**Supplementary material S2: Methodology details**

**Database:**

The NNF records comprise a taxonomic organization that goes up to the lowest possible taxonomic level for each record, i.e. considering the best possible taxonomic identity, geographical coordinates, date, information source type and record authorship. Information regarding possible source region and/or native range of each NNF species was subsequently added when available. Additional NNF occurrence records were compiled from the Global Biodiversity Information Facility (GBIF [www.gbif.org](http://www.gbif.org/)) and the Species Link Network, (SpeciesLink, [www.splink.org.br](http://www.splink.org.br/)). Information in the present study was compared to data from AmazonFish (Jézéquel et al., 2020), which also encompasses data from GBIF. Furthermore, a covariance analysis (ANCOVA) was done to check if temporal trends in NNF species presence and number of occurrence records compiled by this study could represent the historical increase in sampling effort in the different sub-basins of the Amazon. . In this analysis we considered NNF records (i.e. number of individuals per species) per year and compared them to the sampling effort in the Amazon Region (number of samplings obtained from the AmazonFish database). The ANCOVA was carried out comparing years (i.e. year by year, in five and in ten-years periods of time), comparing basins (i.e. Basin A and Basin B) and considering the overall total (i.e. overall sampling). Scientific nomenclature was checked using the Eschmeyer's Catalog of Fishes (Fricke et al., 2021) and the *Fishbase* package in R software (version 4.0.2).

Occurrence records from watersheds adjacent to the Amazon Basin macroregion (see Venticinque et al., 2016 for basins delimitation) were also included to identify possible current and historical invasion sources and propagules pressure.

**Spatial and Temporal representation:**

The graphical representation of the spatial distribution of records of selected NNF species was performed in ArcGIS (v 10.4) using shapefiles provided by Venticinque et al. (2016) (fig 1). The spatial representation shows the point density (blue to red color scale) of occurrence records per basin and the isolated points (gray) in its adjacent area. This representation for the isolated points was chosen due to proximity of several NNF that can represent a threat due to propagule pressure.

For sub-basins representations the level 2 from Venticinque et al. (2016) was used. We also plotted in chropletic scales the NNF species richness (absolute values) for each sub-basin and the NNF species richness per sub-basin area (proportional values). The NNF richness per country was also represented in order to illustrate the data in the geopolitical divisions.

The temporal representations were presented in cumulative graphs for NNF occurrence records and species richness (Vitule et al., 2021), for each sub-basin (fig 2) and country (fig 3) using *ggplot2* package (Wickham, 2016) in R version 4.0.3 (R Core Team 2020).

**References**

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