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Cross-ocean patterns and processes in fish biodiversity on coral reefs through the lens of eDNA metabarcoding

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Abstract

Increasing speed and magnitude of global change threaten the world's biodiversity and particularly coral reef fishes. A better understanding of large-scale patterns and processes on coral reefs is essential to prevent fish biodiversity decline but it requires new monitoring approaches. Here, we use environmental DNA metabarcoding to reconstruct well-known patterns of fish biodiversity on coral reefs and uncover hidden patterns on these highly diverse and threatened ecosystems. We analyzed 226 eDNA seawater samples from 100 stations in 5 tropical regions (Caribbean, Central and Southwest Pacific, Coral Triangle and Western Indian ocean) and compared those to 2,047 underwater visual censuses from the Reef Life Survey (RLS) in 1,224 stations. Environmental DNA reveals a higher (16%) fish biodiversity, with 2,650 taxa, and 25% more families than underwater visual surveys. By identifying more pelagic, reef-associated and crypto-benthic species, eDNA offers a fresh view on assembly rules across spatial scales. Nevertheless, RLS identified more species than eDNA in 47 shared families, which can be due to incomplete sequence assignment, possibly combined with incomplete detection in the environment, for some species. Combining eDNA metabarcoding and extensive visual census offers novel insights on the spatial organization of the richest marine ecosystems.

1. Introduction

Coral reefs host the highest fish diversity on earth despite covering less than 0.1% of the ocean's surface (1,2). They are also severely threatened (3), with near future outlooks predominantly pessimistic (4). Data syntheses over decades of surveys estimate the total number of coral reef fishes from 2,400 to 8,000 species (5,6), distributed among approximately 100 families (7). Typically, coral reef biodiversity displays clear spatial patterns, including longitudinal and latitudinal gradients outwards the Indo-Australian Archipelago (8,9), also known as the 'Coral Triangle', hosting the world's highest level of marine biodiversity (10). The exceptional biodiversity in the Coral Triangle has recently been suggested to strongly relate to higher diversity among fish families that feed on plankton (11). Other trophic groups are also very important on coral reefs but are often undetected because they are transient or hidden (12,13). Intriguingly, the proportions of fish species among families are shown to be strongly conserved across the Indo-Pacific (8). The spatial patterns of coral reef fishes are also marked by strong variations in taxonomic composition (species turnover or β diversity), often due to isolation (14). Many species on coral reefs are geographically localized, but can sometimes be locally abundant, while others are widespread (15).

Coral reef fishes have evolved in a physically complex environment and present a wide range of forms and functions (16). Small cryptic species, hereafter called crypto-benthic, that live inside the reef structure, can be very difficult to sample or survey using non-destructive methods (17), yet represent half of the fish diversity on coral reefs (13). Even though fishes are among the best-studied taxa inhabiting coral reefs (18), our knowledge of their biodiversity is only partial (19), the taxonomy is complex, uncertain for many species (5), and countless species remain undescribed.

Environmental DNA (eDNA) metabarcoding, a method retrieving and analyzing DNA naturally released by organisms in their environment (20), provides an opportunity to not only better understand classical biodiversity patterns, but also uncover novel ones hidden by our incomplete taxonomic and biogeographic coverage (21). Environmental DNA is particularly powerful in aquatic ecosystems (22) and is now well established for marine microorganisms (23,24). By contrast, its potential to provide an integrated biodiversity assessment of macroorganisms, including vertebrates of all trophic levels (from crypto-benthic to large pelagic fish species), is only shown at local (25) and regional (26–30) scales but not yet at spatial scales including more than one biogeographic region or multiple ocean basins.

Here, we investigate how a cross-ocean basin snapshot of eDNA sampling could describe the distribution of fish biodiversity on coral reefs, reveal unknown patterns, and challenge well-established assembly rules. From 226 eDNA seawater samples (2,712 PCR replicates) collected in 100 stations at 26 sites covering five tropical regions (Southeast Polynesia, Tropical Northwestern Atlantic, Tropical Southwestern Pacific, Western Indian Ocean and Western Coral Triangle) across the Indian, Pacific and Atlantic Oceans (figure S1-S2), we produced a final dataset of 189,350,273 mitochondrial 12S rRNA gene sequence reads (see Methods), clustered into 2,023 molecular operational taxonomic units (MOTUs), and assigned to Actinopterygii (bony fishes) and Chondrichthyes (cartilaginous fishes) taxa (tables S1- S2). We then compared fish biodiversity patterns obtained from eDNA to those observed from 2,047 standardized visual surveys of reef fishes in 1224 stations at 219 sites within 24 tropical regions (31).

2. Results

(a) Global estimates of fish biodiversity on coral reefs

104 We estimated total fish diversity on coral reefs using the asymptote of a multi-model accumulation
105 curve for both eDNA MOTUs (32) and visual census species (Methods). The asymptote estimated
106 from 100 eDNA stations distributed in five regions sampled over a 28-month period reaches 2,650
107 MOTUs (figure 1a). This detectable fish MOTUs diversity, including also MOTUs unassigned at
108 the species-level, is 16% higher than the estimate from visual census data, which reaches an
109 asymptote at 2,268 fish species from 2,047 tropical transects surveyed during 13 years (figure 1b).
110 The asymptotic estimation of family richness obtained with eDNA reaches 147 families, 25% more
111 than the asymptotic number of families estimated with visual census data (118 families, figure 1c-
112 d). Among the 71 families shared between both datasets, 24 have a higher number of MOTUs from
113 eDNA survey than species from visual survey while 47 have more species from visual survey than
114 MOTUs from eDNA survey (figure 1e). Families with more taxa identified using eDNA include
115 those often associated with reef-adjacent habitats such as mangroves or soft sediments like
116 Mugilidae (e.g. *Mugil rubrioculus*), Elopidae and Gerreidae (33, e.g. *Gerres oyena*), and crypto-
117 benthic species that live hidden in crevices (e.g. Gobiidae) or nocturnal fish species (34, e.g.
118 Congridae). Families with more taxa with visual census include Acanthuridae, Chaetodontidae,
119 Blenniidae, Labridae, Pomacentridae and Scaridae. Fifty-five families are detected only with
120 eDNA, including Myctophidae, Engraulidae, Atherinidae and Exocoetidae, while 24 families are
121 detected only by visual census, including Caesionidae, Chaenopsidae, Labrisomidae and
122 Microdesmidae. Environmental DNA estimates a diversity of crypto-benthic species 13% higher
123 than with visual census, and, among many others, includes species such as the elegant firefish
124 (*Nemateleotris decora*), which lives on the outer reef slope between 25 to 70 m (figure 2a). Yet,
125 the difference in fish diversity assessment between the two methods is the strongest for pelagic
126 and wide-ranging species, for which eDNA reveals more than 7 times higher richness than with

visual census. These species mainly belong to Scombridae (e.g. *Katsuwonus pelamis*), Clupeidae, Carcharhinidae (e.g. *Carcharhinus leucas*, *Sphyrna lewini*) and Belonidae (figure 2b).

MOTU richness per fish family retrieved with eDNA is strongly correlated with fish species richness within families recorded in visual census data (Pearson correlation = 0.84, $p < 0.001$, $n = 71$, figure 1e). Highly diverse families seen on coral reefs are also well represented in eDNA samples, with Gobiidae, Labridae and Pomacentridae containing more than 100 MOTUs each, together representing about 20% of MOTUs (figure 1f, figures S3- S4). The slope of the log-log relationship between MOTUs richness per family and species richness per family is equal to 0.8 showing that the relationship is not proportional but saturating. The richest fish families contain more MOTUs detected with eDNA than species detected with visual surveys.

(b) Biogeography of eDNA sequences

The spatial distribution of MOTUs follows clear biogeographic patterns, with a peak in the coral triangle and lower values of MOTU richness toward Southeast Polynesia (figure S5). The richest region (West Papua, Indonesia, Western Coral Triangle) contains ~50% of the global pool of fish MOTUs while the poorest region (Fakarava, French Polynesia, Southeast Polynesia) contains only 9% of the global pool (figures S6-S7 and table S2). Distance-based Redundancy Analysis (dbRDA) was performed on fish family proportions at each site (i.e. number of MOTUs or species assigned to each family in each site, see Methods) for eDNA and visual surveys with the region and the site MOTU/species richness as explanatory variables, including their interaction (figure 3, table S3). For eDNA, the dbRDA explains up to 42% of variation in family proportions between pairs of sites with region and MOTU/species richness both having significant effects ($F = 4.1$ and 5.7 , respectively, $p < 0.001$), but no significant interaction ($F = 1.99$, $p > 0.05$). The partial dbRDA

on eDNA showed a significant effect of region while controlling for MOTU richness ($F = 2.79$, $p < 0.001$). The first axis explains 17.2% of variation in family proportions and separates the Western Coral Triangle from other regions (figure 3*a-b*). The first axis shows a higher proportion of Lutjanidae but lower proportions of Labridae and Gobiidae in sites of the Western Coral Triangle. It also confirms the longitudinal diversity gradient from the Coral Triangle. The second axis explains 11.2% of variation and discriminates the Tropical Northwestern Atlantic from the Western Indian Ocean, due to a higher proportion of Clupeidae and Carangidae in the Atlantic Ocean and a higher proportion of Acanthuridae in the Indian Ocean. The dbRDA performed on visual census data explained greater variation ($R^2 = 0.5$, $p < 0.001$) and the region also had a significant, albeit weaker than for MOTUs, effect on fish family proportions ($F = 17.7$, $p < 0.01$), while species richness and interaction between the two variables also had significant effects ($F = 6.28$ and 2 , $p < 0.01$ respectively). The first axis explains 41.6% of variance in family proportions and separates the Tropical Northwestern Atlantic from the other regions with a higher proportion of Gobiidae and Serranidae. The second axis explains 5.7% of variance in family proportions and separates the Southeast Polynesia from Indo-Pacific regions, and is mostly driven by the higher proportion of Pomacentridae in the Indo-Pacific (figure 3*c-d*).

(c) Global patterns of fish turnover and rarity

Our eDNA survey shows that a majority of MOTUs are geographically restricted, with 85% of the MOTUs detected in only one region (figure 4*a*), and 35% in only one site (figure S8). Geographic restriction is one aspect of species rarity but is shown to play a primary role in determining extinction risk while local abundance and habitat specialization have secondary roles (35). We hierarchically partitioned the global MOTU diversity (γ_{global}) into additive diversity components

(i.e. dissimilarity) due to difference between regions ($\beta_{inter-region}$), mean difference between sites within regions ($\bar{\beta}_{inter-site}$), mean difference between stations within sites ($\bar{\beta}_{inter-station}$) and mean station diversity ($\bar{\alpha}_{station}$) (36). As a consequence of the geographic restriction of most MOTUs to one region, the total fish MOTU (γ) diversity is mainly due to inter-region β -diversity (~74%) followed by inter-site (14.8%) and inter-station (5.9%) β -diversity (figure 4b). The same partitioning using different site delineations (10 and 20 km) provides similar results (table S4). Diversity partitioning of crypto-benthic fish MOTUs only or pelagic fish MOTUs only reveals similar patterns (table S5). The partitioning diversity of species detected by visual census also revealed similar patterns but with a stronger effect of $\beta_{inter-region}$ (84%) and lower ($3\times$) $\bar{\beta}_{inter-site}$ and $\bar{\beta}_{inter-station}$ (table S5, figure S9).

Beyond the hierarchical partitioning of diversity, we compared the distribution of fish MOTUs and species visual occurrences independently of the survey method and sampling effort using global species abundance distributions (gSAD) (37). We fitted the fish MOTU and species visual occurrences to three distributions (log-series, Pareto and Pareto with exponential finite adjustment, *i.e.* Pareto Bended, see Methods) and estimated the parameters by maximum likelihood. For the visual census gSAD, the best fit was obtained with the log-series and Pareto distributions (table S6) with a slope of -0.95 (confidence interval at 95% [-0.98;-0.92]) (figure S10). This suggests a distribution of geographically restricted or rare species close to the neutral theory (β close to -1). By contrast, the best fit for fish MOTUs was obtained with the Pareto Bended distribution with a slope $\beta = -0.76$ (confidence interval at 95% [-0.85;-0.65]) and then with the log-series distribution, suggesting a lower prevalence of rarity than under the neutral theory, in agreement with previous tests based on species distributions on coral reefs (38).

3. Discussion

Environmental DNA allows the detection and identification of more taxa than traditional techniques (26,39), but further offers novel insights on the spatial organization of the richest marine ecosystem at large scale. Over a timespan of 2.3 years, in major tropical ocean basins, eDNA metabarcoding reveals a higher proportion of crypto-benthic, pelagic and soft-sediment-associated fishes on coral reefs than detected in the most extensive visual census over 13 years. We found a high local MOTU turnover, but we were not able to conclude if it is due to an insufficient sampling at the station level, or if it suggests that differences in fish species composition may exist between adjacent reefs that are not detected by visual surveys (40), so that fish biodiversity is more patchy than previously thought on coral reefs.

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We were also able to retrieve well-known patterns of fish diversity on coral reefs such as the biogeographic boundaries between the Atlantic and Pacific oceans, the longitudinal diversity gradient from the center of the Coral Triangle, with Southeast Polynesia being the least diverse region and Western Coral Triangle the richest, and that Gobiidae, Labridae, Pomacentridae and Apogonidae are the most diverse fish families on coral reefs (8). We found a lower proportion of rare MOTUs than expected under the neutral theory with eDNA, which is in agreement with the findings of a previous study from coral reefs in the Indo-Pacific (38), while visual census data suggests higher rarity close to that predicted from the neutral theory. More surprising, our study calls into question the pattern of fish family stability composition across the Indo-Pacific that was revealed more than 20 years ago (8), and the recent finding that planktivore families drive fish biodiversity patterns on coral reefs (11). We found significant effects of species richness and region on family composition, which appears less stable than previously thought.

221

222 Environmental DNA identified many pelagic, deepwater and crypto-benthic species not seen by
223 divers. Among the pelagic species identified with eDNA, many belong to the Scombridae and
224 Carcharhinidae families, which likely avoid divers or are not permanent residents on coral reefs
225 so can be missed in visual surveys (41). Some crypto-benthic or reef-associated species, hidden in
226 the reef, can also be missed by divers so were also more represented in eDNA than in visual
227 surveys. Crypto-benthic species also have a crucial role for coral reef functioning, by promoting
228 biomass production and fueling the reef trophodynamics (42), but their diversity has been
229 underestimated so far (13). Transient, pelagic and deep-water species may be very important for
230 reef functioning, through pelagic larval stages or nocturnal migration up the reef slope (12,43,44),
231 but their presence and role need further investigation. In contrast, visual census also detected many
232 families not detected, or not identified, by eDNA, such as Acanthuridae, Blenniidae, Caesionidae,
233 Chaenopsidae, Chaetodontidae, Labrisomidae, Labridae or Microdesmidae. This limited
234 identification by eDNA can be due to the very low representation of these families in 12S reference
235 databases (between 0 and 12%), or to the low resolution of the teleo marker for species of these
236 families, so several species can share the same sequence and be grouped under the same MOTU.
237 Environmental DNA may also be inappropriate to detect these species in the environment.

238

239 The finding of a strong regional effect on both species composition (figure 3) and species
240 differentiation (figure 4) at a large scale is in agreement with visual surveys and previous
241 knowledge (45), while the suggestion of a strong turnover at the local scale may be an unexpected
242 result for coral reef fishes. This predominant role of large-scale bioregional differentiation explains
243 the exceptional fish diversity on coral reefs, probably associated with long-term geological
244 isolation (2). Overall, the Tropical Northwestern Atlantic region has a very distinct MOTU

composition compared to the four other regions (figure 3) with only 1.2% of MOTUs being shared between the Tropical Northwestern Atlantic and any other region, while 20% of MOTUs are shared between at least two Indo-Pacific regions (figure 4a). The isolation of the Tropical Northwestern Atlantic region can be explained by the hard vicariant barrier of the Isthmus of Panama (14,46), and a limited suitable area for coral reefs during the past quaternary glaciation. By contrast, the Indo-Pacific maintained extensive coral reef refuges that have served as centers of survival during ice-age periods (9).

The greater local compositional dissimilarity of reef fishes among adjacent stations with eDNA than with visual census may correspond to local environmental or habitat differences, to stochastic or random processes (47), or may be due to an insufficient sampling at the station level (Supplementary Analyses Fig. 6). A higher number of replicates per station would be necessary to characterize exhaustively the diversity at the station level and more confidently conclude on the local turnover hypothesis.

While our results confirm the potential of eDNA to monitor biodiversity in marine ecosystems, some limitations should be addressed in the future to fully exploit this potential. Completing public reference databases would improve the accuracy of taxonomic assignment, which is essential for a better estimation of biodiversity patterns. At such a large spatial scale, reference databases are far from exhaustive with only up to 13% of fish species sequenced on our marker (52), preventing assignment to the species level for 81% of our eDNA sequences. Using multiple markers is an alternative to the database limitation (53,54), but it is much more expensive. For these reasons, we used MOTUs curated by a combination of a clustering algorithm and conservative abundance-based post-clustering filters. While un-curated MOTUs are prone to overestimate real diversity (55) and a given MOTU can represent several species within one cluster or several MOTUs

269 belonging to one species, MOTUs with conservative curation have been shown to reflect the true
270 level of fish diversity across scales in streams (56,57). Additionally, some species share the same
271 barcode sequence due to insufficient genetic differentiation on such a small mitochondrial marker
272 (54). This lack of taxonomic resolution combined with a conservative curated MOTUs pipeline
273 can underestimate MOTUs richness. Moreover, some crypto-benthic or rare fish families are still
274 underrepresented in public databases, and their diversity is potentially underestimated with eDNA
275 (i.e. Blenniidae, Gobiesocidae, Chaenopsidae, Aploactinidae).

276 Differences in sampling method and in sample size might influence the detected biodiversity with
277 eDNA. The lower volume of water sampled in the Western Coral Triangle region (2L per sample,
278 so 4L per station using point-sampling instead of 2-km transect with 30L elsewhere), could
279 underestimate fish biodiversity. However, previous studies show that MOTU accumulation curves
280 based on this dataset were close to the total fish diversity reported in this region (32). Furthermore,
281 β -diversity between samples within stations in each region indicates that dissimilarity between
282 samples is not greater in the Western Coral Triangle than in other regions (figure S11). To account
283 for differences in sample size and obtain a balanced design, we performed sensitivity analyses by
284 rarefying our complete dataset to i) 4 stations for all sites and ii) 4 sites per region after removing
285 the lowest sampled region (Southeast Polynesia) (Supplementary Analyses Fig. 1-4). We obtained
286 similar patterns even after subsampling stations or sites. However, our site-based and station-based
287 accumulation curves do not reach plateaus suggesting that our sampling effort was not sufficient
288 to exhaustively estimate fish biodiversity for each site (Supplementary Analyses Fig. 5) and station
289 (Supplementary Analyses Fig. 6). Twenty-five replicates (so, 12 stations in case of field
290 duplicates) could accurately estimate biodiversity regionally due to high local turnover (58). A
291 higher number of eDNA samples would be necessary here to reach MOTU accumulation per site
292 and station.

The transport and degradation of eDNA can also impact species detection. As some evidence suggests that eDNA from pelagic fishes degrades slower than from inshore species (59), we cannot exclude that eDNA from pelagic and deep-water families (*e.g.* Myctophidae) might disperse sufficiently with sea currents such that species living close to reef habitats are detected. Environmental DNA transport could also explain the detection of some freshwater fish families (*i.e.* Centrarchidae, Osphronemidae or Channidae) in a few samples located near an estuary or in an enclosed bay with freshwater inputs.

Better understanding and anticipating the effects of multiple threats to the marine environment depends on the temporal and spatial extent of our monitoring capacity in the vast ocean. Environmental DNA is a powerful tool to investigate biodiversity patterns at large scale and monitor biodiversity, but still benefits from the combination with complementary approaches as visual methods for an exhaustive biodiversity survey across space and time to keep pace with ongoing changes.

4. Methods

(a) Environmental DNA collection and sample processing

Environmental DNA seawater samples were collected between 2017 and 2019, following a hierarchical pattern. A total of 226 eDNA samples (filters) were collected in 100 stations (gathering of replicates at the same location) located in 26 sites (groups of stations separated by at least 35 km) distributed across five tropical regions (figure S1-S2). Three different sampling methods were used comprising a 2km-long sampling transect of 30L (surface or bottom depth) or point samples of 2L (table S7 and Methods S1), and between 12 and 64 samples were collected by

region. Filtration was performed with Polyethersulfone (PES) filters, 0.2 µm pore size. For each sampling campaign, a strict contamination control protocol was followed in both field and laboratory stages (39). Negative field controls were performed in multiple sites, and revealed no contamination from the boat or samplers.

(b) eDNA extraction, amplification and sequencing

DNA extraction was performed in a dedicated DNA laboratory (SPYGEN, www.spygen.com) equipped with positive air pressure, UV treatment and frequent air renewal. Decontamination procedures were conducted before and after all manipulations. Detailed protocols of DNA extraction, amplification and sequencing can be found in Method S2 and in (32,39). A teleost-specific 12S mitochondrial rRNA primer pair (teleo, forward primer - ACACCGCCCGTCACTCT, reverse primer – CTTCCGGTACACTTACCATG (39)) was used for the amplification of metabarcode sequences. As we analysed our data using MOTUs as a proxy for species to overcome genetic database limitations, we chose to amplify only one marker. Teleo marker has been shown to be the most appropriate for fish, owing to its high interspecific variability, and its short size allowing us to detect rare and degraded DNA reliably (39,54,60,61). Twelve DNA amplifications PCR per sample were performed.

(c) Bioinformatic analysis

Following sequencing, reads were processed using clustering and post-clustering cleaning to remove errors and estimate the number of species using Molecular Operational Taxonomic Units (MOTUs) (56). First, reads were assembled using *VSEARCH* (62), then demultiplexed and trimmed using *CUTADAPT* (63) and clustering was performed using *SWARM* v.2 (64) with a

minimum distance of 1 mismatch between clusters. Taxonomic assignment of MOTUs was carried out using the Lower Common Ancestor (LCA) algorithm *ecotag* implemented in the OBITOOLS toolkit (65) and the European Nucleotide Archive (ENA) as a reference database (release 143, March 2020). Details on the bioinformatics analysis can be found in Methods S3. Taxonomic assignments obtained from the LCA algorithm at the species level were accepted if the percentage of similarity with the reference sequence was 100%, at the genus level if the similarity was between 90 and 99%, and at the family level if the similarity was > 85% following previous studies (32,66). If these criteria were not met, the MOTU was left unassigned. Only 21% of assigned MOTUs are assigned to the family level with a similarity between 85 and 90% (Table S8).

(d) Visual census data

The visual census survey data used here is a subset (2047 transects, in 219 sites, figure S1) of the complete visual census data (3027 transects) provided by the RLS (31), and comprises all species observed on standardized 50 m surveys at sites in tropical biogeographic realms between 2006 and 2017 (Methods S4) (67). We selected only the most recent survey for each station and only transects with more than five percent of coral cover. Two different sampling protocols were adapted to detect both reef and crypto-benthic fishes.

(e) Statistical analysis

More details on the statistical analysis are available in Methods S5. Accumulation curves were calculated for species per 500 m² transect, MOTUs per eDNA sample, and families per transect and sample. We used the functions “specaccum” and “fitspecaccum” from the R package “vegan” which calculates the expected species accumulation curve using a

sample-based rarefaction method and fit a nonlinear accumulation model. In order to assess the impact of the irregular sampling on the estimates measured with accumulation curves, we subset randomly half of the transects in the 3 most sampled regions in Australia, and calculated again the accumulation curves for species and families (figure S12). The results were unchanged.

Linear regression models were fitted between the number of MOTUs per family in the eDNA dataset and the number of species per family in the visual census dataset, after $\log(x+1)$ transformation (figure 1e).

Accumulation curves were also calculated by sub-setting MOTUs belonging to crypto-benthic orders, or to pelagic families, for both datasets (figure 2). The asymptote was calculated as described above.

We performed distance-based Redundancy Analysis (dbRDA) on family proportions, with *region* and *site richness* as explanatory variables, using the function *capscale* from the *vegan* package. We subset the Visual Census to select only the 68 sites that fell into the 5 regions in common with the eDNA dataset. Total dbRDA provided the effects of each of the variables and their interaction. We then calculated partial dbRDA to measure the effect of the Region while correcting for the effect of site richness (figure 3, table S3).

We applied an additive partitioning framework (68) to separate the total MOTUs diversity at the global scale (γ global) into contributions at smaller scales from regions to local richness : $\gamma_{global} = \beta_{inter-region} + \text{mean } \beta_{inter-site} + \text{mean } \beta_{inter-station} + \text{mean } \bar{\alpha}_{station}$. In this additive framework, the three levels of biodiversity (69) (i.e. α , β and γ) are expressed with the same unit and consequently the contribution of α and β diversity to total diversity (γ) can be directly compared (70).

383 We analyzed the distribution of fish MOTU and species occurrences using global species
384 abundance distribution (gSAD) which plots, on a log-log scale, the number of species as a function
385 of the number of observations (37).

386

387

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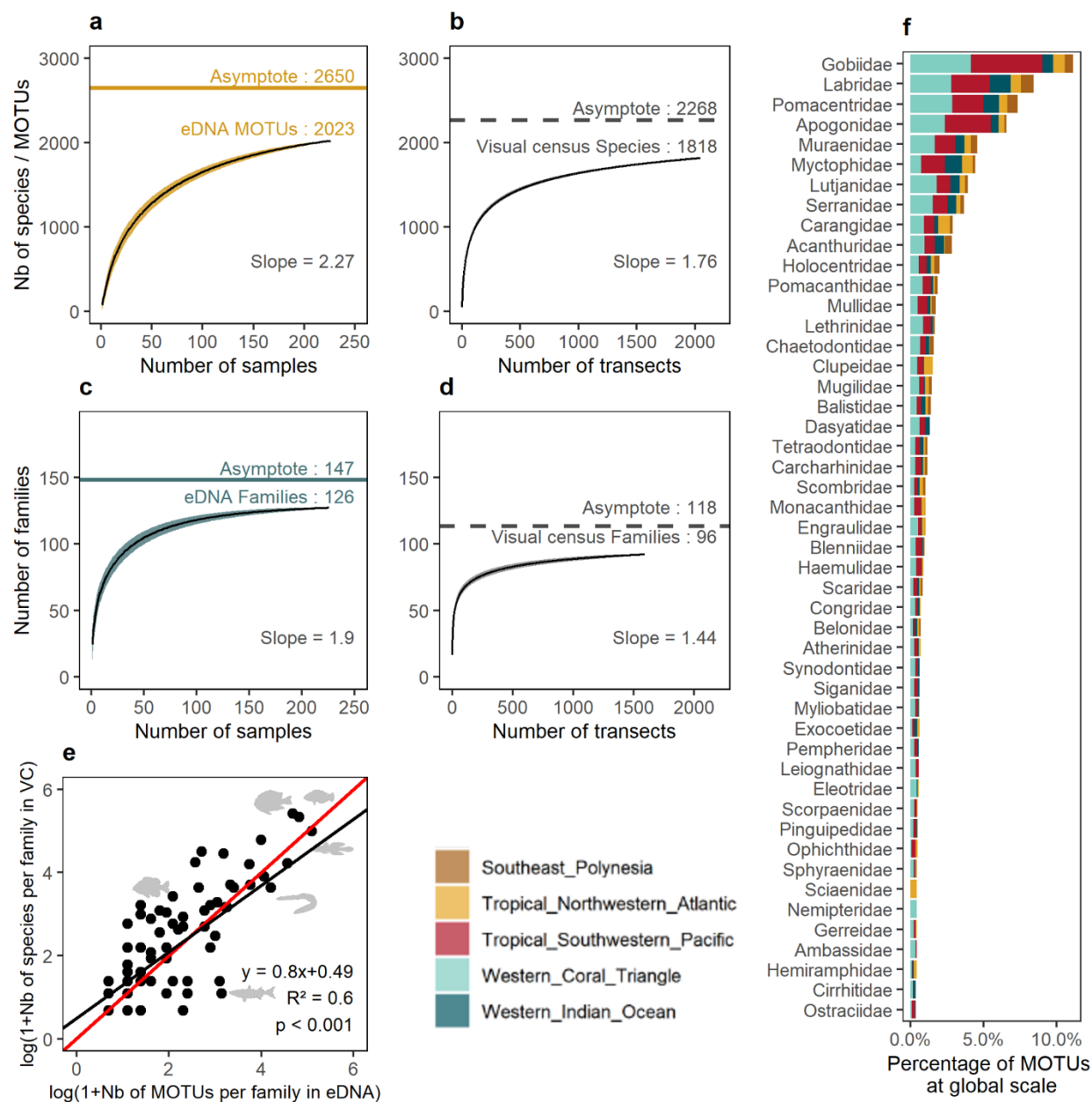
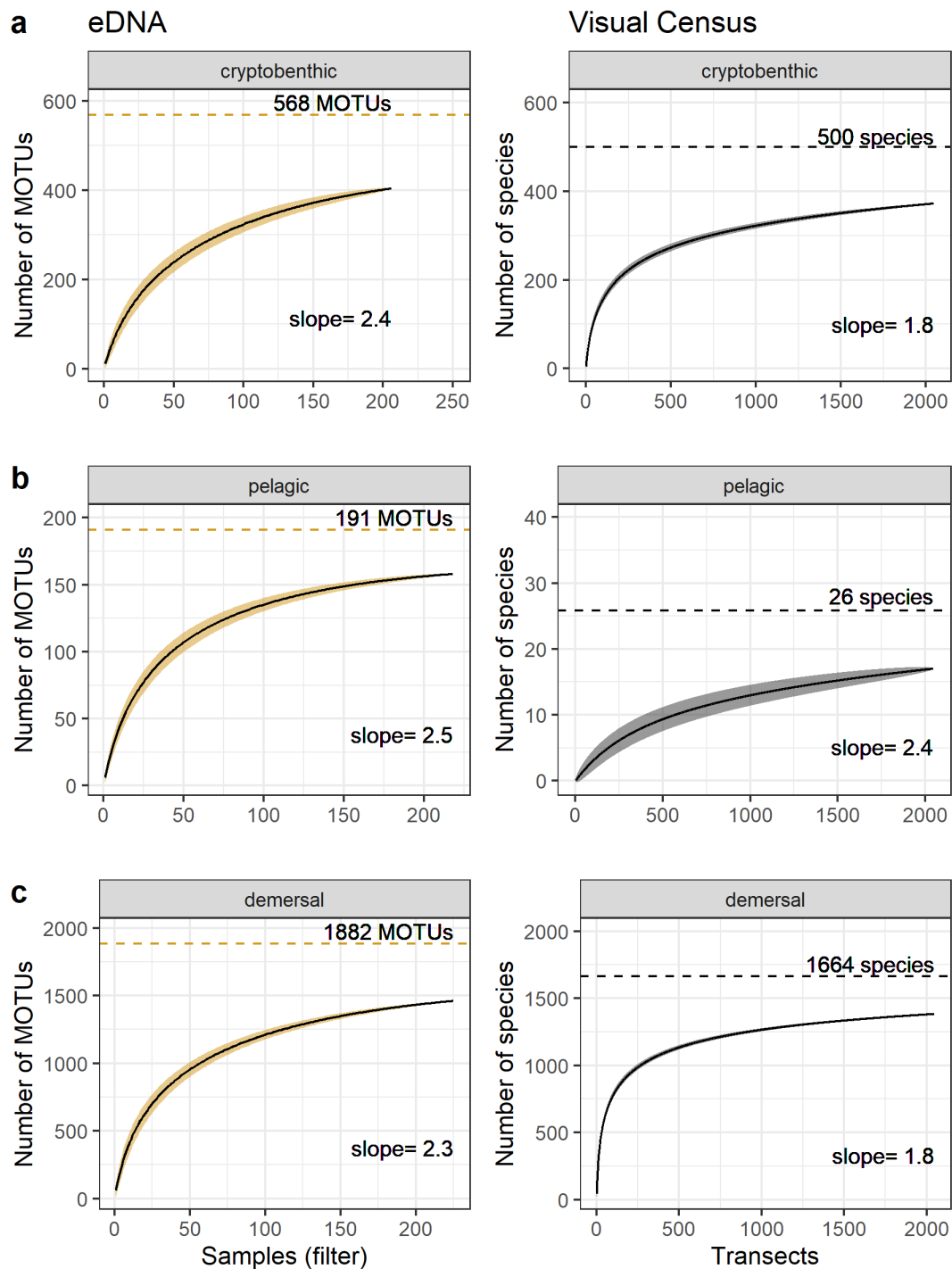


Figure 1. Estimates of overall fish richness from environmental DNA (eDNA) and visual census. (a) accumulation curve of molecular operational taxonomic units from eDNA (eDNA MOTUs), (b) accumulation curve of species from the visual census database, (c) accumulation curve of eDNA families, (d) accumulation curve of visual census families. For (a-d), Species accumulation model is fitted according to Lomolino method (see methods). (e) linear regression (black line) between the number of species per family in visual census data and the number of MOTUs per family in eDNA (log(x+1) transformation) over $n = 77$ families. Each point is a family. Red line is $x=y$. (f) percentage of MOTUs assigned to each family at global scale, and proportion in each region.



579

580 **Figure 2. Estimates of overall fish richness from eDNA and visual census across habitat**
 581 **categories.** (a) accumulation curve of crypto-benthic eDNA MOTUs (left) and visual census
 582 species (right), (b) accumulation curve of pelagic MOTUs (left) and visual census species (right),
 583 c, accumulation curve of demersal MOTUs (left) and visual census species (right). Accumulation
 584 model is fitted with a nonlinear Lomolino model (see Methods).

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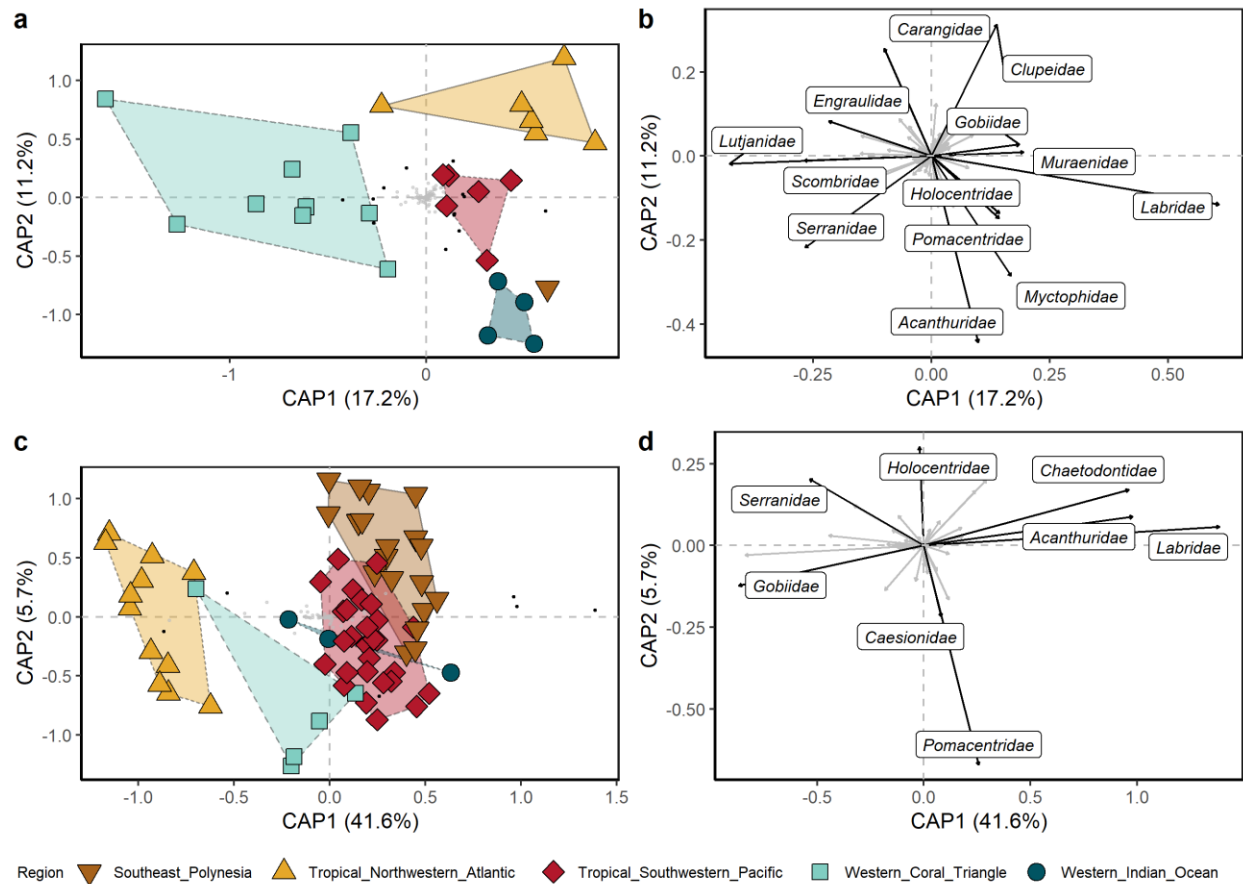


Figure 3. Partial Distance-based Redundancy analysis of MOTU proportions of each family in each site. (a) dbRDA on eDNA dataset, with 133 families in 26 sites ($R^2=0.21$, $F=3.11$, $p=0.001$), (b) families with scores > 95% of scores distribution on each axis for eDNA, (c) dbRDA on a subset of Visual Census dataset to select only the sites in the same regions as in the eDNA dataset, with 76 families in 68 sites ($R^2=0.5$, $F=15.8$, $p=0.001$), (d) families with scores > 95% of scores distribution on each axis for Visual Census. Axis labels indicate the percentage of variance explained by the 2 first dbRDA dimensions (CAP1 and CAP2).

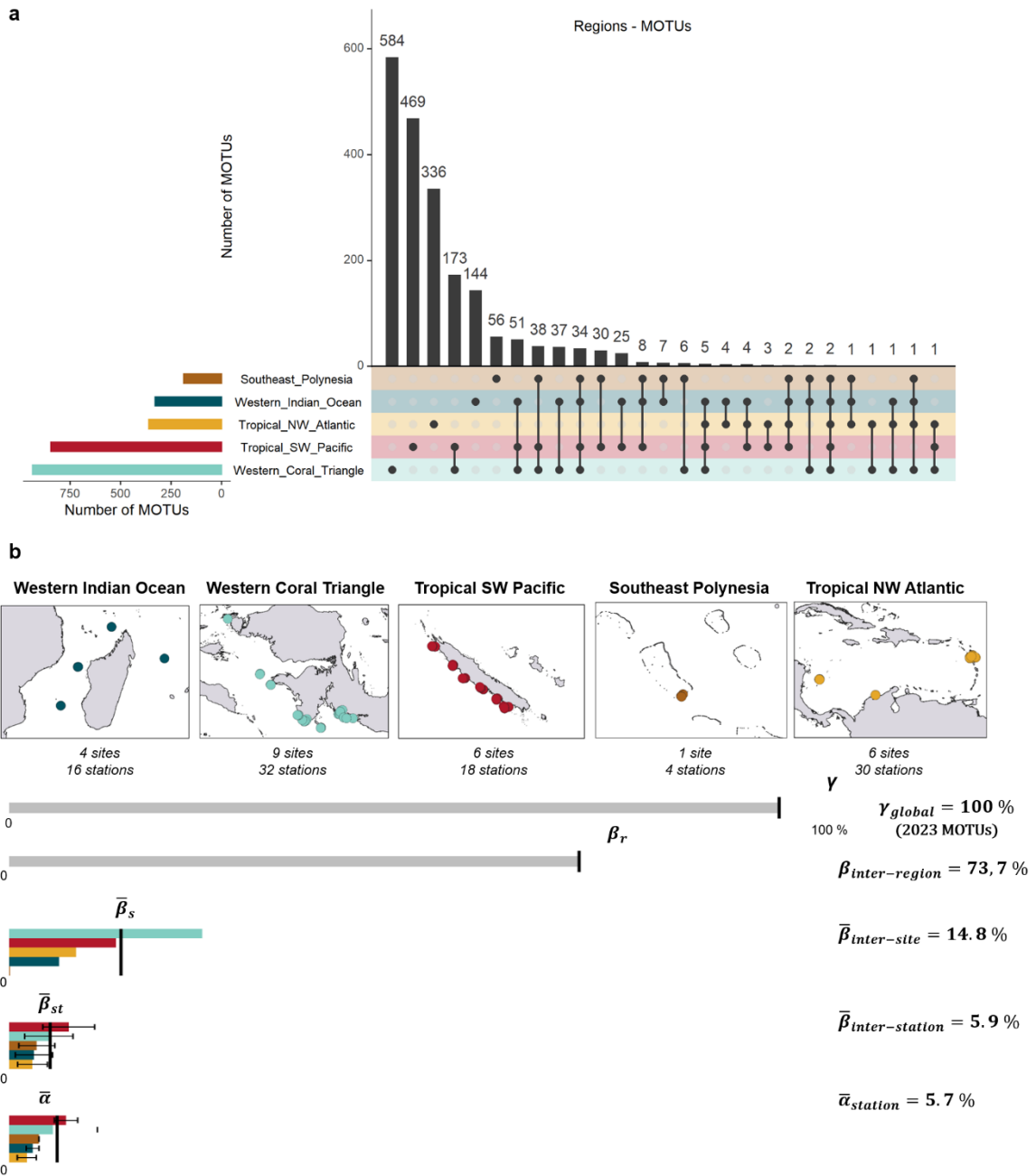


Figure 4. Hierarchical partitioning of MOTU occurrences across spatial scales. (a) Number of MOTUs found in only one region, or shared between 2, 3, 4 or all 5 regions. Histograms indicate the number of MOTUs present in all the regions identified by the dots in the lower part. (b) Global fish diversity (γ_{global}) is partitioned into $\beta_{inter-region}$ + mean $\beta_{inter-site}$ + mean $\beta_{inter-station}$ + mean $\bar{\alpha}_{station}$. Mean values at global scales are indicated with the black vertical segments. For $\beta_{inter-site}$, $\beta_{inter-station}$ and $\bar{\alpha}_{station}$, mean values are given for each region (colored bars) with the standard errors. $\beta_{inter-region}$ contributes the highest to gamma global (73.7%).