Stingrays, Parasites, and Neotropical Biogeography: A Closer Look at Brooks et al.'s Hypotheses Concerning the Origins of Neotropical Freshwater Rays (Potamotrygonidae)

Nathan R. Lovejoy

The reconstruction of evolution. Heredity 18:553. (Abstr.)

FARRIS, J. S. 1986. On the boundaries of phylogenetic systematics. Cladistics 2:14–27.

FARRIS, J. S., AND A. G. KLUGE. 1986. Synapomorphy, parsimony, and explanatory power. Taxon 35:298–306.

FARRIS, J. S., A. G. KLUGE, AND M. J. ECKARDT. 1970. A numerical approach to phylogenetic systematics. Syst. Zool. 19:172–179.

FELSENSTEIN, J. 1982. Numerical methods for inferring evolutionary trees. Q. Rev. Biol. 57:379–404.

FELSENSTEIN, J. 1984. The statistical approach to inferring phylogeny and what it tells us about parsimony and compatibility. Pages 169–191 in Cladistics: Perspectives on the reconstruction of evolutionary history (T. Duncan and T. F. Stuessy, eds.). Columbia Univ. Press, New York.

HENNIG, W. 1950. Grundzüge einer Theorie der phylogenetischen Systematik. Deutscher Zentralverlag, Berlin.

HENNIG, W. 1966. Phylogenetic systematics. Univ. Illinois Press, Urbana.

HENNIG, W. 1981. Insect phylogeny. John Wiley and Sons, New York.

HENNIG, W. 1983. Stammesgeschite der Chordaten. Verlag Paul Parey, Hamburg.

KLUGE, A. G., AND J. S. FARRIS. 1969. Quantitative phyletics and the evolution of anurans. Syst. Zool. 18:1–32.

MICKEL, J. T. 1962. A monographic study of the fern genus Anemia, subgenus Ceratophyllum. Iowa State Univ. J. Sci. 36:349–482.

RICHTER, S., AND R. MEIER. 1994. The development of phylogenetic concepts in Hennig's early theoretical publications (1947–1966). Syst. Biol. 43:212–221.

SNEATH, P. H. A. 1995. Thirty years of numerical taxonomy. Syst. Biol. 44:281–298.

SOKAL, R. R., AND P. H. A. SNEATH. 1963. Principles of numerical taxonomy. Freeman, San Francisco.

WAGNER, W. H. 1980. Origin and philosophy of the groundplan-divergence method of cladistics. Syst. Bot. 5:173–193.

Stingrays, Parasites, and Neotropical Biogeography: A Closer Look at Brooks et al.'s Hypotheses Concerning the Origins of Neotropical Freshwater Rays (Potamotrygonidae)

NATHAN R. LOVEJOY

Section of Ecology and Systematics, Corson Hall, Cornell University, Ithaca, New York 14853-2701, USA;
E-mail: nrl2@cornell.edu

The exploration of host/parasite coevolution has been invigorated in recent years, in part by the application of explicit methods of phylogeny estimation to parasitic organisms (Klassen, 1992). A pioneering study in the field was Brooks et al.'s (1981b) investigation of South American stingray biogeography and phylogeny, based on cladograms for helminth parasites (hereinafter referred to as Brooks et al.). The frequent reiteration of the results (e.g., Brooks, 1988, 1992, 1995; Brooks and Deardorff, 1988; Brooks and McLennan, 1991, 1993a, 1993b) has turned this "novel contribution to biogeographic analysis" (Straney, 1982:337) into an "old friend"— a great example to use in teaching (Blair, 1994:296). However, the Brooks et al. analysis has occasionally been questioned. Straney (1982) (and to a lesser extent Simberloff, 1987) voiced concerns about the biogeographic inferences drawn from the presented parasite cladograms. More recently, Caira (1990, 1994) cautioned that several of Brooks et al.'s character data sets and analyses remain unpublished, implying that there may be problems with the parasite data themselves. In this paper, I focus on another aspect of this widely known study, the methods used by Brooks et al. to infer host biogeography and phylogeny from parasite cladograms and distributions.

The exclusively freshwater stingray family Potamotrygonidae ranges throughout the major Atlantic drainages of South

America (including the Atrato, Maracaibo, Orinoco, Essequibo, Amazon, Parana, and de la Plata [Rosa, 1985]). This distribution represents an anomaly within stingrays (suborder Myliobatidoidei), an otherwise predominantly marine taxon. With the goal of indirectly inferring the origins of potamotrygonids, Brooks et al. examined the phylogenies of several groups of helminth parasites inhabiting freshwater and marine stingrays (for parasite cladograms, see Brooks et al., 1981a; Deardorff et al., 1981). Although the presence of potamotrygonids in Atlantic drainages might appear to suggest that Neotropical freshwater stingrays were derived from one or more lineages of Atlantic marine stingrays, Brooks et al. instead suggested that the parasites of potamotrygonids were most closely related to those of coastal Pacific rays (of the genus *Urolophus*). This counterintuitive result was combined with South American paleogeographic data to generate a novel biogeographic scenario for the entry of marine stingrays into Neotropical freshwater. Brooks et al. suggested that the Cretaceous–Miocene orogeny of the Andes gradually blocked a Pacific-draining proto-Amazon, trapping marine and estuarine taxa in a shallow inland sea. Taxa surviving in this progressively freshwater environment (e.g., potamotrygonid ancestors) subsequently spread through South America and were isolated in various river drainages. The scenario explains the apparent relationship between the parasites of stingrays in Atlantic drainages and those of Pacific Coast rays.

In the original paper, Brooks et al. proposed a test of their hypothesis: a phylogeny of stingrays, including potamotrygonids and their relatives, should show a pattern similar to that of their parasites. Such a study was recently completed (Lovejoy, 1996), building on previous systematic investigations of stingrays (Rosa, 1985; Nishida, 1990). The resultant phylogenetic and biogeographic patterns differ from those proposed by Brooks et al. based on parasites. The marine sister group to potamotrygonids was determined to be a clade of Pacific and Caribbean *Himantura* species, rather than Pacific *Urolophus* as proposed by Brooks et al. *Urolophus* (hereinafter referred to as *Urobatis* for amphi-American forms; after Lovejoy, 1996; McEachran, in press) was relatively basal in the stingray cladogram and represented the sister clade of *Urotrygon*. Constraining monophyly between Pacific *Urobatis* and the Potamotrygonidae yielded considerably longer trees (five additional steps required, >8% increase in tree length [Lovejoy, 1996]). The disparities between stingray and parasite patterns prompted a closer examination of the parasite-based analyses and conclusions.

Brooks et al. used their parasite phylogeny data to address five questions, or "test points," about the evolution and origins of potamotrygonids: (1) Are potamotrygonids monophyletic or derived from multiple ancestral lineages? (2) Was their geographic origin freshwater or marine? (3) Are they derived from Atlantic, Pacific, or continental ancestors? (4) What is their sister taxon? (5) Did they invade freshwater habitats (dispersal) or were they "trapped" in them by vicariant processes? Brooks et al. suggested that their answers to these points clearly support a monophyletic origin of potamotrygonids from Pacific *Urobatis* stingrays by vicariance. However, there are problems with the methods used by Brooks et al. (and more recently by Brooks and Deardorff, 1988; Brooks and McLennan 1991, 1993b; Brooks, 1992) to test these points. In some cases, problems are specific to the stingray/parasite system; however, others (e.g., the use of parasite monophyly to infer host monophyly) may apply more generally to host/parasite coevolutionary investigations. Here, I discuss the difficulties with Brooks et al.'s approaches to each of their original test points and demonstrate that an alternative biogeographic hypothesis is better supported.

POINT 1: MONOPHYLETIC OR POLYPHYLETIC

If potamotrygonids are descended from a single ancestral lineage that entered South America, they should represent a monophyletic group within stingrays.

Likewise, Brooks et al. suggested that the monophyly/polyphyly of potamotrygonid parasites should indicate the monophyly/polyphyly of their hosts. Twenty-four species of parasites have been recorded from potamotrygonids. Of these, four also inhabit teleosts (*Paravitellotrema overstreeti, Terranova edcaballeroi*) or crocodilians (*Leiperia gracile, Brevimulticaecum* sp.), presumably representing incidental host shifts, and one (*Megapriapus ungriai*) a unique elasmobranch-infecting species whose ancestry is not known (Brooks and Amato, 1992; Brooks and McLennan, 1993b). The remaining 19 potamotrygonid parasite species are members of eight separate elasmobranch-infecting clades. Three clades contain multiple species infecting potamotrygonids (*Acanthobothrium*, 4 spp.; *Potamotrygonocestus*, 3 spp.; *Rhinebothroides*, 7 spp.), and the other five clades each contain a single species infecting potamotrygonids (*Eutetrarhynchus, Rhinebothrium, Paraheteronchocotyle, Potamotrygonocotyle, Echinocephalus*) (Brooks and McLennan, 1993b).

Brooks et al. considered several of these clades (*Acanthobothrium, Potamotrygonocestus, Rhinebothroides, Rhinebothrium, Eutetrarhynchus, Echinocephalus*) and found in nearly all cases that the species infecting freshwater potamotrygonids comprise monophyletic groups relative to sister taxa infecting marine elasmobranchs (the only exception being *Potamotrygonocestus*, whose putative marine sister group could not be identified). Thus, according to Brooks et al., freshwater *Acanthobothrium* parasites of potamotrygonids make up a monophyletic group that is most closely related to a clade of two species that infect marine elasmobranchs (*Acanthobothrium cartegenensis, A. holorhini*), and freshwater *Rhinebothroides* compose a monophyletic group that is most closely related to a clade of two different marine taxa (*Phyllobothrium kingae, P.* cf. *kingae*). This pattern of monophyly is taken to indicate the monophyly of potamotrygonids themselves. Brooks (1992: 589) stated, "It would appear that the helminth fauna of potamotrygonids is an assemblage of monophyletic groups and sin-
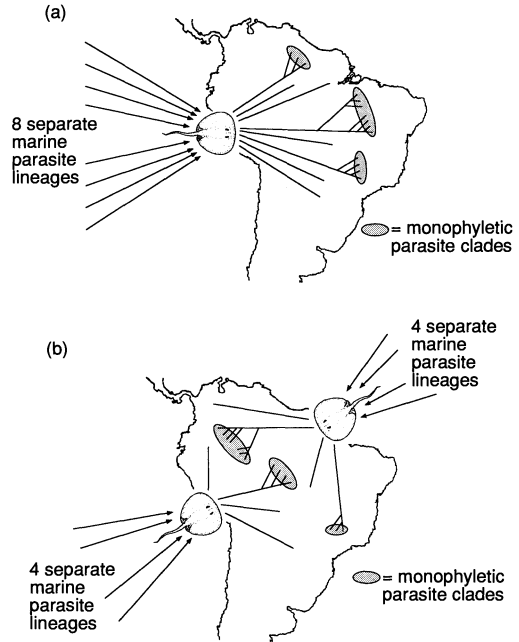

FIGURE 1. Representation of stingray/parasite invasion of South American freshwater. All lines represent parasite lineages; lines within South America represent freshwater parasite lineages. Monophyletic freshwater parasite clades result under both scenarios; therefore, assemblages of monophyletic parasite clades do not necessarily indicate host monophyly. (a) Invasion by single stingray host taxon infected with eight separate parasite lineages: Brooks et al.'s (1981b) hypothesis. (b) Invasion by two stingray host taxa, each infected with four separate parasite lineages.

gle species, supporting the hypothesis that potamotrygonids arose from a single invasion of freshwater habitats in South America."

However, the evaluation of potamotrygonid monophyly should not be based solely on knowledge of parasite relationships and monophyly. Two additional requirements that Brooks et al. neither presented nor discussed include (1) data indicating which parasite species and lineages occur within different potamotrygonid hosts and (2) estimates of factors (such as host switching) that might obscure the original coevolutionary patterns.

Consider two alternative scenarios for the invasion of South America (Fig. 1). In one (Brooks et al.'s hypothesis), a single ancestral stingray lineage carries a fauna

of eight separate parasite lineages into freshwater South America (Fig. 1a); in the other, independently invading stingray lineages each carry their own separate parasite faunas into South America (Fig. 1b). In both cases, monophyletic freshwater parasite groups may be produced; therefore, the monophyly of assemblages of South American stingray parasites is insufficient to demonstrate the monophyly of stingrays in South America. To distinguish between these alternatives, the distributions of parasite species and lineages within different freshwater stingray hosts needs to be taken into account. Further consideration of Figure 1 shows why this is the case. If all stingray parasite lineages in South America were present in a single ancestral stingray invader (Fig. 1a), all descendant freshwater stingrays should host representatives of each of the eight parasite lineages (assuming no extinction or sampling error). However, two freshwater stingray clades descended from independently invading ancestors (Fig. 1b) would host distinct groups of monophyletic parasite clades (assuming, most importantly, no host switching). In simpler terms, if all freshwater stingray species are observed to host a common set of parasite lineages, monophyly of the stingrays is a possible explanation; however, if many freshwater stingray species are sampled and appear to fall into different groups characterized by nonoverlapping sets of parasite lineages, nonmonophyly of the hosts is the possible cause.

Unfortunately, the coevolutionary patterns of parasite lineages within different hosts may be obscured by several phenomena. Host switching by parasites will tend to homogenize the distribution of parasite lineages among unrelated hosts, thereby biasing patterns in favor of overall host monophyly. Extinction of parasites and sampling error will result in parasites being recorded as missing from certain hosts, potentially creating an artificial pattern of nonmonophyly of hosts. An understanding of the possible extent of these phenomena is important for the interpretation of parasite distributions in various

hosts. Although Brooks et al. did not address this issue, the most recent host–parasite list for helminths inhabiting potamotrygonids (Brooks and Amato, 1992) indicates that some of these phenomena may be common. Each species of *Potamotrygon* hosts an average of four different parasite lineages (rather than the eight predicted by Brooks et al.'s hypothesis), suggesting either that extinction and sampling error are rampant or that Brooks et al.'s hypothesis of host monophyly is not accurate.

Without supporting evidence (of distributions of parasite species and lineages within host species and of the potential frequency of host switching, extinction, and sampling error), the monophyly of parasite assemblages is insufficient to demonstrate host monophyly. In the case of potamotrygonids, Brooks et al. did not provide this information, thus determination of potamotrygonid monophyly based on parasites is tenuous. Current morphological evidence for stingrays indicates the monophyly of Potamotrygonidae, but not completely unambiguously; if characters associated with freshwater adaptation are removed from the stingray analysis, a clade composed exclusively of potamotrygonids is absent in two of the three shortest trees (Lovejoy, 1996).

POINT 2: FRESHWATER OR MARINE

Most freshwater potamotrygonid parasites represent derived groups or individual taxa nested within parasite clades infecting marine stingrays (Brooks et al.). This pattern is paralleled by the stingrays, i.e., the freshwater habitat optimized on the stingray cladogram suggests that freshwater tolerance is a derived condition (Lovejoy, 1996). Also, potamotrygonids possess vestigial rectal glands, the salt excretion organs present in marine stingrays (Thorson et al., 1978). Thus, phylogenies for both stingrays and parasites suggest an ancestral marine origin for Neotropical freshwater representatives.

POINT 3: AREA OF DERIVATION

Although a marine origin of potamotrygonids and their parasites is relatively
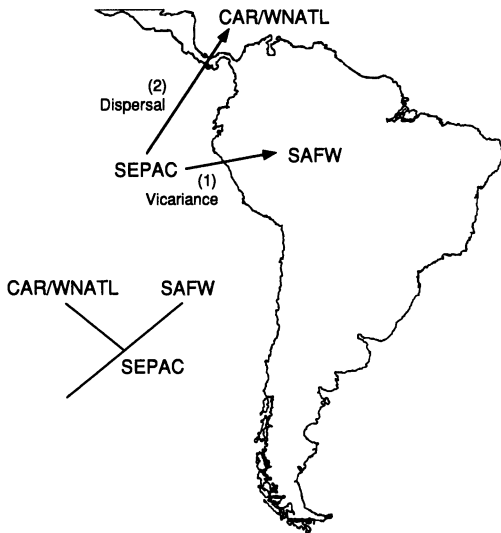
FIGURE 2.   Brooks et al.'s (1981b) biogeographic hypothesis for relationships of areas CAR/WNATL (Caribbean/northern West Atlantic), SAFW (South American freshwater), and SEPAC (southeastern Pacific). Entire tree is not shown; an additional CAR/WNATL component is proposed to have dispersed from the northeastern Pacific (see Brooks et al., 1981b: fig. 16).

clear, the specific area of derivation of these groups is more ambiguous. To approach this question, Brooks et al. (1981b: fig. 16) generated a "cladogram depicting historical relationships of various circum-Pacific and Caribbean/Atlantic areas" based on several clades of stingray parasites. The Caribbean (CAR) and northern West Atlantic (WNATL) are grouped as sister areas to South American freshwater (SAFW), and the node leading to this clade is labeled southeastern Pacific (SEPAC) (see Fig. 2). This part of the topology was explained by Brooks et al. as follows: (1) South American parasites of potamotrygonids (SAFW) are derived from SEPAC marine ancestors, and (2) CAR/WNATL parasites are subsequently derived from dispersals of SEPAC taxa. Because the SAFW and CAR/WNATL areas were both independently derived from the Pacific, "the history of the Atlantic–Caribbean species could not pertain directly to events producing the species of helminths inhabiting potamotrygonids" (Brooks et al., 1981b:158). This conclusion, however, is

problematic in terms of both biogeographic methodology and the geological history of the areas.

Brooks et al. did not specify the method used to construct the cladogram of relationships among areas. Figure 3 shows the parasite cladograms used by Brooks et al. to generate the overall cladogram of area relationships (geographic distributions added: from Brooks et al., 1981b: figs. 6–15, appendix 1). The SEPAC element, which Brooks et al. proposed has given rise to the SAFW element, is not represented in any of these cladograms.

The separate parasite area cladograms (Fig. 3) were reanalyzed using Brooks's (1981) parsimony method (BPA of Wiley, 1988; cospeciation analysis of Brooks and McLennan, 1991) and component methods (Nelson and Platnick, 1981) as implemented by Component 1.5 (Page, 1989). Since Brooks et al.'s paper, the original cladogram for *Rhinebothrium* (Fig. 3c) has been modified (Fig. 3d; Brooks and Deardorff, 1988); I conducted separate analyses using both the new and old cladograms. The cladogram for *Echinocephalus* has also been altered (Brooks and Deardorff, 1988); however, the relevant area cladogram is not significantly different as a result.

BPA produces a composite representation of area relationships from multiple independent area cladograms, using taxa (parasites in this case) and their relationships as characters. The coded matrix is shown in Table 1. Following Wiley (1988), in clades with no representatives for particular areas, those areas were coded as missing/unknown data (?); e.g., the *Rhinebothroides / Phyllobothrium kingae* clade was coded as unknown for the NEPAC (northeastern Pacific) and IWP (Indo-West Pacific) areas (making this clade uninformative as a two-taxon statement). Sympatric species in the *Rhinebothrium* clades (Figs. 3c, 3d) were coded as present in their respective areas. Other methods (e.g., Brooks's [1990] procedure of dividing areas with sympatric taxa into separate units for analysis) did not alter the results. The single tree produced (Fig. 4a; branch and bound analysis with PAUP 3.1; Swofford,

IWP SAFW CAR/WNATL NEPAC

urolophi FW clade cartagenensis holorhini

1

2

(a) *Acanthobothrium*

SAFW CAR/WNATL CAR/WNATL

FW clade Phyllobothrium Phyllobothrium
(*Rhinebothroides*) kingae cf. kingae

3

(b) *Rhinebothroides* +
sister group

NEPAC NEPAC SAFW CAR/WNATL

urobatidium ditesticulum paratrygoni margaritense spinicephalum tetralobatum

4

5

(c) *Rhinebothrium*

IWP NEPAC SAFW CAR/WNATL NEPAC CAR/WNATL

out urobatidium paratrygoni margaritense ditesticulum tetralobatum biorchidum spinicephalum

(d) *Rhinebothrium* (2)

IWP SAFW CAR/WNATL NEPAC

sinensis daileyi diazi pseudouncinatus

6

7

(e) *Echinocephalus*

IWP NEPAC SAFW CAR/WNATL

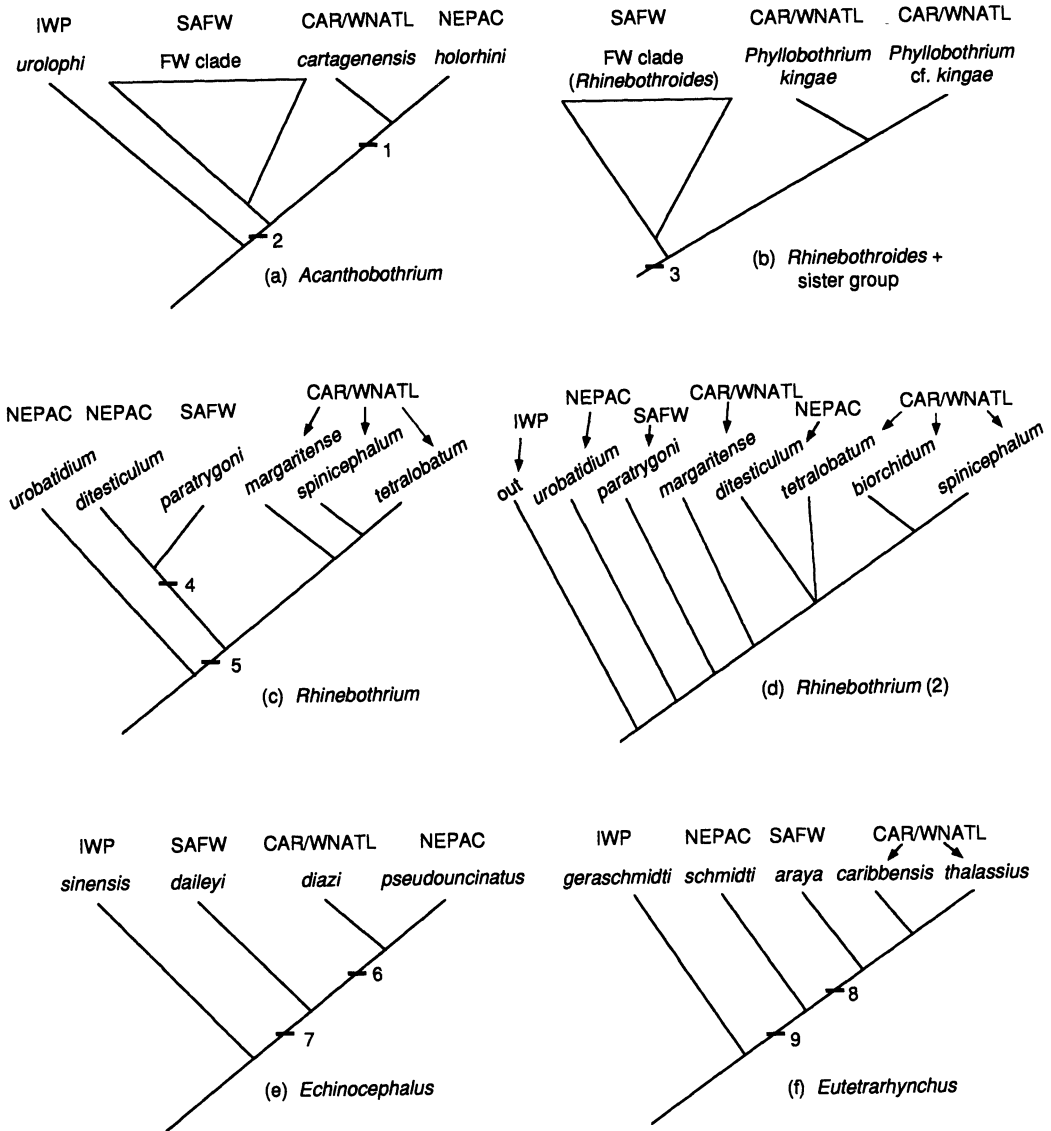geraschmidti schmidti araya caribbensis thalassius

8

9

(f) *Eutetrarhynchus*

FIGURE 3. Phylogenies for potamotrygonid parasites and their marine relatives, with geographic distributions added, from Brooks et al. (1981b). Informative branches coded for area by parasite parsimony analysis. NEPAC = northeastern Pacific; IWP = Indo-West Pacific (may also include Mediterranean species); out = *Rhinebothrium hawaiiensis, R. walga,* and *R. euzeti* (see Figure 2 for other abbreviations). *Rhinebothrium margaritense* was listed as *R.* sp. by Brooks et al. (1981b) but was corrected by Brooks and Deardorff (1988). Newer phylogeny for *Rhinebothrium* (d) is from Brooks and Deardorff (1988).

1993) shows SAFW to be most closely related to a clade composed of CAR/WNATL and NEPAC. The topology is irrespective of the use of the old or new cladogram for *Rhinebothrium*.

Component methods (Nelson and Plat-nick, 1981; Humphries and Parenti, 1986; Page 1988, 1990) were also used. Resolved area cladograms were generated for each parasite clade (using assumption 1 or assumption 2; see Page, 1990, for definitions) and compared by searching for intersect-

TABLE 1. Area × parasite (character) matrix for Brooks parsimony analysis (only informative characters included).

Area[a]	Characters[b]								
	1	2	3	4	5	6	7	8	9
IWP	0	0	?	?	?	0	0	0	0
SAFW	0	1	1	1	1	0	1	1	1
CAR/WNATL	1	1	1	0	1	1	1	1	1
NEPAC	1	1	?	1	1	1	1	0	1

[a] IWP = Indo-West Pacific; SAFW = South American freshwater; CAR/WNATL = Caribbean/northern West Atlantic; NEPAC = northeastern Pacific.
[b] Characters are numbered in Figure 3.

ing topologies or by generating consensus trees. All results either supported the topology in Figure 4a or were unresolved. Page's (1994a, 1994b) newer approaches of reconciling/superimposing "associate" with "host" trees were also explored. Using TreeMap (see Page, 1994b), parasite taxon–area cladograms were mapped (by maximizing codivergence between species and areas while allowing dispersal) onto all possible trees for the four areas. The Figure 4a topology provided the greatest amount of codivergence, as summed over
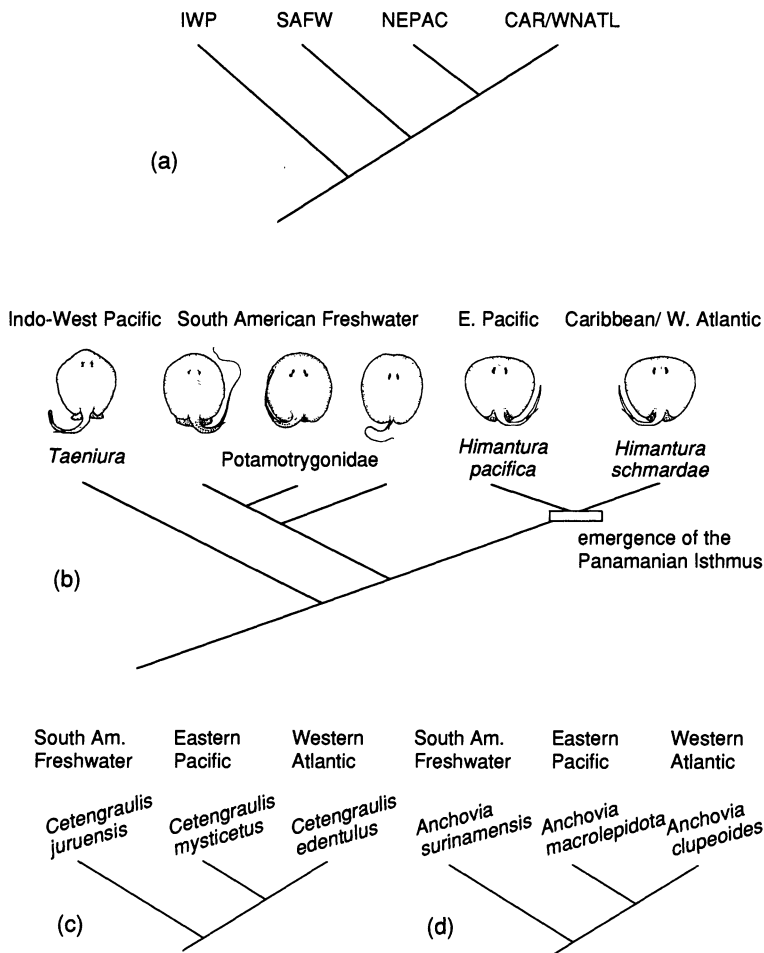

FIGURE 4. Area cladograms for amphi-American and freshwater South American taxa. (a) Parasite area cladogram produced by parsimony analysis of area by parasite matrix (see Table 1); length = 8 steps, consistency index = 0.88. IWP = Indo-West Pacific; SAFW = South American freshwater; NEPAC = northeastern Pacific; CAR/WNATL = Caribbean/northern West Atlantic. (b) stingrays (from Lovejoy, 1996). (c, d) Anchovies (after Nelson, 1984).

all clades (TreeMap was designed for host/parasite systems; the appropriateness of codivergence as an optimality criterion in biogeography, although advocated by Page [1994a], remains to be fully discussed).

This analysis of Brooks et al.'s data indicates that both the eastern Pacific and Caribbean/Atlantic are closely related to the South American freshwater area, a point also appreciated by Straney (1982: 337–338): "Brooks et al. seem to have slightly misinterpreted their data ... it would seem that the freshwater rays have their closest associations with north-Pacific–Caribbean ancestors." Brooks et al. dismissed the possibility of a biogeographic connection between the South American freshwater and Caribbean/Atlantic faunas by postulating that both originated independently from the Pacific, the freshwater fauna by vicariance (orogeny of the Andes) and the Atlantic fauna by dispersal (Fig. 2). However, no rationale was given for the preference of dispersal over vicariance as the mode of origin of the Caribbean/Atlantic fauna; the assumption of vicariance is a cornerstone of historical biogeography because any distribution can be explained by dispersal (Nelson and Platnick, 1981; Humphries and Parenti, 1986). In this case, the repeated pattern of SAFW as sister to CAR/WNATL and NEPAC in separate parasite groups makes a vicariance explanation for the Caribbean/Atlantic and Pacific split more defensible. Additionally, the (SAFW(EPAC, CAR/WNATL)) topology in Figure 4a is congruent with area cladograms for stingrays (Lovejoy, 1996) and two separate anchovy clades (Nelson, 1984), providing further support for the vicariance scenario (Figs. 4b–d).

Brooks et al.'s endeavor to differentiate between a Pacific and an Atlantic origin for potamotrygonids was likely inappropriate given the recency of the emergence of the Panamanian isthmus (Rosa, 1985). Before the Pliocene, the eastern Pacific and western Atlantic marine areas were connected (White, 1986), making it unlikely that a marine potamotrygonid ancestor would be distributed in only one of the two areas. The area cladograms in Figure 4 suggest that the ancestors of the freshwater South American stingrays and parasites and of anchovy taxa were distributed in both what are now the Atlantic and Pacific oceans. After giving rise to freshwater forms, they were split by the isthmus of Panama, giving rise to Atlantic/Pacific sister pairs (Nelson, 1984; Parenti, 1991; Lovejoy, 1996).

Although the branching of freshwater South American forms before the Atlantic/Pacific split indicates a preisthmian origin and the repetition of biogeographic pattern in multiple clades suggests that a vicariant event was responsible, the exact location and timing of the freshwater entry remains unclear. The distribution of amphi-American *Himantura* species (the marine sister taxon to potamotrygonids), however, provides additional clues (Lovejoy, 1996). *Himantura schmardae* is broadly distributed in the Caribbean, but *H. pacifica* is known only from western central America (Costa Rica) to Mexico (see Lovejoy, 1996: fig. 17). These ranges suggest that the marine ancestor of potamotrygonids was distributed along the northern coast of South America rather than along the western coast, as Brooks et al. suggested.

Several authors have discussed the possibility of marine introgressions from the northern coast of South America and biogeographic connections between the Caribbean and the upper Amazon (e.g., Nuttall, 1990; Hoorn, 1993). The gradual retraction of such marine intrusions may have provided appropriate conditions for the isolation of marine/estuarine fauna (including stingrays) in developing freshwater habitats. More recently, Hoorn et al. (1995) suggested that prior to the late Miocene, the upper Amazon was drained by a paleo-Orinoco River system to a delta in the Lake Maracaibo region (see also Lundberg and Chernoff's [1992] evidence for a connection between the Amazon and Magdalena regions). When Andean tectonics shifted the flow of the Orinoco to its present course, estuarine taxa may have been isolated in newly formed drainages, lead-
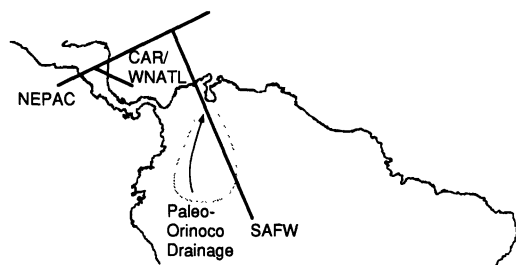
FIGURE 5. An alternative biogeographic scenario for the origin of Neotropical freshwater stingrays, stingray parasites, and anchovies. The basal separation of South American freshwater clades from northern South American marine groups may have been the result of marine incursions or drainage alterations of a paleo-Orinoco River system. The later separation of Pacific and Atlantic marine taxa was likely due to orogeny of the isthmus of Panama. Arrow indicates direction of paleo-Orinoco flow (after Hoorn et al., 1995). SAFW = South American freshwater; NEPAC = northeastern Pacific; CAR/WNATL = Caribbean/northern West Atlantic.

ing to their subsequent radiation in freshwater (Fig. 5). Amphi-American *Himantura* species (the hypothesized sister group to potamotrygonids based on stingray morphology) are extremely tolerant of brackish and freshwater, whereas *Urobatis* (the sister group to potamotrygonids hypothesized by Brooks et al.) appears to be an obligate marine genus (Thorson et al., 1983).

POINT 4: WHAT IS THE SISTER GROUP

To infer the sister group of potamotrygonids, Brooks et al. attempted to quantify how often different marine stingrays hosted parasites that were closely related to those of freshwater rays. For each of the parasite clades shown in Figure 3, the number of times particular marine stingray taxa were found to host "helminth species closely-related to those occurring in potamotrygonids" (Brooks et al., 1981b: 159) was counted. The most frequently counted taxon was *Urolophus* (*Urobatis*), with 11 instances where it was found to host parasites closely related to those of potamotrygonids; other taxa counted were *Dasyatis* (3 instances), *Himantura* (2 instances), *Myliobatis* (2 instances), and *Aetobatus* (2 instances) (see Brooks et al.,

1981b: table II). Brooks et al. were persuaded that potamotrygonids and at least some urolophids are probably sister taxa. Their procedure, however, is flawed in two respects: an explicit protocol was not provided for selecting "closely related" helminth taxa, and the results will be biased by amounts of speciation in parasites.

The title of Brooks et al.'s table II indicates that the "closest genealogical relatives of helminth parasites inhabiting fresh-water stingrays" were included in their tabulation. However, the true criterion appears to be ambiguous, depending on how much phylogenetic information is available for members of each clade in question. In one case (*Rhinebothroides*), only the sister-group species (*Phyllobothrium kingae*, *P.* cf. *kingae*) were considered "closely related" (Fig. 3b). In other cases, both the sister group and more basal taxa were considered closely related, e.g., for *Eutetrarhynchus*, *E. caribbensis*, *E. thalassius*, *E. schmidti*, and *E. geraschmidti* were all included in the tabulation (Fig. 3f). Presumably, only sister-group relationships (like derived characters in phylogenetic analysis) should be of interest here; parasites basal to those infecting potamotrygonids could be expected to infect stingrays basal to potamotrygonids rather than infecting the sister group of freshwater stingrays.

Simply counting the number of stingray taxa with closely related parasites will result in more species-rich parasite clades being accorded greater importance in determining the overall result. In *Rhinebothrium*, for example, *R. ditesticulum*, adds 1 to the score of its host group, *Urobatis* (Fig. 3c). In *Echinocephalus*, however, *E. diazi* and *E. pseudouncinatus* add 2 to the score for *Urobatis* (Fig. 3e). The speciation of *Echinocephalus diazi* and *E. pseudouncinatus*, however, has no bearing on the group's relationship to potamotrygonids.

To avoid the difficulties discussed above, the Brooks et al. tabulation procedure can be modified as follows: (1) only members of the sister group of the parasites infecting potamotrygonids are counted, and (2) the presence of parasites belonging to the sister group of a particular host taxon is

recorded only once. The application of this tabulation system to the Brooks et al. data (with the substitution of the newer phylogeny for *Rhinebothrium* [Brooks and Deardorff, 1988]) yielded the following results: four Atlantic *Urobatis*, two Pacific *Urobatis*, two Atlantic *Dasyatis*, two Atlantic *Himantura*, and two Pacific *Myliobatis*. Numbers represent the number of times that a particular stingray group hosts parasites belonging to the sister group of potamotrygonid parasites (out of a total of five parasite clades for which marine sister groups have been hypothesized).

When the tabulation is done in this way, strong ties are evident between potamotrygonid and Caribbean/western Atlantic parasites, consistent with the hypothesis of a northern South American (Caribbean?) origin for potamotrygonids. Atlantic *Urobatis* (a single species, *U. jamaicensis*) scores the highest value, indicating genealogical ties between its parasites and those of potamotrygonids. However, the signal is not as strong as Brooks et al. originally presented it; in general, potamotrygonid parasites reflect mixed affinities to the parasites of several different marine stingray groups. Furthermore, even this improved tabulation method is rather crude; ideally, the potamotrygonid sister group would be ascertained from a cladogram for stingrays generated from phylogenies of several groups of their parasites (using BPA or component analysis). Unfortunately, attempts in this direction have resulted in unresolved trees, largely because the parasite clades contain conflicting information about the relationships of their hosts. The low value determined for *Himantura* may result from the fact that helminths of species such as *Urobatis jamaicensis* have been investigated by a number of authors in localities throughout the Caribbean (e.g., Schmidt, 1978; Brooks and Mayes, 1980; Kovacs and Schmidt, 1980; Huber and Schmidt, 1985), whereas only a single specimen of *Himantura pacifica* has been investigated for parasites (Marques et al., 1996).

POINT 5: VICARIANCE OR DISPERSAL

Brooks et al. interpreted the biogeographic patterns of potamotrygonid parasites as consistent with vicariance/area of endemism scenarios for South American fishes (as discussed by Gery, 1969) and other organisms (Croizat, 1964). This interpretation indicates that potamotrygonids entered South America early enough to be affected by the same processes that affected other components of the aquatic Neotropical fauna (i.e., mid-Miocene or earlier). Brooks (1992) and Brooks and McLennan (1991, 1993b) have made several paleogeological claims concerning a Miocene origin of potamotrygonids. They stated that prior to the mid-Miocene Africa and South America were joined (thus, there was no Atlantic Ocean at the current mouth of the Amazon) and that the Amazon River flowed into the Pacific, leading "to the startling conclusion that if potamotrygonids are a relatively old component of neotropical freshwater diversity east of the Andes, they must have come from the Pacific Ocean, which is west of the Andes!" (Brooks and McLennan, 1991:245, emphasis in original).

However, the statement that the pre-Miocene Amazon drained west into the Pacific is incomplete. Although a number of authors have suggested that before the complete uplift of the Andes, the upper Amazon flowed west, they also posited that the middle (from Iquitos) or lower (from Manaus) Amazon drained to the Atlantic during this time (e.g., Beurlen, 1970; Putzer, 1984). More recently, Hoorn et al. (1995) proposed that between the late Oligocene and late middle Miocene, the upper Amazon (paleo-Orinoco) drained towards what is presently the Caribbean. The opening of the Atlantic had likely begun by the Jurassic/Triassic, with Africa and South America separating by the early Cretaceous (100 million years ago) (Smith et al., 1981; Parrish, 1993). Thus, marine ancestors of potamotrygonids could have entered South America from the Atlantic or Caribbean some 80 million years before the Miocene. Despite Brooks's (1992)

claims to the contrary, South American pa-leogeological data are equally consistent with pre-Miocene vicariance and with dispersal hypotheses, involving either the Atlantic or the Pacific Oceans (or both; see Lovejoy, 1996, for further discussion).

CONGRUENCE

The stingray phylogeny generated by Lovejoy (1996) initially suggested discordance with the patterns indicated by helminth parasites. Closer examination revealed that these disparities are mainly artifacts of Brooks et al.'s interpretation of data. Rather than indicating a solely Pacific origin for potamotrygonids, helminths show a pattern of relationships reiterated by stingrays and clades of anchovies: freshwater South American groups are basal to clades of Pacific and Atlantic taxa. Although parasites appear to show a relationship between potamotrygonids and *Urobatis jamaicensis*, the link is ambiguous; several marine stingray taxa seem to have contributed parasite ancestors to the potamotrygonid community. Further descriptions of marine stingray parasites (particularly from *Himantura pacifica*, a member of the sister group to potamotrygonids) will be necessary before sampling effects can be discounted. Monophyly has been hypothesized for the Potamotrygonidae based on stingray morphology, but not without some uncertainty. Conclusions of potamotrygonid monophyly based solely on parasite monophyly are incomplete. Caira's (1990, 1994) warning that Brooks et al.'s complete parasite cladograms, matrices, and tree statistics remain unpublished is a clear invitation for critical circumspection of the parasite character data themselves.

The elucidation of host relationships and biogeographic history from parasite phylogenies may be a powerful procedure. However, the stingray example suggests that some attempts should be viewed with caution until independent analyses of the hosts or close examination of the particular methods used to extract host history from parasite data are undertaken. Brooks et al.'s study remains a landmark for its scope and the wealth of hypotheses on Neotropical biogeography it has generated.

REFERENCES

BEURLEN, K. 1970. Geologie von Brasilien. Gebrüder Borntraegen, Berlin.

BLAIR, D. 1994. So many parasites and so little time! Review of *Parascript: Parasites and the Language of Evolution*, by D. R. Brooks and D. A. McLennan. Syst. Biol. 43:296–298.

BROOKS, D. R. 1981. Hennig's parasitological method: A proposed solution. Syst. Zool. 30:229–249.

BROOKS, D. R. 1988. Scaling effects in historical biogeography: A new view of space, time, and form. Syst. Zool. 37:237–244.

BROOKS, D. R. 1990. Parsimony analysis in historical biogeography and coevolution: Methodological and theoretical update. Syst. Zool. 39:14–30.

BROOKS, D. R. 1992. Origins, diversification, and historical structure of the helminth fauna inhabiting Neotropical freshwater stingrays (Potamotrygonidae). J. Parasitol. 78:588–595.

BROOKS, D. R. 1995. Neotropical freshwater stingrays and their parasites: A tale of an ocean and a river long ago. J. Aquaric. Aquat. Sci. 7:52–61.

BROOKS, D. R., AND J. F. R. AMATO. 1992. Cestode parasites in *Potamotrygon motoro* (Natterer) (Chondrichthyes: Potamotrygonidae) from southwestern Brazil, including *Rhinebothroides mclennenae* n. sp. (Tetraphyllidea: Phylobothriidae), and a revised host–parasite checklist for helminths inhabiting Neotropical freshwater stingrays. J. Parasitol. 78:393–398.

BROOKS, D. R., AND T. L. DEARDORFF. 1988. *Rhinebothrium devaneyi* n. sp. (Eucestoda: Tetraphyllidea) and *Echinocephalus overstreeti* Deardorff and Ko, 1983 (Nematoda: Gnathostomatidae) in a thorny back ray, *Urogymnus asperrimus*, from Enewatak Atoll, with phylogenetic analysis of both species groups. J. Parasitol. 74:459–465.

BROOKS, D. R., AND M. A. MAYES. 1980. Cestodes in four species of euryhaline stingrays from Colombia. Proc. Helminthol. Soc. Wash. 47:22–29.

BROOKS, D. R., M. A. MAYES, AND T. B. THORSON. 1981a. Systematic review of cestodes infecting freshwater stingrays (Chondrichthyes: Potamotrygonidae) including four new species from Venezuela. Proc. Helminthol. Soc. Wash. 48:43–64.

BROOKS, D. R., AND D. A. McLENNAN. 1991. Phylog-

eny, ecology, and behavior: A research program in comparative biology. Univ. Chicago Press, Chicago.

BROOKS, D. R., AND D. A. McLENNAN. 1993a. Historical ecology: Examining phylogenetic components of community evolution. Pages 267–280 in Species diversity in ecological communities (R. E. Rickleffs and D. Schluter, eds.). Univ. Chicago Press, Chicago.

BROOKS, D. R., AND D. A. McLENNAN. 1993b. Parascript: Parasites and the language of evolution. Smithsonian Institution Press, Washington, D.C.

BROOKS, D. R., T. B. THORSON, AND M. A. MAYES. 1981b. Freshwater stingrays (Potamotrygonidae) and their helminth parasites: Testing hypotheses of evolution and coevolution. Pages 147–175 in Advances in cladistics, proceedings of the first meeting of the Willi Hennig Society (V. A. Funk and D. R. Brooks, eds.). New York Botanical Garden, New York.

CAIRA, J. N. 1990. Metazoan parasites as indicators of elasmobranch biology. Pages 71–96 in Elasmobranchs as living resources: Advances in the biology, ecology, systematics, and behavior, and the status of fisheries (H. L. Pratt, S. H. Gruber, and T. Taniuchi, eds.). NOAA Tech. Rep., NMFS 90. U.S. Department of Commerce, Seattle.

CAIRA, J. N. 1994. Parascript: Paragon or parody. Review of Parascript: Parasites and the Language of Evolution, by D. R. Brooks and D. A. McLennan. BioScience 44:771–773.

CROIZAT, L. 1964. Space, time, form: The biological synthesis. Published by the author, Caracas, Venezuela.

DEARDORFF, T. L., D. R. BROOKS, AND T. B. THORSON. 1981. Two species of Echinocephalus (Nematoda: Gnathostomidae) from Neotropical stingrays. J. Parasitol. 67:433–439.

GERY, J. 1969. The freshwater fishes of South America. Pages 828–848 in Biogeography and ecology in South America (E. J. Fittkau, J. Illeis, G. H. Schwabe, and H. Sioli, eds.). W. Junk, The Hague.

HOORN, C. 1993. Marine incursions and the influence of Andean tectonics on the Miocene depositional history of northwestern Amazonia: Results of a palynostratigraphic study. Palaeogeogr. Palaeoclimatol. Palaeoecol. 105:267–309.

HOORN, C., J. GUERRERO, G. A. SARMIENTO, AND M. A. LORENTE. 1995. Andean tectonics as a cause for changing drainage patterns in Miocene northern South America. Geology 23:237–240.

HUBER, P. M., AND G. D. SCHMIDT. 1985. Rhinebothrium biorchidium n. sp., a tetraphyllidean cestode from a yellow-spotted stingray, Urolophus jamaicensis, in Jamaica. J. Parasitol. 71:1–3.

HUMPHRIES, C. J., AND L. R. PARENTI. 1986. Cladistic biogeography. Clarendon Press, Oxford, England.

KLASSEN, G. J. 1992. Coevolution: A history of the macroevolutionary approach to studying host–parasite associations. J. Parasitol. 78:573–587.

KOVACS, K. J., AND G. D. SCHMIDT. 1980. Two new species of cestode (Trypanorhyncha, Eutetrarhynchidae) from the yellow-spotted stingray, Urolophus jamaicensis. Proc. Helmintol. Soc. Wash. 47:10–14.

LOVEJOY, N. R. 1996. Systematics of myliobatoid elasmobranchs: With emphasis on the phylogeny and biogeography of Neotropical freshwater stingrays (Potamotrygonidae). Zool. J. Linn. Soc. 117:207–257.

LUNDBERG, J. G., AND B. CHERNOFF. 1992. A Miocene fossil of the Amazonian fish Arapaima (Teleostei: Arapaimidae) from the Magdalena River region of Colombia—Biogeographic and evolutionary implications. Biotropica 24:2–14.

MARQUES, F., D. R. BROOKS, AND H. M. UREÑA. 1996. Two new species of tetraphyllidean cestodes in Himantura pacifica (Chondrichthyes: Myliobatiformes: Dasyatidae) from the northwest coast of Costa Rica. J. Parasitol. 82:302–306.

McEACHRAN, J. D. In press. Urolophidae. In FAO species identification sheets for the central eastern Pacific. FAO, Rome.

NELSON, G. J. 1984. Identity of the anchovy Hildebranchthyes setiger with notes on relationships and biogeography of the genera Engraulis and Cetengraulis. Copeia 1984:422–427.

NELSON, G. J., AND N. PLATNICK. 1981. Systematics and biogeography: Vicariance and dispersal. Columbia Univ. Press, New York.

NISHIDA, K. 1990. Phylogeny of the suborder Myliobatidoidei. Mem. Fac. Fish. Hokkaido Univ. 37:1–108.

NUTTALL, C. P. 1990. A review of the Tertiary nonmarine molluscan faunas of the Pebasian and other inland basins of north-western South America. Bull. Br. Mus. Nat. Hist. (Geol.) 45:165–371.

PAGE, R. D. M. 1988. Quantitative cladistic biogeography: Constructing and comparing area cladograms. Syst. Zool. 37:254–270.

PAGE, R. D. M. 1989. Component, release 1.5. Univ. Auckland, Auckland.

PAGE, R. D. M. 1990. Component analysis: A valiant failure? Cladistics 6:119–136.

PAGE, R. D. M. 1994a. Maps between trees and cladistic analysis of historical associations among genes, organisms, and areas. Syst. Biol. 43:58–77.

PAGE, R. D. M. 1994b. Parallel phylogenies: Reconstructing the history of host–parasite assemblages. Cladistics 10:155–173.

PARENTI, L. R. 1991. Ocean basins and biogeography of freshwater fishes. Aust. Syst. Bot. 4:137–149.

PARRISH, J. P. 1993. The palaeogeography of the opening south Atlantic. Pages 8–27 in The Africa–South America connection (W. George and R. Lavocat, eds.). Clarendon Press, Oxford, England.

PUTZER, H. 1984. The geological evolution of the Amazon basin and its mineral resources. Pages 15–83 in The Amazon. Limnology and landscape ecology of a mighty tropical river and its basin (H. Sioli, ed.). W. Junk, Boston.

ROSA, R. S. 1985. A systematic revision of the South American freshwater stingrays (Chondrichthyes: Potamotrygonidae). Ph.D. Dissertation, College of William and Mary, Williamsburg, Virginia.

SCHMIDT, G. D. 1978. Phyllobothrium kingae sp. n., a tetraphyllidean cestode from a yellow-spotted stingray in Jamaica. Proc. Helminthol. Soc. Wash. 45:132–134.

SIMBERLOFF, D. 1987. Calculating the probabilities

that cladograms match: A method of biogeograph-
ical inference. Syst. Zool. 36:175–195.

SMITH, A. G., A. M. HURLEY, AND J. C. BRIDEN. 1981.
Phanerozoic paleocontinental world maps. Cam-
bridge Univ. Press, Cambridge, England.

STRANEY, D. O. 1982. Review of *Advances in Cladistics:
Proceedings of the First Meeting of the Willi Hennig
Society*, edited by V. A. Funk and D. R. Brooks. Syst.
Zool. 31:337–341.

SWOFFORD, D. L. 1993. PAUP: Phylogenetic analysis
using parsimony, version 3.1. Illinois Natural His-
tory Survey, Champaign.

THORSON, T. B., D. R. BROOKS, AND M. A. MAYES.
1983. The evolution of freshwater adaptation in
stingrays. Natl. Geogr. Soc. Res. Rep. 15:663–694.

THORSON, T. B., R. M. WOTTON, AND T. A. GEORGI.
1978. Rectal gland of freshwater stingrays, *Potamo-
trygon* spp. (Chondrichthyes: Potamotrygonidae).
Biol. Bull. 154:508–516.

WHITE, B. N. 1986. The isthmian link, antitropicality
and American biogeography: Distributional history
of the Atherinopsinae (Pisces: Atherinidae). Syst.
Zool. 35:176–194.

WILEY, E. O. 1988. Parsimony analysis and vicariance
biogeography. Syst. Zool. 37:271–290.

Limitations on the Use of Compatibility Methods for Polarizing and Ordering Characters

MARK WILKINSON

*School of Biological Sciences, University of Bristol, Bristol BS8 1UG, England;
E-mail: mark.wilkinson@bris.ac.uk*

Sharkey (1994) recently introduced a suite of new compatibility measures and a new method of tree construction, the reduction routine, that uses his character discriminate compatibility measure as a basis for differential weighting of characters. These new measures and the reduction routine require that characters be polarized and that they be binary or, if multistate, that they be ordered so that they can be represented by an analytically equivalent set of binary factors. Because much systematic data comprises unordered multistate characters (e.g., DNA or protein sequences) and polarity is often uncertain, the applicability of these new methods would seem to be severely limited. However, Sharkey (1994) also proposed that his discriminate character compatibility measures be used to polarize characters of uncertain polarity and to order unordered multistate characters.

Sharkey's techniques for ordering and polarizing characters are conceptually the same. In each case, all possible data sets comprising alternative polarizations or or-

derings are compared, and his data set discriminate compatibility measure or average data set discriminate compatibility measure are determined for each. The polarity and/or ordering implied by the data sets that achieve the highest values for these indices are adopted, and the ordered, polarized data are input into the reduction routine. Sharkey (1994) provided examples of this approach, but his examples are very simple. In assessing polarity, he considered the case of a data set of three binary characters, one of which is nonpolar. Alternative polarities produce just two data sets to be evaluated using the discriminate compatibility measure. For ordering multistate characters, he considered a data set of four binary characters and a single three-state character, requiring nine alternative data sets to be evaluated.

Unfortunately, computational problems will arise as increased demands are made of these methods. When used to polarize binary characters, the number of alternative data sets to be compared is 2^n, where n is the number of such characters. Thus,