Systematics and biogeography of the genus *Donax* (Bivalvia: Donacidae) in eastern North America

S. Laura Adamkewicz[1] and M. G. Harasewych[2]

[1]Department of Biology, George Mason University, Fairfax, Virginia 22030, U. S. A.
[2]Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D. C. 20560, U. S. A.

Abstract: Recent work with RAPD DNA markers (Adamkewicz and Harasewych, 1994) revealed the absence of fixed differences between the subspecies *Donax variabilis variabilis* Say, 1922, and *D. variabilis roemeri* Philippi, 1849, confirmed that *D. parvulus* Philippi, 1849, is distinct from *D. texasianus* Philippi, 1847, and established that *D. parvulus* is more closely related to *D. variabilis* than to *D. texasianus*, which it otherwise resembles. A fresh examination of RAPD markers in the remaining two Carolinian nominate species has shown that *D. fossor* Say, 1822, is indistinguishable from *D. parvulus* for all markers studied. As the name *D. fossor* has priority, *D. parvulus* should be considered its synonym. Similarly, *D. dorotheae* Morrison, 1971, was indistinguishable from *D. texasianus*, and should therefore be placed in the synonymy of *D. texasianus*. Thus, the biogeography of *Donax* is considerably simplified. *D. variabilis*, the larger, more intertidal species, occurs on both the Atlantic and Gulf coasts. Its division into two subspecies separated by the mouth of the Mississippi River cannot be justified based on RAPD data. *D. variabilis* shares each coast with a smaller, subtidal species, *D. fossor* on the Atlantic coast and *D. texasianus* in the Gulf of Mexico, with the Florida peninsula separating the subtidal species. We suggest that the emergence of Florida during the Cenozoic served as a barrier that led to the differentiation of *D. fossor* and *D. texasianus*. *D. variabilis* apparently evolved as an offshoot from *D. fossor* and subsequently entered the Gulf of Mexico, perhaps when it was connected to the Atlantic by the Suwanee Strait.

Key words: *Donax*, RAPD, systematics, biogeography, Carolinian Province

Determining the status of closely related taxa with allopatric distributions is always difficult and represents one of the most useful applications of molecular techniques to systematic problems. Having used randomly amplified polymorphic DNA (RAPD) markers to examine the relationships of sympatric pairs of *Donax* species (Adamkewicz and Harasewych, 1994), we next applied the technique to study allopatric species in the same complex. In his revision of the western Atlantic species of *Donax*, Morrison (1971) recognized six taxa, representing two adaptive strategies, as occurring along the Atlantic and Gulf coasts of North America (Fig. 1A). Two of these taxa, the subspecies *Donax variabilis variabilis* Say, 1822, and *D. variabilis roemeri* Philippi, 1849, display one adaptive strategy: they are fairly large (15-20 mm), with flattened, triangular shells, and occupy the middle intertidal zone, migrating actively with the tide. *D. variabilis* usually occurs sympatrically with a member of the second adaptive complex: these clams are smaller (5-8 mm), with smoother, more inflated shells, have an intertidal to subtidal distribution, and little tendency to migrate with the tide. Morrison recognized four allopatric species in this second group, referred to here as the subtidal species complex. These are *D. fossor* Say, 1822, which ranges from New Jersey to Cape Hatteras, *D. parvulus* Philippi, 1849, occurring from Cape Hatteras to eastern Florida, *D. dorotheae* Morrison, 1971, from the Gulf coast of Florida to Louisiana, and *D. texasianus* Philippi, 1847, reported from Louisiana to Vera Cruz, Mexico.

Distinguishing young, therefore small, specimens of *Donax variabilis* from any member of the subtidal species complex is quite difficult because the morphological distinctions are few and largely subjective (Morrison, 1971). This difficulty has led to controversies regarding the validity of several of the species in the subtidal species complex (Loesch, 1957; Chanley, 1969; Abbott, 1974) and prompted our original effort to find molecular markers that would resolve these questions (Adamkewicz and Harasewych, 1994). The RAPD molecular markers discovered in that study confirmed the distinctions between *D. variabilis* and two members of the subtidal species complex, and supported the validity of three of the North American taxa recognized by Morrison: *D. parvulus, D. texasianus*, and *D. variabilis*. However, no diagnostic markers were found to distinguish between the subspecies *D. variabilis variabilis* and *D. variabilis roemeri*, nor were any markers identified that were unique to either *D. parvulus* or *D. texasianus*. We now report the results of continuing research to further characterize these taxa using additional RAPD markers and to assess the relationships of *D. fossor* and *D. dorotheae*.

Data on the degree of differentiation among these donacid taxa can also shed light on the significance of

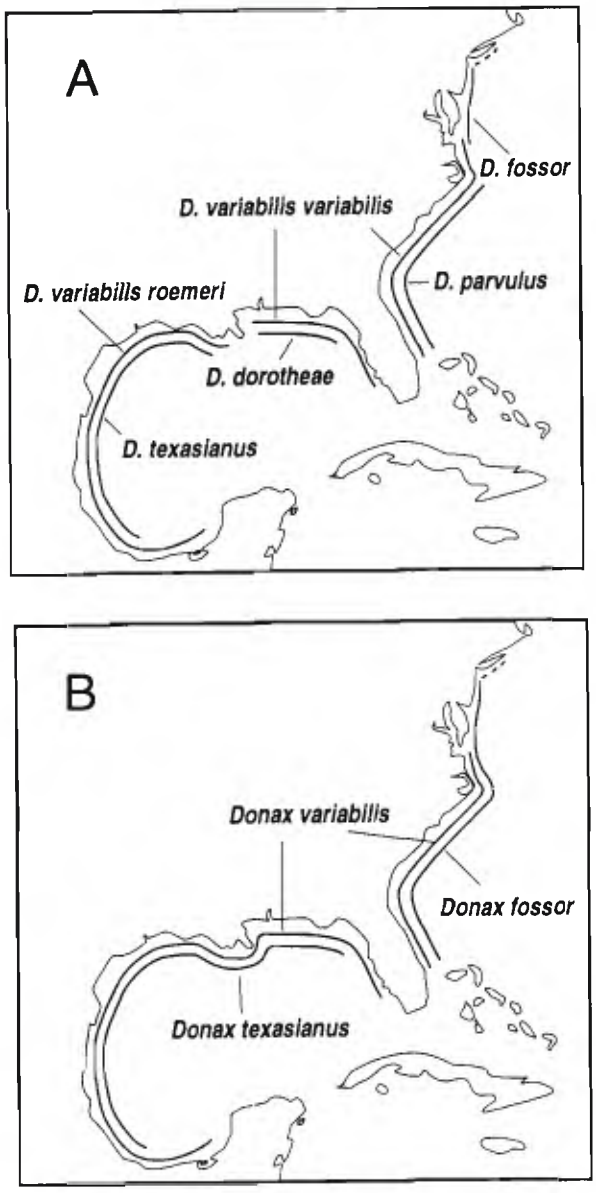

Fig. 1. A. Geographic distributions of the six *Donax* taxa recognized by Morrison (1971). **B.** Geographic distribution of *Donax* based on the findings of the present study.

major and minor geographic barriers along the coastal southeastern United States as isolating mechanisms for shoreline marine organisms with planktonic larvae. The mouths of two great rivers - the Chesapeake Bay complex and the Mississippi River - can create barriers to the dispersal of marine organisms, and were thought by Morrison (1971) to have a significant impact on the distribution of *Donax*. In his scheme (Fig. 1A), the northern limit of *D. variabilis variabilis* is the mouth of the Chesapeake, while Cape Hatteras, with its changes in currents, marks the boundary between *D. fossor* and *D. parvulus*. The mouth of the Mississippi River is the geographical boundary between *D. variabilis variabilis* and *D. variabilis roemeri* as well as between *D. dorotheae* and *D. texasianus*. Another major barrier, the peninsula of Florida, separates *D. parvulus* from *D. dorotheae*, and interrupts the distribution of *D. variabilis variabilis*.

The degree to which the Florida peninsula imposes a biogeographic boundary between the fauna of the southern Atlantic coast of the United States and the fauna of the northern Gulf of Mexico, both components of the Carolinian Province, has been a topic of considerable interest. Several molecular techniques have been used to assess genetic differentiation between Gulf and Atlantic populations of a variety of taxa. Restriction fragment length polymorphism (RFLP) analyses of the mitochondrial genomes of horseshoe crabs, toadfish, black sea bass, and diamondback terrapins have revealed significant differences in populations separated by the Florida peninsula (Avise, 1992), as did allozyme studies of a sea anemone (McCommas, 1982) and a marsh crab (Felder and Staton, 1994). Although no evidence of differentiation was found in allozyme studies of the oyster *Crassostrea virginica* (Gmelin, 1791) (see Buroker, 1983) or the periwinkle *Littorina irrorata* (Say, 1822) (see Dayan and Dillon, 1995), investigations of Atlantic and Gulf coast oyster populations using mitochondrial (Reeb and Avise, 1990) and nuclear (Karl and Avise, 1992) DNA, revealed genetic divergences.

MATERIALS AND METHODS

PREPARATION OF DNA SAMPLES

Specimens of *Donax fossor* were collected by one of us (MGH) in Wildwood Crest, New Jersey, immediately frozen and transported back to the laboratory at George Mason University where they were stored at -60°C. Specimens of *D. dorotheae* were collected from their type locality at Alligator Point, Florida, by Gulf Specimen Marine Laboratories, Inc. (P. O. Box 237, Panacea, Florida 32346) and shipped alive to the laboratory, where their identification was confirmed before they were stored at -60°C. Voucher material is deposited at the National Museum of Natural History, Smithsonian Institution (*D. fossor,* USNM 888672; *D. dorotheae,* USNM 888673). For collection localities and voucher information on the remaining species of *Donax* used in this study, see Adamkewicz and Harasewych (1994: table 1).

To obtain DNA from these specimens, foot muscle was dissected from thawed animals and extracted using a modification of the method of Doyle and Doyle (1987) communicated to us by Andrew McArthur, and further

modified in our laboratory. This method has proven both simple and superior to most others in terms of the quality of the DNA produced. Approximately 100-600 µg of tissue was ground in 300 µl of heated (60°C) buffer composed of 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, and 2% Hexadecyltrimethylammonium bromide (CTAB). As a modification to prevent co-isolation of contaminants, we included 2% polyvinyl pyrolidone in the extraction buffer. Immediately prior to use, we added 0.2% mercaptoethanol to the buffer. The macerate was incubated at 60°C for at least 30 min, then shaken with 300 µl of chloroform/ isoamyl alcohol 24:1 and spun at 14,000 x g for 5 min. The upper, aqueous phase was transferred to a clean tube and treated again with chloroform/isoamyl alcohol. After the aqueous phase was again transferred to a clean tube, the DNA was precipitated by adding 25 µl of 3 M sodium acetate and 600 µl of 70% ethyl alcohol. The mixture was spun at 14,000 x g for 10 min and the supernatant discarded. The DNA pellet was washed twice in 300 µl of 70% ethyl alcohol and dried at 60°C before being dissolved in 50-100 µl of TE buffer (10 mM Tris, 1 mM EDTA) and stored frozen.

PRODUCTION OF RAPD MARKERS

Aliquots of DNA were amplified by the polymerase chain reaction (PCR) according to our earlier RAPD protocol (Adamkewicz and Harasewych, 1994: 53). When the amplification products were separated on a 1.4% agarose gel, this procedure produced DNA fragments of several sizes for each primer, the relative sizes being determined by comparison to DNA markers of known sizes run on the same gel. Our original study (Adamkewicz and Harasewych, 1994) screened 60 primers and found five that were informative about relationships among five species of *Donax*. The present study assayed 20 additional primers, of which one proved to be informative. With two exceptions, the 80 primers used in this study were obtained from Operon Technologies (1000 Atlantic Avenue, Alameda California 94501) and are identified by OP followed by the kit identification letter and number. The remaining two primers were synthesized by the Laboratory for Molecular Systematics, National Museum of Natural History, Smithsonian Institution, and are identified by LMS followed by their identification number.

In the original study, each primer was tested on three specimens of each taxon, and chosen for further use only if it met certain criteria for amplification quality, reproducibility, and distribution of markers among taxa. At that time, our screening criteria emphasized fragments that were shared by multiple taxa, and no primer was studied further unless it generated a marker that occurred in more than one sample of *Donax*. Therefore, taxon-specific markers were identified only for the two species, *D. variabilis* and *D. denticulatus* Linné, 1758, that were represented in our study by multiple populations. To remedy this bias, the present study sought to identify additional taxon-specific markers by several methods. First, we assayed nine individuals each of *D. fossor* and *D. dorotheae* for the 17 markers produced by the five primers identified in our previous study. In addition, we screened these new taxa plus *D. parvulus* and *D. texasianus* samples from our previous study for an additional 20 primers from Operon Kit A. The principle criterion in this screening was that a primer should produce a DNA fragment present in most or all members of one or more taxa. This search identified one new primer which was then used on all samples from the earlier study. Finally, we re-examined photographs of the screening gels from our original study in order to identify any primers that would have met this new standard. This search added two new markers from previously tested primers to the data set. The sequences of old and new primers, and the sizes of informative markers they produced, are summarized in Table 1.

Table 1. DNA primers used in this study. For each primer, the table shows its sequence and the sizes in kilobases of those DNA amplification products used as markers. Primers designated OP A and OP E are from Operon Technologies Kits A and E respectively. Primers designated LMS P were provided by the Laboratory for Molecular Systematics, National Museum of Natural History, Smithsonian Institution. Undesignated fragments are those used in our earlier study (Adamkewicz and Harasewych, 1994). Fragments marked with an asterisk (*) are newly reported in this study.

PRIMER	SEQUENCE	USEFUL RAPD MARKERS (sizes in kb)				
OP A07	5'-GAAACGGGTG	0.3*				
OP E07	5'-AGATGCAGCC	0.6	1.5			
OP E16	5'-GGTGACTGTG	0.3	0.5	0.6	0.9	1.1
OP E18	5'-GGACTGCAGA	0.5	0.6	0.9		
LMS P01	5'-TGGTCAGTGA	0.5	0.6*	1.0	1.2	2.0*
LMS P56	5'-AGATCTGCAG	0.3	0.6	1.1	1.2	

PHYLOGENETIC ANALYSES

Data from nine populations, including all six Recent nominal species and subspecies of Carolinian *Donax* were used in this study. A sample of *D. denticulatus* from Negril, Jamaica, and a sample of *D. striatus* Linné, 1767, from Black River, Jamaica, were used as outgroups, based on a previously published phylogeny (Adamkewicz and Harasewych, 1994). As in our earlier study, each RAPD marker was treated as a separate character without regard to the primer used to produce it or whether other bands produced by the same primer co-occurred with it. Markers were scored as absent (0), polymorphic (1), *i. e.* present in some, but not all, members of a population, or fixed (2), *i. e.* present in all members of a population. Maximum parsi-

mony trees, bootstrap values (1000 replicates), and tree diagnostics were calculated using PAUP version 3.1 (Swofford, 1993). Characters were treated as ordered (0 ↔ 1 ↔ 2), because this models the way in which alleles enter, become established, and leave populations. Branch support values (*b*) and the total support index (*ti*) were calculated using procedures outlined by Bremer (1994: 300). Data were entered and character evolution on the resulting trees analyzed using MacClade version 3.01 (Maddison and Maddison, 1992).

RESULTS

RAPD DNA MARKERS

Both sets of screening criteria were successful in identifying taxon-specific RAPD markers among the *Donax* species of our original study. Of the new primers tested, one was found to produce a marker (OP A07 0.3 kb) unique to *D. texasianus* and *D. dorotheae*. A re-analysis of previous data revealed one marker (LMS P01 2.0 kb) unique to *D. parvulus* that was also found to occur in *D. fossor*, and another marker (LMS P01 0.6 kb) that was unique to *D. striatus*. Thus, each of the five species in our 1994 study can now be distinguished by the presence of one or more unique markers (Table 2). Of the 20 markers used in the present study, eight are common to all the Carolinian populations examined. Two are unique to *D. variabilis*, which shares another two markers with the two nominal, Atlantic, subtidal taxa, *D. fossor* and *D. parvulus*. The Atlantic *D. fossor/D. parvulus* pair and the Gulf coast *D. texasianus/D. dorotheae* pair are each distinguished by a unique marker. Although unique markers were sought for each taxon, none were found that would distinguish between the two members of either of these pairs.

Table 2. RAPD DNA markers diagnostic for species of northwestern Atlantic Donacidae. Markers preceded by a dagger (†) are fixed, the remaining markers are present in most but not all members of their respective taxa. See Table 3 for frequencies of markers.

TAXON	DIAGNOSTIC MARKER(S)
Donax fossor (+ *D. parvulus*)	LMS P01 2.0 kb
D. variabilis variabilis (+ *D. variabilis roemeri*)	OP E16 0.3 kb; †OP E18 0.5 kb
D. texasianus (+ *D. dorotheae*)	OP A07 0.3 kb
D. denticulatus	†OP E16 0.6 kb; OP E18 0.6 kb; LMS P01 1.0 kb
D. striatus	LMS P01 0.6 kb

The marker unique to the *Donax texasianus/D. dorotheae* pair, a *ca.* 300 bp fragment produced by primer OP A 07, was present in eight of nine *D. texasianus* individuals examined, as well as in six of nine *D. dorotheae* analyzed. Similarly, the 2 kb fragment produced by primer LMS P01 was present in all nine individuals of *D. parvulus* and in eight of nine individuals of *D. fossor*. After a screening with 80 primers and a detailed examination with six primers, only very minor differences in marker frequencies separated the members of these two pairs. Such differences have little meaning when sample sizes are small, nine individuals per taxon, and differences as large or larger were found among the species represented by multiple populations, *D. variabilis* and *D. denticulatus* (Table 3).

PHYLOGENETIC ANALYSIS

A single most parsimonious tree (length = 36, ci = ri = 0.92, ti = 0.42), shown in Fig. 2, was produced using the data matrix given in Table 4 and the Exhaustive Search command, which guarantees that all minimum length trees will be found (Fig. 2). Three additional steps would be required to unite the members of the subtidal species complex in a clade. Of the 20 markers, four (markers 14, 15, 17, and 20) were phylogenetically uninformative, appearing only in terminal taxa (autapomorphies). All but three of the remaining 16 markers had a consistency index of 1.0. Two of the exceptions (marker 6, ci = 0.667; 12, ci = 0.500), were apparent reversals from a fixed to a polymorphic state in the *D. fossor/D. parvulus* pair. The remaining exception (marker 10, ci = 0.667) was accounted for by an independent (homoplastic) fixation of the marker in *D. denticulatus* and in the *D. fossor/D. parvulus* pair.

DISCUSSION

The four nominal taxa of the subtidal species complex replace each other geographically, but neighboring taxa do not differ morphologically in any clearly recognizable way and all occupy the same ecological niche. Although the binomen *Donax fossor* is among the oldest to be applied to American donacids, the systematic relationships of this taxon are among the most poorly understood. Jacobson and Emerson (1961) noted the sporadic occurrence of these animals in Long Island, and suggested that this taxon represented juvenile or stunted specimens of *D. variabilis* that were recruited from larvae swept north of the sustainable range of this species. They further reported that *D. fossor* does not survive the winter in this area. Chanley (1969) reiterated this hypothesis on the basis of his studies of seasonal distribution of *Donax* in the mid-Atlantic region, and speculated that "minor conchological

Table 3. Distribution of the RAPD DNA markers among sample populations. For each RAPD marker, the band designation identifies the primer, the approximate size of the marker in kilobases and a sequential number to identify the marker. Each entry in the matrix shows the number of individuals in which the RAPD marker was detected over the number of individuals tested (*e. g.* 3/9). In a few cases, the number of individuals tested was fewer than nine either because fewer DNA samples were available or because we were unable to score an individual for a particular marker. When no individuals produced a marker, the entry is marked "–" rather than 0/n. Data are grouped to emphasize affinities among sample populations, rather than by primer. Abbreviations (DDB, DDM, DDN, DVF, DVG, DVR) as in Adamkewicz and Harasewych (1994).

PRIMER:	LMS P01	OP E18	OP E16	OP E16	LMS P56	OP E16	OP E16	LMS P01	LMS P56	OP E18	OP E07	OP E07	OP A07	OP E18	LMS P01	LMS P01	OP E16	LMS P56	LMS P56	LMS P01
SIZE (kb):	2.0	0.5	0.3	0.9	0.3	0.5	1.1	0.5	0.6	0.9	0.6	.5	0.3	0.6	1.0	1.2	0.6	1.1	1.2	0.6
NUMBER:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Donax fossor	8/9	–	–	4/9	6/9	7/9	2/9	9/9	9/9	9/9	3/9	4/9	–	–	–	–	–	–	–	–
D. parvulus	9/9	–	–	7/9	6/9	7/9	2/9	9/9	9/9	9/9	2/9	6/9	–	–	–	–	–	–	–	–
D. v. variabilis (DVF)	–	9/9	2/9	5/9	8/9	9/9	3/9	9/9	9/9	9/9	9/9	9/9	–	–	–	–	–	–	–	–
D. v. variabilis (DVG)	–	9/9	4/9	5/9	9/9	9/9	49	9/9	9/9	9/9	9/9	9/9	–	–	–	–	–	–	–	–
D. v. roemeri (DVR)	–	9/9	1/9	5/9	6/9	9/9	6/9	9/9	9/9	9/9	9/9	9/9	–	–	–	–	–	–	–	–
D. dorotheae	–	–	–	–	–	7/7	7/7	4/9	9/9	2/9	1/9	9/9	6/9	–	–	–	–	–	–	–
D. texasianus	–	–	–	–	–	9/9	9/9	3/9	9/9	2/9	2/9	9/9	8/9	–	–	–	–	–	–	–
D. denticulatus (DDB)	–	–	–	3/3	–	–	3/3	–	–	3/3	2/3	2/3	–	3/3	3/3	1/3	2/3	3/3	1/3	–
D. denticulatus (DDM)	–	–	–	9/9	–	–	9/9	–	–	9/9	5/9	2/9	–	9/9	8/9	1/9	8/9	6/6	5/8	–
D. denticulatus (DDN)	–	–	–	9/9	–	–	9/9	–	–	9/9	7/9	7/9	–	9/9	9/9	1/9	8/9	5/9	6/9	–
D. striatus	–	–	–	6/6	–	–	6/6	–	–	–	–	5/6	–	–	–	6/6	–	4/6	6/6	8/9

Table 4. Data matrix for cladistic analyses. Markers listed in Table 2 are scored as: absent (0), polymorphic (1), fixed (2), or unknown (?). Abbreviations as in Table 3.

MARKER #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Donax fossor	1	0	0	1	1	1	1	2	2	2	1	1	0	0	0	0	0	0	0	0
D. parvulus	2	0	0	1	1	1	1	2	2	2	1	1	0	0	0	0	0	0	0	0
D. v. variabilis (DVF)	0	2	1	1	1	2	1	2	2	2	2	2	0	0	0	0	0	0	0	0
D. v. variabilis (DVG)	0	2	1	1	2	2	1	2	2	2	2	2	0	0	0	0	0	0	0	0
D. v. roemeri (DVR)	0	2	1	1	1	2	1	2	2	2	2	2	0	0	0	0	0	0	0	0
D. dorotheae	0	0	0	0	2	2	1	2	1	1	2	1	0	0	0	0	0	0	0	0
D. texasianus	0	0	0	0	0	2	2	1	2	1	1	2	1	0	0	0	0	0	0	0
D. denticulatus (DDN)	0	0	0	2	0	0	2	0	0	2	1	1	0	2	2	1	1	1	1	0
D. striatus	0	0	0	2	0	0	2	0	0	0	0	1	0	0	0	2	0	1	2	1

differences" between *D. fossor* and *D. variabilis* were likely ecophenotypic. Most (*e. g.* Abbott, 1974; Emerson and Jacobson, 1976), but not all (Morrison, 1971) subsequent workers have treated *D. fossor* as a synonym of *D. variabilis*. *D. parvulus* was also regarded as an offshore ecological form of *D. variabilis* by some authors (*e. g.* Abbott, 1974).

Implicit in the hypotheses of seasonal northern range extensions (in the case of *Donax fossor*) or offshore ecophenotypes (in the case of *D. parvulus*) is the assumption that these populations comprise subsets of the genetic variation to be found in *D. variabilis*. Our RAPD data demonstrate clearly that, although *D. fossor* and *D. parvulus* cannot be distinguished from each other, both are genetically distinct from *D. variabilis*. Because the ranges of *D. fossor* and *D. parvulus*, as suggested by Morrison (1971), are allopatric, it is difficult to be certain that this indistinguishable pair is, in fact, the same species. However, a careful search for significant differences in their gene pools has produced entirely negative results. As *D. fossor* is the older name, it should be applied to all members of the subtidal species complex living along the Atlantic coast of the United States, and *D. parvulus* treated as its synonym. This taxon is characterized by the presence of a 2.0 kb marker produced by the LMS P01 primer in most members of its populations. Our coarse sampling of two populations from near the extremes of the range of this species failed to uncover differences that might justify even a subspecific distinction of these taxa.

Similarly, patterns of RAPD markers in *Donax dorotheae* do not differ in any meaningful way from those in *D. texasianus*. Only the most minor differences in frequencies of polymorphic markers were found, and these were well within the range of inter-population variation in *D. variabilis*. The name *D. texasianus* has priority, and should be applied to all Gulf coast members of the subtidal species complex. *D. texasianus* may be diagnosed by the presence, in the majority of the individuals in its populations, of a 0.3 kb marker generated by the OP A07 primer.

Additional data from the present study have failed to support the subspecific division of *Donax variabilis* that Morrison suggested. Differences between the Atlantic and eastern Gulf populations appear to be of similar type and magnitude to those between the eastern and western Gulf populations, and are within the range of variation of three populations of *D. denticulatus* from around Jamaica (Table 3), as well as of a single population of *D. denticulatus* sampled at different times (Adamkewicz and Harasewych, 1994: table 3).

If the above changes are adopted, the distribution of *Donax* along the Atlantic and Gulf coasts of North America changes from that proposed by Morrison (Fig. 1A) to the one shown in Fig. 1B.

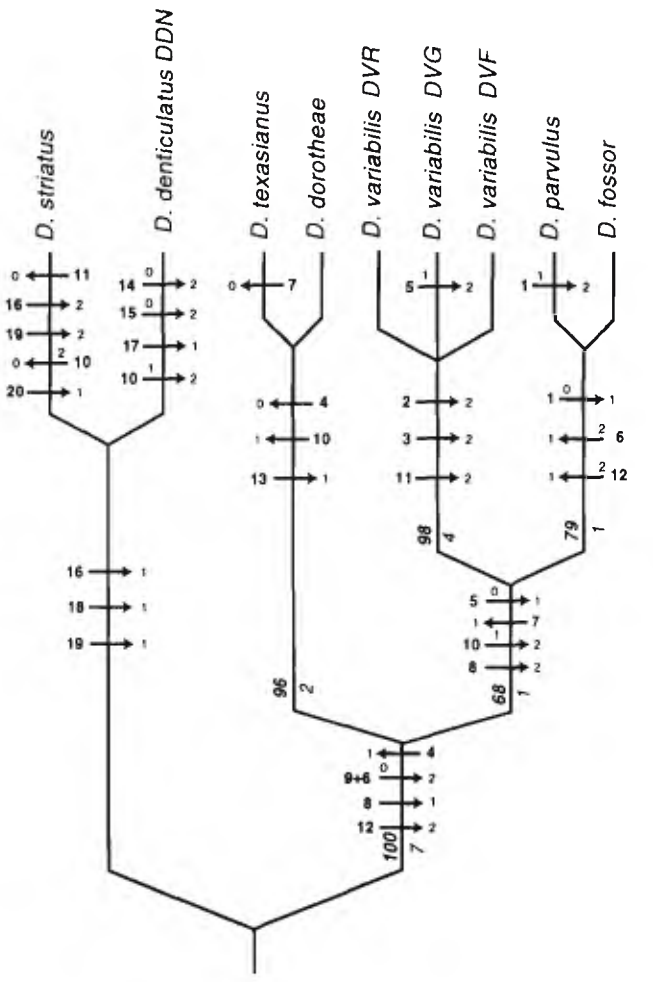

Fig. 2. Phylogenetic relationships of the six *Donax* taxa recognized by Morrison (1971) and two additional *Donax* taxa identified as outgroups by Adamkewicz and Harasewych (1994). Relationships are inferred from the distribution of the 20 RAPD markers shown in Table 3.

The inclusion of additional taxa and characters in our data matrix produced a single most parsimonious tree (Fig. 2) identical in topology to that from our previous study (Adamkewicz and Harasewych, 1994: figs 2-3). The topology of the phylogenetic tree indicates that the small size and non-migratory subtidal habitat of *Donax fossor* and *D. texasianus* are ancestral conditions, while the larger size, elongated shell morphology and migratory, intertidal habitat of *D. variabilis* are more recently evolved. The difficulty in discriminating juvenile *D. variabilis* from *D. fossor* using morphological characters, and the resulting confusion regarding their taxonomy, are likely a consequence of the sister-group relationship between these taxa. *D. variabilis*, the younger species, retains the morphology of its

smaller ancestors at a comparable body size but, as an adult, develops a larger size and more derived shell form, possibly adapted to its migratory habitat.

Of particular interest is the lack of correspondence in the distributions and biogeographic boundaries of *Donax variabilis* and the members of the subtidal species complex. Peninsular Florida separates the Atlantic and Gulf species of the subtidal complex but does not appear to have caused discernible differentiation in populations of *D. variabilis*. *D. fossor* inhabits the Atlantic coast of the United States from the extreme north of the range for any donacid species to central Florida. Jacobson and Emerson (1961) and Chanley (1969) were likely correct in their conjecture that northernmost populations of *D. fossor* are ephemeral range extensions during seasonally favorable conditions of a species with a more southern distribution, but erred in regarding them to be conspecific with *D. variabilis*. Our admittedly coarse samples, which span both Chesapeake Bay and Cape Hatteras, reveal few differences between populations and suggest that neither of these geographical features are barriers to gene flow in *D. fossor*. Similarly, our samples of *D. texasianus*, the Gulf member of the subtidal complex, span the Mississippi Delta and also demonstrate the absence of any barrier to gene flow within the Gulf of Mexico. For this genus, the major rivers apparently do not impede gene flow. In contrast, the Florida peninsula separates *D. fossor* and *D. texasianus* but has not produced qualitative differences among populations of *D. variabilis*, which it also separates. Because studies surveying variation in the mitochondrial genome have been most successful in discovering genetic differentiation between Gulf and Atlantic conspecific populations (Avise, 1992), perhaps the application of such techniques would reveal differentiation among populations of *D. variabilis*. Our RAPD study does not do so.

The different ages of these taxa could contribute to the differences in their geographic distributions. The emergence of peninsular Florida could have contributed to the vicariant differentiation of *Donax fossor* and *D. texasianus*. *D. variabilis* appears to have evolved from *D. fossor* along the Atlantic coast, although by what mechanism we have no evidence. *D. variabilis* could then have invaded the Gulf of Mexico through the Suwanee Straits (Huddlestun *et al.*, 1988).

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