Shifts in morphological covariation and evolutionary rates across multiple acquisitions of the trap-jaw mechanism in *Strumigenys*

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A long-standing question in comparative biology is how the evolution of biomechanical systems influences morphological evolution. The need for functional fidelity implies that the evolution of such systems should be associated with tighter morphological covariation, which may promote or dampen rates of morphological evolution. I examine this question across multiple evolutionary origins of the trap-jaw mechanism in the genus *Strumigenys*. Trap-jaw ants have latch-mediated, spring-actuated systems that amplify the power output of their mandibles. I use Bayesian estimates of covariation and evolutionary rates to test the hypotheses that the evolution of this high-performance system is associated with tighter morphological covariation in the head and mandibles relative to nontrap-jaw forms and that this leads to shifts in rates of morphological evolution. Contrary to these hypotheses, there is no evidence of a large-scale shift to higher covariation in trap-jaw forms, while different traits show both increased and decreased evolutionary rates between forms. These patterns may be indicative of many-to-one mapping and/or mechanical sensitivity in the trap-jaw LaMSA system. Overall, it appears that the evolution of trap-jaw forms in *Strumigenys* did not require a correlated increase in morphological covariation, partly explaining the proclivity with which the system has evolved.

KEY WORDS: trap-jaw ants, biomechanics, evolutionary rates, covariation.

The tree of life is characterized by variation in rates of phenotypic evolution across clades (Simpson 1944; Gingerich 2009). Potential underlying causes of this unevenness can be intrinsic (genetic or developmental) or extrinsic (ecological or environmental). One potential influence on the rate of phenotypic evolution is the appearance of novel morpho-functional systems. Such functional systems may act as "key innovations", allowing organisms to exploit new niches and resulting in potential bursts of speciation (Heard and Hauser 1995). However, if the new morpho-functional system has tight mechanical constraints on performance, it may have an equally constraining influence on its evolution, potentially dampening rates of phenotypic evolution (Raup and Gould 1974). Teasing apart this relationship requires understanding how the biomechanical demands of morpholog-ical systems influence their evolution (Arnold 1992; Schaefer and Lauder 1996; Alfaro et al. 2005; Breuker et al. 2006; Wainwright 2007; Holzman et al. 2012; Collar et al. 2014; Anderson and Patek 2015; Munoz et al. 2017; Munoz et al. 2018). Here, I explore how the repeated evolution of a high-performance biomechanical system affects evolutionary covariation between morphological traits and subsequent rates of morphological evolution.

High-performance biomechanical systems have garnered a great deal of interest in biology, due to both their charismatic nature and lingering questions on how they evolve. I define "high performance" as a biomechanical system that results in extreme speed or power beyond what is normally available via muscle action alone. One common example of such a system
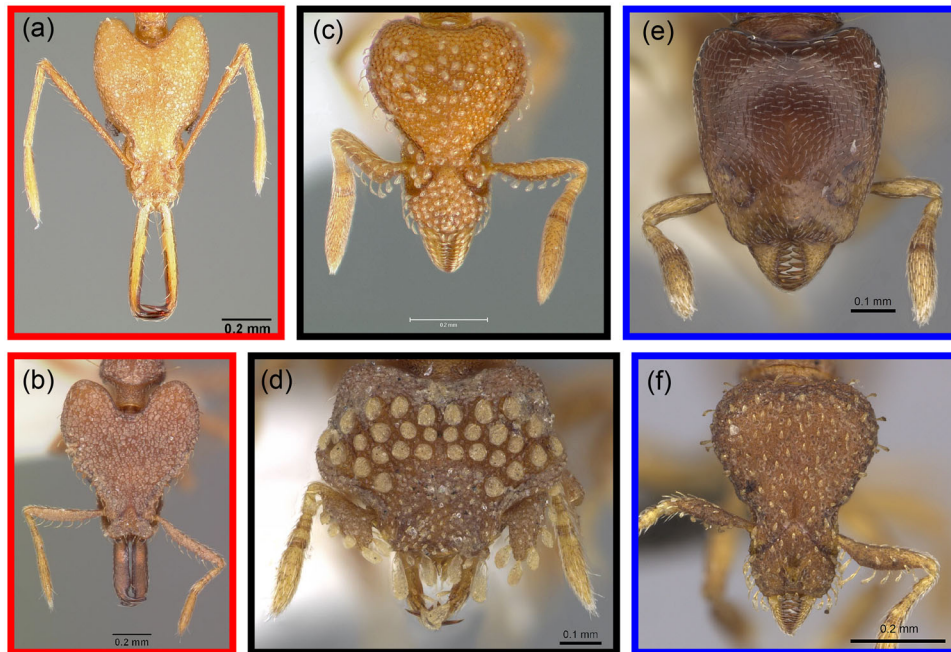
Figure 1. A small sample of the diversity of *Strumigenys* species used for this study. The red boxes are long-mandible trap-jaw species (L-Trap): (A) *S. bibliona*, ID # CASENT0005494 (photo by April Nobile). Scale bar = 0.2 mm. (B) *S. ignota* ID #CASENT0102610 (photo by April Nobile). Scale bar = 0.2 mm. Black boxes are nontrap-jaw species (GRP): (C) *S. ambatrix* ID #CASENT0005990 (photo by April Nobile). Scale bar = 0.2 mm. (D) *S. roomi* ID #CASENT0914713 (photo by Zach Lieberman). Scale bar = 0.1 mm. Blue boxes are short-mandible trap-jaw species (S-Trap): (E) *S. azteca* ID #CASENT0281949 (photo by Shannon Hartman). Scale bar = 0.1 mm. (F) *S. truncatidens* ID #CASENT0235691 (photo by Will Ericson). Scale bar = 0.2 mm. All photos are from antweb.org.

is the power amplification that results from a latch-mediated spring actuation (LaMSA) mechanism (Longo et al. 2019). It has been hypothesized that the evolution of such biomechanical systems will be associated with tight covariation across morphology (Raup and Gould 1974). This association may be due to biomechanical systems requiring morphological covariation less small, uncorrelated changes reduce the functionality of the system; or simply that increased morphological covariation is necessary to facilitate the evolution of biomechanical systems to begin with. Regardless, strong morphological covariation associated with these biomechanical systems has been suggested to enhance evolutionary change by creating strong morphological pathways for diversification, leading to higher rates of morphological evolution than would be expected if the covariation was weaker (Alfaro et al. 2005; Collar and Wainwright 2006; Holzman et al. 2012; Munoz et al. 2017). In contrast, strong morphological covariations have also been argued to reduce the freedom of morphology to vary, potentially leading to lower rates of morphological evolution (Schaefer and Lauder 1996; Collar et al. 2014). While work on biomechanical systems has illustrated evidence for both arguments, they all start from the assumption that the evolution of biomechanical systems is associated with strong morphological covariation.

There is growing evidence that multipart biomechanical systems need not necessarily be tightly integrated in terms of morphological variation. Work on fish feeding mechanics reveals that similar performance can often be obtained from multiple morphological configurations (many-to-one mapping) (Hulsey and Wainwright 2002; Alfaro et al. 2004; Alfaro et al. 2005; Wainwright et al. 2005;). Further work on four-bar linkages across multiple groups has shown that not all components of the system contribute equally to variation in performance (mechanical sensitivity) (Anderson and Patek 2015; Munoz et al. 2017; Hu et al. 2017). In the latter case, some components of the biomechanical system will be tightly correlated with performance, while others may be free to evolve in response to other demands. To understand how the origin of multipart biomechanical systems can influence overall morphological evolution, it is necessary to examine the evolution of morphological traits that are associated with the system but may not be directly part of it.

To test how the acquisition of a biomechanical system influences the evolution of associated morphological traits, I focus on the ant genus *Strumigenys* (Fig. 1). Studies on the evolution of high-performance systems are often constrained by a lack of replication; the mechanism in question evolved only once within

a group. However, the trap-jaw mechanism has evolved independently at least 7 times in the genus *Strumigenys* (Booher et al. 2021), allowing for repeated tests for patterns of morphological covariation and evolutionary rates within a single clade. The trap-jaw mechanism found in this and other ant groups is a LaMSA system consisting of a latch that holds the mandibles in an open position, while enlarged mandibular closure muscles load elastic strain energy into a spring, the apodeme. Once the spring is fully loaded, specialized trigger muscles remove the latch, releasing the stored energy and allowing the mandibles to snap shut at exceedingly high speeds (upward of 60 m/s) (Larabee and Suarez 2014; Larabee et al. 2017; Gibson et al. 2018; Booher et al. 2021).

A recent review of the genus *Strumigenys* has identified repeated evolution of potentially two distinct trap-jaw forms (Booher et al. 2021). Traditionally, *Strumigenys* was broadly cataloged into two morphotypes: one with short, triangular mandibles suited for gripping but with no associated LaMSA system (GRP) and the other characterized by long mandibles with a LaMSA system allowing for fast strikes (L-Trap). However, Booher et al. (2021) identify a third group: those with short, triangular mandibles that have the same latching mechanism as the long mandible forms and similarly redesigned head musculature associated with the presence of the LaMSA system (S-Trap) (Booher et al. 2021). Booher and colleagues hypothesize that the transition from GRP forms (well supported as the basal form for the clade) was initially through the S-Trap forms, some of which only slightly differ in morphology from the GRP forms. Notably, the S-Trap forms evolved directly from GRP forms several times but also evolved via a secondary shortening of the mandible from L-Trap forms (Booher et al. 2021). A high-performance biomechanical system evolving repeatedly across a single clade allows rigorous testing of the influence of this system on the morphological evolution of the head capsule and mandibles.

I test for shifts in patterns of trait covariation and evolutionary rates across multiple evolutionary origins of the trap-jaw system in the ant genus *Strumigenys*. The main objective is to examine whether the evolution of this multipart, high-performance mechanism is associated with higher covariation in head capsule and mandibular morphology and whether this covariation results in higher or lower rates of morphological evolution. Specifically, I test the following paired hypotheses: H1: The evolution of the trap-jaw system results in tighter morphological covariation across the head capsule and mandibles. This is a long-standing prediction in terms of the evolution of high-performance systems. If this hypothesis is rejected, it could indicate that many-to-one mapping or mechanical sensitivity may be at play. H2: The evolution of the trap-jaw mechanism is accompanied by shifts in the rates of morphological evolution relative to nontrap taxa. If the trap-jaw taxa show increased rates, it may indicate that the

trap-jaw mechanism provides a strong morphological pathway for adaptation. If they show decreased rates, it may indicate that functional requirements in the trap-jaw system constrain the rate of morphological evolution across the head and mandibles.

Methods

TAXA USED

All *Strumigenys* images used for the project were sourced from antweb.org (Antweb), an online repository of photographs of museum specimens maintained by Brian Fisher at the California Academy of Sciences. Species were selected for inclusion in the study based on whether they were included in the phylogeny used (see below) and whether images of the head capsule with visible mandibles in a closed orientation and a scale bar were available (Fig. 1). Measures were taken from three individuals per species (all workers) when available (some species only had 1 or 2 individuals available on the site). This resulted in 782 individuals from 351 species of *Strumigenys*, accounting for ~41% of the genus's known diversity.

For the phylogenetic analyses, I used the species-level phylogeny of Booher et al. (2021). Species were sorted into three, nonmonophyletic groups based on their identifications in Booher et al. (2021): nontrap-jaw ants (the ancestral state) that lack the LaMSA mechanism and have standard "gripping" mandibles [GRP], long-mandible trap-jaw forms [L-Trap] and short-mandible trap-jaw forms [S-Trap] (Fig. 1). These three classifications are based on the presence of a latching mechanism in the mandibles as a sign of a LaMSA system. As previously noted, none of these three groups are monophyletic, and both types of trap-jaw mechanisms have evolved multiple times in the clade (Booher et al. 2021). In total, the dataset contains 95 GRP species, 204 L-Trap species, and 52 S-Trap species. A full list of specimens used and associated metadata can be found in the Appendix (SI_data.xlsx).

MORPHOLOGICAL TRAITS

The goal of this study was to observe broad patterns of evolutionary change in morphology across multiple evolutionary origins of the trap-jaw system. I utilized data collected from photographs of the external morphology of the head capsule and mandibles to examine how the presence of the biomechanical system influences overall morphology. Focusing on external morphology also allowed for a large dataset (350+ species), offering a broad-scale view of morphological trends across the group. While the external morphology of the head capsule in ants does not necessarily correspond directly with the components of the trap-jaw system, the specific traits used are associated with aspects of the trap-jaw system (as detailed below).
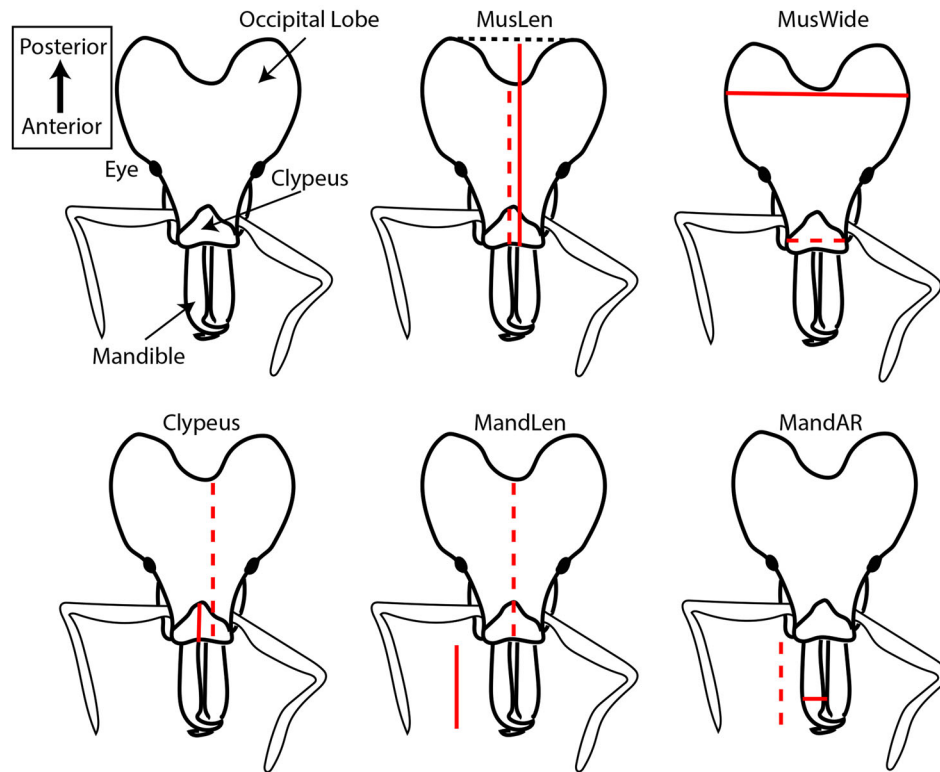
Figure 2. Sketches illustrating the five morphological traits used in this study. The outline is based on the *S. ignota* image from figure 1. For all traits, the length shown as a solid line is regressed against the length shown with a dotted line. Full measurement definitions are in the main text. Anterior-posterior orientation based on Bolton 1994 and Boudinot et al. 2013.

Figure 2 shows a sketch of an ant head with the measurements taken for the morphological traits. The use of anterior and posterior in Figure 2 and the following descriptions is based on previous descriptions of ant head morphology (Bolton 1994; Boudinot et al. 2013). Each trait is regressed against a standard length measure using phylogenetic regression techniques, and the extracted residuals are used in subsequent analyses. The five traits used here are as follows: (1) *MusLen*, the length from the posterior-most point of the clypeus to a line delineated by the posterior tips of the occipital lobes regressed against the length from the posterior of the clypeus to the occipital margin of the head between the lobes. These lobes are formed via an expansion of the occipital margin, so this trait gives an indication of how expanded the back of the head capsule is, which is associated with the space available for mandibular adductor muscles (Gronenberg 1996). These muscles have also been shown to undergo structural changes between GRP and LaMSA forms in the clade (Booher et al. 2021). (2) *MusWide*, The greatest width in the head posterior to the clypeus regressed against the greatest width of the clypeus itself. This also gives a sense of how enlarged the posterior portion of the head capsule is. (3) *Clypeus*, The anterior-posterior length of the clypeus regressed against the same head length used for MusLen. This gives a measure of how large the clypeus is relative to the overall head size. The

clypeus overlies the actual spring and latch mechanism (Gronenberg 1996; Booher et al. 2021), so we might expect it to show tighter covariations with other morphological traits in the LaMSA groups. (4) *MandLen*, the length of the mandible divided by the same head length as MusLen. This measurement represents the relative length of the mandible, the accelerating body of the trap-jaw system. It is important to note that the joint between the mandible and head is obscured by the clypeus, resulting in an underestimate of the total mandible length from these photographs. To ensure that these measures still have comparative value, only mandibles in the fully closed position were used, and the estimate for length was made from the anterior margin of the clypeus to the anterior tip of the mandible. (5) *MandAR*, Maximum length of the mandible regressed against the maximum mandible width. This gives a sense of the overall robustness of the mandible.

MusLen, Clypeus, and MandLen all use the same standard length measure to regress the trait in question (Fig. 2). However, MusWide and MandAR use different measures. As MusWide is used to estimate the lateral expansion of the head, it makes more sense to compare it to a parallel measure from a different portion of the head, in this case the width of the clypeus. Similarly, MandAR is meant to estimate the aspect ratio of the mandibles. Since ratios are problematic in statistical tests (Albrecht et al. 1993;

Baur and Leuenberger 2011), the length is regressed against the width instead.

COMPARATIVE ANALYSES

All seven length measures used for defining the five morphological traits were tested for normality using a Shapiro–Wilks normality test and log transformed. For each trait, phylogenetic regressions were run using the R package caper (Orme et al. 2012) to extract residuals to be used in lieu of ratios for the five traits.

To examine morphological differences between the three types of *Strumigenys*, I tested for differences in the means of all five traits between the three groups using a phylogenetic ANOVA (Garland et al. 1993) as implemented in phytools (Revell 2012). For the ANOVA test, 1000 simulations were run, and post hoc tests were conducted to calculate pairwise differences between the three groups. I predicted that all three groups would show significant differences in means from each other with the possible exception of the mandible traits. The mandibles in GRP and S-Trap forms are likely highly similar given how the three forms are defined (see discussion above).

I tested whether the individual morphological traits evolve in similar patterns across the three forms by applying maximum-likelihood techniques to test alternative adaptive models of trait evolution. I modeled trait evolution using functions in the R package OUwie (Beaulieu et al. 2012), which fits various likelihood models for continuous characters evolving under discrete selective regimes. I compared six distinct models of evolution: 1) a Brownian motion model with a single rate of evolution for all three forms, 2) a Brownian motion model with separate rates of evolution for GRP and the two trap-jaw forms grouped together, 3) a Brownian motion model with three separate rates of evolution, 4) a single-peak Ornstein–Uhlenbeck (OU) model (Brownian motion pulled toward a single adaptive peak for each parameter), 5) an OU model that was set to pull toward two adaptive peaks, one for GRP species and one for trap-jaw forms (treating L-Trap and S-Trap as a single group), and 6) an OU model that was set to pull toward three adaptive peaks: GRP, L-Trap and S-Trap. To map the different groups to the tree, I utilized the make.simmap function in phytools. I performed 100 stochastic mapping simulations using an ARD model of transition rates. The estimations were run twice, once for the two-group simulations (BM2 and OU2 models) and once with all three groups (BM3 and OU3 models). Models were compared and evaluated utilizing maximum likelihood and Akaike information criterion corrected for finite sample sizes (AICc), with $\Delta AICc > 4$ considered strong support (Burnham and Anderson 2002; Whittingham et al. 2006; Mundry and Nunn 2009).

Finally, I utilized the package ratematrix (Caetano and Harmon 2017; Caetano and Harmon 2019) to evaluate potential shifts in evolutionary rates and patterns of morphological trait covariation between the three groups. Ratematrix uses Markov chain Monte Carlo (MCMC) techniques to implement a Bayesian estimate for the evolutionary rate matrix (**R**). **R** is a covariance matrix that includes the evolutionary rates for each trait along the diagonal and the patterns of covariation between pairwise traits in the off-diagonal. The ratematrix package allows the estimation of **R** for each taxon group across the same tree while incorporating uncertainty associated with parameter estimates and ancestral state reconstructions (Caetano and Harmon 2017). This allows for both the evolutionary rates and covariation patterns of morphological traits to be compared between different subgroups across the tree. The model was set to estimate an **R** for each taxon group: GRP, L-Trap, and S-Trap. A pool of 100 stochastic maps was created for the MCMC to account for uncertainty associated with ancestral state estimation (for an example simmap, see figure S1). I ran four independent MCMC chains of 20 million generations each and used a random sample from the prior as the starting point of each chain. I set a uniform prior on the correlation matrices, discarded 25% of each MCMC chain as burnin and checked for convergence using the potential scale reduction factor (Gelman and Rubin 1992).

Since the ratematrix is a Bayesian method, it outputs posterior distributions of the fitted rate matrices **(R)** as opposed to single estimates. Differences in the posterior distributions of the evolutionary rates and covariations among GRP, L-Trap, and S-Trap forms were visualized with box plots. To quantify the extent of differences in the posterior distributions of rates and covariations between the three forms, I calculated the proportion of overlap between the distributions as implemented in the ratematrix. This proportion of overlap is presented as similar to a significance value. For instance, when the proportion of overlap is listed as 0.05, the posterior distributions of the estimated rates/covariations do not overlap more than 5% between forms (Caetano and Harmon 2017; Caetano and Harmon 2019). Note that although they appear similar, these test statistics are *not* p values, as they do not estimate a probability of deviance from a null distribution. They are simply used to quantify the overlap between posterior distributions between types; the assumption being that if the posterior distribution of a parameter does not overlap between types, then there is a relevant difference in the parameters between those types.

Results

pANOVA AND TRAIT EVOLUTION

The results from the phylogenetic ANOVA show that for all five traits, L-Trap taxa are significantly different from both GRP and S-Trap taxa (Table S1). L-Trap taxa generally have longer, narrower heads, smaller clypei, and longer and narrower mandibles (Fig. 1). It is important to note that since the L-Trap

Table 1. Evolutionary model comparisons for the five morphological traits across *Strumigenys* phylogeny. BM2 and OU2 treat all trap-jaw ants as a single group in comparison with nontrap-jaws. BM3 and OU3 separate long-mandible (L-Trap) and short-mandible (S-Trap) trap-jaws. *Italicized** AICc values indicate the best-fit model for each trait.

	BM		BM2		BM3	
Traits	Loglik	AICc	loglik	AICc	loglik	AICc
MusLen	655.70	−1307.36	657.50	−1308.93	659.44	−1310.77
MusWide	298.028	−592.021	298.93	−591.78	299.28	−590.43
Clypeus	281.36	−558.68	281.54	−557.012	281.86	−555.60
MandLen	19.2	−34.366	35.9	−65.724	45.41	−82.704
MandAR	−22.488	49.011	−20.747	47.562	−14.828	37.772

	OU1		OU2		OU3	
Traits	Loglik	AICc	loglik	AICc	loglik	AICc
MusLen	658.301	−1310.53	666.49	−1324.86	670.25	*−1330.32**
MusWide	298.028	−589.99	300.97	−593.81	316.29	*−622.40**
Clypeus	285.61	−565.15	308.23	−608.34	323.40	*−636.622**
MandLen	19.2	−32.331	27.88	−47.653	64.983	*−119.79**
MandAR	−22.488	51.046	−17.77	43.67	36.136	*−63.729**

and S-Trap forms are heavily differentiated based on mandible length, it is not surprising that the pANOVA shows a significant difference. However, the other four traits are not used for differentiating the two trap-jaw forms. GRP and S-Trap taxa only differ in two traits, with the S-Trap taxa having both shorter clypei and thicker mandibles (Fig 1).

The results from the OUwie analyses further support different evolutionary patterns for the three taxon groups (Table 1). For all five traits, the best supported evolutionary model is OU3, where all three groups are assumed to evolve toward separate adaptive peaks. The difference in support was strong (ΔAICc > 4) for all traits. Interestingly, while BM3 is often a better fit than the other BM models for the two mandible traits, there is little difference in the other traits, potentially indicating not much difference in evolutionary rates between those traits. Regardless, all of these results point toward distinct evolutionary patterns for the GRP, L-Trap, and S-Trap *Strumigenys* taxa.

RATEMATRIX RESULTS

The Bayesian estimate of the evolutionary rate matrix (**R**) for *Strumigenys* lineages showed good convergence in all four MCMC chain runs (Figs. S2 and S3). The posterior distributions for both trait covariations and evolutionary rates for each trait are plotted as box plots to illustrate potential overlap between taxon groups (Figs. 3–5). A summary figure showing all posterior distributions as histograms is included in the supplementary information (Fig. S4).

The posterior distributions of the covariations between traits show a complex pattern (Figs. 3 and 4; Table 2). However, what is clear is that there is no overall shift to higher covariation in trap-jaw forms relative to the nontrap-jaw forms, as evidenced by the vast majority of posterior overlaps being greater than 10% (Table 2). That said, there are individual examples of potential shifts in covariation, dependent on the type of trap-jaw form. L-Trap species show an increase in MusLen-MusWide covariation compared to both GRP and S-Trap forms (Fig. 3; Table 2). On the other hand, S-Trap forms show an increase in MusWide-MandAR covariation relative to the other two forms (Fig. 4, Table 2). A few other boxplots appear to show distinct shifts, but not always in the expected direction. For instance, L-Trap forms actually show a reduction in MandLen-MandAR covariation relative to both S-Trap and GRP forms (Fig. 4). However, across all comparisons, there was no overall trend toward higher or lower trait covariation in trap-jaw forms.

Figure 5 shows the posterior distributions of the evolutionary rates of each trait for each taxon group. L-Trap taxa show visible rate shifts for three out of the five traits relative to both GRP and S-Trap taxa. However, the direction of the shift is not consistent. L-Trap taxa show an apparent increase in rates of evolution for MusLen but decrease in rates of evolution for MandLen and MandAR. These trends are supported by the overlap statistics presented in Table 3, although only the MandLen and MandAR comparisons show overlaps <10%.
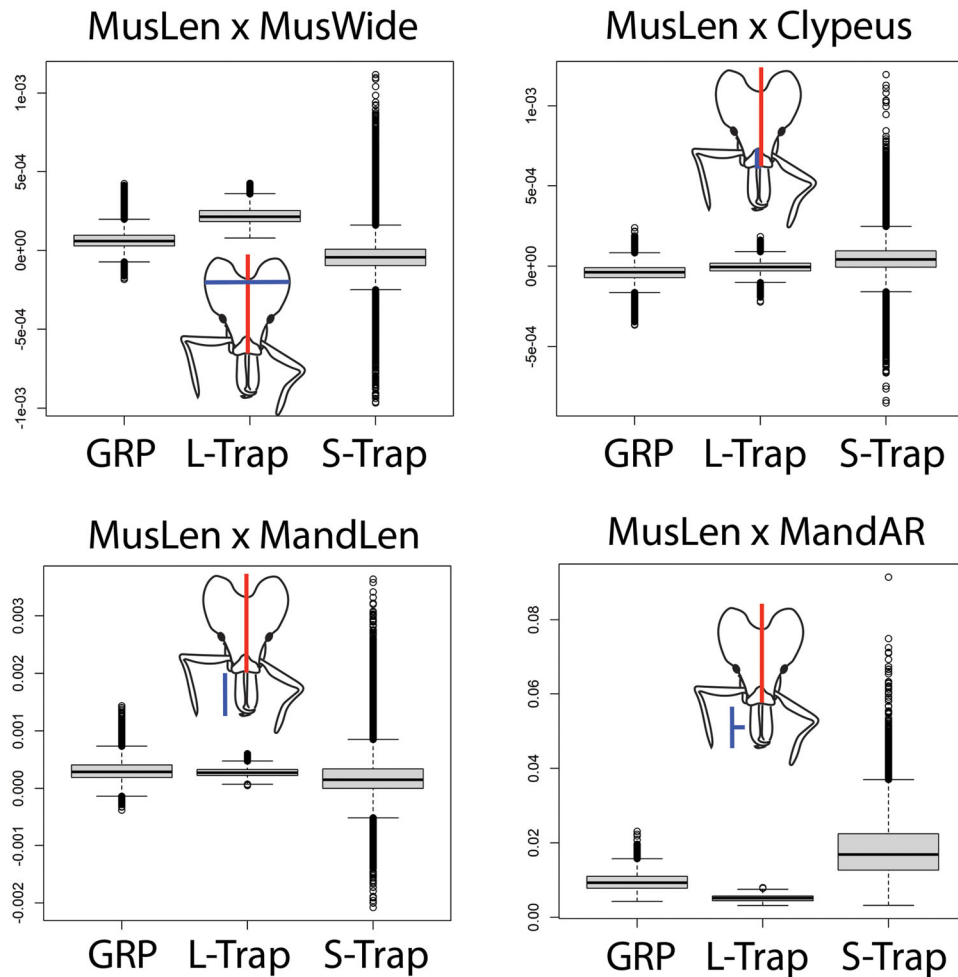
Figure 3. Comparison of the posterior distributions of estimated covariation between MusLen and the other four traits across the three types of *Strumigenys* species. There was no overall trend toward higher covariations in the trap-jaw groups. However, some individual shifts can be seen: L-Trap species show an increase in covariation between MusLen and MusWide compared to other forms. On the other hand, L-Trap forms show a reduction in MandLen-MandAR covariation.

Table 2. The proportion of overlap in posterior densities of estimated covariation between traits for the three types of *Strumigenys*. Overlap proportions are calculated in the package ratematrix for R. A value of 0.05 indicates 5% overlap between distributions. Note: these are NOT *p*-values but simply proportions of overlap in the distribution of rate estimates. Denisty overlaps <10% are indicated with an *.

|	GRP vs. L-Trap	GRP vs. S-Trap	L-Trap vs. S-Trap
Muslen versus MusWide	0.0123*	0.2642	0.0097*
Muslen versus Clypeus	0.5349	0.3323	0.5590
Muslen versus Mandlen	0.3914	0.6233	0.2984
Muslen versus MandAR	0.2433	0.6981	0.7230
MusWide versus Clypeus	0.2326	0.3022	0.7630
MusWide versus Mandlen	0.7852	0.4285	0.3208
MusWide versus MandAR	0.9818	0.106	0.0981*
Clypeus versus Mandlen	0.7071	0.4129	0.2723
Clypeus versus MandAR	0.5098	0.1264	0.2457
Mandlen versus MandAR	0.6167	0.1209	0.2197
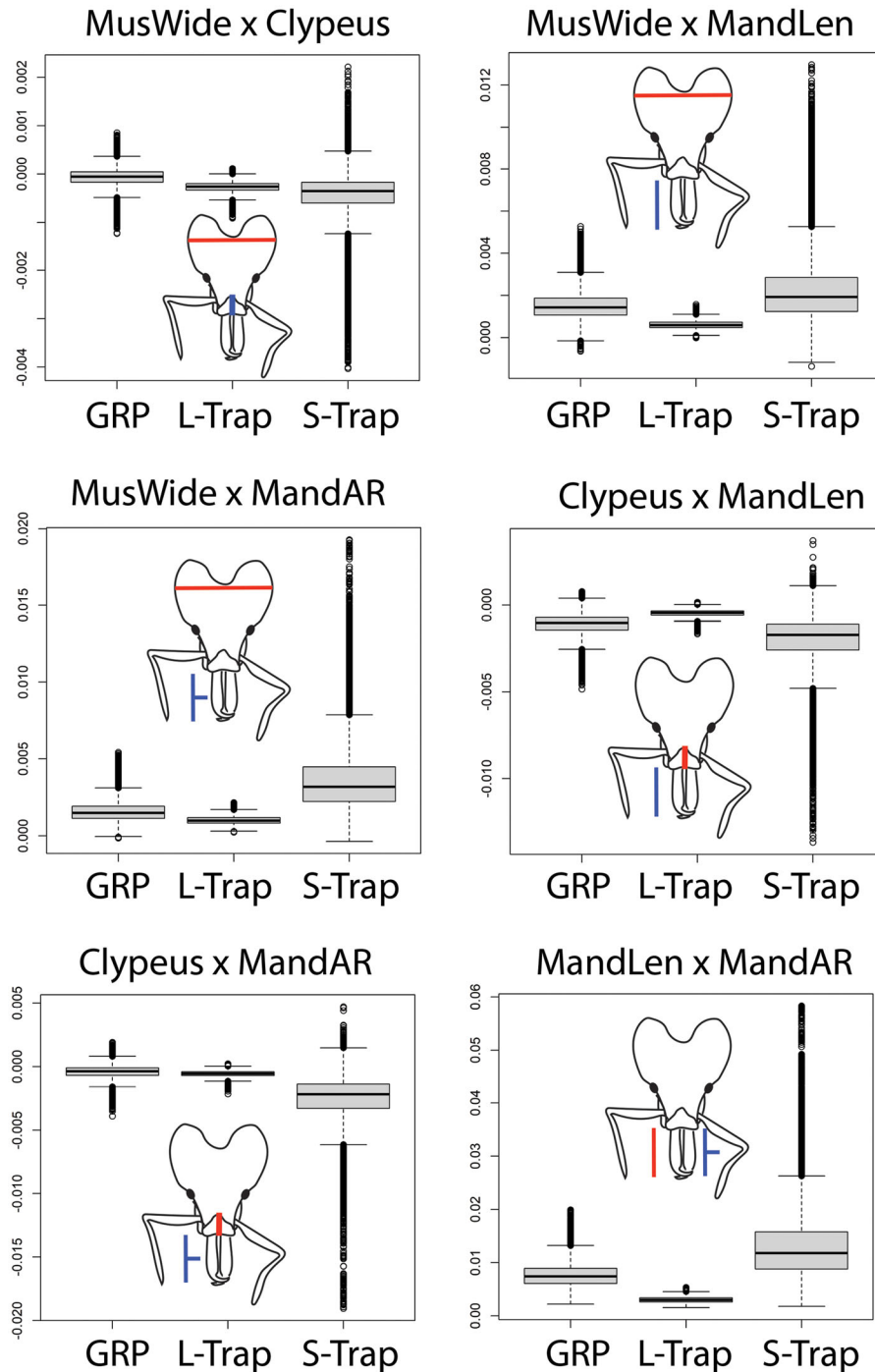
Figure 4. Comparison of the posterior distributions of estimated covariation for the remaining six potential trait pairs across the three types of *Strumigenys* species. There was a general lack of increased covariation in the trap-jaw groups. However, comparing MusWide and MandAR, S-Trap forms show an increase in covariation relative to the other two forms.

Discussion

Functional fidelity in a biomechanical system can either promote morphological evolution (Holzman et al. 2012; Munoz et al. 2017) or act as a constraint (Schaefer and Lauder 1996; Collar et al. 2014). I tested whether the evolution of a high-performance biomechanical system was associated with higher covariation in head capsule and mandibular morphology in trap-jaw ants and whether this covariation resulted in higher or lower rates of morphological evolution. I hypothesized that (1) the trap-jaw forms would show tighter covariation between morphological traits of the head capsule and mandibles relative to
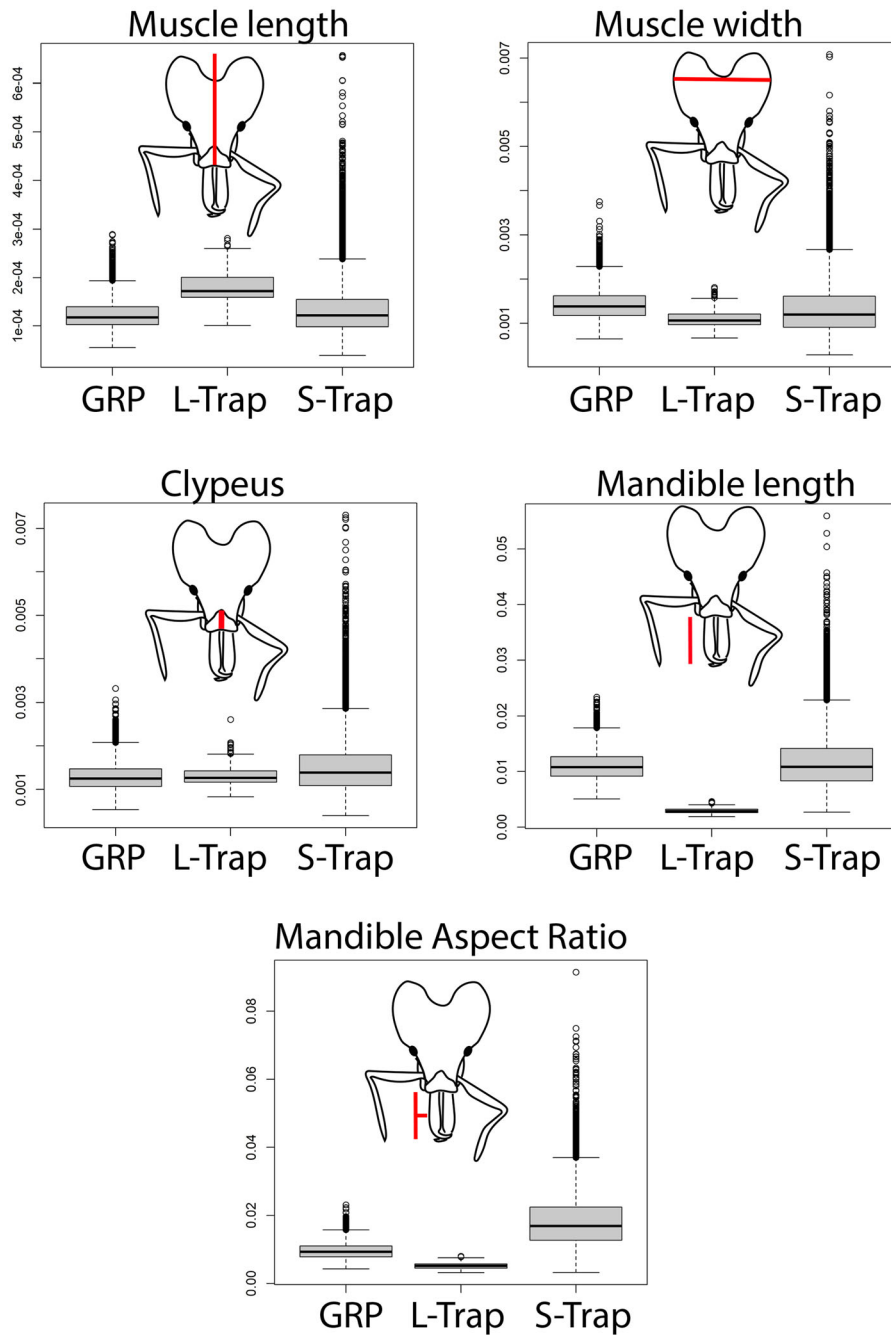
Figure 5. Comparison of the posterior distributions of estimated evolutionary rates between three forms of *Strumigenys* across all five morphological traits. Although L-Trap shows significant shifts in evolutionary rate compared with both GRP and S-Trap taxa for 3 out of 5 traits, the direction of the shift is not consistent. L-Traps have higher rates for MusLen but lower rates for MandLen and MandAR.

nontrap-jaws and (2) trap-jaw forms would therefore show either increased or decreased rates of morphological evolution for each trait. The results for the ant genus *Strumigenys* did not support either hypothesis. The evolution of this LaMSA system was not associated with an overall increase in covariation between morphological traits. Furthermore, shifts in the rate of morphological evolution did not necessarily correspond to shifts in tighter co-variation, and the direction of rate shift (increase or decrease)

varied with trait. Below, I discuss what these results suggest concerning the evolution of the LaMSA system in *Strumigenys* as well as larger questions of morphological evolution related to high-performance systems.

STRUCTURAL EVOLUTION

The ant genus *Strumigenys* presents a unique opportunity to examine the repeated evolution of a high-performance LaMSA

Table 3. The proportion of overlap in posterior densities of estimated evolutionary rates between the three types of *Strumigenys*. Overlap proportions are calculated in the package ratematrix for R. A value of 0.05 indicates 5% overlap between distributions. Note: these are NOT p values but simply proportions of overlap in the distribution of rate estimates. Overlaps in density that are <10% are indicated with an *.

	GRP vs. L-Trap	GRP vs. S-Trap	L-Trap vs. S-Trap
Muslen	0.1859	0.9336	0.3466
MusWide	0.4047	0.7743	0.8302
Clypues	0.8997	0.8014	0.8535
Mandlen	*0.0000**	0.9922	*0.00062**
MandAR	*0.0502**	0.2093	*0.01318**

system within a single clade. A recent large-scale phylogeny and biomechanic reconstruction of the clade identified two distinct types of trap-jaws—long mandible (L-Trap) and shortmandible (S-Trap)—and illustrated how they have each evolved multiple times across all biogeographic regions occupied by the clade (Booher et al. 2021). This paper was the first to identify the short-mandible trap-jaw forms (S-Trap) based on the ability of the latch mechanism to hold the mandibles in place. In doing so, they discovered that the variation across trap-jaw forms was more extensive than previously thought, with variation in traits such as mandible length and the angle at which latched mandibles are held overlapping with nontrap-jaw forms.

Despite the observed continuous variation across forms, phylogenetic ANOVA results mostly support L-Trap, S-Trap, and nontrap-jaw "gripping" forms (GRP) as distinct morphotypes in terms of head and mandible morphology with some overlap between the GRP and S-Trap forms. Differences in mandible length between L-Trap and S-Trap forms are expected given how the groups are defined (Booher et al. 2021). However, differences in other traits, such as clypeus length, are not necessarily expected, and these results help verify morphological differences between the classifications used. Furthermore, the best-supported model of evolution for all five traits (determined by maximum likelihood analysis) is a multiple OU model with all three morphotypes evolving toward distinct optimal values for each trait. These results support the idea that L-Trap and S-Trap forms are not only distinct morphotypes but may also evolve under different selective pressures.

Mantis shrimp, which use a LaMSA mechanism to actuate raptorial appendages for capturing prey, show a similar pattern of morphotypes evolving under differing selective pressures (Anderson et al. 2014). All mantis shrimp have the same LaMSA components, but there are two distinct functional morphologies in the raptorial appendages related to their behaviors. Smashers break open hard-shelled prey with blunt appendages, while spearers capture move evasive prey with harpoon-like appendages. *Similar to subgenetics*, mantis shrimp morphotypes show evolution toward differing optima related to their function: force ampli-

fication for smashers and displacement amplification for spearers (Anderson et al. 2014).

However, unlike the mantis shrimp example, we know little about the performance of one of the groups: the S-Trap forms (Booher et al. 2021). As described in the methods, S-Trap forms were originally identified based on the presence of a functional latch, but there are no direct measures of their performance. Therefore, it is not clear how morphological differences between L-Trap and S-Trap forms might impact the performance. To further complicate the picture, S-Traps may not be monolithic in terms of their form and function (Booher et al. 2021). Some S-Trap taxa evolve from GRP clades, while other S-Trap taxa evolve from L-Trap clades (Fig. S1). It is unclear whether there are distinct differences between S-Trap taxa arising from these different origins. Further dividing the S-Trap taxa for the analyses presented here would sacrifice too much statistical power, as the S-Trap group is already the smallest. Further work on the function/performance of short-mandible forms is required to truly tease these questions apart.

While lacking direct biomechanical data, Booher et al. hypothesize a series of evolutionary steps illustrating how the three forms relate to each other: GRP forms transition to S-Trap and then L-Trap. They specifically note that differences in labrum morphology between GRP and the most ancestral S-Trap forms can be extremely subtle, with GRP *Strumigenys* possessing morphological elements normally found solely in trap jaws (Booher et al. 2021). They argue that it only takes a minor morphological shift for the GRP form to gain the LaMSA mechanism, potentially helping to explain the repeated evolutions of the mechanism across the clade.

The covariation results obtained by the Bayesian analyses performed here help to expand and reinforce Booher et al.'s scheme for trap-jaw evolution within the clade. As noted, a shift to high covariation in this system might be expected if the trap-jaw mechanism required tight covariation to evolve in the first place or if the constituent parts of the system were required to evolve in a correlated fashion to maintain trap-jaw performance. Taken in this light, the lack of a large-scale trend toward tighter

covariation in *Strumigenys* (Figs. 3 and 4, Table 2) potentially signifies that the evolution of this trap-jaw mechanism does not require greater morphological constraint and that the existing level of covariation in GRP *Strumigenys* is sufficient to maintain trap-jaw performance. This reinforces the idea that only a minimum morphological shift is required for *nontrap-jaw strains* to evolve the trap-jaw mechanism, leading to repeated evolution of the mechanism across the genus. The lack of increased morphological covariation is also consistent with the observation that most of the diversification within *Strumigenys* appears to occur *after* the evolution of the trap-jaw mechanism (Booher et al. 2021).

It is important to consider that the lack of increased covariation between traits could also be due to other factors. It is possible that the traits utilized here are not closely associated enough with the underlying mechanism to be constrained. The muscle length and muscle width traits are measurements of the external cuticle rather than the muscle itself and may show more variation than the latter. However, anatomical observations have shown that the posterior half of the head in these ants is typically filled by the muscle, making the external dimensions a fairly good proxy (Booher et al. 2021). Meanwhile, the mandible traits, which are directly related to the trap-jaw mechanism, still show an overall lack of increased covariation. The lack of increased covariation may also be due to intrinsic features of multipart biomechanical systems, such as functional redundancy and mechanical sensitivity. These concepts, identified in other biomechanical systems, can negate the need for greater morphological covariation to maintain performance and are explored further in the next section.

EVOLUTION OF HIGH-PERFORMANCE SYSTEMS

Previous studies have argued that morphological covariation associated with high-performance biomechanical systems could either promote morphological evolution or constrain it. Strong covariation could drive evolution along specific morphological pathways by maintaining a strong connection between form and function (Alfaro et al. 2005; Collar and Wainwright 2006; Holzman et al. 2012; Munoz et al. 2017). Conversely, weaker covariation may allow for greater freedom of morphological diversification (Schaefer and Lauder 1996; Collar et al. 2014). However, the *Strumigenys* dataset presented here shows a different pattern: repeated evolution of the high-performance trap-jaw system is not associated with shifts in morphological covariation, yet there are still shifts in the evolutionary rates of specific morphological traits across the groups.

The evolution of a high-performance biomechanical system may not require a correlated increase in covariation between components if multiple configurations of those components provide equivalent performance, a phenomenon referred to as many-to-one mapping (Wainwright et al. 2005). Many-to-one mapping, or mechanical redundancy, has been identified in several biomechanical systems, including fish jaws (Wainwright et al. 2005), mantis shrimp appendages (Anderson et al. 2014; Anderson and Patek 2015), and frog locomotion (Moen 2019). There is evidence for many-to-one mapping in *Strumigenys*: two distinct forms of the trap-jaw mechanism have evolved with very different head capsule and mandible morphologies (L-Trap and S-Trap). This indicates that there are multiple morphological configurations of the head and mandibles that can comprise the same biomechanical system. Furthermore, the pattern of increased diversification after the evolution of the trap-jaw system across the clade (Booher et al. 2021) is similar to what has been predicted to occur in the linkage system found in fish oral jaws (Alfaro et al. 2004; Alfaro et al. 2005).

While the hypothesis of an overall increase in morphological covariation across *Strumigenys* trap-jaws is not supported, the Bayesian analysis does show shifts in evolutionary rates of individual morphological traits regardless of covariation between them. Specifically, L-Trap taxa show both increased and decreased shifts in evolutionary rates depending on the trait examined. There are even a few examples of individual traits showing increased covariation in certain groups (e.g., MusWide-MandAR in S-Trap forms), but these are generally NOT accompanied by shifts in evolutionary rates for the trait. Across the *Strumigenys* dataset used here, different head capsule and mandible traits show different shifts in covariation and rate between trap-jaw and nontrap-jaw forms.

The pattern of varying covariation and rate shifts by trait could be indicative of mechanical sensitivity in the underlying mechanical system. Mechanical sensitivity refers to the differential effects of changes to morphology on performance in biomechanical systems (Anderson and Patek 2015). In any multipart biomechanical system, there will be components whose morphological variation strongly influences output performance and those components whose variation does not (Koehl 1996; Anderson and Patek 2015; Baumgart and Anderson 2018). Previous work on both mantis shrimp and fish jaws has shown that patterns of mechanical sensitivity lead certain components of a system to show correlated evolution with performance across clades, while others do not (Anderson and Patek 2015; Hu et al. 2017). Those same systems have shown varying evolutionary rates between different components of the system (Munoz et al. 2017; Munoz et al. 2018), not unlike the pattern seen in *Strumigenys*. Strong patterns of mechanical sensitivity within a biomechanical system may complicate standard hypotheses of the relationships between morphological covariation and evolutionary rates. If the system's output shows varying sensitivity to different morphological components of the system, it could result in distinct patterns of covariation and evolutionary rate shifts between different components. This is reminiscent of the larger concept of mosaic evolution, where different components of entire organisms evolve at varying rates through time (different rates for different traits)

(Gould 1977). How well that concept scales down to the level of a specific anatomical system such as head capsule morphology in trap-jaw ants is unclear, but it does present potential avenues for future work.

Conclusions

Bayesian estimation of the evolutionary rate matrix for head capsule and mandibular morphology in *Strumigenys* shows inconsistent patterns of evolutionary covariation and evolutionary rates between trap-jaw and nontrap-jaw forms. Two distinct forms of the trap-jaw mechanism have each evolved multiple times within the clade. There is no broad shift to higher levels of morphological covariation in either trap-jaw form, although some individual shifts occur in certain traits. Furthermore, while there are shifts in evolutionary rate with the evolution of long-mandible trap-jaw forms, the direction of these shifts varies based on the trait examined, and the patterns do not coincide with specific shifts in morphological covariation. This runs contrary to predictions and patterns seen in other studies, where a shift to higher levels of morphological covariation is associated with shifts in evolutionary rates (Alfaro et al. 2005; Collar and Wainwright 2006; Holzman et al. 2012; Munoz et al. 2017).

These results illustrate that the relationship among form, function, and evolutionary processes is not straightforward. For a single biomechanical system, associated morphological features may show distinct patterns of covariation and evolutionary rate shifts. To truly tease this apart, future work requires a detailed examination of the evolution of the mechanism itself, such as what has been done extensively on four-bar linkage systems (Hulsey and Wainwright 2002; Alfaro et al. 2004, Alfaro et al. 2005; Wainwright et al. 2005; Anderson and Patek 2015; Hu et al. 2017; Munoz et al. 2017, Munoz et al. 2018;). The results presented here show that the trap-jaw mechanism is a potentially useful system to explore these questions further. By combining detailed analysis of the mechanical components (such as muscle, latch, and spring) and their relation to performance (many-to-one mapping and mechanical sensitivity) along with comparative methods such as those used here, we can more fully tease apart the relationship among form, function, and evolution in these high-performance systems.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Description of length measures. See Fig 2 in the main text for a visual guide.

Figure S1: Example simmap showing the mapping of the three taxon types onto the Strumigenys phylogeny.

Table S1: Phylogenetic ANOVA results for the five morphological traits.

Figures S2-S3: Trace plots of the log likelihood and acceptance ratios for the MCMC chains.

Figure S4: Posterior distribution of the evolutionary rate matrix presented as a series of histograms.