Environmental influences on the Indo-Pacific octocoral *Isis hippuris* Linnaeus 1758 (Alcyonacea: Isididae): genetic fixation or phenotypic plasticity?

Sonia J Rowley, Xavier Pochon, Les Watling

As conspicuous modular components of benthic marine habitats, gorgonian (sea fan) octocorals have perplexed taxonomists for centuries through their shear diversity, particularly throughout the Indo-Pacific. Phenotypic incongruence within and between seemingly unitary lineages across contrasting environments can provide the raw material to investigate processes of disruptive selection. Two distinct phenotypes of the Isidid *Isis hippuris* Linnaeus 1758 partition between differing reef environments: long-branched bushy colonies on degraded reefs, and short-branched multi/planar colonies on healthy reefs within the Wakatobi Marine National Park (WMNP), Indonesia. Multivariate analyses reveal phenotypic traits between morphotypes were likely integrated primarily at the colony level with increased polyp density and consistently smaller sclerite dimensions at the degraded site. Sediment load and turbidity, hence light availability, primarily influenced phenotypic differences between the two sites. This distinct morphological dissimilarity between the two sites is a reliable indicator of reef health; selection primarily acting on colony morphology, porosity through branching structure, as well as sclerite diversity and size. ITS2 sequence and predicted RNA secondary structure further revealed intraspecific variation between *I. hippuris* morphotypes relative to such environments (Φ_{ST} = 0.7683, P <0.001). This evidence suggests – but does not confirm - that *I. hippuris* morphotypes within the WMNP are two separate species, however to what extent and taxonomic assignment requires further investigation across its full geographic distribution. Incongruence between colonies present in the WMNP with tenuously described *Isis* alternatives (*Isis reticulata* Nutting 1910, *Isis minorbrachyblasta* Zou, Huang and Wang 1991), questions the validity of such assignments. Furthermore, phylogenetic analyses confirm early taxonomic suggestion that the characteristic jointed axis of the Isididae is in fact a convergent trait. Thus the polyphyletic nature of the Isididae lies in its type species *I. hippuris,* being unrelated to the rest of its family members.

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Abstract As conspicuous modular components of benthic marine habitats, gorgonian (sea fan) octocorals have perplexed taxonomists for centuries through their shear diversity, particularly throughout the Indo-Pacific. Phenotypic incongruence within and between seemingly unitary lineages across contrasting environments can provide the raw material to investigate processes of disruptive selection. Two distinct phenotypes of the Isidid *Isis hippuris* Linnaeus, 1758 partition between differing reef environments: long-branched bushy colonies on degraded reefs, and short-branched multi/planar colonies on healthy reefs within the Wakatobi Marine National Park (WMNP), Indonesia. Multivariate analyses reveal phenotypic traits between morphotypes were likely integrated primarily at the colony level with increased polyp density and consistently smaller sclerite dimensions at the degraded site. Sediment load and turbidity, hence light availability, primarily influenced phenotypic differences between the two sites. This distinct morphological dissimilarity between the two sites is a reliable indicator of reef health; selection primarily acting on colony morphology, porosity through branching structure, as well as sclerite diversity and size. ITS2 sequence and predicted RNA secondary structure further revealed intraspecific variation between *I. hippuris* morphotypes relative to such environments (Φ_{ST} = 0.7683, P <0.001). This evidence suggests – but does not confirm - that *I. hippuris* morphotypes within the WMNP are two separate species, however to what extent and taxonomic assignment requires further investigation across its full geographic distribution. Incongruence between colonies present in the WMNP with tenuously described *Isis* alternatives (*Isis reticulata* Nutting, 1910; *Isis minorbrachyblasta* Zou, Huang & Wang, 1991), questions the validity of such assignments. Furthermore, phylogenetic analyses confirm early taxonomic suggestion that the characteristic jointed axis of the Isididae is in fact a convergent trait. Thus the polyphyletic nature of the Isididae lies in its type species *I. hippuris,* being unrelated to the rest of its family members.

Introduction

Reef biodiversity reflects that of its environment, those within the Indo-Pacific Coral Triangle the most diverse of all. Intense competition in such environments may lead to niche partitioning through resource acquisition, leading to ecological divergence. Such diversification may occur with or without extrinsic barriers to gene flow and is particularly marked in sessile modular organisms such as cnidarians, far from passive to their environment (Cossins et al., 2006) with respect to growth form and biochemical composition. However, delimitation between closely related species across steep environmental gradients on coral reefs may be confounded by phenotypic plasticity, homoplasy, cryptic or sibling taxa (Knowlton, 1993). It is, therefore, necessary to define environmentally driven divergent mechanisms on select phenotypic traits to accurately assess species biodiversity and endorse effective conservation management strategies (Ladner & Palumbi, 2012).

Gorgonian corals (Cnidaria: Anthozoa: Octocorallia) are conspicuous modular organisms, the greatest diversity occurring within the Indo-Pacific coral triangle yet remarkably 'poorly known' (van Ofwegen, 2004). Intra- and inter-specific morphological variability in gorgonians is influenced by environmental factors such as light, temperature, sedimentation and flow rates. However, little is known about the responses of gorgonian taxa to environmental parameters within the Coral Triangle. Distinct morphotypes of the isidid gorgonian *Isis hippuris* LINNAEUS 1758 exist between healthy (Ridge 1) and degraded (Sampela) reefs within the Wakatobi Marine National Park (WMNP), SE Sulawesi, Indonesia; short-branched multi/planar colonies, and long-branched bushy colonies, respectively (Rowley, 2014; Rowley & Watling, in press). Whether such morphological differentiation is a consequence of a capacity to be plastic, plasticity as an adaptation (Hoogenboom et al., 2008), or has become genetically fixed leading to two species, is unclear.

Isis hippuris within the WMNP may represent 'robust' canalisation where morphotypes are in fact two previously diverged species through disruptive (in sympatry) selection on traits between environments (Schluter, 2001). Alternatively, physiological developmental constraints may have become decanalised through an acute perturbation (e.g., Bergman & Siegel, 2003). In the first scenario, existence in low water velocity, high turbid environments typical of lagoon, semi-lagoon or sea-grass beds, as seen in Sampela, gave rise to an accumulation of pre- or postzygotic isolation between populations leading to separate adaptive fitness peaks representing ecological niches of long standing. Divergent morphotypes would therefore be robust to environmental change, maintaining native phenotypic traits. In the second scenario, cryptic variation more likely to be adaptive than random mutations, facilitate rapid mutation, as can acute perturbations (Flatt, 2005). Either case can be accelerated by pleiotropy, linkage disequilibrium or concerted evolution (Sánchez & Lasker, 2003) leading to population level genetic assimilation of a particular phenotype and thus provides a testable level of emergent trait integration (i.e., characters behaving as a unit). Moreover, phenotypic variation can be largely attributed to rapid evolutionary events (Eldredge & Gould, 1972; Simpson, 2013).

The phenomenon of species delimitation in closely related modular organisms can be investigated through models of integration (Magwene, 2001). Growth persists through the iterative addition of modules (e.g., polyps, branching properties), which may develop independently or in concert (by trait integration, see Magwene, 2001; Sánchez & Lasker, 2003; Sánchez et al., 2007) leading to differential integration in response to environmental perturbations. The co- and multi-variance of certain phenotypic traits may differ due to inextricably linked developmental (e.g., heterochrony; Sánchez, 2004) or functional integration leading to patterns of diversity through plasticity or divergent selection directly (extrinsic) or indirectly (intrinsic) on traits between populations or subpopulations (Schluter, 2001). By measuring five morphological traits in twenty-one Caribbean gorgonian species, Sánchez &

Lasker (2003) revealed integration within both branching and polyp dynamics yet were independent of each other. Furthermore, colony form and growth via branching were interconnected through the ratio of 'mother' branches to 'daughter' branches (Sánchez & Lasker, 2004). Whether this is replicated across all gorgonians i.e. from different regions and habitats, is unclear, however species-specific trait integration, particularly in response to environmental change, has been shown in other taxonomic groups (e.g., plants; Xu, Schooler & Van Llinken, 2012).

Isis hippuris may simply possess high capacity for plasticity, itself an adaptation facilitating considerable physiological tolerance to environmental heterogeneity, not uncommon in gorgonians (West, Harvell & Walls, 1993; West, 1997; Skoufas, 2006). Long-branched bushy, porous colonies reduce sediment settlement and maximise light capture through increased surface area and decreased self-shading in reduced light and water flow environments as seen in the scleractinian *Stylophora pistilata* Esper, 1797 (Shaish, Abelson & Rinkevich, 2006). Whereas the densely packed short branches of planar colonies in high light and water flow, coupled with greater densities of small micro-skeletal elements (sclerites) provide mechanical strength (West et al., 1993; Kim et al., 2004; but see Skoufas, 2006). Sclerites are key characters for species delineation within Octocorallia, those of the coenenchyme (soft tissue or 'rind') surface and polyps being most susceptible to environmental variation (Bayer & Stefani, 1987). Reduced polyp density with depth as a function of light in zooxanthellate taxa (West et al., 1993; Kim et al., 2004; Prada, Schizas & Yoshioka, 2008; Prada & Hellberg, 2013) increases photosynthetic gain through surface area, yet polyp dimensions decouple integration by remaining independent of branching dynamics in Caribbean taxa (Sánchez & Lasker, 2003; Sánchez et al., 2004; 2007). A broad trait assessment including genetic analyses would therefore provide further insight into the relationship between *I. hippuris* and its environment. Are such trait patterns commensurate with those described in other gorgonians, and thus fixed within the

phylogenetic group (e.g., Lasker & Sánchez, 2003; Sánchez, 2004; Sánchez et al., 2007)?

A single representative of its genus, *I. hippuris* is taxonomically problematic (Watling et al. 2012), having a recognised plasticity (Wright & Studer, 1889; Simpson, 1906; Thomson & Simpson, 1909; Bayer & Stefani, 1987; Fabricius & Alderslade, 2001), which may obscure any possible species boundaries. In order to fully elucidate the nature of *I. hippuris* phenotypic variation among reef sites, a brief taxonomic and historical account is presented (see also Supporting Information) with subsequent investigation into potential adherence to previously documented lower taxonomic assignments.

The family Isididae Lamouroux, 1812 [nom. correct. Kükenthal, 1915 (pro Isidae Lamouroux, 1812)], currently placed within the sub-Order Calcaxonia, is characterised by a unique axis of alternating calcareous internodes and proteinaceous (gorgonin) nodes giving a bamboo appearance. Calcareous internodes can be hollow or solid and are not sclerobastic (i.e., consisting of fused sclerites *sensu* the sub-group Scleraxonia). The Isididae was further subdivided into four currently accepted subfamilies (see Alderslade, 1998; under revision, Watling et al., in prep.) based primarily on polyp retractability and sclerite composition and arrangement. Pertinent to this study, the sub-family Isidinae Lamouroux, 1812 (*sensu* Studer, 1887) is distinguished by small, warty and usually irregular sclerite forms and contains the genera *Isis* Linnaeus, 1758 and *Chelidonisis* Studer, 1890. Within the *Isis* genus 20 species have been assigned with currently only *I. hippuris* Linnaeus, 1758, the type species for the Isididae, Isidinae and genus *Isis* being widely accepted (Bayer & Stefani, 1987; Fabricius & Alderslade, 2001). *I. reticulata* Nutting, 1910 and *I. minorbrachyblasta* Zou, Huang & Wang, 1991 have occasional reference but with taxonomic misgivings (see Bayer & Stefani, 1987; but also Mai-Bao-Thu & Domantay, 1971). Diagnostic traits are, therefore, summarised here (see also Supplementary Material) to compare with those found within the WMNP.

Primarily, *I. hippuris* colonies are arborescent, planar or bushy with varying branch length and diameter, which can be swollen at the tip. Polyps are distributed all around the branches. A diversity of sclerites within the coenenchyme includes small warty clubs and spindles, asymmetric capstans, doubleheads and occasional crosses (e.g., Bayer & Stefani, 1987). Colonies of *I. reticulata* are slender, planar, with long slim branches, and asymmetrically distributed polyps. Sclerites are generally smaller, and their warts, when present, are arranged symmetrically (e.g., Nutting, 1910). *I. minorbrachyblasta* colonies are bushy with densely aggregated short, fine branches bearing equally distributed polyps. Sclerites are also variable in form and articulation. In sum, diagnostic phenotypic traits tend mostly to place *I. minorbrachyblasta* as an intermediate between *I. hippuris* and *I. reticulata*.

Isis *within the Wakatobi - Isis* morphotypes found within the WMNP bear only partial adherence to those previously described (see Supplementary Material) at the colony level. The long-branched bushy colonies on degraded reefs, and short-branched multi/planar colonies on healthy reefs may represent *I. reticulata* and *I. hippuris* respectively, or simply the widely accepted plasticity of the latter (Wright & Studer, 1889; Simpson 1906; Thomson & Simpson, 1909; Bayer & Stefani, 1987; Fabricius & Alderslade, 2001), likely through an integration effect (Magwene, 2001; Sánchez & Lasker, 2003).

Clearly *Isis* taxonomy is in a state of flux, compromising conservation efforts due to difficulties in species assignment. A thorough examination of *Isis* specimens throughout its distribution is both necessary and underway, yet, outside of the scope of this study. Therefore, given the tenuous nature of previously described *Isis* species bar *I. hippuris*, from here on in all speciments will remain assigned to *I. hippuris*, unless specified otherwise.

Here an assessment of morphotypes found within the WMNP relative to reef health is presented, with brief comparisons to those previously described. Firstly we ask: are populations of *I. hippuris*

morphotypes phenotypically and genetically subdivided due to contrasting reef environments within the WMNP, Indonesia? Secondly, do the *I. hippuris* morphotypes represent previously described species, or a single species with highly variant, integrated phenotypic traits? Therefore, this study aims to: (1) investigate morphological variability in the zooxanthellate gorgonian *I. hippuris* between contrasting coral reef environments within the WMNP, SE Sulawesi, Indonesia; (2) identify patterns of genetic variability relative to such morphotypes using population genetics and predicted RNA secondary structure of the nuclear ribosomal Internal Transcribed Spacer region 2 (ITS2), (3) to subsequently infer mechanisms of speciation or phenotypic plasticity as a consequence of environmental change, and (4) investigate the currently assigned phylogenetic position of *I. hippuris* within the Octocorallia using the ITS2 region.

Materials and Methods

Study area

The Wakatobi Marine National Park (WMNP) is a remote archipelago of ca. 13,900 km^2 in S.E. Sulawesi, Indonesia. The epicentre of the Coral Triangle and Indonesia's second largest marine park, the WMNP comprises ca. 600 km^2 of the most biodiverse coral reefs on earth. Such marine biodiversity sustains >100,000 people with alarming human population expansion and consequential marine resource dependence and destructive commercial fisheries (Clifton, 2013). Coral reefs within the Wakatobi range from low current, high turbidity lagoons to highly exposed sites with strong water currents and high nutrient deep-water upwellings. Therefore, strong environmental gradients of natural and anthropogenic disturbance exist across reefs within the Wakatobi, providing a novel natural laboratory for studies of environmentally induced change on reef components.

Research was conducted during July and August 2010, between two sites spanning 5 km of anthropogenic and natural environmental gradients (Figure 1C). Ridge 1 (healthy) is an exposed reef ridge with high nutrient upwellings and water currents; Sampela (impacted) is a semi-lagoonal reef with low water flow and high turbidity. Sampela is situated ca. 400 m from a Bajo (sea gypsy) village of ca. 1600 people and subject to continuous marine resource exploitation and community waste disposal. Environmental variables likely to influence the distribution of morphotypes for the two study sites (summarized in Table 1) were selected for further analyses. Light ($K_{d(PAR)}$) was measured using HOBO® data loggers; turbidity (NTU, expressed as inverse values), and chlorophyll-*a* (µg L^{-1}) were measured using an RBR® XR-420 CTD data logger; a General Oceanics® flow meter was used to measure water flow velocity; and sediment grain size was estimated using Retsch Technology® test sieves, with logarithmically converted diameters expressed as *phi* (Φ) and classified using the Wentworth scale (Wentworth, 1922). Environmental variables, with the exception of latitude and longitude, were edited visually with significant outliers removed, and entered into statistical models as raw values.

Sample collection

Isis hippuris colonies were sampled from the healthy, site Ridge 1 (n = 24), and the anthropogenically impacted site, Sampela (n = 24; total n = 48), where the two distinct morphotypes at densities of 18 and 6 colonies per 10 m^{-2} respectively were previously documented (Rowley, 2014; Rowley & Watling, in press; Figure 1A, B). A further twelve clippings were taken from five additional sites (Blue Bowl n = 2; Pak Kasim's n = 2; Buoy 3 n = 4; Kaledupa n = 2; Sea Grass beds n = 2; Figure 1C) to investigate and compare genetic differences between colonies within the study area. Sample numbers for molecular analyses were low due to financial constraints, yet provide valuable insights for further

study. All colonies were randomly selected within 2 - 5 m depth and a minimum of 10 m distance apart to avoid sampling asexual clone mates. Each colony was subject to *in situ* scaled digital photography using a Canon IXUS 900Ti, WP-DC7 u/w housing and INON UWL-105 AD x 0.51 lens, with duplicate samples preserved in 95% EtOH and Guanidinium solution for morphological and molecular analyses respectively. Colonies were photographed both parallel and overhead for planar and bushy colonies as appropriate with a ruler for scale.

Data analyses

Morphological Measurements: Population comparisons of morphological traits were conducted on 24 *I. hippuris* colonies from both Ridge 1 and Sampela (total 48 colonies). A total of 57 morphological traits were quantified and divided into 32 macro- and 25 micro-morphological traits (Figure 2; S2). Due to the variable nature of *I. hippuris* colonies (planar, multiplanar or bushy), particularly between sites, whole colony height [H], mean width [W] taken equidistant apart, and colony spread [CS] were measured with CS as the mean of two measurements taken above the colony. Branch tips [T], mid main branch [M] and base [B] width were also recorded but limited access to the latter meant data were omitted from further analyses (n = 13 and 5 for Ridge 1 and Sampela respectively). Colony sub-sections of ~20 cm in height were selected for further comparable macro-morphometric analyses: sub height [sH], mean width [sW], and projected sub-colony area [PA] estimated by sH x sW. PA was then used to calculate sub-colony porosity [Po] as a ratio of PA and the projected branch area [PBA] (total branch length multiplied by the mean branch thickness; see below). Branch articulation was assessed using a hierarchical generation ordering system (Lasker et al., 2003; Sánchez & Lasker, 2003; Sánchez et al., 2003), where each branch was ascribed as either a ‘mother’ branch or ‘daughter’ branch, the latter emerging from the former. As the colony develops, daughter branches may also become mother branches (e.g., second generation mother branch; see Figure 2) quantified as follows: mother branch

219 length [sML], mean mother branch width [sMW], daughter branch length [sDL], mean daughter
220 branch width [sDW], total branch number [sTB#], total branch length [sTBL], and mean branch width
221 [MBW]. Branch surface area was calculated on the geometric approximation of a cylinder from branch
222 length and mean width as the radius, with subsequent polyp density [PD] per cm^2. Twenty random
223 measurements were made of both polyp diameter ([pD] mean of 2 measurements see Figure 2) and
224 inter-polyp distance [ID]. All polyp, branch cross-section and canal [C#/Cd; Cd see Figure 2]
225 quantification were visualised under an Olympus SZX16® stereomicroscope at 10x magnification with
226 0.5x objective.
227
228 *Isis hippuris* has a considerable diversity of sclerite form (Simpson, 1906; Bayer & Stefani, 1987;
229 Fabricius & Alderslade, 2001). For consistency, only those represented in all test colonies were
230 selected for quantitative analyses. Length and mean width of three measurements were made on 20
231 randomly selected sclerites per sclerite type; surface clubs [CL1/2, CW1/2]; and sub-surface capstans
232 [7-radiates: CaL/W] and spindles [SL/W] (Figure 2). Additional sclerite diversity is shown in S3.
233 Sclerites were removed by dissolving the surrounding tissue in 5% sodium hypochlorite solution and
234 visualized using optical microscopy (Olympus BX51®) and scanning electron microscopy (SEM),
235 which was performed on a Hitachi S-800 SEM at the University of Hawai'i at Mānoa, USA. All micro-
236 morphological measurements and sclerite preparation were taken 2 cm below the branch tip to avoid
237 underdeveloped traits due to sub-apical branch growth (Lasker et al., 2003) and photographed using an
238 Olympus 3.3MPX™ camera and Rincon software (ImagingPlanet®). All macro- and micro-
239 morphological characteristics were measured using ImageJ64 (Abràmoff, Magalhaes & Ram, 2004).

240 Phenotypic traits were analysed using routines within the PRIMER-E v6.1.12 statistical package
241 (Clarke & Gorley, 2006), with PERMANOVA+ v1.02 extension (Anderson, 2001). Specifically,

character traits (untransformed) were simultaneously correlated in a Draftsman plot to eliminate uninformative traits ($P > 0.95$) and to establish appropriate transformation for downstream analyses (Clarke & Ainsworth, 1993). Informative phenotypic trait data were subsequently standardized and a 'zero-adjusted' Bray-Curtis similarity matrix (Clarke, Somerfield & Chapman, 2006) constructed for tests of morphological divergence between the two sites; Ridge 1 and Sampela. A single-factor model with 9999 permutations (PERMANOVA; Anderson, 2001) was performed and further visualised utilizing constrained canonical analysis of principal coordinates (CAP; Anderson & Willis, 2003). Informative traits contributing most to the dissimilarities between sites, thus specific morphotypes were investigated using similarity percentages (SIMPER; Clarke, 1993) and displayed as a vector overlay on the CAP ordination.

The relationship between *I. hippuris* morphotypes and their environment was investigated using nonparametric multivariate regression (McArdle & Anderson, 2001) with the DISTLM*forward* routine (Anderson, 2003). Based on a Euclidean distance matrix, all raw environmental variable data (Table 1) were normalised and significance tested using 9999 permutations (Anderson, 2001).

Molecular Analyses: Genomic DNA of *I. hippuris* were extracted from 28 colonies, 8 from each of the two test sites (n = 16) and 12 from additional site populations for area and morphotype comparison as described above. Approximately 2 - 3 mm of fresh soft tissue was immediately cut and stored in 400 µl Guanidinium lysis buffer (4 M guanidinium isothiocyanate, 0.05 M Tris pH 7.6, 0.01 M EDTA, 0.07 M Sarkosyl, β-mercaptoethanol 1% v/v) (Pochon et al., 2001) for 14 days at room temperature during transit from the field, with subsequent storage at 4ºC. Preserved samples were incubated at 72ºC for 20 min, vortexed prior, during and after incubation, then centrifuged at 16,000 *g* for 5 min. The resulting DNA-containing supernatant was precipitated with an equal volume of 100% isopropanol, vortexed

and stored over night at -20ºC. DNA was pelleted via centrifugation at 16,000 *g* for 15 min, washed with 70% EtOH, centrifuged for 10 min, dried and resuspended in 0.1 M Tris Buffer pH 8. The DNA solution was placed on ice for 1 hour with frequent vortexing and stored at -20ºC. DNA was visualized on 1% agarose gel. PCR amplifications of the ITS2 rDNA marker were conducted using the primers itsD (forward; 5'-GTGAATTGCAGAACTCCGTG-3') and ITS2Rev2 (reverse; 5'-CCTCCGCTTACTTATATGCTT-3') (Pochon and Gates 2010). Total PCR volume was 50 µl constituting: 5.0 µL of 10x PCR Buffer (Bioline), 2.0 µL of $MgCl_2$ (2 mM), 1.0 µL of each primer (10 mM), 1 µL (2.5 mM of each dATP, dCTP, dGTP, and dTTP), 0.2 µL of Hotstart Immolase *Taq* polymerase (Bioline Incl., London, UK), 1.0 µL of DNA, and 39 µL of sterile water. Touchdown amplification was conducted as follows: denaturation at 95ºC for 10 min, 25 cycles at 94ºC then 35 s at 65ºC (reduction in annealing temperature of 0.5ºC per cycle), and 2 min at 72ºC. A further 14 cycles of 30 s at 94ºC, 35 s at 52ºC, 2 min at 72ºC, and a final 10 min extension at 72ºC. All amplicons were purified using the QIAquick™ PCR Purification Kit (Qiagen), and separated by cloning for haplotype verification. Purified products were ligated into the pGEM®-T Easy vector™ (Promega), transformed into α-Select Gold Efficiency™ competent cells (Bioline), with subsequent positive inserts verified by PCR using plasmid specific primers (M13). Positive inserts (8-12 per library) were purified with an ExoSAP-IT kit, sequenced in both directions using the ABI Prism Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit and run on an ABI 3100 Genetic Analyzer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) at the University of Hawai'i at Mānoa, USA.

ITS2 clone libraries from 28 individuals were aligned using ClustalW2 (Thompson, Gibson & Higgins, 2002) and manually edited in Geneious Pro v.5.6.2 (Biomatters Ltd., NZ). A selection criterion of identical sequences from two or more clone libraries was established to minimize the effect of

287 intragenomic variation and/or PCR artefacts on further analyses. On average 4 - 6 host clones were
288 recovered per library due to simultaneous recovery of both host and endosymbionts.

289 Estimates of genetic differentiation relative to morphotype were investigated via an analysis of
290 molecular variance (AMOVA) with pairwise population comparisons (Φ_{ST}) between sites using
291 ARLEQUIN v.3.5 (Excoffier & Lischer, 2010). Haplotype (h_d), nucleotide diversity (π) and
292 substitution rate (JC) were calculated with DNAsp v.5.0 (Librado & Rozas, 2009). A parsimony
293 haplotype network with a 95% confidence level and gaps treated as a fifth state was constructed using
294 Network v.4.6.1.1 on sample sequences only.

295 *ITS2 Predicted RNA Secondary Structure:* ITS2 RNA secondary structures were predicted to further
296 investigate haplotype differences specifically between Ridge 1 and Sampela at a more conserved level.
297 *Alcyonium digitatum* Linnaeus, 1758 (Genbank Acc. # AF262347; McFadden et al., 2001) was used as
298 a template for conserved motif identification with subsequent constraints implemented into MFOLD
299 (Zuker, 2003) using default parameters. RNA was folded at 37°C and structures with the highest
300 negative free energy values, thus stability, were selected, manually edited in 4SALE (Seibel et al.,
301 2006; 2008) and visually annotated in VARNA (Darty, Denise & Ponty, 2009).
302
303 Phylogenetic reconstructions between *I. hippuris* haplotypes and twenty octocoral ITS2 outgroups
304 obtained from GenBank (see S4) were conducted using the plugins PHYML 2.1.0 (Guindon &
305 Gascuel, 2003) and MrBayes v3.2.2 (Huelsenbeck & Ronquist, 2001) within Geneious. Indels
306 (insertion and deletion mutations) were considered phylogenetically informative and treated as separate
307 characters using the 'simple indel coding' gap method (Simmons & Ochoterena, 2000) in GapCoder
308 v.1.0 (Young & Healy, 2003). Maximum Likelihood (ML) phylogeny was conducted using the best-fit

model (JC) of nucleotide substitution as selected in jModelTest 2 (Darriba et al., 2012) through Akaike Information Criterion (AIC). Bayesian Inference (BI) phylogeny was made with a JC69 substitution model. Analyses were initiated from a random tree of four chains with two runs of Metropolis-coupled Markov Chain Monte Carlo (MCMCMC), including 1,100,000 generations and subsampling every 10 generations. Chains converged within 0.2 generations in all cases with a burn-in of 100,000. Phylogenetic trees were rooted with *Paragorgia kaupeka* Sánchez, 2005 and node support values set at ≥ 70% for both ML and BI.

Results

Morphometrics

Of the 57 morphological traits measured (59,328 individual measurements), 32 were selected for further analyses (41,265 individual measurements, S2). Based on these traits, PERMANOVA revealed significant differences between morphotypes across the two study sites Sampela and Ridge 1 (pseudo-$F = 14.489$; $P < 0.0001$), further corroborated by the CAP analysis ($\delta^2 = 0.995$, $P = <0.0001$; Figure 3) with 89% variance (% var.) as the total variance explained by the first *m* PCO axes. Prominent morphological traits contributing most to dissimilarities between sites were primarily at the colony level (TW, W, H, PBA, Po, and sTB#) with the exception of a higher polyp density (PD) at Sampela (Figure 3). From both Figure 3 and S2 it is clear that larger colonies present at Sampela have a reduction in branch density, yet larger colony size and spread (PBA, sTB#, Po, and H, W, TW respectively). Branches, including the branch tips, were also consistently longer and thicker, at Sampela, however polyp parameters were relatively invariable despite significantly high polyp density. It is noteworthy that all sclerite trait measurements were consistently smaller at Sampela (S2 S3), particularly capstans (S3f.iii, iv and Figure 2 for variability), which are variable throughout *Isis hippuris* distribution (Simpson, 1906; Bayer & Stefani, 1987; Fabricius & Alderslade, 2001).

Irrespective of pre-treatment, the magnitude of differences between sclerite measurements were not that of the colony level. Nevertheless, separation and re-analyses under the same models for macro (e.g., colony and sub-colony: pseudo-F = 15.255; P < 0.0001; CAP $\delta 2$ = 0.976, P = <0.0001, 91% var.) and micro (e.g., sclerite: pseudo-F = 11.727; P < 0.001; CAP δ^2 = 0.996, P = <0.0001, 85% var.) measurements did not significantly alter results from the full model, demonstrating a lack of redundancy in selected character traits.

Results from the distance-based nonparametric regression (DISTLM*forward*) revealed that turbidity and sediment load explained 27.31% (pseudo-F = 5.100; P < 0.001) of morphotype differences between the two sites.

ITS2 Sequence diversity

From the 28 *Isis* samples 120 clones were recovered: Ridge (34), Sampela (29), Sea Grass (9), Kaledupa (12), Buoy 3 (18), Pak Kasim's (10), and Blue Bowl (8); GenBank accessions KP265675-265702. ITS2 sequences revealed five haplotypes: 1-3 per sample population with up to 8 substitutions (S5, Figure 4). In keeping with morphological traits, colonies found at Sampela were significantly different from all other sample sites (Table 2, Figure 4). Haplotype diversity was greatest across Hoga Island with overall haplotype (h_d) and nucleotide diversity (π) measured as 0.780 and 0.0197 respectively with just (JC) 0.0313 substitutions per site. Population division was strongly inferred by all AMOVA models (Table 2) and haplotype network analysis, the latter showing no evidence of reticulation through homoplasy (Figure 4). Curiously, the single haplotype present in the sea-grass beds (D) shared no nucleotide differences with Sampela (A) despite its relatively close proximity, however little can be determined without greater sampling effort. Pairwise Φ_{ST} estimates of ITS2 sequences from Sampela ranged from 1.000 (Sampela *vs*. Sea Grass, Kaledupa, Pak Kasim's, Blue Bowl, and

354 Buoy 3) to 0.843 (Sampela *vs*. Ridge 1; $P < 0.0001$ in all cases), and from 0.467 (Ridge 1 *vs*. Pak
355 Kasim's; $P < 0.05$) to no structure (Ridge 1 *vs.* Blue Bowl and Buoy 3) at Ridge 1. Note that such
356 values, in particular the P – values, should be treated with caution. Low sample sizes reduce fine-scale
357 structure detection. Thus more data would likely provide greater insight into the level of haplotype and
358 nucleotide diversity observed across sites and also increase taxonomic certainty.

359 ITS2 predicted RNA secondary structure analyses revealed minimal variation between haplotypes with
360 the exception of Sampela (Figure 4) providing greater confidence in phenotypic trait differences.
361 Clones were collapsed into haplotypes per sample for phylogenetic analyses. Phylogenetic topologies
362 using ML and BI algorithms were very similar including all outgroups and unambiguously identical
363 with regard to WMNP haplotypes (Figure 5). Branch support was typically stronger with BI
364 particularly regarding outgroup species where recognised taxonomic suborders and groups were
365 distinct. Phylogenetic uncertainty leading to the addition of multiple outgroups, confirmed *I. hippuris*
366 sequences from the WMNP were not grouped with morphologically described sister taxa within the
367 Isididae (highlighted red, Figure 5). Reducing the outgroup number did not alter the integrity of the
368 phylogenetic signal, in fact irrespective of model or selected root *Alcyonium digitatum* consistently
369 positioned directly above *Isis* haplotypes.

370 ## Discussion

371 *Isis hippuris* morphotypes were clearly defined both morphologically and genetically between the two
372 sites Ridge 1 and Sampela (Φ_{ST} = 0.7683, P <0.001). Even with a reduced ITS2 sample size, molecular
373 differences were consistent with morphometric results indicating that divergence has or is taking place,
374 the nature of which is unclear. Multivariate trait integration at the colony level (including branching
375 parameters), polyp density and sclerite size define significant differences between morphotypes

indicative of trait interdependency, yet polyp dimension and canal width appear canalised (genetically fixed). Nevertheless, inherent phenotypic plasticity and/or disruptive selection may enhance the success of two phenotypes particularly between contrasting environments. Trophic-level interaction through differential light and nutrient exposure may drive such phenotypic differences, further reinforced by population structure due to asexual fragmentation and external brooding (Rowley, 2014). Taxonomic assignment may be tenuous, however, considering the partial adherence of morphotypes to previously described species within the *Isis* genus in addition to polyphyly within the Isididae.

Of the 48 colonies (28 used for genetic analyses) studied here (and 1094 ecologically surveyed in Rowley, 2014; Rowley & Watling, in press) it cannot be said with confidence that *I. hippuris* morphotypes at either site within the WMNP adhere to the descriptions as outlined for *Isis reticulata* (Nutting, 1910; Kükenthal, 1919; 1924; Stiasny, 1940; Mai-Bao-Thu, 1971) or *Isis minorbrachyblasta* (Zou et al., 1991). The various morphotypes may simply represent phenotypic response(s) to water depth in those previously described. *I. hippuris* contrasts with *I. reticulata* on the basis of short thick branches in the former, and long thin branches with smaller, coarsely articulated sclerites in the latter. *I. minorbrachyblasta* has bushy colonies with short densely packed branches, but considering both the lack of sampling for this taxon, documented panmixia and noted phenotypic plasticity of *I. hippuris* (e.g., Simpson, 1906; Thomson & Simpson, 1909; Bayer & Stefani, 1987), this latter taxonomic assignment is treated with extreme caution and may simply be an intermediate form. The two morphotypes of *I. hippuris* presented here have but partial adherence to those previously described (summarised in Supporting Information). The short-branched predominantly planar colonies at Ridge 1 are more akin to *I. hippuris* whereas the more open, long-branched colonies at Sampela resemble *I. reticulata* but with thick branches as opposed to thin. Swollen branch tips characteristic of *I. hippuris* were observed in both morphotypes, and is not a reliable trait. Such swollen branch tips were more

399 prevalent at Sampela. This was the only site where external brooding was observed at the time of the
400 study (Rowley, 2014), and therefore, swollen branch tips may pertain to the presence of eggs within the
401 polyps.

402 ***Isis hippuris* phenotypic variability**

403 Measuring a broad range of phenotypic traits between *I. hippuris* morphotypes highlights trait
404 integration, canalisation and thus those traits acted on by selection which may differ from those
405 previously described for other gorgonian taxa (e.g., Sánchez & Lasker, 2003; Sánchez et al., 2003;
406 2007; Sánchez, 2004; Dueñas & Sánchez, 2009). Here clear patterns of colony (therefore branching),
407 integration coupled with sclerite-level traits and polyp density are consistent between the two
408 morphotypes. Specifically, branching dynamics and colony size (colony porosity as a function of total
409 branch number and size [projected branch area]) appear to have a negative association with sclerite
410 size. Whether these traits are negatively associated as emergent properties or of longstanding would
411 necessitate further investigation using reciprocal transplant experimentation and population
412 coalescence (Prada, Schizas, & Yoshioka, 2008; Prada & Hellberg, 2013). In either case, differential
413 light attenuation and nutrient components between the two sites are not unnatural phenomenon, which
414 may or may not be exacerbated by reef resource dependent anthropogenic influence from Sampela.

415 Colony surface area and metabolism are intrinsically linked whereby a cascade effect of concomitant
416 variations in branching, polyp, canal and sclerite dynamics would be expected. However, disintegration
417 or canalisation (fixation) was evident in both polyp and canal dimensions consistent with previous
418 work (Sánchez, 2004). Responses to variations in water quality, thus heterotrophic feeding capacity,
419 are incurred through polyp density as opposed to size, yet both canal number and dimensions remained
420 unchanged in both morphotypes. The exact function of stem canals is unclear (Cadena et al., 2010),

421 although suggested to circulate and exchange water and nutrients throughout the coral colony (Ellis &
422 Solander, 1786). Canalisation/fixation at this level further suggests that photosynthetic gain with
423 nutrient translocation at the cellular level between endosymbionts and host, are likely the primary
424 trophic resource. Optimal allocation theory posits an increase in the uptake of resource(s) that are most
425 limiting growth (Weiner, 2004). Moreover, the same genotype can show resource allocation plasticity
426 (Sebens, 1997) in alternate environments consistent with the 'partitioning' hypothesis (Weiner, 2004).
427 Plasticity as a response is an emergent property of divergence (Pigliucci, 2005). Therefore, to further
428 elucidate energy allocation patterns between morphotypes, physiological tests coupled with
429 morphological and genetic analyses on reciprocal transplants between reefs would establish phenotypic
430 trait plasticity - thus a capacity for plasticity - or ecological divergence through disruptive selection
431 (Schluter, 2001) in *I. hippuris*.

432 Sclerite composition can vary with light intensity and/or water motion (West et al., 1993; Kim et al.,
433 2004; Skoufas, 2006; Clavico et al., 2007; Prada, Schizas, & Yoshioka, 2008; Prada & Hellberg, 2013).
434 The presence of numerous small, articulate interlocking sclerites could provide additional structural
435 support for larger colonies found at Sampela, which lack the close branching structure present at Ridge
436 1. Smaller sclerites may mitigate mechanical constraints on the axis of increased colony size and bushy
437 morphology through long thick branches, and provide greater soft tissue support as surface area
438 increases (Clavico et al., 2007). Small clubs increase both flexion and torsion capacity in less exposed
439 areas of *Eunicella singularis* Esper, 1791 whereas larger spindles were prevalent in the exposed
440 peripheral branches. Nonetheless, *Eunicella cavolinii* Koch, 1887 showed no selective difference
441 between the two (Skoufas, 2006). A decrease in sclerite size with increased density in shallow
442 conspecifics has been shown (e.g., Prada, Schizas, & Yoshioka, 2008), typically due to increased water
443 flow (West et al., 1993; West, 1997; Kim et al., 2004). Here, regardless of both morphotypes

444 containing high densities of small sclerites, the consistency in small size at Sampela coupled with
445 thicker longer branches and higher polyp density likely increases photosynthetic gain through greater
446 surface area, as well as heterotrophic feeding. The lack of variability in canal size or number, as well as
447 polyp dimensions represents canalisation, and further suggests that photosynthetic gain from
448 dinoflagellate endosymbionts is the primary resource for *I. hippuris*.

449 Sclerites are key characters for species identification yet susceptible to environmental perturbation and
450 selection. All sclerites were consistently smaller in colonies from Sampela. The overall dimensions
451 between the two morphotypes from both sites were within the range of those described for *I. hippuris*
452 with regards clubs and radiates from both *I. reticulata* and *I. minorbrachyblasta*. Sclerite differences
453 between morphotypes compared to those published appeared inconclusive with notable overlap. For
454 example, bent spindles characteristic of *I. reticulata* were present in colonies from Ridge 1, themselves
455 bearing closer resemblance to *I. hippuris*. Interestingly, sclerite diversity was greater at Sampela with
456 closer resemblance to *I. reticulata,* particularly considering sclerite asymmetry and crosses. No
457 sclerites were found within the polyps or tentacles in either morphotype in this study, unlike in *I.*
458 *reticulata*. However, the small rods (0.07 x 0.01 mm) of Bayer & Stefani (1987) were present, but their
459 precise location within *I. hippuris* soft tissue could not be determined.

460 Enhanced fitness through an individual's (genotype) capacity to respond to environmental
461 heterogeneity - specific morphotypes predominating in certain habitats - maximises survival through
462 resource acquisition and minimises metabolic costs. Most corals are polymorphic under varying
463 environmental conditions (West et al., 1993), with differential phenotypic expression of a genotype as
464 a consequence of astogeny (colony development), itself genetically and/or environmentally mediated
465 (Sánchez & Lasker, 2003). Environmental influences on larval settlement, such as high sedimentation

466 rates at Sampela or competition and high water flow at Ridge 1, may lead to developmental
467 adaptational responses. Moreover, *I. hippuris* colonies survive and replicate through external brooding
468 and asexual fragmentation with a propensity for philopatry and upward growth (Dauget, 1992),
469 increasing population structure and expansion on such degraded reefs over time.

470 ***Isis hippuris* genetic variability**

471 Phenotypic divergence and biological success of *I. hippuris* within the WMNP may be a consequence
472 of intraspecific polymorphism due to a high capacity for plasticity with no barriers to gene flow
473 between morphotypes. This, in part, can be a consequence of epigenetic effects, which may be
474 heritable and become fixed through genetic assimilation if conditions persist. This, particularly in the
475 presence of evolutionary capacitance whereby cryptic variation becomes functionally overwhelmed or
476 initiated by the environment, can exert pleiotropic effects on significant developmental processes
477 (Rice, 2008). Such non-additive genetic covariance wields a stronger influence on mutation than
478 random drift, itself much stronger in small populations typical of brooding and asexual taxa. Thus,
479 phenotypic divergence as seen in *I. hippuris* across sites within the WMNP, may be a consequence of
480 hidden genetic variation leading to emergent environmentally mediated fixation accelerated by
481 anthropogenic impact. Peripheral haplotypes reveal emergent lineages (Forsman, 2003), with those at
482 Sampela differing by up to seven base pairs between primary ITS2 sequence comparisons, none of
483 which are shared with other haplotypes within the region. Furthermore, shared haplotypes and thus
484 gene flow at the remaining test sites, (with the exception of haplotype D [sea grass]), suggests
485 assortative mating with the onset of reproductive isolation at Sampela. Thus the more frequent and
486 broadly adapted remaining haplotypes are likely ancestral. Greater sampling with genetic and
487 coalescent analyses is required to confirm such supposition, particularly considering a minority
488 presence of opposing morphotypes at each site (SJ Rowley, pers obs).

The consistent mutational differences both within (clones) and between sequenced samples, renders PCR or base-calling errors unlikely. Given the renowned caveats associated with the ITS2 region such as intragenomic variation, the secondary structure of each haplotype confirmed molecular morphometric differences. The most notable difference was between Sampela (haplotype A) and the remaining sites, yet strong sequence similarities were present between the remaining haplotypes. Furthermore, lack of network reticulation suggests no indication of hybridization, validating confidence in two species taxonomic assignment, emergent or previously diverged. Yet, hybridization at this juncture cannot be overlooked. *I. hippuris* morphotypes across its distributional range may also represent an Indo-Pacific syngameon as seen in the notoriously diverse and polymorphic scleractinian *Acropora* Oken, 1815 (Ladner & Palumbi, 2012), with widespread gene flow through introgression (Vollmer & Palumbi, 2002; Ladner & Palumbi, 2012). However, any species delimitation within the *Isis* genus in addition to *I. hippuris* is necessary before further inference can be made.

It is clear that pertinent overlap exists between previously described *Isis* taxa and those present within the WMNP. It is tempting to conclude that *I. hippuris* is a single species with an extensive phenotypic and geographical range, or that only *I. hippuris* are present in the Wakatobi with other taxa within the genus elsewhere. Environmentally tolerant taxa tend to possess wide geographic distributions compared to those that are not (Calosi et al., 2010). However, the historically perceived panmixia of *I. hippuris* is likely more than a single species and not that of a complex when considering similar repetitive phenotypic trait differences across its distributional range. Previous alternative taxonomic assignments are therefore questionable. The standard error of phenotypic variance would be greatly improved by assessing differences between *I. hippuris* morphotypes with increased specimen analyses from throughout its distributional range; a beneficial strategy when dealing with highly polymorphic taxa. Again, tests of coalescence on numerous independent highly polymorphic markers (SNPs; Ladner

512 & Palumbi, 2012) would be required in order to fully elucidate convergent genotypic-by-environment
513 effects in *I. hippuris* across its distributional range.

514 **Isididae polyphyly**

515 Phylogenetic analyses confirm haplotype differences as well as polyphyly within the Isididae; a
516 phenomenon recently reported using the putative octocoral mitochondrial marker *msh1* (Watling et al.,
517 2012). Even as far back as the earliest part of the last century, Kükenthal (1919) considered the Isididae
518 to be polyphyletic, the subfamilies within as independent groups and the colony axis a "convergent
519 phenomenon." Furthermore, *I. hippuris,* itself the type species of this family and the subfamily
520 Isidinae, appears to have minimal phenotypic similarities to virtually all other isidid taxa with the
521 exception of the axis, yet even this has been shown to be scleritic (consist of fused sclerites; Milne-
522 Edwards & Haime, 1857; Kükenthal, 1919; 1924; Bayer, 1955; Watling et al., 2012; but see Nutting,
523 1910). Such evidence naturally brings into question the validity of *I. hippuris* in its current
524 classification. Polyphyly within gorgonian groups across bathymetry is not unknown (McFadden et al.,
525 2006). *I. hippuris* is the only shallow and zooxanthellate representative of the Isidinae and Isididae
526 respectively, the remainder being characteristic of the deep ocean.

527 The scleritic composition of the *I. hippuris* axis further sets it apart from both the Isididae and the
528 suborder Calcaxonians, which are more closely affiliated with the Alcyoniinan-Holaxonian clade as
529 phylogenetically determined by Bernston et al., (2001) and McFadden et al., (2006). However, this
530 convergent trait holds significant evolutionary intrigue. The fused scleritic internodes with gorgonin
531 nodes of the *I. hippuris* axis, ensures flexibility and durability under high water energy conditions. Yet
532 what is the selective advantage of a jointed axis in deep-sea isidids? Empirically, this is undetermined
533 but it is not unreasonable to propose that the jointed axis is a relictual anachronism consequential of

534 geological (e.g., opening of the Drake Passage) as well as later glacio-eustatic sea-level changes
535 resulting in bathymetric refugia from turbulent shallow coastal waters (Helm & Schülke, 2003). Thus,
536 the functional significance of an articulated axis at depth is still a mystery; however longer internodes
537 in the colonies at Sampela – like those seen in the benign deep ocean Isidids - compared to Ridge 1
538 were observed but not quantified (SJ Rowley, pers obs). Interestingly, deep-sea low flow specialists
539 *Isidella* Gray, 1857, have long elegant calcareous internodes compared to the larger much more robust
540 internodes of *Keratoisis* Wright, 1869, characteristic of moderate flow environments in the deep-sea,
541 yet with no appreciable flexibility. A deep divergence with stabilizing selection regards a non-sclerite
542 axis in deep-sea isidids may have occurred. Whether the *I. hippuris* axis is a consequence of
543 convergent evolution based on ecological necessity in heterogeneous environments typical of shallow
544 reefs or deep inheritance is unclear and under investigation.

545 ## Conclusion

546 In closing, the two distinct *I. hippuris* morphotypes within the WMNP are phenotypically segregated
547 through trait integration between healthy and degraded reefs, likely reinforced through reproductive
548 strategy. The co-variability of light, sediment and water flow between sites fortify directional trait
549 selection (Feder, 1998); colony, branching dynamics, polyp density, sclerite size and diversity all vary
550 significantly between sites. Moreover, polyp and nutrient canals appear canalized due to the additive
551 effect of modules to the colony as opposed to an increase in size. Greater polyp density may lead to an
552 increase in photosynthetic yield and heterotrophy, in turn mitigating and capitalizing on environmental
553 conditions, particularly at Sampela. Diverse phenotypic trait assessment through character trait
554 integration using reciprocal transplant experiments across the two sites would undoubtedly be
555 insightful, particularly as shifts in metabolic function are subject to selection at opposite ends of
556 environmental gradients (Feder, 1998). Selection acts on phenotypic variation (reflecting variation in

gene expression), which may have become fixed over time leading to ecological divergence. *I. hippuris* morphotypes, tentatively confirmed by ITS2 sequences and secondary structure analyses, have only partial adherence to previously described taxa. Species assignements cannot be prudently made at this time, requiring classical and genomic taxonomic analyses across the distributional range. Furthermore, compelling phylogenetic evidence not only confirms *I. hippuris* morphotype differences, but also reveals its disassociation within the Isididae. Phylogenetic discernment investigating congruence between skeletal structure, multi-locus next-generation sequencing and coalescence modelling (Puritz et al., 2012), will assist unresolved hypotheses within this turbulent group.

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Figure Legends

779 **Figure 1.** *Isis hippuris* morphotypes: (A) short branched predominantly planar or multiplanar colonies
780 at the healthy site Ridge 1, and (B) long branched bushy colonies at the impacted site Sampela, with
781 additional (C) collection localities within the WMNP. Sample number in brackets for molecular and
782 asterisk for morphological analyses.

783 **Figure 2.** *Isis hippuris* morphological trait measurements of the (A) colony; (B, C, D) canal and polyp
784 dynamics; (E) sub-colony (branching) dynamics; (F) sclerites site/morphotype comparisons of i and ii
785 spindles, iii and iv capstan 7-radiates, v – vi and vii - viii clubs from Ridge 1 and Sampela respectively.
786 All abbreviations are described in text.

787 **Figure 3.** Constrained ordinations (CAP) of *Isis hippuris* character traits between Ridge 1 [◆] and
788 Sampela [▲]. Vector abbreviations as described in text.

789 **Figure 4.** *Isis* haplotype network with corresponding ITS2 RNA predicted secondary structure relative
790 to haplotype (A – E, see also Figure 5) and enthalpy values according to MFOLD. Roman numerals (I
791 – IV) represent helices; red and black arrows indicate point mutations and loop differences,
792 respectively. Coloured bases according to transitions (red), transversions (yellow) and gaps (lilac), S5.
793 Haplotype circle diameters are proportional to identical clone sequences.

794 **Figure 5.** Phylogram based on Maximum Likelihood (ML) analyses of the ITS2 region from twenty
795 Octocoral taxa in GenBank and *Isis* haplotypes within the WMNP. Branch numbers represent ML
796 bootstrap support and BI posterior probabilities, respectively, with low values expressed as a hyphen (-
797 -) ≤ 70% and asterisk (*) indicative of differences from MrBayes phylogenetic inference. Letters Sc =
798 Scleraxonia, Ca = Calcaxonia, Ho = Holaxonia, Al = Alcyoniina, and A – E represent *Isis* haplotypes
799 as depicted in Figure 4.

Tables and Legends

Table 1. Environmental characteristics of the two study sites in the WMNP, Indonesia. All values expressed as mean (± SE) with the exception of diurnal temperature range (˚C), light ($K_{d(PAR)}$) and sediment grain size (Φ).

Parameter Recorded	Mean value ± SE or range	
Site	**Sampela**	**Ridge 1**
Latitude (S)	005˚ 29'01"	005˚ 26'57"
Longitude (E)	123˚45'08"	123˚45'38"
Temperature (˚C min-max)	25.61 – 29.36	24.06 – 28.07
Light ($K_{d(PAR)}$ min-max)	0.31 – 3.14	0.1 – 1.56
Flow (cm/s)	5.02 ± 2.18	30.54 ± 2.61
Chlorophyll-*a* (µg l^{-1})	0.3 ± 0.01	0.35 ± 0.03
Turbidity (NTU)	4.38 ± 1.80	0.17 ± 0.33
Sedimentation (g d^{-1}, n = 12)	3.28 ± 0.26	1.16 ± 0.07
Sediment grain size (Φ, n = 12)	5 [31.25–62.5 µm]	1 [0.5–1 mm]

Table 2. AMOVA of genetic structure between sites within the WMNP from both cloned and sample sequences. R1 denotes Ridge 1; S denotes Sampela. *$P < 0.001$ significant.

Source of Variation	*df*	SS	Variance Component	Variance %	Φ_{ST}
7 Populations: Clones					
Among populations	6	122.248	$V_a = 1.237$	80.97	0.80974*
Within populations	113	32.844	$V_b = 0.291$	19.03	
Total	119	155.092	1.528		
7 Populations: Samples					
Among populations	6	33.768	$V_a = 1.401$	76.83	0.76831*
Within populations	21	8.875	$V_b = 0.423$	23.17	
Total	27	42.643	1.824		

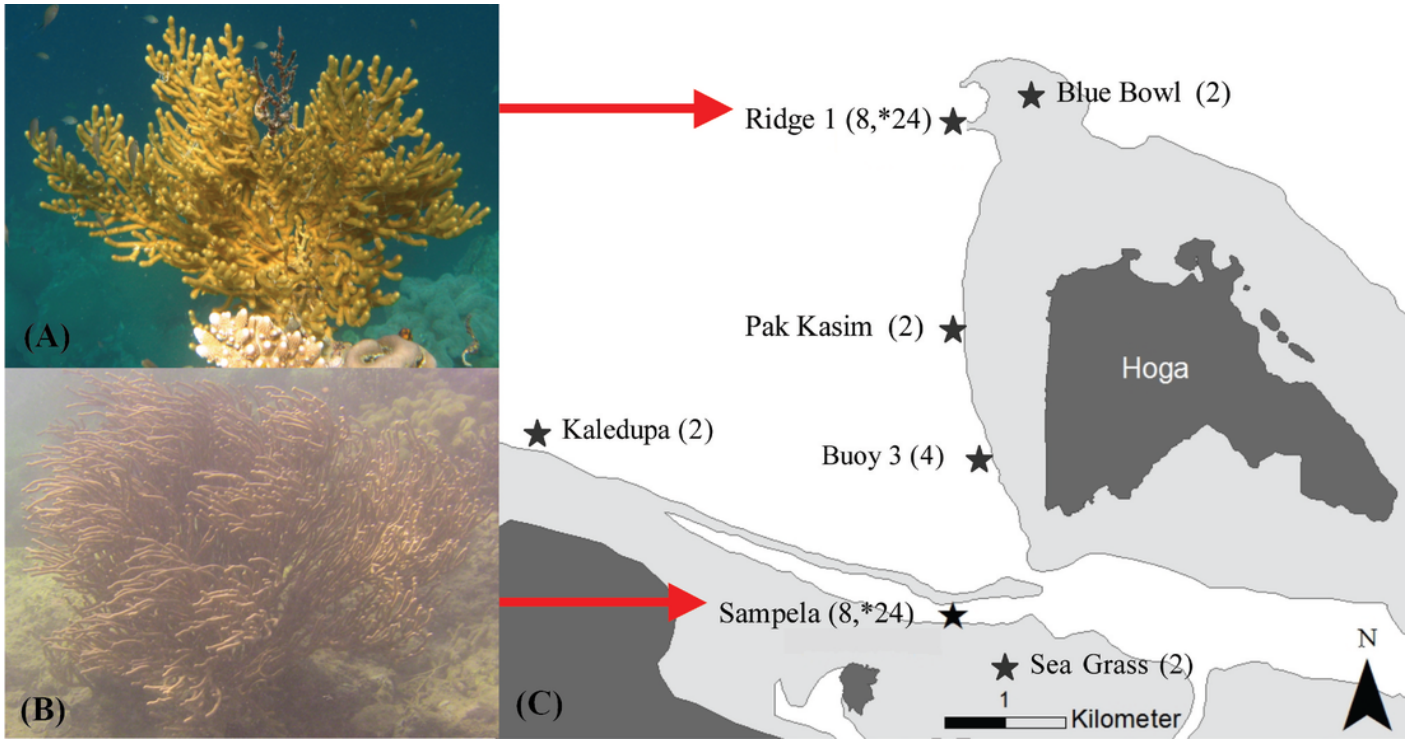
2 Populations (R1 & S): Samples					
Among populations	1	17.688	$V_a = 2.161$	84.32	0.84321*
Within populations	14	5.625	$V_b = 0.402$	15.68	
Total	15	23.312	2.563		

808

1

Isis hippuris morphotypes and location map of the Wakatobi Marine National Park (WMNP), SE Sulawesi, Indonesia.

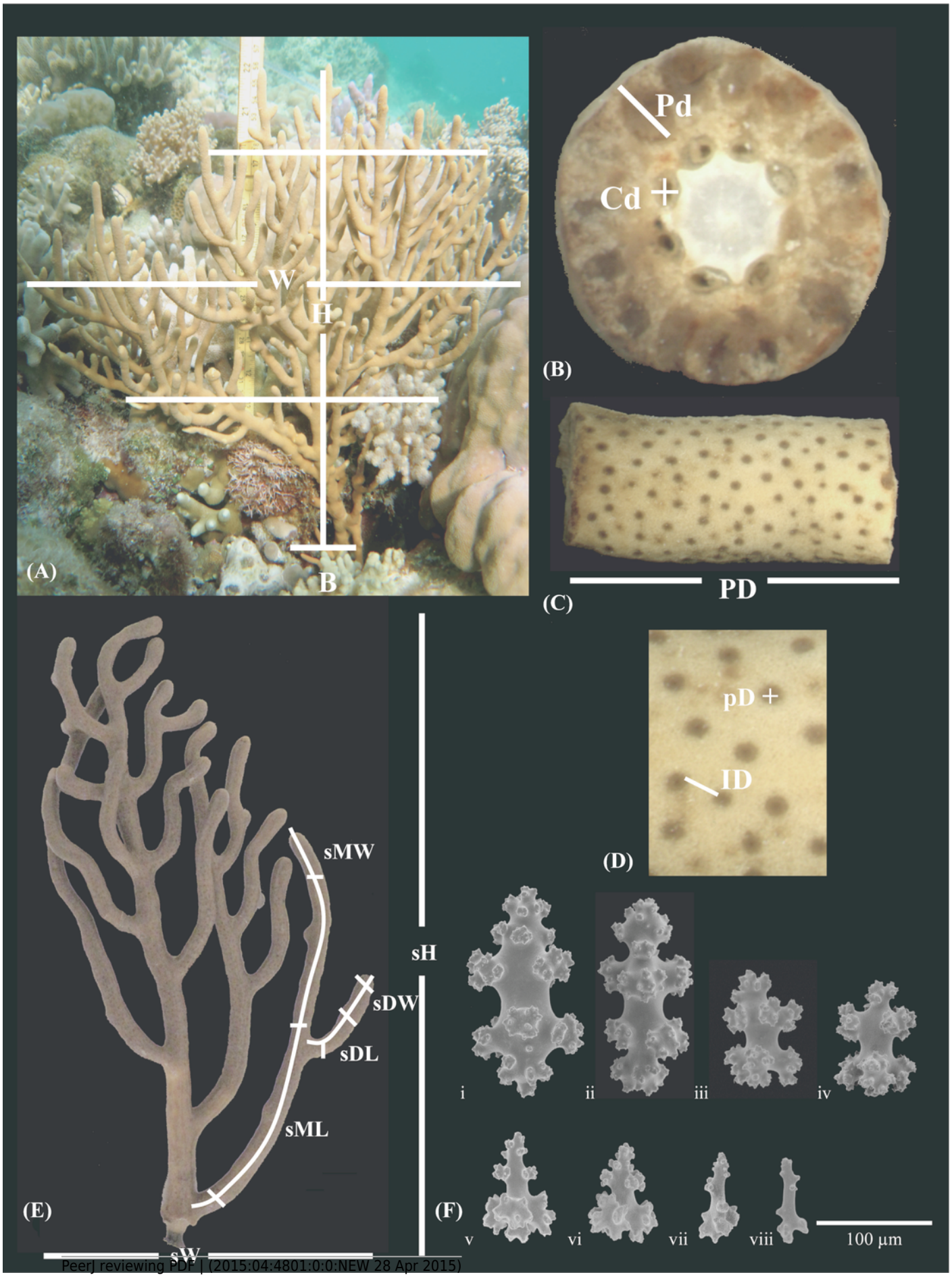
Isis hippuris morphotypes: (A) short branched predominantly planar or multiplanar colonies at the healthy site Ridge 1, and (B) long branched bushy colonies at the impacted site Sampela, with additional (C) collection localities within the WMNP. Sample number in brackets for molecular and asterisk for morphological analyses.



2

Isis hippuris morphological trait measurements plate.

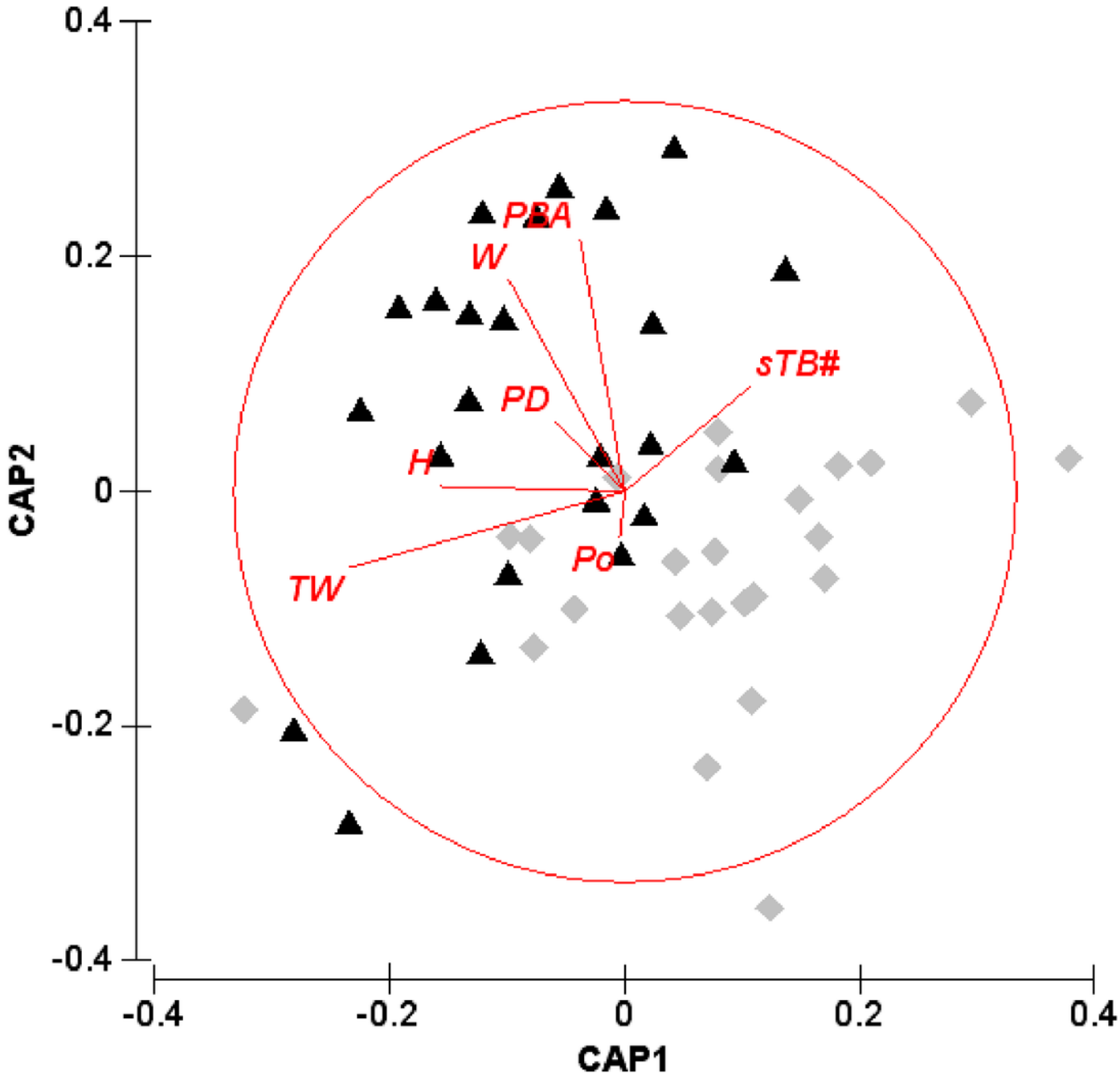
Isis hippuris morphological trait measurements of the (A) colony; (B, C, D) canal and polyp dynamics; (E) sub-colony (branching) dynamics; (F) sclerites site/morphotype comparisons of i and ii spindles, iii and iv capstan 7-radiates, v – vi and vii - viii clubs from Ridge 1 and Sampela respectively. All abbreviations are described in text.


W
H
B
(A)
Pd
Cd
(B)
(C)
PD
pD
ID
(D)
sMW
sH
sDW
sDL
sML
sW
(E)
i
ii
iii
iv
v
vi
vii
viii
(F)
100 µm

3

Constrained ordinations (CAP) of *Isis hippuris* character traits.

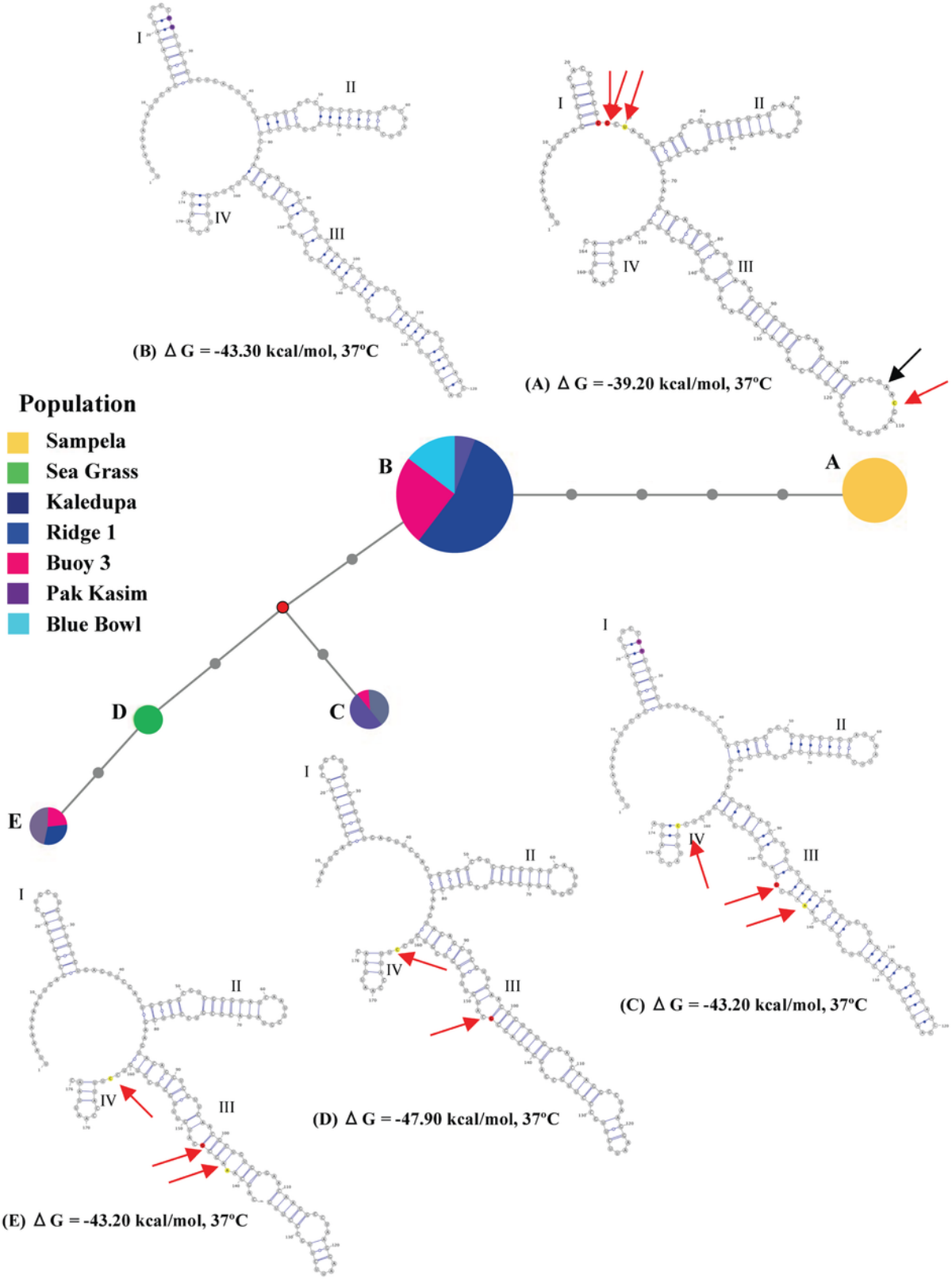
Constrained ordinations (CAP) of *Isis hippuris* character traits between Ridge 1 [◆] and Sampela [▲]. Vector abbreviations as described in text.



4

Isis haplotype network and ITS2 RNA predicted secondary structure.

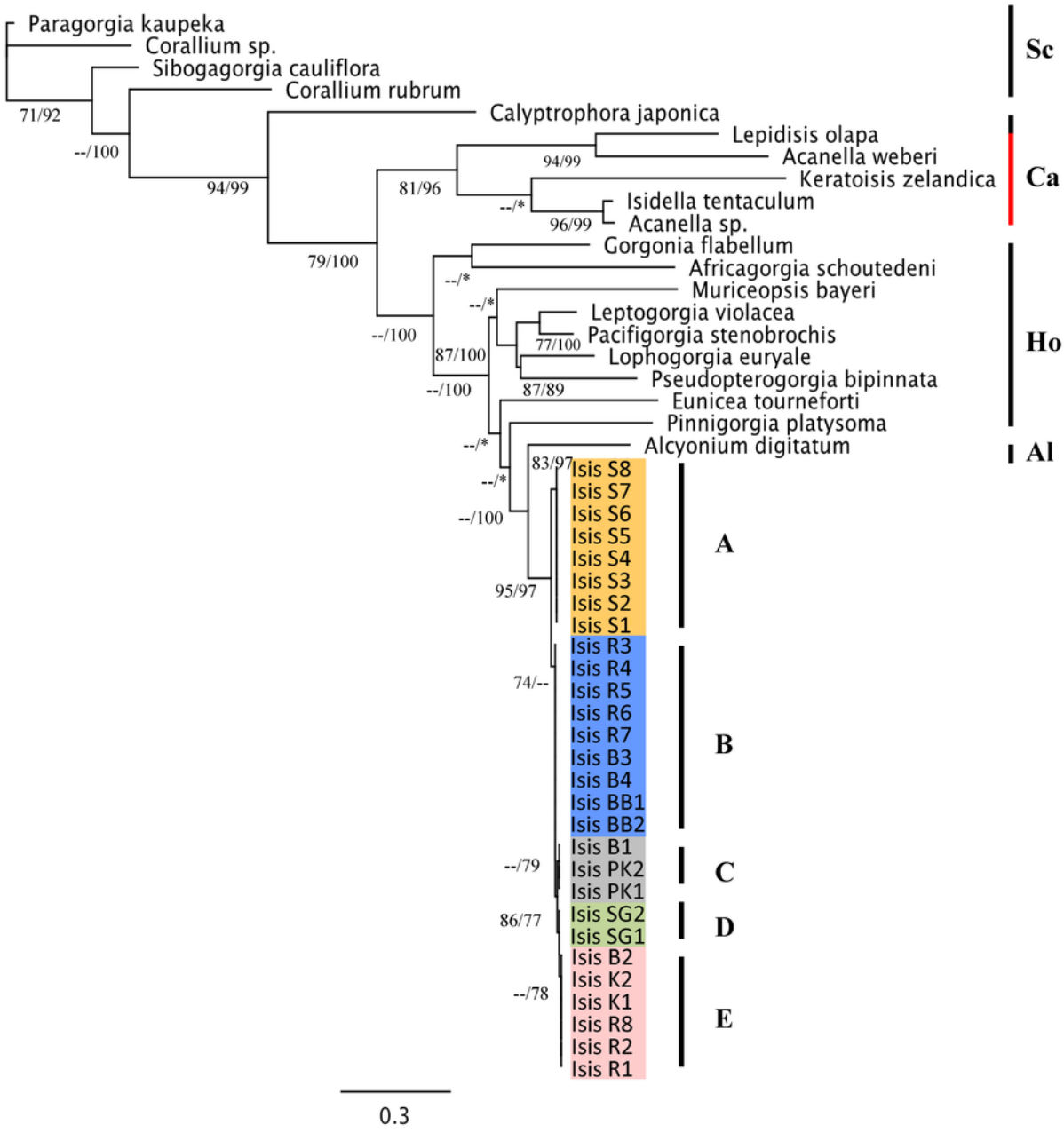
Isis haplotype network with corresponding ITS2 RNA predicted secondary structure relative to haplotype (A – E, see also Figure 5) and enthalpy values according to MFOLD. Roman numerals (I – IV) represent helices; red and black arrows indicate point mutations and loop differences, respectively. Coloured bases according to transitions (red), transversions (yellow) and gaps (lilac), S5. Haplotype circle diameters are proportional to identical clone sequences.


Population
Sampela
Sea Grass
Kaledupa
Ridge 1
Buoy 3
Pak Kasim
Blue Bowl
A
B
C
D
E
I
II
III
IV
(A) Δ G = -39.20 kcal/mol, 37°C
(B) Δ G = -43.30 kcal/mol, 37°C
(C) Δ G = -43.20 kcal/mol, 37°C
(D) Δ G = -47.90 kcal/mol, 37°C
(E) Δ G = -43.20 kcal/mol, 37°C

5

Phylogram of *Isis* haplotypes and Octocoral out groups.

Phylogram based on Maximum Likelihood (ML) analyses of the ITS2 region from twenty Octocoral taxa in GenBank and *Isis* haplotypes within the WMNP. Branch numbers represent ML bootstrap support and BI posterior probabilities, respectively, with low values expressed as a hyphen (--) ≤ 70% and asterisk (*) indicative of differences from MrBayes phylogenetic inference. Letters Sc = Scleraxonia, Ca = Calcaxonia, Ho = Holaxonia, Al = Alcyoniina, and A – E represent *Isis* haplotypes as depicted in Figure 4.


Paragorgia kaupeka
Corallium sp.
Sibogagorgia cauliflora
Corallium rubrum
Calyptrophora japonica
Lepidisis olapa
Acanella weberi
Keratoisis zelandica
Isidella tentaculum
Acanella sp.
Gorgonia flabellum
Africagorgia schoutedeni
Muriceopsis bayeri
Leptogorgia violacea
Pacifigorgia stenobrochis
Lophogorgia euryale
Pseudopterogorgia bipinnata
Eunicea tourneforti
Pinnigorgia platysoma
Alcyonium digitatum
Isis S8
Isis S7
Isis S6
Isis S5
Isis S4
Isis S3
Isis S2
Isis S1
Isis R3
Isis R4
Isis R5
Isis R6
Isis R7
Isis B3
Isis B4
Isis BB1
Isis BB2
Isis B1
Isis PK2
Isis PK1
Isis SG2
Isis SG1
Isis B2
Isis K2
Isis K1
Isis R8
Isis R2
Isis R1
71/92
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81/96
--/*
96/99
94/99
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87/100
77/100
--/100
87/89
--/*
--/*
--/100
83/97
95/97
74/--
--/79
86/77
--/78
Sc
Ca
Ho
Al
A
B
C
D
E
0.3