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SYSTEMATIC ANALYSIS OF *SPHAEROPLEA* (CHLOROPHYCEAE)[1]

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ABSTRACT

A systematic investigation of the genus Sphaeroplea *was conducted using cladistic analyses of both structural and isozyme characters for the same set of taxa. The structural data were not able to fully resolve some of the taxa while the isozyme data did produce a tree in which all nodes were supported by data. The structural characters were relatively consistent with one another, whereas the isozyme characters were much less internally consistent. Results from independent, cladistic analyses of both data sets support the concept that among those* Sphaeroplea *species investigated,* S. fragilis *Buchheim et Hoffman had an early divergence. The two data sets differed primarily*

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in that the structural data support monophyly of the genus Sphaeroplea *and the isozyme data do not. The greater relative consistency of the structural data suggests better support for trees inferred from its analysis. Furthermore, searches for character congruence between the two data sets revealed isozyme data which support monophyly of the genus* Sphaeroplea, *but had been overwhelmed by conflicting isozyme characters.*

Key index words: Atractomorpha; *Chlorophyceae; cladistic analysis; isozymes; morphology; phylogeny;* Sphaeroplea; *Sphaeropleaceae; systematics*

Studies by Fritsch (1929), Palik (1950), and Ramanathan (1964) represent the most comprehensive taxonomic analyses of the genus *Sphaeroplea*. Species diagnoses in all three of these studies was based primarily on comparative zygote morphology (including differences in zygote size, shape, and cell wall ornamentation), septal morphology, cell width and length, and cytoplasm-chloroplast morphology. Of these characters, zygote morphology was cited as the structural feature most likely to reflect species differences (Fritsch 1929) and was consistently utilized as the single most important means to distinguish species.

More recent investigations describing new taxa (Sarma 1974, Tracanna and Couté 1982, Buchheim and Hoffman 1985, 1987) have expanded the known range of structural variation among species of *Sphaeroplea*. The green algal family Sphaeropleaceae had been considered monotypic prior to these studies, consisting only of the genus *Sphaeroplea*. The description of a new sphaeropleacean genus, *Atractomorpha*, has substantially altered the taxonomy of the family (Hoffman 1983, 1984). Furthermore, scanning electron microscopy (SEM) has led to the characterization of previously undetected features of the zygote wall (Sarma 1974, Tracanna and Couté 1982, Buchheim and Hoffman 1985, 1987, Hoffman 1986, Cambra and Couté 1988, Hoffman and Buchheim 1989).

Addition of new taxa and accumulation of new information regarding morphological variation in *Sphaeroplea* and the Sphaeropleaceae justified a systematic re-examination of the genus *Sphaeroplea* as a whole. However, the assessment of phylogenetic relationships among microscopic organisms presents several challenges. The often small number of morphological features available for study leads to the need for supplementary data sets to help resolve relationships. A biochemical approach frequently used to address systematic and phylogenetic questions at the level of genus and species has been analysis of isozyme and allozyme banding patterns. Although such analyses have often been employed for vascular plants (Gottlieb 1977, Crawford 1983), relatively few systematic investigations of algae have utilized this methodology. The few studies of algal systematics and genetics using isozymes employed a phenetic identity measure to assess similarity between algal taxa (Cheney and Babbel 1978, Blair et al. 1982, Hayhome and Pfiester 1983, Francke and Coesel 1985, Coesel and Menken 1988). We employed here a cladistic analysis of variation in isozyme patterns obtained from haploid vegetative cells in the Sphaeropleaceae to develop hypotheses regarding phylogenetic relationships in *Sphaeroplea*. We could then directly compare cladistic hypotheses from analyses of morphological and isozyme characters derived from the same isolates of the same sphaeropleacean taxa.

MATERIALS AND METHODS

Structural data. The taxa included in this systematic study and their sources are presented in Table 1. Structural characters (Table 2) for each of the taxa are based on published sources (Fritsch 1929, Rieth 1952, Ramanathan 1964, Hoffman 1983, 1984, 1986, 1987, Buchheim and Hoffman 1985, 1987, Hoffman and Buchheim 1989) and more recent original observations of living cells in culture (Buchheim 1988). Techniques of light microscopy (including brightfield, anoptral contrast, and interference contrast optics) were employed to study important vegetative and reproductive features in living material. Living material of *Sphaeroplea africana* Fritsch (Fritsch 1929), *S. chapmanii* Sarma (Sarma 1974), *S. tricarinata* Gauthier-Lièvre (Ramanathan 1964)

TABLE 1. *Sphaeropleacean taxa studied.*

Taxon and authority	Abbreviation	No. isolates	Source and citations
Sphaeroplea annulina (Roth) Agardh	SAN	6	L. R. Hoffman (Denmark): Fritsch (1929), Ramanathan (1964).
S. fragilis Buchheim et Hoffman	SFR	1	R. C. Starr (Lemoncove, CA): Buchheim and Hoffman (1987).
S. robusta Buchheim et Hoffman	SRO	2	H. C. Bold and R. C. Starr (Llano, TX): Buchheim and Hoffman (1985).
S. soleirolii (Duby) Montagne ex Kützing	SSO	4	L. Kies (Germany): Ramanathan (1964).
S. soleirolii var. *crassisepta* (Rieth) Ramanathan	SSC	6	M. Pocock (South Africa): Rieth (1953), Ramanathan (1964).
S. wilmani Fritsch et Rich	SWIU	1	UTEX LB 59 (S. Africa): Fritsch (1929), Ramanathan (1964), Rieth (1952), Starr (1978).
S. wilmani Fritsch et Rich	SWIA	6	E. J. Cáceres (Argentina): Fritsch (1929), Ramanathan (1964), Rieth (1952), Parodi and Cáceres (1986).
Atractomorpha echinata Hoffman	AEC	4	V. Proctor (New Deal, TX): Hoffman (1983).
A. porcata Hoffman	APO	4	R. C. Starr (Lemoncove, CA): Hoffman (1984).
A. sp. 195	A195	1	L. R. Hoffman (S. Africa): Hoffman (1987).
A. sp. 196	A196	1	L. R. Hoffman (S. Africa): Hoffman (1987).

TABLE 2. *Structural characters for* Sphaeroplea *and* Atractomorpha. *See Table 1 for full scientific names for the taxa.*

	SAN	SFR	SRO	SSO	SSC	SWI	AEC	APO	A195	A196
Plant body type	1	1	1	1	1	1	0	0	0	0
Sexual reproduction	2	2	2	2	2	2	0	1	1	1
Site of fertilization	2	2	2	2	2	2	0	1	0	0
Asexual reproduction	1	1	1	1	1	1	0	0	0	0
Cytoplasm/chloroplast	0	0	0	0	1	0	0	0	0	0
Septal type	0	0	0	0	2	1	0	0	0	0
Antheridial type	0	1	1	0	0	0	0	0	0	0
Primary membrane type	0	0	0	0	0	0	0	1	1	1
Zygote wall ornamentation	1	1	1	2	2	1	0	2	1	0
Zygote shape	0	0	0	1	0	0	0	1	0	0
Maximum zygote diameter	0	0	2	1	1	0	0	0	0	0
Zygote appendages	0	1	0	0	0	0	0	1	1	1

and *S. tenuis* Fritsch (Fritsch 1929) was not available. Since data from the literature could not be corroborated by examination of living material, these taxa were deemed inappropriate for inclusion in this investigation.

Electrophoretic data. All taxa (Table 1) were maintained in unialgal culture for use in the isozyme investigation. The algal isolates were maintained at 19–28° C as stock cultures in 125 mL Erlenmeyer flasks containing 60–75 mL of sterile, modified Pocock's medium (Stanker and Hoffman 1979). Cool-white fluorescent lamps supplied a photon flux density of 180–240 $\mu E \cdot m^{-2} \cdot s^{-1}$ on a 12:12 h LD cycle. Stock cultures were transferred into fresh medium each week. Each isolate was sub-cultured into flasks containing fresh, sterile medium 12–14 days prior to protein extraction. Algal material from each flask was apportioned equally into two flasks of fresh, sterile medium 6–7 days prior to extraction. In this manner, large amounts of actively growing algal material with a standard culture history could be harvested for protein extraction.

Vegetative filaments of *Sphaeroplea* were collected by pipet (2–4 g fresh weight), washed once in sterile medium, blotted dry on paper towels, and placed with an equal amount (weight/volume) of a pH 7.5 extraction buffer containing the following: 0.2 M tris, 7 mM boric acid, 2 mM sodium sulfate, 50 mM Na ascorbic acid, 0.4 mM EDTA, 4 mM Na diethyldithiocarbamic acid, 1% (weight/volume) PVP-40, and 0.1% (volume/volume) 2-mercaptoethanol. Each sample of vegetative unicells of *Atractomorpha* was collected by centrifugation (100 × *g* for 5 min at room temperature), washed once in sterile medium, concentrated again by centrifugation, and suspended in equal volumes of the extraction buffer.

Cell material plus cold extraction buffer (2–3 mL) were placed in a conical centrifuge tube and disrupted using an ultrasonicator. Five to ten pulses of 5 sec duration, alternated with 5 sec cooling intervals, were applied to each sample. Cell debris was pelleted by centrifugation (10,000 × *g* for 15 min) at 0° C. The supernatant was decanted and stored at −100° C. Extracts stored for three months could be used without loss of enzyme activity and without detectable changes in isozyme band mobility or intensity.

Isozymes were resolved on either the citrate-morpholine system (pH 6.5 for the gel and pH 6.1 for the electrode buffer) of Nickrent (1986), or the LiOH gel buffer system (pH 8.3) of Selander et al. (1971). Standard methods of starch gel electrophoresis were employed (Shaw and Prasad 1970).

The following enzyme systems were used (each followed by its abbreviation and number, IUBNC 1984): aldolase (ALD; 4.1.2.13), aspartate amino transferase (AAT; 2.6.1.1), isocitrate dehydrogenase (IDH; 1.1.1.42), leucine aminopeptidase (LAP; 3.4.11.-), malate dehydrogenase (MDH; 1.1.1.37), glucose phosphate isomerase (PGI; 5.3.1.9), and shikimate dehydrogenase (SKDH; 1.1.1.25). Standard enzyme staining procedures were adapted from Soltis et al. (1983). Isocitrate dehydrogenase and malate dehydrogenase were resolved on the citrate-morpholine gel buffer; the remaining enzyme systems were resolved on the LiOH gel buffer system.

Absolute mobilities of all isozyme bands were measured and converted to relative mobilities by comparison with a standard (*Sphaeroplea fragilis*) run on each gel. A single band was detected for ALD and LAP. For IDH, PGI and SKDH, two bands were detected in *S. fragilis* and the most anodal (fast) band was used as the standard. For AAT and MDH, the middle of three bands was designated the "100" band.

Data analysis. Cladistic analyses of structural and electrophoretic data from sphaeropleacean taxa were undertaken using PAUP version 2.4 (phylogenetic analysis using parsimony; Swofford 1985). Trees derived from analysis of all systematic data were generated by applying the "branch and bound" algorithm (Hendy and Penny 1982) which guarantees finding the shortest tree or trees.

The ingroup taxa in the cladistic analyses are species of *Sphaeroplea* (Table 1). *Atractomorpha* species were designated the outgroup for cladistic analysis of *Sphaeroplea* (the ingroup) since *Atractomorpha* represents the only sister taxon in the family Sphaeropleaceae (*sensu* Mattox and Stewart 1984). Species of *Atractomorpha* possess structural features which are most likely ancestral for the Sphaeropleaceae (Hoffman 1983) implying possible paraphyly for *Atractomorpha*. These features include the production of zoospores, a unicellular plant body type, and anisogamous sexual reproduction (Hoffman 1983, 1984). Thus, without direct evidence for monophyly of *Atractomorpha*, we have taken a conservative stance in designating all *Atractomorpha* taxa as outgroups, and do not attempt to resolve relationships among these taxa. All trees were rooted using the outgroup taxa.

CHARACTER DATA

Structural characters and character states. Some of the structural data were coded employing ordered characters which involves the assumption of a transformation series. For example, two evolutionary steps are required in a change from the first to the third character state of an ordered, three-state character. During each search, PAUP determines the most parsimonious explanation of character state polarities in a transformation series, hence the resulting tree is not influenced by previously specified incorrect polarities. This is especially valuable where insufficient evidence exists to polarize the characters or where outgroup taxa show heterogeneity for certain character states.

A comparison of the primary features utilized to distinguish sphaeropleacean taxa (i.e. zygote morphology, cytoplasm/chloroplast morphology, and

septal morphology) is shown in Table 2. Character states for a total of 12 vegetative or reproductive structural characters were employed including the most recent observations of zygote wall morphology and cytoplasm/chloroplast morphology (Buchheim 1988). All structural characters were given equal weight in the analysis.

1. *Plant body type.* Each taxon was scored as either unicellular (0) or filamentous (1). The typical condition of the mature vegetative state was considered when scoring this character since all taxa germinate from zygotes as spindle-shaped unicells.

2. *Sexual reproduction.* Three character states, regularly anisogamous (0), anisogamy and oogamy both occurring with regularity (1), and regularly oogamous (2), were employed to describe sexual reproduction in the Sphaeropleaceae. Oogamy and anisogamy are the only forms of sexual reproduction observed among sphaeropleacean species. Only *A. echinata* is regularly anisogamous (produces motile male and female gametes). The remaining *Atractomorpha* species are frequently anisogamous but occasionally exhibit oogamy (produce motile male gametes and non-motile female gametes). *Sphaeroplea soleirolii* and *S. soleirolii* var. *crassisepta* may rarely produce motile female gametes; however, oogamy is the typical form of sexual reproduction in these two taxa and in all other species of *Sphaeroplea.* An evolutionary progression from anisogamy to oogamy through the combined condition (or vice versa) represents a reasonable assumption; thus, the three states of this character have been ordered.

3. *Site of fertilization.* Fertilization in *A. echinata, A.* sp. 195, and *A.* sp. 196 typically occurs outside the female gametangia (0). In contrast, fertilization occurs inside oogonia (2) in all species of *Sphaeroplea* (with the possible exception of *S. tenuis,* Fritsch 1929). *Atractomorpha porcata* represents an intermediate of the two extremes in that fertilization regularly occurs both outside and inside female gametangia (Hoffman 1984). We assumed a progression from external fertilization to internal fertilization through the combined condition (or vice versa) in the cladistic analysis.

4. *Asexual reproduction.* Asexual reproduction in the Sphaeropleaceae is accomplished either by the production of zoospores (0) or through fragmentation (1). Zoospores were reported to occur in *S. wilmani* (Rieth 1952), but Smith (1955) suggested that the motile cells observed by Rieth were actually female gametes. No other reports of zoospore production in *S. wilmani* have been published and zoospore production was not detected in the isolates of *S. wilmani* utilized for this investigation. Thus, asexual reproduction in *S. wilmani* was scored as occurring through fragmentation.

5. *Cytoplasm/chloroplast morphology.* Each taxon was scored as typically producing either distinct bands of cytoplasm with large, discrete chloroplasts equivalent to the "annular chloroplasts" of Fritsch's (1929) terminology (0) or a reticulum of cytoplasm with a corresponding reticulum of chloroplasts (1).

6. *Septal type.* Three types of septa have been identified as characterizing various species in *Sphaeroplea*: simple septa (0), thickened septa (1), and thickened and lobed septa (2). These three states have been ordered since a progression from simple, to thickened, to thickened and lobed septa represents a logical assumption. Although rare, simple septa occur in *Atractomorpha* (Hoffman, unpubl. observ.); thus, *A. echinata, A. porcata, A.* sp. 195 and *A.* sp. 196 were scored as producing simple septa.

7. *Antheridial type.* Only *S. fragilis* and *S. robusta* have been described (Buchheim and Hoffman 1985, 1987) as producing modified (short) antheridia (1). Since the literature provides no specific mention of modified antheridia in other taxa, we assumed that all other taxa produce unmodified antheridia (0). This assumption is also consistent with our observations on antheridia of these taxa maintained in culture (Table 1).

8. *Primary membrane.* The primary membrane surrounding the maturing zygote is one of the key features which distinguishes the Sphaeropleaceae from most other groups of green algae. Two types of primary membrane, smooth (0) and with bristles (1), occur in the Sphaeropleaceae (Hoffman 1984). Primary membranes with bristles are only known to occur in *A. porcata, A.* sp. 195 and *A.* sp. 196 (Hoffman 1984, 1987).

9. *Zygote wall ornamentation.* Ultrastructural studies of the zygote wall have led to the identification of three principal states for this character. The presence of true spines (0) is found only in zygotes of *A. echinata* and *A.* sp. 196. A reticulate pattern of raised ridges (1) is found on the zygote wall of most *Sphaeroplea* taxa and *A.* sp. 195. Of the taxa studied, the third pattern, where zygote walls produce parallel ridges or wings (2), occurs only in *A. porcata, S. soleirolii* and *S. soleirolii* var. *crassisepta.* At present, there is insufficient evidence to support the ordering of these character states.

10. *Zygote shape.* Zygotes are either spherical (0) or ellipsoidal (1).

11. *Maximum zygote diameter.* Zygotes with a maximum diameter ranging from 21–40 μm were scored as (0), 41–60 μm as (1), and 61–80 μm as (2). We assumed a progression from small to large zygotes (or vice versa) in ordering this character.

12. *Zygote wall appendages.* Zygotes of each sphaeropleacean taxon were scored as either lacking curled appendages termed cirri (0) or with cirri present (1) (Hoffman and Buchheim 1989). The only taxa now recognized to produce zygotes with cirri are *S. fragilis, A. porcata, A.* sp. 195 and *A.* sp. 196.

Isozyme characters. Genetic analyses of electrophoretic data in vascular plants typically rely on isozyme banding patterns derived from diploid tissue and information on the inheritance and subcellular compartmentalization of isozymes (Gottlieb 1982). In

contrast with vascular plants, the only diploid stage of many green algae is a single-celled zygote. Since zygotes are often thick-walled (making protein extraction difficult) and are rarely available in quantity, electrophoretic investigations of most groups of green algae have exclusively employed haploid material.

Isozyme banding patterns derived from haploid cells present several problems relating to genetic interpretation and data analysis. Haploid cells cannot produce heterozygotes; thus, multiple bands seen in a particular isozyme system may represent (1) products from different subcellular compartments (nuclear vs. chloroplast, Gottlieb 1982), (2) products derived from different allelic or nonallelic genes (Scandalios 1969), and/or (3) protein variants resulting from post-transcriptional or post-translational events. To differentiate among these possibilities, formal genetic analyses are required which are beyond the scope of this investigation. As a consequence, it is difficult or impossible to analyze our electrophoretic data in terms of gene frequencies (Swofford and Berlocher 1987).

An alternative approach to the analysis of electrophoretic data derived from haploid cells would be to collectively regard all of the bands that are visualized for a given enzyme system as a single character. A consequence of this treatment of the data is that much systematic information is lost.

If the assumption is made that each band derived from a haploid cell represents the product of a presumptive locus, then these bands can be treated as characters for systematic analysis (Buth 1984). We adopted this assumption for our cladistic analysis of electrophoretic data in the Sphaeropleaceae. We identified each isozyme band as a binary character and its presence or absence the states of the character. Each isozyme character was given equal weight.

RESULTS

Cladistic analysis of structural characters. A cladistic analysis of the structural characters resolved three most parsimonious trees each with a total length of 23 steps (consistency index [CI] of informative characters only = 0.73). A strict consensus tree (Fig. 1) based on these three trees was computed utilizing Swofford's (1985) CONTREE program which employs the methods of Rohlf (1982). The strict consensus tree, which supports monophyly of the genus *Sphaeroplea,* places *S. fragilis* at the base of the *Sphaeroplea* clade as sister taxon to the remaining *Sphaeroplea* species. The two taxa with winged zygotes, *S. soleirolii* and *S. soleirolii* var. *crassisepta,* were hypothesized to be sister taxa and *S. robusta* was hypothesized to be a sister taxon to the "*soleirolii*" clade. Insufficient structural data were available to resolve the relationships between *S. annulina, S. wilmani,* and the "*robusta-soleirolii*" clade.

Cladistic analysis of electrophoretic characters. A total of 54 bands were observed for the seven enzyme

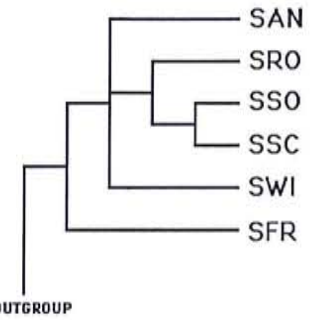

FIG. 1. Most parsimonious tree (rooted using outgroup taxa, length = 23 steps, CI = 0.73) derived from cladistic analysis of morphological character data. The node joining SAN, SRO, SSO, SSC, and SWI is not resolved by the structural data.

systems. The relative mobilities of all bands are presented for each taxon in Table 3. Of the seven taxa represented by more than one clonal isolate (Table 1), variation in isozyme patterns occurred among clones of *S. robusta, S. soleirolii* var. *crassisepta,* and *A. echinata.* Two PGI phenotypes (PGI-89, PGI-100 and PGI-93) were observed for the two clones of *S. robusta.* Similarly, two ALD phenotypes (ALD-100 and ALD-110) were seen in two different clones of *S. soleirolii* var. *crassisepta.* Three unique SKDH phenotypes (SKDH-80, SKDH-83, SKDH-88) were observed in three different clones of *A. echinata.* Other autapomorphic characters for this taxon include ALD-86, ALD-95, and PGI-85. The other sphaeropleacean taxa with multiple clones (*S. annulina, S. soleirolii, S. wilmani* and *A. porcata*) did not exhibit any variation in isozyme patterns.

Three equally parsimonious trees, each with total length equaling 84 steps (CI of informative characters only = 0.51), were generated from the data matrix of isozyme band characters (Table 3). Monophyly of the genus *Sphaeroplea* was not supported by a cladistic analysis of electrophoretic data, and thus the trees could not be rooted. Of these three trees, only one is fully resolved (Fig. 2). A *Sphaeroplea* clade and an outgroup (*Atractomorpha*) clade can be identified in the fully resolved tree (Fig. 2). The latter clade includes all outgroup taxa plus the two electromorphs of *S. soleirolii* var. *crassisepta.* The *S. fragilis* isolate occupies the base of the "*Sphaeroplea*" clade as sister taxon to all *Sphaeroplea* taxa except *S. soleirolii* var. *crassisepta.* Of the remaining *Sphaeroplea* taxa, the two "*wilmani*" electromorphs are sister taxa and form a clade with *S. soleirolii. Sphaeroplea annulina* forms a clade with the "*wilmani-soleirolii*" clade. The *S. robusta* electromorphs are hypothesized to be sister taxa to the "*annulina-wilmani-soleirolii*" clade.

Character congruence between the two data sets. As a means to identify character congruence between the two data sets, the matrices were combined (Kluge 1989), cladistically analyzed, and strict consensus analysis performed. Two most parsimonious trees (112 steps, CI = 0.54) were found. Analysis of the

TABLE 3. *Presence/absence matrix of electrophoretic characters in* Sphaeroplea *and* Atractomorpha. *See Table 1 for full scientific names of the taxa.*

Enzyme	Relative mobility number (locus)	SAN	SFR	SRO1	SRO2	SSO	SSC1	SSC2	SWIA	SWIU	AEC	APO	A195	A196
ALD	86	0	0	0	0	0	0	0	0	0	1	0	0	0
	95	0	0	0	0	0	0	0	0	0	1	0	0	0
	100	1	1	1	1	1	1	0	0	0	0	0	0	0
	106	0	0	0	0	0	0	0	0	0	0	1	1	1
	110	0	0	0	0	0	0	1	1	1	0	0	0	0
AAT	50	0	0	0	0	0	0	0	0	0	0	0	0	1
	60	0	0	0	0	0	0	0	0	1	0	0	0	0
	74	0	1	0	0	0	0	0	0	0	0	0	0	0
	100	0	1	0	0	0	0	0	1	0	0	0	0	0
	104	0	0	1	1	0	0	0	0	0	0	0	0	0
	107	0	0	0	0	0	0	0	1	0	0	1	0	0
	117	1	0	0	0	0	0	0	0	0	0	0	0	0
	121	0	1	0	0	0	0	0	0	0	1	0	1	0
	129	0	0	0	0	0	1	1	0	0	0	0	0	0
	137	0	0	0	0	1	0	0	0	0	0	0	0	0
IDH	6	0	0	0	0	0	0	0	0	0	0	1	0	1
	20	0	0	1	1	0	0	0	0	0	0	0	0	0
	27	0	0	0	0	0	1	1	0	0	1	0	1	0
	35	0	0	0	0	0	0	0	0	0	0	0	0	1
	41	0	0	1	1	0	0	0	0	0	0	0	0	0
	45	1	0	0	0	1	0	0	1	1	0	0	0	0
	51	0	0	0	0	0	1	1	0	0	1	0	1	0
	71	1	0	0	0	1	0	0	1	1	0	0	0	0
	78	0	1	0	0	0	0	0	0	0	0	0	0	0
	100	0	1	0	0	0	0	0	0	0	0	0	0	0
LAP	80	0	0	0	0	0	0	0	0	0	0	0	1	0
	100	0	1	0	0	0	0	0	0	1	0	0	0	0
	105	0	0	0	0	1	0	0	1	0	0	0	0	0
	109	0	0	0	0	0	1	1	0	0	1	0	0	1
	115	0	0	0	0	0	0	0	0	0	0	1	0	0
	118	1	0	1	1	0	0	0	0	0	0	0	0	0
MDH	67	1	1	1	1	1	1	1	1	0	1	1	1	1
	75	0	0	0	0	0	0	0	1	0	0	0	0	0
	83	0	0	0	0	0	0	0	1	1	0	0	1	0
	88	0	0	0	0	0	0	0	1	0	0	1	0	0
	92	0	0	1	1	0	0	0	0	0	0	0	0	0
	97	1	0	0	0	1	0	0	1	0	1	0	0	0
	100	0	1	0	0	0	1	1	1	1	0	0	1	1
PGI	53	0	0	0	0	0	0	0	0	1	0	0	0	0
	62	0	0	0	0	0	0	0	0	0	1	1	0	1
	85	0	0	0	0	0	0	0	0	0	1	0	0	0
	89	1	1	1	0	1	0	0	1	1	0	1	0	1
	93	0	0	0	1	0	1	1	0	0	0	0	0	0
	97	0	0	0	0	0	0	0	0	0	1	1	1	0
	100	1	1	1	0	1	0	0	1	0	0	0	0	1
	104	0	0	0	0	0	1	1	0	1	0	0	1	0
SKDH	80	0	0	0	0	0	0	0	0	0	1	0	0	0
	83	0	0	0	0	0	0	0	0	0	1	0	0	0
	88	0	0	0	0	0	0	0	0	0	1	0	0	0
	95	0	1	0	0	0	0	0	0	0	0	0	0	0
	98	0	0	0	0	0	0	0	0	0	0	1	0	0
	100	0	1	0	0	1	0	0	0	0	0	0	0	0
	105	0	0	0	0	0	0	0	0	0	0	0	1	1
	109	1	0	1	1	0	1	1	1	1	0	0	0	0

combined data sets (Fig. 3) supports the monophyly of *Sphaeroplea*. The "*wilmani*," "*annulina*," and "*soleirolii*" isolates form an unresolved trichotomy. The "*robusta*" isolates and "*soleirolii* var. *crassisepta*" isolates form two sister clades to the "*wilmani-annulina-soleirolii*" clade. Topologically, *S. fragilis* is the sister taxon to the unresolved trichotomy from which all other *Sphaeroplea* species are derived.

DISCUSSION

Several key points can be identified to summarize the results of cladistic analyses of structural and isozyme data. The structural data support monophyly of the genus *Sphaeroplea*, and *S. fragilis* is hypothesized to be sister taxon to all other species of *Sphaeroplea* included in the study. Although three groups of taxa could not be resolved by the structural data

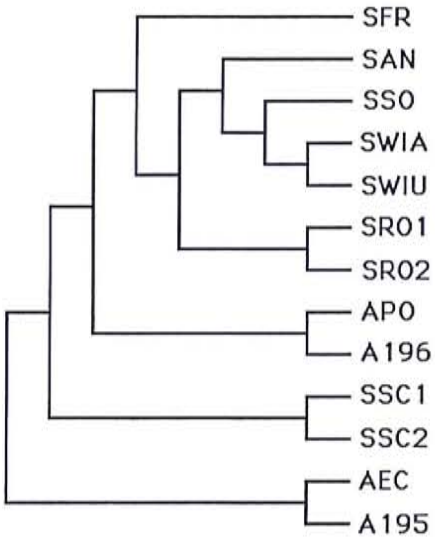

FIG. 2. The only fully resolved tree of three most parsimonious trees (length = 84 steps, CI = 0.51) derived from cladistic analysis of isozyme character data. This tree is unrooted since monophyly of the ingroup is not supported by the isozyme character data.

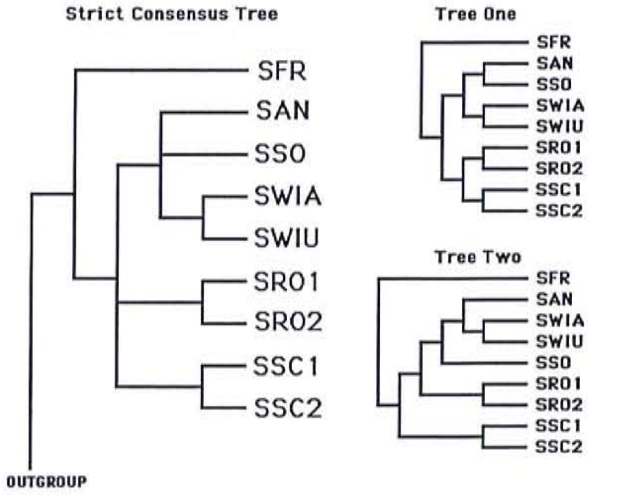

FIG. 3. Strict consensus tree (on the left) and the two most parsimonious trees (on the right) derived from cladistic analysis (length = 112 steps, CI = 0.54) of the combined data. The trees were rooted using outgroup data. Ingroup monophyly is supported as is the basal position of *S. fragilis* on the *Sphaeroplea* clade.

at hand, the data are relatively consistent (CI=0.73) with the hypothesized trees. In contrast to the structural data, cladistic analysis of the isozyme data did not support monophyly of the genus *Sphaeroplea.* Cladistic analysis of the isozyme data produced a fully resolved tree (Fig. 2), but the isozyme data were much less consistent (CI = 0.51) than the structural data.

Although cladistic analyses of the two data sets produced different hypotheses regarding the phylogenetic relationships of *Sphaeroplea* species, congruence between the data sets can be found. Independent analyses of both data sets place *S. fragilis* as the sister taxon to most other *Sphaeroplea* species. Furthermore, a strict consensus analysis of the combined data supports the monophyly of *Sphaeroplea* and the resolution of three groups of *Sphaeroplea* taxa (Fig. 3).

Monophyly of Sphaeroplea. The question of monophyly of the genus *Sphaeroplea* represents a point of fundamental importance. The structural data overwhelmingly support monophyly of the genus *Sphaeroplea* as does analysis of the combined data sets. Therefore, we studied the electrophoretic data in order to find a possible explanation to account for the differing hypothesis regarding monophyly of *Sphaeroplea* inferred from the electrophoretic data.

Cladistic analysis of the electrophoretic data placed the two isolates of *S. soleirolii* var. *crassisepta* together on the outgroup (*Atractomorpha*) clade. The topological position of these taxa either reflects actual phylogenetic divergence (this is the interpretation inferred from the isozyme data) or the topology may be influenced by incorrect interpretations of the protein variants themselves. If the interpretation of the proteins is in error, the problem is one of homology. Isozymes with the same relative mobilities on a gel may or may not represent homologous proteins. However, without additional information one must assume homology for bands which have identical migration distances. For example, IDH-27 in *S. soleirolii* var. *crassisepta* may or may not be genetically homologous to the band scored as IDH-27 in *Atractomorpha echinata.*

Within the isozyme data alone (Table 3), we can find characters that support placement of *S. soleirolii* var. *crassisepta* with the ingroup (ALD-100, ALD-110, PGI-93, PGI-104, and SKDH-109). These (homoplasious) characters are only narrowly outweighed by the characters that do not support *Sphaeroplea* monophyly (IDH-27, IDH-51, LAP-109, MDH-67, and MDH-100). When the isozyme data are cladistically analyzed with the structural data, the former homoplasies become synapomorphies with the ingroup. This reversal when the two data sets are combined indicates that the non-monophyly of the genus *Sphaeroplea* inferred from the isozyme data is not strongly supported.

We recognize that coding isozyme data as the presence or absence of bands coupled with inadequate taxon sampling can also contribute to misleading phylogenetic hypotheses (Swofford and Berlocher 1987). For example, clones from distinct species might share one of many different alleles present in both species. This synapomorphy would not be representative of the spectrum of alleles these two taxa do or do not share. Although this type of error may be present in our analyses, we have utilized all known sources to provide living material for this investigation. As more sphaeropleacean taxa are placed in

culture, the study of isozyme variation can be expanded.

Conclusions. The inter-relationships of some species of *Sphaeroplea* remain ambiguous given the lack of structural data and the inconsistency in the isozyme data. As additional taxa (e.g. *S. chapmanii, S. tenuis, S. africana,* and *S. tricarinata*) and more character data (e.g. ultrastructural character data) are added to the matrices presented in this investigation, we hope to address this question.

Although the relationships of some taxa remain ambiguous, this phylogenetic study of *Sphaeroplea* represents a framework for further systematic study of the genus and the family Sphaeropleaceae. Furthermore, this investigation represents one of the first to undertake a cladistic analysis of electrophoretic data from green algal material. Unlike many studies which compare cladistic analyses of two very different data sets, our data are based on the same clones of the same taxa. Although cladistic analyses of the two data sets yield hypotheses that differ in some respects, the character data from both are sufficiently congruent that strict consensus analysis of the combined data reveals a significant amount of resolution (Fig. 3). Monophyly of the genus *Sphaeroplea* is supported by the structural data and by analysis of the combined data sets. Independent analyses of both structural and isozyme data place *S. fragilis* as sister taxon to the other *Sphaeroplea* species in the study.

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