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CHARACTERS OF FRUITBODIES, BASIDIOSPORES AND CULTURES USEFUL FOR RECOGNIZING AMYLOSTEREUM AREOLATUM AND A. CHAILLETII

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Abstract

The fruitbodies and cultures of *A. areolatum* and *A. chailletii* may be recognized by using hymenial colours, basidiospore size, colour of mycelial mat on PDA, and presence or absence of oidia in culture. Basidiospore size is just as useful a characteristic for separating fruitbodies of *A. areolatum* and *A. chailletii* as presence or absence of oidia is for cultures. Conflicting information from previous literature is discussed.

Introduction

Amylostereum areolatum (Fr.) Boidin and *A. chailletii* (Pers.:Fr.) Boidin are two fungi that have attracted much interest, both in relation to their symbiotic association with siricid woodwasps and in their own right as members of the Corticiaceae. Fruitbodies of *A. areolatum* and *A. chailletii* have generally been considered to be similar and difficult to distinguish morphologically (e.g. Skovsted, 1956; Jahn, 1971; Breitenbach & Kränzlin, 1986). However, certain characteristics of fruitbodies and cultures may aid in delimiting the species.

The basidiospore size and length/width ratio of basidiospores is considered a key character for distinguishing these species by Boidin & Lanquetin (1984). They list spore sizes of 4,8-6,5 x 2,8-3,8 µm for *A. areolatum* and 6,2-8 x 3-4 µm for *A. chailletii*, with a length/width ratio for basidiospores of *A. areolatum* less than 2, and for *A. chailletii* equal to or greater than 2. However, varying spore sizes are given in other sources.

Another trait considered important is the colour of the hymenium. *Amylostereum areolatum* fruitbodies are described as having brown-violet hymenia with purple or lilac tints, whereas the hymenial colours of *A. chailletii* are said to be grey-brown, cinnamon, nut-brown, or ochre (Jahn, 1971; Breitenbach & Kränzlin, 1986). In addi-

tion, a few sources mention the presence of a thin, dark line separating trama and tomentum in *A. areolatum* (Eriksson & Ryvarden, 1973; Jahn, 1971, 1979; Breitenbach & Kränzlin, 1986).

With respect to cultures, both *A. areolatum* and *A. chailletii* are characterized by possessing numerous clamp connections and by occurrence of encrusted cystidia after a few weeks incubation. However, the most important character is the presence of oidia in the cultures of *A. areolatum*. Because *A. chailletii* does not produce oidia in culture, this difference has long been considered the best way to differentiate cultures of these species (Siepmann & Zycha, 1968; Jahn, 1971; Stalpers, 1978; Boidin & Lanquetin, 1984). Separation of *A. areolatum* and *A. chailletii* based on the colour of mycelial mats has not been described previously.

The presence or absence of oidia in culture is helpful in distinguishing between these fungi as symbionts of siricid woodwasps. Cultures obtained from the wasps themselves or from the wood near larval tunnels can be determined from cultural characteristics alone. However, the difference applies only to the cultures. Within the intersegmental sacs (mycetangia) of woodwasps, both *A. areolatum* and *A. chailletii* produce oidia.

This article describes characteristics of fruitbodies, basidiospores and cultures useful in separating *A. areolatum* from *A. chailletii*. The aim is to compile and elaborate on characters described previously in different sources. The concept of basidiospore measurements as key character is discussed in relation to earlier works on *A. areolatum* and *A. chailletii*.

Material and methods

Fruitbodies of both *A. areolatum* and *A. chailletii* were collected from various places in Denmark, mostly within 100 km of Copenhagen. The colour of the hymenial layer was recorded, and basidiospore prints were taken on microscope slides. The prints were flooded with lactophenol (equal parts lactic acid, phenol, glycerol, and distilled water). A cover glass was added, and the combination stored for later study, as observations were not possible immediately for many of these basidiospore prints. Basidiospore measurements were made on a Reichert OPTIGRA light microscope at 10 x 63 times magnification. For each basidiospore print, 25 spores were measured, and the average length and width and the ranges were calculated. For each species a single average size and range was then calculated on basis of the measurements.

To culture from the fruitbodies small pieces of the tramal layer were removed with a sterile razor blade and placed aseptically on 4% potato-dextrose agar (PDA) or 5% malt extract agar (MEA) in 90 mm Petri dishes. After three to four weeks the colour of the mycelial mat was noted, and the presence or absence of oidia in the cultures were used to identify the isolates unequivocally as *A. areolatum* or *A. chailletii*. Colour names reported in quotations marks or with numerical codes (e.g. 6C4) are those of Kornerup & Wanscher (1981). Others are the authors vernacular. Black and white photos were made with the aid of a Nikon Optiphot with a microflex PFX attachment, in differential interference contrast at 10 x 40 magnification.

Most basidiospore measurements were made on spore prints that were several months old. Fresh spores suspended in lactophenol under cover glasses were found to drift and move slightly, thus making measurements difficult. When left for a couple of months the lactophenol dried out (albeit slowly), so that the cover glasses adhered more tightly to the microscope slides. However, measurements on three fresh spore prints of each *Amylostereum* species were also made, in order to compare the results.

Results

Only thirteen fruitbodies of *A. areolatum* were found, but in addition isolates were obtained from eleven woodwasps. All the finds were from *Picea abies* or from woodwasps inhabiting *P. abies*. Of the fruitbody finds, eight were confirmed as *A. areolatum* through the presence of oidia in culture. Spore prints were obtained from six of the fruitbodies confirmed through culturing and from two fruitbodies identified by hymenial colouring, and these prints were used for basidiospore measurements. (Table 1 & 2).

Fruitbodies of *A. chailletii* were much more abundant, occurring on *P. sitchensis*, *Larix decidua*, *Abies grandis*, *A. nordmanniana*, *A. procera*, *A. alba* and *Pseudotsuga menziesii*, but especially on *Picea abies*. Thirtythree of the fruitbodies catalogued as *A. chailletii* from fruitbody characters were positively identified through cultural characters. Of the collections identified through culturing, eight spore prints from the fruitbodies were used for measurements. (Table 1 & 2).

Hymenial colours

Amylostereum areolatum and *A. chailletii* both produce resupinate and effuso-reflexed fruitbodies. The fruitbodies used for description of hymenial colours were all confirmed as to identity through culturing and observing the presence or absence of oidia.

Most fruitbodies of *A. areolatum* had the deep brown-violet colour typical for this species on all or part of the hymenium ("ruby" 12E8 to "dark ruby" 12F7, Kornerup & Wanscher (1981)). One of the fruitbodies showed a purple to violet tinge over most of the hymenium ("deep violet" 16E8). These violet patches were also found on other fruitbodies. In addition, some parts of the fruitbodies were red-brown or paler, becoming (dark) greyish brown or even leather-brown. However, all *A. areolatum* fruitbodies could be recognized by the prevalent brown-violet colour and the quite wide, white margin around the edges. Breitenbach & Kränzlin, (1986, p 179) provide a photo showing the violet colouration of *A. areolatum*. The "deep ruby" colour resembles the photo of *Chondrostereum purpureum* (Fr.) Pouz. (ibid., p 181). Both hymenial colour types of *A. areolatum* fruitbodies may also be seen in Thomsen & Koch (1993).

The hymenial colours of *A. chailletii* are more variable than those of *A. areolatum*. They range from cream and grey ("orange grey to greyish orange" 5B2-5, Kornerup & Wanscher (1981)) over pale leather-brown (= 6C4) to "reddish-brown" (8E8) or "brick red " (7D7). However, the pale colours are most common and can be found

even on specimens where red-brown colours dominate. The margin of the fruitbody may be white, but is narrower than that of *A. areolatum*. Often, typical *A. chailletii* fruitbodies resemble *Stereum sanguinolentum* (Alb. & Schw.: Fr.) Fr. The "reddish-brown" hymenial colour may be seen in Breitenbach & Kränzlin (1986, p 181) and in Thomsen & Koch (1993), whereas the greyish colours are illustrated in Rymann & Holmåsen (1992, p 87).

Basidiospore measurement

The basidiospores from *A. areolatum* and *A. chailletii* showed a distinct difference in size. The spores of *A. areolatum* were within the range 3,6-5,6 x 2-3,2 µm, and the average spore size was 4,5 x 2,4 µm. The spores of *A. chailletii* were in the range 4,8-8 x 2,4-4 µm, and in average 6,1 x 3,1 µm wide. Thus the basidiospores of *A. areolatum* are noticably smaller that those of *A. chailletii*, and the difference can be recognized by visual observation of spore prints without measuring.

Storing the basidiospore prints caused the spores to shrink slightly. The length of the basidiospores in fresh prints did not approach the lower limits of the range seen in stored prints. Thus fresh basidiospores were never less than 4 µm long for *A. areolatum* nor 5,2 µm for *A. chailletii*. In addition, the width of fresh *A. areolatum* basidiospores stayed at or above 2,4 µm. The ranges and averages of basidiospore sizes for the two fungi is summarized in Table 1.

The length/width ratios given by Boidin & Lanquetin (1984) did not always fit for a single spore, but in average for 25 spores they were found to be precise in twelve out of sixteen cases (Table 2). In fresh sporeprints of both *Amylostereum* species the ratios held for most single basidiospores and always for the average basidiospore size. In stored sporeprints, many spores of *A. areolatum* conformed to the ratio associated with *A. chailletii*, i.e. spore length twice the width or longer. However, in average the length was less than two times the width in all cases except one (Table 2). In stored sporeprints of *A. chailletii* the average basidiospore length was generally twice the width, but some notable exceptions occured (Table 2) which is reflected in the length/width ratio for stored spores (Table 1).

Table 1 Summary of basidiospore sizes (fresh and preserved prints) for *A. areolatum* and *A. chailletii* measured in lactophenol.

	A. areolatum	*A. chailletii*
Average, fresh Length/width ratio Range, fresh	4,6 x 2,6 µm 1,77 4,0-5,6 x 2,4-3,2 µm	6,2 x 3,1 µm 2,00 5,2-8,0 x 2,4-4,0 µm
Average, stored Length/width ratio Range, stored	4,5 x 2,4 µm 1,88 3,6-5,6 x 2,0-3,2 µm	6,1 x 3,1 µm 1,97 4,8-8,0 x 2,4-4,0 µm
Boidin & Lanquetin (1984)	4,8-6,5 x 2,8-3,8 µm	6,2-8,0 x 3,0-4,0 µm

Table 2 Average and range of basidiospore size for *A. areolatum* and *A. chailletii* based on measurements of 25 basidiospores in sporeprints stored for at least 3 months. The averages marked with * do not conform to the length/width ratios given by Boidin & Lanquetin (1984). The identity of *A. areolatum* was confirmed by presence of oidia in culture except for imt 137 and 141.

Species	ID number	Average spore size	Spore size range
A. areolatum	imt 109	4,6 x 2,6 µm	4,0-5,6 x 2,4-3,2 µm
A. areolatum	imt 137	5,0 x 2,6 µm	4,0-5,6 x 2,4-3,2 µm
A. areolatum	imt 141	4,5 x 2,3 µm	3,6-5,6 x 2,0-2,8 µm
A. areolatum	imt 146	4,1 x 2,2 µm	3,6-4,8 x 2,0-2,4 µm
A. areolatum	imt 155	4,6 x 2,3 µm *	3,6-5,6 x 2,0-2,4 µm
A. areolatum	imt 156	4,5 x 2,4 µm	4,0-5,6 x 2,0-2,8 µm
A. areolatum	imt 159	4,5 x 2,3 µm	4,0-5,6 x 2,0-2,8 µm
A. areolatum	imt 162	4,4 x 2,3 µm	4,0-5,2 x 2,0-2,4 µm
Total (stored)		4,5 x 2,4 µm	3,6-5,6 x 2,0-3,2 µm
A. chailletii	imt 122	6,3 x 3,5 µm *	5,6-8,0 x 2,4-4,0 µm
A. chailletii	imt 128	6,1 x 2,9 µm	5,6-6,8 x 2,4-3,6 µm
A. chailletii	imt 138	5,9 x 3,3 µm *	5,6-6,4 x 2,8-4,0 µm
A. chailletii	imt 140	6,0 x 3,2 µm *	5,6-6,4 x 2,8-4,0 µm
A. chailletii	imt 142	5,9 x 2,7 µm	4,8-7,2 x 2,4-3,2 µm
A. chailletii	imt 144	6,1 x 2,8 µm	4,8-8,0 x 2,4-3,6 µm
A. chailletii	imt 145	6,2 x 2,8 µm	5,6-7,2 x 2,4-3,2 µm
A. chailletii	imt 163	6,2 x 3,1 µm	5,6-7,2 x 2,4-4,0 µm
Total (stored)		6,1 x 3,1 µm	4,8-8 x 2,4-4 µm

Amylostereum areolatum basidiospores were consistently smaller than *A. chailletii* - spores. *A. chailletii* spores were more variable in size, although their average length did not vary much. The average spore width of *A. chailletii* was much more variable than the average spore length (fig. 6). Single basidiospores of *A. chailletii* might approach the smaller size of *A. areolatum* spores, but in average (of 25 spores) they were always longer and wider (fig. 6). As a rule, *Amylostereum chailletii* spores are nearly always 5 µm or longer, whereas *A. areolatum* spores may be as short as 4 µm and only rarely exceed 5,6 µm in length. *Amylostereum areolatum* spores are rarely wider than 3 µm, but *A. chailletii* spores may sometimes reach 4 µm in width.

Cultural Characters

In the first cultures from fruitbodies or woodwasps grown on PDA and on MEA, the growth and colours of the mycelial mats of *A. areolatum* differed markedly from that of *A. chailletii*.

A. areolatum: Mycelial mat pellicular or very thinly felty; aerial hyphae appressed in both marginal and older parts; "yellowish brown" (5C8, Kornerup & Wanscher (1981)) to "rust brown" (6E8) or "leather brown" (6E6) with a tinge of curry-yellow

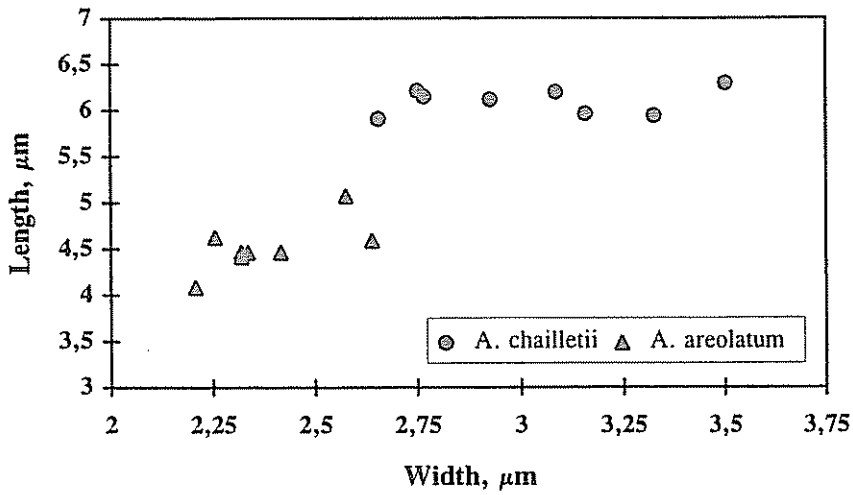
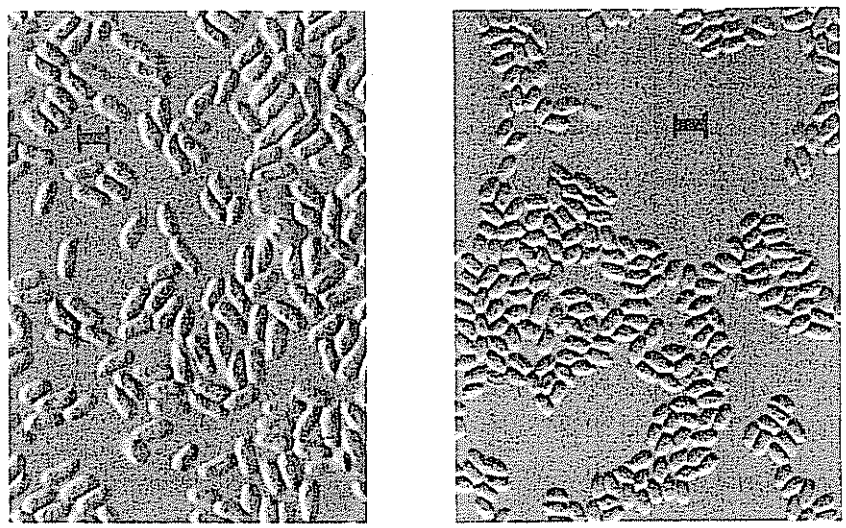


Fig. 1 Measurements of basidiospores from *Amylostereum areolatum* and *A. chailletii* in lactophenol. Each marker represents the average of 25 basidiospores from one fruitbody.



right left

Fig. 2 Basidiospores of (~~left~~) *Amylostereum areolatum*, imt 112, and (~~right~~) *A. chailletii*, CP 1850. Bar = 5 µm. Photos J. Koch 1993.

("orange-yellow" 4B8), especially when originally isolated; reverse darkening within three weeks, especially on MEA. Sometimes changes in growth-rate and colour developed resulting in cinnamon-colour or cream-colour patches with slightly more aerial hyphae.

A. chailletii: Mycelial mats felty or woolly with felty patches, aerial hyphae not appressed in older parts but somewhat appressed in margin; cream coloured, "pale yellow" (4A3) or "yellowish white" (4A2), after 3-4 weeks with light brown specks, patches or overall tinge of "golden wheat" (4B5) or "clay" (5D5); reverse darkening slowly and in patches. The darker curry-yellow and rust brown colours typical of *A. areolatum* were never observed in *A. chailletii* isolates.

Both species are also characterized by clamps at all septa and by the appearence of encrusted cystidia after five or six weeks. Repeated transfers from *A. areolatum* isolates typically resulted in altered mat colouration, so that the colours resembled *A. chailletii*. However, the reverse colouration did not change, and small patches of the culture would retain the leather brown colour. For *A. chailletii* repeated transfers did not cause any obvious alterations.

Further descriptions of the cultural characteristics of *A. chailletii*, including colour of mycelial mat, may be found in Basham (1959), whose description was made by Nobles, and in Nakasone (1990); descriptions of cultures of *A. areolatum* may be found in Talbot (1964) and Siepmann & Zycha (1968), however none of these mention the mycelial mat colours described here. Stalpers (1978) does not distinguish between *A. areolatum* and *A. chailletii* by the colour of the mycelial mat.

Discussion

The frequency of *A. chailletii* fruitbodies found in this study showed that *A. chailletii* is correctly considered as common in coniferous forests in Denmark (Petersen & Vesterholt, 1990). The presence of *A. areolatum* (as fruitbodies) in Denmark was previously considered as probable, but records were lacking (Petersen & Vesterholt, 1990; Koch & Thongjiem, 1989). Yet the first find of *A. areolatum* as fruitbody later confirmed by the presence of oidia in culture was actually made in 1985 (Koch, deposited in the culture collection CP, Copenhagen). However, the occurence of *A. areolatum* fruitbodies was not published until 1993 with a list of finds, all deposited in CP at that time (Thomsen & Koch, 1993). Thus *A. areolatum* may now be designated as present but rare in Denmark.

Hymenial colour is a key character for distinguishing between *A. areolatum* and *A. chailletii* fruitbodies. The deep brown-violet and purple colours of *A. areolatum* are never found in *Amylostereum chailletii* specimens. For *A. chailletii* the easiest fruitbody specimens to recognize are those which look like *Stereum sanguinolentum*. Thus, the absence of a bleeding reaction on the hymenium of a *S. sanguinolentum*-like fruitbody on conifers indicates that *A. chailletii* has been found. In addition, the presence of woodwasp emergence holes near such fruitbodies also points to *Amylostereum*. Although *S. sanguinolentum* was once thought to be associated with woodwasps, this is actually not the case, even though the misconception still persists in

many places. Another common hymenial colour seen in *A. chailletii* is brick red or red-brown. In general, fruitbodies of *A. chailletii* are very variable in hymenial colour, and therefore not so easy to determine in the field.

Of the four characteristics studied, the colour of the mycelial mat in *A. areolatum* was the least trustworthy, as it changed according to the media used or with reinoculation of the cultures. However, the first culture of *A. areolatum* on PDA or MEA from a fruitbody or woodwasp usually had the typical colours. Yellow-brown or red-brown colours and pellicular or felty growth were only associated with *A. areolatum* and never with *A. chailletii*. The fast and complete darkening of the reverse side of the culture may be considered as an additional aid in identifying *A. areolatum*, as this process occurs even if the colours are not typical. Thus, the macroscopic characters described above should suggest *A. areolatum* to the observer, whether the culture was retrieved from fruitbodies, woodwasps or wood.

In contrast to *A. areolatum*, cultures of *A. chailletii* only darkened the reverse side slowly and in patches, if at all. The mycelial mats of *A. chailletii* were consistently white, cream or light brown, and the growth was much thicker. This was true of both fruitbody and woodwasp isolates. Although not as characteristic as *A. areolatum*, cultures of *A. chailletii* can at least be easily distinguished from the former, if not recognized directly. However, distinguishing unequivocally between isolates of *A. areolatum* from *A. chailletii*, whether from fruitbodies, wood or woodwasps, requires microscopic characteristics. The presence or absence of oidia is the ultimate criterion for separating *A. areolatum* and *A. chailletii*. If possible, all finds of fruitbodies considered to be either species should have the identity confirmed through culturing and checking for the oidia present in *A. areolatum*.

In addition, *A. areolatum* and *A. chailletii* fruitbodies can be accurately distinguished by the size of their respective basidiospores. As already shown in the key of *Amylostereum* species provided by Boidin & Lanquetin (1984), the basidiospores of *A. areolatum* are clearly smaller than the basidiospores of *A. chailletii*. Thus it may be recommended that sporeprints should routinely be taken of *Amylostereum* specimens collected. There is no doubt that basidiospore size can be used to identify the species just as effectively as presence or absence of oidia in culture. Compared to Boidin & Lanquetin (1984), basidiospore measurements in this study depict *A. areolatum* with smaller spores, resulting in the spore size range having lower limits (table 1). For *A. chailletii*, only the lower limits of spore lenght and width in this study differed by more than 1 µm from Boidin & Lanquetin (1984). However, it is not the exact spore size range which is most important, but the obvious and consistent difference in basidiospore size, *A. areolatum* spores being invariably smaller than *A. chailletii* spores.

An important aspect of the consistent difference in basidiospore size is that some literature concerning *A. areolatum* and *A. chailletii* should perhaps be reinterpreted. For instance, most authors agree that Pilát (1931) was mistaken in his identification of *A. chailletii* and had really described *A. areolatum*. However, the range for basidispore size given by Pilát (6-7,5 x 3-4 µm) fits *A. chailletii* rather than *A. areolatum*. In addition, the hymenial colours described by Pilát are also consistent

with those typical of *A. chailletii*. It is therefore likely that he did indeed describe *A. chailletii* and thus named it correctly.

Reinterpretations of older sources according to basidiospore measurements would also change the perception of Bourdot & Galzin (1921, 1927) and Skovsted (1956). They identified their decribed fungi as *A. areolatum*, but the basidiospore sizes given are clearly too large to fit *A. areolatum*. The range is 7-9 x 3,5-4,5 µm for Bourdot & Galzin, and 5,5-8,5 x 2,5-4 µm for Skovsted. In all of these sources the hymenial colours described for *A. areolatum* could easily be applied to *A. chailletii* instead.

In the case of Jahn (1971) an attempt at reevaluation based on basidiospore size encounters severe difficulties. In his descriptions of *A. areolatum* and *A. chailletii* Jahn (1971) was the first to place great emphasis on the dark purple-violet or blue-violet hymenial colours which are characteristic for *A. areolatum*. In addition, Jahn had some of the cultures from fruitbodies checked for the presence or absence of oidia. Based on these characteristics, Jahn undoubtedly identified the species correctly. But the ranges of basidiospore sizes were said to be 5-8,5 x 2,4-4 µm for *A. areolatum* and 5-8 x 2,5-4 µm for *A. chailletii*. In other words the spores of *A. areolatum* were considered to be as long as or longer than those of *A. chailletii*. Unless there is a printing mistake in the paper, the size of *A. areolatum* basidiospores given by Jahn seriously conflicts with both Boidin & Lanquetin (1984) and the results found in the present study. It is difficult to explain the discrepancy, especially because the average basidiospore size is not given. Thus, one possible explanation could be that a few specimens of *A. chailletii* had been classified as *A. areolatum* and not checked by culturing. Thereby the range for *A. areolatum* would come to overlap that of *A. chailletii*. This would have been revealed in the average if included, as *A. areolatum* spores would probably still have been markedly smaller than those of *A. chailletii*.

Other sources of basidiospore sizes give measurements with ranges within those described by Boidin & Lanquetin (1984) or within the results of the present paper. Examples are : Talbot (1964) 4-6,3 x 2,5-3 µm for *A. areolatum*, Eriksson & Ryvarden (1973) 6-7 x 2,5-3 µm for *A. chailletii*, Martin & Gilbertson (1980) 6-8,5 x 2-3 µm for *A. chailletii*, Breitenbach & Kränzlin (1986) 5-6 x 2,5-3 µm for *A. areolatum* and 5,5-7 x 2,5-3 µm for *A. chailletii*, and Rymann & Holmåsen (1992) 6-8 x 2,5-3 µm for *A. chailletii*.

Conclusion

It may be concluded that the four characteristics studied in this investigation are very helpful in identifying *A. areolatum* and *A. chailletii*. The presence or absence of oidia in culture and basidiospore size in fruitbodies are the best way to distinguish the two species. The advantage of basidiospore measurement is that is does not require culturing, but only access to a light microscope. Hymenial colour is useful as field characteristic, especially for *A. areolatum*. When working with isolates from wood or woodwasps, macroscopic characteristics of cultures may be helpful, although the presence or absence of oidia is infallible in making the distinction.

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