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Global analysis reveals that cryptic diversity is linked with habitat but not mode of life

R. POULIN* & G. PÉREZ-PONCE DE LEÓN†

*Department of Zoology, University of Otago, Dunedin, New Zealand
†Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad Universitaria, México, D.F., México

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Abstract

The ubiquity of genetically distinct, cryptic species is limiting any attempt to estimate local or global biodiversity as well as impeding efforts to conserve species or control pests and diseases. Environmental factors or biological traits promoting rapid diversification into morphologically similar species remain unclear. Here, using a meta-analysis of 1230 studies using DNA sequences to search for cryptic diversity in metazoan taxa, we test two hypotheses regarding the frequency of cryptic taxa based on mode of life and habitat. First, after correcting for study effort and accounting for higher taxonomic affinities and biogeographical region of origins, our results do not support the hypothesis that cryptic taxa are more frequent among parasitic than free-living taxa. Second, in contrast, the results support the hypothesis that cryptic taxa are more common in certain habitats than others: for a given study effort, more cryptic taxa are found in freshwater than in terrestrial or marine taxa. These findings suggest that the greater heterogeneity and fragmentation of freshwater habitats may promote higher rates of genetic differentiation among its inhabitants, a general pattern with serious implications for freshwater conservation biology.

Introduction

Mounting evidence indicates that cryptic species, that is genetically distinct but morphologically similar species, are everywhere (Bickford *et al.*, 2007). Their existence challenges all current estimates of total biodiversity (Scheffers *et al.*, 2012; Loxdale *et al.*, 2016). Cryptic species may also have distinct ecological traits, show divergence in their ecological niches and play functionally different roles in ecosystems (Bickford *et al.*, 2007; Rissler & Apodaca, 2007). The presence of cryptic species masks host–symbiont specificity and limits our ability to assemble accurate species interaction networks (Poulin & Keeney, 2008; Prada *et al.*, 2014; Nilsson *et al.*, 2016). Our inability to distinguish among different biological units can also have serious practical implications. For instance, it could lead to misdirected efforts to conserve species, eradicate invasive species or control pests and diseases (Armstrong & Ball, 2005; Pringle *et al.*, 2005; Witt *et al.*, 2006; Pérez-Ponce de León & Nadler, 2010; Arthur *et al.*, 2011; Nadler & Pérez-Ponce de León, 2011; Burger *et al.*, 2014). However, there has been no serious attempt at identifying the lifestyles or environmental conditions propitious for the formation of cryptic taxa.

The occurrence of cryptic species within a genus may represent morphological stasis among related species experiencing similar environmental conditions, but it may also reflect frequent, recent and/or rapid speciation (Cooke *et al.*, 2012). This is a plausible assumption, as the degree of morphological differentiation between related species is generally proportional to the time since their divergence and often minimal between recently formed sister species or during incipient speciation (e.g. Knowlton, 1993; Bond *et al.*, 2001; Derkarabetian *et al.*, 2011). Therefore, the occurrence of cryptic diversity may be associated with various factors driving broadscale patterns of diversification. These factors include intrinsic properties of organisms and

Correspondence: Robert Poulin, Department of Zoology, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand.
Tel.: +643 479 7983; fax: +643 479 7584;
e-mail: robert.poulin@otago.ac.nz

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environmental variables that can act to promote diversification in certain taxa more than in others (Bloom *et al.*, 2013). For instance, the well-established latitudinal gradient in species diversity (Willig *et al.*, 2003; Hillebrand, 2004; Chaudhary *et al.*, 2016) may result in cryptic species being more frequent among tropical taxa than among those of colder regions. However, the spatial distribution of reports of cryptic species, once corrected for area and study effort, is quite homogeneous among biogeographical regions of the world, at least for metazoans (Pfenninger & Schwenk, 2007). Additionally, the vast differences in body sizes and life history traits among higher taxa may cause variation in rates of diversification and possibly also in the occurrence of cryptic taxa. Indeed, some have argued that cryptic species are particularly common in certain taxa, such as mites (Skoracka *et al.*, 2015) and nematodes (Palomares-Rius *et al.*, 2014). There certainly is some heterogeneity among metazoan higher taxa in the rates at which cryptic species are reported, after accounting for their known species richness and for study effort (Poulin, 2011; Pérez-Ponce de León & Poulin, 2016).

Here, we test two hypotheses regarding the determinants of cryptic species occurrence, both based on the assumption that cryptic diversity generally results from rapid and/or recent speciation not yet detectable phenotypically. First, we hypothesize that organisms with a parasitic mode of life include more cryptic taxa than free-living ones. Inevitably, parasites are smaller than their hosts and have shorter generation times, which should promote higher rates of diversification (de Meeûs *et al.*, 1998). Their dispersal abilities are at most equal to those of the most mobile of their hosts, for example a vertebrate host in the case of helminths with complex life cycles. Although not true in all cases, the mobility of parasites is usually lower, increasing the likelihood of genetic structure among spatially separated populations (Mazé-Guilmo *et al.*, 2016). Also, within a diverse host community, individuals of a parasite population may undergo specialization for different host species. This phenomenon is more likely to serve as a prelude to speciation than diet specialization in free-living animals due to the much more intimate association between hosts and parasites than that typical of other trophic relationships, although undersampling may overestimate the host specificity of parasites (Costello, 2016). A study on mites found no difference between the probability of cryptic species occurrence between mite superfamilies that contain at least some parasitic or symbiotic species and those that do not (Skoracka *et al.*, 2015). However, a larger-scale test of the hypothesis is lacking.

Second, we hypothesize that the habitat in which organisms live will influence the frequency of cryptic species formation. More specifically, we expect that cryptic taxa are more common among freshwater organisms than among marine ones. Disparities in species richness between habitats suggest that ecological factors influence net rates of diversification (May, 1994; Grosberg *et al.*, 2012; Wiens, 2015). For instance, freshwater, terrestrial and marine environments have very different total areas (approximately 2%, 28% and 70% of Earth's surface, respectively), yet for many higher taxa like fishes, freshwater and marine environments have similar overall species richness (Wiens, 2015). This is true for free-living species, but may also apply to at least some types of parasites, with numbers of species per genus being higher in freshwater than marine parasite assemblages (Poulin, 2016). Marine habitats are open, with no hard barriers to dispersal, which generally, although not always, promotes weak genetic differentiation across large scales (Palumbi, 1994). In contrast, the fragmented nature of freshwater habitats, leading to greater habitat heterogeneity and geographical isolation, may explain the disproportionately high diversification of many, although not all, freshwater taxa (Grosberg *et al.*, 2012; Bloom *et al.*, 2013; Wiens, 2015). If the nature of freshwater habitats enhances diversification rates, then we may expect a greater frequency of recently diverged species with weak morphological differentiation in freshwater than in marine habitats. Terrestrial habitats, with their greater net primary productivity but weaker barriers to dispersal (Grosberg *et al.*, 2012; Wiens, 2015), may lie between marine and freshwater habitats with respect to frequency of cryptic taxa.

Here, we test these two hypotheses by conducting a meta-analysis of all published studies that used DNA sequences to search for cryptic species of metazoans. Our analysis controls for study effort and taxonomic and biogeographical influences, to reveal the effect of habitat and mode of life on the occurrence of cryptic taxa and provide the first global test of two potentially fundamental correlates of unrecognized biodiversity.

Materials and methods

The general definition of cryptic species, that is genetically distinct but morphologically similar species, is here replaced by a more precise operational definition. We define 'cryptic species' as genetically distinct taxa previously not recognized and first uncovered through genetic analysis. The level of genetic difference (i.e. % base-pair differences) required for a taxon to be considered as a 'cryptic species' distinct from already known species varies across studies, based on the higher group under study or the authors' personal views. The number and type of genetic markers used and the approaches followed also varied across studies. Here, we assumed that any such differences are randomly distributed across studies, and we accepted the expertise of the authors of the original studies and used their estimate of the number of cryptic taxa in their samples. Whether or not morphological differences were later

found between cryptic taxa and the originally known ones, and whether or not the cryptic taxa were subsequently described formally and given Latin names, by the same or different authors, is not relevant to our study, and no effort was made to chase this information. Many species do remain in taxonomic crypsis by never receiving a proper description (see Schlick-Steiner *et al.*, 2007); however, our focus is strictly on how many 'cryptic species' are revealed at the time of their discovery via genetic analysis.

We conducted an exhaustive search of the literature in the main collection of the ISI Web of Science™ for the period 1978–2015, to gather data from published studies on cryptic species, with an initial search using the terms: 'cryptic speci*' OR 'cryptic linea*' OR 'cryptic tax*' OR 'sibling species' in either the title, abstract or keywords of papers. The total of 7551 entries recovered was further narrowed down to 6054 articles by refining the search to only retain studies on members of the kingdom Animalia (= Metazoa) (see Supporting Information for further search details). Each record was then checked individually to eliminate all remaining nonrelevant articles and retain only true reports of cryptic species. For a study to qualify, it had to use DNA sequence data to discover or recognize cryptic species. We retained both explicit prospecting studies where searching for cryptic species was the main goal and other studies where the discovery of cryptic species was a by-product of genetic analyses that had other purposes. Also, we retained studies where an attempt was made to identify cryptic taxa using sequence data, but none were found. The final data set includes 1230 studies, published between 1991 and 2015 (available from the Dryad Digital Repository, doi: 10.5061/dryad.1hj46).

For each study, we also recorded (i) the species, genus or family targeted by the study; (ii) the higher taxon to which it belonged, with those consisting mostly of phyla but also classes in the case of diverse groups (Table 1); (iii) the habitat in which it is found, either predominantly terrestrial, freshwater or marine; (iv) its mode of life, either free-living or parasitic, with parasites here including various types of worms (acanthocephalans, cestodes, trematodes, monogeneans, nematodes, leeches, nematomorphs), ectoparasitic arthropods (mites, ticks, fleas, lice, copepods), unionid bivalves (whose juveniles are parasitic on fish gills) and parasitoid wasps and flies, but not phytophagous or haematophagous insects as these have only brief and less intimate interactions with their hosts; (v) the biogeographical region where the study was conducted, that is Afrotropical, Antarctic, Australasian, Boreal, Indo-Malayan, Nearctic, Neotropical, Oceanic, Palearctic or more than one of them; (vi) the year in which the study was published; (vii) the numbers of mitochondrial genes, sequences and base pairs used; (viii) the numbers of nuclear genes, sequences and base pairs used; (ix) whether or not additional molecular markers were used, for instance allozyme electrophoresis, random amplified polymorphic DNA or RAPDs, microsatellites, etc.; and finally, (x) the number of cryptic species detected beyond the originally known species. In other words, the number of cryptic species found was taken as the number of species uncovered excluding the originally named species that were investigated and refers to the net addition to known diversity resulting from the study (see Supporting Information for details). For example, when we report the number of cryptic species in a study as 1, this means that in the original study, what the authors believed represented a single species was in fact a complex of two cryptic species. As one of them retains the species name, the net addition to known diversity is 1 cryptic species. All continuous variables (except the year of publication) were log-transformed prior to analysis.

The number of sequences represents the sum of sequences obtained across different markers, including multiple copies of the same haplotype or genotype, calculated separately for mitochondrial and nuclear genes; it captures both the number of markers and the number of individuals from which DNA was obtained and is used here as a measure of sequencing effort instead of the number of individuals, as the latter varies across markers. We found that the number of genes, the number of sequences and the number of base pairs are all positively correlated with each other across all studies, for both mitochondrial and nuclear genes (see Fig. S1 in Supporting Information). For this reason, we use only the number of sequences to control for study effort in subsequent analyses, as the number of cryptic taxa found tends to increase with the level of effort put into their search (Poulin, 2011).

We tested the determinants of the number of cryptic species found per study (response variable) using generalized linear mixed models with Poisson's error structure. The fixed factors were our two main predictors, habitat (terrestrial, freshwater or marine) and mode of life (free-living or parasitic), as well as the number of sequences obtained as a measure of study effort. Initially, we also included the year of publication and whether or not additional molecular markers were used as fixed factors, but they had no effects of their own and did not change the estimated effects of the other predictors; therefore, they were excluded from the final models. To account for any idiosyncrasies of particular higher taxa and for the fact that cryptic species are reported relatively more frequently in some taxa than others (Pérez-Ponce de León & Poulin, 2016), the higher taxa to which the species studied belonged was included as a random factor. In addition, the biogeographical region where each study was conducted was also included as a random factor, to account for possible spatial variation in the formation of cryptic taxa (Pfenninger & Schwenk, 2007). All analyses were conducted

Table 1 Numbers of studies included in the data set, numbers of cryptic species found, their mode of life and habitat for each higher taxon.

Taxon	No. of studies	No. of cryptic species per study (range)	Total no. of cryptic species	Mode of life*	Habitat†
Acanthocephala	2	2–5	7	P	FW
Amphibia	85	1–16	254	FL	T
Annelida	43	0–9	116	FL, P	FW, M, T
Arachnida	54	0–26	189	FL, P	FW, T
Ascidiacea	9	1–5	22	FL	M
Aves	35	0–24	107	FL	T
Bivalvia	25	1–13	82	FL, P	FW, M
Bryozoa	10	1–10	26	FL	FW, M
Cephalochordata	1	2	2	FL	M
Cephalopoda	7	1–4	10	FL	M
Chaetognatha	4	1–4	9	FL	M
Cnidaria	29	0–9	73	FL	M
Collembola	1	1	1	FL	T
Crustacea	146	0–40	597	FL, P	FW, M, T
Cycliophora	2	2	4	FL	M
Diplopoda	3	1–3	6	FL	T
Echinodermata	23	0–6	63	FL	M
Fish‡	125	0–42	434	FL	FW, M
Gastropoda	56	0–20	147	FL	FW, M, T
Gastrotricha	3	1–4	6	FL	FW, M
Insecta	234	0–31	623	FL, P	FW, T
Mammalia	77	0–12	170	FL	M, T
Merostomata	1	1	1	FL	M
Nematoda	49	0–9	83	FL, P	FW, M, T
Nematomorpha	1	7	7	P	FW
Nemertea	5	1–8	17	FL	M
Onychophora	6	2–10	25	FL	T
Platyhelminthes	62	0–14	165	FL, P	FW, M, T
Porifera	13	0–8	27	FL	M
Pycnogonida	2	1–4	5	FL	M
Reptilia	92	0–9	221	FL	FW, M, T
Rotifera	17	0–34	131	FL	FW, M
Sipuncula	4	2–3	11	FL	M
Tardigrada	4	1–32	38	FL	M, T

*FL, free-living; P, parasitic.

†Habitat refers to that of species investigated only; FW, freshwater; M, marine; T, terrestrial.

‡Fish = Actinopterygii + Elasmobranchii + Holocephali + Sarcopterygii.

in JMP version 11.0 (SAS Institute Inc., Cary, NC, USA).

We ran the above model on three subsets of the data: only studies using mitochondrial markers, only studies using nuclear markers and all studies. In the latter case, the number of sequences obtained was calculated as the sum of mitochondrial and nuclear sequences. Note that among studies using both types of markers, the number of mitochondrial sequences and the number of nuclear sequences were strongly correlated ($r = 0.595$, $N = 424$, $P < 0.0001$).

Results

Of the 1230 studies in the data set, 1079 were on free-living organisms and 151 on parasites, all of them representing 34 higher taxa (Table 1). With regard to the habitat in which the organisms lived, 650 studies focused on terrestrial taxa, 217 on freshwater ones and 363 on marine ones. From the perspective of the type of molecular markers used, 1141 studies used mitochondrial markers and 513 used nuclear markers, with 424 studies using both types of markers. In total, the 1230 studies uncovered 3679 cryptic taxa, ranging from none to 42 per study.

The annual number of published studies using DNA sequences to search for cryptic species has increased markedly over the past two decades (Fig. 1). In parallel, the number of DNA sequences obtained per study has also increased over the years (Fig. 1). The same trend is seen for the number of genes used and number of base pairs investigated per study, separately for both mitochondrial and nuclear genes (Fig. S2). Not surprisingly, this increasing search effort over time has been accompanied by an increase in the number of cryptic taxa uncovered per study (Fig. 1).

There are no obvious differences in the frequency distributions of raw numbers of cryptic taxa uncovered per study between organisms with different modes of life or from different habitats (Fig. 2). Most studies typically find from 0 to 4–5 cryptic species, and very few find more than 10.

The mixed-model results reveal a strong effect of study effort, measured as the number of sequences obtained, on the number of cryptic species found, whatever the subset of studies used (Table 2). Studies that obtain more DNA sequences from many markers and/or many individuals tend to uncover more cryptic species. Model results find no effect of mode of life, that is no evidence that independently of study effort cryptic species tend to occur more frequently in parasitic than free-living taxa (Table 2). Even when focusing only on the seven higher taxa comprising both free-living and parasitic species (Fig. S3), there are no strong and consistent differences in numbers of cryptic species detected between studies on organisms with those two modes of life. In contrast, the habitat in which organisms live influenced how many cryptic species were found: for any increase in study effort, that is any increase in the number of sequences obtained, more cryptic taxa are found in freshwater organisms than in terrestrial or marine ones (Fig. 3). However, this effect depends on the subset of studies used for analysis: it is seen for all studies combined and for only those using mitochondrial markers, but not for only those using nuclear markers (Table 2). Also, the result seems to be mostly driven by taxa such as crustaceans, bryozoans and annelids, as either there are no habitat differences in other taxa or they run counter to the main result of

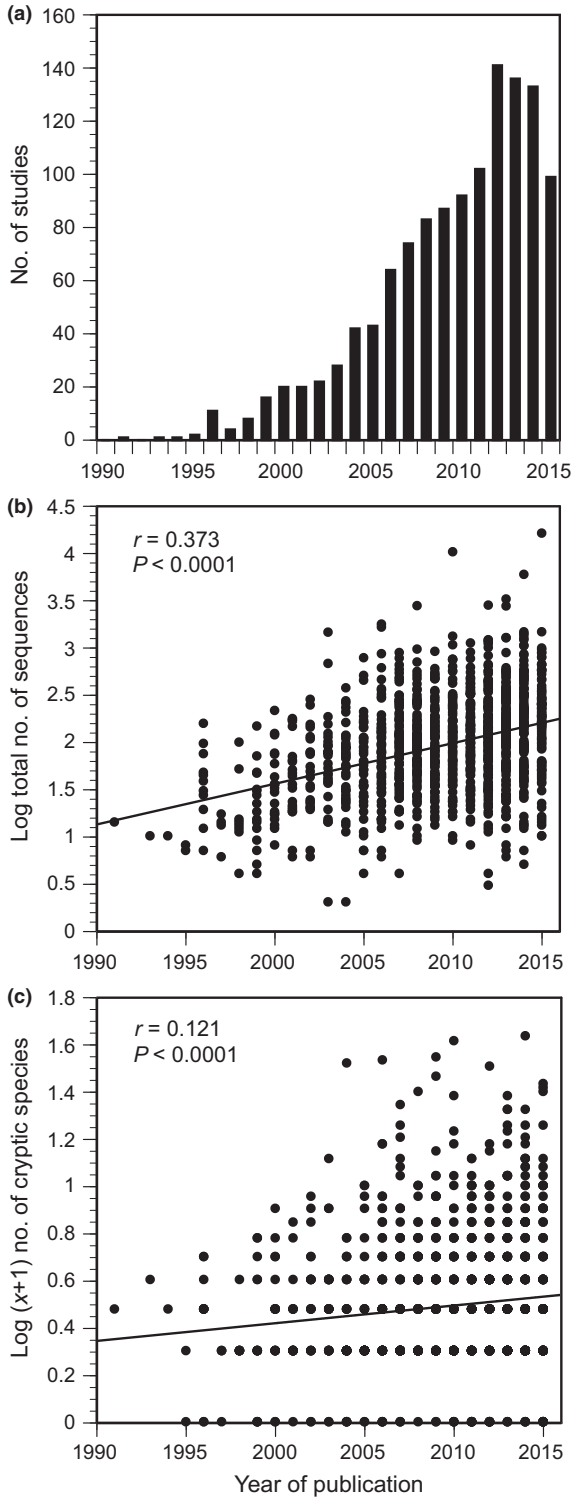

Fig. 1 Annual number of studies searching for cryptic species (a), total number of DNA sequences, summed across mitochondrial and nuclear markers, obtained per study (b), and number of cryptic species found per study (c) as a function of the year of publication. Correlation coefficients and best-fit lines are also shown.

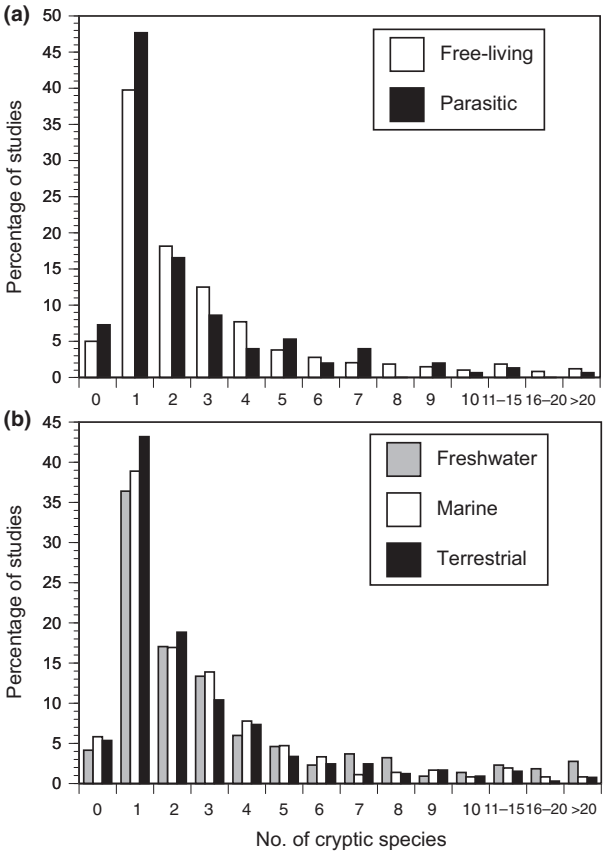

Fig. 2 Frequency distributions of numbers of cryptic species found per study based on either mode of life (a) or habitat (b). Note the contraction of the scale on the x-axis towards high values.

the analysis (Fig. 4). Finally, the higher taxon to which organisms belonged and the biogeographical region where they occurred did not account for more than about 3% of the remaining variance not explained by fixed factors (Table 2), suggesting little influence of these variables on the likelihood of finding cryptic taxa.

Discussion

The implications of ubiquitous cryptic diversity anticipated years ago (Bickford *et al.*, 2007) are now a real and present concern. Here, we provide evidence that the frequency of cryptic taxa is, at least in part, driven by habitat differences, hinting at general underlying patterns in rates of diversification.

The rising number of studies searching for cryptic species using molecular approaches in the past couple of decades reflects the growing interest in this hidden component of biodiversity. At the same time, the increasing number of cryptic species found per study is more a consequence of the increased effort put in the search for these species in recent years, as measured by

Table 2 Results of the mixed-effects models with the number of cryptic species found per study as response variable, showing the effects of the main predictors and the proportion of the remaining variance accounted for by the random factors. Models were run across all studies by computing the total number of sequences (mitochondrial plus nuclear) obtained and separately using only studies using mitochondrial sequences or only studies using nuclear sequences. Significant main effects are shown in bold.

Fixed factors	Estimate	Std error	*t*-value	*P*	Random factors	% variance
Mitochondrial + nuclear markers (n = 1230)						
Intercept*	0.0726	0.0326	2.23	0.0277	Higher taxon	0.65
Log no. of sequences	**0.2174**	**0.0142**	**15.30**	**< 0.0001**	Biogeographical region	1.75
Mode of life (free-living)	0.0005	0.0126	0.04	0.9672		
Habitat (freshwater)	**0.0402**	**0.0140**	**2.87**	**0.0047**		
Habitat (marine)	**−0.0256**	**0.0122**	**2.09**	**0.0377**		
Mitochondrial markers (n = 1141)						
Intercept*	0.0817	0.0348	2.34	0.0203	Higher taxon	0.23
Log no. of sequences	**0.2348**	**0.0158**	**14.85**	**< 0.0001**	Biogeographical region	1.67
Mode of life (free-living)	−0.0201	0.0141	1.43	0.1556		
Habitat (freshwater)	**0.0452**	**0.0141**	**3.21**	**0.0016**		
Habitat (marine)	−0.0216	0.0125	1.73	0.0849		
Nuclear markers (n = 513)						
Intercept*	0.2276	0.0510	4.46	< 0.0001	Higher taxon	2.99
Log no. of sequences	**0.1569**	**0.0254**	**6.18**	**< 0.0001**	Biogeographical region	3.10
Mode of life (free-living)	0.0294	0.0179	1.63	0.1079		
Habitat (freshwater)	0.0182	0.0234	0.78	0.4388		
Habitat (marine)	−0.0143	0.0205	0.70	0.4870		

*Parasitic mode of life and terrestrial habitat are included in the intercept.

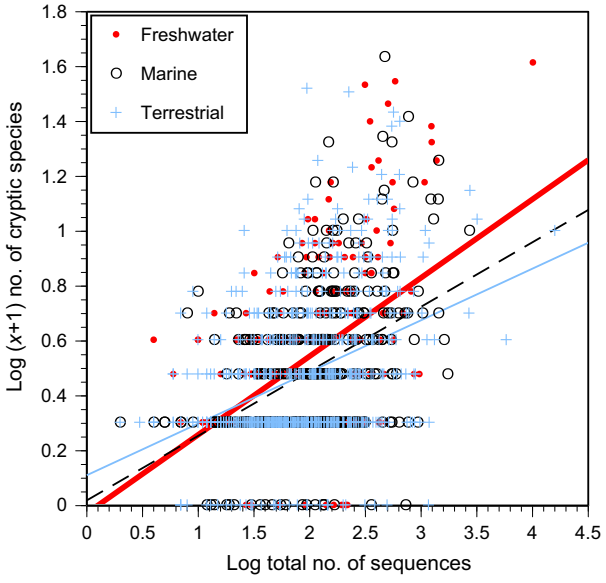

Fig. 3 Number of cryptic species found as a function of the total number of DNA sequences obtained (summed across mitochondrial and nuclear markers) across 1230 studies, with separate lines of best fit shown for taxa from freshwater (thick solid line), marine (broken line) and terrestrial (thin solid line) habitats.

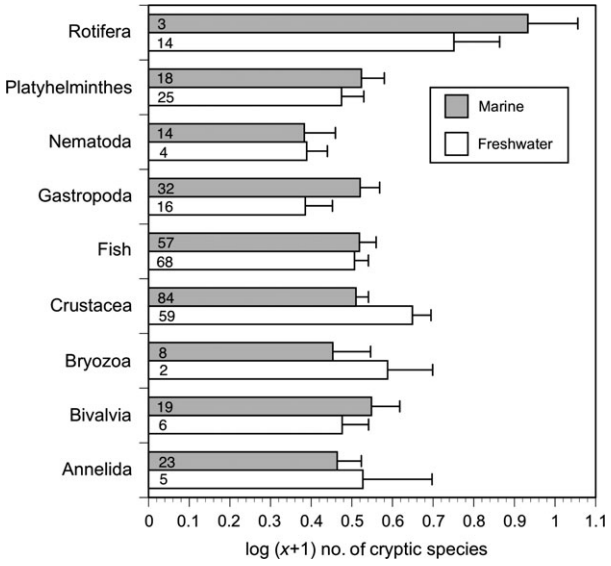

Fig. 4 Mean (±SE) number of cryptic species found per study in nine higher taxa, shown separately for freshwater and marine species. Numbers of studies in each category are shown at the base of the bars.

either the number of markers used, the number of individuals sequenced and thus the number of sequences obtained, and the number of base pairs analysed. In addition, more sophisticated methods of species delineation using DNA sequences have been implemented recently (e.g. Puillandre *et al.*, 2012; Carstens *et al.*, 2013; Flot, 2015), adding rigour and power to the discrimination of unique genetic lineages. The upshot is that biodiversity assessments will need revision in the light of our increasing knowledge of cryptic diversity

(Bickford *et al.*, 2007; Scheffers *et al.*, 2012). Cryptic species are being found not only in well-documented urban regions (Feinberg *et al.*, 2014), but also in well-researched, charismatic animals such as giraffes (Brown *et al.*, 2007), penguins (Grosser *et al.*, 2015) and crocodiles (Shirley *et al.*, 2014). The convenient binning of indistinguishable species as single entities is a thing of the past, raising important issues for conservation biology. For taxonomists and systematists, the task ahead is huge because the vast majority of cryptic taxa uncovered to date have received no formal description and no scientific name, a missing but critical step in the identification of new species (Schlick-Steiner *et al.*, 2007; Pante *et al.*, 2015).

Our main results support one of our original hypotheses: for a given study effort, cryptic taxa occur more frequently among freshwater metazoans than among marine ones and, to a lesser extent, terrestrial ones. These findings align with expectations based on habitat differences in heterogeneity, openness and primary productivity, all factors that can affect rates of genetic differentiation (Grosberg *et al.*, 2012; Wiens, 2015). It has been suggested that discrimination among cryptic species should be easier in taxa with complex morphology than in those with limited morphological complexity; this is the case for crustaceans, where previously cryptic species are very often distinguishable morphologically and later described formally (Appeltans *et al.*, 2012). The inclusion of the higher taxon to which target species belonged as a random factor in our analyses should account for such disparities. When comparing habitats within higher taxa (Fig. 4), there nevertheless appears to be contrasting patterns: among the better studied taxa, studies on freshwater crustaceans tend to uncover more cryptic species than studies on marine ones, but there is no difference for fish. The latter result contrasts with previous findings on freshwater vs. marine fish (Bloom *et al.*, 2013); this may be due in part to the fact that several marine studies have sampled fish across larger spatial scales than freshwater studies. Nevertheless, the tendency for cryptic diversity to be more common in freshwaters than in other habitats may depend in part on the taxa studied.

The results of our study do not support our second hypothesis: we found no evidence that cryptic taxa are more common among parasitic than free-living metazoans. The shorter generation times and possibly greater resource specialization of parasites do not seem to lead to higher rates of diversification and thus to more cryptic species found per study effort, than in free-living species. A long-held view in parasitology, known as Manter's first rule, is that parasites evolve more slowly than their hosts (Brooks & McLennan, 1993). In some molecular studies of host–parasite cophylogenies, it has been found that parasite speciation lags behind host speciation during codivergence events (Hafner & Nadler, 1988), but this is far from the rule (Clayton *et al.*, 2015). Resource specialization may lead to evolutionary conservatism in parasites, possibly negating other factors such as generation time and limited dispersal and causing them to have a potential for rapid diversification not much different from that of their hosts. The lower-than-expected cryptic diversity revealed by studies on parasites may also explain why much fewer parasite species are known and described than the global species richness extrapolated from estimates of host specificity and host species richness (Costello, 2016).

The clear effect of study effort, measured as the number of sequences obtained and thus dependent on the number of markers used and individuals sampled, was apparent whatever subset of studies was used. The harder one looks, the more cryptic taxa are found (Poulin, 2011). However, the higher occurrence of cryptic taxa among freshwater organisms than among marine and, to a lesser extent, terrestrial ones was only revealed by analyses including either all studies or only those using mitochondrial DNA sequences and not by analysis of only those studies that included nuclear sequences. One reason for this may be that there were fewer studies using nuclear sequences. Also, the proportional representation of different higher taxa among studies using nuclear markers differed a little from that among all studies or among those using mitochondrial markers, which may have influenced the results. In addition, the different authors of the original studies surveyed here used various criteria and approaches to decide how DNA variation was representative of cryptic diversity; subtle nuances in how this was done for mitochondrial vs. nuclear markers may also explain why we found patterns based on the former and not the latter. Finally, a more likely reason is that mitochondrial genes tend to evolve faster than nuclear ones and are generally thought to be more sensitive for distinguishing among closely related species (Morgan & Blair, 1998; Blouin, 2002; Locke *et al.*, 2010; Nadler & Pérez-Ponce de León, 2011). This is true of the cytochrome *c* oxidase subunit 1 (CO1), the most widely used mitochondrial marker among the studies in our data set, compared to the internal transcribed spacer regions (ITS-1 and ITS-2), the most widely used nuclear markers (Morgan & Blair, 1998; Blouin, 2002; Locke *et al.*, 2010). Therefore, analyses including studies based on mitochondrial markers are more likely to detect the subtle effect of freshwater environments on the occurrence of cryptic diversity.

In conclusion, we find that both the number of reports of cryptic species and the number of cryptic species found per report have been rising rapidly in the past two decades. We also find support for the hypothesis that for a given study effort, more cryptic taxa tend to be found in freshwater than in marine or terrestrial metazoans. Although this pattern depends somewhat

on the taxonomic group involved, it suggests that environmental effects modulate rates of genetic diversification. These findings have important implications for freshwater conservation biology, given the high biodiversity of freshwater habitats and how heavily they are impacted by species invasions and human activities (Abell, 2002). Inaccurate identification of target species can mislead management efforts (Cook *et al.*, 2008), and the possibility of cryptic species will require special attention in freshwaters.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Appendix S1 Additional methodological details.
Figure S1 Pairwise correlations between number of genes, number of sequences, and number of base pairs per study.
Figure S2 Number of genes, number of sequences, and number of base pairs per study vs. year of publication.
Figure S3 Number of cryptic species found per study for free-living and parasitic animals in seven higher taxa.

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