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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

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

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 1*e*). 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 1*f*, 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 ($\overline{\beta}_{inter-site}$), mean difference between stations within sites ($\overline{\beta}_{inter-station}$) and mean station diversity ($\overline{\alpha}_{station}$) (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 4*b*). 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 (3x) $\overline{\beta}_{inter-site}$ and $\overline{\beta}_{inter-station}$ (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 β = -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.

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.

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

The finding of a strong regional effect on both species composition (figure 3) and species differentiation (figure 4) at a large scale is in agreement with visual surveys and previous knowledge (45), while the suggestion of a strong turnover at the local scale may be an unexpected result for coral reef fishes. This predominant role of large-scale bioregional differentiation explains the exceptional fish diversity on coral reefs, probably associated with long-term geological 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 4*a*). 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

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

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

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 (ɣ global) into contributions at smaller scales from regions to local richness : $\gamma_{global} = \beta_{inter\text{-}region} + \text{mean } \beta_{inter\text{-}site} + \text{mean } \beta_{inter\text{-}station} + \text{mean } \bar{\alpha}_{station}$. 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 (ɣ) can be directly compared (70).

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

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References

1. Parravicini V, Kulbicki M, Bellwood DR, Friedlander AM, Arias-Gonzalez JE, Chabanet P, et al. Global patterns and predictors of tropical reef fish species richness. Ecography (Cop). 2013;36(12):1254–62.
2. Cowman PF, Bellwood DR. The historical biogeography of coral reef fishes: Global patterns of origination and dispersal. J Biogeogr. 2013;40(2):209–24.
3. Cinner JE, Zamborain-mason J, Gurney GG, Graham NAJ, Macneil MA, Hoey AS, et al. Meeting fisheries, ecosystem function, and biodiversity goals in a human-dominated world. Science (80-). 2020;368(April):307–11.
4. Hoegh-Guldberg O, Jacob D, Taylor M, Guillén Bolaños T, Bindi M, Brown S, et al. The human imperative of stabilizing global climate change at 1.5°C. Science (80-). 2019;365(6459).
5. Victor BC. How many coral reef fish species are there? Cryptic diversity and the new molecular taxonomy. In: Ecology of fishes on coral reefs Cambridge University Press, Cambridge. 2015. p. 76–88.
6. Siqueira AC, Morais RA, Bellwood DR, Cowman PF. Trophic innovations fuel reef fish diversification. Nat Commun. 2020;11(1):1–11.
7. Bellwood D, Wainwright P. The history and biogeography of fishes on coral reefs. In: Sale P, editor. Coral Reef Fishes: Dynamics and Diversity in a Complex Ecosystem. Academic P. San Diego; 2002.
8. Bellwood DR, Hughes TP. Regional-scale assembly rules and biodiversity of coral reefs. Science (80-). 2001;292(5521):1532–4.
9. Pellissier L, Leprieur F, Parravicini V, Cowman PF, Kulbicki M, Litsios G, et al. Quaternary coral reef refugia preserved fish diversity. Science (80-). 2014;344(6187):1016–20.
10. Veron J, Devantier LM, Turak E, Green AL, Kininmonth S, Stafford-Smith M, et al. Delineating the Coral Triangle. Galaxea, J Coral Reef Stud. 2009;11(2):91–100.
11. Siqueira AC, Morais RA, Bellwood DR, Cowman PF. Planktivores as trophic drivers of global coral reef fish diversity patterns. PNAS. 2021;118(9).
12. Morais RA, Bellwood DR. Pelagic Subsidies Underpin Fish Productivity on a Degraded Coral Reef. Curr Biol. 2019;29(9):1521–7.
13. Brandl SJ, Goatley CHR, Bellwood DR, Tornabene L. The hidden half : ecology and evolution of cryptobenthic fishes on coral reefs. Biol Rev. 2018;93:1846–73.
14. Bender MG, Leprieur F, Mouillot D, Kulbicki M, Parravicini V, Pie MR, et al. Isolation drives taxonomic and functional nestedness in tropical reef fish faunas. Ecography (Cop). 2017;40(3):425–35.
15. Hughes TP, Bellwood DR, Connolly SR, Cornell H V., Karlson RH. Double jeopardy and global extinction risk in corals and reef fishes. Curr Biol. 2014;24(24):2946–51.
16. Mouillot D, Villéger S, Parravicini V, Kulbicki M, Arias-González JE. Functional over-redundancy and high functional vulnerability in global fish faunas on tropical reefs. PNAS. 2014;111(38):13757–62.
17. Alzate A, Zapata FA, Giraldo A. A comparison of visual and collection-based methods for assessing community structure of coral reef fishes in the Tropical Eastern Pacific. Rev Biol Trop. 2014;62(February):359–71.
18. Bellwood D, Renema W, Rosen BB. Biodiversity hotspots, evolution and coral reef biogeography: a review. In: Biotic Evolution and Environmental Change in Southeast Asia Cambridge: Cambridge University Press. 2012. p. 216–45.
19. Mora C. Ecology of fishes on coral reefs. Ecology of Fishes on Coral Reefs. 2015. 1–374 p.
20. Taberlet P, Coissac E, Hajibabaei M, Rieseberg LH. Environmental DNA. Mol Ecol. 2012;21(8):1789–93.
21. Boulanger E, Loiseau N, Valentini A, Arnal V, Boissery P, Dejean T, et al. Environmental DNA metabarcoding reveals and unpacks a biodiversity conservation paradox in Mediterranean marine reserves, Dryad, Dataset. Proc R Soc B. 2021;288(20210112).
22. Harrison JB, Sunday JM, Rogers SM. Predicting the fate of eDNA in the environment and implications for studying biodiversity. Proc R Soc B Biol Sci. 2019;286(1915):1–9.
23. Cordier T, Alonso-Saez L, Apothéloz-Perret-Gentil L, Aylagas E, Bohan DA, Bouchez A, et al. Ecosystem monitoring powered by environmental genomics: A review of current strategies with an implementation roadmap. Mol Biol Evol. 2020;may:1–22.
24. De Vargas C, Audic S, Henry N, Decelle J, Mahé F, Logares R, et al. Eukaryotic plankton diversity in the sunlit ocean. Science (80-). 2015;348(6237):1–11.
25. Valdivia-Carrillo T, Rocha-Olivares A, Reyes-Bonilla H, Domínguez-Contreras JF, Munguia-Vega A. Integrating eDNA metabarcoding and simultaneous underwater visual surveys to describe complex fish communities in a marine biodiversity hotspot. Mol Ecol Resour. 2021;(March):1558–74.

26. West K, Travers MJ, Stat M, Harvey ES, Richards ZT, DiBattista JD, Newman SJ, Harry A, Skepper CL, Heydenrych M, Bunce M. Large-scale eDNA metabarcoding survey reveals marine biogeographic break and transitions over tropical north-western Australia. Diversity and Distributions. 2021 Oct;27(10):1942-57.
27. Kume M, Lavergne E, Ahn H, Terashima Y, Kadowaki K, Ye F, et al. Factors structuring estuarine and coastal fish communities across Japan using environmental DNA metabarcoding. Ecol Indic [Internet]. 2021;121(November 2020):107216. Available from: https://doi.org/10.1016/j.ecolind.2020.107216
28. Fraija-Fernández N, Bouquieaux MC, Rey A, Mendibil I, Cotano U, Irigoien X, et al. Marine water environmental DNA metabarcoding provides a comprehensive fish diversity assessment and reveals spatial patterns in a large oceanic area. Ecol Evol. 2020;10(14):7560–84.
29. Aglieri G, Baillie C, Mariani S, Cattano C, Calò A, Turco G, et al. Environmental DNA effectively captures functional diversity of coastal fish communities. Mol Ecol. 2020;(August):1–13.
30. DiBattista JD, Berumen ML, Priest MA, De Brauwer M, Coker DJ, Sinclair-Taylor TH, et al. Environmental DNA reveals a multi-taxa biogeographic break across the Arabian Sea and Sea of Oman. Environ DNA. 2021;(December 2020):1–16.
31. Edgar GJ, Stuart-Smith RD. Systematic global assessment of reef fish communities by the Reef Life Survey program. Sci Data. 2014;1:1–8.
32. Juhel JB, Utama RS, Marques V, Vimono IB, Sugeha HY, Kadarusman, et al. Accumulation curves of environmental DNA sequences predict coastal fish diversity in the coral triangle. Proceedings Biol Sci. 2020;287(1930):1–10.
33. Castellanos-Galindo GA, Krumme U, Rubio EA, Saint-Paul U. Spatial variability of mangrove fish assemblage composition in the tropical eastern Pacific Ocean. Rev Fish Biol Fish. 2013;23(1):69–86.
34. Willis TJ, Anderson MJ. Structure of cryptic reef fish assemblages: Relationships with habitat characteristics and predator density. Mar Ecol Prog Ser. 2003;257:209–21.
35. Harnik PG, Simpson C, Payne JL. Long-term differences in extinction risk among the seven forms of rarity. Proc R Soc B Biol Sci. 2012;279(1749):4969–76.
36. Crist TO, Veech JA. Additive partitioning of rarefaction curves and species-area relationships: Unifying α-, β- and γ-diversity with sample size and habitat area. Ecol Lett. 2006;9(8):923–32.
37. Enquist BJ, Feng X, Boyle B, Maitner B, Newman EA, Jørgensen PM, et al. The commonness of rarity: Global and future distribution of rarity across land plants. Sci Adv. 2019;5(11):1–14.
38. Dornelas M, Connolly SR, Hughes TP. Coral reef diversity refutes the neutral theory of biodiversity. Nature. 2006;440(7080):80–2.
39. Valentini A, Taberlet P, Miaud C, Civade R, Herder J, Thomsen PF, et al. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. Mol Ecol. 2016;25(4):929–42.
40. West K, Travers MJ, Stat M, Harvey ES, Richards ZT, Dibattista JD, et al. Large-scale eDNA metabarcoding survey reveals marine biogeographic break and transitions over tropical north- western Australia. Divers Distrib. 2021;(00):1–16.
41. Boussarie G, Kiszka JJ, Mouillot D, Bonnin L, Manel S, Kulbicki M, et al. Environmental DNA illuminates the dark diversity of sharks. Sci Adv. 2018;4(5):1–8.
42. Brandl SJ, Tornabene L, Goatley CHR, Casey JM, Morais RA, Côté IM, et al. Demographic dynamics of the smallest marine vertebrates fuel coral-reef ecosystem functioning. Science (80-). 2019;(May):799–802.
43. Kimmerling N, Zuqert O, Amitai G, Gurevich T, Armoza-Zvuloni R, Kolesnikov I, et al. Quantitative species-level ecology of reef fish larvae via metabarcoding. Nat Ecol Evol. 2018;2(2):306–16.
44. Beckley LE, Holliday D, Sutton AL, Weller E, Olivar MP, Thompson PA. Structuring of larval fish assemblages along a coastal-oceanic gradient in the macro-tidal, tropical Eastern Indian Ocean. Deep Res Part II. 2019;161(March 2018):105–19.
45. McLean M, Stuart-Smith RD, Villéger S, Auber A, Edgar GJ, MacNeil MA, et al. Trait similarity in reef fish faunas across the world's oceans. Proc Natl Acad Sci. 2021;118(12):e2012318118.
46. Gaboriau T, Leprieur F, Mouillot D, Hubert N. Influence of the geography of speciation on current patterns of coral reef fish biodiversity across the Indo-Pacific. Ecography (Cop). 2018;41(8):1295–306.
47. Ahmadia GN, Tornabene L, Smith DJ, Pezold FL. The relative importance of regional, local, and evolutionary factors structuring cryptobenthic coral-reef assemblages. Coral Reefs. 2018;37(1):279–93.
48. Coker DJ, DiBattista JD, Sinclair-Taylor TH, Berumen ML. Spatial patterns of cryptobenthic coral-reef fishes in the Red Sea. Coral Reefs. 2018;37(1):193–9.
49. Goatley CHR, González-Cabello A, Bellwood DR. Reef-scale partitioning of cryptobenthic fish assemblages across the Great Barrier Reef, Australia. Mar Ecol Prog Ser. 2016;544(February):271–80.
50. Wang S, Loreau M. Biodiversity and ecosystem stability across scales in metacommunities. Ecol Lett. 2016;19:510–8.

51. Benkwitt C, Wilson S, Graham NAJ. Biodiversity increases ecosystem functions despite multiple stressors on coral reefs. Nat Ecol Evol. 2020;4:916–26.
52. Marques V, Milhau T, Albouy C, Dejean T, Manel S, Mouillot D, et al. GAPeDNA : Assessing and mapping global species gaps in genetic databases for eDNA metabarcoding. 2020;(April):1–13.
53. Ruppert KM, Kline RJ, Rahman MS. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. Glob Ecol Conserv. 2019;17:e00547.
54. Polanco A, Richards FE, Flück B, Valentini A, Altermatt F, Jean- SB, et al. Comparing the performance of 12S mitochondrial primers for fish environmental DNA across ecosystems. Environ DNA. 2021;(June):1–15.
55. Brandt MI, Trouche B, Quintric L, Günther B, Wincker P, Poulain J, et al. Bioinformatic pipelines combining denoising and clustering tools allow for more comprehensive prokaryotic and eukaryotic metabarcoding. Mol Ecol Resour. 2021;21(6):1904–21.
56. Marques V, Guérin PÉ, Rocle M, Valentini A, Manel S, Mouillot D, et al. Blind assessment of vertebrate taxonomic diversity across spatial scales by clustering environmental DNA metabarcoding sequences. Ecography (Cop). 2020;43:1–12.
57. Sales NG, Wangensteen OS, Carvalho DC, Deiner K, Praebel I, McDevitt A, et al. Space-time dynamics in monitoring neotropical fish communities using eDNA metabarcoding. bioRxiv. 2020;1–38.
58. Stauffer S, Jucker M, Keggin T, Marques V, Andrello M, Bessudo S, et al. How many replicates to accurately estimate fish biodiversity using environmental DNA on coral reefs ? Authors : bioRxiv. 2021;
59. Collins RA, Wangensteen OS, O'Gorman EJ, Mariani S, Sims DW, Genner MJ. Persistence of environmental DNA in marine systems. Commun Biol. 2018;1(185):1–12.
60. Collins RA, Bakker J, Wangensteen OS, Soto AZ, Corrigan L, Sims DW, et al. Non-specific amplification compromises environmental DNA metabarcoding with COI. Methods Ecol Evol. 2019;10(11):1985–2001.
61. Zhang S, Zhao J, Yao M. A comprehensive and comparative evaluation of primers for metabarcoding eDNA from fish. Methods Ecol Evol. 2020;2020(January):1609–25.
62. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: a versatile open source tool for metagenomics. PeerJ. 2016;4:1–22.
63. Martin. M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal. 1994;17(1):10–2.
64. Mahé F, Rognes T, Quince C, de Vargas C, Dunthorn M. Swarm v2: highly-scalable and high-resolution amplicon clustering. PeerJ. 2015;3:1–12.
65. Boyer F, Mercier C, Bonin A, Le Bras Y, Taberlet P, Coissac E. obitools: A unix-inspired software package for DNA metabarcoding. Mol Ecol Resour. 2016;16(1):176–82.
66. Polanco Fernández A, Marques V, Fopp F, Juhel J, Borrero-Pérez GH, Cheutin M, et al. Comparing environmental DNA metabarcoding and underwater visual census to monitor tropical reef fishes. Environ DNA. 2020 Oct 2;1–15.
67. Spalding MD, Fox HE, Allen GR, Davidson N, Ferdaña ZA, Finlayson M, et al. Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas. Bioscience. 2007;57(7):573–83.
68. Escalas A, Troussellier M, Yuan T, Bouvier T, Bouvier C, Mouchet MA, et al. Functional diversity and redundancy across fish gut, sediment and water bacterial communities. Environ Microbiol. 2017;19(8):3268–82.
69. Whittaker RH. Evolution and measurement of species diversity. Taxon. 1972;21:213–51.
70. Veech JA, Summerville KS, Crist TO, Gering JC. The additive partitioning of species diversity: Recent revival of an old idea. Oikos. 2002;99(1):3–9.

Figures

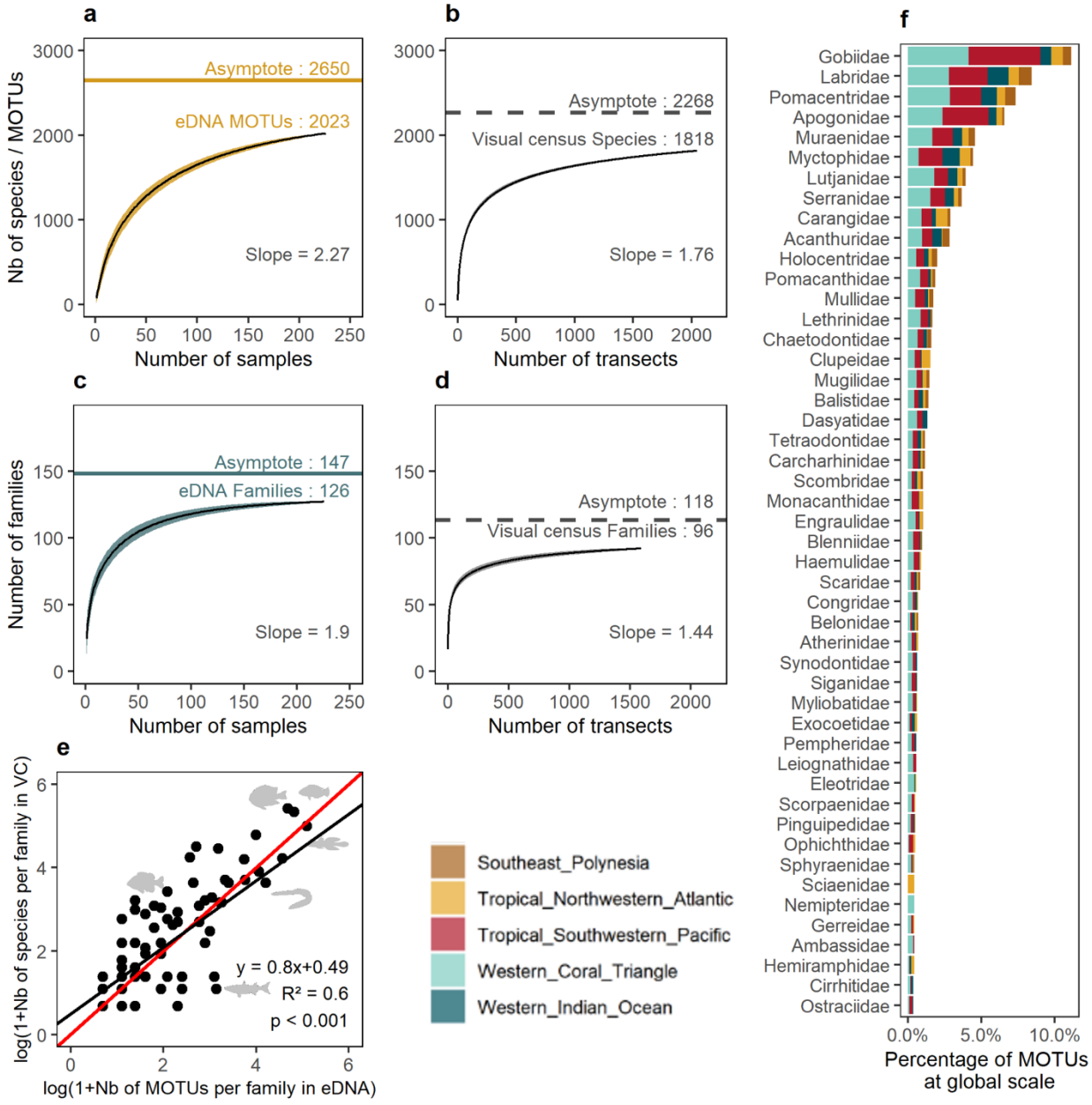
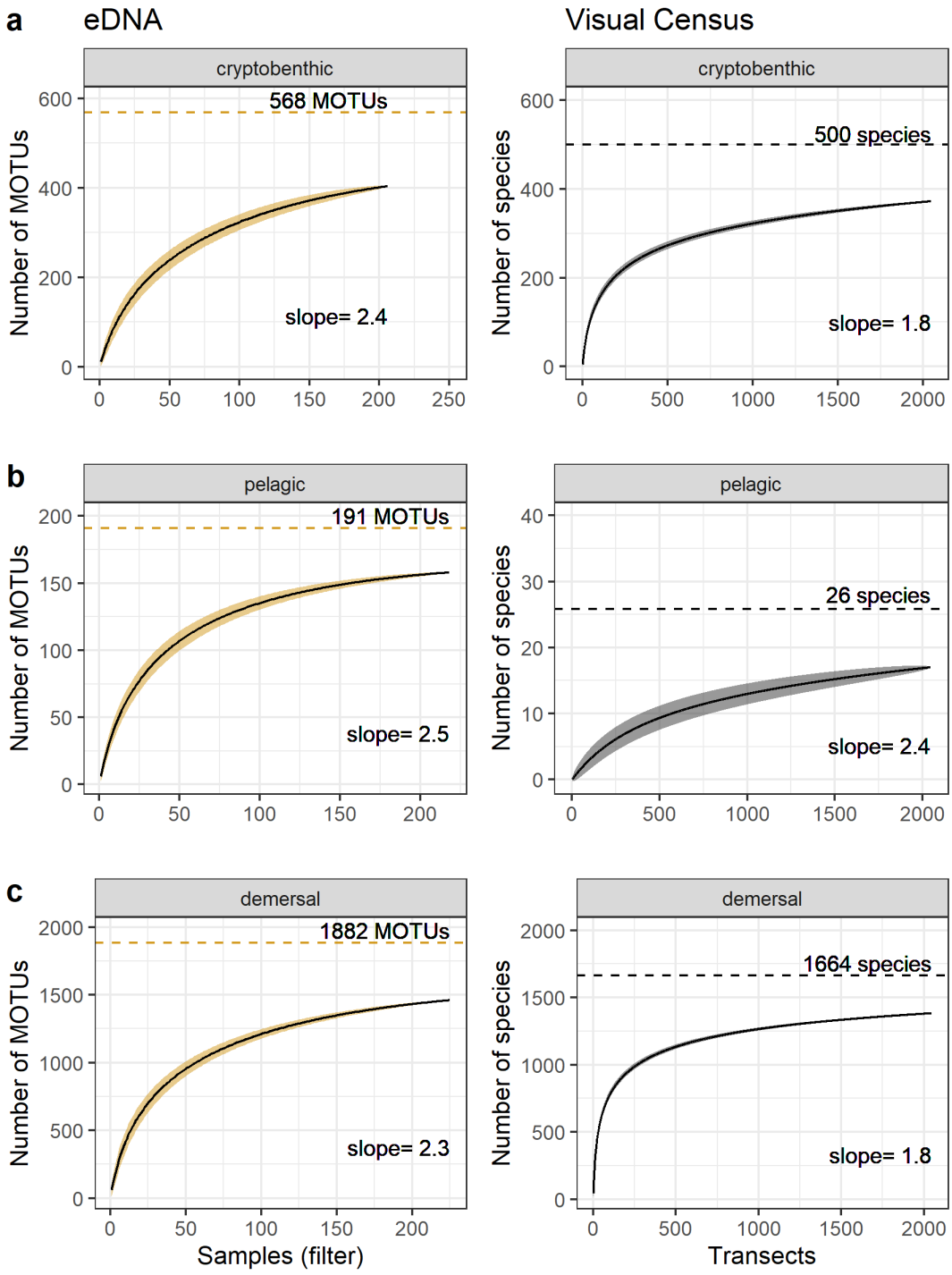


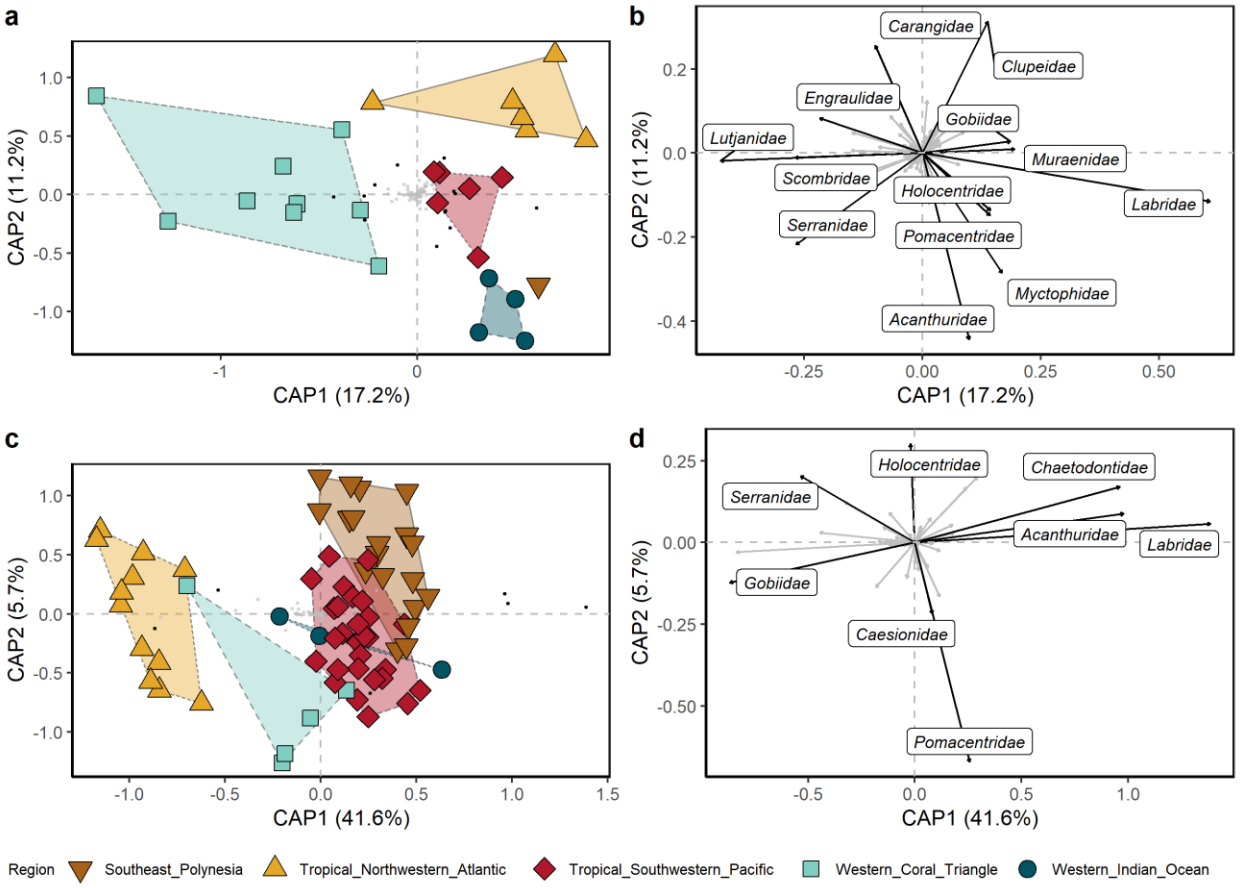
Figure 1. Estimates of overall fish richness from environmental DNA (eDNA) and visual census. (*a*) accumulation curve of molecular operational taxonomic units from eDNA (eDNA MOTUs), (*b*) accumulation curve of species from the visual census database, (*c*) accumulation curve of eDNA families, (*d*) 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).

585



586

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

594

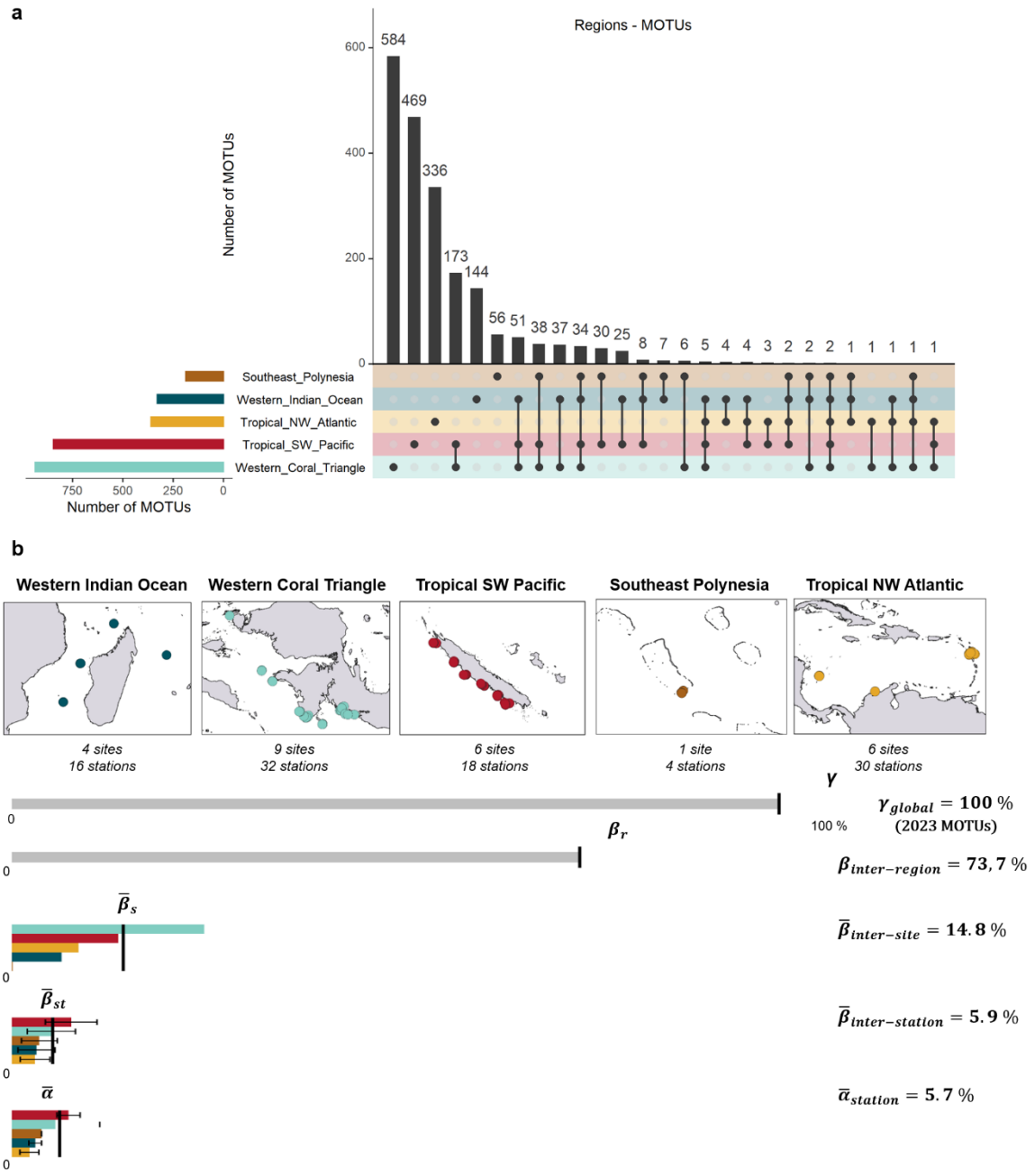


Figure 4. Hierarchical partitioning of MOTU occurrences across spatial scales. (*a*) Number of MOTUs found in only one region, or shared between 2, 3, 4 or all 5 regions. Histograms indicate the number of MOTUs present in all the regions identified by the dots in the lower part. (*b*) Global fish diversity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