A preliminary survey of zoantharian endosymbionts shows high genetic variation over small geographic scales on Okinawa-jima Island, Japan

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Symbiotic dinoflagellates (genus *Symbiodinium*) shape the responses of their host reef organisms to environmental variability and climate change. To date, the biogeography of *Symbiodinium* has been investigated primarily through phylogenetic analyses of the ribosomal internal transcribed spacer 2 (ITS2) region. Although the marker can approximate species-level diversity, recent work has demonstrated that faster-evolving genes can resolve otherwise hidden species and population lineages, and that this diversity is often distributed over much finer geographical and environmental scales than previously recognized. Here, we use the noncoding region of the chloroplast psbA gene ($psbA^{ncr}$) to examine genetic diversity among clade C *Symbiodinium* associating with the common reef zoantharian *Palythoa tuberculosa* on Okinawa-jima Island, Japan. We identify four closely related *Symbiodinium* $psbA^{ncr}$ lineages including one common generalist and two potential specialists that appear to be associated with particular microhabitats. The sea surface temperature differences that distinguish these habitats are smaller than those usually investigated, suggesting that future biogeographic surveys of *Symbiodinium* should incorporate fine scale environmental information as well as fine scale molecular data to accurately determine species diversity and their distributions.

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Abstract

Symbiotic dinoflagellates (genus *Symbiodinium*) shape the responses of their host reef organisms to environmental variability and climate change. To date, the biogeography of *Symbiodinium* has been investigated primarily through phylogenetic analyses of the ribosomal internal transcribed spacer 2 (ITS2) region. Although the marker can approximate species-level diversity, recent work has demonstrated that faster-evolving genes can resolve otherwise hidden species and population lineages, and that this diversity is often distributed over much finer geographical and environmental scales than previously recognized. Here, we use the noncoding region of the chloroplast psbA gene ($psbA^{ncr}$) to examine genetic diversity among clade C *Symbiodinium* associating with the common reef zoantharian *Palythoa tuberculosa* on Okinawa-jima Island, Japan. We identify four closely related *Symbiodinium* $psbA^{ncr}$ lineages including one common generalist and two potential specialists that appear to be associated with particular microhabitats. The sea surface temperature differences that distinguish these habitats are smaller than those usually investigated, suggesting that future biogeographic surveys of *Symbiodinium* should incorporate fine scale environmental information as well as fine scale molecular data to accurately determine species diversity and their distributions.

29

30 **Introductions**

31 *Symbiodinium* is an important genus of dinoflagellate photosymbionts found in tropical
32 and subtropical marine ecosystems. These 'zooxanthellae' transfer energy to their invertebrate
33 hosts in nutrient-poor environments, enhancing the growth of reef-building corals and other reef
34 organisms such as zoantharians (Muscatine and Cernichiari 1969; Baker 2003). They also serve a
35 key role in establishing the thermal tolerance of coral colonies and shaping the adaptive response
36 of reef organisms to climate change (Sampayo et al. 2008; Thornhill et al. 2014). As climate
37 change intensifies and diversity patterns are expected to alter, it is increasingly important to map
38 marine species distributions on scales both large (McClanahan et al. 2014; Reimer et al. 2017)
39 and small (Mieszkowska and Lundquist 2011).

40 The biogeography of *Symbiodinium* is determined by many factors, including host
41 species distributions and environmental parameters (LaJeunesse et al. 2004). To determine
42 *Symbiodinium* biogeography, it is critical to use DNA markers with the resolving power to
43 delineate between and among species (LaJeunesse and Thornhill 2011). Our ability to discern
44 different lineages of *Symbiodinium* has improved in tandem with the resolution of the molecular

markers used to identify them. Originally, highly divergent lineages or 'clades' designated A-I were confirmed via the sequencing of 18S and 28S ribosomal DNA (Rowan and Powers 1991; Pochon and Gates 2010), followed by the delineation of numerous subclades via phylogenetic analyses of the internal transcribed spacer regions (e.g. ITS2; LaJeunesse 2001). More recently, sequences from domain V of the chloroplast large subunit (cp23S) ribosomal DNA (Santos et al. 2003; Kirk et al. 2009; LaJeunesse and Thornhill 2011; LaJeunesse et al. 2012) and the non-coding region of the plastid minicircle ($psbA^{ncr}$; Takishita et al. 2003; LaJeunesse and Thornhill 2011) have provided even greater resolution, often corresponding to the species level (*e.g.* LaJeunesse et al. 2012). Microsatellite markers have also been developed to investigate fine-scale diversity in *Symbiodinium* (e.g. Pettay and LaJeunesse 2007; Grupstra et al. 2017).

As a result of the development and implementation of finer resolution molecular markers, it is apparent that the extent of *Symbiodinium* diversity may be much greater than previously recognized based on studies using only internal transcribed spacer regions. For example, in examinations of *Symbiodinium* within the common reef zoantharian *Palythoa tuberculosa* (Esper, 1805) across a latitudinal gradient of 800 km in the northern Red Sea, $psbA^{ncr}$ sequences revealed up to four unique lineages, despite ITS2 results showing only one

single "subclade" lineage (Reimer et al. 2017). The distribution of these individual psbA^{ncr} lineages strongly correlated with sea surface temperature differences of approximately 1°C. Such results demonstrate a need to re-examine previously reported *Symbiodinium* diversity with high-resolution markers, as fine scale, ecologically important differences may have been missed.

In biogeography, isolation by distance (IBD) generally refers to how genetic differences between individuals or populations increase with increasing geographical distance due to limitations on dispersal. In *Symbiodinium* species, other factors in addition to IBD that may act as drivers of evolution include host species associations (LaJeunesse et al. 2004), UV light levels (correlates of turbidity or chlorophyll-*a* concentrations; LaJeunesse et al. 2010; Tonk et al. 2014), and ocean temperatures (LaJeunesse et al. 2010; Tonk et al. 2014). Correspondingly, differences in *Symbiodinium* associations have been noted over large oceanic or latitudinal gradients (LaJeunesse and Trench 2000; LaJeunesse et al. 2004; Reimer et al. 2017), within different host species (Frade et al. 2008a; Thornhill et al. 2014), at different light levels (Frade et al. 2008b; Sampayo et al. 2008; Kamezaki et al. 2013), and in areas of extremely high (Hume et al. 2013) or low ocean temperatures (Chen et al. 2003).

Okinawa-jima Island is ~1200 km^2 in area, just over 100 km in length, and 3-30 km in

77 width. Although it is not ‘large’ when compared to oceanic or regional scales, the surrounding
78 marine environment encompasses a variety of ecosystems including large bays, muddy tidal flats,
79 patch reefs within lagoons, and fringing reefs (Fujita et al. 2015). Additionally, recent population
80 genetic studies on scleractinian corals (Shinzato et al. 2015), sea cucumbers (Soliman et al.
81 2016), and amphipods (White et al. 2016) have described unexpected genetic structure among
82 locations around Okinawa-jima Island, particularly between the Kuroshio-influenced west coast
83 and the more isolated east coast.

84 Based on ITS2 sequence analyses of *Symbiodinium* within *P. tuberculosa* in southern
85 Japan (including Okinawa), we previously reported that subclade C1 or closely related types are
86 dominant (Reimer et al. 2006). Here we use psbA^{ncr} sequences to re-examine how much
87 variation exists within *Symbiodinium* C1 associating with *P. tuberculosa*, specifically focusing
88 on Okinawa-jima Island’s shallow coral reef environments. We additionally explore potential
89 associations between observed diversity and the environment. This study is intended to
90 complement our recent work in the Red Sea (Reimer et al. 2017) by focusing on the same host
91 species and describing the extent of symbiont diversity on a much smaller geographical and
92 environmental scale.

93

94 **Materials and Methods**

95 *Environmental data*

96 Satellite-derived sea surface temperature (SST) and chlorophyll-*a* (chl-*a*) data for the
97 waters around Okinawa-jima Island and nearby Amami Oshima Island were acquired from
98 National Aeronautic and Space Administration Giovanni database
99 (http://gdata1.sci.gsfc.nasa.gov/; Acker and Leptoukh 2007), developed and maintained by the
100 NASA Goddard Earth Sciences Data and Information Services Center. Error ranges of the
101 MODIS Aqua data were approximately ±0.25°C for SST, and ±40% for chl-*a*. Yearly average
102 SST (SST^{avg}) and chl-*a* data and maps used in this study were derived from 4 km resolution data
103 from the Moderate Resolution Imaging Spectroradiometer (MODIS) Aqua database. These
104 generated maps provided the basis for estimating annual average SST and chl-*a* at each sampling
105 location. Data from Feb-2000 to May-2015 were used for SST^{avg} analyses, and from Jul-2002 to
106 May-2015 for chl-*a*. To examine yearly winter minimum (SST^{min}) and summer maximum SSTs
107 (SST^{max}), we averaged monthly data from February and from August, respectively (2000-2014,
108 n=14 each).

Specimen collection

The zooxanthellate cnidarian species *Palythoa tuberculosa* is the most common zoantharian on coral reefs surrounding Okinawa-jima Island (Irei et al. 2011), and is easily identifiable (Hibino et al. 2014). Previous work in southern Japan has also ascertained that the taxon does not contain cryptic species (Reimer et al. 2007), making it a simple and reliable species for research and citizen science (Parkinson et al. 2016).

Specimens of *P. tuberculosa* were collected from Jan-2012 to Nov-2015 from eight locations around Okinawa-jima Island and one location on Amami Oshima Island, Kagoshima, to the north of Okinawa in the Middle Ryukyus (Table 1). All specimens were collected from the low intertidal zone (0-2 m depths depending on tides) via snorkeling. Small portions of colonies were collected and fixed in 70-99.5% ethanol for further molecular analyses. The collections were limited by the low numbers of *P. tuberculosa* present at some sites (n = 3-11).

DNA extraction, PCR, and phylogenetic analyses

DNA was extracted from preserved colony samples using a DNeasy Blood and Tissue Kit (Qiagen, Tokyo, Japan) following the manufacturer's protocol. We amplified two *Symbiodinium* DNA markers; the internal transcribed spacer 2 (ITS2) in the ribosomal DNA

125 array, and a portion of the plastid minicircle non-coding region (psbA^{ncr}). ITS2 sequences were
126 obtained to place our new specimens within the phylogenetic framework of this well reported
127 marker, and with past research on *Symbiodinium* within *P. tuberculos*a in southern Japan
128 (Reimer et al. 2006), while psbA^{ncr} sequences were obtained to examine finer scale phylogenetic
129 patterns (*e.g.* Reimer et al. 2017). ITS2 was amplified using the primers zITSf (5'-CCG GTG
130 AAT TAT TCG GAC TGA CGC AGT-3') and ITS4 (5' –TCC TCC GCT TAT TGA TAT GC-
131 3') (White et al. 1990; Rowan and Powers 1992; Hunter et al. 1997). psbA^{ncr} was amplified using
132 the primers 7.4-Forw (5'-GCA TGA AAG AAA TGC ACA CAA CTT CCC-3') and 7.8-Rev
133 (5'-GGT TCT CTT ATT CCA TCA ATA TCT ACT G- 3') (LaJeunesse and Thornhill 2011).
134 Reaction mixes contained 1.0 µl of genomic DNA, 7.0 µ1 of Milli-Q water, 10.0 ul of
135 HotStarTaq Plus Master Mix, and 1.0 µl of each primer (10 pmol). Thermocycler conditions
136 were as follows: for ITS2: 95.0°C for 5 min; 35 cycles of 94.0°C for 30 s, 51.0°C for 45 s, and
137 72.0°C for 2 min; 72.0°C for 10 min; and for psbA^{ncr}: 95.0°C for 5 min; 40 cycles of 94.0°C for
138 10 s, 55.0°C for 30 s, and 72.0°C for 2 min; 72.0°C for 10 min. Products were directly sequenced
139 by Fasmac (Kanagawa, Japan). Novel sequences are deposited in GenBank under Accession
140 Numbers XXXXX-XXXXX.

141 The nucleotide sequences for ITS2 and psbA^{ncr} were separately aligned within
142 Geneious v9.1.3 (Biomatters Limited, Auckland, New Zealand). Alignments were inspected
143 manually, and primer regions and short sequences were excluded. Because the long plastid non-
144 coding region rarely sequenced completely, we used only the forward psbA^{ncr} reads. The ITS2
145 alignment contained 21 sequences of 216 bp, while the psbA^{ncr} forward alignment contained 61
146 sequences of 300 bp. Previously reported sequences from GenBank were incorporated into the
147 ITS2 alignment for reference (DQ480631, DQ480639, DQ889741, DQ889743 - all
148 *Symbiodinium* subclade C1 or closely related from *P. tuberculosa* from southern Japan; and
149 AB207184 - *Symbiodinium* subclade C15 related from *Zoanthus* sp. in southern Japan), while the
150 psbA^{ncr} alignment contained only novel sequences (no previously reported sequences bore strong
151 similarity).
152 Both alignments were analyzed using maximum likelihood (ML), neighbor-joining
153 (NJ), maximum parsimony (MP), and Bayesian inference (BI) methods. ML analyses for both
154 datasets were performed using PhyML (Guindon et al. 2010) with input trees generated by
155 NJPlot (Perriere and Gouy 1996) under automatic model selection by smart model selection with
156 Akaike Information Criterion. Both datasets were analyzed under the HKY85 model (Hasegawa

et al. 1985) with the transition/transversion ratio estimated, the proportion of invariable sites fixed at 0.0, and the number of substitution rate categories as 1. PhyML bootstrap trees were made using the same parameters as the individual ML tree. The distances were calculated using a Kimura's two-parameter model (Kimura 1980). NJ analyses for both alignments were run within Geneious on default settings under the HKY85 model. MP analyses were performed in Paup* 4.0a147 (Swofford 2000) with indels included as a fifth character state. All trees were run with 1000 bootstraps. Bayesian posterior probabilities were calculated with the software Mr. Bayes (Huelsenbeck and Ronquist 2001) using the HKY85 substitution model and default parameters (chain length = 1,000,000; burn-in = 250,000). Genetic distances between and within lineages were calculated in MEGA6 (Tamura et al. 2013) using the Maximum Composite Likelihood Model (Tamura et al. 2004).

Results

Environmental data

Yearly average SST (SST^{avg}) showed southern sites to be warmer than northern sites, with a difference of 0.95°C between Wase on Amami Oshima Island (24.6°C) compared to Kyan

and Odo on the southern tip of Okinawa-jima Island (25.35°C) (Table 1). For summer maximum SST (SST^{max}), Wase was lowest (28.88±0.93°C) while Uken on the east coast of Okinawa-jima Island was highest (29.43±0.76°C) (Table 1). For winter minimum SST (SST^{min}), Wase (20.35±0.43°C) was coldest, with highest SST^{min} at Odo (21.42±0.60°C). The highest observed SST in any year was at Uken (30.9°C in 2001), and the lowest was at Bise (18.7°C in 2015) and Oku (18.7°C in 2008) on the northwest and north coasts of Okinawa-jima Island, respectively (Table 1). Yearly average chl-*a* concentration values were generally low at all sampling sites, ranging from a low of 0.08 mg/m^3 at Odo to a high of 0.50 mg/m^3 at Uken (Table 1).

Phylogenetic analyses

Two different *Symbiodinium* ITS2 types were detected. The first type (n=17) matched 100% with previously reported *Symbiodinium* subclade C1 from *P. tuberculosa* in southern Japan (DQ889743; DQ889741) (Fig. 1a). The other type (n=5) differed by one base pair and was also 100% identical to previously reported *Symbiodinium* from *P. tuberculosa* in southern Japan (DQ480639). This second type formed a subclade within C1 (ML=99%, NJ=74%, MP=61%, BI=0.90) (Fig. 1a). These two types formed a large, moderately supported clade separate from

188 subclade C3 (ML=69%, NJ=83%, MP=64%, BI=0.70), so we considered all of our sequences to
189 be subclade C1 or 'C1-related' (Fig. 1a; Electronic Supplementary Material Table S1).

190 Sixty-three unique *Symbiodinium* psbA^{ncr} forward sequences were recovered. Two
191 sequences were too short to be included in the final alignment but were long enough to identify
192 to lineage (described below, Electronic Supplementary Material Table S1). The resulting psbA^{ncr}
193 ML tree showed 33 specimens within a large, well-supported clade (ML=100%, NJ=100%,
194 MP=100%, BI=1.00) that we designated as 'lineage 1' (Fig. 1b). Specimens belonging to lineage
195 1 (n = 33) were recovered from all 9 locations. Another 19 specimens from Oku, Teniya, and
196 Mizugama formed a separate monophyly ('lineage 2'; ML=76%, NJ=93%, MP=100%, BI=0.98).
197 Additionally, 7 specimens from Wase, Nerome, Bise, Mizugama and Odo formed a monophyly
198 ('lineage 3'; ML=75%, NJ=100%, MP=100%, BI=0.77). Finally, 2 specimens from Bise and
199 Nerome formed another monophyly ('lineage 4'; ML=100%, NJ=100%, MP=100%, BI=1.00).
200 The between-lineage molecular distances for psbA^{ncr} ranged from 0.105-0.256, while the within-
201 lineage distances were much smaller, ranging from 0.003-0.021 (Table 2).

202 *Lineage distributions*

We next examined the distribution of *Symbiodinium* lineages across locations (Fig. 2). Wase, Amami Oshima Island was dominated by lineage 1 (4/5 *P. tuberculosa* colonies), as was Bise (9/11), Uken (3/3), Kyan (4/4), and Odo (6/7). Lineage 2 was dominant at Oku (8/10), Teniya (5/7), and Mizugama (6/8), while lineage 3 was dominant at Nerome (5/8) and also appeared at Bise (2/7). Lineage 4 members only appeared in one colony each at Wase, Mizugama, and Odo.

Finally, we examined the range of environments in which each lineage could be found (Fig. 3). *Symbiodinium* lineage 1 appeared at all sites and thus all environments in this study (SST^{avg} = 24.6°C-25.35°C; SST^{max} = 29.43°C; SST^{min} = 20.35°C; chl-*a* 0.08 mg/m^3 - 0.50 mg/m^3). Lineage 2 was only observed at Oku, Teniya, and Mizugama, where SST^{avg} ranged between 24.95°C to 25.15°C, with SST^{min} of 20.69°C (Oku) and SST^{max} of 29.16°C (Teniya, Mizugama), and chl-*a* ranged between 0.08 mg/m^3 to 0.25 mg/m^3. Lineage 3 was only found at Bise and Nerome, with SST^{avg} of 24.85°C-24.95°C, SST^{min} of 20.47°C, SST^{max} of 29.33°C (both Nerome), and chl-*a* of 0.15 mg/m^3 to 0.30 mg/m^3. Lineage 4 members were only observed once each at three locations, but these stretched across the geographic range of this study (Wase, Mizugama, Odo).

Discussion

Using the high-resolution psbA^{ncr} marker, we identify four *Symbiodinium* lineages associated with *Palythoa tuberculosa* on Okinawa-jima Island. The lineages feature surprisingly unique distributions over a small geographic scale, and would be considered at most two entities based on lower-resolution ITS2 data (Fig. 1). Because the between-lineage molecular distances for psbA^{ncr} (0.105-0.256; Table 2) are greater than those reported between the psbA^{ncr} sequences of two divergent *Symbiodinium* ITS2 types (~0.045 for C26a vs. C31; LaJeunesse and Thornhill 2011), these lineages likely represent reproductively isolated species rather than populations within a species.

Lineage 1 appears to be a widely distributed generalist, at least over the range of this study in the Central Ryukyus (Fig. 2). It is found at all sites and often occupies the majority of colonies at a given site. Lineage 3 is observed only at the two sites on the northwestern coast of Okinawa-jima Island, and thus appears to have narrow geographical and environmental components to its distribution (SST^{avg} = 24.85°C-24.95°C; chl-*a* = 0.15– 0.30 mg/m^3) (Fig. 3). Lineage 4 is present in very low numbers (n=3) across the latitudinal range of the study, making it difficult to infer its environmental niche.

Lineage 2 has a somewhat restricted range, found at only three locations (where it also

235 occupied the majority of colonies): Oku, Teniya, and Mizugama. These three sites are located
236 near river outflows, suggesting this *Symbiodinium* lineage may be able to tolerate changes in
237 salinity more effectively than the others. Unfortunately, fine-scale salinity data are not yet
238 available to investigate this trend further. Chl-*a* levels at these sites are generally low (< 0.25
239 mg/m^3), while the SST^{avg} range is intermediate (24.95°C - 25.15°C). Lineage 2 is absent at other
240 locations within this SST^{avg} range; for example, at Bise and Nerome, where only lineages 1 and 3
241 are present, and at Uken, where only lineage 1 is detected. Although Uken's SST^{avg} is firmly in
242 the middle of the SST range investigated in this study, Uken's SST^{min} and SST^{max} are generally
243 more extreme than those at other sites (Table 1) due to Uken's position within shallow southern
244 Kin Bay, which is isolated from the stabilizing temperature effects of the open ocean (Montani
245 1996). Additionally, chl-*a* levels at Uken are higher than those of all other locations (0.50
246 mg/m^3).

247 By focusing on a very small area of the northwest Pacific, and by using a rapidly
248 evolving molecular marker, we could resolve a much finer scale of *Symbiodinium* biogeography
249 than has previously been recognized in the region. Some psbA^{ncr} lineages appear to be
250 partitioned on the basis of temperature (SST^{max}) differences on the scale of 0.1~0.3°C, much

lower than the 0.7°C-1.0°C observed in previous studies (Baums et al. 2014; Reimer et al. 2017). It also appears SST stability (e.g. differences between SST^{max} and SST^{min}), as well as fine scale salinity differences (not measured here) may play an important role in the distribution of *Symbiodinium* diversity. Chl-*a* levels are generally low at all locations, although it should be noted that only generalist lineage 1 is found in Uken (although n=3), the site with the highest chl-*a* levels. Overall, the contribution of chl-*a* (a proxy for turbidity) is not clear, as all *P. tuberculosa* specimens were collected from very shallow waters (0-2 m).

It is surprising that such biogeographic patterns could be uncovered given the low numbers of host *P. tuberculosa* at some locations. However, these low numbers also caution that the symbiont species' distributions are unlikely to have been completely resolved. Another issue is that the fine-scale environmental variation among sites falls below the margin of error in the satellite datasets; future research of this nature must utilize more precise instrumentation. We did not examine host *P. tuberculosa* population genetics, and it remains to be seen if fine-scale host structure may also play a role in the observed patterns. Previous studies indicate cnidarian host and symbiont genetic structure can be mismatched (e.g. Baums et al. 2014; Leydet and Hellberg 2016), although clear cases of matching genetic structure have also been observed (e.g.

267 Bongaerts et al. 2010; Prada et al. 2014).

268 As our ability to discern between different lineages of *Symbiodinium* has increased, so
269 too has our understanding of the complexity and nuances of *Symbiodinium* diversity and
270 distribution. The present work supports previous studies that show *Symbiodinium* evolution is
271 driven largely by specialization to different environmental niches (e.g. Frade et al. 2008b;
272 LaJeunesse et al. 2010; Kamezaki et al. 2013; Reimer et al. 2017), and that specialization may
273 occur on much finer micro-environmental scales than usually addressed. Further characterization
274 of the *Symbiodinium—Palythoa tuberculosa* symbiosis within the Ryukyus or comparable island
275 chains should be carried out to confirm similar symbiont structuring based on fine-scale
276 environmental heterogeneity.

277 The *Symbiodinium* diversity patterns on Okinawa-jima Island highlight three major
278 considerations for future investigations of this kind. First, it is advantageous for researchers to
279 obtain fine-scale environmental data of the study area if available, so as to better characterize
280 niches that might otherwise be deemed homogenous. Second, researchers should refrain from
281 considering pooled specimens or results from different nearby locations as representative of a
282 larger area without thoroughly checking for fine scale environmental patterns. Third, as has been

283 suggested elsewhere (e.g. LaJeunesse and Thornhill 2011; Reimer et al. 2017), researchers
284 should incorporate both ITS2 data (to tie to past work) as well as rapidly evolving markers (to
285 uncover hidden diversity) when investigating *Symbiodinium* biogeography, as a failure to address
286 fine-scale niche adaptation could lead to a misinterpretation of results. These suggestions should
287 improve the design of *Symbiodinium* studies on all geographic scales, from local to regional to
288 global.
289

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293

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Figure 1(on next page)

Figure 1. Phylogenies of clade C Symbiodinium isolated from Palythoa tuberculosa around Okinawa-jima Island and Amami Oshima Island, Japan.

Maximum Likelihood (ML) trees are depicted for **(a)** the internal transcribed spacer 2 (ITS2) and **(b)** the chloroplast psbA noncoding region ($psbA^{ncr}$). Sequences from previous studies are included with GenBank accession numbers, host species, location, and subclade names *sensu* LaJeunesse (2001). Values at nodes represent ML, neighbour joining (NJ), and maximum parsimony (MP) bootstrap percentages, as well as Bayesian inference (BI) posterior probabilities, respectively. Specimen abbreviations are as in Electronic Supplementary Material Table S1.

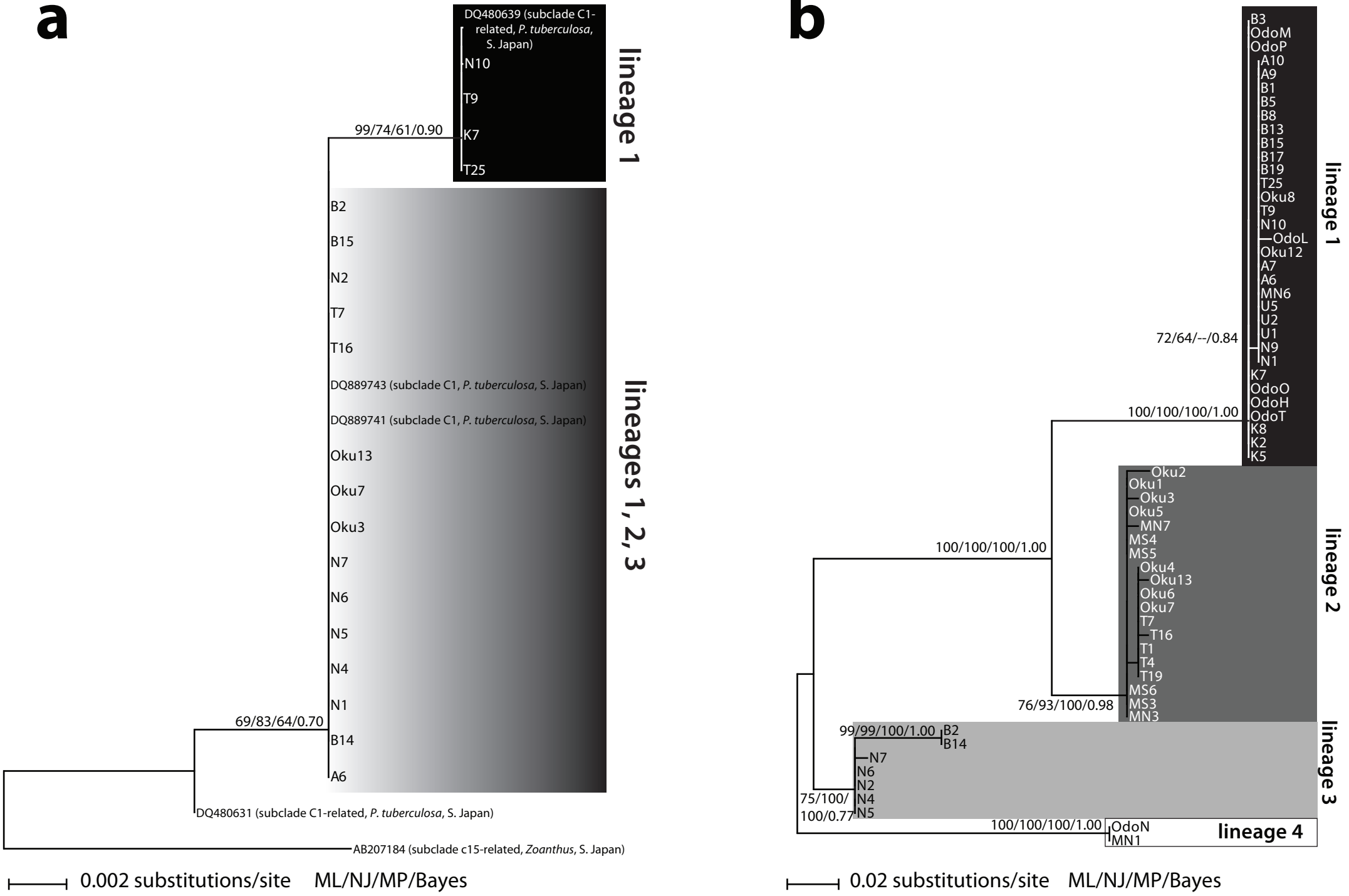
a
DQ480639 (subclade C1-related, P. tuberculosa, S. Japan)
N10
T9
K7
T25
lineage 1
99/74/61/0.90
B2
B15
N2
T7
T16
DQ889743 (subclade C1, P. tuberculosa, S. Japan)
DQ889741 (subclade C1, P. tuberculosa, S. Japan)
Oku13
Oku7
Oku3
N7
N6
N5
N4
N1
B14
A6
lineages 1, 2, 3
69/83/64/0.70
DQ480631 (subclade C1-related, P. tuberculosa, S. Japan)
AB207184 (subclade c15-related, Zoanthus, S. Japan)
0.002 substitutions/site ML/NJ/MP/Bayes
b
B3
OdoM
OdoP
A10
A9
B1
B5
B8
B13
B15
B17
B19
T25
Oku8
T9
N10
OdoL
Oku12
A7
A6
MN6
U5
U2
U1
N9
N1
K7
OdoO
OdoH
OdoT
K8
K2
K5
lineage 1
72/64/--/0.84
100/100/100/1.00
Oku2
Oku1
Oku3
Oku5
MN7
MS4
MS5
Oku4
Oku13
Oku6
Oku7
T7
T16
T1
T4
T19
MS6
MS3
MN3
lineage 2
100/100/100/1.00
76/93/100/0.98
99/99/100/1.00
B2
B14
N7
N6
N2
N4
N5
lineage 3
75/100/
100/0.77
100/100/100/1.00
OdoN
MN1
lineage 4
0.02 substitutions/site ML/NJ/MP/Bayes

Figure 2

Map of Amami Oshima Island (A) and Okinawa-jima Island (B) with average sea surface temperature (SSTavg) and *Symbiodinium* psbA^{ncr} lineage ratios at each site investigated.

Map of Amami Oshima Island (A) and Okinawa-jima Island (B) with average sea surface temperature (SST^{avg}) and *Symbiodinium* psbA^{ncr} lineage ratios at each site investigated. Note thermal distortions near coastlines were ignored in all SST analyses as these are generated by influence of terrestrial portions of islands within the 4-km resolution of satellite data.

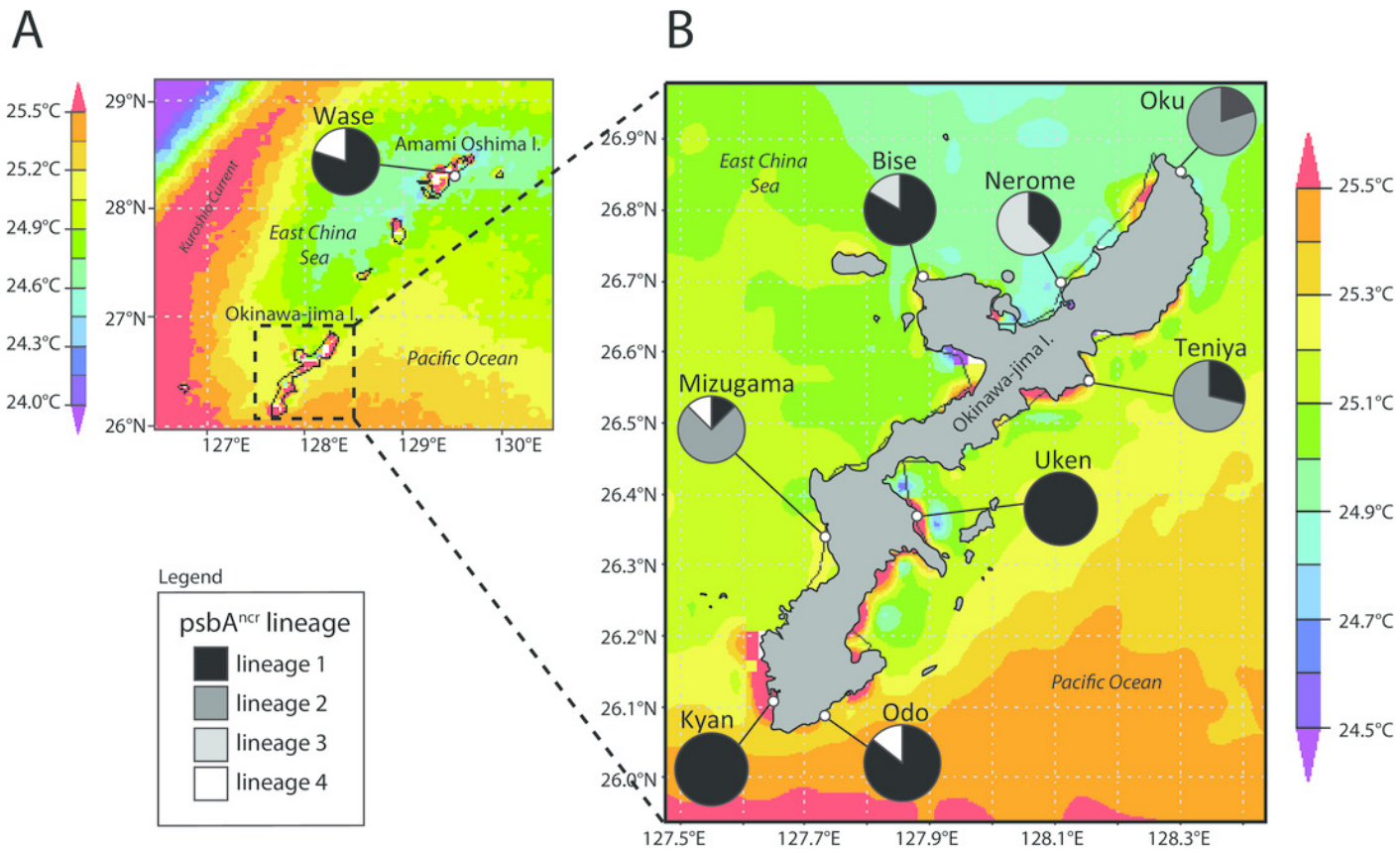

Figure 3(on next page)

Figure 3. *Symbiodinium* psbA^{ncr} lineage distribution by environment.

Distributions are represented as **(a)** proportions of each lineage in each sampling site; or as ranges (dotted lines) with respect to **(b)** SST^{avg} values, **(c)** SST^{max} values, **(d)** SST^{min} values, and **(e)** chl-*a* values. All colors correspond to sample site designations in **(a)**.

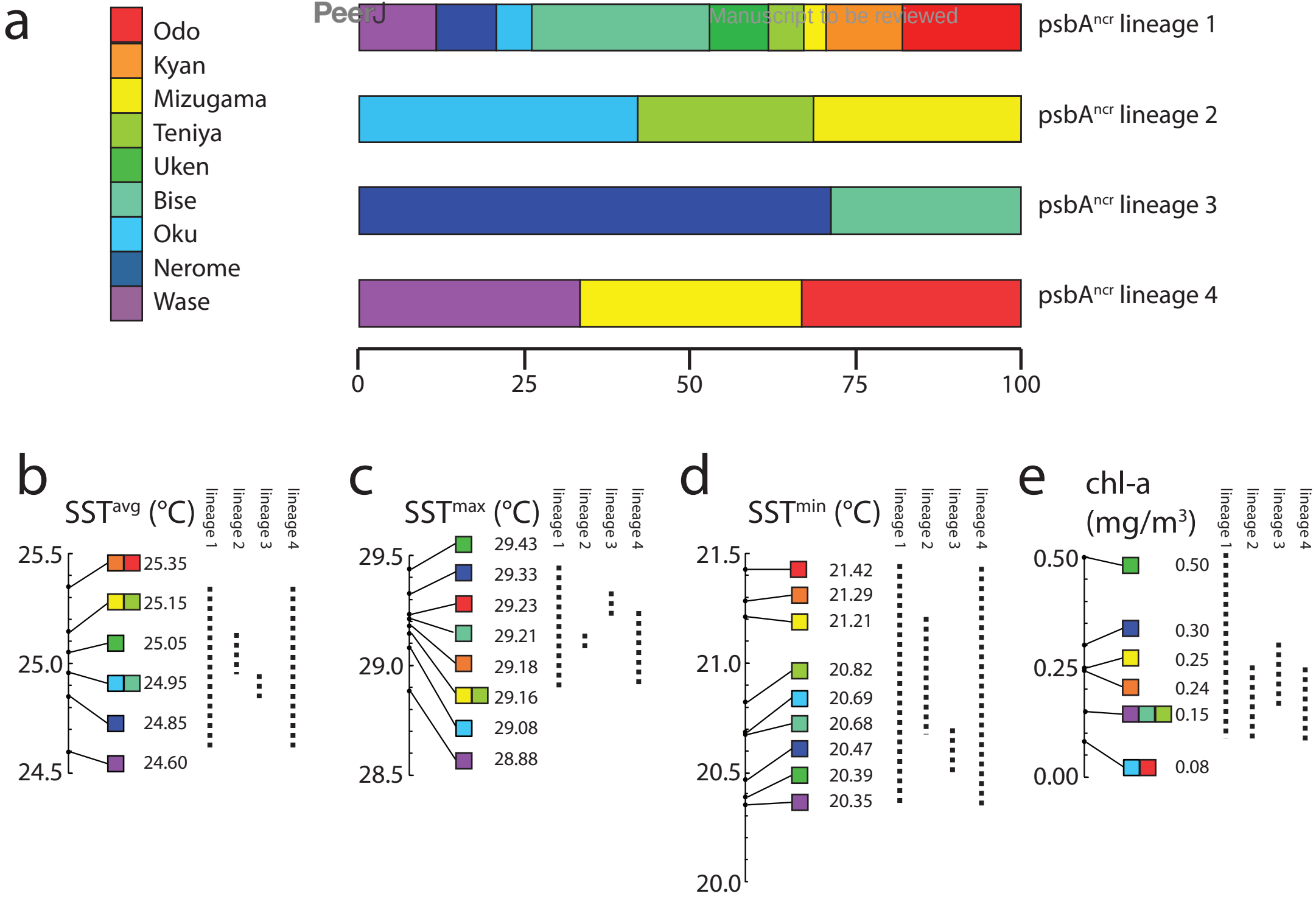

Table 1(on next page)

Sites from which *Palythoa tuberculosa* specimens were collected in this study

Sites from which *Palythoa tuberculosa* specimens were collected in this study to examine *Symbiodinium* spp., and information on numbers of specimens, sea surface temperature (SST), and chlorophyll-a (chl-a) concentrations.

Table 1. Sites from which *Palythoa tuberculosa* specimens were collected in this study to examine *Symbiodinium* spp., and information on numbers of specimens, sea surface temperature (SST), and chlorophyll-a (chl-a) concentrations.

Site name	Latitude, longitude	# of specimens	SST^{avg} (°C)[1]	SST^{max} ±SD (°C)[2]	SST^{min} ± SD (°C)[3]	High August SST (year(s))	Low February SST (year)	Chl-a (mg/m^3)[4]
Wase (Amami)	28°17’37’’ N, 129°28’27’’ E	5	24.60	28.88±0.93	20.35±0.43	30.10 (2001, 2013)	19.50 (2009)	0.15
Oku	26°50’53’’ N, 128°17’14’’ E	10	24.95	29.08±0.60	20.69±0.73	30.30 (2001)	18.70 (2008)	0.08
Nerome	26°41’36’’ N, 128°6’28’’ E	8	24.85	29.33±0.53	20.47±0.59	30.45 (2001)	19.10 (2008)	0.30
Bise	26°42’39’’ N, 127°52’52’’ E	11	24.95	29.21±0.54	20.68±0.46	30.30 (2001)	19.80 (2015)	0.15
Teniya	26°33’51’’ N, 128°8’28’’ E	7	25.15	29.16±0.57	20.82±0.50	30.15 (2001)	20.25 (2002, 2009)	0.15
Uken	26°22’46’’ N, 127°52’47’’ E	3	25.05	29.43±0.76	20.39±0.45	30.90 (2001)	19.55 (2011)	0.50
Mizugama	26°21’35’’ N, 127°44’19’’ E	8	25.15	29.16±0.51	21.21±0.42	30.15 (2001)	20.70 (2015)	0.25
Kyan	26°5’40’’ N, 127°39’10’’ E	4	25.35	29.18±0.58	21.29±0.62	30.15 (2001)	20.40 (2015)	0.24
Odo	26°5’11’’ N, 127°42’37’’ E	7	25.35±	29.23±0.51	21.42±0.60	30.15 (2001)	20.60 (2011)	0.08

[1]Generated by Giovanni data (see Materials and Methods), average of all SST measurements taken May 2000 – May 2015; value from generated map (standard deviation not available).

[2]Average of highest SST observed in August each year (2000-2014).

[3]Average of lowest SST observed in February each year (2000-2015).

[4]Generated by Giovanni data (see Materials and Methods), average of all chl-a measurements taken July 2002 – May 2015; value generated from generated map (standard deviation not available).

Table 2(on next page)

Pairwise genetic distances among *Symbiodinium* psbA^{ncr} lineages

Pairwise genetic distances among *Symbiodinium* psbA^{ncr} lineages isolated from *Palythoa tuberculosa* in southern Japan. Shaded diagonal values represent within-lineage distances.

Table 2. Pairwise genetic distances among *Symbiodinium* psbA^{ncr} lineages isolated from *Palythoa tuberculosa* in southern Japan. Shaded diagonal values represent within-lineage distances.

	Lineage 1	Lineage 2	Lineage 3	Lineage 4*
Lineage 1	0.003			
Lineage 2	0.137	0.005		
Lineage 3	0.184	0.128	0.021	
Lineage 4*	0.256	0.198	0.105	0.000*

*Lineage 4 was represented by two identical sequences.