Title

In Love and War: The Morphometric and Phylogenetic Basis of Ornamentation in the Livebearer Genus Poecilia

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In Love and War: the Morphometric and Phylogenetic Basis of Ornamentation in the Livebearer Genus *Poecilia*

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of the requirements for the degree of

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by

Daniel Lorenz Goldberg

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ABSTRACT OF THE THESIS

In Love and War: the Morphometric and Phylogenetic Basis of Ornamentation in the Livebearer Genus *Poecilia*

by

Daniel Lorenz Goldberg

Master of Science, Graduate Program in Evolution, Ecology, and Organismal Biology
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Dr. David Reznick, Chairperson

In many species, exaggerated secondary sexual characters are used for both competition with rivals and courtship. One possible explanation for this association is the pre-existing trait hypothesis – ornamental characters initially evolve for male contests, but become co-opted to be evaluated by female choice. Comparative studies of a variably decorated group of animals can test the applicability of this hypothesis. Here I apply a phylogenetic approach to study the evolution of enlarged dorsal fins in male poeciliid fish of the subgenera *Mollienesia* and *Limia*, which have repeatedly developed a sailfin phenotype and use an erect-fin display for both intrasexual and intersexual purposes. These subgenera include species that show considerable male-male aggression without any elaborate courtship displays toward females, as well as species that show frequent male displays to both sexes. In contrast to prior categorical assessments of poeciliid adornments, I propose that dorsal fin size can be measured as a continuous index of ornamentation. I construct a new phylogeny to examine relationships between multiple subgenera of *Poecilia*, with Bayesian inference and two maximum likelihood methods robustly supporting the sister grouping of *Limia* and *Pamphorichthys* to the exclusion of *Mollienesia*, in accord with previous studies. Bayesian tests provide strong evidence for correlated evolution of the ornamentation index and several behavioral and morphological traits. The results of phylogenetic logistic and generalized least-squares regressions indicate that a high ornamentation index is significantly associated with the presence of exaggerated traits and component postures of courtship displays, but not with sexual dichromatism or variability in male mating tactics. Larger species with male-biased sexual size dimorphism have increased dorsal fin height relative to body length, body depth, and dorsal fin length. Ancestral state reconstructions and correlation analyses based on Bayesian Markov chain Monte Carlo simulations reveal that variability in male reproductive behavior has often appeared in courting species, and that two components of the courtship display have evolved together. The aggressive form of the display was present near the base of *Poecilia* well before the appearance of the mating display, suggesting that this trait originated for male-male competition and has become co-opted for use in courting females.

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INTRODUCTION

Traits under Intrasexual and Intersexual Selection

Many organisms exhibit flamboyant and exaggerated characters and behaviors that appear counterproductive to their survival. In his theory of sexual selection, Darwin (1871) outlined the adaptive benefits of these displays by dividing them into two distinct processes. Traits can be used by members of one sex to directly compete over a mate (intrasexual selection), or they can be chosen by one sex to procure a mate among the displaying sex (intersexual selection). Darwin also noted that the competitive sex is most often male, the choosy sex is most often female, and the two sexes may be dimorphic in structures and coloration associated with sexual selection (1871). The exact mechanism of sexual selection has been debated ever since; for example, Wallace (1889) argued that female choice is negligible compared to male combat, and cannot explain the evolution of extravagant traits. Nevertheless, a huge body of observational and experimental evidence from a wide range of taxa has strongly supported both processes of sexual selection at work, and it is now seen as a major evolutionary force (Andersson 1994).

Darwin (1871) initially suggested that ornamental traits used to attract females, which are purely for display, are different from ornaments used in competition between males, which he defined as weapons. He attributed the first type to intersexual selection, and the second to intrasexual selection. However, ornaments have long been recognized to function in both courtship and combat (Fisher 1930; Noble 1938; Zahavi 1975). Male structures that actually act as offensive weapons can visually signal the fighting ability of their bearers, to deter rivals and resolve contests without physical violence (Berglund et al. 1996; Borgia and Coleman 2000). In addition, male battles and displays to females are types of social interactions, and ornaments can function as status badges in contests and dominance hierarchies independent of mate choice, which occur within and between the sexes over resources such as food or territories (West-Eberhard 1979).

Comparative studies of dual-function displays have put forward a model for their origin, known as the armament-ornament or pre-existing trait hypothesis (Berglund et al. 1996; Borgia and Coleman 2000). This holds that elaborate male characters can initially evolve for competitive purposes, either as status badges or weapons, and subsequently become adapted for courtship. Although males may initially use their displays to indicate their health to rival males, these traits can become equally useful to females as signals of vitality and genetic or proximate benefits of potential mates (Berglund et al. 1996; Borgia and Coleman 2000). Few studies, however, have actually assessed the validity of this hypothesis in a phylogenetic context. Borgia and Coleman (2000) determined that the agonistic "skrraa" call of male bowerbirds has become adapted for mate attraction in the genus *Chlamydera*, due to the unique frequency of the mating call, and male modulation of calling intensity to entice females. Regaledo (2015) used a qualitative model and tests of behavior to suggest that monochromatic and irregularly dichromatic *Sphaerodactylus* dwarf geckos have co-opted an arched-back threat posture for use in courtship. Yet a reverse case of ornament co-option has also been found: Morris et al. (2007) showed that dark vertical bars on the bodies of northern swordtail fish (*Xiphophorus*) initially served as a display for female mate choice, but gained a new function in male contests, perhaps as a result of eavesdropping. Here males may have exploited the correlation between boldness in courtship displays and aggressiveness in agonistic encounters, which has been found in numerous organisms (Morris et al. 2007).

An implicit assumption of the pre-existing trait hypothesis is that male ornaments evolve for competition prior to the development of a female preference for them (Borgia and Coleman 2000), consistent with other theories of sexually selected character evolution through mate choice, such as Fisherian runaway selection and indicator models like the good-genes hypothesis (Berglund et al. 1996). On the other hand, the pre-existing bias hypothesis predicts that instead of females gaining from exploitation of male signals, it is males who exploit sensory or perceptual biases of females (Ryan and Keddy-Hector 1992; Ryan and Cummings 2013). This theory holds that female preference for a male ornamental trait should exist prior to the appearance of the trait itself, which evolves to take advantage of the bias and increase male mating success. Yet signals must be tuned to the sensory capacities of the species expressing them, and a pre-existing bias that allows females to assess male signals may simply reflect the perceptual ability of both sexes that enabled males to evolve these traits in the first place (Berglund et al. 1996). Male sensory biases also exist, and may shape ornament evolution in an aggressive context, so the two hypotheses need not be mutually exclusive (Ryan and Cummings 2013). While several lineages of frogs and fish do appear to show a pre-existing female bias that has driven the evolution of male traits (Ryan and Keddy-Hector 1992; Basolo 1996), the huge diversity of ornamental traits used for courtship and competition implies that both models may be valid (Berglund et al. 1996). Species in which the sexes differ in perceptual constraits, as a result of intersexual versus intrasexual selection, may provide test cases to examine if a male trait has evolved for an initial competitive advantage or for exploiting an ancestral female bias (Ryan and Cummings 2013).

If female sensory biases are responsible for the origin and exaggeration of a male trait, comparative studies of closely related and variably ornamented taxa will only favor this hypothesis if speciation events occur between the evolution of the preference and the emergence of a display to exploit it (Fuller et al. 2005). Most sensory bias models predict that male displays evolve rapidly, and this could give the appearance of coevolution with a female preference whether sensory bias is responsible or not (Fuller et al. 2005). But if the appearance of a display is shown to precede any sort of female preference, then the signal may have first evolved for male-male contests, and only later have been co-opted for mate attraction (Berglund et al. 1996). To uncover the order of events that have driven the evolution of ornamental characters, behavior and morphology must be correlated in a phylogenetic context. Phylogenetic methods can serve to assess historical patterns of trait evolution, conduct character state reconstructions in ancestral taxa, and correlate multiple organismal features to infer their temporal changes relative to each other (Pagel 1999). If male-male aggressive displays can be shown to predate courtship, and trait exaggeration is found to follow, this should lend support to the pre-existing trait hypothesis. A shift to a display-based mating system could allow female preferences to become important in mate selection, which in turn should maintain the male ornaments used in these displays.

Courtship and Alternative Reproductive Strategies in Poeciliid Fishes

Teleost fishes are an excellent system to study the evolution of courtship display traits, due to their vast diversity of reproductive modes and sexual dimorphism (Noble

1938; Taborsky 1994). A variety of mating behaviors are also found within species, as bony fishes have frequently evolved alternative reproductive strategies such as group spawning, nest piracy, female mimicry, mate-choice copying, and rapid, covert, or forced insemination (Gross 1984; Taborsky 1994; Henson and Warner 1997). These strategies can be evolutionarily stable, can be maintained through frequency-dependent selection (Gross 1984), and are usually chosen based on individual size or condition to maximize fitness and fertilization success (Gross 1996). Male fish often show two types of mating behaviors. Colorful or large courting males invest in conspicuous traits to attract females, who then choose a mate based on the quality of his signal; whereas small, drab, or mimic males will simply copulate without displaying, which bypasses female choice (Ryan and Causey 1989; Taborsky 1994). The bright colors and elaborate displays of courting males are used for both attracting females and intimidating rivals, indicating that these traits are often under intersexual and intrasexual selection (Kodric-Brown 1990, 1998). Given the existence of alternative pre-copulatory tactics, it can be difficult to determine whether male-male aggression or female choice has been more important in driving display trait evolution in species under strong sexual selection (Hamilton 2001).

The family Poeciliidae offers a promising system to study the evolution of ornate characters because males in several genera exhibit various types of colorful adornments. Two well-known examples are the elongated caudal fins of the swordtails (*Xiphophorus*), and the enlarged dorsal fins of the sailfin mollies (*Mollienesia*), used for both courtship and competition (Noble 1938; Parzefall 1969; Baird 1974; Bisazza 1993; Berglund et al. 1996). Poeciliids are small freshwater fishes, native to the Neotropics, which mate via internal fertilization and are primarily viviparous, hence their common name of livebearers (Rosen and Bailey 1963). Male poeciliids possess a modified anal fin, and associated internal anatomy called a gonopodial suspensorium, that functions as a mobile intromittent organ (Rosen and Gordon 1953; Rosen and Tucker 1961; Chambers 1987). Male traits associated with female choice have repeatedly evolved in different poeciliid genera (though not always simultaneously); these include bright coloration, ornamental structures, and a courtship display of variable swimming movements in front of a female with fins fully spread (Meffe and Snelson 1989; Bisazza 1993; Evans et al. 2011).

The two mating strategies of poeciliids exemplify the typical teleost dichotomy of courting versus covert males. Most species lack courtship, with males relying solely or predominantly on gonopodial thrusting for stealthy and forcible copulation (Farr 1989; Bisazza 1993). Alternatively, males of some species do use displays to attract mates, and may switch between the two tactics depending on size, dominance, and female receptivity (Farr 1989; Bisazza 1993). Furthermore, differences in poeciliid pre-copulatory behavior have been linked to significant variation in sexual size dimorphism, relative gonopodium length, and the strength of sexual selection (Bisazza 1993; Bisazza et al. 1996; Pollux et al. 2014). In species reliant on gonopodial thrusting, the sexes tend to be equally dull in coloration (Farr 1989), and males are much smaller than females, perhaps to increase the success of covert copulation (Bisazza 1993; Bisazza and Pilastro 1997). Sneaker males frequently have gonopodia that measure up to half their body length (Rosen and Gordon 1953; Rosen and Tucker 1961) and often have unadorned tips, perhaps to allow quick and shallow penetration of the female gonopore (Greven 2005); these males can see the tip of their gonopodium and hence aim it when trying to inseminate females (Chambers 1987). Conversely, in a multitude of courting poeciliids, males are often more similar in size to females (Bisazza et al. 1996). The gonopodia of these species tend to be short relative to total body length (Rosen and Tucker 1961; Chambers 1987), and often have hooked or spiny tips, or sometimes what appear to be sensory papillae, which are thought to provide tactile cues to assist penetration of the female gonopore (Greven 2005). Courting species may exhibit striking sexual dichromatism, and a few lineages have evolved extravagant sexually selected ornaments and displays in males (Rosen and Bailey 1963; Farr 1989). Moreover, larger or more colorful males are preferred by females in numerous courting species (Bisazza 1993; Bisazza et al. 1996).

Different theories have been put forward to explain the evolution of mating tactics and sexual dimorphism in poeciliids. Long gonopodia and covert copulation are believed to be ancestral traits in the family (Bisazza 1993; Bisazza et al. 1997; Ptacek and Travis 1998), and the evolution of short gonopodia has been proposed to precede a transition to courtship (Martin et al. 2010). Male displays increase female receptivity toward mating, which may allow for more efficient sperm transfer (Rosen and Tucker 1961), or promote cooperation despite potential injuries to females caused by the accessory structures of reduced gonopodia (Wang et al. 2015). Behavioral studies have also found evidence that female choice and male-male competition can influence evolution of mating displays and exaggerated traits in poeciliids (Farr 1989; Bisazza 1993). For example, in males of the green swordtail, *Xiphophorus helleri*, increased sword length and body size are positively correlated with success in both contests and mate attraction (Benson and Basolo 1996), consistent with a general female preference for victors in male battles (Andersson 1994). Although swordtails are cited as a classic example of the pre-existing bias hypothesis at work, since a female preference for swords exists in species of *Xiphophorus* and the sister genus *Priapella* that lack this trait (Basolo 1996), a competitive advantage indicates that the pre-existing trait hypothesis may at least partly explain sword evolution. Exaggerated fins and courtship displays have also been lost or reduced repeatedly in different poeciliid lineages (Heinrich and Schröder 1986; Meyer et al. 1994; Ptacek et al. 2011; Kang et al. 2013), and in some of these species, females show a preference for male courtship but disdain for ornaments (Ryan and Keddy-Hector 1992; Rosenthal et al. 2002; Wong and Rosenthal 2006). This suggests that female ancestral biases may be for male signals that utilize ornamental fins, and not for the fins themselves. Such a preference could provide a mechanism for males to co-opt their displays from competition to courtship.

One problem that can arise in studies of ornamental traits is that display structures may not be uniform in size. In contrast to behavioral traits such as courtship, which can be easily dichotomized if most species in a clade lack them, morphological traits like fins often vary continuously. The length of the caudal fin in *Xiphophorus* differs considerably between species and individuals (Meyer et al. 1994), and the size and shape of the dorsal fin in *Mollienesia* is similarly variable (Ptacek 2005). Yet the use of quantitative traits such as fin sizes for phylogenetic analysis has been the subject of debate (Rae 1998), with little agreement on how data should be coded as discrete character states. Comparative studies of livebearers typically categorize ornamental morphology as categorical, instead of continuous. Exaggerated fins are listed as either present or absent (Meyer et al. 1994;

Martin et al. 2010; Kang et al. 2013; Pollux et al. 2014), or as a numerical sum of binary pigmentation and shape components (Bisazza 1993; Basolo 1996). However, the great diversity of trait sizes observable in mollies and swordtails indicates that a better method may be to consider the size of an ornament as a continuum, rather than as either present or absent. I will use a phylogenetic approach to compare a continuous trait index with behavior, morphology, and sexual dimorphism. This analysis of evolutionary history should determine the context in which ornamentation originated and how it may be used, whether for male-male competition, courtship of females, or both types of displays.

Mollies and Limia as Paradigms of Trait Evolution

To study the relative influence of male-male aggression and courtship of females in driving ornament evolution, and how this display trait may be correlated with behavior and morphology, I will focus on two poeciliid subgenera, *Mollienesia* and *Limia*. Mollies are found along the Gulf Coast of North America and throughout Central America, while Limia are native exclusively to islands of the Caribbean and have a close South American relative, *Pseudolimia heterandia* (Rosen and Bailey 1963; Hrbek et al. 2007; Weaver 2015). These two poeciliid clades are ideal model organisms for numerous reasons. They are closely related, which simplifies the number of taxa required for a comparative phylogenetic study; they have independently evolved diverse and labile male adornments, used in comparable displays for courtship and competition; and they are highly variable in pre-copulatory behaviors as well as the extent of their sexual dimorphism.

The close affinity of mollies and Limia is well supported, as prior phylogenetic research has robustly combined *Mollienesia*, *Limia*, and the subgenus *Pamphorichthys* in a monophyletic group within the genus *Poecilia* (Rodriguez 1997; Hamilton 2001; Hrbek et al. 2007; Meredith et al. 2010, 2011; Pollux et al. 2014; Weaver 2015). An enlarged dorsal fin has convergently evolved in each subgenus: once in the sailfin mollies, with a unique loss of this trait evident in the short-finned Tamesí molly, *Poecilia latipunctata* (Ptacek and Breden 1998); and once in the humpback Limia, *Limia nigrofasciata* (Farr 1984; Cruz and Munger 1999). Courtship display behavior has evolved at least twice in the mollies – once in the sailfin molly group (including *P. latipunctata*) (Ptacek et al. 2011), and once in the shortfin *P. mexicana* and *P. limantouri* (Parzefall 1989; Ptacek 1998) – and at least three times in different clades of Limia (Farr 1984; Hamilton 2001; Weaver 2015). *Limia* and *Mollienesia* each show a wide range of sexual dichromatism and reproductive tactics: some species have bright courting males and drab females, and are less reliant on gonopodial thrusting, while others have nearly identical sexes, and males predominantly use gonopodial thrusting (Hamilton 2001; Ptacek 2005). In both subgenera, male displays are also used in agonistic interactions (Parzefall 1969; Balsano et al. 1985; Woodhead and Armstrong 1985; Parzefall 1989; Bierbach et al. 2013; Holz 2015). Shortfin mollies generally form dominance hierarchies, in which large and high-ranking males will attack subordinate males and exclude them from mating with females (Ptacek 1998). Male-male competition is important for reproductive success in these mollies (Ptacek 2005), as well as in Limia, since aggression is commonly seen in most species, whereas courtship is rare (Hamilton 2001; Holz 2015). This implies that male displays in both *Mollienesia* and *Limia* have primarily evolved for competitive uses, and have seldom become adapted for mate attraction.

General Hypotheses

The goals of the present study are twofold. First, I will examine the relationship between ornamentation and a suite of morphological and behavioral characters in *Limia* and *Mollienesia*, using Bayesian tests for correlated trait evolution, and phylogenetic logistic and least-squares regressions. Second, I will use ancestral state reconstructions to map the evolution of the ornamentation index, sexual selection, and display behavior onto a new phylogeny of these subgenera. I predict that a high index of ornamentation should be positively correlated with sexual dichromatism, sexually selected male characters, multi-component courtship displays, and variability in male reproductive tactics, which have all been observed in sailfin mollies and the humpback Limia. The ornamentation index should be inversely correlated with relative gonopodium length and female-biased sexual size dimorphism, since long intromittent organs and larger females are often seen in species without display traits. The use of courtship versus the ancestral covert mating strategy should be variable among males of courting species, since alternative tactics may result from dominance heirarchies, distinct size classes, or different environmental factors between populations. Finally, the use of two display components (dorsal fin erection and sigmoid body postures) in male-male competition should precede the co-option of both traits for attracting females, as aggression appears more common than courtship in both mollies and Limia. I will use these analyses to test the applicability of the pre-existing trait hypothesis in a phylogenetic context, to elucidate the origin and evolution of ornaments in the genus *Poecilia*.

MATERIALS AND METHODS

Morphological Measurements

Two sets of images were obtained to measure body and fin dimensions for 14 species of *Limia*, 14 species of *Mollienesia*, and 4 other species in *Poecilia*. One set was taken from preserved specimens on loan from six museum collections, and a second set, taken of anesthetized or recently preserved individuals from seven *Mollienesia* species, was acquired from the laboratory collections of Clemson University, Kentucky State University, and Florida State University (Table 2). Specimens of *Pamphorichthys* were not available in museum collections for measurements to be made, so these species were excluded from the analysis. Mature males and females were selected from the preserved collections for imaging. Lateral photographs of individual specimens were taken with a Pentax K200D single-lens reflex digital camera, using the macro setting for close-up images. Each fish was laid out on its left side above a ruler to facilitate measurement. When necessary, insect pins were used to raise the dorsal fin and separate the anal fins from the ventral fins, so that their insertion points would be visible. The crumpled state of the dorsal fins meant that they could not be fully spread, lest they be damaged. Digital images were saved in JPEG file format and copied to a laptop computer for analysis. Measurements from all photographs were obtained in the processing program ImageJ (Schneider et al. 2012; Rasband 2014).

The standard length of each fish was measured (to the nearest 0.01 mm) from the tip of the lower jaw to the base of the caudal fin at the caudal peduncle. Gonopodium length was measured as the distance from the fin base to its distal tip, and this value was

Table 1. Collection data for all preserved specimens examined in this study, including the museum where specimens were stored, accession numbers, species, number of males photographed, number of females photographed, preparation type, country of origin, municipality information, locality information, date of collection, and individual collector(s).

Museum Code	Accession Number	Species	*N* M	*N* F	Preservative	Country	Municipality	Locality	Collection Date	Collector(s)
FMNH	104641	*Limia caymanensis*	5	7	70% ethanol	Cayman Islands	Grand Cayman, West Bay	West Bay, Meteorological Station AGRO-MET - MRCU-WMO	11/25/1980	D. W. Greenfield, T. A. Greenfield
UF	47434	*Limia caymanensis*	14	34	70% ethanol	Cayman Islands	Grand Cayman, Newlands	Cow well 1.92 km west of Newlands Road crossroads	8/171987	R. Franz, S. Franz
UF	165812	*Limia dominicensis*	22	36	70% ethanol	USA	Florida	Silver Bay	7/14/1957	Silver Bay personnel
UF	30389	*Limia dominicensis*	27	42	70% ethanol	Haiti	Sud	Les Cayes at Hotel Relais	1/28/1978	L.R. Franz, F.G. Thompson
USNM	220524	*Limia grossidens*	17	24	75% ethanol	Haiti	Sud	North end of Lake Miragoane, Dept. De L'Ouest, Haiti	3/7/1979	L. R. Rivas
UF	98159	*Limia melanogaster*	22	36	70% ethanol	Jamaica	Cornwall, Hanover Parish	Silver Spring at road from Savanna-La-Mar to Green Island Harbour (Station 25)	12/27/1949	L.R. Rivas, O.C. Rivas
UF	98151	*Limia melanogaster*	29	42	70% ethanol	Jamaica	Cornwall, Westmoreland Parish	Camonte Pond River, 4 km west of Savanna-La-Mar (Station 24)	12/27/1949	L.R. Rivas, O.C. Rivas
UF	110059	*Limia melanonotata*	17	34	70% ethanol	Dominican Republic	Barahona	Canos, 200 m northwest of El Penon, Lago Rincon	11/6/1991	G.H. Burgess et al.
UF	110060	*Limia melanonotata*	44	44	70% ethanol	Dominican Republic	Independencia	Lago Enriquillo at El Zufrada, 4 km east of La Descubierta	11/9/1991	G.H. Burgess et al.
UF	110964	*Limia nigrofasciata*	60	51	70% ethanol	Haiti	Sud	Northeast end of Lake Miragoane	4/12/1951	L.R. Rivas, L. Bonnefil, S.Y. Lin
UF	29823	*Limia pauciradiata*	20	30	70% ethanol	Haiti	Nord	Grand Riviere du Nord at town of Grand Riviere	4/10/1951	L.R. Rivas, E. Garnier

UF	110859	*Limia perugiae*	101	81	70% ethanol	Dominican Republic	Trujillo	Rio Nigua at San Cristobal	4/20/1949	L.R. Rivas, B.P. Hunt
UF	28007	*Limia rivasi*	9	17	70% ethanol	Haiti	Ile de la Gonave	Dept. de L'Ouest, mangrove swamp, 1 km southeast of Anse a Galet	1/27/1980	R. Franz
UF	110075	*Limia sulfurophila*	59	53	70% ethanol	Dominican Republic	Independencia	Balneario la Zurza, 5 km west-northwest of Diverge	11/9/1991	G.H. Burgess et al.
UF	118808	*Limia tridens*	42	36	70% ethanol	Haiti	Artibonitã	Spring at Dessalines	4/9/1951	L.R. Rivas, Garuier
UF	110074	*Limia tridens*	84	35	70% ethanol	Dominican Republic	Barahona	Tierra Blanca, out of Cabral on road to Polo, 0.4 km from intersection of road Barahona-Cabral	11/5/1991	G.H. Burgess et al.
UF	110080	*Limia versicolor*	11	36	70% ethanol	Dominican Republic	San Cristobal	Arroyo Jibana, 9 km south of Madrigal	6/23/1977	F.G. Thompson
UF	92415	*Limia vittata*	21	30	70% ethanol	Cuba	Villa Clara	East of Central Resulta, Sagua La Grande	8/10/1945	L.R. Rivas
UF	23964	*Limia zonata*	11	62	70% ethanol	Dominican Republic	Maria Trinidad Sanchez	Ojo de Agua, Rio Cana Azul, 2 km northwest of Cano Claro	2/2/1977	L.R. Franz, F.G. Thompson
FMNH	84056	*Allopoecilia caucana*	47	36	70% ethanol	Colombia	Atlantico	Magdalena, at 2 km south Malambo Road to Sabanagrande	11/16/1973	T.T. Thomerson, D. W. Greenfield
UF	15260	*Poecilia butleri*	34	56	70% ethanol	Mexico	Guerrero	Lagoon 21.44 km southeast of Puerto Marques	6/23/1966	F.G. Thompson
UMMZ	192217	*Poecilia chica*	24	29	70% ethanol	Mexico	Jalisco	Tributary, 2.56 km from W Hwy. 80 on road to Purificacion, Pacific	4/24/1969	C.D. Barbour, R.J. Douglass
USNM	293416	*Poecilia gilli*	7	17	75% ethanol	Panama	Guna Yala	Guna Yala, Rio Carti Grande at Carti Road 3 km from Carti, Atlantic	3/1/1985	W. Starnes et al.
LSUMZ	15018	*Poecilia gillii*	2	15	70% ethanol	Honduras	Colon	Laguna Bacalar	11/28/2010	J.C. Carrasco

LSUMZ	15700	*Poecilia hondurensis*	13	14	70% ethanol	Honduras	Atlantida	Rio Blanco at San Patricio, La Ceiba	9/14/2011	C. McMahan, W. Matamoros
UMMZ	193309	*Poecilia orri*	17	51	70% ethanol	Belize	Belize District	Drains and canals in northern suburbs of Belize City	1/2/1973	R.E. Norris
UMMZ	181821	*Poecilia salvatoris*	19	70	70% ethanol	El Salvador	Santa Ana	El Salvador, north shore of island in Lake Coatepeque	1/19/1958	P.L. Clifton
LSUMZ	17583	*Poecilia sphenops*	2	18	70% ethanol	Mexico	Chiapas	3.2 km north of Puerto Arrista at ridge that crosses marsh and bayou	7/21/1976	B. Hanks, G. McQuown, G. Serarese
TCWC	1864.02	*Poecilia sphenops*	8	32	70% ethanol	Mexico	Chiapas	6.4 km northeast of Arriaga, Hwy 195 at km marker 41	6/10/1966	J.R. Dixon, T. D. Meyer
UMMZ	184716	*Poecilia sulphuraria*	42	43	70% ethanol	Mexico	Tabasco	Arroyo del Azufre at Banos de Azufre, 6.4 km west of Teapa; Rio Grijalva, Atlantic drainage	2/15/1959	R.R. Miller, R.J. Schultz
USNM	247412	*Poecilia vivipara*	18	45	75% ethanol	Brazil	Ceara	Brazil, Reservoir At Pentecoste	8/14/1966	J. Dendy, V. Franca
UF	25049	*Psychro-poecilia hispaniolana*	41	37	70% ethanol	Dominican Republic	La Vega	Rio Yaque del Norte, 9 km southwest of Jarabacoa	6/24/1977	F.G. Thompson
UMMZ	186919	*Pseudolimia heterandria*	17	39	70% ethanol	Venezuela	Carabobo	Rio Guaiguaza, 3 km west of Puerto Cabello, 2 km from mouth of river	1/15/1938	F.F. Bond

Museum Key: *FMNH* Field Museum of Natural History, Chicago, IL, USA; *LSUMZ* Louisiana State University Museum of Natural Science, Baton Rouge, LA, USA; *TCWC* Texas A&M Biodiversity Teaching Collections, College Station, TX, USA; *UF* Florida Museum of Natural History, Gainesville, FL, USA; *UMMZ* University of Michigan Museum of Zoology, Ann Arbor, MI, USA; *USNM* National Museum of Natural History, Washington, D.C., USA

Table 2. Collection data for all live and recently preserved specimens whose photographs were measured in this study, including the university campus where stocks were kept (Clemson University, Florida State University, or Kansas State University), species, number of males photographed, number of females photographed, preparation type (if dead), country of origin, municipality information, locality information, date of collection, and individual collector(s).

Campus	Species	*N* Males	*N* Females	Preservative	Country	Municipality	Locality	Collection Date	Collector(s)
FSU	*Poecilia latipinna*	12 live	47 live	None	USA	Florida	Wacissa River, Jefferson County Steve's Ditch, Dickerson Bay, Wakulla County	2015	A. Landy
FSU	*Poecilia latipinna*	20 preserved	2 preserved	70% ethanol	USA	Florida	St. Marks National Wildlife Refuge, near Tallahassee, Wakulla County	2014	A. Landy
Clemson	*Poecilia latipunctata*	45 live	4 live, 8 preserved	95% ethanol	Mexico	Tamaulipas	El Nacimiento, tributary to Rio Tamesí, near Ciudad Mante	3/17/2003	M.B. Ptacek
Clemson	*Poecilia limantouri*	21 live	0	None	Mexico	San Luis Potosi	Los Anteojitos, Rio Verde	3/19/2002	M.B. Ptacek
KSU	*Poecilia mexicana*	10 preserved	25 preserved	75% isopropanol	Mexico	Chiapas	Arroyo Rosita, tributary to Rio Pichucalco, near Pichucalco	6/12/2012	M. Tobler
Clemson	*Poecilia mexicana*	63 live	0	None	Mexico	Campeche	Cuidad del Carmen, Champóton	5/31/2003	M.B. Ptacek
KSU	*Poecilia mexicana*	0	28 preserved	75% isopropanol	Mexico	Tamaulipas	Rio La Bomba, west of Cuauhtemoc, Tampico	9/20/2010	M. Tobler
Clemson	*Poecilia orri*	36 live	0	None	Mexico	Quintana Roo	Near Villa las Estrellas, Tulum	6/7/2003	M.B. Ptacek
Clemson	*Poecilia petenensis*	58 live	41 live	None	Mexico	Campeche	Cuidad del Carmen, Champóton	5/31/2003	M.B. Ptacek & S. Hankison
Clemson	*Poecilia velifera*	27 live	21 live, 4 preserved	95% ethanol	Mexico	Yucatan	Roadside ditch along Mérida-Progreso, Hwy 261	4/14/2002	M.B. Ptacek & S. Hankison
Clemson	*Poecilia velifera*	39 live	3 preserved	95% ethanol	Mexico	Campeche	Cuidad del Carmen, Champóton	5/31/2003	M.B. Ptacek & S. Hankison

then divided by standard length to calculate relative anal fin size. Dorsal fin height was taken as the distance from the tip of the longest fin ray to the base of that ray. Dorsal fin length was measured as the distance between the anterior and posterior insertion points of that fin. Finally, body depth was measured from the highest point on the dorsal surface (usually right before the dorsal fin anterior insertion) to the lowest point on the abdomen (Fig. 1). Sexual size dimorphism of standard length, dorsal fin length, and body depth was calculated as the natural logarithm of the size ratio between females and males (SSD = ln(female size/male size)) (Lovich and Gibbons 1992; Smith 1999). A separate size dimorphism index was also quantified as the ratio of the larger to the smaller sex, minus one (SDI = (larger sex/smaller sex) – 1) (Lovich and Gibbons 1992). This index was given a negative value if males are larger and a positive value if females are larger. Body and fin measurements were natural-log transformed for all statistical analyses (Table 3).

Ornamentation Index

The degree of display trait exaggeration was estimated as a continuous index of dorsal fin height: a standardized size value to correct for males and females differing in length, used to compare between species. This was defined as the difference between the adjusted least-squares mean values of each sex (male and female) for the natural-log-transformed dorsal fin height, estimated at the grand mean of the natural-log-transformed standard length. Differences between the average dorsal fin height of males and females were calculated with an ANCOVA implementing ln(length) as the continuous covariate, sex as the categorical independent variable, and ln(dorsal fin height) as the continuous dependent variable. This standardized estimation across species with different body sizes,

Table 3. Species measured for morphological analysis, ornamentation index (OI), number of males measured (NM), number of females measured (NF), relative gonopodium length (PROPGL), natural-log-transformed male standard length (LNMSL), natural-log-transformed female standard length (LNFSL), natural-log-transformed male body depth (LNMBD), natural-log-transformed female body depth (LNFBD), natural-log-transformed male dorsal fin length (LNMDL), natural-log-transformed female dorsal fin length (LNFDW), size dimorphism index standard length (SDISL), sexual size dimorphism standard length (SSDSL), size dimorphism index body depth (SDIBD), sexual size dimorphism body depth (SSDBD), size dimorphism index dorsal fin length (SDIDL), and sexual size dimorphism dorsal fin length (SSDDL).

Species	OI	NM	NF	PROP GL	LN MSL	LN FSL	LN MBD	LN FBD	LN MDL	LN FDL	SDISL	SSDSL	SDIBD	SSD BD	SDI DL	SSD DL
Limia caymanensis	0.3540	19	41	0.2878	3.2853	3.4282	2.3295	2.4610	1.5113	1.4326	0.1586	0.1472	0.1460	0.1363	-0.0806	-0.0775
Limia dominicensis	0.0857	47	78	0.2665	3.3465	3.5343	2.3022	2.3693	1.3448	1.6328	0.2221	0.2006	0.1203	0.1136	0.3579	0.3059
Limia grossidens	0.2645	17	24	0.2784	3.5020	3.4955	2.5528	2.4564	1.7315	1.6316	-0.0029	-0.0029	-0.0952	-0.0909	-0.0996	-0.0950
Limia melanogaster	0.1933	51	78	0.2287	3.4626	3.4580	2.3278	2.1534	1.3733	1.3527	-0.0076	-0.0076	-0.1982	-0.1808	-0.0271	-0.0267
Limia melanonotata	0.3767	61	78	0.2671	3.3293	3.3863	2.2050	2.1216	1.4837	1.4189	0.0557	0.0542	-0.0952	-0.0909	-0.0762	-0.0735
Limia nigrofasciata	0.6683	60	51	0.2353	3.6603	3.5388	2.8271	2.5558	2.0819	1.5841	-0.1269	-0.1195	-0.3076	-0.2682	-0.6489	-0.5001
Limia pauciradiata	0.3356	20	30	0.2586	3.2015	3.4903	2.1910	2.3915	1.1820	1.3200	0.3481	0.2987	0.2439	0.2182	0.1517	0.1412
Limia perugiae	0.3643	101	81	0.2540	3.4032	3.5794	2.3927	2.5296	1.5376	1.5488	0.2009	0.1831	0.1576	0.1464	0.0117	0.0117
Limia rivasi	0.1928	9	17	0.2902	2.9205	3.1260	1.9322	2.1075	0.7208	0.9798	0.2399	0.2151	0.2035	0.1852	0.3287	0.2842
Limia sulfurophila	0.3536	59	53	0.2568	3.4514	3.4130	2.4200	2.0935	1.3432	1.3029	-0.0358	-0.0352	-0.3842	-0.3251	-0.0402	-0.0394
Limia tridens	0.3172	126	71	0.2914	3.2862	3.2936	2.2552	1.9735	1.2921	1.1923	0.0046	0.0046	-0.3240	-0.2807	-0.1081	-0.1026
Limia versicolor	0.0961	11	36	0.2577	3.3880	3.6388	2.2613	2.5404	1.4852	1.6558	0.2901	0.2547	0.3314	0.2862	0.1854	0.1701
Limia vittata	0.1768	21	30	0.2675	3.5037	3.8217	2.5388	2.8491	1.9031	2.0709	0.4011	0.3373	0.3902	0.3294	0.2147	0.1945
Limia zonata	-0.1130	11	62	0.2774	3.2100	3.4376	2.0857	2.2902	1.2725	1.2502	0.2613	0.2321	0.2373	0.2130	-0.0219	-0.0216
Allopoecilia caucana	0.1355	47	36	0.2484	3.0530	3.1476	1.9928	1.8628	0.7079	0.8488	0.1018	0.0970	-0.1372	-0.1286	0.3201	0.2777
Poecilia butleri	0.1341	34	56	0.2606	3.1805	3.2789	2.2046	2.2912	1.4267	1.3456	0.1046	0.0995	0.0913	0.0874	-0.0915	-0.0875

Poecilia chica	0.2316	24	29	0.2133	3.1623	3.3168	2.1691	2.3412	1.2756	1.2435	0.1750	0.1613	0.1983	0.1809	-0.0239	-0.0236
Poecilia gilli	0.4335	9	32	0.2111	3.8727	3.8867	2.8898	2.8930	2.1824	2.0179	0.0052	0.0052	0.0021	0.0021	-0.1931	-0.1765
Poecilia hondurensis	0.1260	13	14	0.2080	3.7982	3.9702	2.7228	2.8330	1.9500	2.0341	0.1965	0.1794	0.1249	0.1177	0.0904	0.0865
Poecilia latipinna	0.5967	32	48	0.2329	3.7544	3.5913	2.6358	2.4341	2.6647	2.2502	-0.2224	-0.2008	-0.2897	-0.2544	-0.6001	-0.4701
Poecilia latipunctata	0.4069	45	12	0.2154	3.5013	3.5780	2.3955	2.4215	1.7328	1.5746	0.0842	0.0808	0.0299	0.0294	-0.1859	-0.1705
Poecilia limantouri	0.0930	31	25	0.1990	3.7877	3.9410	2.6184	2.8675	1.9017	1.9546	0.1696	0.1566	0.2816	0.2481	0.0604	0.0587
Poecilia mexicana	0.4190	63	28	0.1910	4.0746	3.8201	3.0349	2.7987	2.3510	1.9042	-0.2896	-0.2543	-0.2657	-0.2356	-0.5664	-0.4488
Poecilia orri	0.2834	53	51	0.1798	4.0387	3.8722	3.0837	2.8398	2.4660	2.3817	-0.1926	-0.1761	-0.2844	-0.2503	-0.0884	-0.0847
Poecilia petenensis	1.0716	58	41	0.2006	4.1745	4.0441	3.1146	2.9990	3.3369	2.9268	-0.1430	-0.1336	-0.1325	-0.1245	-0.5266	-0.4230
Poecilia salvatoris	0.1680	19	70	0.1867	4.0424	3.9562	3.0581	2.9295	2.3777	2.1214	-0.0793	-0.0763	-0.1230	-0.1160	-0.2969	-0.2600
Poecilia sphenops	0.4071	10	50	0.2230	3.5830	3.8105	2.5793	2.8179	1.6576	1.8167	0.2518	0.2246	0.2609	0.2318	0.1353	0.1269
Poecilia sulphuraria	0.1302	42	43	0.2175	3.3779	3.5277	2.3336	2.4893	1.4419	1.4096	0.1668	0.1543	0.1813	0.1666	-0.0269	-0.0265
Poecilia velifera	0.8677	66	28	0.1883	4.0138	3.8906	2.9811	2.9404	3.2572	2.9216	-0.1741	-0.1605	-0.0870	-0.0834	-0.5049	-0.4087
Poecilia vivipara	0.2242	18	45	0.1931	3.3473	3.5246	2.3556	2.5384	1.0013	1.0178	0.1948	0.1780	0.2040	0.1856	0.0159	0.0158
Psychro-poecilia hispaniolana	0.0209	41	37	0.2188	3.5465	3.5806	2.4782	2.4211	1.6935	1.6284	0.0384	0.0377	-0.0572	-0.0556	-0.0635	-0.0615
Pseudolimia heterandria	0.2599	17	39	0.2437	2.8621	3.0289	1.7275	1.9715	1.0804	1.0311	0.1805	0.1660	0.2836	0.2496	-0.0484	-0.0472

different allometric slopes of fin height for each sex, or different levels of heterogeneity in slope. This served as a conservative, robust estimator of sexual dimorphism calculated at the average body length of each species, and could objectively compare species that have isometric dorsal fin heights with those in which male dorsal fin heights are strongly allometric relative to females. Levels of species-specific dimorphism measured by the difference in adjusted means are not correlated with the grand mean itself, thus removing a potential source of systematic bias from the model. Table 4 summarizes the values used in the ANCOVA calculation of the ornamentation index.

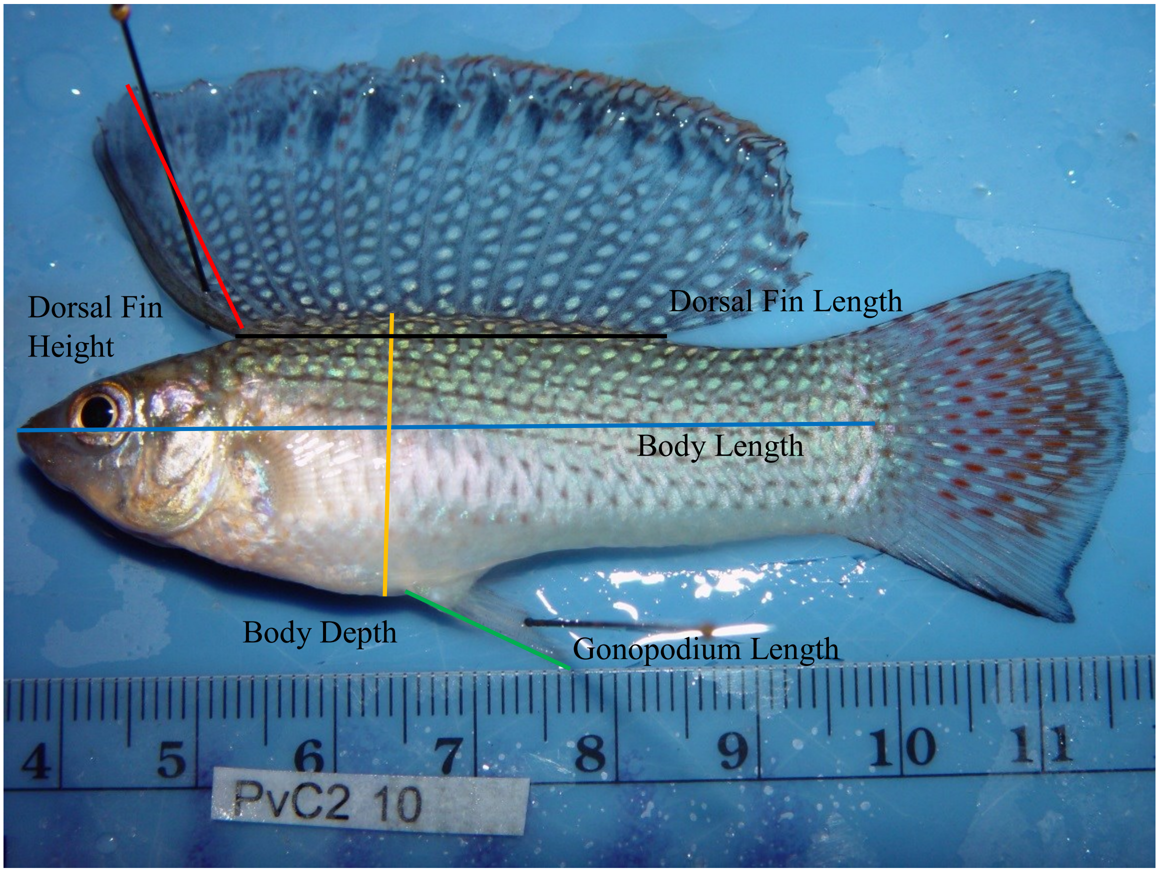

Figure 1. A male of *Poecilia petenensis* with reference lines drawn to indicate the body markers used to measure each specimen. Image courtesy of Margarek Ptacek, Clemson University.

Table 4. Species values used to make the Ornamentation Index. F = females, M = males.

Species	Grand mean of ln(Length)	Adjusted F mean from ANCOVA	Adjusted M mean from ANCOVA	Difference
Limia caymanensis	3.38296	1.060794	1.414742	0.3539486
Limia dominicensis	3.46277	1.571433	1.657108	0.0856750
Limia grossidens	3.49821	1.575510	1.840039	0.2645286
Limia melanogaster	3.45981	1.688024	1.881358	0.1933341
Limia melanonotata	3.36127	1.718377	2.095049	0.3766725
Limia nigrofasciata	3.60448	1.448284	2.116569	0.6682854
Limia pauciradiata	3.37475	1.160438	1.495996	0.3355579
Limia perugiae	3.48161	1.532419	1.896765	0.364346
Limia rivasi	3.05483	1.038937	1.231726	0.1927889
Limia sulfurophila	3.43320	1.708739	2.062328	0.3535889
Limia tridens	3.28889	1.608905	1.926090	0.3171847
Limia versicolor	3.58009	1.517210	1.613331	0.0961214
Limia vittata	3.69073	1.852267	2.029109	0.1768421
Limia zonata	3.40328	1.394654	1.281698	-0.112956
Allopoecilia caucana	3.09405	1.446005	1.581475	0.1354696
Poecilia butleri	3.24172	1.23856	1.37269	0.1341301
Poecilia chica	3.24681	1.002915	1.234532	0.2316166
Poecilia gilli	3.88364	1.982528	2.416025	0.4334976
Poecilia hondurensis	3.88742	1.976703	2.102698	0.1259952
Poecilia latipinna	3.65652	1.405272	2.002005	0.5967328
Poecilia latipunctata	3.51747	1.388446	1.795307	0.4068615
Poecilia limantouri	3.85614	2.055776	2.148790	0.0930136
Poecilia mexicana	3.99631	1.987395	2.406375	0.4189795
Poecilia orri	3.95708	2.075912	2.359317	0.2834045
Poecilia petenensis	4.12051	1.803439	2.873639	1.070201
Poecilia salvatoris	3.97460	1.971395	2.139409	0.1680145
Poecilia sphenops	3.77261	1.757275	2.164375	0.4070994
Poecilia sulphuraria	3.45367	1.368996	1.499229	0.1302332
Poecilia velifera	3.97713	1.818943	2.686602	0.8676587
Poecilia vivipara	3.47391	1.473294	1.697504	0.2242095
Psychropoecilia hispaniolana	3.56265	1.563516	1.584441	0.0209250
Pseudolimia heterandria	2.97826	0.8633016	1.1231849	0.2598833

Dorsal fin height, rather than length, was chosen to represent trait elaboration for all species, because the enlarged fin of *Limia nigrofasciata* is formed from a tall set of dorsal fin rays that emerge from a relatively narrow base, in contrast to the long fin base of the sailfin mollies. Shortfin species, in which male and female dorsal fins are roughly the same size, have ornamentation index values lower than 0.5. The humpback Limia and the sailfin mollies have ornamentation index values greater than 0.5, fitting the pattern of male dorsal fins much larger than those of females. Thus, the size of male ornamentation for each species remains continuous while still falling into a range of values expected for a given phenotype. An increase in the ornamentation index past the cutoff point of 0.5 can be used to pinpoint exactly when a lineage began to evolve an elaborate dorsal fin.

Behavioral Information

I searched the published literature for information on sexual dichromatism, male ornamentation, pre-copulatory and aggressive male behaviors, and male variability for 40 species in *Poecilia* used in the phylogeny (see below). Dichromatism states for these livebearers were first assessed based on colored images and desciptions from Wischnath (1993). For species not included in this source, color differences between the sexes were recorded from new species descriptions or species revalidations (Rivas and Fink 1970; Menzel and Darnell 1973; Miller 1975; Rivas 1978, 1980; Franz and Burgess 1983; Costa 1991; Miller 1994; Poeser 2003, 2011; Casatti et al. 2006). Comparative behavioral studies also provided some descriptions of male and female coloration (Farr 1984, 1989; Hamilton 2001; Ptacek 2005; Pollux et al. 2014). In each species examined, this character was coded as 0 = monochromatic sexes from a human perspective, or 1 = dichromatic sexes, if color of the dorsal and/or caudal fin differed in males and females over at least 33% of the fin surface. Two types of sexually selected male traits were coded as either absent or present in all taxa: the enlarged dorsal fin of the sailfin mollies and humpback Limia, and the sensory filamentous “moustache” seen in some males of the Mexican molly, *Poecilia sphenops* (Schlupp et al. 2010).

Male pre-copulatory behavior in livebearers can be divided into three distinct categories (Liley 1966; Parzefall 1969): 1. Nibbling (also called nipping or nudging), in which a male closely follows a female and touches his snout to the female’s gonopore; 2. Thrusting, in which a male orients behind a female, swings his gonopodium forward with support from his pelvic fins, and attempts to forcibly insert it into the female’s gonopore, usually in conjunction with nibbling; and 3. Courtship displays, in which a male positions himself laterally in front of a female and raises his dorsal fin to become fully spread; he may curve his body and fan the female with his fin (Farr 1984; Ptacek 1998). Although courtship varies from species to species, the typical pattern is a fully spread dorsal fin to show off conspicuous pigmentation or an elaborate fin shape (Bisazza 1993). Of the three behaviors, only the use of courtship varied among species, and hence was coded as either present or absent; while nibbling and thrusting were excluded for being phylogenetically uninformative. I also defined a pre-copulatory sexual selection index for each species, as did Pollux et al. (2014), giving a value of 1 to sexual dichromatism, sexually selected male traits, and courtship, if they were present, and then calculating the sum. The SSI ranged from 0 (all three traits were absent) to 3 (all three traits were present), and was listed with all other behavioral data (Table 5).

Antagonistic behavior between males can take many forms (Liley 1966; Parzefall 1969), such as ramming, biting, tail flicking, and chasing. Competing males may further adopt a display consisting of a sigmoid body curvature, or S-posture, in which they swim parallel or anti-parallel to another male with their back arched and their dorsal fin fully spread (Liley 1966; Parzefall 1969). While this behavior has often been observed in aggressive male encounters (Parzefall 1969; Bierbach et al. 2013), it has also been seen in courtship displays toward females (Liley 1966; Farr 1989; Ptacek 2005). Thus, dorsal fin erection and sigmoid curvature of the body were coded as absent or present for every species, with each recipient sex coded separately. However, dorsal fin erection to males was found to occur in all species in which this behavior had been studied, suggesting an ancestral status, but it could not be properly incorporated into a correlation analysis. I predict that both displays originated for male-male competition and became co-opted for courting females, consistent with the pre-existing trait hypothesis. Finally, the study species of *Poecilia* were all coded as lacking or possessing male variability in the form of alternative reproductive tactics, which may be preferred by males of distinct size classes, dominance ranks, or populations under different environmental stressors. Little data could be gathered for *Pamphorichthys* (Costa 1991; Casatti et al. 2006; Pollux et al. 2014) since comparative behavioral studies of this subgenus have not yet been conducted.

Behavioral Observations

For species that were not well documented in the literature, I was able to obtain behavioral information from personal observations and personal correspondence. Study populations were kept in single-species mixed-sex stocks maintained in the Department

Table 5. Species examined with behavioral traits labeled as absent (0), present (1) or unknown (?). The traits are the ornamentation index (OI), sexual dichromatism (SD), male sexually selected traits (MST), courtship display behavior (CRT), sexual selection index (SSI), sigmoid posture to females (SPF), sigmoid posture to males (SPM), dorsal fin erection to females (FEF), dorsal fin erection to males (FEM) and male behavioral variability (MBV).

Species	OI	SD	MST	CRT	SSI	SPF	SPM	FEF	FEM	MBV	References
Limia caymanensis	0.3539	1	0	0	1	0	0	0	1	0	Rivas & Fink 1970, Hamilton 2001, Allen Wood and Pablo Weaver‡
Limia dominicensis	0.0857	1	0	0	1	0	0	0	1	0	Farr 1984, Cruz & Cruz 1994, Hamilton 2001, Allen Wood‡
Limia garnieri	-	0	0	?	0	?	?	?	?	?	Rivas 1980
Limia grossidens	0.2645	0	0	?	0	?	?	?	?	?	Rivas 1980
Limia melanogaster	0.1933	1	0	1	2	0	0	1	1	1	Farr 1984, Bisazza 1993, Hamilton 2001, Samantha Cohen and Allen Wood‡
Limia melanonotata	0.3767	1	0	1	2	1	0	1	1	1	Pablo Weaver‡
Limia nigrofasciata	0.6683	1	1*	1	3	1	1	1	1	1	Farr 1984, Cruz & Munger 1999, Hamilton 2001, Holz 2015, Pablo Weaver‡
Limia pauciradiata	0.3356	1	0	0	1	0	0	0	1	0	Rivas 1980, Hamilton 2001
Limia perugiae	0.3643	1	0	1	2	1	0	1	1	1	Farr 1984, Erbelding-Denk et al. 1994, Hamilton 2001, Pablo Weaver‡
Limia rivasi	0.1928	0	0	?	0	?	?	?	?	?	Franz & Burgess 1983
Limia sulfurophila	0.3536	1	0	0	1	0	1	0	1	0	Rivas 1980, Bierbach et al. 2013
Limia tridens	0.3172	1	0	0	1	0	1	0	1	0	Farr 1989, Bierbach et al. 2013
Limia versicolor	0.0961	1	0	0	1	0	0	0	1	0	Hamilton 2001, Pablo Weaver‡
Limia vittata	0.1768	1	0	0	1	0	0	0	1	0	Farr 1984, Hamilton 2001
Limia zonata	-0.113	1	0	0	1	0	0	0	1	0	Farr 1984, Hamilton 2001
Allopoecilia caucana	0.1355	1	0	0	1	?	?	0	1	0	de Jong 1992, Meyer & Radda 2000
Poecilia butleri	0.1341	1	0	0	1	?	?	?	?	?	Farr 1989
Poecilia catemaconis	-	1	0	?	1	?	?	?	?	?	Miller 1975
Poecilia chica	0.2316	1	0	1	2	1	1	1	1	0	Miller 1975, Brett & Grosse 1982, Kees de Jong, Henk Plomp, and Bruno Kaubisch‡
Poecilia gilli	0.4335	1	0	0	1	0	1	0	1	0	Farr 1989, Pollux et al. 2014, This study
Poecilia hondurensis	0.1260	1	0	?	1	?	?	?	?	?	Poeser 2011

Poecilia latipinna	0.5967	1	1*	1	3	1	1	1	1	1	Parzefall 1969, Baird 1974, Woodhead & Armstrong 1985, Farr et al. 1986, Ptacek & Travis 1996
Poecilia latipunctata	0.4069	1	0	1	2	1	1	1	1	0	Niemeitz et al. 2002, Ptacek et al. 2005
Poecilia limantouri	0.0930	1	0	1	2	1	1	1	1	1	Menzel & Darnell 1973, Balsano et al. 1985, Ptacek 1998, Ptacek 2002, Bierbach et al. 2013
Poecilia mexicana	0.4190	1	0	1	2	1	1	1	1	1	Menzel & Darnell 1973, Parzefall 1979, Parzefall 1989, Parzefall 2001, Loveless et al. 2009, Bierbach et al. 2012
Poecilia orri	0.2834	1	0	0	1	0	1	0	1	0	Farr 1989, Ptacek 1998, Bierbach et al. 2013
Poecilia petenensis	1.0717	1	1*	1	3	1	1	1	1	0	Farr 1989, Hankison & Ptacek 2007
Poecilia salvatoris	0.1680	1	0	1	2	1	1	1	1	1	Miller 1994, Manfred Schartl‡
Poecilia sphenops	0.4071	1	1†	0	2	0	1	0	1	0	Parzefall 1969, Woodhead & Armstrong 1985, Schlupp et al. 2010
Poecilia sulphuraria	0.1302	1	0	0	1	0	1	0	1	0	Bierbach et al. 2012, David Bierbach‡
Poecilia thermalis	-	1	0	0	1	?	?	?	?	0	Palacios et al. 2013, Michael Tobler‡
Poecilia velifera	0.8677	1	1*	1	3	1	1	1	1	1	Parzefall 1969, Bildsoe 1988, Parzefall 1989, Farr 1989
Poecilia vivipara	0.2242	1	0	0	1	0	1	0	1	0	Liley 1966, Pollux et al. 2014
Psychropoecilia hispaniolana	0.0209	1	0	0	1	?	?	0	?	?	Rivas 1978, Pablo Weaver‡
Pseudolimia heterandria	0.2599	1	0	0	1	0	1	0	1	0	Poeser 2003, Pollux et al. 2014, Samantha Cohen‡, This study
Pamphorichthys hollandi	-	1	0	?	1	?	?	?	?	?	Casatti et al. 2006
Pamphorichthys araguaiensis	-	1	0	?	1	?	?	?	?	?	Costa 1991
Pamphorichthys hasemani	-	?	0	?	?	?	?	?	?	?	No data
Pamphorichthys scalpridens	-	0	0	?	0	?	?	?	?	?	Pollux et al. 2014
Pamphorichthys minor	-	0	0	?	0	?	?	?	?	?	Pollux et al. 2014

Type of male ornaments: * Enlarged dorsal fin (“sailfin”); † sensory filaments on snout (“moustache”). ‡Obtained from personal communication.

of Evolution, Ecology and Organismal Biology at the University of California Riverside. *Poecilia gilli* were collected by Andrew Furness from the Rio Ceibo, Costa Rica, and have been housed in a 190 liter tank since 2013. *Pseudolimia heterandria* were collected by Dieter Bork in Puerto Cabello, Venezuela, were obtained from Manfred Schartl at the *Xiphophorus* Genetic Stock Center at Texas State University in San Marcos, Texas, and were housed in a 114 liter tank at UCR. Both stock tanks contained a gravel bed with live aquatic plants, and were kept at room temperature under a 12:12 hour light-dark cycle. Fish were fed *ad libitum* twice daily with commercial flake food.

From each stock tank, a set of focal males was selected (N = 3 for *P. gilli*, N = 2 for *P. heterandria*). I observed each focal male for a period of 10 minutes, and recorded all pre-copulatory and aggressive behaviors that he performed during this period: the number of dorsal fin erections and sigmoid postures to females and to other males, plus the number of attacks on other males in the tank. These observations were used to qualify the existence of certain behaviors for the study species, which were recorded in Table 5.

Taxon Sampling and DNA Alignments

For phylogenetic reconstructions, I focused on 40 species in the genus *Poecilia* that have been used in previous systematic studies. This included 15 species of *Limia*, 16 species of *Mollienesia*, 5 species of *Pamphorichthys*, and 4 close relatives of these three subgenera. *Pseudolimia heterandria* was once grouped in *Limia* (Poeser 2003), but has been recently recognized to be a monotypic subgenus closely allied to them, based on morphological synapomorphies and robust phylogenetic support (Meredith et al. 2011; Pollux et al. 2014). The South American *Allopoecilia caucana* was once considered a molly (Ptacek and Breden 1998), but it is now placed within its own subgenus that lies basal to the molly clade (Ho et al. 2016). *Psychropoecilia hispaniolana*, meanwhile, is a member of a Caribbean clade that is closely allied with *Limia* (Alda et al. 2013; Weaver et al. 2015; Ho et al. 2016; Palacios et al. in press). The former subspecies *limantouri* of *Poecilia mexicana* (Menzel and Darnell 1973) was listed as a distinct species following Palacios et al. (in press), as it is the sister taxon to two mollies endemic to sulfide springs, *P. sulphuraria* and *P. thermalis*, instead of *P. mexicana* (Tobler et al. 2011; Palacios et al. 2013; Pollux et al. 2014). Lastly, *Poecilia vivipara* served as the outgroup, a position supported by multiple studies (Hamilton 2001; Meredith et al. 2011; Pollux et al. 2014; Weaver 2015; Ho et al. 2016; Palacios et al. in press). This South American species has also been considered a member of *Mollienesia* in the past (Ptacek and Breden 1998), and is placed in the monotypic subgenus *Poecilia*.

To study the relationships of these 40 species of *Poecilia*, I identified molecular sequence data through the NCBI Taxonomy browser. Geneious Version 5.5.4 (Kearse et al. 2012) was used to compile seven nuclear and four mitochondrial genes from GenBank that have been suitable in past phylogenetic studies (Ptacek and Breden 1998; Hamilton 2001; Hrbek et al. 2007; Meredith et al. 2010, 2011; Alda et al. 2013; Pollux et al. 2014). The seven nuclear genes sampled were: exon 2 of ectodermal-neural cortex 1-like protein (*ENC1*); exon 2 of glycosyltransferase (*Glyt*); exon 1 of myosin heavy polypeptide 6 (*Myh6*); exon 3 of recombination activating gene-1 (*Rag1*); part of the 7 transmembrane receptor region of rhodopsin (*Rh*); exon 1 of the SH3 and PX domain 3 (*SH3PX3*); and two partial exons (8 and 10), the entire exon 9, and two introns (8 and 9) of the tyrosine kinase gene (*X-src*). The four mitochondrial genes sampled were complete copies of ATP synthase 8 and 6 (*ATPase 8/6*), cytochrome B (*Cytb*), cytochrome oxidase subunit 1 (*COI*), and NADH dehydrogenase subunit 2 (*ND2*). Species and genes used in the phylogeny, and all GenBank accession numbers, are provided in Table S1.

For each sequence, I removed extraneous base pairs from flanking gene segments, as well as gaps where reading frames overlapped. Protein coding genes were translated into amino acids in Se-Al Version 2.0a11 (Rambaut 1996) to verify that these sequences were not interrupted by gaps and did not contain stop codons, and hence were unlikely to be pseudogenes. All sequences were aligned with MUSCLE (Edgar 2004) and manually adjusted in Se-Al. SequenceMatrix 1.7.8 (Vaidya et al. 2011) was used to concatenate the final alignments into a supermatrix for the 40 poeciliid taxa (9825 base pairs total), with the four mitochondrial genes grouped as a single sequence (3683 bp), and the combined data set was exported as a NEXUS file (Maddison et al. 1997). PartitionFinder 1.1.1 (Lanfear et al. 2012) was used to determine the appropriate partitioning scheme and the best-fit nucleotide substitution models for the combined data set. Branch lengths were linked, and substitution models were examined with the greedy search algorithm. Models combining invariant sites and gamma distributed rates across sites were excluded from the search, since the Γ distribution can account for rate heterogeneity on its own (Yang 2006). The corrected Akaike Information Criterion served as the model selection metric, as it generally performs better than the AIC (Lanfear et al. 2014). 13 unique evolutionary models were chosen to best fit the sequence data for each codon position, and these were used in all subsequent phylogenetic analyses (Table 6).

Table 6. Molecular evolution models, subsets, partitions, and sites chosen to best fit each codon position of all nuclear and mitochondrial genes, as determined by PartitionFinder.

Best Model	**Subset**	**Subset Partitions**	**Subset Sites**
F81	1	*ENC1* Codon 1, *X-src* Codon 2	1-846, 9284-9428
TrN	2	*ENC1* Codon 2, *SH3PX3* Codon 2, *X-src* Exon 1 Codon 3	2-846, 8561-9282, 9285-9428
TVM+Γ	3, 7	*ENC1* Codon 3, *Glyt* Codon 3, *Myh6* Codon 3, *SH3PX3* Codon 3, *X-src* Exon 3 Codon 3	3-846, 849-1731, 1734-2496, 8562-9282, 9726-9825
TIM+I	4, 6	*Glyt* Codon 1, *Rag1* Codon 1, *Myh6* Codon 1, *SH3PX3* Codon 1, *X-src* Exon 3 Codon 1	847-1731, 1732-2496, 6181-7740, 8560-9282, 9724-9825
K81uf+I	5	*Glyt* Codon 2, *Myh6* Codon 2	848-1731, 1733-2496
GTR+Γ	8, 17	Combined Mitochondrial Codon 1, *X-src* Intron 1, *X-src* Intron 2	2497-6180, 9429-9529, 9607-9723
TrN+I	9, 13	Combined Mitochondrial Codon 2, *Rh* Codon 1	2498-6180, 7741-8559
TrN+Γ	10	Combined Mitochondrial Codon 3	2499-6180
TVM+I	11	*Rag1* Codon 2, *X-src* Exon 3 Codon 2	6182-7740, 9725-9825
TIM+Γ	12, 16	*Rag1* Codon 3, *X-src* Exon 1 Codon 1, *X-src* Exon 2 Codon 2	6183-7740, 9283-9428, 9531-9606
TIM+I	14	*Rh* Codon 2, *X-src* Exon 2 Codon 1	7742-8559 , 9530-9606
HKY+Γ	15	*Rh* Codon 3	7743-8559
TrNef+I	18	*X-src* Exon 2 Codon 3	9532-9606

Phylogenetic Analyses

Phylogenetic reconstructions were performed on the combined partitioned data set with maximum parsimony, maximum likelihood, and Bayesian inference. In all analyses, gaps were treated as missing data. Cladistic analysis with maximum parsimony (MP) was conducted in PAUP 4.0a147 (Swofford 2002), beginning with a heuristic search to find the shortest tree(s). Bootstrap analyses with 1000 replications were then employed, with unordered characters of equal weight, 1000 randomized stepwise addition sequences, and the tree bisection and reconnection (TBR) algorithm for branch swapping. From these replicates, a strict consensus tree and 50% majority rule support values were estimated.

Maximum likelihood (ML) analyses were achieved with two methods. RAxML-VI-HPC, Version 8.1.11 (Stamatakis 2006, 2008), operated in the Cyberinfrastructure for Phylogenetic Research (CIPRES) Science Getaway of the NSF XSEDE server (Miller et al. 2010), was first used to conduct rapid bootstrapping followed by a search for the best ML tree. The 13 distinct models and data partitions were incorporated with joint branch length optimization, and all free model parameters were estimated by RAxML. A general time reversible model with gamma rate heterogeneity (GTR+Γ) was implemented, since RAxML cannot choose simpler models. This analysis employed 500 bootstrap iterations, randomized MP starting trees, and the fast hill-climbing algorithm. GARLI 2.01 (Zwickl 2006) was also used for ML analysis. For search replicates ran for 5 million generations, based on starting randomized trees generated by a fast stepwise addition algorithm, with 100 attachments evaluated for each taxon. The default value of 20,000 generations was set as running limit before topology improvement, and the maximum score improvement required for termination was set to 0.05. Support values were obtained with the GARLI Web Service (Bazinet et al. 2014) with 500 bootstrap replicates of 5 million generations, with the limit of 10,000 generations run before encountering a better tree topology. The SumTrees utility of the DendroPy computing library (Sukamaran and Holder 2010) was used to summarize bootstrap support for clades onto the best likelihood phylogeny.

Bayesian inference (BI) was done in MrBayes Version 3.2 (Ronquist et al. 2012) using Metropolis-coupled Markov chain Monte Carlo sampling (MCMC). To estimate the posterior probability distribution, two runs of four Markov chains (three heated and one cold) were employed with random starting trees and default priors, under the GTR+Γ model of rate variation across sites. The chains were sampled every 50,000 generations, and analyses were terminated once the average standard deviation of split frequencies reached 0.001, after 14 million generations. Burn-in, or the number of samples discarded before a stationary posterior distribution is reached, was set to the default value of 25% of trees from the cold chain (3.5 million generations). Tracer 1.6 (Rambaut et al. 2014) was used to confirm that potential scale reduction factors (PSRF) approximated 1, and that effective sample sizes (ESS) exceeded 200, indicating a good sample from the posterior distribution. Trees obtained with all four methods were exported from FigTree 1.4.0 (Rambaut 2012), and edited in Adobe Illustrator CC Version 17.0.0. In all phylogenetic analyses, I considered nodes to be highly supported when Bayesian posterior probabilities (BPP) and bootstrap support percentages (BPP) exceeded 95% and well-supported when these values exceeded 75%.

Bayesian Inference of Correlated Trait Evolution

To test for correlated evolution between the ornamentation index and behavioral and morphological traits in *Poecilia,* BayesTraits Version 2.0 (Pagel and Meade 2014) was used to analyze trait evolution in a generalized least-squares (GLS) framework. Two models of evolution were compared with the BayesTraits Continuous module: dependent, in which traits are assumed to show correlated evolution on the phylogeny, and independent, in which the *testcorrel* command set zero correlation between traits. Two different sets of analyses were performed for each model: the first set used the RAxML maximum likelihood phylogram, and the second set used ten trees sampled at intervals of 50 from the GARLI bootstrap analysis (which returned the same topology as RAxML), to control for phylogenetic uncertainty. The BayesTraits Continuous Random Walk module treats all traits, including binary traits, as continuous variables. Analyses were run with a reversible-jump MCMC method, which tests alternative evolutionary scenarios for the data by simultaneously estimating each model's posterior distribution of rate parameters. Each run was set for 5 million generations and was sampled every 1,000 generations, with a burn-in of 50,000, to ensure that likelihood and probability values converge. The scaling parameter lambda (λ) was estimated to determine if the phylogeny correctly predicted patterns of similarity between species for each trait. It takes the value of 0 for phylogenetic independence, and the value of 1 for phylogenetic dependence, consistent with a constant-variance model of Brownian motion (Pagel 1999). A schedule file was produced to monitor acceptance values of the transition rate parameter (*ratedev*), which are required to be at 20–40% for correct mixing of the MCMC chain. BayesTraits uses an automatic tuning function to set a 35% acceptance rate (Pagel and Meade 2014).

Correlated evolution between behavioral traits in *Poecilia* was also evaluated, using the BayesTraits Discrete module (Pagel and Meade 2006). All recorded behavioral traits are binary in nature: sexual dichromatism, sexually selected male traits, courtship, dorsal fin erections to females, sigmoid curvature to females, sigmoid curvature to males, male-male aggression, and male behavioral variability. They were coded as either 0 (absent) or 1 (present) for every species in Table 5. The presence or absence of courtship was treated as its own category because some sources do not describe whether males of certain *Poecilia* species display sigmoid body postures or dorsal fin erections toward females (Meffe and Snelson 1989; Bisazza 1993). The RAxML maximum likelihood phylogram was pruned to remove taxa with missing data, and this was employed for the BayesTraits Discrete analyses using reversible-jump MCMC. Courtship was compared with male behavioral variability, and dorsal fin erection to females was compared to sigmoid postures to females, under both dependent and independent models of evolution. For each trait, two Markov chains were run for 5 million generations, with sampling every 1,000 steps and a burn-in of 50,000. Prior parameter values were set with a hyper-prior that seeded the mean and variance of the gamma prior from a uniform distribution in an interval from 0 to 10 (Pagel and Meade 2014), which reduces uncertainty and difficulty of prior choice.

From all BayesTraits results, the best fitting models of trait evolution were determined from the logarithm of each Bayes factor, calculated as 2[log(harmonic mean of the dependent model) – log(harmonic mean of the independent model)] (Kass and Raftery 1995). Negative log(Bayes factors) support an independent model of evolution, and positive values indicate a dependent model of evolution. log(Bayes factors) greater than 2 offer positive evidence for correlated evolution, values greater than 5 offer strong evidence, and values greater than 10 offer very strong evidence (Pagel and Meade 2014).

Finally, a second set of comparisons was implemented in Mesquite Version 3.03 (Maddison and Maddison 2015), using Pagel's (1994) correlation method to examine the relationship between the evolution of courtship and male variability, and between dorsal fin erections and sigmoid displays to females. This analysis also tests the likelihood of evolutionary models that assume independence versus dependence of binary traits under study. To estimate statistical significance of the relationship between pairs of traits, 10,000 simulation replicates were run for each, with 10 iterations used for a more intense likelihood search.

Phylogenetic Least-Squares and Logistic Regressions

Once a well-supported phylogram was obtained, it was used for two methods of phylogenetic regression analysis in MatLab (Mathworks) 7.5.0. The best tree, from the RAxML maximum likelihood analysis, was exported in Newick format as a NEXUS file and converted into a phylogenetic variance–covariance matrix in the APE package of R (Paradis et al. 2004). Morphological and behavioral traits were likewise coded into a tip data file of comparative species values in APE.

Phylogenetic generalized least-squares regressions were done in Regressionv2.m Version 14 March 2011 (Lavin et al. 2008), to determine linear relationships between the ornamentation index and the sexual selection index, relative gonopodium length, sexual size dimorphism, size dimorphism indices, and natural-log-transformed male and female standard lengths, body depths, and dorsal fin lengths. In each analysis, the output of three regression models was compared: an ordinary linear least-squares regression with the assumption that the tree topology fits a star phylogeny; a phylogenetic generalized least-squares regression with the assumption of a Brownian motion process of trait evolution; and a phylogenetic generalized least-squares regression with the assumption that trait evolution fits an Ornstein–Uhlenbeck process of stabilizing selection toward an optimum (Garland et al. 1993; Blomberg et al. 2003). If the PGLS models fit the data better than the OLS model, based on the AIC, then residual variation in the dependent variable will exhibit significant phylogenetic signal, the tendency of closely-related species to have similar phenotypes (Freckleton et al. 2002; Blomberg et al. 2003). Regressionv2.m obtains a value for this phylogenetic signal in the optimal OU transformation parameter *d*, using restricted maximum likelihood (REML). As Ornstein–Uhlenbeck regression allows branch lengths to vary, a *d* value of 0 indicates that the star phylogeny of OLS fits the data best; a value of 1 indicates that the starting tree of PGLS fits the data best; and a value between 0 and 1 indicates that branch lengths intermediate between the two provide the best fit (Lavin et al. 2008). A bootstrapping run of 2,000 simulations, generating confidence intervals with the default alpha value of 0.05, was used for each regression model to test for statistical significance of the slope of the regression line.

From the output of these runs, two methods were used to select the best fitting regression model. First, differences in the corrected Akaike Information Criterion (AICc), or Δi, were assessed ($\Delta i = \text{AICc}_i - \text{AICc}_{\text{min}}$), with AICc_{min} as the best model (having the lowest AICc value) and AICc_i as the alternative model *i*. Δi serves as a heuristic measure of the fit of the alternative model relative to the best model's fit, with $\Delta i < 2$ providing substantial support for the alternative model; $4 < \Delta i < 7$ offering less support; and $\Delta i > 10$ showing that the alternative model is extremely unlikely (Burnham and Anderson 2002). Second, when one model is a nested subset of another model (i.e. the Ornstein-Uhlenbeck model has one more parameter estimated than the OLS and PGLS models), the models can be compared with likelihood ratio tests (LRTs). Two times the difference in ln likelihoods between models (D = -2 [maximum likelihood for the best model – maximum likelihood for the alternative model]) is assumed to fit an asymptotic X^2 distribution with degrees of freedom equal to the difference in parameters between the two models. Likelihood ratio tests were also used to compare phylogenetic generalized least-squares and ordinary linear least-squares models (Burnham and Anderson 2002). While these comparisons have zero degrees of freedom, as the number of parameters is identical for each model, a difference in likelihoods that exceeds 3.841 (the ninety-fifth percentile of the X^2 distribution assuming one degree of freedom) signals a significant difference ($P < 0.05$) in the fit of the two models (Lavin et al. 2008). From the regression parameters and likelihood ratio tests, all *P* values were obtained with an online calculator (Soper 2016).

Phylogenetic logistic regressions to compare the ornamentation index with all behavioral traits were performed in PLogReg.m Version 7 September 2012 (Ives and Garland 2010), which can analyze binary dependent variables that may be shared between closely-related species. As the independent variable, the ornamentation index was standardized to have a mean of 0 and a standard deviation of 1, so that regression coefficients represent the magnitudes of its effect sizes (Ives and Garland 2010). The model of phylogenetic regression implemented the Firth correction, to reduce bias of the regression coefficients away from zero; and phylogenetic signal in the distribution of binary behavioral variables across the tree was corrected for with an initial estimate of -1. Parametric bootstrapping was run with 2,000 simulations and the default alpha value of 0.05, to obtain confidence intervals and test for statistical significance of the regression model slope. The GEE (generalized estimating equations) approximation was used to generate parameters of the logistic regression and standard errors of the estimates (Ives and Garland 2010), which converged properly in all runs.

Ancestral State Reconstructions

Patterns of evolution for the ornamentation index and several key behavioral traits were reconstructed on the RAxML maximum likelihood cladogram using two different methods. First, the index of ornamentation was plotted onto the tree topology in Mesquite with the Trace Character History command, which maps ancestral states for a continuous trait using maximum parsimony. Second, the BayesTraits Multistate module (Pagel et al. 2004) was used with the most recent common ancestor (MRCA) approach in a reversible-jump MCMC framework, to map the evolutionary history of male behavioral variability relative to courtship (N = 27 taxa), as well as the individual components of the display to males and females relative to each other (N = 26 taxa). All state changes were restricted to equal probability, reflecting the one-parameter Mk1 model of trait evolution, and a gamma hyper-prior was set to find suitable values of prior parameters under a uniform distribution. Commands setting the MRCAs were generated in TreeGraph 2 Version 2.9.2 (Stöver and Müller 2010) for the pruned RAxML phylogram bearing only taxa known to possess each trait, and MRCAs were exported in Nexus file format to BayesTraits. For each of the five traits analyzed, Markov chains were run for 5 million generations apiece, with a burn-in of 50,000 and sampling performed every 1000 generations, to achieve adequate mixing and stationarity. The resulting posterior probabilities for ancestral states at internal nodes were imported into TreeGraph 2 to be visualized as pie chart labels onto the phylogeny.

RESULTS

Phylogenetic Analyses

The three different methods – maximum parsimony, maximum likelihood, and Bayesian inference – yielded phylogenies with mostly congruent topologies. There was robust support for all major clades within *Mollienesia* and *Limia*, which were found to be topologically identical between the three methods. However, relationships among species of *Pamphorichthys*, and between each of the subgenera, were less in agreement. The MP phylogenies grouped *Limia* with *Mollienesia*, to the exclusion of *Pamphorichthys*, while the two ML trees and the BI tree placed *Limia* and *Pamphorichthys* together as sister taxa. All trees were rooted with the outgroup *Poecilia vivipara* after Pollux et al. (2014).

The most parsimonious tree (4499 steps) obtained with PAUP for the combined data set is shown in Fig. S1, and the strict consensus tree from PAUP (4539 steps) is shown in Fig. S2 with BSPs written above branches. The maximum likelihood phylogram obtained with RAxML-VI-HPC on CIPRES for the combined data set, with 13 best-fitting models, is shown in Fig. 2. Lastly, Fig. 3 shows the cladogram obtained with ML and BI methods of RAxML, GARLI, and MrBayes, with BSPs and BPPs written above branches. Weak support for a sister-group relationship between *Limia* and *Mollienesia* was recovered in the MP analysis (BSP = 50%), whereas the sister-group relationship of *Limia* and *Pamphorichthys* found with ML and BI had greater support (BSP = 55–65%, BPP = 74%). The relationships within *Pamphorichthys* also differed between the two sets of trees. *P. araguaiensis* and *P. hollandi* were placed together unequivocally (BSP and BPP = 100%), and *P. minor* and *P. scalpridens* had a consistent association as well (BSP

= 85–97%, BPP = 100%). However, MP supported the grouping of *P. araguanensis/P. hollandi* + *P. minor/P. scalpridens* (BSP = 97%), to the exclusion of *P. hasemani* (BSP = 74%), while ML and BI found *P. hasemani* was basal to *P. araguaensis* and *P. hollandi* (BSP = 64–81%), to the exclusion of *P. minor* and *P. scalpridens* (BSP = 85%, BPP = 100%). In addition, there was a discrepancy between the methods in the placement of *Psychropoecilia hispaniolana* and *Pseudolimia heterandria*. Although these two taxa were found to be basal to *Limia* in all analysis, the MP tree put *P. hispaniolana* closest to *Limia* (BSP = 100%), followed by *P. heterandria* (BSP = 57%), whereas the ML and BI trees placed *P. heterandria* as basal to *Limia* (BSP = 53%, BPP = 61%), with *P. hispaniolana* as the next closest taxon (BSP = 94–96%, BPP = 100%).

Limia as a subgenus was recovered as monophyletic (BSP = 97–99%, BPP = 100%), and a basal split separated the Jamaican *L. melanogaster* from all other species (BSP = 97–99%, BPP = 100%). A Hispaniolan clade comprising the slender-bodied species *L. zonata* and *L. versicolor* was the next to branch off (BSP and BPP = 100%), followed by a clade linking the Cuban *L. vittata* as the sister taxon of *L. caymanensis*, from the Cayman Islands (BSP and BPP = 100%). The remaining *Limia* are all found in Hispaniola, beginning with the next species pair to branch off, *L. pauciradiata* and *L. dominincensis* (BSP = 94–99%, BPP = 100%). Two species complexes were recovered with strong support of 99–100% BSP and BPP: a clade linking *L. nigrofasciata* with the sister taxa of *L. garnieri* and *L. grossidens*, and a clade of *L. sulfurophila* + *L. perugiae* + *L. melanonotata* + *L. rivasi* + *L. tridens*. These groupings were concordant in all analyses even though some support values for nodes within each complex were less robust.

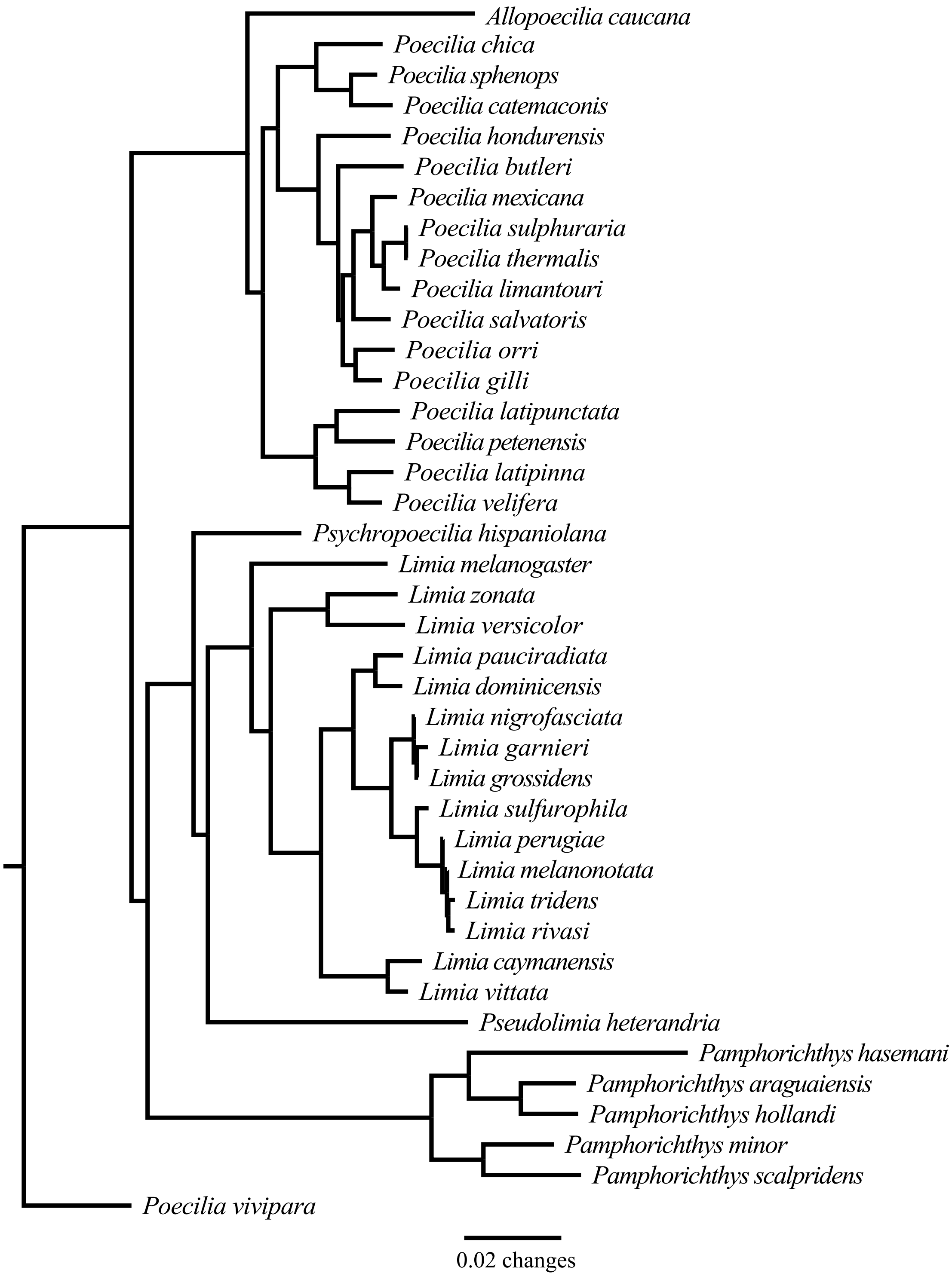

Figure 2. ML phylogram (ln likelihood = -34650.360931) obtained with RAxML-VI-HPC on CIPRES for the combined data set with 13 best-fitting models.

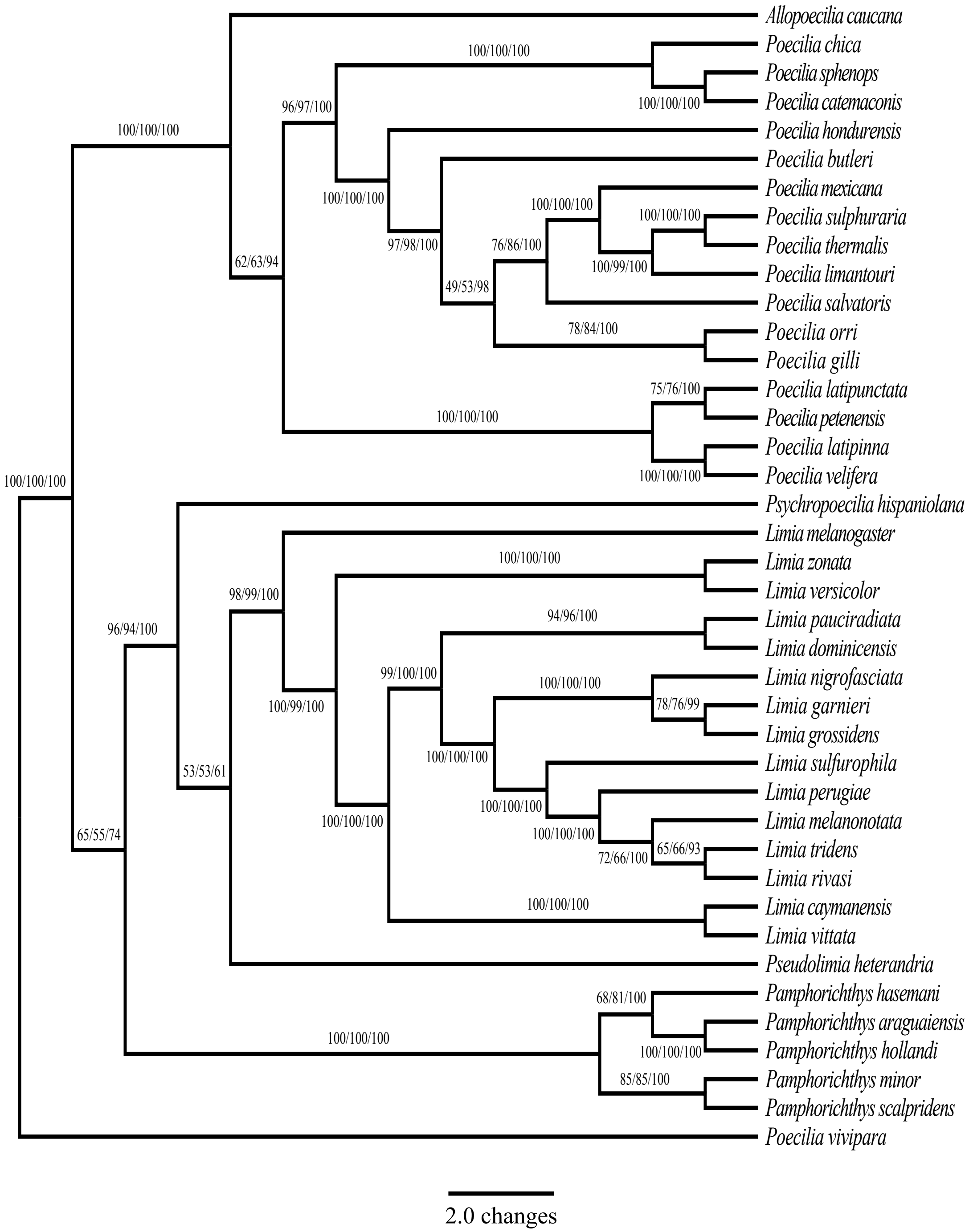

Figure 3. Cladogram obtained with RAxML, GARLI, and MrBayes for the combined data set with 13 best-fitting models. Values by each branch correspond to ML bootstrap support percentages obtained with RAxML (first), ML bootstrap support percentages obtained with GARLI (second), and Bayesian posterior probabilities obtained with MrBayes (third), respectively.

The sampled *Mollienesia* formed a single well-supported clade (BSP and BPP = 100%). *Allopoecilia caucana* was basal to this group, which could be divided into two monophyletic clades, the sailfin mollies (BSP = 98–100%, BPP = 100%), and the shortfin mollies (BSP = 89–97%, BPP = 100%). The sailfin mollies were found to split into the pairings of *P. velifera* + *P. latipinna* and *P. petenensis* + *P. latipunctata*, although the former group was more highly supported. A separation of two major species complexes was also discovered for the shortfin mollies. One linked *P. chica* with the sister taxa of *P. catemaconis* + *P. sphenops* (BSP and BPP = 100%), while the other progressively narrowed from *P. hondurensis* to *P. butleri* to *P. gilli* + *P. orri* (a pair with 78–84% BSP and 100% BPP) to *P. salvatoris* and lastly, to a robust clade of sulfide-associated mollies. Within this group, *P. limantouri* was closest to the sister taxa of *P. sulphuraria* and *P. thermalis* (BSP and BPP = 100%), and *P. mexicana* was basal to these species.

Bayesian Inference of Correlated Trait Evolution

The results of the BayesTraits Continuous analysis indicate that the ornamentation index has evolved in a correlated fashion with multiple behavioral and morphological traits within *Poecilia* (Tables 7 and 8). Furthermore, the results of BayesTraits Discrete analysis and Pagel's method reveal that behavioral traits have also evolved in a correlated fashion (Tables 9 and 10). In pairwise comparisons, there was very strong evidence for a correlation between evolution of courtship and male behavioral variability (log(Bayes factor) = 10.6983, $P = 0.0003$), and between evolution of dorsal fin erection and sigmoid postures toward females (log(Bayes factor) = 10.9254, $P < 0.0001$), offering evidence that these two behaviors have evolved together.

Table 7. BayesTraits results indicating the strength of evidence for correlated evolution between the ornamentation index and behavioral and morphological traits within the genus *Poecilia*, with a single phylogenetic tree used.

Trait	Number of Taxa	Log(Harmonic Mean) of Dependent Model*	Log(Harmonic Mean) of Independent Model†	Log(Bayes Factor)
Sexual Dichromatism	32	-1.8976	-1.8085	-0.1782[a]
Sexual Selection Index	32	-30.4665	-38.4076	15.8820[d]
Male Sexually Selected Traits	32	-1.7078	-13.1450	22.8743[d]
Courtship	29	-21.4374	-23.2469	3.6190[b]
Sigmoid Posture to Females	26	-19.8684	-21.4340	3.1318[b]
Sigmoid Posture to Males	26	-13.0275	-13.5367	1.01843[a]
Dorsal Fin Erection to Females	27	-20.6781	-23.1852	5.0141[c]
Male Behavioral Variability	27	-21.5011	-21.9961	-0.9900[a]
Relative Gonopodium Length	32	76.0383	71.4848	9.1071[c]
ln(Male Standard Length)	32	-3.5864	-7.2685	7.3642[c]
ln(Female Standard Length)	32	1.5037	-0.3161	3.6395[b]
ln(Male Body Depth)	32	-3.0483	-9.1280	12.1598[d]
ln(Female Body Depth)	32	-3.5562	-5.1279	3.1643[b]
ln(Male Dorsal Fin Length)	32	-16.3139	-27.0224	21.4171[c]
ln(Female Dorsal Fin Length)	32	-15.0640	-20.5851	11.0422[d]
Size Dimorphism Index Standard Length	32	14.7235	10.8498	7.7474[c]
Sexual Size Dimorphism Standard Length	32	19.6663	14.0239	11.2848[d]
Size Dimorphism Index Body Depth	32	3.6894	1.4529	4.4730[b]
Sexual Size Dimorphism Body Depth	32	8.1815	5.3302	5.7026[c]
Size Dimorphism Index Dorsal Fin Length	32	7.4598	-3.1981	21.3159[d]
Sexual Size Dimorphism Dorsal Fin Length	32	11.9060	3.7478	16.3164[d]

*The dependent model assumes a correlation between traits. †The independent model sets the correlation to zero. Evidence for a correlation ranges from weak (a) to positive (b) to strong (c) to very strong (d).

Table 8. BayesTraits results indicating the strength of evidence for correlated evolution between the ornamentation index and behavioral and morphological traits within *Poecilia*. A subset of ten trees was used to control for phylogenetic uncertainty.

Trait	Number of Taxa	Log(Harmonic Mean) of Dependent Model*	Log(Harmonic Mean) of Independent Model†	Log(Bayes Factor)
Sexual Dichromatism	32	-1.8832	-1.9856	0.2048[a]
Sexual Selection Index	32	-33.3492	-41.9853	17.2721[d]
Male Sexually Selected Traits	32	-1.5308	-15.5234	27.9852[d]
Courtship	29	-22.8765	-26.0971	6.4412[c]
Sigmoid Posture to Females	26	-22.4859	-24.1102	3.2485[b]
Sigmoid Posture to Males	26	-20.2499	-22.5510	4.6022[b]
Dorsal Fin Erection to Females	27	-22.2054	-26.9851	9.5594[c]
Male Behavioral Variability	27	-22.5080	-23.1564	1.2968[a]
Relative Gonopodium Length	32	67.2211	65.7003	3.0415[b]
ln(Male Standard Length)	32	-8.7467	-14.1220	10.7506[d]
ln(Female Standard Length)	32	-3.2049	-5.4953	4.5808[b]
ln(Male Body Depth)	32	-9.1111	-14.6619	11.1015[d]
ln(Female Body Depth)	32	-10.7541	-11.7418	1.9754[a]
ln(Male Dorsal Fin Length)	32	-22.7058	-32.3330	19.2543[d]
ln(Female Dorsal Fin Length)	32	-19.8091	-25.4755	11.3329[d]
Size Dimorphism Index Standard Length	32	13.8746	7.3067	13.1359[d]
Sexual Size Dimorphism Standard Length	32	17.7437	10.0291	15.4293[d]
Size Dimorphism Index Body Depth	32	2.4734	-0.5416	6.0301[c]
Sexual Size Dimorphism Body Depth	32	6.1246	2.5775	7.0942[c]
Size Dimorphism Index Dorsal Fin Length	32	4.2195	-7.3370	23.1130[d]
Sexual Size Dimorphism Dorsal Fin Length	32	8.5661	-0.0341	17.2005[d]

*The dependent model assumes a correlation between traits. †The independent model sets the correlation to zero. Evidence for a correlation ranges from weak (a) to positive (b) to strong (c) to very strong (d).

Table 9. BayesTraits results indicating the strength of evidence for correlated evolution in *Poecilia* between courtship and male behavioral variability, and between fin erection to females and sigmoid curvature to females.

Pairs of Traits	Number of Taxa	Log(Harmonic Mean) of Dependent Model*	Log(Harmonic Mean) of Independent Model†	Log(Bayes Factor)
Courtship and Male Behavioral Variability	27	-35.5778	-40.926944	10.6983[d]
Dorsal Fin Erection to Females and Sigmoid Posture to Females	26	-36.0751	-41.5378	10.9254[d]

*The dependent model assumes a correlation between traits. †The independent model sets the correlation to zero. Evidence for a correlation ranges from weak (a) to positive (b) to strong (c) to very strong (d).

Table 10. Results of Pagel's correlation test in Mesquite to investigate the relationship between courtship and male behavioral variability, and between fin erection to females and sigmoid curvature to females. *P* values were obtained through 10,000 simulations with 10 likelihood iterations per replicate.

Pairs of Traits	Number of Taxa	ln(Likelihood) of Dependent Model	ln(Likelihood) of Independent Model	Difference	*P*
Courtship and Male Behavioral Variability	27	-27.2969	-34.5911	7.2942	0.0003*
Dorsal Fin Erection to Females and Sigmoid Posture to Females	26	-25.5828	-34.3785	8.7957	< 0.0001*

*Indicates dependent evolution of the two traits.

Phylogenetic Least-Squares and Logistic Regressions

Based on the results of Regressionv2.m analyses, the AICc, Δi, and LRT values indicated that the RegOU model fit the data best for relative gonopodium length and the natural-log-transformed size variables, while OLS was the best-fitting model for the sexual size dimorphism and size dimorphism indices (Tables 11 and 12). Phylogenetic generalized least-squares regressions showed a significant negative correlation between relative gonopodium length and the index of ornamentation, demonstrating that species with larger dorsal fins tend to have shorter intromittent organs ($F = 23.141$, $P < 0.001$, Fig. 5A). All other variables had a significant positive correlation with the index of ornamentation. In species with taller dorsal fins, males exhibit larger size relative to females for standard length ($F = 14.7912$, $P = 0.0006$, Fig. 4A), body depth ($F = 6.659$, $P = 0.015$, Fig. 4C), and dorsal fin length ($F = 29.4654$, $P < 0.0001$, Fig. 4E). The decrease in female-biased sexual size dimorphism, for both indices examined, is linked to an increased index of ornamentation in these species ($F = 26.4708$, $P < 0.001$, Fig. 5B). Overall, a positive allometric trend toward greater size can be seen in highly ornamented species, for both males (ln(standard length): $F = 14.6619$, $P = 0.0006$; ln(body depth): $F = 20.9762$, $P < 0.0001$; ln(dorsal fin length): $F = 29.0842$, $P < 0.0001$; Fig. 4G, H, I blue lines) and females (ln(standard length): $F = 4.4858$, $P = 0.0426$; ln(body depth): $F = 4.6476$, $P = 0.0392$; ln(dorsal fin length): $F = 10.193$, $P = 0.0033$; Fig. 4G, H, I red lines). This relationship is evident in the two largest poeciliids sampled – the sailfin mollies *P. velifera* and *P. petenensis* – which exhibit the longest and deepest bodies, and the longest dorsal fin bases, in both sexes.

Table 11. Statistical tests, implemented in Regressionv2.m, to study variation in the sexual selection index and morphological traits within the genus *Poecilia*, as a function of the ornamentation index. The best-fitting model for each trait is listed in bold.

Regression Parameters							Fit Parameters			
Model		Coeff.	SE	df	*F*	*P*	r^2	*d*	ln(ML)	AICc
Sexual Selection Index										
OLS	**Y-int.**	**0.7744**	**0.1711**	**30**	**20.4935**	**< 0.0001**	**0.4688**		**-27.741**	**62.338†**
	Slope	**2.2965**	**0.4463**	**30**	**26.4708**	**< 0.0001**				
PGLS	Y-int.	-0.1628	1.1970	30	0.0185	0.8927	0.7313		-51.546	109.949
	Slope	5.9513	0.6452	30	85.0836	< 0.0001				
RegOU	Y-int.	0.7715	0.1716	30	20.2259	< 0.0001	0.4695	6.9389 x 10^{-21}	-27.837	65.156†
	Slope	2.3063	0.4476	30	26.5478	< 0.0001				
Relative Gonopodium Length										
OLS	Y-int.	0.2480	0.0095	30	688.1656	< 0.0001	0.0802		64.925	-122.993
	Slope	-0.0399	0.0246	30	2.6171	0.1162				
PGLS	Y-int.	0.2267	0.0251	30	81.4417	< 0.0001	0.5685		72.101	-137.344
	Slope	-0.0086	0.0137	30	39.5219	< 0.0001				
RegOU	**Y-int.**	**0.2542**	**0.0108**	**30**	**548.7172**	**< 0.0001**	**0.4355**	**0.5615**	**74.113**	**-138.744***
	Slope	**-0.0776**	**0.0016**	**30**	**23.1410**	**< 0.0001**				
ln(Male Standard Length)										
OLS	Y-int.	3.2876	0.0859	30	1.4644 x 10^3	< 0.0001	0.2534		-5.701	18.258
	Slope	0.7152	0.2242	30	10.1799	0.0033				
PGLS	Y-int.	3.2484	0.2897	30	125.7001	< 0.0001	0.3520		-6.151	19.159
	Slope	0.6367	0.1577	30	16.2966	0.0003				
RegOU	**Y-int.**	**3.2556**	**0.0913**	**30**	**1.2704 x 10^3**	**< 0.0001**	**0.3283**	**0.3925**	**-0.787**	**11.056***
	Slope	**0.6778**	**0.1770**	**30**	**14.6619**	**0.0006**				
ln(Female Standard Length)										
OLS	Y-int.	3.4653	0.0732	30	2.2439 x 10^3	< 0.0001	0.1079		-0.557	7.971
	Slope	0.3636	0.1909	30	3.6285	0.0664				
PGLS	Y-int.	3.4690	0.2750	30	159.1784	< 0.0001	0.1017		-4.476	15.809
	Slope	0.2758	0.1497	30	3.3958	0.0752				
RegOU	**Y-int.**	**3.4631**	**0.0750**	**30**	**2.1307 x 10^3**	**< 0.0001**	**0.1301**	**0.2489**	**2.401**	**4.679***
	Slope	**0.3489**	**0.1647**	**30**	**4.4858**	**0.0426**				
ln(Male Body Depth)										
OLS	Y-int.	2.2328	0.0880	30	643.7733	< 0.0001	0.2779		-6.469	19.796
	Slope	0.7802	0.2296	30	11.5456	0.0019				
PGLS	Y-int.	2.1952	0.2790	30	61.8904	< 0.0001	0.4704		-4.948	16.753

	Slope	0.7841	0.1519	30	26.6459	< 0.0001				
RegOU	**Y-int.**	**2.1649**	**0.0967**	**30**	**501.6426**	**< 0.0001**	**0.4115**	**0.4572**	**-0.483**	**10.447***
	Slope	**0.7889**	**0.1723**	**30**	**20.9762**	**< 0.0001**				
ln(Female Body Depth)										
OLS	Y-int.	2.3461	0.0876	30	718.0225	< 0.0001	0.1218		-6.307	19.472
	Slope	0.4659	0.2284	30	4.1592	0.0503				
PGLS	Y-int.	2.4234	0.3699	30	42.9223	< 0.0001	0.0749		-13.968	34.794
	Slope	0.3138	0.2014	30	2.4281	0.1297				
RegOU	**Y-int.**	**2.3686**	**0.0874**	**30**	**734.8452**	**< 0.0001**	**0.1341**	**0.1023**	**-4.163**	**17.808***
	Slope	**0.4433**	**0.2056**	**30**	**4.6476**	**0.0392**				
ln(Male Dorsal Fin Length)										
OLS	Y-int.	1.1637	0.1336	30	75.8771	< 0.0001	0.4739		-19.828	46.513
	Slope	1.8119	0.3486	30	27.0205	< 0.0001				
PGLS	Y-int.	0.9498	0.4642	30	4.1856	0.0496	0.4913		-21.236	49.330
	Slope	1.3601	0.2527	30	28.9702	< 0.0001				
RegOU	**Y-int.**	**1.1945**	**0.1458**	**30**	**67.1191**	**< 0.0001**	**0.4923**	**0.3343**	**-15.977**	**41.435***
	Slope	**1.5381**	**0.2852**	**30**	**29.0842**	**< 0.0001**				
ln(Female Dorsal Fin Length)										
OLS	Y-int.	1.2717	0.1201	30	112.1143	< 0.0001	0.3471		-16.421	39.670
	Slope	1.2515	0.3134	30	15.9506	0.0004				
PGLS	Y-int.	1.1567	0.4555	30	6.4476	0.0165	0.0852		-20.631	48.119
	Slope	0.4145	0.2480	30	2.7950	0.1050				
RegOU	**Y-int.**	**1.3797**	**0.1271**	**30**	**117.8727**	**< 0.0001**	**0.2536**	**0.2144**	**-14.963**	**39.407***
	Slope	**0.9099**	**0.2850**	**30**	**10.1930**	**0.0033**				
Size Dimorphism Index Standard Length										
OLS	**Y-int.**	**0.2068**	**0.0416**	**30**	**24.7000**	**< 0.0001**	**0.3302**		**17.500**	**-28.143†**
	Slope	**-0.4175**	**0.1086**	**30**	**14.7912**	**0.0006**				
PGLS	Y-int.	0.2460	0.1887	30	1.6994	0.2023	0.3480		7.569	-8.280
	Slope	-0.4111	0.1027	30	16.0142	0.0004				
RegOU	Y-int.	0.2084	0.0432	30	23.2128	< 0.0001	0.3453	0.1055	18.131	-26.780†
	Slope	-0.4086	0.1027	30	15.8212	0.0004				
Sexual Size Dimorphism Standard Length										
OLS	**Y-int.**	**0.1865**	**0.0368**	**30**	**25.7224**	**< 0.0001**	**0.3422**		**21.448**	**-36.038†**
	Slope	**-0.3791**	**0.0960**	**30**	**15.6050**	**0.0004**				
PGLS	Y-int.	0.2245	0.1668	30	1.8110	0.1885	0.3635		11.512	-16.166
	Slope	-0.3759	0.0908	30	17.1318	0.0003				
RegOU	Y-int.	0.1878	0.0382	30	24.2105	< 0.0001	0.3584	0.1022	22.091	-34.700†

	Slope	-0.3718	0.0908	30	16.7585	0.0003				
Size Dimorphism Index Body Depth										
OLS	**Y-int.**	**0.1419**	**0.0588**	**30**	**5.8146**	**0.0222**	**0.1816**		**6.416**	**-5.976†**
	Slope	**-0.3961**	**0.1535**	**30**	**6.6590**	**0.0150**				
PGLS	Y-int.	0.2647	0.3287	30	0.6487	0.4269	0.2492		-10.189	27.235
	Slope	-0.5646	0.1789	30	9.9563	0.0036				
RegOU	Y-int.	0.1460	0.0589	30	6.1505	0.0190	0.1819	1.9379 x 10^{-6}	6.510	-3.538†
	Slope	-0.3940	0.1525	30	6.6715	0.0149				
Sexual Size Dimorphism Body Depth										
OLS	**Y-int.**	**0.1269**	**0.0519**	**30**	**5.9885**	**0.0205**	**0.1852**		**10.450**	**-14.044†**
	Slope	**-0.3533**	**0.1353**	**30**	**6.8183**	**0.0140**				
PGLS	Y-int.	0.2350	0.2932	30	0.6425	0.4291	0.2411		-6.529	19.915
	Slope	-0.4927	0.1596	30	9.5328	0.0043				
RegOU	Y-int.	0.1301	0.0519	30	6.2794	0.0179	0.1845	6.7902 x 10^{-7}	10.526	-11.570†
	Slope	-0.3506	0.1346	30	6.7870	0.0142				
Size Dimorphism Index Dorsal Fin Length										
OLS	**Y-int.**	**0.1573**	**0.0546**	**30**	**8.3019**	**0.0072**	**0.4955**		**8.818**	**-10.778†**
	Slope	**-0.7730**	**0.1424**	**30**	**29.4654**	**< 0.0001**				
PGLS	Y-int.	0.2798	0.2251	30	1.5442	0.2236	0.7833		1.922	3.013
	Slope	-1.2763	0.1225	30	108.4661	< 0.0001				
RegOU	Y-int.	0.1654	0.0546	30	9.1614	0.0050	0.5282	6.4808 x 10^{-4}	9.138	-8.794†
	Slope	-0.8110	0.1399	30	33.5839	< 0.0001				
Sexual Size Dimorphism Dorsal Fin Length										
OLS	**Y-int.**	**0.1273**	**0.0458**	**30**	**7.7229**	**0.0093**	**0.4751**		**14.428**	**-21.999†**
	Slope	**-0.6227**	**0.1195**	**30**	**27.1509**	**< 0.0001**				
PGLS	Y-int.	0.2175	0.1937	30	1.2611	0.2703	0.7448		6.731	-6.605
	Slope	-0.9868	0.1055	30	87.5703	< 0.0001				
RegOU	Y-int.	0.1478	0.0469	30	9.9158	0.0037	0.5479	0.0359	14.752	-20.022†
	Slope	-0.6983	0.1158	30	36.3583	< 0.0001				

* From Δ_i and likelihood ratio test (LRT) values, the RegOU model is statistically significantly better than one or both alternative models (see Table 12).

† From Δ_i and LRT values, there is no statistically significant difference in the fit between the OLS and RegOU models. Both are statistically significantly better than the PGLS model (see Table 12).

Table 12. Comparisons between models for all traits analyzed in Regressionv2.m, to find the best-fitting linear regression model for each trait.

Trait	Best vs. Alternative Model	Δi‡	X^2 for LRT§	df	*P* for LRT
Sexual Selection Index	OLS vs PGLS	47.611^{c}	$47.610^{\#}$	$0^{\#}$	$< 0.05^{\#}$
	OLS vs. RegOU	2.818	0.192	1	0.6613
Relative Gonopodium Length	RegOU vs. OLS	15.751^{c}	18.376	1	< 0.0001
	RegOU vs. PGLS	1.400^{a}	4.024	1	0.0449
ln(Male Standard Length)	RegOU vs. OLS	7.202	9.828	1	0.0017
	RegOU vs. PGLS	8.103	10.728	1	0.0011
ln(Female Standard Length)	RegOU vs. OLS	3.292	5.916	1	0.0150
	RegOU vs. PGLS	11.130^{c}	13.754	1	< 0.0001
ln(Male Body Depth)	RegOU vs. OLS	9.349	11.972	1	< 0.0001
	RegOU vs. PGLS	6.306^{b}	8.930	1	0.0028
ln(Female Body Depth)	RegOU vs. OLS	1.664^{a}	4.288	1	0.0384
	RegOU vs. PGLS	16.986^{c}	19.610	1	< 0.0001
ln(Male Dorsal Fin Length)	RegOU vs. OLS	5.078^{b}	7.702	1	0.0055
	RegOU vs. PGLS	7.895	10.518	1	0.0012
ln(Female Dorsal Fin Length)	RegOU vs. OLS	0.263^{a}	2.916	1	0.0877
	RegOU vs. PGLS	8.712	11.336	1	< 0.0001
Size Dimorphism Index Standard Length	OLS vs PGLS	19.863^{c}	$19.862^{\#}$	$0^{\#}$	$< 0.05^{\#}$
	OLS vs. RegOU	1.363^{a}	1.262	1	0.2613
Sexual Size Dimorphism Standard Length	OLS vs PGLS	19.872^{c}	$19.872^{\#}$	$0^{\#}$	$< 0.05^{\#}$
	OLS vs. RegOU	1.338^{a}	1.286	1	0.2568
Size Dimorphism Index Body Depth	OLS vs PGLS	33.211^{c}	$33.21^{\#}$	$0^{\#}$	$< 0.05^{\#}$
	OLS vs. RegOU	2.438	0.188	1	0.6646
Sexual Size Dimorphism Body Depth	OLS vs PGLS	33.959^{c}	$33.958^{\#}$	$0^{\#}$	$> 0.05^{\#}$
	OLS vs. RegOU	2.474	0.152	1	0.6966
Size Dimorphism Index Dorsal Fin Length	OLS vs PGLS	13.791^{c}	$13.792^{\#}$	$0^{\#}$	$< 0.05^{\#}$
	OLS vs. RegOU	1.984^{a}	0.640	1	0.4237
Sexual Size Dimorphism Dorsal Fin Length	OLS vs PGLS	15.394^{c}	$15.394^{\#}$	$0^{\#}$	$< 0.05^{\#}$
	OLS vs. RegOU	1.977^{a}	0.648	1	0.4208

‡When comparing models, the model with the lowest AICc is considered the best. The difference in AICc between models ($\Delta_i = \mathrm{AICc}_i - \mathrm{AICc}_{\mathrm{min}}$) indicates support for the alternative model *i*. Support may be substantial, if $\Delta i < 2$ (a); considerably less, if $4 < \Delta_i < 7$ (b); or essentially zero, if $\Delta i > 10$ (c) (Burnham and Anderson 2002).

§When one model is a nested subset of another, they can be compared with LRTs. Two times the difference in ln likelihoods between models (D = -2 [maximum likelihood for the best model – maximum likelihood for the alternative model]) is assumed to have an asymptotic X^2 distribution with degrees of freedom equal to the difference in the number of parameters in the two models.

#PGLS and OLS models have the same number of parameters. In this comparison with 0 degrees of freedom, a difference in likelihoods > 3.8414 (the ninety-fifth percentile of the X^2 distribution with 1 df) is used to indicate a significant difference ($P < 0.05$) in the fit of the two models.

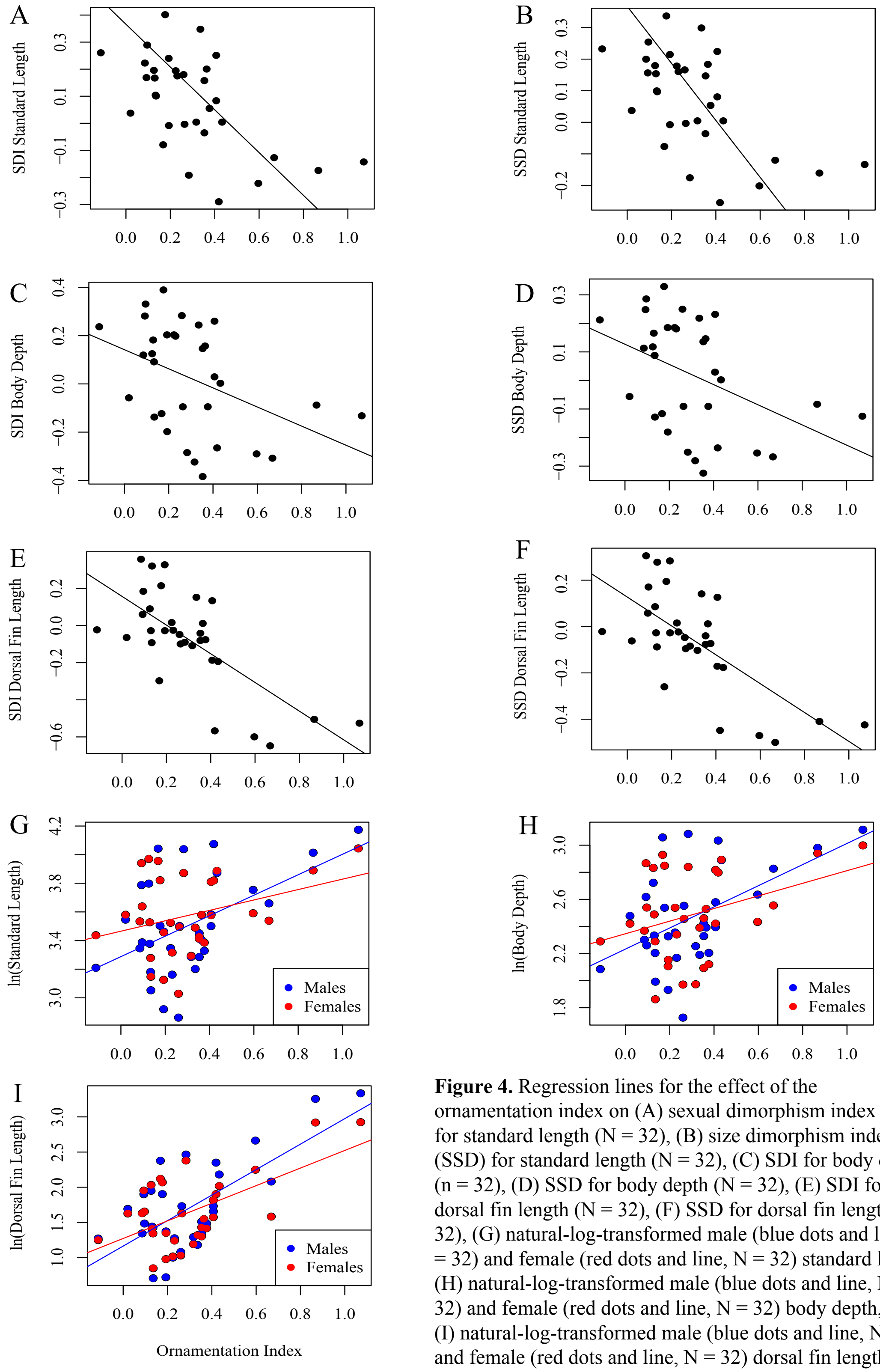

Figure 4. Regression lines for the effect of the ornamentation index on (A) sexual dimorphism index (SDI) for standard length (N = 32), (B) size dimorphism index (SSD) for standard length (N = 32), (C) SDI for body depth (n = 32), (D) SSD for body depth (N = 32), (E) SDI for dorsal fin length (N = 32), (F) SSD for dorsal fin length (N = 32), (G) natural-log-transformed male (blue dots and line, N = 32) and female (red dots and line, N = 32) standard length, (H) natural-log-transformed male (blue dots and line, N = 32) and female (red dots and line, N = 32) body depth, and (I) natural-log-transformed male (blue dots and line, N = 32) and female (red dots and line, N = 32) dorsal fin length.

The PLogReg.m results (Table 13) indicate that sexual dichromatism was not significantly associated with a high ornamentation index ($b_1 = 0.4166$, $P = 0.6004$, Fig. 5D), as sex-based differences in coloration are common in both shortfin and large-finned species. Likewise, there was no relationship between male behavioral variability and the possession of an enlarged dorsal fin ($b_1 = 0.4503$, $P = 0.2358$, Fig. 5I), indicating that maintenance of alternative reproductive strategies is not correlated with exaggeration of ornamental traits. However, there was a significant positive correlation between a high ornamentation index and the presence of courtship ($b_1 = 1.1493$, $P = 0.004$, Fig. 5C), the presence of sexually selected male traits ($b_1 = 3.0418$, $P = 0.001$, Fig. 5E), the use of dorsal fin erection to females ($b_1 = 1.0921$, $P = 0.008$, Fig. 5F), and the use of sigmoid postures to males ($b_1 = 1.0909$, $P = 0.025$, Fig. 5G) and females ($b_1 = 1.1438$, $P = 0.002$, Fig. 5H). As expected, species with the largest dorsal fins have been classified as bearing a sailfin phenotype, and these ornamented species also tend to exhibit display behaviors associated with both courtship and male-male aggression.

Ancestral State Reconstructions

Within *Poecilia*, there have been repeated shifts toward an exaggerated dorsal fin, as shown in the evolutionary history of the continuous ornamentation index (Fig. 6). The tallest fins appeared in the sailfin molly lineage, reaching index values of roughly 0.87 in *Poecilia petenensis*, and 1.1 in *Poecilia velifera*, species in which male dorsal fins dwarf those of females. Among the other sailfins, *P. latipinna* has an ornamentation index of 0.6, whereas *P. latipunctata* has a value of 0.41, consistent with its predicted loss of the enlarged dorsal fin phenotype (Ptacek and Breden 1998). Therefore, the ancestral state

Table 13. Parameter estimates for phylogenetic logistic regression analyses, implemented in PLogReg.m, to study variation in sexual dichromatism and binary behavioral traits within the genus *Poecilia*, as a function of the ornamentation index.

Parameter	Estimate	SE	Bootstrap mean	Bootstrap 95% CI	Bootstrap *P*
Sexual Dichromatism					
Phylogenetic Logistic Regression with the Firth Correction					
a	-4.0000		-2.2262	(-4.0000, 4.0000)	0.3644
b_0 (Y-int.)	2.4812	0.7115	1.6630	(0.2378, 2.8458)	0.0166
b_1 (OI)	0.4166	0.8250	0.3608	(-0.4985, 2.2768)	0.6004
Courtship Behavior					
Phylogenetic Logistic Regression with the Firth Correction					
a	-4.0000		-3.1117	(-4.0000, -0.7381)	0.5305
b_0 (Y-int.)	-0.4387	0.4311	-0.4349	(-1.3407, 0.3559)	0.2750
b_1 (OI)	1.1493	0.5742	1.1556	(0.2692, 2.7669)	0.0040
Sexually Selected Male Traits					
Phylogenetic Logistic Regression with the Firth Correction					
a	4.0000		2.6537	(-4.0000, 4.0000)	0.0866
b_0 (Y-int.)	-1.6575	1.5807	-1.2322	(-2.5170, 1.5400)	0.3345
b_1 (OI)	3.0418	1.3341	3.3426	(1.5792, 7.5508)	0.0010
Sigmoid Posture to Females					
Phylogenetic Logistic Regression with the Firth Correction					
a	-4.0000		-3.0237	(-4.0000, -0.6039)	0.5190
b_0 (Y-int.)	-0.5260	0.4580	-0.5000	(-1.389, 0.3612)	0.2380
b_1 (OI)	1.1438	0.6075	1.1595	(0.2912, 2.9068)	0.0020
Sigmoid Posture to Males					
Phylogenetic Logistic Regression with the Firth Correction					
a	-4.0000		-2.9598	(-4.0000, -0.3844)	0.4662
b_0 (Y-int.)	0.6909	0.4669	0.6688	(-0.2020, 1.7531)	0.1342
b_1 (OI)	1.0909	0.6665	1.1275	(0.0927, 3.2183)	0.0250
Dorsal Fin Erection to Females					
Phylogenetic Logistic Regression with the Firth Correction					
a	-4.0000		-3.0759	(-4.0000, -0.7584)	0.5260
b_0 (Y-int.)	-0.4002	0.4337	-0.3761	(-1.2886, 0.4538)	0.3690
b_1 (OI)	1.0921	0.5657	1.1118	(0.2347, 2.7362)	0.0080
Male Behavioral Variability					
Phylogenetic Logistic Regression with the Firth Correction					
a	-3.9999		-2.8921	(-4.0000, -0.5260)	0.4305
b_0 (Y-int.)	-0.8184	0.4398	-0.7677	(-1.6521, -0.0146)	0.0462
b_1 (OI)	0.4503	0.4122	0.4567	(-0.3315, 1.4397)	0.2358

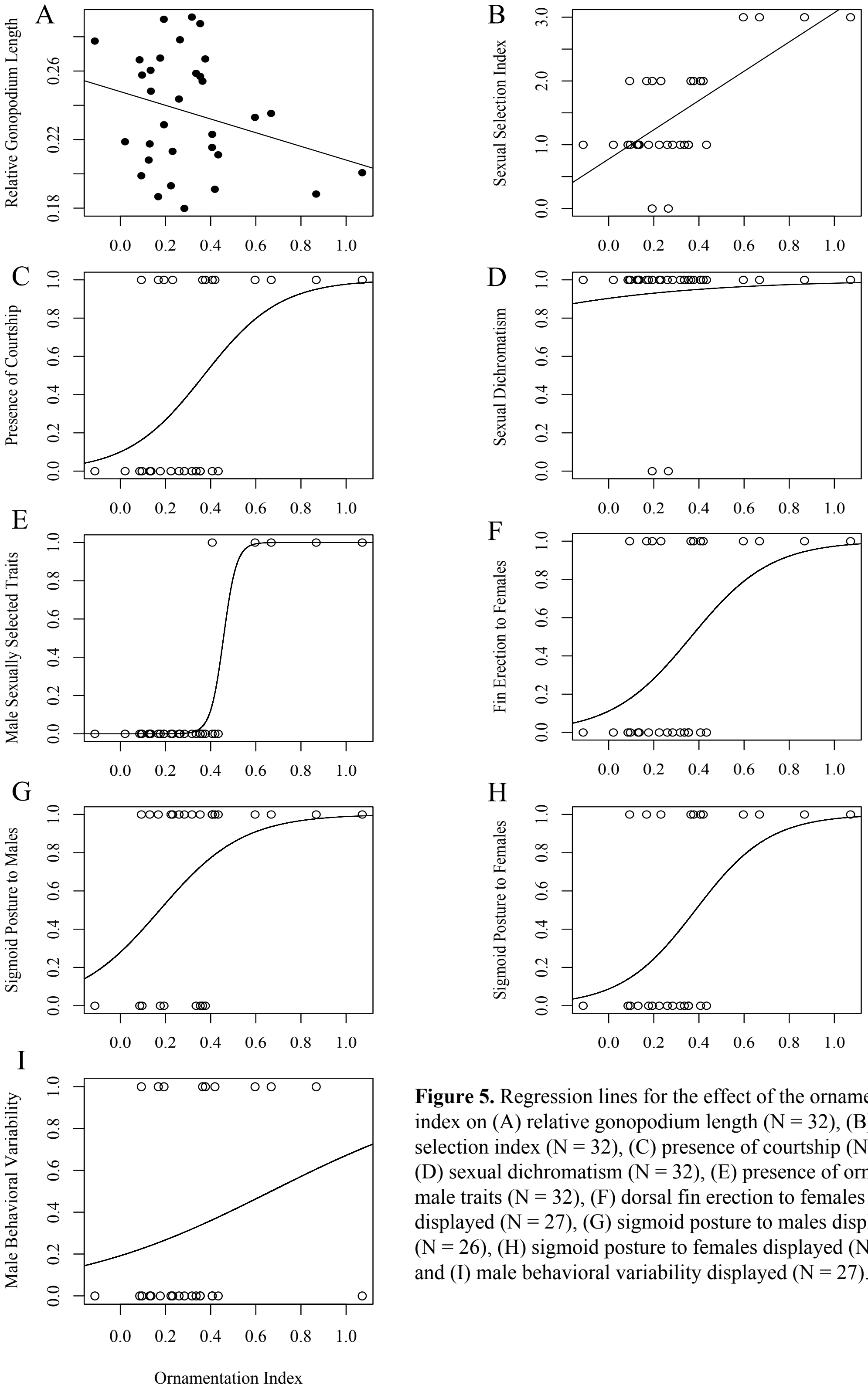

Figure 5. Regression lines for the effect of the ornamentation index on (A) relative gonopodium length (N = 32), (B) sexual selection index (N = 32), (C) presence of courtship (N = 29), (D) sexual dichromatism (N = 32), (E) presence of ornamental male traits (N = 32), (F) dorsal fin erection to females displayed (N = 27), (G) sigmoid posture to males displayed (N = 26), (H) sigmoid posture to females displayed (N = 26), and (I) male behavioral variability displayed (N = 27).

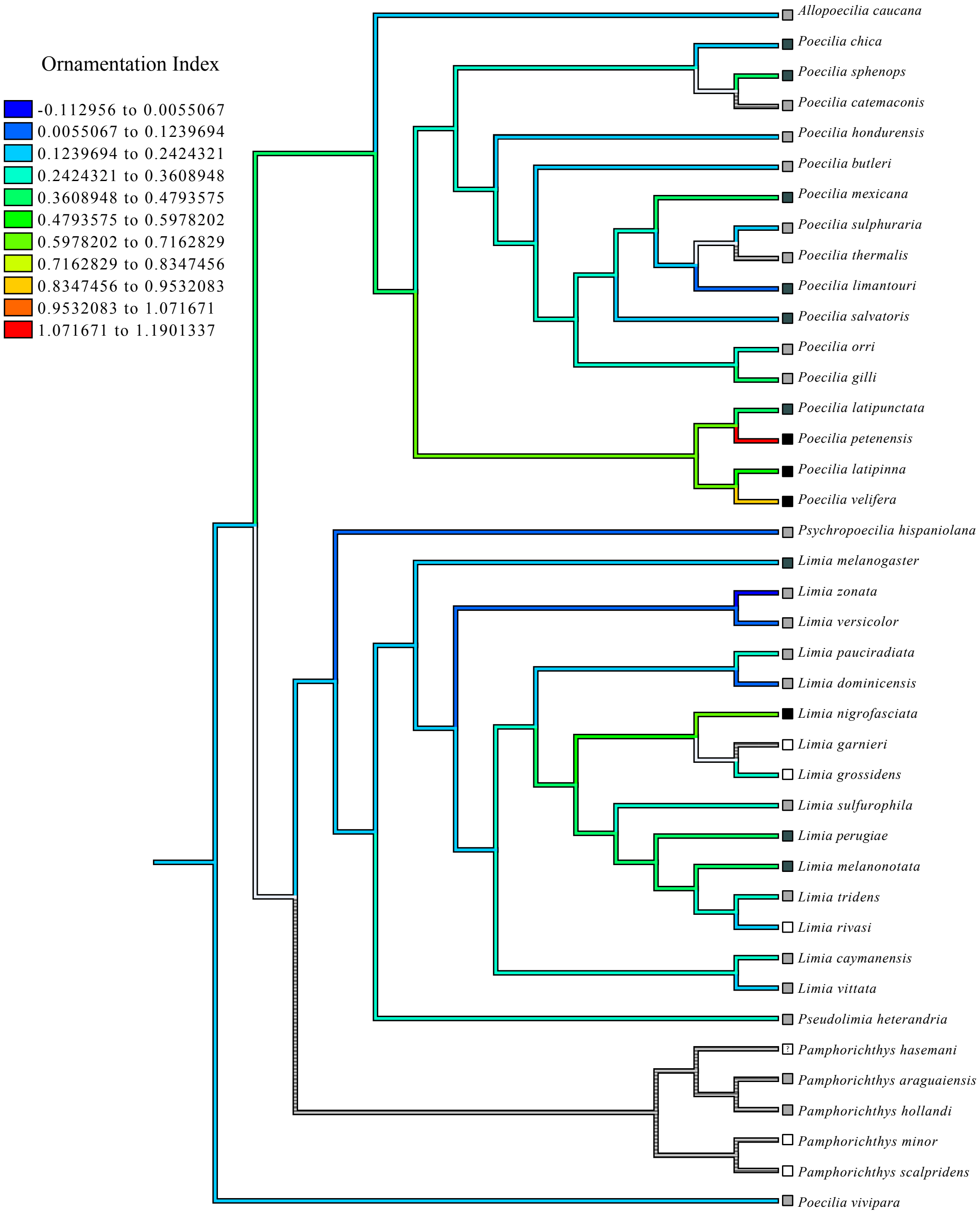

Figure 6. Ultrametric tree for 40 species of livebearers within the genus *Poecilia*. Boxes at branch tips are colored for the male sexual selection index: black = 3, dark gray = 2, light gray = 1, and white = 0. The question mark within the box for *Pamphorichthys hasemani* indicates incomplete information (see Table 5). Branch colors correspond to a maximum parsimony reconstruction of the ornamentation index, mapped in Mesquite.

reconstruction of this ornamental trait in the sailfin mollies is of a moderately enlarged fin that became hypertrophied in the two largest species, and that shrank in the smallest species. Among the shortfin species of *Mollienesia*, the ornamentation index is highest in one courting species (*P. mexicana*, OI = 0.42), and two non-courting species (*P. gilli*, OI = 0.43; and *P. sphenops*, OI = 0.41). An exaggerated dorsal fin has also evolved in *Limia nigrofasciata*, (OI = 0.66), although other courting species within the subgenus, such as *L. perugiae* (OI = 0.36) and *L. melanonotata* (OI = 0.38), have smaller fins. The lowest value (OI = -0.11) is found in *L. zonata*, indicating that female dorsal fins are actually larger than male fins in this species. From a predicted ancestral value of 0.22 (in *P. vivipara*), male dorsal fin height in *Poecilia* has reached huge sizes on two separate occasions, and has diminished to reverse sexual dimorphism in a single case.

The ancestral character state estimation for courtship and alternative reproductive tactics revealed that these two male traits fit a highly synchronous pattern of evolution across the phylogeny (Fig. 7). As shown in Table 14, a shift to courtship was recovered twice in *Mollienesia* (in the sailfin molly clade, posterior probability = 0.88; and in the *P. mexicana* complex, PP = 0.92), and twice in *Limia* (in *L. nigrofasciata*, PP = 0.81; and in the *L. perugiae* complex, PP = 1.0). Although *L. melanogaster* males court as well, there was not sufficient support in favor of a transition at this node. The evolution of courtship display components (sigmoid postures and dorsal fin erection) was reconstructed with similar posterior probabilities for each of the clades mentioned above (Fig. 8). Male behavioral variability had comparable posterior probabilities to courtship for most of the same nodes, but two losses were found in the *P. sphenops* complex (PP = 0.72) and the pair of *P. latipunctata* + *P. petenensis* (PP = 0.73). All of these traits were estimated to be absent at the root of *Poecilia*. The strong phylogeny-trait associations lend support to at least four separate origins of courtship within *Mollienesia* and *Limia*, while phylogenetic studies have validated a fifth in *L. melanogaster* (Hamilton 2001).

On the other hand, the ancestral character state estimation reconstructed sigmoid postures to males as present at the root of *Poecilia* (PP = 0.62). This display was found to be the ancestral state for the sailfin and shortfin *Mollienesia* (PP = 0.92), and was further recovered at every node within this subgenus that had available behavioral data (Fig. 8). The opposite case was predicted for *Limia*, in which a lack of sigmoid postures to males was found to be ancestral (PP = 0.93). Despite an apparent loss in the common ancestor of *Limia*, this trait reappeared in *L. nigrofasciata* (PP = 0.85), a species that also uses sigmoid postures to females; and in two non-courting species within the *L. perugiae* complex. The two courting species in this clade (*L. perugiae* and *L. menanonotata*) both perform sigmoid postures to females, but not to males. These results are not surprising, given that male-male dorsal fin erections were observed in every species with behavioral data available, indicating that this aggressive display preceded the appearance of dorsal fin erections to females, and is not linked to the use of sigmoid curvature in male-male agonistic encounters. There is thus evidence of an ornament transition from male-male competition to courtship of females in *Mollienesia*, occurring for dorsal fin erections and body curvature at around the same time. In *Limia*, the evidence only favors co-option of dorsal fin erections, as the history of sigmoidal body postures is unclear for the few species that have evolved them.

Table 14. BayesTraits posterior probabilities of ancestral state reconstructions for courtship, male behavioral variability, and display components to females and males. Higher values for a given trait for each clade are listed in bold.

Clade	Courtship		Male Behavioral Variability		Dorsal Fin Erection to Females		Sigmoid Posture to Females		Sigmoid Posture to Males	
	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present
Poecilia (Root)	**0.5644**	0.4356	**0.5540**	0.4460	**0.5695**	0.4305	**0.5705**	0.4295	0.3828	**0.6172**
Poecilia (Mollienesia) + Limia + Pamphorichthys	0.4688	**0.5312**	0.4981	**0.5019**	0.4740	**0.5260**	0.4760	**0.5240**	**0.5973**	0.4027
Mollienesia + Allopoecilia caucana	0.3321	**0.6679**	0.4699	**0.5301**	-	**-**	-	**-**	-	**-**
Poecilia (Mollienesia)	0.2858	**0.7142**	0.4519	**0.5481**	0.3027	**0.6973**	0.3067	**0.6933**	0.0753	**0.9247**
Poecilia sphenops complex + *Poecilia mexicana* complex	0.4300	**0.5700**	0.5242	**0.4758**	0.4486	**0.5514**	0.4524	**0.5476**	0.1060	**0.8940**
P. chica + P. sphenops + P. catemaconis	0.4576	**0.5424**	**0.7184**	0.2816	0.4714	**0.5286**	0.4738	**0.5262**	0.1057	**0.8943**
P. orri + P. gilli + P. salvatoris + P. mexicana	0.3927	**0.6073**	0.2947	**0.7053**	0.4193	**0.5808**	0.4234	**0.5766**	0.0148	**0.9852**
P. orri + P. gilli	**0.9005**	0.0995	**0.8665**	0.1335	**0.9038**	0.0962	**0.9032**	0.0968	0.0374	**0.9626**
P. salvatoris + P. mexicana	0.0794	**0.9206**	0.0599	**0.9401**	0.0863	**0.9137**	0.0875	**0.9125**	0.0301	**0.9699**
P. mexicana + P. limantouri + P. sulphuraria + P. thermalis	0.1049	**0.8951**	0.0740	**0.9260**	0.1151	**0.8849**	0.1166	**0.8834**	0.0149	**0.9851**
P. limantouri + P. sulphuraria + P. thermalis	0.3297	**0.6703**	0.2593	**0.7407**	0.3486	**0.6514**	0.3513	**0.6487**	0.0160	**0.9840**

P. latipunctata + *P. petenensis* + *P. latipinna* + *P. velifera*	0.1246	**0.8754**	0.3447	**0.6553**	0.1321	**0.8679**	0.1339	**0.8661**	0.0597	**0.9403**
P. latipunctata + *P. petenensis*	0.1688	**0.8312**	**0.7294**	0.2706	0.1764	**0.8236**	0.1783	**0.8217**	0.0991	**0.9009**
P. latipinna + *P. velifera*	0.0863	**0.9137**	0.0718	**0.9282**	0.0918	**0.9082**	0.0930	**0.9070**	0.0492	**0.9508**
Limia + *Pseudolimia heterandria*	**0.5354**	0.4646	**0.5072**	0.4928	**0.5444**	0.4556	**0.5458**	0.4542	**0.8193**	0.1807
Limia	**0.5482**	0.4518	**0.5025**	0.4975	**0.5614**	0.4386	**0.5634**	0.4366	**0.9320**	0.0680
L. zonata + *L. versicolor* + Ingroup	**0.6609**	0.3391	**0.6042**	0.3958	**0.6703**	0.3297	**0.6711**	0.3289	**0.9295**	0.0705
L. zonata + *L. versicolor*	**0.7313**	0.2687	**0.6848**	0.3152	**0.7368**	0.2632	**0.7368**	0.2632	**0.9418**	0.0582
L. caymanensis + *L. vittata* + Ingroup	**0.6770**	0.3230	**0.6038**	0.3962	**0.6912**	0.3088	**0.6924**	0.3076	**0.9193**	0.0807
L. caymanensis + *L. vittata*	**0.9266**	0.0734	**0.8988**	0.1012	**0.9253**	0.0707	**0.9287**	0.0713	**0.9912**	0.0088
L. pauciradiata + *L. dominicensis* + Ingroup	**0.6090**	0.3910	**0.5232**	0.4768	**0.6295**	0.3705	**0.6322**	0.3678	**0.8112**	0.1888
L. pauciradiata + *L. dominicensis*	**0.9224**	0.0776	**0.8932**	0.1068	**0.9252**	0.0748	**0.9246**	0.0754	**0.9907**	0.0093
L. nigrofasciata complex + *L. perugiae* complex	0.1890	**0.8110**	0.1434	**0.8566**	0.2037	**0.7963**	0.2062	**0.7938**	0.1516	**0.8484**
L. sulfurophila + Ingroup	**0.5950**	0.4050	**0.5104**	0.4896	**0.6151**	0.3849	**0.6178**	0.3822	**0.5112**	0.4888
L. perugiae + Ingroup	0.0003	**0.9997**	0.0002	**0.9998**	0.0003	**0.9997**	0.0003	**0.9997**	**1.0000**	0.0000
L. melanonotata + *L. tridens* + *L. rivasi*	0.0023	**0.9977**	0.0015	**0.9985**	0.0026	**0.9974**	0.0026	**0.9974**	**0.9982**	0.0018

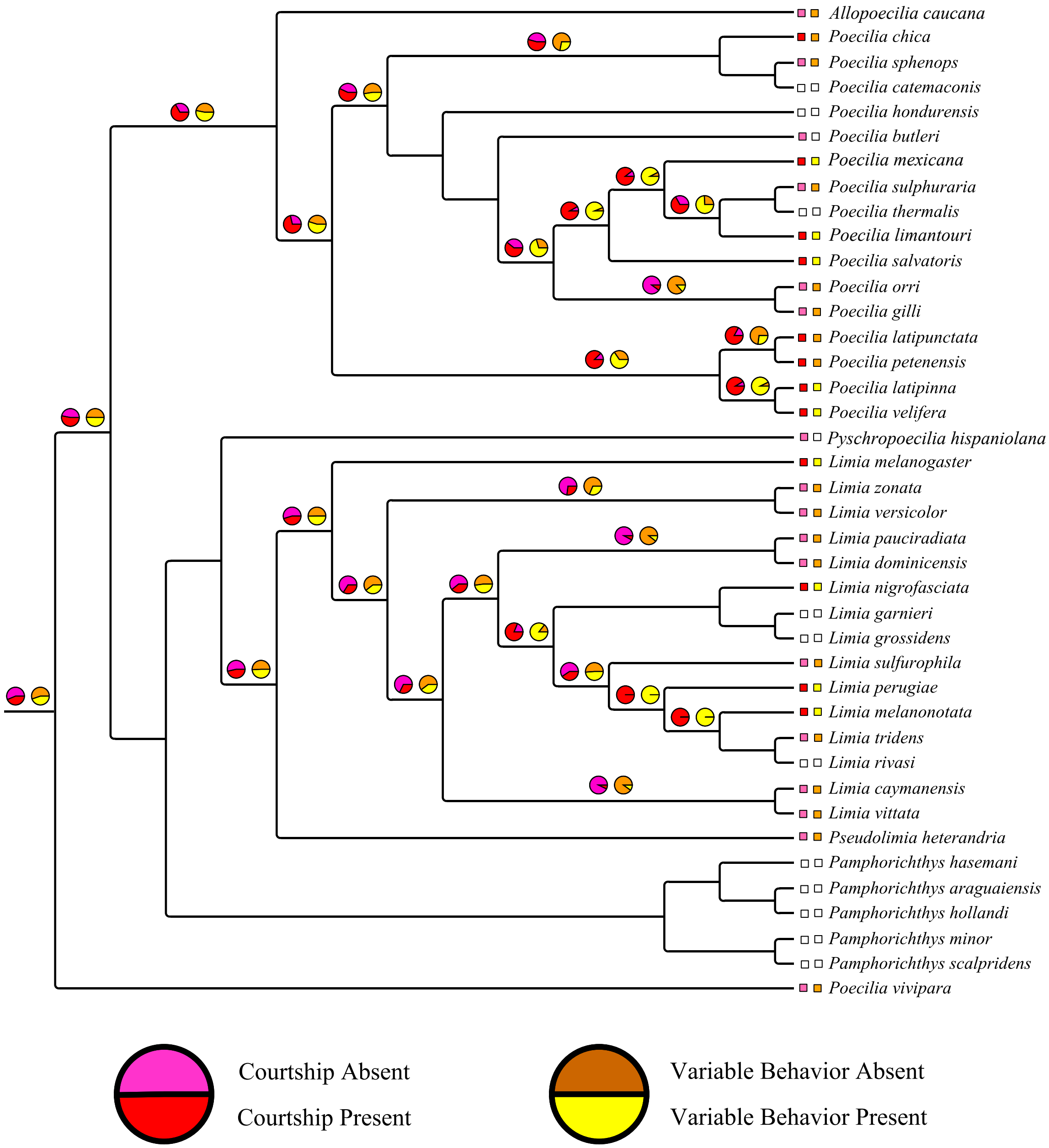

Figure 7. Ancestral character state reconstruction for the presence of display behavior in the subgenera *Mollienesia* and *Limia*: courtship (above the branch left) and male behavioral variability (above the branch right). The pie charts plotted on the maximum likelihood cladogram represent posterior probabilities of the occurrence of each state. Boxes at branch tips represent states for each taxon; white boxes indicate that no behavioral data is available. Courtship is classified as absent (pink) or present (red). Alternative reproductive tactics are classified as absent (orange) or present (yellow).

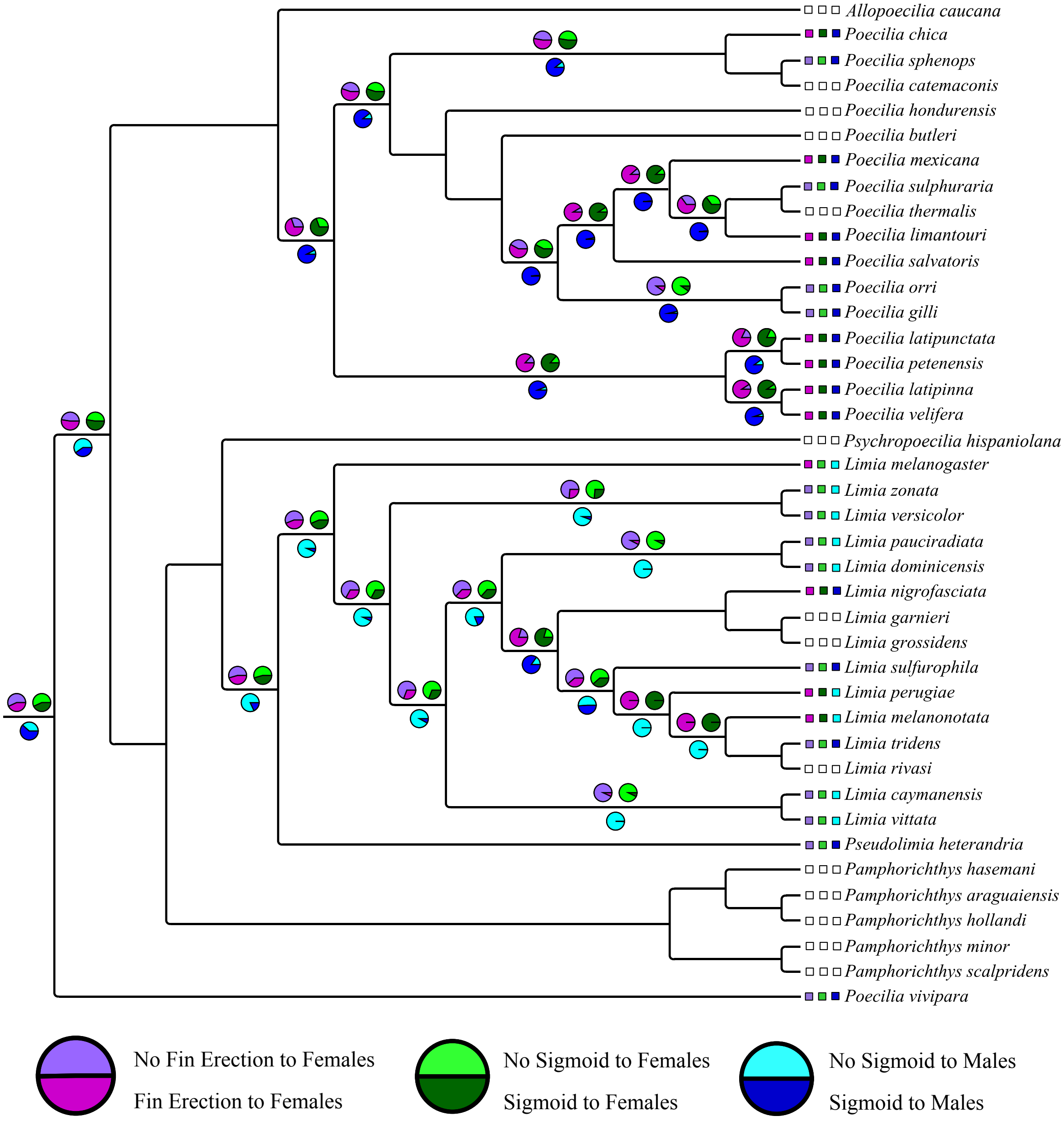

Figure 8. Ancestral character state reconstruction for the presence of display behavior in the subgenera *Mollienesia* and *Limia*: dorsal fin erection to females (above the branch left), sigmoid posture to females (above the branch right), and sigmoid posture to males (below the branch). The pie charts plotted on the maximum likelihood cladogram represent posterior probabilities of the occurrence of each state. Boxes at branch tips represent states for each taxon; white boxes indicate that no behavioral data is available. Fin erection is classified as absent (lavender) or present (violet). Sigmoid posture to females is classified as absent (lime) or present (emerald). Sigmoid posture to males is classified as absent (azure) or present (cobalt).

DISCUSSION

Patterns of morphological and behavioral characteristics in species of *Mollienesia*, *Limia*, and their close relatives support the hypothesis that an increase in dorsal fin height has evolved in conjunction with a reduction in gonopodium length and female-biased size dimorphism, an increase in overall body size, and the development of courtship. Species that exhibit courtship tend to include males that preferentially copulate covertly, whether they form a distinct size class, are subordinate, or are found in populations under different environmental factors. In *Mollienesia*, two components of male-male aggressive displays (dorsal fin erection and sigmoid body postures) show evidence of co-option for courtship, while *Limia* only shows evidence for dorsal fin erection as the ancestral state. These results were achieved through the use of a well-resolved molecular phylogeny, and a continuous index of ornamentation derived from the height of male dorsal fins.

Phylogenetic Relationships within *Poecilia*

Trees based on the combined data set provided robust support for all clades within the subgenera *Mollienesia* and *Limia*. Three major clades were recovered in the mollies – the sailfin mollies and two shortfin sister groups, the *Poecilia sphenops* and *P. mexicana* complexes – which are well established by prior phylogenetic studies (Ptacek and Breden 1998; Alda et al. 2013; Bagley et al. 2015; Ho et al. 2016; Palacios et al. in press). The basal position of the Jamaican *L. melanogaster*, and the separation of the Cuban *L. vittata* and Cayman Islands *L. caymanensis* from the Hispaniolan *L. nigrofasciata* and *L. perugiae* complexes, also fits previous phylogenies of Limia (Hamilton 2001; Pollux et al. 2014; Weaver 2015). When tree topologies differed, those obtained with maximum likelihood and Bayesian inference gave stronger support than maximum parsimony for the sister grouping of *Limia* + *Pamphorichthys*, instead of *Mollienesia* + *Pamphorichthys*, as corroborated by the results of three different programs (RAxML, GARLI, MrBayes) versus a single program (PAUP). These also gave better support for relationships among the five species of *Pamphorichthys*, and placement of *Pseudolimia heterandria* followed by *Psychropoecilia hispaniolana* as successive outgroups to *Limia*. The combination of *Limia* and *Pamphorichthys* as close relatives within the genus *Poecilia* corresponds with the findings of past phylogenetic studies (Hrbek et al. 2007; Meredith et al. 2010; Pollux et al. 2014; Weaver 2015). It should be noted that one other study (Meredith et al. 2010) arrived at the combination of *Mollienesia* + *Limia* using maximum parsimony in PAUP, but these authors subsequently determined that maximum likelihood could better describe a sister-group pairing between *Limia* and *Pamphorichthys*, a position that my analysis supports to yield the most probable relationship.

Utility of the Ornamentation Index for Poeciliid Traits

By applying a continuous index of ornamentation to dorsal fin height in *Poecilia*, I could incorporate the wide range of species-specific sizes exhibited by this trait. Other studies of sexual selection in livebearers have overlooked this variation because they used either a categorical definition (Meyer et al. 1994; Martin et al. 2010; Pollux et al. 2014), or a discrete numerical sum of trait components (Bisazza 1993; Basolo 1996). Despite the fact that morphology varies continuously, there has been much dispute over the best way to code discrete character states for quantitative data, and the applicability of these data for phylogenetic analysis (Rae 1998). A continuous ornamentation index offers a solution to this problem, since a trait is converted from binary categories to a spectrum that varies considerably among different species. Moreover, the index serves as a standardized value for each species: the actual level of sexual dimorphism in dorsal fin height, calculated at the average body length. This value holds true for all members of *Poecilia* under study, regardless of body size and any differences in size or allometry between the sexes.

Whether height, length, or depth is taken into account for a particular display trait, a continuous ornamentation index may be appropriate to study many sexually selected morphological ornaments. In the family Poeciliidae alone, several other body structures are good candidates for this application. For instance, the caudal fin extension seen in the genus *Xiphophorus* ranges from sixty to over one hundred percent of male body length in various species of swordtails, and exists as short protrusions in two species of platyfish (Meyer et al. 1994; Basolo 1996). In one unique sword-less species, *X. birchmanni*, males possess enlarged and brightly colored dorsal fins that are raised in courtship displays to females (Robinson et al. 2011). This remarkable phenotypic convergence with the sailfin mollies and humpback Limia suggests that dorsal fins in *Xiphophorus* may be sexually dimorphic and fit a range of sizes suitable for analysis with an ornamentation index. Even the gonopodium itself, which ranges from about ten to almost fifty percent of male body length in poeciliids (Pollux et al. 2014), may be swung outward in a display favored by sexual selection. Long gonopodia are preferred by females in the mosquitofish *Gambusia holbrooki* (Kahn et al. 2010), *G. affinis*, and *G. hubbsi* (Langerhans et al. 2005); and in the metallic livebearer, *Girardinus metallicus*, males of the dominant black morph utilize a novel courtship display by presenting their long gonopodia and ventral pigmentation to females (Kolluru et al. 2014; Kolluru et al. 2015). These are just a few examples of traits that can be modeled with an ornamentation index and correlated with display behavior.

Ornamentation and Display Behaviors in *Poecilia*

The significant positive correlation of the ornamentation index with the presence of male courtship, as well as its two component behaviors, supported the hypothesis that exaggerated dorsal fin height has evolved primarily for courtship, instead of male-male aggression. Not all courting species exhibit a large male dorsal fin, but all species with an ornamentation index above 0.5 were found to court. This robust correlation also applied to the two displays used together in courtship, dorsal fin erection to females and sigmoid posture to females. Prior studies such as Pollux et al. (2014) have designated five species in *Poecilia* as bearing sexually selected traits in males, including the unique "moustache" in *P. sphenops* (Schlupp et al. 2010). This species has a moderate ornamentation index, while all species labeled with a sailfin phenotype had elevated indices, indicating that the designation is an appropriate indicator of truly large dorsal fins. In addition, the index was positively correlated with sigmoid postures to males, suggesting that courtship and aggressive displays can each be enhanced by an enlarged fin size. This is consistent with the hypothesis that highly exaggerated ornaments may arise secondarily to advertise male motor performance in displays (Byers et al. 2010). Since agonistic dorsal fin erection was present in every species with behavioral data, it was a constant character that could not be accurately compared to the ornamentation index or sigmoid curvature, but its universal use implies that it may be ancestral. Behavioral studies have often reported that males use the aggressive S-posture while swimming with all unpaired fins erect (Ptacek et al. 2005;

Bierbach et al. 2013), but use of both displays in unison was only found in *Mollienesia*, *Poecilia vivipara*, *Pseudolimia heterandria*, and three *Limia* species.

The absence of a significant correlation between the ornamentation index and sexual dichromatism can be explained by differences in male and female coloration that are present even in species of *Poecilia* with small male dorsal fins. Indeed, all but three of the taxa examined (*Limia garnieri*, *grossidens*, and *rivasi*) exhibit pigmentation in males that is absent in females. Sexual dichromatism is generally uncommon among poeciliids (Pollux et al. 2014), but it can be found to some degree in many species, even those that lack courtship or male ornaments. The relative importance of fin coloration in female choice and male-male competition remains difficult to determine (Kodric-Brown 1998; Hamilton 2001). Finally, no significant correlation was discovered between the index of ornamentation and male variability in reproductive tactics, indicating that species with an exaggerated dorsal fin do not always include individuals that mostly copulate covertly, in addition to those that mostly court. For example, in the sailfin mollies *P. latipunctata* and *P. petenensis*, males of all sizes show no differences in their rates of courtship displays compared to gonopodial thrusting (Ptacek et al. 2005; Hankison and Ptacek 2007).

Ornamentation, Gonopodium Length, and Sexual Size Dimorphism in *Poecilia*

Recent studies of fin morphology in poeciliids have shown that the appearance of a shorter gonopodium may precede courtship evolution (Martin et al. 2010), and that intromittent organ length is negatively correlated with courtship display rates (Loveless et al. 2010). The use of courtship may increase the effectiveness of sperm transfer from a reduced gonopodium (Rosen and Tucker 1961). It may also promote female receptivity toward mating in the face of sexually antagonistic structures, as the hooks and spines facilitating insemination in these species have been found to occasionally injure females (Wang et al. 2015). In addition, courtship has evolved primarily in species that rely on pre-copulatory maternal provisioning, the ancestral trait in poeciliids (Pollux et al. 2014; Haig 2014). *Mollienesia* and *Limia* (but not *Pamphorichthys*) fall into this lecithotrophic category, and have short gonopodia compared to body size, based on Rosen and Tucker's (1961) definition of less than a third of male standard length. Indeed, courting species of *Poecilia* showed the lowest relative intromittent organ size of all species studied. While Ptacek and Travis (1998) found that *P. latipinna* males have longer gonopodia than do shortfin molly males, their comparison was based on absolute anal fin length, which if divided by standard length would yield a reduced relative value for the sailfin molly.

A shift toward male-biased sexual dimorphism with an increasing ornamentation index may be due to the effects of sexual as well as natural selection. Although females are the larger sex in most poeciliids, occasionally reaching more than twice the length of males (Bisazza et al. 1996), this study offers evidence that in *Mollienesia* and *Limia* with ornamentation and courtship, males have evolved to surpass females. Reduced male size may offer an advantage in covert copulation, whereas increased male size provides more success in courtship and competition; and so sexual size dimorphism has been thought to vary from most female-biased in species with gonopodial thrusting only, to least female-biased in species with courtship (Bisazza and Pilastro 1997). Both measures of sexual dimorphism used in the present study reveal that this contrast occurs in *Poecilia*: females tend to be larger in non-courting species, and males tend to be larger in courting species.

For the three morphological variables compared to the ornamentation index, the greatest size of both sexes occurs in species with exaggerated display traits, which may be explained from observations of mate choice. Males of many poeciliids seek out large females, perhaps because large size indicates enhanced fecundity (Arriaga and Schlupp 2013; Bierbach et al. 2013). Sexually receptive females of multiple species also prefer large males (Bisazza et al. 1996; Bisazza and Pilastro 1997), who can effectively exclude smaller competitors from mating. In sailfin mollies, larger males often show consistently larger dorsal fin sizes and higher courtship rates (Ptacek and Travis 1996, 1997). Both direct and indirect advertisement of fertility, through visual or chemical cues, may allow females to attract large males, who focus display efforts on contested females and restrict access of diminutive rivals to mates through inter-male competition (Farr and Travis 1986; Sumner et al. 1994; MacLaren and Rowland 2004). On the other hand, studies of association between the sexes in mollies have found that both males and females prefer to swim near large members of the opposite sex (Gabor 1999), perhaps as a result of natural selection for shoaling in response to predation pressure. If both types of selection operate in certain lineages, a general preference for greater size may have become linked between the sexes and driven the coevolution of increased body size in both males and females. In the sailfin mollies, for instance, this process could account for long dorsal fins in females, although the heights of these fins are short compared to males.

One possible method by which a preference for large size could have originated is through an ancestral sensory bias (Basolo 1996). Although major caudal fin extensions have evolved only in swordtails, both males and females of sword-less poeciliids (such as sailfin mollies and platyfish) prefer this trait in conspecifics of either sex (Basolo 2002; Makowicz et al. 2015). A preference for large size, in the form of lateral projection area, has also been found in female sailfin and shortfin mollies (Ptacek 1998; MacLaren and Rowland 2004, 2006; Kozak et al. 2008), suggesting that enlarged dorsal fins have evolved as a way for males to exploit the female preference and appeal to potential mates. Even in the unisexual Amazon molly *P. formosa* (a gynogenic hybrid of *P. mexicana* and *P. latipinna*), females prefer large males, yet they obtain no genetic material from them (Marler and Ryan 1997). An ancestral bias for large size in swordtails may constitute the female preference for elongated caudal fins, which add to lateral projection area when viewed horizontally (Rosenthal and Evans 1998). It could further explain why shortfin molly females prefer large dorsal fins found naturally on sailfin males or superimposed artificially on males of their own species (Ptacek 1998; Jordan et al. 2006). The influence of biases for sexually selected traits in poeciliids may go well beyond lateral projection: females of some molly species have at least a weak preference for the "moustache" of male *P. sphenops*, implying an emergent preference that has led to selection for this novel trait (McCoy et al. 2011). In the case of ornament elaboration, a pre-existing female bias could have resulted in an extreme dorsal fin phenotype that increases the apparent body size of males. But a preference for larger conspecifics could result from natural selection as well (Gabor 1999), which does not produce a conclusive phylogenetic pattern if both types of selection influence a bias toward greater body size (Fuller et al. 2005). It remains to be seen whether a female preference is responsible for the enlarged dorsal fin, deep body, and pronounced hump of male *Limia nigrofasciata*, although the laterally flattened shape of these males has been attributed to natural selection for greater maneuverability (Hamilton 2001).

Courtship Evolution and Alternative Reproductive Tactics

The significant correlation between the presence of courtship and male behavioral variability in *Poecilia* also fit the predicted relationship between these two behaviors, as variation in male reproductive strategies only appeared in courting species. This has been suggested to evolve in social systems in which a few individual males achieve the most mating success (Henson and Warner 1997); and if females preferentially mate with larger or socially dominant males, subordinate or small males may attain higher fitness through covert copulation (Ryan and Causey 1989). In *Mollienesia* and *Limia*: males that rely on gonopodial thrusting may indeed form a distinct diminutive size class, as in *P. velifera* (Hankison and Ptacek 2007), may be low-ranking, as in *P. limantouri* (Balsano et al. 1985), or both, as in *L. nigrofasciata* (Holz 2015). Intermediate males can also form a size class (*L. perugiae*: Farr 1984; Erbelding-Denk et al. 1994; Schröder et al. 1996; *P. latipinna*: Farr et al. 1986; Swanbrow Becker et al. 2012), and may be phenotypic plastic in their use of covert copulation instead of courtship. Males of *P. latipinna* change their behavior depending on the size of nearby males, either by courting to mirror large rivals when in groups (Swanbrow Becker et al. 2012), or by switching to gonopodial thrusting when in groups (Travis and Woodward 1989; Fraser et al. 2014). In *L. perugiae*, the reproductive success of dominant males is negatively correlated with population size, as small (Schartl et al. 1993a; Schartl et al. 1993b) or intermediate subordinates (Erbelding-Denk et al. 1994) have the highest mating success in larger groups.

Although sexual selection has been proposed to maintain alternative reproductive tactics and their associated phenotypes in livebearers (Bisazza and Pilastro 1997), several genetic factors may also be accountable. In poeciliids, male growth slows substantially at maturity (Swanbrow Becker et al. 2012), and variation in male size may be controlled by a patrilinearly inherited Y-linked allelic series, with different alleles present in males of different sizes (Ryan and Causey 1989; Travis 1989, 1994; Erbelding-Denk et al. 1994). In hybrids of shortfin and sailfin mollies, the presence of these paternal genes increases male courtship rates significantly, perhaps due to autosomal interactions (Ptacek 2002; Loveless et al. 2009). Moreover, in *Xiphophorus*, copy number variation at the Y-linked melanocortin 4 receptor (*mc4r*) locus has been correlated with male body size and mating strategies, and this size-dependence spans both courtship and aggression (Lampert et al. 2010; Smith et al. 2015). A similar allelic pathway of growth and ultimate size at maturity has recently been discovered in *P. latipinna* (Lange 2013), indicating that *mc4r* genotype regulation of alternative mating tactics may be widespread in poeciliids.

However, the similar rates of courtship and thrusting that have been observed in species inferred to lack a genetic polymorphism for male body size (such as *P. petenensis* and *P. latipunctata*) do not fit the pattern seen in most species that use mating displays. In these two mollies, environmental constraints may have contributed to the loss of variable male behavior. Females of *P. petenensis* do not have a preference for larger males (Kozak et al. 2008), and though females of *P. latipunctata* prefer larger males, they have no bias for the sailfin phenotype (Ptacek et al. 2011). This could be influenced by differences in flow rate and predation pressure between the habitats of the two sailfin molly groups, as the polymorphic and variable *P. velifera* and *P. latipinna* are found in coastal marshes and tidal pools, while the invariant *P. petenensis* and *P. latipunctata* are found in streams and rivers with dissimilar predation regimes (Hankison and Ptacek 2007; Ptacek et al. 2011). In turbid waters of fast-flowing streams, *P. petenensis* females may cue in on male courtship to reliably recognize mates, which could explain the absence of small males and behavioral variability in this species (Kozak et al. 2008). As *P. latipunctata* is found in a restricted habitat, its narrow range of male sizes may have been caused by a genetic bottleneck in isolated populations (Ptacek et al. 2011). Small size in mollies may also be shaped by selective predation, since wading birds are known to target larger individuals (Trexler et al. 2004). If predation is found to effectively select for small males, then these two factors together may drive diminished size variation in the Tamesí molly (Ptacek et al. 2011), and the loss of a male size class that relies primarily on covert copulation.

Differences in habitat and environmental stressors may further drive a shift away from courtship in a few extremophile populations of *Mollienesia* and *Limia* species. In *L. melanonotata* inhabiting a hypersaline lagoon, mature males are small and exhibit fewer courtship displays and less sexual dichromatism than freshwater conspecifics (Weaver et al. unpubl. data). In *P. mexicana*, males from cave populations rely far less on courtship displays than those from surface populations, and have a correlated increase in rates of gonoporal nibbling, which may act as an alternative tactic for attracting mates (Parzefall 1989, 2001; Plath et al. 2004). Males from both subterranean and sulfide habitats show decreased courtship rates and increased thrusting and nibbling (Plath 2008; Bierbach et al. 2013). Although male-male aggression is highly reduced in cave mollies (Bierbach et al. 2012), and males do not monopolize females, a female bias for large males remains (Plath et al. 2003). Adaptation to the extreme conditions of these caves (such as darkness, hypoxia, and high sulfide levels) may be responsible for the absence of size-dependent mating tactics in cave mollies: unlike in surface populations, small males do not copulate covertly at greater rates than large males (Riesch et al. 2006). The complete loss of courtship in *P. sulphuraria* could have occurred over its longer history of isolation in a few sulfidic stream populations compared to cave *P. mexicana* (Bierbach et al. 2012), and this may also have occurred in its equally isolated sister taxon *P. thermalis* (Palacios et al. 2013), a species with currently undescribed pre-copulatory behavior.

Aggressive Displays and Co-Option for Courtship

Surveys of the literature for wide variety of taxa that possess secondary sexual traits, used as ornaments for agonistic contests and courtship, have led to the pre-existing trait hypothesis: dual-function characters originated for male-male competition and were later co-opted for mate attraction (Berglund et al. 1996; Borgia and Coleman 2000). Based on previous assessments of courtship and male aggression in *Poecilia* suggesting a link between sigmoidal body curvature and dorsal fin erections (Liley 1966; Parzefall 1969; Ptacek 2005; Bierbach et al. 2013), the pre-existing trait hypothesis predicts an aggression-to-courtship behavioral transition that could explain evolution of elaborate fin morphology. However, the present study shows that this scenario may only have occurred in *Mollienesia*, since both sigmoidal postures and dorsal fin erections for male contests appear to be ancestral displays that are found in every species examined. In *Limia*, on the other hand, only dorsal fin erection is an ancestral trait used in male-male aggressive encounters, and the order of evolution is equivocal for sigmoid curvature. The taxonomic distribution of male competitive displays is much broader than the distribution of female courting displays, indicating that male-male aggression came first, and that co-option for mate attraction has occurred in select lineages of both *Mollienesia* and *Limia*. A sailfin phenotype is present in males of three species of *Mollienesia* and one species of *Limia*, and can be inferred to have evolved well after the appearance of courtship. This supports the pre-existing trait hypothesis: mating displays precede the development of ornamental morphology (Berglund et al. 1996). Extreme dorsal fin height in sailfin mollies and the humpback Limia may have arisen secondarily to enhance the vigor of male aggressive and courtship displays (Byers et al. 2010), and this scenario has been proposed for male dorsal fin enlargement in *Xiphophorus birchmanni* as well (Robinson et al. 2011).

The joint use of dorsal fin erection and sigmoid postures in male-male aggression has evolved three times in *Limia*: once in *L. nigrofasciata*, and two times in non-courting shortfin species, *L. sulfurophila* and *L. tridens*. All three Limia belong to a large species complex (Hamilton 2001; Weaver 2015), and agonistic sigmoid curvature may have re-evolved in each species, rather than evolving in their common ancestor and disappearing in the six or so other species in the complex. Behavioral analyses of the few species that have not been studied (*L. garnieri*, *grossidens*, and *rivasi*) could offer support for this scenario. For example, the newly-discovered tiger Limia (*L. isla*), the closest relative of *L. nigrofasciata*, is completely reliant on gonopodial thrusting, does not display sigmoid postures or dorsal fin erections to females, and shows low levels of male-male aggression (Keeney 2013; Weaver 2015). A potential loss of sigmoid curvature in courtship may be correlated with a loss in agonistic interactions as well, if the display is not present in the remaining species in the complex. This pattern is already known from one other species, *L. melanogaster*, in which courtship consists solely of dorsal fin erection.

If dorsal fin erection and sigmoid body curvature indeed originated for male-male competition, then a complete behavioral assessment of the diverse members of *Poecilia* could help determine when these displays initially evolved. The monophyletic clade used in the present study is the sister group to the twin subgenera of *Acanthophacelus* and *Micropoecilia* (Meredith et al. 2010; Meredith et al. 2011; Pollux et al. 2014; Palacios et al. in press). Aggressive use of dorsal fin erection and sigmoid postures among males are known to occur in species of each subgenus, but only the guppy (*Poecilia reticulata*) uses both display components together in courtship (Liley 1966). While *Acanthophacelus* and *Micropoecilia* males lack elaborate dorsal fins, they bear conspicuous colorful spots, and in *P. reticulata* these have been proposed to act as ornaments for male-male competition and for courting females (Kodric-Brown 1993; Berglund et al. 1996). In guppies and their relatives, color patterns occur in discrete patches, and are polymorphic in size and shape (*P. reticulata*: Brooks and Caithness 1995; Houde 1997; *P. picta*: Lindholm et al. 2015; *P. parae*; Hurtado-Gonzales and Uy 2009, 2010; *P. wingei*: Poeser et al. 2005). Courtship has been observed in members of both subgenera (Liley et al. 1966; Poeser et al. 2005; Řežucha and Reichard 2014). This suggests that color patterns may fit a continuous index of ornamentation and function generally in male-male aggression and mating displays in *Micropoecilia* and *Acanthophacelus*. Future studies should investigate whether the pre-existing trait hypothesis can explain the evolution of male color patterns in guppies and their relatives, as the behaviors of a few species may not characterize the entire clade.

Conclusions

The pre-existing trait hypothesis proposes that ornament co-option from contests to courtship is a major driving force behind the evolution of exaggerated display traits in many animal taxa. However, for livebearing fish of the family Poeciliidae, these variable characters are usually scored by categorical values instead of a more accurate continuous index. In this study, I apply an index of ornamentation based on height of the male dorsal fin to members of the subgenera *Mollienesia* and *Limia*, and show that increased fin size is positively correlated with larger body size and use of courtship displays, and negatively correlated with female-biased sexual dimorphism and relative length of the intromittent organ that facilitates covert copulation. Ancestral state reconstructions reveal that the two component behaviors used in both courtship and male-male aggression were present in an agonistic form at the base of *Poecilia*, and only later became adapted for mate attraction. Although *Mollienesia* clearly fit the expected pattern for both display components, my analysis suggests that the common ancestor of *Limia* lacked the use of sigmoid posture in male-male interactions, and then re-evolved it. This scenario requires future investigation to determine the order in which traits have appeared. In poeciliids, sexually selected characters can also fit a transition from courtship to male-male competion (Morris et al. 2007). Comparative studies of a diversity of display traits, appropriate for a continuous index of ornamentation, may elucidate the evolution of ornamental phenotypes and their associated behaviors in livebearers and many other taxa with extreme sexual dimorphism.

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Table S1. Species used in the phylogenetic analysis, genes sampled, and GenBank accession numbers. N/A = not present.

Species	*ATPase 8/6*	*Cytb*	*COI*	*ENC1*	*Glyt*	*Myh6*
Poecilia vivipara	N/A	HQ857430	GU701911	HQ857472	HQ857466	HQ857460
Limia caymanensis	N/A	KJ696810	N/A	KJ696902	KJ697012	KJ697122
Limia dominicensis	N/A	EF017533	N/A	GU179170	GU179199	GU179245
Limia garnieri	N/A	KJ696811	N/A	KJ696903	KJ697013	KJ697123
Limia grossidens	N/A	KJ696812	N/A	KJ696904	KJ697014	KJ697124
Limia melanogaster	N/A	EF017534	N/A	GU179171	GU179200	GU179246
Limia melanonotata	JX968645	KJ696813	JX968692	KJ696905	KJ697015	KJ697125
Limia nigrofasciata	N/A	KJ696814	N/A	KJ696906	KJ697016	KJ697126
Limia pauciradiata	N/A	KJ696815	N/A	KJ696907	KJ697017	KJ697127
Limia perugiae	N/A	KJ696816	N/A	KJ696908	KJ697018	KJ697128
Limia rivasi	N/A	KJ696817	N/A	KJ696909	KJ697019	KJ697129
Limia sulfurophila	N/A	KJ696818	N/A	KJ696910	KJ697020	KJ697130
Limia tridens	N/A	EF017535	N/A	KJ696911	KJ697021	KJ697131
Limia versicolor	N/A	KJ696819	N/A	KJ696912	KJ697022	KJ697132
Limia vittata	JX968641	KJ696820	FN545667	KJ696913	KJ697023	KJ697133
Limia zonata	N/A	KJ696821	N/A	KJ696914	KJ697024	KJ697134
Pamphorichthys araguaiensis	N/A	GU179195	N/A	GU179181	GU179210	GU179257
Pamphorichthys hasemani	N/A	HQ857427	N/A	HQ857469	HQ857463	HQ857457
Pamphorichthys hollandi	N/A	HQ857428	HM405174	HQ857470	HQ857464	HQ857458
Pamphorichthys minor	N/A	GU179196	N/A	GU179182	GU179211	GU179257
Pamphorichthys scalpridens	N/A	HQ857429	N/A	HQ857471	HQ857465	HQ857459
Allopoecilia caucana	JX968639	EF017540	JX968686	GU179183	GU179212	GU179258
Poecilia butleri	JX968561	KJ696829	JX968651	KJ696928	KJ697038	KF276642
Poecilia catemaconis	JX968568	KF276610	JX968654	N/A	N/A	KF276639
Poecilia chica	N/A	KJ696830	N/A	KJ696929	KJ697039	KJ697149

Poecilia gilli	JX968634	FJ446169	JX968681	KJ696930	KJ697040	KJ697150
Poecilia hondurensis	JX968599	N/A	JX968667	N/A	N/A	N/A
Poecilia latipinna	N/A	KJ696833	HQ557463	KJ696932	KJ697042	KJ697152
Poecilia latipunctata	N/A	EF017539	JQ935927	GU179184	GU179213	GU179259
Poecilia limantouri	JX968566	KJ696834	N/A	KJ696933	KJ697043	KF276663
Poecilia mexicana	JX968571	KJ696835	JX968656	KJ696934	KJ697044	KF276658
Poecilia orri	JX968603	KJ696836	JQ840650	KJ696935	KJ697045	KJ697155
Poecilia petenensis	N/A	KJ696837	EU751941	KJ696936	KJ697046	KJ697156
Poecilia salvatoris	N/A	KJ696839	N/A	KJ696938	KJ697048	KJ697158
Poecilia sphenops	JX96853	HQ677898	JX968657	KJ696939	KJ697049	KF276640
Poecilia sulphuraria	N/A	KF276623	N/A	N/A	N/A	KF276653
Poecilia thermalis	N/A	KF276617	N/A	N/A	N/A	KF276646
Poecilia velifera	N/A	KJ696841	JQ667585	KJ696940	KJ697050	KJ697160
Psychropoecilia hispaniolana	JX968644	N/A	JX968690	N/A	N/A	N/A
Pseudolimia heterandria	N/A	HQ857426	N/A	HQ857468	HQ857462	HQ857456

Species	*ND2*	*Rag1*	*Rh*	*SH3PX3*	*X-src*
Poecilia vivipara	HQ857454	HQ857448	HQ857442	HQ857424	HQ857436
Limia caymanensis	AF353192	KJ697291	KJ697385	KJ697495	KJ697601
Limia dominicensis	AF353195	EF017431	GU179273	GU179216	GU179154
Limia garnieri	N/A	KJ697292	KJ697386	KJ697496	KJ697602
Limia grossidens	N/A	KJ697293	KJ697387	KJ697497	KJ697603
Limia melanogaster	AF353202	EF017432	GU179274	GU179217	GU179155
Limia melanonotata	JX968738	KJ697294	KJ697388	KJ697498	KJ697604
Limia nigrofasciata	AF031391	KJ697295	KJ697389	KJ697499	KJ697605
Limia pauciradiata	AF353196	KJ697296	KJ697390	KJ697500	KJ697606
Limia perugiae	AF031392	KJ697297	KJ697391	KJ697501	KJ697607

Limia rivasi	N/A	KJ697298	KJ697392	KJ697502	KJ697608
Limia sulfurophila	N/A	KJ697299	KJ697393	KJ697503	KJ697609
Limia tridens	N/A	KJ697230	KJ697394	KJ697504	KJ697610
Limia versicolor	AF353193	KJ697231	KJ697395	KJ697505	KJ697611
Limia vittata	JX968734	KJ697232	KJ697396	KJ697506	KJ697612
Limia zonata	AF353194	KJ697233	KJ697397	KJ697507	KJ697613
Pamphorichthys araguaiensis	GU179241	GU179269	GU179284	GU179227	GU179165
Pamphorichthys hasemani	HQ857451	HQ857445	HQ857439	HQ857421	HQ857433
Pamphorichthys hollandi	HQ857452	HQ857446	HQ857440	HQ857422	HQ857434
Pamphorichthys minor	GU179242	GU179270	GU179285	GU179228	GU179165
Pamphorichthys scalpridens	HQ857453	HQ857447	HQ857441	HQ857423	HQ857435
Allopoecilia caucana	JX968732	EF017437	GU179286	GU179229	PCXSRC
Poecilia butleri	KJ697229	KJ697310	KJ697411	KJ697521	KJ697627
Poecilia catemaconis	KF276668	KF276697	KF276726	N/A	N/A
Poecilia chica	KJ697230	KJ697311	KJ697412	KJ697522	KJ697628
Poecilia gilli	JX968727	KJ697312	KJ697413	KJ697523	KJ697629
Poecilia hondurensis	JX968713	N/A	N/A	N/A	N/A
Poecilia latipinna	KF276667	KJ697314	KJ697415	KJ697525	KJ697631
Poecilia latipunctata	AF080489	EF017436	GU179287	GU179230	GU179167
Poecilia limantouri	KJ697232	KJ697316	KJ697416	KJ697526	KJ697632
Poecilia mexicana	KJ697233	KJ697317	KJ697417	KJ697527	KJ697633
Poecilia orri	JX968716	KJ697318	KJ697418	KJ697528	KJ697634
Poecilia petenensis	KJ697234	KJ697319	KJ697419	KJ697529	KJ697635
Poecilia salvatoris	KJ697236	KJ697321	KJ697421	KJ697531	KJ697637
Poecilia sphenops	KF276669	KF276699	KJ697422	KJ697532	KJ697638
Poecilia sulphuraria	AF080490	KF276713	KF276740	N/A	N/A
Poecilia thermalis	KF276676	KF276708	KF276734	N/A	N/A

Poecilia velifera	KJ697237	KJ697323	KJ697423	KJ697533	KJ697639
Psychropoecilia hispaniolana	JX968736	N/A	N/A	N/A	N/A
Pseudolimia heterandria	HQ857450	HQ857444	HQ857438	HQ857420	HQ857432

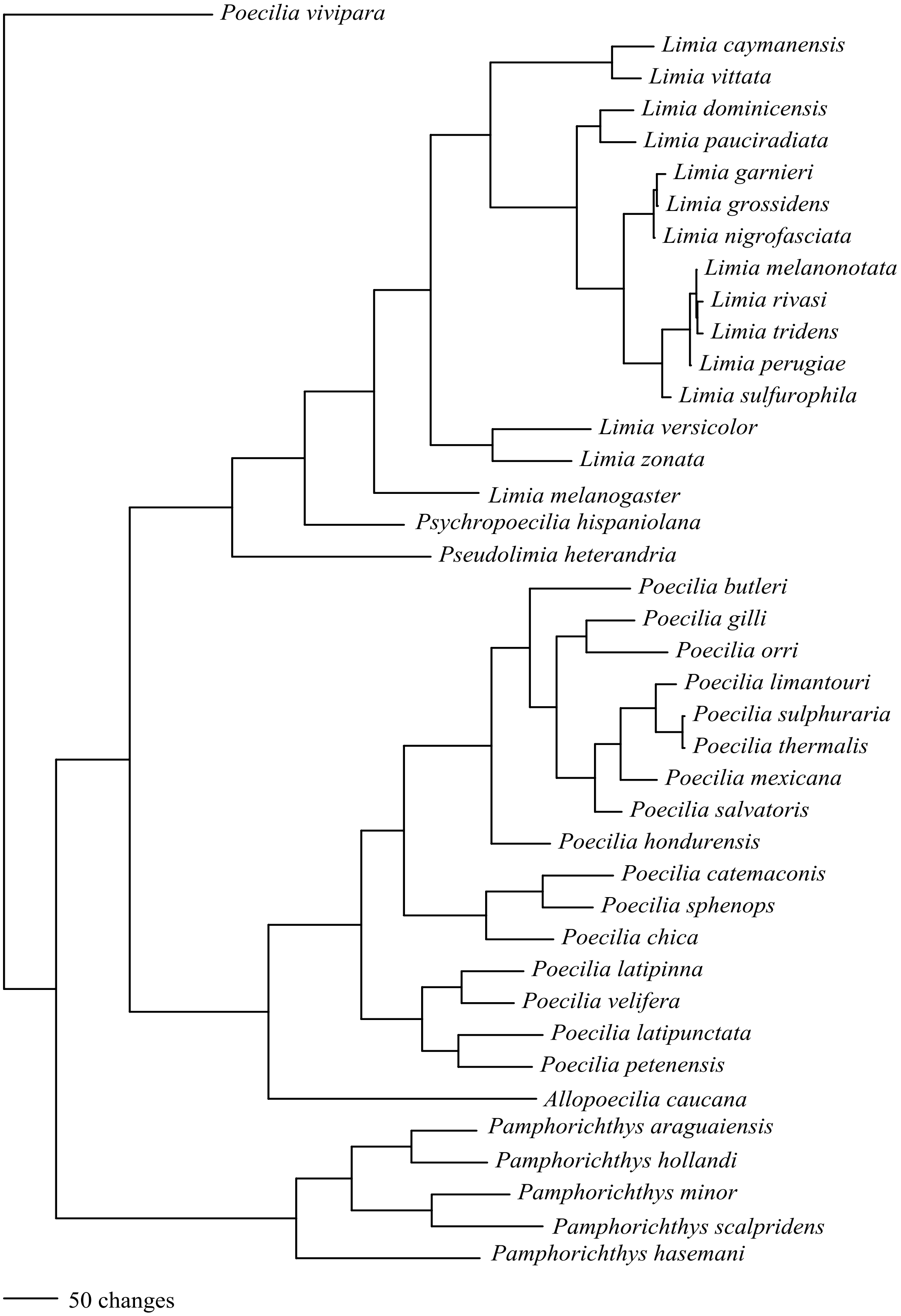

Figure S1. The shortest MP tree (4499 steps) for the combined data set obtained with PAUP.

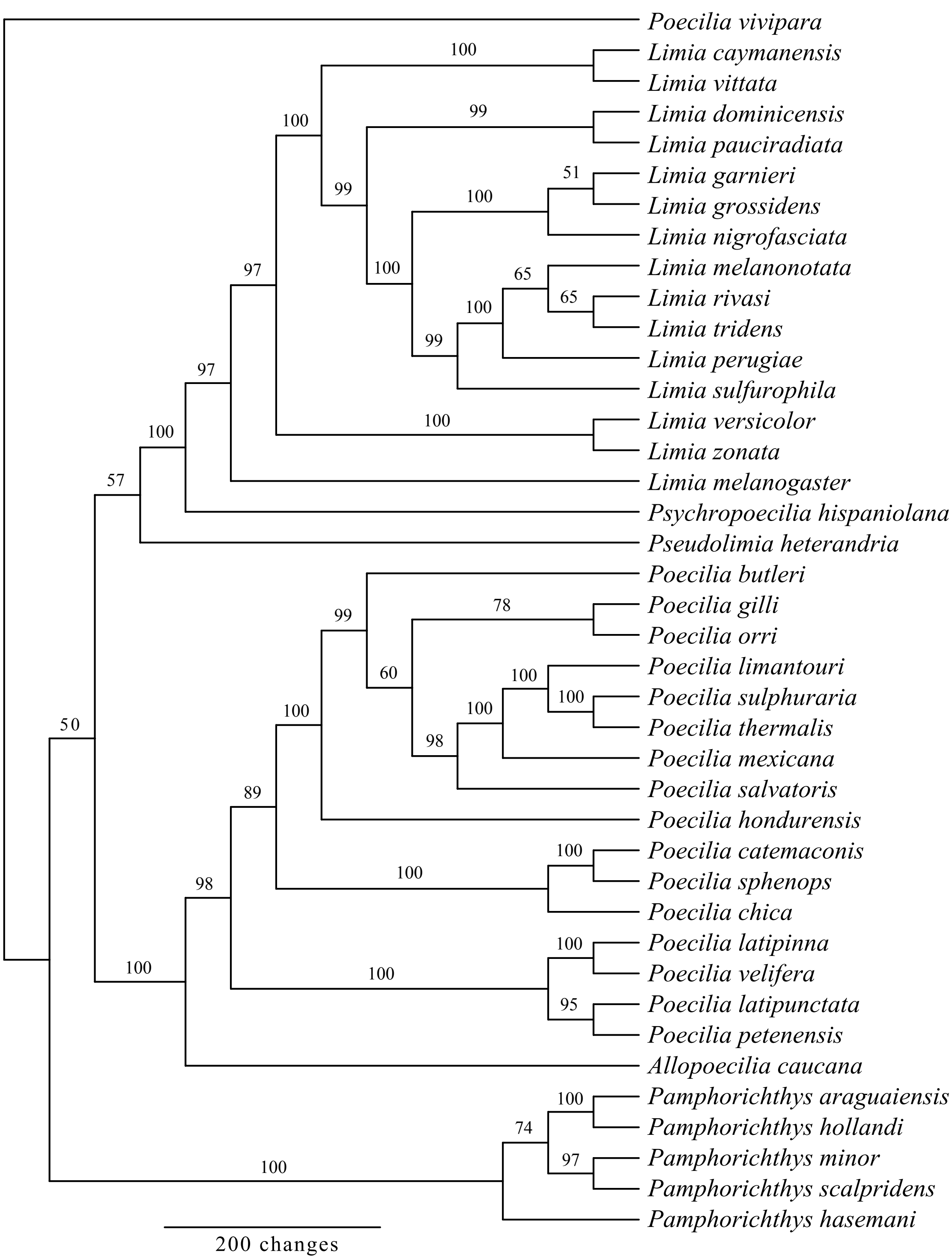

Figure S2. Strict consensus tree (4539 steps) for the combined data set obtained with PAUP. Values above each branch correspond to MP bootstrap support percentages.