Correspondence

Polyplacotoma mediterranea is a new ramified placozoan species

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The enigmatic phylum Placozoa is harboring an unknown number of cryptic species and has become a challenge for modern systematics. Only recently, a second species has been described [1], while the presence of more than a hundred additional species has been suggested [2]. The original placozoan species *Trichoplax adhaerens* [3], the second species *Hoilungia hongkongensis* [1] and all yet undescribed species are morphologically indistinguishable (i.e. no species diagnostic characters are available [4]). Here, we report on a new placozoan species, *Polyplacotoma mediterranea* gen. nov., spec. nov., which differs from other placozoans in its completely different morphological habitus, including long polytomous body branches and a maximum body length of more than 10 mm. *Polyplacotoma mediterranea* also necessitates a different view of placozoan mitochondrial genetics. *P. mediterranea* harbors a highly compact mitochondrial genome with overlapping mitochondrial tRNA and protein coding genes. Furthermore, the new species lacks typical placozoan features, including the *cox1* micro exon and *cox1* barcode intron. As phylogenetic analyses suggest a sister group relationship of *P. mediterranea* to all other placozoans, this new species may also be relevant for studies addressing the relationships at the base of the metazoan tree of life.

Our extensive sampling efforts in the Mediterranean Sea — a diversity hotspot for placozoans — resulted in the identification of a highly unusual placozoan species,

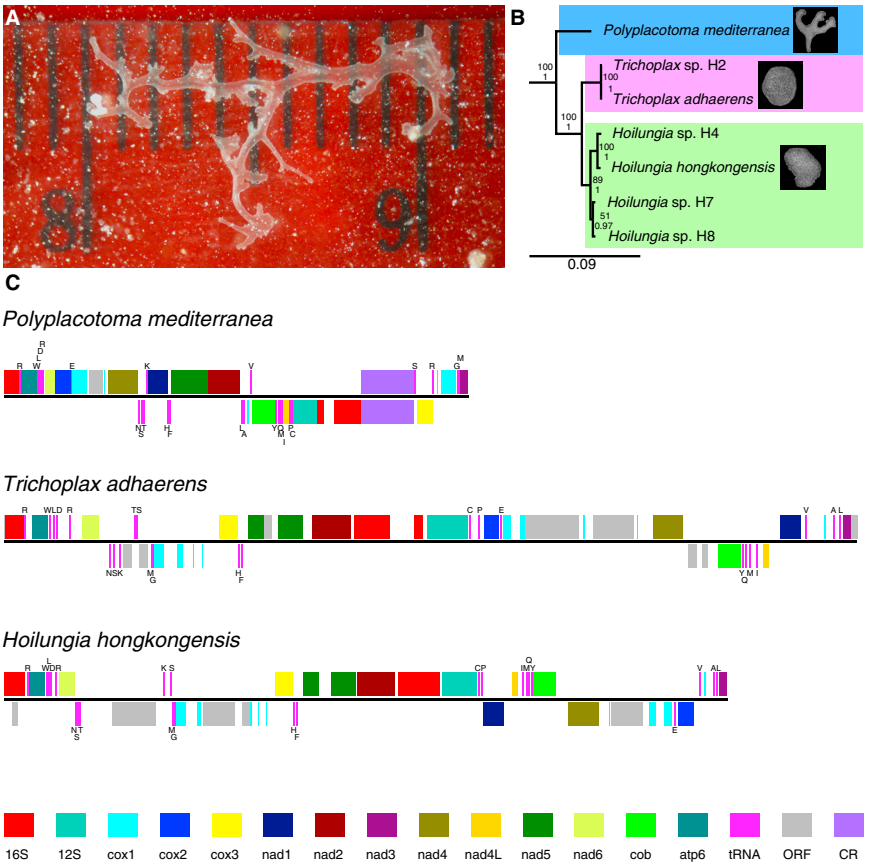

Figure 1. Habitus, phylogenetic placement and mitochondrial genome of *Polyplacotoma mediterranea* gen. nov., spec. nov.
(A) Light microscopy image of *P. mediterranea* (clone "Alassio", haplotype H0) in a culture petri dish. Single individuals can enlarge their body length to a size of more than 10 mm with multiple branches of several millimeters in length. One unit ("8" to "9") equals 10 mm. (B) Phylogenetic relationships within Placozoa based on Maximum Likelihood (ML) and Bayesian (BA) phylogenetic analyses using concatenated 18S/28S rDNA sequence data. The analyses reveal *P. mediterranea* as the sister to all other placozoans. Bootstrap support values (ML, top) and posterior probabilities (BA, bottom) are given at the nodes of the Maximum Likelihood subtree (see also Supplemental Figure S2A). Life images of the three placozoan species are not to scale. (C) Linearized mitochondrial genomes of *Polyplacotoma mediterranea* H0 (23.5 kb), *Trichoplax adhaerens* H1 (43.1 kb) and *Hoilungia hongkongensis* H13 (36.5 kb). The size of the schematic genomes and encoded genes are to scale (except for the *cox1* micro exon and the transfer RNA genes). Protein coding genes (*cox1-3*, *nad1-6*, *nad4L*, *cob*, *atp6*), ribosomal RNAs (12S and 16S), transfer RNAs (tRNAs), open reading frames (ORFs) as well as the putative mitochondrial control region (CR) are color-coded as rectangles. Information on the corresponding amino acid for tRNA genes is provided by the single letter amino acid code. The transcriptional orientation of genes is indicated by their relative positioning (above = 5'–3'; below = 3'–5'). Multiple *cox1*, *nad5* and 16S at different positions indicate the fragmentation of these genes into different exons.

P. mediterranea. The most obvious feature of *P. mediterranea* is its ability to adopt ramified body shapes (Figure 1A), which have never been observed before in placozoans. Single individuals of *P. mediterranea* can enlarge their body to a length of more than 10 mm. While no structural differences to other placozoans are detectable through light microscopy, we do not know yet whether ultrastructural differences related to the ramified habitus are present; so far, such analyses have been hampered by the exceptional fragility and very limited availability of this species. *P. mediterranea* cannot be cultured on established standard placozoan algae diets [2] and mostly shows unusually slow growth and division rates, even when cultured under presumably favorable

conditions, i.e. on natural substrates from the sampling site.

The new placozoan species, *P. mediterranea*, differs from all other known placozoans not only in its unique habitus, but also in its mitochondrial genetics. Previously reported placozoan mitogenomes are characterized by a large genome size (>30 kb), a high GC content (>39%), the absence of a single large non-coding control region, large intergenic spacers, non-overlapping tRNA/protein coding genes, a constant number of 24 tRNAs, a *cox1* micro exon, a conserved intron in the *cox1* 'Folmer' barcode region [5] and an unusual "GTG" start codon in the *nad4L* gene [6–8]. By contrast, the compact mitochondrial genome of *P. mediterranea* (23,462 bp) is by far the smallest placozoan mitogenome (Figure 1C; Figure S1 in Supplemental Information). The low GC content (32.9%), the small intergenic spacer regions (mostly <100 bp) and 25 (instead of 24) tRNA genes are likewise remarkable deviations. Two tRNA genes overlap with protein coding genes, highlighting the compact architecture of this mitogenome. In detail, the 3' end of *cob* and the 3' end of trnVal, as well as the 5' end of *cox3* and the 5' end of the unique trnArg, are overlapping by 7 and 19 base pairs, respectively. This is the first observation of overlapping mitochondrial protein coding and tRNA genes in the phylum Placozoa. The *cox1* gene differs from all other placozoans in two remarkable features: first, the *cox1* micro exon [9] is linked to the adjacent exon, representing the ancestral state of this gene; second, the *cox1* intron in the 'Folmer' barcode region is missing. Interestingly, instead of an unusual GTG start codon, the *nad4L* gene in *P. mediterranea* possesses a typical (presumably ancestral) ATG start codon. A 2.6 kb mitogenome fragment located between the 16S gene and trnSer does not encode any gene or open reading frame of more than 100 amino acids. This region, however, harbors several putative stem-loops as well as one perfect 39-mer direct repeat and one imperfect 27-mer triple repeat. These features are characteristics of typical animal mitochondrial control regions. As a conclusion, we prefer the hypothesis that this region is a putative mitochondrial control region over the alternative that *P. mediterranea* keeps more than 11% of non-functional DNA in its otherwise highly compact mitogenome. Detailed analyses of the nuclear genome of *P. mediterranea* as well as comprehensive metazoan phylogenies based on whole nuclear genome data are beyond the scope of this paper and will be reported elsewhere.

In order to further clarify the relationship of *P. mediterranea* to other extant placozoans, we performed phylogenetic analyses using nuclear ribosomal RNA and mitochondrial protein coding genes, which have been known to robustly resolve inner placozoan systematics [6,10]. Both data sets provide strong support for a sister group relationship between *P. mediterranea* and all other placozoans (Figure 1B; Figure S2A,B). This sister group relationship in our view justifies the erection of the new placozoan genus *Polyplacotoma*.

SUPPLEMENTAL INFORMATION

Supplemental Information including experimental procedures, taxonomic diagnosis and two figures can be found with this article online at https://doi.org/10.1016/j.cub.2019.01.068.

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AUTHOR CONTRIBUTIONS

Conceptualization: H.J.O., B.S.; Data curation: H.J.O., K.K., B.S.; Formal analysis: H.J.O., K.K., B.S.; Investigation: H.J.O., S.R., R.H., K.K., B.S.; Field work: S.R., R.H., B.S.; Resources: B.S.; Funding acquisition: B.S.; Writing: H.J.O., S.R., R.H., K.K., B.S.; Visualization: H.J.O., K.K., B.S.; Supervision: B.S.; Project administration: B.S.

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