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Vertical structure of Caribbean deep-reef fishes from the altiphotic to deep-sea boundary

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40 many shallow-reef species extended down to mesophotic depths. Depth segregation among species of a
41 genus was found across ten reef-fish genera and likely constitutes one of the mechanisms driving
42 community distinctiveness and fish diversity across depths.

43

44 **Main text**

45 Tropical and temperate reefs are amongst the best described marine ecosystems, yet their study
46 has largely been restricted to shallow depths down to 40 m, corresponding to the traditional limits of
47 non-technical SCUBA diving, depicting a very skewed representation of these ecosystems^{1,2}. The recent
48 widespread adoption of closed-circuit rebreather diving coupled with the increased use of remotely
49 operated vehicles (ROVs), baited remote underwater videos, and human-occupied submersibles has
50 enabled a surge in the description of reefs below 40 m, highlighting their diversity and taxonomic
51 distinctiveness with respect to their shallow counterparts³⁻⁵. Despite these advances, our understanding
52 of the ecology of mesophotic (40-150 m), and even more so of rariphotic (150-300 m), reef communities
53 lags behind that of their shallow counterparts.

54 Most tropical reef communities surveyed across the world display decreases in fish abundance,
55 biomass and richness with depth⁶⁻⁸. While many shallow species have wide depth ranges that extend
56 down to the mesophotic⁹, deep-reef fish species (below 40 m) tend to be depth-endemic and therefore
57 are not found on shallow reefs^{6,10,11}. The number of distinct fish communities occurring along the reef
58 slope, as well as the depths of associated faunal breaks, varies regionally¹² depending on temperature,
59 light availability, and other environmental factors¹³⁻¹⁵. Similarly, the composition of deep-reef fish
60 assemblages at a given depth is known to vary within and between regions^{16,17}. Regional gradients of
61 reef-fish richness have been found to dampen with depth, likely a result of increased environmental
62 constraints that lead to convergent filtering of species composition¹⁶.

63 Despite recent advances, several data gaps still hamper our understanding of deep-reef fish
64 community structure and its regional variability. First, the limited number of deep-reef survey locations
65 constitutes an important barrier to evaluating global patterns of deep-reef fish diversity and levels of
66 deep-reef fish endemism^{16,18}. The limited number of study sites also restricts regional comparisons,
67 which are essential for identifying environmental drivers of deep-reef fish diversity patterns and, in turn,
68 increase our capacity to predict the spatial distribution and composition of deep-reef communities^{19,20}.
69 Such information is necessary to reveal evolutionary and demographic processes at play on deep reefs
70 and to adequately place and design conservation tools that protect the full range of reef diversity²¹.

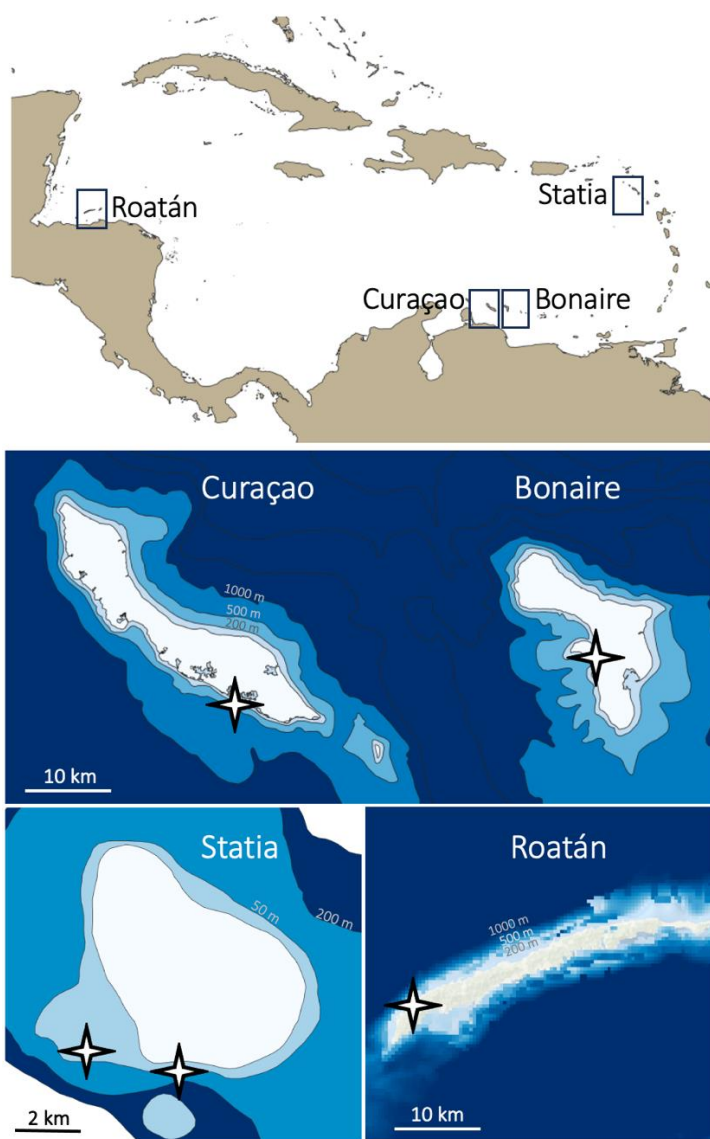
71 Second, technical limitations of rebreather diving and the scarcity and cost of submersibles and ROVs
72 have restricted most deep-reef fish studies to the upper range of deep reefs (40-120 m⁵; leading to the
73 continued discovery of new species below those depths^{2,10,22} even in the most heavily sampled sites²³.
74 While submersible research at several locations in the tropical western Atlantic has established that
75 deep-reef fishes extend down to at least 300 m^{4,7,10,11}, the lower depth limit of deep-reef fish distribution
76 and their transition to deep-sea fish communities remains largely unknown.

77 Here, we advance the understanding of deep-reef fish community structure using one of the most
78 extensive datasets on deep-reef fish communities, comprising observations down to 480 m collected by
79 submersible diving at four sites in the Caribbean. Using hierarchical clustering and similarity profile
80 analyses, we first test whether distinct communities occur across depths, identify the depth of associated
81 faunal breaks, and quantify the relative role of nestedness and turn-over in explaining the dissimilarity
82 between these communities. We then evaluate the relative contribution of reef-associated vs. deep-sea
83 families to total abundance and richness across depths, and test for depth segregation between closely-
84 related species. Lastly, we perform a cross-site similarity analysis to test whether vertical structure and
85 community composition are stable at the regional scale.

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90 **Figure 1:** Locations of study sites. The bathymetric maps of Curaçao, Bonaire and Statia were obtained
 91 from the Dutch Caribbean Biodiversity Database, and the bathymetric map of Roatán from the General
 92 Bathymetric Chart of the Ocean. Rendering was obtained using QGIS.

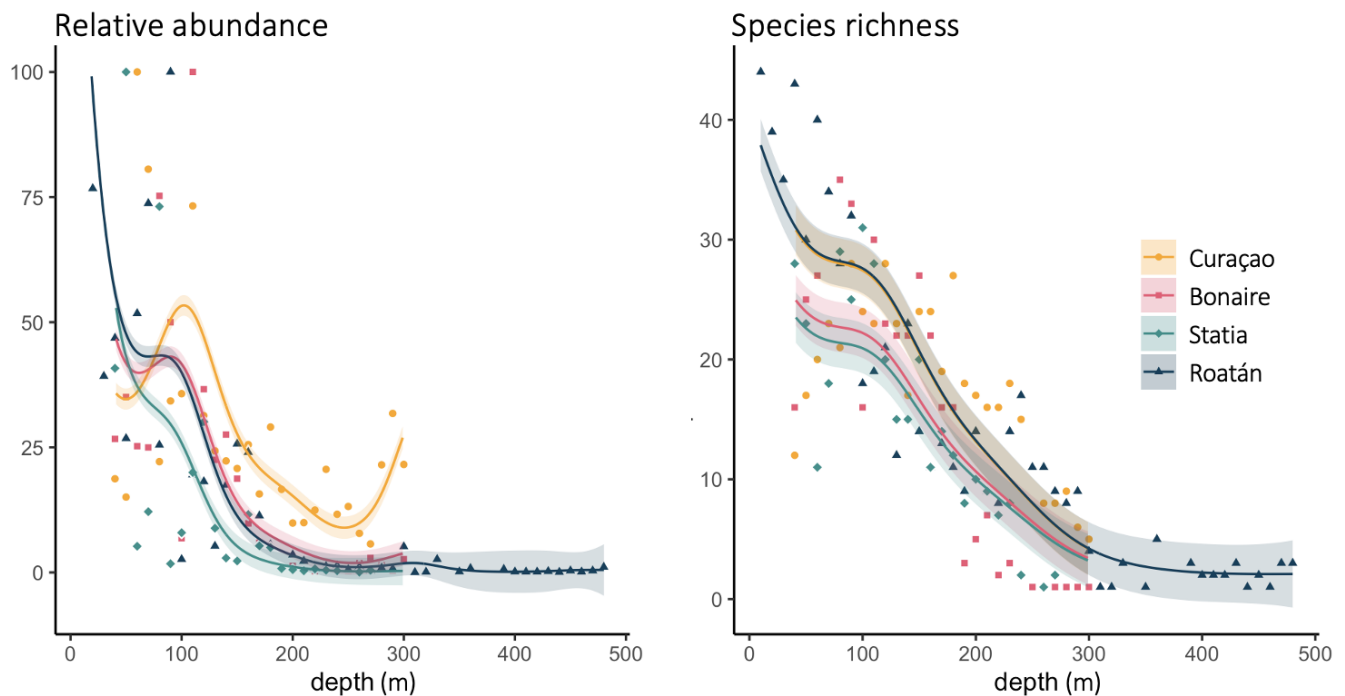
93

94 **Results**

95 *Abundance and richness across depth.* A total of 33,873 individuals representing 225 fish species were
 96 observed across depths ranging from 10 to 480 m, including 38 deep-reef species that were new to
 97 science at the time of their observation (Table S1). Rarefaction curves indicate that sampling effort was
 98 sufficient to sample most of the fish diversity in Curaçao, but that additional sampling effort would have
 99 likely resulted in additional species observed in Bonaire, Statia, and Roatán (Fig. S1). Fish abundance

100 and species richness decreased with depth at all sites ($p < 0.001$, Fig. 2). Abundance decreased steeply
101 below 80 m depth and plateaued at all sites at 0-10 % of initial abundance values below 200 m. The
102 exception was Curaçao, where abundance remained at or above 10% and even increased back to ~25 %
103 between 270 and 300 m (Fig. 2). Abundance remained stable from 300 to 480 m in Roatán, at 0-3 % of
104 initial abundance. Total fish species richness across depth was 73 species in Curaçao, 119 species in
105 Bonaire, 107 species in Statia, and 145 species in Roatán (see Table S1 for complete list of species at
106 each site). At all sites, species richness decreased mostly linearly with depth from 40 to 300 m (Fig. 2).
107 Below 260 m depth, species richness remained below 10 species at all sites, with values ranging
108 between 8-10 species in Curacao and Roatán, and 0-2 species in Bonaire and Statia. Species richness
109 halved again below 300 m in Roatán, and plateaued at 2-6 species between 300 and 480 m.

110



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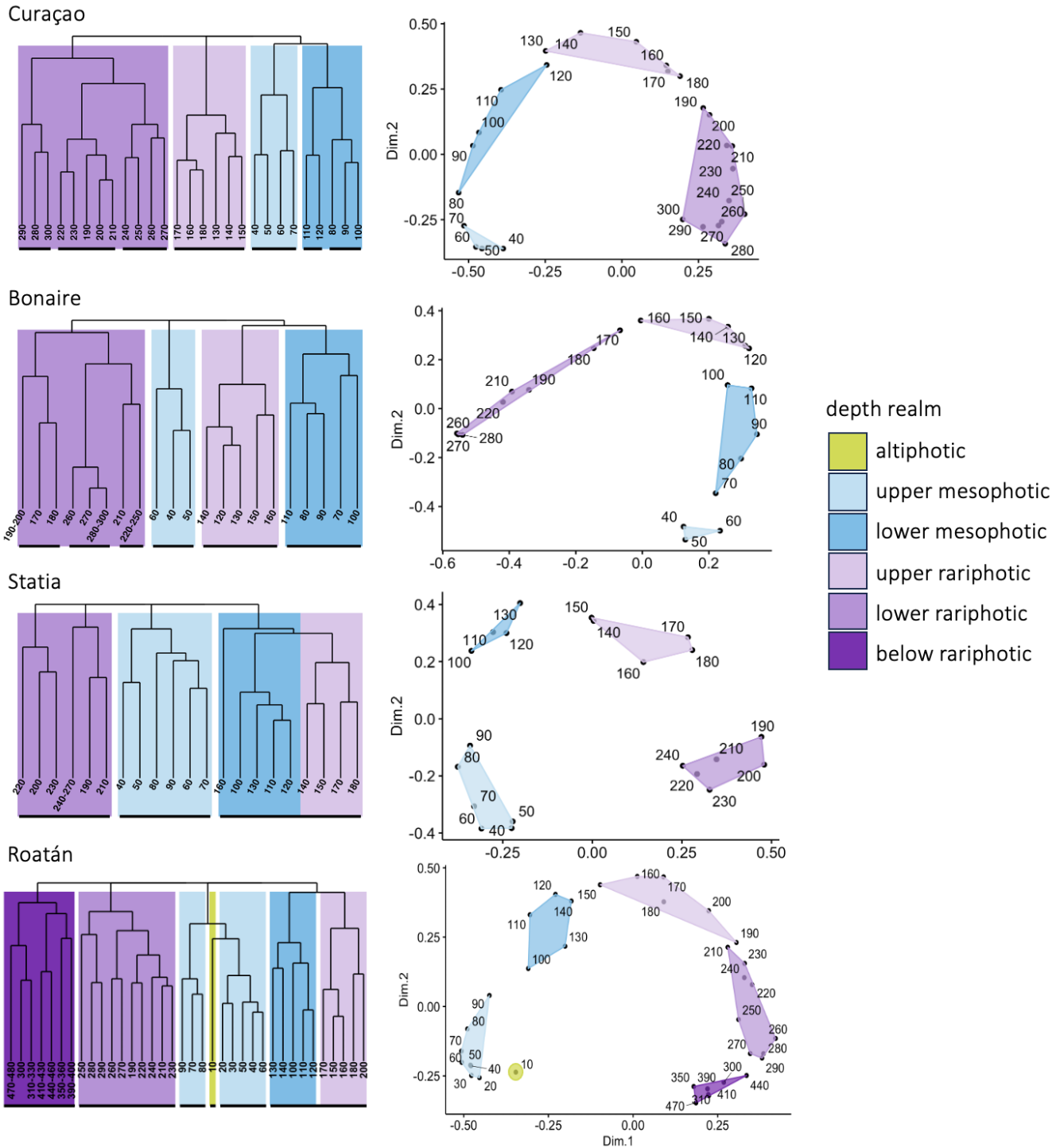
112 **Figure 2:** Relative abundance (left) and species richness (right) across depth at the four study sites.

113 Relative abundances were normalized by depth-specific sampling effort and are given as a proportion of
114 the site-specific maximal abundance. Points represent observed relative abundance and richness at each
115 10 m depth bin, lines represent predicted values from a generalized additive model and shaded areas
116 represent 95% confidence intervals. Note that for depths greater than 300 m and shallower than 40 m,
117 predictions are based on observations from Roatán alone.

118

119 *Vertical structure of fish communities.* Similarity profile (SIMPROF) analyses based on species
120 composition and abundance at each 10 m depth bin revealed between three (Statia) and seven (Curaçao)
121 distinct fish community clusters between 40 and 300 m (denoted by black thick horizontal lines in Fig.
122 3). With the exception of the 190 m community in Roatán, which pooled with the 210-300 m
123 communities, all clusters comprised communities from continuously ordered depth bins. Based on main
124 branching events of hierarchical clustering dendrograms (Fig. 3) and on previous analyses of reef-fish
125 depth zonation (Baldwin et al., 2018), SIMPROF clusters were pooled into four depth zones: upper and
126 lower mesophotic, and upper and lower rariphotic. Depth zones formed a posteriori by pooling
127 SIMPROF clusters displayed significantly different fish communities (PERMANOVA, p -value $<$
128 0.001), and explained between 46% (Statia) and 60% (Curaçao) of the total variance between fish
129 communities at a given site (Fig. 3).

130 The upper mesophotic zone corresponded to a single cluster starting at 40 m and ending at 70-90
131 m in Bonaire, Curaçao, and Statia. In Roatán, two sister clusters from 20 to 100 m were considered to
132 form the upper mesophotic. The lower mesophotic corresponded to a single cluster in Bonaire and
133 Roatán and to two sister clusters in Curaçao, and spanned from 70-90 to 120-150 m. In Statia, a single
134 large cluster spanned from 100 to 190 m. For comparative purposes, this cluster was divided into a
135 lower mesophotic and upper rariphotic zone, with a depth break at 140 m corresponding to the main
136 branching event within this cluster (Fig. 3). At all other sites, the upper rariphotic corresponded to a
137 single cluster spanning from 120-150 to 170-210 m. Lastly, the lower rariphotic corresponded to a single
138 cluster in Statia and Roatán, and to three adjacent clusters in Curaçao and Bonaire. Community breaks
139 (i.e., boundaries between depth zones) occurred at similar depths in Curaçao and Bonaire: ~70-80 m,
140 ~120-130 m, and ~170-190 m. At Statia and Roatán, community breaks were skewed towards deeper
141 depths at 100 m, ~140-150 m, and ~190-210 m. Based on observations from Roatán, we found two
142 additional community breaks: one separating the shallowest 10 m depth bin from the next
143 altiphotic/upper mesophotic cluster (20-60 m), and one at 300 m separating the lower rariphotic from a
144 deeper fish community that formed a single cluster down to 470 m (Fig. 3).



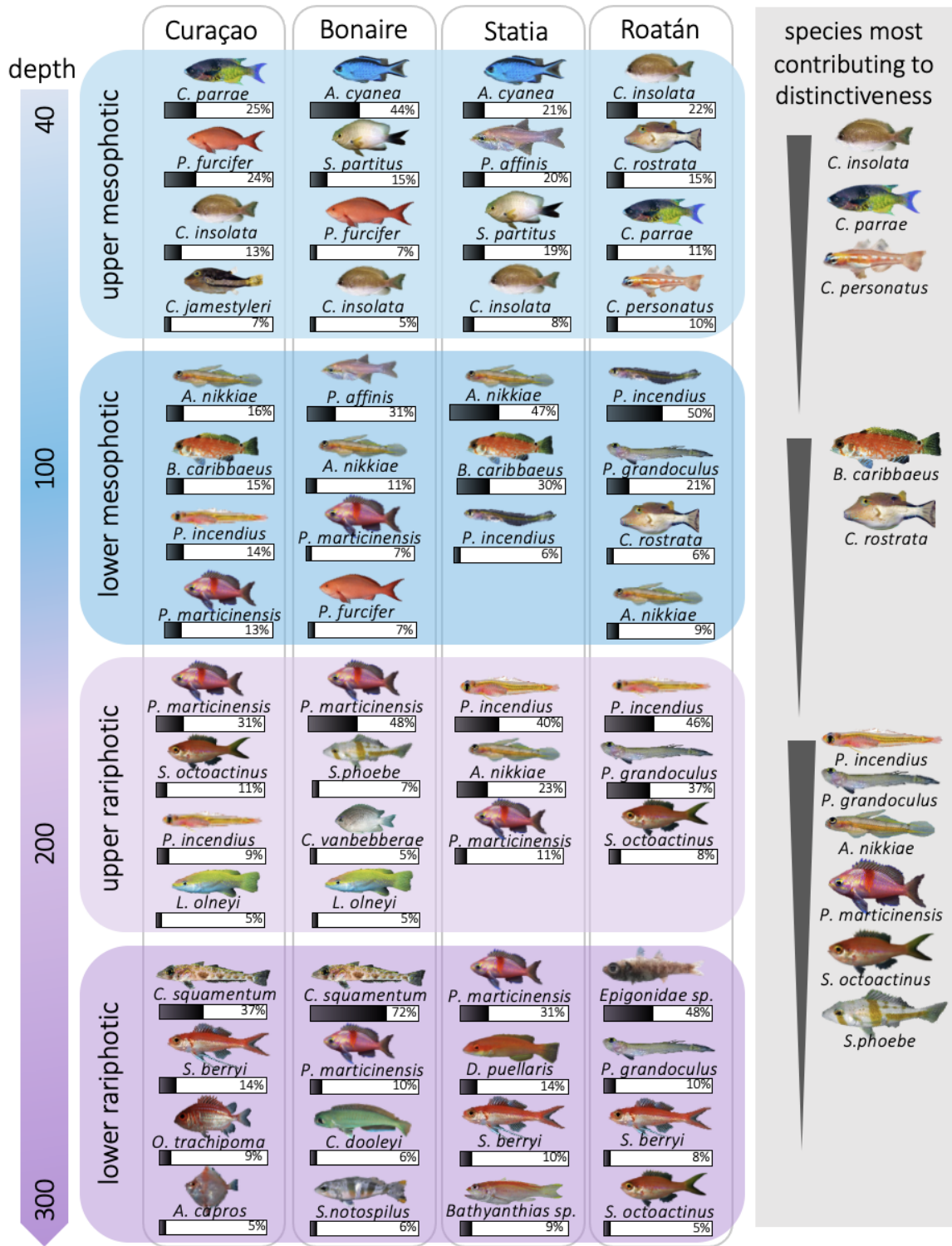
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146 **Figure 3:** Site-specific dissimilarity analysis of reef-fish community from 40 to 300 m depth. Left
 147 panels display hierarchical clustering dendrograms, right panels display nonmetric multidimensional
 148 scaling ordination (MDS) plots derived from Bray-Curtis dissimilarity analysis. Clusters with
 149 significantly distinct composition (SIMPROF analyses) are indicated on dendrograms by thick

150 continuous horizontal lines below depth bin values. Color scale indicates the depth zones that clusters
151 obtained from SIMPROF analyses were pooled into. Depth-bins are labeled with the minimum depth of
152 each 10 m depth interval (e.g., “100 m” = 100-109 m).

153

154 *Indicator species across depth zones.* The top five most abundant fish species per site and per depth
155 zones represented 46 to 91% of the total community abundance in that depth zone. Most abundant
156 species changed between depth zones but were often identical across several sites (Fig. 4). Species that
157 significantly contributed to differences in fish communities between depth zones (SIMPER analyses)
158 were a subset of the most abundant species (Fig. 4). A decrease in the abundance of *Clepticus parrae*,
159 *Chromis insolata*, and *Coryphopterus personatus* marked the transition between the upper and lower
160 mesophotic; a decrease in the abundance of *Canthigaster rostrata* and *Bullisichthys carribaeus* marked
161 the transition between the lower mesophotic and upper rariphotic; and a decrease in the abundance of
162 *Serranus phoebe*, *Pronotoqrammus martinicensis*, *Symphysanodon octoactinus*, *Palatogobius incendius*,
163 *Palatogobius grandoculus*, and *Antilligobius nikkiae* marked the transition between the upper and lower
164 rariphotic. In Roatán, an increase in the abundance of *Hollardia hollardi*, *Epigonidae sp.*, *Chrionema*
165 *squamentum*, *Phenascoscorpius nebrius*, and *Neoepinnula americana* marked the transition between the
166 lower rariphotic and the next deeper community.



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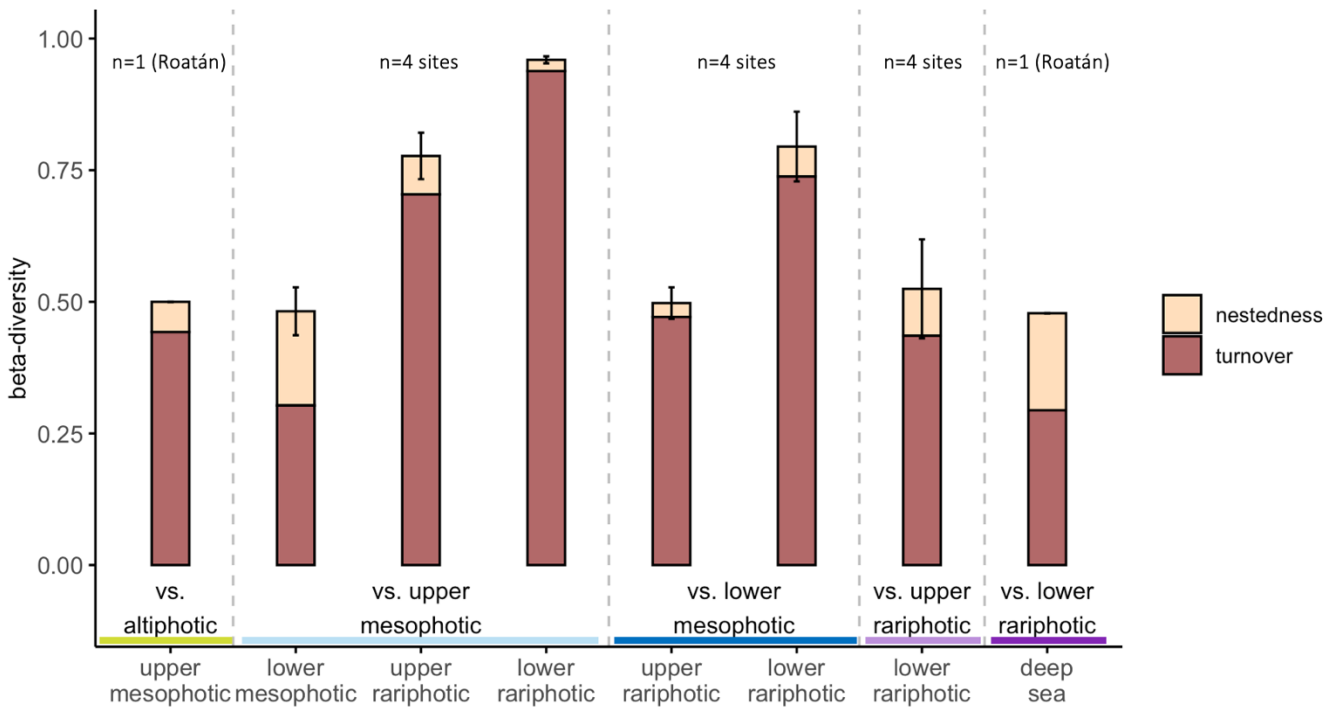
168 **Figure 4:** Most common species by depth zone and site. Species displayed are the top four most
 169 common species by depth zone and sites, representing at least 5% of the total abundance. The relative
 170 abundance of each species per depth zone per site is indicated in %. Species most contributing to

171 differences between depth zones (SIMPER contribution > 5% and p-value < 0.05) are displayed in the
 172 right grey panel, with abundance of these species being higher in the shallower of the two depth zones.
 173 Full genus names are available in Table S1.

174
 175

176 *Beta-diversity across depth zones.* Dissimilarity (i.e., total beta-diversity) between depth zones increased
 177 with depth disparity, with the highest dissimilarity (~100 %) occurring between the upper mesophotic
 178 and the lower rariphotic (Fig. 5, Fig. S3). Dissimilarity between adjacent depth zones was ~ 50 % across
 179 depths and sites and mostly resulted from species turnover. However, higher contribution of nestedness
 180 (40 %) between the upper and lower mesophotic communities, and between the lower rariphotic and the
 181 next deeper depth zone in Roatán, suggest that species drop-out plays an important role in these faunal
 182 transitions.

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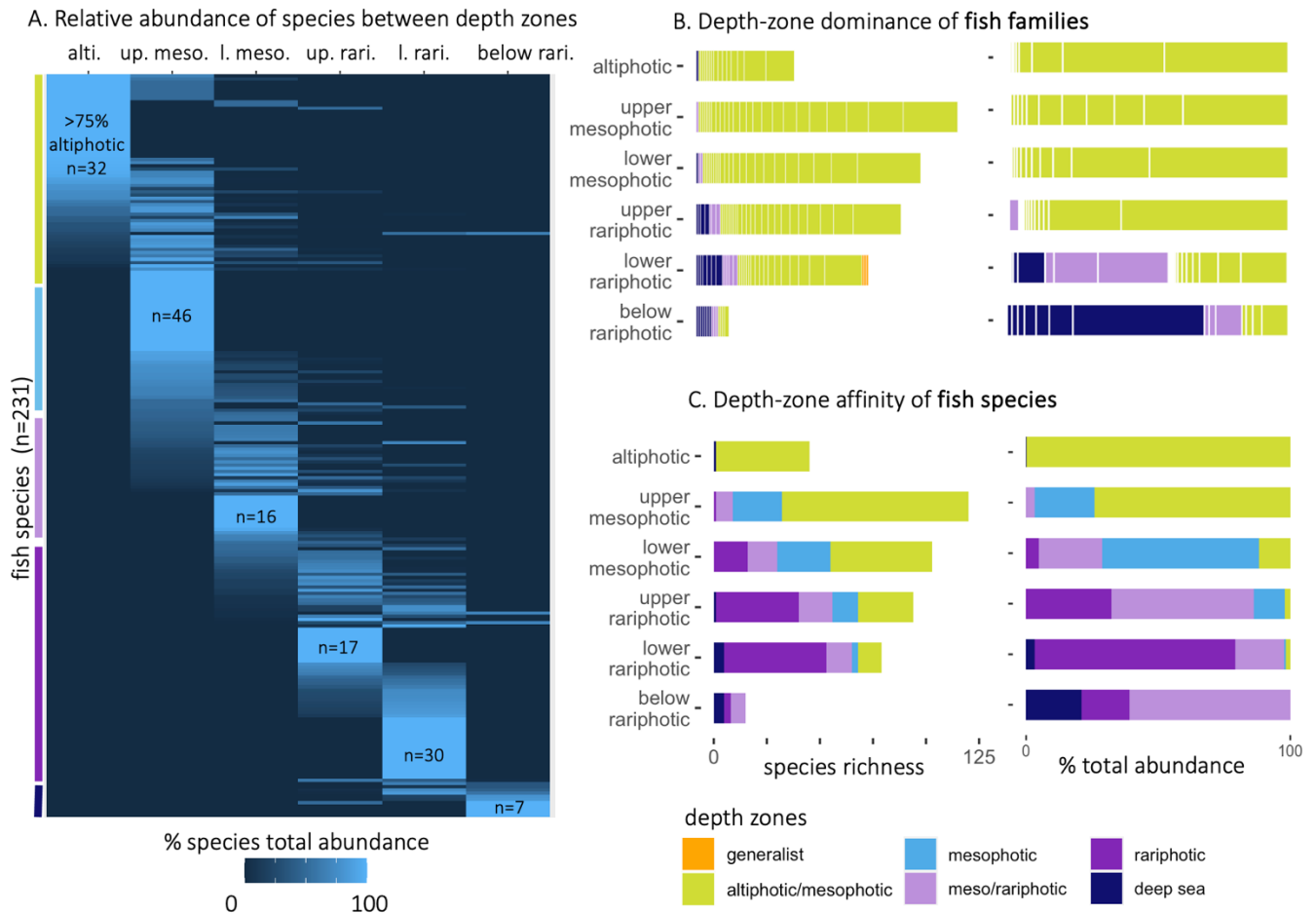
186 **Figure 5:** Beta diversity and its components, nestedness and turnover of reef-fish communities across
 187 depth zones. Values are shown as the average of values across the four sites, and error bars indicate
 188 standard error. “Altiphotic vs. upper mesophotic” and “lower rariphotic vs. below rariphotic” values

189 were only available for Roatán. Coloring of bars indicate the relative contribution of nestedness (beige)
190 and turnover (red).

191

192 *Depth affinities of species.* We found that 151 out of 225 species were depth specialists with >75% of
193 their observed abundance occurring in a specific depth zone: n=35 in the altiphotic, n=44 in the upper
194 mesophotic, n=16 in the lower mesophotic, n=17 in the upper rariphotic, n=31 in the lower rariphotic
195 and n=8 below the rariphotic (Fig. 6A). When considering depth-zone predominance at the family level,
196 we found that reef-fish families dominated all depth zones in abundance and richness from the altiphotic
197 to the lower rariphotic. This result stood true when pooling observations across sites (Fig. 6B), and when
198 considering sites individually (Fig. S6). Species from altiphotic/mesophotic families dominated in
199 abundance down to the upper rariphotic and in richness down to the lower rariphotic, but rariphotic taxa
200 dominated in abundance in the lower rariphotic. Below the rariphotic (300-480 m), fish communities
201 were dominated in abundance and richness by species from deep-sea families (e.g., Epigonidae,
202 Triacanthodidae, Trachichthyidae; Table S6). However, when considering depth affinity at the species
203 level (Fig. 6C), deep-sea fishes were only present in the lower rariphotic and below and represented a
204 minority of total abundance and richness even in the 300-480 m depth zone. The altiphotic, upper
205 mesophotic, and lower rariphotic were dominated by species from a single depth-zone affinity group
206 (altiphotic/mesophotic and rariphotic, respectively), while the lower mesophotic and upper rariphotic
207 were characterized by overlaps between four depth-zone affinity groups (Fig. 6C). Peaks in the richness
208 of depth-zone affinity groups occurred at different depths among sites and tended to match site-specific
209 depth breaks identified from hierarchical clustering (Fig. S5).

210



211

212 **Figure 6:** Depth affinity of reef-fish species and families across depth zones. Distribution (% total

213 abundance) of fish species among depth zones (A). Each line displays the relative abundance of a given

214 species across five depth zones from the “altiphotic” to “below rariphotic” (300-480 m). Vertical

215 colored lines indicate species falling under each depth affinity category given their depth distribution.

216 The number of depth specialists of each depth zone (>75% of total abundance in a given depth zone) is

217 indicated on the figure (“n=”). Depth-zone dominance of fish families (B) and depth-zone affinity of

218 species (C) in each depth zone, and contribution of depth affinity groups to species richness (left panel)

219 and abundance (right panel). White lines in barplots of panel C distinguish species from different

220 families. Filling colors indicate the depth affinity of species, from the altiphotic to the deep sea. Results

221 are shown pooled across the four study locations, except for altiphotic and below rariphotic zones for

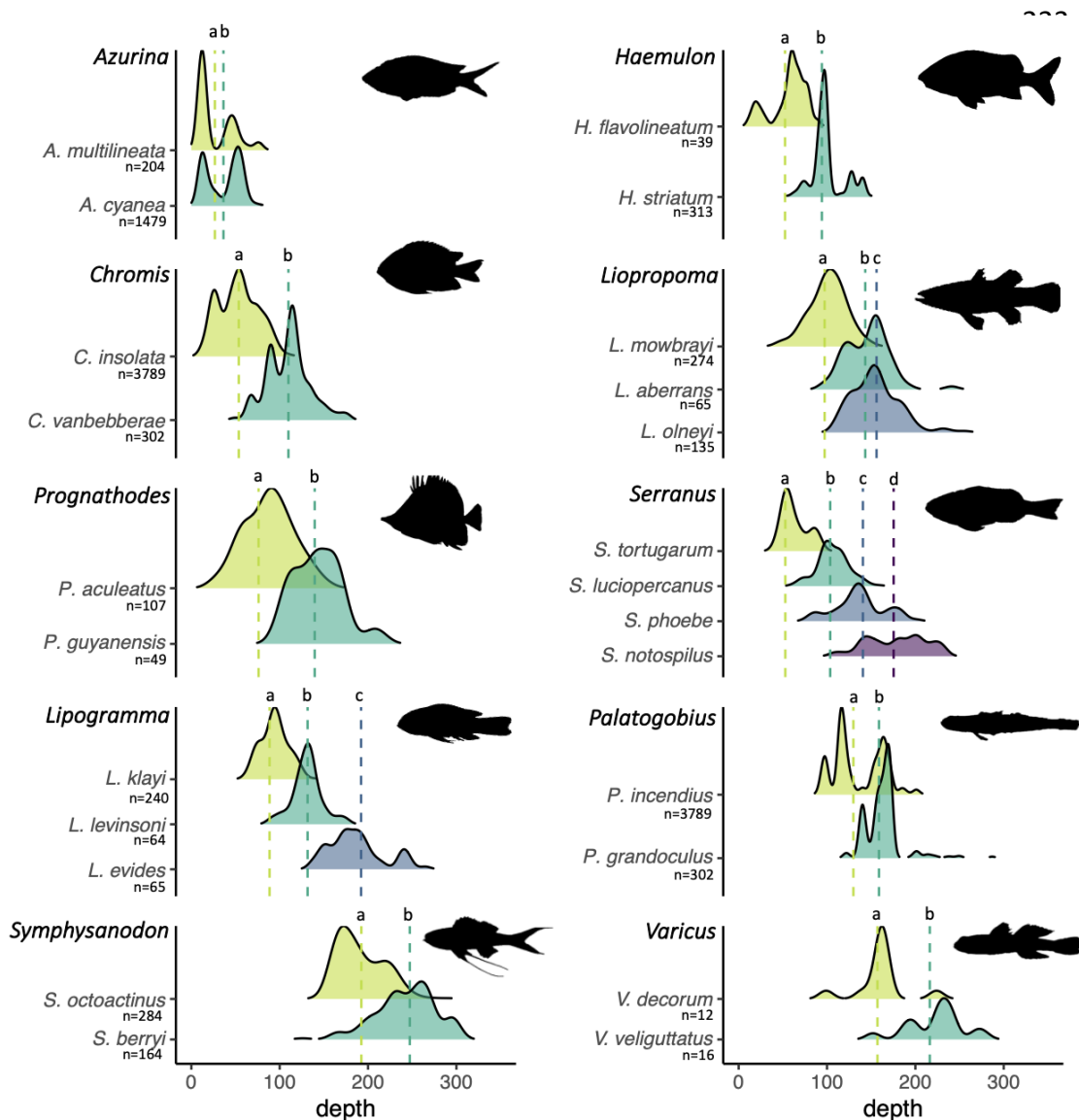
222 which data was only collected in Roatán.

223

224 *Habitat segregation by depth.* We found strong evidence for depth segregation between species of the

225 same genus (Fig. 7). Within all ten genera tested, species was a significant factor influencing mean

226 depth of occurrence (Wilcoxon and Kruskal-Wallis tests, p -values <0.01), and all pairwise comparisons
 227 of mean depths between species of a same genera were statistically significant (p -values <0.04 , Table S4
 228 and S5). Depth segregation occurred both between and within depth zones. For instance, three
 229 *Lipogramma* species partitioned across the upper mesophotic, lower mesophotic and upper rariphotic,
 230 while *Azurina multilineata* and *A. cyanea* displayed depth segregation within the upper mesophotic (Fig.
 231 7). The depth distributions of species varied across sites, reflecting in some instances the variations in
 232 depth zones' boundaries between sites (e.g., see *Palatogobius incendius*, Fig. S2).



254 **Figure 7.** Depth segregation among congeners. Dashed vertical lines indicate the mean depth of
 255 occurrence of each species. Differently colored abundance curves represent different species within each
 256 genus. Statistically different mean depths within a genus are indicated by different letters (Table S4). A

257 comparison of depth distributions observed at our sampling sites vs. global depth ranges is provided in
258 Fig. S7.

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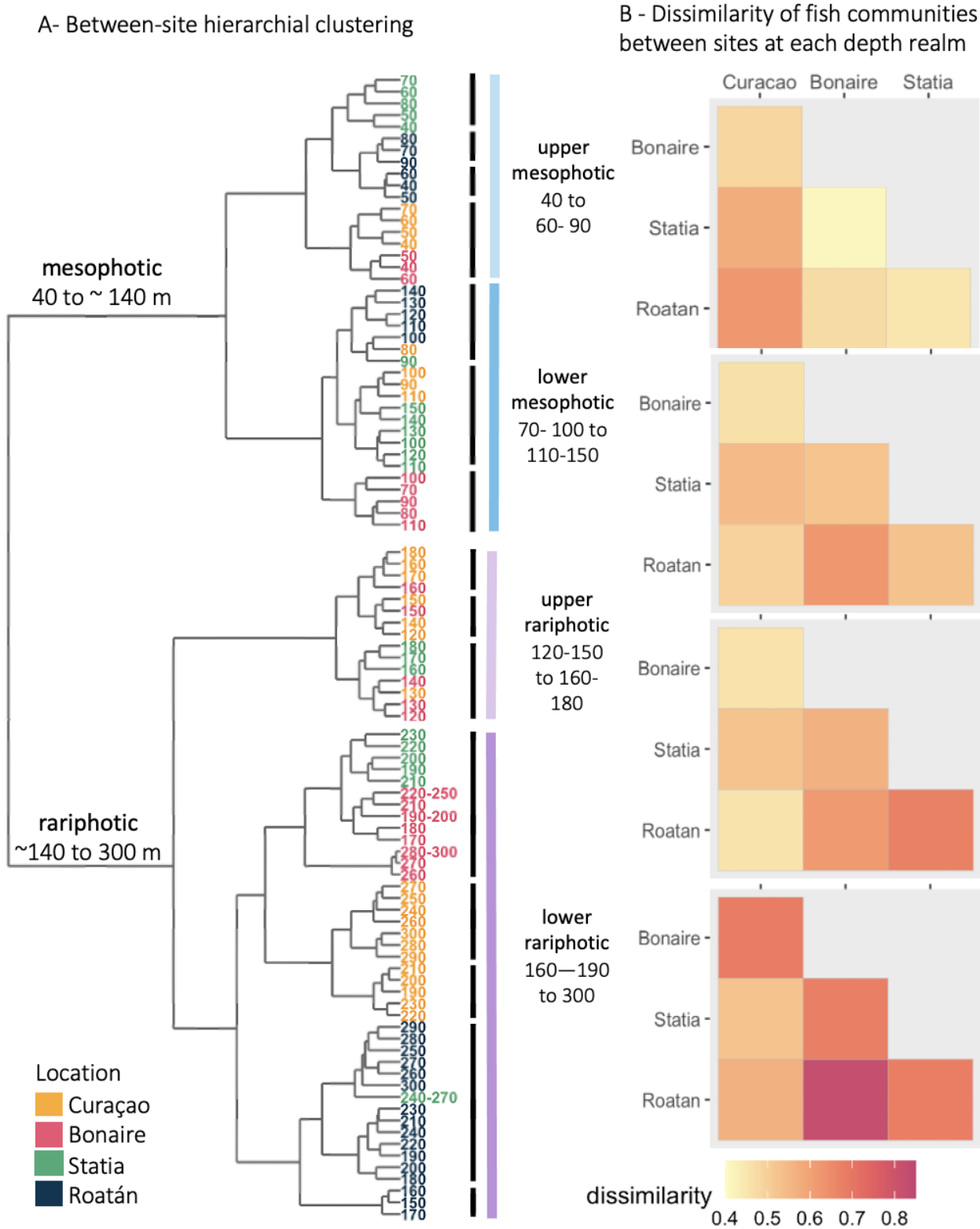
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261 *Cross-site community analysis.* We performed a cross-site hierarchical clustering of deep-reef fish
262 communities between 40 and 300 m using a Ward linkage algorithm (Fig. 8A). We found that the 96
263 fish communities corresponding to distinct sites and depth bins formed 15 significant clusters
264 (SIMPROF based on Bray-Curtis distance, $\alpha = 10^{-6}$). The total number of significant clusters was
265 unchanged when adding communities observed at 10-40 m and 300-480 m in Roatán (Fig. S4). The two
266 first levels of branching of the dendrogram (Fig. 8A) reproduced the four depth zones identified at the
267 site-level (Fig. 3). The first level of branching corresponded to a depth break at 120-160 m depending on
268 site, marking the transition between the mesophotic and the rariphotic. The second level of branching
269 within the mesophotic branch (40 to 120-160 m) formed two groups with a depth break at 70-100 m
270 depending on site, marking the transition between the lower and upper mesophotic. The second level of
271 branching within the rariphotic branch (120-160 to 300 m) formed three groups: one group
272 encompassing all rariphotic depths from Roatán (150-309 m), and two groups separating the lower and
273 upper rariphotic depth zones from Curaçao, Bonaire and Statia, with a depth break at 170-190 m. The
274 depth of community breaks found in the cross-site clustering (Fig. 8A) were similar to those found in
275 site-specific clustering (Fig. 3).

276 We found that depth zone, site location, and the interaction between these two factors had a
277 significant influence, in this order, on fish community structure (PERMANOVA, Table S7). In the upper
278 mesophotic, hierarchical clustering indicated greater similarities between Curaçao and Bonaire on one
279 hand, and Roatán and Statia on the other (Fig. 8A). However, in the lower mesophotic, fish communities
280 from Bonaire and Roatán pooled separately, while Statia and Curaçao pooled together. In the upper
281 rariphotic, communities from Curaçao, Bonaire, and Statia were pooled together while fish communities
282 from Roatán were in a separate cluster. Beta-diversity based on the presence/absence of species
283 confirmed that similarity between sites varied across depth, with Roatán tending to display higher
284 dissimilarity to other sites (Fig. 8B). Cross-site beta-diversity values remained within similar ranges
285 from the upper mesophotic to the upper rariphotic but were higher in the lower rariphotic.

286

287



288

289 **Figure 8:** Cross-site dissimilarity analysis of deep-reef fish communities. (A) Cross-site hierarchical
 290 clustering analysis of deep-reef fish communities from 40 to 300 m. Length of branches in the

291 dendrogram is commensurate to the dissimilarity between depth bins based on Bray-Curtis distance.
292 Font color indicates sites. Significant clusters (SIMPROF analyses, Ward linkage) are indicated by thick
293 black vertical lines. (B) Dissimilarity of deep reef-fish communities between sites at the four depth
294 zones defined in this study, based on the presence/absence of fish species.

295

296

297 **Discussion**

298 *Vertical zonation of reef-fish communities*

299 The present study provides an extensive analysis of the vertical structure of reef fishes based on
300 continuous, direct visual observations of communities from 40 to 300 m depth at four sites from the
301 Caribbean, with additional data from 10 to 480 m depth at one site. The number of species observed per
302 site (73-145) is within the range found by previous deep-reef fish studies in the Caribbean (e.g., 103 in
303 Puerto Rico ²⁴). The patterns of decreased richness and abundance with depth documented in this study
304 are consistent with trends observed in the Caribbean ²⁵ and other regions of the world ^{6,7}. While we
305 found that the number of distinct community clusters and their depth boundaries varied between sites
306 (Fig. 3), we identified four main depth zones occurring at all sites: the upper mesophotic (~40-90 m), the
307 lower mesophotic (~100-140 m), the upper rariphotic (~150-190 m), and the lower rariphotic (~ 200-300
308 m). This depth zonation was found both using site-specific hierarchical clustering (Fig. 3) and cross-site
309 hierarchical clustering (Fig. 8). The only exceptions were the deep-reef fish communities from Statia for
310 which the distinction between lower mesophotic and upper rariphotic was more ambiguous. Greater
311 similarity between the upper and lower mesophotic on one hand, and between the upper and lower
312 rariphotic on the other hand, was supported by the branching hierarchy of the cross-site dendrogram
313 (Fig. 8A). Our results support a two-tier vertical zonation nomenclature for deep reefs as established in
314 Baldwin et al., (2018), with a first level comprising two zones (the mesophotic and rariphotic) separated
315 by a strong community break, and a secondary level separating “upper” and “lower” communities within
316 these zones. While the 300 m lower boundary of the lower rariphotic was originally set by the depth
317 limitations of submersibles rather than by actual ecological observations ^{7,10}, the depth break found at
318 300 m in Roatán between the lower rariphotic and a deeper fish community that extends down to at least
319 480 m now supports the ecological reality of this boundary. To the best of our knowledge, the only other
320 study that tested the lower limit of the rariphotic identified a comparable faunal break depth at 320 m in
321 Hawaii ⁸.

322 The depth of community breaks we identified match those of previous studies conducted locally,
323 especially by Pinheiro et al., (2016) ¹⁷ who documented a strong fish species turnover at 80 m in
324 Curaçao, matching the upper to lower mesophotic transition found here. The few studies conducted
325 outside of the Caribbean over a comparable depth range have also reported successions of three to five
326 distinct reef-fish assemblages ^{8,26}, but differences in the number of distinct assemblages and in the depth
327 of faunal breaks suggest important regional variability. Many more studies have investigated the vertical
328 zonation of deep-reef communities, but most have been limited to a maximal depth of 60 to 100 m; as
329 such, they have only captured the upper range of deep reefs ^{9,12}. Conversely, some studies have extended
330 below 300 m depth but did not describe fish communities continuously across depths, hindering depth-
331 zonation analyses ^{11,27}. As a result, our understanding of the number of depth zones and their boundaries
332 across oceanic regions remains limited. Given that the shallowest deep-reef fish community break
333 generally occurs around 60-100 m ^{10,26,28}, surveys extending down to at least 150 m are necessary to
334 adequately locate deep community breaks. In that regard, submersible surveys combined with the use of
335 ichthyocides or anesthetics offers unparalleled means to describe deep-reef fish communities. In
336 particular, only submersibles allow for direct visual observation and fish collection below ~150 m, the
337 lower limit of rebreather diving. Such sampling techniques are necessary to document cryptic species, a
338 major component of deep-reef fish communities that cannot be readily observed using remotely operated
339 vehicles or underwater video systems ^{4,29}.

340

341 *Shifts in fish community structure across depths*

342 We found high and relatively constant values of dissimilarity (~ 50%) between fish communities
343 of adjacent depth zones resulting mostly from species turnover (Fig. 5), corroborating existing evidence
344 for the ecological uniqueness of deep-reef fish communities ^{2,22,26}. While previous studies have reported
345 important taxonomic turnover between deep-reef fish communities ^{22,26}, only one study has tested this
346 down to rariphotic depths ²⁶. While ²⁶ reported lower rates of turnover at rariphotic than at mesophotic
347 depths, we found the opposite trend, with the upper to lower mesophotic transition having the lowest
348 rate of species turnover.

349 We found that “altiphotic/mesophotic” species contributed to ~60% of total diversity and ~80%
350 of total abundance in the upper mesophotic (Fig. 6C). This aligns with previous studies demonstrating
351 that a large proportion (~64%) of shallow reef fishes occur down to 60 m ⁹ and suggests that mesophotic
352 fish communities are largely composed of shallow reef fishes at the extremity of their depth distribution,

353 especially in the upper mesophotic. The ecological continuum between altiphotic and upper mesophotic
354 fish communities was further supported by SIMPROF analyses which identified a single cluster from 20
355 to 60 m in Roatán (Fig. 2). These results suggest that the upper mesophotic boundary, typically
356 considered at around 30-40 m in the literature, might reflect conventional SCUBA limits, as well as
357 breaks in habitat-forming communities rather than actual fish community breaks. By contrast, the high
358 rates of rariphotic specialists in the rariphotic depth zones (Fig. 6C) combined with the important species
359 turnover found between mesophotic and rariphotic communities (Fig. 5) confirm the ecological
360 uniqueness of rariphotic fish communities, a depth zone only recently described by Baldwin et al.,
361 (2018). Together, these results suggest that the faunal break between the upper and lower mesophotic is
362 numerical (i.e., decrease in abundance and loss of species) rather than compositional (i.e., species
363 turnover), and represents a weaker faunal break than the mesophotic to rariphotic transition. We also
364 confirm that the rariphotic zone is dominated by reef-affiliated families by showing this pattern across
365 four sites of the Caribbean (Fig 6B), a result previously documented only in Curaçao¹⁰. Lastly, we
366 determine that the lower boundary of the rariphotic occurs at 300 m in Roatán and corresponds to the
367 depth limit of communities dominated by reef-affiliated fish families. From 300 to 480 m, we found a
368 single community dominated by rariphotic specialists from deep-sea families rather than true deep-sea
369 species.

370

371 *Depth specialization and segregation in reef fish taxa*

372 We found that the majority of fish species observed at our study sites occur predominantly
373 within a single depth zone (Fig. 6A). Eighty-five percent of deep-reef species (i.e., excluding altiphotic
374 and deep-sea affiliated species) were depth specialists, meaning that > 75% of their total abundance was
375 confined to either mesophotic or rariphotic depths. Furthermore, our study documents depth segregation
376 among ten reef-fish genera, with up to four species of a given genus occurring at distinct average depths
377 (Fig. 7). These results demonstrate that habitat segregation by depth is an important ecological
378 mechanism driving the zonation patterns observed on deep reefs. Moreover, depth segregation may be
379 important in driving speciation in reef fish, or at the very least, an important factor in maintaining
380 species boundaries. A substantial part of deep-reef fish diversity originates from speciation of shallow-
381 reef fish having adapted to deep-reef environments^{10,30}. Depth segregation has already been
382 demonstrated on deep reefs for the family Gobiidae^{30,31}, the genus *Lipogramma*, and the genus
383 *Lipropoma*^{32,33}, or the genus *Bathypterois* found on seamounts¹¹. Importantly, large differences

384 between global depth distributions and locally realized depth distribution (Fig. S9) suggests that depth
385 segregation processes are underestimated when working with global datasets.

386

387 *Regional variability of deep-reef fish communities*

388 We found that fish communities from a given depth zone shared many characteristics across
389 sites. The most abundant species at a given depth zone were usually found at several sites and
390 participated in driving community distinctiveness between depth zones (Fig. 4). Some of these key
391 species have already been identified at different Caribbean sites ^{7,11}, in particular *Pronotogrammus*
392 *martinicensis* and *Serranus phoebe* as indicators of rariphotic communities, and *Paranthias furcifer* as
393 an indicator of mesophotic communities. Such indicator species could be useful to more promptly
394 identify vertical boundaries of depth zones at new study sites, or to test whether depth boundaries at a
395 given site are changing through time. The limited coastal extent (~1 km) sampled at each island
396 precludes us from explaining differences in fish communities between sites through a biogeographic
397 lens. Rather, variations in vertical zonation and in fish-community structure are likely due to local
398 characteristics of study sites, such as irradiance, temperature, nutrient availability ³⁴, and habitat
399 characteristics ^{6,7,13}. Local geomorphological specificities of study sites, such as the location of reef
400 walls and reef flats, could explain a large part of the variability in vertical zonation and community
401 structure of deep-reef fish ^{15,35}. Human impacts, particularly fishing pressure and coastal development,
402 could also explain differences observed between sites ³⁶. Notably, the survey site at Bonaire occurs
403 within a marine protected area ³⁷ while the study site in Curaçao is located in front of a resort complex
404 and experiences fishing and shoreline artificialization. More research is needed to document how such
405 pressures affect deep-reef fish communities ^{21,38}.

406

407 *Increased endemism in the rariphotic?*

408 The higher regional beta-diversity in the lower rariphotic revealed in this study could be due to
409 (1) lower fish abundance, which might have amplified biases associated with incomplete sampling effort
410 by artificially increasing differences between rariphotic communities described, (2) greater influence at
411 rariphotic depths of local environmental variables and micro-habitats on community structure, (3) higher
412 rates of endemism at rariphotic depths, or (4) a combination of these factors. While assessments of
413 endemism rates at rariphotic depths are unprecedented, higher rates of endemism on mesophotic reefs
414 than on shallow reefs have been documented by an increasing body of literature ^{18,39,40}, and surveys of

415 deep reefs across the Pacific found that deep-reef fishes tend to have smaller geographic ranges than
416 shallow-reef fishes^{39,41}. The greater environmental stability of deep reefs, in particular in relation to sea-
417 level change associated with glacial-interglacial cycles⁴², has been hypothesized to promote higher rates
418 of endemism at depth³⁹, and could explain how endemism occurs on Caribbean deep reefs despite
419 extremely low regional levels of endemism on Caribbean shallow reefs⁴³. While close to half (48 out of
420 109) of mesophotic and rariphotic species observed in this study were found at a single site, it is
421 currently unclear whether these species are locally endemic. Extending deep-reef studies to new sites
422 across ocean basins will help us to determine whether increased rates of endemism are indeed a
423 characteristic of deep-reef fish communities or rather reflect decreasing sampling efforts with depth.

424

425 *Accounting for depth in biogeographic studies*

426 Decades of biogeographic research have attempted to divide the world's tropical reef regions into
427 homogeneous and distinct ecological units^{43,44}. The resulting classifications are based almost
428 exclusively on the distribution of shallow-reef species and, as such, only account for ~20% of reef
429 biodiversity. Given the growing evidence that deep-reef communities exhibit distinct diversity patterns
430 relative to shallow reefs¹⁶ and could be influenced by different diversification mechanisms³⁹, we
431 suggest that the delimitation of marine realms and ecoregions should shift from a two-dimensional (2D)
432 to a 3D approach. Adding depth boundaries to marine ecological units would reflect that several
433 ecologically distinct marine communities co-occur at a given location at different depths. For example,
434 “depth zones” corresponding to distinct ecological communities, and “depth realms” corresponding to
435 distinct taxonomic communities (e.g., reef-affiliated vs. deep-sea affiliated) could constitute two levels
436 of subdivision of marine units across depth. At minima, we suggest that biogeographic studies revisit
437 current classification by incorporating the rapidly evolving data on the distributional range of deep-reef
438 and deep-sea species⁴⁵. Similarly, biogeographic theories developed to explain spatial patterns of reef-
439 fish diversity, such as the center of overlap, origin, and accumulation hypotheses^{46,47} have also been
440 restricted to explaining diversity patterns of shallow-reef fishes. Testing whether these theories could be
441 transposed to explain diversity gradients across both space and depth could represent the next step for
442 reef-fish biogeographic studies.

443

444 **Conclusion**

445 Continuous observations from the surface to 480 m at four sites in the Caribbean suggest that six
446 vertically-stratified fish communities occur across these depths, including five reef-fish dominated
447 communities down to 300 m. Vertical community zonation was comparable across sites suggesting
448 regional stability of this pattern, although we observed variations in the dominating species and in the
449 exact depth of community breaks, likely a result of local environmental conditions. Our results
450 demonstrate that several ecologically distinct deep-reef fish communities occur below shallow reefs and
451 support the growing literature calling to extend conservation actions beyond shallow ecosystems to
452 ensure that the full succession of marine diversity benefits from protection.

453

454 **Methods**

455 1. Sites

456 Data collection occurred at four locations in the Caribbean (Fig. 1): two neighboring western Caribbean
457 islands that are part of the Lesser Antilles (Bonaire and Curaçao), an eastern Caribbean island that is part
458 of the Leeward Islands (Saint Eustatius, hereafter Statia), and a western Caribbean island off the coast of
459 mainland Honduras (Roatán). See Supplementary Information for a more detailed description of islands'
460 environmental and ecological specificities.

461

462 2. Data collection

463 *Submersible transects.* All fish data were collected using human-occupied submersibles as part of the
464 Smithsonian Deep Reef Observation Project (DROP). Fishes were identified by two trained observers
465 (among Luke Tornabene, Ross Robertson, and Carole Baldwin) in the submersible as it slowly
466 descended (first half of dives) or ascended (second half of dives) the reef slope. Ascents were conducted
467 on a different trajectory than descending transects. Some fishes, with emphasis on cryptobenthic fishes,
468 were opportunistically collected using anesthetics and a suction hose attached to the submersible, either
469 for identification purposes or for systematic projects. See Baldwin et al., (2018) for additional details.
470 In **Curaçao** (site coordinates: 12.083197N, 68.899058W) fish data were collected between 2011 and
471 2016 for a total of > 100 submersible dives ranging from 40 to 310 m using the Curasub. Surveys
472 covered ~ 1,000 m on both sides of the drop-off site. In **Bonaire**, fish data were collected in January
473 2017 during 11 submersible dives. Submersible dives were performed between Klein Bonaire Island and
474 the South of Punt Vierkant by Belnem (drop-off coordinates: 12.094974N, 68.296635W) between 40 to
475 300 m depth using the Curasub. Each dive averaged 3 to 4 hours in duration and altogether covered ~

476 1,000 m on both sides of the drop-off site. Depths between 40 and 150 m were systematically covered,
 477 five dives extended down to 180-230 m, and one dive reached 300 m. In **Statia**, fish data were collected
 478 in April 2017 during 12 submersible dives between 40 to 305 m depth using the Curasub. Different
 479 locations off the southwestern edge of the island were sampled using the R/V Chapman (Robertson et
 480 al., 2020), including Gallows Bay (17.474734N, 62.987370W) and Kay Bay (17.467653N,
 481 62.978778W). Dives were conducted off the southwestern edge of the island's 200 m platform and
 482 lasted 5 to 6 hours. In **Roatán** (drop-off coordinates: 16.305557N, 86.597669W), fish data were
 483 collected between 2016 and 2018 during 17 dives from 0 to 480 m using the submersible Idabel. Each
 484 dive averaged 3 to 4 hours in duration, and altogether covered ~ 500 m on both sides of the drop-off site.
 485 *Guidelines for the Use of Fishes in Research* co-established by the American Society of Ichthyologists
 486 and Herpetologists⁴⁸ were followed for all field-collecting activities, and fish specimens collected as
 487 part of this study were done so under Smithsonian Animal Care and Use Committee (ACUC) approval
 488 to C. C. Baldwin (ACUC #2011–07 and #2014–13). No In Vivo Experiments were conducted as part of
 489 this research.

490

491 3. Data analysis

492 All data analyses were performed using R version 4.4.1⁴⁹ in R Studio.

493 *Abundance and species richness.* Fish abundance was reported as the total number of individuals
 494 observed at each 10 m depth bin from 40 to 300 m (e.g., all observations from 40 to 49 m were grouped
 495 into the 40 m depth bin) across all submersible dives at a given site. Because sampling effort t (hours
 496 spent diving) per depth bin x varied depending on technical and environmental factors (e.g., steepness of
 497 the slope), abundance values were normalized using coefficients denoting the relative time spent at each
 498 depth bin x and site z (Baldwin et al., 2018):

499

500 $abundance\ normalized_{x,z} = \square\square\square\square\square\square\square\square_{x,z} \times \square\square\square\square\square\square\square\square\square\square\square\square\square\square_{x,z}$ (1)

501

502 with sampling coefficient at depth x of site z being calculated as:

503

504 $\square\square\square\square\square\square\square\square\square\square\square\square\square\square_{x,z} = \frac{\max_x(t_{x,z})}{t_{x,z}}$ (2)

505

506 Because total sampling time also varied between sites, we transformed normalized abundance values
507 into relative abundance values when comparing data between sites (see Fig. 2):

508

$$509 \quad \text{Relative Abundance} (x) = \frac{\text{Normalized Abundance}_x}{\max_{x \in [40, 300]} (\text{abundance normalized}_x)} \times 100 \quad (3)$$

510

511 Models of abundance and species richness were fitted using generalized additive models (`mgcv`
512 package, `gam()` function) accounting for depth and location (abundance ~ depth + location. We used
513 Poisson error distributions to account for the non-normal data structure, accounting for depth and
514 location (abundance ~ depth + location).

515

516 *Community structure.* The depth structure of fish assemblages was examined using multivariate
517 hierarchical clustering methods. First, we calculated the Bray-Curtis dissimilarity matrix, which
518 accounts for species presence and their relative abundance within the community. To avoid
519 overemphasizing the importance of rare species in our analysis, while also controlling for extremely
520 abundant species that sometimes occur in large schools, we applied a square-root transformation to
521 abundance data before performing cluster analyses. Additionally, depth bins for which fewer than five
522 individuals were observed were pooled with the next deeper 10 m depth bin until a collective total of
523 five individuals was reached. This was done to counter biases arising from the fact that there are
524 inherently fewer similarities between communities with fewer individuals, as in ⁵⁰. We used a complete-
525 linkage clustering algorithm, which seeks to maximize distance between clusters based on the two most
526 distant objects in each cluster, to group depth bins based on species composition. Hierarchical cluster
527 dendrograms and nonmetric multidimensional scaling ordinations (MDS) based on Bray-Curtis
528 distances were used to visualize community structure. The number of significantly distinct depth clusters
529 was determined using Analysis of Similarity Profiles (SIMPROF) ⁵¹ based on a complete linkage
530 algorithm, Bray-Curtis distances, and a conservative value of alpha = 10⁻⁷ as in ^{11,52}. Based on main
531 branching events in dendrograms, we pooled adjacent clusters into six depth zones: altiphotic, upper
532 mesophotic, lower mesophotic, upper rariphotic, lower rariphotic, and “below rariphotic” (see Fig. S8).
533 We confirmed the distinctiveness of these depth zones a posteriori using PERMANOVA ⁵³. We used a
534 similarity percentage analysis (SIMPER) ⁵³ to determine which species contributed most to differences
535 between adjacent depth zones. Lastly, we tested for faunal depth breaks across sites by performing a

536 hierarchical clustering and SIMPROF analyses on depth bins across all four sites based on a Ward
537 linkage (which forms clusters by minimizing variance within clusters), Bray-Curtis distances, and alpha-
538 value = 10^{-7} .

539

540 *Depth segregation.* To test whether depth is a driver of niche segregation between closely related
541 species, we compared the depth distribution of species belonging to the same genus. This was performed
542 for all genera for which at least two species had been observed on deep reefs, with each species
543 represented by a minimum of ten individuals across study sites. Because the depth distribution of species
544 from most genera tested (eight out of ten) did not follow a normal distribution (Shapiro-Wilk normality
545 test, $p < 0.05$) we performed Kruskal-Wallis tests followed by pair-wise Wilcoxon tests to assess the
546 correlation between species and depth (depth~species). Statistical tests were performed on original depth
547 observations (non-transformed for sampling effort), but density curves and means represented in Fig. 7
548 display abundance corrected for sampling effort (as in equation 1).

549

550 *Depth affinities of fishes.* We defined five categories of depth affinities at the species level:
551 “altiphotic/mesophotic”, “mesophotic”, “mesophotic/rariphotic”, “rariphotic”, and “deep-sea”. A species
552 was determined to have an affinity for a given depth zone when $\geq 75\%$ of observations¹⁰ occurred in
553 that zone. We classified species as belonging to the “altiphotic/mesophotic” category when $\geq 75\%$ of
554 observations in this study occurred at mesophotic depths, but the species was also known to commonly
555 occur above 40 m depth^{54,55}. Similarly, the “deep-sea” depth affinity was attributed to species typically
556 associated with deep-sea ecosystems, but also observed in the present study (e.g., *Synagrops spp.* ,
557 *Bathyclupea sp.* , *Epigonidae sp.*; see Table S1 for complete list). When determining in which depth
558 zones a species was observed, we used the site-specific depth breaks from hierarchical clustering
559 analyses (Figure 2).

560 We further defined four categories of depth dominance at the family level based on published fish
561 databases⁵⁴⁻⁵⁶ and expert knowledge: “altiphotic/mesophotic”, “rariphotic”, “deep-sea”, and “depth
562 generalists” (Table S1). We classified families as “altiphotic/mesophotic” when the depth ranges of their
563 members were predominantly shallower than 130 m, as rariphotic when the depth ranges of their
564 members were predominantly between 130 and 500 m, and as deep-sea affiliated when the depth ranges
565 of their members were predominantly below 500 m. In addition to these three categories first defined by

566 Baldwin et al., (2018), we classified families as depth generalists when their members spanned multiple
567 depth zones, which was the case for some non-reef affiliated families (e.g., Paralichthyidae).

568

569 *Dissimilarity across depths and between sites.*

570 We calculated the β -diversity index β_{sor} ⁵⁷ between depth zones to assess whether the distinctiveness
571 of deep-reef fish communities is predominantly driven by changes in species composition (i.e., turnover)
572 or by loss in diversity (i.e., nestedness⁵⁸):

573
$$\beta_{sor} = \frac{b+c}{2a+b+c} \quad (4),$$

574 where a is the number of species common to both groups (site or depth bin), b is the number of species
575 that occur in the first group but not in the second, and c is the number of species that occur in the second
576 group but not in the first. This index varies between 0 and 1, with 1 indicating no species in common
577 between sites and 0 indicating identical species composition between sites.

578

579 We calculated the turnover component of dissimilarity using the Simpson dissimilarity index (β_{sim}),
580 which calculates the amount of dissimilarity due to the presence of distinct species in both groups:

581
$$\beta_{sim} = \frac{\min(b,c)}{a+\min(b,c)} \quad (5).$$

582 β_{sim} varies between 0 and 1, with 1 denoting that no species are shared between the two groups, and 0
583 denoting that all species from a group correspond to a sub-sample of the species from the other group.

584

585 The nestedness component of pairwise dissimilarity, i.e., the component of dissimilarity linked to
586 species loss rather than species turnover, is calculated by subtracting β_{sim} from β_{sor} :

587
$$\beta_{nes} = \beta_{sor} - \beta_{sim} \quad (6).$$

588 Taxonomic dissimilarity, nestedness, and turnover components were computed in R using the {betapart}
589 package⁵⁹.

590

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733

734 **Author contribution**

735 Tornabene L, Carole B, McFarland E, and Barrett J collected the data. Jacquemont J and Brandl S
736 performed data analysis. Jacquemont J wrote the manuscript. Tornabene L, Claudet C, Baldwin C,
737 McFarland E, and Brandl S revised the manuscript.

738

739 **Data availability statement**

740 All data and R scripts used for analyses are available through the author's personal GitHub repository
741 (<https://github.com/jjacquemont/Deep.Reef.Fish.Carib>).

742

743 **Conflict of interest statement.**

744 The authors declare no competing financial interests.

745

746 **Ethics approval statement**

747 Fish collections in Curaçao and Bonaire were conducted under research permits granted to Substation
748 Curaçao. Permits for fieldwork in Roatán were approved under research permit number DG/PMM 010-
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759 **Figure legends**

760 **Figure 1:** Locations of study sites. The bathymetric maps of Curaçao, Bonaire and Statia were obtained
761 from the Dutch Caribbean Biodiversity Database, and the bathymetric map of Roatán from the General
762 Bathymetric Chart of the Ocean. Rendering was obtained using QGIS.
763
764
765

766 **Figure 2:** Relative abundance (left) and species richness (right) across depth at the four study sites.
767 Relative abundances were normalized by depth-specific sampling effort and are given as a proportion of
768 the site-specific maximal abundance. Points represent observed relative abundance and richness at each
769 10 m depth bin, lines represent predicted values from a generalized additive model and shaded areas
770 represent 95% confidence intervals. Note that for depths greater than 300 m and shallower than 40 m,
771 predictions are based on observations from Roatán alone.
772

773 **Figure 3:** Site-specific dissimilarity analysis of reef-fish community from 40 to 300 m depth. Left
774 panels display hierarchical clustering dendrograms, right panels display nonmetric multidimensional
775 scaling ordination (MDS) plots derived from Bray-Curtis dissimilarity analysis. Clusters with
776 significantly distinct composition (SIMPROF analyses) are indicated on dendrograms by thick
777 continuous horizontal lines below depth bin values. Color scale indicates the depth zones that clusters

778 obtained from SIMPROF analyses were pooled into. Depth-bins are labeled with the minimum depth of
779 each 10 m depth interval (e.g., “100 m” = 100-109 m).

780

781 **Figure 4:** Most common species by depth zone and site. Species displayed are the top four most
782 common species by depth zone and sites, representing at least 5% of the total abundance. The relative
783 abundance of each species per depth zone per site is indicated in %. Species most contributing to
784 differences between depth zones (SIMPER contribution >5% and p-value < 0.05) are displayed in the
785 right grey panel, with abundance of these species being higher in the shallower of the two depth zones.

786

787 **Figure 5:** Beta diversity and its components, nestedness and turnover of reef-fish communities across
788 depth zones. Values are shown as the average of values across the four sites, and error bars indicate
789 standard error. “Altiphotic vs. upper mesophotic” and “lower rariphotic vs. below rariphotic” values
790 were only available for Roatán. Coloring of bars indicate the relative contribution of nestedness (beige)
791 and turnover (red).

792

793 **Figure 6:** Depth affinity of reef-fish species and families across depth zones. Distribution (% total
794 abundance) of fish species among depth zones (A). Each line displays the relative abundance of a given
795 species across five depth zones from the “altiphotic” to “below rariphotic” (300-480 m). Vertical
796 colored lines indicate species falling under each depth affinity category given their depth distribution.
797 The number of depth specialists of each depth zone (>75% of total abundance in a given depth zone) is
798 indicated on the figure (“n=”). Depth-zone dominance of fish families (B) and depth-zone affinity of
799 species (C) in each depth zone, and contribution of depth affinity groups to species richness (left panel)
800 and abundance (right panel). White lines in barplots of panel C distinguish species from different
801 families. Filling colors indicate the depth affinity of species, from the altiphotic to the deep sea. Results
802 are shown pooled across the four study locations, except for altiphotic and below rariphotic zones for
803 which data was only collected in Roatán.

804

805 **Figure 7.** Depth segregation among congeners. Lines indicate the mean depth of occurrence of each
806 species. Colors of abundance areas indicate species. Statistically different mean depths within a genus
807 are indicated by different letters (Table S4). A comparison of depth distributions observed at our
808 sampling sites vs. global depth ranges is provided in Fig. S7.

809

810 **Figure 8:** Cross-site dissimilarity analysis of deep-reef fish communities. (A) Cross-site hierarchical
811 clustering analysis of deep-reef fish communities from 40 to 300 m. Length of branches in the
812 dendrogram is commensurate to the dissimilarity between depth bins based on Bray-Curtis distance.
813 Font color indicates sites. Significant clusters (SIMPROF analyses, Ward linkage) are indicated by thick
814 black vertical lines. (B) Dissimilarity of deep reef-fish communities between sites at the four depth
815 zones defined in this study, based on the presence/absence of fish species.