Molecular Phylogenetics and Evolution 124 (2018) 151–161

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Phylogenetic analysis of trophic niche evolution reveals a latitudinal herbivory gradient in Clupeoidei (herrings, anchovies, and allies)

Joshua P. Egan[a,b,*], Devin D. Bloom[c,d], Chien-Hsien Kuo[e], Michael P. Hammer[f], Prasert Tongnunui[g], Samuel P. Iglésias[h], Marcus Sheaves[i], Chaiwut Grudpan[k], Andrew M. Simons[b,l]

[a] Conservation Sciences Graduate Program, University of Minnesota, 135B Skok Hall, 2003 Upper Buford Circle, St. Paul, MN 55108, USA
[b] Bell Museum of Natural History, University of Minnesota, 100 Ecology, 1987 Upper Buford Circle, St. Paul, MN 55108, USA
[c] Department of Biological Sciences, Western Michigan University, Kalamazoo, MI, USA
[d] Institute of the Environment and Sustainability, Western Michigan University, Kalamazoo, MI, USA
[e] National Chiayi University, Department of Aquatic Biosciences, No. 300, University Road, Chiayi City, Taiwan, Republic of China
[f] Museum and Art Gallery of the Northern Territory, GPO Box 4646, Darwin, NT 0801, Australia
[g] Faculty of Science and Fisheries Technology, Rajamangala Institute of Technology, Sikao, Trang 92150, Thailand
[h] Institut Systématique Evolution Biodiversité (ISYEB), Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, Station Marine de Concarneau, Place de la Croix, 29900 Concarneau, France
[i] College of Science and Engineering, James Cook University, Townsville, Qld 4811, Australia
[k] Department of Fisheries, Faculty of Agriculture, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand
[l] Department of Fisheries, Wildlife and Conservation Biology, University of Minnesota, 135B Skok Hall, 2003 Upper Buford Circle, St. Paul, MN 55108, USA

ARTICLE INFO

Keywords:
Trophic niche
Diet transition
Herbivory
Clupeoidei
Phylogenetics
Latitudinal gradient

ABSTRACT

Biotic and abiotic forces govern the evolution of trophic niches, which profoundly impact ecological and evolutionary processes and aspects of species biology. Herbivory is a particularly interesting trophic niche because there are theorized trade-offs associated with diets comprised of low quality food that might prevent the evolution of herbivory in certain environments. Herbivory has also been identified as a potential evolutionary "dead-end" that hinders subsequent trophic diversification. For this study we investigated trophic niche evolution in Clupeoidei (anchovies, sardines, herrings, and their relatives) and tested the hypotheses that herbivory is negatively correlated with salinity and latitude using a novel, time-calibrated molecular phylogeny, trophic guilds delimited using diet data and cluster analysis, and standard and phylogenetically-informed statistical methods. We identified eight clupeoid trophic guilds: molluscivore, terrestrial invertivore, phytoplanktivore, macroalgivore, detritivore, piscivore, crustacivore, and zooplanktivore. Standard statistical methods found a significant negative correlation between latitude and the proportion of herbivorous clupeoids (herbivorous clupeoid species/total clupeoid species), but no significant difference in the proportion of herbivorous clupeoids between freshwater and marine environments. Phylogenetic least squares regression did not identify significant negative correlations between latitude and herbivory or salinity and herbivory. In clupeoids there were five evolutionary transitions from non-herbivore to herbivore guilds and no transitions from herbivore to non-herbivore guilds. There were no transitions to zooplanktivore, the most common guild, but it gave rise to all trophic guilds, except algivore, at least once. Transitions to herbivory comprised a significantly greater proportion of diet transitions in tropical and subtropical ($< 35°$) relative to temperate areas ($> 35°$). Our findings suggest cold temperatures may constrain the evolution of herbivory and that herbivory might act as an evolutionary "dead-end" that hinders subsequent trophic diversification, while zooplanktivory acts as an evolutionary "cradle" that facilitates trophic diversification.

1. Introduction

Trophic niche evolution can profoundly impact ecological and evolutionary processes, including phenotypic evolution, speciation, and community assembly (Kalko et al., 1998; Duda and Palumbi, 2004; Day et al., 2011; Pekár et al., 2011; Davis et al., 2012; Chubaty et al., 2014;

---

* Corresponding author at: Conservation Sciences Graduate Program, University of Minnesota, 135B Skok Hall, 2003 Upper Buford Circle, St. Paul, MN 55108, USA.
E-mail address: eganx149@umn.edu (J.P. Egan).

https://doi.org/10.1016/j.ympev.2018.03.011
Received 15 November 2017; Received in revised form 8 March 2018; Accepted 8 March 2018

Goldman-Huertas et al., 2015; Burin et al., 2016). Understanding biotic and abiotic forces that govern the evolution of trophic niches offers critical insight into biogeographic patterns (Futuyma and Moreno, 1988; Floeter et al., 2005; Slatyer et al., 2013; Brown, 2014). Herbivory is a particularly interesting trophic niche because there are theorized trade-offs associated with diets containing large quantities of low quality food (little energy per unit mass) and it has been identified as a potential evolutionary "dead-end" that hinders subsequent trophic diversification (Gaines and Lubchenco, 1982; Floeter et al., 2005; Lobato et al., 2014; Burin et al., 2016; Sanchez and Trexler, 2016). If trade-offs restrict the evolution of herbivory in certain environments and herbivory constrains trophic diversification, there may be predictable geographic patterns of herbivory and trophic evolution (Floeter et al., 2005, 2004; González-Bergonzoni et al., 2012; Chubaty et al., 2014; Sanchez and Trexler, 2016).

There are trade-offs associated with herbivory. To meet metabolic demands, herbivores may spend more time foraging, have reduced activity levels, slower digestion, and higher energy allocation to digestive tissues, relative to species consuming primarily high quality prey (Ralston and Horn, 1986; Elliott and Bellwood, 2003; Floeter et al., 2005; Sanchez and Trexler, 2016). Proposed advantages of herbivory include increased prey encounter rates, little energy required to capture prey, and utilization of suboptimal habitats (Floeter et al., 2005). Herbivorous fishes are abundant in many marine and freshwater aquatic communities (Nakamura et al., 2002; Ibañez et al., 2009; González-Bergonzoni et al., 2012; Hundt et al., 2014; Egan et al., 2017) and consume low quality prey such as detritus, algae, macrophytes, and phytoplankton (Wilson et al., 2003; Heck et al., 2008; Hundt et al., 2014).

Abiotic environmental gradients might determine geographic patterns of herbivory. High salinity and cold temperature may decrease the probability of herbivory arising in fishes by preventing them from obtaining enough energy to meet metabolic demands (Gaines and Lubchenco, 1982; Floeter et al., 2005; González-Bergonzoni et al., 2012). Cold temperatures slow production of detritus and algae and decrease digestion rates, which may limit the evolution of herbivory (Gaines and Lubchenco, 1982; Floeter et al., 2005; Behrens and Lafferty, 2007; Clements et al., 2009; González-Bergonzoni et al., 2012). The influential "digestion constraint" hypothesis (Gaines and Lubchenco, 1982) states that in ectotherms energy requirements are difficult to meet at low temperatures when low quality materials comprise a substantial portion of the diet because digestion rate decreases more quickly than metabolic rate with declining temperature (Brett and Higgs, 1970; Horn and Gibson, 1990; Floeter et al., 2005). There may be low availability of detrital, algal, and plant matter in marine relative to freshwater habitats (Winemiller and Lesli, 1992), a scenario that predicts a negative relationship between salinity and herbivory. Previous studies found negative correlations between herbivory and salinity and herbivory and latitude in fishes, supporting the existence of environmental constraints on herbivory, although herbivores are present in both marine and temperate areas (Floeter et al., 2005; González-Bergonzoni et al., 2012).

Evolutionary transition rates between trophic niches are variable and different trophic niches can have distinct consequences for subsequent ecological diversification (Price et al., 2012; Burin et al., 2016). Some trophic niches may act as "cradles" of diversity from which different trophic niches frequently evolve while others may act as evolutionary "dead-ends" that, once evolved, rarely give rise to additional trophic diversity (Dennis et al., 2001; Price et al., 2012; Lobato et al., 2014; Santini et al., 2015; Burin et al., 2016). Studies describing the evolution of diet in bony fishes find that herbivory may be an evolutionary dead-end because there are frequent transitions to herbivory, but infrequent transitions from herbivory to other diets (Davis et al., 2012; Price et al., 2012; Lobato et al., 2014; Santini et al., 2015; Burin et al., 2016; Lavoué et al., 2017a). Only a handful of studies have investigated the consequences of herbivory for diversification, and few studies have focused on actinopterygian fishes (Lobato et al., 2014).

For this study we investigated trophic niche evolution in Clupeoidei (anchovies, sardines, herrings, and their relatives). Clupeoidei contains over 30 herbivorous species and freshwater, marine, temperate, and tropical species (Whitehead et al., 1988; Lavoué et al., 2013; Bloom and Lovejoy, 2014). Recent studies have identified strongly supported lineages within Clupeodei, but failed to resolve relationships among these lineages, in part because they used a small number of loci and relied heavily on mitochondrial DNA (Bloom and Lovejoy, 2012; Lavoué et al., 2013, 2017b,c; Bloom and Lovejoy, 2014). The most comprehensive phylogenetic hypothesis for Clupeoidei contains 153 of approximately 400 clupeoid species (Bloom and Lovejoy, 2014). This phylogeny contains robust sampling of South American taxa, but poor sampling of several trophically diverse Indo-Pacific lineages. For example, the herring genus *Herklotsichthys* (12 species) is entirely missing and the diverse anchovy genera *Stolephorus* (20 species) and *Thryssa* (24 species) and sardines in the genus *Sardinella* (22 species) are each represented by only three species.

In this study, we investigated the evolution of herbivory and associations between herbivory and habitat in clupeoid fishes. Our first objective was to use an updated molecular dataset to reconstruct a new clupeoid phylogeny with more representative sampling of Indo-Pacific trophic diversity. We then used this phylogeny to estimate the history of trophic niche evolution in clupeoids and test the hypotheses that herbivory is negatively correlated with salinity and latitude (proxy for temperature).

2. Materials and methods

2.1. Taxon sampling and molecular data collection

This study adhered to the Lavoué et al. (2014) classification of Clupeoidei and revisions suggested for the genus *Encrasicholina* (Hata and Motomura, 2017), genus *Sardinella* (Stern et al., 2017), and genera *Pseudosetipinna*, *Setipinna*, and *Lycothrissa* (Lavoué and Ho, 2017). We acquired DNA sequences for 191 individuals from 190 clupeoids and the denticle herring *Denticeps clupeoides* to serve as an out-group (Supplementary Table 1). Our sampling included all major clupeoid lineages and 67 of 82 genera. We downloaded sequences from GenBank and generated additional sequences from specimens we collected. We extracted total genomic DNA using Qiagen® DNAeasy Blood and Tissue Kits (Qiagen, Valencia, CA) following the manufacturer's protocol. We used polymerase chain reaction (PCR) to amplify four nuclear (*rag1*, *rag2*, *slc*, *zic1*) and two mitochondrial loci (*cytb*, *16s*) that have been used extensively for actinopterygian systematics (Li et al., 2007, 2010; Near et al., 2012). PCR reactions contained 2.75 µl water, 1.5 µl genomic DNA, 6.25 µl GoTaq® Green Master Mix (Promega, Madison, WI), 1.0 µl primers and were conducted using published PCR cycling protocols and amplification primers (López et al. 2004; Li et al., 2007, 2010; Bloom and Lovejoy, 2012). We used Exosap to remove excess primers and nucleotides from PCR products (Werle et al., 1994). We sequenced purified PCR products using ABI Prism® BigDye Terminator version 3.1 chemistry (Applied Biosystems, Foster City, CA) at the University of Minnesota Biomedical Genomics Center DNA Sequencing and Analysis Facility. We edited sequences, produced contigs and consensus sequences, and aligned consensus sequences using the MUSCLE algorithm (Edgar, 2004) in Geneious v. 6.0.3 (www.geneious.com; Biomatters Ltd., Auckland, New Zealand). We confirmed the quality of alignments by visual inspection of sequences and their amino acid translation and comparing our alignments to alignments previously published by Bloom and Lovejoy (2014), then trimmed sequences to the following lengths (in base pairs): *rag1* 1571, *rag2* 1269, *slc* 770, *zic1* 902, *cytb* 1143, *16s* 1480.