

1 **REGULAR PAPER**

2

3 **DNA reconciles morphology and colouration in the drunk blenny genus**  
4 ***Scartichthys* (Teleostei: Blenniidae) and provides insights into their**  
5 **evolutionary history**

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35

36 **Abstract**

37 The blenniids of the genus *Scartichthys* represent key herbivore species of central and south  
38 American Pacific coastal reefs. Yet *Scartichthys* spp. remain difficult to identify in the  
39 field, especially across the c.a 6000 km where three of the four currently accepted species  
40 are known to occur in sympatry. The main diagnostic characters from traditional taxonomy  
41 that have been used to revise this genus are indeed elusive. At the same time, species can  
42 display multiple colour patterns in the field, depending on the ontogenetic stage, habitat  
43 association, and/or reproductive behaviour. Overall, molecular characterization is  
44 warranted to help address these issues. Here, we used a combination of colouration,  
45 morphological and molecular data for the first time, including specimens representative of  
46 the four currently valid species and seven described colour patterns. Our integrative  
47 approach revealed that only three of the four species should be considered as valid;  
48 *Scartichthys gigas* (Steindachner, 1876), *S. variolatus* (Valenciennes, 1836) and *S. viridis*  
49 (Valenciennes, 1836); while *S. crapulatus* Williams 1990 should be synonymized with *S.*  
50 *viridis*. In the same way, our analyses show that one of the colour patterns attributed so far  
51 to *S. gigas* is characteristic of the juvenile stages of *S. viridis*. Our time-calibrated  
52 phylogeny shows that this genus is relatively young, with an estimated time of divergence  
53 between *Scartichthys gigas* and *S. viridis* of around 1.71 Ma. In comparison, the  
54 Desventuradas and Juan Fernandez Islands endemic *S. variolatus* diverged about 1.95 Ma.  
55 Our results help to clarify the taxonomy of the *Scartichthys* genus.

56

57 **Keywords** biogeography, Chile, integrative taxonomy, kelp forests, molecular phylogeny,  
58 species delimitation.

## 59 1 | INTRODUCTION

60 Species are the core units of any analysis in ecological, biogeographical, conservation, or  
61 evolutionary studies. Taxonomists used to describe and name species using solely  
62 morphological characters, being the only tool available until the development of modern  
63 molecular biology tools (Teletchea, 2010). The recent development of molecular biology  
64 with the use of DNA sequences data, combined with global initiative such as the Barcode  
65 of Life initiative (Hebert & Gregory, 2005) have offered new tools and framework to not  
66 only complete taxonomic description but also challenge how species are described. If  
67 molecular approaches were at the beginning the subject of debate in the taxonomists  
68 community (Will et al., 2005), it is now recognized that the combination of molecules and  
69 morphology can improve and accelerate the process of describing new species (Kekkonen  
70 & Hebert, 2014a; Pante et al., 2015). In this context, the integrative taxonomy framework  
71 has been proposed to examine the congruence of the diversity of data available such as  
72 morphology, colouration, behaviour, and molecules to help delimiting species (Padial et  
73 al., 2010).

74

75 The blenniids of the genus *Scartichthys* are one of the most abundant herbivorous  
76 fishes of intertidal and shallow subtidal rocky environments along the Pacific coast of  
77 South America from western Panama to the latitude 33°S in Chile. They also occur at the  
78 Juan Fernández Archipelago and the Desventuradas Islands (Stepien, 1990; Pérez-Matus et  
79 al., 2017a, 2017b). They are famous among fishermen for the unfounded side effects  
80 associated with its consumption leading to its common name “borrachillas” [the drunken  
81 ones] (Williams, 1990; Méndez-Abarca & Mundaca, 2016). Yet, these giants among  
82 blenniids, adults reaching up to 300 mm total length, remain difficult (Pérez-Matus et al.,  
83 2007; Riquelme-Pérez et al., 2019) or even impossible to identify at the species level in the  
84 field (Villegas et al., 2019), especially across the 6000 km where three of them are known  
85 to occur in sympatry. Elusive characters such as the number of dental incisors (DI) and  
86 colour patterns described mainly from preserved specimens, the only material available at  
87 that time (Williams 1990), are indeed the main diagnostic characters for the four currently  
88 valid species: (1) *Scartichthys variolatus* (Valenciennes, 1836) is endemic to the islands of  
89 the Juan Fernández Archipelago (33°SL) and the San Ambrosio and San Félix Islands



90 (26°SL); (2) *S. gigas* (Steindachner, 1876) is distributed from Panama (9 ° NL) to Northern  
91 Chile (Antofagasta, 23°SL) and in contrast to the three other species, display less than 73  
92 DI and typically 17 dorsal rays; (3) *S. viridis* (Valenciennes, 1836) with a geographic  
93 distribution from Peru (Independence Bay, 14°SL) to Central Chile (Valparaíso, 33 ° SL),  
94 display more than 73 DI while the distinct colour patterns of preserved specimen (“*Tiny,*  
95 *dark-brown (rarely pale) spots on posterior half of body*”) have been commonly used to  
96 described it; (4) *S. crapulatus* Williams 1990, as a species endemic to Central Chile,  
97 reported only from Central Chile (Barquito (26°S) and Valparaiso (33°S)) where it occurs  
98 in sympatry with *S. gigas* and *S. viridis*.

99

100           Using colouration patterns solely to identify species can be problematic and can  
101 lead to confusion in the field. Five different colour patterns based on live specimens were  
102 reported (Méndez-Abarca & Mundaca, 2016) for two species, *S. viridis* and *S. gigas*. A  
103 clear link has been made between the “reticulated colour pattern” reported by Williams  
104 (1990) from preserved specimens of *S. gigas* and three live colour patterns reported by  
105 Méndez-Abarca & Mundaca (2016), respectively the “two-bar front head covered”, the  
106 “two-bar front head uncovered”, and the “reticulated bar-stained”. In addition to these three  
107 colour patterns, a new colour pattern has been attributed to juveniles of *S. gigas*; the  
108 “uniform orange-brown”. This last colour pattern is problematic because it is very similar  
109 to one of the colours in life described for *S. crapulatus* (“reddish-brown to golden”) by  
110 Williams (1990). However, *S. crapulatus* are also presenting “orange-brown dots on  
111 posterior half of the body” (Williams, 1990) difficult to see on the field. A “dark-light  
112 bluish green” pattern has been attributed to juveniles and adults of *S. viridis* (Méndez-  
113 Abarca & Mundaca, 2016). Together with the “circular red spots in head and body” pattern  
114 of *S. variolatus*, a total of seven different colour patterns have been described for the four  
115 species of this genus. The diversity of live colour patterns has been attributed to ontogeny,  
116 habitat association, and/or reproductive behavior. Unfortunately, no explicit references  
117 have been made to classic morphological characters such as the number of DI for the new  
118 colour patterns described. It is worth noting that assessing these characters are of particular  
119 complexity for juveniles.

120

121 Finally, the recent phylogeny of blenniids (Hundt & Simons, 2018) represents the  
122 first attempts to reconstruct the evolutionary history of *Scartichthys* including one  
123 representative of the four currently valid species based on five nuclear markers (refer to  
124 Supplementary Figure S1 in Hundt & Simons (2018)). This molecular phylogeny  
125 confirmed the monophyly of the genus *Scartichthys*. Molecular evidence (Hundt et al.,  
126 2014; Hundt & Simons, 2018) confirmed that *Scartichthys* was sister to *Ophioblennius* as  
127 formerly hypothesized by Williams (1990) using morphological data. Interestingly, a  
128 minimal divergence between *S. crapulatus* and *S. viridis* was retrieved, questioning whether  
129 this divergence was of the order of inter or intraspecific divergence and paralleling the  
130 concern expressed by Stepien (1990) regarding the validity of *S. crapulatus*.

131  
132 The elusive diagnostic morphological characters, the controversy regarding the  
133 validity of *S. crapulatus*, the lack of diagnostic molecular data, and the multitude of colour  
134 patterns described so far for the genus *Scartichthys* called for a reappraisal of the different  
135 diagnostic characters available so far. This study aims at clarifying the taxonomy of  
136 *Scartichthys* using for the first-time colouration, morphological and molecular data in  
137 combination. We reconstructed a phylogeny, including the different live colour patterns  
138 described so far for the four currently valid species of this genus to investigate the validity  
139 of the species described so far and reconstruct the evolutionary history of this genus.

140

## 141 **2 | MATERIALS AND METHODS**

142

### 143 **2.1 | Ethical statement sampling**

153

154 Fishes were collected according to Chilean environmental laws through R.EX 2231, R.EX  
155 556 and R.EX 1489 permits, and procedures for collection, maintenance, and analyses of  
156 fishes followed the international guidelines for animal experiments through ethical permits  
157 of Universidad de Valparaiso and Pontificia Universidad Católica de Chile.

158

### 159 **2.2 | Taxon sampling**

160

161 We analysed 66 specimens from 8 locations, covering most of the four species' geographic  
162 range composing the *Scartichthys* complex (Figure 1a, Table S1). Fifty-five specimens  
163 were sampled using hand nets and fishing lines between 0 and 15 m depth from December  
164 2018 to February 2019 for this study. Also, eleven specimens (early juveniles) were  
165 captured between Montemar, 33°SL, and El Quisco, 33°SL, between September 2015 and  
166 February 2017 using Ecocean light traps (CARE, Ecocean, Montpellier, France) and  
167 preserved in 96% EtOH (specimens from Díaz-Astudillo *et al.* (2019)). All specimens were  
168 euthanized with an overdose of benzocaine under bioethical standards before preservation.  
169 A small piece of pectoral fin tissue from each specimen was preserved in 96% EtOH at -  
170 20°C.

171

### 172 **2.3 | Morphological analyses**

173

174 Counts of incisor teeth and dorsal fin rays followed Williams (1990) and were taken using a  
175 Leica model EZ4 binocular. Specimens were photographed using a Nikon D90 and a Canon  
176 EOS T5 digital cameras, upon collection when possible, and in the laboratory to identify  
177 their fresh colouration patterns, following (Williams, 1990) and (Méndez-Abarca &  
178 Mundaca, 2016). The total counts of dentary incisors (DI) are diagnostic for only two of the  
179 four species of this genus (*S. gigas* and *S. variolatus*), while the seven colour patterns in life  
180 currently reported so far allow distinguishing in the field the four currently described  
181 *Scartichthys*; one for *Scartichthys variolatus*, one for *S. viridis*, one for *S. crapulatus* and  
182 four for *S. gigas*. Only part of morphological data (colouration) was collected for  
183 specimens collected in San Juan de Marcona (SJM), Perú. No morphological data could be  
184 obtained from specimens kept in alcohol upon capture (Table S1).

185

### 186 **2.4 | DNA Extraction, amplification and sequencing**

187

188 Whole genomic DNA was extracted from fin tissue preserved in 96% EtOH. DNA  
189 extraction was performed following the HotSHOT method (Truett *et al.*, 2000), using  
190 50mM NaOH and 1M Tris-HCL. For each specimen, we amplified a fragment of 652 bp of  
191 the mitochondrial gene coding for cytochrome C oxidase subunit I (COI) with the primers

192 F2 and R2 designed by (Ward et al., 2005). Fragments were amplified using PCR protocols  
193 as described by (Williams et al., 2012), with modifications in the final reactions (10 µl),  
194 containing 5 µl of KAPAG Fast Multiplex Mix multiple mixing solution (KAPA2G Fast  
195 HotStart DNA Polymerase (1 U per 25 µL reaction), KAPA2G Buffer A (1.5X at 1X),  
196 dNTPs (0.2 mM each dNTP at 1X), MgCl<sub>2</sub> (3 mM at 1X) and stabilizers), 1 µl of a mixture  
197 of F2 and R2 primers (2mM each primer), 3.0 µl H<sub>2</sub>O, and 1 µl of genomic DNA. After  
198 PCRs, 1 µl of each PCR product (mixed with 1 µl of Red Gel dye) was separated by  
199 electrophoresis on a 1% agarose gel at 100 V for 30 minutes and visualized with a UV  
200 transilluminator. When the PCR products showed a clear and unique band of the correct  
201 expected length, all PCR products were purified by adding 1.3 µl of alkaline exonuclease  
202 phosphatase and then placed in the thermal cycler at 37°C for 60 min and then at 85°C for  
203 15 min. The sequencing was performed bi-directionally with the same PCR primers using a  
204 BigDye™ Terminator v3.1 cycle sequencing kit and an ABI 3500 XL Applied Biosystems  
205 sequencer. Sequences were aligned with Clustal W (Thompson et al., 1994) and edited  
206 using GENEIOUS 9.0.5 (<http://www.geneious.com>, (Kearse et al., 2012)). All generated  
207 sequences were deposited in GenBank (Accession numbers: *forthcoming*).

208

## 209 **2.5 | Phylogenetic analyses**

210

211 We first constructed a haplotype network using the haplonet function of the package  
212 “pegas” (Paradis, 2010) in the R statistical environment (R Core Team, 2020) to visualize  
213 the relationships between haplotypes of *Scartichthys* spp. among the different sampling  
214 localities. We then implemented Neighbour-Joining (NJ), Maximum Likelihood (ML), and  
215 Bayesian inference (BI) methods to reconstruct phylogenetic relationships of *Scartichthys*  
216 spp. The NJ analysis based on the Kimura 2-parameter (K2P) model of sequence evolution  
217 (Kimura, 1980) was conducted using the software package MEGA 6 (Tamura et al., 2013).  
218 Confidence in topology was evaluated by bootstrap analysis with 1000 replicates  
219 (Felsenstein, 1985). The Maximum Likelihood (ML) analysis was performed using the  
220 online version of IQ-TREE (Minh et al., 2013; Nguyen et al., 2015) available at  
221 <http://iqtree.cibiv.univie.ac.at> (Trifinopoulos et al., 2016). ModelFinder implemented in  
222 IQ-TREE was used to assess the best model of evolution using the Bayesian Information

223 Criterion (BIC) prior to the construction of the ML tree (Kalyaanamoorthy et al., 2017).  
224 The ultrafast bootstrap approximation (UFboot) (Minh et al., 2013) and the SH-like  
225 approximate likelihood ratio test (SH-aLRT), both with 1,000 bootstrap replicates  
226 (Guindon et al., 2010) was conducted to evaluate the reliability of the nodes. Sequences of  
227 *Ophioblennius macclurei* (KF930203), *Cirripectes variolosus* (MH707881), *Cirripectes*  
228 *polyzona* (HQ168554) and *Exallias brevis* (MF409572) were downloaded from GenBank  
229 and used to root the trees in all analyses.

230

231 Finally, a time-calibrated phylogeny (BI) of *Scartichthys* spp. using one specimen  
232 per species based on our mitochondrial marker (COI) and five nuclear markers (ENC1,  
233 myh6, ptr, sreb2 and tbr1) was constructed with the software BEAST2 2.5.2 (Bouckaert et  
234 al., 2019). We used COI sequences representatives of *S. viridis*, *S. gigas* and *S. variolatus*  
235 together with nuclear sequences corresponding to these three species produced by Hundt &  
236 Simons (2018) (Table S2; *Scartichthys crapulatus* discarded given results from all previous  
237 analyses (see Results)). Blenniids are relatively rare and fragmented in the fossil record  
238 (Bannikov 1998), but see Methods and Supplementary Information section of Liu *et al.*  
239 (2018) for a recent review), preventing their use as reliable calibration point. Deep  
240 secondary calibrations have thus been generally used for Blenniids (e.g. Lin & Hastings,  
241 2013; Liu *et al.*, 2018), which can lead to overestimation of divergence times among taxa  
242 that have recently diverged (Ho et al., 2008) or in such small scale survey. We thus chose to  
243 use an informative prior for the evolutionary rate of COI based on the substitution rate of  
244 1.2 % per million years commonly used for fishes for this marker (e.g. Bermingham,  
245 McCafferty, & Martin, 1997; Lessios, 2008; Tea *et al.*, 2019). We assumed a strict clock  
246 for each of the six markers, with the relative rates of ENC1, myh6, ptr, sreb2 and tbr1 being  
247 inferred in our analyses, a Birth-Death model as tree prior, with a chain length of 30  
248 million generations. ModelFinder implemented in IQ-TREE was used to assess the best  
249 model of evolution for each marker using the Bayesian Information Criterion (BIC). Trees  
250 and parameters were sampled every 3000 generations, and the first 10% of the samples  
251 were discarded as burn-in. We assessed the convergence and appropriate burn-in of each  
252 analysis using TRACER 1.5 (Drummond & Rambaut, 2007). Three independent analyses  
253 were run to ensure convergence. A maximum clade credibility tree was constructed using

254 TreeAnnotator 2.5.2 (Bouckaert et al., 2019) to get median ages and 95% highest posterior  
255 density (HPD) intervals for each node. The 95% HPD represents the smallest interval that  
256 contains 95% of the posterior probability and can be loosely thought of as a Bayesian  
257 analog to a confidence interval (Gelman et al., 2013).

258

## 259 **2.6 | Sequence-based species delimitation analysis**

260

261 As each sequence-based species delimitation method is susceptible to pitfalls, we used a  
262 50% consensus to produce a robust delimitation scheme among five different methods  
263 (Kekkonen & Hebert, 2014b; Hubert & Hanner, 2015; Kekkonen et al., 2015): (1)  
264 Automatic Barcode Gap Discovery (ABGD; (Puillandre et al., 2012)) available at  
265 <https://bioinfo.mnhn.fr/abi/public/abgd/>, Poisson Tree Process (PTP, Zhang *et al.*, 2013) in  
266 its (2) single (sPTP) and (3) multiple rate version (mPTP) available at [https://mptp.h-](https://mptp.h-its.org/#/tree)  
267 [its.org/#/tree](https://mptp.h-its.org/#/tree) and General Mixed Yule-Coalescent (GMYC) in its (5) single rate version  
268 (sGMYC) and (6) multiple rate version (mGMYC) as implemented in the R package Splits  
269 1.0-19 (Fujisawa & Barraclough, 2013). ABGD need a DNA alignment as input while we  
270 used the ML tree as input for PTP. Finally, the ultrametric and fully resolved tree needed to  
271 conduct GMYC analyses was reconstructed using the Bayesian approach implemented in  
272 BEAST 2.5.2. Two Markov chains of 10 million were run independently using a Yule pure  
273 birth model tree prior, a strict-clock model of 1.2% of genetic distance per million years.  
274 Trees were sampled every 1,000 states after an initial burn-in period of 1 million, both runs  
275 were combined using LogCombiner 2.5.2, and the maximum credibility tree was  
276 constructed using TreeAnnotator 2.5.2 (Bouckaert et al., 2019). Duplicated sequences were  
277 pruned prior to the Bayesian analysis.

278

## 279 **3 | RESULTS**

280

281 The total length (TL) of specimens collected ranged from 50 to 246 mm (Figure 2, Table  
282 S1). Specimen displaying the “uniform orange-brown” and the “orange-brown dots on  
283 posterior half of the body” colour patterns were the smallest, with mean size 80 mm ( $\pm$  24  
284 mm) and 106 mm ( $\pm$  20 mm), respectively. In contrast, specimens displaying the reticulated



285 patterns were the largest with a mean size 156 mm ( $\pm$  44 mm). The number of dorsal rays  
286 retrieved for the “reticulated bar stained” and the “two bar front head covered” colour  
287 patterns were typically 17 while the five other colour patterns displayed typically 18  
288 (Figure 2). The number of dentary incisors (DI) was counted for 66 of the specimens and  
289 ranged from 52 to 123 (Figure 2). Among the three-colour pattern attributed to *S. gigas*,  
290 only the “reticulated” and the “two-bar front head covered” displayed a number of DI in  
291 accordance with the diagnostic description. Specimens with the “uniform orange-brown”  
292 colour pattern presented up to 118 DI. Interestingly, red/orange spots (dots) that are usually  
293 a diagnostic character of *S. crapulatus* were found on specimens with the “uniform orange-  
294 brown” colour pattern and all specimens of dark-light bluish-green colouration when  
295 observed under the binocular (see Figure S1). *S. variolatus* displayed a unique colour  
296 pattern (circular red spots in head and body) and DI number (80-93). Both characters can be  
297 used to distinguish this island species from the remaining continental species. We retrieved  
298 DI numbers in accordance with the diagnostic report. Finally, the colour pattern is the only  
299 character distinguishing *S. crapulatus* from *S. viridis*; we retrieved similar DI numbers for  
300 both colour patterns described so far for these two species (Figure 2). It is worth noting that  
301 the range of values retrieved for specimen displaying the “uniform orange-brown” colour  
302 pattern attributed so far to *S. gigas* correspond with those of *S. crapulatus* and *S. viridis*.

303

304 Specimen displaying the “uniform orange-brown” and the “orange-brown dots on  
305 posterior half of the body” colour patterns were the smallest, with mean size 80 mm ( $\pm$  24  
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307 patterns were the largest with a mean size 156 mm ( $\pm$  44 mm). The number of dorsal rays  
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322 both colour patterns described so far for these two species (Figure 2). It is worth noting that  
323 the range of values retrieved for specimen displaying the “uniform orange-brown” colour  
324 pattern attributed so far to *S. gigas* correspond with those of *S. crapulatus* and *S. viridis*.

325

326 For the molecular analyses, we worked from an alignment of 652 base pairs from  
327 the mitochondrial COI region. While specimens of all currently four valid *Scartichthys*  
328 species were sampled, our molecular analysis only shows the existence of three well  
329 supported and highly divergent clades. The haplotype network analysis shows 3 distinct  
330 groups, separated by 36 and 26 mutations, respectively: a first one composed of all  
331 Robinson Crusoe specimens, a second one composed of specimens collected in the two  
332 northernmost sampling localities (San Juan de Marcona (Peru) and Antofagasta (Chile)),  
333 and finally a third group composed of specimens caught in continental Chile (Figure 1b).  
334 The best nucleotide substitution model using the Bayesian information criterion (BIC) was  
335 HKY+I+G. The NJ and ML produce the same tree topology with strong bootstrap support  
336 (Figure 3). Similarly, the Bayesian analysis produced the same tree topology (Figure 3)  
337 across all three runs with high posterior probabilities (PP) and parameters that reached  
338 effective sample sizes higher than 200.

339

340 All three methods revealed that *Scartichthys* is a monophyletic group composed of  
341 three well supported and highly divergent clades: (1) the first clade is composed of all  
342 specimen from Robinson Crusoe Island identified as *S. variolatus*; (2) the second clade is  
343 composed of 11 out of the 20 specimens identified as *S. gigas*; representing all specimens  
344 with the “reticulated” and the “two-bar front head covered” colour pattern and (3) the third  
345 clade is composed of not only all specimens identified as *S. viridis*, but also of all  
346 specimens identified as *S. crapulatus*, and all specimens displaying the “uniform orange-



347 brown” colour pattern, described so far as a juvenile colour pattern for *S. gigas* (Figure 3).  
348 Species delimitation analyses provided concordant number of Molecular Operational  
349 Taxonomic Units (MOTUs) among the different methods: 3 for ABGD, PTP, mPTP,  
350 GMYC and 4 for mGMYC, leading to a consensus delimitation scheme of 3 MOTUs. It is  
351 worth noting that the fourth MOTU delimited by mGMYC correspond to a single  
352 specimen among the *S. gigas* clade presenting a “reticulated bar-stained” colour pattern.

353

354 The same topology has been retrieved in our time-calibrated phylogeny based on  
355 six markers, on mitochondrial (COI) and five nuclear (ENC1, myh6, ptr, sreb2 and tbr1)  
356 (Figure 4). All but one node (*S. gigas* – *S. viridis* ; 0.50) were well supported (above 0.9).  
357 We found that *Scartichthys* and *Ophioblennius* diverged 7.84 Ma (6.20 – 9.41, 95% HPD).  
358 The estimated time of divergence between the *S. variolatus* (Clade 1) and the other  
359 *Scartichthys* spp. is approximately 1.95 Ma (1.41- 2.49, 95% HPD; Figure 4) while *S. gigas*  
360 (Clade 2) and *S. viridis* (Clade 3) diverged 1.71 Ma (1.00 - 1.97, 95% HPD, Figure 4).

361

## 362 **4 | DISCUSSION**

363

364 The present study represents an updated phylogeny of the *Scartichthys* genus. Using  
365 morphological, colouration, and molecular evidence in combination, we show that this  
366 genus is composed of three species. Our integrative approach revealed indeed that the  
367 colour pattern used to diagnose *S. crapulatus* and the “uniform orange-brown” colour  
368 patterns recently attributed to juveniles of *S. gigas* are both juvenile colour patterns of *S.*  
369 *viridis*. Finally, we show that the diversification of this genus is relatively recent, beginning  
370 around 1.95 Ma.

371

372 A diversity of information has been produced so far to describe and characterize  
373 species of the genus *Scartichthys*. Our study proposed to use for the first time this broad  
374 array of evidence in an integrative approach by congruence to clarify the taxonomy of  
375 *Scartichthys*. Five lines of evidence taken together indicate that the “small dark-brown  
376 spots on the posterior half of the body” colour pattern, used to characterize *Scartichthys*  
377 *crapulatus*, is also a colour pattern of juveniles of *S. viridis* and that the “uniform orange-

378 brown” colour pattern is also a colouration of *S. viridis* juveniles, not of *S. gigas* as  
379 previously proposed. These conclusions are based on:

380 (1) *Size*: the “small dark-brown spots on the posterior half of the body” and the  
381 “uniform orange-brown” specimens observed in the field and collected  
382 measured respectively 106 mm ( $\pm$  20 mm) and 80 mm ( $\pm$  24 mm), substantially  
383 smaller than what *Scartichthys* adults usually are. These two colour patterns are  
384 thus likely characteristic of juveniles. Stepien (1990) first suggested that the  
385 colour pattern attributed to *S. crapulatus* (“small dark-brown spots on the  
386 posterior half of the body”) was actually one of the juvenile forms of an already  
387 existing species (*S. viridis*). Indeed, both juveniles and adults of *S. viridis* can be  
388 found displaying a “Dark-light bluish green” pattern ;

389 (2) *Colouration*: we found small dark-brown spots on the posterior half of the body  
390 not only on adult specimens presenting the “Dark-light bluish green” pattern  
391 characteristic of *S. viridis*, similarly to Méndez-Abarca & Mundaca (2016), but  
392 also on specimen displaying the “uniform orange-brown” pattern (so far  
393 attributed to juveniles of *S. gigas*). The main diagnostic of *S. crapulatus* is thus  
394 not anymore diagnostic, three colour patterns sharing this character.  
395 Interestingly, Williams (1990) mentioned that colour in life of *S. crapulatus*  
396 were highly variable, with “Body colours [ranging] from green or reddish brown  
397 to golden with small brownish orange or brown spots on posterior half of body  
398 and on segmented-ray portion of dorsal fin.”, and it is possible that specimen  
399 presenting an “uniform orange-brown” pattern were actually included in *S.*  
400 *crapulatus* at that time. It should be emphasized that the “two bar front head  
401 uncovered” colour pattern described by Méndez-Abarca and Mundaca (2016)  
402 and attributed specifically to *S. gigas* corresponds to the “Orange-brown dots on  
403 posterior half of body” colour pattern described by Williams (1990) and is used  
404 in the present study as a character of *S. viridis* (Figure 5). If Méndez-Abarca &  
405 Mundaca (2016) did not mention orange-brown dots, we found that specimens  
406 with these two colour pattern have the two dark bars and the front head  
407 uncovered, and it is likely that dots were not easily noticeable on live specimens  
408 (see live specimens in Figure 5).

409 (3) *Dental incisors and dorsal rays*: The three colour patterns sharing the “small  
410 dark-brown spots on the posterior half of the body” (“dark-light bluish green”,  
411 “uniform orange-brown” and “orange-brown dots on posterior half of the body”  
412 patterns) also share a similar number of DI, clearly higher than the number of DI  
413 observed in *S. gigas* (Figure 2) and generally higher than the number of DI  
414 observed in *S. variolatus*. They also share similar a higher number of dorsal  
415 rays, typically 18, compared to the number of dorsal rays observed typically in  
416 *S. gigas*. The more variable number of DI for the “uniform orange-brown”  
417 retrieved here ( $109 \pm 8$ ), but as low as 58 in small specimens and the variable  
418 number of dorsal rays (typically 18, but as low as 17) might have led (Méndez-  
419 Abarca & Mundaca, 2016) to attribute this colour pattern to *S. gigas*, the species  
420 displaying the lowest number of DI and dorsal rays in this complex.

421 (4) *Molecular data*: Molecular analyses showed a lack of reciprocal mtDNA  
422 monophyly for the “small dark-brown spots on the posterior half of the body”,  
423 the “uniform orange-brown” and “Dark-light bluish green” patterns. Indeed,  
424 phylogenetic analyses (distance-based, Maximum Likelihood and Bayesian) and  
425 sequence-based delimitation methods all showed that these three-colour patterns  
426 were part of a single clade.

427 (5) *Distribution*: Our extensive sampling allowed us to find the juvenile “uniform  
428 orange-brown” patterns in a geographic region where *S. gigas* has not yet been  
429 recorded in central Chile (Valparaiso region). No *S. gigas* adults have been  
430 observed, excluding the possibility of a range extension of *S. gigas* and  
431 reinforcing the hypothesis that the “uniform orange-brown” is one of a juvenile  
432 colour patterns in *S. viridis*. The same reasoning applies for the “two bar front  
433 head uncovered” colour pattern. Specimens displaying the “uniform orange-  
434 brown” colour pattern are frequently observed in the subtidal kelp, *Lessonia*  
435 *trabeculata*, down to 20 m depth, and we thus hypothesize that this colouration  
436 is involved in camouflage (Gaither et al., 2020). In line with Gaither et al.  
437 (2020), different colour morphs of *S. viridis* have been observed together  
438 inhabiting the same environments. This colour polymorphism could thus be  
439 related to either juvenile stages or microhabitat preferences as both colour

440 morphs are more algae associated (Pérez-Matus *et al.*, 2017; Riquelme-Pérez *et*  
441 *al.*, 2019).

442

443 Our study also allowed us to depict the evolutionary history of the *Scartichthys*  
444 genus. The phylogenetic analyses all retrieved the monophyly of the genus *Scartichthys*, as  
445 previously proposed using a single representative per species (Hundt & Simons, 2018).  
446 Based on multiple specimens per colour patterns and currently valid species, we show here  
447 that the extremely shallow divergence Hundt & Simons (2018) observed between *S. viridis*  
448 and *S. crapulatus* can be attributed to intraspecific divergence. Indeed, all our analysis  
449 converged in the existence of three and not four well supported clades within *Scartichthys*,  
450 and resulted in trees presenting the same topology whether analysis were performed on COI  
451 only or on the mitochondrial marker and the five nuclear markers.

452

453 The topology retrieved here differs from the one previously proposed based on  
454 nuclear markers only in that *S. gigas* was early diverging from the other *Scartichthys*  
455 (Hundt & Simons, 2018). Mitochondrial markers such as COI indeed often possess  
456 numerous informative sites to untangle the relationship within species complex but are  
457 more prone to saturation and homoplasy than conserved nuclear markers. These conserved  
458 markers are often preferred to untangle relationships between genera (Clabaut *et al.*, 2005;  
459 Dornburg *et al.*, 2014). *Scartichthys* is sister to *Ophioblennius*, which are found in the  
460 Atlantic (*O. atlanticus*, *O. macclurei*, and *O. trinitatis*) and in Eastern Pacific (*O.*  
461 *clippertonensis* and *O. steindachneri*) (Froese & Pauly, 2019). Our estimates for the  
462 divergence between these two genera (7.84 Ma (6.20 – 9.41, 95% HPD)) are in agreement  
463 with the first estimations of Liu *et al.* (2018) (around 12 Ma (5 – 19 Ma) a secondary  
464 calibration (age of the crown Blenniidae: 66 Mya). *Scartichthys* and *Ophioblennius* genera  
465 diverging from Indo-Pacific *Cirripectes* and *Exallia*, an Eastward dispersal, from the Indo-  
466 West Pacific to Eastern Pacific to finally reach the Atlantic is a likely dispersal hypothesis  
467 at the origin of the emergence of *Scartichthys* in the Eastern Pacific (Duncan *et al.*, 2006;  
468 Hou & Li, 2018).

469

470 Three latitudinal biogeographic regions have been described for the continental  
471 Chilean species based on species geographic range distributions that inhabit intertidal  
472 habitats which coincide with the ecoregions delineated within the Warm Temperate  
473 Southeastern Pacific Province (Spalding et al., 2007); the central Chile that ranges from 20°  
474 to 36° SL, the Araucanian ecoregion from 39° to 43° SL, and the Chiloense region from  
475 45° to 55° SL (Spalding et al., 2007; Navarrete et al., 2014). Here, the two closely related  
476 continental species *Scartichthys viridis* and *S. gigas* are both distributed within this first  
477 biogeographic region. However, with *S. gigas* occurring from 9 ° NL to 23 ° SL and *S.*  
478 *viridis* from 14 ° SL to 33 ° SL, the two species occur in sympatry only from 14°SL to  
479 23°SL. They differ indeed in their success to colonize warmer waters, *S. viridis* being  
480 restricted to the Humboldt Current system and found only up to 14 ° SL while *S. gigas* can  
481 be found not as south as *S. viridis* but as north as 9 ° NL, outside of the Humboldt Current  
482 system, within the Tropical Eastern Pacific Province.

483  
484 The Desventuradas and Juan Fernández Islands represent a distinct biogeographic  
485 unit (Dyer & Westneat, 2010), a hotspot of endemism with more than 42 % of the species  
486 observed in its water occurring nowhere else in the world (Dyer & Westneat, 2010). Their  
487 origin and the processes that led to their emergence remain somehow a mystery.  
488 Biogeographic analyses based on species distribution showed that the Desventuradas  
489 Islands were grouped with Easter Island, and Sala y Gomez (Kulbicki et al., 2013) and  
490 related to the “Hawaiian Archipelago” and the “South Western Pacific Ocean” province,  
491 extending from western Australia all the way to the Kermadec islands (Kulbicki et al.,  
492 2013). Phylogenetic analysis including Juan Fernandez endemics are scarce and showed  
493 that Juan Fernandez endemic species were related so far to (a) either southwest Pacific  
494 species; with Juan Fernandez endemics *Chironemus bicornis* (Steindachner, 1898), *C.*  
495 *delfini* (Porter, 1914), *Amphichaetodon melbae* Burgess & Caldwell, 1978 and *Girella*  
496 *albostrigata* Steindachner, 1898 being all closely related to Australian / New Zealand / Lord  
497 How Island species (Burrige et al., 2006; Cowman & Bellwood, 2011; Gaboriau et al.,  
498 2018; Delrieu-Trottin et al., 2019; Beldade et al., 2020),(b) or southern Pacific species,  
499 occurring from Australia to Easter Island, with *Pseudolabrus gayi* (Valenciennes, 1839)  
500 being closely related to *P. fuentesi* (Regan, 1913) (Delrieu-Trottin et al., 2019). Our

501 phylogenetic analyses showed however that Juan Fernandez endemic species could also be  
502 related to continental Chile. The distinct origins so far retrieve call for a more extensive  
503 study including a larger number of Juan Fernandez endemic species.

504

505 The time tree produced here showed that the divergence time *Scartichthys*  
506 *variolatus* is around 1.95 Ma (1.41- 2.49, 95% HPD) and can be considered as a  
507 Neoendemic, being younger than the geological age of the Juan Fernandez and  
508 Desventuradas Islands (Santa Clara: 5.8 Ma, Robinson Crusoe: 3.7 Ma (Clouard &  
509 Bonneville, 2005; Lara et al., 2018)). Since Desventuradas Islands are younger, with San  
510 Ambrosio being 2.9 million years old (Clouard & Bonneville, 2005), it is likely that  
511 speciation occurred in Juan Fernandez Island followed by a colonization of the  
512 Desventuradas Islands. Similar divergence time have been retrieved for *Pseudolabrus gayi*,  
513 dated around 2.35 Ma (1.34-3.63, 95% HPD) (Delrieu-Trottin et al., 2019) while  
514 divergence estimates are older than the geological age of Juan Fernandez islands for  
515 *Amphichaetodon melbae* (7.26 Ma (4.23-10.97, 95% HPD)) (Delrieu-Trottin et al., 2019).  
516 A comprehensive survey of the divergence estimates of Juan Fernandez endemics would  
517 provide a better insight into the origin of this unique fauna.

518

## 519 **5 | CONCLUSION**

520

521 This study presents evidence that *S. crapulatus* should be synonymized with *S. viridis*,  
522 resolving a 30-year-old discord among taxonomists. We also showed that specimens  
523 displaying the colouration newly pattern described in 2016 (Méndez-Abarca & Mundaca,  
524 2016) are juvenile of *S. viridis*, using different habitats (mainly occupying subtidal kelp  
525 sporophytes) than adult, greenish individuals. This study is a novel example of how  
526 molecular genetics can help to address the problem of species delimitation. We contribute  
527 to the clarification of the systematics of the *Scartichthys* genus, reconciling morphological,  
528 distributional, colouration and molecular evidence. We also showed that the Juan  
529 Fernandez endemic species is relatively young and likely to be a neoendemic species.

530

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539

#### 540 **SUPPORTING INFORMATION**

541 Supplementary material is available at <https://doi.org/xxx>.

542

#### 543 **AUTHOR CONTRIBUTIONS**

544 EDT, HHS, PSA, MFL, and APM conceived the study; PSA, MFL, and APM acquired the  
545 funding; HHS, MFL, and APM collected the field data; EDT, HHS, PSA, and APM  
546 produced the data; EDT, HHS, PSA, and APM analysed the data; and all authors  
547 contributed to the writing and approved the final version of the manuscript.

548

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554

#### 555 **SIGNIFICANCE STATEMENT**

556 The blenniids of the genus *Scartichthys* represent key herbivore species of central and south  
557 American Pacific coastal reefs. Yet, they remain difficult to identify in the field. Here we  
558 provide an updated phylogeny of this genus, comparing for the first time morphological,  
559 coloration, and molecular data in combination, resolving a 30-year-old discord among  
560 ecologists and taxonomists.

561

562

563 **REFERENCES**

- 564 Bannikov, A. F. (1998) New Blennioid Fishes of the Families Blenniidae and Clinidae  
565 (Perciformes) from the Miocene of the Caucasus and Moldova. *Paleontologicheskii*  
566 *Zhurnal*.
- 567 Beldade, R., Longo, G. C., Clements, K. D., Robertson, D. R., Perez-Matus, A., Itoi, S.,  
568 Sugita, H. & Bernardi, G. (2020) Evolutionary Origin of the Atlantic Cabo Verde Nibbler  
569 (Girella Stuebeli), a Member of a Primarily Pacific Ocean Family of Antitropical  
570 Herbivorous Reef Fishes. *Molecular Phylogenetics and Evolution*.
- 571 Bermingham, E., McCafferty, S. & Martin, A. P. (1997) Fish Biogeography and Molecular  
572 Clocks: Perspectives from the Panamanian Isthmus. In *Molecular systematics of fishes*  
573 (Kocher, T. D., Stepien, C. A., eds), pp. 113–128 San Diego: CA Academic Press.
- 574 Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M.,  
575 Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., De Maio, N., et al. (2019) BEAST 2.5:  
576 An Advanced Software Platform for Bayesian Evolutionary Analysis. *PLoS Computational*  
577 *Biology*.
- 578 Burridge, C. P., Meléndez C, R. & Dyer, B. S. (2006) Multiple Origins of the Juan  
579 Fernández Kelpfish Fauna and Evidence for Frequent and Unidirectional Dispersal of  
580 Cirrhitoid Fishes across the South Pacific. *Systematic Biology* **55**, 566–578.
- 581 Clabaut, C., Salzburger, W. & Meyer, A. (2005) Comparative Phylogenetic Analyses of the  
582 Adaptive Radiation of Lake Tanganyika Cichlid Fish: Nuclear Sequences Are Less  
583 Homoplasious but Also Less Informative than Mitochondrial DNA. *Journal of Molecular*  
584 *Evolution*.
- 585 Clouard, V. & Bonneville, A. (2005) Ages of Seamounts, Islands, and Plateaus on the  
586 Pacific Plate. *Special Paper 388: Plates, plumes and paradigms* **388**, 71–90.
- 587 Cowman, P. F. & Bellwood, D. R. (2011) Coral Reefs as Drivers of Cladogenesis:  
588 Expanding Coral Reefs, Cryptic Extinction Events, and the Development of Biodiversity  
589 Hotspots. *Journal of Evolutionary Biology* **24**, 2543–2562.
- 590 Delrieu-Trottin, E., Brosseau-Acquaviva, L., Mona, S., Neglia, V., Giles, E. C., Rapu-  
591 Edmunds, C. & Saenz-Agudelo, P. (2019) Understanding the Origin of the Most Isolated  
592 Endemic Reef Fish Fauna of the Indo-Pacific: Coral Reef Fishes of Rapa Nui. *Journal of*  
593 *Biogeography* **46**, 723–733.



- 594 Díaz-Astudillo, M., Landaeta, M. F., Bernal-Durán, V., Castillo, M. I., Alvarado-Niño, M.  
595 & Alarcón, D. (2019) The Influence of Regional and Local Oceanography in Early Stages  
596 of Marine Fishes from Temperate Rocky Reefs. *Marine Biology* **166**, 42.
- 597 Dornburg, A., Townsend, J. P., Friedman, M. & Near, T. J. (2014) Phylogenetic  
598 Informativeness Reconciles Ray-Finned Fish Molecular Divergence Times. *BMC*  
599 *Evolutionary Biology* **14**, 169.
- 600 Drummond, A. J. & Rambaut, A. (2007) BEAST: Bayesian Evolutionary Analysis by  
601 Sampling Trees. *BMC Evolutionary Biology* **7**, 214.
- 602 Duncan, K. M., Martin, A. P., Bowen, B. W. & De Couet, H. G. (2006) Global  
603 Phylogeography of the Scalloped Hammerhead Shark (*Sphyrna Lewini*). *Molecular*  
604 *Ecology* **15**, 2239–2251.
- 605 Dyer, B. S. & Westneat, M. W. (2010) Taxonomy and Biogeography of the Coastal Fishes  
606 of Juan Fernández Archipelago and Desventuradas Islands, Chile. *Revista de biología*  
607 *marina y oceanografía* **45**, 589–617.
- 608 Felsenstein, J. (1985) Phylogenies and the Comparative Method. *The American Naturalist*  
609 **125**, 1–15.
- 610 Froese, R. & Pauly, D. (2019) FishBase [www.fishbase.org](http://www.fishbase.org).
- 611 Fujisawa, T. & Barraclough, T. G. (2013) Delimiting Species Using Single-Locus Data and  
612 the Generalized Mixed Yule Coalescent Approach: A Revised Method and Evaluation on  
613 Simulated Data Sets. *Systematic Biology* **62**, 707–724.
- 614 Gaboriau, T., Leprieur, F., Mouillot, D. & Hubert, N. (2018) Influence of the Geography of  
615 Speciation on Current Patterns of Coral Reef Fish Biodiversity across the Indo-Pacific.  
616 *Ecography* **41**, 1295–1306.
- 617 Gaither, M. R., Coker, D. J., Greaves, S., Sarigol, F., Payet, S. D., Chaidez, V., Sinclair-  
618 Taylor, T. H., DiBattista, J. D. & Berumen, M. L. (2020) Does Color Matter? Molecular  
619 and Ecological Divergence in Four Sympatric Color Morphs of a Coral Reef Fish. *Ecology*  
620 *and Evolution*.
- 621 Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010)  
622 New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing  
623 the Performance of PhyML 3.0. *Systematic biology* **59**, 307–321.
- 624 Hebert, P. D. N. & Gregory, T. R. (2005) The Promise of DNA Barcoding for Taxonomy.

- 625 *Systematic Biology* **54**, 852–859.
- 626 Ho, S. Y. W., Saarma, U., Barnett, R., Haile, J. & Shapiro, B. (2008) The Effect of  
627 Inappropriate Calibration: Three Case Studies in Molecular Ecology. *PLoS ONE*.
- 628 Hou, Z. & Li, S. (2018) Tethyan Changes Shaped Aquatic Diversification. *Biological*  
629 *Reviews* **93**, 874–896.
- 630 Hubert, N. & Hanner, R. (2015) DNA Barcoding, Species Delineation and Taxonomy: A  
631 Historical Perspective. *DNA Barcodes* **3**, 44–58.
- 632 Hundt, P. J. & Simons, A. M. (2018) Extreme Dentition Does Not Prevent Diet and Tooth  
633 Diversification within Combtooth Blennies (Ovalentaria: Blenniidae). *Evolution* **72**, 930–  
634 943.
- 635 Hundt, P. J., Iglésias, S. P., Hoey, A. S. & Simons, A. M. (2014) A Multilocus Molecular  
636 Phylogeny of Combtooth Blennies (Percomorpha: Blennioidei: Blenniidae): Multiple  
637 Invasions of Intertidal Habitats. *Molecular Phylogenetics and Evolution* **70**, 47–56.
- 638 Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., Von Haeseler, A. & Jermin, L. S.  
639 (2017) ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates. *Nature*  
640 *Methods* **14**, 587–589.
- 641 Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S.,  
642 Cooper, A., Markowitz, S., Duran, C., et al. (2012) Geneious Basic: An Integrated and  
643 Extendable Desktop Software Platform for the Organization and Analysis of Sequence  
644 Data. *Bioinformatics* **28**, 1647–1649.
- 645 Kekkonen, M. & Hebert, P. D. N. (2014a) DNA Barcode-Based Delineation of Putative  
646 Species: Efficient Start for Taxonomic Workflows. *Molecular Ecology Resources* **14**, 706–  
647 715.
- 648 Kekkonen, M. & Hebert, P. D. N. (2014b) DNA Barcode-Based Delineation of Putative  
649 Species: Efficient Start for Taxonomic Workflows. *Molecular Ecology Resources*.
- 650 Kekkonen, M., Mutanen, M., Kaila, L., Nieminen, M. & Hebert, P. D. N. (2015)  
651 Delineating Species with DNA Barcodes: A Case of Taxon Dependent Method  
652 Performance in Moths. *Plos One* **10**, e0122481.
- 653 Kulbicki, M., Parravicini, V., Bellwood, D. R., Arias-González, E., Chabanet, P., Floeter,  
654 S. R., Friedlander, A., McPherson, J., Myers, R. E., Vigliola, L., et al. (2013) Global  
655 Biogeography of Reef Fishes: A Hierarchical Quantitative Delineation of Regions. *PLoS*

- 656 *ONE* **8**.
- 657 Lara, L. E., Reyes, J., Jicha, B. R. & Díaz-Naveas, J. (2018)  $^{40}\text{Ar}/^{39}\text{Ar}$  Geochronological  
658 Constraints on the Age Progression along the Juan Fernández Ridge, SE Pacific. *Frontiers*  
659 *in Earth Science* **6**, 194.
- 660 Lessios, H. A. (2008) The Great American Schism: Divergence of Marine Organisms After  
661 the Rise of the Central American Isthmus. *Annual Review of Ecology, Evolution, and*  
662 *Systematics* **39**, 63–91.
- 663 Lin, H.-C. & Hastings, P. A. (2013) Phylogeny and Biogeography of a Shallow Water Fish  
664 Clade (Teleostei: Blenniiformes). *BMC Evolutionary Biology* **13**, 210.
- 665 Liu, S. Y. V., Frédérick, B., Lavoué, S., Chang, J., Erdmann, M. V., Mahardika, G. N. &  
666 Barber, P. H. (2018) Buccal Venom Gland Associates with Increased of Diversification  
667 Rate in the Fang Blenny Fish *Meiacanthus* (Blenniidae; Teleostei). *Molecular*  
668 *Phylogenetics and Evolution*.
- 669 Méndez-Abarca, F. & Mundaca, E. A. (2016) Colouration Patterns of Two Species of the  
670 Genus *Scartichthys* (Blenniidae: Perciformes) in the Coastal Area of Northern Chile.  
671 *Revista de biología marina y oceanografía* **51**, 475–481.
- 672 Minh, B. Q., Nguyen, M. A. T. & Von Haeseler, A. (2013) Ultrafast Approximation for  
673 Phylogenetic Bootstrap. *Molecular Biology and Evolution* **30**, 1188–1195.
- 674 Navarrete, A. H., Lagos, N. A. & Ojeda, F. P. (2014) Latitudinal Diversity Patterns of  
675 Chilean Coastal Fishes: Searching for Causal Processes. *Revista Chilena de Historia*  
676 *Natural*.
- 677 Nguyen, L. T., Schmidt, H. A., Von Haeseler, A. & Minh, B. Q. (2015) IQ-TREE: A Fast  
678 and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies.  
679 *Molecular Biology and Evolution* **32**, 268–274.
- 680 Padial, J. M., Miralles, A., De la Riva, I. & Vences, M. (2010) The Integrative Future of  
681 Taxonomy. *Frontiers in Zoology* **7**, 16.
- 682 Pante, E., Schoelink, C. & Puillandre, N. (2015) From Integrative Taxonomy to Species  
683 Description: One Step Beyond. *Systematic Biology* **64**, 152–160.
- 684 Paradis, E. (2010) Pegas: An {R} Package for Population Genetics with an Integrated--  
685 Modular Approach. *Bioinformatics* **26**, 419–420.
- 686 Pérez-Matus, A., Ferry-Graham, L. A., Cea, A. & Vásquez, J. A. (2007) Community

687 Structure of Temperate Reef Fishes in Kelp-Dominated Subtidal Habitats of Northern  
688 Chile. *Marine and Freshwater Research* **58**, 1069–1085.

689 Pérez-Matus, A., Carrasco, S. A., Gelcich, S., Fernandez, M. & Wieters, E. A. (2017a)  
690 Exploring the Effects of Fishing Pressure and Upwelling Intensity over Subtidal Kelp  
691 Forest Communities in Central Chile. *Ecosphere* **8**, e01808.

692 Pérez-Matus, A., Ospina-Alvarez, A., Camus, P. A., Carrasco, S. A., Fernandez, M.,  
693 Gelcich, S., Godoy, N., Patricio Ojeda, F., Pardo, L. M., Rozbaczylo, N., et al. (2017b)  
694 Temperate Rocky Subtidal Reef Community Reveals Human Impacts across the Entire  
695 Food Web. *Marine Ecology Progress Series* **567**, 1–16.

696 Puillandre, N., Lambert, A., Brouillet, S. & Achaz, G. (2012) ABGD, Automatic Barcode  
697 Gap Discovery for Primary Species Delimitation. *Molecular Ecology* **21**, 1864–1877.

698 R Core Team. (2017) R: A Language and Environment for Statistical Computing. R  
699 *Foundation for Statistical Computing, Vienna, Austria*. 2017, {ISBN} 3-900051-07-0,  
700 doi:<http://www.R-project.org/>.

701 Riquelme-Pérez, N., Musri, C. A., Stotz, W. B., Cerda, O., Pino-Olivares, O. & Thiel, M.  
702 (2019) Coastal Fish Assemblages and Predation Pressure in Northern-Central Chilean  
703 *Lessonia Trabeculata* Kelp Forests and Barren Grounds. *PeerJ* **7**, e6964.

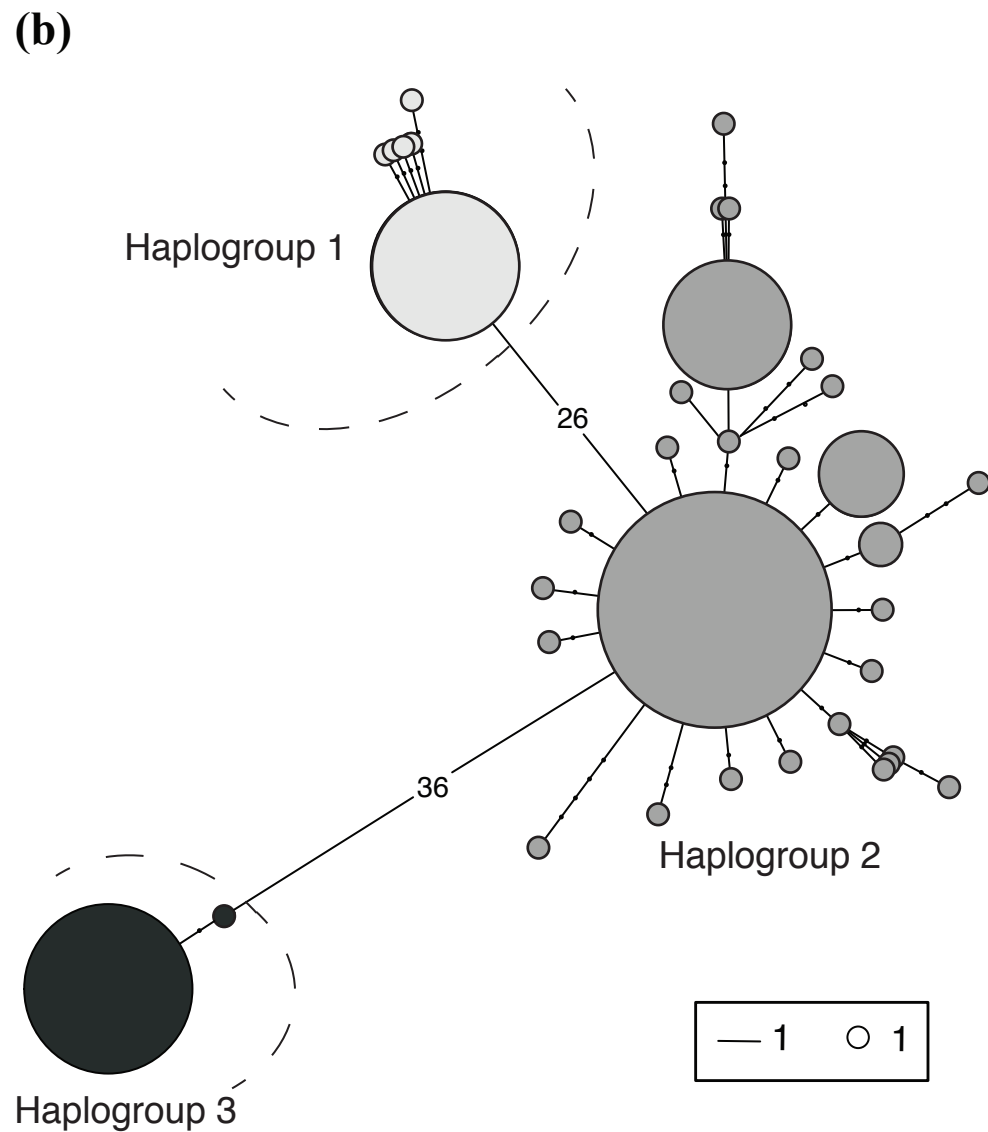
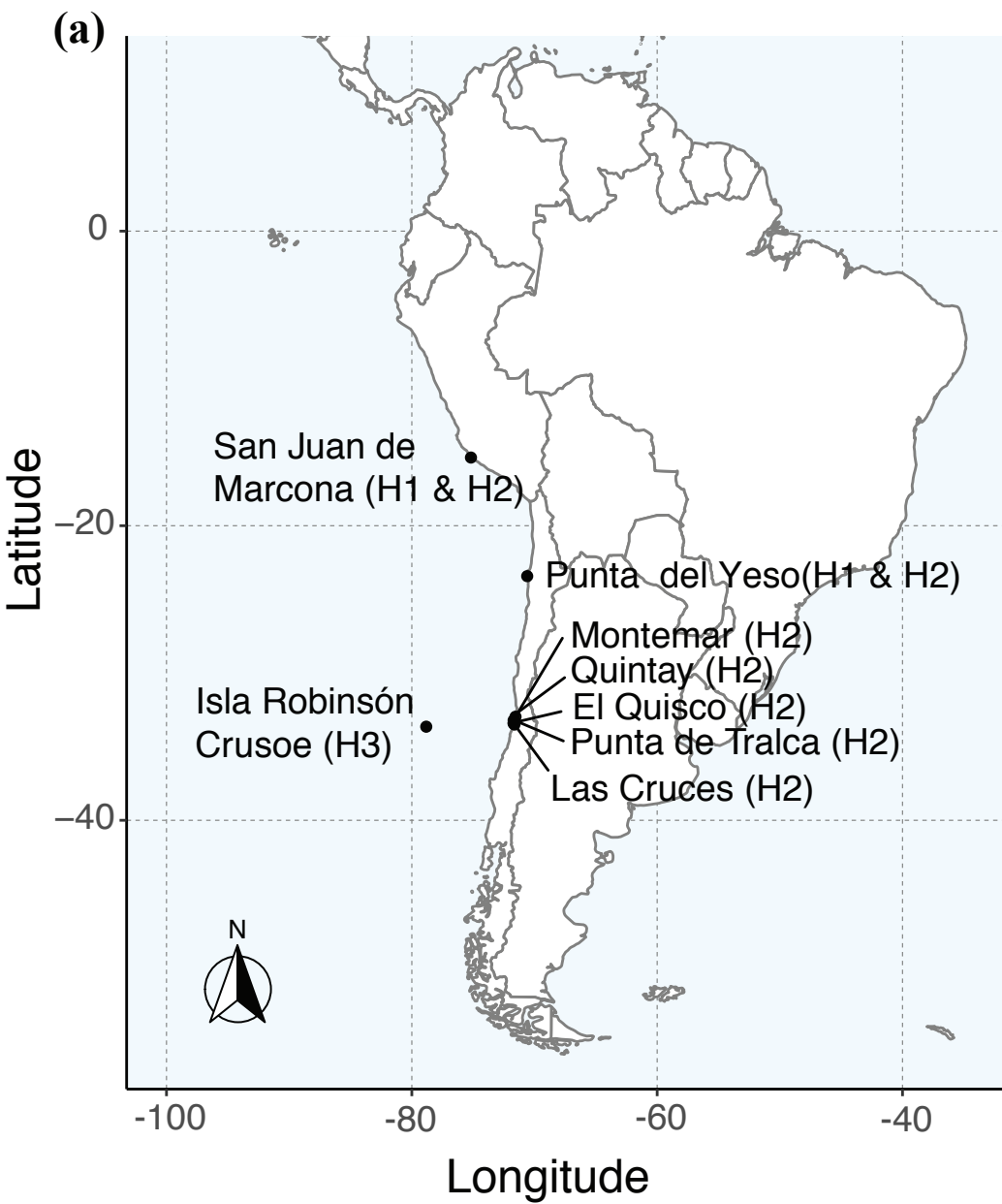
704 Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z. A., Finlayson, M.,  
705 Halpern, B. S., Jorge, M. A., Lombana, A., Lourie, S. A., et al. (2007) Marine Ecoregions  
706 of the World: A Bioregionalization of Coastal and Shelf Areas. *BioScience*. July 2007,  
707 573–583, doi:10.1641/B570707.

708 Stepien, C. A. (1990) Population Structure, Diets and Biogeographic Relationships of a  
709 Rocky Intertidal Fish Assemblage in Central Chile: High Levels of Herbivory in a  
710 Temperate System. *Bulletin of Marine Science* **47**, 598–612.

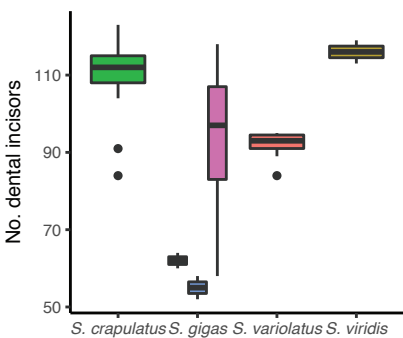
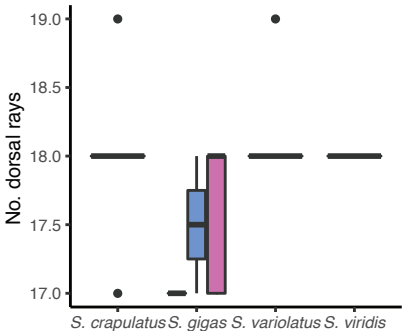
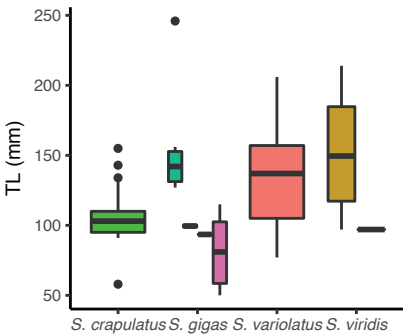
711 Tamura, K., Stecher, G., Peterson, D., Filipiński, A. & Kumar, S. (2013) MEGA6: Molecular  
712 Evolutionary Genetics Analysis Version 6.0. *Molecular biology and evolution* **30**, 2725–  
713 2729.

714 Tea, Y. K., Van Der Wal, C., Ludt, W. B., Gill, A. C., Lo, N. & Ho, S. Y. W. (2019)  
715 Boomeranging around Australia: Historical Biogeography and Population Genomics of the  
716 Anti-Equatorial Fish *Microcanthus Strigatus* (Teleostei: Microcanthidae). *Molecular*  
717 *Ecology*.

- 718 Teletchea, F. (2010) After 7 Years and 1000 Citations: Comparative Assessment of the  
719 DNA Barcoding and the DNA Taxonomy Proposals for Taxonomists and Non-  
720 Taxonomists. *Mitochondrial DNA* **21**, 206–226.
- 721 Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) CLUSTAL W: Improving the  
722 Sensitivity of Progressive Multiple Sequence Alignment through Sequence Weighting,  
723 Position-Specific Gap Penalties and Weight Matrix Choice. *Nucleic Acids Research* **22**,  
724 4673–4680.
- 725 Trifinopoulos, J., Nguyen, L.-T., von Haeseler, A. & Minh, B. Q. (2016) W-IQ-TREE: A  
726 Fast Online Phylogenetic Tool for Maximum Likelihood Analysis. *Nucleic Acids Research*  
727 **44**, W232–W235.
- 728 Villegas, M., Laudien, J., Sielfeld, W. & Arntz, W. (2019) Effect of Foresting Barren  
729 Ground with *Macrocystis Pyrifera* (Linnaeus) C. Agardh on the Occurrence of Coastal  
730 Fishes off Northern Chile. *Journal of Applied Phycology* **31**, 2145–2157.
- 731 Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R. & Hebert, P. D. N. (2005) DNA  
732 Barcoding Australia’s Fish Species. *Philosophical Transactions of the Royal Society B:*  
733 *Biological Sciences* **360**, 1847–1857.
- 734 Will, K. W., Mishler, B. D. & Wheeler, Q. D. (2005) The Perils of DNA Barcoding and the  
735 Need for Integrative Taxonomy. *Systematic Biology*. 2005, 844–851,  
736 doi:10.1080/10635150500354878.
- 737 Williams, J. T. (1990) Phylogenetic Relationships and Revision of the Blennioid Fish Genus  
738 *Scartichthys*. *Smithsonian Contributions to Zoology* **492**, 1–30.
- 739 Williams, J. T., Delrieu-Trottin, E. & Planes, S. (2012) A New Species of Indo-Pacific  
740 Fish, *Canthigaster Criobe*, with Comments on Other *Canthigaster* (Tetraodontiformes:  
741 Tetraodontidae) at the Gambier Archipelago. *Zootaxa* **3523**, 80–88.
- 742 Zhang, J., Kapli, P., Pavlidis, P. & Stamatakis, A. (2013) A General Species Delimitation  
743 Method with Applications to Phylogenetic Placements. *Bioinformatics* **29**, 2869–2876.  
744  
745





**(a) Following current species names****(b) Following proposed revision**