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OPINION PAPER

# Progress in glass sponge phylogenetics: a comment on Kersken et al. (2018)

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**Abstract** Kersken et al. (Hydrobiologia 811:283–293, 2018) recently reported a phylogenetic analysis of ribosomal DNA of glass sponges (Porifera: Hexactinellida) including new specimens from the NE Pacific. Their study is important as it significantly increased the taxon sampling of this important deep-sea group. Unfortunately, there are several issues with these authors' dataset assembly and choice of phylogenetic analysis methods. Furthermore, they did not consider previous literature that is highly relevant to interpretation of their results. Here I provide critically constructive comments on their paper and present an alternative analysis that utilizes established methodological techniques. I show that several of Kersken et al.'s findings and claims are unsustainable, and provide an extended discussion of the updated glass sponge phylogeny.

**Keywords** Hexactinellida · COI · Phylogenetic analysis · Ribosomal DNA · Secondary structure · Taxon sampling

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## Introduction

In a recent paper in *Hydrobiologia*, Kersken et al. (2018) (KEA hereafter) presented a molecular phylogenetic analysis of glass sponges (Porifera: Hexactinellida), including newly generated ribosomal DNA (rDNA) sequences from several thus far unsequenced species collected from polymetallic nodule fields of the NE Pacific Clarion-Clipperton Fracture Zone, adding to a set of previously published sequences. Clearly, these authors' efforts to genetically characterize this fauna and increase taxon sampling for molecular phylogenetics of these fascinating and ecologically important deep-sea animals are laudable. Unfortunately, however, KEA's analysis was not conducted according to currently established standards in the field, and their paper lacks a thorough discussion of, and comparison with, the latest literature on the subject (Dohrmann et al., 2017). This hinders comparability of their results with previous work and obfuscates how addition of the newly sequenced species advances our understanding of glass sponge phylogeny and systematics. Below I present a reanalysis of KEA's data using a methodological framework that was previously established for molecular phylogenetics of Hexactinellida (Dohrmann et al., 2008, 2012b, 2017) and compare the results with those of KEA and Dohrmann et al. (2017). Before I discuss methodological issues of KEA however, I draw attention to a number of errors and omissions in their Introduction and Results sections.

## Comments on Kersken et al.'s introduction and results sections

In their Introduction (p. 284), KEA give an account of previously published work on the molecular phylogenetics of glass sponges, conducted within the last decade. While they correctly cite Dohrmann et al. (2008, 2009, 2012a) and Reiswig & Dohrmann (2014), they erroneously cite Dohrmann et al. (2012b) as "Dohrmann et al. (2011)" and omit the actual study by Dohrmann et al. (2011). A more serious omission is the lack of consideration of the study of Dohrmann et al. (2017) (DEA17 hereafter), which would have provided the most up-to-date and comprehensive account of glass sponge phylogeny available to KEA at that time. While KEA included sequence data published by DEA17 in their phylogenetic analysis, this work was neither referred to nor discussed.

KEA then describe the outcomes of some of those earlier papers, stating for Dohrmann et al. (2008) that, "Monophyly of the orders Hexasterophora, Lyssacinosida and Sceptrulophora was not supported". This is nonsensical as firstly, Hexasterophora is a subclass not an order, and its monophyly was highly supported (as KEA correctly state in the preceding sentence)—presumably they meant the order Hexactinosida, monophyly of which was indeed rejected by Dohrmann et al. (2008). Secondly, monophyly of Lyssacinosida was dependent on the RNA substitution model used, so perhaps not fully supported but rather, ambiguous. Finally, one of the main outcomes of Dohrmann et al. (2008) was that monophyly of Sceptrulophora was indeed maximally supported.

In KEA's Results section, it then appears that they misinterpreted parts of their own phylogenetic tree (their Fig. 1). On p. 287 they state that monophyly of Lyssacinosida, Aphrocallistidae, Dactylocalycidae, and Leucopsacidae was maximally supported by Bayesian posterior probability (BPP). However, these groups are clearly not recovered as clades in their Fig. 1: Dactylocalycidae (Hexasterophora *incertae sedis*) is firmly nested within Lyssacinosida, rendering the order paraphyletic, and is itself a paraphyletic group, with *Dactylocalyx* and *Iphiteon* forming successive sister groups to Euplectellidae; likewise, Aphrocallistidae and Leucopsacidae appear polyphyletic as "*Aphrocallistes vastus* MCZDNA105724" and "*Oopsacas* sp. (SMF 12068)" firmly group outside of the remaining representatives of their families. Although the authors later (p. 290) acknowledge non-monophyly of Aphrocallistidae, Dactylocalycidae, and Leucopsacidae in their tree (further discussed below), it is important to point out their earlier misrepresentation as inconsistent with later statements. On the other hand, non-monophyly of Lyssacinosida, which—if true—would have far-reaching consequences for understanding skeletal evolution of glass sponges (cf. Dohrmann et al., 2017), goes entirely undetected by KEA—apparently they assume that Dactylocalycidae belongs in that order, which is incorrect (see Dohrmann et al., 2017; van Soest et al., 2018).

Regarding the relationships within the lyssacinosidan family Rossellidae, KEA state on p. 290 that the small subfamily Acanthascinae (*Acanthascus*, *Rhabdocalyptus*, and *Staurocalyptus*; cf. Reiswig and Stone, 2013) is not recovered monophyletic because "*Rhabdocalyptus dawsoni* Collins, 1998" (represented by 18S) does not group with the two *Acanthascus* spp. they included—"*Acanthascus* sp. (SMF12080)" (16S, 18S) + "*Acanthascus dawsoni* (Dohrmann et al., 2008)" (16S). While this is technically true, KEA overlooked that firstly, *Rhabdocalyptus dawsoni* and *Acanthascus dawsoni* are one and the same species—the latter, used by Dohrmann et al. (2008), is short notation for *Acanthascus (Rhabdocalyptus) dawsoni*. 5 years later the subgenus *Rhabdocalyptus* was elevated (back) to genus level (Reiswig and Stone, 2013). Hence, KEA's conclusion should have been that the species *R. dawsoni* is polyphyletic. Secondly, however, the 18S sequence published by Collins (1998) was actually used by Dohrmann et al. (2008) and subsequent studies. Thus, KEA should have concatenated this sequence with the 16S sequence of Dohrmann et al. (2008) instead of including it as a separate terminal taxon. Although the position of "*Rhabdocalyptus dawsoni* Collins, 1998" as sister to *Crateromorpha meyeri* in KEA's tree remains somewhat mysterious, it is clear from the above that discussions of non-monophyly of Acanthascinae or misidentification of the Collins specimen (as suggested by KEA: p. 290) are obsolete.

Finally, on p. 290 KEA further state that they did not recover monophyly of Farreidae, which is incorrect. Monophyly of that family is maximally supported in KEA's Fig. 1—it is only the type genus *Farrea* that is paraphyletic (further discussed below).

Comments on Kersken et al.'s methodological approach

DEA17 presented the most comprehensive molecular phylogeny of glass sponges at that time (their Fig. 7) and made the underlying alignments of 16S, 28S, and 18S rDNA, as well as cytochrome oxidase subunit 1 (COI) publicly available as a resource for future studies (Additional files 5-8 of DEA17, available at https://doi.org/10.6084/m9.figshare.3120130.v3). Instead of adding their new sequences to these carefully curated alignments, KEA built their dataset from scratch, by automatically aligning their new sequences with sequences downloaded from GenBank. However, they did not use RNA secondary structure information to aid alignment, as was done for 18S and 28S in DEA17 and previous studies (consensus structures are included in the alignments of DEA17). KEA also did not incorporate secondary structure information in their phylogenetic analysis, although it has been comprehensively shown that taking co-evolution of paired sites into account is important in sponge rDNA phylogenetics (Dohrmann et al., 2006, 2008; Erpenbeck et al., 2007; Voigt et al., 2008). Furthermore, it appears that KEA did not estimate substitution model parameters independently for each gene (partitioning), which is a standard procedure nowadays and was also used in DEA17 and previous studies. Finally, KEA state that they were able to amplify COI sequences for only seven specimens, but unfortunately they did not include or publish these. Instead, they excluded the COI partition altogether, which further reduces comparability with previous work (Dohrmann et al., 2012b, 2017).

## Materials and methods

Here I provide an alternative analysis following the protocol of DEA17. Firstly, I retrieved the new sequences of KEA, as well as some other previously published sequences used by KEA but not DEA17 from GenBank. Inspection of KEA's Supplementary Table revealed that the authors did not include the following publicly available sequences: 18S of *Atlantisella* sp., *Euryplegma auriculare*, and *Tretodictyum reiswigi*, 28S of "*Dactylocalyx pumiceus* RMNHPOR9215", *Nodastrella nodastrella*, *Acanthascus dawsoni*, and *Cyrtaulon sigsbeei*, 16S and 28S of "*Sympagella nux* Dohrmann et al., 2008" [which should be "2012" since Dohrmann et al. (2008) did not include any sequences from that species], and 16S of *Tabachnickia* sp. and *Lophophysema eversa* (Haen et al., 2014; Zhang et al., 2016). Furthermore, *Heterorete* sp. and "*Sympagella nux* Dohrmann et al., 2012" do not appear in KEA's tree although they are listed in their Supplementary Table. These sequences are included here as they are part of DEA17's alignments (except *T. reiswigi*, which is from Boury-Esnault et al., 2017). I also included 28S sequences of several *Rossella* spp. from Vargas et al. (2017). These were not available in GenBank at the time of writing and hence not used by KEA (who only included the corresponding 16S sequences), but kindly provided to me by S. Vargas.

I manually aligned all new sequences (including COI, where available) to the curated alignments provided by DEA17, using AliView (Larsson, 2014). During that process, it turned out that the sequence of "*Aphrocallistes vastus* MCZDNA105724" (18S; from Riesgo et al., 2014) was very difficult to align; a BLAST search (Altschul et al., 1990) revealed that it is a demosponge sequence erroneously uploaded under the name *Aphrocallistes vastus*, so its placement within Hexactinellida is entirely random and discussions of non-monophyly of Aphrocallistidae (see above) are obsolete. Besides this sequence I also excluded the sequences of *Sympagella nux* and *Iphiteon panicea* published by Adams et al. (1999) since they contain numerous missing or undetermined bases and obvious sequencing errors and are therefore of insufficient quality for phylogenetic inference; furthermore, I did not use the sequence (18S) of *Farrea occa* published by West & Powers (1993); see Dohrmann et al. (2009) for justification. All updated alignments are available at https://doi.org/10.6084/m9.figshare.5951965.

After trimming unalignable regions, I concatenated the individual alignments (including COI) with SeaView (Gouy et al., 2010), and subjected the resulting supermatrix (available at https://doi.org/10.6084/m9.figshare.5951965) to partitioned maximum-likelihood analysis in RAxML v. 8.0.26 (Stamatakis, 2014), using the -f a option. I assigned independent GTR + G models (Lanave et al., 1984; Yang, 1994) to COI, 16S, and unpaired sites of 18S and 28S, and the S16 + G model (see Savill et al., 2001) to paired sites of 18S + 28S (note that a reliable consensus secondary structure for the 16S fragment cannot be

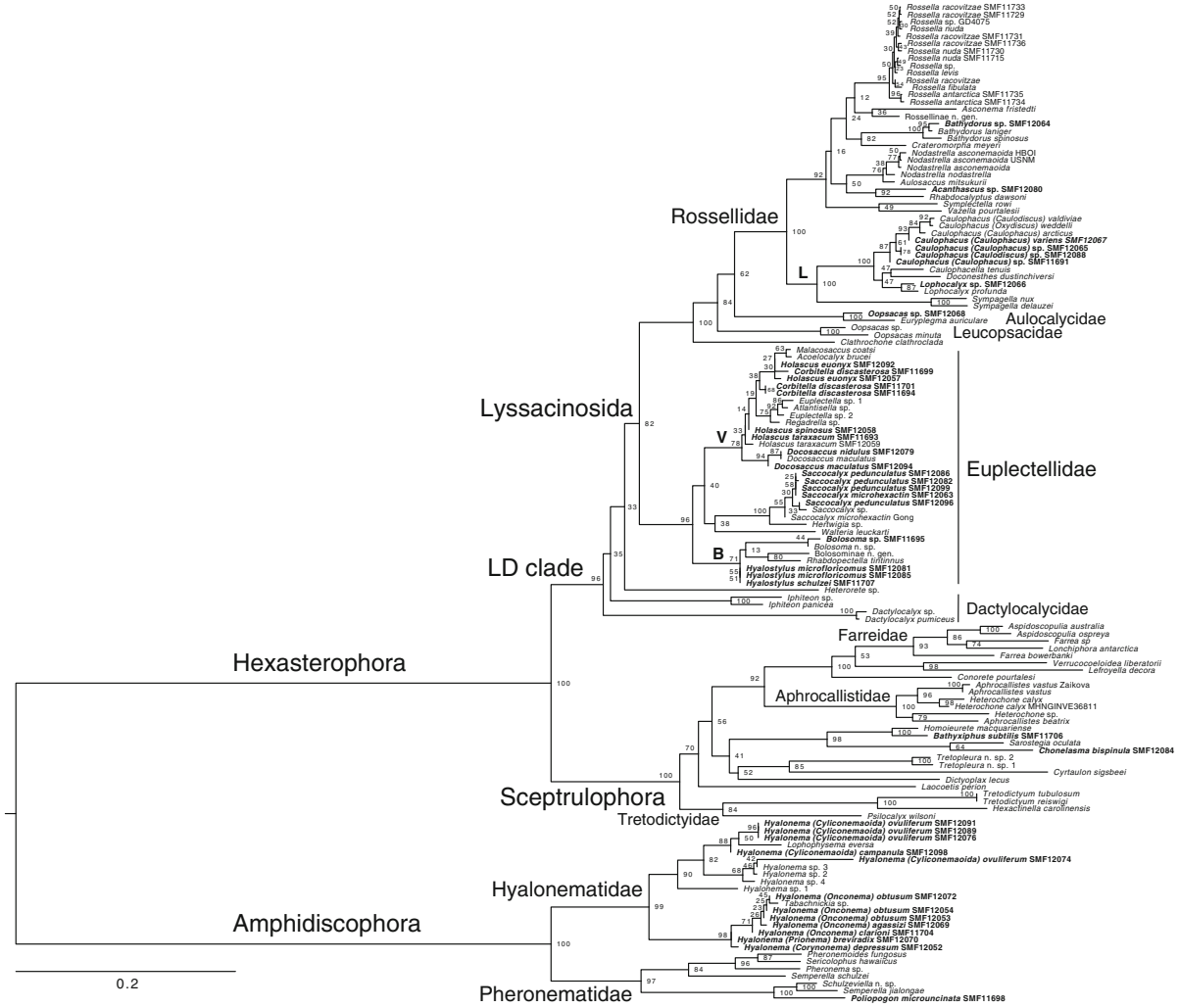


**Fig. 1** Maximum-likelihood phylogeny of Hexactinellida inferred using the protocol of DEA17 (see Materials and methods section). Specimens that were newly sequenced by KEA are displayed in bold font. *L* Lanuginellinae, *B* Bolosominae sensu stricto, *V* Venus Flower Basket clade sensu lato (see text for explanations). Numbers at branches are non-parametric rapid bootstrap values (Felsenstein, 1985; Stamatakis et al., 2008) based on 450 pseudoreplicates as determined by autoMRE bootstopping (Pattengale et al., 2010). Scale bar, expected number of substitutions per site

predicted). The resulting tree is shown in Fig. 1 (available in Newick format at https://doi.org/10.6084/m9.figshare.5951965) and discussed in the following.

## Results and discussion

### Deep phylogeny of Hexactinellida

As in KEA and DEA17, hexactinellid phylogeny is composed of three major clades: Amphidiscophora, Sceptrulophora, and a clade containing Lyssacinosida and Dactylocalycidae ("LD clade" of DEA17); Sceptrulophora and the LD clade together form the Hexasterophora. Monophyly of Dactylocalycidae is not recovered, which is also congruent with these two studies. However, in KEA paraphyly of this family was maximally supported by BPP, whereas here it has very low bootstrap support (BS), as in DEA17 (BS and BPP $< 50$). More strikingly, the dactylocalycids (*Dactylocalyx* and *Iphiteon*) are here and in DEA17 recovered outside of a monophyletic Lyssacinosida, whereas KEA recovered them nested within that order

with maximal BPP, rendering it paraphyletic (see above). Notably, support for monophyly of Lyssacinosida here is higher than in DEA17 (82 vs. 65% BS), possibly due to the increased taxon sampling. Branching order of *Iphiteon*, *Dactylocalyx*, and *Heterorete* (Hexasterophora *inc. sed.*, not included by KEA) differs from that obtained in DEA17, but the low support values basically render the exact positions of these three genera unresolved (for further discussion see DEA17).

Within Lyssacinosida, the position of *Clathrochone clathroclada* (*inc. sed.*) as sister to Leucopsacidae + Aulocalycidae + Rossellidae agrees with DEA17, whereas KEA recover this species as sister to Leucopsacidae, a hypothesis that was first proposed by Dohrmann et al. (2008) but could not be corroborated by follow-up studies (Dohrmann et al., 2009, 2012b).

Amphidiscophora: Pheronematidae

The topology within Pheronematidae recovered here is the same as in KEA (note however that *Pheronemoides fungosus* and *Semperella jiaolongae* were named by KEA "Pheronematidae gen. sp. (Gong et al., 2016)" and "*Semperella crosnieri* (Gong et al., 2015)", respectively, based on outdated GenBank entries). As noted, but not further discussed by KEA, the genus *Semperella* appears diphyletic, with *S. jialongae* (not included by DEA17) sister to *Schulzeviella*, and *S. schulzei* sister to *Pheronema* + *Sericolophus* + *Pheronemoides*. Given the high BS and unsuspicious branch lengths, this is unlikely to be a phylogenetic reconstruction artefact. Rather, the assignment of the then new species *jialongae* by Gong et al. (2015) to *Semperella* might have been erroneous (at least to me it appears ambiguous); alternatively, morphological distinction of pheronematid genera, which is largely based on body shape (Tabachnick and Menshenina, 2002), might require refinement. In any case, more species of definitive *Semperella* spp. need to be sequenced to further test the status of this genus.

A representative of *Poliopogon* (*P. microuncinata*) was included in a molecular phylogenetic study for the first time by KEA but, unfortunately, its position within the family not further discussed. DEA17 found *Schulzeviella* alone as sister to the remainder of the family, a hypothesis that is now overturned by recovery of *S. jialongae* and *P. microuncinata* as successive sister groups to *Schulzeviella* n. sp.; the position of *Poliopogon* based on molecular data refutes morphology-based hypotheses of DEA17, which supported a more nested position of that genus within Pheronematidae.

Amphidiscophora: Hyalonematidae

KEA recovered *Hyalonema* as monophyletic, which is not surprising because they did not include any of the other genera of that family. However, mitochondrial sequence data of *Lophophysema* (Zhang et al., 2016) and *Tabachnickia* (Haen et al., 2014) are available in GenBank and included here; in accordance with DEA17, these genera are nested within *Hyalonema*, rendering the latter paraphyletic. Based on their more limited taxon sampling of the mega-genus *Hyalonema*, DEA17 suggested that this might indicate that at least some of the 12 subgenera should be elevated to genus level. However, as revealed by KEA's significant additions, the situation might be more complicated: *Lophophysema* is nested within *H. (Cyliconemaoida)* and *Tabachnickia* is nested within *H. (Onconema)*.[1] At first glance, this achieves fairly good support (88 and 71% BS); on the other hand, the positions of *Lophophysema* and *Tabachnickia* within these clades are poorly resolved (BS $\leq$ 50%), leaving open the possibility that they are sister to the respective subgenera of *Hyalonema*. More precise placement of *Lophophysema* and *Tabachnickia* might be achieved with the addition of nuclear data. In any case, a thorough integrative taxonomic revision is required to clarify the systematics of Hyalonematidae.

[1] Some clarifications are in order here: First, KEA state that they found *H. (Onconema)* paraphyletic; however, this is incorrect because they erroneously named *H. (Onconema) clarioni*, *H. (Corynonema) clarioni*. Second, they state that they found *H. (Cyliconemaoida)* monophyletic; this is true but was only poorly supported (0.6 BPP). Here I found "*H. (Cyliconemaoida) ovuliferum* SMF12074" grouping with "*Hyalonema* sp. 3" instead of its conspecifics, suggesting paraphyly of the subgenus and the species. However, this is clearly an artefact of poor gene coverage: SMF12074 is only present in the 28S partition, whereas all the other specimens of the subgenus are only sequenced for 16S, such that the dataset is indecisive regarding monophyly of *H. (Cyliconemaoida)*.

Sceptrulophora: Farreidae

As mentioned above, monophyly of the type genus of this family is not recovered, which is not further discussed by KEA who only (erroneously) state that they found the family to be paraphyletic (see above). The position of *F. bowerbanki* (18S from Boury-Esnault et al., 2017; not included by DEA17) as sister to the remaining farreids instead of sister to "*Farrea* sp." implies that *Lonchiphora* and *Aspidoscopulia* are mere derived members of *Farrea*. This scenario appears entirely plausible since besides the presence of some unusual types of sceptrules (or absence of sceptrules altogether) in the few species not currently assigned to the large genus *Farrea* (Reiswig, 2002; Duplessis and Reiswig, 2004; Dohrmann et al., 2011), all farreids are morphologically quite similar. Probably the five currently recognized genera should be synonymized; comprehensive integrative taxonomic revision might then provide some support for the erection of subgenera.

Sceptrulophora: "Euretidae"

Euretidae is a "waste-bin" family and therefore sequencing of more genera is needed to revise its classification (Reiswig and Dohrmann, 2014; Dohrmann et al., 2017). KEA added 16S sequences of *Bathyxiphus* and *Chonelasma* and found them to group with *Homoieurete* and *Sarostegia*, respectively, in a well-supported clade, which is confirmed here. *Homoieurete* and *Sarostegia* were formerly placed in Euretidae but are currently treated as *incertae sedis* because they did not group with the other euretids *Conorete*, *Verrucocoeloidea*, and *Lefroyella*, which are closely related to Farreidae (Reiswig and Dohrmann, 2014; Dohrmann et al., 2017). Thus, there currently seem to be two unrelated groups of euretid genera; placement of the type genus *Eurete* will be required to decide which one becomes the new "core" of a revised Euretidae. Interestingly, *Bathyxiphus* and *Chonelasma* were resolved closer to the second group in the "total evidence" analysis of DEA17, which demonstrates the limitations of morphological data for resolving euretid relationships.

Lyssacinosida: Euplectellidae

KEA increased taxon sampling of this large family by 10 species and 3 genera. Below I briefly sketch similarities and differences of KEA's and my results regarding their placement and discuss some implications.

*Bolosoma* is monophyletic in KEA (with strong BPP) and here (with weak BS). *Saccocalyx* is recovered here as monophyletic (albeit with weak support), whereas in KEA, it was found paraphyletic (with *Hertwigia* sp. nested within it). Resolution within *Saccocalyx* is poor and the monophyly of the species not resolved (note that "*S. pedunculatus* (Gong et al., 2015)" in KEA = "*S. microhexactin* Gong" here; the name used by KEA is based on an outdated GenBank entry).

By addition of *Docosaccus nidulus* and another specimen of *D. maculatus*, monophyly of *Docosaccus* is confirmed in KEA and the present study. The 16S sequence of *D. nidulus* is practically identical to the one of the previously published *D. maculatus*, so the odd result that *D. maculatus* appears paraphyletic is most parsimoniously explained by unintended swap or mislabelling of specimens SMF12079 and SMF12094 or the corresponding DNA extracts.

Monophyly of *Corbitella discasterosa* and *Holascus* is not resolved here, which is similar to KEA's result, who found only very weak support for monophyly of the former and also recovered the latter as polyphyletic. However, support values are generally very low in this part of the tree, so conclusions about the status of these taxa are premature based on the present data. On the other hand, non-monophyly of the "difficult" genus *Holascus* is not implausible since further taxonomic revision is necessary to determine its status (Tabachnick, 2002a).

The placement of the previously unsequenced genera *Corbitella* and *Holascus* in a clade with taxa such as *Euplectella*, *Regadrella*, and *Docosaccus* corroborates the concept of a "VFB sensu lato" clade suggested by DEA17 based on total-evidence analysis (i.e. a grouping of all genera with the iconic venus flower basket or similar body shapes). Similarly, *Hyalostylus* is placed within the Bolosominae sensu stricto clade as predicted by DEA17, although monophyly of the genus is only weakly supported here and in KEA.

Lyssacinosida: Leucopsacidae

Before I discuss the position of the newly sequenced "*Oopsacas* sp. (SMF12068)", it is necessary to consider two problems: Firstly, KEA's terminal taxa "*Oopsacas minuta* (Borchiellini et al., 2001)" and "*Oopsacas minuta* (Dohrmann et al., 2008)" should have been concatenated because the 28S sequence published by Borchiellini et al. (2001) was actually used by Dohrmann et al. (2008). Secondly, during ongoing research it became apparent to me that "*Leucopsacus* sp. (BX12/6)", first published by Dohrmann et al. (2008), must have been misidentified and is very likely an *Oopsacas*. Hence, there are three *Oopsacas* in Fig. 1 instead of three *Oopsacas* and one *Leucopsacus* in KEA's tree.

KEA found that their "*Oopsacas* sp. (SMF12068)" grouped firmly with *Euryplegma auriculare* (Aulocalycidae) instead of the other leucopsacids, which I confirm here. KEA do not discuss this further, simply stating that they have found *Oopsacas* to be paraphyletic (polyphyletic would be more correct). I find this result highly dubious (a phylogenetic reconstruction artefact appears unlikely to me) and I suspect that SMF12068 was misidentified and is actually a species of Aulocalycidae. Aulocalycidae are characterized by a distinct type of fused skeleton (aulocalycid dictyonal framework) that is unique among Lyssacinosida, but there are certain affinities between Aulocalycidae and Leucopsacidae (see DEA17 for discussion). Although *Oopsacas* has little similarity to *Euryplegma*, other aulocalycids can easily be mistaken for being "normal" lyssacinosidans because their dictyonal frameworks are very delicate and can be hard to detect, being hidden among tissue layers with loose spicules, especially in young specimens. Regarding the microscleres, there are no diagnostic spicule types for *Oopsacas*, the only reliable feature being absence of spicules other than discohexasters, which can also occur in other families, including Aulocalycidae.

Lyssacinosida: Rossellidae

Among Rossellidae, KEA added another species each of *Bathydorus*, *Acanthascus*, and *Lophocalyx*, as well as four species of *Caulophacus* from two different subgenera. They corroborate monophyly of *Bathydorus* and *Lophocalyx*, which is confirmed here. *Acanthascus* groups with *Rhabdocalyptus dawsoni* (see discussion above regarding KEA's confusion of names and sequences). It is unclear if this corroborates monophyly of Acanthascinae or only of *Rhabdocalyptus*—KEA do not state if they use the name *Acanthascus* sensu Tabachnick (2002b), which could mean that their specimen belongs to *Acanthascus (Rhabdocalyptus)*, or if they identified their specimen as *Acanthascus* sensu Reiswig & Stone (2013), which would be *Acanthascus (Acanthascus)* sensu Tabachnick. *Caulophacus* is not recovered monophyletic in KEA, but only because they accept *Caulophacella* as a subgenus of that genus, following Boury-Esnault et al. (2015), an interpretation that is not supported in DEA17 (cf. their Table 2). Hence, *Caulophacus* as previously defined (Tabachnick, 2002b) remains monophyletic. However, as correctly observed by KEA, the subgenera *C. (Caulophacus)* and *C. (Caulodiscus)* are not recovered as monophyletic groups, indicating that subgeneric classification of this large genus needs to be revised (or abandoned). Finally, KEA recover two other genera as paraphyletic although they do not mention it: *Sympagella* and *Nodastrella*. Paraphyly of *Sympagella* is not trustworthy since KEA included the sequence of Adams et al. (1999), which I have excluded here (see above); *Nodastrella* is here recovered as monophyletic with weak support while inclusion of *Aulosaccus mitsukurii* within that genus was highly supported by KEA.

## Conclusions

In conclusion, although several results of Kersken et al. (2018) are confirmed here, many of their findings and claims about the phylogenetic relationships of certain taxa are unsustainable (Table 1), as shown by

**Table 1** Main differences between Kersken et al. (2018) (KEA) and this study

| KEA claim or result | This paper | Comment |
|---|---|---|
| Lyssacinosida paraphyletic | Lyssacinosida monophyletic | Paraphyly not noticed by KEA |
| *Clathrochone* sister to Leucopsacidae | *Clathrochone* sister to Leucopsacidae + Aulocalycidae + Rossellidae | |
| Leucopsacidae polyphyletic | Leucopsacidae likely monophyletic | Likely misidentification of SMF 12068; confirmation required |
| Dactylocalycidae paraphyly highly supported | Dactylocalycidae paraphyly weakly supported | Dactylocalycidae supported by morphology (Dohrmann et al., 2017) |
| Aphrocallistidae/ *Aphrocallistes* polyphyletic | Aphrocallistidae/*Aphrocallistes* monophyletic | Dataset assembly error (inclusion of misnamed sequence) |
| Farreidae not monophyletic | Farreidae monophyletic | Misinterpretation of KEA's own results |
| Acanthascinae polyphyletic | Acanthascinae monophyletic | Dataset assembly error; unclear if only applicable to *Rhabdocalyptus* |
| *Hyalonema* monophyletic | *Hyalonema* paraphyletic | Dataset assembly error (exclusion of relevant taxa) |
| *Saccocalyx* paraphyletic | *Saccocalyx* monophyletic | |
| *Sympagella* paraphyletic | *Sympagella* monophyletic | Dataset assembly issue (inclusion of low-quality sequence) |
| *Nodastrella* paraphyletic (high support) | *Nodastrella* monophyletic (weak support) | |

reinterpretation based on careful dataset assembly and the use of established analytical procedures.

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