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多様な形質に基づくイワスナギンチャク Palythoa 属（刺胞動物門：花虫綱：スナギンチャク目）近縁種間における種の境界

Integrative delimitation of species boundaries in closely related *Palythoa* species (Cnidaria: Anthozoa: Zoantharia)
多様な形質に基づくイワスナギンチャク *Palythoa* 属（刺胞動物門：花虫綱：スナギンチャク目）近縁種間における種の境界

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Resume

Sympatric speciation is thought to be a major generator of marine biodiversity. However, it is still controversial and relatively unknown how sessile coral reef organisms living in shallow waters speciate due to the presence of few geographic barriers for marine organisms that have highly dispersive planktonic larvae. Zoantharians are such sessile marine invertebrates (Cnidaria: Anthozoa: Zoantharia). Despite their abundant biomass, accurate identification of zoantharians is often not easy, and utilizes genetic markers combined with morphological characters. One zooxanthellate zoantharian genus, *Palythoa,* has four very closely related putative species; *P. tuberculosa*, *P.* sp. 'yoron', *P.* aff. *mutuki*, and *P. mutuki*, inhabiting sympatrically in intertidal reefs of southern Japan (Okinawa-jima I., Yoron-to I., Okinoerabu-jima I. and Tokunoshima I.). In this study, I targeted this *Palythoa* species group, to answer the following questions: 1) Do these species have the chance to achieve in situ interspecific cross-fertilization?, 2) Where are the species boundaries in this *Palythoa* species group?, and 3) If they are different species, how have they speciated sympatrically? I utilized an integrative approach using morphological, molecular phylogenetic, and ecological datasets. In Chapter 2, I compared datasets of colony morphology, nematocysts, spawning timing, and molecular phylogenetics, and from this the group was considered to include four distinct species based on morphological differentiation and distinct spawning periods (Figure 1), even though no molecular marker was successful in dividing these species into their own monophyletic clades (Figure 2). In Chapters 3 and 4, by comparison of genetic compositions of endosymbiotic Symbiodiniaceae algae and the trophic niches between host species and symbionts, I detected micro-scale geographic variations of the Symbiodiniaceae among these putative *Palythoa* species within a single coral reef (Table 1) and also observed trophic niche partitioning among sympatric *Palythoa* species (Figure 3). Hence, the integrative species delimitation approach utilized in this thesis with multiple lines of evidence succeeded in providing robust evidence of species delineation of four closely related *Palythoa* species. Furthermore, this study indicates that 1) even marine organisms with high dispersal ability that have few geographical barriers that appear to be sympatric may live in different microenvironments within the same narrow reef area, and 2) these microenvironmental differences in intertidal reefs may play a role in generating species biodiversity of coral reef organisms.

Table 1. Composition of monophyletic clade for ITS-rDNA and psbA^ncr^ sequences of Symbiodiniaceae from *Palythoa* species used in this study and microenvironments of host habitats. Significances were tested by Fisher's Exact Test and V value represents Cramer's coefficient of association.

		Symbiodiniaceae genotype (ITS-rDNA region)			Symbiodiniaceae lineage (psbA^ncr^ reverse region)	
		Genotype01	Genotype02	Genotype03	clr1	clr2
Host species	*P. tuberculosa*	20	7	0	13	2
	P. sp. yoron	20	1	8	14	1
	P. mutuki	3	13	2	2	8
	P. aff. *mutuki*	5	1	0	-	-
	Total	48	22	10	29	11
		$p < 0.01$, V = 0.477			$p < 0.01$, V = 0.682	
Host habitats of *P. tuberculosa*	Reef edge	8	0			
	Reef flat	7	2			
	Backreef moat	2	4			
	Total	17	6			
		$p < 0.05$, V = 0.508				

Note: *P.* aff. *mutuki* was removed from statistical analysis for psbA^ncr^ region due to its few number of specimen.

Figure 1. Monthly change of ratio of number of polyps possessing developing and/or developed ovaries (N) on total number of examined polyps (Nt) in **A**, 2010 and **B**, 2011. Color indicates *Palythoa* species; red, *P. tuberculosa*; yellow, *P.* sp. 'yoron'; blue, *P. mutuki*; green, *P.* aff. *mutuki*.
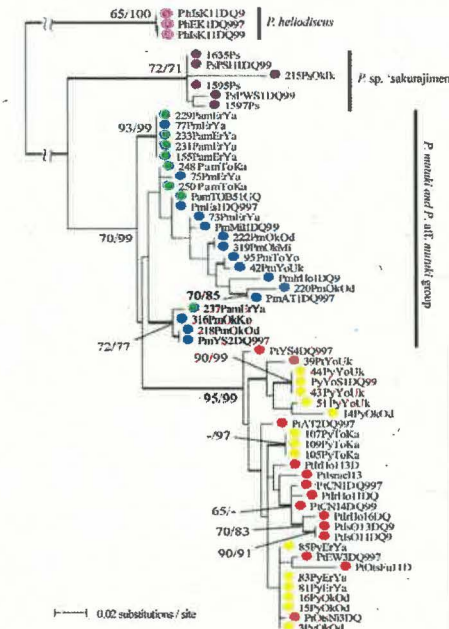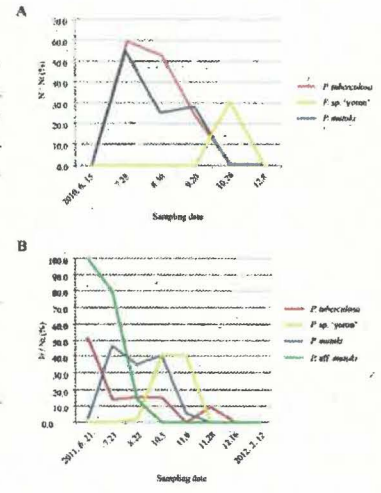

Figure 2. Maximum likelihood (ML) tree of internal transcribed spacer of ribosomal DNA (ITS-rDNA) region. Values at branches represent ML and NJ bootstrap probabilities, respectively (>50%). Bayesian posterior probabilities of >0.95 are represented by thick branches. Colored circles indicate *Palythoa* species; red, *P. tuberculosa*; yellow, *P.* sp. 'yoron'; blue, *P. mutuki*; green, *P.* aff. *mutuki*; purple, *P.* sp. 'sakurajimensis'; pink, *P. heliodiscus*.
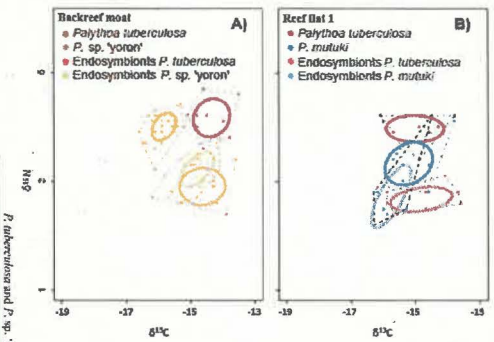

Figure 3. Isotopic niches of four *Palythoa* populations and associated Symbiodiniaceae across two reef habitats of Tokunoshima I.: **A.** backreef moat (*P. tuberculosa* + *P.* sp. 'yoron'), **B.** reef flat 1 (*P. tuberculosa* + *P. mutuki*).

Related publications:

1) **Mizuyama, M.**, Masucci, G. D., & Reimer, J. D. (2018). Speciation among sympatric lineages in the genus *Palythoa* (Cnidaria: Anthozoa: Zoantharia) revealed by morphological comparison, phylogenetic analyses and investigation of spawning period. *PeerJ*, 6, e5132.
2) **Mizuyama, M.**, Iguchi, A., Iijima, M., Gibu, K., & Reimer, J. D. (2020). Comparison of Symbiodiniaceae diversities in different members of a *Palythoa* species complex (Cnidaria: Anthozoa: Zoantharia)—implications for ecological adaptations to different microhabitats. *PeerJ*, 8, e8449.