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SPECIES DELINEATION AND THE IDENTIFICATION OF EVOLUTIONARILY SIGNIFICANT UNITS: LESSONS FROM THE FRESHWATER MUSSEL GENUS *POTAMILUS* (BIVALVIA: UNIONIDAE)

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ABSTRACT Accurate identification of biological entities is critical to the timely and efficient preservation of biodiversity. Concepts that define segments of biological diversity—species and evolutionarily significant units (ESUs)—should reflect our current knowledge of the biological world. Conflation of different hierarchical definitions of taxa has the potential to obscure distinct biological entities in need of protection. The concept of the ESU has been criticized because it includes within its definition distinct biological entities that otherwise would be recognized as species. Herein we evaluate several versions of the evolutionary significant unit concept and provide as a case study an analysis of geographic variation of a species of freshwater mussel, the inflated heelsplitter *Potamilus inflatus*. We demonstrate that as currently formulated, the ESU overlaps considerably with many biological definitions of species and therefore includes, in addition to distinctive populations, entities that would be recognized as species under many contemporary species concepts. Conflation of these two hierarchically distinct entities results in the ambiguous application of these concepts and inaccurate estimations of biological diversity. Continued use of the ESU concept has ramifications for the protection of invertebrate taxa under the Endangered Species Act of 1973 as amended in 1978. Recommendations for modification of the ESU concept are presented.

KEY WORDS: species, evolutionarily significant units, *Potamilus*, freshwater mussels, unionids, cytochrome oxidase I

INTRODUCTION

Accurate identification of biological diversity is considered critical to its conservation. As stated by Mayden and Wood (1995) if ". . . the ability to identify diversity correctly is impaired, then . . . our efforts to conserve and understand these entities further will be ineffective." Species play a prominent role in biological sciences from ecological studies to molecular systematics. Unfortunately, our ability to perceive differences that exist between species often exceeds our ability to accurately define such entities. Since the term "species" has become part of the scientific vocabulary, its definition has changed from typological, to nominalistic, to the contemporary definitions in use today. A typological definition requires that species are relatively static entities and rejects the variation known to occur in natural populations. In a nominalistic definition, species are artificial constructs created by humans and only the individual organism is real. Alternatively, contemporary definitions recognize the variation that is known to exist and that historical ancestor-descendant relationships exist among individuals as well as higher taxa (species).

The purpose of this paper is to evaluate several concepts of the evolutionary significant unit (ESU) with regard to the degree to which they overlap conceptually with existing definitions of species. Herein, we compare the entities defined under the ESU concept to those defined under several contemporary species concepts. We will demonstrate that as currently formulated, the ESU overlaps considerably with many biological definitions of species and therefore includes, in addition to distinctive populations of species, entities that would normally be recognized as species. Current federal law restricts the protection of ESUs to vertebrate populations under the Endangered Species Act of 1973 as amended in 1978 (Public Law 95-632 (1978), 92 Stat. 3751), and we feel that the use of the freshwater mussel *Potamilus inflatus* as a case study highlights another major shortcoming of the ESU concept: the exclusion of invertebrate taxa from legal protection.

Many species concepts have been proposed over the past 50 years. They differ from earlier versions in that they include, to a greater or lesser degree, a historical component that more accurately reflects our current knowledge of the natural world. These concepts include, but are not limited to, the biological species concept (BSC) (Mayr and Ashlock 1991), the phylogenetic species concept (PSC) (Cracraft 1983, Wheeler and Platnick 1997), the recognition species concept (RSC) (Paterson 1993), and the evolutionary species concept (ESC) (Simpson 1961, Wiley 1978). The various criteria for each of these concepts are presented in Table 1. It is not the purpose of this paper to evaluate the various species concepts included here, as that has been done more thoroughly elsewhere (e.g., Wiley 1981, Mayden and Wood 1995, Mayden 1997), but rather we propose for the purpose of this study that entities satisfying most (or all) of the above concepts should be considered species, both biologically and legally.

Whereas the ESA is understood to be a legal document and not a conceptual definition of species, it includes within its definition of "species" those entities (populations) that do not conform to any of the contemporary biological definitions of species that are in use today. In addition to biological species, the ESA includes ". . . any subspecies of fish or wildlife or plants, and any *distinct population* segment of any species of *vertebrate* fish or wildlife which interbreeds when mature" (emphasis ours).

Ryder (1986) was the first to use the term "ESU," "[o]ut of a sense of frustration with the limitations of current mammalian taxonomy in determining which named subspecies actually represent significant adaptive variation." According to Ryder (1986), ESUs represent "subsets of the more inclusive entity species, which possess genetic attributes significant for the present and future generations of the species in question." Waples (1991) noted that whereas the ESA allowed listing of distinct vertebrate populations as "species" it gave no guidelines on how population distinctiveness was to be evaluated. In an effort to clarify species determination for populations under the ESA, Waples defined a vertebrate population to be distinct and therefore a species under the ESA if "... the population represents an *evolutionarily significant unit* of the biological species" (emphasis ours). The term "ESU" was defined by Waples as a population (or group of populations) that (1) is substantially reproductively isolated from other conspecific population units, and (2) represents an important component in the evolutionary legacy of the species.

The ESU concept was conceived as a replacement for the class "subspecies" by Ryder (1986) because of problems associated with the application of that concept (see also Cracraft 1992). Since Waples formalized ESUs as subspecific entities, several other definitions have appeared (Moritz 1994, Vogler and DeSalle 1994). Each of these definitions increases the diagnosability of ESUs by more clearly delineating what an ESU is, however, a conceptual problem arises because all of the definitions proposed for ESUs are virtually identical to preexisting definitions for species. This fact has been recognized by several authors (Moritz 1994, Vogler and DeSalle 1994, Mayden and Wood 1995), although only Mayden and Wood saw this development as problematic. The problem is that there are currently two conceptual entities: ESUs and species, the former a subset of the latter, yet both are defined using the same criteria. Ultimately, in order to allow for the accurate delineation and protection of biological diversity, we feel that biologically sound and unambiguous definitions of biological entities must serve as the basis for the recognition of species and other taxa both conceptually and legally.

Examination of the various ESU and species concepts (Table 1) will serve to more clearly illustrate this point. A comparison of the ESU concept of Waples (1991) and the biological species concept of Mayr and Ashlock (1991) reveals a great similarity, and the crux of the problem. Both concepts rely primarily on the concept of reproductive isolation and because both are based on the same criteria, it seems inevitable that they would also identify the same biological entities. An argument could be made that the words "conspecific" and "... of the species" serve to distinguish Waples' ESUs from species. However, what criteria do we use to determine what a species is? If reproductive isolation is used to distinguish between species, as is required by the BSC, can we also use it to separate ESUs within that species? Moritz's (1994) attempt to more rigorously define the ESU concept succeeded in perhaps increasing the diagnosability of ESUs, but did not correct the overlap with contemporary species concepts. The reliance of the ESU concept of Moritz on reciprocal monophyly is the source of the conceptual ambiguity. By definition, taxa that are reciprocally monophyletic must also have diagnostic characters, which would equate ESUs with species under the PSC (Table 1). Vogler and DeSalle (1994) recommended the use of diagnostic characters to define ESUs, and therefore their definition is also synonymous with species under the PSC. They submitted that the critical step in distinguishing ESUs from nonconservation units was to distinguish characters from traits. Characters (*sensu* Nixon and Wheeler 1990) are those differences that define phylogenetic lineages (fixed attributes), whereas traits (variable attributes) are those differences that indicate tokogenetic relationships. Vogler and DeSalle state that "... only characters are relevant in determining conservation units" (ESUs). Application of this criterion, while consistent with a phylogenetic framework, also fails to discriminate between ESUs (*sensu* Vogler and DeSalle) and species that are also phylogenetic lineages defined by fixed attributes.

If allowed to persist, this philosophically untenable situation will result, and most likely already has resulted in recognizing "real" species as subspecific taxa (ESUs). This would in turn result in an underestimation of biological diversity since it is species that are enumerated when biotic surveys are conducted. The conflation of species and ESUs should be of great concern to those interested in the conservation of invertebrate taxa. Protection as endangered species under the ESA is conferred only to vertebrate ESUs. For example, populations of freshwater mussels that have been identified as ESUs under any definition, would gain no protection under the ESA.

A CASE STUDY

In an investigation of the phylogenetic relationships of the freshwater mussel genus *Potamilus*, Roe and Lydeard (1997) identified two phylogenetically distinct populations of the federally threatened inflated heelsplitter (*Potamilus inflatus*) (Federal Register 1992). *Potamilus inflatus* was once distributed across a sub-

TABLE 1.

Criteria for ESU and species concepts

Evolutionarily Significant Units	
Waples (1991)	A population or group of populations that (1) is substantially reproductively isolated from other conspecific population units and (2) represents an important component in the evolutionary legacy of the species.
Moritz (1994)	ESUs should be reciprocally monophyletic for mt DNA alleles and show significant divergence of allele frequencies at nuclear loci.
Vogler and DeSalle (1994)	Populations that do not overlap in the composition of the members are diagnosibly distinct, and represent seperate ESU's. Attributes that confer these distinctions are diagnostic characters.
Species Concepts	
Biological Species Concept	
Mayr and Ashlock (1991)	A species is a group of interbreeding natural populations that is reproductively isolated from other such groups.
Phylogenetic Species Concept	
Cracraft (1983)	The smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent.
Wheeler & Platnick (in press)	The smallest aggregation of populations or lineages diagnosable by a unique combination of character states.
Recognition Species Concept	
Paterson (1993)	A species is that most inclusive population of individual, biparental organisms that have a common fertilization system.
Evolutionary Species Concept	
Simpson (1961), Wiley (1978)	An entity composed of organisms that maintains its identity from other such lineages and has its own independent evolutionary tendencies and historical fate.

stantial portion of the southeastern United States. Originally described from the Alabama River (Lea, 1831), specimens have also been collected from the Coosa, Black Warrior, and Tombigbee rivers in Alabama. In Alabama, known populations of *P. inflatus* are limited to the Black Warrior River below the Oliver Lock and Dam, although a single live specimen was recovered from the Sipsey River, a tributary of the Tombigbee River (S. McGregor, pers. comm.). In Mississippi, *P. inflatus* was last reported from the Pearl River in 1911 (Frierson 1911). Recently, "fresh dead" shells of *P. inflatus* have been recovered from the Pearl River (George et al. 1995) but to date no live animals have been found. In Louisiana, *P. inflatus* occurred in both the Amite and Tangipahoa rivers, but it is now restricted to the lower and middle portions of the Amite River. The U.S. Fish and Wildlife Service currently recognizes *P. inflatus* as a threatened species because of the reduction in the range of this species due to habitat degradation and continued threats to the remaining populations (U. S. Fish and Wildlife Service 1992).

As part of a phylogenetic analysis of the genus *Potamilus* based on ~600 base pair portion of the first subunit of the mitochondrial cytochrome oxidase c gene, Roe and Lydeard (1997) also assessed the degree of genetic differentiation in the remaining populations of *P. inflatus*. Such information was deemed useful for conservation efforts aimed at preserving the inflated heelsplitter in those rivers where it persisted. The analysis included 24 individuals. Two specimens of each species of *Potamilus* were included, with the exception of *P. inflatus* which was represented by four individuals from the Amite and Black Warrior rivers, respectively. Limited numbers of *P. inflatus* were available from the USFWS because of their threatened status. The results of the study indicated that the Amite and Black Warrior populations of *P. inflatus* represented phylogenetically and evolutionarily distinct entities. Table 2 shows that both populations of *P. inflatus* form reciprocally monophyletic groups.

Bootstrap values based on 200 replicates indicate a high degree of support for those nodes that support the Amite and Black Warrior populations as distinct (Fig. 1). In an effort to characterize the degree of differentiation observed, genetic differentiation was assessed using the number and kind of nucleotide substitutions as well as genetic distances. Examination of nucleotide substitution patterns indicates that populations of *P. inflatus* differ from each other by a total of 12 substitutions (Fig. 1). Closer inspection

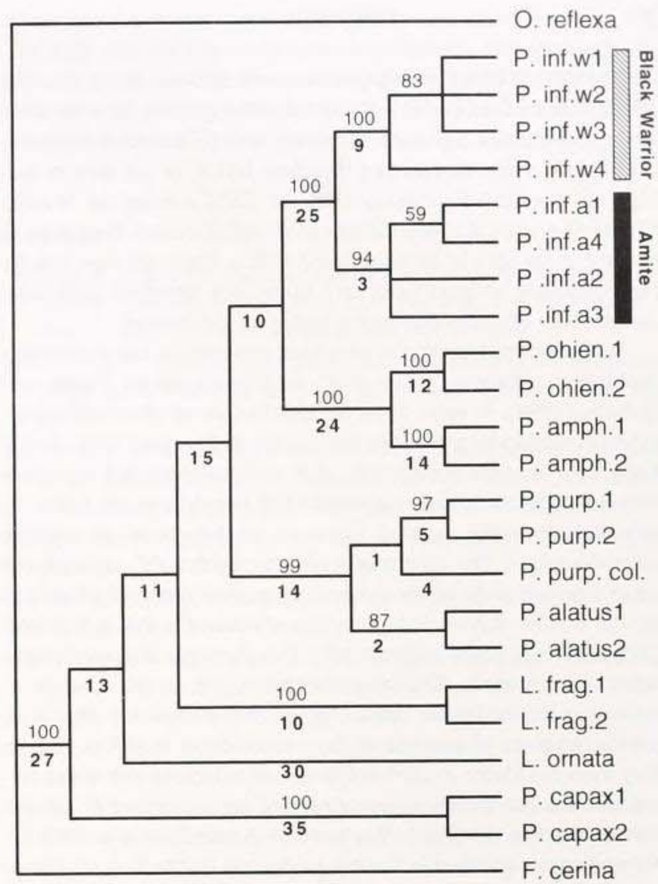

Figure 1. One of two equally parsimonious cladograms based on maximum parsimony analysis weighting transversions 2X transitions at the third codon position. Numbers above the branches correspond to the percentage of bootstrap replicates where the clade was found (200 total replications). Only values greater than 50% are shown. Boldface numbers below the branches correspond to the number of nucleotide substitutions at those nodes. The two trees differ only in the placement of *P. p. coloradoensis* relative to *P. alatus* and *P. purpuratus*. Taxon abbreviations: P. inf. w1-4, *Potamilus inflatus*-Black Warrior River; P. inf. a1-4, *Potamilus inflatus*-Amite River; P. purp. 1-2, *Potamilus purpuratus;* P. purp. col., *Potamilus purpuratus coloradoensis;* P. alatus 1-2, *Potamilus alatus;* P. capax1-2, *Potamilus capax;* P. ohien. 1-2, *Potamilus ohiensis;* P. amph. 1-2, *Potamilus amphichaenus;* L. frag. 1-2, *Leptodea fragilis;* L. ornata, *Lampsilis ornata;* O. reflexa, *Obliquaria reflexa;* F. cerina, *Fusconaia cerina.*

TABLE 2.

Diagnosable nucleotide substitutions for Amite and Black Warrior river populations of *P. inflatus*

Taxa	Sites											
	36	66	105	144	147	258	279	282	315	483	498	513
Warrior 1	A	G	G	G	A	A	G	A	A	G	A	G
Warrior 2	.	.	.	.	.	.	.	.	.	?	.	.
Warrior 3	.	.	.	.	.	.	.	.	.	.	.	.
Warrior 4	.	.	.	.	.	.	.	.	.	.	.	.
Amite 1	T	A	A	A	G	G	A	G	C	A	G	C
Amite 2	T	A	A	A	G	G	A	G	C	A	G	C
Amite 3	T	A	A	A	G	G	A	G	C	A	G	C
Amite 4	T	A	A	A	G	G	A	G	C	A	?	C

? indicates missing data.

reveals that all of these substitutions represent diagnostic characters (Table 2). When substitution patterns are examined across the cladogram we observe that the number of substitutions that support the distinctness of the Amite and Black Warrior populations of *P. inflatus* are intermediate between those that distinguish *P. amphichaenus* and *P. ohiensis* and those that support *P. alatus* and *P. purpuratus* as distinct entities. Examination of genetic distances based on Kimura's "two parameter" model also reveal that the Amite and Black Warrior *P. inflatus* are more genetically distinct from each other (1.93–2.62%) than are *P. alatus* and *P. purpuratus* (1.22–1.40%), which are generally recognized as valid species.

Because of the relatively high degree of genetic differentiation observed between what were considered two populations of the same species and the presence of diagnostic characters, Roe and Lydeard (1997) recommended that both the Amite and Black Warrior forms of *P. inflatus* be recognized as separate species.

DISCUSSION

The degree of differentiation observed between the Amite and Black Warrior forms of *P. inflatus* raises the question of what these biological entities represent. Are they well differentiated populations of the same species and therefore ESUs, or are they in fact separate species? Examination of the ESU concept of Waples (Table 1) reveals that populations of *P. inflatus* meet those criteria and therefore should be considered ESUs. They are functionally reproductively isolated from each other, and represent significant components of the evolutionary legacy of the species.

Reproductive isolation of allopatric populations has presented a problem to adherents of the BSC, as pointed out by Vogler and DeSalle (1994). In most cases the distribution of observable characters is used to infer reproductive isolation. In regard to the Amite and Black Warrior populations of *P. inflatus,* potential reproductive isolation has not been assessed. The populations are functionally reproductively isolated, however, as they occur in separate river drainages. The unique reproductive cycle of *P. inflatus* and other unionacean bivalves involves the parasitization of a fish host by the bivalve larvae. It is therefore conceivable that a fish host carrying larvae could facilitate gene flow between allopatric populations of unionids. The only host identified in the case of *P. inflatus* is the freshwater drum (*Aplodinotus grunniens*) (Roe et al. 1997). Absence of records of freshwater drum from the Mobile Bay system (Metee et al. 1996) indicate a lack of salt water tolerance, and that therefore, movement of drum carrying *P. inflatus* larvae between the Black Warrior and Amite Rivers is unlikely. Reproductive isolation is further evidenced by the lack of shared mitochondrial haplotypes.

Although we have not tested for reproductive isolation in the strict sense, we have established an absence of gene flow to the degree typically required to infer the species status of allopatric populations under the BSC. The criteria of Vogler and DeSalle (1993) (Table 1) is also met in that both populations are defined by diagnosable characters and are therefore ESUs under their definition. Moritz (1994) proposed genetic criteria for determining if populations represented ESUs (Table 1). Examination of nuclear loci has not been performed on these taxa and therefore whether or not they show significant divergence for the nuclear genome remains unknown. Both populations are, however, reciprocally monophyletic for mitochondrial alleles. Based on these findings we feel a strong case can be made for the recognition of these two entities as ESUs.

Application of the criteria of the several contemporary species concepts used in this paper (Table 1) reveals that an equally strong case can be made for recognizing each population as a separate species. The Amite and Black Warrior *P. inflatus* satisfy four of the five species concepts presented here, the sole exception being the recognition species concept of Patterson (1993). The Amite and Black Warrior *P. inflatus* are reproductively isolated as required by the BSC; they are also the smallest clusters of individual organisms that are diagnosable by unique sets of character combinations and therefore satisfy the criteria of the PSC concepts included here. Additionally, they meet the criteria of the ESC: both populations have maintained their identity over time and space as evidenced by DNA sequence differences and because of their geographic isolation have their own independent evolutionary tendencies and historical fate. At the present time the presence of a "common fertilization system" has not been assessed; therefore, whether or not this criterion of the RSC is met remains unknown.

The case study presented highlights the importance of a phylogenetic perspective in identifying natural groups (i.e., species). In the absence of a phylogenetic analysis that includes other species of *Potamilus,* recognition of the Amite and Black Warrior populations as distinct evolutionary entities would be more problematic, because the assumption that they were a single species would remain untested. Recognition of these two populations as separate species does nothing to alter their levels of protection under the ESA, as *P. inflatus* is already listed as threatened by the U.S. Fish and Wildlife Service. However, as stated earlier, protection of ESUs is not extended to invertebrate populations under the ESA. Therefore, the only way to provide this level of legal protection to invertebrates in general is to recognize them as species.

The conflation of the ESU and species concepts presents a difficult problem to solve and still retain both concepts. Before we attempt to correct the problems associated with the ESU concept we should first ask, as Mayden and Wood (1995) did ". . . is there really a need for a 'biolegal' ESU concept when, by the nature of its conceptualization, the entities termed ESUs actually qualify as species?" If the answer to this question is no, then any conceptual problems are immediately solved. If on the other hand, the answer is yes, modification of the ESU so that it applies strictly to non-species level taxa is required. One solution would be to use criteria such as arbitrary values of genetic differentiation over which populations are recognizable as ESUs. The problem associated with such "cutoff" values is that not all lineages evolve at similar rates and a high index of genetic differentiation for one group of taxa may reflect an inconsequential degree of differentiation for another.

Perhaps the simplest solution, which allows retention of the ESU concept is to abandon the attempt to define ESUs as formal taxa and to restrict its application to those organisms for which it was originally defined (i.e., salmon stocks and other organisms with life history characteristics that make them particularly vulnerable to extinction). A modified definition of the ESU might explicitly include stocks of anadromous fishes, which spend large portions of their lives at sea where regulations regarding fishing limits are more difficult to enforce, and which have shown a consistent decline in number of individuals in consecutive years. Other characteristics might include, but are not limited to, delayed age to sexual maturity as seen in long-lived organisms such as turtles, or reliance on a rare host species for completion of their life cycle, as observed in unionid mussels.

As biodiversity continues to decline it becomes increasingly critical that we intelligently and efficiently direct resources to those problems where they are most needed and to where the most benefit can be gained. Because taxonomic/systematic classifications often determine priorities for protecting endangered species, accurate identification of biological entities is critical for the intelligent use of limited resources allocated for preserving biodiversity. Neglect of distinct taxa, whether through ignorance or poor legislation, may lead to their extinction, as is likely the case of the tuatara *Sphenodon punctatus reischeki* Wettstein in New Zealand (Daugherty et al. 1990). Because of conflation with several species concepts, application of the ESU concept, however well intended, has the potential to hinder rather than aid in the recognition of biodiversity by treating two or more distinct biological entities as a single species. The incorrect identification of biological entities can serve only to bias our efforts to protect, understand, and preserve the biological diversity of this planet which we hold in trust for future generations.

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LITERATURE CITED

Cracraft, J. 1983. Species concepts and speciation analysis. pp. 159–187. *In:* R. F. Johnson (ed.). Current Ornithology. Plenum Press, New York.

Cracraft, J. 1992. The species of the birds-of-paradise (Paradisacidae): applying the phylogenetic species concept to complex patterns of diversification. *Cladistics* 8:1–43.

Daugherty, C. H., A. Cree, J. M. Hay & M. B. Thompson. 1990. Neglected taxonomy and continuing extinctions of tuatara *(Sphenodon). Nature* 347:177–179.

Frierson, L. S. 1911. A comparison of the Unionidae of the Pearl and Amite rivers. *Nautilus* 24:134–136.

George, S. G., D. D. Dickerson & K. J. Reine. 1995. Rediscovery of the Inflated Heelsplitter mussel, *Potamilus inflatus*, from the Pearl River Drainage. *J. Freshwater Ecol.* 11:245–246.

Lea, I. 1831. Observations on the naiades and descriptions of new species of that and other families. *Trans. Am. Phil. Soc.* 4:63–121.

Mayden, R. L. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. pp. 381–424. *In:* M. F. Claridge, H. A. Dawah & M. R. Wilson (eds.). *Species: The Units of Biodiversity.* Chapman & Hall.

Mayden, R. L. & R. M. Wood. 1995. Systematics, species concepts, and the evolutionarily significant unit in biodiversity and conservation biology. *American Fisheries Society Symposium* 17:58–113.

Mayr, E. & P. D. Ashlock. 1991. *Principles of Systematic Zoology.* McGraw-Hill, New York.

Metee, F. M., P. E. O'Neil & J. M. Pierson. 1996. Fishes of Alabama and the Mobile Basin. Monograph 15, geological survey of Alabama, Tuscaloosa, Alabama.

Moritz, C. 1994. Defining evolutionarily significant units. *Trends Ecol. Evol.* 9:373–375.

Nixon, K. C. & Q. D. Wheeler. 1990. An amplification of the phylogenetic species concept. *Cladistics* 6:212–223.

Paterson, H. E. H. 1993. *Evolution and the Recognition Species Concept of Species, Collected Writings of H. E. H. Paterson.* S. F. McEvey (ed.) Johns Hopkins University Press, Baltimore, Maryland.

Roe, K. J. & C. Lydeard. 1997. Molecular systematics of the freshwater mussel genus *Potamilus* (Bivalvia: Unionidae). *Malacologia* 39:195–205.

Roe, K. J., A. M. Simons & P. Hartfield. 1997. Identification of a fish host of the Inflated Heelsplitter *Potamilus inflatus* (Bivalvia: Unionidae) with a description of its glochidium. *Am. Midl. Nat.* 138:48–54.

Ryder, O. A. 1986. Species conservation and systematics: the dilemma of subspecies. *Trends Ecol. Evol.* 1:9–10.

Simpson, G. G. 1961. *Principals of Animal Taxonomy.* Columbia University Press, New York.

U.S. Fish and Wildlife Service, 1992. Inflated Heelsplitter, (*Potamilus inflatus*) Technical Agency Draft Recovery Plan. U.S. Fish and Wildlife Service. Jackson, Mississippi. 20 pp.

Vogler, A. P. & R. DeSalle. 1994. Diagnosing units of conservation management. *Conservation Biology* 8:354–363.

Waples, R. S. 1991. Pacific salmon, *Oncorhynchus* spp., and the definition of "Species" under the endangered species act. *Mar. Fish. Rev.* 53:11–22.

Wheeler, Q. D. & N. I. Platnick. 1997. The argument for phylogenetic species. *In:* Q. D. Wheeler and R. Meier (eds.). *Species Concepts and Phylogenetic Theory: A Debate.* Columbia University Press, New York.

Wiley, E. O. 1978. The evolutionary species concept reconsidered. *Syst. Zoo.* 27:17–26.

Wiley, E. O. 1981. *Phylogenetics: The Theory and Practice of Phylogenetic Systematics.* John Wiley and Sons, New York.