

Who is moving where? Molecular evidence reveals patterns of range shift in the acorn barnacle *Hexechamaesipho pilsbryi* in Asia

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ABSTRACT: Global warming is predicted to cause a shift in the geographic ranges of marine organisms. Such shifts have already been observed with the polar retreat of temperate species and northern expansion of more tropical species. *Hexechamaesipho pilsbryi* is an intertidal acorn barnacle first identified by Hiro in 1936. Hiro reported the southern limit of *H. pilsbryi* as southern Kyushu Island, Japan. However, *H. pilsbryi* has recently been recorded in Taiwan as well as Maritime Southeast Asia — suggesting a recent southward range extension into tropical waters that would be contrary to general predictions. To test this hypothesis, we compared the mitochondrial COI gene sequences of 200 individuals sampled from two sites in the previously reported range, and nine sites in the newly reported localities. Two highly diverged lineages were found: a northern lineage, predominantly in Japan and Okinawa, and a southern lineage, primarily in Taiwan and Southeast Asia. The unimodal mismatch distribution and the star-like shaped haplotype network suggest recent demographic expansion in the southern lineage. The molecular data, therefore, rejected the southward extension hypothesis, and instead supported an alternative scenario of a northward range shift of the southern lineage from Southeast Asia to Taiwan. Our results reveal that range shifts in cryptic or poorly studied marine taxa may confound the identification of species' distributions and consequent interpretation of possible changes in ranges and sources of invasion. The application of molecular data to identify and monitor such changes can address these problems and permits a rapid and sensitive confirmation of such events.

KEY WORDS: Global warming · Intertidal · Biogeography · Cryptic species · Population genetics · Chthamalidae

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INTRODUCTION

The predicted increases in temperature and changes in environmental conditions of global climate change will impose new physiological stresses and selection regimes on organisms. The geographic distribution ranges of many species have shifted in association with increased temperature, with the poleward retreat of temperate species and range extension of more thermally tolerant, tropical species (Parmesan & Yohe 2003, Helmuth et al. 2006, Parme-

san 2006). Understanding and forecasting climate-driven changes in patterns of biodiversity is therefore becoming increasingly important for the conservation and management of biological communities (Hannah et al. 2002, Botkin et al. 2007). In the marine realm, rocky shores represent one of the harshest habitats, with strong selection pressure from both physical (e.g. heat stress, desiccation) and biological (e.g. competition, predation) factors (Somero 2002). Organisms inhabiting this system are subject to environmental changes from both aquatic and terrestrial

climatic realms (Thompson et al. 2002, Rivadeneira & Fernandez 2005, Helmuth et al. 2006, Hawkins et al. 2008, Jones et al. 2009, Somero 2010). In addition, rocky shores are well studied and relatively easy to access which make them ideal model ecosystems to detect early signals of biotic responses to climate change (Helmuth et al. 2006, Hawkins et al. 2008). Species inhabiting the high shore, such as barnacles in the family Chthamalidae, are good candidates for use in detecting such impacts as they are thought to be living close to their physiological limits and are expected to show a low capacity for further tolerance to thermal stress (Somero 2010).

The poleward range shift of intertidal organisms is widely acknowledged in American and European waters (reviewed in Helmuth et al. 2006, Hawkins et al. 2008, 2009), where comparatively comprehensive historic records of patterns of species abundance and distribution are available (reviewed in Hawkins et al. 2009). Such changes in distribution are predicted to have potentially large impacts on community structure and functioning (Grosholz 2005, Hawkins et al. 2009). Corresponding changes in the distribution of species in the Indo-West Pacific, however, remain poorly understood, despite the rich marine biodiversity in this region (Hughes et al. 2002, Tittensor et al. 2010). This lack of information is the result of inadequate alpha taxonomy, and also the poor documenta-

tion of records of species distribution in the region (Bouchet 2006).

Hexechamaesipho pilsbryi (Hiro 1936) (Cirripedia: Chthamalidae) is a high shore barnacle, first identified as *Chthamalus pilsbryi* by Hiro (1936) in Wakayama, Japan. In his extensive surveys of the distribution of barnacles in southern Japan and Taiwan, Hiro (1939) and Utinomi (1954) (Hiro and Utinomi are the same author, see Newman 1981) noted that *H. pilsbryi* was absent from Taiwan, reported the southern limit of *H. pilsbryi* as the Tokara Islands (southern waters of Kyushu) and hypothesized a faunal break between the northern Palaearctic and southern Oriental biogeographic zones (Utinomi 1954, see Fig. 1). Chan et al. (2008a), however, recently recorded *H. pilsbryi* in Taiwan and Okinawa, and suggested this may represent a possible southward range extension of this species. This hypothesis was based on the fact that the *H. pilsbryi* populations in Okinawa and Taiwan showed an overlap in their cohort patterns, but the population from Okinawa had a greater number of cohorts, indicating that individuals in this population were older, and therefore suggesting the younger population in Taiwan had originated from Okinawa (Chan et al. 2008a).

In recent ecological surveys, *Hexechamaesipho pilsbryi* has also been recorded at various sites in Southeast Asia (Fig. 1) where it can occupy ~30% of

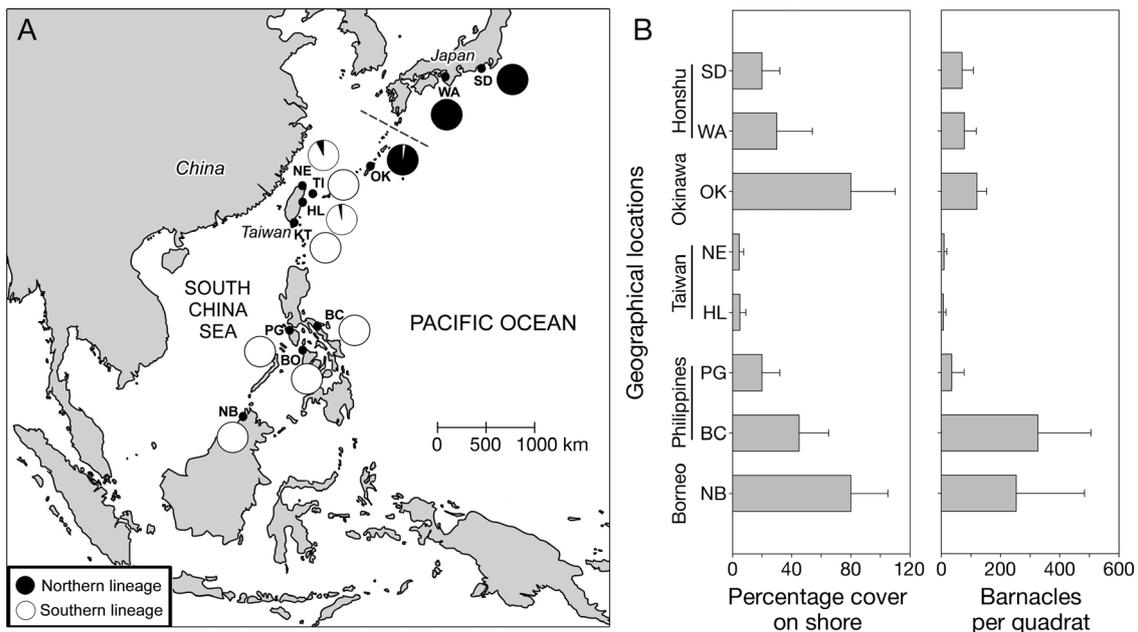


Fig. 1. *Hexechamaesipho pilsbryi*. (A) Sampling locations showing the relative abundance of the two COI lineages. See Table 1 for abbreviations of different sites and sample size analyzed. The dashed line represents the distribution range limit of *H. pilsbryi* suggested by Hiro (1939). (B) Variation in mean percentage cover and density (no. of barnacles per 15×15 cm² quadrat, \pm SD, n = 20) of *H. pilsbryi* in Japan, Taiwan and SE Asian localities. No quantitative quadrat samplings were conducted at the sites at BO, TI and KT

the rock surface on the high shore. The occurrence of this species in Maritime Southeast Asia questions the validity of the hypothesized southward range extension for this species, as it seems unlikely that a northern species can extensively colonize and survive in a more tropical environment (Somero 2010). An alternative hypothesis is that the individuals found in Southeast Asia are a long-standing native population rather than recent colonizers. These populations might not have been recorded previously due to the paucity of thorough ecological surveys in Southeast Asia and the confused alpha taxonomy of the chthamaliid barnacles (see Southward & Newman 2003). This, if correct, would have important implications in determining the origin of the population in Taiwan, and thus the direction of range shift (i.e. from the northern Japanese populations, or from the southern Asian populations). Moreover, this may also imply that species range changes in Southeast Asia may be common but remain undetected due to the poor quality of primary ecological/taxonomic data.

Molecular markers represent a powerful tool to investigate such issues as they take into account the cryptic diversity which can be overlooked by morphological studies and refine our knowledge about species diversity and species' distribution ranges (Carr et al. 2011, Hubert et al. 2012, Tsang et al. 2012a,b). More importantly, molecular markers can determine patterns of gene flow and identify source populations of invasive taxa (e.g. Zardus & Hadfield 2005, Geller et al. 2008, Tsang et al. 2008, Estoup & Guillemaud 2010). Here, we compare the sequences of the mitochondrial cytochrome *c* oxidase subunit I (COI) of samples collected from Japan, Taiwan and nine newly reported Southeast Asian locations of *Hexechamaesipho pilsbryi* to identify the origin of the newly re-

ported populations and distinguish between the two alternative hypotheses which could explain the present day distribution pattern of *H. pilsbryi*.

MATERIALS AND METHODS

Sample collection

Adult *Hexechamaesipho pilsbryi* individuals were collected from the high shore (>1.75 m above Chart Datum, C.D., see Chan et al. 2008a) at 11 locations in the west Pacific region from 2007 to 2009 (Table 1, Fig. 1). Sites in Honshu, Okinawa and Taiwan were the same as those in Chan et al. (2008a). Wakayama (WA) and Shimoda (SD), Honshu, are within the range of the species as reported by Hiro (1939). Individuals were also obtained from nine other localities where *H. pilsbryi* is newly recorded (Chan et al. 2008a, B. K. K. Chan unpubl. data). These include 1 site in Okinawa (OK), 4 sites in Taiwan and 4 sites in Southeast Asia (Table 1, Fig. 1). All samples were preserved in 95% ethanol prior to laboratory analyses.

Geographical variation in the abundance of *Hexechamaesipho pilsbryi*

Field surveys of the abundance of *Hexechamaesipho pilsbryi* followed Chan et al. (2008a). Data on the percentage cover of *H. pilsbryi* at Wakayama, Shimoda, Okinawa and Taiwan were extracted from Chan et al. (2008a), while field surveys in all other locations were conducted between 2009 and 2010. At all sampling locations, *H. pilsbryi* occurs at the high shore level. At each site, a 10 m stretch of shoreline

Table 1. *Hexechamaesipho pilsbryi*. Sample localities, abbreviations (Abbr.), sample sizes (n) and genetic diversity of COI, including number of haplotypes (Na), haplotype diversity ($h \pm SD$), nucleotide diversity ($\pi \pm SD$), Tajima's *D* and Fu's F_S for each population. p values: *p < 0.05, **p < 0.01, ***p < 0.001; na: not applicable

Population	Abbr.	n	Na	h	π	Tajima's <i>D</i>	Fu's F_S
Shimoda, Japan	SD	35	33	0.995 ± 0.009	0.0135 ± 0.0071	-1.38	-24.90***
Wakayama, Japan	WA	14	14	1.000 ± 0.027	0.0118 ± 0.0066	-1.31	-8.51**
Okinawa, Japan	OK	37	32	0.988 ± 0.011	0.0138 ± 0.0073	-1.69*	-22.04***
Turtle Island, Taiwan	TI	4	1	0.000 ± 0.000	0.0000 ± 0.0000	0	na
Da Xiang Lang, Taiwan	NE	26	13	0.757 ± 0.091	0.0101 ± 0.0055	-2.02**	-1.46
Shi Ti Ping, Taiwan	HL	30	22	0.947 ± 0.033	0.0072 ± 0.0041	-2.25**	-14.92***
Kenting, Taiwan	KT	14	8	0.824 ± 0.098	0.0032 ± 0.0022	-1.76*	-3.51**
Puerto Galera, Philippines	PG	3	3	1.000 ± 0.272	0.0055 ± 0.0048	0	-0.08
Tiwi, Bicol, Philippines	BC	20	15	0.921 ± 0.055	0.0029 ± 0.0020	-2.26**	-15.444***
Boracay, Philippines	BO	4	3	0.833 ± 0.222	0.0017 ± 0.0016	-0.71	-0.89*
Nexus Beach, Kota Kinnabalu, Sabah, Malaysia	NB	13	9	0.936 ± 0.051	0.0034 ± 0.0023	-1.48*	-5.21***

was chosen and 10 m transects were set up at four tidal levels (2.5, 2.25, 2.0 and 1.75 m above C.D.), which cover the whole vertical range of the species. At each level, 10 random 15 × 15 cm quadrats were photographed, from which the number of individuals and percentage cover of *H. pilsbryi* were subsequently scored.

Sequence data collection

Total genomic DNA was extracted from whole soft tissues of individual barnacles using the commercial QIAamp DNA Mini Kit (QIAGEN). Partial sequences of mitochondrial COI were amplified using the universal primers LCO1490 and HCO2198 (Folmer et al. 1994), or a newly designed forward primer Trp-F2 5'-TAA CCT ATA GCC TTC AAA G-3', binding to the conserved region of the tryptophan tRNA sequence flanking COI and HCO2198. Amplifications were conducted in a reaction mix containing 1 µl of 10× diluted template DNA, 1× PCR reaction buffer, 3 mM MgCl₂, 200 nM of each primer, 200 µM dNTPs, 1.5 units of *Taq* polymerase (TaKaRa) and ddH₂O to a total volume of 50 µl. The PCR profile was as follows: 3 min at 94°C for initial denaturation, then 35 cycles of 30 s at 94°C, 30 s at 47°C, 1 min at 72°C with a final extension for 3 min at 72°C. The PCR products were then purified using the Millipore Montage PCR96 Cleanup Kit. Sequences were generated using the forward primer (Trp-F2 or LCO1490) on an Applied Biosystems (ABI) 3700 automated sequencer using the ABI Big-dye Ready-Reaction mix kit, following the standard cycle sequencing protocol. Sequence quality was assessed by visualizing the chromatograms with ContigExpress in Vector NTI advance 8 (Invitrogen). Sequencing reactions using the reverse primer were also conducted when ambiguous bases were present in the chromatogram by using the forward primer.

Phylogenetic and population analysis

Nucleotide sequences were aligned using CLUSTAL W (Thompson et al. 1994) and alignments were confirmed by translating the sequences into amino acid sequences. jModeltest v.0.1 (Posada 2008) selected the Tamura & Nei (TrN) model with gamma-distributed rate heterogeneity ($\gamma = 0.328$) and the proportion of invariant sites estimated to be 0.629 as the best fit model of nucleotide substitution using the AIC criterion. These model parameters were applied

in subsequent analyses. Phylogenetic relationships between individuals were determined by neighbour-joining (NJ) based on analysis of TrN distance using PAUP* v.4.0b10 (Swofford 2002) with two *Chthamalus* species, *Chthamalus moro* and *C. challenger* (GenBank Accession nos. EU304363 and EU304447, respectively) as outgroups. The support for individual nodes was evaluated by 1000 bootstrap replicates. The genealogical relationships among haplotypes were inferred by haplotype network constructed using the 95% parsimony criterion as implemented in TCS v.1.21 (Clement et al. 2000).

Haplotype diversity (h) and nucleotide diversity (π) were calculated for each population and lineage using ARLEQUIN v.3.0 (Excoffier et al. 2005). Tajima's D test (Tajima 1989) and Fu's F_S test (Fu 1997) in ARLEQUIN were used to test for departure from mutation and drift equilibrium expected for a selectively neutral marker. Mismatch distribution analysis (Rogers & Harpending 1992) was conducted with the same software to explore any signal of demographic expansion. When a signature of rapid expansion (a unimodal distribution) was detected, we computed the parameter τ , which reflects the location of the mismatch distribution crest, to compare the approximate timing of population expansion among clades and populations (Rogers & Harpending 1992, Schneider & Excoffier 1999). We did not attempt to calculate the absolute timing of population expansion according to the formula $t = \tau/2\mu$ (where μ is the mutation rate per site) as it is widely acknowledged that evolutionary rate varies greatly across species. Therefore, adopting a divergence rate from another species would likely be erroneous, especially when the expansion happened over a very narrow time scale, as in the present study. Pairwise Φ_{ST} values were calculated to estimate the level of gene flow among populations, using the same mutation model and parameters as described above. We also attempted to test for a reduced gene flow among the different geographic regions using an analysis of molecular variance (AMOVA, Excoffier et al. 1992) in ARLEQUIN with 10 000 random permutations to test for statistical significance.

RESULTS

Abundance and population structure of *Hexechamaesipho pilsbryi*

In Shimoda and Wakayama, coverage of *Hexechamaesipho pilsbryi* was sparse (~20% surface cover,

Fig. 1B) on the high shore and was found above *Chthamalus challenger*. In Okinawa, *H. pilsbryi* was very common on the high shore, reaching 80% cover, and was occasionally sympatric with *Nesochthamalus intertextus*. Okinawa individuals were relatively larger (rostral-carinal basal diameter of individuals ~11 mm) than those from Honshu populations (~7–8 mm), and thus the number of individuals per quadrat in Okinawa was similar to those observed in the 2 Honshu populations although the Okinawa populations had a higher percentage cover. Rostral-carinal basal diameter of *H. pilsbryi* populations in Taiwan, Puerto Galera, Bicol and Sabah were similar, ranging from 8 to 9 mm. In Taiwan, coverage of *H. pilsbryi* was very sparse (<10% cover) and it co-existed with *Chthamalus malayensis* at the same tidal height. In Puerto Galera, Philippines, *H. pilsbryi* had ~25% cover on the shores, and co-existed with the highly abundant *C. malayensis* (>70% cover) on the high shore. The abundance of *H. pilsbryi* increased from Bicol, Philippines (40% cover) to a maximum in Sabah, Malaysia (up to 80% cover) where it formed a monospecific zone on the high shore at both sites (Fig. 1B).

Genetic divergence and diversity of *Hexechamaesipho pilsbryi* in the NW Pacific

The COI gene was sequenced from a total of 200 individuals of *Hexechamaesipho pilsbryi* (Table 1). As PCR products generated from the two primer sets varied in length, the dataset was trimmed to unify the sequence length and minimize the amount of missing data. The final aligned sequence dataset comprised 603 bp with 118 variable sites of which 67 were parsimony informative. 128 haplotypes were identified and their sequences were deposited in GenBank (Accession nos. KC896158–KC896285).

Neighbour-joining analysis revealed that *Hexechamaesipho pilsbryi* individuals were clustered into two strongly supported lineages, hereafter referred to as northern and southern lineages (Fig. 2). Individuals from the 2 lineages were differentiated by 4.7 to 6.7% COI sequence divergence with a net sequence divergence of 4.2% (between-lineage mean divergence subtracted by intra-lineage mean divergence). The 2 lineages exhibited little overlap in their geographic distribution (Fig. 1). The original reported range of *H. pilsbryi* (Honshu, Japan) was only occupied by the northern lineage, which was also dominant in Okinawa. Only 1 and 2 northern lineage individuals were found in eastern and northern Taiwan,

respectively (Fig. 1A). In contrast, individuals collected from the newly recorded locations belonged exclusively to the southern lineage, with the exception of the 3 Taiwan individuals mentioned above (Fig. 1). A single individual of the southern lineage was also recorded in Okinawa.

Genetic diversity was high for the northern lineage ($h = 0.993$, $\pi = 0.0127$) but was comparatively much lower in the southern lineage ($h = 0.869$, $\pi = 0.0037$; Table 2). Both of the 2 lineages exhibited significant negative D and F_S values, which indicate an excess of low-frequency haplotypes and can be attributed to selection and/or demographic expansion. The haplo-

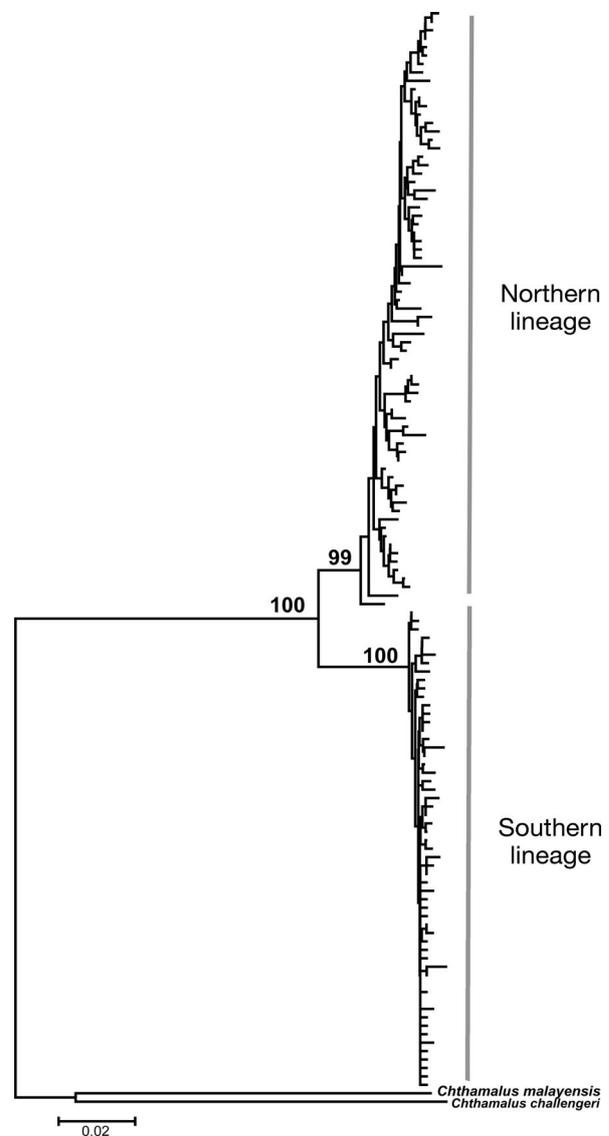


Fig. 2. *Hexechamaesipho pilsbryi*. Neighbour-joining tree of COI haplotypes. Percentage of bootstrap support is shown on the corresponding branch for all values ≥ 75 . The scale bar denotes substitution per site

Table 2. *Hexechamaesipho pilsbryi*. Genetic diversity of the 2 lineages. Number of haplotypes, haplotype diversity ($h \pm SD$), nucleotide diversity ($\pi \pm SD$), Tajima's D , and Fu's F_S are shown. p values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

	N	Na	h	π	Tajima's D	Fu's F_S
Northern lineage	88	72	0.993 ± 0.003	0.0127 ± 0.0066	-1.76*	-24.88***
Southern lineage	112	56	0.869 ± 0.031	0.0037 ± 0.0023	-2.52***	-27.15***
Taiwan populations	72	37	0.828 ± 0.047	0.0048 ± 0.0028	-2.60***	-26.56***
Southeast Asian populations	40	25	0.933 ± 0.027	0.0038 ± 0.0024	-2.28***	-25.82***

type network for the northern lineage revealed no apparent clustering of haplotypes with respect to locations and most haplotypes were only represented by a single individual (Fig. 3, Table A1 in Appendix 1). In contrast, the southern lineage displayed a star-like shaped haplotype dominated by a major haplotype (40 out of 112 individuals, ~36%) which was found in all southern lineage populations except one (Bicol, Philippines, which had only 4 sequenced individuals) (Fig. 3, Table A2 in Appendix 1). A majority of the other haplotypes in the southern lineage differed by 1 to 3 mutations compared to the dominant haplotype (Fig. 3). Furthermore, most of the haplotypes which occurred in multiple individuals were shared among the Taiwan and Southeast Asian populations. The mismatch distribution analyses revealed unimodal distribution for the 2 lineages

(Fig. 4), indicating a rapid demographic expansion. These observations were consistent with the significant and negative values from Tajima's and Fu's tests. However, the values of τ suggested more recent expansion in the southern ($\tau = 1.889$) than in the northern lineage ($\tau = 7.984$), corroborating the star-like shaped haplotype network observed in the former and in contrast to the lack of obvious pattern seen in the haplotype network of the latter. Within the southern lineage, the populations in Taiwan were inferred to have experienced a more recent demographic growth compared to the Southeast Asian populations ($\tau = 1.316$ vs. 2.387, Fig. 4C, D). The shorter evolutionary history of the populations in Taiwan resulted in lower haplotype diversity ($h = 0.828$) than the samples in Southeast Asia ($h = 0.933$).

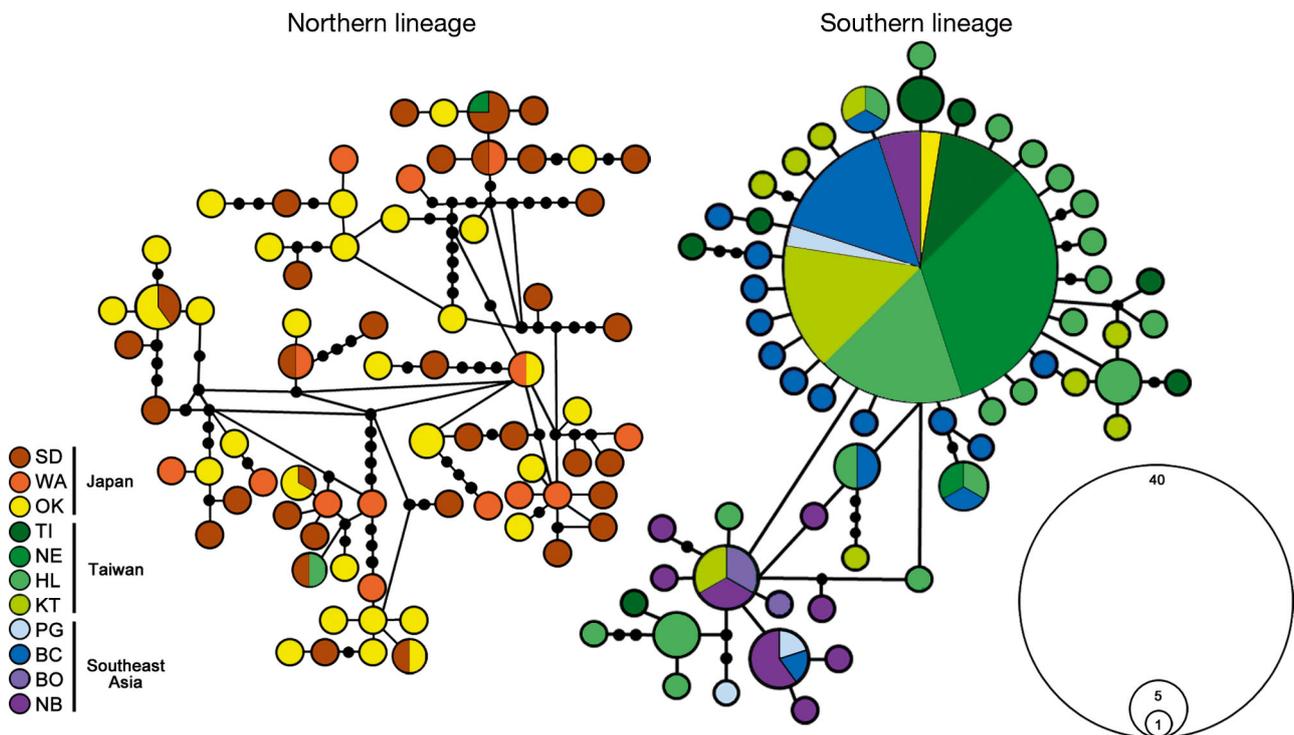


Fig. 3. *Hexechamaesipho pilsbryi*. COI haplotype network. Nodes along each branch designate the number of base pair differences among haplotypes. COI haplotypes are represented proportional to their frequency. See Table 1 for abbreviations of sampling localities

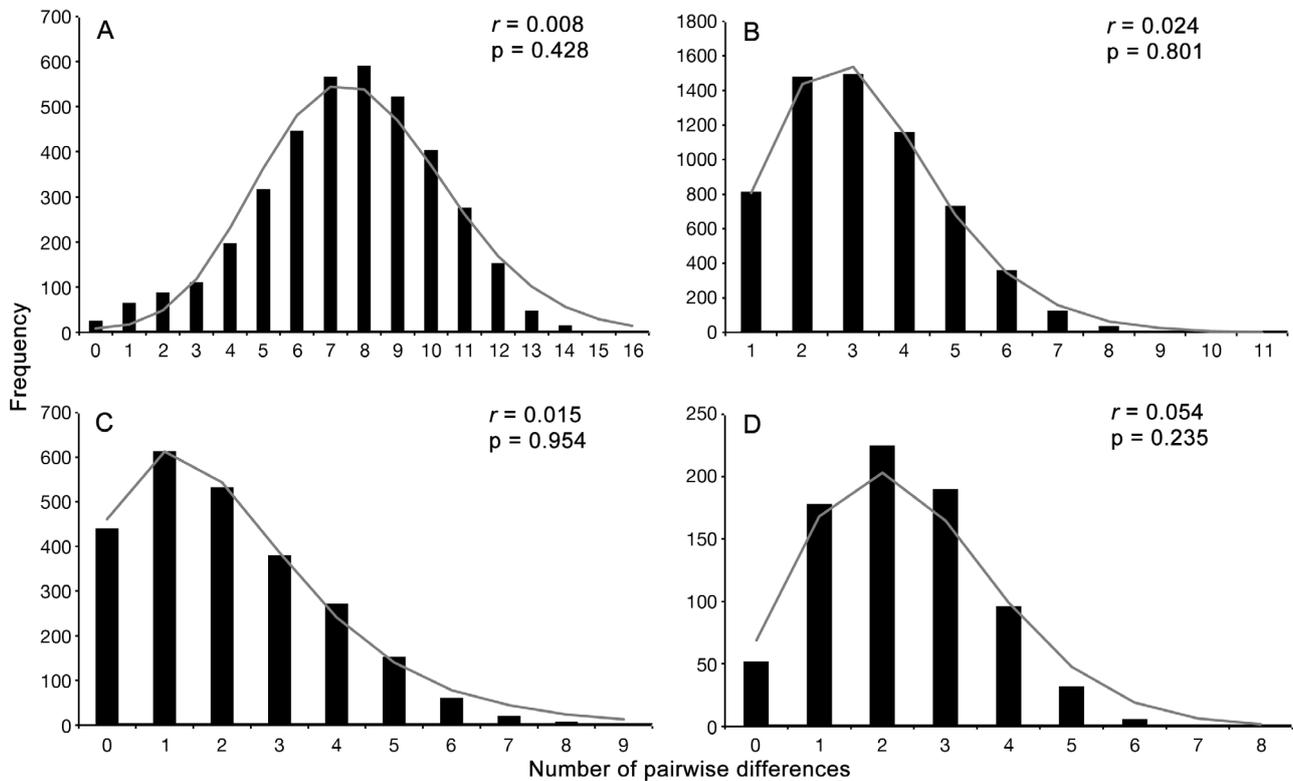


Fig. 4. *Hexechamaesipho pilsbryi*. The observed mismatch distributions (bars) and the expected mismatch distributions under the model of sudden demographic expansion (solid line) for the COI haplotypes in (A) the northern lineage, (B) the southern lineage, (C) Taiwanese populations of the southern lineage, and (D) Southeast Asian populations of the southern lineage. Harpending's raggedness index (r) and associated p -values are shown for each lineage and population group

Little population genetic structuring was observed in the northern lineage (mostly non-significant pairwise Φ_{ST} values within this lineage, Table 3), with the only significant difference occurring between Shimoda and Okinawa in Japan ($p = 0.023$). On the other hand, a number of pairwise Φ_{ST} comparisons showed significant differences between populations of the southern lineage, especially when comparing the

Malaysian site and Bicol, Philippines to the other sampling locations (Table 3). However, the generality of these results may be limited by the relatively low number of individuals analyzed for most of the Southeast Asian populations. To attain a larger sample size for AMOVA analysis, samples of the southern lineage were divided into 3 groups by combining populations according to both geographic proximity

Table 3. *Hexechamaesipho pilsbryi*. Pairwise Φ_{ST} values between populations based on COI sequences. p values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. See Table 1 for abbreviations of sampling localities

	SD	WA	OK	TI	NE	HL	KT	PG	BC	BO
WA	-0.00932	-								
OK	0.02343*	-0.00083	-							
TI	0.79702***	0.83613***	0.77089***	-						
NE	0.77242***	0.78934***	0.74807***	-0.11439	-					
HL	0.80768***	0.83563***	0.78491***	-0.10704	-0.01516	-				
KT	0.82***	0.86641***	0.79603***	-0.09973	-0.00823	-0.01097	-			
PG	0.77498***	0.80394**	0.74575***	0.27287	-0.06076	-0.04173	0.14189	-		
BC	0.83569***	0.88345**	0.81361***	-0.12724	0.01018	0.01726	0.0253*	0.23535*	-	
BO	0.78677***	0.82339***	0.75823***	0.66759*	0.03532	0.06276	0.2807**	-0.02768	0.36068***	-
NB	0.81116***	0.85689***	0.78515***	0.28519*	0.09959*	0.11207**	0.23896***	-0.01691	0.31423***	-0.08818

as well as the estimated Φ_{ST} value among populations: the Taiwan group, the Philippines group, and the Malaysia group. None of the comparisons among the 3 regions showed significant Φ_{CT} values, which summarized the degree of genetic differentiation among geographic regions (i.e. group of populations), and most of the variation identified was contributed by within-population variation (data not shown). The populations in the Philippines and Malaysia were also combined as a Southeast Asian group to compare with the Taiwan samples, but again no significant structuring was detected.

DISCUSSION

Origin of the newly recorded populations

Using the mitochondrial COI gene sequences, we attempted to trace the origin of the newly recorded *Hexechamaesipho pilsbryi* in Taiwan and Southeast Asia. Surprisingly, we found pronounced phylogenetic structure over the range of this species. The samples from Taiwan and Southeast Asia are highly differentiated from the 'native' populations in Japan. The genetic data clearly reject the hypothesis of a southward range extension of *H. pilsbryi* from Japan to Taiwan, but provide strong evidence of the shared ancestry of the Taiwan and Southeast Asian populations. This result therefore suggests the occurrence of a long-standing stock of a southern *H. pilsbryi* lineage in Southeast Asia which has not been previously reported.

In a detailed survey of barnacle diversity in Taiwan (covering locations in Taiwan and its outlying islands including Lanyu, Green Island and Panghu), Hiro (1939) recorded 30 species — of which two were new to science — but did not record *Hexechamaesipho pilsbryi*. Since Hiro was the authority on *H. pilsbryi*, it is highly unlikely that he missed this species in Taiwan, especially given its considerable present-day abundance (~5–10% coverage, Chan et al. 2008a). One possible explanation is that the species only occurred in very low abundance, and so was overlooked by Hiro in the 1930s, and has only experienced recent demographic growth during the last few decades. However, we believe a more plausible explanation is that the individuals found in Taiwan represent recent colonizers from the south, based on the large number of shared haplotypes and high genetic similarity observed between the Taiwanese and Southeast Asian populations. In comparison with Japan and Taiwan, ecological and taxonomic studies

in Southeast Asia are limited (see Bouchet et al. 2009). The alpha taxonomy of barnacles, and indeed the occurrence of many species in the region, is poorly known (Southward & Newman 2003). Moreover, the morphology of chthamalid species is highly variable and many mis-identifications have been reported, even for common species (see the case of *C. malayensis* in Southward & Newman 2003, Tsang et al. 2012b). *H. pilsbryi* was only described relatively recently (in 1939) and so it is possible that previously this species was mis-identified as another chthamalid species in Southeast Asia. Rosell (1972), for example, conducted a detailed barnacle survey in Puerto Galera, Philippines, and reported the occurrence of a temperate European species, *Chthamalus stellatus*. We have failed to identify any *C. stellatus* in several surveys in Southeast Asia, and Rosell's description and illustrations of the cirri of '*C. stellatus*' show features similar to the diagnostic characteristics of *Hexechamaesipho pilsbryi* (Chan et al. 2008a), suggesting that the record of '*C. stellatus*' in the Philippines probably represents *H. pilsbryi*.

The southern lineage of *Hexechamaesipho pilsbryi* may therefore be native but not formally recorded in Southeast Asia, and it may have recently spread northwards and established a new population in Taiwan. The genetic and ecological data support this hypothesis. Reconstruction of the species' demographic history clearly reveals population growth in the southern lineage of *H. pilsbryi*, with the expansion of populations in Taiwan inferred to be more recent. Moreover, the samples from the 2 regions shared most of the common haplotypes, suggesting common ancestry and a considerable level of connectivity. Hence, *H. pilsbryi* in Southeast Asia is a long-standing population while the individuals in Taiwan most likely represent founders associated with the recent establishment of the population there. This proposal is also supported by our observation that although *H. pilsbryi* can be found along the entire northeast and east coastline of Taiwan, its coverage is the lowest among all the sampling locations (~10% mean cover in Taiwan vs. >30% in Southeast Asia and Japan, Chan et al. 2008a) and its abundance increases gradually from Puerto Galera to Borneo. Hence, both the genetic and demographic evidence supports a recent colonization of Taiwan by *H. pilsbryi*.

Assuming that *Hexechamaesipho pilsbryi* has only recently settled in Taiwan from Southeast Asia, it is interesting to speculate on its route of invasion. There are 2 clear possibilities: a northward range extension driven by ocean currents and/or environmental factors; or transport through anthropogenic

activities, such as shipping. *H. pilsbryi* is a natural rocky shore inhabitant, and is seldom reported as fouling ship hulls (Otani et al. 2007) or other man-made structures such as piers. Moreover, if the species was transported to Taiwan by anthropogenic activities, we would expect the species to be also transported elsewhere as reported for most alien barnacle species, e.g. *Chthamalus proteus* (Zardus & Hadfield 2005) and *Balanus glandula* (Geller et al. 2008). Yet, to date there is no record of this species outside our study region. Therefore, we believe a northward range extension driven by the Kuroshio Current, which originates from the North Equatorial Current and bifurcates at the west of the Philippines (Nitani 1972, Toole et al. 1990) is the most plausible explanation. The strong Kuroshio Current accounts for the high connectivity of a wide variety of marine fauna across latitudes from Southeast Asia to Taiwan and Japan (Mann & Lazier 2006). Larvae of southern *H. pilsbryi* could have been transported north by the Kuroshio Current, resulting in settlement and survival in Taiwan as environmental conditions became more favourable for this lineage as a result of recent increases in temperature (see below).

Further north, we also recorded *Hexechamaesipho pilsbryi* in Okinawa, whereas Utinomi (1949, 1954) stated that *H. pilsbryi* was absent from this area. Yet Utinomi's (1949) personal collections were only from Kyushu and its adjacent islands, and most of the specimens he examined from other locations were museum collections, suggesting the Okinawa barnacle fauna was not thoroughly examined by Utinomi himself. Utinomi (1954) also recorded the presence of some barnacle species in Okinawa and Ishigaki Jima as 'uncertain', probably because he had not examined the Okinawa fauna in detail. Therefore, we cannot confirm whether *H. pilsbryi* was already present in Okinawa during the 1940–50s. In any case, our molecular data show that *H. pilsbryi* in Okinawa are affiliated with those in Japan and thus, unlike the population in Taiwan, the populations in Okinawa do not result from a range shift of *H. pilsbryi* from Southeast Asia. It is most likely that the Okinawa individuals represents an unreported long-standing population rather than a southern range extension.

Northward range extension and its potential consequences

Elevated sea surface temperature and shifts in ocean circulation regimes are known to have altered the dispersal pattern of coastal marine taxa (Munday

et al. 2009, Banks et al. 2010). For instance, the blue mussel *Mytilus edulis* has recently been recorded to have traversed an historic oceanographic barrier in the Arctic Ocean, extending its distribution from the Norwegian mainland by 1000 km north to the Svalbard archipelago since 1977 (Weslawski et al. 1997, Berge et al. 2005). This poleward extension of the mussel's northern distribution limit is suggested to have arisen due to the enhanced dispersal of planktonic larvae from a source population on the Norwegian mainland arising from an anomalously large northward transport of warm Atlantic water to the Greenland Sea in recent years (Berge et al. 2005).

In Taiwan, air temperature data from the past 100 yr show that the mean lowest annual temperature in east Taiwan (Tai Tung) has increased from 19°C to 20.5°C between 1901 and 1960 (Lay 2004). Between 1960 and 2000, the lowest mean annual air temperature showed a further increase from 20.5°C to 22°C, and there have been significantly fewer cold days (<10°C) in the past 50 yr (Central Weather Bureau, Taiwan, ROC). The data indicate that Taiwan is becoming warmer and has had longer periods of high temperatures (i.e. more days with temperatures >30°C yr⁻¹, Fig. 5) in the past 50 yr. The present-day occurrence of *Hexechamaesipho pilsbryi* in northeast and east Taiwan therefore probably results from the northward expansion of the southern lineage from Southeast Asia, due to successful colonization of the more suitable (i.e. warmer) local habitats in Taiwan over several decades.

Whether newly established populations continue to increase in size and out-compete native species depends on the adaptability of the alien species, the accommodation of the native species, and the community structure of the invaded region (Briggs 2010). Increases in environmental temperature may negatively affect more cold-water adapted species, and hence play a role in mediating competition between barnacle species (e.g. Wethey 1983, Southward & Crisp 1954, 1956) and determining the abundance and range of species (Wethey 1983, 2002). Similar to other species in the family Chthamalidae, *Hexechamaesipho pilsbryi* is a high shore resident, and these species tend to exhibit distinct geographic distributions in the West Pacific (Southward & Newman 2003). *Chthamalus challengerii*, for example, is common in the temperate waters of Japan and northern China; *Chthamalus malayensis* is dominant in the warmer regions along the coast of Taiwan and southern China while *Chthamalus moro* is largely confined to the outer island regions, such as the Philippines, Okinawa and Taiwan. The distinct ranges of

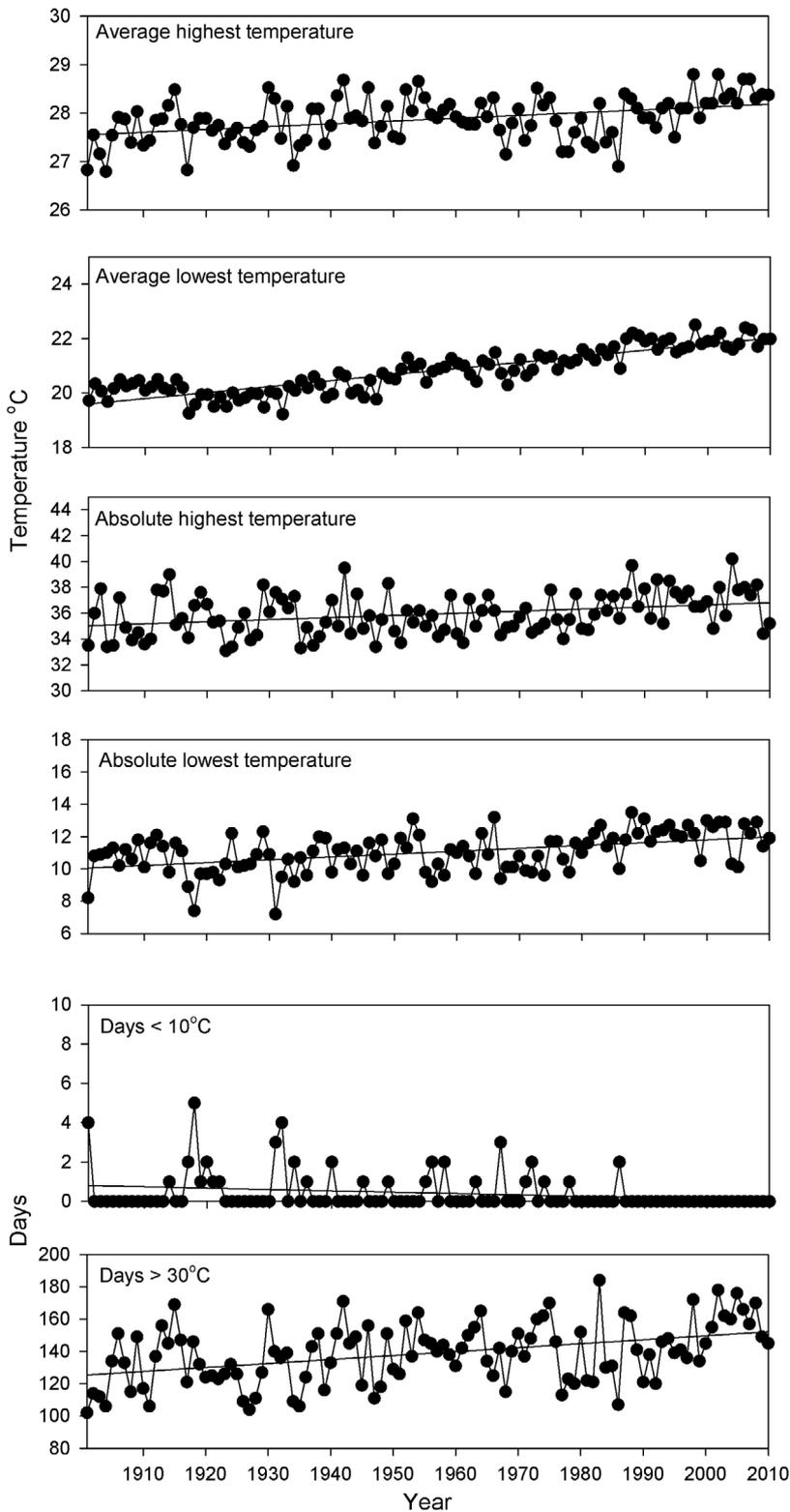


Fig. 5. Variation in climatic conditions (average annual highest temperature, average annual lowest temperature, absolute highest temperature, absolute lowest temperature, number of days $<10^{\circ}\text{C}$ yr^{-1} and number of days $>30^{\circ}\text{C}$ yr^{-1} for the past 100 yr in Taitung ($121^{\circ}08'48''\text{E}$, $22^{\circ}45'15''\text{N}$), on the eastern coast of Taiwan. Data supplied by the Central Weather Bureau, Taiwan, ROC

these species are probably due to local adaptation to different environmental regimes and independent larval supply pools (Southward & Newman 2003, Chan et al. 2008b). In Taiwan, *H. pilsbryi* occurs at the same tidal height as *C. malayensis* and *C. moro* and may compete with these native species.

Implications for ecological monitoring studies

Climate change and anthropogenic activities are both contributing to the global transport of alien species which may threaten the survival of native biota (Sala et al. 2000, Clavero & García-Berthou 2005). The biological consequences of these threats at the community level however remain largely unknown (Folke et al. 2004, Grosholz 2005, Hawkins et al. 2009). Monitoring and management of changes in species ranges are therefore becoming of increasing interest and importance (reviewed in Heller & Zavaleta 2009). In order to achieve early detection of any changes in species range, baseline information on the distribution and relative abundance of the local fauna and flora across a wide temporal and spatial spectrum is an important prerequisite. However, this kind of data is often deficient and highly biased both in terms of geographic region and taxonomic coverage (reviewed in Ruiz et al. 2000, Bouchet 2006). Such information is reasonably comprehensive in western countries where ecological research has a long history (e.g. England has records of local intertidal rocky shore organisms dating back to the 1950s, Hawkins et al. 2009). In other parts of the world, such as the Indo-West Pacific region, such studies are limited, making any comparisons with baseline information difficult or even impossible (see Bouchet et al. 2009).

In the present study, we found that the Taiwanese population of *Hexechamaesipho pilsbryi* seems to emanate

from the northward colonization of a previously unreported, long-standing population in Southeast Asia rather than a southward range extension from Japan. The northward colonization is likely due to local changes in environmental conditions involving an increase in local temperature, as has been observed elsewhere (e.g. Helmuth et al. 2006, Hawkins et al. 2009). This unexpected finding is largely attributed to the lack of reliable biodiversity surveys and alpha taxonomic studies in Southeast Asia. This situation is gradually improving, and recent more comprehensive surveys have refined our understanding of species distribution patterns (e.g. Bouchet et al. 2009, de Forges et al. 2009) as well as 'discovering' many new species even in widespread and common taxa, including economically important species such as *Penaeus japonicus* (Tsoi et al. 2005) and *Portunus pelagicus* (Lai et al. 2010). These findings not only confirm the extraordinary marine biodiversity in the Indo-West Pacific region, but also highlight the need for integrated studies involving morphological, ecological and molecular approaches to help identify biogeographic patterns. The high shore, chthamalid intertidal acorn barnacles demonstrate a high degree of morphological variation that has led to confusion and errors in their field identification (see Southward & Newman 2003, Tsang et al. 2012b). Adopting an integrated approach to the study of these barnacles (i.e. genetic, morphological, and ecological responses) allows us to identify the scales at which climate change may impact natural populations and communities.

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Table A2. *Hexechamaesipho pilsbryi*. Haplotype distribution among populations of the southern clade

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25
OK	1																								
TI	4																								
NE	13	1	1	1	1	1	1	1	1	2	1														
HL	7			1											1				1	1	1	1	1	1	2
KT	6											1	1	1	1	2	1	1							
PG	1																								
BC	6			1											1										
BO																	2								
NB	2															2									

	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47	S48	S49	S50
OK																									
TI																									
NE																									
HL	2	1	1	1	1	1	1	1	1	1	1														
KT																									
PG											1									1					
BC			1																						
BO												1										1	1	1	1
NB												3	1	1	1	1	1	1		1					

	S51	S52	S53	S54	S55	S56
OK						
TI						
NE						
HL						
KT						
PG						
BC	1	1	1	1	1	1
BO						
NB						

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