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Short Communication

The criterion of reciprocal monophyly and classification of nested diversity at the species level

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1. Introduction

The concept of reciprocal monophyly was defined in the context of a model of mitochondrial evolution that explains how incongruence between gene trees and species trees might result (Avise et al., 1983; Neigel and Avise, 1986; Avise and Ball, 1990; Avise, 2000). Under this model, phylogenetic analyses of mtDNA nucleotide sequences from exemplars representing early periods in the history of sister species may yield trees that suggest paraphyly for one or both species (Fig. 1A). Only after sufficient complementary haplotype extinction will species be recovered as monophyletic with respect to each other (= reciprocal monophyly) and gene trees mirror species history (Fig. 1B); this process is referred to as lineage sorting.

Based on this model, Moritz (1994) proposed operational criteria for recognition of Evolutionary Significant Units (ESUs; Ryder, 1986) including the requirement that mtDNA trees of such entities exhibit reciprocal monophyly; however, Moritz (1994) also recognized that nested units of diversity might be overlooked when the criterion of reciprocal monophyly (CRM) is not met. This concern was reiterated by Paetkau (1999) and Crandall et al. (2000); the latter recommended abandoning the ESU and disregarding history in favor of a species concept that focuses on gene flow and adaptive diversity. Although originally conceived to address intraspecific issues, subsequent workers have extended application of the CRM to decisions about species boundaries where it continues to receive wide consideration. Herein, we discuss defi-

ciencies of the CRM further and classification alternatives when species paraphyly is implied.

2. Theoretical and additional practical concerns

The concern about the CRM expressed by Moritz (1994), Paetkau (1999), and Crandall et al. (2000) reflects an inherent bias against nested entities that is illustrated in the following hypothetical example. In Fig. 1B, "a" and "b" form clades satisfying the criterion of reciprocal monophyly and, therefore, the entities possessing them are eligible for recognition (e.g., ESU, species). When "b" is monophyletic and "a" is not (Fig. 1A), however, the criterion argues against recognition of the entity possessing haplotype "b" because samples possessing "a" do not form a clade. Therefore, implementing the CRM implies that the status of an entity is contingent on the status of another entity. For example, Upton and Murphy (1997) relegated the insular species *Uta lowei*, *Uta encantadae*, and *Uta tumidarostra* to subspecies of *Uta stansburiana* because the latter is demonstrably paraphyletic with respect to the insular species; i.e., they regarded the status of insular entities as contingent on the status of the mainland species *U. stansburiana*. This line of reasoning, however, disregards what those entities represent with respect to a theoretical model of species. Species concepts do have a relational component built in, for example, when speciation by peripheral isolation yields two new species (e.g., Hennig, 1966), the "ancestral" species is regarded as new because of its relationship to the peripheral isolate. However, the new relationship is a function of status of the peripheral isolate, which then bears on the status of the mainland entity. We think that the status of
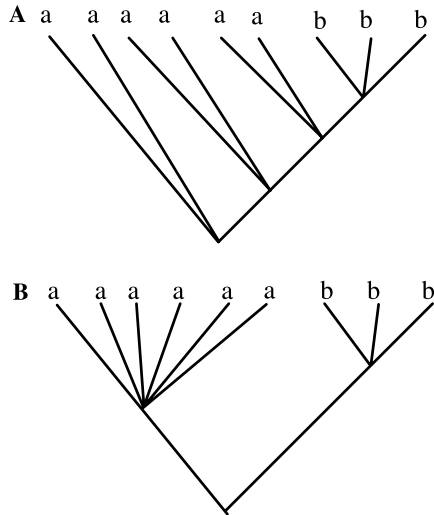
Fig. 1. Hypothetical haplotype trees in which (A) haplotype "a" is paraphyletic with respect to "b" and (B) haplotypes "a" and "b" exhibit reciprocal monophyly.

mainland and insular *Uta*, for example, should be evaluated with respect to a theoretical model of species rather than the relational issue implied by the CRM.

In cases in which the CRM is not met and available names exist, authors are obliged to discuss why nested entities are not being recognized (i.e., placed in synonymy; e.g., Upton and Murphy, 1997), however, in cases where available names do not exist, nested diversity could be discovered without attention being drawn to it (Table 1). Stated another way, because newly discovered entities are generally discussed only when the criterion is satisfied, its implementation can result in a "silent" bias against nested units of diversity when the criterion is not

satisfied. Therefore, implementation of the CRM may result in conservation risks for peripheral isolates with restricted distributions and in underestimation of instances of speciation by peripheral isolation.

The CRM is also frequently invoked without demonstrating that the conditions of the theoretical model (e.g., Avise, 2000) have been satisfied. An essential initial condition of Avise's model is the presence of greater ancestral haplotype diversity, some of which is subsequently lost in descendant lineages.

It should also be noted that the CRM is superfluous if one is faithful to the conviction that only monophyletic groups be recognized. In other words, the criterion does not cover anything not already addressed by a general concern for monophyly—although the concept is harmless with respect to supra-specific groupings, for which it merely reinforces that concern.

Finally, the CRM does not logically derive from Avise's model. Indeed, if what is learned from the model is how two species could exist despite the apparent absence of haplotype sorting then, clearly, all discovered clades should be considered as possibly representing species, even when the CRM is not met (e.g., clade "b", Fig. 1A).

3. Alternative treatments of species paraphyly in mtDNA trees

While avoiding paraphyletic taxa is essential in the erection of meaningful classifications, forsaking diversity "to avoid paraphyly" is counter to a fundamental goal of biodiversity research, namely, to recognize units of diversity. It is ironic that lineages may be unrecognized because more is known about them (i.e., phylogenetic

Table 1
Relationships between the criterion of reciprocal monophyly and diversity implied by haplotype trees

Haplotype Tree	CRM satisfied?	# Species implied	Species type
	No	1	1 exclusive
	No	2	1 exclusive, 1 non-exclusive
	Yes	2	2 exclusive

relationships) and that those studying diversity systematically conceal that which they ostensibly seek to reveal. Various theoretical models and classification schemes have been implemented to account for mtDNA trees that suggest species paraphyly, some of which are discussed below.

Crandall et al. (2000) recognized the bias against nested units of diversity (specifically ESUs) and proposed disregarding historical information (in favor of information about gene flow and adaptive diversity) in recognizing units of diversity. However, it is essential to recognize historical entities if diversity is to be understood in an evolutionary context and, in any case, history need not be sacrificed for nested units to be recognized (see below).

Some regard paraphyly as an unavoidable consequence of research at the species level and retain paraphyletic binominals in their classifications (e.g., Harrison, 1998; Marko, 1998). Although it is true that one might expect to *discover* that recognized species are paraphyletic when population-level history is inferred, paraphyletic taxa are actually *created* by associating a name with neighboring lineages that do not comprise historical groups. Thus, they are artificial constructs and easily avoided.

Some authors use the subspecies category to accomodate paraphyly at the level of species. (e.g., Campbell and Lamar, 1989; Ashton and de Queiroz, 2001; Nice and Shapiro, 2001; Piaggio and Spicer, 2001; Upton and Murphy, 1997). One of the problems with this strategy is that it merely results in paraphyly at a different taxonomic rank. Justification for this strategy via the argument that monophyly and paraphyly do not apply at the species level may just be semantics, particularly in cases in which an "intraspecific" issue is defined on the basis of an assumed classification rather than the status of the entities with respect to a theoretical model of species.

Naming all lineages is a philosophically unassailable strategy that can be implemented to avoid recognition of paraphyletic species. However, it may not be desirable, practical, or productive to name every lineage, especially when they are weakly differentiated (e.g., Fig. 2) and it may be problematic for entities lacking unique apomorphies (e.g., lineages possessing apomorphies that diagnose a species complex, but do not possess unique features).

In some cases, paraphyletic species have been hypothesized (usually in an ad hoc fashion) to correspond to non-exclusive entities (Graybeal, 1995; e.g., Wiens and Penkrot, 2002). To be consistent with that model, however, panmixis must be demonstrated or reasonably assumed for the hypothesized non-exclusive entity. For example, Lovich (2001) concluded that *Xantusia henshawi* is a non-exclusive entity because it is paraphyletic with respect to *Xantusia gracilis* in phylogenetic analyses of mtDNA sequences. However, because *X. henshawi*
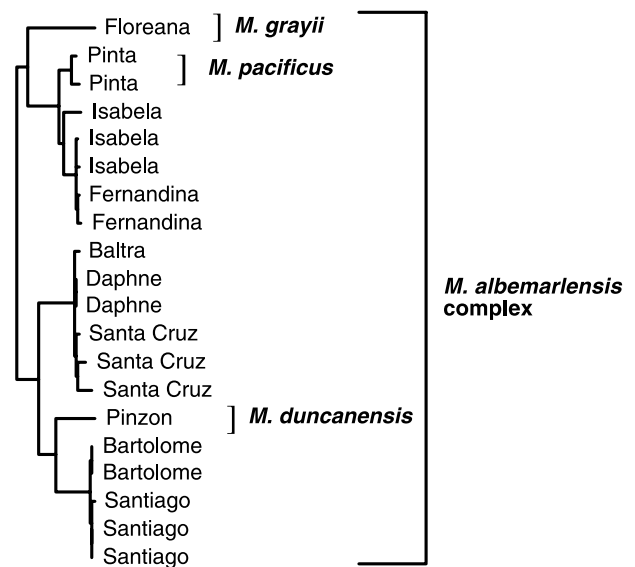

Fig. 2. Phylogenetic tree and classification of some Lava Lizards (*Microlophis*) from the western Galapagos islands (Kizirian et al., in press). The nested species (*Microlophus duncanensis*, *Microlophus grayii*, *Microlophus pacificus*) are morphologically distinguishable from the remaining lineages, which are weakly divergent (0–6%) with mtDNA data. Island names are associated with terminal branches. Under the criterion of reciprocal monophyly the nested species would not be recognized. Under the proposed system binominals refer to species and other names (*Microlophis*, *Microlophis albemarlensis* complex) represent clades. N.B. The binominal "*Microlophis albemarlensis*" is not recognized in this classification.

consists of multiple (and diagnosable) lineages that are isolated from each other by ecological and geological barriers (Lovich, 2001), it does not exhibit the reproductive integrity required of non-exclusive entities.

Finally, informal nomenclature systems (e.g., Moritz, 1994; Vogel and DeSalle, 1994) allow recognition of nested units of diversity without creating paraphyletic binominals and are easily implemented, partly because the units (e.g., Evolutionary Significant Units, Management Units; Moritz et al., 1995) can be unambiguously defined. However, when such units are not associated with a unique theoretical model of biological organization (e.g., molecule, cell, organ, organ system, tokogenetic array), the status of the recognized entities is unclear. Although effective for achieving short term conservation goals, in the long run, the theoretical ambiguity might not be the best thing for diversity.

4. Phylogenetic classification at the species level

One consequence of implementing the CRM is that taxonomic concerns (e.g., monophyly of binominals) may be prioritized over recognition of diversity, especially when a strict Linnaean system is used. Phylogenetic nomenclature (De Queiroz and Gauthier, 1990) on the other

hand, is ideally suited for reflecting nested historical relationship, in part, because it is unconcerned with taxonomic ranks. Currently, however, there is no consensus on the treatment of species-level entities (Cantino et al., 1999). Nevertheless, it is possible to implement the principles of De Queiroz and Gauthier (1990) in conjunction with Latin binominals to erect classifications that more faithfully reflect the nested relationships at the species level. For example, Kizirian et al. (in press) discussed two possible explanations for variation in Lava Lizards (*Microlophus albemarlensis*) from the western islands of the Galapagos (Fig. 2). One possibility is that "*M. albemarlensis*" is actually a complex of weakly divergent lineages, each endemic to a single island. Alternatively, *M. albemarlensis* (or some portion thereof) might represent a non-exclusive entity (e.g., Graybeal, 1995) that occurs on multiple islands, where reproductive connectedness is maintained by vegetative mats that transport lizards among islands. Additional ambiguity exists regarding Lava Lizards on Isabela, where more than one species may exist. To represent the new understanding as well as the ambiguity, Kizirian et al. (in press) recommended an indented classification in which binominals correspond to tokogenetic systems (= species) and other names (genera and species complexes) correspond to clades (Table 2; Fig. 2; because "*M. albemarlensis* complex" is a clade, the name could be replaced by a uninominal clade name, if desired). The salient difference between this and a strict Linnaean classification is the absence of the unappended binominal "*M. albemarlensis*," which had been used for Lava Lizards occurring on most of the western islands in the Galapagos (e.g., Fernandina, Isabela, Santa Cruz, and Santiago; Wright, 1983). We see no benefit in retaining the paraphyletic taxon "*M. albemarlensis*" and prefer the indented classification because it best accommodates multiple interpretations of the available data, allows for recognition of nested units of diversity, and includes only monophyletic groups.

One advantage of the proposed system is that it will do a better job of drawing attention to diversity. For example, whereas "*Rana mucosa*" suggests that only a single unit of biological diversity exists (Macey et al., 2001), "*R. mucosa* complex" more accurately reflects the diversity of this group (there are four major lineages hypothesized to have been isolated for 1.4–2.2 million years and indicates to biologists and non-biologists that there is more than one unit of diversity to be considered

when making management decisions about frogs that are currently facing extinction. Furthermore, under the proposed system, additional lineages could be named without creating a paraphyletic group.

In summary, while the concept of reciprocal monophyly is useful for describing some patterns of haplotype evolution, as a criterion in recognizing units of diversity it possesses theoretical deficiencies and results in an arbitrary and systematic bias against nested units of diversity (Crandall et al., 2000; Moritz, 1994; Paetkau, 1999). Classification employing the principles of De Queiroz and Gauthier (1990) is recommended because it more faithfully represents nested lineage diversity at the species level (especially when highly divergent entities are nested within complexes of weakly divergent lineages or non-exclusive entities) and readily accommodates situations characterized by multiple equally parsimonious explanations and unnamed lineage diversity.

Table 2
Phylogenetic classification of some Lava Lizards in the western Galapagos (Kizirian et al., in press)

Microlophus occipitalis group
M. albemarlensis complex
M. pacificus
M. duncanensis
M. grayii

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