The LINNEAN SOCIETY of London ZOOLOGICAL Journal of the Linnean Society

Zoological Journal of the Linnean Society, 2016, **178**, 730–736. With 3 figures

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

PAUL J. BARTELS[1]*, J.J. APODACA[1], CAMILO MORA[2] and DIANE R. NELSON[3]

[1]*Department of Biology, Warren Wilson College, Asheville, NC, 28815, USA*
[2]*Department of Geography, University of Hawaii, Honolulu HI, 96822, USA*
[3]*Department of Biological Sciences, East Tennessee State University, Johnson City TN, 37614, USA*

Received 16 August 2015; revised 8 February 2016; accepted for publication 24 March 2016

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.

doi: 10.1111/zoj.12441

ADDITIONAL KEYWORDS: asymptotic models – limnoterrestrial – marine – species richness – tardigrades – terrestrial – water bears.

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

*Corresponding author. E-mail: pbartels@warren-wilson.edu

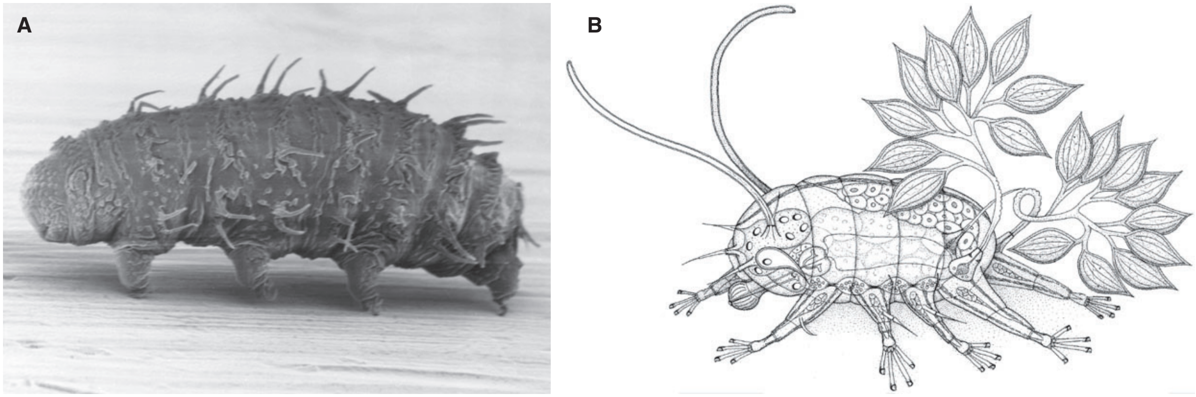

Figure 1. Images of a limnoterrestrial and marine tardigrade: A, scanning electron micrograph (SEM) of the limnoterrestrial eutardigrade *Calohypsibius ornatus* (Richters, 1900) (photo courtesy of Diane R. Nelson); B, drawing of the marine heterotardigrade *Tanarctus bubulubus* Jørgensen & Kristensen, 2001. Used with permission of the authors and publisher.

digits, and two eversible stylets in their buccal apparatus. Ecdysozoans that comprise a sister group to the arthropods (Garey *et al.*, 1996, 1999; Garey, 2001; Edgecombe *et al.*, 2011), tardigrades are ubiquitous in marine, freshwater, and terrestrial interstitial 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 attention (Guil & Cabrero-Sañudo, 2007). For a summary of their biology, see Kinchin (1994), Nelson (2002), and Nelson, Guidetti & Rebecchi (2015).

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 estimates 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 studied?

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 maintained by taxonomic experts (Guidetti & Bertolani, 2005; Degma, Bertolani & Guidetti, 2009-2013). 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 species described through the year 2004, and showed that an asymptotic model would not fit the accumulation 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 methodologies for species estimates have been developed (Bebber *et al.*, 2007; Mora *et al.*, 2011). Appeltans *et al.* (2012) estimated the number of marine tardigrades 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 marine 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 (version 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 Arthrotardigrada; all species in family Echiniscoididae, order Echiniscoidea; and four species in class Eutardigrada, order Parachela [*Halobiotus arcturulius* Crisp & Kristensen, 1983; *Halobiotus crispae* Kristensen, 1982; *Halobiotus stenostomus* (Richters, 1908); *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 tallies 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 hierarchically organized taxa we also performed generalized least squares analysis assuming autocorrelated regression errors. The numbers of species extrapolated from all models were averaged using multimodel 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 limnoterrestrial 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 limnoterrestrial habitats (Fig. 3; Guil & Cabrero-Sañudo, 2007).

Our numbers could be underestimates. The completeness estimate for global limnoterrestrial tardigrades (87.2%; Table 1) is very similar to the findings 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 stability 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. *Echiniscoides sigismundi* (M. Schultze, 1865), *Hypsibius convergens* (Urbanowicz, 1925), *Hypsibius dujardini* (Doyère, 1840), *Macrobiotus harmsworthi* Murray, 1907, and *Pseudechiniscus suillus*

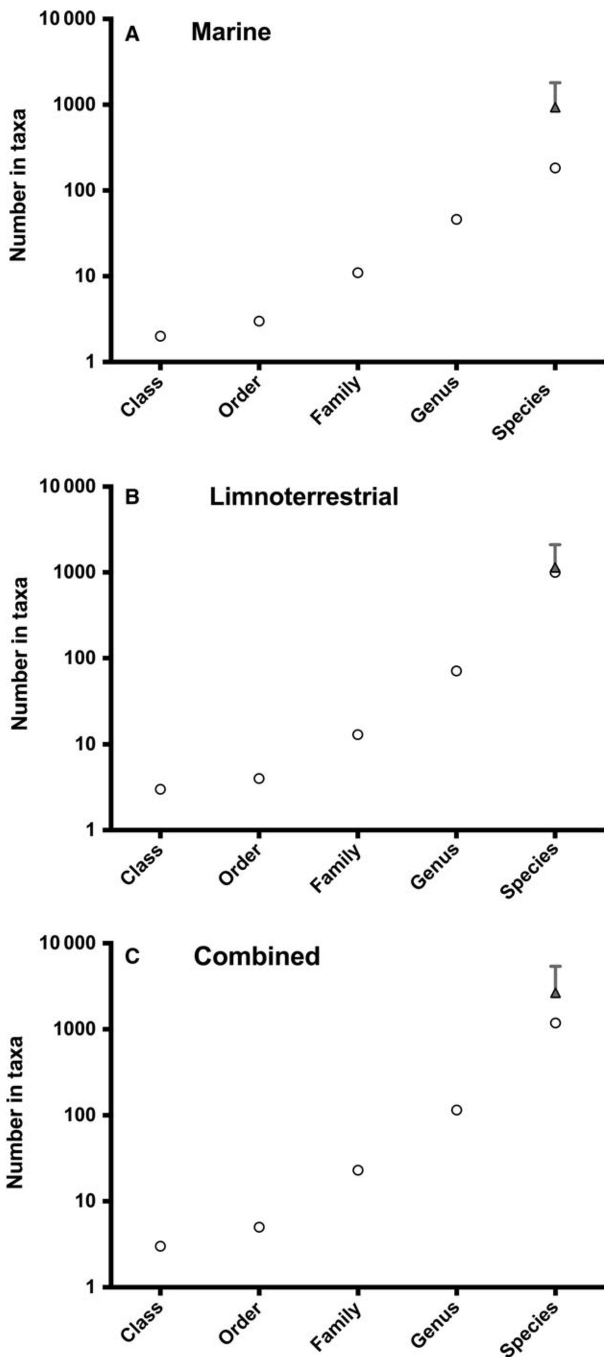

Figure 2. Estimated global tardigrade diversity; ○, known numbers of taxa; ▲, estimated species diversity; error bars are 95% confidence intervals; lower bars are not included as these are lower than the number of known species; *y*-axis is a logarithmic scale. A, marine species; B, limnoterrestrial species; and C, combined species.

(Ehrenberg, 1853)]. *Milnesium tardigradum* Doyère, 1840 was once considered a single species but has now been split into over a dozen species (Michalczyk *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; Faurby *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.*

Table 1. Number of known tardigrade species, estimated total species, and lower and upper 95% confidence intervals of estimates for marine, limnoterrestrial, and combined species. Percent completeness is the number of known species divided by the total estimated species

	Known species	Total estimated species	Lower 95% CI	Upper 95% CI	Percent completeness
Marine	182	936	148	1803	19.4
Limnoterrestrial	1000	1145	320	2101	87.3
Combined	1182	2654	582	5407	44.5

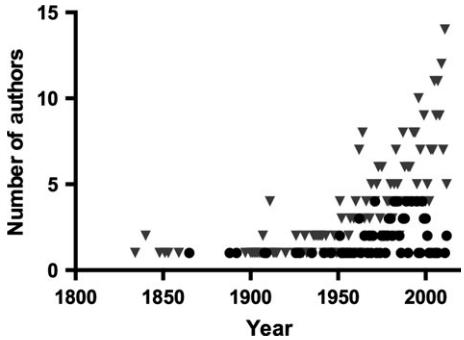

Figure 3. Number of distinct first-author surnames for taxonomic papers listed in the checklist for tardigrades as a function of year; ▼, terrestrial; ●, marine.

(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.

ACKNOWLEDGEMENTS

We would like to thank Discover Life in America (http://www.dlia.org) and the dedicated biologists of the Great Smoky Mountains National Park for the long-term support of our work on the phylum Tardigrada for the All Taxa Biodiversity Inventory. We also appreciate the research support of East Tennessee State University and Warren Wilson College. Aslak Jørgensen kindly provided permission for the use of the figure of *Tanarctus bubulubus* Jørgensen & Kristensen, 2001. This article was greatly improved by comments on an earlier draft by Łukasz Kaczmarek and three anonymous reviewers.

REFERENCES

Appeltans W, Ahyong ST, Anderson G, Angel MV, Artois T, Bailly N, Bamber R, Barber A, Bartsch I, Berta A, Błażewicz-Paszkowycz M, Bock P, Boyko CB, Brandão SN, Bray RA, Bruce NL, Cairns SD, Chan T-Y, Cheng L, Collins AG, Cribb T, Curini-Galletti M, Dahdouh-Guebas F, Davie PJF, Dawson MN, De Clerck O, Decock W, De Grave S, de Voogd NJ, Domning DP, Emig CC, Erséus C, Eschmeyer W, Fauchald K, Fautin DG, Feist SW, Fransen CHJM, Furuya H, Garci-Alvarez O, Gerken S, Gibson D, Gittenberger A, Gofas S, Gómez-Daglio L, Gordon D, Guiry MD, Hernandez F, Hoeksema BW, Hopcroft RR, Jaume D, Kirk P, Koedam N, Koenemann S, Kolb JB, Kristensen RM, Kroh A, Lambert G, Lazarus D, Lemaitre R, Longshaw M, Lowry J, Macpherson E, Madin LP, Mah C, Mapstone G, McLaughlin PA, Mees J, Meland K, Messing CG, Mills CE, Molodtsova TN, Mooi R, Neuhaus B, Ng PKL, Nielsen C, Norenburg J, Opresko DM, Osawa M, Paulay G, Perrin W, Pilger JF, Poore GCB, Pugh P, Read GB, Reimer JD, Rius M, Rocha RM, Saiz-Salinas JI, Scarabino V, Schierwater B, Schmidt-Rhaesa A, Schnabel KE, Schotte M,

Schuchert P, Schwabe E, Segers H, Self-Sullivan C, Shenkar N, Siegel V, Sterrer W, Stohr S, Swalla B, Tasker ML, Thuesen EV, Timm T, Todaro MA, Turon X, Tyler S, Uetz P, van der Land J, Vanhoorne B, van Ofwegen LP, van Soest RWM, Vanaverbeke J, Walker-Smith G, Walter TC, Warren A, Williams CG, Wlson SP, Costello MJ. 2012. The magnitude of global marine species diversity. *Current Biology* **22:** 2189–2202.

Balmford A, Green RE, Jenkins M. 2003. Measuring the changing state of nature. *Trends in Ecology and Evolution* **18:** 326–330.

Bartels PJ, Nelson DR. 2007. An evaluation of species richness estimators for tardigrades of the Great Smoky Mountains National Park, Tennessee and North Carolina, USA. *Journal of Limnology* **66**(Suppl. 1)**:** 104–110.

Bebber DP, Marriott FHC, Gaston KJ, Harris SA, Scotland RW. 2007. Predicting unknown species numbers using discovery curves. *Proceedings Royal Society B* **274:** 1651–1658.

Bertolani R, Rebecchi L, Giovannini I, Cesari M. 2011. DNA barcoding and integrative taxonomy of *Macrobiotus hufelandi* C.A.S. Schultze 1834, the first tardigrade species to be described, and some related species. *Zootaxa* **2997:** 19–36.

Bertolani R, Guidetti R, Marchioro T, Altiero T, Rebecchi L, Cesari M. 2014. Phylogeny of Eutardigrada: new molecular data and their morphological support leading to a new systematics of Eutardigrada. *Molecular Phylogenetics and Evolution* **76:** 110–126.

Brown JH, Lomolino MV. 1998. *Biogeography*, 2nd edn. Sunderland, Massachusetts: Sinauer.

Carbayo F, Marques AC. 2011. The costs of describing the entire animal kingdom. *Trends in Ecology and Evolution* **26:** 154–155.

Costello MJ, May RM, Stork NE. 2011. Can we name Earth's species before they go extinct? *Science* **339:** 413–416.

Degma P, Bertolani R, Guidetti R. 2009-2013. Actual checklist of Tardigrada species. Ver 23: 15-07-2013. http://www.tardigrada.modena.unimo.it

Dirzo R, Raven PH. 2003. Global state of biodiversity and loss. *Annual Review of Environmental Resources* **28:** 137–167.

Edgecombe GD, Giribet G, Dunn CW, Hejnol A, Kristensen RM, Neves RC, Rouse GW, Worsaae K, Sørensen MV. 2011. Higher-level metazoan relationships: recent progress and remaining questions. *Organisms Diversity and Evolution* **11:** 151–172.

Erwin TL. 1982. Tropical forests: their richness in Coleoptera and other arthropod species. *Coleopterists Bulletin* **36:** 74–75.

Faurby S, Jönsson KI, Rebecchi L, Funch P. 2008. Variation in anhydrobiotic survival of two eutardigrade morphospecies: a story of cryptic species and their dispersal. *Journal of Zoology* **275:** 139–145.

Faurby S, Jørgensen A, Kristensen RM, Funch P. 2012. Distribution and speciation in marine intertidal tardigrades: testing the roles of climatic and geographical isolation. *Journal of Biogeography* **39:** 1596–1607.

Garey J. 2001. Ecdysozoa: the relationship between Cycloneuralia and Panarthropoda. *Zoolgischer Anzeiger* **240:** 321–330.

Garey J, Krotec M, Nelson DR, Brooks J. 1996. Molecular analysis supports a tardigrade-arthropod association. *Invertebrate Biology* **115:** 79–88.

Garey J, Nelson DR, Mackey L, Li J. 1999. Tardigrade phylogeny: congruence of morphological and molecular evidence. *Zoolgischer Anzeiger* **238:** 205–210.

Grenyer R, Orme CDL, Jackson SF, Thomas GH, Davies RG, Davies TJ, Jones KE, Olson VA, Ridgely RS, Rasmussen PC, Ding T-S, Bennett PM, Blackburn TM, Gaston KJ, Gittleman JL, Owens IPF. 2006. Global distribution and conservation of rare and threatened vertebrates. *Nature* **444:** 93–96.

Guidetti R, Bertolani R. 2005. Tardigrade taxonomy: an updated checklist of the taxa and a list of characters for their identification. *Zootaxa* **845:** 1–46.

Guidetti R, Altiero T, Rebecchi L. 2011. On dormancy strategies in tardigrades. *Journal of Insect Physiology* **57:** 567–576.

Guil N. 2008. New records and within−species variability of Iberian tardigrades (Tardigrada), with comments on the species from the *Echiniscus blumi-canadensis* series. *Zootaxa* **1757:** 1–30.

Guil N, Cabrero-Sañudo FJ. 2007. Analysis of the species description process for a little known invertebrate group: the limnoterrestrial tardigrades (Bilateria, Tardigrada). *Biodiversity and Conservation* **16:** 1063–1086.

Guil N, Giribet G. 2009. Fine scale population structure in the *Echiniscus blumi-canadensis* series (Heterotardigrada, Tardigrada) in an Iberian mountain range – when morphology fails to explain genetic structure. *Molecular Phylogenetics and Evolution* **51:** 606–613.

Jørgensen A, Kristensen RM. 2001. A new tanarctid arthrotardigrade with buoyant bodies. *Zoolgischer Anzeiger* **240:** 425–439.

Kaczmarek Ł, Bartels P, Roszkowska M, Nelson DR. 2015. The zoogeography of marine Tardigrada. *Zootaxa* **4037:** 1–189.

Kinchin IM. 1994. *The biology of tardigrades*. London: Portland Press.

Lambshead J. 1993. Recent developments in marine benthic biodiversity research. *Oceanis* **19:** 5–24.

MA (Millennium Ecosystem Assessment). 2005. *Ecosystems and human well-being: synthesis*. Washington DC: Island Press.

Marley NJ, McInnes SJ, Sands CJ. 2011. Phylum Tardigrada: a reevaluation of the Parachela. *Zootaxa* **2819:** 51–64.

Michalczyk Ł, Wełnicz W, Frohme M, Kaczmarek Ł. 2012. Redescriptions of three *Milnesium* Doyère, 1840 taxa (Tardigrada: Eutardigrada: Milnesiidae), including the nominal species for the genus. *Zootaxa* **3154:** 1–20.

Møbjerg N, Hallberg KA, Jørgensen A, Persson D, Bjørn M, Ramlov H, Kristensen RM. 2011. Survival in extreme environments – on the current knowledge of adaptations in tardigrades. *Acta Physiologica* **202:** 409–420.

Mora C, Tittensor DP, Myers RA. 2008. The completeness of taxonomic inventories for describing global diversity and distribution of marine fishes. *Proceedings Royal Society B* **274:** 149–155.

Mora C, Tittensor DP, Adl S, Simpson AGB, Worm B. 2011. How many species are there on Earth and in the ocean? *PLoS Biology* **9:** e1001127.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca G, Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403:** 853–858.

Nelson DR. 2002. Current status of the Tardigrada: evolution and ecology. *Integrative and Comparative Biology* **42:** 652–659.

Nelson DR, Guidetti R, Rebecchi L. 2015. Chapter 17 Phylum Tardigrada. In: Thorp JH, Covich AP, eds. *Thorp and Covich's freshwater invertebrates, Fourth Edition. Volume 1, ecology and general biology*, Chapter 17. Oxford, UK: Elsevier, 347–380.

Pilato G, Binda MG. 2010. Definition of families, subfamilies, genera and subgenera of the Eutardigrada, and keys to their identification. *Zootaxa* **2404:** 1–54.

Poore G, Wilson G. 1993. Marine species richness. *Nature* **361:** 597–598.

Roberts CM, McClean CJ, Veron JEN, Hawkins JP, Allen GR, McAllister DE, Mittermeir CG, Schueler FW, Spalding M, Wells F, Vynne C, Werner TB. 2002. Marine biodiversity hotspots and conservation priorities for tropical reefs. *Science* **295:** 1280–1284.

Sands CJ, Convey P, Linse K, McInnes SJ. 2008a. Assessing meiofaunal variation among individuals utilizing morphological and molecular approaches: an example using the Tardigrada. *BMC Ecology* **8:** 7.

Sands J, McInnes SJ, Marley NJ, Goodall-Copestake WP, Convey P, Linse K. 2008b. Phylum Tardigrada: an "individual" approach. *Cladistics* **24:** 861–871.

Scheffers BR, Joppa LN, Pimm SL, Laurance WF. 2012. What we know and don't know about Earth's missing biodiversity. *Trends in Ecology and Evolution* **27:** 712–713.

Vicente F. 2010. Micro-invertebrates conservation: forgotten biodiversity. *Biodiversity and Conservation* **19:** 3629–3634.

Vicente F, Bertolani R. 2013. Considerations on the taxonomy of the Phylum Tardigrada. *Zootaxa* **3626:** 245–248.

Welnicz W, Grohme MA, Kaczmarek Ł, Schill RO, Frohme M. 2011. Anhydrobiosis in tardigrades – The last decade. *Journal of Insect Physiology* **57:** 577–583.

Whittaker RJ, Arau′jo MB, Paul J, Ladle RJ, Watson JEM, Willis KJ. 2005. Conservation biogeography: assessment and prospect. *Diversity and Distributions* **11:** 3–23.

Zhang Z-Q. 2011. Animal biodiversity: an introduction to higher-level classification and taxonomic richness. *Zootaxa* **3148:** 7–12.