THE EVOLUTION AND FUNCTIONAL MORPHOLOGY OF TRAP-JAW ANTS

BY

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DISSERTATION

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ABSTRACT

Key innovations are traits that allow organisms to interact with their environment in novel ways and are thought to facilitate adaptive radiation. By providing access to previously untapped resources, key innovations allow organisms to move into new ecological niches and can promote morphological diversification and speciation. I am interested in the evolution of form and function of one particular morphological innovation in the diversification of "trap-jaw" ants: power-amplified mandibles used for prey capture, nest defense, and individual escape from predators.

Insects are the most diverse and numerically abundant animal group on the planet. One feature that contributed to their evolutionary success was the diversification their mouthparts. From an ancestral mandibulate condition (still found in many extant taxa), insect mouthparts have diversified into many specialized forms such as the piercing-sucking mouthparts of true bugs and various parasites, the sponging mouthparts of flies, and the extendible proboscis of butterflies and moths. This diversity has allowed insects to occupy a variety of dietary niches, including predation, herbivory, liquid feeding, and parasitism. An understanding of the relationship between structure and function of insect mouthparts is, therefore, critical for understanding their ecological success.

My dissertation consists of four chapters and investigates the evolution and functional morphology the highly specialized mouthparts of trap-jaw ants. In Chapter 1, I review the current literature on trap-jaw ant taxonomy, phylogenetics, and biomechanics. The trap-jaw morphology has independently evolved at least four times in the ant family Formicidae, and, in this chapter, I highlight the areas of convergence among the four trap-jaw ant lineages.

The most well studied lineage of trap-jaw ants are found in the subfamily Ponerinae, and consist of the sister genera *Anochetus* and *Odontomachus*. In Chapter 2, I present my findings from the first comprehensive worldwide phylogeny for these two trap-jaw ant genera. Using molecular sequence from four nuclear and one mitochondrial gene, I establish a phylogenetic framework for approximately half of the currently described species. Specifically, I confirm that the two genera are monophyletic sister groups, and found support for seven monophyletic clades. These trap-jaw ants diversified approximately 30 million years ago predominately in Southeast Asia, with multiple dispersal events to Australasia, the Afrotropics, and South America.

Size often determines the output of animal performance systems, and examples of these scaling relationships are common throughout nature. What is unclear is if scaling relationships in musculoskeletal systems are shared within and between species. To answer this question, I examined morphological and performance scaling relationships between different sized trap-jaw ants and within a polymorphic species. I found that among species of *Anochetus* and *Odontomachus*, there is a strong and significant negative relationship between speed and body size, with larger and having longer snap durations and lower peak speeds. Contrasting with interspecific scaling relationships, the speed of mandible strikes within the polymorphic species *Odontomachus turneri* did not show any relationship with body size. Instead the peak kinetic energy of mandibles within and among *Odontomachus* species scaled with body size, suggesting that there may be stabilizing selection acting on mandible speed, but that strike energy may be determined by body size constraints.

In Chapter 4, I examine the biomechanics, morphology and kinematics of the trap-jaw ant, *Myrmoteras barbouri*. A member of the ant subfamily Formicinae, *Myrmoteras* trap-jaw ants have received relatively little attention compared to other trap-jaw ant lineages and the

mechanism of their spring-loaded mandibles have previously been unstudied. Using high-speed videography, I measured mandible strikes that occur in less than 1 millisecond and peak speeds of 2.6×10^4 $\mathrm{rad \cdot s^{-1}}$. These speeds are faster than can be explained by direct muscle contraction, and confirm that *Myrmoteras* jaws are spring-loaded. The spring that stores the potential energy required for the strikes is a modification of the occipital margin, which bends during mandible loading. Compared with other trap-jaw ants, *Myrmoteras* jaws reach similar peak velocities, but accelerate over a much longer period of time, which is likely a reflection of their unique mandible mechanism.

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CHAPTER 1: REVIEW OF TRAP-JAW ANT EVOLUTION AND MORPHOLOGY[1]

Abstract

I review the biology of trap-jaw ants whose highly specialized mandibles generate extreme speeds and forces for predation and defense. Trap-jaw ants are characterized by elongated, power-amplified mandibles and use a combination of latches and springs to generate some of the fastest animal movements ever recorded. Remarkably, trap-jaws have evolved at least four times in three subfamilies of ants. In this review, I discuss what is currently known about the evolution, morphology, kinematics, and behavior of trap-jaw ants, with special attention to the similarities and key differences among the independent lineages. I also highlight gaps in our knowledge and provide suggestions for future research on this notable group of ants.

Introduction

Mandibles are critical to the biology of ants, being the primary structures they use to physically interact with their environment during activities like foraging, predation, food processing, defense, nest excavation, and brood care (HÖLLDOBLER & WILSON 1990, LACH & al. 2010). Although these essential functions constrain their morphology, ant mandibles display a remarkable amount of diversity, with elaborate examples of specialization including the pitchforks of *Thaumatomyrmex*, the sickles of *Polyergus*, the hooks of *Eciton* soldiers, and the vampiric fangs of *Amblyopone* (WHEELER 1927, GOTWALD 1969, HÖLLDOBLER & WILSON 1990). One of the most extreme specializations of ant mandibles can be found among trap-jaw

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ants, whose long, linear, spring-loaded mandibles snap shut at some of the fastest speeds ever recorded for an animal movement (PATEK & al. 2006). Remarkably, the trap-jaw morphology has independently evolved at least four times across the ant tree of life. Each lineage of trap-jaw ant has converged on a common catapult mechanism for mandible closure, but collectively they display a great amount of diversity in body size, diet, nesting habits, and foraging strategies (Figure 1.1).

While trap-jaw ants are frequently cited in reviews on animal speed or ant predation (PATEK & al. 2011, CERDÁ & DEJEAN 2011, HIGHAM & IRSCHICK 2013), there has never been an attempt to summarize their overall biology. The purpose of this review is to synthesize the literature on trap-jaw ant biology, especially focusing on their evolution and biomechanics, and the behavioral consequences of having trap-jaws. I limit my discussion to those ants whose mandibles insert close to the midline of the head and use a catapult mechanism to shut their mandibles from an open position. Consequently, I exclude from this review "snapping ants" (for example the genera *Mystrium* and *Plectroctena*), which also have power-amplified mandibles but shut their widely set mandibles from a closed position, snapping them past each other (MOFFETT 1986a, GRONENBERG & al. 1998, DEJEAN & al. 2002). I also omit discussion of ants with linear mandibles that are not power-amplified, such as the genera *Harpegnathos* or *Myrmecia*, because their rapid mandible movements are the result of direct muscle action (PAUL 2001). Because of their small size and cryptic habits, less is known about the biology of trap-jaw ants from the Myrmicinae and Formicinae relative to the larger species in the subfamily Ponerinae. Consequently, much of this review will focus on the genus *Odontomachus*, where more information is available on their functional morphology, foraging behavior, and systematics.

Taxonomy/Systematics

The term "trap-jaw ant" does not describe a monophyletic taxon. CREIGHTON (1930) used it to discuss how several distantly related lineages of ants have converged to possess long, linear mandibles whose rapid closure results from the release of a latch mechanism and is triggered by long hair-like cuticular mechanoreceptors ("trigger hairs"). This trap-jaw condition has evolved once each in the subfamilies Ponerinae (*Anochetus* and *Odontomachus*) and Formicinae (*Myrmoteras*), and at least twice in the subfamily Myrmicinae (tribe Dacetini) (Figure 1.2). Trap jaws may have also evolved in other lineages, including *Protalaridris armata* in the myrmicine tribe Basicerotini and the fossil genus *Haidomyrmex* (BARDEN & GRIMALDI 2012). Without detailed studies of their functional morphology or behavior, however, it is difficult to confidently define these groups as trap-jaw ants, and so I do not include them in this review.

Subfamily Ponerinae

Two ponerine genera possess trap-jaw mandibles: *Anochetus* and *Odontomachus*, containing 110 and 69 extant species, respectively (BOLTON 2013). These genera are distributed worldwide in the tropics and subtropics but are most diverse in the Neotropics and South East Asia (BROWN 1976). The last worldwide revision was by BROWN (1976, 1977, 1978), but a number of recent studies have described new species and clarified the taxonomy of these genera in specific regions (DEYRUP & al. 1985, DEYRUP & COVER 2004, FISHER & SMITH 2008, SORGER & ZETTEL 2011, SHATTUCK & SLIPINSKA 2012, ZETTEL 2012). Like other ponerines (PEETERS 1997, SCHMIDT 2013), they display a suite of characteristics that are often considered ancestral in ants, including small colony size, monomorphic workers, little differentiation between the workers and queen, and solitary foraging (BROWN 1976, BROWN 1978). The body size of *Anochetus* is generally much smaller than *Odontomachus*, although there is some overlap.

Within and between genera, nesting preferences vary widely, including soil, leaf litter, rotten logs, and even the canopy (RAIMUNDO & al. 2009, CERQUERA & TSCHINKEL 2010, SHATTUCK & SLIPINSKA 2012, CARMAGO & OLIVEIRA 2012).

Molecular phylogenetics strongly supports grouping the clade containing *Odontomachus* and *Anochetus* in the Odontomachus genus group, one of several large multi-generic clades found in the Ponerinae (SCHMIDT 2013). Other genera in the group include *Leptogenys*, *Odontoponera*, *Phrynoponera,* and a number of *Pachycondyla* "subgenera," but it is still unclear which of these is sister to the ponerine trap-jaw ants. Molecular divergence dating estimated that the Odontomachus group rapidly radiated between 50 and 45 million years ago, with the trap-jaw clade arising somewhat more recently (approximately 30 million years ago). Nine fossil species of *Anochetus* and three of *Odontomachus* have been described, mostly from Dominican Amber (but one compression fossil of *Odontomachus* from the Most Basin (WAPPLER & al. 2013)), with ages ranging between 23 and 19 million years (BARONI URBANI 1980, MACKAY 1991, DE ANDRADE 1994).

Most recent morphological and molecular phylogenetic studies have strongly supported monophyly for the clade containing *Anochetus* and *Odontomachus* (BRADY & al. 2006, MOREAU & al. 2006, SPAGNA & al. 2008, KELLER 2011, MOREAU & BELL 2013, SCHMIDT 2013), but whether they are monophyletic sister groups is still unclear. From the morphology of male genitalia and petiole, BROWN (1978) hypothesized that *Odontomachus* arose from within a paraphyletic *Anochetus*. Data from karyotypes (SANTOS & al. 2010) and adductor muscle morphology (GRONENBERG & EHMER 1996) corroborate this scenario, with *Anochetus* possessing ancestral states of both characters. However, preliminary molecular phylogenetic analyses have been hampered by small and unequal taxon sampling and have been unable to reject alternative relationships, including the two genera being exclusive sister groups, or *Odontomachus* being paraphyletic with respect to *Anochetus* (SPAGNA & al. 2008, SCHMIDT 2009).

Subfamily Myrmicinae

The subfamily Myrmicinae has, by far, the most species of trap-jaw ants, all currently classified as members of the tribe Dacetini (which includes over 900 described species) (BOLTON 2013). Although not all dacetine species are trap-jaw ants, a large portion of the genus *Strumigenys* and all members of the genera *Acanthognathus*, *Daceton*, *Epopostruma*, *Microdaceton*, and *Orectognathus* display trap-jaw morphology. Most of these genera are predominantly tropical or subtropical with the genus *Strumigenys* being found worldwide, *Acanthognathus* and *Daceton* limited to the Neotropics, *Microdaceton* only found in the Afrotropics, and *Epopostruma* and *Orectognathus* limited to Australasia (BOLTON 1999, BOLTON 2000). Dacetine mandibles are remarkably variable, with some species clearly displaying long linear trap-jaw mandibles that open at least 180° (kinetic mandibles *sensu* BOLTON 1999), whereas others (many *Strumigenys*, and all *Colobostruma* and *Mesostruma*) have triangular (long or short), forcep-like, or plier-like mandibles that can not open beyond 60–90° (static mandibles *sensu* BOLTON 1999). Each mandibular form is correlated with discrete predatory modes of action (use of sting and speed of attack) (BOLTON 1999). Despite the variation in mandible morphology, body size, and foraging behavior, most dacetines are relatively small bodied and form small colonies in leaf litter or rotten logs (WILSON 1953, BOLTON 1999, DEYRUP & COVER 2009). They can often be locally abundant and it is difficult to find a Berlese or Winkler sample of tropical forest leaf litter that does not contain at least one dacetine species (WARD 2000).

It is beyond the scope of this review to thoroughly cover the taxonomic history of the Dacetini, but to say that the generic classification of the tribe is unstable is an understatement (Baroni Urbani & de Andrade 2006a, b, Bolton 2006a, b). Early generic and species-level revisions were conducted by Brown (1948, 1953, 1961, 1962, 1969, and containing references). More recent studies by Baroni Urbani & de Andrade (1994, 2007) and Bolton (1983, 1998, 1999, 2000), based on extensive comparative morphology, attempted to bring order to the tribe and resulted in major, and sometimes contradictory, rearrangements of genus- and tribe-level groups. Due to the quality of morphological characters used in many of these studies, many questions remain about the classification of dacetines and the relationships between genera.

Given the uncertainty of Dacetini classification, it is not surprising that the evolutionary origin of the trap-jaw morphology within the tribe is also unclear. One possible scenario is that the common ancestor of all dacetines was an epigaeic trap-jaw ant from which hypogaeic short-mandible forms have been derived multiple times (Brown & Wilson 1959). Despite some support for this scenario from a cladistic analysis of dacetine morphology (Bolton 1999), most recent studies favor the alternative hypothesis that the trap-jaw morphology has evolved multiple times from a short-mandible non-trap-jaw ancestor (Bolton 1999, Baroni Urbani & de Andrade 2007). A recent comprehensive molecular phylogenetic analysis of the subfamily Myrmicinae by Ward and colleagues strongly supports *Strumigenys* (*sensu* Baroni Urbani & de Andrade 2007) as sister to the Phalacromyrmecini, rendering the tribe Dacetini (*sensu* Bolton 2000) paraphyletic (Ward personal communication). This would reinforce the hypothesis that the trap-jaw morphology has evolved at least two times within the subfamily: once in *Strumigenys* and at least once in the remaining dacetine genera.

Subfamily Formicinae

The least species-rich trap-jaw ant group is the genus *Myrmoteras*, with only 34 described extant species (BOLTON 2013). A recurring theme in the *Myrmoteras* literature is how rarely workers are collected and how little is known about their general biology. The paucity of *Myrmoteras* collections may partially be explained by their relatively limited distribution (South East Asia) (AGOSTI 1992) and small nests that are primarily located in leaf litter (MOFFETT 1986b). The majority of *Myrmoteras* species (> 20) have been described over the last three decades (MOFFETT 1985, ZETTEL & SORGER 2011, BUI & al. 2013), as standardized methods for sampling leaf litter arthropods have become the primary tool used to quantify ant biodiversity (AGOSTI & al. 2000). With continued efforts to intensively sample leaf litter worldwide, the likelihood of additional species discoveries and the opportunity to study their ecology and behavior will increase.

The morphology of *Myrmoteras* is exceptional even among trap-jaw ants, with long, slender, and dentate mandibles, large eyes, and a small head relative to other trap-jaw ants (AGOSTI 1992). The genus is divided into two subgenera based on the presence of trigger hairs: *Myrmoteras* and *Myagroteras* (MOFFETT 1985). The subgenus *Myagroteras* lacks trigger hairs on the labrum, which may have interesting implications for its trap-jaw mechanism and foraging behavior (see below). Early myrmecologists easily placed them in their own tribe (Myrmoteratini) (WHEELER 1922), but a combination of ancestral and derived traits made the relationship of *Myrmoteras* to other genera within Formicinae more difficult. Based on their large eyes (WHEELER 1922) and simplified proventriculus (GREGG 1954), the genus had been thought to be the remnant of an early branch of the formicine tree. More recently, AGOSTI (1992) placed them in the *Formica* genus-group based on the simple form of the helcium, and molecular phylogenetic studies have suggested they are sister to the tribe Camponotini (BRADY & al. 2006, MOREAU & al. 2006, MOREAU & BELL 2013).

Biomechanics

Animals have repeatedly evolved suites of morphological and behavioral traits that allow them to overcome the physical and biological constraints of muscle speed. The record-breaking jumps of froghoppers (BURROWS 2003, BURROWS 2006), the rapid predatory strikes of stomatopods (PATEK & al. 2004, PATEK & al. 2007), and the ballistic tongues of chameleons (DE GROOT & VAN LEEUWEN 2004) all display movements that are many times faster than the maximum contraction speed of most skeletal muscles (JAMES & al. 2007). Like each of these cases, trap-jaw ants utilize a catapult mechanism that uses latches and elastic elements to amplify the speed and power of appendage movement. In this section, I will survey the functional morphology and kinematics of trap-jaw ants, with an emphasis on the independently derived strategies each lineage uses to amplify speed.

Morphology

Like in most other insects, two muscles are primarily responsible for “normal” mandible movement in ants: the mandible opener (abductor) and the mandible closer (adductor) muscles (SNODGRASS, 1928, CHAPMAN, 1995). The mandible moves as a simple hinge, with the closer and opener muscle attaching, respectively, to the medial and lateral portion the mandible base. The closer muscle is the largest muscle found in ant workers and is composed of fast (but weak) and slow (but forceful) muscle fibers arranged in discrete bundles of a single fiber type (GRONENBERG & al. 1997). Species have varying absolute and relative amounts of each fiber type with varying angles of attachment to the mandible via an apodeme, and these species-specific traits often correlate with the ecological use of the mandible (GRONENBERG & al. 1997, PAUL & GRONENBERG 1999, PAUL 2001). In contrast, the mandible opener muscle is much smaller and usually consists of just a single fiber type.

Trap-jaw ants have modified the basic ant mandible plan by inserting specialized latch, spring and trigger structures that together enable the catapult mechanism. This mechanism allows muscles to build up power over the course of seconds and then release it in less than a millisecond (GRONENBERG 1996a, PATEK & al. 2011, HIGHLAND 2013). A latch keeps the mandibles open even when the mandible closer muscle contracts (GRONENBERG 1995a, JUST & GRONENBERG 1999), allowing potential energy to slowly be stored in a spring until a specialized "trigger muscle" releases the latch and the mandibles shut nearly instantaneously (GRONENBERG 1995b, JUST & GRONENBERG 1999). All trap-jaw ants use this same basic mechanism, but the structures that comprise the individual components (the latch, spring, and trigger) vary between lineages. An initial mechanism was proposed by BARTH (1960) for the mandible snap of *Odontomachus chelifer*, but most of the details of trap-jaw functional morphology and neurophysiology were described by GRONENBERG in the 1990s (GRONENBERG & al. 1993, GRONENBERG & TAUTZ 1994, GRONENBERG 1995a, b, GRONENBERG & EHMER 1996).

In the genera *Odontomachus* and *Anochetus* the latch, spring and trigger all derive from modifications of the mandible joint and closer muscle (GRONENBERG 1995a, GRONENBERG & EHMER 1996). Contraction of the mandible opener muscle moves the ventral base of the mandible into a notch at the base of the mandible joint. This notch acts as the latch, keeping the mandibles securely open even when the relatively large mandible closer muscle contracts. Contraction of the mandible closer muscle builds up potential energy in a spring (GRONENBERG 1995a, b). The anatomical structures that serve as the spring have not yet been definitively described but are likely heavily sclerotized cuticular elements of the mandible, apodeme and anterior head capsule (GRONENBERG 1995a). To release a strike, the small trigger muscle attached to the closer apodeme pulls the mandible laterally out of the notch and allows the mandibles to snap shut. A comparison of *Anochetus* and *Odontomachus* trigger muscle morphology led GRONENBERG & EHMER (1996) to conclude that the trigger muscle is derived from the mandible closer muscle. As already noted, *Anochetus* are, on average, smaller than *Odontomachus* which may significantly affect the speed and acceleration of their mandible strikes (see below). Other notable differences between these two genera include the maximum mandible gape in *Anochetus* often surpasses 180°, in *Anochetus* the trigger and mandible closer muscles are attached to their apodemes via fibers, but in *Odontoamchus* they are directly attached.

Reflecting their complex evolutionary history (BOLTON 2000, BARONI URBANI & DE ANDRADE 2007, WARD personal communication), dacetine trap-jaw ants display multiple power amplification mechanisms. In *Daceton armigerum* and at least some *Strumigenys* species, the latch and trigger are formed by modifications of the labrum (GRONENBERG 1996b). Lateral projections of the "T-shaped" labrum engage with basimandibular processes, locking the mandibles open even when the large mandible closer muscle contracts. Potential energy is likely stored in cuticular elements of the head, but, like the ponerine trap-jaw ants, the spring has not yet been identified. The strike is released when the trigger muscle, derived from the labral adductor, pulls the labrum inward, disengaging from the basimandibular process and allowing the mandibles to close (GRONENBERG 1996b).

Ants in the genus *Acanthognathus* have an extremely reduced labrum (BOLTON 1999, BOLTON 2000) and their mandible-locking mechanism is completely different from other dacetine trap-jaws ants (DIETZ & BRANDÃO 1993, GRONENBERG & al. 1998). In this genus, the latch is formed by long, curved basimandibular processes. As the mandibles open, they rotate about their longitudinal axis, which positions the processes so that their forked apices interlock with each other. In this position, and like all other trap-jaw ants, the mandible closer muscles can contract without closing the mandibles. The trigger muscle is a distinct group of fibers derived from the mandible closer muscle that attach only on the dorsal and lateral sides of the "Y-shaped" mandible closer apodeme. Because of their asymmetrical position, contraction of the trigger muscles applies a torque to the heavily sclerotized arm of the mandible closer apodeme. This reverses the rotation of the mandibles, frees the basal processes, and allows the mandibles to snap shut. Until more information on the evolutionary history of dacetine ants is available, it is unclear if the morphology of *Acanthognathus* is derived from another trap-jaw mechanism like that in *Daceton* or if it is an independent origin from a short-mandible ancestor.

The mandibles of dacetine trap-jaw ants are dramatically different from those of non-trap-jaw dacetines, like some *Strumigenys* (formerly in the genus *Pyramica*), and all species of *Colobostruma*, and *Mesostruma* (BOLTON 2000, BARONI URBANI & DE ANDRADE 2007). Short-mandible static-pressure dacetines are also specialized predators, with large muscle-filled heads and fast mandible strikes (see below) (MASUKO 1984), but the functional morphology of their mandibles and muscles has not been studied in any detail. It is unclear if they use a power amplification mechanism different from the mechanism employed by trap-jaw ants, or if, like *Myrmecia*, *Harpegnathos*, and other predatory ants with rapid mandibles, they rely on the direct action of fast-contracting mandible closer muscles alone (GRONENBERG & al. 1997, PAUL & GRONENBERG 1999).

The convergence among trap-jaw ants extends beyond the morphological structures forming the latches, springs, and triggers. There is also convergence in the physiology of the trap-jaw mechanism, especially in the muscles and neurons controlling the reflex. In every group studied, these muscles and neurons show similar strategies for maximizing the speed of the mandible strike. The large mandible closer muscle that directly powers the trap-jaw is made up of tubular fibers with very long sarcomeres (5–11.4 µm), which characterize slowly contracting muscles. In contrast, the trigger muscle is composed of fibers with many short sarcomeres (1.8–3.0 µm) with large core diameters (2.4–8 µm), evidence of fast muscles (GRONENBERG &. al 1997). Likewise, the sensory neurons that receive stimuli from the trigger hairs and the motor neurons that innervate the trigger muscle have some of largest diameters among insects, (GRONENBERG & TAUTZ 1994, GRONENBERG 1996b , GRONENBERG & al. 1998), which reflect the incredibly fast speed of the trap-jaw reflex.

Despite what their name implies, trigger hairs are not solely responsible for eliciting mandible strikes. They clearly serve a sensory function; they are physically associated with giant sensory cells in the mandible or labrum (depending on lineage), and mechanical stimulation of the trigger hair results in electrophysiological signals in these sensilla (GRONENBERG & TAUTZ 1994, GRONENBERG 1995b, GRONENBERG 1996b, GRONENBERG 1998). However workers will often touch nestmates with their trigger hairs without eliciting a strike, and ablation of the hairs does not prevent *Odontomachus* workers from releasing strikes (CARLIN & GLADSTEIN 1989, personal observations). Indeed, the *Myrmoteras* subgenus *Myagroteras* is defined by the complete absence of trigger hairs, and they might use visual cues to release the strike (MOFFETT 1985). Given the correlation between trigger hair and mandible length (BOLTON 2000) and observations of workers waiting until prey touch the trigger hairs (DEJEAN and BASHINGWA

1985, DEJEAN 1986, GRONENBERG 1998), it is likely that the ants use trigger hairs to judge the distance of the target. A combination of factors, including tactile and chemical signals and even the "motivational state" of the ant together probably determines when a strike will be released.

Kinematics

The speed of trap-jaw ants has been noted by myrmecologists for decades, but it has only been recently that researchers have been able to accurately measure the mandible strike speed. Early investigations relied on phototransducers or high-speed videography (~ 400 frames per second (fps)) that could only estimate minimum strike duration (< 0.3ms–2.5ms) because the mandibles would often shut between frames (GRONENBERG 1995, GRONENBERG 1996b, GRONENBERG & al. 1998). With recent advances in videography, PATEK & al. (2006) were able to film mandible strikes of *O. bauri* at frame rates of 50,000 fps and showed that an entire mandible snap occurs within 0.13 ms (fastest 0.06 ms). These snaps had a mean linear velocity at the tip of the mandible of 38 $m \cdot s^{-1}$ (maximum 64.3 $m \cdot s^{-1}$) and an angular velocity ranging from 2.85×10^4 to 4.73×10^4 $rad \cdot s^{-1}$. These results rank the mandible strikes of trap-jaw ants as one of the fastest animal movements ever recorded, comparable to the velocity attained by the mandibles of snapping termites (*Termes panamaensis*), albeit through a different mechanism (SEID & al. 2008).

There is significant variation in mandible strike performance among species, which is not surprising considering their morphological and ecological diversity. A comparative study of eight species of *Odontomachus*, covering much of the range in body size displayed by the genus, found that average maximum strike speed ranged from 36 $m \cdot s^{-1}$ to 49 $m \cdot s^{-1}$and average maximum angular acceleration ranged from 1.3×10^9 radians/s^2 to 3.9×10^9 radians/s^2 (SPAGNA & al. 2008). Strike acceleration and the estimated resulting strike force scaled negatively and positively with body size, respectively, even when accounting for the effects of shared ancestry. The head geometry (head width, head length, and mandible length) of the included species scaled isometrically with body size, providing the basis for predictive model of strike force based on body size. Based on this model, large trap-jaw ants are predicted to have slow but more forceful mandible strikes compared with smaller ants (SPAGNA & al. 2008). Other morphological features, more directly related to mandible function like muscle volume, angle of muscle attachment, or spring characteristics, may more accurately predict strike performance. Considering the tremendous amount of morphological diversity within and between lineages, additional comparative studies could help generate a mathematical model relating head and mandible morphology to strike performance and contribute to understanding the patterns of trap-jaw morphological evolution.

Predation and Other Behavioral Consequences of Trap-Jaws

The relative speed of predators and prey often determines the outcome of their interactions. Consequently, many predators have specialized morphologies and behaviors that increase their speed during prey capture or handling, while many prey have evolved rapid escape mechanisms to evade predators (ALEXANDER 2003, PATEK & al. 2011). The unique morphology and record-breaking speed of trap-jaw ant mandibles clearly mark these ants as specialized predators (WHEELER 1900, CREIGHTON 1930), and numerous studies have confirmed that trap-jaws are fast enough to capture insects with rapid predator escape mechanisms or chemical defenses. However, trap-jaw mandibles can also be used in defense or escape during interactions with competitors or predators (CARLIN & GLADSTEIN 1989, PATEK & al. 2006). In this section I summarize what is known about the predatory behavior of trap-jaw ants and also discuss how their mandibles are used in defense.

Foraging and Predation

Some aspects of foraging behavior and predation sequence display similarities across all trap-jaw ant lineages and these may reflect further layers of convergence beyond just the morphology of the trap-jaw. With the exception of *Daceton armigerum* (HÖLLDOBLER & al. 1990, DEJEAN & al. 2012), workers are not known to recruit nestmates to food sources, but some species of *Odontomachus* display a simple recruitment behavior, increasing forager activity when food is successfully returned to the nest (EHMER & HÖLLDOBLER 1995, MOFFETT 1986b). With the high speed and force generated by their mandibles, foragers of all trap-jaw species are efficient, if solitary, predators. Foragers search for prey haphazardly on the forest floor, in leaf-litter, in rotting wood, or even in the canopy (WILSON 1953, WILSON 1962, EHMER & HÖLLDOBLER 1995, RAIMUNDO & al. 2009, CARMARGO & OLIVEIRA 2012, DEJEAN & al. 2012), usually with their mandibles in an open position, presumably in anticipation of striking prey. After detecting prey with their antennae, foragers approach with varying speed, depending on species, but all trap-jaw species appear to use their trigger hairs to position their prey in striking range of the apical teeth of their mandibles. After striking, often multiple times, foragers may also sting struggling prey before carrying it back to the nest (DE LA MORA & al. 2008, SPAGNA et al. 2009).

The role vision plays in the predation sequence varies among trap-jaw ant lineages. Many of the dacetines, for example, are cryptobiotic and have reduced or missing eyes, instead relying on olfactory and tactile cues to find prey (DEJEAN 1986, GRONENBERG 1996b). There is some evidence that larger species, however, have a great deal of visual acuity. Workers of

Odontomachus ruginodis use their eyes to detect prey from a distance, but rely on their antennae and trigger hairs to successfully aim strikes at nearby prey items (CARLIN & GLADSTEIN 1989). With their relatively large eyes, *Myrmoteras* workers likely use visual cues to detect, localize and catch prey, but their visual abilities have not been studied in detail (MOFFETT 1986b). Interestingly, the subgenus *Myrmoteras* (*Myagroteras*) lacks trigger hairs, and may use their eyes for detection, localization, and even for release of the strike. These ants were found to most commonly catch small non-springtail arthropods, which may indicate that relying solely on vision may limit the speed of prey that they can catch (MOFFETT 1986b).

There is considerable variation in prey type captured and degree of diet specialization displayed among trap-jaw ant genera. The mandibles of small trap-jaw ants (dacetines and formicines) are fast enough to capture springtails (Collembola), minute leaf-litter dwelling hexapods whose rapid predator escape jumps can occur in less than a millisecond (CHRISTIAN 1978). Field observations and cafeteria experiments have demonstrated that many species of *Strumigenys*, *Myrmoteras*, *Microdaceton*, and, possibly *Acanthognathus* feed mainly on entomobryid and isotomid springtails; however, these and other dacetine species will also accept other small-bodied litter arthropods (WILSON 1953, BROWN & WILSON 1959, BROWN & KEMPF 1969, MOFFETT 1986b, DIETZ & BRANDÃO 1993, BOLTON 1999, BOLTON 2000). The arboreal *Daceton armigerum*, which is much larger than other myrmicine trap-jaw ants, feeds on a variety of arthropods and will also tend honeydew-excreting insects (BROWN & WILSON 1959, WILSON 1962, DEJEAN & al. 2012). Foragers of the polymorphic myrmicine *Orectognathus versicolor* will also accept a wide variety of food items (CARLIN 1981).

The larger ponerine trap-jaw species are also active predators, however there are several differences in their predation sequence and prey preferences relative to smaller trap-jaw ants. In general, *Odontomachus* foragers do not approach prey as slowly as smaller species (CREIGTON 1937), in some species forgoing antennation of the prey prior to the strike (DEJEAN & BASHINGWA 1985, DE LA MORA & al. 2008). Foragers may strike prey items multiple times, using their strikes to break up large items into more manageable fragments (personal observation in *Odontomachus*). Across species, use of the sting may be related to the size of the worker relative to the prey item, with smaller individuals stinging more frequently than larger individuals (BROWN 1976 , DEJEAN & BASHINGWA 1985, SPAGNA & al. 2009). In quantitative studies of foraging preference, *Odontomachus chelifer* and *O. bauri* foragers were found to significantly prefer termites, including chemically defended species of *Nasutitermes* (FOWLER 1980, EHMER & HÖLLDOBLER 1995, RAIMUNDO & al. 2009). In the arboreal species *Odontomachus hastatus*, workers collected termites much less frequently, instead returning with dipterans, lepidopterans, and other ants (CARMAGO & OLIVEIRA 2012). However, foragers of *Odontomachus* accept a wide variety of food including other ants and insects (WHEELER 1900, BROWN 1976, FOWLER 1980, EHMER & HÖLLDOBLER 1995, DE LA MORA & al. 2008, RAIMUNDO & al. 2009), insect frass (personal observation, CERQUERA & TSCHINKEL 2010), plant material (PIZO & OLIVEIRA 2001, PASSOS & OLIVEIRA 2004), honey-dew from tending Hemipterans (EVANS & LESTON 1971), and even juvenile vertebrates (FACURE & GIARETTA 2009). Very little is known about *Anochetus* prey preferences, but at least one species, *Anochetus traegordhi*, is a specialist on *Nasutitermes* termites. This species is found nesting in the same rotten logs as termite colonies, and even retrieves termite worker prey in preference over soldier caste prey (SCHATZ & al. 1999). The colonies of several other *Anochetus* species are also found in termite nests (BROWN 1976, SHATTUCK 1999), but they will accept many different arthropods in the lab, including termites, fruit flies, and springtails (GRONENBERG & EHMER 1996, personal observation).

Trap-jaw ants are not unique among insects that specialize on fast or chemically defended prey. Workers of *Myrmica rubra*, for example, actively catch springtails without use of a trap-jaw, instead using a stereotypical jumping attack (REZNIKOVA & PANTELEEVA 2001). Likewise several species of beetles are springtail specialists. The carabid, *Notiophilus biguttatus*, is a visual hunter that relies on the accuracy of judging the distance and direction of prey to successfully capture springtails (BAUER 1981). The diverse genus *Stenus* (Staphylinidae: Coleoptera) are specialized collembolan predators that use an adhesive secretion on the distal end of their elongated labium to capture their prey. These beetles also employ a power amplification mechanism to rapidly (3–5 ms) extend their labium before a springtail can escape (BETZ & KÖLSCH 2004). No studies have been conducted on the relative capture efficiency or prey preference of these specialized predators compared with trap-jaw ants, and so it is unclear what their competitive interactions would be in areas where their distributions overlap.

Defensive Behaviors

Just as the sting and other predatory weapons can be used in both predation and defense, the mandible strike of trap-jaw ants can also be used for colony or individual defense. The major workers in the polymorphic *Orectognathus versicolor* (CARLIN 1981) as well as workers in the monomorphic *Odontomachus ruginodis* (CARLIN & GLADSTEIN 1989) and *Myrmoteras* spp. (MOFFETT 1986b) wait at nest entrances with open mandibles and act as “bouncers,” snapping their mandibles at would-be invaders and pushing them away. Additional observations have been made of trap-jaw ants attacking predators or potential competitors with their mandible strikes, often dismembering them without bringing them back to the nest as food (CREIGHTON 1937, MOFFETT 1986b, EHMER & HÖLLDOBLER 1995, SPAGNA & al. 2009).

One consequence of producing such large forces and snapping at prey, predators, and competitors is that, occasionally, individuals strike something much larger than themselves, resulting in the trap-jaw ant itself being launched into the air. This behavior was defined as "retrosalience" (backward jumping) by WHEELER (1900, 1922) who reviewed the natural history literature of a number of jumping *Odontomachus* species from the late 1800s and early 1900s. Later authors documented retrosalience in a number of other lineages including *Anochetus*, *Orectognathus, Strumigenys*, *Myrmoteras* and largely concluded that this behavior was an accidental by-product of striking a hard surface with high force (CREIGHTON 1930, CREIGHTON 1937, BROWN 1953, CARLIN & GLADSTEIN 1989). The reported distance travelled by the ants as a consequence of their mandible strikes can quite large ranging from 20–25 cm in a dacetine ant (WHEELER 1922) to over 40 cm in *Odontomachus bauri* (PATEK & al. 2006). The escape jumps powered by trap-jaw ant mandibles are comparable to the record-breaking jumps of froghoppers, fleas and other jumping arthropods that use modified legs (BURROWS 2006, SUTTON & BURROWS 2011).

Recent research suggests that, in some instances, jumping may be an intentional predator avoidance behavior (PATEK & al. 2006, SPAGNA & al. 2009). PATEK & al. (2006) distinguished two different jumping behaviors in *Odontomachus bauri* based on their trajectory: horizontal "bouncer" jumps (not to be confused with bouncer behavior *sensu* CARLIN 1981) resulting from striking a large object and vertical "escape" jumps, resulting from striking the substrate. Using four species of *Odontomachus*, SPAGNA & al. (2009) demonstrated that escape jumps rarely occurred during interaction with prey but were more likely when a focal ant was surrounded by heterospecifics. Predators that *Odontomachus* workers may use the escape jump against include, but are not limited to, a number of specialist or generalist predatory ants. For example, *Formica archboldi* is thought to be a specialist on *Odontomachus brunneus* (DEYRUP & COVER 2004), and the diurnal forager *Pachycondyla striata* occasionally takes as prey or even robs the prey of *Odontomachus chelifer* (RAIMUNDO & al. 2009). More research is still needed, however, to examine how often escape jumps are used in natural contexts and whether the behavior actually improves individual survival.

Trap-jaws As Key Morphological Innovation

The trap-jaw apparatus is a dramatic example of morphological innovation, where a structural novelty (latch and trigger muscle) has facilitated the evolution of a completely new function (power amplification), but it is still unclear why this morphology would evolve convergently so many times in a single insect family. It is possible that trap-jaws enable their owners to catch fast or dangerous prey that are largely inaccessible to other predators. If so, power-amplified mandibles may have facilitated access to a previously untapped dietary source and caused an increase in speciation and morphological evolution (HEARD & HAUSER 1995, HUNTER 1998a) and would fit the definition of a key morphological innovation: traits that allow organisms to interact with their environment in a new way.

Two recent studies provide some evidence that the lineages that contain the ponerine and myrmicine trap-jaw ants are each associated with significant increases in diversification rate (PIE & TSCHÁ 2009, MOREAU & BELL 2013), consistent with the hypothesis that the trap-jaw is a key innovation. Key innovations have been used to explain patterns of diversity in many animal groups (HUNTER 1995 PRICE & al. 2010 DUMONT & al. 2012), but establishing causality of proposed key innovations can be difficult (MASTERS & RAYNER 1998 HUNTER 1998b). In addition to demonstrating a shift in diversification rate, linking trap-jaws to patterns of species diversity will require showing that trap-jaw ants have entered new adaptive zones compared to closely related non-trap-jaw ant species and that trap-jaws quantitatively improve the ecological performance of lineages that have them. For example, *Odontomachus bauri* has been shown to be quantitatively better at disabling *Nasutitermes* soldiers than other ants by using a "strike and recoil" strategy (TRANIELLO 1981). However little is known about predation efficiency for the majority of trap-jaw ant species. More research is needed on the diet, ecology, and macroevolution of trap-jaw ants before any conclusions can be drawn about their importance in trap-jaw ant diversification.

Conclusions

With so much of their biology still unknown, trap-jaw ants should serve as excellent study organisms for future students of functional morphology, behavior, evolution, and development. In many cases, I still know very little about basic natural history and functional morphology, especially in the genus *Myrmoteras*. Accurate estimates of the kinematic capabilities (speed, acceleration, and force) for the vast majority of trap-jaw ants are still unavailable. Paired with mandible performance data, dietary preferences could provide insights into predator-prey arms races. Future efforts should also focus on identifying what structure act as a spring and stores the elastic strain energy that makes power amplified mandibles possible. Only with this information will I be able to derive a predictive model that relates morphology to strike performance.

Beyond stabilizing their classification, working out the phylogenetic relationships among trap-jaw ant genera and their closest non-trap-jaw relatives, especially in the subfamilies Ponerinae and Myrmicinae, will be critical for correctly understanding the evolution of this extreme condition. The tribe Dacetini, as currently defined, is ideal for a careful synthesis of systematics, morphology, and behavior to understand the transition from short, muscle driven mandibles to the power-amplified mandibles of true trap-jaw ants.

Finally, modern genomic and evolutionary development tools will enable research on the developmental patterning of trap-jaw mandibles and insights into the comparative morphology of ant mouthparts. Recent research has provided insight into the genetics and development of insect mouthparts (Angelini & Kaufman 2005) and established a foundation for studying the mechanisms responsible for producing morphologically specialized structures like trap-jaw mandibles. Combined with careful phylogenetic methods, future research will be able to reveal the homology of trap-jaw mandibles across each lineage and study the convergent evolution of morphological innovations at the levels of genetics and development.

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Table 1.1. Summary information on four independent origins of “trap-jaw” power amplified mandibles in ants. Each origin is listed under the subfamily heading. See text for more information.

Subfamily	**Genera**	**Number of Species**	**Distribution**	**Lock**	**Spring**	**Trigger Muscle**
Ponerinae	*Odontomachus* *Anochetus*	183	New & Old World Tropics	Mandible Joint	Adductor Apodeme?	Mandible Adductor
Formicinae	*Myrmoteras*	39	South East Asia	?	?	?
Myrmicinae	*Acanthognathus*	7	New World Tropics	Mandibular Processes	Adductor Apodeme?	Mandible Adductor
	Daceton	2	New World Tropics	Labrum	Adductor Apodeme?	Labral Adductor
	Orectognathus *Epopostruma* *Microdaceton*	51	Old World Tropics	?	?	?
Myrmicinae	*Strumigenys*	834	Temperate & Tropics Worldwide	Labrum	Adductor Apodeme?	Labral Adductor

Figure 1.1. Representative trap-jaw ant species. (A) Two species illustrating the extremes of size variation among different lineages: *Odontomachus chelifer*, in the subfamily Ponerinae, is one of the largest trap-jaw ant species, whereas *Strumigenys* sp., in the subfamily Myrmicinae, is one of the smallest. (B) *Anochetus fauri*. (C) *Odontomachus latidens*. (D) *Myrmotera iriodum*. (E) *Strumigenys rogeri*. (F) *Microdaceton* sp. (G) *Acanthognathus ocellatus*. Images (B-G) © Alex Wild, used by permission.

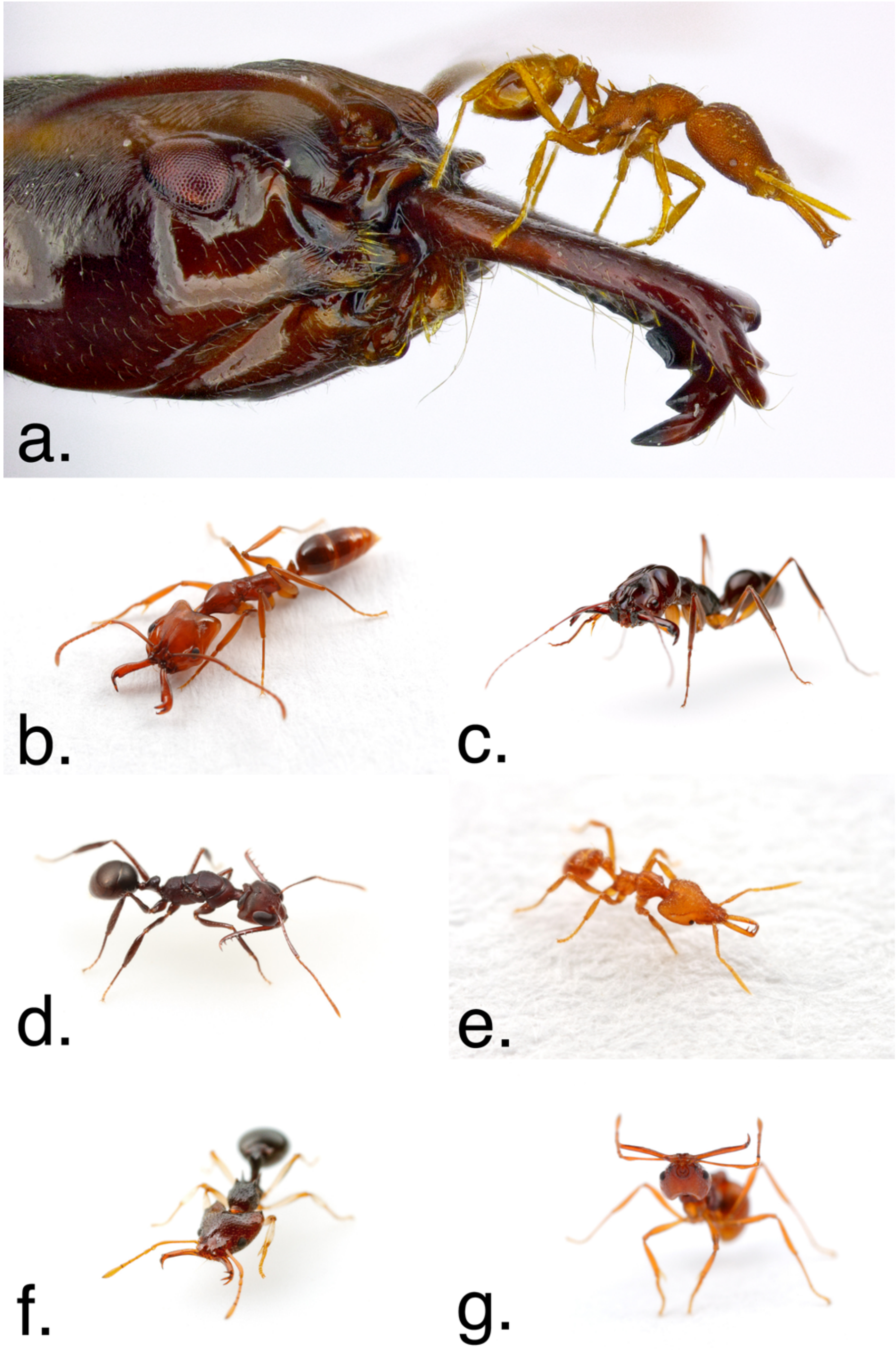
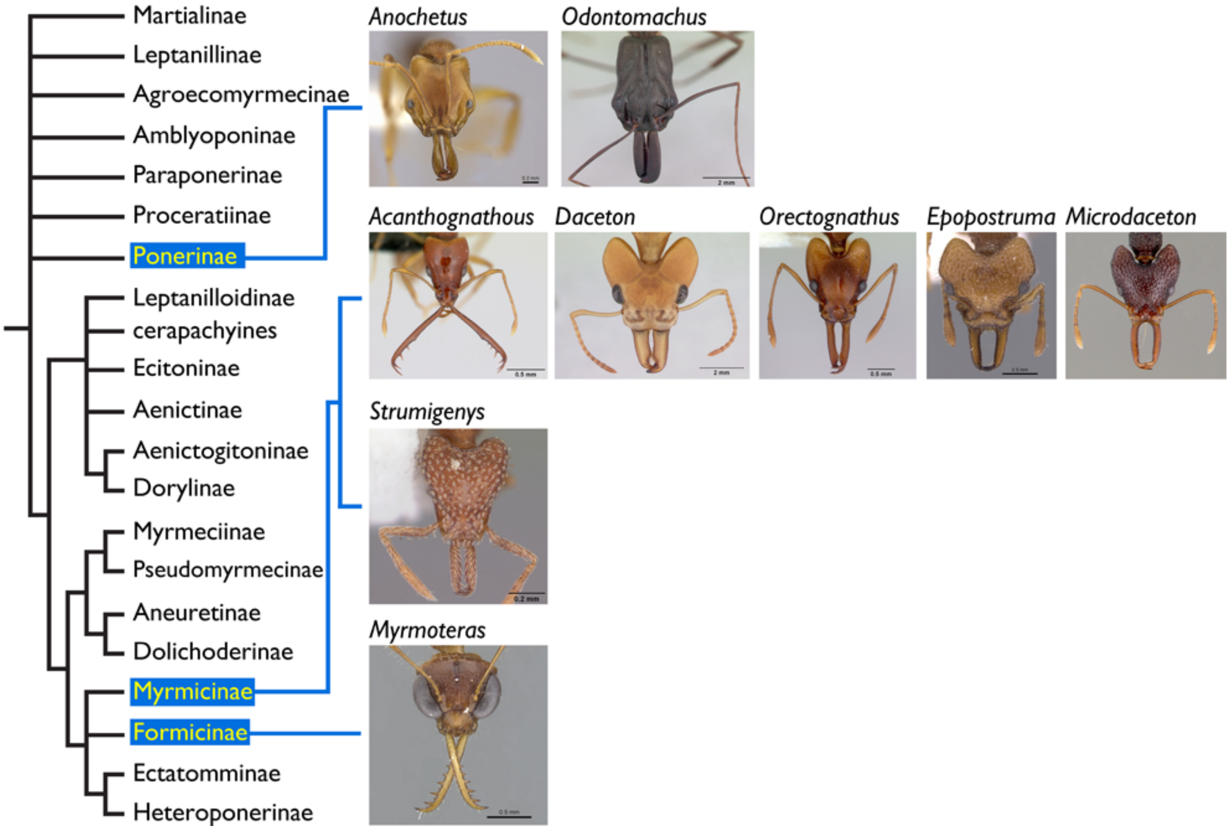


Figure 1.2. Independent origins of trap-jaw ants. A phylogeny showing the well supported relationships of the 21 extant ant subfamilies based on Moreau & al. (2006), Brady & al. (2006), and Moreau & Bell (2013). Ant genera with trap-jaw morphologies have evolved at least four times, once in each of the subfamilies Ponerinae and Formicinae, and twice in the subfamily Myrmicinae. Cladogram modified from Ward (2010). Ant images are courtesy of AntWeb at www.antweb.org.



CHAPTER 2: MOLECULAR PHYLOGENETICS OF TRAP-JAW ANTS IN THE GENERA *ANOCHETUS* AND *ODONTOMACHUS* (HYMENOPTERA: FORMICIDAE)

Abstract

The subtribe Odontomachiti (*Anochetus* and *Odontomachus*) is one of the largest groups in the subfamily Ponerinae, and one of four lineages of ants possessing spring-loaded "trap-jaws." Here I present results from the first global species-level molecular phylogenetic study of these trap-jaw ant genera, reconstructed from one mitochondrial and four nuclear protein-coding genes. Bayesian and likelihood analyses strongly support reciprocal monophyly for the genera *Anochetus* and *Odontomachus*. Additionally, I found strong support for seven deeply divergent clades (four in *Anochetus* and three in *Odontomachus*) mostly concordant with biogeographic patterns. Bayes Factor hypothesis testing suggests the genus *Pseudoneoponera* is the closest non-trap-jaw relative of *Anochetus* and *Odontomachus.* Ponerine trap-jaw ants evolved in the late Eocene (28–48 Mya) likely in Southeast Asia, and subsequently dispersed multiple times to Africa, Australia, and Central and South America where they have radiated rapidly in the last 17 million years. These results will guide future taxonomic work on the group and act as a phylogenetic framework to study the macroevolution of extreme ant mouthpart specialization.

Introduction

Ants are one of the most successful insect groups in terrestrial ecosystems, and have become important models for the study of competition, social evolution, community ecology, mutualisms, and invasion biology (HÖLLDOBLER & WILSON 1990; LACH ET AL. 2010). Because of their high species number and morphological diversity, ants are also an excellent system in which to answer questions about morphological macroevolution. The utility of ants as models in evolutionary biology, however, is dependent on having a stable classification system and an accurate understanding of their evolutionary relationships. In recent years, molecular phylogenetics has been used to clarify the evolutionary relationships of ants at all taxonomic ranks, and has led to major revisions of ant classification (BRADY ET AL. 2006; MOREAU ET AL. 2006; WILD 2009; WARD ET AL. 2010; BRANSTETTER 2012; BLAIMER 2012; MOREAU ET. AL. 2013; SCHMIDT 2013; WARD ET. AL 2014). The aim of the present study is to use molecular phylogenetics to better understand the species-level relationship in one morphologically specialized ant group, trap-jaw ants in the genera *Anochetus* and *Odontomachus*.

Anochetus and *Odontomachus* comprise a monophyletic lineage in the subfamily Ponerinae and represent the second most diverse lineage of trap-jaw ants (114 and 66 species, respectively; BOLTON 2013). They display a suite of traits that are common among other ponerines, including small colony size, monomorphic workers, little differentiation between the workers and queen, and solitary foraging (BROWN 1976; BROWN 1977; BROWN 1978). Within and between genera, nesting preferences vary widely, including leaf litter, rotten logs, open ground and even arboreal nests (CAMARGO & OLIVEIRA 2012; SHATTUCK & SLIPINSKA 2012; HART & TSCHINKEL 2011; RAIMUNDO ET AL. 2009). Most are active predators, feeding on arthropods of various sizes, but some species also tend aphids and collect seeds (EVANS & LESTON 1971). Like all species of trap-jaw ants, *Anochetus* and *Odontomachus* species use their rapid mandible strikes during predatory interactions with prey of all kinds, including fast and chemically defended insects (DEJEAN ET AL. 2002; EHMER & HÖLLDOBLER 1995). In some cases, the ants also use the strikes for nest defense by “bouncing” intruders away from nest entrances

(CARLIN & GLADSTEIN 1989), and for individual escape by striking the substrate and launching themselves away from potential predators (SPAGNA ET AL. 2009; LARABEE AND SUAREZ 2015).

A well-supported phylogeny is a prerequisite for correctly interpreting trait evolution, and developing a comprehensive species-level phylogeny of *Anochetus* and *Odontomachus* will address two outstanding issues about the group. First, to understand how trap-jaw morphology evolved from the ancestral mandibulate condition, it is crucial to identify the closest living non-trap-jaw ant genus. Second, determining whether *Anochetus* and *Odontomachus* are reciprocally monophyletic will have a substantial impact on the classification of the group and also influence the interpretation of macroevolutionary patterns of morphology, performance, and diversification.

To date, published and preliminary phylogenetic analyses of *Anochetus* and *Odontomachus* have been hampered by small and unequal taxon sampling (SPAGNA ET AL. 2008; SCHMIDT 2009), leaving the evolutionary relationships between and within each genus unclear. A preliminary unpublished molecular phylogeny of the two genera was unable to distinguish between a scenario where both genera are reciprocally monophyletic, and a scenario where *Odontomachus* is paraphyletic with respect to *Anochetus* (SCHMIDT 2009). Contrasting with those results, morphological and karyotype data have suggested that *Anochetus* is paraphyletic with respect to *Odontomachus* (BROWN 1976; SANTOS ET AL. 2010). Additionally, several recent multi-gene phylogenetic analyses focusing on ant genus relationships each recovered four different sister groups to the clade comprising *Odontomachus* and *Anochetus* (BRADY ET AL. 2006; MOREAU ET AL. 2006; SPAGNA ET AL. 2008; KELLER 2011; MOREAU 2013). Therefore, reconstructing the phylogenetic relationships of trap-jaw ants is a necessary first step in exploring the origin and subsequent macroevolution of the trap-jaw apparatus.

The goals of the present study are to use molecular phylogenetics to reveal the evolutionary history of spring-loaded mandibles in the subtribe Odontomachiti. Specifically, I answer whether *Anochetus* and *Odontomachus* are sister monophyletic genera, and clarify the species level relationships within each genus. Also, to better understand how the specialized trap jaws evolved from unspecialized mandibles, I identify the closest living non-trap-jaw ant relative in the Odontomachus Genus Group. Finally, using fossil calibrations, I estimate the timing and biogeography of trap-jaw ant diversification.

Materials and Methods

Taxon Sampling

Species were selected to cover a wide taxonomic and morphological breadth of ponerine trap-jaw ants, and were obtained from my own fieldwork, myrmecology collaborators, and museum collections. I assembled a set of 51 species of *Anochetus* and 46 of *Odontomachus* from across their worldwide distribution, representing approximately one-third of the named species of the group (Table 1) (BROWN 1976, 1978). Ants were identified primarily using the keys of BROWN (1976; 1978) and with help from a reference collection (Smithsonian Institution National Museum of Natural History).

Taxa for outgroup comparison were selected based on a recent revision of the subfamily Ponerinae, with special attention to species within the Odontomachus Genus Group (SCHMIDT 2013; SCHMIDT & SHATTUCK 2014). This large clade includes 20 genera and displays a wide range of mandible morphology. The genus *Leptogenys*, for example, has long linear mandibles that could reflect the ancestral condition of trap-jaw ants. Other genera, such as *Phrynoponera*, *Odontoponera*, or *Pseudoneoponera*, have more generalized triangular mandibles of varying lengths. In total, 40 non-trap-jaw ant species were included in the analyses as outgroups, with most sequences coming from previous studies (SCHMIDT 2013). Vouchers of all samples have been deposited at the Smithsonian Institution National Museum of Natural History or in the collection of Andrew Suarez at the University of Illinois, Urbana-Champaign.

Gene Sampling and Molecular Techniques

Sequence data were generated from five gene fragments using a strategy similar to that found in SCHMIDT (2013): the mitochondrial gene *cytochrome oxidase I* (COI), three nuclear protein-coding genes *wingless* (Wg), *long-wavelength rhodopsin* (LWR, including introns), and *rudimentary* (CAD), and the nuclear large subunit ribosomal RNA gene (28S). Genes were selected to include both rapid (COI, introns) and slowly (28S) evolving sequences (SIMON ET AL 1994) and based on their usefulness in resolving relationships at deep and shallow taxonomic levels in previous ant phylogenetic studies, including the subfamily Ponerinae (BRADY ET AL. 2006; MOREAU ET AL. 2006; WILD 2009; WARD ET AL. 2010; BRANSTETTER 2012; BLAIMER 2012; MOREAU ET. AL. 2013; SCHMIDT 2013; WARD ET. AL 2014). The aligned and concatenated data matrix was 4812 bp in length and 80% complete for the ingroup taxa. Sequence characteristics for each gene are listed in Table 2.2.

Genomic DNA was extracted from one leg of an adult female from each collection event using a DNEasy Blood and Tissue Kit (Qiagen Inc., Velencia, California) according to the manufacture's protocol, and diluted in 150 ml nuclease-free water. Sequence fragments were amplified using polymerase chain reaction (PCR) using the primers listed in Table 2.3. PCR was performed in reaction volumes of 50 ml and contained 1 ml genomic DNA template, 100 nM primer (Integrated DNA Technologies, Coralville, IA), and 1X GoTaq DNA Polymerase Master Mix (Promega, Madison, WI). PCR conditions for all genes started with an initial melting step at

94° C (5 min.), followed by 10 cycles of 94° (30 sec.), 60° C (30 sec., decreasing by 1°C per cycle), and 72° C (30 sec.), followed by 30 cycles with the same conditions but an annealing temperature of 50° C, and a final extension of 72° C (5 min.). PCR products were cleaned with ExoSAP-IT (USB Corporation, Cleveland, OH), and amplicon size was verified using gel electrophoresis and GelRed DNA Stain (Biotium, Hayward, CA). Cycle-sequencing reactions were performed using PCR primers and BigDye Terminator ver 3.1, and were analyzed on an ABI 3730 Sequencer (Life Technologies, Grand Island, NY) at the Laboratories of Analytical Biology at NMNH.

Sequences were assembled and edited in GENEIOUS v7.1 (Biomatters, Auckland, New Zealand) and aligned in MUSCLE v3.8 (EDGAR, 2004). Ambiguously aligned regions of 28S, and introns from CAD and LWR for outgroup taxa were removed from the data analysis. The sequences generated for this study will be deposited in GenBank, and the aligned data matrix will be deposited in TreeBase.

Phylogenetic Analysis

Three methods were used to infer the molecular phylogeny of trap-jaw ants: partitioned Bayesian analyses (BI) in MrBayes 3.2.2 (RONQUIST ET AL. 2012), partitioned relaxed clock Bayesian analysis in BEAST 2.2.1 (BOUCKAERT ET AL. 2014), and partitioned maximum likelihood (ML) in RAxML (STAMATAKIS 2006). Analyses were run either on the CIPRES Science Gateway (Miller et al 2010), or on the Smithsonian NMNH Topaz computing cluster.

The partitioning scheme and model of nucleotide substitution for the DNA alignment were simultaneously selected using PartitionFinder 1.1.1 (LANFEAR ET AL 2012). The concatenated alignment of 5 loci was first divided into 10 subsets by gene, expression pattern, (exon, intron), and codon position. Bayesian Information Criterion was then used to select the optimal scheme of three partitions, each with an individual model of nucleotide substitution (Table 2.4).

MrBayes analyses were run with default priors, with the exception of the branch length prior, which was set to brlenspr = unconstrained:Exp(100). Base frequencies, substitution rates, and gamma shape parameters were unlinked across partitions, but topology, branch lengths, and branch length multipliers were linked. Each analysis consisted of two simultaneous Markov Chain Monte Carol (MCMC) runs with four chains per run for 10-40 million generations. The first 25% of sampled trees were discarded as burn-in. Several methods were used to confirm that the runs had converged: (1) MCMC analyses were run until the average split frequencies of standard deviation was below 0.01, (2) the potential scale reduction factor of all parameters were close to 1.00, (3) confirmed that the effective sample sizes of all parameters were above 200, and (4) the likelihood and sample parameters were checked with Tracer 1.6 to confirm they had reached stationarity (RAMBAUT ET AL. 2014). All analyses were also run under the prior without sequence data to check for overly influential priors on the posterior probability distribution. Bayesian analyses were also run without substitution models selected *a priori* using reversible jump MCMC (rjMCMC) in MrBayes. This approach samples the posterior probability of all models in the GTR substitution family (HUELSENBECK ET AL 2004). Trees were summarized as majority rule consensus trees in R 3.1.3 using the 'ape' and 'phytools' packages (PARADIS ET AL 2004; REVELL 2012; R CORE TEAM 2015).

Maximum likelihood analyses were conducted using the rapid bootstrapping algorithm (1000 replicates) combined with a ML tree search RAxML (STAMATAKIS 2014). The same partition scheme from the BI analysis was used for the ML analysis, each with a GTR + Γ model of nucleotide substitution.

Constraint Analyses

To test topological hypotheses on the monophyly of trap-jaw ants and for different trap-jaw ant sister group relationships, I used Bayes Factors (BF) to test the relative support for different topological models (BERGSTEN 2013). In MrBayes, groups under question were constrained to be monophyletic with topology priors (Table 2.5), and then the marginal likelihood of each model was estimated using the stone-stepping sampling method (XIE ET AL 2011). Two runs with four chains each were sampled for 20 million generations, with 2 million generations discarded as burn-in. The ln-BF statistics were calculated as:

$$\text{ln-BF}\ (M_0, M_1) = \ln[P(X \mid M_0)] - \ln[P(X \mid M_1)]$$

where $\ln[P(X \mid M)]$ is the marginal log-likelihood estimate for the model M. The strength of support for a given model was based on the interpretation of BF suggested by KASS & RAFTERY (1995). ln-BF that were 0–0.5 were interpreted as weak or no evidence for the alternative model, M_1, over the null model, M_0. When ln-BF were above 0.5, the alternative model M_1 was supported over the null model M_0, with the strength of support proportional to the ln-BF.

Divergence Estimation

Trap-jaw ant diversification dates were estimated by generating fossil calibrated trees in BEAST 2.2.1 (BOUCKAERT ET AL. 2012). I used an uncorrelated lognormal relaxed clock model with a birth-death process tree prior. Data partitions and models of nucleotide substitution were the same as those used in the MrBayes analyses. The ages of four nodes were calibrated using exponential priors based on fossil ages listed in Table 6. The age for the root node (the subfamily Ponerinae) was calibrated with a prior of 92 million years with a normal distribution based on previous dated trees (SCHMIDT 2013). Two independent MCMC analyses were run each with a length of 200 million generations with parameters sampled every 5000 generations. Convergence was assessed by monitoring parameter estimated sample sizes and parameter posterior probabilities in Tracer 1.6. The first 50 million generations were discarded as burn-in, and the maximum clade credibility tree was summarized in TreeAnnotator 2.2.1 (DRUMMOND ET AL. 2012).

Ancestral Range Reconstruction

The ancestral biogeography of trap-jaw ants was estimated using the likelihood-based program Lagrange v20130526, which implements a dispersal, extinction, and cladogenesis model (REE AND SMITH 2008; REE ET AL 2005). As input, I used the maximum clade credibility tree from the BEAST analysis with all outgroups species removed. Each species of *Anochetus* and *Odontomachus* was assigned to one or more of five previously defined biogeographic regions (COX 2001): Neotropics, Nearctic, Afrotropics, Indomalaya (including southeast Asia and the Pacific Islands west of Wallace's Line), and Australiasia (including New Zealand and Papua New Guinea). The Paleoarctic biogeographic region was not considered in this analysis because only a few species of *Anochetus* are known from this region, none of which were sampled in my dataset. Historic migration between biogeographic regions was weighted with an adjacency matrix, which modeled the instantaneous transition between geographic ranges (Table 2.7). Dispersal between neighboring regions was weighted more favorably (1.0) than non-neighboring regions (0.5) or those separated by oceans (0).

Results and Discussion

Phylogenetics

All analyses resulted in trees that supported essentially the same phylogenetic relationships, especially within the trap-jaw ant genera *Anochetus* and *Odontomachus* (Figure

2.1-2.4). The broad scale topological features were retained across different phylogenetic inference methods, partitioning schemes, and gene sampling. The rjMCMC analysis converged on a similar number of nucleotide substitution parameters for each partition as was found by PartitionFinder (Table 2.4). In the following discussion (unless otherwise noted), support values refer to the Bayesian Posterior Probabilities (BPP) of the MrBayes analysis with an *a priori* specified substitution model and the Bootstrap Support (BS) values of the RAxML Maximum Likelihood analysis of the full, concatenated dataset.

Individual Bayesian analyses of the genes LWR, Wg, and CAD yielded results similar to the concatenated analyses, but with much lower support values (Figure 2.5). Trees based on individual analyses of COI and 28S, however, had very different topologies from the concatenated alignment. Consistent with its slower mutation rate, 28S trees consisted mostly of polytomies with very low support values, especially within trap-jaw ants (Figure 2.5a). The faster evolving COI, on the other hand, resulted in trees with poor resolution among outgroup genera, but relatively similar species relationships within trap-jaw ant genera (Figure 2.5a). In the analysis of COI, *Odontomachus* was found to be paraphyletic with respect to *Anochetus*, but this node had weak support (BPP = 0.59). Analyses of the concatenated dataset that omitted either 28S or COI had much weaker resolution and support values (data not shown).

Monophyly and Placement of Trap-jaw Ants

I found modest support for the Odontomachus Genus Group (BPP= 0.93, BS = 85), consistent with other previous molecular analyses (MOREAU 2013; SCHMIDT 2013). My analysis lacked several genes found in other studies that had higher support for that clade (SCHMIDT 2013), indicating that the weaker support in my analysis was due to gene sampling. All genera sampled with multiple species were strongly inferred to be monophyletic. Like previous studies, relationships within the Odontomachus Genus Group were poorly supported at the genus level and consisted mostly of polytomies (Figure 2.2).

Trap-jaw ants (*Odontomachus* and *Anochetus*) form a monophyletic clade within the Odontomachus Genus Group with very strong support (BPP=1.0, BS=100). This clade is also strongly supported by previous morphological and most molecular studies (BRADY ET AL 2006; MOREAU ET AL 2006; SPAGNA ET AL 2009; KELLER 2011; SCHMIDT 2013; MOREAU 2013). *Odontomachus* and *Anochetus* were also strongly supported to be reciprocally monophyletic sister genera in all analyses (*Odontomachus*: BPP=1.0, BS=96; *Anochetus*: BPP=1.0, BS=97) except some individual gene phylogenies.

Hypothesis testing with Bayes Factors also strongly supported reciprocal monophyly of *Anochetus* and *Odontomachus* (Table 2.5). Constrained models that force monophyletic *Anochetus* and *Odontomachus* genera to be nested in a monophyletic *Anochetus* + *Odontomachus* clade had an estimated marginal ln-likelihood of -69019.77. Models that prevented *Anochetus* and *Odontomachus* monophyly had estimated marginal ln-liklihoods of -69149.97 and -69119.97, respectively. The resulting Bayes Factors were 129.79 (*Anochetus*) and 100.2 (*Odontomachus*), strongly supporting the models constraining trap-jaw ant genera monophyly. Additionally, when alternative placement of the Old World clade of *Odontomachus* was tested, Bayes Factors always supported monophyly of *Odontomachus*.

My analysis failed to resolve the closest extant non-trap-jaw ant relative, and the inferred sister group relationships were dependent on gene sampling. In the full concatenated analysis, *Pseudoneoponera* was sister to trap-jaw ants, but with modest support from the MrBayes analysis (BPP=0.88) and very weak support from maximum likelihood (BS=22). Omitting COI from the analysis found *Phrynoponera* sister to trap-jaw ants (BPP=0.70). This relationship was also recovered in the recent molecular phylogeny of Ponerinae, which also did not sample COI in its data matrix (SCHMIDT 2013). When these two topological hypotheses were constrained during independent stepping-stone MCMC analyses, the estimated marginal log-likelihood of the model constraining (*Odontomachus* + *Anochetus*) + *Pseudoneoponera* was -6,9033.30 and for the model constraining (*Odontomachus* + *Anochetus*) + *Phrynoponera* was -6,9048.35. The ln-Bayes Factor (BF) test statistic comparing these two models was 15.05, strongly favoring *Pseudoneoponera* as sister to the trap-jaw ants. When these two models were compared with the model constraining the long-mandible genus *Leptogenys* as sister to trap-jaw ants, the ln-BF still strongly supported *Pseudoneoponera* as sister to trap-jaw ants (Table 2.5). Additional taxa in the Odontomachus Genus Group and possibly additional molecular markers will be required to resolve these relationships with high confidence.

Genus Odontomachus

Odontomachus is a monophyletic genus with three strongly supported clades, generally corresponding to their biogeography: (1) Clade A (BPP=1.0, BS= 100) consisting of species in the Afrotropics and Southeast Asia, (2) Clade B (BPP=0.981, BS= 61) consisting of species almost entirely found in Central and South America, and (3) Clade C (AC) (BPP=1.0, BS=65), with species located in Australia and Melanesia. Analyses of all concatenated datasets found Clade A to be basal to the sister clades Clade B and AC. Many of the species groups defined by BROWN (1976) were recovered as monophyletic within these clades, in particular the *tyrannicus*, *saevissimus*, *ruficeps*, and *haematodus* groups (Figure 2.1).

Clade A was recovered as seven species found in Africa, Madagascar, and continental Asia and display significant morphological variation. The Malagasy species *O. coquereli*, for example, is distinctive with its well-developed subapical teeth, conical head lacking any temporal prominences, and long petiole. Other species, such as *O. assiniensis* or *O. rixosus*, are more typical of the genus, with finely serrate teeth along the mandibular border, well developed temporal prominences, and more node-like petioles. Despite Clade A having very strong support in my phylogenetic analyses, the relationships among species in clade were not well resolved. The *rixosus* species group, for example, which Brown defined as *O. rixosus*, *O. monticola*, and *O. latidens* and suggested might be geographical variants of the same species, was found to be polyphyletic (Figure 2.1), although with low support.

Clade B corresponds to the large *haematodus* species group complex (Brown 1976). These species are joined morphologically by well-developed temporal prominences, relatively short mandibles with blunt apical teeth, and node-like petiole. The large species *O. chelifer* was recovered as sister to the rest of Clade B (BPP=0.98, BS=66), agreeing with Brown's morphological hypothesis. Interestingly, the Afrotropical species *O. troglodytes* and the Polynesian species *O. simillimus* were consistently recovered as members of the *haematodus,* supporting hypotheses that these may be tramp species moved around by human activity (BROWN 1976; FISHER 2008). Many of the relationships among *haematodus* group are poorly resolved from my analysis, possibly suggesting a rapid radiation or inadequate mutation rates in my chosen markers.

The species of Clade C have distributions in Australia and Melanesia, and one representative in Central/South America. The widespread Neotropical species, *O. hastatus*, was recovered as sister to the rest of the clade (BPP=0.98, BS=65). This close relationship is consistent with the morphological similarity between *O. hastatus* and members of the *saevissimus* and *tyrannicus* species group. The rest of the group has strong support (BPP=1.0, BS=100), and consists of several well-supported clades, including the *ruficeps* (BPP=1.0, BS=99), *saevissimus* (BPP=1.0, BS=99), and *tyrannicus* species groups (BPP=1.0, BS=100). *Odontomachus animosus* and *O. papuanus*, which were placed in the *infandus* group by Brown (1976) based on morphology, were found to be sister to the *saevissimus* group but with only modest support (BPP=0.74, BS=62).

In his revision of the genus, BROWN (1976) considered ancestral mandibular traits of *Odontomachus* to consist of long mandibles relative to head length and long sharp apical teeth. These traits were also associated with the lack of well-defined temporal prominences. Species fitting this description include *O. coquereli*, *O. hastatus*, and members of the *O. tyrannicus* species group, found in both the Clade A and Clade C. This may be evidence of parallel evolution in the two clades of more derived mouthpart and head traits.

Genus Anochetus

The genus *Anochetus* was also found to be monophyletic, and contained four well-supported deep divergent clades: (1) Clade D (BPP=1.0, BS=100), (2) Clade E (BPP=1.0, BS=91), a Neotropical Clade F (BPP=1.0, BS=100), and Clade G (BPP=1.0, BS=100). Similar to *Odontomachus*, many of the *Anochetus* species groups that had been inferred by morphology were recovered as monophyletic. Additionally, most species-level relationships were recovered with very high support.

All analyses of the full, concatenated dataset recovered Clade D as sister to the rest of the *Anochetus*. This group contains just two species, *A. altisquamis* and *A. orchidocola* (the *altisquamis* species group), and range from southern Mexico to northern Argentina. These species are typical of the genus as a whole, being relatively small robust ants that nest in leaf litter. BROWN (1976) hypothesized that the most basal lineage of *Anochetus* were large epigaeic ants in the *gladiator* group, which he thought gave rise to the genus *Odontomachus.* My taxonomic sampling did not include any species from the *gladiator* group, but it is likely that large bodied *Anochetus* evolved multiple times independently in the genus. Among the remaining *Anochetus* clades, there was strong support for Clade G being sister to Clade E + Clade F.

Species found in Clade E are medium sized ants that are found mostly in tropical forest habitats throughout the islands of Southeast Asia. It is comprised of member of the *risii, rugosus*, and *cato* species groups. Although the *rugosus* group was recovered as monophyletic, the sister relationship between *A.* cato and *A. peracer*, renders the *cato* group paraphyletic. At the root of this clade is the undescribed species *Anochetus* sp. 2921, which is notable for its large medial tooth on the inner margin of its mandible. The trait is uncommon in the genus and is not found in the rest of Clade E.

Clade F is a neotropical group consisting of the *emarginatus*, *mayri*, *inermis*, and *bispinosus* species groups. The clade displays a great deal morphological and ecological variation, with large arboreal species such as *A. horridus* and *A. emarginatus*, and also tiny, hypogaeic species such as *A. minans, or A. mayri*. I found the *inermis* species group to be paraphyletic with the sister relationship between *A. targionii* and the *mayri* species group, but with modest statistic support (BPP= 0.94, BS=65). The placement of *A. neglectus* and *A. minans* renders *A. mayri*, paraphyletic. However, *A. mayri* is incredibly variable, and likely is an assemblage of many cryptic species (BROWN 1978).

Like Clade F, Clade G is incredibly variable in terms of morphology, however it is spread across the Afrotropics, Australasia, and throughout Southeast Asia. The Australian *rectangularis* and African *africanus* species groups were the only of BROWN'S morphological groups recovered as monophyletic. My analysis included a number of undescribed species, emphasizing the need for more collecting and revision work on the clade.

Divergence Estimation and Biogeography

Analysis of the full, concatenated alignment in BEAST recovered the same phylogenetic relationships as the analysis in MrBayes (Figure 2.6). The age the Odontomachus Genus Group was estimated to be approximately 60 million years ago (Mya) (95% highest posterior density (HPD) 72-54 Mya). The most recent common ancestor of *Anochetus* and *Odontomachus* originated in the late Eocene (35 Mya, 95% HPD 28–48 Mya), and the crown group ages were estimated to be 31 My *Anochetus* and 28 My for *Odontomachus*. The subgeneric clades are all much younger, having diversified between 16 and 20 Mya. The very short branch lengths of the *haematodus* species group of *Odontomachus*, in particular, suggests that it may have undergone a rapid radiation within the last 8 My. The lineage through time plot (LTT) analysis is shown in Figure 2.7. Diversification in the trap-jaw ant clade fit a null pure-birth model of diversification with a constant birth rate of 0.09, but there is evidence of a shift in diversification rate approximately 17 Mya, coinciding with the radiation of most of the major trap-jaw ant clades (Figure 2.6). However, LTT plots should be considered a preliminary examination of patterns of trap-jaw ant diversification because of incomplete sampling of extant taxa (PYBUS & HARVEY 2000).

The ages in these clades are slightly older than those estimated in other studies on ant diversification. SCHMIDT (2013) estimated the age of crown group *Odontomachus* Genus Group to be approximately 42–54 Myo, and the trap-jaw ant clade to be 28–34 Myo. MOREAU ET AL. (2013) estimated an even younger age for the Odontomachus Genus Group (approximatey 34

Myo). Although those estimates are within the 95% HPD of my divergence analysis, the discrepancy may be explained by differences in fossil sampling. My analysis included more fossil constraints within the Odontomachus Genus Group than previous studies, specifically for the genus *Leptogenys* and additional fossils for *Anochetus* and *Odontomachus*. Increased fossil sampling has been shown to increase age estimates when using fossils to constrain the minimum age of nodes (NEAR ET AL. 2005; BENTON AND DONOGHUE 2007; RUTCHMANN ET AL. 2007). More accurate estimates of node ages may be estimated using a fossilized birth-death model (HEATH ET AL. 2014), which uses extinct species as part of the phylogenetic estimation and are more robust to differences in fossil sampling.

Results from the ancestral range reconstruction analysis LAGRANGE are shown in Figure 2.8 and Table 2.8. The majority of extant trap-jaw ant clades originated in regions that reflect their current distributions, mostly in the Neotropics or Indomalaya. The descendant branches of crown *Anochetus* are reconstructed as having inherited a Neotropical and Indomalayan distribution. Multiple lineages subsequently migrated to the Afrotropics and Australasia. Clade G, in particular, likely colonized Australia, Africa and Madagascar multiple times. The ancestral range at the root of *Odontomachus* was ambiguous (no scenario had more than 0.6 relative probability), but likely originated in Southeast Asia. There was at least one migration to Central and South America, where Clade B has rapidly radiated 16 Mya. *Odontomachus hastatus* appears to be a relict of an ancient migration to South America prior to 18 Mya. The most recent common ancestor of all ponerine trap-jaw ants was also ambiguous, but likely included Southeast Asia as part of its range, which has been shown to be important in the evolution of other members of the Odontomachus Genus Group (SCHMIDT 2013).

Conclusions

My molecular phylogenetic analyses confirmed that *Anochetus* and *Odontomachus* are monophyletic sister groups that evolved in the late Eocene in predominantly in Southeast Asia. *Anochetus* and *Odontomachus* are composed of four and three, respectively, well-supported clades that colonized the rest of the worldwide tropics multiple times. There is evidence that both genera have radiated rapidly in the past 15-20 My, especially in the Neotropics and Australasia. Of the 21 species groups that were previously defined morphologically (BROWN 1976; 1978), I recovered only 10 as monophyletic clades in my phylogenetic analyses. This emphasizes the need for additional phylogenetic analyses and taxonomic revisions for this and other ponerine genera. Although there is still some ambiguity about the identity of the closest living non-trap-jaw ant relative, it is most likely not a genus with long linear mandible like trap-jaws or those of *Leptogenys*. This leaves uncertainty about the sequence of morphological changes that led to the evolution of trap-jaws.

This study is the first species-level molecular phylogenetic analysis of the trap-jaw ant genera *Anochetus* and *Odontomacus*. Genera in the subfamily Ponerinae have received much less attention from molecular systematists than the other “big four” ant subfamilies (Myrmicinae, Formicinae, and Dolichoderinae). Because of their combination of ancestral and derived traits, Ponerinae ant genera, such as *Anochetus* and *Odonotomachus*, will be excellent systems to study the evolution of eusociality and to understand how specialized traits have contributed to the evolutionary success of ants. Trap-jaw ants, in particular, will be a useful system to study patterns of morphological macroevolution because of their highly specialized spring-loaded mandibles. Future studies should be able to use my phylogenetic hypothesis as a framework for answering questions about trap-jaw ant evolution in a phylogenetic context.

Table 2.1. Taxa Used in Molecular Phylogeny. Species identification are based on Brown (1976, 1977, 1978), Shattuck and Slipinska (2012), and Sorger and Zettel (2011). Sequence numbers refer to GenBank accession numbers or personal sample identifiers.

Species	**Locality**	**Sample Number**	**COI**	**Wg**	**LWR**	**CAD**	**28S**
Anochetus africanus	Uganda	AVS4184	FL051COI	FL051Wg	FL051LWR	FL051CAD	FL05128S
Anochetus alae	Australia	FL715	FL122COI	FL122Wg	FL122LWR	FL122CAD	FL12228S
Anochetus altisquamis	Brazil	JCH592	FL052COI	FL052Wg	FL052LWR	FL052CAD	FL05228S
Anochetus bequaerti	CAR	CS0093	CS0093COI	CS0093Wg	CS0093LWR	CS0093CAD	CS009328S
Anochetus bispinosus	Peru	AVS4279	FL054COI	FL054Wg	FL054LWR	FL054CAD	FL05428S
Anochetus boltoni	Madagascar	CASENT0487895	FL055COI	FL055Wg	FL055LWR	FL055CAD	FL05528S
Anochetus cf cato	Papua New Guinea	RAPI092	FL056COI	FL056Wg	FL056LWR	FL056CAD	FL05628S
Anochetus emarginatus	Trinidad	CS0010	CS0010COI	CS0010Wg	CS0010LWR	CS0010CAD	CS001028S
Anochetus goodmani	Madagascar	CASENT0053884	FL047COI	FL047Wg	FL047LWR	FL047CAD	FL04728S
Anochetus graeffei	Malaysia	CS0094	CS0094COI	CS0094Wg	CS0094LWR	CS0094CAD	CS009428S
Anochetus graeffei	Australia	PSW15290	FL042COI	FL042Wg	FL042LWR	FL042CAD	FL04228S
Anochetus grandidieri	Madagascar	CASENT0157523	FL045COI	FL045Wg	FL045LWR	FL045CAD	FL04528S
Anochetus horridus	Guyana	USNM449691	FL060COI	FL060Wg	FL060LWR	FL060CAD	FL06028S
Anochetus incultus	Malaysia	AVS4360	FL176COI	FL176Wg	FL176LWR	FL176CAD	FL17628S
Anochetus katonae	Uganda	AVS4174	FL061COI	FL061Wg	FL061LWR	FL061CAD	FL06128S
Aochetus madagascarensis	Madagascar	CASENT0136590	FL046COI	FL046Wg	FL046LWR	FL046CAD	FL04628S
Anochetus cf madaraszi	Cambodia	AVS4102	FL167COI	FL167Wg	FL167LWR	FL167CAD	FL16728S
Anochetus cf graeffei	Malaysia	MAL015-2	FL178COI	FL178Wg	FL178LWR	FL178CAD	FL17828S
Anochetus mayri	Trinidad	CS0049	CS0049COI	CS0049Wg	CS0049LWR	CS0049CAD	CS004928S
Anochetus mayri	Peru	AVS4268	FL040COI	FL040Wg	FL040LWR	FL040CAD	FL04028S
Anochetus mayri	Nicaragua	Wa-D-07-2-46	FL113COI	FL113Wg	FL113LWR	FL113CAD	FL11328S
Anochetus minans	Honduras	Wa-C-07-2-25	FL062COI	FL062Wg	FL062LWR	FL062CAD	FL06228S
Anochetud modicus	Malaysia	CS0095	CS0095COI	CS0095Wg	CS0095LWR	CS0095CAD	CS009528S
Anochetus neglectus	Brazil	JCH588	FL063COI	FL063Wg	FL063LWR	FL063CAD	FL06328S
Anochetus peracer	Papua New Guinea	AL0556-01	FL059COI	FL059Wg	FL059LWR	FL059CAD	FL05928S
Anochetus obscuratus	Uganda	AVS4204	FL064COI	FL064Wg	FL064LWR	FL064CAD	FL06428S

Table 2.1 (cont.)

Species	Locality	Sample Number	COI	Wg	LWR	CAD	28S
***Anochetus* sp 174**	Malaysia	MAL015-1	FL174COI	FL174Wg	FL174LWR	FL174CAD	FL17428S
Anochetus orchidicola	Costa Rica	JTL6865-s	FL041COI	FL041Wg	FL041LWR	FL041CAD	FL04128S
Anochetus paripungens	Australia	AVSBH	FL065COI	FL065Wg	FL065LWR	FL065CAD	FL06528S
Anochetus princeps	Indonesia	CS0096	CS0096COI	CS0096Wg	CS0096LWR	CS0096CAD	CS009628S
Anochetus nr risii	Malaysia	JTL7191	FL038COI	FL038Wg	FL038LWR	FL038CAD	FL03828S
Anochetus pubescens	Comoros	CASENT0135206	FL048COI	FL048Wg	FL048LWR	FL048CAD	FL04828S
Anochetus rectangularis	Australia	AVS3017	FL110COI	FL110Wg	FL110LWR	FL110CAD	FL11028S
Anochetus rugosus	Malaysia	AVS4346	FL161COI	FL161Wg	FL161LWR	FL161CAD	FL16128S
***Anochetus* sp sc01**	Seychelles	CASENT0159871	FL112COI	FL112Wg	FL112LWR	FL112CAD	FL11228S
Anochetus simoni	Venezuela	JTL6471-s	FL039COI	FL039Wg	FL039LWR	FL039CAD	FL03928S
Anochetus cf simoni	Venezuela	JTL6450-s	FL050COI	FL050Wg	FL050LWR	FL050CAD	FL05028S
***Anochetus* sp 70**	Malaysia	Peetersi_01	FL070COI	FL070Wg	FL070LWR	FL070CAD	FL07028S
***Anochetus* sp 2921**	Malaysia	2921	FL118COI	FL118Wg	FL118LWR	FL118CAD	FL11828S
***Anochetus* sp BS01**	Malaysia	BS01	FL120COI	FL120Wg	FL120LWR	FL120CAD	FL12028S
Anochetus cf targionii	Malaysia	AVS4275	FL133COI	FL133Wg	FL133LWR	FL133CAD	FL13328S
Anochetus risii	Taiwan	TW01	FL164COI	FL164Wg	FL164LWR	FL164CAD	FL16428S
Anochetus isolatus	Papua New Guinea	MJ7709	FL187COI	FL187Wg	FL187LWR	FL187CAD	FL18728S
Anochetus isolatus	Papua New Guinea	MJ14979	FL188COI	FL188Wg	FL188LWR	FL188CAD	FL18828S
Anochetus* cf *cato	Papua New Guinea	MJ15124	FL189COI	FL189Wg	FL189LWR	FL189CAD	FL18928S
Anochetus cato	Papua New Guinea	MJ14775	FL190COI	FL190Wg	FL190LWR	FL190CAD	FL19028S
Anochetus subcoecus	China	CL04207	FL105COI	FL105Wg	FL105LWR	FL105CAD	FL10528S
Anochetus targionii	Guyana	USNM520401	FL068COI	FL068Wg	FL068LWR	FL068CAD	FL06828S
Anochetus turneri	Australia	FL644	FL124COI	FL124Wg	FL124LWR	FL124CAD	FL12428S
Anochetus victoriae	Australia	FL631	FL117COI	FL117Wg	FL117LWR	FL117CAD	FL11728S
***Anochetus* sp yt01**	Madagascar	CASENT0147171	FL153COI	FL153Wg	FL153LWR	FL153CAD	FL15328S
Bothroponera variolosa	South Africa	CASENT0260241	-	JN419154	JN675459	JX310638	JQ023530
Bothroponera wasmannii	Madagascar	CASENT0260242	DQ176299	JN419153	JN675461	JX310640	JQ023529
Brachyponera atrata	Indonesia	CASENT0260243	CS0041	JN419155	JN675435	JX310612	JQ023531

Table 2.1 (cont.)

Species	**Locality**	**Sample Number**	**COI**	**Wg**	**LWR**	**CAD**	**28S**
Brachyponera chinensis	USA	CASENT0260244	GQ264572	JN419156	JN675439	JX310616	AB126802
Brachyponera sennaarensis	Qatar	CASENT0260245	-	JN419157	JN675450	JX310628	JQ023532
Buniapone amblyops	Malaysia	CASENT0260263	-	JN419178	JN675432	JX310609	-
Diacamma rugosum	Indonesia	CASENT0260213	HQ853328	JN419131	JN675414	JX310580	-
Euponera sikorae	Madagascar	CASENT0260249	DQ176229	JN419150	EF013612	JX310629	EF013032
Hagensia havilandi	South Africa	CASENT0260250	-	JN419162	-	JX310622	JQ023537
Hypoponera opacior	USA	CASENT0260218	CS004COI	EU155464	EU155445	JX310587	EU155410
Hypoponera sakalava	Madagascar	CASENT0260219	-	JN419137	EF013577	JX310588	JQ023515
Leptogenys attenuata	South Africa	CASENT0260223	CS0011COI	EU155465	EU155446	JX310592	EU155411
Leptogenys crassicornis	Malaysia	CASENT0260224	-	JN419145	JN675423	JX310593	JQ023522
Leptogenys diminuta		CASENT1060101	-	EF013708	EF013580	-	EFOI3000
Leptogenys falcigera	USA	CASENT0260225	-	JN419142	JN675424	JX310594	JQ023519
Leptogenys iridescens	Malaysia	CASENT0260226	-	JN419141	KC006064	JX310595	-
Leptogenys occidentalis	CAR	CASENT0260227	-	JN419143	JN675425	JX310596	JQ023520
Leptogenys **sp 4004**	Malaysia	AVS4004	FL094COI	FL094Wg	FL094LWR	FL094CAD	FL09428S
Leptogenys **sp 0339**	Cambodia	SDG0339	FL137COI	FL137Wg	FL137LWR	FL137CAD	FL13728S
Megaponera analis	Sudan	CASENT0260251	CS0064COI	JN419165	JN675433	JX310610	JQ023540
Mesoponera ingesta	CAR	CASENT0260255	-	JN419168	JN675446	JX310624	-
Myopias lobosa		RA0261	-	DQ353130	DQ353246	-	DQ353650
Myopias **sp**	Malaysia	CASENT0260230	-	JN419147	JN675428	JX310599	JQ023524
Myopias tenuis	Australia	CASENT0260231	-	JN419148	JN675429	.IX310600	JQ023525
Odontomachus angulatus	Fiji	EPE54	FL108COI	FL108Wg	FL108LWR	FL108CAD	FL10828S
Odontomachus animosus	Papua New Guinea	LEA016	FL031COI	FL031Wg	FL031LWR	FL031CAD	FL03128S
Odontomachus assiniensis	Uganda	AVS4172	FL026COI	FL026Wg	FL026LWR	FL026CAD	FL02628S
Odontomachus banksi	Philippines	MJ13485	MJ13485COI	MJ13485Wg	MJ13485LWR	MJ13485CAD	MJ1348528S
Odontomachus banksi	Philippines	PH0011	FL106COI	FL106Wg	FL106LWR	FL106CAD	FL10628S
Odontomachus bauri	Ecuador	CS 0098	CS0098COI	CS0098Wg	CS0098LWR	CS0098CAD	CS009828S
Odontomachus brunneus	USA	FL572	FL021COI	FL021Wg	FL021LWR	FL021CAD	FL02128S

Table 2.1 (cont.)

Species	**Locality**	**Sample Number**	**COI**	**Wg**	**LWR**	**CAD**	**28S**
Odontomachus caelatus	Guyana	USNM525222	FL029COI	FL029Wg	FL029LWR	FL029CAD	FL02928S
Odontomachus cephalotes	Papua New Guinea	RAPII002-61	FL098COI	FL098Wg	FL098LWR	FL098CAD	FL09828S
Odontomachus chelifer	Honduras	Wm-C-08-2-07	FL002COI	FL002Wg	FL002LWR	FL002CAD	FL00228S
Odontomachus circulus	China	CL04401	FL102COI	FL102Wg	FL102LWR	FL102CAD	FL10228S
Odontomachus clarus	USA	AVS2766	FL024COI	FL024Wg	FL024LWR	FL024CAD	FL02428S
Odontomachus coquereli	Madagascar	CASENT0134224	FL011COI	FL011Wg	FL011LWR	FL011CAD	FL01128S
Odontomachus erythrocephalus	Costa Rica	Pollet05/115	FL028COI	FL028Wg	FL028LWR	FL028CAD	FL02828S
Odontomachus haematodus	St. Thomas	JW273	FL019COI	FL019Wg	FL019LWR	FL019CAD	FL01928S
Odontomachus hastatus	Costa Rica	JTL6865.1	FL005COI	FL005Wg	FL005LWR	FL005CAD	FL00528S
Odontomachus infandus	Philippines	CS0104	CS0104COI	CS0104Wg	CS0104LWR	CS0104CAD	CS010428S
Odontomachus insularis	Peurto Rico	JW248	FL020COI	FL020Wg	FL020LWR	FL020CAD	FL02028S
Odontomachus laticeps	Nicaragua	Wa-D-07-2-30	FL008COI	FL008Wg	FL008LWR	FL008CAD	FL00828S
Odontomachus latidens	Malaysia	JD06-480	FL037COI	FL037Wg	FL037LWR	FL037CAD	FL03728S
Odontomachus malignus	Philippines	HYM-01593	FL184COI	FL184Wg	FL184LWR	FL184CAD	FL18428S
Odontomachus meinerti	Guyana	USNM525480	FL017COI	FL017Wg	FL017LWR	FL017CAD	FL01728S
Odontomachus cf opaciventris	Peru	AVS4264	FL027COI	FL027Wg	FL027LWR	FL027CAD	FL02728S
Odontomachus saevissimus	Papua New Guinea	AL0612	FL032COI	FL032Wg	FL032LWR	FL032CAD	FL03228S
Odontomachus monticola	Taiwan	CCY1_1	FL031COI	FL031Wg	FL031LWR	FL031CAD	FL03128S
Odontomachus opaciventris	Mexico	RSA2008-032	FL003COI	FL003Wg	FL003LWR	FL003CAD	FL00328S
Odontomachus opaculus	Papua New Guinea	RAPI065	FL034COI	FL034Wg	FL034LWR	FL034CAD	FL03428S
Odontomachus panamensis	Costa Rica	Pollet05/LaSelva	FL004COI	FL004Wg	FL004LWR	FL004CAD	FL00428S
Odontomachus papuanus	Papua New Guinea	AL0615-01	FL097COI	FL097Wg	FL097LWR	FL097CAD	FL09728S
Odontomachus relictus	USA	FL107	FL022COI	FL022Wg	FL022LWR	FL022CAD	FL02228S
Odontomachus rixosus	Malaysia	CS0108	CS0108COI	CS0108Wg	CS0108LWR	CS0108CAD	CS010828S
Odontomachus rixosus	Malaysia	JD06-479	FL036COI	FL036Wg	FL036LWR	FL036CAD	FL03628S
Odontomachus ruficeps	Australia	CS0119	C0119COI	C0119Wg	C0119LWR	C0119CAD	C011928S
Odontomacus ruginodis	Honduras	Ba-C-08-3-02-06	FL093COI	FL093Wg	FL093LWR	FL093CAD	FL09328S
Odontomachus imperator	Papua New Guinea	AL0514-02	FL099COI	FL099Wg	FL099LWR	FL099CAD	FL09928S

Table 2.1 (cont.)

Species	Locality	Sample Number	COI	Wg	LWR	CAD	28S
Odontomachus scalptus	Guyana	USNM521459	FL018COI	FL018Wg	FL018LWR	FL018CAD	FL01828S
Odontomachus saevissimus	Indonesia	MCZ-0093	MJ13494COI	MJ13494Wg	MJ13494LWR	MJ13494CAD	MJ1349428S
Odontomachus simillimus	Seychelles	CASENT0159290	FL012COI	FL012Wg	FL012LWR	FL012CAD	FL01228S
Odontomachus tensus	China	BDB086.13	FL107COI	FL107Wg	FL107LWR	FL107CAD	FL10728S
Odontomachus testaceus	Papua New Guinea	MJ13285	COI	Wg	LWR	CAD	28S
Odontomachus troglodtyes	Madagascar	CASENT0116074	FL001COI	FL001Wg	FL001LWR	FL001CAD	FL00128S
Odontomachus turneri	Australia	874-ODO1-1	COI	Wg	LWR	CAD	28S
Odontomachus turneri	Australia	BHA2	FL126COI	FL126Wg	FL126LWR	FL126CAD	FL12628S
Odontomachus tyrannicus	Papua New Guinea	AL0634-02	FL095COI	FL095Wg	FL095LWR	FL095CAD	FL09528S
Odontomachus testaceus	Papua New Guinea	MJ13288	COI	Wg	LWR	CAD	28S
Odontomachus yucatecus	Nicaragua	Wa-D-07-1-22	FL009COI	FL009Wg	FL009LWR	FL009CAD	FL00928S
Odontoponera transversa	Indonesia	CASENT260235	DQ353399	EU155478	EUI55459	JX310607	EU155422
Ophthalmophone berthoudi	South Africa	CASENT0260261	CS0087COI	JN419175	JN675436	JX310613	-
Mesoponera rubra	Malaysia	CASENT0260256	-	JN419169	JN675448	JX310626	-
Bothroponera sp	Tanzania	JSL110324-01	FL135COI	FL135Wg	FL135LWR	FL135CAD	FL13528S
Pseudoneoponera sp	Malaysia	JM01	FL159COI	FL159Wg	FL159LWR	FL159CAD	FL15928S
Neoponera villosa	Brazil	CASENT0260260	-	JN419174	JN675460	JX310639	JQ023548
Paltothyreus tarsata	South Africa	CASENT0260262	-	JN419176	JN675457	JX310636	-
Phrynoponera bequaerti	CAR	CASENT0401941	-	FL092	-	-	DQ401009
Phrynoponera gabonensis	Uganda	AVS4194	FL109COI	FL109Wg	FL109LWR	FL109CAD	FL10928S
Phrynoponera gabonensis	CAR	CASENT0260268	CS008COI	JN419183	JN675462	JX310641	JQ023552
Platythyrea punctata	USA	CASENT0260201	HQ440172	JN419122	EF013620	JX310643	JQ023504
Plectroctena ugandensis	Gabon	CASENT0260270	-	EUI55480	EU155461	JX310647	EU155424
Pseudoneoponera ruficeps	India	CASENT0260239	CS0029COI	JN419151	JN675449	JX310627	JQ023527
Pseudoponera stigma	Dominica Republic	CASENT0260265	CS0030COI	JN419179	JN675456	JX310635	DQ353617
***Simopelta* sp Ecu2**	Ecuador	CASENT0260276	-	JN419190	JN675468	JX310651	JQ023558
Streblognathus peetersi	South Africa	CASENT0260277	CS0016COI	JN419191	JN675470	JX310653	JQ023559

Table 2.2. Summary of sequence characteristics and sampling. Ingroup excludes non-trap-jaw ant species. Percentages indicate how many of taxa sequenced for each gene. VS = Variable sites. PIS= Parsimony Informative Sites.

		All Taxa			Ingroup		
	Length	**Complete (%)**	**VS**	**PIS**	**Complete (%)**	**VS**	**PIS**
COI	1488	60.1	849	705	67.7	737	560
Wg	442	98.5	215	172	98.1	149	110
LWR	570	85.6	297	250	91.4	245	193
CAD	1522	79	802	654	81.2	622	452
28S	792	84	237	102	83.1	173	66
ALL	4814	76.6	2396	1882	80.1	1922	1382

Table 2.3. Primers used in PCR amplification and Sequencing.

Gene	Primer	Sequence (5’ to 3’)	Source
COI	LepF1	ATTCAACCAATCATAAAGATATTGG	Hebert et al. (2004)
	LepR1	TAAACTTCTGGATGTCCAAAAAATCA	Hebert et al. (2004)
	MLepF1[1]	GCTTTCCCACGAATAAATAATA	Haijibabaei et al (2004)
	MLepR1[1]	CCTGTTCCAGCTCCATTTT	Haijibabaei et al (2006)
Wg	Wg550F	ATG CGT CAG GAR TGY CAY GGY ATG TC	Wild and Maddison (2008)
	Wg578F[1]	TGC ACN GTG AAR ACY TGC TGG ATG CG	Ward and Downie (2005)
	Wg1032R	ACY TCG CAG CAC CAR TGG AA	Abouheif and Wray (2002)
LWR	LR134F	ACM GTR GTD GAC AAA GTK CCA CC	Ward and Downie (2005)
	LR140F[1]	GTW GAC AAA GTK CCA CCN GAN ATG	Schmidt (2013)
	LR639ER	YTT ACC GRT TCC ATC CRA ACA	Ward and Downie (2005)
CAD	CD847F	ATG AAT TAC GGY AAT CGC GGY CAY AAY CAR CC	Schmidt (2013)
	CD1267F	GAR TTY GAY TAT TCR GGS TCG CAR GCG	Schmidt (2013)
	CD1421F[1]	AGG TAA TAC RAT CRG ARA GRC DCG ACG G	Schmidt (2013)
	CD1679F	TGG GTT ATC CTG TTA TGG CNC GYG	Schmidt (2013)
	CD1821F[1]	AGG YTG GAA RGA RGT VGA RTA YGA RGT	Schmidt (2013)
	CD1459R[1]	GCA RTT DAG AGC GGT YTG YCC RCC RAA YGT	Schmidt (2013)
	CD1465R	GCA ATT AAG AGC RGT YTG YCC RCC	Schmidt (2013)
	CD1879R	TGG ATR CCG AGR GGA TCG ACR TTY TCC ATR TTR CAY AC	Schmidt (2013)
	CD2362R	GAC CAT CCT CAA AGC CTT YTG RAA RGC	Schmidt (2013)
28S	28S3318F	CCC CCT GAA TTT AAG CAT AT	Schmitz and Moritz (1994)
	28S4068R	TTG GTC CGT GTT TCA AGA CGG G	Belshaw and Quicke (1997)

[1] - Primers used for nested PCR when the primary strategy failed to produce amplicons.

Table 2.4. Partitioning scheme used in Phylogenetic Analyses. Partition schemes and models of nucleotide substitutions were identified by PartitionFinder and used for analyses on full concatenated sequence alignments (4184 bp). The mean k_revmat statistic indicates the number of unique substitution rate values chosen by the reversible jump MCMC analysis in MrBayes.

Partition	Blocks	Model	Mean k_revmat
p1	COI pos3	GTR+I+G	5.13
p2	COI pos12, LWR pos12, Wg pos12, 28S	SYM+I+G	4.5
p3	Wg pos1, LWR intron, LWR pos3, CAD pos3, CAD intron	K80+I+G	3.16

Table 2.5. Constraint Models Used in Hypothesis Testing. Clades constrained to be monophyletic are set in parentheses. Brackets indicate negative constraints where clades were forced to be non-monophyletic. Estimates of Marginal log-likelihoods were estimated with stone stepping MCMC in MrBayes and were used to calculate Bayes Factors.

Constraint Model	**Marginal ln-liklihood**
(*Anochetus*)	-69026.74
(*Odontomachus*)	-69028.38
(*Anochetus*+*Odontomachus*)	-69037.03
((*Anochetus*)+(*Odontomachus*))	-69019.77
({*Anochetus*}+(*Odontomachus*))	-69149.56
((*Anochetus*)+{*Odontomachus*})	-69119.97
((*Anochetus*+OW *Odontomachus*)+(NW *Odontomachus*))	-69215.38
(({*Anochetus*}+(OW *Odontomachus*))+(NW *Odontomachus*))	-69213.69
((*Anochetus*)+(OW *Odontomachus*)+(NW *Odontomachus*))	-69225.62
(*Anochetus*+*Odontomachus*)+(*Phrynoponera*)	-69048.35
(*Anochetus*+*Odontomachus*)+(*Pseudoneoponera*)	-69033.3
(*Anochetus*+*Odontomachus*)+(*Leptogenys*)	-69054.05

Table 2.6. Fossil Calibrations For Divergence Estimation.

Taxon	Age (Mya)	Notes
Ponerinae	92	Divergence Date Estimate (SCHMIDT 2013)
Hypoponera	44.1	Baltic Amber (WHEELER 1915)
Leptogenys	16	Domican Amber (ZHANG 1989)
Anochetus	16	Dominican Amber (MACKAY 1991)
Odontomachus	20	Czech Impression Fossil (WAPPLER ET AL 2013)

Table 2.7. Adjacency Table For Ancestral Range Reconstruction. T= Neotropics, N=Nearctic, E=Afrotropics, O=Indomalaya, and A=Australasian. Values indicate probability of dispersal between regions. 1=high probability of dispersal between adjacent regions. 0.5=modest probability of dispersal between distant regions or regions with small barriers. 0=zero probability of dispersal between regions separated by large oceans.

	T	N	E	O	A
T	-	1.0	0.5	0	0.5
N	1.0	-	0	0	0
E	0.5	0	-	1.0	1.0
O	0	0	1.0	-	1.0
A	0.5	0	1.0	1.0	-

Table 2.8. Ancestral range reconstruction from LAGRANGE analysis. Inheritances are displayed as left|right splits where the "left" and "right" are the ranges inherited by descendant upper and lower branches, respectively, on the dated phylogeny (**Figure 2.7**). T=Neotropical, N=Nearctic, E=Afrotropical, O=Indomalayan, and A=Australasian.

Clade	Split	Rel. Prob.
Odontoamchus + Anochetus	TO|O	0.587
Odontoamchus + Anochetus	TO|T	0.368
Odontomachus	A|O	0.521
Odontomachus	T|E	0.350
Clade A	E|O	0.743
Clade A	O|O	0.217
Clade B	T|T	0.999
Clade C	A|T	0.789
Anochetus	TO|T	0.905
Clade D	T|T	0.996
Clade E	O|O	0.911
Clade F	T|T	0.994
Clade G	O|O	0.874

Figure 2.1. Consensus trap-jaw phylogeny from a partitioned Bayesian analysis of the full, concatenated dataset. Nodal support is given in both Bayesian Posterior Probabilities (BPP) and Maximum Likelihood Bootstrap Percentages (BS). Morphologically inferred species groups that were recovered as monophyletic are marked with blue boxes.

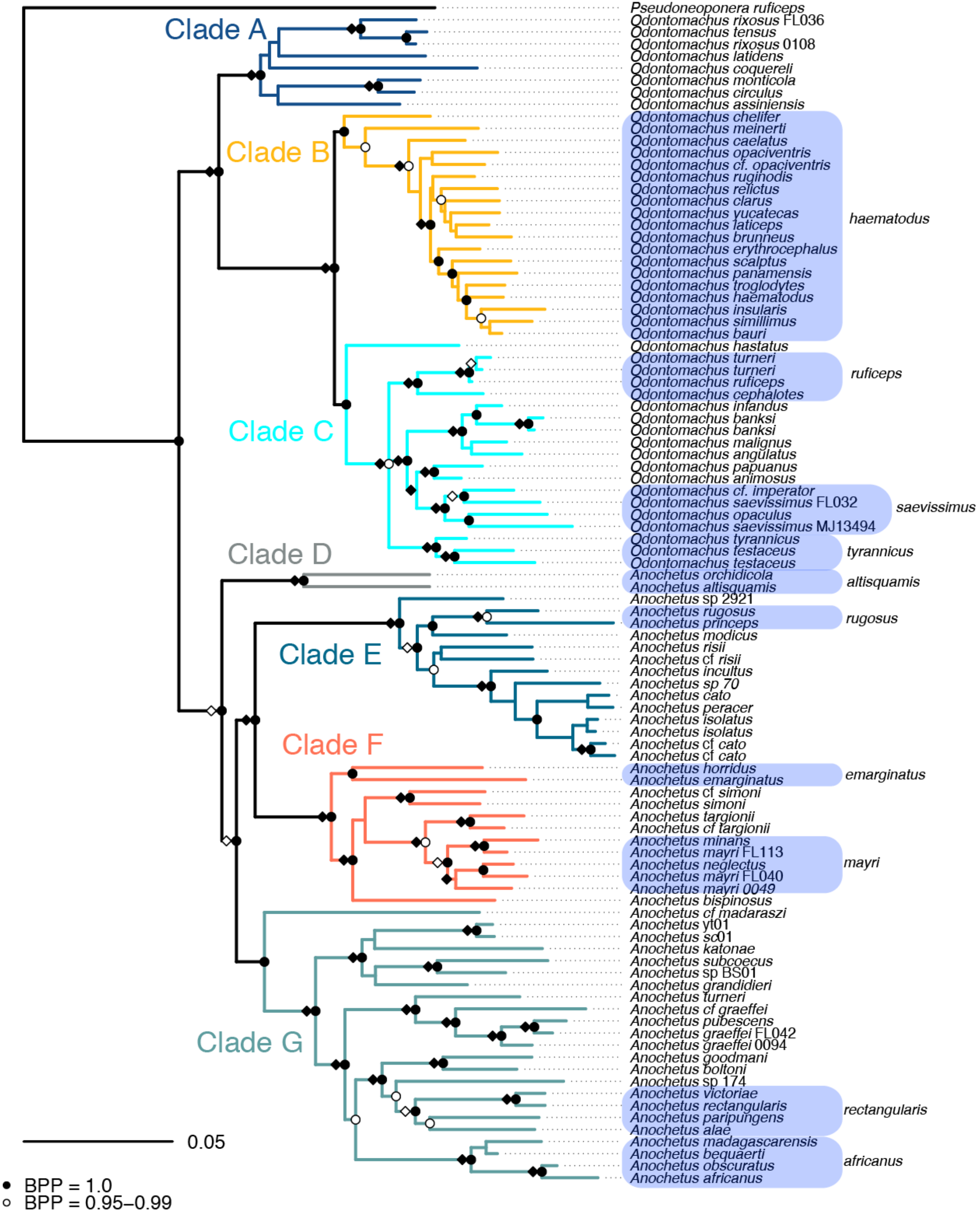


Figure 2.2. Consensus phylogeny of the MrBayes analysis with the full concatenated partitioned dataset. Nodes are labeled with posterior probability support values.

Figure 2.3. **Consensus phylogeny from reversible jump Multichain Monte Carlo analysis.** Posterior probabilities are listed at each node.

Figure 2.4. Consensus phylogeny of maximum likelihood analysis. Nodal support is provided as Bootstrap Support Values.

Figure 2.5. a–e. Consensus phylogenies for Bayesian analyses on single gene datasets. a. *cytochrome oxidase I* (COI).

Figure 2.5. (cont.) b. *wingless* (Wg).

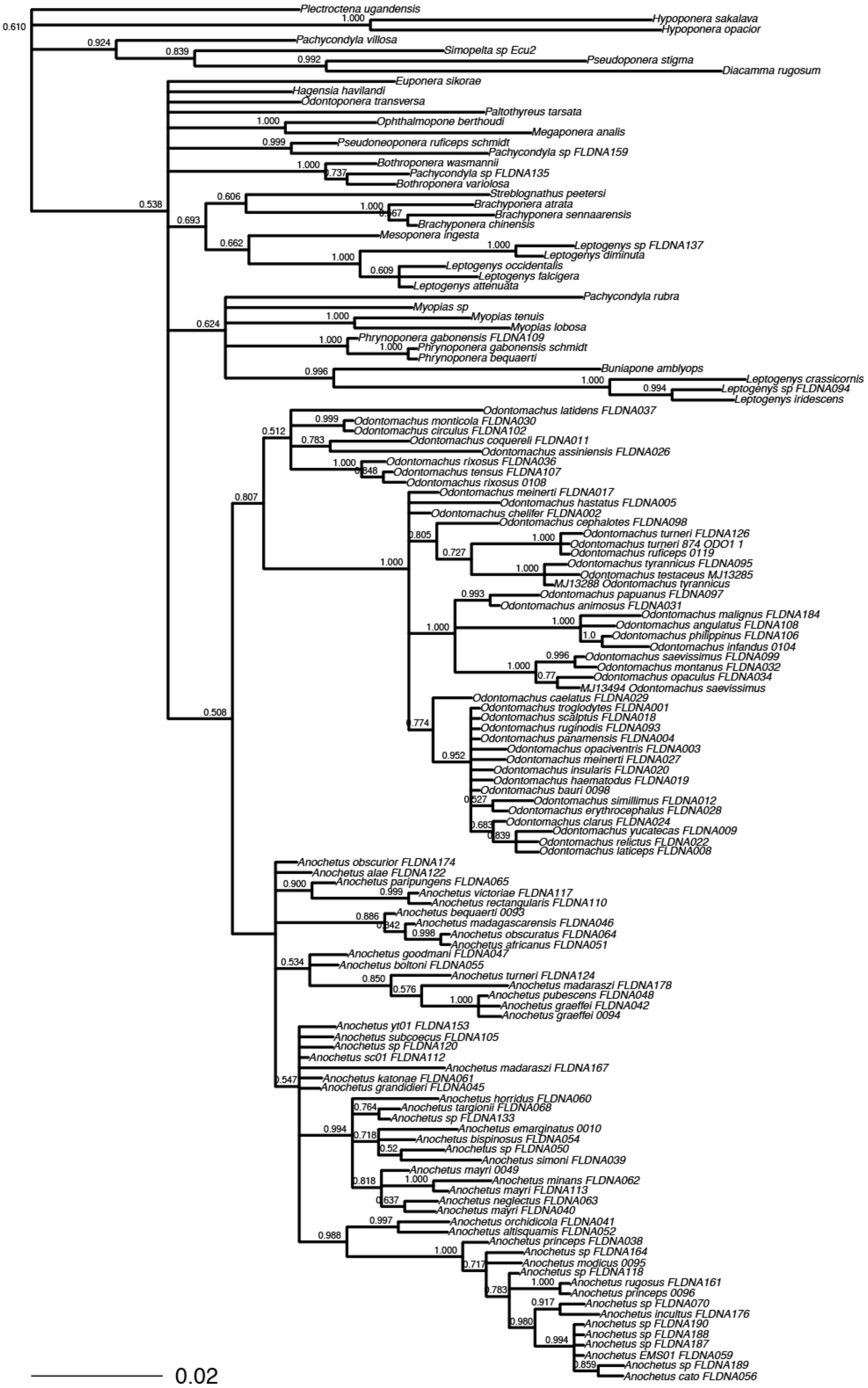


Figure 2.5. (cont.) c. *Long wavelength rhodopsin* (LWR)

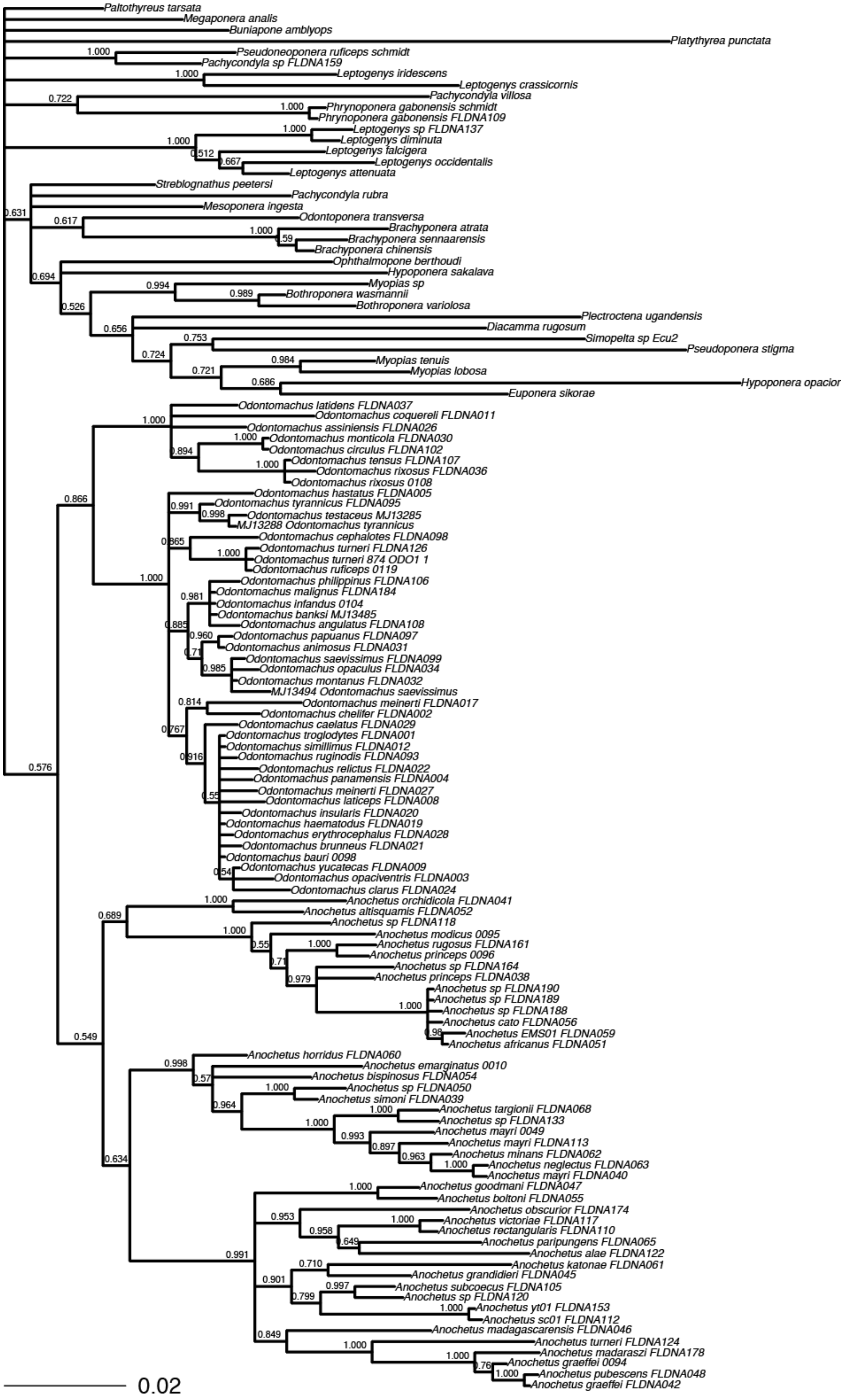


Figure 2.5. (cont.) d. *Rudimentary* (CAD).

Figure 2.5. (cont.) e. 28S rDNA.

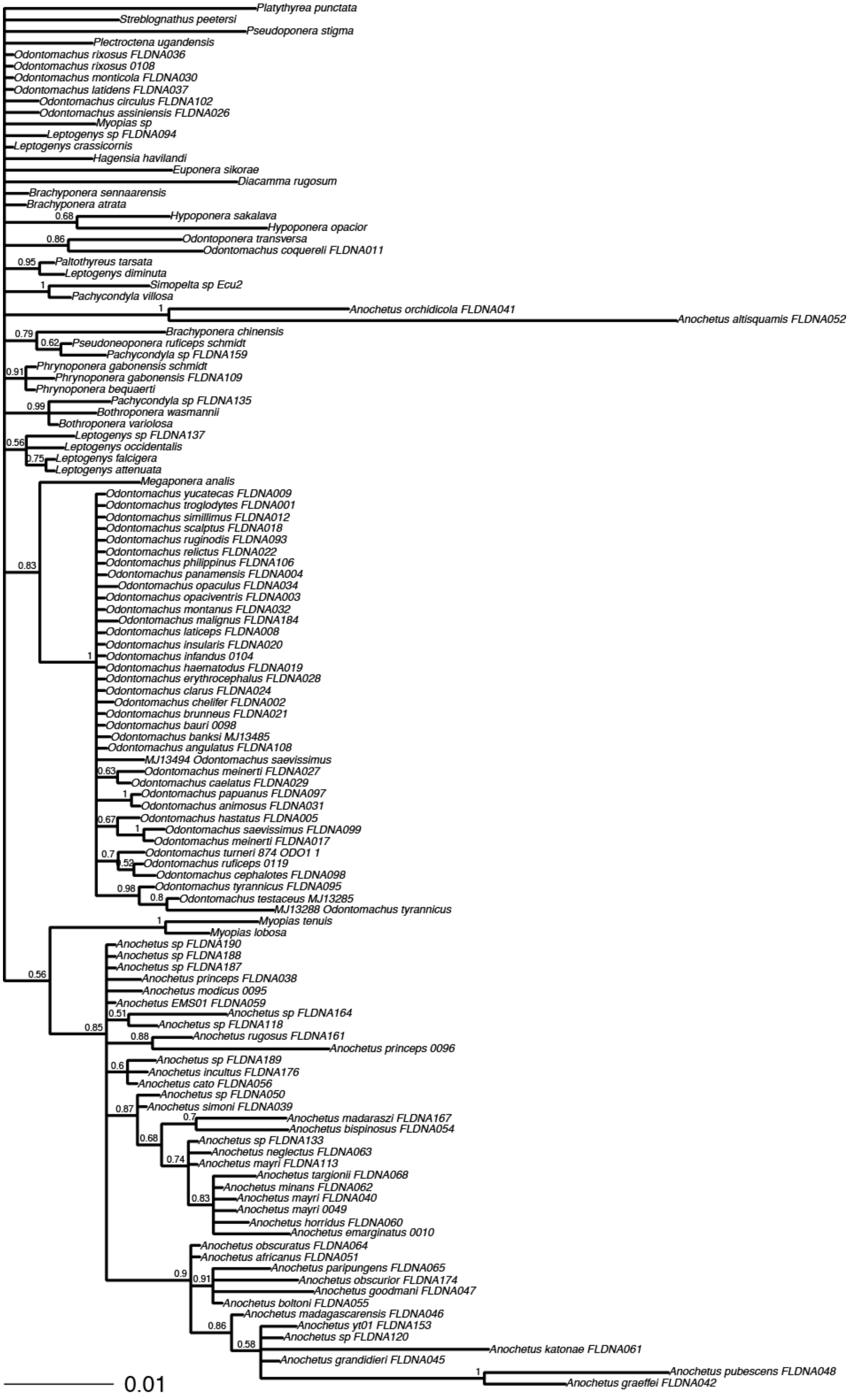


Figure 2.6. Dated phylogeny of trap-jaw ants. Maximum clade credibility tree with mean node ages and 95% highest density probability. Node support is indicated with circles, with black circles having BPP ≥ 0.99 and white circles having $0.95 \geq$ BPP < 0.99.

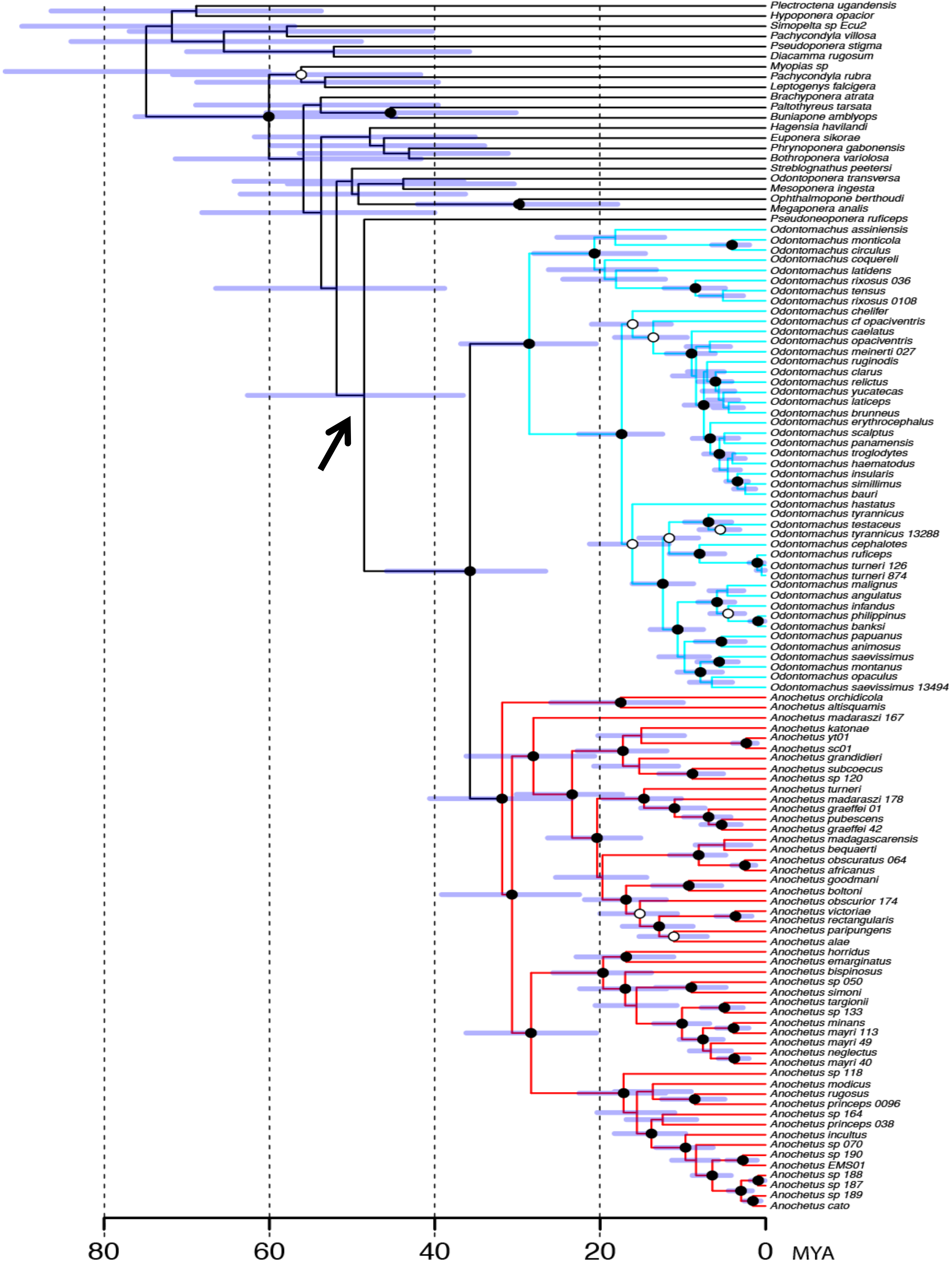


Figure 2.7. Lineage diversification of *Anochetus* and *Odontomachus* clade. Lineage through time plot was generated with the dated tree from the BEAST analysis. The solid line represents the accumulation of trap-jaw ant species over time. The shaded area represents the 95% confidence interval of lineage diversification under a pure birth model with a λ = 0.09.

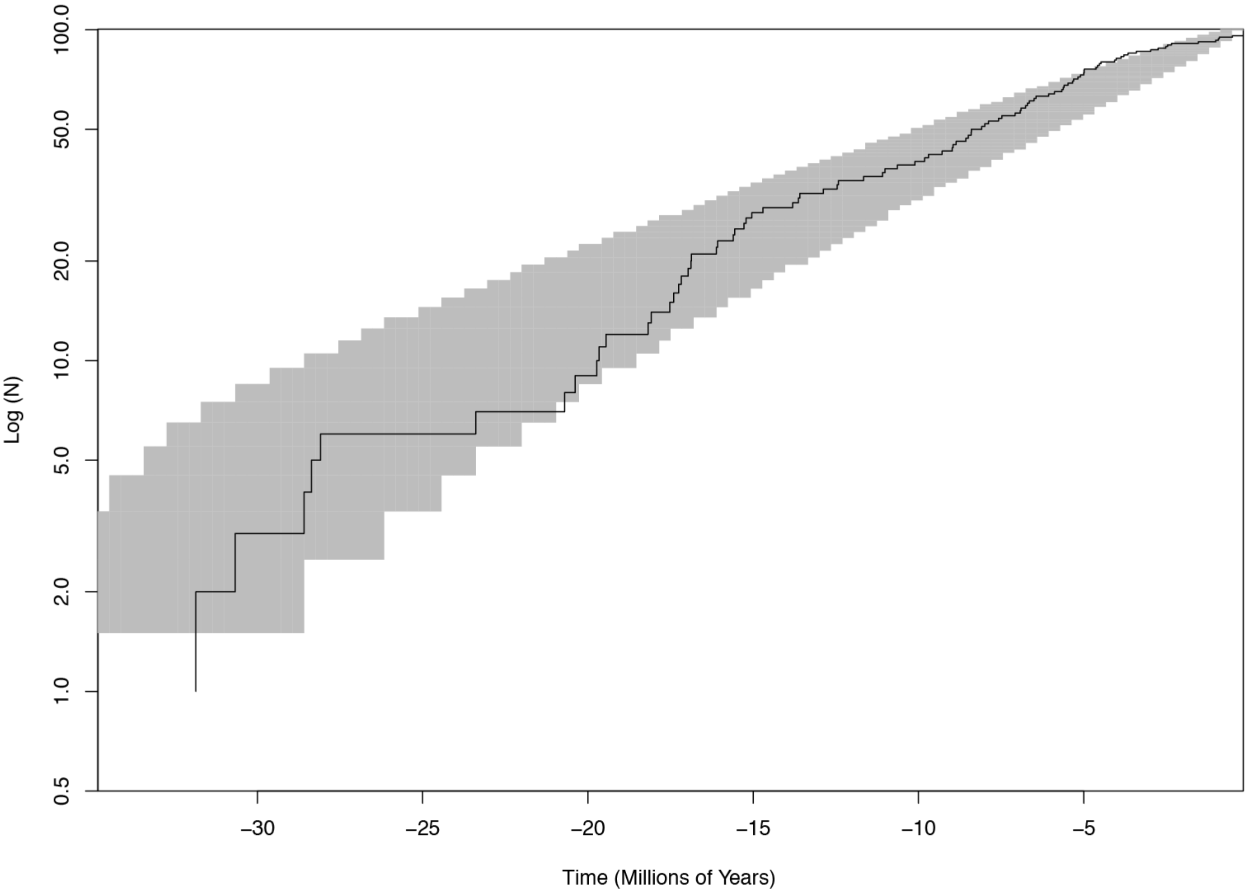
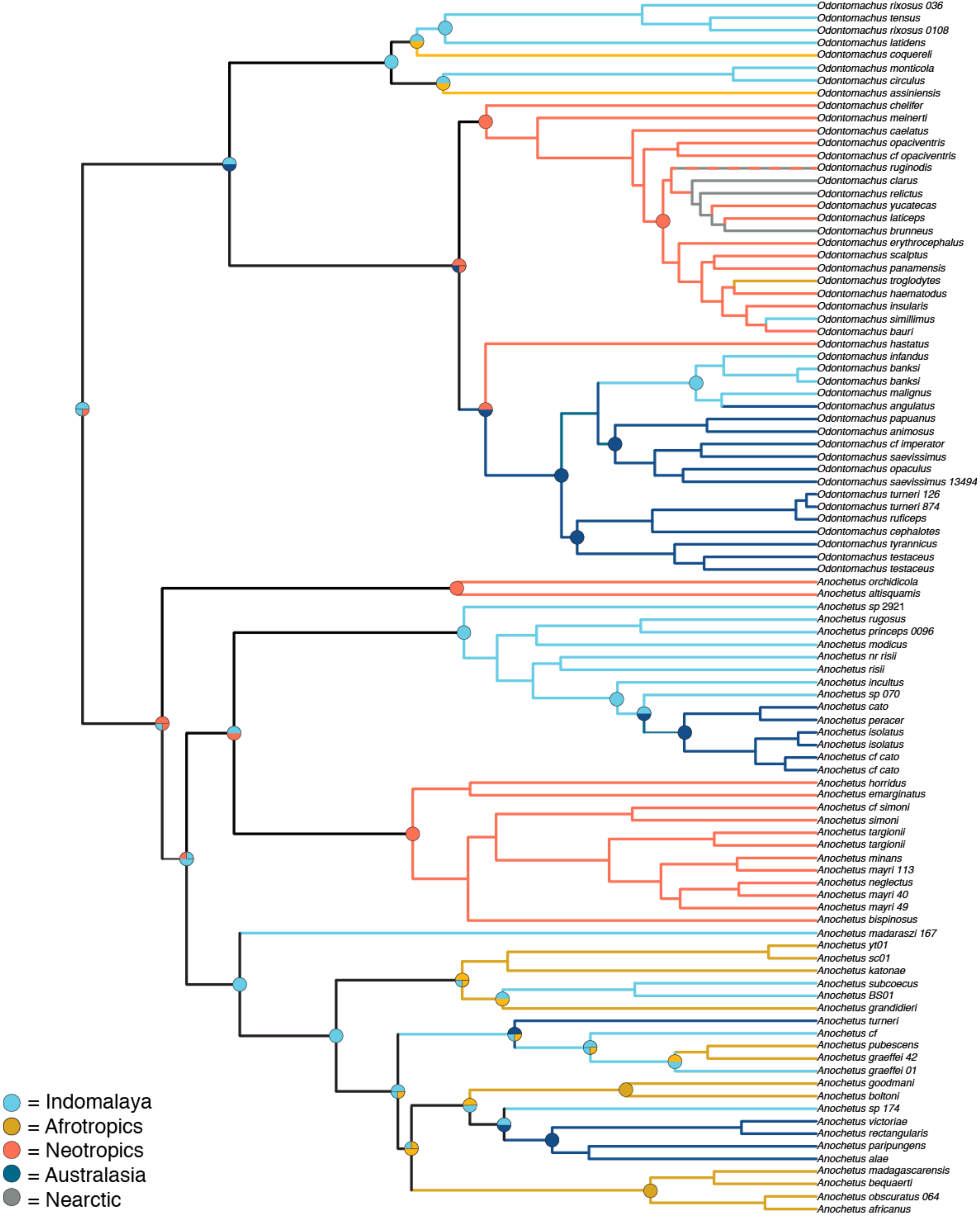


Figure 2.8. Biogeography of trap-jaw ants inferred with LAGRANGE. Maximum clade credibility tree from divergence estimation is presented with inferred ancestral nodes labeled. The upper and lower half of the colored circle indicates the inherited range for the upper and lower branch of the descending branch, respectively. Split halves, indicate that the descending branch inherits a range spanning multiple biogeographic regions.



CHAPTER 3: PHYLOGENETIC VERSUS DEVELOPMENTAL ALLOMETRY OF MANDIBLE PERFORMANCE IN TRAP-JAW ANTS IN THE GENERA *ANOCHETUS* AND *ODONTOMACHUS*

Abstract

Scaling relationships are common in biological systems, but it is unclear if developmental allometry within a species is reflected across phylogenetic allometry across species. Here we examine this question in a phylogenetic context in trap-jaws ants in the sister genera *Anochetus* and *Odontomachus.* I examined head morphology (using geometric morphometrics) and kinematic performance of 25 species of trap-jaw ant species from across their phylogeny, and examined the relationship between morphology and strike performance. Additionally, I compared interspecific relationships with intraspecific scaling relationships in the polymorphic species *Odontomachus turneri*. I found significant relationships between strike speed and body size across species, with larger ants having longer strike durations but higher peak angular velocities. These relationships deviated strongly from isometry, with larger species being much slower than if strike speed was dependent on muscle cross-sectional area. I also found a very strong isometric relationship for peak kinetic energy experienced by the mandibles during strikes, with large ants producing more energy proportional to their body size. Speed did not scale with body size in the polymorphic species, and smaller individuals outperformed similar sized conspecifics. Together these results suggest that strike performance is species-specific and may be under selection, and developmental plasticity within species can create forms that deviate from interspecific tradeoffs in performance.

Introduction

Body size is a critical life history trait that influences nearly every aspect of an organism's biology, from morphology and metabolism to locomotion and longevity (SCHMIDT-NIELSON 1984). How traits scale with body size can reveal how evolution optimizes functional design within physical or biological constraints (GOULD 1966; EMLEN & NIJHOUT 2000). Metabolic rate (GLAZIER 2005), muscle output (ALEXANDER 1981; ALEXANDER 1985), and flight power (CASEY 1985; DARVEAU 2005) all scale non-linearly (allometrically) with body size, reflecting how performance operates differently across size scales. Allometry may be displayed within a species during ontogenetic growth, or across species, but it is unclear whether developmental allometry always matches phylogenetic allometry. The goal of this study is to examine how morphology and performance scale with body size within and among species of trap-jaw ants in the genera *Anochetus* and *Odontomachus*.

An excellent model for studying these scaling relationships can be found in the specialized mandibles of trap-jaw ants in the genera *Anochetus* and *Odontomachus* (reviewed in LARABEE & SUAREZ 2014). These ants possess long, spring-loaded mandibles that snap shut at some of the fastest speeds ever recorded for an animal movement. Members of the genus *Odontomachus*, for example, are capable of snapping their mandibles as fast as 60 m/s and generate forces over 300 times their total body mass (PATEK ET AL. 2006). These rapid mandible movements are used to capture fast or dangerous prey (DE LA MORA 2008; MOFFETT 1986), and to escape from predators (LARABEE & SUAREZ 2015).

Although the ponerine trap-jaw ant genera are closely related and share a common trap-jaw mechanism, across species they display wide variation in head and mandible morphology as well as overall body size. Force production was found to scale positively with body mass among eight species of *Odontomachus* (SPAGNA ET AL 2009), but this sample represented a limited amount of the total morphological variation in the clade and these species are all monomorphic with very narrow ranges of head morphology and body size. It is unclear if mandible performance in trap-jaw ants is species- or size-dependent. The polymorphic species *Odontomachus turneri* provides a "natural experiment" to test if the scaling relationships observed across the genus are developmentally or evolutionarily constrained. Body size in this Australian species is continuously polymorphic and spans nearly the entire range of size displayed across the entire genus *Odontomachus*. If strike performance is simply a physical developmental byproduct of body size, one would expect to see the same scaling relationship in *O. turneri* as across the genus.

The aims of this study are to contrast inter- and intra-specific scaling relationships between mandible performance and body size in the trap-jaw genera *Anochetus* and *Odontomachus*. To accomplish this, I (1) estimate strike performance and test for scaling relationships in a wide range of *Anochetus* and *Odontomachus* species while accounting for phylogenetic non-independence, (2) use geometric morphometrics to quantify head variation within and between these two genera, (3) test whether these inter-specific scaling relationships are also observed within the polymorphic species *Odontomachus turneri*.

Materials and Methods

Study Species

For kinematic studies, live nests of 24 species of *Anochetus* and *Odontomachus* were collected from numerous field sites in North, Central and South America, Africa, Southeast Asia, and Australia (Table 3.1 and Figure 3.2). Between one and ten nests were collected for each species, and most of these colonies contained queens. In the lab, artificial nests were kept in plastic boxes coated with Fluon (Northern Products), and plaster-filled petri dishes for nest chambers. Ants were given water and sugar *ad libitum* and fed live termites or frozen crickets three times a week. All ants were kept at 25° C and a 12-hour light-dark cycle.

For morphometric analyses, field collections were supplemented with museum collections (the National Museum of Natural History (NMNH) and the California Academy of Sciences) and loans from numerous collaborators (Table 2.1). The specimen list for my morphometric analysis is the same as the taxonomic sampling for my worldwide species-level phylogeny with duplicate species removed (Table 2.1). All collections have been vouchered on point mounts and in 100% ethanol and will be stored at the NMNH and the University of Illinois, Urbana-Champaign.

Kinematics

Mandible strike performance was measured with high-speed videography using a modified protocol of SPAGNA ET AL (2008). Ants were restrained to the end of a #3 insect pin on the dorsal surface of the head with dental wax (Kerr Laboratory Products, Orange, CA). The pin was fitted onto a micromanipulator and the ant was positioned under a Zeiss SteREO Discovery V20 stereomicroscope and backlit with an LED light (Visual Instrumentation Corp.). Magnified trap-jaw strikes were recorded with a Phantom v9.1 high-speed camera (Vision Research) with frame rates between 65,000–90,000 frames per second (fps). All filming took place at the Beckman Institute Imaging Technology Group at the University of Illinois, Urbana-Champaign. Three to five strikes were recorded for each individual, and 3–40 individuals were filmed for each species.

Following strike recordings, individual ants were immediately stored in a -20º C freezer. Within one week of filming, several morphological traits were measured for each specimen: whole-body wet mass, head length (from clypeus to posterior-most margin), head width (including eyes), wet mandible mass, and mandible length. All linear measurements were made with a Semprex Micro-DRO digital stage micrometer (Semprex Corporation) connected to a Leica MZ 12.5 stereomicroscope. Masses were measured with a UMX2 microbalance (Mettler-Toledo).

Kinematic parameters were estimated in MATLAB R2013a (Mathworks, Natick, MA). The positional coordinates of the mandible tip were tracked manually using a custom script (Spagna et al 2009), providing the displacement information of the mandible during its trajectory. Minimum strike duration and peak angular velocity were derived from this displacement data. Peak rotational kinetic energy, $E_{rotation}$, was calculated for each mandible during a strike, by modeling the mandible as a thin rod with a moment of inertia, I, of ($1/3MR^2$):

$$E_{rotation} = \frac{1}{6}MR\omega^2$$

where M is the mass of the mandible, R is the length of the mandible, and ω is the angular velocity of the mandible. The strike characteristics for *O. bauri*, *O. cephalotes*, *O. haematodus*, and *O. ruginodis* were taken directly from PATEK ET AL (2006) and SPAGNA ET AL (2008).

Geometric Morphometrics

Geometric morphometric methods were used to quantify head shape and examine the relationship between shape, body size, and snap performance. (ROHLF & MARCUS 1993; ADAMS ET AL. 2004; ADAMS ET AL. 2013). Two-dimensional landmark coordinates were digitized from focus-stacked images of heads for 51 species of *Anochetus* and 46 species of *Odontomachus* trap-jaw ants. Head shape was defined by 10 fixed and 28 semi-landmarks (Figure 3.1). Coordinate digitization was performed in ImageJ v1.46 (SCHNEIDER ET AL 2012).

Digitized landmark coordinates were aligned using Procrustes superimposition, which accounts for differences in orientation, relative position, and size among specimens (ROHLF AND SLICE 1990). Semi-landmarks provide a better quantification of curves than fixed landmarks alone, and were allowed to slide during superimposition using the minimal bending energy method (BOOKSTEIN 1997). The resulting set of shape variables (56 dimensions) were used in subsequent multivariate statistical tests. Depending on analysis, body size was estimated with either wet body mass or the $\log_{10}$-transformed centroid size of head shape. All morphometric analyses were performed in R 3.2.0 (R Development Core Team, 2014) with the package 'geomorph' (ADAMS & OTÁROLA-CASTILLO 2013).

Data Analysis

To summarize overall head shape variation, I performed a principle component analysis (PCA) of shape variables. Shape was defined as the multivariate Procrustes residuals. Scaling relationships and differences in shape between the genera *Anochetus* and *Odontomachus* were tested with a non-parametric multivariate analysis of variance (Procrustes MANOVA) with a randomized residual permutation procedure (1,000 permutations) (DRAKE & KLINGENBERG 2008) in the 'geomorph' package of R. The Procrustes residuals were treated as the multivariate response variable and size, genus, and their interaction as model effects. Allometric relationships were visualized using scores from a multivariate regression of head shape versus centroid size, and also predicted values from genus-specific multivariate regressions. Thin-plate spline (TPS) deformation grids were generated for shape means of each genus to visualize shape differences between genera.

Two strategies were used to analyze the relationship between strike performance and body size across species: ordinary least squares and phylogenetically corrected generalized linear squares (pGLS). All strike performance parameters were log-transformed and wet whole body mass was used as the independent variable. Phylogenetic signal was estimated for each trait using Pagel's λ using a molecular phylogeny for the two genera (see Chapter 2) and used to scale the pGLS test in the package 'phytools'.

Ordinary least squares was also used to test the scaling relationships of trap-jaw strike performance within the polymorphic species *Odontomachus turneri*. Scaling relationships were tested for 40 individuals in the same way as for interspecific relationships. To test for differences in slope between inter- and intra-species relationships, analysis of covariance (ANCOVA) was used. Polymorphic status was used as a categorical factor with two levels (polymorphic or not) with body size as the independent variable and strike performance as the covariate.

Results

Strike Kinematics

I found a significant amount of variation in strike performance across species of *Anochetus* and *Odontomachus* (Table 3.1 and 3.2). The shortest strike duration was observed in *A. mayri* ($6.0 \cdot 10^{-5}$ ms) and the *O. hastatus* had the longest minimum strike duration ($4.2 \cdot 10^{-4}$ ms). Peak angular velocity averaged $2.95 \cdot 10^{4}$ rad·s^{-1} and ranged between $1.24 \cdot 10^{4}$ rad·s^{-1} (*O. coquereli*) and $7.79 \cdot 10^{4}$ rad·s^{-1} (*A. graeffei*). *Odontomachus* mandibles experienced higher peak rotational energy (36.3 mJ ± 22.4) than *Anochetus* (9.6 mJ ± 7.2). The most energetic mandibles belong to *O. turneri*, which experienced peak rotational energy of 84.1 mJ, whereas the least energetic belonged to the undescribed Malaysian species *Anochetus* sp. BS01 (2.9 mJ).

Among the 25 species of trap-jaw ants that I measured, there was a significant association between strike performance and body size (Table 3.2 and Figure 3.3). Larger ants had significantly longer strike duration and lower peak angular velocities than small ants. Because of their larger mass, however, ant mandibles experience higher peak rotational kinetic energy. Mandibles scale isometrically with body size (slope = 0.97). All strike characteristics showed very strong phylogenetic signal (l > 0.88 for all traits), but even when incorporating shared ancestry, all scaling relationships were still significant. Indeed, the slops of the pGLS analyses were not different from the OLS analyses.

Head Morphometrics

Head shape also displayed significant morphometric variation among within and among trap-jaw ant genera (Figure 3.3 and 3.4). The results of the PCA on the Procrustes shape residuals are shown in Figure 3.3. I found significant differences (statistically and visually) in head shape between each genus (MANOVA shape x genus: F =151.0, R^2= 0.632, P=0.001). *Odontomachus* ants have longer, narrower heads than *Anochetus,* but broader heads at the anterior near the mandibles. Ants in the genus *Anochetus* are also characterized by a deep groove defining the occipital lobes, whereas *Odontomachus* occipital lobes are squared. Two significant outliers from the PCA, *A. horridus* and *A. emarginatus*, have heads that are much similar to *Odontomachus* than *Anochetus*.

Head shape also displayed a significant scaling relationship with log centroid size (Figure 3.4). Head shape scaled with head size, but the allometric slopes did not differ among *Anochetus* and *Odontomachus* (MANOVA: shape x size: F = 135.4, P=0.0001; size x genus: F= 4.40, P=0.198). Regression of phylogenetic independent contrasts on multivariate shape variables also displayed a significant scaling relationship with body size (F = 3.4, P = 0.038).

Intraspecific Variation

The intraspecific scaling relationships of strike traits in the polymorphic species *O. turneri* are significantly different from the interspecific relationships among species (Table 3.3). The results of the ANCOVA analysis showed a significant interaction between body size and group for each strike trait. Strike duration and peak velocity did not have a significant correlation with body size (ω: slope=-0.106, P=0.156; t: slope = 0.068, P=0.197) (Figure 3.5). The peak kinetic energy did show a significant scaling relationship with body size (slope = 1.03, $P <$ 0.001), and was even stronger than the relationship among monomorphic species.

Discussion

The large mandible adductor (closer) muscle is responsible for generating the power behind trap-jaw ant strikes (GRONENBERG 1996; PAUL 1999). If adductor muscle size is the main factor that determines strike performance, an isometric relationship between strike speed and body size should scale to the power of 0.67 (muscle cross-sectional area scaling with body volume). My kinematic data for 25 species of *Anochetus* and *Odontomachus* revealed a large amount of variation in strike performance and statistically significant scaling relationships, but did not scale isometrically. Minimum strike duration scaled to the power of 0.28 and peak angular velocity scaled to the power of -0.36, meaning larger ants had much slower strikes than would be expected if speed scaled proportional to body size. One explanation for this relationship is that strike speed in this system is also heavily influenced by the elastic properties of the spring in the system (ALEXANDER 1988; PATEK ET AL 2013). The spring structure of *Odontomachus* trap-jaw ants is thought to be the cuticle of the head capsule and the apodeme that connects the adductor to the base of the mandible (GRONENBERG 1995; PATEK ET AL 2006), but the mechanics of trap-jaw springs have not been studied in any detail.

Unlike strike speed, the peak kinetic energy of the mandibles during a strike did scale isometrically with body size (log-log coefficient = 0.64). This relationship may be due to a tight correlation between strike energy and mandible mass, which this study and previous research has shown to be proportional to body size (0.98) (SPAGNA ET AL 2008). In the absence of direct measures of mandible bite force, kinetic energy is arguably a more important measure of mandible performance than strike speed because it incorporates aspects of both mandible speed and mass. Future research on the structure-function relationship of trap-jaws would greatly benefit from measuring the elastic mechanisms of the trap-jaw spring and measuring strike forces.

With my recently generated phylogeny (Chapter 2), I was able to correct for shared ancestry in the regression analyses. Mandible strike traits had very high estimates of phylogenetic signal (Pagel's λ), suggesting that they follow a simple Brownian motion model of trait evolution. Under Brownian motion, morphological evolution is random and proportional with time, causing closely related species to resemble each other because they share a relatively recent common ancestor (FELSENSTEIN 1985; PAGEL 1999). All scaling relationships I found using OLS were still supported using pGLS, despite the high amount of phylogenetic signal. This may be a result of the broad taxonomic sampling from most of the major clades in the group.

Results from my analysis of the polymorphic trap-jaw ant species *O. turneri* suggest that strike performance traits may be a species-specific trait and under stabilizing selection in *Odontomachus*. Interestingly, although *O. turneri* displays nearly as much body size polymorphism as the rest of the genus, I did not find a significant relationship between strike speed and body size; larger individuals of *O. turneri* produced faster strikes than predicted from the inter-specific relationship. The relationship between kinetic energy and body size was significant, and stronger than the interspecific relationship (coefficient was 1.08), driven mostly by the strong relationship between body and mandible size. There are two other polymorphic *Odontomachus* species, *O. cephalotes* and *O. ruficeps*, whose mandible performance has not been examined in detail. Most other studies on selection of whole-animal performance have not found evidence for stabilizing selection, but instead find directional selection for increased speed (MILES 2004; HUSAK ET AL 2006). Alternatively, there could be some other morphological trait that explains strike performance better than body size.

My geometric analysis of head shape also yielded interesting results. I found a significant difference in head shape, corresponding to head length and the morphology of the occipital lobes. The occipital lobes of *Anochetus* may be more pronounced in order to efficiently house as much pennate muscle possible (PAUL 1999). Two interesting morphological outliers are the sister species, *A. horridus* and *A. emarginatus*, which have long slender heads more similar to *Odontomachus* than to other *Anochetus* species. Unlike most other *Anochetus* trap-jaw ants that are hypogeaic foragers, *A. horridus* and *A. emarginatus*, are both arboreal ants that forage above ground and have well developed eyes. My phylogenetic analysis suggests that this lineage descended from hypogaeic ancestors, providing evidence that foraging behavior or nesting ecology strongly influence the evolution of trap-jaw head and mandible morphology.

This study did not result in any new speed records for animal movement. The fastest trap-jaw strike in my dataset belonged to one of the smallest species: *A. mayri*. Strikes by that species shut as fast as 0.06 ms, which matches the duration of the much larger *O. bauri* (PATEK ET AL 2006). Similarly, the highest peak angular velocity I measured was $7.79 \cdot 10^4$ rad$\cdot$s^{-1} by *A.*

graeffei, which translates to approximately 40 m/s, well below currently established records (*O. brunneus* peak speeds are 60 m/s). Most species I recorded were approximately half as fast as these outliers, which probably reflects trade-offs between mandible speed and the energetic costs of powering such large mandible adductor muscles.

Future work on the structure-function relationship of the trap-jaw mechanism should attempt to disentangle the complex relationship between size and other factors that influence performance, such as metabolism, spring mechanics, and muscle geometry. Ultimately, the goal of studying key innovations is to relate presence of a novel morphology or performance of that structure to species divergence rates. My recently generated phylogeny and newly developed phylogenetic comparative methods should facilitate testing the hypothesis that trap-jaws are associated with higher rates of morphological or species evolution.

Table 3.1. Summary of trap-jaw ant species and strike kinematics. All means are ± standard deviation.

Species	Locality	N	Body Mass (mg)	Mandible Mass (mg)	Mandible Length (mm)	Min. Strike Duration (ms)	Mean Strike Duration (ms)	Max Velocity ($\mathrm{rad\cdot sec^{-1}}$)	Mean Velocity ($\mathrm{rad\cdot sec^{-1}}$)	Max E_{max} (J)	Mean E_{max} (J)
A. alae	Australia	4	3.82 ±0.22	46.2 ±2.31	1.26 ±0.132	0.125	0.136 ±0.014	38978	32794 ±4447	0.013	0.006 ±0.001
A. graeffei	Malaysia	4	0.76 ±0.08	8.51 ±1.05	0.57 ±0.014	0.075	0.091 ±0.019	77892	62266 ±10875	0.006	0.010 ±0.002
A. mayri	USA	1	0.53 ±0.04	5.58 ±0.96	0.54 ±0.068	0.060	0.065 ±0.008	75316	70192 ±6737	0.003	0.003 ±0.002
A. paripungens	Australia	5	1.62 ±0.1	16.69 ±0.84	0.82 ±0.028	0.090	0.097 ±0.007	55014	50416 ±5639	0.007	0.002 ±0.001
A. risii	Taiwan	3	3.38 ±0.03	41 ±1.31	1.27 ±0.021	0.144	0.173 ±0.025	33927	30450 ±3013	0.010	0.008 ±0.001
A. rugosus	Cambodia	3	4.48 ±0.45	48.77 ±5.3	1.26 ±0.066	0.114	0.133 ±0.018	37886	34717 ±3250	0.016	0.012 ±0.003
A. sp 2921	Malaysia	3	1.83 ±0.09	23.17 ±1.53	0.95 ±0.026	0.131	0.165 ±0.038	42738	33609 ±8496	0.006	0.004 ±0.002
A. sp BS01	Malaysia	4	0.5 ±0.04	7.06 ±0.78	0.45 ±0.021	0.063	0.077 ±0.013	72646	62298 ±11945	0.003	0.002 ±0.001
A. turneri	Australia	4	3.1 ±0.16	33.25 ±3.66	0.97 ±0.029	0.113	0.122 ±0.012	69848	45528 ±17751	0.027	0.012 ±0.010
A. victoriae	Australia	4	1.12 ±0.09	12.25 ±1.89	0.63 ±0.034	0.075	0.091 ±0.017	62511	53875 ±5914	0.005	0.004 ±0.001
O. bauri[b]	Ecuador	8	11.6 ±0.20	131 ±13	1.29 ±0.07	0.060	0.015 ±0.019	37291	30698 ±4713	0.051	0.035 ±0.012
O. brunneus	USA	5	7.65 ±0.73	68.74 ±4.09	1.35 ±0.048	0.111	0.111 ±0	41280	35124 ±6029	0.026	0.019 ±0.006
O. cephalotes[a]	Australia	20	16.71 ±2.17	162.2 ±28.06	1.58 ±0.08	0.110	0.134 ±0.014	26819	22598 ±4010	0.031	0.022 ±0.007
O. chelifer[a]	Trinidad	4	24.57 ±2.8	310.3 ±32.6	2.28 ±0.216	0.122	0.161 ±0.078	20805	16920 ±3404	0.052	0.035 ±0.016
O. coquereli	Madagascar	3	17.03 ±2.86	146.7 ±13.89	2.31 ±0.057	0.216	0.243 ±0.023	12350	12049 ±352	0.009	0.008 ±0.001

Table 3.1 (cont.)

Species	Locality	N	Body Mass (mg)	Mandible Mass (mg)	Mandible Length (mm)	Min. Strike Duration (ms)	Mean Strike Duration (ms)	Max Velocity (rad·sec^{-1})	Mean Velocity (rad·sec^{-1})	Max $E_{\max}$ (J)	Mean $E_{\max}$ (J)
O. hastatus	Brazil	6	20.01 ±2.36	55.6 ±9.94	1.39 ±0.198	0.083	0.111 ±0.019	44246	36687 ±5094	0.021	0.017 ±0.003
O. haematodus[a]	Ecuador	4	6.03 ±0.79	107.2 ±11.85	2.21 ±0.066	0.208	0.232 ±0.018	18331	15914 ±2056	0.014	0.010 ±0.003
O. latidens	Malyasia	4	28.82 ±2.22	383.3 ±36.6	2.18 ±0.127	0.192	0.216 ±0.016	20917	18476 ±1775	0.075	0.049 ±0.018
O. meinerti	Peru	7	7.4 ±0.67	75.8 ± 8.1	1.15 ± 0.05	0.128	0.128 ±0.013	46536	46536 ±5422	0.045	0.045 ±0.002
O. monticola	Taiwan	5	16.9 ±1.24	210.7 ±16.8	1.86 ±0.03	0.175	0.189 ±0.014	23638	21036 ±2667	0.033	0.029 ±0.006
O. relictus	USA	5	6.03 ±0.67	53.0 ±4.55	1.19 ±0.051	0.090	0.101 ±0.013	48626	43335 ±6534	0.027	0.021 ±0.007
O. ruginodis[a]	USA	5	5.66 ±0.54	51.8 ±6.94	1.05 ±0.024	0.100	0.104 ±0.009	42805	35757 ±5325	0.018	0.012 ±0.004
O. troglodytes	South Africa	10	4.91 ±0.45	58 ±0	1.57 ±0.067	0.113	0.134 ±0.016	36585	32547 ±2496	0.020	0.016 ±0.003
O. turneri	Australia	40	12.8 ±5.3	133.8 ±63.95	1.47 ±0.152	0.101	0.129 ±0.021	40529	30566 ±5806	0.084	0.032 ±0.021

[a] = Data from SPAGNA ET AL 2008 [b] = Data from PATEK ET AL 2006

Table 3.2. Scaling relationships between mandible strike characteristics and body size among species in the genera *Anochetus* and *Odontomachus*. Relationships were calculated with mean species values with ordinary least squares (OLS) and phylogenetically corrected generalized least squares (pGLS) with the indicated lambda.

	pGLS				OLS		
	lambda	**Intercept**	**Coefficient**	**P-value**	**Intercept**	**Coefficient**	**P-value**
Mandible mass	1	2.43	0.97	<<0.001	2.43	0.96	<<0.001
ω	1	11.11	-0.36	<<0.001	11.07	-0.34	<<0.001
E_{max}	0.893	-5.14	0.64	<<0.001	-5.15	0.66	<<0.001
t	0.957	-9.45	0.28	<<0.001	-9.46	0.22	<<0.001

ω = peak angular velocity, E_{max} = peak rotational energy, t = minimum strike duration

Table 3.3. ANCOVA of mandible strike scaling relationships within and between genera. The factor group has two levels, *O. turneri* and non-polymorphic trap-jaw ants.

response variable	Model	SS	F	P
ω	body size	4.53	90.281	<0.00001
	group	0.001	0.012	0.91194
	body size x group	0.371	7.392	0.00859
t	body size	1.7231	56	<0.00001
	group	0.0338	1.1	0.2986
	body size x group	0.1657	5.386	0.0238
E_{max}	body size	0.2	1.294	0.2598
	group	76.23	501.977	<0.00001
	body size x group	1.04	6.849	0.0112

Figure 3.1. Landmarks used for geometric morphometrics. (a)Yellow dots mark fixed landmarks and white dots mark sliding landmarks. (b) and (c) aligned landmarks for *Anochetus* and *Odontomachus*, respectively, after Procrustes superimposition.

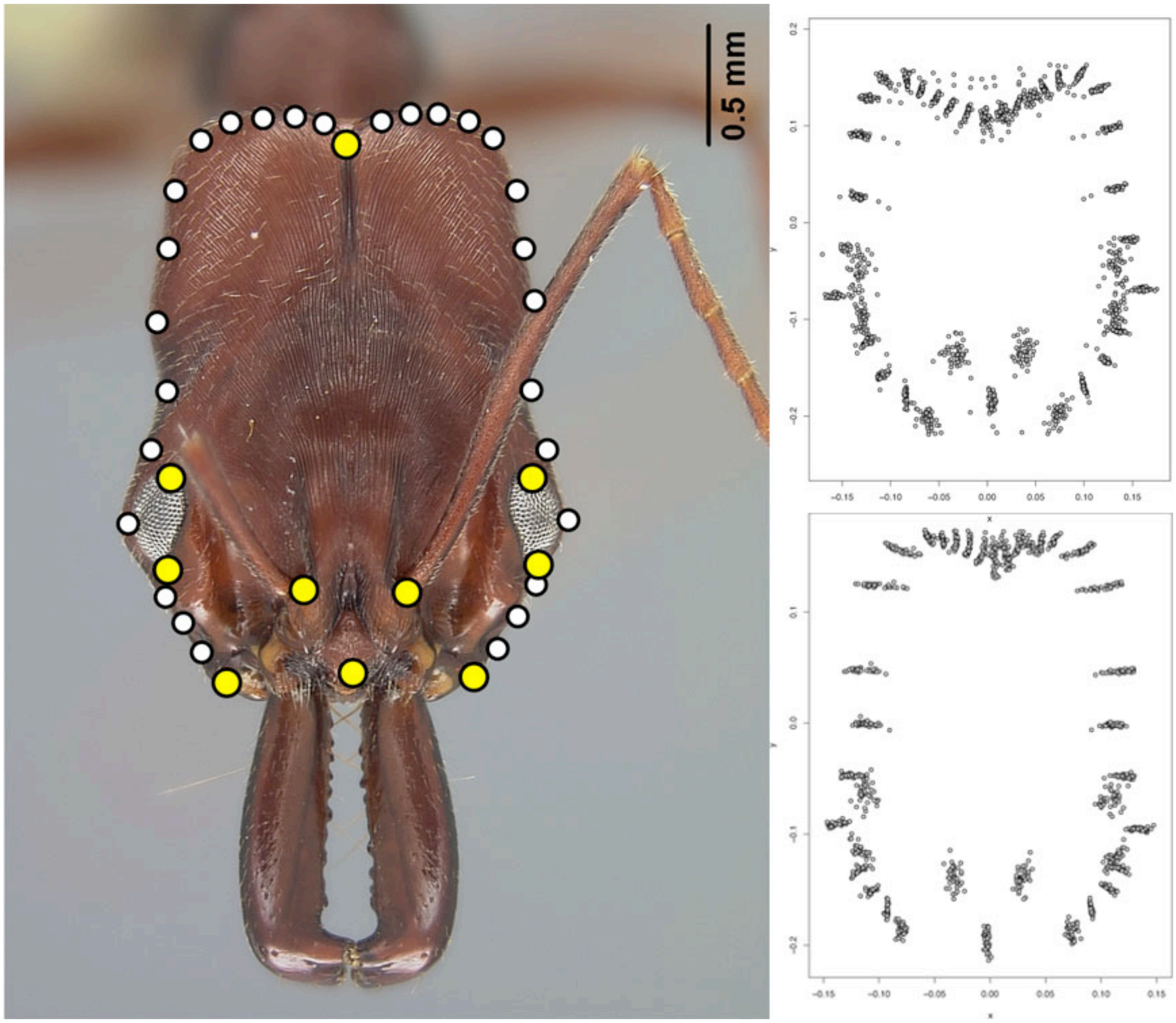


Figure 3.2. Phylogenetic tree used in comparative analyses. Trimmed version of the dated maximum clade credibility tree generated in Chapter 2.

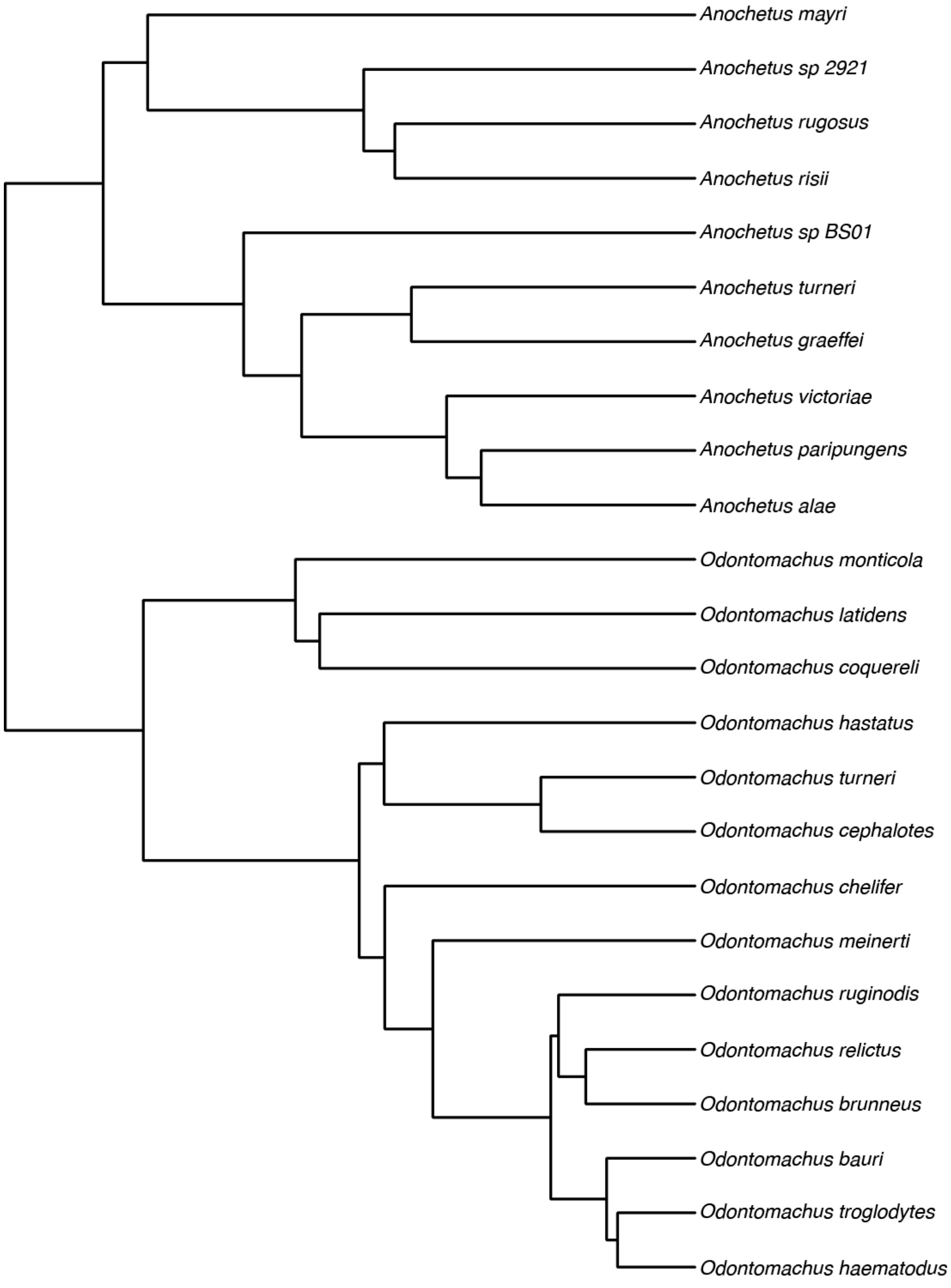


Figure 3.3. Scaling of mandible strike characteristics across species. Each graph shows mean values for each species. Blue dots are *Anochetus* and red dots are *Odontomachus*.

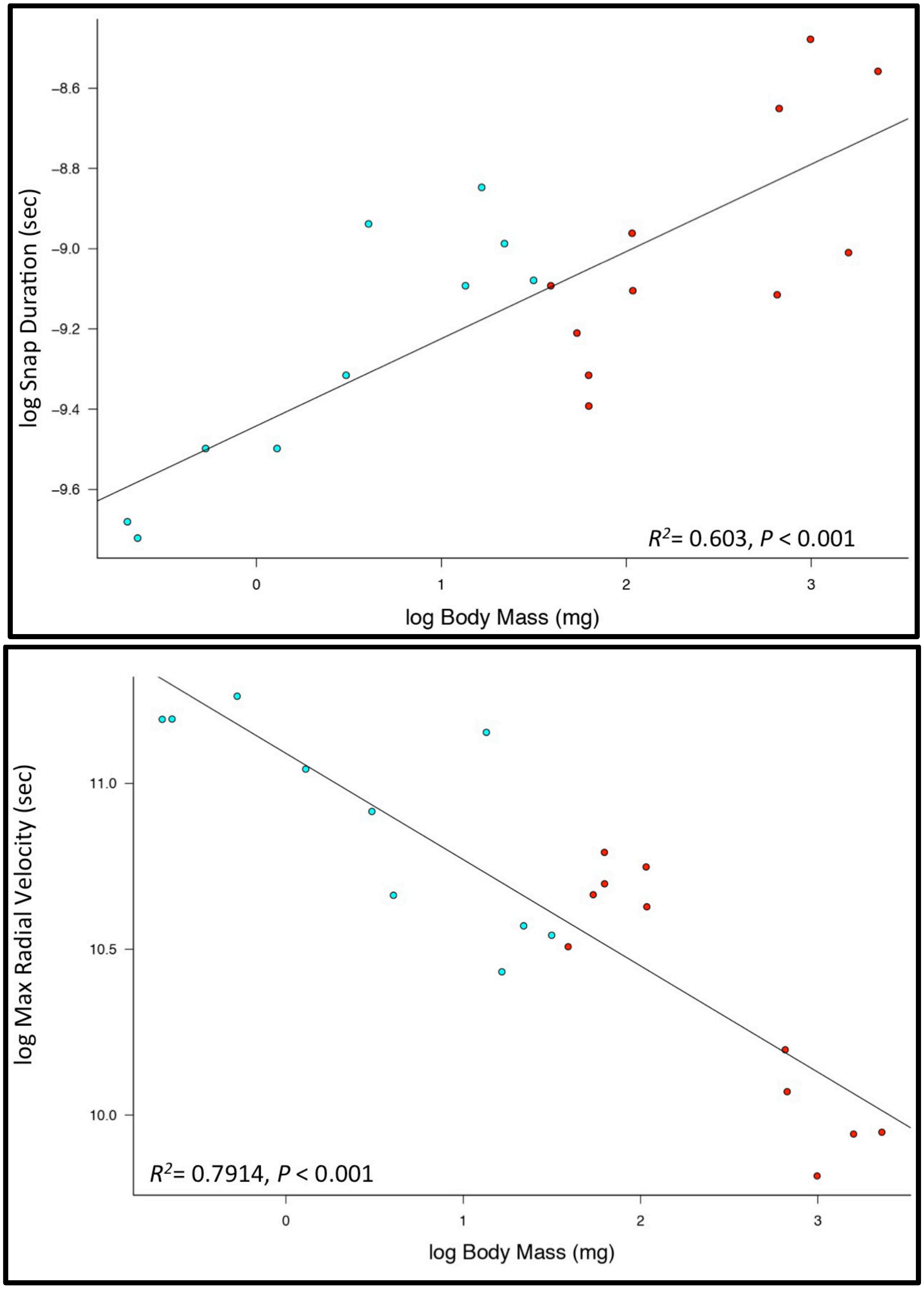


Figure 3.3 (cont.)

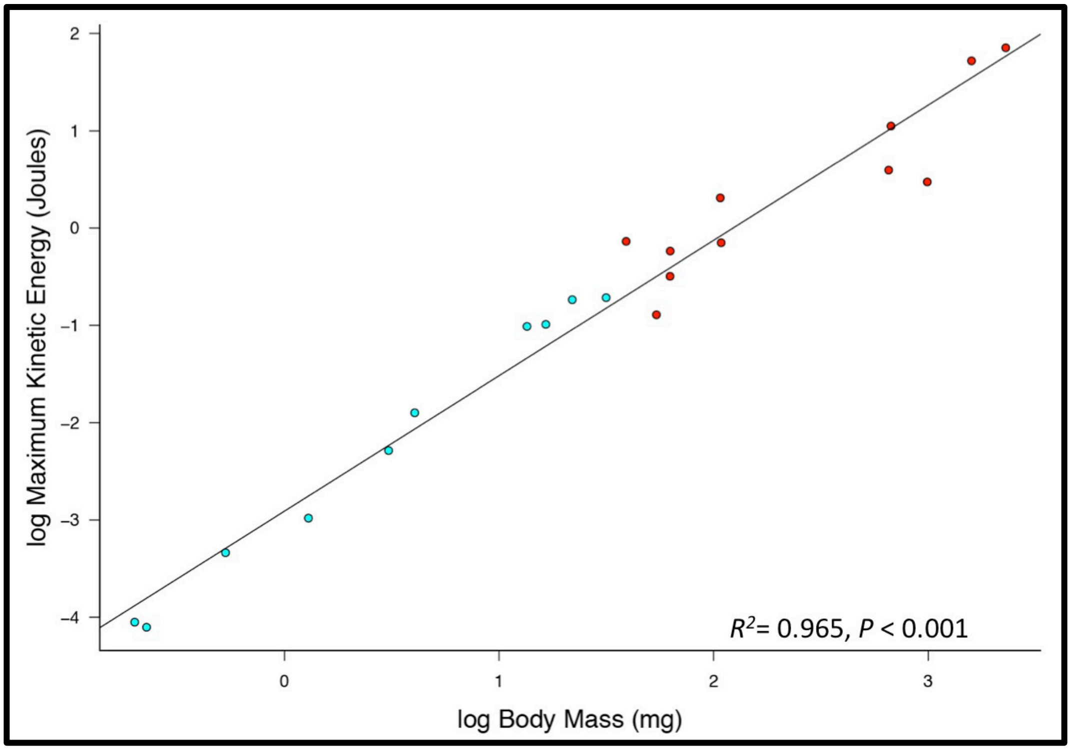


Figure 3.4. Morphological scaling relationships. (a) Principle component analysis of Procrustes residual, defining shapespace. TPS grids of extremes of PC1 are provided. (b) Allometric relationship of shape with body size (centroid size). Blue dots indicate *Anochetus* and red dots indicate *Odontomachus.*

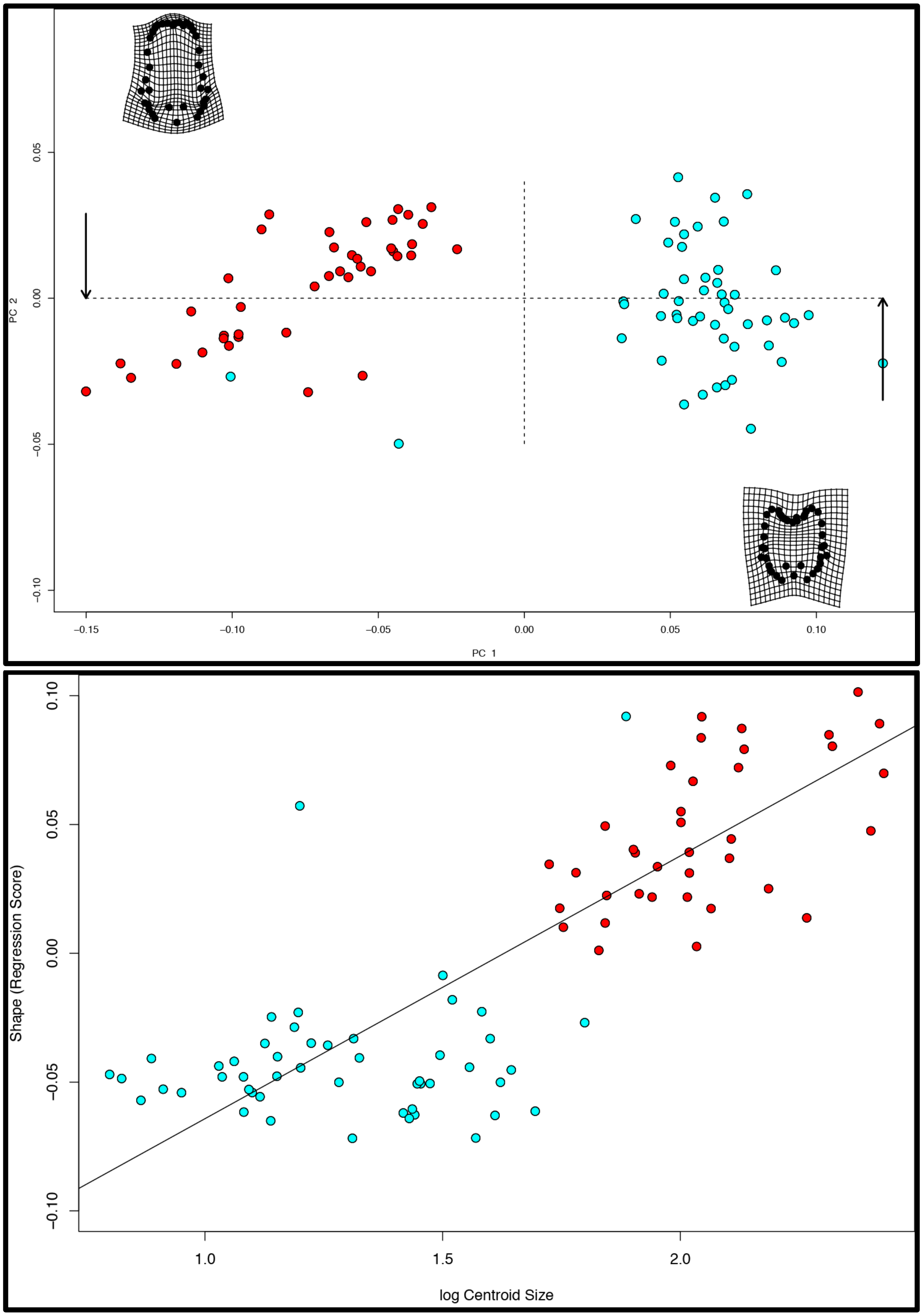


Figure 3.5. **Intraspecifc scaling relationships in *Odontomachus turneri*.** Log-transformed strike characteristics are plotted against log-transformed body mass. Trend lines are not shown in (a) or (b) because those relationships were not significant. In (c) the solid line is the trend line for *O. turneri* and the dashed line is for the interspecific relationships.

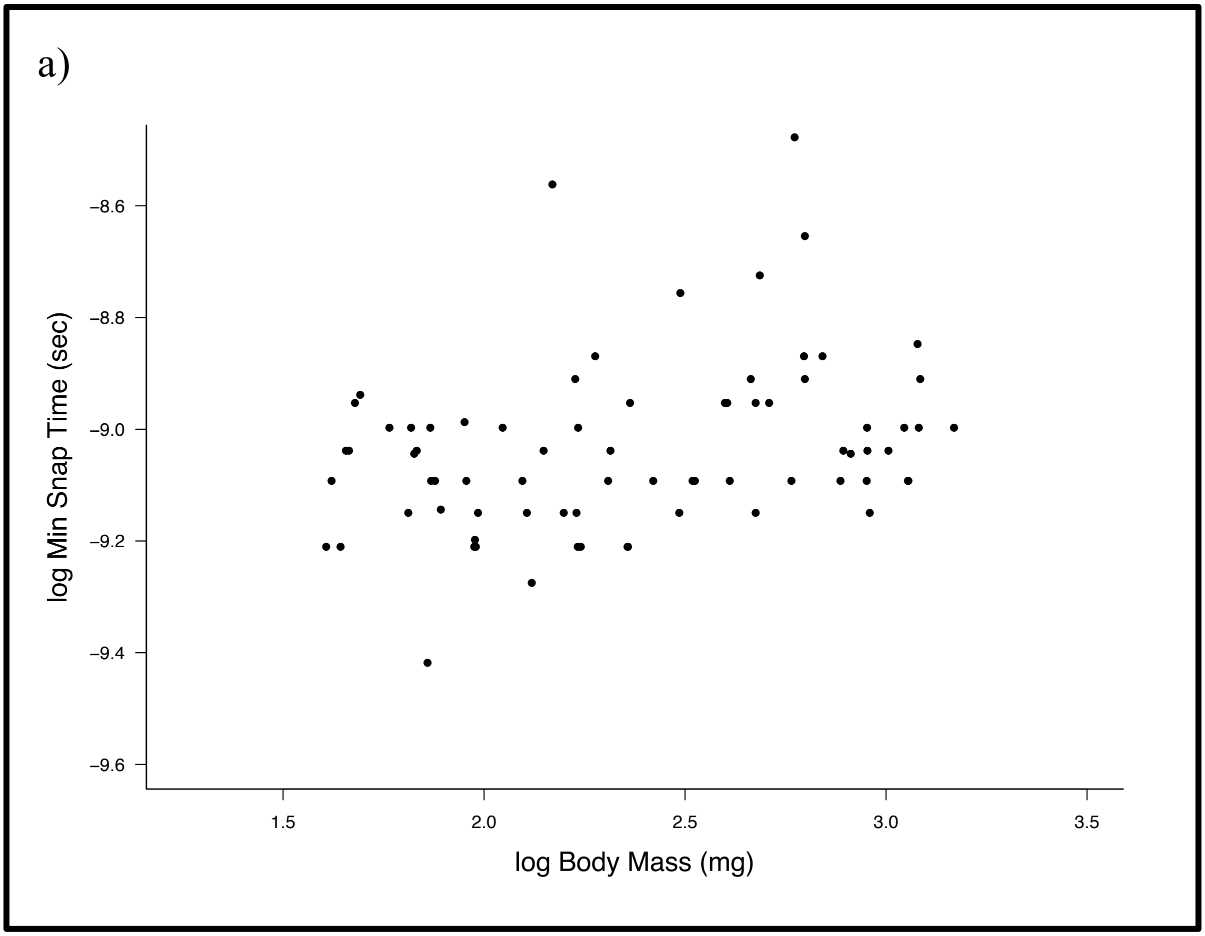
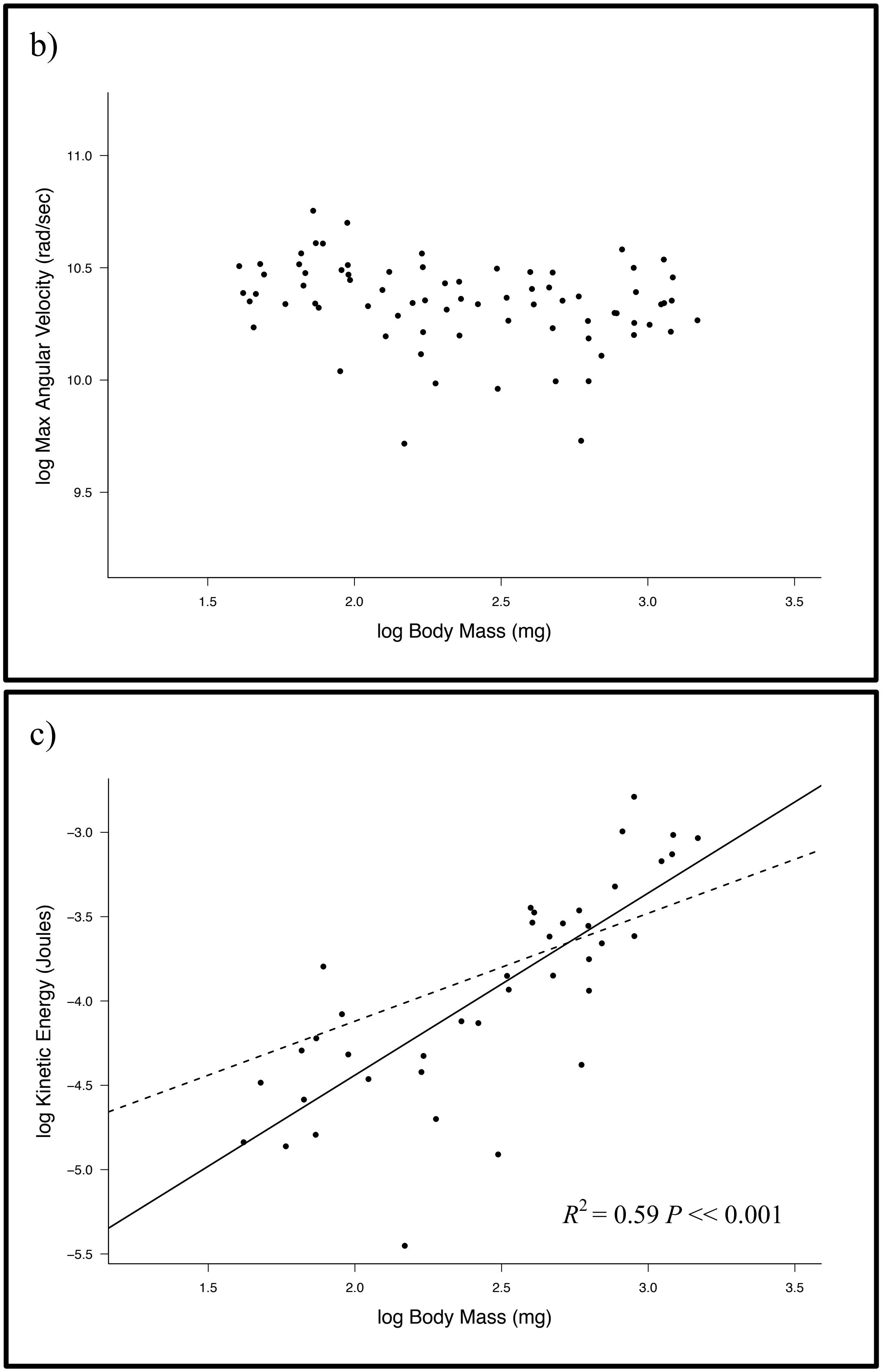


Figure 3.5. (cont.)



CHAPTER 4: THE SPRING-LOADED MANDIBLES OF THE TRAP-JAW ANT *MYRMOTERAS BARBOURI* (HYMENOPTERA: FORMICIDAE)

Abstract

Animals have convergently evolved spring-loaded appendages to overcome the limits on muscle contraction speed many times. Here, I describe the morphology and mechanism of another lineage of spring-loaded insects: trap jaws in the ant genus *Myrmoteras*. Like other trap-jaw ants, *Myrmoteras* have long thin mandibles that they snap shut to catch prey. Mandible strikes occur as fast as 0.50 ms and are powered by a very large mandible adductor muscle. The modified occipital lobe serves as a spring that stores potential energy when it is deformed during muscle loading. While the specific latch structure that keeps the mandibles open is still unknown, it may involve a modification of the mandible joint. The trigger that releases the strike is a subgroup of the closer muscle that is composed of fast fibers and rotates the mandibles out of the latch. *Myrmoteras* strikes are slower and less energetic than strikes of other trap-jaw ants in the genera *Anochetus* and *Odontomachus*, highlighting its unique and convergently evolved trap-jaw mechanism.

Introduction

Animals have repeatedly converged on similar design solutions for biomechanical problems, such as independently evolving wings for flight, eyes for image formation, and fins for swimming. One of the more spectacular examples of convergence is the repeated evolution of spring-loaded systems to overcome the limits of muscle contraction speed (GRONENBERG 1996a; PATEK ET AL. 2011; HIGHAM & IRSCHICK 2013). By incorporating latches and elastic elements into their appendage systems, many animals build up potential energy over the course of seconds or minutes, and release it almost instantaneously. These innovations allow animals to exceed the speed of direct muscle action and can determine the outcome of many predator-prey interactions (SCHARF ET AL. 2006; CARD 2012; DEVRIES ET AL. 2012). The champions of spring-loaded fast movements are the "trap-jaw" ants and termites that generate the highest velocity body movements ever recorded for an animal (PATEK ET AL 2006; SEID ET AL 2008; SPAGNA ET AL 2009).

Trap-jaws have evolved at least four times among three different ant subfamilies, and are used for predation and defense (reviewed in LARABEE & SUAREZ 2014). All trap-jaw ant lineages have evolved the same basic mechanism of power amplification, but have adapted different structures to act as the latch, spring, and trigger. Ants in the genera *Strumigenys* and *Daceton* from the subfamily Myrmicinae have a specialized labrum that blocks and releases the mandibles (GRONENBERG 1996). Ponerine trap-jaw ants in the genera *Anochetus* and *Odontomachus* have a modified mandible insertion that locks the jaws open during muscle loading, and a specialized fast trigger muscle that unlocks the mandibles from the joint and allows them to swing shut (GRONENBERG 1995A; GONENBERG 1995B). In the subfamily Formicinae, a single genus, *Myrmoteras*, has evolved trap-jaws. Despite some early fieldwork on *Myrmoteras* diet and behavior (MOFFETT 1985; MOFFETT 1986b), very little is known about their trap jaw mechanism because of difficulty of finding colonies and maintaining them in the lab. The goal of this study is to examine the mandible mechanism of the formicine trap-jaw ant*, Myrmoteras*, whose internal morphology and biomechanics are unknown.

Species of *Myrmoteras* display a combination of traits that are similar to and very different from other trap-jaw ants, obscuring the mechanism they use to spring-load their jaws —

assuming that they are indeed trap-jaw ants. They are small-bodied ants and have long thin mandibles that are equipped with well-developed teeth along the inner margin and can be opened beyond 180° (Figure 4.1). Studies on their foraging behavior and diet preference confirm that *Myrmoteras* use these mandibles like other trap-jaw ants to catch fast prey such as springtails (Collembola) (MOFFETT 1985; MOFFETT 1986b). However, their heads are small relative to their body size, suggesting that, unlike other trap-jaw ant groups, the proportional volume of their mandible adductor muscles is small (PAUL 2001). Their large eyes indicate that they are visual hunters, unlike most other springtail specialists that rely on chemical and mechanical cues (WILSON 1953; GRONENBERG 1996A). Finally, an entire subgroup of *Myrmoteras* is defined by the absence of trigger hairs, which are used by other trap-jaw ants to help release mandible strikes. Here, I present data on the morphology, strike behavior, and strike kinematics of *Myrmoteras barbouri* to quantify performance and elucidate the mechanism of power amplification. I also compare its strike kinematics to two species from other ant genera (*Odontomachus* and *Anochetus*) with similar performance but very different head morphology.

Materials and Methods

Study Organism

Four colonies of *Myrmoteras barbouri* were collected from Maliau Basin Conservation Area, in Sabah, Malyasia in August 2014. Colonies ranged from 10 to 25 workers and three included queens. In the lab, artificial nests were kept in plastic boxes coated with Fluon (Northern Products), and plaster-filled petri dishes or water-filled test tubes plugged with cotton served as nest chambers. Ants were given water and sugar *ad libitum* and fed live termites or frozen crickets three times a week. All ants were kept at 25° C and a 12-hour light-dark cycle.

Morphological Examination

X-Ray Microtomography (microCT) was used to examine the internal and external morphology of *Myrmoteras* head and mandible apparatus. MicroCT allows efficient and non-destructive imaging of internal organization of muscles, and cuticular structures, as well as 3D reconstruction of biological structures. Ants were fixed in alcoholic Bouin's solution for two days, gradually dehydrated in an ethanol series (70, 80, 90, 95 and 100% with 20 minutes between changes), and finally critical-point dried. Heads were affixed to wooden dowels with low melting point dental wax (Kerr Laboratory Products). MicroCT was performed with an Xradia MicroXCT-400 (Carl Zeiss X-ray Microscopy, Inc., Pleasanton, CA). Between 720 and 745 images were acquired with a connected CCD camera (exposure 10 seconds per image) while rotating the specimen 180º. The voltage and power of the x-ray beam were set to 40 keV and 4 W, respectively. Tomographic reconstruction was performed in Xradia XMReconstructor 8.1. Volume renderings, cross-sections, and surface models were produced in Amira 5.5.0 (FEI, Hillsboro, OR). All microCT imaging and image processing was conducted in the Imaging Technology Group at the Beckman Institute (University of Illinois, Urbana-Champaign).

High-Speed Videography

Mandible strike performance was measured with high-speed videography (see Chapter 3). Ants were restrained to the end of a #3 insect pin on the dorsal surface of the head with dental wax. Care was taken to avoid gluing any part of the posterior portion of the head, based on initial observations that the occipital lobe may be involved in the power-amplification mechanism. The pin was fitted onto a micromanipulator and filmed with one of two camera arrangements. To record the loading phase of the snap, ants were filmed at 1000 frames per second with a Photron 512-PCI camera using a Canon macro lens. To measure the kinematics of the mandibles during a strike, the ants were magnified with a Zeiss SteREO Discovery V20 stereomicroscope and backlit with an LED light (Visual Instrumentation Corp.). Magnified trap-jaw strikes were recorded with a Phantom v9.1 high-speed camera (Vision Research) with frame rates between 2–6x10^5 frames per second.

Ants were frozen at -20º C and their wet mass was recorded within one week of filming. Additionally, the wet mass of each mandible, head width and length, and mandible length were recorded. Linear measurements were made with a Semprex Micro-DRO digital stage micrometer (Semprex Corporation) connected to a Leica MZ 12.5 stereomicroscope. Masses were measured with a UMX2 microbalance (Mettler-Toledo).

Seven individuals were filmed and 3–6 strikes were recorded from each individual. The kinematic parameters of each strike were estimated in MATLAB R2013a (Mathworks, Natick, MA) (SPAGNA ET AL 2009, see Chapter 3). Positional coordinates of the mandible tip were used to derive the instantaneous velocity of the mandible throughout its snap trajectory. The minimum strike duration (in msec) and peak angular velocity were then estimated for each individual. Additionally, peak rotational kinetic energy, $E_{rotation}$, for each mandible during strike was calculated by modeling the mandible as a thin rod with a moment of inertia, I, of ($1/3MR^2$):

$$E_{rotation} = \frac{1}{6}MR\omega^2$$

where M is the mass of the mandible, R is the length of the mandible, and ω is the angular velocity of the mandible.

Species means for minimum strike duration, peak angular velocity, and peak kinetic energy were used for comparing the kinematic properties of *Myrmoteras* with *Odontomachus hastatus* and *Anochetus alae* (See Chapter 3). Differences among species were tested with a single-factor ANOVA with a post-hoc Tukey's test after log-transforming species means to meet the assumption of equal variance. All statistical tests were performed in R 3.1.3.

Results

Strike Observations and *External Morphology*

Observations in the lab confirmed other reports that the long linear mandibles of *Myrmoteras* are spring-loaded (MOFFETT 1986b). When fed small termites or disturbed with forceps, the ants open their jaws approximately 270º, and then smash them shut faster than could be detected by eye. Filming the jaw strike at 1000 fps confirmed that these jaw strikes occur in less than 1 ms, faster than can be accounted for by direct muscle action (Figure 4.2, Appendix 1) (GRONENBERG 1996a).

The mandibles are loaded from the open position approximately 5–10 seconds prior to a strike. Loading was detected in the high-speed videos by deformation of the occipital lobe and the slight dorsal rotation of the mandibles around the long axis of the body. The lateral side of the mandible base has a deep notch that accommodates an enlarged pleurostoma (Figure 4.1 (c) and (e), Figure 4.3 (f) and (g)) when the mandibles are fully open. Movement of the pleurostoma or another structure in the mandibular notch may be responsible for locking the mandibles open and their pre-strike rotation. As a result of contraction of the large mandible adductor (closer) muscle, the occipital lobe deflects inward, compressing approximately 8% (Figure 4.2). The adductor muscle originates on a thick sulcus that surrounds the occipital lobe (Figure 4.1.c). Deformation of this structure suggests that the occipital lobe serves as a spring for the mandible strike.

Internal Morphology

Despite the small size of the head of *M. barbouri*, the mandible adductor (closer) muscle takes up a significant volume of the head capsule (approximately 50 x 10^6 μm^3) (Figure 4.3). It originates from the posterior, ventral and lateral walls of the head and completely fills the occipital lobe. Like other ants, it inserts on the medial side of the mandible base via a thick apodeme, and contraction results in pulling a very short lever arm to rotate the mandibles closed. By virtually dissecting the mandible adductor muscles with microCT, I found that it composed of two different muscle groups whose contraction would pull the mandible in two different directions. The larger muscle group (*ad* in Figure 4.3 (d) and (e)) pulls down the long access of the head, much like other ant mandible adductor muscles. The smaller muscle group (*tr* in Figure 4.3 (d) and (e)) pulls ventro-laterally, and its action could result in a rotation of the mandible. This smaller muscle group also has much shorter sarcomere lengths (W. GRONENBERG, personal communication), suggesting that they are fast contracting muscles. Instead of acting as adductor muscles, this distinct muscle group may actually be trigger muscles that release the mandible from a latch.

The mandible abductor (opener) muscle, in contrast, is much smaller and occupies the ventral portion of the head capsule. It attaches to the mandible on the lateral side of the mandible base, and contraction results in opening the mandibles a full 270°. Reflecting the large size of their eyes, the optic lobes of *M. barbouri* also take up a large portion of the head volume. MicroCT does not have high enough resolution or contrast to visualize most neural tissue, and so it was not possible to reproduce the neuromorphology of *Myrmoteras*.

Kinematics

The average duration of a *Myrmoteras barbouri* strike was 0.58 ms (SD ± 0.27) with a minimum observed duration of 0.12 ms (Figure 4.4 and Appendix 2). Peak angular velocities ranged from 9.23×10^3 rad·s^{-1} to 2.58×10^4 rad·s^{-1} and the estimated peak accelerations were on the order of $10^4 \times g$. The peak rotational kinetic energy ranged from 4.12×10^{-10} J to 6.58×10^{-9} J (SD ± 2.23×10^{-9}). The mandibles accelerated very slowly through the beginning of the strike until they reached peak velocity at midline about three quarters through the trajectory, where they decelerated rapidly (Figure 4.4 (b)).

There was a significant difference in strike duration and peak velocity among the three trap-jaw ant species tested (ANOVA duration: $F = 11.4$, $P < 0.001$; velocity: $F = 13.7$, $P < 0.001$). Tukey post-hoc analysis showed that *Myrmoteras* strikes were significantly longer than both the large-bodied *Odontomachus hastatus* (P=0.045) and the similarly sized *Anochetus alae* (P<0.001) (Figure 4.x). However the longer strikes resulted in lower peak strike speeds only compared with *A. alae* (P<0.001). There was no difference in speed between *Myrmoteras* and *O. hastatus* (P=0.31). Although I detected significant differences in mandible mass and peak rotational kinetic energy among the three species (ANOVA mass: F=434.1, $P \ll 0.001$; $E_{rotation}$: F=86.5, P<0.001), *Myrmoteras* strikes are more similar to *A. alae* (Figure 4.6). Both *Myrmoteras* and *A. alae* mandibles are about one quarter of the size of *O. hastatus*. Consequently, they also experience significantly less peak kinetic energy (P<0.001), although not significantly different from each other (P=0.996).

Discussion

These data confirm that ants in the genus *Myrmoteras* are, indeed, trap-jaw ants, and that their spring-loaded mandibles move much faster than can be explained by direct muscle action. Although not all aspects of the mandible strike are clear, I suggest that the following descriptive model for *Myrmoteras* mandible strikes. In preparation for a strike, the ants open their jaw to a full 270°, where the pleurostoma slides into the mandibular notch and is responsible for locking the mandibles open by an as-yet-unknown mechanism. While locked open, the large mandible adductor slowly contract, deforming the occipital lobe, which acts as a spring and stores the potential energy to power the jaw strike. The mandibles are released by mechanical stimulation of the trigger hairs or some other visual cue, which activates the small fast trigger muscle that disengages the pleurotomal lock.

My kinematic data demonstrate that *Myrmoteras* mandible strikes do not display the same allometric relationship as *Anochetus* and *Odontomachus*. The speed and energy of ponerine trap-jaw strikes display a negative and positive, respectively, allometric relationship with body size (SPAGNA ET AL 2009; Chapter 3). Minimum strike duration was much longer for *M. barbouri* than both *A. alae* and *O. hastatus*. However peak velocity in *Myrmoteras* was lower than the similarly sized *Anochetus* and much less energetic than the equally fast *Odontomachus*. This difference is not entirely surprising because the spring-loaded mandibles of *Myrmoteras* were independently derived, whereas all ponerine trap-jaw ants use the same catapult mechanism. The slower speeds of *Myrmoteras* may be a result of their smaller adductor muscles, which take up only 20% of their head volume (vs. 70% in *Odontomachus bauri* (GRONENBERG ET AL 1997)). Also, *Myrmoteras* jaws accelerate more slowly than the ponerine trap jaw, likely because of a difference in efficiency of the unlatching mechanism. Despite their less energetic strikes and slower strikes, *Myrmoteras* jaws are still fast enough to catch springtails, whose escape jumps range in speed from 0.6 ms to 16 ms (CHRISTIAN 1978).

The difficulty of finding *Myrmoteras* ants in the field limited the number of live colonies that were collected. As a consequence there are several aspects of the mandible function that I could not examine, in particular the neural control and muscle activity of a strike. In other trap-jaw lineages the large mandible closer muscle contracts slowly in prior to a strike. The strike is released when trigger hairs are stimulated mechanically, which activates a fast trigger muscle responsible for unlocking the mandibles and allowing them to rotate shut (GRONENBERG 1996b; GRONENBERG ET AL. 1998; JUST & GRONENBERG 1999). The trigger muscles are regulated by large-diameter sensory and motor neurons and form very fast a monosynaptic reflex. My high-speed videos and morphological examination suggests that the large adductor muscle is responsible for mandible loading, and it is likely that *Myrmoteras* mandible control is similar to other trap-jaw ants. However, without thorough electrophysiological measurements of the muscle and neural components, the details of *Myrmoteras* trap-jaw control are still an open question.

The unique mandible mechanism employed by *Myrmoteras* trap-jaw ants highlights the evolutionary success of spring-loaded appendages. Trap-jaws have evolved at least four times in ants, and at least two other lineages (*Mystrium* and *Plectrotena*) employ spring-loaded “snapping jaws” to amplify the force of their mandibles (GRONENBERG ET AL 1998; DEJEAN ET AL 2002). It is unclear why so many independent lineages have converged on this same basic predatory strategy in a single insect family. One possibility is that terrestrial foraging in ants (rather than aerial predation, obligate herbivory, or pollination) has opened up niches that are more easily exploited with fast mouthparts than other strategies. Given the number of insects that employ rapid escape behaviors as antipredation adaptations (e.g. crickets, springtails, click-beetles), it is not surprising that predators have evolved specializations to counter act these adaptations. In addition to revealing the remaining details of trap-jaw mandible biomechanics, future research should focus on the selective pressures that have lead to the repeated evolution of power-amplified mandibles in ants.

Figure 4.1. Photomicrographs of *Myrmoteras barbouri*. (a) individuals forage with mandibles open beyond 180° (b) full face view of a worker with mandibles and occipital lobe visible (c) lateral view of head illustrating the size of the occipital lobe (d) and (e) display the whole mandible and mandible base, respectively. ol = occipital lobe, mn= mandibular notch, pl = pleurostoma. (a) used with permission from Alex Wild.

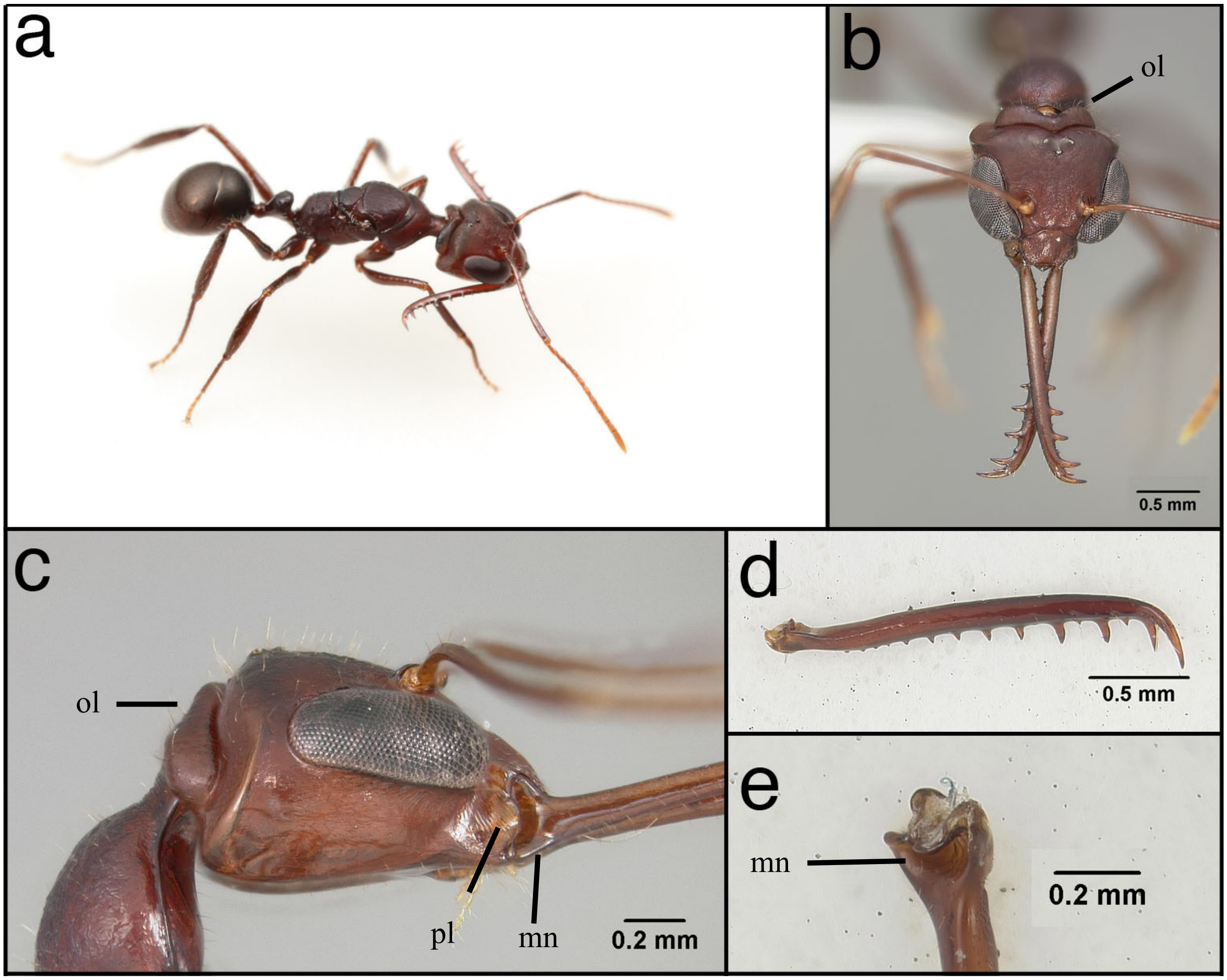


Figure 4.2. Stills from high-speed video of *Myrmoteras barbouri* mandible strike. Dorsal view of posterior margin of head, one frame before (a) and one frame after (b) strike is released. Arrow head indicates location of occipital lobe, which deforms during muscle loading. White line in (b) indicates where occipital sulcus was during loading in (a).
Scale bar = 1 mm.

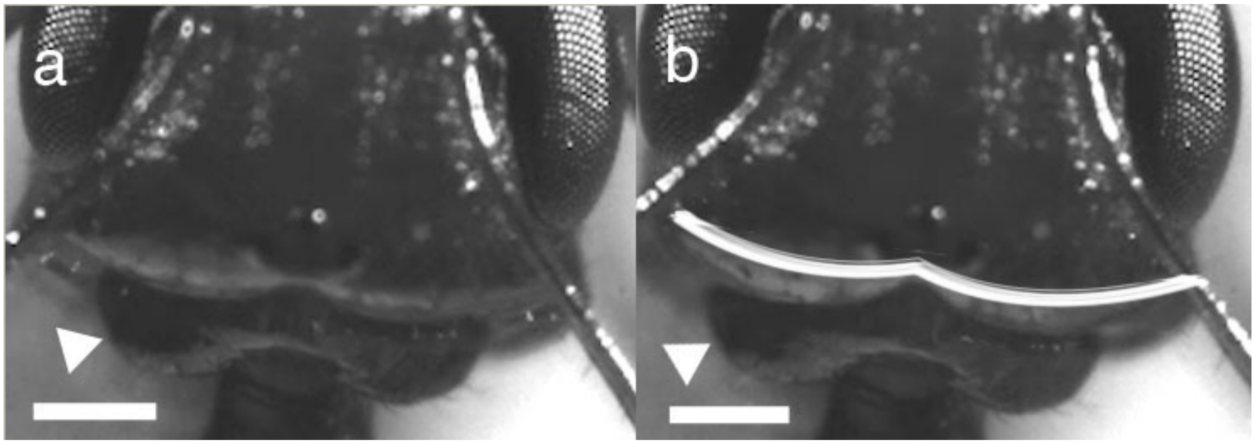


Figure 4.3. MicroCT images of *Myrmoteras barbouri* displaying the general organization of the mandibule adductor muscles. (a) virtual frontal section of the head. (b) volume rendering of the head, sectioned at the same location as (a). The mandible and its adductor muscle is highlighted in yellow. (c) surface model of the head exterior. (d) and (e) are volume renderings of virtual dissections of the mandible and adductor muscle in a dorsal (d) and transverse (e) orientation. (f) virtual frontal section with mandibles in the open position, showing the pleurostoma engaged with the mandibular notch.(g) volume rendering of mandible base illustrating the mandibular notch. ad= adductor muscle, ap = apodeme, t = trigger muscle, pl= pleurostoma.

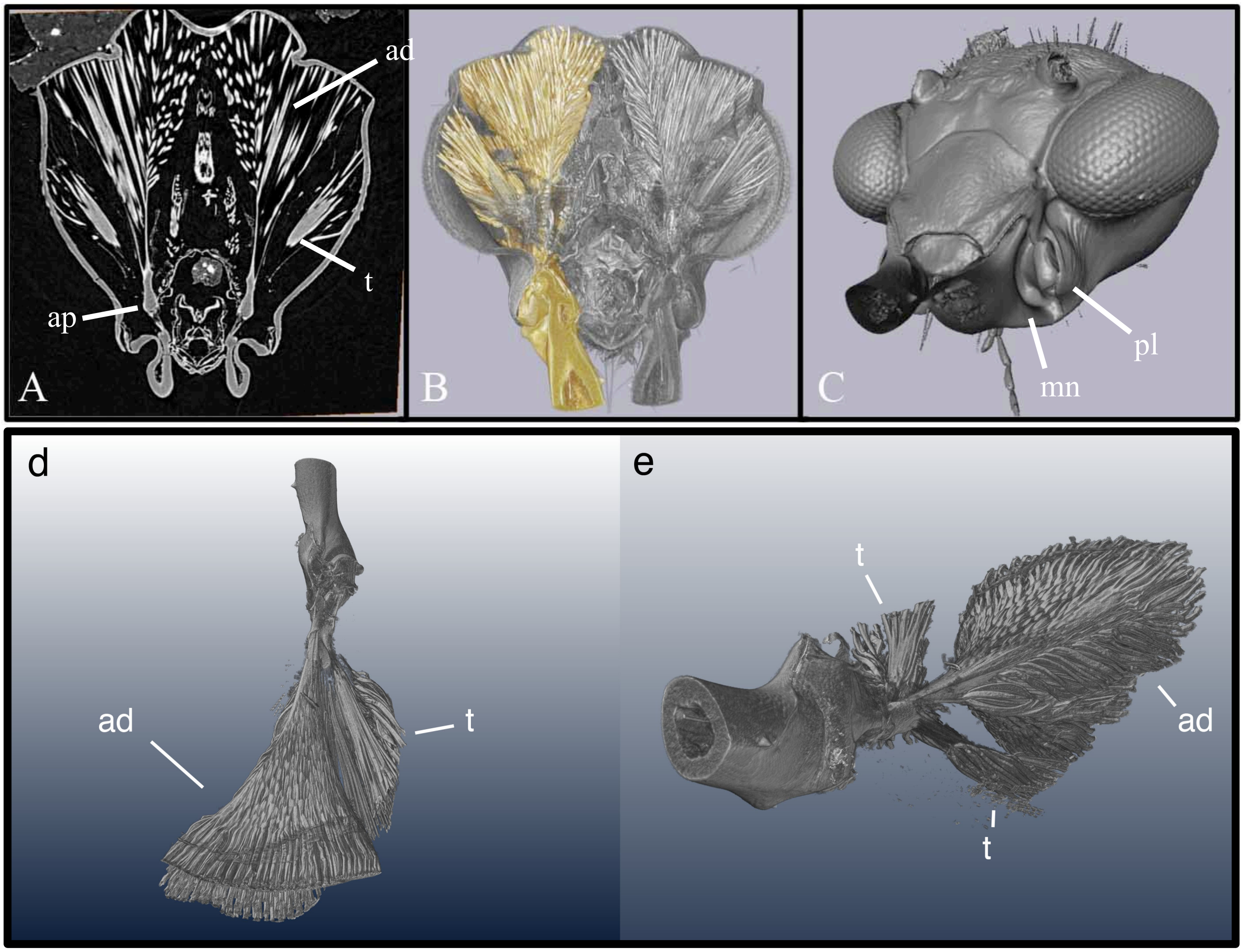


Figure 4.3. (cont.)

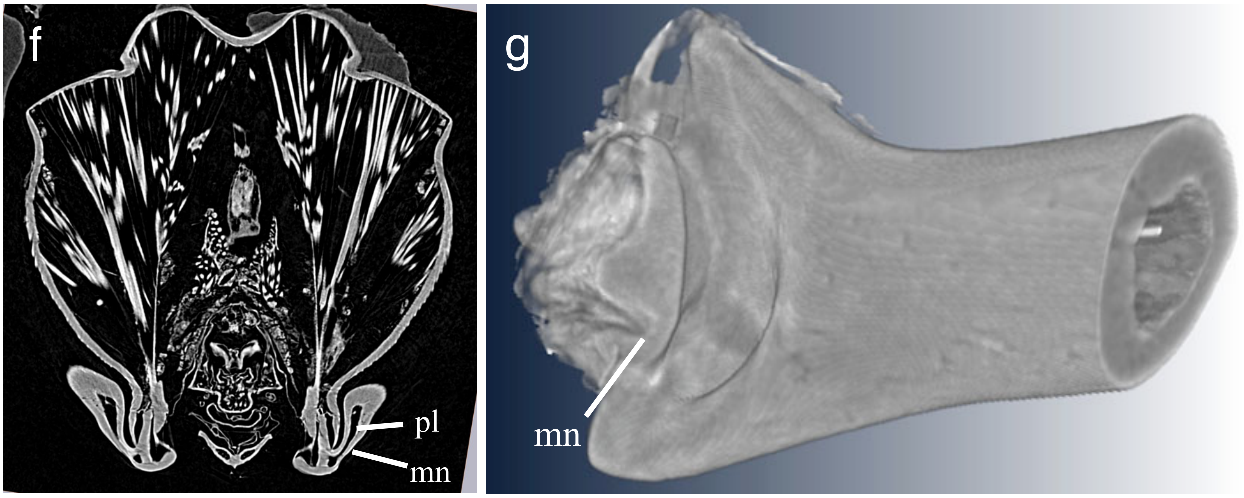


Figure 4.4. Trap-jaw strike of *Myrmoteras* mandible strike. (a) Stills from a high-speed video (5 x 10^4 fps). Scale bar = 1mm. (also see Appendix). (b) plot of absolute radial velocity over time.

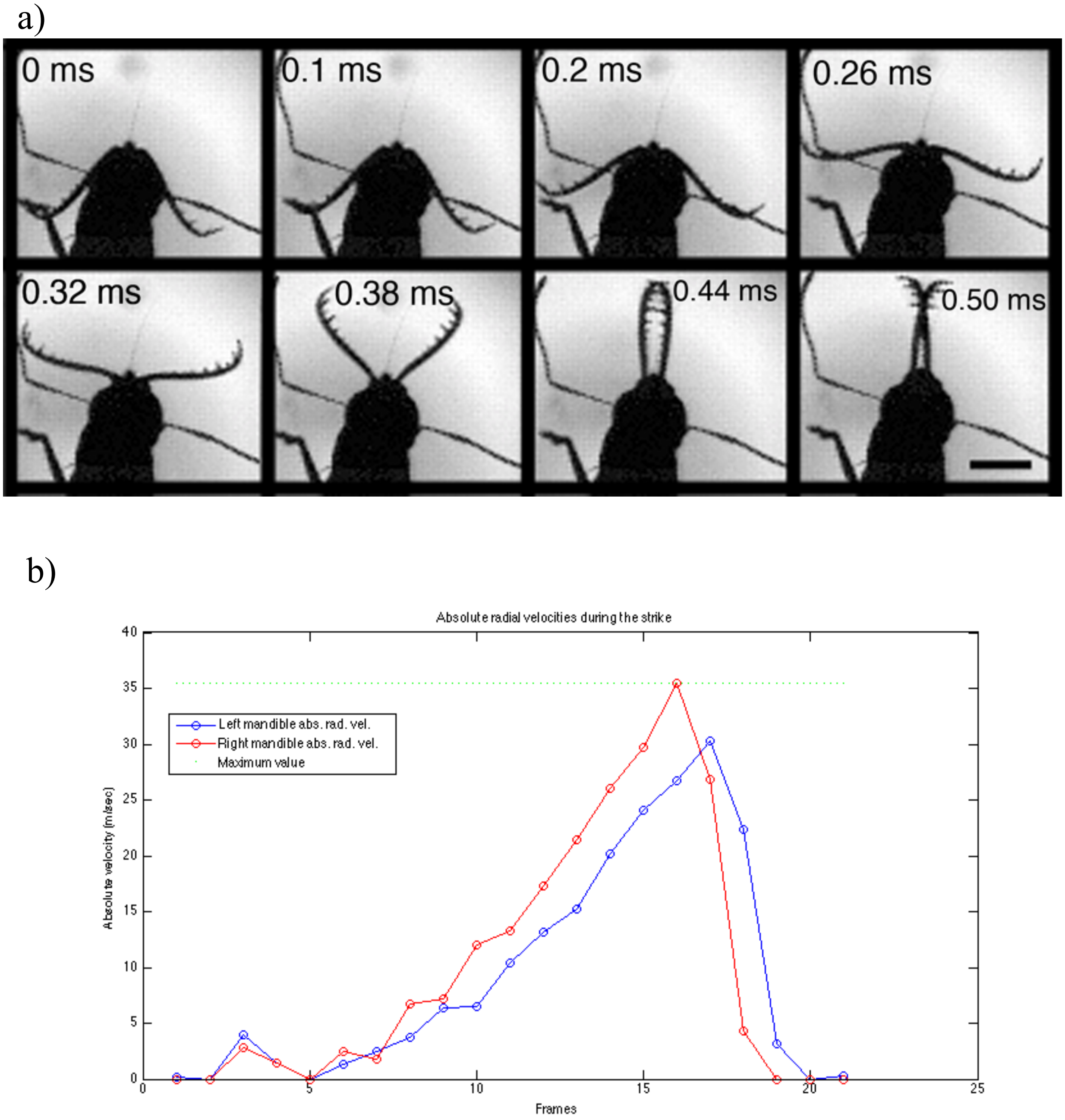


Figure 4.5. Comparison of strike duration and peak velocity among the trap-jaw ants *M. barbouri*, *A. alae,* and *O. hastatus.* Different letters correspond to significant differences between sample means at the 0.05 level.

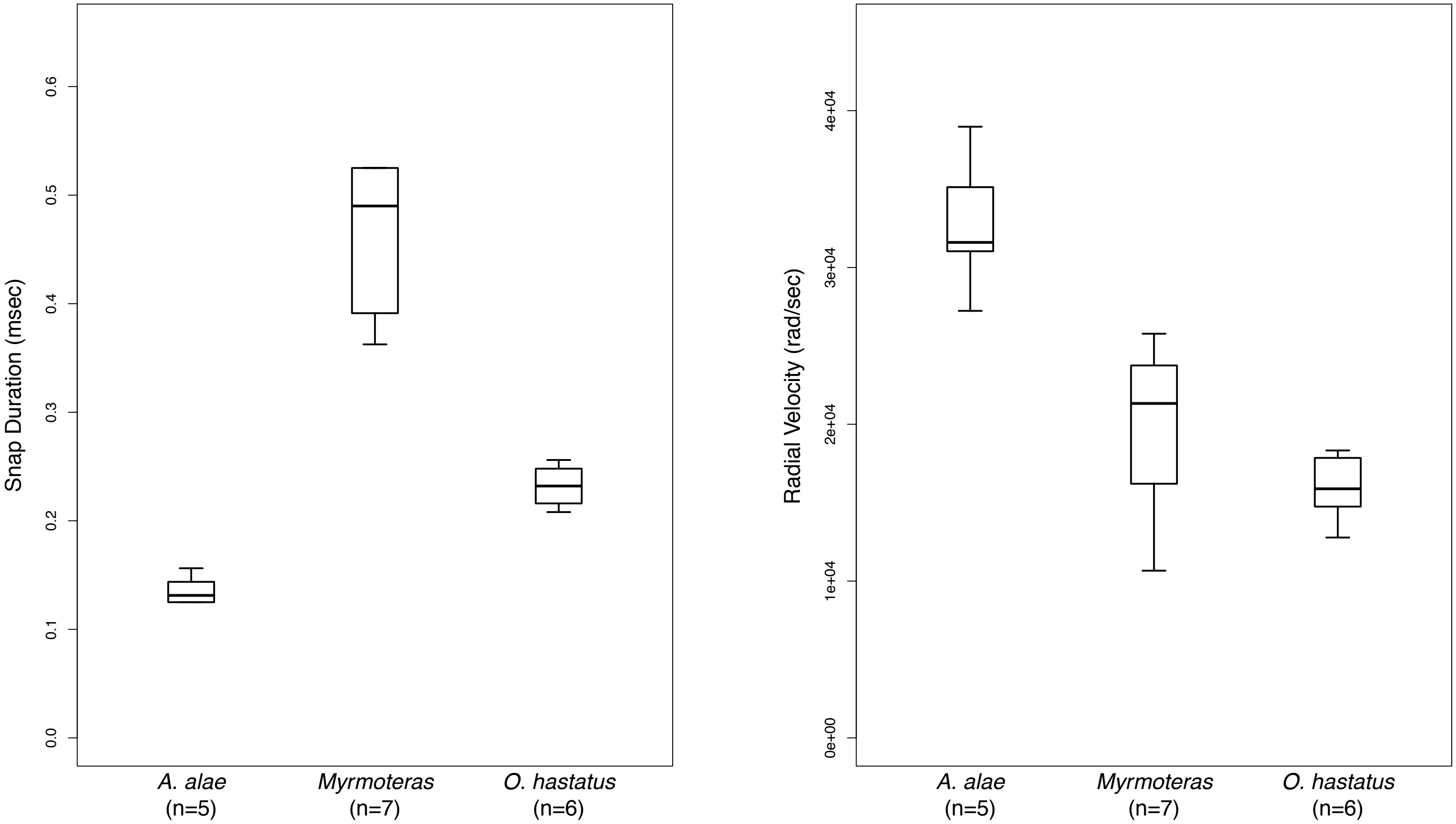
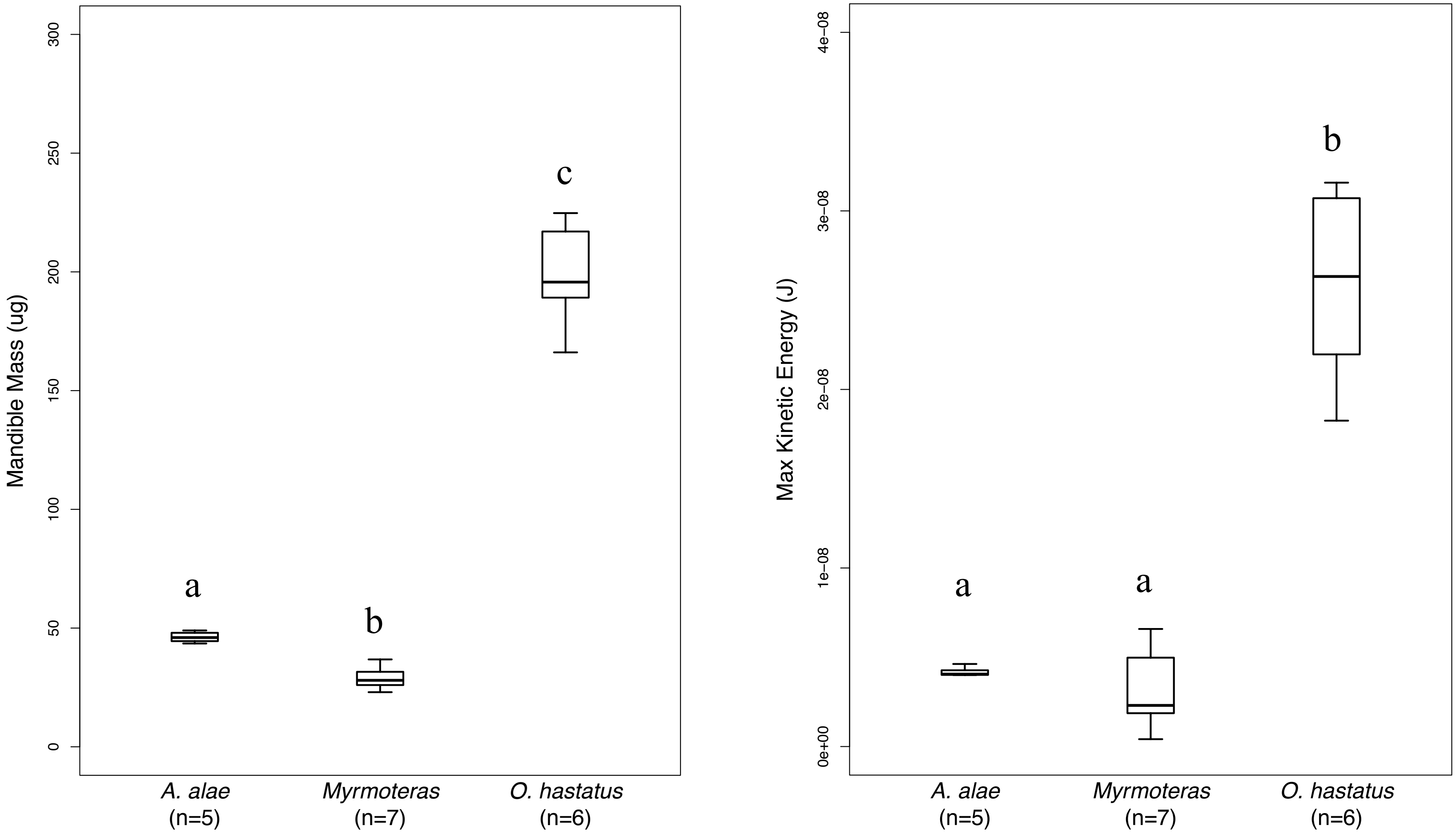


Figure 4.6. Comparison of mandible masses and maximum kinetic energy among the trap-jaw ants *M. barbouri*, *A. alae,* and *O. hastatus.* Different letters correspond to significant differences between sample means at the 0.05 level.



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APPENDIX: SUPPLEMENTAL VIDEOS

Appendix: Supplemental Video 1. High-speed video of *Myrmoteras barbouri* illustrating the loading of the post-occipital lobe prior to a strike. Filmed at 1000 fps. Can be found in the supplemental file **larabee_appendix_1.mov.**

Appendix: Supplemental Video 2. High-speed video of a mandible strike of *Myrmoteras barbouri* used to quantify kinematic traits. Filmed at 50000 fps. Can be found in the supplemental file **larabee_appendix_2.mov.**