Journal of the Marine Biological Association of the United Kingdom

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Assessing the core microbial symbionts of jellyfish in Indonesian and Vietnamese marine lakes

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Research Article

Cite this article: Ferreira MRS, Cleary DFR, Bat NK, Polónia ARM, Gomes NCM (2022). Assessing the core microbial symbionts of jellyfish in Indonesian and Vietnamese marine lakes. *Journal of the Marine Biological Association of the United Kingdom* **102**, 486–495. https://doi.org/10.1017/S0025315422000777

Received: 12 October 2021
Revised: 28 July 2022
Accepted: 23 August 2022
First published online: 12 October 2022

Key words:
Anchialine systems; *Cassiopea*; Illumina; *Mastigias*; microbial composition

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Abstract

Jellyfish are a well-known component of marine ecosystems. Here, we aimed to assess whether populations of the jellyfish species *Mastigias* cf. *papua* and *Cassiopea ornata* inhabiting different marine lakes and jellyfish species from open water habitat host 'core' symbionts and if there is evidence of species-specific host-microbial associations. Compositionally, jellyfishes hosted prokaryotic communities distinct from those found in water samples. All jellyfish samples across habitats and species exhibited a core OTU, assigned to the genus *Endozoicomonas*. This OTU was particularly abundant (>90% of all sequences) in *C. ornata* from one Papuan marine lake. Additionally, an OTU assigned to the Entomoplasmatales order was found in all but two jellyfish specimens, and was particularly abundant in marine lake specimens from Berau and Papua, Indonesia. Given the well-known relationship between *Endozoicomonas* and *Symbiodinium* spp., we tested for *Symbiodinium* presence in pooled specimens of *M. papua* from Berau. Our results showed that OTUs assigned to the genus *Symbiodinium* accounted for >99% of all sequences in jellyfish-associated microeukaryotic communities; these were closely related to organisms from *Symbiodinium* clade C. These results suggest the existence of a widespread and abundant jellyfish core symbiont, which may interact with symbiotic *Symbiodinium* populations to influence host fitness.

Introduction

Jellyfish (Medusozoa, Helm 2018), have a relatively simple anatomy, but with features that make them particularly interesting to study host-microbial symbioses. Jellyfish account for only 2–3% of cnidarian species (Brekhman *et al.*, 2015), but differ in their morphology, size, ecology and distribution, which may lead to significant differences in their symbiotic relationships. Only a few studies have assessed the prokaryotic communities associated with jellyfish species. These have shown that jellyfish prokaryotic communities are distinct from those found in the surrounding marine environment (Daley *et al.*, 2016; Kramar *et al.*, 2018). Lee *et al.* (2018) also identified a possible core community of 16 OTUs of the jellyfish species *Chrysaora plocamia*, which were shared with the jellyfish species *Aurelia aurita*. This consisted of a range of OTUs in the Nitrospira, Alpha-, Beta-, Gamma- and Deltaproteobacteria related to organisms involved in nitrogen and sulphur cycling. OTUs assigned to the phyla Actinobacteria, Bacteroidetes, Planctomycetes and Proteobacteria were also observed in the prokaryotic communities of *Chrysaora plocamia* and *Aurelia aurita* (Weiland-Bräuer *et al.*, 2015; Daley *et al.*, 2016; Kramar *et al.*, 2018; Lee *et al.*, 2018). Jellyfish are also known to contain endosymbiotic dinoflagellates of the genus *Symbiodinium* (Mellas *et al.*, 2014; Klein *et al.*, 2017; Newkirk *et al.*, 2018), which provide nutrients to the host (Awai *et al.*, 2012; Mellas *et al.*, 2014; Klein *et al.*, 2017). Another remarkable aspect of jellyfish is their prevalence in a range of marine habitats, including the deep sea (Kawabata *et al.*, 2013). One of the most interesting habitats for jellyfish are marine lakes where they can become highly abundant and may develop distinctive morphological, behavioural and genetic characteristics depending on the lake they inhabit (Hamner & Hauri, 1981; Hamner *et al.*, 1982; Dawson, 2005*a*). One of the most widespread and abundant jellyfish species in marine lakes is *Mastigias* cf. *papua*, which can form high density populations (Dawson *et al.*, 2001). These populations are morphologically, behaviourally and genetically distinct from non-lake populations (Dawson, 2005*b*). This suggests an evolutionary adaptation over time to their habitat thereby increasing fitness (Cimino *et al.*, 2018). In the present study, our main goals were to assess whether populations of jellyfish inhabiting geographically distant marine lakes and from open water habitat host the same 'core' prokaryotic symbionts and identify if there is evidence of species-specific host-microbe associations. In addition to the above, the presence of *Symbiodinium* in pooled specimens of *M. papua* from Berau was also evaluated. To achieve these goals, we assessed the microbial communities of two jellyfish species, *Mastigias* cf. *papua* and *Cassiopea ornata*, collected from marine lakes in the Berau region of Borneo (Becking *et al.*, 2011), the Misool region, Raja Ampat, West Papua, Indonesia (Purba *et al.*, 2018) and Ha Long Bay, Vietnam

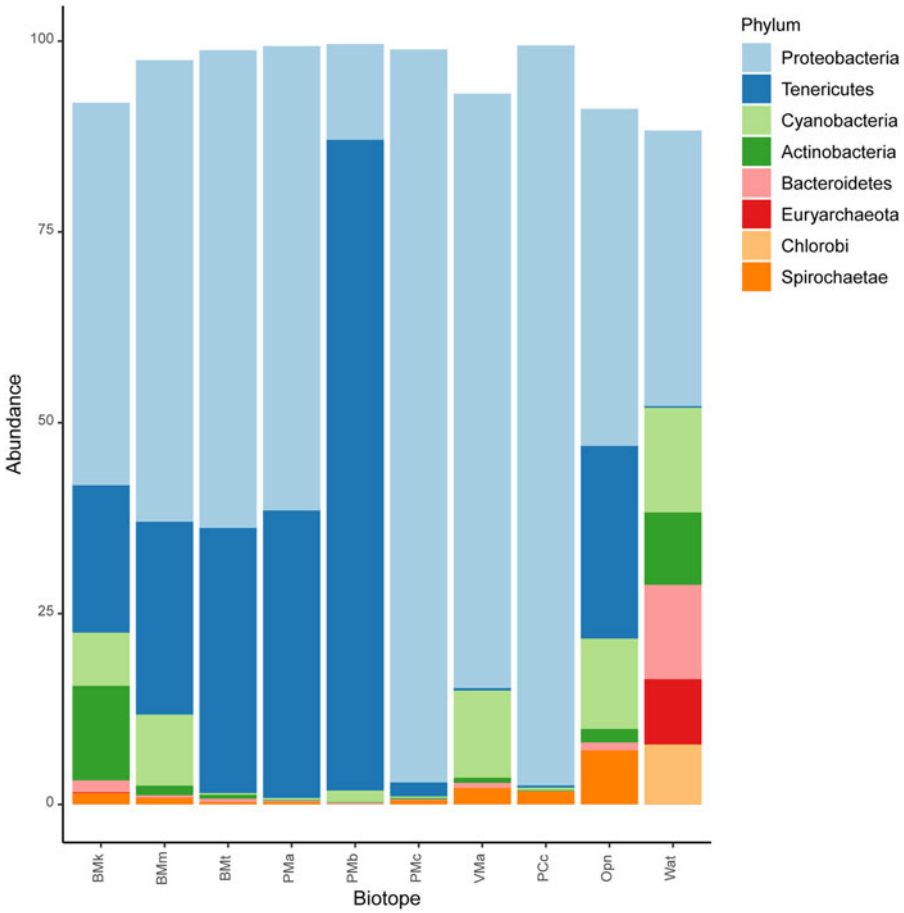

Fig. 1. Stacked barplots showing the relative abundances of the eight most abundant phyla in each group of *M. papua* samples from marine lakes in Berau: marine lake Haji Buang (BMm), marine lake Kakaban (BMk) and marine lake Tanah Bamban (BMt), Papua: (Kr02: PMa), (Ms01: PMb), (Ms17: PMc), in Vietnam (Bui Xam: VMa), for *C. ornata* samples from marine lake Ms17 in Papua (PCc), for jellyfish species sampled in open-water habitat in Taiwan and Vietnam (Opn), and from water samples (Wat).

(Azzini *et al.*, 2007). In addition to this, we also sampled water and jellyfish species from open-water habitat in Vietnam and Taiwan.

Materials and methods

Location and sampling

Jellyfish species, *Mastigias* cf. *papua* Lesson, 1830 and *Cassiopea ornata* Haeckel, 1880, were sampled by snorkelling and scuba diving in the Berau region of Borneo, East Kalimantan Province, Indonesia, the Misool region, Raja Ampat, West Papua Province, Indonesia, and in Ha Long Bay, on the north-eastern coast of Vietnam. Detailed descriptions of the environmental conditions found in the marine lakes of each region can be found in Tomascik & Mah (1994), Cerrano *et al.* (2006), Azzini *et al.* (2007), Becking *et al.* (2011, 2015), Cleary *et al.* (2013, 2015, 2016, 2018, 2020), Cleary & Polónia (2018), and Maas *et al.* (2018). Additional information including the GPS coordinates of the sample sites is provided in Electronic Supplementary Material 1. Jellyfish specimens were collected from marine lakes in Berau (21–28 August 2012), from Papuan marine lakes (15–20 September 2013) and from a Vietnamese marine lake (16–17 August 2013). In total 22 specimens of *Mastigias* cf. *papua* and three specimens of *Cassiopea ornata* were sampled from all three regions. In addition to this, five specimens belonging to different jellyfish species were sampled outside of marine lakes. Four of these specimens were collected in Vietnam from 16–17 August 2013 and one in Taiwan on 29 July 2014. These specimens were identified as *Chrysaora* sp. (2 samples), *Chrysaora* aff. *colorata*, *Catostylus townsendi* Mayer, 1915 and *Cyanea* sp. (from Taiwan). All specimens were collected from relatively shallow water (< 10 m depth) and preserved in 96% EtOH. During the jellyfish survey in Papua, water samples were also collected by filtering 1 l of seawater through a Millipore® White Isopore Membrane Filter (GTTP04700, 47 mm diameter, 0.22 µm pore size). The samples were kept cool (<4°C) immediately after collection and transport. In the laboratory, samples were stored at −20° C until DNA extraction.

DNA extraction

PCR-ready genomic DNA was isolated from water and jellyfish samples with the FastDNA SPIN Kit for soil (MPbiomedicals, Santa Ana, CA, USA) following the manufacturer's instructions. This is an extraction method frequently used for this purpose (Urakawa *et al.*, 2010; Costa *et al.*, 2013).

Previous studies have shown that different jellyfish compartments house distinct prokaryotic communities (Weiland-Bräuer *et al.*, 2015; Lee *et al.*, 2018). We, therefore, included fragments of the bell and tentacles of adult medusa, which were thoroughly mixed (Cleary *et al.*, 2016). Briefly, the whole membrane filter (for water samples) and ± 500 mg of jellyfish specimens were cut into small pieces and transferred to Lysing Matrix E tubes containing a mixture of ceramic and silica particles. The microbial cell lysis was performed in the FastPrep Instrument (Q Biogene, Inc., CA, USA) for 80 s at speed 6.0 ms^{-1}. Extracted DNA was eluted into DNase/Pyrogen-Free Water to a final volume of 50 µl and stored at − 20°C until use. The 16S rRNA gene V3V4 variable region PCR primers 341F 5′-CCTACGGGNGGCWGCAG-3′ and 785R 5′-GACTACHVGGGTATCTAATCC-3′ (Klindworth *et al.*, 2013) with barcode on the forward primer were used, to generate product fragments with expected amplicon size of 444 bps, in a 30 cycle PCR assay using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 min, followed by 28 cycles of 94°C for 30 s, 53°C for 40 s and 72°C for 1 min, after which a final elongation step at 72°C for 5 min

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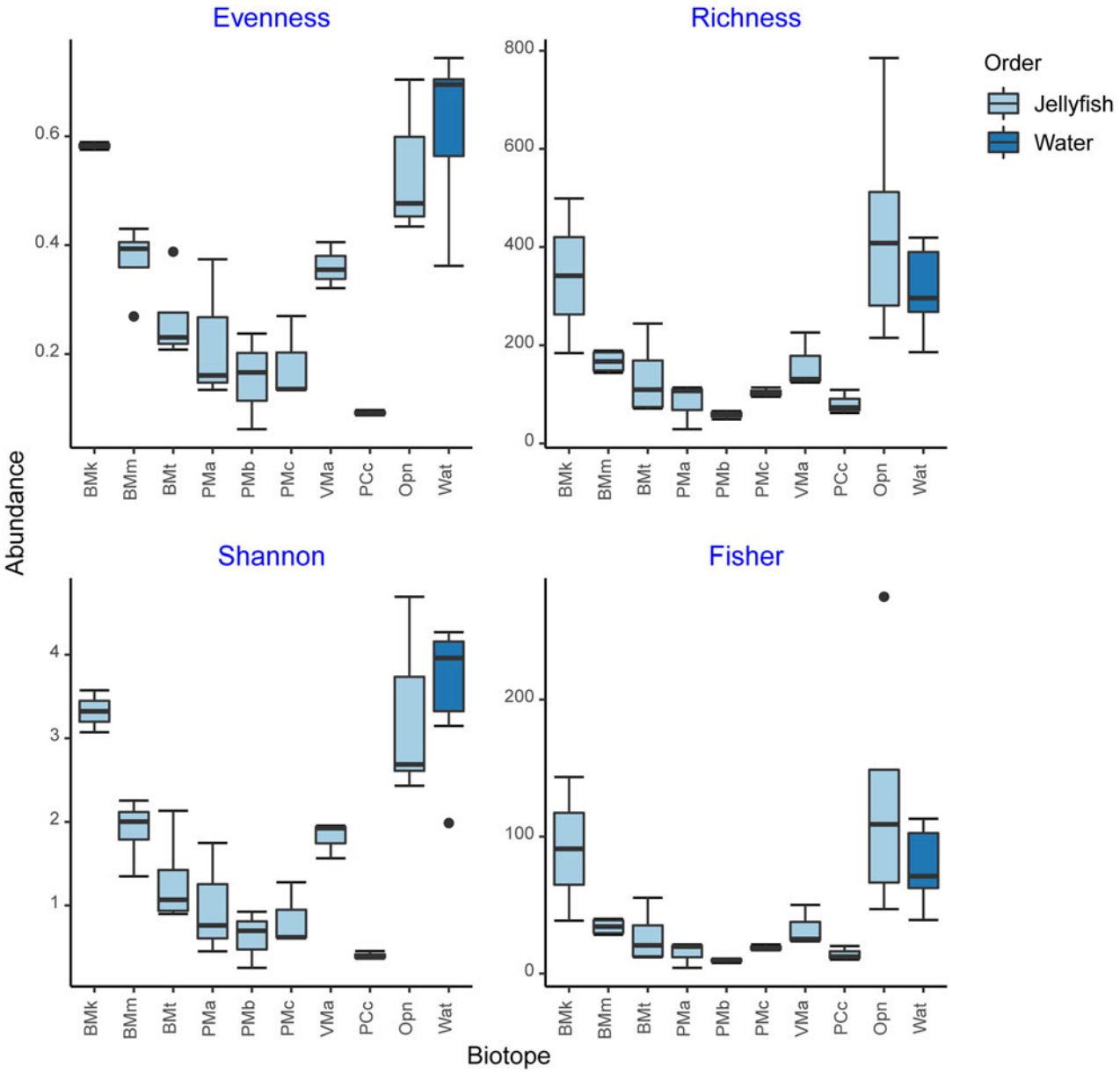

Fig. 2. Values of (a) evenness, (b) richness, (c) Shannon's 'H and (d) Fisher's alpha diversity indices of *M.* cf. *papua* samples from marine lakes in Berau, Indonesia: Kakaban lake (BMk), Haji Buang lake (BMm) and Tanah Bamban lake (BMt), in Papua: (Kr02: PMa), (Ms01: PMb), (Ms17: PMc), and in Vietnam (Bui Xam: VMa), for *C. ornata* samples from marine lake Ms17 in Papua (PCc), for jellyfish species sampled in open water habitat in Taiwan and Vietnam (Opn), and for water samples (Wat).

was performed. After amplification, PCR products were checked on a 2% agarose gel to determine the success of amplification and the relative intensity of bands. Samples were purified using calibrated Ampure XP beads and purified PCR product was used to prepare the DNA library following the Illumina TruSeq DNA library preparation protocol. Next-generation, paired-end sequencing was performed at MRDNA (Molecular Research LP; http://www.mrdnalab.com/; last checked 18 November 2016) on an Illumina MiSeq device (Illumina Inc., San Diego, CA, USA) following the manufacturer's guidelines. Sequences from each end were joined following Q25 quality trimming of the ends followed by reorienting any 3′–5′ reads back into 5′–3′ and removal of short reads (<150 bp). The resultant files were analysed using QIIME (Quantitative Insights Into Microbial Ecology; Caporaso *et al.*, 2010; http://www.qiime.org/).

In addition to this, we assessed microeukaryotic composition from two pooled samples of *M. papua* from lakes Kakaban and Haji Buang. The 18S small ribosomal subunit was amplified, generating product fragments of ~400 bps (Cook *et al.*, 2005), using the primers SSUF_FO4 (5′-GCTTGTCTCAAAGATTAA GCC-3′) and SSU_R22 (5′-GCCTGCTGCCTTCCTTGGA-3′) following previously published methods (Fonseca *et al.*, 2010; Coelho *et al.*, 2016). Equimolar concentrations of the 18S small subunit rRNA gene fragments were then sequenced using GS 454 FLX Titanium chemistry according to the manufacturer's instructions (Roche, 454 Life Sciences, Brandford, CT, USA).

16S rRNA gene sequencing analysis

For a detailed description of the sequence analysis, see Coelho *et al.* (2018) and Cleary *et al.* (2018). Briefly, we used QIIME (Caporaso *et al.*, 2010) and UPARSE (Edgar, 2013) with OTU selection at 97% similarity cut-off. Taxonomy was assigned using a fasta file containing reference sequences from the SILVA_132_QIIME_release (Quast *et al.*, 2012). We used the make_otu_table.py script in QIIME to generate a square matrix

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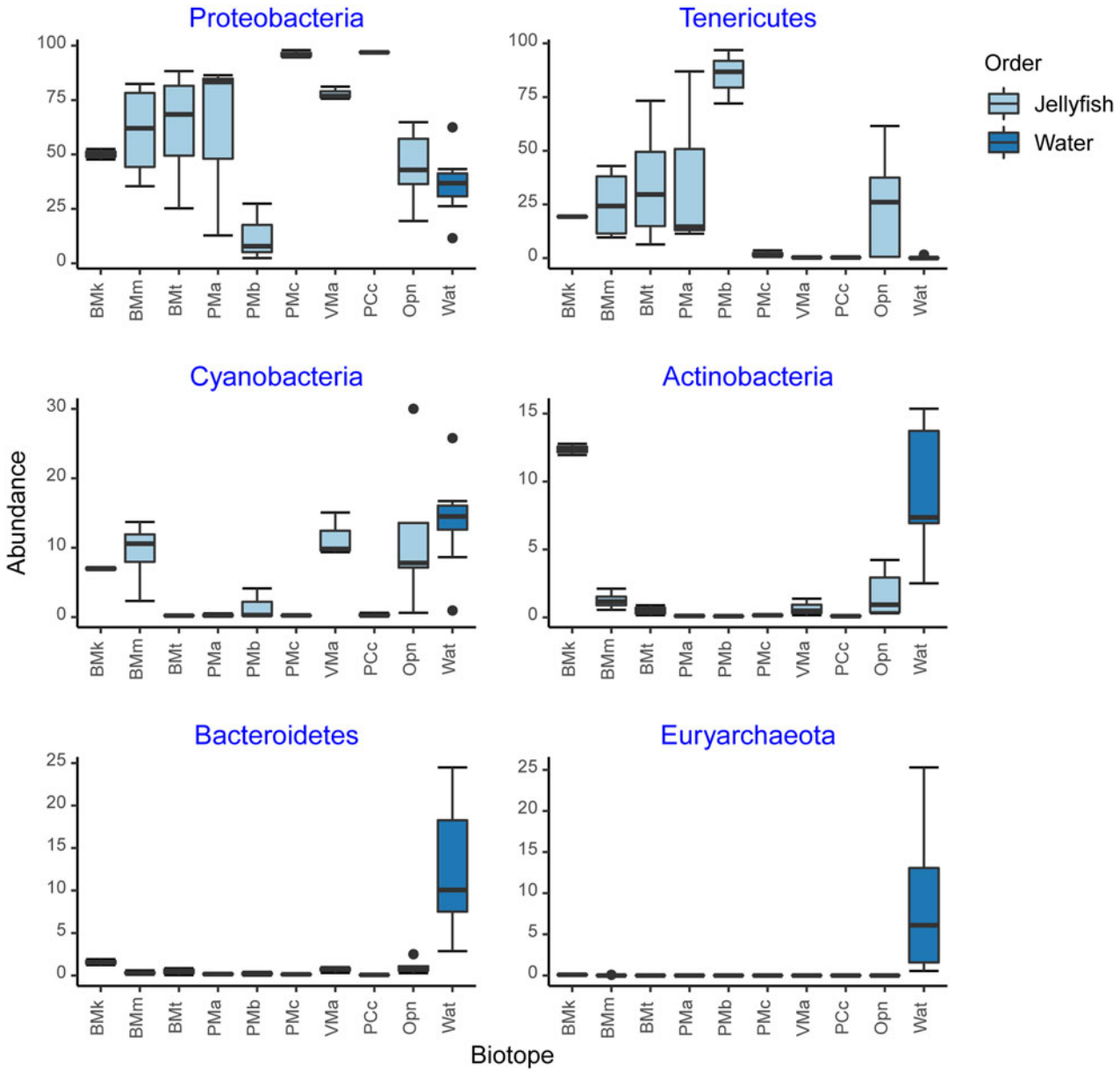

Fig. 3. Relative abundance of the most abundant prokaryotic phyla of *M.* cf. *papua* samples from marine lakes in Berau, Indonesia: Kakaban lake (BMk), Haji Buang lake (BMm) and Tanah Bamban lake (BMt), in Papua: (Kr02: PMa), (Ms01: PMb), (Ms17: PMc), and in Vietnam (Bui Xam: VMa), for *C. ornata* samples from marine lake Ms17 in Papua (PCc), for jellyfish species sampled in open-water habitat in Taiwan and Vietnam (Opn), and for water samples (Wat).

of OTUs × SAMPLES. For the OTU table, OTUs not classified as Bacteria or Archaea or classified as chloroplasts or mitochondria were removed prior to statistical analysis. The resultant table was subsequently rarefied to 4500 sequences per sample with the single_rarefaction.py script.

Statistical analysis

All statistical analyses were performed in the R environment (R Core Team, 2013). For a more detailed description of the sequencing and statistical analyses, see Coelho *et al.* (2018), Cleary *et al.* (2018) and Cleary & Polónia (2018).

A phylogenetic tree including OTUs assigned to *Symbiodinium* sp. and sequences from *Amoebophrya* and *Polarella* (related to *Symbiodinium* sp. and included as outgroups) was constructed using the MEGAX program (http://www.megasoftware.net/; last checked 2019/09; Tamura *et al.*, 2011) and inferred using the maximum likelihood method and General Time Reversible model (Nei & Kumar, 2000) with discrete Gamma distribution and invariant sites. Branches reproduced in <50% of the bootstrap replicates were collapsed.

Results

The dataset in the present study consisted of 175,500 sequences, assigned to 3765 OTUs after quality control, OTU picking and removal of chimeras, chloroplasts and mitochondria. The most abundant taxa were Proteobacteria (97,080 sequences; 1700 OTUs) and Tenericutes (35,231 sequences; 35 OTUs) (Electronic Supplementary Material 2) (Figure 1). Of the proteobacterial OTUs, 70,358 were assigned to the Gammaproteobacteria class and of these 52,142 to the Oceanospirillales order (Figure 1). OTU richness and evenness (Figure 2) were both significantly higher in open water jellyfish and *M. papua* from lake Kakaban than *M. papua* and *C. ornata* from the remaining marine lakes, although the difference was at the margin of significance for *M. papua* from Bui Xam, Vietnam (Electronic Supplementary Material 3). *Mastigias* cf. *papua* from Berau and the open-water

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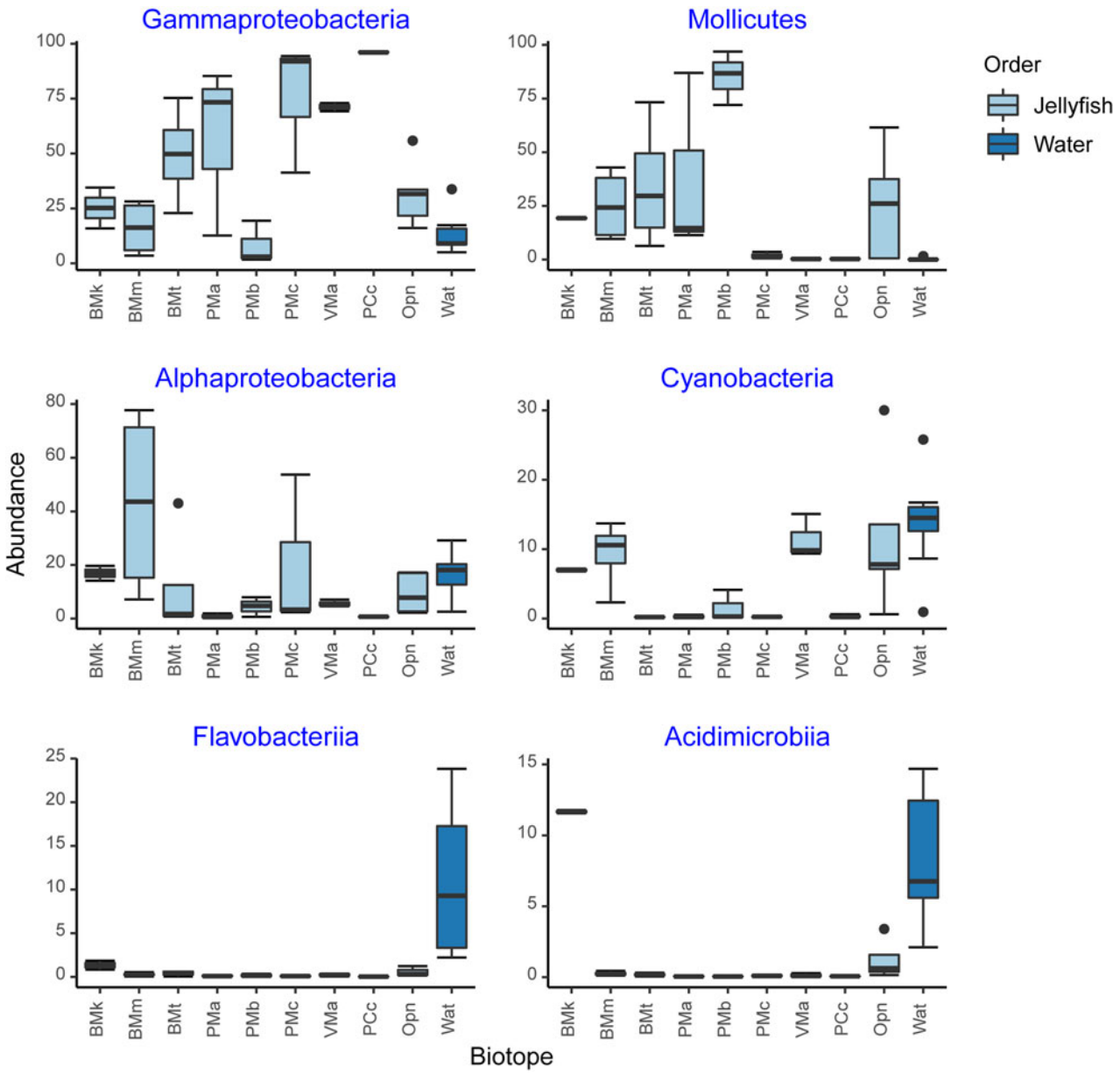

Fig. 4. Relative abundance of the most abundant prokaryotic classes of *M.* cf. *papua* samples from marine lakes in Berau, Indonesia: Kakaban lake (BMk), Haji Buang lake (BMm) and Tanah Bamban lake (BMt), in Papua: (Kr02: PMa), (Ms01: PMb), (Ms17: PMc), and in Vietnam (Bui Xam: VMa), for *C. ornata* samples from marine lake Ms17 in Papua (PCc), for jellyfish species sampled in open-water habitat in Taiwan and Vietnam (Opn), and for water samples (Wat).

jellyfish also had significantly higher relative abundances of Actinobacteria, Bacteroidetes, Deltaproteobacteria and Rhodospirillales than remaining marine lake populations of *M. papua* and *C. ornata* (Figures 3 and 4). This difference was most pronounced and significant for the Actinobacteria. There were also pronounced differences in the relative abundance of Tenericutes among jellyfish populations (Electronic Supplementary Material 3). The relative abundance of Tenericutes was highest in the *M. papua* population from lake Ms01 in Papua at 85.24 ± 12.51% and lowest in the *M. papua* and *C. ornata* populations from lake Ms17 in Papua and the *M. papua* population from Bui Xam, Vietnam (<2%) (Figure 1).

There were significant differences in composition among groups (adonis: $F_{9,29} = 4.39$, $R^2 = 0.577$, $P < 0.001$). The first PCO axis separated jellyfish samples from water samples while the second axis separated samples of jellyfish from Papua, Vietnam and open water from *M. papua* samples from Berau (Figure 5). Note that jellyfish populations formed distinct clusters based on habitat (e.g. marine lake *vs* open water) and lake origin. *M. Papua* from all Berau lakes had high relative abundances of OTU-5 as did *M. papua* from lake Ms01 in Papua. (Figures 5 and 6). This OTU, assigned to the order Entomoplasmatales, was recorded in 28 of 30 jellyfish samples, but was most abundant (>19%) in *M. papua* specimens from all Berau lakes and lake Ms01 in Papua (85.01 ± 12.48%). In the other lakes, its relative abundance varied from 0.02 ± 0.02% in Bui Xam lake to 1.40 ± 1.52% in lake Ms17 in Papua. OTU-5 had 96% sequence similarity to an organism obtained from the jellyfish *Cotylorhiza tuberculata* and 92% sequence similarity to an organism from a jellyfish enrichment experiment (Electronic Supplementary Material 4). The other dominant Tenericutes OTU, OTU-9, had a highly variable abundance, but was most abundant in *M. papua* from lake Kr02 (32.67 ± 47.20%) and open water jellyfish (18.04 ± 26.11%) compared with the other jellyfish populations in which it accounted for less than 0.09% of sequences; it was also not recorded in any of the Berau populations. This OTU only had 88.9% sequence similarity to an organism obtained from a chiton and 88.6% sequence similarity to an organism

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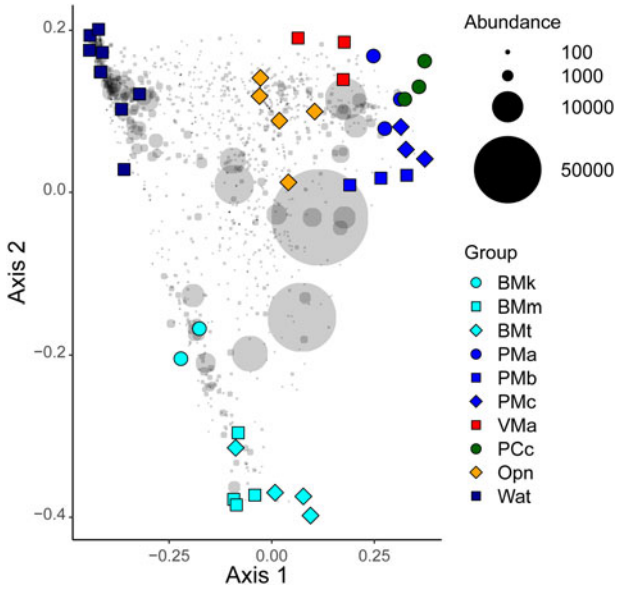

Fig. 5. Principal coordinates analysis (PCO) ordination of the first two axes. Coloured symbols represent the sample sites for *M.* cf. *papua* samples from marine lakes in Berau, Indonesia: Kakaban lake (BMk), Haji Buang lake (BMm) and Tanah Bamban lake (BMt), in Papua: (Kr02: PMa), (Ms01: PMb), (Ms17: PMc), in Vietnam (Bui Xam: VMa), for *C. ornata* samples from marine lake Ms17 in Papua (PCc), for jellyfish species sampled in open-water habitat in Taiwan and Vietnam (Opn), and for water samples (Wat). The circle size of the OTU is proportional to their abundance (number of sequences) as indicated by the symbol legend in the bottom.

obtained from an anemone. Of the abundant OTUs, the lowest sequence similarity (<86%) was obtained for OTU-175, assigned to the genus *Wolbachia*, and related to organisms obtained from a tick and various nematode species. This OTU was present in all specimens from lakes Bui Xam and Ms01 (Electronic Supplementary Material 4).

Of all the OTUs, OTU-2, assigned to the genus *Endozoicomonas*, was, by far, the most abundant with 49,441 sequence reads. It was the only OTU recorded in every jellyfish sample, thus across sampling sites and species. It reached its greatest abundance in *M. papua* and *C. ornata* from lake Ms17 in Papua at 73.17 ± 29.73% and 94.40 ± 0.39% of all sequences, respectively. Its mean abundance exceeded 6% in all other jellyfish populations. In Berau, it increased in abundance from 6% in lake Kakaban to 13% in Haji Buang and 44% in lake Tanah Bamban. In the open water jellyfish, it had a mean abundance of 7.67 ± 8.75%. It was also recorded in water where it had a relatively low abundance of 0.05 ± 0.09%. It had very high sequence similarity (>99%) to bacterial symbionts obtained from a range of hosts including an anemone, octocoral, cuttlefish, lionfish, and comb pen (Electronic Supplementary Material 4).

In addition to the prokaryotic analyses, we also assessed microeukaryotic composition from samples of *M. papua* and *C. ornata* from lakes in Berau. In both populations, more than 99% of all sequences were assigned to OTUs classified as *Symbiodinium* including a dominant OTU. This OTU had 100% sequence similarity to a number of organisms including an organism identified as *Symbiodinium* sp. clade C from the corals *Montastraea franksi*, *M. digitata*, *Siderastrea siderea* and *Stylophora pistillata*. All three of these were closely related (sequence similarities varying from 98.05 to 98.77%) to the same organism identified as *Symbiodinium* (*Cladocopium*) sp. clade C clone N60.3.4_JS626 obtained from the coral *Montipora digitata* at Heron Island, Australia. The phylogenetic tree (Figure 7) supported the BLAST sequence similarities with OTUs from our study clustering in a main group with sequences from *Symbiodinium* sp. clade C and one from clade D. Sequences from *Symbiodinium* sp. from other clades (A, B, D and E) all formed small clusters for each clade.

Discussion

In the present study, we assessed the prokaryotic communities associated with populations of *Mastigias* cf. *papua* inhabiting geographically distant marine lakes, the marine lake jellyfish species *Cassiopea ornata*, and jellyfish species from open water habitat. The two dominant bacterial taxa observed in this study, Gammaproteobacteria and Mollicutes, were already reported as dominant in *M. papua* specimens from other marine lakes in the Berau region of north-eastern Borneo, Indonesia (Cleary *et al.*, 2016). Gammaproteobacterial members have been associated with different jellyfish species including *Aurelia aurita* (Weiland-Bräuer *et al.*, 2015; Daley *et al.*, 2016), *Aurelia solida* (Kramar *et al.*, 2019), *Nemopsis bachei* (Daley *et al.*, 2016) and *Chrysaora plocamia* (Lee *et al.*, 2018). The presence of Mollicutes members was only observed in *Aurelia aurita* specimens, and it was suggested that they may be part of jellyfish-specialist bacterial lineages (Weiland-Bräuer *et al.*, 2015; Daley *et al.*, 2016). For example, Daley *et al.* (2016) observed a higher abundance of Gamma- and Alphaproteobacteria and Bacteroidetes in the hydrozoan *Nemopsis bachei* (class: Hydrozoa), while Cyanobacteria, Tenericutes and unclassified bacteria were more abundant in the moon jellyfish *Aurelia aurita*; note that these two species belong to different classes within the Cnidaria.

In this study, the most abundant OTU (OTU-2), was assigned to the family Endozoicimonaceae (Oceanospirillales order). Members of this family have also been observed in several marine organisms, e.g. annelids, cnidarians, fish, molluscs, Porifera and tunicates (Neave *et al.*, 2016, 2017*a*, 2017*b*; van de Water *et al.*, 2018), and have been considered as 'core' symbionts of corals (Neave *et al.*, 2017*b*; van de Water *et al.*, 2018). Here, this OTU may also be considered a 'core' symbiont, given its presence in all samples from all jellyfish hosts inside and out of marine lakes in Vietnam, Papua and Taiwan. Despite their abundance and distribution, the function of *Endozoicomonas* members is still uncertain, and it has been suggested that depending on the host, the function could differ (Neave *et al.*, 2017*a*). These bacteria may be involved in three major roles: nutrient acquisition and provision, modulation of the host microbiome (influencing bacterial colonization) and influencing host health (Neave *et al.*, 2016, 2017*a*, 2017*b*; Shiu & Tang, 2019). Previous studies also suggested that *Endozoicomonas* members may establish relationships with algal symbionts such as *Symbiodinium* spp., which may be mutualistic or antagonistic (Morrow *et al.*, 2012; Neave *et al.*, 2017*b*). *Symbiodinium* members are known to provide, through photosynthesis, a large part of the energy required by the host (Neave *et al.*, 2017*b*).

In the present study, OTUs assigned to *Symbiodinium* sp. were recorded in jellyfish from marine lakes, which also had high abundances of *Endozoicomonas* OTU-2. This finding suggests that in line with other marine organisms (e.g. corals, Neave *et al.*, 2017*a*), *Endozoicomonas*–*Symbiodinium* interactions may also contribute to jellyfish fitness. It is important to note that sequences related to the jellyfish host were removed from the eukaryotic dataset and that other eukaryotes may have remained undetected due to a high number of *Symbiodinium* reads. Here, selected OTUs had high sequence similarities (>99%) to sequences in the NCBI database assigned to *Symbiodinium* sp. clade C. In the phylogenetic tree, these OTUs all clustered together inside a major cluster with organisms assigned to *Symbiodinium* sp. clade C, supporting the BLAST sequence similarity results and with one sequence from *Symbiodinium* sp.

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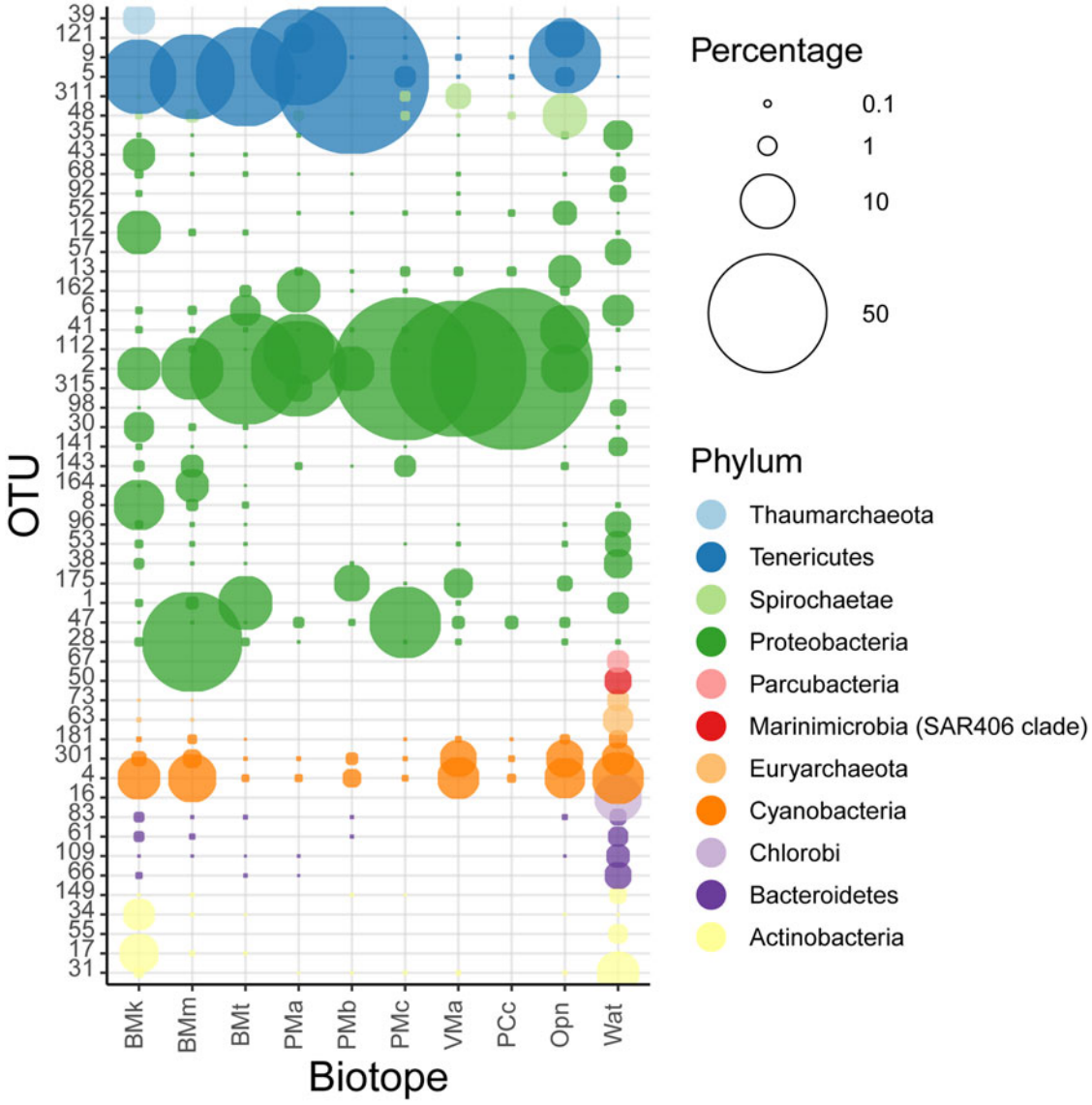

Fig. 6. Relative abundance of significantly discriminating OTUs ($P < 0.01$) identified using Simper. Symbols are colour-coded according to prokaryote phylum. Abbreviations represent the sample sites for *M.* cf. *papua* samples from marine lakes in Berau, Indonesia: Kakaban lake (BMk), Haji Buang lake (BMm) and Tanah Bamban lake (BMt), in Papua: (Kr02: PMa), (Ms01: PMb), (Ms17: PMc), in Vietnam (Bui Xam: VMa), for *C. ornata* samples from marine lake Ms17 in Papua (PCc), for jellyfish species sampled in open-water habitat in Taiwan and Vietnam (Opn), and for water samples (Wat). The circle size of the OTU is proportional to the mean percentage of sequences per biotope. The *y*-axis shows the OTU id number.

clade D. Previous studies have also identified *Symbiodinium* spp. in *M. papua* (Shirahama & Kakinuma, 1985) and *Cassiopea* spp. (Freeman *et al.*, 2017). Zooxanthelate jellyfish are also known to depend on their symbiotic dinoflagellates for nutrition and metabolic function (Pitt *et al.*, 2009). However, given the short length of the sequences used in the present study, a more in-depth study will be needed to confirm the suggested relationship between *Endozoicomonas* and *Symbiodinium* in jellyfish hosts. Previous studies, however, have also described this association in coral hosts, and observed that OTUs assigned to *Endozoicomonas* co-occurred with *Symbiodinium* from both clades C and D (Bernasconi *et al.*, 2019).

In lake Ms01 in Papua, OTU-5 was the most abundant OTU (Mollicutes class, Entomoplasmatales order). The Mollicutes comprises small bacteria devoid of cell walls, which are widespread commensals of eukaryotic organisms, and may be pathogens of plants, animals and humans (Weiland-Bräurer *et al.*, 2015; Chernov *et al.*, 2018). The Entomoplasmatales order and genus *Mycoplasma* (Mollicutes class) have previously been associated with jellyfish, namely with *M. papua* and *Aurelia aurita* species (Weiland-Bräuer *et al.*, 2015; Cleary *et al.*, 2016). In addition to this, OTU-9 was one of the most abundant OTUs in *M. papua* from Kr02. OTU-9 had low sequence similarity (88.86%) to known sequences in the NCBI database. Cleary *et al.* (2016) suggested that an OTU assigned to the Entomoplasmatales order observed in *M. papua* from marine lakes in Berau may be a species-specific jellyfish symbiont (Cleary *et al.*, 2016). OTU-175, assigned to the genus *Wolbachia*, also had low sequence similarity (<86%) to known sequences in the NCBI database and was detected in all jellyfishes in Lakes Bui Xam and Ms01. These results indicate that this taxon may represent a novel lineage within the order that may be specific to *Mastigias* jellyfishes. Members of the genus *Wolbachia* are known as obligate intracellular bacteria infecting arthropods and nematodes, which can establish parasitic, mutualistic or commensal relationships with their hosts (Werren *et al.*, 2008). It should, however, be noted that taxonomic assignment to the genus level is unreliable given the small fragment size of the

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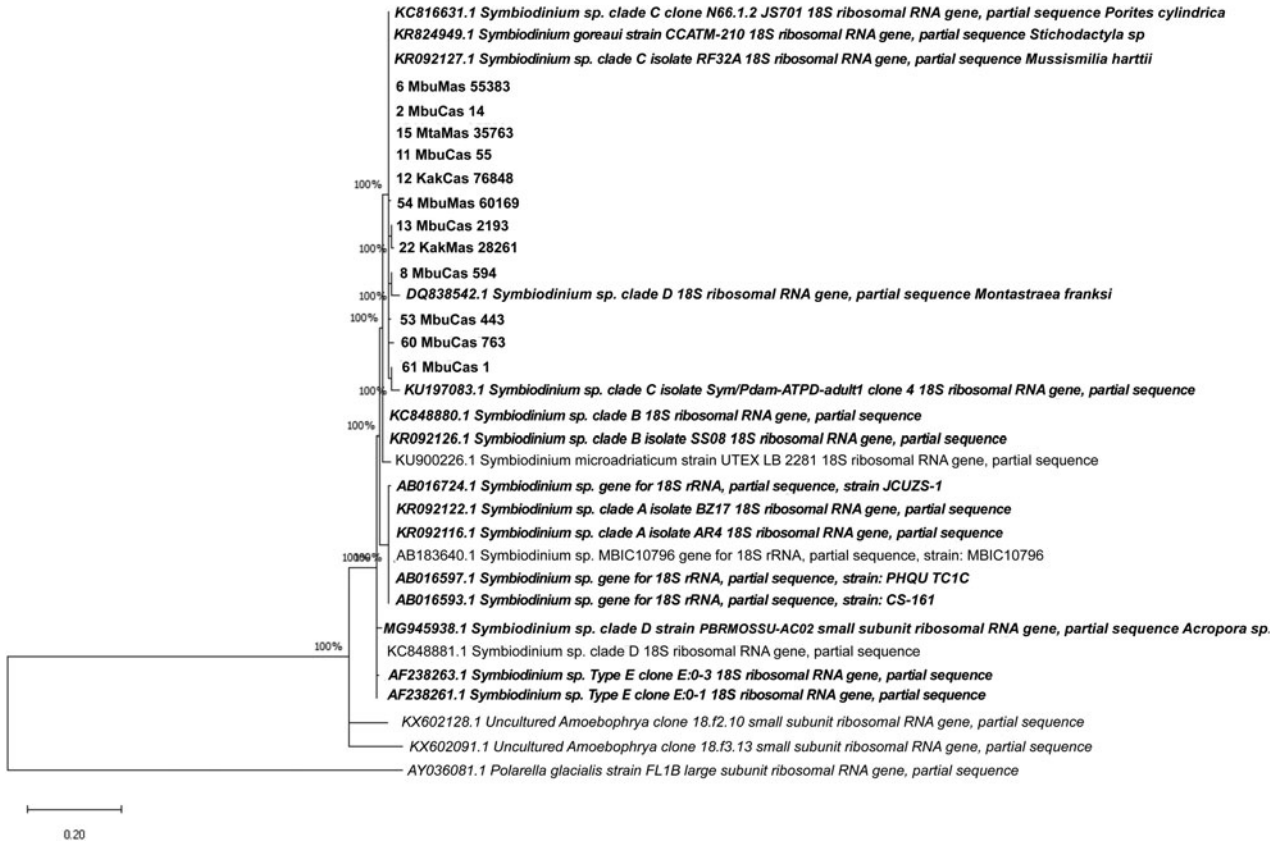

Fig. 7. Phylogenetic tree inferred using the maximum likelihood method and General Time Reversible model with aligned 18S sequences of *Symbiodinium* OTUs recovered from the studied biotope (jellyfish). The bootstrap values are shown next to each branch when this exceeds 50%. This value represents the percentage of replicate trees in which the associated taxa clustered together. *Symbiodinium* sequences retrieved from this study are highlighted in bold, sequences from invertebrate hosts (e.g. corals, anemones, bivalves) are in bold italic, and sequences from outgroups are in italic.

16S rRNA gene used in the present study. More detailed research is needed to obtain a more reliable taxonomic assignment of this organism.

Conclusion

Our results showed that samples from jellyfish species collected outside marine lakes housed more diverse prokaryotic communities than samples collected inside marine lakes, with the exception of *M. papua* from lake Kakaban. The most dominant members of the jellyfish prokaryotic communities were assigned to the classes Gammaproteobacteria and Mollicutes. Our results also identified a single 'core' OTU, which was found in every jellyfish sample. This OTU was assigned to the genus *Endozoicomonas* and closely related to other symbiotic bacterial OTUs previously detected in a range of different marine organisms including specimens of cuttlefish, octocoral, lionfish, anemone and comb pen. Interestingly, a preliminary analysis of the microeukaryotic community also revealed high relative abundances of *Symbiodinium* sp. members. The co-occurrence of *Endozoicomonas* and *Symbiodinium* sp. has been recently reported in other marine organisms (e.g. corals), however, this is the first study, to our knowledge, in which this relationship is reported in jellyfish. Further studies are needed to confirm possible symbiotic interactions between *Endozoicomonas* and *Symbiodinium* in jellyfish and if they jointly provide fitness benefits to their hosts.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0025315422000777

Acknowledgements. We are grateful to students and colleagues from the Department of Marine Biodiversity and Conservation, Research Institute for Marine Fisheries in Vietnam that assisted in the field. We are grateful to the Indonesian State Ministry of Research and Technology (RISTEK) for providing research permits. We would like to thank Lisa Becking for collecting the Papua specimens in the field and Ristek and LIPI, Indonesia, for supporting the fieldwork.

Author contributions. Marina R.S. Ferreira contributed to laboratory work and writing the manuscript. Daniel F.R. Cleary came up with the idea for the manuscript, and contributed to fieldwork, data analysis, and writing the manuscript. Nguyen K. Bat contributed to fieldwork. Ana R.M. Polónia contributed to laboratory work and writing the manuscript. Newton C.M. Gomes contributed to writing the manuscript.

Financial support. This work was a contribution to the LESS CORAL [PTDC/AAC-AMB/115304/2009] and the Ecotech-Sponge [PTDC/BIAMIC/6473/2014 – POCI-01-0145-FEDER-016531] projects funded by FEDER, through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES. FCT/MCTES contributed financial support to CESAM (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020), through national funds. Marina R.S. Ferreira was supported by a PhD scholarship (SFRH/BD/114809/2016) and Ana R.M. Polónia was supported by a postdoctoral scholarship (SFRH/BPD/117563/2016) funded by the Portuguese Foundation for Science and Technology (FCT)/national funds (MCTES) and by the European Social Fund (ESF)/EU.

Conflict of Interest. The authors declare that they have no conflicts of interest.

Data. The DNA sequences generated in this study can be downloaded from the NCBI SRA: PRJNA479655 (SRP153146) and PRJNA397182 (SRP133415).

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