

## Inconsistent estimates of diversity between traditional and DNA taxonomy in bdelloid rotifers

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### Abstract

Microscopic animals offer great potential in the analysis of spatial patterns of diversity, as they may provide different scenarios for biogeography and macroecology, but understanding diversity of microscopic animals is hampered by lack of comprehensive data on species distribution and by unreliable taxonomy. DNA taxonomy may prove useful in obtaining reliable data in the future, but we still do not know to what extent traditional and DNA taxonomy can be comparable for microscopic organisms. In this paper, we compare analyses and estimates of diversity at the level of species assemblage between traditional and DNA taxonomy for a group of moss-dwelling microscopic animals, bdelloid rotifers. The results are straightforward: Traditional species identification underestimates diversity by factors of 2 at the local and 2.5 at the regional scale. We discuss the results in the framework of current hypotheses on the distribution of microscopic animals.

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### Introduction

Analyses of patterns of diversity are common and form the empirical and theoretical bases for macroecology and biogeography both in terrestrial and aquatic environments (Brown 1999; Kent 2005; Hawkins et al. 2007). For macroscopic organisms, species identification may be considered reliable, and detailed distribution maps have been used for analyses of spatial patterns (e.g. Orme et al. 2006; Abell et al. 2008). Microscopic

animals, on the contrary, are not often used for macroecological purposes; nevertheless, they could offer great potential in the analysis of diversity, as they may provide different scenarios for biogeography and macroecology, due to their small size, desiccation resistance and dispersal abilities (Fenchel and Finlay 2004; Fontaneto et al. 2006, Guil et al. in press). Unfortunately, our understanding of diversity of microscopic animals is still hampered by lack of comprehensive data on species distribution and by unreliable taxonomy. More faunistic works need to be carried out to obtain useful distribution data, but lack of taxonomic expertise and unreliable taxonomy for most study groups are problematic.

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Molecular phylogeny and recent advances in DNA taxonomy offer new possibilities to overcome the taxonomic impediments of compiling reliable species lists. For macroscopic organisms, the use of DNA taxonomy is becoming a common tool in identifying species (Pons et al. 2006; Vogler and Monaghan 2007). DNA sequence analyses have also proven useful at the community level (Janzen et al. 2005; Lahaye et al. 2008), as well as to identifying otherwise unrecognisable larval stages (e.g. Scarabaeidae: Ahrens et al. 2007; Chironomidae: Pfenninger et al. 2007).

However, for microscopic animals all taxa studied in detail were shown to be composed of cryptic species complexes (e.g. Casu and Curini-Galletti 2006; Suatoni et al. 2006; Heethoff et al. 2007; Fontaneto et al. 2008b), which highlights that diversity may be largely underestimated. Furthermore, most if not all studies on microscopic organisms focus on a single morphospecies, and we still cannot predict the effect of such hidden diversity on the analysis of diversity at the level of species assemblages. Moreover, while cryptic species in macroscopic organisms are usually allopatric or at least partition their habitat (e.g. Highton 1995; Nicholls and Racey 2006), in microscopic organisms more cryptic species seem to be able to live together in the same species assemblage (Ciros-Pérez et al. 2001; Ricci 2001; Fontaneto et al. 2008b), and traditional taxonomy may actually underestimate both their local and global diversity.

The aim of the present study, therefore, is to compare diversity estimates from traditional species identification and from DNA taxonomy, using moss-dwelling bdelloid rotifers as a test case. The results confirm the hypothesis that traditional species identification underestimates diversity at all levels. We discuss the results in the framework of current hypotheses on distribution of microscopic animals.

## Material and methods

### Traditional taxonomy

We collected six dry mosses, three in Turkey and three in the UK, cutting 5 cm<sup>2</sup> each from the central part of the moss patch. In the lab, we rehydrated in a petri dish with distilled water 1 cm<sup>2</sup> cut from the central part of the sample; after 30 min, we aimed to collect between 40 and 50 living animals recovered from desiccation. Species identification was performed on these animals at 400–1000× following Donner (1965).

### DNA taxonomy

The same animals identified from traditional taxonomy were processed for DNA taxonomy. DNA was

extracted from single animals in 35 µl of chelex (InstaGene Matrix, Bio-Rad); for each individual, a part of the cytochrome *c* oxidase subunit I (COI) gene was PCR-amplified using optimised primers LCOI (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCOI (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al. 1994). Cycle conditions comprised initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 90 s, and a final extension step at 72 °C for 7 min. Cycle sequencing reactions were set up using PCR primers and the ABI Big Dye Terminator v1.1 kit, and run on an ABI 3770 automated sequencer. The sequences were checked and assembled using FINCHTV 1.4.0 (<http://www.geospiza.com/finchtv>), aligned and edited by eye with MACCLADE 4.07 (Maddison and Maddison 2005). The number of haplotypes per species was computed using DNASP 4.0 (Rozas et al. 2003).

Genetic differentiation among COI sequences was assessed by testing for the presence of clusters, evidence for independently evolving entities (Pons et al. 2006). An initial phylogenetic tree reconstruction was performed, using one single sequence for each haplotype: A Bayesian analysis was run in MRBAYES 3.1.1 (Ronquist and Huelsenbeck 2003) for 5 million generations with two parallel searches, using a GTR + invgamma model, which has been formally suggested by MRMODELTEST (Nylander 2004) as the best model for COI evolution. As outgroups, we used 22 COI sequences obtained from GenBank: 16 monogonont rotifers, one seisonid rotifer, and five acanthocephalans.

The protocol developed by Pons et al. (2006) under coalescent theory was used to detect independently evolving entities in bdelloids, optimising a threshold age, *T*, such that nodes before the threshold are considered as diversification events, with branching rate and scaling parameter estimated from the tree. Branches crossing the threshold define *k* clusters, each obeying a separate coalescent process. Using an R script written by TGB, models were fitted to an ultrametric tree (a rooted additive tree with terminal nodes equidistant from the root). The latter had been obtained by rate-smoothing the tree built by Bayesian analysis, using penalised likelihood in R8S and cross-validation to choose the optimal smoothing parameter (Sanderson 2002), which resulted equal to one. For further details on this procedure applied to bdelloid phylogenetic trees, refer to Fontaneto et al. (2007, 2008b).

### Alpha, beta, and gamma diversity

Alpha diversity was evaluated as the number of entities (traditional species or phylogenetic entities) identified from the animals collected from the moss.

Analysing about 50 animals from each sample might not be enough for standard analyses of diversity, but since our aim was to test differences between taxonomic methods, such differences can be seen using the same number of animals for each analysis. Beta diversity was computed as Bray–Curtis dissimilarity indexes between each pair of samples, for entities identified both by traditional and by DNA taxonomy. Gamma diversity was evaluated as the total number of entities from traditional and DNA taxonomy overall. The Chao algorithm (Chao 1987) was used to estimate the number of entities potentially present, for alpha and gamma diversity, and from traditional and DNA taxonomy.

We checked whether the number of entities from DNA taxonomy was a function of the number of analysed specimens, using a generalised linear model (GLM). To test whether the number of entities from DNA taxonomy was significantly higher than that from traditional taxonomy, we used a mixed effects model (MEM). GLM and MEM were fitted in R 2.5 (R Development Core Team 2007), using the functions `glm`, package `stats`, and `lmer`, package `lme4`, respectively, assuming Poisson distributions for count data, and using sample as a random factor for comparison of estimates of richness from the same sample. Correlations were performed using Kendall's  $\tau$  for paired data. A Mantel test was performed to compare matrices of dissimilarities.

## Results

### Traditional vs. DNA taxonomy

Overall, among 264 animals sorted from six mosses, 22 traditional species were found. Seven of them are probably new species. Nevertheless, we could place all of them into separate, still unnamed, morphospecies; five belonged to the family Habrotrochidae, one to the order Philodinida, and one to the genus *Philodina* (Table 1).

The same 264 animals identified from traditional taxonomy were processed for amplification of partial COI gene (GenBank accession numbers EU751023–EU751286; Table 2). Overall, 117 haplotypes were found, and the molecular phylogenetic analysis provided evidence of 57 independently evolving entities (Fig. 1).

Among species with at least seven individuals, only *Adineta barbata*, *Macrotrachela multispinosa*, and *Philodina duplicalcar* comprised just one phylogenetic entity; *Macrotrachela latior* had four phylogenetic entities, belonging to a single highly supported monophyletic clade; all other species were represented by more, non-monophyletic phylogenetic entities (Fig. 1). The number of phylogenetic entities found within each traditional species was a function of the number of analysed specimens (GLM with the Poisson distribution,  $r^2 = 0.95$ ,  $p < 0.0001$ ). Nevertheless, within each traditional species, phylogenetic diversification was not

**Table 1.** Number of individuals of bdelloid rotifers analysed per species and sample, number of haplotypes, and number of independently evolving entities (from DNA taxonomy based on partial COI sequences).

Species	TU1	TU2	TU3	UK1	UK2	UK3	Haplotypes	Entities
<i>Adineta barbata</i> Janson, 1893	—	—	—	—	7	—	1	1
<i>Adineta steineri</i> Bartoš, 1951	—	—	—	—	—	2	2	1
<i>Adineta vaga</i> (Davis, 1873)	—	16	2	25	2	—	17	6
Bdelloid nd	—	—	11	—	—	—	7	5
Habrotrochidae nd 1	—	—	—	—	—	5	3	3
Habrotrochidae nd 2	1	—	—	—	—	—	1	1
Habrotrochidae nd 3	—	14	—	—	—	—	6	1
Habrotrochidae nd 4	—	—	2	—	—	—	2	2
Habrotrochidae nd 5	—	—	—	4	—	—	4	4
<i>Macrotrachela ehrenbergii</i> (Janson, 1893)	—	—	—	—	23	—	2	2
<i>Macrotrachela habita</i> (Bryce, 1894)	—	—	—	—	1	—	1	1
<i>Macrotrachela latior</i> Donner, 1951	—	—	19	—	—	—	12	4
<i>Macrotrachela multispinosa</i> Thompson, 1892	—	—	—	—	—	14	5	1
<i>Macrotrachela quadricornifera</i> Milne, 1886	—	—	—	10	7	5	7	5
<i>Philodina acuticornis</i> Murray, 1902	—	9	—	—	—	—	6	2
<i>Philodina duplicalcar</i> (de Koning, 1947)	—	9	—	—	—	—	3	1
<i>Philodina plena</i> (Bryce, 1894)	45	—	—	—	—	—	25	7
<i>Philodina</i> sp.	—	—	—	—	1	—	1	1
<i>Philodina vorax</i> (Janson, 1893)	—	1	—	—	—	—	1	1
<i>Pleuretra lineata</i> Donner, 1962	—	—	—	1	1	—	2	1
<i>Rotaria rotatoria</i> (Pallas, 1766)	—	—	—	—	1	—	1	1
<i>Rotaria sordida</i> (Western, 1893)	5	—	—	1	2	18	8	6

**Table 2.** List of all analysed individuals, with corresponding samples of origin, species identifications, and GenBank accession numbers for the partial COI sequence.

Specimen	Sample	Species	Acc. no.
A.barb.UK.a	UK2	<i>A. barbata</i>	EU751029
A.barb.UK.a.1	UK2	<i>A. barbata</i>	EU751023
A.barb.UK.a.2	UK2	<i>A. barbata</i>	EU751024
A.barb.UK.a.3	UK2	<i>A. barbata</i>	EU751025
A.barb.UK.a.4	UK2	<i>A. barbata</i>	EU751026
A.barb.UK.a.5	UK2	<i>A. barbata</i>	EU751027
A.barb.UK.a.6	UK2	<i>A. barbata</i>	EU751028
A.stei.UK.a	UK3	<i>A. steineri</i>	EU751080
A.stei.UK.b	UK3	<i>A. steineri</i>	EU751081
A.vaga.TU.a	TU2	<i>A. vaga</i>	EU751167
A.vaga.TU.a.1	TU2	<i>A. vaga</i>	EU751164
A.vaga.TU.a.2	TU2	<i>A. vaga</i>	EU751165
A.vaga.TU.a.3	TU2	<i>A. vaga</i>	EU751166
A.vaga.TU.b	TU2	<i>A. vaga</i>	EU751168
A.vaga.TU.c	TU2	<i>A. vaga</i>	EU751168
A.vaga.TU.d	TU2	<i>A. vaga</i>	EU751170
A.vaga.TU.e	TU2	<i>A. vaga</i>	EU751171
A.vaga.TU.f	TU2	<i>A. vaga</i>	EU751172
A.vaga.TU.g	TU2	<i>A. vaga</i>	EU751173
A.vaga.TU.h	TU2	<i>A. vaga</i>	EU751174
A.vaga.TU.i	TU2	<i>A. vaga</i>	EU751175
A.vaga.TU.j	TU2	<i>A. vaga</i>	EU751176
A.vaga.TU.k	TU2	<i>A. vaga</i>	EU751177
A.vaga.TU.l	TU2	<i>A. vaga</i>	EU751178
A.vaga.TU.m	TU2	<i>A. vaga</i>	EU751179
A.vaga.TU.n	TU3	<i>A. vaga</i>	EU751213
A.vaga.TU.o	TU3	<i>A. vaga</i>	EU751214
A.vaga.UK.a	UK2	<i>A. vaga</i>	EU751078
A.vaga.UK.a.1	UK2	<i>A. vaga</i>	EU751077
A.vaga.UK.b	UK1	<i>A. vaga</i>	EU751271
A.vaga.UK.b.1	UK1	<i>A. vaga</i>	EU751247
A.vaga.UK.b.10	UK1	<i>A. vaga</i>	EU751256
A.vaga.UK.b.11	UK1	<i>A. vaga</i>	EU751257
A.vaga.UK.b.12	UK1	<i>A. vaga</i>	EU751258
A.vaga.UK.b.13	UK1	<i>A. vaga</i>	EU751259
A.vaga.UK.b.14	UK1	<i>A. vaga</i>	EU751260
A.vaga.UK.b.15	UK1	<i>A. vaga</i>	EU751261
A.vaga.UK.b.16	UK1	<i>A. vaga</i>	EU751262
A.vaga.UK.b.17	UK1	<i>A. vaga</i>	EU751263
A.vaga.UK.b.18	UK1	<i>A. vaga</i>	EU751264
A.vaga.UK.b.19	UK1	<i>A. vaga</i>	EU751265
A.vaga.UK.b.2	UK1	<i>A. vaga</i>	EU751248
A.vaga.UK.b.20	UK1	<i>A. vaga</i>	EU751266
A.vaga.UK.b.21	UK1	<i>A. vaga</i>	EU751267
A.vaga.UK.b.22	UK1	<i>A. vaga</i>	EU751268
A.vaga.UK.b.23	UK1	<i>A. vaga</i>	EU751269
A.vaga.UK.b.24	UK1	<i>A. vaga</i>	EU751270
A.vaga.UK.b.3	UK1	<i>A. vaga</i>	EU751249
A.vaga.UK.b.4	UK1	<i>A. vaga</i>	EU751250
A.vaga.UK.b.5	UK1	<i>A. vaga</i>	EU751251
A.vaga.UK.b.6	UK1	<i>A. vaga</i>	EU751252
A.vaga.UK.b.7	UK1	<i>A. vaga</i>	EU751253
A.vaga.UK.b.8	UK1	<i>A. vaga</i>	EU751254
A.vaga.UK.b.9	UK1	<i>A. vaga</i>	EU751255

**Table 2. (continued)**

Specimen	Sample	Species	Acc. no.
Bdello.TU.a	TU3	Bdelloid nd	EU751223
Bdello.TU.b	TU3	Bdelloid nd	EU751227
Bdello.TU.b.1	TU3	Bdelloid nd	EU751224
Bdello.TU.b.2	TU3	Bdelloid nd	EU751225
Bdello.TU.b.3	TU3	Bdelloid nd	EU751226
Bdello.TU.c	TU3	Bdelloid nd	EU751228
Bdello.TU.d	TU3	Bdelloid nd	EU751229
Bdello.TU.e	TU3	Bdelloid nd	EU751231
Bdello.TU.e.1	TU3	Bdelloid nd	EU751230
Bdello.TU.f	TU3	Bdelloid nd	EU751236
Bdello.TU.g	TU3	Bdelloid nd	EU751237
Habro.TU.a	TU1	Habrotrochidae nd 2	EU751118
Habro.TU.b	TU2	Habrotrochidae nd 3	EU751180
Habro.TU.c	TU2	Habrotrochidae nd 3	EU751189
Habro.TU.c.1	TU2	Habrotrochidae nd 3	EU751181
Habro.TU.c.2	TU2	Habrotrochidae nd 3	EU751182
Habro.TU.c.3	TU2	Habrotrochidae nd 3	EU751183
Habro.TU.c.4	TU2	Habrotrochidae nd 3	EU751184
Habro.TU.c.5	TU2	Habrotrochidae nd 3	EU751185
Habro.TU.c.6	TU2	Habrotrochidae nd 3	EU751185
Habro.TU.c.7	TU2	Habrotrochidae nd 3	EU751187
Habro.TU.c.8	TU2	Habrotrochidae nd 3	EU751188
Habro.TU.d	TU2	Habrotrochidae nd 3	EU751190
Habro.TU.e	TU2	Habrotrochidae nd 3	EU751191
Habro.TU.f	TU2	Habrotrochidae nd 3	EU751192
Habro.TU.g	TU2	Habrotrochidae nd 3	EU751193
Habro.TU.h	TU3	Habrotrochidae nd 4	EU751240
Habro.TU.i	TU3	Habrotrochidae nd 4	EU751242
Habro.TU.j	UK3	Habrotrochidae nd 1	EU751083
Habro.UK.a.1	UK3	Habrotrochidae nd 1	EU751082
Habro.UK.b	UK3	Habrotrochidae nd 1	EU751085
Habro.UK.b.1	UK3	Habrotrochidae nd 1	EU751084
Habro.UK.c	UK3	Habrotrochidae nd 1	EU751086
Habro.UK.d	UK1	Habrotrochidae nd 5	EU751272
Habro.UK.e	UK1	Habrotrochidae nd 5	EU751273
Habro.UK.f	UK1	Habrotrochidae nd 5	EU751274
Habro.UK.g	UK1	Habrotrochidae nd 5	EU751275
M.ehre.UK.a	UK2	<i>M. ehrenbergii</i>	EU751050
M.ehre.UK.a.1	UK2	<i>M. ehrenbergii</i>	EU751030
M.ehre.UK.a.10	UK2	<i>M. ehrenbergii</i>	EU751039
M.ehre.UK.a.11	UK2	<i>M. ehrenbergii</i>	EU751040
M.ehre.UK.a.12	UK2	<i>M. ehrenbergii</i>	EU751041
M.ehre.UK.a.13	UK2	<i>M. ehrenbergii</i>	EU751042
M.ehre.UK.a.14	UK2	<i>M. ehrenbergii</i>	EU751043
M.ehre.UK.a.15	UK2	<i>M. ehrenbergii</i>	EU751044
M.ehre.UK.a.16	UK2	<i>M. ehrenbergii</i>	EU751045
M.ehre.UK.a.17	UK2	<i>M. ehrenbergii</i>	EU751046
M.ehre.UK.a.18	UK2	<i>M. ehrenbergii</i>	EU751047
M.ehre.UK.a.19	UK2	<i>M. ehrenbergii</i>	EU751048
M.ehre.UK.a.2	UK2	<i>M. ehrenbergii</i>	EU751031
M.ehre.UK.a.20	UK2	<i>M. ehrenbergii</i>	EU751049
M.ehre.UK.a.3	UK2	<i>M. ehrenbergii</i>	EU751032
M.ehre.UK.a.4	UK2	<i>M. ehrenbergii</i>	EU751033
M.ehre.UK.a.5	UK2	<i>M. ehrenbergii</i>	EU751034
M.ehre.UK.a.6	UK2	<i>M. ehrenbergii</i>	EU751035
M.ehre.UK.a.7	UK2	<i>M. ehrenbergii</i>	EU751036

**Table 2.** (continued)

Specimen	Sample	Species	Acc. no.
M.ehre.UK.a.8	UK2	<i>M. ehrenbergii</i>	EU751037
M.ehre.UK.a.9	UK2	<i>M. ehrenbergii</i>	EU751038
M.ehre.UK.b	UK2	<i>M. ehrenbergii</i>	EU751052
M.ehre.UK.b.1	UK2	<i>M. ehrenbergii</i>	EU751051
M.habi.UK.a	UK2	<i>M. habita</i>	EU751072
M.lati.TU.a	TU3	<i>M. latior</i>	EU751220
M.lati.TU.a.1	TU3	<i>M. latior</i>	EU751215
M.lati.TU.a.2	TU3	<i>M. latior</i>	EU751216
M.lati.TU.a.3	TU3	<i>M. latior</i>	EU751217
M.lati.TU.a.4	TU3	<i>M. latior</i>	EU751218
M.lati.TU.a.5	TU3	<i>M. latior</i>	EU751219
M.lati.TU.b	TU3	<i>M. latior</i>	EU751222
M.lati.TU.b.1	TU3	<i>M. latior</i>	EU751221
M.lati.TU.c	TU3	<i>M. latior</i>	EU751233
M.lati.TU.c.1	TU3	<i>M. latior</i>	EU751232
M.lati.TU.d	TU3	<i>M. latior</i>	EU751234
M.lati.TU.e	TU3	<i>M. latior</i>	EU751235
M.lati.TU.f	TU3	<i>M. latior</i>	EU751238
M.lati.TU.g	TU3	<i>M. latior</i>	EU751239
M.lati.TU.h	TU3	<i>M. latior</i>	EU751241
M.lati.TU.i	TU3	<i>M. latior</i>	EU751243
M.lati.TU.j	TU3	<i>M. latior</i>	EU751244
M.lati.TU.k	TU3	<i>M. latior</i>	EU751245
M.lati.TU.l	TU3	<i>M. latior</i>	EU751246
M.mult.UK.a	UK3	<i>M. multispinosa</i>	EU751090
M.mult.UK.a.1	UK3	<i>M. multispinosa</i>	EU751087
M.mult.UK.a.2	UK3	<i>M. multispinosa</i>	EU751088
M.mult.UK.a.3	UK3	<i>M. multispinosa</i>	EU751089
M.mult.UK.b	UK3	<i>M. multispinosa</i>	EU751095
M.mult.UK.b.1	UK3	<i>M. multispinosa</i>	EU751091
M.mult.UK.b.2	UK3	<i>M. multispinosa</i>	EU751092
M.mult.UK.b.3	UK3	<i>M. multispinosa</i>	EU751093
M.mult.UK.b.4	UK3	<i>M. multispinosa</i>	EU751094
M.mult.UK.c	UK3	<i>M. multispinosa</i>	EU751097
M.mult.UK.c.1	UK3	<i>M. multispinosa</i>	EU751096
M.mult.UK.d	UK3	<i>M. multispinosa</i>	EU751099
M.mult.UK.d.1	UK3	<i>M. multispinosa</i>	EU751098
M.mult.UK.e	UK3	<i>M. multispinosa</i>	EU751100
M.quad.UK.a	UK2	<i>M. quadricornifera</i>	EU751055
M.quad.UK.a.1	UK2	<i>M. quadricornifera</i>	EU751053
M.quad.UK.a.2	UK2	<i>M. quadricornifera</i>	EU751054
M.quad.UK.b	UK2	<i>M. quadricornifera</i>	EU751074
M.quad.UK.b.1	UK2	<i>M. quadricornifera</i>	EU751073
M.quad.UK.c	UK2	<i>M. quadricornifera</i>	EU751076
M.quad.UK.c.1	UK2	<i>M. quadricornifera</i>	EU751075
M.quad.UK.d	UK3	<i>M. quadricornifera</i>	EU751101
M.quad.UK.e	UK3	<i>M. quadricornifera</i>	EU751105
M.quad.UK.e.1	UK3	<i>M. quadricornifera</i>	EU751102
M.quad.UK.e.2	UK3	<i>M. quadricornifera</i>	EU751103
M.quad.UK.e.3	UK3	<i>M. quadricornifera</i>	EU751104
M.quad.UK.f	UK1	<i>M. quadricornifera</i>	EU751284
M.quad.UK.f.1	UK1	<i>M. quadricornifera</i>	EU751284
M.quad.UK.f.2	UK1	<i>M. quadricornifera</i>	EU751277
M.quad.UK.f.3	UK1	<i>M. quadricornifera</i>	EU751278
M.quad.UK.f.4	UK1	<i>M. quadricornifera</i>	EU751279
M.quad.UK.f.5	UK1	<i>M. quadricornifera</i>	EU751280
M.quad.UK.f.6	UK1	<i>M. quadricornifera</i>	EU751281

**Table 2.** (continued)

Specimen	Sample	Species	Acc. no.
M.quad.UK.f.7	UK1	<i>M. quadricornifera</i>	EU751282
M.quad.UK.f.8	UK1	<i>M. quadricornifera</i>	EU751283
M.quad.UK.g	UK1	<i>M. quadricornifera</i>	EU751285
P.acut.TU.a	TU2	<i>Ph. acuticornis</i>	EU751195
P.acut.TU.a.1	TU2	<i>Ph. acuticornis</i>	EU751194
P.acut.TU.b	TU2	<i>Ph. acuticornis</i>	EU751198
P.acut.TU.b.1	TU2	<i>Ph. acuticornis</i>	EU751196
P.acut.TU.b.2	TU2	<i>Ph. acuticornis</i>	EU751197
P.acut.TU.c	TU2	<i>Ph. acuticornis</i>	EU751199
P.acut.TU.d	TU2	<i>Ph. acuticornis</i>	EU751200
P.acut.TU.e	TU2	<i>Ph. acuticornis</i>	EU751201
P.acut.TU.f	TU2	<i>Ph. acuticornis</i>	EU751202
P.dupl.TU.a	TU2	<i>Ph. dupicalcar</i>	EU751203
P.dupl.TU.b	TU2	<i>Ph. dupicalcar</i>	EU751208
P.dupl.TU.b.1	TU2	<i>Ph. dupicalcar</i>	EU751204
P.dupl.TU.b.2	TU2	<i>Ph. dupicalcar</i>	EU751205
P.dupl.TU.b.3	TU2	<i>Ph. dupicalcar</i>	EU751206
P.dupl.TU.b.4	TU2	<i>Ph. dupicalcar</i>	EU751207
P.dupl.TU.c	TU2	<i>Ph. dupicalcar</i>	EU751211
P.dupl.TU.c.1	TU2	<i>Ph. dupicalcar</i>	EU751209
P.dupl.TU.c.2	TU2	<i>Ph. dupicalcar</i>	EU751210
P.plen.TU.a	TU1	<i>Ph. plena</i>	EU751117
P.plen.TU.a.1	TU1	<i>Ph. plena</i>	EU751113
P.plen.TU.a.2	TU1	<i>Ph. plena</i>	EU751114
P.plen.TU.a.3	TU1	<i>Ph. plena</i>	EU751115
P.plen.TU.a.4	TU1	<i>Ph. plena</i>	EU751116
P.plen.TU.b	TU1	<i>Ph. plena</i>	EU751123
P.plen.TU.b.1	TU1	<i>Ph. plena</i>	EU751122
P.plen.TU.c	TU1	<i>Ph. plena</i>	EU751127
P.plen.TU.c.1	TU1	<i>Ph. plena</i>	EU751124
P.plen.TU.c.2	TU1	<i>Ph. plena</i>	EU751125
P.plen.TU.c.3	TU1	<i>Ph. plena</i>	EU751126
P.plen.TU.d	TU1	<i>Ph. plena</i>	EU751128
P.plen.TU.e	TU1	<i>Ph. plena</i>	EU751130
P.plen.TU.e.1	TU1	<i>Ph. plena</i>	EU751129
P.plen.TU.f	TU1	<i>Ph. plena</i>	EU751131
P.plen.TU.g	TU1	<i>Ph. plena</i>	EU751134
P.plen.TU.g.1	TU1	<i>Ph. plena</i>	EU751132
P.plen.TU.g.2	TU1	<i>Ph. plena</i>	EU751133
P.plen.TU.h	TU1	<i>Ph. plena</i>	EU751135
P.plen.TU.i	TU1	<i>Ph. plena</i>	EU751136
P.plen.TU.j	TU1	<i>Ph. plena</i>	EU751138
P.plen.TU.j.1	TU1	<i>Ph. plena</i>	EU751137
P.plen.TU.k	TU1	<i>Ph. plena</i>	EU751139
P.plen.TU.l	TU1	<i>Ph. plena</i>	EU751144
P.plen.TU.l.1	TU1	<i>Ph. plena</i>	EU751140
P.plen.TU.l.2	TU1	<i>Ph. plena</i>	EU751141
P.plen.TU.l.3	TU1	<i>Ph. plena</i>	EU751142
P.plen.TU.l.4	TU1	<i>Ph. plena</i>	EU751143
P.plen.TU.m	TU1	<i>Ph. plena</i>	EU751145
P.plen.TU.n	TU1	<i>Ph. plena</i>	EU751150
P.plen.TU.n.1	TU1	<i>Ph. plena</i>	EU751146
P.plen.TU.n.2	TU1	<i>Ph. plena</i>	EU751147
P.plen.TU.n.3	TU1	<i>Ph. plena</i>	EU751148
P.plen.TU.n.4	TU1	<i>Ph. plena</i>	EU751149
P.plen.TU.o	TU1	<i>Ph. plena</i>	EU751151
P.plen.TU.p	TU1	<i>Ph. plena</i>	EU751152

**Table 2.** (continued)

Specimen	Sample	Species	Acc. no.
P.plen.TU.q	TU1	<i>Ph. plena</i>	EU751153
P.plen.TU.r	TU1	<i>Ph. plena</i>	EU751154
P.plen.TU.s	TU1	<i>Ph. plena</i>	EU751155
P.plen.TU.t	TU1	<i>Ph. plena</i>	EU751156
P.plen.TU.u	TU1	<i>Ph. plena</i>	EU751157
P.plen.TU.v	TU1	<i>Ph. plena</i>	EU751158
P.plen.TU.w	TU1	<i>Ph. plena</i>	EU751159
P.plen.TU.x	TU1	<i>Ph. plena</i>	EU751160
P.plen.TU.y	TU1	<i>Ph. plena</i>	EU751163
P.sp.UK.a	UK2	<i>Ph. sp.</i>	EU751056
P.vora.TU.a	TU2	<i>Ph. vorax</i>	EU751212
P.line.UK.a	UK2	<i>Pl. lineata</i>	EU751079
P.line.UK.b	UK1	<i>Pl. lineata</i>	EU751286
R.rota.UK.a	UK2	<i>R. rotatoria</i>	EU751057
R.sord.TU.a	TU1	<i>R. sordida</i>	EU751121
R.sord.TU.a.1	TU1	<i>R. sordida</i>	EU751119
R.sord.TU.a.2	TU1	<i>R. sordida</i>	EU751120
R.sord.TU.b	TU1	<i>R. sordida</i>	EU751162
R.sord.TU.b.1	TU1	<i>R. sordida</i>	EU751161
R.sord.UK.a	UK2	<i>R. sordida</i>	EU751059
R.sord.UK.a.1	UK1	<i>R. sordida</i>	EU751058
R.sord.UK.b	UK2	<i>R. sordida</i>	EU751071
R.sord.UK.b.1	UK3	<i>R. sordida</i>	EU751060
R.sord.UK.b.10	UK3	<i>R. sordida</i>	EU751069
R.sord.UK.b.11	UK3	<i>R. sordida</i>	EU751070
R.sord.UK.b.2	UK3	<i>R. sordida</i>	EU751061
R.sord.UK.b.3	UK3	<i>R. sordida</i>	EU751062
R.sord.UK.b.4	UK3	<i>R. sordida</i>	EU751063
R.sord.UK.b.5	UK3	<i>R. sordida</i>	EU751064
R.sord.UK.b.6	UK3	<i>R. sordida</i>	EU751065
R.sord.UK.b.7	UK3	<i>R. sordida</i>	EU751066
R.sord.UK.b.8	UK3	<i>R. sordida</i>	EU751067
R.sord.UK.b.9	UK3	<i>R. sordida</i>	EU751068
R.sord.UK.c	UK3	<i>R. sordida</i>	EU751109
R.sord.UK.c.1	UK3	<i>R. sordida</i>	EU751106
R.sord.UK.c.2	UK3	<i>R. sordida</i>	EU751107
R.sord.UK.c.3	UK3	<i>R. sordida</i>	EU751108
R.sord.UK.d	UK3	<i>R. sordida</i>	EU751110
R.sord.UK.e	UK3	<i>R. sordida</i>	EU751111
R.sord.UK.f	UK3	<i>R. sordida</i>	EU751112

Abbreviations of genus names: *A.* = *Adineta*, *M.* = *Macrotrachela*, *Ph.* = *Philodina*, *Pl.* = *Pleuretra*, *R.* = *Rotaria*.

consistent locally: for example, all 25 individuals of *Adineta vaga* found in one British sample had the same haplotype, whereas 13 haplotypes belonging to three phylogenetic entities were found among 16 *A. vaga* individuals from a Turkish sample (Fig. 1).

**Fig. 1.** Phylogenetic relationships inferred from COI mtDNA dataset, after collapsing all sequences of a given haplotype into a single entry. Consensus of 70,000 sampled trees from Bayesian analysis, displaying all compatible groupings, with average branch lengths proportional to numbers of substitutions per site under a GTR + invgamma substitution model. Posterior probabilities  $>0.75$  shown, except for very short terminal branches. Solid circles indicate clusters and singletons identified as independently evolving entities by phylogenetic analysis. Labels at terminal ends identify specimens, including information on the respective species, country of origin, and haplotype (number of corresponding sequences between brackets); for further details, see Tables 1 and 2. Scale bar = 0.1 substitutions/site.

## Alpha diversity

Locally, DNA taxonomy provided higher observed and estimated richness than traditional taxonomy. The number of species in each sample ranged from 3 to 9; the number of phylogenetic entities was on average two times higher than the number of traditional species and ranged from 8 to 12 (Fig. 2). The number of phylogenetic entities was significantly higher (mixed effect model with the Poisson distribution,  $p = 0.002$ ) than, but not significantly correlated with, the number of traditional species across samples (Kendall's  $\tau = 0.08$ ,  $p = 0.83$ ).

Estimated richness (using Chao) was not significantly higher than the observed richness in the case of traditional taxonomy ( $p = 0.71$ ), but the difference was significant from DNA taxonomy ( $p = 0.02$ ) (Fig. 2). Moreover, for traditional species estimated richness was positively correlated with observed richness ( $\tau = 0.92$ ,  $p = 0.014$ ), whereas for phylogenetic entities it was not ( $\tau = 0.29$ ,  $p = 0.43$ ).

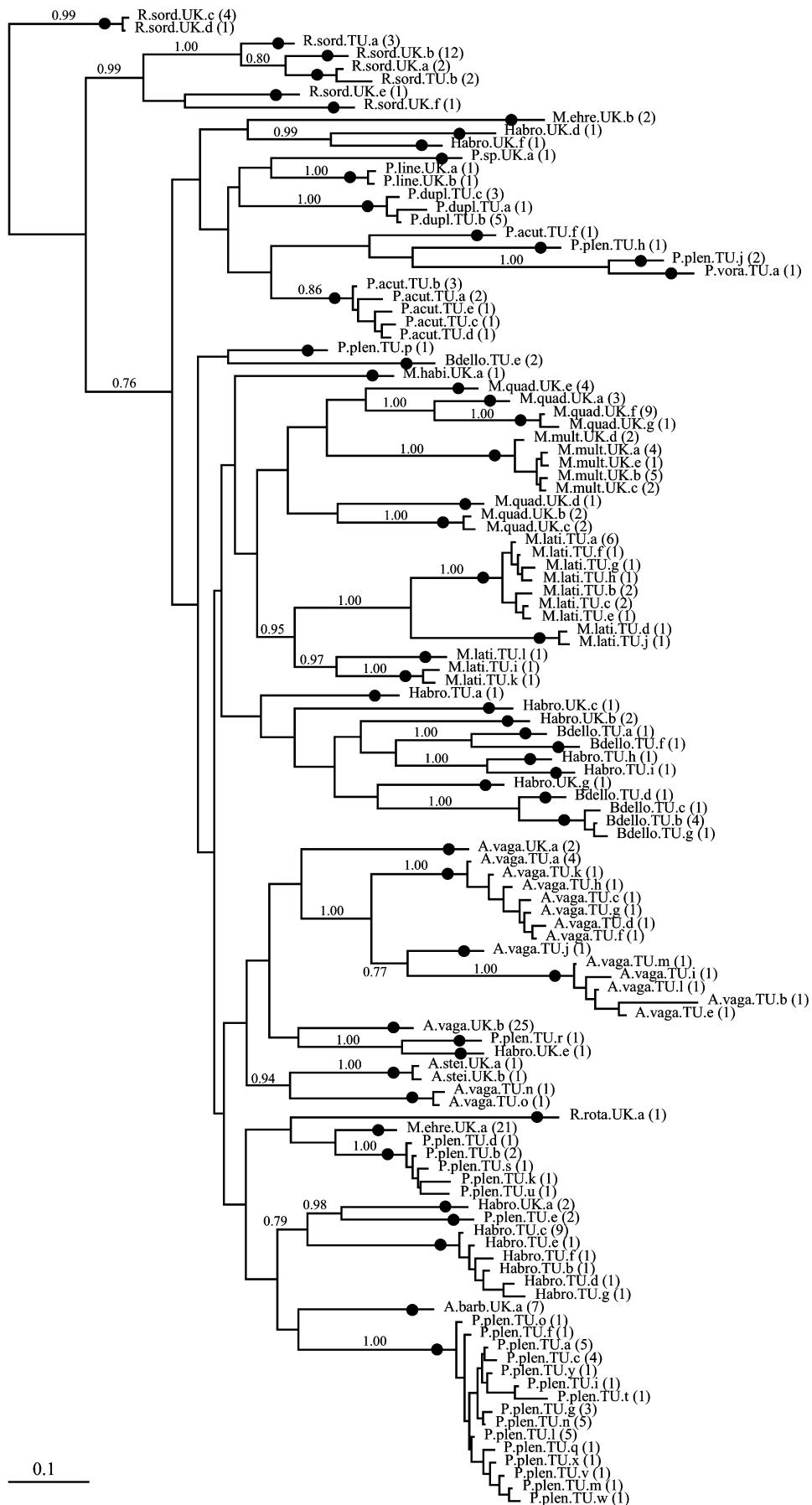
## Beta diversity

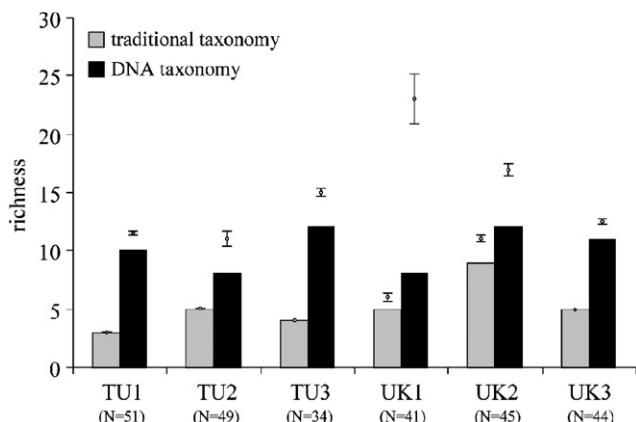
Molecular phylogeny, resolving more groups within each traditional species, was expected to reduce similarities between samples, and that expectation was fulfilled: the beta diversities obtained as Bray–Curtis dissimilarity indexes from traditional and DNA taxonomy are not significantly correlated (the Mantel test:  $r = 0.36$ ,  $p = 0.12$ ), with higher dissimilarity values from molecular phylogeny (Fig. 3).

Overall, with traditional species identification, four taxa out of 22 (18%) were present in more than one sample: *Adineta vaga* and *Rotaria sordida* were shared between Turkey and the UK, and *Macrotrachela quadricornifera* and *Pleuretra lineata* between different UK samples. Using phylogenetic entities, only three taxa out of 57 (5%) were present in more than one sample: two taxa were present at two British samples (*P. lineata* and a taxon in *R. sordida*), one single taxon in *R. sordida* was shared between Turkey and the UK.

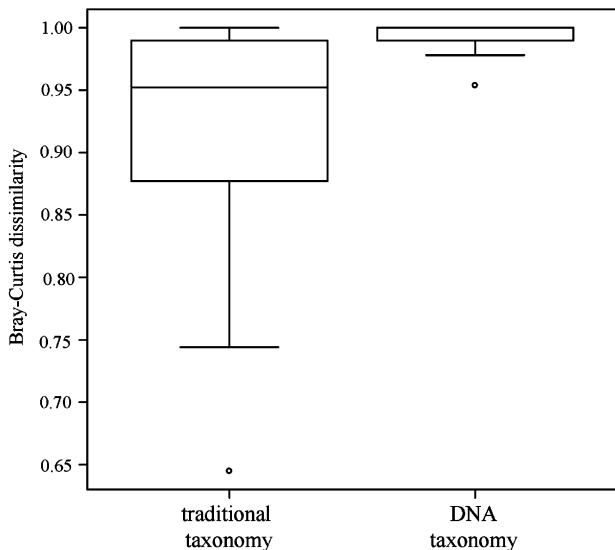
## Gamma diversity

The overall number of entities was higher from molecular phylogeny (57) than from traditional species identification (22). The estimated species richness,





**Fig. 2.** Observed number of species according to traditional taxonomy (grey bars), and independently evolving entities according to DNA taxonomy (black bars); Chao-estimated values provided above each bar as dots  $\pm 1$  SE.



**Fig. 3.** Box plot of distribution of Bray–Curtis dissimilarity indexes between species assemblages as obtained from traditional and DNA taxonomy.

according to the Chao algorithm, was much higher from molecular phylogeny ( $78.2 \pm 0.45$  (1 SE)) than from traditional taxonomy ( $24.5 \pm 0.13$ ). Moreover, 24 out of 57 (42%) phylogenetic entities were represented by single individuals.

## Discussion

Lack of taxonomic expertise makes testing macroecological hypotheses challenging for microscopic organisms. One way to overcome the problem could

be the application of DNA taxonomy to environmental samples to obtain the necessary distribution data, as has been done for bacteria and protists (Rusch et al. 2007; Strom 2008). Unfortunately, we demonstrate that analyses of diversity through traditional species identification and through DNA taxonomy are not congruent. They are quite different, especially if we compare the observed and estimated number of taxa both locally and globally. Using traditional taxonomy, the number of estimated taxa is not significantly higher than the observed one, whereas using DNA taxonomy estimated richness always results much higher than the observed one. This outcome, resulting from different resolution and lumping, would also affect the reliability of sampling procedure for each kind of taxonomical analysis: 50 animals could be considered enough for community analyses using traditional taxonomy, as observed and estimated diversity are not different. The same number of animals will not be enough to describe diversity using DNA taxonomy, as the estimated number is much higher than the observed one.

If we assume the results from DNA taxonomy to be a more reliable proxy for ‘true’ diversity, the traditional species identification approach currently used to support global patterns of distribution in the framework of the ‘everything-is-everywhere’ hypothesis (Fenchel and Finlay 2004; Fontaneto et al. 2006; Guil et al. in press) may be misleading.

DNA taxonomy and specifically DNA barcoding have been claimed to be highly accurate in species identification in macroscopic organisms (Hebert et al. 2004; Pons et al. 2006; Clare et al. 2007). However, DNA taxonomy is not always so efficient (Boyer et al. 2007; Elias et al. 2007; Whitworth et al. 2007). Problems in DNA taxonomy for macroscopic organisms usually arise when different populations are analysed, as more diversity than expected tends to fill the gap between species. Moreover, according to the monopolisation hypothesis (De Meester et al. 2002), within-population genetic divergence in freshwater microscopic animals tends to be much lower than between-population divergence. We found evidence for this trend, but also for the exact opposite. For example, populations of *A. vaga* conformed to the monopolisation hypothesis, with no shared entities between populations. On the other hand, populations of *R. sordida* hosted more phylogenetic entities shared between samples, even between Turkey and the UK. The outcome for the latter species contrasts with the idea that it may be a confounding effect of within-populations similarity that affects DNA taxonomy.

It has been demonstrated already that some microscopic organisms such as bdelloids can potentially achieve global distribution (Fontaneto et al. 2008a). Nevertheless, spatial patterns of diversification can be

completely different for different species even between closely related taxa. Therefore, no generalisation is possible for microscopic organisms, other than that DNA taxonomy is providing much higher estimates of species richness than traditional taxonomy, especially for gamma diversity. Such higher estimates from DNA taxonomy are already known also in macroscopic organisms; the phenomenon has been termed ‘taxonomic inflation’ (Padial and De la Riva 2006). Taxonomic inflation is notorious in conservation biology, to the extreme that each population may be considered as an independent unit of concern from DNA taxonomy (de Guia and Saitoh 2007). Nevertheless, in our system, spatially isolated populations were not always consistent with independently evolving entities from DNA taxonomy. The difference between macroscopic and microscopic organisms is that for the former, inflation due to DNA taxonomy is considered responsible for only a small fraction of the whole increase in species numbers (Padial and De la Riva 2006), while for the latter the number of cryptic species uncovered by DNA taxonomy may be overwhelming (Suatoni et al. 2006; Heethoff et al. 2007; Fontaneto et al. 2008b). Differences between traditional and DNA taxonomy will affect macroecological hypotheses. Analyses of spatial patterns of diversity implicitly assume species as discrete equivalent entities; thus, different approaches to identifying such entities may lead to different results. For microscopic organisms, the mere existence of most traditional species remains undiscovered, and we are far from unravelling the amount of cryptic species and their relationships. Much work still needs to be performed before reliable tests of macroecological hypotheses can be performed for microscopic organisms.

One caveat of our results is that bdelloid rotifers are obligate parthenogens; thus their species reality and spatial patterns may be completely different from other microscopic organisms. We cannot completely rule out that possibility, but morphological and evolutionary patterns of diversification in bdelloids have been demonstrated to be broadly equivalent to those found in sexual groups (Fontaneto et al. 2007), and the amount of hidden diversity within traditional species is comparable in sexual and asexual microscopic organisms (Suatoni et al. 2006; Derycke et al. 2007; Heethoff et al. 2007; Fontaneto et al. 2008b).

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