



Contents lists available at ScienceDirect

## Molecular Phylogenetics and Evolution

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## Short Communication

## Insights into the evolution of freshwater sponges (Porifera: Demospongiae: Spongillina): Barcoding and phylogenetic data from Lake Tanganyika endemics indicate multiple invasions and unsettle existing taxonomy

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## ARTICLE INFO

## Article history:

Received 21 January 2011

Revised 3 May 2011

Accepted 30 May 2011

Available online xxxxx

## Keywords:

Freshwater sponges

Porifera

Lake Tanganyika

Ancient lakes

Species radiations

Barcoding

## ABSTRACT

Sponges are a conspicuous element in many benthic habitats including in Africa's oldest, deepest lake, Lake Tanganyika. Despite their prevalence and pivotal ecological role as filter feeders, knowledge of the evolutionary history of sponges is in its infancy. Here, we provide the first molecular analysis targeting the evolution of sponges from Lake Tanganyika. Independent markers indicate the occurrence of several colonisation events which have shaped the current Tanganyikan lacustrine sponge biodiversity. This is in contrast to a range of previously studied organisms that have diversified within the lake from single lineages. Our tree reconstructions indicate the presence of two genera, *Oncosclera* and *Eunapius*, which are globally distributed. Therefore, we reject the hypothesis of monophyly for the sponges from Lake Tanganyika and challenge existing higher taxonomic structure for freshwater sponges.

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## 1. Introduction

Evolutionary studies of sponges are challenging received wisdom for major questions in biology. Despite seemingly simple morphology, apparently little modified since the Cambrian, their success in wide ranging habitats (from abyssal marine to running freshwaters), their high species diversity (>8000 Recent taxa described to date, Van Soest et al., 2011) and the nascent stage of phylogenetic knowledge, research on sponges continues to provide important insights into animal evolution.

We are extending research on sponges to Lake Tanganyika (LT), the largest of the East African rift lakes and a classic island system that has revealed contrasting model systems in evolutionary radiations of organisms as varied as cichlid fish (Salzburger et al., 2002), cerithioid snails (Michel, 2004), and platyhelminthid crabs (Marijnissen et al., 2006). In each well-documented case, invasion

of the rift lake has been followed by species-rich radiations, but with differences in triggers, timing and scale of diversification. It has been proposed that after the Tanganyika rift formed 9–12 million years ago, and evolved to something resembling its current mega-lake form several million years later (Cohen et al., 1997), it provided a series of novel niches, subsequently exploited by diversification-prone taxa and a cradle of origin for taxa now found in surrounding, more ephemeral freshwater systems (see e.g. Wilson et al., 2004 and references therein).

The Tanganyikan sponge fauna appears to be highly endemic including five species in two endemic genera (Manconi and Pronzato, 2008). These include animals with diverse growth forms living in a wide range of habitats (see Supplementary Figure), making this an ideal model assemblage for exploring patterns of sponge evolution in a single lake system. While in some habitats sponges are cryptic, for example in deepwater rocky habitats, in other habitats they are common, encrusting large surfaces of shallow rock walls or forming thickets of small branching sponges on shell beds, thus they are likely to play important ecological roles. However, until now, our knowledge of the Tanganyikan sponge fauna has been largely restricted to the original systematic descriptions, each

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based on very limited material. Eight species from six genera have so far been described from Lake Tanganyika, all from Burundi, Zambia and the Democratic Republic of Congo (Manconi and Pronzato, 2009). Despite their pivotal ecological function as filter feeders, existing studies of African freshwater sponges in general are scarce and contain little information beyond taxonomic descriptions (see Manconi and Pronzato, 2009 for a current review).

The taxonomy of freshwater sponges is currently challenged by molecular data (e.g. Meixner et al., 2007). There are currently about 250 species divided into six extant described families, and one extinct family. Similarities in the morphology of their skeletal elements have caused them to traditionally be assigned to the class Demospongiae, order Haplosclerida, a very diverse and successful group. The freshwater members all have been placed in the suborder Spongillina (Manconi and Pronzato, 2002). However, recent molecular phylogenetic analyses favour a non-monophyletic Haplosclerida and suggest independent evolution of marine and freshwater lineages (see e.g. Boury-Esnault, 2006). Nonetheless, until recently, all molecular data have suggested that the freshwater sponges are monophyletic (e.g. Addis and Peterson, 2005; Redmond et al., 2007).

Colonisation from marine to limnic systems has been suggested to have taken place in the Jurassic, with freshwater sponges now inhabiting a diverse range of habitats throughout all continents except Antarctica (see for details Manconi and Pronzato, 2002). Most freshwater sponges have gemmulae, globular bodies containing totipotent cells protected by an armoured layer of specialised spicules (gemmulothecae). Gemmulae facilitate dispersal and also function as resting bodies that may endure unfavourable abiotic conditions, which affect limnic more frequently than marine systems (Frost et al., 1982; Pronzato and Manconi, 1994, 1995). Presence or absence of gemmulae and differences in gemmular morphology varies at the family level and is considered to have had a major impact on dispersal success of the freshwater taxa (Manconi and Pronzato, 2002), leading to divergent geographical distribution patterns of freshwater sponge families. The families with a complex and variable gemmular bauplan are widespread; species in the family Spongillidae inhabit almost all regions, ranging from boreal to tropical, and species in the Metaniidae are widespread from temperate to tropical regions. In contrast, families with a simple gemmular bauplan are mostly geographically more restricted; members of the Potamolepidae are Afro- and Neotropical only, and the family Palaeospongillidae is extinct and known only from southern Patagonia. The families lacking gemmulae are known exclusively from ancient limnic systems, including the Lubomirskiidae (Lake Baikal region), Metschnikowiidae (Caspian Sea) and Malawispongiidae (endemic to various ancient lakes worldwide) (reviewed in Manconi and Pronzato, 2002).

While the freshwater sponge fauna of Lake Baikal in Siberia, the largest and oldest freshwater lake in the world, has been the focus of a series of evolutionary studies (e.g. Meixner et al., 2007), the species richness, systematic diversity, and evolutionary history of the sponge fauna in Lake Tanganyika remains effectively unknown. Until recently, only very limited and opportunistic collecting has been undertaken; most species are known from one or a few poorly labelled specimens lacking geographic or habitat information.

In addition, there are difficulties in the unambiguous systematic assignment of taxa due to a paucity of complex morphological characters (see e.g. Boury-Esnault, 2006), a problem in most lineages of sponges, which hinders taxonomic assignments, and complicates evolutionary conclusions. This means that until we make progress through both substantially intensifying collection efforts and refining taxonomic tools, our poorly developed taxonomic knowledge will restrict the description of species, hamper analysis

of radiation patterns and render biodiversity assessments grossly incomplete for ancient lakes. A potential solution is provided by molecular approaches. DNA barcoding was initially introduced as a method for diagnosis of known species but likewise finds application in the assessment of biodiversity patterns and cryptic speciation in sponges (e.g. Pöppe et al., 2010). These gene fragments used for barcoding also harbour considerable phylogenetic signal, which in many studies showed considerable congruence to ribosomal data (e.g. 18SrDNA, see Lavrov et al., 2008). Comparative phylogenetic analyses between the mitochondrial cytochrome oxidase subunit 1 and ribosomal genes resulted in largely congruent gene tree topologies for freshwater sponges from a wide range of families (Itskovich et al., 2007; Meixner et al., 2007).

We have sequenced the barcoding region of COI and intron 2 of ITS2 for a selection of sponges from Lake Tanganyika. To our knowledge this is the first molecular analysis of a diverse set of African ancient lake sponges. We use these to derive preliminary insights into phylogenetic relationships and to assess patterns of evolutionary diversification. By combining our results with those from other freshwater sponges, we begin to assess (1) whether the Tanganyikan sponges radiated after a single colonisation event, or resulted from repeated colonisation events from different basal taxa and (2) whether radiations are restricted to endemic genera, as seen in most other Tanganyikan endemics, or include wide-ranging genera.

## 2. Material and methods

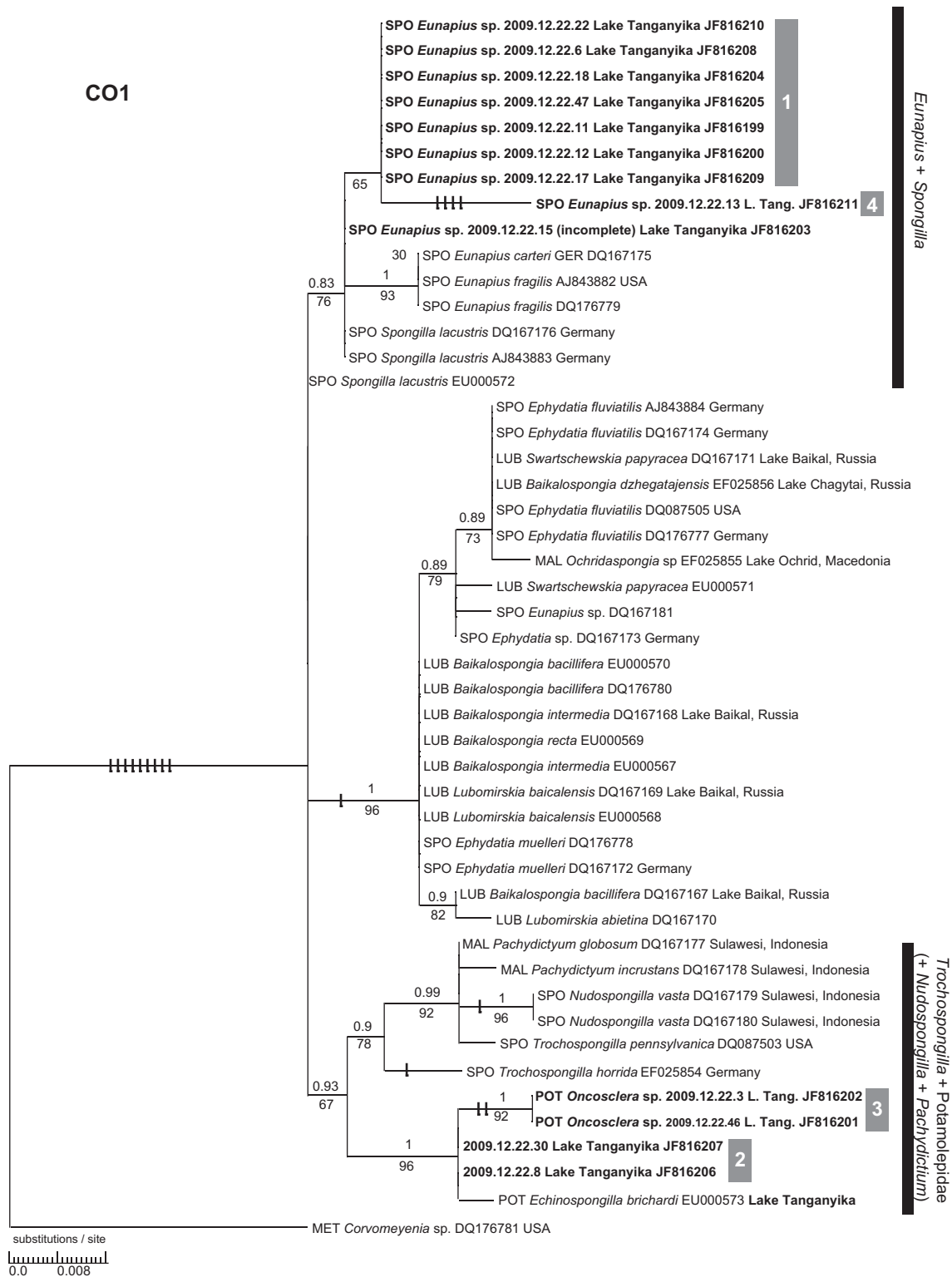
We amplified the DNA for the COI barcoding fragment and intron 2 of the nuclear ribosomal cistron (ITS2) from sponge specimens collected during series of underwater surveys of sponges at nine sites near Kigoma (Kigoma Region, Tanzania) of Lake Tanganyika (Weier, 2005 and additional collections by EM and JT). After inclusion of several additional spongillid COI sequences from GenBank ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)), gene trees were reconstructed.

For detailed material and methods please refer to [Supplementary Material](#).

## 3. Results and discussion

The reconstructed gene trees for COI and ITS2 from the Lake Tanganyika specimens have similar phylogenetic relationships (Figs. 1 and 2). The COI sequences of the Lake Tanganyika specimens collected during this study produced four haplotypes. In the COI gene tree Haplotype 1 and Haplotype 4 (both morphologically identified as *Eunapius* sp., in the family Spongillidae) form a monophyletic group with *Eunapius* species from other locations outside Lake Tanganyika. Together they form a sister group with *Spongilla lacustris*. Haplotype 2 (morphologically not unambiguously identified) and Haplotype 3 (identified as *Oncosclera* sp., Potamolepidae) cluster with another potamolepid *Echinospongilla brichardi*. They are a sister group to a clade consisting of *Nudospingilla* and *Trochospongilla* (Spongillidae) and *Pachydictyum* (Malawispongiidae).

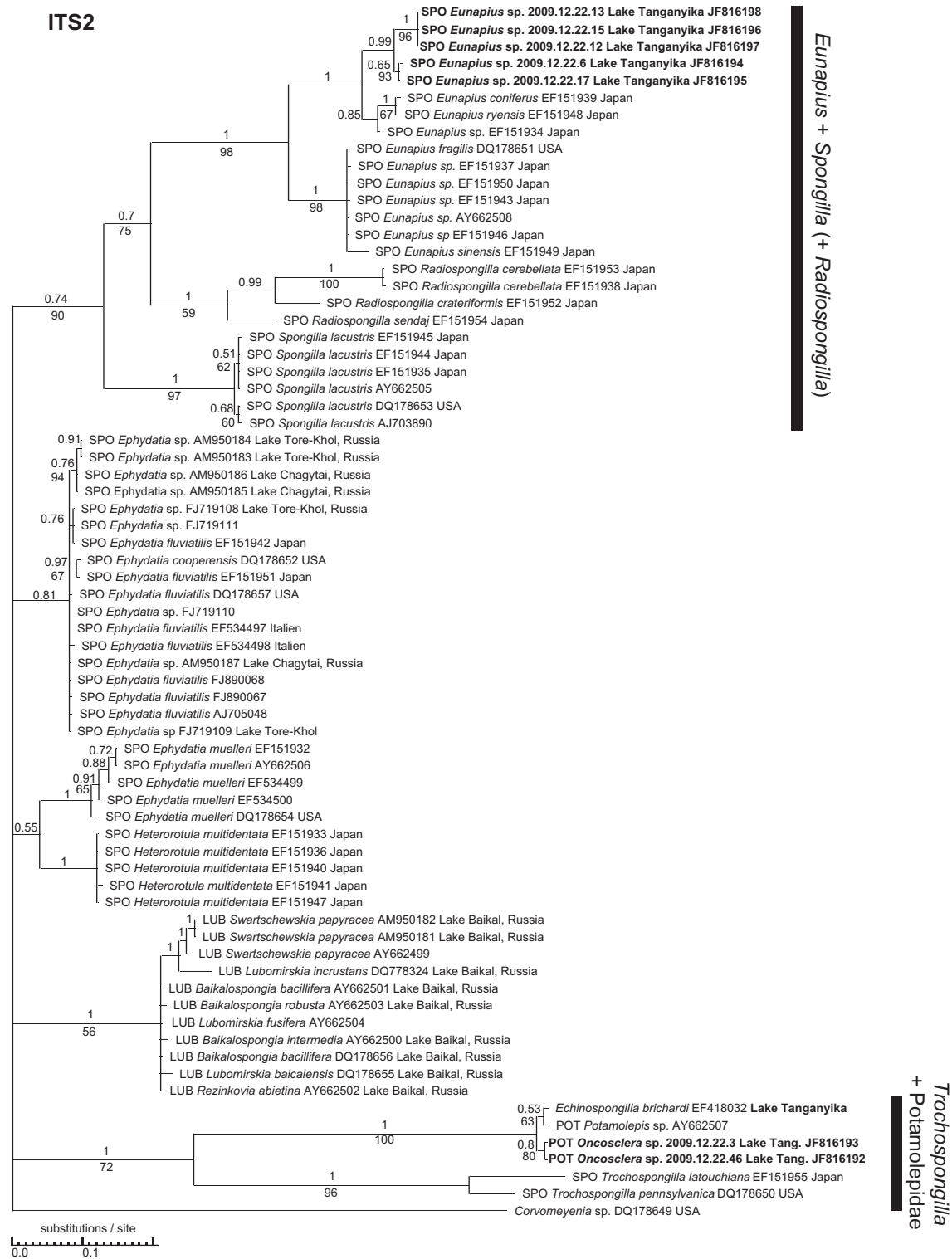
ITS2 phylogenetic reconstructions result in a topology for the Lake Tanganyika samples that is congruent with the COI gene tree. The Lake Tanganyika *Eunapius* (Haplotypes 1 and 4 in COI) form a monophyletic group with other *Eunapius* species and again form a sister group to *Spongilla* (and *Radiospongilla*). ITS2 amplifications of specimens with a COI Haplotype 2 were unsuccessful, but *Oncosclera* (COI Haplotype 3) specimens again cluster with *E. brichardi* (and a *Potamolepis* sp.), resulting in a Potamolepidae clade as a sister group to the spongillid *Trochospongilla* species in congruence with COI.



**Fig. 1.** Phylogram of phylogenetic relationships based on COI reconstructed with Maximum Likelihood (RAxML) and Bayesian Inference methods (MrBayes). Numbers indicate Bayesian posterior probabilities above the branches and the bootstrap probabilities below. Taxon names comprise Spongillina family abbreviations (LUB = Lubomirskidae, MAL = Malawispongiidae, MET = Metaniidae, POT = Potamolepidae, SPO = Spongillidae), Genbank Accession number, NHMUK accession code and sampling site (if known from the literature). White numbers in grey boxes indicate COI haplotypes. Vertical bars at the branches indicate amino acid changes. Images of selected representatives of sequenced LT sponges are presented in the [Supplemental Figure](#).

The 26 autapomorphic and 16 informative COI characters display non-synonymous substitutions along the branches to two Lake Tanganyika haplotypes, resulting in four and two amino-acid

substitutions respectively (Haplotype 1: Q > E, F > L, L > F, I > N, Haplotype 3: M > I, A > S). Non-synonymous substitutions are rare among the Spongillina sequences known to date (see [Fig. 1](#)). All



**Fig. 2.** Phylogram of phylogenetic relationships based on ITS2 reconstructed with Maximum Likelihood (RAxML) and Bayesian Inference methods (MrBayes). Numbers indicate Bayesian posterior probabilities above the branches and the bootstrap probabilities below. Taxon names comprise Spongillina family abbreviations (LUB = Lubomirskidae, MAL = Malawispongiidae, MET = Metaniidae, POT = Potamolepidae, SPO = Spongillidae), Genbank Accession number, NHMUK Zoology Department collection registration number and sampling site (if known from the literature).

amino acid changes are apomorphic for the haplotypes and consequently provide no internal structure to the phylogenetic relationships in this study.

All four haplotypes are different from previously assessed haplotypes for Spongillina and therefore appear to be endemic to Lake

Tanganyika, with the important caveat that molecular sampling from elsewhere in Africa is non-existent. This is in contrast to lubomirskiid sponges from the Lake Baikal region, whose presumed endemic species share identical COI haplotypes with widespread species collected from other continents. For example, *Lubomirskia*

*baikalensis*, *Baikalospongia intermedia*, *Baikalospongia erecta*, and some specimens identified as *Baikalospongia bacillifera* have COI haplotypes identical to the more widespread spongillid *Ephydatia muelleri* collected in Germany or North America (Fig. 1). Likewise, one specimen identified as the endemic lubomirskiid *Swartschewskia papyracea* (DQ167171) shares its COI haplotype with a widespread species identified as *Ephydatia fluviatilis* for specimens collected in Germany and identified as the junior synonym *Clypeatula cooperensis* for specimens collected in North America (Fig. 1, N.B.: the lubomirskiid nature of *Baikalospongia dzhegatajensis* is questioned, see e.g. Maikova et al., 2010). This observation led to speculations that *Ephydatia* is not monophyletic and that species belonging to Lubomirskiidae may have evolved from one of these “*Ephydatia*” lineages (Addis and Peterson, 2005; Meixner et al., 2007). Likewise the COI data rejects monophyly for the endemic family Lubomirskiidae, similar to 18S rDNA analyses (e.g. Itskovich et al., 2007; Meixner et al., 2007). Nevertheless, support in these 18S trees is low and in contrast to ITS2 data (Fig. 2 and Itskovich et al., 2008, N.B.: the alignment with other taxa necessitates the exclusion of many lubomirskiid synapomorphies).

Our analyses reject monophyly of the Lake Tanganyika sponges and cast further uncertainty on the existing higher taxonomic structure for freshwater sponges. The tree reconstructions derived from both independent loci and under different reconstruction methods (Maximum Likelihood and Bayesian Inference) unequivocally indicated that *Eunapius* (Haplotypes 1 and 4) from Lake Tanganyika are more closely related to widespread species of *Eunapius* and *Spongilla* collected from Europe and North America than to the other Tanganyikan sponges in this study. Similarly, *Oncosclera* (Haplotype 3) and the Haplotype 2 specimens form a clade with *E. brichardi* (plus *Potamolepis* in ITS2) (Potamolepidae, Itskovich et al., 2006), which are endemic to Lake Tanganyika and which again is sister to a clade to spongillids (and malawispongiids in CO1). This underscores the non-monophyly of the nominal and widespread freshwater sponge family Spongillidae and supports earlier speculations that several endemic freshwater lineages arose from members of this family (discussed in Meixner et al., 2007, see also Addis and Peterson, 2005; Harcet et al., 2010; Itskovich et al., 1999; Redmond et al., 2007). Spongillidae currently consists of 23 valid genera (Van Soest et al., 2011), making it one of the most genus-rich families within the class Demospongiae. Like many other sponge taxa, definition of Spongillidae as a clade is difficult, as membership is mostly defined by a combination of characters rather than unambiguous autapomorphies; this is additionally hampered by a wide plasticity of the morphological characters and their combinations. Thus, it is plausible that spongillid lineages are sister groups to the several endemic freshwater sponge taxa and radiations. Meixner et al. (2007) remarked that morphological definition of Malawispongiidae (Manconi and Pronzato, 2002) is weak, as it relies on the absence of characters. This makes it doubtful that there is a close phylogenetic relationship among malawispongiid genera restricted to hydrologically separated tectonic lakes in Africa, Europe and Asia, despite the antiquity of these water bodies. Meixner et al. (2007) showed that several genera were more closely related to members of Spongillidae than to their own presumed confamilials, which is also evident in the molecular analysis we present here (see also the remarks of Harcet et al. (2010) on this topic).

The COI Haplotype 1 of *Eunapius* sp. displays a remarkable accumulation of substitutions compared to its congeners (Fig. 1). Porifera and other diploblastic animals possess a reduced substitution rate (Shearer et al., 2002). In marine sponges, nucleotide diversities among populations of *Crambe crambe* (Poecilosclerida) and *Astrosclera willeyana* (Agelasida) collected from locations several thousand kilometres apart were found to be as low as  $\pi = 0.00049$  and  $\pi = 0.0006$ , respectively (Duran et al., 2004;

Wörheide, 2006). This was congruent with current views that geographic ranges of sponge species are frequently overestimated (Klautau et al., 1999) and the number of distinct and endemic species is higher than currently estimated (Bierne et al., 2003; Palumbi et al., 1997). Likewise, intraspecific variation in demosponge COI is generally low (and occasionally absent) and therefore insufficient for DNA barcoding with the standard barcoding fragment (see e.g. Erpenbeck et al., 2006). In this respect Haplotype 1 could indicate a separate *Eunapius* lineage, however, this finds no support with our ITS2 data.

Our sampling reveals genetic diversity in an ecologically diverse genus (see also Manconi et al., 2008). *Eunapius* was previously unrecorded from Lake Tanganyika, though present in surrounding waterbodies, including the Malagarasi River and elsewhere in the Congo drainage, and widespread in Africa (eight taxa) (Manconi and Pronzato, 2008) as well as other continents. Further collecting and molecular characterisation of *Eunapius* species from surrounding waterbodies and elsewhere in Africa are in progress to unravel the *Eunapius* intralacustrine radiation. *Eunapius* then may be one of the very few widespread taxa that successfully radiated within Lake Tanganyika (others include *Gomphocythere* ostracods and haplochromine cichlids).

## Acknowledgments

D.E. acknowledges support from a SYNTHESYS Grant No. GB-TAF-4186. Fieldwork by T.W., E.M. and J.A.T. was aided by George Kazumbe and Kimberly Smith, and supported by US NSF DBI-0353765, The Nyanza Project, with thanks to University of Dar es Salaam and TAFIRI-Kigoma for research permits and field station infrastructure. G.W. acknowledges funding from the German Science Foundation (DFG). Support, advice and access to museum collections were generously provided by Clare Valentine and Emma Sherlock (Natural History Museum, London), Rob van Soest (University of Amsterdam) and Andrew Cohen (University of Arizona). Benjamin Läubli, Simone Schätzle and Gabriele Büttner are thanked for help in the lab, and Joanna Griffin for her help with specimen curation. We also thank two anonymous reviewers for their constructive suggestions to improve earlier versions of this manuscript.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympcv.2011.05.021.

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