

Sparassoid ascocarps in Pezizales and Tuberales*

Richard P. Korf**

Abstract

Sparassoid ascocarps are known in four groups of the Pezizales. In the Pezizaceae sparassoid development may occur in the genus *Peziza*, in which *P. proteana* f. *campbellii* is now recognized on the basis of recent collections as distinct from *P. proteana* f. *sparassoides*; three other sparassoid species of the genus are critically reviewed. In the Helvellaceae a somewhat similar developmental process results in the gyromitroid pileus in *Gyromitra* and in *Discina* (*Neogyromitra*), and true sparassoid development is reported here in a new species, *Helvella astieri*. In the Pyronemataceae, tribe Otideae, such development occurs in *Ascosparassis*, the monotype species of which is now to be placed in *Otidea*. In one instance sparassoid development occurs in a hypogean rather than an epigeal environment in this order, in *Geopora* (*Sepultaria*), a genus of the Pyronemataceae, tribe Mycolachneae. Sparassoid fructifications are widespread in the Tuberales, and a comparison is made of members of the Tuberales and Pezizales with such fruitbodies. Hypotheses are advanced to account for sparassoid development in epigeal and hypogean fungi.

While the great majority of Operculate Discomycetes which have been referred to the Pezizales have fructifications which are simple cups, from which they derive their common name, cup-fungi, various other shapes of ascocarps are known in this order. The purpose of this paper is to examine one such developmental form, the sparassoid fruitbody. The term sparassoid is derived from the generic name *Sparassis*, applied to a genus of the hymenomycetous Basidiomycetes which forms a more or less spherical ball of interlaced, torn, flabelliform branches. Sparassoid development is now known in four distinct groups in the Pezizales, and we shall examine each of these in some detail. Finally, we shall turn our attention to the evolutionary significance of sparassoid development in both the Pezizales and in the Tuberales.

I. Pezizaceae

The development of a sparassoid form of an otherwise typically operculate species, *Peziza proteana* (BOUDIER) SEEVER, was noted by BOUDIER (1899), who gave a varietal epithet to the sparassoid form. Until now this remains the only species of *Peziza* in which both the cupulate and sparassoid forms have been and apparently often are collected together at the same place, usually a burn site. This has facilitated direct comparison

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** Plant Pathology Herbarium, Cornell University, Ithaca, N.Y. 14850, U.S.A.

of the two forms, the one a shallow cup a few centimeters in diameter, the other a cabbage-head-like mass of chambered locules sometimes 30 centimeters in diameter (and reported to me by Dr. D.P. ROGERS to be very much larger at times). Other than the bizarre development, no microscopic differences are evident between *P. proteana* forma *proteana*, the cupulate element, and *P. proteana* forma *sparassoides* (BOUDIER) KORF, the sparassoid element. As I much earlier pointed out (KORF 1956), this did not prevent authors who collected the sparassoid form from redescribing it. It was given specific rank (in the genus *Gyromitra*) by MASSEE (1895), and transferred to the genus *Underwoodia* by BÁNHEGYI (1937). The same fungus was made the type of a new genus, *Durandiomyces*, by SEAVER (1928). The genera *Gyromitra*, *Underwoodia*, and *Durandiomyces* were all referred to the Helvellaceae at the time of the description of these sparassoid forms, far removed from the Pezizaceae where Boudier had correctly referred his fungus. The same fungus was, moreover, described as a member of the Tuberales. SETCHELL (1924) called it a new species of a new genus (*Daleomyces*) of the truffle order; that generic name was gratuitously renamed *Napomyces* by CLEMENTS and SHEAR (1931).

How is it possible for major workers in the Discomycetes and Tuberales to differ so widely in their assignment of this fungus to families as disparate as the Tuberales, Pezizaceae, and Helvellaceae? The answer lies in the bases upon which these groups were originally recognized. The Pezizaceae were conceived of, in early classifications, as simple cups, only rarely stipitate. The Helvellaceae, on the other hand, were thought of as usually stipitate and with a highly developed pileus which might assume the form of a saddle in a typical *Helvella*, a contorted, gyrose pileus in *Gyromitra*, an internally chambered clavula in *Underwoodia*, or an externally chambered, hollow fruitbody in *Morchella*. Even though practically none of the microscopic characters of the sparassoid ascocarp resemble those of any member of the Helvellaceae (inclusive of Morchellaceae), authors such as MASSEE (1895), SACCARDO (1909), SEAVER (1928, 1942) and BÁNHEGYI (1937) were convinced that such a highly developed fructification as seen in this sparassoid form could only be accommodated in the Helvellaceae. It was gross morphology of the ascocarp which led them to assign the fungus there. Those authors (SETCHELL 1924; CLEMENTS & SHEAR 1931; FISCHER 1938; GILKEY 1939) who have assigned this sparassoid form to the Tuberales were likewise influenced by the gross anatomy, a chambered mass of tissues lined by an apparently labyrinthine hymenium not unlike that exhibited by many hypogean Tuberales. What these authors failed to notice was that the asci in this fungus are still provided with a functional operculum, and that the ascospores are forcibly discharged into the air, a feature unknown in the Tuberales.

The evidence of the relationship of "the cabbage-head fungus" with its normally discoid and much smaller "typical" form has made its assignment to the genus *Peziza* (called by a few authors *Galactinia*) a thoroughly accepted position for all modern disco-systematists.

A second, far less sparassoid, species of *Peziza* was also described as a member of the Tuberales, *Hydnocystis convoluta* McALP. in McALPINE and RODWAY (1896). It was

characterized as an "irregularly convoluted, hollow sac, externally roughened, internally smooth, about 2×2 inches, and narrowing towards the ground." McLENNAN (1961) transferred this epigeal 'truffle' to *Hydnotrya* of the same family. On the basis of BURDSALL's (1968) examination of the type specimen, the fungus was shown to be a member of the Pezizaceae. It was necessary to rename it, as *Peziza jactata* BURDSALL & KORF in BURDSALL, since the epithet 'convoluta' is preoccupied in *Peziza* by another fungus.

To McALPINE's original description of the spores as "spherical or oval, slightly verrucose, about 9 μm in diameter or 10~11×8.5 μm," McLENNAN merely added that "spore measurements of the co-type material from RODWAY's collection are approximately 12×8 μm, and for the Victorian samples 12×8.4 μm," and that the original description "adequately fits" the Australian specimens. BURDSALL (1968) reported that the asci blue strongly in iodine, but did not comment either on spore size or kind of ornamentation present. My examination of the type specimen (RODWAY 21, CUP 48853) shows ascospores 11.3~14×8.1~11.3 μm, and that when stained in lactic acid cotton blue they are uniguttulate and very clearly reticulate (Fig. 17). I consider this kind of convoluted, enclosed ascocarp as representing the initial stage in development of a sparassoid fructification, perhaps best termed subsparassoid. The species is included in this discussion primarily because the ascocarp is sufficiently sparassoid or convoluted to have been confused by at least four authors with that of the Tuberales, and scarcely recalls that of a Discomycete.

A few years later, a third sparassoid species of *Peziza* was described by HENNINGS (1900) from Java, which he called *Aleuria? sparassiformis* HENN. Through the kindness of Dr. Mien A. RIFAI of the Herbarium Bogoriense, I have been able to examine pickled material of the only known remaining collection of HENNINGS' species. This was collected by RACIBORSKI, actually a year earlier than the collection by FLEISCHER which had served as HENNINGS' type material, and is the collection reported by PENZIG and SACCARDO (1902). Since all of HENNINGS' original material is apparently lost, I hereby designate the RACIBORSKI (PENZIG, tubo 3) material, BO 10110, as the NEOTYPE of *Peziza sparassiformis* (HENN. in WARB.) SACC. & SYD. in SACC. The specimen may also be the same as that referred to in the Herbarium Bogoriense under a different accession number, BO 5334, though that number is no longer associated with the pickled material nor any dried specimen which can be located. Some slides and photographs serving as an ISONEOTYPE are on deposit in CUP 52756. The RACIBORSKI collection agrees well in nearly every respect with the description by HENNINGS, except that the paraphyses are not pointed as illustrated by him, but swollen apically as one would expect in the genus *Peziza*. The fruitbodies are much smaller than in *P. proteana* f. *sparassoides*, 3.5 cm high and 2 cm broad according to HENNINGS and much the same in the RACIBORSKI collection (Fig. 7: a, b). The ascospores are narrow for a *Peziza*, biguttulate, and completely smooth (Figs. 6 & 15). I was unable to demonstrate a blueing reaction of the ascus apices in iodine, but considering that the fungus has been preserved in a fluid for over 70 years, this is no surprise. The tissues are not in a good state of preservation, but appear to be typical of a *Peziza*.

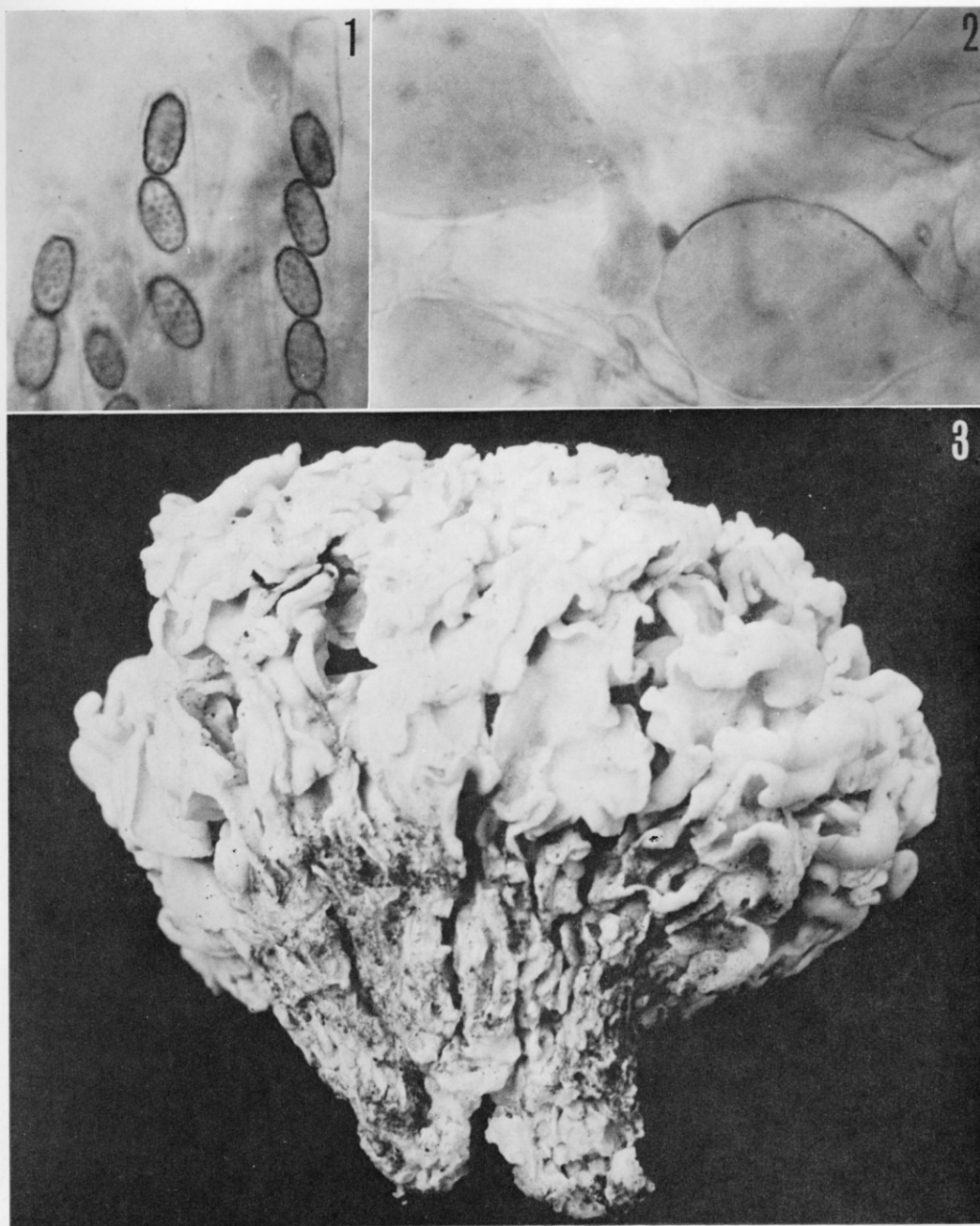
The fourth known sparassoid species of the Pezizaceae was described by GILKEY (1939)

in the Tuberales, as *Daleomyces shearii* GILKEY. In my earlier report (Korf 1956), I transferred this species to *Peziza* as *P. shearii* (GILKEY) Korf. It differs widely from the three previously discussed species in its very nearly globose ascospores (Figs. 5: a, b & 16). The type material was embedded in paraffin wax, but on dissolving the wax from a portion of the type specimen graciously sent to me by Dr. Gilkey, I was able to demonstrate that the asci turn blue in iodine, are operculate, and that some of the ascospores turn brownish-yellow at maturity. I suspect that *P. shearii* is closely allied to the *P. trachycarpa* Currey complex of species still needing intensive study. Some authors recognize a separate genus, *Plicaria*, for species around *Peziza trachycarpa*, but I find such a genus quite unacceptable (Korf 1961, 1972). The fact that sparassoid development is known in both 'genera' reinforces my belief that these cannot be separated effectively. The excipular tissues of *P. shearii* contain the same large, globose to pyriform cells which characterize the oval-spored species of *Peziza*.

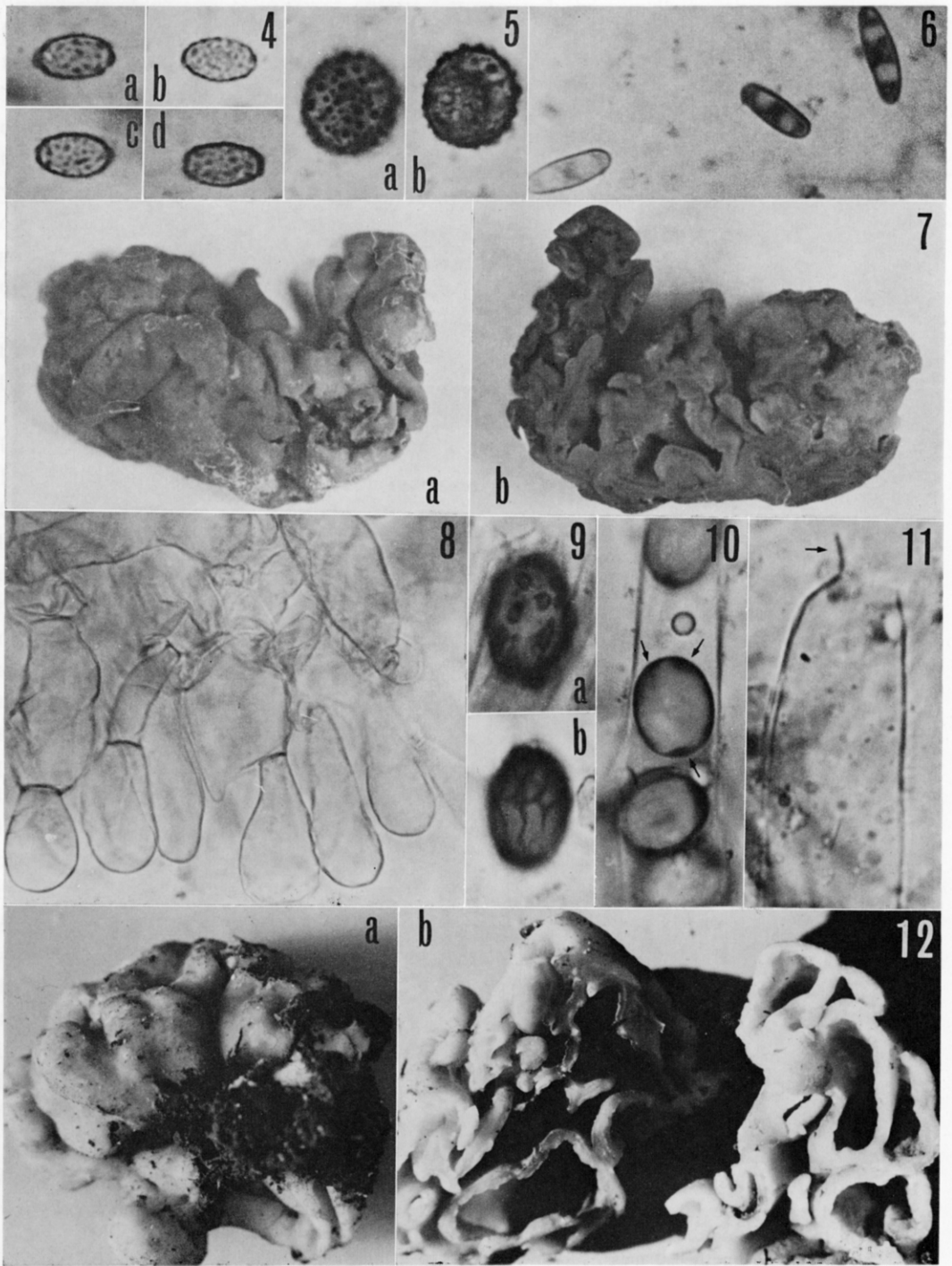
Recently I was sent material of a sparassoid *Peziza* by Dr. R.W. Lichtwardt of the University of Kansas, to whom it had been sent by the collector, Mrs. John Banninger. I assumed at first that the collection represented *P. proteana* f. *sparassoides*, but it proved to have larger, more regularly spaced, and more rounded spore markings (Figs. 1 & 13: b, c) than do the type and other collections of *P. proteana* f. *sparassoides* (Figs. 4 & 14). A critical reexamination of all of the material at my disposal revealed to me that the type specimen of *Underwoodia campbellii* Saccardo (Campbell, s.n., Saccardo Herb., PAD) has spores matching Mrs. Banninger's collection (Fig. 13a). She and her grandson then went back and collected fresh material of the fungus. She dried some according to my suggestions, and preserved one specimen in alcohol (Fig. 3). This collection agreed in possessing the same, larger warts, and is much better preserved. The ectal excipulum has the large, pyriform-globose cells (Fig. 2) which are expected in the genus *Peziza*. Though I earlier had considered *U. campbellii* to be a synonym of *P. proteana* f. *sparassoides*, the consistently different spore markings have convinced me that these are different taxa. The spore dimensions are so nearly the same, and the differences in spore markings so subtle, that I choose not to recognize Saccardo's taxon at specific rank, but shall treat it as a forma of *Peziza proteana*. Both of the new collections were made on sandy soil around and under a felled, partly burned tree, Lovers' Lane (Cemetery Road), 1/2 mile east of Solomon, Kansas. Mrs. Banninger's first collection, July 1972, is deposited as CUP 52743. The second collection, Mrs. John Banninger and Michael Eugene Banninger, 17 August 1972, is on deposit as CUP 52625. I propose to call this taxon ***Peziza proteana*** (Boud.) Seaver forma ***campbellii*** (Saccardo) Korf, comb. nov. [basonym: *Underwoodia campbellii* Sacc., (as '*campbellii*'), Ann. Mycol. 7: 433. 1909].

The following key will serve to separate the five sparassoid taxa known thus far in *Peziza*:

1. Ascospores cylindrical (l/w=2.5~2.9), 11.0~12.5×3.7~4.4 μm, biguttulate, smooth
..... *P. sparassiformis*
- 1'. Ascospores broader, with cyanophilic markings 2



Figs. 1~3. *Peziza proteana* f. *combellii* (CUP 52625). Fig. 1. Asci and ascospores showing evenly spaced cyanophilic tuberculations, mounted in cotton-blue lactic-acid. $\times 1,050$. Fig. 2. Pyri-form to subglobose cells of the ectal excipulum, mounted in cotton-blue lactic-acid. $\times 700$. Fig. 3. Sparassoid apothecial mass, specimen preserved in alcohol. $\times 2$.



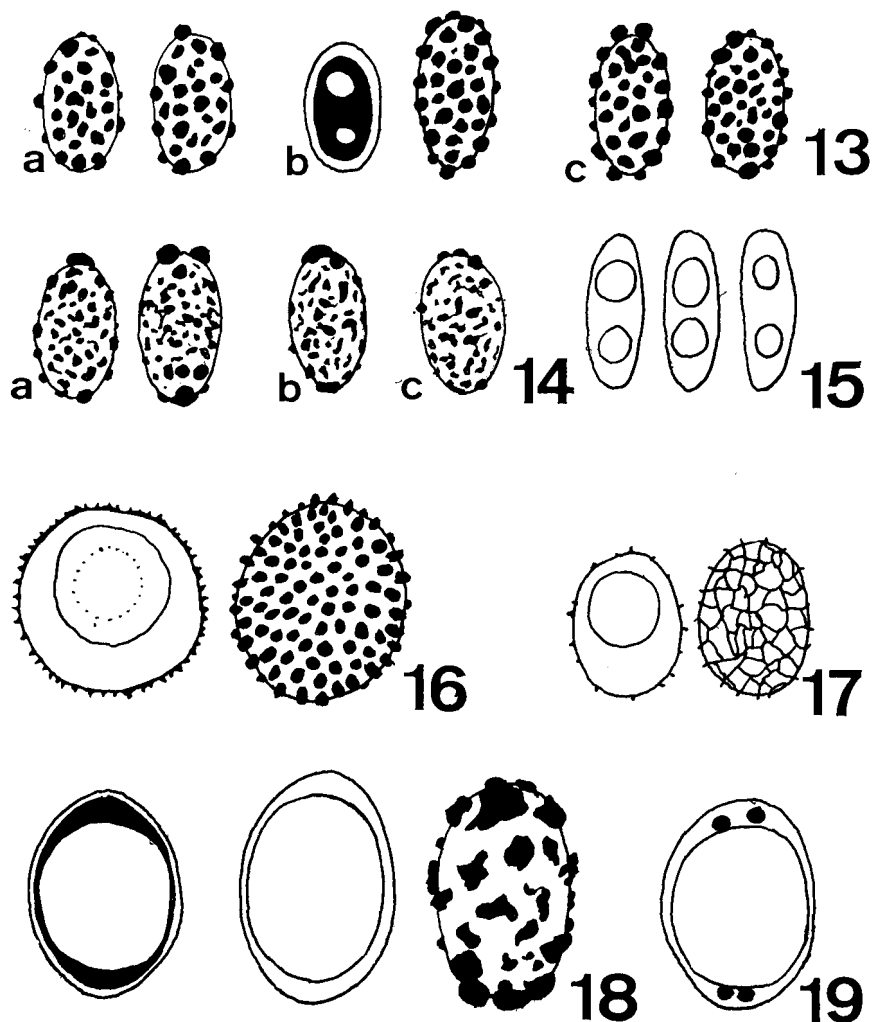
2. Ascospores elongate ($1/w=1.6\sim 2.0$), $9.5\sim 11.0\times 4.7\sim 6.2\ \mu\text{m}$, biguttulate 3
 2'. Ascospores subglobose to broadly ellipsoid, uniguttulate 4
 3. Ascospore markings of isolated, large, rounded warts which only rarely fuse.....
*P. proteana* f. *campbellii*
 3'. Ascospore markings of more densely placed, lower warts and ridges which frequently
 anastomose, with larger crests or warts at the ends, appearing sometimes apiculate
*P. proteana* f. *sparassoides*
 4. Ascospores subglobose ($1/w=1.1\sim 1.2$), $13.5\sim 16.2\times 11.0\sim 14.0\ \mu\text{m}$, markings tubercules
*P. shearii*
 4'. Ascospores broadly ellipsoid ($1/w=1.3\sim 1.4$), $11.3\sim 14.0\times 8.1\sim 11.3\ \mu\text{m}$, reticulate
*P. jactata*

II. Helvellaceae

Let us now turn our attention to the pileus in the gyrose-capped species often placed in *Gyromitra* and in *Discina* (some authors keep this name for cupulate species, and would use *Neogyromitra* and even additional generic names for those with a gyrose pileus). In the very early stage of development the pileus is a cup. This grows marginally, folds down against the stipe, with which it may fuse, and then begins to proliferate, becoming folded and eventually cerebriform. The folded hymenial condition is termed saccate, and when these folds are examined they do not differ fundamentally from the back-to-back excipular structure seen when sectioning a typical sparassoid Discomycete. Even in some species of *Helvella* with a saddle-shaped pileus, portions of the pileus may fuse with each other, back-to-back, or back-to-stipe. In all these cases there remains enough visible, external morphology, a stipe and a pileus, that I prefer not to call such development sparassoid, and reserve the terms saccate and gyromitroid for them.

Recently, at the Congress of the Mycological Society of France held in Corsica, Mr. Joseph ASTIER showed some kodachrome transparencies of a peculiar sparassoid fungus he and his friend, Mr. Jean-Claude DONADINI, had collected near Marseille (Fig. 12: a, b). The fungus has broadly ellipsoid ascospores with a very large central guttule, recalling to the

Figs. 4~12. *Peziza* spp. and *Helvella astieri*. Fig. 4. *Peziza proteana* f. *sparassoides*, holotype (PC, BOUDIER s.n.), four ascospores (a~d) showing cyanophilic warts and crests. Fig. 5. *Peziza shearii*, isotype (CUP 52758), an ascospore in face view (a) showing cyanophilic tuberculations, and (b) the same spore at a lower focal plane showing the height of the warts. Figs. 6 & 7. *Peziza sparassiformis*, neotype (BO 10110). Fig. 6. Completely smooth ascospores showing two oil guttules. Fig. 7. Portion of an ascocarp preserved in liquid (a) in face view, and (b) viewed from the cut surface. $\times 2$. Figs. 8~12. *Helvella astieri*, holotype (PC, ASTIER & DONADINI s.n.). Fig. 8. Excipular palisade of sterile cells, mounted in Belling's iron acetocarmine stain. $\times 700$. Fig. 9. Mature ascospores with cyanophilic markings, in (a) with irregular, large warts, in (b) with a partial reticulum. Fig. 10. Nearly mature ascospores after prolonged treatment in Belling's iron acetocarmine stain, one spore with three of its four nuclei visible (arrows), the fourth nucleus not in focus. $\times 1,050$. Fig. 11. Mature ascus which has discharged its ascospores, the thrown back operculum visible (arrow). Fig. 12. Sparassoid ascocarp viewed (a) from the outside and (b) viewed from the two cut surfaces. $\times 2$. (Figs. 4, 5, 6, 9 & 11 in cotton-blue lactic acid. $\times 1,050$).



Figs. 13~19. Ascospores drawn with the aid of a Wild drawing tube, all at $\times 1,700$. Figs. 13~18. Mounts in cotton-blue lactic acid. Fig. 13. *Peziza proteana* f. *campbellii*, (a) from holotype (PAD, CAMPBELL, s.n.), (b) from CUP 52625, immature and mature, (c) from CUP 52743. Fig. 14. *Peziza proteana* f. *sparassoides*, (a) from holotype (PC, BOUDIER, s.n.), (b) from holotype of *Gyromitra phillipsii* (BM=K, BECK s.n.), (c) from holotype of *Daleomyces gardneri* (UC, PARKS 1412). Fig. 15. *Peziza sparassiformis*, from neotype (BO 10110). Fig. 16. *Peziza shearii*, young spore in optical section showing central guttule with two refringent zones, mature spore in face view, from holotype (OSC, SHEAR s.n., GILKEY 170). Fig. 17. *Peziza jactata*, one ascospore in optical section showing central guttule, one spore in face view, from holotype (CUP 48853, RODWAY 21). Figs. 18 & 19. *Helvella astieri* from holotype (PC, ASTIER & DONADINI s.n.). Fig. 18. Young spore with cyanophilic cytoplasm surrounding the large central guttule, mature and smooth spore with cytoplasm not cyanophilic, a third spore, also mature, but with large, irregular warts as seen in face view. Fig. 19. Ascospore in optical section after prolonged mounting in BELLING's acetocarmine stain, showing location of the four nuclei in relation to the central guttule.

collectors those of the genus *Helvella*. Mr. ASTIER sent me dried material of the fungus, and I was able to confirm their suspicion that it was correctly to be assigned to the Helvellaceae. While most of the ascospores remain smooth, some asci contain all or only some of the ascospores with large, irregular warts (Figs. 9: a, b & 18), exactly the condition that obtains in the tribe Helvelleae. Moreover, I was able to stain the nuclei in certain spores after long exposure to BELLING's iron-acetocarmine stain, and to demonstrate that the ascospores are tetranucleate (Fig. 10), a feature characteristic only of the Helvellaceae among members of the Pezizales. The excipular tissues (Fig. 8) are also exactly those of a *Helvella*, the outermost layer made up of a palisade of clavate cells.

At the Congress, some mycologists suggested that the species might belong in *Geoporella*, a genus of the Tuberales. My investigation of Mr. ASTIER's fungus showed, however, that the asci have a functional operculum (Fig. 11), and that this is surely not a *Geoporella*. I then reexamined those materials reported by BURDSALL (1968) which are on deposit in CUP of the monotype species of the genus, *G. michaelis* (FISCHER) SOEHNER. While these show some similarities in both ascospore markings and tissue structure to Mr. ASTIER's fungus, I could neither demonstrate an operculum in that material nor stain nuclei in any of the ascospores.

Great variability in ascocarp shape is known in *Helvella*, when the genus is taken in the modern, wide sense of NANNFELDT, recently the subject of important papers by DISSING (1966) and others. Erection of a new genus to accommodate this sparassoid species seems, however, foolish. Mr. DONADINI joins me in proposing this fungus as a new species of *Helvella*. Which of the described species of that genus is closest to the new species is most difficult to determine. The basis for the sections recognized by DISSING are primarily macroscopic features. Whether the stipe is solid, hollow, or chambered, whether it is sulcate or smooth, whether and how far sulcate ribs extend up onto the outer surface of the pileus, and whether the pileus remains cupulate or assumes a saddle-shape are some of the key characters of the sections, none of which can be determined for such a sparassoid species. The formal diagnosis of the new species is as follows:

***Helvella astieri* KORF & DONADINI, sp. nov.**

Ascocarpi clausi, tuberiformes (Fig. 12a), intus loculo-sparassiformes (Fig. 12b), sessiles, sporaosiformes ca. 3 cm diam., bubalini, *Raphanum* odorem redolentes. Excipulum externum e cellulis clavatis (Fig. 8). Asci ca. $240 \times 15.5 \sim 19 \mu\text{m}$, ex unciis ennati, cylindrici, 8-sporei, operculati, in iodo non caerulescentes (Fig. 11). Ascosporae hyalinae, uniguttulatae, tetranucleatae, laeves vel tuberculatae, raro reticulatae, ($13.7 \sim$) $15.5 \sim 16.5 \times 10.2 \sim 11.3 \mu\text{m}$ (Figs. 9, 10, 18 & 19). Paraphyses filiformes, ascos aequans, inferne $2.8 \mu\text{m}$ diam., ad apicem $4.2 \sim 5.6 \mu\text{m}$ diam.

TYPE SPECIMEN: Joseph ASTIER & Jean-Claude DONADINI, s.n., 17. IX. 1972, on soil under *Quercus pubescens*, près de l'Hostellerie, Sainte Baume, Var, France. HOLOTYPE: PC. ISOTYPE: CUP 52755.

III. Pyronemataceae, tribe Otideae

KOBAYASI (1960) erected the genus *Ascosparassis* for a single new species, *A. shimizuensis* KOBAYASI, collected in Japan. He assigned his sparassoid genus to the Sclerotiniaceae, but KORF (1963) showed that it has operculate asci, and reported five additional collections from Java; he placed the genus close to *Otidea* FUECKEL. NANNFELDT (1966), ECKBLAD (1968), and KORF (1972, 1973) all accepted the genus as closely related to *Otidea*, based upon its great similarities in ascospores, paraphyses, and sterile tissues.

The sparassoid fruitbodies of *A. shimizuensis* may be only slightly lobed and branched, as drawn by VAN OVEREEM and reproduced in KORF's (1963) paper, or may form a much more solid, sparassoid mass as illustrated by KOBAYASI (1960). Inasmuch as I can find only this one character—that of fruitbody shape—to differentiate KOBAYASI's species from the genus *Otidea*, I feel that to maintain a separate genus for it is both unreasonable and scientifically unsound. I propose, therefore, its formal transfer, as **Otidea shimizuensis** (KOBAYASI) KORF, comb. nov. (basonym: *Ascosparassis shimizuensis* KOBAYASI, Bull. Natl. Sci. Mus. 5: 45. 1960).

IV. Pyronemataceae, tribe Mycolachneae

In this fourth group of the Pezizales, sparassoid development occurs in the genus *Geopora* HARKN. The type species of the genus, *G. cooperi* HARKN., originally described as belonging to the Tuberales, was shown by BURDSALL (1965) to have operculate asci, and was later transferred to the Pezizales (BURDSALL 1968). Here sparassoid development differs somewhat from that we have seen previously: it occurs in a hypogean environment, and the fructification retains a nearly spherical shape despite the infolding of the ectal excipulum and the production of a highly contorted and lacunose interior. BURDSALL recognized a second wholly hypogean species, *G. clausa* (TUL. & TUL.) BURDSALL, which is much smaller and shows few or no hymenial convolutions. Similarities in spores, excipular hairs, and tissues of the ascocarps led BURDSALL to place in synonymy with *Geopora* the younger generic name *Sepultaria* (COOKE) BOUD., long used in the Pezizales for species with partially buried apothecia. These, too, begin their development as nearly closed spheres, but rupture on maturity, splitting into *Geastrum*-like rays, to expose the hymenium and to allow for forcible ascospore discharge. A saccate or subsparassoid hymenial condition is known in at least one of the species formerly placed in *Sepultaria*, *G. longii* (SEEVER) BURDSALL & KORF in BURDSALL, as illustrated by SEEVER (1915, 1928, 1942).

Most of the epigeous, or at least not completely hypogeous, species assigned to *Sepultaria* have not yet been transferred to *Geopora*. Neither Burdsall nor I have been prepared to make these transfers without proceeding first to a monographic investigation of these species. We contented ourselves (BURDSALL 1968) with transferring only the very handsome *G. sepulta* (FR.) KORF & BURDSALL in BURDSALL. For other species, concepts vary so widely among authors, and specific variation is so poorly understood in this genus, that transfer of other specific epithets now would be premature.

V. Some conclusions and hypotheses on sparassoidism and the origin of the Tuberales

The origin of the Tuberales from Operculate Pezizales has been argued by many authors, and it is not my intent to restate their arguments here except insofar as it is necessary in a consideration of sparassoid development in both orders. The purported origin of the Tuberales from Basidiomycetes seems to me too remote a possibility to consider. But that the Tuberales *sensu lato* may be polyphyletic I am quite prepared to accept. The unlikelihood of the Elaphomycetaceae having a close affinity with the other families of the Tuberales is clear (KORF 1973), and they may be much closer to the Eurotiales in their ancestry.

What actually distinguishes the two orders, Tuberales and Pezizales? It is, in the final analysis, only the possession of a functional operculum in the latter, and its absence in the former. We may well question whether this is a taxonomic feature worthy of recognizing at the ordinal rank. That it is a fundamental biological feature cannot be denied. A fungus which depends upon air dispersal of its ascospores to reproduce effectively has, as in the Pezizales, strong evolutionary pressure to retain those mechanisms which allow such spore dispersal. The cylindrical ascus shape, the palisade arrangement of such asci, delimitation of an apical operculum which must split open along a predetermined line, resulting in an ascostome of the correct size to allow passage of the squirted ascospores, capable of contracting the right amount as each spore passes through to give an additional push to the spore projectile, maintenance of turgor in the epiplasm — all these are complex phenomena doubtless under the control of a multitude of genes. Mutation of any one of these genes would probably result in failure of the whole discharge mechanism to operate. That such deleterious gene mutations must regularly occur is clear; likewise, such non-discharging mutant strains will never get their spores into the air, and the mutant will be self-eliminating. Selection pressure maintains the mechanism and eliminates all such mutant forms for so long as that is the only way a spore can be disseminated.

In the Tuberales, however, where asci do not discharge their spores, air dispersal of ascospores is not the means of fungal reproduction. The evidence is strong that the major role in dispersal is by animals, insects, perhaps annelids, and above all, for the larger species, by rodents and other burrowing animals. Moreover, the ascospores of many Tuberales are thick-walled and capable of withstanding the action of digestive juices. (I have examined the stomach contents of voles in New York State which consisted almost wholly of the spores and tissues of Tuberales and of Endogonaceae). We find, further, that the great majority of Tuberales (and of hypogeous Basidiomycetes for that matter) emit strong odors, allowing for the detection of subterranean fruitbodies by such animals through the fragrances they emit.

When we examine the ascospores themselves, or sterile tissues of members of the Tuberales, we find many parallels with those in Pezizales at the microscopic level. To the naked eye, the "simplest" Tuberales are hollow spheres lined with a hymenium of asci

and paraphyses, closely resembling a still closed apothecium of, e.g., an epigeal species of *Geopora* (*Sepultaria*). More complex fruitbodies are found, often in the same species or genera, where an infolding of the hymenium occurs in the tuberculate fruitbody, eventually resulting in a sparassoid hymenial tissue filling the sphere. We should also examine what is happening to two microscopic structures, the paraphyses and the asci. The former are, in the simplest forms, about as long as the asci, just as in Pezizales. Sometimes they may be much shorter than the asci (e.g., *Labyrinthomyces steenisii* BOEDIJN), or, far more frequently, they may be longer than the asci, often branching apically and forming an epithelial tissue which may fill in the hollow cavities between adjacent hymenial folds of the cerebriform interior of the ascocarp. Such an epithecium does not occur in the Pezizales, where it would only impede ascus discharge. The asci, on the other hand, also show marked variation from that of the Pezizales. Not only do we no longer find much, if any, sign of a vestigial mechanism at the apex which would delimit an operculum, but the asci in the more advanced forms lose the cylindrical shape which is characteristic of the pezizalean ascus. Such asci assume a pyriform or even globose shape, becoming soon displaced so that their original arrangement in a distinct hymenium in youth may no longer be evident at maturity. The genus *Tuber* represents such an extreme form. Here, as in other advanced Tuberales, even spore number frequently has become reduced and irregular; spore size also becomes variable among spores within the same ascus. In the Pezizales, on the other hand, spore number is usually under rigid genetic control, since spore size — which is directly correlated with spore number — must be maintained within certain limits if the operculum on the ascus is to function effectively.

The upper surface of a smooth hymenium in the Pezizales is essentially a series of opercula ready to open and interspersed among paraphysis apices (whose function is yet another story). This appears to be the most efficient arrangement for a member of this order. Any gross irregularity or folding of the hymenium, and above all a sparassoid development, can only be biologically inefficient, since then a porportion of the asci will be unable to discharge their spores into the air, but instead will do so against another hymenial surface where the spores will stick. This explains, I believe, why sparassoid development is so rare among Pezizales. But in a member of the Tuberales, where spore discharge is no longer a controlling selection pressure, contortion and folding of the hymenium is not a disadvantage. It may, and I believe usually is, on the contrary a biological advantage, and will be selected for in the processes of natural selection. If we examine the simplest Tuberales, the hollow spheres with few or no hymenial contortions, we find that mostly these are very small fructifications. Large ascocarps, on the contrary, are almost all sparassoid internally. For a hypogean fruitbody to enlarge, it must develop sufficient forces to push the soil particles apart during the growth phase. A hollow sphere is clearly less efficient at such a process than is a compact ball of tissues. Further, given a ball of certain size, two features will argue for a selection towards a sparassoid rather than a hollow interior: the solid will have more tissues capable of emitting an attractant odor, and the solid will be biologically more efficient in its sterile to fertile tissue ratio,

producing more reproductive propagules (spores) per unit weight.

From these considerations, it follows that I believe the Tuberales to represent a biological unit rather than a phylogenetic one. Several distinct lines of the Pezizales have presumably given rise to members of the Tuberales, independently, by loss of functional opercula. At what point does a member of the Pezizales cease to be that, and become a truffle? That point is, I believe, the one at which it loses its functioning operculum and successfully depends upon its new way of spore dispersal. Such a derived truffle is then free to evolve into additional species and genera of the Tuberales.

Geopora cooperi thus represents a member of the Pezizales, even though it grows below the surface of the soil. It is collected by mycologists astute enough to follow rodent diggings. Partially eaten ascocarps on the surface of the soil allow him to dig up others nearby, undiscovered by the squirrels or other rodents that play a role in the distribution of this fungus. Here, however, there is no evidence that the spores can or do pass through the digestive tract. On the contrary, the maintenance of the delicate mechanism of forcible spore discharge can only tell us that air dispersal of the spores is still the biologically significant method of reproduction in the species. The animal bites or breaks open the ascocarp, and a cloud of ascospores results as the asci (nearly) simultaneously eject their spores. Here is a prime example of a "truffle-in-the-making," but it has not reached that point where loss of the discharge mechanism would be insignificant in future evolution. When it does adopt a new method for getting its spores disseminated, it will lose that discharge mechanism rapidly by random mutations of the many controlling genes, and then *Geopora* will indeed have given rise to a member of the Tuberales. It is interesting to note that this species has also, independently, followed the tenets set down above for development of a hypogeous fungus, i.e., it is sparassoid interiorly as a result of selection pressures for developing a large sphere in a hypogean environment.

All of the other sparassoid Discomycetes I have discussed in this paper differ from the hypogean species of *Geopora* in being above ground. What evolutionary advantage, indeed, can one ascribe to such epigeal development by a fungus which must discharge its ascospores into the air? I, for one, can see no such advantage, and would point again to the very obvious disadvantage, already mentioned above, that a large number of the asci will fail to function. If we examine again the case of *Peziza proteana*, we may find some clues to solve this paradox. For here is a typically cupulate species which, apparently regularly and under some unknown stimulus, produces in addition its monstrous, cabbage-head form(s). We have no data to support any logical hypothesis, but two explanations, neither far-fetched, immediately spring to mind. One is that a (fairly frequent) mutational event occurs which results in the formation of a sparassoid mass instead of a normal cup. The mutant may be somewhat self-eliminating, since most of the ascospores bearing such a mutant gene obviously never get into the air, though many do. The frequency with which the sparassoid form is found in association with the normal cups suggests that a single-gene mutation would be a perfectly likely explanation. Another, equally plausible hypothesis is that the sparassoid form develops because of an infection, perhaps viral. We

know now that true viruses can infect species of *Peziza*. Electron microscopy of the cytoplasm of the cupulate and sparassoid forms would help settle the question of whether a virus is responsible for the change in morphology which we observe.

That sparassoid members of the Pezizales have been confused with Tuberales clearly rests upon the similarity of their sparassoid forms, when we consider epigean Pezizales. In this instance sparassoidism is a mere convergence of features with no apparent phylogenetic significance.

This discussion has rejected, at the outset, the concept that the Tuberales could be closely related to Basidiomycetes. But what of the possibility that Tuberales are derived from or gave rise to other orders of the Discomycetes than the Pezizales? On the basis of the kinds of ascospores produced, their markings, the microanatomy of sterile tissues, and their chemistry as far as we know it, I can only indicate that there is very little similarity between members of the Tuberales and any order of the Inoperculate Discomycetes, or to the Cyttariales, whether that order be considered as Inoperculate or Operculate. The Pezizales offer many points of similarity, on the contrary.

Thus far, also, this discussion has admitted only the concept that the Tuberales are derived from the Pezizales, and not the reverse. If, as we believe now, there are several distinct points of evolutionary contact between the two orders, to argue that the Tuberales gave rise several times to the Pezizales means that each time such origin occurred the same mechanism — development of a spore capable of air dispersal and of the complex operculum system — was developed independently. This stretches credulity too far. Loss of a character, but only after its functional value in evolution has been supplanted by another pathway — here animal dispersal — is easily explained and wholly consistent with genetic evidence in other evolutionary systems.

It is, however, by no means certain that all of the species now ascribed to the Tuberales belong there. Some may be, as *Geopora* was before BURDSALL's (1965) discovery, Pezizales that live below ground. I look with suspicion at any member of the Tuberales possessing paraphyses which do not greatly exceed in length cylindrical, 8-spored asci. How many of them are Pezizales in hiding?

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