and had a somewhat fuzzy appearance. The inner layer continued in the wall of the haustorial neck. The material of the outer layer seemed to form the more electron-dense swelling which could be observed at the base of most haustorial necks (Figs. 8, 9). In most species material of the outer layer could not be traced more distally at the neck. In *C. euphrasiae*, however, the outer layer persisted, albeit very thin, and became more prominent again proximally to the neckband (Fig. 10).

In all species a neckband formed at about one third or half of the length of the neck. In section the neckband was short, \pm homogeneously electron-dense and in most cases extended farther up and down along the binding plasmalemma (Fig. 6). Beyond the neckband the diameter of the neck slightly enlarged (Figs. 5, 9, 11).

Discussion

Occurrence of velopedunculate D-haustoria

Velopedunculate D-haustoria were described from *Cronartium* spp. by Gray & al. (1982), Khan & al. (1982), and Longo & Bruscazioni (1986). This haustorial type has since been found in species of *Calyptospora*, *Hyalospora*, *Milesina*, *Pucciniastrum* (*p.p.*), and *Uredinopsis* (Berndt & al., 1994; Berndt & Oberwinkler, 1995). All these genera belong to Melampsoraceae (*sensu* Sydow & Sydow, 1915). Hitherto, velopedunculate haustoria have not been found in non-melampsoraceous species. Velopedunculate D-haustoria thus should be considered a special feature of Melampsoraceae. Because of the assumed close systematic position of *Cronartium* and *Coleosporium* it was not unexpected to find velopedunculate haustoria also in the latter genus. Although only 7 *Coleosporium* spp. were investigated for haustorial ultrastructure, the similarity of D-haustoria of *Coleosporium* spp. from both "primitive" and more "advanced" host plants suggests that velopedunculate D-haustoria are a character common to all *Coleosporium* spp.

Morphology and ultrastructure of D-haustoria

The haustorial body of D-haustoria of *Milesina* spp. and *Uredinopsis filicina* (Niessl) Magnus was branched apically. The *Coleosporium* haustoria were morphologically similar to the D-haustoria of *Cronartium*, *Hyalopsora*, and *Pucciniastrum* (p. p.) which are unbranched (Berndt, 1993; Colley, 1918). The ultrastructure of D-haustoria of *Coleosporium* spp. was similar to that of velopedunculate D-haustoria of *Cronartium*, *Hyalopsora*, *Pucciniastrum* (p.p.), and *Uredinopsis filicina* (Berndt & al., 1994; Berndt & Oberwinkler, 1995; Longo & Brusaglioni, 1986). D-haustoria of *Milesina* spp. and associated *Uredo* spp. differ from other velopedunculate D-haustoria by the occurrence of complex neckbands with two clearly distinguishable electron-dense neckrings (Berndt & al., 1994). A two-layered fungal wall within the penetration peg as revealed in *Coleosporium* spp. also could be found in *Milesina* spp., *Uredo* spp. and *Uredinopsis filicina*. The fuzzy appearance of the outer fungal wall layer in the penetration peg of *C. ipomoeae* haustoria is quite similar to the fibrillar wall layer in *Milesina blechni* Sydow (Berndt & al., 1994). The fuzzy or fibrillar structure of that wall layer may be determined by the fibrillar structure of the surrounding host cell wall. Thus the electron-dense outer "layer" could rather be regarded as a \pm indistinct halo much influenced by the structure of the host cell wall than a distinct fungal-determined layer.

As most *Coleosporium* spp. revealed a rather similarly shaped neck fold, and as there was some variation in the form and extension, the neck fold usually could not be used as a character to distinguish species. In *C. euphrasiae*, however, the shape of the fold was very characteristic and may distinguish the species from other *Coleosporia*.

Beside the neck fold velopedunculate D-haustoria do not differ essentially from gymnopedunculate D-haustoria (Harder & Chong, 1984; Harder & Chong, 1991 and references therein).

Ontogeny of velopedunculate haustoria

No early ontogenetic stages of haustorium formation could be found in *Coleosporium* spp. although numerous haustoria were investigated in each species. It is not clear, therefore, how the neck fold is formed. Probably, the fold is formed early in ontogeny, as it was already fully developed in several haustoria when the nuclei still had not passed into the haustorial body. Formation of the fold may thus be contemporaneous with the inflation of the haustorial body, the process which precedes migration of the nuclei.

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