

Mandible strike kinematics of the trap-jaw ant genus *Anochetus* Mayr (Hymenoptera: Formicidae)

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Abstract

High-speed power-amplification mechanisms are common throughout the animal kingdom. In ants, power-amplified trap-jaw mandibles have evolved independently at least four times, including once in the subfamily Ponerinae which contains the sister genera *Odontomachus* and *Anochetus*. In *Odontomachus*, mandible strikes have been relatively well described and can occur in <0.15 ms and reach speeds of over 60 m s⁻¹. In contrast, the kinematics of mandible strikes have not been examined in *Anochetus*, whose species are smaller and morphologically distinct from *Odontomachus*. In this study, we describe the mandible strike kinematics of four species of *Anochetus* representative of the morphological, phylogenetic, and size diversity present within the genus. We also compare their strikes to two representative species of *Odontomachus*. We found that two species, *Anochetus targionii* and *Anochetus paripungens*, have mandible strikes that overall closely resemble those found in *Odontomachus*, reaching a mean maximum rotational velocity and acceleration of around 3.7 × 10⁴ rad s⁻¹ and 8.5 × 10⁸ rad s⁻², respectively. This performance is consistent with predictions based on body size scaling relationships described for *Odontomachus*. In contrast, *Anochetus horridus* and *Anochetus emarginatus* have slower strikes relative to the other species of *Anochetus* and *Odontomachus*, reaching mean maximum rotational velocity and acceleration of around 1.3 × 10⁴ rad s⁻¹ and 2 × 10⁸ rad s⁻², respectively. This variation in strike performance among species of *Anochetus* likely reflects differences in evolutionary history, physiology, and natural history among species.

Introduction

Speed is an important trait for the success of many animals. The ability to move quickly to evade predators or capture prey is a significant selection pressure. How fast animals can move depends, in part, on how quickly they can contract muscles to accelerate their limbs. For small animals such as arthropods, the distance they move their limbs is short, requiring higher acceleration values to obtain peak velocities similar to larger animals (Alexander, 1988). In addition, muscle fibers are limited by how quickly they can contract (Ellington, 1985; Gronenberg, 1996a; Askew & Marsh, 2002), reducing the ability of organisms to achieve high accelerations via direct muscle contraction. To overcome these constraints, many animals have evolved catapult mechanisms that use a combination of springs, levers, and latches to store and transfer elastic and mechanical energy, allowing them to accelerate their limbs more quickly than possible through muscle contraction alone

(Gronenberg, 1996a; Patek, Dudek & Rosario, 2015). These power amplification or impulse mechanisms have evolved repeatedly in a diverse range of taxa and result in some of the fastest movements known in the animal kingdom (Gronenberg, 1996a; Patek *et al.*, 2015).

In ants, power-amplifying 'trap-jaw' mandibles have evolved independently for prey capture at least four times across three subfamilies (Larabee & Suarez, 2014) and possibly repeatedly in a single myrmicine genus (Ward *et al.*, 2014). While each group has different morphological adaptations to store and amplify energy, most have structures that perform similar functions (Gronenberg, Tautz & Hölldobler, 1993; Gronenberg & Tautz, 1994; Gronenberg, 1996b; Gronenberg & Ehmer, 1996; Gronenberg *et al.*, 1998; Just & Gronenberg, 1999; Larabee, Gronenberg & Suarez, 2017). Elongated mandibles are locked open with a latch mechanism, allowing elastic strain energy to be stored within the head capsule as enlarged mandible closer (adductor) muscles contract. When inter-mandibular

mechanosensory trigger hairs are stimulated, the latch is released by the contraction of fast-twitch muscles, allowing the mandibles to close extremely rapidly (Patek *et al.*, 2006; Spagna *et al.*, 2008; Larabee, Gronenberg & Suarez, 2017).

Most research on the functional morphology and strike kinematics of trap-jaw ants has focused on the genus *Odontomachus* Latreille. In this genus, the mandibles are held open by a latch formed through interactions between the basal condyle of the mandibles and the mandible sockets (Gronenberg *et al.*, 1993). Elastic energy for the mandible strike is likely stored, in part, within enlarged mandibular apodemes, although the exact location of energy storage remains unknown (Gronenberg *et al.*, 1993). Strikes are stimulated by contact with trigger hairs on the mandibles that connect to ‘giant’ sensory neurons (Gronenberg & Tautz, 1994). During a strike, *Odontomachus* mandibles reach peak maximum velocities between 35 and 67 m s⁻¹, accelerate tens to hundreds of thousands times faster than gravity, and generate forces over 300 times the mass of an individual ant (Patek *et al.*, 2006; Spagna *et al.*, 2008). Due to the high force-to-body mass ratio of these mechanisms, some *Odontomachus* species use their mandibles to repel small intruders out of their nests (Carlin & Gladstein, 1989) and to perform horizontal or vertical jumps, which can increase survivorship during encounters with predators such as antlions (Patek *et al.*, 2006; Spagna *et al.*, 2009; Larabee & Suarez, 2015).

Anochetus Mayr is the sister genus to *Odontomachus* and together they represent a single evolutionary transition to power-amplified mandibles within the subfamily Ponerinae (Larabee *et al.*, 2016). Relative to *Odontomachus*, ants in the genus *Anochetus* are generally smaller, have a distinct head morphology, and frequently ambush and sting their prey when hunting. In contrast, *Odontomachus* species typically rely on the crushing force of their mandibles alone for prey capture (Brown, 1976, 1978; De la Mora, Pérez-Lachaud & Lachaud, 2008). Performance of mandible strikes is correlated with body size in *Odontomachus* (Spagna *et al.*, 2008), with smaller species generally producing faster but less forceful strikes. While the performance properties of *Anochetus* mandible strikes have never been quantified, they are likely to have similar scaling relationships between performance and body size to species in the genus *Odontomachus*.

In this study, we describe the mandible strike kinematics of four species of *Anochetus*. In addition to quantifying interspecific variation within *Anochetus*, we compare their strike performance to two species of *Odontomachus*. While *Odontomachus* are generally larger than *Anochetus*, one of our species (*A. emarginatus*) is similar in size to smaller *Odontomachus* (Larabee *et al.*, 2016). We use these data to test the following hypotheses based on the scaling of body size with performance seen in *Odontomachus*: (1) *Anochetus* mandibles will achieve higher accelerations and velocities, and (2) *Anochetus* mandibles will generate less kinetic energy relative to larger species of *Odontomachus*. We also used X-ray microtomography (microCT), a method of visualizing three-dimensional morphological structures (Friedrich *et al.*, 2014; Wipfler *et al.*, 2016) to characterize the internal morphology of *Anochetus*'s trap-jaw mechanism.

Materials and methods

Colony collection and maintenance

We selected four species of *Anochetus* representative of the morphological, phylogenetic, and size diversity within the genus (Fig. 1): two species, *Anochetus horridus* and *Anochetus emarginatus*, from the *emarginatus* species group (Brown, 1976, 1978; clade F in Larabee *et al.*, 2016); one species, *Anochetus targionii*, from the *mayri* species group (Brown, 1976; Brown, 1978; clade F in Larabee *et al.*, 2016); and one species, *Anochetus paripungens*, from the *rectangularis* species group (Brown, 1976, 1978; clade G in Larabee *et al.*, 2016). We compared the kinematics of *Anochetus* to two species of *Odontomachus* chosen to represent the large variation in size seen in this genus – *Odontomachus chelifer* (one of the largest; Fig. 1e) and *O. ruginodis* (one of the smallest; Fig. 1f). The kinematics of *O. ruginodis* mandible strikes were originally described in Spagna *et al.* (2008); videos recorded for that study were reanalyzed using the methods described below.

Filming and video analysis

We filmed mandible strikes with a Phantom V9.1 high-speed camera (Vision Research Co., Wayne, NJ, USA) connected to

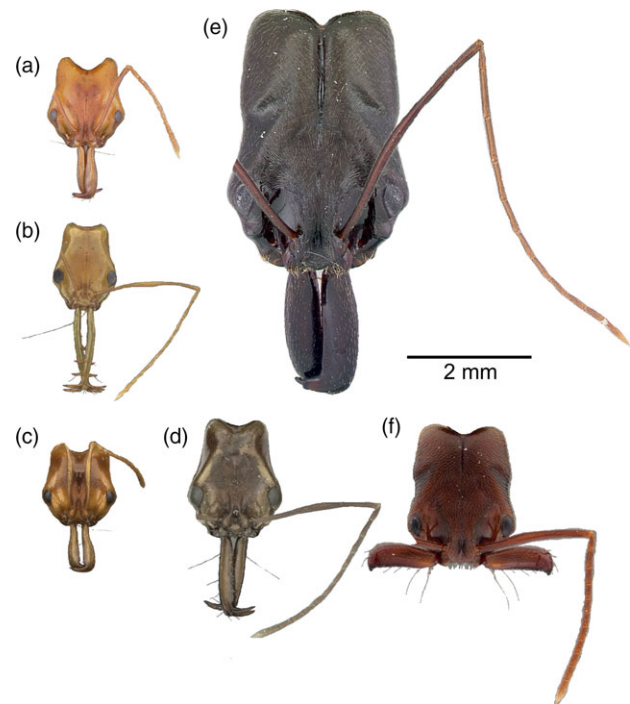


Figure 1 Representative images of study species displaying the morphological and size variation present within *Anochetus* and *Odontomachus*. Images are to scale. (a) *Anochetus targionii*. (b) *Anochetus horridus*. (c) *Anochetus paripungens*. (d) *Anochetus emarginatus*. (e) *Odontomachus chelifer*. (f) *Odontomachus ruginodis*. All images are from Antweb.org. [Colour figure can be viewed at zslpublications.onlinelibrary.wiley.com]

a Zeiss SteREO Discovery V20 Microscope (Carl Zeiss Inc., Oberkochen, Germany). Three individuals of *A. horridus* were filmed using a Phantom Miro eX4 camera (Vision Research Co.) instead of the V9.1. Individual ants were restrained by attaching a size three insect pin (Bioquip, Rancho Dominguez, CA, USA) to the vertex of their head with utility wax (Kerr Laboratory Products, Orange, CA). This pin was placed in a custom micromanipulator to position the ant's mandibles into frame prior to striking. Strikes were stimulated by either blowing on the ant or touching the trigger hairs/antennae with an insect pin. Videos were filmed at a rate of 80 000–100 000 frames per second (the three *A. horridus* filmed with the Miro eX4 were filmed at 58 823 fps). We recorded between three and five individuals for each species and between 4 and 20 strikes per individual (Table 1). After recording, each ant was preserved in a -20°C freezer for at least 24 h. Within 1 week of filming, the mass of each ant was measured with a UMX2 ultra microbalance (Mettler Toledo, Columbus, OH). Ants were then imaged using a Leica M205 C stereo microscope (467-nm resolution) attached to a five-megapixel Leica DFC 425 digital microscope camera, and the head width, head length, and mandible lengths measured. The mandibles were then removed and weighed individually using the microbalance.

To digitize mandibles strikes, we tracked the tips of each mandible in each video in MATLAB (release 2014a; MathWorks Inc., Natick, MA, USA) using a custom script taken from Spagna *et al.* (2008). The cumulative displacement of the mandibles was then calculated trigonometrically from the x-y coordinates of the mandible tips generated by this script. These values were then exported to R (ver3.3.3) where the cumulative angular displacement was smoothed using a quintic spline via the penalized smoothing splines package (pspline). Instantaneous rotational velocity and rotational acceleration were calculated by taking the first and second derivatives of displacement, respectively, and used to determine the maximum value for each variable. Maximum linear velocity and acceleration were calculated by multiplying the maximum rotational velocity and acceleration by the length of the mandible. Rotational kinetic energy (K) was calculated by modeling each

mandible as a thin rod of uniform density rotating about one end, using the equation:

$$K = \frac{1}{6}mr^2w^2$$

where m is the mandible mass, r is the mandible length, and w is the maximum rotational velocity. Maximum rotational kinetic energy and strike duration were used to estimate maximum power output (P) for each strike, using the equation:

$$P = \frac{K}{tm_a}$$

where t is the duration of the strike and m_a is the estimated mass of the mandible closer muscle. This estimate assumes a combined mandible closer muscle mass equal to one-fourth that of the ant's body mass based on the relationship between mandible closer's size and body mass in other odontomachine species (Larabee unpublished data) and is a conservative estimate of the maximum power output of the mechanism. Our goal in calculating power output is not to obtain absolute measurements for each species, but rather estimates that will allow us to determine if they are using a power-amplification mechanism. Time delay between the release of each mandible during strikes was calculated by subtracting the frame number of the first mandible's release from the frame number of the second and dividing the result by the frame rate.

MicroCT

To examine the internal arrangement of head musculature in *Anochetus*, one individual of *A. horridus* was examined using X-ray microtomography (microCT). The ant was fixed in alcoholic Bouin's solution (Sigma Aldrich Corporation, Steinheim, Germany) for 24 h, after which it was washed in 70% ethanol and dehydrated through an ethanol series (at least 20 min each at 70%, 80%, 90%, 95%, and two changes of 100%). The ant was stained overnight in I2E (2% iodine w/v in 100% ethanol) to increase contrast of muscle tissue and then washed in 100% ethanol. To preserve microstructure of soft tissue, the specimen was critical point dried (AutoSamdri-931.GL Supercritical Point

Table 1 Summary of *Anochetus* and *Odontomachus* measurements

Species	Locality	n	i	Head width (mm)	Body mass (mg)	Mandible length (mm)	Mandible mass (μg)	Estimated closer muscle mass (mg)
<i>A. targionii</i>	Peru	17	4	1.07 ± 0.013	1.17 ± 0.001	0.80 ± 0.01	13.65 ± 0.64	0.29 ± 0.02
<i>A. horridus</i>	French Guiana	41	5	1.03 ± 0.015	1.39 ± 0.09	1.33 ± 0.03	12.99 ± 6.3	0.35 ± 0.02
<i>A. paripungens</i>	Australia	32	5	1.17 ± 0.013	1.62 ± 0.1	0.81 ± 0.04	16.69 ± 1.9	0.41 ± 0.03
<i>A. emarginatus</i>	French Guiana	22	3	1.72 ± 0.03	5.85 ± 0.31	1.93 ± 0.1	72.72 ± 5.7	1.46 ± 0.08
<i>O. ruginodis</i>	Florida, USA	15	5	1.62 ± 0.1	5.66 ± 0.05	1.08 ± 0.03	52.33 ± 6.9	1.42 ± 0.13
<i>O. chelifera</i>	Brazil	15	3	2.68 ± 0.17	30.1 ± 5.6	2.34 ± 0.13	366.33 ± 93	7.53 ± 1.4

Values in each column are species means for a given trait \pm standard deviation. Species are listed by genus top to bottom by increasing body mass. Mandible closer muscle mass is estimated by multiplying the total body mass by 0.25. n , number of strikes; i , number of individuals. *Anochetus targionii* was collected from leaf litter at the Villa Carmon Biological Research station in Peru (12.89474°S $71.403850^{\circ}\text{W}$) in August 2013; *A. horridus* was collected from under a log and a colony of *A. emarginatus* was collected from the roots of epiphytes at the Nouragues National Nature Reserve, French Guiana (3.982411°S $52.563872^{\circ}\text{W}$) in March 2016; *A. paripungens* was collected from the soil in Humpty Doo, NT, Australia ($12^{\circ}34'21.37''\text{S}$ $131^{\circ}5'2.43''\text{E}$) in April 2010; *O. chelifera* was collected from Viçosa, Minas Gerais, Brazil ($20^{\circ}45'28''\text{S}$ $42^{\circ}51'46''\text{W}$) in October 2014; *O. ruginodis* was collected in October 2005 from Archbold Biological Station in Florida ($27^{\circ}11'5.61''\text{N}$ $81^{\circ}20'19.64''\text{W}$).

Dryer, Tousimis Research Corporation, Rockville, MD) and stored in a desiccator for approximately 24 h before scanning. The head of the ant was placed in an appropriately sized pipette tip which was sealed with cotton and scanned using an Xradia MicroXCT-400 scanner (Carl Zeiss, Oberkochen, Germany) set at 25-kV voltage and 5W power with an exposure time of 3 s. A total of 1441 images were taken at different rotations spanning 180°. Tomographic reconstruction was performed in Xradia XMReconstructor 8.1. Volume renderings and surface models were produced in Amira 5.6.0 (FEI, Hillsboro, OR) and used to calculate the relative volumes that the mandible abductor, adductor, and trigger muscles occupy in the head capsule.

Statistics

To compare the kinematic properties of mandible strikes between species, only the fastest of the left and right mandible were used for each strike. Kinematic properties of mandible

strikes were compared between species using an ANOVA and post hoc Tukey HSD test with strikes treated as a random effect nested within individuals. All stats were performed in R version 3.3.3 (CRAN Studios) using the package nlme (Pinheiro *et al.*, 2017).

Results

The species of *Anochetus* examined in this study exhibited a fivefold difference in body size between the smallest and largest species (Table 1; Fig. 1). The mass-specific power of *Anochetus* strikes ranged from 4.3×10^4 to 1.9×10^5 W kg⁻¹, with all species producing strikes that had power output values far above those possible through direct muscle contraction alone (Josephson, 1993). The kinematic profiles of *A. targionii* and *A. paripungens* resembled those of *O. ruginodis*, the smaller of the two *Odontomachus* species examined in this study (Fig. 2, black curves). *Anochetus*

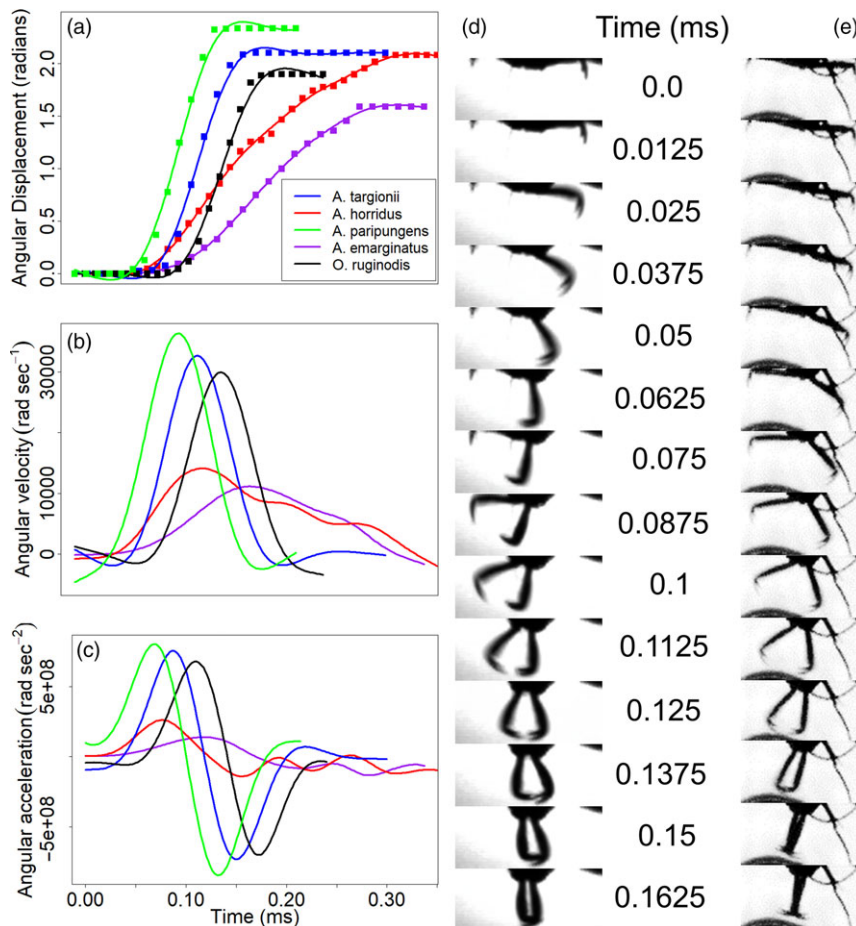


Figure 2 Representative kinematic profiles of *Anochetus* mandible strikes. (a) Angular displacement versus time. Points represent raw cumulative displacement data, curves are the spline fitted to those points. (b) Angular velocity versus time. (c) Angular acceleration versus time. (d) Representative frames of the *A. targionii* mandible strike described in a–c. (e) Representative frames from the video of the *A. emarginatus* mandible strike described in a–c. For panels a–c, species are indicated by color: Blue: *A. targionii*. Red: *A. horridus*. Purple: *A. emarginatus*. Black: *Odontomachus ruginodis*. *Odontomachus chelifer* closely resembles *O. ruginodis* and was not included in these graphs. Numbers between d and e indicate the time in milliseconds relative to the start of the mandible strike. Strikes depicted here are shown in Videos S1–S4. [Colour figure can be viewed at zslpublications.onlinelibrary.wiley.com]

horridus and *A. emarginatus*, in contrast, had different strike kinematic profiles from the other *Anochetus* species examined (Fig. 2).

Mean strike durations for *A. horridus* (mean \pm SD: 0.251 ± 0.11 ms) and *A. emarginatus* (0.22 ± 0.08 ms) were longer than *A. targionii* (0.101 ± 0.04 ms) and *A. paripungens* (0.103 ± 0.04 ms) and were similar to strike durations recorded for the larger *O. ruginodis* (0.19 ± 0.09 ms) and *O. chelififer*

(0.24 ± 0.09 ms; Fig. 3a). *Anochetus targionii* and *A. paripungens* both had similar maximum rotational velocities ($37\,000 \pm 3000$ rad s⁻¹ and $36\,500 \pm 3000$ rad s⁻¹, respectively, rounded to the nearest 500) that were significantly higher than those seen in *Odontomachus* ($31\,500 \pm 6000$ rad s⁻¹ for *O. ruginodis* and $17\,000 \pm 3500$ rad s⁻¹ for *O. chelififer*; Fig. 3b). *Anochetus horridus* and *A. emarginatus* had maximum rotational velocity values that were similar to each other

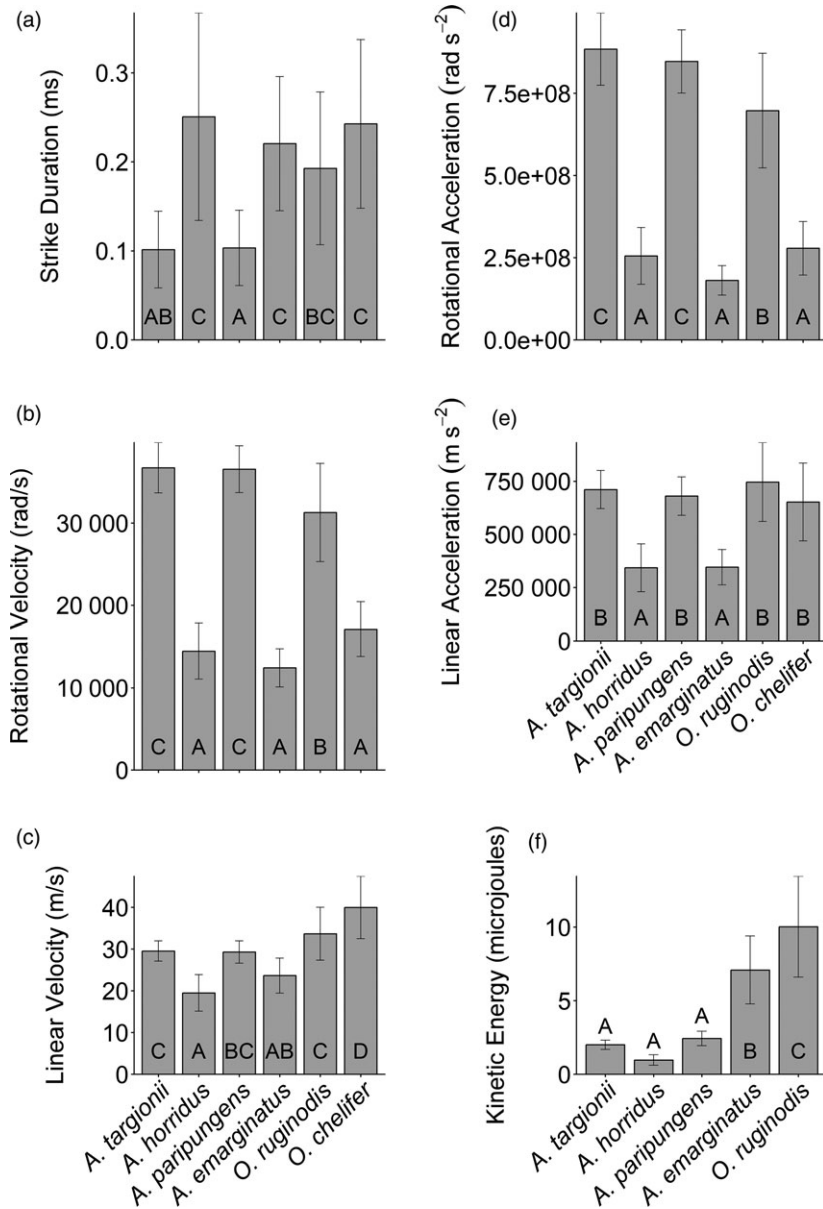


Figure 3 Comparative mandible strike kinematics for *Anochetus* and *Odontomachus* species included in this study. Values shown are means of the maximum values for the left and right mandibles of each strike. Species are arranged by body mass, with the smallest species on the left and the largest species on the right. Error bars denote one standard deviation from the mean. All variables examined differed significantly between species (Nested ANOVA, d.f. = 5, $P < 0.05$). Bars that share letters at their bases are not statistically distinguishable from one another based on a Tukey HSD test at the $P = 0.05$ level. (a) Strike duration; (b) maximum rotational velocity; (c) maximum linear velocity; (d) maximum rotational acceleration; (e) maximum linear acceleration; (f) maximum kinetic energy (with an average kinetic energy value of 100 ± 35 μ J, *O. chelififer* was excluded from graph due to the large difference from other species).

and were the slowest of all species examined in this study ($14\,500 \pm 3000 \text{ rad s}^{-1}$ and $12\,500 \pm 2500 \text{ rad s}^{-1}$, respectively). Maximum rotational velocity was attained at approximately the same time for all species, but *A. horridus* and *A. emarginatus* accelerated and decelerated at a slower rate than other *Anochetus* and *O. ruginodis* (Fig. 2b,c). Mean linear velocity was lowest in the mandible strikes of *A. horridus* ($19.9 \pm 4.3 \text{ m s}^{-1}$) and *A. emarginatus* ($23.6 \pm 4.2 \text{ m s}^{-1}$) and highest in *O. chelififer* ($40.0 \pm 7.5 \text{ m s}^{-1}$), with all other species obtaining intermediate maximum velocities (*A. targionii*: 29.4 ± 2.4 , *A. paripungens*: $29.0 \pm 2.7 \text{ m s}^{-1}$, *O. ruginodis*: 33.7 ± 6.3 ; Fig. 3c).

Maximum rotational acceleration was lowest in *A. horridus* ($2.6 \times 10^8 \pm 8.4 \times 10^7 \text{ rad s}^{-2}$) and *A. emarginatus* ($1.8 \times 10^8 \pm 4.5 \times 10^7 \text{ rad s}^{-2}$), both of which were similar in value to the larger *O. chelififer* ($2.8 \times 10^8 \pm 8.1 \times 10^7 \text{ rad s}^{-2}$). All other species of *Anochetus* and *Odontomachus* examined obtained peak rotational accelerations two to three times that of *A. horridus* and *A. emarginatus* (*A. targionii*: $8.8 \times 10^8 \pm 1.1 \times 10^8 \text{ rad s}^{-2}$, *A. paripungens*: $8.5 \times 10^8 \pm 9.6 \times 10^7 \text{ rad s}^{-2}$, *O. ruginodis*: $7.0 \times 10^8 \pm 1.7 \times 10^8 \text{ rad s}^{-2}$; Fig. 3d). Linear acceleration followed the same pattern, with *A. horridus* and *A. emarginatus* reaching the lowest peak linear acceleration ($3.5 \times 10^5 \pm 1.1 \times 10^5 \text{ m s}^{-2}$ and $3.5 \times 10^5 \pm 8.3 \times 10^4 \text{ m s}^{-2}$, respectively) and all other species reaching peak linear accelerations approximately two times greater than *A. horridus* and *A. emarginatus* (*A. targionii*: $7.1 \times 10^5 \pm 8.9 \times 10^4 \text{ m s}^{-2}$, *A. paripungens*: $6.8 \times 10^5 \pm 9.0 \times 10^4 \text{ m s}^{-2}$, *O. ruginodis*: $7.5 \times 10^5 \pm 1.9 \times 10^5 \text{ m s}^{-2}$, *O. chelififer*: $6.5 \times 10^5 \pm 1.8 \times 10^5 \text{ m s}^{-2}$; Fig. 3e).

Maximum kinetic energy was positively associated with body size (Fig. 3f). The smallest three *Anochetus* species had the lowest kinetic energy values (*A. targionii*: $2.0 \pm 0.31 \mu\text{J}$, *A. horridus*: $0.93 \pm 0.35 \mu\text{J}$, *A. paripungens*: $2.4 \pm 0.49 \mu\text{J}$), while *A. emarginatus* and *O. ruginodis*, the two medium sized species, had intermediate kinetic energy values (*A. emarginatus*: $7.1 \pm 2.3 \mu\text{J}$, *O. ruginodis*: $10 \pm 3.4 \mu\text{J}$). *Odontomachus chelififer*, the largest species, had the most energetic strike of all species examined ($100 \pm 35 \mu\text{J}$).

In most strikes examined, the mandibles were not released simultaneously (*A. horridus*: 40 of 41 strikes; *A. emarginatus*: 18 of 22 strikes; *A. targionii*: 14 of 17 strikes; *A. paripungens*: 28 of 32 strikes); lag times between each mandible starting its acceleration averaged from 0.03 to 0.12 ms among species (Fig. 4). This variation was significant (nested ANOVA, $F = 6.7$, d.f. = 5, $P < 0.001$) and paired comparisons revealed that *A. horridus* had longer lag times between mandibles than *A. targionii* and *A. paripungens* (Tukey HSD, $P < 0.001$; other paired comparisons $P > 0.05$). Maximum rotational and linear velocity did not differ significantly between the fastest and slowest mandible within a strike for any species except *A. paripungens* (paired *t*-test, rotational velocity: $t = 6.9$, d.f. = 62, $P < 0.0001$; linear velocity: $t = 4.3$, d.f. = 62, $P < 0.0001$). In *A. targionii*, the first mandible to be released tended to reach a higher maximum rotational velocity than the second (exact binomial test, number of successes = 13, number of trials = 14, $P = 0.001$). This was not the case for *A. paripungens* (exact binomial test, number of successes = 9,

number of trials = 28, $P = 0.08$), *A. horridus* (exact binomial test, number of successes = 14, number of trials = 31, $P = 0.72$), or *A. emarginatus* (exact binomial test, number of successes = 5, number of trial = 13, $P = 0.58$). In these species, there was no relationship between which mandible was released first and which obtained a higher velocity. As in *Odontomachus bauri* (Patek *et al.*, 2006), *Anochetus* mandibles began to decelerate before crossing the midline (Fig. 4b,c).

The microCT scan of *A. horridus* revealed the mandible closer (adductor) muscles (AdM) occupy most of the ventral and posterior sections of the head capsule and account for at least 20% of the total head capsule volume (Fig. 5). Contraction of these muscles when the mandibles are locked open likely deforms the mandible apodemes, allowing elastic energy to be stored prior to a strike. The trigger muscles (fAdM) are specialized sections of the mandible closer muscle that consist of a small group of muscle fibers inserting on the mandible adductor apodeme ventrolaterally and originating at the ventrolateral side of the postgenal section of the head capsule. They occupy 0.17% of the head capsule volume and account for 0.9% of the volume of the mandible adductor muscle. These fibers are likely fast-twitch muscle fibers based on previous studies of this muscle in other species of trap-jaw ants, and their contraction rotates the mandibles out of their locked position and allows the mandibles to swing shut when the elastic energy stored in the apodemes is released. The mandible opener (abductor) muscles (AbM) are much smaller than their closer counterparts, occupying 2.5% of the total head capsule volume. The mandible abductor muscles insert near the base of the mandible and attach to the medial portion of the postgena in between the closer muscles. Contraction of these muscles opens the mandibles and, if contracted far enough, locks the mandibles into the open position.

Discussion

Influence of body size and morphology on strike performance

Our estimates of mass-specific power output for each species exceed those possible through muscle contraction alone (Josephson, 1993), confirming that *Anochetus* mandible strikes are indeed power amplified. All species followed a pattern of increasingly energetic strikes with larger body mass. This relationship is likely driven primarily by the increase in mandible mass as body size increases. *Anochetus targionii* and *A. paripungens* mandible strikes closely resemble those of *Odontomachus ruginodis*, although they consistently outperform *O. ruginodis* in many aspects of their strike kinematics, on average obtaining higher peak rotational velocities and accelerations. As both species are considerably smaller than *O. ruginodis*, these results are consistent with the relationship between strike performance and body size found in *Odontomachus* (Spagna *et al.*, 2008). In contrast, *A. horridus* and *A. emarginatus* (both from the *emarginatus* species group) have similar mandible strikes that consistently underperform other *Anochetus* and *Odontomachus* species, on average obtaining lower peak velocities and accelerations. This relationship cannot be explained by relative differences in body size, as the body mass of

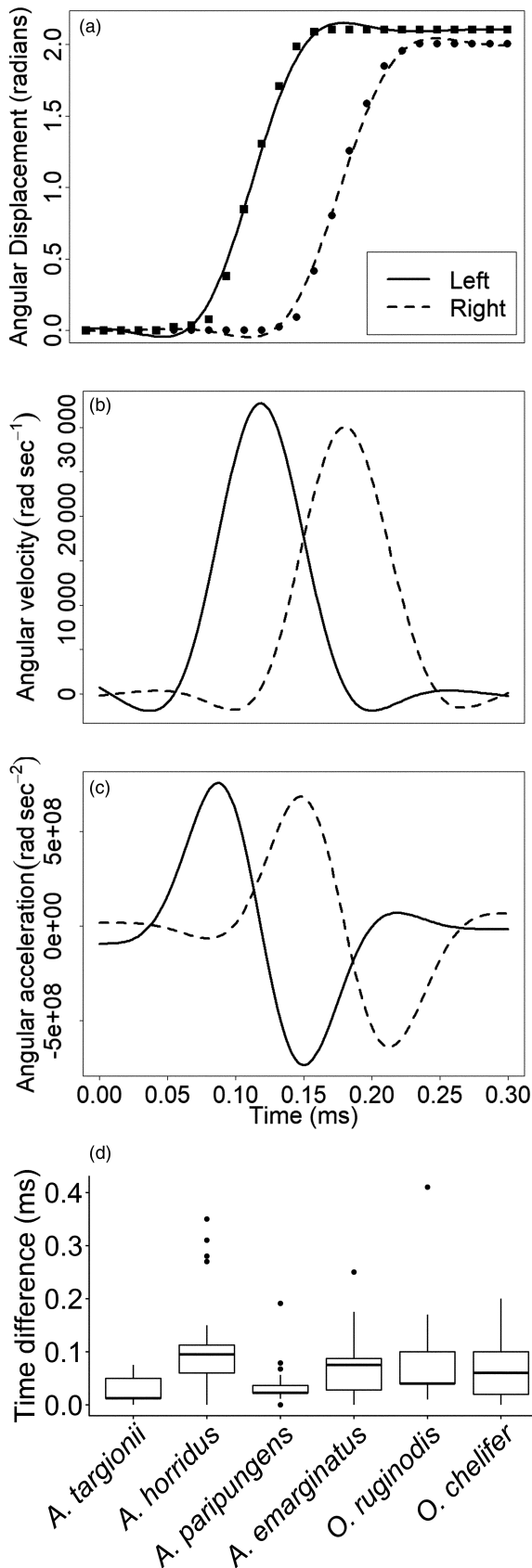


Figure 4 Delay in mandible release (the time difference between when the first and second mandible begin to move) is present in all *Anochetus* spp. examined. (a–c) Graphs displaying kinematic profiles of the left and right mandibles during a representative *A. targionii* strike (the same strike depicted in Figure 2). Points represent the raw displacement data used to calculate the spline. Solid lines represent the left mandible; dotted lines represent the right mandible. (a) Angular displacement versus time; (b) angular velocity versus time; (c) angular acceleration versus time; (d) box and whisker plot depicting the time delay in mandible release between the left and right mandibles for all mandible strikes examined.

A. horridus is intermediate between *A. targionii* and *A. paripungens*, and one-fifth that of *A. emarginatus*. Other factors, such as similar head and mandible morphology, differing life-history strategies, or evolutionary constraints (Larabee *et al.*, 2016), may partially explain differences in strike performance of *A. horridus* and *A. emarginatus* relative to other *Anochetus* species.

Both *A. horridus* and *A. emarginatus* have relatively long, thin mandibles with respect to their head width compared with the other *Anochetus* species. The average mandible length to head width ratio is 1.32 and 1.12 for *A. horridus* and *A. emarginatus*, respectively, compared with 0.74 and 0.69 for *A. targionii* and *A. paripungens*. These differences in relative mandible length, however, do not translate to differences in relative mass; the average mandible mass to body mass ratio is 0.011 and 0.012 for *A. horridus* and *A. emarginatus* compared with 0.011 and 0.010 for *A. targionii* and *A. paripungens*. Having more elongate mandibles increases the minimum distance needed between the ant and its prey for a strike to be successful, possibly increasing the chances of successfully capturing prey. The reduced strike performance seen in *A. horridus* and *A. emarginatus* may be an adaptation to prevent their mandibles from fracturing under the increased stresses of a more forceful strike. The reduced strike performance in this species group could, therefore, be the result of a trade-off between the probability of successfully capturing prey and the amount of internal stress the mandible experiences when biting, an idea that warrants future study.

Influence of physiology on strike performance

Differences in muscle geometry and composition might explain differences in *A. horridus* and *A. emarginatus* strike performance (see, e.g., David *et al.*, 2016). However, the muscle arrangement within the head capsule of *A. horridus* we report here is similar to that of *A. graeffei* as described by Gronenberg & Ehmer (1996). In ants, muscle attachment angle and the proportion of mandible closer muscle consisting of fast and slow twitch muscle fibers both influence bite performance (Gronenberg *et al.*, 1997; Paul & Gronenberg, 1999). *Anochetus* species do not possess fast-twitch fibers in their mandible closer muscle and rely on stored elastic energy (likely in the muscle apodeme or cuticle of the head) to provide the speed needed for their strikes (Gronenberg & Ehmer, 1996).

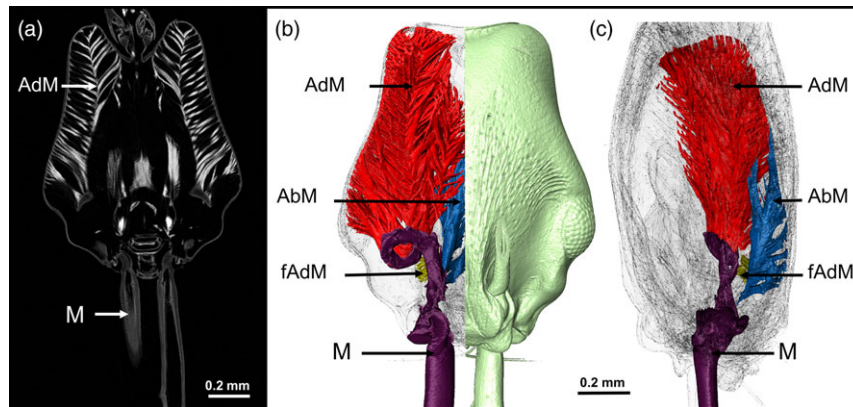


Figure 5 Reconstructed model of *Anochetus horridus* head capsule from a micro-CT scan. (a) 2D coronal section of head capsule. (b) Dorsal view, with the left side of the head capsule removed to show the arrangement of muscles relevant to the trap-jaw mechanism and the right side showing the external features of the head. (c) Lateral view cut away to display the same structures labeled in A. AdM, mandible adductor muscle; AbM, mandible abductor muscle; fAdM, fast-twitch adductor muscle (trigger muscle); M, mandible and mandible apodeme. Voxel size: $2.0 \mu\text{m}^3$. [Colour figure can be viewed at zslpublications.onlinelibrary.wiley.com]

Differences in strike kinematics are, thus, likely not due to underlying physiological differences in mandible closer muscle fibers between species, and likely reflect differences in the material properties of the elastic energy storing component of the mechanism. Determining the specific structures responsible for elastic energy storage within the head capsule of *Odontomachus* and *Anochetus* prior to mandible release and examining species level differences in the material properties of those structures may help to explain performance differences in future studies.

The venom potency of trap-jaw ants is another aspect that may influence the mandible strike performance. Both mandibles and venom are important features for prey capture and one may be negatively correlated to the other. This is well illustrated in scorpions where scorpions with more potent venom possess relatively slender claws (Van der Meijden, Herrel & Summers, 2010). Although little data on the potency and the toxicity of ant venoms are available, both *A. horridus* and *A. emarginatus* have unusual venom compositions that vastly contrast with venoms of *Odontomachus* and other ponerine ants (Touchard *et al.*, 2015). Furthermore, the investigation of the *A. emarginatus* venom resulted in the discovery of a novel family of neurotoxins that reversibly paralyse insects (Touchard *et al.*, 2016). Nevertheless, further venom investigations extended to other *Anochetus* species are needed to support this hypothesis.

All four *Anochetus* exhibited a lag between the release of the first and second mandibles during a strike. These lag times were similar to those observed in both *Odontomachus* species, and there was no difference in performance between the first and second mandible to be released with the exception of *A. targionii*, in which the first mandible to be released consistently reached a higher maximum rotational velocity compared with the second. This contrasts with the findings of Patek *et al.* (2006), who found that in *O. bauri* the second mandible to be released usually reached a higher maximum velocity. A delay between mandibles in their release may be the result of the time it takes to transmit a signal from sensory neurons to

the corresponding motor neurons (Patek *et al.*, 2006). In *A. graeffei*, the sensory neurons attached to trigger hairs do not cross over to the opposing side of the subesophageal ganglion like they do in *Odontomachus* (Gronenberg & Tautz, 1994; Gronenberg & Ehmer, 1996), suggesting that *Anochetus* should have longer lag times compared with its sister genus. Our results are not consistent with this pattern, possibly because the difference in lag time is so minute that we were not able to detect it at the frame rates used in this study. It is also possible that this neuronal configuration is restricted to certain species like *A. graeffei*, and in the species examined in this study crossing over of the sensory trigger neurons does occur in a similar manner to *Odontomachus*. Future studies using histological methods to examine how neurophysiology varies between species and examining the performance of *A. graeffei* mandible strikes will be able to answer this question. Lag time between mandible release is likely restricted to ponerine trap-jaw ants; in many myrmicine trap-jaw ants (*Daceton*, *Orectognathus*, *Epopostruma*, and *Strumigenys*), the labrum is modified to act as the latch, allowing the mandibles to be released simultaneously (Gronenberg, 1996b).

In all species examined, both mandibles begin to decelerate before crossing the midline, similar to *Odontomachus* mandible strikes (Patek *et al.*, 2006). This premature deceleration is thought to be adaptive as it would prevent the mandibles from crashing into each other at full force if the ant misses its target. Examining the kinematics of more phylogenetically independent trap-jaw mechanisms (e.g. in other ant genera) will determine if this deceleration is a universal characteristic of trap-jaw mandibles to reduce mandible wear or if it is restricted to certain taxa. Most myrmicine trap-jaw ant genera, for example, are smaller bodied and likely generate less force than ponerine trap-jaw ants which have more massive mandibles. Preventing mandibles from colliding at full force may be less of an issue in smaller species.

Gronenberg & Ehmer (1996) described the trap-jaw mechanism in *A. graeffei*, noting that the morphology, musculature,

and neurophysiology of the mechanism are generally similar to *Odontomachus* but differ in a few key characteristics. *Anochetus graeffei* has a trigger muscle composed of fibers that attached indirectly to the mandible apodeme via short, thin filaments, while in *Odontomachus* the trigger muscle fibers all attach to the apodeme directly (Gronenberg & Ehmer, 1996). The mandible closer muscle of *A. graeffei* also contains more filament-attached muscle fibers compared with the closer muscle in *Odontomachus* (Gronenberg & Ehmer, 1996). This interspecific variation in physiology may account for some of the differences we see in kinematics between *Anochetus* and *Odontomachus*. The microCT scan of *A. horridus* suggests that muscle arrangement is likely consistent across species of *Anochetus* as muscle arrangement in *A. horridus* closely resembles that of *A. graeffei* at the level of detail discernable via MicroCT scans. More detailed studies comparing head capsule musculature and innervation at the cellular level are needed to confirm this.

Influence of phylogeny on strike performance

The confounding effect of shared evolutionary relatedness may also partially explain the strike performance patterns we observed. *Anochetus horridus* and *A. emarginatus* are closely related, both being members of the *emarginatus* species group (Larabee *et al.*, 2016). In contrast, *A. targionii* and *A. paripungens* originate from the phylogenetically and geographically distinct *mayri* and *rectangularis* species groups (distributed throughout the new and old world tropics, respectively; Larabee *et al.*, 2016). The strong similarities in their strike performance suggest that morphology and body size are a large component of what determines strike performance in this genus. However, the scope of this study does not allow for large-scale comparisons of the relative roles of phylogeny and morphology on strike performance.

Conclusion

Our results suggest that phylogeny, physiology, morphology, and natural history of trap-jaw ant species may each play an important role in determining strike performance, although understanding the relative role of each will require describing the kinematics of a larger number of species in an explicit phylogenetic context. The repeated evolution of power-amplified mandibles in trap-jaw ants has been implicated in increased diversification rates and lineage persistence (Pie & Tschá, 2009; Moreau & Bell, 2013; Larabee & Suarez, 2014), although this idea has not yet been tested empirically. Future work should focus on describing the strike performance of additional *Odontomachus* and *Anochetus* species and combine these data with phylogenetic (Larabee *et al.*, 2016), natural history (Brown, 1976, 1978), and morphological information to examine the relative importance of these factors on strike performance. Future studies that study the performance of other independent evolutions of trap-jaw mechanisms, such as those in the subfamily Myrmicinae, will also shed light on which

kinematic properties of mandible strikes, if any, are universal to all trap-jaw ants.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Video S1. Representative mandible strike of *Anochetus targionii*.

Video S2. Representative mandible strike of *Anochetus horridus*.

Video S3. Representative mandible strike of *Anochetus paripungens*.

Video S4. Representative mandible strike of *Anochetus emarginatus*.