Freshwater sponge (Porifera: Spongillidae) distribution across a landscape: environmental tolerances, habitats, and morphological variation.

Karen L. Evans and David J. S. Montagnes

Institute of Integrative Biology, University of Liverpool, Crown Street, Liverpool, UK

Correspondence
Karen Evans, Institute of Integrative Biology, University of Liverpool, Crown Street, Liverpool, UK, L69 7ZB.
E-mail: karenevans2000@gmail.com

Abstract

Freshwater sponges are important to ecosystem functioning; however, information about their biogeography and interspecific variation is fragmentary, limiting our ability to assess their role. Although the specific epithets of two common species suggest that sponges found in lentic habitats are *Spongilla lacustris*, and those found in lotic habitats are *Ephydatia fluviatilis*, the number of sponge species in the UK is unresolved. We sampled sponges in a variety of habitats and used both morphological and molecular (D3 domain of 28S rDNA) methods to identify six species, including the first record of *Trochospongilla horrida*. We contrasted species in terms of their environmental tolerances, habitats, and variation, and we expanded on the limited information available about these sponges . In our study, both common and rarer sponges colonized a variety of substrates, but exhibited different distributions. The most widespread sponge, *S. lacustris*, was present at lower mean water temperatures and was more often located above a latitude of 55°N. *Ephydatia fluviatilis* was the most common species in rivers, but was also located in lentic habitats. Salinity in anthropogenic habitats was not a significant factor for the presence of *E. fluviatilis* or the more patchily distributed species *Eunapius fragilis*. Instead, these species occurred more frequently at sites with negative oxidation–reduction potential. Sponge biodiversity may be affected by substrate availability in anthropogenic habitats, invasive species, and improved ability to recognize sponge taxa. Crucially, we provide foundation data as a prerequisite for future ecological evaluation.

KEYWORDS
biodiversity, biogeography, canals, morphology, redox

1 INTRODUCTION

Despite sponges being important to freshwater ecosystem functioning (Manconi and Pronzato 2008), studies on their distribution and biodiversity, and associated drivers of these, typically consist of fragmentary records relating to a single habitat or a limited number of microhabitats (e.g. Parfitt 1868; Carter 1868; Annandale 1908; Mellanby 1953; Clegg 1979; Waterston & Lyster 1979; Waterston, 1981). Given the apparent ubiquity of sponges and the growing appreciation of their ecological role (De Santo & Fell 1996; Økland & Økland 1996; Gugel 2001), there is a need to more accurately assess the distribution patterns of freshwater sponges. To this end, we examined distributional patterns across a model landscape: the UK. Specifically, we first test (and reject) the hypothesis that freshwater sponges in the UK comprise a small number of ubiquitous taxa, and second, explore the environmental pressures that dictate distributional patterns.

One reason for the paucity of freshwater sponge documentation is the lack of clear morphological features enabling recognition. To resolve this, based on our observations and guided by the literature (e.g. Hooper & van Soest 2002), we have developed a key to all freshwater sponges found in the UK, and the major taxa found across Western Europe. However, in doing so, we recognized that species identification can be problematic. We therefore applied molecular methods, sequencing a section of 28S rDNA including the variable D3 region (Alvarez et al. 2000; Lopp et al. 2007), to assist identification and quantify pairwise genetic divergence rates. In this study we have resolved issues associated with a fragmented collection of reports, established the extent of sponge biodiversity across a broad landscape, and indicated factors that govern their distribution.

2 METHODS

2.1 Sampling and environmental conditions

A total of 230 sites from 9 habitat types (canal, lake, river, reservoir, pond, stream, broad, mere, moss) were sampled between 2008 and 2012 (Figure 1A; Supporting Information, Table S1). Surveys were conducted at three levels. First, canals in the northwest UK were surveyed under bridges (n = 100, each with three sub-sites), where sponge colonies were characterized, and specimens (bearing gemmules where possible) were collected to a depth of 40 cm; this method is appropriate to assess species richness (Evans 2016). We assessed these sites ≤10 times, and recorded measurements of water quality variables (YSI multiprobe, Ohio, USA): temperature, pH, salinity, dissolved oxygen, conductivity, total dissolved solids (TDS), and oxidation–reduction potential (ORP). Second, a UK-wide survey (n = 80), with more limited measurement of water quality variables , was conducted. Sponge samples from both the first and second survey were stored in 100% ethanol. Third, UK-wide sponge samples (n = 50) were obtained from the Natural History Museum, London.

2.2 Taxonomy

2.2.1 Microscopy

Nitric acid digestion of a 1-cm^3 tissue sample was used to separate siliceous spicules in preparation for microscopy. After three washes in water and resuspension in 100% ethyl alcohol, spicules were transferred to slides. Spicules were examined using a Zeiss Axiovert microscope at 200× magnification. Images were captured with a video camera (JVC model KY-F55B, 3-CCD, 750 horizontal × 480 vertical line resolution) interfaced with a Pentium II PC equipped with an image analysis capturing program (Scion Image for Windows, Scion Corp., MD) and a high–resolution frame grabber (CG-7, Scion Corp., MD). Up to three types of siliceous spicules were analyzed for sponge identification: megascleres, which make up the framework of a sponge skeleton; microscleres, which are spicules with a smaller size range; and gemmuloscleres, which surround the gemmules. Megascleres, microscleres, and gemmuloscleres were examined, and minimum, maximum, and mean (±*SE*) length and width of the spicules were determined from >25 spicules of each type from several specimens (except for *T. horrida*, in which ~20 were measured). Diagnostic traits (colony morphology, macro- and micromorphology of skeletal mega- and microscleres, gemmule arrangement) were compared with data from Pronzato & Manconi (2001).

2.2.2 Sequencing

The D3 domain of 28S rDNA with ~150 bp of the 3′ core sequence (303 bp) was amplified using the primers of Lopp et al. (2007). A DNA extraction kit (DNeasy, Qiagen Inc, Hilden, Germany) was used following the manufacturer's instructions, except that after incubation in lysis buffer the tubes were briefly centrifuged to remove spicules. PCRs were set up in a 20 µl final reaction volume containing ~10 ng genomic DNA, plus 10 µl 2X GoTaq Green (Promega, Southampton, UK), 0.4 pmol of each primer (Eurofins MWG Operon, Germany), and 2.0 µl 10X BSA buffer (New England Biolabs, UK). Thermal cycling conditions were: 94°C for 4 min, followed by 35 cycles at 94°C for 45 s, 45°C for 30 s, 72°C for 45 s, and a final 72°C for 8 min. PCR products were then purified with 0.15 µl shrimp alkaline phosphatase (1000 U/ml; USB, UK) and 0.03 µl Exonuclease I (20000 U/ml; New England Biolabs, UK), following manufacturer's protocol. Sequencing was performed using BigDye v.3.1 chemistry (Applied Biosystems, Life Technologies, UK), with ~10 ng of PCR products and 1.6 pmol of primer in each reaction. Sequencing products were cleaned by ethanol precipitation and separated by capillary electrophoresis on an ABI3100xl. Forward and reverse sequences were checked by eye in Geneious v.6.1.2 (Kearse et al. 2012) to produce a consensus. Species' pairwise genetic distances were calculated using Kimura's two-parameter (K2P) model (Kimura 1980) using MEGA v.6 (Tamura et al. 2013). Phylogenetic relationships among species were inferred from sequences using maximum likelihood (Kimura 1980), with support for nodes calculated using a bootstrap test with 500 replications. The analysis was performed with a heuristic strategy using Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach. Sequence data from *Corvomeyenia* sp. (Metaniidae) taken from GenBank (Table S2) was used as an out-group, because this group does not have a European distribution (Manconi & Pronzato, 2008), and it appeared to be the sister group to other freshwater sponge taxa in former phylogenetic analyses (see Lavrov et al.2012, mtDNA; Itskovich et al. 2007, cytochrome oxidase subunit I; Meixner et al. 2007, 18S rDNA, cytochrome oxidase subunit I, ITS2 rDNA).

2.3 Data analyses

To test for ubiquitous species presence, Cochran's Q test (for dichotomous variables; Zar 2010) was performed, followed by a post hoc Bonferroni correction (Zar 2010), contrasting coexisting sponge species at sites. With a heterogeneous distribution (see Results), the degree of species coexistence was assessed by cluster analysis on the overall Jaccard coefficient (*J*),

which is given by: $J = a / (a + b + c)$, where a is the number of species present at two sub-sites, and b and c are the total number of species occurring at each sub-site but not the other (see Clifford & Stephenson 1975; Plafkin et al. 1989). Then, to identify water quality variables that exhibited significant associations with sponge presence, data from the first survey (see "Sampling and environmental conditions," above) were analyzed. To this end, we applied *t*-tests for independent samples together with Levene's test to examine the assumption of equal variances (SPSS v. 22, IBM Corp, 2013; Zar 2010). Finally, to provide means for UK species determination, a spicule-based dichotomous key was constructed.

3 RESULTS

3.1 Species identification

Based on morphological and molecular data (Figures 2, 3; Tables 1, 2; Figure S1), six freshwater species were observed across the landscape. We provide the first UK record of *Trochospongilla horrida* (WELTNER 1893); the other five species had been recorded in the UK previously: *Spongilla lacustris* (LINNAEUS 1759), *Ephydatia fluviatilis* (LINNAEUS 1759), *Eunapius fragilis* (LEIDY 1851), *Ephydatia muelleri* (LIEBERKÜHN 1856), and *Racekiela ryderii* (POTTS 1882). Colonies of single or coexisting species were most commonly on brick, rock, or concrete (Figure 4), and there were few observations of larvae (<5% of colonies). Consensus rDNA 28S sequence data were obtained for five species (Figure S1, Table S2), with differences at 13 sites and low pairwise sequence divergence across all sequences (mean distance 0.007, Table 3). Sequence data for *T. horrida* were not obtained due to a limited sample and contamination, so a sequence from GenBank was substituted for phylogenetic analysis (Table S2). Using these consensus rDNA sequences, phylogenetic analysis suggests that UK sponge species are closely related, with short distances between groups (Figure 5). The percentage of trees in which the sponge species clustered together is fairly consistent (61–70%). All internal relationships between species were unresolved (*Ephydatia* spp.), or were supported by low bootstrap percentages.

Colonies of *Spongilla lacustris* were generally found at sites from year to year; however, colonies of *E. fluviatilis* was absent at sites where they had previously been abundant. At the base of colonies of *Eu. fragilis*, gemmules were present, with foramina that were always directed upward from a pavement layer or outward from a cluster (Figure 2A).

Some distinctive green colonies of *S. lacustris* suggested the presence of algal symbionts (Reiswig et al. 2009) (Figure 2B), whereas colonies in shaded sites were white (Figure 2E). *Trochospongilla horrida* was found at one southerly site, coexisting with *E. muelleri*.

The presence of microscleres permitted identification of *S. lacustris* (Figure 3A, Table 2). Although the number of rotule rays and the degree of indentation was irregular, gemmuloscleres of *E. fluviatilis* could be discrimination from *E. muelleri* by the presence of occasional spined shafts (Figure 3B). *Racekiela ryderii* was the only species with two distinct classes of gemmuloscleres (birotules and longer pseudobirotules). Distinctive gemmuloscleres of *T. horrida* were birotulates with disk-like rotules (Figure 3F).

3.2 Distribution patterns

Species distributions were heterogeneous (Figure 1) with significant differences in species' presence at the different sites ($\chi^2_{(5)} = 115.6$, $p < 0.01$; Table 4). These data show that some species have a wide biogeographic range (*S. lacustris*), and others have a more patchy distribution (*E. muelleri*, *Eu. fragilis*) ($p < 0.01$). *Racekiela ryderii* was rare, occurring in significantly fewer sites (in lakes; Figure 6) ($p < 0.01$, J ranged 0.02–0.04; Table 4), and never in anthropogenic habitats. In addition to lakes, *S. lacustris* also occurred in canals (Figure 2E). *Ephydatia fluviatilis* was the most common species in rivers. However, *E. muelleri* and *Eu. fragilis* were also occasionally (<10% of sites examined) found in rivers (Figure 6). Across all types of sites, the most frequently coexisting pair of species was *Eu. fragilis–E. muelleri*, ($J = 0.24$). *Eunapius fragilis* also occurred with *S. lacustris* and *E. fluviatilis* (Table 4), especially at canal sites.

Seasonal increase in colony growth of sponges was associated with significantly higher water temperature (mean = 12.1°C, $SE \pm 0.3$, $p > 0.01$) and lower dissolved oxygen levels (mean = 10.56 mg/L, $SE \pm 0.43$, $p > 0.01$) (Table 5). The most widespread sponge, *S. lacustris,* was present at lower mean temperatures and was more often located at latitudes >55°N. Salinity was a significant factor associated with the presence of *S. lacustris* and *E. muelleri*, despite the species' significantly different distributions, and both species occurred at sites with lower average salinity, conductivity, and TDS (Table 5). *Spongilla lacustris* occurred most often at sites when ORP levels were significantly more positive (mean = 4.71 mV, $SE \pm 3.47$, $p > 0$ .05) and water conductivity was significantly lower (mean = 180.48 µS/cm, $SE \pm 24.04$, $p > 0.01$). By contrast, salinity in anthropogenic habitats was not a

significant factor for the presence of colonies of either *Ephydatia fluviatilis* or *Eu. fragilis*, and these species occurred more frequently at sites with negative ORP (Table 5).

4 DISCUSSION

Freshwater sponges have important ecological roles (Reiswig *et al*., 2009), constituting a food source for certain invertebrates (Bērzinš, 1950; Resh 1976; Williamson, 1979), and are inhabited by a variety of epibionts and symbionts (Gugel, 2001; Kamaltynov *et al*., 1993; Matteson and Zacobi, 1980; Traveset, 1990; Wilkinson 1978). Importantly, the capacity of sponges to remove suspended particles (<5 µm) from the water column (Reiswig *et al*., 2009; Frost, 1976, 1980) contributes to both water quality and nutrient cycling (Reiswig *et al*., 2009).

Although freshwater sponges are reported to be widely distributed (Manconi and Pronzato, 2008), to date, distributional records of freshwater sponges have been mostly based on serendipitous finds, with no systematic examination of species richness or habitat tolerances. Furthermore, records of species may have been based on habitat assumptions (e.g., if a sponge is in a lake, then it must be *S. lacustris*). With fewer sponge species than that of many other encrusting freshwater invertebrate taxa (e.g., 19 species of bryozoans; Wood and Okamura 2005), the possibility arises that sponge species are eurytopic (see Manconi & Pronzato 2008). Here, we tested the hypothesis that species were homogeneously distributed, and given that they were not, we then recognized factors that appear to drive occurrence.

Paralleling, and indeed arising from, this study, we recognized the need for a morphology-based key (Figure 7), one that can also apply broadly to sponge species across Western Europe. The key relies primarily on megasclere characteristics, because they are always present in colonies, but this approach, although useful, has limitations (e.g., there are few differences between *E. fluviatilis* and *Eu. fragilis*). To accurately assess biodiversity, we used the D3 expansion segment of 28S rDNA; several studies have indicated that this is a robust method to provide distinct and effective sequences for sponge species identification (e.g. Alvarez et al. 2000; Lopp et al. 2007; Itskovich et al. 2013). Consensus 28S rDNA sequences required analysis of thirteen key bases for conclusive identification. Using genetic diversity to assess biodiversity is useful for invertebrates (Dudgeon *et al*., 2006), especially in taxa that are understudied, or have complicated life cycles. The main K2P genetic divergence

values were relatively low compared with other sponge studies (Itskovich et al. 2013), suggesting closely related species and their recent genetic radiation.

From this rigorous analysis that combined morphological and molecular methods, we demonstrate that freshwater sponge species are not ubiquitous across a landscape. Although the literature suggests the importance of pH in determining species distributions (Ricciardi & Reiswig 1993; Økland & Økland 1996; Gugel 2000), we instead show that temperature, dissolved oxygen, salinity and ORP have a significant impact on sponge presence. Lakes and canals with colonies of *S. lacustris* exhibited low conductivity, TDS, and salinity levels, suggesting that ions contributing to salinity (and indirectly to conductivity and TDS values; Radojevic & Bashkin 2006) restrict this species' distribution (see Harrison 1974). However, tolerance to lower temperatures contributed to a more northern persistence of *S. lacustris* than other UK species. Although the specific name of the other common species, *E. fluviatilis* (22% of sites) indicates occurrence in rivers, it was more ubiquitous than other species. In common with other surveys (Stephens 1920; Gugel 1999; Lucey & Cocchiglia 2014), some colonies did not form gemmules, thus reducing the potential for regrowth (Manconi & Pronzato 2008), restricting dispersal ability (Freeland et al. 2000), and possibly explaining the disappearance of colonies of *E. fluviatilis* from some sites.

The patchy distribution of *Eu. fragilis* and *E. muelleri* could possibly arise due to colony characteristics that increase the likelihood of a species being overlooked (Dröscher & Waringer 2007); for example, colonies of *Eu. fragilis* were smaller and disintegrated earlier in the year than other species (Tendal 1967; Gugel 2001; Dröscher & Waringer 2007). However, following an initial assessment of the optimum level of sampling required for an unbiased survey (Evans 2016), we are confident that species were not overlooked, and our work supports observations of a patchy distribution of *E. muelleri* throughout Europe (Stephens 1920; Tendal 1967; Waterston 1981; Manconi & Pronzato 2008).

Here we provide the first record of *T. horrida* in the UK, an extension of its range from central Europe. The record of *T. horrida* at a southern coastal canal site is consistent with the species' distribution in warm, brackish water (Sharapova et al. 2014; Poirrier 1969), its presence in navigable canals (Schletterer & Eggers 2006), and rarity (Gugel 2000; Richelle-Maurer et al. 1994). With no previous UK records of any *Trochospongilla* taxa, we might speculate that *T. horrida* is a recent invasive species from the continent, and climate change may contribute to newly suitable conditions. To investigate this conjecture, subsampling of colonies of *E. muelleri* along the south coast of the UK, and sampling in July, when *T. horrida* is abundant in Germany (Gugel 2000), may yield additional data.

We provide more comprehensive distribution data for the other rare species, *R. ryderii*, which was previously known from remote sites and now includes an expanded range in the UK (Annandale 1908; Waterston & Lyster 1979; Waterston 1981). Perhaps this biogeography is not surprising, because *R. ryderii* is common along a similar latitudinal range in the Republic of Ireland (Stephens 1920; Pronzato & Manconi 2001; Lucey & Cocchiglia 2014). Although misidentification of *R. ryderii* may occur because of spicule variability (as documented by Stephens 1920; Ricciardi & Reiswig 1993), our identification methods should alleviate confusion, and the data suggest that if lakes contain colonies of *S. lacustris,* then *R. ryderii* may also be present. We, therefore, suggest that this species is not invasive; instead previous taxonomic difficulties have hindered proper identification of this species.

Clearly, the apparent absence of freshwater sponge records because of insufficient searching is problematic in establishing biogeographical patterns (Smith & Wilkinson 2007; Evans 2016). However, here, in common with other research (see De Santo & Fell 1996), ~50% of environmentally similar sites remained unoccupied. Sponges may be absent from an otherwise suitable freshwater habitat because of substrate characteristics (Minshall 1984). For example, broad areas of solid substrate (e.g., brick and concrete in anthropogenic habitats) rather than substrates composed of small, loose particles (e.g., gravel in rivers) may impact colonization (Gugel 1999), increasing the likelihood of gemmule settlement. Therefore, sponges may be widely distributed, but local conditions dictate their occurrences.

Our data suggest that an oxidizing environment has a significant effect on *S. lacustris* presence (Table 5). Oxidative–reductive potential may directly affect sponges by impacting physiological processes and indirectly affect them by altering the abundance of food such as bacteria (Barton & Northrup 2011). It might be assumed that salinity is the principle driving factor for all freshwater species, but salinity had a significant effect on the presence of *S. lacustris* and *E. muelleri* only. Stenotopic responses to water quality variables (temperature, salinity, ORP) lowered coexistence rates between *S. lacustris* and the other widespread species, *E. fluviatilis*. The growth of sponge colonies during spring and summer is reflected in the significance of water temperature. Areas with an abundance of bacteria may lead to the presence of lower oxygen levels as bacteria use available dissolved oxygen (Radojevic & Bashkin 2006). A significant negative association between dissolved oxygen level and presence of *E. fluviatilis* indicates that there is greater tolerance for lower dissolved oxygen level in this species than in other species, and this may allow exploitation of greater bacterial food supply and presence in a variety of habitat types. By contrast, in *Eu. fragilis* there are fewer factors that are significantly associated with the species' presence, which suggests a

eurytopic response characteristic for this species with a cosmopolitan distribution (Pronzato & Manconi 2001).

In summary, a landscape pattern of freshwater sponges is more complex than expected. Our study demonstrates morphological and genetic distinctions among species, clarifies distributional patterns, and identifies factors associated with species' presence. Overall, the distribution of sponges across the landscape seems to be regulated by both habitat and substrate characteristics, as well as water quality tolerances of each species. Furthermore, by rigorously evaluating an extensive and diverse landscape, our guidance for identification and data collection provide a foundation for the continued evaluation of sponge biodiversity, both at local and global scales. Critically, we have shown that all the species in the present study have wider distributions than previously appreciated.

Across Europe sponge species have disappeared from water bodies because of changes in water chemistry, potentially due to anthropogenic pollution that increases conductivity (Dröscher & Waringer 2007). We might speculate that, with climate change, southern European species may invade more northern sites, such as the UK. Consequently, the continued assessment of sponges, as we have done, should provide useful indication of how our changing environment will alter freshwater biodiversity.

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REFERENCES

Alvarez B, Crisp MD, Driver F, Hooper JNA, & van Soest RWM 2000. Phylogenetic relationship of the family Axinellidae (Porifera: Demospongiae) using morphological and molecular data. Zool. Scr. 29: 169–198.

Barton LL & Northup DE 2011. Microbial Ecology. Wiley-Blackwell, Hoboken. 440 pp.

Bērzinš B 1950. Observations on rotifers on sponges. Trans. Am. Micro. Soc.69: 189-193.

Carter HJ 1868. On a variety of *Spongilla meyeni* from the river Exe, Devonshire. Ann. Mag. Nat. Hist. 4: 247-250.

Clegg J 1979. Pond and stream life. Blandford Press, London. 108 pp.

Clifford HT & Stephenson W 1975. An introduction to numerical classification. Academic Press, Cambridge. 229 pp.

De Santo EM & Fell PE 1996. Distribution and ecology of freshwater sponges in Connecticut. Hydrobiolgia 341: 81-89.

Dröscher I & Waringer J 2007. Abundance and microhabitats of sponges (Spongillidae) in a Danubean floodplain in Austria. Freshwater Biol. 52: 998-1008.

Dudgeon D, Arthington A H, Gessner M O, Kawabata Z, Knowler D J, Lévêque C, Naiman R J, Prieur-Richard A, Soto D, Stiassny M L J & Sullivan CA 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. 81: 163–182.

Evans KL 2016. Biodiversity of freshwater sponges in the UK: taxonomy, distribution, and ecology. PhD Thesis, University of Liverpool, Liverpool. 236 pp.

Freeland JR, Romualdi R, & Okamura B 2000. Gene flow and genetic diversity: a comparison of freshwater bryozoan populations in Europe and North America. Heredity 85: 498-508.

Gugel J 1999. Ecological adaptations of a freshwater sponge association in the River Rhine, Germany (Porifera: Spongillidae). Mem. Qld. Mus. 44:215-224.

——— 2000. High abundance of *Trochospongilla horrida* (Porifera, Spongillidae) in the Rhine (Germany) 1992–1995. Hydrobiologia 421:199-207.

——— 2001. Life cycles and ecological interactions of freshwater sponges (Porifera, Spongillidae) in the River Rhine in Germany. Limnologica 31:185-98.

Harrison FW 1974. Sponges (Porifera: Spongillidae). In: Pollution ecology of freshwater invertebrates. Hart CW &Fuller SLH, eds., pp. 29-66. Academic Press, New York.

Hooper JNA & van Soest RWM 2002. Class Demospongiae Sollas, 1885. In: Systema Porifera: a guide to the classification of sponges, Vol. 1. Hooper JNA & van Soest RWM, eds., pp. 15-18. Kluwer Academic, New York.

Itskovich V, Belikov S, Efremova S, Masuda Y, Perez T, Alivon E, & Boury-Esnault N 2007. Phylogenetic relationships between freshwater and marine Haplosclerida (Porifera, Demospongiae) based on the full length 18S rRNA and partial COXI gene sequences. In Porifera Research: Biodiversity, Innovation and Sustainability. Custódio MR,

Lôbo-Hajdu G, Hajdu E, Muricy G, eds., pp. 383-391. Série Livros Museu Nacional, Rio de Janeiro.

Itskovich V, Kaluzhnaya O, Ostrovsky I & McCormack G 2013. The number of endemic species of freshwater sponges (Malawispongiidae; Spongillina; Porifera) from Lake Kinneret is overestimated. J. Zool. Syst. Evol. Res. 51: 252-257.

Kamaltynov RM, Chernykh VI, Slugina ZV & Karabanov EB 1993. The consortium of the sponge *Lubomirskia baicalensis* in Lake Baikal, East Siberia. Hydrobiologia. 271: 179-189.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C & Thierer T 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28: 1647-1649.

Kimura M 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16: 111-120.

Lavrov DV, Maikova OO, Pett W & Belikov SI 2012. Small inverted repeats drive mitochondrial genome evolution in Lake Baikal sponges. Gene. 505: 111-120.

Lopp A, Reintamm T, Vallmann K, Päri M, Mikli V, Richelle-Maurer E & Kelve M 2007. Molecular identification, characterization and distribution of freshwater sponges (Porifera: Spongillidae) in Estonia. Arch. Hydrobiol. 168: 93-103.

Lucey J & Cocchiglia L 2014. Distribution of sponges (Porifera: Spongillidae) in southern Irish rivers and streams. Proc Roy Ir Acad. 114: 89-100.

Manconi R & Pronzato R 2008. Global diversity of sponges (Porifera: Spongillina) in freshwater. Hydrobiologia. 595: 27-33.

Matteson JD & Zacobi G 1980. Benthic macroinvertebrates found on the freshwater sponge *Spongilla lacustris*. Great Lakes Entomologist**.** 13:169-172.

Mellanby H 1953. Animal life in freshwater: a guide to freshwater invertebrates. Methuen, London. 308 pp.

Meixner MJ, Luter C, Eckert C, Itskovich V, Janussen D, von Rintelen T, Bohne AV, Meixner JM, Hess WR, 2007. Phylogenetic analysis of freshwater sponges provides evidence for endemism and radiation in ancient lakes. Mol Phylogenet Evol. 45:875–886.

Minshall GW 1984. Aquatic insect-substratum relationships. In: The ecology of aquatic insects. Resh VH & Rosenberg DM eds., pp. 358-400. Praeger Scientific, New York.

Økland KA & Økland J 1996. Freshwater sponges (Porifera: Spongillidae) of Norway: distribution and ecology. Hydrobiologia 330: 1-30.

Parfitt E1868. On the marine and freshwater sponges of Devonshire. Trans. Devon. Assoc. Adv. Sci. 2: 443-462.

Plafkin JL, Barbour MT, Porter KD, Gross SK & Hughes RM 1989. Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish. United States Environmental Protection Agency, Washington, D.C. 181 pp.

Poirrier MA 1969. Louisiana fresh-water sponges: taxonomy, ecology, and distribution. PhD Thesis, Louisiana State University, Louisiana. 173 pp.

Pronzato R & Manconi R 2001. Atlas of European freshwater sponges. Ann. Mus. Civ. Stor. Nat. Ferr. 4: 3-64.

Radojvic M & Bashkin V N 2006. Practical Environmental Analysis. RSC Publishing, UK. 480 pp.

Reiswig HM, Frost TM & Ricciardi A 2009. Porifera. In: Ecology and classification of North American freshwater invertebrates. Thorp J H & Covich AP, eds., pp. 95-124. Academic Press, New York.

Resh VH 1976. Life cycles of invertebrate predators of freshwater sponge. In: Aspects of sponge biology, Harrison FW & Cowden RR, eds., pp. 299-314. Academic Press, New York.

Ricciardi A & Reiswig HM 1993. Freshwater sponges (Porifera, Spongillidae) of eastern Canada: taxonomy, distribution, and ecology. Can. J. Zool. 71: 665-682.

Richelle-Maurer E, Degoudenne Y, van de Vyver G & Dejonghe L 1994. Some aspects of the ecology of Belgian freshwater sponges. In: Sponges in time and space. Van Soest RWM, van Kempen TMG & Braekman JC, eds., pp. 341-350. Balkema, Rotterdam.

Schletterer M & Eggers TO 2006. Evidence of Freshwater Sponges (Porifera: Spongillidae) in the Upper Volga River (Russia). Ber. Nat. Med. Vers. Inn. 93: 73-84.

Sharapova TA, Trylis VV, Ivanov SN & Ilyushina VV 2014. Composition and distribution of sponges (Porifera) in continental waters of western Siberia. Contemp. Probl. Ecol. 7: 543-550.

Smith HG & Wilkinson DM 2007. Not all free-living microorganisms have cosmopolitan distributions: The case of *Nebela* (*Apodera*) *vas* Certes (Protozoa: Amoebozoa: Arcellinida). J. Biogeogr. 34: 1822-1831.

Stephens J 1920. The fresh-water sponges of Ireland. Proc. Roy. Ir. Acad. B. 35: 205-254.

Tamura K, Stecher G, Peterson D, Filipski A, & Kumar S 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30: 2725-2729.

Tendal OS 1967. On the freshwater sponges of Denmark. Vid. Med. Dansk. Nat. For. 130: 173-178.

Traveset A 1990. Notes on Iberian freshwater sponges. In: New Perspectives in Sponge Biology. Rützler K, ed., pp.320-322. Smithsonian Institution Press, Washington, D. C.

Waterston AR 1981. Present knowledge of the non-marine fauna of the Outer Hebrides. Proc. Roy. Ir. Acad. B. 79: 215-321.

Waterston AR & Lyster IHJ 1979. The macrofauna of brackish and fresh waters of the Loch Druidibeg National Nature Reserve and its neighbourhood, South Uist. Proc. Roy. Soc. Edinb. B. 77: 353-376.

Wilkinson CR 1978. Microbial associations in sponges. Mar Biol. 49: 169-176.

Williamson CE 1979. Crayfish predation on freshwater sponges. Am Mid Nat, 101:245-246.

Wood TS & Okamura B 2005. A new key to the freshwater bryozoans of Britain, Ireland and Continental Europe, with notes on their ecology. Freshwater Biological Association, Ambleside. 111pp.

Zar JH 2010. Biostatistical analysis. Prentice-Hall, Upper Saddle River. 944 pp.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information tab for this article.

Table S1 Survey sites (with latitude, longitude, and habitat type) comprising canal survey sites, UK survey sites, and sites of Natural History Museum (MS) samples. Following preliminary rarefaction sampling (see Evans, 2016), data collection for canal survey sites were performed in shaded areas underneath or near canal bridges along 15 canals at 100 stations. All stations were located along the water line and under a bridge; three stations were equally spaced along a 5-m stretch

Table S2 Accession numbers for nucleotide sequences analyzed. Accession numbers of 28S rDNA sequences for *Trochospongilla horrida and Corvomeyenia sp*. from GenBank.

Figure S1 Alignment of the extended 28S rDNA D3 domains from *Ephydatia fluviatilis*, *E. muelleri*, *Eunapius fragilis*, *Spongilla lacustris*, and *Racekiela ryderii*. Identities have been indicated by dots, and gaps by hyphens

FIGURE LEGENDS

Figure 1 Distribution of freshwater sponges in the UK. **A.** All sites, showing locations of sponge occurrence, sampling areas, and data sources used to determine sponge distribution. Sites where water quality data were collected are shaded symbols, sites generally without water data are open symbols, and sites for which data from the Natural History Museum were available are dark symbols. **B.** Distribution of *Spongilla lacustris*. **C.** Distribution of *Ephydatia fluviatilis*. **D.** Distribution of *E. muelleri*. **E.** Distribution of *Eunapius fragilis*. **F.** Distribution of *Racekiela ryderii* and *Trochospongilla horrida* (star). UK islands not included on maps were not sampled.

Figure 2 Examples of freshwater sponges from the UK. **A.** Gemmular arrangement of *Eu. fragilis*, showing "carpet" formation with foramina facing upwards, and a group of four gemmules (lower left) with foramina facing away from the cluster. **B.** *Spongilla lacustris*, with astrorhiza surface. **C.** Side view of a colony of *E. muelleri* covering large area of vertical substrate. **D.** Brown and white gemmules in degenerating colony of *E. muelleri*. **E.** Side view of a colony of *S. lacustris* on vertical brick substrate. Scale bars: A = 1 mm; B,D,E = 10 mm; C = 50 mm

Figure 3 Spicule ultrastructure of freshwater sponges. **A.** *Spongilla lacustris*. **B.** *Ephydatia fluviatilis*. **C.** *Eunapius fragilis*. **D.** *Ephydati*a *muelleri*. **E.** *Racekiela ryderii* with two classes of gemmuloscleres. **F.** *Trochospongilla horrida*. Scale bars = 10 µm. g, gemmulosclere; M, megasclere; m, microsclere; r, rotule

Figure 4 Frequency of sponge presence (white) on different substrate types (rock; rock with zebra mussels; brick; brick plus rocks; concrete; concrete and bricks; wood with other plant materials) at sites in canal survey (sampling method 1; see Methods). Percentages above each

bar indicate the proportion of survey sites at which each substrate type occurred. On average, sponges were absent from 51% of survey sites

Figure 5 Phylogenetic analysis of 28S rDNA sequences for freshwater sponge species using *Corvomeyenia sp*. as an outgroup. Genus names *Ephydatia*, *Eunapius*, *Racekiela*, *Spongilla*, and *Trochospongilla* have been abbreviated. Bootstrap values (500 replications) for the percentage of trees in which the associated taxa clustered together using maximum likelihood method are indicated to the left of a node. The tree with the highest log likelihood (-465.2500) is drawn to scale, with branch lengths measured in the number of substitutions per site.

Figure 6 Species presence at canal, river, or lake habitats. Species were recorded alone at a site, or co-occurring with other species. Number of habitat sites sampled shown below axis. a, *Spongilla lacustris*; b, *Ephydatia fluviatilis*; c, *E. muelleri*; d, *Eunapius fragilis*; e, *Racekiela ryderii*; f, *Trochospongilla horrida*

Figure 7 Key to six freshwater sponge species found in the UK, using megasclere morphology as an initial determinant

Figure 1.

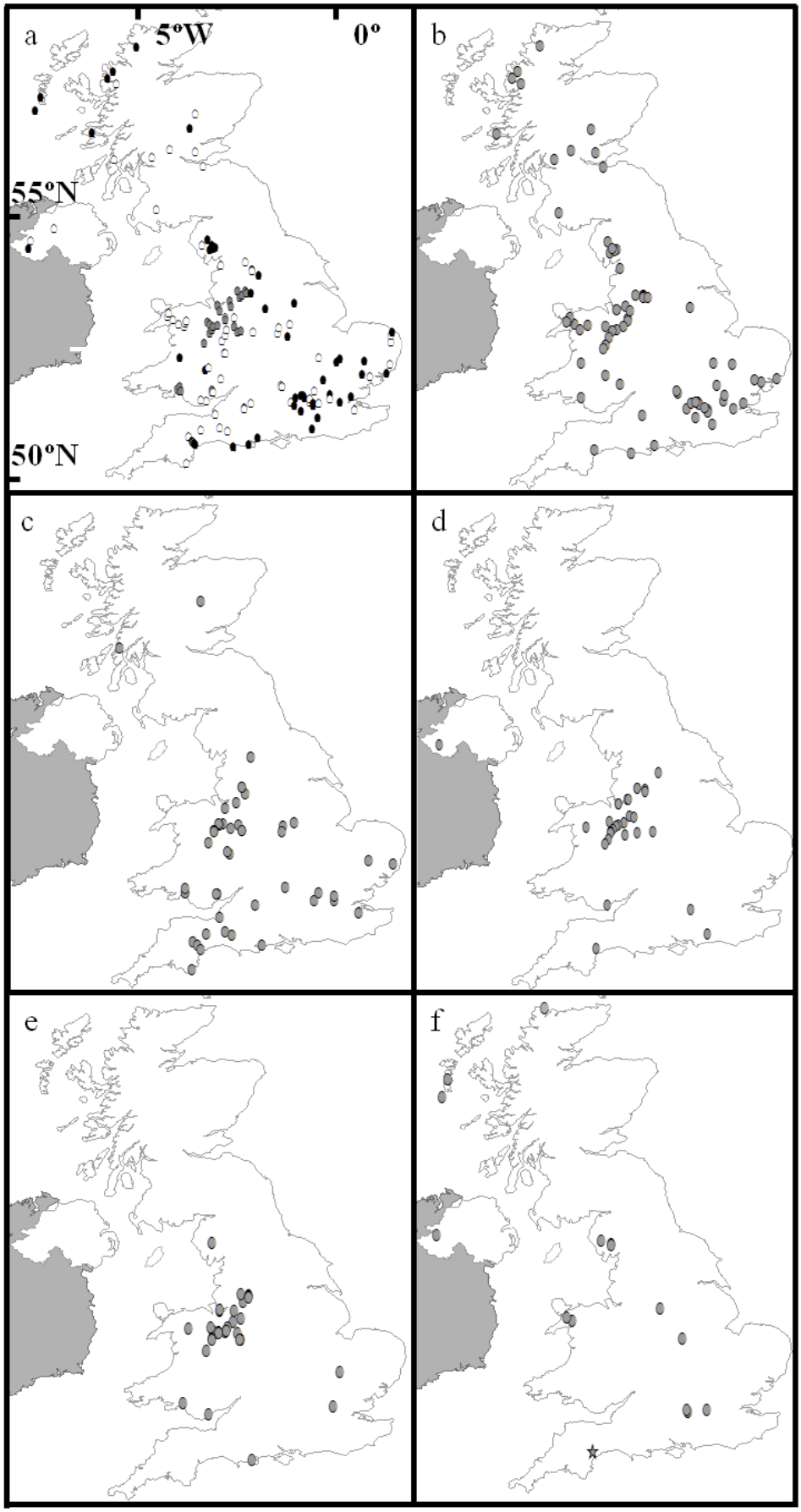

Fig. 2

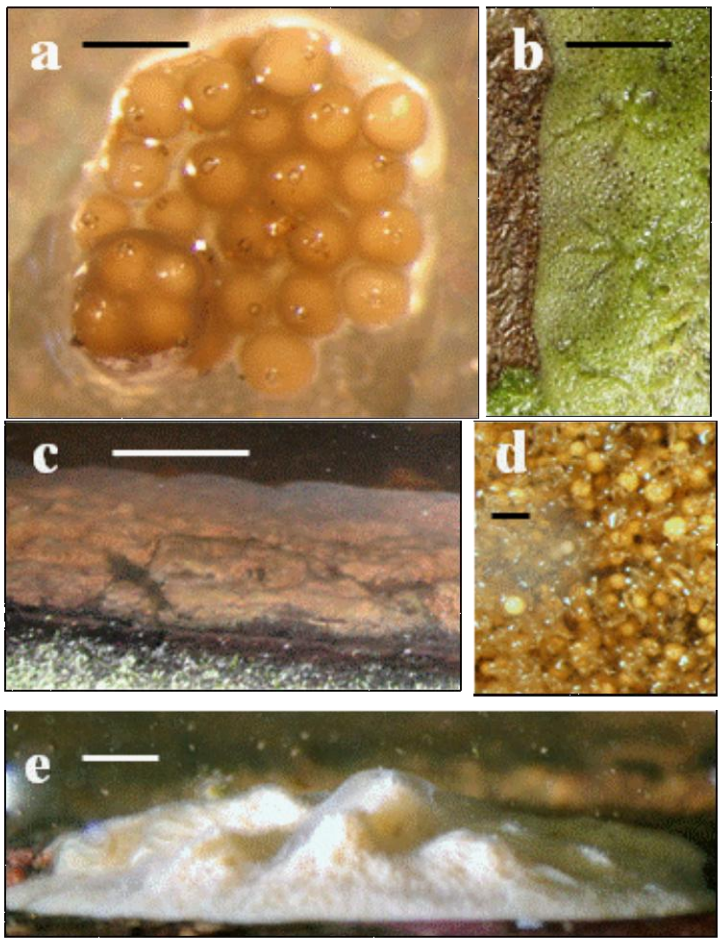

Figure 3.

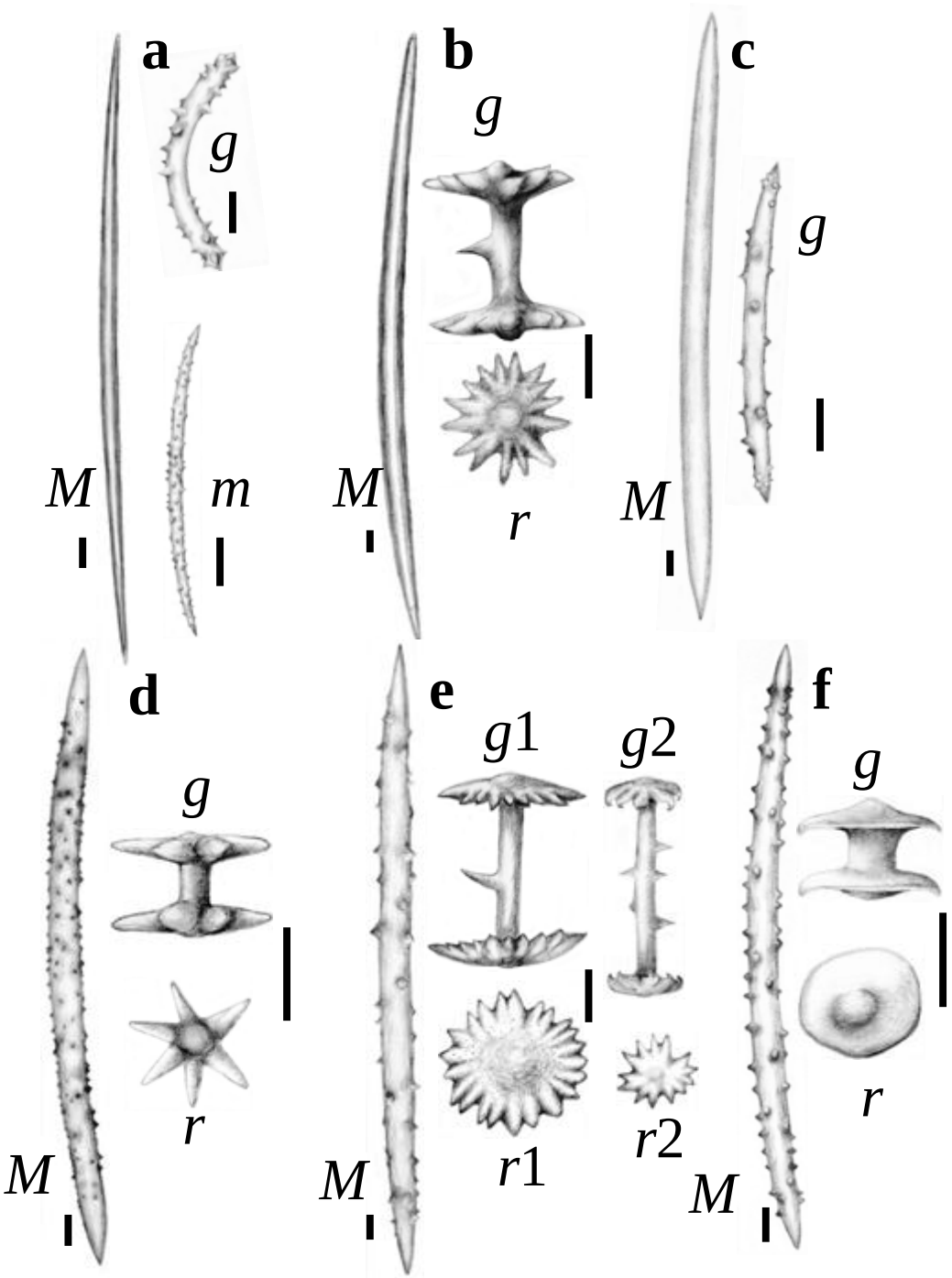

Figure 4.

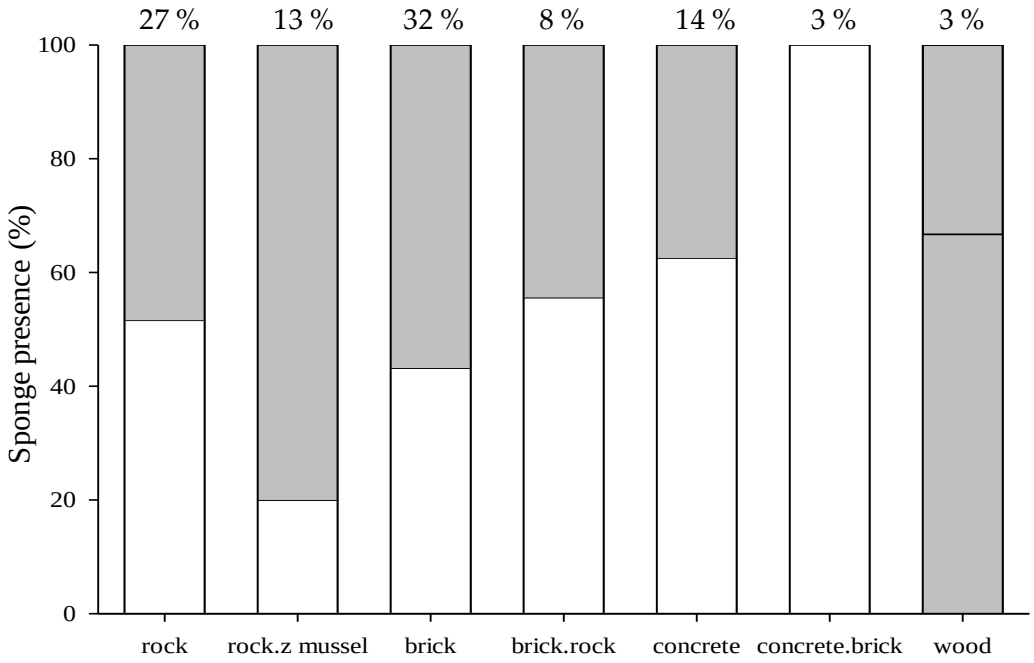
27 %
13 %
32 %
8 %
14 %
3 %
3 %
100
80
60
40
20
0
Sponge presence (%)
rock
rock.z mussel
brick
brick.rock
concrete
concrete.brick
wood

Figure 5.

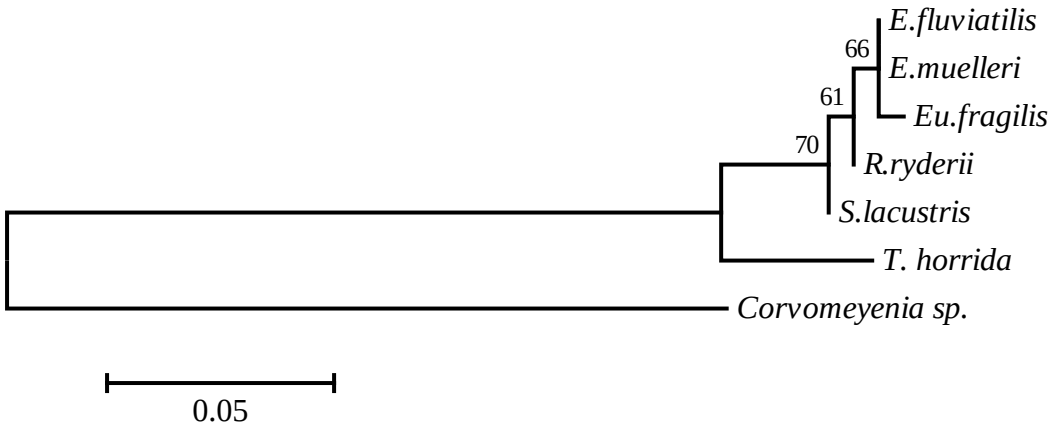
E.fluviatilis
66
E.muelleri
61
Eu.fragilis
70
R.ryderii
S.lacustris
T. horrida
Corvomeyenia sp.
0.05

Figure 6.

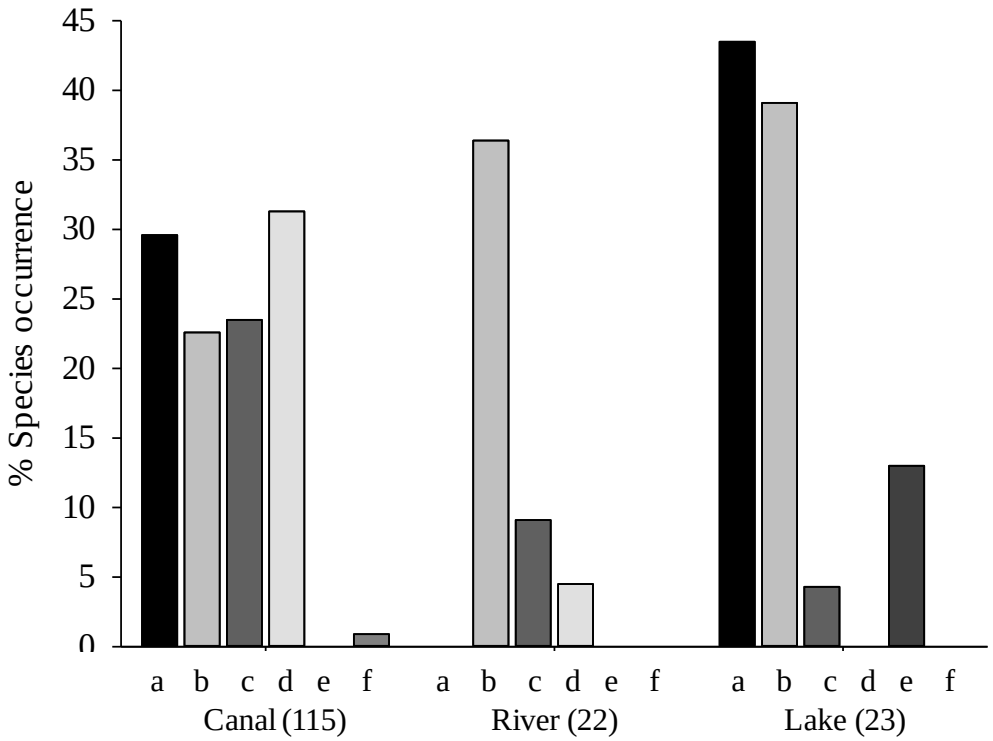
% Species occurrence
45
40
35
30
25
20
15
10
5
0
a b c d e f
Canal (115)
a b c d e f
River (22)
a b c d e f
Lake (23)

Figure 7.

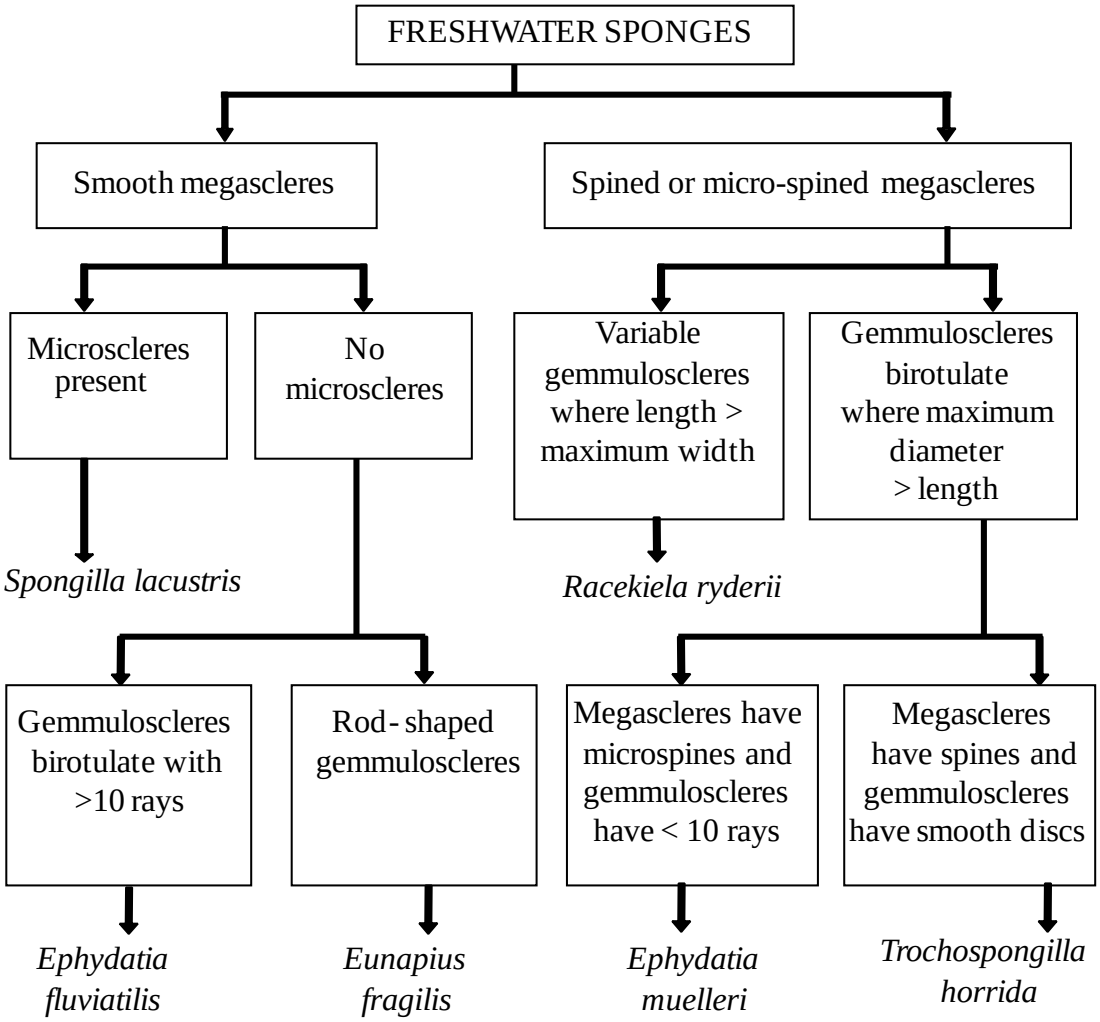
FRESHWATER SPONGES
Smooth megascleres
Spined or micro-spined megascleres
Microscleres present
No microscleres
Variable gemmuloscleres where length > maximum width
Gemmuloscleres birotulate where maximum diameter > length
Spongilla lacustris
Racekiela ryderii
Gemmuloscleres birotulate with >10 rays
Rod-shaped gemmuloscleres
Megascleres have microspines and gemmuloscleres have < 10 rays
Megascleres have spines and gemmuloscleres have smooth discs
Ephydatia fluviatilis
Eunapius fragilis
Ephydatia muelleri
Trochospongilla horrida

Table 1. Distribution patterns and colony characteristics of the species identified in this study.

Sponge species	Distribution	Colony characteristics
Spongilla lacustris	Widely distributed	Encrusting colonies (l to 2.5 cm thick) with irregular, rounded edges. Occasionally large colonies (> 1 m long) between June and August. Occasionally formed arborescent projections (10 x 1 cm). Often green with astrorhiza or ribbed surface, disintegrating in Oct to Nov, leaving white or brown gemmules in former basal parts. This species occasionally (< 33% of sites) occurred with or on zebra mussels (*Dreissena polymorpha*).
Ephydatia fluviatilis	Widely distributed	Encrusting colonies with an irregular outline and rounded edges. Occasionally large green, lobate colonies (>0.3 m long) formed in canals. The most common species in rivers, where colonies were smaller and ecru (<10 cm diameter). Some colonies lacked gemmules.
Eunapius fragilis	Patchily distributed	Colonies formed low crusts (~ l-2 cm thick), with an irregular, rounded outline. Surface was ribbed, with noticeable oscula. Numerous, small (rarely >20 cm), ecru colonies were recorded at some sites, disintegrating in Sep to Oct. Gemmules in a pavement layer ("carpets"), or in individual clusters (2-4).
Ephydatia muelleri	Patchily distributed	Firm, thickly encrusting (2-3 cm thick) colonies, with irregular, rounded edges, peaking between Jul and Aug especially at undisturbed sites. Colonies were yellow, brown, or pale grey and often hispid. Colonies partially disintegrated in winter, leaving gemmules in former basal parts.
Racekiela ryderii	Rare	Colonies formed thin hemispherical encrustations (<1 cm thick), with rounded edges and a hard, brittle consistency. Growth in lakes was on the top surface of rocks, and peaked during Jul to Aug, with only a few gemmules present.
Trocho-spongilla horrida	Rare	Colonies formed thin (<5 mm), flat, light yellow encrustations.

Table 2. Variation in spicule dimensions (n >25) with comparison to available data from Pronzato and Manconi (2001), indicating shorter gemmulosclere sizes in *Spongilla lacustris*, *Eunapius fragilis*, *Ephydatia muelleri*, and *Racekiela ryderii*.

	Spicules		**Length (µm)**		**Width (µm)**		**Rotule diameter (µm)**	
			Max	Min	Max	Min	Max	Min
Spongilla lacustris	Megascleres slightly curved, smooth	This study	278	140	15	4	___	___
		P - M	300	90	18	2	___	___
	Microscleres slightly curved with microspines	This study	91	35	7	2	___	___
		P - M	178	25	8	2	___	___
	Gemmuloscleres curved, spined	This study	89	29	9	2	___	___
		P - M	130	21	10	1	___	___
Ephydatia fluviatilis	Megascleres slightly curved, smooth	This study	419	244	16	5	___	___
		P - M	400	210	19	6	___	___
	Gemmulosclere birotulate > 10 rays	This study	33	23	6	2	25	17
		P - M	30	26	n/a	n/a	21	18
Eunapius fragilis	Megascleres slightly curved, smooth	This study	360	175	21	2	___	___
		P - M	270	180	15	4	___	___
	Gemmuloscleres slightly curved or straight, with spines	This study	87	54	8	3	___	___
		P - M	140	75	8	3	___	___
Ephydatia muelleri	Megascleres slightly curved with micro-spines	This study	270	158	16	4	___	___
		P - M	350	200	20	9	___	___
	Gemmulosclere birotulate < 10 rays	This study	14	7	17	2	23	12
		P - M	10	5	n/a	n/a	20	12
Racekiela ryderii	Megascleres variable with spines	This study	352	160	14	4	___	___
		P - M	431	141	26	12	___	___
	Birotule gemmulosclere	This study	23	25	5	3	21	15
		P - M	49	28	8	3	29	20
	Pseudo-birotule gemmulosclere	This study	41	25	6	2	17	9
		P - M	92	47	10	5	23	17
Trochospongilla horrida	Megascleres with spines that have rounded tips	This study	230	187	10	7	___	___
		P - M	235	170	15	11	___	___
	Gemmuloscleres birotulate, with smooth margins	This study	20	8	5	2	17	9
		P - M	n/a	n/a	n/a	n/a	n/a	n/a

Table 3. The number of base substitutions per site as K2P (Kimura 2 parameter) between 28S sequences of sponge species (*Spongilla lacustris*, *Ephydatia muelleri*, *E. fluviatilis*, *Eunapius fragilis*, *Racekiela ryderii*) are shown.

K2P	*E. muelleri*	*E. fluviatilis*	*Eu. fragilis*	*R. ryderii*
S. lacustris	0.007	0.007	0.017	0.003
E. muelleri		0.000	0.010	0.003
E. fluviatilis			0.010	0.003
Eu. fragilis				0.014

Table 4. Occurrence and co-existence of sponges across sites. Variations in percentage of sites occupied (%) indicated a significant difference in presence of sponge species (*Spongilla lacustris*, *E. fluviatilis*, *Eunapius fragilis*, *Ephydatia muelleri*, *Racekiela ryderii* and *Trochospongilla horrida*) at sites. On the top diagonal are Bonferroni corrected pairwise comparisons between species, where significant differences ($p < 0.01$) in distribution were found. On bottom diagonal are the Jaccard coefficient measures of mean similarity, assessing co-existence of sponge species.

	%	***S. lacustris***	***E. fluviatilis***	***Eu. fragilis***	***E. muelleri***	***R. ryderii***	***T. horrida***
S. lacustris	34.1		Not different	different	different	different	different
E. fluviatilis	22.0	0.09		Not different	Not different	different	different
Eu. fragilis	18.4	0.17	0.17		Not different	different	different
E. muelleri	16.6	0.15	0.09	0.24		different	different
R. ryderii	5.8	0.04	0.00	0.00	0.02		different
T. horrida	0.4	0.00	0.00	0.00	0.03	0.00	

Table 5. Independent t-test analyses of differences in water quality variables including temperature (°C), conductivity (μScm^{-1}), total dissolved solids salinity ($g\ l^{-1}$), salinity (psu), oxygen ($mg\ l^{-1}$), pH, and oxidation reduction potential (mV; ORP) at sites where all sponge species were present or all were absent, and at sites where one species was present or absent. Note:*p<0.05, **p<0.01.

	Significant abiotic factor	**Presence range**	**Mean values ±SE**	
			Presence	Absence
Overall sponge presence or absence	Temperature**	7.18 -18.12	12.10 ± 0.33	9.69 ± 0.56
	Conductivity	60.00 – 921.67	223.76±25.51	271.25± 22.43
	TDS	0.05 – 0.74	0.19±0.02	3.02±2.35
	Salinity*	0.04 - 0.57	0.15 ± 0.02	0.26 ± 0.04
	Oxygen**	4.84 - 17.97	10.56 ± 0.43	13.01 ± 0.41
	pH	6.12 – 8.87	7.62±0.07	7.83±0.96
	ORP	-64.80 – 94.10	-0.45±3.60	-5.02±2.61
Spongilla lacustris	Temperature *	7.18 - 18.12	11.97 ± 0.41	10.42 ± 0.45
	Conductivity**	60.00 - 687.67	180.48 ± 24.04	275.35 ± 21.15
	TDS	0.05 – 0.67	0.16±0.02	2.24±1.69
	Salinity**	0.04 - 0.46	0.12 ± 0.02	0.24 ± 0.03
	Oxygen*	4.91 - 15.60	11.06 ± 0.49	12.41 ± 0.38
	pH	6.75 – 8.87	7.60±0.08	7.79±0.08
	ORP*	-19.05 - 47.07	4.71 ± 3.47	-5.91 ± 2.71
Ephydatia muelleri	Temperature**	9.34 - 16.00	12.50 ± 0.45	10.36 ± 0.41
	Conductivity	68.50 – 921.67	195.32±34.60	264.32 ±19.31
	TDS	0.05 – 0.74	0.16±0.03	2.10 ±1.57
	Salinity*	0.04 - 0.57	0.12 ± 0.02	0.23 ± 0.03
	Oxygen**	4.88 - 15.60	10.19 ± 0.55	12.56 ± 0.35
	pH	6.12 – 8.87	7.63±0.10	7.76 ±0.07
	ORP	-53.23 - 94.10	6.20±5.67	-5.49 ±2.25
Ephydatia fluviatilis	Temperature *	7.86 - 18.12	12.43 ± 0.58	10.52 ± 0.39
	Conductivity	79.83 – 921.67	322.93±55.32	231.41±16.44
	TDS	0.07 – 0.74	0.27±0.05	1.95±1.47
	Salinity	0.05 – 0.57	0.22±0.04	0.20 ± 0.02
	Oxygen**	4.84 - 14.73	9.83 ± 0.64	12.51 ± 0.33
	pH	6.77 – 8.48	7.64±0.09	7.75±0.07
	ORP	-64.80 – 47.07	-5.09±6.73	-2.24 ± 2.25
Eunapius fragilis	Temperature**	7.18 - 18.12	12.35 ± 0.49	10.36 ± 0.42
	Conductivity	61.00 – 921.67	233.42±41.73	253.08 ±18.01
	TDS	0.05 – 0.74	0.20±0.04	2.13 ±1.61
	Salinity	0.04 – 0.57	0.15±0.03	0.22 ±0.03
	Oxygen	4.84 – 17.97	11.26±0.62	12.28 ±0.36
	pH	6.77 – 8.48	7.65±0.08	7.76 ±0.07
	ORP	-64.80 – 47.07	-2.70±5.20	-2.80 ±2.39

Supporting Information

Table S1. Survey sites (with latitude, longitude, and habitat type) comprising Canal survey sites, UK survey sites and sites of Natural History Museum samples. Following preliminary rarefaction sampling (see Evans, 2016), data collection for Canal survey sites were performed in shaded areas underneath or near canal bridges along 15 canals at 100 stations. All stations were located along the water line and under a bridge; three stations were equally spaced along a 5 m stretch. Natural History Museum samples obtained from Darwin centre (catalogue number: 1938.5.4.1., 2005.06.15.01, 1936.1.22.2, 25.11.1., 1954.9.16, 2005.06.08.01/02, 32.10.24.10, 1965.9.21.1, 1937.06.15.01., 32.12.24.4., 25.11.1.1598.01/02/03, 1936.1.21.1, 1955.2.4.1., 2004.10.16, 2005.06.08.04), sponge slide collections (shelves 1- 5), and dried sponge specimens (drawers 484-501).

	Lat	**Long**	**Habitat type**	**Site Name**
1	52.803	-2.301	Canal	Norbury Junction
2	52.844	-2.400	Canal	Park Heath Bridge
3	52.847	-2.416	Canal	Soundley Bridge
4	52.851	-2.422	Canal	Fox Bridge
5	52.861	-2.439	Canal	Goldstone Bridge
6	52.887	-2.459	Canal	Tyrley Bridge
7	52.901	-2.482	Canal	Market Drayton
8	52.913	-2.478	Canal	Victoria Bridge
9	52.958	-2.495	Canal	Hawsmoor Bridge
10	52.975	-2.510	Canal	Bagley Lane Bridge
11	53.043	-2.541	Canal	Baddington Bridge
12	53.105	-2.574	Canal	Bremilow Bridge
13	53.139	-2.756	Canal	Crows Nest Bridge
14	53.150	-2.778	Canal	Golden Nook Bridge
15	53.172	-2.816	Canal	Egg Bridge
16	53.282	-2.889	Canal	Stanlow Bridge
17	53.286	-2.891	Canal	Powells Bridge
18	52.906	-2.757	Canal	Starks Lift
19	52.903	-2.756	Canal	Dobsons Bridge
20	53.036	-2.588	Canal	Swanley Ridge
21	53.069	-2.575	Canal	Wrenbury Heath Bridge
22	53.028	-2.613	Canal	Wrenbury Church Bridge
23	53.007	-2.667	Canal	Marbury Church Bridge
24	53.730	-2.686	Canal	Quoisley
25	52.980	-2.710	Canal	Grindley Bridge
26	52.971	-2.708	Canal	Whitchurch
27	52.968	-2.707	Canal	Chemistry, Whitchurch

28	52.952	-2.720	Canal	Duddleston Bridge
29	52.949	-2.726	Canal	Canbrian Railway Bridge
30	52.947	-2.723	Canal	Blackoe Bridge
31	52.923	-2.729	Canal	Platt Lane Bridge
32	52.914	-2.805	Canal	Bettisfield Bridge
33	52.904	-2.818	Canal	Hampton Bank Bridge
34	52.902	-2.831	Canal	Lyneal Lane Bridge
35	52.896	-2.835	Canal	Little Mill Bridge
36	52.900	-2.876	Canal	Ellesmere Tunnel
37	52.894	-2.913	Canal	White Mill Bridge
38	52.880	-2.936	Canal	Peters Bridge
39	52.890	-2.958	Canal	Maestermyn House
40	52.890	-2.990	Canal	Hindford
41	52.920	-3.046	Canal	Gledrid Bridge
42	52.927	-3.055	Canal	Monks Bridge
43	52.930	-3.070	Canal	Chirk Tunnel
44	52.957	-3.065	Canal	Whitehouse Bridge
45	52.959	-3.064	Canal	Irish Bridge
46	52.970	-3.120	Canal	Plas Ifan
47	52.970	-3.140	Canal	Wenffrwd
48	52.980	-3.180	Canal	Pentrefelin
49	52.927	-3.055	Canal	Monks Bridge
50	52.880	-2.937	Canal	G. Palmer Lock
51	52.818	-3.020	Canal	Maesbury Marsh
52	52.817	-3.033	Canal	Gronwyn Bridge
53	52.780	-3.090	Canal	Llanymynech
54	52.780	-3.095	Canal	Walls Bridge
55	52.770	-3.110	Canal	Carreghofa Locks
56	52.760	-3.090	Canal	Four Crosses
57	52.719	-3.119	Canal	Bank Lock
58	52.632	-3.169	Canal	Sweeps Bridge
59	52.612	-3.184	Canal	Brithdir
60	52.586	-3.192	Canal	Garthmyl Bridge
61	52.578	-3.198	Canal	Near Garthmyl Aqueduct
62	52.560	-3.229	Canal	Glanhafren Bridge
63	52.842	-2.965	Canal	Heath House Bridge
64	52.839	-2.972	Canal	Corbett's Bridge
65	52.834	-2.982	Canal	A5 Bridge
66	52.910	-3.021	Canal	St Martin's Moor Bridge
67	52.910	-3.036	Canal	Belmont Bridge
68	52.772	-2.380	Canal	Newport Central
69	52.771	-2.384	Canal	Newport Channel
70	52.772	-2.389	Canal	Tickethouse Lock
71	53.354	-2.632	Canal	Golborne
72	53.362	-2.602	Canal	Walton
73	53.367	-2.579	Canal	London Road, Grappenhall
74	53.373	-2.549	Canal	Grappenhall Bridge

75	53.431	-2.312	Canal	Whites Bridge
76	53.451	-2.301	Canal	Trafford Park
77	53.552	-2.169	Canal	Oldham Road Bridge
78	53.549	-2.169	Canal	Chadderton
79	53.527	-2.160	Canal	White Gate Bridge
80	53.509	-2.158	Canal	Failsworth Railway Bridge
81	53.130	-2.374	Canal	Wheelock
82	53.171	-2.418	Canal	Sandbach
83	53.200	-2.457	Canal	Middlewich
84	53.270	-2.539	Canal	Soote Bridge
85	53.480	-2.264	Canal	Middlewood Lock
86	53.479	-2.262	Canal	Margaret Fletcher Lock
87	53.558	-2.380	Canal	Farnworth
88	53.554	-2.375	Canal	Bridge14
89	53.556	-2.378	Canal	Opposite Carlisle Close
90	53.560	-2.387	Canal	Near Hall Lane
91	51.652	-3.816	Canal	Crown Food, Metal Box
92	51.650	-3.820	Canal	Tricks bridge
93	51.647	-3.825	Canal	Neath Junction Rail Bridge
94	51.694	-3.898	Canal	Pont John
95	51.737	-3.824	Canal	Ynysmeudwy
96	51.742	-3.814	Canal	Cilmaengwyn
97	51.737	-3.824	Canal	Cilmaengwyn Lock
98	52.974	-3.139	Canal	Sebastopol Panteg bridge
99	51.678	-3.025	Canal	Crown bridge
100	51.642	-3.027	Canal	Ty Coch Cwmbran
101	53.949	-2.016	Canal	Horse Close Bridge
102	53.940	-2.012	Canal	Snaygill Stone Bridge
103	53.848	-1.830	Canal	Bingley, stretch 18
104	53.115	-2.548	Canal	Cholmondeston
105	53.142	-2.493	Canal	Church Minshall
106	50.668	-3.468	Canal	Turf Inn Lock
107	52.716	-2.694	Canal	Kiln Bridge
108	52.704	-2.694	Canal	Preston Tunnel
109	52.700	-2.679	Canal	Berwick Wharf
110	54.044	-2.801	Canal	Lancaster
111	51.432	-0.326	Canal	Teddington
112	51.510	-0.538	Canal	Iver
113	51.655	-0.425	Canal	Rickmansworth
114	52.686	-1.102	Canal	Leicester Line
115	51.513	-0.036	Canal	Regent’s Canal
116	52.924	-1.051	Canal	Cotgrave
117	51.319	-2.210	Canal	Hilperton Marina
118	51.762	-1.270	Canal	Walton Well
119	51.762	-1.270	Canal	Rugby
120	51.267	-0.781	Canal	Basingstoke Canal
121	53.507	-2.039	Canal	Huddersfield Canal

122	53.319	-0.941	Canal	Retford Town Lock
123	55.930	-3. 233	Canal	Union Canal, Edinburgh
124	50.689	-3.488	Canal	Countess Wear Bridge
125	52.405	-2.744	River	River Corve, Stanton Lacy
126	52.373	-2.721	River	River Corve, Ludlow
127	52.836	-2.673	River	River Roden, Lee Brockhurst
128	52.817	-2.657	River	River Roden, Moreton View
129	52.801	-2.634	River	River Roden, Mill Farm
130	52.867	-1.336	River	Trent Bridge
131	52.757	-1.358	River	Grace Dieu Wood
132	50.780	-3.624	River	River Creedy
133	50.914	-3.290	River	River Culm
134	52.170	1.472	River	River Alde, Langham Bridge
135	52.872	-3.733	River	Afon Lliw, Llanwchllyn
136	52.948	-3.688	River	Afon Tryweryn, Frongoch
137	52.782	-1.971	River	River Trent
138	52.199	0.114	River	River Cam, Newnham Millpond
139	50.764	-1.872	River	River Stour, Bournemouth
140	50.634	-3.438	River	River Exe, Near Exeter
141	51.993	1.390	River	River Deben, Suffolk
142	51.536	-0.900	River	Henley-on-Thames
143	51.568	-0.712	River	River Thames, Cookham
144	51.523	-0.702	River	River Thames, Maidenhead
145	51.382	-0.456	River	River Thames, Weybridge
146	51.567	-0.773	River	River Thames, Marlow Lock
147	52.054	-3.178	River	River Wye
148	51.380	-0.954	River	Swallowfield River, Reading
149	51.972	0.774	River	River Stour At Burs, Suffolk
150	51.319	-2.210	River	River Biss, Trowbridge
151	51.208	-2.939	River	River Brue, Bason Bridge
152	52.110	-3.106	River	River Wye, Clifford
153	51.890	-2.801	River	River Morrow, Skenfrith
154	51.490	-3.294	River	River Ely, St Fagans
155	50.949	-0.502	River	River Arun, Pulborough
156	53.478	-2.259	River	River Lock
157	52.984	-3.665	Lake	Llyn Hesgyn
158	52.916	-3.870	Lake	Llyn Hiraethlyn
159	53.053	-4.224	Lake	Llyn Nantlle Uchaf
160	53.015	-4.016	Lake	Llyn Llagi
161	53.072	-4.151	Lake	Llyn Cwellyn
162	53.126	-4.129	Lake	Llyn Padarn
163	51.640	-3.006	Lake	Cwmbran boating lake
164	56.058	-5.499	Lake	Dunardry Burn
165	56.914	-3.441	Lake	Baddoch Burn
166	55.093	-4.431	Lake	Round Loch of Glenhead
167	57.491	-5.431	Lake	Loch Coire, Fionnaraich
168	54.543	-7.989	Lake	Mallybreen Lough

169	54.100	-2.165	Lake	Malham Tarn
170	54.430	-3.262	Lake	Burnmoor Tarn
171	50.276	-3.655	Lake	Slapton Ley
172	52.291	-0.315	Lake	Grafham Water
173	51.437	-1.018	Lake	Holybrook
174	51.739	-1.258	Lake	Oxford
175	50.963	-2.811	Lake	Lambrook
176	51.432	-2.031	Lake	Calne
177	54.343	-3.071	Lake	Coniston Water, Lake District
178	54.372	-2.991	Lake	Priest's Pot, Lake District
179	50.609	-2.501	Lake	Little Sea Lagoon, Dorset
180	52.229	0.839	Lake	Drinkstone Park, Suffolk
181	57.229	-7.347	Lake	South Uist
182	56.969	-7.494	Lake	Barra, Outer Hebrides
183	56.549	-6.066	Lake	Isle of Mull
184	54.351	-7.647	Lake	Lough Erne, Enniskillen
185	51.878	-0.210	Lake	Knebworth Park, Hertfordshire
186	54.363	-2.986	Lake	Esthwaite, Lake District
187	54.410	-2.890	Lake	Bletham Beach, Lake District
188	54.469	-3.087	Lake	Codale Tarn, Lake District
189	50.649	-2.093	Lake	Little Pea Lagoon, Dorset
190	52.262	0.203	Lake	Mere Fen Road, Waterbeach
191	56.632	-3.577	Lake	Loch Ordie
192	54.535	-3.122	Lake	Watendlath Tarn, Lake District
193	54.430	-3.010	Lake	Loughrigg Tarn, Lake District
194	51.145	-0.347	Lake	Vann Lake, Ockley
195	54.374	-2.938	Lake	Windermere, Lake District
196	57.710	-5.531	Lake	Loch Maree
197	58.178	-4.937	Lake	Loch Fleodach Coire
198	57.579	-5.669	Lake	Upper Diabaig,
199	51.437	0.223	Lake	Brooklands Lake, Kent
200	54.740	-7.006	Lake	Coney Glen Burn
201	56.080	-4.532	Lake	Loch Lomond
202	56.240	-4.102	Lake	Loch Mahaick
203	56.200	-3.439	Lake	Loch Leven
204	51.462	-3.166	Lake	Cardiff Bay Wall
205	52.634	-2.774	Stream	Cound Brook, Stapleton
206	52.487	-2.777	Stream	Byne Brook, Wolverton
207	52.497	-2.763	Stream	Eaton Brook, Harton
208	52.497	-2.754	Stream	Eaton Brook, New Hall Farm
209	52.506	-2.739	Stream	Eaton Brook, Eaton
210	52.372	-2.723	Stream	Linney Brook, Ludlow
211	52.366	-2.684	Stream	Ledwyche Brook
212	52.474	-2.816	Stream	Quinny Brook, Craven Arms
213	52.873	-2.664	Stream	Soulton Brook
214	51.929	0.978	Reservoir	Ardleigh
215	50.900	-2.639	Reservoir	Sutton Bingham

217	51.590	-0.046	Reservoir	Walthamstow Waterworks
218	51.417	-0.460	Reservoir	Conduit Littleton Reservoir
219	52.594	1.492	Broad	Buckenham Hassingham
220	52.716	1.461	Broad	Burntfen Broad
221	52.723	1.606	Broad	Heigham Sound
222	52.665	1.537	Broad	Upton Little Broad
223	52.723	1.515	Broad	Cromes Broad
224	52.766	1.520	Broad	Stalham Broad
225	50.709	-3.526	Pond	Salmon Pool, Exeter
226	51.554	0.495	Pond	Fish Farm Tanks, Haselmere
227	51.450	-0.554	Pond	Wraysbury gravel pit
228	52.648	-3.150	Pond	Welshpool fishing pool
229	53.052	-2.688	Mere	Deer Park Mere, Cheshire
230	53.048	-2.460	Moss	Wybunbury

TableS2. Accession numbers for nucleotide sequnces analysed.

	28S rDNA nucleotide sequence accession number
Ephydatia fluviatilis	MK423202
Ephydatia muelleri	MK423203
Eunapius fragilis	MK423204
Spongilla lacustris	MK423205
Racekiela ryderii	MK423206
Trochospongilla horrida	MH569483
Corvomeyenia sp.	DQ178649

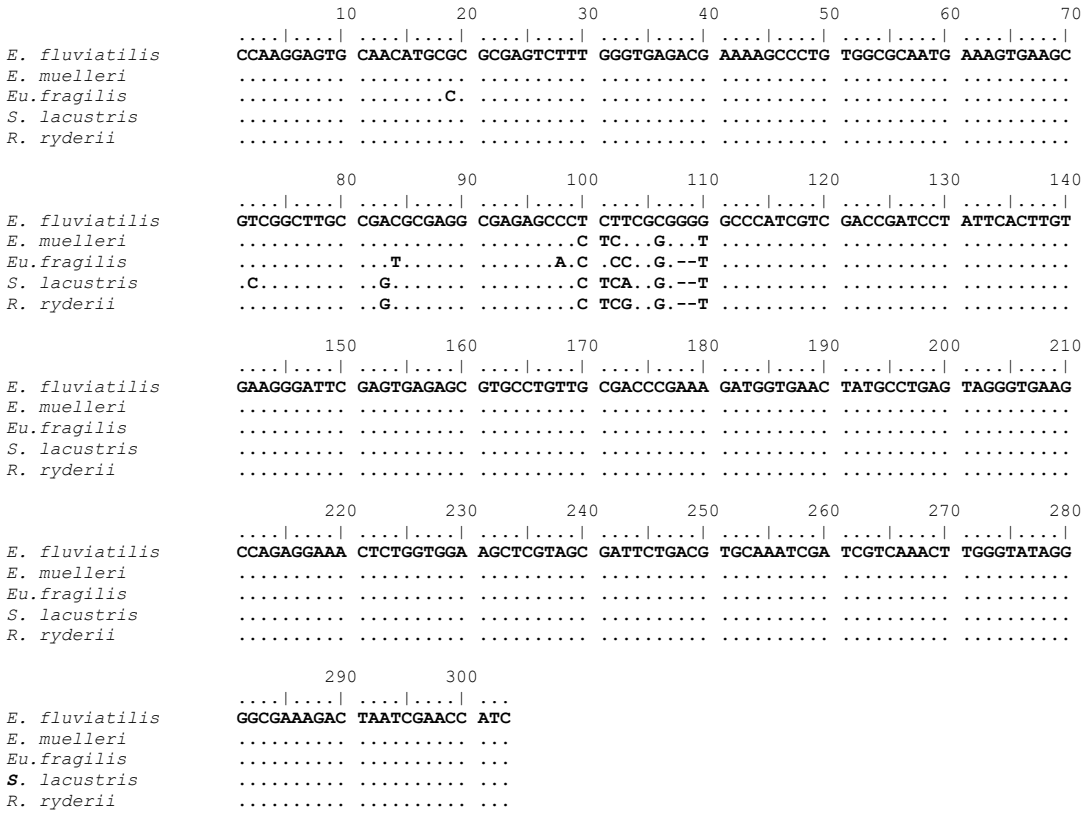

Figure S1. Alignment of the extended 28S rDNA D3 domains from *Ephydatia fluviatilis*, *E. muelleri*, *Eunapius fragilis*, *Spongilla lacustris* and *Racekiela ryderii*. Identities have been indicated by dots and hyphens.