

# THE EVOLUTION OF THE SEXUALLY SELECTED SWORD IN *XIPHOPHORUS* DOES NOT COMPROMISE AEROBIC LOCOMOTOR PERFORMANCE

Christopher E. Oufiero,<sup>1,2,3</sup> Robert W. Meredith,<sup>1,4</sup> Kristine N. Jugo,<sup>1</sup> Paulina Tran,<sup>1</sup> Mark A. Chappell,<sup>1</sup> Mark S. Springer,<sup>1</sup> David N. Reznick,<sup>1</sup> and Theodore Garland Jr.<sup>1</sup>

<sup>1</sup>Department of Biology, University of California, Riverside, California 92521

<sup>2</sup>Current Address: Department of Biological Sciences, Towson University, Towson, Maryland 21252

<sup>3</sup>E-mail: [coufiero@towson.edu](mailto:coufiero@towson.edu)

<sup>4</sup>Current Address: Department of Biology and Molecular Biology, Montclair State University, Upper Montclair, New Jersey 07043

Received October 9, 2012

Accepted February 17, 2014

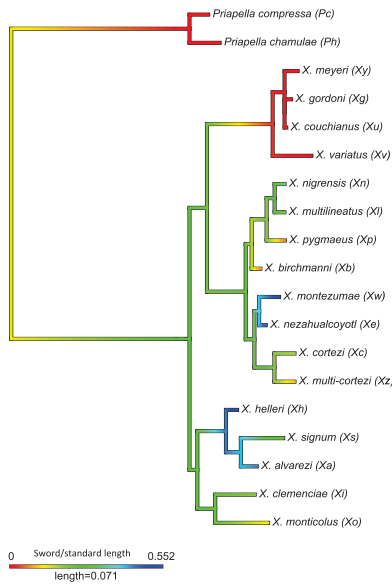
Sexual selection can increase morphological diversity within and among species. Little is known regarding how interspecific variation produced through sexual selection affects other functional systems. Here, we examine how morphological diversity resulting from sexual selection impacts aerobic locomotor performance. Using *Xiphophorus* (swordtail fish) and their close relatives ( $N = 19$  species), we examined whether the evolution of a longer sexually selected sword affects critical swimming speed. We also examined the effect of other suborganismal, physiological, and morphological traits on critical swimming speed, as well as their relationship with sword length. In correlation analyses, we found no significant relationship between sword length and critical swimming speed. Unexpectedly, we found that critical swimming speed was higher in species with longer swords, after controlling for body size in multiple regression analyses. We also found several suborganismal and morphological predictors of critical swimming speed, as well as a significant negative relationship between sword length and heart and gill mass. Our results suggest that interspecific variation in sword length is not costly for this aspect of swimming performance, but further studies should examine potential costs for other types of locomotion and other components of Darwinian fitness (e.g., survivorship, life span).

**KEY WORDS:** Costs, locomotion, phylogenetic comparative analysis, sexual selection, suborganismal traits, swimming performance.

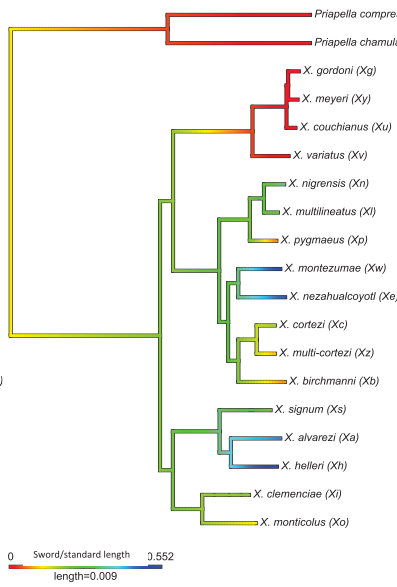
Interspecific morphological variation results from both natural (Wainwright and Reilly 1994; Price et al. 2011) and sexual selection (Kazancioglu et al. 2009). Sexual selection can cause an increase in the rates of evolution of morphological diversity as well as result in the evolution of exaggerated structures (Emlen 2001); and often results in interspecific variation among closely related species in the size, shape, and expression of the sexually selected structures (e.g., eye span in stalk-eyed flies [Baker and

Wilkinson 2001], horns in beetle [Emlen et al. 2005], tail feathers in birds [Balmford et al. 1994], claws in fiddler crabs [Rosenberg 2002], and fins in fish [Bisazza et al. 1997]). Since Darwin (1871) first puzzled over such exaggerated traits, biologists have been interested in the effect of these seemingly “unnatural” structures on various components of Darwinian fitness (Andersson 1994). Although these sorts of traits typically increase reproductive success, they are assumed to hinder other functional abilities.

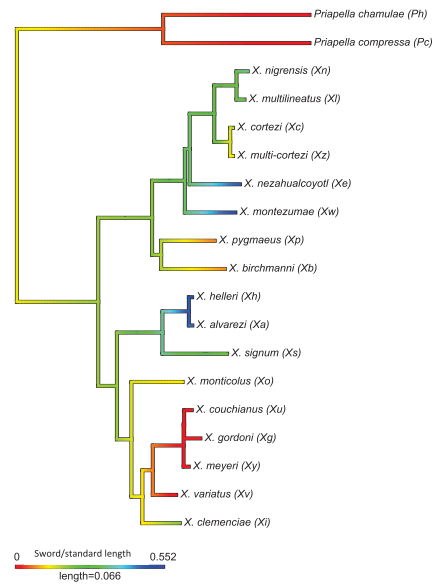
**A** Nuclear tree from this study



**B** Transcriptome tree based on Cui et al. 2013 figure 2A



**C** Mitochondrial tree based on Cui et al. 2013 figure S2



**Figure 1.** Phylogenetic hypotheses used for analysis based on the phylogeny in Figure S1, the total evidence nuclear phylogeny of Cui et al. (2013), and the mitochondrial phylogeny of Cui et al. (2013). Branch colors represent relative sword length (sword length/standard length) mapped using the contMapp function (Revell 2013) in the phytools package for R (Revell 2012).

However, many studies have found little to no effect of the exaggerated structures on functional abilities (Kotiaho 2001; Oufiero and Garland 2007). Although this may be due in part to the evolution or development of compensatory traits (Kirkpatrick 1987; Balmford et al. 1994; Møller 1996; Husak and Swallow 2011), it may also be due to a lack of understanding of the form-function and evolutionary relationships of exaggerated morphological traits. That is, how does morphological diversity that arises through sexual selection affect functional diversity?

The purpose of this study was to determine how the evolution of a sexually selected trait affects one important aspect of functional diversity. Specifically, using swordtails, platyfish and their close relatives (*Xiphophorus* and *Priapella*), as well as well-supported hypotheses of phylogenetic relationships, we examined the effect of sword length, a sexually selected trait, on aerobic swimming performance. However, because locomotion is a complex trait that involves many underlying components, we also examined the effect of suborganismal, morphological, and physiological traits on aerobic locomotion, in addition to their relationship with the sexually selected structure.

*Xiphophorus* is a genus of live-bearing freshwater fish, found throughout Mexico and parts of Central America; and are an excellent group to examine the effect of sexual selection on functional diversity for multiple reasons. Among the 26 closely related species (Cui et al. 2013; Kang et al. 2013), variation in caudal fin shape appears to have evolved due to females in some species pre-

ferring males with an elongation along the lower edge of the caudal fin, termed the sword (Basolo 1990, 1995), whereas females of other species apparently do not show such preference (Wong and Rosenthal 2006). This variation in the caudal fin (Fig. 1) is directly coupled to the propulsive system of the fish, and intraspecific studies have found mixed results regarding the effect of variation in the sword on functional abilities, potentially due to variation in species, methods used, and performance traits analyzed (Ryan 1988; Basolo and Alcaraz 2003; Royle et al. 2006; Kruesi and Alcaraz 2007; Baumgartner et al. 2011; Oufiero et al. 2012).

Based on previous studies and biomechanical models, three hypotheses can be made for the effect of interspecific variation in the sexually selected sword on aerobic locomotion. (1) The evolution of the sword among males will compromise aerobic locomotion and be a cost. Intraspecific experimental reductions of sword length in *Xiphophorus montezumae* have demonstrated an increase in critical swimming speed, a standard measure of aerobic locomotor capacity (Kruesi and Alcaraz 2007), and a decrease in routine and courtship oxygen consumption (Basolo and Alcaraz 2003). Therefore, species without a sword may exhibit an increase in aerobic performance compared to conspecifics with long swords, due to the potential drag and energetic burden associated with bearing a sword. (2) The evolution of the sword among males will have no effect on aerobic locomotion. Previous intraspecific studies of natural variation have found no effect of sword length

on aerobic performance (Ryan 1988; Oufiero et al. 2012a). Similarly, interspecific studies examining the impact of variation in a sexually selected trait on other aspects of fitness have found no significant effects (Promislow et al. 1992). Although Basolo and Alcaraz (2003) found a negative effect of the sword on routine and courtship oxygen consumption, they suggest no effect of the sword on steady swimming in a current. Furthermore, kinematic studies have demonstrated no difference in tail beat kinematics among four species of *Xiphophorus* that vary naturally in sword length (Oufiero et al. 2014). Thus, interspecific variation in sword length may not be related to interspecific variation in aerobic locomotion and the evolution of the sword may have no effect on aerobic locomotor abilities. (3) The evolution of the sword among males has a positive effect on aerobic locomotion. The sword has been shown to have a positive effect on c-start burst speed in *X. hellerii* (Royle et al. 2006). Furthermore, biomechanical studies of fish with asymmetric caudal fins (as occur in swordtails) have demonstrated variation in the flow shed off the caudal fin during swimming (Wilga and Lauder 2004; Flammang et al. 2011), and biorobotic and simulation models have suggested that this alteration of flow is beneficial to locomotion (Lauder et al. 2011; Borazjani and Daghooghi 2013). Similarly, species of fish with trailing edges (asymmetric) on their pectoral fins may have increased aerobic swimming performance (Walker and Westneat 2002). Furthermore, because the sword is coupled to the propulsive system, it may increase the caudal fin surface area, and thus the amount of thrust during swimming. We therefore might expect an elongation of the lower margin of the caudal fin to increase aerobic swimming performance.

## Methods

### ANIMAL CARE AND HUSBANDRY

We measured a total of 280 male fish from 17 species of *Xiphophorus* and two species of *Priapella*; which is closely related to *Xiphophorus* and was used by Basolo (1995) to demonstrate the preexisting sensory bias in females (Fig. 1, Table S1). Several adult mating pairs for each species were obtained from laboratory populations (e.g., The *Xiphophorus* Genetic Stock Center, <http://www.xiphophorus.txstate.edu/>, see Table S1) and bred to obtain F1s and F2s for analyses. Species were reared separately under common garden conditions (Garland and Adolph 1991). When born, fry were isolated from parent fish (to avoid cannibalism) and reared in 19, 38, or 151L aquaria (depending on the size and number of fish). F1 and F2 fish were fed brine shrimp, liver paste, freeze-dried bloodworms, and flake food ad libitum throughout development. At the first sign of maturation (the development of the gonopodium), males, chosen at random, were isolated from stock tanks, housed individually in 9L aquaria, and fed ad libitum. All fish from a species were tested at various times,

and several species were tested at once to minimize any effects of time of year. Fish were allowed to grow after maturation for up to six months, to reach larger sizes and to grow the sword (Marcus and McCune 1999; Oufiero et al. 2012). Prior to critical swimming speed and  $VO_{2max}$  measures, fish were fasted for 24 h.

Mature fish were tested and processed for performance measures in batches of 20–30 individuals of mixed species. Critical swimming speed was tested first, followed by maximum oxygen consumption several days later. After all individuals in a given batch were measured for performance, a digital photo was taken, and the following day the fish were euthanized and size traits measured (see below).

### CRITICAL SWIMMING SPEED ( $U_{CRIT}$ )

Critical swimming speed (Table S2) is defined as the maximum velocity a fish maintains for a specific period of time ending in fatigue (Brett 1964; Beamish 1978). It is a ramped velocity test that estimates the aerobic locomotor performance of fish (Brett 1964; Beamish 1978; Plaut 2001). Critical swimming speed was measured using the setup and procedures outlined in previous studies (Oufiero and Garland 2009; Oufiero et al. 2011b, 2012). In short, fish were acclimated to a flow tunnel for 15 min at a low flow velocity ( $\sim 3 \text{ cm}\cdot\text{s}^{-1}$ ) to orient them to the direction of flow. After acclimation, the velocity of the water was increased  $4.5 \text{ cm}\cdot\text{s}^{-1}$  every 5 min until the fish was fatigued. Fatigue was determined as the point when the fish would not remove itself from the back grating after gently prodding with a net and tapping on the sides of the flow tunnel.  $U_{crit}$  was calculated following the formula presented by Brett (1964) and Beamish (1978).  $U_{crit}$  was measured at room temperature ( $23.024^\circ\text{C} \pm 0.873 \text{ SD}$ , range  $20.0\text{--}25.3^\circ\text{C}$ ); temperature did not significantly affect  $U_{crit}$  and was not included in the final statistical analyses (analysis of covariance [ANCOVA] on individual values with species as factor, standard length (SL) and water temperature as covariates: water temperature  $F_{1,268} = 1.351$ ,  $P = 0.246$ ; SL  $F_{1,268} = 46.914$ ,  $P < 0.0001$ ; species  $F_{19,268} = 11.925$ ,  $P < 0.0001$ ). This performance has been shown to be repeatable in this experimental setup in *Poecilia reticulata* (Oufiero and Garland 2009) and *X. maculatus* (Oufiero 2010).

### MAXIMUM OXYGEN CONSUMPTION ( $VO_{2MAX}$ )

Details of maximum oxygen consumption ( $VO_{2max}$ ) measurements can be found in Appendix S1. In short,  $VO_{2max}$  (Table S2) was measured through forced exercise in a closed system, similar to methods outlined in Chappell and Odell (2004). After a 15-min acclimation to the swim respirometer, the fish was sealed in and the velocity of flow was increased every 3 min, until the fish was fatigued. A fish was considered fatigued when it no longer maintained its position in the respirometer and was carried by the flow of the water at least three consecutive turns. The maximum

oxygen consumption was obtained from the recorded trials using LabAnalyst software (<http://warthog.ucr.edu/>). The highest continuous 60-sec average oxygen consumption throughout the trial was found and used as maximum oxygen consumption ( $VO_{2max}$ ). This method of eliciting  $VO_{2max}$  was significantly repeatable (see Appendix S1).

## BODY SIZE AND SUBORGANISMAL MORPHOLOGICAL TRAITS

When  $U_{crit}$  and  $VO_{2max}$  trials were complete, fish were euthanized in a lethal dose of buffered MS-222 (tricaine methanesulfonate) and measured for several size traits (Table S3): Body mass (to the nearest 0.001 g), total length (TL: from tip of snout to end of the caudal fin, to the nearest 0.01 mm), SL (from the most anterior portion to the insertion [base] of the caudal fin, to the nearest 0.01 mm), and body depth (BD: at the insertion of the dorsal fin, to the nearest 0.01 mm). Sword length was measured (to the nearest 0.01 mm) from the most posterior edge of the caudal fin (the base of the sword) to the tip of the sword, representing how much the sexually selected structure extends beyond the caudal fin. Caudal fin length was calculated as  $TL - SL$ , and relative sword length was calculated as  $SW/SL$ . The fish was then dissected from the insertion of the anal fin to the insertion of the dorsal fin to separate the caudal peduncle. The posterior section (caudal peduncle) was weighed to the nearest 0.001 g, flash frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  until enzyme assays (see below). The anterior portion of the fish was preserved in 5% formalin. At a later date, the hearts and gills were dissected from all fish (Table S3). The entire heart was removed, blotted dry, and weighed to the nearest 0.01 mg; the gills were separated from surrounding cartilage, ensuring only gill tissue was weighed, blotted dry, and weighed to the nearest 0.01 mg.

## GEOMETRIC MORPHOMETRICS

Each fish was photographed laterally while alive (not anesthetized). We digitized 17 landmarks, using a combination of body landmarks (Langerhans et al. 2004) and sliding landmarks (Hendry et al. 2006) to capture the detailed shape in the caudal peduncle region (Fig. S2). We used the tpsDig2 program to digitize landmarks on lateral photos of live fish (<http://life.bio.sunysb.edu/morph/>); to minimize error only one person (P. Tran) digitized all photos. We then used the tpsRelW program to first obtain the consensus shape of males within each species based on the 17 digitized landmarks (<http://life.bio.sunysb.edu/morph/soft-tps.html>). The consensus shapes for each species were then combined (19 species consensus shapes) to obtain the consensus shape among the 19 species. We next extracted 18 principal components (PCs) of shape variation among the 19 species. We used the first four PCs of shape, which explained 92.52% of the variation in shape (PC1: 37.39%,

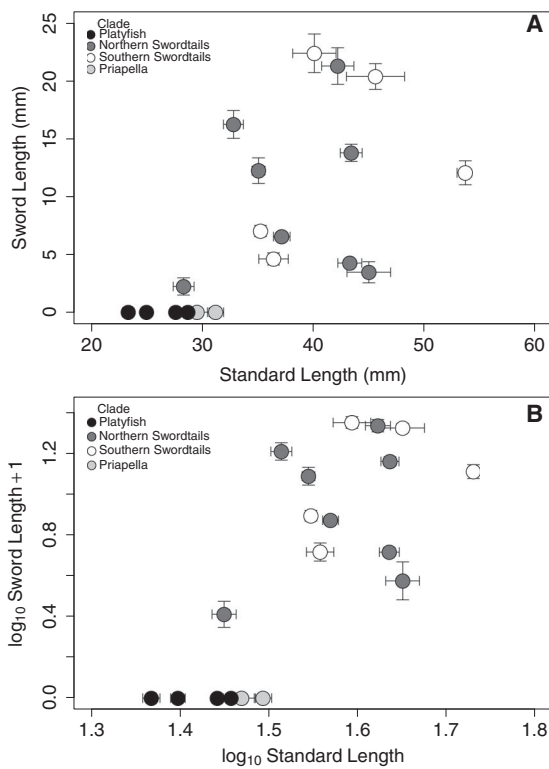
PC2: 29.31%, PC3: 16.79%, and PC4: 9.04%), as an indication of shape and potential compensatory traits.

## CITRATE SYNTHASE AND LACTATE DEHYDROGENASE ACTIVITY

Details on citrate synthase (CS) and lactate dehydrogenase (LDH) methods are provided in Appendix S1. In short, a kinetic assay was used for both, obtaining the  $V_{max}$  of enzymatic activity, following methods outlined in Odell et al. (2003). The entire tail muscle for each fish was homogenized in a buffer solution. After 10 min of centrifuging, 1 mL of the supernatant was removed and used for assays. CS activity was measured at a wavelength of 412 nm and LDH at 340 nm. Both were measured in triplicate with a control, with 10  $\mu\text{L}$  of tissue supernatant added to 100  $\mu\text{L}$  of assay mixture. The absorbance of the reaction was recorded at  $23^{\circ}\text{C}$  (to match the temperature of the performance trials).  $V_{max}$  was recorded for each sample, obtained as the slope of the line of time versus optical density for the first 10 points of the line and was expressed as milliunits  $\text{min}^{-1}$ . For both enzymes, we corrected  $V_{max}$  for the control and the assay batch (Table S1). We used the least squares means from each species in final analyses. Because LDH  $V_{max}$  is negative ( $V_{max}$  for LDH is expressed negatively because the reaction changes from a colored to a colorless solution), we reversed the sign so the values are positive.

## PHYLOGENY

We examined the relationships among traits (see statistical analysis below) using three phylogenetic hypotheses for *Xiphophorus* (Fig. 1). First, we generated our own phylogenetic hypothesis. Taxonomic sampling included 23 *Xiphophorus* spp. Two species of *Priapella* were chosen as the outgroup based on a previous molecular study (Hrbek et al. 2007). The internal branch connecting *Priapella* and *Xiphophorus* was arbitrarily midpoint rooted prior to performing phylogenetic generalized least squares (PGLS) analyses (see below). Genomic DNA extractions, PCR amplifications, and DNA sequencing were performed with protocols outlined in Meredith et al. (2010). Portions of seven nuclear gene regions were newly amplified in this study: (1) two partial exons (8 and 10), all of exon 9, and two introns (8 and 9) of the tyrosine kinase gene (*X-src*); (2) exon 1 of myosin, heavy polypeptide 6 (*myh6*); (3) exon 2 of ectodermal-neural cortex 1 like protein (*ENC1*); (4) exon 2 of glycosyltransferase (*Glyt*); (5) exon 1 of SH3 and PX domain containing 3 (*SH3PX3*); (6) a portion of the 7 transmembrane receptor region of rhodopsin (*Rh*); and (7) exon 3 of recombination activating gene-1 (*Rag1*). These gene segments were combined with previously published nuclear markers D2, D8, D29, and T36 (Meyer et al. 2006). Accession numbers for the new sequences are KJ525772-KJ525911.



**Figure 2.** Plots of the relationship between standard length and sword length (A) and  $\log_{10}$  standard length and  $\log_{10}$  (sword length + 1) (B). Points represent species means  $\pm$  standard error; color represents clade.

Sequences were manually aligned using Se-AL (Rambaut 1996). Visual inspection of the alignment identified alignment-ambiguous regions in x-src (intron 9, 13 bp) and D8 (15 bp), which were subsequently removed from all analyses. The concatenated alignment length was 7872 bp (without ambiguous regions). The dataset was partitioned into 12 segments (X-src introns, X-src exons, ENC1, Glyt, myh6, SH3PX3, Rh, Rag1, D2, D8, D29, T36) each of which was given its own model of sequence evolution (GTR + I +  $\Gamma$ ) based on the results of Modeltest 3.06 (Posada and Crandall 1998). Gaps were treated as missing data in all analyses. The maximum likelihood (ML) analyses were performed using RAxML 7.2.7 (Stamatakis 2006) and employed 500 bootstrapped replicates, randomized maximum parsimony starting trees, and the fast hill-climbing algorithm with all other free parameters estimated.

Second, we used two phylogenetic hypotheses based on Cui et al.'s recent study (Cui et al. 2013). We used their total nuclear evidence phylogeny based on transcriptome data (their Fig. 2A), as well as their mitochondrial tree, which included *Priapella* (their Fig. S2). All trees were subsequently modified for our analyses (Fig. 1, Appendices S2 and S3). First, we removed all species from the three phylogenies for which we did not have data (*X. andersi*, *X. continens*, *X. malinche*, *X. milleri*, *X. maculatus*, *X. evelynae*,

*X. xiphidium* [our phylogenetic hypothesis], and *X. mayae*, *Pseudoxiphophorus jonesi* [Cui et al.'s phylogeny]). Second, because our two populations of *X. cortezi* had no detectable genetic differences between them, resulting in branch lengths of zero, we only used the population from the *Xiphophorus* stock center (Appendix S2), which had the larger sample size. Third, because one of our species is a potential hybrid (what we are calling *X. multi-cortezi*), we added this species as a sister to *X. cortezi* because of its morphological similarity. We added *X. multi-cortezi* halfway up the *X. cortezi* branch, giving the two sister taxa equal branch lengths, each half the original branch length of *X. cortezi* (Oufiero et al. 2011a). Lastly, because Cui et al.'s phylogenies only included one species of *Priapella*, we grafted a second species of *Priapella* (*Priapella chamulae*) onto their trees following the same procedures that were used to add *X. multi-cortezi*.

## STATISTICAL ANALYSES

To determine if the evolution of a longer sword affects critical swimming speed ( $U_{crit}$ ) and the effects of any other significant predictors of  $U_{crit}$ , we examined correlations and multiple regressions of residual trait values (residuals from regressions on  $\log_{10}$  SL) using both conventional and phylogenetically informed statistical models (Rezende and Diniz-Filho 2012). We first  $\log_{10}$  transformed all traits except enzyme activities (for sword length, we used  $\log_{10}$  sword length + 1, as some species have a zero value for the trait); we also included a ratio of sword size (sword length/SL). Using these trait values, we determined by ANCOVA (with  $\log_{10}$  SL as the covariate) that there was statistically significant variation among species for all traits (see Table S4).

Because body size may affect most if not all traits used in this study, we examined allometric equations for each trait based on the phylogenetic hypothesis from this study. Although all of our size traits were significantly positively correlated, we used  $\log_{10}$  SL as an index of size. Body mass may have been more appropriate for allometric relationships with mass measures, but because we were interested in the effects of sword length, which is a linear measure, we used SL for all traits. We used the Matlab Regressionv2.m program (Lavin et al. 2008) to obtain the slope, intercept, and 95% confidence intervals (from parametric bootstrapping) for each allometric equation under three models of evolution: nonphylogenetic (ordinary least squares [OLS]; equivalent to using a star phylogeny), PGLS with our branch lengths (as shown in Fig. 1A and presented in Appendix S2), and a phylogenetic regression model that uses an Ornstein–Uhlenbeck transformation to model the residuals as subject to stabilizing selection (Ornstein–Uhlenbeck regression [RegOU], with restricted maximum likelihood [REML] estimation of “d” [the tree transformation parameter, related to the strength of the restraining force in an Ornstein–Uhlenbeck (OU) model]

**Table 1.** Allometric equations with  $\log_{10}$  standard length (SL) from Regressionv2.m—three models of evolution (OLS, PGLS, RegOU) based on the phylogeny depicted in Figure 1A.

Comparison	OLS	PGLS	RegOU	Chi-square RegOU versus OLS	Chi-square RegOU versus PGLS
$\log_{10} U_{crit}$ on $\log_{10}$ SL					
$\beta$	0.3080	0.2028	0.2310		
$P$	0.2539	0.4272	0.3876		
CI (lower, upper)	-0.179, 0.805	-0.261, 0.658	0.019, 0.932		
Intercept	0.8760	1.1269	1.0320		
ln maximum likelihood	16.36	17.13	18.14	3.08	2.02
$d$			0.766		
$r^2$	0.0758	0.0375	0.0442		
$\log_{10} VO_{2max}$ on $\log_{10}$ SL:					
$\beta$	1.245	1.4607	1.3911		
$P$	<0.001	<0.001	<0.001		
CI (lower, upper)	0.935, 1.546	1.085, 1.816	1.039, 1.662		
Intercept	-0.8502	-1.2069	-1.0830		
ln maximum likelihood	25.40	21.12	26.70	2.60	11.16 <sup>2</sup>
$d$			0.131		
$r^2$	0.7764	0.7546	0.7938		
$\log_{10} VO_{2max}$ on $\log_{10}$ mass					
$\beta$	0.4035	0.4739	0.4432		
$P$	<0.001	<0.001	<0.001		
CI (lower, upper)	0.313, 0.499	0.355, 0.589	0.321, 0.526		
Intercept	1.0689	1.0547	1.0653		
ln maximum likelihood	25.14	21.61	25.95	1.62	8.68 <sup>2</sup>
$d$			0.141		
$r^2$	0.7703	0.7668	0.7762		
$\log_{10}$ heart mass on $\log_{10}$ SL <sup>1</sup>					
$\beta$	2.7497	2.8630	2.7878		
$P$	<0.001	<0.001	<0.001		
CI (lower, upper)	2.101, 3.399	1.989, 3.702	2.115, 3.489		
Intercept	-7.7163	-7.7932	-7.7720		
ln maximum likelihood	11.56	5.95	11.60	0.08	11.30 <sup>2</sup>
$d$			0.003		
$r^2$	0.8018	0.7012	0.7907		
$\log_{10}$ gill mass on $\log_{10}$ SL <sup>1</sup>					
$\beta$	2.4079	3.1478	3.1276		
$P$	<0.001	<0.001	<0.001		
CI (lower, upper)	1.672, 3.194	2.371, 3.764	2.273, 3.859		
Intercept	-6.0103	-7.1003	-7.0657		
ln maximum likelihood	7.76	9.56	10.32	5.12 <sup>2</sup>	1.52
$d$			0.793		
$r^2$	0.6920	0.8071	0.7902		
CS $V_{max}$ on $\log_{10}$ SL					
$\beta$	-139.129	-154.865	-119.656		
$P$	0.0538	0.0858	0.1403		
CI (lower, upper)	-263.1, -192.0	-328.7, -9.320	-271.6, 352.4		
Intercept	354.853	377.052	325.787		
ln maximum likelihood	-89.11	-93.66	-89.06	0.10	9.2 <sup>2</sup>
$d$			0.195		
$r^2$	0.2016	0.1636	0.1234		

(Continued)

**Table 1. Continued.**

Comparison	OLS	PGLS	RegOU	Chi-square RegOU versus OLS	Chi-square RegOU versus PGLS
LDH $V_{\max}$ on $\log_{10}$ SL					
$\beta$	13.2699	-95.640	-33.183		
$P$	0.8484	0.1686	0.6478		
CI (lower, upper)	-112.7, 143.2	-212.6, 37.981	-168.2, 108.3		
Intercept	108.280	267.259	180.285		
ln maximum likelihood	-89.44	-89.02	-87.32	4.24 <sup>2</sup>	3.4
$d$			0.518		
$r^2$	0.0022	0.1085	0.0126		
$\log_{10}$ sword length on $\log_{10}$ SL					
$\beta$	4.1723	0.8044	1.1381		
$P$	<0.001	0.3614	0.2170		
CI (lower, upper)	2.562, 5.727	-0.252, 2.648	-0.457, 3.145		
Intercept	-5.7665	-0.7903	-1.3056		
ln maximum likelihood	-6.00	-6.35	-5.28	1.44	2.14
$d$			0.781		
$r^2$	0.5885	0.0492	0.0882		
$\log_{10}$ mass on $\log_{10}$ SL					
$\beta$	3.0057	3.0467	3.0564		
$P$	<0.001	<0.001	<0.001		
CI (lower, upper)	2.710, 3.310	2.754, 3.259	2.745, 3.391		
Intercept	-4.6314	-4.7179	-4.7385		
ln maximum likelihood	26.08	27.03	28.54	4.92 <sup>2</sup>	3.02
$d$			0.683		
$r^2$	0.9560	0.9614	0.9586		
$\log_{10}$ tail mass on $\log_{10}$ SL					
$\beta$	3.3335	3.0361	3.1415		
$P$	<0.001	<0.001	<0.001		
CI (lower, upper)	3.019, 3.640	2.643, 3.345	2.704, 3.463		
Intercept	-5.5478	-5.0864	-5.2645		
ln maximum likelihood	24.86	21.99	25.07	0.42	6.16 <sup>2</sup>
$d$			0.387		
$r^2$	0.9592	0.9357	0.9395		
$\log_{10}$ caudal fin length on $\log_{10}$ SL					
$\beta$	1.1846	1.0399	1.1217		
$P$	<0.001	<0.001	<0.001		
CI (lower, upper)	1.073, 1.301	0.907, 1.187	0.987, 1.257		
Intercept	-0.8473	-0.6214	-0.7474		
ln maximum likelihood	44.16	39.43	44.79	1.26	10.72 <sup>2</sup>
$d$			0.132		
$r^2$	0.9577	0.9146	0.9435		
$\log_{10}$ body depth on $\log_{10}$ SL					
$\beta$	1.0415	1.1988	1.1951		
$P$	<0.001	<0.001	<0.001		
CI (lower, upper)	0.841, 1.237	1.199, 1.335	1.018, 1.384		
Intercept	-0.6020	-0.8230	-0.8246		
ln maximum likelihood	33.41	35.87	36.19	5.56 <sup>2</sup>	0.64
$d$			0.948		
$r^2$	0.8495	0.9073	0.9042		

<sup>1</sup>These equations also included preservation time, effect not shown.

Chi-squares (2 × difference in ln maximum likelihoods) reported for RegOU versus OLS, and RegOU versus PGLS.

<sup>2</sup>Significant difference in models ( $\chi^2 > 3.841$ ).

and the regression parameters; see Lavin et al. 2008). To correct the traits for associations with body size, we used residuals from the allometric equations under the OLS model (Table 1, note: for heart and gill mass, preservation time was included in allometric equations). These OLS residuals were used for bivariate correlations and multiple regression analyses. We performed subsequent analyses on OLS residuals, not generalized least squares (GLS) residuals, to retain a clear interpretation of the results. In GLS estimation, the computed residuals are not associated with specific taxa, and therefore any explanation of the residual variance cannot be traced back to traits of species. Even for phylogenetically correlated data, the OLS residuals are statistically well defined; OLS gives unbiased estimates of the mean and regression coefficients, with the difference between OLS and GLS being that GLS gives the correct uncertainty in the estimates (e.g., standard errors). Because we are interested in the (possibly phylogenetic) patterns exhibited by the residuals rather than the confidence we have in the estimates of parameters in the initial regressions on body size, there are no statistical problems or inconsistencies introduced by our approach.

Because allometric equations for LDH changed qualitatively among models (i.e., a change in sign of the partial regression coefficient), we did not correct this trait for size. Similarly, the allometric relationship for sword length changed among models (see Results), so we used relative sword length (SW/SL) as a size-corrected measure of sword length; this effectively assumes an allometric slope of unity, which is justified based on analyses of the data (see Results). Finally, we used raw PCs of shape variables as an indication of shape because none of our four PCs of shape were significantly related to size in OLS regressions with centroid size (PC1  $P = 0.126$ ; PC2  $P = 0.0814$ ; PC3  $P = 0.710$ ; PC4  $P = 0.0699$ ); and only one of our four PCs of shape was marginally significantly related to size in OLS regressions with  $\log_{10}$  SL (PC1  $P = 0.122$ ; PC2  $P = 0.0867$ ; PC3  $P = 0.688$ ; PC4  $P = 0.0457$ ).

We used several methods to test for relationships between sword length and other potential predictors of  $U_{crit}$ . (1) We first performed four correlation analysis for all traits under four models of evolution: (a) nonphylogenetic, (b) phylogenetic based on the phylogeny obtained from this study (Fig. 1A), (c) phylogenetic based on the total evidence nuclear phylogeny of Cui et al. (2013; our Fig. 1B), and (d) phylogenetic based on the mitochondrial phylogeny of Cui et al. (2013; our Fig. 1C). If the sword has a negative effect on performance and compensatory traits have evolved to offset the potential cost of the sword, then we would expect a significant negative correlation between relative sword length and  $U_{crit}$ , a positive correlation between relative sword length and size-corrected suborganismal, physiological, and shape predictors, and a positive effect of suborganismal, physiological, and shape predictors on  $U_{crit}$ . (2) Because of the multivariate nature of

the dataset, we also used phylogenetic principal component analyses (PCAs) across the three phylogenies in Figure 1 (Clobert et al. 1998; Revell 2009) to determine if there are patterns of diversity in lower level traits that associated with sword length. This procedure allows for the determination of the major axis of diversity in relation to sword length, suborganismal traits, and PCs of shape.

To determine if sword length is a significant predictor of critical swimming speed taking into account uncertainty in phylogenetic relationships and other potential  $U_{crit}$  predictors, we examined phylogenetic multiple regression models under the four models of evolution listed above for correlation analyses: the three phylogenetic hypotheses presented in Figure 1 as well as a nonphylogenetic multiple regression (OLS). We first examined a full multiple regression model. This multiple regression model included residual  $\log_{10} U_{crit}$  as the dependent variable, with independent variables of SW/SL, LDH, PC of shape axes 1–4 (PC1–4, see Geometric Morphometrics above), residual CS, and residual  $\log_{10}$ :  $VO_{2max}$ , heart mass, gill mass, BD, tail mass, and caudal fin length. However, because of small taxonomic sample size, the potential of overfitting of the full models, and to determine if there were any unique combinations of predictors for  $U_{crit}$ , we performed a backwards elimination procedure (sequentially removing the predictor with the lowest  $F$ -value) under the four models of evolution (three phylogenetic and one nonphylogenetic). Taken together, these models let us determine the important predictors of critical swimming speed when body size and phylogenetic information are taken into account. If morphological diversity resulting from sexual selection is important, then relative sword length should be retained in the models. Although automated algorithms (such as backwards elimination) for finding “best” multiple regression models can perform poorly, other procedures yielded similar models, with similar levels of statistical significance for relative sword length in both OLS and GLS (based on the phylogeny of this study, Fig. 1A) analyses (Appendix S4). Multiple regressions were performed in R using the `gls` function, with the “`corPagel`” correlation structure for each of the three phylogenies to perform a PGLS regression.

## Results

### SWORD LENGTH

In the 13 species that exhibited a sword, sword length ranged from 7% of SL in *X. pygmaeus* to 55% in *X. hellerii*, and varied significantly among species (Fig. 1, Table S3). Bivariate scatterplots suggest a generally positive relation between sword length and SL (Fig. 2), but these plots are misleading. The apparently positive relation is driven by the fact that species lacking swords are mostly small in body size (see Table S3), and restricted to two lineages (clades): the two species of *Priapella* and the four *Xiphophorus* species at the top of the phylogeny shown in Figure 1A. The slope



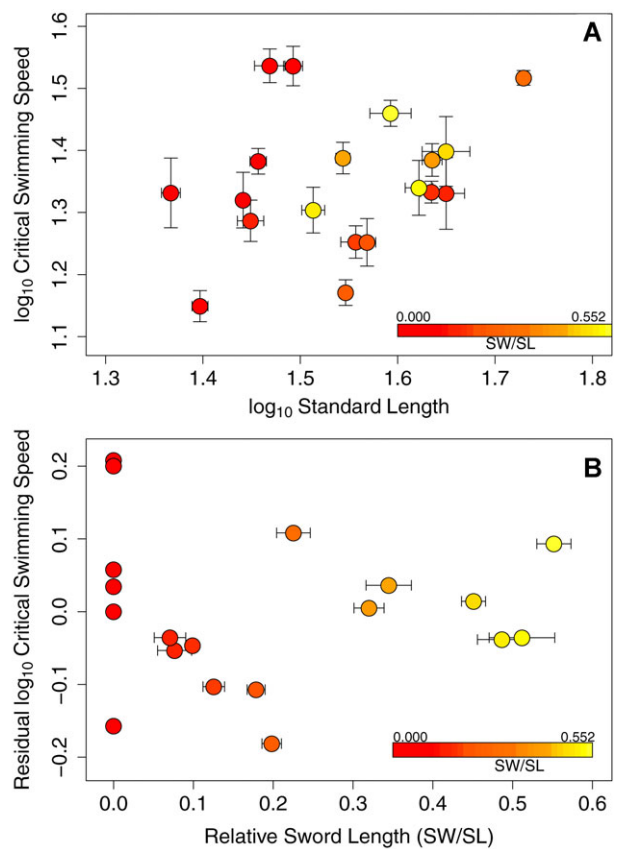
for  $\log_{10}$  sword length on  $\log_{10}$  SL changed from 4.17 under the OLS model, where it was significantly different from 1.00, to 0.80 and 1.06 under the PGLS and RegOU models, respectively, where it was not significantly different from 1.00 (Table 1). Further analyses indicated that a “grade shift” (e.g., see Garland et al. 2005) in sword length was responsible for the discrepancy between the OLS and phylogenetic models, that is, some entire lineages have swords whereas others do not (Fig. 1). Adding a clade variable (1 = platyfish, 2 = Northern swordtails, 3 = Southern swordtails, 4 = *Priapella*) significantly improved the OLS model (ln ML increased from  $-6.00$  to  $1.31$ , ln likelihood ratio test  $\chi^2 = 14.63$ ,  $df = 3$ ,  $P = 0.002$ ; partial  $F$  for clade =  $5.41$ ,  $df = 3, 14$ ,  $P = 0.011$ ) and changed the slope to  $1.21$  (95% bootstrap CI =  $-1.38$  to  $3.86$ ). As a result of this “grade shift,” the OLS allometric model erroneously suggests a positive relation between  $\log_{10}$  sword length and  $\log_{10}$  SL, whereas the PGLS and RegOU models indicate no statistically significant relation (Table 1). Allometric models for the other traits were similar across phylogenetic models; therefore, grade shifts were not apparent in these traits (Table 1).

### CRITICAL SWIMMING SPEED ( $U_{\text{CRIT}}$ )

Mean  $U_{\text{crit}}$  varied significantly among species (Table S4,  $P < 0.0001$ ), ranging from  $14.46 \text{ cm}\cdot\text{s}^{-1}$  in *X. meyeri* to  $35.60 \text{ cm}\cdot\text{s}^{-1}$  in *P. compressa* (Table S2). This interspecific variation was not significantly related to  $\log_{10}$  SL (Table 1, Fig. 3A, OLS:  $\beta = 0.3080$ ,  $P = 0.2539$ ; PGLS:  $\beta = 0.2028$ ,  $P = 0.4272$ ; RegOU:  $\beta = 0.2310$ ,  $P = 0.3876$ ); and  $r^2$  values (not comparable across models) were small (OLS  $r^2 = 0.076$ , PGLS  $r^2 = 0.038$ , RegOU  $r^2 = 0.035$ ).

Correlation results (Tables 2 and 3) reveal no significant correlation between  $U_{\text{crit}}$  and relative sword length across phylogenies, supporting hypothesis 2 that interspecific variation in sword length has no effect on endurance. However, there were some consistent correlates of  $U_{\text{crit}}$  across phylogenies. BD was positively correlated and PC shape 3 negatively correlated to  $U_{\text{crit}}$  in all analyses and significant in three of the four phylogenetic analyses. Heart mass was positively correlated to  $U_{\text{crit}}$  in the analyses using the two phylogenies from Cui et al. (2013). Similar results were obtained in PCA results (Tables S5–S7), with sword length and  $U_{\text{crit}}$  not loading significantly on the same axes.

Results from multiple regression analyses based on the full model revealed a significant positive effect of relative sword length in three of the four models of evolution (Table S8). Furthermore, in backwards elimination procedures for each phylogeny, relative sword length was retained and had a significant positive effect on residual  $\log_{10} U_{\text{crit}}$  (Table 4, Fig. 3B). Thus, as species evolved longer swords, they had an increase in  $U_{\text{crit}}$ , consistent with hypothesis 3. Across phylogenies, we also found several significant suborganismal and shape predictors of  $U_{\text{crit}}$ . As expected from functional relationships, heart mass and BD had significant



**Figure 3.** Plots of the relationship between  $\log_{10}$  standard length and  $\log_{10}$  critical swimming speed (A) and relative sword length and residual  $\log_{10}$  critical swimming speed (B). Points represent species means  $\pm$  standard error; color represents relative sword length.

positive effects on  $U_{\text{crit}}$  (Table 4). We also found a significant positive effect of PC shape 2 (Fig. 4A) and a significant negative effect of PC shape 3 (Fig. 4B). High values of PC shape 2 indicate a shape that is similar to the shape of fish from *Priapella*, with a dorsal fin that is more posterior, and a slightly narrower caudal peduncle (Fig. 5). Low values of PC shape 3 are indicative of fish with the indentation at the posterodorsal end of head (point 2) further back, representing a more elongate head (Fig. 5).

### RELATIONSHIPS AMONG SWORD LENGTH, SUBORGANISMAL TRAITS, AND SHAPE PREDICTORS

Results from nonphylogenetic (Table 2) and phylogenetic (Tables 2 and 3) pairwise correlations, as well as the phylogenetic PCA (Tables S5–S7) revealed no consistent significant positive associations of relative sword length with other potential performance predictors of  $U_{\text{crit}}$ . However, under some phylogenetic hypotheses, relative sword length was positively associated with other traits that were not related to  $U_{\text{crit}}$ , such as caudal fin length and PC shape 4 (Tables 2, 3, and S5). Furthermore, results from

**Table 2.** Pearson's correlation coefficient of residuals (except SW/SL, LDH, and principal components of shape [PC1–4]) from each trait on log<sub>10</sub> standard length.

	$U_{crit}$	SW/SL	VO <sub>2max</sub>	Heart	Gill	CS $V_{max}$	LDH $V_{max}$	Body depth	Tail Tail mass	Caudal fin length	PC1	PC2	PC3	PC4
$U_{crit}$		-0.053	-0.367	0.226	0.165	-0.147	-0.125	<b>0.499</b>	0.157	-0.062	-0.327	<b>0.517</b>	<b>-0.560</b>	0.018
SW/SL	0.185		-0.060	<b>-0.470</b>	-0.405	0.025	0.150	-0.204	0.019	0.292	-0.140	-0.414	0.241	<b>0.571</b>
VO <sub>2max</sub>	-0.005	-0.042		-0.073	0.093	0.349	0.354	-0.154	-0.025	-0.127	0.194	-0.019	<b>0.476</b>	-0.204
Heart	0.380	<b>-0.600</b>	0.025		<b>0.880</b>	0.072	0.040	<b>0.536</b>	0.247	-0.452	-0.050	-0.068	-0.244	<b>-0.556</b>
Gill	0.099	<b>-0.610</b>	0.055	<b>0.820</b>		0.021	0.164	<b>0.521</b>	0.188	<b>-0.598</b>	0.014	-0.122	-0.207	<b>-0.657</b>
CS $V_{max}$	0.333	0.035	0.202	0.434	0.134		<b>0.560</b>	-0.114	0.013	0.039	0.260	0.069	0.294	-0.025
LDH $V_{max}$	0.174	0.185	0.002	0.082	0.028	0.304		-0.275	-0.233	-0.021	0.386	0.163	0.215	0.002
Body depth	0.331	-0.094	0.182	0.391	0.446	0.146	-0.163		<b>0.748</b>	0.004	<b>-0.674</b>	0.093	-0.078	<b>-0.510</b>
Tail mass	0.238	0.031	0.267	0.263	-0.032	<b>0.634</b>	0.151	0.318		0.374	<b>-0.662</b>	-0.104	0.128	-0.188
Caudal fin length	-0.196	0.315	-0.115	<b>-0.574</b>	<b>-0.685</b>	-0.244	0.063	-0.075	0.166		-0.184	0.123	0.316	0.416
PC1	0.053	-0.117	-0.326	-0.004	-0.165	-0.129	0.190	<b>-0.710</b>	-0.269	0.080		0.000	0.000	0.000
PC2	0.297	-0.028	0.184	0.014	-0.139	-0.092	0.433	-0.128	0.000	0.262	<b>0.459</b>		0.000	0.000
PC3	-0.452	0.229	<b>0.472</b>	-0.256	-0.159	-0.001	-0.197	0.231	0.170	0.252	-0.361	0.067		0.000
PC4	0.015	0.373	-0.142	-0.449	-0.490	-0.039	0.149	<b>-0.610</b>	-0.146	0.347	<b>0.511</b>	0.289	-0.149	

Pearson's  $r$  from OLS is in upper diagonal, and Pearson's  $r$  from PGLS correlation based on the phylogenetic hypothesis of this study is in lower diagonal (Fig. 1A). Significant ( $P < 0.05$ ) correlations are in bold, uncorrected for multiple comparisons. PC1–4 are from geometric morphometric data.

**Table 3.** Pearson's correlation coefficient of residuals (except SW/SL, LDH, and principal components of shape [PC1–4]) from each trait on log<sub>10</sub> standard length.

	$U_{crit}$	SW/SL	VO <sub>2max</sub>	Heart	Gill	CS $V_{max}$	LDH $V_{max}$	Body depth	Tail mass	Caudal fin length	PC1	PC2	PC3	PC4
$U_{crit}$		0.071	0.019	<b>0.595</b>	0.369	0.271	0.124	<b>0.563</b>	0.135	−0.351	−0.039	0.355	<b>−0.470</b>	−0.086
SW/SL	0.056		0.189	<b>−0.526</b>	<b>−0.498</b>	0.099	0.149	−0.047	0.060	0.152	−0.221	−0.074	0.365	0.371
VO <sub>2max</sub>	−0.219	0.349		−0.208	−0.165	0.021	0.064	0.062	0.137	−0.092	−0.263	0.198	0.411	−0.168
Heart	<b>0.594</b>	<b>−0.582</b>	<b>−0.559</b>		<b>0.804</b>	0.393	0.102	<b>0.484</b>	0.235	−0.442	0.064	0.093	<b>−0.488</b>	−0.335
Gill	0.132	<b>−0.696</b>	<b>−0.468</b>	<b>0.771</b>		0.152	0.136	<b>0.474</b>	0.022	<b>−0.581</b>	−0.067	−0.084	−0.445	−0.418
CS $V_{max}$	<b>0.462</b>	−0.129	<b>−0.517</b>	<b>0.671</b>	0.295		0.248	0.315	<b>0.579</b>	−0.132	−0.264	−0.191	0.004	−0.070
LDH $V_{max}$	−0.018	0.137	0.251	0.001	0.061	<b>0.556</b>		−0.148	0.092	0.063	0.217	0.428	−0.147	0.267
Body depth	<b>0.477</b>	<b>−0.472</b>	<b>−0.482</b>	<b>0.824</b>	<b>0.757</b>	0.363	−0.157		0.430	−0.074	<b>−0.631</b>	−0.063	0.029	<b>−0.596</b>
Tail mass	0.087	−0.208	−0.014	0.394	0.396	−0.206	0.172	<b>0.519</b>		0.281	−0.419	−0.138	0.159	−0.258
Caudal fin length	−0.077	<b>0.526</b>	0.343	<b>−0.575</b>	<b>−0.814</b>	<b>−0.477</b>	0.100	<b>−0.480</b>	−0.186		0.017	0.149	0.363	0.217
PC1	0.052	0.234	0.382	−0.363	−0.416	−0.429	0.266	<b>−0.608</b>	−0.395	0.364		<b>0.456</b>	−0.279	<b>0.467</b>
PC2	0.131	0.273	<b>0.655</b>	−0.325	−0.412	−0.272	<b>0.533</b>	−0.378	−0.095	0.442	<b>0.629</b>		0.039	0.237
PC3	<b>−0.615</b>	0.200	<b>0.541</b>	−0.443	−0.222	−0.174	0.149	−0.224	0.126	0.243	−0.035	0.318		−0.124
PC4	−0.036	<b>0.678</b>	0.221	<b>−0.577</b>	<b>−0.760</b>	<b>0.556</b>	0.205	<b>−0.710</b>	−0.413	<b>0.591</b>	0.432	0.385	0.008	

Pearson's  $r$  from PGLS correlation based on phylogenetic hypothesis of transcriptome data (Cui et al., Fig. 2A; our Fig. 1B) is in upper diagonal, and Pearson's  $r$  from PGLS correlation based on the phylogenetic hypothesis of mitochondrial data (Cui et al., Fig. S2; our Fig. 1C) is in lower diagonal. Significant ( $P < 0.05$ ) correlations are in bold, uncorrected for multiple comparisons. PC1–4 are from geometric morphometric data.

**Table 4.** Comparison of models from backwards elimination procedure under four models of evolution: nonphylogenetic (OLS), PGLS based on the tree from this study (Fig. 1A), PGLS based on the total evidence nuclear tree from Cui et al. (Fig. 1B), and PGLS based on the mitochondrial tree of Cui et al. (Fig. 1C).

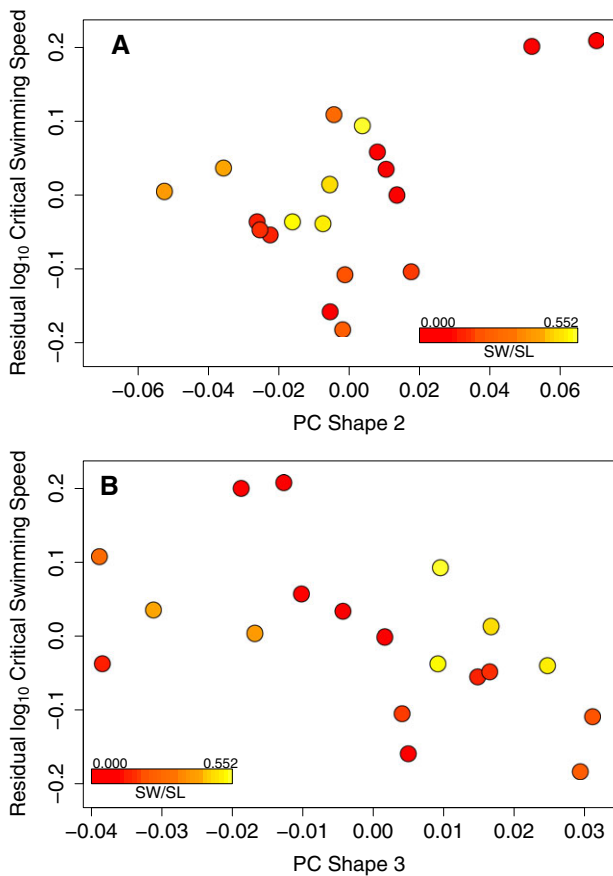
Backwards elimination (OLS)			
	$\beta$	$F_{1,14}$	$P$ -value
Sword/standard length	0.2554	29.2165	<0.0001
Residual $\log_{10}$ body depth	1.1889	38.7853	<0.0001
Morphometric PC2	2.4776	63.0568	<0.0001
Morphometric PC3	-3.1190	65.0001	<0.0001
Backwards elimination (our tree, Fig. 1A)			
	$\beta$	$F_{1,11}$	$P$ -value
Sword/standard length	0.4055	45.0714	<0.0001
Residual $\log_{10}$ $VO_{2\max}$	0.2666	5.0953	0.0188
Residual $\log_{10}$ heart mass	0.3045	18.3865	0.0045
LDH $V_{\max}$	-0.0012	9.8651	0.0003
Residual $\log_{10}$ body depth	0.7274	11.2658	0.0093
Morphometric PC2	3.3401	26.4023	<0.0001
Morphometric PC3	-3.3993	49.5645	<0.0001
Backwards elimination (Cui et al.'s transcriptome, Fig. 1B)			
	$\beta$	$F_{1,11}$	$P$ -value
Sword/standard length	0.2578	17.9385	0.0014
Residual $\log_{10}$ heart mass	0.2916	12.2508	0.0050
Residual $\log_{10}$ gill mass	-0.3431	12.2981	0.0049
Residual $\log_{10}$ body depth	1.5636	44.9285	<0.0001
Residual $\log_{10}$ tail length	-1.2109	11.8064	0.0056
Morphometric PC2	2.4537	33.9540	0.0001
Morphometric PC3	-2.7908	38.8105	0.0001
Backwards elimination (Cui et al.'s mitochondrial tree, Fig. 1C)			
	$\beta$	$F_{1,13}$	$P$ -value
Sword/standard length	0.4729	43.6672	<0.0001
Residual $\log_{10}$ heart mass	0.5557	90.2507	<0.0001
LDH $V_{\max}$	-0.0017	21.0974	0.0005
Morphometric PC2	3.3026	50.9082	<0.0001
Morphometric PC3	-2.5640	38.0322	<0.0001

the backwards elimination procedures revealed several significant predictors of  $U_{\text{crit}}$  (as discussed above), suggesting that although they have not coadapted with relative sword length, these traits have evolved, at least in part, to increase stamina. Furthermore, in pairwise correlations and phylogenetic PCA, we found a significant negative association of residual heart and gill mass with relative sword length and tail length, suggesting a possible trade-off between these organ masses and relative sword and/or tail length.

## Discussion

Our results suggest that the exaggeration of a morphological trait, which is used for propulsion, through sexual selection does not constrain aerobic locomotion; species that evolved longer swords do not have a reduction in critical swimming speed. These results are the first to show the effect of interspecific variation in

a sexually selected trait on functional abilities. The sword found in several species of *Xiphophorus* was one of the first examples of sexual selection proposed by Darwin (1871). Sword evolution is partly the result of a preexisting female bias (Basolo 1990, 1995), and expression of the sword varies throughout the genus (Fig. 1). Theory predicts that sexually selected traits will impose costs (Kotiaho 2001; Oufiero and Garland 2007), and intraspecific experimental evidence suggests that the presence of the sword increases energetic demands (Basolo and Alcaraz 2003) and reduces  $U_{\text{crit}}$  (Kruesi and Alcaraz 2007). Additionally, the absence of swords in females and immature individuals suggests that the exaggeration of a structure used for propulsion may reduce locomotor capabilities (and hence does not appear except where needed for reproductive success, as in adult males). Conversely, interspecific variation in a sexually selected trait may have no effect on locomotion. Consistent with that perspective, some



**Figure 4.** Relationship between shape and residual  $\log_{10}$  critical swimming speed. (A) Principal component of shape 2 versus residual  $\log_{10}$  critical swimming speed, high values of PC2 are indicative of species with the insertion of the dorsal fin more posterior, and more narrow caudal peduncle (see Fig. 5). (B) Principal component of shape 3 versus residual  $\log_{10}$  critical swimming speed, low values of PC3 are indicative of shapes with indentation at the posterodorsal end of head more posterior (see Fig. 5). Points represent species means; colors represent relative sword length.

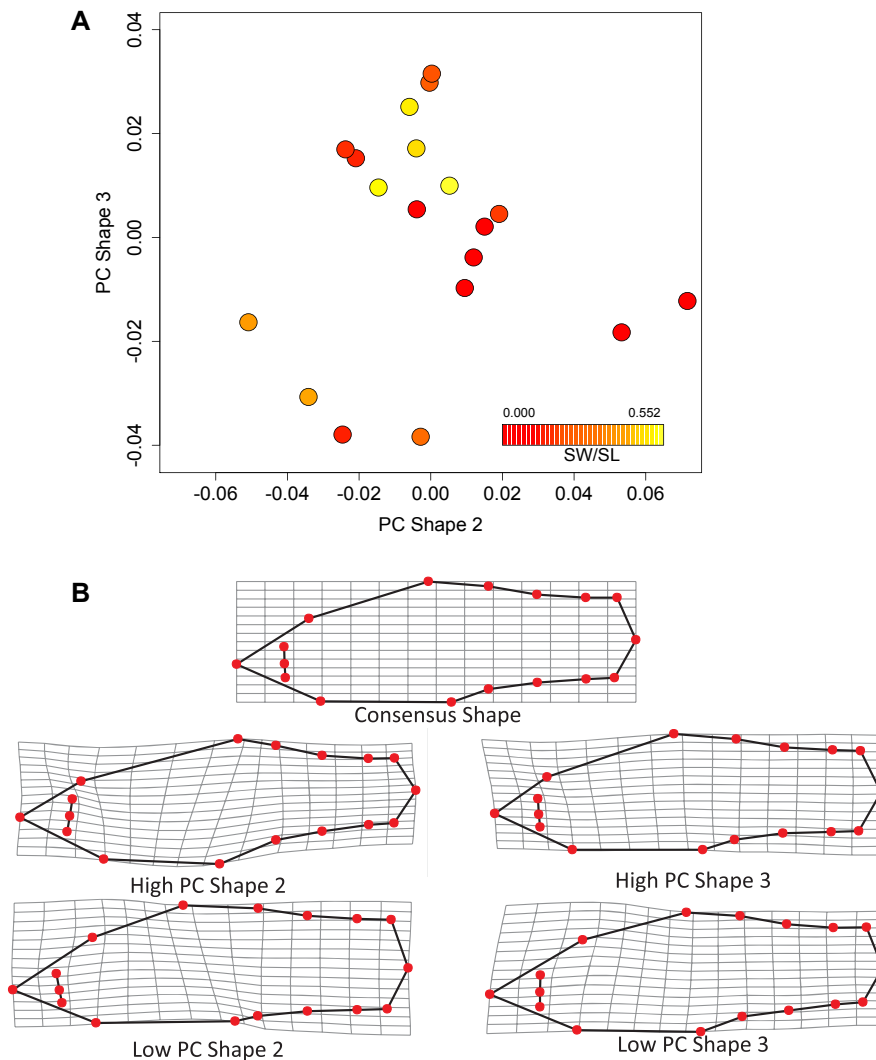
biomechanical models suggest that asymmetric exaggeration of fins may be beneficial to locomotion (Walker and Westneat 2002; Lauder et al. 2011; Borazjani and Daghooghi 2013). Our results support hypothesis 2 that the evolution of a sexually selected trait does not hinder aerobic locomotion; however, it may be beneficial to aerobic locomotion.

Critical swimming speed is a measure of endurance capacity, initially used in fisheries sciences (Brett 1964; Beamish 1978). It has more recently been examined in ecological and evolutionary contexts (Fulton et al. 2005; Claireaux et al. 2007; Fulton 2007; Handelsman et al. 2010; Dalziel et al. 2012), with studies demonstrating that measures of individual differences in  $U_{crit}$  can be statistically repeatable (Oufiero and Garland 2009) and can diverge among populations in relation to differing selective regimes, such as variation in predation (Oufiero et al. 2011b). The Darwinian

fitness benefit of an increased  $U_{crit}$  has not been demonstrated rigorously, but it is reasonable to assume that an increased  $U_{crit}$  among *Xiphophorus* and some poeciliids in general may have fitness benefits. First, several poeciliids, including *Xiphophorus* and *Priapella*, are found in fast-moving streams (e.g., Gordon 1953), where a high  $U_{crit}$  may be beneficial for maintaining position against the current (Nelson et al. 2003). Second, many *Xiphophorus* males court females with complex maneuvers, fin displays, and chasing (Bisazza 1993). An increased  $U_{crit}$  may give males an advantage by allowing them to exhibit increased courtship displays (Basolo and Alcaraz 2003), or may be a trait preferred by females (Byers et al. 2010). Finally, several species of *Xiphophorus* engage in male–male interactions (Morris et al. 1992), and an increased  $U_{crit}$  may provide a competitive advantage, although no studies of fish have examined if an increase in aerobic locomotor performance correlates with mating and/or intraspecific competitive success (cf. Chappell et al. 1997, Perry et al. 2004, and references therein).

Interpretation of our interspecific results for the effect on the sexually selected sword on  $U_{crit}$  is difficult for several reasons. First, most studies of sexual selection have focused on intraspecific variation. The goal of our study was to determine how the evolution of morphological diversity through sexual selection affects functional diversity; whereas intraspecific studies examining the costs of sexually selected traits are concerned with why the trait evolves and what information it may convey to the receiver (e.g., good genes). Few studies have provided information for how interspecific variation in sexual selection or sexual dimorphism may affect the species (Read and Weary 1990; Promislow 1992; Promislow et al. 1992). For example, Promislow et al. (1992) found an increase in sex-biased mortality with increasing male brightness among 28 species of birds (after phylogenetic corrections), suggesting that in species with brighter males, the males suffer higher mortality compared to conspecific females. However, when they examined the sexes separately, they found a significant negative relationship between female mortality and male brightness, and no significant relationship between male brightness and male mortality. These results suggest that the sex-biased mortality is the result of a decrease in female mortality in species with high male brightness and that there is no difference in mortality among males with varying brightness. It has therefore been suggested previously that interspecific variation in a sexually selected trait may not result in interspecific variation in the trait's costliness (e.g., see Lyon and Montgomerie 1985).

Second, the methods to determine costs at the intraspecific level have been variable, and have included experimental manipulation, natural variation, and comparison to the sex that lacks the trait (Kotiaho 2001; Oufiero and Garland 2007). Based on the previous results in *Xiphophorus*, should we expect species with longer swords to incur a cost to locomotion? Intraspecific



**Figure 5.** Plot of principal component of shape 2 versus principal component of shape 3 (A), the two shape variables that were significant predictors of residual  $\log_{10}$  critical swimming speed. (B) Consensus shape and representative shapes for high and low values of PC shapes 2 and 3 included. Although there was no relationship between shape and relative sword length (Tables 2 and 3), species with a high value of PC2 had increased critical swimming speeds, and species with a low value of PC3 had increased critical swimming speeds. Points represent species means for shape; color represents relative sword length.

natural variation in sword length has been shown to have no effect on endurance (Ryan 1988), and a positive effect on c-start burst speed (Royle et al. 2006). Comparing males without swords to males with swords through ontogenetic development revealed no effect on  $U_{crit}$  or sprint speed (Oufiero et al. 2012). Lastly, experimental reduction of sword length has been shown to reduce routine oxygen consumption (Basolo and Alcaraz 2003) and increase  $U_{crit}$  (Kruesi and Alcaraz 2007), but has no effect on c-start burst speed (Baumgartner et al. 2011) or sprint speed (C.E. Oufiero et al., unpubl. data).

Focusing on aerobic locomotion is the sword a significant cost? A difficulty in comparing our results to experimental manipulations is the difference between species evolving to swim with the sword versus individuals adapting to the development of

the sword. Experimentally removing the sword within a species does not necessarily make the males equivalent to a male platyfish without a sword or a female without a sword given that sworded males have developed and evolved with this structure. Removal of the sword may allow males to perform better because of the physiological adaptations that may have developed in conjunction with the sword, such as increased muscle mass. This type of manipulation may be similar to resistance training studies, in which males are able to perform better because of their physiological adaptations (see Oufiero et al. 2012 for a discussion). Recent kinematic work has demonstrated that sword removal enhances a sworded males ability to swim, but no difference among species that vary in sword length in their kinematics (Oufiero et al. 2014). A comparison of tail beat kinematics among four species

of *Xiphophorus* found no difference in tail beat amplitude or frequency among two species with relatively long swords (*X. alvarezii* and *X. nigrensis*) compared to a platy without a sword (*X. meyeri*), but completely removing the sword in *X. alvarezii* increased tail beat amplitude, but not frequency (Oufiero et al. 2014). Thus, removal of the sword alters the kinematics of swimming, but the presence of the sword does not hinder a male's swimming ability. Finally, no studies have compared male's performance to female's performance within *Xiphophorus*, so it is not known if sword removal allows males to perform similar, better, or worse than females. A recent survival study in stalk-eyed flies (where males possess longer eye stalks due to sexual selection) demonstrated an increased survival in males (Worthington and Swallow 2010); therefore, to fully determine how sexually selected traits affect the bearer females should be taken into account. Removal of the sword seems to highlight the potential physiological adaptations that may develop in concert with the development of the sword; but the evolution of the structure does not affect how the fish swim or their whole-organismal performance capabilities. More studies are warranted to fully determine the effect of this structure on locomotion by comparing male's performance to females and determining the potential physiological adaptations of developing the structure.

Although theory often predicts negative effects on function of sexually selected traits, recent intraspecific studies have shown minimal adverse effects of exaggerated structures for locomotion (Clark and Dudley 2009; McCullough et al. 2012; Oufiero et al. 2012). Data from intraspecific studies indicate that the sword increases energetic demands (Basolo and Alcaraz 2003) and reduces  $U_{crit}$  (Kruesi and Alcaraz 2007). By contrast, our results do not show a significant negative effect on  $U_{crit}$  interspecifically, and instead show a potential positive effect when other traits are included in models. Furthermore, although Basolo and Alcaraz (2003) found a reduction in routine and courtship oxygen consumption when the sword is removed, they suggest the sword will have little impact during steady swimming stating, "Thus, it does not appear that the sword is costly when swimming in a current," such as in our  $U_{crit}$  trials. Taken together, the evolution of the sexually selected sword does not hinder aerobic locomotor abilities.

If the evolution of the sword does not negatively affect  $U_{crit}$ , then why might species with longer swords have a potentially increased  $U_{crit}$ ? Although our correlation analyses revealed no significant relationship between relative sword length and  $U_{crit}$ , our multiple regression analyses revealed a significant, consistent positive effect of relative sword length on  $U_{crit}$ . If the sword is not correlated to  $U_{crit}$ , perhaps other factors may be influencing the correlated evolution of relative sword length and  $U_{crit}$ . Variation in ecological factors may lead to natural selection for different aspects of locomotor performance (Nelson et al. 2003;

Fulton et al. 2005; Fulton 2007; Langerhans 2009b; Oufiero et al. 2011b; Careau and Garland 2012); therefore, differences in  $U_{crit}$  may be a result of differences in selection among habitats. For example, if species with longer swords are found in areas with increased stream velocity, then there may be selection for an increased  $U_{crit}$ . As yet, no studies have correlated abiotic habitat characteristics with interspecific variation in sword length. Interspecific variation in behavior may also alter how selection acts on  $U_{crit}$ . For example, if males from species with longer sexually selected swords also engage more in courtship displays or competitive interactions (Bisazza 1993; Basolo and Alcaraz 2003), then selection may favor increased  $U_{crit}$ .

Although we did not find a cost to  $U_{crit}$  among species that vary in sword length, this does not necessarily imply absence of any cost of the sword. The sword may make males more conspicuous to predators (Endler 1980; Rosenthal et al. 2001; Basolo and Wagner 2004), and hence may result in increased mortality despite a lack of effect on locomotor performance. For example, Basolo and Wagner (2004) demonstrated a reduction in sword length in *X. hellerii* in areas with increased predation. Therefore, the natural variation in sword length within species may be partly a result of reducing the conspicuousness of males. Furthermore, there may be differences in sex-biased mortality among species with varying sword lengths. Species with longer sworded males may experience male-biased mortality in the presence of a predator. Whether this mortality is caused by a reduction in locomotor abilities could be addressed by detailed studies of behavior in the field and/or by comparing male and female locomotor performance within species, and examining the relationships in a phylogenetic framework (Oufiero and Garland 2007). Other costs may exist, such as reduced life span or increased energetic demands. However, no studies to date have examined survival or reproductive effort in relation to sword length in *Xiphophorus*.

Previous results have suggested that compensatory traits may also evolve with a sexually selected trait (Kirkpatrick 1987; Møller 1996; Oufiero and Garland 2007; Husak and Swallow 2011). Obviously, however, if the sexually selected trait does not adversely affect locomotor abilities, then selection favoring compensatory traits would not occur. We found no aerobic locomotor cost to the sword; we also found few positive relationships of relative sword length with any of the potential compensatory traits (Tables 2 and 3). However, we did find several significant shape predictors of  $U_{crit}$ . We found significant effects of shape, with fish with longer heads having an increase in  $U_{crit}$  (Fig. 5), similar to previous studies of closely related *Gambusia* from low-predation environments (Langerhans et al. 2004; Langerhans 2009a, b). We also found a significant positive effect of BD on  $U_{crit}$  (Tables 2–4). However, there were few positive relationships between the predictors of  $U_{crit}$  (Tables 2, 3, and S5). These results suggest, similar to other complex functional systems (Wainwright et al.

2005; Garland et al. 2011), that multiple alternative mechanisms may be possible to achieve an increase in aerobic locomotor abilities—expression of a sexually selected may just be one of the possibilities.

The sword in *Xiphophorus* was one of the first sexually selected structures described by Darwin (1871), it is well studied from several disciplinary perspectives, and it has been assumed to impose a cost to locomotor abilities (Darwin 1871; Ryan 1988; Royle et al. 2006; Oufiero and Garland 2007; Baumgartner et al. 2011), with some empirical support (Basolo and Alcaraz 2003; Kruesi and Alcaraz 2007). Our results demonstrate that the sexually selected sword found in males of many species of *Xiphophorus* has not been costly for aerobic locomotor performance during diversification of this lineage, and may be beneficial. Future interspecific comparisons of sexually selected traits should adopt a more integrative approach, examining several candidate traits that may be affected by the sexually selected trait, potential traits that may help compensate for any such effects, and ecological variation that may cause natural selection (Oufiero and Garland 2007). Integrative studies will lead to a better understanding of how a trait that evolves primarily because it increases mating success interacts with the rest of the organism's biology.

#### ACKNOWLEDGMENTS

We thank the labs of M. Ryan, M. Morris, and M. Scharl, as well as the *Xiphophorus* Genetic Stock Center for providing starting populations of *Xiphophorus* and *Priapella*. We also thank K. Dickson for help with enzyme assays, R. Cardullo and his laboratory for help with oxygen electrodes, K. Hammond and her laboratory for providing some essential equipment, H. Schutz for advice on geometric morphometrics, and M. McHenry and A. Summers for use of the flow tunnel. We thank T. Do, T. Yau, D. Wickman, and B. Wang for assistance in the laboratory. Finally, we thank several anonymous reviewers for comments and suggestions that improved the quality and clarity of the manuscript. This work was supported by the UCR Graduate School, UCR Biology Shoemaker Award, and NSF DDIG IOS-0709788 to TG and CEO.

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Associate Editor: M. Alfaro

## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

**Appendix S1.** Detailed methods for maximum oxygen consumption, citrate synthase and lactate dehydrogenase.

**Appendix S2.** Philip file of the phylogeny depicted in Figure 1A.

**Appendix S3.** DSC file corresponding to the phylogenetic tree in Figure 1A.

**Appendix S4.** Alternative methods of multiple regression model selection.

**Table S1.** Species list, sample sizes, and sources for the species used in the study.

**Table S2.** Male means  $\pm$  standard errors for critical swimming speed ( $U_{crit}$ ), sprint speed, maximum oxygen consumption ( $VO_{2max}$ ), citrate synthase activity ( $CS V_{max}$ ), and lactate dehydrogenase activity ( $LDH V_{max}$ ) for each sex among the 19 species/populations.

**Table S3.** Male means  $\pm$  SE for five linear measurements, four mass measurements, and relative sword length (sword length/standard length [SW/SL]) among the 19 species used in the study.

**Table S4.** Results from ANCOVAs for traits used in phylogenetic analyses.

**Table S5.** Principal component loadings from a phylogenetic PCA (Revell 2009) based on the phylogeny from this study as depicted in Figure 1A.

**Table S6.** Principal component loadings from a phylogenetic PCA (Revell 2009) based on the total evidence nuclear phylogeny from Cui et al. (2013) as depicted in Figure 1B.

**Table S7.** Principal component loadings from a phylogenetic PCA (Revell 2009) based on the mitochondrial phylogeny from Cui et al. (2013) as depicted in Figure 1C.

**Table S8.** Full model multiple regressions on four models of evolution.

**Figure S1.** ML phylogram ( $-\ln$  likelihood = 15,090.109907) obtained with RaxML for the combined dataset with 12 partitions.

**Figure S2.** Landmarks used in geometric morphometric analyses.