Description of a new cryptic, shallow-water tonguefish (Pleuronectiformes: Cynoglossidae: Symphurus) from the western North Pacific Ocean

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Combined results based on morphological characters and analyses of partial sequences of the 16s rRNA and col genes confirm the validity of a new, cryptic, symphurine tonguefish from the western North Pacific Ocean. Symphurus leucochilus n. sp., a diminutive species reaching sizes to c. 67 mm standard length, is described from nine specimens that were collected from fish-landing ports and from trawls made at c. 150 m off Taiwan and Japan. Symphurus leucochilus shares many similar features with those of Symphurus microrhynchus and that of several undescribed species that are morphologically similar to S. microrhynchus. Symphurus leucochilus has also been misidentified as Symphurus orientalis in fish collections because of shared similarities in some aspects of their morphology. The new species differs from all congeners by the following combination of meristic, morphological and pigmentation features: a predominant 1–2–2–2–2 pattern of interdigitation of proximal dorsal-fin pterygiophores and neural spines; 12 caudal-fin rays; 89–92 dorsal-fin rays; 49–51 total vertebrae; four hypurals; 75–83 longitudinal scale rows; 32–35 transverse scales; 15–17 scale rows on the head posterior to the lower orbit; absence of a fleshy ridge on the ocular-side lower jaw and a membranous connection between the anterior nostril and lower part of the eye; a narrow interorbital space and dorsal-fin origin anterior to the vertical through the anterior margin of the upper eye; absence of both dermal spots at bases of anterior dorsal-fin rays and melanophores on the isthmus; uniformly yellow to light-brown ocular-side colouration without bands; dorsal and anal fins with alternating series of dark rectangular blotches and unpigmented areas; a uniform white blind side and a bluish-black peritoneum. Despite overall similarities in morphology between S. leucochilus and S. orientalis, as well as between two of the nominal species morphologically similar to S. microrhynchus, analyses of partial 16s rRNA and col gene sequences show that S. leucochilus, S. orientalis and the two other nominal species represent three distinct lineages within the genus Symphurus.

Key words: cryptic species; DNA barcoding; Symphurus leucochilus; Symphurus microrhynchus; Symphurus orientalis.

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INTRODUCTION

Six nominal species of the Indo-West Pacific genus *Symphurus*, characterized by having 12 caudal-fin rays, are considered valid (Munroe, 1992; Munroe & Marsh, 1997; Lee et al., 2013). Most of these nominal species, which include *Symphurus orientalis* (Bleeker 1879), *Symphurus septemstriatus* (Alcock 1891), *Symphurus trifasciatus* (Alcock 1894), *Symphurus microrhynchus* (Weber 1913), *Symphurus luzonensis* Chabanaud 1955 and *Symphurus fallax* Chabanaud 1957, occur in relatively deep water, are rarely collected and are known only from restricted localities, usually in tropical seas. Of these six species, only *S. orientalis*, an inhabitant of the outer continental shelf and upper continental slope in the western North Pacific Ocean, occurs beyond subtropical waters (Chyung, 1961; Amaoka, 1982; Ochiai, 1984, 1987, 1988, 1989; Shen, 1984; Shen et al., 1993; Kim & Choi, 1994; Li & Wang, 1995; Yamada, 2000, 2002; Lee et al., 2013).

Recently, Lee et al. (2013) re-evaluated and re-described *S. orientalis* based on a large series of specimens collected from different parts of this species’ geographic range and they stabilized the species concept of *S. orientalis* by incorporating information from both morphological and molecular analyses. Those authors also called attention to the fact that many unresolved questions exist regarding the identity and status of other populations of nominal Indo-West Pacific species of *Symphurus* possessing 12 caudal-fin rays.

Lee et al. (2013) noted that previously reported (Okada & Matsubara, 1938; Matsubara, 1955; Ochiai, 1959, 1963) ranges of meristic and morphometric features for specimens purported to be *S. orientalis* probably included data for at least two other nominal species whose meristic features are lower than those of *S. orientalis*. Some of the specimens examined by Lee et al. (2013) and M.-Y. Lee & T. A. Munroe (unpubl. data) from the western North Pacific Ocean are similar in their meristic and morphological features to those reported for *S. microrhynchus* (Munroe & Marsh, 1997). Munroe & Marsh (1997), however, did not examine any specimens of *S. microrhynchus* from Japanese waters.

*Symphurus microrhynchus*, as understood by Munroe & Marsh (1997), is a small-sized (maximum size c. 51 mm standard length, $L_s$), shallow-water tonguefish with wide distribution in tropical waters of the Indo-Pacific region, especially those in the Indo-Australian archipelago. Diagnostic features of *S. microrhynchus* (Weber & de Beaufort, 1929; Munroe & Marsh, 1997) include darkly pigmented melanophores in the dermis at the bases of the anteriormost dorsal and anal-fin rays, pigmentation on the isthmus, presence of an obvious fleshy ridge on the posterior half of the ocular-side lower jaw and an anterior placement of the dorsal-fin origin.

Results of morphological and molecular analyses (M.-Y. Lee & T. A. Munroe, unpubl. data) indicate that specimens morphologically similar to *S. microrhynchus* vary significantly among different geographical locations. Results from the DNA sequences, in particular, indicate much higher genetic variation among these populations than would be expected for intraspecific populations (Ward et al., 2005, 2008, 2009; Benziger et al., 2011). These specimens, tentatively identified as *S. microrhynchus*, actually represent a composite mix of several morphologically similar species. The taxonomic status of these nominal species is unresolved.

Unexpectedly, while examining specimens of *S. orientalis* and those tentatively identified as *S. microrhynchus*, a small number of specimens were discovered that appeared
different from any of these other species. Meristic features of these unusual specimens that were captured at several locations in the western North Pacific Ocean overlap nearly completely in the ranges of those found in specimens tentatively identified as *S. microrhynchus*. Both their meristic features and colouration are similar to those of some of the specimens of *S. orientalis* that have been examined (unpubl. obs.). Further analyses of these unusual specimens indicated that they represent an undescribed species featuring subtle morphological differences from those observed in *S. microrhynchus*. They also have slight morphological, but distinct genetic, differences from those of specimens tentatively identified as *S. microrhynchus* and from those of *S. orientalis*. The objective of this study was to provide a formal description of this new species of *Symphurus* based on both morphological and molecular data, and to diagnose it from congener.

**MATERIALS AND METHODS**

A total of nine specimens identified as *Symphurus leucochilus* n. sp., including both preserved specimens in fish collections as well as fresh specimens collected in the field, constitute the basis for data collected in this study. Tissue samples from specimens in field collections were used for DNA analyses. Voucher specimens for all tissue samples analysed were preserved subsequently in 95% ethanol, catalogued and deposited in fish collections. Institutional abbreviations follow those listed by Fricke & Eschmeyer (2014). Comparative materials for all other Indo-Pacific species of *Symphurus* included in this study are listed in the previous studies by Munroe (1992, 2006), Shen *et al.* (1993), Munroe & Amaoka (1998), Krabbenhoft & Munroe (2003), Munroe & Hashimoto (2008), Lee *et al.* (2009a, 2009b, 2013) and Munroe *et al.* (2011).

Methods for counting meristic characters and for measuring morphometric features and general terminology follow those in the study by Munroe (1998). All specimens examined were radiographed. Terminology and formulae for interdigitation patterns of proximal dorsal pterygiophores and vertebral neural spines (ID pattern) follow those in the study by Munroe (1992). Morphometric characters were measured to the nearest 0.01 mm using either dial callipers or a dissecting microscope fitted with an ocular micrometre. Morphometric features are expressed either as proportions in per cent $L_S$ or per cent head length ($L_{H}$).

Description of pigmentation features are based primarily on freshly landed specimens, with supplemental information provided from specimens preserved in formalin and transferred to 75% ethanol. Maturity was estimated by macroscopic examination of the extent of posterior elongation of ovaries and presence of developing ova in the ovaries (both observed by using light transmitted through the body). In species of *Symphurus*, no obvious differences are apparent in testis size between mature and immature males; therefore, estimates of maturity are based entirely on females.

Tissue samples from a total of 13 specimens, including six of *S. cf. microrhynchus* (identification based on Munroe & Marsh, 1997) and seven of *S. leucochilus*, were amplified for both partial 16s RNA and coI gene sequences. To examine genetic divergence in nominal species, some researchers (Hebert *et al.*, 2003; Ward *et al.*, 2005) suggest using sequences of the 5′ region of the mitochondrial *cytochrome c oxidase subunit I* gene (coI or coxI) for species identification, whereas others recommend using 16s rRNA as a good model for assisting taxonomic works (Akimoto *et al.*, 2002; Maretto *et al.*, 2007; Lakra *et al.*, 2009). Both genes were examined in this study for identifying species of *Symphurus* (Lee *et al.*, 2013). Total genomic DNA was extracted from seven individuals of the new species and six individuals of *S. cf. microrhynchus* (from different localities around Taiwan). DNA was extracted using the Genomic DNA Mini Kit (Geneaid; www.geneaid.com). Sequences were amplified using the following primer pair 16sa-L (5′ CGCTGTTTACCAAAACATCGCCT 3′) and 16sb-H (5′ CCGGTCCTGAATCAGTCACT 3′) (Palumbi, 1996) for the 16s rRNA gene. The 16s RNA sequences of the individuals of each species were aligned to yield a final alignment varying from 505 bp (*S. cf. microrhynchus*), to 508 bp (*S. leucochilus*), to 510 bp.
The primer pair *Symphurus-coIf* (‘GGTGCGCCTGAGCHGGRATAATTGGHAC’
3’) and *Symphurus-coIr* (‘TAAATTTITGKGGCCAAAGAATCA’ 3’) was used for the
*coI* gene (Lee et al., 2013). This pair of primers was designed by aligning the universal primer
(Ward et al., 2005) with *coI* sequences of Cynoglossidae, especially species of *Symphurus*,
from GenBank, to focus on amplifying the *coI* sequences from *Symphurus*. The *coI* sequences
of individuals of each species were aligned to yield a final alignment of 639 bp. A polymerase
chain reaction (PCR) was carried out using a thermal cycler (BIO-RAD; www.bio-rad.com)
in 25 μl reaction volumes containing 100 ng of total DNA, 1 μM of each primer, 0-04 mM
of deoxynucleotide triphosphate (dNTP), 1x reaction buffer and 0-05 U of Taq polymerase
( Genomics; www.genomics.sinica.edu.tw.) with denaturation at 94° C for 4 min; this was
followed by 35 cycles of denaturing at 94° C for 30 s, annealing at 48° C for 45 s and extension
at 72° C for 1 min, with a final extension at 72° C for 10 min. The PCR products were
then sequenced bidirectionally and analysed on an ABI3730XL model (Applied Biosystems;
www.appliedbiosystems.com). All sequences were checked against electropherograms and
manually edited using the programme 4Peaks 1.7 (www.mekentosj.com/science/4peaks). In
order to confirm the absence of stop codons in the amplified *coI*, the nucleotide sequences
were translated according to the vertebrate mitochondrial genetic code using EMBOSS-transeq
(EMBL-EBI; www.ebi.ac.uk/Tools/st/emboss_transeq).

All sequences were aligned using ClustalW (Thompson et al., 1994) in MEGA 4.0 (Tamura
et al., 2007), with default settings chosen for parameters. Nucleotide genetic distances, both
the Kimura two-parameter distance (K2P) (Kimura, 1980) and *p*-distance substitution model
including transitions and transversions, complete deletion of gaps and missing data, uniform
rates among sites, and between and within species comparisons, were also calculated by
using MEGA 4.0 (Tamura et al., 2007). Trees based on sequence data were constructed by
the neighbour-joining (NJ) method, K2P substitution model, and evaluated by 10 000 boot-
strapping replications (Felsenstein, 1985) using MEGA 4.0 (Tamura et al., 2007). They were
constructed only to show divergence in genetic sequences among the samples analysed. The
NJ method was used instead of maximum likelihood or maximum parsimony methods because
the approach in this study focused on species identifications, with both *coI* (DNA barcoding)
and 16s rRNA applied for this purpose. Sequences were deposited in GenBank under the
accession numbers KC900860–KC900885 and KF676778–KF676783, and sequences of three
specimens of *S. orientalis* reported in the study by Lee et al. (2013) were also included in the
NJ analysis. Sequences of *S. orientalis* are JN678742, JN678752 and JN678763 for 16s rRNA,
and JN678777, JN678787 and JN678798 for *coI*.

**SYMPHURUS LEUCOCHILUS** N. SP. (**FIGS 1 – 5 AND TABLES I – IV**)

**HOLOTYPE**

USNM 408271: mature female, 58-7 mm *L*<sub>S</sub>, Da-Shi fish market, north-eastern Tai-
wan, 24 August 2011.

**PARATYPES ( *N* = 7; THREE MALES AND FOUR FEMALES)**

ASIZP 72343: adult male, 54-8 mm *L*<sub>S</sub>, Dong-Gang fish market, south-western Tai-
wan, 15 June 2009; ASIZP 72355: immature female, 31-0 mm *L*<sub>S</sub>, Tosa Bay, off Kochi,
Japan, 150 m, 17 June 2009; ASIZP 72357: immature female, 32-9 mm *L*<sub>S</sub>, Tosa Bay,
off Kochi, Japan, 150 m, 17 June 2009; ASIZP 72369: mature female, 55-9 mm *L*<sub>S</sub>,
Dong-Gang fish market, south-western Taiwan, 3 October 2007; NMMB–P 17767:
adult male, 66-9 mm *L*<sub>S</sub>, Da-Shi fish market, north-eastern Taiwan, 24 August 2011;
USNM 408272: mature female, 57-5 mm *L*<sub>S</sub>, Da-Shi fish market, north-eastern Taiwan,
24 August 2011; and USNM 408273: adult male, 47-4 mm *L*<sub>S</sub>, Da-Shi fish market,
north-eastern Taiwan, 24 August 2011.
**SYMPHURUS LEUCOCHILUS, NEW TONGUEFISH SPECIES**

**Fig. 1.** *Symphurus leucochilus* n. sp., holotype USNM 408271, mature female, 58.7 mm standard length, north-east Taiwan: (a) ocular-side pigmentation of freshly caught specimen and (b) blind-side colouration of same specimen.

**NON-TYPE SPECIMEN**

ASIZP 72356: juvenile (unknown sex), 21.7 mm *L*₅, Tosa Bay, off Kochi, Japan, 150 m, 17 June 2009.

**DIAGNOSIS**

*Symphurus leucochilus* is distinguished from all congeners by the following combination of characters: 1–2–2–2 ID pattern; 12 caudal-fin rays; nine abdominal and 49–51 total vertebrae; four hypurals; 89–92 dorsal-fin rays; 76–80 anal-fin rays; 75–83 longitudinal scale rows; 32–35 transverse scales; 15–17 scale rows on the head posterior to the lower orbit; uniformly yellow to light-brown ocular side; uniform white blind side; bluish-black peritoneum; entire lengths of dorsal and anal fins with alternating series of dark rectangular blotches (extending from base to tip of fin) and unpigmented areas; no dermal spots at bases of anterior dorsal-fin rays; no pigmented spots on the isthmus; no fleshy ridge on ocular-side lower jaw; upper and lower eyes separated from each other by a narrow interorbital space; lower eye without a membranous connection to anterior nostril; and dorsal-fin origin at the vertical through anterior margin of the upper eye.
Fig. 2. Geographic distribution of *Symphurus leucochilus* n. sp. based on specimens examined in this study. ★, capture location of holotype; ■, capture location of other specimens. The numbers indicate the number of specimens collected from locations.

**DESCRIPTION**


Proportions of morphometric features are presented in Table II. Body relatively deep; maximum depth in anterior one-third of body usually at point between anus and eighth anal-fin ray and body depth tapering rapidly posterior to midpoint. Preanal length usually smaller than body depth. Head moderately long and wide; head width ($W_H$) slightly shorter than body depth, and much greater than head length ($W_H$: $L_H = 1.07–1.29$, $\bar{X} = 1.20$). Upper head lobe wider than lower head lobe ($L_{UH}/L_{LH} = 1.05–1.37$, $\bar{X} = 1.22$); slightly shorter than postorbital length. Upper lobe of ocular-side opercle wider than lower opercular lobe; posterior margin of lower lobe projecting slightly
Fig. 3. Kimura two-parameter (K2P) and neighbour-joining tree of 19 sequences from the genus *Symphurus* [TW, *Symphurus cf. microrhynchus* (TW); VN, *S. cf. microrhynchus* (VN)]: (a) 16s rRNA sequences and (b) *col* sequences. Number at nodes indicates bootstrap values for 10,000 replications and the scale bar represents the K2P distance.
Fig. 4. Comparison of the ocular side of the head of two species of Indo-West Pacific Symphurus: (a) Symphurus cf. microrhynchus (TW), ASIZP 72370, adult male, 57.0 mm standard length ($L_S$), north-east Taiwan and (b) Symphurus leucochilus n. sp., holotype USNM 408271, mature female, 58.7 mm $L_S$, north-east Taiwan.

beyond posterior margin of upper opercular lobe or both lobes equal with posterior margins reaching similar points along the same vertical plane. Snout moderately long, slightly round to obliquely blunt anteriorly, its length ($L_{SN}$) much greater than eye diameter ($D_E$) ($L_{SN}:D_E = 1.47–2.09$, $\bar{X} = 1.83$). Dermal papillae present, but not well developed, on blind side of snout. Ocular-side anterior nostril tubular and short, usually not reaching anterior margin of lower eye when depressed posteriorly. Ocular-side posterior nostril a small, rounded tube located on snout just anterior to interorbital space. Blind-side anterior nostril tubular and short, easily distinguishable from dermal papillae; blind-side posterior nostril a shorter, and wider, posteriorly directed tube situated posterior to vertical at posterior margin of jaws. Jaws long and slightly arched; upper jaw length equal to, or slightly longer than, snout length; posterior margin of upper jaw usually extending to point between verticals through anterior margin of eye and anterior margin of pupil of lower eye. Ocular-side lower jaw without fleshy ridge. Cheek depth shorter than snout length. Eyes moderately large and oval, separated by two to three rows of small ctenoid scales in narrow interorbital space. Eyes usually equal in position, or with anterior margin of upper eye slightly in advance of that of lower eye. Pupillary operculum absent. Dorsal-fin origin located at point between verticals through anterior margin of upper eye and anterior margin of pupil of upper eye; predorsal length moderately short. Anteriormost dorsal-fin rays slightly shorter than more posterior fin rays. Scales absent on both sides of dorsal and anal-fin rays. Pelvic fin moderately long; longest pelvic-fin ray, when extended posteriorly, usually reaching base of first to fourth anal-fin ray. Posteriormost pelvic-fin ray connected to
**SYMPHURUS LEUCOCHILUS**, NEW TONGUEFISH SPECIES

Fig. 5. Lateral views comparing the bases of the anteriormost part of the dorsal fin and isthmus in two species of Indo-West Pacific *Symphurus*: (a, b) *Symphurus cf. microrhynchus* (TW), ASIZP 67658, adult male, 53.3 mm standard length (*L*<sub>S</sub>), north-east Taiwan and (c, d) *Symphurus leucochilus* n. sp. holotype USNM 408271, mature female, 58.7 mm *L*<sub>S</sub>, north-east Taiwan.

anal fin by delicate membrane. Caudal fin relatively long, with several rows of ctenoid scales on base of fin. Both sides of body with numerous, strongly ctenoid scales.

Teeth present and recurved slightly inwards on all jaws; better developed on blind-side jaws. Ocular-side premaxilla and dentary with single row of sharply pointed, well-developed teeth. Blind-side premaxilla with three to four rows of sharp, recurved teeth. Blind-side lower jaw with four to six rows of well-developed teeth.

**PIGMENTATION (FIG. 1).**

Ocular-side background colouration of body, head and most of external surface of opercle generally light-yellow to light-brown, sometimes also with irregular, darkly shaded areas. External surface overlying abdominal area usually darker bluish-black due to dark peritoneal pigment visible through abdominal wall. Posterior margin of opercle darker brown than more lightly pigmented anterior regions. Outer surface of ocular-side isthmus without conspicuous melanophores. Inner surface of ocular-side opercle and isthmus unpigmented. Ocular-side lips and chin region light-yellow to light-brown; margins of lips with numerous small black dots. Ocular-side anterior nostril light-yellow to brown. Upper aspects of eyes and eye sockets light blue to bluish-green; pupils bluish-black. No conspicuous melanophores on head region posterior to eyes. No conspicuous dermal spots or melanophores at bases of anterior dorsal-fin rays. Blind side generally white to light yellow with darkly pigmented
Table I. Frequency of meristic characters of *Symphurus leucochilus*. Counts for the holotype (USNM 408271) are indicated (*). Sample size nine fish except where indicated, +, when sample size was eight fish.

<table>
<thead>
<tr>
<th>Meristic character</th>
<th>$n$</th>
<th>Meristic character</th>
<th>$n$</th>
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<tbody>
<tr>
<td>ID pattern</td>
<td></td>
<td>Longitudinal-scale count</td>
<td></td>
</tr>
<tr>
<td>$1{\text{--}}2{\text{--}}2{\text{--}}2{\text{--}}2^*$</td>
<td>8</td>
<td>12*</td>
<td>9</td>
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<tr>
<td>$1{\text{--}}2{\text{--}}1{\text{--}}2{\text{--}}2$</td>
<td>1</td>
<td>75</td>
<td>1</td>
</tr>
<tr>
<td>Longitudinal-scale count</td>
<td></td>
<td>76*</td>
<td>1</td>
</tr>
<tr>
<td>75</td>
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<td>76*</td>
<td>1</td>
</tr>
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<td>77</td>
<td>2</td>
<td>77</td>
<td>1</td>
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<td>1</td>
<td>78</td>
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<td>80</td>
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<td>80</td>
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</tr>
<tr>
<td>81</td>
<td>–</td>
<td>81</td>
<td>1</td>
</tr>
<tr>
<td>Head-scale count</td>
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<td>82</td>
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</tr>
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<td>15</td>
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<td>83</td>
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</tr>
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<td>16</td>
<td>3</td>
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<td>17*</td>
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<td>15</td>
<td>2</td>
</tr>
<tr>
<td>16</td>
<td>3</td>
<td>16</td>
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</tr>
<tr>
<td>17*</td>
<td>4</td>
<td>17*</td>
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<td>Lateral-scale count</td>
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<td>9</td>
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<td>3</td>
</tr>
<tr>
<td>35*</td>
<td>1</td>
<td>35*</td>
<td>2</td>
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bluish-black region overlying peritoneum. Background colouration of outer surface of blind-side opercle white to light yellowish; inner surface unpigmented. Small specimens with a streak of black colouration on vertebrae distinctly visible externally through body musculature.

Colouration of alcohol-preserved specimens similar to that of freshly caught fish, except bluish-black colouration of pupil of freshly caught specimens blending more gradually and eventually fading into white background colouration. Fin rays of dorsal, anal and pelvic fins uniformly yellow to brown; basal regions of fin rays and membranes covering fin rays light yellow, with diffuse scattering of yellow to brown melanophores covering entire fin membranes on both sides of fins. Entire dorsal and anal fin with a series of dark streaks alternating with lighter areas on fins. Basal margins of blind sides of dorsal and anal-fin rays and associated fin membranes light yellow to light brown.

SIZE AND SEXUAL MATURITY

Nine specimens range in size from 21.7 to 66.9 mm $L_S$. Two fish (31.0 and 32.9 mm $L_S$) are immature juvenile females with little elongation of the ovaries. Three other specimens (57.5–55.9 mm $L_S$) are non-gravid, mature females with elongated ovaries. There were no gravid females of this species in the collections studied. Males ($n = 3$, 47.4–66.9 mm $L_S$) attain similar sizes to those of mature females. The sex of the smallest juvenile (21.7 mm $L_S$) could not be determined macroscopically.

11. Prising thelandings at both fish ports where fish species that generally live at about 100–200 m. The assemblages of fishes containing this species were collected also contained an assemblage of fishes at Da-Shi fish port, north-eastern Taiwan, and Dong-gang fish port, south-western Taiwan, and in the South China Sea off Dong-Gang, Taiwan (Fig. 2).

The only documented depth record for *Symphurus leucochilus* is the capture of three specimens at 150 m off Tosa Bay, Japan. Other specimens examined were retrieved from fish landings at Da-Shi fish port, north-eastern Taiwan, and Dong-gang fish port, south-western Taiwan. Landings in which these species were collected also contained an assemblage of fish species that generally live at or about 100–200 m. The assemblages of fishes comprising the landings at both fish ports where *Symphurus leucochilus* were found suggest that the depth range inhabited by this species is on the outer continental shelf or maybe even on the upper continental slope. More accurate information on exact localities, bathymetric distribution and substrata, where this species is found, is needed to understand the ecology of this diminutive species of tonguefish.

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**Table II.** Morphometrics for the holotype (USNM 408271), examined specimens of *Symphurus leucochilus* and an atypical juvenile specimen (ASIZP 72356). Characters 2–15 in % of standard length (LS); 16–23 in % of head length (LH).

<table>
<thead>
<tr>
<th>Characters</th>
<th>Holotype</th>
<th>n</th>
<th>Range</th>
<th>Mean ± s.d.</th>
<th>ASIZP 72356</th>
</tr>
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<tbody>
<tr>
<td>1. LS (mm)</td>
<td>58.7</td>
<td>8</td>
<td>31.0–66.9</td>
<td>50.64 ± 12.71</td>
<td>21.7</td>
</tr>
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<td>2. Body depth</td>
<td>32.3</td>
<td>8</td>
<td>26.3–32.3</td>
<td>28.76 ± 1.86</td>
<td>22.7</td>
</tr>
<tr>
<td>3. Trunk length</td>
<td>82.2</td>
<td>8</td>
<td>79.4–83.0</td>
<td>81.45 ± 1.14</td>
<td>81.5</td>
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<tr>
<td>4. Predorsal length</td>
<td>4.6</td>
<td>8</td>
<td>3.4–4.6</td>
<td>3.97 ± 0.41</td>
<td>6.4</td>
</tr>
<tr>
<td>5. Preanal length</td>
<td>24.4</td>
<td>7</td>
<td>24.3–26.0</td>
<td>25.29 ± 0.70</td>
<td>30.7</td>
</tr>
<tr>
<td>6. Dorsal-fin length</td>
<td>95.5</td>
<td>8</td>
<td>95.5–96.6</td>
<td>96.04 ± 0.40</td>
<td>93.7</td>
</tr>
<tr>
<td>7. Anal-fin length</td>
<td>75.6</td>
<td>7</td>
<td>74.0–75.7</td>
<td>74.72 ± 0.70</td>
<td>69.3</td>
</tr>
<tr>
<td>8. Pelvic-fin length</td>
<td>6.8</td>
<td>7</td>
<td>5.1–7.9</td>
<td>6.46 ± 0.92</td>
<td>5.0</td>
</tr>
<tr>
<td>9. Pelvic to anal length</td>
<td>4.6</td>
<td>7</td>
<td>2.7–4.6</td>
<td>3.62 ± 0.63</td>
<td>4.7</td>
</tr>
<tr>
<td>10. Caudal-fin length</td>
<td>11.4</td>
<td>7</td>
<td>10.2–11.6</td>
<td>10.76 ± 0.62</td>
<td>9.0</td>
</tr>
<tr>
<td>11. LH (mm)</td>
<td>20.4</td>
<td>8</td>
<td>18.7–21.8</td>
<td>20.39 ± 1.07</td>
<td>23.6</td>
</tr>
<tr>
<td>12. Head width</td>
<td>26.0</td>
<td>8</td>
<td>22.4–26.0</td>
<td>24.36 ± 1.34</td>
<td>20.5</td>
</tr>
<tr>
<td>13. Postorbital length</td>
<td>13.4</td>
<td>8</td>
<td>12.6–14.7</td>
<td>13.78 ± 0.70</td>
<td>14.3</td>
</tr>
<tr>
<td>14. Upper head lobe width</td>
<td>14.6</td>
<td>8</td>
<td>12.8–14.6</td>
<td>13.54 ± 0.57</td>
<td>11.0</td>
</tr>
<tr>
<td>15. Lower head lobe width</td>
<td>10.9</td>
<td>8</td>
<td>9.9–12.3</td>
<td>11.12 ± 0.94</td>
<td>9.3</td>
</tr>
<tr>
<td>16. Predorsal length</td>
<td>22.3</td>
<td>8</td>
<td>17.6–22.3</td>
<td>19.45 ± 1.84</td>
<td>26.9</td>
</tr>
<tr>
<td>17. Postorbital length</td>
<td>65.6</td>
<td>8</td>
<td>65.6–69.5</td>
<td>67.61 ± 1.50</td>
<td>60.5</td>
</tr>
<tr>
<td>18. Snout length</td>
<td>23.4</td>
<td>8</td>
<td>18.4–23.4</td>
<td>20.65 ± 1.94</td>
<td>28.5</td>
</tr>
<tr>
<td>19. Upper jaw length</td>
<td>23.6</td>
<td>8</td>
<td>19.6–23.6</td>
<td>21.89 ± 1.42</td>
<td>24.0</td>
</tr>
<tr>
<td>20. Eye diameter</td>
<td>11.7</td>
<td>8</td>
<td>10.4–12.5</td>
<td>11.30 ± 0.76</td>
<td>12.0</td>
</tr>
<tr>
<td>21. Chin depth</td>
<td>20.9</td>
<td>8</td>
<td>15.5–20.9</td>
<td>17.97 ± 1.87</td>
<td>16.1</td>
</tr>
<tr>
<td>22. Lower opercular lobe</td>
<td>27.4</td>
<td>8</td>
<td>26.8–34.4</td>
<td>28.96 ± 2.65</td>
<td>21.4</td>
</tr>
<tr>
<td>23. Upper opercular lobe</td>
<td>26.4</td>
<td>8</td>
<td>23.2–27.2</td>
<td>25.20 ± 1.43</td>
<td>20.1</td>
</tr>
<tr>
<td>24. WH/LH</td>
<td>1.27</td>
<td>8</td>
<td>1.07–1.29</td>
<td>1.20 ± 0.07</td>
<td>0.87</td>
</tr>
<tr>
<td>25. Pupil:Eye diameter</td>
<td>44.6</td>
<td>8</td>
<td>41.9–55.9</td>
<td>49.33 ± 5.64</td>
<td>50.3</td>
</tr>
</tbody>
</table>

n, sample size; WH, head width; LH, head length.
Table III. Catalogue and GenBank information for specimens of *Symphurus* included in construction of the neighbour-joining trees.

<table>
<thead>
<tr>
<th>Species</th>
<th>Catalogue number</th>
<th>Locality</th>
<th>16s rRNA</th>
<th>coI</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Symphurus orientalis</em></td>
<td>ASIZP 72372</td>
<td>Da-Shi, NE Taiwan</td>
<td>JN678742</td>
<td>JN678777</td>
</tr>
<tr>
<td><em>Symphurus orientalis</em></td>
<td>ASIZP 72556</td>
<td>Dong-Gang, SW Taiwan</td>
<td>JN678752</td>
<td>JN678787</td>
</tr>
<tr>
<td><em>Symphurus orientalis</em></td>
<td>ASIZP 72358</td>
<td>Tosa Bay, Japan</td>
<td>JN678762</td>
<td>JN678797</td>
</tr>
<tr>
<td><em>Symphurus cf. microrhynchus (VN)</em></td>
<td>ASIZP 72365</td>
<td>Nha Trang, Viet Nam</td>
<td>KF676778</td>
<td>KF676781</td>
</tr>
<tr>
<td><em>Symphurus cf. microrhynchus (VN)</em></td>
<td>ASIZP 72366</td>
<td>Nha Trang, Viet Nam</td>
<td>KF676779</td>
<td>KF676782</td>
</tr>
<tr>
<td><em>Symphurus cf. microrhynchus (VN)</em></td>
<td>ASIZP 72367</td>
<td>Nha Trang, Viet Nam</td>
<td>KF676780</td>
<td>KF676783</td>
</tr>
<tr>
<td><em>Symphurus cf. microrhynchus (TW)</em></td>
<td>ASIZP 72361</td>
<td>Dong-Gang, SW Taiwan</td>
<td>KC900860</td>
<td>KC900873</td>
</tr>
<tr>
<td><em>Symphurus cf. microrhynchus (TW)</em></td>
<td>ASIZP 72362</td>
<td>Dong-Gang, SW Taiwan</td>
<td>KC900861</td>
<td>KC900874</td>
</tr>
<tr>
<td><em>Symphurus cf. microrhynchus (TW)</em></td>
<td>ASIZP 72363</td>
<td>Dong-Gang, SW Taiwan</td>
<td>KC900862</td>
<td>KC900875</td>
</tr>
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<td><em>Symphurus cf. microrhynchus (TW)</em></td>
<td>ASIZP 72370</td>
<td>Da-Shi, NE Taiwan</td>
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<td>KC900876</td>
</tr>
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<td><em>Symphurus cf. microrhynchus (TW)</em></td>
<td>ASIZP 72371</td>
<td>Da-Shi, NE Taiwan</td>
<td>KC900864</td>
<td>KC900877</td>
</tr>
<tr>
<td><em>Symphurus cf. microrhynchus (TW)</em></td>
<td>ASIZP 67658</td>
<td>Nanfang-Ao, NE Taiwan</td>
<td>KC900865</td>
<td>KC900878</td>
</tr>
<tr>
<td><em>Symphurus leucochilus</em></td>
<td>ASIZP 72355</td>
<td>Tosa Bay, Japan</td>
<td>KC900866</td>
<td>KC900879</td>
</tr>
<tr>
<td><em>Symphurus leucochilus</em></td>
<td>ASIZP 72356</td>
<td>Tosa Bay, Japan</td>
<td>KC900867</td>
<td>KC900880</td>
</tr>
<tr>
<td><em>Symphurus leucochilus</em></td>
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<td>KC900868</td>
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<tr>
<td><em>Symphurus leucochilus</em></td>
<td>USNM 408271</td>
<td>Da-Shi, NE Taiwan</td>
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<td>KC900882</td>
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<td><em>Symphurus leucochilus</em></td>
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<td><em>Symphurus leucochilus</em></td>
<td>USNM 408273</td>
<td>Da-Shi, NE Taiwan</td>
<td>KC900871</td>
<td>KC900884</td>
</tr>
<tr>
<td><em>Symphurus leucochilus</em></td>
<td>NMMB–P 17767</td>
<td>Da-Shi, NE Taiwan</td>
<td>KC900872</td>
<td>KC900885</td>
</tr>
</tbody>
</table>

**Etymology**

The name *leucochilus* is derived from the Greek, *leuco* meaning white, and *chilus* meaning border, in reference to the whitish border without dermal spots along bases of the anteriormost dorsal-fin rays and absence of pigmented spots on the isthmus.
Table IV. Diagnostic sites between haplotype of *Symphurus leucochilus* and other congener species distributed in the Indo-Pacific area, based on the alignment of 624 homologous positions of the *coI* mitochondrial gene.

<table>
<thead>
<tr>
<th>Species</th>
<th>coI diagnostic sites</th>
<th>GenBank number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Symphurus leucochilus</em></td>
<td>3 3 5 9 4 4 1 1 2 6 6 7 5 0 0 1 4 5</td>
<td>KC900882</td>
</tr>
<tr>
<td><em>Symphurus cf. microrhynchus</em> (TW)</td>
<td>3 6 9 3 8 3 4 8 1 0 6 5 2 1 7 3 0 5</td>
<td>KC900883</td>
</tr>
<tr>
<td><em>Symphurus cf. microrhynchus</em> (VN)</td>
<td>3 6 9 3 8 3 4 8 1 0 6 5 2 1 7 3 0 5</td>
<td>KC900884</td>
</tr>
<tr>
<td><em>Symphurus orientalis</em></td>
<td>3 6 9 3 8 3 4 8 1 0 6 5 2 1 7 3 0 5</td>
<td>KC900873</td>
</tr>
<tr>
<td><em>Symphurus hondoensis</em></td>
<td>3 6 9 3 8 3 4 8 1 0 6 5 2 1 7 3 0 5</td>
<td>KC900874</td>
</tr>
<tr>
<td><em>Symphurus megasomus</em></td>
<td>3 6 9 3 8 3 4 8 1 0 6 5 2 1 7 3 0 5</td>
<td>KC900875</td>
</tr>
<tr>
<td><em>Symphurus strictus</em></td>
<td>3 6 9 3 8 3 4 8 1 0 6 5 2 1 7 3 0 5</td>
<td>KC900876</td>
</tr>
<tr>
<td><em>Symphurus thermophilus</em></td>
<td>3 6 9 3 8 3 4 8 1 0 6 5 2 1 7 3 0 5</td>
<td>KC900877</td>
</tr>
</tbody>
</table>
REMARKS

Some previous studies dealing with specimens purported to be *S. orientalis* possibly also included specimens of *S. leucochilus*. This idea is based on the fact that reported ranges for meristic features of specimens purported to be *S. orientalis* in these studies are lower than corresponding ranges recently reported (Lee et al., 2013) for specimens of *S. orientalis* that were collected throughout the geographic range of the species. Given the overall similarities between *S. leucochilus* and *S. orientalis* in their general morphologies, it is easy to understand why earlier researchers might have overlooked this species in their studies. Meristic information appearing in the redescription of *S. orientalis* by Ochiai (1959) probably included data for *S. leucochilus*. Ochiai’s work (1959, 1963) has been widely cited, and the incorrect information reported for *S. orientalis* in this study has been perpetuated throughout much of the subsequent literature from Japan, Korea and Taiwan that has dealt with this species (Chyung, 1961; Ochiai, 1987, 1988, 1989; Shen et al., 1993; Sakamoto, 1997; Yamada, 2000, 2002). Now that reliable diagnostic information is available for distinguishing *S. leucochilus* from *S. orientalis*, the process of documenting geographic and bathymetric distributions and gathering other life-history information can begin for this poorly known species.

One of the three juveniles examined showed substantial differences in its morphological features compared with those of the other specimens (Table II). Most of the differences represent ontogenetic variation in morphometric features. For example, this specimen has a shallower body (22.7, 26.3–32.3% *L* in other specimens), and longer predorsal (6.4 v. 3.4–4.6% *L*), preanal (30.7 v. 24.3–26.0% *L*) and head (23.6 v. 18.7–21.8% *L*) in other specimens) lengths. Head width of this specimen is also smaller than its head length (28.0 v. 1.07–1.29 in other specimens), whereas its snout length is much longer than that of other specimens (28.5 v. 18.4–23.4% *L*). This specimen also has a correspondingly shorter postorbital length (*L* = 60.5 v. 65.6–69.5% *L*). The intestine and peritoneum are relatively elongated and the anal-fin origin is located at the vertical through the midpoint of the peritoneum (v. anal-fin origin usually at, or slightly in advance of, the vertical through the posteriormost region of the peritoneum). The differences in morphology of this specimen are attributed to the fact that at time of its capture it had just recently completed metamorphosis from the larval stage (evidenced by loop formed from previously protruding gut mass of the larval stage having just been incorporated within ventral body profile). This specimen is considered to be a transitional stage early juvenile.

MOLECULAR DATA

*Symphurus leucochilus* has many similar morphological features, including 12 caudal-fin rays, to those observed in *S. orientalis* and the specimens from Taiwan (TW) and Viet Nam (VN) that are tentatively identified in this study as *S. microrhynchus*. Analysis of genetic divergence for the partial 16s rRNA gene sequence indicates that *S. leucochilus* differs distinctly from *S. orientalis* by having a mean ± s.d. K2P distance of 10.96 ± 0.20% and a *p*-distance of 10.13 ± 0.17%. *Symphurus leucochilus* differs from *S. cf. microrhynchus* (TW) by a 23.91 ± 0.43% K2P distance and 20.19 ± 0.30% *p*-distance. From *S. cf. microrhynchus* (VN), *S. leucochilus* also differs distinctly with a 25.71 ± 0.18% difference in K2P distance and a 21.38 ± 0.12% difference in *p*-distance. Similar differences were also apparent in the analysis of the partial *coI* gene, where a mean ± s.d. K2P distance of 18.30 ± 0.23% and a *p*-distance
Comparisons included all known sequences from Symphurus nominal species of 16s FJ858987 for (the longer partial sequences with enough numbers of bp: FJ858984, FJ858985 and clades for these three taxa. Results clearly show separate clades for these three taxa.

K2P and p-distances were also compared between S. leucochilus and several nominal species of Symphurus characterized by having 14 caudal-fin rays. These comparisons included all known sequences from Symphurus hondoensis Hubbs 1915 (Lee et al., 2013), Symphurus megasomus Lee, Chen & Shao 2009 (Lee et al., 2013) and Symphurus strictus Gilbert 1905 (Lee et al., 2013), and some of the sequences (the longer partial sequences with enough numbers of bp: FJ858984, FJ858985 and FJ858987 for 16s rRNA; FJ863018, FJ863021 FJ863024–26 for coI sequences) from Symphurus thermophilus Munroe & Hashimoto 2008 (Tunnicliffe et al., 2010). Symphurus leucochilus differs distinctly from S. hondoensis (11·23 ± 0·15% K2P distance and 10·37 ± 0·13% p-distance), S. megasomus (14·04 ± 0·19% K2P distance and 12·68 ± 0·15% p-distance), S. strictus (13·87 ± 0·17% K2P distance and 12·55 ± 0·14% p-distance) and S. thermophilus (14·26 ± 0·15% K2P distance and 12·82 ± 0·13% p-distance) in partial 16s rRNA gene sequences. The coI sequences were shortened from 639 to 624 bp to include sequences of S. thermophilus. Again, differences in K2P and p-distance are also distinct between S. leucochilus and these species: S. hondoensis (24·22 ± 0·28% K2P distance and 20·27 ± 0·18% p-distance), S. megasomus (21·49 ± 0·22% K2P distance and 18·37 ± 0·16% p-distance), S. strictus (23·61 ± 0·21% K2P distance and 19·82 ± 0·14% p-distance) and S. thermophilus (18·93 ± 0·18 % K2P distance and 16·49 ± 0·13% p-distance). Symphurus leucochilus is distinguished by 19 diagnostic sites when compared with selected 624 bp coI sequences (Table IV) from these nominal species inhabiting the Indo-Pacific region. Also, there were no nucleotide insertions or deletions in the sequence alignment in this study.

Alignment results of NJ trees, and sequences for comparing diagnostic sites between S. leucochilus and the other nominal species of Symphurus from Indo-Pacific areas were deposited at DRYAD (Dryad Digital Repository; http://datadryad.org/).

Comparisons

Among Indo-Pacific Symphurus, six described species in addition to S. leucochilus have 12 caudal-fin rays (Alcock, 1891; Chabanaud, 1955, 1957; Munroe & Marsh, 1997; Munroe, 2001). Of these, only two, S. microrhynchos and S. trifasciatus, have similar meristic features to those of S. leucochilus, including numbers of dorsal-fin rays (85–92), anal-fin rays (70–80) and total vertebrae (usually <52).

Symphurus leucochilus has low meristic features, relatively small adult size ($L_\text{S} < 70$ mm), and also inhabits relatively shallow depths, which are morphological and ecological characteristics that it shares with those of another diminutive species, S. microrhynchos. Symphurus microrhynchos was previously considered to be a widely distributed tonguefish of the tropical and sub-tropical Indo-Pacific (Munroe & Marsh, 1997). Data (M.-Y. Lee & T. A. Munroe, unpubl. data), however, indicate that S. microrhynchos as presently conceived actually represents a complex of at
least seven, morphologically similar species, all of which, except *S. microrhynchus*, are undescribed. Meristic and most morphometric features of *S. leucochilus* nearly completely overlap those of *S. microrhynchus*, but several morphological characters easily separate *S. leucochilus* from *S. microrhynchus* and other nominal species that are morphologically similar to *S. microrhynchus*. For example, *S. leucochilus* lacks a fleshy ridge on its ocular-side lower jaw (v. fleshy ridge present in *S. microrhynchus* and morphologically similar nominal species); its dorsal-fin origin (at vertical through anterior margin of upper eye) is situated more anteriorly than that in others (e.g. dorsal-fin origin at, or beyond, vertical through posterior margin of eye) and the anterior margins of the eyes of *S. leucochilus* are equal, or nearly equal, in position (v. anterior margin of upper eye usually noticeably in advance of anterior margin of lower eye in *S. microrhynchus* and morphologically similar nominal species). Additionally, in *S. leucochilus*, the eyes are separated by a narrow interorbital space and the anterior nostril is not contiguous with the eyes (v. usually both upper and lower eyes contained within the same fleshy membrane and with flap of skin connecting anterior nostril with eye membrane in *S. microrhynchus* and morphologically similar nominal species; see Fig. 4). *Symphurus leucochilus* also has different colouration compared with that of *S. microrhynchus* and these other nominal species (Fig. 5). In particular, it lacks dermal spots or melanophores at bases of the anterior dorsal-fin rays and lacks melanophores on the head region posterior to the eyes and on the ocular-side isthmus (v. *S. microrhynchus* and morphologically similar nominal species with pepper dots or dermal spots at bases of anterior dorsal and anal-fin rays and with melanophores on the head region posterior to the eyes and also on the isthmus). Small specimens of *S. leucochilus* also have a streak of black pigment along the caudal portion of their vertebral column that is visible through the body musculature on the blind side, whereas *S. microrhynchus* and morphologically similar nominal species have uniform whitish colouration along their blind-side vertebrae without this dermal pigment streak (Fig. 6).

*Symphurus leucochilus* differs obviously from *S. trifasciatus*, a deeper-dwelling tonguefish (ranging between c. 220 and 737 m depth) with an allopatric distribution in the Indian Ocean, in some meristic characters and in many other aspects of its morphology. For example, *S. leucochilus* has larger scales than those of *S. trifasciatus*, as reflected in the much lower number of scale rows on the head posterior to the lower orbit (15–17 v. 22–25 in *S. trifasciatus*) and by the fewer transverse scales (32–35 v. 35–38 in *S. trifasciatus*). *Symphurus leucochilus* also differs from *S. trifasciatus* by having a more anterior point of insertion of its dorsal fin, which is reflected in a shorter predorsal length (3.4–4.6 v. 5.2–9.7% Lₕ in *S. trifasciatus*). *Symphurus leucochilus* also has shorter head (18.7–21.8 v. 23.8–27.2% Lₕ in *S. trifasciatus*) and postorbital lengths (12.6–14.7 v. 16.4–20.4% Lₕ in *S. trifasciatus*), a wider upper head lobe (LₕH 12.8–14.6 v. 8.1–10.8%) and narrower lower head lobe (LₕL 9.9–12.3 v.13.7–17.1%) compared with corresponding features in *S. trifasciatus*. The anterior profile of the snout of *S. leucochilus* is rounded to slightly blunt v. the more pointed (usually) snout in *S. trifasciatus*. Another difference between these species occurs in shape of their opercles. In *S. leucochilus*, the lower opercular lobe (OPLL) is equal to, or slightly wider than, the upper opercular lobe (OPLL 26.8–34.4 v.23.2–27.2% LₕH), whereas in *S. trifasciatus* the lower opercular lobe is narrower than the upper opercular lobe (OPLL 22.1–29.7 v. 30.3–37.6% LₕH). In *S. leucochilus*, the posterior margin of the lower opercular lobe also projects slightly beyond, or is
equal to, the vertical at the posterior margin of the upper opercular lobe. In contrast, in *S. trifasciatus*, the posterior margin of the upper opercular lobe usually extends well beyond the posterior border of the lower opercular lobe. The ocular-side pigmentation of *S. leucochilus* (light yellow to light brown without crossbands) is distinctly different from the greyish ocular-side background colouration with three to four, prominent, wide (five to 10 scales) crossbands of *S. trifasciatus*. *Symphurus leucochilus* appears to be a smaller species (largest specimen attaining only c. 67 mm *L*<sub>S</sub>) that matures at smaller sizes (females mature at c. 47 mm *L*<sub>S</sub>) compared with the larger *S. trifasciatus*, which reaches sizes to 116 mm *L*<sub>S</sub> and possibly also matures at larger sizes (two known mature females are c. 90 mm *L*<sub>S</sub>) than that noted for *S. leucochilus*.

*Symphurus orientalis* is another species of *Symphurus* characterized by 12 caudal-fin rays that has a geographical distribution sympatric with that of *S. leucochilus*. Features
of the ocular-side pigmentation of \textit{S. leucochilus} are similar to those of specimens of \textit{S. orientalis} that lack crossbands on their ocular side, which hampers identification of these species. \textit{Symphurus leucochilus} is easily distinguished from \textit{S. orientalis} by having 89–92 dorsal-fin rays (v. 96–101 in \textit{S. orientalis}), 76–80 anal-fin rays (v. 82–89), 49–51 total vertebrae (v. 52–55), 75–83 longitudinal scale rows (v. 87–99 in \textit{S. orientalis}), 32–35 transverse scales (v. 37–42) and 15–17 scale rows on the head posterior to the lower orbit (v. 18–22 scale rows in \textit{S. orientalis}).

Five other Indo-Pacific species of symphurine tonguefishes, \textit{Symphurus macrophthalmus} Norman 1939, \textit{Symphurus monostigmus} Munroe 2006, \textit{Symphurus multimaculatus} Lee, Munroe & Chen 2009, \textit{Symphurus schultzi} Chabanaud 1955 and \textit{Symphurus thermophilus}, have counts of dorsal and anal-fin rays, and abdominal and total vertebrae that overlap with those of \textit{S. leucochilus}. \textit{Symphurus leucochilus} differs distinctly from all of these species by having 12 caudal-fin rays and four hypurals (v. 14 caudal-fin rays and five hypurals in others).

**DISCUSSION**

Recent advances in molecular sequencing methods have contributed to taxonomic studies by discriminating morphologically similar species, discovering cryptic species (Victor, 2007; Diaz de Astarloa \textit{et al.}, 2008; Ward \textit{et al.}, 2008; Baldwin \textit{et al.}, 2011; Luchetti \textit{et al.}, 2011) and by resolving issues concerning the validity of questionable nominal species for which previous taxonomic uncertainty has existed (Byrkjedal \textit{et al.}, 2007; Dooley & Jimenez, 2008; Victor, 2008; Allen \textit{et al.}, 2010; Last \textit{et al.}, 2010; Benziger \textit{et al.}, 2011). These applications are also utilized in resolving taxonomic issues of tonguefishes. One study examining sequence divergences in western Pacific tonguefishes (Tunnicliffe \textit{et al.}, 2010) observed significant differences in partial 16s rRNA (9.0%) and coI (14.2%) gene sequences among allopatric populations that had previously been considered to be one species, \textit{S. thermophilus} (Munroe & Hashimoto, 2008). Based on these genetic differences, Tunnicliffe \textit{et al.} (2010) suggested that their results showed the presence of a cryptic species with similar morphological features to those of \textit{S. thermophilus} among their studied specimens. Another study (Lee \textit{et al.}, 2013) examining sequence divergence from both partial 16s rRNA and coI genes between two nominal tonguefish species, \textit{Symphurus novemfasciatus} Shen & Lin 1984 and \textit{S. orientalis} (Bleeker 1879), found only minor sequence differences (0-21% K2P distance in 16s rRNA and 0-26% K2P distance in coI) between these two nominal species. In combination with results from morphological evidence, Lee \textit{et al.} (2013) concluded that \textit{S. novemfasciatus} was the junior synonym of \textit{S. orientalis}.

Results from the present investigation show that \textit{S. leucochilus} differs significantly in its genetic composition from other species with which it shares many meristic and morphological similarities. For example, \textit{S. leucochilus} differs in K2P distances of 16s rRNA and coI sequence from both that of \textit{S. orientalis} and the nominal species tentatively identified as \textit{S. cf. microrhynchus}. Additionally, the NJ algorithm shows that \textit{S. leucochilus}, \textit{S. orientalis} and the two nominal species identified as \textit{S. cf. microrhynchus} clearly belong to three different clades (Fig. 3). Based on the algorithm, \textit{S. leucochilus} is neither in the group, nor is the sister group of the nominal species tentatively identified as \textit{S. microrhynchus}. Rather, \textit{S. leucochilus} is the sister group to \textit{S. orientalis}. Although morphologically most similar to the nominal species tentatively identified
as *S. microrhynchus*, the genetic divergences between *S. leucochilus* and *S. orientalis*, and between *S. leucochilus* and the other nominal species possessing 14 caudal-fin rays, are much smaller than are those between *S. leucochilus* and the two populations tentatively identified as *S. microrhynchus*. Smaller sequence divergences (3.87 ± 0.33% K2P distance and 3.74. ± 0.31% p-distance in partial 16s rRNA gene; 8.66 ± 0.09% K2P distance and 8.03 ± 0.08% p-distance in partial col gene) are found between members of these two populations, *S. cf. microrhynchus* (VN) and *S. cf. microrhynchus* (TW), than between them and *S. leucochilus*. Morphological characters show significant differences between *S. leucochilus* and each of the nominal species that are tentatively identified as *S. microrhynchus*. Evidence from both morphological and molecular approaches indicates that *S. leucochilus* is not a member of the clade containing the two nominal species tentatively identified as *S. microrhynchus*.

Approaches that examine DNA sequences can facilitate identifications of morphologically distinct specimens and this capability becomes especially important when study material is limited, as was the case for *S. leucochilus*, where only one recently settled juvenile was available for study. Most species of Pleuronectiformes metamorphose at relatively small sizes (between 10 and 25 mm body length, \( L_B \); Hensley & Ahlstrom, 1984), and are morphologically quite distinct from juveniles and adults of the species. For species of *Symphurus* where larval stages are known (Kurtz & Matsuura, 1994; Aceves-Medina et al., 1999; Evseenko & Shtaut, 2000; Saldierna et al., 2005; Farooqi et al., 2006), transformation also occurs at smaller sizes (usually < 20 mm \( L_B \)). Morphometric features of symphurine larvae and early stage juveniles are different from those of later stage juveniles and adults. Also, meristic features such as caudal, dorsal and anal-fin rays and scales do not complete their development until late in the larval stage. Because of these factors, previous studies attempting to identify larvae and juveniles of these fishes, most of which have been conducted on species occurring in Atlantic and eastern Pacific Oceans, have had to rely extensively on differences in combinations of short and long anterior dorsal-fin rays, on ID patterns or pigmentation patterns, and also on the presence or absence of specialized structures, such as a conical appendix on the abdominal wall (Ahlstrom et al., 1984; Kurtz & Matsuura, 1994; Munroe et al., 2000; Farooqi et al., 2006; Munroe & Krabbenhoft, 2010). In the Indo-West Pacific region, where species diversity of *Symphurus* may be greater than that in other regions and with new species continuing to be discovered, larvae of most of these species are unknown. Furthermore, unlike symphurine species occurring in the Atlantic and eastern Pacific Oceans, species in the Indo-West Pacific region display less variation in key diagnostic features, such as ID pattern and fin-ray counts, that have facilitated identifications of symphurine larvae in these other geographic areas (Kurtz & Matsuura, 1994; Aceves-Medina et al., 1999; Saldierna et al., 2005; Farooqi et al., 2006; Munroe & Krabbenhoft, 2010). With fewer meristic features available for identifying early life-history stages of Indo-West Pacific species of *Symphurus*, especially when cryptic species and species complexes are involved, other approaches to help with identifications will be required. Incorporation of DNA sequence data in these studies offers great promise in assisting with identifications of these early life-history stages within the subfamily Symphurinae, as well as in other flatfish larvae, and should be explored further.

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**Electronic Reference**