Contributions to Zoology

The stoloniferous octocoral, *Hanabira yukibana*, gen. nov., sp. nov., of the southern Ryukyus has morphological and symbiont variation

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Supplementary material



FIGURE S1Maximum likelihood phylogenetic reconstruction of gene region 28S rDNA of
Hanabira yukibana, gen. nov., sp. nov., specimens from Okinawa and Iriomote
Islands and octocoral references from 120 genera, including outgroup Cornularia
spp.



FIGURE S2 Maximum likelihood phylogenetic reconstruction of gene region COI of *Hanabira yukibana*, gen. nov., sp. nov., specimens from Okinawa and Iriomote Islands and octocoral references from 120 genera, including outgroup *Cornularia* spp.



FIGURE S3 Maximum likelihood phylogenetic reconstruction of gene region mtMutS of *Hanabira yukibana*, gen. nov., sp. nov., specimens from Okinawa and Iriomote Islands and octocoral references from 120 genera, including outgroup *Cornularia pabloi*.



0.04

FIGURE S4Maximum likelihood phylogenetic reconstruction of gene region 28S rDNA of
Hanabira yukibana, gen. nov., sp. nov. specimens from Okinawa and Iriomote
Islands, Clavularia spp., Knopia octocontacanalis and outgroup taxa
Parasphaerasclera rotifera.



FIGURE S5Maximum likelihood phylogenetic reconstruction of gene region COI of Hanabira
yukibana, gen. nov., sp. nov. specimens from Okinawa and Iriomote Islands,
Clavularia spp., Knopia octocontacanalis and outgroup taxa Eleutherobia grayi and
Parasphaerasclera rotifera.



FIGURE S6Maximum likelihood phylogenetic reconstruction of gene region mtMutS of
Hanabira yukibana, gen. nov., sp. nov. specimens from Okinawa and Iriomote
Islands, Clavularia spp., Knopia octocontacanalis and outgroup taxa Eleutherobia
grayi and Parasphaerasclera rotifera.



FIGURE S7 Maximum likelihood phylogenetic reconstruction of gene region ND6 of *Hanabira yukibana*, gen. nov., sp. nov. specimens from Okinawa and Iriomote Islands and outgroup taxa *Clavularia* spp.



 FIGURE S8 Maximum likelihood phylogenetic reconstruction of gene region ITS of Symbiodiniaceae hosted by *Hanabira yukibana*, gen. nov., sp. nov. specimens from Okinawa and Iriomote Islands and reference taxa obtained from GenBank; *Durusdinium* sp. and *Cladocopium* sp. (accession numbers: AF396631 and EU074892, respectively) and outgroup sister taxa, *Gerakladium* sp. (=former *Symbiodinium* 'Clade G', accession number: KC597694).



FIGURE S9Phylogenetic relationships among 134 species of octocorals, including Hanabira
yukibana, gen. nov., sp. nov. specimens, using the combined 28S
rDNA+COI+mtMutS dataset. The best maximum likelihood tree is shown, with all
values at branches representing bootstrap probabilities and Bayesian posterior
probabilities, respectively. Values are not shown when 100%/1.00. Stoloniferous
species are highlighted in grey boxes. Cornularia spp. were used as outgroup.



FIGURE S10Sclerite types seen in *Clavularia inflata.*, OKA170530-02; a) spindles of calyx (scale
bar 0.1 mm), b) spindles of anthocodiae (scale bar 0.1 mm), c) anthocodial and
tentacular platelets (0.01 mm), d) anthocodial and tentacular rodlets (scale bar 0.01
mm), e) anthocodial and tentacular rods (scale bar 0.1 mm).



FIGURE S11Sclerite types seen in *Clavularia* sp., IRI170727-74; a) anthocodial and tentacular
rods (scale bar 0.01 mm), b) spindles of stolon (scale bar 0.1 mm), c) anthocodial
and tentacular platelets (scale bar 0.01 mm).

TABLE S1Number of base differences per site from averaging over all sequence pairs between
Hanabira groups (1-3) is shown (uncorrected p, expressed as percentage) for gene
regions COI and mtMutS. Standard error estimates (S.E.) are shown above the
diagonal. Analysis involved 22 nucleotide sequences for both COI and mtMutS. All
positions containing gaps and missing data were eliminated. There were a total of
638 and 717 positions in the final dataset for COI and mtMutS, respectively.
Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016)

COI	between groups	Hanabira yukibana 1	Hanabira yukibana 2	Hanabira yukibana 3
	Hanabira yukibana 1		0.0133	0.1468
	Hanabira yukibana 2	0.0131		0.1470
	Hanabira yukibana 3	0.1698	0.1567	
mtMutS	between groups	Hanabira yukibana 1	Hanabira yukibana 2	Hanabira yukibana 3
	Hanabira yukibana 1		0.0000	0.0000
	Hanabira yukibana 2	0.0000		0.0000
	Hanabira yukibana 3	0.0000	0.0000	

TABLE S2Estimates of average evolutionary divergence over sequence pairs within groups for
gene regions COI and mtMutS. The numbers of base differences per site from
averaging over all sequence pairs within each group (d) are shown (uncorrected *p*,
expressed as percentage). Standard error estimates (S.E.) are shown in the second
column and were obtained by a bootstrap procedure (1000 replicates). Analyses
involved 22 nucleotide sequences for both COI and mtMutS for *Hanabira yukibana*
specimens and nine and six nucleotide sequences for COI and mtMutS,
respectively, for *Clavularia* spp. specimens. All positions containing gaps and
missing data were eliminated. There were a total of 638 and 717 positions in the
final dataset for COI and mtMutS, respectively, for *Clavularia* spp.
specimens and 777 and 541 positions for COI and mtMutS, respectively, for *Clavularia* spp.
specimens. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016)

	COI		mtMutS	
Within groups	d	S.E.	d	S.E.
Hanabira yukibana	0.09	0.07	0.00	0.00
<i>Clavularia</i> spp.	0.00	0.00	0.00	0.00

TABLE S3Number of base differences per site from averaging over all sequence pairs between
Clavularia spp. is shown (uncorrected *p*, expressed as percentage) for gene regions
COI and mtMutS. Standard error estimates (S.E.) are shown above the diagonal.
Analysis involved nine and six nucleotide sequences for COI and mtMutS,
respectively. All positions containing gaps and missing data were eliminated. There
were a total of 777 and 541 positions in the final dataset for COI and mtMutS,
respectively. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).

COI	between groups	Clavularia inflata	<i>Clavularia</i> sp.
	Clavularia inflata		0.25
	<i>Clavularia</i> sp.	0.59	
mtMutS	between groups	Clavularia inflata	<i>Clavularia</i> sp.
	Clavularia inflata		0.00
	<i>Clavularia</i> sp.	0.00	