

Cryptic species as a window on diversity and conservation

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The taxonomic challenge posed by cryptic species (two or more distinct species classified as a single species) has been recognized for nearly 300 years, but the advent of relatively inexpensive and rapid DNA sequencing has given biologists a new tool for detecting and differentiating morphologically similar species. Here, we synthesize the literature on cryptic and sibling species and discuss trends in their discovery. However, a lack of systematic studies leaves many questions open, such as whether cryptic species are more common in particular habitats, latitudes or taxonomic groups. The discovery of cryptic species is likely to be non-random with regard to taxon and biome and, hence, could have profound implications for evolutionary theory, biogeography and conservation planning.

Cryptic species: the biodiversity wildcard

Increasing worldwide destruction and disturbance of natural ecosystems are precipitating catastrophic extinctions of species [1]. Given that most species remain undescribed, efforts to catalogue and explain biodiversity need to be prioritized. Research on cryptic species has increased exponentially over the past two decades (Figure 1), fuelled in large part by the increasing availability of DNA sequences. Identifying cryptic species (Box 1; see Glossary) challenged biologists and naturalists even before the Linnaean classification system was adopted [2]. Most species descriptions conform with what can be regarded as the morphological or typological species concept (Box 1) [3], because they predate Mayr's classic *Animal Species and Evolution* [4], which articulated the first incarnation of his 'biological species concept' now adopted by many biologists. Because speciation is not always accompanied by morphological change, the true number of biological species is likely to be greater than the current tally of nominal species, most of which are delineated on purely morphological grounds.

Unexpected genetic diversity within species throughout the tree of life prompts several questions about possible regional and taxonomic biases in our estimates of diversity. For example, are tropical biomes home to more cryptic

species than are temperate biomes? Do large, varied groups such as insects and fungi hide unknown numbers of new species? Genetic diversity within a 'species' also implicates underappreciated mechanisms of morphologically static cladogenesis (i.e. diversification of new species without morphological change). Moreover, misidentification of medically and economically important species in cryptic complexes [5–11] can have serious negative consequences (e.g. in fisheries management and pest control), and implications of cryptic species complexes for conservation [12,13] are sobering.

Here, we summarize the burgeoning literature on cryptic and sibling species (Figure 1) and describes its salient trends. Our emphasis on metazoan animals reflects the intended focus of this review and the existing bias in the literature, although we suspect that our conclusions apply more broadly across the tree of life.

Glossary

Allopatric: populations or species with geographically separated ranges.

Alpha taxonomy: the description and naming of organisms.

Clade: a monophyletic group of organisms.

Cladogenesis: the evolutionary splitting of groups of organisms or, literally, the generation of a clade.

Cryptic species: two or more distinct species that are erroneously classified (and hidden) under one species name.

Karst: irregular (often limestone) formations often including structures such as caves, sink holes, underground water ways and abrupt outcroppings.

Koinobiont: parasitoids that allow their hosts to develop normally, often not killing the host for a relatively long time period, and therefore coexisting with the host.

Monophyly: the grouping together of an organism and all of its descendants in a clade.

Morphological stasis: lack of change in characteristics of gross external anatomy

Parapatric: populations or species with contiguous, nonoverlapping geographical ranges.

Sister taxa or species: two taxa that are derived from the same immediate common ancestor and are therefore.

Sibling species: a cryptic sister species; two species that are the closest relative of each other and have not been distinguished from one another taxonomically.

Species flock: a monophyletic group of closely related species all living in the same ecosystem; sometimes called a 'species swarm'.

Sympatric: populations or species with the same or overlapping geographical ranges.

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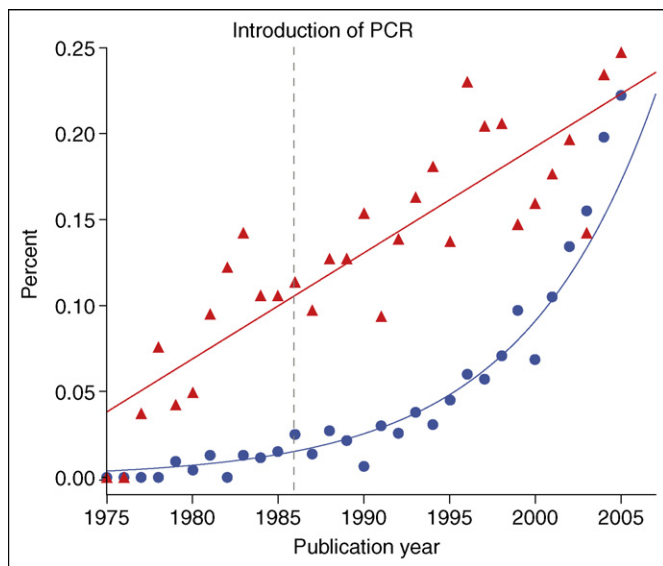


Figure 1. Increased recognition of cryptic species. Although the concept of 'cryptic' species has existed for hundreds of years, their study has increased exponentially over the past two decades. The percent of peer-reviewed publications in Zoological Record Plus (CSA) that mention 'cryptic species' (circles) or 'sibling species' (triangles) in the title, abstract, or keywords has increased dramatically since the advent of PCR. Similar positive trends are observed in absolute number of publications per year, and in publications cited in other searchable databases of biological literature, including Science Citation Index (ISI) and Biosis Previews (Biological Abstracts) (OVID).

What are cryptic species?

The literature is brimming with definitions of cryptic or hidden species. Most authors regard cryptic species to be synonymous with 'sibling species' [16], whereas others specify that 'sibling' connotes more recent common ancestry than does 'cryptic', implying a sister-species relationship [17]. Although not yet universally adopted, we feel that differentiating the term 'sibling species' in this way gives the phrase enhanced utility. Some advocate that sibling species graduate to the category of pseudo-sibling species once diagnosable characters are found [16,18]. Adding confusion, some authors refer to camouflaged or secretive species as being 'cryptic species' [19].

We consider two or more species to be 'cryptic' if they are, or have been, classified as a single nominal species because they are at least superficially morphologically indistinguishable. Some authors further stipulate that species designated as 'cryptic' should be recently diverged, separable only with molecular data, occur in sympatry, or be reproductively isolated [14]; however, we do not regard these as essential features of cryptic species. We acknowledge that there is no single best species concept (Box 1) [15] and therefore exclude the latter qualification of reproductive isolation to disentangle definition of cryptic species from the quagmire surrounding species concepts.

The frequency with which cryptic species are uncovered with DNA sequence data (and often subsequently confirmed with morphological and/or ecological data) suggests that molecular data should be incorporated in the research of alpha taxonomists (i.e. biologists that discover, describe, and name new species) as a matter of routine and/or that genetic material should be preserved so that subsequent molecular analysis is possible.

Box 1. DNA, species concepts and cryptic species

The prevalence of cryptic species impairs biodiversity estimation, but the magnitude of our ignorance depends, at least in part, on how species are defined. The biological species concept (BSC) is perhaps the most popular species concept and stipulates that species are actually or potentially interbreeding populations isolated from other such groups. Under the BSC, new species are formed when they are reproductively isolated. Because mechanisms of reproductive isolation differ among taxa, the BSC offers no universal yardstick to delimit species. Detailed, taxon-specific methods and knowledge are therefore needed to discriminate species.

Although copious DNA sequence data have encouraged a new era in species discovery, DNA has not emerged as a panacea for species description or delimitation. Generating and analysing molecular data requires no specialized knowledge of the anatomy, ecology, behaviour or biogeography of the taxa involved. When genetic distances among individuals or populations appear to be great (some authors advocate a threshold percentage [67]), some authors would call the genetic entities putative species [68]. However, the notion that anyone with a thermal cycler and DNA sequencer can act as a taxonomist for any group of organisms, however appealing the notion might be, is overly optimistic and biologically specious [69,70].

The presumed neutrality of some molecular markers, in conjunction with phylogenetic methods, offer at least two new classes of information that provide a new perspective on the species problem: hierarchical relatedness and relative rates of evolution (i.e. molecular clocks) [71]. In the interest of creating a species concept that can be applied unambiguously to molecular investigations of species limits, several authors have coined phylogenetic or genealogical species concepts that define species in terms of monophyly without explicit regard to reproductive barriers, thus side-stepping the difficulties associated with diagnosing reproductively isolated species [72,73].

In practice, it is often difficult to determine whether populations that have not exchanged genes in the recent past actually cannot exchange genes. Whereas DNA data can reveal population structure and a lack of interbreeding between two groups, the analysis of neutral genetic markers says little about whether these taxa could exchange genes if given the chance. Many reported cryptic species complexes are sympatric, providing strong indirect evidence that these distinctly derived entities do not and cannot exchange genetic material [22,46,74,75]. Genetic differentiation of allopatric populations, however, might be ascribable to local adaptation or genetic drift.

Description and discovery of undetected diversity

Large genetic distances within traditionally recognized species, often in combination with morphological, geographical and other subtle differences, have revealed cryptic species in most types of organism and habitat, from deep-sea clams [20] to freshwater fish [21], and from tropical butterflies [22] to arctic plants [23]. We surveyed literature referenced in ISI Web of Science (<http://scientific.thomson.com/products/wos/>) and Zoological Record Plus (<http://www.csa.com/factsheets/zooclust-set-c.php>) containing the phrases 'cryptic species' or 'sibling species' in the title, abstract or keywords, and found >3500 references from the past 50 years (Figure 1). The most striking bias in these data is the preponderance of studies on animals. Even in light of inherent discrimination of our data sources and current numbers of described species in different taxonomic groups, we found surprisingly few papers reporting cryptic species in higher plants or microbes.

The need for fresh material for molecular studies has motivated the collection of new specimens of many species, and the discovery of cryptic species is often a byproduct of

Box 2. Key targets for cryptic species investigations

Although many small clades of organisms (<100 species) have been thought to harbour cryptic species complexes, there are few larger groups that have received thorough taxonomic treatment from investigators attempting to uncover cryptic diversity. Here, we detail four research areas awaiting study.

Tropical rainforests and marine habitats

Tropical rainforest (Figure 1a)* and marine (Figure 1b) habitats might be breeding grounds of cryptic speciation because they are the most species-rich habitats on Earth [27] and because many of those organisms are involved in specialized interspecific interactions. Most publications on cryptic and sibling species concern organisms from temperate regions, and many workers in the tropics feel that tropical ecosystems have many undescribed cryptic species [22,76].

Fungi

Fungi (Figure 1c) are among some of the least well-studied organisms. Their importance for ecosystem functioning and nutrient cycling is instrumental, yet we know little about their species recognition and mating systems, making them another group that is most likely to harbour cryptic species. Commercially important as pests, fermenters, decomposers and pathogens, we might also be missing out on many 'valuable' aspects of their biodiversity.

Frogs

Frogs and many other organisms have species recognition and mate choice systems that rely on non-morphological characteristics (e.g. advertisement calls, cuticular hydrocarbons and other pheromones) and might be storehouses of tremendous cryptic diversity [46]. In addition, species that have not attracted attention because of their small size, inaccessible habitats, or have been ignored because they have no commercial value (e.g. Figure 1d, *Litoria iris* from Papua New Guinea) are even less likely to be studied and, hence, hiding cryptic species diversity.

Arthropods

Arthropods are another group expected to contain many new cryptic species and are well represented in the cryptic species literature. The recognition and description of cryptic species and speciation in this group has serious implications for human health (e.g. *Anopheles* malaria-transmitting mosquitoes; Figure 1e), pest management (different species have variable pesticide resistance), and studies of coevolution and species interactions.

* Figure 1 images copyright David Bickford (a,c,d); (b) from <http://www.photos.com>; (e) from CDC (<http://phil.cdc.gov>).

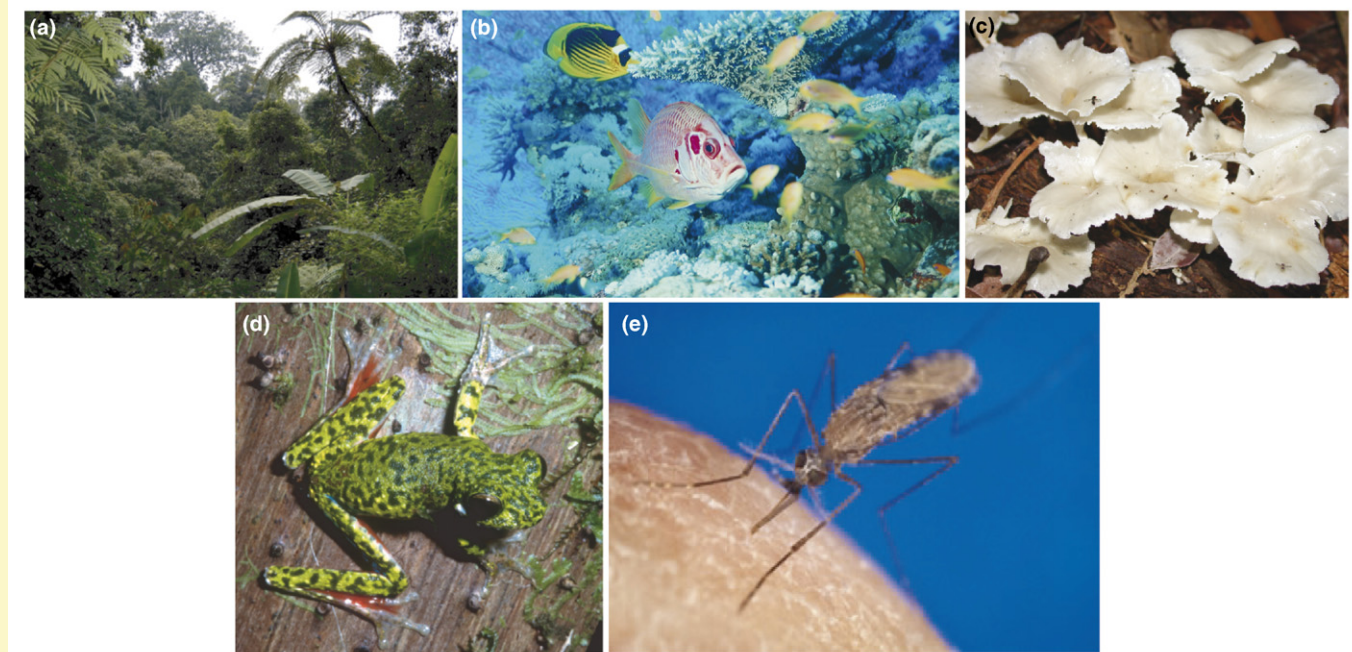


Figure 1.

research on other biological problems associated with study organisms. Thus, taxonomic favouritism in the literature does not necessarily reflect a real bias in affinity for certain taxa to conceal cryptic species in nature. In addition, the history of taxonomic activity in particular groups appears to influence the rate at which cryptic species are found [24]. Taxonomists of moss, for example, have tended to be 'lumpers', with most nominal moss species being distributed across more than one continent [25]. By contrast, shells of marine molluscs, which are easily collected and manifest a high degree of phenotypic plasticity, have long attracted the taxonomic attention of amateur and professional 'splitters' alike. Molecular investigations of molluscan species limits have tended to

invalidate species that were described without any unambiguous differentiating characters [26].

Although two-thirds of all described species reside in the tropics [27], roughly half of the cryptic and sibling species studies that we found focus on temperate organisms. This discrepancy appears to reflect the preponderance of biologists located in temperate climates rather than biological reality. Whether the tropics harbour more cryptic species than do temperate environments is a question that awaits systematic study (Box 2).

Why are cryptic species cryptic?

Most sensory information processed by the human brain is visual, perhaps explaining why morphological characters

feature more prominently than chemical and auditory characters in our classification of the natural world. Integrative taxonomists look for concordant changes in more than one characteristic of an organism, and corroboration from independent data (e.g. molecules, morphology or mating signals) is frequently deemed to be good evidence for separating species [4] (Box 1). However, there are several reasons why morphological change might not be correlated with species boundaries or might not be useful in discriminating species, and there appear to be two general and recurrent themes: cryptic species are either differentiated by nonvisual mating signals and/or appear to be under selection that promotes morphological stasis.

Nonvisual mating signals

Cryptic species can often be discriminated by differences in mating pheromones or mating calls, but the morphological machinery needed to produce different acoustic or olfactory signals need not differ appreciably. Taxa that differ in these ways are thus likely to escape the notice of visually oriented taxonomists.

Acoustic mating signals have been used to discriminate closely related cryptic species in a variety of insects [28], and vertebrates, including frogs [29], bats [30] and birds [31]. Pheromones of sibling species are often distinct, although many differ in subtle ways, such as in the chirality of compounds [32] or differences in ratios of compounds in a pheromone blend [33]. In the words of Mayr [4] 'Sibling species are apparently particularly common in those kinds of species in which chemical senses (olfactory and so on) are more highly developed than the sense of vision. Although indistinguishable to the eye of man, these sibling species are evidently dissimilar to each other. Sibling species are apparently rarest in organisms such as birds that are most dependent on vision in the role of epigamic characters.'

Given that nonvisual communication might be a key to discovering cryptic species, particular focus on environments that hamper transmission of visual signals could be fruitful (Box 2). For example, Knowlton [26] has argued that cryptic species might be more common in marine taxa because marine animal taxonomists rarely have the chance to see live organisms (and therefore lack knowledge of behavior that students of terrestrial organisms take for granted), and because marine organisms often rely on chemical signals for gamete recognition [34] and mate choice [35]. Unlike signals of most visually oriented terrestrial organisms, interspecific differences in these features rarely leave a morphological imprint.

Morphological stasis

Extreme environmental conditions might impose stabilizing selection on morphology, reducing or eliminating morphological change that can accompany speciation. Schröngge and colleagues [36] have argued that species experiencing strong selection on behavioral or physiological characters for adaptation to a specific host might not be expected to show morphological changes among species. These include myrmecophiles inhabiting ant nests, endoparasites and koinobiont parasites, which have prolonged relationships with their hosts. Evolving

under severe environmental extremes can also limit changes in morphology. For example, because there are a limited number of ways in which an organism can adapt to harsh conditions [37], extremophiles are expected to converge in physical characteristics and speciate via habitat fragmentation and subsequent drift [38] or through other mechanisms that are more associated with cryptic speciation, such as pheromonal or behavioral differentiation. Large numbers of cryptic species found in Arctic tundra [23], underwater karst [39] and deep-sea environments [20] are consistent with this hypothesis.

Correcting cryptic assumptions

Several common but perhaps incorrect assumptions about cryptic species pervade the literature (Figure 1). One of the most common assumptions is that most cryptic species result from speciation that is so recent that morphological traits or other diagnosable features have not yet evolved. Although undoubtedly true for some taxa such as coccolithophores (see Ref [16]), the view that cryptic species are recent is challenged by studies of bonefish [40], amphipods [39] and copepods [41], which show apparently ancient divergences among cryptic species.

Another common misunderstanding in the discussion of cryptic species stems from presumed mechanisms of speciation. Can diversification of cryptic species, sometimes termed 'cryptic speciation', be attributed to a particular evolutionary mechanism? This seems unlikely, owing simply to the complexity and diversity of speciation mechanisms, but perhaps new mechanisms can be identified or the range of possibilities narrowed. Strong divergent natural or sexual selection are thought to be primary drivers behind rapid morphological divergence with little accompanying genetic differentiation (the equivalent of anti-cryptic speciation). This phenomenon is well documented in species flocks of African cichlid fish and several other groups, including drosophilid flies [42]. Is it possible that directional selection could be ruled out as a significant force in the evolution of cryptic species? Not necessarily. Directional selection on ecological, behavioral, or reproductive traits that have no observed morphological correlates might foster cryptic speciation and be a mechanism that drives cryptic diversification [43,44]. Whereas morphological stasis through cladogenesis might be expected under several models of speciation, certain organismal features have been posited to predispose particular groups towards cryptic speciation (Box 2). Among the simplest explanations is that many cryptic species are morphologically austere (e.g. sponges and nematodes), lacking features that can be used to distinguish species effectively [24,45]. Organisms that communicate reproductive signals via nonvisual means (e.g. sound, vibration, pheromones or electrical signals) are perhaps most likely to harbour cryptic species because changes in signals conveyed in these modalities need not involve morphological change. Well-studied examples include insects [28] frogs [44,46] and fish [21]. Although we have not yet explicitly identified new mechanisms that are responsible for cryptic speciation, analysis of evolutionary trends in the

evolution of cryptic species is a field that is ripe for study and could reveal previously underappreciated characters that are frequently involved in cryptic species formation.

Paradigm shifts in ecology

Newly discovered cryptic species complexes have overturned traditional notions about ecological specialization in interspecific interactions. Investigations of many antagonistic interactions have found that some exploitative species tend to have more specialized diets than was previously thought. Studies of herbivorous beetles [47], Lepidoptera [22] and whole guilds of other herbivorous insects [48] have shown that presumed dietary generalists are complexes of dietary specialists. Similarly, dipteran [49] and hymenopteran [50] parasites of Lepidoptera are more specialized because so-called 'generalist' species are in fact cryptic complexes of specialists. Conversely, investigations of mutualistic interactions tend to find less ecological specialization than was previously thought. Molbo *et al.*'s conservative estimate that 50% of figs are pollinated by more than one fig wasp species contradicts accepted dogma that each fig is pollinated by a single fig wasp species [51]. Similarly, obligate ant mutualists of the Australian lycaenid butterfly *Jalmenus evagoras* were thought to comprise primarily two *Iridomyrmex* ant species [52]. However, a recent molecular investigation has revealed at least seven co-distributed cryptic species [53].

What we don't know might hurt us

Several lines of evidence underscore the importance of recognizing cryptic species. Conservation planning, bio-prospecting, biological control and the treatment of diseases and snakebites all hinge on the identification of species in cryptic complexes. An inability to identify biologically important species therefore hampers our efforts to conserve, study, contain or utilize them.

Cryptic species recognition

Accurate species identifications are often crucial for the implementation of biological control, diagnosis and prevention of disease and the identification of invasive and pest species. Recent molecular investigations have uncovered species complexes in human [7,9] and crop plant [10,54] pathogens. Failure to recognize cryptic species of pathogens might complicate efforts toward eradication, given that separate species might respond to control measures differently.

Cryptic species complexes have been uncovered in entomopathic fungi [5] and parasitic wasps [55] that are used for biological control. Scientists developing biological control measures for crop pests and invasive species exploit generally species-specific interactions between parasites or pathogens and their hosts. Failing to recognize cryptic species limits the effectiveness of these programs and could cause rejection of potentially valuable species as control agents because host breadth was misinterpreted. Pest 'species' themselves could also be species complexes, whose members differ in their resistance to insecticide [8].

Research on malaria has benefited from the study of mosquito vector taxonomy. Investigation of the *Anopheles gambiae* species complex, the primary malarial vector in

Africa, has uncovered seven cryptic species that vary in their habitat and host preferences. More importantly, some of these newly recognized species attack only nonhuman animals, posing no threat to human health [56]. If efforts are focused on human-specific malarial vectors, resources could be spent more economically, without unnecessarily detrimental effects on nontarget species.

Searches for new pharmaceuticals can also benefit from the intensified study of species limits in medicinally valuable organisms. Natural products derived from plants, sponges and other organisms are a natural library of novel chemicals with potential medicinally valuable properties, such as antibiotic or antiviral activity. Because these compounds vary among species [57], the existence of undetected cryptic species complexes can mask potentially valuable sources of medicinal substances [58].

The importance of recognizing cryptic venomous snake species is obvious in both initial snakebite treatment and the production of antivenin serum. Yet, even within groups that cause significant human fatalities, such as vipers (Viperidae) and Asian cobras *Naja* spp., taxonomically undescribed cryptic species complexes exist [59,60]. Venom composition can vary greatly among congeners [6], and systematic recognition of all taxa within each group is essential for successful snakebite treatment [11].

Conservation, management and cryptic diversity

How accurate are estimates of diversity? What environments or life histories might promote cryptic diversity? Through systematic studies that try to estimate cryptic species in particular taxa and environments, it might be possible to determine the extent of our ignorance. The identification and description of cryptic species have important implications for conservation and natural resource protection and management. For example, the common blue mussel *Mytilus edulis* is a commercially valuable and biologically important indicator species that is currently used to monitor pollution. This 'species' comprises three different cryptic species with different characteristic growth rates [61]. Because this cryptic species complex has been used to monitor pollution, the inaccuracy of results from monitoring could compromise human safety from consumption of heavy metals and/or other pollutants. Species differ in their responses to the environment, and utilizing a single species as a bioindicator of pollution, heavy metal contamination or environmental degradation will be a more accurate metric than relying on a suite of species in a cryptic complex.

The importance of identifying cryptic species complexes for conservation should also not be underestimated. Introduced invasive species are one of the primary causes of population declines among native species in wild habitats, implicated as the most common cause of bird and other vertebrate extinctions [62], and invasions by previously allopatric cryptic species, such as the common blue mussel discussed earlier, can cause a native species to decline before invasion is detected [61].

Molecular work has revealed at least 14 frog species within two nominal species [46], a revelation that could have ramifications for amphibian conservation. Whereas the cryptic frog species complexes had broad geographical

ranges, actual biological species in those complexes have more limited distributions, making each more prone to extinction. Preventing habitat loss is perhaps the greatest challenge for the conservation of global biodiversity, and prioritizing habitats for conservation often relies on estimation of species richness and endemism. The discovery of geographical and habitat-related patterns in distribution of cryptic species could therefore uncover as-yet-unknown pockets of endemism and diversity that might warrant reconsideration for particular habitats or sites for conservation. The explosive radiation of rhacophorine frogs in Sri Lanka provides an excellent example. An integrative taxonomic study using morphological, ecological, bioacoustical, and genetic data increased the number of species from 18 to over 100 [63].

Cryptic species require special consideration in conservation planning because the prevalence of cryptic complexes in already endangered nominal species presents a dual problem: (i) species already considered endangered or threatened might be composed of multiple species that are even more rare than previously supposed; and (ii) the different species might require different conservation strategies [36]. Molecular evidence has revealed that several already endangered species are cryptic species complexes, making them a collection of even more critically endangered species with fewer numbers and smaller distributions. For example, the distinctiveness of critically endangered Kemp's ridley *Lepidochelys kempii* and olive ridley *Lepidochelys olivacea* sea turtles was supported on the basis of genetic evidence [12]. Similarly, mitochondrial DNA and karyotypic evidence indicates that one of the four described subspecies of endangered northern sportive lemur *Lepilemur septentrionalis* is a distinct species with a restricted range, ~150 individuals, and no legal protection status [13].

Prevalence of cryptic species might even call into question estimates of the number of species on Earth. Early extrapolations of global diversity relied heavily upon estimating dietary specialization of the most diverse guild on Earth, tropical phytophagous insects [64]. Subsequent empirical work suggested that host plant specificity is not as narrow as was previously assumed, and global estimates of insect diversity were reduced accordingly [65]. However, the prevalence of cryptic species complexes in the tropics is an important unknown. Recent studies of cryptic species in temperate zones indicate that so-called 'generalist' herbivores are complexes of host-specialists [22,47,66]. The discovery of a similar trend in the tropics could re-inflate estimates of tropical diversity and, thus, reflect the true diversity of life on Earth. Understanding and quantifying biological diversity is imperative if we want to be able to explain and, ultimately, conserve it.

Summary

Molecular techniques (primarily DNA sequencing) have transformed the ability of scientists to describe and define biological diversity. Although they are not a panacea for species delimitation, molecular data are important and useful when combined with other types of data. Studies using non-morphological characters, such as chemical and auditory signals, to discriminate otherwise indistinguish-

able species are being published at an increasing rate. These newly discovered cryptic species present opportunities to study important mechanisms of speciation, mate recognition and conservation management. High priorities for future research include discovering characters that natural selection acts upon and studying non-morphological characters that can be used to differentiate species. Similarly, investigating novel mechanisms of speciation, conservation planning with new data on cryptic species, and projecting taxonomic, regional and global diversity indices are worthwhile avenues for future research.

Many unresolved questions still remain, including: what evolutionary and ecological processes lead to genetic divergence and reproductive isolation in the absence of morphological differentiation? Do some taxonomic groups have a greater propensity for cryptic speciation? Is cryptic speciation more common in allopatry, parapatry, or sympatry? Are cryptic species more common in the tropics, if so, why? How do different species concepts influence the assessment of cryptic speciation? What kinds of genetic evidence are necessary for demonstrating and formally describing cryptic species? Clearly, more research on cryptic species is urgently needed.

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