

# Low mitochondrial genetic diversity of *Engraulis ringens* compared to its congeners from coastal upwelling systems



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## Introduction

The Southeastern Pacific Ocean is characterized by highly variable environmental conditions, exhibiting oceanic features that challenge its marine inhabitants. Fluctuations in the abundance of the Peruvian anchovy (*Engraulis ringens*), in response to both natural and anthropogenic pressures, suggest that the species possesses a high capacity for adaptation. By exploring how genetic variation is distributed within and among related taxa across space and time (i.e., phylogeography), we can gain insights into evolutionary history and infer some of the factors driving genetic diversity. Although mitochondrial DNA has limitations in reconstructing the complete history of a species, it provides a preliminary view of global or regional patterns, drawing on public databases enriched with mitochondrial DNA sequences collected over several years in different regions around the world.

### OBJECTIVE:

This study employs a phylogeographic approach using mitochondrial DNA (mtDNA), to compare the genetic diversity of *E. ringens* with related taxa across global upwelling ecosystems. By leveraging large-scale public datasets, we interpret these genetic patterns in light of species-specific demographic histories and habitat characteristics.

## Material and methods

### Sampling

Individuals were collected from the northern, central, and southern Peru (Fig. 1), from 2014 to 2016. Sampling was performed using the scientific purse seine operations conducted by IMARPE. Additionally, ten specimens of Argentine anchovy (*Engraulis anchoita*) were analyzed.

### DNA, PCR & Sequencing

Total DNA were extracted from muscle tissues. The 5' mtDNA control region (CR) and complete Cytochrome b gene (Cytb) were amplified. A total of 236 individuals were sequenced for Cytb (913 bp), and 445 for CR (420 bp).

### Data analysis

#### Genealogy

Haplotype networks were obtained using the Statistical Parsimony method (TCS) implemented in PopArt. Published DNA sequences of five nominal *Engraulis* species were included to evaluate genealogies: *E. encrasicolus*, *E. japonicus*, and *E. australis* from the Old World lineage; and *E. mordax*, and *E. anchoita* from the New World lineage.

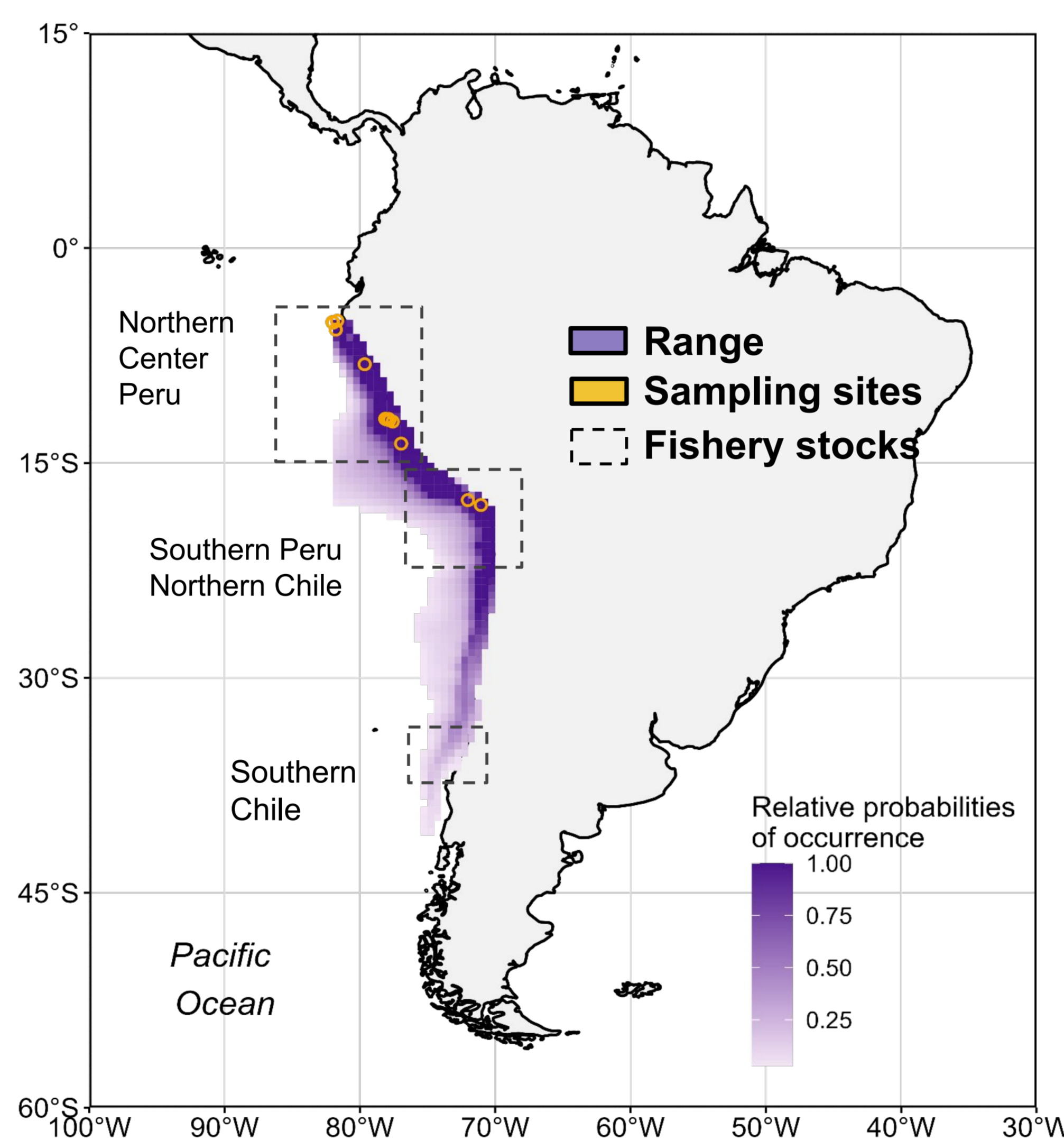
#### Genetic diversity

Haplotype diversity (Hd) and nucleotide diversity ( $\pi$ ) were calculated with DnaSP<sup>2</sup>. Genetic diversity was compared with published values of eight nominal species of the genus. A total of 13 studies were consulted to compile the genetic diversity indexes.

#### Neutrality Test

Tajima's D test was performed using DnaSP<sup>2</sup> and statistical significance was assessed using the beta approximation<sup>3</sup>.

**Figure 1.** Peruvian anchovy distribution range from AquaMaps<sup>1</sup>, showing modeled occurrence probability. Sampling sites are shown in yellow. Fishery stocks are depicted schematically as approximate reference areas.



## Results

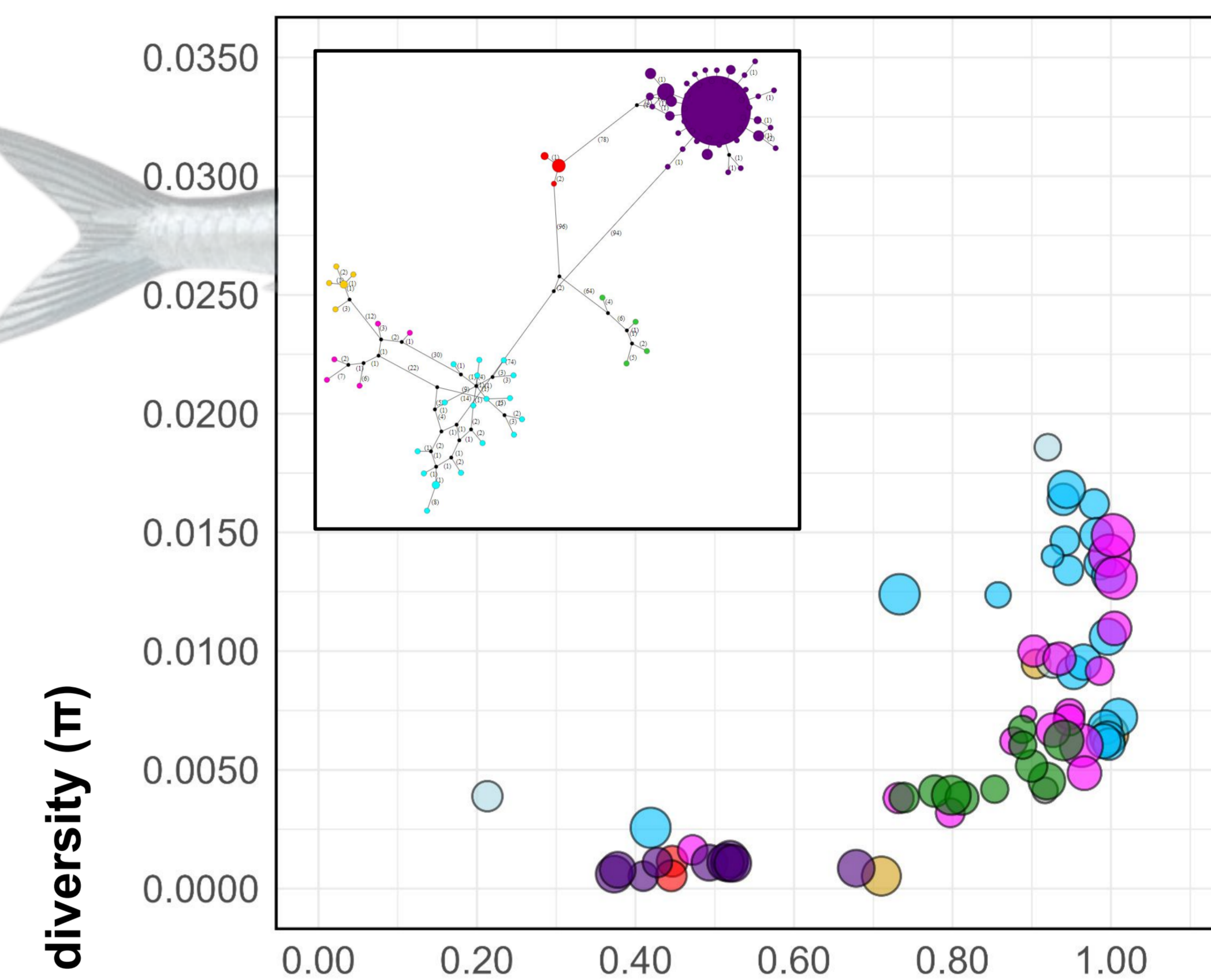
### A

#### Species

- *E. anchoita*
- *E. australis*
- *E. capensis*
- *E. encrasicolus*
- *E. eurystole*
- *E. japonicus*
- *E. mordax*
- *E. ringens*

#### Sample size

- 10
- 20
- 30
- 40



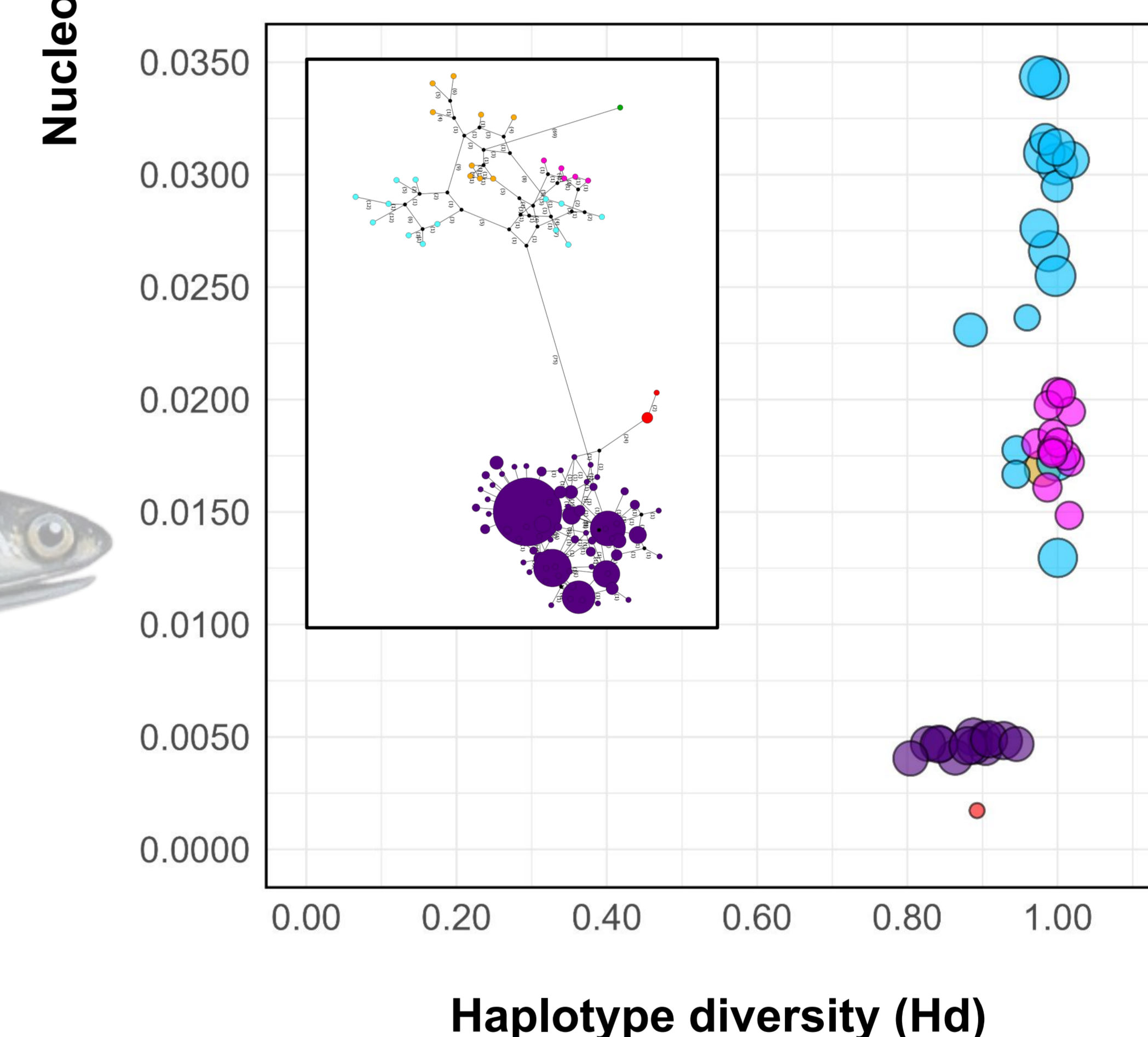
### B

#### Species

- *E. anchoita*
- *E. australis*
- *E. encrasicolus*
- *E. japonicus*
- *E. ringens*

#### Sample size

- 10
- 20
- 30
- 40
- 50



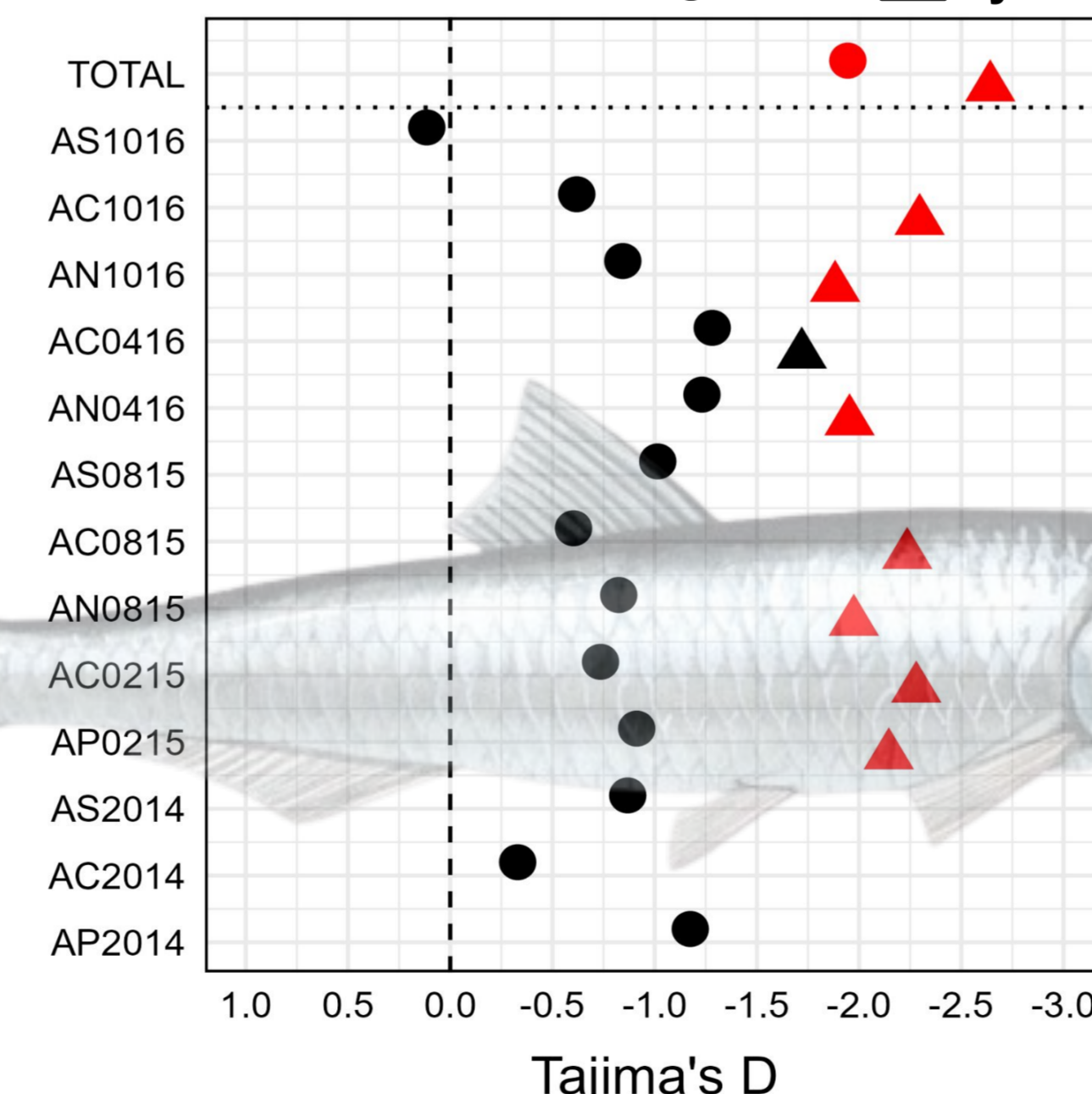
**Figure 2.** Scatter plots of haplotype and nucleotide diversity of *Engraulis* species, based on Cytb (A) and CR (B). Statistical parsimony networks are displayed within each plot.

## Results and discussion

### Cytb showed signals of non-neutral processes

Tajima's D contrasts two estimators of genetic diversity, nucleotide diversity ( $\pi$ ) and the number of segregating sites, which are expected to be approximately equal ( $D \approx 0$ ) under conditions of neutrality and demographic equilibrium. Significant negative values of D indicate an excess of low-frequency variants, a pattern consistent with either recent population expansion or purifying selection<sup>3</sup>. In the case of population expansion, these rare variants arise from recent mutations in a rapidly growing population; under purifying selection, they correspond to deleterious alleles maintained at low frequencies by selection. Cytb consistently showed negative D values in *E. ringens*, both per sample and Total (Fig. 3). In contrast, CR showed no significant deviation from 0, except when all sequences were analyzed as Total. Assuming stable demography, this result suggests the action of selection on Cytb, which may display a stronger signal than CR due to its functional constraints. However, because few-loci analyses often confound selection with demography, this pattern may instead reflect historical population shifts. Disentangling demography from selection requires further study, as the population dynamics of Peruvian anchovy are characterized by short-term demographic fluctuations, while its large population size and highly variable habitat provide fertile ground for an effective role of selection.

Genetic marker: ○ CR △ Cytb



**Figure 3.** Tajima's D statistic for each *E. ringens* sample and overall, calculated from Cytb and CR datasets. Red symbols indicate significance deviation from neutral expectation ( $P < 0.05$ ).

### South American species showed lower mtDNA diversity than Old World lineages

Cytb (Fig. 2A) indicates that the Peruvian anchovy exhibits markedly lower genetic diversity, with consistently reduced Hd and  $\pi$  values; this contrast is strongest relative to *E. japonicus* and *E. encrasicolus*, and less pronounced, though still evident, in comparison with its Northeast Pacific congener *E. mordax*. The control region (Fig. 1B) supports the same pattern: *E. ringens* shows comparable Hd but lower  $\pi$  relative to Old World species, consistent with the higher polymorphism expected in a non-coding region compared with Cytb. The Brazilian–Argentine anchovy, *E. anchoita*, displays a similarly low-diversity pattern to *E. ringens*; however, limited sampling for this species warrants caution in interpretation. Genealogies reveal shallow coalescence within South American species (haplotypes separated by only 1–2 mutations) despite deep interspecific divergence, whereas Old World species exhibit deeper genealogies, with more mutational steps among haplotypes within species but lower interspecific divergence. Notably, Asian and European anchovies show a broader range of nucleotide diversity than *E. ringens* and *E. mordax*, reflecting greater spatial heterogeneity in nucleotide diversity across their geographic ranges.

### References

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