We conducted the first ever survey of xeniid octocorals in the Indonesian Archipelago, centre of the highly biodiverse Coral Triangle region of the Indo-Pacific. Among 48 xeniid specimens collected from Lembeh Strait, North Sulawesi, we identified 26 morphospecies belonging to six genera based on assessment of the morphological characters traditionally used for xeniid taxonomy. Multilocus DNA barcodes obtained from 23 morphospecies clustered into 21 molecular operational taxonomic units (MOTUs) separated by average genetic distance values >0.3%. The overall concordance between morphospecies and MOTUs was 91%; just one pair and one trio of morphospecies were not distinguished by the DNA barcodes. A molecular phylogenetic reconstruction of family Xeniidae based on four loci (COI, mtMutS, ND2, 28S rDNA) supported the distinction of Anthelia and Cespitularia plus Efflatounaria from all other xeniid genera. Although the remaining genera for which molecular data were available (Asterospicularia, Heteroxenia, Ovabunda, Sansibia, Sarcothelia, Sympodium, Xenia) belonged to a single, well-supported clade, the phylogenetic relationships among them were poorly resolved. Species of Xenia were distributed among three different sub-clades within which they were paraphyletic with Ovabunda (clade X1), Heteroxenia (clade X2) and Sansibia plus Sarcothelia (clade X3). No morphological characters have yet been identified that differentiate these three phylogenetically distinct clades of Xenia. Use of molecular barcodes to discriminate species will facilitate future ecological studies of Xeniidae, a group that has been shown to opportunistically monopolize disturbed reef habitat.

Key words: 28S rDNA, Cnidaria, COI, coral reef, molecular phylogenetics, mtMutS, ND2, Xenia
distinguish species in most other groups of octocorals, are homogeneous and simple in structure, usually consisting solely of minute oval platelets less than 20 μm in diameter (Fabricius & Alderslade, 2001). As a result of this lack of variation in sclerite form, xenid taxonomy has relied on a handful of soft tissue characters, such as the size of colonies, polyps and tentacles, numbers of rows of pinnules and numbers of pinnules per row on the tentacles, as well as characters such as colour and pulsation behaviour that can only be assessed in live material (Halász et al., 2013). All of these characters are variable within species, overlap in range among species, and the degree to which they are influenced by the environment has never been assessed (Reinicke, 1997). There is growing recognition that the crystalline ultrastructure of the sclerite surface may be taxonomically important (Alderslade, 2001; Janes, 2008; Benayahu, 2010; Aharonovich & Benayahu, 2011; Halász et al., 2013), but this character can only be evaluated with the use of scanning electron microscopy.

Increasingly, molecular data are being used to evaluate species boundaries and to assist in the identification of groups such as octocorals in which morphological characters often do not distinguish species reliably. DNA barcoding has been proposed as a tool to facilitate identification of species (Hebert et al., 2003), and more controversially (e.g. DeSalle et al., 2005; Will et al., 2005; Rubinoff et al., 2006), to discover new species. As a consequence of their unusually slow rates of mitochondrial gene evolution (Shearer et al., 2002; Hellberg, 2006; Huang et al., 2008; Chen et al., 2009), however, the development of species-specific DNA barcodes for anthozoan cnidarians has lagged behind that of other groups (Bucklin et al., 2010). Nonetheless, several recent studies have identified multilocus barcodes that distinguish morphospecies of octocorals with relatively high (70–80%) success (McFadden et al., 2011; Baco & Cairns, 2012). In particular, a barcode that combines cytochrome oxidase I (COI) plus an adjacent intergenic region (igrl), the octocoral-specific mitochondrial mutS gene (mtMutS) and a fragment of the nuclear 28S ribosomal RNA gene has been shown to distinguish molecular operational taxonomic units (MOTUs; Floyd et al., 2002) that are in agreement with >70% of morphospecies identifications across a wide taxonomic range of shallow-water octocorals (McFadden et al., 2014). In most octocoral taxa, we cannot currently determine if the ~30% of cases in which there is disagreement between molecular and morphological species identifications are due to inadequacies of the molecular markers or instead to flawed interpretations of morphologically defined species boundaries.

Both the ecology and taxonomy of Xenidae have been widely studied within the Red Sea, with 29 species belonging to five genera having been recorded there (Reinicke, 1997). In contrast, relatively little is known of xenid diversity in the Coral Triangle, that area centred on the Indonesian Archipelago within which the species diversity of zooxanthellate scleractinian corals reaches its maximum (Veron et al., 2009). Schenk (1896) described eight species of Xenia Lamarck, 1816 and one Anthelia Lamarck, 1816 from Ternate Is. in eastern Indonesia. Working from existing museum collections, Roxas (1933) later recorded approximately 40 species of xenids in five genera from Puerto Galera, Mindoro, Philippines. The only modern-day biodiversity surveys from the Coral Triangle region that have identified xenids to species are from the Bismarck Sea (13 species in five genera; Ofwegen, 1996) and Taiwan (11 species in six genera; Benayahu et al., 2004). Although a few other recent studies have documented the occurrence of particular species of Xenidae in Indonesia (Verseveldt, 1960; Imahara, 1996; Stemmer et al., 2013), to date no systematic biodiversity surveys of the family have been conducted anywhere within the Indonesian Archipelago. Here we document a diverse assemblage of Xenidae found in Lembeh Strait, North Sulawesi. We use both morphological and molecular criteria to estimate species richness, discuss cases of non-concordance between the two types of evidence, and present the first molecular phylogenetic reconstruction of the family Xenidae to include representatives from 10 of the 17 known genera.

**Materials and methods**

**Collection and morphological assessment**

Xenid octocorals were collected in May 2009 from Lembeh Strait, between Lembeh Island and the coast of North Sulawesi, Indonesia. Dives were made to a maximum depth of 30 m using SCUBA at 28 stations spanning approximately 5 km of coastline on either side of the northern half of the Strait. Colonies were photographed in situ prior to being collected; they were preserved in 90% EtOH immediately following collection. Vouchers of all material have been deposited at the California Academy of Sciences (CASIZ).

Specimens were identified to morphospecies based on assessment of the following character set typically used for taxonomy of Xenidae (e.g. Reinicke, 1997; Halász et al., 2013): overall colony size and growth form; length of polyps and tentacles, numbers of rows of pinnules on the tentacles, and numbers of pinnules in the aboral row; size, shape and crystalline ultrastructure of sclerites in the polyps and colony stalk; and colour in life (Table 1). To facilitate counting pinnules, polyps were stained with acid fuchsin and mounted on glass microscope slides using Durecupan AMC mounting medium (Fabricius & Alderslade, 2001). Sclerites were obtained by dissolving tissue in 10% sodium hypochlorite (household bleach). Sclerites were rinsed well with deionized water, dried, and mounted on stubs for SEM. They were imaged using a JEOL 6480LV Scanning
Table 1. Morphological characters of xeniid species collected from Lembeh Strait, North Sulawesi, Indonesia. # p/s = relative abundance of sclerites in polyp and stalk (+ high, – low)

| Species | Colony form | Pinnules | Sclerites |
|---------|-------------|----------|-----------|
|         | # of rows | # in outer | In situ colour | # of forms | Polyp Max | Base Max | # p/s | Shape | Ultra-structure |
|          |          | outer row | polyp/stalk | | L x W (µm) | L x W (µm) | | |
| Genus Anthelia: polyp clusters united by thin membrane or stolons, rod-like sclerites |
| A. philippinense Roxas, 1933 | Basal membrane | 2 | 10–13 | Tan | 1 | 60 × 11 | 60 × 11 | +/- | Flattened cigar | Herringbone points |
| A. ternatana Schenk, 1896 | Basal membrane | 1 | 36–45 | Creamish pink | 1 | 60 × 13 | 60 × 13 | +/- | Flattened cigar | Herringbone points |
| Anthelia sp. 1 | Ribbons | 1 | 7–8 | Pinkish white | 1 | 36 × 13 | 55 × 12 | +/- | Flattened cigar | Herringbone scales |
| Anthelia sp. 2 | Basal membrane | 1 | 19–21 | Light pink | 1 | 63 × 10 | 60 × 13 | +/- | Flattened cigar | Herringbone points |
| Genus Cespitularia: soft, branched colonies with polyps distributed on branches |
| C. simplex Thomson & Dean, 1931 | Branched lobes | 1 | 12–15 | Pink/purple-pink | 1 | 25 × 25 | 31 × 31 | +/- | Spherical | Ultra-fine papillate |
| Genus Heteroxenia: branched or unbranched stalks with polyps on distal capitulum, polyps dimorphic |
| H. elizabethae Köllicher, 1874 | Unbranched stalks | 3(4) | 18–26 | Light brown/cream | 1 | 22 × 15 | 25 × 18 | +/- | Oval platelets | Fine papillate |
| H. mindorensis Roxas, 1933 | Unbranched stalks | 3 | 21–24 | Brown/whitish | 1 | 21 × 15 | 22 × 16 | +/- | Oval platelets | Fine papillate |
| Heteroxenia sp. 1 | Unbranched stalks | 2(3) | 21–24 | Brown/cream | 1 | 22 × 19 | 25 × 18 | +/- | Oval to irregular | Tooth-like |
| Genus Sansibia: small, non-retractile polyps arising directly from ribbon-like basal membrane |
| Sansibia sp. 1 | Ribbons | 2 | 4–5 | Light blue/white | 1 | 23 × 19 | 23 × 22 | +/- | Oval platelets | Loose papillate |
| Genus Sympodium: small, retractile polyps arising from encrusting membrane |
| S. caeruleum Ehrenberg, 1834 | Basal membrane | 2 | 4–6 | Brown/light blue | 1 | 20 × 18 | 18 × 15 | +/- | Oval platelets | Fine papillate |
| Genus Xenia: branched or unbranched stalks with polyps on distal capitulum, polyps monomorphic |
| X. felicianoi Roxas, 1933 | Unbranched stalks | 3–4 | 28–32 | Brown/white | 1 | 18 × 13 | 18 × 13 | +/- | Oval platelets | Coarse crust-like |
| X. fisheri Roxas, 1933 | Unbranched stalks | 2(3) | 16–21 | Pink/white | 1 | 23 × 13 | 25 × 15 | +/- | Oval platelets | Fine papillate |
| X. kusimotoensis Utinomi, 1955 | Low branched stalks | 2 | 10–12 | Blue-grey/whitish | 1 | 22 × 16 | 22 × 16 | +/- | Oval platelets | Fine papillate |
| X. lepida Verseveldt, 1971 | Branched or unbranched stalks | 3 | 28–31 | Cream/whitish | 1 | 22 × 17 | 22 × 17 | +/- | Oval platelets | Fine papillate |
| X. lillieae Roxas, 1933 | Branched stalks | 2 | 10–12 | Brown/yellow | 2 | 15 × 11 | none | 0 | Oval platelets | Fine papillate |
| X. membranacea Schenk, 1896 | Branched stalks | 3(4) | 23–27 | Tan/cream | 1 | 25 × 17 | 26 × 18 | +/- | Oval platelets | Fine papillate |
| X. puerto-galerae Roxas, 1933 | Branched stalks | 2 | 17–20 | Tan/pinkish | 1 | 21 × 16 | 22 × 18 | +/- | Oval platelets | Coarse papillate |

(continued)
Electron Microscope at 10 kV, an FEI XL30 Environmental Scanning Electron Microscope at 15 kV and a JEOL JSM840A Scanning Electron Microscope at 25 kV.

Molecular phylogenetic analyses

Extraction of DNA from ethanol-preserved tissue samples, PCR amplification and sequencing of the mitochondrial mtMutS (msh1), COI + igr1 and ND2 genes followed the protocols published in McFadden et al. (2006, 2011). In addition, we sequenced an approximately 750 nt fragment of the 28S nuclear ribosomal gene using primers 28S-Far and 28S-Rar (McFadden & Ofwegen, 2013). Sequences were aligned using the L-INS-i method in MAFFT (Katoh et al., 2005), and pairwise genetic distances (Kimura 2-parameter) among specimens were calculated using the DNADist program in PHYLIP v. 3.69 (Felsenstein, 2005). MOTHUR v. 1.29 (Schloss et al., 2009) was used to cluster sequences into MOTUs based on an average neighbour distance threshold of 0.3%, a value that has been shown previously to yield a high concordance between molecular and morphological identifications in other octocoral families (McFadden et al., 2014). The concordance of species identifications was estimated as the percentage of specimens for which molecular and morphospecies classifications were in agreement. For example, if a MOTU included three specimens of morphospecies A and one specimen of morphospecies B it would be counted as three concordant identifications and one non-concordant identification for a total concordance of 75%. If a fourth individual of morphospecies A belonged to a different MOTU it would also be counted as a non-concordant identification, for an overall concordance of 60% among the five specimens.

GARLI 2.0 (Zwickl, 2006) was used to construct Maximum Likelihood trees following selection of appropriate models of evolution using Modeltest 3.0 (Posada & Crandall, 1998). Because of a lack of congruence between mitochondrial and nuclear gene trees, we constructed two separate trees, one for 28S rDNA (GTR+I+G model) and another for the three mitochondrial genes (mtMutS, COI + igr1, ND2) concatenated (HKY+I+G model). Analyses were run for 1000 bootstrap replicates. Bayesian analyses of both datasets were conducted separately with MrBayes v. 3.2.1 (Ronquist et al., 2012) using either the GTR+I+G (28S) or HKY+I+G (mt genes) models of evolution; analyses were run for 2 million generations (until standard deviation of split partitions < 0.005) with a burn-in of 25% and default Metropolis coupling parameters (i.e. 2 runs, 4 chains (3 heated), sample frequency = 500 generations). Several xeniid sequences published previously by the first author (McFadden et al., 2006) were included in the phylogenetic analyses to increase the representation of xeniid genera (Appendix S1, see online supplemental material, which is available from the...
article’s Taylor & Francis Online page at http://dx.doi.org/10.1080/14772000.2014.902866. Trees were rooted using the genera Coelogorgia Milne-Edwards & Haime, 1857, Para-lemnalia Kükenthal, 1913 and Rhytisma Alderslade, 2000 as outgroup taxa; previous molecular phylogenetic analyses have identified these taxa as the sisters to Xeniiidae (McFadden et al., 2006).

Results

Morphospecies identifications

Among the 48 xeniid specimens collected, 26 distinct morphospecies belonging to six genera were identified (Table 1). These included 16 morphospecies of Xenia, four morphospecies of Anthelia, three morphospecies of Heteroxenia Kolliker, 1874, and one morphospecies each of Cespitularia Milne-Edwards & Haime, 1857, Sanssibia Alderslade, 2000 and Sympodium Ehrenberg, 1834. Fifteen morphospecies were tentatively identified to nominal taxa by comparison to original species descriptions or, in a few cases, to type material (Table 1). Eleven morphospecies, however, could not be matched to existing species accounts, and are provisionally considered to represent new, undescribed taxa. A complete taxonomic treatment of the material will be published elsewhere (Janes et al., pers. comm.).

Species discrimination using DNA barcodes

DNA sequences were obtained from 44 specimens representing 24 of the 26 morphospecies collected in Lembeh; complete 4-locus genotypes (~2996 bp) were obtained for 35 specimens (Appendix S1, see supplemental material online). No DNA sequences were obtained from the morphospecies identified as Xenia felicianoi Roxas, 1933 and Anthelia sp. 2, and only ND2 was obtained from Sympodium caeruleum Ehrenberg, 1834. ND2 exhibited less variation than the other three loci, and consequently was less informative as a species-specific marker (Table 2). For example, eight morphospecies belonging to three different genera all shared an identical ND2 haplotype. Among the various combinations of markers and genetic distance thresholds we tested (Table 2), both species richness accuracy (MOTUs: morphospecies ratio) and concordance were highest using the combined mt + 28S barcode (mt = COI + igr1 + mtMutS; McFadden et al., 2011) at an average genetic distance threshold of 0.3%. This DNA barcode distinguished 21 MOTUs among the 23 morphospecies for which we had complete or partial sequence data. Morphospecies identified as Xenia sp. 1, X. sp. 2 and X. sp. 6 belonged to the same MOTU, as did Xenia sp. 3 and X. sp. 4. One of the seven specimens identified as X. membranacea Schenk, 1896 (CASIZ 184563) belonged to a separate MOTU from the remaining six. In all other cases, morphospecies identifications were concordant with MOTUs, for an overall concordance of 91% (Table 2).

Molecular phylogenetic analysis

Phylogenetic reconstructions using maximum likelihood and Bayesian methods yielded similar tree topologies, and differed only in relative support for some of the deeper nodes within the tree (Figs 1, 2). In general, support values from maximum likelihood tended to be lower than those from Bayesian analyses. The separate mitochondrial and 28S rDNA gene trees supported most of the same clades, and differed only in the phylogenetic placement of a few species. All analyses supported three well-defined, major clades within Xeniiidae: a monophyletic Anthelia, further distinguished from all other genera by a unique 6-bp insertion in the intergenic region just upstream of ND2 and a unique amino acid insertion in mtMutS (Fig. 1); Cespitularia plus Efflatounaria Gohar, 1939; and a third large clade comprising all of the remaining genera. Within the latter clade, five distinct sub-clades were resolved, although levels of support for these sub-clades

Table 2. Summary of MOTUs estimated using different barcode markers and detection thresholds (average genetic distance values, Kimura 2-parameter). S = number of morphospecies for which barcode was obtained; #MOTU = number of molecular operational taxonomic units separated by specified genetic distance threshold; SA (species richness accuracy) = #MOTU/S; concordance = fraction of specimens for which MOTU and morphospecies assignments are in agreement (see text). Bold font: barcode with highest SA and concordance values.

| Barcode   | S     | #MOTU | SA  | concordance | #MOTU | SA  | Concordance |
|-----------|-------|-------|-----|-------------|-------|-----|-------------|
| mtMutS    | 21    | 15    | 0.71| 0.77        | 12    | 0.57| 0.67        |
| 28S       | 22    | 18    | 0.82| 0.84        | 17    | 0.77| 0.79        |
| ND2       | 23    | 10    | 0.43| 0.54        | 7     | 0.30| 0.47        |
| mt         | 22    | 16    | 0.73| 0.81        | 14    | 0.64| 0.71        |
| mt + 28S  | 23    | 21    | 0.91| 0.91        | 16    | 0.70| 0.70        |
| mt + 28S + ND2 | 24 | 19 | 0.79 | 0.89 | 13 | 0.54| 0.66        |

a mt = mtMutS + igr1 + COI
differed among analyses, and the phylogenetic relationships among them were unresolved. The genera *Sympodium* and *Asterospicularia* Utinomi, 1951, each represented by only a single individual in our analyses, both fell out as unique sub-clades. The other three sub-clades (X1, X2, X3) each included a mix of *Xenia* species plus members of other genera (Figs 1, 2).

Clade X1 included *X. fisheri* Roxas, 1933, *X. lepida* Verseveldt, 1971, *X. viridis* Schenk, 1896, *X. hicksoni* Ashworth, 1899 and *X. sp. 7* along with members of genus *Ovabunda* Alderslade, 2001. Strong support for this clade was provided by a unique 24-bp deletion in the intergenic region upstream of the ND2 coding region that was shared only by these species (Fig. 1). Both mt and 28S gene trees positioned the small clade comprising *Xenia* sp. 3 and *X. sp. 4* as the sister to clade X1, although the 28S tree supported that relationship only weakly and neither of those two species shared the 24-bp ND2 deletion with clade X1 (Fig. 1). The two gene trees also differed with respect to the position of *X. ternatana* Schenk, 1896, which lay within clade X1 as a sister to *X. fisheri* in the mt gene tree, but outside of that clade in the 28S tree. *Xenia ternatana* does share the 24-bp deletion with other clade X1 species, supporting its position within rather than outside of that clade (Fig. 1).

Clade X2 comprised all four species of *Heteroxenia*, plus *X. puerto-galerae* Roxas, 1933 and *Xenia* sp. 1, *X. sp. 2* and *X. sp. 6*. The 28S analysis also included *X. lillieae* Roxas, 1933 within this clade, but with lower overall support values for the clade. All nine of these species, however, shared a unique single amino acid deletion in mtMutS, a synapomorphy that supports the inclusion of *X. lillieae* in clade X2 (Fig. 1). The mt gene tree provided strong support for the monophyly of *Heteroxenia* as the
sister to the Xenia species within the clade, while the 28S tree only supported the monophyly of three of the four Heteroxenia species, excluding H. elizabethae Kolliker, 1874 (Fig. 2).

Clade X3 encompassed X. membranacea Schenk, 1896, X. kusimotoensis Utinomi, 1955 and Xenia sp. 5, a group whose monophyly was well supported by the 28S analyses (Fig. 2). The mt gene tree weakly supported the inclusion of these species in a larger clade with members of Sansibia and Sarcothelia, two genera for which 28S sequences were unavailable (Fig. 1).

In addition to the phylogenetic positions of X. ternatana, X. lillieae and H. elizabethae, the relationship between the family Xenidae and the outgroup taxa also differed between 28S and mt gene trees. The mt tree strongly supported the monophyly of Xenidae (Fig. 1). In the 28S tree, however, Coelogorgia palmosa (family Coelogorgiidae) fell within Xenidae as the sister taxon to Cespitularia, albeit with low maximum likelihood bootstrap support (Fig. 2).

Discussion
DNA barcoding in Xenidae

The concordance between morphospecies identifications and molecular classification into MOTUs obtained here using the mt + 28S DNA barcode is the highest yet achieved in any application of DNA barcoding to octocorals. McFadden et al. (2011) were able to distinguish 69% of morphospecies using the mt barcode (COI + igr1 + mtMutS) in a biodiversity study of Red Sea octocorals. Concordance between morphospecies and molecular identifications was a slightly higher 74% when the combined mt + 28S barcode was used to discriminate species in a taxonomically comprehensive survey of octocorals in Palau (McFadden et al., 2014). Baco & Cairns (2012) resolved 83% of morphospecies belonging to the deep-water octocoral genus Narella using an extended mitochondrial gene barcode of mt + ND2. The 91% concordance we obtained here using the mt + 28S barcode is not, however, directly comparable to our earlier estimates.
using the mt and mt + 28S markers (McFadden et al., 2011, 2014) which may have underestimated the concordance between morphospecies and MOTUs. Both of those studies were conducted blind (morphospecies were assessed independently of MOTUs by separate researchers) and no attempt was made to subsequently reconcile any disagreements between methods. In the present study, however, we both re-evaluated morphospecies assignments and re-sequenced some specimens in an attempt to reconcile the two different types of evidence for species boundaries. Only in the cases of *Xenia* sp. 1, 2 and 6, and *Xenia* sp. 3 and 4 were we unable to bring the morphological and molecular data into agreement.

A recent study by Stemmer et al. (2013) tested the ability of two different loci, a fragment of the mitochondrial ND6/ND3 region and the nuclear SRP54 gene, to discriminate species of xeniid octocorals. Their tree-based analysis is difficult to compare directly to our results, but if their ‘distinct clades’ are considered to represent MOTUs, they found that ND6/ND3 discriminated only six MOTUs and SRP54 discriminated nine MOTUs among the 14 morphospecies they identified. For SRP54, it appears that only two morphospecies were fully concordant with MOTUs – all other morphospecies were either divided among multiple MOTUs or multiple morphospecies belonged to the same MOTU. The much higher concordance between morphospecies and MOTUs we found among the samples from Lembeh suggests that mt + 28S is a more reliable species-specific barcode for xenidi than the more variable SRP54 gene.

Three unidentified morphospecies of *Xenia* from Lembeh, designated *X* sp. 1, *X* sp. 2 and *X* sp. 6, belonged to the same MOTU, sharing identical mtMutS and 28S sequences, and differing from one another by genetic distances of only 0.1% at COI. These three morphospecies are, however, morphologically quite distinct: *X* sp. 6 has two rows of pinnules plus a partial third row, whereas *X* sp. 1 and *X* sp. 2 both have only a single row of pinnules. *X* sp. 1 and *X* sp. 2 in turn differ from one another in average numbers of pinnules (18–20 vs. 28–31), and in the density as well as the ultrastructure of sclerites in the colony (Table 1). Two additional unidentified morphospecies, *Xenia* sp. 3 and *X* sp. 4, also belonged to a single MOTU, sharing identical genotypes at all loci. Although these two morphospecies both have two rows and similar numbers of pinnules, they differ markedly in sclerite complement: *X* sp. 4 has sclerites distributed densely throughout the colony, whereas *X* sp. 3 lacks sclerites entirely (Table 1). Based on the morphological characters traditionally used for xeniid taxonomy, each of these five morphospecies would be considered distinct. Further integrative taxonomic studies are needed to determine if these are indeed different species that have not yet diverged genetically at the particular loci we sequenced, or, alternatively, if the morphological differences we observed could be the result of morphological plasticity or ontogenetic variation within species. Support for the latter case will necessitate a radical reinterpretation of 150 years of xeniid taxonomy.

### Phylogenetic relationships within Xeniiidae

The molecular phylogeny presented here is the first to be published for Xeniiidae that includes more than one or a small number of species from more than just a few genera (McFadden et al., 2006, 2011; Stemmer et al., 2013). The results from the mitochondrial gene analyses strongly support the monophyly of the family. Although the earlier analyses of McFadden et al. (2006) using just mtMutS and ND2 placed *Anthelia* outside of Xeniiidae, the one *Anthelia* specimen sequenced for that study is now suspected to have been either misidentified or a contaminant. Neither the ND2 nor mtMutS sequences from that individual fall within the clade of *Anthelia* presented here (C.S. McFadden, unpubl. data). The molecular phylogeny also supports the monophyly of the genera *Anthelia* and *Cespitularia*+*Efflatounaria*, and positions them as sister clades to all remaining xeniid genera. The morphological distinctions between *Cespitularia* and *Efflatounaria* are unclear (Fabricius & Alderslade, 2001; Benayahu & McFadden, 2011), therefore it is not surprising that there also appears to be little or no phylogenetic distinction between them. The phylogenetic relationships among the six other genera of xenidi included in our analyses are poorly resolved, although both *Sympodium* and *Asterospicularia* appear to be well differentiated genetically from other genera (Fig. 1, 2). *Sympodium* is unique among xeniiids in having fully retractile polyps, while the stellate sclerites of *Asterospicularia* are so different from those typical of other Xeniiidae that until recently it was placed in its own family (Alderslade, 2001).

Neither the mitochondrial nor the 28S rDNA gene trees support the monophyly of *Xenia*, with both analyses instead suggesting that this genus comprises at least three distinct clades that may be paraphyletic with other genera. The most strongly supported clade, X1, is paraphyletic with *Ovabunda*, a genus that was recently split from *Xenia* on the basis of its distinctive sclerite microstructure (Alderslade, 2001). There is, however, relatively little genetic differentiation between *Ovabunda* species and several species of *Xenia* from the Red Sea, represented in our analyses by *X. hicksoni* (Haverkort-Yeh et al., 2013). A second clade of *Xenia*, X2, appears to be paraphyletic with *Heteroxenia*, a genus that differs from *Xenia* by having dimorphic polyps (i.e. siphoneozooids in addition to autozooids). This trait may, however, vary seasonally or ontogenetically (Gohar, 1940; Achituv & Benayahu, 1990; Fabricius & Alderslade, 2001), and when siphoneoizooids are absent in *Heteroxenia* the two genera are
indistinguishable. A third clade of *Xenia*, X3, may be paraphyletic with *Sansibia* and *Sarcothelia*, two similar genera in which polyps arise from a membrane rather than a stalk. The relationships among clades X1, X2, X3, *Symposium*, *Asterospecificaria* and several additional species of *Xenia* that do not fall cleanly into any one of the clades (e.g. *X.* sp. 3, *X.* sp. 4) are, however, poorly resolved in both gene trees. Additional evidence will be necessary to confirm and explore further the apparent polyphyly of *Xenia*, as well as the relationships among *Xenia*, *Heteroxenia*, *Ovabunda*, *Sansibia* and *Sarcothelia*. At present, no obvious morphological differences distinguish the three clades of *Xenia* from one another; they all include stalked colonies with monomorphic polyps situated on a distinct capitulum, whose sclerites are typical oval forms with a microstructure composed of dendritic rods (Table 1).

The molecular phylogeny presented here does not include representatives of an additional seven genera of Xeniidae for which DNA samples are not currently available, including *Bayerxenia* Alderslade, 2001, *Fascicilia* Janes, 2008, *Funginus* Tixier-Durivault, 1978, *Ixion* Alderslade, 2001, *Ingotia* Alderslade, 2001, *Orangaslzia* Alderslade, 2001 and *Yamazatum* Benayahu, 2010. Six of these genera have been described only recently to accommodate species that have unique sclerite microstructure and/or multiple sclerite forms within a colony. Their phylogenetic relationships to other genera remain unknown. *Ixion*, *Ingotia* and *Orangaslzia* all share a similar colony growth form with *Anthelia* but harbour unique sclerite forms (Alderslade, 2001), *Fascicilia* has a stalked growth form similar to *Xenia* but with sclerites resembling those of *Anthelia* (Janes, 2008), and *Yamazatum* also resembles *Xenia* but has a unique sclerite form (Benayahu, 2010). Finally, *Bayerxenia* shares a stalked growth form and polyp dimorphism with *Heteroxenia*, but differs from that genus by having multiple distinct sclerite forms within a colony (Alderslade, 2001). Stammer et al. (2013) included three morphospecies they identified as *Bayerxenia* in their molecular analyses, and could not distinguish them phylogenetically from other species of *Heteroxenia* and *Xenia*. Because they sequenced a different set of genes, we were unable to include their material in our analyses for comparison. The species we identified as *Heteroxenia* sp. 1 has sclerites with microstructure similar to that of *Bayerxenia* but lacks the multiple sclerite forms that are an important diagnostic character of that genus (Table 1). *Heteroxenia* sp. 1 was not distinguishable phylogenetically from other species of *Heteroxenia*. Although sclerite ultrastructure, which can be observed only by using SEM, has been proposed recently to be an important genus-level taxonomic character in Xeniidae (Alderslade, 2001; Janes, 2008; Benayahu, 2010; Aharonovich & Benayahu, 2011; Halász et al., 2013), the phylogenetic distinction of species with unique sclerite ultrastructures has yet to be demonstrated.

**Xenid biodiversity in the Coral Triangle**

To date, information on the xenid fauna of the central Indo-Pacific has been limited, especially compared with the extensive studies that have been carried out on this family in the Red Sea (Gohar, 1940; Benayahu, 1990; Reinicke, 1995, 1997; Halász et al., 2013) and the western Indian Ocean (Benayahu et al., 2003; Janes, 2008). The results of our survey of Lembeh Strait suggest that this one very small area within the Indonesian Archipelago supports as many species as the entire Red Sea. Although 29 species of Xenidae have been recorded within the Red Sea (and unverified records exist for another five species; Reinicke, 1997), recent taxonomic work has synonymized several species in genus *Ovabunda* (Halász et al., 2013), thereby reducing the estimated species richness of that region by four. Our estimates of the richness of xenids in Lembeh Strait based on a combination of morphological and molecular data range from 23 to 26 species. It is likely that more extensive surveys over a broader geographic scale within the Coral Triangle will discover many additional species of xenids. For instance, only two of the nine species described by Schenk (1896) from nearby Ternate Is. and only 10 of the ~40 species recorded by Roxas (1933) from the Philippines were found in Lembeh Strait. Eleven of the 26 morphospecies we collected could not be matched to any nominal species of Xenidae and may represent yet undescribed species. Taken together, the results of our survey plus previous records of Xenidae suggest the potential for a high species richness within this understudied family in the Coral Triangle.

The high diversity of xenid species on Indo-Pacific reefs in turn suggests the need for more detailed taxon-specific ecological studies. Studies that have reported instances of xenids opportunistically recruiting to disturbed reef habitat and either inhibiting or facilitating the recovery of scleractinians have typically only identified the family (Wood & Dipper, 2008; Tilott et al., 2008) or genus (Fox et al., 2003). It is unknown, therefore, if a single opportunistic species of xenid is responsible for monopolizing space on disturbed reefs, or if a diverse assemblage of ecologically similar species contributes to the high cover of xenids in such situations. DNA barcoding using the mt + 28S marker promises to greatly facilitate the discrimination and identification of species in this taxonomically confusing group, and provide needed insights into the community dynamics of disturbed reef habitats.

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References

ACHITUV, Y. & BENAYAHU, Y. 1990. Polyp dimorphism and functional, sequential hermaphroditism in the soft coral *Heteroxenia fuscescens* (Octocorallia). *Marine Ecology Progress Series* 64, 263–269.

AHARONOVICH, D. & BENAYAHU, Y. 2011. Microstructure of octocoral sclerites for diagnosis of taxonomic features. *Marine Biodiversity*. ALDERSLADE, P. 2001. Six new genera and six new species of soft corals, and some proposed familial and subfamilial changes within the Alcyonacea (Coeleenterata: Octocorallia). *Bulletin of the Biological Society of Washington* 10, 15–65.

BACO, A.R. & CARNS, S.D. 2012. Comparing molecular variation to morphological species designations in the deep-sea coral *Narella* reveals new insights into seamount coral ranges. *Public Library of Science One* 9, e45555.

BENAYAHU, Y. 1999. Xenidiidae (Cnidaria: Octocorallia) from the Red Sea, with the description of a new species. *Zoologische Mededelingen Leiden*, 64, 113–120.

BENAYAHU, Y. 2010. A new genus of a soft coral of the family Xenidiidae (Cnidaria: Octocorallia) from Japan. *Galaxea, Journal of Coral Reef Studies* 12, 53–64.

BENAYAHU, Y. & LOYA, Y. 1977. Space partitioning by stony corals, and some proposed familial and subfamilial changes within the Alcyonacea (Coeleenterata: Octocorallia). *Annals of the Entomological Society of America* 70, 541–552.

BENAYAHU, Y. & LOYA, Y. 1981. Competition for space among reef sessile organisms at Eilat, Red Sea. *Bulletin of Marine Science* 31, 514–522.

BENAYAHU, Y. & LOYA, Y. 1985. Settlement and recruitment of a soft coral: why is *Xenia macrospiculata* a successful coloniser? *Bulletin of Marine Science* 36, 177–188.

BENAYAHU, Y. & MCFADDEN, C.S. 2011. A new genus of soft coral from the Red Sea, with the description of a new species. *Zoologische Verhandelingen* 345, 49–57.

BENAYAHU, Y., JENG, M-S., PERKOL-FINKEL, S. & DAI, C-F. 2004. Soft corals (Octocorallia: Alcyonacea) from Southern Taiwan, II. Species diversity and distributional patterns. *Zoological Studies* 43, 548–560.

BUCKLIN, A., STENKE, D. & BLANCO-BERCIAL, L. 2010. DNA barcoding of marine metazoans. *Annual Reviews of Marine Science* 3, 471–508.

CHEN, I-P., TANG, C-Y., CHOU, C-Y., HSU, J-H., WEL, N.V., WALLACE, C.C., MUIR, P., WU, H. & CHEN, C.A. 2009. Comparative analyses of coding and noncoding DNA regions indicate that *Acropora* (Anthozoa: Scleractinia) possesses a similar evolutionary tempo of nuclear vs. mitochondrial genomes as in plants. *Marine Biodiversity* 11, 141–152.

DESAULLE, R., EGAN, M.G. & SIDDALL, M. 2005. The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society B*, 360, 1905–1916.

DINESEN, Z.D. 1983. Patterns in the distribution of soft corals across the central Great Barrier Reef. *Coral Reefs* 1, 229–236.

FABRICIUS, K.E. 1997. Soft coral abundance on the central Great Barrier Reef: effects of *Acanthaster planci*, space availability, and aspects of the environment. *Coral Reefs* 16, 159–167.

FABRICIUS, K.E. 1998. Reef invasions by soft corals: which taxa and which habitats? In: GREENWOOD, J.G. & HALL, N.J., Eds., *Proceedings of the Australian Coral Reef Society 75th Anniversary Conference, Heron Island October 1997*. School of Marine Science, The University of Queensland, Brisbane, Australia.

FABRICIUS, K.E. & ALDERSLADE, P. 2001. *Soft Corals and Sea Fans*. Australian Institute of Marine Science, Queensland, Australia.

FELSENSTEIN, J. 2005. *PHYLIP (Phylogeny Inference Package)* version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, Washington, USA.

FLOYD, R., ABEBE, E., PAPERT, A. & BLAXTER, M. 2002. Molecular barcodes for soil nematode identification. *Molecular Ecology* 11, 839–850.

FOX, H.E., PET, J.S., DAIHURI, R. & CALDWELL, R.L. 2003. Recovery in rubble fields: long-term impacts of blast fishing. *Marine Pollution Bulletin* 46, 1024–1031.

GOKHALE, H.A.F. 1940. Studies on the Xeniidae of the Red Sea: their ecology, physiology, taxonomy and phylogeny. *Publications of the Marine Biological Station Ghardaqa (Red Sea)* 2, 25–118.

HALASZ, A., MCFADDEN, C.S., AHIRONOVICH, D., TOONEN, R. & BENAYAHU, Y. 2013. A revision of the octocoral genus *Ovabunda* (Alderslade, 2001) (Anthozoa, Octocorallia, Xenidiidae). *ZooKeys* 373, 1–41.

HAVENKORT-VEEH, R.D., MCFADDEN, C.S., BENAYAHU, Y., BERUMEN, M., HALASZ, A. & TOONEN, R.J. 2013. A taxonomic survey of Saudi Arabian Red Sea octocorals (Cnidaria: Alcyonacea). *Marine Biodiversity* 43, 279–291.

HEIBERT, P.D.N., CYWINSKA, A., BALL, S.L. & DEWAARD, J.R. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B*, 270, 313–321.

HILLBERG, M.E. 2006. No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. *BioMed Central Evolutionary Biology* 6, 24.

HUANG, D., MEIER, R., TODD, P.A. & CHO, L.M. 2008. Slow mitochondrial COI sequence evolution at the base of the metazoan tree and its implications for DNA barcoding. *Journal of Molecular Evolution* 66, 167–174.
Species richness and phylogenetic relationships of Indonesian xenidi

IMAHARA, Y. 1996. Previously recorded octocorals from Japan and adjacent seas. Precious Corals & Octocoral Research 4, 17–44.

JANES, M.P. 2008. A study of the Xenidiidae (Octocorallia, Alcyonacea) collected on the “Tyro” expedition to the Seychelles with a description of a new genus and species. Zoologische Mededelingen Leiden 82, 599–626.

KARLSON, R.H., HUGHES, T.P. & KARLSON, S.R. 1996. Density-dependent dynamics of soft coral aggregations: the significance of clonal growth and form. Ecology 77, 1592–1599.

KATOH, K., KUMA, K., TOH, H. & MIYATA, T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Research 33, 511–518.

McFADDEN, C.S. & OFWEGEN, L.P. VAN 2013. A second, cryptic species of the soft coral genus Incurtus (Anthozoa: Octocorallia: Clavulariidae) from Tierra del Fuego, Argentina, revealed by DNA barcoding. Helgoland Marine Research 67, 137–147.

McFADDEN, C.S., BENAYAHU, Y., PANTE, E., THOMA, J.N., NEVAREZ, P.A. & FRANCE, S.C. 2011. Limitations of mitochondrial gene barcoding in the cnidarian sub-class Octocorallia. Molecular Phylogenetics and Evolution 41, 513–527.

McFADDEN, C.S., OFWEGEN, L.P. VAN 2014. Application of DNA barcoding in biodiversity studies of shallow-water octocorals: molecular proxies agree with morphological estimates of species richness in Palau. Coral Reefs.

OFWEGEN, L.P. VAN 1996. Octocorallia from the Bismarck Sea (part II). Zoologische Mededelingen Leiden 70, 207–215.

POSADA, D. & CRANDALL, K.A. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

REINICKE, G.B. 1995. Xenidiidae des Roten Meeres (Octocorallia, Alcyonacea). Beitrag zur Systematik und Okologie. Essener Okologische Schriften 6, 1–168.

REINICKE, G.B. 1997. Xenidiidae (Coelelterata: Octocorallia) of the Red Sea, with descriptions of six new species of Xenia. Fauna of Saudi Arabia 16, 5–62.

RONQUIST, F., LESLIEKOV, M., VAN DER MARK, P., AYRES, D., DARLING, A., HOSINO, S., LARTIGE, B., LIU, L., SUCHARD, M.A. & HUELENBRECK, J.P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61, 539–542.

ROXAS, H.A. 1933. Philippine Alcyonaria: the families Cornulariidae and Xenidiidae. Philippine Journal of Science 50, 49–110.

RUBINOFF, D., CAMERON, S. & WILL, K. 2006. A genomic perspective on the shortcomings of mitochondrial DNA for “barcoding” identification. Journal of Heredity 97, 581–594.

SCHNITZER, A. 1896. Clavulariiden, Xenien und Alcyonien von Ternate. In: KUKENTHAL, W., Ed., Ergebnisse einer zoologischen Forschungsreise in den Molukken und Borneo, im Auftrage der Senckenbergischen naturforschenden Gesellschaft ausgeführt von Dr Willy Kukenthal, Teil 2, Band 1. Abhandlungen der Senckenberg Gesellschaft für Naturforschung 23, 41–80.

SCHLOSS, P.D., WESTCOTT, S.L., RYABIN, T., HALL, J.R., HARTMANN, M., HOLLISTER, E.B., LESNIEWSKI, R.A., OAKLEY, B.B., PARKS, D.H., ROBINSON, C.J., SAILH, J.W., STRES, B., THALLINGER, G.G., VAN HORN, D.J. & WEBER, C.F. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75, 7537–7541.

SHEARER, T.L., VAN OPPEN, M.H.J., ROMANO, S.L. & WORHEAD, G. 2002. Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). Molecular Ecology 11, 2475–2487.

STEMMER, K., BURGHARDT, I., MAYER, C., REINICKE, G.B., WAGELE, H., TOLLRIAN, R. & LEES, F. 2013. Morphological and genetic analysis of xenid soft coral diversity (Octocorallia: Alcyonacea). Organisms Diversity and Evolution 13, 135–150.

TILOT, V., LEUJAK, W., ORMOND, R.F.G., ASHWORTH, J.A. & MABROUCK, A. 2008. Monitoring of South Sinai coral reefs: influence of natural and anthropogenic factors. Aquatic Conservation: Marine and Freshwater Ecosystems 18, 1109–1126.

VERON, J.E.N., DEVANTIER, L.M., TURAK, E., GREEN, A.L., KINNMONTH, S., STAFFORD-SMITH, M. & PETerson, N. 2009. Delinating the coral triangle. Galaxea Journal of Coral Research Studies 11, 91–100.

VERSEVELD, J. 1960. Octocorallia from the Malay Archipelago (Part I) Biological results of the Snellius Expedition XX. Temminckia 10, 209–251.

WILL, K.W., MISHLER, B.D. & WHEELER, Q.D. 2005. The perils of DNA barcoding and the need for integrative taxonomy. Systematic Biology 54, 844–851.

WOOD, E. & DEPP, F. 2008. What is the future for extensive areas of reefs impacted by fish blasting and coral bleaching and now dominated by soft corals: a case study from Malaysia. 11th International Coral Reef Symposium, Fort Lauderdale, Florida, USA. Abstract [http://www.bova.edu/ncr1/ncr1_lircs/ms12_orals.html, accessed 14 August 2013].

ZWICKL, D.J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. University of Texas, Austin, TX, USA.

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