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MOLECULAR PHYLOGENY OF *CLIBANARIUS* DANA, 1852 FROM THE INDO-WEST PACIFIC: EVOLUTION OF PEREOPOD COLOUR PATTERN AND HABITAT ADAPTATION

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ABSTRACT

Species of hermit crabs in the genus *Clibanarius* Dana, 1852 have adapted to various environments in the intertidal areas, including hard substrates and soft sediments. These species often bear a close morphological resemblance to each other, therefore, the colouration on the pereopods can be one of the reliable characteristics to distinguish the species. However, the evolutionary relationships among species with different colour patterns and relationships between colour patterns and habitat adaptation have not previously been investigated. Therefore, we reconstructed the phylogenetic relationships among 19 species of *Clibanarius* based on mitochondrial [12S rRNA, 16S rRNA and cytochrome oxidase I] and nuclear [histone H3] DNA markers. The results suggest that the striped and solid colour elements have evolved multiple times independently, with the ancestral colour pattern potentially being scattered, bright colour spots with a bright colour band. Our findings also suggest that evolutionary adaptation from hard substrates to mudflats and soft sediments may have occurred at least twice.

Key words. — Intertidal area, adaptation, rocky shore, mudflat, DNA marker

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RÉSUMÉ

Les espèces de bernard-l'ermite du genre *Clibanarius* Dana, 1852, ses sont adaptées à divers environnements dans les zones intertidales, incluant des substrats durs et des sédiments mous. Ces espèces présentent souvent une forte ressemblance entre elles, ainsi la coloration des péréiopodes peut être une caractéristique fiable pour les distinguer. Cependant les relations évolutives entre ces espèces avec différents modèles de couleurs et les interrelations entre répartition de couleurs et l'adaptation à l'habitat n'ont pas été examinées. Ainsi nous avons reconstruit les relations phylogénétiques pour les 19 espèces de *Clibanarius* sur la base des marqueurs mitochondriaux [12S ARNr, 16S ARNr et cytochrome oxydase I] et ADN nucléaire [histone H3]. Les résultats suggèrent que les bandes et les éléments de couleur ont évolué plusieurs fois de façon indépendante, avec un modèle de couleur ancestrale potentiellement dispersé, des tâches de couleurs vives avec des bandes de couleurs vives. Nos résultats suggèrent aussi que l'adaptation évolutive de substrats durs vers des vasières et des sédiments mous a pu se produire au moins deux fois.

Mots clés. — Zones intertidales, adaptation, côte rocheuse, vasière, marqueurs ADN

INTRODUCTION

Body colour is sometimes considered a reliable character for distinguishing species in the morphological study of crustaceans (Knowlton, 1986, 1993; Anker, 2012). In many genera of hermit crabs, species that have very similar morphology have often conspicuously different colour patterns (Asakura & Paulay, 2003; Lemaitre & Poupin, 2003; Komai, 2004; Poupin & Malay, 2009; Malay et al., 2012). However, few molecular studies have been conducted to elucidate the evolutionary history of the development of the colour elements constructing such species-specific colour patterns (Malay & Paulay, 2009).

The hermit crab genus *Clibanarius* Dana, 1852, currently includes 59 species, 37 of which are distributed in the Indo-West Pacific (Osawa & Fujita, 2006; McLaughlin et al., 2007; Marin, 2016). Most species inhabit intertidal areas of rocky shores, coral reefs and mudflats. The species in this genus are morphologically very similar to, or often indistinguishable from, each other. Thus, the colouration on the pereopods can be one of the reliable characteristics to distinguish *Clibanarius* species (Rahayu & Forest, 1993; Negri et al., 2012, 2014; Marin, 2016; Yoshikawa et al., 2018). In these colour patterns, four major elements can be recognized on the dactyli of the second and third pereopods (ambulatory pereopods), i.e., (1) longitudinal stripe(s) on the lateral and mesial faces, (2) scattered bright colour spots on the whole surface, (3) dactyli with a solid colour similar to that of the propodi, (4) dactyli with solid colour distinctly brighter than those of the propodi, as well as four elements on the propodi, carpi, and meri of the same pereopods, i.e., (1) bright coloured transverse band(s), (2) longitudinal stripe(s) on the lateral and mesial faces, (3) scattered bright colour spots on the whole surfaces, and (4) solid colour. The colour patterns of the second and third pereopods of most

Clibanarius species can be expressed by these elements (table I). For example, longitudinal stripes are present on the ambulatory pereopods of *C. ambonensis* Rahayu & Forest, 1993, *C. danai* Rahayu & Forest, 1993, *C. eurysternus* (Hilgendorf, 1879), *C. infraspinatus* (Hilgendorf, 1869), *C. longitarsus* (De Haan, 1849), *C. padavensis* De Man, 1888, *C. striolatus* Dana, 1852, *C. taeniatus* (H. Milne Edwards, 1848) (fig. 1: Group I, fig. 2C, E), *C. rhabdodactylus* Forest, 1953, *C. symmetricus* (Randall, 1840), and *C. vittatus* (Bosc, 1802). Many bright colour spots are present on the ambulatory pereopods of *C. cruentatus* (H. Milne Edwards, 1848) and *C. snelliusi* Buitendijk, 1937 (fig. 2F and G), and, in addition, those of *C. snelliusi* have the longitudinal stripes on each dactylus (fig. 2G); *C. arethusa* De Man, 1888, *C. corallinus* (H. Milne Edwards, 1848), *C. englaucus* Ball & Haig, 1972, *C. rutilus* Rahayu, 1999, *C. virescens* (Krauss, 1843) have solid colour elements, and *C. humilis* (Dana, 1851) and *C. merguienis* De Man, 1888 have a bright colour band on each propodus. In addition, *C. englaucus*, *C. humilis*, *C. merguienis* and *C. virescens* also have a solid-colour band on each dactylus, much brighter than the colour of the propodi. Furthermore, *C. snelliusi*, *C. humilis* and *C. merguiensis* also have a bright colour band on each propodus, carpus and/or merus (fig. 1: Group 1, fig. 2G). However, the phylogenetic relationships and evolutionary history of these colour elements that produce the species-specific colour pattern have, until now, not yet been investigated by using a molecular approach.

Species of *Clibanarius* usually inhabit shallow waters, including both hard substrates (e.g., marine rocky shores and coral reefs) and soft sediments (e.g., mudflats, estuary flats and mangrove forests) (Osawa & Fujita, 2008), making them one of the most successfully adapted groups of intertidal hermit crabs. Moreover, these species can be morphologically separated into two major groups depending on the habitat. The short-dactylus group includes species that have stout second and third pereopods, with the dactyli shorter than the propodi. They are generally found on hard substrates. On the other hand, the long-dactylus group includes species that have slender second and third pereopods with the dactyli as long as, or slightly longer than, the propodi. They are generally found on soft sediments (Asakura, 2005; Osawa & Fujita, 2008; Malay et al., 2018). However, the evolutionary relationships for habitat adaptation in these species have also not yet been clarified.

In the present study, we reconstructed the phylogenetic relationships of *Clibanarius* based on three mitochondrial DNA markers [cytochrome oxidase I (COI), 12SrRNA and 16S rRNA] and a nuclear DNA marker [histone H3] from the perspective of the origin of the different colour elements and the supposed habitat shift.

TABLE I

Comparison of the colour elements on the pereopods of the species of *Clibanarius* considered herein

Species	Colour patterns of pereopods (dactylus colour element + colour element of propodus, carpus, and merus)	Element on dactylus				Element on propodus, carpus, and merus				Phylogenetic group (fig. 2)
		Stripe(s) (ST)	Solid colour (SC)	Bright colour spots (SP)	Brighter colour than colour of propodus (BC)	Stripe(s) (ST)	Solid colour (SC)	Bright colour spots (SP)	Bright colour band (BB)	
Clibanarius ambonensis Rahayu & Forest, 1993	ST + ST	○	–	–	–	○	–	–	–	II
Clibanarius arethusa De Man, 1888	SC + SC	–	○	–	–	–	○	–	–	–
Clibanarius corallinus (H. Milne Edwards, 1848)	SC + SC	–	○	–	–	–	○	–	–	–
Clibanarius cruentatus (H. Milne Edwards, 1848)	SP + SP	–	–	○	–	–	–	○	–	–
Clibanarius demani Buitendijk, 1937	ST + ST	○	–	–	–	○	–	–	–	II
Clibanarius englaucus Ball & Haig, 1972	SC(BC) + SC	–	○	–	○	–	○	–	–	I
Clibanarius eurysternus (Hilgendorf, 1879)	ST + ST	○	–	–	–	○	–	–	–	–
Clibanarius humilis (Dana, 1851)	SC(BC) + BB	–	○	–	○	–	–	–	○	I
Clibanarius infraspinatus (Hilgendorf, 1869)	ST + ST	○	–	–	–	○	–	–	–	–

TABLE I
(Continued)

Species	Colour patterns of pereopods (dactylus colour element + colour element of propodus, carpus, and merus)	Element on dactylus				Element on propodus, carpus, and merus				Phylogenetic group (fig. 2)
		Stripe(s) (ST)	Solid colour (SC)	Bright colour spots (SP)	Brighter colour than colour of propodus (BC)	Stripe(s) (ST)	Solid colour (SC)	Bright colour spots (SP)	Bright colour band (BB)	
Clibanarius longitarsus (De Haan, 1849)	ST + ST	○	–	–	–	○	–	–	–	II
Clibanarius merguiensis De Man, 1888	ST(BC) + BB	○	–	–	○	–	–	–	○	I
Clibanarius padavensis De Man, 1888	ST + ST	○	–	–	–	○	–	–	–	II
Clibanarius rhabdodactylus Forest, 1953	ST + ST	○	–	–	–	○	–	–	–	–
Clibanarius rutilus Rahayu, 1999	SC + SC	–	○	–	–	–	○	–	–	III
Clibanarius snelliusi Buitendijk, 1937	ST(BC) + SP	○	–	–	○	–	–	○	–	–
Clibanarius striolatus Dana, 1852	ST + ST	○	–	–	–	○	–	–	–	II
Clibanarius taeniatus (H. Milne Edwards, 1848)	ST + ST	○	–	–	–	○	–	–	–	III
Clibanarius virescens (Krauss, 1843)	SC(BC) + SC	–	○	–	○	–	○	–	–	I
Clibanarius sp.	ST + ST	○	–	–	–	○	–	–	–	II

Fig. 1. The *Clibanarius* species used in this study and their colour patterns (dactylus colour element + colour elements of propodi, carpi, and meri). The species are grouped according to the results of our phylogenetic analysis (see fig. 3).

MATERIAL AND METHODS

Sample collection

Clibanarius specimens were collected from intertidal areas at various localities in the Indo-West Pacific region. All specimens were identified to species following Miyake (1982), Rahayu & Forest (1993) and Asakura (2005). The coloura-

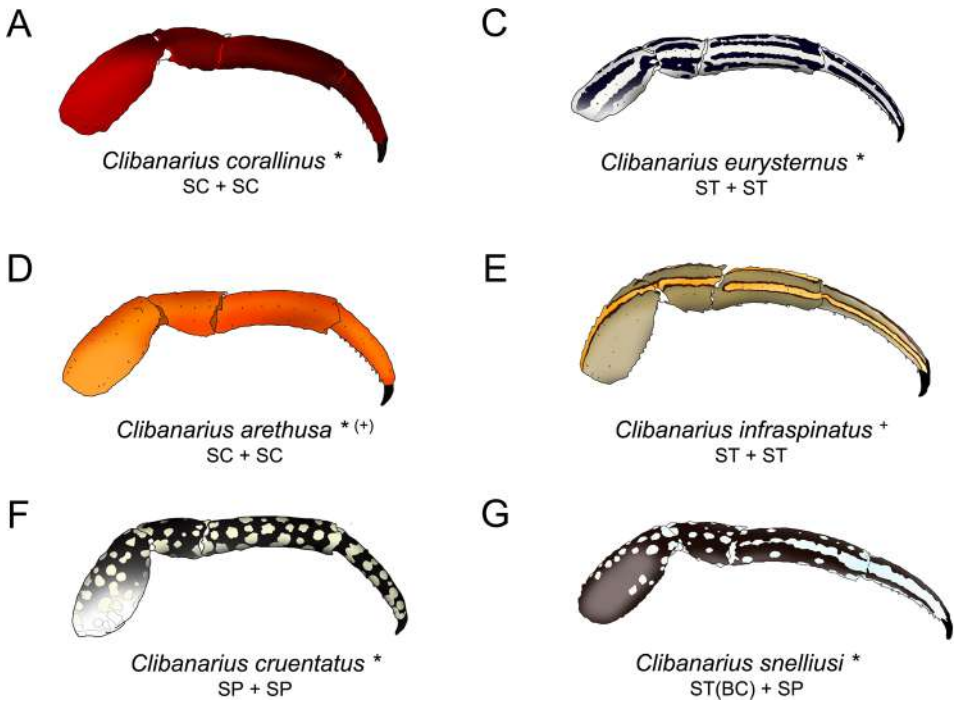

Fig. 2. The *Clibanarius* species used in this study and their colour patterns (dactylus colour element + colour elements of propodi, carpi, and meri). A to G represent the species that each constituted a monophyletic clade, but were not contained in the above-mentioned three groups; the capital letters here used coincide with the positions that are indicated in the same way in the maximum likelihood phylogenetic tree (fig. 3).

tion of the live specimens was recorded before fixing them in 99.5% ethanol. The specimens used in this study were housed in the Seto Marine Biological Laboratory, Kyoto University, Shirahama, Japan, with their catalogued numbers (see Appendix: table AI); the Crustacean Collection of the Lee Kong Chian Natural History Museum, Singapore; the Muséum national d'Histoire naturelle, Paris; and the Museum of Tropical Queensland, Townsville, Queensland.

Molecular methods

Total DNA was extracted from the tissue of each specimen using the High Pure Polymerase Chain Reactions (PCR) Template Kit (Roche) following the manufacturer's recommendations. PCR was used to amplify 600 bp of the COI gene, 500 bp of the 16S rRNA gene, 300 bp of the 12S rRNA gene, and 300 bp of the histone H3 gene. Each amplification was performed in a 25-μl reaction volume consisting of 5.0 μl of forward and reverse primers (2.5 μl each; Appendix: table AI), 2.5 μl of Ex Taq™ buffer, 2.0 μl of deoxyribonucleotide triphosphates

(dNTPs) (2.5 μl each), 0.13 μl of Ex Taq polymerase (TaKaRa, Otsu, Japan) and 14.87 μl of distilled water. The thermal cycling conditions were set at 94°C for 3 min., followed by 35 cycles at 95°C for 45 s, 50°C for 90 s at gene-specific annealing temperatures (COI and histone H3: 55°C; 12S rRNA and 16S rRNA: 45°C) and 72°C for 2 min. and final extension at 72°C for 4 min., and the PCR products were purified using the High Pure PCR Product Purification Kit (Roche). The sequencing reaction was performed using PCR primers and the BigDye® Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, U.S.A.) on an ABI 3130 sequencer (Applied Biosystems).

Phylogenetic analysis

Sequences of the COI, 12S rRNA, 16S rRNA and histone H3 genes were aligned with the program Clustal W (Thompson et al., 1997) implemented in MEGA version 7 (Kumar et al., 2016) using default settings. The sequences were then manually corrected by eye and any poorly aligned regions and all of the indels found in the 16S rRNA and 12S rRNA regions were excluded from the final alignments. Phylogenetic trees were constructed using maximum likelihood (ML) methods based on the combined dataset (COI + 12S rRNA + 16S rRNA + histone H3). Datasets were partitioned by codon, and the robustness of the ML tree was evaluated using 10,000 bootstrap replications. The ML analyses were performed using RAxML (Stamatakis, 2006) as implemented in raxmlGUI 1.31 (Silvestro & Michalak, 2012) and IQ-TREE 1.3.0 (www.iqtree.org; Nguyen et al., 2014). The GTRGAMMA model was implemented in raxmlGUI 1.31 and IQ-TREE 1.3.0. Three species in the most closely related genera in the family Diogenidae Ortmann, 1892 (*Calcinus morgani* Rahayu & Forest, 1999 and *Paguristes ortmanni* Miyake, 1978) and Coenobitidae Dana, 1851 (*Coenobita violascens* Heller, 1862) were used as outgroup species, following the previous studies (Tsang et al., 2011; Yoshikawa et al., 2018).

Ancestral state reconstruction analysis

To assess evolutionary relationships among the colour elements, we used the Trace Character History function of Mesquite v3.03 (Madison & Madison, 2015) with the ML reconstruction algorithm (model Mk1). Ancestral state reconstruction analysis was conducted separately on the dactyli and propodi, carpi, and meri. The colour elements seen on surfaces of the dactyli were categorised into four: stripe(s) (ST), bright colour spots (SP), solid colour (i.e., no stripes nor scattered spots) (SC) and much brighter colour than the colour of propodi (BC) (table I). The colour element seen on surfaces of the propodi, carpi, and meri were categorized similarly as the dactylus into ST, SP, SC and a bright colour band (BB) (table I).

Then, the colour patterns of the ambulatory pereopods of *Clibanarius* species are indicated in the present paper as "dactylus colour element" + "colour element on propodi, carpi, and meri" (e.g., *C. ambonensis*, ST + ST; *C. virescens*, SC(BC) + SC) (table I).

The colour variations were sometimes seen on the ambulatory pereopods in several *Clibanarius* species. For example, the colour band on each of the propodi of the third pereopods is often brighter than that of the second pereopods in *C. englaucus*. In *C. virescens*, specimens with distinctive whitish-coloured propodi, carpi, and meri of the ambulatory pereopods are rarely found (Yoshikawa et al., 2018). Minor colour variation is also seen in *C. virescens*; a vertical dark band or black spots are often seen on the middle of the dactyli (Morgan, 1988). However, because most of the analysed specimens of these two species have solid coloured propodi, carpi, and meri as well as dactyli much brighter coloured than the propodi, we used specimens of *C. virescens* with the typical colour type of the dactyli (without the vertical-dark band or black spots on the middle of the dactyli) for the categorization. Thus, we included these species as SC(BC) + SC patterns in the ancestral state reconstruction analysis (table I). Although five colour variations were recorded on the second and third pereopods of *C. merguiensis*, all of them can fall into the category of bright colour band type (Rahayu & Forest, 1993). Therefore, we categorized *C. merguiensis* as ST(BC) + BB in our analyses (table I).

The types of habitats inhabited by species were obtained during specimen collection and/or decided from previously published studies (Osawa & Fujita, 2008; Malay et al., 2018). We categorized the habitats into two groups: hard-bottom substrate (HS), which includes rocky shores, coral reefs, boulder beaches and dead-coral boulder beaches, and soft-bottom sediments (SS), which includes estuary-mudflats and estuary-mangrove forests. We then recorded the habitats where species were collected in order to discuss the postulated habitat shifts for these species (table II).

All transformations were mapped on the topology of the ML tree derived from the combined molecular data using RAxML (Stamatakis, 2006) as implemented in raxmlGUI 1.31 (Silvestro & Michalak, 2012).

RESULTS

In total, 93 individuals belonging to 22 species, including 19 *Clibanarius* species and three outgroup species (*Ca. morgani*, *Co. violascens* and *P. ortmanni*), were collected from 10 localities in the Indo-West Pacific region. These species were analysed using the combined 1438-bp dataset that included the COI (510 bp),

TABLE II

Species which were used in this study and their colour pattern of pereopods and habitats

Species	Colour patterns of pereopods (dactylus colour element + colour elements of propodus, carpus, and merus)	Phylogenetic group (fig. 2)	Category	Records	Reference of habitats
Clibanarius ambonensis	ST + ST	II	SS	Intertidal, in estuarine river habitats, mangroves and nipa palms	Malay et al. (2018)
Clibanarius arethusa	SC + SC	–	HS + SS	Coral reef, oyster reef and mud flats, intertidal or subtidal	Osawa & Fujita (2008); this study
Clibanarius corallinus	SC + SC	–	HS	Intertidal, reef flats and seagrass beds, on sandy, rocky, and mixed substrates	Malay et al. (2018)
Clibanarius cruentatus	SP + SP	–	HS	Intertidal, reef flats and seagrass beds, on rocky and sandy substrates	Malay et al. (2018)
Clibanarius demani	ST + ST	II	SS	Sand–mud, near river mouth (including mangrove area), 0–0.6 m depth	Osawa & Fujita (2008)
Clibanarius englaucus	SC(BC) + SC	I	HS	Intertidal, reef flats, on sandy and coralline substrates	Malay et al. (2018)

TABLE II
(Continued)

Species	Colour patterns of pereopods (dactylus colour element + colour elements of propodus, carpus, and merus)	Phylogenetic group (fig. 2)	Category	Records	Reference of habitats
Clibanarius eurysternus	ST + ST	–	HS	Sand–rock, reef flat, 0–5 m depth	Osawa & Fujita (2008)
Clibanarius humilis	SC(BC) + BB	I	HS	Intertidal, reef flats, on sandy and coralline substrates	Malay et al. (2018)
Clibanarius infraspinatus	ST + ST	–	SS	Intertidal to subtidal, near river mouths, 0–2 m deep, on mud–sand substrates	Malay et al. (2018)
Clibanarius longitarsus	ST + ST	II	HS + SS (mainly SS)	Brackish intertidal, river mouths, fringing mangroves, and nipa palms, on sandy, rocky, and sand–mud substrates	Malay et al. (2018)
Clibanarius merguiensis	ST(BC) + BB	I	HS + SS (mainly HS)	Intertidal, reef flats, seagrass beds, and mangroves, on rocky, sandy, and sand–mud substrates	Osawa & Fujita (2008)
Clibanarius padavensis	ST + ST	II	SS	Rubble–mud, mangrove area, intertidal	Malay et al. (2018)

TABLE II
(Continued)

Species	Colour patterns of pereopods (dactylus colour element + colour elements of propodus, carpus, and merus)	Phylogenetic group (fig. 2)	Category	Records	Reference of habitats
Clibanarius rhabdodactylus	ST + ST	–	HS	Rock, reef flat, 0–10 m depth	Osawa & Fujita (2008)
Clibanarius rutilus	SC + SC	III	HS	Rock–sand, 0.5–1 m depth	Osawa & Fujita (2008)
Clibanarius snelliusi	ST(BC) + SP	–	HS	Sand–rock, 0–3 m depth	Osawa & Fujita (2008)
Clibanarius striolatus	ST + ST	II	HS + SS (mainly SS)	Intertidal, mangrove areas, nipa palms, and seagrass beds, on sandy, sand–mud, and rocky substrates	Malay et al. (2018)
Clibanarius taeniatus	ST + ST	III	HS	Sand–rock, intertidal	Osawa & Fujita (2008)
Clibanarius virescens	SC(BC) + SC	I	HS + SS (mainly HS)	Intertidal, reef flats, seagrass beds, and mangrove areas, on rocky, sandy, mud–sand and coralline substrates	Malay et al. (2018)

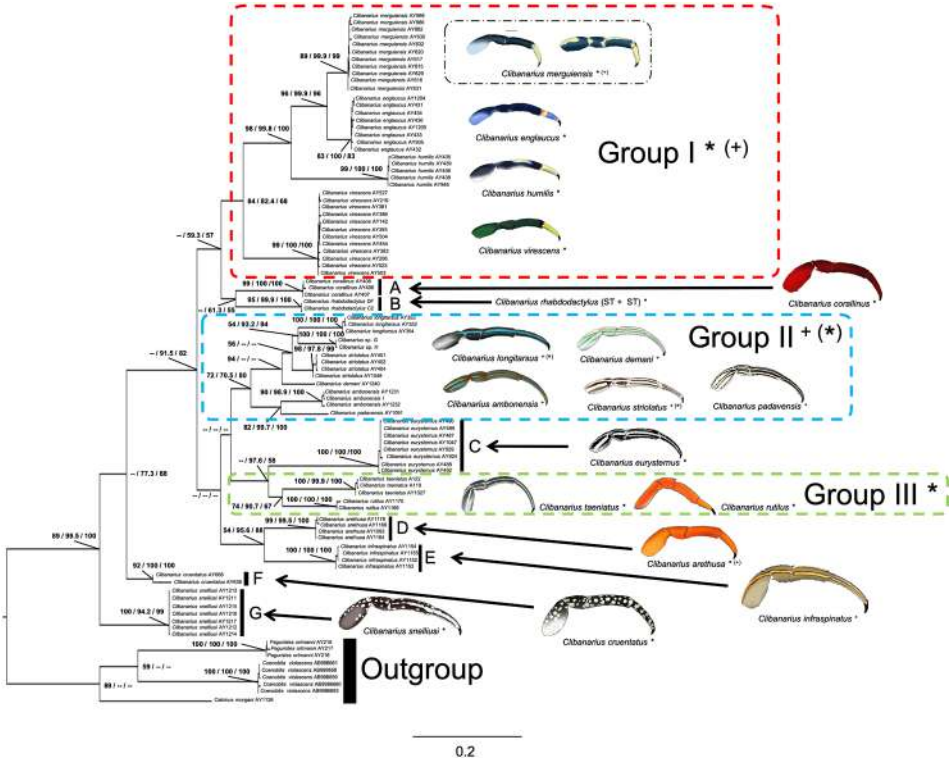

Fig. 3. Maximum likelihood (ML) tree of *Clibanarius* species based on the combined dataset of the genes cytochrome oxidase I (COI) (510 bp), 12S rRNA (269 bp), 16S rRNA (385 bp), and histone H3 (274 bp). Numbers above the branches indicate the bootstrap value, the SH-aLRT support (%) and the ultrafast bootstrap support (%). Red rectangle = Group 1; blue rectangle = Group 2; and green rectangle = Group 3.

12S rRNA (269 bp), 16S rRNA (385 bp) and histone H3 (274 bp) genes. The collection localities of the specimens and DNA accession numbers are listed in the Appendix: table AII.

The bootstrap value (≧50), SH-aLRT support (≧50) and ultrafast bootstrap support (UFboot) (≧60) in our ML phylogenetic tree, are shown in fig. 3. This tree reconstructed the existence of three groups among the *Clibanarius* species (fig. 3): Group I (*C. virescens*, *C. merguiensis*, *C. englaucus* and *C. humilis*), the monophyly of which is highly supported by high BS values (BS = 84); Group II (*C. longitarsus*, *C. striolatus*, *C. demani* Buitendijk, 1937, *C. ambonensis*, *C. padavensis* and *Clibanarius* sp. registered as *C. demani* in the Museum national d'Histoire naturelle, Paris, France), the monophyly of which is highly supported by the relatively high BS and SH-aLRT (BS = 72, SH-aLRT = 87.1); and Group III (*C. taeniatus* and *C. rutilus*), the monophyly of which is supported by relatively high BS and SH-aLRT (BS = 74, SH-aLRT = 90.7). Although the monophyly

of each clade of *C. corallinus*, *C. rhabdodactylus*, *C. eurysternus*, *C. arethusa*, *C. infraspinatus*, *C. cruentatus* and *C. snelliusi* was also highly supported by the BS, SH-aLRT and UFboot values (BS > 95, SH-aLRT > 99.0, UFboot = 100), these species were not contained in the three above-mentioned groups.

Reconstruction of the ancestral state of colour elements

To understand the evolution of the pereopod colour pattern in *Clibanarius* species, we conducted an ancestral state reconstruction analysis using the four colour elements on dactyli and the four colour elements on propodi, carpi, and meri.

In regard of the colouration of the dactyli, because the ancestral state of each colour element was not significantly supported on the basal position of the phylogenetic tree, the ancestral colour element on the dactyli was not unambiguously determined in this analysis. The striped elements have been gained not only in Groups II and III, but also in Group I and other *Clibanarius* species (Appendix: fig. A1). The solid coloured element has also been developed on the several lineages independently (Appendix: fig. A2). In contrast, the bright colour spots were only found on the dactyli of *C. cruentatus* (Appendix: fig. A3). Therefore, the elements of ST, SP and SC have been evolved multiple independent times in several lineages and/or gained in the specific species. However, our analysis did highly support an independent evolution of dactyli with distinctly brighter colour than the propodi (BC) in a common ancestor of Group I (i.e., *C. merguienesis*, *C. englaucus*, *C. humilis* and *C. virescens*) (fig. 4).

With respect to the colouration of the propodi, carpi, and meri, our analysis gave significant support to the ancestral colour pattern being composed of bright colour spots, as seen in *C. cruentatus* and *C. snelliusi* (fig. 5). Thus, the gain and loss (i.e., the SC type) of the ST element occurred independently in *Clibanarius* species (fig. 3, Appendix: figs. A4; A5). The bright colour band has also arisen multiple times independently (Appendix: fig. A6).

Reconstruction of the ancestral state of the habitat substrate

To understand the evolution of the presumed habitat shifts in *Clibanarius* species, we included five substrate types in the ancestral state reconstruction analysis (fig. 6) and categorized the various species into these as follows: *C. ambonensis*, *C. demani*, *C. infraspinatus* and *C. padavensis* in SS; *C. corallinus*, *C. cruentatus*, *C. englaucus*, *C. eurysternus*, *C. humilis*, *C. rhabdodactylus*, *C. rutilus*, *C. snelliusi* and *C. taeniatus* in HS; *C. longitarsus* and *C. striolatus* in HS + SS (mainly SS); *C. merguiensis* and *C. virescens* in HS + SS (mainly HS); and *C. arethusa* in HS + SS (table II). The results suggested that hard substrate

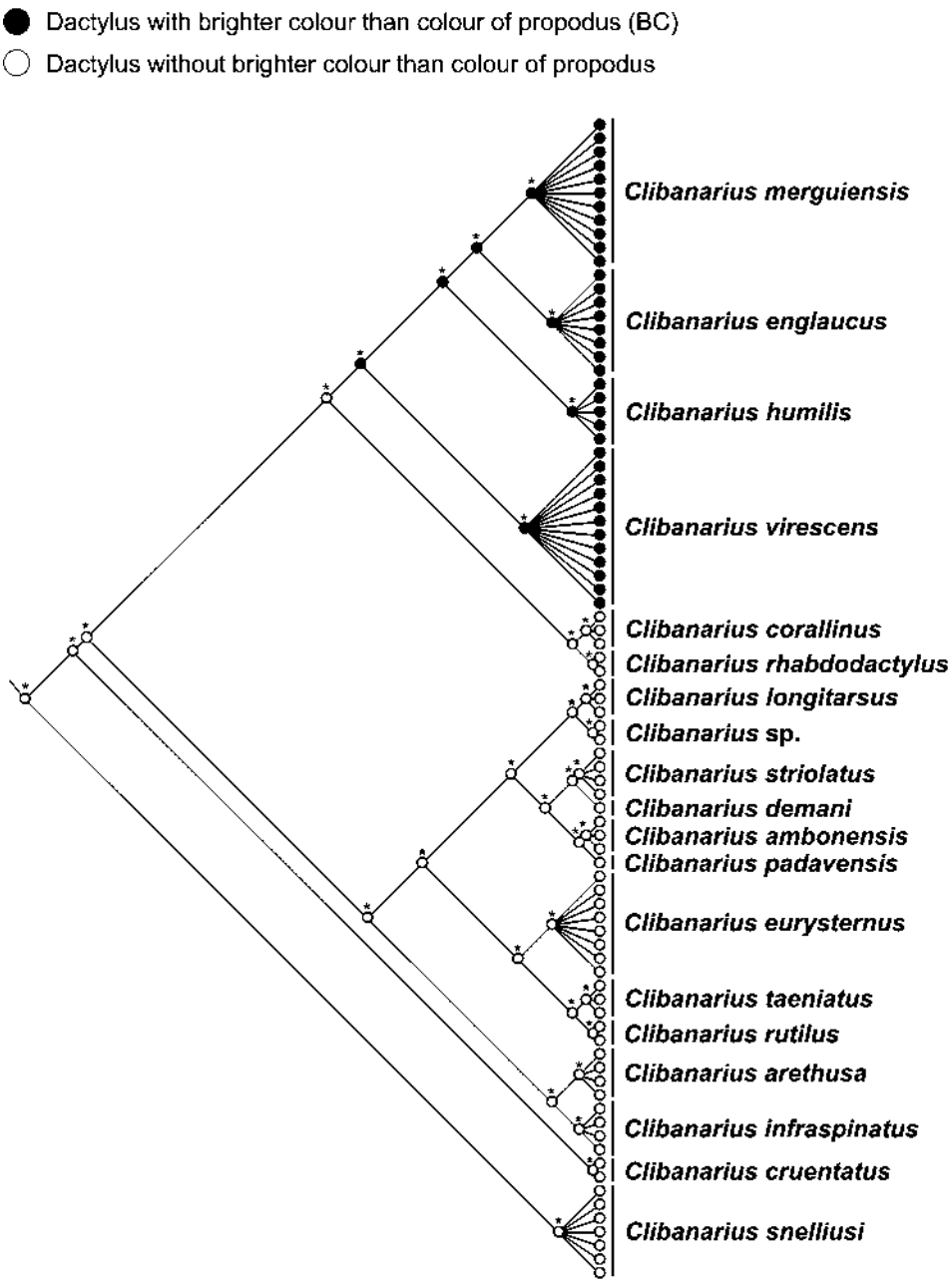

Fig. 4. Maximum likelihood reconstruction of the feature "colour brighter than that of the propodi" (BC) in the dactyli of *Clibanarius* spp., based on the combined dataset of four genes [cytochrome oxidase I (COI) + 12S rRNA + 16S rRNA + histone H3]. Pie charts illustrate the relative likelihoods of the two possible states in each clade. Each asterisk indicates the significantly supported ancestral state.

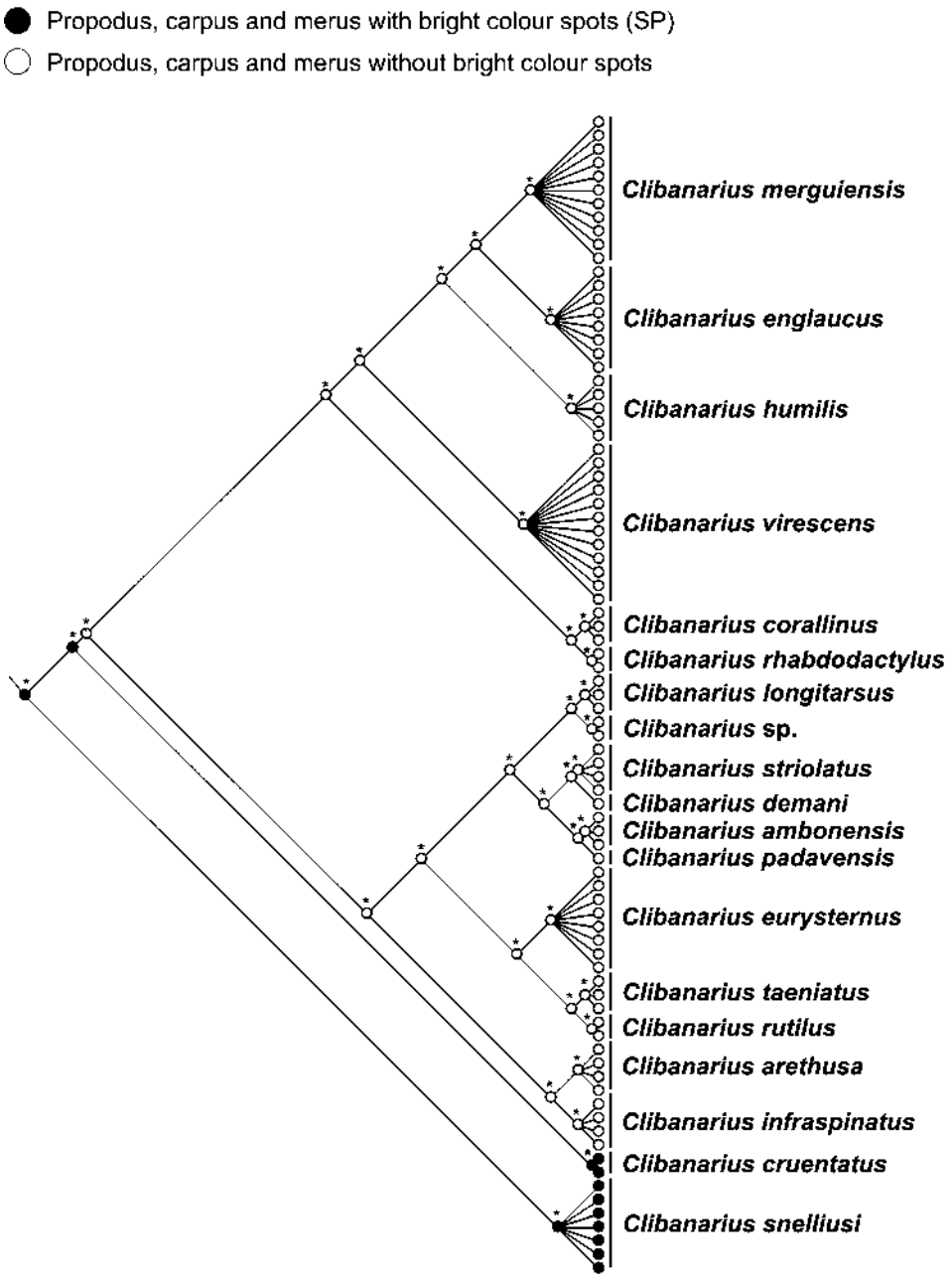

Fig. 5. Maximum likelihood reconstruction of the bright colour spots (SP) on the propodi, carpi, and meri of *Clibanarius* spp. based on the combined dataset of four genes [cytochrome oxidase I (COI) + 12S rRNA + 16S rRNA + histone H3]. Pie charts illustrate the relative likelihoods of the two possible states in each clade. Each asterisk indicates the significantly supported ancestral state.

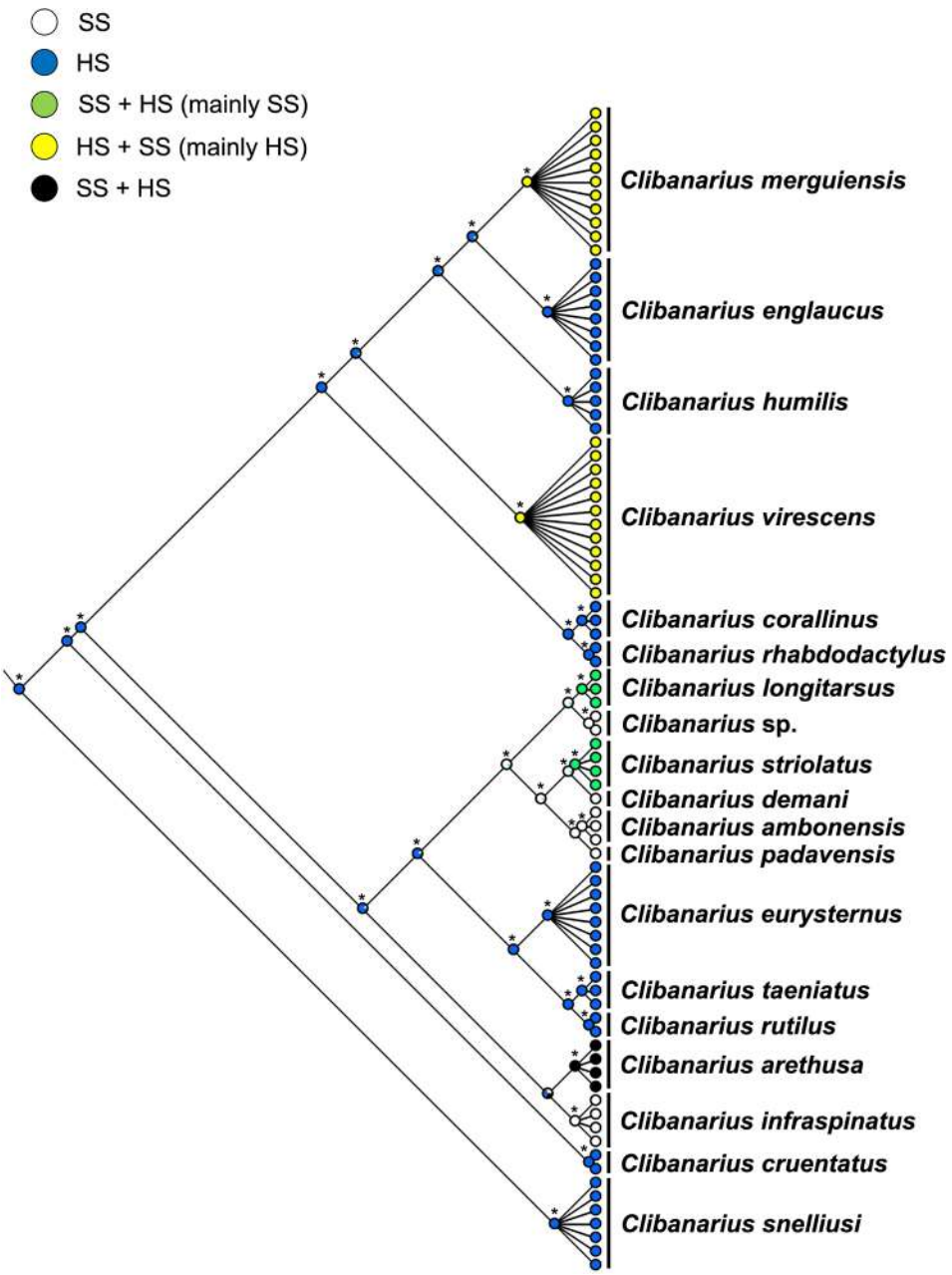

Fig. 6. Maximum likelihood reconstruction of substrate type of the habitat in *Clibanarius*, based on the combined dataset of four genes [cytochrome oxidase I (COI) + 12S rRNA + 16S rRNA + histone H3]. Pie charts illustrate the relative likelihoods of the two possible states in each clade. Each asterisk indicates the significantly supported ancestral state.

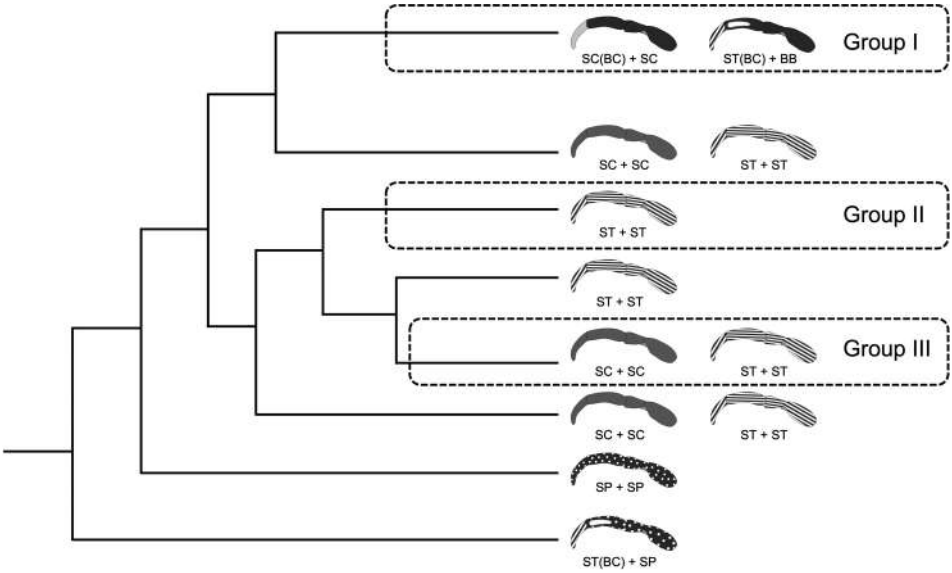

Fig. 7. Diagram of the phylogenetic relationships of *Clibanarius* and evolutionary patterns of the colour patterns on the pereopods (dactylus colour element + the colour elements of the propodi, carpi, and meri). The dotted lines indicate the groups that were significantly supported by our ML analyses.

was the ancestral habitat of *Clibanarius* species and that habitat shifts from this to soft sediments may have occurred independently in the ancestors of the species belonging to Groups II and III, as well as in *C. eurysternus*, *C. arethusa* and *C. infraspinatus* (fig. 7).

DISCUSSION

Reconstruction of the ancestral colour patterns of the propodi, carpi, and meri

According to our phylogenetic reconstruction, colour elements SC and ST developed in Group III, while Group I only exhibited SC and BB, and Group II only exhibited ST. *Clibanarius infraspinatus* typically has stripes on the pereopods and inhabits soft-bottom environments. However, this species was not included in Group II, but rather was a sister clade of *C. arethusa*, with a relatively low bootstrap value (54) but high SH-aLRT values (93.5). Thus, it is suggested that the loss of the element and the development of stripes have occurred at least twice in *Clibanarus* species. The striped element on the propodi, carpi, and meri appears to have been secondarily obtained in the ancestor of *C. rhabdodactylus* and to have been secondarily lost in the ancestor of *C. rutilus* and *C. arethusa* (figs. 3, 7; Appendix: fig. A1).

The analysis also suggested that the most recent common ancestor of *Clibanarius* species had bright colour spots (fig. 5), which were probably similar to those found on the ambulatory pereopods of *C. cruentatus* and *C. snelliusi* (F and G in fig. 3). By contrast, a BB was recognized not only in *C. snelliusi* but also in some other species, such as *C. merguiensis* and *C. humilis*. Since the ancestral state of a brightly coloured dactylus was not significantly supported in our analysis, this colour element also appears to have evolved multiple times independently (Appendix: fig. A3). In addition, five colour variations were recorded on the second and third pereopods of *C. merguiensis*, all of which were based on the BB rather than the typical striped element (Rahayu & Forest, 1993). Therefore, this may be a plastic phenotype for this species.

Reconstruction of the ancestral colour patterns of the dactylus

Group I only included species with the SC colour element, which was a much brighter colour (BC) than that on the propodi, carpi, and meri (i.e., *C. merguiensis*, *C. englaucus*, *C. humilis* and *C. virescens*), while Group II only included species with the ST colour element and Group III included species with both the SC and ST colour elements, which were the same as the elements seen on the propodi, carpi, and meri. The ancestral dactylus colour patterns in the basal position of the phylogenetic tree were not significantly supported in the ancestral reconstruction analysis, which may indicate that all of the colour elements on the dactyli evolved independently, or are very plastic in this genus.

Group I consisted of common species that inhabit rocky shores and coral reefs in the Indo-West Pacific area (Rahayu & Wahyudi, 2008; Hirose et al., 2010) and that often aggregate and cluster on the rocks and reefs during low tide (Gherardi & Vannini, 1989; A. Yoshikawa, pers. obs.), and only the SC (BC) colour pattern was recorded for these species. Furthermore, *C. merguiensis*, *C. englaucus*, *C. humilis*, *C. virescens* and *C. snelliusi* have a sympatric distribution in hard-bottom environments and exhibit dactylus colour patterns different from the colouration of propodi, carpi, and meri. Therefore, it may be probable that a bright colour on the dactyli or a colour pattern different from that on the propodi, carpi, and meri has some adaptive significance for inhabiting hard-bottom environments, such as species recognition during aggregating/clustering in low tidal time and/or for mate guarding.

Evolutionary processes of colour pattern development

As mentioned above, our findings indicate that the ancestral state of the colour pattern on the ambulatory pereopods was a pattern of bright colour spots, and the other colour elements that are observed in *Clibanarius* species may

have evolved or be derived from this element. It is possible that the striped colour element was formed by some bright colour spots extending from the distal margin to the proximal margin of propodi, carpi, and meri. However, our analyses suggest that the striped colour element secondarily evolved in *C. rhabdodactylus*, so the evolutionary process may have been different in this species.

Although *C. corallinus*, *C. rutilus*, *C. arethusa* and the Group I species all have solid colouration on each segment of the ambulatory pereopods, the colouration is the same as on the dactylus in the former three species but relatively brighter on the dactyli than on the propodi, carpi, and meri in the Group I species. Therefore, it appears that the evolutionary process for the formation of a solid colour element differed between Group I and the other species with a solid colour element. It is possible that the extension of the bright colour spots to completely cover the segment resulted in the brighter solid colour element that is observed on the propodi, carpi, and meri of *C. corallinus*, *C. rutilus* and *C. arethusa*, and on the dactyli of Group I species, whereas reduction of the bright colour spots to an extremely small size or expansion of the dark colouration outside the spots to cover the entire segment led to the darker solid colour element that is seen on the propodi, carpi, and meri of *C. englaucus* and *C. virescens*. Further investigations are needed to elucidate the evolutionary processes of colour element formation, which may include a comparison of the gene expression pattern for each colour element.

Clibanarius tricolour (Gibbes, 1850), which is distributed in the intertidal and shallow subtidal zones of the Caribbean Sea and western Atlantic (Provenzano, 1960), expresses complicated colour patterns (a complex combination of many elements) compared to other *Clibanarius* species. The colour of the dactyli of *C. tricolour* is solid/bright-yellow, while the propodi, carpi, and meri are brilliant blue with black spots and the dark colour band; the dactylus' colour is brighter than that of the basic colour of the propodi, carpi, and meri. Because we could not analyse the phylogenetic position of *C. tricolour* and only estimated the evolutional relationships of the major colour elements of the pereopods, the evolutional history of the complicated-colour pattern seen in *C. tricolour* is open to discussion. A more comprehensive molecular analysis of *Clibanarius* species is needed to strengthen and/or verify (or to refute and extend) our conclusion and to reconstruct the evolutionary history of the specific colour patterns of *C. tricolour*. However, at least our molecular and ancestral reconstruction analyses could indicate a new insight into the evolutionary relationships of the major colour elements of the pereopods in *Clibanarius*.

Importance of colour pattern for *Clibanarius* taxonomy

Species in the genus *Clibanarius* are morphologically very similar but can be distinguished by species-specific colouration (Negri et al., 2012, 2014). However, some species exhibit intraspecific variation in colour (Rahayu & Forest, 1993; Yoshikawa et al., 2018), and the colours are very difficult to preserve, readily fading after alcohol fixation, which has often contributed to taxonomic confusion in this genus (Marin, 2016).

Three specimens borrowed from the Muséum national d'Histoire naturelle in Paris, France, that had been identified as *C. ambonensis*, were used in our analysis; these specimens were collected during the Santo Marine Biodiversity Survey. However, our ML analysis showed that two of these (MNHN-IU-2017-2008 and MNHN-IU-2017-2009) were not monophyletic with the clade of *C. ambonensis* or even its sister clade, indicating possibly the existence of a cryptic species that remarkably resembles *C. ambonensis*. Therefore, there is a possibility of the existence of a cryptic population that resembles the colour pattern of *C. ambonensis* in the Indo-West Pacific area. Because after fixation in ethanol, the colouration of the specimens easily fades and comparison with other, live materials becomes difficult, we could not observe the colouration of the preserved material. Therefore, for further taxonomic study of *C. ambonensis* it will be required that not only morphological observation will be performed, but also a detailed description of the live colour patterns.

Habitat adaptation of *Clibanarius* species

According to the results of the ancestral state reconstruction of the habitat substrate, the ancestral habitat analysis suggested that the most recent common ancestor of *Clibanarius* species most likely inhabited hard-bottom environments (the sandy shore, rocky shore and coral reef of the intertidal or subtidal area) (fig. 6). The findings of the present study may also indicate that the adaptation for inhabiting mudflats and mangrove forests arose multiple times independently. The striped species *C. longitarsus*, *C. striolatus*, *C. demani*, *C. ambonensis*, *C. padavensis* and *C. infraspinatus* have mainly been recorded from soft-sediment environments (Osawa & Fujita, 2008; Malay et al., 2018). However, *C. rhabdodactylus*, *C. eurysternus* and *C. taeniatus*, which are also striped, are mainly found in hard-bottom environments (Osawa & Fujita, 2008; Hirose et al., 2010; Malay et al., 2018). Furthermore, although *C. eurysternus*, *C. taeniatus* and *C. arethusa* were sister species to those found in soft-bottom environments, *C. rhabdodactylus* was a sister to *C. corallinus*, and all of these species were closely related to Group I, which mainly comprised HS species. Therefore, *C. eurysternus*, *C.*

taeniatus and *C. arethusa* may have secondarily adapted to hard-bottom environments, while the striped colouration may have secondarily evolved in *C. rhabdodactylus*. Thus, since the striped pattern was found in both SS and HS environments, the adaptive significance of this colour pattern to the habitat remains to be clarified.

Although we categorized the habitats into HS and SS in our analysis, the boundary between these environments is often unclear due to the environmental gradient that occurs in nature. Additionally, some *Clibanarius* species, such as *C. striolatus* and *C. arethusa*, have been recorded from both hard- and soft-bottom habitats (Osawa & Fujita, 2008; Hirose et al., 2010; Malay et al., 2018), which implies that *Clibanarius* species will have experienced habitat shifts several times during the adaptation processes, or an ongoing habitat shift. Therefore, our findings suggest that the ancestors of *C. eurysternus*, *C. taeniatus* and *C. arethusa* may have inhabited soft-bottom environments and secondarily have adapted to hard-bottom environments, whereas *C. merguiensis*, which has been recorded in both environments (Osawa & Fujita, 2008), probably is experiencing an ongoing adaptation shift from hard- to soft-bottom environments.

FUNDING

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We are grateful to Ms. Chie Iwamoto for their help in our experiments and to Mr. Norikazu Kitamura for improving our manuscript and supporting our analysis, and also to all staff members of the Centre for Information Biology, National Institute of Genetics. We are also grateful to all of the staff of the Phuket Marine Biological Centre (PMBC), Thailand for sample collection. Our special thanks are likewise due to those who gave us opportunities to examine the *Clibanarius* specimens held in their respective museums: Dr. Peter Kee Lin Ng and Jose Christopher Escano Mendoza (Lee Kong Chian Natural History Museum, Singapore), Dr. Niel L. Bruce (Museum of Tropical Queensland, Townsville, Queensland) and Dr. Laure Corbari (Muséum national d'Histoire naturelle, Paris, France).

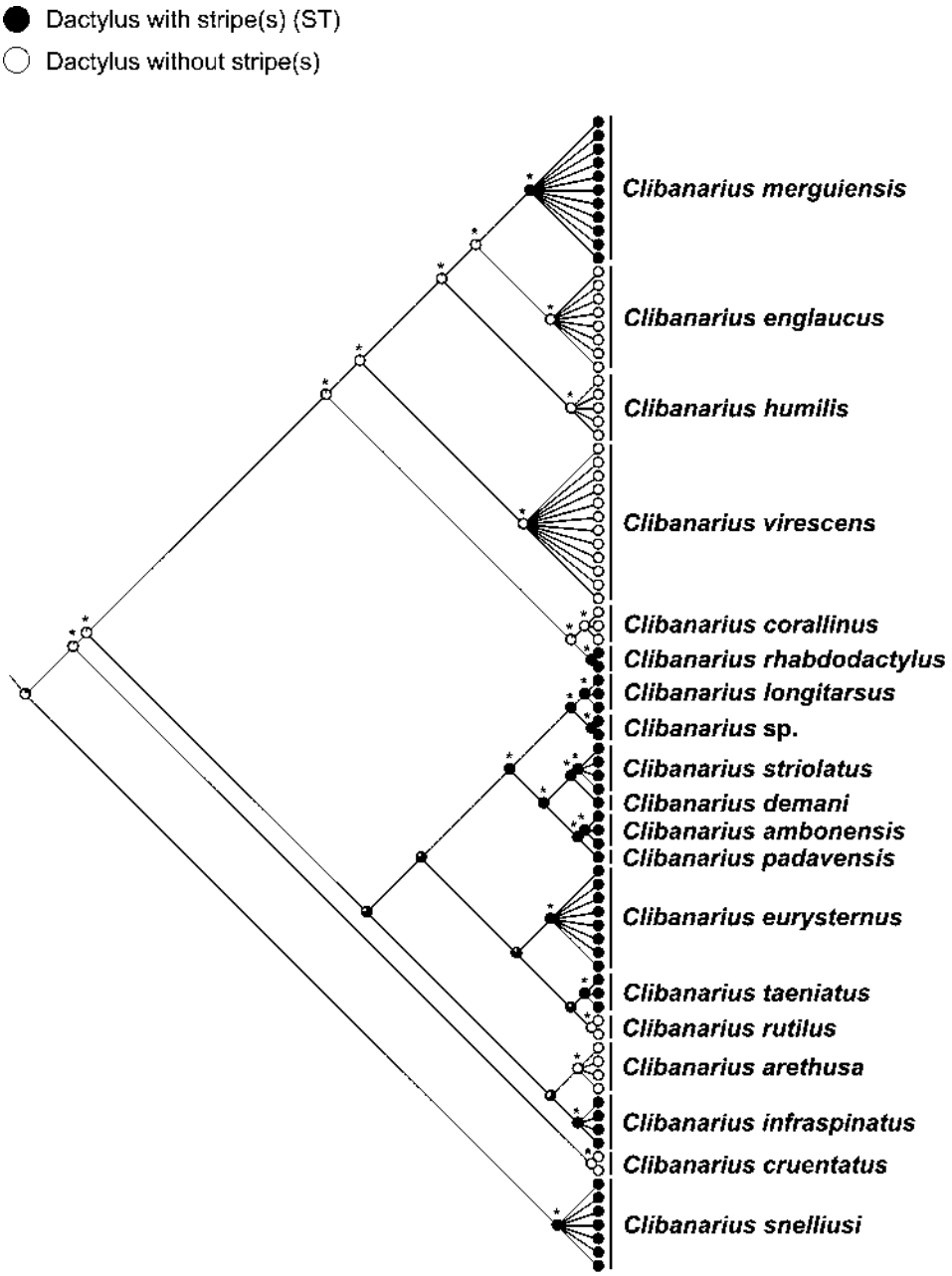

Fig. A1. Maximum likelihood reconstruction of the striped colour element (ST) in the dactyli of *Clibanarius* based on the combined dataset of four genes [cytochrome oxidase I (COI) + 12S rRNA + 16S rRNA + histone H3]. Pie charts illustrate the relative likelihoods of the two possible states in each clade. Each asterisk indicates the significantly supported ancestral state.

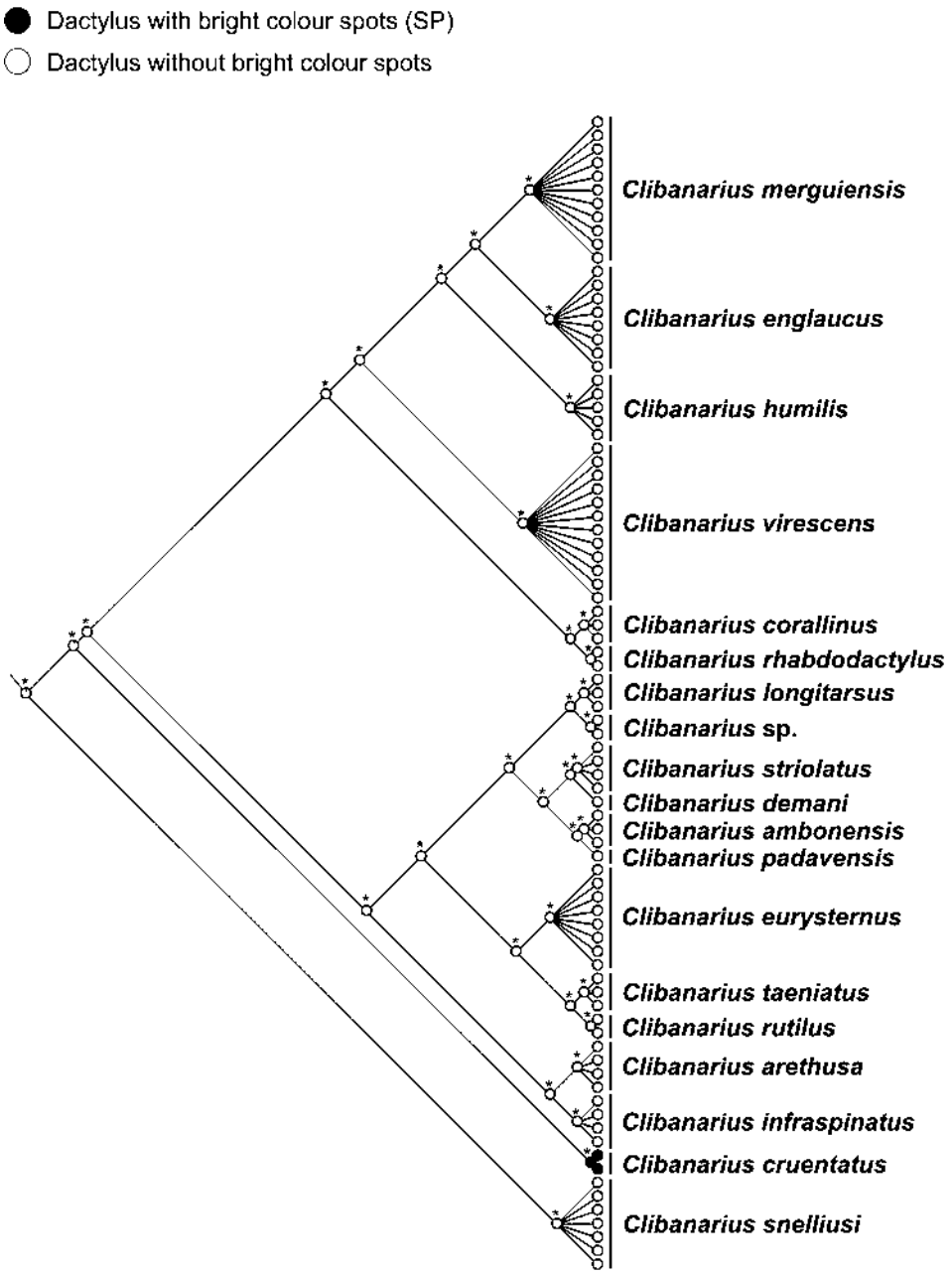

Fig. A2. Maximum likelihood reconstruction of the solid colour element (SC) in the dactyli of *Clibanarius* based on the combined dataset of four genes [cytochrome oxidase I (COI) + 12S rRNA + 16S rRNA + histone H3]. Pie charts illustrate the relative likelihoods of the two possible states in each clade. Each asterisk indicates the significantly supported ancestral state.

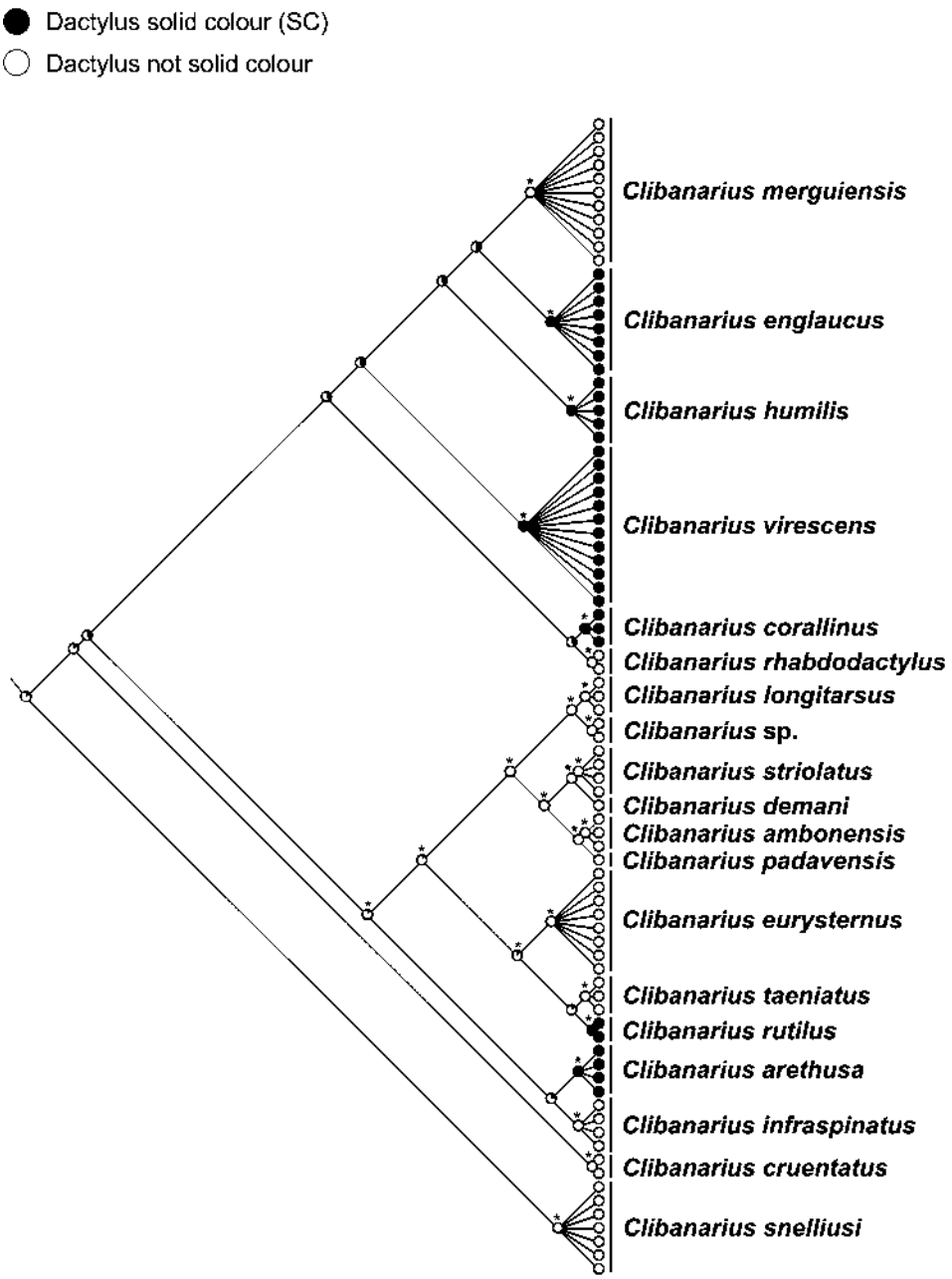

Fig. A3. Maximum likelihood reconstruction of the brightly coloured spots (SP) in the dactyli of *Clibanarius* based on the combined dataset of four genes [cytochrome oxidase I (COI) + 12S rRNA + 16S rRNA + histone H3]. Pie charts illustrate the relative likelihoods of the two possible states in each clade. Each asterisk indicates the significantly supported ancestral state.

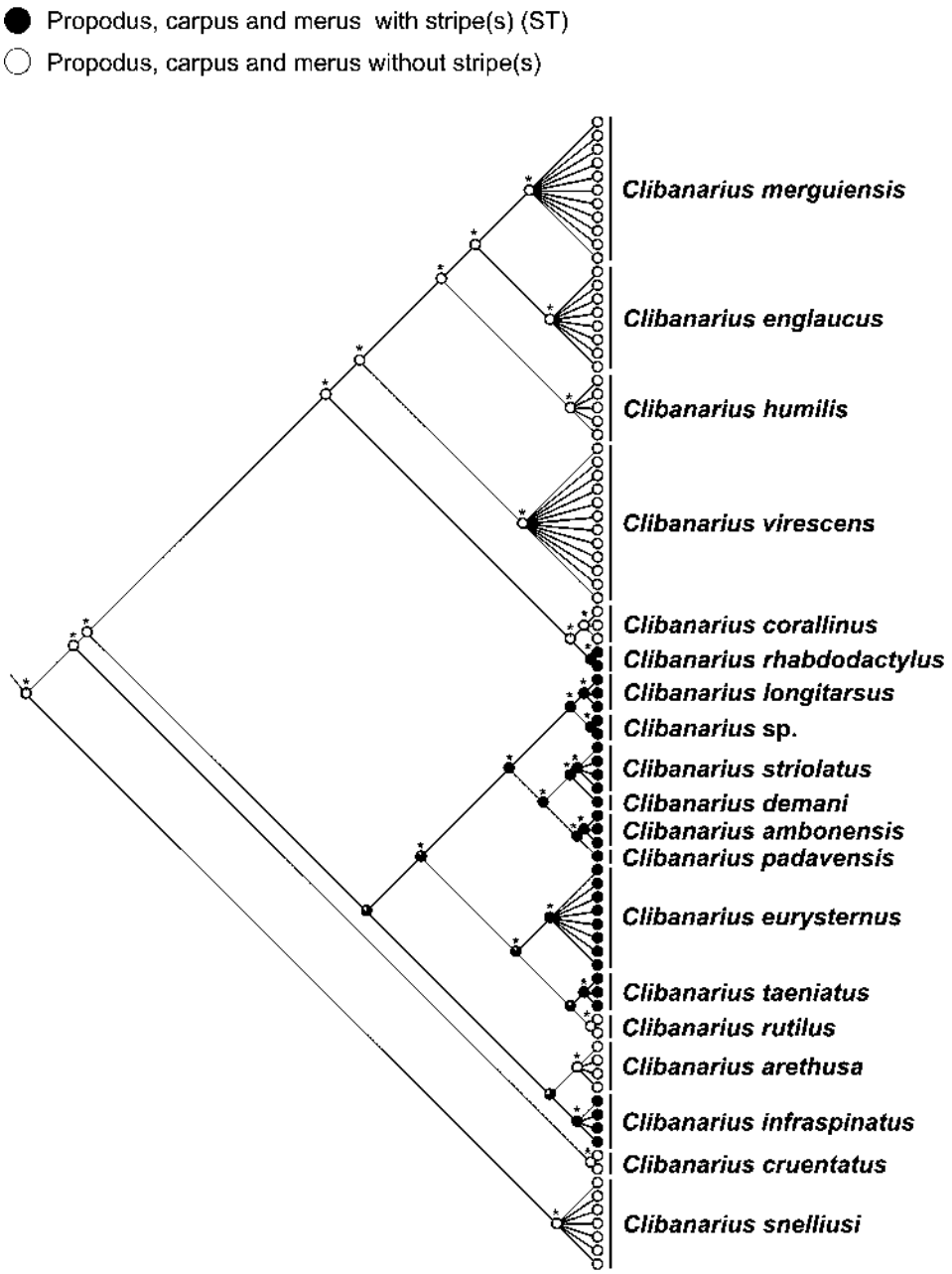

Fig. A4. Maximum likelihood reconstruction of the striped colour element (ST) in the propodi, carpi, and meri of *Clibanarius* based on the combined dataset of four genes [cytochrome oxidase I (COI) + 12S rRNA + 16S rRNA + histone H3]. Pie charts illustrate the relative likelihoods of the two possible states in each clade. Each asterisk indicates the significantly supported ancestral state.

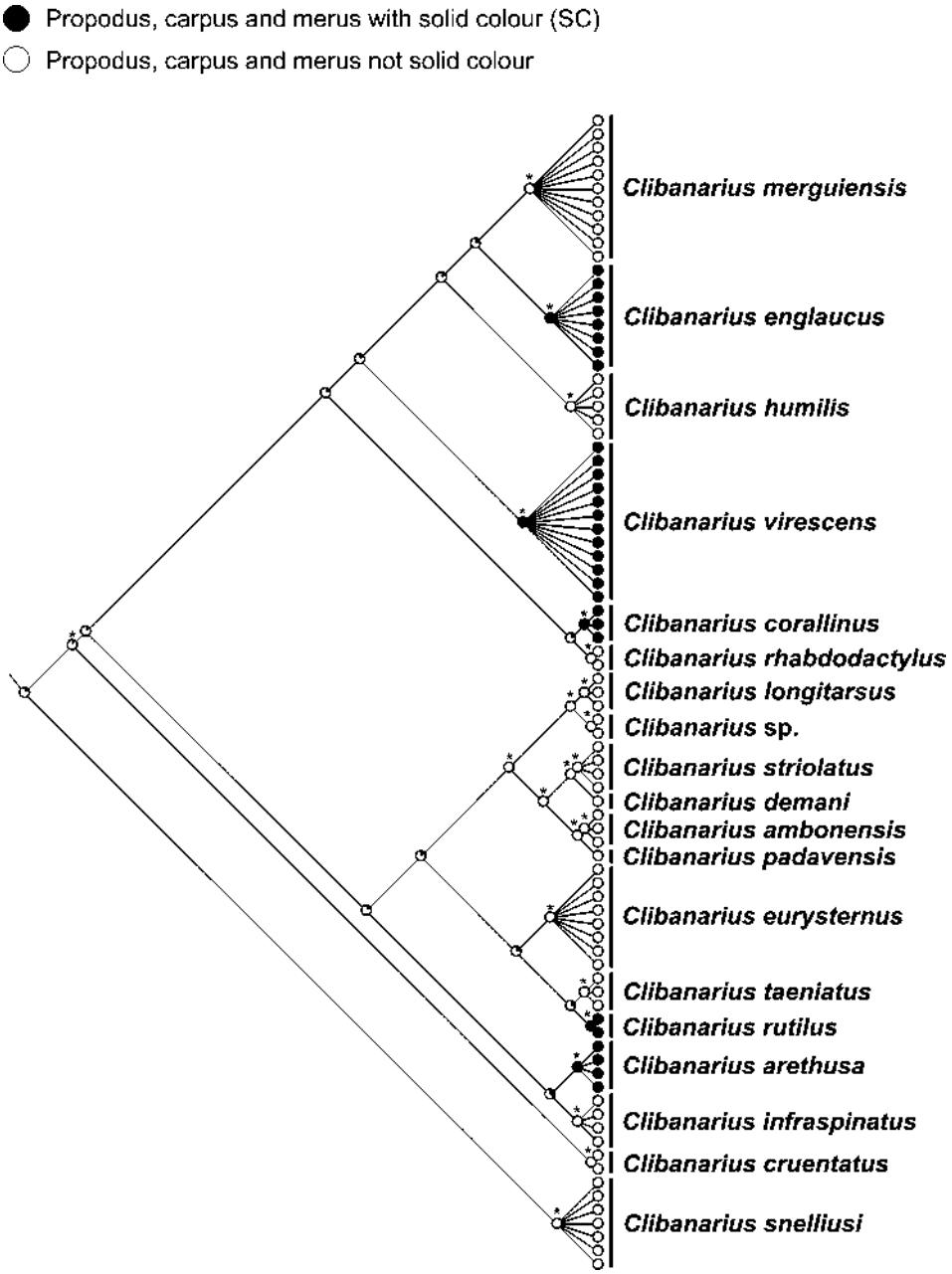

Fig. A5. Maximum likelihood reconstruction of the solid colour element (SC) in the propodi, carpi, and meri of *Clibanarius* based on the combined dataset of four genes [cytochrome oxidase I (COI) + 12S rRNA + 16S rRNA + histone H3]. Pie charts illustrate the relative likelihoods of the two possible states in each clade. Each asterisk indicates the significantly supported ancestral state.

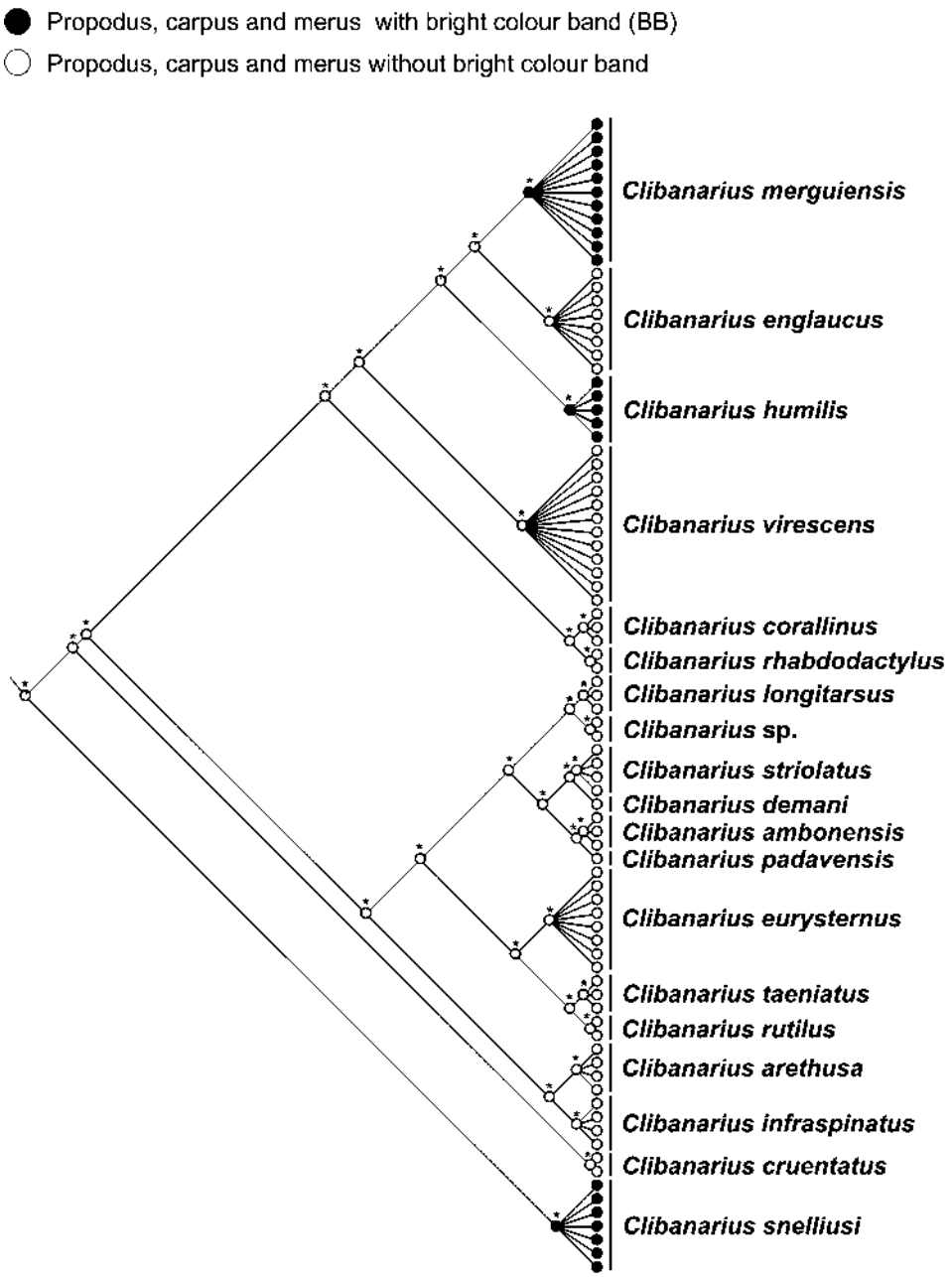

Fig. A6. Maximum likelihood reconstruction of the brightly coloured band (BB) in the propodi, carpi, and meri of *Clibanarius* based on the combined dataset of four genes [cytochrome oxidase I (COI) + 12S rRNA + 16S rRNA + histone H3]. Pie charts illustrate the relative likelihoods of the two possible states in each clade. Each asterisk indicates the significantly supported ancestral state.

TABLE AI
Primers used in this study

Primer	Direction	Sequence (5′ → 3′)	Reference
12S rRNA			
PCR amplification and sequencing			
12 Sma	Forward	CTG GGA TTA GAT ACC CTG TTA T	This study
12 Smb	Reverse	CAG AGA GTG ACG GGC GAT TTG T	This study
H3			
PCR amplification and sequencing			
H3 af	Forward	ATG GCT CGT ACC AAG CAG ACV GC	Colgan et al. (1998)
H3 ar	Reverse	ATA TCC TTR GGC ATR ATR GTG AC	Colgan et al. (1998)
COI			
PCR amplification and sequencing			
LCO1490	Forward	GGT CAA CAA ATC ATA AAG ATA TTG	Folmer et al. (1994)
HCO2198	Reverse	TAA ACT TCA GGG TGA CCA AAA AAT CA	Folmer et al. (1994)
16S rRNA			
PCR amplification and sequencing			
16 SAR	Forward	CGC CTG TTT ATC AAA AAC AT	Malay et al. (2009)
16 SBR	Reverse	GCC GGT CTG AAC TCA GAT CAC GT	Malay et al. (2009)

TABLE AII

Species used in present analyses with sampling localities or sources and GenBank (DDBJ) accession numbers

Species	COI	16S	12S	H3	Specimen ID (fig. 2)	Deposited ID	Collection date	Sampling locality or source	Latitude/ longitude	Reference	Remarks
Clibanarius ambonensis	LC474633	–	LC474737	LC474686	I	MNHN-IU-2017-2010	11 September 2006	Espiritu Santo, Vanuatu	15.5864 S, 167.0232 E	This study	
Clibanarius ambonensis	LC474634	–	LC474738	–	AY1231	SMBL-V0146	5 March 2017	Okinawa, Japan	26.6063 N, 128.1424 E	This study	
Clibanarius ambonensis	LC474635	–	LC474739	–	AY1232	SMBL-V0147	5 March 2017	Okinawa, Japan	26.6063 N, 128.1424 E	This study	
Clibanarius arethusa	LC474636	–	–	–	AY1093	ZRC 2019.0737	22 March 2017	Thiruvananthapuram, India	8.5210 N, 76.8976 E	This study	
Clibanarius arethusa	LC474637	–	–	–	AY1164	ZRC 2019.0738	22 March 2017	Thiruvananthapuram, India	8.5210 N, 76.8976 E	This study	
Clibanarius arethusa	LC474638	–	–	–	AY1166	ZRC 2019.0735	22 March 2017	Thiruvananthapuram, India	8.5210 N, 76.8976 E	This study	
Clibanarius arethusa	LC474639	–	–	–	AY1178	ZRC 2019.0736	22 March 2017	Thiruvananthapuram, India	8.5210 N, 76.8976 E	This study	
Clibanarius corallinus	LC195163	LC474712	LC474740	LC474687	AY406	SMBL-V0148	26 January 2016	Amami, Kagoshima, Japan	28.4162 N, 129.6381 E	Yoshikawa et al. (2018); this study	
Clibanarius corallinus	LC195162	LC474713	LC474741	LC195181	AY407	SMBL-V0149	26 January 2016	Amami, Kagoshima, Japan	28.4162 N, 129.6381 E	Yoshikawa et al. (2018); this study	

TABLE AII
(Continued)

Species	COI	16S	12S	H3	Specimen ID (in fig. 2)	Deposited ID	Collection date	Sampling locality or source	Latitude/ longitude	Reference	Remarks
Clibanarius corallinus	LC195161	–	–	LC474688	AY408	SMBL-V0150	26 January 2016	Amami, Kagoshima, Japan	28.4162 N, 129.6381 E	This study	
Clibanarius cruentatus	–	–	–	LC474689	AY638	SMBL-V0151	25 April 2016	Phuket, Thailand	7.7997 N, 98.4082 E	This study	
Clibanarius cruentatus	LC474640	–	–	LC474690	AY668	SMBL-V0152	25 April 2016	Phuket, Thailand	7.7997 N, 98.4082 E	This study	
Clibanarius demani	LC474641	–	–	–	AY1240	SMBL-V0153	6 April 2017	Phuket, Thailand	7.7997 N, 98.4082 E	This study	
Clibanarius englaucus	LC474642	–	–	LC195180	AY430	SMBL-V0154	26 January 2016	Amami, Kagoshima, Japan	28.4168 N, 129.6338 E	Yoshikawa et al. (2018); this study	
Clibanarius englaucus	LC195160	LC474714	LC474742	LC474691	AY431	SMBL-V0155	26 January 2016	Amami, Kagoshima, Japan	28.4168 N, 129.6338 E	This study	
Clibanarius englaucus	LC195159	LC474715	LC474743	LC195179	AY432	SMBL-V0156	26 January 2016	Amami, Kagoshima, Japan	28.4168 N, 129.6338 E	Yoshikawa et al. (2018); this study	
Clibanarius englaucus	LC195158	LC474716	LC474744	LC195178	AY433	SMBL-V0157	26 January 2016	Amami, Kagoshima, Japan	28.4168 N, 129.6338 E	Yoshikawa et al. (2018); this study	
Clibanarius englaucus	LC195157	LC474717	LC474745	LC195177	AY434	SMBL-V0158	26 January 2016	Amami, Kagoshima, Japan	28.4168 N, 129.6338 E	Yoshikawa et al. (2018); this study	

TABLE AII
(Continued)

Species	COI	16S	12S	H3	Specimen ID (in fig. 2)	Deposited ID	Collection date	Sampling locality or source	Latitude/ longitude	Reference	Remarks
Clibanarius englaucus	–	–	–	LC474692	AY935	SMBL-V0159	14 July 2016	Pangandaran Indonesia	7.7094 S, 108.6498 E	This study	
Clibanarius englaucus	LC474643	–	–	–	AY1204	SMBL-V0160	4 March 2017	Okinawa, Japan	26.2375 N, 127.7972 E	This study	
Clibanarius englaucus	LC474644	–	–	–	AY1205	SMBL-V0161	4 March 2017	Okinawa, Japan	26.2375 N, 127.7972 E	This study	
Clibanarius eurysternus	LC195152	–	–	LC195172	AY487	SMBL-V0162	27 January 2016	Amami, Kagoshima, Japan	28.1485 N, 129.2959 E	Yoshikawa et al. (2018)	
Clibanarius eurysternus	LC195151	–	–	–	AY488	SMBL-V0163	27 January 2016	Amami, Kagoshima, Japan	28.1485 N, 129.2959 E	Yoshikawa et al. (2018)	
Clibanarius eurysternus	LC195150	LC474718	LC474746	LC195171	AY489	SMBL-V0164	27 January 2016	Amami, Kagoshima, Japan	28.1485 N, 129.2959 E	Yoshikawa et al. (2018); this study	
Clibanarius eurysternus	LC195149	LC474719	LC474747	LC474693	AY490	SMBL-V0165	27 January 2016	Amami, Kagoshima, Japan	28.1485 N, 129.2959 E	Yoshikawa et al. (2018); this study	
Clibanarius eurysternus	LC474645	LC474720	LC474748	LC195169	AY492	SMBL-V0166	27 January 2016	Amami, Kagoshima, Japan	28.1485 N, 129.2959 E	Yoshikawa et al. (2018); this study	
Clibanarius eurysternus	LC195145	–	–	–	AY1047	SMBL-V0167	7 August 2016	Lombok, Indonesia	8.4014 S, 116.0805 E	Yoshikawa et al. (2018)	

TABLE AII
(Continued)

Species	COI	16S	12S	H3	Specimen ID (in fig. 2)	Deposited ID	Collection date	Sampling locality or source	Latitude/ longitude	Reference	Remarks
Clibanarius eurysternus	LC195148	–	–	–	AY924	SMBL-V0168	14 July 2016	Pangandaran Indonesia	7.7094 S, 108.6498 E	Yoshikawa et al. (2018)	
Clibanarius eurysternus	LC195147	–	–	–	AY929	SMBL-V0169	14 July 2016	Pangandaran Indonesia	7.7094 S, 108.6498 E	Yoshikawa et al. (2018)	
Clibanarius humilis	–	–	–	LC195176	AY435	SMBL-V0170	26 January 2016	Amami, Kagoshima, Japan	28.4168 N, 129.6338 E	Yoshikawa et al. (2018)	
Clibanarius humilis	LC195156	LC474721	–	LC474694	AY436	SMBL-V0171	26 January 2016	Amami, Kagoshima, Japan	28.4168 N, 129.6338 E	Yoshikawa et al. (2018); this study	
Clibanarius humilis	LC195154	LC474722	–	LC195175	AY438	SMBL-V0172	26 January 2016	Amami, Kagoshima, Japan	28.4168 N, 129.6338 E	Yoshikawa et al. (2018); this study	
Clibanarius humilis	LC195153	LC474723	–	LC195174	AY439	SMBL-V0173	26 January 2016	Amami, Kagoshima, Japan	28.4168 N, 129.6338 E	Yoshikawa et al. (2018)	
Clibanarius humilis	LC195146	–	–	LC474695	AY945	SMBL-V0174	14 July 2016	Pangandaran, Indonesia	7.7094 S, 108.6498 E	Yoshikawa et al. (2018); this study	
Clibanarius infraspinatus	LC474646	LC474724	–	LC474696	AY1152	SMBL-V0175	15 September 2016	Wakaura, Wakayama, Japan	34.1845 N, 135.1760 E	This study	

TABLE AII
(Continued)

Species	COI	16S	12S	H3	Specimen ID (in fig. 2)	Deposited ID	Collection date	Sampling locality or source	Latitude/ longitude	Reference	Remarks
Clibanarius infraspinatus	–	LC474725	–	LC474697	AY1153	SMBL-V0176	15 September 2016	Wakaura, Wakayama, Japan	34.1845 N, 135.1760 E	This study	
Clibanarius infraspinatus	LC474647	LC474726	–	LC474698	AY1154	SMBL-V0177	15 September 2016	Wakaura, Wakayama, Japan	34.1845 N, 135.1760 E	This study	
Clibanarius infraspinatus	–	LC474727	–	LC474699	AY1155	SMBL-V0178	15 September 2016	Wakaura, Wakayama, Japan	34.1845 N, 135.1760 E	This study	
Clibanarius longitarsus	LC474648	LC474728	LC474749	LC474700	AY352	SMBL-V0179	26 January 2016	Amami, Kagoshima, Japan	28.4242 N, 129.6515 E	This study	
Clibanarius longitarsus	LC474649	LC474729	LC474750	LC474701	AY353	SMBL-V0180	26 January 2016	Amami, Kagoshima, Japan	28.4242 N, 129.6515 E	This study	
Clibanarius longitarsus	LC474650	LC474730	LC474751	LC474702	AY354	SMBL-V0181	26 January 2016	Amami, Kagoshima, Japan	28.4242 N, 129.6515 E	This study	
Clibanarius merguiensis	–	–	–	LC474703	AY882	SMBL-V0182	24 July 2016	Seribu Islands, Jakarta, Indonesia	5.5951 S, 106.5620 E	This study	
Clibanarius merguiensis	LC474651	–	–	–	AY886	SMBL-V0183	24 July 2016	Seribu Islands, Jakarta, Indonesia	5.5951 S, 106.5620 E	This study	
Clibanarius merguiensis	LC474652	–	–	–	AY966	SMBL-V0184	14 July 2016	Pangandaran, Indonesia	7.7094 S 108.6498 E	This study	
Clibanarius merguiensis	LC474653	–	–	–	AY615	SMBL-V0185	25 April 2016	Phuket, Thailand	7.7997 N, 98.4082 E	This study	

TABLE AII
(Continued)

Species	COI	16S	12S	H3	Specimen ID (in fig. 2)	Deposited ID	Collection date	Sampling locality or source	Latitude/ longitude	Reference	Remarks
Clibanarius merguiensis	LC474654	–	–	–	AY616	SMBL-V0186	25 April 2016	Phuket, Thailand	7.7997 N, 98.4082 E	This study	
Clibanarius merguiensis	LC474655	–	–	–	AY617	SMBL-V0187	25 April 2016	Phuket, Thailand	7.7997 N, 98.4082 E	This study	
Clibanarius merguiensis	LC474656	LC474731	LC474752	LC474704	AY620	SMBL-V0188	25 April 2016	Phuket, Thailand	7.7997 N, 98.4082 E	This study	
Clibanarius merguiensis	LC474657	LC474732	LC474753	LC474705	AY629	SMBL-V0189	25 April 2016	Phuket, Thailand	7.7997 N, 98.4082 E	This study	
Clibanarius merguiensis	LC474658	LC474733	LC474754	LC474706	AY630	SMBL-V0190	25 April 2016	Phuket, Thailand	7.7997 N, 98.4082 E	This study	
Clibanarius merguiensis	LC474659	–	–	–	AY631	SMBL-V0191	25 April 2016	Phuket, Thailand	7.7997 N, 98.4082 E	This study	
Clibanarius merguiensis	LC474660	–	–	–	AY632	SMBL-V0192	25 April 2016	Phuket, Thailand	7.7997 N, 98.4082 E	This study	
Clibanarius padavensis	LC474661	–	–	–	AY1091	ZRC 2019.0739	22 March 2017	Thiruvananthapuram, India	8.5210 N, 76.8976 E	This study	
Clibanarius rhabdodactylus	LC474662	–	–	–	C2	MNHN-IU-2017-2004	5 November 2009	Dzaoudzi, Mayotte	12.7738 S, 45.2600 E	This study	
Clibanarius rhabdodactylus	LC474663	–	LC474755	–	DF	MNHN-IU-2017-2005	October 1995	–	–	This study	
Clibanarius rutilus	LC474664	–	–	–	AY1169	SMBL-V0193	–	Indonesia	–	This study	
Clibanarius rutilus	LC474665	–	–	–	AY1170	SMBL-V0194	–	Indonesia	–	This study	
Clibanarius snelliusi	LC474666	–	–	–	AY1211	SMBL-V0195	4 March 2017	Okinawa, Japan	26.2375 N, 127.7972 E	This study	

TABLE AII
(Continued)

Species	COI	16S	12S	H3	Specimen ID (in fig. 2)	Deposited ID	Collection date	Sampling locality or source	Latitude/ longitude	Reference	Remarks
Clibanarius snelliusi	LC474667	–	–	–	AY1212	SMBL-V0196	4 March 2017	Okinawa, Japan	26.2375 N, 127.7972 E	This study	
Clibanarius snelliusi	LC474668	–	–	–	AY1213	SMBL-V0197	4 March 2017	Okinawa, Japan	26.2375 N, 127.7972 E	This study	
Clibanarius snelliusi	LC474669	–	–	–	AY1214	SMBL-V0198	4 March 2017	Okinawa, Japan	26.2375 N, 127.7972 E	This study	
Clibanarius snelliusi	LC474670	–	–	–	AY1215	SMBL-V0199	4 March 2017	Okinawa, Japan	26.2375 N, 127.7972 E	This study	
Clibanarius snelliusi	LC474671	–	–	–	AY1216	SMBL-V0200	4 March 2017	Okinawa, Japan	26.2375 N, 127.7972 E	This study	
Clibanarius snelliusi	LC474672	–	–	–	AY1217	SMBL-V0201	4 March 2017	Okinawa, Japan	26.2375 N, 127.7972 E	This study	
Clibanarius sp.	LC474673	–	LC474756	LC474707	G	MNHN-IU-2017-2008	11 September 2006	Espiritu Santo, Vanuatu	15.5864 S, 167.0232 E	This study	Deposited as *Clibanarius ambonensis*
Clibanarius sp.	LC474674	–	LC474757	–	H	MNHN-IU-2017-2009	11 September 2006	Espiritu Santo, Vanuatu	15.5864 S, 167.0232 E	This study	Deposited as *Clibanarius ambonensis*
Clibanarius striolatus	LC474675	–	–	LC474708	AY401	SMBL-V0202	27 January 2016	Amami, Kagoshima, Japan	28.4242 N, 129.6515 E	This study	
Clibanarius striolatus	LC195165	LC474734	LC474758	LC195182	AY402	SMBL-V0203	27 January 2016	Amami, Kagoshima, Japan	28.4242 N, 129.6515 E	Yoshikawa et al. (2018); this study	

TABLE AII
(Continued)

Species	COI	16S	12S	H3	Specimen ID (in fig. 2)	Deposited ID	Collection date	Sampling locality or source	Latitude/ longitude	Reference	Remarks
Clibanarius striolatus	LC195164	–	–	LC474709	AY404	SMBL-V0204	27 January 2016	Amami, Kagoshima, Japan	28.4242 N, 129.6515 E	Yoshikawa et al. (2018); this study	
Clibanarius striolatus	LC474676	–	–	–	AY1048	SMBL-V0205	7 August 2016	Lombok, Indonesia	8.4014 S, 116.0805 E	This study	
Clibanarius taeniatus	LC474677	–	LC474759	–	AY1327	QM W29442	22 August 2017	North Ward, Townsville, Queensland, Australia	19.2400 S, 146.7990 E	This study	
Clibanarius taeniatus	LC474678	–	LC474760	–	A119	QM W29442	22 August 2017	North Ward, Townsville, Queensland, Australia	19.2400 S, 146.7990 E	This study	
Clibanarius taeniatus	LC474679	–	–	–	A122	QM W29442	22 August 2017	North Ward, Townsville, Queensland, Australia	19.2400 S, 146.7990 E	This study	
Clibanarius virescens	LC155092	LC474735	LC474761	LC195185	AY383	SMBL-V0206	26 January 2016	Amami, Kagoshima, Japan	28.4172 N, 129.6330 E	Yoshikawa et al. (2018); this study	
Clibanarius virescens	LC155091	–	LC474762	LC195184	AY386	SMBL-V0207	26 January 2016	Amami, Kagoshima, Japan	28.4172 N, 129.6330 E	Yoshikawa et al. (2018)	

TABLE AII
(Continued)

Species	COI	16S	12S	H3	Specimen ID (in fig. 2)	Deposited ID	Collection date	Sampling locality or source	Latitude/ longitude	Reference	Remarks
Clibanarius virescens	LC155090	–	LC474763	LC195183	AY391	SMBL-V0208	26 January 2016	Amami, Kagoshima, Japan	28.4172 N, 129.6330 E	Yoshikawa et al. (2018); this study	
Clibanarius virescens	LC155089	–	–	LC474710	AY393	SMBL-V0209	26 January 2016	Amami, Kagoshima, Japan	28.4172 N, 129.6330 E	Yoshikawa et al. (2018)	
Clibanarius virescens	LC155095	–	–	LC195173	AY454	SMBL-V0210	26 January 2016	Amami, Kagoshima, Japan	28.4172 N, 129.6330 E	Yoshikawa et al. (2018)	
Clibanarius virescens	LC155088	–	–	LC195168	AY503	SMBL-V0211	10 February 2016	Okinawa, Japan	26.1265 N, 127.7730 E	Yoshikawa et al. (2018)	
Clibanarius virescens	LC155087	–	–	–	AY504	SMBL-V0212	10 February 2016	Okinawa, Japan	26.1265 N, 127.7730 E	Yoshikawa et al. (2018)	
Clibanarius virescens	LC155094	–	–	LC195167	AY523	SMBL-V0213	9 February 2016	Okinawa, Japan	26.3671 N, 127.8775 E	Yoshikawa et al. (2018)	
Clibanarius virescens	LC155093	–	–	LC195166	AY527	SMBL-V0214	9 February 2016	Okinawa, Japan	26.3671 N, 127.8775 E	Yoshikawa et al. (2018)	
Clibanarius virescens	LC474680	–	–	LC474711	AY142	SMBL-V0215	11 September 2015	Reihoku, Amakusa, Japan	32.5386 N, 130.0300 E	This study	
Clibanarius virescens	LC474681	–	–	–	AY219	SMBL-V0216	26 August 2015	Shima, Mie, Japan	34.2772 N, 136.8036 E	This study	

TABLE AII
(Continued)

Species	COI	16S	12S	H3	Specimen ID (in fig. 2)	Deposited ID	Collection date	Sampling locality or source	Latitude/ longitude	Reference	Remarks
Clibanarius virescens	LC155096	–	–	LC195186	AY206	SMBL-V0217	1 December 2015	Shirahama, Wakayama, Japan	33.6917 N, 135.3358 E	Yoshikawa et al. (2018)	
Coenobita violascens	AB998661	–	–	–	AB998661	NCHU-ZOOL 13632	–	Dongsha, Kaohsiung, Taiwan	–	Rhayu et al. (2016)	
Coenobita violascens	AB998663	–	–	–	AB998663	ZRC	–	Kawasan Falls, Cebu, Philippines	–	Rhayu et al. (2016)	
Coenobita violascens	AB998658	–	–	–	AB998658	NCHU-ZOOL 13630	–	Yuanjhong-gang, Kaohsiung, Taiwan	–	Rhayu et al. (2016)	
Coenobita violascens	AB998659	–	–	–	AB998659	NCHU-ZOOL 13630	–	Yuanjhong-gang, Kaohsiung, Taiwan	–	Rhayu et al. (2016)	
Calcinus morgani	LC474682	LC474736	LC474764	–	AY1126	SMBL-V0218	9 February 2016	Okinawa, Japan	26.3666 N, 127.8767 E	This study	
Paguristes ortmanni	LC474683	–	–	–	AY216	SMBL-V0219	26 August 2015	Shima, Mie, Japan	34.2772 N, 136.8036 E	This study	
Paguristes ortmanni	LC474684	–	–	–	AY217	SMBL-V0220	26 August 2015	Shima, Mie, Japan	34.2772 N, 136.8036 E	This study	
Paguristes ortmanni	LC474685	–	–	–	AY218	SMBL-V0221	26 August 2015	Shima, Mie, Japan	34.2772 N, 136.8036 E	This study	

Abbreviations: MNHN, Museum National D'histore Naturelle, ZRC, Lee Kong Chian Natural History Museum, QM, Museum of Tropical Queensland, Queensland Museum; SMBL, Seto Marine Biological Laboratory, Kyoto University, Japan.

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