Cryptic species as a window into the paradigm shift of the species concept

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Abstract

The species concept is the cornerstone of biodiversity science and any paradigm shift in the delimitation of species affects many research fields. Many biologists now are embracing a new ‘species’ paradigm as separately evolving populations using different delimitation criteria. Individual
criteria can emerge during different periods of speciation; some may never evolve. As such, a paradigm shift in the species concept relates to this inherent heterogeneity in the speciation process and species category – which is fundamentally overlooked in biodiversity research. Cryptic species fall within this paradigm shift: they are continuously being reported from diverse animal phyla but are poorly considered in current tests of ecological and evolutionary theory. The aim of this review is to integrate cryptic species in biodiversity science. In the first section, we address that the absence of morphological diversification is an evolutionary phenomenon, a ‘process’ counterpart to the long-studied mechanisms of morphological diversification. In the next section regarding taxonomy, we show that molecular delimitation of cryptic species is heavily biased towards distance-based methods. We also stress the importance of formally naming of cryptic species for better integration into research fields that use species as units of analysis. Finally, we show that incorporating cryptic species leads to novel insights regarding biodiversity patterns and processes, including large-scale biodiversity assessments, geographic variation in species distribution, and species coexistence. It is time for incorporating multi-criteria species approaches aiming to understand speciation across space and taxa, thus allowing integration into biodiversity conservation while accommodating for species uncertainty.

1. INTRODUCTION

Genes, species and ecosystems are three major components of biodiversity. Among them, species has proven the most challenging to conceptualize. Decade-lasting disputes over the species concept yielded no less than 24 species definitions (Mayden, 1997). Defining a species is central to biodiversity science (de Queiroz, 1998, 2005b; Mayden, 1997) because any change in the species concept directly affects many important research fields, including taxonomy, biogeography, ecology, conservation and macroevolution (Agapow et al., 2004).

An important step forward was made with a distinction between conceptual and operational issues, i.e. what a species is and how they should be delimited (Bock, 2004; Hey, Waples, Arnold,
Butlin, & Harrison, 2003). Most authors today agree conceptually (species concept, see Glossary), i.e.
that species are entities emerging from the speciation process as separately evolving segments of
metapopulation lineages (Bock, 2004; de Queiroz, 1998; Hey, 2006; Hey et al., 2003; Mayden, 1997).
Establishing the correspondence between the species concept and species taxa, i.e. elementary units
used in biodiversity research (see Glossary), remains a challenge. From a temporal point of view, a
species is framed by a speciation event, at the origin of this process, and by event putting an end to
this species (further speciation or extinction) (Bock, 2004; Roux et al., 2016). Inference of speciation
depends on different properties that are expressed during separate evolution, such as morphological
distinguishability, reproductive isolation and monophyly. These properties remain the best lines of
evidence to identify lineage divergence and thus delimit species taxa (de Queiroz, 2005a,b, 2007).
However, the evolution of these properties is context dependent and can emerge at different times
and sequential order, if at all (de Queiroz, 2005a, 2007). Consequently, there is no particular
property that would universally delimit a species. Not surprisingly, difficulties inherent to species
delimitation prompted proliferation of species delimitation methods based on different premises
(Fontaneto, Flot, & Tang, 2015; Raxworthy, Ingram, Rabibisoa, & Pearson, 2007; Sites & Marshall,
2003, 2004), whose accuracy depended on the context of speciation (Dellicour & Flot, 2015).
Furthermore, species taxa delimited with these methods should be considered merely as scientific
hypotheses of separately evolving entities (de Queiroz, 2007; Hey et al., 2003), which are iteratively
tested using new methods and data in the procedure called “integrative taxonomy” (Padial, Miralles,
De la Riva, & Vences, 2010; Yeates et al., 2011). The outcome of this conceptual and methodological
development is recognition that species taxa represent a heterogeneous set of hypotheses whose
properties are contingent with the heterogeneous nature of speciation.

A central question raised by this paradigm shift is whether we can make use of the explicit
links between the speciation process and species hypotheses. If heterogeneity in the speciation
process leads to heterogeneity among species hypotheses, can we integrate heterogeneity in
speciation into evolutionary, biogeographic and ecological research? This issue has been partly

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explored in the case of ecological speciation (Schluter, 2000a, 2016; Stroud & Losos, 2016). Ecological speciation is a process where ancestral populations diverge due to resource partitioning and is associated with phenotypic differentiation (Nosil, 2012; Rundle & Nosil, 2005). It takes place when ecologically accessible resources can be evolutionarily exploited (ecological opportunity) (Schluter, 2000b; Stroud & Losos, 2016). Ecological speciation can contribute to speciation rates and can underlie major evolutionary events such as adaptive radiation, but its relative importance depends on ecological opportunities that vary in space and time (Schluter, 2016). Hence, ecological speciation provides a mechanistic explanation between ecological opportunity and global biodiversity patterns (Kozak & Wiens, 2016; Schluter, 2016). Further generalizations require better understanding between environmental and speciation relationships, especially regarding speciation heterogeneity.

Over the last two decades, increased evidence emerged for speciation governed by entirely different mechanisms, leading to so-called sibling or cryptic species. The idea that species can evolve similar morphologies is historical (Mayr, 1942), but the use of molecular delimitation methods has now brought cryptic species to the forefront in many research arenas (Bickford et al., 2007; Knowlton, 1993; Nygren, 2014). Indeed, we are now aware that cryptic species are quite common (M. Adams, Raadik, Burridge, & Georges, 2014) and widespread across most animal phyla (Pérez-Ponce de León & Poulin, 2016; Pfenninger & Schwenk, 2007). They even have been found in mostly overlooked parasite (Pérez-Ponce de León & Nadler, 2010) and nematode species (Derycke et al., 2008), but also among the largest and heavily studied mammalian species like giraffes (Fennessy et al., 2016). In spite of being common, they are still poorly considered in testing ecological and evolutionary theories (Beheregaray & Caccone, 2007). It is timely to intensify research beyond the phenomenological level of exploration and incorporate what we can learn from cryptic species into biodiversity science. The aim of this review is to propose a research agenda for fully integrating cryptic species into biodiversity science, thereby fostering a better understanding of the heterogeneous role of speciation in biodiversity pattern and process.
Towards this goal, we examine three inter-related facets of cryptic species in respect to biodiversity science (Fig. 1). First, we reconsider and define the phenomenon of phenotypic similarity from an evolutionary perspective. We explore three non-mutually exclusive mechanisms explaining the lack of morphological distinguishability among divergent lineages; i.e., recent divergence, niche conservatism, and morphological convergence. Here, we evaluate the existing empirical evidence and suggest novel tests to better elucidate each mechanism. Second, we review the practices in delimiting cryptic species and reconsider them from a taxonomic perspective. We explore other contingent properties of species currently used to identify species boundaries in the absence of morphological distinguishability. Here, we look into the burgeoning fields of DNA taxonomy and integrative taxonomy (Camargo & Sites, 2013; Fontaneto et al., 2015; Padial et al., 2010), highlighting the recurrent need for naming species. We show how integrative taxonomy can improve understanding of morphological cryptic evolution. Third, we discuss how cryptic species have been integrated into biodiversity research and, more specifically, what can be gained by incorporating different hypotheses (or properties) of speciation into biodiversity science. Lastly, we use these facets to identify major gaps in biodiversity research and promising research directions towards enhancing biodiversity science.

The ideas discussed in this review apply to all animal phyla. To provide data-driven evaluation regarding the current research status on cryptic diversity, we focus (but not limit) our literature search to the Amphipoda. Amphipods are an excellent invertebrate model for studying morphologically cryptic species, underlying speciation and its relationship to biodiversity science. They are species rich, ecologically diverse, thrive in most aquatic environments, play a crucial role in ecosystem function, and are often used as biological indicators. Importantly, they comprise numerous cryptic species within different ecological types, and thus are relatively well-studied in eco-evolutionary studies (Box 1, Fig. 2).
2. COMPILATION OF LITERATURE DATA

Cryptic species were often detected as a by-product in phylogeographic studies of amphipods. For this reason, we searched for relevant articles in the ISI Web of Science using keywords (Amphipoda/Amphipods * cryptic species), (Amphipoda/Amphipods * sibling species) and (Amphipoda/Amphipods * phylogeography) and (Amphipoda/Amphipods * molecular taxonomy). The search before the year 2000 yielded no results, hence we analyzed the period between 2000 and 2015 (search was made on September 2015). All titles were checked and duplicates removed, yielding a total of 120 articles. From this search, our review covers 122 morphological species of amphipods or morphospecies, i.e., species that were delimited on a basis of their morphological distinctness. The list of morphospecies contained no subspecies. These morphospecies collectively contained a total of 439 cryptic species. Of 122 nominal species, 13 were found to be synonyms, but the remaining 109 morphospecies represent 304 (295-315, depending on the delimitation method of the 439 total) cryptic species discovered for the first time. Individual articles were searched for several categories of information that were relevant to the three topics below (see also Supporting Information).

2.1. Mechanisms causing cryptic diversity

From these 120 articles, we searched for information regarding three related hypotheses explaining the lack of morphological distinguishability, namely recent divergence, phylogenetic niche conservatism and morphological convergence. The recent divergence hypothesis posits that cryptic species have diverged recently and morphological differentiation presently is not evident (Egea et al., 2016). Here, we analyzed the distribution of age of the cryptic species using reported estimations of node age in phylogenetic trees. Descriptive estimations of age were discarded (e.g., early Miocene), which reduced the number of relevant papers to 29.
The phylogenetic niche conservatism hypothesis (PNC) postulates that niche evolution and hence morphological differentiation across descendant species is constrained by selection, a phenomenon known as morphological stasis (Bravo, Remsen, & Brumfield, 2014; Egea et al., 2016; Smith, Harmon, Shoo, & Melville, 2011). To identify cases of cryptic species potentially matching this hypothesis, we searched for reports of cryptic species among complexes of sister species that were ecologically specialized at the micro-niche level. We assumed that a single origin of narrow specialization in an ancestor parsimoniously explains specialization in sister descendants more than multiple and independent origins.

The morphological convergence hypothesis also is based on selection and assumes that morphological similarity may evolve independently among distantly-related species in response to similar selection pressures (Bravo et al., 2014; Trontelj et al., 2009). Here, we explored whether morphological similarity of cryptic species evolved independently through convergent evolution. For this reason, we quantified the proportion of cryptic species that were not in sister relationships with each other.

2.2 Taxonomic practices in delimiting cryptic species

Authors rarely a priori referred to a well-defined species concept while delimiting species taxa. For this reason, the evaluation what they considered as a species was not possible. The following analysis of past research is based on the premise that species are separately evolving entities, and that species taxa can be delimited using the criteria of genetic isolation.

We reviewed the number and type of molecular markers as well as the methods used to delimit cryptic species. Molecular markers included allozymes, mitochondrial and nuclear genes, microsatellites and single nucleotide polymorphisms (SNPs). DNA taxonomy is a rapidly evolving field such that molecular methods for species delimitation used a decade ago may not be considered...
valid today (compare, for example, the classifications of Sites & Marshall, 2004, 2003 and Flot, 2015; Fontaneto et al., 2015). All methods, albeit on different premises, search for cessation of gene flow between populations. Such genetic isolation is a primary pointer of ongoing speciation (Bock, 2004) and emergence of independently evolving entities (de Queiroz, 2007), and serves for delimitation of corresponding species taxa (see Glossary). The progress in methodology makes any classification using different methods a difficult exercise. For this review, we used four categories for classification: namely, distance-based, tree-based, allele-sharing and population genetics methods (Table 1). Distance-based methods assume that speciation should result in smaller genetic distances within than between species. Species are delimited either using a fixed threshold (Lefébure, Douady, Gouy, & Gibert, 2006) or the best-fitting threshold calculated for the dataset at hand (Puillandre, Lambert, Brouillet, & Achaz, 2012). Tree-based methods search for the most likely transition point between species-level and coalescent processes using single locus (Monaghan et al., 2009; Pons et al., 2006; Reid & Carstens, 2012; J. Zhang, Kapli, Pavlidis, & Stamatakis, 2013), multiple loci (Ence & Carstens, 2011; Grummer, Bryson, & Reeder, 2014; Knowles & Carstens, 2007; O’Meara, 2010; Z. Yang & Rannala, 2010, 2014) and genomic-scale data (Leache, Fujita, Minin, & Bouckaert, 2014). Allele-sharing based methods (Flot et al., 2010) attempt to discern populations that are no longer connected by gene flow and therefore evolved mutual allelic exclusivity. We recognize that population genetic methods (e.g. nested clade analysis, indices of genetic differentiation) are not intended to solely delimit species, but have been used often in the past in respect to species delimitation. In fact, many authors considered genetically distinct populations as putative species.

In addition, we quantified cases when cryptic species were analyzed for differences in morphology, physiology, ecology or behavior. Compositing different data types to delimit species is known as integrative taxonomy (Padial et al., 2010; Wiens & Penkrot, 2002). It can either provide additional support for a species hypothesis (Schlick-Steiner et al., 2010) or detect contradictions among data (Padial et al., 2010); both help link taxonomy with evolutionary biology (Sites & Marshall, 2003, 2004). Finally, we examined whether cryptic species have a binomial name and, if so, it is based
solely on molecular criteria (Cook, Edwards, Crisp, & Hardy, 2010; Jörger & Schrödl, 2013; Renner, 2016). Although naming is not always done, the naming of new species integrates taxonomy with other fields of biology, (Pante, Schoelinck, & Puillandre, 2015).

2.3 Cryptic species in biodiversity research

Although cryptic species are being documented at an ever-increasing rate, they are integrated in quite different ways across fields in biodiversity science. Here, we distinguish between two major ways of incorporating cryptic species in biodiversity research. The first corresponded to studies that explore whether cryptic species might have biased our understanding of biodiversity patterns and thus misled conservation efforts. The second corresponded to studies that only retain DNA-based criteria for delimiting species, thereby inherently using cryptic species as units of analysis.

3. MECHANISMS CAUSING CRYPTIC DIVERSITY

3.1 Recent divergence

The simplest evolutionary model predicts that morphological disparity increases due to stochastic processes alone (see e.g., Adams et al. (2009) and Harmon et al. (2003) for changes in morphology through time). The evolution of morphological distinctness depends only on time since divergence, hence cryptic species are expected to be relatively young (Zúñiga-Reinoso & Benítez, 2015). Diagnostic morphological traits evolve slowly if they are selectively neutral, effective population size is large and ancestral polymorphism is high (Egea et al., 2016; Wiens & Servedio, 2000).

We obtained age estimates for 43 cryptic species of amphipods (Fig. 3). The age distribution was typically skewed left, but only five out of the 43 cryptic species were younger than 1 Myr. This result is in stark contrast to theoretical predictions mentioned above. One explanation may be that this
mechanistic explanation is unlikely. The evolutionary response to environmental selection is rapid (Carroll, Hendry, Reznick, & Fox, 2007; Herrel et al., 2008), thus evolving species quickly attain their morphological distinctness. The alternative explanation invokes methodological biases. First, the methods used for age estimation have (and are still ongoing) changed over time, and current estimated ages are sensitive to a number of poorly understood parameters (dos Reis, Donoghue, & Yang, 2015). Different authors estimated node ages using different methods, optimized for their datasets. Some variation in age estimation among these studies is unavoidable and at times even biased. Second, many evolutionarily young cryptic species might have been simply overlooked. Most of the reviewed studies used coarse delimitation methods that accurately delineate only relatively old species (see Section 3). Consequently, we conclude that recent divergence presently explains morphological similarity of only a small fraction of known cryptic species.

To note, none of the examined studies explicitly tested for recent divergence. This hypothesis has two central predictions: first, cryptic species are young and, second, morphological traits evolve under the neutral model of evolution (Brownian motion, see Glossary) (Felsenstein 1985). Rigorous testing of the predictions should consider three aspects as guidelines for future research. First, testing requires accurate age estimations. This task is not easy and methodological details are beyond the scope of this review; readers should consult more specialized papers (dos Reis et al., 2015). Second, the distinction between “young” and “old” species depends on ancestral polymorphism and population size, therefore it is clade specific. Appropriate evaluation of the hypothesis needs to account for clade specificity. For instance, assessing whether cryptic species are younger than non-cryptic ones requires analysis at the clade level rather than the species pair level. Lastly, models of evolution can be estimated from extant morphology and phylogeny, but a changing selection regime also can produce a pattern similar to neutral evolution and thus lead to erroneous estimation of trait evolution. Fortunately, this issue can be solved using recently developed software that allows for discrimination between models of neutral evolution and changing selective regimes (Clavel, Escarguel, & Merceron, 2015).
3.2 Phylogenetic niche conservatism (PNC)

Recent divergence cannot explain relatively old cryptic species. An alternative hypothesis, so called “morphological stasis”, assumes that morphological differentiation of descendant species is constrained by biological mechanisms (Bickford et al., 2007). Since a species’ morphology is partly related to its ecological niche (e.g., Losos, 2009, Schluter, 2000a), this hypothesis is closely related to the broader concept of phylogenetic niche conservatism (PNC). Initially, PNC was used to explain the tendency for some lineages to retain their ecological niches over evolutionary time. More recently, the concept was elaborated further (Pyron, Costa, Patten, & Burbrink, 2015), but here we refer to its original intention. The biological mechanisms underlying PNC encompass stabilizing selection, gene flow swamping local adaptation, pleiotropic effects constraining adaptation, and lack of genetic variation (Losos, 2008; Wiens, 2004, 2008; Wiens et al., 2010; Wiens & Graham, 2005).

The PNC hypothesis was not explicitly tested in the reviewed studies here. Nevertheless, we found several cases of cryptic species among complexes of sister amphipod species that were ecologically highly specialized, and might support the PNC hypothesis. These include Cyamus species (whale lice) living on distinct parts of a whale’s body (Kaliszewska et al., 2005), Niphargus species colonizing fissures in rock (C. Fišer & Zagmajster, 2009; Meleg, Zakšek, Fišer, Kelemen, & Moldovan, 2013; Švara, Delić, Rada, & Fišer, 2015; Trontelj et al., 2009), some species of Gammarus inhabiting mountain streams (Hou & Li, 2010), and sponge-dwelling species of Leucothoe (Richards, Stanhope, & Shivji, 2012; White, Reimer, & Lorion, 2016). This list is likely longer, especially among deep sea and subterranean amphipods. Regardless, attributing morphological crypsis to PNC for species with unclear ecological specialization is problematic in the absence of explicit testing.

We now discuss three predictions of the PNC to stimulate future research regarding mechanisms causing cryptic diversity (Pyron et al., 2015; Wiens et al., 2010). First, evolution of morphology is constrained. Morphological disparity remains constant through time, as if being constrained by stabilizing selection. The model of morphological evolution inferred from extant morphology and
phylogeny no longer corresponds to neutral Brownian motion but to models simulating non-neutral evolution (but see Losos, 2008). These models include stabilizing selection (Orstein-Uhlenbeck model, see Glossary) or, arguably, constantly moving adaptive optima (white noise model, see Glossary) (Pyron et al., 2015; Smith et al., 2011; Wiens et al., 2010). The preferred models are selected based on likelihood values. Some authors proposed an alternative test using a special model of Brownian motion that allows for different rates of trait evolution across clades. The rates of trait evolution should be lower in niche-conserved than in niche non-conserved clades (O’Meara, Ané, Sanderson, & Wainwright, 2006; Wiens et al., 2010). Second, sister cryptic species are expected to share ecological niches. Equivalency among ecological niches can be tested either implicitly from distributional data (Kozak & Wiens, 2006; Raxworthy et al., 2007; Warren, Glor, & Turelli, 2008) or from experimental data (Kearney, Wintle, & Porter, 2010). Third, PNC promotes allopatric speciation (Kozak & Wiens, 2006; Pyron et al., 2015; Wiens, 2004; Wiens & Graham, 2005). This prediction requires analysis of the evolution of geographic ranges (Matzke, 2013; Ree & Smith, 2008). To note, the third prediction may strengthen arguments supporting PNC, but it is not mandatory. Species can evolve in sympatry along niche axes, e.g. salinity, pH or temperature (Delić, Švara, Coleman, Trontelj, & Fišer, 2017) that are not related to morphology.

3.3 Morphological convergence

The third hypothesis infers that morphological similarity evolved among distantly-related species in response to similar selective regimes (Ingram & Mahler, 2013). Morphological convergence based on our reviewed data was common: 26% of the nominal amphipods examined contained cryptic species that were not sister groups. This proportion is probably a low estimate because detection of convergence depends on completeness of taxon sampling. Cases of morphological convergence among cryptic species were documented in several amphipod genera, where species from the same cryptic complex independently colonized freshwater-semiterrestrial (L. Yang, Hou, & Li, 2013) or
subterranean habitats (Villacorta, Jaume, Oromí, & Juan, 2008) or microhabitats within the subterranean realm (Trontelj et al., 2009; Trontelj, Blejec, & Fišer, 2012).

Evidence from the above studies is descriptive. A central prediction of this hypothesis states that convergent evolution should result from shifts in selective regimes. Appropriate testing must show a correlated evolution between morphology and the environment. The evolution of morphology should correspond to the Ornstein-Uhlenbeck stabilizing selection model (see Glossary) (Butler & King, 2004; Hansen, 1997). Further, Ingram & Mahler (2013) developed a method to map shifts in continuous phenotypic characters towards convergent regimes on a phylogenetic tree and to test whether these shifts are more numerous than expected under a given null model.

3.4 General summary of invisible species diversification

Patterns of cryptic species, i.e. the distribution of cryptic species across space and taxa as well as the occurrence of closely-related / distantly-related cryptic species have been relatively well documented in the amphipod literature and in the literature in general. Yet, there is comparatively much less knowledge about the relative importance of different mechanisms generating cryptic species. Since, as usual in science, patterns alone are not enough to infer mechanism (i.e. different mechanisms can generate the same pattern), there is a need for a shift from pattern-oriented studies of cryptic diversity to mechanism-oriented studies of cryptic diversity. The mechanisms generating cryptic species are clearly diverse. They likely follow the same evolutionary and ecological principles that generated the diversity of morphologically distinguishable species and have fascinated naturalists for centuries (Collar, Schulte, & Losos, 2011; Harmon et al., 2010; Losos, 2011; Nosil, 2012). The lack of morphological diversification is an evolutionary phenomenon, a counterpart to the long-studied mechanisms of morphological diversification such as character displacement (Schluter, 2000a; Stuart & Losos, 2013). Research on morphological similarity is hence closely related
to that on morphological diversification. It requires gathering information on the function and heritability of traits as well as on how trait variation relates to fitness (Schluter, 2000a; Stuart & Losos, 2013). Contrary to morphological diversification, mechanisms causing morphological similarity have received little attention. Most of the reviewed studies focused on revealing cryptic species but did not test for causal mechanisms. Thus even if slightly morphologically different, any species pair satisfying the predictions above might be treated as morphologically cryptic.

Many authors stated that perfect morphological similarity does not exist and that morphological analyses based on molecular species delimitation often yield morphological distinctness. Hence, some researchers consider the concept of cryptic species as overrated (Jugovic, Jalžić, Prevorčnik, & Sket, 2012; Karanovic, Djurakic, & Eberhard, 2016; Knowlton, 1993; Zúñiga-Reinoso & Benítez, 2015). Noteworthy, some authors have already coined a term “pseudo-cryptic” in order to distinguish “completely identical species” from “morphologically diagnosable species” (Achurra, Rodriguez, & Erséus, 2015; Knowlton, 1993; Sáez & Lozano, 2005). This classification may lead to questions such as “When are species similar enough to be considered morphologically cryptic?” (Karanovic et al., 2016; Lajus, Sukhikh, & Alekseev, 2015). This question is at risk to remain without epilogue. Importantly, it could hinder exploration of a rather broadly widespread evolutionary phenomenon and even impede progress in biodiversity science. As discussed further below, the quest for mechanisms can lead taxonomic practices towards delivering species hypothesis that are theoretically grounded in the heterogeneous properties of speciation (section 3), and can better integrate the speciation process into ecology and evolution (section 5).

4. TAXONOMIC PRACTICES IN DELIMITING CRYPTIC SPECIES

4.1 Species delimitation methods

Up to now, most cryptic species of amphipods were delimited using either one or two genes (Fig. 4A). The mitochondrial cytochrome oxidase subunit I (COI) gene was used in 83% of the reviewed...
studies, possibly reflecting the general use and applicability of this marker across most animal phyla (Hebert, Cywinska, Ball, & DeWaard, 2003; Hebert, Ratnasingham, & Waard, 2003) and as a well-developed “barcoding” system for storage and identification of animal species (Ratnasingham & Hebert, 2007). Other genetic markers included allozymes, additional mitochondrial markers (16S and CytB) and nuclear markers (28S rDNA, ITS, H3, EF1α, ND II and 18S rDNA). Guerra-García et al. (2006) used RAPDs to explore intra- and interspecific genetic differentiation in two species of caprellidean amphipods. Microsatellites were developed for numerous amphipods (Baird, Miller, & Stark, 2012; Rewicz, Wattier, Rigaud, Bacela-Spychalska, & Grabowski, 2015; Villacorta, Canovas, Oromi, & Juan, 2009; Weiss & Leese, 2016), but were never used for taxonomic purposes and are not considered here.

Molecular delimitation of cryptic amphipod species most often relied on a single category of methods; only 30% of the examined studies combined two or three methods (Fig. 4B). In most studies, species were delimited using distance-based methods or using a combination of distance-based and population genetics methods (Fig. 4B). There was a substantial lag in the application of tree-based methods. The generalized mixed Yule-coalescent (Pons et al., 2006), multilocus coalescent (Rannala & Yang, 2003; Z. Yang & Rannala, 2010) and Poisson tree process models (J. Zhang et al., 2013) were used in only seven studies (Copilaș-Ciocianu & Petrusek, 2015; Esmaeili-Rineh, Sari, Delić, Moškrič, & Fišer, 2015; Katouzian et al., 2016; King & Leys, 2011; Murphy, King, & Delean, 2015; Weiss, Niklas, Anna, & Florian, 2014; L. Yang et al., 2013). This lag in the use of tree-based methods occurred despite 78% of the studies inferring molecular phylogenies (supporting information 1). Allele-sharing based methods, e.g., haplowebs (Flot, Couloux, & Tillier, 2010), were used in only two studies (Brad, Fišer, Flot, & Sarbu, 2015; Flot et al., 2014).

Use of different molecular markers and species delineation methods revealed conflicting results about the number of species. Alternative species delimitations suggested by different methods were not in conflict with each other, but differed in the degree of splitting; distance methods typically
turned to be more conservative (Copilaș-Ciocianu & Petrusek, 2015; Delić, Švara, et al., 2017; Delić, Trontelj, Rendoš, & Fišer, 2017; Eme et al., 2017; Katouzian et al., 2016; Weiss et al., 2014). In such cases, authors either provided a rough estimate of species number (Copilaș-Ciocianu & Petrusek, 2015; Weiss et al., 2014), opted for a particular species hypothesis by ranking the efficiency of different methods (e.g., conservative ABGD was preferred over PTP and bGMYC; Katouzian et al., 2016), reconsidered the results within spatial context (presence of syntopy (Delić, Trontelj, et al., 2017)), or drew on other studies to justify the choice of a particular threshold value. Unfortunately, cross-referencing is not always appropriate because the value of thresholds depend on how genetic distances are calculated (e.g., uncorrected p, K2P, patristic distances) (Richards et al., 2012). Moreover, the use of thresholds is case-specific because the rate of substitution differs among taxa (but see Lefébure et al., 2006). Oddly, reasons for the discordant number of species number were never explicitly addressed. Inconsistency among different species delimitation methods is well known, arising due to violations of various assumptions required by the different delimitation methods (Carstens, Pelletier, Reid, & Satler, 2013). Molecular species delimitation methods generally yield congruent results when speciation rates are low, population sizes are small, and the molecular markers used have a high mutation rate (Dellicour & Flot, 2015; Fontaneto et al., 2015). On the contrary, evolutionarily young species with large effective population sizes can be a source of dispute (as alluded to above). A highly promising solution for the issue seems to be genome-wide multilocus coalescence (Fujita, Leaché, Burbrink, McGuire, & Moritz, 2012; Leache et al., 2014), and also confrontation with non-molecular characters as discussed in the next section.

4.2 Confronting species criteria

Ideally, species should be delimited using an integrative approach including genetics, morphology, ecology, behavior and geography (Edwards & Knowles, 2014; Guillot, Renaud, Ledevin, Michaux, & Claude, 2012; Padial et al., 2010). Steps towards integrative taxonomy were made in 55 of the 122
(42%) nominal species examined. To note, integrative exploration of cryptic species complexes often occurred in a series of consecutive publications by a research group. Molecular inferences were mostly supplemented with morphological and ecological data as well (Fig. 4C). Reproductive barriers were explicitly inferred from mating behavior in five cryptic species complexes. Differences in genome size were reported for a single species complex. No biological difference between cryptic species, except genetic ones, was detected in only four of the 55 nominal species (Cothran, Henderson, Schmidenberg, & Relyea, 2013; Meleg et al., 2013; Wellborn & Cothran, 2004).

4.3 Naming cryptic species

In most cases, cryptic species were un-named, even when supported by multiple lines of evidence. The 304 (295-315 depending on the method, excluding nominal species) species of amphipods discovered in the period 2000-2015 represented 17% of the 1822 amphipod species described during the same time period (Fig. 5). Yet, only 21 of these cryptic species were described and named. In other words, DNA-complemented taxonomy contributed only 1% to the total number of described amphipod species. Only two studies relied solely upon DNA sequences to diagnose species (Delić, Trontelj, et al., 2017; Murphy et al., 2015) and one study complemented morphological diagnoses with diagnostic COI sequences (King & Leys, 2011).

4.4 Future challenges for the taxonomy of cryptic species

Taxonomic practices can contribute more to the mechanistic understanding of morphological crypsis as well as to the integration of cryptic species into biodiversity research. Aside from technical issues such as development of barcoding libraries (Morinière et al., 2017), we envision three important issues.
First, testing whether cryptic species are evolutionarily young requires using species delimitation methods that are sensitive enough to detect early divergence, e.g., before evolving species reach reciprocal monophyly. Lineage sorting can take a long time in species with large population sizes (Dellicour & Flot, 2015; Knowles & Carstens, 2007). Haploweb and multilocus coalescent methods are both designed to delimit non-monophyletic species. A major impediment to broaden implementation of the two methods is the low number of nuclear markers exhibiting both suitable variability and low homoplasy (Kornobis & Pálsson, 2011, 2013; Nahavandi, Ketmaier, & Tiedemann, 2012). Among amphipods, only two markers (ITS, EF1α) apparently meet these conditions (Flot, Wörheide, & Dattagupta, 2010; Nahavandi et al., 2012). Fortunately, high-throughput sequencing technologies are becoming readily accessible and affordable, thus multilocus species delimitation may become standard within the next few years (Raupach, Amann, Wheeler, & Roos, 2016) and the recent divergence hypothesis may increase in importance (see 3.1).

Second, taxonomy can strengthen the link between species hypotheses and the speciation process or, more generally, between taxonomic practice and evolutionary theory (de Queiroz, 2007). Only practices grounded in theory can fully appreciate the heterogeneous nature of speciation, and appropriately confront multiple data (morphology, ecology, physiology, multiple DNA) to unveil the full spectrum of divergent processes. This strengthening is a major step towards discovery of general rules in the speciation process (Butlin et al., 2012) and linking speciation with other fields in biodiversity science (section 5). Further, understanding why some lineages genetically diverge without morphological difference, or conversely, why some genetically nearly-identical populations ecologically differ (Herrel et al., 2008) can increase taxonomy rigor. Some taxonomists consider species hypotheses supported by multiple lines of evidence as more robust than single line evidence (Padial et al., 2010). Yet incongruences among data can yield species hypotheses as robust as those supported by congruence as long as incongruences have sound evolutionary explanations.
Third, novel species, no matter if morphologically cryptic, can be described and named (Jörger & Schrödl, 2013; Pante et al., 2015). This practice is one way to communicate cryptic species with other fields of biodiversity science that use Linnaean binomials in their research. It is paradoxical that cryptic species remain nameless until described based on morphological criteria. Finding morphological diagnostic traits among morphologically cryptic species is challenging (C. Fišer & Zagmajster, 2009; Schlick-Steiner et al., 2010) and the practical value of such diagnoses may be questionable (Jugovic et al., 2012). Indeed, The International Code of Zoological Nomenclature (http://iczn.org/code, ICZN ) does not limit the diagnosis of species to the use of morphological traits. The idea of diagnosing species using DNA sequences is not new (Goldstein & DeSalle, 2011; Knowlton, 1993) but descriptions relying only on molecular data are generally still rare (Clouse & Wheeler, 2014; Cook et al., 2010; Edgecombe & Giribet, 2008; Halt, Kupriyanova, Cooper, & Rouse, 2009; M. S. Harvey, Berry, Edward, & Humphreys, 2008; Jörger & Schrödl, 2013; Murphy et al., 2015; Renner, 2016; Strand & Sundberg, 2011). All these studies broadly agree that holotypes should be defined and deposited into collections. Technical aspects vary on how species are diagnosed (Goldstein & DeSalle, 2011; Renner, 2016) and recent works recommend two technical details. First, diagnosis should rely on diagnostic substitutions rather than genetic distances (i.e., nodes in phylogeny). Second, detailed alignment construction, sequences and final alignments must be made available to ensure repeatability and continuity in taxonomy (Jörger & Schrödl, 2013; Renner, 2016).

An important corollary of the molecular description of cryptic species is that species retaining the original name need to be molecularly diagnosed as well. This step may require designation of neotypes or co-types that can replace or supplement holotypes if the latter are lost or improperly preserved for DNA analysis. Finally, formal naming sometimes is not considered desirable when species hypotheses need to be strengthened with additional lines of evidence of lineage separation. In such cases, Morard et al. (2016) proposed an interim nomenclature system to codify genetically circumscribed entities, including potential cryptic species, as an effort to communicate data on
5. CRYPTIC DIVERSITY IN BIODIVERSITY RESEARCH

5.1 Biodiversity patterns revisited and consequences for nature conservation

Following the plea by Bickford et al. (2007) to account for cryptic species in biodiversity science, we now revisit a number of biodiversity-related issues. Notably, the number of examined studies strongly depended on the scale of the analysis.

5.1.1 Global-scale analyses

Global-scale analyses are few. Pfenninger and Schwenk (2007) conducted a meta-analysis of cryptic species to test for variation in the proportion of cryptic species across major metazoan phyla and biogeographical regions. The authors found that cryptic species were homogeneously distributed among taxa and regions, and concluded “that cryptic metazoan diversity can be treated as random error in biodiversity assessments”. Their meta-analysis was later criticized for several methodological problems (Trontelj & Fišer, 2009), and additional analyses almost a decade later suggested that cryptic species actually may be heterogeneously distributed across animal phyla (Pérez-Ponce de León & Poulin, 2016). The evidence for homogenous distribution of cryptic species across geographic regions is conflicting (Eme et al., 2017; Gill et al., 2016; Voda, Dapporto, Dinca, & Vila, 2015); perhaps an heterogeneous distribution can be detected only at global geographic scales.

Finding that cryptic species are homogeneously distributed across space does not necessarily imply that the mechanisms causing cryptic species are themselves invariant. These mechanisms can compensate each other across space to produce an even distribution of cryptic species. An untested
hypothesis is that selectively neutral mechanisms (e.g., the recent divergence hypothesis) predominate in regions where recent climatic perturbations fragmented ranges of ancestral species (e.g., Pleistocene glaciations, Fišer et al., 2010), whereas selection-based mechanisms (PNC, morphological convergence) operate more frequently in environments with strong directional selection (Bickford et al., 2007; Trontelj & Fišer, 2009).

It also is unclear why cryptic species are overrepresented in some phyla. Knowlton (1993) suggested that cryptic species may be more frequent in taxa that do not rely on visual information, but we are not aware of a single study that explicitly addressed this question. Analogous to geographic aspects of cryptic diversity, the relative importance of the three mentioned mechanisms causing morphological crypsis in different taxonomic groups awaits to be quantified and explained. In particular, PNC may well explain the morphological similarity in organisms, the taxonomy of which has been based on functional somatic characters related to ecology (e.g., amphipods: Dahl, 1977; Fišer et al., 2009). In contrast, it may be less important in species delimited essentially on sexual characters, such as isopods or spiders.

5.1.2 Geographically and phylogenetically limited analyses

Most studies are limited either in geographic or phylogenetic scope. In the amphipod literature, these include but are not limited to a species geographic range size (Trontelj et al., 2009), the extent of ecological niche overlap and species coexistence (Ž. Fišer, Altermatt, Zakšek, Knapič, & Fišer, 2015), the strength of size-assortative pairing (Galipaud et al., 2015), the effectiveness of conservation measures (Venarsky, Anderson, & Wilhelm, 2009), and the use of biological indicators in ecosystem health assessment (Feckler et al., 2014; Feckler, Schulz, & Bundschuh, 2013; Feckler, Thielsch, Schwenk, Schulz, & Bundschuh, 2012; Major, Soucek, Giordano, Wetzel, & Soto-Adames, 2013; Soucek, Dickinson, Major, & McEwen, 2013; Weston et al., 2013; Zettler et al., 2013). Notably,
all these studies consider cryptic species as a source of error that must be accounted for when documenting and explaining biodiversity patterns.

The question of whether the range size of cryptic species is smaller than that of corresponding morphological species was addressed explicitly in amphipods from Antarctic deep-sea (Baird et al., 2012; Lörz, Maas, Linse, & Coleman, 2009), desert springs Australia and North America (Murphy, Adams, Guzik, & Austin, 2013; Witt, Threlfoll, & Hebert, 2006) and groundwater amphipods from Australia, Canary Islands and Europe (e.g., Bausa-Ribot, Jaume, Fornós, Juan, & Pons, 2011; Bradford et al., 2013; Bradford, Adams, Humphreys, Austin, & Cooper, 2010; Meleg et al., 2013; Trontelj et al., 2009a; Villacorta et al., 2008). In general, large range sizes of morphological species were “symmetrically” divided into a series of smaller ranges for cryptic species. Yet, some studies reported “asymmetrical” fragmentation where the range of at least one cryptic species was as large as that of the morphological species (Havermans, Nagy, Sonet, De Broyer, & Martin, 2011; Lefébure et al., 2006). Whether symmetrical and asymmetrical fragmentations of morphospecies ranges are homogeneously distributed across geographical regions has yet to be determined. One study indicates, however, that consideration of cryptic species does not affect continental scale patterns of range size, the so-called Rapoport effect (i.e., the pattern of increasing range size of morphological species at higher latitudes in the Palearctic region; Stevens, 1989) (Eme et al., 2017).

Another important aspect is whether morphologically cryptic species have the same ecological requirements (i.e., Grinnellian niche) and play similar roles in ecosystems (i.e., Eltonian niche) (Ž. Fišer et al., 2015). Almost all detailed ecological studies showed that cryptic species differed in their requirements along one or more dimensions of their Grinnellian niche (Colson-Proch, Renault, Gravot, Douady, & Hervant, 2009; Eisenring, Altermatt, Westram, & Jokela, 2016; Krebes, Blank, & Bastrop, 2011; Krebes, Blank, Jürrss, Zettler, & Bastrop, 2010; Rock, Ironside, Potter, Whiteley, & Lunt, 2007). Apparently, cryptic species may not be cryptic to other community members. Studies on cryptic Hyalella species imply different vulnerabilities to fish predation (Cothran, Henderson, et al.,
Westram et al. (2011) showed different susceptibilities to acanthocephalan parasites among cryptic species of the *Gammarus fossarum* species complex, and Colson-Proch et al. (2009) found substantial differences in behavioral, metabolic and biochemical responses to cold among cryptic species of the subterranean amphipod *Niphargus rhenorhodanensis*. Ecotoxicological tests further revealed large differences in sensitivity to a number of pollutants across a range of cryptic taxa (Feckler et al., 2012; Feckler et al., 2014, 2013; Major et al., 2013; Soucek et al., 2013; Weston et al., 2013). Interpretation of the magnitude of these differences in an ecological context is highly challenging but studies on co-occurrence of cryptic species indicate they are insufficient to mediate stable coexistence (Box 2).

Cryptic species having a roughly similar role in the ecosystem (Eltonian niche) but differing in their environmental requirements and responses to various stressors may be functionally redundant. If so, cryptic species may be critically important in stabilizing ecosystem functioning (Elmqvist et al., 2003; E. Harvey, Gounand, Ward, & Altermatt, 2017; Scheffer et al., 2015). To our knowledge, a single study using cryptic nematodes as a model system explored differences in the functional role of cryptic species (De Meester, Gingold, Rigaux, Derycke, & Moens, 2016). A microcosm experiment by these authors unveiled species-specific effects on organic matter decomposition, challenging the hypothesis of functional redundancy.

Results from biogeographic and ecological studies are difficult for nature conservation. First, range sizes of many species are much smaller than previously conceived. Species with small range sizes are much more vulnerable to external disturbances than broadly distributed species and even local perturbations can lead to biodiversity loss (Bland & Collen, 2016; Delić, Trontelj, et al., 2017). Second, ignoring the vulnerability of cryptic species to multiple stressors can underestimate threats to local populations and ultimately biodiversity. Niche models forecasting the future of aquatic cryptic species, for example, indicate that global climate change will cause the loss of species along with genetic diversity throughout Europe (Bálint et al., 2011). There is an urgent need for assessing
cryptic diversity to reduce the potential loss from environmental change. One step in this direction may be predictive phylogeographies. These use environmental data and known phylogeographies of co-distributed taxa to predict the presence of cryptic species in other taxa in the same biome (Espíndola et al., 2016). In conjunction with molecular operational units (discussed below), these rapid assessments of cryptic diversity can assist towards improving policies and decision making in conservation.

5.2 Molecular operational taxonomic units

Cryptic species are *de facto* taken into consideration by an increasing number of biodiversity studies that use sequence clusters rather than nominal species as units for taxonomic diversity (Ryberg, 2015). Such clusters, referred to as molecular operational taxonomic units (OTUs, also referred as MOTUs), are delimited using methods presented in Section 2. In the amphipod literature, OTUs were used to explore determinants of diversification rates (Hou, Sket, Fišer, & Li, 2011; Mamos, Wattier, Burzyński, & Grabowski, 2016; L. Yang et al., 2013), the geographic origin of European groundwater fauna (McInerney et al., 2014), and species diversity-environment relationships of local assemblages (Knox et al., 2012). Importantly, failing to account for cryptic diversity in these studies is equivalent to omitting known species; thereby underestimating diversification rates, incorrectly reconstructing ancestral ranges, and undervaluing local diversity (Morvan et al., 2013; Pyron & Burbrink, 2013). Inferences about eco-evolutionary processes and patterns drawn from OTUs are free from biases due to morphological crypsis, although still being dependent on the accuracy of molecular delimitation methods.

The use of OTUs in diversity analyses can be viewed as a logical extension of de Queiroz’s (1999) view that each biologist can select species properties that are most relevant to the question being addressed. OTUs cannot be extended across all fields of biodiversity science with similar success,
although they may become mandatory in ecology in the near future (Gill et al., 2016). Neglecting cryptic species can bias inferences derived from large-scale patterns of phylogenetic diversity and phylogenetic turnover, especially when the proportion of convergently evolved cryptic species is high (Fritz & Rahbek, 2012; Safi et al., 2011). However, applying this approach to conservation biology (Asmyhr, Linke, Hose, & Nipperess, 2014) and local community ecology (Elbrecht & Leese, 2015; Jones, Ghoorah, & Blaxter, 2011) can be risky. OTUs of unknown vulnerability to different threats lack protection, and their ecological role in local assemblages cannot be inferred from phylogenetic relatedness alone (Kembel, 2009; Best & Stachowicz, 2013, 2014; Best, Caulk, & Stachowicz, 2013).

5.3 Making use of different species criteria

Using OTUs and treating cryptic species as a source of accountable bias are complementary approaches for integrating cryptic diversity into biodiversity science. However, neither satisfactorily integrates the various properties of speciation towards a better understanding of biodiversity patterns. We now discuss how a broader use of species properties in testing eco-evolutionary hypotheses can foster our understanding of large-scale biodiversity patterns.

The regional number of species is ultimately determined by three processes – speciation, extinction and dispersal (Wiens, 2011). Further, the contribution of speciation to species richness and the relative importance of different speciation properties can vary across geographical regions and in time (Jablonski, Roy, & Valentine, 2006). Thus, geographic variation in the proportion of morphologically cryptic (OTUs) and morphologically distinguishable species could pinpoint (hypothesis driven) how environmental factors contribute to species richness patterns via variation in species properties during speciation. An underlying hypothesis is that morphological and ecological distinctness can be acquired much faster along a resource gradient with varying selection
pressures (ecological speciation) than from a physical barrier with similar selection pressures in each environment (non-ecological speciation, perhaps driven by PNC; Wiens et al., 2010; Pyron et al., 2015). An important point here is that these hypothesis-driven predictions can be tested by integrating different species criteria into ecological analyses (Fig. 6).

One step towards this testing consists of building occurrence databases in which thousands of specimens collected across large geographic regions can be associated to multiple species hypotheses based on morphology and multiple DNA-based criteria (Fig. 6). Note that species hypotheses can be refined as more data and methods become available (e.g., new genes, ecological data). Ultimately, one can assess the relative importance of different mechanisms explaining species richness patterns for the different species hypotheses. In our example (Fig. 6), the relative importance of spatial heterogeneity, present climate/productivity, and historical climate variability for explaining biodiversity patterns is assessed using multi-model inferences and variance partitioning (see for example Eme et al., 2015, 2017). For illustration, DNA-based criteria increase the proportion of geographic variation in species richness attributed to spatial heterogeneity due to the disproportionately higher number of allopatric cryptic species in highly fragmented regions. In fact, this approach can contribute towards understanding how mechanisms causing cryptic diversity vary in strength relative to each other across taxa and geographical regions.

Hey et al. (2003) used the term “species uncertainty” to denote the ambiguous correspondence between a taxon delimited using any criteria and the evolving entity for which it is used in a hypothesis. The authors argued that “this scientific uncertainty cannot be ‘solved’ or stamped out, but neither need it be ignored or feared”. Recent progress made in conceptualizing and delimiting species notably opens the door for broader consideration of this uncertainty in hypothesis testing of eco-evolutionary theories in biodiversity science.
6. CONCLUSIONS

(1) Cryptic species refer to a heterogeneous and negatively-defined set of species lacking morphological distinguishability. Evolutionary mechanisms leading to morphological similarity are heterogeneous, comprising recent divergence, niche conservatism and morphological convergence. They are as important and fascinating as mechanisms leading organisms to diversify into a multitude of phenotypes. We are only just beginning to understand the ‘invisible’ world of cryptic species. We now need explicit testing of whether morphological traits evolve neutrally, are constrained by stabilizing selection, or converge from distantly related lineages in response to similar directional selection on morphology. At present, the relative importance of different explanations of cryptic diversity remains largely unknown.

(2) Taxonomy can provide evidence for evolutionary explanations if research efforts are oriented towards species hypotheses that are theoretically and empirically grounded in the speciation process. DNA taxonomy provides an ever-increasing variety of methods for delimiting cryptic species, and be used to explore species boundaries related to morphology, ecology, and physiology. Comprehensive analyses of multiple types of data can unveil generalities in the speciation process, improve our understanding of the heterogeneity (species properties) in speciation, and allow better integration in biodiversity science.

(3) Cryptic species are often considered in biodiversity research as evidence of incomplete species inventory or as potential sources of bias. Paradoxically, the majority of known cryptic species are undescribed or un-named, and thus overlooked in research fields relying on species binomials. Studies in evolution and ecology increasingly use molecular operational taxonomic units (OTUs), thereby inherently incorporating cryptic species into their analyses, but no study to date has used multiple criteria for species delineation. Extending multi-criteria approaches to different fields of biodiversity science can better integrate cryptic species in ecological and evolutionary theories, paving the way to new frontiers in biodiversity science. For example, are biodiversity patterns
inferred from morphologically and DNA delimited species discordant? If so, is discrepancy a contingency to ecological and processes generating cryptic species? How do different mechanisms causing cryptic diversity vary in strength relative to each other across geographical space, and environmental gradients? And lastly, do coexistence mechanisms at local scales change as lineage divergence proceeds?

(4) The phenomena of cryptic and ecological species can be parallel. Importantly, cryptic species clearly complement research on speciation. We do envision that with the development of new methodologies, cryptic species pervasiveness within the broader context of biodiversity science will likely fade over time and be viewed by the next generation of scientists as a constructive epiphenomenon towards integrating speciation and its heterogeneity (species properties) into biodiversity science. This integration necessarily must include integrative taxonomy and the use of multiple species criteria, as already evident in basic biodiversity research. Importantly, shifts to integrative taxonomy and multi-criteria have profound implications in terms of forecasting and consolidating new skills and occupations in biodiversity assessment and conservation. In the future, conservationists and policy-makers must be prepared to make informed decisions using a more complex but novel body of knowledge on species diversity and distribution. For example, this information may include continental maps of species richness and geographic range size using different species delimitation methods that can pinpoint regions in method incongruences. These incongruences then will reveal geographic variation in the speciation process. Thus, better understanding of the causes of this variation in speciation can reinforce process-oriented conservation strategies. Lastly, incongruences that cannot be readily explained can promote the development of adaptive conservation principles and public policies that explicitly acknowledge the scientific uncertainty of species entities.
AUTHOR CONTRIBUTION

C.F. gathered the literature data, all authors contributed original ideas and helped draft the paper.

CONFLICT OF INTERESTS

Authors declare no conflict of interest.

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Table 1. Classification of methods used in DNA taxonomy to delimit cryptic species of amphipods

<table>
<thead>
<tr>
<th>Methods</th>
<th>Delimitation criteria</th>
<th>Examples of analyses / programs</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Distance-based</td>
<td>Intraspecific genetic variation &lt;&lt; interspecific variation</td>
<td>Fixed molecular threshold, automated barcoding gap detection</td>
<td>Lefébure et al., 2006; Puillandre et al., 2012</td>
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<td>methods</td>
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<tr>
<td>Tree-based</td>
<td>Point of transition from species-level to population-level processes</td>
<td>GMYC, PTP, BP&amp;P, BFD, BFD*, SpedeSTEM, Brownie</td>
<td>Pons et al., 2006; O’Meara, Ence &amp; Carstens, 2011; Grummer, Bryson &amp; Reeder, 2014; Yang &amp; Rannala, 2010</td>
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<tr>
<td>methods</td>
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<tr>
<td>Allele-sharing</td>
<td>Mutual allelic exclusivity</td>
<td>Haplowebs</td>
<td>Flot et al., 2010</td>
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<td>based</td>
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<tr>
<td>Population</td>
<td>Reduced gene flow among populations</td>
<td>Nested clade analysis, differentiation statistics</td>
<td>Delimit differentiated populations rather than species</td>
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<td>genetics</td>
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GMYC: Generalized mixed Yule-coalescent models; PTP: Poisson tree processes models, BP&P: Bayesian phylogenetics & phylogeography; BFD: Bayes factor delimitation (BFD* for genomic-scale data).
BOX 1 Amphipods as a model for study of cryptic species

Amphipods (Crustacea: Malacostraca: Peracarida) consist of ca. 10,000 species that are classified into six orders (Horton et al., 2016; Väinölä et al., 2008). There is no comprehensive molecular phylogeny of amphipods and clade-specific phylogenetic studies indicate that morphology alone neither accurately depicts species diversity nor phylogenetic relationships (Corrigan, Horton, Fotherby, White, & Hoelzel, 2013; C. Fišer, Sket, & Trontelj, 2008; Hou et al., 2011; Lörz & Held, 2004; Verheyen, Backeljau, & d’Udekem d’Acoz, 2016). Species-level taxonomy is demanding. Amphipods do not have copulatory apparatus and the species were traditionally delimited on a basis of different appendage shapes, body shapes and setal patterns; reproductive barriers were rarely if ever studied. Within-population variation is often high in contrast to small between-species differences.

Amphipods thrive in most aquatic habitats and some even inhabit terrestrial habitats (Ketmaier & Pavesi, 2013) (Fig. 2). Many species (80%) are marine, living in the supra-littoral zone to the hadal zone, where they represent an important part of the community (Jamieson, Fujii, Mayor, Solan, & Priede, 2010). About 45% of freshwater species are exclusive to groundwater (Väinölä et al., 2008), a highly constrained environment that has long been thought to promote morphological stasis and convergence (Lefébure et al., 2006). Most amphipods are benthic, however, and one order (Hyperiida) is pelagic. Benthic species display highly diverse ecologies (e.g., free-roaming species, burrowers, tube builders, sedentary ambush-predators, and commensals on whales, mollusks and bryozoans) and use a broad range of feeding strategies (e.g., filter feeding, shredding, scavenging and predation) (Alexander, Dick, & O’Connor, 2013; Macneil, Dick, & Elwood, 1997). Local abundances can be high; hence they are also a key link in food webs (Pinchuk, Coyle, Farley, & Renner, 2013). Lastly, amphipods are routinely used in ecotoxicological tests (Feckler et al., 2012; Marmonier et al., 2013), the results of which may be biased if model species contain cryptic species with different sensitivities to pollutants.
In the absence of free pelagic larvae, most amphipod species have limited distributional ranges (Myers & Lowry, 2009). Yet, some tens of species, including some cryptic species (Cristescu, Witt, Grigorovich, Hebert, & Maclsaac, 2004; Pilgrim, Blum, Reusser, Lee, & Darling, 2013; Pilgrim & Darling, 2010), have substantially expanded their distributional ranges in recent decades (Grabowski, Bacela, & Konopacka, 2007; Jazdzewski, Konopacka, & Grabowski, 2004). Some amphipods are invasive species and pose a threat to native fauna (Dick & Platvoet, 2000; Dodd et al., 2014).

Amphipods are an ideal model organism for linking evolutionary processes contributing to cryptic diversity with local ecological dynamics, global biodiversity patterns and applied ecology. Cryptic species have been found in all ecological groups of amphipods. The number of cryptic species within nominal species varies up to several tens of species (Havermans, 2016; Katouzian et al., 2016; Mamos et al., 2016; Witt et al., 2006). Ecological diversity of amphipods allows for testing generalities in processes generating cryptic diversity under different ecological settings. Limited distributional ranges at the species level coupled with ubiquitous distribution at the genus level provide natural replicates for analysis. As a key functional group in ecosystems, amphipods naturally link the issue of morphological evolution with local ecological dynamics, ecosystem functioning and nature conservation.

**BOX 2. Co-occurrence of cryptic species**

Knowlton (1993) suggested that sympatry and local co-occurrence is common among cryptic species in the sea. Recent studies from many animal phyla largely support this view; no less than 20% of the cryptic amphipod species were found to live in sympatry or even syntopy.

Assuming that cryptic species significantly overlap in their ecological niche space, niche differentiation cannot explain their coexistence (Chesson, 2000). Several studies addressed this issue using various model organisms, including rotifers (Montero-Pau, Ramos-Rodríguez, Serra, & Gómez,
2011; Montero-Pau & Serra, 2011; Ortells, Gomez, & Serra, 2003), nematodes (De Meester, Derycke, Bonte, & Moens, 2011; De Meester et al., 2016; Derycke et al., 2008), different amphipods (Cothran, Henderson, et al., 2013; Cothran, Noyes, & Relyea, 2015; Cothran, Stiff, Chapman, Wellborn, & Relyea, 2013; Dionne, Vergilino, Dufresne, Charles, & Nozais, 2011; Eisenring et al., 2016; Ž. Fišer et al., 2015; Wellborn & Cothran, 2004), chironomids (Pfenninger & Nowak, 2008), bugs (Saleh, Laarif, Clouet, & Gauthier, 2012), bumble bees (Scriven, Whitehorn, Goulson, & Tinsley, 2016), fig wasps (D. Y. Zhang, Lin, & Hanski, 2004) and bats (Ashrafi, Beck, Rutishauser, Arlettaz, & Bontadina, 2011; Nicholls & Racey, 2006; Rutishauser, Bontadina, Braunisch, Ashrafi, & Arlettaz, 2012). These studies unveiled emerging commonalities in co-occurrence patterns and unsolved issues with important implications for nature conservation.

With little exception, studies showed that co-occurring cryptic species are ecologically differentiated. The degree of species differentiation varies from case to case. One study showed strong differential preferences for foraging habitat (Nicholls & Racey, 2006), whereas other cases found differences are more subtle (Ž. Fišer et al., 2015; Wellborn & Cothran, 2004). Co-occurrence is based on different trade-offs; i.e., i) when species disperse along environmental gradients and locally outcompete each other (Eisenring et al., 2016; Ž. Fišer et al., 2015; Pfenninger & Nowak, 2008; Rutishauser et al., 2012), ii) when temporal fluctuations in environmental factors alter competitive strength of co-occurring species over daily, seasonal or annual scales (Montero-Pau et al., 2011; Montero-Pau & Serra, 2011; Ortells et al., 2003), iii) where selective predation regulates the strength of the stronger competitor (Wellborn & Cothran, 2004, 2007), and iv) when, under various scenarios, heterospecific breeding interference favors population growth when species is rare (Ruokolainen & Hanski, 2016; D. Y. Zhang et al., 2004).

This review brought up a general issue; i.e., whether and how statistically significant differences in biological traits can be translated into ecologically meaningful differences. Some authors attempted to solve the puzzle by testing whether species co-occurrences are random or non-randomly more
frequent/rare with respect to distributional data (Ž. Fišer et al., 2015; Pfenninger & Nowak, 2008; Rutishauser et al., 2012; Saleh et al., 2012). The best evidence for coexistence was a demonstration that a species’ population can grow if initially present in the system at low abundance (Siepielski & McPeek, 2010). This test requires laboratory controlled experiments that are, in the case of cryptic species, quite demanding. We are aware of only two model systems that tested for co-existence under laboratory conditions. In the amphipod *Hyalella*, none of the three co-occurring species showed positive population growth when they were introduced at low abundance in experimental mesocosms (Cothran et al., 2015). In contrast, interactions among species of the nematode *Pseudoditis marina* either facilitated or suppressed population growth (De Meester et al., 2011). An ambiguous relationship between the degree of species differentiation and population growth in a community has important implications for many questions; e.g., can we predict species responses to environmental change (Sinclair et al., 2016). This question extends beyond the issue of co-occurring cryptic species. For example, if local factors mediate long-term co-occurrence of species which outcompete each other in mesocosms, it can be expected that altered environmental conditions may reshape ecological space and intensify competitive interactions between initially non-competing species (Lortie & Callaway, 2006; Poisot, Canard, Mouillot, Mouquet, & Gravel, 2012).

**Glossary**

**Brownian motion:** A model of trait evolution, sometimes called random walk, which assumes neutral and gradual evolution of traits due to genetic drift. Change in mean phenotype is expected to be non-directional and occurs at a constant rate, whereas variance among species is linearly related to the amount of time since divergence.
Orstein-Uhlenbeck model: A model of trait evolution, sometimes also called a “rubber-band”, which is an alternative model describing the response of a phenotype to stabilizing selection. Evolutionary changes are constrained by a constant force towards an adaptive optimum.

White noise model: A model of trait evolution representing a process in which variation of phenotypic traits revolves constantly around moving trait optima, which generates evolutionary phenotypic change that is independent from phylogenetic relationships.

Morphological crypsis: Extreme similarity of morphological phenotypes of two or more species. In this review we use this term for those species whose morphological differentiation cannot be told apart without prior molecular analysis.

Polyphyletic group: A group of organisms derived from different ancestors.

Monophyletic group: A group of organisms composed of a common ancestor and all its descendant species.

Paraphyletic group: A group of organisms derived from a common ancestor, which excludes some descendants.

Grinellian ecological niche. A concept of ecological niche grounded on species requirements for a specific habitat; a niche is parametrized with conditions in which a species persists and breeds.

Eltonian ecological niche: A concept of ecological niche defined by the place a species takes in its biotic environment; i.e., “its relationship towards food and enemies” and the functional role it plays in the ecosystem.

Integrative taxonomy: An interdisciplinary approach in delimiting species, based on confrontation of genetic, morphological, behavioral and ecological evidence of lineage separation.

Species concept vs. species taxon: Several authors argued that much of the confusion related to the term species is of semantic nature. The “species concept” treats species as evolutionary entities. “Species taxon” has practical value. It is an elementary unit in biodiversity
research, and individuated by delimitation methods. The correspondence between species concept and species taxon is unclear, and depends on the context of speciation. For this reason the “species taxon” is a hypothesis of the evolutionary entities emerging through the process of speciation.

Figures

**FIGURE 1** The three sections of a research agenda for integrating cryptic species in biodiversity science. For each section, we provide the main topics addressed (headings) as well as quantitative information retrieved from the literature (italics). Arrows show how the three sections are integrated into biodiversity science. The quest for causal mechanisms directs taxonomic practices. Integrative taxonomy fosters understanding of the evolutionary mechanisms behind morphological crypsis and integration of cryptic species into biodiversity research. The latter may in turn reveal how the three mechanisms contribute to biodiversity across space and environmental gradients.

**FIGURE 2** (as part of BOX 1). A snapshot of Amphipoda diversity. Lyssianasoids (A) live in the deep sea at depths below 5000 m depth; ampeliscids (B) commonly burrow in soft sediments; corophiids (C) are filter feeders that live in self-built tubes; caprellids (D) mimic plants on which they live; leucothoids (E) and cyamids (F), respectively, live in association with sponges and whales; several lines of talitroids (G) successfully colonized semi-terrestrial supra-littoral habitats, while hyperiids (H) live pelagial, often within gelatinose plankton. Cryptic species were found in all these ecological groups. They naturally pose the question whether the relative importance of evolutionary mechanisms underlying morphological crypsis differs across ecological settings. All photos made by Hans Hillewaert and used with permission (licensed under Creative Commons, photos available at https://www.flickr.com/photos/bathyporeia/albums/72157639365477036).
FIGURE 3 Age distribution of 43 cryptic species of amphipods, extracted from 29 out of 120 reviewed publications. Please note that the methods used for estimation of divergence vary between studies, see text.

FIGURE 4 An overview of practices in taxonomy to delimit cryptic species of amphipods. A) Number of molecular markers (n = 76 studies); B) Molecular methods to species delimitation (n = 76 studies); C) Non-molecular data used to test cryptic species hypotheses inferred from DNA taxonomy (n = 55 described species containing cryptic species complexes).

FIGURE 5 Rates of species description and discovery of cryptic species of amphipods. Upper panel shows rates of species description between 2000 and 2015 (source: Amphipoda Newsletters vol. 30-39). Middle panel shows cryptic species discovery rate (source Supplementary Information) over the same time period. Lower panel shows that the proportion of discovered but undescribed cryptic species increases through time.

FIGURE 6 A general methodology for integrating multiple species criteria into the analysis of large-scale biodiversity patterns. Individuals can be assigned to different species hypotheses according to different criteria. Alternative datasets can be mapped in space, and regions where different speciation mechanisms operate can be pinpointed and tested for causal environmental factors using multi-model inferences and variance partitioning. PTP: Poisson tree processes models; GMYC: generalized mixed Yule-coalescent approaches; BP&P: Bayesian phylogenetics & phylogeography; AICc: Akaike’s information criterion corrected for small sample size.
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