

The generic limit between *Otidea* and *Tarzetia* (*Pustularia* auct.)

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The classical differences between *Otidea* (Pers.) Bon. and *Tarzetia* (Cooke) Lamb. (*Pustularia* auct.) are evaluated and shown to be of slight taxonomic value. The structure of the excipulum is found to be identical in the two genera. On the other hand, they are clearly differentiated by certain sporal and cytological characters, an essential part of which are presented for the first time. The *Otidea* and *Tarzetia* spores belong to different types on the basis of the existence versus absence of a cyanophilic perispore-periplasm coating in mature spores. Staining with cotton blue proved to be very helpful in the study. It is concluded that the two genera should be kept separate, as has generally been done up till now, although on different and uncertain grounds.

The justification for separating the very similar genera *Otidea* (Pers.) Bon. (here treated in an amended sense, i.e., excluding *Flavoscypha* Harmaja; see HARMAJA 1974a) and *Tarzetia* (Cooke) Lamb. (*Pustularia* auct.; see ROGERS, DUMONT & KORF 1971) has puzzled me for some time, as the classical differences, used even in the recent literature (e.g. by NANNFELDT 1966 and also those used by KORF 1972, except the carminophily of the *Tarzetia* nuclei) do not seem to be sufficiently important to be diagnostic at generic level. I began to study this point and, on observing that these genera have exactly the same type of excipulum, I at first even considered the possibility of merging them. However, certain differences that I found, together with BERTHET's (1964) observation of the strong carminophily of the nuclei of *Tarzetia* (confirmed by KORF, 1972, and the present study), clearly show that these two genera are distinct.

In the following the three »classical» differences between *Otidea* and *Tarzetia* are considered first (for the carminophily of the

nuclei, see later) and shown to be invalid. The characters observed by me that are of taxonomic interest and common to both genera are then described. Lastly, the truly diagnostic differences between the genera are given, also based exclusively on my own observations, except for certain cytological characters. The following species were studied: *Otidea alutacea* (Fr.) Bres., *O. bufonia* (Fr.) Boud., *O. caligata* (Nyl.) Sacc., *O. indivisa* Vel., *O. leporina* (Fr.) Fuck. coll., *O. onotica* (Fr.) Fuck., *O. platyspora* Nannf., *O. sp.*; *Tarzetia catinus* (Pers.) Korf & Rogers, *T. cupularis* (Fr.) Lamb., *T. spurcata* (Pers.) Harmaja and *T. pusilla* Harmaja (see HARMAJA 1974b).

A. Evaluation of the three classical differences

1. The shape of the fruit body

The *Otidea* species were, and are often still, said to have ear-shaped apothecia, split

on one side, while those of *Tarzetta* are normal and cup-shaped. In the first place, there is a general and justifiable tendency in the modern taxonomy of the *Pezizales* to accord less importance at generic level to macroscopic differences in the ascocarp shape (e.g. in the cases of *Helvella* and *Gyromitra*). In the second, this difference does not always hold; the apothecium of *Otidea indivisa*, otherwise a very typical *Otidea*, is a regular stipitate cup, and *O. apophysata* (Cooke & Phill.) Sacc. also has an entire apothecium. This difference thus only represents a general trend, and does not deserve to be emphasized at the generic level.

2. The hairs of the excipulum

NANNFELDT (1938 and 1966) accords great value to this character; *Tarzetta* is said to possess »long cylindrical, sparingly septate hairs» in contrast to the »short-celled hyphal chains» in *Otidea*. However, my observations show that, if the basal tomentum is excluded, species of both genera may produce septate, hyaline, unspecialized hyphae of variable length, mostly very inconspicuous, as germs of the outermost cells of the *textura angularis* layer (to some degree also from those of the *t. globulosa* or *t. prismatica* layer) of their ectal excipulum (see also below). At the margin of especially young apothecia the hyphae are in fact more distinct and abundant in *Tarzetta* (and at least in *T. spurcata* even finely papillose in places), but I am not inclined to call them specialized hairs. Anyway, as the tendency for the excipulum to produce these hyphae is present in both genera, including their type species, I do not consider this possible small difference a decisive generic feature.

3. The apices of the paraphyses

The apices are said to become curved, at least in age, in *Otidea* and to be straight in *Tarzetta*. Firstly, this difference does not give the impression of being an important generic character. Secondly, in *O. kauffmanii* Kanouse and *O. rainierensis* Kanouse (and also in *O. phlebophora* (Berk. & Broome) Sacc., which is currently ascribed to *Otidea*; see, however, HARMAJA 1974a) the curvature is at most slight. A third and most important fact is that *T. spurcata* and *T. pusilla* possess a

considerable proportion of distinctly curved paraphyses, exactly like those of *O. indivisa* (see HARMAJA 1974b). The present character is thus worthless at the generic level.

B. Important characters common to both genera

1. The anatomy of the excipulum

My studies have demonstrated that the excipulum has exactly the same construction in *Otidea* and *Tarzetta*, which fact has not been reported before. It consists of three distinct layers. The bulk of the context of the apothecium, i.e. the ental part, is composed of typical *textura intricata* with hyphae of a moderate diameter, ca. 2—10 (—15) μm . Strongly cyanophilic »septal collars», or belts (see HARMAJA 1973: 55), occur here and there, whose breadth varies somewhat, commonly reaching about 2.5 μm (infrequently even 5.0 μm). These may be more numerous in *Tarzetta* than in *Otidea*. The middle layer, which is fairly narrow, ca. 35—100 μm , is composed of typical *textura angularis*, more or less sharply delimited on both sides and with rather large cells, ca. 10—50 \times 8—40 μm . The cell walls do not seem to be thicker than elsewhere in the excipulum, but amorphous, strongly (to moderately?) cyanophilic matter is deposited between the walls of adjacent cells, so that in a routine microscopic examination of a cotton blue preparation the walls themselves appear thick (even up to 4.0 μm) and cyanophilic. (According to my studies this layer is also present in e.g. *Sowerbyella* Nannf., but absent in *Flavoscypha* Harmaja.) The *textura angularis* layer thus has the most striking appearance in a section that has absorbed the dye well (when it is not strongly stained, look at the margins of the section or boil the mount once more). Sometimes the outermost cells of this layer give rise to few septate hyphae mostly without cyanophilic walls or deposits (see also above). The third and outermost layer of the excipulum is slightly thinner than the preceding one and is always discontinuous, consisting of more or less conical warts or fascicles of hyphal cells arising from the outermost cells of the preceding layer. It is composed of *textura globulosa* or *textura prismatica* (in *Tarzetta* evidently al-

ways typical *t. globulosa*). This layer is further distinguished from *textura angularis* layer by its smaller cells, these being roughly 8—25 μm in diameter. The walls are also heavily encrusted, which is best seen in KOH mounts, since in the species studied by me the encrusting pigment became colourless when the tissue was boiled in cotton blue. The last and very conspicuous feature distinguishing this layer is that no cyanophilic matter is deposited on the cell walls. The outermost cells of this layer may infrequently give rise to some simple septate hyphae (see also p. 139). In both genera interspecific differences are found in: the thickness of the two outermost excipular layers, the sharpness of the limit between the *t. intricata* and *t. angularis* layers, the exact shape of the exterior, principally conical, warts, the exact size and shape of the cells of the two outermost layers, and the intensity with which the cyanophilic matter absorbs cotton blue. ECKBLAD's (1968) Fig. 38 representing the anatomy of the excipulum of *T. catinus* gives a good general view of the three layers present in both *Tarzetia* and *Otidea* (his Fig. 40, which is supposed to show *O. onotica*, is not representative). The cells of the *t. angularis* layer should, however, be somewhat larger. In his figure the intercellular spaces in that layer are clearly darkened, as if stained with cotton blue. Most previous studies, however, have not distinguished sufficiently between the two layers of the ectal excipulum in these genera, and, as far as I know the very important cyanophily of the intercellular matter in the *t. angularis* layer has not been observed before now.

2. The perispore-periplasm coating in immature spores

At the stage at which they first become discernible, the spores of *Otidea* and *Tarzetia* possess a hyaline and cyanophobic true spore wall, and moderately cyanophilic spore contents. Subsequently a very thin (ca. 0.2—0.3 μm thick when broadest), more or less strongly cyanophilic layer begins to develop evenly all over the wall in both genera. (For the sake of brevity, the word perispore will be used in this paper to mean a structure on the principal spore wall that stains with cotton blue, regardless of whether it may actually be composed of two cyanophilic

parts, an outer film, or perispore, and periplasm interjacent between the perispore and the true spore wall.) Once I observed one young aberrant spore of *O. indivisa* which had a cyanophilic »apiculus» at each end, like those present in immature *Gyromitra* spores. As a rule the perispore in the immature spores is seen as a dark blue margin outlining the spore contour; it is observable at least at a magnification of 1000 x with an oil immersion lens, and can usually even be distinguished at a magnification of 600 x. It is generally smooth, but especially at high magnifications it not infrequently appears very slightly uneven, sometimes even being seen to be slightly detached from the spore wall proper, forming an occasional pustule, but never being regularly warted. The presence of this distinct perispore essentially accounts for the fact that in both genera the somewhat immature spores are as a rule the darkest blue ones in cotton blue mounts, the spores at earlier and later stages being paler.

C. The differentiating characters

1. The perispore-periplasm coating in mature spores

The genera were found to have a most important difference in their mature spores: the perispore described in the preceding section vanishes (or at least ceases to absorb cotton blue) in *Otidea*, whereas in *Tarzetia* the perispore persists in the mature spores. According to HARMAJA (1974c) the spores of the former genus belong to a spore type named after it, i.e., the *Otidea* type, and those of *Tarzetia* belong to the *Peziza* type. As the developing *Otidea* spores have not been observed to shed the cyanophilic matter during maturation, there seem to be two possible explanations of its disappearance: either the perispore dissolves, or it persists but undergoes such physical and/or chemical alterations that it is no longer able to absorb the dye. It is thinner in the mature *Tarzetia* spores than in the immature ones, and very tightly appressed to the true spore wall throughout, so that it is not easy to observe unless the section has been stained well and the spores are carefully examined and compared with those of younger stages. How-

ever, even the mature spores of *Tarzetta* turn blue when heated in cotton blue (more exactly, it appears as if their contents were weakly cyanophilic), whereas the mature spores of *Otidea* are completely colourless, or hyaline, even after repeated boiling. The rôle of the contents in the dye absorption of the spore in *Tarzetta* is not known. The oil drops, however, are always cyanophobic.

2. De Bary gas bubbles in mature spores

For some reason, the fact that the mature spores of the larger members of the *Pezizales* also possess these conspicuous bubbles is scarcely mentioned in the literature (but see HARMAJA 1973 and 1974c), and yet e.g. in *Otidea* they are present in the three usual mountants: KOH, Melzer's reagent and heated cotton blue (their presence in water needs further study) and seem to have definite taxonomic value here as well. In the present study all the *Otidea* species examined displayed single bubbles in the mature or almost mature (still in the asci) spores, whereas the spores of the *Tarzetta* species were devoid of them at every developmental stage. In *Otidea*, depending on the species, they may be present in seemingly all the mature spores, or a majority, or a minority of them. Their position inside the spore may also be dependent on the species, as in some species the majority of them are situated near one or other end of the spore and the rest around the middle, while in a few species the majority lie around the middle, and the remainder at the ends.

3. The carminophily of the nuclei

BERTHET (1964) and BERTHET & KORF (1969) noted that the nuclei of the ascocarp cells are carminophilic in *Tarzetta*, while in *Otidea* they are not. My preliminary studies with a small number of species (*O. indivisa*, *O. onotica*, *T. catinus*, and *T. spurcata*) gave similar results. A section of the dried fruit body was observed first after being put in acetocarmine stain and then after subsequent boiling and pressing. In mature spores, the nuclei may be called carminophilic in *Tarzetta* (discernible after some minutes in the stain even before boiling), and carminophobic in *Otidea* (still not visualized many hours after

repeated boiling!). In young spores, however, especially in *Otidea*, the nuclei were diffuse or dispersed and the chromatin absorbed the stain to a certain extent, although not sufficiently to allow a reliable count of the number of nuclei per spore. However, I found a method for improving the staining of the nuclei of *Otidea* (and certain other genera, such as *Gyromitra*, *Flavoscypha* and *Pseudorhizina*). A section of dry ascocarp was soaked in 5% KOH for some time (e.g. 15 min.), after which sufficient acetocarmine was added to the margins of the cover glass and allowed to replace the potassium hydroxide. Even before boiling the nuclei were fairly readily discernible and boiling deepened the stain. The single nuclei of the mature and almost mature *Otidea* spores (less satisfactorily visualized in the former) that happened to be at the compact phase had exactly the same median position between the two oil drops and near the spore wall as those of the *Tarzetta* spores, being either spherical or elongated.

4. The number of nuclei in the paraphysis cells

The paraphysis nuclei were also studied on dried material of *O. indivisa*, *O. onotica*, *T. catinus*, and *T. spurcata*, both with the simple acetocarmine treatment and with the preceding soaking in KOH described above. The latter method gave better results. The *Otidea* species were found to possess two nuclei in each cell of the paraphyses (in the terminal cell perhaps sometimes even more, e.g. three ones), while in the two *Tarzetta* species the paraphysis cells each contained only one single nucleus. It remains to be ascertained whether this very interesting difference holds true for the rest of the species of these genera (cf. BERTHET 1964).

5. Some less important characters

The three features mentioned at the beginning of this paper can appropriately be listed under this heading. In addition, it is well known that in *Tarzetta* the spores are on the average larger, and the colours of the apothecia paler and less variable than in *Otidea*. The teeth on the margin of young and also older apothecia are more developed in *Tarzetta*. The perispore in immature spo-

res may be slightly thicker in *Tarzetta*, being ca. 0.3 μm when broadest, as opposed to ca. 0.2 μm in *Otidea*, but this observation should be checked by a careful examination of larger material. The two oil drops in mature *Tarzetta* spores very easily merge during boiling in cotton blue, while those of *Otidea* mostly remain separate. I have also observed that the wall in the apical parts of the *Tarzetta* asci is two-layered, the inner layer being cyanophobic and light-refractive while the outer, almost equally thick layer, is very weakly cyanophilic and mat. In the somewhat smaller asci of *Otidea* the walls seem to be slightly thinner and it is difficult to tell

whether they also have a double structure. The occurrence of cyanophilic matter, between the walls of the *textura angularis* cells in the ectal excipulum and in the septal collars in the ental excipulum, seems to be commoner in *Tarzetta*, the matter being perhaps also slightly more strongly cyanophilic. Lastly, the *Tarzetta* species generally fruit earlier than those of *Otidea*.

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