

Distinctiveness of aecia and aeciospores in rust fungi on conifers

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Abstract: Surface structures of aeciospores have been examined with the scanning electron microscope (SEM). Spores in three morphologically different types of aecia have been compared: spores in aecidioid aecia on angiospermous hosts, spores in peridermioid aecia on coniferous hosts, and spores in caeomoid aecia on coniferous hosts. In aecidioid and peridermioid aecia, the correlation between the mode of peridium rupturing and the arrangement of zones with different ornamentation on the spore surface is striking. In aecidioid aecia, the pattern of surface zonation shows a regular sequence from the apex to the base of the spore. The apical spore region is characterized by conspicuous features, i.e. a smooth, round cap or a hole surrounded by an annulus.

Spores in peridermioid aecia are often characterized by a smooth or nearly smooth strip from the apex to the base on one side of the spore. SEM images show these regularly arranged strips as longitudinal overlying structures or as areas with distinctly finer warts. The former have been found in the genera *Chrysomyxa*, *Cronartium* or *Pucciniastrum*, the latter in the genera *Milesina* and *Uredinopsis*.

Spores in caeomoid aecia lack surface zonations and overlying structures. The spore surface ornaments in the genera *Mikronegeria* and *Melampsora* represent morphologically different types. Aeciospore warts of *Melampsora* consist of two elements. In this respect, they resemble rusts with peridermioid aecia. The aeciospore warts in *Mikronegeria* represent a distinctly different type of ornaments compared to those in other rusts which produce aecia on conifers. Implications for anatomy and phylogeny are discussed.

1. Introduction

In many rust fungi, aeciospores are the first dikaryotic spores in their life cycle and initiate the alternation to another host. They originate in a special sorus, the aecium. Aecia are produced on leaves, branches, stems and cones where the fungus often causes bright yellow, reddish or purple-red discoloration and deformation of the surrounding host tissue. In addition, the spores in the aecia are usually bright orange-coloured by carotenes and are, therefore, easily visible. In many instances, however, the understanding of morphological criteria in aecia and aeciospore structure is inadequate. Based on the gross morphology of the peridium, the following main types of aecia are distinguished.

- 1) Aecidioid aecia are usually cup-shaped with a revolute margin (Fig.1a). When mature, their well-developed cylindric peridium is regularly opening at the roundish apex. The peridial cells are \pm rhomboidal. This type of aecia is characteristic for the *Puccinia-Uromyces* complex and can be found on numerous angiosperms except Poaceae, and (at least in Europe) Juncaceae and Cyperaceae.

- 2) Peridermioid aecia are cylindrical, tongue-, sac- or blister-shaped; often the blister is flattened. The peridium consists of relatively long and narrow, thick-walled cells arranged in one or several layers. It is irregularly fragmented at maturity (Fig. 1b). All members of the families Coleosporiaceae, Cronartiaceae and Pucciniastraceae s.l. produce peridermioid aecia. Within the rust fungi, this type of aecia represents a unique character, and its exclusive occurrence on conifers (Pinaceae) leads to special adaptations of the parasites to their host plants.
- 3) Caeomoid aecia lack peridia (Fig. 1c), but some *Melampsora* species have peridial cells adherent to the epidermis of the host plant. In *Mikronegeria*, a single layer of overlying thin-walled cells exists. This layer has little resemblance to a peridium, and breaks up with the overlying host epidermis (PETERSON & OEHRENS 1978). *Melampsora* and *Mikronegeria* produce aecia on conifers, the former also on some angiosperms. Caeomoid aecia on Rosaceae are produced by *Phragmidium* and other Phragmidiaceae; but aecia on this host family are not included here. For further information on these, refer to BEDLAN (1984) and WAHYUNO et al. (2002), who described five different types of echinulate aeciospores on roses.
- 4) Roestelioid aecia and the aeciospore surface have been analysed in detail by LEE and KAKISHIMA (1999) in the genus *Gymnosporangium* and its anamorph *Roestelia*. Therefore, their study is recommended for the interpretation of roestelioid aecia.



Fig. 1a-c: a) *Puccinia urticata* on *Urtica dioica*: aecidioid aecium; b) *Cronartium ribicola* on *Pinus strobus*: peridermioid aecium; c) *Melampsora* cf. *larici-epitea* on *Larix decidua*: caeomoid aecium.

The peridium in peridermioid aecia is formed by the differentiation of the uppermost cells of aeciospore chains into thick-walled peridial cells (COLLEY 1918, LITTLEFIELD & HEATH 1979). This contrasts clearly with the mode of peridium morphogenesis in aecidioid aecia (*Puccinia-Uromyces* complex), where peripheral chains of aeciospores and intercalary cells differentiate to form the tubular peridium around the elongated aecia (FROMME 1914, LITTLEFIELD & HEATH 1979). The lateral wall of the aecial peridium is formed in *Puccinia* and *Uromyces* from the equivalent of the spore mother-cell; but in

most genera of the coniferous rust fungi (Colesporiaceae, Cronartiaceae and Pucciniastraceae s.l.), its cells are the equivalents of aeciospores, and small residual intercalary cells may be encountered. This lateral wall is a structure that arose de novo in the aecium under the necessity for a powerful thrust being exerted upon the epidermis (SAVILE 1955). This author presented an interpretation for the different mode of peridium morphogenesis in peridermioid and aecidioid aecia: “It is not certain that the original gymnospermous hosts had as tough an epidermis or cuticle as *Abies*, on which the contemporary fern rusts produce their aecia”. The very robust peridermioid peridia of many members of the families Colesporiaceae, Cronartiaceae and Pucciniastraceae s.l., however, show “the morphological adaption that was necessary. In the majority of angiospermous hosts, rupture of the epidermis presents no great problem.” In the *Puccinia-Uromyces* complex, the principal function of the peridium has been changed. The peridium has won a new role in intensifying the pressure needed for forcibly discharging the aeciospores.

The question arises, if the mechanical problem of rupturing the epidermis of gymnospermous hosts exclusively characterizes the morphology of the aecial peridium. Peridermioid aecia and aecidioid aecia differ not only in the morphology and morphogenesis of the peridium but also in the morphology of the spores. Therefore, a comparison of spores in aecidioid aecia with spores in peridermioid aecia and spores in caecomoid aecia is appropriate here.

2. Comparison of aeciospores

2.1 Spores in aecidioid aecia

Due to the method of preparation, mature aeciospores are usually not observed in chains but as an assemblage of scattered spores and fragments of chains. ZWETKO and BLANZ (2012) looked inside open cup-shaped aecidioid aecia by SEM. Especially in young aecia, the spores are regularly arranged and show the apex of the uppermost – i.e. oldest – spore of each chain. In aecia of *Puccinia* and *Uromyces* species on several members of Ranunculaceae and Asteraceae, the apical spore region has been found to exhibit conspicuous features, either a smooth, round cap or a hole, surrounded by an annulus. These features are hardly recognizable in a light microscope, and have, therefore, not been described before.

Smooth apical caps have always been found on spores which possess large dehiscent platelets (Fig. 2), but caps could not be found when platelets are lacking. These large platelets have been termed “abfallende Plättchen” by KLEBAHN (1914), “refractive granules” by HOLM (1967), or “pore plugs” by SAVILE (1973). In mature spores the platelets drop off, revealing circular, smooth bulges on the spore surface. Shape and size of these platelets provide valuable taxonomic characters as HOLM (1967), SAVILE (1973) and ZWETKO

(1993) have shown. The positions of the platelets are rather constant; they are placed in a broad belt with coarse warts. This belt surrounds a smooth, circular area, which resembles a polar cap. These caps have a regular shape and seem to cover a small, roundish, finely and densely verrucose area on the spore wall (Fig. 3). ZWETKO and BLANZ (2012) found this finely verrucose area and the broad belt with coarse warts also on spores which lack apical caps and large dehiscent platelets. These spores are characterized by apical holes (figs. 4 and 5). In all specimens examined, an apical hole has been observed whenever no apical cap was present. The size of these holes is about 350 nm in diam. At the base of the spores such holes have never been observed. Further studies in spore ontogeny are needed to understand this pore-like feature, which is not mentioned by GOLD et al. (1979), LITTLEFIELD and HEATH (1979) or SATO and SATO (1982).

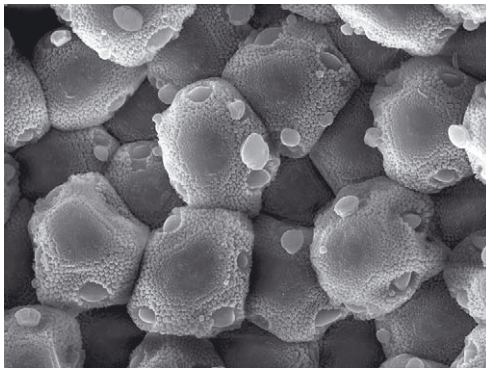


Fig. 2: *Puccinia poae-aposeridis* on *Aposeris foetida*: Spores in an open aecium with smooth apical caps and dehiscent platelets.

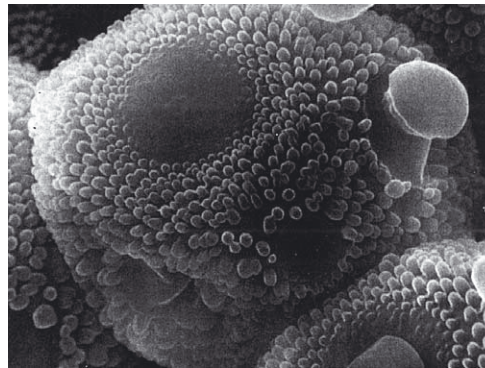


Fig. 3: *Puccinia silvatica* on *Taraxacum officinale* agg.: Aeciospore, apical cap covering a zone with fine warts.

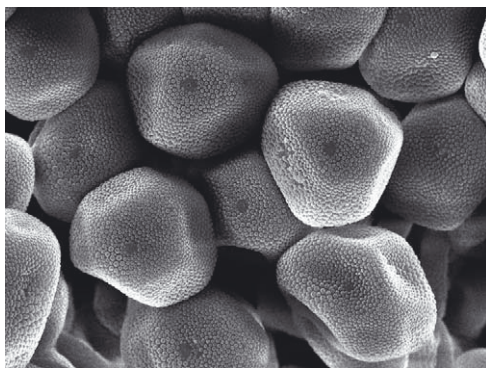


Fig. 4: *Puccinia magnusiana* on *Ranunculus repens*: Spores in an open aecium with pore-like structure at the apex and a belt with coarse warts surrounding the apical zone.

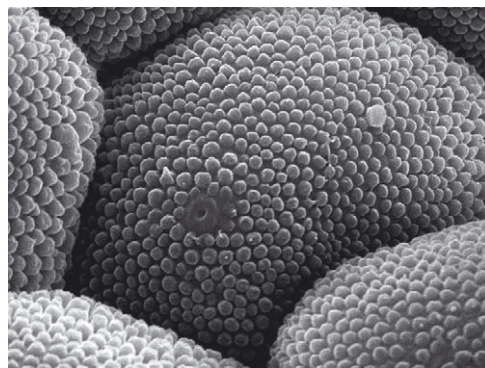


Fig. 5: *Puccinia magnusiana* on *Ranunculus repens*: Pore-like structure in details.

However, these features help to recognize apex and basis of isolated aeciospores. When knowing the position of the apex, the regular structure of the spore surface is evident. It consists of a small, circular, finely verrucose, apical zone, surrounded by a broad belt with coarse warts or with coarse warts and dehiscent platelets, respectively; the surface of the basal hemisphere is finely verrucose as it is at the apex (Fig. 6). When knowing about these three zones one can recognize them even with a light microscope. Their regular arrangement on the spore surface is characteristic for rusts with aecidioid aecia (*Puccinia-Uromyces* complex).

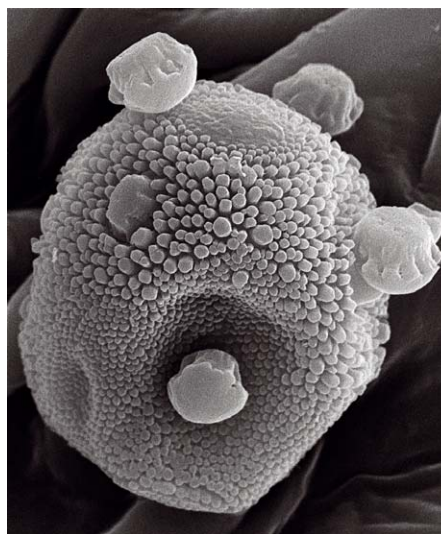


Fig. 6: *Uromyces alpinus* on *Ranunculus* cf. *montanus*: Aeciospore showing three different zones of wall ornamentation and dehiscent platelets.

2.2. Spores in peridermioid aecia

In contrast to the smooth, apical cap on spores of aecidioid aecia, a smooth strip on one side of spores in peridermioid aecia has been observed by previous authors, who examined aecia with a light microscope. KLEBAHN'S (1914) excellent drawings already display such longitudinal smooth strips on aeciospores in the genera *Cronartium*, *Melampsoridium* and *Pucciniastrum* s.l. He supposed that in *Cronartium ribicola*, the smooth strip on the aeciospore surface is caused by the fusion of warts. In *C. flaccidum*, he explained the nearly smooth strip as result of distinctly broader warts and closer spacing (Fig. 7a and 7b). A similar interpretation was presented by HIRATSUKA and SATO (1982). They stated that smooth spots on the aeciospores of some species such as *C. coleosporioides* are made up of "undivided wart layers". These statements are not confirmed by our observations.

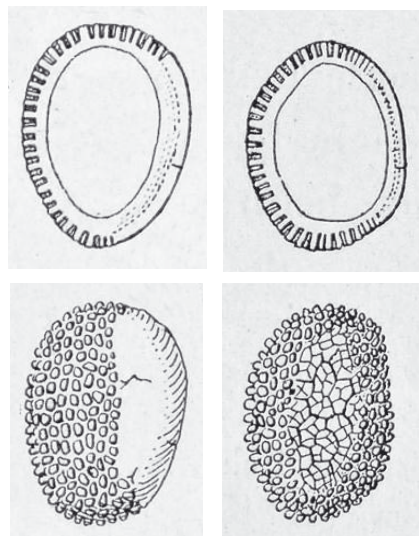
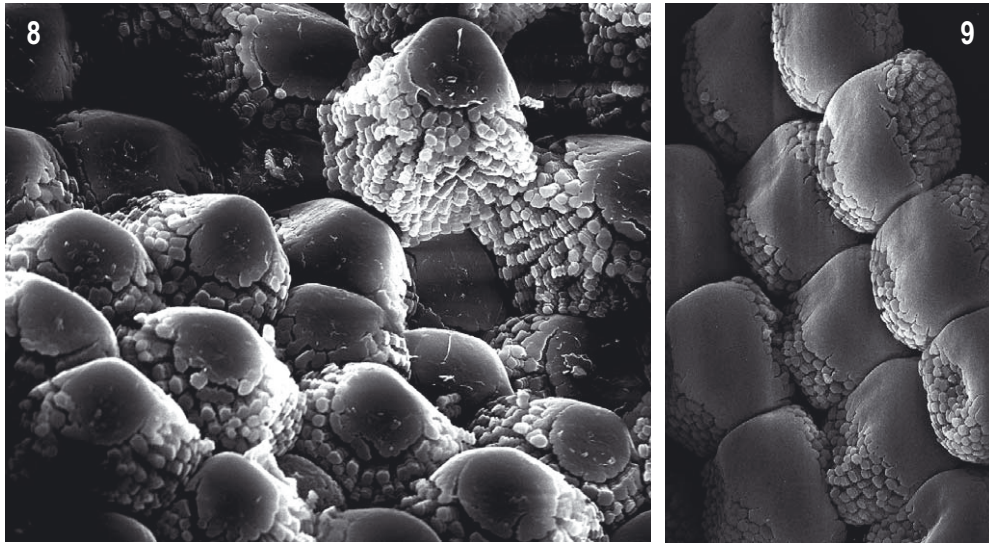


Fig. 7a (left column): *Cronartium ribicola* on *Pinus strobus*: Aeciospore with a smooth strip on one side (after KLEBAHN 1914).

Fig. 7b (right column): *Cronartium flaccidum* on *Pinus sylvestris*: Aeciospore with a nearly smooth strip (after KLEBAHN 1914).



Figs. 8–9: *Pucciniastrum areolatum* on *Picea abies*. Fig. 8: Spore with smooth apical caps in an opened aecium. Fig. 9: Spores arranged in chains with smooth longitudinal overlays from the apex to the base.

The view into a peridermioid aecium of *Pucciniastrum areolatum* after manually opening the peridium shows smooth, apical caps on all spores (Fig. 8). Chains of aeciospores with a longitudinal, smooth strip on one side connecting apex and base (Fig. 9) make clear that this overlying structure is regularly arranged on the spore surface. In figure 8, it covers the warts at the apex and the back side of the spores.

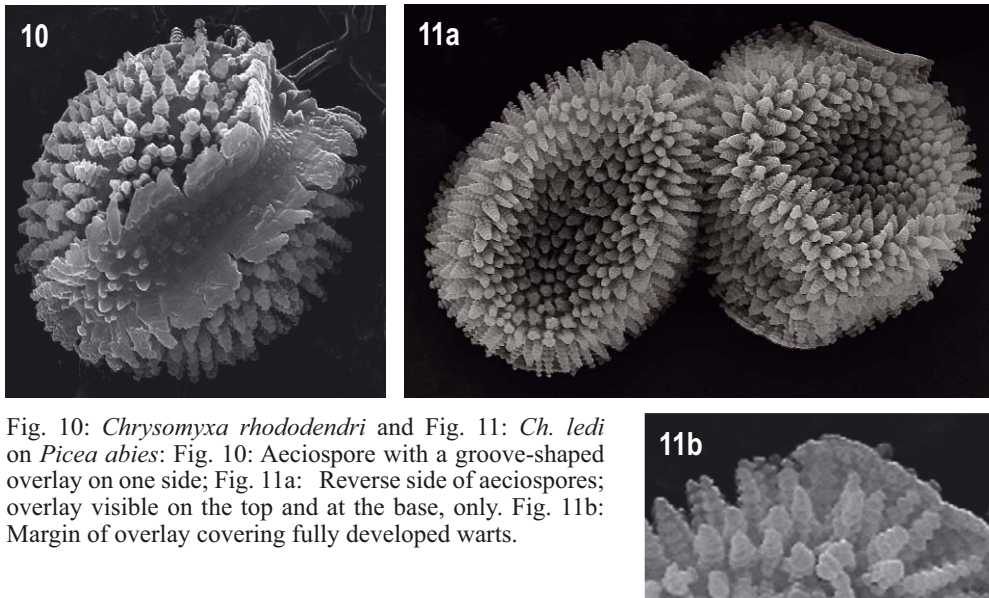


Fig. 10: *Chrysoomyxa rhododendri* and Fig. 11: *Ch. ledi* on *Picea abies*: Fig. 10: Aeciospore with a groove-shaped overlay on one side; Fig. 11a: Reverse side of aeciospores; overlay visible on the top and at the base, only. Fig. 11b: Margin of overlay covering fully developed warts.

In *Chrysomyxa rhododendri*, a similarly arranged longitudinal overlay covers the warts on one side of the aeciospores from the apex to the base. Its margin is curved and full of crevices, so that the longitudinal structure becomes groove-shaped. Bumps are caused by warts underneath the overlay (Fig. 10).

The persistence and regular shape of this overlying structure contrasts clearly with the mode of digestion, reabsorption or otherwise disappearance of the primary wall layer from the embedded ornaments (warts), as described and illustrated by LITTLEFIELD and HEATH (1979). They state that in immature aeciospores of *Melampsora lini*, “a mucilage-like interstitial matrix between warts appears to condense or to break down, exposing ornaments”. BERNDT (1999) showed that warts of immature spores of *Chrysomyxa rhododendri* are almost entirely embedded in the primary wall and protrude only slightly from the spore surface. In older spores the warts have already penetrated the entire primary wall; only rests of the primary wall persist between the warts. In figure 11, the smooth longitudinal structure is on the reverse side of the spores of *Chrysomyxa ledi*. Only its apical and basal ends are visible in the uppermost and basal parts of the spores. It overlies fully developed warts, no remnants of the primary wall are visible between the warts. This can be seen in SEM photos of other aeciospores, for example of aeciospores of *Ch. nagodhii* on *Picea pungens* in CRANE (2001).

The constancy of these overlying structures and their usefulness for distinguishing species within the *Chrysomyxa ledi* complex were demonstrated by CRANE (2001). Three of six species of the *Ch. ledi* complex recognized by CRANE (2001) were fully resolved with the ITS barcode by FEAU et al. (2011).

CRANE (2001) also described and illustrated a broad longitudinal, reticulate area, which covers one side of secondary aeciospores (urediniospores) of *Chrysomyxa reticulata*. Aeciospores of *Cronartium flaccidum* show a very similar structure. In figure 12, fully developed and fully exposed warts appear on one side of the spore and an overlying reticulate structure on the other. This clearly contrasts to the interpretations of KLEBAHN (1914) and HIRATSUKA and SATO (1982) mentioned before; according to them, smooth spots on aeciospores are made up of “undivided wart layers”.



Fig. 12: *Cronartium flaccidum* on *Pinus sylvestris*: Aeciospores with a reticulate overlay on one side.

The presence of these overlying longitudinal structures in several genera with peridermioid aecia, their occurrence only in genera with aecia on conifers, their absence in genera on angiospermous hosts, their regular arrangement on one side of the aeciospore surface and, finally, their regular shape suggest an important function of these structures. They probably protect the spores when the sorus breaks through the tough epidermis and during the following rupture of the robust peridermioid peridium.

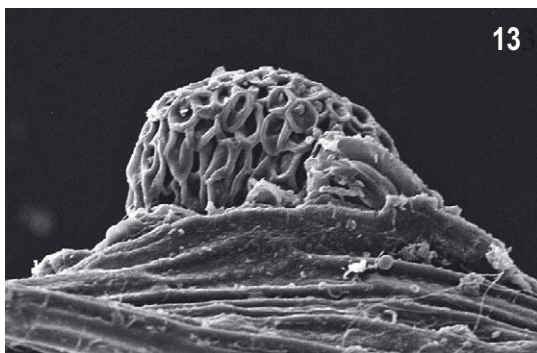
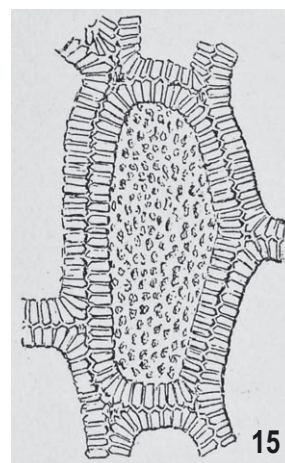
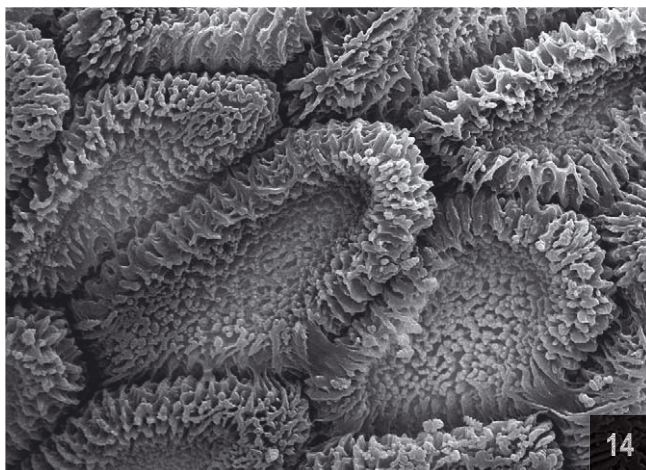


Fig. 13: *Chrysomyxa ledi* on *Picea abies*: young aecium breaking through the epidermis of a spruce needle.

Fig. 14: *Chrysomyxa ledi* on *Picea abies*: inner side of a peridium with interlocking cells.

Fig. 15: *Chrysomyxa ledi* on *Picea abies*: inner side of a peridium with interlocking cells (KLEBAHN 1914).



In figure 13, a young aecium of *Chrysomyxa ledi* breaks through the epidermis of needles of *Picea abies*. The outer wall of the peridial cells is smooth, the inner shallowly verrucose. The side walls are covered with sharp-edged ridges (Fig. 14), which interlock the cells as already shown in KLEBAHN'S (1914) drawing (Fig. 15). SAVILE (1955) emphasized that "it is worth noting the mechanical perfection of the aecial dome, which is closely analogous to that of an Eskimo snow house or, less exactly, the rounded Norman arch".

In aecidioid and peridermioid aecia, the correlation between the mode of peridium rupturing and the arrangement of zones with different ornamentation on the spore surface is striking. Spores in aecidioid aecia exhibit a regular se-

quence of these zones from the apex to the base; apical and basal hemispheres are distinctly different. After regularly opening at the apex their cylindrical peridium becomes cup-shaped with a revolute margin. This points to a pressure in apical direction within the closed aecium. For peridermioid aecia, however, irregular splitting or rupturing above or at the sides, slits and clefts and a torn up peridium, sometimes at length torn to the base, are typical. Spores in these aecia do not exhibit morphologically different apical and basal hemispheres. But in many genera they possess a smooth or nearly smooth strip on one side from the apex to the base.

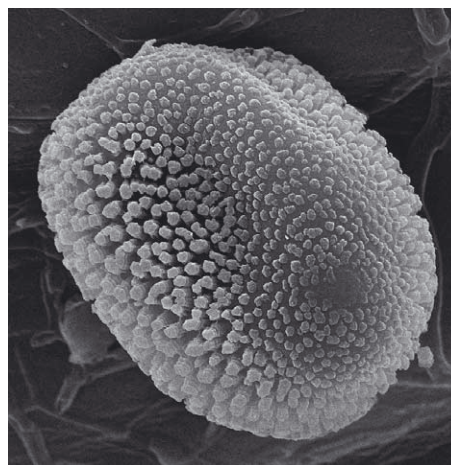


Fig. 16: *Milesina* cf. *exigua* on *Abies alba*: aeciospore with a finely verrucose strip on one side.

The aeciospores of the fern rust genera *Milesina* and *Uredinopsis* are characterized by a special type of nearly smooth strips on one side. SEM images show these strips as areas with distinctly finer warts from the apex to the base (Fig. 16). The contours of these areas resemble those of the overlying structures in the genera *Chrysomyxa*, *Cronartium*, *Pucciniastrum* s.l. These areas with finer warts are considered to be a homologous character; i.e. their resemblance to the overlying structures indicates phylogenetic relationship. The function of these strips of finer warts remains unclear. The fern rust genera *Milesina* and *Uredinopsis* usually occur on humid and shady sites like ravines. In comparison to *Chrysomyxa* spp., *Cronartium* spp. and *Pucciniastrum areolatum*, their aecial peridia are relatively delicate.

Close relationship of the genera *Milesina* and *Uredinopsis* is confirmed by molecular data (MAIER et al. 2003). These data (MAIER et al. 2003, AIME 2006; FEAU 2011) give evidence that the family Pucciniastraceae and also the genera *Chrysomyxa*, *Pucciniastrum* s.str. and *Thekopsora* as accepted by CUMMINS and HIRATSUKA (2003) are polyphyletic. However, AIME (2006) described three suborders within the rust fungi: Uredinineae, Melampsorineae and Mikronegeriineae. All genera listed in the preceding and in the following paragraph belong to the Melampsorineae and produce peridermioid aecia on Pinaceae.

A striking feature in the rust fungi on Pinaceae refers to the structure of warts which are built up of two to many stacked discs, sometimes tapering towards the top. SATO and SATO (1982) defined this surface structure as annulate. In the genera *Chrysomyxa*, *Coleosporium*, *Cronartium*, *Hyalopsora*, *Melampsorella*, *Melampsoridium*, *Milesina*, *Pucciniastrum* s.l. and *Uredinopsis*

these structures are clearly annulate. Without doubt, they represent homologous characters.

In *Milesina exigua*, the annulate ornaments are formed as stacks of discs starting from the base, scarcely tapering towards the top. They exhibit crenate edges with lobes arranged in longitudinal rows (Fig. 17). Aeciospores of *Cronartium ribicola* and *Melampsorella caryophyllacearum* possess similar structures. In *Chrysomyxa rhododendri*, the annulate warts consist of several stacked disks clearly tapering towards the apex, but the edges of their upper discs are scarcely crenate (Fig. 18).

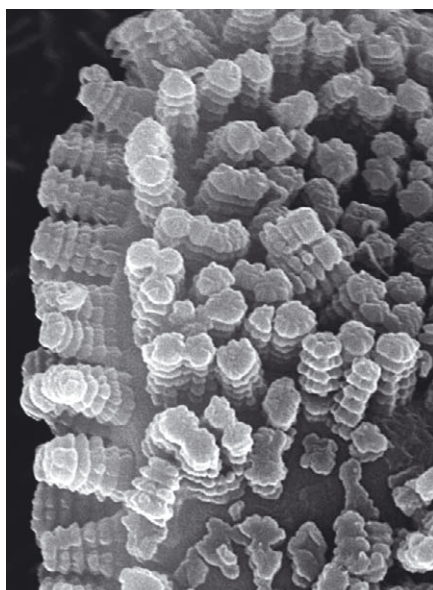


Fig. 17: *Milesina* cf. *exigua* on *Abies alba*: annulate warts of aeciospores.

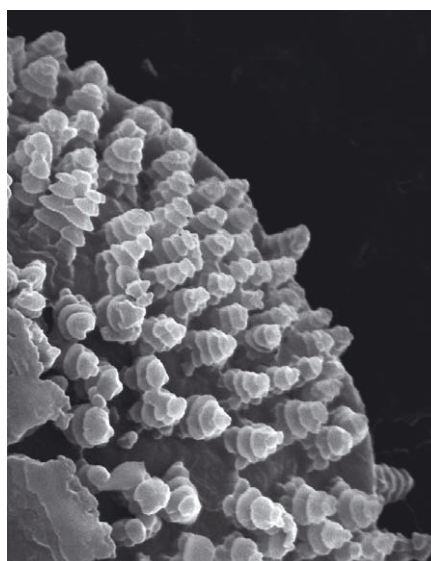


Fig. 18: *Chrysomyxa rhododendri* on *Picea abies*: annulate warts of aeciospores; on the lower left side parts of the overlay can be seen.

LITTLEFIELD and HEATH (1979) already noted that a somewhat different annulate wart structure occurs in *Chrysomyxa pirolata* aeciospores. The warts consist of only two cushion-like discs, one on top of the other. Figure 19a shows that these discs are on a foot with low, longitudinal ridges. Molecular data show that the spruce cone rusts *Chrysomyxa pirolata* and *Ch. monesis* group with the pine needle rusts belonging to the genus *Coleosporium* (FEAU et al. 2011), while most of the other species of the genus *Chrysomyxa* form a separate group.

BERNDT (1999) presented SEM photos of secondary aeciospores (urediniospores) of *Ch. pirolata* and the closely related *Ch. ramischiae*, and stated that their warts are not annulate but dome-shaped with longitudinal ridges. The foot of the primary aeciospores shows similar longitudinal ridges. In other

Chrysomyxa species like *Ch. rhododendri*, the whole wall ornaments of primary and secondary aeciospores look alike. Due to the longitudinal ridges in both types of warts, the feet of the annulate ornaments of the primary aeciospores and the dome-shaped ornaments of the secondary aeciospores in *Ch. pirolata* may have the same origin.

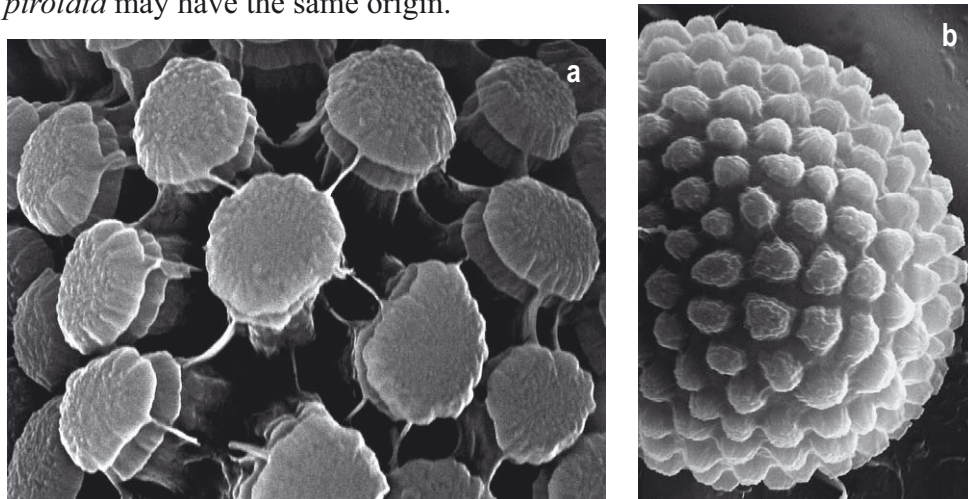


Fig. 19: *Chrysomyxa pirolata*: a) primary aeciospore on *Picea abies* with annulate warts consisting of two discs on a foot; b) secondary aeciospore on *Pyrola rotundifolia* with dome-shaped warts.

2.3. Spores in caeomoid aecia

The aeciospores of *Melampsora cf. larici-epitea* on *Larix decidua* from the European Alps and those of *Mikronegeria fagi* on *Araucaria araucana* from Chile appear to be similar when observed using low magnification, apart from more dense and fine warts in the latter (Figs. 20a and 21a). Both rusts lack zones with different ornaments, as well as overlying structures. When applying higher magnification, the surface ornaments of these rusts differ distinctly. In *Mikronegeria fagi*, the thin warts are connected by relatively high and broad structures, building up a labyrinth as shown in figure 21b. Each wart in *Melampsora cf. larici-epitea* consists of two elements, a broad conical base and an upper element resembling a compressed globe. Some warts are connected by narrow low structures (Fig. 20b).

SATO and SATO (1982) defined different types of ornaments of the aeciospore wall; for only one they used the term verrucose. They found that all species of *Puccinia* and *Uromyces* examined have aeciospores of the verrucose type. But also the ornaments in the species of *Melampsora* studied by them were described as verrucose. However, we find that in *Melampsora cf. larici-epitea*, these structures represent a distinctly different type of ornaments compared to those in *Mikronegeria* and *Puccinia* (Fig. 21). These three genera belong to different phylogenetic groups. Their surface structure of the aeciospores also exhibits basic differences. The warts

of *Puccinia* consist of one element only and have a smooth surface. This can be seen in aeciospores of *P. bromina* subsp. *symphyti-bromorum* with small, hemispherical warts, larger subcylindrical warts and small dehiscent platelets (Fig. 22). Warts of *Mikronegeria* and *Melampsora* are uniform in size and distribution.

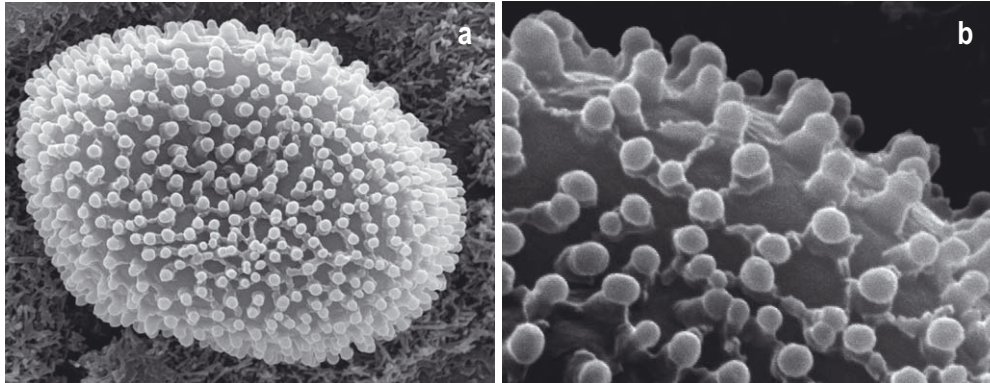


Fig. 20: *Melampsora* cf. *larici-epitea* on *Larix decidua*: a) evenly verrucose aeciospore; b) surface ornamentation of aeciospore in detail.

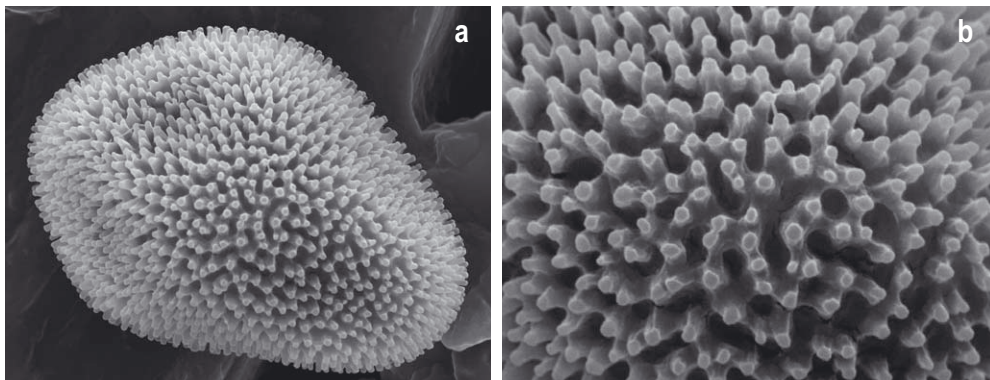


Fig. 21: *Mikronegeria fagi* on *Araucaria araucana*: a) evenly verrucose aeciospore; b) surface ornamentation of aeciospore in detail.

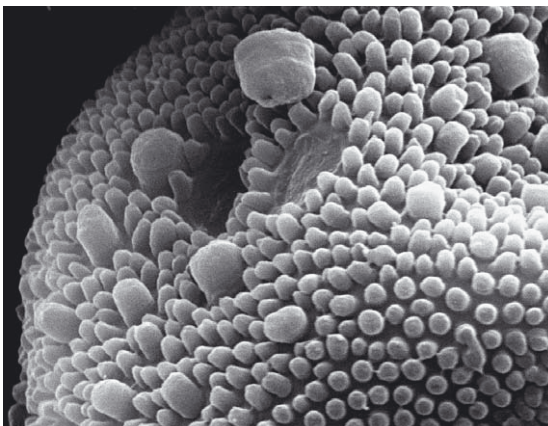


Fig. 22: *Puccinia bromina* subsp. *symphyti-bromorum* on *Pulmonaria australis*: surface ornamentation of aeciospore in detail.

In the definition of SATO and SATO (1982), verrucose warts have a smooth surface, while annulate warts show two or more rings. Aeciospores' warts of *Melampsora* cf. *larici-epitea* do not have a smooth surface but resemble the annulate structure of other rust fungi on conifers; they also consist of more than one element. This resemblance supports one phylogenetic lineage for rusts which produce aecia on conifers. With the exception of *Mikronegeria* and *Caeoma torreyae*, these rusts belong to the suborder Melampsorineae (AIME 2006).

PETERSON and OEHRENS (1978) supposed that the genus *Mikronegeria* displays more "primitive" characteristics than other heteroecious genera of rust fungi. Many previous authors thought the fern rust genus *Uredinopsis* to be the most primitive rust genus. But this genus has elaborate aecia, and its aeciospores are characterized by a complicated surface ornamentation. In comparison, the aecia of *Mikronegeria* and *Caeoma torreyae* are simple; they lack peridia and intercalary cells and their telia are so poorly defined that they barely exist. Of all genera of coniferous rusts, only *Mikronegeria* infects more than one family of conifers – Araucariaceae, Cupressaceae and Podocarpaceae (PETERSON & OEHRENS 1978, CRANE & PETERSON 2007). *Caeoma torreyae* occurs on Taxaceae. In contrast, all genera with peridermioid aecia occur on Pinaceae as does *Melampsora*. Based on molecular data, AIME (2006) concluded that the Mikronegeriaceae and *Caeoma torreyae* are the most basal rusts.

3. Conclusions

- (1) Spores from peridermioid aecia coincide in their main features of wall ornamentation. They differ clearly from spores in aecidioid aecia.
- (2) These facts are reflected also in their restriction to Pinaceae as hosts.
- (3) Spores from caeomoid aecia on conifers do not coincide in their main features of wall ornamentation. Concerning the structure of their two-element-warts, aeciospores from *Melampsora* exhibit similarities with spores from peridermioid aecia. Species of *Melampsora* produce aecia on Pinaceae as well.
- (4) Spores of *Mikronegeria* differ from the other groups in wall ornamentation and host range.
- (5) These correlations of aeciospore morphology and host range correspond to molecular data.

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6. Appendix: Methods and description of specimens used for illustrations

For the study of aeciospore surface ornamentation, air dried aecia on corresponding hosts have been mounted and sputtered with gold. A Philips XL30 ESEM was used for examining aeciospore surface structures. The resulting images have been evaluated with Olympus's Cell A software. Most specimens are deposits of the GZU herbarium. Further specimens have been loans from the herbarium ZT of the ETH Zürich and of the herbarium M of the Bayerische Staatssammlung Munich.

- Chrysomyxa ledi* (ALB. & SCHW.) DE BARY on needles of *Picea abies* (L.) KARST.: Germany, Saxonia, Sächsische Schweiz, leg. W. KRIEGER, 31.07. and 08.08.1891; W. KRIEGER, *Fungi saxonic* No. 663. Figures 11, 13 and 14
- Chrysomyxa pirolata* (KÖRN.) G. WINTER s.str. on cone scales of *Picea abies* (L.) KARST.: Denmark, Bornholm, leg. F.W. NEGER, 20.08.1906; SYDOW, *Uredineen*, No. 2094. Figure 19a
- Chrysomyxa pirolata* (KÖRN.) G. WINTER s.str. on *Pyrola rotundifolia* L.: Austria, Lower Austria, near Windischgarsten, leg. S. WAGNER, 03.04.1990; GZU. Figure 19b
- Chrysomyxa rhododendri* (DC.) DE BARY on needles of *Picea abies* (L.) KARST.: Austria, East Tyrol, Matreier Tauernhaus, leg. F. GRIMS, 02.09.1986; GZU. Figures 10 and 18

P. Zwetko † & P. Blanz

Cronartium flaccidum (ALB. & SCHW.) G. WINTER on branches, erumpent from the bark of *Pinus sylvestris* L.: Austria, Styria, Köflach, leg. M. URBAS, 05.1910; GZU. Figure 12

Melampsora cf. *larici-epitea* KLEB. on needles of *Larix decidua* MILL.: Austria, Styria, Hochschwab area, Seewiesen, leg. P. BLANZ & P. ZWETKO, 19.06.2012; GZU. Figures 20a and 20b

Remarks: Aeciospore dimensions and wall thickness of *M. larici-epitea*, *M. caprearum*, *M. larici-pentandrae* and *M. larici-populina* are very similar, and do not permit save assignment. We found aecia on larch in close vicinity to uredinia on *Salix glabra*. This is the only recent collection of aecia on larch in Austria. We assigned them to *M. larici-epitea*. To our knowledge, no evidence is provided for the occurrence of another *Melampsora* species on *S. glabra*.

Mikronegeria fagi (DIETEL & NEGER) DIETEL auf *Araucaria araucana* (MOLINA) K. KOCH: Chile, Cordillera Nahuelbuta, 1100 m, leg. H. BUTIN, 03.12.1966; ZT Myc 13485. Figures 21a and 21b

Milesina cf. *exigua* (FAULL) FAULL ex HIRATS. f. on needles of *Abies alba* MILL.: Austria, Styria, Höllgraben WNW Stainz, 700 m, ravine, leg. J. POELT & P. ZWETKO; 29.09.1988; Mycotheca Graecensis, Fasc. 19 (2004), in *Fritschiana* 46. Figures 16 and 17

Remarks: The Aecia have been found in close vicinity to uredinia on *Polystichum braunii*. According to the diagnosis of HIRATSUKA (1936), the aeciospores of *M. exigua* are distinctly smaller ($18\text{--}24 \times 14\text{--}22.5 \mu\text{m}$) than those of all other *Milesina* species reported on *Abies* from Europe. In the specimen from Stainz, the dimension of the aeciospores is distinctly smaller ($23\text{--}30 \times 19\text{--}24 \mu\text{m}$) than in other specimens of *Milesina* sp. from Styria ($39\text{--}42 \times 27\text{--}29 \mu\text{m}$). The latter have been found in a ravine “Gesäuse, Hartelsgraben” at 700 m (leg. P. ZWETKO, 15.09.1989). The ornamentation of their spore surface equals that in the specimen from Stainz.

The ornamentation of aeciospore surface in the following specimens of the genus *Uredinopsis* resembles that in *Milesina*:

Uredinopsis americana SYD. (syn. *U. mirabilis* (PK.) MAGNUS) on *Abies balsamea* (L.) MILL.: Canada, New Foundland, Gros Morne Nat. Park, leg. J.A. PARMELEE & B.M. DECARTERET, 31.07.1983; DAOM, GZU. SEM photo not published here.

Uredinopsis pteridis DIETEL & HOLW. in DIETEL on *Abies concolor* (GORD. & GLEND.) LINDL. ex HILDEBR.: USA, California, Head of Concow Creek, ca. 750 m, leg. C.R. QUICK, 24.07.1965; California Fungi No. 1320. SEM photo not published here.

Puccinia bromina ERIKSS. subsp. *symphyti-bromorum* (F. MÜLLER) URBAN & MARKOVÁ (syn. *Puccinia symphyti-bromorum* F. MÜLLER) on *Pulmonaria australis* (MURR.) SAUER: Slovenia, Čičarija, N of Rakitovec, leg. Ch. SCHEUER, 08.06.2006; GZU; Dupla Graecensia Fungorum. Figure 22

P. magnusiana KOERN. on *Ranunculus repens* L.: Austria, Styria, Ennstal near Trautenfels, leg. P. ZWETKO, 17.06.1984; GZU. Figure 5

P. magnusiana KOERN. on *Ranunculus repens* L.: Austria, Burgenland, between Neusiedl am See and Jois, leg. T. BARTA, 14.05.1994; GZU, Dupla Fungorum. Figure 4

Distinctiveness of aecia and aeciospores

P. poae-aposeridis GÄUM. & POELT on *Aposeris foetida* (L.) LESS.: Germany, Bavaria, Schleching, leg. H. & H. DOPPELBAUR, 28.05.1967; M. Figure 2

Puccinia silvatica J. SCHROET. on *Taraxacum officinale* WIGGERS agg.: from P. ZWETKO (1993) image plate No. 1. Figure 3

Pucciniastrum areolatum (FR.) OTH (syn. *Thekopsora areolata* (FR.) MAGNUS) on cone scales of *Picea abies* (L.) KARST.: Austria, Styria, near Judenburg, leg. H. MELZER, 04.1953; GZU. Figures 8 and 9

Uromyces alpinus J. SCHROET. (syn. *Schroeteriaster alpinus* (J. SCHROET.) MAGNUS) on *Ranunculus* cf. *montanus* WILLD.: Switzerland, Valais, Chemin du Rawil, Praz Jean – Les Ravins, leg. CH. TERRIER, 01.06.1947; ZT. Figure 6

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