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Allozyme variation and the taxonomy of *Wolffiella*

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Abstract

Allozyme electrophoresis was carried out to estimate genetic diversity within and assess divergence between the 10 recognized species in three sections of the aquatic angiosperm genus *Wolffiella*. Eleven presumptive loci were used in the calculations. Highest variation was found in *W. lingulata* and *W. oblonga*, two common species with widespread distributions in North and South America. Four of the species showing low allozyme variation include *W. caudata*, *W. denticulata*, *W. neotropica*, and *W. rotunda*, all of which have restricted distributions. *W. hyalina* exhibits low allozyme diversity despite being widely distributed in Africa. Three species with intermediate levels of diversity include: *W. welwitschii*, which is widely distributed on two continents; *W. gladiata*, which occurs widely in North America; and *W. repanda*, which has a restricted distribution in Africa. Genetic identities between species of *Wolffiella* vary from 0.00 (no alleles in common) to over 0.94. *W. lingulata* and *W. oblonga* share the highest identity of any two species. These two species are viewed as most closely related and are difficult to distinguish in some instances. Species within the large sect. *Wolffiella* (incl. *W. caudata*, *W. denticulata*, *W. gladiata*, *W. lingulata*, *W. neotropica* and *W. welwitschii*) have identities ranging from 0.00 to 0.940, whereas identities with species in this section and the two species of sect. *Stipitatae* (incl. *W. hyalina* and *W. repanda*) are mostly 0.000, and the same applies for *W. rotunda*, the only species in sect. *Rotundae*. The two species of sect. *Stipitatae*, *W. hyalina* and *W. repanda*, have an identity of 0.800, which is higher than they share with any other species. Species of sect. *Stipitatae* have higher identities with *W. rotunda* (0.538, 0.504) than they do with any species of sect. *Wolffiella*, and *W. rotunda* is more closely related to sect. *Stipitatae* than to sect. *Wolffiella*.

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Allozyme data support the recognition of sect. *Stipitatae* as now constituted and provide evidence for the circumscription of sect. *Wolffiella* as presently recognized. However, *W. denticulata* is rather isolated within this section. © 1997 Elsevier Science B.V.

Keywords: Lemnaceae; *Wolffiella*; Allozymes; Genetic identity

1. Introduction

The genus *Wolffiella* (Hegelm.) Hegelmaier is a member of the Lemnaceae or duckweed family. Landolt (1986) recognized nine species in three sections, and later (Landolt, 1992) described an additional species. The sections and species are: sect. *Stipitatae* containing *W. hyalina* (Del.) Monod and *W. repanda* (Hegelm.) Monod; sect. *Rotundae* with *W. rotunda* Landolt; and sect. *Wolffiella* consisting of *W. caudata* Landolt, *W. denticulata* (Hegelm.) Hegelm., *W. gladiata* (Hegelm.) Hegelm., *W. lingulata* (Hegelm.) Hegelm., *W. neotropica* Landolt, *W. oblonga* (Phil.) Hegelm., and *W. welwitschii* (Hegelm.) Monod. A recent cladistic analysis based on morphological, micromolecular and anatomical features suggests that the sections of *Wolffiella* represent monophyletic groups, but the genus *Wolffiella* is paraphyletic. However, constraining the trees to make the genus monophyletic adds only a few steps to their length (Les et al., 1997).

The present electrophoretic study of *Wolffiella* was undertaken to ascertain whether allozymic divergence among the species is concordant with their taxonomic disposition in different sections. Also, we wished to see if species within sections viewed as most closely related on the basis of morphology (Landolt, 1986, 1992) show the highest allozymic similarity. Prior electrophoretic studies of two other duckweed genera, *Spirodela* (Crawford and Landolt, 1993) and *Wolffia* (Crawford and Landolt, 1995), indicate that species considered most closely related may show genetic identities lower than 0.50. In many pair-wise species comparisons in these two genera, no alleles were shared at any of the loci examined, and thus the data were not useful for assessing relationships other than to indicate that the taxa are much more divergent allozymically than most congeneric species of flowering plants (Gottlieb, 1977; Crawford, 1990). A secondary objective was to assess genetic variation within each species.

2. Methods

A total of 79 clones (strains) representing all ten recognized species of *Wolffiella* was included in the electrophoretic survey. Because the strains represent single isolates, no assessment of diversity within populations was attempted. The clones studied and the localities of origin are given in Table 1. Clones were selected so that species could be sampled from most of their geographic ranges. The smaller number of samples examined for certain species such as *W. caudata*, *W. denticulata*, *W. repanda*, and *W. rotunda* is a reflection of their rarity and/or restricted geographic distributions. Plant material, either from agar or liquid culture, was supplied and identified taxonomically by

E.L. The grinding buffer was made up of 10% glycerol and was 0.1 M tris-HCl, pH 7.5, with 14 mM 2 mercaptoethanol, 1.0 mM tetrasodium salt of EDTA, 10 mM $MgCl_2$, 10 mM KCl, and 5–10 mg polyvinylpolypyrrolidine per 0.5 ml of buffer (Gottlieb, 1981). Several enzymes were separated in polyacrylamide gels according to the methods of Crawford et al. (1987): alcohol dehydrogenase (ADH, E.C. 1.1.1.1); glutamate dehydrogenase (GDH, E.C. 1.4.1.2); and phosphogluconate dehydrogenase (PGD, E.C. 1.1.1.44). The remaining enzymes were resolved in 12.5% starch gels using two buffer systems. Forms of malate dehydrogenase (MDH, E.C. 1.1.1.37) were separated with an electrode buffer of 0.04 M citric acid adjusted to pH 6.1 with *N*-(3-aminopropyl)-morpholine, and the gel buffer was a 1:19 dilution of the electrode buffer. Forms of glucose-6-phosphate isomerase (GPI, E.C. 5.3.1.1) and triosephosphate isomerase (TPI, E.C. 5.3.1.1) were resolved with an electrode buffer of 0.5 M tris, 0.65 M boric acid, 0.02 M EDTA, pH 8.0, and a 1:9 dilution of this was used for the gel buffer. Staining protocols and nomenclature for all enzymes followed Wendel and Weeden (1989). Several lines of evidence were used to infer the genetic bases of the banding patterns for the enzymes. One useful source of data was the known active subunit composition of the enzymes (Weeden and Wendel, 1989). Additional information included variation seen in banding patterns between clones of the same and/or different taxa, and the expected minimal conserved number of isozymes for diploid plants (Gottlieb, 1982; Weeden and Wendel, 1989). Allelic frequencies were determined for each species and were used to calculate Nei's genetic identity and distance (Nei, 1972). The GeneStat-PC (version 3.3) software (Lewis, 1993) was employed to calculate the statistics. An unweighted pair-group method using arithmetic averages (UPGMA) cluster analysis of genetic identity values was performed using version 1.70 NTSYS-pc (Rolf, 1992). Mean number of alleles per locus and per polymorphic locus, and proportion polymorphic loci were also calculated for each species.

3. Results

Eleven presumptive loci were used to calculate the statistics for the 10 species of *Wolffiella*, *Adh-1*, *Adh-2*, *Gdh*, *Gpi-1*, *Gpi-2*, *Mdh-1*, *Mdh-2*, *Pgd-1*, *Pgd-2*, *Tpi-1*, and *Tpi-2*. Not all loci were scored for every strain because of poor staining and/or resolution.

The mean numbers of alleles per locus and polymorphic locus, and the proportion polymorphic loci are shown in Table 2. No variation was detected between the two clones of *W. denticulata*. The mean numbers of alleles per locus and per polymorphic locus are highest in *W. oblonga* and *W. lingulata* with *W. gladiata* and *W. welwitschii* next highest. The five species *W. caudata*, *W. hyalina*, *W. neotropica*, *W. repanda* and *W. rotunda* show similarly low values for mean numbers of alleles per locus and per polymorphic locus. Proportion polymorphic loci is highest in *W. oblonga*, followed by *W. lingulata*; *W. repanda* and *W. welwitschii* exhibit similar proportions of polymorphic loci (Table 2). The same four species with low mean numbers of alleles per locus (*W. caudata*, *W. hyalina*, *W. neotropica* and *W. rotunda*) also have a low proportion polymorphic loci (Table 2).

Table 1
Collection numbers and geographic origins of *Wolffiella* clones used for enzyme electrophoresis

Species	Collection number [a]		Geographic origin
W. caudata	9155	Bolivia:	Beni, La Pascane Grande
	9158	Bolivia:	Beni, La Pascane Grande
	9165	Bolivia:	Beni, Rurrenabaques
	9173	Bolivia:	Beni, San Pablo
W. denticulata	7454	South Africa:	Natal, Zululand
	8221	South Africa:	Natal, Sordwana Bay
W. gladiata	7173	USA:	Washington, Tacoma
	7590	USA:	Virginia, Dymer Creek
	7595	USA:	Virginia, Brandon
	7852	USA:	Louisiana, East Baton Rouge Parish
	8066	USA:	Texas, Old Ocean
	8261	USA:	Pennsylvania, Conneaut Lake
	8350	USA:	Illinois, Pine Hills Swamp
	8392	USA:	Texas, Austin
	8768	USA:	Florida, Tallahassee
W. hyalina	7376	Egypt:	Mahallet, El Qubba
	7378	Egypt:	Hafr Shoukr, Naim
	8640	Tanzania:	Arusha: Amboseli
W. lingulata	7289	Brazil:	Amazonas, Neptunia
	7292	Brazil:	Amazonas, Rio Negro
	7330	Trinidad:	St. Augustine
	7360	Surinam:	Saramacca River
	7464	Venezuela:	Yaracuy, Marlin
	7655	Mexico:	Tabasco, Villahermosa
	7725	Argentina:	Corrientes, Mburucuya
	8041	USA:	Louisiana, Pecan Island
	8141	USA:	California, Vandenberg AFB
	8175	USA:	California, Lake Thynan
	8237	Paraguay:	Asuncion
	8776	USA:	California, Black Lake Canyon
	8823b	Argentina:	Formosa, Clorinda
	8664	Argentina:	Corrienfes, Empedrado
	8886	USA:	California, Monterey Co.
	8898a	Ecuador:	Guayaquil
	9133	Brazil:	Matto Grosso, Corrumba
W. neotropica	7225	Brazil:	Guanabara, Rio de Janeiro
	7279	Brazil:	Rio de Janeiro, Cabo Frio
	7290	Brazil:	Amazonas, Neptunia
	7609	Brazil:	Espirito-Santo, Heliofila
	8848	Brazil:	Rio de Janeiro, Marico
	8849	Brazil:	Rio de Janeiro, Saquarema
W. oblonga	7164	USA:	Louisiana, New Orleans
	7167	USA:	Louisiana, Norco
	7201	Argentina:	Buenos Aires, Arroyo Burgueño
	7569	Brazil:	Sao Paulo
	7732	Brazil:	Sao Paulo
	7853	USA:	Louisiana, East Baton Rouge Parish

Table 1 (continued)

Species	Collection number [a]		Geographic origin
W. oblonga	7855	USA:	Louisiana, St. James
	7923	Argentina:	Buenos Aires, Arroyo Vitel
	7997	Brazil:	Rio Grande de Sul, Pelotas
	8031	USA:	Louisiana, Rapides Parish
	8072	USA:	Texas, Old Ocean
	8393	USA:	Florida, Immokalee
	8751	Argentina:	Salta, El Rey
	8777	USA:	California, Black Lake Canyon
	8816	Argentina:	Santa Fé, Esperanza
	8828	Argentina:	Formosa, Clorinda
	8881b	USA:	California, Black Lake Canyon
	8984	Columbia:	Cundinamarca, Laguna La Herrera
	9139	Brazil:	Amazonas, Manaus
	9140	Chile:	Quillon, Laguna, Allendaño
	9141	Chile:	Quillon, Laguna Allendaño
W. repanda	9055	Zimbabwe:	Urungwe Safari Area, Chirundu
	9062	Zimbabwe:	Urungwe Safari Area, Chirundu
	9104	Botswana:	South Gate to Moremi
	9107	Botswana:	85 km NNE of Shorobe
	9116	Zimbabwe:	Urungwe Safari Area, Chirundu
	9122	Zimbabwe:	Urungwe Safari Area, Chirundu
W. rotunda	9048	Zimbabwe:	Urungwe Safari Area, Chirundu
	9054	Zimbabwe:	Urungwe Safari Area, Chirundu
	9072	Zimbabwe:	Mana Pools
	9121	Zimbabwe:	Urungwe Safari Area, Chirundu
W. welwitschii	7468	Columbia:	Atlantico, Barranquilla
	7644	Angola:	Benguela, Cubal
	8863	Senegal:	between Saint Louis and Richard Toll
	9086	Botswana:	Daonara, Santantadibe
	9089	Botswana:	Boteti River
	9093	Botswana:	Moremi Wildlife Reserve
	9096	Botswana:	Chobe River, Chubu Lodge

[a] Collection numbers those of Landolt.

Table 2
Number of clones examined and allozymic variation in species of *Wolffiella*

Species	Clones	Alleles per locus (Mean no.)	Alleles per polymorphic locus (Mean no.)	Proportion polymorphic loci
W. caudata	4	1.18	2.00	0.18
W. denticulata	2	1.00	–	0.00
W. gladiata	9	1.46	2.25	0.36
W. hyalina	3	1.10	2.00	0.10
W. lingulata	17	1.64	2.40	0.46
W. neotropica	6	1.09	2.00	0.09
W. oblonga	21	2.00	2.83	0.55
W. repanda	6	1.20	2.00	0.32
W. rotunda	4	1.10	2.00	0.10
W. welwitschii	7	1.44	2.33	0.33

Table 3
Nei's genetic identity between species of *Wolffiella*. Species designations are the first three letters of names given in Table 1

Sect. *Wolffiella*								sect. *Stipitatae*		sect. *Rotundae*
Species	cau	den	gla	lin	neo	obl	wel	hya	rep	rot
Nei's genetic identity between pairs of species										
cau	X									
den	0.000	X								
gla	0.438	0.000	X							
lin	0.467	0.000	0.816	X						
neo	0.113	0.096	0.226	0.116	X					
obl	0.461	0.000	0.845	0.940	0.131	X				
wel	0.338	0.142	0.426	0.430	0.316	0.443	X			
hya	0.000	0.000	0.000	0.000	0.012	0.000	0.000	X		
rep	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.800	X	
rot	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.504	0.538	X

The pair-wise comparisons of genetic identities between species are shown in Table 3, and the dendrogram produced from UPGMA clustering of these identities is shown in Fig. 1. The values range from 0.00 in 22 pair-wise comparisons to 0.940 for *W. lingulata* and *W. oblonga*; these two species also share high identities with *W. gladiata* (Table 3, Fig. 1). Within sect. *Wolffiella* identities vary from the high values for the three aforementioned species down to 0.000 (Table 3). The mean identity value for all species in sect. *Wolffiella* is 0.32. *W. hyalina* and *W. repanda* of sect. *Stipitatae* have an identity of 0.800, which is higher than they share with species in other sections (Table 3, Fig. 1). The highest intersectional identity mean (0.521) occurs between sect. *Stipitatae* and sect. *Rotundae*, the latter section consisting only of *W. rotunda* (Fig. 1). By contrast, these two sections have a mean identity of only 0.001 with sect. *Wolffiella*; except for *W. neotropica*, no species of sect. *Wolffiella* shares any alleles with species in the other two sections (Table 3, Fig. 1).

The two species *W. lingulata* and *W. oblonga*, which are widespread geographically

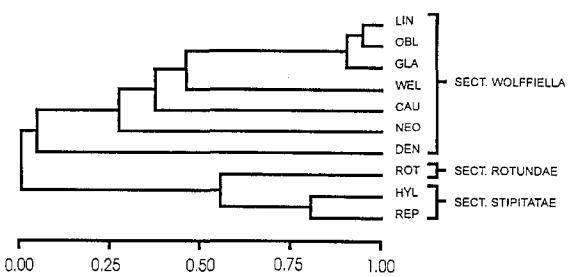

Fig. 1. Unweighted pair-group method using arithmetic averages (UPGMA) dendrogram showing clustering of Nei's genetic identities (shown along bottom) among the ten *Wolffiella* species (abbreviations same as in Table 3). Integrity of taxonomic sections is retained, although several species of sect. *Wolffiella* show very low identities.

and are distributed in both North and South America, also show a particular distribution of alleles at *Gpi-1*. In *W. lingulata*, the allele *f* is found only in South American populations, whereas the two North American populations have allele *g* and one population from Mexico has both alleles. In *W. oblonga*, six of the nine clones from North America have allele *Gpi-1 f* and the three other strains have *Gpi-1 g*. Eight of the 10 clones from South America have *Gpi-1 g*, two have *Gpi-1 f* and another is heterozygous for the latter two alleles.

4. Discussion

Numerous studies have examined genetic diversity within a wide taxonomic sample of flowering plants with various life history attributes, and many of these results have been summarized by Hamrick and Godt (1990). Results from the present study may be compared to the compilations of Hamrick and Godt (1990) as well as the species of two other genera of Lemnaceae (Crawford and Landolt, 1993, 1995). The two most widely distributed and common species of *Wolffiella* are *W. lingulata* and *W. oblonga*, and they are the two most variable species allozymically (Table 2). However, the mean numbers of alleles per locus (2.29 versus 2.00 and 1.64) and proportion polymorphic loci (0.59 versus 0.55 and 0.46) for these two taxa are lower than in other widespread species (Hamrick and Godt, 1990). The one species in which no variation was detected (*W. denticulata*) has one of the smallest geographic distributions of any species of *Wolffiella* (Landolt, 1986, 1992), but it must be emphasized that only two clones from neighboring localities were examined. Three species with low allozyme variation, *W. caudata*, *W. neotropica*, and *W. rotunda*, have restricted distributions on single continents. *W. hyalina* exhibits low diversity but is widely distributed in Africa (Landolt, 1986). Two species with 'intermediate' levels of diversity, *W. gladiata* and *W. welwitschii*, are widely distributed, with the former restricted to North America and the latter present over wide areas in Africa as well as in South America, Central America and in the Caribbean (Landolt, 1986). Another species with 'intermediate' diversity is *W. repanda*, which is narrowly distributed in Africa. Thus, in general, more widely distributed and common species of *Wolffiella* have higher allozyme diversity compared to more restricted taxa.

The lower allozyme variation detected in the rarer species is not an artifact of smaller number of clones sampled. When subsamples of clones of the two most common species, *W. lingulata* and *W. oblonga*, were selected randomly and the values calculated for mean number of alleles per locus, per polymorphic locus and proportion polymorphic loci, the values are much higher than those found for the same number of clones of the rare species. This is particularly true when the clones of *W. lingulata* and *W. oblonga* originate from different continents (North and South America); in some instances the variation is actually higher than when all clones of each species are included in the calculations.

Diversity in species in two other genera of duckweeds, *Spirodela* and *Wolffia* (Crawford and Landolt, 1993, 1995), are compared with *Wolffiella* in Table 4. The genera have very similar mean levels of variation for each of the measures (Table 4).

Table 4
Means of genetic variation compared for species in the genera of Lemnaceae; all known extant species except one have been examined

Genus	Number of species investigated	Mean (and range) number of alleles per locus	Mean (and range) number of alleles per polymorphic locus	Mean (and range) proportion polymorphic loci
Spirodela	3	1.41 (1.13–1.63)	2.17 (2.00–2.25)	0.38 (0.13–0.50)
Wolffia	10 [a]	1.40 (1.07–2.29)	2.28 (2.00–3.00)	0.30 (0.07–0.79)
Wolffiella	10	1.32 (1.00–2.00)	2.20 (2.00–2.83)	0.25 (0.00–0.50)

[a] *W. elongata* was not studied.

The range of values is lower in *Spirodela* than in the other two genera for all measures of variation; this may be a reflection of the fact that there are only three species in this genus as compared to 10 investigated in *Wolffia* and 10 in *Wolffiella*. Within *Spirodela*, the most widespread species, *S. polyrrhiza*, is the least diverse whereas the most restricted species geographically (*S. intermedia*) has the highest level of genetic variation. In this genus, diversity is correlated with greater frequency of flowering and seed set, and not with geographic range (Crawford and Landolt, 1993). In *Wolffia*, the three most allozymically variable species (*W. arrhiza*, *W. columbiana* and *W. globosa*) are also the most geographically widespread taxa with distributions on at least two continents (Landolt, 1994; Crawford and Landolt, 1995). All species of *Wolffia*, except *W. microscopica*, flower with similar frequencies. Therefore, in *Wolffia* geographic distribution is associated with the level of allozyme diversity within species. In *Wolffiella*, flowering frequency is not consistently correlated with allozyme diversity. For example, *W. hyalina*, *W. repanda* and *W. rotunda* are the three species with by far the highest percentage of flowering of any in the genus (Table 5). Yet, *W. hyalina* and *W. rotunda* have very low allozyme diversity and *W. repanda* has just an average diversity (Table 2), although sampling of additional clones of *W. hyalina* may have revealed higher diversity. By contrast, the three most allozymically diverse species of

Table 5
Percentage of flowering in samples of *Wolffiella* species

Species	Number of samples investigated	Flowering percentage of *Wolffiella* species in nature (from herbarium specimens and field observations of EL)
W. caudata	7	0
W. denticulata	9	11
W. gladiata	250	4
W. hyalina	62	32
W. lingulata	236	10
W. neotropica	10	10
W. oblonga	266	5
W. repanda	10	40
W. rotunda	16	56
W. welwitschii	124	9

Wolffiella (*W. gladiata*, *W. lingulata* and *W. oblonga*) have very low flowering frequency (Table 5). Thus, it appears that in *Spirodela* the frequency of flowering is correlated with higher diversity rather than geographic distribution, whereas in *Wolffia* and *Wolffiella* it does not appear that flowering frequency is correlated with (and ostensibly influences) allozyme variation.

Previous studies of allozyme divergence between congeneric species of flowering plants have revealed a wide range of genetic identities, but a mean identity value between 0.65 and 0.70 is common (Gottlieb, 1977; Crawford, 1989, 1990). Given the reduced morphology of Lemnaceae, both in size and number of structures, there are fewer characters to compare than in other terrestrial flowering plants. The taxonomic difficulty in duckweeds could be the result of extreme parallel reduction, or the similarity may reflect close relationships. In the other duckweed genera, *Spirodela* and *Wolffia*, all pair-wise species comparisons revealed very low genetic identities between many species (Crawford and Landolt, 1993, 1995). For example, in *Spirodela* two species share no alleles and the other species pair has an identity of only 0.40. In *Wolffia*, the highest identity between two species is 0.40, and 37 of the 45 pair-wise species comparisons are 0.00, that is, with no alleles in common (Crawford and Landolt, 1995). The results for *Wolffiella* are similar in certain respects to the other two genera because several species (22 of the 45 pair-wise comparisons) share no alleles at the loci examined (Table 3). *Wolffiella* differs from the other two genera, however, because the three species *W. gladiata*, *W. lingulata* and *W. oblonga* have identities of 0.816 or higher, and *W. hyalina* and *W. repanda* share an identity of 0.800 (Table 3). The former three taxa are viewed as closely related (Landolt, 1986); *W. lingulata* and *W. oblonga* are particularly difficult to distinguish morphologically and the two have the very high identity of 0.940 (Table 3), which is comparable to values often obtained for populations of the same species (Gottlieb, 1977; Crawford, 1989, 1990). *W. hyalina* and *W. repanda* are the only two members of sect. *Stipitatae* and their identity of 0.800 (Table 3) is nearly twice as high as found between any species in either of the other two genera of Lemnaceae. The high identities for the species of *Wolffiella* could be attributed to a more recent divergence time and/or hybridization. *W. gladiata* is quite distinct from *W. lingulata* and *W. oblonga*, the two other species with which it shares a high identity. In addition, *W. gladiata* differs from the other two species ecologically and in geographic distribution; it grows in North America in warm temperate regions and overlaps with the other two species only in the very southern United States and in the high plateau of Mexico. *W. lingulata* and *W. oblonga*, which share the highest identity of any two species, are not as well differentiated morphologically as each is from *W. gladiata*. There are some ecological differences, however, with *W. oblonga* more tolerant of lower temperatures. Therefore, it occurs at higher altitudes in the mountains of South America and also farther south than *W. lingulata*. Unlike *W. lingulata*, *W. oblonga* is very rare in warm tropical regions (Landolt, 1986).

To consider whether hybridization or lack of divergence may be the primary factors in producing similarity at allozyme loci, three clones of each species from outside the geographic range of the other species were compared. These include clones *7289*, *7330* and *7360* of *W. lingulata*, and *7201*, *7923*, *9140* of *W. oblonga* (Table 1). Presumably interspecific hybridization would not be a cause of similarity at allozyme loci in these

regions and if consistent differences exist between 'pure' strains of each species they should be seen when comparing these allopatric clones. This is not the case, however, because the same allelic variation at certain loci occurs between clones of the same species in these areas of allopatry, and thus genetic identities between strains of the two species are just as high between the areas of allopatry as they are for those from sympatric areas. It does not appear, therefore, that allozymically 'pure' clones of each species occur in the sense that particular alleles are restricted to one species or the other. On present evidence, it is not possible to determine whether these taxa represent one variable gene pool or distinct species that my hybridize when they come in contact, but the data cast some doubt on the existence of two separate gene pools. However, despite the allozyme evidence one of us (EL) is consistently able (albeit with difficulty) to place clones into one of the species on the basis of morphology. This situation appears similar to *Lemna minima* and *L. valdiviana*, two morphologically similar (nearly indistinguishable) species with a genetic identity of 0.70 at allozyme loci (Crawford et al., 1996). In *Wolffiella*, those species with very low genetic identities presumably represent taxa of ancient divergence, and this includes the majority of recognized species in the genus. At the same time, there are other recognized species with very high identities, and these are likely either recently diverged taxa, taxa with distinct gene pools but with occasional hybridization that effectively homogenizes allelic frequencies, or the two 'taxa' are in reality minor morphological variants of a single species. Additional studies are needed to elucidate the situation. An important point is that morphological similarity may or may not indicate high similarity at allozyme loci.

The systematics of the subfamily Wolffioideae was treated rather differently by various authors within the last 150 yrs. Until Hegelmaier (1868) all species of this group were incorporated into the genus *Wolffia*. Hegelmaier (1868) created a subgenus *Wolffiella* with *W. denticulata*, *W. gladiata*, *W. lingulata* and *W. oblonga*. The main distinguishing character was the asymmetry of the four species. All the other known species with a symmetrical appearance (including *W. hyalina*, *W. repanda* and *W. welwitschii*) he left with the other species of *Wolffia*. Later Hegelmaier (1895) upgraded the subgenus to a genus. Monod (1949) placed all species with flat fronds in the genus *Wolffiella* and the genus comprised in this way also included the three mentioned symmetrical species. In his monograph, Daubs (1965) kept only *W. welwitschii* within the genus *Wolffiella*. *W. hyalina* and *W. repanda* were transferred to *Wolffia* again. Finally, Den Hartog and Van der Plas (1970) placed *W. hyalina* and *W. repanda* in the separate genus *Pseudowolffia*, and *W. welwitschii* in the genus *Wolffiopsis*. *Wolffiopsis* was characterized by two flowers per frond in contrast to only one for the other species, and *Pseudowolffia* was distinguished by the labellum. Landolt (1986), having detected two new species with transitional characters between the three genera of Den Hartog and Van der Plas (1970), included all these species again in the genus *Wolffiella* creating three sections: sect. *Stipitatae* with *W. hyalina* and *W. repanda*; sect. *Rotundae* with *W. rotunda*; and sect. *Wolffiella* with the rest of the species.

The allozyme data provide some support for the morphological affinities of the species. They show that the genus *Wolffiella* can be divided into two groups, which have identities near zero (Fig. 1, Table 3), one group with the three species *W. hyalina*, *W. repanda* and *W. rotunda*, and one with the rest of the species. Whether these two groups

correspond to two genera, subgenera or sections is a matter of opinion and no decision should be made until DNA sequence data have been analyzed. Allozyme data place some doubt on the justification of placing *W. rotunda* in its own section separate from *W. hyalina* and *W. repanda*, though the lack of a labellum is a very conspicuous characteristic. The three species have in common that they flower frequently, surviving the dry period in the form of seeds. On the other hand, within the large sect. *Wolffiella* all species except *W. denticulata* have some level of allozyme similarity (Fig. 1, Table 3). The isolated position of *W. denticulata* within sect. *Wolffiella* seems remarkable; it shares only low identities with *W. welwitschii* and *W. neotropica* (0.142 and 0.096). Additional investigations are needed to determine if the species is best placed in a separate section. The allozyme data confirm the central position of *W. neotropica* within the genus; it is the only species having some identity with each of the other species. It has the morphological characters of both the two groups. In addition, it is the only species of *Wolffiella* which can grow, depending on conditions, either submerged or partly submerged like the species of the sect. *Wolffiella* or floating entirely on the surface as *W. hyalina*, *W. repanda* and *W. rotunda*.

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