

STUDIES ON GRAMINICOLOUS SPECIES OF *PHYLLACHORA* FCKL.

IV. EVALUATION OF THE CRITERIA OF SPECIES

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Summary

Recognition of the large number of graminicolous *Phyllachora* spp. by various authors is attributed to the use of unreliable morphological characters as taxonomic criteria, as well as acceptance of the concept of each *Phyllachora* species being host-specific. It is considered that in fact there are far fewer *Phyllachora* spp. than reported in the literature and an attempt is made to clarify the position by making a study of *Phyllachoras* which occur on grasses.

By considering in turn each character previously used to delimit species and by studying the degree of variation of each character within individual specimens as well as groups of specimens on closely related hosts, and between groups of specimens on unrelated hosts, it is possible to evaluate the usefulness of each character in taxonomy.

It is shown that of all the characters previously used only two are sufficiently stable for taxonomic purposes. These are the length of the ascus pedicel relative to that of the ascospore portion of the ascus, and the morphology of ascospores. Ascospore morphology is particularly useful, but only when all the various shapes that can occur in individual specimens are noted and when ascospore shape, as distinct from outline, is used.

The use of certain other characters of *Phyllachoras* is proposed. These are: appressorium morphology (when appressoria are produced from ascospores germinated on host tissue) and the morphology of the sporophores produced by the *Leptostromella*-states of certain *Phyllachoras*. It is suggested that characters of imperfect states of *Phyllachora* will also provide useful taxonomic characters when these states are known to exist in more species than at present.

As well as the characters listed as reliable for delimiting species, other characters such as ascospore size and arrangement, ascus length, and sporophore size may have limited usefulness when used to confirm a diagnosis, but not to delimit species.

I. INTRODUCTION

Members of the genus *Phyllachora* occur as parasites of plants in many families of angiosperms. The species have previously been delimited with the concept of host specialization as an important criterion. This has led to great confusion in the taxonomy of these fungi as frequently identification is possible only when host species is considered. This, however, is not usually satisfactory because fungi occurring on closely related host species are often identified as different species although morpho-

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logically indistinguishable. The reasons for this are readily understood when the following example of species numbers is considered. Of 68 (possibly more) species described on hosts in the tribe Paniceae, 25 occur on *Panicum* spp. and 18 on *Paspalum* spp. A search through the literature has revealed that there are at least 240 *Phyllachora* spp. listed on grass hosts.

It is most probable that a similar state of affairs exists for species of *Phyllachora* found on hosts in families other than the Gramineae, and although a survey of all members of this genus would be valuable it would involve an extremely wide field of research. Consequently these studies have been limited to graminicolous species.

Fischer and Shaw (1953) considered related problems in work on the smut fungi. They point out that the problem of defining and delimiting species is an old one and that it has gradually been recognized that the valid criteria for delimiting species vary in different groups of organisms. They believe that the greatest taxonomic problems are found in the parasitic fungi because the general tendency in the past was to delimit species on the basis of host, with little regard for morphology. They emphasized, however, that when there are relatively few morphological characteristics available as taxonomic criteria, a classification based on morphology alone would be in danger of grouping unrelated, though morphologically similar species. After considering various alternatives, they proposed the following: "That species should be delimited primarily on a sound morphological basis, but utilising at the same time host specialisation at the host-family level".

It is considered that this concept, while not without its weaknesses, provides a useful approach to the problem. The species of *Phyllachora* on grasses might thus be considered as a group of related fungi, divisible into species when morphological features are used as criteria. The substratum, the host plant in this case, would not be taken into account.

The main emphasis in much of the earlier work, which is reviewed by Orton (1944), was put on fixing the taxonomic position of the genus *Phyllachora*, while little attention was given to criteria for delimiting species. Although Theissen, Sydow, and Sydow (1915) listed 85 graminicolous species of *Phyllachora* they did not indicate which criteria were used for separating them. From the similarities of many of their descriptions it is evident that separations were frequently made by noting differences in host rather than differences in morphology. Similarly Doidge (1942) gave a detailed account of *Phyllachora* spp. occurring in South Africa but omitted to indicate which criteria were used to delimit species. It is apparent, however, that the host species was often relied on and that gross morphological characters which have since been shown to be unreliable (Parbery 1963*b*) were often used.

In a taxonomic account of graminicolous *Phyllachora* spp. occurring in North America, Orton (1944) provided a key to the species he recognized. The criteria used in this key are all morphological characters. On trying to use this key, however, the impression is gained that although morphological terms were used to describe each species, it was delimited originally by host specialization. The key is difficult to use and is unreliable. Fungi in Orton's list are more readily recognized if the host species is taken into account, but this is still not wholly satisfactory. The difficulties (existing

in the use of Orton's key) arise from Orton's use of morphological characters which are too variable to be useful taxonomically. This is partly evident from the key itself since some species needed to be included as many as four times under various categories.

Consequently it is evident that before further attempts are made to revise the taxonomy of this group of fungi a thorough understanding is needed of the reliability of the various morphological characters used as criteria of species.

The purpose of this paper is to evaluate the criteria of species.

II. MATERIALS AND METHODS

Two approaches to the study of the various criteria were taken. Biological studies were undertaken to determine the manner of development of these fungi in their various hosts and to examine the effects that host anatomy might have on the development and ultimate form of morphological features of *Phyllachoras*. These findings have already been recorded (Parbery 1963*b*). Comparative studies were also made to investigate the extent of variation of individual characters which might be used as criteria of species. This was done by noting the amount of variation of particular characters in a single colony and then comparing this with variation found in other colonies on the same host leaf, separate leaves in a single collection, and finally colonies from different collections of a single host species. Collections were made over as wide an area as possible and some host groups were examined from world-wide sources.

Data from 181 different specimens of 140 species in 57 grass genera were compared. These specimens are listed in the systematic account of *Phyllachora* in a thesis from which these data have been extracted (Parbery 1962).

III. EVALUATION OF OLD CRITERIA

(a) *Characters of the Phyllachora Colony*

(i) "Stroma"

The term "stroma" has been used in the past to refer to the part of a *Phyllachora* colony visible as a tar spot. Consequently "stroma" was used instead of "colony" or "clypeus" when reference was being made to the shape, colour, or the distribution of colonies. It is preferable to use the term "clypeus" or "colony" when referring to the visible part of a *Phyllachora* colony.

Sometimes, however, characters of the stroma have been discussed separately from those of the clypeus. Doidge (1942) described the extent of development of the stroma in the leaf, its density, and the diameter of the component hyphae. In none of the specimens examined during the present study, however, was any stromatic tissue apart from the clypeus found. The intracellular hyphae were readily seen ramifying through the leaf away from the perithecium or perithecium initial, but the only dense tissue was the perithecium initial itself. The total diameter range of hyphae in specimens examined was 1.5-4 μ , there being little difference in range between specimens. Consequently hyphal diameter is not regarded as useful for taxonomic purposes.

(ii) *Clypeus*

Characters of the clypeus which have been used by previous authors (Theissen and Sydow 1915; Doidge 1942; Orton 1944) to delimit species were: whether they were amphigenous,* epiphyllous, or hypophyllous; whether they were gregarious and confluent or scattered and discrete; and their shape and dimensions of length, breadth, and thickness.

The side of the leaf in which the clypeus develops most strongly indicates the position of the colony in the leaf. To evaluate the usefulness of the position of the colony in the leaf as a criterion of species, a survey was made to determine whether this character was constant for *Phyllachora* specimens collected on a single host species.

In a collection of *Panicum decompositum* from Dalby, Qld., heavily infected leaves tended to have colonies in all three positions whereas lightly infected leaves contained them in only one or two positions. A second collection from Dalby showed colonies in the hypophyllous position only, while a collection from Jimboomba, Qld., had colonies in only the epiphyllous position. In six other collections of *P. decompositum* colonies occurred in the three positions. In all these collections, however, there was no doubt that only one fungal species was being examined.

Similar findings indicated that colony position in other collections of host species (Parbery 1962), notably *Capillipodium spicigerum* (five collections) and *Themeda australis* (four collections), was largely a matter of chance and not characteristic of a particular fungus species.

The distribution of colonies over a leaf also varied. When there were only a few colonies they were usually scattered and discrete but when there were several they were frequently gregarious and often confluent. It was seen in collections of *P. decompositum* that in a single collection the distribution of colonies on separate leaves, and sometimes on different halves of the one leaf, varied greatly. Differences in the distribution of colonies over a host leaf are more probably the result of differences in the density of spore showers at the time of inoculation than of differences in *Phyllachora* species.

The dimensions and shapes of clypei have already been shown to reflect differences in host anatomy rather than between species of *Phyllachora* (Parbery 1963*b*). It was found that clypeus thickness was directly related to epidermal cell size and that clypeus width was restricted by vascular and associated mechanical tissue to between 0.5 and 1.5 mm in nearly all specimens (see also Parbery 1962, Table VI). Clypeus length was relatively unrestricted and was extremely variable even in individual specimens. Consequently clypeus size is no criterion of differences between species of *Phyllachora*.

If clypeus shape (surface outline) is considered it will be realized that it will vary according to variation in length and breadth. Clypei which are 0.5 by 0.5 mm will be circular to square whereas those 0.5–1 mm or more in length will tend to be oblong or elliptical to elongate. Shape therefore is as variable as size and consequently is valueless as a taxonomic criterion.

* Amphigenous has been used to refer to a single colony of *Phyllachora* which has an upper and lower clypeus, and perithecia opening to each surface of the leaf.

(b) Characters of the Perithecium

Various characteristics of the perithecia of *Phyllachora* spp. have been included in descriptions by various authors. These are: the number of perithecia per colony; the depth to which they are immersed in the leaf; their size and shape; and the thickness of their walls. None of these characters is of any taxonomic value.

Numbers of perithecia per colony is no criterion of species, but merely a reflection on the size of the colony. Generally, it is found that large colonies contain many perithecia whereas smaller ones contain one or only a few. The size and shape of the perithecium have also been shown to have no taxonomic value (Parbery 1963*b*).

Perithecial wall thickness was extremely variable in all groups of specimens examined. This variability between *Phyllachora* specimens on *P. decompositum* from three Queensland localities is shown in the following tabulation:

Locality:	Dalby (1)	Dalby (2)	Jimboomba	Oakey
Wall thickness (μ):	13-16	7-13	7-12	13-23

Also wall thicknesses for *Phyllochora* specimens on *Ischaemum australe* ranged from 13 to 17 μ whilst those on *I. triticeum* ranged from 7 to 13 μ .

The overall range for all specimens examined on *P. decompositum* was 7-23 μ , yet some individual specimens had nearly as wide a range on other hosts, e.g. on *Paspalidium distans* the range was 7-20 μ . These ranges do not take into account the thin areas of the wall where it is appressed to a vascular bundle, or the thick areas in the ostiolar region.

The range of perithecial wall thickness (i.e. 7-23 μ) of *Phyllachora* specimens on *P. decompositum* covers the total range for all the *Phyllachoras* on all the host genera, yet within this total range, there is not necessarily any overlap of ranges for two different specimens on a single host species. Consequently the thickness of the perithecium wall can be excluded as a criterion of species.

(c) Characters of the Ascus

Characters of the ascus which have been used to separate species (Orton 1944) are size, shape, and pedicel length. The present study, however, has shown that generally none of these characters is suitable as a taxonomic criterion.

(i) Size of the Ascus

It is evident (Table 1) that each *Phyllachora* species has recognizable ascus size limits, but at the same time a fairly wide range of variation. Within the ascus size range of most groups of related specimens variation between specimens can be so great that the individual size ranges for two specimens on a single host need not even overlap. For example, the overall size range of asci found in colonies on *Capillipedium spicigerum* was 67-98 μ by 10-20 μ but the ranges found on the specimens collected at Mt. Glorious (1961) and Tallebudgera were respectively 67-77 μ by 10-15 μ and 82-98 μ by 10-20 μ . Similar results were found in specimens on other groups of closely related host species. Variation of similar magnitude was noted between specimens collected on the same host from the one locality in different years, e.g. the

ascus size ranges in specimens collected on *Dichanthium sericeum* at Hirstglen in 1960 and 1961 were respectively 66–82 μ by 10–12 μ and 80–91 μ by 10–13 μ .

Sometimes the variation within a single specimen was the direct result of ascospore arrangement within the ascus. Asci with a monostichous arrangement were usually longer and cylindrical to cylindrical-ellipsoid, whereas asci containing

TABLE 1
VARIATION IN THE LENGTH AND WIDTH OF ASCI FROM COLLECTIONS ON SINGLE
HOST SPECIES AND GROUPS OF CLOSELY RELATED SPECIES

Host	Locality	Ascus Length (μ)	Ascus Width (μ)
<i>Bothriochloa ambigua</i>	Heifer Creek	78–86	12–17
<i>B. decipiens</i>	Mt. Glorious	75–83	11–16
<i>B. intermedia</i>	Brisbane	72–86	10–15
<i>Capillipedium spicigerum</i>	Glasshouse Mts.	72–87	10–14
	Mt. Glorious (1960)	70–77	11–13
	Mt. Glorious (1961)	67–77	10–15
	Tallebudgera	82–98	10–20
	Mt. Coot-tha	68–75	10–12
<i>Dichanthium sericeum</i>	Hirstglen (1960)	66–82	10–12
	Hirstglen (1961)	80–91	10–13
<i>Cymbopogon refractus</i>	Southport	79–89	65–10
	Mt. Glorious	87–99	10–11
	Stradbroke I.	82–90	9–10
<i>Ischaemum australe</i>	Glasshouse Mts.	60–75	13–15
	Noosa Heads	82–89	14
<i>I. triticeum</i>	Noosa Heads	70–89	14–20
	Mt. Mitchell	90–98	9–12
<i>Themeda australis</i>	Mt. Glorious (1960)	82–90	10
	Mt. Glorious (1961)	90–120	14–16
<i>T. avenacea</i>	Darling Downs	80–118	10–11
Total range (20 specimens)		60–120	65–20
<i>Elymus</i> spp. (4)*		65–80	8–10
<i>Arundinella</i> spp. (5)		60–83	6.5–18
<i>Oplismenus</i> , <i>Panicum</i> spp. (4)		45–84	7–13
<i>Pennisetum</i> spp. (5)		65–125	10–18
<i>Paspalum</i> spp. (3)		82–96	9–10
<i>Panicum decompositum</i> (5)		56–77	7–10

* No. of specimens.

spores in a distichous or inordinate arrangement were short and ellipsoid to saccate. In single colonies of *Phyllachora* on *Panicum pygmaeum* two kinds of asci were found. There were short saccate asci measuring 45–56 μ by 7–13 μ in which spore arrangement was distichous and there were cylindrical asci measuring 70–80 μ by 8–10 μ in which spore arrangement was monostichous. A similar observation was made from a specimen on *Oplismenus imbecillus* in which short, distichous, saccate asci were

48–60 μ by 10–13 μ and the long, monostichous, cylindrical ones were 66–70 μ by 10–12 μ . Ascus width did not vary greatly within any group of specimens or between groups even when spore arrangement was variable.

If it is considered that the group of specimens with the shortest asci, i.e. those on *Panicum pygmaeum* and *Oplismenus* spp., has an ascus size range of 48–84 μ by 7–13 μ and the group of specimens with the longest asci, i.e. those on *Pennisetum alopecuroides*, has a size range of 65–125 μ by 10–18 μ , it can be seen that every group of specimens examined contained asci in the range 65–84 μ by 10–13 μ . Consequently even though individual species may have a fairly well-defined ascus size range, there is so great a variation within groups of similar specimens (similar in several characters to be described later, as well as being on the same host) and such wide overlapping between the size ranges of dissimilar specimens that this character is not at all useful for delimiting species although it may in some instances be taken into account to confirm an identification.

(ii) Shape of the Ascus

Ascus shape has already been shown to be a direct reflection of ascospore arrangement. Asci with monostichous arrangement are always cylindrical to ellipsoid whereas distichous, tristichous, and inordinate arrangements give ellipsoid and saccate forms. Therefore ascus shape can be discounted as a criterion of species especially as spore arrangement is not a particularly useful taxonomic character.

(iii) Length of the Ascus Pedicel

The third character of the ascus which has been used in taxonomy is the length of the ascus pedicel. Generally, however, its use has not been justifiable. In nearly all specimens examined, the ascus pedicel varied in length but was shorter than half the length of the ascosporific part of the ascus. In some specimens found on *Muehlenbergia* spp., however, the pedicels were always longer than half the length of the ascosporific part of the ascus and frequently longer than its total length.

The length of the pedicel relative to the ascospore-bearing part of the ascus can be used within certain limitations, as a criterion of species. Only when pedicel length is defined as either shorter than half or up to the same length or longer than the ascosporific portion of the ascus is it useful. Most statements on pedicel length occurring in the literature are meaningless because they make no comparisons.

(d) Characters of the Ascospores

Previous authors have used shape, size, and arrangement of ascospores to delimit species of *Phyllachora*. In the present study, shape has been shown to be a useful criterion of species, and size to be of limited use. In the past the use of these characters has not been particularly satisfactory, largely because of a limited understanding of the nature and extent of their variability.

(i) Shape of Ascospores

In descriptions of species of *Phyllachora* (Theissen, Sydow, and Sydow 1915; Doidge 1942; Orton 1944) usually only one ascospore shape is given for each species. During the present study, however, it was unusual to find a specimen in which the

TABLE 2*
 VARIATIONS IN OUTLINE, SIZE, AND ARRANGEMENT OF ASCOSPORES OF SPECIMENS OF PHYLLACHORA
 Specimens are grouped according to affinities in ascospore shape

Collections of Ascospores from:	Hosts	No. of Specimens	Outline										Shape	Size		Arrangement			
			Fusiform	Oblong	Subcircular	Oval	Elliptical	Ovoid	Ovoid-Truncate	Ovoid-Acuminate	Lacrimiform	Semi-elliptical		Length (μ)	Width (μ)	Monostichous	Distichous		
<i>Agropyron repens</i> <i>A. caninum</i> <i>Elymus</i> spp. (6)† <i>Hystrix patula</i> <i>Poa nemoralis</i> <i>Muehlenbergia</i> spp. (11) <i>Panicum</i> spp.‡ (12)		5				3	1	5	5							8-13	4-7	5	
		1														8-11	4-6	1	
		8	2			5		7	8							7-14	4-7	8	
		4			4	4	3									8.5-14	4-6	4	
		2			1	2	2	2								8.5-13	4-6	2	
		15			13	8	2									7-13	4-6	15	
		18	2		15	3	17									7-12	4-5.5	18	
		3			3	3	2									10-16	5-7	3	
		7			7	4	7									10-17.5	5.5-7	7	
		4			3	1	2									10-16	5-7	4	
<i>Bothriochloa</i> spp. (3) <i>Capillipedium</i> spp. (2) <i>Cymbopogon refractus</i> <i>Dichanthium</i> spp. (3) <i>Ischaemum</i> spp. (2) <i>Themeda</i> spp. (2)		4		3	1	2										10-16.5	4-7	4	1
		6	1		6	1	4									10-21	4-9	6	3
		13	1		6	12	5									10-21	5-8.5	13	2
		5			1	3	2									14-28	6-13	3	4
		2		1		1							4			14-24	5.5-9	2	2
<i>Pennisetum</i> spp. (2) <i>Setaria</i> spp. (2) <i>Oplismenus</i> spp. (3) <i>Panicum</i> spp. (2) <i>Urochloa helopus</i>		4			4	2										11-17	4.5-7	2	4
		2			2								2			12.5-19	5-8.5	1	2
		1			1											11-14	6-7	1	1

TABLE 2 (Continued)

Collections of Ascospores from:	No. of Specimens	Outline									Shape	Size		Arrangement		
		Fusiform	Oblong	Subcircular	Oval	Elliptical	Ovoid	Ovoid-Truncate	Ovoid-Acuminate	Lacrimiform		Semi-elliptical	Length (μ)	Width (μ)	Monostichous	Distichous
<i>Arundinella</i> spp. (3)	6	1		3		5							10-22	5-8.5	4	3
<i>Oryzopsis asperifolia</i>	2		2	2		2							11-15	5.5-7	2	1
<i>Paspalum</i> spp. (3)	5			4	5	2							8.5-13.5	5.5-8.5	5	
<i>Panicum obtusum</i>	1		1	1									9-13	5-7	1	
<i>Imperata cylindrica</i>	1			1	1			1					14-21	6-7.5	1	1
<i>Eremochloa bimaculata</i>	1			1	1	1							12-16	6.5-8	1	
<i>Thysanolaena maxima</i>	1												36-42	7-8		1

* For more detailed data see Parbery 1962, Table VIII.

† No. of species.

‡ *P. decompositum* group.§ Distinctive shape in some specimens on *Themeda* spp.

ascospores were of only one morphological type. For, example, the ascospores taken from a single peritheciium of *Phyllachora* on *Themeda australis* (from Mt. Glorious, Qld.) exhibited five different forms (Table 2) and commonly three or four of these occurred among spores in individual asci.

Similarly ascospores from specimens of *Phyllachora* on species of *Elymus* (*E. europaeus* being the host of the type species of the genus *Phyllachora*, *P. graminis*) exhibited three morphological types, these being oval, ovoid, or ovate-truncate.* In the original description of *P. graminis* the only shape noted, however, was ovoid (Fuckel 1869). Ovoid spores were found in most specimens (Table 2) and consequently their presence did not distinguish one *Phyllachora* specimen from many others. The presence of ovate-truncate spores, however, was noted only in specimens on species of *Agropyron*, *Brachypodium*, *Bromus*, *Cinna*, *Elymus*, *Hystrix*, and *Poa*, and therefore distinguished them from any other group.

It is necessary to realize that spore shape is not always as simple and as symmetrical as the two-dimensional view a microscope tends to convey. It is important to distinguish between outline and shape, yet there is no evidence in the literature to suggest that this has been done. Three different types (in outline) of ascospore in a specimen need not necessarily mean there are three different morphological types (shapes), but simply one which is not symmetrical and consequently has a different outline in each plane of view.

This was readily demonstrated by causing spores to drift and roll while being observed through a microscope, for their outlines often changed considerably. For example, ascospores in specimens on *Bothriochloa*, *Capillipedium*, *Cymbopogon*, *Dichanthium*, *Ischaemum*, and sometimes *Themeda* were commonly oval or ovoid or slightly dumbbell-shaped. By rolling these spores around it was found that they were oval in one longitudinal plane, ovoid in the other, and circular in cross section. Frequently, however, there was an equatorial constriction in one plane (usually the oval plane) which gave them the distinctive dumbbell appearance. Consequently, what at first appeared to be three different spore shapes, proved to be one shape which had various outlines in different planes of view. A similar investigation of other spores from the same source showed, however, that other spore shapes did occur, e.g. ovoid-dumbbell, oval-dumbbell, oval-elliptical, and oval-ovoid.

When examining ascospore shape, therefore, it is important to bear three points in mind: (i) that the visible outline of ascospores does not necessarily give a real indication of its shape, and mere outline is inadequate for taxonomic purposes; (ii) that it is unusual to find only one ascospore shape in a single ascus and most uncommon in a group of specimens; and (iii) that the most common ascospore shape is not necessarily the one which distinguishes a specimen in one group from those in another, as was shown for *Phyllachora graminis*.

An illustration of the importance of these three points can be given in the following example. In specimens of *Phyllachora* on *Pennisetum* spp. the outlines of the ascospores (Table 2) were oval, elliptical, ovoid, and ovoid-truncate. The actual shapes noted, however, were ovate-acuminate-oval, ovate-acuminate-ovoid, and

* Ovate-truncate spores were basically ovoid but the broad end, instead of being rounded, was truncate so as to appear flattened and the general outline of the spore was triangular.

ovoid-ellipsoid. Of the latter two of these shapes which were common to all specimens, only the ovate-acuminate-ovoid form was of taxonomic significance because the ovoid-ellipsoid form occurred commonly in other groups of specimens as well.

Ascospore shape is a valuable taxonomic character providing it is used properly. Individual shapes have to be determined by rolling the individual spores and noting changes in outline. Then each shape, if there is more than one, needs to be taken into account when making comparisons with ascospores from other specimens.

(ii) *Size of Ascospores*

In Table 2 [see Table VIII (Parbery 1962) for more detail] the various characters of ascospores have been recorded so that specimens with similarly shaped ascospores are grouped together and the size and arrangement of the spores is given for each specimen. The size of ascospores of some of these groups was fairly constant but in others it was quite variable. The ascospores of specimens on *Agropyron*, *Elymus*, *Hystrix*, *Poa*, and *Muehlenbergia* were never larger than 14 by 7 μ and were always in the range 7-14 μ by 4-7 μ . Similarly the ascospores of specimens on *Panicum* (*P. decompositum* group) were very uniform in size, being 7-12 μ by 4-5.5 μ . Most specimens on *Bothriochloa*, *Capillipedium*, *Cymbopogon*, *Dichanthium*, and *Ischaemum* had ascospores which were 10-17 μ by 5-7 μ . Some specimens, however, were more variable, the size range being 10-21 μ by 5-9 μ . Consequently there were often considerable differences between ascospore sizes even on the same host and this wide range cut across or included the ranges of size of ascospores in nearly every group examined.

In the past, size of ascospores has been used extensively for delimiting species of *Phyllachora* (e.g. Orton 1944), differences recorded between the maximum and minimum spore length for any species usually only being 3 or 4 μ , or occasionally 5 μ , rarely greater. The differences recorded between species are often no greater than this. During the present study, however, it has been found that differences in the size of ascospores discharged from a single perithecium are often greater than those recorded in the literature for a species. For example, in specimens on *Pennisetum alopecuroides* a difference of 12 μ was recorded between the maximum and minimum lengths of ascospores. The variation in size of ascospores in a single ascus was frequently as great as noted in discharged ascospore masses.

Since the variation among ascospores within an ascus can be as great as among liberated ascospores and because it is evident that many species have been based on measurements of ascospores still retained in asci, it is necessary to consider variation of unliberated spores.

Asci which were as fully developed as was possible to determine visually were selected, the spores were measured, and representative results are recorded in Table 3. The difference between maximum and minimum spore lengths was frequently 3-4 μ and sometimes up to 7 μ , but was not greater than observed among the liberated spores. The observation that similarly sized spores were often arranged in pairs within the ascus in such a way as to suggest genetic segregation, indicates that it may be profitable to consider the extent to which spore size variation is genetically controlled within species.

TABLE 3
 VARIATIONS IN ASCOSPORE SIZE WITHIN INDIVIDUAL ASCI IN SPECIMENS OF PHYLLOCHORA ON THREE COLLECTIONS OF ISCHAEMUM SPP.
 Measurements in microns

Ascospore Number in Ascus	<i>I. triticeum</i> (Stradbroke I.)			<i>I. triticeum</i> (Beerwah)			<i>I. australe</i> (Southport)		
	Ascus 1	Ascus 2	Ascus 3	Ascus 1	Ascus 2	Ascus 3	Ascus 1	Ascus 2	Ascus 3
1	17 × 7	15.5 × 7	14 × 7	17 × 7	17 × 7	14 × 5.5	15 × 6.5	14 × 6.5	14 × 6.5
2	17 × 7	15.5 × 7	15 × 7	14 × 7	15 × 7	12.5 × 6	14 × 7	13 × 7	15 × 6.5
3	15 × 6	18 × 7	14 × 7.5	15.5 × 6	12.5 × 7	10.5 × 6	13 × 7	11 × 6.5	12 × 6.4
4	15 × 7	17 × 7	15 × 7	14 × 7	14.6 × 5	11.5 × 6	13 × 6.5	15 × 8	11 × 6.5
5	21 × 6	14 × 7	16 × 6	14 × 7	15.5 × 6.5	10.5 × 6	13 × 6.5	14 × 7.5	12 × 6.5
6	19 × 7	17 × 7	15.5 × 6	14 × 7	13 × 6	11 × 6	13 × 7	14 × 6.5	12 × 6.5
7	17 × 7	16 × 7	14 × 7	14 × 7	15 × 6.5	13 × 7	14 × 6.5	14 × 7	13 × 6.5
8	17 × 7	21 × 7	15 × 7	17 × 7	16 × 7	13 × 7	14 × 7	15 × 9	12 × 6.5
Size range	15-21 × 6-7	14-21 × 7	14-16 × 6-7.5	14-17 × 6-7	12.5-17 × 6-7	10.5-14 × 5.5-7	13-15 × 6.5-7	11-15 × 6.5-9	11-15 × 6.5
Differences between maxima and minima: Lengths	6	7	2	3	4.5	3.5	2	4	4
Widths	1	0	1.5	1	1	1.5	0.5	2.5	0

It has been noted that the actual range as well as the degree of variation in spore length can differ between specimens. Consequently strict adherence to the size limits noted in the type specimen of a species is not justifiable. In fact it is possible to know the limits of spore length for a species only after several specimens have been seen.

Similar conclusions can be drawn about the usefulness of ascospore width. Generally width is less variable than length, but most specimens have ascospores which are 4–8 μ wide. In some cases, e.g. where ascospores are wider than 8 μ , this character may be of some use.

The ratio of ascospore length to width has sometimes been used (Orton 1944), but this is extremely variable and is only useful if large differences are considered. For example, *P. bambusae* has very long ascospores which have a length to breadth ratio of 5 : 1. Most other species have a range between 1 : 1 and 3 : 1.

TABLE 4
COMPARISON OF THE SIZE OF ASCOSPORES FOUND IN SPECIMENS OF
PHYLLACHORA ON SPECIES OF ISCHAEMUM
Ischaemum arcuatum, *I. latifolium*, *I. laxum*, and *I. brachyatherum* were
the hosts of the type specimens of *Phyllachora bottomleyae*, *P. ichae-*
micola, *P. ischaemi*, and *P. liebenbergii* respectively

Host	Origin	Ascospore Length (μ)	Ascospore Width (μ)
<i>I. arcuatum</i>	South Africa	10–14	5.5–7.5
<i>I. latifolium</i>	Venezuela	12–14	7–9
<i>I. laxum</i>	Burma	12–16	5–6
<i>I. brachyatherum</i>	Uganda	13–17	7–9
Total range		10–17	5–9
<i>I. australe</i>	Queensland	11–18	6–8
<i>I. triticeum</i>	Queensland	10–21	5–9
Total range		10–21	5–9

It is often noted in the literature that the only difference in the descriptions of two fungi regarded as different species is a slight difference in dimensions of the ascospores. An illustration of this can be seen in Table 4 where the sizes of the spores of the four "species" described on various species of *Ischaemum* are compared with each other and with those recorded for *Phyllachora* specimens found on species of *Ischaemum* from Queensland. It can be seen (Table 4) that the range of sizes found in a single specimen on *I. triticeum* exceeded the combined ranges recorded for the descriptions of four species. It is believed that the four fungi referred to in Table 4 are all specimens of a single species.

Although ascospore size is constant in some groups of specimens, it is too widely variable in other groups to be a useful character for species delimitation. A comparison of the ranges of size between the specimens on the various host groups

set out in Table 2, with the exception of the specimen on *Thysanolaena maxima* (*Phyllachora bambusae*), shows that no group is distinct because of the size of its ascospores. The overlap in range between groups is usually so considerable as to render it impossible to say where one group of specimens ends and the next begins. The ascospores of the *Phyllachora* specimens on *Themeda* spp. were 10–21 μ long. Some of the ascospores from nearly every specimen examined during the present study would fit into this range. Therefore, although it may be helpful to know the range of ascospore sizes recorded for a particular species for purposes of confirming an identification, this character cannot be used as a criterion of species.

TABLE 5

RELATIONSHIP OF ASCOPORE SHAPE AND APPRESSORIUM MORPHOLOGY WITHIN GROUPS OF SPECIMENS

Host Genera	Most Common or Distinctive Ascospore Shape	Appressorium Shape	Host Genera	Most Common or Distinctive Ascospore Shape	Appressorium Shape
<i>Elymus</i> <i>Agropyron</i> <i>Hystrix</i>	Oval, ovoid Ovate-truncate	Unknown	<i>Arundinella</i> <i>Oryzopsis</i>	} Oval, ovoid	Ovoid, obclavate
<i>Panicum decompositum</i>	Oval, ovoid	Clavate	<i>Paspalum</i> <i>Panicum</i>		
<i>Bothriochloa</i> <i>Capillipedium</i> <i>Cymbopogon</i> <i>Dichanthium</i> <i>Ischaemum</i>	} Oval, ovoid	} Ovoid (sigmoid), obclavate	<i>Oplismenus</i> <i>Panicum</i> <i>Urochloa</i>	} Ellipsoid	Obclavate Obclavate Unknown
<i>Themeda</i>			Ellipsoid, semi-ellipsoid		
			<i>Imperata</i>	Lacrimiform	Ovoid-obclavate
			<i>Eremochloa</i>	Oval-ovoid	Obclavate

(iii) *Arrangement of Ascospores*

The arrangement of ascospores in the ascus has been used to separate species (Orton 1944). During the present survey, some groups of specimens were noted as having only monostichous ascospore arrangement (Table 2) and the evidence indicates that this is characteristic of some species of *Phyllachora*. Generally, however, this was a variable character. In most specimens found on species of *Capillipedium* and related genera, monostichous ascospore arrangement was found, but in occasional specimens spores were distichous. The only specimens in which only distichous arrangement was observed occurred on *Festuca* and *Dactylis* spp. but too few specimens were seen to evaluate the variability of this. *Phyllachora* specimens on *Pennisetum* and *Setaria* usually exhibited distichous arrangement of ascospores, but monostichous arrangement was not uncommon. Consequently this character cannot be used to delimit species.

IV. PROPOSED NEW CRITERIA

(a) *Appressorium Morphology*

Parbery (1963a) showed that specimens could be grouped according to the morphology of the appressoria produced by their germinated ascospores. These groupings showed that all specimens of *Phyllachora* on a particular group of host species had similarly shaped appressoria and that well-defined differences in morphology occurred between the various groups (Table 5). The individual groupings made in this way corresponded to the groupings made according to ascospore shape listed in Table 2, except that there were not as many types of appressorial form as there were of ascospore shape. Consequently, although it is not suggested that each species of *Phyllachora* has an individual appressorium morphology, it is considered that the appressorium type (Parbery 1963a) is constant for individual *Phyllachora* spp. Therefore, appressorium shape is regarded as a useful criterion for delimiting species.

(b) *Imperfect States*

At present only one species of *Phyllachora*, *P. quadraspora*, is known to produce a conidial state (Parbery and Langdon 1963a). Until more is known about the imperfect states of other *Phyllachora* spp. and some comparative studies have been made, it is not possible to say which particular character of the asexual structures may be useful for taxonomic purposes.

(c) *Scolecosporic States*

Studies on the development of *P. parilis* (Parbery and Langdon 1963b) showed that this fungus produced both ascal and scolecosporic states. It was also shown that several other *Phyllachora* spp. have similar *Leptostromella*-type fungi associated with them and evidence of these various species of *Leptostromella* as also being genetically connected to their respective *Phyllachora* sp. was presented. Tilak (personal communication) has shown that the scolecospores of the *Leptostromella* state of *Phyllachora cynodontis* are in fact spermatia. This supports the view of Parbery and Langdon (1963b).*

The general shape and width of scolecospores are similar in all cases (Parbery and Langdon 1963b) and length is often so variable within groups of specimens (Table 6) that these characters are not suitable taxonomic criteria. In some groups, e.g. the *Leptoloma-Panicum* group and the *Muehlenbergia* group, the respective spore length ranges of 7–10 μ and 8–15 μ were extremely stable.

The shapes of the sporophores of the *Leptostromella* states of *Phyllachora* specimens (illustrated in Parbery and Langdon 1963b) were constant within groups of specimens which already had a common ascospore and appressorium morphology. The general shape of the sporophore, or each branch, the type of apex, and whether

* The authors wish to thank Dr. Tilak for drawing their attention to an error in their recent paper (Parbery and Langdon 1963b, p. 151) in which it was stated that Dr. Tilak (1960) described spermatization in *Phyllachora ambrosiae*. The species studied by Dr. Tilak was *P. actinodaphnes*.

TABLE 6
 CHARACTERS OF THE SCOLECOSPORES AND THEIR SUBTENDING SPOROPHORES ASSOCIATED WITH VARIOUS GROUPS OF PHYLLACHORA SPECIMENS
 + Character present

Host	No. of Specimens	Scolecospore Size (μ)		Sporophore Size (μ)		Sporophore Shape			Sporophore Apex Type			Sporophore Branched	
		Length	Width	Length	Width	Cylindrical	Irregular Cylindrical	Slightly Tapered	Obclavoid	Rounded	Mucronate		Acuminate
<i>Cynodon dactylon</i> <i>Chloris</i> sp.	1	8.5-14	0.5	7-9	1.5	+				+			
	2	8-14	0.5										
<i>Muehlenbergia</i> spp. (7)*	7	8-15	0.5	7-14	1.5	+						+	
	1	16-20	1										
<i>Capillipedium spicigerum</i>	3	8-20	1	7-12	1.5-2			+			+		
<i>Cymbopogon refractus</i>	2	7-20	1	10-13	2			+			+		
<i>Themeda australis</i>													
<i>Arundinella</i> spp. (3)	4	14-30	0.5-1	12-21	2-3		+			+			
<i>Loudetia simplex</i>	1	17-21	0.5-0.7	10-16	2		+			+			
<i>Oryzopsis asperifolia</i>	2	14-17	0.5	14-17	2		+			+			
<i>Paspalum orbiculare</i>	2	8-19.5	0.5-1	7-12	2-3							+	+
<i>Paspalum scrobiculatum</i>	2	8-16	0.5-1	8-16	1.5-2							+	+
<i>Panicum obtusum</i>	1	12-18	0.5-0.7	15-17	2							+	+
<i>Leptoloma cognatum</i>	1	7-10	0.5	7-10	1.5								
<i>Panicum</i> spp. (4)	4	7-10	0.5	6-10	1.5				+	+			
<i>Pennisetum</i> spp. (2)	3	15-28	0.5	16-22	1.5-3								+
<i>Setaria</i> spp. (2)	2	17-24	0.5										
<i>Oplismenus</i> spp. (3)	3	10-16	0.5	7-11	1.5-2								
<i>Panicum</i> spp. (2)	2	11-18	0.5	9-10	1.5-2								+
<i>Urochloa helopus</i>	1	10-14	0.5-0.7										+
<i>Sporobolus</i> spp. (2)	(2)	14-21	0.5	7-14	1.5								+

* No. of species.

† Indicates the morphology of individual branches of the sporophore.

or not the sporophore was branched were all useful criteria (Table 6). The size of the sporophores was not as variable as that of the scolecospores, and within limits may be useful. For example, specimens with sporophores longer than $17\ \mu$ were distinct from others, as were those with sporophores shorter than $10\ \mu$, with the exception of specimens on *Cymbopogon* and *Themeda*. As knowledge of the *Leptostromella* states widens it is probable that sporophore size will prove to be of even more limited use than at present, so that emphasis should not be placed on the use of this character. Sporophore morphology, however, is regarded as a useful taxonomic character.

V. DISCUSSION

The result of this study is that of the numerous characters once used to delimit species of *Phyllachora*, only two are now regarded as valid. These are the length of the ascus pedicel relative to that of the ascospore portion of the ascus, and the morphology of the ascospores. To these have been added two new criteria, the morphology of the appressorium produced by ascospores germinating on their host's leaf surface (Parbery 1963a) and the morphology of the sporophores which bear the scolecospores (Parbery and Langdon 1963b).

It is now possible to delimit species of *Phyllachora* by morphological criteria alone, thus eliminating much of the confusion which has arisen from the use of unreliable morphological criteria and the concept of host specificity among *Phyllachora* spp. *Phyllachora* spp. should be delimited, within the framework of host family, by the morphology of the various taxonomically useful structures of the ascospore and scolecospore states. When more proven imperfect states are known, it is probable that their structures will provide further useful criteria of species.

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