Do colour-morphs of an amphidromous goby represent different species? Taxonomy of *Lentipes* (Gobiiformes) from Japan and Palawan, Philippines, with phylogenomic approaches

KEN MAEDA[1], HIROZUMI KOBAYASHI[2], HERMINIE P. PALLA[3], CHUYA SHINZATO[4], RYO KOYANAGI[1], JAVIER MONTENEGRO[2], ATSUSHI J. NAGANO[5], TOSHIFUMI SAEKI[6], TAIGA KUNISHIMA[7], TAKAHIKO MUKAI[8], KATSUNORI TACHIHARA[2], VINCENT LAUDET[1], NORIYUKI SATOH[1], & KAZUNORI YAMAHIRA[2]

[1]Okinawa Institute of Science and Technology Graduate University, 1919-1 Tancha, Onna, Okinawa 904-0495, Japan
[2]University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903-0213, Japan
[3]College of Fisheries and Aquatic Sciences, Western Philippines University - Puerto Princesa Campus, Puerto Princesa City, 5300 Palawan, Philippines
[4]Atmosphere and Ocean Research Institute, The University of Tokyo, Chiba 277-8564, Japan
[5]Faculty of Agriculture, Ryukoku University, Otsu 520-2194, Japan
[6]Rivus, 2-13-14-203 Oyama, Ginowan, Okinawa 901-2223, Japan
[7]Wakayama Prefectural Museum of Natural History, 370-1 Funoo, Kainan, Wakayama 642-0001, Japan
[8]Faculty of Regional Studies, Gifu University, Gifu 501-1193, Japan

Correspondence to: Ken Maeda. E-mail: goby@live.jp

Running title: Phylogenomics and taxonomy of *Lentipes*

Abstract We document four male colour morphs of the Indo-Pacific goby genus *Lentipes* in Japan and the Philippines. Despite distinctive colour patterns, males of the different morphs could not be distinguished by meristic or morphometric charactres. In contrast, co-occurring females had very similar colouration and could not be sorted into different types. We observed that the four types are not distinguished by mitochondrial genome sequences. On the other hand, genome-wide SNPs analysis clearly separated the four types, suggesting that they indeed represent four independent lineages. We considered that the four lineages could have diverged recently, and therefore, the sorting of mitochondrial haplotypes may not have been completed yet. One of the four lineages is identified as *L. armatus* Sakai & Nakamura, 1979, and the other three are described in this study as new species: *L. kijimuna*, *L. bunagaya*, and *L. palawanirufus*. We observed that males display their species-specific body colourations during courtship. Pre-zygotic isolation due to female preferences for different male body colours is probably the primary mechanism of reproductive isolation between the four species.

Key words: *Lentipes*, goby, colouration, mitochondrial genome, genome-wide SNPs, ddRAD-seq, amphidromy, new species

Introduction

Body colour is one of the major characters to distinguish fish species (Cal et al., 2017; Salis et al., 2019; Yabe et al., 2017). It is often used as an essential criterion to identify fishes at the species level in many taxa (Nakabo, 2002; Puebla et al., 2007). On the other hand, distinctive colour morphs within a single species are often known, e.g., dark (normal) and yellow colour morphs of gobies in the genera *Cryptocentrus* and *Myersina* (see Shibukawa & Satapoomin, 2006; Thacker et al., 2011).

Members of the goby genus *Lentipes* (order Gobiiformes: family Oxudercidae) inhabit freshwater streams on Indo-Pacific islands (Keith et al., 2015; Nelson et al., 2016). They are considered to be amphidromous (i.e., inhabiting freshwater as juveniles and adults, with a marine pelagic larval stage) and exhibit pronounced sexual dimorphism and sexual dichromatism. Males generally exhibit vivid nuptial colours whereas females are greyish (Keith et al., 2015).

During ongoing studies of *Lentipes* in Japan and the Philippines, we found four male types distinguished by colour pattern. Three of the four types are sympatric in Japan, and the other is found only in the Philippines. To date, only one species of *Lentipes* is recognised from each country so far (Keith et al., 2015). They are *Lentipes armatus* Sakai & Nakamura, 1979 from Japan and *Lentipes mindanaoensis* Chen, 2004

from the Philippines. One of the four colour types corresponds to the colour pattern of *L. armatus* (described in Chen et al., 2007; Nakabo, 2018; Sakai & Nakamura, 1979), but the other three exhibit novel male colour patterns that are not known amongst congeners, including *L. mindanaoensis*. These four types could be distinguished only by components of the colour pattern. All females observed by us in this region exhibit a similar colour pattern and could not be sorted into different types.

To further investigate the taxonomic status of the aforementioned male colour morphs of *Lentipes* in Japan and the Philippines, we conducted a broad phylogenetic investigation of the genus using mitochondrial genome sequences as well as single nucleotide polymorphisms (SNPs) detected by a genome-wide screening using double digest restriction-site associated DNA sequence (ddRAD-seq; see details in Peterson et al., 2012). Based on our phylogenetic analyses and our observations on large series of specimens, we discuss the taxonomic status of *Lentipes* in this region of the Indo-Pacific. We provide a redescription for *L. armatus* and descriptions of three new species. We also report aspects of male courtship behaviour, which involve the display of brilliant colour patterns and may play an important role in the reproductive isolation between species of *Lentipes*.

Materials and methods

Sampling

Specimens of *Lentipes* were collected using hand nets on Okinawa Island in the Ryukyu Archipelago, southern Japan, as well as on Palawan Island, western Philippines. After euthanisation with 2-phenoxyethanol, the right pectoral fin was cut off and preserved in 99.5% ethanol for genetic analysis. Specimens were fixed in 10% formalin and preserved in 70% ethanol for morphological examination. Sampling was conducted according to the laws and regulations of the Philippines and Japan. Collections in Palawan were performed with Wildlife Gratuitous Permits (No. 2016-09, 2018-16) provided by the Palawan Council for Sustainable Development and with Prior Informed Consent Certificates from all relevant cities, municipalities, and barangays (villages). The procedures used to handle fish specimens in this study were approved by the Animal Care and Use Committees of the Okinawa Institute of Science and Technology Graduate University.

Mitochondrial DNA analysis

The total genomic DNA of 46 specimens of *Lentipes* and three specimens of *Sicyopus zosterophorus* (outgroup) was extracted from pectoral fins, using DNeasy Blood &

Tissue Kit (Quiagen, Hilden, Germany) or Maxwell RSC Blood DNA Kit (Promega, Fitchburg, Wisconsin, USA).

Whole genome shotgun sequencing libraries were prepared using a KAPA HyperPlus Kit for PCR-free workflows (KAPA Biosystems, Wilmington, Massachusetts, USA) or NEBNext Ultra DNA Library Prep Kit for Illumina (New England BioLabs, Ipswich, Massachusetts, USA). The extracted genomic DNA was enzymatically fragmented into pieces of 200–1000 bp using KAPA Frag (KAPA Biosystems). After repairing the protruding ends and A-tailing, sequencing adaptors were ligated onto both ends of the DNA fragments. The shotgun libraries were then sequenced on one of the following instruments and reagents, following the manufacturer's instructions: Illumina MiSeq sequencer (Illumina, San Diego, California, USA) using a MiSeq Reagent Kit v3 (Illumina), Illumina HiSeq 2500 sequencer in Rapid Run mode using a HiSeq Rapid Cluster Kit v2-Paired-End (Illumina) and a HiSeq Rapid SBS Kit v2 (Illumina), or Illumina HiSeq 4000 sequencer using HiSeq 3000/4000 Cluster Kit (Illumina) and HiSeq 3000/4000 SBS Kit (Illumina).

Sequencing data from each library was assembled using IDBA_UD assembler version 1.1.1 (Peng et al., 2012) with different kmer lengths (60, 80, 100). Identification of complete mitochondrial genomes from assembled contigs was performed by: 1) comparing them with the complete mitochondrial genome of *Stiphodon alcedo* (accession: AB613000.1) (BLASTN e-value B 1e-100); and 2) confirming that 100 bp of both head and tail DNA sequences of a contig were identical, indicating that the sequence was circular. Complete mitochondrial genomes were aligned using MAFFT v7.244 (Katoh & Standley, 2013) and all positions with gaps were removed using trimAl (Capella-Gutiérrez et al., 2009). We performed molecular phylogenetic analyses of the aligned mitochondrial genomes using the GTR+I+Gamma model and performed a maximum likelihood (ML) analysis using RAxML version 8.2.3 (Stamatakis, 2014) with 100 bootstrap replicates. The assembled mitochondrial genome sequences with gene annotations are available in the DNA Data Bank of Japan (DDBJ) under accession numbers: LC564924–LC564972. Accession numbers for each individual are given in the online supplemental material, Table S1. For the molecular phylogenetic analysis of cytochrome oxidase subunit 1 (COI), we first retrieved published COI nucleotide sequences representing six species (Dahruddin et al., 2017; Keith et al., 2011; Taillebois et al., 2014) from the International Nucleotide Sequence Database (accession numbers are given in Table S2). As in the case of the ML analysis of the whole mitochondrial genomes, we aligned the COI sequences of the six species with the assembled mitochondrial genomes using MAFFT v7.244 (Katoh & Standley, 2013), and all

positions with gaps were removed using trimAl (Capella-Gutiérrez et al., 2009). The resulting aligned sequences (629 bp) were used for ML analysis using RAxML version 8.2.3 with 100 bootstrap replicates. Collection data for outgroup specimens are as follows: *Sicyopus zosterophorus*, URM-P 48751, Okinawa Island, Ryukyu Archipelago, Japan, 12 Dec. 2013, coll. K. Maeda; URM-P 48752 and 48753, Okinawa Island, 5 Sep. 2014, coll. K. Maeda.

Genome-wide SNPs analyses

The library for ddRAD-Seq (Peterson et al., 2012) was created using the method described in Sakaguchi et al. (2015) with slight modifications, in which *Bgl*II was used as the first restriction site adjacent to the binding site of the primer to read a single-end sequence, and *Eco*RI was used as the second restriction site adjacent to the binding site to read an index sequence. The library was sequenced with 50-bp single-end reads in an Illumina HiSeq2500 by Macrogen Japan Corporation (Kyoto, Japan). The reads were deposited in DDBJ Sequence Read Archive under the accession number DRA011030. Accession numbers for each individual are given in Table S1. Sequence trimming was performed using Trimmomatic version 0.32 (Bolger et al., 2014) to remove adapter regions from the Illumina reads, using ILLUMICLIP: TruSeq3-SE.fa:2:30:10, LEADING:19, TRAILING:19, SLIDINGWINDOW: 30: 20, and 20, and AVGQUAL:20, MINLEN:51.

Genotyping was conducted using the Stacks version 2.41 software pipeline, i.e., *ustacks*, *cstacks*, and *sstacks*, and *populations* (Catchen et al., 2011, 2013), in which the four male types were regarded as populations (p = 4), and each male was assigned to one of the four. One female from Okinawa and two females from Palawan were assigned to male type 1 and type 4, respectively, following preliminary genotyping. First, the *ustacks* program was run to build loci *de novo* in each sample using default settings except for the minimum of 10 reads (m = 10) to create a 'stack'. Second, using the *cstacks* and *sstacks* programs with default settings, a catalogue of all loci across populations was created, and each sample was matched against the catalogue. Finally, the *populations* program was run to export data in a variant call format (VCF) file using p = 4 (i.e., the minimum number of populations a locus must be present in to process a locus was four), r = 0.75 (i.e., the minimum percentage of individuals in a population required to process a locus for that population was 75%), and the write_single_SNP option (i.e., only the first SNP was filtered out per locus). Exact tests of the Hardy–Weinberg equilibrium (HWE) were performed separately for each male type, using the hwe option in the *populations* program, and the loci that deviated from the Hardy–Weinberg equilibrium (5% significance level) in one or more male types were excluded

from the dataset. This created a dataset of 1,532 SNP. In addition, genotype outputs were created in a PHYLIP file using the *populations* program. Exclusion of loci deviating from HWE resulted in 889 loci, with a total length of 45,485 bp, including both variant and invariant sites.

For the 1,532 SNP dataset, we built individual-based phylogenetic networks with Split Tree version 4.14.8 (Huson & Bryant, 2006). The networks were built using the Neighbour-Net method based on Nei's standard genetic distances between individuals (Nei, 1972), which were calculated from the individual genotype calls using the R package StAMPP version 1.5.1 (Pembleton et al., 2013). For the 45,485-bp concatenated RAD sequences, a neighbour-joining (NJ) tree was reconstructed using *p*-distances. Analysis was performed with MEGA X version 10.8.1 (Kumar et al., 2018), where a bootstrap analysis of 1,000 bootstrap replicates was conducted. The ML phylogeny was also estimated using RAxML version 8.0.0 (Stamatakis, 2014) based on the 45,485-bp dataset. The codon-non-specific GTRCAT model was assigned to the concatenated sequence, and a rapid bootstrap analysis of 1,000 bootstrap replicates was conducted.

A phylogenetic tree among the 24 specimens was also built for the RAD-seq SNP dataset, using the Bayesian method implemented with SNAPP version 1.4.1 (Bryant et al., 2012), an add-on package of BEAST version 2.5.0 (Bouckaert et al., 2014). Backward (U) and forward mutation rates (V) were estimated from the stationary allele frequencies in the data (U = 7.3206, V = 0.5367). Analysis was run using default priors with chainLength = 5,000,000 and storeEvery = 1,000. We discarded the first 10% of the trees as burn-in and visualized the posterior distribution of the remaining 4,500,000 trees using DensiTree version 2.2.6 (Bouckaert, 2010).

To examine population structure, admixture analysis was also conducted using ADMIXTURE version 1.3.0 (Alexander et al., 2009) based on a PED file converted from the VCF file above using PLINK version 1.90b4.6 (Purcell et al., 2007). ADMIXTURE was run for 1–7 clusters (i.e., K=1–7). Statistical support for the different number of clusters was evaluated based on the cross-validation errors (CV errors) method implemented in ADMIXTURE. We also conducted principal component analyses using R package SNPRelate version 1.10.2 (Zheng et al., 2012).

Morphological examination

In addition to the collection we made, specimens collected on Ishigaki Island in the Ryukyu Archipelago in 1993 from the URM fish collection (this collection was established at the University of the Ryukyus, Japan and was transferred to the Okinawa Churashima Foundation Research Center, Japan, in 2011) were also examined.

Localities in the Ryukyu Archipelago are listed at the island scale, as indicating a more precise location (e.g. stream name) often causes a negative impact on the small-scale habitats of these species. Measurements and counts were taken from the left side of each fish, but the arrangement of the sensory canal pores was observed on both sides of the head. Measurements were made point-to-point to the nearest 0.1 mm, using a vernier caliper or a divider under a stereomicroscope, and were expressed as a percentage of standard length (SL). Measurements and counts followed Nakabo (2002), with the following modifications: SL, head length, snout length, predorsal length, and preanal length were measured to the anterior-most point of the protruding snout (PS: Fig. 1a), not to the anterior-most tip of the upper jaw (UJ: Fig. 1a), even if UJ precedes PS. Body depths were measured at the pelvic- and anal-fin origins. The first and second dorsal- and anal-fin lengths were measured from the anterior origin of each fin to the farthest point where the fin was adpressed. Caudal-fin length was measured from the midpoint of the caudal-fin base to the posterior-most part of the caudal fin. The interval between the first and second dorsal-fin bases was measured from the posterior end of the first dorsal-fin base to the second dorsal-fin origin. Longitudinal scales were counted from the middle of the posterior end of the hypurals to behind the pectoral-fin base. Transverse scales were counted along a diagonal line extending posteriorly and ventrally from the nearest scale anterior to the second dorsal fin (on the dorsal midline if available) to the scale along the anal-fin base. Circumpeduncular scales were counted along the circumference of the narrowest point of the caudal peduncle in a zigzag manner. Tooth counts of the upper and lower jaws were taken from the left side of the symphysis. Dentition terms used follow Watson (2008). Abbreviations pertaining to the cephalic sensory pore system follow Nakabo (2002, p. 1269). Symbolic codes used to represent collections and institutions follow Sabaj (2019). Descriptions of colours in life are based on photographs of individuals taken in situ, including those of individuals that were not collected.

Comparative material

Lentipes adelphizonus Watson & Kottelat, 2006: MZB 5933, holotype, 27.0 mm SL, Sungei Okitai, Halmahera, Maluku, Indonesia, Aug. 1994. *Lentipes caroline* Lynch, Keith & Pezold, 2013: MNHN 2012-0213, holotype, 36.6 mm SL, Pohnpei, Federated States of Micronesia, 13 Mar. 2012; MNHN 2012-0214, 7 paratypes, same data as holotype. *Lentipes concolor* (Gill, 1860): URM-P 26610, 2 specimens, 34.7 and 39.3 mm SL, Wailau River, Molokai, Hawaii, 5 Aug. 1991. *Lentipes crittersius* Watson & Allen, 1999: MZB 9222, holotype, 31.4 mm SL, Aiyindor Creek, Biak Island, Papua, Indonesia, 12 Jan. 1997. *Lentipes dimetrodon* Watson & Allen, 1999: MZB 8001,

holotype, 21.2 mm SL, Omamerwai Creek, Papua, Indonesia, 9 Aug. 1995. *Lentipes ikeae* Keith, Hubert, Busson & Hadiaty in Keith et al., 2014: MZB 21477, holotype, 31.3 mm SL, Cisolok, Kab Sukabumi, Java, Indonesia, 13 Dec. 2013. *Lentipes kaaea* Watson, Keith & Marquet, 2002: MNHN 1997-4175, holotype, 25.7 mm SL, North Province, New Caledonia, 13 Nov. 1997; MNHN 2002-0114, 3 paratypes, 21.8–26.8 mm SL, North Province, New Caledonia, 28 Oct. 1999. *Lentipes kolobangara* Keith, Lord, Boseto & Ebner, 2016: MNHN 2015-0473, holotype, 23.8 mm SL, Kolobangara Island, Solomon Islands, 14 Nov. 2015. *Lentipes mekonggaensis* Keith & Hadiaty in Keith et al., 2014: MZB 21473, holotype, 33.2 mm SL, Sungei Tepasa, Wawo, Sulawesi Tenggara Province, Indonesia, 30 Jan. 2011. *Lentipes multiradiatus* Allen, 2001: MZB 10902, holotype, 27.6 mm SL, Danyamo Stream Cyclops Nature Reserve, Papua, Indonesia, 25 Aug. 2000. *Lentipes rubrofasciatus* Maugé, Marquet & Laboute, 1992: MNHN 1992-0116, holotype, 21.9 mm SL, Ua Huka, Marquesas Islands, French Polynesia, 18 Dec. 1986; MNHN 1992-0117, 4 paratypes, 19.0–21.0 mm SL, same data as holotype.

Morphological information obtained from the following references was also used as part of our comparisons: Allen, 1997, 2001, 2004; Chen, 2004; Chen et al., 2007; Gill, 1860; Harrison, 1993; Jenkins et al., 2008; Keith et al., 2014, 2015, 2016; Lynch et al., 2013; Maciolek, 1977; Maugé et al., 1992; Mukerji, 1935; Nakabo, 2018; Sakai & Nakamura, 1979; Watson & Allen, 1999; Watson & Kottelat, 1994, 2006; and Watson et al., 2002.

Results

Sorting of male-colour types

Male specimens were sorted into the four types according to their colour while alive, as detailed below.

Male type 1 (Fig. 2a): body and head are grey or brown. Belly is light blue with three black vertical lines. Snout is often light blue. Second dorsal fin is dark brown proximally and white or light brown distally, with one black spot at the anterior part (sometimes with two or three spots). Anal fin is dark brown proximally and translucent distally.

Male type 2 (Fig. 2b): body is grey or bluish grey with a red head and a broad red band between the bases of the posterior half of the second dorsal and anal fins. Belly is grey or light blue (sometimes with two or three indistinct, blackish vertical lines). Second dorsal and anal fins have a submarginal black stripe and a transparent margin; most of the proximal parts are red.

Male type 3 (Fig. 2c): body is grey or greyish brown with two broad red bands on the posterior part of the body. The red bands are faded on the dorsum. Belly is grey or light blue with three black vertical lines. Second dorsal fin is reddish brown proximally and white distally, with one black spot at the anterior part. Anal fin is white with an obscure reddish-brown base.

Male type 4 (Fig. 2d): body is grey with a broad reddish-brown or dark-red band between the bases of the second dorsal and anal fins. Lateral and ventral sides of the head are red, reddish brown, or dark brown. Belly is grey or light blue, without clear black lines. Second dorsal fin is reddish brown or dark red proximally and white or light yellowish brown distally, with one black spot at the anterior part. Anal fin is reddish brown or dark red proximally and translucent distally.

Several pictures are provided to show variation in the colour pattern within each type in the morphological description section. The basic arrangements of the markings (especially the shape of the black markings on the second dorsal and anal fins and the positions of red or dark-brown markings on the body) are stable within each type and characterise the four types, although the background colours are variable, the red and dark-brown colours often become pink or brown, and the blue colour often fades to grey. Specimens of types 1, 2, and 3 were collected in Okinawa, and specimens of type 4 were collected in Palawan. On Okinawa Island, type 1 was common, type 2 was rare, and type 3 was very rare. Type 4 was abundant at the two sites on Palawan Island where they were collected.

Body colour of females

All females exhibit similar colouring (Fig. 2e): body is greyish brown but whitish ventrally, with some silver and indistinct dusky markings. Fins are translucent. The females observed in Okinawa often had a dusky-grey lateral stripe running from the snout to the caudal peduncle (Fig. 3a,b), while the females in Palawan often had an additional, indistinct stripe dorsally along the stripe, and their dorsum usually had mesh patterns formed by the grey margins of the scales (Fig. 3c,d). Because these differences were, however, not clear enough to distinguish them from each other, the females were sorted by localities (Okinawa or Palawan).

Mitochondrial DNA analysis

In the maximum likelihood phylogenetic tree, using 16,454 bp of aligned mitochondrial genomes, 46 specimens of *Lentipes* were divided into two clades, supported by 82% and 95% bootstrap replicates, respectively (Fig. 4). Male types 2 and 4 and females collected from Palawan occurred in one clade, while male type 3 occurred in another

clade. Meanwhile, male type 1 and females collected from Okinawa were included in both clades. These types were intermixed within the clades, and none of the colour types corresponded to a monophyletic group.

In the ML phylogenetic tree, which used the published sequences of the mitochondrial COI gene (629 bp) of six species in the genus (Dahruddin et al., 2017; Keith et al., 2011; Taillebois et al., 2014) with the addition of the four male types and females of *Lentipes* we collected (Fig. 5), only *L. concolor* and *L. whittenorum* Watson & Kottelat, 1994 were monophyletic. *Lentipes kaaea* was paraphyletic within the *L. whittenorum* lineage. *Lentipes ikeae*, *L. armatus*, and *Lentipes* sp. (sensu Taillebois et al., 2014) were all intermixed within a single clade with the four male types and females in this study. Although most of *L. ikeae* was included in a single subclade, two individuals were located outside of this subclade (Fig. 5). *Lentipes kaaea* (from New Caledonia, Vanuatu, and Bali) and *L. ikeae* (from Bali and West and East Java) were collected from several regions, but neither species formed a geographic structure.

Genome-wide SNPs analysis

Unlike in the mitochondrial phylogeny, the four types were clearly separated from each other in the phylogenetic network (Fig. 6). A female from Okinawa and two females from Palawan belonged to clades of male type 1 and male type 4, respectively. Male type 2 was subdivided into two clades; three males (URM-P 48881, 48884, and 48885) composed one subclade (clade B), and the other five males composed another (clade A). The NJ, ML, and SNAPP trees also revealed that the four male types were separated from each other, but that male type 2 was divided into two subclades; the three males in clade B did not form a clade with the other type 2 males (Fig. S1–S3).

The ADMIXTURE analysis revealed that the four types were clearly distinguished from each other when the occurrence of four clusters (K=4) was assumed (Fig. 7). In the case of K=2 and 3, the three specimens in the clade B (URM-P 48881, 48884, and 48885) of male type 2 exhibited mixed ancestries. These three males were separated from the others when K=5. The CV error was the lowest when K=1 and increased with the number of clusters (Fig. S4). The principal component (PC) analysis revealed that the four male types also tended to be separated from each other in the PC2–PC3 plot (Fig. S5).

Morphological description and taxonomy

The four male types are distinguished clearly by the phylogenetic and population structure inferred from the genome-wide SNPs analyses (Figs. 6, 7) and in this section we describe them as four different species. The two lineages detected within type 2 are

regarded as a single species (see ‘Reproductive isolation and speciation’ in the ‘Discussion’). However, only five specimens belonging to the clade A (same lineage with the holotype) are included in the type series, and the other five specimens (three specimens belonging to the clade B and two specimens that have not been analysed by the genome-wide SNPs analysis) represent non-type material. Because colour pattern and other morphological characteristics of our type 1 corresponded with that of the holotype of *L. armatus* described by Sakai & Nakamura (1979), this type is identified as *L. armatus* and re-described below. Types 2, 3, and 4 are described as the new species, *L. kijimuna*, *L. bunagaya*, and *L. palawanirufus*, respectively. Detailed comparisons with other species are provided in the ‘Discussion’. We examined many female specimens but failed to identify diagnostic characters to distinguish them. We identified one female specimen as *L. armatus* and two other females as representatives of one of our new species via genome-wide SNPs analysis, although all other females without the genome-wide SNPs examination could not be identified at the species level. Therefore, we included only one and two female specimens into the material for the morphological descriptions of *L. armatus* and *L. palawanirufus*, respectively. The following female specimens could not be identified and data obtained from these individuals did not contribute to descriptions of species presented below:

Okinawa Island (n=13): URM-P 48821, 24.6 mm SL, 23 Aug. 2003, coll. N. Hanahara and K. Maeda; URM-P 48833, 21.8 mm SL, 11 Oct. 2010, coll. K. Maeda; URM-P 48840, 30.9 mm SL, 11 Oct. 2012, coll. K. Maeda; URM-P 48841, 31.9 mm SL, 11 Oct. 2012, coll. K. Maeda; URM-P 48845, 31.3 mm SL, 1 Aug. 2013, coll. K. Maeda; URM-P 48848, 32.1 mm SL, 27 Aug. 2013, coll. K. Maeda; URM-P 48851, 25.5 mm SL, 8 Dec. 2013, coll. K. Maeda; URM-P 48858, 33.8 mm SL, 20 Apr. 2016, coll. K. Maeda; URM-P 48864–48866, 48870, and 48874 (n=5), 24.3–32.1 mm SL, 12 Dec. 2018, coll. K. Maeda and H. Kobayashi.

Ishigaki Island (n=21): URM-P 30104 (1 in 5 specimens), 44.0 mm SL, 5 Aug. 1993, coll. H. Yoshigou; URM-P 30105 (9 in 13 specimens), 36.5–45.4 mm SL, 6 Aug. 1993, coll. H. Yoshigou; URM-P 30107 (2 in 7 specimens), 45.8–48.1 mm SL, 8 Aug. 1993, coll. H. Yoshigou; URM-P 30108 (9 in 11 specimens), 41.9–49.1 mm SL), 9 Aug. 1993, coll. H. Yoshigou.

Palawan Island (n=8): URM-P 48909, 46.6 mm SL, Estrella Falls, Narra, 13 May 2016, coll. K. Maeda, T. Kunishima, and H. P. Palla; URM-P 48914, 28.5 mm SL, Olanguan Falls, Puerto Princesa, 16 May 2016, coll. K. Maeda, T. Kunishima, and H. P. Palla; URM-P 48916, 51.1 mm SL, Estrella Falls, 29 May 2018, coll. K. Maeda, H. Kobayashi, and H. P. Palla; WPU-PPC-P 35, 35.1 mm SL, Estrella Falls, 13 May 2016, coll. K. Maeda, T. Kunishima, and H. P. Palla; WPU-PPC-P 39 and 42 (n=2), 22.0–31.2

mm SL, Olanguan Falls, 16 May 2016, coll. K. Maeda, T. Kunishima, and H. P. Palla; WPU-PPC-P 44 and 49 (n=2), 46.9–52.3 mm SL, Estrella Falls, 29 May 2018, coll. K. Maeda, H. Kobayashi, and H. P. Palla.

Counts and measurements obtained from the holotype are indicated with the letter 'h' presented within square brackets in each description.

Lentipes armatus Sakai & Nakamura, 1979

Japanese name: Yoroi-bouzu-haze. English name: Peppermint armour goby or armoured lentipes (Keith et al., 2015) (Figs. 2, 8–13; Tables 1, 2; Figs. S6, S7; Table S3)
Male type 1 - this study.

Material examined: 36 males and one female from Okinawa Island and Ishigaki Island in the Ryukyu Archipelago, Japan. The identification of a female was verified by genome-wide SNPs analysis.

Okinawa Island (n=20): URM-P 48822, male (28.3 mm SL), 15 Aug. 2005, coll. K. Maeda; URM-P 48836, male (26.9 mm SL), 19 Sep. 2012, coll. K. Maeda; URM-P 48837–48839, 3 males (21.2–23.3 mm SL), 11 Oct. 2012, coll. K. Maeda; URM-P 48842–48844, 3 males (27.6–29.9 mm SL), 1 Aug. 2013, coll. K. Maeda; URM-P 48846, male (28.0 mm SL), 27 Aug. 2013, coll. K. Maeda; URM-P 48847, female (32.9 mm SL), 27 Aug. 2013, coll. K. Maeda; URM-P 48852–48854, 3 males (22.2–29.0 mm SL), 8 Dec. 2013, coll. K. Maeda; URM-P 48855 and 48856, 2 males (22.7–26.0 mm SL), 14 Aug. 2014, coll. K. Maeda; URM-P 48857 and 48859, 2 males (26.3–27.9 mm SL), 20 Apr. 2016, coll. K. Maeda; URM-P 48860, male (32.3 mm SL), 19 June 2016, coll. K. Maeda; URM-P 48867 and 48868, 2 males (21.2–24.4 mm SL), 12 Dec. 2018, coll. K. Maeda and H. Kobayashi.

Ishigaki Island (n=17): URM-P 30104, 4 males (32.3–37.2 mm SL) (5 specimens including 1 female were registered under this catalogue number but the female specimen was not used here), 5 Aug. 1993, coll. H. Yoshigou; URM-P 30105, 4 males (32.4–40.4 mm SL) (excluding 9 females), 6 Aug. 1993, coll. H. Yoshigou; URM-P 30107, 5 males (32.5–41.1 mm SL) (excluding 2 females), 8 Aug. 1993, coll. H. Yoshigou; URM-P 30108, 2 males (31.2–43.7 mm SL) (excluding 9 females), 9 Aug. 1993, coll. H. Yoshigou; URM-P 30109, 2 males (33.9–43.8 mm SL), 11 Aug. 1993, coll. H. Yoshigou.

Distribution: This species has been reported from the islands of Tanegashima, Yakushima, Amami-oshima, Okinawa, Ishigaki, and Iriomote in the Ryukyu Archipelago, Japan, and Taiwan, including Lanyu (Chen et al., 2007; Yoshigou, 2014). This species has not been recorded based on specimens from the Philippines, but one

male individual of *L. armatus* was observed in a stream on Cebu Island, Philippines by the staff and customers of a diving shop (Aquarius Inc., Lapu Lapu City, Province of Cebu, Philippines) (Naoshi Suzuki, personal communication). We confirmed that a goby in a photograph shows the typical colouration of the male *L. armatus*.

Diagnosis: *Lentipes armatus* is distinguished from all congeners by having the following combination of characteristics: second dorsal and anal fins usually with one spine and 10 soft rays; pectoral fin usually with 18 or 19 rays; fourth and/or fifth spines longest in first dorsal fin of male; small to no interval between first- and second-dorsal-fin bases in male (usually less than 1/3 of the length of the first-dorsal-fin base; the fin bases are often connected); cephalic sensory pore D single; preopercular sensory canal usually with two pores, M' and O'; no enlarged lobes or projections in front of the urogenital papilla of males; lateral scales reaching anteriorly beyond the area below the origin of the first dorsal fin; scales with large, spike-like ctenii laterally on the trunk in male; and colouration of male without red markings on the body and fins, and with three black vertical lines on the light-blue belly, snout often bearing light blue, second dorsal fin being dark brown proximally and white or light brown distally, with one (sometimes two or three) black spot at the anterior part of the second dorsal fin, and anal fin being dark brown proximally and translucent distally.

Description: Body nearly cylindrical. Head depressed with a snout protruding over upper jaw. Head larger in male than female (head length 25.3–29.3 vs. 24.6% of SL; Fig. 9; Table S3). Anterior nostril short tubular, posterior nostril a pore. Mouth inferior with upper jaw projecting beyond lower jaw. Upper lip thick with a small median cleft. Mouth larger in male than female (upper jaw length 9.9–14.1 vs. 10.3% of SL; Table S3) and larger male with larger jaws (Fig. 9). Male with a row of tricuspid teeth (number of teeth 14–30; Fig. 10) and a row of conical teeth following the tricuspid teeth row on premaxilla; larger male with more conical teeth (number of teeth 1–7, but smallest male, 21.2 mm SL, without the conical teeth; Fig. 10). Female with more tricuspid teeth on premaxilla than male (number of teeth 31; Fig. 10), with no conical teeth following the tricuspid teeth (Fig. 10). In male, dentary with canine-like symphyseal teeth (number of teeth 1–9, but smallest male with none; Fig. 10) and a row of unicuspid horizontal teeth enclosed in fleshy sheath (number of teeth 16–26; Fig. 10). Larger male with more symphyseal teeth. Female with more horizontal teeth than male (number of teeth 27; Fig. 10) and no symphyseal teeth (Fig. 10). Cephalic sensory pore system usually with A', B, C, D(S), F, H', K', L', M', and O' (Fig. 1). Preopercular canal with two pores, M' and O' (Table 1). One specimen with two unpaired-pores at D and one specimen with an additional pore between A' and B. Cutaneous sensory

papillae developed over dorsal, lateral, and ventral surfaces of head (Fig. 1). Urogenital papilla of male triangular and housed in a round hollow together with anus; anus located at centre of the hollow (Fig. 11a,b). Urogenital papilla of female plumper and rectangular with small bulge at each corner of posterior edge; the papilla fitting in a hollow; anus located at anterior end of the hollow (Fig. 11c,d).

Male with larger fins than female (Figs. 9, 12). First dorsal fin with six spines; second dorsal fin usually with one spine and 10 soft rays, but one specimen with one spine and eight soft rays. In female, first dorsal fin rounded, almost semicircular, third spine longest, posterior-most tip not extending to origin of second dorsal fin; first- and second-dorsal-fin bases separated by a wide interval (5.5% of SL; Fig. 9). In first dorsal fin of male, fourth and/or fifth spines longest; posterior-most point of the fin (tip of fifth and/or sixth spines) of males larger than 27 mm SL usually extending to base of spine or first soft ray of second dorsal fin when depressed; that of males larger than 40 mm SL often reaching base of second soft ray. Posterior end of first dorsal-fin base often touch or connect to second dorsal-fin origin in larger males, but always separated in smaller males (<26 mm SL; Fig. 9). Anal fin with one spine and 10 soft rays, but one male with one spine and 11 soft rays. Caudal fin usually with 17 segmented rays, including usually 13 branched rays (n=28), but sometimes 12 (n=2) or 14 (n=6); posterior margin rounded, somewhat truncated in smaller specimens. Pectoral fin usually with 18 or 19 rays, but few with 16, 17, or 20 (Table 2). Pelvic fin with one spine and five soft rays; pelvic fins joined together to form strong, cuplike disk with fleshy frenum.

Ctenoid scales covering posterior half of body, but dorsal and ventral scales and scales on caudal-fin base cycloid. In male, ctenoid scales with 1–6 (usually 3 or 4) large, prominent spike-like ctenii covering lateral sides of anterior half of body with a few cycloid scales, but no or few scales with such ctenii in smaller male (<25 mm SL). Female lacking scales with spike-like ctenii. Instead, cycloid scales covering anterior half of body in female and smaller male. Head, nape, breast, and belly naked. Longitudinal scales 26–37, transverse scales 9–15, circumpeduncular scales 15 or 16 (usually 16).

Colour in preservative: In male, background brown. Caudal peduncle, ventral surface of head, breast, and belly light brown, but with a blackish area surrounding a hollow accommodating anus and urogenital papilla. Trunk with three black vertical lines along sensory-papillae rows. First dorsal fin brown without significant marking. Second dorsal fin brown proximally and light brown distally with one black spot between the first and second soft rays (sometimes with two or three spots on membranes between spine and third soft ray). Size of the black spot equal to or smaller than pupil. Anal fin brown proximally and translucent distally. Caudal and pectoral fins

light greyish brown. Pelvic disk brown proximally and translucent distally.

In female, ventral surface of head and body cream, lateral and dorsal surface of head and body light brown. Snout, upper lip, cheek, and opercular region somewhat dusky. An indistinct dusky longitudinal stripe on middle of pectoral-fin base. Internal dusky grey triangle on neurocranium behind eyes. First and second dorsal, anal, caudal, and pectoral fins almost translucent but spines and rays lightly pigmented by melanophores. Pelvic disk cream.

Colour in life (Fig. 13): In male, body grey or brown with a broad dark-brown band between bases of second dorsal and anal fins. Belly and lateral side of trunk light blue with three black vertical lines along the sensory-papillae rows. Snout often bearing light blue. 3–6 silver patches often along dorsal midline from nape to caudal peduncle. Iris silver ventrally and brown dorsally. Anterior part of first dorsal fin (membrane between first and fourth spines and distal part of membrane between fourth and fifth spines) white or light brown, posterior part dark brown. Second dorsal fin dark brown proximally and white or light brown distally, with one black spot surrounded by light-blue ring on membrane between first and second soft rays (sometimes with two or three spots on membranes between spine and third soft ray). Anal fin dark brown proximally and translucent distally. Caudal fin light grey. Pectoral fin light grey but often whitish proximally. During courtship, male displays a sharper nuptial colour with shining white snout, pectoral fin, sky-blue belly, and whitish caudal peduncle.

In female, body greyish brown dorsally and laterally and whitish ventrally. Dusky markings similar to those of preserved specimens. Surface of abdominal cavity with silver mottles. Iris brown or grey. Colours of fins similar to those of preserved specimen.

Lentipes kijimuna Maeda & Kobayashi, sp. nov.

New Japanese name: Kijimunâ-bouzu-haze. New English name: Kijimuna goby (Figs. 9, 10, 12, 14–16; Tables 1, 2; Figs. S6–S10; Table S3)

Lentipes sp. 2 - Zhou & Gao, 2011: 262–263.

Lentipes sp. - Chang & Tseng, 2014: 252–255.

Male type 2 - this study.

Material examined: Holotype. NSMT-P 136934, male (30.5 mm SL), Okinawa Island, 17 Oct. 2014, coll. K. Maeda.

Paratypes. Okinawa Island (n=5): URM-P 48880, male (21.5 mm SL), 11 Oct. 2010, coll. K. Maeda; URM-P 48882, male (27.2 mm SL), 1 Aug. 2013, coll. K. Maeda; URM-P 48883, male (28.6 mm SL), 17 Nov. 2013, coll. H. Kobayashi; URM-P 48886,

male (30.0 mm SL), 19 June 2016, coll. K. Maeda.

Non-type material. Okinawa Island (n=4): URM-P 48879, male (27.2 mm SL), 30 July 2005, coll. K. Maeda, N. Hanahara, and K. Tachihara; URM-P 48881, male (21.3 mm SL), 19 Sep. 2012, coll. K. Maeda; URM-P 48884, male (30.7 mm SL), 12 Jan. 2015, coll. H. Kobayashi; URM-P 48885, male (27.4 mm SL), 20 Apr. 2016, coll. K. Maeda. Ishigaki Island (n=1): URM-P 48908, male (33.1 mm SL), 5 Aug. 1993, coll. H. Yoshigou.

Distribution: The new species was found in seven streams in Okinawa Island and one stream in Ishigaki Island, both in the Ryukyu Archipelago, Japan. The species is rare in these islands, although we sometimes observed it in situ apart from the 10 specimens collected. *Lentipes kijimuna* was usually alone and surrounded by many *L. armatus* at stream rapids. According to two Taiwanese books, *L. kijimuna* is also distributed in Taiwan, but less abundantly than *L. armatus* (*Lentipes* sp. 2 in Zhou & Gao, 2011, pp. 262–263 and *Lentipes* sp. in Chang & Tseng, 2014, pp. 252–255). Some individuals of *L. kijimuna* were observed in a stream on Cebu Island, Philippines by the staff and customers of a diving shop (Aquarius Inc., Lapu Lapu City, Province of Cebu, Philippines) (Yoshio Suzuki, personal communication). We confirmed that the gobies in the photographs and a movie show the typical colouration of male *L. kijimuna*.

Etymology: *Kijimuna* is a creature in Okinawan mythology. It is regarded as a wood spirit and usually described as a child with red hair or a child whose whole body is red. The new species is named *Lentipes kijimuna* for the characteristically red colour associated with *kijimuna*. The new specific name is a noun in apposition.

Diagnosis: *Lentipes kijimuna* is distinguished from all congeners by having the following combination of characteristics: second dorsal and anal fins usually with one spine and 10 soft rays; pectoral fin with 18 or 19 rays; fourth and/or fifth spines longest in first dorsal fin of male; interval between first- and second-dorsal-fin bases in male less than 1/3 of the length of the first-dorsal-fin base; cephalic sensory pore D single; preopercular sensory canal with two pores, M’ and O’; no enlarged lobes or projections in front of the urogenital papilla of male; lateral scales reaching anteriorly beyond the area below the origin of the first dorsal fin; scales with large, spike-like ctenii laterally on the trunk in male; and colouration of male with a red head, a broad red band between bases of posterior half of second dorsal and anal fins, and red second dorsal and anal fins with a submarginal black stripe and a transparent margin.

Description: Morphology described only based on male specimens. Body nearly cylindrical. Head depressed with a snout protruding over upper jaw. Anterior nostril short tubular, posterior nostril a pore. Mouth inferior with upper jaw projecting beyond lower jaw. Upper lip thick with a small median cleft. Premaxilla with a row of tricuspid

teeth (number of teeth 14–24 [h 18]; Fig. 10) and a row of conical teeth following the tricuspid teeth row (number of teeth 1–6 [h 6], but a small male, 21.5 mm SL, without conical teeth; Fig. 10). Dentary with canine-like symphyseal teeth (number of teeth 2–10 [h 7]; Fig. 10) and a row of unicuspid horizontal teeth enclosed in fleshy sheath (number of teeth 16–25 [h 17]; Fig. 10). Cephalic sensory pore system with A', B, C, D(S), F, H', K', and L' in oculoscapular canal (but one specimen lacking pore B) and two pores, M' and O', in preopercular canal (Fig. 15, Table 1). Cutaneous sensory papillae developed over dorsal, lateral, and ventral surface of head (Fig. 15). Urogenital papilla triangular and housed in a round hollow together with anus (same as *Lentipes armatus* shown in Fig. 11a,b).

First dorsal fin with six spines and second dorsal fin with one spine and 10 soft rays, but one paratype specimen with seven spines in first dorsal fin and one spine and nine soft rays in second dorsal fin. In first dorsal fin, third, fourth and/or fifth spines longest; posterior-most point of the fin (tip of fifth and/or sixth spines) of 3/8 specimens larger than 27 mm SL extending to base of spine of second dorsal fin when depressed. Posterior end of first-dorsal-fin base not connecting to second-dorsal-fin origin (interval between first- and second-dorsal-fin bases 0.7–5.3% [h 1.3%] of SL; Fig. 9). Anal fin with one spine and 10 soft rays. Caudal fin with 17 segmented rays, including 13 branched rays, but 14 branched rays in two paratype specimens; posterior margin rounded or somewhat truncated. Pectoral fin with 18 or 19 rays [h 18 rays] (Table 2). Pelvic fin with one spine and five soft rays; pelvic fins joined together to form strong, cuplike disk with fleshy frenum.

Ctenoid scales covering posterior half of body, but dorsal and ventral scales and scales on caudal-fin base cycloid. Lateral sides of anterior half of body covered by ctenoid scales with 1–6 (majority 3 or 4) large, prominent spike-like ctenii and cycloid scales, but that area covered by cycloid scales in two smallest specimens (21.3 and 21.5 mm SL). Head, nape, breast, and belly naked. Longitudinal scales 29–35 [h 34], transverse scales 9–14 [h 13], circumpeduncular scales 16–18 [h 16].

Colour in preservative: Head and body brown with indistinct light-brown band between bases of posterior half of the second dorsal and anal fins. Ventral surface of head, breast, and belly light brown. First dorsal fin grey or light grey with translucent margin along anterior edge, sometimes with black stripe proximally along the translucent margin. Second dorsal and anal fins with a submarginal black stripe and a translucent margin; proximal part below submarginal black stripe pale brown with grey at the bases. In smallest specimen (21.3 mm SL), black stripe on second dorsal fin indistinct and no black stripe on anal fin. Caudal and pectoral fins light greyish brown. Pelvic disk brown proximally and translucent distally.

Colour in life (Fig. 16): Body grey or bluish grey with a red head and a broad red band connecting bases of posterior half of the second dorsal and anal fins. Trunk grey or light blue sometimes with two or three indistinct, blackish vertical lines along the sensory-papillae rows. Margins of scales with large ctenii dark grey. Posterior half of caudal peduncle light grey. Four to six silver patches often along dorsal midline from snout to caudal peduncle. Iris red or reddish brown. First dorsal fin grey or bluish grey with transparent margin along anterior edge, sometimes with black line sub-proximally along the transparent margin. Second dorsal and anal fins with a submarginal black stripe and a transparent margin; proximal part below submarginal black stripe red with grey at the bases; second dorsal fin sometimes with one black spot surrounded by light-blue ring anterior to the black stripe (observed in a specimen not collected). Caudal fin light grey. Pectoral fin light grey but bluish silver proximally. Red colours on head, body, and fins turning vivid during courtship, but faded (pink or reddish brown) when fish inactive or cautious.

Lentipes bunagaya Maeda & Kobayashi, sp. nov.

New Japanese name: Bunagaya-bouzu-haze. New English name: Bunagaya goby (Figs. 9, 10, 12, 17–19; Tables 1, 2; Figs. S6, S7; Table S3)

Lentipes sp. 1 - Zhou & Gao, 2011: 260–261.

Lentipes armatus? - Chang & Tseng, 2014: 258–259.

Male type 3 - this study.

Material examined: Holotype. NSMT-P 136935, male (22.9 mm SL), Okinawa Island, 11 Oct. 2012, coll. K. Maeda.

Paratype. URM-P 48887, male (24.8 mm SL), Okinawa Island, 16 Dec. 2018, coll. K. Maeda and H. Kobayashi.

Distribution: We found two specimens of this new species in the same stream in Okinawa Island. They were collected at different sites (ca. 150 m apart) in different years (2012 and 2018). Both sites were rapids with strong flows, and *L. armatus* was abundant while *L. bunagaya* was always alone. This new species is very rare in Japan. Although *L. kijimuna* were sometimes found apart from the 10 specimens collected during the past 15 years, we have no experience with finding *L. bunagaya* other than the two type specimens described. This species is also distributed in Taiwan. Gobies in the photographs shown in the two Taiwanese books (Chang & Tseng, 2014; Zhou & Gao, 2011) are identified here as *L. bunagaya* based on the distinctive male colouration. Zhou and Gao (2011) regarded it as *Lentipes* sp. 1 and Chang and Tseng (2014) tentatively classified it as a variation of *L. armatus*.

Etymology: *Bunagaya* is a creature in Okinawan mythology, known especially in Ogimi Village, in the northern part of the island. *Bunagaya* is thought to live in forests and/or streams. It is usually described as a child with red hair or whose whole body is red, like the *kijimuna*. The name of this new species is derived from this Okinawan creature, the *bunagaya*. The new specific name is a noun in apposition.

Diagnosis: *Lentipes bunagaya* is distinguished from all congeners by having the following combination of characteristics: second dorsal and anal fins with one spine and 10 soft rays; pectoral fin with 18 or 19 rays; fourth and/or fifth spines longest in first dorsal fin of male; interval between first- and second-dorsal-fin bases in male less than 1/3 of the length of the first-dorsal-fin base; cephalic sensory pore D single; preopercular sensory canal with two pores, M' and O'; no enlarged lobes or projections in front of the urogenital papilla of male; lateral scales reaching anteriorly beyond the area below the origin of the first dorsal fin; scales with large, spike-like ctenii laterally on the trunk in male; and colouration of male with two broad red bands on the posterior part of the body, three black vertical lines on grey or light-blue belly, grey or greyish-brown head without red marking, second dorsal fin being reddish brown proximally and white distally, a black spot at anterior part of second dorsal fin, and white anal fin with obscure reddish-brown base.

Description: Morphology described only based on male specimens. Body nearly cylindrical. Head depressed with snout protruding over upper jaw. Anterior nostril short tubular, posterior nostril a pore. Mouth inferior with upper jaw projecting beyond lower jaw. Upper lip thick with a small median cleft. Premaxilla with a row of tricuspid teeth (number of teeth 15 or [h 17]; Fig. 10) and a row of conical teeth following the tricuspid teeth row (number of teeth [h 3] or 4; Fig. 10). Dentary with canine-like symphyseal teeth (number of teeth 5 or [h 7]; Fig. 10) and a row of unicuspid horizontal teeth enclosed in fleshy sheath (number of teeth 17 or [h 19]; Fig. 10). Cephalic sensory pore system with A', B, C, D(S), F, H', K', and L' in oculoscapular canal and two pores, M' and O', in preopercular canal (Fig. 18, Table 1). Cutaneous sensory papillae developed over dorsal, lateral, and ventral surface of head (Fig. 18). Urogenital papilla triangular and housed in a round hollow together with anus (same as *Lentipes armatus* shown in Fig. 11a,b).

First dorsal fin with six spines and second dorsal fin with one spine and 10 soft rays. In first dorsal fin, [h fourth] or fifth spine longest. Posterior end of spines and membrane of first dorsal fin not extending to second dorsal-fin origin (interval between first- and second-dorsal-fin bases [h 2.2] or 3.2% of SL; Fig. 9). Anal fin with one spine and 10 soft rays. Caudal fin with 17 segmented rays, including 13 branched rays; posterior margin rounded. Pectoral fin with [h 18] or 19 rays (Table 2). Pelvic fin with

one spine and five soft rays; pelvic fins joined together to form strong, cuplike disk with fleshy frenum.

Ctenoid scales covering posterior half of body, but dorsal and ventral scales and scales on caudal-fin base cycloid. Lateral sides of anterior half of body covered by ctenoid scales with 1–5 (majority 3 or 4) large, prominent spike-like ctenii and cycloid scales. Head, nape, breast, and belly naked. Longitudinal scales 30 or [h 32], transverse scales 6 or [h 11], circumpeduncular scales 16 or [h 17].

Colour in preservative: Background brown, but ventral surface of head, breast, and belly light brown. Trunk with three black, distinct vertical lines along sensory-papillae rows. Grey transverse band connecting middle of second-dorsal- and anal-fin bases. First dorsal fin brown or grey without significant marking. Second dorsal fin brown proximally and light grey distally with one black spot between spine and second soft rays. Size of the black spot equal to or larger than pupil. Anal fin brown proximally and translucent distally. Caudal and pectoral fins light greyish brown. Pelvic disk light brown proximally and translucent distally.

Colour in life (Fig. 19): Body grey or greyish brown with two broad red bands on posterior part. The bands faded on dorsum. Belly grey or light blue with three black, distinct vertical lines along the sensory-papillae rows, but area surrounding anus incorporated into anterior red band. Five silver patches along dorsal midline from nape to caudal peduncle. Iris silver ventrally and reddish brown dorsally. Anterior part of first dorsal fin (membrane between first and fifth spines) white, posterior part reddish brown. Second dorsal fin reddish brown proximally and white distally with one black spot surrounded by light-blue ring on membrane between spine and second soft rays. Anal fin white with obscure reddish-brown base.

Lentipes palawanirufus Maeda & Palla, sp. nov.

New English name: Palawan lentipes goby (Figs. 9, 10, 12, 20–22; Tables 1, 2; Figs. S6, S7; Table S3)

Male type 4 - this study.

Material examined: 18 males and two females from Palawan Island in the Philippines. The identification of the females was verified by genome-wide SNPs analysis.

Holotype. NSMT-P 136936, male (44.8 mm SL), Estrella Falls, Narra, 13 May 2016, coll. K. Maeda, T. Kunishima, and H. P. Palla.

Paratypes. NSMT-P 136937, female (49.2 mm SL), same data as holotype; URM-P 48910–48912, 3 males (25.8–45.9 mm SL), same data as holotype; URM-P 48913, male (31.4 mm SL), Olanguan Falls, Puerto Princesa, 16 May 2016, coll. K.

Maeda, T. Kunishima, and H. P. Palla; URM-P 48915 and 48917–48919, 3 males (33.2–46.3 mm SL) and 1 female (45.2 mm SL), Estrella Falls, 29 May 2018, coll. K. Maeda, H. Kobayashi, and H. P. Palla; WPU-PPC-P 36–38, 3 males (39.4–46.3 mm SL), same data as holotype; WPU-PPC-P 40–41, 2 males (24.5–30.7 mm SL), Olanguan Falls, 16 May 2016, coll. K. Maeda, T. Kunishima, and H. P. Palla; WPU-PPC-P 42–48, 5 males (33.7–47.2 mm SL), Estrella Falls, 29 May 2018, coll. K. Maeda, H. Kobayashi, and H. P. Palla.

Distribution: The new species was found only in Palawan Island, Philippines. We collected it from two streams flowing into the Sulu Sea. One of the sites was Estrella Falls in the municipality of Narra, and another was Olanguan Falls in Puerto Princesa City. In the Estrella Falls, *L. palawanirufus* was commonly observed with many other goby species and two cyprinid species at the reaches below the first waterfall. It was more abundant at the reaches above the waterfall, where *L. palawanirufus* and *Sicyopus zosterophorus* occupied the habitat together. The Olanguan Falls consists of continuous waterfalls along the course of one stream. *Lentipes palawanirufus* co-occurred with many other goby species, a cyprinid species, and two *Kuhlia* species.

Etymology: The new species name is derived from the type locality, Palawan, and the Latin word *rufus*, meaning red, with a connecting-vowel, '*i*'. The name means red *Lentipes* of Palawan. The new specific name is treated as an adjective.

Diagnosis: *Lentipes palawanirufus* is distinguished from all congeners by having the following combination of characteristics: second dorsal and anal fins usually with one spine and 10 soft rays; pectoral fin usually with 18 or 19 rays; fourth and/or fifth spines longest in first dorsal fin of male; no or small interval between first- and second-dorsal-fin bases in male (less than 1/3 of the length of the first-dorsal-fin base; the fin bases are often connected); cephalic sensory pore D single; preopercular sensory canal usually with two (M' and O') or three pores (M', N, and O'); no enlarged lobes or projections in front of the urogenital papilla of males; lateral scales reaching anteriorly beyond the area below the origin of the first dorsal fin; scales with large, spike-like ctenii laterally on the trunk in male; and colouration of male with a broad, reddish-brown or dark-red band between bases of the second dorsal and anal fins, red, reddish-brown, or dark-brown head, belly without clear black lines, second dorsal fin being reddish brown or dark red proximally and white or light yellowish brown distally, one black spot at anterior part of second dorsal fin, and anal fin being reddish brown or dark red proximally and translucent distally.

Description: Body nearly cylindrical. Head depressed with a snout protruding over upper jaw. Head larger in male than female (head length 24.8–28.2 [h 26.3] vs. 24.2–24.8% of SL; Fig. 9). Anterior nostril short tubular, posterior nostril a pore. Mouth

inferior with upper jaw projecting beyond lower jaw. Upper lip thick with a small median cleft. Mouth larger in male than female (upper jaw length 10.1–14.6 [h 12.7] vs. 10.4–10.8% of SL) and larger male with larger jaws (Fig. 9). Male with a row of tricuspid teeth (number of teeth 8–23 [h 11]; Fig. 10) and a row of conical teeth following the tricuspid teeth row on premaxilla; larger male with more conical teeth (number of teeth 1–9 [h 7]; Fig. 10). Female with 19–40 tricuspid teeth on premaxilla (Fig. 10), no conical teeth following them (Fig. 10). In male, dentary with canine-like symphyseal teeth (number of teeth 3–9 [h 6]; Fig. 10) and a row of unicuspid horizontal teeth enclosed in fleshy sheath (number of teeth 14–24 [h 19]; Fig. 10). Larger male with more symphyseal teeth. Female with more horizontal teeth than male (number of teeth 33–40; Fig. 10) and no symphyseal teeth (Fig. 10). Cephalic sensory pore system usually with A', B, C, D(S), F, H', K', and L' in oculoscapular canal and two (M' and O') or three (M', N, and O') pores in preopercular canal (Fig. 21, Table 1), but one specimen lacking pore B, one specimen with an additional pore between B and C, and one specimen with pore G. Cutaneous sensory papillae developed over dorsal, lateral, and ventral surface of head (Fig. 21). Urogenital papilla of male triangular and housed in a round hollow together with anus; anus located at centre of the hollow. Urogenital papilla of female plumper and rectangular with small bulge at each corner of posterior edge; the papilla fitting in a hollow; anus located at anterior end of the hollow (both male and female representing same shape as *Lentipes armatus* shown in Fig. 11).

Male with larger fins than female (Figs. 9, 12). First dorsal fin with six spines; second dorsal fin with one spine and 10 soft rays. In female, first dorsal fin rounded, almost semicircular, second or third spine longest, posterior-most tip not extending to origin of second dorsal fin; first- and second-dorsal-fin bases separated by a wide interval (4.5–5.3% of SL; Fig. 9). In first dorsal fin of male, fourth and/or fifth spines longest; posterior-most point of the fin (tip of fifth and/or sixth spines) of males larger than 35 mm SL extending to base of spine or first soft ray of second dorsal fin when depressed, that of males larger than 43 mm SL often reaching base of second soft ray. Posterior end of first-dorsal-fin membrane of 7 in 16 male specimens larger than 30 mm SL touching or connecting to second-dorsal-fin origin; other males has a small interval (0.3–3.9% [h 0.7%] of SL; Fig. 9). Anal fin with one spine and 10 soft rays. Caudal fin with 17 segmented rays, including 13 (n=10) or 14 (n=10) [h 13] branched rays; posterior margin rounded. Pectoral fin with 16–19 rays (usually 18 or 19 rays) [h 18] (Table 2). Pelvic fin with one spine and five soft rays; pelvic fins joined together to form strong, cuplike disk with fleshy frenum.

Ctenoid scales covering posterior half of body, but dorsal and ventral scales and scales on caudal-fin base cycloid. In male, ctenoid scales with 1–8 (usually 3–5)

large, prominent spike-like ctenii covering lateral sides of anterior half of body with a few cycloid scales, but females and one small male (25.8 mm SL) lacking ctenoid scales on that area and with cycloid scales instead. Head, nape, breast, and belly naked. Longitudinal scales 29–38 [h 34], transverse scales 10–15 [h 14], circumpeduncular scales usually 16 [h 16], but 15 in two males.

Colour in preservative: In male, background brown, but ventral surface of head and breast light brown. First dorsal fin brown without significant marking. Second dorsal fin brown proximally and light brown distally with one black spot on membrane between spine and second soft ray. Size of the black spot variable, being equal to, smaller, or larger than pupil. Anal fin brown proximally and translucent distally, with black stripe on border between the brown base and translucent margin. Caudal and pectoral fins light greyish brown. Pelvic disk brown proximally and translucent distally.

In female, ventral surface of head and body cream, lateral and dorsal surface of head and body light brown. Snout, upper lip, cheek, and opercular region somewhat dusky. An indistinct dusky longitudinal stripe on middle of pectoral-fin base, continuing ventrally along lateral midline of body to caudal peduncle. Another indistinct, longitudinal dusky stripe running dorsally along lateral midline on trunk in one of the two specimens. Internal dusky grey triangle on neurocranium behind eyes. First and second dorsal, anal, caudal, and pectoral fins almost translucent but spines and rays lightly pigmented by melanophores. Pelvic disk cream.

Colour in life (Fig. 22): In male, body grey with a broad reddish-brown or dark-red band between bases of second dorsal and anal fins. Belly and lateral side of trunk light blue or grey. Caudal peduncle grey, light grey, or light bluish grey. Lateral and ventral sides of head red, reddish brown, or dark brown. Three silver or light-grey patches often along dorsal midline from nape to origin of second dorsal fin. Iris red or reddish brown. Anterior part of first dorsal fin (membrane between first and fourth spines and distal part of membrane between fourth and fifth spines) white or light yellowish brown, posterior part dark brown. Second dorsal fin reddish brown or dark red proximally and white or light yellowish brown distally, with one black spot surrounded by light-blue ring on membrane between spine and second soft ray. Anal fin reddish brown or dark red proximally and translucent distally, with black stripe on border between the reddish-brown or dark-red base and translucent margin. Caudal fin light grey or light bluish grey. Pectoral fin light grey but often bluish proximally.

In female, body greyish brown dorsally and laterally and whitish ventrally. Dusky markings similar to those of preserved specimens. Iris brown or grey. Colours of fins similar to those of preserved specimens.

Courtship behaviour

Males of *Lentipes* often display one side of the body when approaching a female, while simultaneously lifting the posterior part of the body and spreading the second dorsal and anal fins (Fig. 23a). Subsequently, the male touches the head of the female with the posterior part of its body (Fig. 23b,c). This behaviour was usually observed when a male displayed intense nuptial colouration with strong contrast and was moving around actively. This behaviour was observed and recorded in males of two species, *L. armatus* and *L. bunagaya*.

Discussion

Comparison

Meristic and morphometric characters of *L. armatus* and the three new species are almost identical. *Lentipes armatus*, *L. kijimuna*, and *L. bunagaya* always have two pores in the preopercular canal, whereas approximately half of the specimens of *L. palawanirufus* have three pores in the preopercular canal on one or both sides of the head (two pores in another side if only one side has three; Table 1). But they cannot be distinguished using pore arrangement because another half of *L. palawanirufus* specimens has two pores on both sides. The four species are, however, distinguished from each other by the colour pattern of respective males, as summarized in 'Sorting of male-colour types' (Fig. 2).

We compare below the morphology of the four species with other species of *Lentipes*. In the genus *Lentipes*, 19 species including *L. armatus* are considered as valid (Keith et al., 2015, 2016). Watson & Allen (1999) and Kottelat (2013) considered *Raogobius andamanicus* Mukerji, 1935 a member of *Lentipes*, and Keith et al. (2015) tentatively followed this assignment. Later, Watson et al. (2001) suggested the possibility that *Raogobius* is a senior synonym of *Smilosicyopus*. Both Watson et al. (2001) and Keith et al. (2015) noted the necessity of more research to clarify the status of *Raogobius*. Whether a synonymy of *Lentipes* or not, *R. andamanicus* is clearly distinguished from *L. armatus* and the three new species by its second dorsal and anal fins with nine and 12 soft rays, respectively (vs. 10 soft rays in both fins in *L. armatus* and the three new species), pectoral fins with 13 rays (vs. usually 18 or 19 rays), and scales on the caudal peduncle only (vs. lateral scales extending anteriorly beyond the area below the origin of first dorsal fin).

Lentipes armatus and the three new species differ from *L. adelphizonus*, *L. argenteus* Keith, Hadiaty & Lord in Keith et al., 2014, *L. caroline*, *L. concolor*, *L. dimetrodon*, *L. ikeae*, *L. kaaea*, *L. mindanaoensis*, *L. rubrofasciatus*, *L. solomonensis*

Jenkins, Allen & Boseto, 2008, *L. watsoni* Allen, 1997, and *L. whittenorum* Watson & Kottelat, 1994 in one or more characteristics (summarised in Table 3). This includes the absence of large lobes in front of the urogenital papilla in males (vs. with two pairs of lobes in *L. rubrofasciatus* and *L. whittenorum*, and with a single pair of lobes in *L. kaaea* and *L. solomonensis*); scales with large, prominent spike-like ctenii laterally on the trunk in males (vs. no such scale in *L. caroline*, *L. concolor*, *L. dimetrodon*, and *L. rubrofasciatus*); the lateral sides of the body below the first dorsal fin scaled (vs. naked in *L. caroline*, *L. concolor*, and *L. rubrofasciatus*); with a single sensory pore D (vs. pore D usually absent in *L. dimetrodon*); preopercular sensory canal with two or three pores (vs. preopercular sensory canal without pore or rarely a single pore in males of *L. caroline*); fourth and/or fifth spine longest in the first dorsal fin of males (vs. sixth spine longest in *L. dimetrodon*); smaller interval between first and second dorsal-fin bases in males, which is usually less than 1/3 of the length of the first dorsal-fin base, and the fin-bases are often connected in males larger than 25 mm SL (vs. interval equal to 1/2 of the first dorsal-fin base in males, even in specimens larger than 45 mm SL in *L. watsoni*); second dorsal and anal fins usually with one spine and 10 soft rays (vs. one spine and nine soft rays in *L. solomonensis* and *L. ikeae*; one spine and nine or 10 soft rays in *L. adelphizonus*, the holotype with one spine and nine soft rays); pectoral fins usually with 18 or 19 rays (vs. usually fewer than 18 rays in *L. adelphizonus*, *L. argenteus*, *L. caroline*, *L. concolor*, *L. dimetrodon*, *L. ikeae*, *L. kaaea, L. mindanaoensis*, *L. rubrofasciatus*, *L. solomonensis*, and *L. watsoni*); and finally several differences in male colour pattern. Watson & Kottelat (2006) reported ‘papillose finger-like projections anterior to urogenital papilla’ in males of *L. adelphizonus*, but we did not observe such a projection on the male holotype, and the structure around the anus and urogenital papilla is identical to those of *L. armatus* and the three new species described in this study (Fig. 11a,b). This suggests that the structure of the male urogenital papilla in *L. adelphizonus* may exhibit variation or that the reported observation may have been based on a misinterpretation (e.g., the finger-like projections reported might actually be something evacuated from the anus). Even without the finger-like projection, fewer counts in the second dorsal, anal, and pectoral fins of *L. adelphizonus* distinguish this species from *L. armatus* and the three new species.

In taxonomic studies of *Lentipes*, characters of the male are typically more useful for diagnosis than those of the females (Keith et al., 2015). As a result, female individuals are generally more difficult to distinguish and identify than male individuals, as we have reported in this study. *Lentipes crittersius* was described from Biak Island in Indonesia based only on the female holotype and no additional material is known so far. Distinguishing *L. crittersius* from other species of *Lentipes* is difficult as

it shares many characters with the other species, and therefore more specimens are required to ascertain the validity of this species (Keith et al., 2015). In fact, no author has compared *L. crittersius* with any other species, although 11 new species have been described in this genus since the original description of *L. crittersius*. According to Watson & Allen (1999), *L. crittersius* lacks scales on the anterior part of the body. Meanwhile, the lateral scales of female *L. armatus* and *L. palawanirufus* reach anteriorly beyond the area below the origin of the first dorsal fin and so can be distinguished from *L. crittersius*. As far as we know, species whose males have scales with prominent spike-like ctenii on the trunk have females with cycloid scales on the anterior part of the body. Although no female specimen is known to date for *L. kijimuna* and *L. bunagaya*, we presume (but can not confirm) that females of these two species have scales on the anterior part of the body, which differs from *L. crittersius* in this feature (Table 3). Therefore, the species that we have described herein are unlikely to be conspecific with *L. crittersius*.

The remaining four species, *L. kolobangara*, *L. mekonggaensis*, *L. multiradiatus*, and *L. venustus* Allen, 2004, share several characteristics with *L. armatus* and the three new species (Table 3), but *L. kolobangara*, *L. mekonggaensis*, and *L. venustus* Allen, 2004 can be distinguished by the colouration of their males, especially by the positions of reddish or brown markings on the body and the shape of black markings on the second dorsal fin (Fig. 24). *Lentipes venustus* has no black spot and no submarginal black stripe on the second dorsal fin, *L. mekonggaensis* has a red caudal peduncle, and *L. kolobangara* has a large red marking on the pectoral-fin base and proximal part of the pectoral fin. *Lentipes multiradiatus* is, however, very similar to *L. armatus* even with regard to male colouration. Colour variation in *L. multiradiatus* is not well known, and more extensive studies are required to determine the differences between *L. armatus* and *L. multiradiatus*. The three new species are clearly different from *L. multiradiatus* by having species-specific reddish markings on the body.

The four species investigated in this study are distributed in Japan, Taiwan, and the Philippines. Another species from this region is *L. mindanaoensis*, known from the islands of Mindanao and Panay, Philippines (Keith et al., 2015). The male colouration of this species differs from that of the four species treated herein in that it has a large red or dark-brown marking on the pectoral-fin base and the proximal part of the pectoral fin (vs. generally grey but often whitish or bluish in the four species) and the broad red or dark-brown band on the posterior part of the body extending anterior to the anus (vs. anterior margin of the red or dark-brown band never extending to the anal and second dorsal-fin origin in *L. kijimuna* and *L. palawanirufus*) (Fig. 24), in addition to fewer pectoral-fin rays (16–17 vs. usually 18–19) (Table. 3).

Reproductive isolation and speciation

The genome-wide SNPs analyses revealed that the four male-colour types are genetically distinguishable (Figs. 6, 7, S1–S3, and S5). In the ADMIXTURE analyses, the four types were completely separated from each other when K=4 was assumed, although the CV error was the lowest when K=1 (Fig. S4). These results indicate that the four types are genetically very close to each other to the extent that the K=1 model has the best CV error, but that they are absolutely distinguishable. However, the four types were not distinguished from each other by the analyses of the mitochondrial DNA sequences (Fig. 4), and *L. ikeae* and *Lentipes* sp. [sensu Taillebois et al. (2014)] were also intermixed with them (Fig. 5). Other species with poor diagnostic characteristics, aside from male colouration (such as *L. kolobangara*, *L. mekonggaensis*, *L. multiradiatus*, and *L. venustus*) might also not be distinguished by the mitochondrial markers. We consider that this reflects incomplete lineage sorting. Probably, these species have diverged recently, and therefore the sorting of mitochondrial haplotypes, as well as many other nuclear genes, may not have been completed yet.

The male colouration of the four species, *L. armatus*, *L. kijimuna*, *L. bunagaya*, and *L. palawanirufus*, is clearly different from each other and from those of other species in *Lentipes*. We observed that males displayed the posterior part of their body to the females. This is considered a courtship behaviour. Although both *L. kijimuna* and *L. bunagaya* have red band(s) on the posterior part of the body, the colour of the area surrounding the anus is completely different: this area is incorporated into the anterior red band in *L. bunagaya* (Fig. 23d), while it is grey or light blue in *L. kijimuna*. This area becomes especially highlighted when the male places the posterior part of the body on the head of the female (Fig. 23b,c) as well as when the male approaches the female while lifting the posterior part of the body. Colouration of the second dorsal and anal fins would also be expected to be important, as the male often opens these fins as part of the display (Fig. 23a). The species-specific male colourations would be one of the major factors leading to reproductive isolation, if females distinguish between them. Pre-zygotic isolation may represent a primary mechanism of reproductive isolation among the four species.

We found three species of *Lentipes* in Okinawa Island, but only *L. armatus* was abundant, whereas *L. kijimuna* and *L. bunagaya* were very rare. Probably the latter two species are rare migrants transported from unknown main habitats as is the case for *Stiphodon niraikanaiensis* and *Stiphodon alcedo* (see Keith et al., 2015; Maeda, 2013; Maeda et al., 2012). Because the species composition and abundance in other islands of the Ryukyu Archipelago and Taiwan seem similar to what we observed in Okinawa

Island (Chang & Tseng, 2014; Keith et al., 2015; Yoshigou, 2014; Zhou & Gao, 2011), and since *Lentipes* has never been reported from any continental landmass, we expect that the main habitats of *L. kijimuna* and *L. bunagaya* are islands in the Philippines and/or Indonesia, and that their larvae are sometimes transported to the Ryukyu Archipelago during their pelagic phase. Indeed, *L. armatus* and *L. kijimuna* have been observed on Cebu Island in the central Philippines (see the 'distribution' of *L. armatus* and *L. kijimuna*), although this needs to be verified with museum vouchered material. Current knowledge of the distribution of *Lentipes* in Southeast Asia is still limited and more information will be needed to circumscribe the geographic distribution of *L. kijimuna* and *L. bunagaya*.

In the genome-wide SNPs analyses, two lineages were detected within *L. kijimuna*. Species delimitation analyses also supported the presence of five species rather than four species (see Supporting Information). However, the colouration was identical between these two (Figs. S8, S9). Given the roles of male colouration suggested above, pre-zygotic isolation between the two lineages in *L. kijimuna* is quite unlikely. How then have the two lineages differentiated and remained genetically isolated? We expect that the two lineages are distributed allopatrically on tropical islands (we do not yet know where), where no gene exchange occurs between them. However, larvae would be transported from each of the allopatric populations to Okinawa. Because they are very rare in Okinawa, it would likely be very difficult for mating to taking place. Even if they could encounter a conspecific mate and spawn, it may be difficult for the larvae to return to the tropical habitats in the south because the strong Kuroshio Current would most likely transport the larvae northward. Competition from *L. armatus* may also prevent members of the two lineages of *L. kijimuna* from becoming established on Okinawa. Nevertheless, we cannot reject another hypothesis that the two lineages are different species with pre-zygotic reproductive isolation by unknown, subtle differences of the colour pattern, behaviour, and/or other traits such as pheromone or microhabitat differences. This issue should be resolved in future research, including intensive surveys of the freshwater fish fauna in Southeast Asia, phylogenetic and morphological studies with more samples, and behavioural and experimental approaches to confirm whether reproductive isolation is really absent or not. We put the two lineages together under the name of *L. kijimuna* tentatively, because no diagnostic characters have been found, except the fixed differences in SNPs between the two lineages.

Situations similar to what we have observed in *Lentipes*, that is, mate choice by the colour pattern thought to limit the gene flow between closely related species, have been reported for several other fish taxa, including both freshwater and marine

taxa. For example, species of Caribbean coral reef fishes in the genus *Hypoplectrus* are not distinguished by mitochondrial markers, but microsatellite analysis demonstrated genetic differences among them (Puebla et al., 2007; Ramon et al., 2003). Each species has been recognized primarily on the basis of colouration, and the colour pattern is considered as a cue for assortative mating (Puebla et al., 2007). Cichlids in East African lakes are also a famous example of rapid speciation. Although some species of cichlid could potentially interbreed as they have not developed a mechanism of post-zygotic isolation, it is suggested that their reproductive isolation is maintained by female preference for the nuptial colouration of conspecific males (Kocher, 2004; Miyagi & Terai, 2013). We consider that the amphidromous sicydiine gobies, in which males generally represent vivid nuptial colouration, have the potential to be an interesting system for the study of reproductive isolation. Sicydiine gobies including *Lentipes* are distributed widely across oceanic islands, which suggest widespread larval dispersal (Keith et al., 2015; Maeda, 2013; Maeda & Saeki, 2018). This is a unique feature of the taxon, which differs from *Hypoplectrus* and cichlids. Widespread larval dispersal is expected to prevent speciation and reduce endemism. However, sicydiine gobies actually harbour a high level of diversity (>100 species) and many species are known to be endemic to small areas, although some species such as *Sicyopterus lagocephalus* are widely distributed nearly throughout the Indo-Pacific islands (Keith et al., 2015). Additional studies are needed to clarify how endemism develops and is maintained in this group.

Acknowledgments

Samplings and surveys in Palawan were conducted under a Memorandum of Agreement for joint research between Okinawa Institute of Science and Technology Graduate University (OIST) and Western Philippines University (WPU). We thank Elsa P. Manarpaac, Lota A. Creencia, Benjamin J. Gonzales (WPU), Jonathan Dorfan, Hiroyo Clemente (OIST), Filipina B. Sotto, and Joeppette J. Hermosilla (University of San Carlos) for their support of this collaboration. We are grateful to Nelson P. Devanadera, Nino Rey C. Estoya, Beth Lagrada, Aira Bayron, and Aiza Nunez (Palawan Council for Sustainable Development, PCSD) for providing Wildlife Gratuitous Permits (No. 2016-09, 2018-16) and Wildlife Export Certifications (No. 16-03, 2018-03); Lucena D. Demaala (Municipality of Narra), Lucilo R. Bayron (Puerto Princesa City), Rosalina Cañaveral (Barangay Estrella Village), and Macario Fabrigas (Barangay Binduyan) for providing Prior Informed Consent Certificates, and all local residents who supported our surveys. We appreciate Gento Shinohara, Masanori Nakae (NSMT), and Kei Miyamoto (Okinawa Churashima Foundation) for loan and registration of the

specimens, Renny K. Hadiaty, Sopian Sauri (MZB), Zara Gabsi, Jonathan Pfliger, and Philippe Keith (MNHN) for their supports during we visited and investigated the collections, Hidenori Yoshigou (Chugai Technos), Nozomi Hanahara (Okinawa Churashima Foundation), and Daiki Ito (University of the Ryukyus) for providing valuable information and/or for their supports to the fieldworks, Konstantin Khalturin, Mariia Khalturina, Miyuki Kanda, and Mayumi Kawamitsu (OIST) for their support to the library preparation and sequencing for mitochondrial DNA analysis, Ayşe Haruka Oshima Açıkbaş and Pei Chen King (OIST) for useful suggestions to improve the manuscript, staff and customers of Aquarius Divers (the late Takumi Shiraishi, Kaori Koromo, Yoshio Suzuki, and Naoshi Suzuki) for providing valuable information on the distribution of *Lentipes* in Cebu, Yasushi Sadoyama (Okinawa, Japan) for providing a picture, and Tetsuo Yoshino (Okinawa Churashima Foundation) for his useful advice and support to our study. We would like to thank all three reviewers and the editors for their valuable comments to improve the manuscript. This study was supported by JSPS KAKENHI Grant Numbers 24780200 and 16K07492 and by the Okinawa Institute of Science and Technology Graduate University.

References

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research, 19,* 1655–1664.

Allen, G. R. (1997). *Lentipes watsoni*, a new species of freshwater goby (Gobiidae) from Papua New Guinea. *Ichthyological Exploration of Freshwaters, 8*, 33–40.

Allen, G. R. (2001). *Lentipes multiradiatus*, a new species of freshwater goby (Gobiidae) from Irian Jaya, Indonesia. *Aqua, Journal of Ichthyology and Aquatic Biology, 4*, 121–124.

Allen, G. R. (2004). Two new species of gobiid fishes (*Lentipes* and *Stenogobius*) from fresh waters of Milne Bay Province, Papua New Guinea. *Fishes of Sahul, 18*, 87–96.

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114-2120.

Bouckaert, R. R. (2010). DensiTree: making sense of sets of phylogenetic trees. *Bioinformatics*, *26*, 1372–1373.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M. A., Rambaut, A., & Drummond, A. J. (2014). BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, *10*, e1003537.

Bryant, D., Bouckaert, R., Felsenstein, J., Rosenberg, N. A., & RoyChoudhury, A. (2012). Inferring species trees directly from biallelic genetic markers: bypassing

gene trees in a full coalescent analysis. *Molecular Biology and Evolution*, *29*, 1917–1932.

Cal, L., Suarez-Bregua, P., Cerdá-Reverter, J. M., Braasch, I., & Rotllant, J. (2017). Fish pigmentation and the melanocortin system. *Comparative Biochemistry and Physiology, Part A, 211*, 26–33.

Capella-Gutiérrez, S., Silla-Martínez, J. M., & Gabaldón, T. (2009). trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics, 25*, 1972–1973.

Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W., & Postlethwait, J. H. (2011). Stacks: building and genotyping loci de novo from short-read sequences. *G3: Genes, Genomes, Genetics, 1*(3), 171–182.

Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: an analysis tool set for population genomics. *Molecular Ecology, 22*(11), 3124–3140.

Chen, I-S. (2004). *Lentipes mindanaoensis*, a new species of freshwater goby (Teleostei: Gobiidae) from southern Philippines. *Platax, 1*, 37–41.

Chen, I-S., Suzuki, T., Cheng, Y-H., Han, C-C., Ju Y-M., & Fang, L-S. (2007). New recoed of the rare amphidromous gobiid genus, *Lentipes* (Teleostei: Gobiidae) from Taiwan with the comparison of Japanese population. *Journal of Marine Science and Technology, 15*, 47–52.

Chang, D-C., & Tseng, W-J. (2014). *Goby Pedia: A handbook for freshwater and brackish species*. Taipei: Fish Magazine Taiwan.

Dahruddin, H., Hutama, A., Busson, F., Sauri, S., Hanner, R., Keith, P., Hadiaty, R., & Hubert, N. (2017). Revisiting the ichthyodiversity of Java and Bali through DNA barcodes: taxonomic coverage, identification accuracy, cryptic diversity and identification of exotic species. *Molecular Ecology Resources*, *17*, 288–299.

Gill, T. (1860). Conspectus piscium in expeditione ad oceanum Pacificum septentrionalem, C. Ringold et J. Rodgers ducibus, a Gulielmo Stimpson collectorum. Sicydianae. *Proceedings of the Academy of Natural Sciences of Philadelphia, 12*, 100–102.

Harrison, I. J. (1993). The West African sicydiine fishes, with notes on the genus *Lentipes* (Teleostei: Gobiidae). *Ichthyological Exploration of Freshwaters, 4*, 201–232.

Huson, D. H., & Bryant, D. (2006). Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution, 23*(2), 254–267.

Jenkins, A. P., Allen, G. R., & Boseto, D. (2008). *Lentipes solomonensis*, a new species of freshwater goby (Teleostei: Gobioidei: Sicydiinae) from the Solomon Islands.

Aqua, International Journal of Ichthyology, 14, 165–174.

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution, 30*, 772–780.

Keith, P., Hadiaty, R. K., Hubert, N., Busson, F., & Lord, C. (2014). Three new species of *Lentipes* from Indonesia (Gobiidae). *Cybium, 38*, 133–146.

Keith, P., Lord, C., Boseto, D. & Ebner, B. (2016). A new species of *Lentipes* (Gobiidae) from the Solomon Islands. *Cybium, 40*, 139–146.

Keith, P., Lord, C., Lorion, J., Watanabe, S., Tsukamoto, K., Couloux, A., & Dettai A. (2011). Phylogeny and biogeography of Sicydiinae (Teleostei: Gobiidae) inferred from mitochondrial and nuclear genes. *Marine Biology*, *158*, 311–326.

Keith, P., Lord, C., & Maeda, K. (2015). *Indo-Pacific Sicydiine gobies: Biodiversity, life traits and conservation*. Paris: Société Française d'Ichtyologie.

Kocher, T. D. (2004) Adaptive evolution and explosive speciation: the cichlid fish model. *Nature Reviews Genetics*, *5*, 288–298.

Kottelat, M. (2013). The fishes of the inland waters of Southeast Asia: a catalogue and core bibliography of the fishes known to occur in freshwaters, mangroves and estuaries. *Raffles Bulletin of Zoology, Supplement 27*, 1–663.

Kumar, S., Stecher, G., Lim, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*, 1547–1549.

Lynch, B., Keith, P., & Pezold, F. L. (2013). A new species of freshwater goby from Pohnpei, Micronesia (Gobioidei: Sicydiinae). *Cybium, 37*, 171–177.

Maciolek, J. A. (1977). Taxonomic status, biology, and distribution of Hawaiian *Lentipes*, a diadromous goby. *Pacific Science*, *31*, 355–362.

Maeda, K. (2013). *Stiphodon niraikanaiensis*, a new species of sicydiine goby from Okinawa Island (Gobiidae: Sicydiinae). *Ichthyological Research,* online first article. doi: 10.1007/s10228-013-0379-2

Maeda, K., Mukai, T., & Tachihara, K. (2012). A new species of amphidromous goby, *Stiphodon alcedo*, from the Ryukyu Archipelago (Gobiidae: Sicydiinae). *Cybium, 35,* 285–298.

Maeda, K., & Saeki, T. (2018). Revision of species in *Sicyopterus* (Gobiidae: Sicydiinae) described by de Beaufort (1912), with a first record of *Sicyopterus longifilis* from Japan. *Species Diversity, 23*, 253–262.

Maugé, A., Marquet, G., & Laboute, P. (1992). Les Sicydiinae (Gobiidae) des eaux douces de la Polynésie française. Description de trois espèces nouvelles [The Sicydiinae (Gobiidae) from French Polynesia freshwaters. Description of three new

species]. *Cybium, 16*, 213–231.

Miyagi, R., &Yohey Terai, Y. (2013) The diversity of male nuptial coloration leads to species diversity in Lake Victoria cichlids. *Genes & Genetic Systems*, *88*, 145–153.

Mukerji, D. D. (1935). Notes on some rare and interesting fishes from the Andaman Islands, with descriptions of two new freshwater gobies. *Records of the Indian Museum, 37*, 259–277.

Nakabo, T. (2002) *Fishes of Japan with pictorial keys to the species* (English ed.). Tokyo: Tokai University Press.

Nakabo, T. (2018). *The natural history of the fishes of Japan*. Tokyo: Shogakukan.

Nei, M. (1972). Genetic distance between populations. *The American Naturalist*, *106*, 283–292.

Nelson, J. S., Grande, T. C., & Wilson, M. V. H. (2016) *Fishes of the world fifth edition*. Hoboken, New Jersey: John Wiley & Sons, Inc.

Pembleton, L. W., Cogan, N. O. I., & Foster, J. W. (2013). StAMPP: an R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. *Molecular Ecology*, *13*, 946–952.

Peng, Y., Leung, H. C. M., Yiu, S. M., & Chin, F. Y. L. (2012). IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. *Bioinformatics, 28*, 1420–1428.

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. *PloS One*, e37125.

Puebla, O., Bermingham, E., Guichard, F., & and Whiteman, E. (2007) Colour pattern as a single trait driving speciation in *Hypoplectrus* coral reef fishes? *Proceedings of the Royal Society B*, *274*, 1265–1271.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., Maller, J. Sklar, P., de Bakker, P., Daly, J. M., & Sham, P. C. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *The American Journal of Human Genetics, 81*(3), 559–575.

Ramon, M. L., Lobel, P. S., & Sorenson, M. D. (2003) Lack of mitochondrial genetic structure in hamlets (*Hypoplectrus* spp.): recent speciation or ongoing hybridization? *Molecular Ecology*, *12*, 2975–2980.

Sakaguchi, S., Sugino, T., Tsumura, Y., Ito, M., Crisp, M. D., Bowman, D. M., Nagano, A. J., Honjo, M. N., Yasugi, M., Kudo, H., Matsuki, Y, Suyama, Y., & Isagi, Y. (2015). High-throughput linkage mapping of Australian white cypress pine (*Callitris glaucophylla*) and map transferability to related species. *Tree Genetics & Genomes*, *11*, 121.

Sabaj, M. H. (2019). Standard symbolic codes for institutional resource collections in herpetology and ichthyology: An Online Reference. Version 7.1 (21 March 2019). Electronically accessible at http://www.asih.org, Washington, DC: American Society of Ichthyologists and Herpetologists.

Sakai, H., & Nakamura, M. (1979). Two new species of freshwater gobies (Gobiidae: Sicydiaphiinae) from Ishigaki Island, Japan. *Japanese Journal of Ichthyology, 26*, 43–54.

Salis, P., Lorin, T., Laudet, V., & Frédérich, B. (2019) Magic traits in magic fish: Understanding color pattern evolution using reef fish. *Trends in Genetics, 35*(4), 265–278.

Shibukawa, K., & Satapoomin, U. (2006). *Myersina adonis*, a New Species of Shrimp-associated Goby (Pisces, Perciformes, Gobiidae) from the Andaman Sea. *Bulletin of the National Science Museum Series A (Zoology), 32*, 29–37.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics, 30*, 1312–1313.

Taillebois, L., Castelin, M., Lord, C., Chabarria, R., Dettaï, A., & Keith, P. (2014). New Sicydiinae phylogeny (Teleostei: Gobioidei) inferred from mitochondrial and nuclear genes: Insights on systematics and ancestral areas. *Molecular Phylogenetics and Evolution*, *70*, 260–271.

Thacker, C. E., Thompson, A. R., & Roje, D. M. (2011). Phylogeny and evolution of Indo-Pacific shrimp-associated gobies (Gobiiformes: Gobiidae). *Molecular Phylogenetics and Evolution, 59*, 168–176.

Watson, R. E. (2008). A new species of *Stiphodon* from southern Sumatra (Pisces: Gobioidei: Sicydiinae). *Zootaxa, 1715*, 43–56.

Watson, R. E., & Allen, G. R. (1999). New species of freshwater gobies from Irian Jaya, Indonesia (Teleostei: Gobioidei: Sicydiinae). *Aqua, Journal of Ichthyology and Aquatic Biology, 3*, 113–118.

Watson, R. E., & Kottelat, M. (1994). *Lentipes whittenorum* and *Sicyopus auxilimentus*, two new species of freshwater gobies from the western Pacific (Teleostei: Gobiidae: Sicydiinae). *Ichthyological Exploration of Freshwaters, 5*, 351–364.

Watson, R. E., & Kottelat, M. (2006). Two new freshwater gobies from Halmahera, Maluku, Indonesia (Teleostei: Gobioidei: Sicydiinae). *Ichthyological Exploration of Freshwaters, 17*, 121–128.

Watson, R. E., Keith, P., & Marquet, G. (2001). *Sicyopus* (*Smilosicyopus*) *chloe*, a new species of freshwater goby from New Caledonia (Sicydiinae). *Cybium, 25*, 41–52.

Watson, R. E., Keith, P., & Marquet, G. (2002). *Lentipes kaaea*, a new species of freshwater goby (Teleostei: Gobioidei: Sicydiinae) from New Caledonia. *Bulletin*

Français de la Pêche et de la Pisciculture, 364, 173–185.

Yabe, M., Kuwamura, T., & Takagi, Y. (2017). *Ichthyology*. Tokyo: Kouseishakouseikaku.

Yoshigou, H. (2014). Annotated checklist and bibliographic records of inland water fishes of the Ryukyu Archipelago, Japan. *Fauna Ryukyuana, 9*, 1–153.

Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics*, *28*, 3326–3328.

Zhou, M., & Gao, R. (2011). *The Freshwater and Estuarine Fishes of Taiwan*. Taichung, Taiwan: Morning Star Publishing.

Associate Editor: Dr Kevin Conway

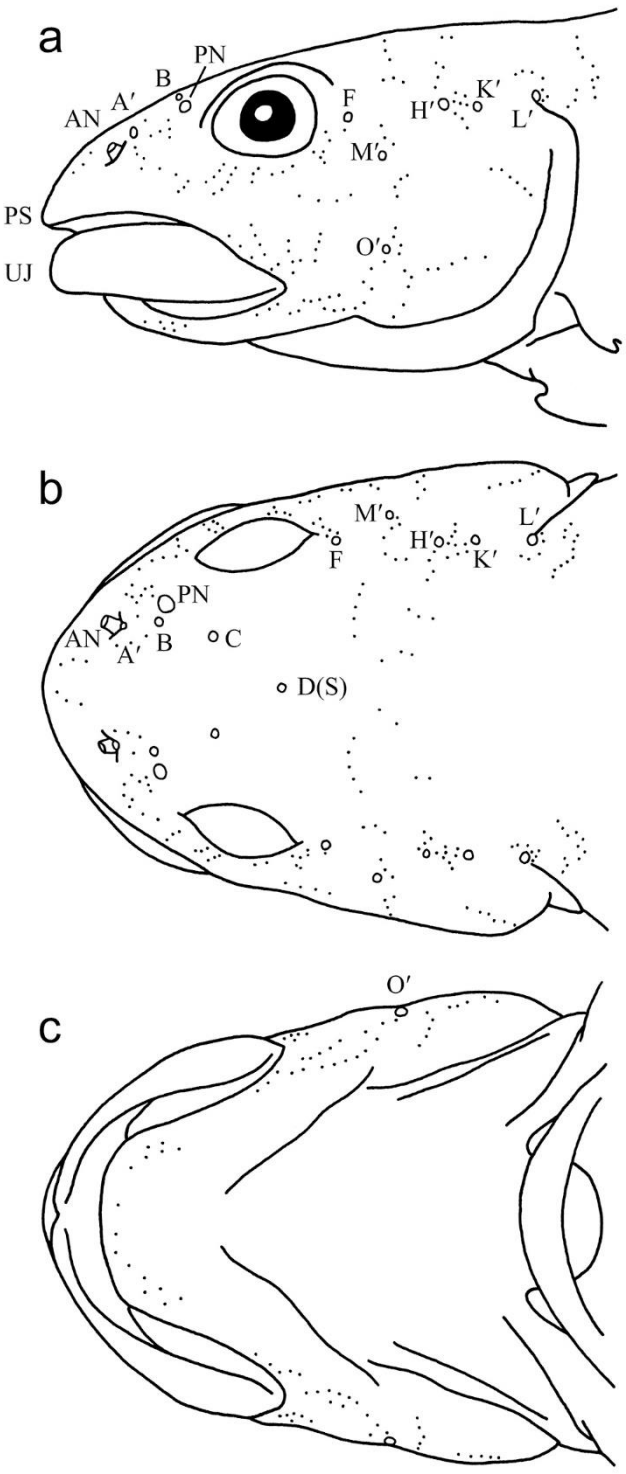

Fig. 1. Arrangement of cephalic sensory pores and cutaneous sensory papillae in *Lentipes armatus* (URM-P 48822). AN anterior naris, PN posterior naris, PS anterior-most point of the protruding snout, UJ anterior-most tip of the upper jaw.

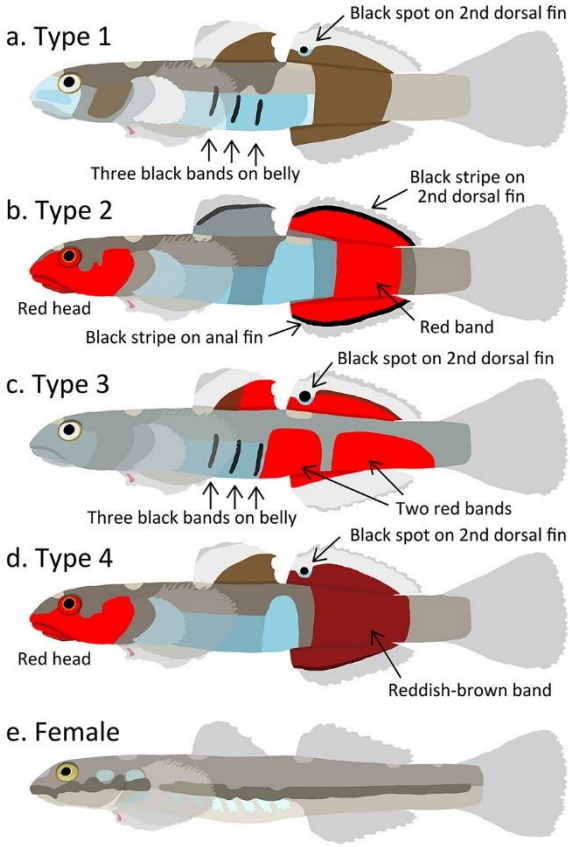

Fig. 2. Four types of male in *Lentipes* and a representative female with notations of the key colouration characteristics.

Fig. 3. In situ underwater photographs of female *Lentipes* observed in Okinawa Island, Japan (a, 8 May 2012; b, 6 Mar. 2010) and in Palawan Island, Philippines (c and d, 29 May 2018) (photo by K. Maeda).

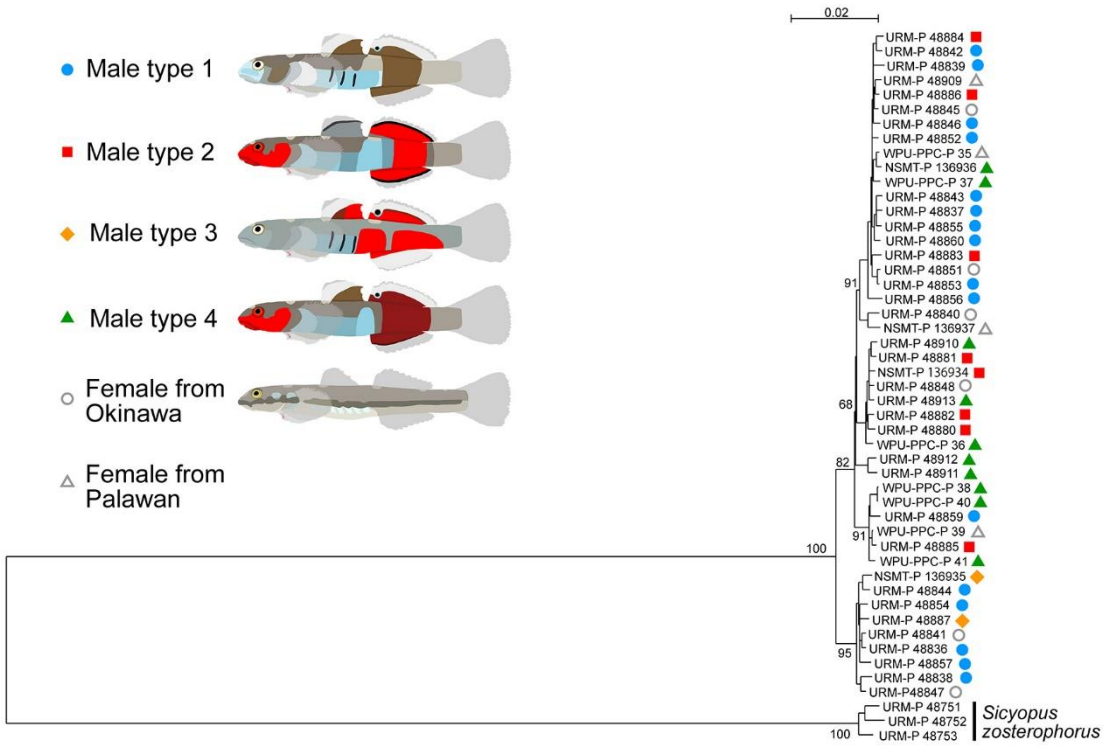

Fig. 4. Maximum likelihood phylogenetic tree with 100 bootstrap using the aligned 16,454 bp of mitochondrial genomes of 46 specimens of *Lentipes* with *Sicyopus zosterophorus* as an outgroup taxon.

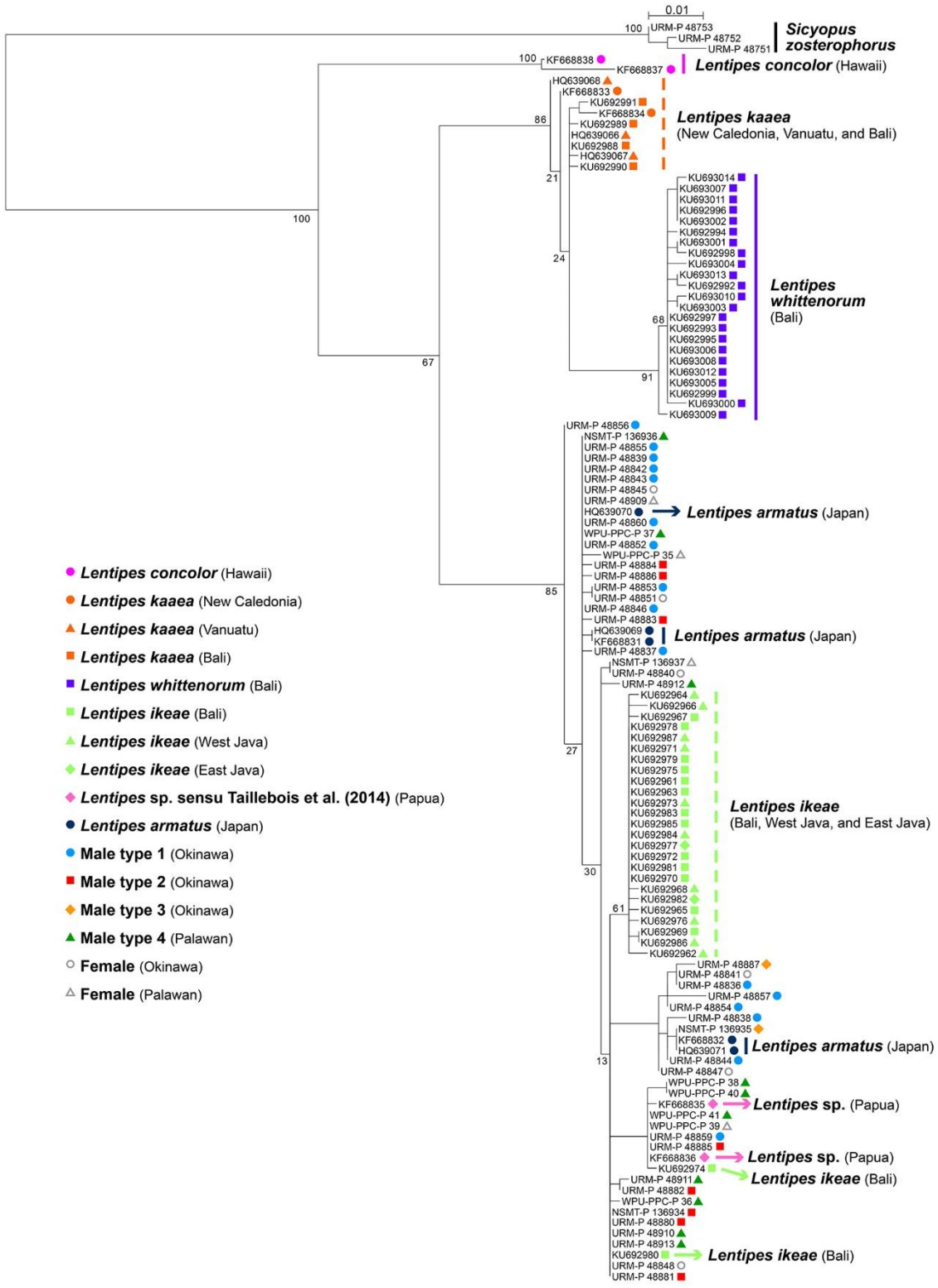

Fig. 5. Maximum likelihood phylogenetic tree with 100 bootstrap using the aligned sequences (629 bp) of a mitochondrial marker, partial cytochrome oxidase subunit 1 (COI), of species in *Lentipes* with *Sicyopus zosterophorus* as an outgroup taxon. Material sequenced in the present study (male types 1–4 and females) are shown with the catalogue numbers of the vouchers (beginning with NSMT-P, URM-P, or WPU-PPC-P) and sequences from the International Nucleotide Sequence Database are shown with the accession numbers

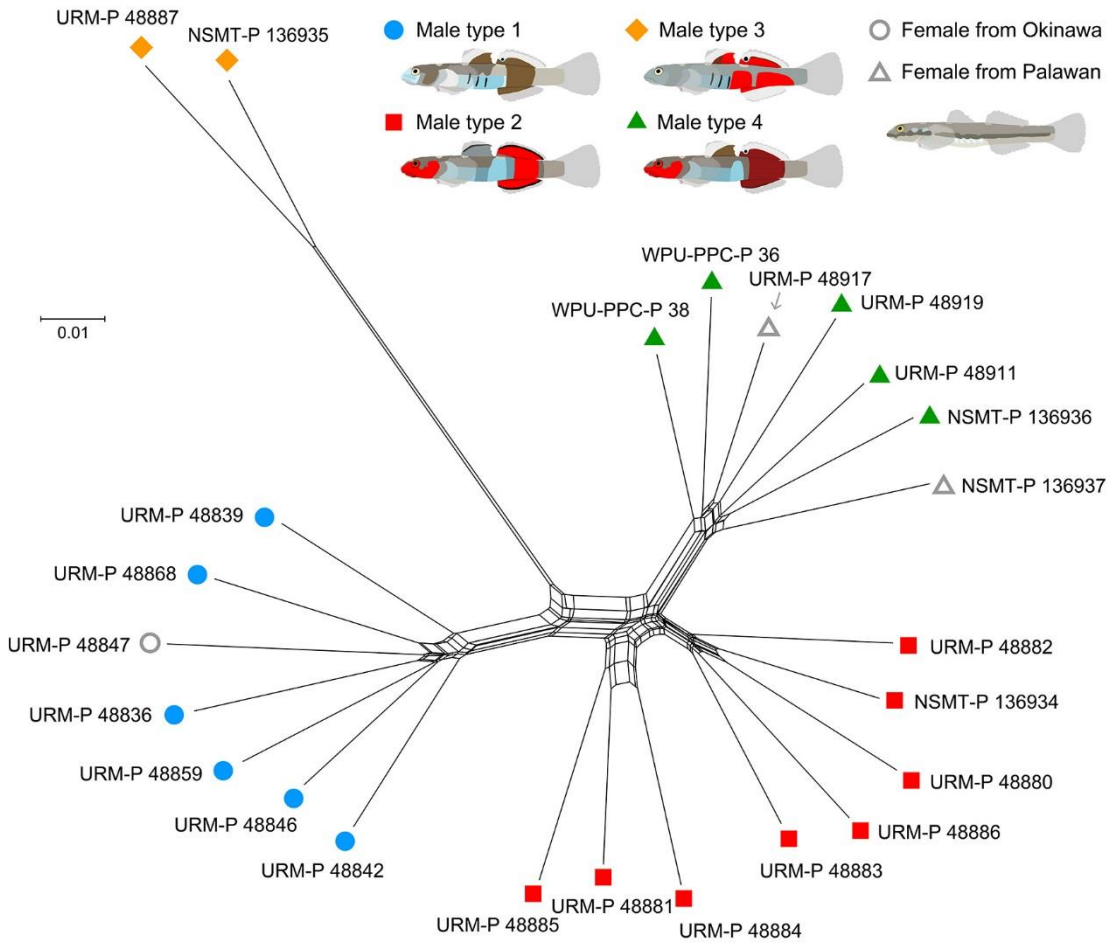

Fig. 6. Neighbour-net phylogenetic networks based on Nei's genetic distances calculated from the 1,556 SNPs. Scale bar indicates substitutions per site.

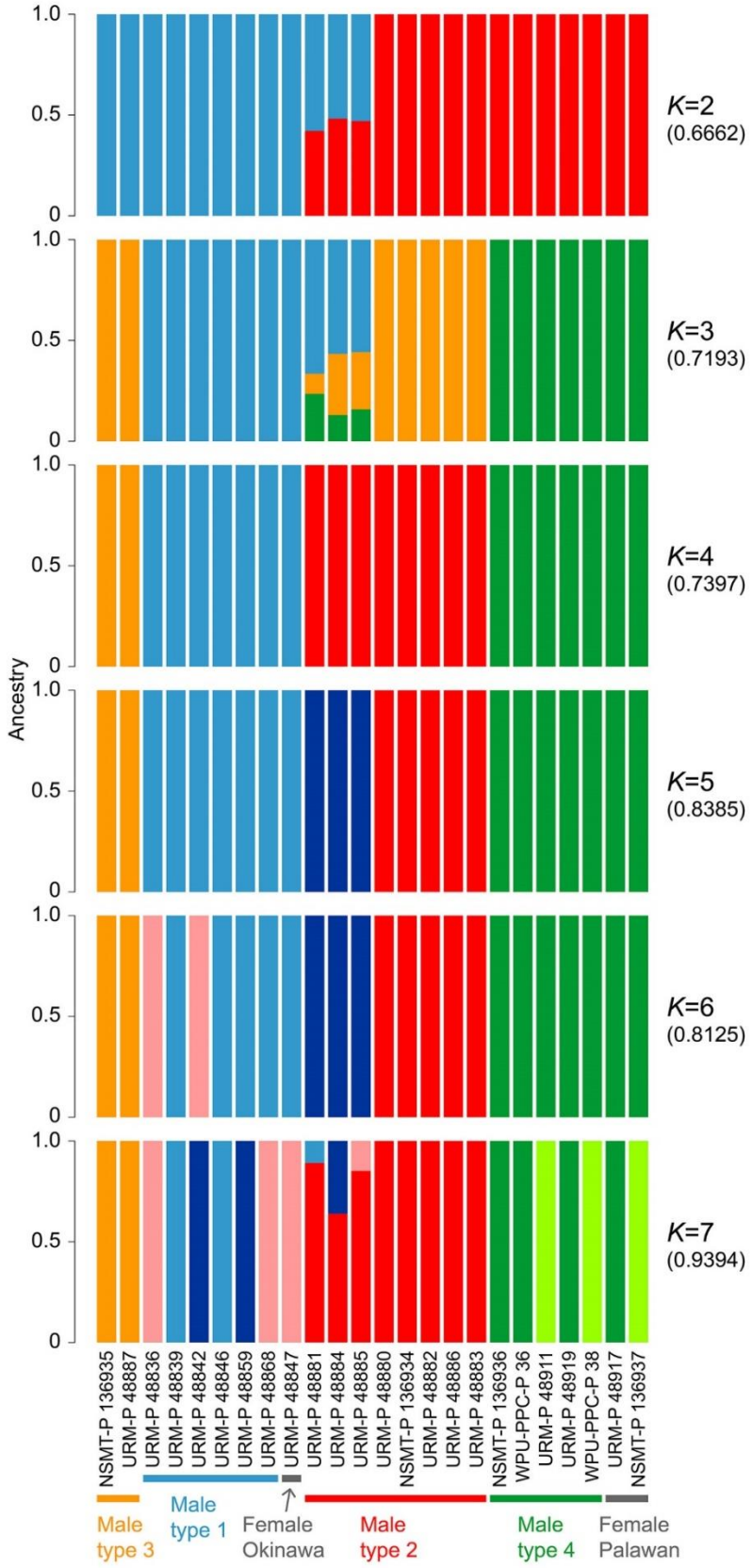

Fig. 7. ADMIXTURE results showing *K*=2–7 genetic clusters based on the 1,556 SNPs among four male types and females. The cross-validation errors are shown in brackets below the *K* values.

Fig. 8. *Lentipes armatus* from Okinawa Island immediately after fixation. (a) URM-P 48868, male, 24.4 mm SL; (b) URM-P 48860, male, 32.3 mm SL; (c) URM-P 48847, female, 32.9 mm SL (photo by K. Maeda).

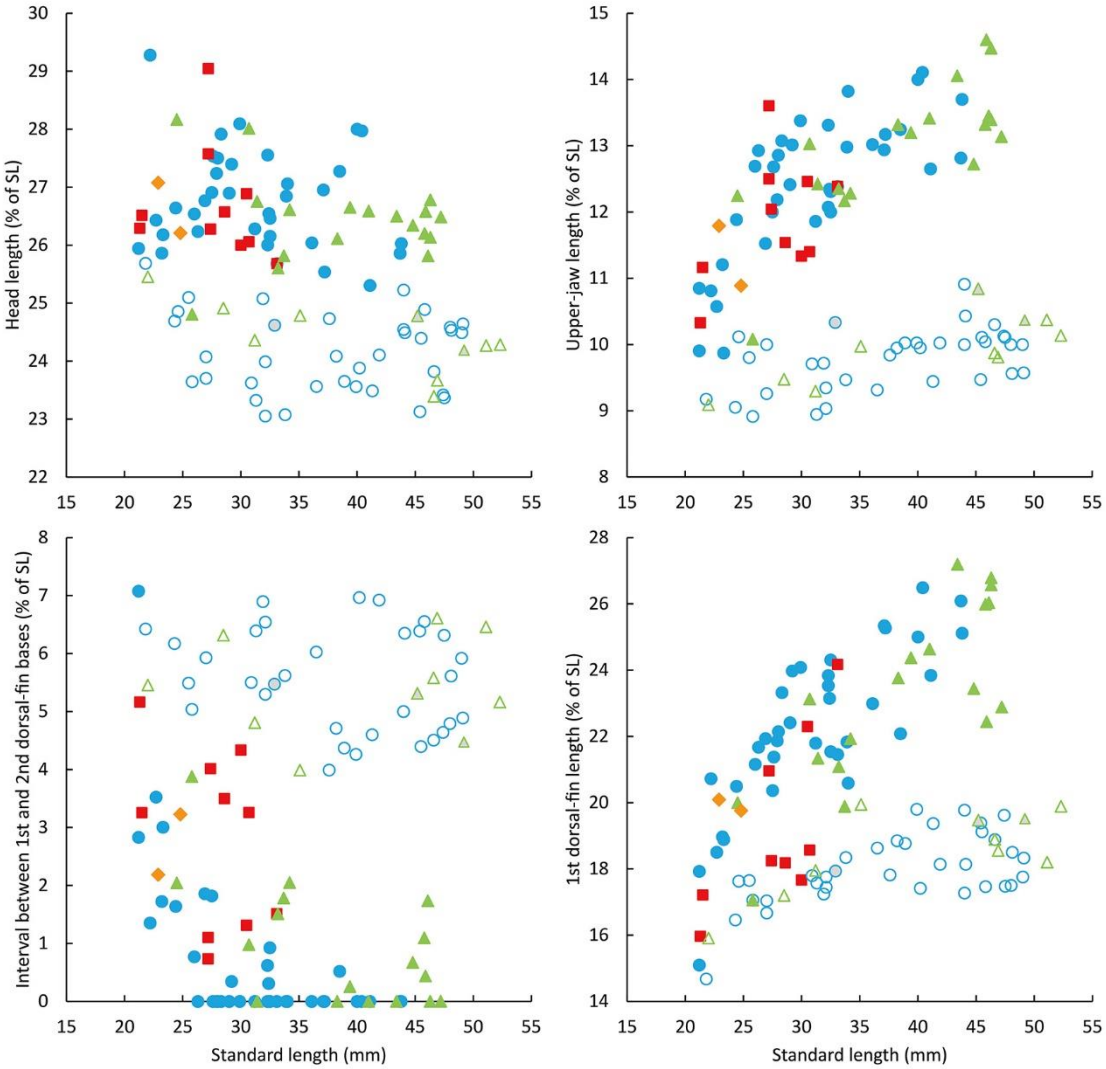

Fig. 9. Head length, upper-jaw length, interval between first and second dorsal-fin bases, and first dorsal-fin length of *Lentipes armatus* males (blue solid circles), *L. armatus* female (blue circles filled with grey), unidentified females from Japan (blue open circles), *L. kijimuna* males (red squares), *L. bunagaya* males (orange diamonds), *L. palawanirufus* males (green solid triangles), *L. palawanirufus* females (green triangles filled with grey), and unidentified females from Palawan (green open triangles).

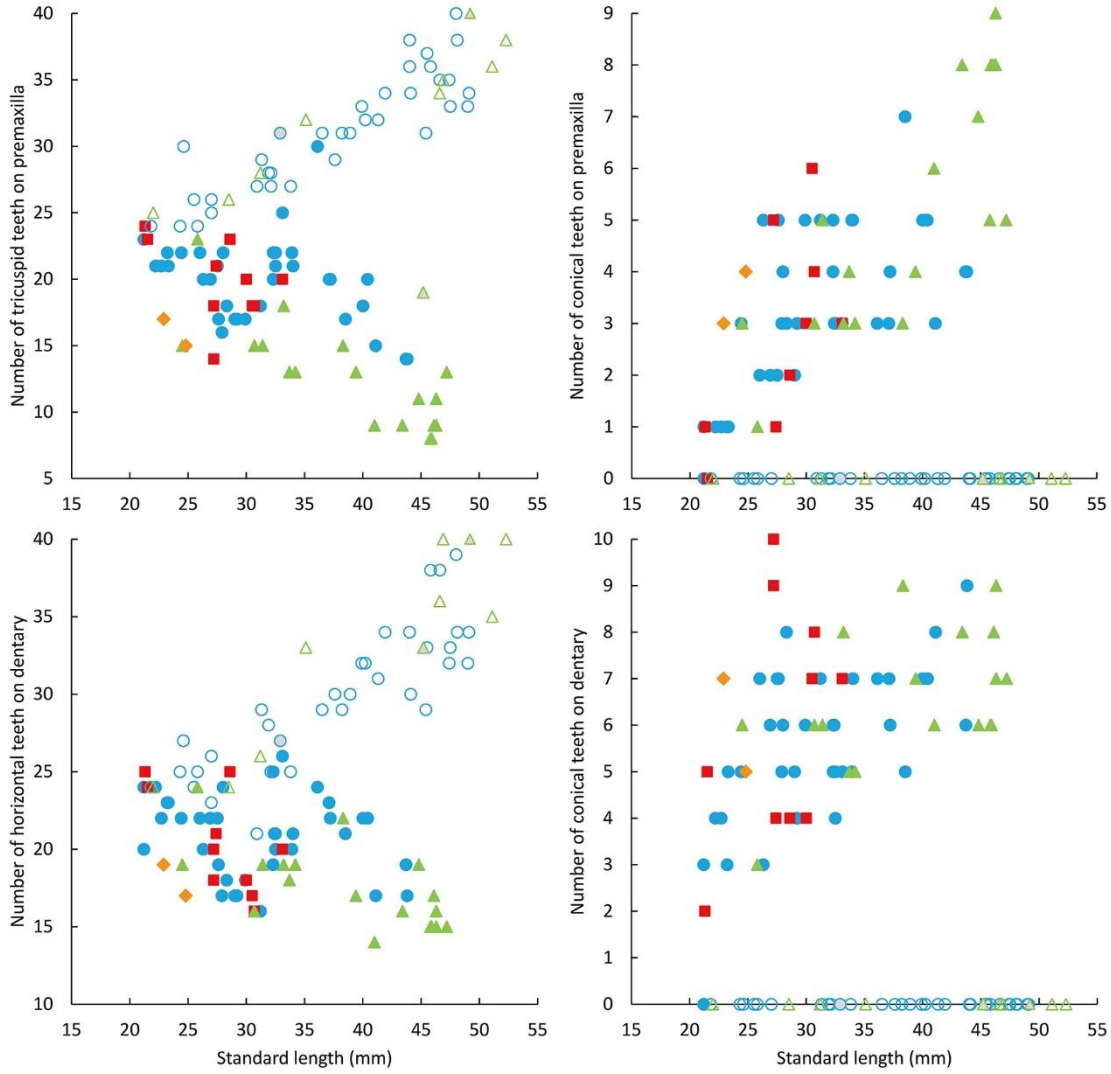

Fig. 10. Number of teeth on premaxilla and dentary of *Lentipes armatus* males (blue solid circles), *L. armatus* female (blue circles filled with grey), unidentified females from Japan (blue open circles), *L. kijimuna* males (red squares), *L. bunagaya* males (orange diamonds), *L. palawanirufus* males (green solid triangles), *L. palawanirufus* females (green triangles filled with grey), and unidentified females from Palawan (green open triangles).

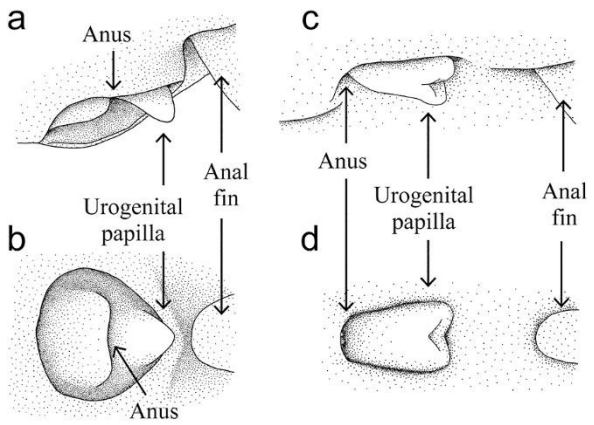

Fig. 11. Urogenital papilla of male (a,b; URM-P 48822) and female (c,d; URM-P 48847) of *Lentipes armatus.*

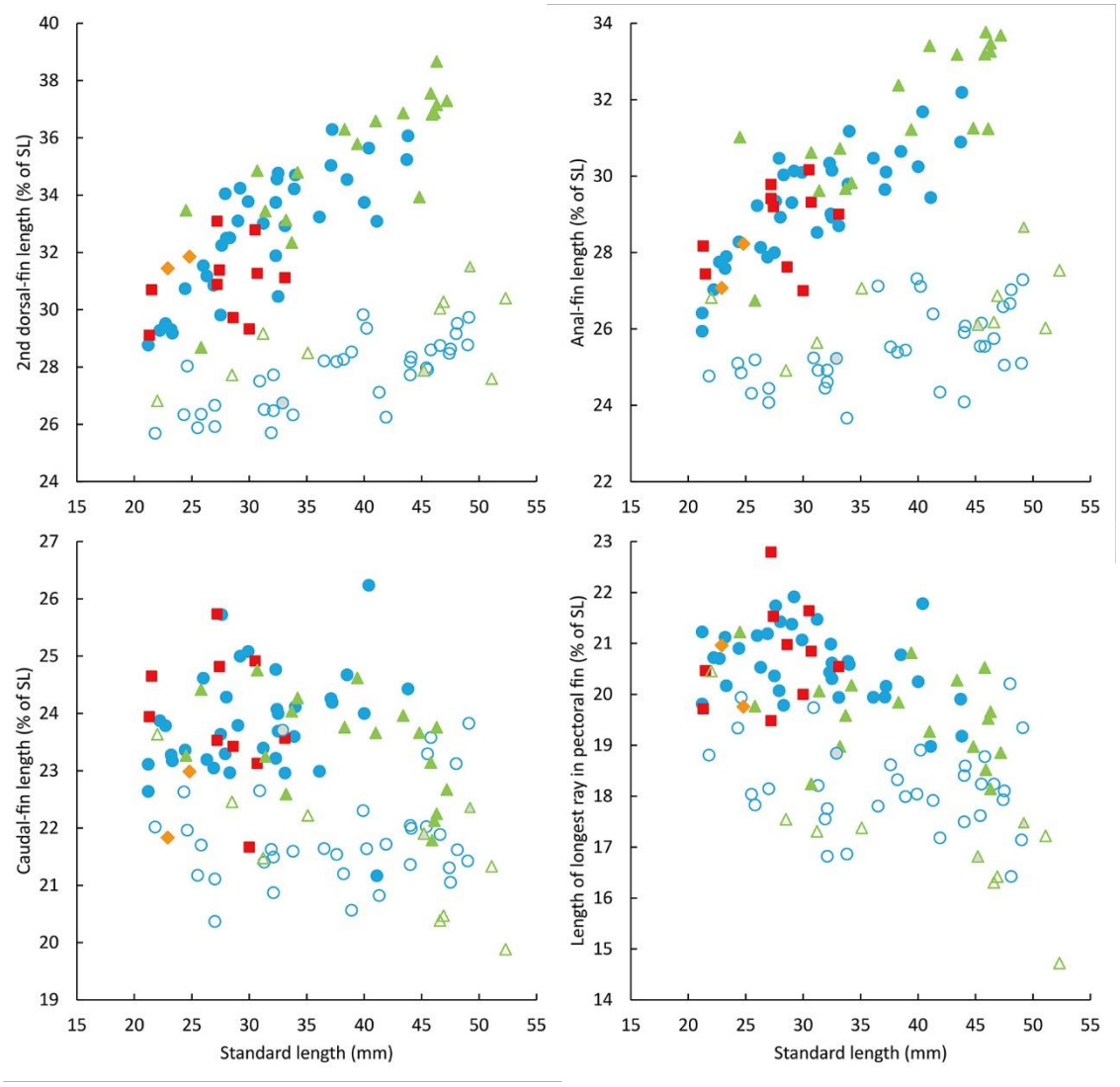

Fig. 12. Second dorsal-fin length, anal-fin length, caudal-fin length, and length of longest ray in pectoral fin of *Lentipes armatus* males (blue solid circles), *L. armatus* female (blue circles filled with grey), unidentified females from Japan (blue open circles), *L. kijimuna* males (red squares), *L. bunagaya* males (orange diamonds), *L. palawanirufus* males (green solid triangles), *L. palawanirufus* females (green triangles filled with grey), and unidentified females from Palawan (green open triangles).

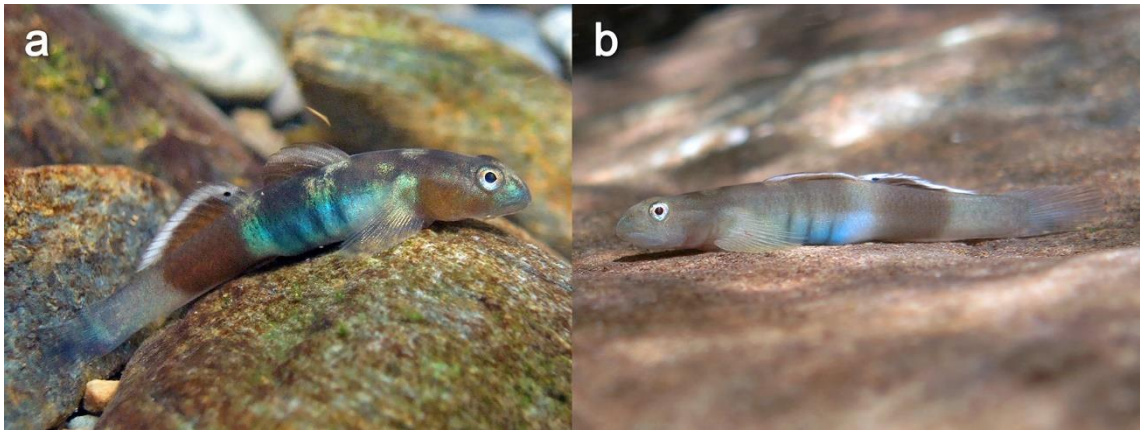

Fig. 13. In situ underwater photographs of *Lentipes armatus* on Okinawa Island, Japan. (a) male, 25 May 2007; (b) male, 19 June 2016 (photo by K. Maeda).

Fig. 14. *Lentipes kijimuna* sp. nov. immediately after fixation. (a) NSMT-P 136934, holotype, male, 30.5 mm SL; (b) URM-P 48880, paratype, male, 21.5 mm SL (photo by K. Maeda).

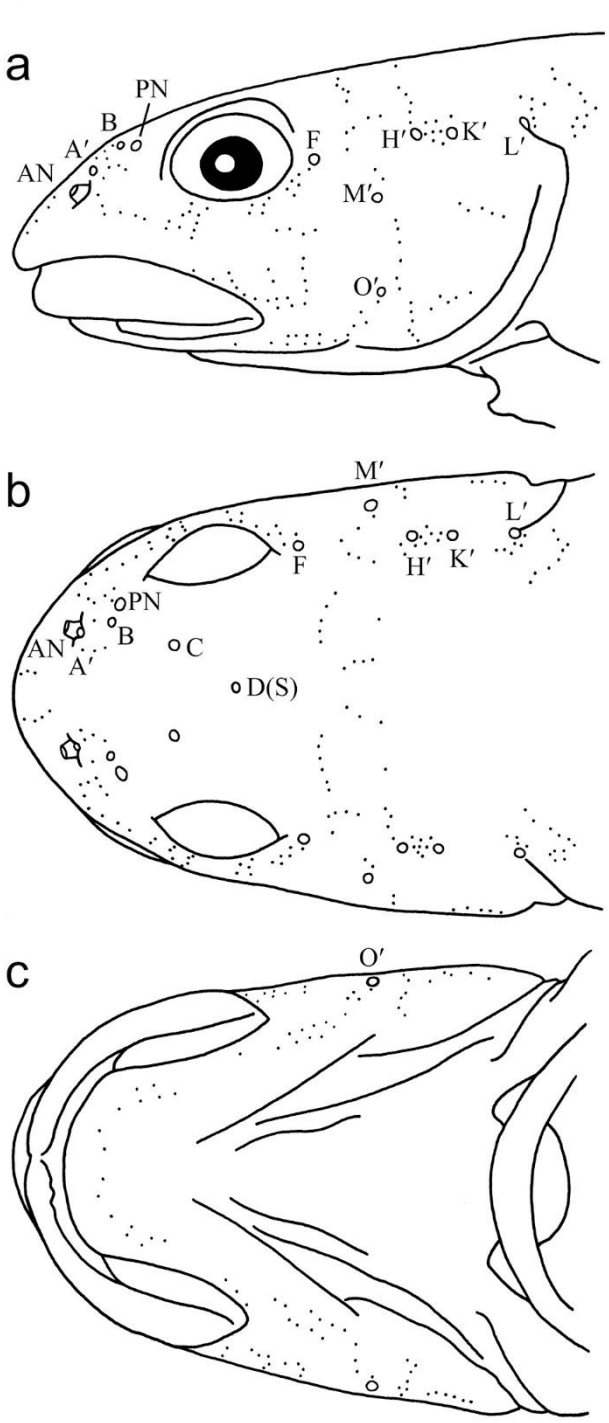

Fig. 15. Arrangement of cephalic sensory pores and cutaneous sensory papillae in *Lentipes kijimuna* sp. nov. (URM-P 48879). AN anterior naris, PN posterior naris.

Fig. 16. In situ underwater photographs of *Lentipes kijimuna* sp. nov. on Okinawa Island, Japan. (a) male, 17 Oct. 2014, (NSMT-P 136934, holotype); (b) male, 25 July 2010 (not collected) (photo by K. Maeda).

Fig. 17. *Lentipes bunagaya* sp. nov. immediately after fixation. (a) NSMT-P 136935, holotype, male, 22.9 mm SL; (b) URM-P 48887, paratype, male, 24.8 mm SL (photo by K. Maeda).

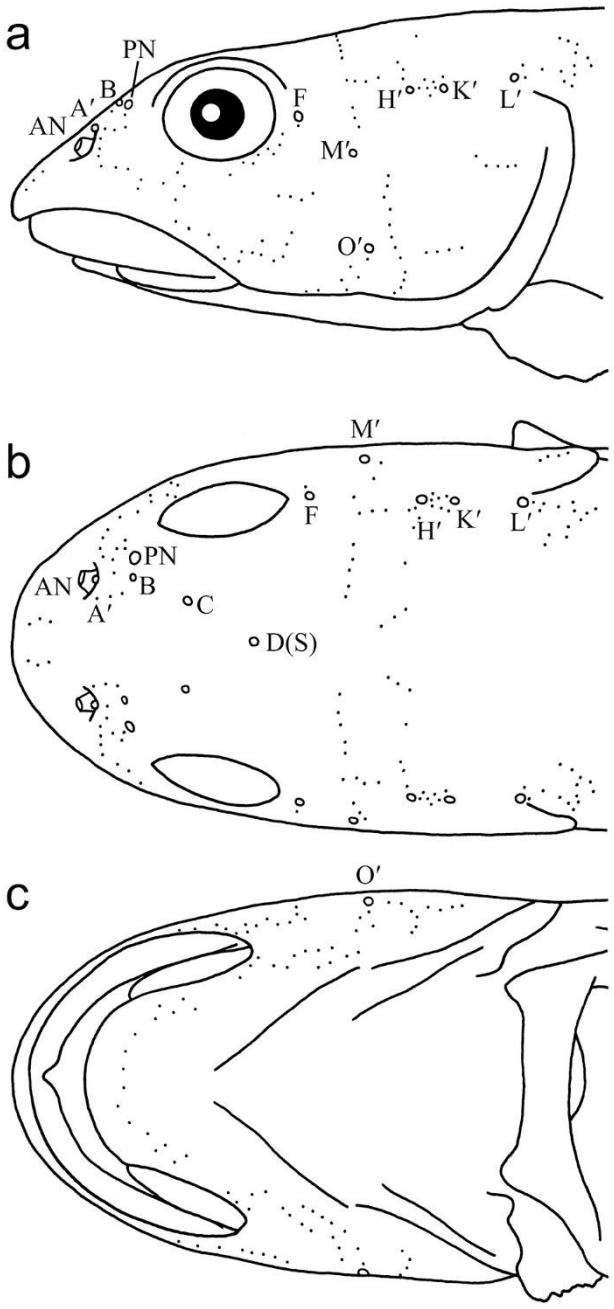

Fig. 18. Arrangement of cephalic sensory pores and cutaneous sensory papillae in *Lentipes bunagaya* sp. nov. (NSMT-P 136935). AN anterior naris, PN posterior naris.

Fig. 19. In situ underwater photographs of *Lentipes bunagaya* sp. nov. on Okinawa Island, Japan. (a) male, 6 Dec. 2012 (NSMT-P 136935, holotype); (b) male, 16 Dec. 2018 (URM-P 48887, paratype) (photo by K. Maeda).

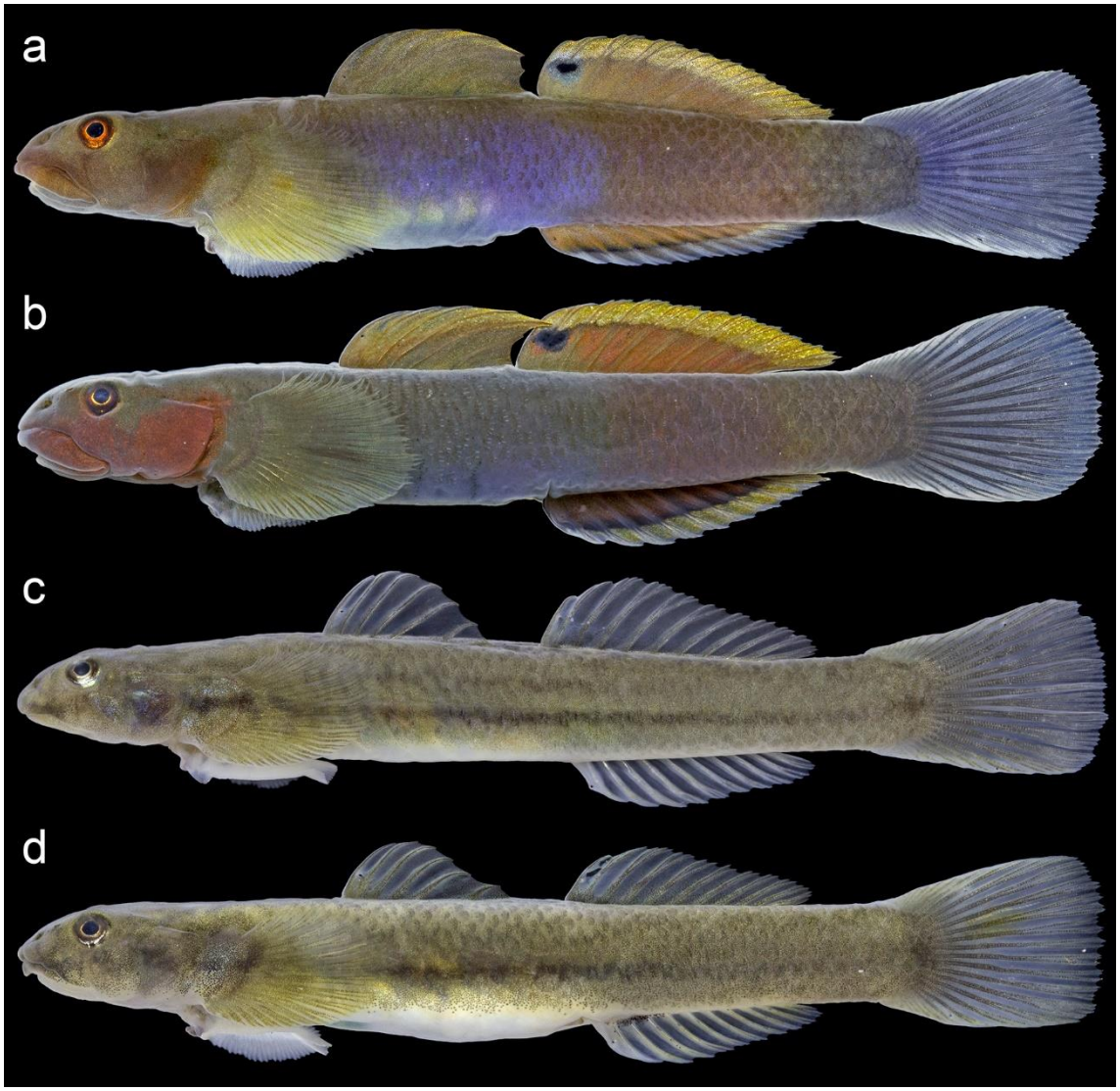

Fig. 20. *Lentipes palawanirufus* sp. nov. immediately after fixation. (a) NSMT-P 136936, holotype, male, 44.8 mm SL; (b) URM-P 48915, paratype, male, 45.8 mm SL; (c) NSMT-P 136937, paratype, female, 49.2 mm SL; (d) URM-P 48917, paratype, female, 45.2 mm SL (photo by K. Maeda).

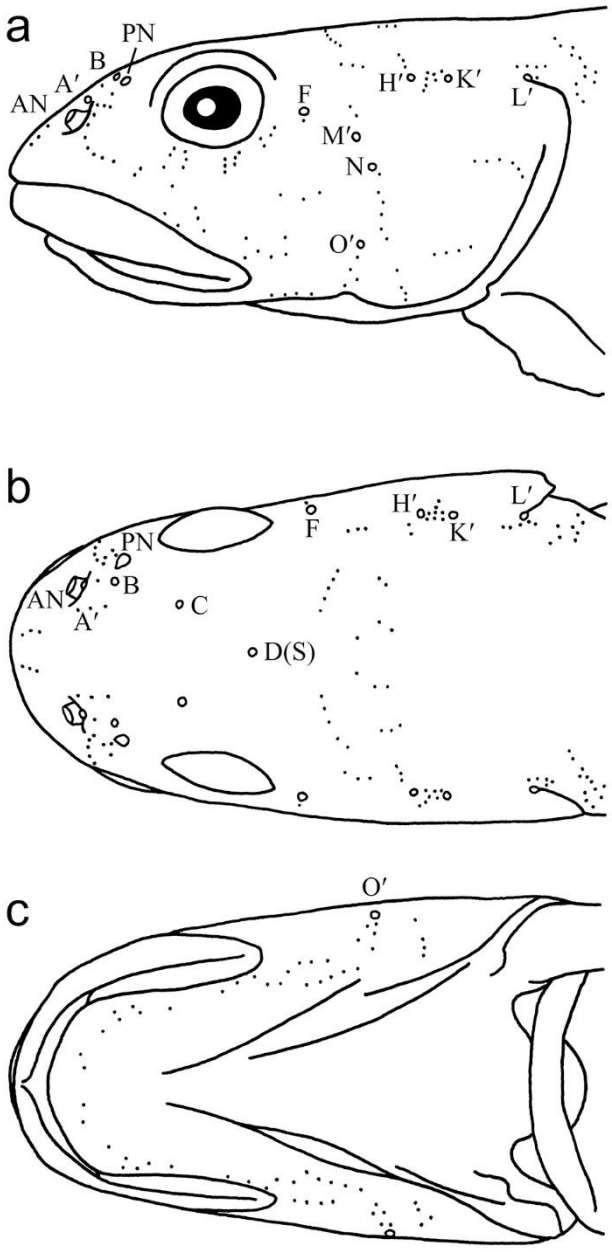

Fig. 21. Arrangement of cephalic sensory pores and cutaneous sensory papillae in *Lentipes palawanirufus* sp. nov. (URM-P 48919). AN anterior naris, PN posterior naris.

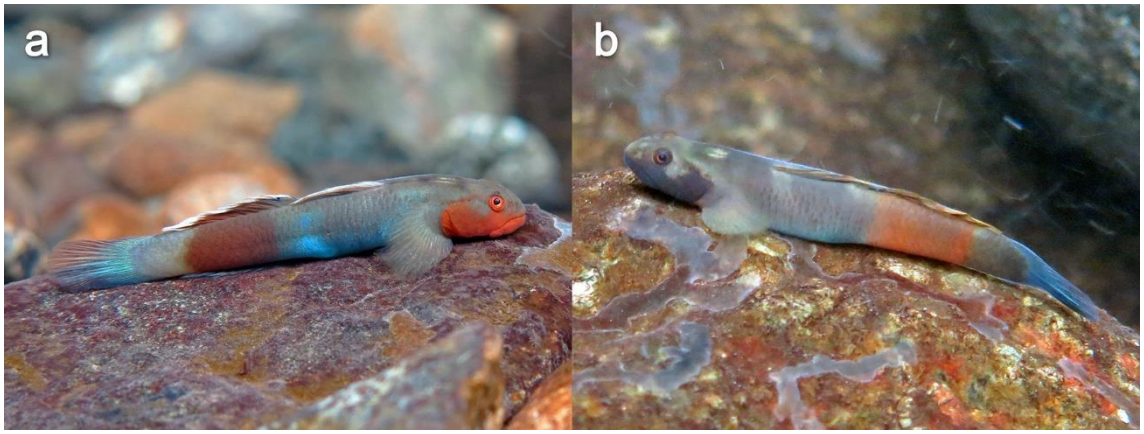

Fig. 22. In situ underwater photographs of *Lentipes palawanirufus* in Palawan, Philippines. (a) male, 28 May 2018; (b) male, 16 May 2015 (photo by K. Maeda).

Fig. 23. Display behaviour of *Lentipes* observed in streams on Okinawa Island (a–c) and a picture of live *L. bunagaya* (NSMT-P 136935, holotype) in ventral view (d). (a) and (b) a male of *L. armatus* to a female (19 Sep. 2015); (c) a male of *L. bunagaya* to a female (6 Oct. 2012) (photo by K. Maeda).

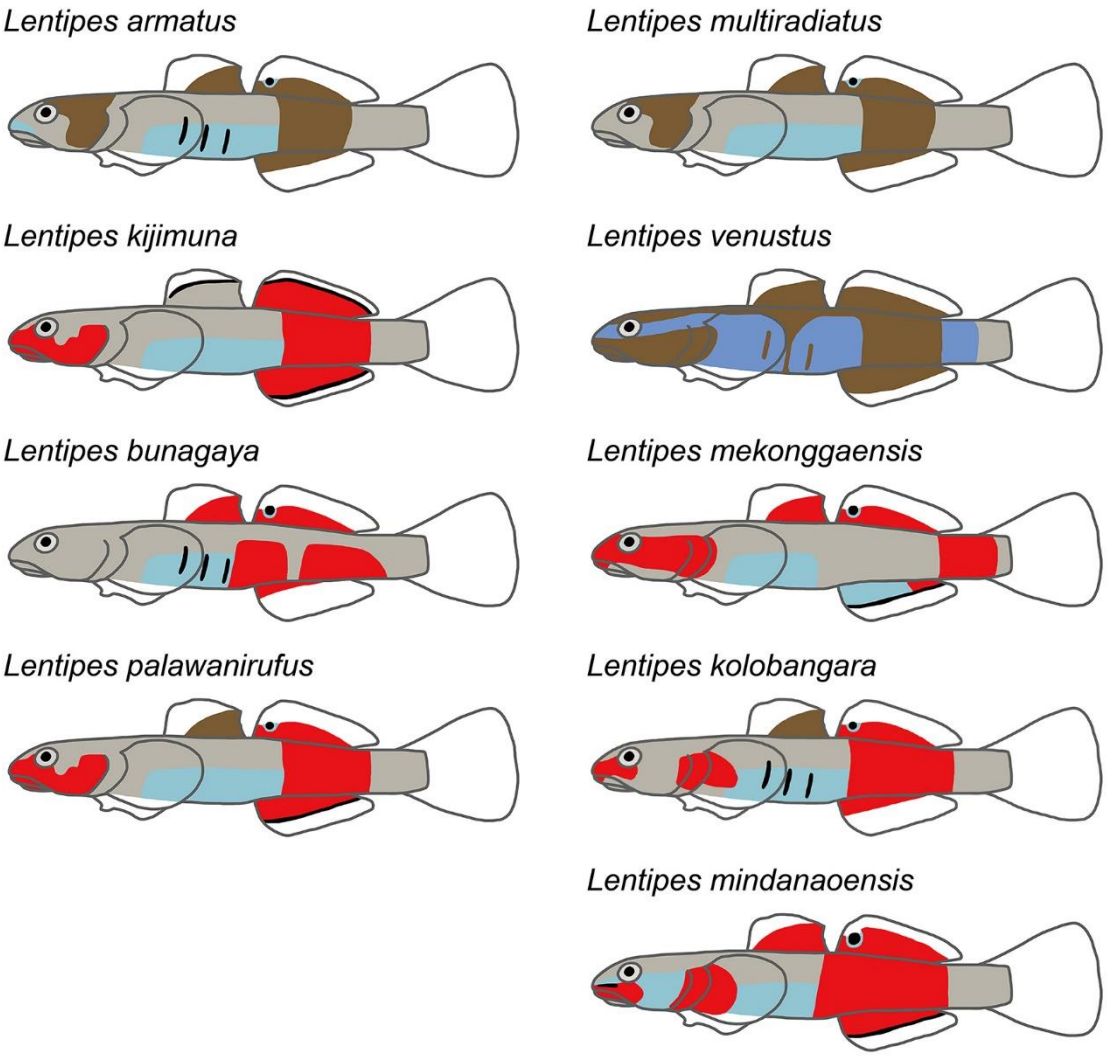

Fig. 24. Schematic illustrations of males in the genus *Lentipes* to show different colour patterns, especially the positions of reddish or brown markings on the body and the shape of black markings on the second dorsal fin.

Table 1. Number of pores in the preopercular canal of *Lentipes* investigated in the present study.

	2 on both sides	2 on left, 3 on right	3 on left, 2 on right	3 on both sides
Lentipes armatus	37	-	-	-
Lentipes kijimuna	5*(5)	-	-	-
Lentipes bunagaya	2*	-	-	-
Lentipes palawanirufus	11*	1	4	4
Unidentified females from Japan	31	2	1	-
Unidentified females from Palawan	4	1	2	1

* Including holotype. Non-type material is shown in brackets.

Table 2. Pectoral-fin ray counts of *Lentipes* investigated in the present study.

	16	17	18	19	20
Lentipes armatus	1	1	14	20	1
Lentipes kijimuna	-	-	2*(4)	3 (1)	-
Lentipes bunagaya	-	-	1*	1	-
Lentipes palawanirufus	1	1	13*	5	-
Unidentified females from Japan	-	1	21	10	2
Unidentified females from Palawan	-	1	7	-	-

* Including holotype. Non-type material is shown in brackets.

Table 3. Diagnostic morphological characters to distinguish *Lentipes armatus* and the three new species (*L. kijimuna*, *L. bunagaya*, and *L. palawanirufus*) from their congeners (excluding colour patterns).

L. armatus and three new species	No enlarged lobes or projections in front of the urogenital papilla in male	Scales present anterior half of body	Having scales with spike-like ctenii in male	Cephalic sensory pore D present	Preopercular sensory canal with 2 or 3 pores	4th and/or 5th spines longest in D1 of male	Interval between D1 and D2 bases <1/3 in length of D1 base in male	10 soft rays in D2	10 soft rays in A	18–19 rays in P1
L. concolor	-	Absent	No such scale	-	-	-	-	-	-	15–17
*R. andamanicus**	-	Absent	No such scale	-	-	-	-	9	12	13
L. rubrofasciatus	With two pairs of lobes	Absent	No such scale	-	-	-	-	-	-	15–17
L. whittenorum	With two pairs of lobes	-	-	-	-	-	-	-	-	-
L. watsoni	-	-	-	-	-	-	Interval 1/2 of D1 base	-	-	16–17
L. crittersius	-	Absent	-	-	-	-	-	-	-	-
L. dimetrodon	-	-	No such scale	Pore D absent	-	6th spine longest	-	-	-	15–16
L. multiradiatus	-	-	-	-	-	-	-	-	-	-
L. kaaea	With single pair of lobes	-	-	-	-	-	-	-	-	17
L. venustus	-	-	-	-	-	-	-	-	-	-
L. mindanaoensis	-	-	-	-	-	-	-	-	-	16–17
L. adelphizonus	With finger-like projections?	-	-	-	-	-	-	9–10	9–10	16–18
L. solomonensis	With single pair of lobes	-	-	-	-	-	-	9	9	16–17
L. caroline	-	Absent	No such scale	-	With no or 1 pore	-	-	-	-	16
L. mekonggaensis	-	-	-	-	-	-	-	-	-	-
L. argenteus	-	-	-	-	-	-	-	-	-	16–17
L. ikeae	-	-	-	-	-	-	-	9	9	16–17
L. kolobangara	-	-	-	-	-	-	-	-	-	-

Characters of the species indistinguishable from *L. armatus* and the three new species, and characters with unknown states are shown as blanks. D1 first dorsal fin; D2 second dorsal fin; A anal fin; P1 pectoral fin.

**Raogobius andamanicus*.