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Morpho-anatomical and molecular reassessments of *Rhodymenia prostrata* (Rhodymeniaceae, Rhodophyta) from Japan support the recognition of *Halopeltis tanakae nom. nov.*

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RUNNING TITLE

Halopeltis tanakae nom. nov. from Japan

ABSTRACT

We collected the deep-water species *Rhodymenia prostrata* (Rhodymeniaceae, Rhodophyta), for the first time in nearly 50 years, from the type locality off Mageshima Island, Japan. Our combined *rbc*L, *cox*1 and 28S rDNA analyses suggested that *R. prostrata* belongs in the genus *Halopeltis* but is distinct from all other *Halopeltis* species. However, the binomial *Halopeltis prostrata* already exists and hence a new epithet was required. Consequently, we propose the new name *Halopeltis tanakae* for *R. prostrata* based on morpho-anatomical and molecular analyses. The occurrence of small intercalating cells in *H. tanakae* is congruent with the generic concept of *Halopeltis. Halopeltis tanakae* is characterized by a procumbent thallus, crispate blades, cystocarps lacking basal constrictions, cystocarps primarily on blade margins, and mucilaginous strands in cystocarp cavities. *Halopeltis tanakae* is the second representative of the genus to be reported from the northwestern Pacific.

KEYWORDS

28S rDNA; *cox*1; Phylogeny; *rbc*L; Rhodymeniales; Taxonomy

INTRODUCTION

The marine red algal genus *Halopeltis* J. Agardh (Rhodymeniaceae, Rhodymeniales) was originally described by J. Agardh in 1854. Nine species of this genus are presently known (Saunders & McDonald 2010; Schneider *et al*. 2012). Agardh (1854) described *Halopeltis* based on *Rhodymenia australis* Sonder. Agardh (1854) originally distinguished *R. australis* from the genus *Acropeltis* Montagne (currently treated as a synonym of *Gelidium* J.V. Lamouroux) by the absence of longitudinal filaments in the medulla in the former taxon. The absence of longitudinal filaments in the medulla is what Agardh (1854) subsequently chose to ascribe specimens to

Halopeltis. Despite having described *Halopeltis*, Agardh (1876) continued to recognize *R. australis* and made no further mention of *Halopeltis*. Under *R. australis*, Agardh (1876, p. 332) placed into synonymy *Acropeltis australis* J. Agardh. Since Agardh's (1854) original treatment, *Halopeltis* had not been mentioned for nearly 150 yr.

Silva *et al*. (1996) pointed out that *R. australis* was an illegitimate name and proposed the new name *R. sonderi* P.C. Silva. They further pointed out that although *R. australis* was an illegitimate name, its synonym, *A. australis* was legitimate (Silva *et al*. 1996). Using molecular data, Saunders & McDonald (2010) subsequently distinguished *R. sonderi* from *Rhodymenia* Greville, and resurrected *Halopeltis*, treating *A. australis* as the basionym of the new combination *H. australis* (J. Agardh) G.W. Saunders. Additionally, Saunders & McDonald (2010) transferred three species of *Rhodymenia* and one of *Rhodymeniocolax* Setchell to *Halopeltis* and described two further species of the genus. Schneider *et al*. (2012) subsequently transferred *Rhodymenia adnata* Okamura to *Halopeltis* and described two new species. Most *Halopeltis* species are distributed in the southern hemisphere. *Halopeltis adnata* (Okamura) G.W. Saunders & C.W. Schneider from Japan and Korea is the only known species of *Halopeltis* occurring in the northwestern Pacific Ocean (Schneider *et al*. 2012).

Rhodymenia prostrata Tak. Tanaka was first described from Japan based on a collection dredged from the seafloor at a depth of 50 m off Mageshima Island, Kagoshima Prefecture (Tanaka 1965). The species has not been reported since Tanaka's (1965) original description. In our survey off Mageshima Island from 2016–2017, we collected two specimens that were morpho-anatomically identified as *R. prostrata*, and analysed their *rbc*L, *cox*1 and 28S rDNA. We also examined the vegetative and reproductive anatomies of the specimens to draw a complete picture of the phylogenetic relationship of this poorly known species.

MATERIAL AND METHODS

Samples morpho-anatomically identified as *R. prostrata* were collected from the seafloor at a depth of 32.7–36.8 m offshore of Mageshima Island, Japan, by dredging (T/S *Nansei-Maru*, Faculty of Fisheries, Kagoshima University; Table S1). Specimens were quickly dried in silica gel for molecular analyses. For morpho-anatomical observations, specimens were frozen; voucher specimens were deposited at the National Museum of Nature and Science, Japan (TNS). The holotype, deposited in the Herbarium, Graduate School of Science, Hokkaido University, Japan (SAP), was also examined. Herbarium acronyms followed Thiers (2021).

Partial *rbc*L, *cox*1 and 28S rDNA were sequenced for molecular phylogenetic analyses. Genomic DNA was extracted and sequenced as described by Suzuki *et al*. (2016). The *rbc*L, *cox*1 and 28S rDNA sequences of two specimens of *R. prostrata* from Japan were sequenced (Table S1). We compiled sequence data available from GenBank for eight *Halopeltis* species, four unnamed *Halopeltis* species and 12 genera within Rhodymeniaceae for the combined *rbc*L, *cox*1 and 28S rDNA analyses. As the Rhodymeniaceae have been resolved as monophyletic (Filloramo & Saunders 2016), three taxa belonging to Champiaceae were designated as outgroup (Table S1). We assembled data for eight *Halopeltis* species and four or five unnamed *Halopeltis* spp. for the *rbc*L, *cox*1 and 28S rDNA individual analyses. The *rbc*L data for *Halopeltis* sp. 1 WA is not recorded in GenBank. Because Saunders & McDonald (2010) and Schneider *et al*. (2012) suggested that *Halichrysis* (J. Agardh) F. Schmitz is a suitable outgroup for *Halopeltis*, two taxa of *Halichrysis* were designated as an outgroup for all individual analyses (Table S1). The 34 *rbc*L, 82 *cox*1 and 33 28S rDNA sequences were aligned using ClustalW (Larkin *et al*. 2007). The 28S rDNA sequence alignments were further refined by eye based on published secondary structures of the 28S rRNA gene of *Palmaria palmata* (Linnaeus) Kuntze (Wuyts *et* *al*. 2002) using SeaView 4.1 (Gouy *et al*. 2010). The ambiguous regions of the alignments and samples with identical nucleotide sequences were removed.

The four datasets, i.e. (i) combined *rbc*L, *cox*1 and 28S rDNA sequences, (ii) *rbc*L individual sequences, (iii) *cox*1 individual sequences, and (iv) 28S rDNA individual sequences, were subjected to maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses. We included 29 *rbc*L (1299 bp), *cox*1 (645 bp) and 28S rDNA (2463 bp) sequences into the combined analyses, and 18 *rbc*L (1233 bp), 29 *cox*1 (624 bp) and 16 28S rDNA (2634 bp) individual sequences in our data matrix. The alignment was partitioned by region and codon, and distinct models were applied to each partition for both ML and BI analyses. The substitution models for ML and BI are summarized in Table S2. ML analyses were performed using RAxML-NG v1.0.1 (Kozlov *et al*. 2019) as described previously (Suzuki & Terada 2021). BI analyses were performed using MrBayes 3.2.7a (Ronquist *et al*. 2012) following the protocols of Suzuki *et al*. (2016). The *p*-distances for each pair of *Halopeltis* species were calculated using PAUP 4.0b10 software (Swofford 2002).

Four DNA-based methods were applied to the *cox*1 dataset to delimit *Halopeltis* species: Poisson Tree Processes (PTP; Zhang *et al*. 2013); Automatic Barcode Gap Discovery (ABGD; Puillandre *et al*. 2012); and single and multiple thresholds Generalized Mixed Yule Coalescent method (GMYC; Pons *et al*. 2006; Reid & Carstens 2012), as previously described (Suzuki *et al*. 2021).

For morpho-anatomical observations, specimens were first thawed and sectioned by hand or using a freezing microtome (MA-101, Komatsu Electronics, Komatsu, Japan). Sections were stained with 1% cotton blue, acidified with 1% HCl and mounted in 50% aqueous Karo syrup. Photomicrographs were taken using a BX51 microscope (Olympus, Tokyo, Japan) with an ATZ digital camera (Kenis, Tokyo, Japan). Drawings were made using the U-DA Drawing Attachment (Olympus, Tokyo, Japan) on a BX51 microscope.

RESULTS

The topology of the ML tree based on the combined *rbc*L, *cox*1 and 28S rDNA sequences was similar to that from the Bayesian inference analyses, except in poorly supported branches (Fig. 1). The 12 *Halopeltis* species formed a monophyletic group that included *R. prostrata* with strong statistical support (BP = 94%; PP = 1.00). Within this *Halopeltis* clade, *R. prostrata* was sister to *H. adnata* with full statistical support (BP = 100%; PP = 1.00). All individual analyses gave topologies similar to that obtained in the combined analyses, except for poorly supported branches, although the statistical support of individual analyses was typically lower than that of the combined analyses (Figs S1–S3).

We used the *cox*1 individual sequence analyses and the four DNA-based species delimitation methods (PTP, ABGD, GMYC single and GMYC multiple), based on the *cox*1 dataset, to examine the relationships between *R. prostrata* and other *Halopeltis* species. The topology of the ML tree based on *cox*1 sequences is shown in Fig. S1. *Rhodymenia prostrata*, *H. adnata*, *H. willisii* Freshwater & G.W. Saunders and *Halopeltis* sp. 1 WA formed a monophyletic group with full statistical support. The *cox*1 sequence divergence between *R. prostrata* and other *Halopeltis* species varied from 3.0% to 14.4%. All DNA-based species delimitation methods showed that *R. prostrata* and the *Halopeltis* species were subdivided into 14 evolutionarily significant units (Fig. S4). Molecular analyses indicated that *R. prostrata* is distinct from all presently described *Halopeltis* species. Since the binomial *Halopeltis prostrata* G.W. Saunders already exists (Saunders & McDonald 2010), a new name, with a new epithet, is required for the species (Turland *et al.* 2018, Art. 11.4 and 53.1). Therefore, we propose the following binomial:

Halopeltis tanakae **Mas. Suzuki & R. Terada** ***nom. nov.***

Figs 2–16

REPLACED SYNONYM: *Rhodymenia prostrata* Tak. Tanaka 1965, *Memoirs of the Faculty of Fisheries, Kagoshima University* 14, p. 58., figs 7, 8. (Priority for *Halopeltis prostrata* G.W. Saunders *in* Saunders & McDonald 2010, p. 662, figs 50–58.)

HOLOTYPE: SAP 52156!, female gametophyte, seafloor at depth of 50 m, collected 7 June 1964 by T. Tanaka, deposited in the Herbarium, Graduate School of Science, Hokkaido University, Japan (SAP).

ADDITIONAL SPECIMENS EXAMINED: **Japan**: Seafloor at depth of 36.8 m offshore of Mageshima Island, Kagoshima Prefecture, 30°41.029′N, 130°50.597′E, *R. Terada*, 18 May 2016, female gametophyte, TNS-AL 209873 (DDBJ Accession numbers: 28S rDNA, LC605141; *cox*1, LC605134; *rbc*L, LC605128); *loc. cit*., depth 32.7 m, 30°41.745′N, 130°52.300′E, *R. Terada*, 20 Jul. 2017, female gametophyte, TNS-AL 209875 (DDBJ Accession numbers: *cox*1, LC605135; *rbc*L, LC605129).

TYPE LOCALITY: Offshore of Mageshima Island, Kagoshima Prefecture, Japan.

ETYMOLOGY: This species is named after Dr Takeshi Tanaka (1907–1997), who first recognized it as a distinct species.

Thalli were procumbent, attached by haptera scattered along the margins and ventral blade surface, radially extended by 3–5-fold pseudodichotomously branched blades, 2–5 cm in lateral spread, yellowish red to deep red, iridescent *in situ*. Blades were flattened, crispate, anastomosing, nearly 5 mm in width (Figs 2–4). The thallus was solid, 175–220 μm thick near the margins, 240–270 μm thick in middle portion, comprising a cortex and medulla. The cortex was composed of two layers of rounded cells. The outer cortical cells were irregularly and sparsely arranged. The medulla usually had two (rarely three) layers of large ovoid to polygonal cells, with one layer of smaller subcortical, ovoid to rounded cells present between the cortex and medulla (Figs 5, 13, 14). The small intercalating cells were sometimes produced in the interstitial spaces between medullary cells (Figs 6, 7, 15, 16). Cystocarps occurred mostly along the margins, but at times on both blade surfaces (Figs 2–4, 8). Cystocarps contained tightly packed carposporangia (Fig. 9). Mature carposporangia were polygonal, 17–25 µm in length and 14–18 µm in diameter. The floor of the cystocarps were lined with nutritive cells (Fig. 10). Mucilaginous strands (the 'réseau muqueux' of Huvé & Huvé 1977) were visible in the cystocarpic cavities (Figs 11, 12). Mature cystocarps were hemispherical and lacked a basal constriction, bearing a thick pericarp penetrated by an ostiole (Figs 8, 12). Male gametophytes and tetrasporophytes remain unknown.

DISCUSSION

Recent collections of *Halopeltis tanakae* (previously *Rhodymenia prostrata*) from its type locality allowed a phylogenetic and morpho-anatomical investigation of this poorly known species. The diminutive size of the holotype specimen of *R. prostrata* precluded examination of its anatomy or genetic sequencing, but the procumbent thalli, crispate blades, cortex and medulla, and positions and shapes of the cystocarps, in our newly collected material closely resembled both the holotype and Tanaka's (1965) original descriptions, supporting the assignment of our specimens to *R. prostrata*. However, our molecular analyses indicated that the specimens we morpho-anatomically identified as *R. prostrata* belong in *Halopeltis* and also that they are distinct from all presently described *Halopeltis* species. The epithet '*prostrata*' is unavailable in *Halopeltis* because of *H. prostrata* G.W. Saunders and thus we formally established the new name *Halopeltis tanakae nom. nov*.

Our combined *rbc*L, *cox*1 and 28S rDNA analyses grouped 13 *Halopeltis* species, including *H. tanakae*, into a monophyletic natural assemblage (with strong statistical support), in agreement with other published tree topologies (Saunders & McDonald 2010; Schneider *et al.* 2012; Filloramo & Saunders 2016). *Halopeltis* is characterized and distinguished from

Rhodymenia anatomically by: 1) the presence of small intercalating cells in the interstitial spaces between medullary cells (except in the western Atlantic *H. pellucida* C.W. Schneider & G.W. Saunders); and 2) by tetrasporangia produced by direct conversion of terminal outer cortical cells (Saunders & McDonald 2010; Schneider *et al*. 2012). *Rhodymenia* is anatomically characterized by a lack of special cells in the medulla and tetrasporangia derived from intercalary cortical cells at the transition between the inner and outer cortical layers (e.g. Sparling 1957; Saunders & McDonald 2010). Although our samples did not include tetrasporophytes, the presence of small intercalating cells in *H. tanakae* is consistent with the anatomical concept of *Halopeltis*. Molecular phylogenetic analyses suggest that *Halopeltis* and *Halichrysis* are sister genera (Saunders & McDonald 2010; Schneider *et al*. 2012; Filloramo & Saunders 2016; this study). *Halopeltis* and *Halichrysis* share anatomical features, such as the presence of small intercalating cells in the interstitial spaces between medullary cells and the origin of tetrasporangia, which are produced by direct conversion of terminal outer cortical cells. However, the presence of the 'réseau muqueux' (a mucilaginous strand produced by stretching existing nutritive and pericarp tissue) in the cystocarpic cavity, is suggested to be a unique characteristic of *Halichrysis* (Huvé & Huvé 1977; Saunders *et al*. 2006). We observed mucilaginous strands in the cystocarpic cavity in *H. tanakae*. Similar strands have also been reported in *H. willisii* as 'remnant connections' (Schneider *et al*. 2012). The presence of a 'réseau muqueux' thus may not be unique to *Halichrysis*. *Halichrysis* species form tetrasporangial nemathecia (Huvé & Huvé 1977; Saunders *et al*. 2006), whereas many *Halopeltis* species lack this structure (Saunders & McDonald 2010; Schneider *et al*. 2012). However, *H. australis* and *H. verrucosa* (Womersley) G.W. Saunders form distinct nemathecia (Womersley 1996), and *H. pellucida* form nemathecia-like sori (Schneider *et al*. 2012). Additional specimens and further investigations of *Halopeltis* and

Halichrysis species are required to clarify the morpho-anatomical distinctions between these genera.

Molecular analyses suggest that *H. tanakae* is closely related to *H. adnata*. The *cox*1 interspecific sequence divergence between *H. tanakae* and *H. adnata* was 3.0%, the lowest interspecific sequence divergence among *Halopeltis* species thus far obtained. Application of DNA-based species delimitation methods to the *cox*1 dataset indicated that *H. tanakae* is distinct from *H. adnata* and other *Halopeltis* species. A comparison of *H. tanakae* with five other *Halopeltis* species bearing prostrate thalli showed that *H. tanakae* is morpho-anatomically similar to both *H. adnata* and *H. willisii* (Table 1). All three species have cystocarps that lack basal constrictions (Tanaka 1965; Lee 2008; Schneider *et al*. 2012). *Halopeltis tanakae* is, however, morpho-anatomically distinguished from *H. adnata* and *H. willisii* by the absence of a decumbent thallus, relatively narrow blades (< 5.0 mm), and the production of most cystocarps on the blade margins, whereas *H. adnata* has a partially decumbent thallus and wider blades (> 12 mm), and *H. willisii* has moderately wider blades (4.0–15 mm) and cystocarps on both blade surfaces and margins (Okamura 1934; Tanaka 1965; Lee 2008; Schneider *et al*. 2012). *Halopeltis tanakae* is morpho-anatomically distinguished from *H. prostrata* by the pseudodichotomous branching, smaller thalli (< 5.0 cm), production of most cystocarps on the blade margins, and the lack of basal constrictions at the cystocarp, whereas *H. prostrata* has dichotomous branching, thalli of up to 10 cm, cystocarps that occur on blade surfaces, and a constriction at the cystocarp base (Saunders & McDonald 2010). *Halopeltis tanakae* is the second *Halopeltis* species reported from the northwestern Pacific region.

Halopeltis tanakae has not been collected in Japan from regions beyond offshore Mageshima Island. All specimens were collected from the seafloor at a depth of more than 30 m by dredging. The distribution of the species seems to be restricted to deep-water habitats. Further investigations focusing on sublittoral to deep-water environments in warm temperate to subtropical regions are required to determine if *H. tanakae* is more widely distributed in Japan and the wider northwestern Pacific region.

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LEGENDS FOR FIGURES

Fig. 1. Maximum likelihood phylogeny based on combined *rbc*L, *cox*1 and 28S rDNA sequences. Numbers below the branches indicate the bootstrap values (BP, left) and Bayesian posterior probabilities (PP, right). Only the BP ≥ 50% and PP ≥ 0.95 are shown. Asterisks (*) indicate BP = 100% and PP = 1.00.

Figs 2–12. Habit, vegetative and reproductive structures of *Halopeltis tanakae nom. nov*.

Fig. 2. Holotype (SAP 52156, female gametophyte) specimen showing cystocarps occurring mostly along the blade margins (arrowheads). Scale bar = 5 mm.

Fig. 3. Female gametophyte (TNS-AL 209873) with cystocarps occurring mostly along the blade margins (arrowheads). Scale bar = 5 mm.

Fig. 4. Herbarium specimen of a female gametophyte (TNS-AL 209875) showing cystocarps occurring mostly along the blade margins (arrowheads). Scale bar = 5 mm.

Fig. 5. Transverse section near upper portion of blade (TNS-AL 209873). Scale bar = 50 µm.

Fig. 6. Transverse section of blade (TNS-AL 209873) showing two intercalating cells (arrowheads) in the interstitial spaces between medullary cells. Scale bar = 50 µm.

Fig. 7. Longitudinal section of blade (TNS-AL 209873) showing an intercalating cell (arrowhead) in the interstitial spaces between medullary cells. Scale bar = 50 µm.

Fig. 8. Close up of a female gametophyte (TNS-AL 209873) with cystocarps occurring along the blade margins (arrowheads). Scale bar = 1 mm.

Fig. 9. Longitudinal section through a mature cystocarp (TNS-AL 209873) showing tightly packed carposporangia (arrowheads). Scale bar = 200 µm.

Fig. 10. Close-up of the floor of a cystocarp (TNS-AL 209873) showing basal nutritive tissue (nt). Scale bar = 50 µm.

Fig. 11. Close-up of the mucilaginous strands on the basal nutritive tissue (arrows) (TNS-AL 209873). Scale bar = 20 µm.

Fig. 12. Longitudinal section through a cystocarp (TNS-AL 209873) showing the pericarp with an apical ostiole (arrowhead) and the remnants of the basal nutritive tissue and mucilaginous strands (arrow). Scale bar = 100 µm.

Figs 13–16. Drawings of the vegetative structures of *Halopeltis tanakae nom. nov.* (TNS-AL 209873). cc = cortical cell; mc = medullary cell; sc = subcortical cell. Scale bars = 50 µm.

Fig. 13. Transverse section at the margin of a blade.

Fig. 14. Longitudinal section of a blade.

Fig. 15. Transverse section of a blade showing two intercalating cells (ic) in the interstitial spaces between medullary cells.

Fig. 16. Longitudinal section of a blade showing an intercalating cell (ic) in the interstitial spaces between medullary cells.

SUPPLEMENTARY INFORMATION

Table S1. Collection locations and details, and INSD (DDBJ/EMBL/GenBank) accession numbers of samples used in the *rbc*L, *cox*1, and 28S rDNA sequence analyses. Accession numbers in bold were determined for this study.

Table S2. Substitution models for the maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses on the basis of four datasets (combined *rbc*L, *cox*1 and 28S rDNA analysis, *rbc*L individual analysis, *cox*1 individual analysis, and 28S rDNA individual analysis).

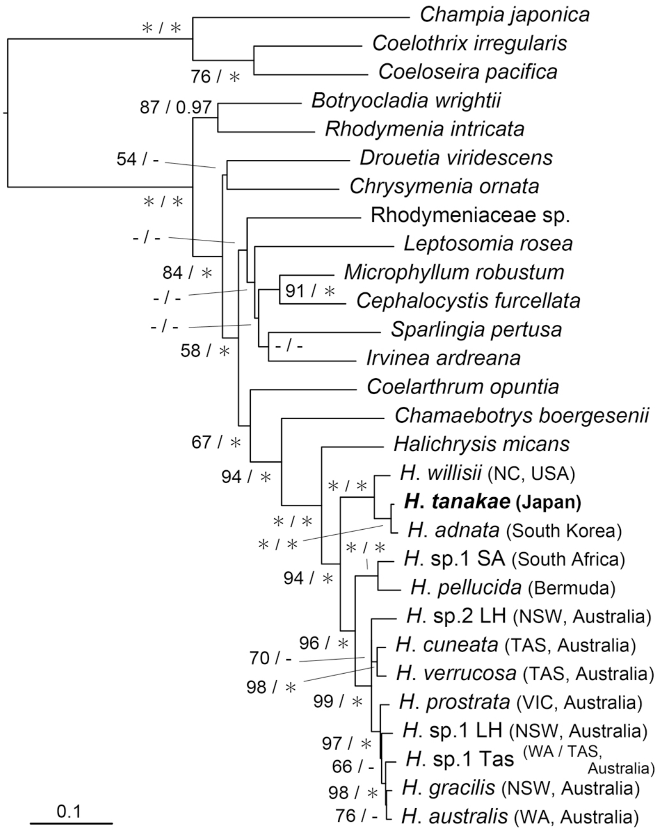
Fig. S1. Maximum likelihood phylogeny based on *cox*1 sequences. Numbers below the branches indicate the bootstrap values (BP, left) and Bayesian posterior probabilities (PP, right). Only the BP ≥ 50% and PP ≥ 0.95 are shown. Asterisks (*) indicate BP = 100% and PP = 1.00.

Figs S2, S3. Phylogenetic trees

Fig. S2. Maximum likelihood phylogeny based on *rbc*L sequences. Numbers below the branches indicate the bootstrap values (BP, left) and Bayesian posterior probabilities (PP, right). Only the BP ≥ 50% and PP ≥ 0.95 are shown. Asterisks (*) indicate BP = 100% and PP = 1.00.

Fig. S3. Maximum likelihood phylogeny based on 28S rDNA sequences. Numbers below the branches indicate the bootstrap values (BP, left) and Bayesian posterior probabilities (PP, right). Only the BP ≥ 50% and PP ≥ 0.95 are shown. Asterisks (*) indicate BP = 100% and PP = 1.00.

Fig. S4. Results of the species delimitation analysis plotted on the ultrametric tree based on *cox*1 sequences. Numbers below the branches indicate the bootstrap values (BP) in maximum likelihood phylogeny. Only the BP ≥ 50% is shown. Asterisks (*) indicate BP = 100%. Bars represent results from the Poisson Tree Processes method (PTP), Automatic Barcode Gap Discovery (ABGD), Generalized Mixed Yule Coalescent (GMYC) method using a single threshold (GMYC single), and GMYC method with multiple thresholds (GMYC multiple).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Coeloseira pacifica
Botryocladia wrightii
Rhodymenia intricata
Drouetia viridescens
Chrysymenia ornata
Rhodymeniaceae sp.
Leptosomia rosea
Microphyllum robustum
Cephalocystis furcellata
Sparlingia pertusa
Irvinea ardreana
Coelarthrum opuntia
Chamaebotrys boergesenii
Halichrysis micans
H. willisii (NC, USA)
H. tanakae (Japan)
H. adnata (South Korea)
H. sp.1 SA (South Africa)
H. pellucida (Bermuda)
H. sp.2 LH (NSW, Australia)
H. cuneata (TAS, Australia)
H. verrucosa (TAS, Australia)
H. prostrata (VIC, Australia)
H. sp.1 LH (NSW, Australia)
H. sp.1 Tas (WA / TAS, Australia)
H. gracilis (NSW, Australia)
H. australis (WA, Australia)
* / *
76 / *
87 / 0.97
54 / -
* / *
- / -
84 / *
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- / -
- / -
- / -
58 / *
67 / *
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* / *
* / *
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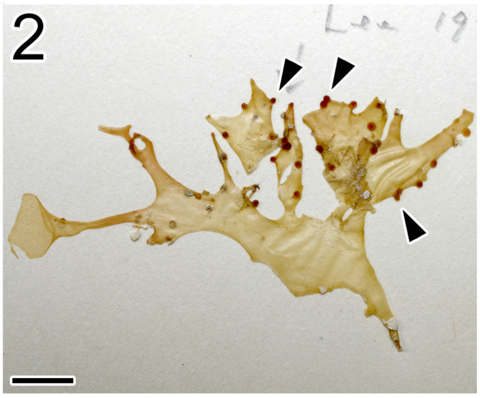
98 / *
76 / -
0.1

2



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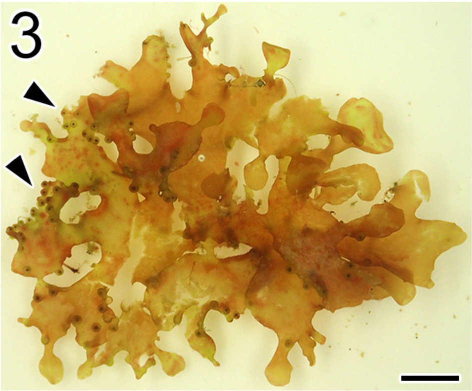

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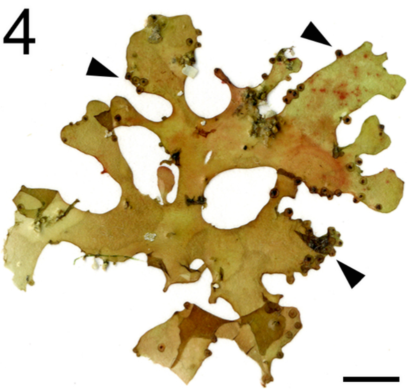

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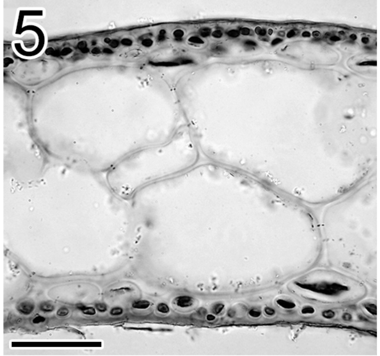

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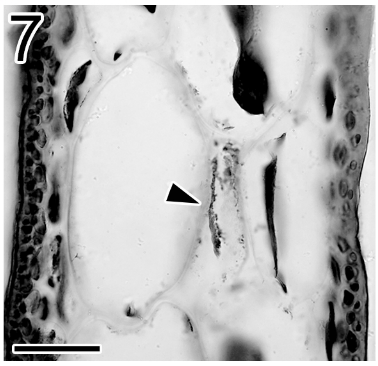

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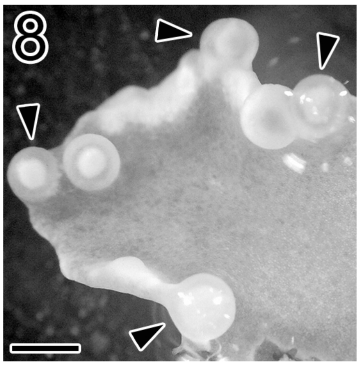

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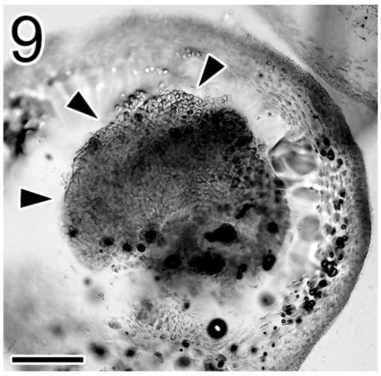

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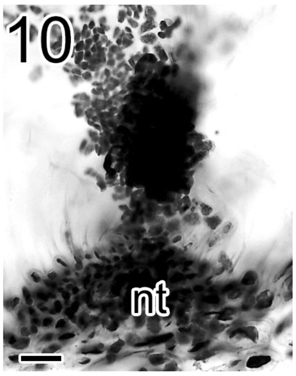

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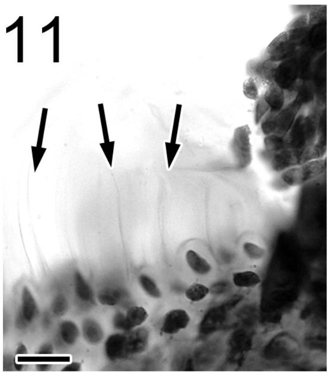

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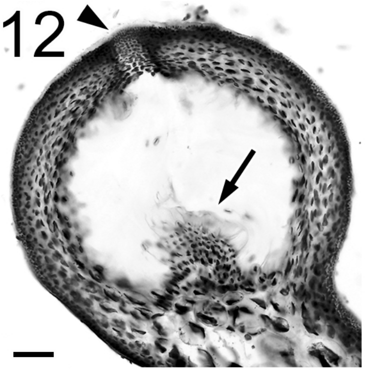

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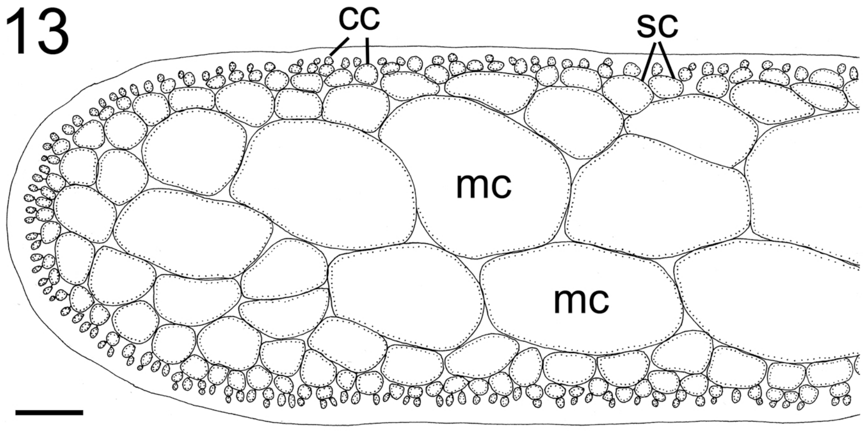

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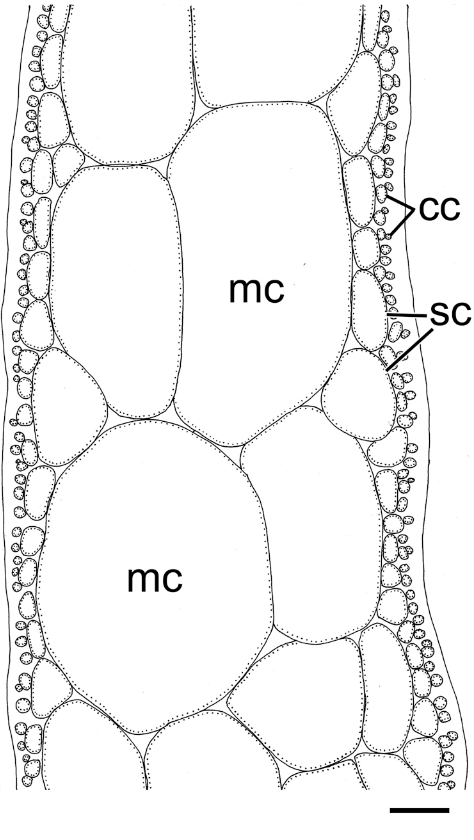

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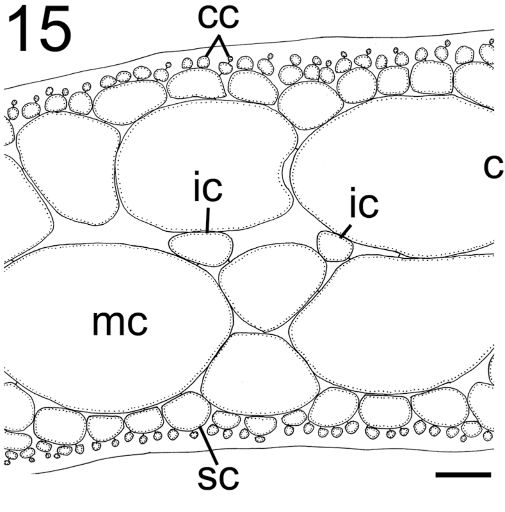

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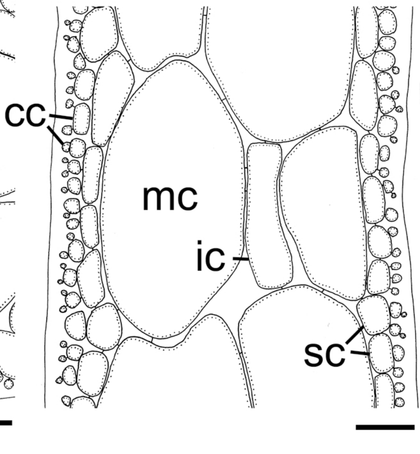

Table S1. Collection locations and details, and INSD (DDBJ/EMBL/GenBank) accession numbers of samples used in the *rbc*L, *cox*1, and 28S rDNA sequence analyses. Accession numbers in bold were determined for this study.

Species	Collection information (locality; date; collector, voucher; Reference)	*rbc*L	*cox*1	28S rDNA
Champiaceae				
Champia japonica Okamura	Futaana (34°38.316' N, 138°54.560' E), Touji, Shimoda City, Shizuoka Pref., Japan; 16 May 2006; M. Suzuki; TNS-AL 163941; Suzuki *et al*. (2010), this study	AB383121	**LC605130**	**LC605138**
Coeloseira pacifica E.Y. Dawson	Tokawa (35°41.391' N 140°51.286' E), Tokawa Town, Choshi City, Chiba Pref., Japan; 9 Mar. 2005; M. Suzuki; TNS-AL 163968; this study	**AB693119**	**LC605131**	**LC605139**
Coelothrix irregularis (Harvey) Børgesen	Wang Li Tung (21°59.441' N, 120°42.235' E), Hengchun Township, Pingdong Co., Taiwan; 18 Mar. 2012; M. Suzuki; TNS-AL 182111; this study	**LC605126**	**LC605132**	
	CL020502; Le Gall *et al*. (2008)			EU624160
Rhodymeniaceae				
Botryocladia wrightii (Harvey) W.E. Schmidt, D.L. Ballantine & Fredericq	Uranohama Beach (39°26' N, 141°58' E), Funakoshi, Yamada Town, Shimohei Co., Iwate Pref., Japan; 23 Jun. 2005; M. Suzuki; TNS-AL 163962; this study	**LC605127**	**LC605133**	**LC605140**
Cephalocystis furcellata (J. Agardh) A.J.K. Millar, G.W. Saunders, I.M. Strachan & Kraft	Queenscliff, Cottage By the Sea Reef, VIC, Australia; 11 Dec. 2000; G.W. Saunders; GWS000958; Schneider *et al*. (2012), Filloramo & Saunders (2016)	JQ907550	KU934268	
	VIC, Australia; G0133; Le Gall & Saunders (2007)			EF033621
Chamaebotrys boergesenii (Weber Bosse) Huisman	Little Beach, WA, Australia; 7 Nov. 2010; G.W. Saunders & K. Dixon; GWS024505; Filloramo & Saunders (2016)	KU726729	KU707876	KU722608
Chrysymenia ornata (J. Agardh) Kylin	Jervis Bay, NSW, Australia; 1 Feb. 1995; A. Millar & P. Richards; G0281; Ballantine *et al*. (2007), Filloramo & Saunders (2016)	KU382073	KU382038	DQ343670
Coelarthrum opuntia (Endlicher) Børgesen	Port Phillip Bay (in the rip), VIC, Australia; 5 Apr. 1995; G.W. Saunders & G.T. Kraft; G0303; Ballantine *et al*. (2007), Filloramo & Saunders (2016)	KU726720	KU707867	DQ343671
Drouetia viridescens Filloramo & G.W. Saunders	Coffs Harbour, Split Solitary Island, NSW, Australia; 9 Dec. 2012; G.W. Saunders & K. Dixon; GWS032624; Filloramo & Saunders (2016)	KU726732	KU707881	KU722609
Halichrysis corallinaria D.L. Ballantine, G.W. Saunders & H. Ruiz	Lajas, Puerto Rico; 10 Jul. 2000; 6268; Saunders (2005), Ballantine *et al*. (2007), Schneider *et al*. (2012)	JQ858279	AY970628	DQ343674

Table S1. Continued.

Species	Collection information (locality; date; collector, voucher; Reference)	*rbc*L	*cox*1	28S rDNA
Halichrysis micans (Hauptfleisch) P. Huvé & H. Huvé	Malabar Reef, Lord Howe Island, NSW, Australia; 14 Mar. 2001; G.W. Saunders; GWS001065; Ballantine *et al*. (2007), Schneider *et al*. (2012), Filloramo & Saunders (2016)	JQ858278	KU934262	DQ343673
Halopeltis adnata (Okamura) G.W. Saunders & C.W. Schneider	Piyangdo Island, Rocky Reef at Lighthouse `Point`, Jeju Island, South Korea; 20 May 2010; G.W. Saunders & H-G. Choi; GWS018249; Schneider *et al*. (2012)	JQ907552	JQ907538	JQ907563
	Channel between Little & Big Munseom Islands; Jeju Island, South Korea; 19 May 2010; G.W. Saunders & H-G. Choi; GWS018549; Schneider *et al*. (2012)		HQ544106	
	Piyangdo Island, Rocky Reef at Lighthouse `Point`, Jeju Island, South Korea; 20 May 2010; G.W. Saunders & H-G. Choi; GWS018224; Schneider *et al*. (2012)		JQ907537	
	Piyangdo Island, Rocky Reef at Lighthouse `Point`, Jeju Island, South Korea; 20 May 2010; G.W. Saunders & H-G. Choi; GWS018248; Schneider *et al*. (2012)		JQ907539	
Halopeltis australis (J. Agardh) G.W. Saunders	Kangaroo Island, SA, Australia; 8 Sep. 1995; M.H. Hommersand & F. Hommersand; WES131; Schmidt *et al*. 2017	HQ400598		
	Tarcoola Beach, WA, Australia; 21 Sep. 1995; M.H. Hommersand & F. Hommersand; WES235; Schmidt *et al*. 2017	HQ400599		
	Wilson Bay, Rottnest Island, WA, Australia; 20 Jul. 1994; J.M. Huisman; G0253; Le Gall *et al*. (2008), Saunders & McDonald (2010); Schneider *et al*. (2012)	JQ858281	HM033026	EU624152
	Carnac Island, WA, Australia; 12 Dec. 2001; J.M. Huisman; GWS001279; Saunders & McDonald (2010)		HM033024	
	Point Lonsdale Reef, VIC, Australia; 7 Dec. 2012; G.W. Saunders; GWS001570; Saunders & McDonald (2010)		HM033025	
	Carnac Island, WA, Australia; 12 Dec. 2001; J.M. Huisman; GWS001280; Saunders & McDonald (2010)		HM033027	
Halopeltis cuneata (Harvey) G.W. Saunders	Arch Rock, south of Hobart, TAS, Australia; 28 Nov. 2002; R. Withall; GWS001521; Saunders & McDonald (2010), Schneider *et al*. (2012)	JQ858283	HM033032	HM033164
	Verona Sands, TAS, Australia; 21 Jan. 2004; G.W. Saunders & R. Withall; GWS001954; Saunders & McDonald (2010)		HM033029	

Table S1. Continued.

Species	Collection information (locality; date; collector, voucher; Reference)	*rbc*L	*cox*1	28S rDNA
	Verona Sands, TAS, Australia; 24 Jan. 2004; G.W. Saunders & R. Withall; GWS001953; Saunders & McDonald (2010)		HM033030	
	Arch Rock, south of Hobart, TAS, Australia; 28 Nov. 2002; R. Withall; GWS001504; Saunders & McDonald (2010)		HM033031	HM033163
Halopeltis gracilis G.W. Saunders	Islands off Balls Pyramid, Lord Howe Island, NSW, Australia; 1 Feb. 2004; G.W. Saunders; GWS002051; Schneider *et al*. (2012), Filloramo & Saunders (2016)	JQ907554	KU934269 (HM915982)	
	Balls Pyramid, Lord Howe Island, NSW, Australia; 1 Feb. 2004; G.W. Saunders; GWS002049; Saunders & McDonald (2010)		HM033033	HM033165
	Balls Pyramid, Lord Howe Island, NSW, Australia; 1 Feb. 2004; R. Withall; GWS002062; Saunders & McDonald (2010)		HM033034	
	Balls Pyramid, Lord Howe Island, NSW, Australia; 1 Feb. 2004; G.W. Saunders; GWS002052; Saunders & McDonald (2010)		HM033035	
Halopeltis pellucida C.W. Schneider & G.W. Saunders	South Shore, Cathedral Rock off Castle Harbour, St George's Parish, Bermuda; 23 Aug. 2010; C.W. Schneider, C. Lane, D. McDevit & T. Popolizio; BDA0369; Schneider *et al*. (2012)	JQ907555	HQ933375	JQ907565
	South Shore, Cathedral Rock off Castle Harbour, St George's Parish, Bermuda; 18 Feb. 2012; T. Popolizio; BDA0886; Schneider *et al*. (2012)		JQ907541	
Halopeltis prostrata G.W. Saunders	Flinders Jetty, VIC, Australia; 9 Dec. 2002; G.W. Saunders; GWS001592; Saunders & McDonald (2010), Schneider *et al*. (2012)	JQ858280	HM033044	HM033166
	Queenscliff, Port Phillip Heads, VIC, Australia; 17 Oct. 2004; G.W. Saunders; GWS002451; Saunders & McDonald (2010)		HM033036	
	Flinders Jetty, VIC, Australia; 16 Oct. 2004; G.W. Saunders; GWS002434; Saunders & McDonald (2010)		HM033037	
	Flinders Jetty, VIC, Australia; 16 Oct. 2004; G.W. Saunders; GWS002433; Saunders & McDonald (2010)		HM033038	
	Flinders Jetty, VIC, Australia; 16 Oct. 2004; G.W. Saunders; GWS002432; Saunders & McDonald (2010)		HM033039	

Table S1. Continued.

Species	Collection information (locality; date; collector, voucher; Reference)	*rbc*L	*cox*1	28S rDNA
	Flinders Jetty, VIC, Australia; 16 Oct. 2004; G.W. Saunders; GWS002431; Saunders & McDonald (2010)		HM033040	
	Flinders Jetty, VIC, Australia; 16 Oct. 2004; G.W. Saunders; GWS002430; Saunders & McDonald (2010)		HM033041	
	Flinders Jetty, VIC, Australia; 16 Oct. 2004; G.W. Saunders; GWS002429; Saunders & McDonald (2010)		HM033042	
	Flinders Jetty, VIC, Australia; 16 Oct. 2004; G.W. Saunders; GWS002428; Saunders & McDonald (2010)		HM033043	
	Cottage By the Sea Reef, Queenscliff, VIC, Australia; 20 Jan. 2005; G.W. Saunders; GWS002558; Saunders & McDonald (2010)		HM033045	
	Verona Sands, TAS, Australia; 23 Jan. 2005; G.W. Saunders; GWS002612; Saunders & McDonald (2010)		HM033046	
	Point Lonsdale Reef, VIC, Australia; 19 Jan. 2005; G.W. Saunders; GWS002542; Saunders & McDonald (2010)		HM033047	
	Williamstown, Port Phillip Bay, VIC, Australia; 20 Oct. 2004; G.W. Saunders; GWS002479; Saunders & McDonald (2010)		HM033048	
	Pennington Bay, Kangaroo Island, NSW, Australia; 9 Feb. 2008; K. Dixon & F. Gurgel; KAD35; Saunders & McDonald (2010)		HM033049	
Halopeltis tanakae Mas. Suzuki & R. Terada *nom*. *nov*.	The seafloor at the depth of 36.8 m offshore of Mageshima Island (30°41.029′N, 130°50.597′E), Kagoshima Pref., Japan; 18 May 2016; R. Terada; TNS-AL 209873; this study	**LC605128**	**LC605134**	**LC605141**
	The seafloor at the depth of 32.7 m offshore of Mageshima Island (30°41.745′N, 130°52.300′E), Kagoshima Pref., Japan; 20 Jul. 2017; R. Terada; TNS-AL 209875; this study	**LC605129**	**LC605135**	
Halopeltis verrucosa (Womersley) G.W. Saunders	Nine Pin Point, TAS, Australia; 23 Jan. 2004; G.W. Saunders & R. Withall; GWS001912; Saunders & McDonald (2010), Schneider *et al.* (2012)	JQ858282	HM033060	

Table S1. Continued.

Species	Collection information (locality; date; collector, voucher; Reference)	*rbc*L	*cox*1	28S rDNA
	Devils Hole, Eaglehawk Neck, TAS, Australia; 27 Nov. 2002; G.W. Saunders; GWS001501; Saunders & McDonald (2010)		HM033062	HM033169
	Warrnambool, VIC, Australia; 17 Jan. 2005; G.W. Saunders; GWS002531; Saunders & McDonald (2010); as *H. austrina*.		HM033028	
	Bicheno, TAS, Australia; 26 Jan. 2004; G.W. Saunders & R. Withall; GWS001981; Saunders & McDonald (2010)		HM033052	
	Bicheno, TAS, Australia; 26 Jan. 2004; G.W. Saunders & R. Withall; GWS001973; Saunders & McDonald (2010)		HM033053	
	Bicheno, TAS, Australia; 26 Jan. 2004; G.W. Saunders & R. Withall; GWS001974; Saunders & McDonald (2010)		HM033054	
	Bicheno, TAS, Australia; 26 Jan. 2004; G.W. Saunders & R. Withall; GWS001972; Saunders & McDonald (2010)		HM033055	
	Bicheno, TAS, Australia; 26 Jan. 2004; G.W. Saunders & R. Withall; GWS001968; Saunders & McDonald (2010)		HM033056	
	Bicheno, TAS, Australia; 26 Jan. 2004; G.W. Saunders & R. Withall; GWS001966; Saunders & McDonald (2010)		HM033057	
	Verona Sands, TAS, Australia; 24 Jan. 2004; G.W. Saunders & R. Withall; GWS001960; Saunders & McDonald (2010)		HM033058	
	Verona Sands, TAS, Australia; 24 Jan. 2004; G.W. Saunders & R. Withall; GWS001958; Saunders & McDonald (2010)		HM033059	
	Arch Rock, south of Hobart, TAS, Australia; 28 Nov. 2002; G.W. Saunders; GWS001508; Saunders & McDonald (2010)		HM033061	
	Point Lonsdale Reef, VIC, Australia; 19 Jan. 2005; G.W. Saunders; GWS002541; Saunders & McDonald (2010)		HM033063	
	Devils Hole, Eaglehawk Neck, TAS, Australia; 27 Nov. 2002; G.W. Saunders; GWS001497; Saunders & McDonald (2010)		HM033064	

Table S1. Continued.

Species	Collection information (locality; date; collector, voucher; Reference)	*rbc*L	*cox*1	28S rDNA
	Point Lonsdale Reef, VIC, Australia; 19 Jan. 2005; G.W. Saunders; GWS002539; Saunders & McDonald (2010)		HM033065	
	Bicheno, TAS, Australia; 26 Nov. 2002; G.W. Saunders; GWS001480; Saunders & McDonald (2010)		HM033066	
	Warrnambool, VIC, Australia; 17 Jan. 2005; G.W. Saunders; GWS002532; Saunders & McDonald (2010)		HM033067	HM033170
	Bicheno, TAS, Australia; 26 Jan. 2004; G.W. Saunders & R. Withall; GWS001986; Saunders & McDonald (2010)		HM033068	
	Bicheno, TAS, Australia; 26 Jan. 2004; G.W. Saunders & R. Withall; GWS001985; Saunders & McDonald (2010)		HM033069	
	Bicheno, TAS, Australia; 26 Jan. 2004; G.W. Saunders & R. Withall; GWS001984; Saunders & McDonald (2010)		HM033070	
Halopeltis willisii Freshwater & G.W. Saunders	Southwest Ledge -1, Onslow Bay, NC, USA; 30 Jun. 2009; A. Poray; GWS011431; Schneider *et al*. (2012)	JQ907553	HM915686	JQ907564
	Offshore LA, USA; 9 Jul. 2006; S. Fredericq; WES48; Schmidt *et al*. (2017)	KT154696		
	Southwest Ledge -1, Onslow Bay, NC, USA; 30 Jun. 2009; A. Poray; GWS011432; Schneider *et al*. (2012)		JQ907540	
Halopeltis sp.1 LH	Roach Wall, Lord Howe Island, NSW, Australia; 22 Nov. 2010; G.W. Saunders, K. Dixon & R. Withall; GWS023017; Schneider *et al*. (2012)	JQ907556	JQ907542	
	Lord Howe Island, NSW, Australia; 31 Jan. 2004; R. Withall; GWS002041; Ballantine *et al*. (2007)		EF101938	DQ343678
Halopeltis sp.1 SA	Cape, third Sister, Riet River, north of Port Alfred, Cape Prov., South Africa; 20 Jul. 1993; M.H., Fran & Eric Hommersand; GWS008826; Schneider *et al*. (2012)	JQ907549	HM918824	JQ907562
Halopeltis sp.1 Tas	Cozy Corner (Knobby Pt.); WA, Australia; 10 Nov. 2010; G.W. Saunders & K. Dixon; GWS024940; Schneider *et al*. (2012)	JQ907557	JQ907543	
	Blowhole, near Eaglehawk Neck, TAS, Australia; 23 Jan. 2005; G.W. Saunders; GWS002596; Saunders & McDonald (2010)		HM033050	HM033167

Table S1. Continued.

Species	Collection information (locality; date; collector, voucher; Reference)	*rbc*L	*cox*1	28S rDNA
Halopeltis sp.1 WA	Abrolhos Island, WA, Australia; 10 Nov. 1995; G.T. Kraft & G.W. Saunders; G0402; Ballantine *et al*. (2007)		EF101939	DQ343679
Halopeltis sp.2 LH	Malabar Reef, Lord Howe Island, NSW, Australia; 21 Nov. 2010; G.W. Saunders, K. Dixon & R. Withall GWS022825; Schneider *et al*. (2012)	JQ907558	JQ907544	
	Balls Pyramid, Lord Howe Island, NSW, Australia; 1 Feb. 2004; G.W. Saunders; GWS002061; Saunders & McDonald (2010)		HM033051	HM033168
Irvinea ardreana (Brodie & Guiry) Guiry	Sines, Portugal; M.D. Guiry & J.A. West; G0173; Withall & Saunders (2006), Schneider *et al*. (2012), Filloramo & Saunders (2016)	JQ907559	KU934258	DQ343714
Leptosomia rosea (Harvey) Womersley	Flinders Jetty; VIC, Australia; 14 May 1993; G.W. Saunders; G0147; Schneider *et al*. (2012), Filloramo & Saunders (2016)	JQ907560	KU934264	
	Seven Mile Beach, WA, Australia; 13 Nov. 1995; G.T. Kraft & G.W. Saunders; G0384; Le Gall *et al*. (2008)			EU624147
Microphyllum robustum G.W. Saunders	Balls Pyramid, Lord Howe Island, NSW, Australia; 1 Feb. 2004; G.W. Saunders; GWS002047; Schneider *et al*. (2012)	JQ858277		
	Balls Pyramid, Lord Howe Island, NSW, Australia; 1 Feb. 2004; G.W. Saunders; GWS002046; Filloramo & Saunders (2016)		KU934274	
	Balls Pyramid, Lord Howe Island, NSW, Australia; 1 Feb. 2004; G.W. Saunders; GWS002057; Saunders & McDonald (2010)			GU997654
Rhodymenia intricata (Okamura) Okamura	Banshobana (31°14.464’ N 130°25.593’ E), Ei Town, Minamikyushu City, Kagoshima Pref., Japan; 31 Mar. 2006; M. Suzuki; TNS-AL 164009; Suzuki *et al*. (2010), this study	AB383120	**LC605136**	**LC605142**
Sparlingia pertusa (Postels & Ruprecht) G.W. Saunders, I.M. Strachan & Kraft	Hamakawame (39°28.571’ N, 141°58.389’ E), Osawa, Yamada Town, Shimohei Co., Iwate Pref., Japan; 3 May 2006; M. Suzuki; TNS-AL 164014; Suzuki *et al*. (2010), this study	AB383123	**LC605137**	**LC605143**
Rhodymeniaceae sp.	Breakwater at harbor, Portland, VIC, Australia; 14 Nov. 2011; G.W. Saunders & K. Dixon; GWS029498; Filloramo & Saunders (2016)	KU726722	KU707871	KU722600

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Table S2. Substitution models for the maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses on the basis of four datasets (combined *rbc*L, *cox*1 and 28S rDNA analysis, *rbc*L individual analysis, *cox*1 individual analysis, and 28S rDNA individual analysis).

	Combined *rbc*L, *cox*1 and 28S rDNA	*rbc*L	*cox*1	28S rDNA
Number of taxa	29	18 (19)[1)]	29 (67)[1)]	16 (18)[1)]
Number of nucleotides (bp)	*rbc*L: 1299, *cox*1: 645, 28S rDNA: 2463	1233	624	2634
Substitution model for ML analyses[2)]	*rbc*L: 1st codons (TIM1+I+G4), 2nd codons (GTR+I), 3rd codons (TPM1uf+I+G4) *cox*1: 1st codons (GTR+I+G4), 2nd codons (TPM3uf+I+G4), 3rd codons (TrN+I+G4) 28S rDNA: GTR+I+G4	1st codons (TrN+I), 2nd codons (GTR+I), 3rd codons (TPM2uf+G4)	1st codons (TIM1+G4), 2nd codons (HKY), 3rd codons (HKY+I+G4)	GTR+I+G4
Substitution model for BI analyses[3)]	*rbc*L: 1st codons (GTR+I+G), 2nd codons (GTR+I+G), 3rd codons (HKY+I+G) *cox*1: 1st codons (GTR+I+G), 2nd codons (HKY+I), 3rd codons (GTR+I+G) 28S rDNA: GTR+I+G	1st codons (GTR+I), 2nd codons (GTR+G), 3rd codons (GTR+G)	1st codons (GTR+I+G), 2nd codons (HKY), 3rd codons (HKY+I+G)	GTR+I+G

[1)] The numbers within parentheses indicate original number of taxa including the samples with identical nucleotide sequences.

[2)] Each substitution model was selected by Akaike's information criterion using ModelTest-NG 0.1.6 (Darriba *et al*. 2019).

[3)] Each substitution model was selected by Akaike's information criterion using MrModeltest 2.3 (Nylander 2004).

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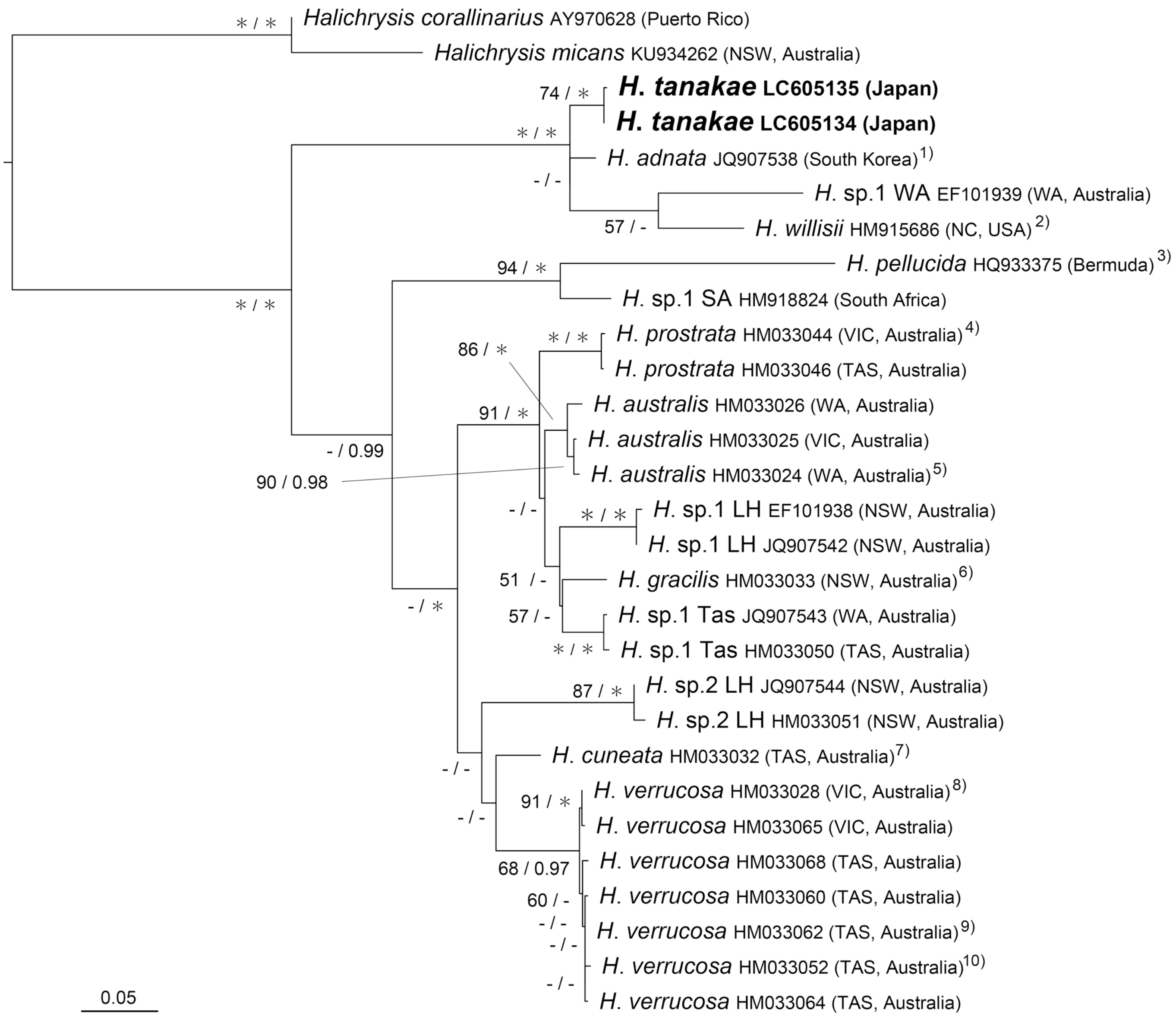
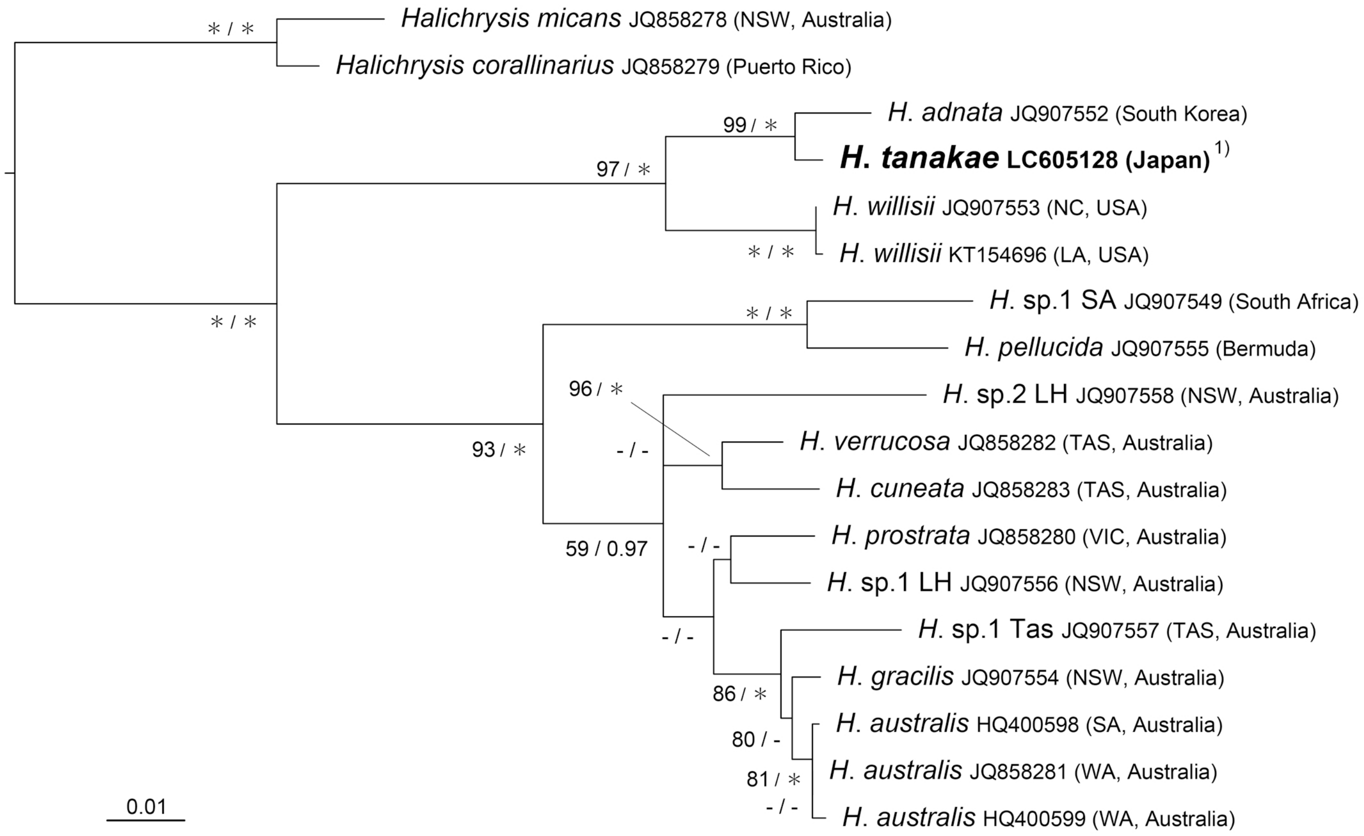


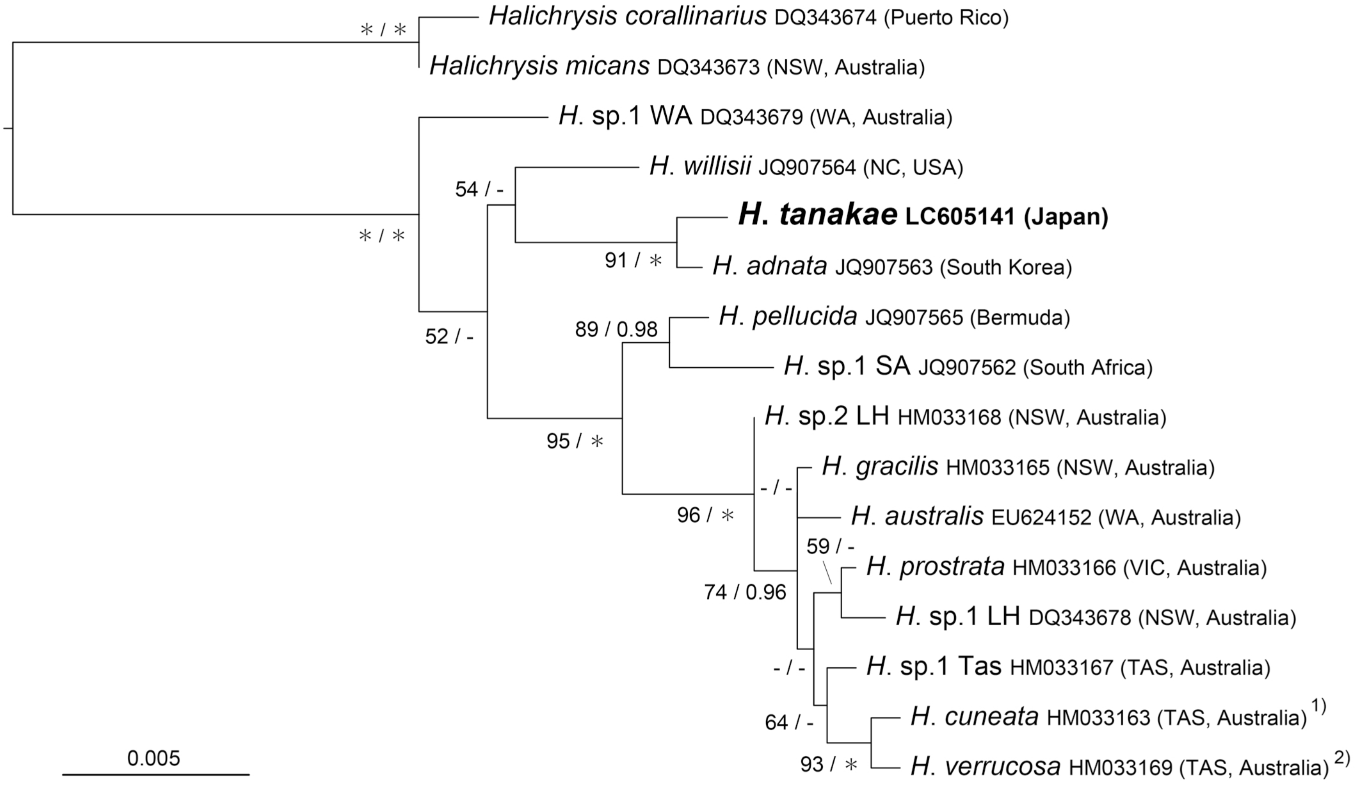
1)HQ544106, JQ907539 (South Korea) had identical sequences. 2)JQ907540 (NC, USA) had identical sequence. 3)JQ907541 (Bermuda) had identical sequence. 4)HM033037-HM033045, HM033047, HM033048 (VIC, Australia), HM033049 (NSW, Australia) had identical sequences. 5)HM033027 (WA, Australia) had identical sequence. 6)HM033034, HM033035, HM915982, KU934269 (NSW, Australia) had identical sequences. 7)HM033030-HM033032 (TAS, Australia) had identical sequences. 8)HM033028, HM033067 (VIC, Australia) had identical sequences. 9)HM033054-HM033056, HM033062, HM033066, HM033069, HM033070 (TAS, Australia) had identical sequences. 10)HM033057-HM033059, HM033061 (TAS, Australia) had identical sequences.

Fig. S1. Maximum likelihood phylogeny based on *cox*1 sequences. Numbers below the branches indicate the bootstrap values (BP, left) and Bayesian posterior probabilities (PP, right). Only the BP ≥ 50% and PP ≥ 0.95 are shown. Asterisks (∗) indicate BP = 100% and PP = 1.00.



[1)]LC605129 (Japan) had identical sequence.

Fig. S2. Maximum likelihood phylogeny based on *rbc*L sequences. Numbers below the branches indicate the bootstrap values (BP, left) and Bayesian posterior probabilities (PP, right). Only the BP ≥ 50% and PP ≥ 0.95 are shown. Asterisks (∗) indicate BP = 100% and PP = 1.00.



[1)]HM033164 (TAS, Australia) had identical sequence. [2)]HM033170 (VIC, Australia) had identical sequence.

Fig. S3. Maximum likelihood phylogeny based on 28S rDNA sequences. Numbers below the branches indicate the bootstrap values (BP, left) and Bayesian posterior probabilities (PP, right). Only the BP ≥ 50% and PP ≥ 0.95 are shown. Asterisks (∗) indicate BP = 100% and PP = 1.00.

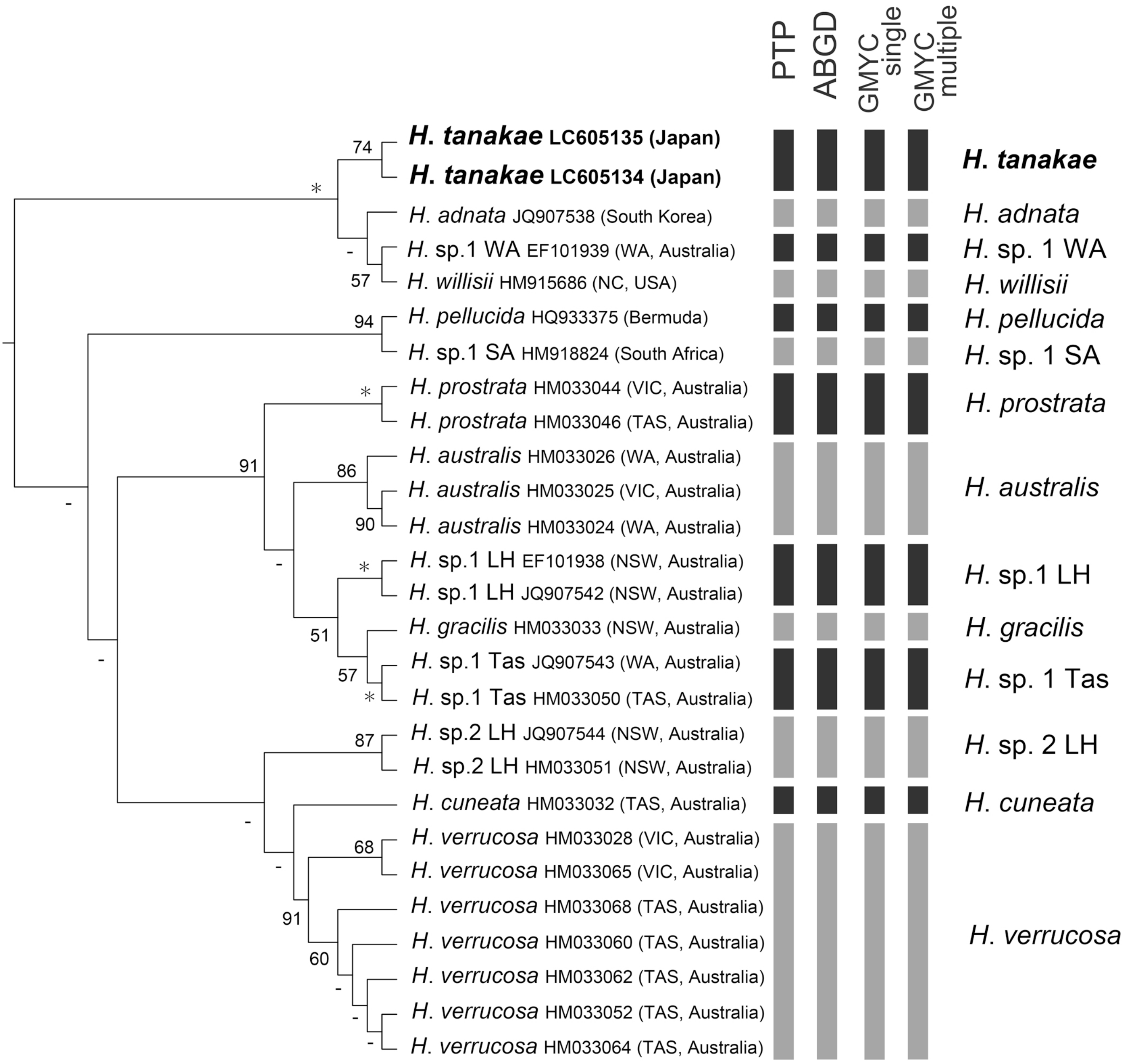


Fig. S4. Results of the species delimitation analysis plotted on the ultrametric tree based on *cox*1 sequences. Numbers below the branches indicate the bootstrap values (BP) in maximum likelihood phylogeny. Only the BP ≥ 50% is shown. Asterisks (∗) indicate BP = 100%. Bars represent results from the Poisson Tree Processes method (PTP), Automatic Barcode Gap Discovery (ABGD), Generalized Mixed Yule Coalescent (GMYC) method using a single threshold (GMYC single), and GMYC method with multiple thresholds (GMYC multiple).