

# Crossing the borders: First record of Bleeding Wrasse, *Polylepion cruentum* Gomon, 1977 (Labriformes, Labridae), in the Northern Peru

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**Abstract.** The Labridae is a species-rich family of colorful fishes distributed globally in different habitats. *Polylepion cruentum* was described based on type material collected from the Gulf of California, and Quepos, Pacific coast of Costa Rica. It has since been reported from several other localities, including Mexico to Nicaragua and the Cocos Island. Recent collecting efforts in Northern Peru yielded an unusual species of labrid with uncertain taxonomic identity. Measurements, counts, coloration (in life and preserved) and DNA barcoding of the specimen agrees well with *Polylepion cruentum*. This represents the first record of the species from Northern Peru (Acapulco, Tumbes Department) and South America. The expansion of the distribution range of this species is perhaps due to the input of warm waters to Northern Peru, increasing especially during El Niño events. This new record is important to include in future marine checklists and consequently evaluate the conservation status of this species in Peru.

**Keywords.** Morphological identification; DNA barcode; *Polylepion*; Rocky fish; Distribution expansion.

## INTRODUCTION

The order Labriformes presents the families Labridae, Odacidae and Scaridae. Labridae is a species-rich family with 68 genera and 562 valid species (Fricke *et al.*, 2021; Nelson *et al.*, 2016). The family comprise colorful fishes characterized by a continuous dorsal fin, thick lips, jaws with well-developed dentition to capture a wide range of prey, and a recognizable labriform locomotion through propulsion of pectoral fins (Bray, 2017; Westneat & Alfaro, 2005). Some species are protogynous hermaphrodites, changing sex from female to male with maturity. The change is usually accompanied by stark sexual dimorphism (Bray, 2017). Species of this family are found in the Atlantic, Indian, and Pacific Oceans in different habitats, such as tidal pools, grassy, rocky or coral reefs, open sand bottoms, with a wide range of water temperatures (Hanel *et al.*, 2002). The genus *Polylepion* Gomon, 1977 comprise two valid species (Fricke *et al.*, 2021), *Polylepion russelli* (Gomon & Randall, 1975) and *Polylepion cruentum* Gomon, 1977. *Polylepion russelli* its distributed from across the Indo-Pacific: Ryukyu Islands, Mariana Islands, Johnston Atoll,

Hawaiian Islands, French Polynesia and Reunion Island (Béarez *et al.*, 2013); and *P. cruentum* from the Tropical Eastern Pacific, ranging from Mexico (Baja California) to Nicaragua, and Cocos Island.

*Polylepion cruentum* is known as the “Bleeding Wrasse”, inhabiting depths ranging from 150 to 200 m and measure up to 25 cm (Gomon, 1997). An unidentified labrid was collected during fieldwork in 2016, and was identified as *Polylepion cruentum* based on morphological and molecular (DNA barcoding) methods. The purpose of this study is to report the occurrence of *P. cruentum* from northern Peru (Acapulco, Tumbes Department), extending the distribution range of this species to include South America.

## MATERIAL AND METHODS

### Taxon sampling and morphological analysis

In July 2016 an unknown labrid was collected from a bottom curtain net in front of 14-16 nautical miles (NM) from Acapulco (Plateros), Tumbes in extreme Northern Peru (03°34'06.08",

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<http://www.revistas.usp.br/paz>

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**Table 1.** Morphometrics of *Polylepidion cruentum* from Peru (LCT 579). Data of *P. cruentum* from Gomon (1977) introduced for comparison. Standard length (SL) is expressed in mm; all other measurements are expressed as percentages of SL, except for subunits of head that are expressed as percentages of head length (HL).

| LCT 579, <i>Polylepidion cruentum</i> |   |             | Gomon (1977) <i>Polylepidion cruentum</i> |    |                   |
|---------------------------------------|---|-------------|---|----|-------------------|
|                                       | n | measurement |   | n  | measurement range |
| Standard length                       | 1 | 233         | Standard length                           | 11 | 56.8-199          |
| <b>Percentages of standard length</b> |   |             | <b>Percentages of standard length</b>     |    |                   |
| Body depth                            | 1 | 26.3        | Body depth                                | 11 | 23.5-29.4         |
| Predorsal length                      | 1 | 33.0        | Predorsal length                          | 11 | 35.7-38.1         |
| Dorsal fin base                       | 1 | 45.9        | Dorsal fin base                           | 11 | 46.9-52.6         |
| First dorsal fin spine length         | 1 | 7.8         | First dorsal fin spine length             | 11 | 7.0-8.6           |
| Eleventh dorsal fin spine length      | 1 | 9.9         | Eleventh dorsal fin spine length          | 11 | 9.1-12.2          |
| Pectoral fin length                   | 1 | 16.3        | Pectoral fin length                       | 11 | 17.3-20.6         |
| Pelvic fin length                     | 1 | 16.9        | Pelvic fin length                         | 11 | 14.7-25           |
| Anal fin base                         | 1 | 25.7        | Anal fin base                             | 11 | 21.8-25.3         |
| First anal fin spine                  | 1 | 7.3         | First anal fin spine                      | 11 | 4.7-6.5           |
| Third anal fin spine                  | 1 | 10.5        | Third anal fin spine                      | 11 | 8.6-10.9          |
| Caudal peduncle depth                 | 1 | 14.2        | Caudal peduncle depth                     | 11 | 11.8-13.6         |
| <b>Percentages of head length</b>     |   |             | <b>Percentages of head length</b>         |    |                   |
| Head Length                           | 1 | 39.0        | Head Length                               | 11 | 38.9-43.8         |
| Snout Length                          | 1 | 10          | Snout Length                              | 11 | 7.6-12.3          |
| Orbital diameter                      | 1 | 8.4         | Orbital diameter                          | 11 | 8.0-12.0          |
| Bony interorbital distance            | 1 | 7.7         | Bony interorbital distance                | 11 | 4.5-6.0           |
| Upper jaw length                      | 1 | 13.0        | Upper jaw length                          | 11 | 9.7-14.2          |

80°56'06.10"). Measurements were taken point-to-point under a stereomicroscope with a digital caliper to the nearest 0.1 mm on the left side of the specimen following Gomon (1977). Measurements are expressed as percent of standard length (SL), except head measurements, which are recorded as percent of head (Table 1).

A piece of muscle was collected from the specimen and preserved in 95% ethanol for molecular analysis. The voucher specimen was fixed in 10% formalin and deposited in the Ichthyology Collection of the Laboratorio Costero de Tumbes of Instituto del Mar de Peru (LCT-IMARPE).

### Molecular analysis

**DNA sequencing:** Total DNA was extracted from ethanol-preserved muscle using the Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin, U.S.A.) following the manufacturer's recommendations. Partial sequence of mitochondrial Cytochrome C Oxidase subunit I (COI) was amplified using polymerase chain reaction (PCR) using the primers Fish F1 and Fish R1 (Ward et al., 2005). All PCR products were sequenced using the BigDye sequencing kit cycle terminators (Applied Biosystems, California, U.S.A.) according to the manufacturer's instructions, and finally the sequences were analyzed using ABI PRISM 3130 Genetic Analyzer (Applied Biosystems).

**Sequence alignment and phylogenetic analyses:** Contigs were assembled and edited in Geneious v5.5.6 (Biomatters, Auckland, New Zealand), and aligned using the Muscle algorithm under default parameters (Edgar, 2004). The sequence was aligned and translated

**Table 2.** Taxon list, specimen and sequence data analyzed in the present study.

| Species                                     | GenBank number COI | Bold Sytem number COI | Reference              |
|---|--------------------|-----------------------|------------------------|
| <i>Polylepidion cruentum</i> Peru           | —                  | FMCT1092-19           | Present study.         |
| <i>Polylepidion cruentum</i> Mexico         | —                  | LIDMA2105-14          | Unpublished            |
| <i>Polylepidion russelli</i> Society Island | JQ432026           | —                     | Hubert et al., 2012    |
| <i>Polylepidion russelli</i> Society Island | JF435093           | —                     | Hubert et al., 2012    |
| <i>Semicossyphus pulcher</i>                | GU440521           | —                     | Unpublished            |
| <i>Decodon melasma</i>                      | MF956643           | —                     | Robertson et al., 2017 |
| <i>Bodianus neoperularis</i>                | KX781948           | —                     | Gomon & Walsh, 2016    |
| <i>Bodianus bimaculatus</i>                 | KX781951           | —                     | Gomon & Walsh, 2016    |
| <i>Abudefduf troschelii</i>                 | MW630825           | —                     | Tang et al., 2021      |

ed using Geneious v5.5.6 (Biomatters, Auckland, New Zealand) to check for the presence of stop codons. The COI gene sequence of *P. cruentum* from Peru was compared with other sequences of *Polylepidion cruentum*, as well as those of *P. russelli*. Species related to *Polylepidion* as proposed by Gomon (1977, 1997) were also included. These were *Bodianus bimaculatus*, *B. neoperularis*, *Decodon melasma*, and *Semicossyphus pulcher*. *Abudefduf troschelii* (Pomacentridae) was used as outgroup. All sequences were retrieved from Genbank and Bold system (Table 2). Analysis of genetic distance according to the Kimura-2-parameter model, including bootstrap analysis (Felsenstein, 1985) with 1,000 replications was performed using MEGA v. X to provide a graphic representation of the pairwise distance among labrid species (Kumar et al., 2018).

Maximum-likelihood (ML) analysis was performed using RAXML Web-Servers Black-Box (Stamatakis et al., 2008) with GTR + G + I as the model. Random starting trees were used for each independent ML tree search, and all other parameters were set at default values.

Topological robustness was investigated using 1,000 nonparametric bootstrap pseudoreplicates (Felsenstein, 1985).

**Institutional acronyms follow Sabaj (2020):** Laboratorio Costero de Tumbes – Instituto del Mar de Perú (LCT-IMARPE).

## RESULTS

### *Polylepidion cruentum* Gomon, 1977 (Fig. 1, Table 1)

#### Bleeding wrasse

**Diagnosis:** *P. cruentum* differs mainly from *P. russelli* by in having the following combination of characters: anal-fin rays III, 12-13 (vs. III, 11), predorsal scales extending forward in advance of anterior nostril (vs. only to anterior extent of orbit), anterior interspinous membranes of dorsal fin black (vs. not dark pigmented), body mostly pink with three narrow wavy yellow stripes on dorsal superior of body and one pink stripe midlaterally; (vs. body alternating broad pink and yellow stripes), caudal peduncle with a large oval red spot in the upper area (vs. oval red and black spot).

**Description:** Morphometric data are presented in Table 1. Body moderately slender, mainly at rear; head large and pointed, forehead and snout profile straight; nape nearly straight in large fish; Head mostly scaled, scales becoming progressively smaller anteriorly on nape and top of head, reaching to midpoint between anterior nostril and snout tip; small scales covering cheek, infra-orbital and lower side of head to corner of the sides of lower jaw; eye large; mouth horizontal, reaching to cen-

ter of eye, lower lip thick; both jaws with 2 pairs of front canines; top jaw with 1 (1-2) large rear canines; lower jaw without large rear canines; gill rakers 15. Dorsal fin XI, 12; anal fin III, 13; pectoral fin II, 17; pelvic fin I, 5. Lateral line complete with 50 scales; no scales on bases of dorsal and anal fins; scales above lateral line 3, scales below lateral line 13. Predorsal scales 8. Caudal fin rays 16.

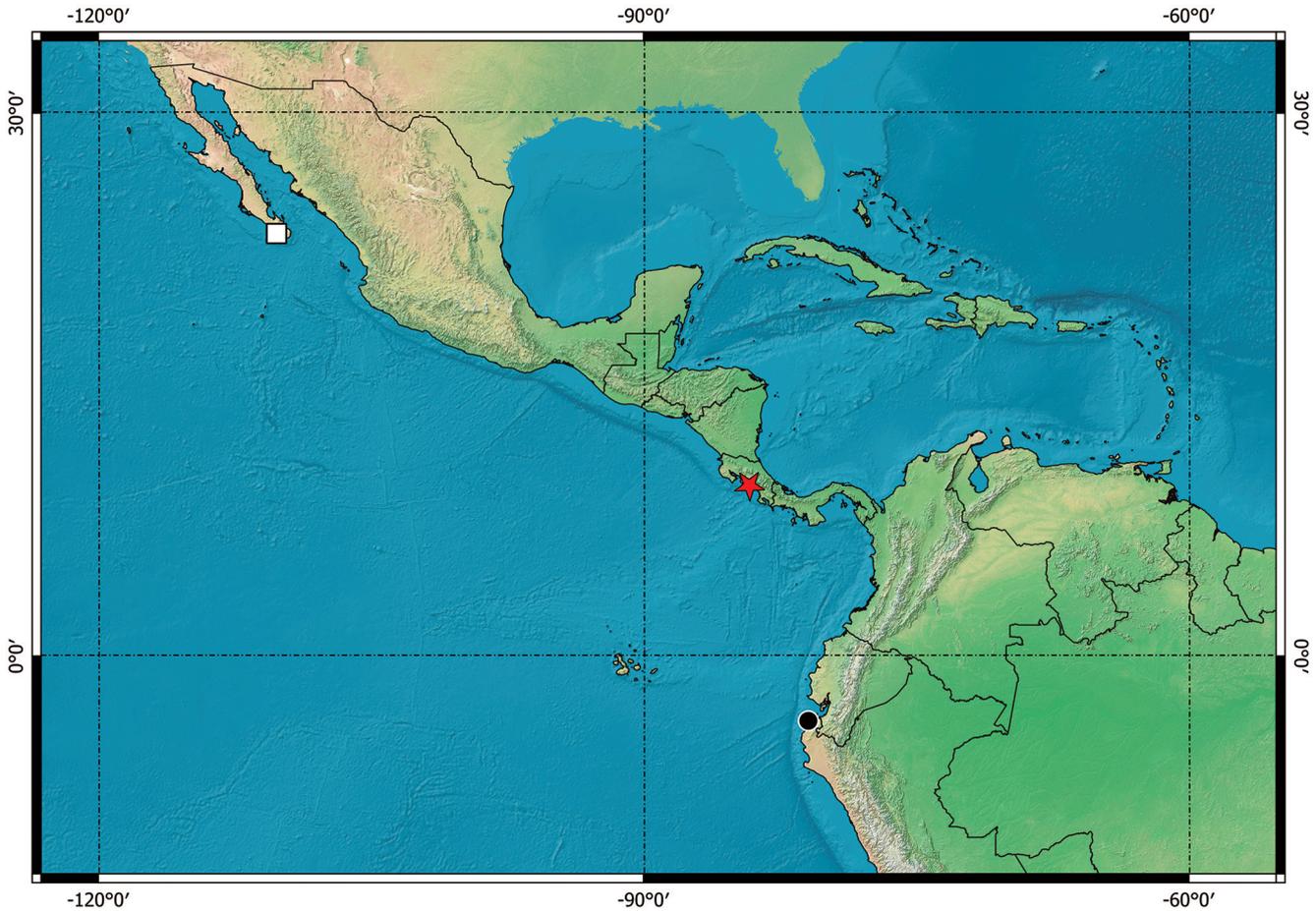
**Color in preserved specimen:** Body pale. Anterior dorsal fin spines with black membranes, posterior dorsal fin rays with gray pigment at the base and along margin. Anal fin with a gray pigment along the margin. Pelvic and pectoral fin pale. Operculum dusky. Freshly preserved specimen with faint irregular narrow dusky stripes on nape and dorsal area of the body. Spot at caudal peduncle fainter. A white pale stripe, from top corner of operculum to flank under 4<sup>th</sup> dorsal soft ray.

**Color in life:** Ground color of the body pink with bright reddish and orange, with the upper portion more reddish and lower body more yellowish; three narrow wavy yellow stripes on dorsal superior of body, one pink stripe midlaterally; underside of head, breast and lower abdomen whitish; a white stripe, from top corner of operculum to flank under 4<sup>th</sup> dorsal soft ray. Snout with yellow spots. Cheek with yellow stripes from the lower eye to operculum. Dorsal fin pink reddish, with distal margin yellowish, and dark gray between front 2-4 spines; membranes of soft dorsal fin yellowish. Anal fin light pinkish with distal margin yellowish. Pelvic fin light pinkish. Pectoral fin light pinkish, with red bar at the base. Caudal fin yellow at the center and pink reddish in the upper and lower edges. Caudal peduncle with a large oval red spot in the upper area.

**Distribution and habitat:** *Polylepidion cruentum* occurs in Eastern Central Pacific Ocean from Mexico to Nicaragua,



**Figure 1.** *Polylepidion cruentum*, LCT 579, 233 mm SL: Peru, Tumbes Department, Plateros-Acapulco, 16 MN into the Pacific Ocean, 09 Aug. 2016. Photo by Carlos Luque.



**Figure 2.** Distribution of *Polylepion cruentum* in the Tropical Eastern Pacific (TEP). Red Star represents Type locality of the species in Costa Rica. Black Circle represents the new record of the species in the Northern Peru (Acapulco, Tumbes Department). White Square represents the specimen from Baja California, Mexico.

**Table 3.** Genetic distance (lower diagonal) and standard error (upper diagonal) according to the Kimura-2-parameter model among species of labrids.

|   | 1     | 2            | 3            | 4            | 5            | 6            | 7            | 8            |
|---|-------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| 1 <i>Polylepion cruentum</i> Tumbes Peru            |       | <b>0.000</b> | <b>0.015</b> | <b>0.015</b> | <b>0.019</b> | <b>0.022</b> | <b>0.020</b> | <b>0.021</b> |
| 2 <i>Polylepion cruentum</i> Baja California Mexico | 0.000 |              | <b>0.015</b> | <b>0.015</b> | <b>0.019</b> | <b>0.022</b> | <b>0.020</b> | <b>0.021</b> |
| 3 <i>Polylepion russelli</i> Society Islands        | 0.111 | 0.111        |              | <b>0.000</b> | <b>0.021</b> | <b>0.023</b> | <b>0.021</b> | <b>0.022</b> |
| 4 <i>Polylepion russelli</i> Society Islands        | 0.111 | 0.111        | 0.000        |              | <b>0.021</b> | <b>0.023</b> | <b>0.021</b> | <b>0.022</b> |
| 5 <i>Semicossyphus pulcher</i>                      | 0.185 | 0.185        | 0.210        | 0.210        |              | <b>0.020</b> | <b>0.019</b> | <b>0.019</b> |
| 6 <i>Decodon melasma</i>                            | 0.231 | 0.231        | 0.232        | 0.232        | 0.213        |              | <b>0.019</b> | <b>0.020</b> |
| 7 <i>Bodianus neoperularis</i>                      | 0.200 | 0.200        | 0.211        | 0.211        | 0.179        | 0.191        |              | <b>0.013</b> |
| 8 <i>Bodianus bimaculatus</i>                       | 0.207 | 0.207        | 0.220        | 0.220        | 0.179        | 0.195        | 0.100        |              |

including Cocos Island (Froese & Pauly, 2021). Here we extend this distribution to Northern Peru (Acapulco, Tumbes Department, Fig. 2). The species occurs over sandy bottoms associated with rocky reefs, at depths of 150-200 meters (Fisherman pers. obs.; Gomon, 1977).

**Conservation status:** *Polylepion cruentum* is categorized as Data Deficient (DD) according to IUCN criteria (Bertoncini, 2010).

**Genetic data:** The *COI* sequence alignment consists of 652 base pairs, with 197 variable nucleotides and 141 parsimony informative sites. The estimated index of substitution saturation (Iss) performed in DAMBE 5.2.31 (Xia & Xie, 2001) showed that the data was not saturated (i.e.,

Iss.c value greater than Iss). Genetic distances (Kimura, 1980) of the *COI* gene between *Polylepion cruentum* and *P. russelli* was  $0.111 \pm 0.015$ ; and between *Polylepion cruentum* from Peru and *P. cruentum* from Mexico was zero (Table 3).

## DISCUSSION

Results from our phylogenetic analyses shows that the clade of *Polylepion* genus (*P. cruentum* and *P. russelli*) are supported by a high bootstrap index and are sister group relationship of *Bodianus* and *Semicossyphus* in the Maximum Likelihood analyses (Fig. 3). The result reinforces overall similarities with Gomon's (1997) phylogeny,

but according to our results, the genus *Polylepidion* is more closely related to genus *Bodianus* and *Semicossyphus*, than to the genus *Decodon*. Gomon (1997), listed several primitive morphological and osteological features found only in the genus *Polylepidion*, when compared to others labrids fishes, such as highest numbers of unbranched pectoral-fin rays, procurrent caudal fin rays, and others; probably related to the ancestors of the family Labridae.

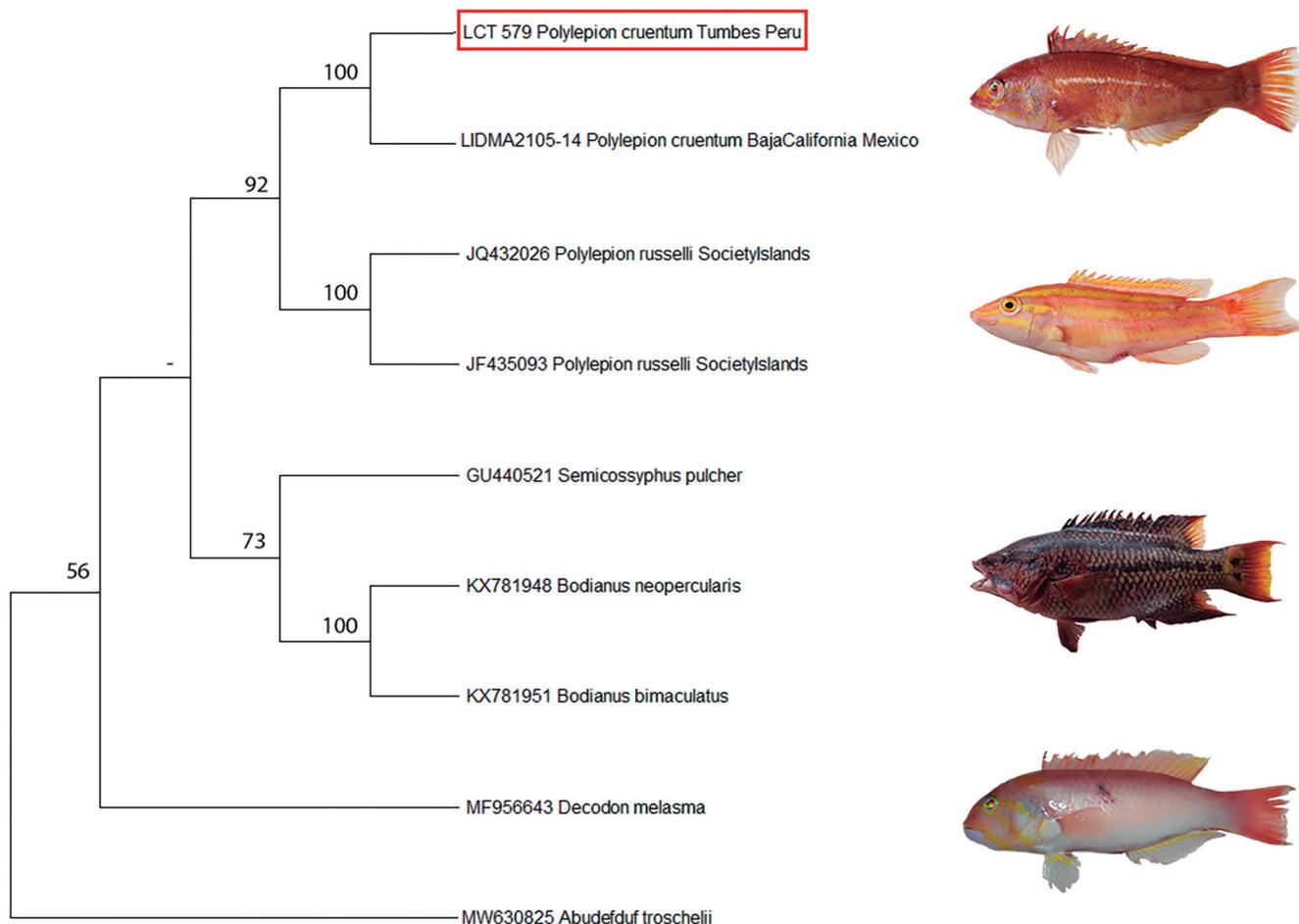
The intraspecific distance was zero between the only two samples of *P. cruentum* tested from Tumbes (Peru) and Baja California (Mexico). This indicates that the Tumbes sample is the same species reported in Baja California, thus extending its distribution range in the Eastern Pacific Ocean (Table 3).

*Polylepidion cruentum* is found over sandy bottoms associated with rocky reefs (150–200 meters of depth). Baldwin et al. (2018) analyzed several fishes that lived in rariphotic zone (below the mesophotic but above the deep aphotic) in the Caribbean Sea: *Lipogramma* (Grammatidae), *Haptoclinus* (Labrisomidae), and *Decodon* plus *Polylepidion* (Labridae). *Polylepidion* sp. from the Caribbean Sea is found in the upper rariphotic zone with 130–189 meters of depth, corroborating the habitat of *P. cruentum* in the Northern Peru. The extreme depths in which *P. cruentum* appear to be confined is, undoubtedly, responsible for their rarity in museum collections

and the absence of records from other areas throughout its distribution.

The Peruvian record of *P. cruentum* also may be explained by the tendency of marine species to be sensitive to changing climate and their greater capacity for colonization, mainly by due to biotic and abiotic factors, for example, the increase in the extent of marine currents, extension of the diet distribution, and others. Also in reef fishes, the early life stage serves as the primary opportunity to expand their range and is strongly influenced by warm currents (Pinsky et al., 2020).

Northern Peru is the end of an extensive coastal marine region, known as the Panamic Province (Hastings, 2000) or Guayaquil Ecoregion (Spalding et al., 2007) with predominantly tropical characteristics, where it presents large bodies of water known as Tropical Surface Waters (TSW), with temperatures close to 30°C and salinities below 33.8 ups; and Equatorial Surface Waters (ESW), with temperatures between 25 and 30°C and salinities between 33.8 and 34.8 ups (Zuta & Guillen, 1970; Takahashi, 2002). Generally, the TSW and ESW remain north between 04 and 06°S respectively, but when projected to the south they carry low salinity (less than 29 ups) up to approximately 06 and 08°S, especially during El Niño (EN) or the arrival of Kelvin waves (Flores et al., 2013). This might account for the expansion of the distribu-



**Figure 3.** Maximum likelihood tree based on mitochondrial Cytochrome oxidase c subunit 1 for several species of labrids, including the new specimen of *P. cruentum*. The series of three numbers (e.g., 100) at each of the main nodes represents the percentage of bootstrap support obtained by Maximum Likelihood (ML) analysis, respectively (1,000 pseudoreplicates). Dashes represent values < 50%.

tion of *P. cruentum* to Northern Peru (Acapulco, Tumbes Department).

The appearances of many fish species might be related to the El Niño event, when warm waters flow to neighboring temperate regions (Chirichigno & Velez, 1998). Studying the family Chaenopsidae, Hastings (2000) comments on barriers to the dispersal of rocky reef fishes due to habitat gaps, like wide expanses of pelagic habitat isolating oceanic islands from the mainland, and long stretches of sandy, muddy, often mangrove lined bottoms, separating coastal hard-bottom reef regions. For some species of fishes, these barriers are ineffective, such as *Mugil setosus* (Britzke et al., 2019) and possibly also for *Polylepidion cruentum*, which inhabits greater depths (150-200 m).

This distribution of *P. cruentum* in Pacific Ocean is similar to the biogeographic region described by Briggs (1974, 1995), named Tropical Eastern Pacific, from southern Baja California to Northern Peru, including the Galapagos, the Revillagigedo archipelago, Clipperton, Cocos, and Malpelo islands. This region presents many highly endemic species and general species richness, which has been associated with the Gulf of California environment; rise of Panama Isthmus; immigration of species for the north, south and west; and presence of non-effective geographical barriers (Robertson & Cramer, 2009). This distribution area of *P. cruentum* is limited in the south by the cold Humboldt Current that flows along to coast of Peru towards the south Galapagos Islands (Penven et al., 2005).

Finally, we suggest that the expansion of the distribution range of *P. cruentum* is due to the input of warm waters in the Northern Peru, especially during El Niño events. Its incidental capture is due to the depths it inhabits which are difficult to access.

## CONCLUSION

The registration of *P. cruentum* in the Northern Peru (Acapulco, Tumbes Department) is important for future checklists of marine fishes found in deep rocky reefs, and consequently to evaluate the conservation status of this species in Peru. Our results also suggest that *COI* barcoding is useful in corroborating initial identifications based on morphological data.

## Comparative Material

*Decodon melasma*: LCT 1720, 1 ex, Perú, Tumbes, Acapulco, frente a Bonanza a 2.5 MN, col. C. Chimbor, R. Asmat, W. Coronado, 06 Feb. 2018.

*Halichoeres nicholsi*: LCT 1165, 1 ex, Perú, Tumbes, Punta Sal, área rocosa frente a Bonanza a 0.2 MN, col. S. Aleman, J. Robles, S. Fernandez, R. Asmat, 19 Feb. 2018.

*Halichoeres dispilus*: LCT 1805 1 ex, Perú, Tumbes, Grau, frente a Mal Paso a 1 MN, col. O. Carrillo, 10 Aug. 2017.

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## AUTHORS' CONTRIBUTIONS

RB, ZRSR, MLGT. Conceptualization, Data curation, Investigation, Methodology, Resources, Writing – original draft, Visualization. RB, ZRSR. Formal analysis, Software, Writing, Review & Editing. All authors actively participated in the discussion of the results, and reviewed and approved the final version of the paper. Authors declare no conflict of interest related to this manuscript.

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