

Species Delimitation: New Approaches for Discovering Diversity

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Systematics can be considered to have two major goals: (1) to discover and describe species and (2) to determine the phylogenetic relationships of these species. But even a quick perusal of titles in *Systematic Biology* throughout most of its 15-year history will confirm an obvious fact: most articles focus on reconstructing phylogenies and on using these phylogenies to address evolutionary or biogeographic questions, with few papers on the methodology underlying this first major goal of systematics research (Fig. 1).

Nevertheless, species delimitation, the process by which species boundaries are determined and new species are discovered, may finally be emerging as a major topic in modern systematics (e.g., Sites and Marshall, 2003). New methods for species delimitation are being developed (e.g., Puerto et al., 2001; Templeton, 2001; Wiens and Penkrot, 2002; Morando et al., 2003; Pons et al., 2006) and compared (e.g., Marshall et al., 2006; Pons et al., 2006). Ambitious proposals have been made, with the goal of accelerating the rate at which new species are discovered and described, including DNA barcoding (e.g., Hebert et al., 2003, 2004), DNA taxonomy (Tautz et al., 2003), and Web-based taxonomy (e.g., Godfray, 2002; Scoble, 2004; Knapp et al., 2007). Controversies are raging in the literature over these proposals (especially over DNA barcoding: Will et al., 2005; Brower, 2006; Hickerson et al., 2006; Meier et al., 2006) and other aspects of species delimitation and over the consequences of new approaches in species delimitation for other fields, such as ecology and conservation (Agapow et al., 2004; Isaac et al., 2004; Padial and de la Riva, 2006). This new emphasis on species delimitation has arisen (at least in part) because of growing concern over threats to biodiversity and the desire to describe as many species as possible as quickly and accurately as possible before they disappear. However, an increased interest in species delimitation is barely reflected in the pages of *Systematic Biology*, if at all (Fig. 1). This disconnect was my major motivation for organizing a symposium for the Society of Systematic Biologists on this topic.

THE SYMPOSIUM

At the evolution meetings in Stony Brook last year (2006), we held the first symposium in the history of the Society of Systematic Biologists dedicated to the topic of species delimitation. After a brief introduction, there were 11 speakers. Six of these led to the papers you see now; presenting but not publishing were Keith Crandall, Paul Hebert, Marshal Hedin, Loren Rieseberg, and Jack

Sullivan. Of course, the field of species delimitation is too large and diverse to be adequately covered by 6 or even 11 papers. Nevertheless, the present papers provide an overview of some of the important ideas and a diversity of cutting-edge research in this area.

We begin with a paper by Kevin de Queiroz on species concepts. Species delimitation obviously depends on having some idea of what species are, and it is easy to imagine a symposium on species delimitation degenerating into endless disagreements about species concepts. Not so. In fact, there has been real progress made in thinking about species concepts, which now makes some general agreement seem possible. Part of this progress comes from recognizing a distinction between what species are and the evidence used to recognize them (e.g., Frost and Kluge 1995; Mayden 1997; de Queiroz 1998). Here, de Queiroz (2007) discusses the underlying unity of species concepts and the implications this has for species delimitation. As evidence of some of this progress, there seems to be general agreement among almost all participants that species are lineages (e.g., de Queiroz, 2007; Knowles and Carstens, 2007; Shaffer and Thomson, 2007; Raxworthy et al., 2007; Rissler and Apodaca, 2007, this paper). This lets us focus on the challenge of how we go about delimiting those lineages.

The paper by Knowles and Carstens (2007) addresses how molecular data (i.e., gene trees from DNA sequence data) can be used in species delimitation. I suspect that this will be the single topic of greatest interest to most readers of *Systematic Biology*. They propose a new method that uses coalescent simulations to test hypotheses about species limits. Their method is particularly valuable in that it can incorporate data from multiple loci and does not require that species have diverged to the point of being reciprocally monophyletic. This paper also showcases the increasing importance of population genetics to species delimitation.

The paper by Shaffer and Thomson (2007) focuses on another topic of considerable interest and practical importance: how can we obtain a large number of unlinked nuclear markers for use in delimiting species? These authors describe an approach for generating SNPs (single nucleotide polymorphisms) using genomic resources and demonstrate this approach with a case study involving Australian turtles. They also describe some of the methods that might be used to apply SNP data in species delimitation. Although Shaffer and Thomson (2007) emphasize nonphylogenetic methods for species delimitation (unlike Knowles and Carstens, 2007), both papers

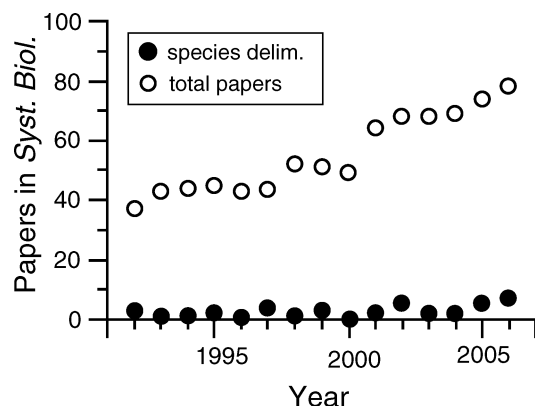


FIGURE 1. The number of papers published in *Systematic Biology* that deal (at least in part) with the topic of species delimitation (closed circles), relative to the total number of papers published in the journal (open circles), from 1992 to 2006. Both major articles and Points of View were considered, but not book reviews, editorial comments, or brief symposium introduction essays. Given that there were so few papers that could potentially be considered as dealing with the theory or practice of species delimitation, I was generous about assigning papers to this category.

clearly agree on the importance of incorporating population genetics into species delimitation.

Two papers (Raxworthy et al., 2007; Rissler and Apodaca, 2007) pioneer a novel use of ecological data in species delimitation. Specifically, they describe how GIS-based analyses of climatic data can be used, in combination with molecular and morphological data, to help delimit species. I find this to be a particularly exciting development in species delimitation. Every species has a geographic range, and the ecological and evolutionary processes that limit the geographic range may be crucial for creating new species, particularly allopatric species (e.g., Wiens, 2004a, 2004b; Wiens and Graham, 2005). Just think about this simple example. One could easily invest hundreds of hours and thousands of dollars into fieldwork and lab work to show that there is no or only limited gene flow between two sets of populations that represent two putative species by using molecular markers. But if the habitat separating these sets of populations is clearly unsuitable and uncrossable for both putative species (e.g., an arid valley separating mesic montane species), then there cannot be ongoing gene flow between them. In theory, this hypothesis could be tested using GIS-based analyses with a few hours work and almost no cost. Although previous authors might have informally considered the geographic distribution of suitable habitats and differences between habitats when making species decisions, the new methods described by Raxworthy et al. (2007) and Rissler and Apodaca (2007) provide a way to test and incorporate this sort of “biological intuition” with rigorous data and statistical analyses.

The final contribution presents a discussion of the idea of Web-based taxonomy (Godfray et al., 2007). These authors argue that taxonomy could be made easier to do, more widely accessible to nonspecialists, and perhaps

even more fundable, by moving information on the taxonomy of every group entirely to the Web. They provide a model for how this might work and discuss the possible pros and cons. Although some more phylogeny-focused readers may be tempted to skip this paper, they should note that these authors present reasonable arguments for dramatic and sweeping changes in the field of systematics.

CONTINUING CHALLENGES IN SPECIES DELIMITATION

Again, I must reiterate that these six chapters cannot possibly cover the entire field of species delimitation. They are merely a sampler. Below, I list some major areas that were not covered and some topics for future research that is needed.

Morphological Data

A major omission in this symposium is the lack of papers on methods for delimiting species using morphological data. Superficially, this may seem trivial. After all, the majority of recognized species presumably have been delimited and described based on morphological differences. Why worry about methodology? In general, species are delimited based on one or more qualitative or quantitative morphological characters that show no overlap with other species. This criterion is very traditional but also makes sense biologically. If two species are consistently distinguished by one or more diagnostic morphological differences, then presumably there is no gene flow between them (given some assumptions, such as the idea that each morphological difference has a genetic basis). But what if one has only a single specimen? How can one be sure that all the individuals of that species share that diagnostic character(s)? Many researchers might hesitate to describe a species based only on a single specimen for this reason. But is having two specimens really that much better than having only one? What about three? With this problem in mind, Wiens and Servedio (2000) examined the sample sizes needed to have statistical confidence that a given diagnostic character is truly fixed (frequency = 100%) within a species. In fact, being reasonably certain (i.e., allowing for a 5% error rate) that a trait is truly fixed within a species is basically impossible, even if hundreds or thousands of individuals are sampled. Even allowing for some level of polymorphism in a diagnostic character (e.g., frequency of 95% rather than 100%), very large sample sizes may still be required to be reasonably certain that the trait is diagnostic at the desired level (Wiens and Servedio, 2000).

What about other approaches to delimiting species with morphology? Using phylogenetic analyses of morphology to make species decisions remains largely unexplored (e.g., Wiens and Penkrot, 2002). Furthermore, the few studies that have compared the results of this approach to those using diagnostic morphological characters found these methods can give quite different species limits in some cases (e.g., Wiens and Penkrot, 2002; Doan and Castoe, 2003).

My point here is not that species limits based on morphological characters are often wrong or that any particular approach is better than any other. My point is simply that our methods for delimiting species with morphological data remain woefully understudied. This is particularly ironic in that many recent papers that have protested the problems of “DNA taxonomy” seem to implicitly assume that species delimitation with morphology is straightforward and uncomplicated.

Molecular Data

The development of methods for delimiting species with DNA data is an active area of research (e.g., Sites and Marshall, 2003; Pons et al., 2006; Knowles and Carstens, 2007). A wish list for such a method might include the following that it considers both incomplete lineage sorting and gene flow among populations, can integrate data from multiple loci, can determine species limits without having those limits defined a priori (i.e., it will allow one to discover unanticipated species from the molecular data), and can allow one to estimate the statistical support for species-level decisions. Clearly, we are not there yet. Most existing methods contain one or more of these elements, but a single method that combines all of them may still be some ways off.

Two critical issues that are particularly relevant to species delimitation with molecular data were brought up during the symposium but did not make it to the publication stage. One is that of dealing with naturally fragmented populations (by Marshall Hedin). The other is making decisions about lineages that are currently introgressing (by Jack Sullivan). Both problems underscore the idea that species delimitation sometimes requires making cut-offs involving continuous processes that are generating (or eliminating) species. Both topics clearly would benefit from further study in the context of species delimitation.

Other Issues

There are dozens of other important and unresolved issues that go beyond what will be covered in this symposium or what I can summarize here. For example, how should asexual species be delimited? Should we even call them species? What insights might we gain from studies of speciation in the field of evolutionary biology (i.e., in terms of the evolution of intrinsic reproductive isolating mechanisms) that can help inform our studies of species delimitation? What is the best way to integrate data from DNA sequences and other types of data (e.g., morphology, behavior, ecology, allozymes, chromosomes) for delimiting species? Given that many, if not most, new species continue to be discovered primarily through new fieldwork, how might we accelerate the rate at which new species are actually encountered in the field? Raxworthy et al. (2003) describe how GIS-based methods facilitated their discovery of new lizard species in Madagascar, but so far these methods have only been used on a relatively small geographic and taxonomic scale. How might we increase the number of systema-

tists who actually work on describing new species (e.g., Rodman and Cody, 2003; Agnarsson and Kuntner, 2007)?

In summary, this symposium highlights some of the exciting research that is going on in the area of species delimitation. But I think that each paper will show that we are only beginning to address the important questions in this field.

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REFERENCES

- Agapow, P. M., O. R. P. Bininda-Emonds, K. A. Crandall, J. L. Gittleman, G. M. Mace, J. C. Marshall, and A. Purvis. 2004. The impact of species concept on biodiversity studies. *Q. Rev. Biol.* 79:161–179.
- Agnarsson, I., and M. Kuntner. 2007. Taxonomy in a changing world: Seeking solutions for a science in crisis. *Syst. Biol.* 56:531–539.
- Brower, A. V. Z. 2006. Problems with DNA barcodes for species delimitation: “Ten species” of *Astraptes fulgerator* reassessed (Lepidoptera: Hesperidae). *Syst. Biodiv.* 4:127–132.
- de Queiroz, K. 1998. The general lineage concept of species, species criteria, and the process of speciation: A conceptual unification and terminological recommendations. Pages 57–75 in *Endless forms: Species and speciation* (D. J. Howard and S. H. Berlocher, eds.). Oxford University Press, Oxford, UK.
- de Queiroz, K. 2007. Species concepts and species delimitation. *Syst. Biol.* 56: This issue.
- Doan, T. M., and T. A. Castoe. 2003. Using morphological and molecular evidence to infer species boundaries within *Proctoporus bolivianus* Werner (Squamata: Gymnophthalmidae). *Herpetologica* 59:433–450.
- Frost, D. R., and A. G. Kluge. 1995. A consideration of epistemology in systematic biology, with special reference to species. *Cladistics* 10:259–294.
- Godfray, H. C. J. 2002. Challenges for taxonomy. *Nature* 417:17–19.
- Godfray, H. C. J., B. R. Clark, I. J. Kitching, S. J. Mayo, and M. J. Scoble. 2007. The Web and the structure of taxonomy. *Syst. Biol.* 56: This issue.
- Hebert, P. D. N., A. Cywinska, S. L. Ball, and J. R. DeWaard. 2003. Biological identifications through DNA barcodes. *Proc. R. Soc. Lond.* 270:313–321.
- Hebert, P. D. N., E. H. Penton, J. Burns, D. J. Janzen, and W. Hallwachs. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly, *Astraptes fulgerator*. *Proc. Natl. Acad. Sci. USA* 101:14812–14817.
- Hickerson, M. J., C. Meyer, and C. Moritz. 2006. DNA barcoding will fail to discover new animal species over broad parameter space. *Syst. Biol.* 55:729–739.
- Isaac, N. J., J. Mallet, and G. M. Mace. 2004. Taxonomic inflation: Its influence on macroecology and conservation. *Trends Ecol. Evol.* 19:464–469.
- Knapp, S., A. Polaszek, and M. Watson. 2007. Spreading the word. *Nature* 446:261–262.
- Knowles, L. L., and B. Carstens. 2007. Delimiting species without monophyletic gene trees. *Syst. Biol.* 56: This issue.
- Marshall, J. C., E. Arévalo, E. Benavides, J. Sites, and J. W. Sites, Jr. 2006. Delimiting species: Comparing methods for Mendelian characters using lizards of the *Sceloporus grammicus* (Squamata: Phrynosomatidae) complex. *Evolution* 60:1050–1065.
- Mayden, R. L. 1997. A hierarchy of species concepts: The denouement in the saga of the species problem. Pages 381–424 in *Species. The units of biodiversity* (M. F. Claridge, H. A. Dawah, and M. R. Wilson, eds.). Chapman and Hall, London.
- Meier, R., R. K. Shiyang, G. Vaidya, and P. K. L. Ng. 2006. DNA barcoding and taxonomy in Diptera: A tale of high intraspecific variability and low identification success. *Syst. Biol.* 55:715–728.

- Morando, M., L. J. Avila, and J. W. Sites, Jr. 2003. Sampling strategies for delimiting species: Genes, individuals, and populations in the *Liolaemus elongatus-kriegi* complex (Squamata: Liolaemidae) in Andean-Patagonian South America. *Syst. Biol.* 52:159–185.
- Padial, J. M., and I. de la Riva. 2006. Taxonomic inflation and the stability of species lists: The perils of ostrich's behavior. *Syst. Biol.* 55:859–867.
- Pons, J., T. G. Barraclough, J. Gomez-Zurita, A. Cardoso, D. P. Duran, S. Hazell, S. Kamoun, W. D. Sumlin, and A. P. Vogler. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Syst. Biol.* 55:595–609.
- Puerto, G., M. G. Salomao, R. D. G. Theakston, R. S. Thorpe, D. A. Warrell, and W. Wuster. 2001. Combining mitochondrial DNA sequences and morphological data to infer species boundaries: Phylogeography of lanceheaded pitvipers in the Brazilian Atlantic forest, and the status of *Bothrops pradoi* (Squamata: Serpentes: Viperidae). *J. Evol. Biol.* 14:527–538.
- Raxworthy, C. J., C. M. Ingram, and R. G. Pearson. 2007. Species delimitation applications for ecological niche modeling: A review and empirical evaluation using *Phelsuma* day gecko groups from Madagascar. *Syst. Biol.* 56: This issue.
- Raxworthy, C. J., E. Martinez-Meyer, N. Horning, R. A. Nussbaum, G. E. Schneider, M. A. Ortega-Huerta, and A. T. Peterson. 2003. Predicting distributions of known and unknown reptile species in Madagascar. *Nature* 426:837–841.
- Rissler, L. J., and J. J. Apodaca. 2007. Ecological niche models and phylogeography help uncover cryptic biodiversity. *Syst. Biol.* 56: This issue.
- Rodman, J. E., and J. H. Cody. 2003. The taxonomic impediment overcome: NSF's Partnerships for Enhancing Expertise in Taxonomy (PEET) as a model. *Syst. Biol.* 52:428–435.
- Scoble, M. J. 2004. Unitary or unified taxonomy? *Phil. Trans. R. Soc. Lond. B* 359:699–710.
- Shaffer, H. B., and R. C. Thomson. 2007. Species, SNPs, and systematics: Defining species in a post-genomic age. *Syst. Biol.* 56: This issue.
- Sites, J. W., Jr., and J. C. Marshall. 2003. Delimiting species: A Renaissance issue in systematic biology. *Trends Ecol. Evol.* 18:462–470.
- Tautz, D., P. Arctander, A. Minelli, R. H. Thomas, and A. P. Vogler. 2003. A plea for DNA taxonomy. *Trends Ecol. Evol.* 18:70–74.
- Templeton, A. R. 2001. Using phylogeographic analyses of gene trees to test species status and processes. *Mol. Ecol.* 10:779–791.
- Wiens, J. J. 2004a. Speciation and ecology revisited: Phylogenetic niche conservatism and the origin of species. *Evolution* 58:193–197.
- Wiens, J. J. 2004b. What is speciation and how should we study it? *Am. Nat.* 163:914–923.
- Wiens, J. J. and C. H. Graham. 2005. Niche conservatism: Integrating evolution, ecology, and conservation biology. *Ann. Rev. Ecol. Evol. Syst.* 36:519–539.
- Wiens, J. J., and T. L. Penkrot. 2002. Delimiting species based on DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). *Syst. Biol.* 51:69–91.
- Wiens, J. J., and M. R. Servedio. 2000. Species delimitation in systematics: Inferring diagnostic differences between species. *Proc. R. Soc. Lond.* 267:631–636.
- Will, K. W., B. D. Mishler, and Q. D. Wheeler. 2005. The perils of DNA barcoding and the need for integrative taxonomy. *Syst. Biol.* 54:844–851.