A POPULATION STUDY OF TULOSTOMA BRUMALE

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The subject of this paper, *Tulostoma brumale* Persoon, is the type species of the genus, which belongs to the class Gasteromycetes. The reason for the study is the existing confusion concerning the definition of species within this genus. The authors collected thirty-five plants (Tiffney and Tiffney, 1965) in 1963. When we attempted to identify our collection we found it possible to relate individual specimens to several species within the genus. After examining specimens in the herbarium of the New York Botanical Garden and in the Farlow Herbarium we concluded that considerable synonomy exists within the genus. This condition has existed for some time. G. H. Cunningham in 1925 stated that ". . . confusion exists in the delimitation of species, and by certain workers the genus is considered to be the most difficult of any included in the Gasteromycetes".

The problem became even more obvious in 1964 and again in 1965 when more specimens were collected from the same location as the 1963 material. We now had 88 specimens taken over three years, all from the same spot, but many differing in appearance sufficiently to raise a question as to their identity. Some were small in a dry year, others larger in a wet year, and all possessed sufficient variations to warrant the belief that certain characteristics were of little value in determining species in the genus. Had we used keys published by several authors or followed identifications suggested on the specimens we examined in various herbaria as our guide we could have reported the collection of several species of the genus *Tulostoma*, all for the first time in the New England area and all from one small location. Such an occurrence appeared to be most unlikely.

We postulated that our collections had to be considered as all belonging to one species but representative of the variation mentioned by White (1901), Lloyd (1906), Cunningham (1925 and 1942) and Long (1947). At the time we first found these plants in 1963 they were limited to a small area of sand dune about 25 feet wide and 70 feet long. They spread to successively wider areas in 1964 and 1965, and by 1968 were found over an area of some five acres. It is probable that the introduction of the original spores occurred only slightly prior to our initial discovery in 1963. This introduction appears to have been an isolated occurrence, perhaps accomplished by a "well-traveled" horse.[1] Long and Ahmad (1947) note that *Tulostoma* spreads rapidly from spores in India. Coker and Couch (1928) note the perennial nature of plants of this genus and report one species as persistent on the same site for 19 years. Perennial and spreading rhizomorphs may also give rise to new plants on the same site. The extensive diversity observed in size and other characteristics in our material probably is due to environmental influence and natural capacity for variation within the population and not to genetic exchange with other *Tulostoma* species. The nearest reported station to ours is Ithaca, New York, by White (1901). Both authors and their students have collected extensively along much of the New England coastline, and although we have been looking for *Tulostoma* ever since the first collection, we have yet to discover a second station; therefore we feel that the Cape Ann material is isolated from other *Tulostoma* populations.

The 88 specimens forming the basis for this study were collected on a public bathing and recreation area at Good Harbor Beach in Gloucester, Essex County, Massachusetts. The plants (Fig. 1) grow in sand dunes near the storm beach, in a band some 150 to 300 feet back from the normal high tide line. The area is only slightly protected from the

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[1]Lloyd (1906) notes that *Tulostoma campestre* is common where "Horses had evidently been in the habit of resting there in the shade". Good Harbor Beach, where our collections were made, is an area used for exercising horses by Cape Ann residents. The spores may have been introduced to the area in the feces of one of these herbivores.

open ocean by the low, 8 to 10 foot fore-dune. The pH in this area ranges from 7.0 to 7.8, undoubtedly resulting from salt spray from ocean storms, as New England soils with but few exceptions are acid. The tolerance of certain members of the genus to basic soils and to salts is well documented by Long and Ahmad (1947) working in India, when they note the presence of "many *Tylostoma* collections" coming from "kallars" or pans whose surfaces may become covered with white incrustations of salt in dry weather. This tendency was mentioned earlier by Long (1944).

The beach faces southeast to east, the direction of many of the severe storm winds of this area. The soil is composed of wind blown sands, derived from the beach area, bound loosely in place by *Ammophila breviligulata* Fernald, a coarse perennial beach-grass. What organic material there is in these sands appears to be derived from the decay of the rhizomes and leaf blades of this grass. Indeed the rhizomorphs of the collected *Tulostoma* frequently penetrated the decayed grass materials. The entire beach is about 10 to 15 acres in extent and rests on a typical estuarine salt marsh of 30 to 40 acres, bounded by a semicircle of low granitic hills, with the exception of the edge facing the sea. To the best of our knowledge the population of *Tulostoma brumale* reported here has not yet migrated out of this relatively limited area.

Priority in the Gasteromycetes begins with the work of C. H. Persoon (Synopsis methodica fungorum, 1801). He assigned the name *Tulostoma brumale* to a form previously recognized by Linnaeus (Species plantarum, 1753) as *Lycoperdon pedunculatum*, an organism apparently of general north European distribution.[2] Later C. Sprengel (Systema vegetabilium, ed. 16, 4 (1) 1827) revised the spelling

[2]Although Lloyd (1906) feels it cannot be found in England, this form is apparently distributed in the Low Countries around the North Sea as well as in Italy, Austria, Switzerland and Hungary. Cunningham (1942) reports it from Australia and New Zealand, and Long and Ahmad (1947) from India, thus it appears to have a cosmopolitan distribution.

Fig. 1. *Tulostoma brumale* Persoon growing in sand dunes 150 to 300 feet back from the storm beach, in the spray zone. The sand is stabilized to some extent by *Ammophila breviligulata* Fernald, which appears to be the source of organic material for the fungus.

of the genus to *Tylostoma* thinking apparently the original to be an orthographic error. As stated in an earlier paper (Tiffney and Tiffney, 1965) we are retaining Persoon's spelling.

We now believe the description of the species must be enlarged to include the considerable variation in size and form possible under varying climatic conditions. It is hoped that this study will aid in reducing the confusion concerning the number of species within the genus. While we do not believe that a study involving even the simple type of statistical functions performed here is required of an investigator each time he collects a species of this genus, we do feel statistical analysis necessary in the current work to describe permissible bounds of variation for the species *Tulostoma brumale* and to establish its position within the genus.

The high degree of variation that exists within species of *Tulostoma* suggests the futility of erecting species based on the examination of a few plants collected over a single growing season. Cunningham (1925) stated this problem, "The difficulty is to decide which are fixed characters in such a variable genus; about half of the species which have been described to date cannot be separated on any recognizable character". Lloyd (1906) notes that species should not be described from single collections, and Cunningham (1942) further states, "About 85 species have been described, of which not more than 30 are valid".

Long (1944) proposed a list of characters he thought necessary to differentiate the species within this genus. As the list appears quite inclusive, we have used it as our standard. We have used two approaches in analyzing this list. The first consists of characters that can be measured to the nearest millimeter or micron, and these have been treated statistically, while the second group of criteria is subjective in nature and requires a percent-presence approach to render them amenable to numerical analysis. The characters measured and subjected to statistical analysis were height of the entire plant, stipe height, sporocarp height and width, and spore diameter. Gross characters were measured to the nearest millimeter, spore diameter to the nearest micron. Spores from each plant were placed on a microscope slide and heated in 5% potassium hydroxide to re-expand them to their original and typical size. This procedure is recommended by Cunningham (1925) and was found to give excellent results when dried material was to be compared with fresh. The average spore diameter is based on a sample of 100 spores from each plant.

The plants were arranged in three groups corresponding to the years of collection: 1963, 1964 and 1965. As these collections constitute a geographically and therefore genetically isolated population of *Tulostoma*, variation may be ascribed to individual genetic diversity inherent within the organism plus variation caused by climatic changes acting within individual growing seasons. Long and Ahmad (1947) have suggested that availability of moisture to the *Tulostoma* plant at the time of stipe elongation and sporocarp expansion has a profound effect on the proportions of the mature plants. Our Cape Ann material apparently expands and appears above ground in late October. The United States Weather Bureau records for nearby Rockport, Massachusetts, show October rainfall for our 1963 collection to be 6.95 inches, for the 1964 collection 5.31 inches and for the 1965 collection only 1.97 inches. The 1965 group had very low moisture available to it during the critical expansion period, while both the 1963 and the 1964 groups had a normal and adequate rainfall.

Table 1 gives comparative values for the range, mean, and standard deviation for each of the three groups and each of the measurable characters within these groups. The table shows that in the character of height of the entire plant the 1965 group is the smallest, while the 1963 group has the greatest range and the 1964 material averages largest. Adequate and similar quantities of rainfall resulted in the larger size of the 1963 and 1964 collections while low precipitation in 1965 is reflected in the small stature of plants for that year. Stipe height figures follow the same pattern; the 1965 material averages smallest, the 1963 plants have the greatest range, and the 1964 plants average largest. However, the character of sporocarp height in the three groups shows less variation as a result of differing rainfall quantities than do the above characters. The 1964 group is unaccountably larger than either the 1963 or the 1965 collections. The same statement holds for sporocarp width. In contrast to the extensive differences noted for gross measurements, spore diameter appears to be a rather consistent figure for the three groups. The greatest range occurs in the 1963 group, 3.88μ to 5.72μ. The mean is slightly higher in 1964 and the overall size variation less in 1965. In general spore size is the most consistent measurable character found in our material.

Table 1

	Group 1, 1963	*Group 2, 1964*	*Group 3, 1965*
		Height Entire Plant	
Mean	49.00 mm	54.24 mm	39.25 mm
Range	21-99	25-72	25-83
Std. Dev.	16.53	10.70	12.92
		Stipe Height	
Mean	43.94 mm	46.21 mm	33.80 mm
Range	17-93	21-65	20-74
Std. Dev.	15.30	10.70	11.83
		Sporocarp Height	
Mean	5.09 mm	8.03 mm	5.45 mm
Range	1-11	4-12	3-12
Std. Dev.	2.37	1.99	1.94
		Sporocarp Width	
Mean	8.34 mm	11.30 mm	7.50 mm
Range	4-14	5-17	5-13
Std. Dev.	3.07	2.59	2.13
		Average Spore Diameter	
Mean	4.81 μ	5.00 μ	4.34 μ
Range	3.88-5.72	4.15-5.66	3.96-5.15
Std. Dev.	0.4459	0.3470	0.3012

Table 1. Comparison of the 35 plants collected in 1963 having 6.95 inches of rainfall during their period of elongation and development with the 33 plants collected in 1964 having 5.31 inches of rainfall and with the 20 plants collected in 1965 having only 1.97 inches of rainfall.

There is a relationship between rainfall during the growing season and size of plants. More rainfall during this critical period gives larger plants, less yields smaller plants. We now wished to find if the ratios of the sizes of measurable parts of these larger and smaller plants were consistent. A coefficient of correlation test was performed on each measurable part of all plants in the three groups. For example, a positive coefficient of correlation result of .60 indicates that the two items measured increase together 60% of the time, while 40% of the time one variable increases and the other either stays the same or decreases. Our results showed that in general a *Tulostoma brumale* plant increases proportionately in size. This relationship holds true between 22% and 99% of the time and averages 63% for our material, based on 30 tests comparing all measurable parts of the plants with all other measurable parts. The least correlation, 41%, was achieved when comparing sporocarp height with stipe height. This is reasonable as the sporocarp collapses with the liberation of the spores. Spore diameter correlated rather poorly with stipe height, 45%, indicating a fairly tenuous relationship between height of the plant and size of spores. Spore diameter correlated fairly well with the height and width of the sporocarp, 62%, indicating that in general larger sporocarps produce larger spores. Macroscopic plant parts (height of the entire plant, stipe height, sporocarp height and width) correlated well, at 68%. The plant, at least internally, is fairly consistent with its parts increasing proportionately 63% of the time.

Coefficient of correlation results indicate that an individual plant is at least reasonably consistent in the relationships of its measurable parts. However the plants of the three yearly collections, taken as groups, appear to differ widely when viewed in the light of range, mean and standard deviation data presented in Table 1. We have suggested that this group variation may be due to differing amounts of rainfall during the various growing seasons.

We wished to test the significance of these yearly group variations statistically. We employed the combination of the "F" test with the Duncan Multiple Range test. The "F" test determines within certain probability ranges if the means of sample groups (in our case the three collections) are sufficiently similar to assume that the sample groups were originally drawn from a single larger aggregate, or if the sample groups are sufficiently dissimilar to assume that they are separate entities. If the first case is true, then the yearly collections are part of a single though variable population, and species in *Tulostoma* may be defined by reasonably narrow ranges of measurable characteristics. If the second case is true, then either the three yearly collections represent three separate species or environmental factors cause much greater variation in measurable portions of the plant than has been previously thought. In this case only the broadest approach to species determination by measurable characters should be used. As before the "F" test and Duncan Multiple Range test were aplied to all measurable parts of the plants, comparisons being made among height of the entire plant, stipe height, sporocarp height and width, and average spore diameter.

The "F" test results confirmed that the second case was true and that the three groups were significantly different. Significance was confirmed among the groups for all measurable characteristics including spores at the 0.005% confidence level.[3]

The "F" test results state that significant differences exist somewhere among the three groups but do not state specifically whether these differences exist between the 1963 and 1964 groups, or between the 1963 and 1965 groups, or between the 1964 and 1965 groups, or all three. With "F" test results available the Duncan Multiple Range Test does just this. The Duncan test shows that significant dif-

[3]The 0.005% confidence level indicates that the conclusion suggested by "F" test results will be correct in 99.5% of all cases. (Pearce, 1965)

ferences at the 0.05% level[4] exist for all characters between all three groups with one exception in the 15 tests performed. The exception is that no significance was seen for spore diameter between the 1963 and 1964 collections.

The Duncan test clearly locates a fact indicated by the "F" test, that with the exception of spore diameter (one out of fifteen tests performed), the 1963, 1964 and 1965 collections are quite different from each other. Had we not possessed complete collection data for these collections, if for example we had been working with scattered herbarium specimens, we could statistically justify erecting three (or at least two) further species in this already over-crowded genus.

The conclusion that we have new species is unacceptable in the light of our complete data. As we stated earlier, the limited area to which these plants are confined, their slow spread from an initial site during the years 1963 to 1968 and our inability to locate other colonies in New England all logically require that our three collections represent a single species. This apparent conflict between statistical and biological evidence does not indicate that either is wrong. Rather than assessing inherent characteristics of plants, the statistical procedures are indicating that variation in some environmental factor, perhaps rainfall, is expressed as variation in each yearly group of *Tulostoma* plants.

The form, size and distribution of *Tulostoma* plants may be potentially affected by other physical and biotic factors. Variation of pH in turn influenced by salt spray blown in high winds may determine the areas of the beach available for colonization as well as the dimensions of the mature plants. Perhaps higher rainfall by its leaching effect would reduce the salt content of the sand. The 1965 plants were subjected not only to a lower rainfall but also to a slightly higher soil-salt content during their growing season. Long

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[4]The 0.05% level indicates that conclusions suggested by the Duncan test will be correct in 95% of all cases. (Duncan, 1955)

and Ahmad (1947) suggest that matted grasses of several species restrict or distort emerging *Tulostoma* plants in India and may preclude growth of plants in areas of thick grass cover. Our material was taken from a well-stabilized area of sand dunes with a characteristic open cover of *Ammophila breviligulata* Fernald. *Tulostoma* does not occur on Good Harbor Beach in areas of dense grass cover. Many plants were collected with their rhizomorphs entangled with or penetrating decaying *Ammophila* rhizomes. This was presumed to be the normal supply of organic material for the fungi. Long and Ahmad (1947) further note that excessively hard soils lead to restricted or atypical development, while excessively soft substrates allow the plants to be dislodged by rain and wind. Our material grew in an intermediate position somewhat back from the fore-dune between the actively moving beach and the well established area. Most of our plants are well developed or at least undistorted. Some few distorted plants were collected from marginal habitats in areas of loose sand or extensive grass cover. In general there is little doubt that environmental factors with rainfall prime among them are responsible for extensive variations in populations of *Tulostoma*.

It is now possible to comment on the reliability of measurable characteristics in regard to separating species in the genus *Tulostoma*. Spore diameter is the most consistent character among those examined. Dramatic differences in spore diameter could, if found, prove useful in determining species; however variation even in this measurement in our material is sufficient to warrant adopting wider boundaries. Gross measurements are highly variable; of these, height characters including height of the entire plant and stipe height exclusive of sporocarp are the most consistent, but dimensions of the sporocarp itself are so variable as to be nearly useless. All these dimensions have been treated in the literature as having diagnostic value (Long, 1944). Gross measurements in general should be used with only the widest boundaries in species determination.

When we compare our measurable characteristics with

Table 2

Published Measurements for *Tulostoma brumale*

	Stipe Height	Sporocarp Height	Sporocarp Width	Spore Diameter
White, 1901	10-50 mm	10-15 mm	10-20 mm	3-5 μ
Lloyd, 1906	—	—	—	5
Coker & Couch, 1928	10-20	—	6-10	3.8-4.5 × 4.0-5.5
Cunningham, 1942	20-40	—	12 max.	4-6
Smith, 1951	10-50	10-15	10-12	3-5
Maximum Dimensions	10-50	10-15	6-20	3-6
Tiffney & Tiffney	17-93	1-12	4-17	3.88-5.72

Table 2. Measurements given by various authors for the described characters of *Tulostoma brumale* plants compared with our results.

those set by other authors (see Table 2) for *Tulostoma brumale* we find that our figures show a wider range for the species but the means appear to be in general agreement with those in the literature. However when we turn to a consideration of those qualities which compose the bulk of most species descriptions in taxonomic publications, the subjective delineation of the form of the plant, color of various parts and vestiture or surface appearance of such structures as the peridium, spores or stipe, we discover that our descriptions and those of the literature are in disagreement. Part of the problem is created by the conventions used in writing such descriptions. While phrases such as "globose to subglobose" are vague but with experience understandable, statements such as "occasionally wanting" and "present or absent" serve no informative function at all. Comparative data of a qualitative nature are necessary for an adequate description.

We have attempted such a qualitative approach below. Characters are listed by their percentage of presence in the specimens examined, using Long's (1944) suggested list of diagnostic characters for the genus. This approach permits a type of quantitative analysis and requires an actual count of all specimens, where previous descriptions have utilized only a "feeling" for the characters, often from a relatively small number of specimens. Moreover our method requires the investigator to note variations occurring over several growing seasons.

Group 1, 1963.

STIPE: mycelial strands mostly lacking (85%); texture variable, lightly ribbed in about half the specimens (43%) and the rest lightly to heavily scaled (57%); rarely with sand-flocci (6%); color mostly light brown (77%), occasionally gray-white (14%), rarely medium brown (9%).

SPOROCARP: shape mostly subglobose (89%); exoperidium texture with sand-flocci aggregates (100%); endoperidium color mostly gray-white (77%), occasionally light yellow or brown (17%), rarely pure white (3%), or dark brown (3%).

MOUTH: mostly tubular (74%), rarely lacerate (8%); occasionally with a small raised mat (20%).

BASE: highly variable, frequently bulbous (69%), a few volvulate (13%) and an equal number with indistinct bases; mostly with both fibrils and rhizomorphs (89%).

Group 2, 1964.

STIPE: mycelial strands mostly present (94%); lightly ribbed in some specimens (18%), mostly lightly scaled (76%), never heavily scaled; about half with sand-flocci (63%); color mostly light brown (79%), occasionally gray-white (18%), rarely medium brown.

SPOROCARP: shape mostly subglobose (81%); exoperidium texture with sand-flocci aggregates (88%); endoperidium color mostly gray-white (85%), occasionally light yellow or brown (15%).

MOUTH: frequently lacerate (45%), occasionally tubular (15%); mostly with a large or small raised mat (82%).

BASE: highly variable, mostly bulbous (69%), a very few volvulate (7%), or with indistinct bases (24%); mostly with both fibrils and rhizomorphs (89%).

Group 3, 1965.

STIPE: mycelial strands mostly lacking (90%); mostly lightly ribbed (80%), occasionally lightly scaled (15%), never heavily scaled; mostly with sand-flocci (80%); color almost equally divided between light brown (55%) and gray-white (40%), with a few dark brown (5%).

SPOROCARP: shape mostly subglobose (70%); exoperidium texture with sand-flocci aggregates (60%); endoperidium color mostly gray-white (85%), occasionally pure white (20%), rarely light yellow or brown (10%).

MOUTH: mostly tubular (85%), occasionally lacerate (10%); rarely with a large or small raised mat (10%).

BASE: highly variable, about half bulbous (55%), almost half volvulate 45%); three-fourths with fibrils (75%), one-fourth with rhizomorphs (25%).

Stipe characteristics within the three groups were so highly variable that they could not be used to distinguish the groups from one another. Only in color was there consistency, and stipe color appears to be a factor of soil staining and subsequent bleaching by sun or leaching by rain. Since all plants grow in a restricted area, the color of their stipes should be similar. Sporocarp shape, presence of sand-flocci on the exoperidium and color of the endoperidium were quite generally similar. Mouth shape and vestiture, considered so important for species diagnosis by Coker and Couch (1928), Cunningham (1942), Long (1944), Long and Ahmad (1947) and others, proved to be a quite variable and confusing characteristic for our plants. Indeed, it was at this point that we were forced to turn to herbaria to determine our species. It should be noted that specimens in the herbaria showed more variation in mouth structure than the keys and descriptions in the literature would lead one to expect. Spore size and vestiture may be of some value but we feel that more study of herbarium material is indicated before a final verdict is rendered.

In the hope of making the systematics of this genus less confusing, we offer the following revised species description for *Tulostoma brumale* Persoon 1801 (Synopsis methodica

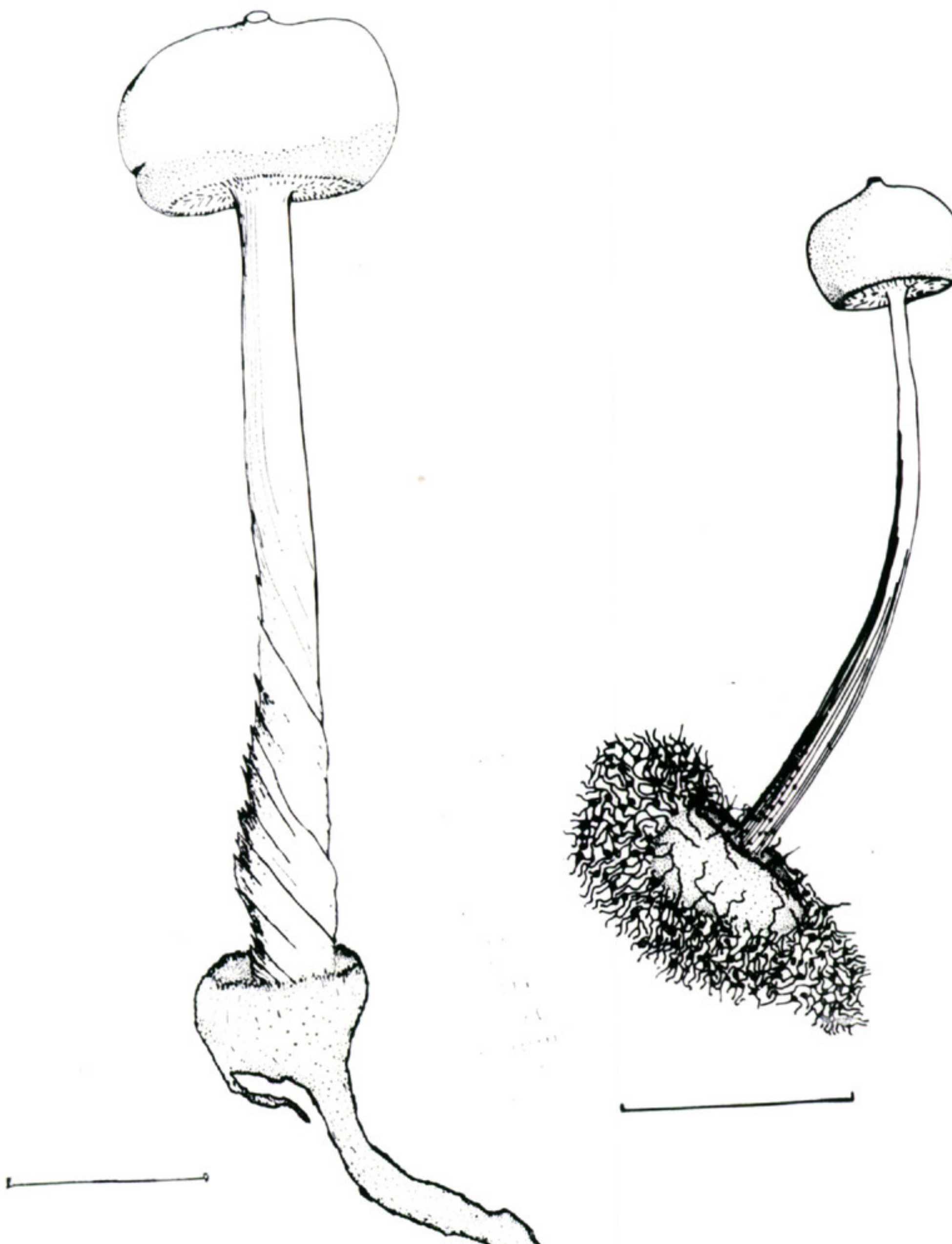

Fig. 2. *Tulostoma brumale*, two plants with bulbous bases, tubular mouths and subglobose sporocarps. The larger plant has an almost smooth stipe lightly scaled at base, the base with a rhizomorph; the smaller plant has a ribbed stipe with the bulb at the base covered with fibrils. The scale lines equal 1 cm. lengths.

fungorum p. 139). We hope it will prove useful in the necessary future taxonomic revision of this group; Figs. 2 and 3 illustrate several features of the species.

The peridium of this organism originates below ground level (hypogeously) at a depth of about 5 to 10 cm. By elongation of a basal stalk it appears above ground (epigeous) in the New England area in mid-October. The early development of spores is in doubt but according to Schroeter (1876) the spores are borne laterally on the basidia. The peridium is composed of two membranes, an outer exoperidium and an inner endoperidium surrounding the spores; numerous unbranched thick-walled sinuous hyphae, the capillitium threads, grow throughout the spore cavity. According to our observations the peridium first appears as a small swelling on the hypogeous mycelium in late August or by mid-September. The exoperidium surface is composed of loosely intertwined slender hyphae which trap the surrounding substrate into the structure, hence the exoperidium is initially the same color as the substrate (5 to 10 cm. deep) in which it developed. It may bleach in sun or wash in rains to a lighter color, sometimes to almost white. The process of emergence of the fruiting body aids in grinding the exoperidium away from the apical portion of the peridium, exposing the endoperidium and leaving the exoperidium persistent only from the equator of the fructification to the insertion of the stalk. The retained portion of the exoperidium is quite fibrous and even in old specimens sand and earth particles of the substrate may be present, coloring this portion of the specimen. Also the exposed exoperidium takes on the soil color through which it has been pushed by the elongation of the stalk. The endoperidium is a tougher, more compact material and lighter in color than the exoperidium. It is a persistent, firm papery structure, smooth to slightly pubescent, and may bleach almost to white in old specimens. Dehiscence is effected by means of a mouth or pore at the apex of the endoperidium. The mouth is usually raised, tubular or umbonate, and may have a smooth or fibrillose area around the opening. In some

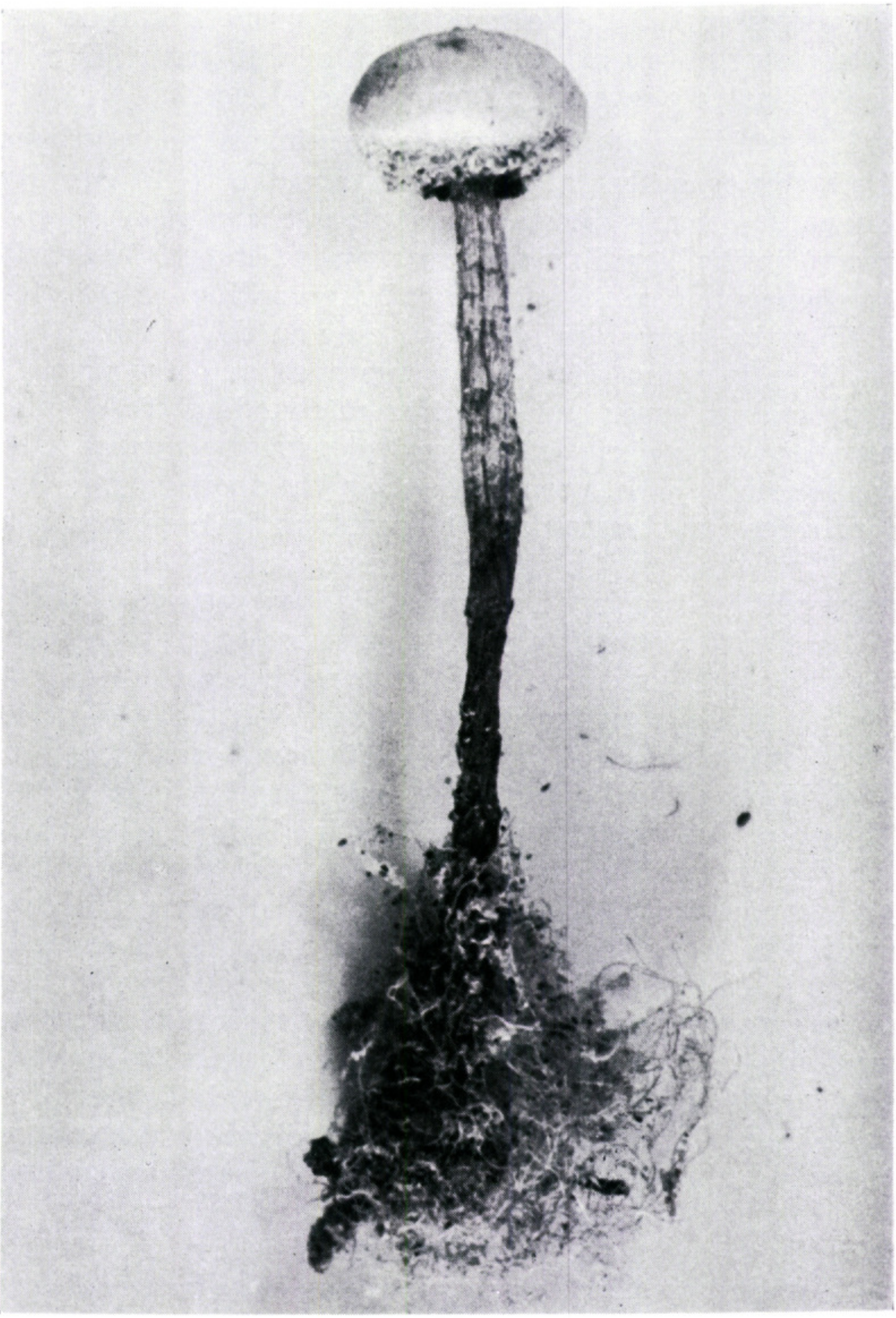

Fig. 3. *Tulostoma brumale* with a rather small bulbous base with both rhizomorphs and fibrils, with a ribbed stipe scaled toward the base. The sporocarp is subglobose with just a hint of a tubular mouth but with a tendency to be lacerate.

old weathered specimens and a few new forms an irregular tear may form the mouth opening, thus the appearance of the mouth is variable. The stem is inserted in a socket at the base of the peridium. It may vary greatly in thickness and length. It is smooth in older specimens, or scaly or striate, and is usually hollow with wefts of mycelia in the central chamber. The stem terminates in a bulb, often with an upturned cup-like structure, or it may produce a mass of coarse mycelia which gradually taper off into the substrate. The spores are hyaline to brownish in color, mostly verrucose to warty in surface vestiture. A sufficient number of spiny to almost smooth spores have been observed to make the authors suggest that there is some variation in this characteristic. The capillitium associated with the spores is abundant in some specimens and less in others. These hyphae are smooth-walled, hyaline structures, swollen at the cross walls. The central lumen of the capillitium cells becomes swollen at the septa and narrows in the mid-region. The dimensions of the plant are: height of the entire plant 2 to 10 cm. (20 to 100 mm.), stipe height 1.5 to 10 cm. (15 to 100 mm.), sporocarp height 0.1 to 1.2 cm. (1 to 12 mm.), sporocarp width 0.4 to 1.7 cm. (4 to 17 mm.), spore diameter 3 to 8μ.

Much of the confusion surrounding *Tulostoma* has arisen because investigators described new species from single plants or from only a few specimens. We suggest that some kind of population study, perhaps similar to the one we have performed here, precede the erection of further species in this genus. We feel that we have demonstrated that the type specimen, although useful as a datum point for the name of a given organism, cannot serve as a typical representative of the population or species from which it was drawn. Much synonymy and confusion have arisen in this genus from regarding the type specimen as typical. Although fungi and flowering plants differ greatly, some of the population techniques proved on vascular material may profitably be applied to the higher fungi.

We are indebted to the curators of the Farlow Herbarium of Harvard University and the Herbarium of the New York Botanical Garden for the opportunity to examine specimens. A large part of the research by the junior author was carried out in association with the Systematics-Ecology Program, Marine Biological Laboratory, Woods Hole. Systematics-Ecology Program Contribution No. 222.

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