

Ultrastructure of the parasitic interface of *Pucciniastrum*, *Thekopsora*, *Naohidemyces*, and *Calyptospora* (Uredinales, Pucciniastraceae) in the dikaryotic stage*

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The ultrastructure of dikaryotic haustoria of seven *Pucciniastrum* species, *Thekopsora galii*, *Naohidemyces vaccinii*, and *Calyptospora goeppertiana* was investigated. *Pucciniastrum actinidiae*, *P. agrimoniae*, *P. pyrolae*, and *Calyptospora goeppertiana* revealed haustoria whose necks were wrapped by a fold of the extrahaustorial matrix. The matrix-fold of *Calyptospora goeppertiana* was characteristically shaped. *Pucciniastrum circaeae*, *P. epilobii*, *P. hikosanense*, *P. styracinum*, *Thekopsora galii*, and *Naohidemyces vaccinii* showed typical haustorial necks which were not sheathed by a matrix-fold. Haustorial necks which were wrapped by a fold of the extrahaustorial matrix were designated "velopedunculate," and those which were naked "gymnopedunculate." The application of haustorial ultrastructure as a character for use in systematics is discussed.

Key Words—D-haustoria; *Pucciniastrum* s.l.; systematics; ultrastructure.

Introduction

The ultrastructure of dikaryotic haustoria of rust fungi has been studied mostly in species of *Puccinia* and *Uromyces*. Investigations of species of other genera are scanty. Mims and Gidewell (1978) investigated haustoria of *Gymnosporangium juniperi-virginianae* Schw. (Pucciniaceae), and Koch et al. (1983) examined those of *Phakopsora pachyrhizi* Sydow (Phakopsoraceae). *Melampsora* (Melampsoraceae s. str.) was studied by Littlefield and Bracker (1972) and Longo and Naldini (1972). D-haustoria of species of *Cronartium* (Cronartiaceae) were described by Gray et al. (1982), Khan et al. (1982) and Longo and Bruscaiglioni (1986). Berndt et al. (1994) investigated haustoria of the genera *Milesina*, *Uredinopsis* and *Hyalopsora* (Pucciniastraceae).

D-haustoria of most genera show a great overall similarity, and Littlefield and Heath (1979) proposed an idealized D-haustorium of the rust fungi. The D-haustoria of *Cronartium*, *Milesina*, *Uredinopsis* and *Hyalopsora*, however, are different from the normal rust haustorium: the necks of the haustoria are covered by an extension of the extrahaustorial matrix, a matrix-fold.

Ultrastructural characters of haustoria have not been used so far for systematics of rust fungi. Only Longo and Bruscaiglioni (1986) raised the question whether haustorial necks covered by a matrix-fold were restricted to the genus *Cronartium*. Berndt et al. (1994), however, showed that this character was also present in species of

Milesina, *Uredinopsis*, *Hyalopsora* and some related *Uredo* species. They considered haustoria with a neck covered by a matrix-fold to be more advanced than haustoria with naked necks and to be indicative of natural relationships.

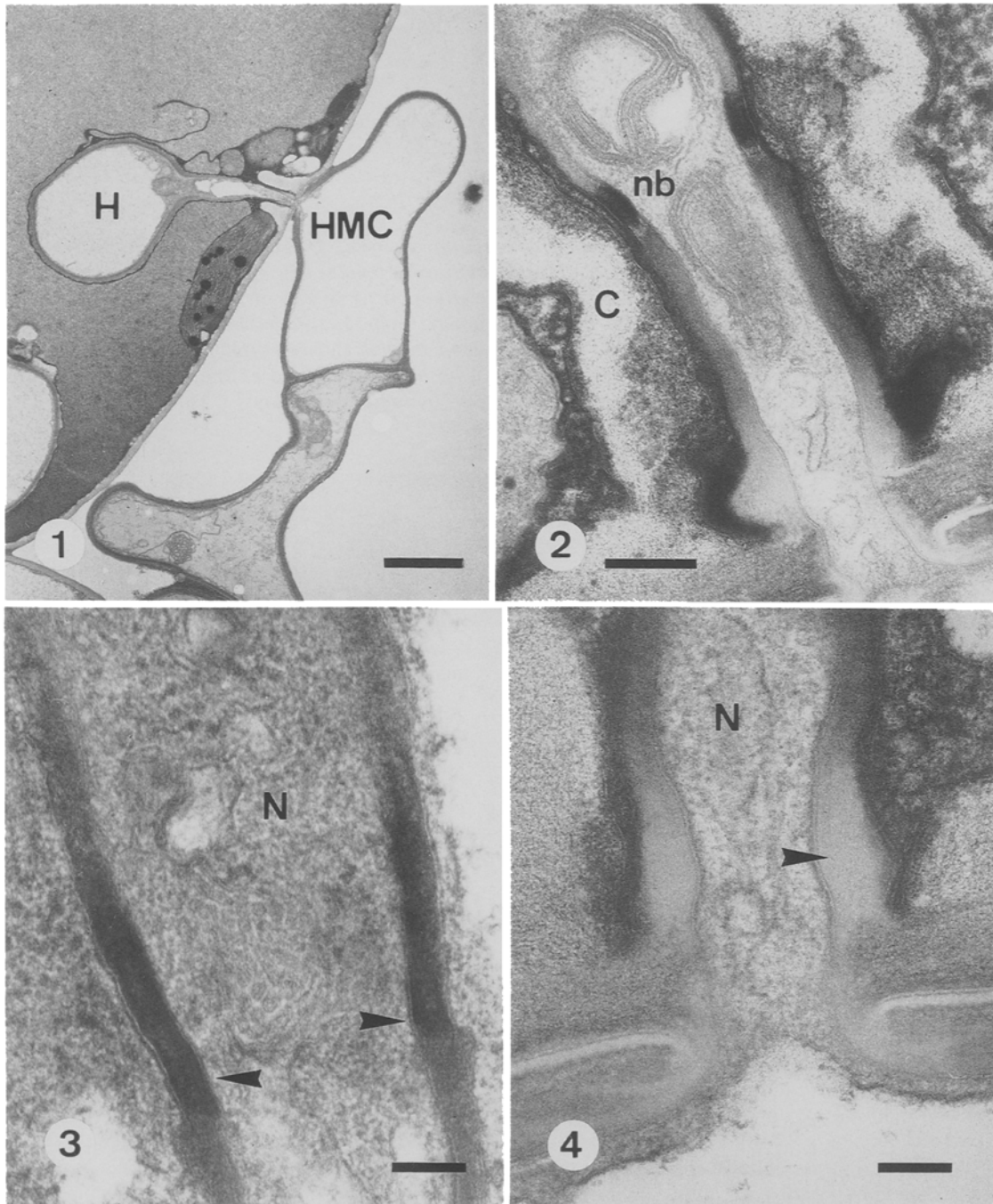
The genera *Pucciniastrum* s. str., *Thekopsora* and *Calyptospora* are closely related and may be united in *Pucciniastrum* s. l. (Cummins and Hiratsuka, 1983). This is also true for the recently established genus *Naohidemyces*. Like the fern rusts *Milesina*, *Uredinopsis* and *Hyalopsora*, these genera belong to the Pucciniastraceae. They were mostly separated by the location of the teliospores and life cycle modifications: in *Pucciniastrum* s. str. small groups of teliospores develop underneath the epidermis; in *Thekopsora* and *Naohidemyces* the teliospores are formed within the epidermal cells of the host (Pady, 1933; Faull, 1938; Sato et al., 1993). Some authors do not consider the site of teliospore formation to be indicative of generic delimitation and include *Thekopsora* in *Pucciniastrum* (Cummins and Hiratsuka, 1983). *Calyptospora goeppertiana* lacks uredinia but closely resembles *Thekopsora* in its intraepidermal teliospores. It may, therefore, be regarded as an *opsis*-form of *Thekopsora* (Gäumann, 1959). *Naohidemyces* differs from *Thekopsora* in its repeating uredinia and the probably different location of the germ pore of the teliospores (Sato et al., 1993).

We were interested to investigate the haustorial ultrastructure of *Pucciniastrum* s. str. and allied genera to look for differences and to compare it with that of the pucciniastraceous fern rusts and the genus *Cronartium*.

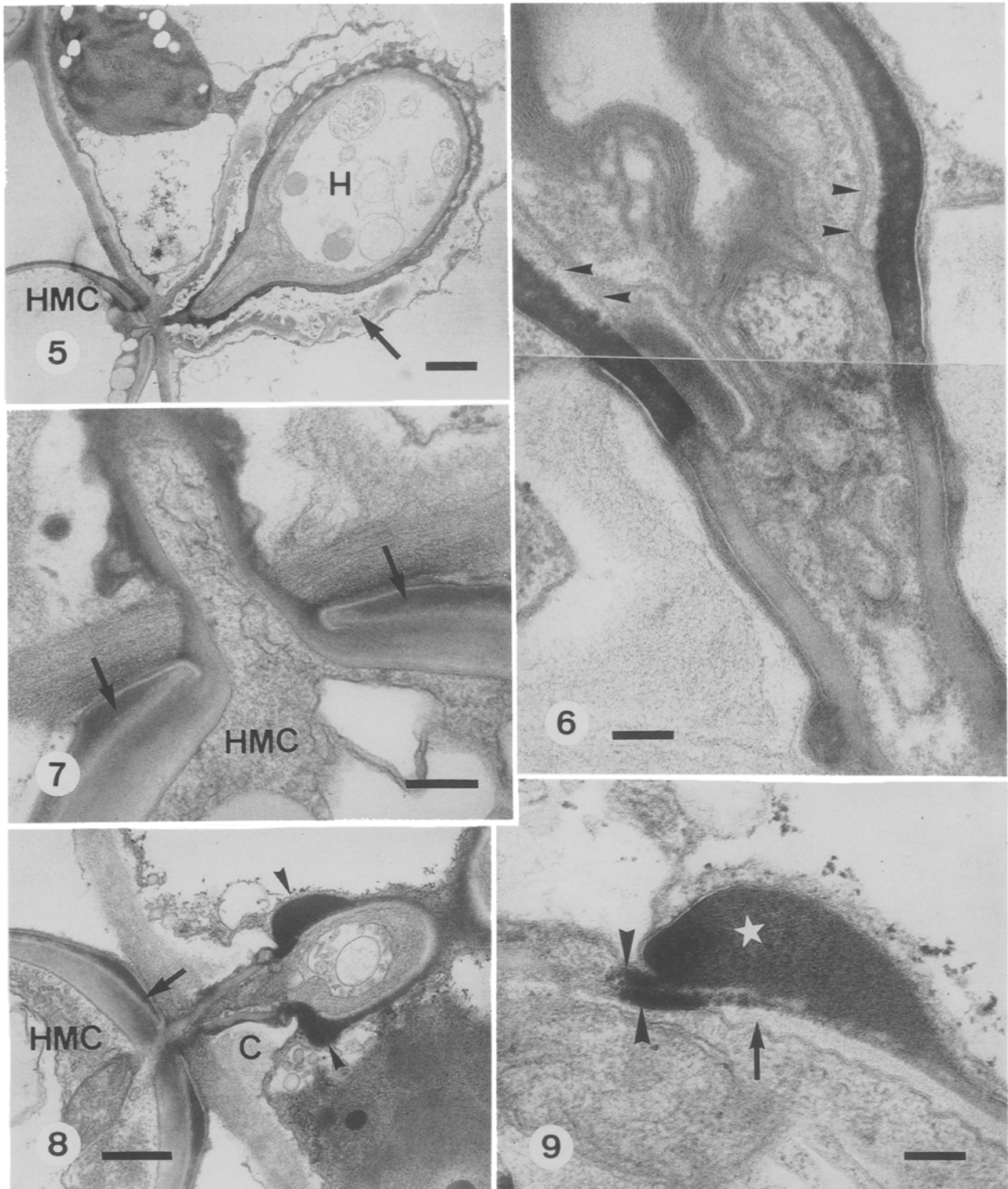
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For the description of the haustoria we follow the terminology of Littlefield and Heath (1979). The part of the host plasmalemma surrounding the haustorium will be called the extrahaustorial membrane. To facilitate descriptions, haustoria whose necks are covered by a matrix-fold will be designated "velopedunculate haustor-

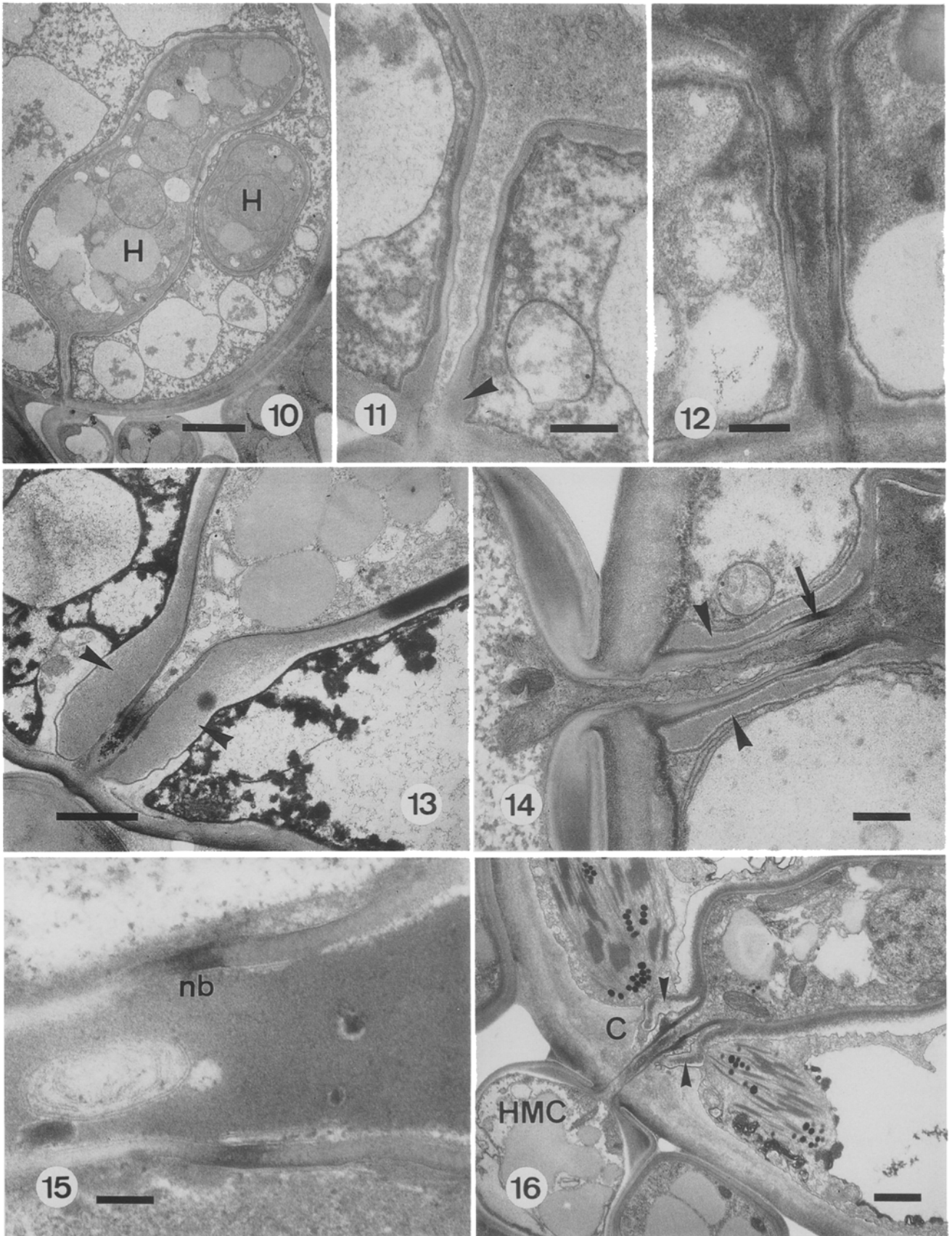
ia," and those with naked necks will be referred to as "gymnopedunculate." Haustorial mother cell will be abbreviated HMC.



Figs. 1-4. *Pucciniastrum epilobii*. 1. Overview of haustorial mother cell (HMC) and vesicular haustorium (H). 2. Haustorial neck. The neck wall stains more densely proximal than distal to the neckband (nb). The base of the neck is less electron-dense. The neck is surrounded by a collar (C) of host origin. 3. Haustorial neck (N) with elongated neckband. The proximal part of the neckband (arrowheads) stains slightly more densely than the distal part. 4. Basal part of the haustorial neck (N) and penetration peg. The haustorial wall is slightly inflated at the base of the neck (arrowhead) and stains less densely than the following part of the wall up to the neckband.



Figs. 5-9. *Pucciniastrum circaeae*. 5. Overview of haustorial mother cell (HMC) and vesicular haustorium (H). The haustorium is surrounded by a collar (arrow) almost entirely. 6. Haustorial neck. The neckband is not sharply delimited distally from the more densely staining distal part of the neck wall. An additional electron-transparent wall layer originates distally to the neckband (arrowheads). 7. The wall of the haustorial mother cell (HMC) reveals several layers at the penetration site. An outer wall layer (arrows) is thickened and enhances the contact between haustorial mother cell and host cell. 8. Haustorial mother cell (HMC) and haustorial neck. Distal to the shallow collar (C) the wall of the neck is inflated and stains deeply electron-densely (arrowheads). Note electron-dense wall layer of the HMC (arrow). 9. Section of the same haustorial neck. Detail of the neckband and distal neck wall. At the neckband the haustorial wall is slightly constricted. The neckband consists of two lateral parts (arrowheads). Distal to the neckband (right hand) the wall of the neck becomes two-layered with an inner, electron-transparent layer (arrow). The outer, densely staining wall layer is inflated blister-like (star). The swelling also comprises the distal part of the neckband.



Materials and Methods

Species investigated:

Calyptospora goeppertiana Kühn. Germany, Black Forest, near Lauterbach, on *Vaccinium vitis-idaea* L., 22. IX. 1993. RB 3330.

Naohidemyces vaccinii (Wint.) Sato, Katsuya & Y. Hiratsuka. Germany, Black Forest, Triberg, on *Vaccinium myrtillus* L., 15. VIII. 1990. RB 2251.

Pucciniastrum actinidiae Hirats. f. Taiwan, Prov. Taichung, Ku Kwan, on *Actinidia* spec., 15. VII. 1990. FO 42728.

Pucciniastrum agrimoniae (Diet.) Tranzsch. Germany, Tübingen, Käsenbachtal, on *Agrimonia eupatoria* L., 27. VIII. 1988. RB 746.

Pucciniastrum circaeae (Wint.) Speg. ex De Toni. Switzerland, Schaffhausen, Randen mountains, near the Schloßranden, on *Circaea lutetiana* L., 12. VIII. 1990. RB 2247.

Pucciniastrum epilobii Otth. Germany, Black Forest, near Schappbach, on *Epilobium angustifolium* L., 15. VIII. 1990. RB 2250.

Pucciniastrum hikosanense Hirats. f. Taiwan, Prov. Nantou, Ho Huan San, near ranger station, on *Acer kawakamii* Koidz., 5. VII. 1990. FO 42273.

Pucciniastrum pyrolae Diet. ex Arth. Austria, Tannheim, Vilsalpsee, on *Moneses uniflora* (L.) A. Gray, 12. IX. 1989. RB 1824.

Pucciniastrum styracinum Hirats. Taiwan, Hui Sun, on *Styrax formosana* Matsum., 22. VI. 1990. RB 3386.

Thekopsora galii De Toni. Germany, Donaueschingen, at the Schellenberg, on *Galium odoratum* (L.) Scop., 28. VIII. 1991. RB 2792.

Specimens are deposited in the authors' herbaria RB and FO.

For electron microscopy small pieces of infected leaves were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer for several hours or days. Following six transfers into 0.1 M cacodylate buffer, the material was postfixed in 1% OsO₄ for 1 h in the dark, washed in distilled water six times and stained with 1% aqueous uranyl acetate for 1 h. After washing out of the uranyl acetate in distilled water (three times), the specimens were dehydrated in an acetone series using 10, 25, 50, 70, 95 and 3 × 100% acetone. The material was embedded in Spurr's resin (Spurr, 1969). Blocks were sectioned on a Reichert-Jung Ultracut-E microtome with diamond knives. Sections, about 85 nm thick, were collected on formvar-coated slot grids and stained with lead ci-

trate (Reynolds, 1963).

Results

Pucciniastrum circaeae and *P. epilobii*—The haustoria of *P. circaeae* and *P. epilobii* were very similar. They were vesicular, i.e., they had a bulb-shaped to allantoid haustorial body (Figs. 1, 5). A neckband was formed at about one third of the length of the neck or half of the way up. In *P. epilobii* there were some haustoria whose neckbands were in the upper third of the neck. At the neckband the wall stained much darker, especially towards the extrahaustorial membrane and the plasmalemma of the fungus (Figs. 2, 3, 6). The extension of the neckband varied. Most often just a narrow band was formed, but there were also broad bands (Fig. 3). In *P. circaeae* the neck wall became two-layered distal to the neckband (Figs. 6, 9). In addition to the more densely staining original wall, an inner, electron-transparent layer was formed. In some haustoria the distal end of the neckband could not be discerned exactly as it blended into the dark-staining distal wall of the haustorium (Fig. 6). The exact end of the neckband could only be determined by the formation of the additional electron-transparent wall layer. In several haustoria the wall was swollen at the neckband, and in one haustorium a prominent, blister-like swelling was formed above the neckband (Figs. 8, 9). The swelling seemed to be part of the outer, darker staining layer of the neck wall and even comprised a part of the neckband. Above the neckband the neck dilated (Figs. 2, 8). It then retained about the same width up to the haustorial body. In *P. epilobii* the neck wall was not clearly layered distal to the neckband, and did not show a swelling (Figs. 2, 3). The fungal wall was sometimes slightly thickened at the base of the neck and more or less electron-transparent (Fig. 2). In *P. epilobii* the wall of the neck gradually stained more densely towards the neckband (Fig. 2). The neckband was separated from the dark wall by a narrow electron-transparent zone (Fig. 2). Distal to the neckband the wall was again electron-transparent (Fig. 2).

Many haustoria of *P. circaeae* were accompanied by collars formed by the host cell. In some cases nearly the whole haustorium was encased (Fig. 5). The HMCs were in broad contact with the wall of the host cell. Their wall was thickened around the penetration site and clearly showed an outer and a thicker inner wall layer (Figs. 4, 7). In some HMCs sublayers could be discerned in the outer wall layer (Fig. 7). A dark-staining sublayer (Fig. 7, arrows) was often thickened. By this thickening

Figs. 10–16. Haustoria of *Pucciniastrum* spp. 10. *P. hikosanense*. Overview of haustorial mother cell and haustorium (H). The haustorial body was sectioned twice. 11. Haustorial neck, detail of Fig. 10. The base of the neck is inflated (arrowhead). A neckband could not be discerned. 12. *P. styracinum*. Haustorial neck. A neckband could not be discerned. 13. *P. actinidiae*. Haustorial neck and part of the haustorial body. The neck is wrapped by a voluminous fold of the extra-haustorial matrix (arrowheads). The neckband is indistinct and can hardly be discerned. Figs. 14–15. *P. agrimoniae*. 14. Penetration peg and haustorial neck. The neck is wrapped by a slender fold of the extra-haustorial matrix (arrowheads). The neckband is formed in the distal part of the neck (arrow). 15. Detail of the neck. The wall of the neck proximal (left hand) to the neckband (nb) stains slightly less electron-densely than the wall of the neck distal to the neckband. 16. *P. pyrolae*. Haustorial mother cell (HMC), neck and basal part of the haustorial body. The haustorial neck is accompanied by a slender matrix-fold (arrowheads). The form of the fold is influenced by the formation of a collar (C) of the host cell.

the surface of the HMC towards the host cell became flatter, and the contact zone with the host cell wall was increased.

Pucciniastrum styracinum—The haustoria were gym-

nopedunculate as in *P. epilobii* and *P. circaeae*. In contrast to all other *Pucciniastrum* spp. investigated, *P. styracinum* possessed haustoria whose haustorial body branched into a few lobes. The wall of the neck was

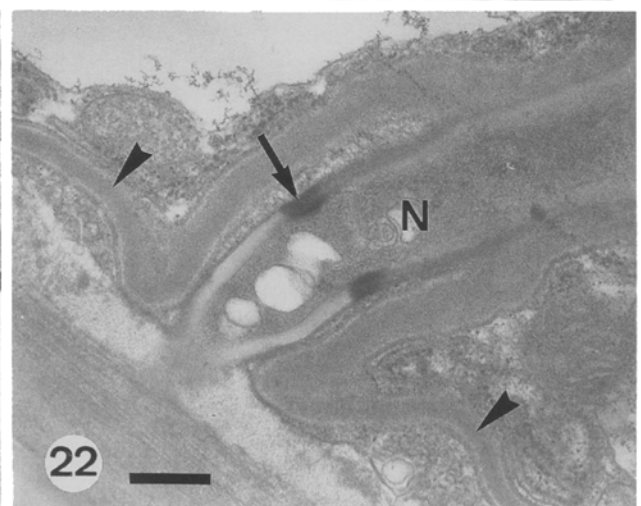
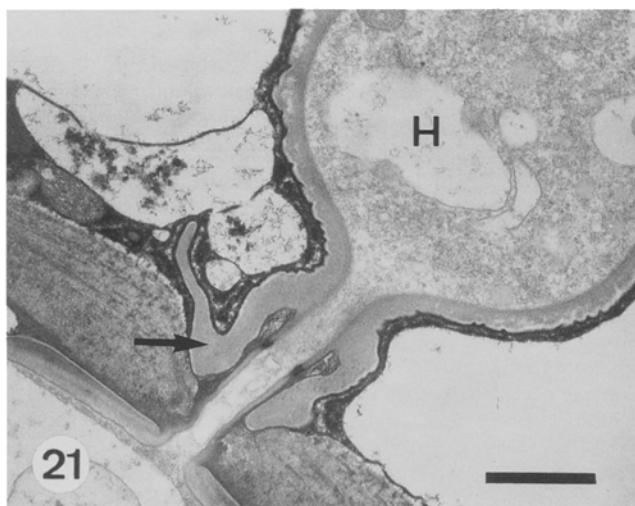
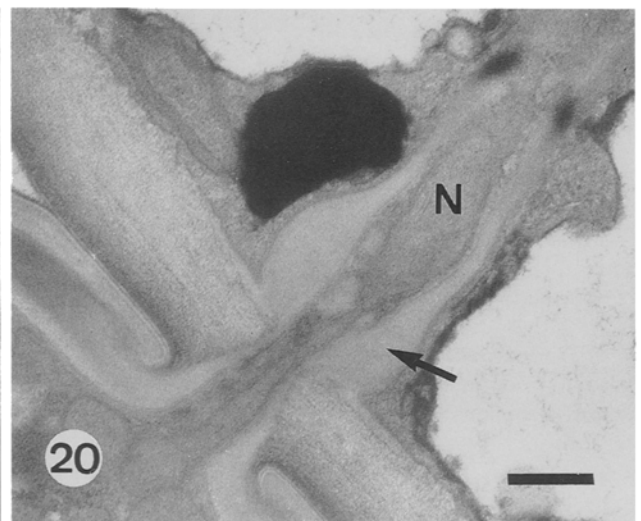
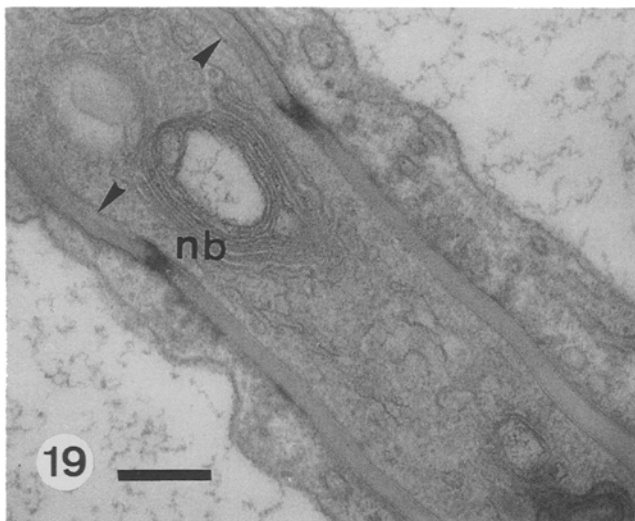
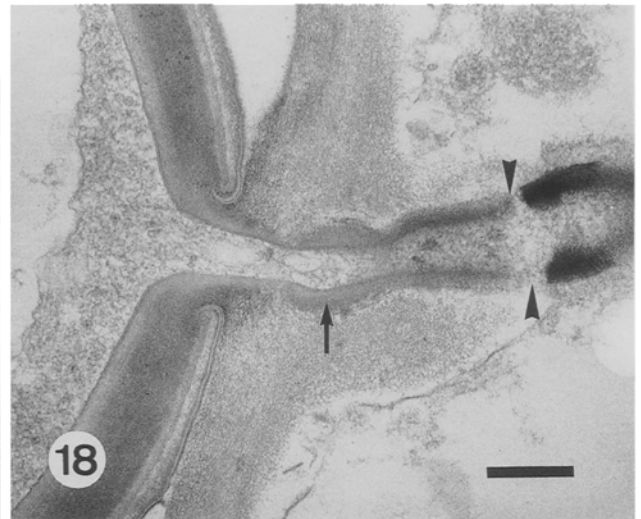
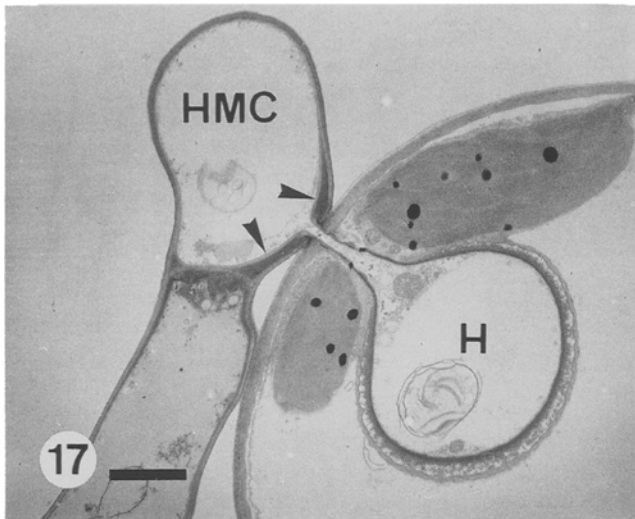


Table 1. Differences between the genera of *Pucciniastrum* s.l.

	Teliospores	Life cycle	Host plant	Pycnidium	Haustorium
<i>Pucciniastrum</i> s. str.	subepiderm.	O/II/III	<i>Abies/Picea</i>	type 3	gymnopedunculate or velopedunculate
<i>Thekopsora</i>	intraepiderm.	O/II/III	<i>Abies/Picea/Tsuga</i>	type 3	gymnopedunculate
<i>Calyptospora</i>	intraepiderm.	O/II/III	<i>Abies</i>	type 3	velopedunculate
<i>Naohidemycetes</i>	intraepiderm.	O/II*/II/III	<i>Tsuga</i>	type 3	gymnopedunculate
<i>Hyalospora</i>	intraepiderm. ¹	O/II/III	<i>Abies</i>	type 2	velopedunculate

* repeating uredinia.

¹ walls of teliospores not pigmented.

slightly inflated basally and did not show a clear neckband (Fig. 12). The HMCs were attached to the host cell only with a small contact zone, and the whole HMC possessed a very thick wall.

Pucciniastrum hikosanense—This species also possessed gymnopedunculate haustoria. As in *P. styracinum*, we could not find neckbands in mature haustoria which stained differently from the remaining neck (Fig. 11). The whole wall of the neck stained almost homogeneously electron-transparent, also in the slightly thick-walled base. The neck was surrounded by a thin layer of dark-staining material of host origin which wedged out, reaching the haustorial body and the host cell wall (Fig. 11). As in *P. styracinum*, the wall of the HMC was extraordinarily thick, especially around the penetration site (Fig. 10).

Pucciniastrum agrimoniae, *P. pyrolae*, and *P. actinidiae*—In contrast to the species described above, the haustorial necks of *P. agrimoniae*, *P. pyrolae*, and *P. actinidiae* were surrounded by a fold of the extrahaustorial matrix (Figs. 13, 14, 16) and were thus designated “velopedunculate.” In this haustorial type the extrahaustorial membrane doubled back upon itself with a sharp bend distal to the neckband. It then formed a fold which wrapped the neck tightly. In *P. agrimoniae* and *P. pyrolae* the matrix-fold was slender but slightly enlarged at its rim. It contained a more-or-less homogeneously granular matrix (Figs. 14, 16). The matrix-fold of *P. actinidiae* was voluminous and also contained granular matrix material (Fig. 13). Except for the neckband, the neck wall stained uniformly electron-transparent over most of its length. Only around the neckband could an area of diffuse higher electron-density be observed. In *P. agrimoniae* the neck wall stained more densely distal than proximal to the neckband. The neckband itself was

almost homogeneously electron-dense and varied only slightly in width (Fig. 15).

Thekopsora galii and *Naohidemycetes vaccinii*—The haustoria of *T. galii* and *N. vaccinii* were gymnopedunculate and closely resembled the haustoria of the gymnopedunculate *Pucciniastrum* species (Figs. 17–20). A neckband was formed at about half of the neck length. The neckband was roughly trapezium- to spool-shaped in section, as it extended farther up along the extrahaustorial membrane and the fungal plasmalemma or only along the extrahaustorial membrane (Fig. 19). The width of the neckband varied only slightly in different haustoria. The whole wall of the neck was moderately electron-dense (Figs. 18, 19). In *T. galii* a small zone of the wall stained less densely just beneath the neckband (Fig. 18). The neck wall was conspicuously swollen at its base in *N. vaccinii* (Fig. 20) and only slightly swollen in *T. galii* (Fig. 18). Most HMCs were formed as terminal cells of the intercellular mycelium. Some intercalary cells of the mycelium of *T. galii*, however, were found to be HMCs as well. In most HMCs the thickened zone of the HMC wall, where penetration of the host cell wall took place, was shifted close to the septum of the HMC (Fig. 17). At the penetration site the walls of the HMCs were thickened and showed various layers (Fig. 18).

Calyptospora goeppertiana—The haustoria of *C. goeppertiana* were velopedunculate and differed from the haustoria of the velopedunculate *Pucciniastrum* spp. by the form of the matrix-fold. The matrix-fold consisted of a more or less cylindrical part which sheathed the haustorial neck, and a broad, disc-shaped rim of the fold which was orientated nearly perpendicularly to the cylindrical part (Figs. 21, 22). In few haustoria the disc-shaped part of the matrix-fold was not developed completely (Fig. 21). The neckband was formed at about a

Figs. 17–22. Haustoria of *Thekopsora galii*, *Naohidemycetes vaccinii* and *Calyptospora goeppertiana*. Figs. 17–18. *T. galii*. 17. Overview of haustorial mother cell (HMC) and haustorium (H). The wall of the mother cell is thickened around the penetration site. The thickening of the HMC wall (arrowheads) is located near the mother cell septum. 18. Penetration peg and basal neck. The haustorial wall is slightly thickened at the base of the neck (arrow). The neckband is separated from the moderately densely staining proximal wall of the neck by a narrow, electron-transparent zone (arrowheads). Figs. 19–20. *N. vaccinii*. 19. Detail of the neck with a narrow neckband (nb). An indistinct additional, electron-transparent wall layer of the haustorial neck originates distal to the neckband (arrowheads). 20. Penetration peg and basal part of the neck (N). The neck wall is conspicuously thickened at the base (arrow). Figs. 21–22. *C. goeppertiana*. 21. Penetration peg, haustorial neck and body (H). The neck is sheathed by a fold of the extra-haustorial matrix which reveals a characteristically bent rim (arrow). 22. Haustorial neck (N). The matrix-fold consists of a more-or-less cylindrical part around the neck and a roughly disc-shaped part (arrowheads) along the host cell wall. The wall of the neck proximal to the neckband (arrow) is less electron-dense than the part of the wall distal to the neckband.

third to half of the neck length. Proximal to the neckband the wall was rather electron-transparent. Distally it stained considerably denser (Fig. 22).

Discussion

Ultrastructure of velopedunculate haustoria—The velopedunculate haustoria of *Pucciniastrum* and *Calyptospora* are similar to those of *Cronartium* (Gray et al., 1982; Khan et al., 1982; Longo and Bruscazioni, 1986) and the fern rusts (Berndt et al., 1994). There are certain differences, however, especially in the matrix-fold and the staining of the fungal wall of the haustorial neck. The matrix-fold varies in extension and form. However, both form and extension could be influenced by collar formation of the host cell. Thus only the very characteristically shaped matrix-fold of *Calyptospora goeppertiana* may prove to be specific. In *Cronartium* (Khan et al., 1982) and in *Milesina*, *Uredinopsis* and *Hyalopsora* spp. (Berndt et al., 1994) the matrix-fold contained granular material which accumulated especially adjacent to the neck. The velopedunculate haustoria of *Pucciniastrum* and *Calyptospora* showed a homogeneously staining content of the matrix-fold. The appearance of the content of the fold, however, may be due to ontogenetical stages, as has been shown for the extra-haustorial matrix surrounding the haustorial body (Zimmer, 1970; Coffey et al., 1972; Harder, 1978; Chong et al., 1981). Gray et al. (1982) reported that in *Cronartium* the breadth of the neckband and also the site where the extra-haustorial membrane began to double back were variable. In the velopedunculate *Pucciniastrum* spp. and *Calyptospora goeppertiana* the neckband did not vary considerably in breadth although the band was sometimes poorly delimited against the distal, more densely staining wall of the neck. There is more variation concerning the staining of the neck walls of velopedunculate haustoria. In *Cronartium* (Khan et al., 1982), in *Calyptospora goeppertiana* and *P. agrimoniae* the neck wall stained more densely distal to the neckband. Unlike these species, the haustoria of *Milesina* had a haustorial wall which stained more densely proximal to the neckband and often revealed an area resembling an additional neckband (Berndt et al., 1994).

Ultrastructure of gymnopedunculate haustoria—The gymnopedunculate haustoria found in *Pucciniastrum*, *Thekopsora* and *Naohidemycetes* are similar to those already described in the literature. *P. hikosanense* and *P. styracinum*, however, did not show a neckband although the haustoria were mature. This may be due to fixation problems. Heath and Heath (1975) showed that in young haustoria of *Uromyces phaseoli* (Pers.) Wint. var. *vignae* (Barcl.) Arth. neckbands did not occur before the haustorial body had reached a diameter of about 2–3 μm . Abu-Zinada et al. (1975) occasionally observed haustoria lacking neckbands in *Uromyces fabae* (Pers.) de Bary. The authors did not comment on the age of such haustoria. Heath and Heath (1971) found haustoria lacking neckbands in *Uromyces phaseoli* var. *vignae* on a resistant host. However, such haustoria were not ma-

ture, and a neckband probably still had not formed at the stage when development of the haustoria ceased.

Collar formation or encapsulation of haustoria as found in *Pucciniastrum circaeae* seem to be a widespread host response to haustorium formation. Similar observations were made by several authors in other rust fungi (e.g., Abu Zinada et al., 1975; Chong and Harder, 1982; Heath, 1972; Taylor and Mims, 1991).

A blister-like swelling of the haustorial wall at and immediately above the neckband has only been observed in a single haustorium of *Pucciniastrum circaeae* and may be connected with collar formation of the host cell. There were many haustoria, however, which showed a slightly swollen wall at the neckband. Fungal walls which are swollen at the neckband have been reported from several rusts (e.g., *Melampsora lini*, Littlefield and Bracker, 1972; *Uromyces appendiculatus* Fr., Hardwick et al., 1971; Müller et al., 1974).

Of the species investigated, only in *P. circaeae* could a clear layering of the fungal wall distal to the neckband be observed. A layering of the wall of the neck and the haustorial body has been observed in various rust fungi. In *Puccinia graminis* Pers. f. sp. *tritici* Erikss. & Henn. the haustorial wall was sometimes two-layered (Ehrlich and Ehrlich, 1963), and, as in *Pucciniastrum circaeae*, the outer layer stained more electron-densely. With Thiéry's staining, however, the haustorial neck of *Puccinia graminis* f. sp. *tritici* revealed a thin, densely-staining inner layer and a broader, more electron-transparent outer layer (Chong et al., 1986). These layers continued around the haustorial body. In *Uromyces appendiculatus* (Pers.) Link (Hardwick et al., 1971) and in *Melampsora lini* (Ehrenb.) Lév. (Coffey, 1976) the haustorial wall was also two-layered distal to the neckband, but with an electron-transparent outer layer.

Distribution of haustorial types, implications for systematics—Velopedunculate haustoria were first discovered in species of the genus *Cronartium* (Gray et al., 1982; Khan et al., 1982; Longo and Bruscazioni, 1986) and later in species of *Uredinopsis*, *Milesina*, *Hyalopsora* and related *Uredo* spp. (Berndt et al., 1994). The results of Berndt et al. (1994) indicate that all species of the investigated genera possess the same haustorial type.

It was therefore quite surprising that in *Pucciniastrum* s. str. there are species with gymno- or velopedunculate haustoria. The haustorial heterogeneity may suggest that *Pucciniastrum* s. str. does not represent a natural group. *Pucciniastrum* s. str. is not only heterogeneous in respect of haustorial ultrastructure but also in the selection of *Abies* and *Picea* as conifer hosts of the haplontic phase. As far as is known, *Pucciniastrum* s. str. and *Thekopsora* are the only genera of the Puccinias-traceae whose species are not restricted to a single host genus in the haplontic phase. Unfortunately it was not possible to bring into line the haustorial ultrastructure of the investigated species of *Pucciniastrum* s. str. and the selection of conifer hosts, as the complete life cycle (Hiratsuka, 1958) and haustorial ultrastructure are only known for a few species.

The genera *Pucciniastrum* s. str., *Thekopsora*, *Calyp-*

tospora, *Naohidemycetes* and the pucciniastraceous fern rust *Hyalopsora* are delimited only for weak characters as position of teliospores, life cycle variation or minor pycnidium characters (Table 1).

Haustorial ultrastructure is probably a conservative character which is not easily influenced by environmental factors and may prove to be more important than teliospore location or subtle pycnidium differences in the Pucciniastraceae. In view of their haustorial ultrastructure, one could speculate that the velopedunculate *Pucciniastrum* spp. are related more closely to the fern rusts than to the gymnopedunculate *Pucciniastrum* spp. These are more similar to *Thekopsora* and *Naohidemycetes*. *C. goepertiana* appears to be related more closely to the velopedunculate *Pucciniastrum* spp. by its haustorial ultrastructure.

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Literature cited

- Abu-Zinada, A.-A. H., Cobb, A. and Boulter, D. 1975. An electron-microscopic study of the effects of parasite interaction between *Vicia faba* L. and *Uromyces fabae*. *Physiol. Plant Pathol.* **5**: 113–118.
- Berndt, R., Bauer, R. and Oberwinkler, F. 1994. Ultrastructure of the host-parasite interface in the fern rusts *Milesia*, *Uredinopsis* and *Hyalopsora* (Pucciniastraceae, Uredinales). *Can. J. Bot.* **77**: 1084–1094.
- Chong, J., Harder, D. E. and Rohringer, R. 1981. Ontogeny of mono- and dikaryotic rust haustoria: Cytochemical and ultrastructural studies. *Phytopathology* **71**: 975–983.
- Chong, J. and Harder, D. E. 1982. Ultrastructure of haustorium development in *Puccinia coronata avenae*: Some host responses. *Phytopathology* **72**: 1527–1533.
- Chong, J., Harder, D. E. and Rohringer, R. 1986. Cytochemical studies on *Puccinia graminis* f. sp. *tritici* in a compatible wheat host. II. Haustorium mother cell walls at the host cell penetration site, haustorial walls, and the extrahaustorial matrix. *Can. J. Bot.* **64**: 2561–2575.
- Coffey, M. D. 1976. Flax rust resistance involving the K-gene: An ultrastructural survey. *Can. J. Bot.* **54**: 1443–1457.
- Coffey, M. D., Palevitz, B. A. and Allen, P. J. 1972. The fine structure of two rust fungi *Puccinia helianthi* and *Melampsora lini*. *Can. J. Bot.* **50**: 231–240.
- Cummins, G. B. and Hiratsuka, Y. 1983. "Illustrated genera of rust fungi," The American Phytopathological Society, St. Paul, Minnesota. 152 p.
- Ehrlich, H. G. and Ehrlich, M. A. 1963. Electron microscopy of the host-parasite relationships in stem rust of wheat. *Am. J. Bot.* **50**: 123–130.
- Faull, J. H. 1938. *Pucciniastrum* on *Epilobium* and *Abies*. *J. Arnold Arboretum* **19**: 163–173.
- Gäumann, E. 1959. "Die Rostpilze Mitteleuropas," Beiträge zur Kryptogamenflora der Schweiz XII. Bümchler, Bern. 1405 p.
- Gray, D. J., Amerson, H. V. and Van Dyke, C. G. 1982. An ultrastructural comparison of monokaryotic and dikaryotic haustoria formed by the fusiform rust fungus *Cronartium quercuum* f. sp. *fusiforme*. *Can. J. Bot.* **60**: 2914–2922.
- Harder, D. E. 1978. Comparative ultrastructure of the haustoria in uredial and pycnial infections of *Puccinia coronata avenae*. *Can. J. Bot.* **56**: 214–224.
- Hardwick, N. V., Greenwood, A. D. and Wood, R. K. S. 1971. The fine structure of the haustorium of *Uromyces appendiculatus* in *Phaseolus vulgaris*. *Can. J. Bot.* **49**: 383–390.
- Heath, M. C. 1972. Ultrastructure of host and nonhost reactions to cowpea rust. *Phytopathology* **62**: 27–38.
- Heath, M. C. and Heath, I. B. 1971. Ultrastructure of an immune and a susceptible reaction of cowpea leaves to rust infection. *Physiol. Plant Pathol.* **1**: 277–287.
- Heath, M. C. and Heath, I. B. 1975. Ultrastructural changes associated with the haustorial mother cell during haustorium formation in *Uromyces phaseoli* var. *vignae*. *Protoplasma* **84**: 297–314.
- Hiratsuka, N. 1958. Revision of taxonomy of the Pucciniastraceae. *Mem. Fac. Agric., Tokyo-Univ. of Education* **5**: 1–167.
- Khan, S. R., Kimbrough, J. W. and Webb, P. G. 1982. The fine structure of septa and haustoria of *Cronartium quercuum* f. sp. *fusiforme* on *Quercus rubra*. *Mycologia* **74**: 809–819.
- Koch, E., Ebrahim-Nesbat, F. and Hoppe, H. H. 1983. Light and electron microscopic studies on the development of soybean rust (*Phakopsora pachyrhizi* Syd.) in susceptible soybean leaves. *Phytopathol. Z.* **106**: 302–320.
- Littlefield, L. J. and Bracker, C. E. 1972. Ultrastructural specialization at the host-pathogen interface in rust-infected flax. *Protoplasma* **74**: 271–305.
- Littlefield, L. J. and Heath, M. C. 1979. "Ultrastructure of rust fungi," Academic Press, New York. 277 p.
- Longo, N. and Bruscazioni, L. 1986. Ultrastructural observations on the dikaryotic haustorium of *Cronartium flaccidum* (Alb. & Schw.) Wint. in *Vincetoxicum hirsutinaria* Med. *Caryologia* **39**: 51–64.
- Longo, N. and Naldini, B. 1972. Osservazioni sull' ultrastruttura dell' austorio di *Melampsora pinitorqua* (A. Br.) Rostr. in cellule di *Pupulus tremula*. *Caryologia* **25**: 383–401.
- Mims, C. W. and Glidewell, D. C. 1978. Some ultrastructural observations on the host-pathogen relationship within the telial gall of the rust fungus *Gymnosporangium juniperi-virginianae*. *Bot. Gaz.* **139**: 11–17.
- Müller, L. Y., Rijkenberg, F. H. J. and Truter, S. J. 1974. Ultrastructure of the uredial stage of *Uromyces appendiculatus*. *Phytophylactica* **6**: 73–104.
- Pady, S. M. 1933. Teliospore development in the Pucciniastraceae. *Can. J. Res.* **9**: 458–485.
- Reynolds, E. S. 1963. The use of lead citrate at a high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* **17**: 208–212.
- Sato, S., Katsuya, K. and Hiratsuka, Y. 1993. Morphology, taxonomy and nomenclature of *Tsuga*-Ericaceae rusts. *Trans. Mycol. Soc. Japan* **34**: 47–62.
- Spurr, A. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26**: 31–43.
- Taylor, J. and Mims, C. W. 1991. Fungal development and host cell responses to the rust fungus *Puccinia substriata* var. *indica* in seedling and mature leaves of susceptible and resistant pearl millet. *Can. J. Bot.* **69**: 1207–1219.
- Zimmer, D. E. 1970. Fine structure of *Puccinia carthami* and the ultrastructural nature of exclusionary seedling-rust resistance of safflower. *Phytopathology* **60**: 1157–1163.