A global biodiversity estimate of a poorly known taxon: phylum Tardigrada

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Knowledge of global biodiversity is essential for a basic understanding of Earth’s ecosystems and for the conservation of life. Although many estimates of species numbers have been attempted using various techniques, many smaller phyla remain poorly known without such estimates. For most of these it is unclear if they are species-poor or just poorly studied. The phylum Tardigrada is one of these phyla. Specialists have created a regularly updated checklist for the known tardigrade species, which as of 15 July 2013 listed 1190 taxa (species and subspecies). Of these, 1008 are limnoterrestrial and 182 are marine. These were the most up-to-date data at the time of our analysis. As species accumulation curves show little sign of levelling out, they do not provide a useful tool for estimating global tardigrade diversity from existing species numbers. A new technique has recently been developed that uses the more complete knowledge of higher taxonomic levels to estimate the asymptotic number of species. We applied this technique to limnoterrestrial and marine tardigrades. We estimate that the global total for limnoterrestrial tardigrades is 1145 (upper 95% CI = 2101), and the global total for marine tardigrades is 936 (upper 95% CI = 1803). This yields 87% completeness for our knowledge of limnoterrestrial tardigrades, and only 19% completeness for our knowledge of marine tardigrades. Thus, although many more marine species remain to be discovered, it appears that tardigrades are both poorly studied and relatively species poor.

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INTRODUCTION

Knowledge of the number of species on Earth, for both particular taxa and in total, is fundamental and imperative for conservation (Balmford, Green & Jenkins, 2003; Dirzo & Raven, 2003; Mora et al., 2011; Scheffers et al., 2012). Many conservation efforts, such as identifying areas of high extinction (Myers et al., 2000; Roberts et al., 2002), and the creation of optimum conservation strategies (Myers et al., 2000; Grenyer et al., 2006), are highly dependent on such basic data (Mora, Tittensor & Myers, 2008). Yet, it is likely that describing even a majority of the diversity on Earth will require billions of dollars and perhaps hundreds of years (Carbayo & Marques, 2011; Scheffers et al., 2012). Thus, if we are to gain a more thorough understanding of global diversity there is an immediate need to derive estimates of total species richness within all taxonomic groups based on existing information. Although there have been many recent estimates of species diversity overall and within a number of lineages (reviewed in Scheffers et al., 2012), many lesser-studied groups have been largely ignored (Vicente, 2010; MA (Millennium Ecosystem Assessment), 2005). One such taxon is the phylum Tardigrada.

Tardigrades (Fig. 1) are micrometazoans, generally ranging in size from 50 μm to 1 mm in body length, although recently a species was found reaching 2 mm in length (Guil, 2008). They have four pairs of lobopodous legs, usually terminating in claws and/or
digits, and two eversible stylets in their buccal appar-atus. Ecdysozoans that comprise a sister group to
the arthropods (Garey et al., 1996, 1999; Garey,
2001; Edgecombe et al., 2011), tardigrades are ubiq-
uitous in marine, freshwater, and terrestrial intersti-
tial communities. Tardigrades are famous for their
ability to survive dehydration, freezing, and other
environmental extremes via cryptobiosis (for reviews
of tardigrade cryptobiosis, see Guidetti, Altiero &
Rebecchi, 2011; Møbjerg et al., 2011; and Welnicz
et al., 2011), but generally this ability is restricted to
terrestrial species. As they have no impact on human
economy, they receive relatively little research atten-
tion (Guil & Cabrero-Sañudo, 2007). For a summary
of their biology, see Kinchin (1994), Nelson (2002),
Tardigrades are one of many animal phyla with
few known species. Zhang (2011) presented a new
evo-Linnean classification for the Kingdom Animalia
listing 40 extant phyla and providing current esti-
mates of all known species. Twenty-seven of the 40
phyla contain less than 2000 reported species, and
our knowledge of total species in these groups is still
far from complete (Zhang, 2011). For many animal
phyla, then, there remains an important unanswered
question: are they species-poor or just poorly stud-
ied?
With 1190 described taxa, as of 15 July 2013,
tardigrades are one of the more numerous of the 27
phyla, but the total number of species has never
been estimated (Nelson et al., 2015). A regularly
updated checklist of recognized valid species is main-
tained by taxonomic experts (Guidetti & Bertolani,
Although a newer version of the checklist exists, in
which some additional taxa have been added, we
used the most up-to-date data available at the time
that we ran our models. Guil & Cabrero-Sañudo
(2007) analysed the cumulative limnoterrestrial spe-
cies described through the year 2004, and showed
that an asymptotic model would not fit the accumu-
lation curve, implying that total species estimates
were not possible given the current rapid rate of new
species descriptions. Biodiversity studies have
increased in recent years, however, and new method-
ologies for species estimates have been developed
(Bebber et al., 2007; Mora et al., 2011). Appeltans
et al. (2012) estimated the number of marine tardi-
grades only, but their model’s estimates were very
low and differed greatly from the predictions made
by their taxonomic expert (see Discussion).
In this article we apply the method of Mora et al.
(2011) to global tardigrade data, including both mar-
ine and limnoterrestrial species. This technique
recognises that although species accumulation curves
often show no sign of levelling out, our knowledge of
numbers at higher taxonomic levels is usually more
complete. The relationship between the numbers of
higher taxa allows us to project an asymptotic value
for species even in poorly studied groups.

MATERIAL AND METHODS
Data for global species estimates were taken from
the most recent available version of the tardigrade
checklist at the time that we ran our models (ver-
sion 23, 15 July 2013; Degma et al., 2009-2013). The
checklist was transferred to a spreadsheet to tally
the counts for each taxon by publication year. In
order to compare taxonomic effort between marine
and limnoterrestrial habitats, the number of distinct
first-author surnames (see Costello, May & Stork,
2011) was also tallied. Marine and limnoterrestrial
species were counted separately. Marine species were
defined as: all species in the class Heterotardigrada, order Arthropodida; all species in family Echiniscoidea, order Echiniscoidea; and four species in class Eutardigrada, order Parachela \( \text{Halobiotus arcturulius} \) Crisp & Kristensen, 1983; \( \text{Halobiotus cris-pae} \) Kristensen, 1982; \( \text{Halobiotus stenostomos} \) (Richters, 1908); \( \text{Thulinius itoi} \) (Tsurusaki, 1980). The class Mesotardigrada is considered dubious by some authors (Nelson et al., 2015), but it is included here because it is included in the tardigrade checklist. We only included species described earlier than 2013 (i.e. from 2012 and earlier), because version 23 of the checklist (Degma et al., 2009-2013) was incomplete for the year 2013 (eight species described in 2013 were excluded). Subspecies were included in the species counts, in keeping with other recent tali-

eyes of known tardigrade species (Appeltans et al., 2012; Vicente & Bertolani, 2013). Thus, the number of taxa included in this analysis was 1182.

The global number of species was estimated following the protocol described in Mora et al. (2011). This method relates the number in each taxa (y-axis) to the numeric rank of the higher taxa (x-axis) (i.e. kingdom = rank 1, phylum = rank 2, class = rank 3, order = rank 4, family = rank 5, and genus = rank 6). The pattern generated follows an upward concave shape that can be fitted with a set of exponential, power, and hyper-exponential models using least-squares regression models, which are then used to extrapolate to the rank of species (rank = 7). As data are not strictly independent across hierarchi-
cally organized taxa we also performed generalized least squares analysis assuming autocorrelated regression errors. The numbers of species extrapo-
lated from all models were averaged using multi-
model averaging with Akaike's information criterion (AIC). This method was applied to marine tardigrade species, limnoterrestrial tardigrade species, and to all species combined. The method yields estimates for the number of species that closely resemble, with a high degree of confidence, the number of species known in well-studied taxa, and it recognizes that higher taxonomy is much better known than the number of species. For example, in Tardigrada the last class was described in 1937, the last order in 1983, and although families and genera are still being described, they are certainly being described at lower rates than new species. Recent phylogenetic analyses (Sands et al., 2008a,b; Pilato & Binda, 2010; Marley, McInnes & Sands, 2011; Bertolani et al., 2014) based on molecular and morphological data have made changes to levels ranging from superfamily to genus, and all of these, excepting those proposed very recently by Bertolani et al. (2014), were included in the current analysis. Regardless, Mora et al. (2011) conducted sensitivity tests for kingdom Animalia and found that even frequent new discoveries of families and genera do not have large effects on predicted species estimates.

RESULTS

Global species estimates are compared with known numbers of taxa in Figure 2. The number of known species, estimated total species, 95% confidence intervals of estimates, and percentages of completeness are shown in Table 1. Our estimated means are 936 for total marine species, 1145 for total limnoter-
restrial species, and 2654 for the grand total of all species. The large discrepancy between marine and limnoterrestrial tardigrade percentages of completeness (19.4% for marine species compared with 87.3% for limnoterrestrial species) is understandable based on the extremely low taxonomic effort made in marine habitats (Fig. 3).

DISCUSSION

No attempt has previously been made to estimate global tardigrade diversity including both marine and limnoterrestrial species. The total number of known tardigrade taxa at the end of 2012, based on the current version of the checklist when we ran our models, was 1182. We estimate that the global total number of species is 2654 (44.5% completeness), although it could be as high as 5407 (21.9% completeness). Optimistically, then, we are nearly halfway down the road of discovery, largely as a result of the increased taxonomic effort in recent years in lim-
noterrestrial habitats (Fig. 3; Guil & Cabrero-
Sanudo, 2007).

Our numbers could be underestimates. The com-
pleteness estimate for global limnoterrestrial tardi-
grades (87.2%; Table 1) is very similar to the find-
ings of Bartels & Nelson (2007) in the extensive, large-scale inventory of the Great Smoky Mountains National Park, Appalachian Mountains, USA. Using species-richness estimators, they predicted 96 species for the park. The current species list for the park is now 81, yielding 84% completeness. The tardigrade fauna of the Smokies is now one of the best known in the world, so our global estimate of 87.2% completeness seems low. Our model depends on the sta-

bility of the number of known higher taxa. As those numbers increase, so will the species numbers, although it is unlikely that the higher taxa numbers will increase drastically. We also know that many current ‘species’ are actually species clusters [e.g. \( \text{Echiniscooides sigismundi} \) (M. Schultze, 1865), \( \text{Hypsi-bius convergens} \) (Urbanowicz, 1925), \( \text{Hypsi-bius dujardini} \) (Doyle, 1840), \( \text{Macrobiotus harmsworthi} \) Murray, 1907, and \( \text{Pseudochiniscus suillus} \) © 2016 The Linnean Society of London, Zoological Journal of the Linnean Society, 2016, 178, 730–736
Milnesium tardigradum Doyère, 1840 was once considered a single species but has now been split into over a dozen species (Michalczyn et al., 2012), and a similar situation occurred with Macrobiotus hufelandi Schultze, 1834 (Bertolani et al., 2011). Additionally, we now know of the existence of cryptic species in both limnoterrestrial and marine tardigrades (Guil & Giribet, 2009; Faury et al., 2008, 2012). Species complexes and cryptic species will certainly increase the number of predicted species. On the other hand, the extent to which synonymies still exist in the current checklist will decrease estimates, and it is prudent to note that expert opinion often errs on the side of exaggeration (Erwin, 1982; Lambshead, 1993; Poore & Wilson, 1993).

Our model indicates that vastly more marine species remain to be discovered. The lack of taxonomic effort for marine tardigrades is well known among tardigrade researchers (Vicente & Bertolani, 2013), but our results allow us to gauge this marine ‘Linnean shortfall’ (Brown & Lomolino, 1998; Whittaker et al., 2005). Figure 3 clearly shows the discrepancy between research effort for marine and limnoterrestrial species. The collection of marine species, especially species from the sublittoral and deeper zones, is costly, and most species are rare and quite small, making the processing more challenging than for limnoterrestrial species. Marine species make up only 15% of currently known species, but by our estimate the actual number of marine species is likely to be very close to the actual number of limnoterrestrial species. Indeed, given the very fragmentary nature of marine tardigrade collections (Kaczmarek et al., 2015) and the high morphological variability within marine tardigrades, it is even possible that the number of marine species will eventually exceed limnoterrestrial species. This likelihood falls within the 95% confidence intervals of our model.

One previous attempt was made to estimate global marine tardigrade diversity: Appeltans et al. (2012) used a logistic model to estimate the total marine species diversity for all marine groups including marine tardigrades, and compared this with ‘taxonomic expert opinion’. They counted 183 known marine tardigrades, but their model predicted only 40–280 species yet to be discovered. The number estimated by marine tardigrade taxonomist Reinhardt Kristensen (based on his experience, and cited in Appeltans et al., 2012) was 1120 yet to be discovered, for a total estimate of 1303. This falls within our predicted mean and upper 95% confidence interval, so our marine estimate is much more congruent with Kristensen’s expert opinion.

It is not unexpected that a logistic discovery model would give unreliable results for poorly studied groups with small sample sizes, in which the temporal species accumulation curves show no sign of levelling off. The logistic model used by Appeltans et al. © 2016 The Linnean Society of London, Zoological Journal of the Linnean Society, 2016, 178, 730–736
(2012) generally gave poor results for the 27 phyla with less than 2000 known species (Zhang, 2011). It was unable to fit the curves for 12 of these phyla, and in eight phyla the model underestimated the expert opinion by a factor of between four and 18. In only two of the 27 phyla did their model reasonably agree with expert opinion. The approach used here is more promising, and Mora et al. (2011) demonstrated that this method outperforms simple extrapolation from species accumulation curves.

Our results suggest that marine tardigrade species diversity, once fully known, will be similar to that of limnoterrestrial tardigrades. They also suggest that the maximum number of tardigrade species is around 5500, although approximately 2500 is more likely, and the overall percentage completeness of our knowledge of tardigrade diversity is 22–45%. What will it take to have a complete inventory of global tardigrade diversity? Around 20 new limnoterrestrial species are described each year. If the rate of new marine species descriptions were equal to that rate, and if the actual percentage completeness is at the upper end of the range (45%), this could be achievable in the next 40 years.

Is phylum Tardigrada species-poor or just poorly studied? According to Zhang (2011), tardigrades are the 17th most speciose of the 40 phyla, and the top ten phyla are higher by orders of magnitude. Even if the total number of tardigrades reaches the upper 95% confidence interval of our estimate, it is unlikely that their relative abundance would change greatly. Given the low taxonomic effort, especially in marine species, it is also fair to say that they are poorly studied. Thus, the answer to the question would appear to be: both.

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