TAXONOMIC STUDIES ON *VAUCHERIA* (VAUCHERIACEAE, CHRYSOPHYTA) IN SOUTH-EASTERN AUSTRALIA

A thesis submitted to
La Trobe University
in fulfilment of the
requirements of the degree of
Doctor of Philosophy.

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"In walking along the banks of the little stream, where, half concealed by more pretentious plants, our humble Vaucheria grows, the average passer-by, if he notices it at all, sees but a tangled tuft of dark-green 'scum'. Yet, when this is examined under the magic tube, a crystal cylinder, closely set with sparkling emeralds, is revealed."

p. 6 of Breckenfeld (1885) The life history of Vaucheria. Am. Mon. microsc. J. 6: 2-6.

SUMMARY

The genus Vaucheria is represented in south-eastern Australia by 24 species; 20 are new records for the continent, including the newly described V. bicornigera and V. gyrogyna. The subsections Racemosae (Walz) Heering and Sessiles (Walz) Heering have been raised to sectional level, and as a prelude to the monographic account, the variability of characters used to delineate species in these two sections has been evaluated. Twenty-two features have been assessed in 56 field populations and in seven culture isolates subjected to twenty-three treatments involving varying combinations of temperature, light, substrate, salinity and nutrient levels. In the section Corniculatae (previously subsection Sessiles), the texture of the oogonial wall, the size and shape of the oogonium and the structure of the oospore wall have been found to be stable taxonomic characters, and as a result V. bursata (O.F.Müller) C.Agardh [including V. repens Hassall, V. clavata sensu Klebs and V. sessilis (Vaucher) de Candolle], V. dillwynii (Weber & Mohr) C. Agardh and V. borealis Hirn are recognised as distinct species. In the section Racemosae, the shape of the oospore, the structure of the oospore wall and the orientation of the oogonium are stable taxonomic characters in those plants with oogonia pendent or perpendicular to the peduncle. As a result, two species, V. prona Christensen and V. frigida (Roth) C. Agardh, are recognised in the south-eastern Australian flora and the status of V. terrestris (Vaucher) de Candolle and V. racemosa (Vaucher) de Candolle as distinct species needs further evaluation. A reassessment of the sectional characters of Vaucheria has led to the recognition of nine sections, six of which are

represented in south-eastern Australia. Historical information on the sections and species of Vaucheria, and experimental studies on the optimum conditions for vegetative growth and induction of gametangia, are also included.

DECLARATION

All the work reported in this thesis was carried out by myself, unless otherwise indicated, and no material has been submitted for any previous degree or diploma. Chapter 3 has been submitted to Phycologia as 'An Evaluation of Taxonomic Characters in the Subsection Sessiles, Section Corniculatae of Vaucheria (Vaucheriaceae, Chrysophyta)'.

Timothy J. Entwisle

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Financial support was provided by a Commonwealth Postgraduate Research Award and Lynda.

ABBREVIATIONS AND FORMAT

Journals cited in the reference list are abbreviated in accordance with the World List of Scientific Periodicals, 4th ed., 1963-65 (Ed. by P. Brown & G.B. Stratton; published by Butterworth's, London). Contractions, abbreviations and spelling are from the Macquarie Dictionary, 1981 (Ed.-in-chief A.Delbridge; published by Macquarie Library, St Leonards, Australia). Authors names are spelt as they appear in the cited publication (e.g. Åberg, Götz) and non-English names are ordered according to their native alphabet. Chapters 3, 4, and 5 have been written to facilitate publication as separate manuscripts and conform to the format used in Phycologia, Journal of the International Phycological Society.

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CHAPTER ONE

INTRODUCTION

The genus Vaucheria (Vaucheriaceae, Chrysophyta) includes over 60 species, most with a reportedly worldwide distribution. Although a number of important taxonomic and/or floristic studies of Vaucheria have been published for North America (Blum 1972, Hoppaugh 1930, Ott & Hommersand 1974), Europe (Christensen 1969, Dangeard 1939b, Simons 1977, Rieth 1980b), North Africa (Gauthier-Lièvre 1955), Iraq (Islam 1984), U.S.S.R. (Zauer 1974) and Asia (Jao 1936; Ley 1944; Li 1936; Saxena 1962; Yamagishi 1959, 1963, 1965), few data are available for Australia and most of these (Bailey 1893; Cribb 1979; Hardy 1906; May 1938; Möbius 1892, 1895; Phillipson 1935; Playfair 1917; Sonder 1881; Watts 1887) represent isolated records of species occurrence which now require confirmation and reassessment. Only one protologue, that of V. glomerata Blum & Womersley (1955), is based on Australian material.*

Historical information on the taxonomy of Vaucheria is provided by Christensen (1968, 1969, 1973) and Rieth (1980b), but certain points require emphasis here. The first published record of Vaucheria (as Byssus) was probably in Ray (1724, p.56). Over seventy years later, de Candolle (1801, p.20) established the name Vaucheria, based on material examined by Pierre Vaucher. Silva (1952, p.256) lectotypified the genus with V. disperma de Candolle, which according to Christensen (1968,

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* Christensen (pers. comm.) is submitting a paper to Aust. J. Bot. which includes two previously undescribed species, V. conifera (see p.213) and V. nanandra (see p.142), based on material from south-eastern Australia.

1969) is a heterotypic synonym of V. canalicularis (Linnæus) Christensen.

Little further work was done on Vaucheria until the last half of the nineteenth century, when a number of taxonomic accounts (e.g. Arechavaleta 1883, Cleve 1863, Crouan & Crouan 1867, Götz 1897, Walz 1866) appeared. Walz (1866), in his world monograph of Vaucheria, devised an infrageneric system of 'groups', later (Heering 1907) termed sections, but confused the nomenclature of species by ignoring some earlier published names and using others for obviously different taxa. Arechavaleta (1883), in a floristic account of Vaucheria in Uraguay, created new names for taxa similar to those described by Vaucher and Walz, thus further confusing the nomenclature at species level. Götz (1897) attempted to clarify species concepts in Vaucheria but misinterpreted some of Vaucher's names (Christensen 1969).

A number of taxonomic accounts appeared in the early twentieth century (e.g. Heering 1907, 1921; Hoppaugh 1930; Randhawa 1939, 1942a) but much of the nomenclature remained confused until the studies of Christensen (1968, 1969, 1973). Rieth (e.g. 1953, 1956a, 1956b, 1961, 1965b) has clarified the circumscription of some species of Vaucheria, based on extensive field collections and studies of plants under constant culture conditions, but the delineation of many species remains unresolved.

Species concepts in the sections Racemosae (Walz) sect. nov. [previously section Corniculatae subsection Racemosae (Walz) Heering *] and Corniculatae (Walz) Heering sens. nov. [previously section Corniculatae subsection Sessiles (Walz) Heering] are largely unresolved

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*See Chapter 5 for evaluation of sectional characters and emendments to sectional classification.

(Blum 1972; Christensen 1969; Rieth 1963b, 1980b). A number of species referable to the sections Racemosae and Corniculatae occur in south-eastern Australia and studies were needed to evaluate the characters used to delineate taxa in these sections.

The present study has been undertaken to provide an analytical monograph of the species of Vaucheria occurring in south-eastern Australia, and where necessary to evaluate the characters used to separate species within the genus. As a prelude to the taxonomic work, studies were carried out to determine the optimum conditions for induction of gametangia and maintenance of isolates in culture (Chapter 2). This is followed by an analysis of the stability and suitability of characters used to delineate certain species in the sections Corniculatae (Chapter 3) and Racemosae (Chapter 4). On the bases of the conclusions reached in Chapters 3 and 4, and from the data obtained from numerous field populations and culture isolates, a monographic account of Vaucheria in south-eastern Australia has been produced (Chapter 5). This is followed by some concluding remarks (Chapter 6) and a list of references.

The remainder of Chapter 1 includes a brief account of the morphological features of Vaucheria and a general statement about species concepts.

BASIC MORPHOLOGICAL FEATURES OF VAUCHERIA

The thallus of Vaucheria consists of more or less tubular, branched SIPHONS, sometimes (Fritsch 1935, Blum 1972, Ott & Hommersand 1974) termed aseptate or siphonous filaments. Strictly, however, the term 'filament' usually implies a septate thallus (e.g. Fritsch 1935, p.18). There are few characters of taxonomic significance associated with the

vegetative thallus, but a specialised terminology has been developed for the reproductive system, and this becomes important in keys and descriptions.

Gametangia may be sessile or pedicellate on the vegetative siphons (Fig. 1.1), or borne on a GAMETOPHORE (Fig. 1.2); the term fruiting branch has been used frequently (e.g. Blum 1972, Christensen 1969) for this latter structure. The gametophore consists of a PEDUNCLE (Fig. 1.2) arising perpendicularly from the vegetative siphon (if other siphons arise perpendicularly from the peduncle, the gametophore is considered to be terminal), usually one ANTHERIDIAL PEDICEL (Fig. 1.2) and one or more OOGONIAL PEDICELS (Fig. 1.2). Sometimes ADVENTITIOUS GAMETOPHORES (Fig. 1.9) arise from some part of the existing gametophore; previously (e.g. Blum 1971, Rieth 1975), these have been termed proliferations. In some species, none of which were found in south-eastern Australia, a differentiated portion of the siphon, termed an ANDROPHORE, gives rise to the antheridium (see Rieth 1980b, fig.48; Ott & Hommersand 1974, figs 1-8). Some antheridia and oogonia are subtended by a WALL-BOUND CAVITY (Fig. 1.5); this has been termed an "empty cell-like space" by Blum (1972).

The antheridia are variously shaped and dehisce through terminal or lateral pores. ANTHERIDIAL LENGTH refers to the length of the longest axis of the antheridia, if straight (Fig. 1.5), or the greatest 'diameter' or extension of the antheridia, if curved or circinate (Figs 1.1,1.3). ANTHERIDIAL DIAMETER is the maximum diameter perpendicular to the long-axis, not including lateral protuberances (Fig. 1.5), or in circinate antheridia, the maximum diameter irrespective of the long-axis (Fig. 1.1). The ANTHERIDIAL SYSTEM LENGTH (Fig. 1.1) refers to the combined length of the antheridial pedicel and antheridium (extending in

the direction of the pedicel).

Oogonia are variously shaped and usually have a single fertilisation pore. OOGONIAL LENGTH is the length of the longest axis of the oogonium, irrespective of its orientation (Fig. 1.1). OOGONIAL DIAMETER is the maximum diameter perpendicular to the long-axis (Fig. 1.1). (The ratio of oogonial length to diameter is abbreviated as L/D.) All measurements are taken from oogonia containing mature oospores. In some species, part of the oogonium is not occupied by the mature oospore; this unoccupied part is termed an OOGONIAL CAVITY. The oogonial cavity can be either proximal (Fig. 1.7), distal (Fig. 1.4) or peripheral (Fig. 1.6); previously (e.g. Blum 1972, Rieth 1980b), a distal oogonial cavity has been termed a 'beak'. The LENGTH of the DISTAL OOGONIAL CAVITY (Fig. 1.4) is the length of a line from midway through the fertilisation pore, to midway through the part of the oospore wall not contiguous with the oogonial wall.

Terminology for the orientation of oogonia depends on whether or not the oogonia are borne on gametophores. The orientation of oogonia not borne on gametophores is described by the OOGONIAL LONG-AXIS ANGLE (Fig. 1.1): i.e. the angle between the long-axis of the oogonia and the attached siphon (or line perpendicular to pedicel, if present), and the OOGONIAL PORE ANGLE (Fig.1.1): i.e. the angle between a line through the fertilisation pore and the attached siphon. The orientation of oogonia in gametophores may be pendent (Fig. 1.8), or erect (Fig. 1.9), or perpendicular or transverse to the peduncle (Fig. 1.10).

Aplanosporangia (Fig. 1.13) and zoosporangia (Fig. 1.12) are generally clavate and borne terminally or on short laterals, and akinetes (Fig. 1.11) are formed by the production of two septa in the siphon. The asexual reproductive system has little known taxonomic

value.

SPECIES CONCEPTS

The species described in this study are based on culture and field studies on south-eastern Australian plants, in conjuction with an examination of the available literature. The relationship between these entities and biological species (sensu Mayr 1963) cannot be assessed until extensive interbreeding experiments have been carried out. Until such time, the species concept used in this study approaches that proposed by Darwin (1859), Bentham (1875) and, more recently, Levin (1979). The adoption of infraspecific categories in Vaucheria is considered unnecessary and cumbersome, and has been avoided in this study. Variants which differ slightly in one or a few characters from a described species are considered worthy of note, but not of formal taxonomic recognition.

Much of the analytical work in this study is based on plants grown under controlled conditions in culture. Most phycologists now accept the view of Pringsheim (1967), that neither culture nor field studies should be revered above the other in their taxonomic implications; the potential polymorphism or phenotypic range of an interbreeding population provides a sound background to the study of field diversity. Furthermore, as many field collections of Vaucheria are infertile, identification must be based on some form of subsequent culturing. Thus the morphological variation of plants in culture must be included in any species definition.

In general, type material has not been available for examination. Consequently, names are applied on the basis of information in the protologue or on the basis of data on type material provided by other

authors. Type material is often of limited value as a reference point for species concepts due to inadequate preservation and/or the absence of critical diagnostic features (see in particular, Christensen 1968, 1973). The circumscription of species in this study is based on observations on collected plants, and on comparisons with previous studies of type material or plants from type localities where available. Generally, taxa are considered to be conspecific only when a comparison has been made between the relevant type material. Christensen (1968,1969,1973), however, has based a number of synonymies on a comparison between type material or subsequent collections from the type locality, and published descriptions. As many of the synonyms are used extensively in the literature, Christensen's conclusions are adopted here.

Figs 1.1-1.5 Morphology of Vaucheria.

Fig. 1.1 Features associated with gametangia in the section Corniculatae, showing angle through which antheridium turns (a.a), antheridial diameter (a.d), antheridial length (a.l), antheridial pedicel length (a.p.l), antheridial system length (a.s.l), long-axis angle (l.a), oogonial diameter (o.d), oogonial length (o.l) and oogonial pore angle (o.p.a),

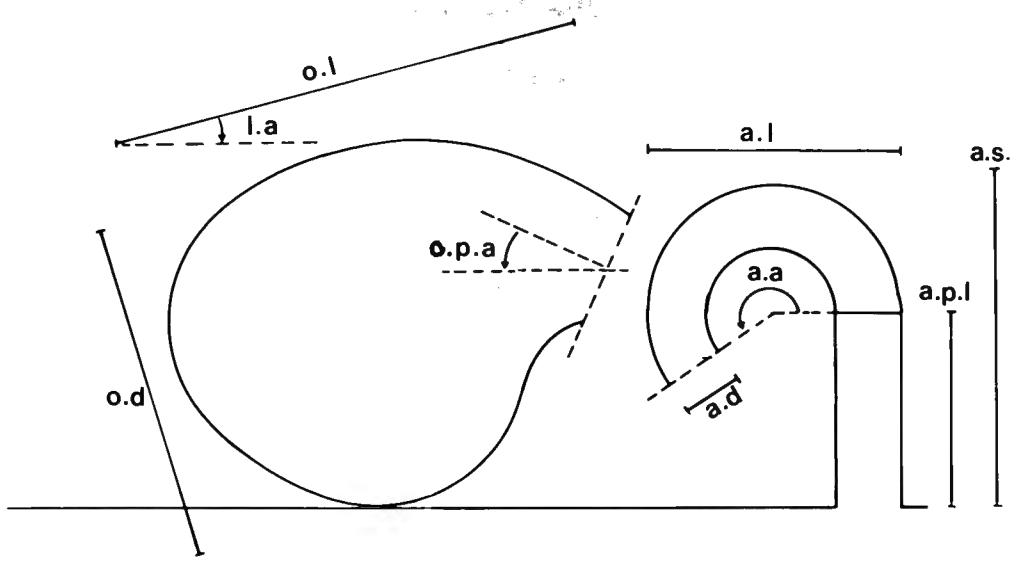
Fig. 1.2 Features associated with gametophores, showing antheridial pedicel length (a.p.l), oogonial pedicel length (o.p.l), peduncle diameter (p.d) and peduncle length (p.l).

Fig. 1.3 Features associated with measuring antheridial curvature, showing angle through which antheridia turns (a.a), antheridial length (a.l), inner radius (r_2) and outer radius (r_1).

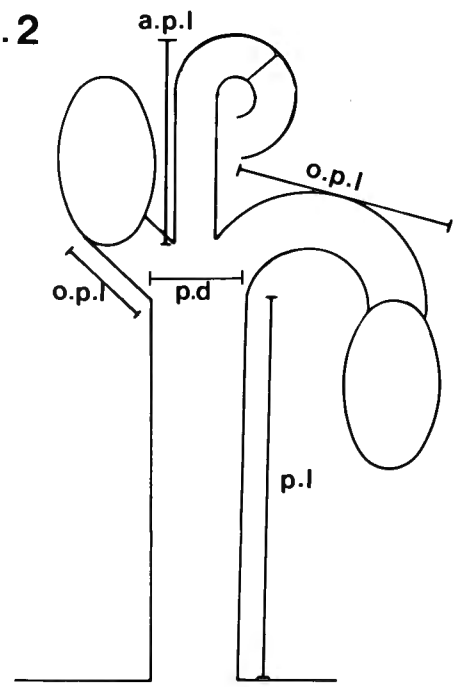
Fig. 1.4 Oogonium with mature oospore and distal oogonial cavity, showing oogonial cavity length (o.c.l).

Fig. 1.5 Antheridium subtended by a wall-bound cavity (w.c), showing antheridial length (a.l) and antheridial diameter (a.d).

1.1


o.l
l.a
a.l
a.s.l
o.p.a
a.a
a.p.l
o.d
a.d

1.2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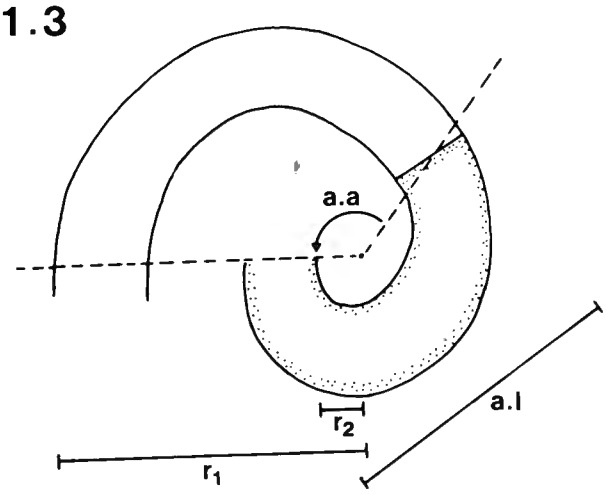
o.p.l
p.d
p.l

1.3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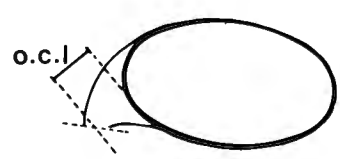
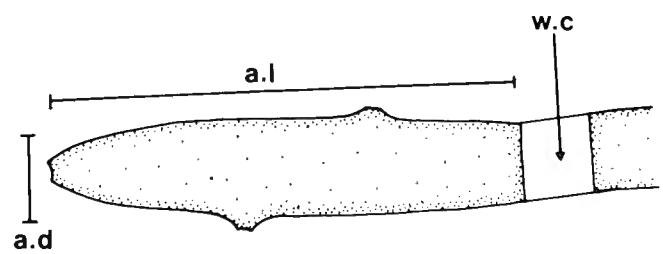
a.l
r_2
r_1

1.4


o.c.l

1.5


w.c
a.l
a.d

Figs 1.6-1.13 Morphology of Vaucheria.

Fig. 1.6 V. aversa Hassall, showing peripheral oogonial cavity. MEL 1049105

Fig. 1.7 V. litorea Hofman & C.Agardh, showing proximal oogonial cavity (arrow). MEL 1049199.

Fig. 1.8 V. prona Christensen, showing pendent oogonia. MEL 1049269.

Fig. 1.9 V. cruciata (Vaucher) de Candolle, showing erect oogonia, and adventitious gametophores (arrow). MEL 1049058.

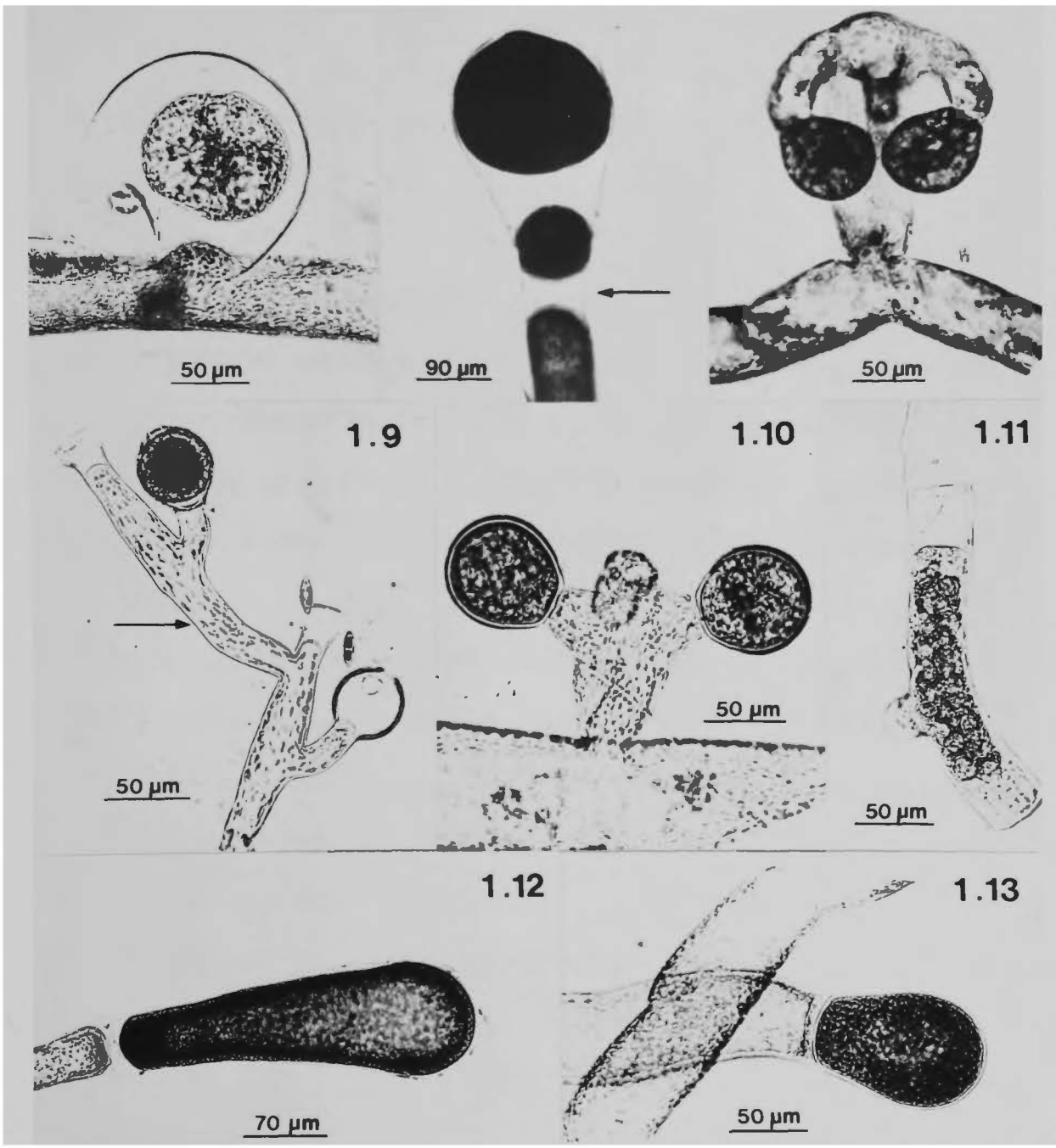
Fig. 1.10 V. gyrogyna Entwisle, showing oogonia in a plane transverse to the peduncle. MEL 1049132.

Fig. 1.11 V. erythrospora Christensen, showing -like fungal infection akinetes. MEL 1049409.

Fig. 1.12 V. bursata (O.F.Müller) C.Agardh, showing sporangium (probably zoosporangium). MEL 1049133.

Fig. 1.13. V. canalicularis (Linnæus) Christensen, showing aplanosporangium. MEL 1049134 p.p.

1.6 1.7 1.8



CHAPTER TWO

GROWTH AND REPRODUCTION OF *VAUCHERIA* (VAUCHERIACEAE, CHRYSOPHYTA) IN CULTURE

INTRODUCTION

Field material of *Vaucheria* (Vaucheriaceae, Chrysophyta) is often sterile, and hence unable to be identified. It is necessary, therefore, to induce reproduction by maintaining plants in culture. There have been few experimental studies, however, on the growth and reproduction of *Vaucheria* in culture. Åberg & Fries (1976) studied the effect of culture conditions on growth rates in plants of *V. dichotoma* (Linnæus) C. Agardh, and Klebs (1896), Andrews (1927), League & Greulach (1955) and Rieth (1959b) have studied the induction of gametangia and zoosporangia in plants referable to the *V. bursata* (O.F. Müller) C. Agardh complex (see Chapter 3). Thus as a prelude to the evaluation of taxonomic characters in culture (Chapters 3 & 4), the optimum conditions for vegetative growth and reproduction in isolates of three species have been determined, and the results are summarised in this chapter.

MATERIALS AND METHODS

Strains of *Vaucheria bursata* (MEL 1049235, Diamond Creek, Eltham, Victoria, *Entwisle*, 28.iv.1983), *V. nanandra* Christensen (MEL 1049044, Lake Corangamite, Victoria, *Entwisle*, 29.viii.1983), and *V. erythrospora*

Christensen (MEL 1049045, Lake Corangamite, Victoria, Entwisle, 29.viii.1983) were subjected to a range of treatments, involving temperature, light, salinity, nutrient and substrate-solidity gradients (Table 2.1).

The culture medium (Table 2.2) used was a salinity variant of ASP-V (see Åberg & Fries 1976). Stock solutions and the trace element mix were all autoclaved separately, while the vitamin mix was not autoclaved, but kept frozen until addition. The medium was solidified with 1% agar unless otherwise indicated. Vitamin B12 has been shown (Parker et al 1963, Åberg & Fries 1976, Mack & Turian 1975) to be essential, or at least stimulatory, for growth in most species of Vaucheria, while the other two vitamins, biotin and thiamine, apparently do not inhibit growth in the quantities used (Åberg & Fries 1976). All experimental isolates were unialgal but not axenic.

Most authors have found that gametangia are relatively easy to induce, generally by placing a crude culture (usually field material kept moist) near a window or under fluorescent lights (Ott & Hommersand 1974, Pecora 1976, Polderman 1974). The most widely used light:dark photoperiods have been 12:12 h (Gallagher & Humm 1981, Simons & Vroman 1973), 14:10 h (Pecora 1976, Simons & Vroman 1968), 16:8 h (Ott & Hommersand 1974, Polderman 1974) and 18:6 h (Åberg & Fries 1976, League & Greulach 1955). Although League & Greulach (1955) found that a photoperiod of 18:6 h induced greater gametangia production than 8:16 h, a wide range of photoperiods seem to have been used successfully. Cullinane (pers. comm.) has found freshwater species in Ireland, where the photoperiod varies widely among seasons, to be opportunists: plants appear after rains in any season of the year. A 14:10 h photoperiod was used in this study as it was closer to that

experienced in the field but with a long enough light period to allow more vegetative growth. Variation in the photoperiod was not tested.

Five replicates were inoculated for each treatment, and three were randomly chosen for analysis from those that survived (in V. erythrospora at temperature $10^{o}C$ and photon flux density 6-10 $\mu mol/m^2/s$, only 2 inocula survived). The initial inoculum was an approximately 1 cm length of vegetative siphon from a two week old culture (see Åberg & Fries 1976). Some variation within treatments may be due to the variat-ion in inoculum size (i.e. $\pm$ 0.25 cm). Eight regularly arranged, but randomly positioned, radii of the culture (Figs 2.1A,B) were measured (area of petri dish was 63.6 cm^2). The frequency of siphons was calculated by randomly locating a grid (in the eyepiece of a dissecting microscope with total magnification X 10) on four of the radii (Nos 1-4 in Fig. 2.1A), and recording the number of times at which a point on the grid hit a siphon (the grid had 121 points and covered 4.3 cm^2 of petri dish). A measure of the vegetative cover of a culture is $\pi r^2 f$ (where r is average radial extension and f the point quadrat frequency). This measure takes into account the density of siphons and the total extension of the culture, giving an approximation of the area covered by vegetative siphons. The number of antheridia (representing a group of gametangia) in 4.3 cm^2 was measured in grids randomly placed on eight radii. Only treatments with an average of > 1 antheridium per grid were plotted. The plotted data include the mean of the three treatment means, and the standard error of this mean (this does not represent the full range of values but allows some generalizations to be made). The Minitab statistical package (Ryan et al 1981) on the university VAX/VMS computer system was used to analyse some of the results.

RESULTS AND DISCUSSION

In Vaucheria bursata and V. nanandra, the average frequency of gametangial groups was weakly correlated with vegetative growth (corr. coeff. of means = 0.64 & 0.67 respectively), and there were fewer gametangia when growth was minimal or maximal. In V. erythrospora, however, vegetative growth was highly correlated with gametangial production (corr. coeff. of means = 0.96). In general, fertility was unpredictable and sometimes only one or two of the five replicates would produce gametangia. Under optimum conditions, however, gametangia usually developed within three weeks (see Figs 2.5A,B).

The photon flux density of 35-50 $\mu mol/m^2/s$ was more suitable than 6-10 or 12-20 $\mu mol/m^2/s$ for the production of gametangia in V. erythrospora (Fig. 2.5A) and V. nanandra (Fig. 2.5B), while 12-20 and 35-50 $\mu mol/m^2/s$ were more suitable in V. bursata (Fig. 2.2A). Higher photon flux densities enhanced vegetative growth in all isolates (Figs 2.2B,2.4A,B), although 110-130 $\mu mol/m^2/s$ resulted in a similar vegetative cover to 35-50 $\mu mol/m^2/s$ in V. bursata. Although photon flux densities approaching that of direct sunlight were not tested (15-75 $\mu mol/m^2/s$ have been reported in the literature as suitable for growing Vaucheria in culture), most species can tolerate full sunlight in the field and appear to be restricted by the moisture content of the substrate. Plants growing in cracks in mud are capable of spreading over the surface when it is moist. The reduced frequency of plants of V. bursata in summer, therefore, is probably more due to dehydrating effects rather than high photon flux density.

The strict winter seasonality of V. erythrospora and V. nanandra, however, may be more dependent on temperature than on photon flux

density. The isolates of V. nanandra and V. erythrospora had greater vegetative growth and reproduction at 10°C and 15°C than at 20°C (see Figs 2.4A,B,2.5A,B). These results correlated well with the field data; V. nanandra and V. erythrospora is found only in the colder months, and were nearly always fertile. The isolate of V. bursata in the current study generally grew faster at 15° and 20°C rather than at 10°C, but produced more gametangia at 10° and 15°C (see Fig. 2.2A,B). Vaucheria bursata was found year round but is often sterile in the field. In the literature, the most commonly used temperatures for inducing vegetative growth and reproduction in culture, have been 12-16°C. Gallagher & Humm (1981) found that in mats dominated by blue green algae (Cyanobacteria), Vaucheria grew and became fertile at 14-19°C, remained sterile at 23-24°C and did not grow at 27-33°C. Åberg & Fries (1976) found that plants of V. dichotoma grew vegetatively at 15-25°C, were killed at 30°C, and grew at minimal rates at 10°C. Patel & Francis (1968) obtained fertile material of V. longicaulis Hoppaugh from cultures grown at 24-28°C. In V. bursata [as V. sessilis (Vaucher) de Candolle], Klebs (1896) found that no growth occurred below 0-3°C or above 26°C (Åberg & Fries 1976). Vegetative growth rate in the isolate of V. bursata, from a freshwater habitat, seemed to be little affected by the salinity range used (Fig. 2.3B), but fertility was stimulated when the salinity was 0.3 o/oo compared with 2.0-6.5 o/oo (Fig. 2.3A). Simons (1974) found that sexuality in the freshwater and brackish water species, V. compacta (Collins) Collins, was stimulated when salinity was 0.5-10 o/oo (in a range of 0-25 o/oo) and the highest growth rates were measured in salinities of 0-17 o/oo.

Vegetative growth was also enhanced in the V. bursata isolate when

liquid culture media were used, and particularly when cultures were aerated. There is no qualitative data on these treatments as they could not be measured using the system devised for cultures on solidified media. The data for the 0.5% agar, when compared with 1% agar, show this trend (Fig. 2.3B) but are not conclusive. Generally, fewer gametangia were produced in liquid culture, and usually after a longer time.

In the current study (Figs 2.3A,B), reduction of the nitrogen and phosphorus concentration by 1/10 of that used in 'standard' ASP-V inhibited both vegetative growth and sexual reproduction in V. bursata. Nutrient levels appear to be important in controlling growth of Vaucheria in the field, and Hoffman et al (1974) showed that phosphorus was one of the most important. Hanatschek (1932) and Åberg & Fries (1976) found that low concentrations of nitrogen, and in the latter case also phosphorus, enhanced gametangia production while League & Greulach (1955) found that increased nutrient supplies promoted gametangia production.

In summary, it appears that a solidified medium of ASP-V with 'standard' nitrogen and phosphorus levels, a temperature of 15^{o}C and a photon flux density of 35-50 $\mu mol/m^2/s$, allows adequate vegetative growth and maximal reproduction in the isolates studied. For V. bursata, from a freshwater habitat, a salinity of 0.3 o/oo is favourable for both growth and reproduction. [Of the isolates maintained in culture for the monograph, V. uncinata Kützing (p.192) and V. aversa Hassall (p.198) would not grow 'normally' in salinities of 2 o/oo or greater.] Field collections, therefore, were grown under the above conditions with variations in salinity as needed. Additional media were found useful for maintaining some species and these are outlined in Chapter 5 (p.107).

Table 2.1 Culture treatments used to assess vegetative growth rates and induction of gametangia.

Table 2.1.

Isolates studied	Temperature (°C)	Photon flux density (μmol/m^2/s)	% Agar	Salinity (o/oo)	Proportion of (P)hosphate and (N)itrate [1]
all	10	6-10	1	6.5	1
all	15	6-10	1	6.5	1
all	20	6-10	1	6.5	1
all	10	12-20	1	6.5	1
all	15	12-20	1	6.5	1
all	20	12-20	1	6.5	1
all	10	35-50	1	6.5	1
all	15	35-50	1	6.5	1
all	20	35-50	1	6.5	1
V. bursata	15-20	110-130	1	6.5	1
V. bursata	15	30-40	1	0.3	1
V. bursata	15	30-40	1	2.0	1
V. bursata	15	30-40	1	4.0	1
V. bursata	15	30-40	1	6.5	1
V. bursata	15	30-40	1	0.3	1/10
V. bursata	15	30-40	1	0.3	1/2
V. bursata	15	30-40	0.5	6.5	1

[1] A proportion of 1 is equivalent to the amount of phosphate and nitrate given in Table 2.2.

Table 2.2 Composition of culture media, based on ASP-V (Åberg & Fries 1976, Fries 1963).

Table 2.2.

Salinity (o/oo)	0.3	2.0	4.0	6.5	16.0	30.0
pH [1]	6.4-7.0	8.5	8.5	8.5	8.5	8.5
NaCl (g)	0	0	2.6	4.5	14.4	29.0
$MgSO_4$ (g)	0.15	1.5	1.5	1.5	1.5	1.5
KCl (mg)	13	130	130	130	130	130
$CaCl_2.H_2O$ (mg)	10	100	100	100	100	100
Trizma HCl (mg)	19	190	190	190	190	190
$NaNO_3$ (mg)	90	90	90	90	90	90
Na_2-β-Glycero-PO_4(mg)	50	50	50	50	50	50
H_2O.distilled (l)	1	1	1	1	1	1

Vitamin Mix:		
	Cyanocobalamin	10 µg
	Biotin	5 µg
	Thiamine	200 µg
	H_2O.distilled	1 ml

Trace Element Mix:		
	Nitrotriacetic Acid	50 mg
	$FeCl_3.6H_2O$	1 mg
	$ZnSO_4.7H_2O$	250 µg
	$MnCl_2.4H_2O$	5 µg
	$CuCl_2.2H_2O$	10 µg
	H_3BO_3	1 mg
	$Na_2MoO_4.2H_2O$	250 µg
	H_2O.distilled	1 ml

[1] All pH values are approximate and taken from liquid culture.

Fig. 2.1 A. Arrangement of eight radii for measuring vegetative cover. Alternate radii (numbered 1-4) were used to measure siphon frequency.

B. Example of calculation of radii of culture. r = 'radius' of culture, v.s = vegetative siphon, p = perimeter of culture.

2.1

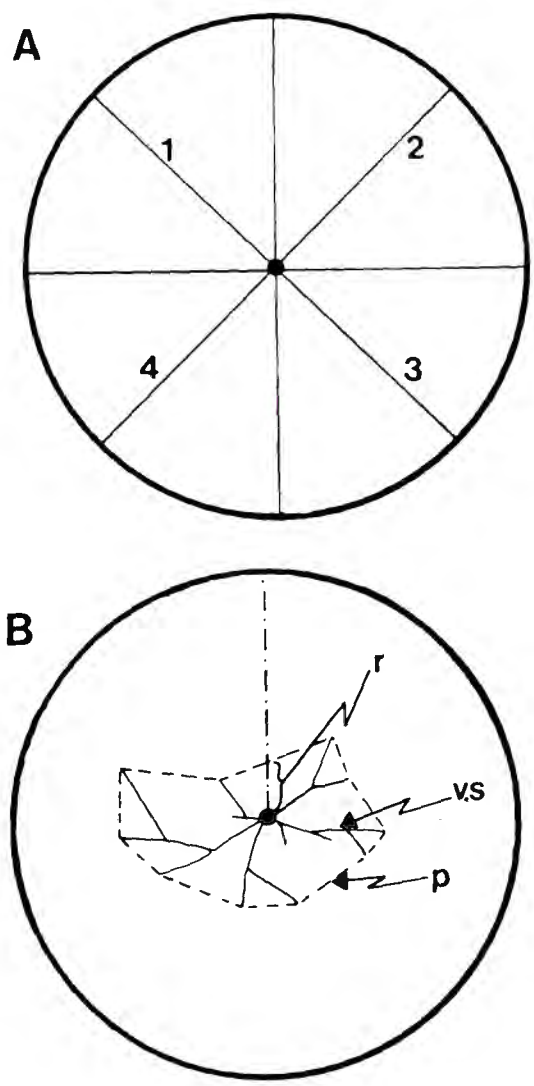


Fig. 2.2 Vaucheria bursata (O.F.Müller) C.Agardh: Gametangial groups per grid (A) and vegetative cover (B) vs temperature; photon flux densities 110-130 µmol/m²/s (◇), 35-50 µmol/m²/s (●), 12-20 µmol/m²/s (■) and 6-10 µmol/m²/s (◆).

2.2

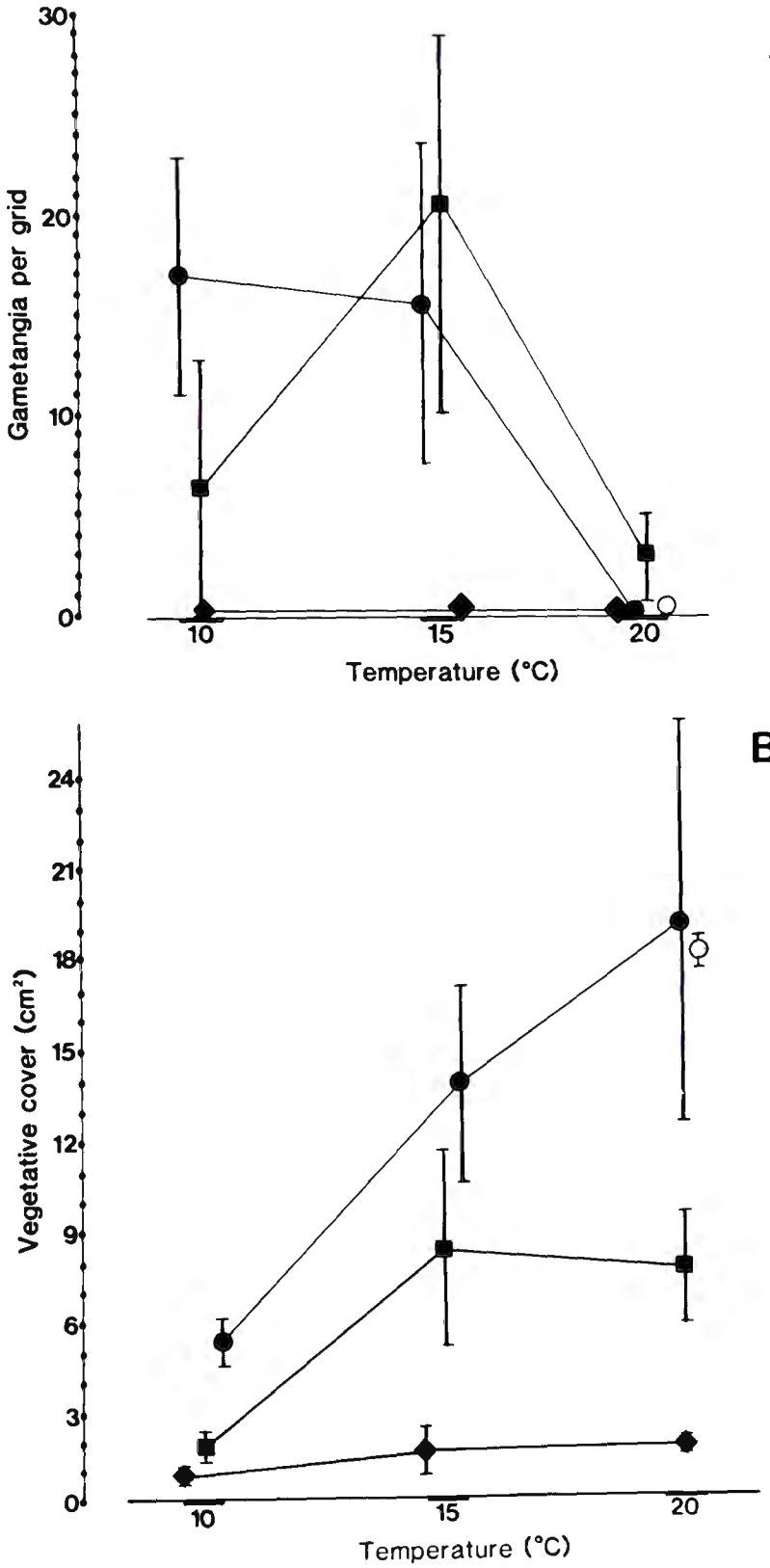

A
30
20
10
0
Gametangia per grid
10
15
20
Temperature (°C)
B
24
21
18
15
12
9
6
3
0
Vegetative cover (cm²)
10
15
20
Temperature (°C)

Fig. 2.3 Vaucheria bursata (O.F.Müller) C.Agardh: Gametangial groups per grid (A) and vegetative cover (B) vs phosphorus and nitrogen (PN; reduced from 0.3 o/oo medium in Table 2.1), salinity and 0.5% agar (others 1%).

2.3

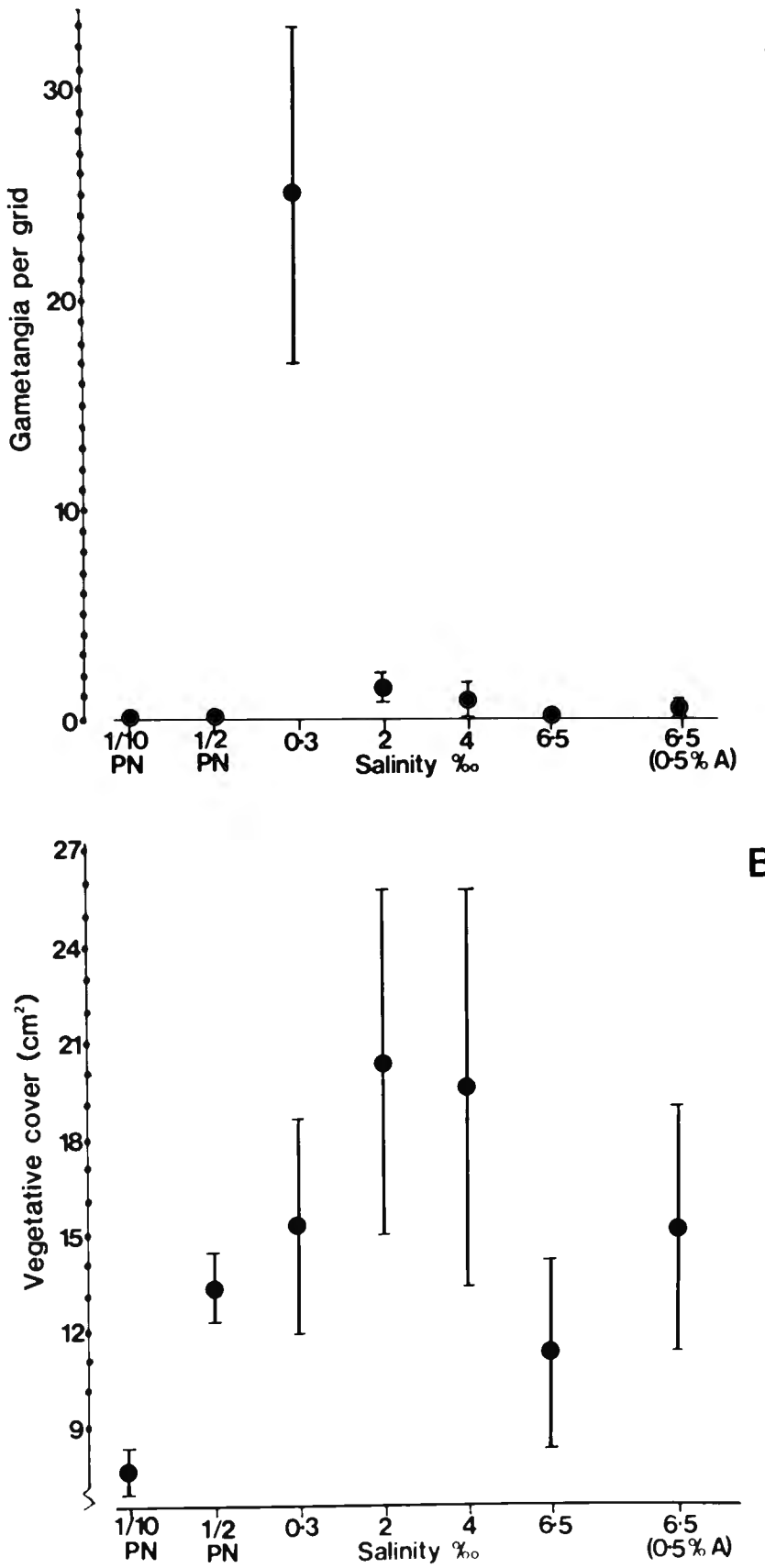

A
Gametangia per grid
30
20
10
0
1/10 PN
1/2 PN
0·3
2
4
6·5
6·5 (0·5% A)
Salinity ‰
B
Vegetative cover (cm²)
27
24
21
18
15
12
9
1/10 PN
1/2 PN
0·3
2
4
6·5
6·5 (0·5% A)
Salinity ‰

Fig. 2.4 Vaucheria nanandra Christensen (A) and V. erythrospora Christensen (B): Vegetative cover vs temperature (symbols for photon flux density variations as in Fig. 2.2).

2.4

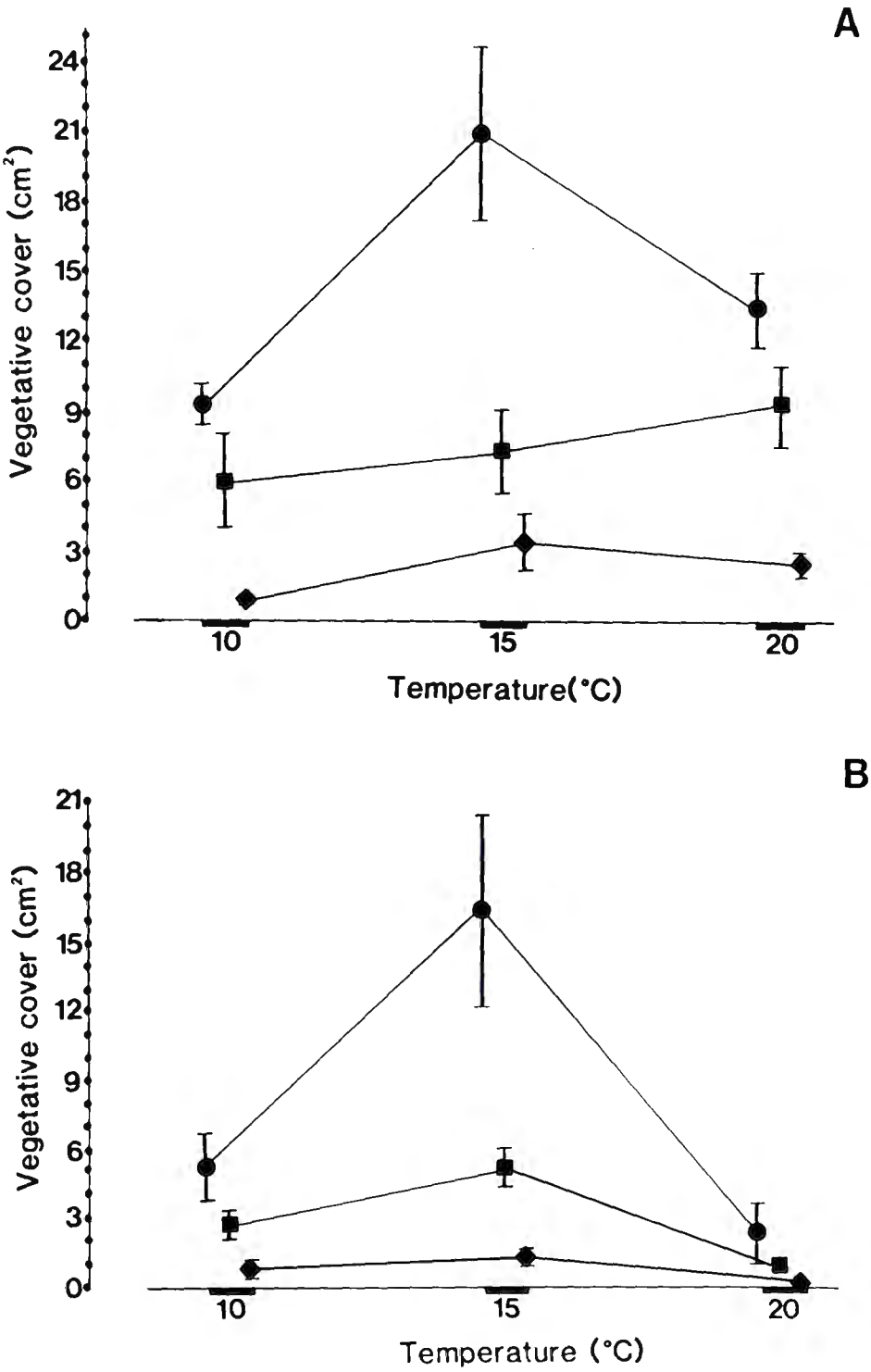
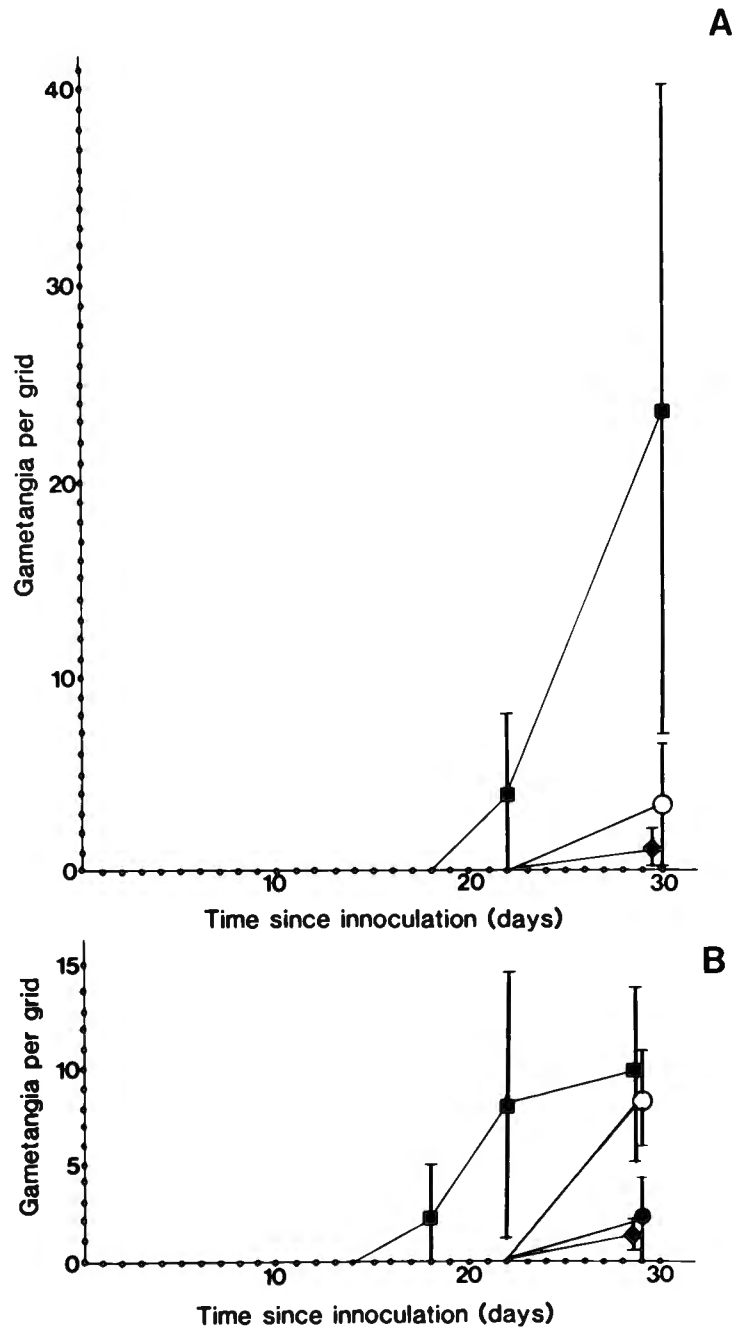

A
Vegetative cover (cm²)
24
21
18
15
12
9
6
3
0
10
15
20
Temperature(°C)
B
Vegetative cover (cm²)
21
18
15
12
9
6
3
0
10
15
20
Temperature (°C)

Fig. 2.5 Vaucheria erythrospora Christensen (A) and V. nanandra Christensen (B): Gametangial groups per grid vs time since inoculation. Culture conditions which induced gametangia in 30 days were: 15°C, 35-50 μmol/m^2/s (■); 15°C, 12-20 μmol/m^2/s (◆), 10°C, 35-50 μmol/m^2/s (○); and 10°C, 12-20 μmol/m^2/s (●).

2.5


A
40
30
20
10
0
Gametangia per grid
10
20
30
Time since innoculation (days)
B
15
10
5
0
Gametangia per grid
10
20
30
Time since innoculation (days)

CHAPTER THREE

AN EVALUATION OF TAXONOMIC CHARACTERS IN THE *VAUCHERIA BURSATA* AND *V. DILLWYNII* COMPLEXES (VAUCHERIACEAE, CHRYSOPHYTA)

INTRODUCTION

The section Corniculatae (Walz) Heering emend. Entwisle of *Vaucheria* (Vaucheriaceae, Chrysophyta), as defined in Chapter 5 (p.125), includes those species with pedicellate antheridia which are not subtended by a wall-bound cavity and are not borne on a gametophore. At least 18 taxa have been referred previously to Corniculatae [as section Corniculatae subsection Sessiles (Walz) Heering] and these have been delineated from one another on differences in antheridial shape, oogonial morphology, oospore wall thickness, oogonial wall texture and the arrangement of gametangia. The stability of these characters, however, has not been evaluated critically in culture, and only limited data are available on variability in field populations (e.g. Rieth 1963b).

It is apparent from the literature (see Rieth 1963b, Christensen 1969, Blum 1972) that some species in this section are poorly defined and that further studies are needed to clarify species concepts. Thus, for example, the number and rank of taxa in the *V. bursata* complex [i.e., *V. bursata* (O.F.Müller) C.Agardh, *V. sessilis* (Vaucher) de Candolle, *V. clavata* sensu Klebs and *V. repens* Hassall] differ among authors and have resulted in three major interpretations: 1) three

species are recognised, primarily on the basis of oogonial orientation and arrangement (Götz 1897, Blum 1972); 2) one species is recognised, but with forms or varieties distinguishable in most collections (Heering 1907, 1921; Hoppaugh 1930; Gauthier-Lièvre 1955; Starmach 1972); or 3) one species is recognised, with no subspecific taxa (Rieth 1963b, Christensen 1969, Islam 1984). The delineation of species in the V. dillwynii complex [i.e., V. dillwynii (Weber & Mohr) C. Agardh and V. borealis Hirn] also varies among authors. Heering (1921), Hoppaugh (1930) and Rieth (1962, 1980b) recognise two distinct species based on differences in antheridial shape and oogonial wall texture, while Blum (1972) recognises only one species. Furthermore, the differences between these two complexes are not well documented in the literature. In south-eastern Australia, plants referable to V. bursata, V. sessilis, V. clavata, V. repens and V. dillwynii have been found. Table 3.1 contains a summary of the currently used diagnostic characters for the species assigned to these complexes.

The aims of this study have been to evaluate the potential variability in taxonomic characters within and between the V. bursata and V. dillwynii complexes and to then reassess species concepts in these complexes.

MATERIALS AND METHODS

PLANTS STUDIED: Four south-eastern Australian isolates were chosen for culture studies (Table 3.2): culture isolate 644 was referable to Vaucheria dillwynii sensu Rieth [1980b, as V. pachyderma Walz; see Christensen (1973) for details of this synonymy], while the other three, isolates 211, 325, and 489, were representative of the range of

plants usually referred to the V. bursata complex. The isolates referable to the V. bursata complex could not be identified unequivocally to species using Blum (1972), as many of the characters used were too variable. On the basis of oogonial orientation, however, isolate 325 was similar to both V. sessilis and V. clavata, and isolates 211 and 489, to V. repens. The plants studied, therefore, are referred to by isolate numbers as names cannot be applied correctly.

Twenty four field populations (Table 3.2) were chosen to include a range of morphological forms and habitats; although termed 'field populations', some were cultured under standard culture conditions (1% agar, 0.3 o/oo salinity, 35-50 $\mu mol/m^2/s$, 15^oC) to induce fertility. Collections 1-21 were referable to the V. bursata complex and collections 22-24, to the V. dillwynii complex. The two collections of V. borealis from Sweden were kindly supplied by Dr Tyge Christensen.

CULTURE CONDITIONS: The culture medium was a salinity variant of ASP-V as outlined in Chapter 2 (see p.11 and Table 2.2). The culture conditions for each treatment are described in Table 3.3 and correspond to the following gradients occurring in the field: salinity, nutrient concentration, solidity of substrate (liquid to solid), light and temperature. The ranges chosen for these variables were similar to those likely to occur in habitats where the algae were collected. The day:night cycle was 14:10 h for all cultures. The light and temperature regimes were achieved with a cross gradient table, and aeration was through a laboratory air supply passing first through a flask of water to remove oil. The lights were cool white fluorescent tubes.

PRESERVATION AND OBSERVATION: Field and culture material was preserved in FAA (100 ml of commercial formalin, 50 ml of glacial acetic acid, 500 ml of 95% ethanol, 350 ml of distilled water), and a portion of each field collection was dried on cellophane. Voucher specimens and microscope slides have been deposited in MEL and to conform with the standard herbarium practice there, a single herbarium number has been affixed to each collection whether it includes one or more species. Plants cited in the text which are part of a mixed herbarium collection have 'p.p.' (pro parte) following the herbarium number.

For light microscopy, FAA preserved material was stained for 30-60 s in 2% aqueous $KMnO_4$ and mounted in 10% "Karo" corn syrup (with 0.25% phenol). Following dehydration of the mounting media, 40% corn syrup (with 1% phenol) was added. Living culture material was photographed *in situ* by placing the inverted petri dish on the microscope stage and photographing the gametangia embedded in agar.

For scanning electron microscopy, liquid preserved material, usually still attached to agar, was either i) dehydrated through 50, 60, 70, 80, 90% ethanol at 30 min intervals, then placed into 100% acetone, or ii) left for 10 h in an acetone desiccator (see Sitte 1962). There seemed to be little difference between the results of these two methods but the latter was simpler. The material was then critically point dried (emersed in CO_2 for 2 h), mounted with "DAG" colloidal carbon (Acheson) and coated with gold prior to viewing in a Siemans autoscan scanning electron microscope.

MEASUREMENTS: The following characters were assessed in culture isolates and field populations: siphon diameter; number of oogonia per

gametangial group; length, diameter, L/D, shape, and orientation of oogonia; oospore wall thickness and texture in transverse section; oogonial wall texture; size and shape of antheridia; antheridial pedicel length; pigment spots in oospores; and zoosporangia production.

Measurements of morphological characters (for definitions of the measured characters see p.3) were made using a calibrated eyepiece micrometer. The angles for oogonial orientation were measured by focusing one eye on a protractor, and the other on the slide material under the microscope.

Where possible, three slides were made from material chosen randomly from each petri dish, and at least ten random measurements made of each character. Three to five replicates of each isolate were placed under each treatment, but not all were measured. Data from isolates studied in culture were plotted only if $>$ 5 values were measured, while all field data were plotted. If $>$ 1 replicate was measured under a treatment, each was plotted independently. The values plotted (mean and standard deviation) are a conservative estimate of the population or sample range (about 66% of values) but provide a useful comparison between isolates and treatments.

Data were analyzed using the Minitab statistical package (Ryan *et al* 1981) on the university VAX/VMS computer system, and standard calculator statistics programs. The means of the treatments for each isolate were normally distributed, as was confirmed by finding a high correlation (correlation co-efficient $>$ 0.95) between the 'normal scores' and the data (Ryan *et al* 1981). All correlation co-efficients were calculated from the means of each treatment and not from all measured values.

In the current study, the range of values was of more importance than the means - it is the overlap of ranges that determines a good or bad character - and two sample T tests on means for most characters indicated a significant difference ($p < 0.05$) among isolates. For this reason, statistical analysis was seldom used while plots of the data clearly illustrated the useful taxonomic characters. Numerical characters were evaluated firstly, on the basis of the plotted data (mean and standard deviation), and then, if disjunctions occurred, on the full range of values measured.

RESULTS AND DISCUSSION

The *Vaucheria bursata* Complex.

SIPHON DIAMETER: The diameter of vegetative siphons has been used by Blum (1972) and Götz (1897) to delineate taxa in the *V. bursata* complex. *Vaucheria repens*, according to Blum (1972), is distinguished from *V. sessilis* and *V. clavata* in having siphons, at least in part, < 32 µm in diameter. Götz (1897) characterised *V. repens* as always having siphons < 49.5 µm in diameter.

In the current study, however, the plotted data for siphon diameter ranged from 29-95 µm (see Fig. 3.1), with no disjunctions either under the range of culture conditions tested or among field populations. All siphons in isolates 211 and 489 were > 32 µm. Isolate 325 had siphons, at least in part, < 32 µm, but had oogonia similar in morphology to those of *V. clavata* and *V. sessilis*, rather than *V. repens*. Moreover, based on other published reports (see Table 3.4), siphon diameter in *V. repens* appears to vary considerably.

Consequently, it appears that siphon diameter is an unreliable taxonomic character for separating species within the V. bursata complex.

NUMBER OF OOGONIA PER GAMETANGIAL GROUP: In the section Corniculatae, gametangia are arranged such that one antheridium occurs either adjacent to one oogonium or between two oogonia. (In Vaucheria arcassonensis, not considered in this study, more than one antheridium occasionally may occur with one or two oogonia.) Within the V. bursata complex, the number of oogonia per gametangial group has been used to delineate taxa. In V. repens, according to Blum (1972) and Hoppaugh (1930), gametangial groups usually contain one oogonium, while in V. clavata and V. sessilis, two oogonia are usually present.

In south-eastern Australian plants, the proportion of gametangial groups with two oogonia varied considerably under the range of culture conditions tested (Fig. 3.2). Under some conditions, particularly 0.1% agar media with low temperatures ($5^{o}C$) or high photon flux density (35-50 $\mu mol/m^2/s$), > 50% of gametangial groups in isolate 325 contained two oogonia, but in other treatments, such as liquid and 0.5% agar media, there were < 20%. Isolate 489 only once ($5^{o}C$ and 35-50 $\mu mol/m^2/s$) had > 50% of gametangial groups with two oogonia, and generally, there were only 0-30%. In all treatments, isolate 211 had 25% of gametangial groups with two oogonia. Thus in all isolates, one or two oogonia per gametangial group occurred, and the proportion of each was influenced by environmental conditions. In addition, although field populations of the V. bursata complex had predominantly single oogonia (Fig. 3.2), some had gametangia orientated similarly to those of V. clavata and V. sessilis. Rieth (1963b) also found that the proportion of gametangial groups with two oogonia, in field populations of the V. bursata

complex, was variable. The number of oogonia included in gametangial groups, therefore, appears to be a poor character for delineating species in the V. bursata complex.

ORIENTATION OF OOGONIA: Most authors (including Heering 1921, Götz 1897, Blum 1972) consider the orientation of oogonia to be of prime importance in delineating taxa in the Vaucheria bursata complex. There are two quantitative aspects to this orientation (see Fig. 1.1): the angle of the oogonial long-axis relative to the siphon and the angle of the oogonial pore relative to the siphon. Blum (1972), for example, described V. clavata as having mostly erect oogonia with a perpendicular pore (i.e. both angles close to 90^{o}), V. repens as having oogonial pores directed horizontally or towards the siphon (i.e. pore angle $< 0^{o}$), and V. sessilis as having oogonial pores forming an acute angle with the siphon (i.e. $0^{o}<$ pore angle 90^{o}). The orientation of oogonia was considered by Heering (1907) to be variable, but still important at subspecific level, and by Rieth (1963b) and Christensen (1969), to be of no use in distinguishing taxa in the V. bursata complex.

The symmetry of the oogonia can be quantified by calculating the difference between the long-axis and pore angle. If the angles are nearly the same, the oogonia are radially symmetrical, and if they differ, the oogonia are bilaterally symmetrical. Blum (1972) has distinguished V. clavata from V. sessilis and V. repens in having radially, rather than bilaterally, symmetrical oogonia.

In the current study, there were some discontinuities in the plotted data for oogonial long-axis angle (Fig. 3.3) in the isolates studied in culture, but none in the data for pore angle (Fig. 3.4) and oogonial symmetry.

The long-axis angle of the oogonium in isolate 489 was generally 30^{o}, and in isolate 211, -6^{o} to 54^{o}. In both isolates, the oogonial pore was usually parallel with, or directed towards, the siphon, but the pore angle ranged from -89^{o} to 34^{o}. Measurements taken from published illustrations of V. repens (see Table 3.5) include values similar to those measured in isolates 211 and 489. Few published illustrations, however, included oogonia with pores as strongly directed towards the siphon as some (e.g. Figs 3.9,3.10) in the south-eastern Australian isolates.

In isolate 325, the long-axes of the oogonium were more erect, 30^{o}, and the pore usually parallel with, or directed away from, the siphon (see Fig. 3.6). Only in aerated liquid cultures (treatment 16) was the pore angle $< -15^{o}$. These cultures often had pedicellate oogonia (Fig. 3.8), sometimes arising from the antheridial pedicels (Fig. 3.5). Cultures in agar topped with liquid (treatment 17), and non-aerated liquid cultures, also had pedicellate oogonia, but the distal cavity was not directed towards the siphon. On the basis of oogonial orientation, isolate 325 was referable to V. sessilis or V. clavata. In published illustrations of V. clavata (see Table 3.5), the long-axis angle is close to 90^{o}. While the plotted data from the current study (mean $\pm$ s.d.) includes no values $> 70^{o}$, some oogonia did have both a long axis and pore angle of 90^{o}. Furthermore, Blum (1972, p.15) noted that the oogonial pore in V. clavata was "...occasionally oblique as in typical V. sessilis;..."

Two growth forms, therefore, were evident among the isolates studied in culture, and among some of the field populations. Plants similar to isolate 325 (Figs 3.5-3.8), usually with the oogonial pore directed away from the siphon, are referable to V. clavata or V.

sessilis, and plants similar to isolate 489 (Figs 3.9,3.10), usually with the pore directed towards the siphon, are referable to V. repens. From the plotted data, isolate 489 can be distinguished from isolate 325 in generally having oogonia with long-axis angles > 30^{o}. The full range of values, however (plotted data includes only mean $\pm$ s.d.), was 30^{o} to 90^{o} in isolate 325, and -10^{o} to 40^{o} in isolate 489. Furthermore, the range of values for long-axis and pore angles in isolate 211 (see also Figs 3.11,3.12), and some field populations, overlapped these two ranges. Therefore, although isolates 325 and 489 are usually distinct, oogonial orientation seems too variable to be a good taxonomic character in the V. bursata complex.

The symmetry of oogonia could not be used to distinguish any isolate studied in culture, or in any field population. Some oogonia observed in isolate 325 were almost radially symmetrical (Fig. 3.7), while others were bilaterally symmetrical (Fig. 3.6): values for 'long-axis angle minus pore angle' ranged from $0-45^{o}$ (to 70^{o} in aerated liquid cultures). In isolates 211 and 489, all oogonia were bilaterally symmetrical (Figs 3.9-3.12), and the 'long-axis minus pore angle' ranged from $25-70^{o}$ and $30-80^{o}$ respectively. Oogonial symmetry, therefore, is considered to be a poor diagnostic character in the V. bursata complex.

These conclusions are supported by the observations of Teodoresco (1907) and Randhawa (1942a), who described populations allied to V. sessilis which included oogonia which were erect or oblique, and radially or bilaterally symmetrical, on the one siphon.

SIZE OF OOGONIA: The length and diameter of oogonia have not been used previously to distinguish taxa in the Vaucheria bursata complex, but Blum (1972) distinguished V. clavata from V. repens and V.

sessilis in having oogonial L/D > 1.5.

There were no disjunctions in the data for oogonial length (Fig. 3.17), diameter (Fig. 3.18) or L/D (Fig. 3.19) from south-eastern Australian plants studied in culture or from field populations. The plotted data (mean ± s.d.) for the diameter of oogonia in isolates 325 and 489 included little overlap, but the full range of values for isolate 489 extended to 67 μm. Furthermore, there is a considerable overlap in the values for oogonial diameter in the published literature (Table 3.6).

Hussain & Faridi (1977) found that a continuous light period reduced oogonial length, but not diameter, in V. sessilis. According to Hussain & Faridi (1977), oogonia were 84-100 μm long in plants subjected to a diurnal photoperiod, and 62-77 μm in continuous light. Growth rates were measured in isolate 211 (see Chapter 2), and the oogonial length was weakly, but positively, correlated with vegetative growth (corr. coeff. of means = 0.68 and 0.54 with agar/salinity and temperature/light gradients respectively), indicating that increased vegetative growth at least does not reduce oogonial length. The diameter of oogonia was also positively correlated with vegetative growth (corr. coeff. of means = 0.68 and 0.95, as above). The smallest oogonia, in both dimensions, were found in cultures subjected to the lower photon flux density of 6-10 $\mu mol/m^2/s$. These results suggest that the continuous light regime studied by Hussain & Faridi (1977) did not decrease the oogonial length by increasing vegetative growth rate.

In some treatments, e.g. low and high photon flux density and temperature, nearly all the oogonia of 325 had L/D > 1.5. There was no correlation, however, between the production of erect oogonia and of oogonia with L/D > 1.5 (corr. coeff. of means = 0.37), two characters

used by Blum (1972) to distinguish V. clavata from V. repens and V. sessilis. Teodoresco (1907) has described a population intermediate between V. sessilis and V. clavata in oogonial orientation with oogonial L/D about 1.5. The length, diameter and L/D of oogonia, therefore, seem to be unreliable taxonomic characters for delineating species in the V. bursata complex.

OOSPORE WALLS: The number of layers in the oospore wall has been used to delineate species in the Vaucheria bursata complex. Reinsch (1887) distinguished V. orthocarpa from V. sessilis in having erect oogonia and a seven, rather than three, layered oospore wall. Hoppaugh (1930) considered all subsequent literature records of V. orthocarpa to be misidentified V. clavata, since the plants described all had only 3 layered oospore walls.

It was difficult to distinguish between layers in the oospore walls examined in this study, and the number of wall layers observed probably depends on the optical system used. The oospore walls of V. dillwynii, described by Walz (1866) and Hoppaugh (1930) as seven layered, were relatively thick and had a 'flaky' texture when viewed in transverse section under light microscopy (Fig. 3.30). Therefore, oospore wall thickness and texture were used to test this character in the V. bursata complex.

The oospore walls in the isolates studied in culture, and in the field populations, were evenly textured in transverse section (Fig. 3.5-3.12) and 4-7 μm thick, with no apparent difference between treatments. The oospore walls of V. orthocarpa were described by Reinsch (1887) as 6-8 μm thick, only a little above the range of values measured in this study. Oospore wall texture (or number of layers) and

thickness were of no taxonomic value in south-eastern Australian representatives of the V. bursata complex, but no plants with erect oogonia and 'flaky' textured walls were examined. Therefore, the value of oospore wall texture, or number of oospore wall layers, as taxonomic characters in the V. bursata complex, remains uncertain.

CURVATURE OF ANTHERIDIA: The antheridia of species in the Vaucheria bursata complex are cylindrical and circinate. Although the curvature of the antheridium has not been used as a taxonomic character in the V. bursata complex, it was measured for comparison with V. nanandra Christensen (p.142), which has antheridia 35-60 µm long which turn through over a full circle. Curvature was measured using antheridial length as defined on p.3 and the angle through which the antheridia turned (Fig. 1.1).

Neither of these features appeared to differ either in the isolates studied in culture, or among the field populations. The length of antheridia in all four isolates and the field populations ranged from 36-89 µm and the angle through which the antheridia turned ranged from 90-320^{o}. No antheridia were seen which turned through a full circle (> 360^{o}), so the curvature of antheridia appears to be useful for distinguishing V. nanandra from the V. bursata complex.

ANTHERIDIAL PEDICELS: Although the length and curvature of the antheridial pedicel have not been used previously as taxonomic characters in the Vaucheria bursata complex, they have been assessed in the current study. If the antheridial pedicel was as long as the entire antheridial system (= antheridia + antheridial pedicel) then the pedicel was curved distally (see Fig. 1.1).

In the current study, there was a continuum in the data for antheridial pedicel length, both in isolates studied in culture, and in field populations (Fig. 3.20). Isolate 489 usually had very short pedicels (Figs 3.9,3.10), similar but broader than those of V. nanandra, but the total length of the antheridial system, up to 77 µm, was similar to that measured in the other isolates. It appeared that long antheridial pedicels were commonly associated with erect oogonia (such as in isolate 325), and vice versa (such as in isolate 489), but the correlation coefficient on the pooled means of all isolates was only 0.56, indicating only a weak correlation between the two features. Antheridial length, therefore, seems to be of no taxonomic value in the V. bursata complex.

Although the section Corniculatae has been defined as having curved antheridial pedicels (Walz 1866, Blum 1972; both as Corniculatae subsection Sessiles), antheridial pedicels measured in this study were frequently straight both in field populations and in isolates 211 and 489. The antheridial system, however, was always distally curved, and the septa separating the antheridium from the pedicel could be proximal or distal to the curvature. The presence of a circinate antheridial system, therefore, is characteristic of the section Corniculatae.

PIGMENT SPOTS IN OOSPORES: Walz (1866) characterised the sections Corniculatae and Racemosae (Walz) Entwisle (as 'subgroups' Sessiles and Racemosae of the 'group' Corniculatae) by the presence of a brown pigment spot in the mature oospore. Götz (1897) found that the oospores of Vaucheria repens contained one to several sepia-brown pigment spots; V. sessilis, one to several red-brown spots; and V. clavata, rarely more than one bright red spot. There has been no

subsequent confirmation of these observations (Rieth 1963b).

While all species in the section Corniculatae have a pigment spot in the oospore, so too do V. aversa Hassall (see Walz 1866) and V. bicornigera Entwisle, both in the section Tubuligerae (Walz) Heering emend. Entwisle, and all species in the sections Vaucheria [syn. Anomalae (Hansgirg) Heering] and Racemosae. Furthermore, the pigment spot was red-brown and blotchy in all plants referable to the V. bursata complex, and thus appeared to be of no use as a taxonomic character.

ZOOSPORANGIA: The quantity, and duration of production, of zoosporangia in standing water, was used by Götz (1897) to distinguish species; thus, according to Götz (1897) few sporangia are produced in V. sessilis, many are produced in V. repens, and many zoosporangia are produced for a month following inoculation, in V. clavata.

This attribute is considered to be of little practical value as a taxonomic character, and in the current study, sporangia were often produced by isolates 489, 325 and 211, in both solid and liquid media. The abundance of sporangia in isolate 489 was almost inversely proportional to the production of gametangia. In agar, the sporangia usually germinated in situ but were similar in shape to the zoosporangia illustrated and described for V. bursata (Christensen 1969). Zoosporangia appeared to be morphologically similar in all species examined.

CONCLUSIONS: None of the characters considered could be used to adequately separate either the three isolates studied in culture, or the field populations from one another. Although isolate 325 can be generally distinguished from isolate 489 in having oogonia < 72 µm in diameter and

with oogonial pore angle $> 0^{o}$ and oogonial long-axis angle $> 30^{o}$, the distinctions are not clearcut. Furthermore, isolate 211 and many field populations cannot be distinguished from either isolate 325 or 489 on the basis of the characters studied. Therefore, all plants from south-eastern Australia referable to the Vaucheria bursata complex are included in a single species, for which V. bursata is the earliest available name (see Christensen 1969, 1973).

The Vaucheria dillwynii Complex

ORIENTATION OF OOGONIA: In Vaucheria dillwynii and V. borealis, according to Hoppaugh (1930, former species as V. pachyderma), the oogonial long-axis is parallel with the siphon (long-axis angle ca 0^{o}), while the pore is directed towards the siphon (pore angle $< 0^{o}$) in V. dillwynii, but is parallel to the siphon in V. borealis.

In the current study, the long-axis angle (Fig. 3.21) was always close to 0^{o} (Figs 3.13,3.33) in both V. dillwynii and V. borealis. Although the oogonial pore in V. dillwynii was directed strongly towards the siphon and in V. borealis, generally less reflexed from the long axis, there was no disjuction in the data (Fig. 3.23). Furthermore, Rieth (1980b) illustrated V. borealis with most oogonia having the pore directed towards the siphon (but not strongly reflexed from the long- axis). The orientation of oogonia, therefore, does not appear to be a useful taxonomic character for delineating species in the V. dillwynii complex.

SIZE OF OOGONIA: The oogonia of Vaucheria borealis have been described by some authors (Hoppaugh 1930, Rieth 1980b) as being generally larger than those of V. dillwynii (as V. pachyderma).

In the current study, there was no disjunction in the data for oogonial length (Fig. 3.22), diameter (Fig. 3.24) or L/D (Fig. 25). Although in the plotted data (mean ± s.d.) the oogonia of V. borealis were always > 145 µm long, and those of V. dillwynii always < 145 µm long, in the actual data there was some overlap. Thus V. borealis had oogonia 137-187 µm long, and V. dillwynii 101-150 µm long. In addition, there is considerable overlap in the values recorded for oogonial length and diameter in the literature (Table 3.7). These features, therefore, seem to be poor characters for distinguishing species in the V. dillwynii complex.

OOGONIAL SHAPE: Walz (1866) described the oogonia of Vaucheria dillwynii (as V. pachyderma) as spherical or ellipsoid, while the oogonia of V. borealis were originally described (Hirn 1900) as oblique-ovoid.

The oogonia and oospores of V. dillwynii observed in the current study (Figs 3.13,3.15), were truncate-napiform (or 'door-knob' shaped), while those of V. borealis were usually ovoid to ovoid- reniform (Fig. 3.33). Vaucheria borealis sometimes had almost ellipsoid oogonia, but never constricted to a relatively narrow attachment area like those of V. dillwynii. Oogonial shape, therefore, is a useful character for distinguishing V. dillwynii from V. borealis.

OOSPORE AND OOGONIAL WALLS: Hoppaugh (1930) described the oospore walls of Vaucheria dillwynii (as V. pachyderma) as seven layered, and those of V. borealis as only three layered. Vaucheria dillwynii is also distinguished from V. borealis in having a rugose, rather than smooth, patterning on the oogonial wall (Heering 1907; Rieth 1962, 1980b; Sarma & Chapman 1975a; former species as V. pachyderma).

The oospore walls in plants of V. dillwynii seen in the current study, often appeared 'flaky' in transverse section (Fig. 3.30), similar to those of V. frigida (Roth) C. Agardh (see Chapter 4). This flakiness, which probably corresponds to the additional layers noted by Walz (1866) and Hoppaugh (1930), was not observed in V. borealis. The structure of the oospore wall in transverse section, therefore, provides a useful feature for characterising V. dillwynii.

In all isolates studied in culture, and all field populations, the oospore walls of V. dillwynii were 8-14 µm thick and those of V. borealis, 5-7.5 µm thick. The disjunction in the data between the two species is negligible, and future studies may show some overlap in the size ranges. Oospore wall thickness, therefore, is considered to be of little use in distinguishing species in the V. dillwynii complex.

The rugose texture of oogonial walls in V. dillwynii was evident with both scanning electron (Fig. 3.37) and light (Fig. 3.31) microscopy. The oogonial walls of V. borealis had a smoother texture under scanning electron microscopy (Fig. 3.38), and no obvious 'pits' under light microscopy. These results are confirmed by Sarma & Chapman (1975a) and Rieth (1962), and oogonial wall texture appears to be useful in characterising V. dillwynii.

SIZE AND SHAPE OF ANTHERIDIA: Vaucheria dillwynii has been distinguished from V. borealis in having saccate rather than cylindrical antheridia (Hoppaugh 1930; Rieth 1962, 1980b; former species as V. pachyderma). Blum (1972), however, could not identify herbarium material on the basis of antheridial shape.

The distal and proximal diameter of antheridia (before dehiscence, but after a septum formed to separate pedicel from antheridium) were

measured here to evaluate antheridial shape as a taxonomic character. The variation in antheridial diameter was difficult to quantify, due to the variability of antheridial orientation and the difficulty in assessing similar stages of development.

There was insufficient data for any comparisons to be made between treatments, but the antheridia of V. borealis were 28-38 µm in diameter, both distally and proximally, and those of V. dillwynii were 24-34 µm in diameter, both distally and proximally. In V. dillwynii, the distal diameter was similar to the proximal diameter before dehiscence, and the distal end did not appear to be inflated (Fig. 3.16). Occasionally, 'antheridial pedicels' with swollen ends were observed in V. dillwynii (Fig. 3.28). These did not seem to produce antheridia or sperm, but to be abnormal formations produced when plants were stressed. The antheridia of V. dillwynii usually flared distally after dehiscence (Fig. 3.15), similar to those described in the literature. Specimens examined in situ (embedded in agar rather than in a permanent slide mount), however, remained almost cylindrical throughout (Figs 3.14,3.29). The agar may have restricted the normal opening mechanism of the antheridia, or fixation and slide preparation may normally disrupt the antheridia. The antheridia of V. dillwynii, therefore, can be described as essentially circinate-cylindrical, but usually disintegrating distally upon dehiscence.

Most antheridia in the plants of V. borealis observed, were disrupted (Fig. 3.33), presumably becoming inflated at maturity like those of V. dillwynii. Rieth (1962, 1980b), however, found that the antheridia of V. borealis were cylindrical throughout their development. Further studies are required on living plants to fully assess antheridial shape as a taxonomic character in V. borealis.

SIPHON DIAMETER: The diameter of siphons has not been used previously to delineate species in the Vaucheria dillwynii complex, and in the current study siphon diameter ranged from 36-91 μm, with no disjunctions among treatments or in the field populations studied (Fig. 3.27).

NUMBER OF OOGONIA PER GAMETANGIAL GROUP: Since all gametangial groups in plants of Vaucheria dillwynii and V. borealis contained one oogonium (see also Christensen 1969, Rieth 1980b), the number of oogonia per gametangial group could not be used to distinguish species in the V. dillwynii complex.

ANTHERIDIAL PEDICELS: Features of the antheridial pedicel have not been used previously to delineate species in the Vaucheria dillwynii complex, but two aspects of antheridial pedicel length were measured here (see p.35 for rationale behind these two measurements). There were, however, no disjunctions in the data for antheridial pedicel length or the length of the entire antheridial system (Fig. 3.26), so neither seems to be a good taxonomic character for distinguishing V. borealis or V. dillwynii.

CONCLUSIONS: The orientation and size of oogonia, antheridial shape, siphon diameter, antheridial pedicel and system length, oospore wall thickness, and the number of oogonia per gametangial group were found to be of little value in distinguishing Vaucheria dillwynii and V. borealis. However, the structure of oospore walls in transverse section, oogonial wall texture, and the shape of oogonia were found to be useful taxonomic characters.

The two species studied, therefore, are considered to be distinct, although V. borealis needs be examined in culture before it can be adequately delineated. From the results of the current study, V. borealis is distinguished from V. dillwynii in having oospore walls evenly textured in transverse section, smooth textured oogonial walls, and ovoid to ovoid-reniform oogonia. Vaucheria dillwynii has oospore walls 'flaky' in transverse section, rugose oogonial walls and truncate-napiform oogonia.

Comparison of V. bursata with V. dillwynii and V. borealis

The Vaucheria bursata complex, now including the single species V. bursata, has been distinguished from the V. dillwynii complex, now including V. dillwynii and V. borealis, in having oogonial long-axes erect or oblique, but not parallel with the siphon (e.g. Blum 1972). In addition, V. bursata has been distinguished from V. dillwynii in having cylindrical rather than saccate antheridia, and smooth, rather than rugose, oogonial walls (Rieth 1980b). Some of these features, however, are of doubtful taxonomic value. For example, the long-axes of oogonia range from perpendicular to parallel with the siphon in plants of V. bursata, and antheridia of V. dillwynii can sometimes be cylindrical. In addition, plants of V. borealis from Sweden had oogonia similar in shape and orientation to those of V. bursata, and similar in size, to those of V. dillwynii. From the results of the current study (Table 3.8), V. bursata appears to be distinct from both V. dillwynii and V. borealis, but some previously used taxonomic characters were too variable to distinguish the plants studied.

Although the orientation of oogonia has been used to distinguish

the V. bursata complex from the V. dillwynii complex (e.g. Blum 1972, Christensen 1969, Hoppaugh 1930), there was no disjunction in the data for long-axis or pore angle in the current study.

The oogonia of V. borealis are said to be > 100 µm in diameter, rather than < 100 µm as in V. bursata (Rieth 1980b, the latter as V. sessilis). In the current study, the oogonia of V. borealis were > 100 µm, and those of V. bursata almost always < 100 µm Vaucheria borealis also had longer oogonia, > 135 µm, than V. bursata, < 135 µm. Published values for oogonial diameter and length (Table 3.7) all show a disjunction betwen these two species, but some ranges overlap the disjunction found in the current study. The length and diameter of oogonia, therefore, can generally be used to distinguish V. borealis from V. bursata, but studies of V. borealis in culture are required before these characters can be fully assessed. The range of values for V. dillwynii overlaps those of V. bursata and seems to be of little taxonomic use in distinguishing these two species.

Vaucheria bursata has ovoid to ovoid-reniform oogonia, similar to those of V. borealis rather than truncate-napiform as in V. dillwynii, and this seems to be a useful distinguishing character.

Vaucheria dillwynii has been distinguished from V. bursata in having a rugose patterning on the oogonial wall (Heering 1907; Rieth 1962, 1980b; Sarma & Chapman, 1975a; as V. pachyderma and V. sessilis respectively) and seven, rather than three, layered oospore walls (see Hoppaugh 1930). From the results of the current study, the oospore wall texture in transverse section, and the oogonial wall external texture are adequate for distinguishing V. bursata (Figs 3.5-3.12, 3.34-3.36), as well as V. borealis, from V. dillwynii. Although there is no overlap in the values for oospore wall thickness between V. bursata and

V. dillwynii, the disjuction seems negligible.

The antheridia of V. bursata were cylindrical throughout their development, and generally thinner than those of V. dillwynii and V. borealis. No antheridia were observed which disintegrated, or flanged distally, after dehiscence like those of V. dillwynii (and V. borealis?). Although Rieth (1980b) considers the antheridia of V. bursata to be cylindrical, and distinct from the saccate antheridia of V. dillwynii, this difference is not always observable (c.f., Figs. 3.6 & 3.14). Antheridial shape, therefore, does not appear to be a useful character in distinguishing V. bursata from V. borealis or V. dillwynii.

Siphon diameter, the number of oogonia per gametangial group and the length of the anthgeridial pedicel have not been used previously to distinguish V. bursata from V. dillwynii or V. borealis, and the results of the current study support this conclusion.

Taxonomic Implications

Based on the results of the current study, three distinct species are recognised (for diagnostic features see Table 3.9), and V. sessilis, V. clavata and V. repens are considered to be conspecific with V. bursata. (The differences between V. bursata and V. borealis, however, should be reassessed after more material is studied, both in culture and in field populations.) Nomenclatural and historical aspects of V. bursata and V. dillwynii are included in Chapter 5, while V. borealis is considered below.

Vaucheria borealis Hirn 1900: 87, fig. 2.

The protologue of V. borealis refers to plants with large oogonia (oospores 148-163 µm long), long-axes parallel with the siphon, thin oospore walls, no apparent sculpturing of the oogonial wall and cylindrical antheridia. Although Hirn's (1900) illustration includes an oogonium only 116 µm long and 88 µm in diameter [Christensen (pers. comm.) suggests that Hirn may not have allowed for the reduction in printing], the other features are diagnostic of this species (Heering 1921, Hoppaugh 1930, Rieth 1980b). The plants of V. borealis observed in the present study (from Sweden) shared most of these characters, but the antheridia were disrupted in all material examined. Furthermore, the orientation of the oogonia, with long-axis parallel to the siphon, was similar to that found in V. dillwynii and some populations of V. bursata from south-eastern Australia. Although apparently clearly delineated from North American and European populations of V. bursata, V. borealis was only distinguished from V. bursata as circumscribed in the current study by the size of the oogonia. As the disjuction in size (see Table 3.8) was relatively small, the stability of taxonomic characters in V. borealis needs to be studied in culture before reliable species concepts can be formulated.

There is some doubt concerning the type elements of V. borealis. A search [Christensen (pers. comm.)] in the Botanical Herbarium, Helsinki, Finland, failed to turn up the collections by Kihlman and Lindén, referred to in the protologue (Hirn 1900). A collection by Hult and Rosberg, however, made at one of the localities listed by Hirn (Lkem. = Lapponia kemensis, Finnish Lapland) at about the same time, was found. According to Dr Hällfors (Christensen, pers. comm.), this

locality is seldom visited by botanists, and it is likely that both collections were made on the same collecting trip. Whether the collection by Hult and Rosberg is an isotype or a topotype is uncertain, but an examination of this material by Christensen confirmed that it is referable to V. borealis. In the absence of isotype material, V. borealis should be typified by the description and illustration of Hirn (1900).

Vaucheria pachyderma var. islandica Børgesen (1898, p.137, fig. 3) was described from plants with large ovoid oogonia (220 µm long and 160 µm in diameter), with long-axis parallel to the siphon and oogonial pore (from the illustration, Børgesen 1898, fig. 3) making an angle of -50^{o} with the siphon. According to Børgesen (1898), it differed from the type variety in having nearly regular ovoid oogonia and long, very curved antheridia.

The antheridia illustrated by Børgesen, and those observed in a photograph of the type material (Fig. 3.32) kindly supplied by Dr Tyge Christensen, are similar to those found in V. bursata. Although the antheridia in the plants of V. borealis examined in this study were disrupted, antheridial shape and curvature were found to be poor specific characters in V. dillwynii and V. bursata. The oogonia illustrated by Børgesen have the pore reflexed a little more than was measured in this study but the shape of the oogonium (see Fig. 3.32) is not truncate-napiform like in V. dillwynii. In addition, although Børgesen illustrated the oogonial wall as roughly textured, in type material of this species (Fig. 3.32) the oogonial wall is relatively smooth and not strongly rugose. Furthermore, the oospore wall seems to be evenly textured, rather than 'flaky', in transverse section. Cedergren (1933, p.96) raised V. pachyderma var. islandica to

species level but it is unlikely that this taxon should be recognised as distinct from V. borealis. A comparison of the actual type collections of the two will probably show the two to be conspecific.

Vaucheria pachyderma var. islandica f. filis crassiusculus Borge (1931, p.23, pl, 1 fig. 7) was distinguished from V. borealis (as V. pachyderma var. islandica f. islandica) in having broad vegetative siphons, 86-108 µm, and a sculptured oogonial wall. This sculpturing was described as irregularly ribbed and in the drawing by Borge (1931, pl. 1 fig. 7) it resembles the pattern observed in scanning electron micrographs of V. borealis (the plants of V. borealis examined in the current study were collected from the type locality of Borge's form). Vaucheria dillwynii, however, has a rugose pattern which appears more pitted under scanning electron microscopy (Fig. 3.37). In addition, Rieth (1962, 1980b) found that vegetative siphon diameter ranged from 34-143 µm in V. borealis. An examination of the type material of this form, therefore, will probably show it to be synonymous with V. borealis.

Table 3.1 Previously used diagnostic features of species in the *Vaucheria bursata* (O.F. Müller) C. Agardh and *V. dillwynii* (Weber & Mohr) C. Agardh complexes.

Table 3.1.

Species	Reference - (B)lum 1972 or (R)ieth 1980b	Oogonial long-axis angle (°)[3]	Oogonial pore angle (°)[3]	Oogonial symmetry - (B)ilateral or (R)adial	Oogonial L/D	Siphon diameter (µm)	Number of oogonia per gametangial group	Oogonial length (µm)	Oogonial diameter (µm)	Oogonial wall texture - (S)mooth or (R)ugose	Antheridial shape - (C)ircinate-cylindrical or (S)accate
V. sessilis [1] (Vaucher)	R	0–130	–40 to 130	B,R	1.1–1.5 (–1.9)	20–135 (–150)	1–2	65–115	52–80	S	C
de Candolle	B	40–70	(0–)40–70	B	< 1.5	42–126	(1–)2	71–104	50–78	S	C
V. repens Hassall	B	0–50	–40 to 40	B	> 1.5	28–72	1(–2)	64–105	50–67	S	C
V. clavata sensu Klebs	B	50–130	50–130	R	1.5–2	49–98	(1–)2	85–113	49–77	S	C
V. dillwynii [2] (Weber & Mohr)	B	≃ 0	–45 to –90	B	?	40–123	1	69–220	69–160	R	C,S
C.Agardh	R	≃ 0	–45 to –90	B	1.1–1.4	20–78	1	(82–)95–140 (–150)	(65–)72–117	R	S
V. borealis Hirn	R	≃ 0	0 to –45	B	1.0–1.6	34–43	1	130–226	99–174	S	C

[1] Vaucheria bursata (O.F.Müller) C.Agardh is the earliest name available for plants referable to the V. bursata complex, but its application to one of the species recognised by Blum (1972) is uncertain. For this reason, therefore, the later name, V. sessilis, is used here. [2] Rieth (1980b) uses V. pachyderma Walz for V. dillwynii. [3] Calculated from illustrations.

Table 3.2 Collection details for field populations and isolates used to evaluate taxonomic characters in the *Vaucheria bursata* and *V. dillwynii* complexes.

Table 3.2.

Collection or isolate number	Herbarium number	Collection details (all collected by Entwisle unless otherwise indicated)
1.	MEL 1049208	Tambo River, Ensay, Victoria, 17.x.1984.
2.	MEL 1049209	Seaspray, Victoria, 30.ix.1983.
3.	MEL 1049210[1]	Seaspray, Victoria, 30.ix.1983.
4.	MEL 1049211	Tambo River, Bark Sheds, Victoria, 17.x.1984.
5.	MEL 1049212	Skenes Creek, Skenes Creek Township, Victoria, 17.x.1984.
6.	MEL 1049213 p.p.	Greendale Reservoir, Victoria, 22.viii.1984.
7.	MEL 1049214 p.p.	Stony Creek, Greens Gully, Victoria, 18.viii.1984.
8.	MEL 1049215 p.p.	La Trobe River, Traralgon, Victoria, 18.viii.1984.
9.	MEL 1049216 p.p.	Fords Creek, Mansfield, Victoria, 22.vii.1984.
10.	MEL 1049217 p.p.	Merri Creek, Clifton Hill, Victoria, 24.vi.1984.
11.	MEL 1049218	Ovens River, Porepunkah, Victoria, 22.vii.1984.
12.	MEL 1049219	Happy Valley Creek, Ovens, Victoria, 22.vii.1984.
13.	MEL 1049220	Lake Guy, Bogong Village, Victoria, 16.x.1984.
14.	MEL 1049221	Five Mile Creek, Woodend, Victoria, 21.i.1984.
15.	MEL 1049222	Lake Guy, Bogong Village, Victoria, 16.x.1984.
16.	MEL 1049223 p.p.	Running Creek, Myrtleford-Mt Beauty Road, Victoria, 16.x.1984.
17.	MEL 1049224	Lake Linlithgow, Hamilton-Chatsworth Road, Victoria, 4.x.1984.
18.	MEL 1049224[1]	Lake Linlithgow, Hamilton-Chatsworth Road, Victoria, 4.x.1984.
19.	MEL 1049226	Werribee River, Bacchus Marsh, Victoria, 27.v.1983.
20.	MEL 1049227	Giffard, Victoria, 30.ix.1983.
21.	MEL 1049228	Corringle Creek, Corringle-Newmerella Road, Victoria, 1.x.1983.
22.	MEL 1049231 p.p.	Lake Wallace, Edenhope, Victoria, 2.x.1984.
23.	MEL 1049232	Morses Creek, Bright, Victoria, 22.vii.1984.
24.	MEL 1049233 p.p.	Royal Botanic Gardens, South Yarra, Victoria, 4.vi.1984.
644.	MEL 1049234	Barwon River, Forrest, Victoria, 28.viii.1984.
211.	MEL 1049235	Diamond Creek, Eltham, Victoria, 28.iv.1983.
325.	MEL 1049236	Onkaparinga River, Clarendon, South Australia, 4.viii.1983.
489.	MEL 1049237	Five Mile Creek, Woodend, Victoria, 23.i.1984.
V.borealis	MEL 1049229	Husklappen, Sweden, Christensen, 10.viii.1955.
V.borealis	MEL 1049230	Morvallen, Sweden, Christensen, 9.viii.1955.

[1]Taken from older culture of previous collection or same collection.

Table 3.3 Culture treatments used to evaluate taxonomic characters in the *Vaucheria bursata* and *V. dillwynii* complexes.

Table 3.3.

Treatment number	Temperature (°C)	Photon flux density ($\mu mol/m^2/s$)	% Agar	Salinity (o/oo)
1.	5	6-10	1	0.3 (isolate 211 - 6.5)
2.	10	6-10	1	0.3 (isolate 211 - 6.5)
3.	15	6-10	1	0.3 (isolate 211 - 6.5)
4.	20	6-10	1	0.3 (isolate 211 - 6.5)
5.	5	12-20	1	0.3 (isolate 211 - 6.5)
6.	10	12-20	1	0.3 (isolate 211 - 6.5)
7.	15	12-20	1	0.3 (isolate 211 - 6.5)
8.	20	12-20	1	0.3 (isolate 211 - 6.5)
9.	5	35-50	1	0.3 (isolate 211 - 6.5)
10.	10	35-50	1	0.3 (isolate 211 - 6.5)
11.	15	35-50	1	0.3 (isolate 211 - 6.5)
12.	20	35-50	1	0.3 (isolate 211 - 6.5)
13.	15-20	110-130	1	0.3
14.	15	30-40	0.5	6.5[1]
15.	15	30-40	0	6.5 (isolate 325 - 4.0)
16.	15	30-40	0[2]	0.3 (isolate 325 - 4.0, isolate 489 - 6.5)
17.	15	30-40	1 & 0[3]	6.5
18.	15	30-40	1	0.3 with $\frac{1}{2}$P & $\frac{1}{2}$N
19.	15	30-40	1	6.5
20.	15	30-40	1	4.0
21.	15	30-40	1	2.0
22.	15	30-40	1	0.3
23.	15	30-40	1	Soil/water media (Stein 1973, p.22)

[1]Isolate 211 had 44 days at 20°C and 30-35 $\mu mol/m^2/s$ before being transferred to the conditions given in table (material was observed from 45-68 day old cultures).

[2]Aerated.

[3]Solid agar media with liquid media on top (approximately 1 cm of each).

Table 3.4 Siphon diameter (μm) of taxa in the *Vaucheria bursata* complex from selected published accounts.

Table 3.5 Oogonial long-axis and pore angle (^{O}) of taxa in the *Vaucheria bursata* complex calculated from selected published illustrations.

Table 3.4.

Reference	V.sessilis	V.repens	V.clavata
Blum 1972	42-126	28-71	49-98
Götz 1897	49.5-82.5	33-49.5	77-110
Hoppaugh 1930 [1]	60-110	48-65	49-78
Jao 1936 [1]	55-110	29-48	49-115
Li 1936 [2]	60-130	35-60	77-110

Table 3.5.

Reference	V.sessilis		V.repens		V.clavata	
	long-axis	pore	long-axis	pore	long-axis	pore
Blum 1972	80-100	80-100	0-50	40-40	80-100	80-100
Götz 1897	50-70	30-40	40	0	90	90
Hoppaugh 1930 [1]	60	40-50	40	20	70-90	70-90
Jao 1936 [1]	60-70	50-70	30	10	90	90

[1] All species referred to as forms of V. sessilis.

[2] V. clavata as V. orthocarpa Reinsch.

Table 3.6 Length and diameter of oogonia (μm) of taxa in the *Vaucheria bursata* complex from selected published accounts.

Table 3.7 Length and diameter of oogonia (μm) from selected published accounts recognising *Vaucheria bursata* [as *V. sessilis* (Vaucher) de Candolle], *V. borealis* and *V. dillwynii* [as *V. pachyderma* Walz] as distinct species.

Table 3.6.

Reference	V.sessilis		V.repens		V.clavata	
	length	diameter	length	diameter	length	diameter
Blum 1972	71-104	50-78	64-105	50-67	85-113	49-77
Gotz 1897	66-99	60.5-77	67-77	55-77.5	66-88.5	49.5-66.5
Hoppaugh 1930 [1]	80-98	60-80	60-70	50-70	78-99	50-75
Jao 1936 [1]	80-102	57-77	70-95	60-80	80-110	50-75
Li 1936 [2]	75-95	70-80	50-80	60-70	65-90	50-65

[1] All species referred to as forms of V. sessilis.

[2] V. clavata as V. orthocarpa.

Table 3.7.

Reference	V.bursata		V.borealis		V.dillwynii	
	length	diameter	length	diameter	length	diameter
Rieth 1980b	70-115	55-80	132-197.5 (-216)	104-156	(82.5)95-140(-150)	(65-)72.5-117
Hoppaugh 1930	60-98	50-80	120-145	90-105	98-120	90-100
Heering 1921	60-99	49.5-77.5	148-163	111-138	69-220	69-160

Table 3.8 Summary of features evaluated in *Vaucheria bursata*, *V. borealis* and *V. dillwynii*.

Table 3.8.

Species	Oogonial long-axis angle (°)	Oogonial pore angle (°)	Oogonial L/D	Siphon diameter (µm)	Oogonial length (µm)	Oogonial diameter (µm)	Oogonial wall texture - (S)mooth or (R)ugose	Oospore wall in transverse section - (F)laky or (E)venly textured	Oospore wall thickness (µm)	% gametangial groups with 2 oogonia	Antheridial pedicel length (µm)	Antheridial diameter (µm)	Oogonial shape - (T)runcate-napiform or (O)void to ovoid-reniform
V.bursata	0-90	⁻90-90	1.0-1.7	29-84(-115)	67-130	53-96(-103)	S	E	2-7	0-100	5-132	17-29	O
V.dillwynii	⁻10-10	⁻70-⁻30	1.2-1.5	(36-)46-72	101-150	79-120	R	F	8-14	0	36-84 (-96)	28-38	T
V.borealis	0-10(-20)	⁻50-0	1.2-1.5	58-91	137-187	(101-)108-132	S	E	5-7.5	0	50-84	24-34	O

Table 3.9 Diagnostic features of *Vaucheria bursata*, *V. borealis* and *V. dillwynii* in the current study.

Table 3.9.

Species	Oogonial length (µm)	Oogonial diameter (µm)	Oospore wall in transverse section	Oogonial wall texture	Oogonial shape
V. bursata	< 135	< 100	evenly textured	smooth	ovoid to ovoid-reniform
V. borealis	> 135	> 100	evenly textured	smooth	ovoid to ovoid-reniform
V. dillwynii	101-150	79-120	flaky	rugose	truncate-napiform

Fig. 3.1 Siphon diameter in culture isolates 211, 325 and 489 (see Table 3.3 for treatment details) and field populations (see Table 3.2 for collection details).

3.1

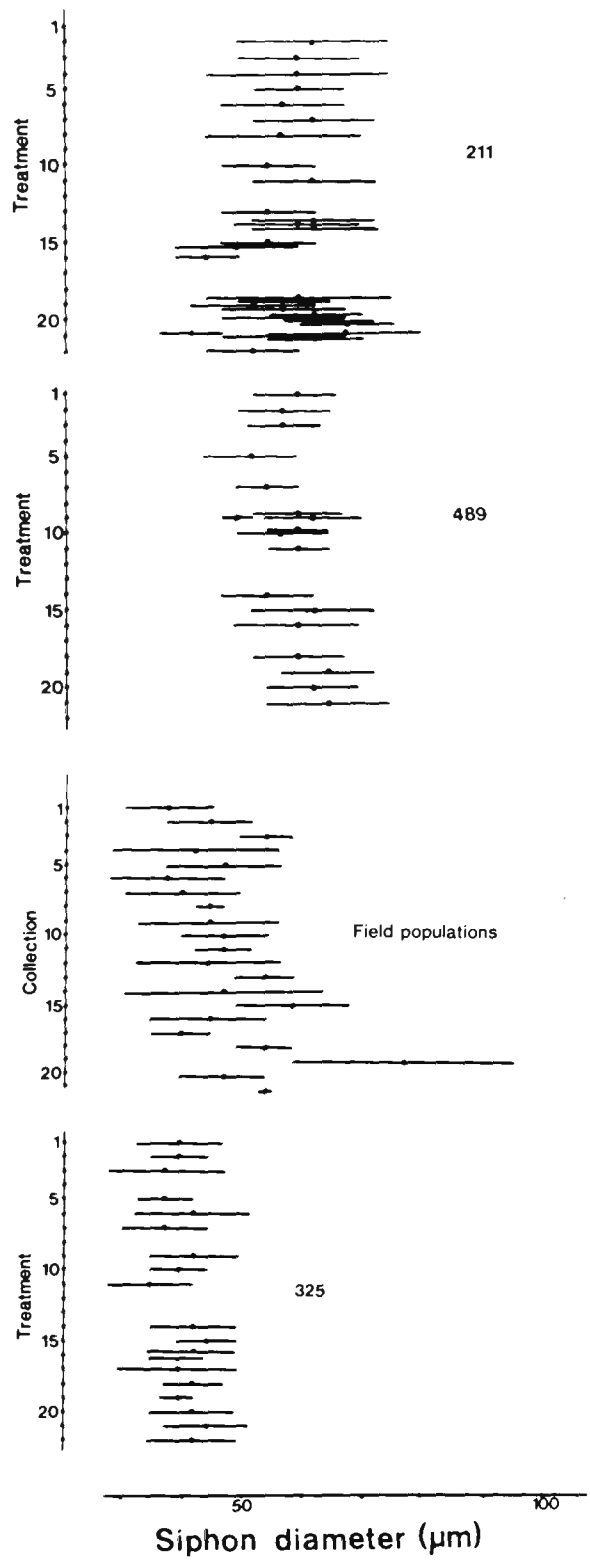

Treatment
1
5
10
15
20
211
Treatment
489
Collection
Field populations
Treatment
325
50
100
Siphon diameter (µm)

Fig. 3.2 Percentage of gametangial groups with two oogonia (remainder have one oogonium) in culture isolates 211 (○), 325 (■) and 489 (◀)(see Table 3.3 for treatment details) and field populations (see Table 3.2 for collection details).

3.2

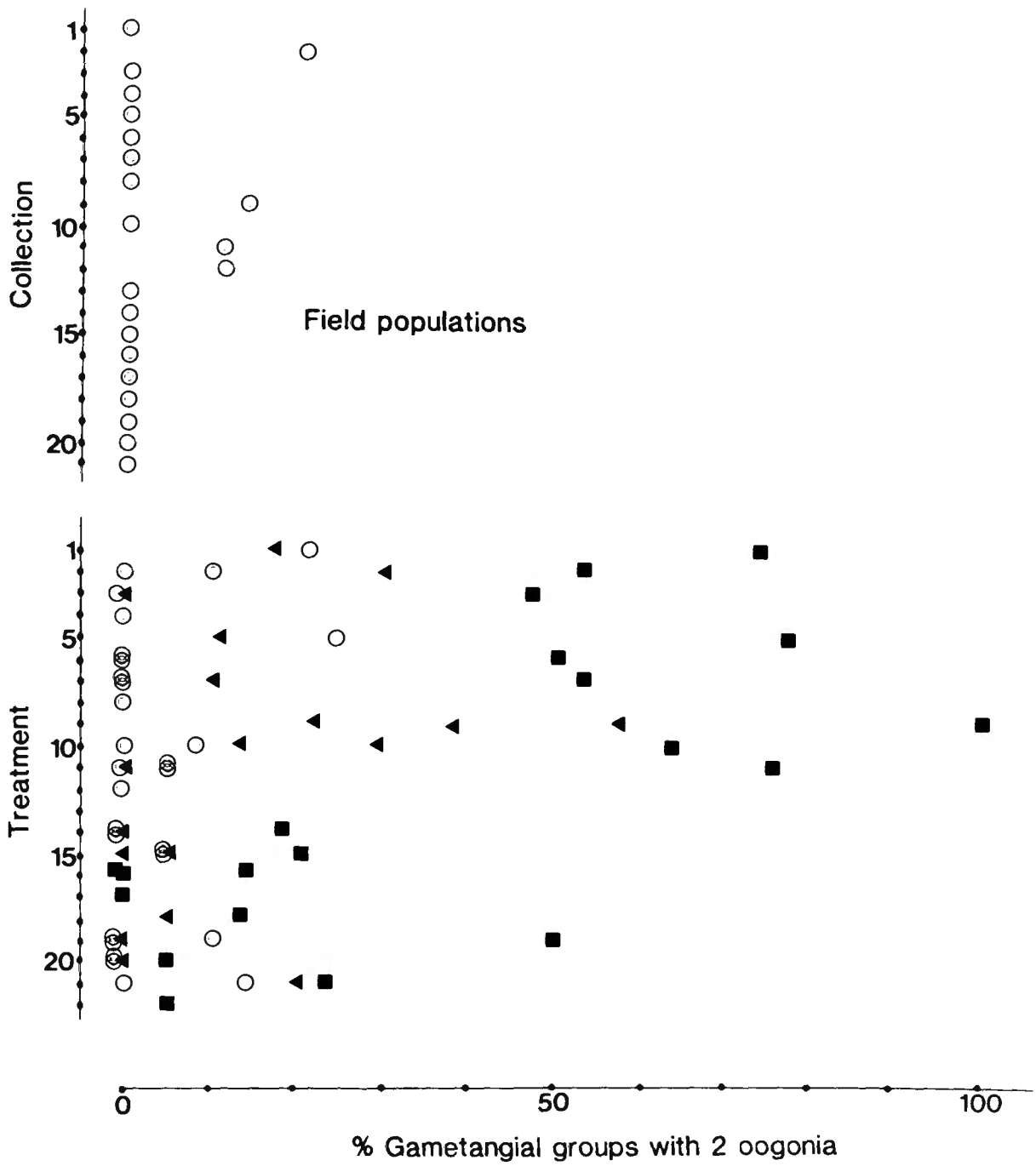

Collection
1
5
10
15
20
Field populations
Treatment
1
5
10
15
20
0
50
100
% Gametangial groups with 2 oogonia

Fig. 3.3 Oogonial long-axis angle in culture isolates 211, 325 and 489 (see Table 3.3 for treatment details) and field populations (see Table 3.2 for collection details).

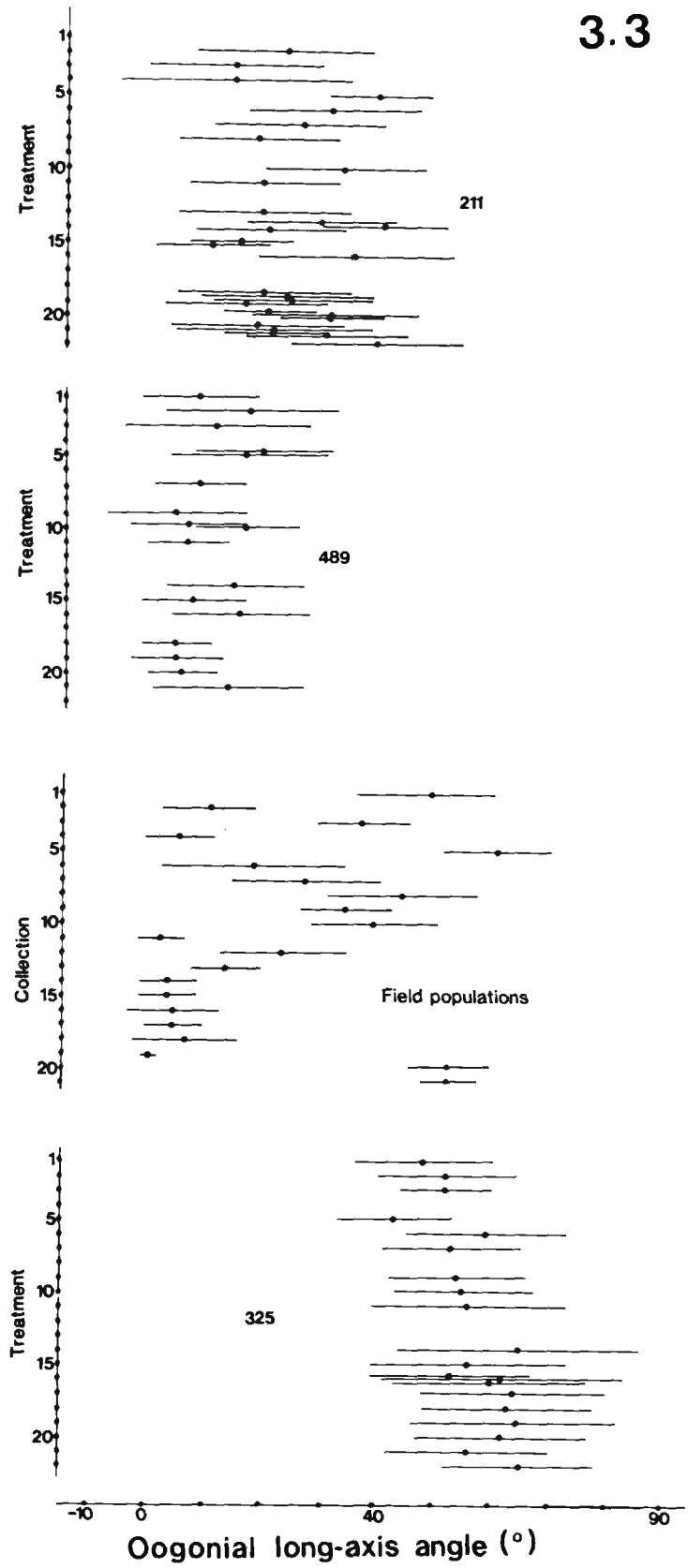
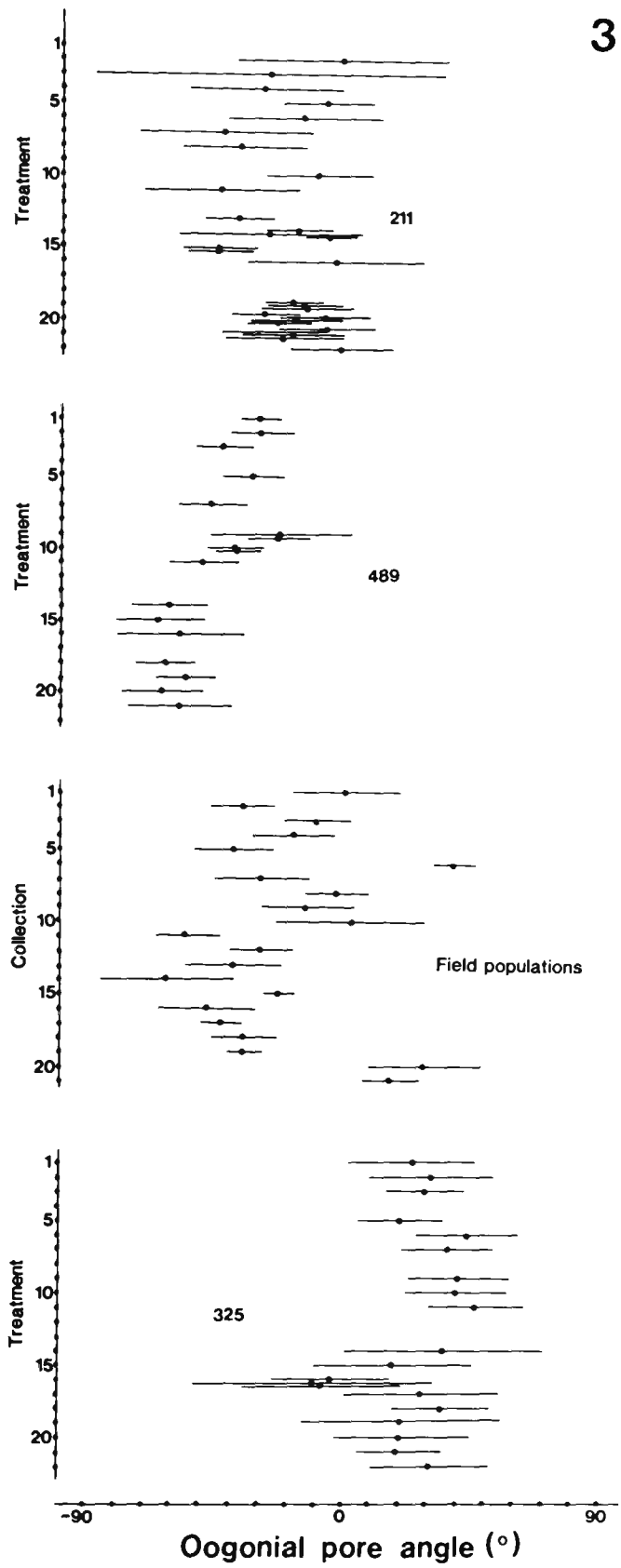
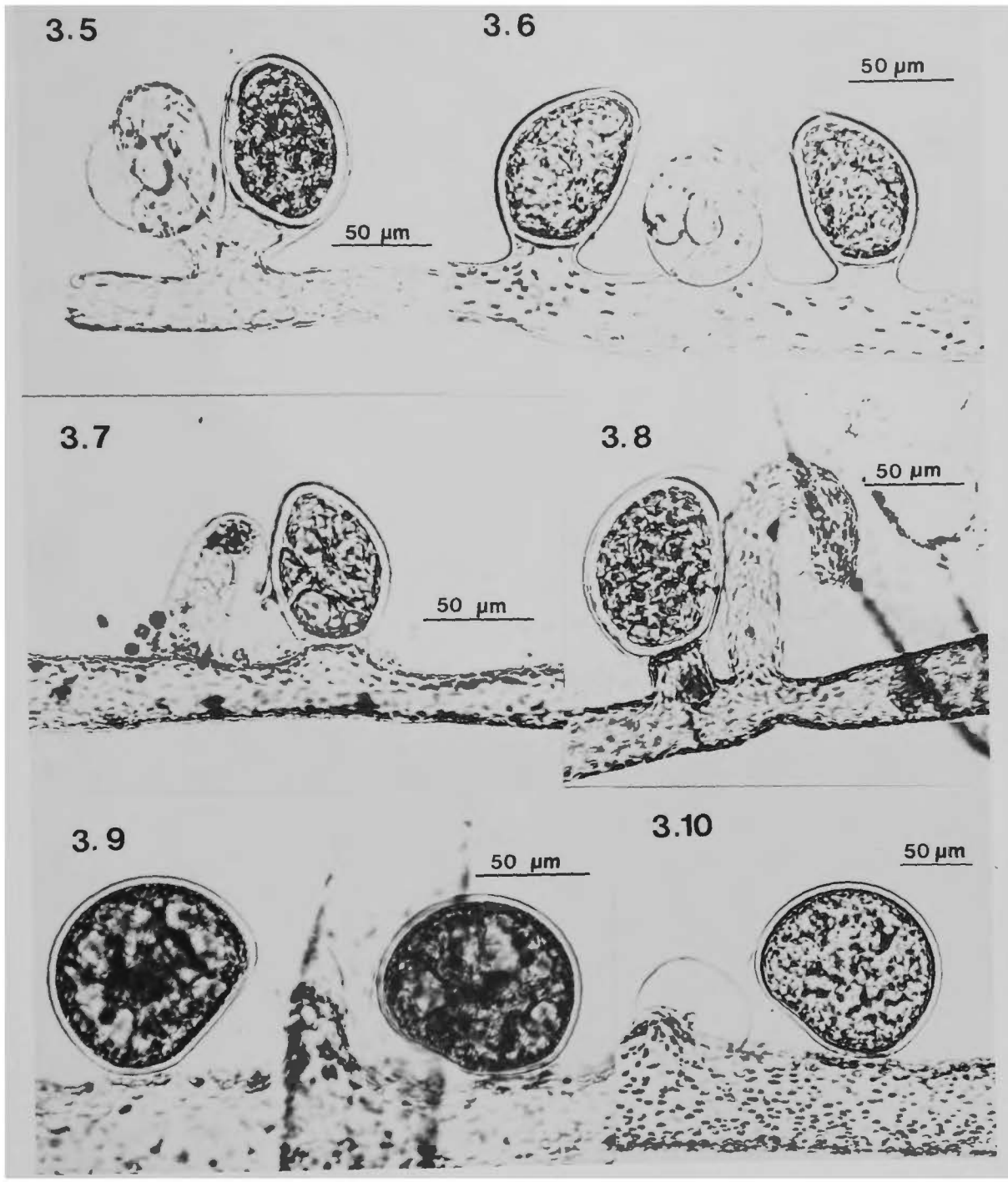

3.3
Treatment
1
5
10
15
20
211
Treatment
489
Collection
Field populations
Treatment
325
-10
0
40
90
Oogonial long-axis angle (°)

Fig. 3.4 Oogonial pore angle in culture isolates 211, 325 and 489 (see Table 3.3 for treatment details) and field populations (see Table 3.2 for collection details).

3.4


Treatment
1
5
10
15
20
211
Treatment
1
5
10
15
20
489
Collection
1
5
10
15
20
Field populations
Treatment
1
5
10
15
20
325
-90
0
90
Oogonial pore angle (°)

Figs 3.5-3.10	Vaucheria bursata (O.F.Müller) C. Agardh.
Figs 3.5-3.8	Isolate 325 in agar.
Fig. 3.5	Pedicellate oogonium arising from antheridial pedicel.
Fig. 3.6	Bilaterally symmetrical oogonium with pore directed away from siphon and with curved antheridial pedicel.
Fig. 3.7	Nearly radially symmetrical oogonium.
Fig. 3.8	Pedicellate oogonium separate from antheridial pedicel.
Figs 3.9-3.10	Isolate 489 in agar.
Fig. 3.9	Oogonia with pores directed towards siphon.
Fig. 3.10	Oogonia with shortly pedicellate antheridium.


3.5
50 µm
3.6
50 µm
3.7
50 µm
3.8
50 µm
3.9
50 µm
3.10
50 µm

Figs 3.11-3.16

Figs 3.11-3.12 Vaucheria bursata (O.F.Müller) C.Agardh. Preserved material of isolate 211.

Fig. 3.11 Oogonium with pore directed towards siphon.

Fig. 3.12 Oogonium with obscured pore (arrow) directed nearly parallel with siphon.

Figs 3.13-3.16 Vaucheria dillwynii (Weber & Mohr) C.Agardh. Isolate 644.

Fig. 3.13 Truncate-napiform oogonium in agar.

Fig. 3.14. Almost cylindrical antheridium near a germinating oospore, in agar.

Fig. 3.15 Truncate-napiform oogonium from preserved material. Note elongation near attachment area and disintegrated antheridium (arrow).

Fig. 3.16 Cylindrical antheridium before dehiscence.

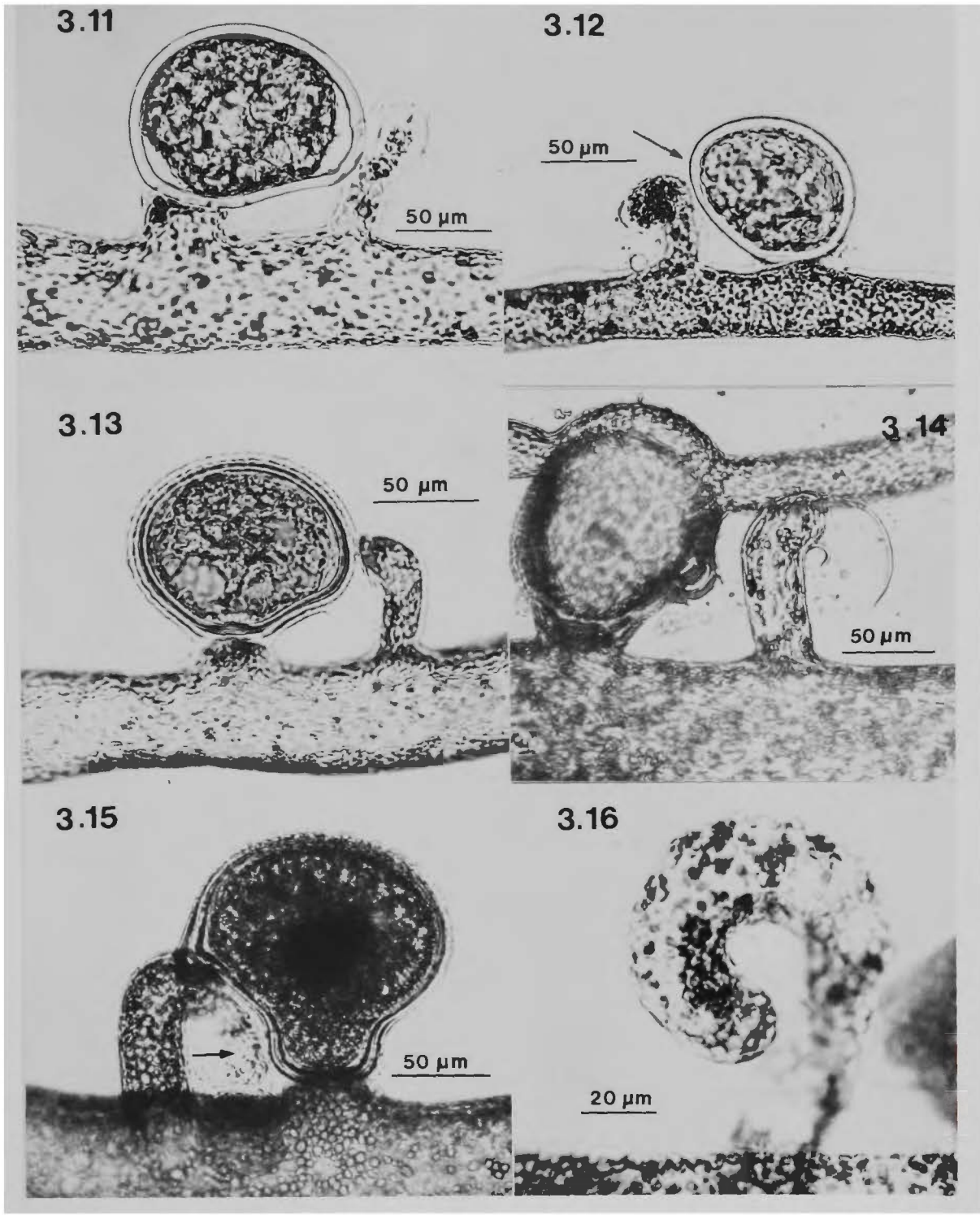

3.11
50 µm
3.12
50 µm
3.13
50 µm
3.14
50 µm
3.15
50 µm
3.16
20 µm

Fig. 3.17 Oogonial length in culture isolates 211, 325 and 489 (see Table 3.3 for treatment details) and field populations (see Table 3.2 for collection details).

3.17

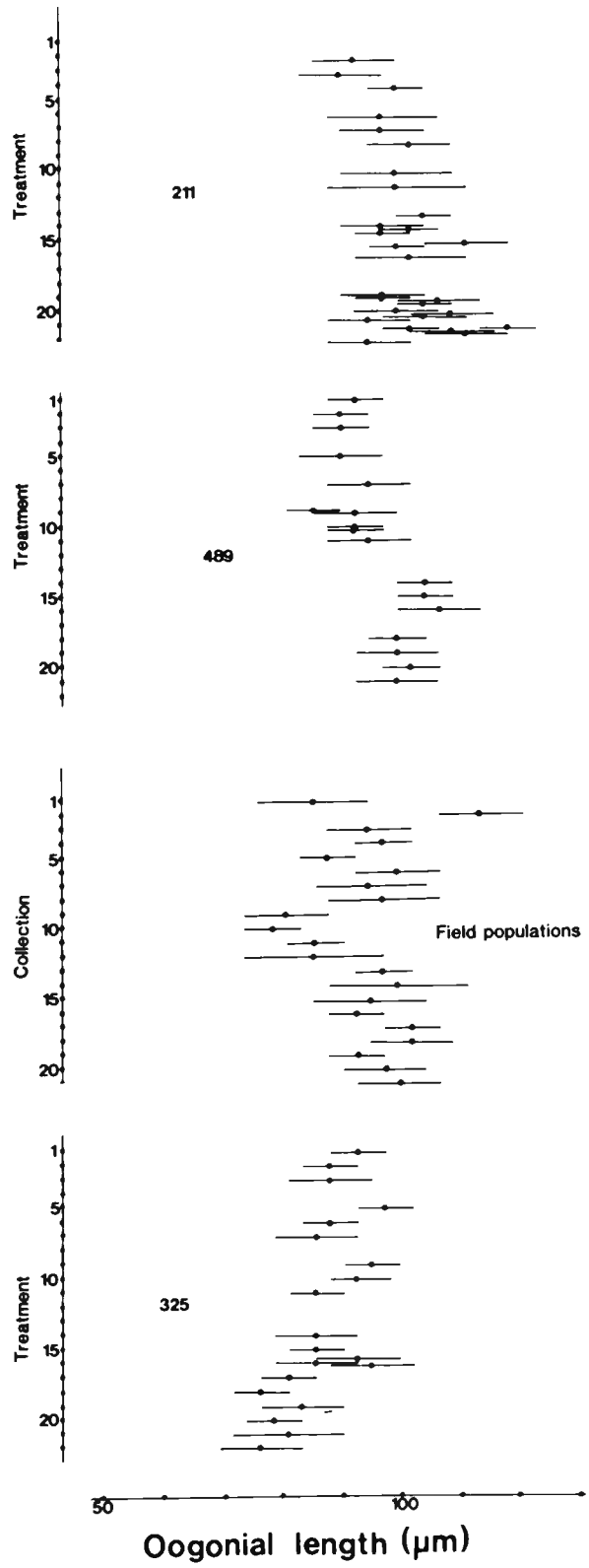

Treatment
1
5
10
15
20
211
Treatment
489
Collection
Field populations
Treatment
325
50
100
Oogonial length (µm)

Fig. 3.18 Oogonial diameter in culture isolates 211, 325 and 489 (see Table 3.3 for treatment details) and field populations (see Table 3.2 for collection details).

3.18

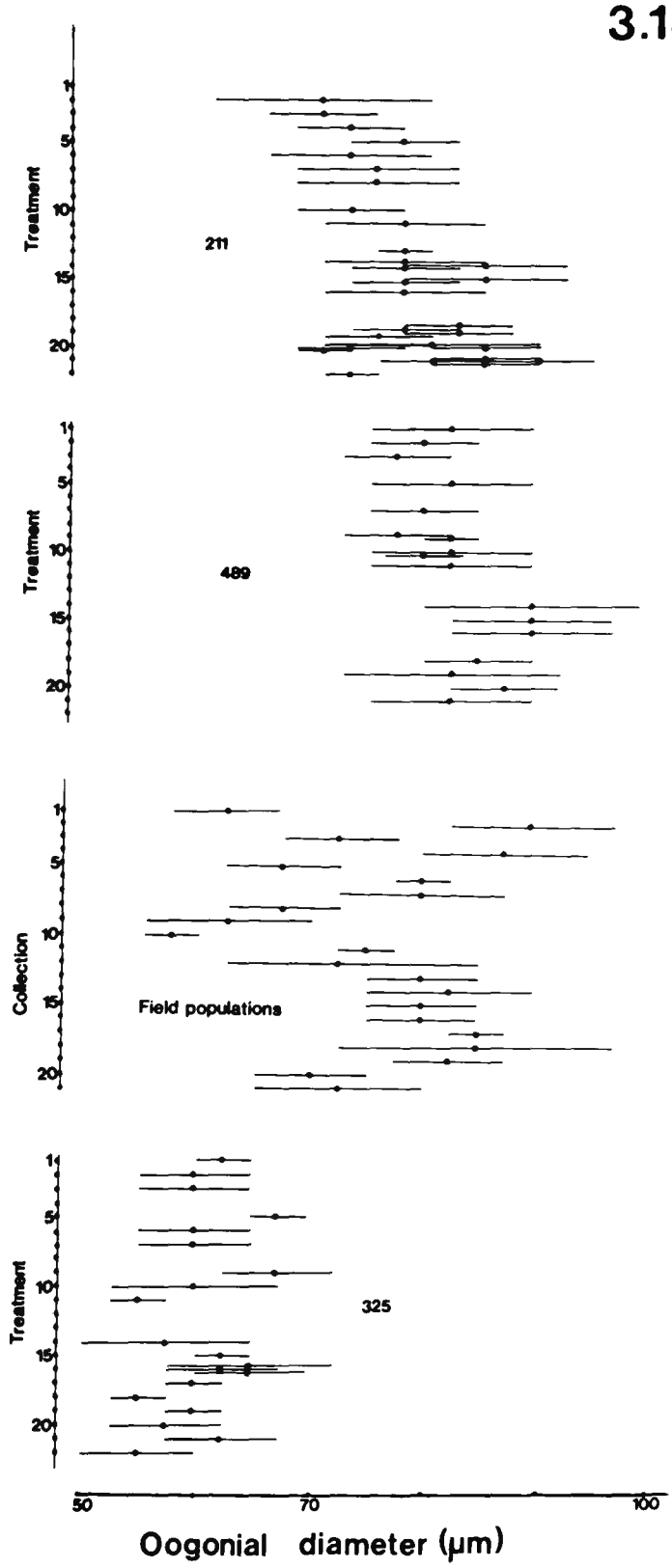

Treatment
1
5
10
15
20
211
Treatment
1
5
10
15
20
489
Collection
1
5
10
15
20
Field populations
Treatment
1
5
10
15
20
325
50
70
100
Oogonial diameter (µm)

Fig. 3.19 Oogonial L/D in culture isolates 211, 325 and 489 (see Table 3.3 for treatment details) and field populations (see Table 3.2 for collection details).

3.19

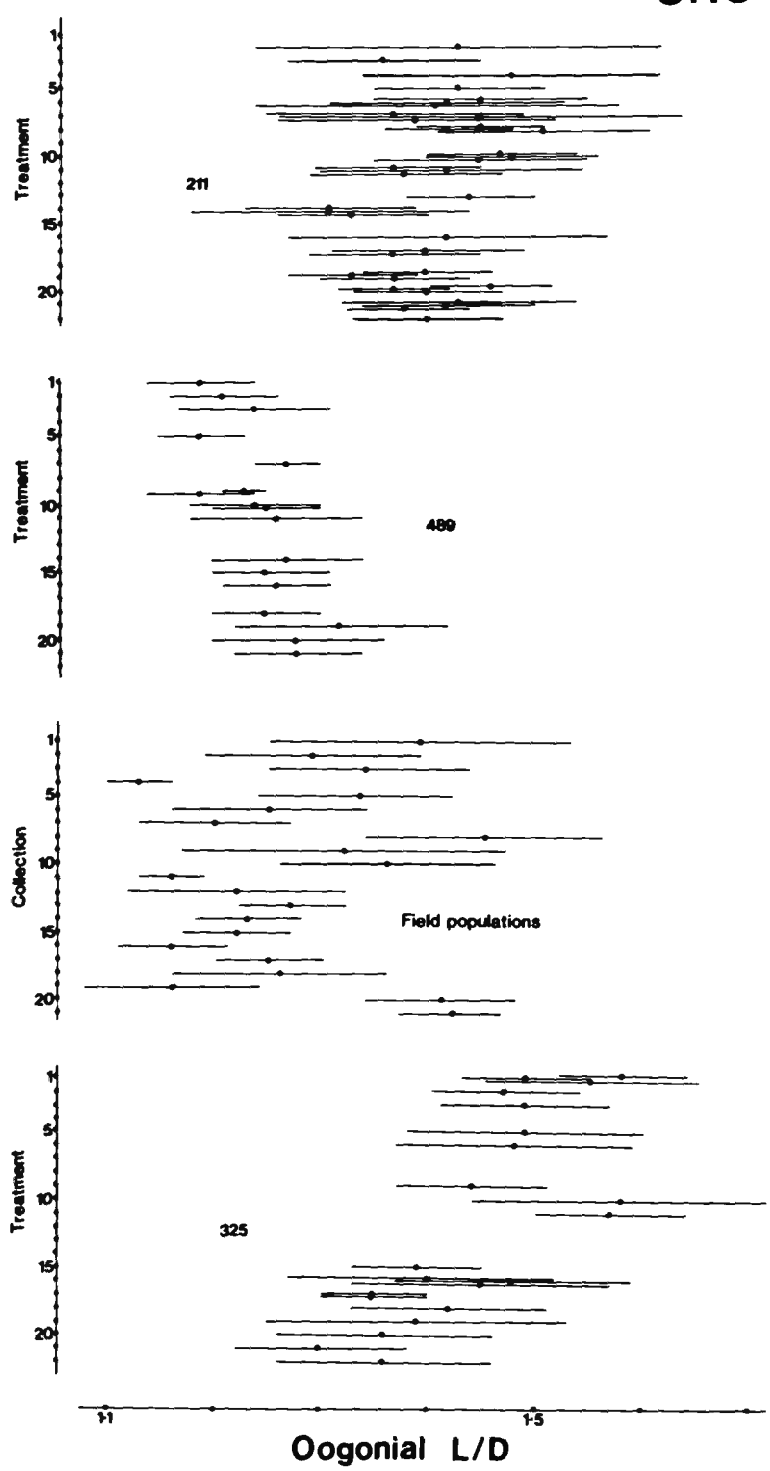
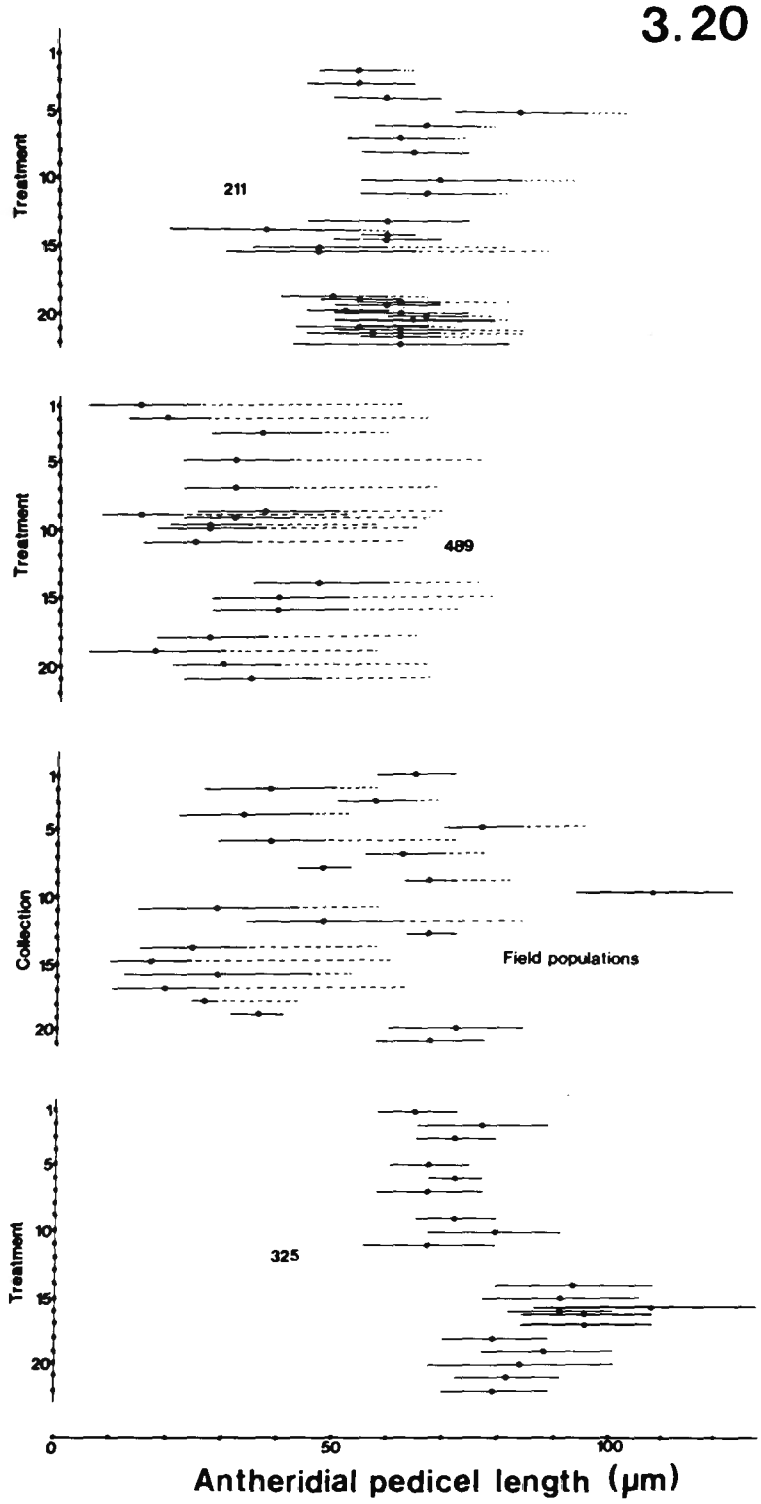

Treatment
1
5
10
15
20
211
Treatment
489
Collection
Field populations
Treatment
325
1·1
1·5
Oogonial L/D

Fig. 3.20 Length of antheridial pedicel (unbroken line) and antheridial system (unbroken and broken line) in culture isolates 211, 325 and 489 (see Table 3.3 for treatment details) and field populations (see Table 3.2 for collection details).

3.20


Treatment
1
5
10
15
20
211
Treatment
489
Collection
Field populations
Treatment
325
0
50
100
Antheridial pedicel length (µm)

Figs 3.21-3.27 Data from Vaucheria dillwynii (Weber & Mohr) C. Agardh (dil.), from culture isolate 644 (see Table 3.3 for treatment details) and field populations (see Table 3.2 for collection details), compared with V. borealis Hirn (bor.).

Fig. 3.21 Oogonial long-axis angle.

Fig. 3.22 Oogonial length.

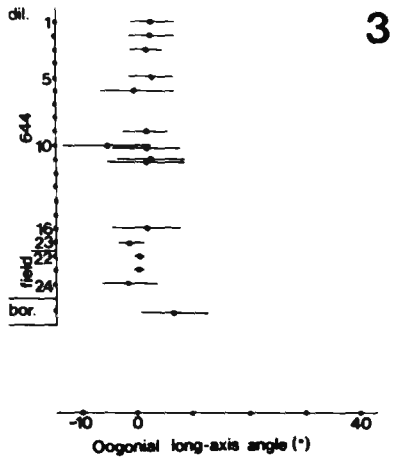
Fig. 3.23 Oogonial pore angle.

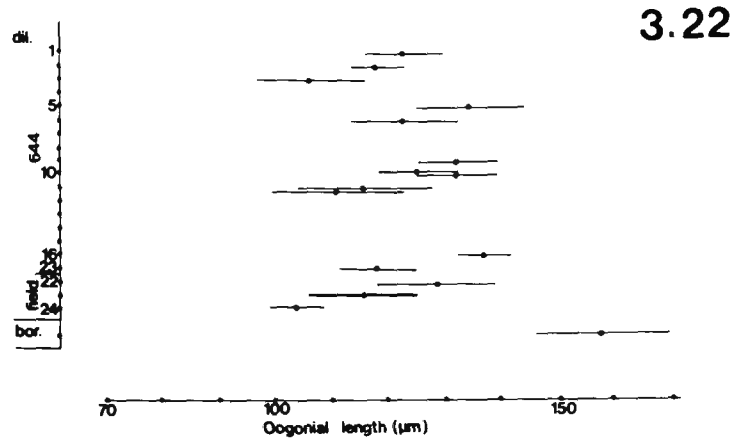
Fig. 3.24 Oogonial diameter.

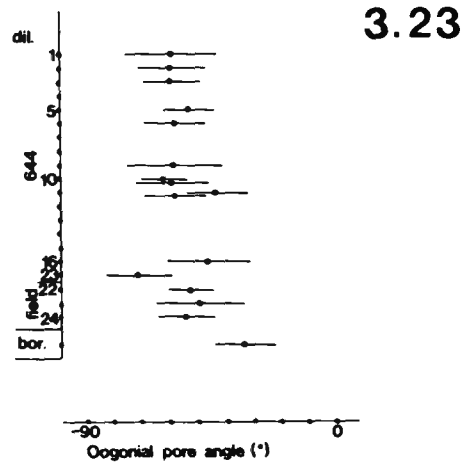
Fig. 3.25 Oogonial L/D.

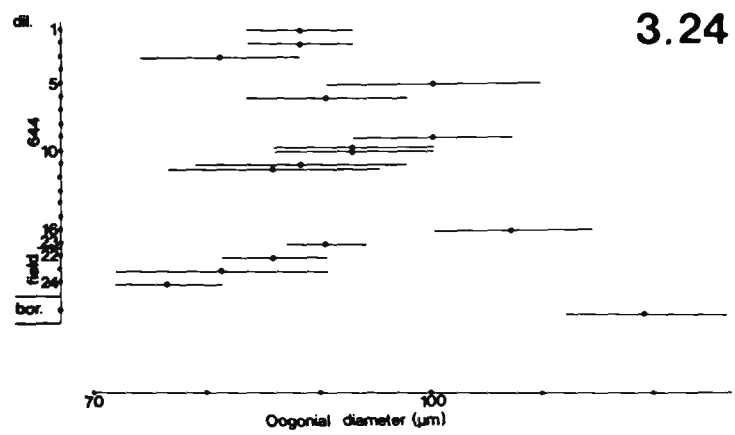
Fig. 3.26 Length of antheridial pedicel (unbroken line) and antheridial system (unbroken and broken line).

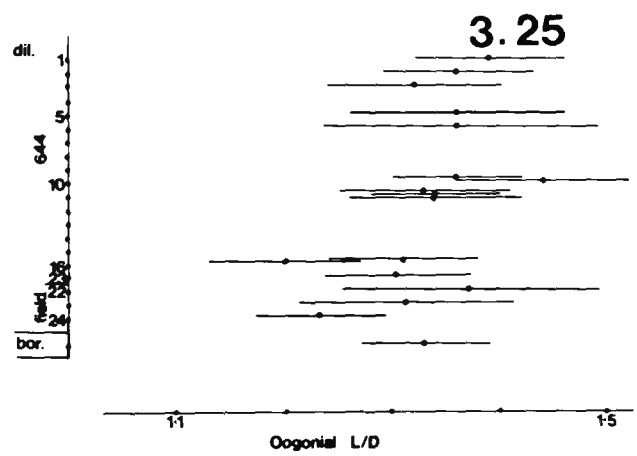
Fig. 3.27 Siphon diameter.

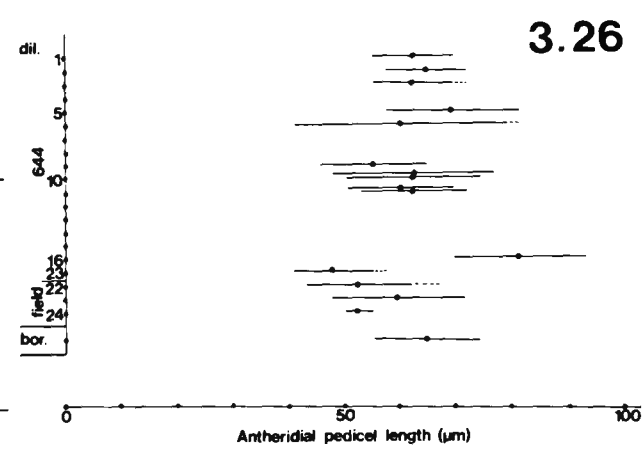

3.21
dil.
644
field
bor.
-10
0
40
Oogonial long-axis angle (°)

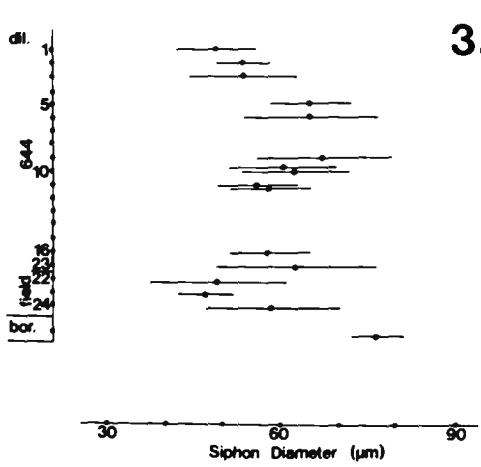

3.22
dil.
644
field
bor.
70
100
150
Oogonial length (µm)


3.23
dil.
644
field
bor.
-90
0
Oogonial pore angle (°)


3.24
dil.
644
field
bor.
70
100
Oogonial diameter (µm)


3.25
dil.
644
field
bor.
1·1
1·5
Oogonial L/D


3.26
dil.
644
field
bor.
0
50
100
Antheridial pedicel length (µm)


3.27
dil.
644
field
bor.
30
60
90
Siphon Diameter (µm)

Figs 3.28-3.34

Figs 3.28-3.31 Vaucheria dillwynii (Weber & Mohr) C. Agardh. Isolate 644.

Fig. 3.28 'Antheridial pedicels' with swollen distal ends but not producing antheridia, from preserved material.

Fig. 3.29 Cylindrical antheridium in agar.

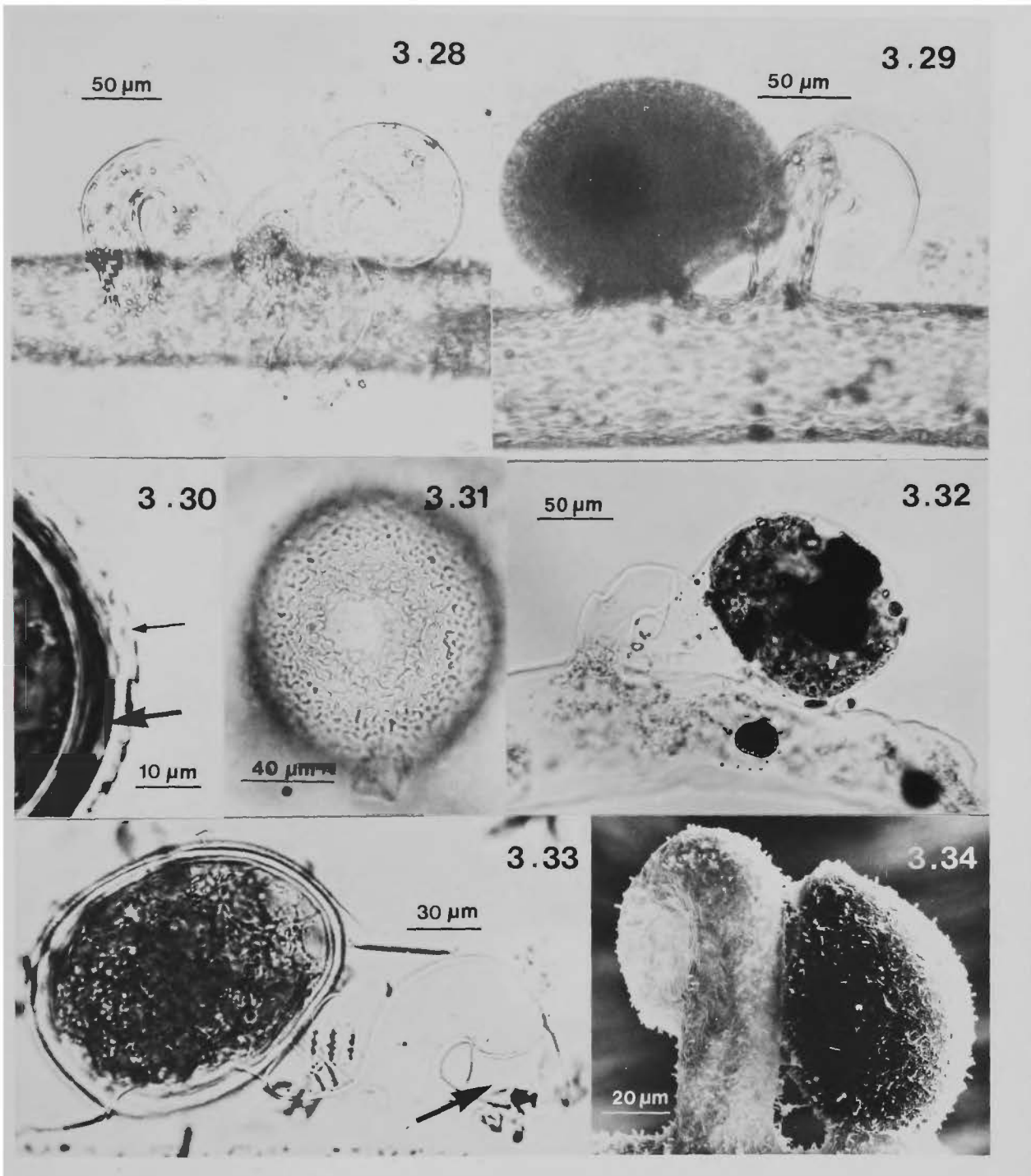
Fig. 3.30 Oogonium from preserved material. Note 'flaky' texture of oospore wall (large arrow) and undulation of oogonial wall (small arrow).

Fig. 3.31 Oogonial wall showing rugose patterning.

Fig. 3.32 Photograph of type material of Vaucheria islandica (Børgesen) Cedergren; photograph kindly supplied by Dr Tyge Christensen. Note relatively smooth oogonial wall, evenly textured oospore wall and cylindrical antheridium.

Fig. 3.33 Vaucheria borealis Hirn, showing ovoid to ovoid-reniform oogonium and disrupted antheridium (arrow). Rehydrated herbarium material of MEL 1049229.

Fig. 3.34 Vaucheria bursata (O.F.Müller) C. Agardh. Isolate 325, showing oogonium and antheridium. Note relatively smooth oogonial wall (some debri is attached).


3.28
50 µm
3.29
50 µm
3.30
10 µm
3.31
40 µm
50 µm
3.32
3.33
30 µm
3.34
20 µm

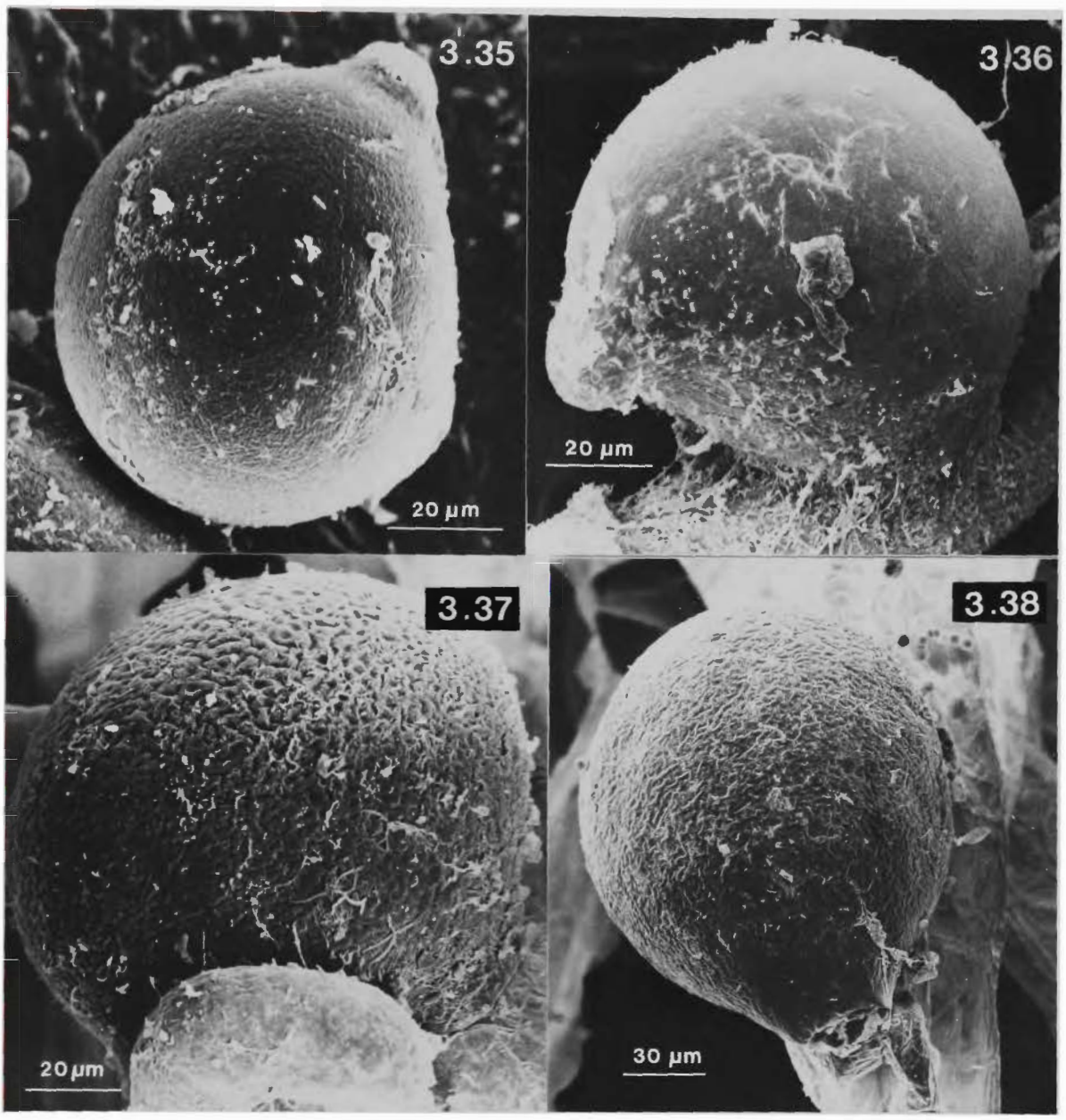
Figs 3.35-3.38

Fig. 3.35 Vaucheria bursata (O.F.Müller) C. Agardh. Isolate 211, showing oogonium with relatively smooth oogonial wall.

Fig. 3.36 Vaucheria bursata. Isolate 489, showing oogonium with relatively smooth oogonial wall.

Fig. 3.37 Vaucheria dillwynii (Weber & Mohr) C. Agardh. Isolate 644, showing oogonium with rugose oogonial wall.

Fig. 3.38 Vaucheria borealis Hirn. MEL 1049229. showing oogonium with wrinkled (from old herbarium material) but not rugose oogonial wall.


3.35
20 µm
3.36
20 µm
3.37
20 µm
3.38
30 µm

CHAPTER FOUR

AN EVALUATION OF TAXONOMIC CHARACTERS IN THE *VAUCHERIA PRONA* COMPLEX (VAUCHERIACEAE, CHRYSOPHYTA)

INTRODUCTION

The section Racemosae (Walz) Entwisle of *Vaucheria* (Vaucheriaceae, Chrysophyta), as defined in Chapter 5 (p.155), includes those species with monoporic antheridia not subtended by a wall-bound cavity and borne on gametophores. Although at least 15 species have been referred to this section, the stability of characters used to delineate these species has not been evaluated critically in culture, and only limited data are available on variability in field populations (Blum 1953, Rieth 1965b, Christensen 1969).

Within the section Racemosae, species concepts in the *V. prona* complex [i.e. *V. prona* Christensen, *V. terrestris* (Vaucher) de Candolle, *V. frigida* (Roth) C. Agardh and *V. racemosa* (Vaucher) de Candolle] are poorly understood in spite of the studies of Rieth (1965b) and Blum (1953). This complex includes all species with oogonia pendent or perpendicular to the peduncle, and with no distal oogonial cavity left by mature oospore.

Christensen (1969) recognised *V. terrestris* as a species distinct from *V. prona* and *V. frigida*. Blum (1972), however, found Christensen's concept of *V. terrestris* difficult to apply to North American plants, and Rieth (1980b) included plants referable to *V. frigida* in both *V. terrestris* var. *terrestris* sensu Götz and *V. terrestris* var. *major* Rieth. Furthermore, in south-eastern Australian

populations referable to V. prona many previously used taxonomic characters (e.g., the orientation of oogonia, the number of oogonia per gametophore, the length of oogonial pedicels) varied considerably. Table 4.1 contains a summary of the published data for the four species included in the V. prona complex.

The aims of this study have been to evaluate taxonomic characters in the V. prona complex and to then formulate more meaningful species concepts for members of that complex.

MATERIALS AND METHODS

Three south-eastern Australian isolates were chosen for culture study and for comparison with 32 field populations (Table 4.2). These plants could not be identified unequivocally to species, but on the basis of the species concepts of Christensen (1969), culture isolate 551 shared characters of both Vaucheria frigida and V. terrestris, 502 shared characters of V. prona and V. racemosa, and 544 shared characters of V. prona and V. terrestris. Isolate numbers, therefore, have been used to refer to plants as names can not be applied correctly. On the same basis, field populations 1-17 included characters found in V. prona, V. racemosa and V. terrestris, while field populations 18-32 included characters found in V. frigida and V. terrestris.

Many authors (e.g. Blum 1953; Heering 1907, 1921; Rieth 1965b, 1980b) have used V. terrestris and V. hamata in the sense of Götz (1897). Christensen (1969), however, concluded that V. terrestris sensu Götz has been applied to plants correctly referable to V. frigida, and V. hamata sensu Götz, to plants correctly referable to V. prona. Christensen (1969) also place V. walzii in synonymy with V.

V. racemosa, and this synonymy is accepted here.

The following characters were assessed in all culture isolates and field populations: siphon diameter and wall thickness; peduncle length, diameter and shape; the number of oogonia per gametophore; the orientation of gametangia; oogonial pedicel length; length, diameter, L/D and shape of oogonia and oospores; oospore wall thickness and texture in transverse section; antheridial shape; and habitat. The gelatinisation of oospore walls and presence or absence of aplanospores (see Götz 1897), were not assessed as they were impractical diagnostic characters or difficult to interpret.

Procedures for the preparation of culture media have been outlined in Chapter 2 (p.11), and culture treatments are described in Table 4.3. Specimen preservation, observation and measurement procedures have been outlined in Chapter 3 (p.26).

The results of two sample T tests on the means of the experimental data showed that the treatment means of certain characters had similar pooled means. It should be noted, that even if treatment means are significantly different ($p < 0.05$), a feature may still be a poor taxonomic character due to the range of values in each treatment. The ranges plotted are mean $\pm$ standard deviation.

RESULTS AND DISCUSSION

SIPHON DIAMETER AND WALL THICKNESS: Christensen (1969) noted that Vaucheria frigida had thicker siphons than V. terrestris: siphons illustrated by Christensen (1969, figs 8,9) were 40-80 µm and 30-50 µm in diameter, respectively. Blum (1972) described the siphons of V. prona as commonly < 70 µm in diameter, and those of V. racemosa as

commonly 70 µm, or greater, in diameter. Blum (1953) also found that the siphons of V. frigida (as V. terrestris sensu Götz) retain their shape in dried material, but soon become tan or brown coloured, while the siphons of V. prona (as V. hamata sensu Götz) collapse, due to the thinner walls, but retain their green colour for many years.

In the current study, siphon diameter ranged from 19-125 µm with no disjunctions in the data among culture isolates or among field populations (Fig. 4.1). The treatment means for siphon diameter in isolates 551 and 544 were not significantly different ($p = 0.08$), and the ranges of all isolates were similar. Although the field populations had siphons from 22-120 µm in diameter, only collection MEL 1049244 had siphons > 85 µm in diameter. Collection MEL 1049244 was similar to isolate 502 in oogonial and gametophore morphology, but had (1-)2 rather than 2-4 oogonia. Vaucheria racemosa has in addition to siphons > 70 µm, > 3 oogonia per gametophore and shorter peduncles than isolate 502 or collection MEL 1049244 (Blum 1972). The range in siphon diameter in isolate 551 overlaps that of V. terrestris and V. frigida illustrated by Christensen (1969), and some field populations with oogonia similar in size and orientation to V. frigida (e.g., MEL 1049259 p.p., MEL 1049261, MEL 1049263) had siphons 25-50 µm in diameter. The diameter of siphons, therefore, appears to be a poor character for delineating species in the V. prona complex.

Dried herbarium material of isolates 551, 544 and 502, was green and wrinkled after 2 years, and the walls of vegetative siphons were ca 1 µm thick. Neither colour of dried plants nor siphon wall thickness, therefore, could be used to distinguish the populations studied.

PEDUNCLES: The length and shape of peduncles both have been used to delineate species in the Vaucheria prona complex. Blum (1972) distinguished V. prona from V. racemosa in having peduncles commonly > 300 µm long rather than frequently < 300 µm long. In 1951, Blum reported that the peduncles of V. prona (as V. hamata sensu Götz) and V. frigida (as V. terrestris sensu Götz) 'average' 80-250 µm long. All peduncles for V. prona illustrated by Blum (1953, 1972), however, are < 200 µm long. Christensen (1969, p.16) described the peduncles of V. frigida as having a "...somewhat elephant-like plumpness, often with irregular bends.", and those of V. terrestris as having "...an elegant straightness in their lines,...". In the accompanying illustrations, the peduncles of V. terrestris are longer (see Table 4.4) and thinner (peduncle diameter 70-80 µm cf. 80-100 µm) than those of V. frigida, and have almost parallel rather than diverging lateral walls.

There were no disjunctions in the data for peduncle length (Fig. 4.2) and distal diameter (Fig. 4.3) among south-eastern Australian culture isolates or field populations. The treatment means for peduncle length in isolates 502 and 544 were not significantly different ($p = 0.28$) and peduncles were similar in length to those illustrated in the literature for V. prona (Table 4.4). Contrary to the findings of Blum (1972), however, most peduncles were < 300 µm long, even in gametophores with 1 or 2 oogonia (see next section). Islam (1965) found that in damp and shady habitats V. prona (as V. hamata) produced longer peduncles than in drier areas exposed to full sunlight. Yet in the current study, the range of photon flux densities tested did not seem to effect peduncle length.

Isolate 551, referable to V. terrestris or V. frigida on the basis of the number of oogonia per gametophore and their orientation, had

peduncles similar in length to those illustrated for all species in the V. prona complex (Table 4.4). The peduncles were up to 430 µm long, but in some treatments (e.g. soil/water and ASP-V 1% agar at photon flux densities of 6-120 µmol/m^2/s) all were < 150 µm long. High photon flux densities and temperatures, liquid media, and high salinities appeared to induce a larger range of values. The length of peduncles, therefore, appears to be a poor character for separating species in the V. prona complex.

The distal diameter of peduncles in isolate 551, 41-80 µm, was similar to that illustrated by Christensen (1969) for V. frigida, but field population MEL 1049888 had peduncles 31-49 µm in diameter and oogonia orientated and shaped like those of V. frigida. There was no correlation (corr. coeff. = 0.24) between peduncle diameter and length in isolate 551; some peduncles had almost parallel lateral walls (Fig. 4.18), like V. terrestris, while most were expanded distally (Fig. 4.15), like V. frigida. The peduncles of isolates 502 and 544 usually had parallel lateral walls (Figs 4.5, 4.6, 4.8, 4.9), but some peduncles (Fig. 4.10) were expanded distally. The shape of peduncles, therefore, cannot be used either quantitatively or qualitatively to distinguish species in the V. prona complex.

NUMBER OF OOGONIA PER GAMETOPHORE: Christensen (1970) characterised Vaucheria racemosa as having (2-3)-4-(-6) oogonia per gametophore, V. prona as normally having two, and V. frigida and V. terrestris as always having one. Despite the variability within some species, Christensen (1970) considered the number of oogonia per gametophore as diagnostic for these taxa. Similarly, Blum (1972)

described the gametophores of V. racemosa as having 2-4(-7) oogonia, those of V. prona as having (1-)2(-3) oogonia, and those of V. frigida as always having one oogonium.

Isolate 551 from south-eastern Australia almost always had one oogonium per gametophore (in over 350 gametophores examined, only three had 2 oogonia), and thus would be referable to V. frigida or V. terrestris on the basis of Christensen (1970). The number of oogonia per gametophore in the other two isolates was more variable (Fig. 4.4): isolate 502 always had 1-2 and isolate 544 had (1-)2-3(-4). These two isolates would be referable to V. prona or V. racemosa, although they rarely had 4 oogonia per gametophore. The number of oogonia per gametophore was not dependent on culture conditions; at 5^{o}C and 12-20 $\mu mol/m^2/s$, for example, replicates of isolate 502 had 100%, 40%, 10% or 0% of gametophores with one oogonium. Both Christensen (1970) and Desroche (1910) reported an increase in the number of oogonia per gametophore in plants referable to the section Racemosae after a period in culture, while Cullinane (1976) found the converse. In the current study, there seemed to be no relationship between time in culture and the number of oogonia per gametophore.

Field populations 18-32, allied with isolate 551 on the basis of oogonial orientation and oospore wall characters, also had constantly one oogonium per gametophore. Of the remaining field populations, some always had one oogonium per gametophore, while others had 1-2, 1-3 or 2-3. The number of oogonia per gametophore, therefore, while characteristic of V. frigida and V. terrestris, seems to have little diagnostic value for species in the V. prona complex.

GAMETANGIAL ORIENTATION: Many authors (see Blum 1953) have used the angle between oogonia and antheridia to distinguish Vaucheria prona from V. frigida. Blum (1953), however, suggested that the structure of the gametophore precluded all but one orientation of oogonia and antheridia. Christensen (1969, 1970) observed that the oogonia in V. prona were initiated laterally from the antheridial pedicel, while the oogonia in V. terrestris and V. frigida were initiated from the dorsal side of the antheridial pedicel and eventually overtopped the antheridium. The arrangement of oogonia in opposite rows along the peduncle in V. racemosa, rather than clustered near the antheridium as in V. prona, is also a widely used taxonomic character (e.g. Christensen 1969; Blum 1972; Rieth 1980b, as V. walzii).

The orientation of gametangia could be used to distinguish two groups of plants in the south-eastern Australian culture isolates and field populations. Care has to be taken, however, when describing oogonial orientation, as oogonia are often displaced during slide preparation. In isolates 502 and 544, one or two oogonia were borne laterally to the antheridium (Figs 4.5, 4.6, 4.8-4.11, 4.14). If present, the third oogonium was lateral (Fig. 4.6) or distal (Fig. 4.13) to the antheridium and the fourth, usually lateral (Fig. 4.7). The oogonial pedicels in isolate 544 were erect to transverse to the peduncle (Figs 4.8, 4.10, 4.14), while those in isolate 502 were pendent (Figs 4.5-4.7, 4.11), transverse to the peduncle (Fig. 4.13), or in the case of a third oogonial pedicel, sometimes erect (Fig. 4.13). The long-axis and fertilisation pore of oogonia in both isolates were directed towards the peduncle: towards the siphon in isolate 544 (Figs 4.8-4.10, 4.14) and usually away from it in isolate 502 (Figs 4.5-4.7, 4.11). Field populations 1-17 had oogonia orientated like those of

isolate 502, isolate 544 or intermediate between the two, and no distinctions could be made among the orientations. Furthermore, the published illustrations of V. prona (Götz 1897, figs 31, 32; Rieth 1980b, fig. 39; both as V. hamata; Blum 1972, figs 53, 55; Christensen 1969, fig. 10) include plants with oogonia orientated similar to those observed in both isolates 502 and 544.

The oogonia in isolate 551 and field populations 18-32 always had the fertilisation pore directed towards the siphon (Fig. 4.15) and often through the middle of the circinate antheridium (Figs 4.16, 4.21). The oogonial long-axis was transverse to the peduncle (Fig. 4.15) or sometimes slightly pendent, but always distal to the antheridium with the oogonial pedicel developing from the dorsal side of the antheridium. In published illustrations of V. frigida (Blum 1972, figs 44, 45; Christensen 1969, fig. 9), the oogonium is always borne distally to the antheridium. The development of an adventitious gametophore, however, can displace the oogonium laterally (see Fig. 4.16). Sometimes a sequence of adventitious gametophores can leave a row of antheridia terminated by a single oogonium (Fig. 4.19). The antheridia seldom curved in a plane parallel to the peduncle (but see Fig. 4.20), as in isolates 502 and 544 (Figs 4.5, 4.9), but were usually almost transverse to the peduncle (Figs 4.15, 4.19).

Vaucheria racemosa has oogonia and oogonial pedicels borne transverse to the peduncle, like those of isolate 502, but in opposite rows along the peduncle (Christensen 1969, fig. 12). In gametophores of isolate 502 with four oogonia (Fig. 4.7), the oogonial pedicels arose from near or distally to the antheridium. No plants examined had > 4 oogonia per gametophore, and only rarely were there 4. Thus the orientation of oogonia in V. racemosa needs to be studied further in

material which regularly has > 3 oogonia per gametophore.

The value of oogonial orientation as a diagnostic character of V. terrestris is also uncertain. According to Christensen (1969), V. terrestris has oogonia and antheridia similarly arranged to those of V. frigida. In illustrations of V. terrestris (Christensen 1969, fig. 8), however, the arrangement of gametangia appears to be intermediate between V. frigida and V. prona. The antheridia are seldom parallel with the peduncle, but the oogonium often tends slightly beyond the antheridium. No south-eastern Australian plants had gametophores similar to those illustrated for V. terrestris, and all collections were referable to V. frigida or V. prona on the basis of gametangial orientation. From the results of the current study, it seems that V. frigida can be distinguished from V. prona in having a single oogonium borne distally rather than laterally to the antheridium.

OOGONIAL PEDICELS: Hoppaugh (1930) used the length of oogonial pedicels to distinguish species in the Vaucheria prona complex, and the two isolates referable to V. prona in the current study appeared to be distinguished by oogonial pedicel length. Cullinane (1976), however, found oogonial pedicel length in some species in the sections Vaucheria [as Anomalae (Hansgirg) Heering] and Racemosae (as Corniculatae subsection Racemosae) to be variable in culture, and Islam (1965) found pedicel length in V. prona (as V. hamata) to be dependent on moisture content and photon flux density in the field.

In the current study the data for pedicel length in the V. prona complex ranged from 10-90 μm, with no disjuctions either in isolates studied in culture, or among field populations (Fig. 4.24). The length of the pedicel did not appear to be dependent on the culture treatment

used. The plotted data (mean ± s.d.) for isolates 502 and 551 overlapped only a little, but the data from isolate 544 extensively overlapped both of the other ranges. Oogonial pedicel length, therefore, seems a poor taxonomic character for distinguishing species in the V. prona complex.

Oogonial pedicel length can also be expressed as a proportion of total oogonial length. Although 544 could generally be distingiushed from 502 using this ratio [oogonial-pedicel-length/oogonial-length = 0.2-0.8-(0.9) and (0.7-)0.8-1.2 respectively], there were no disjunctions in the data from field populations. Isolate 551 had values of 0.1-0.4 for this ratio. The ratio of oogonial pedicel length to oogonial length, therefore, does not appear to be a useful taxonomic character in the V. prona complex.

SIZE AND SHAPE OF OOGONIA AND OOSPORES: The oogonia of Vaucheria frigida were reported by Christensen (1969) to be generally larger than those of V. terrestris, V. prona and V. racemosa, but oogonial or oospore shape has not been used to distinguish species in the V. prona complex.

There were no disjunctions in the data for oogonial length (Fig. 4.25) diameter (Fig. 4.26) or L/D (Fig. 4.27) either in culture isolates or among field populations examined in the current study. Furthermore, the data for oogonial length and diameter overlapped the range of values in the literature (Table 4.5) for all four species in the V. prona complex. None of oogonial length, diameter and L/D, therefore, have any diagnostic value in the V. prona complex. Culture isolates and field populations referable to V. frigida, however, may be characterised by the generally larger oogonia (often > 100 µm). This

is supported by the values given in the published literature (Table 4.5).

The oogonia of isolate 551 were globose to dimidiate-globose, usually with a conical distal protuberance following oospore maturation (Figs 4.15, 4.17). This protuberance probably results from part of the oospore wall extruding from the oogonium (Figs 4.22, 4.23). Rieth (1965b, fig. 33) provided drawings of mature oospores showing this extrusion of the thick oospore wall. Although not all illustrations of V. frigida in the literature (e.g. Christensen 1969, fig. 9; Blum 1972, figs 44, 45) show oogonia with a large distal protuberance, in the current study, this protuberance was much larger than the distal prominence found in isolates 502 and 544 (Fig. 4.12). The oogonia and oospores of 502 and 544 are also less globular; they were usually ovoid-reniform (Figs 4.5,4.10,4.12) and similar to those illustrated for V. prona and V. racemosa in the published literature (e.g. Christensen 1969, figs 10, 12). The oospores of V. terrestris illustrated by Christensen (1969, fig. 8) are dimidiate-globose but have no large distal protuberance; no south-eastern Australian plants had oospores similar to these. The shape of the oospore, therefore, can be used to distinguish isolates referable to V. prona and V. racemosa from those referable to V. frigida.

OOSPORE WALLS: The oospore walls of Vaucheria frigida have been described and illustrated as being thicker than the oospore walls of V. prona (e.g. Blum 1953, Rieth 1980b; both as V. terrestris sensu Götz and V. hamata sensu Götz respectively), and sometimes (Rieth 1980b, fig. 36k) as flaky rather than evenly textured in transverse section.

In the current study, the oospore walls of isolate 551 and field

populations 18-32 were 8-16 µm thick and had a 'flaky' texture in transverse section under light microscopy (Fig. 4.17), while those of isolates 544, 502, and field populations 1-17, were 1-3 µm thick and evenly textured in transverse section (Figs 4.5-4.10). Oospore wall thickness and texture did not seem to vary with culture conditions. No additional oogonial or oospore wall characters which could be used taxonomically were revealed by scanning electron microscopy (see Figs 4.11-4.14, 4.20-4.23).

Vaucheria frigida is characterised by thick and flaky textured oospore walls, while V. prona has thin and smooth textured oospore walls. The oospore wall structure of V. terrestris and V. racemosa has not been described in the literature, but is illustrated (e.g. Christensen 1969, figs 8,12) as relatively thin.

SHAPE OF ANTHERIDIA: At least three methods can be been used to measure the curvature of the circinate antheridial system (= antheridium + antheridial pedicel). The first, considered here to be overly complex, was devised by Blum (1953) and used later by Rieth (1956a, 1965b, 1980b). The curvature is measured as the ratio between the radius of the outer circumference of the antheridial system, r_1 and the radius of the inner circumference, r_2 (see Fig. 1.3). Vaucheria prona (as V. hamata sensu Götz), with tightly coiled antheridia, had a ratio of 3.0-5.0, and V. frigida (as V. terrestris sensu Götz), with laxly coiled antheridia, had a ratio of 1.5-3.0 (Blum 1953). Rieth (1965b), however, found populations of V. frigida (as V. terrestris sensu Götz) with r_1/r_2 = 2-5.

Preliminary measurements from south-eastern Australian plants included values of 2.8-4.7 for isolate 551 and 2.8-5.5(-21) for isolates

544 and 502 (antheridia in isolate 502 was difficult to measure as few were in lateral view). It was often difficult to accurately measure the values r_1 and r_2 consistently, due to the variability in antheridial shape.

From illustrations and descriptions of V. prona and V. frigida, it appears that the length of antheridia (see Fig. 1.3) also gives a measure of the degree of coiling. Blum (1972) reported that V. frigida has antheridia 64-93 µm long, and V. prona 30-65 µm long. [Rieth (1965b) does not give antheridial lengths so the plants with an intermediate r_1/r_2 cannot be compared with these values.] This method was used in the current study, as it is relatively simple to measure and unambiguous. Although it only measures antheridial curvature, the curvature of the antheridium is similar to that of the entire antheridial system.

One requirement for antheridial length to be equivalent to the diameter of the circle through which the antheridium curves is that the antheridia turn through at least 1/2 a circle. The proportion of a circle through which antheridia turn (Fig. 1.3) is the third measure of antheridial curvature. Blum (1972) found that antheridia in V. prona turned through 90-216^{o}, while those of V. frigida turned through 180-270^{o}. This measurement does not adequately distinguish these two species, but indicates that the antheridia of V. prona can turn through less than half a circle (i.e. antheridial length may be shorter than the diameter of the circle through which the antheridia turn). However, as the antheridia of V. prona must be 'shorter' than those of V. frigida if they are more tightly coiled, this will only exaggerate the difference between the two species, possibly making antheridial length a better taxonomic character.

In south-eastern Australian plants, there were no disjunctions in the data for antheridial length either in isolates studied in culture or among field populations (Fig. 4.28). There also seemed to be no other differences between the antheridia (cf., Figs 4.9 & 4.15), and thus antheridial curvature and shape seems to be a poor specific character in the V. prona complex.

HABITAT: Vaucheria terrestris was described as Ectosperma terrestris Vaucher (1803) from plants growing on moist soil. Christensen (1969) found that V. terrestris grew mostly mixed with V. prona on arable land in the area surrounding Geneva, Switzerland (studied earlier by Vaucher). Vaucheria frigida and V. prona, however, were amphibious and occurred in both aquatic and terrestrial habitats. According to Christensen (1969), the aquatic habitat of plants referable to V. frigida would have been sufficient in 1803 for Vaucher to separate them from V. terrestris. No collections made by Christensen (1969) contained both V. terrestris and V. frigida. Blum (1953) confirmed that V. frigida (as V. terrestris sensu Götz) was usually aquatic, and found that V. prona (as V. hamata sensu Götz) was more commonly found on moist soil.

In the current study, plants referable to V. frigida came from both terrestrial to aquatic habitats, while plants referable to V. prona were more often found in terrestrial habitats (including farmland, garden beds and soil well above the high water-level of rivers and drains) than aquatic ones. On the basis of the characters considered previously, V. terrestris is a doubtful species which, if distinct from V. frigida, does not occur in south-eastern Australia. In any case, habitat seems to be a poor taxonomic character since moisture level was

variable at most collection sites.

CONCLUSIONS AND TAXONOMIC IMPLICATIONS: A summary of the results is given in Table 4.6. The length and diameter of peduncles, the diameter of vegetative siphons and the thickness of their walls, habitat, oogonial pedicel length and the curvature of antheridia have all been used previously to distinguish taxa in the Vaucheria prona complex, but none were found to be of taxonomic value in the current study. The length of oogonia had no diagnostic value, but may be useful in a key including V. frigida. The number of oogonia per gametophore was also found to be characteristic of V. frigida, but again of no diagnostic value in the V. prona complex. The thickness and texture of oospore walls in transverse section, oogonial orientation and oospore shape, however, were found to be useful distinguishing characters in the V. prona complex.

As a result of these studies, therefore, two species, V. prona and V. frigida, are recognised in south-eastern Australia (for diagnostic features see Table 4.7). Vaucheria prona includes plants which range between two growth forms. The first, represented by isolate 544, has shorter oogonial pedicels, usually < 0.8 times the length of the oogonium, and 1-2 oogonia per gametophore (Figs 4.8-4.10,4.14). The second, represented by 502, has oogonial pedicels usually > 0.8 times as long as the oogonium, and (1-)2-3(-4) oogonia per gametophore (Figs 4.5-4.7, 4.11-4.13). On the basis of intermediate field populations and the variation of characters in culture, however, these two growth forms are not considered worthy of taxonomic recognition.

Vaucheria frigida includes plants with a large variation in the size of oogonia and peduncles, but only one distinct entity was

recognised in the south-eastern Australia flora (Figs 4.15-4.23). The status of V. terrestris and V. racemosa is still unclear as plants including the full variation reported for these species were not found in south-eastern Australia.

Nomenclatural and historical aspects of V. frigida and V. prona are considered in Chapter 5 [including the disposition of V. terrestris (p.166) and V. racemosa (p.186)].

Table 4.1 Previously used diagnostic features from published accounts (Blum 1953, 1972; Christensen 1969) of species in the *Vaucheria prona* Christensen complex.

Table 4.1.

Species	Siphon diameter (µm)	Peduncle length (µm)	Peduncle diameter (µm)[1]	Number of oogonia per gametophore	Oogonial pedicel length (µm)[1]	Oogonial length (µm)	Oogonial diameter (µm)	Antheridial length (µm)	Oogonial long-axis orientation[1]- (T)ransverse to peduncle or (P)endent	Oogonial pedicel orientation[1]- (T)ransverse to peduncle or (E)rect	Oospore wall thickness (µm)	Oogonial L/D
V. prona Christensen	28-60 (-110)	usually > 300	25-40	(1-)2 (-3)	25-70	43-94	42-78	30-65	T,P	T,E	1-2	1.0-1.4(1.5)[1]
V. terrestris[1] (Vaucher) de Candolle	30-50	70-350	30-45	1	20-50	90-100	65-75	≈ 60	T(,P)	T,E	?	1.1-1.3
V. frigida (Roth) C.Agardh	42-120	65-100[1]	50-65	1	30-50	106-165	80-135	64-93	T	E	2-5	1.1-1.2[1]
V. racemosa (Vaucher) de Candolle	42-126	usually < 300	25-40	2-6 (-7)	25-65	63-93	55-78	48-88	H,P	T(,E)	?	1.0-1.3

[1] Calculated from illustrations.

Table 4.2 Collection details for field populations and isolates used to evaluate taxonomic characters in the *Vaucheria prona* complex.

Table 4.2.

Collection or isolate number	Herbarium number	Collection details (all collected by Entwisle)
1.	MEL 1049238 p.p.	Gunbower Creek, Gunbower, Victoria, 20.vii.1985.
2.	MEL 1049239 p.p.[1]	Gunbower Creek, Gunbower, Victoria, 20.vii.1985.
3.	MEL 1049240 p.p.	Salt Creek, Berri, South Australia, 2.x.1984.
4.	MEL 1049241	Falls Creek Township, Victoria, 16.x.1984.
5.	MEL 1049242	Royal Botanic Gardens, South Yarra, Victoria, 31.viii.1983.
6.	MEL 1049243	Iguana Creek, Bairnsdale-Cobbannah Road, Victoria, 18.x.1984.
7.	MEL 1049244	Livingstone Creek, Omeo, Victoria, 17.x.1984.
8.	MEL 1049245	Bulga National Park, Victoria, 19.viii.1984.
9.	MEL 1049246	Clifton Hill, Victoria, 2.vi.1984.
10.	MEL 1049247	Yarriambiack Creek, Warracknabeal, Victoria, 5.viii.1984.
11.	MEL 1049248 p.p.	Diamond Creek, Diamond Creek Township, Victoria, 28.iv.1983.
12.	MEL 1049249 p.p.	Lake Glenmaggie, Victoria, 18.viii.1984.
13.	MEL 1049250 p.p.	Bool Lagoon tributrary, Apsley, Victoria, 2.x.1984.
14.	MEL 1049251	Murray River, Renmark, South Australia, 2.x.1984.
15.	MEL 1049252	Boundary Creek Swamp, Gellibrand-Colac Road, Victoria, 28.viii.1984.
16.	MEL 1049253	German Creek, Mt Beauty-Bright Road, Victoria, 16.x.1984.
17.	MEL 1049254 p.p.	Charlies Creek, Lavers Hill-Colac Road, Victoria, 28.viii.1984.
18.	MEL 1049255 p.p.	Irrigation channel, Sea Lake-Woomalang Road, Victoria, 4.viii.1984.
19.	MEL 1049256	Irrigation channel, Swan Hill-Sea Lake Road, Victoria, 4.viii.1984.
20.	MEL 1049257	Third Lake, Kerang, Victoria, 20.vii.1984.
21.	MEL 1049258 p.p.	Glenelg River, Nelson, 3.x.1984.
22.	MEL 1049259 p.p.	Running Creek, Happy Valley, Victoria, 16.x.1984.
23.	MEL 1049260	Murray River, Merbein-Mildura Road, Victoria, 2.x.1984.
24.	MEL 1049261	Lake Corangamite, Wool Wool, Victoria, 28.viii.1984.
25.	MEL 1049262	Lake George, Beachport, Victoria, 3.viii.1983.
26.	MEL 1049263	Lake Connewarre, Barwon Heads, Victoria, 15.vi.1983.
27.	MEL 1049888	Piccaninnie Ponds, South Australia, 3.viii.1983.
28.	MEL 1049889	The Coorong, Woods Well, South Australia, 8.viii.1983.
29.	MEL 1049264	Campbells Creek, Yapeen, Victoria, 9.viii.1983.
30.	MEL 1049265	Lake Beeac, Victoria, 29.viii.1983.
31.	MEL 1049266	Corringle Creek, Corringle-Newmerella Road, Victoria, 1.x.1983.
32.	MEL 1049267	Campbells Creek, Yapeen, Victoria, 17.ix.1983.
551.	MEL 1049268	Avoca River, Elmhurst-Ampitheatre Road, Victoria, 12.vi.1984.
502.	MEL 1049269	Flowerdale Mineral Springs, Victoria, 24.i.1984.
544.	MEL 1049233 p.p.	Royal Botanic Gardens, South Yarra, Victoria, 4.vi.1984.

[1]Taken from older culture of previous collection.

Table 4.3 Culture treatments used to evaluate taxonomic characters in the *Vaucheria prona* complex.

Table 4.3.

Treatment number	Temperature (°C)	Photon flux density ($\mu mol/m^2/s$)	% Agar	Salinity (o/oo)
1.	5	6-10	1	0.3
2.	10	6-10	1	0.3
3.	15	6-10	1	0.3
4.	20	6-10	1	0.3
5.	5	12-20	1	0.3
6.	10	12-20	1	0.3
7.	15	12-20	1	0.3
8.	20	12-20	1	0.3
9.	5	35-50	1	0.3
10.	10	35-50	1	0.3
11.	15	35-50	1	0.3
12.	20	35-50	1	0.3
13.	15-20	110-130	1	0.3
14.	15	30-40	0.5	2.0
15.	15	30-40	0	2.0 (isolate 551 - 0.3)
16.	15	30-40	0[1]	2.0 (isolate 551 - 0.3)
17.	15	30-40	1 & 0[2]	2.0 (isolate 551 - 0.3, isolate 502 - 6.5)
18.	15	30-40	1	0.3 with $\frac{1}{2}$P & $\frac{1}{2}$N
19.	15	30-40	1	6.5
20.	15	30-40	1	4.0
21.	15	30-40	1	2.0
22.	15	30-40	1	0.3
23.	15	30-40	1	Soil/water media (Stein 1973, p.22)

[1] Aerated.

[2] Solid agar media with liquid media on top (approximately 1 cm of each).

Table 4.4 Peduncle length (μm) of species in the Vaucheria prona complex from selected published accounts.

Table 4.5 Length and diameter of oogonia (μm) in the Vaucheria prona complex from selected published accounts.

Table 4.4.

Reference	V.prona	V.terrestris	V.frigida	V.racemosa
[1]Blum 1953	40–167	–	67–200	7–80
Blum 1972	usually > 300	–	–	usually < 300
[1,2] Rieth 1965b	100–200	–	50–150	50–100(–250)
[1]Christensen 1969, 1970	50–200	150–400	50–150	50–200

Table 4.5.

Reference	V.prona		V.terrestris		V.frigida		V.racemosa	
	length	diameter	length	diameter	length	diameter	length	diameter
Blum 1972	43–94	42–78	–	–	106–165	80–135	63–93	55–78
[2]Hoppaugh 1930	55–100	50–95	–	–	80–165	70–160	–	–
[3]Heering 1907	75–90	60–80	–	–	82.5–211	60.5–103	71.5–90	63–77
[1]Christensen 1969	–	–	90–100	70–80	100–130	80–100	–	–

[1] From illustrations.

[2] V.frigida as V.terrestris sensu Götz, V.racemosa as V.walzii, V. prona as V. hamata sensu Götz.

[3] V.frigida as V.terrestris Lyngbye emend Walz, V.prona as V.hamata, V.racemosa as V.uncinata (see Christensen 1969 for details on these synonymies).

Table 4.6 Summary of features evaluated in *Vaucheria prona* and *V. frigida*.

Table 4.6.

Species	Siphon diameter (µm)	Peduncle length (µm)	Peduncle diameter (µm)	Number of oogonia per gametophore	Oogonial pedicel length (µm)	Oogonial length (µm)	Oogonial diameter (µm)	Antheridial length (µm)	If one oogonium; (D)istal or (L)ateral to antheridium ?	Oospore wall thickness (µm)	Oogonial L/D	Oospore wall in transverse section - (F)laky or (E)venly textured	Oospore shape
V. prona	19-125	48-432 (-685)	24-79 (-96)	1-3 (-4)	12-96 (-115)	48-101	(36-) 48-82 (-94)	38-65 (-77)	L	1.5-4	1.0-1.5	E	ovoid-reniform with small distal prominence
V.frigida	29-62 (-86)	20-340 (-670)	(31-) 41-72 (-85)	1	12-38 (-48)	72-158	58-144	38-67 (-79)	D	8-16	1.0-1.4	F	dimidiate-globose with large conical protuberance

Table 4.7 Diagnostic features of *Vaucheria*. *prona* and *V. frigida* (Roth) C. Agardh from the current study.

Table 4.7.

Species	Oospore wall		If one oogonium per gametophore; lateral or distal to antheridium ?	Oospore shape
	Transverse section	Thickness (µm)		
V. prona	evenly textured	< 5	lateral	ovoid-reniform with small distal prominence
V. frigida	flaky	> 5	distal	dimidiate-globose with large conical protuberance

Fig. 4.1 Siphon diameter in culture isolates 502, 544 and 551 (see Table 4.3 for treatment details) and field populations (see Table 4.2 for collection details).

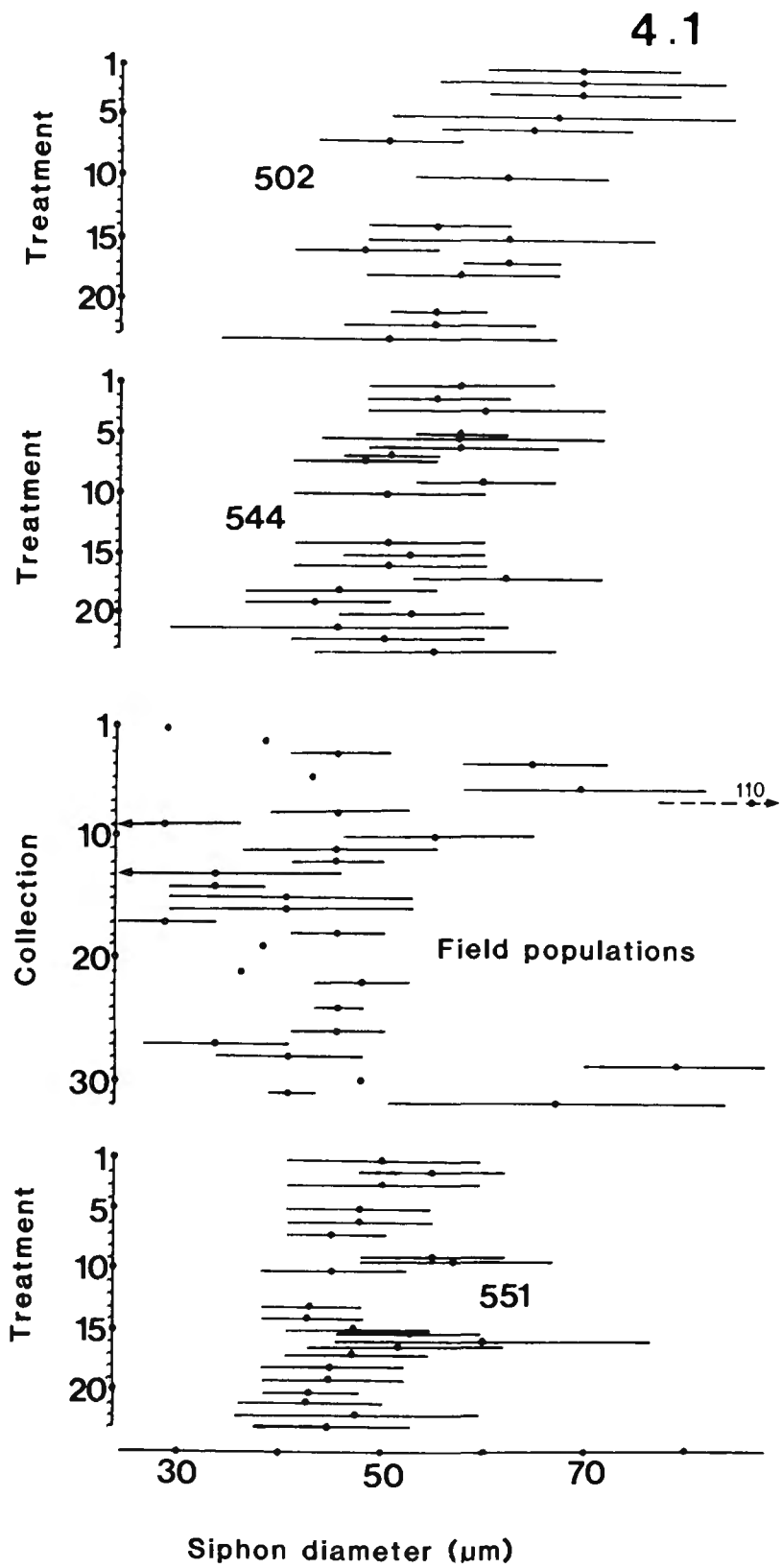

4.1
Treatment
1
5
10
15
20
502
Treatment
544
Collection
110
Field populations
30
Treatment
551
30
50
70
Siphon diameter (µm)

Fig. 4.2 Peduncle length in culture isolates 502, 544 and 551 (see Table 4.3 for treatment details) and field populations (see Table 4.2 for collection details).

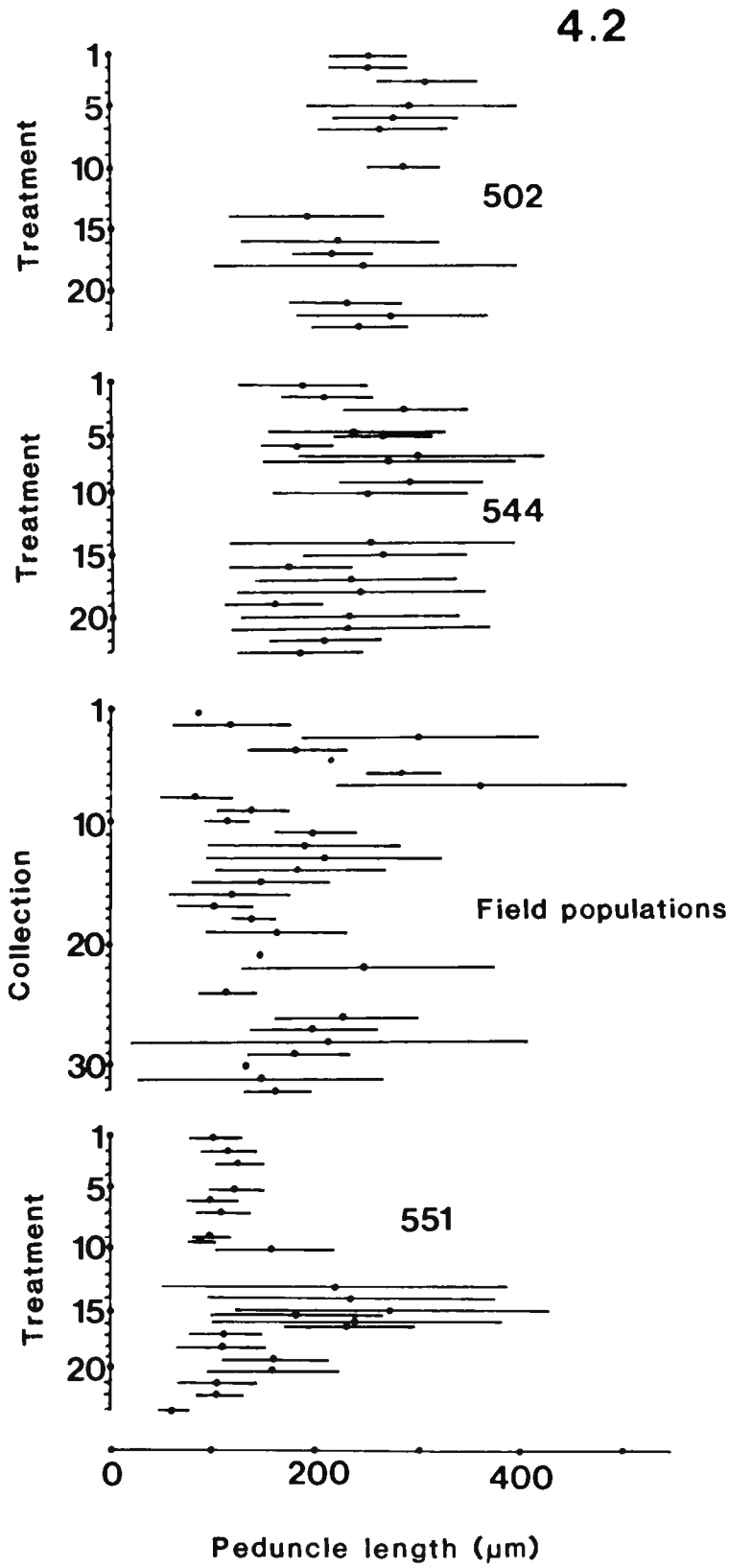

4.2
Treatment
1
5
10
15
20
502
Treatment
544
Collection
30
Field populations
551
0
200
400
Peduncle length (μm)

Fig. 4.3 Peduncle diameter in culture isolates 502, 544 and 551 (see Table 4.3 for treatment details) and field populations (see Table 4.2 for collection details).

4.3

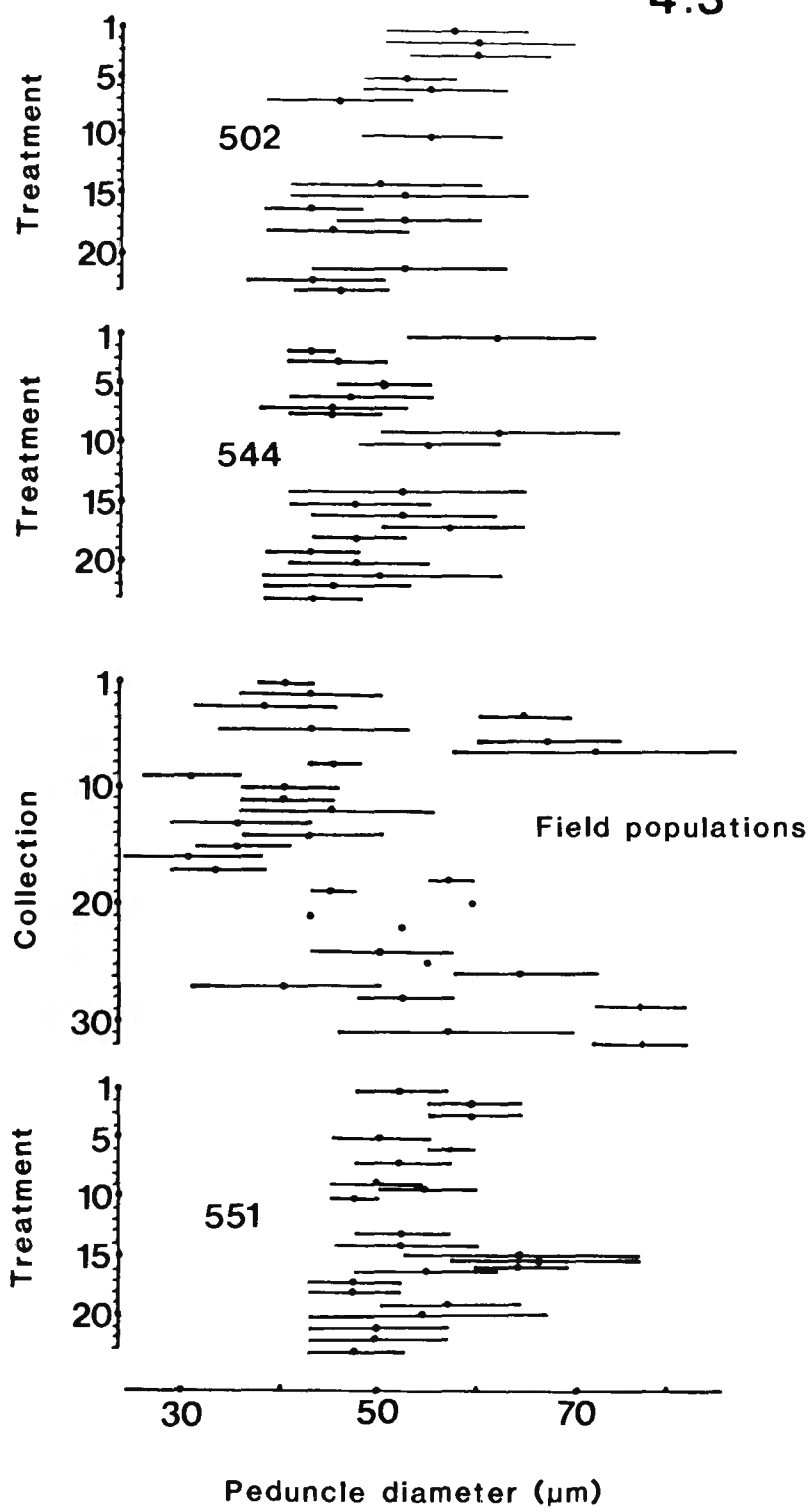
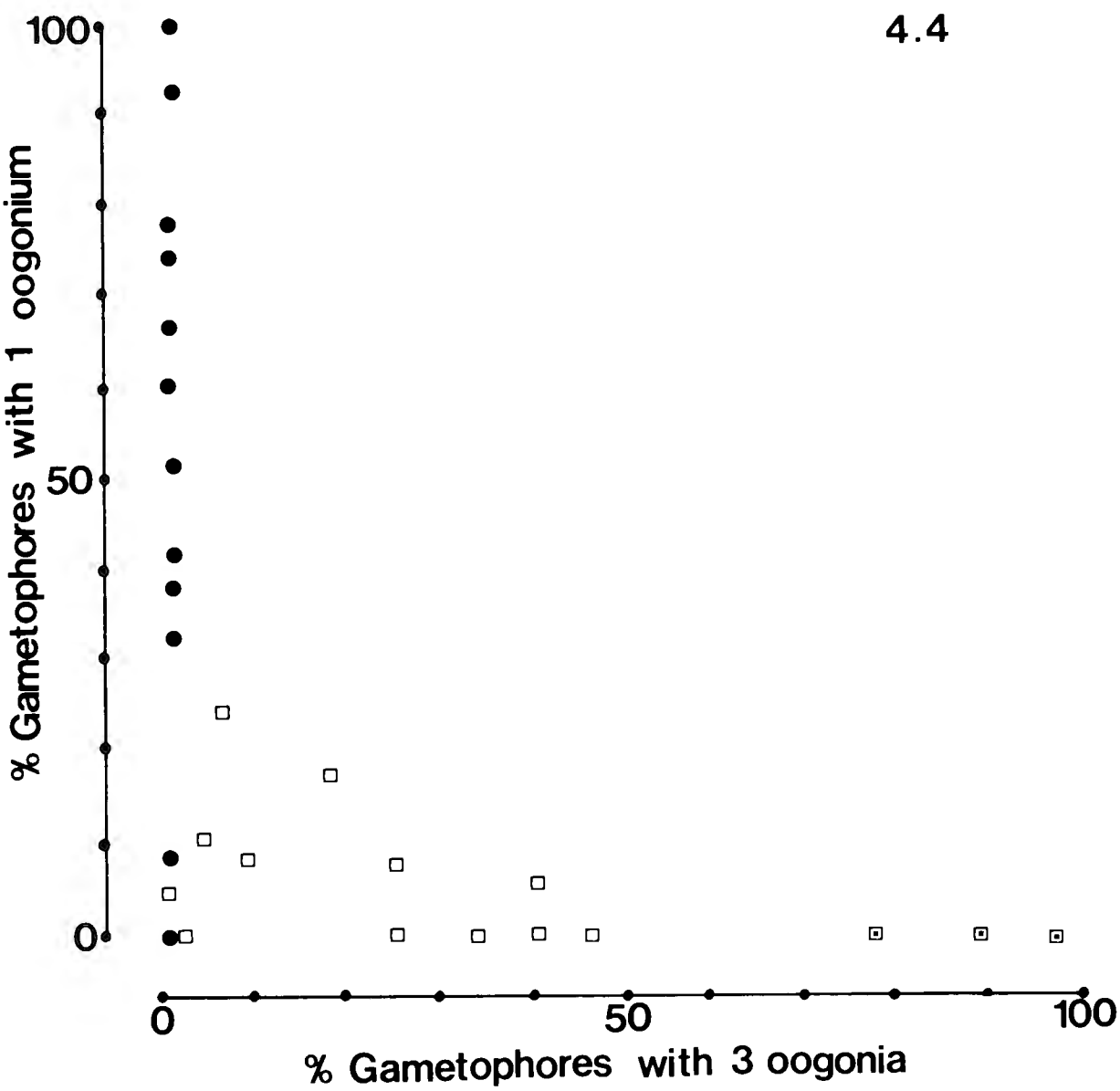

Treatment
1
5
10
15
20
502
Treatment
544
Collection
30
Field populations
551
30
50
70
Peduncle diameter (μm)

Fig. 4.4 Percentage of gametophores with one and three oogonia (remainder have two oogonia) in isolates 502 (□) and 544 (●) studied in culture. In some treatments (⧈), isolate 502 had 6-26% of gametophores with four oogonia (included in the percentage of gametophores with three oogonia).

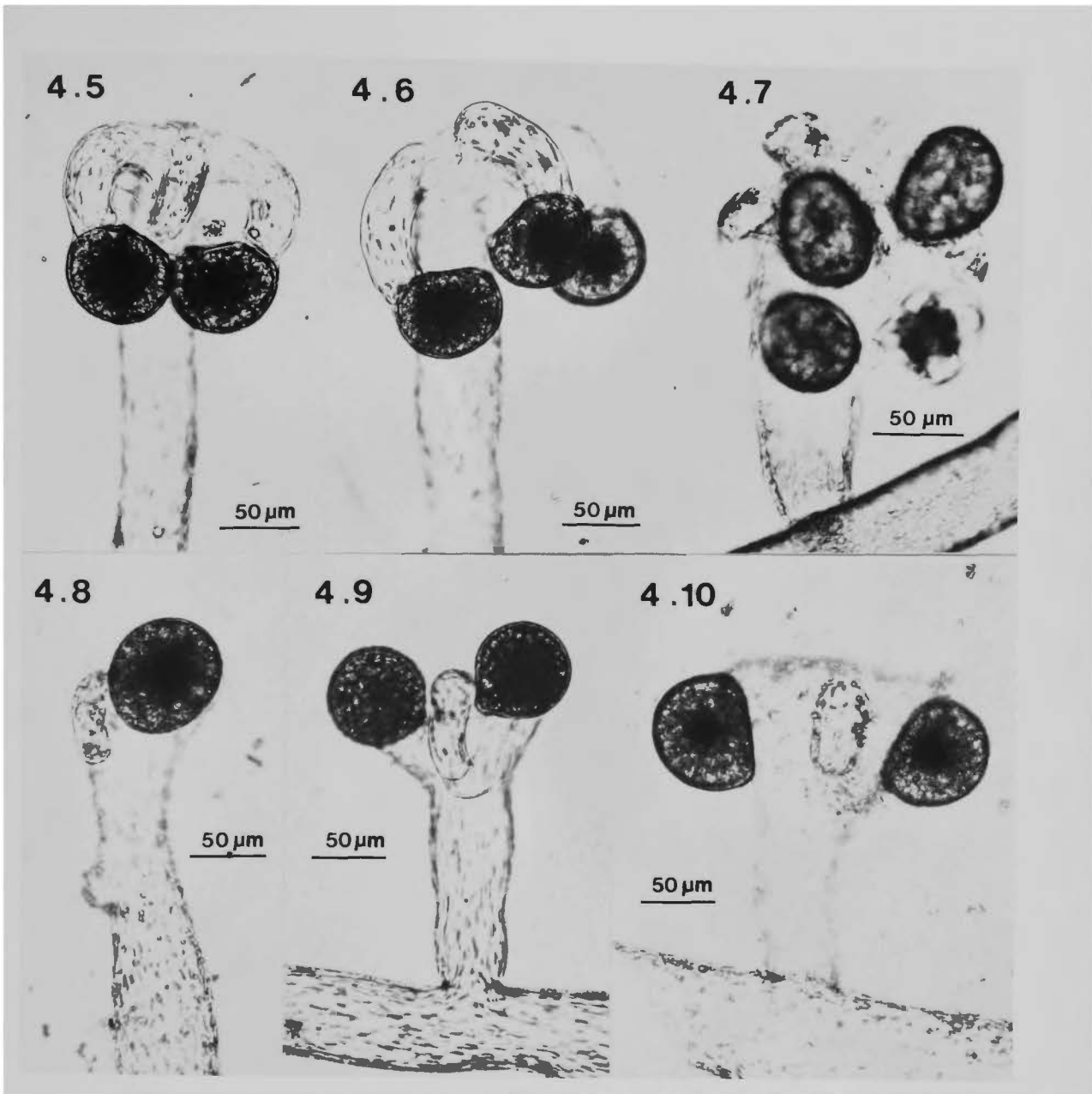

4.4
100
50
0
% Gametophores with 1 oogonium
0
50
100
% Gametophores with 3 oogonia

Figs 4.5-4.10 Vaucheria prona Christensen. Note the variation in orientation of oogonia and oogonial pedicels, and thin evenly textured oospore walls.

Figs 4.5-4.7 Gametophores from isolate 502.

Figs 4.8-4.10 Gametophores from isolate 544.

Fig. 4.10 Gametophore also shows peduncle expanded distally.


4.5
50 µm
4.6
50 µm
4.7
50 µm
4.8
50 µm
4.9
50 µm
4.10
50 µm

Figs 4.11-4.14 Vaucheria prona Christensen.

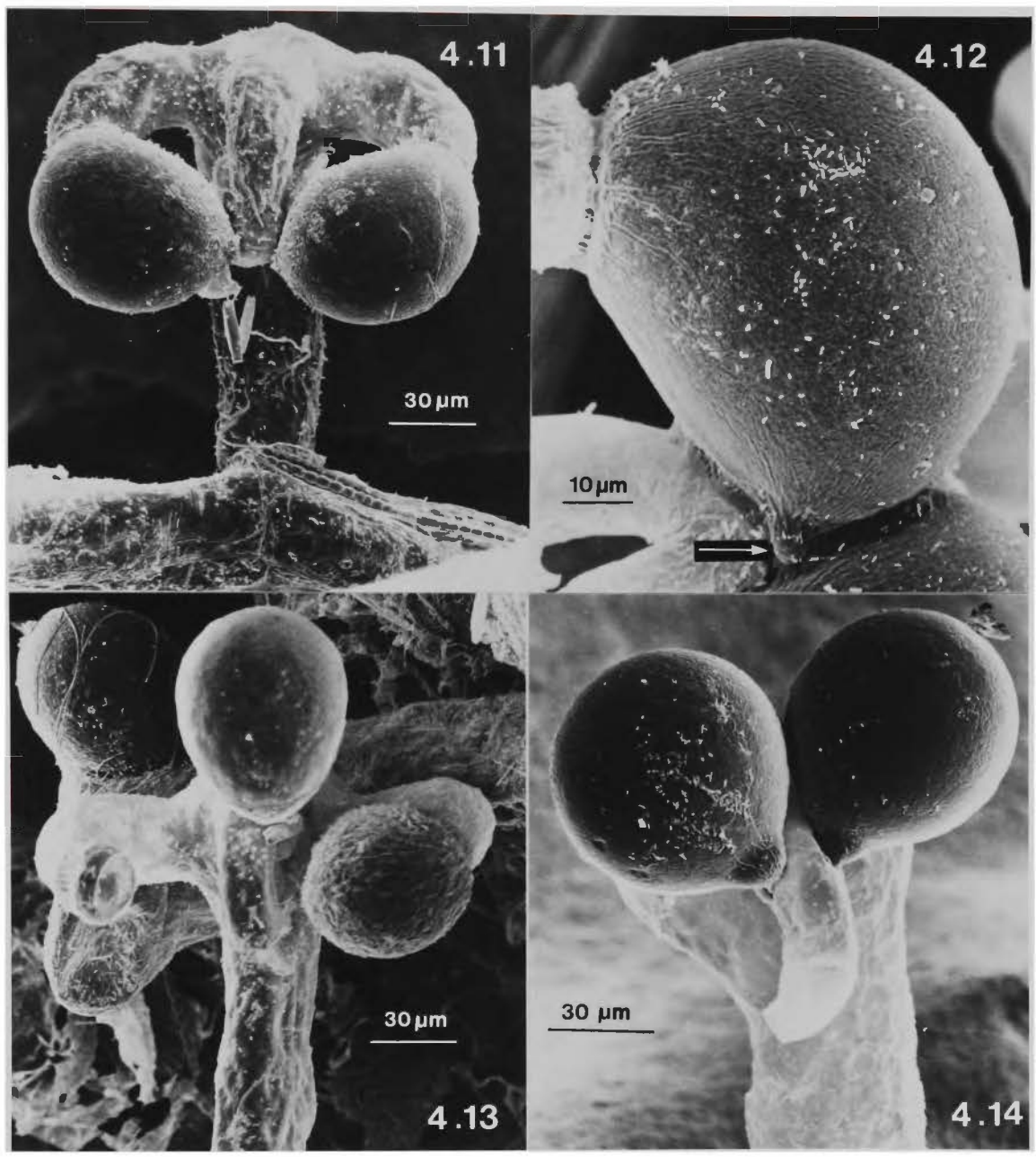
Figs 4.11-4.13 Isolate 502.

Fig. 4.11 Gametophore showing orientation of oogonia and oogonial pedicels.

Fig. 4.12 Ovoid-reniform oogonium with small distal prominence (arrow).

Fig. 4.13 Gametophore showing oogonial and oogonial pedicel orientation.

Fig. 4.14 Isolate 544, showing oogonial orientation.


4.11
30 µm
4.12
10 µm
30 µm
4.13
30 µm
4.14

Figs 4.15-4.19 *Vaucheria frigida* (Roth) C. Agardh.

Figs 4.15,4.16 Isolate 551 in agar.

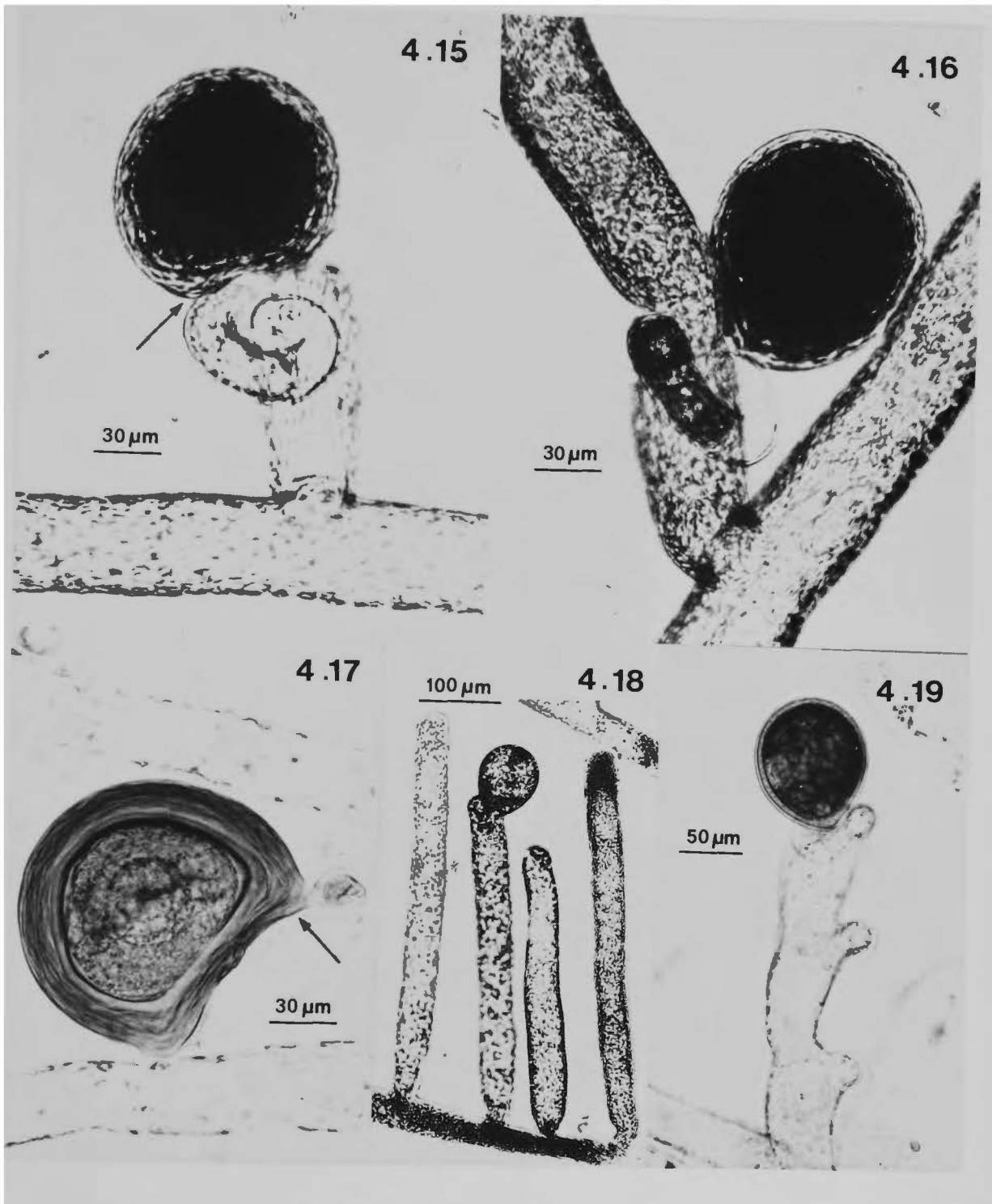
Fig. 4.15 Gametophore showing oogonium borne distally to antheridium. Note antheridium is almost transverse to the peduncle and oogonial pore (arrow) directed towards siphon.

Fig. 4.16 Gametophore showing oogonia pushed laterally by adventitious gametophore.

Fig. 4.17 Dimidiate-globose oospore showing flaky texture of oospore wall in transverse section and distal conical protuberance (arrow). MEL 1049888.

Fig. 4.18 Gametophore showing peduncle with almost parallel lateral walls. Isolate 644 in agar.

Fig. 4.19 Two adventitious gametophores with only a terminal oogonium still attached. MEL 1049889.


4.15
30 µm
4.16
30 µm
4.17
30 µm
100 µm
4.18
4.19
50 µm

Figs 4.20-4.23 Vaucheria frigida (Roth) C. Agardh. Isolate 551.

Fig. 4.20 Gametophore showing oogonial pedicel arising from dorsal side of antheridium.

Fig. 4.21 Gametophore showing oogonium with distal conical protuberance directed through the middle of a circinate antheridium.

Fig. 4.22 Oogonium showing distal conical protuberance of oospore. Note what is probably the oogonia wall (large arrow) and the oospore wall (small arrow).

Fig. 4.23 Oogonium showing slightly reflexed distal protuberance of oospore.

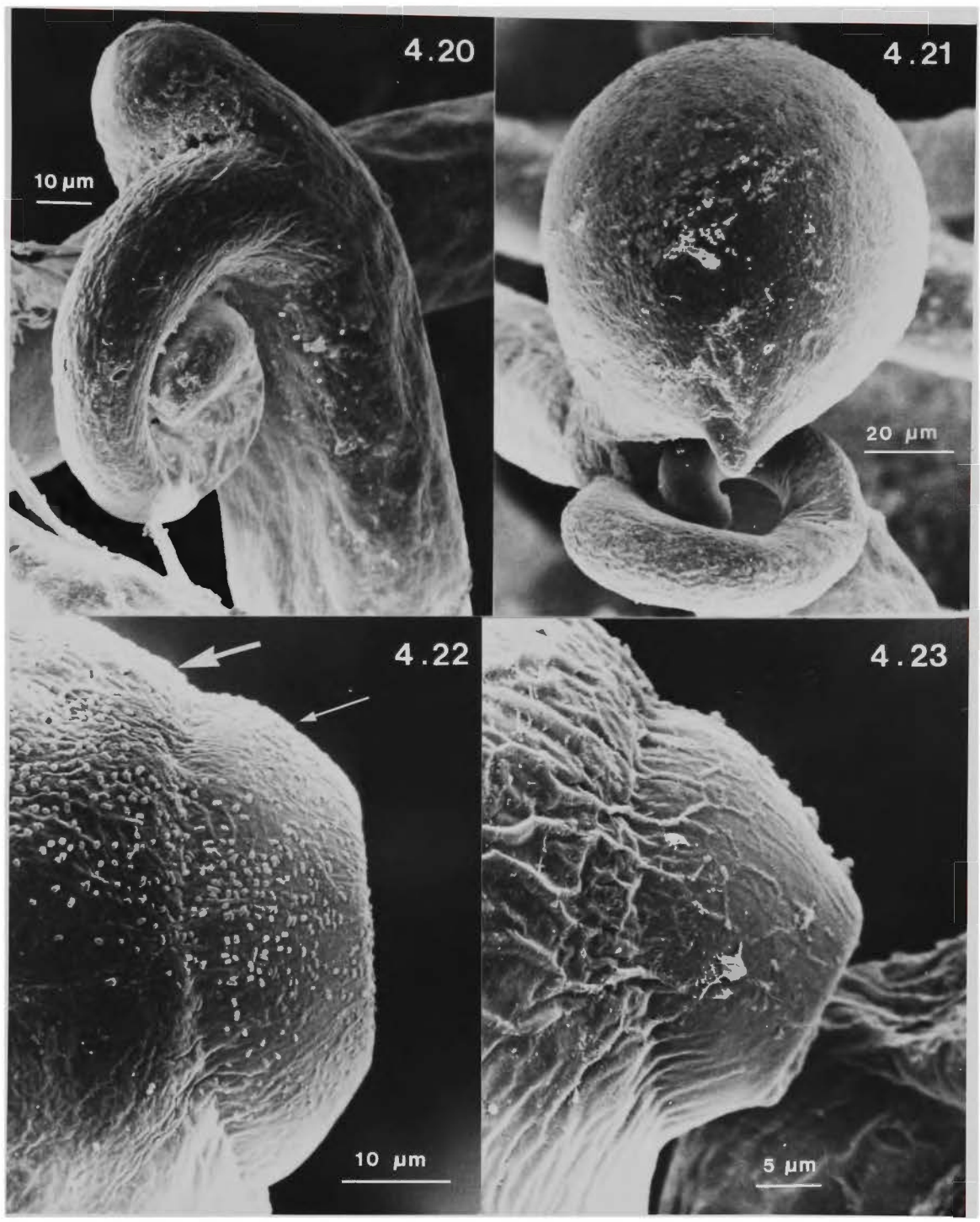

4.20
10 µm
4.21
20 µm
4.22
10 µm
4.23
5 µm

Fig. 4.24 Oogonial pedicel length in culture isolates 502, 544 and 551 (see Table 4.3 for treatment details) and field populations (see Table 4.2 for collection details).

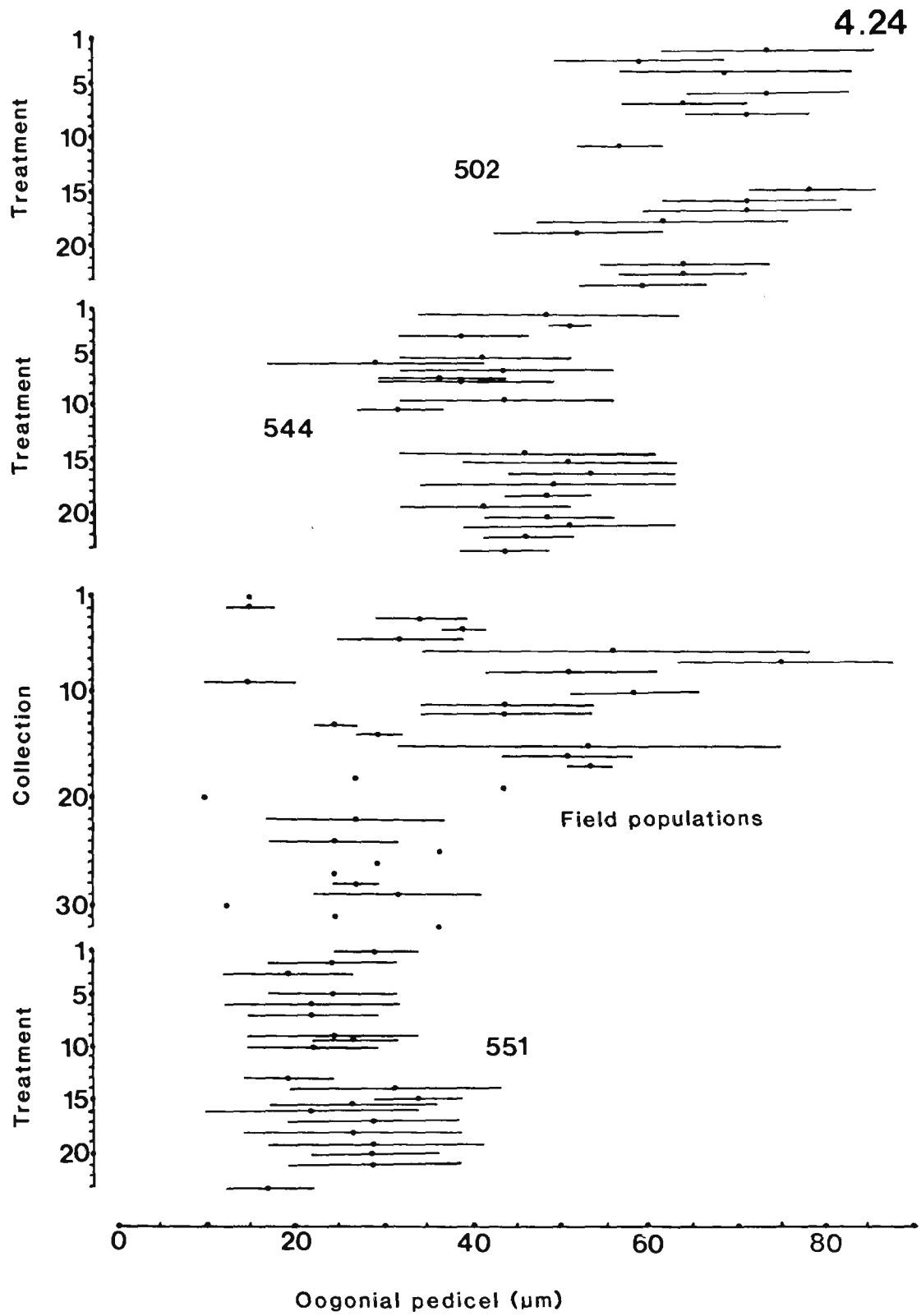

4.24
Treatment
1
5
10
15
20
502
Treatment
1
5
10
15
20
544
Collection
1
10
20
30
Field populations
Treatment
1
5
10
15
20
551
0
20
40
60
80
Oogonial pedicel (µm)

Fig. 4.25 Oogonial length in culture isolates 502, 544 and 551 (see Table 4.3 for treatment details) and field populations (see Table 4.2 for collection details).

4.25

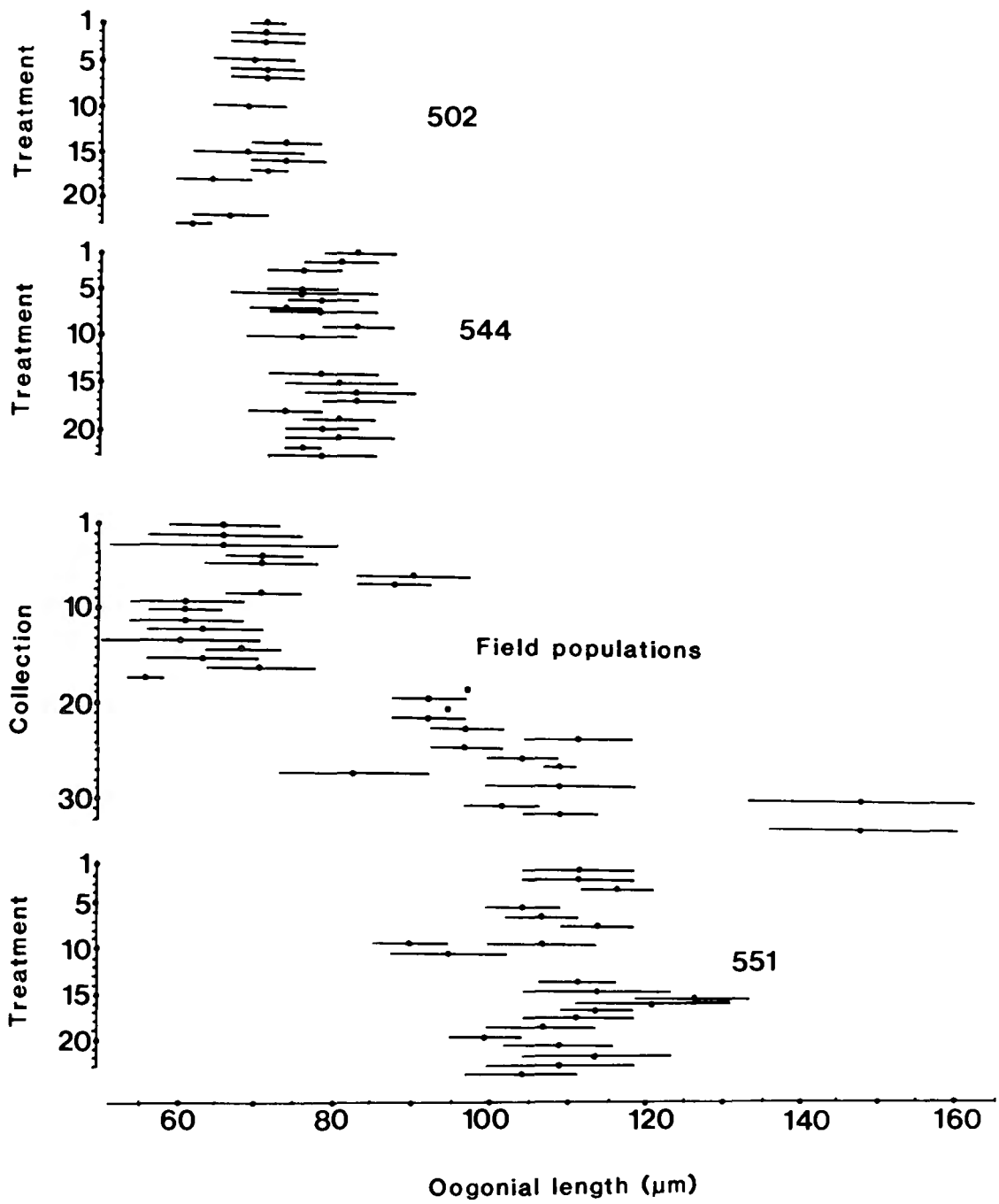

Treatment
1
5
10
15
20
502
Treatment
1
5
10
15
20
544
Collection
1
10
20
30
Field populations
Treatment
1
5
10
15
20
551
60
80
100
120
140
160
Oogonial length (µm)

Fig. 4.26 Oogonial diameter in culture isolates 502, 544 and 551 (see Table 4.3 for treatment details) and field populations (see Table 4.2 for collection details).

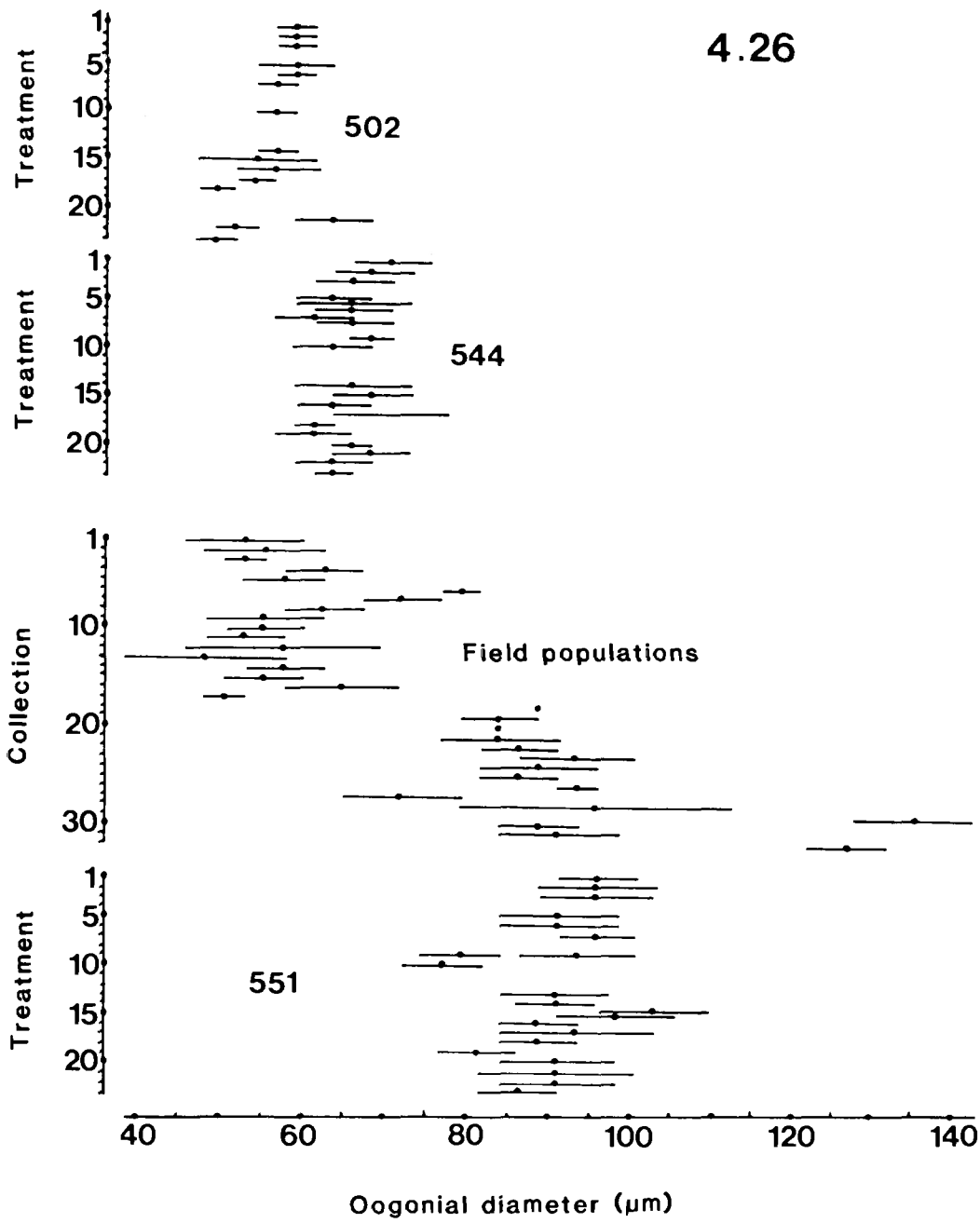

4.26
Treatment
1
5
10
15
20
502
Treatment
1
5
10
15
20
544
Collection
1
10
20
30
Field populations
Treatment
1
5
10
15
20
551
40
60
80
100
120
140
Oogonial diameter (µm)

Fig. 4.27 Oogonial L/D in culture isolates 502, 544 and 551 (see Table 4.3 fortreatment details) and field populations (see Table 4.2 for collection details).

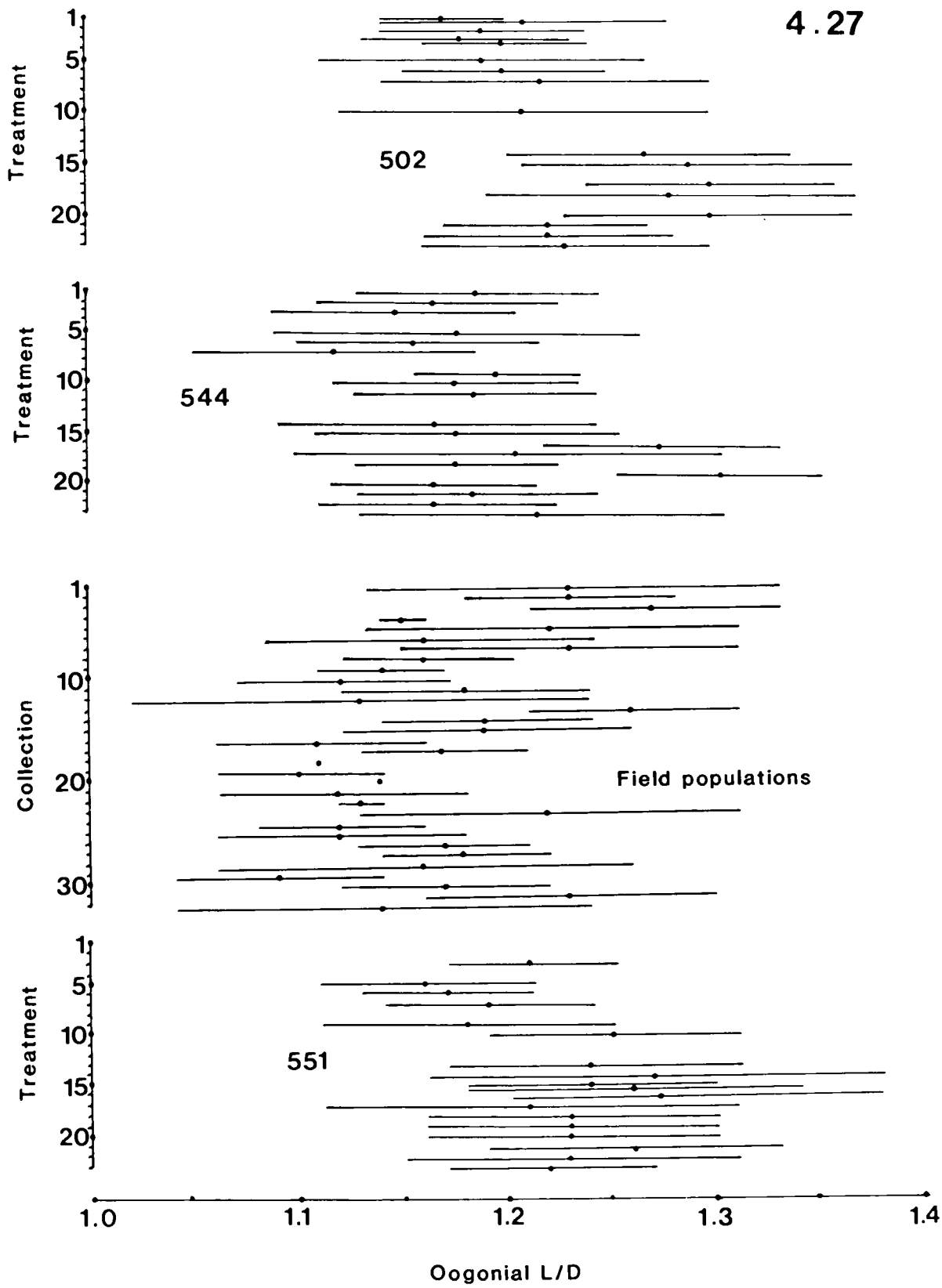
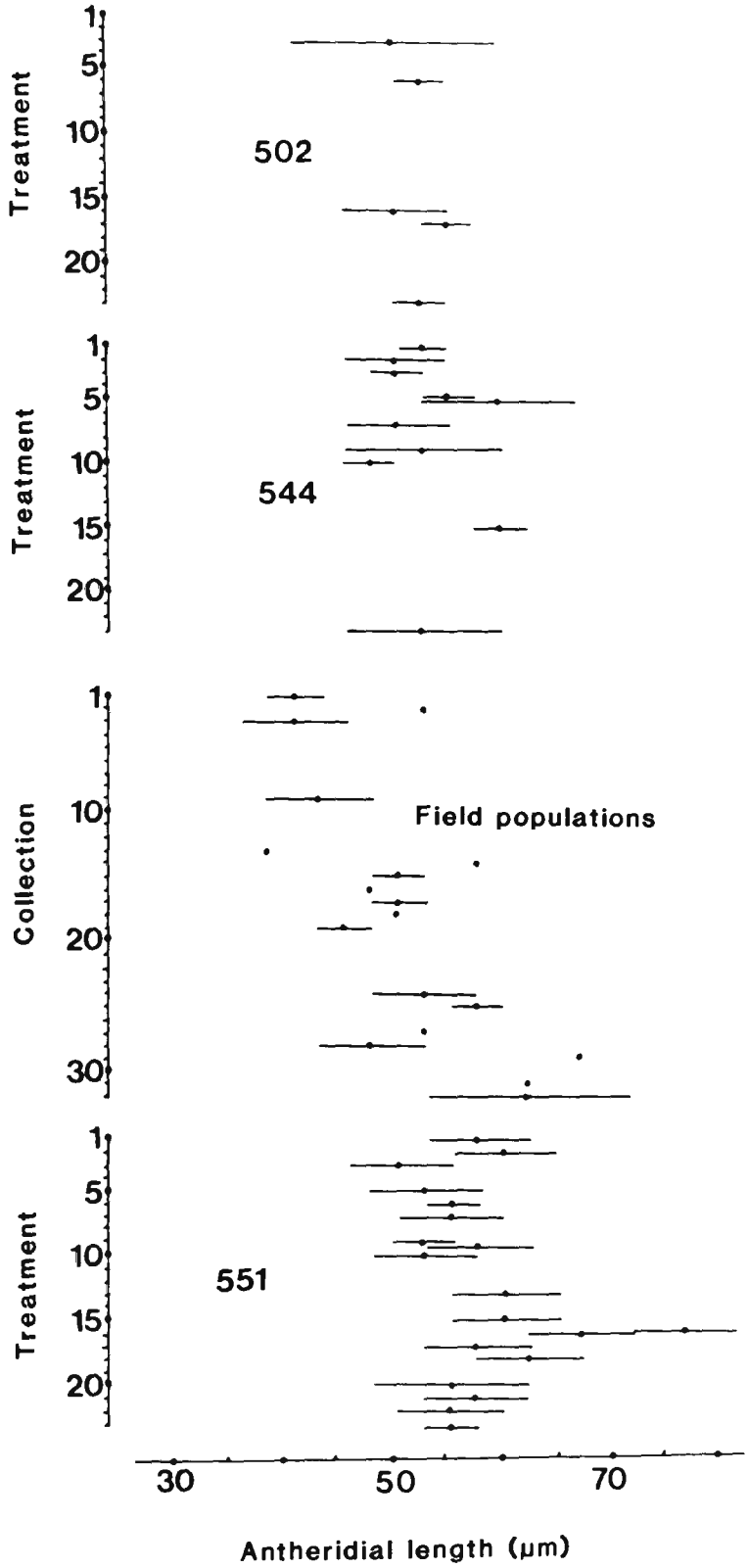

4.27
Treatment
1
5
10
15
20
502
Treatment
544
Collection
30
Field populations
551
1.0
1.1
1.2
1.3
1.4
Oogonial L/D

Fig. 4.28 Antheridial length in culture isolates 502, 544 and 551 (see Table 4.3 for treatment details) and field populations (see Table 4.2 for collection details).

4.28


Treatment
1
5
10
15
20
502
Treatment
1
5
10
15
20
544
Collection
1
10
20
30
Field populations
Treatment
1
5
10
15
20
551
30
50
70
Antheridial length (µm)