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The cryptic cornucopia revisited– an extended analysis of the COI gene in the gobiid fish genus *Trimma* (Percomorpha: Gobiiformes)

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Abstract

We present the results of a Neighbor-joining analysis of the partial mitochondrial 5' cytochrome c oxidase I gene (COI) from 849 samples of the gobiid fish genus *Trimma*, representing 87 of the 106 species currently regarded as valid (82%). We compare these results with those of Winterbottom et al. (2014c) who analyzed sequences of 473 specimens of this genus assigned to 46 of the 73 valid species at the time (63%). That study found 94 COI genetic lineages (as arbitrarily defined by ≥ 2% sequence divergence) as opposed to the 155 such lineages recognized here. Many of the multiple haplogroups grouped under a single species name appear to be genovariants, although in some cases color or morphologically congruent characters are known. Even though we are missing genetic samples from many localities a given species has been recorded from based on museum specimens, we found that 35 species (40% of the total species sampled) had a single haplogroup, with one or more samples collected from a single general locality. Another 25 species (29%) had a single haplogroup but with samples from two or more geographic localities (up to 6). The remaining 27 species (31%) exhibited two or more haplogroups (up to 8 in *T. erdmanni*), with a mean of 3.5 haplogroups/species. Each haplogroup occurred at a mean of 1.7 localities. In most cases, these haplogroups were separated geographically (although often not by large distances). An apparently similar situation in another small but very speciose Indo-Pacific goby, *Eviota*, is known but needs formal documentation. Future comparisons of the Indo-Pacific complexes with those from the Caribbean, where a somewhat similar situation occurs in at least one goby (*Elacatinus*), as well as certain labrisomid blennioids (e.g. *Starksia*), are suggested.

Key words: ichthyology, taxonomy, coral-reef fishes, DNA barcodes, cryptic species, Indo-West Pacific Ocean.

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Introduction

Descriptions of new species of the small (average 2 cm SL), often colorful, Indo-Pacific pygmy gobies (*Trimma*) have exploded during the last 35 years, from 17 species recognized as being valid prior to Winterbottom's (1984) publication on the *Trimma* of the Chagos Archipelago to the 106 valid species recognized by Winterbottom (2019)– an average of 2.5 new species described each year. Numerous other species have been recognized as morphologically distinct from those already described and await publication. Additionally, almost all collections of fishes from below about 50 m on Indo-Pacific tropical reefs result in yet more undescribed species of *Trimma*.

As a contribution to the Fish Barcode of Life (FISH-BOL) project (Ward et al. 2009), a genetic analysis of the partial mitochondrial 5' cytochrome c oxidase I gene (DNA barcode, abbreviated to COI) of the gobiid *Trimma* was published by Winterbottom et al. (2014c). That study contains a summary of our previous knowledge of the contribution of this locus to our understanding of the biodiversity of these small cryptic reef fishes. Since that paper was published, little has been produced on the subject. The current state of our knowledge of cryptic diversity was, however, well summarized by Victor (2015), and the literature pertaining to *Trimma* specifically was summarized by Winterbottom (2019). In the Winterbottom et al. (2014c) study, COI sequences of 473 specimens were assigned to 46 (not 52 as stated in that paper) morphological species of the 73 formally described, valid species at the time (63%). This number included four known, but not formally named, species, designated as "*T.* RW sp xx", the numbers having been previously assigned to them. Those four species have now been formally described (*Trimma* RW sp 24 = *T. xanthum* Winterbottom & Hoese, 2015; *T.* RW sp 32 = *T. trioculatum* Winterbottom et al., 2015; *T.* RW sp 97 = *T. bathum* Winterbottom, 2017; and *T.* RW sp 98 = *T. kardium* Winterbottom et al., 2015). The 2014 study used a Neighbor-joining analysis which revealed the presence of 94 COI genetic lineages, as arbitrarily defined by ≥ 2% sequence divergence (Steinke & Hanner 2011), and referred to as "haplogroups" in this paper. A very detailed morphological/color pattern analysis of a portion of one of the nesting haplogroups (called Section A in Winterbottom et al. [2014c]) was undertaken as a result of the COI findings (Winterbottom 2016). The four targeted haplogroups included in the 2014 study that were provisionally identified on the basis of morphological characters as being *T. tevegae* Cohen & Davis, 1969 were included, together with *T. caudomaculatum* Yoshino & Araga, 1975. As a result, three new species were described (*T. corerefum* Winterbottom, 2016, identified as *T. tevegae* Group 1 in Fig. 1 of Winterbottom et al. (2014c); *T. burridgeae* Winterbottom, 2016, as Group 5, and *T. hollemani* Winterbottom, 2016, as Group 4) based on morphological/coloration differences (Winterbottom 2016). Only one of the other haplogroup complexes identified as a single species (*T. xanthochrum* Winterbottom, 2011) has been examined in a similar manner to date, but that analysis failed to find any physical attributes complementing the COI analysis results, and the results have not been published. In this paper we add 376 samples and 41 described species to the results presented by Winterbottom et al. (2014c), and provide some (but incomplete) evidence of haplogroup separation based on color, as well as speculation of possible mechanisms involved in the evolution of these small fishes.

Materials and Methods

The present dataset is derived from 849 samples representing 87 of the 106 species (82%) currently regarded as valid. The Barcode Index Number (BIN) (Ratnasingham & Hebert 2013) algorithm suggests that there are 160 separate groupings in this data set. Using only the arbitrary definition of ≥ 2% sequence divergence to define a haplogroup (for ease of comparison with Winterbottom et al. [2014c]), we found 155 such groupings in the current results. The ROM specimens used in our analysis (catalogue number prefixed by "T") were collected using various anaesthetics (e.g. clove oil, quinaldine), and the whole fish was placed in a vial containing either a saturated salt solution (Seutin et al. 1990; specimens collected prior to 1998) or 95% ethyl alcohol (after 1998). These specimens were subsampled for genetic analysis. In collections made prior to 2006, the samples retained for genetic analysis were seldom photographed immediately after collection, although photographs of a specimen from the same lot as the tissue specimen were frequently taken. In collections after 2006, every effort was made to photograph the live/freshly collected coloration pattern of the specimens retained for genetic analysis. The samples used in the previous analysis (Winterbottom et al. 2014c) were included in this analysis. Almost all of the

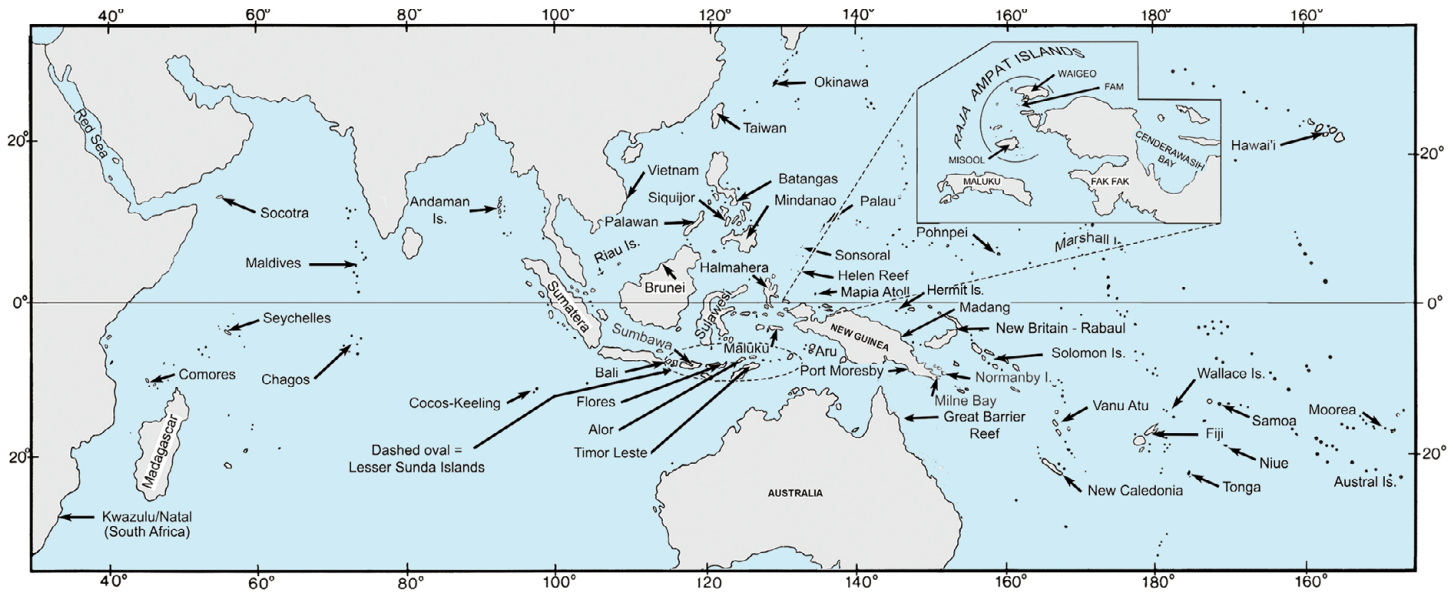


Figure 1. Map of the Indo-Pacific Ocean showing most localities from which samples were collected; inset shows detail of the Raja Ampat archipelago and the Bird's Head Seascape of West Papua, Indonesia on the island of New Guinea. Dashed oval denotes the extent of the Lesser Sunda Islands; Marquesas and Easter Islands, east of the Society Islands, are not shown.

localities mentioned here are included in the map of the Indo-Pacific region (Fig. 1), and the abbreviations used for these localities are included in the legend for Fig. 2 part 1.

Voucher specimen information and digital images (where applicable) were deposited in the Barcode Of Life Database (BOLD; http://www.barcodedsystems.org; Ratnasingham & Hebert 2007) following recommendations of the FISH-BOL campaign (Ward et al. 2009) and related collaborators protocol (Steinke & Hanner 2011) in an accessible data project (titled "RWTRM + new data post 2012", code "DS-*Trimma*18").

DNA was extracted using a Qiagen DNeasy Blood & Tissue Kit (QIAGEN) following the manufacturer's instructions with some exceptions: after adding AW2, spin columns were dried through a final centrifugation at 17,000× g for 5 minutes; sample DNA was eluted with 50 µL of AE buffer and centrifuged at 6,000× g for 1 minute, and the same 50 µL of AE buffer was then re-eluted with a final centrifugation at 6,000× g for 1 minute in order to increase the DNA concentration. Each 12.5 µL PCR reaction consisted of 2 µL of template DNA, 6.25 µL 10% trehalose, 2 µL ddH2O, 0.625 µL MgCl2 [50 mM], 0.0625 µL dNTPs [10 mM], 0.06 µL Platinum Taq (Invitrogen), 0.10 µL [0.01 mM] each of the universal fish COI cocktail primers C_FishF1t1 and C_FishR1t1 (Ivanova et al. 2007) and 1.25 µL 10X PCR buffer (Invitrogen). PCR thermocycling conditions were an initial hot start of 94° C for 2 min, 25 cycles of denaturation at 94° C for 30 s, annealing at 52° C for 40 s and extension at 72° C for 1 min, with a final extension at 72° C for 10 min. PCR products were visualised using a 2% agarose gel E-Gel96 Pre-cast Agarose Electrophoresis System (Invitrogen). Only amplicons with single, intense bands were sequenced.

Each sequencing reaction consisted of 1 µL of PCR product along with 1 µL BIG DYE 3.1 reagent (Applied Biosystems, Inc), 1 µL M13F/M13R primer (Messing 1983), 10 µL ddH20 and 1 µL 5X sequencing buffer (Invitrogen). The thermocycling profile was an initial hot start 96° C for 2 min, followed by 30 cycles of denaturation at 96° C for 30 s, annealing at 55° C for 15 s, and an extension at 60° C for 4 min. PCR products were bidirectionally sequenced and run on an ABI 3730xl capillary sequencer (Applied Biosystems, inc.). Sequencher 4.05 (GeneCodes) was used to trim primers, assemble and manually edit bidirectional contigs from raw trace files.

Certain samples did not sequence well using the above method. These specimens were subsequently analyzed at the ROM by one of us (KC) using the following protocols. DNA was eluted with 100 µL of AE buffer. Each 25 µL PCR reaction consisted of 1 µL of template DNA, 18.89 µL ddH2O, 0. 56µL dNTPs [10 mM], 0.05 µL Platinum Taq (Invitrogen), 1 µL [0.01 mM] each of the universal COI primers LCO1490 and HCO2198 (Folmer et al. 1994) and 2.5 µL 10X PCR buffer (Invitrogen). PCR thermocycling conditions were an initial hot start of 94° C for 1 min, 5 cycles of denaturation at 94° C for 30 s, annealing at 42° C for 40 s and extension at 72° C for 1 min, then 35 cycles of denaturation at 94° C for 30 s, annealing at 46° C for 40 s and extension at 72° C for 1 min,

with a final extension at 72° C for 5 min. PCR products were visualised using 1% agarose gel. Only amplicons with single, intense bands were sequenced. Each sequencing reaction consisted of 2 µL of PCR product along with 0.5 µL BIG DYE 3.1 reagent (Applied Biosystems, Inc), 0.5 µL LCO1490/HCO2198 primer, 5 µL ddH20 and 2 µL 5X sequencing buffer (Invitrogen). PCR products were bidirectionally sequenced and run on an ABI 3730 capillary sequencer (Applied Biosystems). Bioedit was used to trim primers, assemble and manually edit bidirectional contigs from raw trace files.

Sequence assemblies and supporting electropherogram "trace" files were uploaded to BOLD (and submitted to GenBank, accession numbers: MT999488–MT999854 in addition to those listed in Winterbottom et al. 2014) and combined with other available *Trimma* sequences using a Hidden Markov Model alignment of translated COI amino acid sequences (Ratnasingham & Hebert 2007). Aligned sequences of >500 bp in length were used to generate pairwise or p-distances to infer a Neighbor-joining phenogram of sequence divergences using BOLD in order to provide a visual depiction of the barcode variation among and between species. Sequence data from an updated data set were also parsed into molecular operational taxonomic units (MOTUs) using the RESL (Refined Single Linkage Analysis) algorithm and subsequently annotated with BINs, as implemented on version 3 of BOLD. This approach combines single linkage clustering and Markov clustering to recognize gaps in sequence space that correlate with species boundaries by optimizing MOTU partitions using the Silhouette index and uniquely labelling each MOTU with a BIN. The value of 2% used here is approximately equivalent to the more sophisticated RESL analysis used to distinguish haplogroup clusters that are subsequently enumerated with BINs as promulgated by Ratnasingham & Hebert (2013; see above).

Results

This section discusses the phenetic relationships of each colored, numerical block of haplogroups (numbered 1 through 29) in Fig. 2. These groupings should in no way be construed as representing clades, because clades (= monophyletic groups) cannot be revealed by purely phenetic analyses such as this study. For our purposes, they are artificial groupings or grades erected for the convenience of discussion only (unless otherwise stated) and have no reality in nature except by chance. Note that the alphabetical assignments for color blocks of haplogroups used in Winterbottom et al. (2014c, Fig. 1) usually bear no or little resemblance/relationship to the numerical blocks used here. The sequences of phenetic relationships of the haplogroups in these analyses are incredibly sensitive to the addition of: a) previously unrepresented taxa, which always seems to result in a significant rearrangement of the taxa in the phenogram; and b) new samples of previously represented taxa (which frequently do the same, especially when from new localities). The small minimum number of base pairs in the analysis (≥ 500) coupled with the number of samples (849) and putative number of species (87) makes this volatility almost inevitable. The comparative nature of this paper necessitates frequent referral to the results of the Winterbottom et al. (2014c) analysis, which was based on far fewer (473 vs. 849) specimens. That paper will, for the sake of brevity, be referred to as "W14" in this section. Geographic ranges given for species here are based only on samples analysed for this paper unless otherwise detailed. Full distributions as currently known for each included species can be found in Winterbottom (2019).

BLOCK 1. This block consists of a single haplogroup (*T. unisquame* Gosline, 1959), with samples from the Austral, Society and Tuamotu islands as well as Easter Island. Within these samples, those from Easter Island (n = 4) group together with 0.4% variance and are minimally (0.3%) different from the samples from three different localities in French Polynesia (n = 3, within-group variation = 0.0%). It seems potentially interesting that samples separated by about 2,000 km show no difference in CO1, whereas those from a single small island differ by 0.4% of that same gene. Specimens identified as *Trimma unisquame* (type locality Hawai'i) have been found at the Comoros Islands (some 18,000 kms west in the western Indian Ocean), but no tissue samples are available from anywhere west of French Polynesia. This morphologically distinctive species is also very well differentiated in its COI sequences from all other species of *Trimma*, and the minimum distance from the closest such species is 26.7% (from *T. meranyx* Winterbottom et al., 2014b). The species was not included in W14 because no samples were available for analysis at that time.

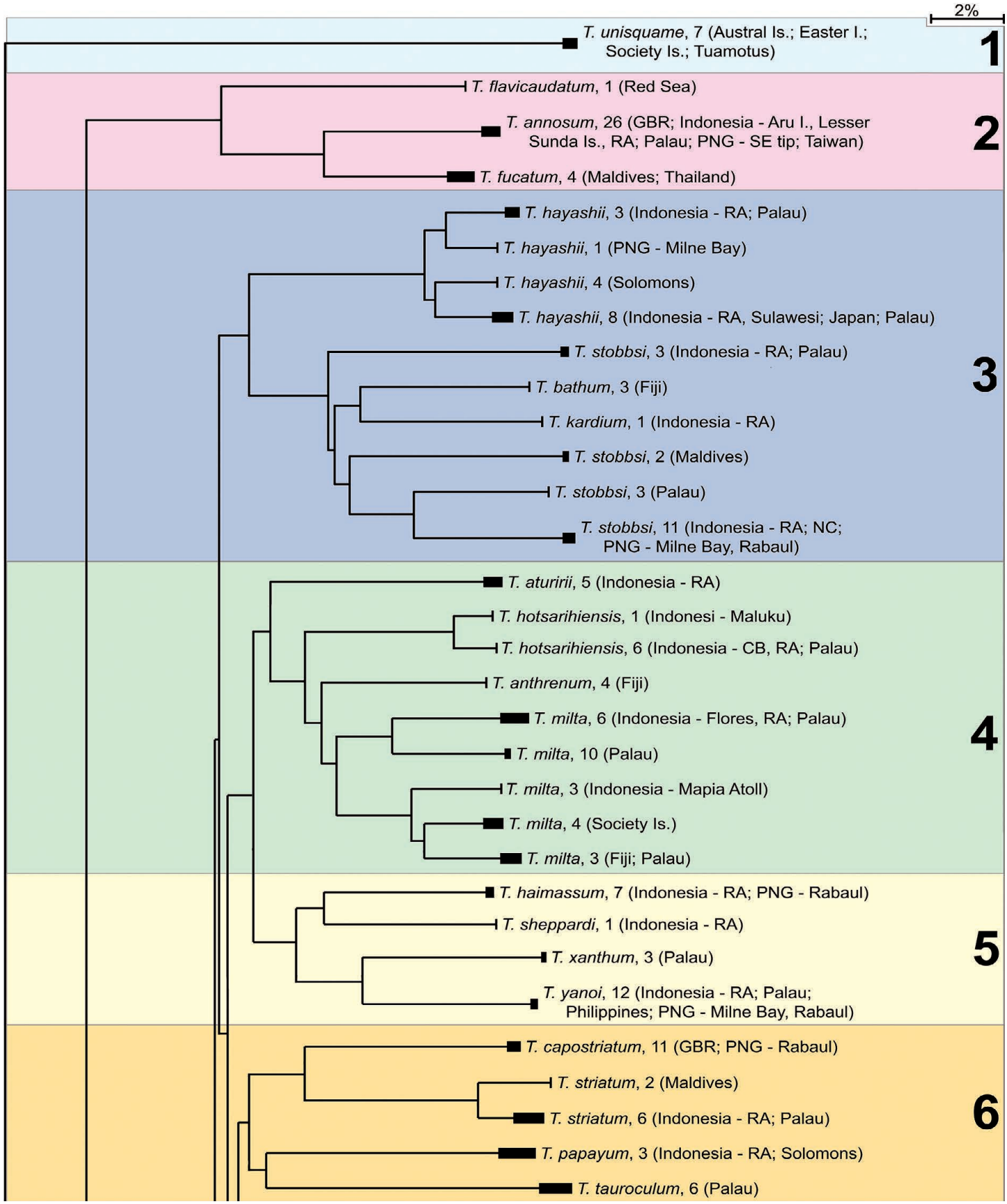


Figure 2 part 1 (Groups 1–6). Condensed Neighbour-joining network of the COI gene based on an analysis of 849 specimens of *Trimma*. Solid bars at tips of branches represent approximate within-group variation. Species names are followed by the number of specimens with locality(ies) of the specimens in parentheses. Colored blocks and numerical notations refer to the sequential groups discussed in the text, additional groups continue on subsequent pages. Scale bar is 2% COI genetic distance. Abbreviations are: Indonesia– Cenderawasih Bay (CB) Great Barrier Reef, Australia (GBR), New Caledonia (NC), Papua New Guinea (PNG), Indonesia– Raja Ampat (RA).

BLOCK 2. Three species are included here. *Trimma flavicaudatum* (Goren, 1982) (Red Sea only, one sample) differs from *T. annosum* Winterbottom, 2003 (26 samples, 0.6% within-group variation, western Pacific from the GBR and the Lesser Sunda Islands to Taiwan) by 13.2%, and from *T. fucatum* Winterbottom & Southcott, 2007 (4 samples, 0.8% within-group variation, Maldives and Thailand) by 13%. The latter two species differ from each other by 7.4% of the COI base pairs, with no samples available from the type locality of *T. annosum* (Fiji), but such samples analysed for *T. fucatum* (Thailand). No geographic pattern is discernable in the variation of either of the last two species. No specimens of *T. flavicaudatum* were available for the W14 study. An additional sample of *T. fucatum*, from the Maldives is included here, and two additional specimens of *T. annosum* extend the geographic sample range for that species to Aru Island and to the SE tip of Papua New Guinea.

BLOCK 3. Ten haplogroups divided among four named species are included in Block 3 (Fig. 3, Table 1). Four of the haplogroups are currently identified as *T. hayashii* Hagiwara & Winterbottom, 2007, and another four are labelled as *T. stobbsi* Winterbottom, 2001. Note that the other two species (*T. bathum* and *T. kardium*) are nested within the four haplogroups identified as *T. stobbsi* (Fig. 3, Table 1).

Among the *T. hayashii*, three specimens from Mapia Atoll and Palau were separated from a single specimen from Milne Bay (PNG) by 3.2%, and four specimens from the Solomon Islands were separated from a group of 8 samples from Indonesia (Raja Ampat and Sulawesi), Japan (= type locality) and Palau by 3.5%. Thus, two general areas (Raja Ampat + Mapia Atoll, and Palau) each had two haplogroups (1a and 1d) present. However, in the case of 1a, the Palauan samples were from Helen Reef in the South West Islands, and the sample from Indonesia was from Mapia Atoll, an isolated oceanic atoll about 360 kms to the southeast of Helen Reef (see Fig. 2). In the other case (1d), samples were from the main Palauan islands and from Raja Ampat (including FakFak) and Sulawesi, in addition to the two samples from Japan (the type locality of the species). Two haplogroups of *T. hayashii* were recognized in W14. Their Group 1 corresponds to our 1d (above). Three samples have been added to this group, including one from Sulawesi, a new locality, and intra-group variance has increased from 0.4% to 1.0%. Group 2 of W14, which corresponds to our Group 1a (above), contained only the two samples from the South West Islands of Palau. The additional specimen of this haplogroup from Mapia Atoll is the source of the intra-group variation (0.5%; there is no difference between the two Palau samples). The minimum variance between these two haplogroups remains at 4.7%. Table 1 gives the intergroup minimum variations of the four haplogroups, which vary between 3.2–4.7%. As is so frequently the case in *Trimma*, further morphological work is necessary to see if differences can be found which support the genetic differentiation in CO1.

The remaining 6 haplogroups are assigned to *T. stobbsi* (4), *T. kardium* and *T. bathum*, with the latter two

TABLE 1

Distance summary barcode analysis of 39 *Trimma* specimens in Block 3 & Fig. 3
(minimum distance between groups)
Var.= maximum variation within group

Group		location	n	Var.	1b	1c	1d	2a	3	4	2b	2c	2d
1a	*T. hayashii*	Indonesia- Mapia Atoll, Palau- Helen Reef	3	0.5	3.2	3.2	4.7	15.2	14.6	16.0	16.8	17.6	17.0
1b	*T. hayashii*	PNG- Milne Bay	1	0.0	–	4.2	4.7	14.6	15.2	16.0	16.8	16.8	16.5
1c	*T. hayashii*	Solomon Islands	4	0.0		–	3.5	14.6	13.9	14.4	16.2	16.4	16.8
1d	*T. hayashii*	Indonesia- RA & Sulawesi, Japan, Palau	8	1.0			–	14.7	14.0	14.6	16.2	17.3	16.3
2a	*T. stobbsi*	Indonesia- RA, Palau	3	0.3				–	11.6	12.5	13.3	12.4	12.4
3	*T. bathum*	Fiji	3	0.0					–	9.6	10.7	11.1	11.7
4	*T. kardium*	Indonesia- RA	1	0.0						–	11.2	11.9	13.1
2b	*T. stobbsi*	Maldives	2	0.2							–	11.2	12.0
2c	*T. stobbsi*	Palau	3	0.0								–	7.9
2d	*T. stobbsi*	RA, NC, PNG- Milne Bay & Rabaul	11	0.5									–

nestled within the former (Fig. 3). Generally, these forms are part of the "deeper reef" fauna (>50 m), although the *T. stobbsi* haplogroups can also frequently be found in shallower water (least depth recorded = 3.7 m). The phenetically most basal of the haplogroups (2a, *T. stobbsi*) contains three samples from Raja Ampat and Palau with a within-group variation of 0.3%, and it differs minimally by 11.6% from the remaining five haplogroups. *Trimma kardium* (n = 1) and *T. bathum* (n = 3, invariant) differ from each other by a minimum of 9.6%. The minimum distances between these two and the remaining three haplogroups varies between 10.7–13.1%. *Trimma stobbsi* (2b, Maldives, 0.2% with-in group variance) differs from three samples from Palau (2c, northern of the South West Islands only, no variation) by 11.2% and from a total of 11 samples from Raja Ampat (Indonesia), New Caledonia– the type locality– and from Milne Bay and Rabaul in Papua New Guinea (2d, 0.5% intra-group variation) by 12.0%. Groups 2c and 2d differ by 7.9%. Again, further studies need to be undertaken to determine if there are any morphological characters to support the genetic differentiation detailed above.

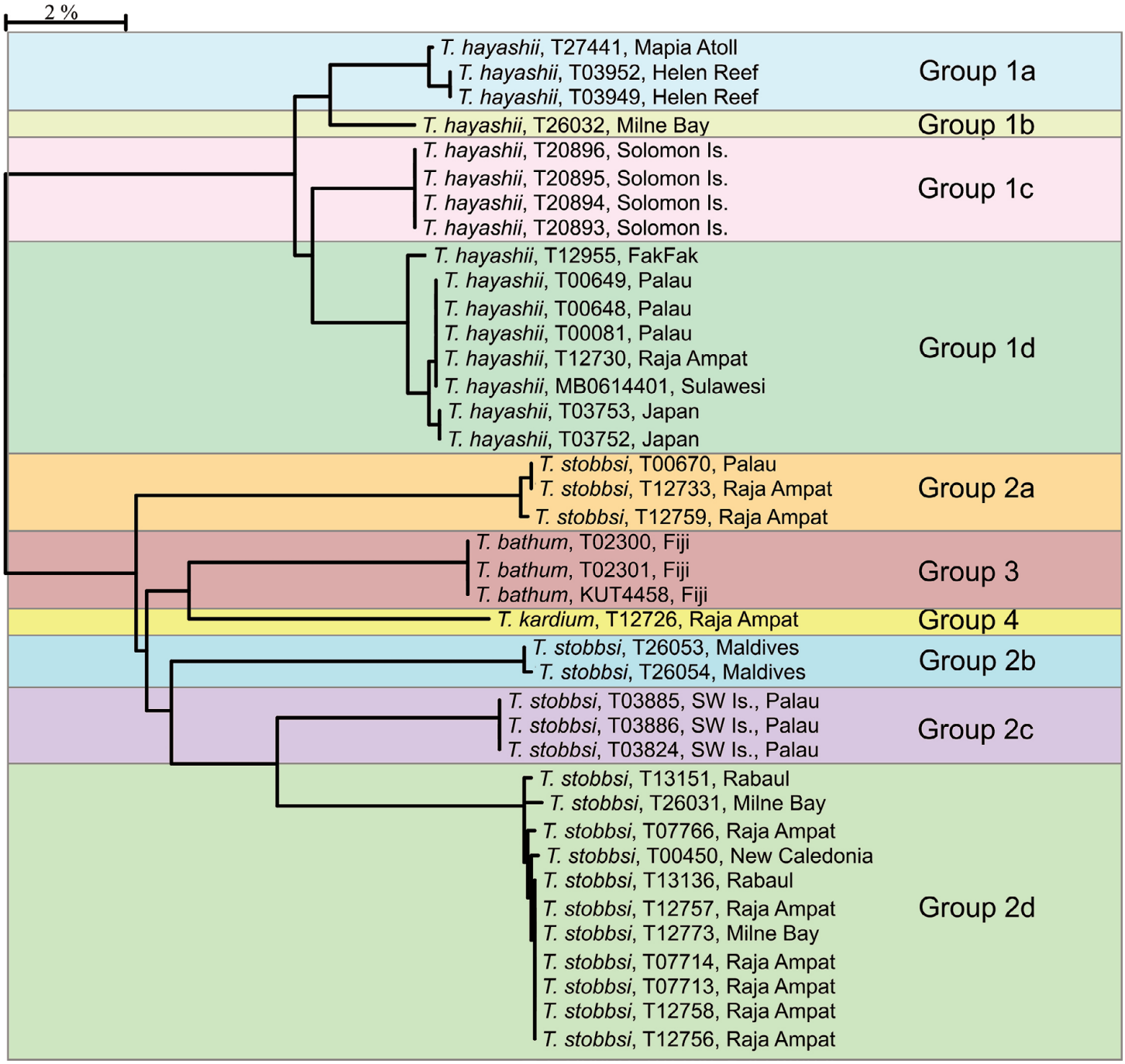


Figure 3. Phenetic relationships of individual sequences of the 39 members of the *Trimma hayashii* and *Trimma stobbsi* haplogroups (Block 3 of Fig. 2; group designations follow Table 1). Scale bar is 2% COI genetic distance.

A further potentially fruitful path of enquiry would be a detailed comparison of the live color patterns of the four haplogroups of *T. stobbsi*. In a previous study on the *T. tevegae* complex, Winterbottom (2016) found live color pattern to be very important in distinguishing amongst morphologically similar haplogroups of *Trimma*. Although we have yet to analyse the situation in detail, and we are thus uncertain of the constancy of color among specimens of *T. stobbsi* from various localities, Figure 4 provides us with some idea of the potential of color for differentiating groups. A freshly collected paratype from New Caledonia (Fig. 4A, type locality) is a light brown fish with darkened scale pockets with golden spots at the apices of the scales on the ventral and posterior parts of the body and a discontinuous cerise ring around the pupil with a very narrow yellow ring bordering the pupil. A specimen from the Philippines (Fig. 4B, no COI sample) is a darker brown with dark brick red spots at the scale apices and a brownish pink ring around the pupil; while a live specimen from FakFak, Indonesia has a rosy-grey body with a lighter, almost yellow head (Fig. 4C, no COI sample). A specimen from the Maldives (Fig. 4D, Group 2b) is overall red with a darker dorsum and elongate yellow spots on the scale apices and a diffuse reddish circle around the pupil which is darkest ventrally. The sample from the South West Islands of Palau (Sonsorol I., Fig. 4E, Group 2c) is light brown on the body and upper head, grading to yellowish-brown on the snout and cheek with no particular color at the scale apices, although the scale pockets are still strongly outlined with melanophores, and a black ovoid opercular spot. Specimens from Rabaul (Fig. 4F, Group 2d) are light brown posteriorly grading

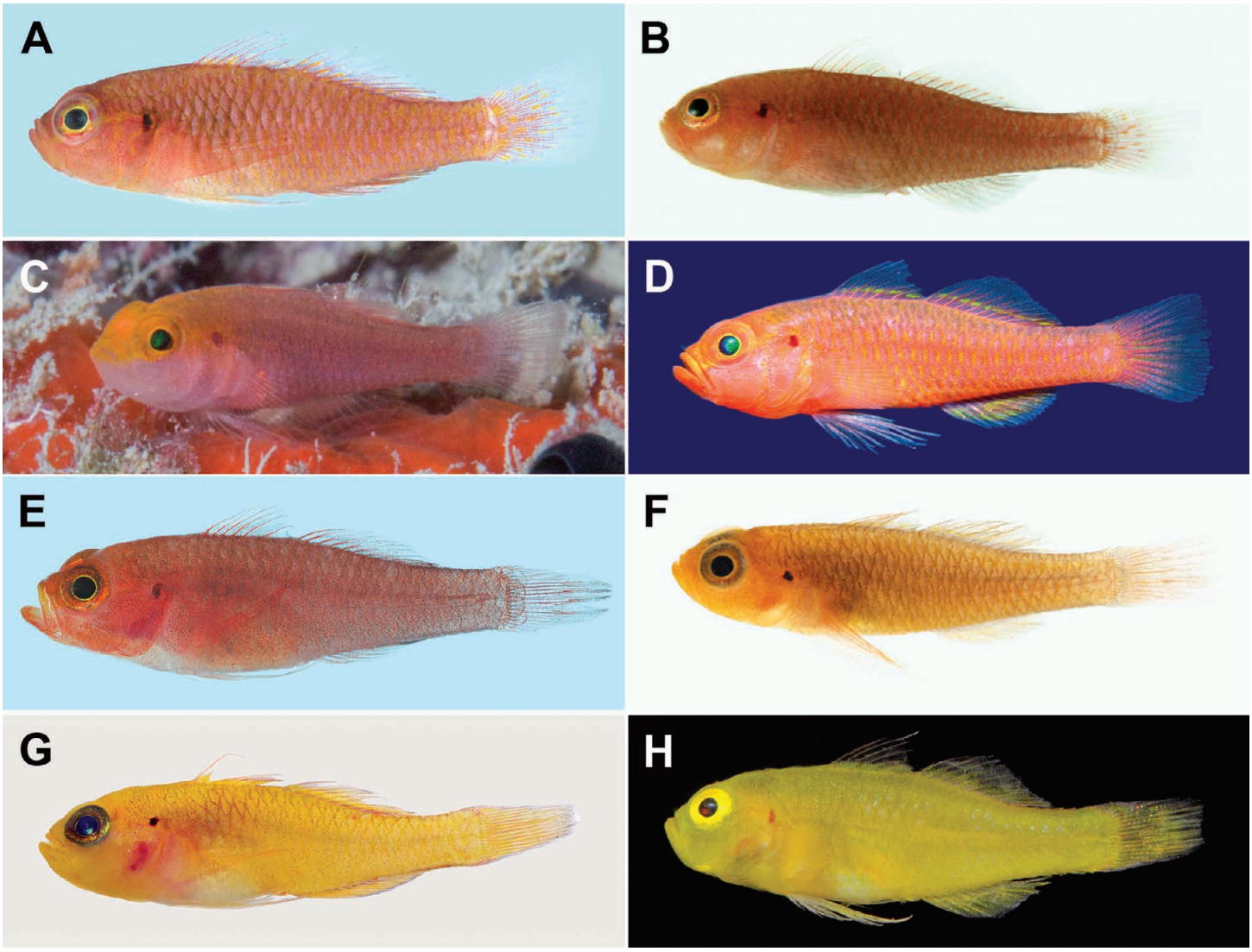


Figure 4. *Trimma stobbsi* from different localities to show variation in coloration (left lateral view): A) 20.3 mm SL paratype, ROM 63938, New Caledonia; B) 17.0 mm SL, ROM 49231, Mactan, Philippines; C) FakFak, Indonesia (specimen not collected); D) 15.1 mm SL, ROM 107896 Maldives; E) 14.9 mm SL, ROM 92100, Rabaul, PNG; F) 17.4 mm SL, ROM 82982, Sonsorol I., South West Islands, Palau; G) 15.3 mm SL, ROM 94024, Timor-Leste; H: ROM 94176, SE Maluku. Photographs A & B, E & F (R. Winterbottom); C & D, G & H (M.V. Erdmann).

to orangish-red on the head, with the apices of some scale pockets reddish brown and a vertically oval opercular spot. In samples from southeastern Indonesia and Timor-Leste, the head and body are predominantly yellow, with a sample from Timor-Leste (Fig. 4G, no COI sample) having the scale pockets on the dorsum outlined with melanophores and a round black spot above the rear of the operculum, while a sample from SE Maluku (Fig. 4H, no COI sample) is plain yellow with a vertically elongate red spot above the operculum. Further study on the degree to which these color differences might correspond to the various *T. stobbsi* haplogroups defined above is clearly warranted.

W14 found three haplogroups of what they identified as *T. stobbsi*, which formed a coordinate group with *T.* RW sp 97 and *T.* RW sp 98 (subsequently described and named as *T. bathum* and *T. kardium* respectively). Those two species are within a larger grouping containing the three haplogroups of *T. stobbsi* in this study. Compared to the W14 results, their Group 1 (= 2a in Table 1) has an extra sample from RA; their Group 2 (= 2c in Table 1) consisted of three samples from Palau with 0.0% variation (no change); and their Group 3 (= 2d in Table 1) consisted of 6 samples from the same localities with a within-group variance of 0.3%). There is now an additional haplogroup based on samples from the Maldive Islands (= 2b).

BLOCK 4. This block of haplogroups contains two single haplogroups each representing a described species, another species with two haplogroups, and a fourth, *T. milta* Winterbottom, 2002, with five discrete haplogroups. These fishes are primarily found in clear water on steep walls and drop-offs (with the notable exception of the types of *T. milta*, which were collected from somewhat silty lagoonal reefs). All four described species are separated by over 10% of the COI except for *T. anthrenum*, where the separation between it and *T. milta* groups

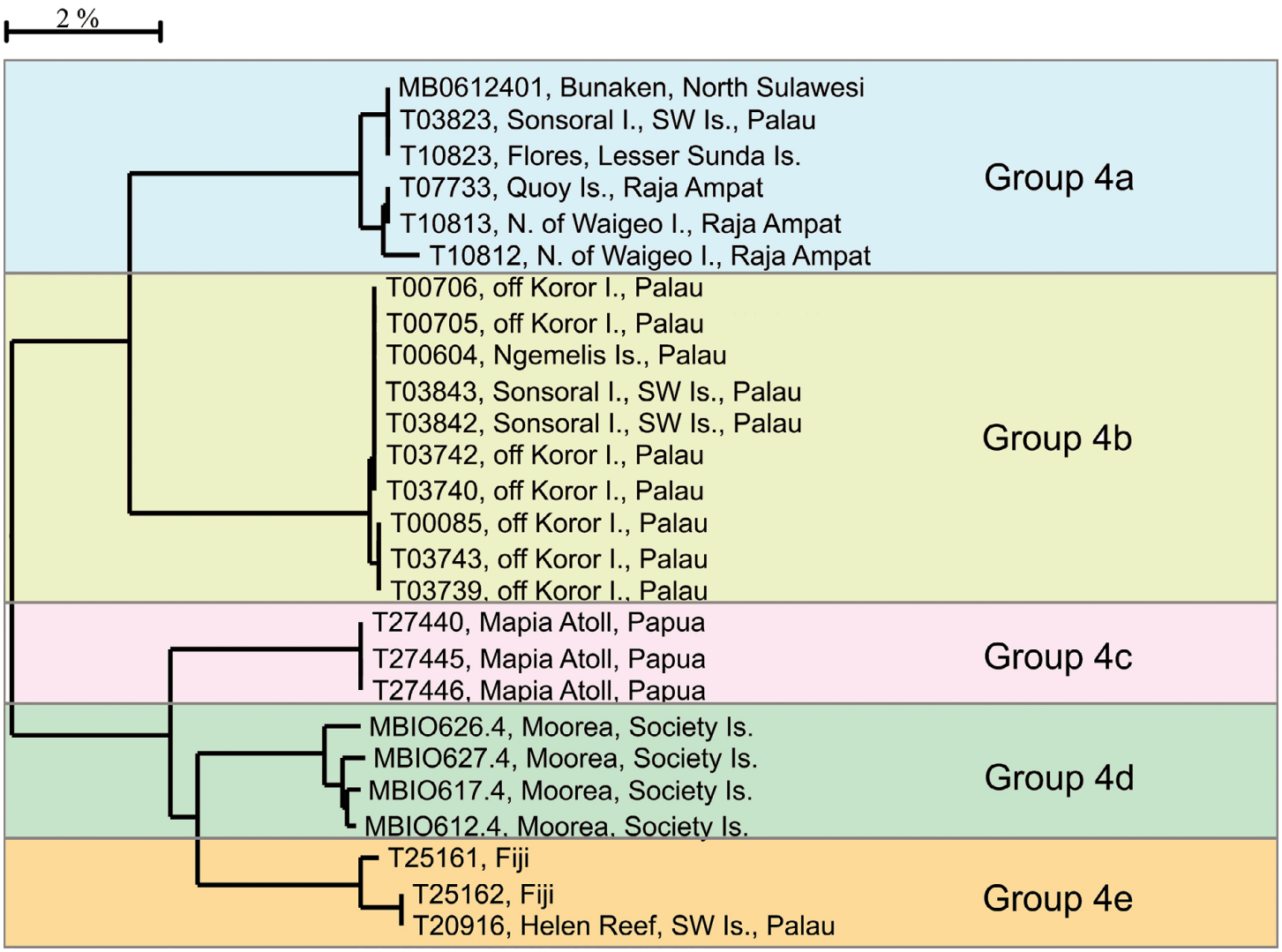


Figure 5. Phenetic relationships of individual sequences of the 26 members of the *Trimma milta* haplogroup (Block 4 of Fig. 2; group designations follow Table 2). Scale bar is 2% COI genetic distance.

TABLE 2

Distance summary barcode analysis of 39 *Trimma* specimens in Block 4 & Fig. 5
(minimum distance between groups)
Var.= maximum variation within group

Group		location	n	Var.	2a	2b	3	4a	4b	4c	4d	4e
1	*T. aturirii*	Indonesia- RA	5	1.4	10.7	10.6	13.3	14.8	14.6	14.5	14.2	15.8
2a	*T. hotsarihiensis*	Indonesia- Cenderawasih Bay & RA, Palau	6	0	–	2.2	9.3	12.6	12.5	11.8	11.7	12.1
2b	*T. hotsarihiensis*	Indonesia- Maluku Islands	1	n/a		–	9.2	12.5	12.4	11.7	11.6	12.0
3	*T. anthrenum*	Fiji	5	0			–	11.0	10.6	8.6	9.1	10.1
4a	*T. milta*	Indonesia- Flores & RA, Palau	6	1.3				–	6.1	9.1	9.0	9.5
4b	*T. milta*	Palau	10	0.2					–	9.1	7.9	8.3
4c	*T. milta*	Indonesia- Mapia Atoll	3	0						–	4.1	6.8
4d	*T. milta*	Society Islands	4	1.1							–	3.9
4e	*T. milta*	Fiji, Palau	3	1.6								–

4c and 4d is 8.6% and 9.1% respectively (see Table 2). There is no within-group variation in either haplogroup of *T. hotsarihiensis* Winterbottom, 2009. The samples from Cenderawasih Bay and from Helen Reef (SW Islands of Palau) show no variation between them, and are 2.2% different from the Maluku specimen. This suggests that a close comparison of the morphology of the two haplogroups may prove informative.

The five haplogroups identified as *T. milta* (Fig. 5) are all well separated from each other by 3.9–9.5%, including samples from the type locality (Moorea, Society Islands, 4d). These latter samples are phenetically closest to 4e, from Fiji and the South-West Islands of Palau, which has a within-group variance of 1.6%, with one of the two Fiji samples identical in COI to the Palauan sample (Fig. 4). One haplogroup (4c) is apparently confined to the offshore Mapia Atoll north of Cenderawasih Bay (aka Pulau Bras and Pulau Pengun), and which is 4.1% different from samples from the type locality. The largest sample (Group 4b, 10 specimens) is only from Palau, and has a very low within-group variation (0.2%). Interestingly, samples are from the main Palauan islands and the northernmost of the South West Islands, and the latter locality also yielded a sample of Group 4a. The southernmost of these islands (Helen Reef) has a representative of Group 4e. Group 4b is phenetically closest to Group 4a (minimum difference = 6.1%), a haplogroup which is widely spread from northern Sulawesi and Flores eastward to Raja Ampat and Sonsoral I. (one of the two northernmost of the South-West Islands of Palau). Detailed morphological and color studies of representatives of these five haplogroups are needed to assess these genetic differences in more detail.

W14 contained two samples of *T. hotsarihiensis*, both from Palau, which were phenetically closest to the *T. milta* complex of haplogroups. The additional five samples reported here are all from Indonesia (see above), and an extra haplogroup for the sample from the Maluku Islands is present. No specimens of *T. anthrenum* Winterbottom, 2006 or *T. aturirii* Winterbottom et al., 2015 were available at the time W14 was published. The W14 paper documented four haplogroups of *T. milta*. Their Group 2 (Fiji) has had a sample from Palau added (Group 4e), and a new haplogroup, Group 4c, from Mapia Atoll north of Cenderawasih Bay is added here.

BLOCK 5. There are four discrete haplogroups in this block (Fig. 2, Table 3). *Trimma haimassum* Winterbottom, 2011 (n = 7, variance = 0.3%) pairs phenetically with *T. sheppardi* Winterbottom, 1984 (n = 1), the two being separated by 9.3%, while *T. xanthum* (n = 3, variance = 0.2%) is phenetically closest to *T. yanoi* Suzuki & Senou, 2008 (n = 12, variance 0.3%) from which it is separated by a minimum of 9% (see Table 3). The haplogroup pairs are separated by about 11% of the COI bases. We note that all the species in this group are deeper bodied than other *Trimma*, (25% SL or more) and have accessory scales on the body (see, e.g. Winterbottom 2011:147, Fig. 16). These results are virtually identical with those reported in in W14 (where they were not discussed separately)

TABLE 3

Distance summary barcode analysis of 23 *Trimma* specimens in Block 5
(minimum distance between groups)
Var.= maximum variation within group

Group		location	n	Var.	2	3	4
1	*T. haimassum*	Indonesia- RA, PNG- Rabaul	7	0.3	9.3	11.2	11.2
2	*T. sheppardi*	Indonesia- RA	1	n/a	–	13.6	10.9
3	*T. xanthum*	Palau	3	0.2		–	9.0
4	*T. yanoi*	Indonesia- RA, PNG-Milne Bay & Rabaul, Palau, Philippines	12	0.3			–

with the exceptions: i) that *T.* RW sp 24 has now been formally named (as *T. xanthum*), ii) there is an additional specimen of *T. haimassum* but no new locality, and iii) there are an additional 6 samples of *T. yanoi*, with an increase of within-group variance to 0.3% and two new localities (Philippines and Milne Bay, PNG). Only in the case of *T. haimassum* are tissue samples available from the type locality.

BLOCK 6. There are five haplogroups divided among four recognized valid species in this block (Table 4). *Trimma striatum* (Herre, 1945) has two haplogroups, separated by a minimum of 3%, one from the Maldives (n = 2, no within-group variation) and the other from Palau and Raja Ampat (n = 6, within-group variation 1.3%). These two haplogroups are phenetically closest to *T. capostriatum* (Goren, 1981) (n = 11, 0.3% within-group variation), from which they are separated by a minimum of 10.4%. Also included in the block are *T. papayum* Winterbottom, 2011 (n = 3; 1.9% within-group variation primarily between the Solomon Island sample and the two from Raja Ampat), and *T. tauroculum* Winterbottom & Zur, 2007 (n = 6; within-group variation 1.1%) from Palau only. These two differ from each other by 14.3% and differ from the *T. striatum*/*T. capostriatum* haplogroups by a similar amount.

Essentially similar results for these species were reported by W14, although they did not have the Maldives samples of *T. striatum* and thus had only a single haplogroup under that name. Additionally, their two specimens of *T. papayum* were only from Raja Ampat, with no sample from the Solomon Islands.

BLOCK 7. The members of this assemblage of 10 haplogroups are currently identified as either *T. erdmanni* Winterbottom, 2011 or *T. chledophilum* Allen, 2015. Morphological differences between these two species consist primarily of minor variations in color pattern, and these are not visible in preserved specimens. However, one of us (MVE) has found subtle live color and/or habitat differences between several of these haplogroups. Differences in COI between the haplogroups are relatively trenchant (4.5–11.3% – see Table 5 and Fig. 6) except for the last four haplogroups, where they are between 2.4–3.3%.

TABLE 4

Distance summary barcode analysis of 28 *Trimma* specimens in Block 6
(minimum distance between groups)
Var.= maximum variation within group

Group		location	n	Var.	2a	2b	3	4
1	*T. capostriatum*	Australia- GBR, PNG- Rabaul	11	0.3	12.6	10.4	14.7	14.4
2a	*T. striatum*	Maldives	2	0	–	3.0	16.3	16.8
2b	*T. striatum*	Indonesia- RA, Palau	6	1.3		–	15.1	15.2
3	*T. papayum*	Indonesia- RA, Solomon Islands	3	1.9			–	14.3
4	*T. tauroculum*	Palau	6	1.1				–

Two groupings are of significant interest here. The haplogroups identified as *T. chledophilum* (Groups 2a and 2b) form a phenetic group with a haplogroup identified as *T. erdmanni* (Group 1d), with 7.7–8.6% differences between them. Two of these groups (2a and 2b) are syntopic (from the same crevice in the reef) in Milne Bay, PNG. The third haplogroup, phenetically closest to 2b, is also from PNG (Rabaul). No other haplogroups have been found in PNG to date. Two haplogroups (1a and 1b) are confined to the northern part of the island of New Guinea (Raja Ampat and Cenderawasih Bay), and a third (1c) is present in Palawan, Philippines and the Riau archipelago (northwestern Indonesia). Of the remaining four haplogroups, three (Groups 1e, 1f and 1g) are confined to the Lesser Sunda Islands, with syntopy between Groups 1e and 1g near the town of Bima, on the north coast of Sumbawa. Group 1e has also been found at Lembata, a scant 100 kms west of the location of Group 1g on Alor. Group 1g has also been found on the northwest coast of Bali. The relatively small genetic differences between these three groups (2.4–2.7%), coupled with their virtually continuous distribution along the Lesser Sunda island chain suggests caution in drawing any conclusions, and is further confused by the syntopy between

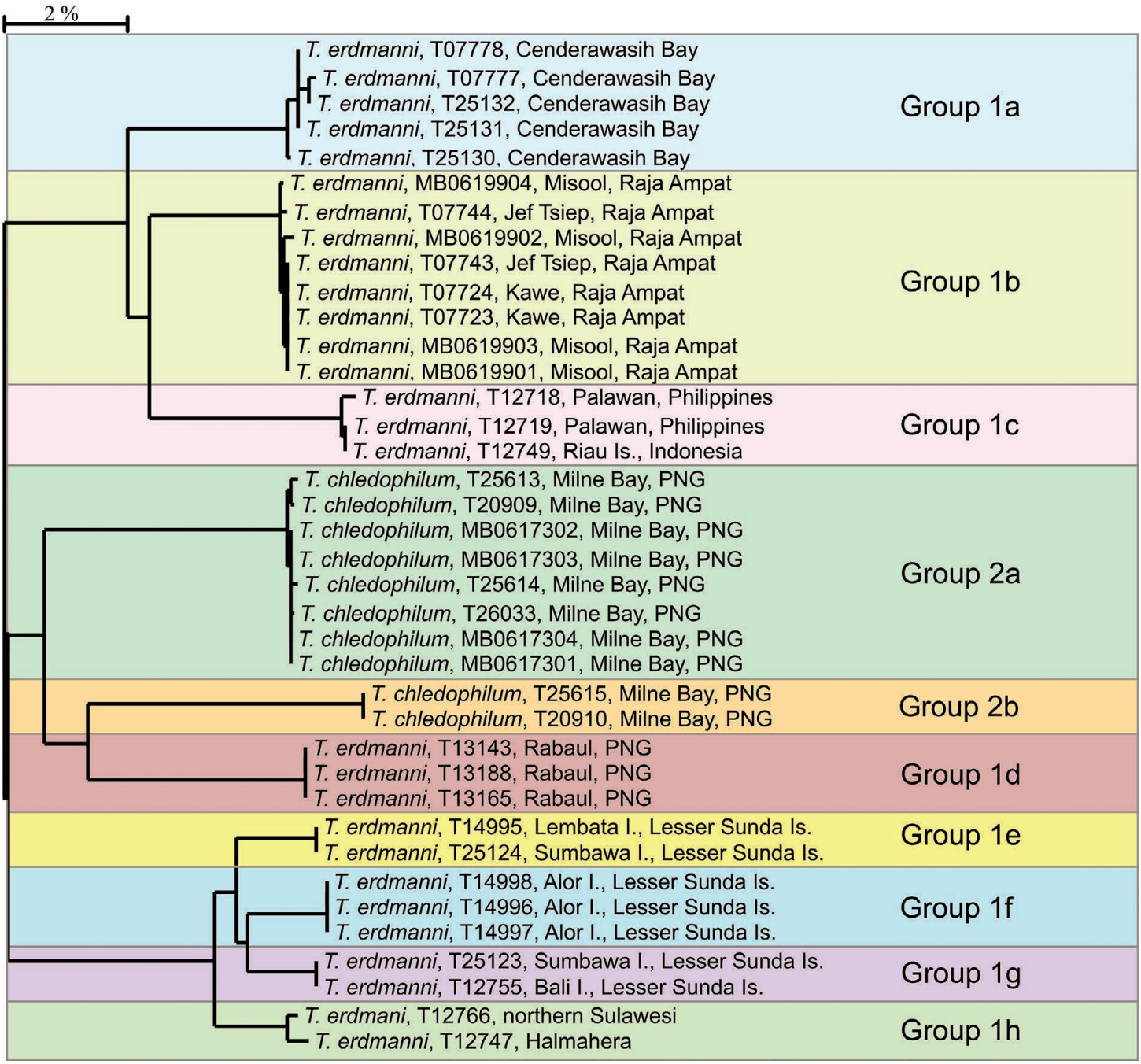


Figure 6. Phenetic relationships of individual sequences of the 38 members of the *Trimma erdmanni* and *T. chledophilum* haplogroups (Block 7 of Fig. 2; group designations follow Table 5). Scale bar is 2% COI genetic distance.

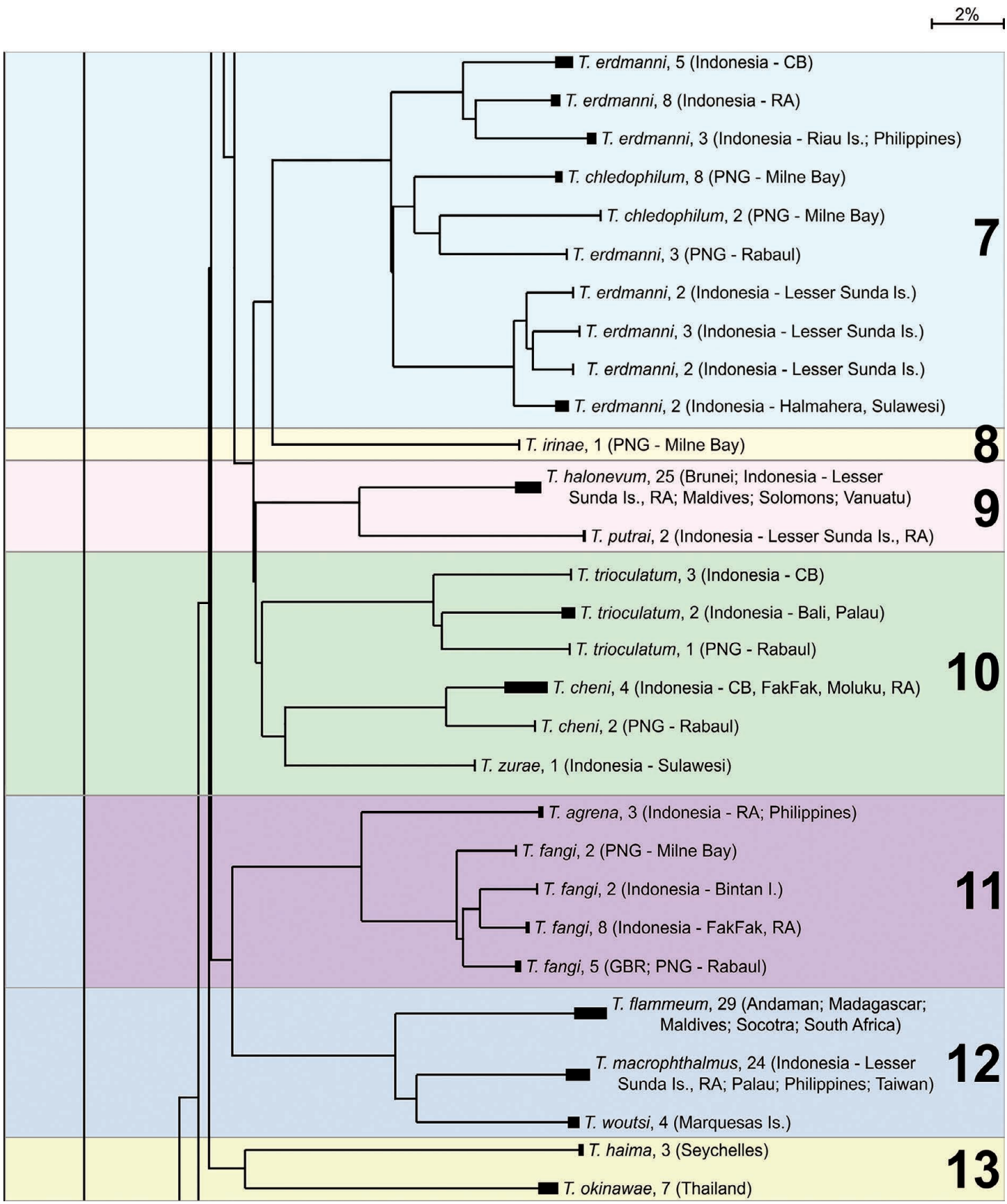


Figure 2 part 2 (Groups 7–13). Condensed Neighbour-joining network of the COI gene based on an analysis of 849 specimens of *Trimma*. Solid bars at tips of branches represent approximate within-group variation. Species names are followed by the number of specimens with locality(ies) of the specimens in parentheses. Colored blocks and numerical notations refer to the sequential groups discussed in the text, additional groups continue on subsequent pages. Scale bar is 2% COI genetic distance.

TABLE 5

Distance summary barcode analysis of 38 *Trimma* specimens in Block 7 & Fig. 6
(minimum distance between groups)
Var.= maximum variation within group

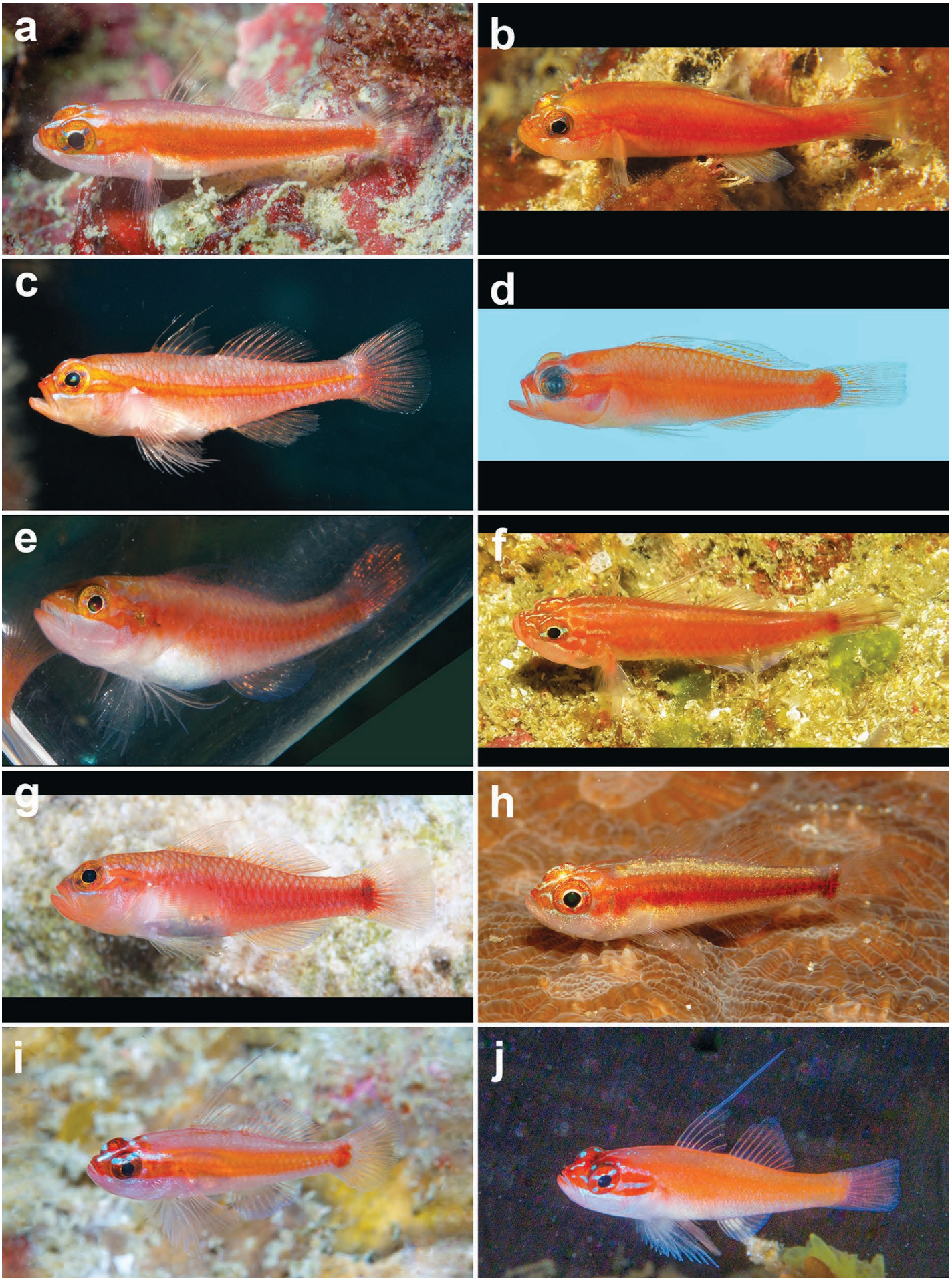
Group		location	n	Var.	1b	1c	2a	2b	1d	1e	1f	1g	1h
1a	*T. erdmanni*	Indonesia- Cenderawasih Bay	5	0.5	4.5	6.5	9.8	9.8	10.1	10.2	9.9	9.8	9.3
1b	*T. erdmanni*	Indonesia- RA	8	0.3	–	5.1	9.1	7.5	8.4	9.1	8.4	9.3	8.8
1c	*T. erdmanni*	Indonesia- Riau, Philippines	3	0.3		–	11.0	9.5	10.6	11.0	10.6	11.2	10.3
2a	*T. chledophilum*	Papua New Guinea- Milne Bay	8	0.5			–	8.6	8.1	9.1	8.8	8.4	8.6
2b	*T. chledophilum*	Papua New Guinea- Milne Bay	2	0				–	7.7	11.3	10.9	11.3	11.1
1d	*T. erdmanni*	Papua New Guinea- Rabaul	3	0					–	10.1	10.0	9.5	9.5
1e	*T. erdmanni*	Indonesia- Lesser Sunda I.- Lembata & Sumbawa	2	0						–	2.7	2.5	3.3
1f	*T. erdmanni*	Indonesia- Lesser Sunda I.- Alor	3	0							–	2.4	2.8
1g	*T. erdmanni*	Indonesia- Lesser Sunda I.- Sumbawa & Bali	2	0								–	2.5
1h	*T. erdmanni*	Indonesia- Halmahera, Sulawesi	2	0.5									–

Groups 1e and 1g. The final group (Group 1h) is very similar to these three haplogroups (2.5–3.3% different), and is geographically situated nearby on the NE coast of Sulawesi, and at Halmahera.

In the previous study (W14), four haplogroups were recovered. Group 1 (= Group 1b here) consisted of four samples from Raja Ampat (now 8 samples), Group 2 (= Group 1a) consisted of two samples (now 5) from Cenderawasih Bay; there were two samples from Palawan in their Group 3 (= Group 1c here, where an additional sample from the Riau Islands of Indonesia has been added), and their Group 4 from Rabaul (our Group 1d) consisted, as here, of three samples. Thus, this paper adds another 6 haplogroups. Two of these were identified as *T. chledophilum*, and this represents an additional named valid species described after W14 was published. The other four consist of the three haplogroups from the Lesser Sunda Islands (Groups 1d, 1e and 1f) and the group from Sulawesi/Halmahera (Group 1h). Clearly a huge amount of work, both morphological and genetic, needs to be undertaken, and many further samples from the geographic range of this block of haplogroups are needed before we can have any chance of forming a clear idea of its diversity, evolution and biogeography.

One of us (MVE) has observed certain color and habitat differences that appear to hold up in our analysis (Fig. 7). We include his observations here as an example of the potential power of live/fresh coloration to help separate haplogroups among *Trimma*. For example, there is considerable variation in the form and coloration of the light stripes on the head and the degree to which the scale pockets on the body are outlined in haplogroups that are well separated in the BOLD analysis (Fig. 7, a–d). The situation in the coloration of the two samples labelled as *T. chledophilum* here (Groups 2a and 2b) has been analysed in great detail by MVE, based on over 100 underwater photographs of the live fishes. He found that the true *T. chledophilum* (Group 2a) has only one light "stripe" across the iris, always above the pupil, and almost always in the form of two separated iridescent white/blue spots on either side above pupil, whereas *T. chledophilum* (Group 2b) has a complete iridescent white/blue stripe above the pupil (often times intersecting the pupil), and another below the pupil. The completeness of these two stripes is

Figure 7 (opposite page). Images of the haplogroups of the *Trimma erdmanni-T. chledophilum* complex (Block 7). a) *T. erdmanni* Group 1a, Cenderawasih Bay, Indonesia; b) *T. erdmanni* Group 1b, Kawe Island. Raja Ampat, Indonesia; c) *T. erdmanni* Group 1c, Riau Islands., Indonesia; d) *T. erdmanni* Group 1d, Rabaul, New Britain, Papua New Guinea; e) *T. erdmanni* Group 1e, Lembata, Lesser Sunda Islands, Indonesia; f) *T. erdmanni* Group 1f, Alor, Lesser Sunda Islands, Indonesia; g) *T. erdmanni* Group 1g, Bali, Lesser Sunda Islands, Indonesia; h) *T. erdmanni* Group 1h, Halmahera, Indonesia; i) *T. chledophilum* Group 2a, Sideia Reef, Milne Bay, Papua New Guinea; j) *T. chledophilum* Group 2b, Sideia Reef, Milne Bay, Papua New Guinea. All photographs by M.V. Erdmann, except d (R. Winterbottom) and f (J. Eyre).


a
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variable, but there is always a bottom stripe in Group 2b, while it is never present in Group 2a. Both species have a darkened red/orange pair of stripes extending to the operculum that all members of the *T. erdmanni* complex seem to have, but in Group 2a the two stripes converge into a "V", and have at most a tiny bit of iridescent blue/white coloration in the "V", whereas in Group 2b the two stripes are generally parallel and do not converge, or if they do converge there is always a definitive iridescent white/blue stripe between the two red stripes. Finally, if one looks head-on or down on the head, in Group 2b, there is always a thin center stripe (iridescent white/blue) that is uninterrupted and runs from snout to level with the back of the eye, and there are always additional small iridescent white spots on snout lateral to the midline. In Group 2a, the center stripe is variable– in some cases it is broken into several "blobs", but more often than not it starts out relatively wide, narrows significantly between the eyes, and then ends. Further posteriorly, behind the eye, is a separate and slightly wider dark blotch. Group 2a never has additional iridescent spots on the snout lateral to the single midline. It is unfortunate that we do not currently have images of live specimens of *T. erdmanni* Group 1d (from Rabaul, Papua New Guinea) which is closer phenetically to *T. chledophilum* Group 2b than either is to Group 2a, for comparison of the detailed coloration of the head.

In addition, MVE has observed that the habitats of *T. erdmanni* in the Lesser Sunda Islands consist of murky water bays with freshwater influx separated by long stretches of exposed coastline, and the samples which are found there (Fig. 7, e–g) are marginally differentiated in their COI (2.4–2.7%, Table 5). Initially we thought these shallow divisions might represent very recent/incomplete speciation events, being driven by the apparent genetic isolation generated by the habitat allopatry. While that hypothesis remains viable and testable, we note that one of the two samples from Sumbawa I., Lesser Sunda Is. in Group 1e (T25124) was collected in the same small cave at the same time as one of the two samples in Group 1g (T25123). Furthermore, in each case the Sumbawa specimens have an identical sequence of base pairs to the other sample in their respective groups (T14995 from Lembata I. in Group 1e, and T12755 from Bali in Group 1g). Thus, if Groups 1e and 1g do represent different species, it would represent the third example of true syntopy known in *Trimma*. And if the situation represents incipient speciation, then it would suggest assortative mating, because the speciation process is incomplete. The situation questions the validity of the separation of Groups 1 e–g as taxonomic units, and suggests they may form a single, highly variable haplogroup. Obviously, much more collecting and documenting of *T. erdmanni*-like fishes throughout the Lesser Sunda Islands and adjacent areas is necessary before we can have any chance of solving this issue.

BLOCK 8. This block consists of a single haplogroup (and a single sample), *T. irinae* Winterbottom, 2014. The specimen is phenetically well separated from all other species, the closest being *T. erdmanni* Group 1b from Raja Ampat. *Trimma irinae* is separated from that haplogroup by about 14% of the COI base pairs.

BLOCK 9. Two named species, each of a single haplogroup, constitute this block. *Trimma halonevum* Winterbottom, 2000 (n = 25, within-group variation = 1.6%) differs by 10.8% from *T. putrai* Winterbottom et al., 2019 (n = 2, within-group variation = 0.2%). Although the samples of *T. putrai* available were both from Raja Ampat, those of *T. halonevum* were from widely separated localities, with possible geographic structure. Geographic sampling includes the Maldives (n = 3, no variation), Vanuatu (1), and 21 samples from Brunei; Indonesia in Bali, Cenderawasih Bay, Flores, Raja Ampat, and Sumbawa; the Solomon Islands; and Timor-Leste. The Maldives samples differ from the Vanuatu sample by 1.2%, and by 1.1% from the remaining samples. It is not clear whether there is any geographic significance to the Vanuatu sample differences, although that evinced by the Maldives samples seems correlated with several other haplogroup differentiations.

W14 reported on only 8 samples from Bali and Raja Ampat, Indonesia, Timor-Leste and Vanuatu, so all additional categories for *T. halonevum* listed above are new. No samples of *T. putrai* were included, as that species had not yet been described.

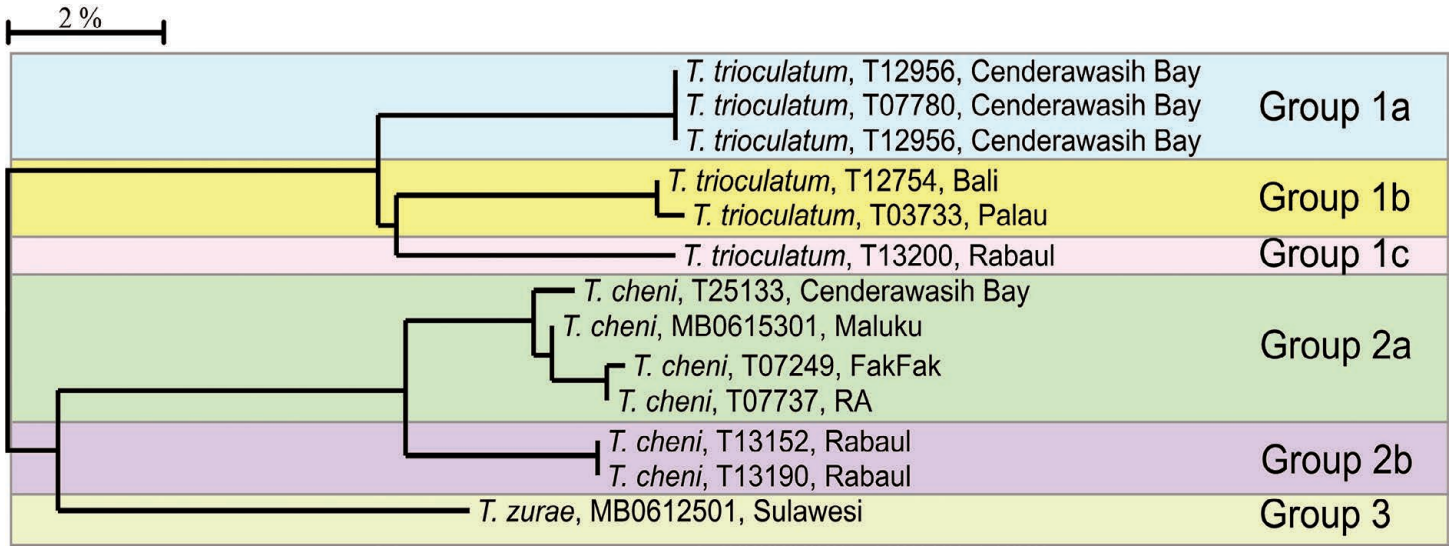

Figure 8. Phenetic relationships of individual sequences of the 13 members of the *Trimma trioculatum, T. cheni* and *T. zurae* haplogroups (Block 10 of Fig. 2; group designations follow Table 6). Scale bar is 2% COI genetic distance.

BLOCK 10. Specimens in this block were identified as *T. trioculatum*, with three haplogroups, *T. cheni* Winterbottom, 2011 with two, and *T. zurae* Winterbottom et al., 2014b with a single haplogroup (Table 6).

Trimma trioculatum consists of three haplogroups, with one from Cenderawasih Bay, Indonesia (Group 1a, n = 3, variation = 0.0%) separated from a second haplogroup from Bali and Palau (Group 1b, n = 2, variation = 0.3) by 7.4% and from the third haplogroup from Rabaul, Papua New Guinea (Group 1c, n = 1) by 7.7%. Groups 1b and 1c are separated by 7% (Fig. 8). The species is separated by 14.1–15.6% from the other two species in this block. These three haplogroups match up well with the color differences described for samples identified as this species in the original description (Winterbottom et al. 2015). *Trimma cheni* consists of two haplogroups separated by 4.0%, one confined to Rabaul, Papua New Guinea (Group 2b, n = 2, 0% variance), the other consisting of four samples (2% variance) all from around the Bird's Head region of Indonesian New Guinea (Maluku, FakFak, Raja Ampat and Cenderawasih Bay; Fig. 8).

In W14, *T. trioculatum* is referred to as *T.* RW sp 32. An additional sample of our Group 1a (their Group 1) from Cenderawasih Bay is included here, as well as a sample from Bali in Group 1b (their Group 2). Only three samples of *T. cheni* were analysed in W14, and the same two haplogroups were present. Two of these samples represent the Rabaul specimens above, and the single sample was from Raja Ampat (the type locality). This paper adds three samples and three new localities to the latter, and decreases the minimum difference between the two haplogroups from 4.3% to 4.0%. No specimens of *T. zurae* were included in W14.

TABLE 6

Distance summary barcode analysis of 13 *Trimma* specimens in Block 10 & Fig. 8
(minimum distance between groups)
Var.= maximum variation within group

Group		location	n	Var.	1b	1c	2a	2b	3
1a	*T. trioculatum*	Indonesia- Cenderawasih Bay	3	0	7.4	7.7	14.1	15.6	15.3
1b	*T. trioculatum*	Indonesia- Bali, Palau	2	0.3	–	7.0	14.6	15.0	15.0
1c	*T. trioculatum*	Papua New Guinea- Rabaul	1	n/a		–	15.1	15.0	15.0
2a	*T. cheni*	Indonesia- Cenderawasih Bay, FakFak, Maluku Is. & RA	4	2.0			–	4.0	12.3
2b	*T. cheni*	Papua New Guinea- Rabaul	2	0				–	11.7
3	*T. zurae*	Indonesia- Sulawesi	1	n/a					–

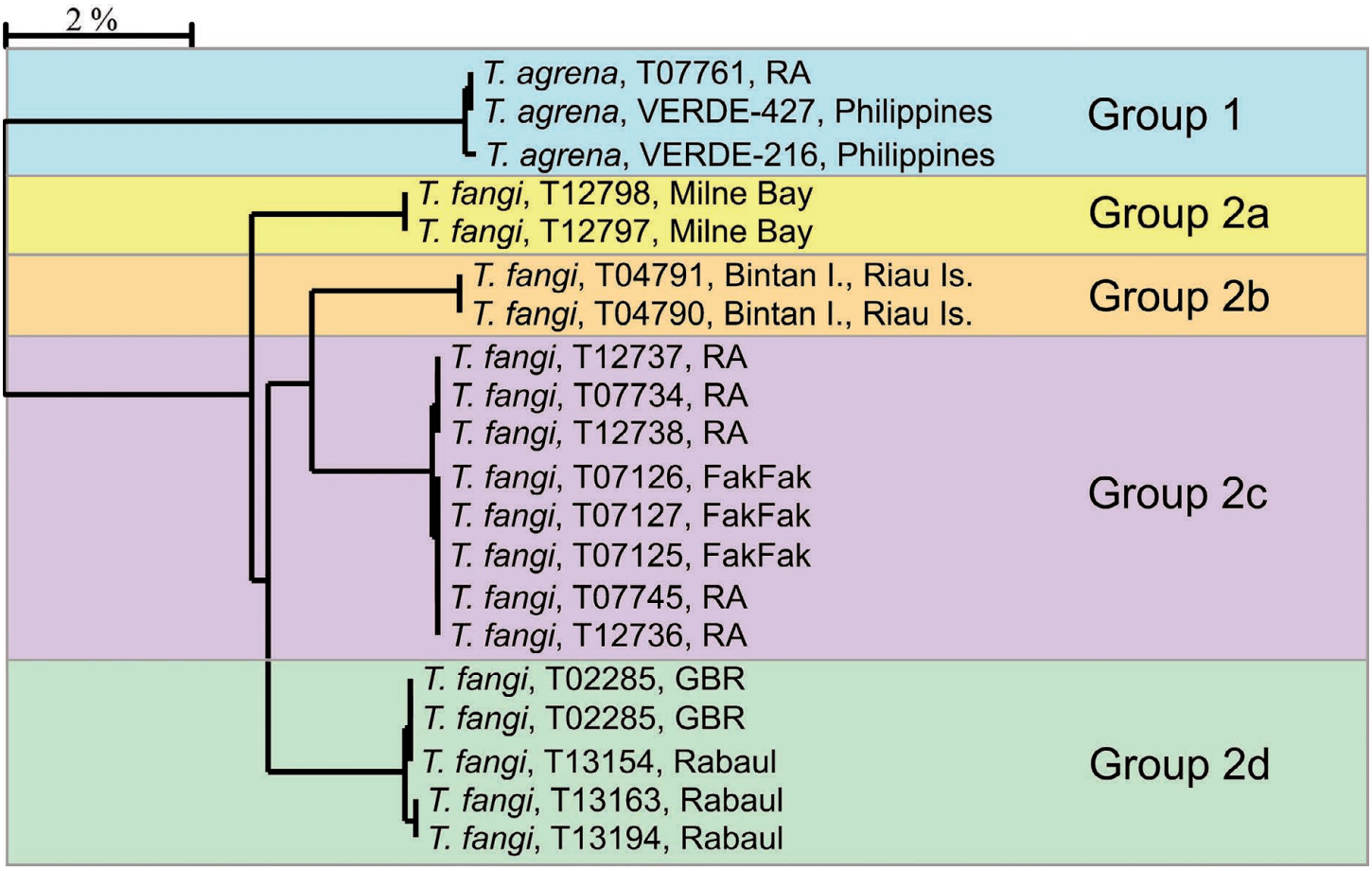

Figure 9. Phenetic relationships of individual sequences of the 20 members of the *Trimma agrena* and *T. fangi* haplogroups (Block 11 of Fig. 2; group designations follow Table 7). Scale bar is 2% COI genetic distance.

BLOCK 11. Five haplogroups are divided into two species, *T. agrena* Winterbottom & Chen, 2004 with one, and *T. fangi* Winterbottom & Chen, 2004 with four. Within-group variation in these group is minimal (a maximum of 0.2%; see Table 7). *Trimma agrena* differs from the four haplogroups of *T. fangi* by 8.6–9.6%. Differentiation between those four haplogroups varies from 2.8–3.7%. Although the differences between the *T. fangi* haplogroups is relatively low, the small amount of within-group variation even where more than one locality is involved (see, e.g., Groups 2c and 2d, Fig. 9) may be correlated with genetic isolation, and needs to be tested against the morphology. At least some color differences have been noted by MVE between Groups 2a and 2c.

W14 found two haplogroups of *T. fangi*, one consisting of the five samples from Raja Ampat (our Group 2c) and the other of the five samples from the Great Barrier Reef plus Rabaul (our Group 2d). This study adds a further three samples from FakFak to Group 2c, as well as two new haplogroups (Groups 2a and 2b). Although we do not have samples from the type locality itself (Pulau Bajau, Riau Islands), Group 2b is from its general vicinity (about 300 kms southwest).

TABLE 7

Distance summary barcode analysis of 20 *Trimma* specimens in Block 11 & Fig. 9
(minimum distance between groups)
Var.= maximum variation within group

Group		location	n	Var.	2a	2b	2c	2d
1	*T. agrena*	Indonesia- Raja Ampat, Philippines	3	0.2	9.6	9.1	9.3	8.6
2a	*T. fangi*	Papua New Guinea- Milne Bay	2	0	–	3.2	3.7	3.5
2b	*T. fangi*	Indonesia- Riau Islands	2	0		–	2.8	3.3
2c	*T. fangi*	Indonesia- Raja Ampat & FakFak Peninsula	8	0.2			–	3.4
2d	*T. fangi*	Australia- Great Barrier Reef, Papua New Guinea- Rabaul	5	0.2				–

TABLE 8

Distance summary barcode analysis of 56 *Trimma* specimens in Block 12
(minimum distance between groups)
Var.= maximum variation within group

Group		location	n	Var.	2	3
1	*T. flammeum*	Andaman Islands, Madagascar, Maldives, Socotra, South Africa	29	1.1	9.0	10.6
2	*T. macrophthalmus*	Indonesia: Raja Ampat & Komodo & Sumbawa & Wetar, Palau, Philippines, Taiwan	23	0.6	–	7.8
3	*T. woutsi*	Marquesas Islands	4	0.5		–

BLOCK 12. The three species listed here form three well separated haplogroups (between 7.8–10.6%; Table 8). While within-group variation seems fairly high, there are large numbers of samples for each of the first two haplogroups, and 0.3% of the total variance of 1.1% for *T. flammeum* (Smith, 1959) is derived from a single sample from the Seychelles (one of two samples from that locality). Similarly, two samples of *T. macrophthalmus* (Tomiyama, 1936), one from Raja Ampat, the other from Taiwan, contribute nearly 0.4% of the overall within-group variance of 0.6%.

No specimens of *T. woutsi* Winterbottom, 2002 were available for the W14 study, which included only the first two species (27 and 15 samples respectively). The two additional specimens of *T. flammeum* are from the new localities of Socotra and the Maldives, while the additional specimens of *T. macrophthalmus* are from the Philippines and from the Lesser Sunda Islands. The degree of separation between these two species remains an identical 9%. W14 mentioned morphological differences involving color between the latter two species (as unpublished results). Those findings are now available (Winterbottom & Hoese 2015).

BLOCK 13. There are two discrete haplogroups in this block, with little in common morphologically. Three samples of *T. haima* Winterbottom, 1984 from the Seychelles have a within-group variance of 0.2% and are separated by 17.4% from 7 samples identified as *T. okinawae* (Aoyaga, 1949) from Thailand (variance of 0.9%). Seven additional haplogroups identified as *T. okinawae* or *T. readerae* Winterbottom & Hoese, 2015 occur elsewhere in the analysis (Block 17). Current but uncompleted studies by RW of the comparative morphology of this haplogroup currently assigned to *T. okinawae* demonstrate that it has several anatomical/color differences from samples from the type locality (Japan), and the Thailand samples are in the process of being formally described as a new species.

BLOCK 14. This block consists of four haplogroups all identified morphologically as *T. benjamini* Winterbottom, 1996 (Fig. 10 & Table 9). Two of the haplogroups (Groups 1a and 1b) consist of samples from a single locality each. Group 1a (2 samples, 0.25% variance) is from Upolu, Samoa and differs from the other three haplogroups by between 7.2–8.2%. Group 1b, with four samples and 0.5% variance is so far confined to the steep outer reefs of the main islands of Palau. The remaining two haplogroups occur in several different localities, but are allopatric

TABLE 9

Distance summary barcode analysis of 30 *Trimma* specimens in Block 14 & Fig. 10
(minimum distance between groups)
Var.= maximum variation within group

Group		location	n	Var.	1b	1c	1d
1a	*T. benjamini*	Samoa	2	0.3	7.2	8.1	8.2
1b	*T. benjamini*	Palau	4	0.5	–	3.4	5.5
1c	*T. benjamini*	Indonesia- Raja Ampat & Sumbawa, Philippines	13	2.1		–	5.1
1d	*T. benjamini*	Australia- GBR, Indonesia- Mapia Atoll, NC, PNG- Milne Bay & Rabaul, Palau	11	1.3			–

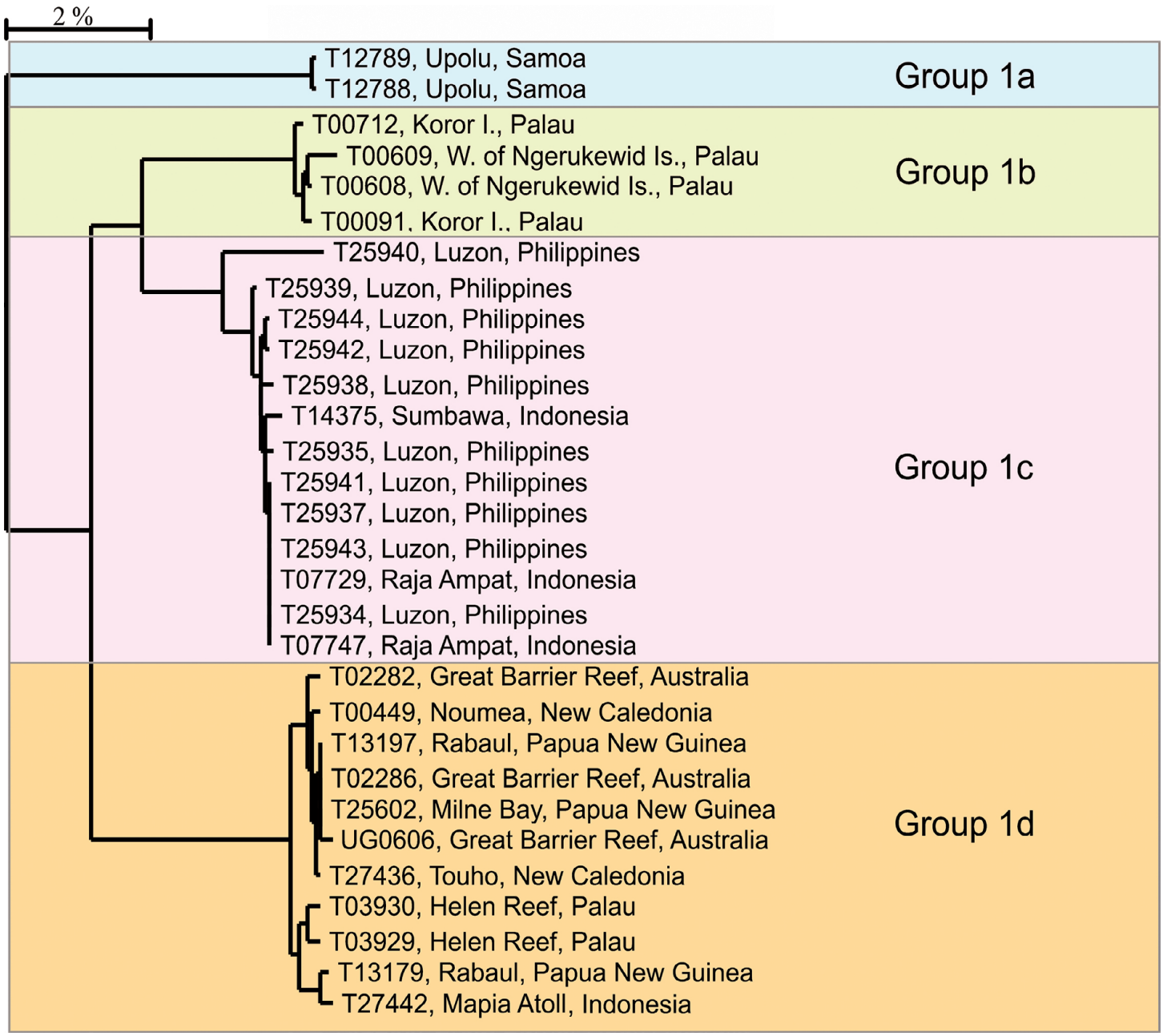

Figure 10. Phenetic relationships of individual sequences of the 30 members of the *Trimma benjamini* haplogroups (Block 12 of Fig. 2; group designations follow Table 9). Scale bar is 2% COI genetic distance.

distributed. Group 1c (13 samples, 2.1% variance) is found from the area of Luzon in the Philippines southwest to Sumbawa and east from there to Raja Ampat. Note that most of the within-group variance in this haplogroup can be attributed to a single sample from Luzon (Fig, 8, T25940). Group 1d (11 samples, 1.3% variance) occurs from Helen Reef in the South-West Islands of Palau via Mapia Atoll (north of Cenderawasih Bay) to Rabaul, New Caledonia, the Great Barrier Reef and Milne Bay.

In W14, there were no samples from Samoa (our Group 1a). Our Group 1b was present as their Group 1 with all the same samples. Their Group 2 (our Group 1c) consisted solely of two samples from Raja Ampat, and lacked any specimens from the Philippines. We obtained samples of *T. benjamini* from the vicinity of Luzon, which fall into a group with the specimens from Sumbawa (Lesser Sunda Islands) and Raja Ampat. The type locality for the species is Siquijor Island, south of Oriental Negros, and about 550 kms southeast of the Philippine genetic samples. Nonetheless, it appears that Group 1c most probably represents the type species. W14's Group 3 is equivalent to our Group 1d, to which we have added samples from Mapia Atoll (north of Cenderawasih Bay) and Milne Bay (Papua New Guinea).

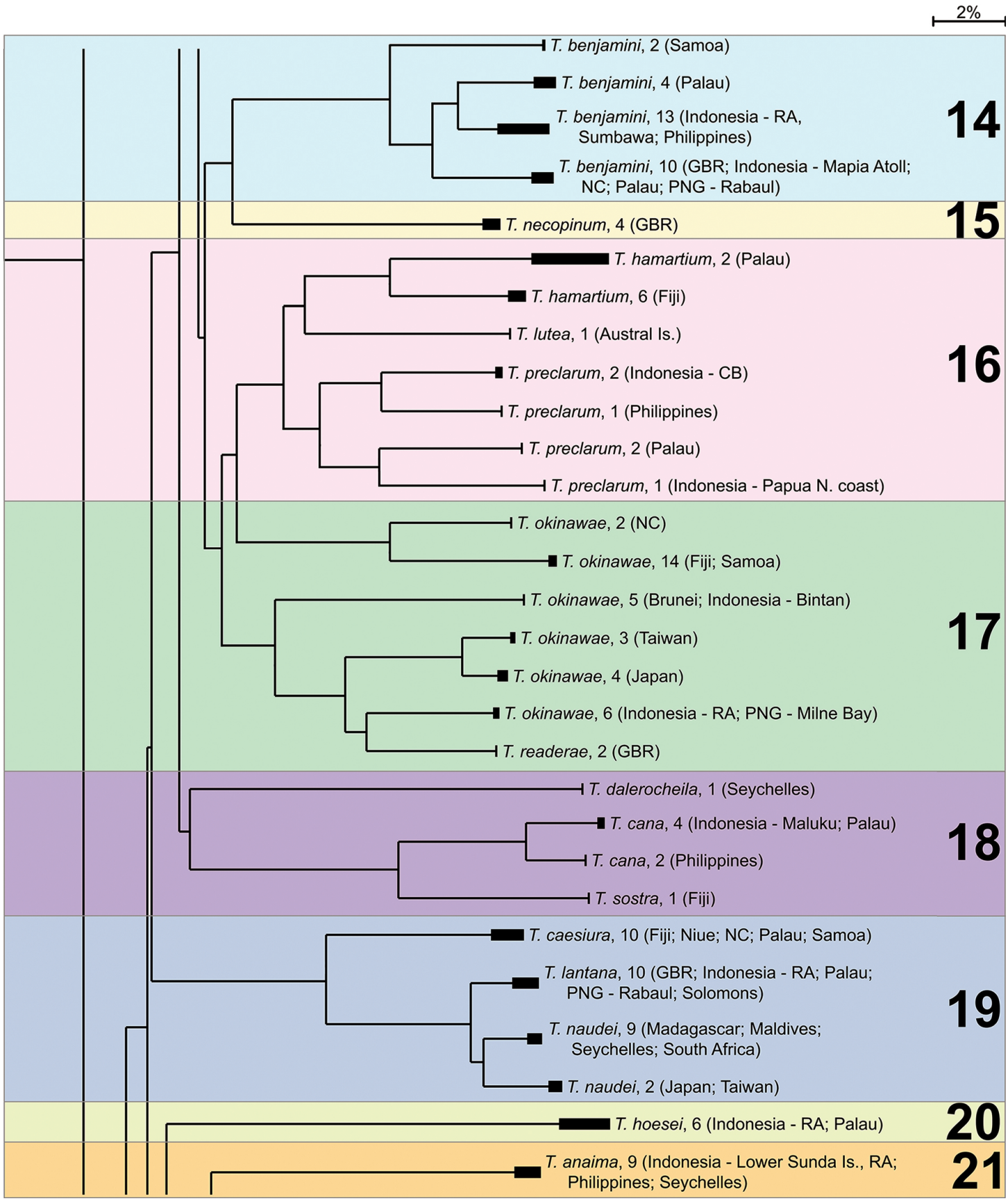

Figure 2 part 3 (Groups 14–21). Condensed Neighbour-joining network of the COI gene based on an analysis of 849 specimens of *Trimma*. Solid bars at tips of branches represent approximate within-group variation. Species names are followed by the number of specimens with locality(ies) of the specimens in parentheses. Colored blocks and numerical notations refer to the sequential groups discussed in the text, additional groups continue on subsequent pages. Scale bar is 2% COI genetic distance.

BLOCK 15. This block consists of a single haplogroup and species, *T. necopinum* (Whitley, 1959), from the Great Barrier Reef. The same four samples as utilized by W14 are included here, with the same within group variance of 0.5%.

BLOCK 16. Seven haplogroups (see Table 10) were found among samples identified as *T. hamartium* Winterbottom, 2018 (2), *T. lutea* Viviani et al., 2016 (1), and *T. preclarum* Winterbottom, 2006 (4). The samples of *T. hamartium* from the South-West Islands of Palau were found among specimens originally preserved for otolith studies (see Winterbottom et al. 2011). Five specimens were utilized, but only two returned sequences. All of these samples were originally preserved in 80% ethanol. This may explain the limited length of sequences retrieved (510bp and 596bp) and the high degree of ambiguity, up to about 11.3% of the base pairs in T20915, and hence the large within-group variance (2.4%). For this reason, the two samples are not regarded as separate haplogroups despite the 2.4% difference between them. These samples differ from those from Fiji (n = 6, variance 0.8%) by 8.2%. They differ from *T. lutea* by about 11–13%. The latter species comes out phenetically closest to *T. emeryi* Winterbottom, 1985 in Viviani et al.'s (2016) analysis– a relationship not found here.

Our samples identified as *T. preclarum* consist of four haplogroups. Two samples from the main islands of Palau correspond to the type locality of the species. The other haplogroups are from the Philippines, Cenderawasih Bay, Indonesia and from Depapre Bay on the northeast coast of Papua, Indonesiaabout 600 kms due east of Cenderawasih Bay. These haplogroups differ from each other by 6.6–11.2%.

W14 included only the two samples identified as *T. preclarum* from the main Palauan Islands in their analysis. The present results thus include two species new to the analysis (one with two haplogroups, and both of which were described after W14 was published), as well as three new haplogroups identified morphologically as *T. preclarum*.

TABLE 10

Distance summary barcode analysis of 15 *Trimma* specimens in Block 16
(minimum distance between groups)
Var.= maximum variation within group

Group		location	n	Var.	1b	2	3a	3b	3c	3d
1a	*T. hamartium*	Palau	2	2.4	8.2	12.8	12.5	12.7	16.4	10.6
1b	*T. hamartium*	Fiji- Lau Islands	6	0.8	–	10.9	11.0	10.8	12.4	11.8
2	*T. lutea*	Austral Islands	1	n/a		–	11.2	14.0	14.2	14.4
3a	*T. preclarum*	Indonesia- Cenderawasih Bay	2	0.3			–	6.6	10.4	10.6
3b	*T. preclarum*	Philippines	1	n/a				–	11.2	10.9
3c	*T. preclarum*	Palau	2	0					–	8.5
3d	*T. preclarum*	Indonesia- Papua- Depapre Bay	1	n/a						–

BLOCK 17. This block contains 7 haplogroups, 6 identified as *T. okinawae*, the other as *T. readerae* Winterbottom & Hoese, 2015 (Table 11). The two species are difficult to tell apart morphologically. We note here that a seventh haplogroup was identified as *T. okinawae* based on morphology. That haplogroup falls out into Block 13 (see above). Two additional aspects of the results seem worthy of comment: 1) the within-group variation for all is relatively low given the number of samples (0–0.3%); 2) the distances between haplogroups are mostly well over 10%, with the exception of group 1d (Taiwan) versus 1e (Japan), where the distance is 2.4%.

W14 also reported a total of 8 haplogroups identified as *T. okinawae*, based on 28 samples, with their Group 2 from Thailand again linked phenetically to *T. haima* and well separated from the other samples. At the time of W14's study, *T. readerae* (our Group 2 above) had not yet been formerly named, and was referred to as *T. okinawae* Group 8. Their Group 3, based on four samples from Fiji, has here been expanded to 10 samples from

TABLE 11

Distance summary barcode analysis of 36 *Trimma* specimens in Block 17
(minimum distance between groups)
Var.= maximum variation within group

Group		location	n	Var.	1b	1c	1d	1e	1f	2
1a	*T. okinawae*	New Caledonia	2	0	7.4	16.2	14.6	14.8	15.6	15.3
1b	*T. okinawae*	Fiji, Samoa	14	0.2	–	18.0	16.3	16.8	16.6	7.1
1c	*T. okinawae*	Brunei, Indonesia- Bintan Island	5	0		–	13.3	13.7	13.1	11.6
1d	*T. okinawae*	Taiwan	3	0.3			–	2.4	8.9	7.8
1e	*T. okinawae*	Japan	4	0.3				–	8.9	7.9
1f	*T. okinawae*	Indonesia- Raja Ampat, Papua New Guinea- Milne Bay & Rabaul	6	0.2					–	7.1
2	*T. readeri*	Australia- Great Barrier Reef	2	0.2						–

Fiji plus four from Samoa (as our Group 1b). Their Group 3, from Brunei only, has its range expanded to include a sample from Bintan Island, Indonesia (our Group 1c). Finally, their Group 7, from Raja Ampat, Indonesia and Rabaul, Papua New Guinea, was found to also occur at Milne Bay at the southern tip of Papua New Guinea (our Group 1f).

This block represents another case where a huge amount of work needs to be undertaken in order to determine whether morphological and/or color differences can be found that are congruent with the COI data. In addition, genetic samples are sorely needed from the several Pacific plate localities from which *T. okinawae* has been recorded (see Winterbottom & Hoese 2015, Fig. 38). Samples identified here as *T. okinawae* could consist of up to 6 undescribed species in addition to the samples from the type locality (Japan), plus *T. readerae*. We have already dealt separately above with samples identified as *T. okinawae* from the west coast of Thailand (Block 13).

BLOCK 18. Three named species and four haplogroups are included in this block (Table 12). *Trimma dalerocheila* Winterbottom, 1984 (n = 1) is confined to the central and western Indian Ocean, and is very different in COI base pair composition from the other three haplogroups (20.8–22.3%). *Trimma cana* Winterbottom, 2004 consists of two haplogroups, one from Halmahera, Indonesia, and Palau (n = 4, variance = 0.5%) which is 3.6% different from a haplogroup from the Philippines (n = 2, variance = 0.2%). These two differ by between 10.0–11.2% from *T. sostra* Winterbottom, 2004 (n = 1) from Fiji.

Only the three samples of *T. cana* (variance = 0.2%) from Palau were included in W14, and thus all the other species/haplogroups/localities cited above are new to this study.

TABLE 12

Distance summary barcode analysis of 8 *Trimma* specimens in Block 18
(minimum distance between groups)
Var.= maximum variation within group

Group		location	n	Var.	2a	2b	3
1	*T. dalerocheila*	Seychelles	1	n/a	23.3	22.3	20.8
2a	*T. cana*	Indonesia- Halmahera, Palau	4	0.5	–	3.6	11.2
2b	*T. cana*	Philippines	2	0.2		–	10.0
3	*T. sostra*	Fiji	1	n/a			–

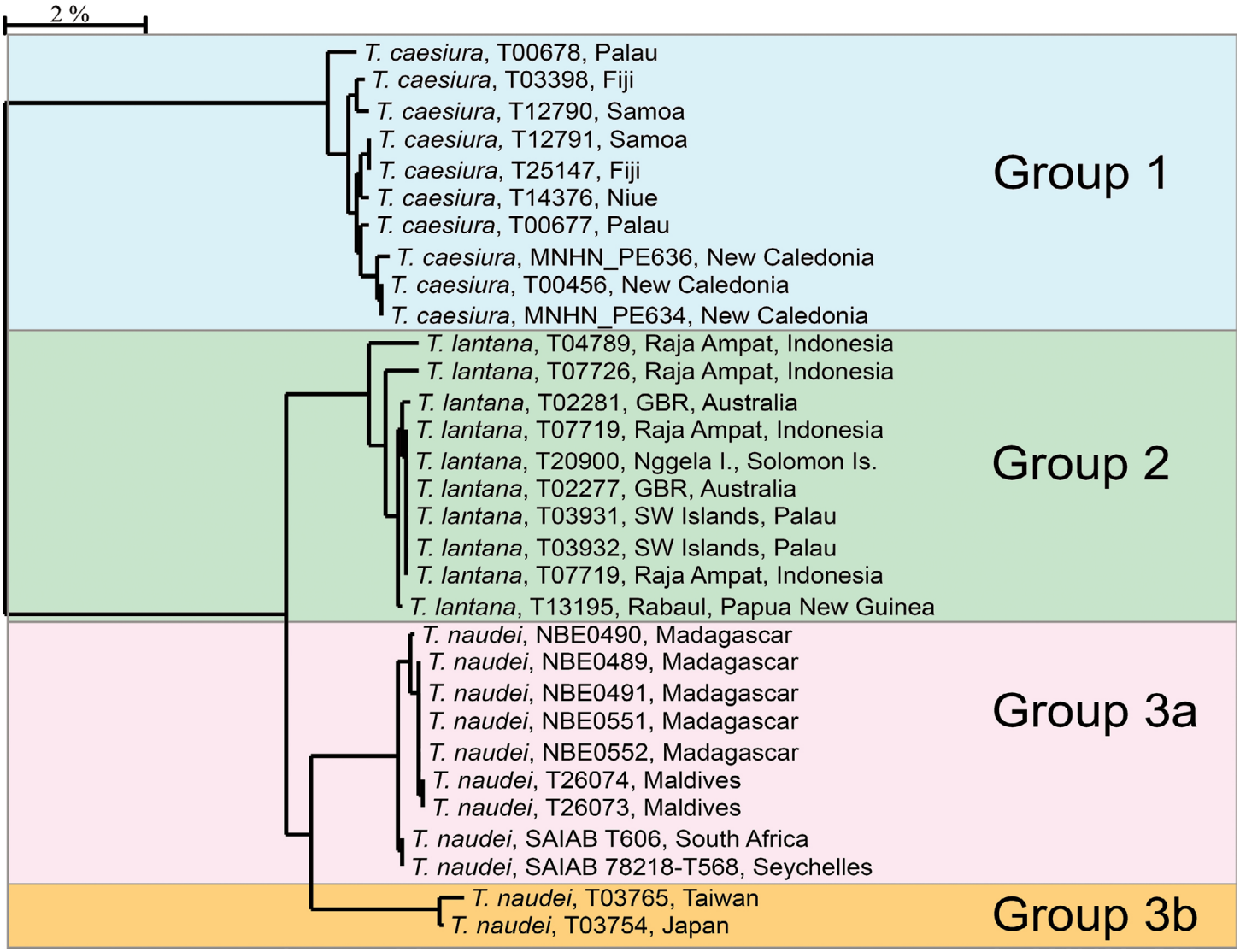

Figure 11. Phenetic relationships of individual sequences of the 31 members of the *Trimma caesiura, T. lantana* and *T. naudei* haplogroups (Block 19 of Fig. 2; group designations follow Table 13). Scale bar is 2% COI genetic distance.

BLOCK 19. There are also three species and four haplogroups included in this block (Table 13), with *T. naudei* Smith, 1957 consisting of two haplogroups. *Trimma caesiura* Jordan & Seale, 1906, with 10 samples including two from the type locality (Upolu Island, Samoa) and a variance of 1.2%, is well separated from all the other haplogroups (9.7–10.7%). The difference between *T. lantana* Winterbottom & Villa, 2003 and *T. naudei* Group 3b is greater than that between Group 3 b and *T. naudei* Group 3a (4% vs. 3%), and the least distance (2.3%) is between *T. lantana* (confined to the western Pacific) and *T. naudei* Group 3a (confined to the Indian Ocean; a sample from the type locality of the Seychelles included here). Group 3b is currently only known from Taiwan

TABLE 13

Distance summary barcode analysis of 31 *Trimma* specimens in Block 19 & Fig. 11
(minimum distance between groups)
Var.= maximum variation within group

Group		location	n	Var.	2	3a	3b
1	*T. caesiura*	Fiji, NC, Niue, Palau, Samoa	10	1.2	9.7	10.5	10.7
2	*T. lantana*	Australia- GBR, Indonesia- RA, Palau- South West Islands, PNG- Rabaul, Solomon Islands	10	1.4	–	2.3	4.0
3a	*T. naudei*	Madagascar, Maldives, Seychelles, South Africa	9	0.5		–	3.0
3b	*T. naudei*	Japan, Taiwan	2	0.4			–

and Japan. However, specimens identified as *T. naudei* have been recorded from intermediate areas such as the Philippines, Indonesia and Vietnam, and no samples of *T. lantana* are available from western Australia or the Lesser Sunda Islands (see Winterbottom & Hoese (2015) for a detailed discussion of possible color pattern and meristic value differences). Obviously further samples and more morphological work need to be undertaken to try and resolve the conflicts and confusion surrounding *T. naudei* and *T. lantana* (see variation in Fig. 11). Note that *T. caesiura* T00678, from Palau, provides almost half of the recorded variance.

These results are essentially the same as those reported by W14. The samples of *T. caesiura* reported here include an additional five samples from three new localities (Fiji, Niue, and the type locality, Samoa), with a 0.3% increase in variance. In *T. lantana*, sample size has increased by three, with two additional localities represented (Rabaul, Papua New Guinea; and the Solomon Islands) and a 0.6% increase in variance. Group 3a (*T. naudei*) contains two additional samples from a new locality (Maldives) with no change in variance.

BLOCK 20. *Trimma hoesei* Winterbottom, 1984 constitutes the sole member of this block. Six samples, from Raja Ampat and Palau, are included (Fig. 12). The variance is much greater than is usually found within a haplogroup (= 2.5%), but the majority of this is due to a single sample from Misool Island, Raja Ampat (MB0619601). Although this is the most southwesterly of the locations represented, the identical barcode from samples from Wofoh and Penemu Islands in Raja Ampat with one of the Helen Reef, Palau samples, which are separated by a much greater distance, would suggest this may be intra-specific variation or an anomaly rather than evidence of a separate haplogroup.

W14 also found that this species was well separated from all others, based on four samples from the same two areas. They recorded a variance of 0.6%, but their samples did not include the Misool Island specimen (see above). More genetic samples from the rest of the geographic range (Chagos/Maldives to the Philippines, Great Barrier Reef, Solomons and Fiji), but especially from the type locality (Chagos Archipelago, Indian Ocean) are needed to test more broadly for variation in haplotypes.

Figure 12. Phenetic relationships of individual sequences of the 6 members of the *Trimma hoese* haplogroup (Block 20 of Fig. 2). Scale bar is 2% COI genetic distance.

BLOCK 21. There are three described species and three haplogroups in this assemblage, all with large differences both between each other and from other species of the genus (Table 14 and Fig. 2). Much of the 1.5% variance in *T. anaima* Winterbottom, 2000 was found in a sample from Misool, Raja Ampat, and a single sample from the western Indian Ocean (Seychelles). Specimens identified here as *T.* cf *imaii* Suzuki & Senou, 2009 are probably not that species (which may be restricted to Japanese waters), but there is insufficient material to try to decide morphologically, and no samples are currently available from Japan for sequencing comparisons.

TABLE 14

Distance summary barcode analysis of 13 *Trimma* specimens in Block 21
(minimum distance between groups)
Var.= maximum variation within group

Group		location	n	Var.	2	3
1	*T. anaima*	Indonesia- Lesser Sunda Isl. & Mapia Atoll & RA, Philippines, Seychelles	9	1.5	19.0	18.4
2	*T.* cf. *imaii*	Indonesia- Bali, Timor-Leste	2	0.9	–	17.4
3	*T. erwani*	Marquesas Islands	2	0.0		–

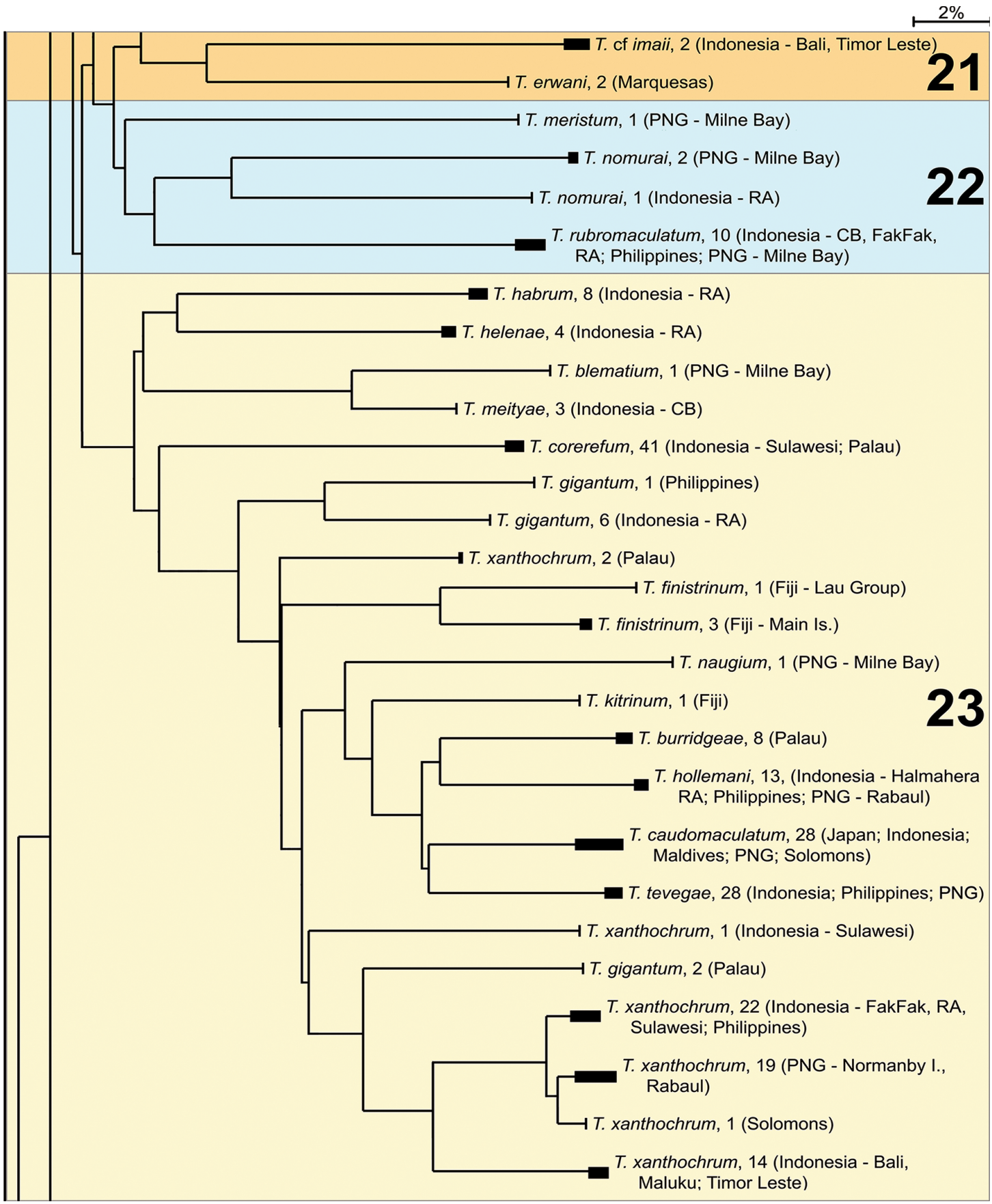

Figure 2 part 4 (Groups 21–23). Condensed Neighbour-joining network of the COI gene based on an analysis of 849 specimens of *Trimma*. Solid bars at tips of branches represent approximate within-group variation. Species names are followed by the number of specimens with locality(ies) of the specimens in parentheses. Colored blocks and numerical notations refer to the sequential groups discussed in the text, additional groups continue on subsequent pages. Scale bar is 2% COI genetic distance.

W14 reported on two samples of *T. anaima* (from Raja Ampat and the LSI) and a single sample identified as *T. imaii* from Bali (Fig. 2). Thus the present paper expands the range of samples of the former species substantially (westward to the Indian Ocean), but adds only a single sample to the latter. No samples of *T. erwani* Viviani et al., 2016 were available to W14, as the species had not been described at the time of publication

BLOCK 22. Three widely divergent species are included here (Table 15), one of which, *T. nomurai* Suzuki & Senou, 2007 consists of two haplogroups separated by 15.9%. One possible morphological correlation has been noticed (number and size of opercular scales), but this has not been vigorously pursued, in part because there are only a few specimens available, and because there are currently no genetic samples of this species available from the type locality (Japan). *Trimma meristum* Winterbottom & Hoese, 2015 also lacks genetic samples from the type locality (Fiji). The sample we have available is from Milne Bay, Papua New Guinea, and was described as a different species, *T. christianeae* Allen, 2019. A careful morphological examination did not reveal any trenchant differences, although COI sequences from the type locality are needed to test the assumption that there is only one species/haplogroup. Samples of *T. rubromaculatum* Allen & Munday, 1995 cover a wide geographic range, with a sample from Rabaul, New Britain being geographically closest to the type locality (Kimbe Bay, New Britain, Papua New Guinea). More than half the variance of 1.6% derives from a single sample from Misool, Raja Ampat – there are other samples from the same collection which do not show this variance.

W14 had no samples of the first two species in their analysis, and only two samples of *T. rubromaculatum*, both from Raja Ampat. This study thus adds 8 additional samples from four new localities for this species, plus the two previously unrepresented species.

TABLE 15

Distance summary barcode analysis of 14 *Trimma* specimens in Block 22
(minimum distance between groups)
Var.= maximum variation within group

Group		location	n	Var.	2a	2b	3
1	*T. meristum*	Seychelles	1	n/a	23.2	20.3	19.9
2a	*T. nomurai*	Papua New Guinea- Milne Bay	2	0.2	–	15.9	20.2
2b	*T. nomurai*	Indonesia- Raja Ampat archipelago	1	n/a		–	19.7
3	*T. rubromaculatum*	Indonesia- Cenderawasih Bay & FakFak & RA, PNG- Milne Bay, Philippines	10	1.6			–

BLOCK 23. This is by far the largest grouping in our analysis (22 haplogroups with a total of 208 samples, see Table 16), at least in part because there seemed to be no logical way to split it up without producing numerous single-haplogroup blocks. Also influencing our decision to keep the group intact is that most of these taxa were treated together as the "*Trimma tevegae* group" (or as section A in Fig. 1) in W14, and the sharing by all members of a broad interorbital width, modified and/or enlarged haemal arches around the transition area between the abdominal and caudal vertebrae, and the propensity to swim in small to large schools in a head-up position in the water column. These character states are thought by one of us (RW) to probably be derived within the genus, and are shared by only a few other groups of *Trimma* (e.g. see Blocks 26 and 27 in Figure 2 part 5) that are not included in Block 23.

TABLE 16

Distance summary barcode analysis of 208 *Trimma* specimens in 22 haplogroups in Block 23 (minimum distance between groups)
Var.= maximum variation within group

	Group	location	n	Var.	2	3	4	5	6a	6b	7a	8a	8b	9	10	11	12	13	14	7b	6c	7c	7d	7e	7f
1	*T. habrum*	Indonesia- Raja Ampat archipelago	8	0.7	14.3	17.8	17.3	18.0	17.4	16.4	21.7	21.6	19.9	21.8	22.0	20.9	20.5	18.8	22.0	21.8	17.3	20.5	20.3	20.6	20.3
2	*T. helenae*	Indonesia- Raja Ampat archipelago	4	0.6	–	17.3	16.1	17.3	18.0	17.0	18.9	23.1	20.5	23.5	20.9	22.3	21.3	20.5	22.0	19.7	17.8	18.0	17.8	18.2	18.5
3	*T. blematium*	Papua New Guinea- Milne Bay	1	n/a		–	7.3	21.4	17.4	18.9	19.2	21.2	22.4	24.2	22.8	23.8	23.4	20.4	23.8	22.5	21.8	22.8	22.9	23.6	23.9
4	*T. meityae*	Indonesia- Cenderawasih Bay	3	0			–	18.4	16.5	15.7	18.1	21.9	21.0	20.9	20.2	21.3	21.3	20.3	23.2	20.8	19.9	21.0	21.2	22.4	21.3
5	*T. corerefum*	Indonesia- Sulawesi, Palau	41	0.7				–	16.7	18.1	19.7	22.4	20.2	21.6	20.2	21.1	22.0	20.6	22.4	21.7	21.0	17.7	19.3	19.3	20.3
6a	*T. gigantum* a	Philippines	1	n/a					–	9.7	16.6	15.2	15.2	16.4	16.2	16.7	18.0	16.0	16.3	16.6	14.3	15.8	15.2	15.6	16.8
6b	*T. gigantum* b	Indonesia- Raja Ampat archipelago	6	0						–	15.9	18.3	17.5	17.6	17.2	18.5	17.0	16.8	16.8	16.6	16.7	15.2	14.6	15.2	17.3
7a	*T. xanthochrum* a	Palau	2	0.2							–	15.1	16.1	16.8	13.6	14.9	15.8	14.0	15.2	15.4	15.6	16.0	15.8	16.6	16.3
8a	*T. finistrinum* a	Fiji- Lau Islands	1	n/a								–	8.9	17.2	16.1	16.9	17.9	15.4	15.8	15.8	15.6	18.2	17.1	17.7	18.5
8b	*T. finistrinum* b	Fiji- Main Islands	3	0.5									–	18.1	16.2	17.5	17.9	15.5	15.4	16.9	18.9	17.7	16.0	18.9	18.3
9	*T. nauagium*	Papua New Guinea- Milne Bay	1	n/a										–	15.0	14.4	15.1	14.7	14.3	17.8	16.6	18.1	17.9	17.9	18.2
10	*T. kitrinum*	Fiji	1	n/a											–	11.0	13.5	9.6	11.4	12.5	14.1	14.2	14.8	15.0	14.8
11	*T. burridgeae*	Palau	8	0.5												–	9.2	9.1	9.5	13.7	14.7	14.0	14.6	14.8	16.0
12	*T. hollemani*	Indonesia- Raja Ampat & Halmahera, PNG- Rabaul, Philippines	13	0.2													–	10.0	10.3	14.4	15.1	14.8	15.8	16.5	15.8
13	*T. caudomaculatum*	Japan, Maldives, PNG- Milne Bay & Rabaul & Pt Moresby, Solomons, Timor-Leste	28	2.2														–	7.7	13.9	13.7	13.5	14.4	14.7	13.9
14	*T. tevegae*	Indonesia- RA & CB & Halmahera & Malaku, PNG- Rabaul, Philippines	28	0.5															–	14.1	14.5	14.2	15.0	14.4	16.2
7b	*T. xanthochrum* b	Indonesia- Sulawesi	1	n/a																–	14.3	13.7	13.7	13.7	14.4
6c	*T. gigantum* c	Palau	2	0																	–	10.7	10.3	10.9	12.0
7c	*T. xanthochrum* c	Indonesia- Raja Ampat & FakFak & Sulawesi, Philippines	22	3.2																		–	2.0	2.0	7.7
7d	*T. xanthochrum* d	PNG- Rabaul & Normanby Island	19	1.6																			–	1.6	7.7
7e	*T. xanthochrum* e	Solomon Islands	1	n/a																				–	8.1
7f	*T. xanthochrum* f	Indonesia- Bali & Maluku, Timor-Leste	14	0.5																					–

The first two species at the base of the network, *T. habrum* Winterbottom, 2011 (n = 8, var. = 0.7%) and *T. helenae* Winterbottom et al., 2014a (n = 4, var. = 0.6%) each consist of a single haplogroup, and are separated by 14.3% of the COI. In W14, the former consisted of 6 samples, and the latter had not yet been described or recognized. These two are about 16–17% different from their nearest phenetic congener, *T. meityae* Winterbottom & Erdmann, 2018, and our samples of both species are confined to the Raja Ampat region of Indonesia although both have been recorded (non-tissue) from Sulawesi.

The next two species, *T. blematium* Winterbottom & Erdmann, 2018 (n = 1) and *T. meityae* (n = 3, var. = 0%), both have predominantly blue eyes and occur at 50–70 m water depth. The former has been collected only at Milne Bay, PNG, while the latter was found at Cenderawasih Bay, Indonesia. The two are genetically separated by 7.3%, and the latter is phenetically nearest to the two species listed above. Neither of these species had been described or were recognized when W14 was published.

Next in the phenetic sequence is *T. corerefum*, described based on collections from the main islands of Palau. Within-group variance among the 41 samples is 0.7%, and the minimum distance between this species and any other member of Block 23 is 16.7%. In W14, this species was designated as "*T. tevegae* Group 1", based on 38 samples from the main islands of Palau. We have added three specimens from northern Sulawesi near Manado here.

Following this are two haplogroups, both identified as *T. gigantum* Winterbottom & Zur, 2007 (entries 6a and 6b in Table 16). The single sample from the Philippines is separated from 6 samples (0.0% variance) from Raja Ampat, Indonesia by 9.7%. The former sample represents a new haplogroup, but the latter 6 samples were included in W14 under the name "*Trimma gigantum* Group 9".

These two haplogroups are followed by a single haplogroup consisting of two samples from Palau identified as *T. xanthochrum* Group 2c by W14, with a variance of 0.2%. A photograph of a freshly collected specimen indicates that the body color of this form is more brown than yellow, and it could be the same species as *T. yoshinoi* Suzuki et al., 2015 from Japan. Unfortunately, no tissue samples of the latter species are currently available.

The ensuing pair of haplogroups are both identified as *T. finistrinum* Winterbottom, 2017 in Fig. 2. A single sample from the Lau Islands, Fiji (8a in Table 16) is separated by 8.9% of the COI from three samples (8b; var. = 0.5%) from the main complex of the Fijian islands. The species was described after W14 was published. We are unable to comment further on the presence of two haplogroups of what look like the same species in proximate localities (although it is not an uncommon situation in *Trimma*). The samples from the Lau Islands were not available when the original description of *T. finistrinum* was published, and there is currently no comparison of the morphology or color between samples from the two sites available.

The remaining samples form two phenogroups. In the first of these two groups, the first species separating off is the single sample of *T. nauagium* Allen, 2015 from Milne Bay, Papua New Guinea, which differs from all other Block 23 members by a minimum of 14.3%. It is followed by *T. kitrinum* Winterbottom & Hoese, 2015 (n = 1) from Fiji, which differs by a minimum of 9.6% from any other haplogroup/species. Neither of these species had been described at the time W14 was published. Both lack the dark caudal spot characteristic of the following four species and of all samples identified here as *T. xanthochrum*. The last four species (Groups 4–7 in W14) retain the same relationships as reported in that paper. *Trimma burridgeae* Winterbottom, 2016, called *T. tevegae* Group 5 in W14, remains the same in terms of content as in W14. *Trimma hollemani* Winterbottom, 2016, designated as *T. tevegae* Group 4 in W14, now includes two additional genetic samples, from Halmahera and from the Philippines. The presence of this species in the Philippines, suggested by Winterbottom (2016) based on morphology, is now confirmed by the genetic analysis. An additional 7 samples of "the true" *T. tevegae* (Group 6 in W14) have been analysed. Within-group variance is now 0.5% (vs. 0.4% in W14), and geographic coverage has expanded to include Cenderawasih Bay, Halmahera and the Maluku Islands, Indonesia. The number of samples of *T. caudomaculatum* Yoshino & Araga, 1975 has doubled from 14 in the W14 analysis to 28 here, the variance has increased from 1.6% to 2.2%, and the geographic representation has significantly increased (from Japan and Rabaul to now include the Maldives, Milne Bay and Port Moresby in Papua New Guinea, the Solomon Islands, and Timor-Leste). Although the 2.2% overall variance could imply more than one species/haplogroup is present in our data, Figure 13 suggests that, while some differentiation by locality is apparent, there seems to be sufficient within-group variance (see especially Group 1) to suggest that such a subdivision would be premature, especially given the rather spotty and

wide-spread distribution of collecting localities. However, it may be significant that Groups 1 and 2 are in the southern hemisphere while Groups 3 and 4 are in the northern hemisphere (see Fig. 13).

The co-ordinate phenetic grouping to the above assemblage consists of five haplogroups identified as *T. xanthochrum*, and a single haplogroup representing the type specimens of *T. gigantum* from Palau. We have already dealt above with two other haplogroups of *T. gigantum* from the Philippines and Raja Ampat, Indonesia, and one of *T. xanthochrum* from Palau. The first of this series is a single sample from northern Sulawesi (7b in Table 16), which is 13.7% or more different from all other haplogroups in Group 23. This sample was not available for the W14 study. Next are two samples of *T. gigantum* from Palau (the type locality for the species). They are 10.3% or more different from all other haplogroups and are the only member of this series of haplogroups to lack a large dark spot on the caudal peduncle (as 6c here, or Group 8 of W14).

The next three haplogroups (Fig. 14), all identified as *T. xanthochrum*, consist of 22 samples of Group c (Group 2 in W14) from a triangle bordered by the Philippines in the north, Sulawesi in the southwest, and Raja Ampat to the southeast, Group d (Group 2b in W14) consists of 19 samples divided between Normanby Island and Rabaul, Papua New Guinea, and Group e is a single sample from the Solomon Islands (not available to W14). The variance for Group c (3.6%) is beyond our arbitrary cut-off of 2%, but an examination of Fig. 14 reveals that the Philippine sample is only marginally different from the Indonesian samples, which are divided into three subgroups, one (n = 12) from various islands around Raja Ampat, including the type locality, a lone sample from Sulawesi (a new locality for the haplogroup), and a third comprising all the samples from the FakFak area, plus a single sample from Raja Ampat (Kepotsol Island, just south of Misool). The sample from Misool may indicate that it and the FakFak specimens form a discrete population from those in the more northern part of Raja Ampat and from the Sulawesi sample, but further samples are needed from Batanta and Kofiau Islands to test this possibility. A somewhat similar situation pertains to the Papua New Guinea samples forming Group d (n = 19, variance = 1.6%) which are divided into two subgroups, one from Normanby Island and the other from Rabaul, at the eastern tip of New Britain (the source of Group b in W14). However, Groups c, d and e may form a single species because the distance between Group c and the other two is exactly 2%, while the difference between Groups d and e is even smaller (at 1.6%). Examination of this part of Fig. 2 provides a visualization of our interpretation of what might constitute haplogroups in this exceedingly complex grade of fishes.

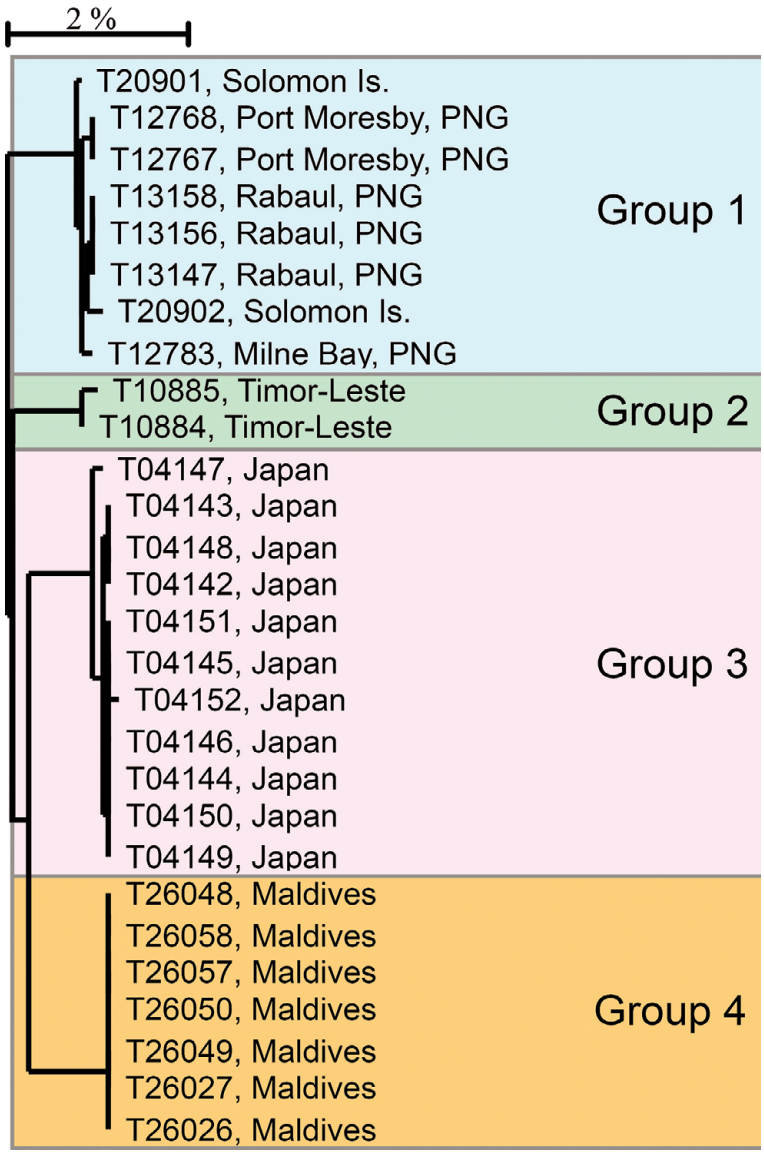

Figure 13. Phenetic relationships of individual sequences of the 28 members of the *Trimma caudomaculatum* haplogroups (from the single lineage midway within Block 23 of Fig. 2). Scale bar is 2% COI genetic distance.

The final haplogroup in Block 23, also identified as *T. xanthochrum*, consists of 14 samples (variance = 0.5%) ranging from Bali to Maluku and Timor-Leste (Group 7f in Table 16, called Group 2a in W14, where it consists only of the 11 samples from Maluku).

Extensive anatomical data (up to 70 characters/specimen) taken from about 50 specimens identified as *T. xanthochrum* from Bali (n = 3) plus Maluku (n = 6; Group 7f) to Palau (n = 7; Group 7a) and south to Raja Ampat (n = 22) plus Sulawesi (n = 10; Group 7c) has failed to reveal any characters that could be used to separate them (RW, unpublished data). There are several localities (e.g. Fiji) from which we have formalin-fixed preserved specimens but no tissue samples, which would undoubtedly further increase the COI variation of the 6 haplogroups in this grade.

BLOCK 24. The 7 haplogroups that constitute this grade are unusual for *Trimma* in their morphology, having the posterior nares adnate to the anterior margin of the eye, a deep and complete posterior interorbital trench (also found in the *T. caesiura* cluster, Block 19), and, except for *T. quadrimaculatum* Hoese et al., 2015, the cephalic sensory papilla line b is broken into short anterior and posterior sections of about 2 or 3 papillae each, separated by a larger, papilla-less gap (see Winterbottom, 2019). The group probably includes *T. winterbottomi* Randall & Downing in Randall et al., 1994 (Socotra and Oman east to Thailand), which shares all three characteristics listed above, but we were unable to obtain tissue samples of this species. The species are all restricted to the Red Sea and the Indian Ocean, reaching eastward only as far as the west coast of Thailand if *T. winterbottomi* is included, and in most cases they differ by >15% from all other members of this Block.

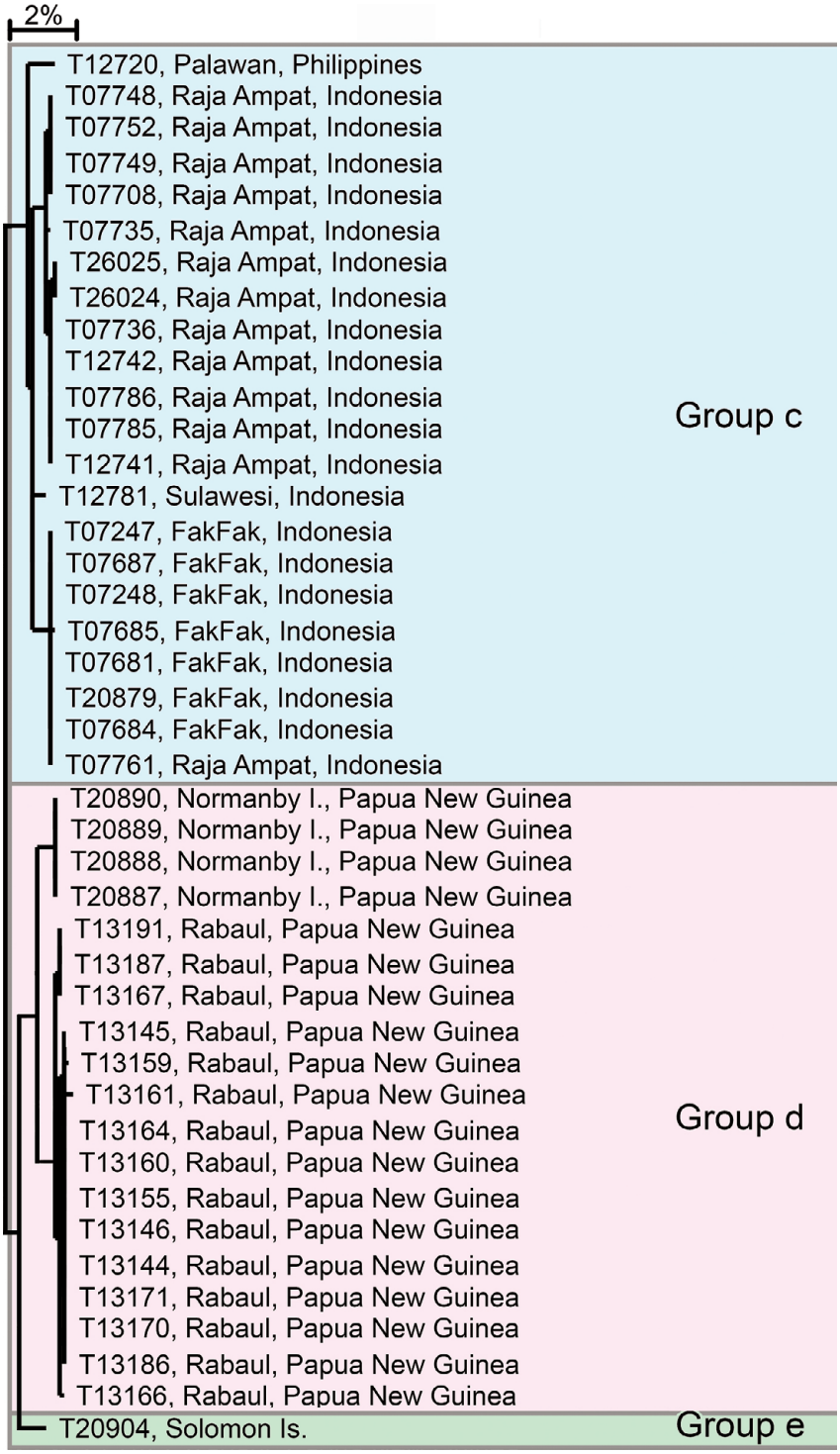

Figure 14. Phenetic relationships of individual sequences of the 44 members of the three *Trimma xanthochrum* haplogroups (from within Block 23 of Fig. 2). Scale bar is 2% COI genetic distance.

The enormous differences of *T. quadrimaculatum* from the other haplogroups (19–23%) may suggest that it does not belong in this assemblage (or perhaps not even within *Trimma*, as was discussed in the original description of the species). *Trimma omanense* ? is also very different (over 20%) from everything else in this block except for *T. corallinum* (11.6%). There are two haplogroups of *T. mendelssohni* (Goren, 1978) in our analysis, one from the Red Sea (the type locality, n = 7, variance = 0.9%), the other from the Seychelles (n = 3, variance = 0.5%), separated by 8.5% sequence divergence of the barcode region of the COI gene (see Table 17). *Trimma avidori* (Goren, 1978) and *T. filamentosus* Winterbottom, 1995 form two sequential grades leading to *T. corallinum* (Smith, 1959) and *T. omanense*? Winterbottom, 2000 (see Fig. 2). We have no explanation for the situation regarding the latter two nominal species. Our results clearly suggest that the former

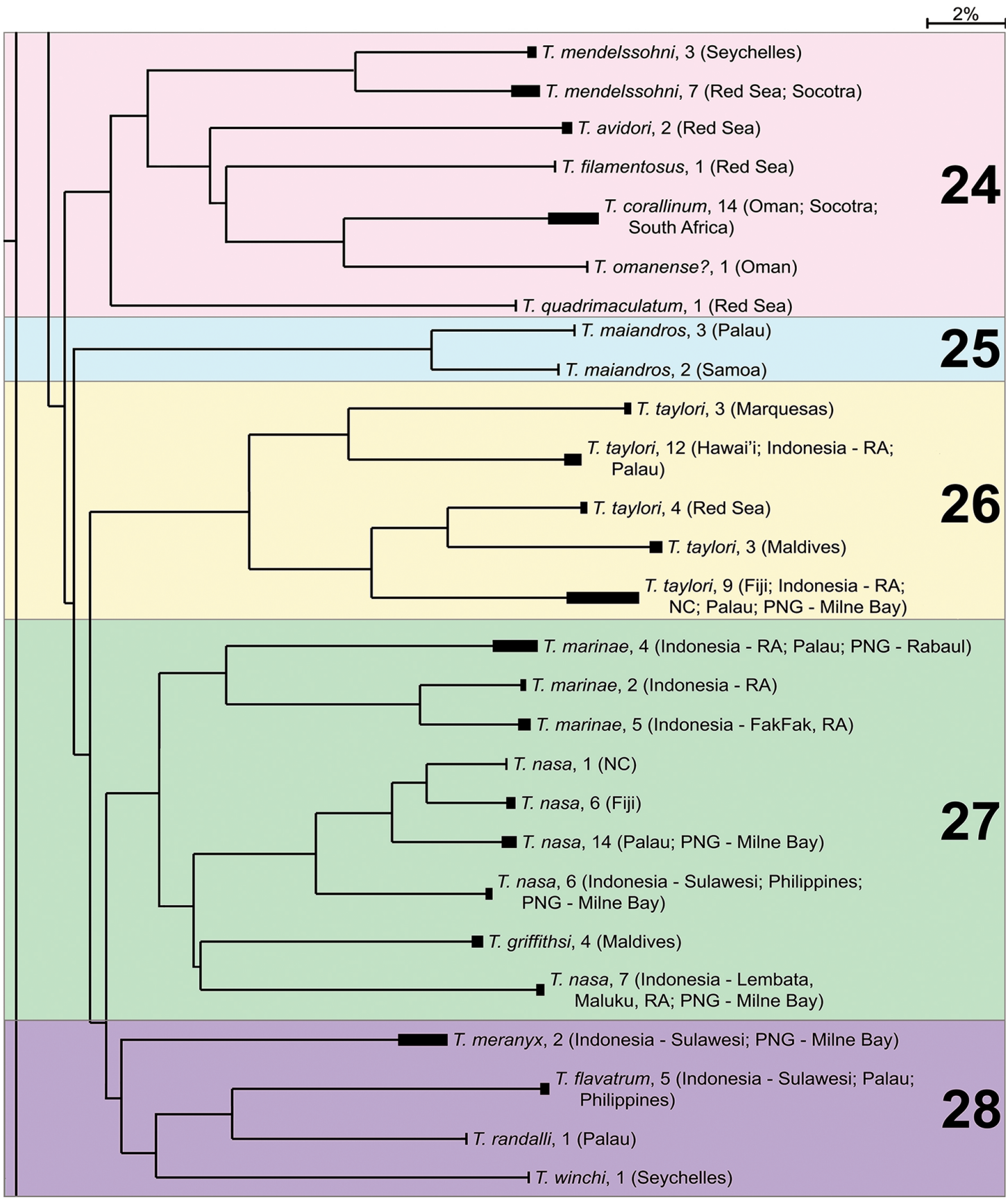

Figure 2 part 5 (Groups 24–28). Condensed Neighbour-joining network of the COI gene based on an analysis of 849 specimens of *Trimma*. Solid bars at tips of branches represent approximate within-group variation. Species names are followed by the number of specimens with locality(ies) of the specimens in parentheses. Colored blocks and numerical notations refer to the sequential groups discussed in the text, additional groups continue on subsequent pages. Scale bar is 2% COI genetic distance.

species extends all the way from KwaZulu-Natal in South Africa to Oman. However, one of the samples from the latter region is separated by 11.6% from the 14 samples of *T. corallinum*. Four of the latter were collected at the same locality and at the same time as this outlier. Careful examination of the morphology of the specimen yielding the tissue sample of the outlier failed to reveal any observable differences from the other specimens. In addition, the morphological characters given in the original description to separate the two species have been found to be invalid. While it seems very likely that *T. corallinum* is the senior synonym of *T. omanense*, we retain the two names here pending more extensive sampling in the area around Oman, and especially from the type locality of *T. omanense* in the vicinity of Sur, Oman.

The W14 analysis included only the three samples of *T. mendelssohni* from the Seychelles, and three samples of *T. corallinum* from KwaZulu, South Africa. The other species had either not yet been described, or samples for genetic analysis were unavailable.

TABLE 17

Distance summary barcode analysis of 29 *Trimma* specimens in Block 24
(minimum distance between groups)
Var.= maximum variation within group

Group		location	n	Var.	1b	2	3	4	5	6
1a	*T. mendelssohni* a	Seychelles	3	0.5	8.5	17.8	22.7	21.8	25.9	21.9
1b	*T. mendelssohni* b	Red Sea	7	0.9	–	17.3	21.5	21.1	22.9	21.4
2	*T. avidori*	Red Sea	2	0.3		–	17.7	16.2	20.2	19.2
3	*T. filamentosus*	Red Sea	1	n/a			–	14.1	20.3	23.1
4	*T. corallinum*	Oman, Socotra, South Africa	14	2.8				–	11.6	21.6
5	*T. omanense* ?	Oman	1	n/a					–	22.4
6	*T. quadrimaculatum*	Red Sea	1	n/a						–

BLOCK 25. The two haplogroups in our data base under the name *T. maiandros* Hoese et al., 2011 consist of three samples from Palau (variance = 0.0%) and two from Samoa (variance = 0.0%). They are separated by 6.7% of the COI base pairs. The samples from Samoa are new to this analysis. It is unfortunate that so few areas are represented in our sampling, as the original description of the species by Hoese et al. (2011) listed several areas of morphological variation, and a distribution from throughout the western Pacific and Mariana Islands, and onto the Pacific plate (Enewetak Atoll, Marshall Islands). The authors also quoted a 27% difference in COI between the Palauan samples and a sample from the Great Barrier Reef (2011:109). We have been unable to find any further references to the latter record, nor have we been able to relocate the original record.

BLOCK 26. The block consists of 31 samples in five haplogroups, all identified as *T. taylori* Lobel, 1979 (Table 18). Samples range from the Red Sea all the way east to the Marquesas Islands, and it is likely that several species are involved.

The haplogroups are well separated from each other by 8.4–20.6%, and the variance within each haplogroup is generally below 1%. However, in Group 1e, which is widespread in the western Pacific, the variance is 3.3%, primarily due to the inclusion of the samples from Fiji in this haplogroup, and the variation provided by one of the samples from Palau (see Fig. 15 A). The type locality for *T. taylori* is the Hawaiian Islands, and we believe that Group 1b represents the form that the name should apply to should it become advisable to recognize additional species from among the haplogroups.

A single sample identified as *T. taylori* from the Seychelles failed to amplify fully, despite several attempts to do so. The best we were able to do was to extract 293 bp from this sample. It is unclear to us at this stage whether this is the result of a nuclear mitochondrial pseudogene (see Song et al., 2008). While thus not truly comparable, the inclusion of this sample in an analysis based on samples with 200 or more bp showed that it slotted in between

TABLE 18

Distance summary barcode analysis of 31 *Trimma* specimens in Block 26
(minimum distance between groups)
Var.= maximum variation within group

Group		location	n	Var.	1b	1c	1d	1e
1a	*T. taylori*	Marquesas Islands	3	0.2	12.6	18.5	20.6	17.4
1b	*T. taylori*	Hawaiian Islands, Indonesia- Raja Ampat, Palau	12	0.8	–	16.6	17.1	17.6
1c	*T. taylori*	Red Sea	4	0.3		–	8.4	12.5
1d	*T. taylori*	Maldives	3	0.4			–	11.0
1e	*T. taylori*	Fiji, Indonesia- Raja Ampat, New Caledonia, PNG- Milne Bay, Palau	9	3.3				–

the Red Sea and the Maldives specimens, being separated from them by 8.5–9.3% of the COI (Fig. 15 B). These three haplogroups are phenetically closest to *T. taylori* Group 1e from the western Pacific. An analysis of samples from the Chagos Archipelago, central Indian Ocean, where this 'species' is very common (especially in caves below about 30 m on the outer, east-facing reefs) should prove especially informative.

In W14, no samples of *T. taylori* were available from the Marquesas, Maldives, Seychelles or Red Sea, and each of these localities has yielded its own haplogroup in this analysis. In addition, W14 recognized the Fiji samples (their Group 1) as distinct from the samples from New Caledonia, Palau and Raja Ampat (their Group 2). We have combined these two as our Group 1e, and have added a sample from Milne Bay, Papua New Guinea. Our Group 1b is identical to group 3 of W14. Clearly further taxonomic work on this complex of haplogroups is sorely needed. It is already known that there are morphological and color differences between the Red Sea population and those from elsewhere (S. Bogorodsky, pers.comm. to RW), and that this haplogroup differs by 8–19% sequence divergence in the COI barcode from all the other haplogroups (Table 18).

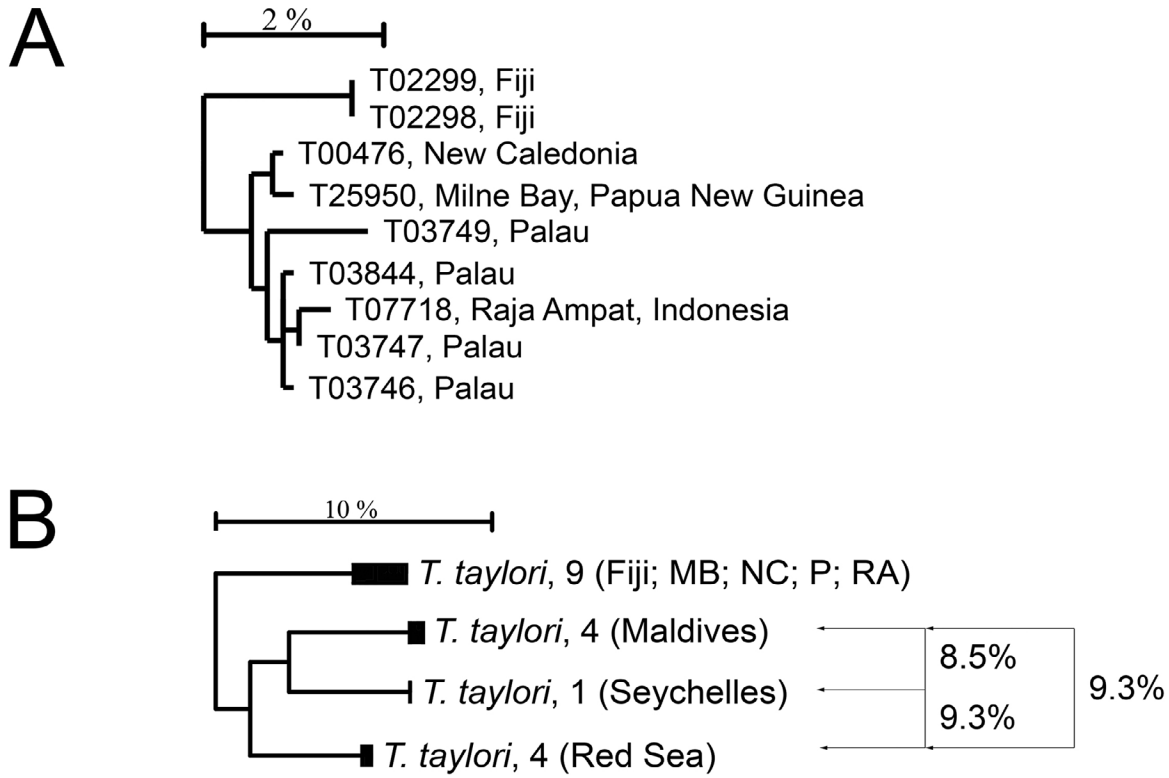

Figure 15. A) Phenetic relationships of individual sequences of the 9 members of the *Trimma taylori* Group 1e (from a single lineage within Block 26 of Fig. 2); scale bar is 2% COI genetic distance. B) Phenetic relationships of individual sequences of the 18 members of the Indian Ocean *Trimma taylori* Group 1c & d (from within Block 26 of Fig. 2) plus Seychelles. Scale bar is 2% COI genetic distance.

BLOCK 27. This block contains three valid species and 9 haplogroups (Table 19). Three of these haplogroups were identified as *T. marinae* Winterbottom, 2005 based on the presence of an open nasal sac and a lack of pigmentation in the form of a black caudal spot. Of these three, the most widely distributed includes samples from the type locality in Palau, as well as samples from Rabaul, Papua New Guinea and Raja Ampat, Indonesia. Although the variance for this haplogroup (*T. marinae* Group a) is 2%, that variance is scattered among the localities, so that the samples are linked by a continuum of difference. A second haplogroup occurs at FakFak and at Misool, Raja Ampat, Indonesia, while the third is confined to northern Raja Ampat. In W14, Groups 3 (Palau) and 4 (Rabaul) are amalgamated as our Group a. The additional sample from Raja Ampat (T25129) decreases the 2% difference between these groups, and lowers the number of haplogroups of *T. marinae* from four to three.

We found five haplogroups identified as *T. nasa* Winterbottom, 2005, two from a single location each (New Caledonia, Group a and Fiji, Group b), with *T. griffithsi* Winterbottom, 1984 phenetically linked to *T. nasa* Group 2e (Fig. 2). In samples from Ka Point, on the north coast of Milne Bay Province, Papua New Guinea, the same collection yielded four samples: two in haplogroup *T. nasa* Group c. The other two did not sequence entirely (T25951 with 349 and T26028 with 467 bp respectively, again possibly confounded by pdeudogenes as in the Seychelles sample of *T. taylori*), but both were included in haplogroup *T. nasa* 2e when analysed using a ≥ 200bp criterion. (These two samples are not included in the main analysis presented here because they have less than 500 bps). Group 2c is separated from Group 2e by 16.6% of the COI (Table 19). Thus these two haplogroups are completely syntopic, at least at one location. Our *T. nasa* Group 2d, which contains samples from the Philippines, Rabaul and Sulawesi may represent the type species, but our Philippine sample is from Batangas rather than from the type locality (Siquijor Island). W14 included four haplogroups, lacking samples from Fiji, and in addition there were no samples of *T. griffithsi* available. Their Group 1 (our *T. nasa* Group 2e) did not have the samples from Halmahera and Milne Bay that we include here; their Group 3 (our *T. nasa* Group 2c) included only the Palauan samples, and their Group 4 (our *T. nasa* Group 2d) consisted only of the two Rabaul samples.

TABLE 19

Distance summary barcode analysis of 41 *Trimma* specimens in Block 27
(minimum distance between groups)
Var.= maximum variation within group

Group		location	n	Var.	1b	1c	2a	2b	2c	2d	3	2e
1a	*T. marinae*	Indonesia- Cenderawasih Bay, PNG- Rabaul, Palau	4	2.0	14.4	14.7	18.3	18.6	17.1	19.5	15.3	19.7
1b	*T. marinae*	Indonesia- Raja Ampat archipelago	2	0.2	–	4.8	15.8	16.7	16.5	17.8	17.0	17.3
1c	*T. marinae*	Indonesia- FakFak & Raja Ampat	5	0.5		–	17.7	17.3	16.7	18.2	17.6	18.1
2a	*T. nasa*	New Caledonia	1	n/a			–	4.1	5.5	9.0	14.7	16.0
2b	*T. nasa*	Fiji	6	0.5				–	5.5	9.1	14.3	16.7
2c	*T. nasa*	Papua New Guinea- Milne Bay, Palau	14	0.5					–	8.9	13.7	16.6
2d	*T. nasa*	Indonesia- Sulawesi, PNG- Rabaul, Philippines	6	0.2						–	15.6	15.7
3	*T. griffithsi*	Maldives	4	0.6							–	15.6
2e	*T. nasa*	Indonesia- RA & Halmahera & Lembata, PNG- Milne Bay	7	0.3								–

BLOCK 28. This block contains four haplogroups, separated by 14–19% of the COI from each other (Table 20), and each with its own species name. It is possible that the two samples assigned to *T. meranyx* in fact represent two haplogroups, since they are separated by 2.4%, and circumstantial support for this is provided in the form of the different geographic locations of the samples (Sulawesi, Indonesia and Milne Bay, Papua New Guinea). However, the species is quite distinctive in its morphology and color pattern, and we are loathe to create yet another haplogroup without more samples and firmer evidence. The species is relatively deep dwelling (45–70 m) and rare but seems to be widely distributed throughout the western Pacific. Neither *T. meranyx* nor *T. winchi* Winterbottom, 1984 were represented in the W14 analysis. The provenance of the samples of *T. flavatrum* Hagiwara & Winterbottom 2007 is increased here to include the Philippines and Sulawesi, Indonesia.

TABLE 20							
Distance summary barcode analysis of 9 *Trimma* specimens in Block 28 (minimum distance between groups) Var.= maximum variation within group							
Group		location	n	Var.	2	3	4
1	*T. meranyx*	Indonesia- Sulawesi, Papua New Guinea- Milne Bay	2	2.4	17.6	18.5	17.2
2	*T. flavatrum*	Indonesia- Sulawesi, , Palau, Philippines	5	0.3	–	13.7	17.9
3	*T. randalli*	Palau	1	n/a		–	18.6
4	*T. winchi*	Seychelles	1	n/a			–

BLOCK 29. The final block contains four named species and 10 haplogroups, 6 of which are currently identified as *T. emeryi*, two as *T. pajama* Winterbottom et al., 2014b, and one each as *T. pentherum* Winterbottom & Hoese, 2015 and *T. wangunui* Winterbottom & Erdmann, 2019 (Table 21).

With the exception of the 7.4% difference between *T. emeryi* Groups a (Cenderawasih Bay) and b (FakFak and Palau), all haplogroups in this block are separated by a minimum distance of 13.8% (*T. emeryi* Groups e and f) or more than 15% (all others). Such huge differences are difficult to understand given how very similar in color and morphology the 6 haplogroups of *T. emeryi* are. It is unfortunate that no samples are available from the type locality of this species (Chagos Archipelago, central Indian Ocean). An enormous amount of basic research is needed to even begin to try and unravel what is occurring in these fishes. For example, both Group 1a and Group 1e are currently only known from Cenderawasih Bay, and are 20.8% different. Yet nobody has as yet been able to find the time to look for morphological/color differences that might support their separate status.

The two haplogroups of *T. pajama*, which has a distinctive striped color pattern, are separated by 15.8% of the COI. *Trimma wangunui*, which is very similar to *T. emeryi* in color and in morphometric and meristic values, differs from all other haplogroups in Block 29 by 18–22%. The degree of genetic separation of the haplogroups is virtually unprecedented, even within other complexes currently assigned to *Trimma*, and would seem to argue for deep divergences and the potential for subsequent dispersal to form secondary sympatry.

W14 recognized five haplogroups among 8 samples of *T. emeryi* (the other two species had not been described at the time and were not included in the analysis). The three samples in our Group a from Cenderawasih Bay, Indonesia constitute a new haplogroup, and a sample from FakFak has been added to our Group b (Group 1 of W14).

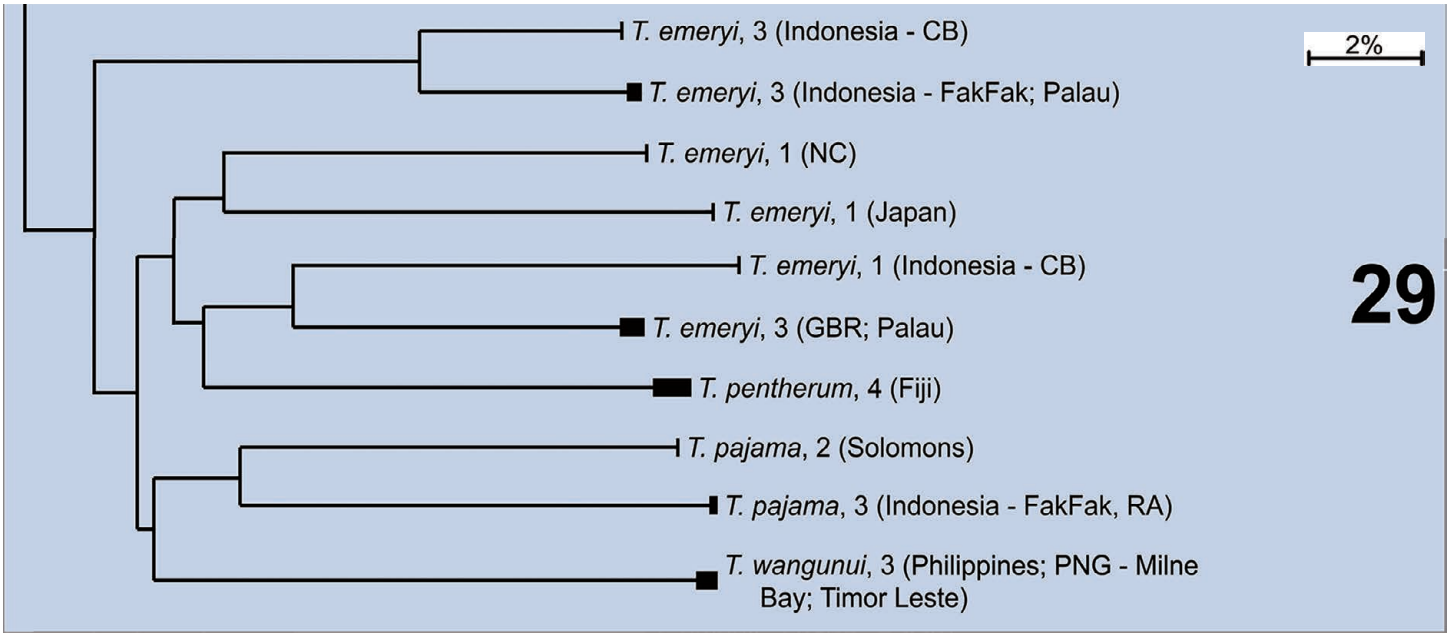

Figure 2 part 6 (Group 29). Condensed Neighbour-joining network of the COI gene based on an analysis of 849 specimens of *Trimma*. Solid bars at tips of branches represent approximate within-group variation. Species names are followed by the number of specimens with locality(ies) of the specimens in parentheses. Colored blocks and numerical notations refer to the sequential groups discussed in the text. Scale bar is 2% COI genetic distance.

TABLE 21

Distance summary barcode analysis of 24 *Trimma* specimens in Block 29
(minimum distance between groups)
Var.= maximum variation within group

Group		location	n	Var.	1b	1c	1d	1e	1f	2	3a	3b	4
1a	*T. emeryi* a	Indonesia- Cenderawasih Bay	3	0	7.4	17.7	20.9	20.8	19.8	19.7	19.6	18.3	20.9
1b	*T. emeryi* b	Indonesia- FakFak, Palau	3	0.5	–	20.4	22.1	20.0	18.0	20.0	17.7	16.5	20.6
1c	*T. emeryi* c	New Caledonia	1	n/a		–	16.1	17.2	18.8	16.6	20.8	21.0	19.9
1d	*T. emeryi* d	Japan	1	n/a			–	18.3	19.3	17.8	21.9	22.0	21.0
1e	*T. emeryi* e	Indonesia- Cenderawasih Bay	1	n/a				–	13.8	18.2	21.6	21.6	22.2
1f	*T. emeryi* f	Australia- Great Barrier Reef, Palau	3	0.8					–	16.3	19.6	16.3	18.4
2	*T. pentherum*	Fiji	4	1.4						–	18.8	18.0	18.2
3a	*T. pajama* a	Solomon Islands	2	0							–	15.8	18.0
3b	*T. pajama* b	Indonesia- FakFak & Raja Ampat	3	0.2								–	19.8
4	*T. wangunui*	PNG- Milne Bay, , Philippines, Timor-Leste	3	0.5									–

Discussion

Our new study has added representatives of 41 species and 62 haplogroups to the 46 species and 94 haplogroups in W14 for a total representation of 87 species (of 106) and 156 haplogroups. The differences are attributable to subsequently obtaining samples of the relevant species through targeted collecting, or because new areas/microhabitats were sampled producing previously unrecorded species/haplogroups.

In his review of the "DNA taxonomy" of reef fishes, Victor (2015: 82) pointed out that analyses of mtDNA sequences can demonstrate "the breaking up of species into pairs or complexes of lineages (frequently parapatric, but often with sympatric members)". He added (op.cit.: 84) that "The most troublesome problem is what to do with "genovariants", divergent lineages which show no apparent morphological, meristic, or color differences". We find that genovariant populations are frequent among *Trimma*, while "phenovariant" species, i.e. species that are morphologically distinct but share mtDNA sequences, have not (as yet) been encountered. For example, our analysis revealed 156 haplogroups (defined for our purposes as mtDNA lineages differing by more than 2%) of *Trimma* among the 87 valid described species of the genus available to us. (We note in passing that our analysis reported 160 BINs in our data set). Since we currently do not have sufficient empirical information on the phenotypes (morphological, meristic, or color differences) of most of these haplogroups, it is unclear to us how many of these genovariants will prove to have morphological differences sufficient to support valid species status in the eyes of most systematists. Putative genovariants, where many, perhaps most, systematists would be reluctant to ascribe them to separate species, may be overestimated since absence of evidence is not necessarily evidence of absence, especially with small sample sizes or older or preserved specimens (essentially Type II errors, i.e. systematist 'A' may not be successful in finding any phenotypic difference to separate two haplogroups, but systematist 'B' may be able to document such differences). Should all our haplogroups prove to be distinct species, then, based on the ratio of 156 haplogroups among 87 valid species (= 1.79), there potentially could be a total of 190 *Trimma* species included among the 106 presently described species. However, the actual total could, on one hand, be much higher, since there are still numerous known but undescribed species of *Trimma*, and more can be expected as we more thoroughly sample the biodiversity of reefs below 50 m. On the other hand, this potential total number of species could be much lower, if the majority of genovariants remain undefinable as discrete species.

We found that our data set consisted of 35 species for which we had a single haplogroup and one or more samples from a single general locality. Fourteen (16%) of these may be true endemic species, since they have, to date, only been collected at or in the vicinity of the type locality. The other 21 species (24%) are represented

by a single sample but have been identified (by morphological criteria) to also occur in other locations, and we simply lack genetic samples from those localities. Another 25 species (29%) have a single haplogroup but with samples from two or more localities (up to 6). In one of these cases (*T. anaima*), samples were from both the western Indian Ocean and the western Pacific Ocean but only a single haplogroup was found. The remaining 27 "species" (i.e. specimens identified as such, 31%) exhibited two or more haplogroups (up to 8 in *T. erdmanni*). In most cases, the haplogroups were separated geographically (although often not by large distances). However, there were three cases where two haplogroups were syntopic, being collected in exactly the same place at the same time: *T. chledophilum*, Groups 2a and 2b, separated by 8.6% of the COI base pairs; *T. erdmanni*, Groups 1 e and 1g, separated by 2.5% of COI,; and *T. nasa* Group 2c and Group 2e, separated by 16.6% of the COI. (Note that this last example is a little more suspect because of the difference in number of base pairs recovered, see under Block 27). It is potentially interesting that in the three cases where syntopy has been found to date, the percentage difference in COI are so disparate, covering much of the variation found in the whole genus (2.5 through 8.6 to 16.6%). The average number of haplogroups per species among the 27 morphological species discussed above was 3.5, and the average number of localities a given haplogroup was documented from was 1.7.

Currently, the only reasonable attempt at a phylogenetic analysis of *Trimma* species was published by Sunobe et al. (2017). Their hypothesis was based on a single gene complex (the mitochondrial ND4/ND5 region), and their analysis contained 31 species of *Trimma*. We declined to attempt such an analysis based on our own COI data, as the resultant gene tree would be unlikely to be particularly informative, and would certainly be unreliable. This is because the limitations affecting the instability of the phenetic results (number of samples, number of base pairs, number of species– see first paragraph of Results) would undoubtedly have an equally negative affect on any attempt at a phylogenetic analysis of this data set.

The advent of barcoding, the BOLD platform and BINs have been of tremendous help in unravelling the astounding amounts of COI diversity among certain members of at least this genus of small reef fishes. While cryptic species are certainly present in the other small, extremely diverse, genus of Indo-Pacific reef gobies, *Eviota*, the phenomenon of barcode haplogroups has not yet been explored to the same degree that it has for *Trimma*. Although with a somewhat different emphasis (phylogenetic analyses), the illuminating paper by Tornabene et al. (2015) concentrated on the *Eviota nigriventris* and *E. bifasciata* complexes, and demonstrated that both contain multiple genetically distinct, geographically restricted color morphs that are indicative of recently diverged species. Greenfield (2017) suggested that details of the coloration of the iris in *Eviota* may be revealing multiple cryptic species of *E. sigillata*, and added that the genetics of the different populations needed to be examined in detail. A preliminary analysis by Tornabene (pers.comm.) revealed at least five COI haplogroups currently identified as *E. sigillata* and which are scattered amongst several other 'species' in the tree. In terms of COI, Tornabene has further found a similar ratio of number of described species (65) to number of haplogroups (109) in that genus (1.7 haplogroups for every described species, versus the 1.8 for *Trimma* we found in this paper). Whether these two instances will be expanded and found in other groups of Indo-Pacific marine organisms, perhaps forming part of a general pattern and thus needing a general explanation, is currently unknown. Multiple cryptic haplogroups do not appear to be present in Caribbean gobies, although Victor (2015) did record 20 described species and 30 DNA lineages in *Elacatinus*. A similar phenomenon may be present among one or more labrisomid blennioids occurring there, e.g. *Malacoctenus* (Victor, pers.comm., 23 described valid species), and is known and documented for *Starksia* (Baldwin et al. 2011), which has some 38 described valid species. This area of research seems to be full of opportunities and potential evolutionary insights. For example, rapid advances in DNA sequencing technology facilitate going beyond the COI barcode to include additional mitochondrial and nuclear gene sequences. This information could corroborate the patterns observed here, test for potential hybridization among haplogroups/species and also recover a more robust phylogenetic signal to assess patterns of cladogenesis within this enigmatic taxon.

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References

Allen, G.R. (2015) Four new species of *Trimma* (Pisces: Gobiidae) from Papua New Guinea and Indonesia. *Journal of the Ocean Science Foundation*, 14, 28–48. https://doi.org/10.5281/zenodo.1037603

Allen, G.R. (2019) *Trimma christianeae*, a new species of goby (Teleostei: Gobiidae) from Milne Bay Province, Papua New Guinea. *Journal of the Ocean Science Foundation*, 32, 39–46. https://doi.org/10.5281/zenodo.2551810

Allen, G.R. & Munday, P. (1995) Description of four new gobies (Gobiidae) from the western Pacific. *Revue Française d'aquariologie*, 22 (3–4), 99–104.

Aoyagi, H. (1949) Studies on the coral reef fishes of the Riu-Kiu Islands. V. *The Zoological Magazine, The Zoological Society of Japan (Tokyo)*, 58 (9), 171–173.

Baldwin, C.C., Castillo, C.I., Weigt, L.A. & Victor B.C. (2011) Seven new species within western Atlantic *Starksia atlantica, S. lepicoelia,* and *S. sluiteri* (Teleostei, Labrisomidae), with comments on congruence of DNA barcodes and species. *ZooKeys*, 79, 21–72. https://doi.org/10.3897/ZooKeys.79.1045

Cohen, D.M. & Davis, W.P. (1969) Vertical orientation in a new gobioid fish from New Britain. *Pacific Science*, 23 (3), 317–324.

Folmer, O., Black, M.B., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3 (5), 294–299.

Goren, M. (1978) A new gobiid genus and seven new species from Sinai coasts. *Senckenbergiana biologica*, 59 (3/4), 191–203.
Goren, M. (1981) Three new species and three new records of gobies from New Caledonia. *Cybium, 3e ser.*, 5 (3), 93–101.
Goren, M. (1982) *Quisquilius flavicaudatus*, a new gobioid fish from the coral reefs of the Red Sea. *Zoologische Mededelingen*, 56 (11), 139–142.
Gosline, W.A. (1959) Four new species, a new genus, and a new suborder of Hawaiian fishes. *Pacific Science*, 13 (1), 67–77.
Greenfield, D.W. (2017) An overview of the dwarfgobies, the second most speciose coral-reef fish genus (Teleostei: Gobiidae: *Eviota*). *Journal of the Ocean Science Foundation*, 29, 32–54. https://doi.org/10.5281/zenodo.1115683
Hagiwara, K. & Winterbottom, R. (2007) Two new species of *Trimma* (Gobiidae) from the western Pacific. *Bulletin of National Science Museum, Series A*, Supplement 1, 163–174.
Herre, A.W.C.T. (1945) Notes on fishes in the Zoological Museum of Stanford University. XIX.– Two new Philippine gobies, with key to the genera of gobies with vomerine teeth. *Proceedings of the Biological Society of Washington*, 58, 77–82.
Hoese, D.F, Bogorodsky, S.V. & Mal, A.O. (2015) Description of a new species of *Trimma* (Perciformes, Gobiidae) from the Red Sea, with a discussion of the generic separation of *Trimma* and *Priolepis*, with discussion of sensory papillae terminology. *Zootaxa*, 4027 (4), 538–550. https://doi.org/10.11646/Zootaxa.4027.4.4
Hoese, D.F., Winterbottom, R. & Reader, S. (2011) *Trimma maiandros*, a new species of pygmy goby (Gobiidae) from the Indo–west Pacific. *Aqua, International Journal of Ichthyology*, 17 (2), 145–152.
Ivanova, N.V., Zemlak, T.S., Hanner, R.H. & Hebert, P.D.N. (2007) Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, 7, 544–548. https://doi.org/10.1111/j.1471-8286.2007.01748.x
Jordan, D.S. & Seale, A. (1906) The fishes of Samoa. Description of the species found in the archipelago, with a provisional check–list of the fishes of Oceania. *Bulletin of the Bureau of Fisheries*, 25, 173–455, [for 1905].
Lobel, P.S. (1979) Description of a new Hawaiian gobiid fish of the genus *Trimma*. *Breviora, Museum of Comparative Zoology*, (456), 1–15.
Randall, J.E., Downing, N., McCarthy, L.J., Stanaland, B.E. & Tarr, A.B. (1994) Fifty–one new records of fishes from the Arabian Gulf. *Fauna Saudi Arabia*, 15, 220–258.
Ratnasingham, S. & Hebert, P.D.N. (2007) BOLD: The Barcode of Life Data System. (www.barcodinglife.org). *Molecular Ecology Notes*, 7, 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x
Ratnasingham, S. & Hebert, P.D.N. (2013) A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System. *PLoS ONE*, 8 (8), e66213. https://doi.org/10.1371/journal.pone.0066213
Seutin, G., Bagley, P. & White, B.N. (1990) Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology*, 69, 82–90. https://doi.org/10.1139/z91-013
Smith, J.L.B. (1957) The fishes of Aldabra. Part VI. *Annals and Magazine of Natural History, Series 12*, (9), 817–829.
Smith, J.L.B. (1959) Gobioid fishes of the families Gobiidae: Periophthalmidae, Trypauchenidae, Taenioididae and Kraemeriidae of the western Indian Ocean. *Ichthyological Bulletin, Department of Ichthyology, Rhodes University*, 13, 185–225.
Song, H., Buhay, J.E., Whiting, M.F. & Crandall, K.A. (2008) Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. *Proceedings of the Academy of Natural Sciences*, 105 (36), 3486-13491. https://doi.org/10.1073/pnas.0803076105
Steinke, D. & Hanner, R.H. (2011) The FISH-BOL collaborator's protocol. *Mitochondrial DNA*, 22 (S1), 10–14. https://doi.org/10.3109/19401736.2010.536538
Sunobe, T., Sado, T., Hagiwara, K., Manabe, H., Suzuki, T., Kobayashi, Y., Sakurai, M., Dewa, S., Matsuoka, M., Shinomiya, A., Fukuda, K. & Miya, M. (2017) Evolution of bidirectional sex change and gonochorism in fishes of the gobiid genera *Trimma*, *Priolepis*, and *Trimmatom*. *The Science of Nature*, 104, 1–11. https://doi.org/10.1007/s00114-017-1434-z

Suzuki, T. & Senou, H. (2007) Two new species of the gobiid fish genus *Trimma* (Perciformes, Gobioidei) from Southern Japan. *Bulletin of the National Museum of Natural Science, Series A*, Supplement 1, 175–184.
Suzuki, T. & Senou, H. (2008) Two new species of the gobiid fish genus *Trimma* (Perciformes, Gobioidei) from Southern Japan. *Bulletin of the National Museum of Natural Science, Series A*, Supplement 2, 97–106.
Suzuki, T. & Senou, H. (2009) Two new species of the genus *Trimma* (Perciformes, Gobioidei) from Japan and Palau. *Bulletin of the Kanagawa Prefectual Museum (Natural Science)*, (38), 107–112.
Suzuki, T., Yano, K. & Senou, H. (2015). *Trimma yoshinoi*, a new gobiid fish from Japan (Perciformes, Gobiidae). *Journal of the Ocean Science Foundation*, 14, 66–73. https://doi.org/10.5281/zenodo.1037593
Tomiyama, I. (1936) Gobiidae of Japan. *Japanese Journal of Zoology*, 7, 37–112.
Tornabene, L., Valdez, S., Erdmann, M.V. & Pezold, F. (2015) Support for a 'Center of Origin' in the Coral Triangle: Cryptic diversity, recent speciation, and local endemism in a diverse lineage of reef fishes (Gobiidae: *Eviota*). *Molecular Phylogenetics and Evolution*, 82, 200–210. http://dx.doi.org/10.1016/j.ympev.2014.09.012
Victor, B.C. (2015) How many coral reef fish species are there? Cryptic diversity and the new molecular taxonomy. Chapter 8. *In*: Mora, C. (Ed) *Ecology of Fishes on Coral Reefs*. Cambridge University Press, Cambridge, UK, pp. 76–87.
Viviani, J., Williams, J.T. & Planes, S. (2016) Two new pygmygobies (Percomorpha: Gobiidae: *Trimma*) from French Polynesia. *Journal of the Ocean Science Foundation*, 23, 1–11. https://doi.org/10.5281/zenodo.159615
Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. & Hebert, P.D.N. (2005) DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 360, 1847–1857. https://doi.org/10.1098/rstb.2005.1716
Ward, R.D., Hanner, R. & Hebert, P.D.N (2009) The campaign to DNA barcode all fishes, FISH-BOL. *Journal of Fish Biology*, 74 (2), 329–356. https://doi.org/10.1111/j.1095-8649.2008.02080.x
Whitley, G.P. (1959) Ichthyological snippets. *The Australian Zoologist*, 12 (4), 310–323.
Winterbottom, R. (1984) A review of the gobiid fish genus *Trimma* from the Chagos Archipelago, central Indian Ocean, with the description of seven new species. *Canadian Journal of Zoology*, 62, 695–715. https://doi.org/10.1139/z84-101
Winterbottom, R. (1985) Two new fish species (in *Priolepis* and *Trimma*) from the Chagos Archipelago central Indian Ocean. *Canadian Journal of Zoology*, 63, 748–754. https://doi.org/10.1139/z85–109
Winterbottom, R. (1995) Red Sea gobiid fishes of the genus *Trimma*, with the description of two new species. *Revue Française d'aquariologie*, 22, 93–98.
Winterbottom, R. (1996) A new species of gobiid fish, *Trimma benjamini*, from the western Pacific. *Revue Française d'aquariologie*, 23, 57–60.
Winterbottom, R. (2000) Four new species of *Trimma* (Gobiidae), from the Indian and western Pacific oceans. *Aqua, Journal of Ichthyology and aquatic Biology*, 4 (2), 57–66.
Winterbottom, R. (2001) Two new gobiid fish species in *Trimma* and *Trimmatom* (Teleostei, Gobiidae) from the Indian and Western Pacific Oceans. *Aqua, Journal of Ichthyology and aquatic Biology*, 5 (1), 19–24.
Winterbottom, R. (2002) Two new species of *Trimma* (Gobiidae) from the central, western, and south Pacific. *Aqua, Journal of Ichthyology and aquatic Biology*, 5 (2), 45–52.
Winterbottom, R. (2003) A new species of the gobiid fish *Trimma* from the western Pacific coral reefs, with a description of its osteology. *Zootaxa*, 218, 1–24. https://doi.org/10.11646/Zootaxa.218.1.1
Winterbottom, R. (2004) Three new species of *Trimma* (Pisces; Gobiidae) from the central, western and south Pacific. *Aqua, Journal of Ichthyology and aquatic Biology*, 9 (1), 7–16.
Winterbottom, R. (2005) Two new species of the *Trimma tevegae* species group from the western Pacific (Percomorpha; Gobiidae). *Aqua, Journal of Ichthyology and aquatic Biology*, 10 (1), 51–56.
Winterbottom, R. (2006) Two new species of the gobiid fish *Trimma* from the coral reefs of western Pacific Ocean (Pisces; Perciformes; Gobioidei). *Zootaxa*, 1331, 55–68. https://doi.org/10.11646/*Zootaxa*.1331.1.3
Winterbottom, R. (2009) A new species of the genus *Trimma* (Percomorpha; Gobiidae) from Helen Reef, South–West Islands of Palau. *Aqua, Journal of Ichthyology and aquatic Biology*, 15 (2), 109–116.

Winterbottom, R. (2011) Six new species of the genus *Trimma* (Percomorpha; Gobiidae) from the Raja Ampat Islands, Indonesia. *Aqua, International Journal of Ichthyology*, 17 (3), 127–162.
Winterbottom, R. (2014) *Trimma irinae*, (Pisces; Gobioidei) a new species of gobiid fish from Milne Bay Province, Papua New Guinea. *Zootaxa*, 3802 (2), 209–216. https://doi.org/10.11646/Zootaxa.3802.2.4
Winterbottom, R. (2016) *Trimma tevegae* and *T. caudomaculatum* revisited and redescribed (Acanthopterygii, Gobiidae), with descriptions of three new similar species from the western Pacific. *Zootaxa*, 4144 (1), 1–53. https://doi.org/10.11646/Zootaxa.4144.1.1
Winterbottom, R. (2017) Two new species of *Trimma* (Pisces; Gobiidae) from Fiji, south-western Pacific Ocean. *Zootaxa*, 4269 (4), 559–570. https://doi.org/10.11646/Zootaxa.4269.4.9
Winterbottom, R. (2018) A new species of *Trimma* (Pisces; Gobiidae) from the South–West Islands of Palau, western Pacific Ocean. *Zootaxa*, 4370 (2), 123–136. https://doi.org/10.11646/Zootaxa.4370.2.2
Winterbottom, R. (2019) An illustrated key to the described valid species of *Trimma* (Teleostei: Gobiidae). *Journal of the Ocean Science Foundation*, 34, 1–61. https://doi.org/10.5281/zenodo.3525430
Winterbottom, R., Alofs, K.M. & Marseu, M. (2011) Life span, growth and mortality in the western Pacific goby *Trimma benjamini*. *Environmental Biology of Fishes*, 91, 295–301. https://doi.org/10.1007/s10641-011-9782-6
Winterbottom, R. & Chen, I.-S. (2004) Two new species of *Trimma* (Teleostei, Gobiidae) from the western Pacific ocean. *Raffles Bulletin, Zoology*, Supplement 11, 103–106.
Winterbottom, R. & Erdmann, M.V. (2018) Two new species of blue-eyed *Trimma* (Pisces; Gobiidae) from New Guinea. *Zootaxa*, 4444 (4), 471–483. https://doi.org/10.11646/Zootaxa.4444.4.7
Winterbottom, R. & Erdmann, M.V. (2019) A new species of *Trimma* (Pisces; Gobiidae) from the Western Pacific Ocean. *Zootaxa*, 4577 (3), 571–578. https://doi.org/10.11646/Zootaxa.4577.3.10
Winterbottom, R., Erdmann, M.V. & Cahyani, N.K.D. (2014a) *Trimma helenae* (Pisces; Gobioidei), a new species of gobiid fish from Indonesia. *Zootaxa*, 3760 (3), 420–428. https://doi.org/10.11646/Zootaxa.3760.3.8
Winterbottom, R., Erdmann, M.V. & Cahyani, N.K.D. (2014b) Three new species of *Trimma* (Pisces; Gobioidei) from Indonesia. *Zootaxa*, 3838 (3), 367–384. https://doi.org/10.11646/Zootaxa.3838.3.7
Winterbottom R., Erdmann M.V. & Cahyani N.K.D. (2015) New species of *Trimma* (Actinopterygii; Gobiidae) from Indonesia, with comments on head papillae nomenclature. *Zootaxa*, 3973 (2), 201–226. https://doi.org/10.11646/Zootaxa.3973.2.1
Winterbottom, R., Erdmann, M.V. & Mambrasar, R. (2019) A new species of *Trimma* (Teleostei: Gobiidae) from Indonesia and Timor-Leste. *Journal of the Ocean Science Foundation*, 32, 47–55. https://doi.org/10.5281/zenodo.2557888
Winterbottom R., Hanner R.H., Burridge, M. & Zur, M. (2014c) A cornucopia of cryptic species—a DNA barcode analysis of the gobiid fish genus *Trimma* (Percomorpha, Gobiiformes). *ZooKeys*, 381, 79–111. https://doi.org/10.3897/ZooKeys.381.6445
Winterbottom, R. & Hoese D.F. (2015) A revision of the Australian species of *Trimma* (Actinopterygii, Gobiidae), with descriptions of six new species and redescriptions of twenty-three valid species. *Zootaxa*, 3934 (1), 1–102. https://doi.org/10.11646/Zootaxa.3934.1.1
Winterbottom, R. & Southcott, L. (2007) Two new species of the genus *Trimma* from western Thailand. *Aqua, Journal of Ichthyology and aquatic Biology*, 13 (2), 69–76.
Winterbottom, R. & Villa, C.A. (2003) A new species of the *Trimma caesiura* complex (Gobiidae: Teleostei) from the north-eastern margin of the Australian Plate, with a redescription of the other nominal species in the complex. *Aqua, Journal of Ichthyology and aquatic Biology*, 7 (1), 13–28.
Winterbottom, R. & Zur, M. (2007) Three new species of the *Trimma* from Palau, Western Pacific (Percomorpha; Gobiidae). *Aqua, Journal of Ichthyology and aquatic Biology*, 13 (1), 13–24.
Yoshino T. & Araga, C. (1975) *Trimma caudomaculata* Yoshino and Araga. *In*: Masuda, H., Araga, C. & Yoshino, T. (Eds.) *Coastal Fishes of Southern Japan*. Tokai University Press, Tokyo, Japan, pp 272.