

**THE EVOLUTIONARY ORIGINS OF VIBRATORY SIGNALS IN DREPANIDAE  
CATERPILLARS: A COMPARATIVE STUDY ON MORPHOLOGY,  
PHYLOGENETICS AND BEHAVIOUR**

by

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## ABSTRACT

Animal communication signals can be highly elaborate, and researchers have long sought explanations for their evolutionary origins. Animal communication theory holds that many signals evolved from non-signalling behaviours through the process of ritualization. Empirical evidence for ritualization is limited, as it is necessary to examine living relatives with varying degrees of signal evolution within a phylogenetic framework. I examined the origins of vibratory signals in Drepanidae caterpillars using comparative and molecular phylogenetic methods. I demonstrated that variation exists in morphology of signalling structures, general life-history characteristics related to signalling, and territorial behaviour by studying morphology in 19 species and behaviour in 11 species. I developed a molecular phylogeny of the Drepanidae onto which these characters could be mapped to test specific hypotheses on to the origin of signalling. These hypotheses included: 1) anal scraping derives from crawling towards an intruder; and 2) mandible scraping derives from lateral head hitting. My results support these hypotheses based on morphological, behavioural and kinematic data, thereby providing strong empirical evidence for the origins of communication signals. My thesis also demonstrates that vibratory communication is widespread and variable in this group of caterpillars, adding much needed information on this mode of communication in larval insects. Finally, I provide several lines of evidence to suggest that larvae that invest in leaf shelters defend these shelters from conspecifics using vibratory communication, which contributes to a growing body of information on this topic in caterpillars.

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## LIST OF PUBLICATIONS

### **This thesis forms the following published manuscripts:**

1. Scott, J. L., Matheson, S. M. & Yack, J. E. (2010). Variation on a theme: Vibrational signalling in the rose hook-tip moth caterpillar, *Oreta rosea*. *Journal of Insect Science* **10**, 54; available online: [insectscience.org/10.54](http://insectscience.org/10.54)

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3. Scott, J. L. & Yack, J. E. (2012). Vibratory territorial signals in caterpillars of the poplar lutestring, *Tethea or* (Lepidoptera: Drepanidae). *European Journal of Entomology*, **109**: 411-417.

*Statement of Contribution:* J. Scott collected and analyzed all of the data, prepared the figures and helped write the paper; and J. Yack developed the concepts and helped write the paper.

### **This thesis will also form the following manuscripts:**

4. Scott, J. L. & Yack, J. E. Caterpillars talk their walk: How vibratory signals evolved from crawling movements in caterpillars (Lepidoptera: Drepanidae) (in preparation for submission to the *Journal of Experimental Biology* in October, 2012)

*Statement of Contribution:* J. Scott collected and analyzed all of the data, prepared the figures and helped write the paper; and J. Yack developed the concepts and helped write the paper.

5. Scott, J. L., Kawahara, A. K., Skevington, J. H., Yen, S. -H., Sami, A., Smith, M. L. & Yack, J. E. Molecular phylogeny of Drepanidae (in preparation, journal to be decided)

*Statement of Contribution:* J. Scott sequenced most of the taxa, helped performed phylogenetic analyses, prepared the figures and helped write the paper; A. Kawahara helped with taxa and gene choice, provided specimens, helped with phylogenetic analysis, and helped write the paper; J. Skevington helped with phylogenetic analysis; S.-H. Yen provided specimens and helped with logistics; A. Sami helped sequence some of the taxa; M. Smith contributed to molecular data collection and phylogenetic analysis; and J. Yack helped develop concepts and write the paper.

## LIST OF ABBREVIATIONS

<b>Abbreviation</b>	<b>Full Name</b>
A	<i>abdominal segment</i>
ANOVA	<i>analysis of variance</i>
AS	<i>anal scraping</i>
B	<i>buzzing</i>
BS	<i>Bremer support</i>
CAD	<i>gene that encodes carbamoyl phosphate synthase II, aspartate carbamoyltransferase, and dihydroorotase</i>
CI	<i>consistency index</i>
COI	<i>cytochrome oxidase I</i>
COII	<i>cytochrome oxidase II</i>
CTAB	<i>ctrimonium bromide</i>
D (1, 2)	<i>dorsal seta (1 or 2)</i>
dB	<i>decibel</i>
df	<i>degrees of freedom</i>
dH <sub>2</sub> O	<i>distilled water</i>
DL	<i>dorsal longitudinal muscles</i>
DNA	<i>deoxyribonucleic acid</i>
dNTP	<i>deoxyribonucleotide triphosphate</i>
EDTA	<i>ethylenediaminetetraacetic acid</i>
EF-1 $\alpha$	<i>elongation factor 1 alpha</i>
EtOH	<i>ethanol</i>
GTR + G	<i>generalized time reversible + gamma model</i>
GTR + I + G	<i>generalized time reversible + proportion invariant + gamma model</i>
HMDS	<i>hexamethyldisilazane</i>
I	<i>intruder</i>
JKS	<i>jackknife support</i>
L	<i>treelength</i>
L (1-3)	<i>lateral seta (1, 2, or 3)</i>
LDV	<i>laser doppler vibrometer</i>
LHH	<i>lateral head hitting</i>
LT	<i>lateral tremulation</i>
LTH	<i>lateral tail hitting</i>
M	<i>molar</i>
MD	<i>mandible drumming</i>
mg	<i>milligrams</i>
MgCl <sub>2</sub>	<i>magnesium chloride</i>
mL	<i>millilitres</i>
min	<i>minutes</i>
mm	<i>millimetres</i>
MPT	<i>most parsimonious tree</i>
MS	<i>mandible scraping</i>

<i>n</i>	<i>sample size</i>
NaCl	<i>sodium chloride</i>
NADH	<i>nicotinamide adenine dinucleotide</i>
ND1	<i>NADH-dehydrogenase subunit 1</i>
nt3	<i>third codon position</i>
<i>P</i>	<i>probability</i>
PCR	<i>polymerase chain reaction</i>
PP	<i>posterior probability</i>
PP1	<i>posterior proctor seta 1</i>
PRM	<i>planta retractor muscle</i>
rDNA	<i>nuclear ribosomal DNA</i>
R	<i>resident</i>
rel.	<i>relative</i>
RI	<i>retention index</i>
rRNA	<i>ribosomal ribonucleic acid</i>
s	<i>seconds</i>
SEM	<i>scanning electron micrograph</i>
SD	<i>standard deviation</i>
SD (1, 2)	<i>sub-dorsal seta (1 or 2)</i>
SPL	<i>sound pressure level</i>
SV (1-4)	<i>sub-ventral seta (1, 2, 3, or 4)</i>
TBR	<i>tree bisection-reconnection</i>
V1	<i>ventral seta 1</i>
VL	<i>ventral longitudinal muscles</i>
28S D2	<i>D2 expansion segment of the 28S rRNA gene</i>
μL	<i>microlitre</i>
μm	<i>micrometres</i>
°C	<i>degrees Celsius</i>

**CHAPTER 1**

**GENERAL INTRODUCTION**

### *1.1 The evolutionary origins of animal communication signals*

Communication can play an important role in the survival and reproduction of all animals. Although there has been significant debate on a formal definition of animal communication (reviewed in Scott-Phillips, 2008; Carazo & Font, 2010), most authors agree that it should center around an adaptationist approach (as opposed to an informational approach; e.g. Dawkins & Krebs, 1978; Krebs & Dawkins, 1984; Grafen, 1990; Krebs & Davies, 1993; Hasson, 1994; Bradbury & Vehrencamp, 1998; Greenfield, 2002; Maynard Smith & Harper, 2003; Scott-Phillips, 2008) and that it must involve the transmission of a signal from a sender to a receiver. The most widely used definition of a signal was put forth by Maynard Smith & Harper in 2003 as: 'any act or structure which alters the behaviour of other organisms, which evolved because of that effect, and which is effective because the receiver's response has also evolved'. Recent reviews have suggested that this standard definition should be modified to include the stipulation that the signal is effective because the effect (the response) has evolved to be affected by the act or structure (and not simply evolved due to other factors) (Scott-Phillips, 2008) to distinguish communication from other phenomena, and that it is effective because it transfers (functional) information to receivers (Carazo & Font, 2010) to incorporate an informational approach.

Distinguishing signals from 'cues' has also been a topic of debate. A cue can be defined as 'any feature of the world, animate or inanimate, that can be used by an animal as a guide to future action' (Hasson, 1994). Authors distinguish cues from signals in three main ways: i) some believe cues are permanently 'on', while signals can be switched 'on' and 'off' depending on the circumstances (Hauser, 1996); and ii) that once a cue has been

produced, it costs nothing extra to express it, whereas signalling can impose additional costs (Hauser, 1996); iii) others agree that cues have not evolved to alter the behaviour of other animals (Galef & Giraldeau, 2001). This latter distinguishing feature concurs with Maynard-Smith and Harper's (2003) definition of a signal in that 'it has evolved for that effect' and suggests that signals are intentional, while cues are not. Signals that are conspicuous, highly redundant, stereotyped, and carrying alerting components (Wiley, 1983; Johnstone, 1997) are said to have undergone 'ritualization', an evolutionary process whereby cues are converted to signals (Tinbergen, 1952). These characteristics make signals more efficient by increasing the reliability of detection (Wiley, 1983; Johnstone, 1997). An increase in conspicuousness, such as an increase in the amplitude of an acoustic signal, can improve the chance a receiver will detect a signal, even in noisy environments. High redundancy, which can involve repeating a signal or using multiple signals for the same function, can reduce errors in the detection and recognition. An increase in stereotypy, such as a reduction in the variation of the duration of a signal, allows receivers to better distinguish signals from other similar behaviours. Finally, ritualized signals are often preceded by alerting components, a conspicuous component that alerts the receiver of the impending signal. For example, orangutans will violently throw tree branches to the ground, making a loud noise, before calling to conspecifics (Galdikas, 1979), and many lizards will begin head-bobbing signals with large amplitude, fast movements, followed by more subtle, species-specific movements (Fleishman, 1992).

Ethologists have been interested in the evolutionary origins of communication signals since Darwin's seminal book "The Expression of the Emotions in Man and

Animals". This paper motivated early ethologists such as Lorenz, Tinbergen and Huxley to start thinking about how animal communication signals have originated and evolved. It is hypothesized that many signals are derived from non-signalling behaviours, or cues, that have undergone ritualization (Tinbergen, 1952; Johnstone, 1997; Bradbury & Vehrencamp, 1998; Maynard Smith & Harper, 2003). Animals can provide cues to other individuals in a variety of contexts, and receivers may pick up on those associated with both physiological (thermoregulation, respiration, urination and defecation, pupil dilation, and yawning) and behavioural (intention movements, protective movements, redirection, and displacement behaviours) states of the signaler (Morris, 1956; Brown, 1975; Bradbury & Vehrencamp, 1998; Maynard Smith & Harper, 2003). For example, cues associated with preparing for flight in birds are often ritualized into many different types of signals, including alerting the flock of an imminent attack (Maynard Smith & Harper, 2003).

The process of signal evolution can be thought of as an evolutionary arms race between the signaler and receiver. Krebs and Dawkins (1984) suggested the notion of mind-reading and manipulation, whereby, simply put, the receiver acts as a mind-reader, anticipating the future behaviours of the sender using behavioural cues. Manipulation evolves as a response to mind-reading, whereby the sender exploits the mind-reading capabilities of the receiver to alter their behaviour. For example, a dog tends to uncover its teeth in preparation for a bite. If receivers are able to pick up on this cue, no matter how subtle, they can predict the future behaviour of the sender (an attack) and retreat. The sender, or manipulator, can then alter the behaviour of the receiver, by causing the receiver to retreat by simply baring its teeth. As such, baring teeth evolves as a signal of

aggression in dogs, and becomes more conspicuous, redundant and stereotyped through ritualization.

Many signals have been traced back to their non-signalling origins through a comparative analysis of behaviours. Early reports on signal origins tend to be quite anecdotal, where similarities in movements between a behaviour and a signal, often involving comparisons within a species, would suffice as evidence. For example, threat displays in Herring gulls, *Larus argentatus*, have been hypothesized to originate from behaviours associated with physical aggression. By comparing movements within a single species, it has been proposed that the 'upright threat posture' is derived from movements associated with striking an opponent, including a downward pointing of the bill and slightly raised wings, and movements involved in appeasement (Tinbergen, 1959). Researchers then began to expand their comparative analysis to include multiple closely-related species. One such study focuses on fiddler crabs from the genus *Uca*, which employ a variety of threat displays, involving the major cheliped, to defend burrows from conspecifics (Crane, 1966). By highlighting similarities between movements associated with territoriality between species, Crane suggests that fiddler crab threat displays derive from grasping movements, more specifically behaviours associated with seizing food, prey, or predators. Another study examining the evolutionary origins of a signal by looking at the variation in behaviours between closely-related species concentrated on the tail-fan display of the peacock, *Pavo cristatus*, which functions in courtship (Schenkel, 1956). Schenkel observed variation in courtship displays between species of Phasianidae, ranging from ground-pecking and offering food to females, mock-pecking and manipulation of food without presenting it to females, rhythmical

pecking followed by posing with the head bowed and tail feathers fanned, to a low bow and tail-fan with extreme tail elongation, as seen in peacocks. Although these behaviours do not constitute an evolutionary series as the phylogenetic relationship between species is unknown, it has been hypothesized that the peacock tail-fan display derives from pecking at the ground and offering food to females, and that multiple intermediate stages exist between the basal behaviour and ritualized signal. The former two studies provide more concrete evidence for the origins of a signal by comparing behaviours across many closely-related species, but specifics on the phylogenetic relationships between taxa were unknown. Phylogenetic information is important for studying the evolutionary origins of a signal, as it provides a framework onto which one can trace the evolutionary history of a behaviour. Most studies of this nature also do not attempt to characterize and compare kinematics of movements in any detail between signals and their basal behaviours, relying on superficial similarities to hypothesize on signal origins. Therefore, studies focusing on the evolutionary origins of signals that combine phylogenetic analysis with detailed comparisons of movements and behaviours across closely-related taxa are currently needed.

### ***1.2 Drepanoidea as a model system for studying signal origins***

The superfamily Drepanoidea, a large assemblage of moths containing more than 1400 described species (Minet & Scoble, 1999), provides an excellent model system for studying the origin and evolution of communication signals. In a previous study it was shown that the larvae of one species, *Drepana arcuata*, use vibratory signalling to resolve territorial disputes with conspecifics over silken leaf shelters (Yack et al., 2001). Solitary

late instar caterpillars occupying shelters produce three distinct signals - mandible drumming, mandible scraping and anal scraping - that escalate as the intruder approaches the resident. This was the first experimental study to demonstrate that caterpillars employ acoustic signals to advertise ownership of a territory. Although vibratory signals have only been studied in one Drepanidae species to date, there is abundant indirect evidence from various descriptive morphological reports (Nakajima, 1970; 1972; I. Hasenfuss, personal communication) and behavioural observations (Dyar, 1895; Federley, 1905; Bryner, 1999; Sen & Lin, 2002; I. Hasenfuss, personal communication; personal observations) that signalling and signalling structures are both widespread and highly variable in the Drepanidae. Previously documented territorial behaviours range from physical aggression, including biting and hitting (I. Hasenfuss, personal communication) to complex signalling, as in *D. arcuata* (Yack et al., 2001). This range of territorial behaviours has led me to the hypothesis that vibratory signals in the Drepanidae are derived from movements associated with more physically aggressive behaviors, including hitting, biting and pushing, perhaps to avoid the costs of physical damage. Due to the purported high degree of variation in behaviour and morphology within the Drepanidae, this system provides an excellent opportunity for testing hypotheses on the ultimate and proximate origins of communication signals. Drepanidae larvae are also ideal study organisms as they are widely distributed, several species have proven to be relatively easy to rear, and many build open shelters allowing for observations to be made without disturbance.

### *1.3 Acoustic communication in larval Lepidoptera*

An additional goal of this research is to provide some much needed general information on acoustic communication in caterpillars. Lepidoptera are highly successful constituents of most terrestrial ecosystems and include some of the most effective pests of economically important plants (Stamp & Casey, 1993). In order to fully understand the extent of their success, it is important to study all aspects of their biology, including how they communicate with other individuals in their environment. Caterpillars rely on communication at some point in their development to facilitate behaviours associated with foraging, defense, aggregation, shelter building, and/or competition for resources (Costa & Pierce, 1997; Fitzgerald & Costa, 1999; Cocroft, 2001; Costa, 2006). Despite the importance of communication, surprisingly little is known about the mechanisms used to broadcast and receive information in caterpillars (Costa & Pierce, 1997). There is evidence that several species, particularly those travelling in processions, use chemical and tactile cues for communication (e.g. Fitzgerald, 1995; Ruf et al., 2001; Fitzgerald & Pescador-Rubio, 2002; Colasurdo & Despland, 2005; Pescador-Rubio et al., 2011). Vision is unlikely to play an important role, as caterpillars have fairly simple eyes capable of discerning crude images only (Warrant et al., 2003). Lepidopteran larvae have also been shown to be capable of discriminating colours (Castrejon & Rojas, 2010), suggesting that they may use this sense to locate hostplants. However, a recent study demonstrates that this is not the case in the larvae of the Apollo butterfly, *Parnassius apollo* (Fred & Brommer, 2010). One sensory modality that remains relatively unexplored in caterpillars is an acoustic sense, and in particular, vibratory communication.

Acoustic communication in adult Lepidoptera has been broadly studied and serves a variety of social and defensive functions (Minet & Surlykke, 2003). Research on acoustic communication in larval Lepidoptera is currently limited, but there is increasing evidence that caterpillars use airborne communication during interactions with heterospecifics. Some caterpillars are capable of using filiform sensilla, sensitive to particle displacement, to perceive near-field airborne sounds produced by the wing-beats of approaching predators and parasitoids (Minnich, 1936; Tautz & Markl, 1978; Taylor, 2009 and references therein). Less is known about sound production in caterpillars, but recent studies and anecdotal reports have shown that silk and hawkmoth (Bombycoidea) caterpillars are capable of producing a variety of airborne sounds (Reed, 1868; Sanborn, 1868; Heinrich, 1979; Brown, 2006; Brown et al., 2007; Bura et al., 2009; Bura, 2010; Bura et al., 2010). These sounds can be produced using a number of mechanisms (reviewed in Bura, 2010) and have been found to function in predatory defense - as acoustic aposematism (e.g. *Antheraea polyphemus*: Brown et al., 2007; *Saturnia pyri*: Bura et al., 2009) or to startle vertebrate predators (e.g. *Amorpha juglandis*: Bura et al., 2011).

Acoustic signals communicated through solids (vibrations) are widespread in small herbivorous insects and are reported in at least 18 orders to date (Cocroft, 2001; Virant-Doberlet & Cokl, 2004; Cocroft & Rodriguez, 2005; Hill, 2009). These vibrations are mostly inaccessible to humans without specialized recording equipment, and therefore many vibratory signals in insects have yet to be described. In larval Lepidoptera there is increasing experimental evidence for vibrational communication in a number of species from different taxa. The functions of these signals include facilitating mutualistic

relationships with ants (Lycaenidae and Riodinidae butterfly larvae: (DeVries, 1990; 1991; Travassos & Pierce, 2000; Pierce et al., 2002) and advertising territorial ownership (Tortricidae: *Sparganothis pilleriana* (Russ, 1969); Drepanidae: *D. arcuata* (Yack et al., 2001), *Falcaria bilineata* (Bowen et al., 2008); and Gracillariidae: *Caloptilia serotinella* (Fletcher et al., 2006)). Beyond these examples, there is abundant inferential evidence for vibrational communication in caterpillars (e.g. Packard, 1890; Federley, 1905; Dumortier, 1963; Hunter, 1987), and the phenomenon is thought to be widespread. More research in this field is required to determine the extent and variation of vibrational communication in caterpillars.

#### **1.4 Thesis objectives**

The overarching goals of my research are two-fold: 1) to use the superfamily Drepanoidea to study the proximate and ultimate mechanisms involved in the evolution of communication signals; and 2) to provide novel information on vibratory signalling in different species of caterpillars. In order to test hypotheses on the evolutionary origins of signalling in the Drepanoidea, it is necessary to first gain an understanding of the extent of variation in territorial behaviour, signalling and signalling structures in this group. This will be the primary focus of Chapter 2, which will also provide much needed information on vibratory signalling in caterpillars. In Chapter 3, I will present a molecular phylogeny of the Drepanoidea that will be used in later chapters to test hypotheses related to the evolutionary origins of signals. By comparing morphology, behaviours, movements, and signal characteristics within a phylogenetic context, Chapters 4 and 5 will respectively test the hypotheses that the anal scraping signal derives from crawling, and that mandible

and other anterior body signals derive from physically aggressive movements involving the head and mouthparts. Finally, Chapter 6 will examine some of the ultimate questions that arose throughout the course of my studies, including: What is the function of signalling? Why produce more than one type of signal? Why signal instead of using physical aggression?

This study will be the first to resolve phylogenetic relationships within the *Drepanoidea* using molecular markers, and to use a combination of molecular phylogenetic, behavioural, and morphological data to provide evidence for the mechanisms underlying the evolution and ritualization of a signal from non-signalling origins. It will also advance our knowledge of the function and evolution of vibratory signalling in caterpillars in general, since little is known to date about this form of communication in larval holometabolous insects.

**CHAPTER 2**

**VARIATION IN MORPHOLOGY AND BEHAVIOUR ASSOCIATED WITH**

**VIBRATORY SIGNALLING IN DREPANIDAE CATERPILLARS**

**Parts of this chapter are included the following manuscripts:**

Scott, J. L., Matheson, S. M. & Yack, J. E. (2010). Variation on a theme: Vibrational signalling in the rose hook-tip moth caterpillar, *Oreta rosea*. *Journal of Insect Science* **10**, 54; available online: [insectscience.org/10.54](http://insectscience.org/10.54)

Scott, J. L., Kawahara, A. K., Skevington, J. H., Yen, S. -H., Sami, A., Smith, M. L. & Yack, J. E. (2010). The evolutionary origins of ritualized acoustic signals in caterpillars. *Nature Communications* **1**, 4; doi: 10.1038/ncomms1002.

Scott, J. L. & Yack, J. E. (2012). Vibratory territorial signals in caterpillars of the poplar lutestring, *Tethea or* (Lepidoptera: Drepanidae). *European Journal of Entomology*, **109**: 411-417.

Scott, J. L. & Yack, J. E. Caterpillars talk their walk: How vibratory signals evolved from crawling movements in caterpillars (Lepidoptera: Drepanidae) (in preparation for submission to the *Journal of Experimental Biology* in October, 2012)

## 2.1 Introduction

As indicated in Chapter 1, the purpose of this thesis is to study the evolutionary origins of vibratory communication in Drepanoidea caterpillars, as well as to expand the knowledge of the prevalence of vibrational signalling in larval Lepidoptera, since at present, little is known about this mode of communication in larval holometabolous insects. A previous study demonstrated that one species of Drepanoidea, *Drepana arcuata*, produces three signals (mandible drumming, mandible scraping, and anal scraping) during interactions with conspecifics; these signals function in territorial defense of silken leaf shelters (Yack et al., 2001). Based on my own preliminary observations, and indirect evidence from literature, there is evidence to suggest that vibratory signalling is not only widespread, but also highly variable within the Drepanoidea (see references below). I hypothesize that vibratory signals derive from more physically aggressive behaviours. The first step, and the goal of this chapter, is to characterize the diversity of behaviour and morphological characters related to signalling (or lack thereof) in representative species of the Drepanoidea.

The Drepanoidea comprises two families, Drepanidae and Epicopeiidae, that include species distributed throughout the Northern Hemisphere, but mostly in Palearctic Asia and the Orient (Minet & Scoble, 1999). The Drepanidae is a large assemblage of moths with approximately 120 genera including three subfamilies: Drepaninae, Thyatirinae, and Cyclidiinae (Minet & Scoble, 1999). Drepanidae larvae are mostly arboreal feeders that may be gregarious when young (Minet & Scoble, 1999). Various descriptive morphological reports (Nakajima, 1970, 1970; I. Hasenfuss, personal communication) and behavioural observations (Dyar, 1895; Federley, 1905; Bryner,

1999; Riegler, 1999; Sen & Lin, 2002; I. Hasenfuss, personal communication) have suggested that other species, in addition to *D. arcuata*, may produce vibratory signals, while some appear to lack the structures associated with at least one form of signalling. For example, based on morphological descriptions, it appears that the anal prolegs (those occurring on the last abdominal segment), can be fully formed, bearing crochets used for grasping the substrate; reduced, but still bearing crochets; or completely absent (Minet & Scoble, 1999), as we see in *D. arcuata*, which uses its anal appendage instead for signalling (Yack et al., 2001). Also, the morphology of a seta, used for signal production in *D. arcuata*, appears to vary between taxa (Nakajima, 1970, 1972; I. Hasenfuss, personal communication). Although mandibles have been implicated in signalling in some species (Yack et al., 2001; Sen & Lin, 2002; Bowen et al., 2008; I. Hasenfuss, personal communication), morphology of mandibles has not been described or compared between species. Finally, based on these preliminary reports and my own behavioural observations, there appears to be very interesting variation with respect to how different species interact with conspecifics; while *D. arcuata* exhibits vibration-mediated territorial behaviour (Yack et al., 2001), other species appear to be more physically aggressive (e.g. hitting, biting) (I. Hasenfuss, personal communication).

The variation in the morphology and behaviour associated with signalling or territorial encounters has not been formally documented in most Drepanoidea species, and to do so will be the purpose of this chapter (Chapter 2). This chapter is necessarily descriptive in nature, but forms an important basis for testing hypotheses in later chapters. The information from this chapter will be used in subsequent chapters (Chapters 4 and 5) that focus on the evolutionary origins of signals produced by the anal segment

and anterior segments, respectively. In this chapter I will also describe some life-history characters that may be relevant to territorial behaviour (e.g. egg-laying, gregarious or solitary behaviour of early and late instars, and shelter-building), and this information will be used in Chapter 6 to begin to answer ultimate questions on the evolution of signalling in Drepanidae larvae. In addition, the external morphology of setae on the abdominal prolegs, suggested to be putative vibration receptors (I. Hasenfuss, personal communication), has been noted, since one would expect variation in these structures to differ between those species that do and do not use vibrational communication.

I have collected information on as many species as possible, representing all three subfamilies, from both my own experiments with live caterpillars, and from collections of preserved specimens, as well as from previous literature cited in this introduction. Due to the large amount of data collected on multiple species, I have selected four species that represent all three subfamilies, and exhibit the range of morphology and behaviours that were documented in different species across this study, to describe in detail. Specific details of other species are summarized in tables within this chapter, in subsequent chapters that focus on the origins of different signals, as well as in an appendix (Appendix A). This chapter will be mainly descriptive, and the results will be used to test hypotheses in other chapters.

## ***2.1 Methods***

### ***Animals***

Living and preserved larvae used in this study were obtained from a variety of sources (Table 2.1); as well, some information was obtained from the literature (see

Tables 2.2, 2.3). When species were reared from eggs, gravid females were collected from the wild at ultraviolet collecting lights and females oviposited on cuttings of their respective hostplant. Larvae were reared indoors on cuttings of their hostplant under a L:D 18:6 h photoperiod at 21-26°C in an insect rearing facility. When possible, early instars (1-2) were studied for life-history traits. Late instars (3-5) were studied for life-history traits, as well as their morphological and behavioural characteristics.

#### *General life history observations relevant to conspecific interactions*

Selected life history traits were documented if they were deemed to be relevant to conspecific interactions. These included notes on egg-laying behaviour (whether adults lay eggs in rows, groups or singly), gregariousness as early or late instars, shelter-building behaviours as late instars (type of shelter, including no shelter, mat of silk, folded/rolled leaf, or two leaves sewn together), and hostplants. In 11 species I obtained most of this information from live specimens, and in others, from the literature (see Table 2.2).

#### *Morphology*

External morphology of the anal segment (abdominal segments 7-10), mandibles, head, and abdominal prolegs were examined in larvae preserved in 80% ethanol in 19 species (using between one and five specimens per species). Drawings of the anal segment and abdominal prolegs were made using a drawing tube (attached to a Wild Heerbrugg M7A microscope; Aargau, Switzerland). Setae of the anal segment were identified and labeled following the nomenclature described by Stehr (1987).

**Table 2.1.** Sources of living and preserved specimens used for morphological and behavioural data.

Taxon	Morphology		Live Larvae for Behaviour	
	Origin	Collector	Origin	Collector
<b>INGROUP TAXA</b>				
<b>Cyclidiinae</b>				
<i>Cyclidia substigmata</i> <i>substigmata</i>	Chuncheon, Gugok-Pokpo, Gangwong Province, Korea	J. C. Sohn	China	S-H. Yen
<b>Drepaninae</b>				
<i>Cilix glaucata</i>	Erlangen, Northern Bavaria, Germany	I. Hasenfuss	NA	NA
<i>Drepana arcuata</i>	Ottawa, Canada	J. Yack	Ottawa, Canada	various collectors
<i>Drepana curvatula</i>	Netherlands	S. Corver & T. Muus	Netherlands	S. Corver & T. Muus
<i>Drepana falcata</i>	Erlangen, Northern Bavaria, Germany	I. Hasenfuss	Netherlands	S. Corver & T. Muus
<i>Falcaria bilineata</i>	Ottawa, Canada	J. Yack	Ottawa, Canada	various collectors
<i>Falcaria lacertinaria</i>	Erlangen, Northern Bavaria, Germany	I. Hasenfuss	NA	NA
<i>Oreta rosea</i>	Ottawa, Canada	L. Scott	Ottawa, Canada	L. Scott
<i>Tridrepana flava</i>	Taiwan	J. Heppner	NA	NA
<i>Watsonalla binaria</i>	Erlangen, Northern Bavaria, Germany	I. Hasenfuss	NA	NA
<i>Watsonalla cultraria</i>	Erlangen, Northern Bavaria, Germany	I. Hasenfuss	Switzerland	J. Miall
<i>Watsonalla uncinula</i>	Boulu, Pyrenees, France	H. Beck	NA	NA
<b>Thyatirinae</b>				
<i>Euthyatira pudens</i>	Unknown	Unknown	NA	NA
<i>Habrosyne pyritoides</i>	Erlangen, Northern Bavaria, Germany	I. Hasenfuss	NA	NA
<i>Ochropacha duplaris</i>	Sipoo, Finland	K. Silvonen	Sipoo, Finland	K. Silvonen
<i>Pseudothyatira cymatophoroides</i>	Unknown	Unknown	NA	NA
<i>Tethea or</i>	Sipoo, Finland	K. Silvonen	Sipoo, Finland	K. Silvonen
<i>Tetheela fluctuosa</i>	Sipoo, Finland	K. Silvonen	Sipoo, Finland	K. Silvonen
<i>Thyatira batis</i>	Sipoo, Finland	K. Silvonen	Sipoo, Finland	K. Silvonen

Photographs were obtained with an Olympus dissection microscope (SZX12; Olympus, Japan) equipped with a Zeiss camera (AxioCam MRc5; Zeiss, Germany), or with a digital camera (various models; Nikon, Japan). Whole caterpillars, anal segments, and mandibles were prepared for scanning electron microscopy by air drying, critical point drying (Bio-Rad Polaron Division; Watford, England), or using HMDS (hexamethyldisilazane) (Rumph & Turner, 1998). Dried specimens were sputter-coated with gold-palladium and examined using a JEOL (JSM-6400; Tokyo, Japan) or a Tescan Vega-II scanning electron microscope (XMU VPSEM; Brno, Czech Republic). Morphological characters for another 20 species were obtained from the literature (see Table 2.3). Although plasticity between individuals was observed for some of these morphological characters, characters were assigned to each species using the best of my knowledge and information was confirmed in the literature when possible.

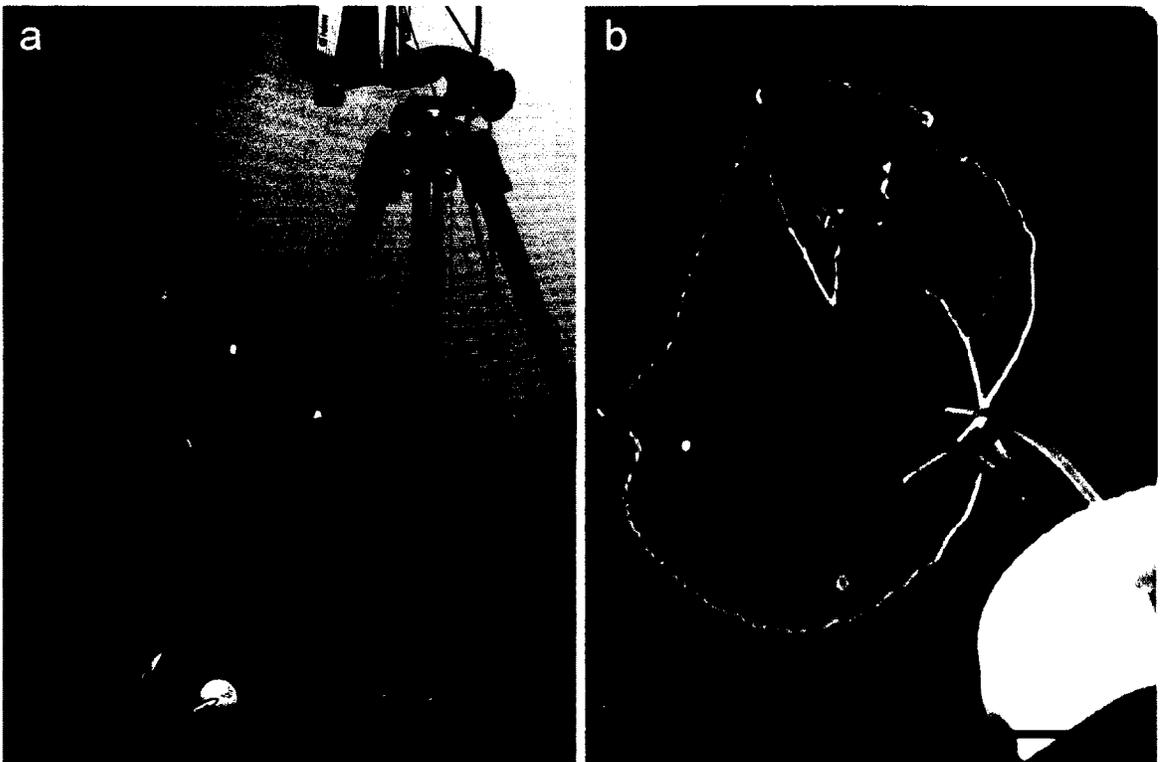
#### *Behavioural trials between conspecifics*

In order to document the diversity of behaviours and associated vibrations that occur during interactions with conspecifics, encounters were staged between a resident and an introduced conspecific intruder in 11 species (using between 3 and 50 individual residents, depending on the species) representing all three sub-families of Drepanidae (Table 2.1). A late instar larva was selected at random and matched with another larva of approximately the same size, as it was shown in a previous study (Yack et al., 2001) that differences in resident and intruder weights affects the outcome of trials. A 'resident' was placed on a leaf of a twig and left undisturbed for at least 60 minutes prior to the trial to construct a shelter. Leaves were selected based on size and the absence of feeding scars,

or other types of leaf damage. A fresh leaf was used for each trial. Once the caterpillar was established, the twig of the caterpillar's hostplant was stripped of all leaves except the occupied leaf, and the twig was cut to a length of 8-12 cm and placed in a water-filled vial through a hole in its lid. The resident was left to settle for a minimum of 10 min immediately before the trial. During the trial, the vial containing the twig and occupied leaf was held in position with a clamp such that the larval interaction could be viewed with a video camera (Fig. 2.1a). In species whose leaf shelters were made between two leaves, which prevented me from observing behaviour directly, a light was shone through the leaves to observe the outlines of the residents (Fig. 2.1b). Prior to the trial, intruders were isolated in a container with bare twigs for 15-20 min. Residents were videotaped for at least 1 min before the intruders were introduced to determine if signals were produced in the absence of an intruder. Using a paintbrush, an intruder was carefully transferred to the twig a few cm below the point where the petiole attaches to the twig, minimizing mechanical disturbance. Trials were videotaped until 1 min after one contestant left the leaf (i.e. when one contestant 'won' the encounter). If there was no winner within 30 min, the trial was deemed a "tie". This time was chosen based on previous trials with another species, *D. arcuata* (Yack et al., 2001). Caterpillars were not reused in subsequent trials. All trials were monitored simultaneously with a Sony High Definition Handicam (HDR-HC7; Tokyo, Japan) and remote Sony audio microphone (ECM-MS907) placed 1-2 cm behind the leaf and/or a laser-doppler vibrometer (LDV; Polytec PDV 100; Walbronn, Germany). Behaviour for one species, *Drepana arcuata*, was collected and analyzed in a previous paper (Yack et al., 2001).

In addition to direct recordings of live species, some behavioural characters were

**Figure 2.1.** Experimental set-up for behavioural trials. **(a)** General set-up with LDV. The leaf (arrow), in a water-filled vial is held in place by a clamp, and recorded with a videocamera and LDV (scale bar = 6 cm). **(b)** Trial set-up in a species that lives in the space between two leaves. A light is shone through the leaf in order to see the outline of the resident (arrow; scale bar = 2.5 cm).



obtained through personal communication (I. Hasenfuss) for three ingroup taxa (*Watsonalla binaria*, *W. uncinula* and *Falcaria lacertinaria*) and from the literature (*Accinctapubes albifasciata*: Solis & Styer, 2003; *Epicopeia hainesii*: Yen et al., 1995) for two outgroup taxa.

Videotapes of behavioural interactions, along with daily observations, were used to determine the types of territorial behaviour produced by each species. Videotapes were also analyzed to measure the durations and outcomes of contests, and to monitor change in behaviour rates in both residents and intruders throughout each trial, in order to test a prediction on ritualization for hypotheses concerning the origin of signals (Chapters 4 and 5) and to help answer questions on the evolution of signalling (Chapter 6). Trial durations were measured from the moment the intruder's head crossed the leaf-petiole junction to when one of the caterpillars crossed that junction while exiting the leaf. To determine how signalling and other territorial behaviours changed with respect to distance between individuals, rates were measured at three stages of intruder approach— FAR (when the head of the intruder passed the junction of the petiole), MID (the mid-way point between the far and close distances) and CLOSE (the point when the intruder first made contact with the resident, or if the intruder did not make contact, when it came within 0.5 mm of the resident). Rates were measured by counting the number of behaviours over a 20 second period from the beginning of the stage and calculated as the number of events per 5 seconds. Grand means of rates for each behaviour type at each distance category were calculated, were checked for normal distribution using the Shapiro-Wilk W test, and were compared accordingly using an ANOVA for normal data and a Kruskal-Wallis one-way analysis of variance for non-normal data to test for changes in signal rates as the intruder

approached. *Post hoc* analyses were either performed using pair wise a Tukey-Kramer HSD (normal data) or pair wise Wilcoxon Rank Sum Tests (non-normal data). The number of trials in which intruders signaled was also counted for each species, in order to test a prediction on the function of signalling in Chapter 6. Finally, distance between the head of the intruder and closest point of the resident was measured at the time of the first signal using ImageJ software (1.40g; National Institute of Mental Health, Maryland, U.S.A.) to test another prediction on the function of signalling in Chapter 6.

#### *Recording and analysis of vibrations*

The data from my vibration analysis will be used in three main ways: i) to provide information on vibrational signalling in caterpillars; ii) to compare vibrations between behaviours in order to test for signal ritualization in Chapters 4 and 5; and iii) to test a hypothesis on why caterpillars produce more than one signal type (Chapter 6). Vibrations were measured with the LDV by reflecting the laser beam from a circular disc of reflective tape (2.0 mm) positioned on the leaf within 1 cm of the resident's leaf shelter, or within 1 cm of the resident's resting position in species that did not produce a leaf shelter. Laser signals were digitized and recorded onto a Marantz Professional portable solid-state recorder (PMD 671; Kanagawa, Japan; 44.1 kHz sampling rate). LDV and microphone recordings in conjunction with videotapes were analyzed to determine if vibrations were associated with different behaviours, and to measure temporal characteristics, and information on signal content of bouts. A bout was defined as any combination of signals that was preceded or followed by feeding, walking or at least 1 s of inactivity. Spectral characteristics were measured using LDV recordings only. Power spectra were generated (15892-point Fourier transform; Hann window), and dominant

frequencies and bandwidths around the dominant peak (at -3 dB SPL and -10 dB SPL) were calculated from 5 individuals (5 signals per individual) per taxa when possible. Amplitudes of vibrations associated with each behaviour were measured relative to background levels. All signals were analyzed using Raven Bioacoustics Research Program (Cornell Laboratory of Ornithology; New York, U.S.A.) and recordings were conducted in an acoustic chamber (Eckel Industries, Massachusetts, U.S.A.).

### **2.3 Results**

In total, I collected morphological information from 19 species and behavioural information from 11 species, and was able to obtain some information from the literature for 43 species (whether it be morphological, behavioural, or both) (see Tables 2.2, 2.3). I noted variation with respect to the following: i) life-history characters associated with territoriality (egg-laying behaviour, gregariousness, and shelter-building); ii) the morphology of the anal segment, with respect to the anal prolegs and modification of the dorsal region; iii) modifications of setae on the abdominal prolegs and anal segment; iv) morphology of the mandibles; and iv) behaviours associated with encounters between conspecifics. This information is summarized below, in Tables 2.2 - 2.13, and in Appendix A. The following results will begin by focusing on four species of Drepanidae representing all three subfamilies (Drepaninae: *D. arcuata* and *Oreta rosea*; Thyatirinae: *Tethea or*; and Cyclidiinae: *Cyclidia substigmata*). I will then generally describe the variation observed in other species of Drepanidae. Further details can be found in Appendix A, and Chapters 4, 5 and 6.

*Drepana arcuata* (Drepaninae)

I am using *D. arcuata* as a representative of the Drepaninae subfamily. It illustrates the following conditions, which may or may not be found in other species of this group: it builds a shelter, lacks anal prolegs, possesses modified setae on the anal segment, and produces vibrational signals during encounters with conspecifics.

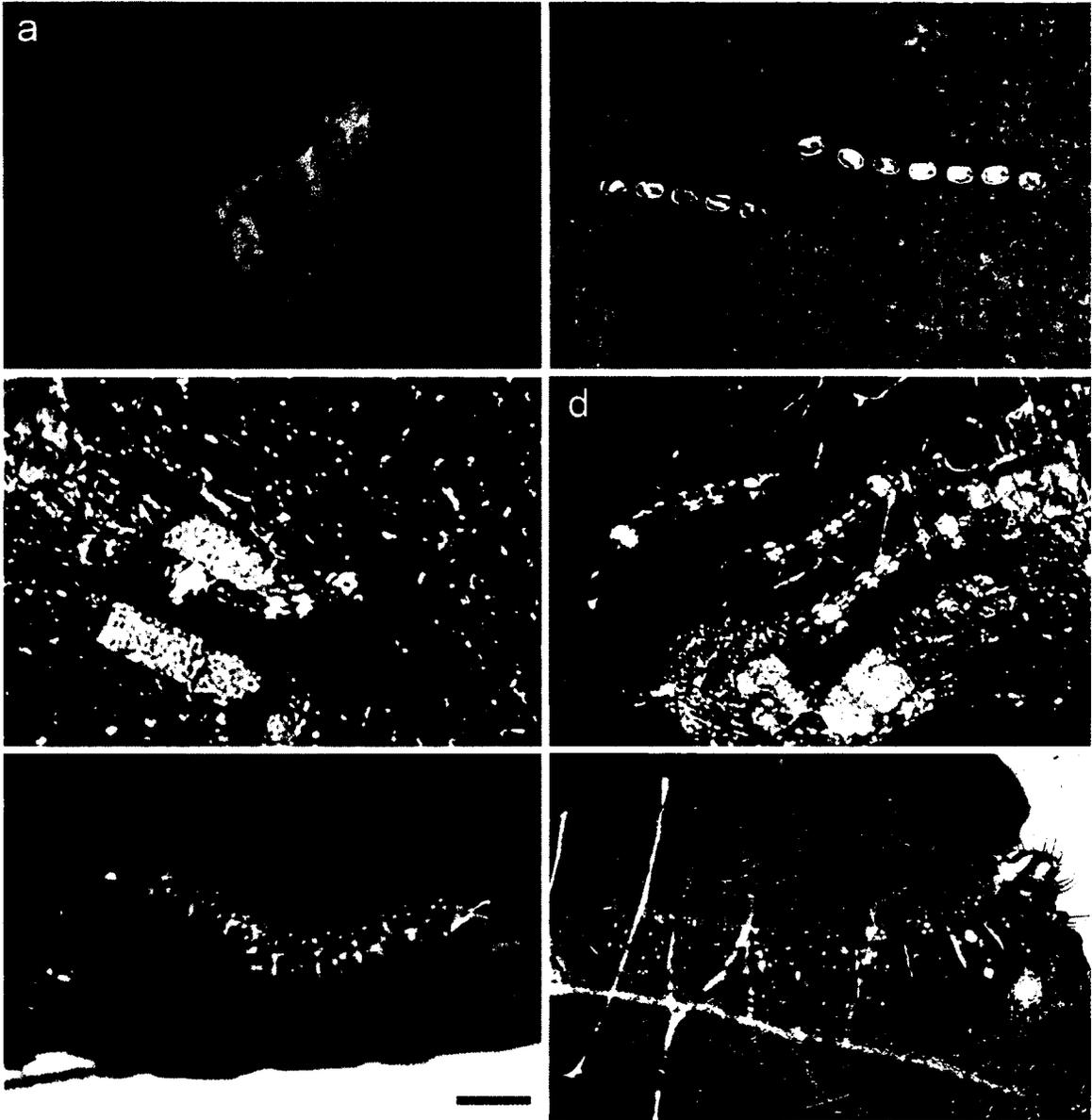
General life history observations relevant to conspecific interactions

Previous observations (summarized in Table 2.2) have shown that adult females of the arched hook-tip moth, *Drepana arcuata* Walker (Fig. 2.2a), oviposit in rows (Yack et al., 2001; Fig. 2.2b) on species of birch (*Betula*) and alder (*Alnus*) (Dyar, 1895; Beutenmüller, 1898). Early instars (Fig. 2.2c) typically form communal silk shelters within which they feed, expanding the nest as they grow (Yack et al., 2001; Fig. 2.2d). As the leaf is consumed, late instar caterpillars (Fig. 2.2e) establish solitary folded leaf shelters by tying leaf edges with silk threads and laying a silk mat on the leaf surface (Yack et al., 2001; Fig.2.2f).

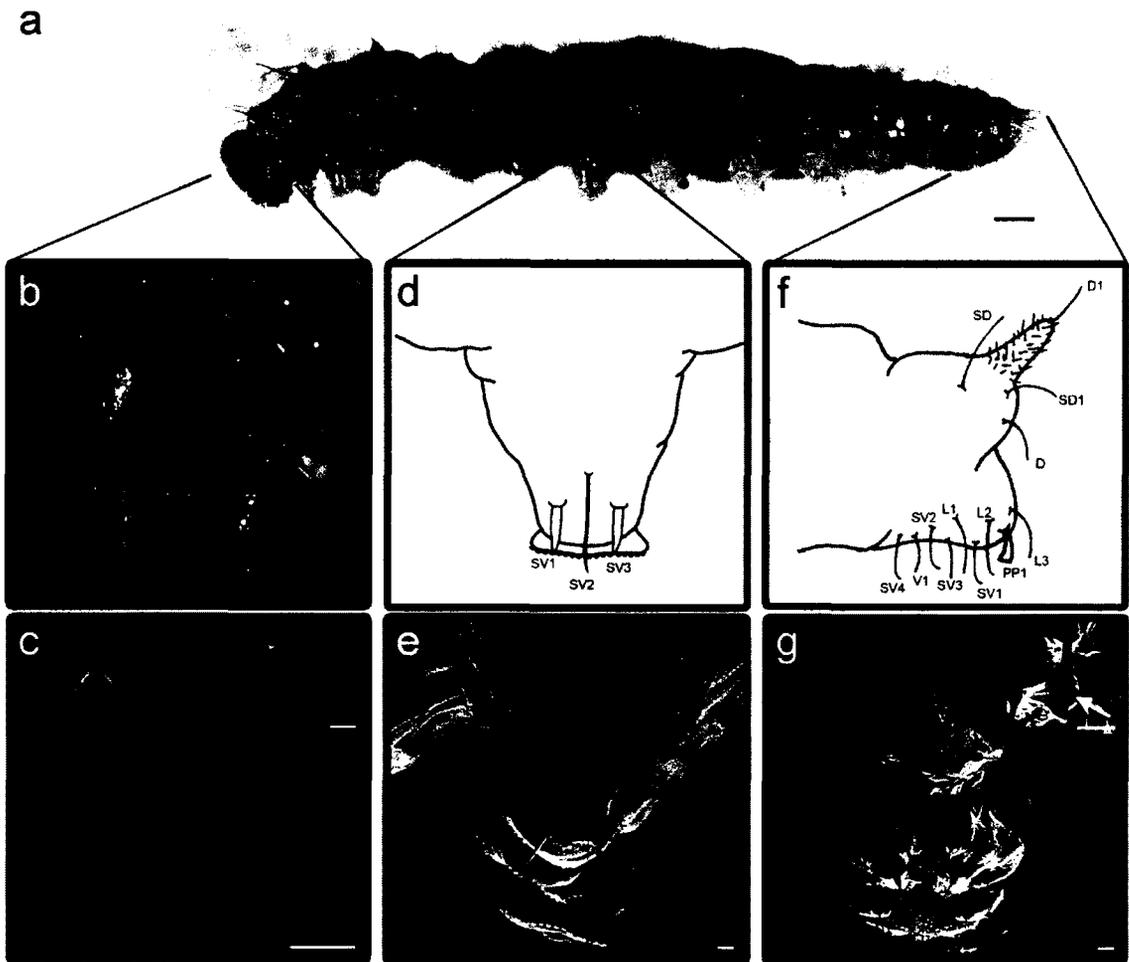
Morphology

The head capsule of late instar larvae is not flattened dorsally (Fig. 2.3a,b) and the mandibles possess six distal teeth on the incisor region with two ridges on the oral surface (Fig. 2.3c). The outer planta region of the abdominal prolegs (excluding the anal prolegs) bears three setae (SV1, SV2, SV3; Fig. 2.3d,e). SV1 and SV3 are modified into a peg shape, with the SV2 seta unmodified. Larvae lack prolegs on the terminal abdominal segment (Fig. 2.3a,f,g). The pair of PP1 setae (used for scraping the leaf) on

**Figure 2.2.** Photographs demonstrating life-history characteristics relevant to territorial behaviour in the arched hook-tip moth, *Drepana arcuata*. Photo credits: J. Yack. **(a)** Dorsal view of two adult moths in resting position (scale bar = 1 cm). **(b)** Row of eggs laid on a leaf of *Betula papyrifera* (scale bar = 3 mm). **(c)** Dorsal view of an early instar larvae (scale bar = 1 mm). **(d)** Whole leaf view of a group of early instar caterpillars on a skeletonized feeding spot (scale bar = 2 mm). **(e)** Lateral view of a late instar caterpillar in resting position (scale bar = 3 mm). **(f)** Late instar caterpillar in a leaf-shelter with a mat of silk (scale bar = 3 mm).



**Figure 2.3.** Morphological characters related to territorial behaviour in *Drepana arcuata*. **(a)** Lateral view of the whole caterpillar (scale bar = 1 mm). **(b)** Anterior view of the head capsule (scale bar = 500  $\mu\text{m}$ ). **(c)** SEMs of lateral and ventral (inset) views of the mandibles (scale bars = 100  $\mu\text{m}$ ; photo credit: J. Yack). **(d)** Drawing of a lateral view of the proleg on the third abdominal segment (A3). **(e)** SEM of a lateral view of the proleg on A3 (scale bar = 100  $\mu\text{m}$ ; photo credit: T. Nevills). **(f)** Drawing of a lateral view the terminal abdominal segment (A10) with named setae. **(g)** SEM of a posterior view of A10 showing the location of the PP1 seta (arrow) with a close-up of the PP1 modified seta (inset; arrow) (scale bars = 100  $\mu\text{m}$ ; photo credit: J. Yack).



the anal segment is modified into an oar shape (Figure 2.3f,g). All other setae are normal to the group (Fig. 2.3f). Details on morphological characters are summarized in Table 2.3.

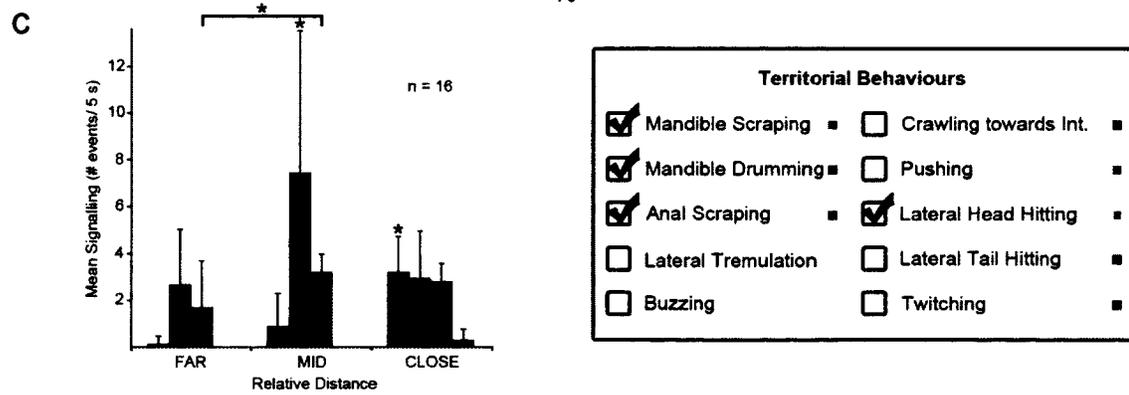
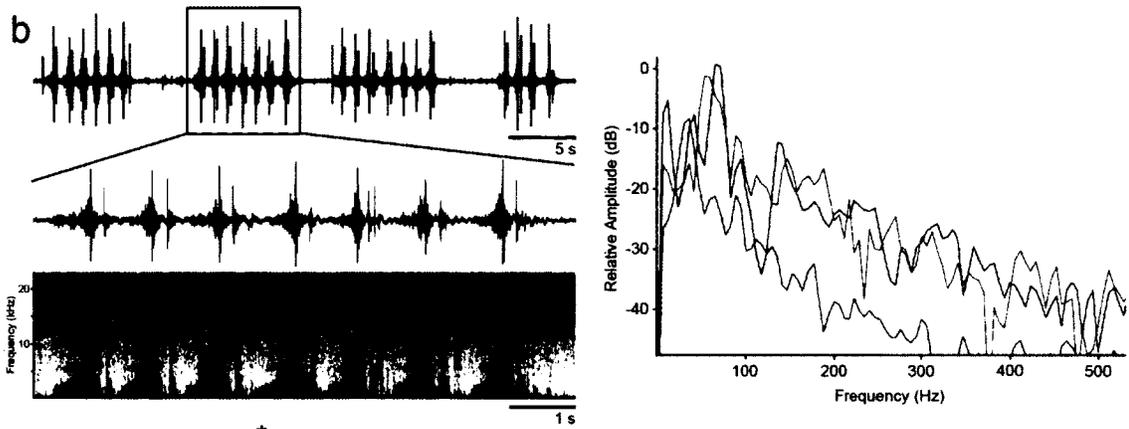
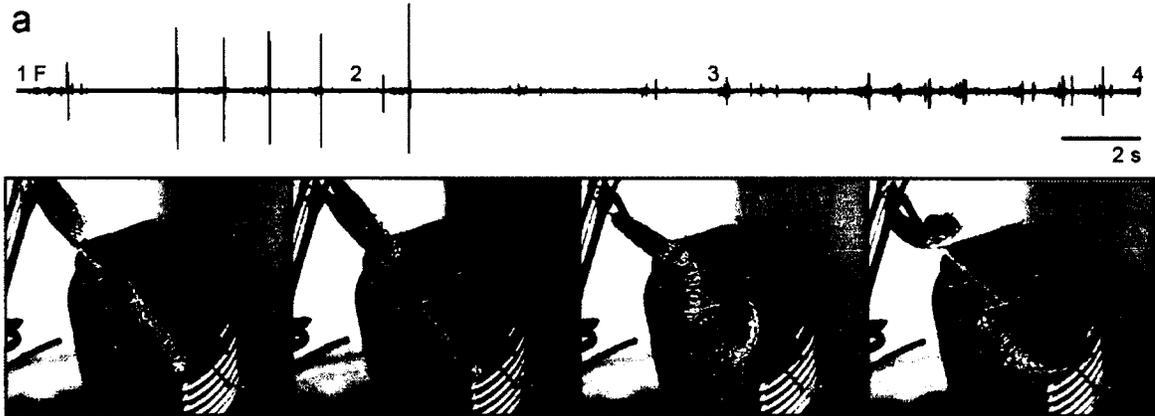
#### Behavioural trials between conspecifics

Information on behavioural trials, including mean trial duration, outcome of trials, intruder signalling and distance between the resident and intruder at first signal is summarized in Table 2.4 below. In brief, a total of 53 encounters were staged between a resident and an intruder of similar weight (Yack et al., 2001). The resident produced four types of behaviours during encounters with conspecifics, including mandible drumming, mandible scraping, anal scraping and lateral head hitting (Fig. 2.4; described briefly below, and in detail in Chapters 4 and 5). Residents won 86.8% of the trials, intruders won 7.5% and in three trials, intruders built shelters on the occupied leaf (Yack et al., 2001). Residents were silent until they detected an intruder (Fig. 2.4a). The resident typically began an encounter with anal scraping followed by signalling with the mandibles (see Chapter 4 for details). The rate of all behaviours changes significantly as the intruder approached the resident (Fig. 2.4b; see Table A.1 for details). Intruders signaled in 37.7% of trials.

#### Analysis of vibrations

Late instar larvae produce vibrations associated with four types of behaviours during encounters with conspecifics - mandible scraping, mandible drumming, anal

**Figure 2.4.** Vibration characteristics and territorial behaviour in *Drepana arcuata*. **(a)** Microphone trace (exported from a video file) of an entire behavioural trial with corresponding video frames below. Numbers correspond in both the trace and the video frames, illustrating the approach of the intruder (1 = FAR, 2 = MID, 3 = CLOSE, 4 = Intruder leaves, F = First resident signal; scale bar = 4 mm; video credit: J. Yack). **(b)** Laser vibrometer trace illustrating a series of bouts, with an enlargement of single bout and corresponding spectrogram below. Power spectra demonstrating the dominant frequencies of each vibration (right panel). **(c)** Mean (+SD) behavioural rates of residents at three stages of intruder approach (FAR, MID, CLOSE). Asterisks denote significant differences within each behaviour between stages of the encounter. All colours throughout the figure correspond to those in the box describing territorial behaviours.



scraping and lateral head hitting (Fig. 2.4c). Mandible scraping, mandible drumming and anal scraping typically occur in bouts that comprise many behaviours.

Details on vibration characteristics, including mean duration, mean relative amplitude, mean dominant frequency and mean bandwidths at -3 dB SPL and -10 dB SPL are summarized in Table A.1. Mandible scraping involves a movement of the head, thorax and first two abdominal segments in a lateral arc in one direction, while dragging the mandibles across the leaf surface to produce a scratching sound. Mandible drumming is produced by striking the leaf with the serrated edges of open mandibles to create a short, percussive vibration (Fig. 2.4c). Anal scraping is produced by dragging modified PP1 setae across the leaf surface (Fig. 2.4c). Lateral head hitting is similar in movement to mandible scraping, but the mandibles do not make contact with the leaf surface, and the head makes contact with the intruder. Lateral head hitting typically occurs after the intruder has made contact with the anterior end of the resident's body. Vibrations produced by lateral head hitting could not be analyzed spectrally for *D. arcuata* because this behaviour was rarely observed during encounters.

*Oreta rosea* (Drepaninae)

I am using *O. rosea* as a representative of the Drepaninae subfamily. This species illustrates the following conditions which may or may not be found in other species in this group: it does not build a shelter, does not possess anal prolegs, does not possess modified setae on the anal segment and during encounters with conspecifics produces vibrational signals with the mandibles only.

### General life history observations relevant to conspecific interactions

Personal observations (summarized in Table 2.2) demonstrate that adult females (Fig. 2.5a) of the rose hooktip moth, *Oreta rosea* Walker 1855 lay eggs singly or in small rows on the upper and under surface of the leaf (Fig. 2.5b). All instars live solitarily on the leaf. Early instars (Fig. 2.5c) occupy individual feeding areas at leaf edges, skeletonizing the leaf surface (Fig. 2.5d). Late instar larvae (Fig. 2.5e) occupy their own leaf and will lay down a mat of silk on the leaf surface, but make no shelter (Fig. 2.5f).

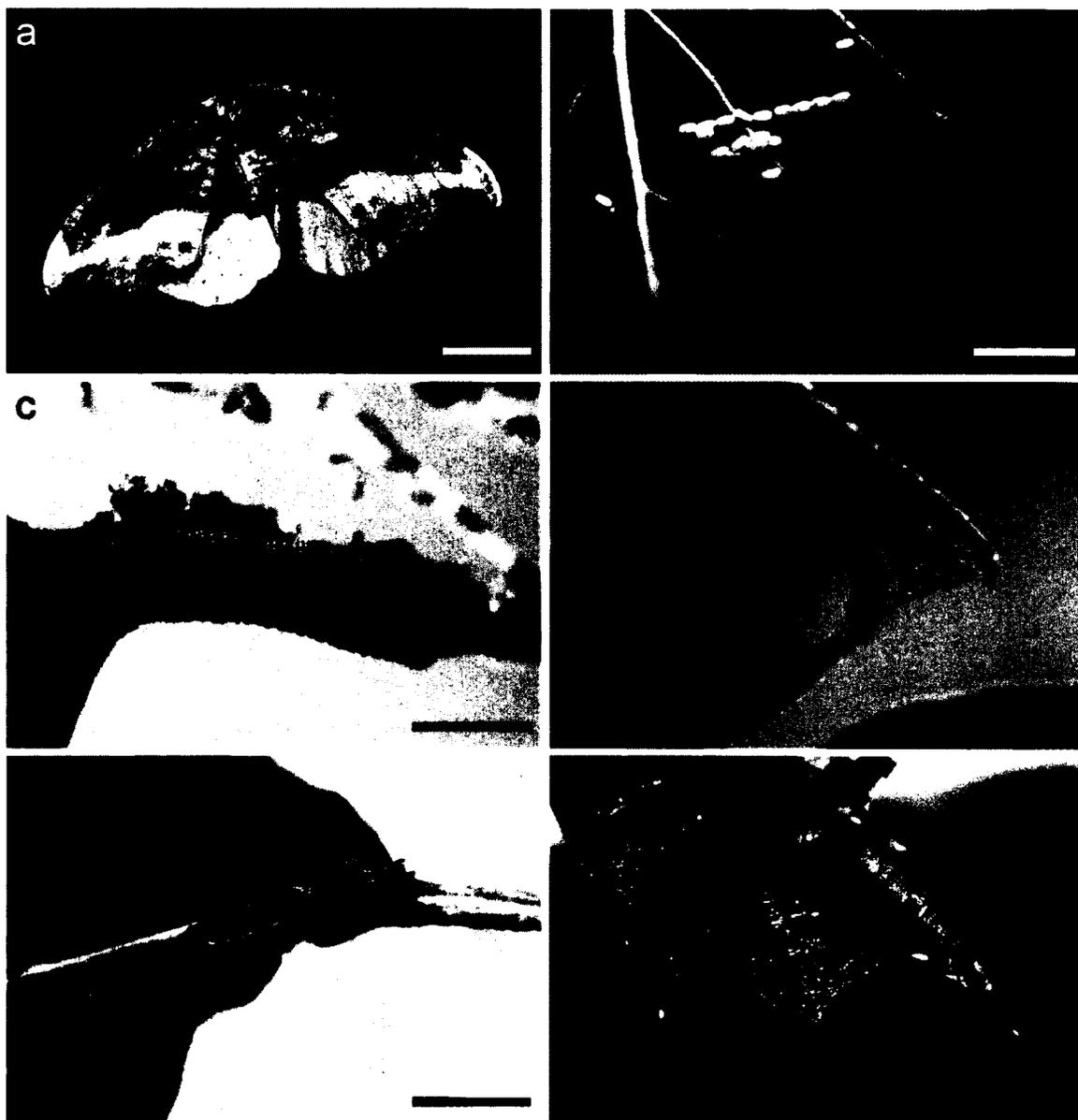
### Morphology

The head capsule of late instar larvae is not flattened dorsally (Fig. 2.6a,b) and the mandibles have six distal teeth on the incisor region with a small ridge on the oral surface (Fig. 2.6c). The outer planta region of the abdominal prolegs (except the anal prolegs) bears many secondary setae, with no modified primary setae (Fig. 2.6d,e). Larvae do not possess prolegs on the terminal abdominal segment and have a long, fleshy caudal process protruding from their anal shield (Fig. 2.6a). They possess many small secondary setae on the anal segment, with no modified primary setae (Fig. 2.6f,g). The primary setae on the anal segment are normal to the group, except for the pair of SD2 setae, which is absent (Fig. 2.6f,g). Details on morphological characters are summarized in Table 2.3.

### Behavioural trials between conspecifics

Detailed information on behavioural trials is summarized in Table 2.4. A total of 22 encounters were staged between a resident and an intruder of equal weight. Residents produced four types of behaviours during trials with conspecifics, including mandible

**Figure 2.5.** Photographs demonstrating life-history characteristics relevant to territorial behaviour in the rose hooktip moth, *Oreta rosea*. **(a)** Dorsal view of an adult moth in resting position (scale bar = 5 mm). **(b)** Eggs laid on the underside of a *Viburnum lentago* leaf (scale bar = 5 mm; photo credit: J. Yack). **(c)** Dorso-lateral view of an early instar larvae (scale bar = 3 mm). **(d)** Whole leaf view of an early instar caterpillar on a skeletonized feeding spot (scale bar = 10 mm). **(e)** Lateral view of a late instar caterpillar in resting position (scale bar = 10 mm). **(f)** Late instar caterpillar on a mat of silk (scale bar = 10 mm).



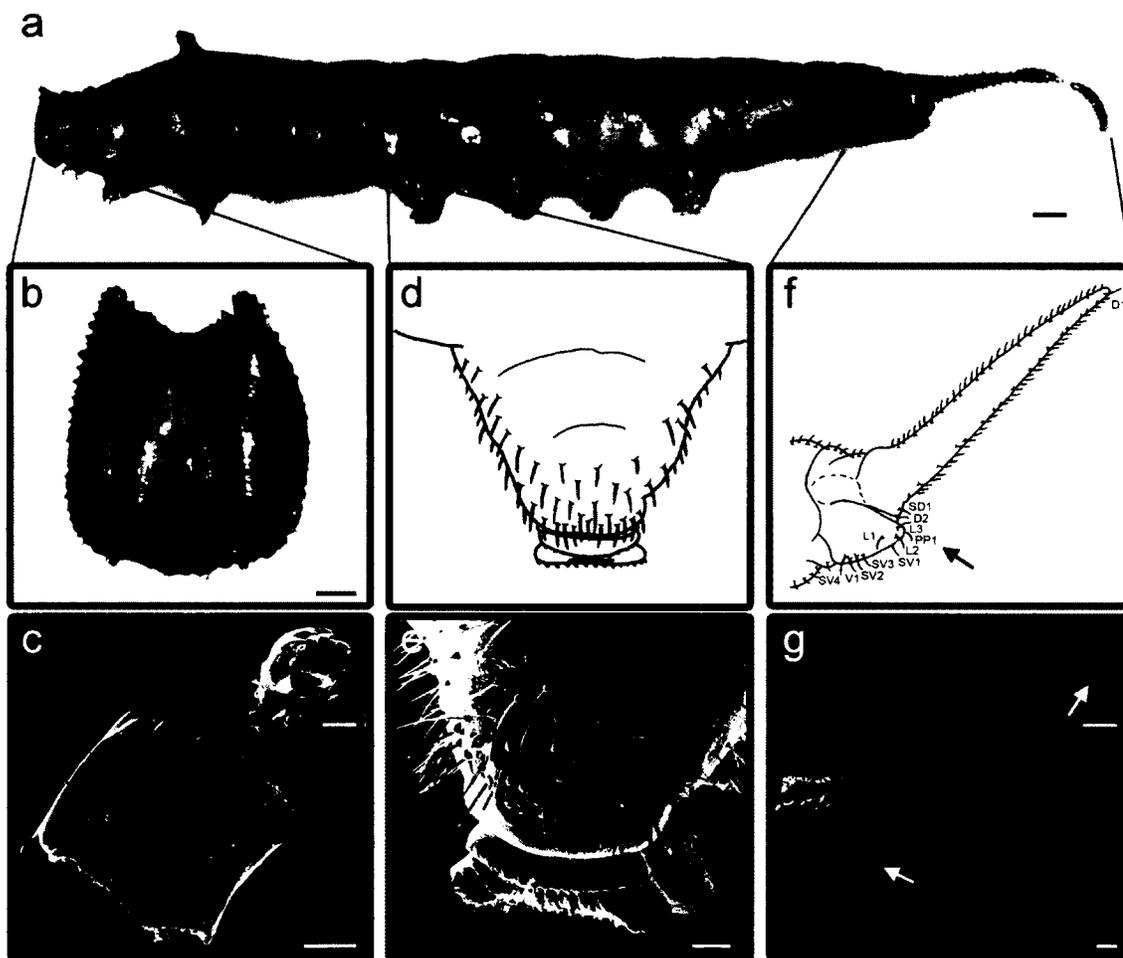
scraping, mandible drumming, lateral tremulation and lateral tail hitting (Fig. 2.7; described briefly below, and in detail in Chapters 4 and 5). Residents won 91.0% of trials, intruders won 4.5%, and 4.5% were ties. Residents remained silent until they detected an intruder and remained in the same approximate position on the leaf during trials (Fig. 2.7a). The rate of mandible scraping, mandible drumming and lateral tail hitting increased significantly as the intruder approached the resident; however the rate of lateral tremulation did not change with distance between the resident and intruder (Fig. 2.7b; see Table A.1 for details). Intruders signaled in about half of the trials where signalling occurred, but at a significantly lower rate (paired  $t$ -test,  $t = -3.84$ ,  $P = 0.001$ ,  $n = 21$ ).

#### Analysis of vibrations

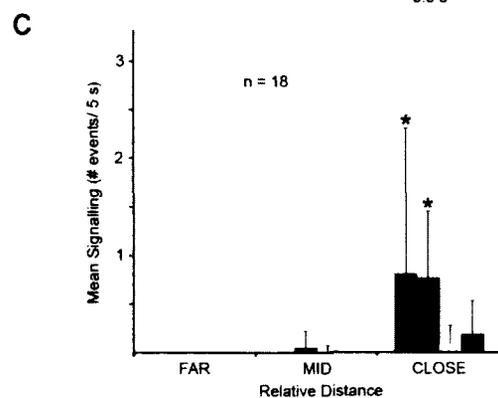
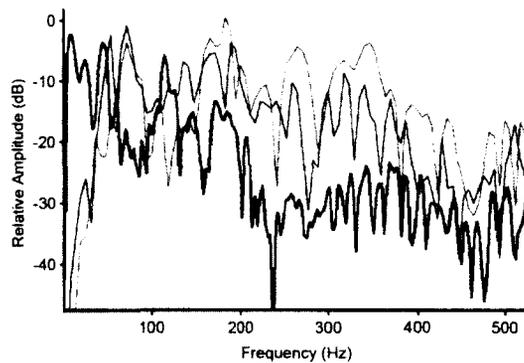
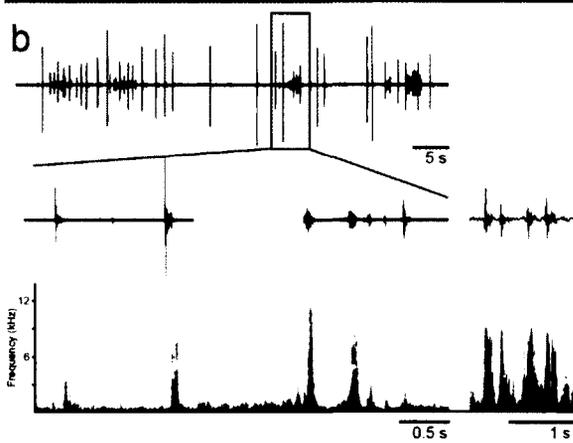
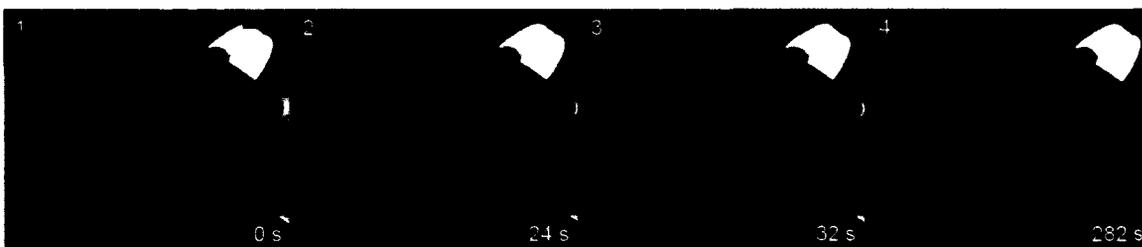
Microphone and LDV recordings revealed that *O. rosea* larvae produce vibrations associated with four types of behaviours during interactions with conspecifics - mandible scraping, mandible drumming, lateral tremulation and lateral tail hitting (Fig. 2.7c). Mandible scraping, mandible drumming and lateral tremulation typically occur in bouts, beginning with a lateral tremulation event followed by any combination of behaviours, with time intervals between bouts being highly variable (see Table 2.6 for details).

Details on temporal and spectral characteristics of vibrations are summarized in Table A.1. Lateral tremulation was only observed in about half the individuals (in 40.9% of trials) and consists of quick, short, successive lateral movements of the head and thorax while the rest of the body remains motionless. A lateral tremulation event is distinguished from a mandible scrape by its much shorter, highly repetitive lateral

**Figure 2.6.** Morphological characters related to territorial behaviour in *Oreta rosea*. **(a)** Lateral view of the whole caterpillar (scale bar = 1 mm). **(b)** Anterior view of the head capsule (scale bar = 500  $\mu\text{m}$ ). **(c)** SEMs of lateral and ventral (inset) views of the mandibles (scale bars = 100  $\mu\text{m}$ ). **(d)** Drawing of a lateral view of the proleg on the third abdominal segment (A3). **(e)** SEM of a lateral view of the proleg on A3 (scale bar = 100  $\mu\text{m}$ ; photo credit: T. Nevills). **(f)** Drawing of a lateral view the terminal abdominal segment (A10) with named setae. **(g)** SEM of a lateral view of A10 showing the location of the PP1 seta (arrow) with a close-up of the PP1 seta (inset; arrow) (scale bars = 100  $\mu\text{m}$ ).



**Figure 2.7.** Vibration characteristics and territorial behaviour in *Oreta rosea*. **(a)** Laser vibrometer trace of an entire behavioural trial with corresponding video frames below. Numbers correspond in both the trace and the video frames, illustrating the approach of the intruder (1 = FAR, 2 = MID, 3 = CLOSE, 4 = Intruder leaves, F = First resident signal; scale bar = 10 mm). **(b)** Laser vibrometer trace illustrating a series of bouts, with an enlargement of single bout and corresponding spectrogram below. Power spectra demonstrating the dominant frequencies of each vibration (gray) (right panel). **(c)** Mean (+SD) behavioural rates of residents at three stages of intruder approach (FAR, MID, CLOSE). Asterisks denote significant differences within each behaviour between stages of intruder approach. All colours throughout the figure correspond to those in the box describing territorial behaviours.



Territorial Behaviours		
<input checked="" type="checkbox"/> Mandible Scraping	<input type="checkbox"/> Crawling towards Int.	<input type="checkbox"/>
<input checked="" type="checkbox"/> Mandible Drumming	<input type="checkbox"/> Pushing	<input type="checkbox"/>
<input type="checkbox"/> Anal Scraping	<input type="checkbox"/> Lateral Head Hitting	<input type="checkbox"/>
<input checked="" type="checkbox"/> Lateral Tremulation	<input checked="" type="checkbox"/> Lateral Tail Hitting	<input type="checkbox"/>
<input type="checkbox"/> Buzzing	<input type="checkbox"/> Twitching	<input type="checkbox"/>

movement, where the mandibles never touch the leaf surface. Finally, lateral tail hitting was observed in 31.8 % of trials and involves a quick lateral movement of the elongated caudal projection, usually directed towards the intruder. Lateral tail hitting is typically observed when the intruder touches the resident near its abdominal end, and the resident swings its caudal projection back and forth multiple times, making contact with the intruder. Spectral properties of lateral tail hitting could not be analyzed because it was rare and I do not have laser files of this behaviour.

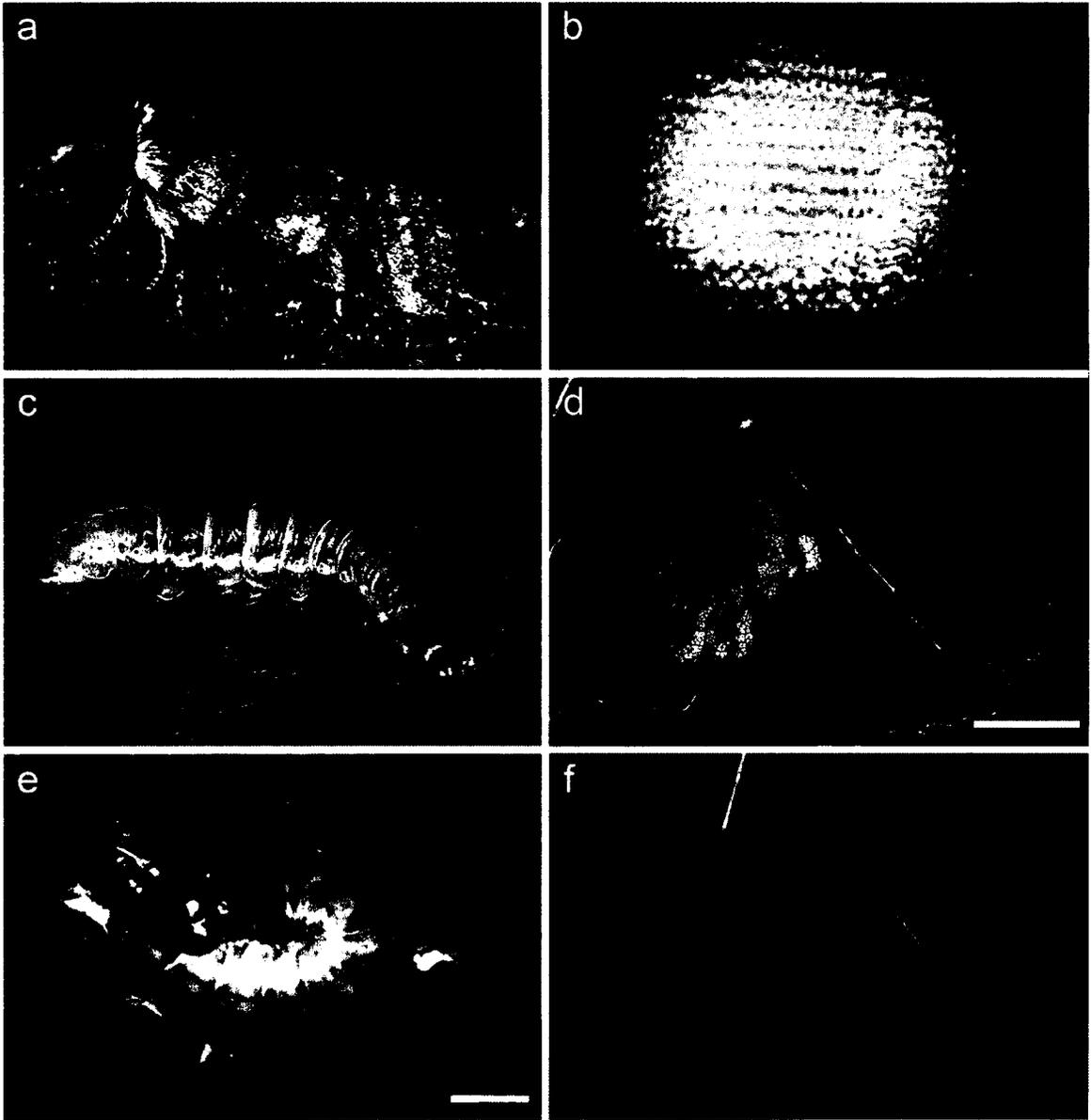
#### *Tethea or (Thyatirinae)*

*Tethea or* is being used as a representative of the Thyatirinae subfamily. It demonstrates the following conditions that may or may not be found in other species of this group: it builds a leaf shelter, possesses anal prolegs, lacks modified setae on the terminal abdominal segment and produces vibrational signals and other territorial behaviours during interactions with conspecifics.

#### General life history observations relevant to conspecific interactions

Previous observations (summarized in Table 2.2) have shown that adult females of the poplar lutestring moth, *Tethea or* Denis & Schiffermüller 1775 (Fig. 2.8a), lay eggs singly or in small groups on the underside of leaves on poplar (*Populus* spp.) (Newman, 1884; Stokoe et al., 1948; Riegler, 1999; Fig. 2.8a,b). Caterpillars of all instars are solitary and build a shelter by tying two leaves together with silk (Theakston, 1866; Newman, 1884; Stokoe et al., 1948; Riegler, 1999; personal observations; Fig. 2.8c-f). Late instars rest inside their shelters in a U-shaped position (Fig. 2.8e; personal observations). Riegler (1999) also noted that when disturbed, caterpillars shake within

**Figure 2.8.** Photographs demonstrating life-history characteristics relevant to territorial behaviour in the poplar lutestring moth, *Tethea or.* **(a)** Lateral view of an adult moth in resting position (scale bar = 0.5 mm; photo credit: J.C. Schou, leps.it). **(b)** Dorsal view of a single egg (scale bar = 0.2 mm; photo credit: R. Fry, ukleps.org). **(c)** Lateral view of an early instar larvae (scale bar = 1 mm; photo credit: R. Fry, ukleps.org). **(d)** View of an early instar shelter with skeletonized feeding spot (scale bar = 2 cm; photo credit: A. Watson Featherstone, treesforlife.org.uk). **(e)** Dorsal view of a late instar caterpillar in resting position (scale bar = 3 mm). **(f)** Late instar caterpillar leaf-shelter (scale bar = 1 cm).



their shelters, making a "sifflement" (whistling) or "raclement" (scraping) noise.

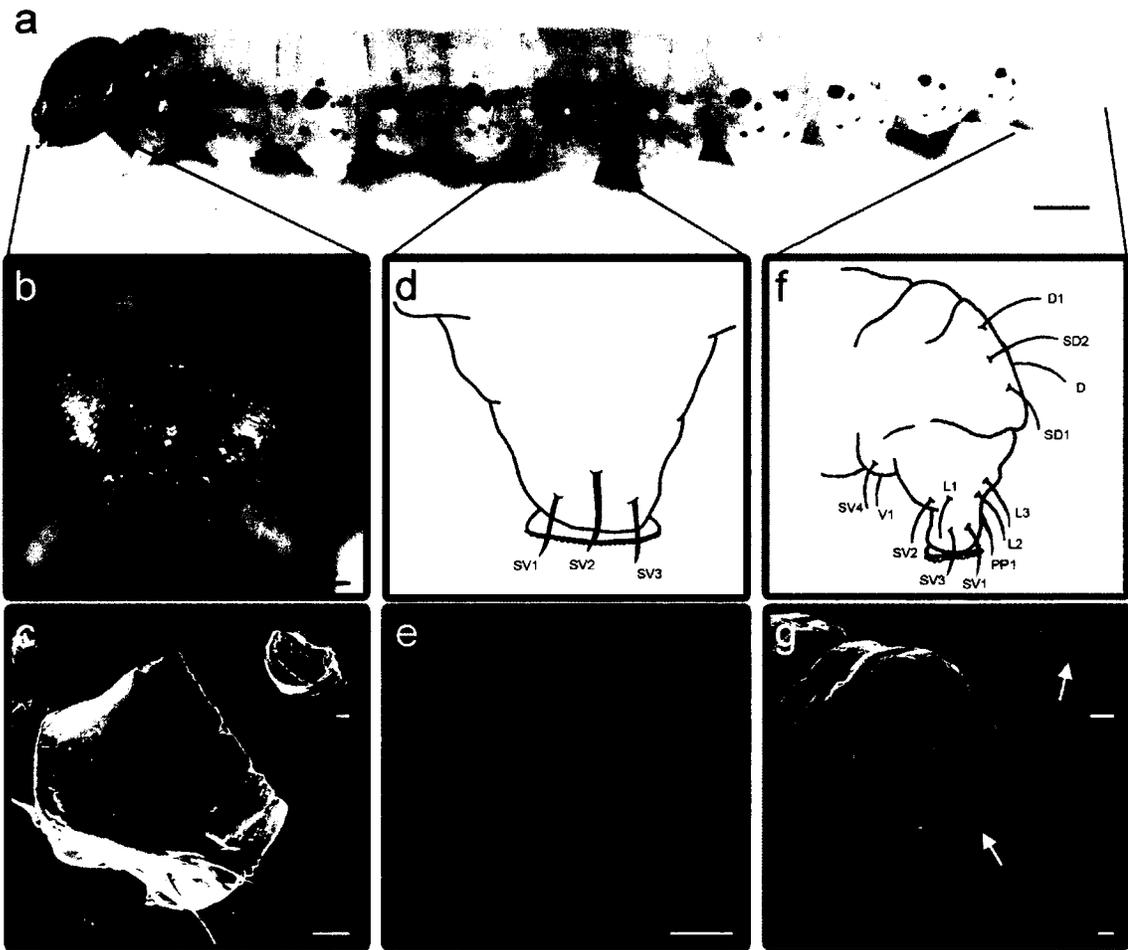
### Morphology

The head capsule of late instar larvae is flattened dorsally (Fig. 2.9a,b). Mandibles have four rounded distal teeth on the incisor area and no ridges on the oral surface (Fig. 2.9c). The abdominal prolegs (excluding the anal prolegs) bear three unmodified setae on the outer planta region (SV1, SV2, SV3; Fig. 2.9d,e). Larvae possess reduced prolegs on the terminal abdominal segment (smaller than the abdominal prolegs) that bear crochets (Fig. 2.9a,f,g). On the anal segment there are no modified primary setae and all setae are normal to the group (Fig. 2.9f,g). Morphological characters are summarized in Table 2.3.

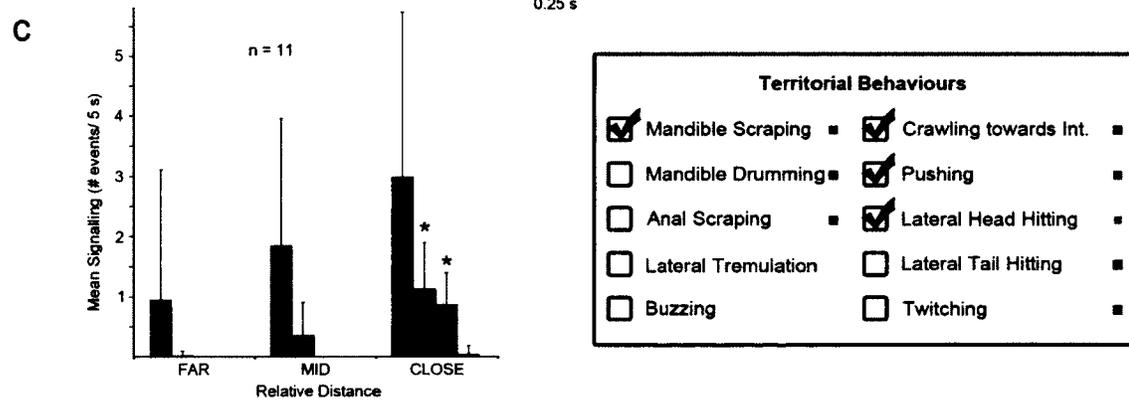
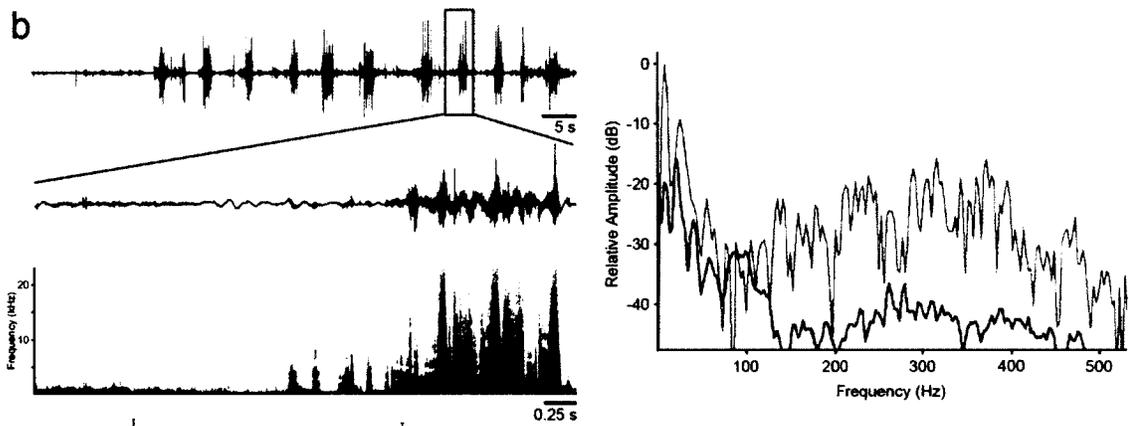
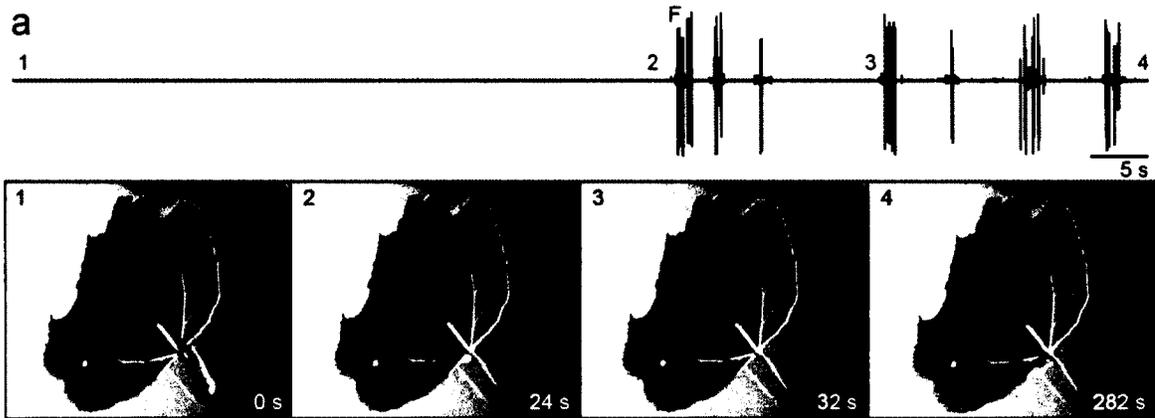
### Behavioural trials between conspecifics

Details on encounters with conspecifics are summarized in Table 2.4. A total of 11 encounters were staged between a resident and an intruder of similar size. Residents produced four types of behaviours during encounters, including mandible scraping, crawling towards the intruder, pushing and lateral head hitting (Fig. 2.10; described briefly below, and in detail in Chapters 4 and 5). In all trials the intruder left the leaf (i.e. the resident 'won' the trial). Residents did not respond until the intruder crossed the leaf-petiole junction (Fig. 2.10a). A typical behavioural sequence begins with the resident crawling toward the intruder, followed by head movements including pushing and mandible scraping (see Chapter 4 for details). The rate of resident behaviours, including mandible scraping, crawling towards the intruder, and pushing changed significantly as

**Figure 2.9.** Morphological characters related to territorial behaviour in *Tethea or.* **(a)** Lateral view of the whole caterpillar (scale bar = 1 mm). **(b)** Anterior view of the head capsule (scale bar = 500  $\mu\text{m}$ ). **(c)** SEMs of lateral and ventral (inset) views of the mandibles (scale bars = 100  $\mu\text{m}$ ). **(d)** Drawing of a lateral view of the proleg on the third abdominal segment (A3). **(e)** SEM of a lateral view of the proleg on A3 (scale bar = 100  $\mu\text{m}$ ; photo credit: T. Nevills). **(f)** Drawing of a lateral view the terminal abdominal segment (A10) with named setae. **(g)** SEM of a lateral view of A10 showing the location of the PP1 seta (arrow) with a close-up of the PP1 seta (inset; arrow) (scale bars = 100  $\mu\text{m}$ ).



**Figure 2.10.** Vibration characteristics and territorial behaviour in *Tethea or.* **(a)** Laser vibrometer trace of an entire behavioural trial with corresponding video frames below. Numbers correspond in both the trace and the video frames, illustrating the approach of the intruder (1 = FAR, 2 = MID, 3 = CLOSE, 4 = Intruder leaves, F = First resident signal; scale bar = 1 cm). **(b)** Laser vibrometer trace illustrating a series of bouts, with an enlargement of single bout and corresponding spectrogram below. Power spectra demonstrating the dominant frequencies of each vibration (right panel). **(c)** Mean (+SD) behavioural rates of residents at three stages of intruder approach (FAR, MID, CLOSE). Asterisks denote significant differences within each behaviour between different stages of intruder approach. All colours throughout the figure correspond to those in the box describing territorial behaviours.



the intruder approached the resident (Fig. 2.10b; see Table A.1 for details). Residents ceased mandible scraping within a few seconds after the intruder left the leaf. When two caterpillars encountered each other on a leaf without a shelter, no signalling or physically aggressive behaviours were observed. Intruders were never observed to mandible scrape during formal trials or during general observations of interactions.

#### Analysis of vibrations

Plant-borne vibrations are associated with four behaviours in late instar larvae during conspecific interactions - mandible scraping, crawling, pushing and lateral head hitting (Fig. 2.10c). Mandible scraping typically occurs in bouts with about 4 signals per bout, ranging between 0.10 - 4.07 s ( $n = 48$  bouts from 11 individuals) (more details in Table 2.6).

Details on temporal and spectral characteristics of vibrations are summarized in Table A.1. Mandible scraping involved scraping the mandibles laterally back and forth on the leaf surface. Forward crawling was performed during encounters as residents crawled towards intruding conspecifics. Pushing is a variation of crawling, where the head of a resident makes physical contact with another caterpillar; therefore the temporal and spectral characters are the same for both behaviours.

#### *Cyclidia substigmata* (Cyclidiinae)

I am including *Cyclidia substigmata* as a representative of the Cyclidiinae subfamily. It illustrates the following conditions which may or may not be found in other species in this group: it does not build a shelter, possesses fully formed anal prolegs, does

not have modified setae on the anal segment and is gregarious as late instars.

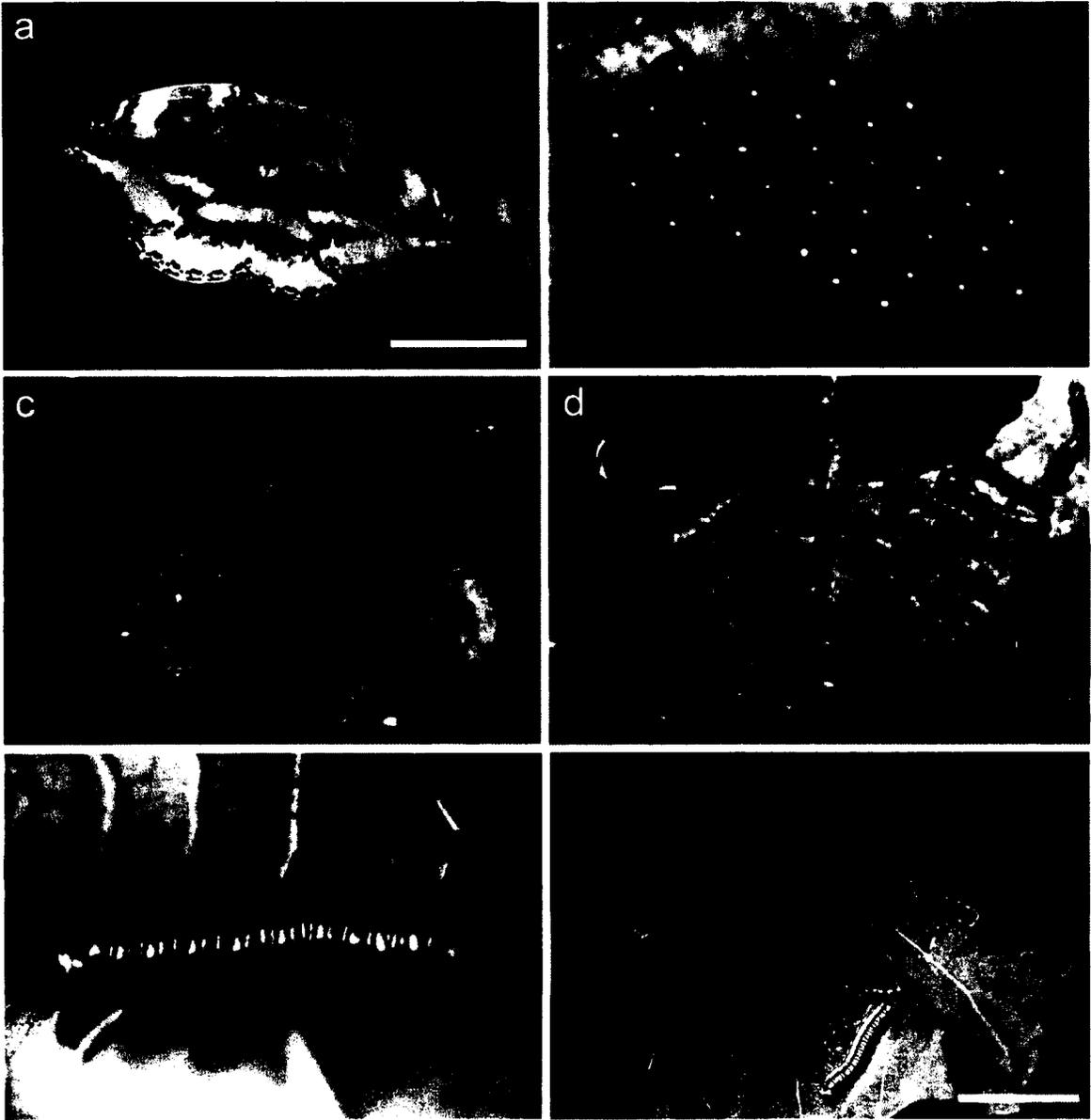
#### General life history observations relevant to conspecific interactions

Previous studies and personal observations (summarized in Table 2.2) show that adult females of *Cyclidia substigmara* Hübner 1831 (Fig. 2.11a), lay eggs in large groups of around 30 eggs (S.-H. Yen, personal communication; Fig. 2.11b) on *Alangium platanifolium* (Minet & Scoble, 1999). Early instars (Fig. 2.11c) are gregarious (S.-H. Yen, personal communication; Fig. 2.11d). Late instar larvae (Fig. 2.11e) were also observed to be gregarious, living in small groups of 3-5 on a single leaf (Fig. 2.11f). Late instars did not construct a shelter or lay a silk mat (Fig. 2.11f).

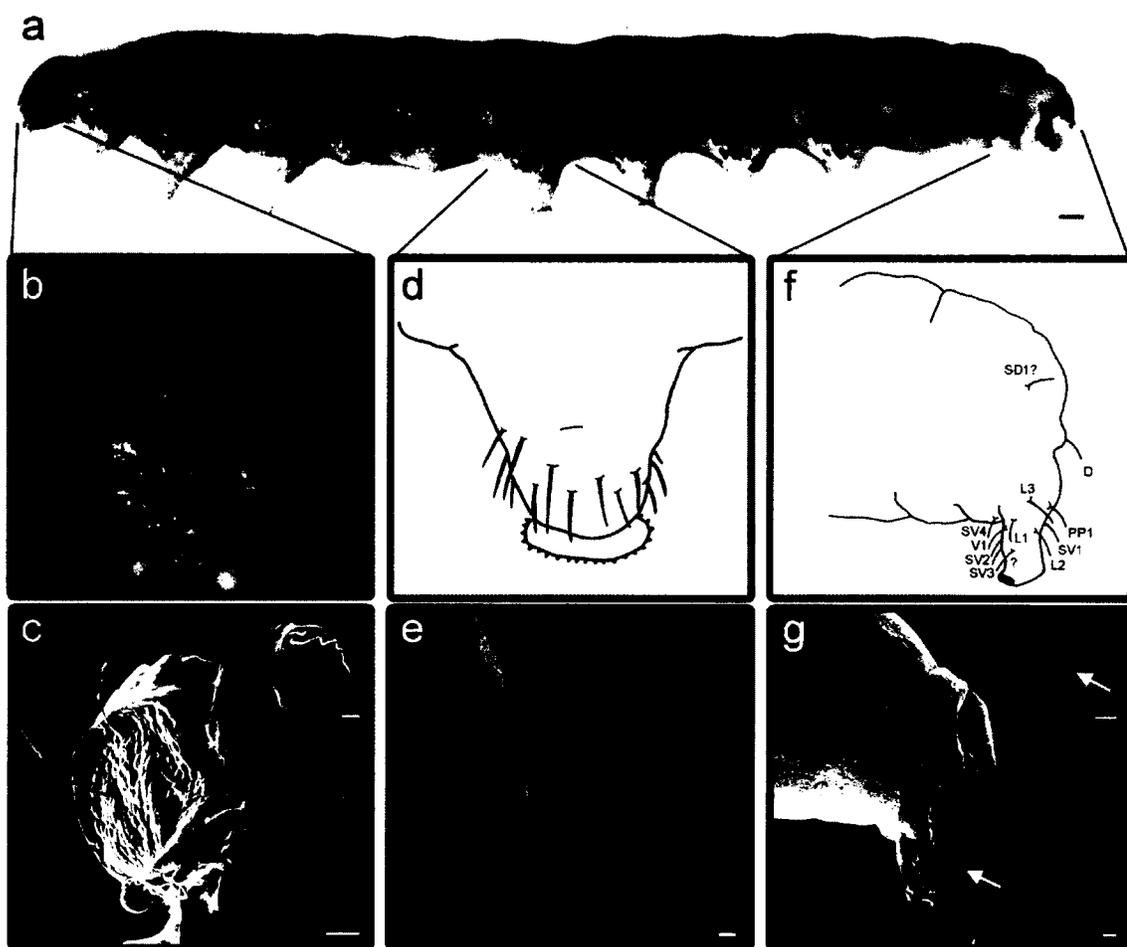
#### Morphology

The head capsule of late instar larvae is not flattened dorsally (Fig. 2.12a,b). Mandibles are small and have three rounded distal teeth on the incisor region and three ridges on the oral surface (Fig. 2.12c). The outer planta region of the abdominal prolegs (excluding the anal prolegs) bears many small secondary setae with no modified primary setae (Fig. 2.12d,e). Larvae possess fully formed prolegs on the terminal abdominal segment (equal in size to the other abdominal prolegs and bearing crochets; Fig. 2.12a). There are two absent dorsal/subdorsal setae, one extra subventral seta, and no modified primary setae on the anal segment (Fig. 2.12f,g). Details on morphology are summarized in Table 2.3.

**Figure 2.11.** Photographs demonstrating life-history characteristics of *Cyclidia substigmata*. **(a)** Dorsal view of an adult moth in resting position (scale bar = 2 cm; photo credit: jpmoth.org). **(b)** Dorsal view of a group of eggs (scale = unknown; photo credit: S.-H. Yen). **(c)** Dorsal view of early instar larvae (scale = unknown; photo credit: S.-H. Yen). **(d)** A group of early instar caterpillars on a leaf (scale = unknown; photo credit: S.-H. Yen). **(e)** Lateral view of a late instar caterpillar (scale bar = 1 cm). **(f)** A group of late instar caterpillars (scale bar = 4 cm).



**Figure 2.12.** Morphological characters related to territorial behaviour in *Cyclidia substigmara*. **(a)** Lateral view of the whole caterpillar (scale bar = 1000  $\mu\text{m}$ ). **(b)** Anterior view of the head capsule (scale bar = 500  $\mu\text{m}$ ). **(c)** SEMs of lateral and ventral (inset) views of the mandibles (scale bars = 100  $\mu\text{m}$ ). **(d)** Drawing of a lateral view of the proleg on the third abdominal segment (A3). **(e)** SEM of a lateral view of the proleg on A3 (scale bar = 100  $\mu\text{m}$ ; photo credit: T. Nevills). **(f)** Drawing of a lateral view the terminal abdominal segment (A10) with named setae. **(g)** SEM of a lateral view of A10 showing the location of the PP1 seta (arrow) with a close-up of the PP1 setae (inset; arrow) (scale bars = 100  $\mu\text{m}$ ).



### Behavioural trials between conspecifics

A total of 7 encounters were staged between a resident and a conspecific of similar size. Residents did not produce any behaviours during interactions with conspecifics (Fig. 2.13). Intruding caterpillars always crawled towards the resident and remained resting beside the resident for the duration of the trial (Fig. 2.13a). Neither the resident nor the intruder left the leaf.

### Analysis of vibrations

Late instar larvae do not produce any vibrations specifically in the context of encounters with conspecifics (Fig. 2.13). They do however, like all other species, produce vibrations while crawling on the leaf. These vibrations are described in Fig. 2.13b and Table A.1.

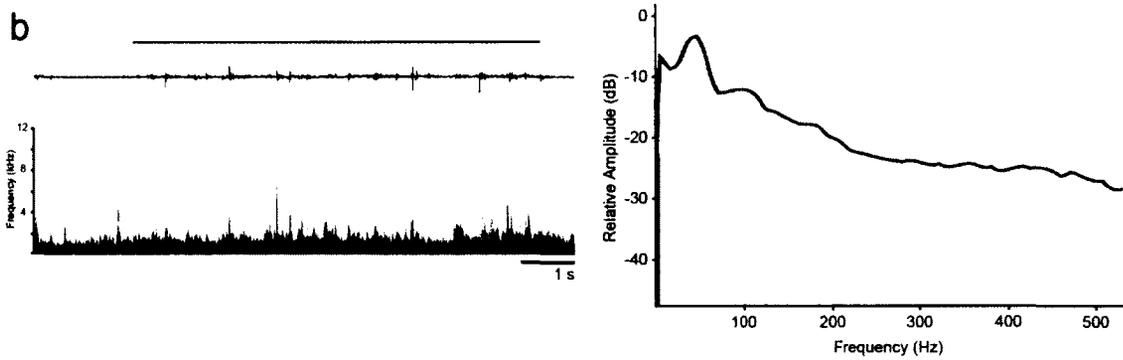
### *A summary of morphology and behaviour observed in all studied species*

#### General life history observations relevant to conspecific interactions

Variation in life-history traits such as egg-laying habit, gregariousness, shelter-building behaviour and hostplants was observed between species included in this study (Table 2.2). Thirteen out of 21 species were found to lay eggs singly, whereas 16 species were found to lay in small groups or rows of up to 11 eggs. Five species were observed to live in small groups of up to 29 as early instars, while 8 species are solitary at this stage. Only one species (*Cyclidia substigmara*) is gregarious as a late instar. Two out of 18 species do not build any type of leaf shelter, while the rest either only lay a silk mat (5 species), fold or roll a single leaf and attach it with silk strands (6 species), or tie two

**Figure 2.13.** Vibration characteristics and territorial behaviour in *Cyclidia substigmata*.

**(a)** Laser vibrometer trace of an entire behavioural trial with corresponding video frames below. Numbers correspond in both the trace and the video frames, illustrating the approach of the intruder (1 = FAR, 2 = MID, 3 = CLOSE, 4 = Intruder leaves; scale bar = 1.5 cm). **(b)** Laser vibrometer trace illustrating vibrations produced by general context with corresponding spectrogram below. Power spectra demonstrating the dominant frequencies of general context crawling (right panel).



Territorial Behaviours		
<input type="checkbox"/> Mandible Scraping	<input type="checkbox"/> Crawling towards Int.	<input type="checkbox"/>
<input type="checkbox"/> Mandible Drumming	<input type="checkbox"/> Pushing	<input type="checkbox"/>
<input type="checkbox"/> Anal Scraping	<input type="checkbox"/> Lateral Head Hitting	<input type="checkbox"/>
<input type="checkbox"/> Lateral Tremulation	<input type="checkbox"/> Lateral Tail Hitting	<input type="checkbox"/>
<input type="checkbox"/> Buzzing	<input type="checkbox"/> Twitching	<input type="checkbox"/>

Table 2.2. Life-history traits relevant to territorial behaviour in Drepanidae species.

Taxon	Egg Laying	Early instar Gregariousness	Late instar Gregariousness	Leaf Shelter	Hostplant
<b>Cyclidiinae</b>					
<i>Cyclidia substigmatica</i>	Groups of approx 30 <sup>1</sup>	Large groups <sup>1</sup>	Groups of 3-5	None	<i>Alangium platanifolium</i> <sup>2</sup>
<b>Drepaninae</b>					
<i>Ausaris palleolus</i>	Singly <sup>3</sup>	-	-	-	<i>Rhus trichocarpa</i> , <i>R. ambigua</i> <sup>4</sup> ; <i>R. succedanea</i> , <i>R. amigus</i> <sup>3</sup>
<i>Auzata superba</i>	Rows of 4-11 <sup>4</sup>	-	-	-	<i>Cornus controversa</i> <sup>4</sup>
<i>Cilix glaucata</i>	Singly <sup>5</sup>	-	-	None <sup>5</sup>	<i>Prunus</i> spp., <i>Crataegus</i> spp., <i>Malus</i> spp., <i>Pyrus</i> spp., <i>Sorbus aucuparia</i> , <i>Rubus fruticosus</i> s. l. <sup>5</sup>
<i>Drepana arcuata</i>	Rows of 2-10	Groups of 3-29	Solitary	Folded leaf	Birch <sup>6</sup> ; Birch, alder <sup>7</sup>
<i>D. curvatula</i>	Rows of 4-11 <sup>4</sup> ; Rows up to 12 <sup>5</sup>	Small groups <sup>5</sup>	Solitary	Folded leaf	<i>Alnus hirsuta</i> var. <i>sibirica</i> <sup>4</sup> ; Birch, alder, sometimes oak and willow <sup>5</sup>
<i>D. falcataria</i>	Short rows <sup>5</sup>	Solitary <sup>5</sup>	Solitary	Folded leaf	Birch, alder, oak, willow, poplar, but raised successfully on birch <sup>5</sup>
<i>Falcaria bilineata</i>	Rows of 2-10	Solitary	Solitary	Silk mat	Birch <sup>8</sup>
<i>F. lacertinaria</i>	Groups of 2 or rows of 3-5 <sup>5</sup>	-	-	Silk mat <sup>9</sup>	Birch, alder <sup>5</sup>
<i>Oreta loochooana</i>	Singly <sup>3</sup>	-	-	-	<i>Viburnum odoratissimum</i> , <i>V. luzonicus</i> var. <i>formosanum</i> <sup>3</sup>
<i>O. rosea</i>	Short rows or singly	Solitary	Solitary	Silk mat	<i>Viburnum</i> <sup>7</sup>
<i>Sabra harpagula</i>	Singly <sup>5</sup>	Solitary <sup>5</sup>	Solitary <sup>5</sup>	Folded leaf <sup>5</sup>	Linden-tree ( <i>Tilia</i> ), oak, alder, birch <sup>5</sup>
<i>Tridrepana flava</i>	Singly <sup>3</sup>	-	-	-	<i>Eurya japonica</i> <sup>3</sup>
<i>T. unispina</i>	Singly <sup>3</sup>	-	-	-	<i>Castanopsis formosana</i> <sup>3</sup>
<i>Watsonalla binaria</i>	Singly or in groups of 2-5 <sup>5</sup>	Small groups of 2-5 <sup>5</sup>		Folded leaf <sup>5</sup>	Oak, beech, alder, birch <sup>5</sup>
<i>W. cultraria</i>	Singly or in groups of 2-4 <sup>5</sup>	Small groups of 2-5 <sup>5</sup>	Solitary	Silk mat	Beech <sup>5</sup>
<i>W. uncinula</i>	-	Small groups of 2-5 <sup>5</sup>	-	-	Oak <sup>5</sup>

Taxon	Egg Laying	Early instar Gregariousness	Late instar Gregariousness	Leaf Shelter	Hostplant
<b>Thyatirinae</b>					
<i>Euthyatira pudens</i>	-	-	-	Loose shelters or none <sup>10</sup>	-
<i>Habrosyne pyritoides</i>	Small groups <sup>11</sup>	-	-	Two leaves <sup>11</sup>	<i>Rubus</i> spp. especially <i>Rubus idaeus</i> and <i>R. fruticosus</i> <sup>11</sup>
<i>Ochropacha duplaris</i>	Singly or in groups of 2-3 <sup>11</sup>	Solitary <sup>11</sup>	Solitary	Two leaves	<i>Betula pendula</i> , <i>Alnus glutinosa</i> , <i>Alnus viridis</i> , <i>Quercus</i> spp., <i>Populus</i> spp. <sup>11</sup>
<i>Pseudothyatira cymatophoroides</i>	-	-	-	Folded leaf or two leaves <sup>9</sup>	-
<i>Tethea or</i>	Singly or in small groups <sup>11</sup>	Solitary	Solitary	Two leaves	<i>Populus</i> spp., <i>Salix</i> spp. <sup>14, 15</sup>
<i>Tetheela fluctuosa</i>	Singly or rarely in pairs <sup>11</sup>	Solitary <sup>11</sup>	Solitary	Two leaves	<i>Betula pendula</i> , <i>Populus tremula</i> , <i>Alnus glutinosa</i> <sup>11</sup>
<i>Thyatira batis</i>	Small groups <sup>11</sup> ; Singly or in pairs <sup>11</sup>	Solitary <sup>11</sup>	Solitary	Silk mat	<i>Rubus</i> spp. especially <i>Rubus idaeus</i> <sup>11</sup>

<sup>1</sup>S.-H. Yen, personal communication; <sup>2</sup>(Minet & Scoble, 1999); <sup>3</sup>(Sen & Lin, 2002); <sup>4</sup>(Nakajima, 1970); <sup>5</sup>(Bryner, 1999); <sup>6</sup>(Beutenmüller, 1898); <sup>7</sup>(Dyar, 1895); <sup>8</sup>(Dyar, 1884); <sup>9</sup>(Newman, 1884); <sup>10</sup>(Wagner, 2005); <sup>11</sup>(Riegler, 1999)

leaves together with silk strands, living in the space between (5 species). Often, species that fold leaves or tie two leaves together will also lay a silk mat. Hostplants were highly variable between species, with birch (*Betula* spp.) and alder (*Alnus* spp.) being the most common.

### Morphology

Morphology of the mandibles, setae on the abdominal prolegs on A3-6, and morphology of the anal segment varied across taxa (Table 2.3). The distal edge of the incisor region of mandibles was found to be either completely smooth (four species) or have between three to eight teeth (14 species). The oral surface was also found to be completely smooth (four species) or contain between one and four ridges (12 species). Species that had smooth distal edges did not necessarily have smooth oral surfaces and vice-versa. Two species (*O. rosea* and *C. substigmata*) had a group of small secondary setae on the outer planta region of the abdominal prolegs (excluding the anal prolegs), while 16 species had only three setae (SV1, SV2 and SV3). In nine of those species, the outer two (SV1 and SV3) were modified (wider than the SV2 setae), and SV2 was unmodified. All species that had modified seta were from the Drepaninae subfamily. The anal prolegs were categorized as being fully formed (equal in area (width at the widest part multiplied by total length from the body to the crochets) to the other abdominal prolegs and bearing full crochets; 8 species), reduced (smaller than the abdominal prolegs, but still bearing crochets; 12 species), or absent (and bearing no crochets; 23 species). Anal segments also varied in the presence or absence of a caudal projections (a single projection from the dorsal anal segment), and these were classified as being short

**Table 2.3.** Morphology characteristics relevant to vibrational communication in Drepanidae larvae and outgroups included in my study.

Taxon	Mandibles		Abdominal Prolegs (A3)	Anal Segment		
	Distal Teeth	Ridges on Oral Surface	SV1 & SV3 Setae	Anal Prolegs	Caudal Projection	PP1 Setae
<b>INGROUP TAXA</b>						
<b>Cyclidiinae</b>						
<i>Cyclidia substigmata</i>	3	3	Unmodified	Full	None	Unmodified
<b>Drepaninae</b>						
<i>Agnidra scabiosa scabiosa</i>	-	-	-	None	Long, filiform	Unmodified
<i>Ausaris micacea</i>	-	-	-	None	Short, fleshy	Unmodified
<i>Ausaris palleolus</i>	-	-	-	None <sup>1</sup>	Short, fleshy <sup>1</sup>	Unmodified <sub>1</sub>
<i>Auzata superba</i>	-	-	-	None <sup>1</sup>	Short, fleshy <sup>1</sup>	Unmodified <sub>1</sub>
<i>Cilix glaucata</i>	0	4	Modified	None	Short, fleshy	Double
<i>Drepana arcuata</i>	6	2	Modified	None	Short, fleshy	Oar
<i>Drepana curvatula</i>	4	0	Modified	None	Short, fleshy	Oar
<i>Drepana falcataria</i>	5	2	Modified	None	Short, fleshy	Oar
<i>Falcaria bilineata</i>	3	2	Modified	None	Short, fleshy	Rectangle
<i>Falcaria lacertinaria</i>	6	2	Modified	None	Short, fleshy	Rectangle
<i>Macrauzata maxima</i>	-	-	-	None <sup>1</sup>	Short, filiform <sup>1</sup>	Unmodified <sub>1</sub>
<i>Microblepsis acuminata</i>	-	-	-	None <sup>1</sup>	Long, filiform <sup>1</sup>	Peg <sup>2</sup>
<i>Nordstromia grisearia</i>	-	-	-	None <sup>2</sup>	Short, fleshy <sup>2</sup>	Peg <sup>2</sup>
<i>Oreta loochooana</i>	-	-	-	None <sup>3</sup>	Long, fleshy <sup>3</sup>	Unmodified <sub>3</sub>
<i>Oreta pulchripes</i>	-	-	-	None <sup>1</sup>	Long, fleshy <sup>1</sup>	Unmodified <sub>1</sub>
<i>Oreta rosea</i>	6	1	Unmodified	None	Long, fleshy	None
<i>Oreta turpis</i>	-	-	-	None <sup>1</sup>	Long, fleshy <sup>1</sup>	Unmodified <sub>1</sub>
<i>Sabra harpagula</i>	-	-	-	None <sup>1</sup>	Short, fleshy <sup>1</sup>	Obtuse <sup>1</sup>
<i>Tridrepana flava</i>	-	-	-	None	Long, fleshy	Thick
<i>Tridrepana unispina</i>	-	-	-	None <sup>3</sup>	Long, fleshy <sup>3</sup>	Laminate <sup>3</sup>
<i>Watsonalla binaria</i>	0	0	Modified	None	Short, fleshy	Unmodified
<i>Watsonalla cultraria</i>	0	0	Modified	None	Short, fleshy	Unmodified
<i>Watsonalla uncinula</i>	0	1	Modified	None	Short, fleshy	Unmodified

Taxon	Mandibles		Abdominal Prolegs (A3)	Anal Segment		
	Distal Teeth	Ridges on Oral Surface	SV1 & SV3 Setae	Anal Prolegs	Caudal Projection	PP1 Setae
<b>Thyatirinae</b>						
<i>Euparyphasma maxima</i>	-	-	-	Reduced <sup>4</sup>	None <sup>4</sup>	-
<i>Euthyatira pudens</i>	Y*	-	-	Reduced	None	Unmodified
<i>Habrosyne aurorina</i>	-	-	-	Reduced <sup>4</sup>	None <sup>4</sup>	-
<i>Habrosyne pyrroides</i>	8	2	Unmodified	Reduced	None	Unmodified
<i>Neodaruma tamanukii</i>	-	-	-	Reduced	None	Unmodified
<i>Ochropacha duplaris</i>	6	1	Unmodified	Reduced	None	Unmodified
<i>Pseudothyatira cymatophoroides</i>	Y	-	Unmodified	Reduced	None	Unmodified
<i>Tethea consimilis</i>	-	-	-	Reduced <sup>4</sup>	None <sup>4</sup>	-
<i>Tethea oberthuri</i>	-	-	-	Reduced <sup>4</sup>	None <sup>4</sup>	-
<i>Tethea or</i>	4	0	Unmodified	Reduced	None	Unmodified
<i>Tetheela fluctuosa</i>	6	3	Unmodified	Reduced	None	Thick
<i>Thyatira batis</i>	5	3	Unmodified	Reduced	None	Unmodified
<b>OUTGROUP TAXA</b>						
<i>Accinctapubes albifasciata</i> (Pyalidae: Epipaschiinae)	-	-	-	Full <sup>2</sup>	None <sup>5</sup>	None
<i>Ennomos autumnaria</i> (Geometridae: Ennominae)	-	-	-	Full <sup>6</sup>	None <sup>6</sup>	-
<i>Epicopeia hainesii</i> (Epicopeiidae)	-	-	-	Full <sup>7</sup>	None <sup>7</sup>	-
<i>Jodis putata</i> (Geometridae: Geometrinae)	-	-	-	Full <sup>6</sup>	None <sup>6</sup>	None
<i>Lyssa zampa zampa</i> (Geometridae: Uraniinae)	-	-	-	Full <sup>8</sup>	None <sup>8</sup>	-
<i>Psychostrophia melanargia</i> (Epicopeiidae)	-	-	-	Full <sup>9</sup>	None <sup>9</sup>	-
<i>Nothus lunus</i> (Sematuridae: Sematurinae)	-	-	-	Full <sup>4</sup>	None <sup>4</sup>	-

\*In the distal teeth category, if the number of teeth could not be counted, a Y represents the presence of teeth and an N represents no teeth (or smooth).

<sup>1</sup>(Nakajima, 1970); <sup>2</sup>(Nakajima, 1972); <sup>3</sup>(Sen & Lin, 2002); <sup>4</sup>(Stehr, 1987); <sup>5</sup>(Solis & Styer, 2003); <sup>6</sup>(Skou, 1986); <sup>7</sup>(Yen et al., 1995); <sup>8</sup>(Holloway, 1998); <sup>9</sup>S.-H. Yen, personal communication

(17 species) or long (6 species) (long was quantified as longer than the length of A7-A10), fleshy (20 species) or filiform (3 species) (as characterized by Nakajima, 1970, 1972, where fleshy represents thicker projections, and filiform represents projections that resemble a thread, or filament), or absent. In addition to these variables, the condition of the pair of posterior proctor (PP1) setae (one on each side) found on the anal segment differed between taxa, where the PP1 setae were broadly classified as unmodified (no wider than the surrounding seta found on the anal segment (L2, L3 and SV1); 17 species), thickened (cylindrically shaped, but wider than the L2, L3 and SV1 setae; 4 species), or paddle-shaped (four-sided with unequal adjacent sides, and wider than the L2, L3 and SV1 setae; 8 species). Within these general categories for PP1, further variation was observed, including two variations in thickened setae and six variations in paddle-shaped setae (described in more detail in Chapter 4).

#### Behavioural trials between conspecifics

The following data will be used to test the hypothesis that vibratory signals function for territoriality in Drepanidae caterpillars. Outcomes and details of contests varied between species (Table 2.4). On average, residents won  $63.9 \pm 38.7\%$  of trials, intruders won  $1.7 \pm 3.0\%$ , and  $34.4 \pm 39.2\%$  ended in ties ( $N = 7$ ). In one species, all trials were won by the resident (*Tethea or*;  $n = 11$ ), and in two others, all trials ended with both contestants remaining on the leaf (*Cyclidia substigmara*,  $n = 7$ ; *Thyatira batis*,  $n = 7$ ). Residents of all species remained silent until they detected an intruder. Residents signaled at a mean distance of  $12.85 \pm 10.86$  mm ( $n = 6$ ) from the intruder's head to the closest point on the resident's body. Resident signalling rates increased as the intruder

**Table 2.4.** Outcomes and details of trials in 10 species of Drepanidae.

Taxon	# of Trials	Mean Trial Duration (s)	# of Trials Won by R	# of Trial Won by I	# of Ties	# of Trials in which R Signaled	# of Trials in Which I Signaled	Distance at First Signal (mm)
<b>Drepaninae</b>								
<i>Drepana arcuata</i>	53	339.2 ± 381.06 (n = 44)**	43	1	9	53	20	23.9 ± 12.4 (n = 10)
<i>D. curvatula</i>	11	574.3 ± 466.3 (n = 7)	7 (n = 10)	0 (n = 10)	3 (n = 10)	11	8	28.1 ± 19.7
<i>D. falcataria</i>	3	412.2 ± 65.9	3	0	0	3	0	12.3 ± 13.9
<i>Falcaria bilineata</i>	54	370.4 ± 327.8 (n = 37)	33	4	17	46	20	2.4 ± 1.6 (n = 43)
<i>Oreta rosea</i>	22	457.4 ± 330.7 (n = 21)	20	1	1	16	9	7.7 ± 9.1 (n = 19)
<i>Watsonalla cultraria*</i>	3	NA	NA	NA	NA	3	1	NA
<b>Thyatirinae</b>								
<i>Ochropacha duplaris*</i>	6	NA	NA	NA	NA	6	0	NA
<i>Tethea or</i>	11	127.9 ± 104.3	11	0	0	10	0	23.8 ± 50.2 (n = 10)
<i>Tetheela fluctuosa</i>	5	125.2 ± 0.0 (n = 1)	1 (n = 1)	0 (n = 1)	0 (n = 1)	2	0	2.8 ± 0.0 (n = 1)
<i>Thyatira batis</i>	7	NA	0	0	7	7	1	NA

\*No full trials were examined for *W. cultraria* and *O. duplaris*, therefore, some of the data for these species could not be measured; \*\*Sample sizes are included in individual columns only when they differed from the total number of trials found in Column 2.

approached in most species, where anal scraping was produced at the highest rates at CLOSE ( $2.29 \pm 0.50$  signals/5 s;  $n = 4$ ), and lateral tremulation being produced at the lowest rate ( $0.33 \pm 0.49$  signals/5 s;  $n = 6$ ). Specific details on behavioural rates can be found in Tables A.1. Intruders signaled in  $22.0 \pm 25.69\%$  of trials where signalling occurred, signalling first in  $6.5 \pm 8.6\%$  of trials.

#### Analysis of vibrations

Caterpillars produced vibrations on the leaf during territorial interactions by mandible scraping, mandible drumming, anal scraping, lateral tremulation, buzzing (similar to lateral tremulation but in the vertical direction), crawling towards the intruder, lateral head hitting, lateral tail hitting, twitching, and pushing. Each species had its own repertoire of vibration producing behaviours (Table 2.5). All caterpillars also produced vibrations by crawling in a general context. Mandible scraping, mandible drumming, anal scraping, lateral tremulation, and buzzing were produced in bouts. Properties of vibrations, including temporal and spectral characteristics, and relative amplitudes varied across taxa (Table A.1). Durations, amplitudes and spectral properties will be compared between behaviours in detail in Chapters 4 and 5. Vibration properties for lateral tail hitting could not be analyzed due to a lack of laser files of this behaviour. Properties for pushing are the same as for crawling towards the intruder, and in some cases properties of anal scraping could not be measured as they were always accompanied by lateral tremulation events.

**Table 2.5.** Behavioural repertoires in species of Drepanidae studied to date.

<b>Taxon</b>	<b>Behaviour(s) Produced</b>
<b>Drepaninae</b>	
<i>Drepana arcuata</i>	AS, LHH, MD, MS
<i>D. curvatula</i>	AS, B, LHH, MD, MS
<i>D. falcataria</i>	AS, B, LHH, MD, MS
<i>Falcaria bilineata</i>	AS, LHH, LTH, MD
<i>Oreta rosea</i>	LT, LTH, MD, MS
<i>Watsonalla cultraria</i>	AS, LHH, LT, LTH, MD
<b>Thyatirinae</b>	
<i>Ochropacha duplaris</i>	C, LHH, LTH, MS, P
<i>Tethea or</i>	C, LHH, MS, P
<i>Tetheela fluctuosa</i>	AS, C, LHH, LT, LTH, MS
<i>Thyatira batis</i>	AS, LHH, LT, LTH, T
<b>Cyclidiinae</b>	
<i>Cyclidia substigmatica</i>	None

AS = anal scraping; B = buzzing; C = crawling towards intruder; LHH = lateral head hitting; LT = lateral tremulation; LTH = lateral tail hitting; MD = mandible drumming; MS = mandible scraping; P = pushing; T = twitching

## 2.4 Discussion

My results demonstrate that variation exists in life-history traits, morphology, territorial behaviours and vibrations produced during encounters with conspecifics in caterpillars of the Drepanidae. Life-history, morphological, behavioural and vibration information obtained in this chapter will be used to test specific hypotheses in later chapters concerning the evolutionary origins of these signals (Chapters 4 & 5) and answer questions on the evolution of signalling (Chapter 6).

### *Variation in vibrational signalling in caterpillars*

An additional goal of this chapter was to provide some much needed information on vibratory signalling in caterpillars. Vibratory signals are widespread in small herbivorous insects and are reported in at least 18 orders to date (Cocroft, 2001; Virant-Doberlet & Cokl, 2004; Cocroft & Rodriguez, 2005; Hill, 2009). Drumming with the head or mandibles in a communicatory context is reported in a number of other insects, including termites (Röhrig et al., 1999; Rosengaus et al., 1999), death-watch beetles (Birch & Keenlyside, 1991) and carpenter ants (Fuchs, 1976). In caterpillars, drumming has been described formally in two species of Drepaninae to date, *D. arcuata* (Yack et al., 2001) and *F. bilineata* (Bowen et al., 2008), and one species of Tortricidae, *Sparganothis pilleriana* (Russ, 1969). Behavioural observations of mandible drumming have been noted in six Drepaninae species to date (*D. falcataria*: Bryner, 1999, I. Hasenfuss, personal communication; *D. lacertinaria*, *W. binaria*, *W. uncinula*: I. Hasenfuss, personal communication; *Nordstromia lilacina* and *Tridrepana arikana*: Sen & Lin, 2002), and I have added behavioural information on four species (*D. curvatula*, *D.*

*falcataria*, *O. rosea*, and *W. cultraria*), suggesting that drumming may be ubiquitous in this subfamily of caterpillars.

Mandible scraping has been less frequently reported in insects, being noted in the larvae of the oriental hornet, *Vespa orientalis*, where they function as hunger signals (Ishay et al., 1974), and in only a few species of caterpillars to date. In larval Lepidoptera, mandible scraping has been experimentally tested and characterized in two species, *D. arcuata* (Yack et al., 2001) and the cherry leaf roller, *Caloptilia serotinella* (Fletcher et al., 2006). Behavioural observations of scraping have been noted in a few other Drepanidae species (*D. falcataria*, *F. lacertinaria*, *W. binaria* and *W. uncinula*: I. Hasenfuss, personal communication). In this thesis I observed and characterized mandible scraping in six species of Drepanidae (*D. curvatula*, *D. falcataria*, *O. rosea*, *O. duplaris*, *T. fluctuosa*, and *T. or*), which suggests that it may represent an important mechanism of vibrational signalling in this subfamily.

Anal scraping signals have been observed in larvae of the sawfly, *Hemicroa crocea* (possibly to orient other larvae to high-quality feeding sites (Hoegraefe, 1984)), and in some species of ants and caddisflies (reviewed in Virant-Doberlet & Cokl, 2004). Other species of insects drum the tip of the abdomen on the substrate to produce percussion signals, such as in some sawfly larvae (*Perga* spp.) that tap a sclerotized portion of the abdominal tail on the substrate for group coordination (Carne, 1962; Fletcher, 2007). Anal scraping has been implicated from behavioural observations in a few other Drepanidae species to date (*D. falcataria*: Federley, 1905; Bryner, 1999, I. Hasenfuss, personal communication; *D. curvatula*: Federley, 1905; *D. lacertinaria*, *W. binaria*, and *W. uncinula*: Federley, 1905; I. Hasenfuss, personal communication; *D.*

*arcuata*: Yack et al., 2001; *N. lilacina* and *T. arikana*: Sen & Lin, 2002; *F. bilineata*: Bowen et al., 2008), and I have added behavioural information on five species (*D. curvatula*, *D. falcataria*, *W. cultraria*, *T. fluctuosa*, and *T. batis*).

Tremulation (i.e. vibrating) is believed to be one of the most simple and widespread vibrational signal production mechanisms in insects (Virant-Doberlet & Cokl, 2004). Tremulation has been reported in a number of insect orders, including species of Orthoptera, Plecoptera, Neuroptera, Coleoptera, Diptera, Trichoptera, Lepidoptera, Hymenoptera, and many others (reviewed in Virant-Doberlet & Cokl, 2004). Tremulation has been reported in one other species of caterpillar, *C. serotinella* (Fletcher et al., 2006). In this thesis, I demonstrate tremulation occurs in at least six species of Drepanidae, in two different forms: lateral tremulation (lateral direction; *O. rosea*, *W. cultraria*, *T. fluctuosa*, and *Thyatira batis*) and buzzing (vertical direction; *D. curvatula* and *D. falcataria*).

#### *Potential sensory structures*

There is some evidence that Drepanidae caterpillars are able to detect vibrations on the leaf surface. For example, in *D. arcuata*, when leaves are cut leaving the resident and intruder on opposite sides, the resident does not produce territorial signals, but begins to signal if the leaf sections are taped back together (Guedes et al., 2012). This finding suggests that the resident is able to detect the vibrations produced by the crawling movements of the approaching intruder. Intruders also take over empty nests, or nests that contain a recently-killed resident, providing evidence that it is the signals produced by the resident that deters intruders (Yack et al., 2001). Although it is clear that vibrational communication plays an important role during territorial interactions in these

caterpillars, it is currently unknown how they receive these vibrations. It has been suggested that setae present on the abdominal prolegs on A3-6 are putative receptor structures, as chordotonal organs have been identified in these prolegs in at least two species of Drepanidae larvae (*D. arcuata* and *W. uncinula*; I. Hasenfuss, personal communication). As such, I have examined the morphology of setae on the proleg on A3 and found that some species possess modified SV1 and SV3 setae, which are thicker than the SV2 setae, and sclerotized. These setae are in contact with the substrate while the caterpillar is at rest. Modified SV1 and SV3 setae are present in all species of Drepaninae that I examined, except *O. rosea*, and in none of the Cyclidiinae and Thyatirinae species. Since the Thyatirinae species also produce (and presumably receive vibrations), the role of these sclerotized setae in vibration reception remains unclear. Future studies should concentrate on locating other possible vibration receptors in these caterpillars, by ablating setae or other putative receptor structures and testing for loss of vibration reception.

#### *Territorial behaviour in caterpillars*

In concordance with some Drepanidae species, other species of caterpillars have been found to defend territories using physical aggression, and in some cases, this can escalate to serious injury or death to one of the contestants (Weyh & Maschwitz, 1982; Okuda, 1989; Berenbaum et al., 1993). Shelters are valuable to own, providing a more stable microclimate, protection from predators and displacement, and enhanced quality of food (Fukui, 2001). Shelters are also costly, requiring both time and energy to build (Ruggiero & Merchant, 1986; Fitzgerald et al., 1991; Berenbaum et al., 1993; Cappuccino, 1993; Fitzgerald & Clark, 1994). Therefore, caterpillars defend these shelters against intruding conspecifics using either physical aggression or ritualized

signaling. Physically aggressive territorial behaviours observed in other caterpillars include striking with the head (e.g. Oecophoridae: *Depressaria pastinacella*; Berenbaum et al., 1993), biting (e.g. Noctuidae: *Busseola fusca*; Okuda, 1989) and even killing opponents (e.g. Pieridae: *Anthocharis cardamines*; Baker, 1983). If physical aggression is costly, leading to serious injury or death to either the resident or the intruder, ritualized signalling may have evolved in some species to avoid those costs. Physically aggressive behaviours, therefore, are likely candidates for the behavioural origins of signals. This, along with preliminary comparisons of characters studied in this chapter, has led me to the hypothesis that ritualized signals in the Drepanidae derive from physically aggressive behaviours. This will be tested using a comparison of morphological, behavioural, and vibrational data between species, within a phylogenetic framework in Chapters 4 & 5 of this thesis. Before testing these hypotheses, however, it is first necessary to develop a phylogeny of the Drepanidae in order to provide an evolutionary framework, which is the focus of Chapter 3.

### CHAPTER 3

#### MOLECULAR PHYLOGENY OF THE DREPANIDAE

**This chapter will form the following manuscript:**

Scott, J. L., Kawahara, A. K., Skevington, J. H., Yen, S. -H., Sami, A., Smith, M. L. & Yack, J. E. Molecular phylogeny of Drepanidae (in preparation, journal to be decided)

**The phylogeny was first introduced in the following manuscript:**

Scott, J. L., Kawahara, A. K., Skevington, J. H., Yen, S. -H., Sami, A., Smith, M. L. & Yack, J. E. (2010). The evolutionary origins of ritualized acoustic signals in caterpillars. *Nature Communications* 1, 4; doi: 10.1038/ncomms1002.

### ***3.1 Introduction***

In order to formally test hypotheses on the evolutionary origins of animal communication signals, it is beneficial to understand the phylogenetic relationships between species used for behavioural comparisons. Phylogenetic trees provide a framework onto which behavioural and morphological characters can be mapped to determine the evolutionary history of a trait. Previous studies focusing on the origin of signals have often lacked this phylogenetic framework. For example, although Schenkel (1956) proposed through behavioural comparisons between species of Phasianidae that the peacock tail-fan display derives from pecking at the ground and offering food to females, he did not provide evidence that pecking behaviour and tail-fanning represent the basal and derived conditions, respectively. This study, and many others (see General Introduction), would have benefitted greatly from a solid phylogenetic framework to provide further evidence for their hypotheses. Since a major goal of my thesis is to determine the evolutionary origins of vibratory signals in the Drepanoidea, it is important to first gain an understanding of the relationships between species in this group.

Until very recently, relationships of Drepanoidea moths were not very well understood. The Drepanoidea is believed to be most closely related to the Geometroidea (Minet & Scoble, 1999), and was even previously placed within the Geometroidea due to the presence of abdominal tympanal organs in the adults (Imms, 1934). The Drepanoidea was thought to comprise two families, Drepanidae and Epicopeiidae, Drepanidae being further divided into Drepaninae, Thyatirinae and Cyclidiinae subfamilies (Minet, 1991; Minet & Scoble, 1999). In the past, authors have also considered Drepanidae, Thyatiridae and Cyclidiidae to be separate families (Inoue, 1954; Nakamura, 1981). Epicopeiidae was

assigned to the Drepanoidea by Minet (1991), based on four autapomorphies, including the following: (i) setae of the larval mandible are inserted on a large, flat, lateral area delimited ventrally by a projecting line; (ii) at least one secondary seta is associated with L3 on the abdominal segments 1-8 of the larva; (iii) the femur of the pupal foreleg is concealed or very slightly exposed; and (iv) the adult abdomen has a lateral complete prespiracular sclerite, interconnecting the first sternite with the lateral bar of the first tergite, which is modified into tympanal organs in the Drepanidae. Despite the fact that other authors have placed Epicopeiidae within the Uranoidea (Imms, 1934; Inoue, 1954; Zhu & Wang, 1991; Kuznetsov & Stekolnikov, 2001), Minet's (1991) definition of the Drepanoidea has been widely accepted (Scoble, 1992; Holloway, 1998; Minet & Scoble, 1999; Holloway et al., 2001; Kristensen et al., 2007). Contrary to this definition, recent molecular studies suggest that Epicopeiidae be placed either next to or within the Geometroidea, as a sister-group to Sematuridae (Regier et al., 2009) or Uraniidae (Wu et al., 2009). A molecular phylogenetic study by Mutanen et al. (2010) found that Epicopeiidae forms a sister-group with the Lasiocampoidea, which may also have some support from morphological findings. The taxonomic status of Epicopeiidae is therefore still under consideration, and needs to be further validated by other molecular phylogenetic studies.

There has been some debate on whether Drepaninae should be further divided into subgroups based on adult body colour, proboscis and frenulum, forewing colour and shape, hind tibial spurs, larval secondary setae, and supracoxal vesicle (reviewed in Wu et al., 2009). Based on these characters, many authors believe that Drepaninae should be either divided into two subfamilies, Drepaninae and Oretinae (Inoue, 1962; Nakajima,

1970; Wilkinson, 1972; Zhu & Wang, 1991; Smetacek, 2002), or into two subgroups at the tribal level, Drepanini and Oretini (Watson, 1965; Watson, 1967; Minet, 1985; Scoble, 1992; Holloway, 1998). More recent studies have even divided Drepaninae into three tribes, Nidarini, Oretini and Drepanini (Minet & Scoble, 1999). Further investigation into the taxonomic status of the *Oreta* group is therefore still necessary.

A preliminary phylogenetic study of the Drepanoidea using two molecular markers, EF-1 $\alpha$  and COI was conducted by Wu et al. (2009) to resolve some of the uncertainties surrounding the taxonomic status of the Drepanoidea and its groups. Although this study was deemed 'a pilot study' and included only 18 taxa, the results provided good support for the monophyly of each of Drepaninae, Thyatirinae, and Cyclidiinae subfamilies, and validated the sister relationship between Drepaninae and Thyatirinae as suggested by Minet (2002). Using molecular phylogenetic analysis, as well as morphological characters, Wu et al. (2009) suggested that *Oreta* should be separate from Drepaninae, with Oretinae restored as a sister-group, under the caveat that analysis of taxa that are not limited to China are required to provide further support. In terms of the taxonomic placement of Epicopeiidae, Wu et al. (2009) showed with both molecular and morphological support that Epicopeiidae has a closer phylogenetic relationship to Geometridae than to Drepanidae, and should therefore be placed within Geometroidea. This conclusion, however, was not supported by all of their molecular phylogenetic analyses, and still requires further consideration.

In order to further test some of the hypotheses on relationships within Drepanoidea, and also to surmise the evolutionary origin of vibratory signals in later chapters, I have chosen to construct a molecular phylogeny of Drepanoidea using three

genes. There are several useful molecular markers for determining the relationships between groups of caterpillars and other insects. Some of the most widely used include the 16S rDNA, 18S rRNA, 28S rRNA, elongation factor-1 $\alpha$  (EF-1 $\alpha$ ), and cytochrome oxidases (COI and COII) (Caterino et al., 2000). Several studies in Lepidoptera have highlighted the importance of combined analyses of nuclear and mitochondrial genes, due to improved resolution of nodal support at both higher and intermediate systematic categories of divergence (Caterino et al., 2001; Monteiro, 2001; Wahlberg, 2003; Kandul et al., 2004; Zakharov et al., 2004). As such, I have chosen to create my trees using a combination of three genes that have proven to be useful in Lepidoptera, including one mitochondrial (ND1) and two nuclear genes (28S, CAD). The mitochondrial ND1 gene has been found to be useful below the superfamily level in Lepidoptera (Pashley & Ke, 1992; Weller et al., 1994; Weller & Pashley, 1995; Abraham et al., 2001). This gene is located between the 16S rRNA and the cytochrome *b* genes in the Lepidoptera mitochondrial genome (Liao et al., 2010) and codes for a subunit of the enzyme NADH-dehydrogenase, one of three enzymes responsible for the transport of electrons from NADH to oxygen to eventually form water (Weiss et al., 1991). The D2 expansion segment of the 28S rRNA gene was chosen due to its high mutation rate and its known utility in insect phylogenetics (Gillespie, 2005). In insects, the 28S rRNA molecule consists of a set of conserved core elements, with 13 interspersed expansion segments (Hancock & Dover, 1988; Hancock et al., 1988; Tautz et al., 1988). Unlike the core segments, the expansion segments are highly variable among insect orders (Hwang et al., 1998; Gillespie, 2005). The nuclear CAD gene, has also proven to be very informative for recovering deep relationships above the tribe level in other Lepidopteran taxa

(Kawahara et al., 2009; Regier et al., 2009; Cho et al., 2011; Kawahara et al., 2011).

CAD is a fusion protein that encodes for three enzymes of the de novo pyrimidine biosynthetic pathway and was first described for its utility in dipteran phylogenetics (Moulton & Wiegmann, 2004).

The main goal of this chapter is to create a molecular phylogeny of the Drepanoidea using regions of the aforementioned three genes (28S, ND1 and CAD) including species that I will be studying in later chapters of this thesis to test hypotheses related to the origin of signals. Additionally, this chapter will also discuss, in light of this molecular phylogeny, some of the taxonomic issues still surrounding the Drepanoidea, including the status of Epicopeiidae and *Oreta*.

### **3.2 Methods**

#### *Specimens*

Adult moths and larvae were obtained from a variety of sources (Table 3.1). Table 3.1 also specifies the collection localities, type of specimen and GenBank accession numbers for all sequences. Sequences from 35 ingroups in three sub-families of Drepanidae and 13 genera were obtained for phylogenetic analysis. Two representatives of Epicopeiidae, three representatives of Geometridae, and one representative each of Sematuridae and Pyralidae were used as outgroups. Outgroups were chosen based on the placement of Drepanoidea in morphological (Minet & Scoble, 1999) and molecular (Regier et al., 2009) phylogenetic studies of greater lepidopteran relationships. The pyralid, *Accinctapubes albifasciata*, was used to root all trees, given the placement of Pyralidae in Lepidoptera (Regier et al., 2009; Mutanen et al., 2010; Cho et al., 2011).

**Table 3.1.** Specimen collection data, voucher specimen data and GenBank accession numbers for 43 taxa used for phylogenetic analysis. Numbers in brackets represent different specimens.

Taxon	Specimen Data				Voucher Specimen		GenBank Accession Number		
	Adult/ Larvae	Country	Locality	Collector	Location	Accession Number	CAD	28S D2	ND1
<b>INGROUP TAXA</b>									
<b>Cyclidiinae</b>									
<i>Cyclidia substigmata</i>	Adult	Taiwan	Uncertain	A. Kawahara	AToLep	AYK-04-0779-08	GU174162	GU174201	GU174237
<i>substigmata</i>									
<b>Drepaninae</b>									
<i>Agnidra scabiosa scabiosa</i>	Adult	Japan	Honshu	A. Kawahara	AToLep	AYK-06-7252	GU174158	GU174197	GU174233
<i>Ausaris micacea</i>	Adult	Taiwan	Nantou, Shanlinshi	A. Kawahara	AToLep	AYK-04-0889-05	GU174160	GU174199	GU174235
<i>Ausaris palleolus</i>	Adult	Japan	Shizuoka Pref., Fujinomiya, Mt. Fuji, Second level trail	A. Kawahara	AToLep	AYK-06-7263	GU174161	GU174200	GU174236
<i>Auzata superba</i>	Adult	Taiwan	Uncertain	A. Kawahara	AToLep	AYK-041013-10	GU174159	GU174198	GU174234
<i>Cilix glaucata</i>	-	-	-	-	-	-	-	AF178907 <sup>1</sup>	AF178859 <sup>1</sup>
<i>Drepana arcuata</i>	Adult	USA	Mathias, Hardy County, West Virginia	C. Mitter	AToLep	CWM-96-0578	GU174163	GU174202	GU174238
<i>Drepana curvatula</i>	Adult	Japan	Yamanashi, Motosu- shistugen, Osaka Trail	A. Kawahara	Carleton (1) AToLep (2)	D0078 (1) AYK-04-5230 (2)	GU174165 (1) GU174166 (2)	GU174204 (1) GU174205 (2)	GU174240 (1) GU174241 (2)
<i>Drepana falcata</i>	Adult	Germany	Erlangen, Northern Bavaria	I. Hasenfuss	Carleton	D0069	GU174167	AF178908 <sup>1</sup>	AF178860 <sup>1</sup>
<i>Falcaria bilineata</i>	Adult	Canada	Ottawa, Ontario	J. Yack	Carleton	D0084	GU174164	GU174203	GU174239
<i>Falcaria lucertinaria</i>	-	-	-	-	-	-	-	AF178906 <sup>1</sup>	AF1788658 <sup>1</sup>
<i>Macrauzata maxima maxima</i>	Adult	Japan	Shizuoka, Fujinomiya, Nonaka	A. Kawahara	AToLep	AYK-04-5709	GU174174	GU174213	GU174246
<i>Microblepsis acuminata</i>	Adult	Japan	Shizuoka Pref., Fujinomiya, Mt. Fuji, Second level trail	A. Kawahara	AToLep	AYK-06-7266	GU174175	GU174214	-
<i>Nordstromia grisearia</i>	Adult	Taiwan	Uncertain	A. Kawahara	AToLep	AYK-04-0819-04	GU174176	-	GU174248

Taxon	Specimen Data				Voucher Specimen		GenBank Accession Number		
	Adult/ Larvae	Country	Locality	Collector	Location	Accession Number	CAD	28S D2	ND1
<i>Oreta loochooana</i>	Adult	Japan	Yamanashi, Motosu-shistugen, Osaka Trail	A. Kawahara	Carleton	D0079	GU174178	GU174217	GU174250
<i>Oreta pulchripes</i>	Adult	Japan	Yamanashi, Motosu-shistugen, Osaka Trail	A. Kawahara	AToLep	AYK-04-5328	GU174179	GU174218	GU174251
<i>Oreta rosea</i>	Adult	USA	Mathias, Hardy County, West Virginia	C. Mitter	AToLep	CWM-95-0466	GU174180	GU174219	GU174252
<i>Oreta turpis</i>	Adult	Japan	Yamanashi, Motosu-shistugen, Osaka Trail	A. Kawahara	AToLep	AYK-04-5733	GU174181	-	GU174253
<i>Sabra harpagula</i>	Adult	Japan	Yamanashi, Motosu-shistugen, Osaka Trail	A. Kawahara	Carleton	D0080	GU174184	GU174222	-
<i>Tridrepana flava</i>	Adult	Malaysia	Pahang, Genting Highlands	A. Kawahara	AToLep	AYK-04-0833-01	GU174192	GU174228	GU174260
<i>Tridrepana unispina</i>	Adult	Japan	Shizuoka, Shizuoka-shi, Honkawane, Kaminagao	A. Kawahara	AToLep	AYK-04-5363	GU174193	GU174229	GU174261
<i>Watsonalla binaria</i>	Adult	Germany	Erlangen, Northern Bavaria	I. Hasenfuss	Carleton	D0023	GU174194	GU174230	GU174262
<i>Watsonalla cultraria</i>	Adult	Switzerland	CABI	J. Miall	Carleton	D0086	GU174195	-	GU174263
<i>Watsonalla uncinula</i>	Adult	France	Boulu, Pyrenees	H. Beck	Carleton	D0017	GU174196	GU174231	-
<b>Thyatirinae</b>									
<i>Euparyphasma maxima</i>	Adult	Japan	Shizuoka Pref., Fujinomiya, Mt. Fuji, Second level trail	A. Kawahara	AToLep	AYK-04-5376	GU174170	GU174208	GU174244
<i>Euthyatira pudens</i>	Adult	Canada	Ottawa, Ontario	L. Scott	Carleton	D0081	GU174171	GU174209	-
<i>Habrosyne aurorina aurorina</i>	Adult	Japan	Shizuoka Pref., Fujinomiya, Mt. Fuji, Second level trail	A. Kawahara	AToLep	AYK-06-7260	-	GU174210	-
<i>Habrosyne pyritoides</i>	Adult	Japan	Yamanashi, Motosu-shistugen, Osaka Trail	A. Kawahara	AToLep	AYK-04-5350	GU174172	GU174211	-
<i>Neodaruma tamanukii</i>	Adult	Japan	Uncertain	K. Eda	Carleton	D0074	-	GU174215	GU174247
<i>Ochropacha duplaris</i>	Larva	Finland	Sipoo	K. Silvonen	Carleton	D0100	GU174177	GU174216	GU174249
<i>Pseudothyatira cymatophoroides</i>	Adult	USA	Clarkesville, Maryland	C. Mitter	AToLep	CWM-94-0380	GU174182	GU174220	-
<i>Tethea consimilis</i>	Adult	Japan	Shizuoka, Shizuoka-shi, Honkawane, Kaminagao	A. Kawahara	AToLep	AYK-04-5374	GU174186	GU174224	-

Taxon	Specimen Data				Voucher Specimen		GenBank Accession Number		
	Adult/ Larvae	Country	Locality	Collector	Location	Accession Number	CAD	28S D2	ND1
<i>Tethea oberthuri taiwana</i>	Adult	Taiwan	Taitung, Lijia	A. Kawahara	AToLep	AYK-04-0786-06	GU174187	GU174225	GU174256
<i>Tethea or</i>	Larva	Finland	Sipoo	K. Silvonen	Carleton	D0099	GU174188	GU174226	GU174257
<i>Tetheela fluctuosa</i>	Larva	Finland	Sipoo	K. Silvonen	Carleton	D0103	GU174189	GU174227	GU174258
<i>Thyatira batis</i>	Adult (1) Larvae (2)	Germany (1) Finland (2)	Erlangen, Northern Bavaria (1) Sipoo (2)	I. Hasenfuss (1) K. Silvonen (2)	Carleton	D0007 (1) D0097 (2)	GU174190 (1) GU174191 (2)	-	GU174259 (1)
<b>OUTGROUP TAXA</b>									
<i>Accinctapubes albifasciata</i> (Pyrallidae: Epipaschiinae)	unknown	unknown	unknown	unknown	AToLep	06-srmp-3517	GU174157	-	GU174232
<i>Ennomos autumnaria</i> (Geometridae: Ennominae)	unknown	unknown	unknown	A. Kawahara	AToLep	AYK-06-7362	GU174168	GU174206	GU174242
<i>Epicopeia hainesii hainesii</i> (Epicopeiidae)	Adult	Japan	Yamanashi Prefecture, Motosu	A. Kawahara	AToLep	AYK-06-7220	GU174169	GU174207	GU174243
<i>Jodis putata</i> (Geometridae: Geometrinae)	-	-	-	-	-	-	-	AF178920 <sup>1</sup>	AF178872 <sup>1</sup>
<i>Lyssa zampa zampa</i> (Geometridae: Uraniinae)	unknown	unknown	unknown	A. Kawahara	AToLep	A-0581	GU174173	GU174212	GU174245
<i>Psychostrophia melanargia</i> (Epicopeiidae)	Adult	Japan	Yamanashi, Motosu- shistugen, Osaka Trail	A. Kawahara	AToLep	AYK-06-7206	GU174183	GU174221	GU174254
<i>Nothus lunus</i> (Sematuridae: Sematurinae)	unknown	unknown	unknown	R. Hutchings	AToLep	RWH-96-0877	GU174185	GU174223	GU174255

<sup>1</sup> Sequence obtained from Abraham et al. (2001)

Sequences for 28S and ND1 for four taxa were obtained from the literature (Abraham et al., 2001) (Table 3.1). Two individuals each (from two different sources) of two species (*Drepana curvatula* and *Thyatira batis*) were sequenced for at least one gene (Table 3.1).

In nearly all cases, live adults were collected at light traps and legs were removed and stored in 100% ethanol at -80°C. Adults were kept as voucher specimens. In some cases, adult females oviposited on cuttings of their hostplant and larvae were reared from eggs. Late instar larvae were injected with 100% ethanol and stored in 100% ethanol at -80°C. Voucher specimens were deposited at either the AToLep Collection at the University of Maryland, USA, or in the Biology Department at Carleton University, Canada (Table 3.1). Wing voucher images for all adult exemplars located at the AToLep Collection are posted at [http://www.leptree.net/voucher\\_image\\_list](http://www.leptree.net/voucher_image_list).

#### *DNA Extraction, Amplification and Sequencing*

Total genomic DNA was extracted from 43 taxa. A single leg of an adult, or the head capsule of a larva, was ground and digested at 58°C for 1 hour with 20 mg/mL proteinase K in 250 µL of 0.1 M Tris (pH 8.0), 10 mM EDTA, and 2% SDS. Samples were then incubated at 65°C for an additional 10 minutes in a solution containing 5 M NaCl and 10% CTAB. The solutions were extracted using standard phenol-chloroform protocols and DNA was precipitated overnight in 95% EtOH.

DNA fragments were amplified by polymerase chain reaction (PCR) in a Biometra TGradient® thermocycler (Goettinger, Germany) using specific primers for three genes – CAD, ND1 and 28S. Amplifications were done in 20 µL volumes containing 3 µL of each primer, 2.0 µL 10X *Taq* buffer - MgCl<sub>2</sub> (Bioshop, Ontario,

Canada), 1.2  $\mu\text{L}$   $\text{MgCl}_2$  (Bioshop), 0.4  $\mu\text{L}$  of an equimolar solution of dNTPs (Invitrogen, California, USA), 0.5  $\mu\text{L}$  of Taq polymerase (Bioshop), 7.9  $\mu\text{L}$   $\text{dH}_2\text{O}$ , and 2  $\mu\text{L}$  DNA template. Negative controls were carried out using 2  $\mu\text{L}$   $\text{dH}_2\text{O}$  in place of the DNA template.

A typical PCR procedure for all genes consisted of 3 min denaturation at 95°C, followed by 30 cycles including denaturation (30 s at 95°C), annealing (30 s, temperatures given in Table 3.2) and extension (30 s at 72°C). An additional 10 min extension step at 72°C was included in the final cycle. Primers and specific annealing temperatures are listed in Table 3.2. It was often necessary to follow the initial amplification with a second amplification using nested primers for CAD (791F and 963R; see Table 3.2). We also designed Drepanidae-specific primer pairs based on Weller et al. (1994) for ND1 (Table 3.2). PCR products and controls were verified by agarose gel electrophoresis and either purified using a GenElute PCR Clean-Up Kit (Sigma-Aldrich, Missouri, USA) for direct sequencing, or cloned using a TOPO Cloning Kit (Invitrogen) prior to sequencing multiple clones of each fragment.

Both sense and anti-sense strands of gel-purified or cloned DNA fragments were sequenced with an Applied Biosystems 3730 DNA Analyzer (California, USA) at the Ottawa Health Research Institute (Ottawa, Canada), or at the McGill University and Genome Québec Innovation Centre (Montréal, Canada). Sequences of 28S and NDI from 20 taxa were obtained by A. Sami (unpublished).

### *Sequence Alignment*

Sequence contigs were viewed, assembled, and edited using DNA Baser

**Table 3.2.** Primers used for PCR amplification of selected gene fragments.

<b>Gene Locus</b>	<b>Primer</b>	<b>Sequence</b>	<b>Reference</b>	<b>Annealing Temperature</b>
<b>28S</b>	28S D2F	AGAGAGAGTTCAAGAGTACGTG	(Belshaw & Quicke, 1997)	59°C
	28S D2R	TTGGTCCGTGTTTCAAGACGGG	(Belshaw & Quicke, 1997)	
<b>CAD</b>	743nF	GGNGTNACNACNGCNTGYTTYGARCC	(Regier, 2008)	50°C
	1028R	TTRTTNGGNARYTGNCNCCCAT	(Regier, 2008)	
	791F	TTYGARGARGCNTTYCARAARGC	(Regier, 2008)	50°C
	963R	GCRACCARTCRAAYTC	(Regier, 2008)	
<b>ND1</b>		GAGCCAGGTTGGTTTCTATC	Based on (Weller et al., 1994)	53°C
		GAATTAGAAGATCAACCAGCAA	Based on (Weller et al., 1994)	

(HeracleSoftware). Sequences were aligned in MAFFT (Kato, 2009) using the E-INS-I setting with 1000 iterations, and verified by eye. Alignments of protein coding genes (CAD and ND1) were also checked for stop codons. Primer ends were removed from all genes, and 181 characters were removed from a non-coding region of the ND1 gene. Final character numbers and number of informative characters for each gene are included in Table 3.3. Sequence alignment data is included in Appendix B.

### *Phylogenetic Analysis*

#### Parsimony

Parsimony analyses were conducted using the concatenated data set in PAUP\* 4.0 (Swofford, 2003) with all characters treated as unordered. A heuristic search with tree bisection-reconnection (TBR) branch swapping in a random stepwise addition of taxa was repeated 1000 times. The information value of third positions of codons in the CAD gene and ND1 were evaluated by using two weighting schemes, the first with equal weighting and the second with the third codon position (nt3) weighted to zero. Gap treatment was also evaluated by treating them as either missing or as a fifth state. Based on these evaluations, nt3 were not weighted, and gaps were treated as missing data, as these led to the least number of most parsimonious trees with the shortest tree lengths (see Results for details). Node support for the concatenated data set was determined by jackknife resampling with 36% of characters excluded and 100 random replicates. Total Bremer support values were also calculated using TreeRot v3 (Sorenson and Franzosa, 2007) and PAUP\* 4.0 (Swofford, 2003) using a heuristic search and 1000 random replicates.

## Bayesian Inference

The optimal nucleotide substitution model for each gene partition was determined with the Akaike Information Criterion (Akaike, 1973) as implemented in ModelTest 3.7 (Posada & Crandall, 1998). The best-fit models from ModelTest for each gene partition were GTR+G (28S) and GTR+I+G (CAD, ND1). Bayesian analysis was conducted on the concatenated data set in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) with 8 chains, sampling every 1000 generations with a heating temperature of 0.12. The Markov chain was terminated at 25 M generations, determined by examining trace plots in AWTY (Wigenbusch et al., 2004).

### **3.3 Results**

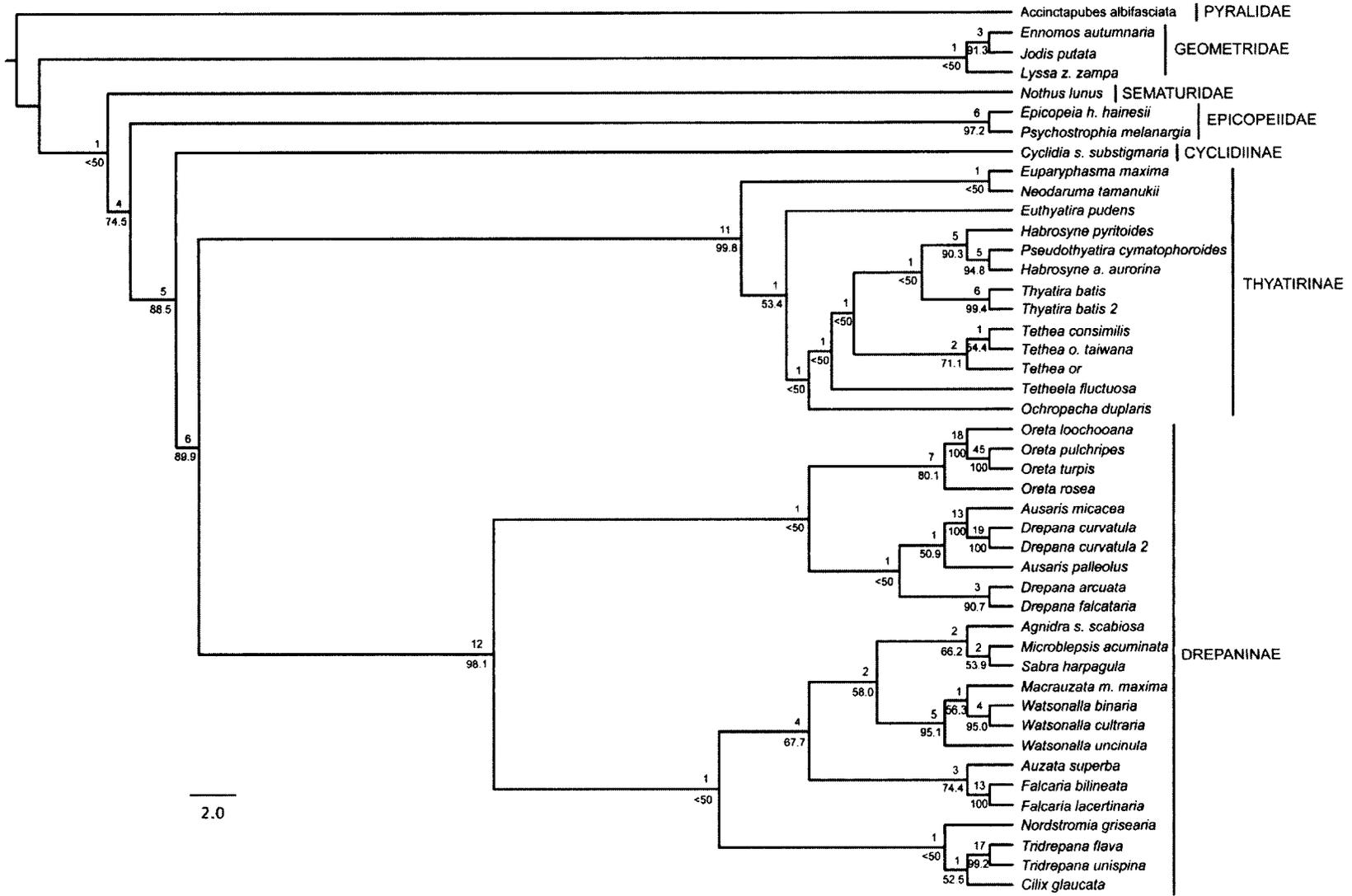
#### *Parsimony*

The concatenated dataset, comprised 43 taxa and 1617 characters, including 662 (40.9%) constant characters and 760 (47%) parsimony informative characters. Values for number of informative characters, number of most parsimonious trees, treelengths, consistency index, and retention index are shown for each gene separately and for combined analysis with different weighting schemes in Table 3.3. Parsimony analysis recovered one most parsimonious tree (MPT) with a length of 3793 steps when third codon positions were excluded and gaps were treated as missing data (CI = 0.373, RI = 0.504) (Fig. 3.1). This tree is well supported with jackknife support (JKS) values above 50% for 30 out of 41 nodes and values of 100% for 5 nodes. Bremer support (BS) values are high (>5) for 16 nodes. Drepanidae and Epicopeiidae are both recovered as monophyletic with high support values (Drepanidae: JKS = 88.5, BS = 5; Epicopeiidae:

**Table 3.3.** Summary of results for individual and concatenated gene partitions from parsimony analysis. Equal - equal weighting; nt3=0 - nt3 weighted to 0; missing - gaps treated as missing; 5th - gaps treated as 5th state.

	Individual Genes			Concatenated			
	CAD	ND1	28S	Equal/missing	Equal/5th	nt3=0/missing	nt3=0/5th
# taxa included	40	37	40	45	45	45	45
# characters analyzed	638	450	529	1617	1617	1617	1617
% characters constant	62.1	57.8	53.1	40.9	40.9	45.3	45.3
% characters informative	37.9	42.2	46.9	47.0	47.0	42.1	42.1
Average nucleotide frequencies							
A	37.4	33.2	21.7				
C	13.0	5.7	23.9				
G	17.2	12.2	25.4				
T	32.5	49.0	29.0				
# most parsimonious trees	2	10	5	1	1	29	128
Length of shortest tree(s)	1469	906	1142	3793	4213	1774	2171
Consistency Index (CI)	0.322	0.397	0.511	0.373	0.399	0.484	0.512
CI excluding uninformative characters	0.299	0.354	0.447	0.331	0.363	0.415	0.462
Retention Index (RI)	0.558	0.522	0.601	0.504	0.514	0.585	0.595

**Figure 3.1.** Single most parsimonious tree of the Drepanoidea from combined 28S, ND1 and CAD sequences (treelength = 3793, CI = 0.373, RI = 0.504). Third codon positions not weighted and gaps treated as missing data. Numbers above nodes represent Bremer support value, numbers below nodes represent jackknife support value.



JKS = 97.2, BS = 6). Epicopeiidae forms a sister-group with Drepanidae with support values of JKS = 74.5 and BS = 4). Cyclidiinae, Thyatirinae and Drepaninae are also recovered as monophyletic with high support values (Thyatirinae: JKS = 99.8, BS = 11; Drepaninae: JKS = 98.1, BS = 12), with Drepaninae and Thyatirinae emerging as a sister-group with high support (JKS = 89.9, BS = 6). *Oreta* is monophyletic with high support (JKS = 80.1, BS = 7), but forms a monophyletic group with the *Drepana* + *Ausaris* clade with weak support (JKS = <50, BS = 1). *Drepana*, *Ausaris*, *Watsonalla*, and *Habrosyne* are found to be paraphyletic. For two taxa in which sequences from two individuals were obtained (*Thyatira batis* and *Drepana curvatula*), individuals of the same species form monophyletic clades in both cases, with high support values (*Thyatira batis*: JKS = 99.4, BS = 6; *Drepana curvatula*: JKS = 100, BS = 19).

Parsimony analysis with gaps coded as a fifth state also recovers one most parsimonious tree with the same topology (L = 4213, CI = 0.399, RI = 0.514). Analysis with nt3 weighted as zero and gaps coded as missing recovers 29 MPTs (L = 1774, CI = 0.484, RI = 0.585), and with nt3 weighted as zero and gaps treated as a fifth base recovers 128 MPTs (L = 2171, CI = 0.512, RI = 0.595). Topologies for these two alternate analyses (not shown) differ from the tree shown in Fig. 3.1. All key clades (i.e. Epicopeiidae, Drepanidae, Drepaninae, Thyatirinae) are recovered as monophyletic in both cases; however, there are more paraphyletic genera in trees from these two alternate analyses than in the tree shown in Fig. 3.1.

### *Bayesian Inference*

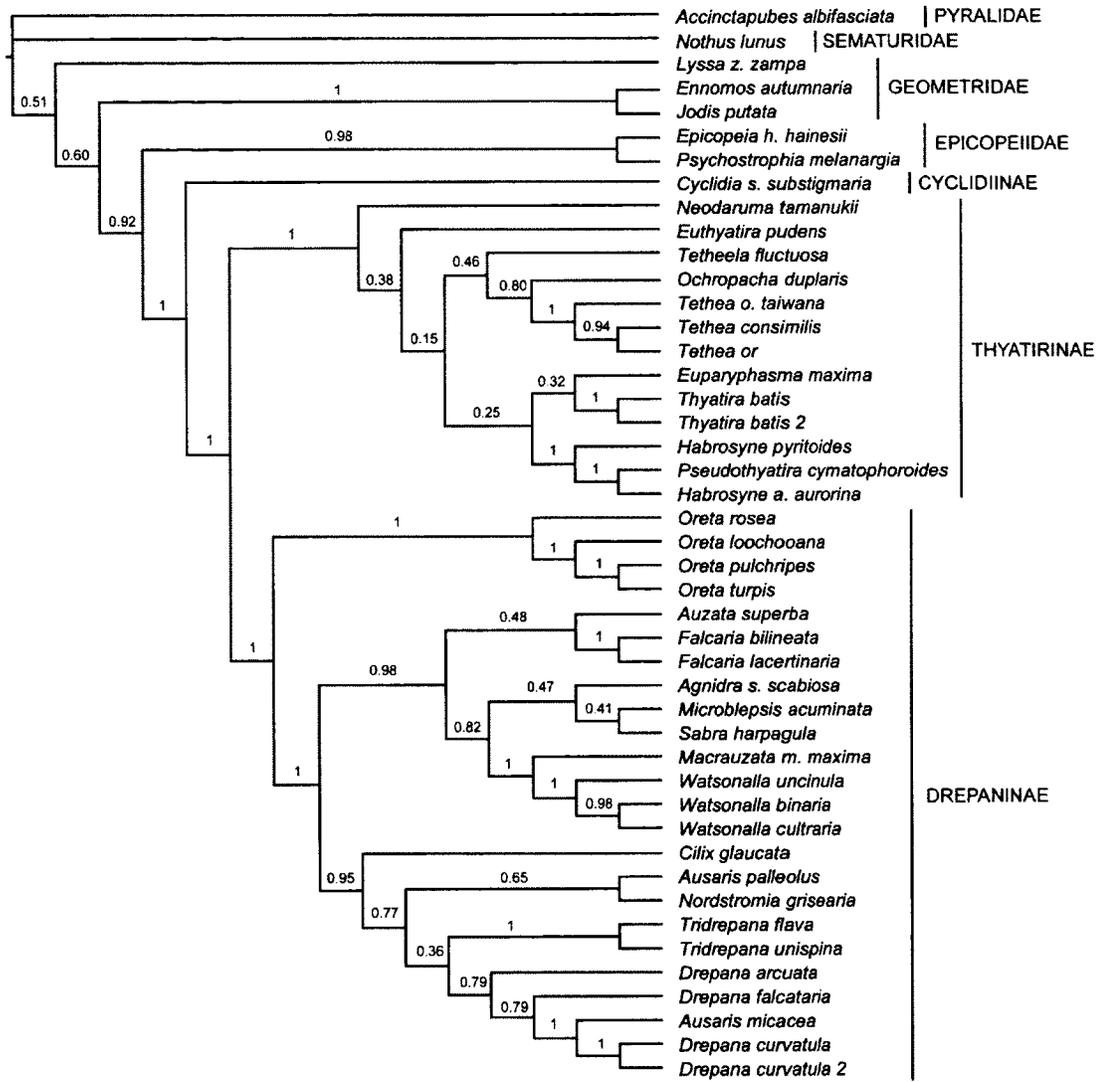
The tree generated from Bayesian inference differs from that of the parsimony MPT and has 100% posterior probabilities (PP) at 19 of 42 nodes (Fig. 3.2). Drepanidae is recovered as monophyletic with high posterior probability (PP = 100%) with the Drepaninae and Thyatirinae emerging as sister-groups (PP = 100%). This tree also supports each of Cyclidiinae, Drepaninae and Thyatirinae as monophyletic, with strong support (PP = 100% in all cases). *Oreta* is monophyletic with robust support (PP = 100%), forming a sister relationship to the rest of Drepaninae (PP = 100%). Finally, Epicopeiidae is monophyletic with high posterior probability (PP = 98%) and is closer to the Drepanidae than to the Geometridae (PP = 92%). Most of the genera within the Thyatirinae and Drepaninae are monophyletic, except *Ausaris* (Drepaninae). In *Ausaris*, *A. micacea* emerges with *Drepana* and *A. palleolus* emerges with *Nordstromia*, both with relatively high support (PP = 100% and 65%, respectively). In addition, *Habrosyne* (Thyatirinae), includes *Pseudothyatira cymatophoroides* with a high support value (PP = 100%). Individuals of the same species form monophyletic clades, with high posterior probabilities (PP = 100% in both cases) for both taxa in which sequences from two individuals were obtained (*T. batis* and *D. curvatula*).

### **3.4 Discussion**

#### *Preferred Tree*

Recovery and strong support for the monophyly of previously defined taxonomic groups is used to measure the strength of an analysis (Wild and Maddison, 2008; Winterton et al., 2007; Yoder et al., 2001). Therefore, in this study, recovery of

**Figure 3.2.** Majority-rule consensus Bayesian tree (25 M generations) of the Drepanoidea reconstructed using combined 28S, ND1 and CAD sequences. Bayesian posterior probabilities are shown above the branches.



congeneric and confamilial nodes with high support values has been taken as evidence of a well-resolved tree. Both Bayesian and parsimony analyses recovered Epicopeiidae and Drepanidae as monophyletic sister-groups, as well as the monophyletic sub-families of Drepanidae (Cyclidiinae, Thyatirinae and Drepaninae) with strong branch support. Both types of analyses also grouped taxa in which two samples were available (*Thyatira batis* and *Drepana curvatula*) into monophyletic clades with high support, giving support to both types of analyses. However, the parsimony tree did not resolve relationships as well at the generic level, with four genera recovered as paraphyletic (compared to three in the Bayesian tree). As such, the Bayesian tree was chosen as the preferred tree. Since three of the paraphyletic genera (*Drepana*, *Ausaris*, and *Habrosyne*) are congruent between the parsimony and Bayesian analyses and have high support values, this suggests that the concepts for these genera require a subsequent revision.

### *Epicopeiidae*

The taxonomic status of Epicopeiidae has long been debated and its position in the Drepanoidea remains uncertain. The results of my phylogenetic analysis reveal that Epicopeiidae is most closely related to Drepanidae (Cyclidiinae + Thyatirinae + Drepaninae) using both parsimony and Bayesian analyses, with high support values. This finding suggests that Minet (1999) was correct in placing Epicopeiidae within the Drepanoidea based on morphology. However, this is in direct contrast to recent molecular studies on ditrysian phylogenetics that recommended that Epicopeiidae be removed from the Drepanoidea and placed next to Sematuridae (Regier et al., 2009) or Lasiocampoidea (Mutanen et al., 2010). It also differs from the pilot study of Wu et al. (2009) that

suggests Epicopeiidae is most closely related to Geometridae. The findings of Wu et al. (2009) may be due to low taxon and gene sampling, and future studies that include more taxa and genes may ultimately reveal the taxonomic position of Epicopeiidae.

### *The Oreta Group*

In their paper on the phylogeny of the Drepanoidea, Wu et al. (2009) suggested based on molecular data that the *Oreta* group should be excluded from Drepaninae to form a separate subfamily, Oretinae. This also agrees with past morphological studies which suggested that Drepaninae be split into Drepaninae and Oretinae (Inoue, 1962; Nakajima, 1970; Wilkinson, 1972; Zhu & Wang, 1991; Smetacek, 2002). Wu et al. (2009) however, cautioned that their molecular findings were limited due to taxon sampling as their taxa were selected based on their availability in China. They suggested that further studies, covering more genera and molecular markers were needed to test the strength of the support for Oretinae and Drepaninae. My study includes taxa found in a number of localities around the world, including North America, Europe and Asia and I have examined three additional genes. My results concur with the findings of Wu et al. (2009), in that *Oreta* forms an independent clade with robust support in both parsimony and Bayesian analyses and is separate from the rest of Drepaninae in the Bayesian tree. Based on this finding, as well as morphological evidence discussed in Wu et al. (2009), I suggest that *Oreta* be separated from Drepaninae and form its own subfamily, Oretinae.

The molecular phylogeny created in this chapter serves two purposes. First, it extends previous studies of the Drepanoidea phylogeny by using further molecular markers and more taxon sampling. In doing so, the results of this molecular phylogeny

shed some light on the issues surrounding the taxonomic status of the Drepanoidea, including the placements of Epicopeiidae and *Oreta*. Second, I have determined the relationship between species that I will be examining for morphological and behavioural characters in subsequent chapters of this thesis. These morphological and behavioural characters will be mapped to test hypotheses on the evolutionary origin of vibratory signals in these caterpillars, more specifically that anal scraping derives from crawling (Chapter 4) and that mandible signals derive from aggressive behaviours involving the head (Chapter 5). The phylogenetic tree created in this chapter will provide a solid foundation to test these hypotheses.

## CHAPTER 4

### FROM WALKING TO TALKING: THE EVOLUTIONARY ORIGIN OF ANAL SCRAPING SIGNALS IN DREPANIDAE CATERPILLARS

**This chapter forms the following manuscripts:**

Scott, J. L., Kawahara, A. K., Skevington, J. H., Yen, S. -H., Sami, A., Smith, M. L. & Yack, J. E. (2010). The evolutionary origins of ritualized acoustic signals in caterpillars. *Nature Communications* 1, 4; doi: 10.1038/ncomms1002.

Scott, J. L. & Yack, J. E. Caterpillars talk their walk: How vibratory signals evolved from crawling movements in caterpillars (Lepidoptera: Drepanidae) (in preparation for submission to the *Journal of Experimental Biology* in October, 2012)

Note: Although these manuscripts differ in the amount of detail presented, they do not differ in major conclusions or findings. This chapter follows the format of the more detailed manuscript (Scott & Yack, in prep).

#### ***4.1 Introduction***

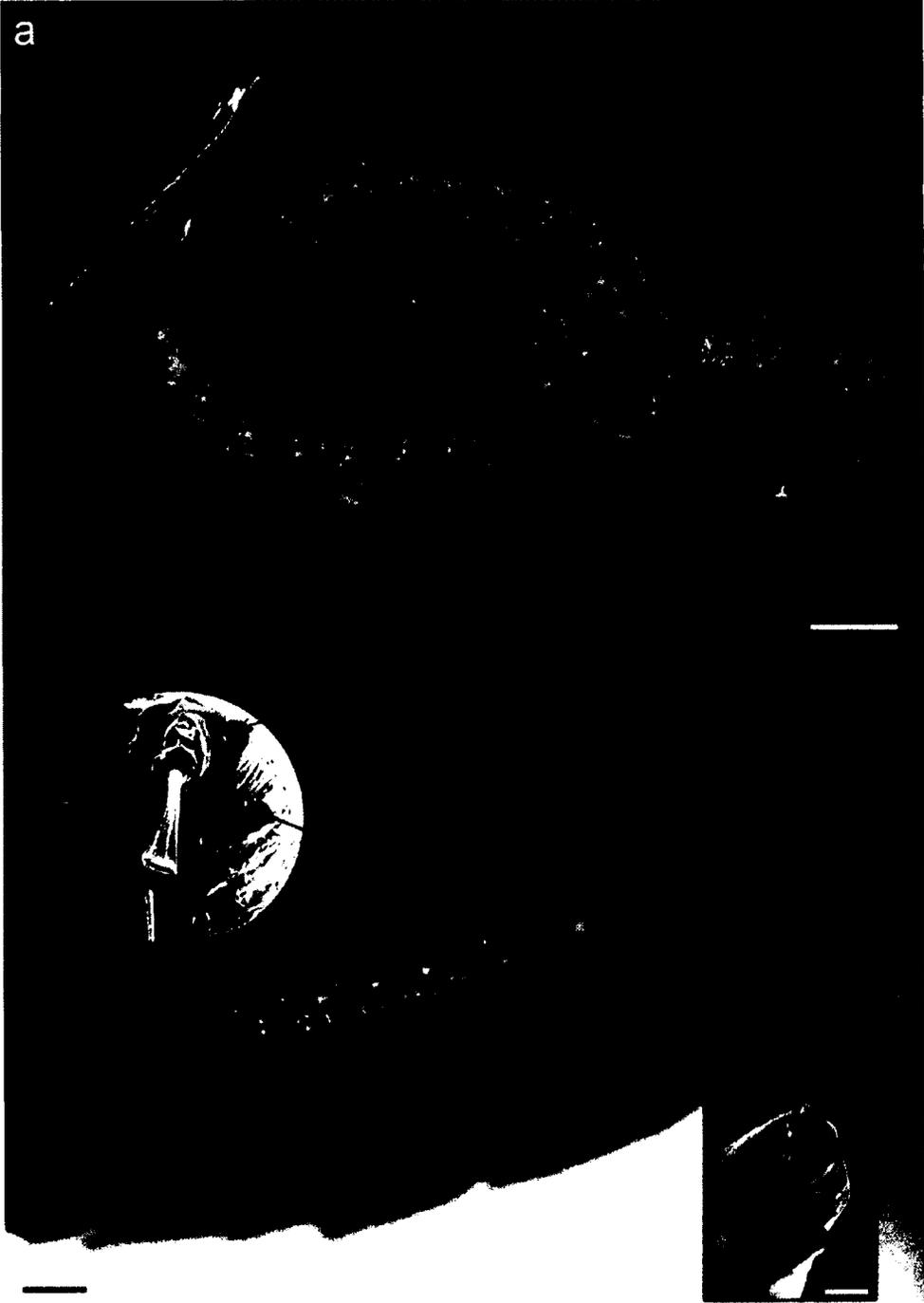
Since Darwin's seminal paper "The Expression of the Emotions in Man and Animals", many scientists have sought explanations for the evolutionary origins of communication signals. Tinbergen (1952), Lorenz (1966) and Huxley (1966) hypothesized that signals are ultimately derived from behaviours previously unconnected with communication through the process of ritualization. During this process, cues generated by intention movements, displacement behaviours or physiological states are modified to enhance their efficacy through enhanced conspicuousness (e.g. increasing the amplitude of the signal), redundancy (e.g. repeating the signal or elements of the signal), stereotypy (e.g. a reduction in variation of the signal), and alerting components (e.g. a conspicuous component at the beginning of the signal) (Cullen, 1966; Wiley, 1983; Johnstone, 1997). Support for hypotheses on signal origins is generally based on comparisons of movement patterns and associated morphology within or between species. As explained in Chapter 1 of this thesis multiple species comparisons have been used to provide support for the hypothesis that the peacock tail-fan display originated from males leaning forward to present food to females (Schenkel, 1956). Although most comparative studies pose interesting hypotheses on signal origins, they are limited in that they lack a phylogenetic framework onto which one can trace the evolutionary history of behaviours (Brooks & McLennan, 2002). Many studies have used a phylogeny to examine other aspects of signal evolution (e.g. Weller et al., 1999; Patek & Oakley, 2003; Martins et al., 2004; Symonds et al., 2009), but studies on the origin of signals that base their results upon a phylogenetic framework are currently non-existent. Therefore, the

major goal of my thesis is to provide evidence for the origin and ritualization of a communication signal using a combination of morphology, behaviour and phylogenetics.

Caterpillars of the family Drepanidae provide an excellent model system to study the evolutionary origin of signals because, as I have shown in Chapter 2 of this thesis, they show wide variation in morphology and behaviour associated with signalling across species. Furthermore, by knowing the relationships between species (see Chapter 3), one can gain a better understanding of the basal behaviours and transitional stages that play a role in the evolution of the signal. In the Drepanidae, certain species use vibration signals in the context of territory defence. For example, solitary late instar caterpillars of *D. arcuata* construct and occupy leaf shelters (Fig. 4.1a), and when approached by a conspecific, residents will produce territorial vibration signals (mandible scraping, mandible drumming, anal scraping) (Yack et al., 2001) (Fig. 4.1b). Variation has been noted in both the signalling behaviours and the morphology of structures involved in signal production associated with anal segments where some species lack signals and signalling structures altogether, while others produce large repertoires of signals and possess highly developed signalling structures (see Chapter 2).

The major goal of this chapter (Chapter 4) is to test the hypothesis that one of these signals, anal scraping, evolved from movements associated with crawling. The results of this chapter will be the first published empirical evidence for the origin and ritualization of a communication signal. To test my hypothesis, I will test the following predictions: 1) the possession of anal prolegs (used for crawling) represents the basal condition; 2) signalling structures found on the anal segment derive from unmodified PP1 setae; and 3) movements associated with anal scraping derive from crawling. I will test

**Fig. 4.1.** An example of signalling in *Drepana arcuata*. **(a)** Two caterpillars within a partially made leaf shelter during a territorial encounter (scale bar = 6 mm; photo credit: J. Yack). **(b)** The signalling repertoire of *D. arcuata*, including anal scraping (AS), mandible drumming (MD) and mandible scraping (MS), with scanning electron micrographs of signal-producing structures (anal oar, left, scale bar = 50  $\mu\text{m}$ ; and mandible, right, scale bar = 100  $\mu\text{m}$ ; photo credits: J. Yack) (scale bar = 1.5 mm; photo credit: S. Matheson).



these predictions by mapping signalling and territorial behaviours, as well as anal segment morphology onto the phylogeny I created in Chapter 3, and by examining basal and derived characters. I also predict: 4) that the vibrations produced by anal scraping will show an increase in conspicuousness, redundancy and stereotypy, and the presence of alerting components when compared to crawling vibrations. I will test this prediction by comparing vibration characteristics between anal scraping and crawling. Finally, I will test the prediction: 5) that anal scraping and crawling will be in the same order of events in a typical sequence of behaviours during encounters with conspecifics in anal scraping and non-anal scraping species. This will be tested by comparing sequences of behaviours between two representative species. Once basal and derived conditions are established, the second goal of this chapter is to propose a model on the evolutionary transition between crawling and anal scraping. This model will be used to explain how anal scraping evolved from a non-signalling behaviour and will be based on the results of mapping behaviour and morphology onto the phylogenetic tree. I will also propose a model for the changes in physiology and mechanisms that may have accompanied the transition from crawling to signalling. This model will be based on a detailed comparison of movements between two representative species- one that crawls and one that anal scrapes.

## ***4.2 Methods***

### *Phylogenetic mapping of anal segment behaviour and anatomy*

Anal segment morphology, including the condition of the prolegs and caudal projection, shape of the PP1 setae, as well as the behaviours associated with the anal

segment have been described previously in Chapter 2 of this thesis. Variability of these traits were further categorized (see Results of this chapter), coded as discrete characters, and mapped onto the existing phylogeny of the Drepanidae (Chapter 3) in Mesquite (Maddison & Maddison, 2009). All behaviours were scored as presence/absence binary characters. Behaviours were said to be present if they were observed at least one time in trials with conspecifics, except for crawling towards the intruder. Since crawling towards the intruder may occur periodically during an encounter just by chance, taxa were said to aggressively crawl towards the intruder only when this behaviour occurred at a frequency of at least 5% of all events recorded. Ancestral conditions of the anal prolegs, caudal projection and PP1 setae were inferred for selected nodes with high posterior probabilities in Mesquite using the Mk1 model (Lewis, 2001), and ancestral behaviours were inferred for all nodes on a reduced phylogeny that included only those taxa for which behavior was known, using parsimony reconstruction ( $n = 13$ ). BayesDiscrete, in BayesTraits (Pagel & Meade, 2006), was used to determine whether morphological and behavioural characters were correlated over the phylogeny following the method outlined in Pagel and Meade (2006).

#### *Comparison of vibrations to assess signal ritualization*

Characteristics of ritualization (conspicuousness, redundancy, stereotypy and alerting components) were assessed for each type of anal segment behaviour by recording and comparing features of their associated vibrations. Conspicuousness was assessed by comparing the relative amplitude of vibrations associated with crawling and anal scraping within trials for 3 taxa (those that anal scrape - *D. arcuata*, *D. curvatula* and *D.*

*falcataria*;  $n = 13$ ) using a paired  $t$ -test, as amplitudes could not be compared between taxa or even between recordings due to differences in leaf structure and size of individuals. Dominant frequencies and bandwidths were also compared between crawling ( $n = 10$ ) and anal scraping ( $n = 3$ ) using Kruskal-Wallis one-way analyses of variance to determine if a shift in dominant frequency may have accompanied the shift to signalling (to increase signal to noise ratio, and thus conspicuousness). Redundancy was assessed by comparing rates per 5 s of each anal segment behaviour type within the 20-s period following the time of closest contact between the resident and intruder during encounters using an ANOVA ( $n = 10$ ). *Post hoc* analyses were performed using Tukey-Kramer HSD, where higher rates indicated high repetition of signals, and thus high redundancy. Stereotyped behaviours are those that vary little between events. In this chapter, I am testing for stereotypy of duration, or the variability in duration within a behaviour. Stereotypy of duration was measured as the inverse of variability, where variability was measured as the coefficient of variation, defined as the ratio between the standard deviation and the mean, expressed in percent of the mean. Stereotypy of duration was then compared between anal scraping and crawling behaviours using a Kruskal-Wallis one-way analysis of variance ( $n = 10$ ) to determine whether anal scraping is a more stereotyped behaviour, which would support the prediction that it is a ritualized signal. Alerting components were assessed by examining signalling bout data (see Chapter 2) per species and determining if any of the anal segment behaviours are typically preceded by any other behaviour. All data were calculated as a mean per individual using 5 behaviours/vibrations per individual when possible. Grand means were then calculated per taxa and finally per behaviour type, to compare between behaviours, except for

amplitude comparisons. All statistical comparisons used an alpha level of 0.05, and data were checked for normal distribution using the Shapiro-Wilk W test.

#### *Comparison of behavioural sequences*

The typical sequence of behaviours during encounters were compared between species to test the prediction that anal scraping and crawling would be found in the same order of events between anal scraping and non-anal scraping species. Behaviours were scored using a computerized event recorder in 10 taxa (J-Watcher; Blumstein et al., 2006). Discrete time sequential analysis was performed to quantify the frequency and transition probabilities between behaviours (with accompanying z-scores and P-values; Blumstein et al., 2006). Transition diagrams were created using only transition probabilities of 0.10 or higher.

#### *Kinematics and musculature of anal segment movement*

The kinematics of movement patterns associated with the anal segments were compared in species representing ancestral non-signalling and derived signalling conditions to propose a model for the physiological changes that may have accompanied the transition from crawling to anal scraping. Movement patterns of segments A6-A10 were analyzed in *D. arcuata* (signalling species;  $n = 9$ ) and *Tethea or* (non-signalling species;  $n = 7$ ) using standard and high-speed videography. High-speed videos were recorded using a Lightning RDT high-speed camera (High Speed Imaging, Inc., Ontario, Canada) at 500 frames per second and MiDAS 2.0 software (Xcitex, Massachusetts, U.S.A.). Videos were analyzed in MiDAS to provide quantitative descriptions of the relative timing, duration, direction, displacement and velocity of each movement

component. Surface points corresponding to the modified setae in *D. arcuata* (PP1) and the midline of the distal edge of the anal prolegs in *T. or* were placed in MiDAS and tracked manually through video frames. The mean duration, displacement and velocity for each movement was calculated for each individual (5 movements per individual) and the mean for all individuals was calculated to as a grand mean. Between species comparisons of duration, displacement and velocity were made using two-tailed independent *t*-tests and absolute values for displacements and velocities were used. These comparisons were made to help determine which parts of the movements were homologous in order to develop a model for the transition between crawling and anal scraping. All displacement values were normalized by the rest length of the sixth abdominal segment calculated as a mean in quiescent animals to correct for size differences between species.

### **4.3 Results**

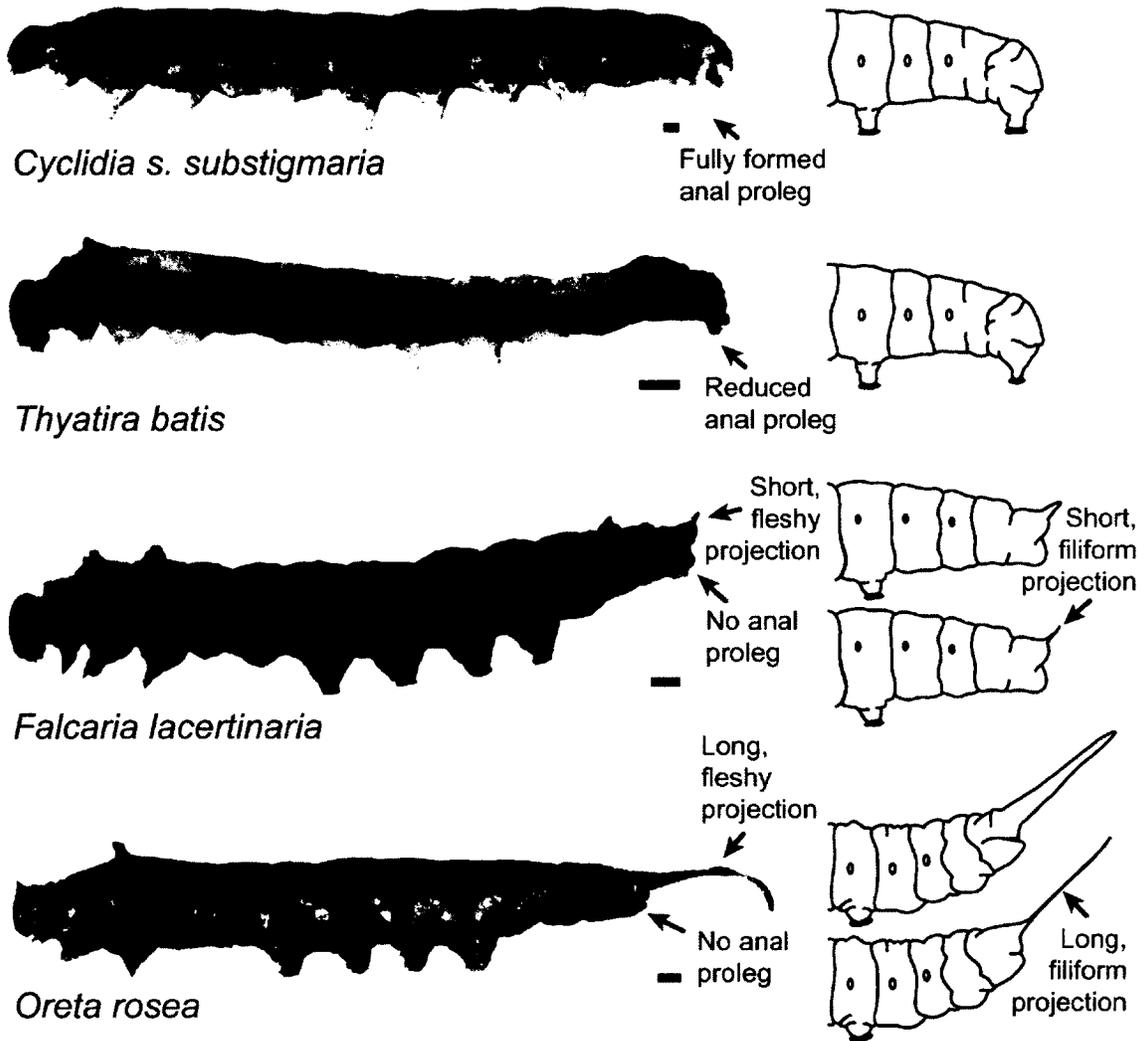
Variation in anatomy and behaviour of the anal segment, as described in Chapter 2, was further characterized in the current chapter in order to map these characters onto the phylogeny.

#### *Comparative anatomy of the anal segment for mapping*

Variation in anal segment morphology is described with respect to the anal proleg condition, PP1 setae morphology, and caudal projections (Fig. 4.2, 4.3).

The **anal prolegs** were categorized as being fully formed (equal in area (width at the widest part multiplied by total length from the body to the crochets) to the abdominal

**Fig. 4.2.** The variation in general morphology of the anal segment in Drepanidae caterpillars. Left panel: Representative photos of the main morphological conditions. Scale bars = 1 mm. Right panel: Drawings of the main morphological conditions and the variations that occur in different species.



prolegs on A3-A6 and bearing full crochets), reduced (smaller than the abdominal prolegs on A3-A6, but still bearing crochets), or absent (and bearing no crochets) (Fig. 4.2).

**Caudal projections** are single projections from the dorsal anal segment, and these were classified as being short or long (long was quantified as longer than the length of A7-A10), fleshy or filiform (as characterized by Nakajima, 1970, 1972, where fleshy represents thicker projections, and filiform represents projections that resemble a thread, or filament), or absent (Fig. 4.2). This character was included in my study because it was a prominent feature of the anal segment that varied across species, and I was curious to see if variability in its morphology was correlated with any of the territorial behaviours of the anal segment (described below).

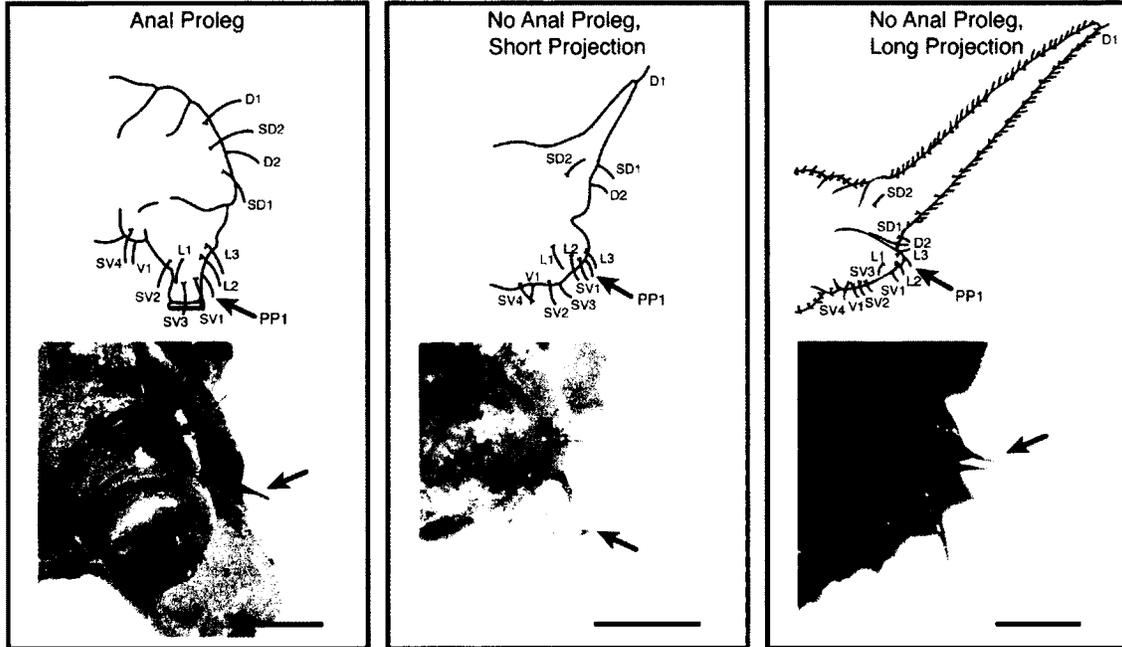
The **posterior proctor (PP1) setae** (one on each side) were broadly classified as unmodified (no wider than the surrounding seta found on the anal segment (L2, L3 and SV1)), thickened (cylindrically shaped, but wider than the L2, L3 and SV1 setae), or paddle-shaped (four-sided with unequal adjacent sides, and wider than the L2, L3 and SV1 setae) (Fig. 4.3). Within these general categories for PP1, further variation was observed, including two variations in thickened setae and six variations in paddle-shaped setae (Table 4.1 and Fig. 4.3).

#### *Phylogenetic mapping of anal segment anatomy*

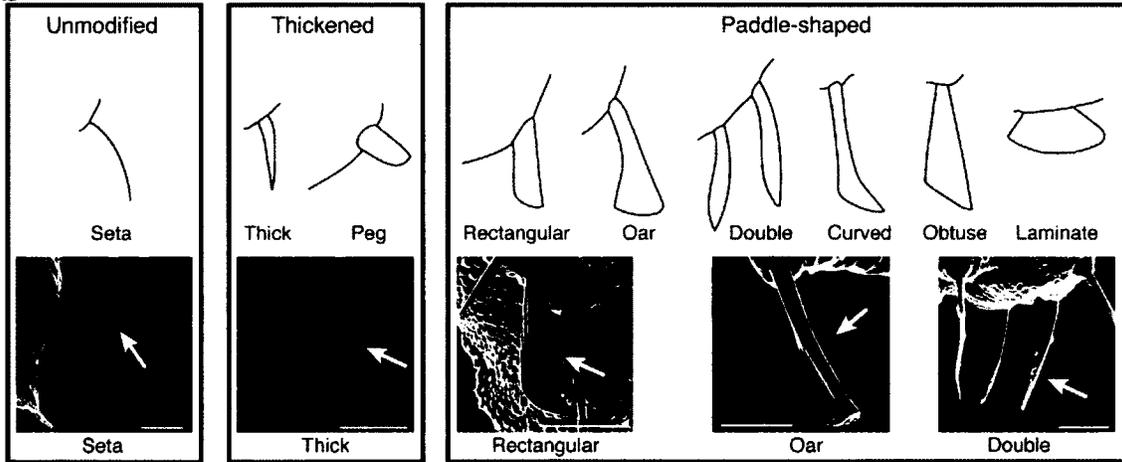
Mapping morphological traits of the anal segment shows that fully formed anal prolegs represent the basal condition, that they were reduced once in the common ancestor of the Thyatirinae clade (Fig. 4.4, Node A) and were subsequently lost in the

**Fig. 4.3.** The variation in PP1 setae morphology observed in Drepanidae larvae. **(a)** The location of the PP1 setae on a species with reduced anal prolegs (left; *Tetheela fluctuosa* (which has a thickened seta); scale bar = 500  $\mu\text{m}$ ), no anal prolegs (middle; *Drepana curvatula* (which has a paddle-shaped seta); scale bar = 500  $\mu\text{m}$ ), and no anal prolegs with a caudal projection (right; *Oreta rosea* (which has an unmodified seta); scale bar = 250  $\mu\text{m}$ ). **(b)** Schematics showing three categories of PP1 setae and their respective variations in morphology with representative scanning electron micrograph (SEM) images below. SEMs include, from left to right, *Tethea or* (unmodified seta), *T. fluctuosa* (thickened seta), *Falcaria bilineata* (rectangular seta), *Drepana arcuata* (oar-shaped seta), and *Cilix glaucata* (double paddle-shaped seta). All scale bars for SEMs = 100  $\mu\text{m}$ .

**a**



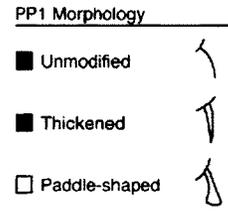
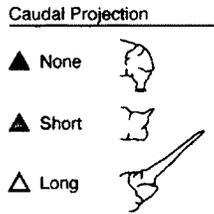
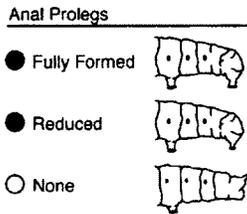
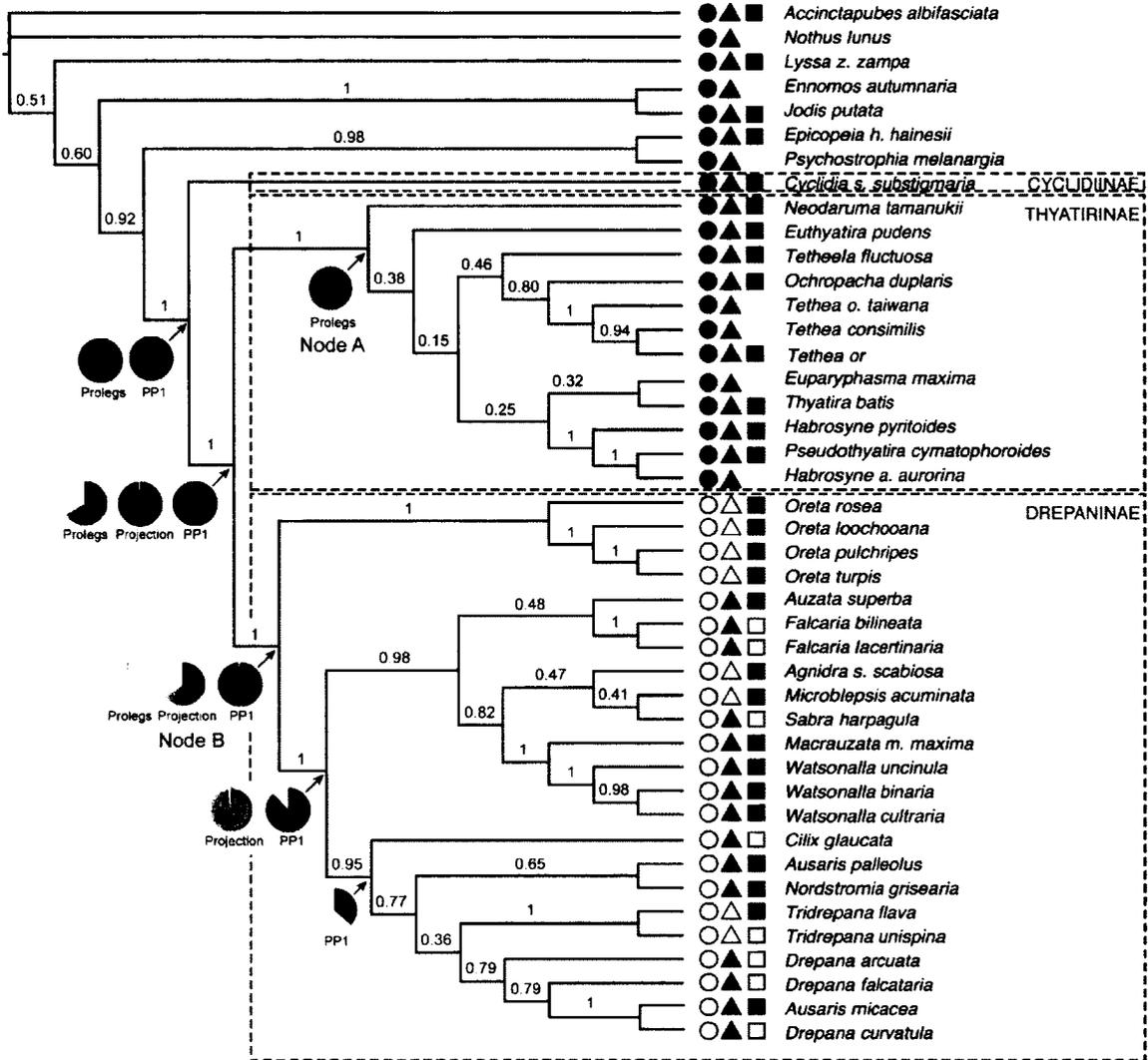
**b**



**Table 4.1.** Definitions of the variation in shape of PP1 setae observed in the Drepanidae.

<b>General Shape</b>	<b>Variation</b>	<b>Description</b>
Unmodified		typical filiform seta
Thickened	Thick seta	shaped like typical filiform seta, pointed at the distal end, but thicker
	Peg	wider and shorter than a typical seta with a blunt edge at the distal end
Paddle-shaped	Rectangular	shaped like a rectangle with one curved distal corner
	Obtuse	shaped like an obtuse triangle with the shortest edge at the distal end
	Curved	composed of a slender shaft with a right-angle curve at the distal end
	Oar	shaped like the oar of a boat, with a slender shaft that widens at the distal end
	Laminate	equilateral triangular shape with one point at the proximal end and a rounded corners on the distal edge
	Double	two modified setae on each side of the body

**Fig. 4.4.** Bayesian tree showing the ancestral and derived conditions of the anal prolegs, caudal projection and PP1 setae morphology. Posterior probabilities are shown above branches. Pie charts are maximum likelihood probabilities of ancestral states at selected nodes.



monophyletic Drepaninae (Node B). The caudal projection evolved once in the common ancestor of Drepaninae (Fig. 4.4, Node B) and variations in length and width evolved multiple times within Drepaninae (Fig. 4.4). Unmodified PP1 setae represent the basal condition, occurring in all outgroup species and most species of the basal Drepanidae subfamilies (Cyclidiinae and Thyatirinae) (Fig. 4.4). The one exception is in *Tetheela fluctuosa* (Thyatirinae) that has a thickened seta (see Fig. 4.3). Modified setae evolved independently multiple times and were subsequently lost multiple times within Drepaninae (excluding the Oretini tribe, the basal group of Drepaninae, which have unmodified setae). Variations in the shape of modified setae were usually restricted to independent evolutionary origins, where rectangular-shaped setae evolved once in the *Falcaria* clade, oar-shaped setae evolved once in the *Drepana* + *Ausaris micacea* clade (being subsequently lost in *A. micacea*), and peg, obtuse, double and laminate-shaped setae evolved independently in *Microblepsis acuminata*, *Sabra harpagula*, *Cilix glaucata*, and *Tridrepana unispina*, respectively. Thickened setae, on the other hand, evolved at least three times independently, in *T. fluctuosa*, *Nordstromia grisearia*, and *Tridrepana flava* (Fig. 4.4, Table 4.1).

Several correlations between morphological characters were observed (Fig. 4.4). Paddle-shaped setae were only observed in taxa that lacked anal prolegs. Species that possessed fully formed or reduced anal prolegs did not possess modified PP1 setae, except for one species, *T. fluctuosa*, which has a thickened seta and reduced anal prolegs. However, the condition of the anal proleg (reduced vs. absent) was not significantly correlated with the condition of the PP1 setae (unmodified vs. modified) over the phylogeny using BayesDiscrete analysis (lnL difference = 6.12, DF = 4, p = 0.19). The

presence or absence of caudal projections, on the other hand, was significantly correlated with anal proleg condition (presence vs. absence) over the phylogeny (lnL difference = 11.41, DF = 4,  $p = 0.022$ ), where caudal projections were only found in taxa that lacked anal prolegs altogether (i.e. only in Drepaninae).

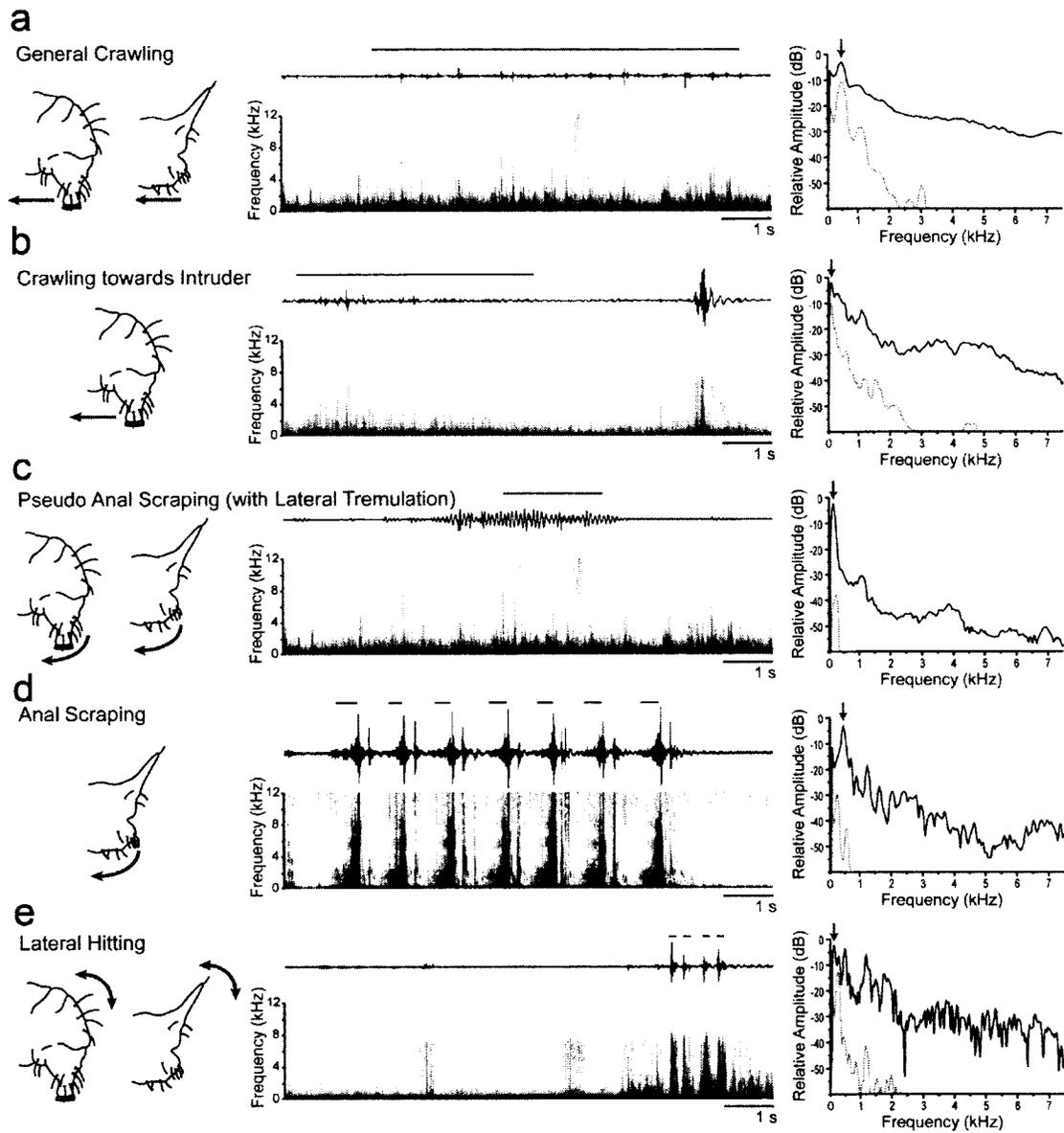
#### *Comparative behaviour of the anal segment during conspecific interactions*

In Chapter 2, four notable behaviours involving the anal segment were identified during conspecific interactions in 11 species, and information on the behaviour of two outgroup species was obtained from the literature. These include: general crawling, crawling toward an intruder (in an aggressive context), anal scraping movement, and lateral tail hitting. Each of these behaviours was described in detail in Chapter 2 with respect to which species produce them, the general context in which they are performed, as well as characteristics of the behaviours, movements, and vibrations on a species by species basis. In this Chapter, I will map behaviour associated with the anal segment onto the phylogenetic tree and will provide further details on the vibrational and kinematic characteristics of each behaviour. Mapping results and comparisons between movements and vibrations will be used to test predictions for the hypothesis that anal scraping derives from crawling and to propose a model for the evolutionary transitions between crawling and anal scraping. Representatives of all 4 behaviours are shown in Figure 4.5.

#### General Crawling

Crawling was performed in a general context to move forward and occurred in both species that possessed and did not possess anal prolegs (Fig. 4.5a). Crawling

**Fig. 4.5.** The variation in anal segment behaviours observed in the Drepanidae. Schematics summarizing the movements (and possible variations in anal proleg and PPI setae morphologies (left panel)), representative oscillograms and spectrograms of the vibrations produced by each behaviour (black lines above traces show when each behaviour occurs) (middle panel), and representative power spectra (black line) with background noise (gray line) included for comparison (right panel) for general context crawling **(a)**, aggressive crawling towards an intruder **(b)**, 'pseudo' anal scraping (accompanied by lateral tremulation) **(c)**, anal scraping **(d)**, and lateral hitting **(e)**.



movements differed between anal segment morphologies where species with fully formed anal prolegs (*Cyclidia substigmata*) used them to grasp the substrate during crawling; species with reduced prolegs (*T. or.*, *T. fluctuosa*, *Ochropacha duplaris* and *Thyatira batis*) did not use them to grasp the substrate, but they were placed upon the substrate during each crawl cycle; and species that lacked anal prolegs altogether (*D. arcuata*, *D. curvatula*, *D. falcataria*, *W. cultraria*, *O. rosea*, and *F. bilineata*) lowered the anal segment slightly to the leaf surface during each crawl cycle. Crawling vibrations were similar in duration (Kruskal-Wallis one-way analysis of variance;  $K = 2.53$ ,  $DF = 2$ ,  $p = 0.28$ ), dominant frequency (Kruskal-Wallis one-way analysis of variance;  $K = 5.71$ ,  $DF = 2$ ,  $p = 0.057$ ) and bandwidth (Kruskal-Wallis one-way analysis of variance; at -3 dB:  $K = 2.42$ ,  $DF = 2$ ,  $p = 0.30$ ; at -10 dB:  $K = 3.00$ ,  $DF = 2$ ,  $p = 0.22$ ) between species with fully formed, reduced and absent anal prolegs. Characteristics of crawling and associated vibrations are presented in Table 4.2.

#### Crawling towards an intruder

Crawling was also performed in a territorial context in three species (*T. or.*, *T. fluctuosa* and *O. duplaris*), all with reduced prolegs, where the resident crawled aggressively towards the intruder (Fig. 4.5b). Crawling towards the intruder was typically followed by other territorial behaviours including biting, pushing, lateral head hitting and mandible scraping. Characteristics of crawling towards the intruder and its associated vibrations were the same as those for general crawling (Table 4.2).

**Table 4.2.** Average vibration and rate data for anal segment signals.

Behaviour	Vibration Characteristics					Rate at CLOSE (events/5 s)
	Relative Amplitude (times the baseline)	Dominant Frequency (Hz)	Bandwidth at -10 dB (Hz)	Bandwidth at -3 dB (Hz)	Duration (ms)	
Crawling	5.2 ± 1.9 (n = 9)	18.9 ± 8.7 (n = 9)	7.2 ± 3.2 (n = 9)	17.7 ± 8.4 (n = 9)	1838.2 ± 1216.1 (n = 11)	0.63 ± 0.48 (n = 3; crawling towards the intruder only)
Anal scraping	11.6 ± 1.5 (n = 3)	41.5 ± 9.4 (n = 3)	7.9 ± 1.2 (n = 3)	18.6 ± 2.9 (n = 3)	779.3 ± 550.6 (n = 3)	1.5 ± 1.1 (n = 3)
Lateral tail hitting	No laser recordings	No laser recordings	No laser recordings	No laser recordings	207.8 ± 59.3 (n = 6)	0.11 ± 0.083 (n = 6)

### Anal scraping movement

Anal scraping movements were observed in 7 species during territorial encounters with conspecifics (Fig. 4.5c,d). The anal scraping movement involves scraping the terminal segment anteriorly on the leaf surface, and is typically followed by signals produced with the anterior body parts (mandible drumming, mandible scraping, lateral vibration and buzzing). Anal scraping was performed in species that had reduced anal prolegs (*T. fluctuosa* and *T. batis*), no anal prolegs (*D. arcuata*, *D. curvatula*, *D. falcataria*, *F. bilineata*, and *W. cultraria*), with modified setae (*D. arcuata*, *D. curvatula*, *D. falcataria*, and *F. bilineata*) and without modified setae (*T. fluctuosa*, *T. batis*, and *W. cultraria*). The anal scraping movement in species that lacked modified setae (with or without anal prolegs) was categorized as 'pseudo' anal scraping, as these species did not possess the structures necessary to produce the anal scraping signal. 'Pseudo' anal scraping did not differ significantly from anal scraping in duration (Wilcoxon Rank Sum Test,  $Z = 1.24$ ,  $DF = 2$ ,  $p = 0.22$ ). Amplitudes, dominant frequencies and bandwidths could not be measured for 'pseudo' anal scraping as these movements were always accompanied by signals involving the head or mandibles. Characteristics of anal scraping and its associated vibrations can be found in Table 4.2.

### Lateral tail hitting

Lateral tail hitting was observed in 6 species (*T. fluctuosa*, *O. duplaris*, *T. batis*, *F. bilineata*, and *W. cultraria*) when the intruder made contact with the posterior part of the resident's body (Fig. 4.5e). Lateral hitting was performed by species that had reduced anal

prolegs and no anal prolegs, with short and long caudal projections. Characteristics of lateral tail hitting are summarized in Table 4.2.

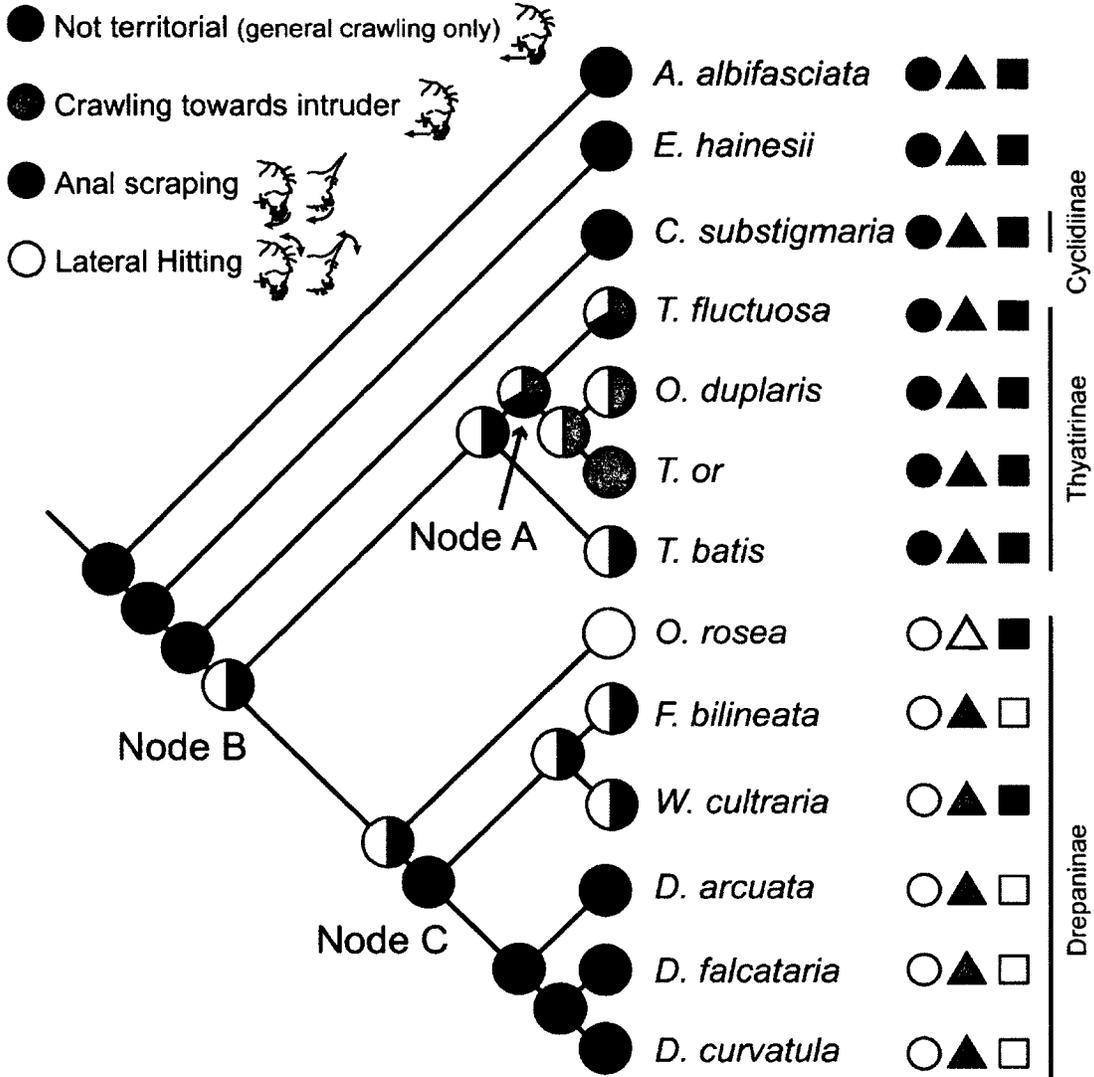
#### *Mapping of behavioural characters*

Behaviours associated with the anal segment as described above in the presence of a conspecific were mapped on to the phylogeny to test the hypothesis that anal scraping derives from crawling. The results indicate that general context crawling represents the ancestral condition and that crawling towards the intruder in a territorial context evolved once in the Thyatirinae at Node A (Fig. 4.6). The anal scraping movement evolved once at the node joining the Thyatirinae and Drepaninae clades (Node B), being subsequently lost twice independently in *O. rosea* and in the ancestor of *O. duplaris* + *T. or* (Fig. 4.6). Finally, lateral hitting evolved at least twice (Fig. 4.6, at Node B, and in the ancestor of *F. bilineata* + *W. cultraria*), being lost twice (Fig. 4.6, at Node C and in *T. or*). None of the territorial behaviours were mutually exclusive, and one species, *T. fluctuosa*, was observed to produce all three types of behaviours during encounters with conspecifics (Fig. 4.6). Other species had varying repertoires of behaviours (Fig. 4.6).

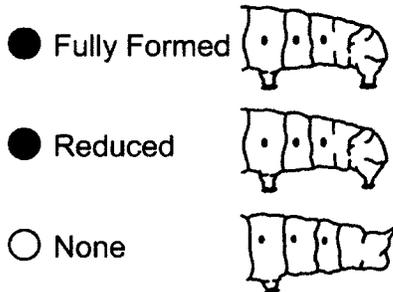
Morphological conditions of the anal segment were also mapped onto the phylogeny to assess if they were correlated with any of the territorial behaviours (Fig. 4.6). Looking at the phylogeny, it appears that crawling towards the intruder is only performed by taxa that have reduced prolegs, do not possess a caudal projection, and do not have paddle-shaped setae. Also, species that possess modified setae always anal scrape to produce signals. However, none of the territorial behaviours were found to be

**Fig. 4.6.** Reduced Bayesian tree including only those species for which territorial behaviour is known, showing the ancestral and derived conditions of anal segment morphology, PP1 setae morphology, and anal segment behaviour. Pie charts are maximum parsimony probabilities of ancestral behaviours at all nodes.

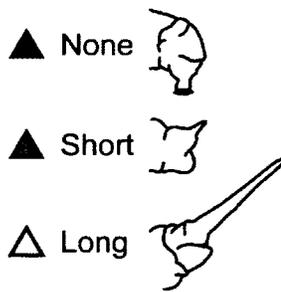
Territorial Anal Segment Behaviours



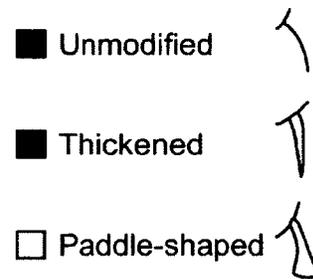
Anal Prolegs



Caudal Projection



PP1 Morphology



significantly correlated with anal proleg, caudal projection or PP1 setae condition over the phylogeny using BayesDiscrete analysis ( $p > 0.05$ ,  $DF = 4$ ).

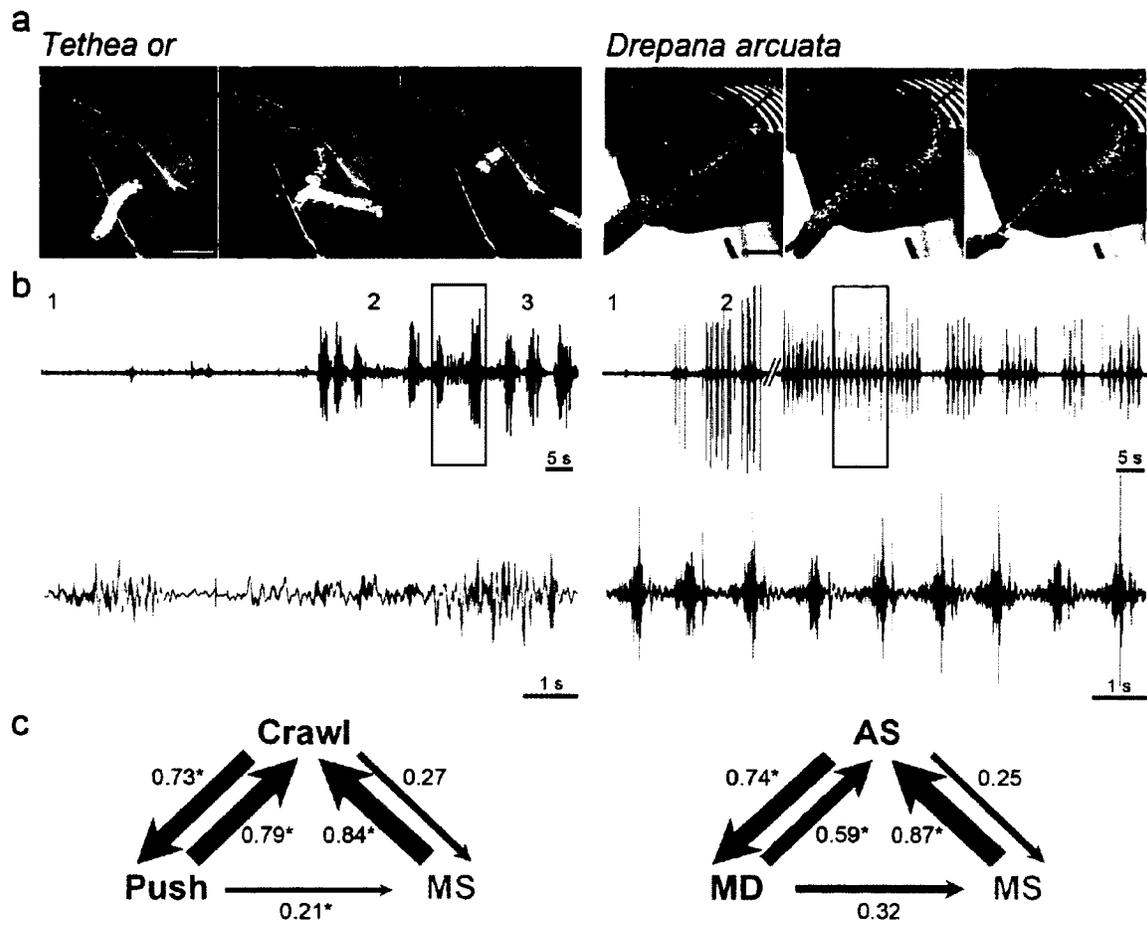
#### *Comparison of vibrations to assess ritualization*

My hypothesis is that anal scraping is a ritualized form of crawling movements, and therefore I predict that anal scraping demonstrates characteristics commonly found in ritualized signals, including high conspicuousness, redundancy, and stereotypy, and contains alerting components. Anal scraping is indeed more conspicuous than crawling, producing significantly higher vibration amplitudes than general crawling in within trial comparisons (paired t-test;  $t = -3.60$ ,  $DF = 12$ ,  $p = 0.004$ ) (Fig. 4.5b). Anal scraping also has a significantly higher dominant frequency than crawling (Wilcoxon Rank Sum Test,  $Z = 2.04$ ,  $DF = 1$ ,  $p = 0.042$ ), making it more conspicuous over background noise (Fig. 4.5b). However, bandwidths did not differ between anal scraping and crawling (Wilcoxon Rank Sum Tests; -3 dB:  $Z = 0.74$ ,  $DF = 1$ ,  $p = 0.46$ ; -10 dB:  $Z = 0.92$ ,  $DF = 1$ ,  $p = 0.35$ ). Vibrations produced by 'true' anal scraping (with no anal prolegs and the possession of modified setae) is also highly redundant, being repeated at a significantly higher rate per 5 s within the 20-s immediately following resident-intruder closest contact than crawling towards the intruder, and 'pseudo' anal scraping (ANOVA;  $F = 17.68$ ,  $DF = 9$ ;  $p = 0.002$ ; Fig. 4.5). Tests of stereotypy of duration, however, do not show any significant differences between anal scraping, 'pseudo' anal scraping and crawling (Kruskal-Wallis one-way analysis of variance;  $K = 2.93$ ,  $DF = 2$ ,  $p = 0.23$ ). Finally, I did not observe any alerting components that immediately preceded any of the anal segment behaviours.

*Comparison of behavioural sequences to test the hypothesis that anal scraping derives from crawling*

To test the prediction that anal scraping and crawling will be in the same order of events in a typical sequence of behaviours during encounters with conspecifics, I examined behavioural patterns during territorial encounters in 10 taxa and compared these sequences in two representative species, *T. or* (Thyatirinae), and *D. arcuata* (Drepaninae) (Fig. 4.7). The typical behavioural sequence in *T. or* begins with the resident crawling toward the intruder, followed by head movements including pushing and mandible scraping (Fig 4.7b, c left panel). Crawling towards the intruder is followed by pushing the intruder with the head (72.7% probability,  $z = 9.60$ ,  $P = <0.001$ ,  $n=7$ ), which is in turn followed by crawling (79.2%,  $z = 6.18$ ,  $P = <0.001$ ,  $n=7$ ). Occasionally the caterpillar will follow the push with a mandible scrape (20.8%,  $z = -0.26$ ,  $P = 0.79$ ,  $n=7$ ), which is followed by a return to crawling (83.9%,  $z = 5.21$ ,  $P = <0.001$ ,  $n=7$ ). In *D. arcuata*, the resident begins an encounter with anal scraping followed by signalling with the mandibles (Fig 4.7b, c right panel). Anal scraping is followed by mandible drumming (74.3%,  $z = 17.0$ ,  $P = <0.001$ ,  $n = 13$ ), which is followed by another anal scrape (58.9%,  $z = 5.53$ ,  $P = <0.001$ ,  $n = 13$ ). After a mandible drum, caterpillars will occasionally mandible scrape (32.5%,  $z = 4.01$ ,  $P = <0.001$ ,  $n = 13$ ) before returning to an anal scrape (87.7%,  $z = 10.9$ ,  $P = <0.001$ ,  $n = 13$ ). Physical aggression (biting, pushing, hitting) was never observed in *D. arcuata* trials. When comparing behavioural sequences between these two species (Fig. 4.7c), my results indicate that both anal scraping and crawling occur in the same position in the behavioural sequences during an encounter (prior to movements involving the head and mandibles).

**Fig. 4.7.** Sequences of behaviour in *Tethea or* and *Drepana arcuata*. **(a)** Video frames illustrating a typical encounter between a resident and intruder, whereby the intruder approaches the leaf shelter (1), the resident then approaches the intruder while hitting, pushing, or mandible scraping (*T. or*, scale bar = 9 mm ) or employs ritualized signal (*D. arcuata*, scale bar = 7 mm; video credit: J. Yack) (2), and the intruder leaves (3). **(b)** Laser vibrometer traces of the vibrations on the leaf during such an encounter. Numbers correspond to frames in (a). Relative amplitudes are equal between species. The box encloses part of the trace that is enlarged in the colored segment below, which shows the vibrations generated by the resident when the two individuals are close together. Colors correspond to cues or signals in (c). **(c)** Transition diagrams showing the probability of one type of behaviour following another. Asterisks denote significantly more probable transitions (*D. arcuata*:  $n = 13$ ; *T. or*:  $n = 7$ ;  $P < 0.05$ ). MS (mandible scraping); AS (anal scraping); MD (mandible drumming).



*Kinematics of anal segment movements in Tethea or (crawling) and Drepana arcuata (signalling)*

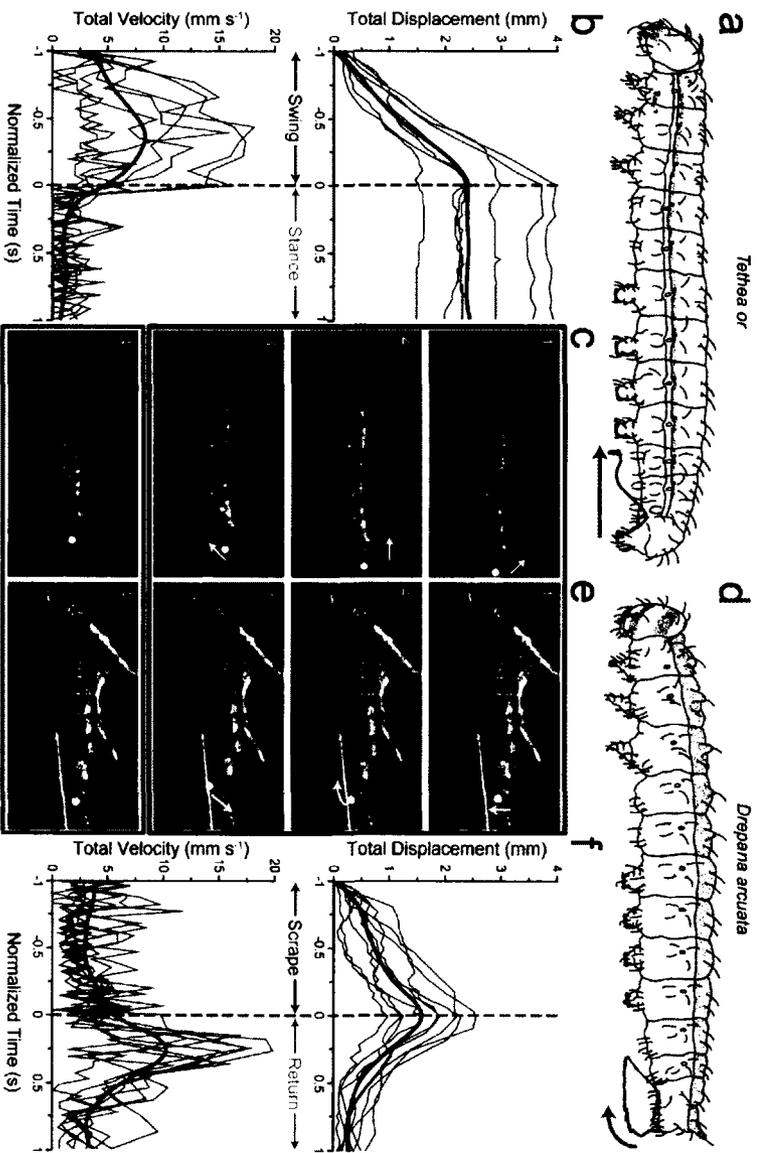
In this section I will describe and compare movement patterns of the anal segment during crawling in a basal species (*T. or*), and a derived signalling species (*D. arcuata*). Differences in movement patterns observed between the two representative species will be used to propose a model to explain the mechanistic changes that occurred to convert from ‘walking’ to ‘talking’.

*Crawling in Tethea or*

Forward crawling in *T. or* corresponds to that described in *Manduca sexta*, where crawling has been described in detail (Dominick and Truman, 1986; Belanger and Trimmer, 2000a, 2000b). In brief, a full crawl cycle in *T. or* involves a wave of longitudinal contractions that begins in the posterior end of the caterpillar and travels towards the anterior end. As the wave passes, each segment is lifted and carried forward using longitudinal contractions (swing phase). In segments bearing prolegs, the prolegs are then lowered and planted on the substrate (stance phase). During a crawl cycle the head can move from side to side or not at all, and telescopes forward as the wave reaches the most anterior part of the body. One entire crawl cycle in the anal segment lasts  $1.4 \pm 0.53$  s ( $n = 7$ ).

I will now describe movements specific to the terminal abdominal segments (A6-10) (Fig. 4.8a-c). At the beginning of the swing phase, the anal prolegs are firmly planted onto the substrate via the crochets (Fig. 4.8c, frame 1). The crochets are released, the prolegs are lifted and carried forward (Fig. 4.8c, frame 2), and then lowered towards the

**Fig. 4.8.** A comparison of crawling movements in *Tethea or* and anal scraping movements in *Drepana arcuata*. **(a)** Schematic showing the paths of a single crawling movement in the anal prolegs in *T. or*. The distance between points represents an equal amount of time. Arrow indicates the direction of motion. **(b)** Total displacement (i.e. in all directions) (top) and total velocity (bottom) of the anal proleg versus normalized time of crawling movement in *T. or*. Time was normalized between trials by making the point where the swing phase changes to the stance phase equal to time 0 s. Gray lines represent different individuals. The black line shows the trend of all the lines. **(c)** A series of high-speed video frames showing the sequence of movements of a single crawling cycle in *T. or*. Frame 1, the caterpillar's position before the crawl. Frame 2, the caterpillar lifts its anal prolegs and moves them forward. Frame 3, the caterpillar lowers its anal prolegs to the substrate while continuing to move them forward. Frame 4, the caterpillar plants its anal proleg on the substrate. Dots represent the position of the PP1 setae, arrows represent the direction of motion. **(d)** Schematic showing the paths of movement of the anal oar during a single anal scrape in *D. arcuata*. **(e)** A series of video frames showing the sequence of movements of a single anal scrape in *D. arcuata*. Frame 1 shows the caterpillar's position before the anal scrape. Frame 2, the caterpillar lowers its terminal abdominal segment to the leaf surface. Frame 3, the caterpillar scrapes its anal oars on the leaf surface and lifts its head in preparation for a mandible drum. Frame 4, the caterpillar returns its terminal abdominal segment and head to their original positions. **(f)** Total displacement (top) and total velocity (bottom) of the modified setae versus normalized time of anal scraping movement in *D. arcuata*. Time was normalized between trials by making the point where the scrape phase changes to the return phase equal to time 0 s. Throughout the figure, red shows the swing phase in *T. or* and the scrape phase in *D. arcuata*, while blue represents the stance phase in *T. or* and the return phase in *D. arcuata*.



substrate (Fig. 4.8c, frame 3). The crochets are engaged, everting the plantae slightly, and the prolegs are planted on the substrate (Fig. 4.8c, frame 4). The swing phase lasts on average  $505 \pm 204$  ms, and carries the proleg anteriorly by  $2.7 \pm 0.8$  mm at a mean horizontal velocity of  $6.5 \pm 3.8$  mm s<sup>-1</sup> ( $n = 7$ ) (Fig. 4.8b). The mean duration of the stance phase, while the prolegs are firmly planted, is  $848 \pm 469$  s ( $n = 7$ ), and the anal prolegs remain in the same approximate position during this phase (Fig. 4.8b). The unmodified PP1 setae, located on the anal proleg were never observed to make contact with the substrate.

#### Signalling in *Drepana arcuata*

The anal scraping movement in *D. arcuata* involves the modified PP1 setae being lowered towards the substrate and scraped anteriorly to produce a vibration on the leaf surface (scrape phase) (Fig. 4.8d-f). The anal segment then returns at a high velocity to its original position (return phase) (Fig. 4.8d-f). The mean duration of the entire anal scrape is  $1.3 \pm 0.35$  s ( $n = 9$ ) (Fig. 4.8f). The rest of the body remains relatively motionless with the head moving slightly in no discernable pattern during the anal scrape, except when a signal produced with the mandibles (mandible drumming) accompanies the anal scrape.

This paragraph will now focus on specific movements of the terminal abdominal segments (A6-10) during an anal scrape. Before the anal scrape, the modified PP1 setae and terminal abdominal segments are raised off the substrate (Fig. 4.8e, frame 1). The scraping phase begins with a slight posterior extension and lowering of the anal segment to the substrate (Fig. 4.8e, frame 2). The anal segment is then curled under slightly so that the PP1 setae make contact with the leaf and is then dragged anteriorly across the leaf

surface (Fig. 4.8e, frame 3). As this occurs, the anal segment is curled under so that the caudal projection almost makes contact with the substrate. The setae are scraped for  $597 \pm 235$  ms, moving a total horizontal distance of  $1.6 \pm 0.45$  mm at a horizontal velocity of  $2.9 \pm 1.1$  mm s<sup>-1</sup>, producing a scratching sound on the leaf ( $n = 9$ ). The setae are then quickly lifted off the substrate and re-extended approximately back to their original position at high velocity ( $3.6 \pm 3.0$  mm s<sup>-1</sup>) during the return phase, to start the next anal scrape ( $n = 9$ ) (Fig. 4.8e, frame 4).

#### *Comparison between crawling and signalling*

In summary, the crawling movement (with respect to the terminal abdominal segments (A6-10)) involves a swing phase, where the anal prolegs are lifted off the substrate, carried forward and lowered back to the substrate, and a stance phase, where the anal prolegs are firmly planted on the substrate (Fig. 4.8a-c). The anal scraping movement involves a scrape phase, where the anal segment is lowered and carried forward along the substrate, and a return phase, where the anal segment returns to its original position at high velocity. Overall, the series of movements do not differ in duration (independent t-test, two-tailed,  $t = 0.30$ ,  $DF = 10$ ,  $p > 0.05$ ), but do differ in other respects. I suggest that the swing phase of crawling is homologous to the scrape phase of anal scraping based on the fact that both movements involve the anal segment being carried forward in the same manner, and that the displacement does not differ between behaviours (independent t-test, two-tailed,  $t = 1.05$ ,  $DF = 11.5$ ,  $p > 0.05$ ) (Fig. 4.8b,f, swing vs. scrape phase). However, there are some differences between these two movements: i) during the swing phase of crawling, the anal segment is lifted off the

substrate before being carried forward, and is then lowered at the end of the phase, where as during anal scraping, the scrape phase begins with the anal segment being lowered towards the substrate (as it begins in a raised position) (see Fig. 4.8c,e, frames 1-3 for a side-by-side comparison of the movements); and ii) the mean horizontal velocity is significantly lower during the scrape phase of the anal scraping movement (independent t-test, two-tailed,  $t = 3.56$ ,  $DF = 7$ ,  $p = 0.01$ ) (Fig. 4.8,b,f, swing vs. scrape phase). The stance phase of crawling differs from the return phase of anal scraping with respect to direction of motion (Fig. 4.8c,e, frames 4), as well as displacement and velocity (Fig. 4.8b,f, stance vs. return phase), where during the stance phase of crawling, the anal segment remains motionless, and during the return phase of anal scraping, the anal segment returns to its original raised position at high velocity. These similarities and differences will be used to propose a model (see Discussion) for how the anal scraping movement was modified from crawling.

#### ***4.4 Discussion***

The goal of this chapter was two-fold: 1) to test the hypothesis that anal scraping derives from crawling, and 2) to propose a model for the transition between crawling and anal scraping. The results of this chapter provide the first empirical evidence for the origin and ritualization of a communication signal using a combination of morphological, behavioural and phylogenetic data.

*Testing the hypothesis that anal scraping derives from crawling*

The hypothesis that anal scraping derives from crawling is supported by the following lines of evidence: 1) crawling with fully formed prolegs and unmodified PP1 setae represents the basal condition when mapped onto the phylogeny; 2) kinematic analysis demonstrates that crawling and anal scraping involve similar movement patterns (see discussion below on comparison of kinematics); 3) vibration analysis suggests that anal scraping shows more characteristics of ritualization than crawling; and 4) aggressive crawling towards an intruder and anal scraping occur in the same position in a typical sequence of behaviours between representative species. The following paragraphs will discuss each line of evidence individually, as well as the interesting variation seen between species.

Comparative morphology of the anal segment

The anal prolegs varied in morphology from fully formed to reduced to completely absent. The results of our phylogenetic mapping demonstrate that fully formed prolegs represent the basal condition, and were reduced once before being lost completely. Reduced or absent prolegs are common in other larval Lepidoptera, especially leaf-mining species which are adapted to living within the tissue of their hostplant and are no longer needed to grasp the substrate, and species that have adopted a 'looping' method of locomotion, such as in the Geometridae (Hinton, 1955). To date, there has been no hypotheses to explain the function of reduced or absent prolegs in Drepanidae, since they are neither leaf-miners nor have an alternate mode of locomotion. However, many Thyatirinae caterpillars create shelters by sewing two leaves together,

and live in the space between (Minet & Scoble, 1999; Riegler, 1999). These shelters are similar to leaf mines, as they have limited vertical space, and anal proleg reduction may have occurred as a result of this mode of living. Another possibility is that the anal prolegs were reduced and finally lost to facilitate anal scraping (see model below).

The anal segment also varied in the presence or absence of the caudal projection, which could be fleshy or filiform in shape. Projections from the terminal segment have been noted in a few groups of caterpillars, mainly species of Notodontidae (Hinton, 1955; Stehr, 1987), Sphingidae (Scoble, 1992) and Drepanidae (Stehr, 1987). These projections have been shown to function for defense, being eversible and used in startle (Hinton, 1955; White et al., 1983), or possibly for mimicry, mimicking the chemical-emitting osmeterium of other species (Chow & Tsai, 1988) in Notodontidae. Since my results suggest that these structures are not correlated with any type of territorial behaviour associated with the anal segment (including lateral tail hitting), their function is currently unknown in Drepaninae. My results also demonstrate that all Drepanidae caterpillars that lack anal prolegs possess these structures, suggesting that they are modified prolegs, as in the Notodontidae (Hinton, 1955). However, the caudal projections in Drepaninae do not arise from the location of the anal prolegs, but from the more dorsal anal plate, and the planta retractor muscles in the anal segment (used to control the prolegs during crawling) do not insert on the caudal projection (see Appendix C for diagrams of muscles). Therefore, it is unlikely that they are modified prolegs.

The PP1 setae varied in shape from unmodified seta, to thickened seta, to various paddle-shaped modifications in Drepaninae. Non-modified PP1 seta are found in most caterpillar species, and function for tactile reception (Stehr, 1987). Modifications in the

shape and size of the PP1 setae evolved multiple times within Drepanidae, where each variation was usually restricted to an independent evolutionary origin event. Since different shapes typically evolved only once, and shapes differed between evolutionary events, this suggests that it is the sclerotization of the seta, and not specifically the shape, that is important for enhancing signalling. It is also possible that the different shapes of modified setae are related to the type of hostplant used by the resident, as leaf shape and size are known to alter the vibrational properties of the leaf (Cocroft et al., 2006).

#### Comparative Behaviour of Anal Segment

Behaviours associated with the anal segment included general context crawling, crawling towards the intruder during territorial encounters, anal scraping, 'pseudo' anal scraping, and lateral tail hitting. Some other examples of insects that use their anal segments for vibrational signalling include: larvae of the sawfly, *Hemicroa crocea*, which anal scrape to orient other larvae to high-quality feeding sites (Hoegraefe, 1984), some sawfly larvae (*Perga* spp.) that drum a sclerotized portion of the abdomen on the substrate to produce percussion signals for group coordination (Carne, 1962; Fletcher, 2007), and some species of ants and caddisflies (reviewed in Virant-Doberlet & Cokl, 2004) which scrape their abdomens to produce vibrations. My phylogeny indicates that general context crawling represents the basal condition and that the anal scraping movement evolved once in the common ancestor of the Thyatirinae, which supports the hypothesis that anal scraping derives from crawling.

### Comparison of vibrations to assess ritualization

For a signal to be effective, it must be detected and distinguished as a signal by the intended receiver. As such, ritualized signals include features that enhance their ability to be detected (increased conspicuousness, redundancy and alerting components) and recognized (stereotypy) (Cullen, 1966; Wiley, 1983; Johnstone, 1997; Bradbury & Vehrencamp, 1998; Maynard Smith & Harper, 2003). Increased conspicuousness, such as an increase in the amplitude of an acoustic signal, can improve the chance a receiver will detect a signal, even in noisy environments (Wiley, 1983). High redundancy, which can involve repeating a signal or using multiple signals for the same function, can reduce errors in the detection and recognition of a signal (Wiley, 1983). Both of these characteristics have been shown to play important roles in the tidbitting displays of male fowl, *Gallus gallus*, where females recognize the signal as distinctly different from the similar basal behavioural precursor (Smith & Evans, 2011). In the Drepanidae, I predicted that if anal scraping derives from crawling, anal scraping will possess characteristics of a ritualized signal. I found that anal scraping was more conspicuous, having a significantly higher relative amplitude and dominant frequency than crawling, and more redundant, having a significantly higher repetition rate at close distance between the resident and the intruder. I did not, however, find evidence for increased stereotypy, perhaps because crawling is also a highly stereotyped behaviour. I also did not find evidence for an alerting component, although since anal scraping is produced first in the typical sequence of behaviours, and usually precedes signals produced by the anterior body parts, it may itself be an alerting component. Overall, my results support

the prediction that anal scraping contains characteristics commonly observed in ritualized signals.

#### Sequences of behaviour

My final prediction to support the hypothesis that anal scraping derives from crawling was that anal scraping and crawling would be in the same position in a typical sequence of resident behaviour between an anal scraping and non-anal scraping species. I found that in two representative species, one that anal scrapes (*D. arcuata*; derived condition) and one that crawls towards the intruder (*T. or*; basal condition), crawling and anal scraping were both first in the typical sequence of behaviour, followed by movements involving the anterior body segments. This finding supports my hypothesis that anal scraping derives from crawling.

#### *The evolutionary transition from crawling to signalling*

Based on my results, I propose two possible scenarios for the evolutionary transition from crawling to anal scraping in the Drepanidae (Fig. 4.9). Both scenarios suggest that anal scraping ultimately derives from crawling movements as supported by the previous lines of evidence. The first scenario proposes that general context crawling with fully formed anal prolegs and unmodified setae transitioned to 'pseudo' anal scraping with reduced prolegs and unmodified setae (Fig. 4.9a). 'Pseudo' anal scraping then transitioned to both aggressive crawling towards the intruder with reduced anal prolegs and unmodified setae (in the Thyatirinae) and 'true' anal scraping with no anal prolegs and modified setae in the Drepaninae (Fig. 4.9a). This scenario is supported by my

behavioural mapping results, which indicate that the anal scraping movement evolved in the common ancestor of the Thyatirinae and Drepaninae, before aggressive crawling evolved in the Thyatirinae (Fig. 4.6, Node B).

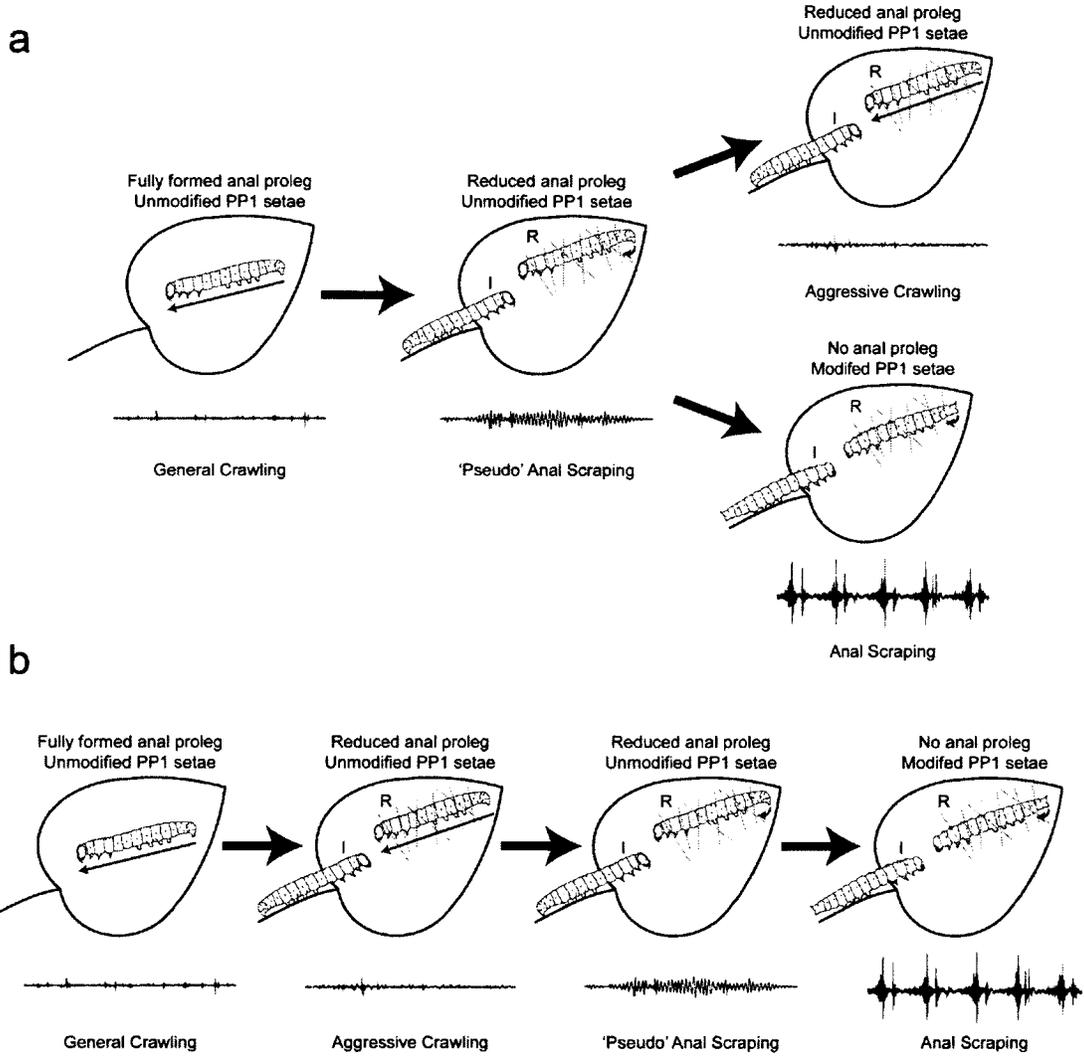
In this scenario, the loss of anal prolegs and evolution of modified setae may have occurred to enhance the signal. If the presence of anal prolegs reduced the efficacy of the signal, by not allowing the PP1 setae to make contact with the leaf (thereby lowering the amplitude of the signal), or by attaching to the substrate between scrapes (thereby not allowing for high repetition rates), selection would have acted to reduce the size of the anal prolegs. Modification of the PP1 setae may have evolved to increase the amplitude of vibrations or to increase the dominant frequency of the vibrations to render the signal more conspicuous over low frequency vibrations generated by background noise (e.g. wind and rain; see Barth et al., 1988; Caldwell et al., 2009; Guedes et al., 2012). Indeed, my results indicate that anal scraping with modified setae and no anal prolegs shows more characteristics of ritualization, having significantly higher repetition rates, and producing significantly higher amplitude vibrations with higher dominant frequencies than crawling (which increase signal to noise ratio, see Fig. 4.5). 'Pseudo' anal scraping, without these specialized setae or the loss of anal prolegs, may represent a transitional stage where modified setae and anal proleg loss has not yet evolved.

This first scenario, however, does not adequately explain why 'pseudo' anal scraping evolved in the first place. The second scenario proposes that general crawling with fully formed anal prolegs and unmodified PP1 setae first transitioned to aggressively crawling towards the intruder with reduced prolegs and unmodified PP1 setae, before transitioning to 'pseudo' anal scraping with reduced prolegs and unmodified setae, and

**Fig. 4.9.** Schematic of the evolution of anal scraping signals in Drepanidae caterpillars.

**(a)** Scenario 1: General crawling (with fully formed anal prolegs and unmodified PP1 setae) transitions to 'pseudo' anal scraping (with reduced anal prolegs and unmodified PP1 setae), which then transitions to either aggressive crawling towards the intruder (with reduced anal prolegs and unmodified PP1 setae) (top) or anal scraping (with no anal prolegs and modified PP1 setae) (bottom) depending on the subfamily. Traces under diagrams show representative oscillograms of the vibrations produced by each behaviour.

**(b)** Scenario 2: General crawling (with fully formed anal prolegs and unmodified PP1 setae) transitions to aggressive crawling towards the intruder (with reduced anal prolegs and unmodified PP1 setae), which transitions to 'pseudo' anal scraping (with reduced anal prolegs and unmodified PP1 setae), and finally to anal scraping (with no anal prolegs and modified PP1 setae).



finally to anal scraping with no anal prolegs and modified setae (Fig. 4.9b). Although this scenario does not strictly follow my behaviour mapping results (suggesting that the anal scraping movement evolved before aggressive crawling), I offer it as an alternative because it is in line with the intention movement hypothesis of signal origin (Tinbergen, 1952; Brown, 1975; Bradbury & Vehrencamp, 1998; Maynard Smith & Harper, 2003). The results of my comparison between behavioural sequences also supports this hypothesis. The intention movement hypothesis of signal origin states that signals can be derived as intention movements, showing the sender's intention to perform a certain behaviour. For example, it is believed that foot drumming in kangaroo rats is derived from the intention to chase (Randall, 2001). In the Drepanidae, I have evidence that some species use crawling in a territorial context, where the resident crawls towards an intruder to perform physical damage (biting, hitting, pushing). If crawling towards the intruder with the intention to physically harm was common, intruders could have potentially exploited the vibrations produced by crawling to avoid physical damage by leaving the territory. The crawling behaviour became ritualized over time, because the reaction of intruder also benefitted the resident (i.e. the resident no longer had to perform a costly behaviour in order for the intruder to leave its territory). Thus, residents began to walk on the spot, or 'pseudo' anal scrape, manipulating the intruder into believing that it was crawling toward it. This 'pseudo' anal scraping then transitioned to 'true' anal scraping through the evolution of modified setae and the complete loss of anal prolegs. In this case, the initial reduction in anal prolegs before the evolution of the anal scraping movement may be attributed to the mode of living of the Thyatrinae (which live between

two leaves, as discussed above). Future studies that include behavioural information on more taxa are needed to determine which evolutionary scenario is more likely.

#### Alternative evolutionary transitions

In a final alternative scenario, it is possible that another behavioural step existed between crawling and anal scraping. Many species of caterpillars, especially those travelling in processions, use chemical signals for communication (e.g. Fitzgerald, 1995; Ruf et al., 2001; Fitzgerald & Pescador-Rubio, 2002; Fitzgerald, 2003; Colasurdo & Despland, 2005; Costa, 2006; Pescador-Rubio et al., 2011). Many of those species have been shown to deposit pheromones by brushing the ventral surface of the tip of the abdomen against the substrate (Fitzgerald, 1995; Ruf et al., 2001; Fitzgerald & Pescador-Rubio, 2002; Fitzgerald, 2003; Costa, 2006; Pescador-Rubio et al., 2011), and pheromones appear to be secreted from glandular setae found on the proximal regions of the anal prolegs and venter (Fitzgerald & Pescador-Rubio, 2002). Therefore, the dispersal of pheromones from the anal segment may have been a transitional stage between crawling and anal scraping in Drepanidae. However, I have found no evidence of glandular setae on the anal segment in caterpillars of Drepanidae and the original function of the PP1 setae was for tactile reception (Stehr, 1987). I have also not found any evidence for the use of pheromones for marking territories in Drepanidae. In one species, *D. arcuata*, it was found that the presence of the resident was crucial for intruder retreat, as intruders would readily establish residency in shelters where previous residents had been removed (Yack et al., 2001). This suggests that intruders are not deterred by pheromones, but by the resident's presence/vibratory signals. Therefore, current evidence

suggests that pheromone dispersal is not a transition stage between crawling and anal scraping.

*Mechanistic transition from walking to talking*

I have discussed how the behaviours may have transitioned from crawling to anal scraping, and will now focus on how this transition may have occurred in a mechanistic point of view. My results demonstrate that crawling in the anal segment comprises two phases: a swing phase, where the anal prolegs are released from the substrate, lifted, carried forward and brought back down, and a stance phase where the crochets are engaged and grasp the substrate. The anal scraping movement also comprises two phases: the scrape phase, where the anal segment is lowered, curled under and scraped along the substrate, and the return phase, where the anal segment is returned to its original raised position at high velocity. I propose that the swing phase of crawling is homologous to the scrape phase of anal scraping, and that the stance phase of crawling is homologous to the return phase of anal scraping. Evidence for homology between the swing and scrape phase includes the facts that both involve the anal segment being carried forward in the same manner and the distance the anal segment travels is statistically equal between both behaviours. Differences between these two phases include a difference in initial direction of motion (the anal prolegs are raised in crawling whereas the anal segment is lowered in anal scraping), and a significantly lower velocity in the anal scrape. Differences in the direction of motion may be explained by changes in the timing of muscular contractions. During crawling, dorsal longitudinal muscles that run horizontally along the dorsal area in each segment contract sequentially, starting at the posterior end of the animal, to raise

each segment (Belanger & Trimmer, 2000a) (see Appendix C for diagram of pertinent muscles in *T. or*). Contraction in the next anterior segment causes each posterior segment to be carried forward (Belanger & Trimmer, 2000a). Finally, the ventral longitudinal muscles, found horizontally along the ventral area of each segment, contract sequentially to lower each segment (Belanger & Trimmer, 2000a). During anal scraping, however, the anal segment begins in a raised position, suggesting that the dorsal longitudinal muscles begin in a contracted position (see Appendix C for diagram of pertinent muscles in *D. arcuata*). The ventral longitudinal muscles then contract sequentially (as in crawling) to lower the anal segment and scrape it along the substrate. Essentially, the order of contraction remains the same between the swing phase of crawling and the scrape phase of anal scraping, except that in anal scraping the sequence has been modified to support a sustained initial contraction of the dorsal longitudinal muscles. Finally, the lower horizontal velocity of the scrape as compared to the swing phase of crawling can simply be attributed to the physical drag created by scraping the modified setae along the substrate.

I also propose that the stance phase of crawling is homologous to the return phase of anal scraping. Although the direction of motion, displacement and velocity may differ between both movements, where the prolegs remain motionless during crawling, and the anal segment returns to its original raised position at high velocity during anal scraping, I argue that muscle activity remains the same between behaviours. Before crawling, the crochets found on the ventral surface of the prolegs attach the prolegs to the substrate. The crochets are released through a contraction of the planta retractor muscles (which arise on the ventral region of the tergum near the middle of the segment, posterior to the

spiracle, and insert on the tendon of the planta within the proleg) (Belanger & Trimmer, 2000b; Mezoff et al., 2004). When the planta retractor muscles contract, this pulls the planta into the proleg slightly, releasing the crochets (Belanger & Trimmer, 2000b; Mezoff et al., 2004). During the stance phase, the planta retractor muscles relax, and the planta everts, inflates medially and fans out into a broad lobe due to hydraulic pressure, re-engaging the crochets in the new position (Belanger & Trimmer, 2000b; Mezoff et al., 2004). If a similar sequence of muscle contraction occurs during anal scraping at the end of the scrape phase, the hydraulic pressure caused by the contraction of the planta retractor muscles would return the anal segment to its original position at high velocity (along with the sustained contraction of the dorsal longitudinal muscles). This would occur because the lack of anal prolegs and crochets would render the caterpillar unable grasp the substrate with the anal segment. Therefore, both phases can be achieved using the same muscle contraction sequence, despite the differences observed in direction of motion, displacement and velocity.

Another major difference between crawling and anal scraping is that crawling movements involve the entire length of the caterpillar's body, where anal scraping movements are isolated to the terminal abdominal segments (A7-10). Results from previous studies provide insight into how crawling movements can be isolated in the terminal abdominal segments. These studies have shown that locomotor patterns in caterpillars are controlled by the interaction of segmental neural pattern generators that can be modified on the basis of sensory input (Dominick & Truman, 1986). Each segmental ganglion receives both activating and coordinating input from higher neural centers, including the brain and subesophageal ganglion (Dominick & Truman, 1986).

Since crawling behaviours can occur in the first thoracic segment in larvae in which the connectives posterior to this ganglion have been severed, it is assumed that each segmental ganglion can independently produce motor patterns (Dominick & Truman, 1986). Reversible deactivation of crawling activity has also been evoked by atropine application in isolated nerve cords, where crawling has been induced using pilocarpine, suggesting that the response is simply mediated by muscarinic-like acetylcholine receptors (Johnston & Levine, 1996). Therefore, the deactivation of crawling motor patterns in all segments up to the sixth abdominal segment in anal scraping is a plausible scenario and may be a product of an inhibition of central pattern generators in preceding ganglia by higher neural centers. Future studies examining the activity of muscles and neurons in the anal segment during crawling and anal scraping are needed to test this hypothesis.

The present chapter focuses on the evolutionary origin of anal scraping in the Drepanidae. However these caterpillars are known to also produce vibratory signals with the mandibles and anterior body segments, including mandible drumming, mandible scraping, and lateral tremulation during encounters with conspecifics. The evolutionary origins of these signals will be the focus of Chapter 5.

**CHAPTER 5**

**FROM HITTING TO SCRAPING: THE EVOLUTIONARY ORIGIN OF MANDIBLE**

**SCRAPING SIGNALS IN DREPANIDAE CATERPILLARS**

### ***5.1 Introduction***

Chapter 4 provided support for the hypothesis that vibratory signals produced by anal scraping with modified PP1 setae derive from movements associated with crawling. Results from Chapter 2 demonstrate that some species also produce vibrations associated with movements of the mandibles and anterior body segments. The present chapter (Chapter 5) will focus on the origin and evolution of these anterior body behaviours. Behavioural observations from 11 species reveal that different species of Drepanidae caterpillars can produce at least eight types of behaviours involving the head and mandibles during encounters with conspecifics. These behaviours include mandible drumming, mandible scraping, twitching, lateral tremulation, buzzing, lateral head hitting, pushing and biting (as described in Chapter 2). Mandibles were also found to vary in structure in 18 species, with respect to the number of distal teeth and ridges on the oral surface (see Chapter 2). This variation in morphology and behaviour associated with the anterior segments poses another opportunity to develop and test hypotheses on the evolutionary origins of vibratory signals in these caterpillars.

The purpose of this chapter, therefore, is to begin to elucidate the evolutionary origins of some of these behaviours. I will start by mapping mandible morphology and anterior body behaviour onto the phylogeny created in Chapter 3, to develop hypotheses on the evolution of signals. This will allow me to assess which behaviours are derived, and if any particular features associated with the mandibles are correlated with behaviour. The second goal of this chapter is to specifically test the hypothesis that mandible scraping derives from lateral head hitting. This hypothesis is based on the observation that both behaviours involve similar movement patterns, where the head and first few

anterior body segments move in a lateral arc to either hit another caterpillar (lateral head hitting), or to scrape the mandibles on the surface of the leaf (mandible scraping). I predict: 1) that lateral head hitting will be basal to mandible scraping, and will test this by mapping behaviours onto the phylogeny; 2) movements will be similar with respect to direction of motion, displacement and velocity, and will test this by comparing properties of lateral head hitting and mandible scraping within and between species; and 3) vibrations produced by mandible scraping will show more features of ritualization (conspicuousness, redundancy and stereotypy, and will contain alerting components) when compared to lateral head hitting. I will test this by directly comparing these features of ritualization between species. The final goal of this chapter is to propose some of the evolutionary transitions between the other anterior segment behaviours. I will do this by mapping all anterior body behaviours onto the phylogeny, and by comparing properties of movements, as well as vibrations between behaviours.

## ***5.2 Methods***

### *Phylogenetic mapping of anterior segment behaviour and mandible morphology*

Mandible morphology, including the condition of the distal edge and oral surface, as well as the behaviours associated with the anterior body segments were previously described in Chapter 2. Variability of these traits were further characterized (see Results of this chapter), coded as discrete characters, and mapped onto the existing phylogeny of the Drepanidae (Chapter 3) in Mesquite (Maddison & Maddison, 2009). All behaviours were scored as presence/absence binary characters. Behaviours were said to be present if they were observed at least one time in trials with conspecifics. Ancestral behaviours

were inferred for all nodes in Mequite (Maddison & Maddison, 2009) using parsimony analysis on a reduced phylogeny that included only those taxa for which behavior was known ( $n = 14$ ). BayesDiscrete, in BayesTraits (Pagel & Meade, 2006), was used to determine whether behaviours were correlated with mandible condition over the phylogeny following the method outlined in Pagel and Meade (2006).

*Comparison of kinematics of movements between anterior body behaviours*

Movements associated with each anterior body behaviour observed during encounters with conspecifics were compared on the basis of direction of motion, total displacement, and total velocity in 10 species. Behaviours were recorded using both standard and high-speed videography. High-speed videos were recorded using a Lightning RDT high-speed camera (High Speed Imaging, Inc., Ontario, Canada) at 500 frames per second using MiDAS 2.0 software (Xcitex, Massachusetts, U.S.A.). Regular videos were analyzed using ImageJ software (version 1.42q; Maryland, USA) to provide detailed quantitative descriptions of each movement. Surface points corresponding to the anterior edge of the head at the midline were placed in ImageJ and tracked manually through video frames. To determine the direction, total displacement, total duration and total velocity of movements, surface points were compared between the starting position and the end position of the head (except for mandible drumming). Surface points were compared between the starting position, the position when the head reached its maximum height, and the end position for mandible drumming. The number of oscillations (changes in direction) per second were also measured for lateral tremulation and buzzing to determine the repetition rates of those movements. The mean duration, displacement and

velocity for each movement was calculated for each individual (5 movements per individual) and the mean for all individuals was calculated to as a grand mean. To test the hypothesis that mandible scraping derives from lateral head hitting, total duration, displacement and velocity were compared between mandible scraping and lateral head hitting, both within (in species that exhibited both behaviours) and between species using paired t-tests (within species) and independent t-tests (between species). All other behaviours were also compared between species on the basis of duration, displacement and velocity to help propose a model for the evolutionary transitions between behaviours. Between species comparisons were performed using ANOVAs or independent *t*-tests and *post hoc* analyses were done using a Tukey-Kramer HSD. To correct for size differences between species, all displacement values were normalized by the rest length of the sixth abdominal segment. All statistical comparisons used an alpha level of 0.05, and data were checked for normal distribution using the Shapiro-Wilk W test.

#### *Comparisons of vibrations to assess signal ritualization*

Characteristics of ritualization (conspicuousness, redundancy, stereotypy and alerting components) were assessed for each type of anterior body behaviour by recording and comparing features of their associated vibrations. These characteristics were first compared between lateral head hitting and mandible scraping to test the hypothesis that mandible scraping is a ritualized signal derived from lateral head hitting. Conspicuousness was assessed by comparing the relative amplitude of vibrations associated with lateral head hitting and mandible scraping within trials using a Wilcoxon Signed Rank Test ( $n = 7$ ), as amplitudes could not be compared between taxa or even

between recordings due to differences in leaf structure and size of individuals. Dominant frequencies and bandwidths at -3 dB and -10 dB were also compared between mandible scraping (n = 7) and lateral head hitting (n = 4) using Wilcoxon Rank Sum Tests to determine if a shift in dominant frequency may have accompanied the shift to signalling (to increase signal to noise ratio, and thus conspicuousness). Redundancy was assessed by comparing rates per 5 s of lateral head hitting and mandible scraping within the 20-s period following the time of closest contact between the resident and intruder during encounters using a t-test (n = 9). The stereotypy of duration was also compared between lateral head hitting and mandible scraping using a t-test. Stereotypy was measured as the inverse of variability, where variability was measured as the coefficient of variation, defined as the ratio between the standard deviation and the mean, expressed in percent of the mean. Stereotypy of duration was then compared between lateral head hitting and mandible scraping using a t-test to determine which behaviour is more stereotyped. Alerting components were assessed by examining signalling bout data (see Chapter 2) per species and determining if mandible scraping or lateral head hitting is typically preceded by any other behaviour. All data were calculated as a mean per individual using 5 behaviours/vibrations per individual when possible. Grand means were then calculated per taxa and finally per behaviour type, to compare between behaviours, except for amplitude comparisons. All statistical comparisons used an alpha level of 0.05, and data were checked for normal distribution using the Shapiro-Wilk W test.

Vibrations were also compared on the basis of conspicuousness, redundancy, stereotypy and alerting components between all other anterior body behaviours to help propose a model for the evolutionary transitions between behaviours. Conspicuousness

was assessed by comparing the relative amplitude of vibrations associated with each anterior body behaviour within trials using paired *t*-tests. Dominant frequencies and bandwidths were also compared between behaviours using Kruskal-Wallis one-way analyses of variance. Redundancy was assessed by comparing rates per 5 s of each anterior body behaviour within the 20-s period following the time of closest contact between the resident and intruder during encounters using an ANOVA. Additionally, the number of oscillations per second for lateral tremulation and buzzing were considered as separate events, and were compared to the other behavioural rates using an ANOVA to determine whether each oscillation contributed to the redundancy of the signal. *Post hoc* analyses were performed using Tukey-Kramer HSD. The stereotypy of duration was compared between each anterior body behaviour using an ANOVA. Alerting components were assessed by examining signalling bout data (see Chapter 2) for each species, and determining if any of the anterior body behaviours are typically preceded by another behaviour. All data were calculated as a mean per individual using 5 behaviours/vibrations per individual when possible. Grand means were then calculated per taxa and finally per behaviour type, to compare between behaviours, except for amplitude comparisons.

### **5.3 Results**

Variation in anatomy and behaviour of the anterior body segments, as described in Chapter 2, was further characterized in the current chapter in order to map these characters onto the phylogeny.

*Comparative morphology of the mandibles for mapping*

Variation in the mandibles is described with respect to the distal edge and the oral surface (Fig. 5.1). The distal edge was categorized as being smooth (having no teeth) or toothed (having at least two teeth). The oral surface was also categorized as smooth (having no ridges) or ridged (having at least one ridge).

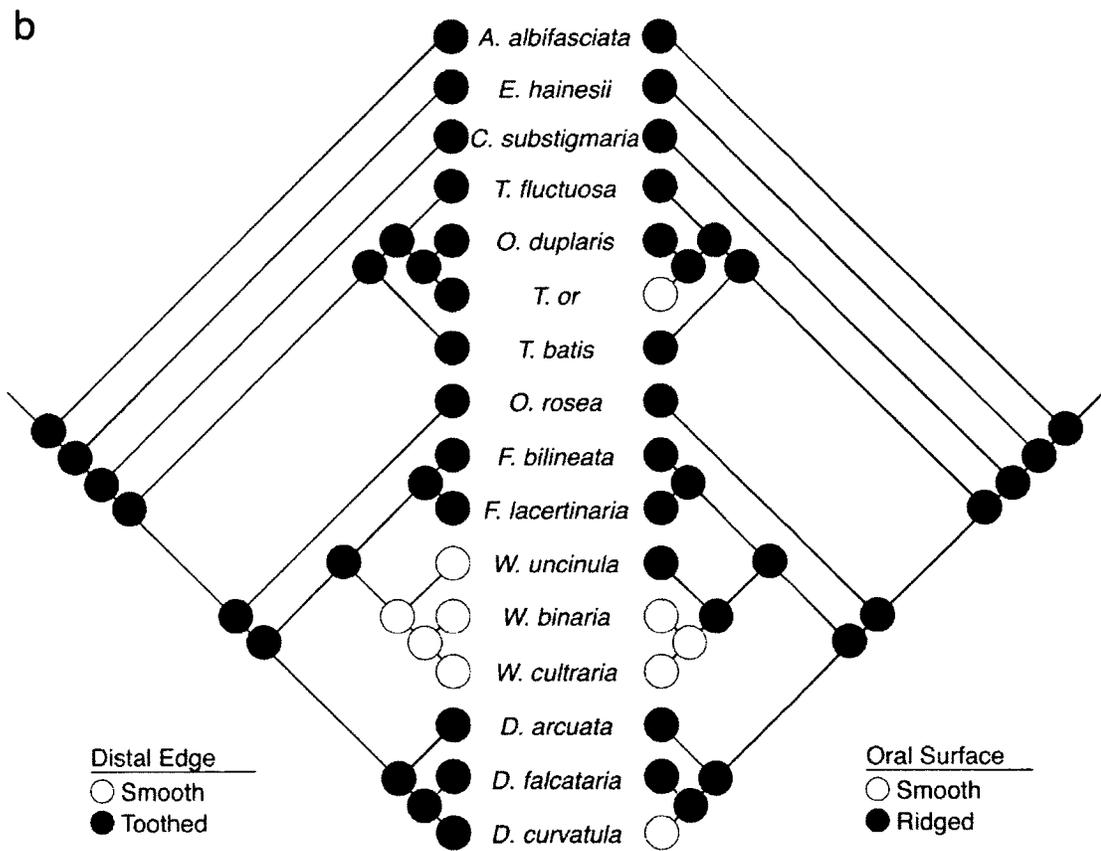
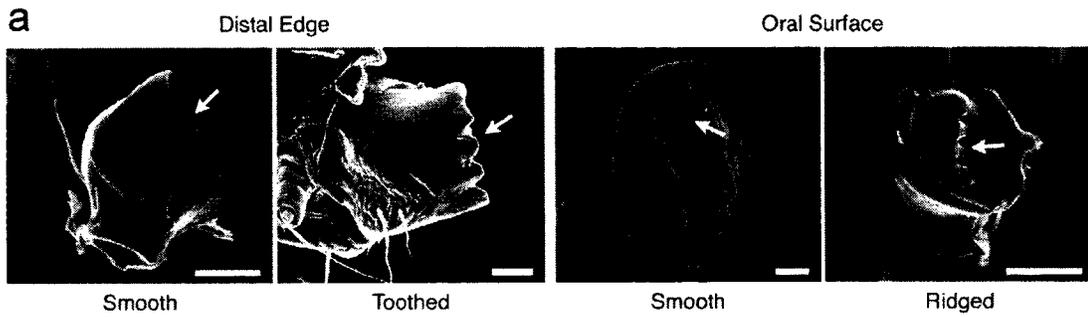
*Phylogenetic mapping of mandible morphology*

Results from mapping the condition of the distal teeth and oral surface of the mandibles onto the phylogeny suggests that having a toothed distal edge and ridged oral surface represents the basal condition (Fig 5.1). Smooth distal edges evolved once in the common ancestor of *Watsonalla* and smooth oral surfaces evolved at least three times in *Tethea* or, *Drepana curvatula*, and the common ancestor of *Watsonalla binaria* + *Watsonalla cultraria* (Fig. 5.1).

*Comparative behaviour of the anterior body segments during conspecific interactions*

In Chapter 2, eight notable behaviours involving the anterior body segments were identified during conspecific interactions in 11 species. These include: mandible drumming, mandible scraping, twitching, lateral tremulation, buzzing, lateral head hitting, pushing and biting. Each of these behaviours was described in detail in Chapter 2 with respect to which species produce them, the general context in which they are performed, as well as characteristics of the behaviours, movements, and vibrations on a species by species basis. In this Chapter, I will map behaviours onto the phylogenetic tree and will provide details on the average characteristics of each behaviour across all

**Fig. 5.1.** Comparative morphology and mapping of mandibles onto the phylogenetic tree of Drepanidae. **(a)** Morphological variation observed in the distal edge (smooth or toothed) and oral surface (smooth or ridged) of the mandibles (scale bars = 100  $\mu\text{m}$ ). **(b)** Reduced Bayesian tree including only those species for which territorial behaviour is known, showing the ancestral and derived conditions of the morphology of the distal edge and oral surface of the mandibles. Pie charts are maximum parsimony probabilities of ancestral characters at all nodes.



species. Mapping results as well as comparisons between movements and vibrations will be used to provide evidence for the hypothesis that mandible scraping derives from lateral head hitting and to propose evolutionary transitions between behaviours. Representatives of all 4 behaviours are shown in Figure 5.2. Detailed characteristics for each behaviour, including movement and vibration properties are presented in Table 5.1.

### Lateral Head Hitting

Lateral head hitting was observed in 8 species (*Tetheela fluctuosa*, *Ochropacha duplaris*, *T. or*, *Thyatira batis*, *Watsonalla cultraria*, *Drepana arcuata*, *Drepana falcataria* and *Drepana curvatula*) and involves a quick, lateral movement of the head, thorax and first two abdominal segments directed to a nearby caterpillar (Fig. 5.2a).

Hitting typically occurs when certain species are lightly touched on the anterior end by an intruding caterpillar. The movement begins with a swinging of the head in a lateral arc towards the posterior end, typically making contact with another caterpillar, with the head slightly raised off the surface of the leaf.

### Mandible Scraping

Mandible scraping also involves a lateral movement of the head, thorax and first two abdominal segments (Fig. 5.2b), and was observed in 8 species (*T. fluctuosa*, *O. duplaris*, *T. or*, *Oreta rosea*, *Falcaria lacertinaria*, *D. arcuata*, *D. falcataria*, and *D. curvatula*). The caterpillar begins by lowering its mandibles to the leaf surface, and quickly swings the head in a lateral arc towards its posterior end. The rest of the body remains in the same approximate position during the mandible scrape, typically no other

**Fig. 5.2.** The variation in anterior body behaviours observed in larvae of the Drepanidae. Schematics summarizing the movements (left panel), representative oscillograms and spectrograms of the vibrations produced by each behaviour (black lines above traces show when each behaviour occurs) (middle panel), and representative power spectra (black line) with background noise (gray line) included for comparison (right panel) for lateral head hitting (**a**), mandible scraping (**b**), mandible drumming (**c**), twitching (**d**), lateral tremulation (**e**), and buzzing (**f**).



**Table 5.1.** Average kinematic, vibration and rate data for anterior body signals.

Behaviour	Kinematics			Vibration Characteristics				Rate at CLOSE (events/5 s)	
	Duration of Movement (ms)	Head Displacement (mm)	Head Velocity (mm s <sup>-1</sup> )	Relative Amplitude (times the baseline)	Dominant Frequency (Hz)	Bandwidth at -10 dB (Hz)	Bandwidth at -3 dB (Hz)		Duration of vibration (ms)
Lateral head hitting	159.6 ± 40.6 (n = 5)	9.4 ± 2.2 (n = 5)	72.1 ± 25.6 (n = 5)	28.9 ± 23.3 (n = 5)	36.5 ± 26.4 (n = 5)	21.7 ± 9.9 (n = 5)	7.8 ± 3.1 (n = 5)	173.2 ± 34.9 (n = 9)	0.37 ± 0.35 (n = 9)
Mandible scraping	135.9 ± 20.7 (n = 7)	8.4 ± 6.1 (n = 7)	62.0 ± 33.7 (n = 7)	29.9 ± 24.3 (n = 7)	25.5 ± 17.2 (n = 7)	14.4 ± 6.3 (n = 7)	6.7 ± 1.4 (n = 7)	145.3 ± 79.0 (n = 7)	1.42 ± 1.21 (n = 7)
Mandible drumming	161.5 ± 39.7 (n = 3)	0.87 ± 0.2 (n = 3)	8.6 ± 1.0 (up) 26.9 ± 5.5 (down) (n = 3)	39.5 ± 20.4 (n = 4)	47.7 ± 28.7 (n = 4)	27.6 ± 15.5 (n = 4)	10.3 ± 4.1 (n = 4)	47.6 ± 23.2 (n = 6)	1.24 ± 1.22 (n = 6)
Twitching	13.6 ± 6.8 (n = 1)	1.7 ± 0.8 (n = 1)	19.4 ± 8.5 (n = 1)	18.8 ± 15.8 (n = 1)	12.9 ± 3.5 (n = 1)	12.7 ± 4.3 (n = 1)	6.8 ± 1.3 (n = 1)	91.20 ± 6.89 (n = 1)	2.71 (n = 1)
Lateral tremulation	666.9 ± 1066.5 (n = 3)	2.2 ± 1.2 (n = 4)	41.6 ± 10.5 (n = 4)	39.3 ± 24.9 (n = 4)	24.8 ± 26.7 (n = 4)	14.5 ± 4.8 (n = 4)	6.7 ± 1.5 (n = 4)	1252.4 ± 661.0 (n = 4)	0.11 ± 0.06 (n = 4)
Buzzing	988.7 ± 23.7 (n = 2)	0.52 ± 0.2 (n = 2)	15.4 ± 5.0 (n = 2)	30.8 ± 9.5 (n = 1)	64.9 ± 23.0 (n = 1)	17.5 ± 3.4 (n = 1)	8.1 ± 1.0 (n = 1)	718.4 ± 24.5 (n = 2)	0.79 ± 0.76 (n = 2)
Pushing	689.0 ± 125.0 (n = 2)	1.6 ± 1.7 (n = 2)	5.3 ± 3.2 (n = 2)	See crawling (Chapter 5)	See crawling (Chapter 5)	See crawling (Chapter 5)	See crawling (Chapter 5)	See crawling (Chapter 5)	0.64 ± 0.32 (n = 2)

signals occur during the movement. During the scrape, the mandibles are dragged across the leaf surface to produce a vibration on the leaf. Often, the caterpillar will scrape in the other lateral direction immediately after the first scrape during signalling bouts (mean =  $1.87 \pm 1.33$  scrapes/bout;  $n = 7$ ).

### Mandible Drumming

Mandible drumming was observed in 7 species (*O. rosea*, *Falcaria bilineata*, *F. lacertinaria*, *W. cultraria*, *D. arcuata*, *D. falcataria*, and *D. curvatula*) and involves the head, thorax and first two abdominal segments being lifted and then quickly lowered to strike the leaf surface (Fig. 5.2c), producing a vibration. The abdominal prolegs do not move during the mandible drum and the terminal segment may concurrently perform an anal scrape in some species. Mandible drumming can be highly repetitive, with a mean of  $2.3 \pm 3.0$  drums per bout ( $n = 4$ ).

### Twitching

Twitching was only observed in one species (*T. batis*) and involves a quick, short, lateral movement of the head and thorax (Figure 5.2d). The rest of the body contracts slightly during the twitch.

### Lateral Tremulation

Lateral tremulation was observed in 4 species (*T. fluctuosa*, *T. batis*, *O. rosea*, and *W. cultraria*) and involves quick, successive lateral movements of the head, thorax and first two abdominal segments (Fig. 5.2e) to produce a vibration. Occasionally, the head

makes contact with the leaf surface during the lateral tremulation event. The abdominal prolegs remain firmly planted during the movement, but the anal segment may perform an anal scrape or 'pseudo' anal scrape at the same time.

### Buzzing

Buzzing is similar to lateral tremulation, but occurs in the opposite direction (Fig. 5.2f). Buzzing was observed in 2 species, both belonging to *Drepana* (*D. falcataria* and *D. curvatula*). The head and thorax is lifted off the leaf surface during this movement, and the mandibles do not make contact with the leaf. The head moves in the vertical direction only, the abdominal prolegs remain firmly planted during the movement, and the anal segment always performs an anal scrape during the buzz.

### Pushing

Pushing with the head was observed in 2 species of Thyatirinae (*O. duplaris* and *T. or*). Each push is accompanied with a forward crawl where the head makes contact with the intruder's body at the end of the crawl and pushes the intruder forward.

### Biting

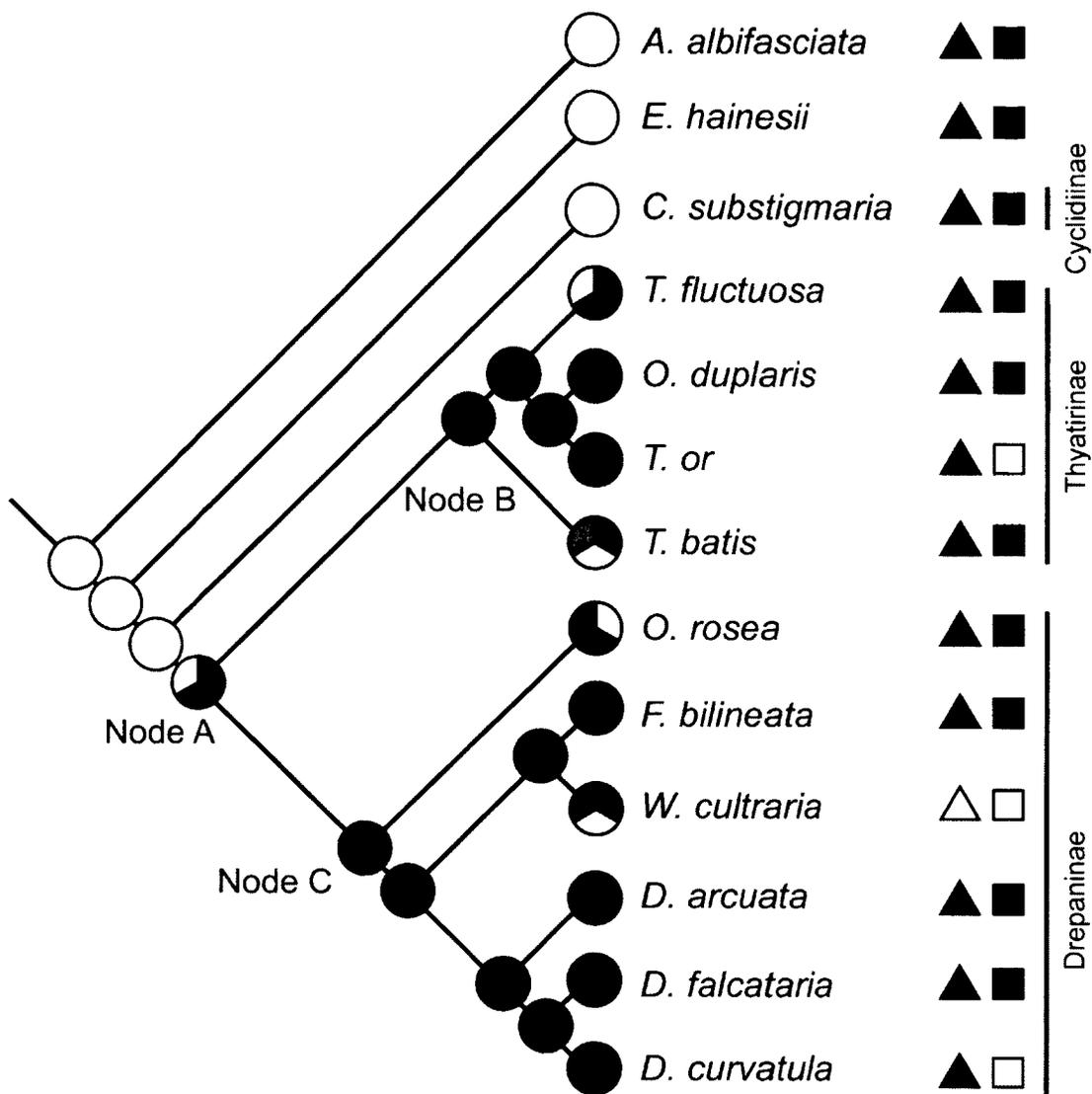
Biting was only observed in one species (*T. or*). Biting was difficult observe and quantify in this species, since they live concealed between two leaves. Therefore, properties of biting are not described in detail.

*Phylogenetic mapping of behavioural characters*

Maximum parsimony analysis demonstrates that territorial behaviours involving the mandibles and/or anterior body segments evolved for the first time in the common ancestor of the Thyatirinae and Drepaninae subfamilies (Fig. 5.3, Node A). The results show that both mandible drumming and lateral head hitting evolved at this node. Mandible drumming was then lost in the common ancestor of Thyatirinae (Fig. 5.3, Node B), and lateral head hitting was lost in the common ancestor of Drepaninae (Fig. 5.3, Node C). Mapping results show that twitching evolved once in *T. batis*, and that lateral tremulation, pushing, buzzing, mandible scraping, and lateral head hitting evolved multiple times within both the Thyatirinae and Drepaninae groups. Results provide support for the hypothesis that mandible scraping derives from lateral head hitting, as lateral head hitting is found at the most basal node of the Thyatirinae + Drepaninae (Node A). Mandible drumming was also found to be a basal behaviour, and at present, the transitional stages between behaviours are unclear.

By mapping mandible structure onto the phylogeny (Fig. 5.3), I was able to determine whether smooth or toothed distal edged, or smooth or ridged oral faced mandibles were correlated with any of the mandible behaviours over the phylogeny. Results from this analysis suggest that mandible morphology is not correlated with mandible behaviours, as none of the mandible structure categories were significantly correlated with any of the behaviours over the phylogeny using BayesDiscrete analysis ( $p > 0.05$ ,  $DF = 4$ ).

**Fig. 5.3.** Reduced Bayesian tree including only those species for which territorial behaviour is known, showing the ancestral and derived conditions of the morphology of the distal edge and oral surface of the mandibles, and anterior body behaviour. Pie charts are maximum parsimony probabilities of ancestral behaviours at all nodes.



Anterior Body Behaviour

- None
- Twitching
- Lateral Tremulation
- Pushing
- Mandible Drumming
- Biting
- Mandible Scraping
- Lateral Hitting

Mandibles - Distal Edge

- △ Smooth
- ▲ Toothed

Mandibles - Oral Face

- Smooth
- Ridged

### *Comparison of movements between behaviours*

I will begin this section by first comparing the movements associated with lateral head hitting and mandible scraping to provide support for my hypothesis that mandible scraping derives from lateral head hitting. I will then compare all other movements on the basis of direction of motion, duration, displacement and velocity to help propose the evolutionary transition of behaviours.

#### Comparison of mandible scraping and lateral head hitting movements

Between species comparisons demonstrated that lateral head hitting and mandible scraping did not differ significantly in terms of normalized displacement (independent t-test, two-tailed,  $t = 0.45$ ,  $DF = 8.1$ ,  $p = 0.67$ ) or velocity (independent t-test, two-tailed,  $t = 1.20$ ,  $DF = 9.0$ ,  $p = 0.26$ ). They did, however differ in duration (independent t-test, two-tailed,  $t = -6.10$ ,  $DF = 4.2$ ,  $p = 0.0030$ ), with lateral head hitting being longer in duration. Within species comparisons (in species that performed both behaviours) had similar results (paired t-tests, two-tailed; displacement:  $t = -1.14$ ,  $DF = 3$ ,  $p = 0.33$ ; velocity:  $t = -0.29$ ,  $DF = 3$ ,  $p = 0.79$ ; duration:  $t = 3.98$ ,  $DF = 3$ ,  $p = 0.028$ ).

#### Comparison of all anterior segment movements

Lateral hitting, mandible scraping, lateral tremulation and twitching all involve lateral movements of the head and anterior body segments. These movements did not differ significantly in displacement (ANOVA,  $F = 2.81$ ,  $DF = 4$ ,  $p = 0.081$ ) or velocity (ANOVA,  $F = 1.63$ ,  $DF = 4$ ,  $p = 0.23$ ), but the duration of lateral head hitting was significantly longer than the other behaviours (ANOVA,  $F = 34.6$ ,  $DF = 3$ ,  $p < 0.001$ ).

These movements also differed in the placement of the head - in lateral hitting, lateral tremulation and twitching, the head is lifted off the leaf at the beginning of the movement, and does not make contact with the leaf during the lateral movement. In mandible scraping, however, the mandibles are placed on the leaf and scraped laterally on the leaf surface during the movement.

Buzzing and mandible drumming differed from these four behaviours as they occur in the opposite direction, with the head being lifted and lowered to the leaf surface. One buzzing oscillation and the upwards movement of mandible drumming was not significantly different in terms of duration, normalized displacement or velocity (independent *t*-test, two-tailed;  $p > 0.05$ ), but the downward movement of mandible drumming occurred at a significantly higher velocity than the upwards movement (independent *t*-test, two-tailed;  $t = 5.62$ ;  $p = 0.01$ ;  $DF = 2.12$ ). The major difference between buzzing and mandible drumming is that the buzzing movement is more repetitive, with 14.7 oscillations per second (see above), and the mandibles do not make contact with the leaf surface. Pushing involves mainly the movement of the entire body during a crawling cycle and is therefore fundamentally different from all other head movements. As mentioned above, biting could not be quantified in terms of kinematic properties.

#### *Comparisons of vibrations to assess ritualization*

Vibrations were compared on the basis of conspicuousness, redundancy, stereotypy and alerting components between lateral head hitting and mandible scraping to test the hypothesis that mandible scraping derives from lateral head hitting. Mandible

scraping was found to be significantly higher in amplitude than lateral head hitting (Wilcoxon Signed Rank Test,  $S = 14.0$ ,  $DF = 6$ ,  $p = 0.016$ ), but did not differ from lateral head hitting in terms of dominant frequency and bandwidths at -3 dB and -10 dB (conspicuousness) (Wilcoxon Rank Sum Tests,  $p > 0.05$ ), rates at close distance (redundancy) (independent  $t$ -test, two-tailed,  $t = 2.20$ ,  $p = 0.064$ ) and stereotypy of duration (stereotypy) (independent  $t$ -test, two-tailed,  $t = 1.29$ ,  $p = 0.22$ ). By examining data on signalling bouts, mandible scraping was typically preceded by anal scraping (in anal scraping species) or mandible drumming. Lateral head hitting was not typically preceded by any one behaviour.

To help determine the evolutionary transition between behaviours, vibrations were compared between all anterior body behaviours based on the characteristics of ritualization. None of the behaviours differ significantly based on dominant frequency and bandwidths at -3 dB and -10 dB (conspicuousness), rates at close distance (redundancy) and stereotypy of duration, normalized displacement and velocity (stereotypy) (ANOVAs;  $p > 0.05$ ). Amplitude comparisons between behaviours (conspicuousness), within trials, demonstrate that mandible drumming is significantly higher in amplitude than mandible scraping (paired  $t$ -test, two-tailed;  $t = -3.65$ ;  $p = 0.008$ ;  $DF = 7$ ), lateral tremulation (paired  $t$ -test, two-tailed;  $t = -4.28$ ;  $p = 0.025$ ;  $DF = 2$ ), and lateral head hitting (paired  $t$ -test, two-tailed;  $t = -13.67$ ;  $p = 0.005$ ;  $DF = 2$ ). Mandible scraping is also significantly higher in amplitude than lateral head hitting (see above). When the number of oscillations per second were factored into the rates of lateral tremulation and buzzing, these movements have significantly higher rates than all other behaviours (redundancy) (ANOVA;  $F = 219.2$ ;  $p < 0.001$ ;  $DF = 7$ ). By examining bout

data, mandible drumming, lateral tremulation and buzzing were typically preceded or accompanied by anal scraping (in species which anal scraped). Crawling, pushing, twitching and lateral head hitting (see above) were not typically preceded by a signal.

#### ***5.4 Discussion***

The major goals of this chapter were: 1) to map morphology and behaviour of the mandibles and anterior body segments onto the phylogeny to determine which characters are basal and derived; 2) to test the hypothesis that mandible scraping derives from lateral head hitting; and 3) to propose a model for the evolutionary transition between anterior body behaviours.

##### *Mapping mandible morphology and anterior body behaviours*

###### Comparative morphology of the mandibles

The mandibles varied in morphology from smooth to toothed on the distal edge, and smooth or ridged on the oral surface. The results of the phylogenetic mapping demonstrate that a toothed distal edge and ridged oral surface represents the basal condition. Mandible morphology was also not correlated with the presence or absence of any of the anterior body behaviours. This agrees with Bura (2009; 2010), who demonstrated that although mandible structure is linked to certain types of signals in some Bombycoidea caterpillar species that produce acoustic warning sounds by clicking their mandibles, it does not predict the ability to produce these signals. Studies on acridids have found that head and mandible size, and mandible morphology is closely related to the type of food consumed (reviewed in Clissold, 2007). For example, species

that feed on hard grasses have larger heads and mandibles, and blunt, or smooth distal edged mandibles (reviewed in Clissold, 2007). These trends are not as clear in lepidopteran larvae, and mandible morphology may be more closely aligned with taxonomic relationships (Bernays, 1991). However, there are some apparent morphological similarities in mandible structure in caterpillars based on diet: those that feed on grasses have smooth distal edges, while those that feed on forbs have toothed distal edges (Brown & Dewhurst, 1975); and species that feed on plants with reticulated veins have longer, toothed, more complexly ridged mandibles than those that feed on old, tough leaves (Bernays & Janzen, 1988). In the Drepanidae, there does not appear to be a relationship between signalling with the mandibles and mandible structure. Since the mandibles are also used for feeding, there is perhaps less of a selection pressure to modify them for signalling, and their structure depends more on taxonomic status and diet.

#### Comparative behaviour of the anterior segments

Behaviours associated with the anterior body during encounters with conspecifics included mandible drumming, mandible scraping, twitching, lateral tremulation, buzzing, lateral head hitting, pushing and biting. Drumming with the head or mandibles has been described in other species of insects, including termites (Röhrig et al., 1999; Rosengaus et al., 1999), death-watch beetles (Birch & Keenlyside, 1991), carpenter ants (Fuchs, 1976) and in a few species of caterpillars, *D. arcuata* (Yack et al., 2001), *Sparganothis pilleriana* (Russ, 1969), *Drepana falcataria* (Bryner, 1999, I. Hasenfuss, personal communication), *Falcaria lacertinaria* (I. Hasenfuss, personal communication), and

*Nordstromia lilacina* and *Tridrepana arikana* (Sen & Lin, 2002). I observed mandible drumming in 6 species of Drepaninae, including *D. arcuata*, *D. curvatula*, *D. falcataria*, *F. bilineata*, *O. rosea* and *W. cultraria*. Interestingly, I did not observe mandible drumming in any of the Thyatirinae species, perhaps because many of them live in shelters made by sewing two leaves together, and there is a lack of vertical space to mandible drum. I did, however, observe mandible scraping in Thyatirinae caterpillars, as this signal uses minimal vertical space. Mandible scraping is less frequently reported in insects, being noted in the larvae of the oriental hornet, *Vespa orientalis*, where they function as hunger signals (Ishay et al., 1974) and in a few species of larval Lepidoptera, *D. arcuata* (Yack et al., 2001), *Caloptilia serotinella* (Fletcher et al., 2006), *D. falcataria*, and *F. lacertinaria* (I. Hasenfuss, personal communication). My results demonstrate that mandible scraping is performed by 7 of 11 species of Drepanidae caterpillars I studied (*D. arcuata*, *D. curvatula*, *D. falcataria*, *O. rosea*, *O. duplaris*, *T. fluctuosa*, and *T. or*), suggesting that it may represent an important form of signalling in the group.

Tremulation (e.g. lateral tremulation or buzzing), believed to be one of the most simple and widespread vibrational signal production mechanisms in insects (Virant-Doberlet & Cokl, 2004) and have been reported in one species of caterpillar to date, *C. serotinella* (Fletcher et al., 2006). Lateral tremulation and buzzing was observed in 6 out of the 11 species I studied (*D. curvatula*, *D. falcataria*, *O. rosea*, *T. batis*, *T. fluctuosa* and *W. cultraria*), and may be common in these species as it does not require the use of specialized signaling structures. Finally, physically aggressive behaviours, including striking with the head, and biting have been described in two other caterpillar species to date (*Depressaria pastinacella*: Berenbaum et al., 1993; and *Busseola fusca*: Okuda,

1989; respectively). My results demonstrate that physically aggressive behaviours is common in the Drepanidae, with 9 out of 11 species studied employing lateral head hitting during encounters with conspecifics. The results of phylogenetic mapping suggest that lateral head hitting and mandible drumming represent the basal behaviours and that lateral tremulation, pushing, buzzing, and mandible scraping evolved multiple times within both the Thyatirinae and Drepaninae groups. Many of the Drepanidae caterpillars studied to date have a repertoire of different signals and aggressive behaviours, but why produce more than one signal for the purpose of territoriality? This question, and others, will be explored in Chapter 6 of this thesis.

*Testing the hypothesis that mandible scraping derives from lateral head hitting*

The hypothesis that mandible scraping derives from lateral head hitting is supported by the following lines of evidence: 1) lateral head hitting represents the basal condition when mapped onto the phylogeny; 2) kinematic analysis suggests that mandible scraping and lateral head hitting involve similar movement patterns; and 3) vibration analysis provides evidence that mandible scraping has more features of ritualization than lateral head hitting, including increased conspicuousness, redundancy, stereotypy and alerting components. One can also observe this transition in behaviour by examining hitting and mandible scraping in certain species of Drepanidae. For example, *F. bilineata*, a species that only lateral head hits will sometimes scrape the mandibles on the surface of the leaf during a lateral head hit. The incidental vibration produced by this movement could represent the beginning stages of the evolution of mandible scraping in this species. The shift from aggressive behaviour (lateral head hitting) to ritualized signalling

(mandible scraping) may function to reduce the costs of physical aggression in these caterpillars. Why produce ritualized signals instead of physical aggression is another question that will be explored in Chapter 6.

#### Alternative hypotheses

The hypothesis that mandible scraping derives from lateral hitting, a physically aggressive behaviour, suggests that mandible scraping is an intention movement, showing the intention of the signaler to physically harm the receiver. However, signals are also proposed to evolve from protective movements, displacement activities or redirection (Morris, 1956; Brown, 1975; Bradbury & Vehrencamp, 1998; Maynard Smith & Harper, 2003). It is therefore possible that mandible scraping evolved from movements not associated with interactions between conspecifics. Two likely candidates for the evolutionary precursor of mandible scraping, based on similarity of movement, would be laying silk, and deterring predators. If mandible scraping is derived from laying silk, this would be classified as a displacement activity, as the original movement is not performed in the same context. Laying silk involves moving the head laterally from side to side to attach silk to the leaf (Fitzgerald et al., 1991). This lateral silk laying movement has been observed in all larvae of Drepanidae that lay silk mats or create silk shelters (personal observation), and therefore represents a basal behaviour common to this group. Rapid flexing of the body in a lateral motion is also a primitive defensive movement performed by many soft-bodied insects, including lepidopteran, dipteran and coleopteran larvae (reviewed in Brackenbury, 1999). This movement has also been observed in caterpillars of the Drepanidae when lightly touched with a paintbrush (personal observation). Both of

these behaviours are widespread and present in the Drepanidae, and therefore either could potentially represent the evolutionary precursor of mandible scraping. However, it is more likely that these signals evolved as intention movements, because movements mimicking physically aggressive behaviours are more relevant during territorial contests, and may be better indicators of size or fighting ability, allowing contests to be resolved more quickly.

*Proposed evolutionary transitions in behaviour*

The results of this study suggest that all anterior body behaviours are either derived from lateral head hitting or mandible drumming, both of which were present in the common ancestor of Drepaninae and Thyatirinae (Node A). By examining the phylogeny in detail, however, it is likely that the presence of mandible drumming in the common ancestor of the Drepaninae and Thyatirinae (Node A) is an anomaly caused by low sample size in Thyatirinae species. Since Thyatirinae represents the basal subfamily, and none of the species of Thyatirinae studied to date have been found to mandible drum, it is likely that mandible drumming evolved for the first time at Node C, the common ancestor of Drepaninae, instead. Although not suggested by the phylogeny, I believe that mandible drumming also derives from lateral head hitting, as during lateral head hitting, the head would occasionally make contact with the surface of the leaf, possibly being the precursor to mandible drumming. Results from the phylogeny, kinematic analysis and ritualization analysis also suggests that buzzing evolved from mandible drumming in some species of *Drepana*.

It is also likely that mandible scraping, twitching, and lateral tremulation derive from lateral head hitting based on some support from phylogenetic, kinematic and ritualization evidence. The present phylogeny also suggests that these derived behaviours evolved independently multiple times, which may signify the importance of a shift from physically aggressive behaviours to ritualized signalling in some of these caterpillars. If contests between resident and intruder caterpillars often ended in injury or death to one of the opponents, as in other species of caterpillars (e.g. *Depressaria pastinacella* (Oecophoridae; Berenbaum et al., 1993), *Busseola fusca* (Noctuidae; Okuda, 1989) and *Anthocharis cardamines* (Pieridae; Baker, 1983)), signalling may have evolved to reduce the likelihood of physical damage to either opponent. Lateral hitting, a physically aggressive behaviour, however, still persists in most species, but seems to be reserved for high escalation contests, where the intruder makes physical contact with the resident (see Chapter 2 and Appendix A for details on escalation).

#### *Comparison of vibrations to assess ritualization*

Ritualization has been shown to play an important role in the evolution of signals, allowing them to be more detectable and recognizable by receivers (Cullen, 1966; Wiley, 1983; Johnstone, 1997; Bradbury & Vehrencamp, 1998; Maynard Smith & Harper, 2003). The present study found evidence for ritualization in terms of conspicuousness, redundancy, stereotypy and alerting components in vibrations associated with anterior body in caterpillars of the Drepanidae. Mandible scraping was found to possess more features of ritualization than lateral head hitting, producing higher amplitude vibrations (conspicuousness), and being produced in bouts of 3-4 signals per bout (redundancy).

Mandible scraping also was often preceded by an alerting component, anal scraping, during signalling bouts. Lateral tremulation and buzzing were also found to be highly ritualized, being significantly more repetitive during a single signalling event. This repetition of movements makes the signal more redundant, reducing errors made by the receiver in detecting and recognizing the signal (Wiley, 1983). It is also clear by examining the movements associated with mandible scraping, mandible drumming, buzzing and lateral tremulation events, that they are highly ritualized, as there is little variation in the direction of motion (i.e. mandible drums always begin with the head being lifted from the leaf surface and end with the mandibles striking the leaf). Therefore, I provide evidence for ritualization of anterior body behaviours in the Drepanidae.

#### *Future Studies*

In conclusion, my study provides support for the hypothesis that mandible scraping derives from lateral head hitting. The transitions between behaviours, however, are less clear. Future studies that examine the behavioural repertoire of more species (especially Thyatirinae species) will provide further insight into the evolution of anterior body behaviours in these caterpillars.

**CHAPTER 6**

**THE EVOLUTION OF VIBRATORY COMMUNICATION SIGNALS IN  
DREPANIDAE CATERPILLARS: ULTIMATE QUESTIONS**

## ***6.1 Introduction***

The main focus of this thesis was to test hypotheses on the evolutionary origins of signals produced by Drepanidae caterpillars. In previous chapters I have shown that variation exists in territorial behaviours, signals and signal-producing structures across species of Drepanidae. I have also developed a phylogeny and used this phylogeny to answer questions concerning how anal segment and anterior body signals evolved from non-signalling behaviours, thereby focusing on proximate mechanisms of signal evolution. During the course of this study, a number of additional questions have arisen about the ultimate mechanisms of signal evolution in these caterpillars. For example, what is the function of signalling? Why do some species produce more than one type of signal? And finally, why signal instead of using physical aggression? Although seeking answers to these questions was not the original intention of this study, the data collected in previous chapters can be used to develop hypotheses for future studies. The current chapter will focus on developing and refining testable hypotheses to answer these three questions. I have also included a table summarizing the main hypotheses and predictions used to answer each of the three questions (Table 6.1). This chapter is meant to be preliminary in nature, and further analyses are required to formally test these hypotheses.

## ***6.2 What is the function of signalling?***

### ***Background***

Throughout this thesis, I have referred to the signals produced by Drepanidae caterpillars as functioning in territorial defense of leaf shelters or leaves. Yack et al.

**Table 6.1.** Summary of questions, hypotheses and predictions tested in this chapter.

<b>Main Question</b>	<b>Hypothesis</b>	<b>Predictions</b>	<b>Tested?</b>
<b>What is the function of vibratory signalling?</b>	Signals function for territorial defense of leaf shelters/leaves	a) signals will be produced primarily by the resident of the leaf shelter/leaf b) signals will be elicited by the approach of the intruder c) signal rates will escalate as the intruder approaches the resident d) signalling will be followed by the resident leaving the leaf shelter/leaf e) residents with higher investments in the leaf shelter construction (from no shelter to silk mat to rolled leaf to two leaves sewn together), will have higher rates of signalling (and aggressive behaviours) and vice-versa	Y
<b>Why more than one type of signal?</b>	1. Different signal types convey information about the motivation of the resident  2. Different signal types increase the detection and recognition of signals by intruders  3. Different signal types evolved to counteract bluffing  4. Different signal types convey different types of information	Signal types will change as the intruder approaches the resident  Signal types differ in spectral properties including bandwidth and peak frequency and temporal characteristics, including duration	Y  Y  N  N
<b>Why signal instead of using aggression?</b>	1. If the chance of encountering a sibling as a late instar is high, residents will produce more signals and be less aggressive towards intruders  2. The costs of aggressive behaviour affects the behaviour of the resident	a) Species that lay eggs in rows/clusters will have a higher ratio of signals to aggressive behaviours than those that lay eggs singly  b) Species that are gregariousness as early instars will have a higher ratio of signalling to aggressive behaviour  If the costs of aggressive behaviour are high, residents will produce more signals than physically aggressive behaviours	Y  N

(2001) provide strong support that in one species, *Drepana arcuata*, vibratory signals including mandible drumming, mandible scraping and anal scraping function for territoriality based on experimental evidence. What about the other species studied to date? Based on similarities in characteristics of behavioural encounters with conspecifics between *D. arcuata* and other species I have studied to date, I believe that the signals described in previous chapters of this thesis function for territorial defense of leaf shelters or leaves. To further test this hypotheses, I predict that: 1) signals will be produced primarily by the resident of the leaf/leaf shelter; 2) signals will be elicited by the approach of the intruder; 3) signalling rates of the resident will escalate as the intruder approaches; 4) signalling will be often followed by the intruder leaving the leaf/leaf shelter; and 5) residents with higher investments in leaf shelter construction will show higher rates of signalling (and aggressive behaviour) and vice-versa. I will test these predictions by comparing the frequency of signalling between residents and intruders (Prediction 1), rates of signalling before and during the course of encounters with conspecifics (Predictions 2 and 3), the number of trials in which the resident won to the number of trials that ended in ties or losses (Prediction 4), and shelter-building behaviour (no shelter, silk mat, rolled/folded leaf, or two leaves tied together) to rates of signalling and aggressive behaviour (Prediction 5).

### *Methods*

The following methods use data collected from conspecific interactions and general observations as described in Chapter 2. All statistical comparisons used an alpha

level of 0.05, and data were checked for normal distribution using the Shapiro-Wilk W test.

**Prediction 1. Signals will be produced primarily by the resident**

The frequency of intruder signalling was compared to that of the resident using a paired t-test (grouped by species to allow for differences in signalling between species). Frequency of intruder and resident signalling was measured as the number of trials in which the intruder or resident signaled over the total number of trials. Signals included mandible scraping, mandible drumming, anal scraping, lateral tremulation and buzzing.

**Prediction 2. Signals will be elicited by the approach of the intruder**

The average distance between the resident and the intruder at first signal was calculated to demonstrate when residents typically begin signalling. Overall signalling rates (all signals combined) were also compared in the 5 min period before the trial to the signalling rates during the trial (average signalling rates at FAR, MID and CLOSE stages of intruder approach; see Chapter 2 for details on how rates were calculated at different stages of intruder approach) using a Wilcoxon Rank Sum Test to determine whether residents signal more while alone on the leaf or with an intruder.

**Prediction 3. Signal rates will increase as the intruder approaches**

Overall signalling rates (all signals combined) were compared at three stages of intruder approach - FAR, MID and CLOSE, using a repeated measures ANOVA (grouped by species, to account for differences in signalling rates between species) to

determine if signal rates escalate as the intruder approaches. *Post hoc* analyses were done using pair wise paired t-tests. Changes in signal rate of individual signals was also examined by species in Chapter 2 and Appendix A.

Prediction 4. Signalling will be followed by the intruder usually leaving the leaf/leaf shelter.

Data on frequency of resident wins (when the intruder left the leaf) were compared to those of ties (when neither left the leaf) and losses (when the resident left the leaf) using a Kruskal-Wallis one-way analysis of variance. *Post hoc* analysis was done using pair wise Wilcoxon Rank Sum Tests. Only trials in which the resident signaled at least once were used.

Prediction 5. Residents with higher investments in their leaf shelter will have higher rates of signalling (and physical aggression)

Signalling rate was defined as the rate of all signals combined per 5 s at CLOSE distance (the point when the intruder first made contact with the resident). Aggressive behaviour rates and overall behaviour rates (signals + aggressive behaviours) were calculated in the same manner. These rates were then compared to data on shelter building behaviour as described in Chapter 2 (no shelter, silk mat, rolled/folded leaf, or two leaves sewn together) using an ANOVA to determine if there is a relationship between overall/signalling/aggressive rates and shelter-building behaviour. The aggressive behaviour rates did not follow a normal distribution and were therefore compared using a Wilcoxon Rank Sum Test. The number of signal types was also

compared between leaf shelter types using an ANOVA to determine if species with a larger investment in leaf shelter produced more types of signals. *Post Hoc* analyses were completed using a Tukey Kramer HSD.

### *Results*

Prediction 1. Signals will be produced primarily by the resident

Overall, residents signaled at least once in significantly more trials than did intruders (paired t-test,  $t = 7.36$ ,  $DF = 9$ ,  $p < 0.001$ ). Figure 6.1a shows signalling rates of a resident and an intruder in average trials of *Falcaria bilineata*. For further information on the frequency of intruder signalling in other species, see Chapter 2 and Appendix I.

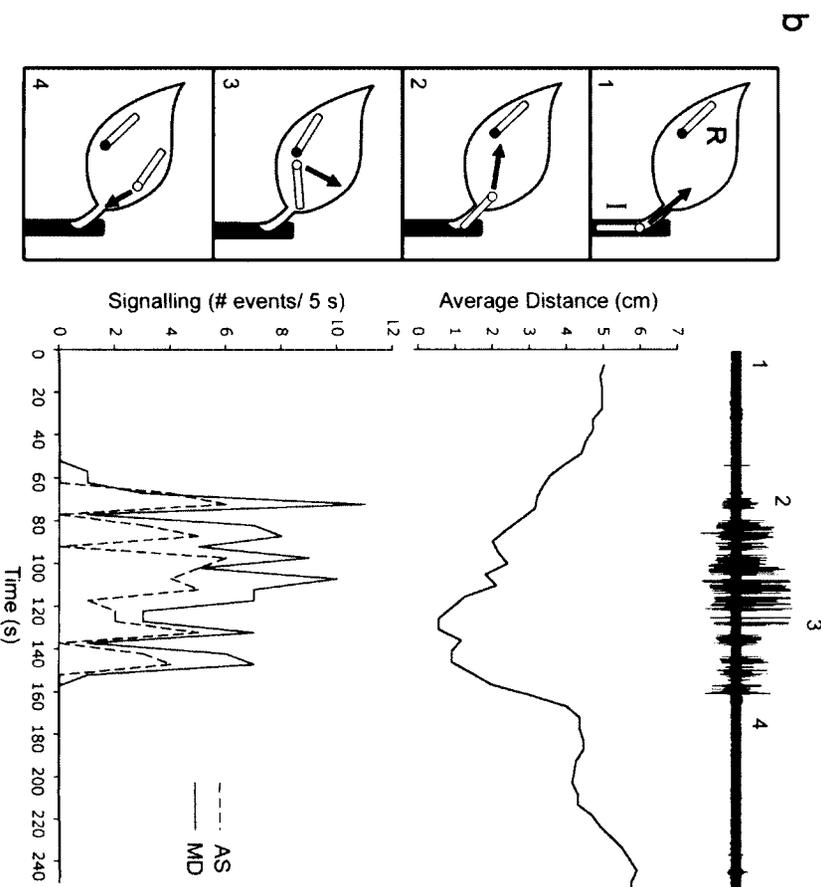
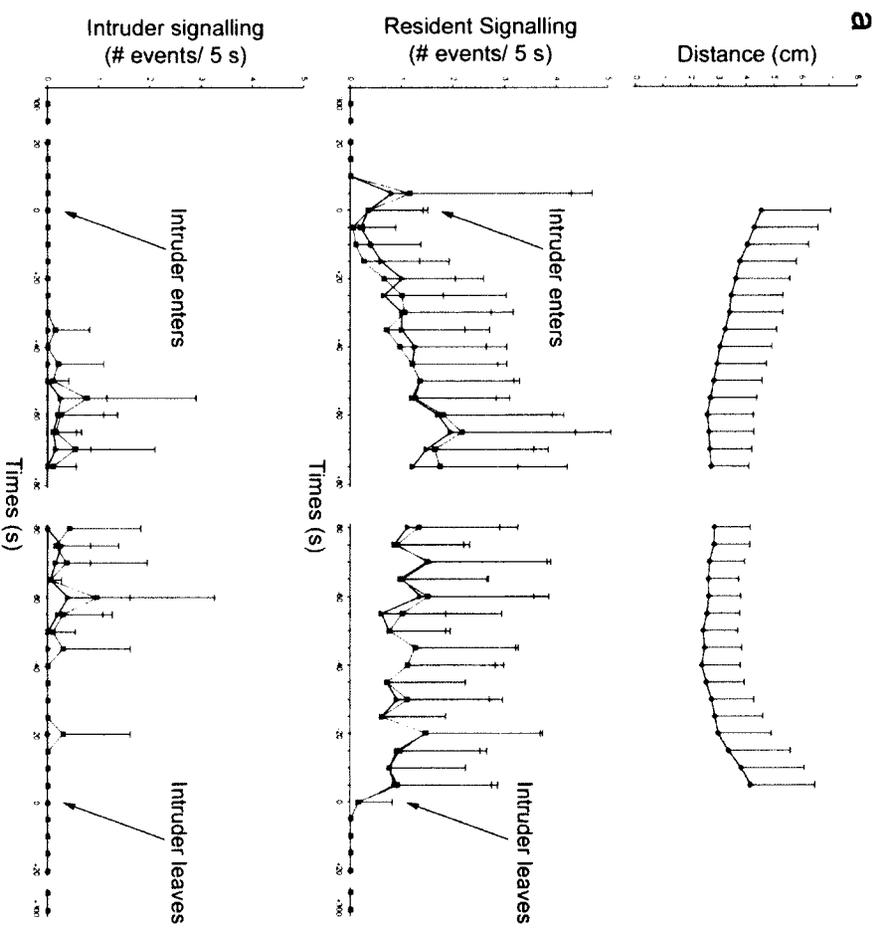
Prediction 2. Signals will be elicited by the approach of the intruder

On average, residents first signaled when the intruder came within  $14.4 \pm 10.7$  mm of the resident. Overall average signalling rates (of all signals combined) were significantly higher after the trial began (average of rates at FAR, MID and CLOSE) than during the 5 min period before the trial ( $0.0 \pm 0.0$  vs.  $2.15 \pm 2.48$ ; Wilcoxon Rank Sum,  $Z = -3.84$ ,  $DF = 1$ ;  $p < 0.001$ ). Figure 6.1b demonstrates a representative trial in *F. bilineata*, showing the distance at first signal and resident signalling rates before and during the trial. For further information on other species, see Chapter 2 and Appendix I.

Prediction 3. Signal rates will increase as the intruder approaches

Overall signalling rates (all signals combined) escalated significantly as the intruder approached the resident, where signalling rates significantly increased from FAR to CLOSE (repeated measures ANOVA,  $F = 8.54$ ,  $DF = 2$ ,  $p = 0.01$ ). Individual signal

**Fig. 6.1.** Average and representative trial data in *Falcaria bilineata*. **(a)** Resident and intruder signalling over 20 encounters. Mean distance (+SD) between resident and intruder larvae at the beginning of each 5-s interval (top graph). Signalling rate of residents (middle graph) and intruders (bottom graph) before and after trials, and for the first 80 s and last 80 s of each trial. Red denotes average mandible drum rate per 5-s interval, and blue denote average anal scrape rate per 5-s interval. **(b)** Resident signalling during a single agonistic encounter (238 s). Schematic of the different stages of the encounter (left panel). Frame 1: the resident (R) is feeding as the intruder (I) moves along the twig toward the leaf before the trial. Frame 2: the resident begins to signal as the intruder enters the leaf. Frame 3: the resident signals continuously as the intruder makes contact. Frame 4: the resident stops signalling as the intruder walks away and eventually leaves the leaf. Arrows indicate the path of the intruder across the leaf. Oscillogram illustrating the vibrational signals made by the resident throughout the encounter (top right). Numbers correspond with frames from (left panel) and timescale corresponds to (bottom right). Mean distance between resident and intruder at the beginning of each 5-s interval (middle right) and the number of mandible drums (MD) and anal scrapes (AS) in consecutive 5-s intervals, including 1 min before the trial, and 1 min after intruder departure (bottom right). Time scale is the same for both distance and signalling graphs.



rates also increased in certain species between different stages of intruder approach (See Chapter 2 and Appendix A). Figure 6.1 demonstrates the changes signalling rates as the intruder approaches the resident in *F. bilineata*. For further information on other species, see Chapter 2 and Appendix I.

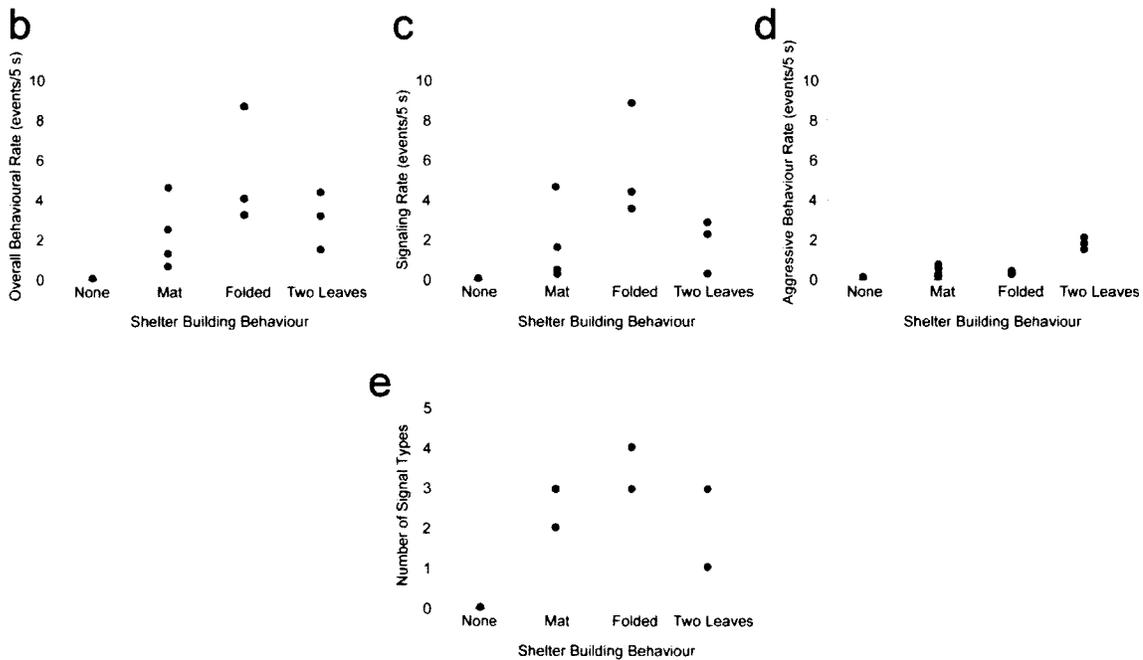
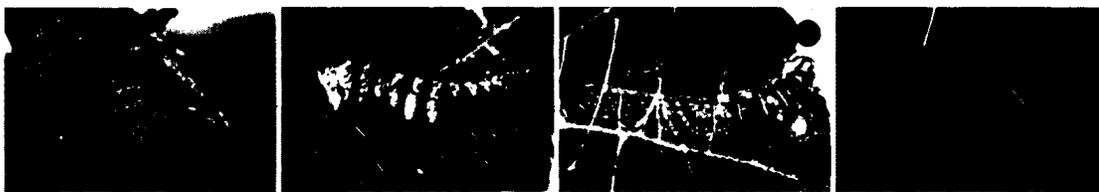
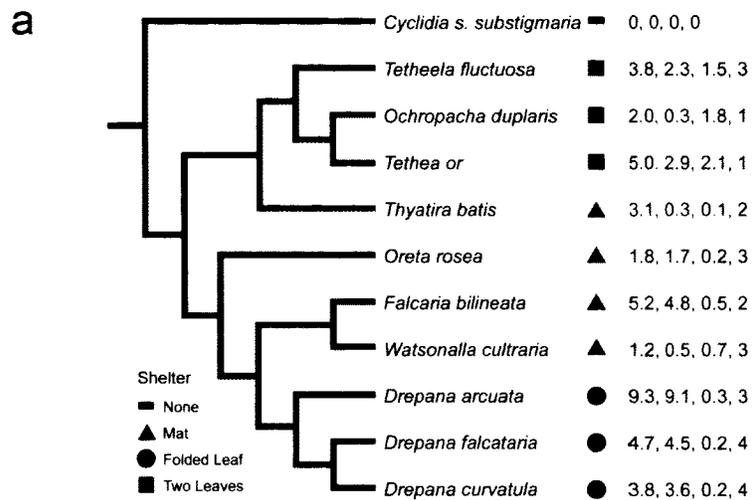
Prediction 4. Signalling will be followed by the intruder leaving the leaf/leaf shelter

Residents had significantly more encounter wins than losses when they signaled at least once (Kruskal-Wallis one-way analysis of variance;  $Z = 9.73$ ,  $DF = 2$ ,  $p = 0.008$ ). Some species, such as *T. or*, never lost an encounter, while in other species, such as *T. batis*, all encounters ended in a tie. See Chapter 2 for details on outcomes of encounters in all species studied to date. Figure 6.1 shows the point at which the intruder leaves in *F. bilineata*.

Prediction 5. Residents with higher investments in their leaf shelter will have higher rates of signalling (and physical aggression)

Signalling, aggressive behaviour, and overall combined rates were compared to shelter building behaviour (mat only, folded leaf, two leaves sewn together) to determine if residents with higher investments in leaf shelters have higher rates in signalling and physical aggression (Fig. 6.2). Rates of signalling, aggressive behaviour and overall combined signalling and aggressive behaviour were not significantly different between shelter types (overall: Fig. 6.2b, ANOVA,  $F = 1.92$ ,  $DF = 2$ ,  $p = 0.22$ ; signalling: Fig. 6.2c, ANOVA,  $F = 3.35$ ,  $DF = 2$ ,  $p = 0.095$ ; aggressive behaviour: Fig. 6.2d, Wilcoxon Rank Sum,  $Z = 5.79$ ,  $DF = 2$ ,  $p = 0.055$ ). Number of signal types was also compared to shelter

**Fig. 6.2.** The relationship between shelter type and overall signalling and aggressive behavioural rates. **(a)** Phylogeny of taxa used in comparative analysis with data for overall (signalling and aggressive rates combined), signalling and aggressive behavioural rates, and number of signal types, respectively, with photographs (from left to right) of no shelter (*O. rosea*; scale bar = 1 cm), silk mat (*F. bilineata*; scale bar = 5 mm; photo credit: J. Yack), rolled leaf (*D. arcuata*; scale bar = 3 mm; photo credit: J. Yack) and two leaves tied together (*T. or*; scale bar = 1 cm). **(b)** The relationship between shelter type and overall behavioural rate. **(c)** The relationship between shelter type and signalling rate. **(d)** The relationship between shelter type and aggressive behavioural rate. **(e)** The relationship between shelter type and number of signal types. Caterpillars that build folded leaf shelter have significantly more signal types (ANOVA,  $F = 4.90$ ,  $DF = 2$ ,  $p = 0.047$ ).



building behaviour to determine if species with higher investments in leaf shelters produce more types of signals. Number of signal types significantly differed between shelter types, with species that fold leaves producing the most signal types (Fig. 6.2e; ANOVA,  $F = 4.90$ ,  $DF = 2$ ,  $p = 0.047$ ).

### *Discussion*

In this section I tested the hypothesis that the signals described in Chapter 2 of this thesis (including mandible scraping, mandible drumming, anal scraping, lateral tremulation and buzzing) function for territorial defense of leaf shelters or leaves in Drepanidae caterpillars. Support for this hypothesis comes from the following lines of evidence. First, signals are produced primarily by residents of leaf shelter or leaves, where residents produced at least one signal in significantly more trials than did intruders. Second, signals are elicited by the approach of an intruder, as residents were never observed to signal while they were alone on the leaf before the trial, and only began signalling once the intruder was presumably close enough to be detected. Third, signals escalate in rate as the intruder approaches, with signalling rates significantly increasing between FAR and CLOSE stages of intruder approach. Gradation in signalling rate may act to express changes in the motivational state of the resident, and is a common feature of protracted territorial encounters (Brown, 1975; Baker, 1983; Maynard Smith & Harper, 2003). Enquist et al. (1990) also argue that contests should begin with less costly, but less informative acts that progress to more costly acts as the risk of threat increases. Thus, when the intruder is at a far distance, it poses little threat and the resident begins with low cost signalling (lower rates) to reserve energy and avoid attracting predators. As the intruder approaches, the risk increases, and the resident uses more costly signals

(increased rates). Higher signal repetition rates as the intruder approaches may also act to ensure the intruder receives the message by increasing the redundancy of the signal. Fourth, resident signalling is followed by the intruder leaving the shelter. Residents retained their shelters or leaves in most trials and the intruder abandoned the occupied leaf in a short amount of time, which is often the case in pair wise contests over an indivisible resource where there exists an asymmetry in ownership (Baker, 1983; Maynard Smith & Harper, 2003).

Why would a caterpillar be territorial against conspecifics? Like many other caterpillars, some Drepanidae caterpillars invests in building a leaf shelter, which provides a more stable microclimate, protection from predators and displacement, and enhanced quality of food (Fukui, 2001). Shelters are also costly, requiring time, energy and material to build (Ruggiero & Merchant, 1986; Fitzgerald et al., 1991; Berenbaum et al., 1993; Cappuccino, 1993; Fitzgerald, 1995). Many caterpillars have evolved ways to maintain the use of their shelters while minimizing their costs, such as using empty shelters, attempting take over of occupied shelters, or by sharing them with con- and heterospecifics (Berenbaum et al., 1993; Cappuccino, 1993; Lill et al., 2007). Shelter sharing, however, often has associated costs and is not always favourable (Cappuccino, 1993; Lill et al., 2007). It is proposed that some caterpillars protect their energetic and time investments by defending their shelters from others using vibratory signals. There have been detailed reports of vibration-mediated territorial signals in 3 species from various families, including the Gracillariidae (*Caloptilia serotinella*: Fletcher et al., 2006), Tortricidae (*Sparganothis pilleriana*: Russ, 1969) and Drepanidae (*D. arcuata*: Yack et al., 2001). The behaviour I have reported in this thesis is consistent with the

behaviours observed in these other species. Therefore, if signals are used for territorial defense, my final prediction was that residents with higher investments in their leaf shelter will have higher rates of signalling and physical aggression. I did not find a significant difference in signalling, aggressive behaviour or overall combined rates with different leaf shelters. However, we do have evidence that *D. arcuata* signals more when living in shelters with more silk (J. Yack, unpublished data), and direct comparisons between *D. arcuata*, *F. bilineata*, and *O. rosea*, sympatric congeners, demonstrate that *D. arcuata* (which builds a folded leaf shelter) signals significantly more than *F. bilineata* and *O. rosea* (which only lay a silk mat). I also show that species that build folded leaf shelters produce more types of signals. This may suggest that caterpillars that have higher investment leaf shelters may have evolved additional signal types to further defend those shelters. However, this is not the case for two-leaf shelters, which are arguably has an even higher investment, requiring residents to locate two suitable leaves close enough to tie together. Since mainly the *Drepana* larvae build folded leaf shelters and produce more types of signals, this result could be simply due to phylogenetic relationships between species and future studies should use the comparative method to determine whether these differences are based solely on phylogeny. Future studies that include behavioural data on more species, and that use additional methods to test for levels of defense are currently required to further test this final prediction. Finally, further studies should also examine whether Drepanidae larvae also respond to heterospecifics, but overall, my current results support the hypothesis that signals are used to advertise ownership of territories.

### 6.3 Why produce more than one type of signal?

#### *Background*

Many Drepanidae caterpillars demonstrate a repertoire of signals during encounters with conspecifics. All six of the Drepaninae species I studied produce at least two, and up to four signal types including any combination of mandible scraping, mandible drumming, anal scraping, lateral tremulation and buzzing. For example, *D. arcuata* produces 3 types of distinct signals (see Chapter 2). Thyatirinae larvae generally produced less signal types, with two of the four species producing more than two types of signals and the other two only producing one type of signal. For example, *Tethea or*, produces only one signal, mandible scraping. Why might these caterpillars use more than one type of signal during territorial interactions? There have been several hypotheses to explain why animals may use such multicomponent or complex signals (Hebets & Papaj, 2005). For example, a series of discrete signals may be used to convey information about motivation of the sender. According to the sequential assessment model (Enquist & Leimar, 1983; 1987), behavioural repertoires are used during contests for assessment of asymmetries between contestants. As such, contests should begin with less costly but less informative acts, and if such acts do not lead either contestant to give up, they will progress to more informative, more costly acts. Therefore, behaviours should change over the course of an interaction. For example, in the cichlid fish, *Nannacara anomala*, fights between conspecifics have distinct phases, beginning with less costly acts, such as visual assessment, and ending in escalated fighting, including circling behaviour (Enquist et al., 1990). Based on this model, I predict that if different signal types convey information about the motivation of the resident, then signal types will change as the intruder

approaches the resident. To test this, I will determine if signal types change within species at FAR, MID and CLOSE stages of intruder approach. The second hypothesis I will be testing is that different types of signals are used to increase the detection and recognition of the signal by receivers. As discussed in previous chapters, signal redundancy can improve the efficacy of a signal by reducing errors in the detection and recognition of a signal (Wiley, 1983). Increasing redundancy can include repeating a signal or producing a complex display with many different elements (or signal types) (Maynard Smith & Harper, 2003). If these redundant signals differ slightly in spectral or temporal properties, it is more likely that the intended receiver will detect and recognize the overall signal. Therefore, if multiple signals evolved in the Drepanidae to increase the detection and recognition of the signal, I predict that signals types will differ in spectral and temporal properties, including bandwidth, peak frequency and duration. I will test this by comparing bandwidth, peak frequency and duration between signal types. A third hypothesis that may explain why there is more than one type of signal, is based on studies performed by Andersson (1980), who proposed that animals produce more than one kind of threat signal due to the evolution of bluffing in the system. Originally, threat signals are reliable indicators of attack, but if cheaters evolve that use the display without the intention of attacking, the signal can lose efficiency. Novel signals may then arise, that are more reliable indicators of attack, and the cycle continues. Competition will arise between signals, and both may persist due to frequency-dependent selection. Andersson (1980) also admits that since the evolution of new displays cannot usually be directly observed or experimentally manipulated, it is difficult to directly test his hypothesis. For this reason, this hypothesis will not be tested in my study. Finally, multiple signal types

may be used to convey different types of information, and each signal may have a unique purpose or context (Maynard Smith & Harper, 2003). The information content of each signal type is difficult to assess with the current data, and will not be tested in this chapter.

### *Methods*

The following methods use data collected from conspecific interactions and vibration recordings as described in Chapter 2. All statistical comparisons used an alpha level of 0.05, and data were checked for normal distribution using the Shapiro-Wilk W test.

Hypothesis 1. Different signal types convey information about motivation - Prediction 1: Signal types will change over the course of an interaction

To test the hypothesis that different signal types convey information about motivation, dominant signal types at three stages of intruder approach (FAR, MID and CLOSE; see Chapter 2 for details) were calculated for each species that produced more than one type of signal (*D. arcuata*, *D. curvatula*, *D. falcataria*, *F. bilineata*, *Oreta rosea*, *Tetheela fluctuosa*, *Thyatira batis*, and *Watsonalla binaria*). Dominant signal types were calculated by taking the signal type with the highest rate/5 s at each stage of approach. These dominant signal types were then compared between stages of intruder approach within species to determine if signal type changes as the intruder approaches the resident.

Hypothesis 2. Different signal types are used to increase detection and recognition -

Prediction 1: Signal types will differ in spectral and temporal properties

Spectral and temporal properties, including dominant frequency, bandwidth at -3 dB and -10 dB, and duration were compared between mandible scraping, mandible drumming, anal scraping, lateral tremulation and buzzing within species (using only species that produced more than one signal and for which I collected LDV recordings) using ANOVAS or independent t-tests. *Post hoc* analyses were done using Tukey Kramer HSDs.

### *Results*

Hypothesis 1. Different signal types convey information about motivation - Prediction 1: Signal types will change over the course of an interaction. Table 6.2 demonstrates the dominant signal type per species that produce more than one type of signal at each stage of intruder approach. Dominant signal types differed between FAR, MID and CLOSE stages of approach in one out of eight of species (*D. arcuata*) that produced more than one type of signal (Table 2; see Chapter 2). Dominant signal types changed between FAR and MID in three out of eight species (*D. arcuata*, *D. curvatula*, and *F. bilineata*), and between MID and CLOSE in five out of eight species (*D. arcuata*, *D. curvatula*, and *F. bilineata*, *O. rosea*, and *T. batis*) (Table 6.2; see Chapter 2 and Appendix A). Dominant signal types did not change at all at different stages of intruder approach in three out of eight species (*D. falcataria*, *O. rosea*, and *W. cultraria*) (Table 6.2; see Chapter 2 and Appendix A).

Hypothesis 2. Different signal types are used to increase detection and recognition -

Prediction 1: Signal types will differ in spectral and temporal properties

Different signal types differed significantly in duration in all five species included in the analysis (*D. arcuata*, *D. curvatula*, *D. falcataria*, *O. rosea*, and *T. fluctuosa*), where lateral tremulation and anal scraping were significantly longer in duration than mandible drumming and mandible scraping, and mandible scraping was significantly longer in duration than mandible drumming. Signal types also significantly differed in dominant frequency in three species (*D. arcuata*, *O. rosea*, and *T. fluctuosa*), where anal scraping had a higher dominant frequency than mandible drumming and mandible scraping in *D. arcuata* (ANOVA,  $F = 4.37$ ,  $DF = 2$ ,  $p = 0.04$ ); mandible scraping had a lower dominant frequency than mandible drumming and lateral tremulation in *O. rosea* (ANOVA,  $F = 9.54$ ,  $DF = 2$ ,  $p = 0.003$ ); and mandible scraping had a higher dominant frequency than lateral tremulation in *O. duplaris* (two-tailed independent t-test,  $t = 17.11$ ,  $DF = 3.86$ ,  $p < 0.001$ ). Bandwidths at -3 dB and -10 dB differed significantly between signal types in two species (*O. rosea* and *T. fluctuosa*), with mandible scraping being the least broadband signal in *O. rosea*, and the most broadband in *T. fluctuosa*. See Figs. 4.5 and 5.2 for examples of signals.

### *Discussion*

The second question I asked in this chapter was: why produce more than one type of signal? Other researchers have proposed hypotheses to account for the presence of such multicomponent signals. Some of these hypotheses include: 1) different signals

**Table 6.2.** Dominant signal types at FAR, MID and CLOSE stages of intruder approach by species (only including species that produce more than one type of signal). AS = anal scraping; MD = mandible drumming; MS = mandible scraping; LT = lateral tremulation.

<b>Species</b>	<b>Stage of Approach</b>	<b>Dominant Signal Type</b>
<i>Drepana arcuata</i>	FAR MID CLOSE	MD AS MS
<i>D. curvatula</i>	FAR MID CLOSE	AS MD AS
<i>D. falcataria</i>	FAR MID CLOSE	AS AS AS
<i>Falcaria bilineata</i>	FAR MID CLOSE	MD AS MD
<i>O. rosea</i>	FAR MID CLOSE	NA MD MS
<i>Tetheela fluctuosa</i>	FAR MID CLOSE	MS MS MS
<i>Thyatira batis</i>	FAR MID CLOSE	NA LT AS
<i>Watsonalla cultraria</i>	FAR MID CLOSE	NA AS AS

convey information about motivation (Enquist & Leimar, 1983; Enquist & Leimar, 1987); 2) to increase the detection and recognition of the signal (Maynard Smith & Harper, 2003); 3) to counteract bluffing (Andersson, 1980); and 4) to convey different types of information (Maynard Smith & Harper, 2003). With the data I collected throughout my research, I was able to preliminarily test the first two hypotheses. If different signal types convey information about the motivation of the resident, then signal types should change over the course of an interaction, as presumably the motivation of the resident changes as the intruder approaches. My results demonstrate that, indeed, signal types changed in some species as the intruder approached the resident, although this trend was not observed in all species. This was perhaps due to low sample sizes in some species, and this prediction needs to be examined in more detail in future studies. Overall, there was also no one type of signal that was used more often at FAR, MID or CLOSE stages of intruder approach over all species.

The second hypothesis I tested was that different signal types enhance the detection and recognition of the signal by intruders. If this were so, signal types should differ in their temporal and spectral characteristics. Indeed, I found that signals did differ in temporal and spectral characteristics within species. This suggests that temporal characteristics (including duration) and spectral characteristics (including dominant frequency, and bandwidth at -3 dB and -10 dB) may play a role in signal detection and processing in these caterpillars, and having more than one signal type may act to increase redundancy in the system to ensure that receivers detect and recognize the overall message.

#### ***6.4 Why signal instead of using physical aggression?***

##### *Background*

Why do some species use mostly physically aggressive behaviours, while others use ritualized signals during encounters with conspecifics? Fighting can be costly in terms of time and energy, and can sometimes lead to serious injury and death (Harper, 1991). Whether to settle a contest with fighting or signalling depends on a variety of factors, namely the value of the resource and the costs of fighting (Harper, 1991). Kin-selection can also play a role in the decision to be physically aggressive. Reducing hostility and physical aggression towards related individuals is believed to enhance an individual's inclusive fitness (Hamilton, 1964), and many studies have found a decrease in physical aggression towards siblings and other related individuals. For example, Markman et al. (2009) demonstrated that aggression and associated injuries decreased as genetic similarity increased among groups of fire salamander (*Salamandra atra*) larvae. Dobler and Kölliker (2009) also found that unrelated individuals were cannibalized earlier and more often than related individuals in nest-mates of the European earwig (*Forficula auricularia*). In the Drepanidae, although kin recognition has not yet been studied, two life history characteristics, egg laying behaviour and gregariousness as early instars, may have an effect on the dispersal of siblings. If eggs are laid in rows instead of singly, we would expect that sibling larvae would not disperse as far, and the chances of encountering a sibling later in life would be high. The same principle can be applied to gregarious living as early instars. Some species maintain small groups (presumably siblings) as early instars, which then disperse as late instars. Again, the chances of encountering a sibling as a late instar would be higher for those

that lived in small groups as early instars. Based on this, I predict that species that lay eggs in rows and/or are gregarious as early instars will have a higher ratio of signalling to aggressive behaviour types as late instars. This will be tested by comparing egg-laying behaviour and gregariousness as early instars with the ratio of signal to aggressive behaviour types. The cost of physical aggression may also play an important role in the decision to be physically aggressive, where if the costs of physical aggression are high, the resident will signal more. Unfortunately, I was unable to properly assess the costs of physical aggression in Drepanidae caterpillars, and therefore this will not be tested in the current study.

### *Methods*

The following methods use data collected from conspecific interactions and general observations as described in Chapter 2. All statistical comparisons used an alpha level of 0.05, and data were checked for normal distribution using the Shapiro-Wilk W test.

Predictions 1 and 2. Species that lay eggs in rows, and/or are gregarious as early instars will have a higher ratio of signalling to aggressive behaviour

The ratio of signal to aggressive behaviour types was calculated per species by dividing the total number of signal types produced (mandible scraping, mandible drumming, anal scraping, lateral tremulation and buzzing) by the total number of aggressive behaviours (crawling towards intruder, pushing, lateral head hitting, lateral tail hitting and biting). This ratio was then compared to data on egg laying behaviour (laid

singly/in small groups or in rows), and gregariousness as early instars (solitary or gregarious) using independent t-tests to determine if there is a relationship between ratio of signalling to aggressive behaviour and either of these life history characteristics. The categories for eggs laid singly and in small groups had to be combined because many species, such as *O. rosea* were found to either lay eggs singly or in short rows.

### *Results*

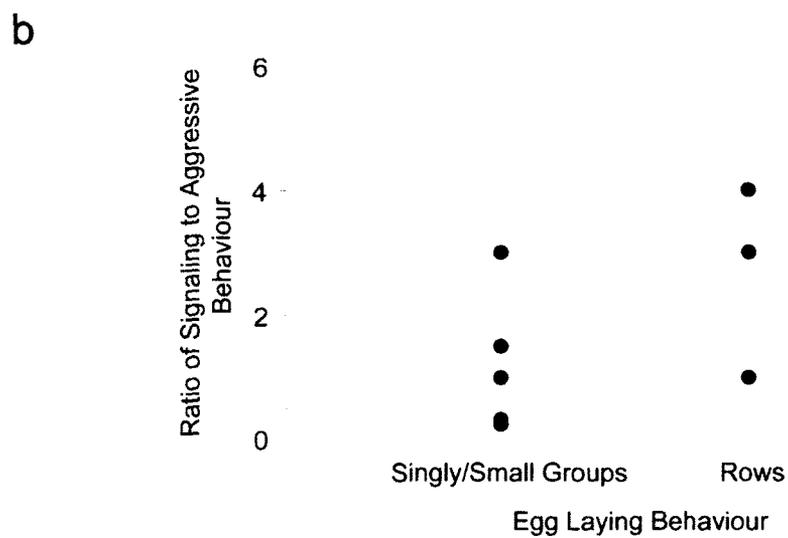
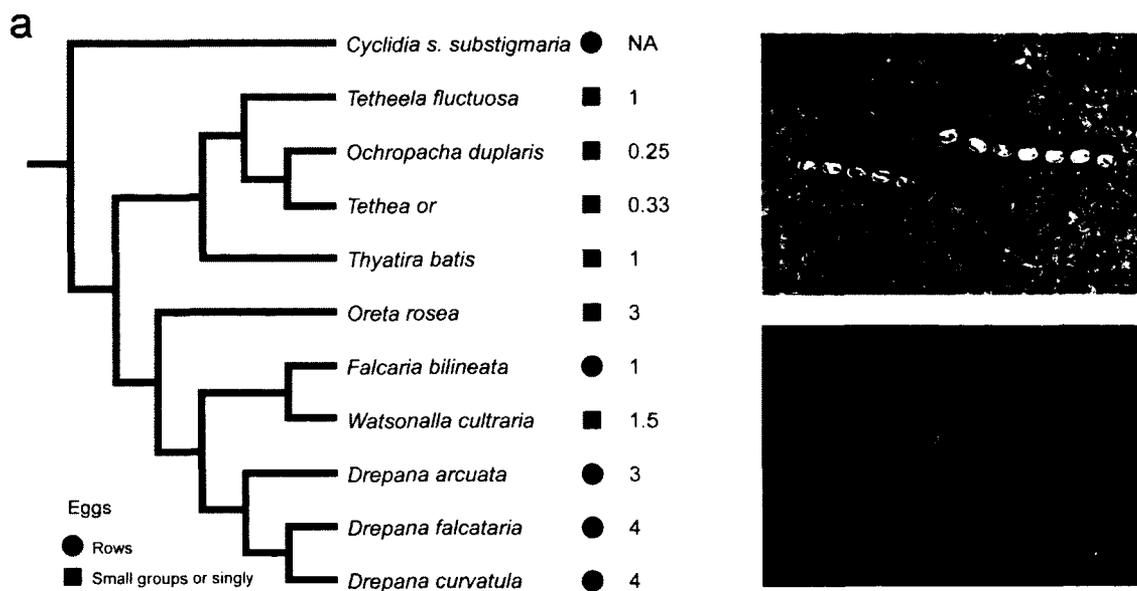
Predictions 1 and 2. Species that lay eggs in rows, and/or are gregarious as early instars will have a higher ratio of signals to aggressive behaviours.

There was no significant difference in ratio of signal to aggressive behaviour types between species that laid eggs in rows and those that laid them singly/in small groups (independent t-test, two-tailed;  $t = -2.22$ ,  $DF = 5.02$ ,  $p = 0.076$ ) (Fig. 6.3). My data do demonstrate, however, that in general, the Thyatirinae caterpillars lay eggs singly, and do not have large repertoires of signals. There was also no significant difference in ratio of signal to aggressive behaviours types between species that were gregarious as early instars and those that were solitary as early instars (independent t-test, two-tailed;  $t = -0.83$ ,  $DF = 7.12$ ,  $p = 0.43$ ) (Fig. 6.4).

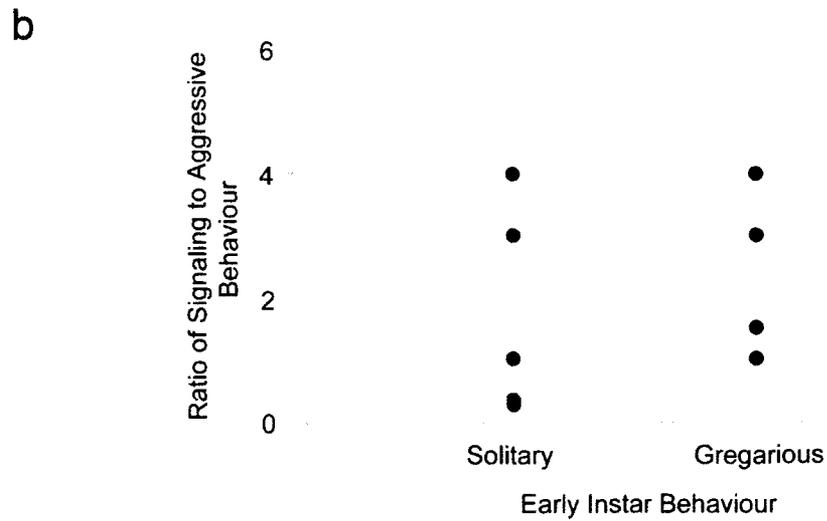
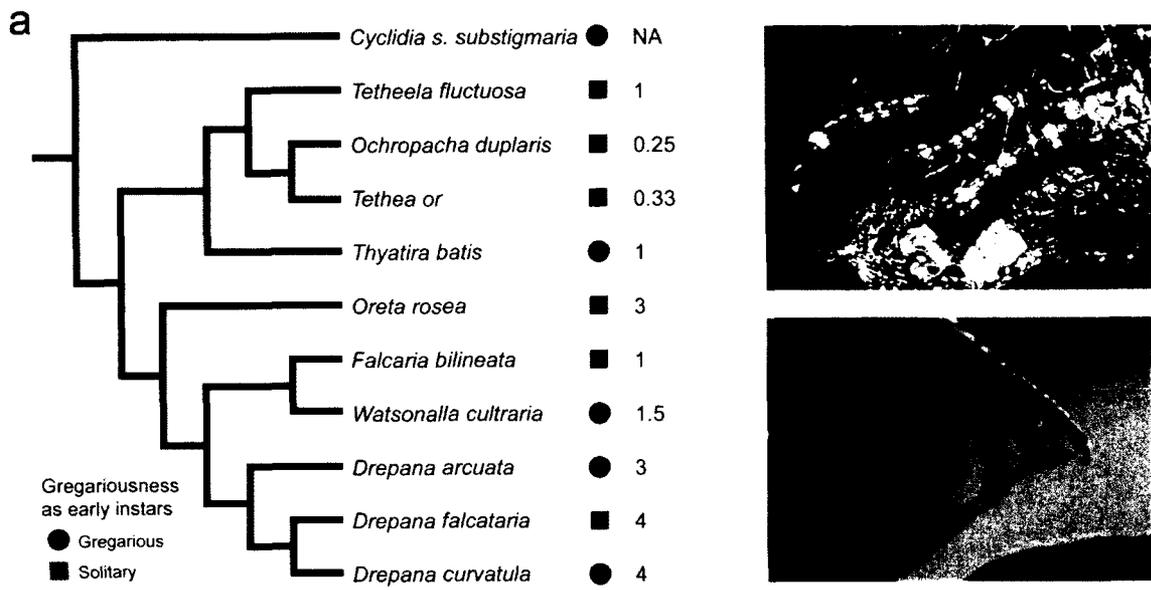
### *Discussion*

The final question I asked was: why signal instead of using physical aggression during encounters with conspecifics? I hypothesized that kin-selection and the costs of fighting may play a role in the decision to signal in the Drepanidae. To test my first hypothesis, I predicted that species of Drepanidae that lay eggs in groups and/or were

**Fig. 6.3.** The relationship between egg-laying behaviour and ratio of signalling to aggressive behaviour in late instars. **(a)** Phylogeny of taxa used in comparative analysis with data for ratio of signalling to aggressive behaviour, with photographs of eggs laid in rows (top; *D. arcuata*; scale bar = 3 mm; photo credit: J. Yack) and singly (bottom; *W. cultraria*; photo credit: ukleps.org; scale bar = 1 mm). **(b)** The relationship between egg-laying behaviour and ratio of signalling to aggressive behaviour.



**Fig. 6.4.** The relationship between gregariousness as early instars and ratio of signalling in late instars to aggressive behaviour. **(a)** Phylogeny of taxa used in comparative analysis with data for ratio of signalling to aggressive behaviour, with photographs of early instars living in groups (top; *D. arcuata*; scale bar = 1 mm; photo credit: J. Yack) and alone (bottom; *O. rosea*; scale bar = 3 mm). **(b)** The relationship between gregariousness as early instars and ratio of signalling to aggressive behaviour.



gregarious as early instars would have a higher ratio of signalling to aggressive behaviours due to low dispersal rates from sibling groups. I did not find any evidence to suggest that egg laying behaviour and/or gregariousness as early instars are related to the ratio of signal to aggressive behaviour type in these caterpillars. This finding contrasts with other studies that found a relationship between species-relatedness and reduced rates of aggression (e.g. Dobler & Kölliker, 2009; Markman et al., 2009). It is possible that Drepanidae larvae are able to disperse adequately from sibling groups after hatching, or after leaving early instar groups, and the probability of encountering a sibling later in life is no higher than encountering a non-sibling in species. More studies are needed to determine the dispersal abilities of larval Drepanidae in order to re-evaluate this hypothesis. My results do indicate that the Thyatirinae species generally have lower ratios of signalling to aggressive behaviours than do Drepaninae species. Further studies should look into other possible reasons for this difference between groups. My second hypothesis, that the cost of fighting affects whether to signal or use physical aggression, may provide a better explanation to answer the question, why signal instead of using physical aggression? Unfortunately because my data was not collected to specifically answer this question, I was unable to test this particular hypothesis. Future studies should collect more information on the costs of fighting, incorporating both the energetic costs and the frequency and severity of injury that results from encounters with conspecifics in order to explore this question in more detail.

### *General Summary*

In this chapter, I preliminarily examined three ultimate questions on the evolution of signalling in Drepanidae caterpillars (Table 6.3). First, I asked: what is the function of signalling? I provided evidence that vibratory signals in Drepanidae caterpillars function for territorial defense of leaf shelters, as they are produced primarily by the resident, are elicited by the approach of an intruder, show increasing signaling rates as the intruder approaches, and the resident signalling is followed by the intruder leaving the leaf. The second question I asked was: Why produce more than one type of signal? I demonstrated that producing more than one type of signal may function to alert the intruder of the motivation of the resident, or to ensure that the intruder detects and recognizes the signal. Finally, I asked: Why signal instead of using physical aggression? I provide evidence to suggest that kin-selection does not explain why these caterpillars signal instead of using physical aggression, and propose that future studies should focus on the costs of fighting.

**Table 6.3.** Summary of questions, hypotheses, predictions and findings in this chapter.

<b>Main Question</b>	<b>Hypothesis</b>	<b>Predictions</b>	<b>Supported by data?</b>
<b>What is the function of vibratory signalling?</b>	Signals function for territorial defense of leaf shelters/leaves	a) signals will be produced primarily by the resident of the leaf shelter/leaf b) signals will be elicited by the approach of the intruder c) signal rates will increase as the intruder approaches d) signalling will be followed by the resident leaving the leaf shelter/leaf e) residents with higher investments in the leaf shelter construction (from no shelter to silk mat to rolled leaf to two leaves sewn together), will have higher rates of signalling (and aggressive behaviours) and vice-versa	Y Y Y Y N
<b>Why more than one type of signal?</b>	1. Different signal types convey information about the motivation of the resident  2. Different signal types increase the detection and recognition of signals by intruders  3. Different signal types evolved to counteract bluffing  4. Different signal types convey different types of information	Signal types will change as the intruder approaches the resident  Signal types differ in spectral properties including bandwidth and peak frequency and temporal characteristics, including duration	Y  Y  Not tested  Not tested
<b>Why signal instead of using aggression?</b>	1. If the chance of encountering a sibling as a late instar is high, residents will produce more signals and be less aggressive towards intruders  2. The costs of aggressive behaviour affects the behaviour of the resident	a) Species that lay eggs in clusters will have a higher ratio of signals to aggressive behaviours than those that lay eggs singly  b) Species that are gregariousness as early instars will have a higher ratio of signalling to aggressive behaviour  If the costs of aggressive behaviour are high, residents will produce more signals than physically aggressive behaviours	N  N  Not tested

**CHAPTER 7**

**GENERAL SUMMARY AND CONCLUSIONS**

The overarching goal of this thesis was to study the evolutionary origins of communication signals from non-signalling behaviours in Drepanidae caterpillars. Biologists have been interested in the evolutionary origin of signals since the time of Darwin, yet there is currently little direct evidence to support hypotheses on the evolution of signals in other model systems. This may be because it is necessary to find a system with sufficient variation in the behaviour of interest, with known phylogeny between species. I provide experimental evidence on the origin of signals using Drepanidae caterpillars as a model system. Previously, vibratory signalling in Drepanidae larvae had only been experimentally examined in one species (*Drepana arcuata*; Yack et al., 2001), but there was indirect evidence suggesting that vibratory signalling is widespread and variable in this group (Dyar, 1884; Federley, 1905; Nakajima, 1970; Nakajima, 1972; Bryner, 1999; Sen & Lin, 2002; I. Hasenfuss, personal communication). The two major goals of my thesis were: 1) to test hypotheses on the non-signalling origins of vibratory signals in the Drepanidae; and 2) to provide general information on vibratory signalling in caterpillars, since little is known about this form of signalling in caterpillars to date.

In Chapter 2, I documented variation in morphology and behaviour associated with conspecific interactions in representative taxa of the Drepanidae. I collected morphological data for 19 species using specimens in alcohol, and behavioural data for 11 species using live specimens. I found variation in structures associated with vibratory signalling, including morphology associated with the anal proleg, caudal projection, PPI setae, and mandibles. Variation in behaviour associated with conspecific encounters included physical aggression without signalling (e.g. lateral head hitting, lateral tail hitting, pushing and biting) as well as vibratory signals (e.g. mandible drumming,

mandible scraping, anal scraping, lateral tremulation and buzzing), or a lack of territorial behaviour altogether. Information on the variation in morphology, movements, vibration properties and sequences of behaviour collected in this chapter was used in subsequent chapters to test hypotheses on signal origins and to propose models on the transition from aggressive behaviour to signalling.

In order to elucidate the evolutionary origins of a signal, it was necessary to understand the phylogenetic relationships between species to provide a framework onto which variation in morphology and behaviour could be mapped. The goal of Chapter 3 was to create a phylogeny of the Drepanoidea using molecular markers, as previous phylogenies of this group had been created using only morphological data (e.g. Minet, 1991; Minet & Scoble, 1999; Wu et al., 2009). In this chapter, I created a robust phylogeny using three genes (CAD, ND1 and 28S) onto which characters could be mapped to study the evolutionary origin of signalling. I confirmed that the Drepanoidea comprises two families, Drepanidae and Epicopeiidae, and that Drepanidae is further divided into three subfamilies, Drepanidae, Thyatirinae, and Cyclidiinae. Based on my results, I have also suggested that a third subfamily, Oretinae, be created, which concurs with other phylogenetic studies on the group.

Chapter 4 focused on testing hypotheses on the evolutionary origin of the anal scraping signal. I hypothesized that anal scraping derives from movements involved in crawling. This hypothesis was supported by the following lines of evidence: 1) crawling with fully formed prolegs and unmodified PP1 setae represented the basal condition when mapped onto the phylogeny; 2) kinematic analysis demonstrated that crawling and anal scraping involve similar movement patterns; 3) vibration analysis suggested that anal

scraping has more features of ritualization than crawling; and 4) aggressive crawling towards an intruder and anal scraping occurred in the same position in a typical sequence of behaviours between representative species. I also proposed two models for the evolutionary transition between crawling and anal scraping, one that focused on the evolutionary transition between behaviours and another that focused on the muscular and neural changes that may have accompanied this transition. I found two possible scenarios for the behavioural transition from crawling to anal scraping based on the results I collected. In the first scenario, general crawling transitioned to aggressively crawling towards the intruder followed by 'pseudo' anal scraping and finally by anal scraping. In the second scenario, general crawling transitioned to 'pseudo' anal scraping followed by either aggressive crawling or anal scraping. I also provided data to propose that the swing phase of crawling is homologous to the scrape phase of anal scraping, using the same sequence of muscle contractions.

Chapter 5 tested the hypothesis that mandible scraping derives from lateral head hitting. Evidence to support this hypothesis included: 1) lateral head hitting represented the basal condition when mapped onto the phylogeny; 2) kinematic analysis suggested that mandible scraping and lateral head hitting involve similar movement patterns; and 3) vibration analysis provided some evidence that mandible scraping shows more features of ritualization than lateral head hitting. I also mapped all other behaviours performed by the anterior body segments and compared behaviours based on characteristics of the movement and vibrations to propose a model for the evolutionary transition between behaviours.

Although my thesis research focused mainly on the proximate mechanisms of signal evolution, during the course of this study many interesting questions concerning ultimate mechanisms of signal evolution arose. I dedicated Chapter 6 to develop hypotheses to answer some of these questions, including: What is the function of signalling? Why produce more than one type of signal? Why signal instead of using physical aggression? Although the results of this chapter are preliminary, I was able to use my data set to provide some initial tests of hypotheses associated with each question. I provided evidence that vibratory signals in Drepanidae caterpillars function for territorial defense of leaf shelters or leaves, that these caterpillars produce more than one signal type possibly to increase the detection and recognition of the signal by intruders, and I suggest that future studies should focus on the costs of fighting to explain why these caterpillars use signals instead of physical aggression.

My research was the first to provide a robust molecular phylogeny of the Drepanoidea and to study the evolutionary origins of communication signals from non-signalling behaviours using a combination of behavioural, morphological and phylogenetic data. In addition, my results have contributed further information on the characteristics and function of vibratory signals in caterpillars. My thesis research has culminated in 4 published papers to date in *Nature Communications*, *Physiological Entomology*, *Journal of Insect Science*, and the *European Journal of Entomology*, as well as two manuscripts in preparation.

Future studies should focus on sampling more taxa for phylogenetic study in order to resolve some of the relationships within the Drepanoidea, including the placements of the Epicopeiidae and Oretini which would allow us to properly place these groups

phylogenetically. My research on the evolutionary origin of anal scraping would benefit from future studies that collect and compare electromyographic and neural data during crawling and anal scraping to provide further support for the hypothesis that anal scraping derives from crawling, and to explore the evolutionary changes to neural circuits. Another important question that still needs to be answered is how do these caterpillars detect and discriminate vibrations on the leaf. In Chapter 2, I examined the morphology of possible vibration receptors on the abdominal prolegs in these caterpillars, but to date it is unknown how vibration reception works in lepidopteran larvae. This would be an avenue that would greatly benefit from future research. Finally, future research on the morphology and behaviour of more species included in the phylogeny would help to explain why variation exists in the territorial behaviour of these species, why they signal at all, and what factors are important in determining the territorial behavioural repertoires in this interesting group of caterpillars.

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## APPENDIX A: GENERAL LIFE-HISTORY, MORPHOLOGY AND BEHAVIOUR OF ADDITIONAL DREPANIDAE SPECIES

This appendix presents information on the general life-history, morphological and behavioural characters of other species that I was able to study during the course of my thesis. Information from this appendix was used to test hypotheses related to the origin of signalling (Chapters 4 and 5), to help begin to answer ultimate questions on the evolution of signalling in the Drepanidae, and to provide general information on vibratory signalling in caterpillars.

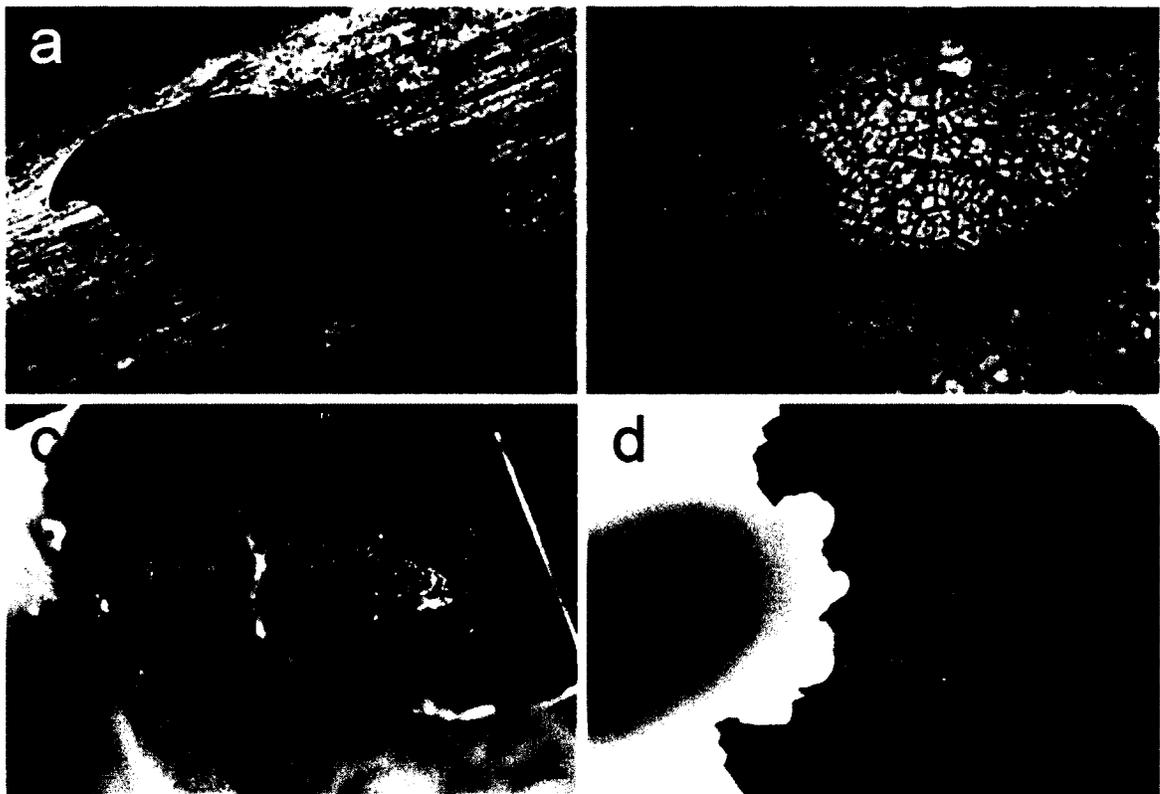
### *Live specimens*

#### *Drepana curvatula (Drepaninae)*

##### General life history observations relevant to conspecific interactions

Previous observations (summarized in Table 2.2) have shown that adult females of the dusky hook-tip moth, *Drepana curvatula* Borkhausen 1790 (Fig. A.1a) oviposit eggs in rows, where the eggs are touching and covered with scales or hairs from the female, with up to a dozen eggs in a row (Bryner, 1999; Fig. A.1b). Eggs are oviposited on species of birch (*Betula* spp.), alder (*Alnus* spp.), and sometimes oak (*Quercus* spp.) or willow (*Salix* spp.; Bryner, 1999). Early instars live in small leaf shelters either solitarily or in small groups (Bryner, 1999; Fig. A.1c,d). Late instars live solitarily in shelters made by rolling the leaf and securing it with 2 or 3 strands of silk (Bryner, 1999; Fig. A.1e,f).

**Fig. A.1.** Photographs demonstrating life-history characteristics relevant to territorial behaviour in the dusky hook-tip moth, *Drepana curvatula*. **(a)** Dorsal view of an adult moth in resting position (scale = unknown; photo credit: [www.leps.nl](http://www.leps.nl)). **(b)** Group of early instar caterpillars on a skeletonized feeding spot (scale = unknown; photo credit: [www.leps.nl](http://www.leps.nl)). **(c)** Lateral view of a late instar caterpillar (scale bar = 5 mm; photo credit: J. Yack). **(f)** Late instar caterpillar in a folded leaf-shelter (scale bar = 2.5 cm; photo credit: J. Yack).



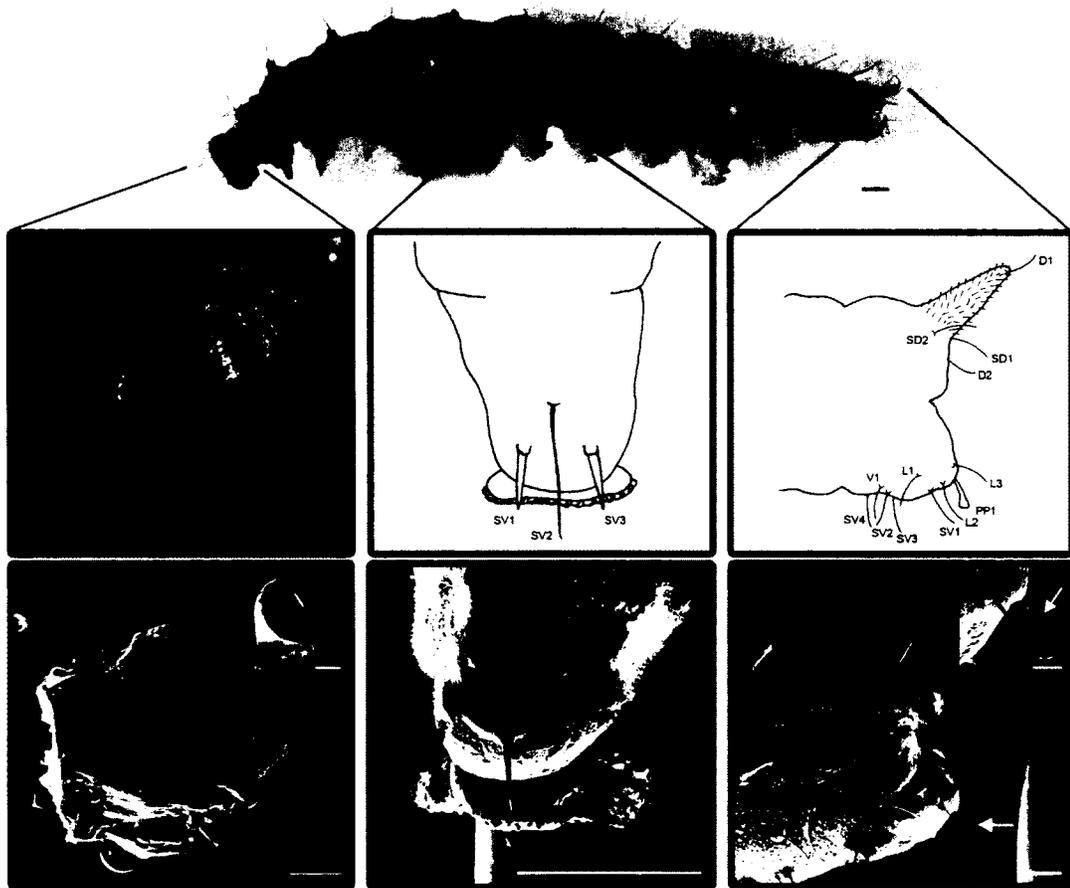
## Morphology

The head capsule of late instar larvae is not flattened dorsally (Fig. A.2a,b). Mandibles have four rounded distal teeth on the incisor area and no ridges on the oral surface (Fig. A.2c). The abdominal prolegs (excluding the anal prolegs) bear three setae on the outer planta region, where SV1 and SV3 are modified (Fig. A.2d,e). Larvae do not possess prolegs on the terminal abdominal segment and the anal segment has a short fleshy caudal projection (Fig. A.2a,f,g). Larvae possess a modified PP1 seta on the anal segment, and no other modified setae on this segment (Fig. A.2f,g). Morphological characters are summarized in Table 2.3.

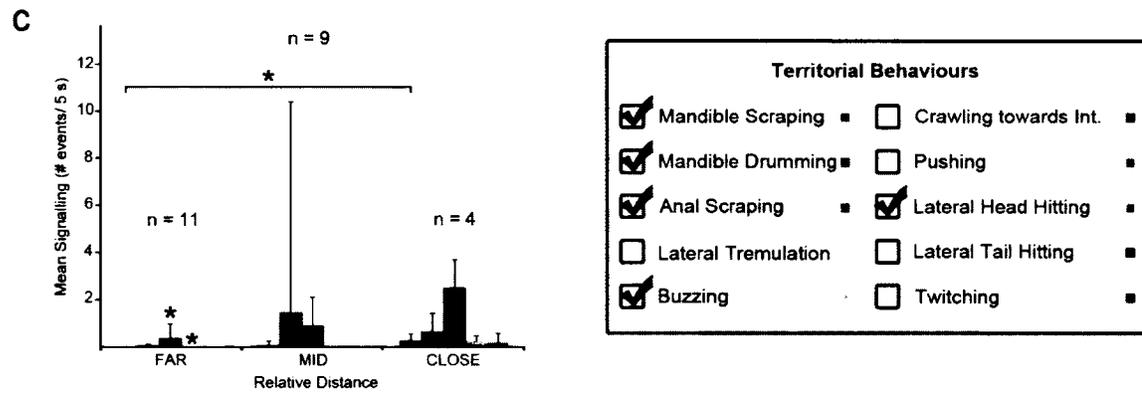
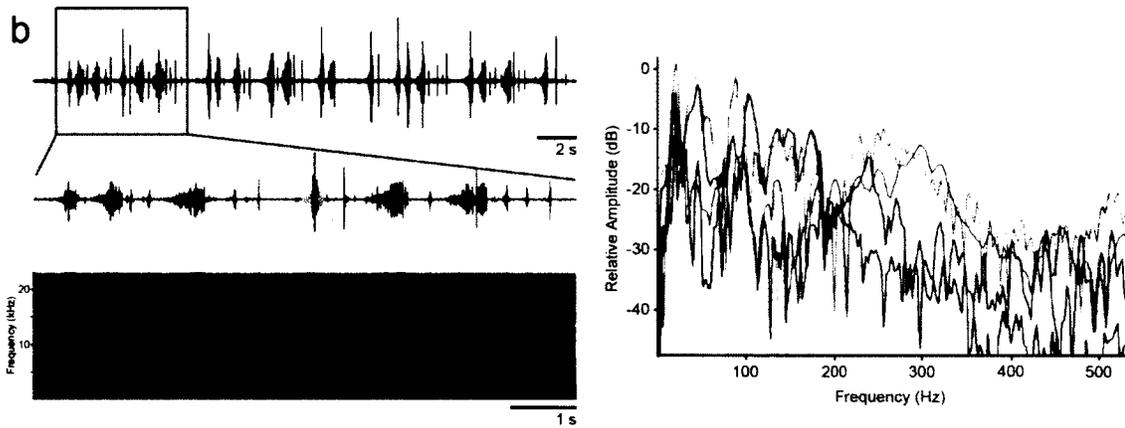
## Behavioural trials between conspecifics

Details on encounters with conspecifics are summarized in Table 2.4. A total of 11 encounters were staged between a resident and an intruder of similar size. Residents produced five types of behaviours during encounters, including mandible scraping, mandible drumming, anal scraping, buzzing, and lateral head hitting (Fig. A.3). Residents won 60.0 % of the trials, intruders won 0 % and 40.0 % of the trials were ties. Residents were silent until they detected an intruder, and signaled at a latency of  $84.1 \pm 131.7$  s ( $n = 9$ ) from the beginning of the trial (Fig. A.3a). The rate of resident behaviours, including mandible scraping, anal scraping and buzzing changed significantly as the intruder approached the resident (Fig. A.3b; see Table A.1 for details). Intruders signaled first in 2 out of the 11 trials, and signaled in all but 3 trials.

**Fig A.2.** Morphological characters related to territorial behaviour in *Drepana curvatula*.  
**(a)** Lateral view of the whole caterpillar (scale bar = 1 mm). **(b)** Anterior view of the head capsule (scale bar = 1 mm). **(c)** SEMs of lateral and ventral (inset) views of the mandibles (scale bars = 200  $\mu$ m). **(d)** Drawing of a lateral view of the proleg on the third abdominal segment (A3) **(e)** SEM of a lateral view of the proleg on A3 (scale bar = 0.5 mm). **(f)** Drawing of a lateral view the terminal abdominal segment (A10) with named setae. **(g)** SEM of a posterior view of A10 showing the location of the PP1 seta (arrow) (scale bar = 0.5 mm) with a close-up of the PP1 seta (inset; arrow) (scale bar = 100  $\mu$ m).



**Fig. A.3.** Vibration characteristics and territorial behaviour in *Drepana curvatula*. **(a)** Laser trace of an entire behavioural trial with corresponding video frames below. Numbers correspond in both the trace and the video frames, illustrating the approach of the intruder (1 = FAR, 2 = MID, 3 = CLOSE, 4 = Intruder leaves, F = First resident signal; scale bar = 1.5 cm). **(b)** Laser vibrometer trace illustrating a series of bouts, with an enlargement of single bout and corresponding spectrogram below. Power spectra demonstrating the dominant frequencies of each vibration (right panel) **(c)** Mean (+SD) behavioural rates of residents at three stages of intruder approach (FAR, MID, CLOSE). Asterisks denote significant differences within each behaviour at different stages of intruder approach. All colours throughout the figure correspond to those in the box describing territorial behaviours.



## Analysis of vibrations

Vibrations are associated with five behaviours in late instar larvae during conspecific interactions - mandible scraping, mandible drumming, anal scraping, buzzing, and lateral head hitting (Fig. A.3c). Details on temporal and spectral characteristics of vibrations are summarized in Tables A.1. Vibrations and movements were similar to those described in other species in Chapter 2.

## *Drepana falcataria* (*Drepaninae*)

### General life history observations relevant to conspecific interactions

Previous observations (summarized in Table 2.2) have shown that adult females of the pebble hook-tip moth, *Drepana falcataria* Linnaeus 1758 (Fig. A.4a) in rows, on species of birch, alder, oak, willow or poplar (Bryner, 1999; Fig. A.4b). Upon hatching, early instars live solitarily or in small groups in shelters made by rolling leaves and sealing them with silk (Bryner, 1999; Fig. A.4c,d). Late instars live solitarily in more open shelters, secured with only a few threads of silk (Bryner, 1999; Fig. A.4e,f). When disturbed, larvae produce a "tic-tac" noise by drumming with their head and thorax against the side of their nest (Bryner, 1999).

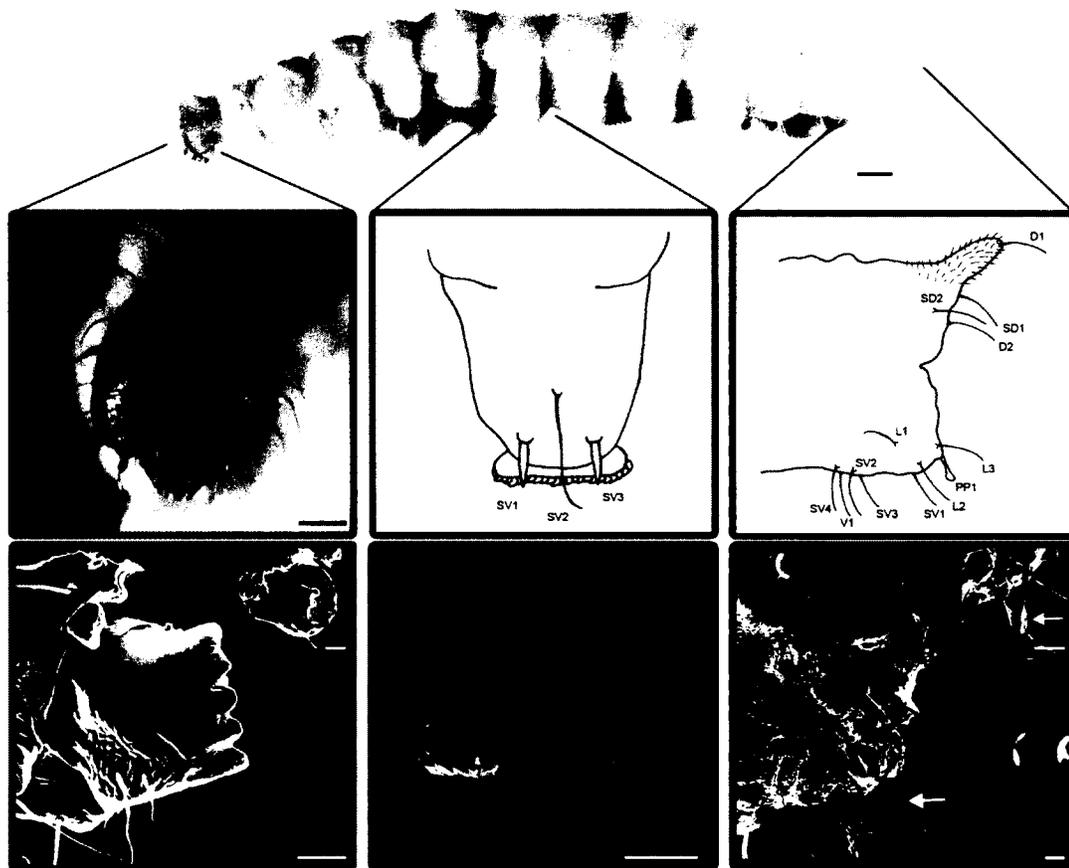
## Morphology

The head capsule of late instar larvae is not flattened dorsally (Fig. A.5a,b). Mandibles have five distal teeth on the incisor area and two ridges on the oral surface (Fig. A.5c). The abdominal prolegs (excluding the anal prolegs) bear three setae on the outer planta region, where SV1 and SV3 are modified (Fig. A.5d,e). Larvae do not

**Fig A.4.** Photographs demonstrating life-history characteristics relevant to territorial behaviour in the pebble hook-tip moth, *Drepana falcataria*. **(a)** Lateral view of an adult moth in resting position (scale bar = 2 cm). **(b)** Eggs covered in scales laid in a small group (scale bar = 2 mm). **(c)** Dorsal view of an early instar larvae (scale bar = 4 mm). **(d)** Solitary early instar caterpillar in a leaf shelter (scale bar = 3 mm). **(e)** Lateral view of a late instar caterpillar (scale bar = 5 mm). **(f)** Late instar caterpillar in a rolled leaf shelter (scale bar = 1.5 cm).



**Fig. A.5.** Morphological characters related to territorial behaviour in *Drepana falcataria*. **(a)** Lateral view of the whole caterpillar (scale bar = 1 mm). **(b)** Anterior view of the head capsule (scale bar = 0.5 mm). **(c)** SEMs of lateral and ventral (inset) views of the mandibles (scale bars = 100  $\mu\text{m}$ ). **(d)** Drawing of a lateral view of the proleg on the third abdominal segment (A3) **(e)** SEM of a lateral view of the proleg on A3 (scale bar = 200  $\mu\text{m}$ ; photo credit: T. Nevills). **(f)** Drawing of a lateral view the terminal abdominal segment (A10) with named setae. **(g)** SEM of a posterior view of A10 showing the location of the PP1 seta (arrow) with a close-up of the PP1 seta (inset; arrow) (scale bars = 100  $\mu\text{m}$ ).



possess prolegs on the terminal abdominal segment and the anal segment has a short fleshy caudal projection (Fig. A.5a,f,g). Larvae possess a modified PP1 seta on the anal segment, and no other modified setae on this segment (Fig. A.5f,g). Morphological characters are summarized in Table 2.3.

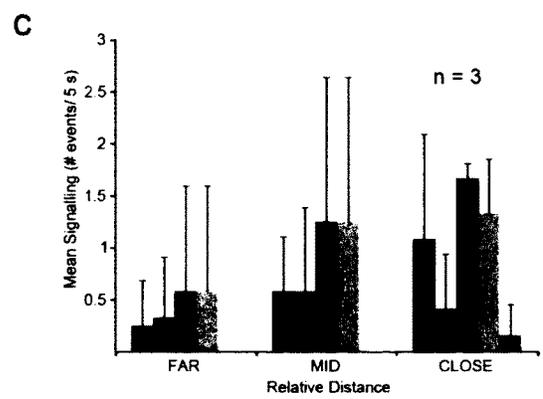
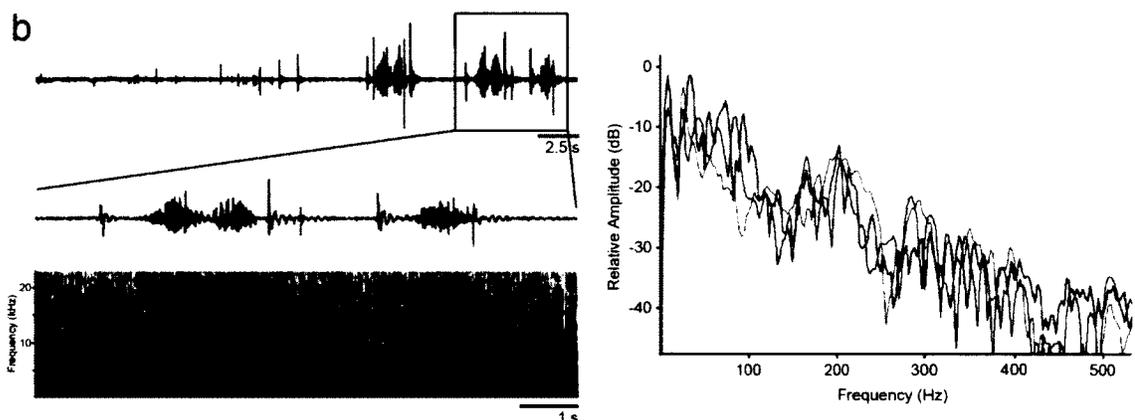
#### Behavioural trials between conspecifics

Details on encounters with conspecifics are summarized in Table 2.4. A total of 3 encounters were staged between a resident and an intruder of similar size. Residents produced five types of behaviours during encounters, including mandible scraping, mandible drumming, anal scraping, buzzing, and lateral head hitting (Fig. A.6). Residents won 100% of the trials and were silent until they detected an intruder (Fig. A.6a). The rate of resident behaviours, including mandible scraping, mandible drumming, anal scraping and buzzing changed as the intruder approached the resident, however none of these changes were significant (Fig. A.6b; see Table A.1 for details). Intruders signaled in one of the three trials.

#### Analysis of vibrations

Vibrations are associated with five behaviours in late instar larvae during conspecific interactions - mandible scraping, mandible drumming, anal scraping, buzzing, and lateral head hitting (Fig. A.6c). Details on temporal and spectral characteristics of vibrations are summarized in Table A.1. Vibrations and movements were similar to those described in other species in Chapter 2.

**Fig. A.6.** Vibration characteristics and territorial behaviour in *Drepana falcataria*. **(a)** Laser trace of an entire behavioural trial with corresponding video frames below. Numbers correspond in both the trace and the video frames, illustrating the approach of the intruder (1 = FAR, 2 = MID, 3 = CLOSE, 4 = Intruder leaves, F = First resident signal; scale bar = 1.5 mm). **(b)** Laser vibrometer trace illustrating a series of bouts, with an enlargement of single bout and corresponding spectrogram below. Power spectra demonstrating the dominant frequencies of each vibration (right panel) **(c)** Mean (+SD) behavioural rates of residents at three stages of intruder approach (FAR, MID, CLOSE). Asterisks denote significant differences within each behaviour at different stages of intruder approach. All colours throughout the figure correspond to those in the box describing territorial behaviours.



Territorial Behaviours			
<input checked="" type="checkbox"/> Mandible Scraping	<input type="checkbox"/> Crawling towards Int.	<input type="checkbox"/>	<input type="checkbox"/>
<input checked="" type="checkbox"/> Mandible Drumming	<input type="checkbox"/> Pushing	<input type="checkbox"/>	<input type="checkbox"/>
<input checked="" type="checkbox"/> Anal Scraping	<input checked="" type="checkbox"/> Lateral Head Hitting	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/> Lateral Tremulation	<input type="checkbox"/> Lateral Tail Hitting	<input type="checkbox"/>	<input type="checkbox"/>
<input checked="" type="checkbox"/> Buzzing	<input type="checkbox"/> Twitching	<input type="checkbox"/>	<input type="checkbox"/>

### *Falcaria bilineata* (*Drepaninae*)

#### General life history observations relevant to conspecific interactions

Personal observations (summarized in Table 2.2) have shown that adult females of the two-lined hooktip moth, *Falcaria bilineata* Packard 1864 (Fig. A.7a) lay eggs in rows of 2 – 10 on the upper leaf surface or on small twigs adjacent to a leaf (Fig. A.7b). Eggs are oviposited on species of birch (*Betula* spp.) or alder (*Alnus* spp). First and second instars occupy individual feeding regions at leaf edges where they skeletonize the upper leaf surface (Fig. A.7c,d). Late instars live solitarily on a silk mat (Fig. A.7e,f).

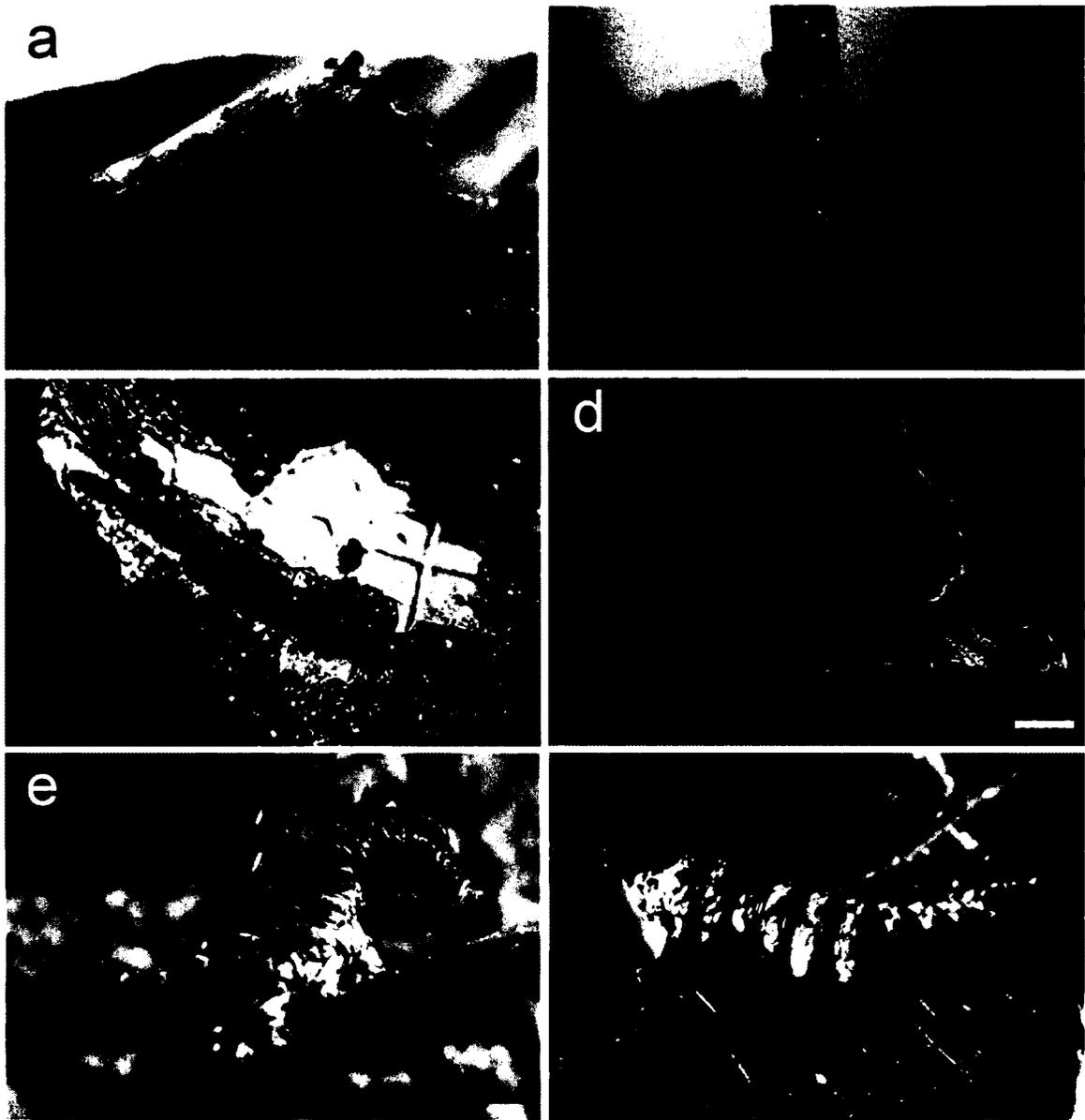
#### Morphology

The head capsule of late instar larvae is not flattened dorsally (Fig. A.8a,b). Mandibles have three distal teeth on the incisor area and two ridges on the oral surface (Fig. A.8c). The abdominal prolegs (excluding the anal prolegs) bear three setae on the outer planta region, where SV1 and SV3 are modified (Fig. A.8d,e). Larvae do not possess prolegs on the terminal abdominal segment and possess a short, fleshy caudal projection (Fig. A.8a,f,g). Larvae possess modified PP1 setae on the anal segment, and no other modified setae on this segment (Fig. A.8f,g). Morphological characters are summarized in Table 2.3.

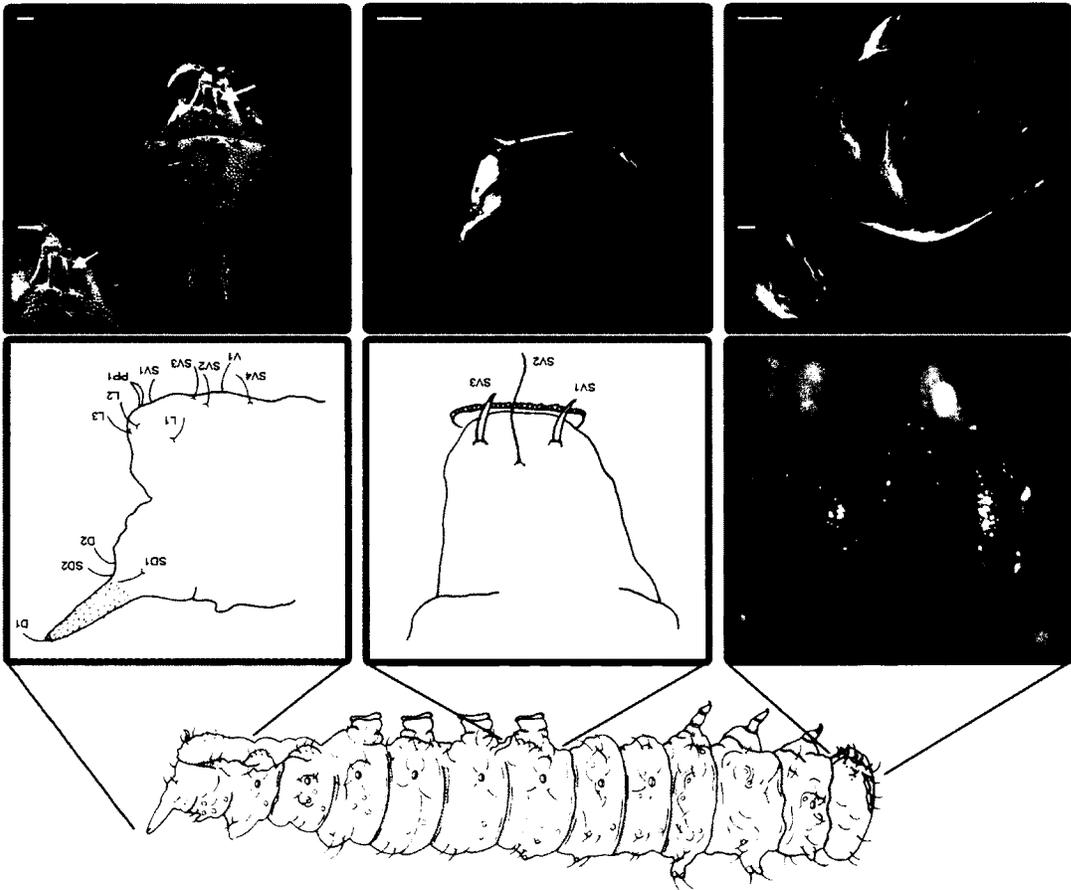
#### Behavioural trials between conspecifics

Details on encounters with conspecifics are summarized in Table 2.4. A total of 53 encounters were staged between a resident and an intruder of similar size. Residents produced four types of behaviours during encounters, including mandible drumming, anal

**Fig A.7.** Photographs demonstrating life-history characteristics relevant to territorial behaviour in the two-lined hook-tip moth, *Falcaria bilineata*. **(a)** Lateral view of an adult moth in resting position (scale bar = 3 mm; photo credit: J. Yack). **(b)** Row of eggs laid on a twig of *Betula papyrifera* (scale bar = 4 mm; photo credit: J. Yack). **(c)** Dorsal view of an early instar larvae (scale bar = 2 mm; photo credit: J. Yack). **(d)** Whole leaf view of a solitary early instar caterpillar on a skeletonized feeding spot (scale bar = 1 cm; photo credit: S. Matheson). **(e)** Lateral view of a late instar caterpillar in resting position (scale bar = 2 mm; photo credit: J. Yack). **(f)** Late instar caterpillar on a mat of silk (scale bar = 2.5 mm; photo credit: J. Yack).



**Fig. A.8.** Morphological characters related to territorial behaviour in *Falcaria bilineata*.  
**(a)** Lateral view of the whole caterpillar. **(b)** Anterior view of the head capsule (scale bar = 1 mm). **(c)** SEMs of lateral and ventral (inset) views of the mandibles (scale bars = 100  $\mu\text{m}$ ; photo credits: J. Yack). **(d)** Drawing of a lateral view of the proleg on the third abdominal segment (A3) **(e)** SEM of a lateral view of the proleg on A3 (scale bar = 100  $\mu\text{m}$ ). **(f)** Drawing of a lateral view the terminal abdominal segment (A10) with named setae. **(g)** SEM of a posterior view of A10 showing the location of the PP1 seta (arrow) with a close-up of the PP1 seta (inset; arrow) (scale bars = 100  $\mu\text{m}$ ; photo credits: J. Yack).

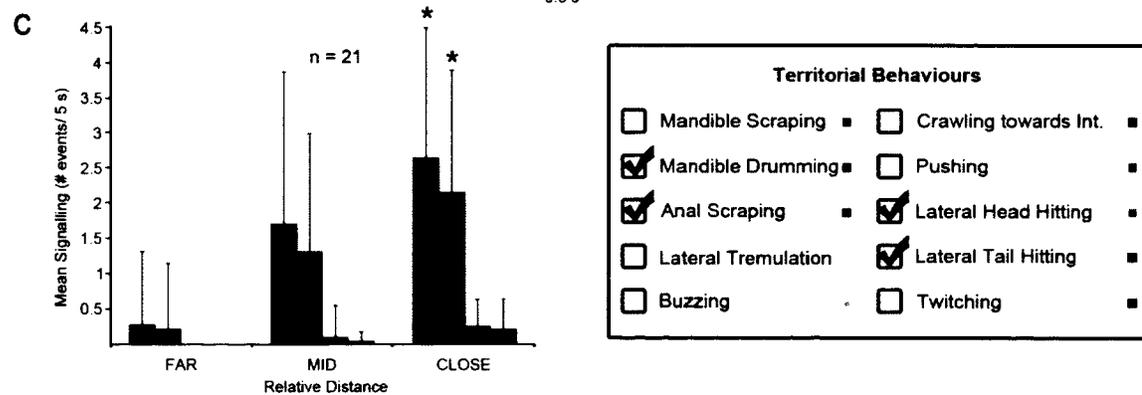
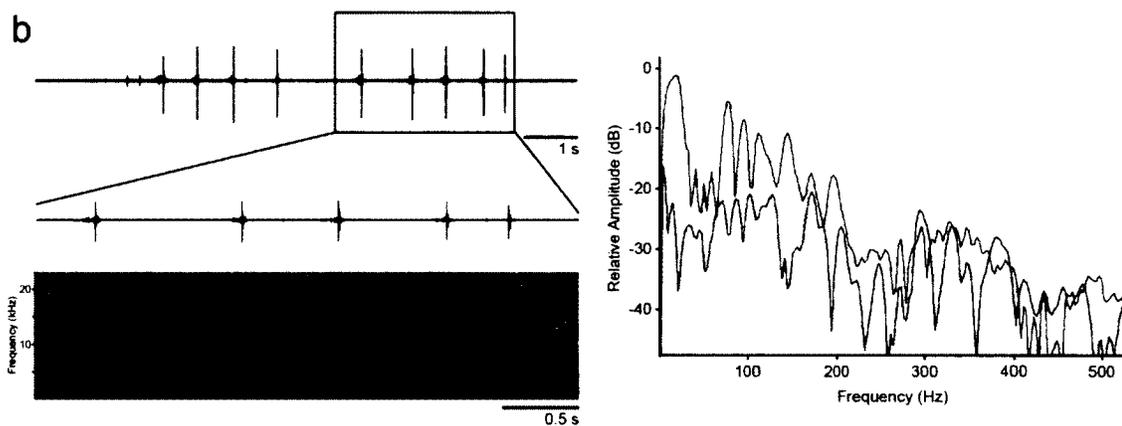
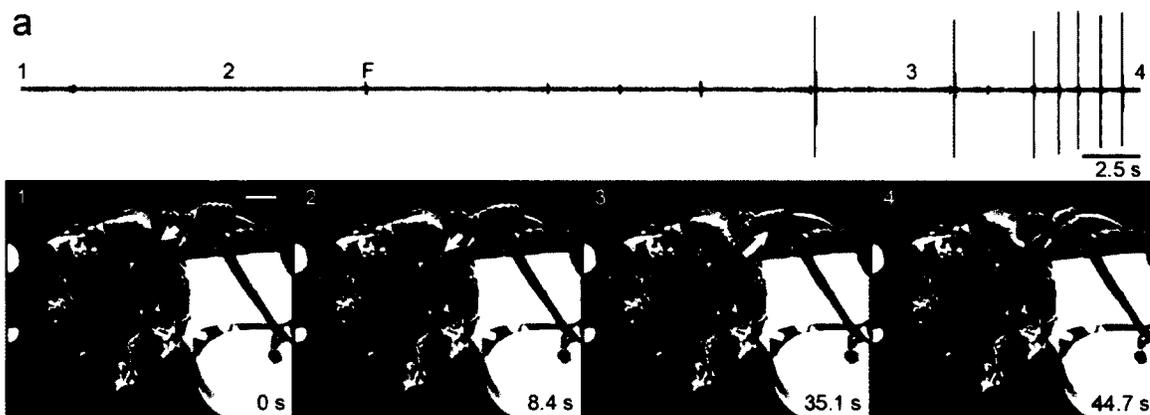


scraping, lateral head hitting, and lateral tail hitting (Fig. A.9). Signalling typically occurs in bouts, each comprising  $8.2 \pm 7.6$  complexes, and lasting  $6.4 \pm 10.2$  s ( $n = 18$  bouts from ten individuals). Each complex is  $223 \pm 315$  ms ( $n = 104$  complexes from ten individuals) in duration, and typically comprises one or two signals. When a mandible drum and anal scrape occur together, the anal scrape almost always precedes the mandible drum. Residents won 61.5% of the trials, intruders won 5.8% of the trials and 32.7% of the contests were ties. Residents were silent until they detected an intruder, and signaled at a latency of  $49.2 \pm 52.3$  s ( $n = 43$ ) from the beginning of the trial (Fig. A.9a). The rate of resident behaviours, including mandible drumming, anal scraping and lateral head hitting changed significantly as the intruder approached the resident (Fig. A.9b; see Table A.1 for details). Residents signalled overall more than intruders, signalling significantly more in the first 80 s (paired  $t$ -test:  $t = -5.066$ ,  $P < 0.001$ ,  $n = 20$ ) and last 80 s of each trial (paired  $t$ -test:  $t = -5.178$ ,  $P < 0.001$ ,  $n = 20$ ) and were the first to signal in 43 of the 52 trials.

#### Analysis of vibrations

Vibrations are associated with four behaviours in late instar larvae during conspecific interactions - mandible drumming, anal scraping, lateral head hitting, and lateral tail hitting (Fig. A.9c). Details on temporal and spectral characteristics of vibrations are summarized in Table A.1. Vibrations and movements were similar to those described in other species in Chapter 2.

**Fig. A.9.** Vibration characteristics and territorial behaviour in *Falcaria bilineata*. **(a)** Microphone trace of an entire behavioural trial with corresponding video frames below. Numbers correspond in both the trace and the video frames, illustrating the approach of the intruder (1 = FAR, 2 = MID, 3 = CLOSE, 4 = Intruder leaves, F = First resident signal; scale bar = 1.5 cm). **(b)** Microphone trace illustrating a series of bouts, with an enlargement of single bout and corresponding spectrogram below. Power spectra demonstrating the dominant frequencies of each vibration (right panel) **(c)** Mean (+SD) behavioural rates of residents at three stages of intruder approach (FAR, MID, CLOSE). Asterisks denote significant differences within each behaviour at different stages of intruder approach. All colours throughout the figure correspond to those in the box describing territorial behaviours.



## *Ochropacha duplaris* (*Thyatirinae*)

### General life history observations relevant to conspecific interactions

Previous observations (summarized in Table 2.2) have shown that adult females of the common lutestring, *Ochropacha duplaris* Linnaeus 1761 (Fig. A.10a) oviposit eggs singly or in groups of 2-3 on species of birch, alder, oak and poplar (Riegler, 1999; Fig. A.10b). Early instars live solitarily (Riegler, 1999; Fig. A.10c,d). Late instars live solitarily in shelters made by tying two leaves together with silk (Riegler, 1999; Fig. A.10e,f).

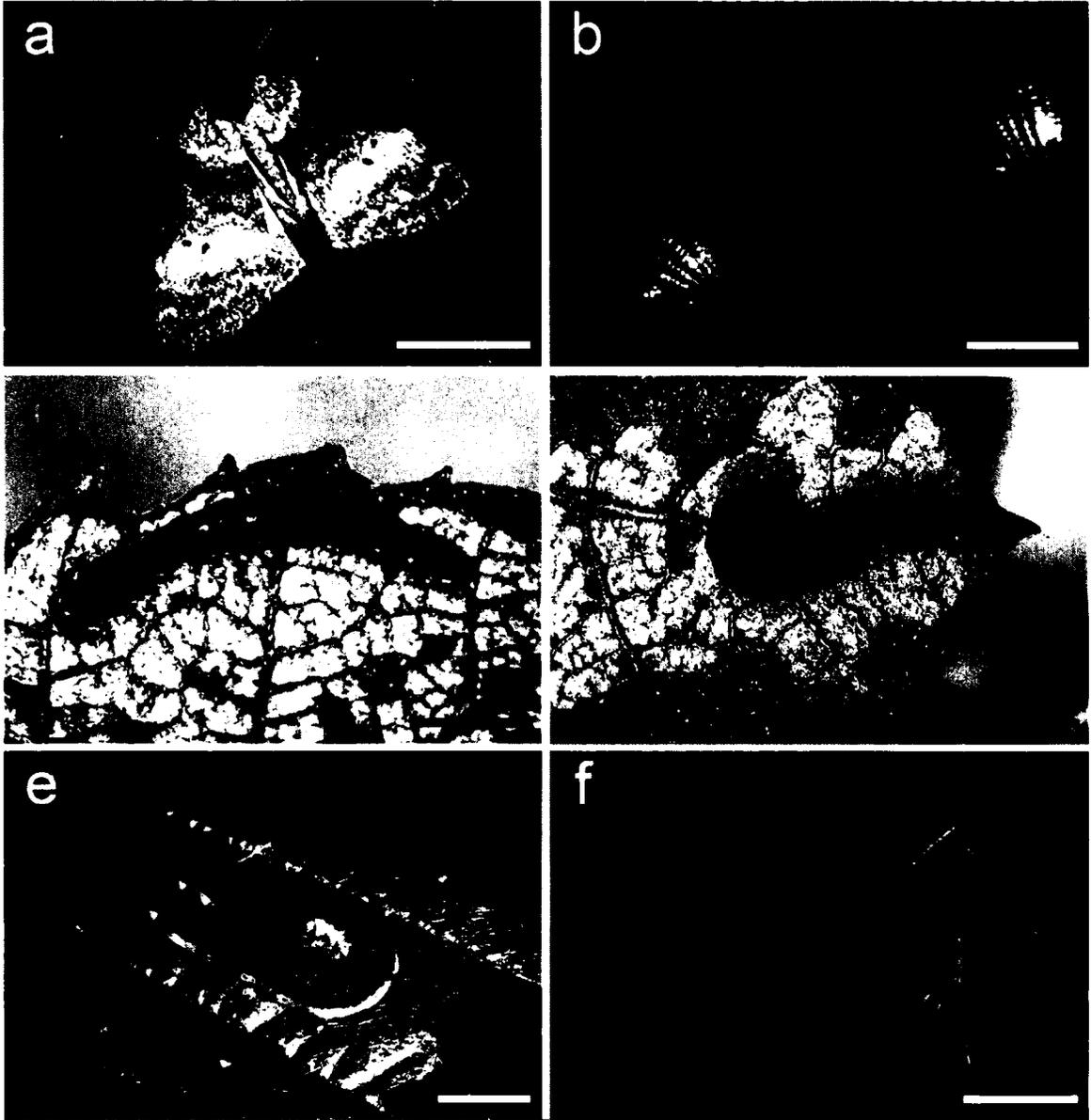
### Morphology

The head capsule of late instar larvae is not flattened dorsally (Fig. A.11a,b). Mandibles have six distal teeth on the incisor area and one ridge on the oral surface (Fig. A.11c). The abdominal prolegs (excluding the anal prolegs) bear three setae on the outer planta region, with no modifications (Fig. A.11d,e). Larvae possess reduced prolegs on the terminal abdominal segment that bear crochets (Fig. A.11a,f,g). Larvae possess no modified setae on the anal segment (Fig. A.11f,g). Morphological characters are summarized in Table 2.3.

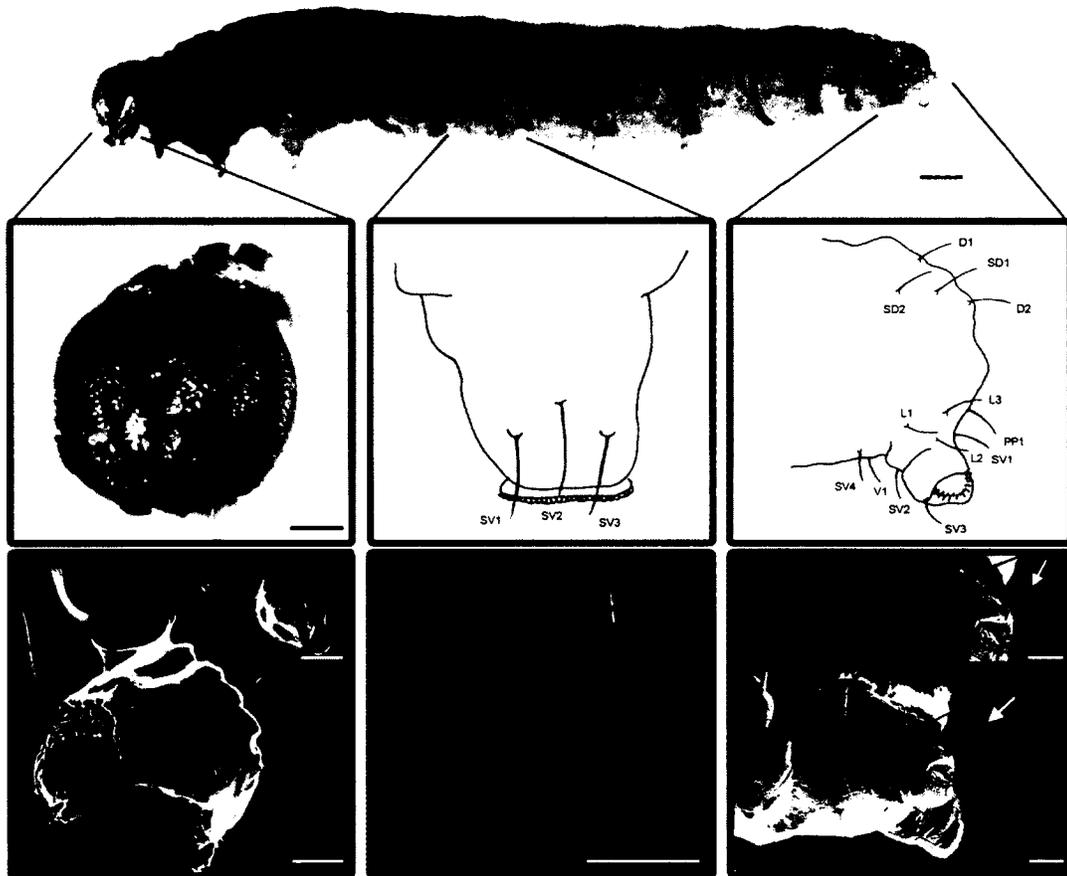
### Behavioural trials between conspecifics

Details on encounters with conspecifics are summarized in Table 2.4. A total of 6 encounters were staged between a resident and an intruder of similar size. Residents produced five types of behaviours during encounters, including mandible scraping, crawling towards the intruder, pushing, lateral head hitting and lateral tail hitting (Fig.

**Fig. A.10.** Photographs demonstrating life-history characteristics relevant to territorial behaviour in the common lutestring, *Ochropacha duplaris*. **(a)** Dorsal view of an adult moth in resting position (scale bar = 1.5 cm; photo credit: lepiforum.de). **(b)** Two eggs laid on a leaf (scale bar = 0.5 mm; photo credit: Karl Rasch, lepiforum.de). **(c)** Dorsal view of an early instar larvae (scale bar = 2 mm; photo credit: ukleps.org). **(d)** Solitary early instar caterpillar on a skeletonized feeding spot (scale bar = 2 mm; photo credit: ukleps.org). **(e)** Lateral view of a late instar caterpillar in resting position (scale bar = 8 mm). **(f)** Late instar caterpillar in a shelter made of two leaves (scale bar = 1 cm).



**Fig. A.11.** Morphological characters related to territorial behaviour in *Ochropacha duplaris*. **(a)** Lateral view of the whole caterpillar (scale bar = 1 mm). **(b)** Anterior view of the head capsule (scale bar = 0.5 mm). **(c)** SEMs of lateral and ventral (inset) views of the mandibles (scale bars = 200  $\mu\text{m}$ ). **(d)** Drawing of a lateral view of the proleg on the third abdominal segment (A3) **(e)** SEM of a lateral view of the proleg on A3 (scale bar = 200  $\mu\text{m}$ ; photo credit: T. Nevills). **(f)** Drawing of a lateral view the terminal abdominal segment (A10) with named setae. **(g)** SEM of a posterior view of A10 showing the location of the PP1 seta (arrow) with a close-up of the PP1 seta (inset; arrow) (scale bars = 200  $\mu\text{m}$ ).



A.12). Data on trial outcomes were not calculated as no full trials were recorded on camera. The rate of resident behaviours, including lateral head hitting changed significantly as the intruder approached the resident (Fig. A.12b; see Table A.1 for details). Intruders were never observed to signal ( $n = 6$ ).

### Analysis of vibrations

Vibrations are associated with five behaviours in late instar larvae during conspecific interactions - mandible scraping, crawling towards the intruder, pushing, lateral head hitting and lateral tail hitting (Fig. A.12c). Details on temporal and spectral characteristics of vibrations are summarized in Tables A.1. Vibrations and movements were similar to those described in other species in Chapter 2.

### *Tetheela fluctuosa* (*Thyatirinae*)

#### General life history observations relevant to conspecific interactions

Previous observations (summarized in Table 2.2) have shown that adult females of the satin carpet moth, *Tetheela fluctuosa* Hübner 1799-1804 (Fig. A.13a) oviposit eggs singly or rarely in pairs on the teeth of leaves of birch, alder, and poplar (Riegler, 1999; Fig. A.13b). Early instars live solitarily between two leaves, feeding at night (Newman, 1884; Riegler, 1999; Fig. A.13c,d). Late instars live solitarily in shelters constructed between two or more leaves or by folding a single leaf and securing it with (Newman, 1884; Riegler, 1999; Fig. A.13e,f).

**Fig. A.12.** Vibration characteristics and territorial behaviour in *Ochropacha duplaris*. **(a)**

Laser trace of an entire behavioural trial with corresponding video frames below.

Numbers correspond in both the trace and the video frames, illustrating the approach of the intruder (1 = FAR, 2 = MID, 3 = CLOSE, 4 = Intruder leaves, F = First resident

signal; scale bar = 8 mm). **(b)** Laser vibrometer trace illustrating a series of bouts, with an

enlargement of single bout and corresponding spectrogram below. Power spectra

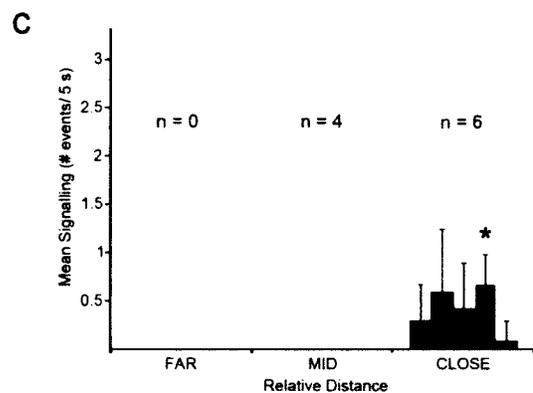
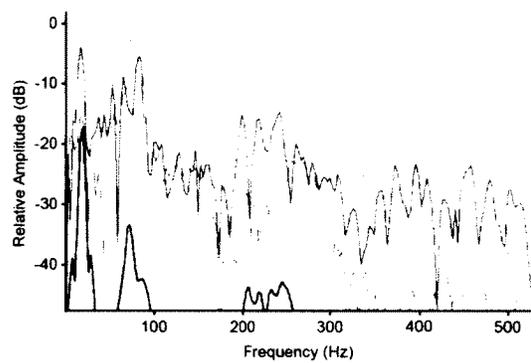
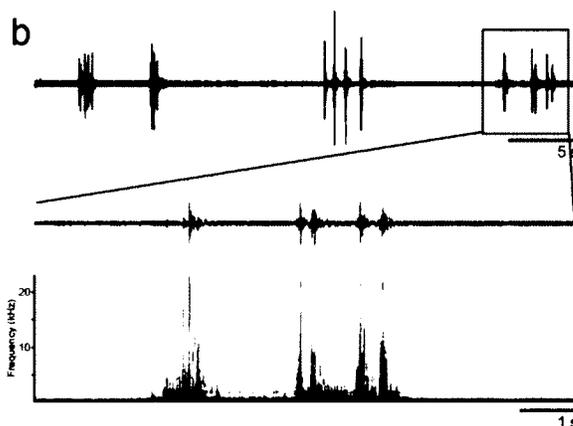
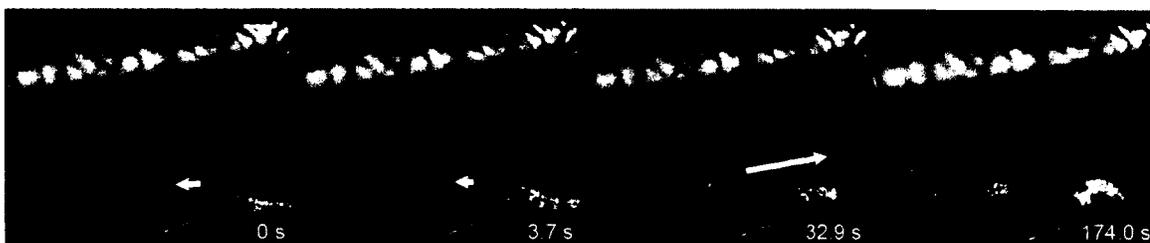
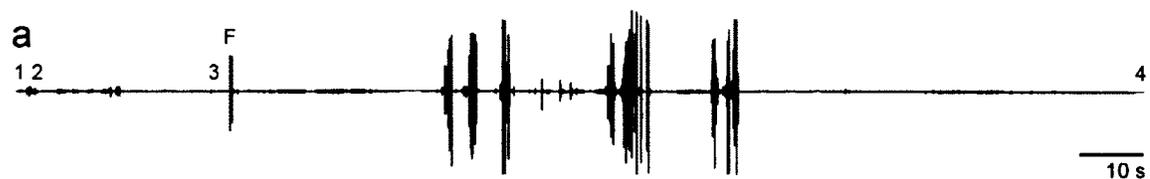
demonstrating the dominant frequencies of each vibration (right panel) **(c)** Mean (+SD)

behavioural rates of residents at three stages of intruder approach (FAR, MID, CLOSE).

Asterisks denote significant differences within each behaviour at different stages of

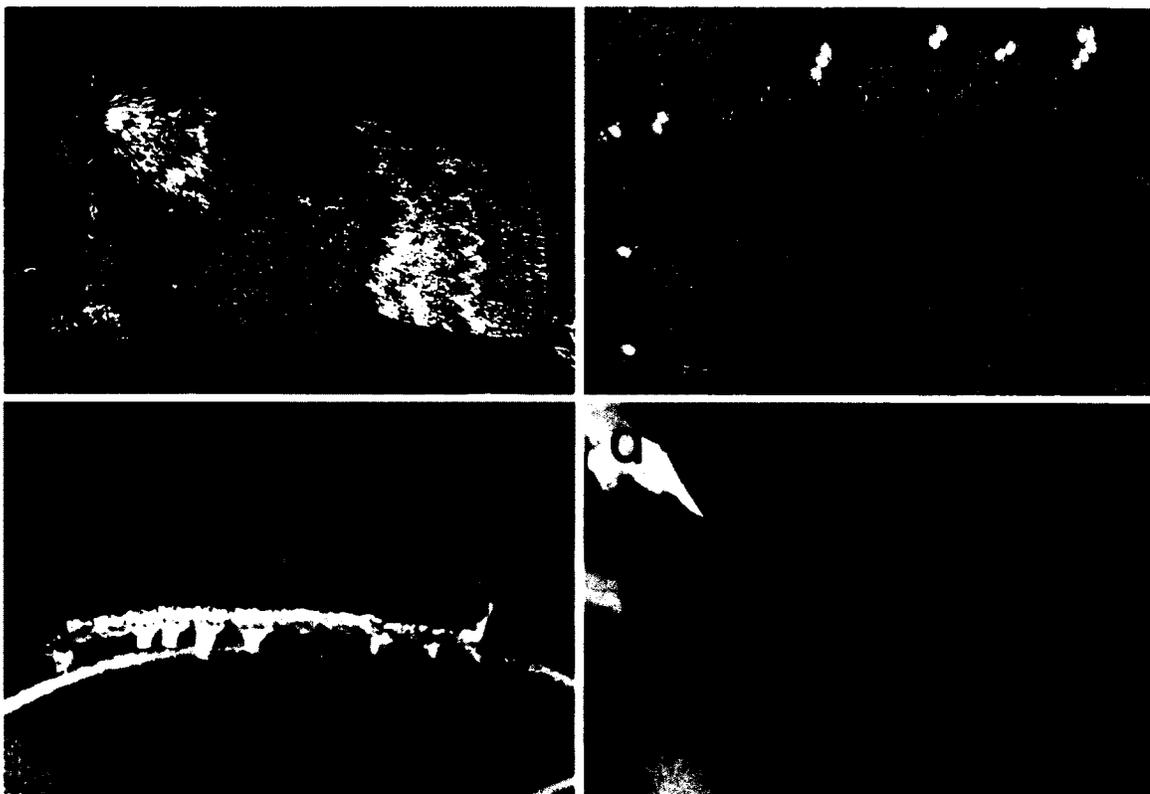
intruder approach. All colours throughout the figure correspond to those in the box

describing territorial behaviours.



- Territorial Behaviours**
- Mandible Scraping
  - Crawling towards Int.
  - Mandible Drumming
  - Pushing
  - Anal Scraping
  - Lateral Head Hitting
  - Lateral Tremulation
  - Lateral Tail Hitting
  - Buzzing
  - Twitching

**Fig. A.13.** Photographs demonstrating life-history characteristics relevant to territorial behaviour in the satin carpet moth, *Tetheela fluctuosa*. **(a)** Lateral view of an adult moth in resting position (scale bar = 7 mm; photo credit: Roy Leverton, ukleps.org). **(b)** Eggs laid singly or in pairs in the teeth of a leaf (scale bar = 5 mm; photo credit: Roy Leverton, ukleps.org). **(c)** Lateral view of a late instar larvae (scale bar = 5 mm). **(d)** Late instar caterpillar in a shelter made between two leaves (scale bar = 1.5 cm).



## Morphology

The head capsule of late instar larvae is not flattened dorsally (Fig. A.14a,b). Mandibles have six distal teeth on the incisor area and three ridges on the oral surface (Fig. A.14c). The abdominal prolegs (excluding the anal prolegs) bear three setae on the outer planta region, with no modifications (Fig. A.14d,e). Larvae possess reduced prolegs on the terminal abdominal segment that bear crochets (Fig. A.14a,f,g). Larvae possess modified PP1 setae on the anal segment and all other setae on this segment are normal to the group (Fig. A.14f,g). Morphological characters are summarized in Table 2.3.

## Behavioural trials between conspecifics

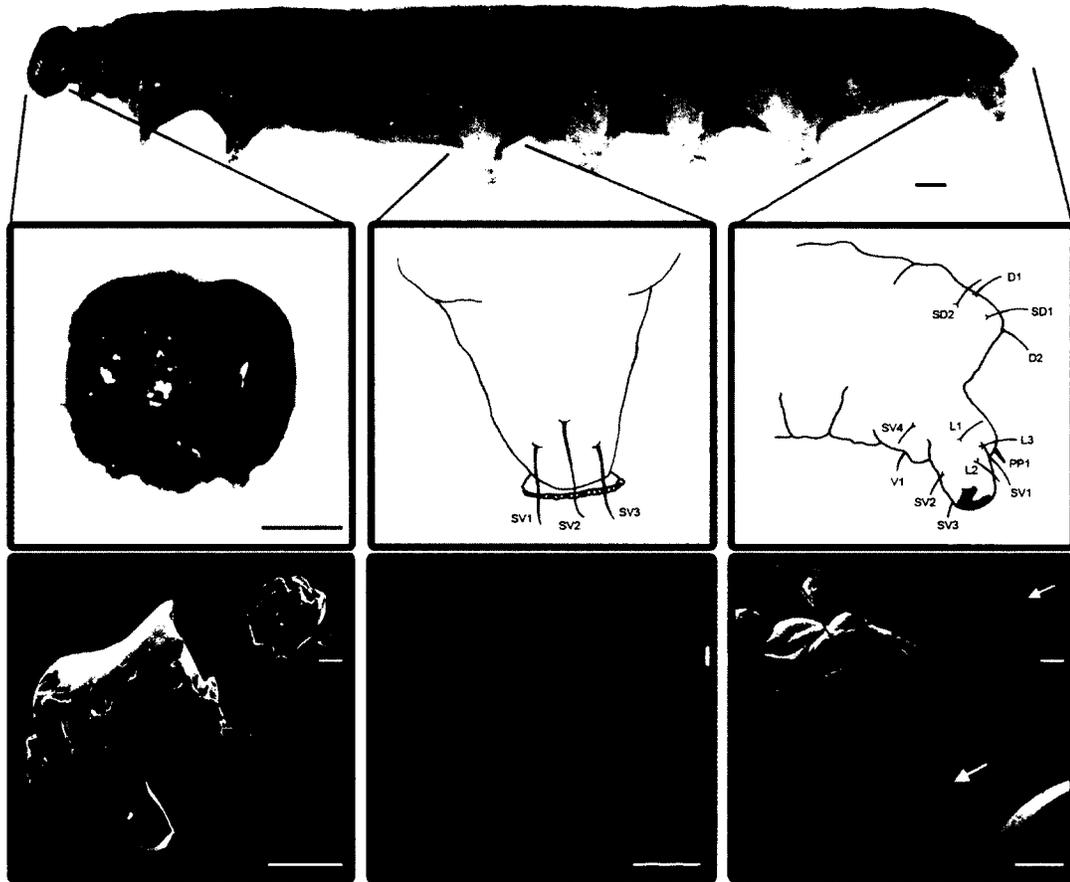
Details on encounters with conspecifics are summarized in Table 2.4. A total of 5 encounters were staged between a resident and an intruder of similar size. Residents produced six types of behaviours during encounters, including mandible scraping, lateral tremulation, anal scraping, crawling towards the intruder, lateral head hitting and lateral tail hitting (Fig. A.15). Residents won 100% of the trials ( $n = 1$ ). Residents were silent until they detected an intruder (Fig. A.15a). The rate of resident behaviours did not change significantly as the intruder approached the resident (Fig. A.15b). Intruders were never observed to signal ( $n = 5$ ).

## Analysis of vibrations

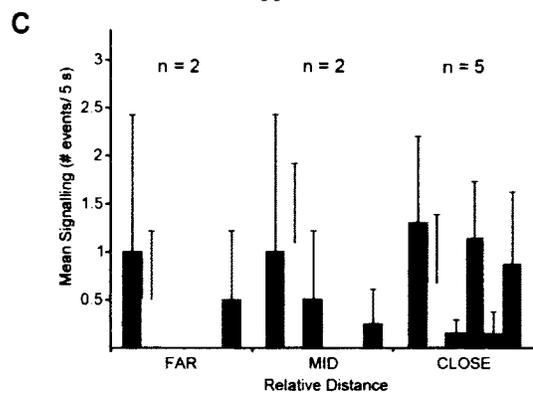
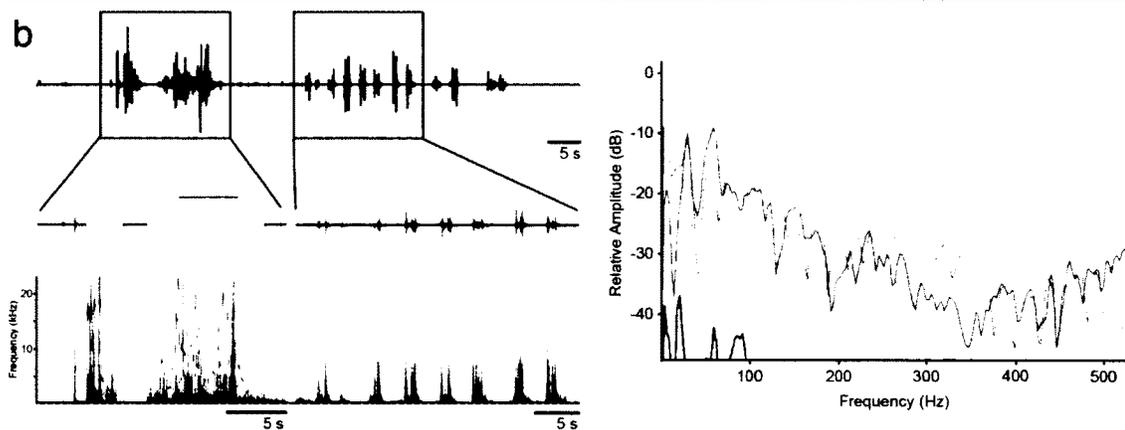
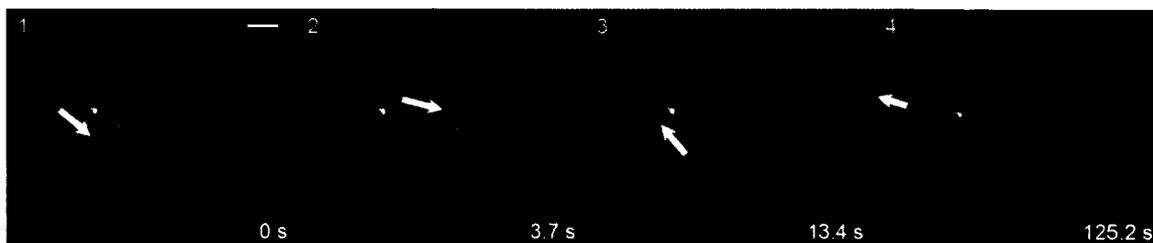
Vibrations are associated with six behaviours in late instar larvae during conspecific interactions - mandible scraping, lateral tremulation, anal scraping, crawling

**Fig. A.14.** Morphological characters related to territorial behaviour in *Tetheela fluctuosa*.

**(a)** Lateral view of the whole caterpillar (scale bar = 1 mm). **(b)** Anterior view of the head capsule (scale bar = 1 mm). **(c)** SEMs of lateral and ventral (inset) views of the mandibles (scale bars = 200  $\mu\text{m}$ ). **(d)** Drawing of a lateral view of the proleg on the third abdominal segment (A3) **(e)** SEM of a lateral view of the proleg on A3 (scale bar = 250  $\mu\text{m}$ ). **(f)** Drawing of a lateral view the terminal abdominal segment (A10) with named setae. **(g)** SEM of a posterior view of A10 showing the location of the PP1 seta (arrow) with a close-up of the PP1 seta (inset; arrow) (scale bars = 250  $\mu\text{m}$ ).



**Fig. A.15.** Vibration characteristics and territorial behaviour in *Tetheela fluctuosa*. **(a)** Laser trace of an entire behavioural trial with corresponding video frames below. Numbers correspond in both the trace and the video frames, illustrating the approach of the intruder (1 = FAR, 2 = MID, 3 = CLOSE, 4 = Intruder leaves, F = First resident signal; scale bar = 1 cm). **(b)** Laser vibrometer trace illustrating a series of bouts, with an enlargement of single bout and corresponding spectrogram below. Power spectra demonstrating the dominant frequencies of each vibration (right panel) **(c)** Mean (+SD) behavioural rates of residents at three stages of intruder approach (FAR, MID, CLOSE). Asterisks denote significant differences within each behaviour at different stages of intruder approach. All colours throughout the figure correspond to those in the box describing territorial behaviours.



**Territorial Behaviours**

<input checked="" type="checkbox"/> Mandible Scraping	<input checked="" type="checkbox"/> Crawling towards Int.	<input type="checkbox"/>
<input type="checkbox"/> Mandible Drumming	<input type="checkbox"/> Pushing	<input type="checkbox"/>
<input checked="" type="checkbox"/> Anal Scraping	<input checked="" type="checkbox"/> Lateral Head Hitting	<input type="checkbox"/>
<input checked="" type="checkbox"/> Lateral Tremulation	<input checked="" type="checkbox"/> Lateral Tail Hitting	<input type="checkbox"/>
<input type="checkbox"/> Buzzing	<input type="checkbox"/> Twitching	<input type="checkbox"/>

towards the intruder, lateral head hitting and lateral tail hitting (Fig. A.15c). Details on temporal and spectral characteristics of vibrations are summarized in Table A.1.

Vibrations and movements were similar to those described in other species in Chapter 2.

### *Thyatira batis* (*Thyatirinae*)

#### General life history observations relevant to conspecific interactions

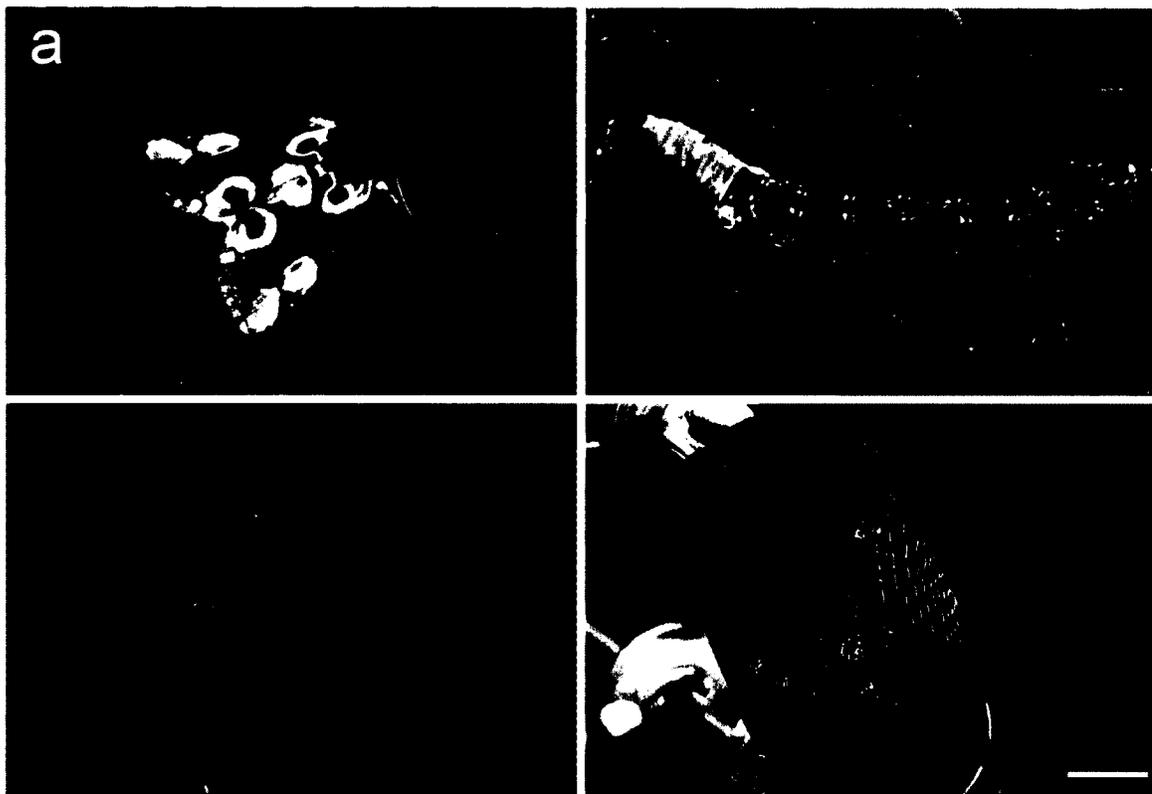
Previous observations (summarized in Table 2.2) have shown that adult females of the peach blossom, *Thyatira batis* Linnaeus 1758 (Fig. A.16a) lay flat, oval eggs either singly, or small groups of 2-3 on species of bramble (*Rubus* spp.; Riegler, 1999; Fig. A.16b). Early instars live solitarily and build a leaf shelter out of silk (Riegler, 1999; Fig. A.16c,d). Late instars live solitarily exposed on the leaf (Riegler, 1999; Fig. A.16e,f).

#### Morphology

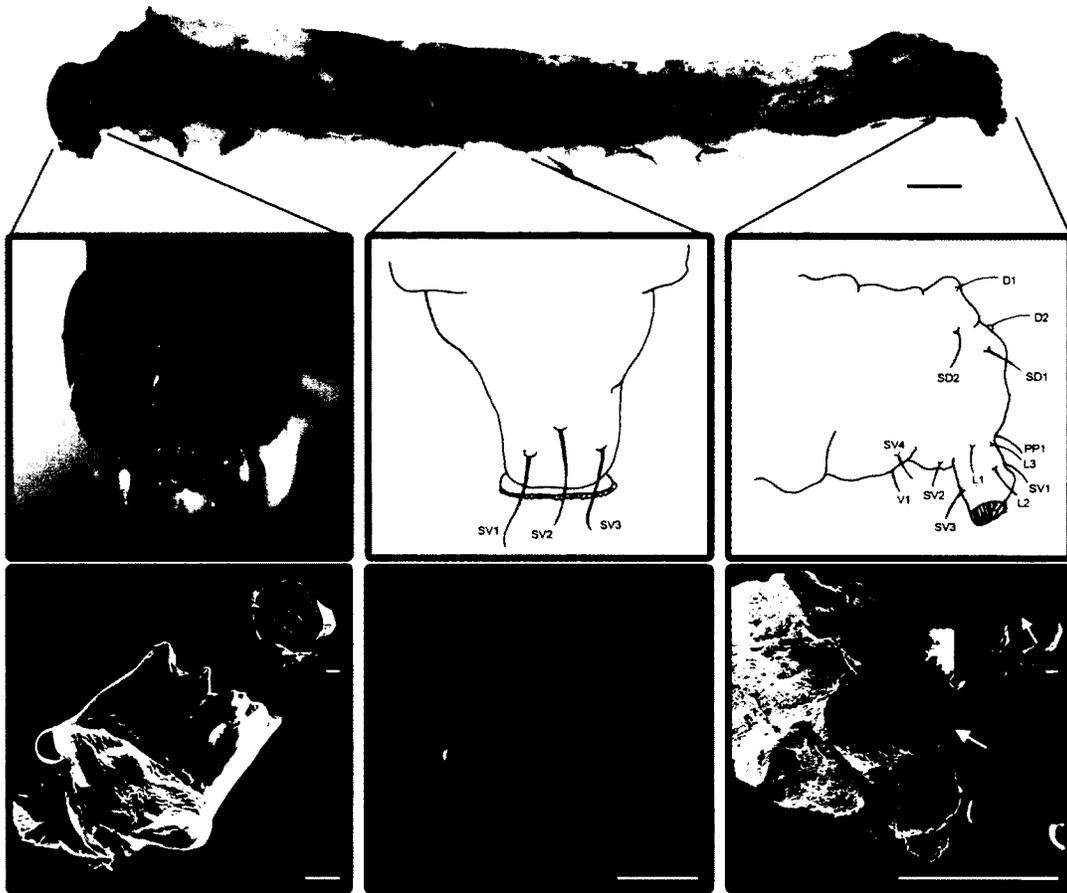
The head capsule of late instar larvae is not flattened dorsally (Fig. A.17a,b). Mandibles have five distal teeth on the incisor area and three ridges on the oral surface (Fig. A.17c). The abdominal prolegs (excluding the anal prolegs) bear three setae on the outer planta region, none of which are modified (Fig. A.17d,e). Larvae possess reduced prolegs on the terminal abdominal segment that bear crochets (Fig. A.17a,f,g). Larvae do not possess any modified setae on the anal segment (Fig. A.17f,g). Morphological characters are summarized in Table 2.3.

#### Behavioural trials between conspecifics

**Fig. A.16.** Photographs demonstrating life-history characteristics relevant to territorial behaviour in the peach blossom, *Thyatira batis*. **(a)** Dorsal view of an adult moth in resting position (scale bar = 1.5 cm). **(b)** Dorsal view of an early instar larvae (unknown scale; photo credit: Claudia Mech, lepiforum.de). **(d)** Dorsal view of a late instar caterpillar in resting position (scale bar = 1 cm). **(f)** Late instar caterpillar on a leaf (scale bar = 3 cm).



**Fig. A.17.** Morphological characters related to territorial behaviour in *Thyatira batis*. **(a)** Lateral view of the whole caterpillar (scale bar = 1 mm). **(b)** Anterior view of the head capsule (scale bar = 1 mm). **(c)** SEMs of lateral and ventral (inset) views of the mandibles (scale bars = 100  $\mu\text{m}$ ). **(d)** Drawing of a lateral view of the proleg on the third abdominal segment (A3) **(e)** SEM of a lateral view of the proleg on A3 (scale bar = 200  $\mu\text{m}$ ; photo credit: T. Nevills). **(f)** Drawing of a lateral view the terminal abdominal segment (A10) with named setae. **(g)** SEM of a posterior view of A10 showing the location of the PP1 seta (arrow; scale bar = 1 mm) with a close-up of the PP1 seta (inset; arrow; scale bar = 100  $\mu\text{m}$ ).



Details on encounters with conspecifics are summarized in Table 2.4. A total of 7 encounters were staged between a resident and an intruder of similar size. Residents produced five types of behaviours during encounters, including anal scraping, lateral tremulation, twitching, lateral head hitting and lateral tail hitting (Fig. A.18). Trials always ended in a tie, with the resident and intruder often sitting side by side. Residents were silent until they detected an intruder (Fig. A.18a). The rate of twitching changed significantly as the intruder approached the resident (Fig. A.18b; see Table A.1 for details). Only one intruder was observed to twitch during a trial, while no other intruder was observed to produce any other territorial behaviours ( $n = 7$ ).

#### Analysis of vibrations

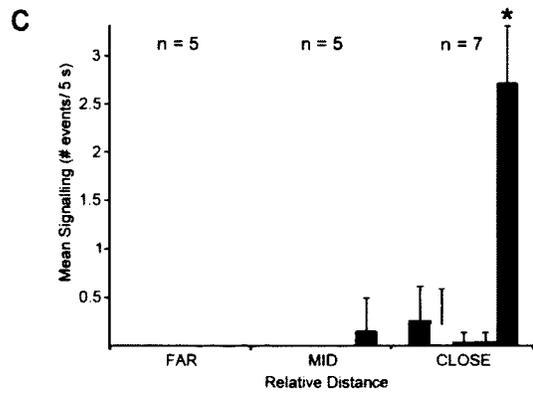
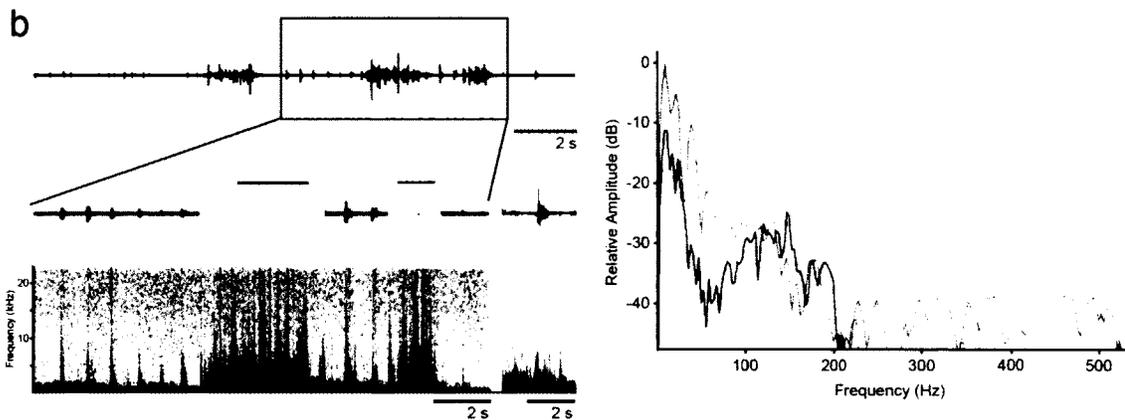
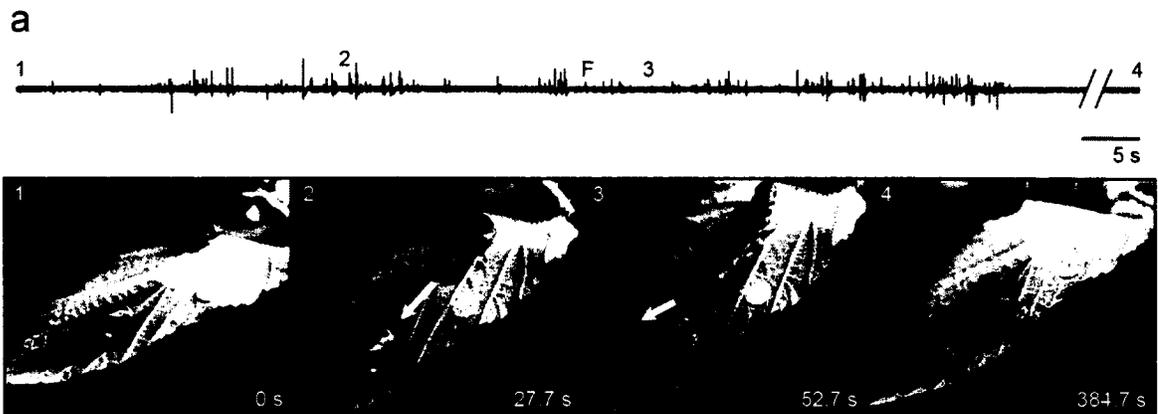
Vibrations are associated with five behaviours in late instar larvae during conspecific interactions - anal scraping, lateral tremulation, twitching, lateral head hitting and lateral tail hitting (Fig. A.3c). Details on temporal and spectral characteristics of vibrations are summarized in Table A.1. Vibrations and movements were similar to those described in other species in Chapter 2.

#### *Watsonalla cultraria* (Drepaninae)

##### General life history observations relevant to conspecific interactions

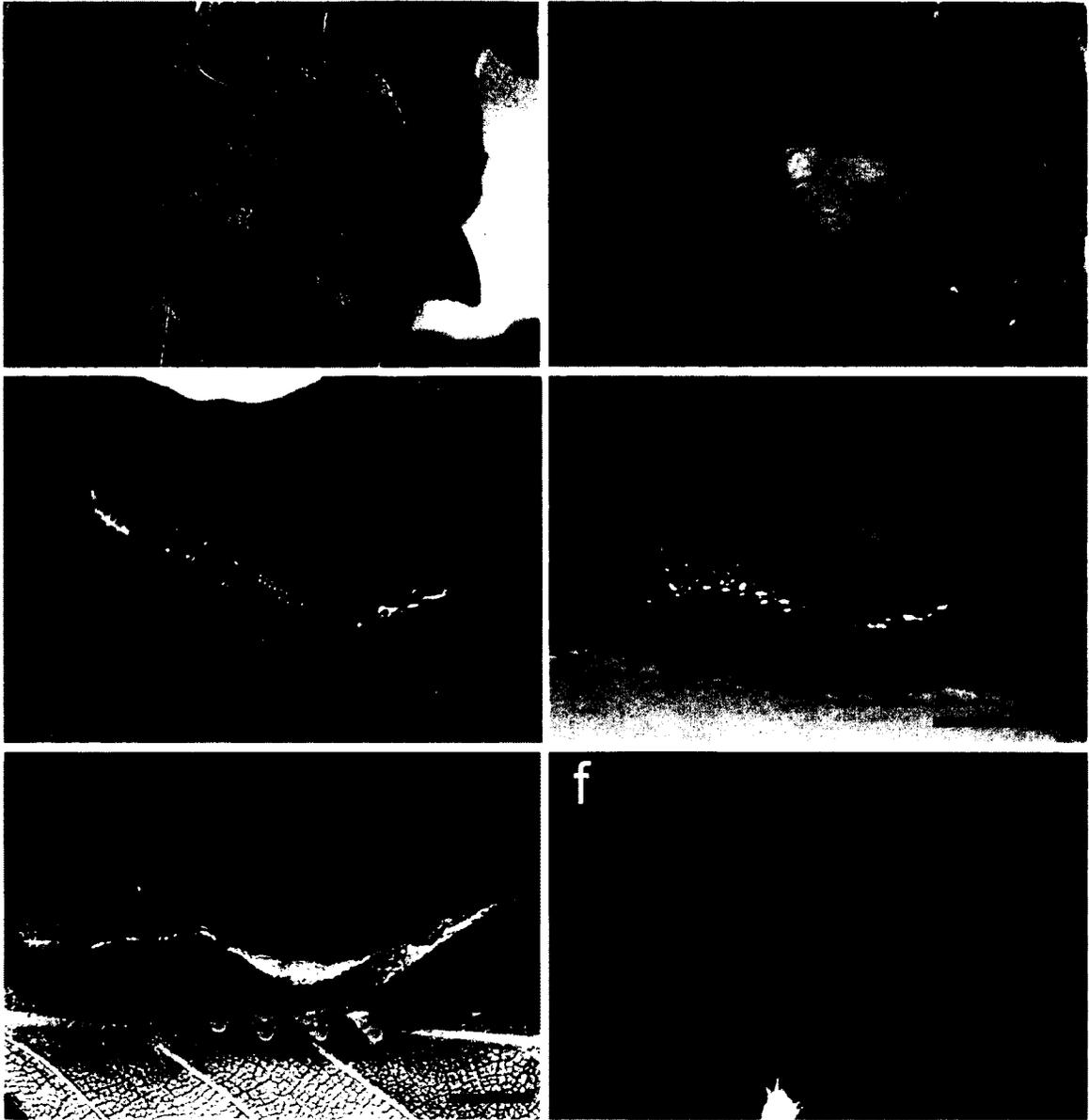
Previous observations (summarized in Table 2.2) have shown that adult females of the barred hook-tip moth, *Watsonalla cultraria* Fabricius 1775 (Fig. A.19a) lay eggs singly or in small groups of 2-4, covered in scales and hairs from the adult, on species of

**Fig. A.18.** Vibration characteristics and territorial behaviour in *Thyatira batis*. **(a)** Laser trace of an entire behavioural trial with corresponding video frames below. Numbers correspond in both the trace and the video frames, illustrating the approach of the intruder (1 = FAR, 2 = MID, 3 = CLOSE, 4 = Intruder leaves, F = First resident signal; scale bar = 1 cm). **(b)** Laser vibrometer trace illustrating a series of bouts, with an enlargement of single bout and corresponding spectrogram below. Power spectra demonstrating the dominant frequencies of each vibration (right panel) **(c)** Mean (+SD) behavioural rates of residents at three stages of intruder approach (FAR, MID, CLOSE). Asterisks denote significant differences within each behaviour at different stages of intruder approach. All colours throughout the figure correspond to those in the box describing territorial behaviours.



- Territorial Behaviours**
- Mandible Scraping
  - Mandible Drumming
  - Anal Scraping
  - Lateral Tremulation
  - Buzzing
  - Crawling towards Int.
  - Pushing
  - Lateral Head Hitting
  - Lateral Tail Hitting
  - Twitching

**Fig. A.19.** Photographs demonstrating life-history characteristics relevant to territorial behaviour in the barred hook-tip moth, *Watsonalla cultraria*. **(a)** Dorsal view of an adult moth in resting position (scale bar = 1.5 cm; photo credit: ukleps.org). **(b)** Dorsal view of a single egg (scale bar = 1 mm; photo credit: ukleps.org). **(c)** Lateral view of an early instar larvae in resting position (scale bar = 2 mm; photo credit: ukleps.org). **(d)** Dorsal view of a solitary early instar caterpillar (scale bar = 2 mm; photo credit: ukleps.org). **(e)** Lateral view of a late instar caterpillar in resting position (scale bar = 5 mm; photo credit: ukleps.org). **(f)** Late instar caterpillar on a leaf (scale bar = 2.5 cm; photo credit: J. Yack).



beech (*Fagus* spp.) or oak (*Quercus* spp.; Bryner, 1999; Fig. A.19b). Early instars live in small groups of 2-5 (Bryner, 1999; Fig. A.19c,d). Late instars live solitarily exposed on the leaf, or in simple shelters they construct with silk (Bryner, 1999; Fig. A.19e,f).

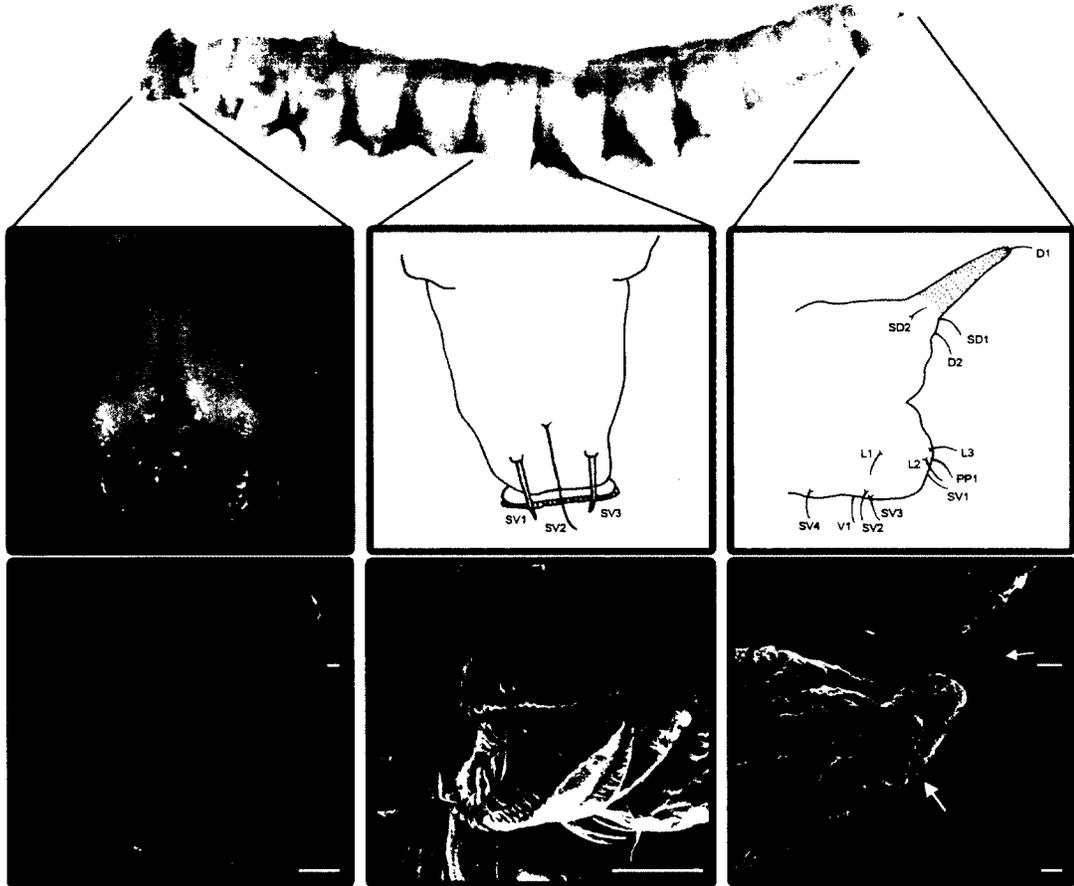
### Morphology

The head capsule of late instar larvae is not flattened dorsally (Fig. A.20a,b). Mandibles have no distal teeth on the incisor area and no ridges on the oral surface (Fig. A.20c). The abdominal prolegs (excluding the anal prolegs) bear three setae on the outer planta region, where SV1 and SV3 are modified (Fig. A.20d,e). Larvae do not possess prolegs on the terminal abdominal segment and possess a short, fleshy caudal projection (Fig. A.20a,f,g). Larvae do not possess any modified setae on the anal segment (Fig. A.20f,g). Morphological characters are summarized in Table 2.3.

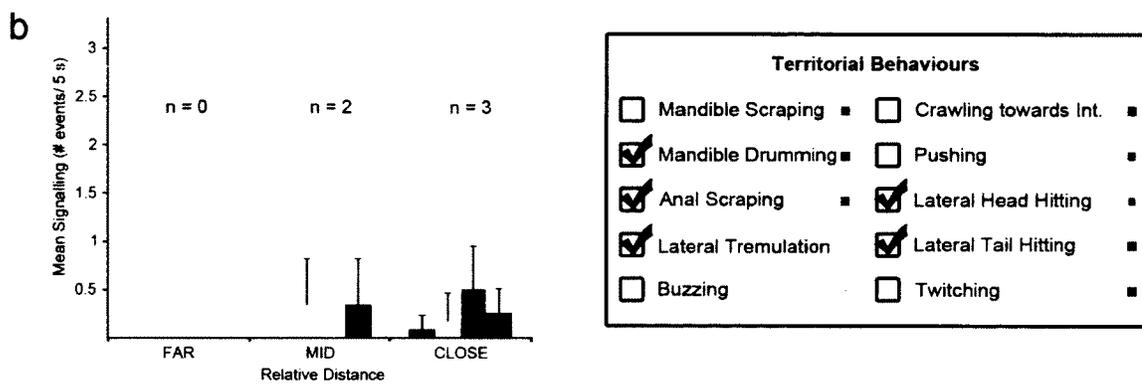
### Behavioural trials between conspecifics

Details on encounters with conspecifics are summarized in Table 2.4. A total of 3 encounters were staged between a resident and an intruder of similar size. Residents produced five types of behaviours during encounters, including mandible drumming, anal scraping, lateral tremulation, lateral head hitting and lateral tail hitting (Fig. A.21). Data on trial outcomes were not calculated as no full trials were recorded on camera. Residents were silent until they detected an intruder (Fig. A.21a). The rate of resident behaviours did not change as the intruder approached the resident (Fig. A.21b). Intruders were observed to signal in one of the two trials (1 lateral tremulation + 1 pseudo anal scrape).

**Fig. A.20.** Morphological characters related to territorial behaviour in *Watsonalla cultraria*. **(a)** Lateral view of the whole caterpillar (scale bar = 2 mm). **(b)** Anterior view of the head capsule (scale bar = 0.5 mm). **(c)** SEMs of lateral and ventral (inset) views of the mandibles (scale bars = 100  $\mu$ m). **(d)** Drawing of a lateral view of the proleg on the third abdominal segment (A3) **(e)** SEM of a lateral view of the proleg on A3 (scale bar = 200  $\mu$ m). **(f)** Drawing of a lateral view the terminal abdominal segment (A10) with named setae. **(g)** SEM of a posterior view of A10 showing the location of the PP1 seta (arrow) with a close-up of the PP1 seta (inset; arrow) (scale bars = 100  $\mu$ m).



**Fig. A.21.** Territorial behaviour in *Watsonalla cultraria*. **(a)** Video frames of an entire behavioural trial. Numbers illustrate the approach of the intruder (1 = FAR, 2 = MID, 3 = CLOSE, 4 = Intruder leaves; scale bar = 2 cm; video credit: J. Yack). **(b)** Mean (+SD) behavioural rates of residents at three stages of intruder approach (FAR, MID, CLOSE). Asterisks denote significant differences within each behaviour at different stages of intruder approach. All colours throughout the figure correspond to those in the box describing territorial behaviours.



## Analysis of vibrations

Vibrations are associated with five behaviours in late instar larvae during conspecific interactions - mandible drumming, anal scraping, lateral tremulation, lateral head hitting and lateral tail hitting (Fig. A.21c). Details on temporal and spectral characteristics of vibrations are summarized in Table A.1. Vibrations and movements were similar to those described in other species in Chapter 2.

## *Specimens in alcohol*

### *Cilix glaucata (Drepaninae)*

#### Morphology

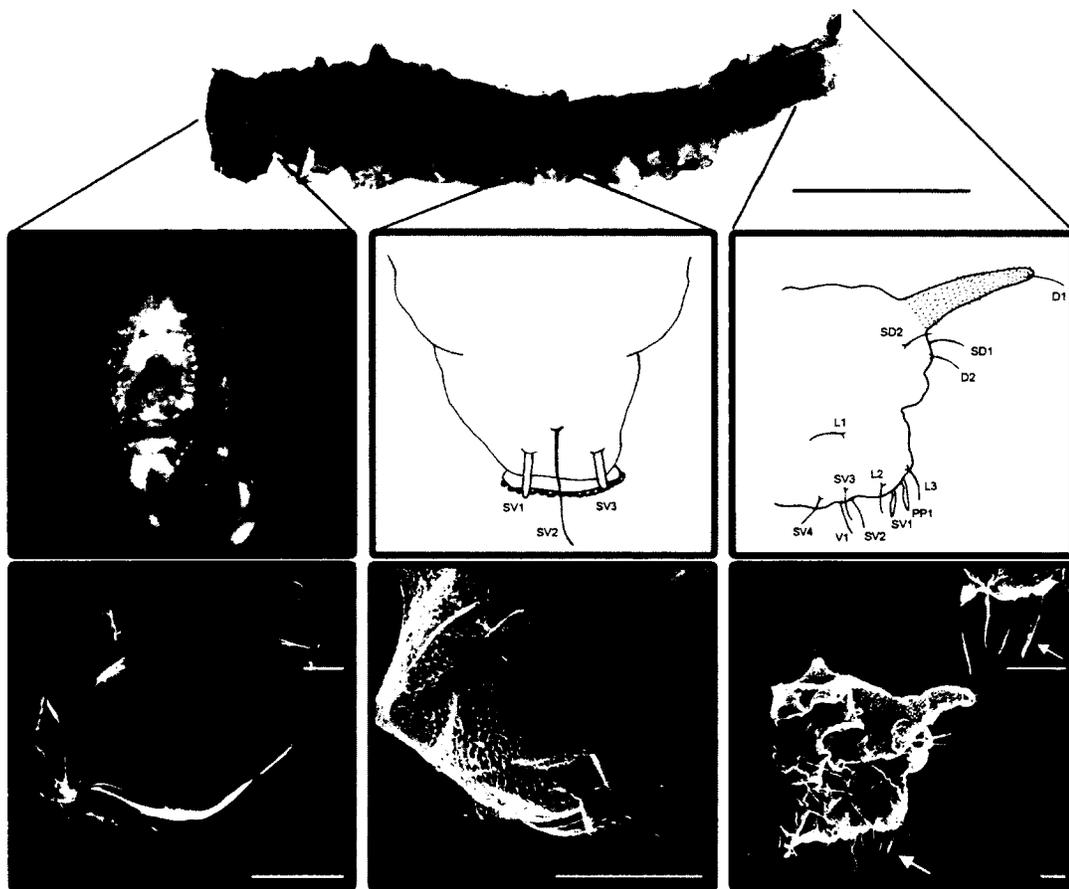
The head capsule of late instar larvae is not flattened dorsally (Fig. A.22a,b). Mandibles have no distal teeth on the incisor area and four ridges on the oral surface (Fig. A.22c). The abdominal prolegs (excluding the anal prolegs) bear three setae on the outer planta region, where SV1 and SV3 are modified (Fig. A.22d,e). Larvae do not possess prolegs on the terminal abdominal segment and possess a short, fleshy caudal projection (Fig. A.22a,f,g). Larvae possess two modified setae on the anal segment (Fig. A.22f,g). Morphological characters are summarized in Table 2.3.

### *Falcaria lacertinaria (Drepaninae)*

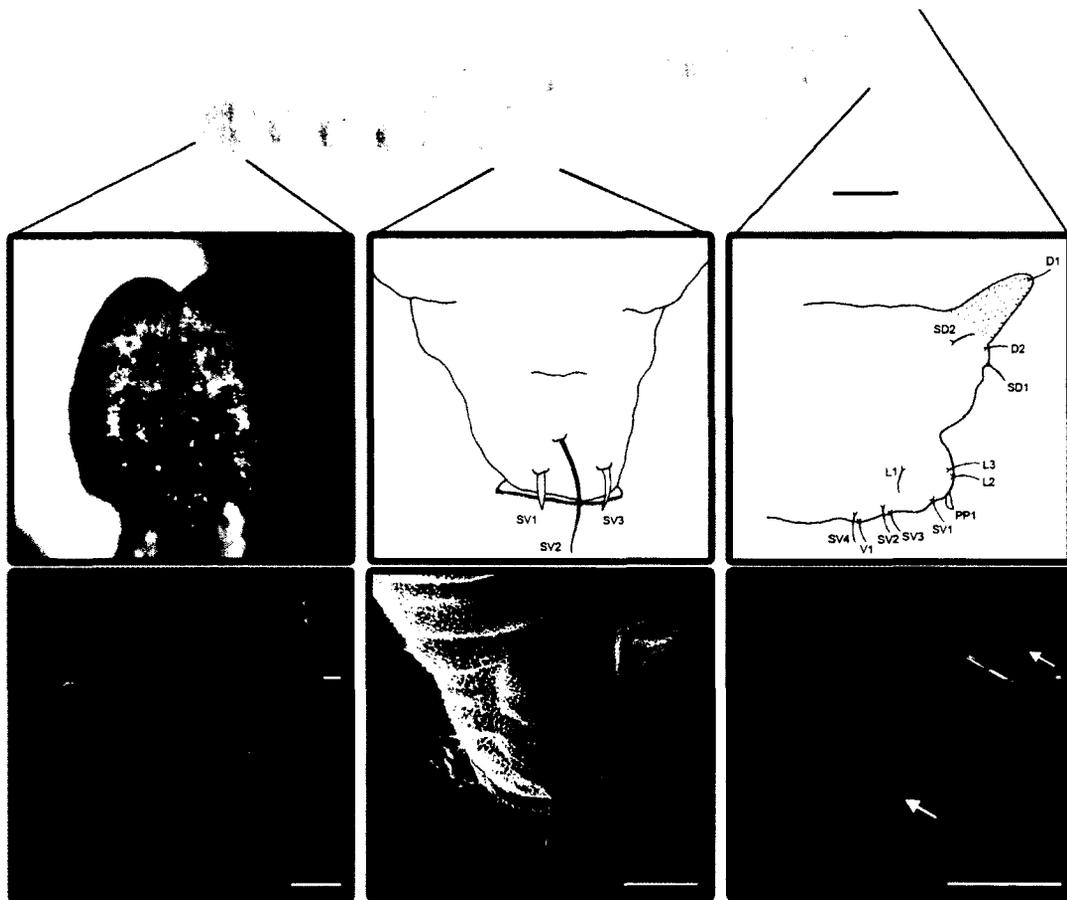
#### Morphology

The head capsule of late instar larvae is not flattened dorsally (Fig. A.23a,b). Mandibles have six rounded distal teeth on the incisor area and two ridges on the oral surface (Fig. A.23c). The abdominal prolegs (excluding the anal prolegs) bear three setae

**Fig. A.22.** Morphological characters related to territorial behaviour in *Cilix glaucata*. **(a)** Lateral view of the whole caterpillar (scale bar = 2 mm). **(b)** Anterior view of the head capsule (scale bar = 0.5 mm). **(c)** SEMs of lateral and ventral (inset) views of the mandibles (scale bars = 100  $\mu$ m). **(d)** Drawing of a lateral view of the proleg on the third abdominal segment (A3) **(e)** SEM of a lateral view of the proleg on A3 (scale bar = 200  $\mu$ m). **(f)** Drawing of a lateral view the terminal abdominal segment (A10) with named setae. **(g)** SEM of a posterior view of A10 showing the location of the PP1 seta (arrow) with a close-up of the PP1 seta (inset; arrow) (scale bars = 100  $\mu$ m).



**Fig. A.23.** Morphological characters related to territorial behaviour in *Falcaria lacertinaria*. **(a)** Lateral view of the whole caterpillar (scale bar = 2 mm). **(b)** Anterior view of the head capsule (scale bar = 0.5 mm). **(c)** SEMs of lateral and ventral (inset) views of the mandibles (scale bars = 100  $\mu\text{m}$ ). **(d)** Drawing of a lateral view of the proleg on the third abdominal segment (A3) **(e)** SEM of a lateral view of the proleg on A3 (scale bar = 200  $\mu\text{m}$ ). **(f)** Drawing of a lateral view the terminal abdominal segment (A10) with named setae. **(g)** SEM of a posterior view of A10 showing the location of the PP1 seta (arrow; scale bar = 1mm) with a close-up of the PP1 seta (inset; arrow; scale bar = 10  $\mu\text{m}$ ).



on the outer planta region, where SV1 and SV3 are modified (Fig. A.23d,e). Larvae do not possess prolegs on the terminal abdominal segment and possess a short, fleshy caudal projection (Fig. A.23a,f,g). Larvae possess modified PP1 setae on the anal segment, and no other modified setae on this segment (Fig. A.23f,g). Morphological characters are summarized in Table 2.3.

*Habrosyne pyritoides* (*Thyatirinae*)

Morphology

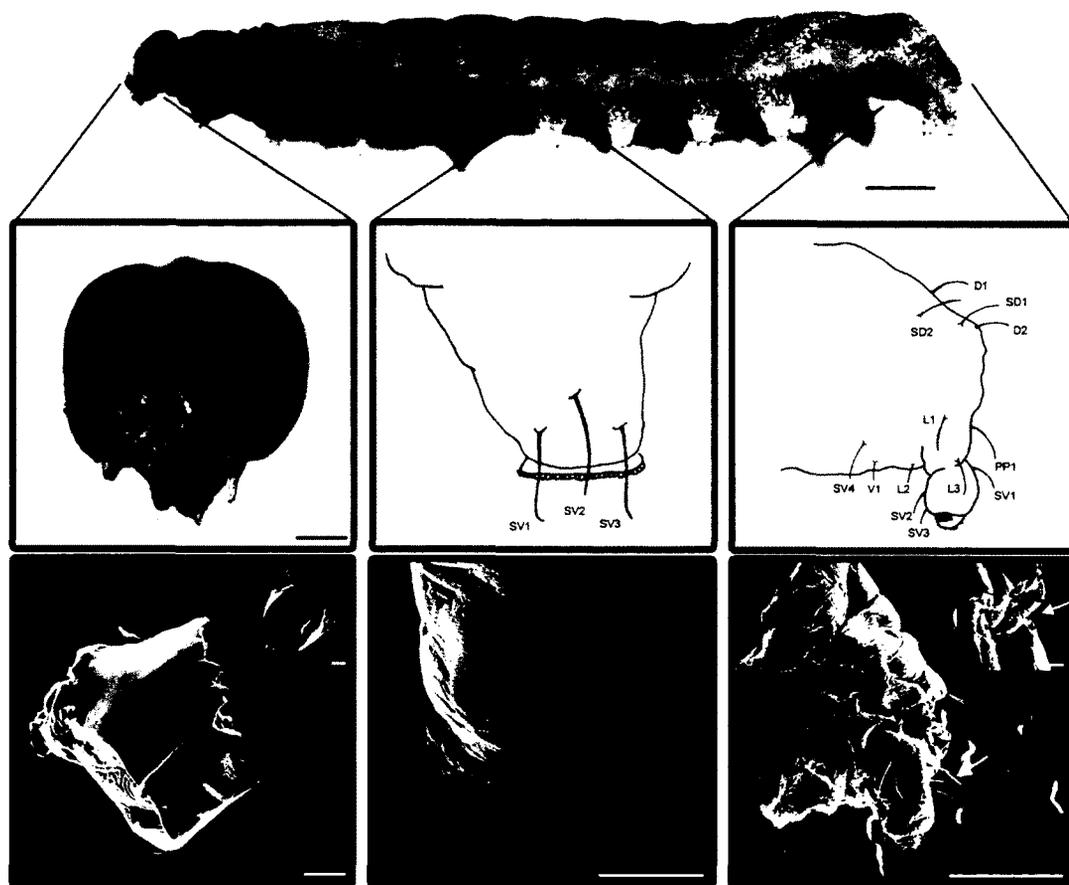
The head capsule of late instar larvae is not flattened dorsally (Fig. A.24a,b). Mandibles have eight distal teeth on the incisor area and two ridges on the oral surface (Fig. A.24c). The abdominal prolegs (excluding the anal prolegs) bear three unmodified setae on the outer planta region (Fig. A.24d,e). Larvae possess reduced prolegs on the terminal abdominal segment that bear crochets (Fig. A.24a,f,g). Larvae do not possess any modified setae on the anal segment (Fig. A.24f,g). Morphological characters are summarized in Table 2.3.

*Watsonalla binaria* (*Drepaninae*)

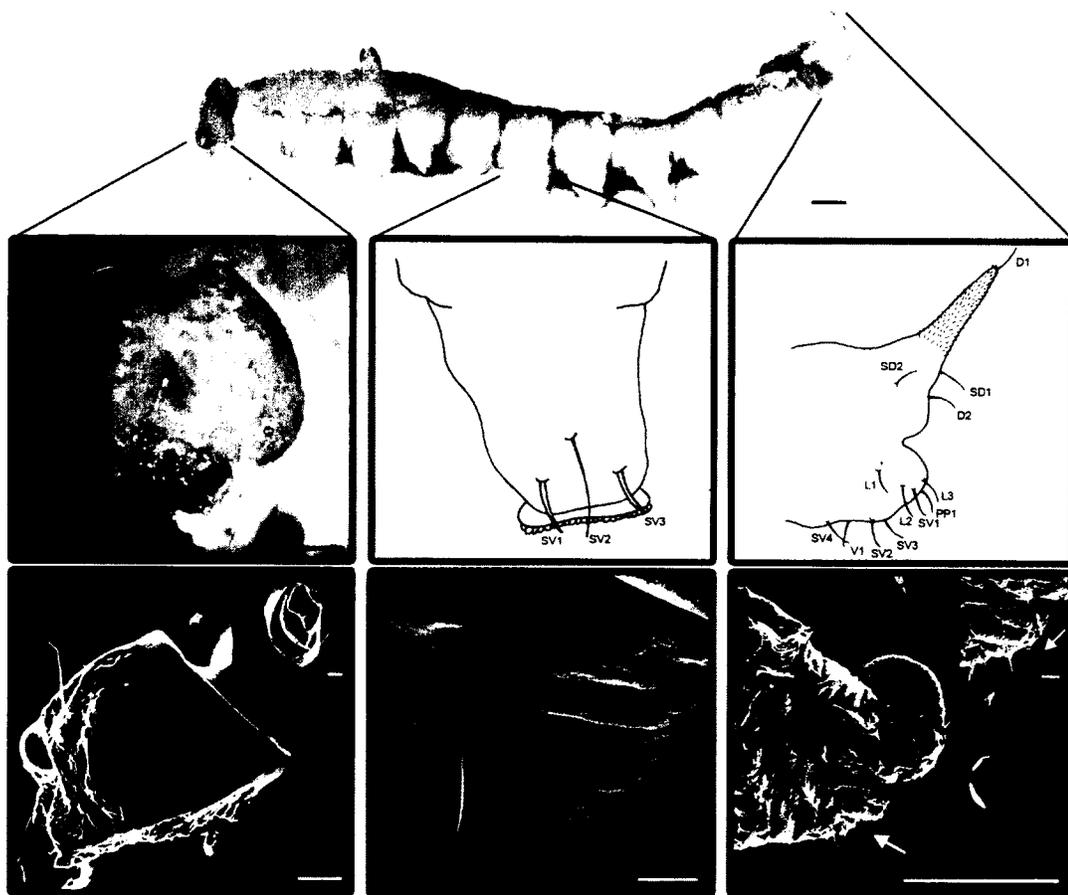
Morphology

The head capsule of late instar larvae is not flattened dorsally (Fig. A.25a,b). Mandibles have no distal teeth on the incisor area and no ridges on the oral surface (Fig. A.25c). The abdominal prolegs (excluding the anal prolegs) bear three setae on the outer planta region, where SV1 and SV3 are modified (Fig. A.25d,e). Larvae do not possess prolegs on the terminal abdominal segment and possess a short, fleshy caudal projection

**Fig. A.24.** Morphological characters related to territorial behaviour in *Habrosyne pyritoides*. **(a)** Lateral view of the whole caterpillar (scale bar = 2 mm). **(b)** Anterior view of the head capsule (scale bar = 0.5 mm). **(c)** SEMs of lateral and ventral (inset) views of the mandibles (scale bars = 100  $\mu\text{m}$ ). **(d)** Drawing of a lateral view of the proleg on the third abdominal segment (A3) **(e)** SEM of a lateral view of the proleg on A3 (scale bar = 200  $\mu\text{m}$ ). **(f)** Drawing of a lateral view the terminal abdominal segment (A10) with named setae. **(g)** SEM of a posterior view of A10 showing the location of the PP1 seta (arrow; scale bar = 1 mm) with a close-up of the PP1 seta (inset; arrow; scale bars = 100  $\mu\text{m}$ ).



**Fig. A.25.** Morphological characters related to territorial behaviour in *Watsonalla binaria*. **(a)** Lateral view of the whole caterpillar (scale bar = 1 mm). **(b)** Anterior view of the head capsule (scale bar = 0.5 mm). **(c)** SEMs of lateral and ventral (inset) views of the mandibles (scale bars = 100  $\mu$ m). **(d)** Drawing of a lateral view of the proleg on the third abdominal segment (A3) **(e)** SEM of a lateral view of the proleg on A3 (scale bar = 200  $\mu$ m). **(f)** Drawing of a lateral view the terminal abdominal segment (A10) with named setae. **(g)** SEM of a posterior view of A10 showing the location of the PP1 seta (arrow; scale bar = 1 mm) with a close-up of the PP1 seta (inset; arrow; scale bar = 100  $\mu$ m).



(Fig. A.25a,f,g). Larvae do not possess modified setae on the anal segment (Fig. A.25f,g).

Morphological characters are summarized in Table 2.3.

#### *Watsonalla uncinula (Drepaninae)*

##### Morphology

The head capsule of late instar larvae is not flattened dorsally (Fig. A.26a,b).

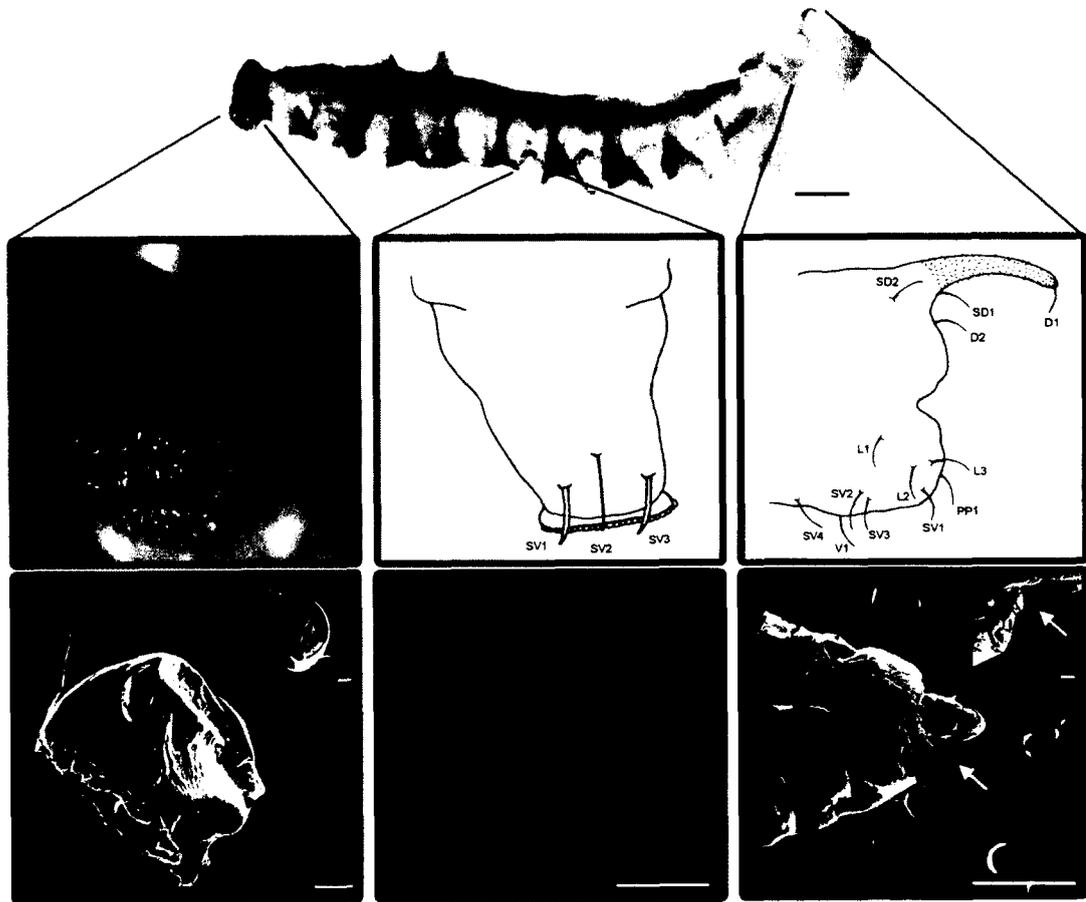
Mandibles have no distal teeth on the incisor area and one ridge on the oral surface (Fig. A.26c). The abdominal prolegs (excluding the anal prolegs) bear three setae on the outer planta region, where SV1 and SV3 are modified (Fig. A.26d,e). Larvae do not possess prolegs on the terminal abdominal segment and possess a short, fleshy causal projection (Fig. A.26a,f,g). Larvae do not possess modified setae on the anal segment (Fig. A.26f,g).

Morphological characters are summarized in Table 2.3.

##### ***Summary of vibration characteristics***

Temporal and spectral characteristics of vibrations by species are summarized in Table A.1.

**Fig. A.26.** Morphological characters related to territorial behaviour in *Watsonalla uncinula*. **(a)** Lateral view of the whole caterpillar (scale bar = 2 mm). **(b)** Anterior view of the head capsule (scale bar = 0.5 mm). **(c)** SEMs of lateral and ventral (inset) views of the mandibles (scale bars = 100  $\mu$ m). **(d)** Drawing of a lateral view of the proleg on the third abdominal segment (A3) **(e)** SEM of a lateral view of the proleg on A3 (scale bar = 200  $\mu$ m; photo credit: T. Nevills). **(f)** Drawing of a lateral view the terminal abdominal segment (A10) with named setae. **(g)** SEM of a posterior view of A10 showing the location of the PP1 seta (arrow; scale bar = 1 mm) with a close-up of the PP1 seta (inset; arrow; scale bars = 100  $\mu$ m).



**Table A.1.** Temporal, spectral, relative amplitude and rate data for all vibrations produced during encounters with conspecifics in 10 species of Drepanidae. Sample sizes are given in number of signals from number of individuals.

Taxon	Duration of Signal (ms)	Rel. Amplitude (Times Baseline)	Dominant Frequency (Hz)	Bandwidth at -3 dB (Hz)	Bandwidth at -10 dB (Hz)	Signals per Bout	Rate at Close (signals/5 s)	Significant Rate Changes
<b>MANDIBLE SCRAPING</b>								
<b>Drepaninae</b>								
<i>Drepana arcuata</i>	59.2 ± 17 (n = 49 from 22)	27.2 ± 17.3 (n = 20 from 4)	17.7 ± 11.9 (n = 20 from 4)	5.2 ± 1.3 (n = 20 from 4)	12.7 ± 3.1 (n = 20 from 4)	2.6 ± 3.2 (n = 25 from 5)	3.23 ± 1.52 (n = 16)	MID to CLOSE (p > 0.001, n = 16)
<i>D. curvatula</i>	101.0 ± 23.8 (n = 25 from 5)	11.7 ± 4.7 (n = 10 from 2)	22.3 ± 5.4 (n = 10 from 2)	6.0 ± 0.1 (n = 10 from 2)	14.0 ± 5.5 (n = 10 from 2)	0.1 ± 0.1 (n = 40 from 9)	0.25 ± 0.29 (n = 11)	FAR to CLOSE (p = 0.011, n = 11)
<i>D. falcataria</i>	94.3 ± 3.1 (n = 15 from 3)	12.9 ± 1.7 (n = 18 from 5)	12.7 ± 6.2 (n = 18 from 5)	5.7 ± 0.8 (n = 18 from 5)	10.6 ± 1.7 (n = 18 from 5)	1.13 ± 0.58 (n = 15 from 3)	1.08 ± 1.01 (n = 3)	None (n = 3)
<i>Oreta rosea</i>	125.6 ± 21.4 (n = 69 from 17)	31.4 ± 14.4 (n = 25 from 5)	26.2 ± 5.1 (n = 25 from 5)	6.8 ± 1.3 (n = 25 from 5)	14.5 ± 3.4 (n = 25 from 5)	1.6 ± 2.0 (n = 71 from 16)	0.81 ± 1.5 (n = 18)	MID to CLOSE (p = 0.001, n = 18)
<b>Thyatirinae</b>								
<i>Ochropacha duplaris</i>	211.2 ± 42.6 (n = 10 from 4)	22.0 ± 7.3 (n = 16 from 5)	32.9 ± 12.0 (n = 16 from 5)	6.6 ± 1.6 (n = 16 from 5)	24.3 ± 8.8 (n = 16 from 5)	2.6 ± 1.7 (n = 11 from 4)	0.29 ± 0.37 (n = 6)	None (n = 6)
<i>Tethea or</i>	136.7 ± 50.8 (n = 40 from 8)	21.6 ± 12.3 (n = 25 from 5)	351.1 ± 65.2 (n = 25 from 5)	17.4 ± 1.6 (n = 25 from 5)	61.2 ± 9.5 (n = 25 from 5)	4 ± 1 (n = 48 from 11)	2.98 ± 2.7 (n = 11)	FAR to CLOSE (p = 0.021, n = 11)
<i>Tetheela fluctuosa</i>	289.1 ± 50.8 (n = 20 from 5)	82.8 ± 28.1 (n = 15 from 3)	59.6 ± 3.3 (n = 15 from 3)	7.5 ± 0.9 (n = 15 from 3)	20.1 ± 2.2 (n = 15 from 3)	1.78 ± 0.81 (n = 22 from 5)	1.3 ± 0.89 (n = 5)	None (n = 5)
<b>MANDIBLE DRUMMING</b>								
<b>Drepaninae</b>								
<i>D. arcuata</i>	15.9 ± 12.5 (n = 180 from 30)	63.03 ± 21.3 (n = 25 from 5)	22.0 ± 16.9 (n = 25 from 5)	7.0 ± 2.1 (n = 25 from 5)	16.3 ± 10.4 (n = 25 from 5)	20.8 ± 9.6 (n = 25 from 5)	2.98 ± 2.0 (n = 16)	FAR to MID (p = 0.015) returned to FAR at CLOSE (n = 16)
<i>D. curvatula</i>	73.5 ± 9.3 (n = 20 from 5)	20.8 ± 11.6 (n = 12 from 3)	26.9 ± 7.9 (n = 12 from 3)	6.8 ± 1.5 (n = 12 from 3)	13.4 ± 1.7 (n = 12 from 3)	6.7 ± 13.1 (n = 40 from 9)	0.63 ± 0.78 (n = 11)	None (n = 11)
<i>D. falcataria</i>	62.0 ± 1.4 (n = 14 from 3)	24.3 ± 12.3 (n = 23 from 5)	83.3 ± 79.0 (n = 23 from 5)	15.3 ± 10.3 (n = 23 from 5)	46.5 ± 39.1 (n = 23 from 5)	0.53 ± 0.76 (n = 15 from 3)	0.41 ± 0.52 (n = 3)	None (n = 3)

Taxon	Duration of Signal (ms)	Rel. Amplitude (Times Baseline)	Dominant Frequency (Hz)	Bandwidth at -3 dB (Hz)	Bandwidth at -10 dB (Hz)	Signals per Bout	Rate at Close (signals/5 s)	Significant Rate Changes
<i>Falcaria bilineata</i>	37.9 ± 13.1 (n = 70 from 14)	No laser files	No laser files	No laser files	No laser files	3.8 ± 1.2 (n = 14 from 5)	2.6 ± 1.9 (n = 21)	MID to CLOSE (p < 0.001, n = 21)
<i>O. rosea</i>	66.9 ± 20.1 (n = 81 from 19)	50.3 ± 19.6 (n = 25 from 5)	59.0 ± 12.4 (n = 25 from 5)	12.2 ± 3.1 (n = 25 from 5)	34.2 ± 15.4 (n = 25 from 5)	2.0 ± 1.2 (n = 71 from 16)	0.76 ± 0.69 (n = 18)	MID to CLOSE (p < 0.001, n = 18)
<i>W. cultraria</i>	29.2 ± 80.4 (n = 3 from 1)	No laser files	No laser files	No laser files	No laser files	0.2 ± 0.3 (n = 6 from 3)	0.083 ± 0.14 (n = 3)	None (n = 3)
<b>ANAL SCRAPING</b>								
<b>Drepaninae</b>								
<i>D. arcuata</i>	366.9 ± 145.4 (n = 112 from 30)	13.37 ± 11.95 (n = 25 from 5)	39.8 ± 3.9 (n = 25 from 5)	7.6 ± 1.7 (n = 25 from 5)	17.2 ± 1.2 (n = 25 from 5)	10.3 ± 2.7 (n = 25 from 5)	2.84 ± 0.75 (n = 16)	FAR to MID (p = 0.033, n = 16)
<i>D. curvatula</i>	855.0 ± 369.2 (n = 42 from 9)	11.70 ± 12.49 (n = 23 from 5)	53.2 ± 26.3 (n = 25 from 5)	9.6 ± 2.0 (n = 25 from 5)	20.0 ± 5.0 (n = 25 from 5)	2.9 ± 2.0 (n = 40 from 9)	2.5 ± 1.17 (n = 11)	FAR to MID (p < 0.001, n = 11)
<i>D. falcataria</i>	536.9 ± 130.7 (n = 11 from 3)	9.72 ± 5.36 (n = 13 from 3)	36.2 ± 6.7 (n = 22 from 5)	7.38 ± 2.0 (n = 22 from 5)	21.5 ± 7.4 (n = 22 from 5)	2.6 ± 0.7 (n = 15 from 3)	1.67 ± 0.14 (n = 3)	None (n = 3)
<i>F. bilineata</i>	125.0 ± 26.7 (n = 65 from 13)	No laser files	No laser files	No laser files	No laser files	4.1 ± 0.8 (n = 14 from 5)	2.15 ± 1.73 (n = 21)	MID to CLOSE (p < 0.001, n = 21)
<i>W. cultraria*</i>	532.5 ± 105.4 (n = 9 from 3)	No laser files	No laser files	No laser files	No laser files	1.7 ± 0.4 (n = 6 from 3)	0.25 ± 0.25 (n = 3)	None (n = 3)
<b>Thyatirinae</b>								
<i>T. fluctuosa*</i>	1513.7 ± 110.5 (n = 20 from 5)	See LT	See LT	See LT	See LT	1.0 ± 1.2 (n = 22 from 5)	0.86 ± 0.75 (n = 5)	None (n = 5)
<i>Thyatira batis*</i>	1524.9 ± 540.5 (n = 20 from 6)	See LT	See LT	See LT	See LT	0.7 ± 0.8 (n = 31 from 7)	0.25 ± 0.35 (n = 7)	None (n = 7)
<b>LATERAL TREMULATION</b>								
<b>Drepaninae</b>								
<i>O. rosea</i>	1966.3 ± 581.0 (n = 32 from 9)	11.1 ± 4.5 (n = 6 from 5)	55.6 ± 18.2 (n = 6 from 5)	8.5 ± 2.1 (n = 6 from 5)	20.0 ± 7.8 (n = 6 from 5)	0.4 ± 0.4 (n = 71 from 16)	0.083 ± 0.19 (n = 18)	None (n = 18)
<i>W. cultraria**</i>	483.3 ± 125.2 (n = 8 from 3)	No laser files	No laser files	No laser files	No laser files	1.33 ± 0.58 (n = 6 from 3)	0.17 ± 0.29 (n = 3)	None (n = 3)
<b>Thyatirinae</b>								
<i>T. fluctuosa**</i>	957.5 ± 162.0 (n = 20 from 5)	58.2 ± 59.0 (n = 17 from 3)	7.8 ± 4.0 (n = 17 from 3)	5.5 ± 0.5 (n = 17 from 3)	10.9 ± 3.2 (n = 17 from 3)	0.76 ± 0.03 (n = 22 from 5)	0.15 ± 0.22 (n = 5)	None (n = 5)
<i>T. batis**</i>	1602.7 ± 1036.9 (n = 22 from 6)	48.8 ± 9.5 (n = 14 from 5)	10.9 ± 1.8 (n = 14 from 5)	6.1 ± 0.9 (n = 14 from 5)	12.7 ± 2.3 (n = 14 from 5)	0.49 ± 0.51 (n = 31 from 7)	0.04 ± 0.10 (n = 7)	None (n = 7)

Taxon	Duration of Signal (ms)	Rel. Amplitude (Times Baseline)	Dominant Frequency (Hz)	Bandwidth at -3 dB (Hz)	Bandwidth at -10 dB (Hz)	Signals per Bout	Rate at Close (signals/5 s)	Significant Rate Changes
<b>BUZZING</b>								
<b>Drepaninae</b>								
<i>D. curvatula</i> **	735.8 ± 356.8 (n = 28 from 6)	30.8 ± 9.5 (n = 13 from 3)	64.9 ± 23.1 (n = 13 from 3)	8.2 ± 1.3 (n = 13 from 3)	18.5 ± 3.5 (n = 13 from 3)	0.8 ± 1.6 (n = 40 from 9)	0.25 ± 0.20 (n = 11)	FAR to MID (p < 0.001, n = 11)
<i>D. falcataria</i> **	701.1 ± 88.2 (n = 15 from 3)	No laser files	No laser files	No laser files	No laser files	-	1.33 ± 0.52 (n = 3)	None (n = 3)
<b>TWITCHING</b>								
<b>Thyatirinae</b>								
<i>T. batis</i>	91.20 ± 6.89 (n = 30 from 6)	18.86 ± 15.81 (n = 25 from 5)	12.99 ± 3.58 (n = 25 from 5)	6.89 ± 1.36 (n = 25 from 5)	12.76 ± 4.34 (n = 25 from 5)		2.71 ± 0.59 (n = 7)	FAR to MID and FAR to CLOSE (p < 0.001, n = 7)
<b>LATERAL HEAD HITTING</b>								
<b>Drepaninae</b>								
<i>D. arcuata</i>	184.0 ± 19.8 (n = 2 from 2)	No laser files	No laser files	No laser files	No laser files		0.34 ± 0.44 (n = 16)	MID to CLOSE (p < 0.001, n = 16)
<i>D. curvatula</i>	206.9 ± 103.4 (n = 6 from 2)	8.0 ± 5.5 (n = 5 from 3)	58.2 ± 44.6 (n = 5 from 3)	12.9 ± 10.6 (n = 5 from 3)	38.6 ± 36.2 (n = 5 from 3)		0.19 ± 0.38 (n = 11)	None (n = 11)
<i>D. falcataria</i>	200.0 ± 47.14 (n = 2 from 2)	No laser files	No laser files	No laser files	No laser files		0.17 ± 0.29 (n = 3)	None (n = 3)
<i>F. bilineata</i>	183.5 ± 23.6 (n = 5 from 2)	No laser files	No laser files	No laser files	No laser files		0.26 ± 0.37 (n = 21)	FAR to CLOSE (p = 0.005, n = 21)
<i>W. cultraria</i>	173.9 ± 25.0 (n = 7 from 3)	No laser files	No laser files	No laser files	No laser files		0.5 ± 0.43 (n = 3)	None (n = 3)
<b>Thyatirinae</b>								
<i>O. duplaris</i>	144.1 ± 34.8 (n = 15 from 5)	19.0 ± 6.5 (n = 9 from 4)	64.3 ± 40.3 (n = 9 from 4)	7.1 ± 2.1 (n = 9 from 4)	17.6 ± 4.7 (n = 9 from 4)		0.67 ± 0.30 (n = 6)	MID and CLOSE (p = 0.003, n = 6)
<i>T. or</i>	100.1 ± 33.4 (n = 6 from 3)	No laser files	No laser files	No laser files	No laser files		0.058 ± 0.12 (n = 11)	None (n = 11)
<i>T. fluctuosa</i>	158.2 ± 54.3 (n = 25 from 7)	37.3 ± 16.4 (n = 13 from 5)	42.3 ± 22.1 (n = 13 from 5)	8.1 ± 1.9 (n = 13 from 5)	17.6 ± 6.6 (n = 13 from 5)		1.15 ± 0.57 (n = 5)	None (n = 5)
<i>T. batis</i>	208.3 ± 11.8 (n = 2 from 1)	65.9 (n = 1 from 1)	8.8 (n = 1 from 1)	6.3 (n = 1 from 1)	22.3 (n = 1 from 1)		0.04 ± 0.10 (n = 7)	None (n = 7)
<b>LATERAL TAIL HITTING</b>								
<b>Drepaninae</b>								
<i>F. bilineata</i>	277.0 ± 32.5 (n = 10 from 3)						0.21 ± 0.43 (n = 7)	None (n = 7)

Taxon	Duration of Signal (ms)	Rel. Amplitude (Times Baseline)	Dominant Frequency (Hz)	Bandwidth at -3 dB (Hz)	Bandwidth at -10 dB (Hz)	Signals per Bout	Rate at Close (signals/5 s)	Significant Rate Changes
<i>O. rosea</i>	264.2 ± 113.6 (n = 10 from 3)						0.18 ± 0.35 (n = 7)	MID to CLOSE (p = 0.01, n = 7)
<i>W. cultraria</i>	187.5 ± 80.4 (n = 3 from 1)						0.0 ± 0.0 (n = 3)	NA
<b>Thyatirinae</b>								
<i>O. duplaris</i>	112.5 ± 17.7 (n = 2 from 1)						0.08 ± 0.20 (n = 6)	None (n = 6)
<i>T. fluctuosa</i>	197.1 ± 24.6 (n = 13 from 5)						0.15 ± 0.22 (n = 5)	None (n = 5)
<i>T. batis</i>	208.3 ± 11.8 (n = 2 from 1)						0.04 ± 0.09 (n = 7)	None (n = 7)
<b>CRAWLING TOWARDS I</b>								
<b>Thyatirinae</b>								
<i>O. duplaris</i>							0.58 ± 0.65 (n = 6)	None (n = 6)
<i>T. or</i>							1.12 ± 0.77 (n = 11)	FAR to MID (p = 0.019, n = 11) and MID to CLOSE (p = 0.018, n = 11)
<i>T. fluctuosa</i>							0.15 ± 0.14 (n = 5)	None (n = 5)
<b>PUSHING</b>								
<b>Thyatirinae</b>								
<i>O. duplaris</i>	600.6 ± 208.4 (n = 8 from 3)						0.42 ± 0.547 (n = 6)	None (n = 6)
<i>T. or</i>	777.4 ± 237.1 (n = 8 from 3)						0.88 ± 0.52 (n = 11)	FAR and MID to CLOSE (p > 0.001, n = 11)

\*represents species that 'pseudo' anal scrape

\*\*represents species that anal scrape or 'pseudo' anal scrape concurrently with lateral tremulation or buzzing



Drepana\_falcataria TTCAACAGAGTGAGCACTAAAATTGGAAGTTCATGAAAAGTGTGGTGA [100]  
 Ennomos\_autumnaria TTTAATAGAGTTAGTACGAAAATTGGAAGTTCATGAAAAGTGTGGAGA [100]  
 Epicopeia\_hainesii TTTAATAGGGTCAGCACAAAATTGGTAGTTCATGAAAAGTGTAGGAGA [100]  
 Euparyphasma\_maxima NNN [100]  
 Euthyatira\_pudens TTTAACAGAGTGAGTACTAAAATTGGAAGTTCATGAAAAGTGTGGTGA [100]  
 Habrosyne\_pyritoides TTTAATAGAGTTAGCACAAAATTGGAAGTTCATGAAAAGTGTGGCGA [100]  
 Lyssa\_zampa NNN [100]  
 Maucrauzata\_maxima TTTAATCGAGTTAGCACGAAAATTGGAAGTTCATGAAAAGTGTGGGAGA [100]  
 Microblepsis\_acuminata TTTAATAGAGTAAGCACAAAATTGGAAGTTCATGAAAAGTGTAGGAGA [100]  
 Nordstromia\_grisearia TTCAATAGAGTGAGTACTAAAATTGGAAGTTCATGAAAAGTGTAGGAGA [100]  
 Ochropacha\_duplaris TTTAACAGAGTAAGCACAAAATTGGAAGTTCATGAAAAGTGTGGCGA [100]  
 Oreta\_loochooana TTTAACAGAGTGAGCACAAAATTGGTAGTTCATGAAAAGTGTAGGCGA [100]  
 Oreta\_pulchripes NNN [100]  
 Oreta\_rosea TTTAACAGAGTAAGCACAAAATTGGAAGTTCATGAAAAGTGTGGTGA [100]  
 Oreta\_turpis TTTAACAGAGTTAGTACTAAAATTGGCAGTTCATGAAAAGTGTGGAGA [100]  
 Pseudothyatira\_cym. TTTAATAGAGTAAGCACAAAATTGGAAGTTCATGAAAAGTGTGGCGA [100]  
 Psychostrophia\_melanargia TTTAATAGAGTAAGCACAAAATTGGAGTTCATGAAAAGCGTGGGGA [100]  
 Sabra\_harpagula TTTAACAGAGTAAGTACTAAAATTGGAAGTTCATGAAAAGCGTAGGAGA [100]  
 Nothus\_lunus NNNNNNNNNNNAGTACTAAAATTGGAAGTTCATGAAAAGTGTGGAGA [100]  
 Tethea\_consimilis TTTAACAGAGTAAGCACAAAATTGGAGTTCATGAAAAGTGTGGGGA [100]  
 Tethea\_taiwana TTTAATAGAGTAAGCACAAAATTGGAGTTCATGAAAAGTGTGGGGA [100]  
 Tethea\_or TTTAACAGAGTAAGCACAAAATTAGGAAGTTCATGAAAAGTGTGGGGA [100]  
 Tetheela\_fluctuosa NNN [100]  
 Thyatira\_batis TTTAACAGAGTAAGTACTAAAATTGGAGTTCATGAAAAGTGTGGTGA [100]  
 Thyatira\_batis2 TTTAACAGAGTTAGTACTAAAATTAGGAAGTTCATGAAAAGTGTGGTGA [100]  
 Tridrepana\_flava TTCAATAGAGTGAGTACTAAAATTGGAAGTTCATGAAAAGTGTGGTGA [100]  
 Tridrepana\_unispina TTTAATCGAGTTAGTACTAAAATTGGAAGTTCATGAAAAGTGTGGGGA [100]  
 Watsonalla\_binaria NNN [100]  
 Watsonalla\_cultraria TTTAACAGAGTGAGCACGAAAATTGGAAGTTCATGAAAAGTGTGGTGA [100]  
 Watsonalla\_uncinula NNN [100]  
 Calix\_glaucata NNN [100]  
 Falcaria\_lacertinaria NNN [100]  
 Habrosyne\_aurorina NNN [100]  
 Jodis\_putata NNN [100]  
 Neodaruma\_tamanukii NNN [100]

{ 110 120 130 140 150 }  
 [ . . . . . ]

Accinctapubes\_albifasciata NNNNNNNNNNNNNNNNNNAATTTGAGGAAGCTTTTCAAAAAGCACTAA [150]  
 Agnidra\_scabiosa AGTTATGTCAATTGGCAGAAACTTTGAAGAAGCTTTTCAGAAAAGCATTAA [150]  
 Auzata\_superba AGTTATGTCTATTGGTAGGAACTTTGAAGAAGCATTCCAAAAAGCATTAC [150]  
 Ausaris\_micacea AGTAATGTCAATTGGTAGAAAATTTGAAGAAGCTTTTCAAAAAGCACTCC [150]  
 Ausaris\_palleola GGTTATGTCCATTGGAAGGAAATTTGAAGAAGCATTTCAAAAGCGTTAC [150]  
 Cyclidia\_substigmatica AGTTATGTCAATTGGTAGAAAATTTGAAGAAGCATTCCAAAAAGCATTAA [150]  
 Drepana\_arcuata AGTTATGTCAATAGGAAGAACTTTGAAGAGGCATTTCAGAAAAGCTTTGA [150]  
 Falcaria\_bilineata AGTCATGTCTAGGTAGAAAATTCGAAGAGGCCTTTTCAAAAAGCACTAC [150]  
 Drepana\_curvatula AGTAATGTCAATTGGTAGAAAATTTGAGGAAGCCTTCCAGAAAAGCACTTC [150]  
 Drepana\_curvatula2 AGTAATGTCAATTGGTAGAAAATTTGAGGAAGCGTTCAGAAAAGCACTTC [150]  
 Drepana\_falcataria AGTAATGTCAATTGGTAGAAAATTTGAGGAAGCCTTCCAGAAAAGCACTTC [150]  
 Ennomos\_autumnaria AGTCATGTCAATCGGACAGAACTTCGAAGAAGCATTTCAGAAAAGCCTTGC [150]  
 Epicopeia\_hainesii AGTTATGTCAATTGGAAGGAAATTTGAGGAGGCATTTCAAAAGCATTAC [150]  
 Euparyphasma\_maxima NNNNNNNNNNNNNNNNNNAAAATTTGAAGAGCGTTCAAAAGCATTGC [150]  
 Euthyatira\_pudens AGTTATGTCAATAGGAAGAAAATTTGAAGAAGCTTTTCAAAAAGCATTAC [150]  
 Habrosyne\_pyritoides AGTAATGTCAATAGGAAGAAAATTTGAAGAAGCCTTTTCAAAAAGCATTGC [150]  
 Lyssa\_zampa NNN [150]  
 Maucrauzata\_maxima AGTTATGTCAATTGGCAGAAACTTTGAAGAAGCTTCCAAAAGCATTAC [150]  
 Microblepsis\_acuminata AGTTATGTCAATTGGCAGAACTTCGAAGAAGCTTTCCAGAAAAGCATTAC [150]  
 Nordstromia\_grisearia AGTTATGTCAATTGGTAGGAACTTTGAAGAAGCTTCCAGAAAAGCATTAC [150]  
 Ochropacha\_duplaris AGTTATGTCAATAGGAAGAAAATTCGAAGAAGCTTTTCAAAAAGCATTGC [150]  
 Oreta\_loochooana AGTTATGTCAATTGGCAGGAACTTTGAAGAAGCTTCCAAAAGCATTAC [150]  
 Oreta\_pulchripes NNNNNNNNNNGATAGGTAGAAAATTCGAAGAGGCCTTTCAAAAAGCACTAC [150]  
 Oreta\_rosea AGTAATGTCAATTGGTAGAAAATTTGAGGAAGCCTTCCAGAAAAGCACTTC [150]  
 Oreta\_turpis AGTCATGTCTAGGTAGAAAATTCGAAGAGGCCTTTTCAAAAAGCACTAC [150]  
 Pseudothyatira\_cym. AGTTATGTCAATAGGAAGAAAATTTGAAGAGGCCTTTTCAAAAAGCATTGC [150]  
 Psychostrophia\_melanargia AGTGATGTCTATTGGCAGAACTTTGAGGAGGCCTTTTCAAAAAGCATTAA [150]  
 Sabra\_harpagula AGTTATGTCAATTGGCAGGAACTTTGAAGAAGCTTCCAGAAAAGCACTAC [150]  
 Nothus\_lunus AGTAATGTCTATTGGTAGAAAATTTGAAGAAGCATTCCAGAAAAGCTTTAC [150]  
 Tethea\_consimilis AGTGATGTCAATAGGAAGAAAATTTGAAGAAGCTTTTCAAAAAGCATTGC [150]  
 Tethea\_taiwana AGTCATGTCAATAGGAAGAAAATTTGAAGAGGCCTTTTCAAAAAGCATTGC [150]  
 Tethea\_or AGTAATGTCAATAGGAAGAAAATTTGAAGAGGCCTTTTCAAAAAGCATTAC [150]  
 Tetheela\_fluctuosa NNNNNNNGTCATAGGAAGAACTTTGAAGAGGCCTTTTCAAAAAGCATTAC [150]  
 Thyatira\_batis AGTGATGTCAATTGGAAGAAAATTTGAAGAAGCTTTTCAAAAAGCATTAC [150]

Thyatira_batis2	AGTGATGTCAATTGGAAGAAATTTGAAGAAGCTTTTCAAAAAGCTTTAC	[ 150 ]
Tridrepana_flava	AGTAATGTCAATTGGAAGAAATTTGAAGAAGCTTTCCAAAAAGCTTTAA	[ 150 ]
Tridrepana_unispina	AGTAATGTCCGATTGGCAGGAACCTTGAAGAAGCTTTCCAAAAAGCATTAA	[ 150 ]
Watsonalla_binaria	AGTTATGTCAATTGGCAGAACTTTGAAGAAGCTTTCCAAAAAGCATTAC	[ 150 ]
Watsonalla_cultraria	NNNTAC	[ 150 ]
Watsonalla_uncinula	AGTTATGTCAATTGGTAGAACTTTGAAGAAGCTTTCCAAAAAGCATTAC	[ 150 ]
Cilix_glaucata	NN	[ 150 ]
Falcaria_lacertinaria	NN	[ 150 ]
Habrosyne_aurorina	NN	[ 150 ]
Jodis_putata	NN	[ 150 ]
Neodaruma_tamanukii	NN	[ 150 ]

[	160	170	180	190	200]
[	.	.	.	.	.]

Accinctapubes_albifasciata	GAATGGTTGACGAAAATGTGAATGGTTTCGATCCATATGCGAAAAAATG	[ 200 ]
Agnidra_scabiosa	GAATGGTTGACGAAAATGTGAATGGTTTCGATCCATATGCAAAAAGATT	[ 200 ]
Auzata_superba	GAATGGTTGATGAAAATGTGAATGGTTTCGATCCATATGCAAAAAGAAAT	[ 200 ]
Ausaris_micacea	GAATGGATGATGAAAATGTGAATGGTTTCGATCCCTACGCGAAAAAGATC	[ 200 ]
Ausaris_palleola	GAATGGTCGATGAAAATGTAAATGGATTTCATCCCTACGCAAAAACCATT	[ 200 ]
Cyclidia_substigmaria	GAATGGTAGATGAAAATGTAAACGGATTTCATCCGAATGCAAAAAGAAATA	[ 200 ]
Drepana_arcuata	GAATGGTCGATGAAAATGTGAATGGTTTCGATCCGATGCAAAAAGAAAT	[ 200 ]
Falcaria_bilineata	GAATGGTTGATGAAAATGTAAATGGTTTCGATCCATACGCGAAAAAAGTT	[ 200 ]
Drepana_curvatula	GTATGGTTGATGAAAATGTGAATGGTTTCGATCCCTACGCGAAAAAGATC	[ 200 ]
Drepana_curvatula2	GTATGGTTGATGAAAATGTGAATGGTTTCGATCCCTACGCGAAAAAGATC	[ 200 ]
Drepana_falcataria	GTATGGTTGATGAAAATGTGAATGGTTTCGATCCCTACGCGAAAAAGATC	[ 200 ]
Ennomos_autumnaria	GTATGGTTGATGAAAACGTAATGGTTTCGACCCTAACGCAAAAAGAAAT	[ 200 ]
Epicopeia_hainesii	GAATGGTCGACGAAAATGTAAATGGTTTCGATCCCAATGCTAAGAAAAAT	[ 200 ]
Euparyphasma_maxima	GCATGGTCGATGAAAATGTAAATGGTTTCGACCCAAACGCTAAGAAAAAT	[ 200 ]
Euthyatira_pudens	GTATGGTCGATGAGAATGTAAATGGTTTCGACCCAAATGCTAAGAAAAAT	[ 200 ]
Habrosyne_pyritoides	GTATGGTTGATGAAAATGTAAATGGTTTCGACCCAAACGCTAAGAAAAAT	[ 200 ]
Lyssa_zampa	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCCCAACGCAAAAAGATT	[ 200 ]
Maucrauzata_maxima	GAATGGTTGACGAAAATGTGAATGGTTTCGACCCGATGCGAAAAAAT	[ 200 ]
Microblepsis_acuminata	GAATGGTTGATGAAAATGTAAATGGTTTCGATCCATATGCAAAAAGAAAT	[ 200 ]
Nordstromia_grisearia	GAATGGTTGATGAAAACGTTAATGGTTTCGACCCGATGCAAAAAGAAAT	[ 200 ]
Ochropacha_duplaris	GTATGGTTGATGAAAATGTAAATGGTTTCGACCCAAATCTAAGAAAAAT	[ 200 ]
Oreta_loochooana	GTATGGTTGACGAAAATGTAAACGGTTTCGACCCATATGCAAAAAGAAAT	[ 200 ]
Oreta_pulchripes	GAATGGTTGATGAAAATGTAAATGGTTTCGATCCGATGCGAAAACAGCTG	[ 200 ]
Oreta_rosea	GTATGGTTGATGAAAATGTGAATGGTTTCGATCCCTACGCGAAAAAGATC	[ 200 ]
Oreta_turpis	GAATGGTTGATGAAAATGTAAATGGTTTCGATCCGATGCGAAAAGCTG	[ 200 ]
Pseudothyatira_cym.	GTATGGTTGATGAAAATGTAAATGGTTTCGACCCAAACGCTAAGAAAAAT	[ 200 ]
Psychostrophia_melanargia	GAATGGTTGACGAAAATGTAAACGGTTTCGATCCCTAATGCTAAGAAGATA	[ 200 ]
Sabra_harpagula	GAATGGTTGACGAAAATGTAAATGGTTTCGACCCATATGCAAAAAGAAAT	[ 200 ]
Nothus_lunus	GAATGGTCGATGAAAATGTAAATGGTTTCGATCCAAACGCTAAGAAAAAT	[ 200 ]
Tethea_consimilis	GCATGGTTGATGAAAATGTAAATGGTTTCGACCCAAACGCTAAGAAAAAT	[ 200 ]
Tethea_taiwana	GCATGGTTGATGAAAATGTAAATGGTTTCGACCCAAACGCTAAGAAAAAT	[ 200 ]
Tethea_or	GCATGGTTGATGAAAATGTAAATGGTTTCGACCCAAACGCTAAGAAAAAT	[ 200 ]
Tetheela_fluctuosa	GTATGGTTGATGAAAATGTAAATGGTTTCGACCCAAATGCTAAGAAAAAT	[ 200 ]
Thyatira_batis	GTATGGTCGATGAAAACGTAATGGTTTCGACCCAAACGCAAAAAGAAAT	[ 200 ]
Thyatira_batis2	GTATGGTCGATGAAAACGTAATGGTTTCGACCCAAACGCAAAAAGAAAT	[ 200 ]
Tridrepana_flava	GAATGGTTGATGAGAATGTAAATGGATTTCGATCCATATGCAAAAAGAAAT	[ 200 ]
Tridrepana_unispina	GGATGGTTGATGAAAACGTAATGGATTTCGATCCGATGCAAAAAGAAAT	[ 200 ]
Watsonalla_binaria	GAATGGTTGACGAAAATGTGAATGGTTTCGACCCGATGCGAAAAAAT	[ 200 ]
Watsonalla_cultraria	GAATGGTTGACGAAAATGTGAATGGTTTCGACCCGATGCGAAAAAAT	[ 200 ]
Watsonalla_uncinula	GAATGGTTGACGAAAATGTAAATGGTTTCGACCCGATGCGAAAAAAT	[ 200 ]
Cilix_glaucata	NN	[ 200 ]
Falcaria_lacertinaria	NN	[ 200 ]
Habrosyne_aurorina	NN	[ 200 ]
Jodis_putata	NN	[ 200 ]
Neodaruma_tamanukii	NN	[ 200 ]

[	210	220	230	240	250]
[	.	.	.	.	.]

Accinctapubes_albifasciata	GGATTTTCTGATAAACAAATCGCTGCAACAATAAAAAGCACTGAAGTTGC	[ 250 ]
Agnidra_scabiosa	GGTTTTTCTGATAAACAAATAGCTGCTGCCATAAAAAGTACAGAATTAGA	[ 250 ]
Auzata_superba	GGTTTTTCTGATAAACAAATGCTGCTGCCATAAAAAGCAGAGAATTGGA	[ 250 ]
Ausaris_micacea	GGATTTTCTGATAAACAAATGCTGCTGCTATTAAGAAGTACAGAGCTGGA	[ 250 ]
Ausaris_palleola	GGTTTTTCTGATAAACAAATGCGGTTGCGGATTAAGAAGTACAGAGCTGGA	[ 250 ]
Cyclidia_substigmaria	GGATTTTCAGATAAACAAATTCCTGCTGCTATTAAGAAGTACAGAGAATTAGC	[ 250 ]
Drepana_arcuata	GGTTTTTCTGATAAACAAATGCTGCTGCCATAAAAAGTACTGAATTGGA	[ 250 ]
Falcaria_bilineata	GGCTACTCCGATAAACAAATGCTGCTGCCATAAAAAGTACAGAGAATTAGA	[ 250 ]
Drepana_curvatula	GGTTTTTCTGATAAACAAATGCTGCTGCTATTAAGAAGTACAGAGAATTGGA	[ 250 ]

Drepana_curvatula2	GGTTTTCTGATAAACAAATTCGAGCTGCTATAAAAAGTACAGAATTGGA	[ 250 ]
Drepana_falcataria	GGTTTTCCGATAAACAAATTCGCTGCTATAAAAAGTACAGAATTGGA	[ 250 ]
Ennomos_autumnaria	GGTTTTCTGATAAACAGATAGCAGCTGCCATAAAGAGCACGGAAGTAGC	[ 250 ]
Epicopeia_hainesii	GGTTTTCTGATAAACAAATAGCAGCTGCCATTAAGAGTACTGAAGTAGC	[ 250 ]
Euparyphasma_maxima	GGATTCCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Euthyatira_pudens	GGATTCCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Habrosyne_pyritoides	GGATTCCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Lyssa_zampa	GGCTTCTCCGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Maucrauzata_maxima	GGCTTCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Microblepsis_acuminata	GGCTTCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Nordstromia_grisearia	GGCTTCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Ochropacha_duplaris	GGTTTTCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Oreta_loochooana	GGCTTCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Oreta_pulchripes	GGCTTCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Oreta_rosea	GGTTTTCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Oreta_turpis	GGCTTCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Pseudothyatira_cym.	GGATTCCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Psychostrophia_melanargia	GGTTTTCTGATAAACAAATAGCAGCTGCCATAAAAAGTACTGAAGTAGC	[ 250 ]
Sabra_harpagula	GGCTTCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Nothus_lunus	GGATTCCTGATAAACAAATAGCAGCTGCCATAAAAAGTACTGAAGTAGC	[ 250 ]
Tethea_consimilis	GGATTCCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Tethea_taiwana	GGCTTCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Tethea_or	GGTTTTCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Tetheela_fluctuosa	GGTTTTCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Thyatira_batis	GGATTCCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Thyatira_batis2	GGATTCCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Tridrepana_flava	GGATTCCTGATAAACAAATAGCAGCTGCCATAAAAAGTACTGAAGTAGC	[ 250 ]
Tridrepana_unispina	GGATTCCTGATAAACAAATAGCAGCTGCCATAAAAAGTACTGAAGTAGC	[ 250 ]
Watsonalla_binaria	GGTTTTCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Watsonalla_cultraria	GGCTTCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Watsonalla_uncinula	GGCTTCTGATAAACAAATTCGCTGCTGCAATAAAAAGTACTGAAGTAGC	[ 250 ]
Cilix_glauca	NN	[ 250 ]
Falcaria_lacertinaria	NN	[ 250 ]
Habrosyne_aurorina	NN	[ 250 ]
Jodis_putata	NN	[ 250 ]
Neodaruma_tamanukii	NN	[ 250 ]

[	260	270	280	290	300]
[	.	.	.	.	.]

Accinctapubes_albifasciata	TGTGAGAAAATAAGAGAAGATAATCAGATTACTCCTTTTGTAAAAAAA	[ 300 ]
Agnidra_scabiosa	CGTGAGGAAGTTGAGAGAAGAAATTAATAATACACCATTGTAACAAA	[ 300 ]
Auzata_superba	TGTAAGGAAGTTAAGAGAAGAAATTAAGATTACACCATTGTTAAACAAA	[ 300 ]
Ausaris_micacea	CGTGAGAAAATTAAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Ausaris_palleola	TGTAAGAAAAGTTAAGGGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Cyclidia_substigmata	GGTAAGAAAAGTTGAGAGAGGAATACAAAATCACACCATTGTTAAACAAA	[ 300 ]
Drepana_arcuata	CGTAAGAAAATTAAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Falcaria_bilineata	TGTGAGAAAAGCTAAGAGAGGAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Drepana_curvatula	CGTGAGAAAATTAAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Drepana_curvatula2	CGTGAGAAAATTAAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Drepana_falcataria	CGTGAGAAAATTAAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Ennomos_autumnaria	CGTAAGAAAACCTAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Epicopeia_hainesii	AGTTAGAAAATTAAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Euparyphasma_maxima	CGTGAGAAAATTAAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Euthyatira_pudens	CGTGAGAAAATTAAGGGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Habrosyne_pyritoides	CGTGAGAAAATTAAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Lyssa_zampa	TGTGAGAAAATAAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Maucrauzata_maxima	TGTGAGAAAATTAAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Microblepsis_acuminata	CGTAAGGAAGTTAAGAGAAGAAATTAAGATAACACCATTGTTAAACAAA	[ 300 ]
Nordstromia_grisearia	CGTGAGAAAATTAAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Ochropacha_duplaris	CGTGCGAAAATTAAGGGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Oreta_loochooana	TGTAAGAAAAGTTAAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Oreta_pulchripes	TGTGCGAAAATTAAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Oreta_rosea	CGTGAGAAAATTAAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Oreta_turpis	TGTGCGAAAATTAAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Pseudothyatira_cym.	CGTGAGAAAATTAAGGGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Psychostrophia_melanargia	TGTTAGAAAATTAAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Sabra_harpagula	TGTGAGAAAAGTTGAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Nothus_lunus	TGTAAGAAAACCTAGAGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Tethea_consimilis	TGTCCGAAAAGTTAAGGGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Tethea_taiwana	TGTGCGAAAATTAAGGGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Tethea_or	TGTGCGAAAAGTTAAGGGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]
Tetheela_fluctuosa	CGTGCGAAAATTAAGGGAAGAAATTAATAATACACCATTGTTAAACAAA	[ 300 ]

Thyatira\_batis CGTAAGAAAATTAAGGGAAGAACACAAAATCACACCATTGTGTCAGCAAAA [ 300 ]  
 Thyatira\_batis2 CGTAAGAAAATTAAGGGAAGAACACAAAATCACACCATTGTGTCAGCAAAA [ 300 ]  
 Tridrepana\_flava CGTGAGAAAATTAAGAGAAGAATTTAAGATTACACCGTTGTGTCAGCAAAA [ 300 ]  
 Tridrepana\_unispina CGTCAGGAAGTTAAGAGAAGAGTTCAAGATTACTCCGTTGTGTAAGCAAAA [ 300 ]  
 Watsonalla\_binaria TGTGAGGAAATTAAGAGAAGAATTTAAAATAACGCCTTTGTGTAAGCAAAA [ 300 ]  
 Watsonalla\_cultraria TGTGAGGAAATTAAGAGAAGAATTTAAAATAACGCCTTTGTGTAAGCAAAA [ 300 ]  
 Watsonalla\_uncinula TGTGAGGAAATTAAGAGAAGAATTTAAAATAACGCCTTTGTGTAAGCAAAA [ 300 ]  
 Cilix\_glaucata NNN [ 300 ]  
 Falcaria\_lacertinaria NNN [ 300 ]  
 Habrosyne\_aurorina NNN [ 300 ]  
 Jodis\_putata NNN [ 300 ]  
 Neodaruma\_tamanukii NNN [ 300 ]

[ 310 320 330 340 350 ]  
 [ . . . . . ]

Accinctapubes\_albifasciata TTGATACGGTGGCTGCGGAATGGCCTGCTTCAACAAATATTGTGATCTA [ 350 ]  
 Agnidra\_scabiosa TTGATACAGTAGCAGCTGAATGGCCTGCATCAACAAATATCTATCTTTG [ 350 ]  
 Auzata\_superba TCGATACAGTGGCAGCAGAATGGCCTGCATCAACAAATATCTATCTTTG [ 350 ]  
 Ausaris\_micacea TTGATACAGTAGCTGCTGAATGGCCGGCATCTACAAATATTGTGACTTG [ 350 ]  
 Ausaris\_palleola TCGATACAGTGGCAGCTGAATGGCCAGCATCAACAAATATTGTGACTTG [ 350 ]  
 Cyclidia\_substigmaria TTGATACAGTGGCAGCAGAATGGCCTGCGTCCACTAATTATCTTTATCTC [ 350 ]  
 Drepana\_arciata TAGACACAGTAGCAGCTGAATGGCCTGCGTCAACTAATATTTATTTG [ 350 ]  
 Falcaria\_bilineata TTGATACAGTGGCAGCTGAATGGCCTGCATCTACTACTACTACTTTG [ 350 ]  
 Drepana\_curvatula TTGATACAGTAGCGGCTGAATGGCCAGCATCAACAAATATCTGTACTTG [ 350 ]  
 Drepana\_curvatula2 TTGATACAGTAGCGGCTGAATGGCCAGCATCAACAAATATTGTGACTTG [ 350 ]  
 Drepana\_falcataria TTGATACAGTAGCGGCTGAATGGCCAGCATCAACAAATATCTGTACTTG [ 350 ]  
 Ennomos\_autumnaria TTGATACAGTGGCAGCTGAATGGCCCGCTCCACAAACTATCTTTATTTG [ 350 ]  
 Epicopeia\_hainesii TTGATACTGTTGCTGCTGAATGGCCAGCATCGACTAATTATCTTTATTTG [ 350 ]  
 Euparyphasma\_maxima TTGATACAGTAGCTGCGGAATGGCCTGCCTTACCAATACCTTTACTTTA [ 350 ]  
 Euthyatira\_pudens TAGATACAGTAGCAGCGGAATGGCCTGCCTTACCAACTACCTTTATTTA [ 350 ]  
 Habrosyne\_pyritoides TTGATACAGTAGCTGCGGAATGGCCTGCCACCCTAATCTTTACTTTA [ 350 ]  
 Lyssa\_zampa TAGATACAGTAGCTGCGGAGTGGCCTGCTTCCACAAATACCTTACTTTA [ 350 ]  
 Maucrauzauta\_maxima TTGATACAGTAGCAGCTGAATGGCCTGCATCAACAAATATCTATACTTG [ 350 ]  
 Microblepsis\_acuminata TTGATACAGTAGCAGCGGAATGGCCTGCATCGACAAATATCTATACTTG [ 350 ]  
 Nordstromia\_grisearia TTGATACAGTAGCTGCGGAATGGCCTGCATCAACAAATATCTGTATTTG [ 350 ]  
 Ochropacha\_duplaris TTGATACTGTAGCAGCGGAATGGCCTGCCTTACCAACTACCTTTATTTA [ 350 ]  
 Oreta\_loochooana TAGACACAGTAGCAGCTGAATGGCCTGCGTCAACAAATATTGTGACTTG [ 350 ]  
 Oreta\_pulchripes TTGATACAGTAGCAGCTGAGTGGCCTGCATCAACGAACTATCTGTACTTG [ 350 ]  
 Oreta\_rosea TTGATACAGTAGCGGCTGAATGGCCAGCATCAACAAATATCTGTACTTG [ 350 ]  
 Oreta\_turpis TTGATACAGTAGCAGCTGAATGGCCTGCATCAACGAACTATCTGTACTTG [ 350 ]  
 Pseudothyatira\_cym. TTGATACAGNN [ 350 ]  
 Psychostrophia\_melanargia TTGACACTGTAGCTGCTGAATGGCCAGCGTCAACTAATCTATCTTATTTA [ 350 ]  
 Sabra\_harpagula TTGATACAGTAGCTGCTGAATGGCCTGCATCAACAAATATCTATACTTG [ 350 ]  
 Nothus\_lunus TAGATACTGTAGCAGCTGAATGGCCGCACTACAAATATTGTGACTTT [ 350 ]  
 Tethea\_consimilis TCGATACAGTAGCAGCGGAATGGCCTGCTTCTACTAATATCTTTACTTTA [ 350 ]  
 Tethea\_taiwana TCGATACAGTAGCAGCGGAATGGCCTGCCTTACTAATCTTTACTTTA [ 350 ]  
 Tethea\_or TCGATACAGTAGCAGCGGAATGGCCTGCCTTACTAATCTTTACTTTA [ 350 ]  
 Tetheela\_fluctuosa TTGATACAGTAGCAGCGGAATGGCCTGCTACTACTAATCTTTACTTTA [ 350 ]  
 Thyatira\_batis TTGACACAGTAGCTGCGGAATGGCCTGCCTCCACTAATCTTTACTTTA [ 350 ]  
 Thyatira\_batis2 TTGACACAGTAGCTGCGGAATGGCCTGCCTCCACTAATCTTTACTTTA [ 350 ]  
 Tridrepana\_flava TAGATACAGTAGCAGCTGAATGGCCTGCATCAACAAATATCTGTACTTG [ 350 ]  
 Tridrepana\_unispina TTGATACCGTAGCAGCGGAATGGCCTGCATCAACAAATATTGTGACTTTA [ 350 ]  
 Watsonalla\_binaria TTGATACAGTAGCAGCTGAATGGCCTGCATCAACAAATATTGTGACTTTG [ 350 ]  
 Watsonalla\_cultraria TTGATACAGTAGCAGCTGAATGGCCTGCATCAACAAATATTGTGACTTTG [ 350 ]  
 Watsonalla\_uncinula TTGATACAGTAGCAGCAGAATGGCCTGCATCAACAAATATTGTGACTTTG [ 350 ]  
 Cilix\_glaucata NNN [ 350 ]  
 Falcaria\_lacertinaria NNN [ 350 ]  
 Habrosyne\_aurorina NNN [ 350 ]  
 Jodis\_putata NNN [ 350 ]  
 Neodaruma\_tamanukii NNN [ 350 ]

[ 360 370 380 390 400 ]  
 [ . . . . . ]

Accinctapubes\_albifasciata ACATATAATGGTAGTACACATGACTTGGATTTCCCTGGAGAGTTCACAAT [ 400 ]  
 Agnidra\_scabiosa ACGTATAACGCAAATCTAATGATTTAGACTTTCCCTGGAAATTTTCATAAT [ 400 ]  
 Auzata\_superba ACGTATAATGGTAATACACATGATTTAGAGTTCCCTGGTAATTTCACTAT [ 400 ]  
 Ausaris\_micacea ACATATAACGGAAATACCCAGCAGCTAGAGTTCCCTGGTAATTTCACTAT [ 400 ]  
 Ausaris\_palleola ACCTACAATGGTACCAGCAGACTTAGAATTTCCCTGGTAATTTCACTAT [ 400 ]  
 Cyclidia\_substigmaria ACATATAACGGTAGCAGCAGCTGATTTAGAATTTCCCTGGAGATTTGTAAAT [ 400 ]  
 Drepana\_arciata ACATATAACGGTAACAGCAGCTGATTTAGATTTCCAGGGAAATTTTCACAAT [ 400 ]  
 Falcaria\_bilineata ACGTATAATGGTAATACACATGATTTAGTGTTCCTGGAAATTTTACTAT [ 400 ]

Drepana_curvatula	ACATATAACGGAAATACATCATGATTTAGAGTTTCCTGGTAATTTCACTAT	[ 400 ]
Drepana_curvatula2	ACATATAACGGAAATACATCATGATTTAGAGTTTCCTGGTAATTTCACTAT	[ 400 ]
Drepana_falcataria	ACATATAACGGAAATACATCATGATTTAGAGTTTCCTGGTAATTTCACTAT	[ 400 ]
Ennomos_autumnaria	ACGTACAATGGCACAACATCATGATTTAGAAATCCAGGCGATTTGACTAT	[ 400 ]
Epicopeia_hainesii	ACTTACAACGGGAGCTCACATGACTTGGAAATCCAGGAGATTTTATCAT	[ 400 ]
Euparyphasma_maxima	ACATACAATGGTAGTACACATGATCTCGAATTCCTGGAAACTTTGTTAT	[ 400 ]
Euthyatira_pudens	ACATATAATGGTAGTACACATGATCTCGAATTCCTGGAAACTTTGTTAT	[ 400 ]
Habrosyne_pyritoides	ACTTATAATGGCAGCACACATGATCTCGAATTCCTGGAAACTTTGTTAT	[ 400 ]
Lyssa_zampa	ACGTACAATGGAAAGTTCGCATGATTTAGATTTCCAGAAAGGTTTCGTTAT	[ 400 ]
Maucrauzata_maxima	ACATATAACGGGAATACCCAGCACTTAGATTTCCCGGGAATTTCACAAT	[ 400 ]
Microblepsis_acuminata	ACATATAACGGGAATACCGCATGACTTGGAAATTCCTGGGAATTTCCAGAT	[ 400 ]
Nordstromia_grisearia	ACATACAATGGGAATTCGCACGATTTAGAGTTTCCTGGAAATTTCACAAT	[ 400 ]
Ochropacha_duplaris	ACTTATAATGGTAGTACACATGACCTTGAATTCCTGGAAACTTTGTTAT	[ 400 ]
Oreta_loochooana	ACATATAATGGGAACCGCATGATTTAGATTTCCAGGGAATTTCACAAT	[ 400 ]
Oreta_pulchripes	ACGTATAACGGTACTACTCAGGATTTAGACTTCCCTGGTACTGCTATAAT	[ 400 ]
Oreta_rosea	ACATATAACGGAAATACATCATGATTTAGAGTTTCCTGGTAATTTCACTAT	[ 400 ]
Oreta_turpis	ACGTATAACGGTACTACTCAGGATTTAGACTTCCCTGGTACTGCTATAAT	[ 400 ]
Pseudothyatira_cym.	NN	[ 400 ]
Psychostrophia_melanargia	ACTTATAATGGCAGCAGCAGCAGCTTGGAAATTCAGGAGAGTTTATAAT	[ 400 ]
Sabra_harpagula	ACTTATAACGGAAACACACAGGATTTGGATTTTCTGGGAATTTACGTAAT	[ 400 ]
Nothus_lunus	ACATATAATGGCAGTACCGCAGCAGCTTAGAGTTTCCTGGCGAGTTTGTAT	[ 400 ]
Tethea_consimilis	ACTTATAATGGTAGTATACACGACCTTGAATTCCTGGAAATTTTGTGAT	[ 400 ]
Tethea_taiwana	ACTTATAATGGCTGTATACACGATCTTGAATTCCTGGAAATTTATGTCAT	[ 400 ]
Tethea_or	ACTTATAACGGTAGTATACACGATGATTTAGATTTCCCTGGAAATTTGTCAT	[ 400 ]
Tetheela_fluctuosa	ACTTATAATGGTTGTACACATGACCTTGAATTCCTGGAAACTTTGTTAT	[ 400 ]
Thyatira_batis	ACTTATAACGGTAGCACACATGATCTTGAATTCCTGGAAATTTATGTAAT	[ 400 ]
Thyatira_batis2	ACTTATAACGGTAGCACACATGATCTTGAATTCCTGGAAATTTATGTAAT	[ 400 ]
Tridrepana_flava	ACATACAACGGCAATACACATGACTTAGACTTTCAGGAAACTTCACTAT	[ 400 ]
Tridrepana_unispina	ACGTACAATGGTAGTACACATGATTTAGATTTCCAGGAAATTTACCAT	[ 400 ]
Watsonalla_binaria	ACATATAACGGGAATACCCAGCACTTAGATTTTCCTGGGAATTTCACAAT	[ 400 ]
Watsonalla_cultraria	ACATATAACGGGAATACCCAGCACTTAGATTTTCCTGGGAATTTCACAAT	[ 400 ]
Watsonalla_uncinula	ACATATAACGGGAATACCCAGCACTTAGATTTTCCTGGGAATTTCACAAT	[ 400 ]
Cilix_glaucata	NN	[ 400 ]
Falcaria_lacertinaria	NN	[ 400 ]
Habrosyne_aurorina	NN	[ 400 ]
Jodis_putata	NN	[ 400 ]
Neodaruma_tamanukii	NN	[ 400 ]
{	410 420 430 440 450]	
{	. . . . .]	
Accinctapubes_albifasciata	GGTTCCTGGATCGGGTGTTCACAGAATAGGTAGTTCTGTGGAAATTTGATT	[ 450 ]
Agnidra_scabiosa	GGTATTTGGGATCAGGTGTATACAGAATAGGAAGCTCGGTTGNNNNNNNNNN	[ 450 ]
Auzata_superba	GGTCCTAGGATCGGGTGTATATAGAATAGGAAGTTCTGTTGAATTCGACT	[ 450 ]
Ausaris_micacea	GGTACTGGATCAGGTGTATACAGAATAGGAAGTTCCGTTGAATTTGATT	[ 450 ]
Ausaris_palleola	GGTTTTGGGATCTGGTGTGTACAGAATAGGTAGTTCTGTGCAATTTGACT	[ 450 ]
Cyclidia_substigmata	GGTTTTAGGATCAGGAGTTTACAGAATAGGAAGCTCTGTTGAATTTGATT	[ 450 ]
Drepana_arcuata	GGTCTTAGGATCAGGAGTTTACAGAATAGGAAGCTCTGTTNNNNNNNNNN	[ 450 ]
Falcaria_bilineata	GGTTCCTAGGTTCTGGTGTATATAGAATAGGAAGCTCAGTTGAATTTGATT	[ 450 ]
Drepana_curvatula	GGTACTGGGATCAGGTGTATACAGAATAGGAAGTTCTGTTGAATTCGACT	[ 450 ]
Drepana_curvatula2	GGTACTGGGATCAGGTGTATACAGAATAGGAAGTTCTGTTGAATTCGACT	[ 450 ]
Drepana_falcataria	GGTACTGGGATCAGGTGTATACAGAATAGGAAGTTCTGTTGAATTCGACT	[ 450 ]
Ennomos_autumnaria	GGTACTTGGATCAGGAGTTTACCAGAAATGGCAGCTCTGTAGAATTTGATT	[ 450 ]
Epicopeia_hainesii	GGTATTAGGATCAGGCGTATACAGAATAGGAAGCTCCGTAGAATTTGATT	[ 450 ]
Euparyphasma_maxima	GGTTTTAGGATCAGGTGTATACAGAATAGGAAGCTCCGTAGAATTTGATT	[ 450 ]
Euthyatira_pudens	GGTTTTAGGATCAGGTGTATATAGAATTTGGGAGCTCCGTAGAATTTGATT	[ 450 ]
Habrosyne_pyritoides	GGTTTTAGGATCAGGTGTATACAGAATAGGAAGCTCAGTAGAATTTGATT	[ 450 ]
Lyssa_zampa	GGTCTCGGATCAGGTGTATACAGAATAGGAAGTTCCGTGGAAATTCGATT	[ 450 ]
Maucrauzata_maxima	GGTTCCTGGGATCAGGTGTCTACAGAATAGGAAGCTCTGTTGAATTTGATT	[ 450 ]
Microblepsis_acuminata	GGTTTTGGGGTCCGGTGTATATAGAATTTGGGAGCTCGGTTGAATTTGACT	[ 450 ]
Nordstromia_grisearia	GGTACTTGGATCAGGTGTATATAGAATAGGAAGCTCAGTTGAATTTGACT	[ 450 ]
Ochropacha_duplaris	GGTTTTAGGATCAGGAGTTTACAGAATAGGAAGCTCAGTTGAATTTGATT	[ 450 ]
Oreta_loochooana	GGTCTTAGGATCAGGAGTTTACAGAATAGGAAGCTCTGTTGAATTTGATT	[ 450 ]
Oreta_pulchripes	GGTCTTAGGATCAGGAGTTTACAGAATAGGAAGCTCTGTTGAATTTGATT	[ 450 ]
Oreta_rosea	GGTACTGGGATCAGGTGTATACAGAATAGGAAGTTCTGTTGAATTTGACT	[ 450 ]
Oreta_turpis	GGTCTTAGGATCAGGAGTTTACAGAATAGGAAGTTCTGTTGAATTTGACT	[ 450 ]
Pseudothyatira_cym.	NN	[ 450 ]
Psychostrophia_melanargia	GGTGTTAGGGTCCGGCTGTATAGAATAGGAAGTTCTGTTGAATTTGATT	[ 450 ]
Sabra_harpagula	GGTCTTAGGATCAGGAGTTTACAGAATAGGAAGCTCAGTTGAATTTGATT	[ 450 ]
Nothus_lunus	GGTTTTAGGATCAGGTGTATACAGAATAGGAAGTTCTGTTGAATTTGATT	[ 450 ]
Tethea_consimilis	GGTTTTAGGATCAGGTGTATATAGAATTTGGGAGCTCCGTAGAATTTGATT	[ 450 ]
Tethea_taiwana	GGTTTTAGGATCAGGTGTATATAGAATTTGGGAGCTCCGTAGAATTTGATT	[ 450 ]
Tethea_or	GGTTTTAGGATCAGGTGTATATAGAATTTGGGAGCTCCGTAGAATTTGATT	[ 450 ]



Falcaria_bilineata	ATCATGGTTAACTACAATCCTGAAACTGTTAGTACTGATTATGATATGAG	[ 550 ]
Drepana_curvatula	ATAATGGTTAACTACAATCCTGAAACTGTTAGTACTGATTATGATATGAG	[ 550 ]
Drepana_curvatula2	ATAATGGTTAACTATAATCCTGAAACTGTTAGTACTGATTATGATATGAG	[ 550 ]
Drepana_falcataria	ATAATGGTTAACTATAATCCTGAAACTGTTAGTACTGATTATGATATGAG	[ 550 ]
Ennomos_autumnaria	ATTATGGTCAATTACAACCCTGAAACTGTGAGTACTGACTATGATATGAG	[ 550 ]
Epicopeia_hainesii	ATAATGGTTAACTACAATCCTGAAACTGTGAGTACTGATTATGATATGAG	[ 550 ]
Euparyphasma_maxima	NN	[ 550 ]
Euthyatira_pudens	ATTATGGTTAACTATAACCCCTGAGACTGTTAGTACCGACTATGACATGAG	[ 550 ]
Habrosyne_pyritoides	ATTATGGTAAATATAACCCCTGAGACGGTAAGTACTGATTATGACATGAG	[ 550 ]
Lyssa_zampa	NN	[ 550 ]
Maucrauzata_maxima	ATAATGGTTAACTATAATCCTGAAACTGTGAGCAGACTATGACATGAG	[ 550 ]
Microblepsis_acuminata	ATAATGGTTAACTATAATCCTGAAACTGTGAGTACTGACTATGATATGAG	[ 550 ]
Nordstromia_grisearia	ATAATGGTTAACTACAACCCTGAAACTGTGAGTACTGACTATGATATGAG	[ 550 ]
Ochropacha_duplaris	ATTATGGTCAATTATAACCCCTGAGACTGTTAGTACTGACTATGACATGAG	[ 550 ]
Oreta_loochooana	ATCATGATTAATTACAACCCTGAAACAGTCACTGATTACGATATGAG	[ 550 ]
Oreta_pulchripes	ATAATGGTAACTACAATCCAGAACTGTAAGCAGACTACGACATGAG	[ 550 ]
Oreta_rosea	ATAATGGTTAACTACAATCCTGAAACTGTTAGTACTGATTATGATATGAG	[ 550 ]
Oreta_turpis	ATAATGGTAACTACAATCCAGAACTGTGAGCAGACTACGACATGAG	[ 550 ]
Pseudothyatira_cym.	NN	[ 550 ]
Psychostrophia_melanargia	ATTATGGTCAACTACAATCCAGAGACCGTCACTGATTACGATATGAG	[ 550 ]
Sabra_harpagula	GTAATGGTTAACTACAATCCAGAACTGTAAGTACTGACTATGACATGAG	[ 550 ]
Nothus_lunus	ATTATGATTAATTATAATCCGAACTGTGAGTACTGACTATGATATGAG	[ 550 ]
Tethea_consutilis	ATCATGGTAAATATAACCCCTGAGACTGTTAGTACTGACTATGACATGAG	[ 550 ]
Tethea_taiwana	ATCATGATAAATATAACCCCTGAGACTGTTAGTACTGATTATGACATGAG	[ 550 ]
Tethea_or	ATCATGGTAAATATAACCCCTGAGACTGTTAGTACTGACTATGACATGAG	[ 550 ]
Tetheela_fluctuosa	ATTATGGTAAATATAACCCCTGAGACTGTTAGTACTGACTATGACATGAG	[ 550 ]
Thyatira_batis	NN	[ 550 ]
Thyatira_batis2	ATTATGGTAAATATAACCCCTGAGACTGTTAGTACTGATTATGACATGAG	[ 550 ]
Tridrepana_flava	ATAATGGTTAACTACAACCAGAACTGTGAGTACTGACTATGACATGAG	[ 550 ]
Tridrepana_unispina	ATAATGGTCAACTACAACCAGAACTGTGAGTACTGACTATGACATGAG	[ 550 ]
Watsonalla_binaria	NN	[ 550 ]
Watsonalla_cultraria	ATAATGGTTAACTATAATCCTGAAACTGTGAGCAGACTATGACATGAG	[ 550 ]
Watsonalla_uncinula	ATCATGGTTAACTATAATCCTGAAACTGTGAGCAGACTATGACATGAG	[ 550 ]
Cilix_glaucata	NN	[ 550 ]
Falcaria_lacertinaria	NN	[ 550 ]
Habrosyne_aurorina	NN	[ 550 ]
Jodis_putata	NN	[ 550 ]
Neodaruma_tamanukii	NN	[ 550 ]

[	560	570	580	590	600]
[	.	.	.	.	.]

Accinctapubes_albifasciata	NN	[ 600 ]
Agnidra_scabiosa	NN	[ 600 ]
Auzata_superba	TGACAGATTGACTTTGAAGAAATTCATTGAGGTTGTAATGGATATTT	[ 600 ]
Ausaris_micacea	NN	[ 600 ]
Ausaris_palleola	TGACCGATTATATTTGGAAGAAATATCTTTGAGGTTGTTATGGATATTT	[ 600 ]
Cyclidia_substigmaria	TGACAGATTGATTTGGAAGAAATTCCTTTGAGGTTGTTATGGATATTT	[ 600 ]
Drepana_arcuata	NN	[ 600 ]
Falcaria_bilineata	TGATCGATTGACTTTGGAAGAAATATCATTGAGGTTGTTATGGATATTT	[ 600 ]
Drepana_curvatula	CGACAGACTGTATTTGGAAGAGATATCATTGAGGTTGTTATGGATATTT	[ 600 ]
Drepana_curvatula2	CGACAGACTGTATTTGGAAGAGATATCATTGAGGTTGTTATGGATATTT	[ 600 ]
Drepana_falcataria	CGACAGACTGTATTTGGAAGAGATATCATTGAGGTTGTTATGGATATTT	[ 600 ]
Ennomos_autumnaria	CGACCGGCTTATTTGGAAGAAATATCCTTTGAGGTTAAANNNNNNNNNN	[ 600 ]
Epicopeia_hainesii	CGACAGATTATACTTTGGAAGAAATTCCTTTGAGGTTGTTATGGATATTT	[ 600 ]
Euparyphasma_maxima	NN	[ 600 ]
Euthyatira_pudens	TGACAGATTGACTTTGGAAGAAATATCCTTTGAGGTTGTTATGGATATTT	[ 600 ]
Habrosyne_pyritoides	TGACAGATTGACTTTGGAAGAAATATCCTTTGAGGTTGTTATGGATATTT	[ 600 ]
Lyssa_zampa	NN	[ 600 ]
Maucrauzata_maxima	TGATCGATTGACTTTGGAAGAAATATCATTGAGGTTGTTATGGATATTT	[ 600 ]
Microblepsis_acuminata	TGATCGATTGATTTGGAAGAAATATCATTGAGGTTGTTATGGATATTT	[ 600 ]
Nordstromia_grisearia	TGATCGATTGACTTTGGAAGAAATATCCTTTGAGGTTGTTATGGATATTT	[ 600 ]
Ochropacha_duplaris	TGACAGGTTGACTTTGGAAGAAATATCCTTTGAGGTTGTTATGGATATTT	[ 600 ]
Oreta_loochooana	TGACCGGTTATACTTGAAGAAATATCATTGAGGTTGTTATGGATATTT	[ 600 ]
Oreta_pulchripes	CGACAGATNN	[ 600 ]
Oreta_rosea	CGACAGACTGTATTTGGAAGAGATATCATTGAGGTTGTTATGGATATTT	[ 600 ]
Oreta_turpis	CGACAGATTATACTTTGGAAGAAATATCCTTTGAGGTTGTTATGGATATTT	[ 600 ]
Pseudothyatira_cym.	NN	[ 600 ]
Psychostrophia_melanargia	TGACAGATTGACTTTGGAAGAAATCTCATTGAGGTTGTTATGGATATTT	[ 600 ]
Sabra_harpagula	CGACCGATTGATTTGGAAGAAATATCATTGAGGTTGTTATGGATATTT	[ 600 ]
Nothus_lunus	TGATAGATTATATTTGGAAGAAANNNNNNNNNNNNNNNNNNNNNNNNNNNNN	[ 600 ]
Tethea_consutilis	TGATAGATTGACTTTGGAAGAAATATCCTTTGAGGTTGTTATGGATATTT	[ 600 ]
Tethea_taiwana	TGATAGATTATATTTGGAAGAAATATCCTTTGAGGTTGTTATGGATATTT	[ 600 ]



*Drepana arcuata* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCACTCCGC [700]  
*Falcaria bilineata* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCACTCCGC [700]  
*Drepana curvatula* AAACCTGCGAAACTCGAATGAGTGAACGGGGAGATTCATCGTAGCTCCG [700]  
*Drepana curvatula2* AAACCTGCGAAACTCGAATGAGTGAACGGGGAGATTCATCGTAGCTCCG [700]  
*Drepana falcataria* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCACTCCGC [700]  
*Ennomos autumnaria* AAACCTGCGAAACTCGAATGAACGAACGGGAGATTCATCGTCAATCCCTC [700]  
*Epicopeia hainesii* GTACCTGCGAA--CTC-GATGAACGAACGGGGAGATTCATCGTATCCGC [698]  
*Euparyphasma maxima* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCAATCCGC [700]  
*Euthyatira pudens* AAACCTGCGAAACTTGAATGAATGAACGGGGAGATTCATCATCATCCGC [700]  
*Habrosyne pyritoides* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCACTCCGC [699]  
*Lyssa zampa* AAACCTGCGAAACTCGAATGAACGAACGGGAGATTCATCGTATCCGC [700]  
*Maucrauzata maxima* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCACTCCGC [700]  
*Microblepsis acuminata* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCACTCCGC [700]  
*Nordstromia grisearia* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCACTCCG [700]  
*Ochropacha duplaris* NNNNNNNNNNNNNNNAATGAACGAACGGGGAGATTCATCGTCAATCCAC [700]  
*Oreta loochooana* AAACCTGCGATACTCGAATGAACGAACGGGGAGATTCATCGTCAATCCGC [700]  
*Oreta pulchripes* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCAATCCGC [700]  
*Oreta rosea* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCAATCCGC [700]  
*Oreta turpis* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCAATCCGC [700]  
*Pseudothyatira cym.* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCAATCCGC [700]  
*Psychostrophia melanargia* GTAC-TGCGAA--CTC-GATGAACGAACGGGGAGATTCATCGTCACTCCGC [697]  
*Sabra harpagula* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCACTCCGC [700]  
*Nothus lunus* AAACCTGCGAAACTCGAATGAACGAACGGGAGATTCATCGTCACTCCGC [700]  
*Tethea consimilis* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCACTCCGC [700]  
*Tethea taiwana* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCACTCCGC [700]  
*Tethea or* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCAATCCGC [700]  
*Tetheela fluctuosa* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCAATCCCTC [700]  
*Thyatira batis* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCAATCCGC [700]  
*Thyatira batis2* NNN [700]  
*Tridrepana flava* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCACTCCGC [700]  
*Tridrepana unispina* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCACTCCGC [700]  
*Watsonalla binaria* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCACTCCGC [700]  
*Watsonalla cultraria* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCACTCCGC [700]  
*Watsonalla uncinula* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCACTCCGC [700]  
*Cilix glaucata* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCAATCCGC [700]  
*Falcaria lacertinaria* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCACTCCGC [700]  
*Habrosyne aurorina* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCAATCCGC [700]  
*Jodis putata* AAACCTGCGAAACTCGAATGAACGAACGGGAGATTCATCGTCAATCCCTC [700]  
*Neodaruma tamanukii* AAACCTGCGAAACTCGAATGAACGAACGGGGAGATTCATCGTCAATCCGC [700]

[ 710 720 730 740 750 ]  
[ . . . . . ]

*Accinctapubes albifasciata* NNN [750]  
*Agnidra scabiosa* GGCGTACGCGCGGCGGCC--TCGATGTCGAA-ACG-----ATCTC [737]  
*Auzata superba* GGCGTACGTCGCGAGCGCA--ACGATGTCG---CCG-----ATCTC [735]  
*Ausaris micacea* GACGTGAGAGCGTGGT---CTGATGCC-----GACTC [729]  
*Ausaris pallaeola* GCCGTGCGAATGTGTG---TTGATGCT-----GTCTC [729]  
*Cyclidia substigmata* GGCGTACGTCGCGCGGCC--TCGATGTC--GTCCG-----ATCTC [736]  
*Drepana arcuata* GGCGTACGTCGCGCGGCC--TCGATGTCGCTTCCGCGTCCGCCCTCGCGGC [748]  
*Falcaria bilineata* GGCGTACGTCGCGCGGCC--ACGATGTC--GTCCG-----ATCTC [736]  
*Drepana curvatula* GGCGTGAGAACGTGGT---TTGATGCC-----GCCTT [729]  
*Drepana curvatula2* GGCGTGAGAACGTGGT---TTGATGCC-----GCCTT [729]  
*Drepana falcataria* GGCGTACGTCGCGCGGCC--TCGATGTCGCGTCCGCGTCCGCCCTCGCGGC [748]  
*Ennomos autumnaria* GGTGCACTTGCCTGCT---TCGATGTT---ATCG-----GTTTC [734]  
*Epicopeia hainesii* GGCGTACGGGCGCGGCC--TCGATGTCG---CCG-----GTTTC [733]  
*Euparyphasma maxima* GGCGTACTGGCGGTACTTTTCGATGTC---ACCG-----GTCTC [737]  
*Euthyatira pudens* GGCATG---GCGGACGACTCTCGATGCTACTATTG----- [732]  
*Habrosyne pyritoides* GGCGTACGTCGCTGCGCC--TCGATGTCGCG--TCG-----GTTTC [736]  
*Lyssa zampa* GGCGGCTGCGACGTCT---GCGATGCG--GTTCC-----GTTTC [735]  
*Maucrauzata maxima* GGCGTACGTCGCGCGGCC--ATCGATGTC---ACCG-----ATTTT [736]  
*Microblepsis acuminata* GGCGTACGTCGCGCGGCC--TCGATGTC---ACCG-----ATCTC [735]  
*Nordstromia grisearia* GGCGTACGCGCGCGGCC--TCGATGTC--TCGCA-----CCCTT [736]  
*Ochropacha duplaris* GGCGTACGGGCGCGGCC--TCGATGTC---ACCG-----ATCTC [735]  
*Oreta loochooana* GGCGTACCGTGGGTGCC---ACGATGTC---ACCG-----ATTTT [735]  
*Oreta pulchripes* GGCGTACCGTGGGTGCC---ACGATGTC---ACCG-----ATTTT [735]  
*Oreta rosea* GGCGTACCGTGGGTGCC---ACGATGTC---GTTCC-----GTTTC [736]  
*Oreta turpis* GGCGTACCGTGGGTGCC---ACGATGTC---ACCG-----ATTTT [735]  
*Pseudothyatira cym.* GGCGTACGTCGCGCGGCC--TCGATGTCG--TCG-----ACTTC [737]  
*Psychostrophia melanargia* GGCGTACGAGCGCGGCC--TCGATGCC--G-CCG-----GTTTC [732]  
*Sabra harpagula* GGCGTACGTCGCGCGGCC--ACGATGTC---ACCG-----ATCTC [735]  
*Nothus lunus* GGCGGACCGGGCGTCTC---TCGATGCC--GGTCG-----GTCTC [736]  
*Tethea consimilis* GGCGTACGGGCGCGGCC--TCGATGTC---ACCG-----ATCTC [735]



Cyclidia\_substigmaria TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 830 ]  
Drepana\_arcuata TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 845 ]  
Falcaria\_bilineata TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 830 ]  
Drepana\_curvatula TGT ACTTCTCCTTTAGTAAACATCGCCACTCGTTTAAAGTACGTCCAAG [ 821 ]  
Drepana\_curvatula2 TGT ACTTCTCCTTTAGTAAACATCGCCACTCGTTTAAAGTACGTCCAAG [ 821 ]  
Drepana\_falcataria TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 845 ]  
Ennomos\_autumnaria TGC ACTTCTCCTTTAGTAAACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 826 ]  
Epicopeia\_hainesii TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 827 ]  
Euparyphasma\_maxima TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 831 ]  
Euthyatira\_pudens TGC ACTTTTCCCTCAGTAATACATCGCGACCTGTTTATGATGCCGGTCTAAG [ 825 ]  
Habrosyne\_pyritoides TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 830 ]  
Lyssa\_zampa TGC ACTTCTCCTTTAGTAAACATCGCGACCCGTTTAGATGTCGGTCTAAG [ 829 ]  
Maucrauzata\_maxima TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 830 ]  
Microblepsis\_acuminata TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 829 ]  
Nordstromia\_grisearia TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 834 ]  
Ochropacha\_duplaris TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 829 ]  
Oreta\_loochooana TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 829 ]  
Oreta\_pulchripes TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 829 ]  
Oreta\_rosea TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 830 ]  
Oreta\_turpis TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 829 ]  
Pseudothyatira\_cym. TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 831 ]  
Psychostrophia\_melanargia TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 826 ]  
Sabra\_harpagula TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 829 ]  
Nothus\_lunus TGC ACTTCTCCTTTAGTAAACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 832 ]  
Tethea\_consimilis TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 829 ]  
Tethea\_taiwana TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 829 ]  
Tethea\_or TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 829 ]  
Tetheela\_fluctuosa TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 829 ]  
Thyatira\_batis TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 829 ]  
Thyatira\_batis2 NNN [ 850 ]  
Tridrepana\_flava TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 830 ]  
Tridrepana\_unispina TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 830 ]  
Watsonalla\_binaria TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 829 ]  
Watsonalla\_cultraria TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 829 ]  
Watsonalla\_uncinula TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 829 ]  
Cilix\_glaucata TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 831 ]  
Falcaria\_lacertinaria TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 830 ]  
Habrosyne\_aurorina TGC ACTTCTCCTTTAGTAAACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 837 ]  
Jodis\_putata TGC ACTTCTCCTTTAGTAAACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 826 ]  
Neodaruma\_tamanukii TGC ACTTCTCCCTCAGTAATACATCGCGACCCGTTTCGATGTCGGTCTAAG [ 829 ]

[ 860 870 880 890 900 ]  
[ . . . . . ]

Accinctapubes\_albifasciata NNN [ 900 ]  
Agnidra\_scabiosa CGCCGTTCCGGGAGCCCATTTGT-----GCCTCTCG-GGGT---CACTTT [ 872 ]  
Auzata\_superba CGCCGTTCCGGGAGCCCATTTGT-----ACCTTTCG-GGGT---CACTTT [ 869 ]  
Ausaris\_micacea TGTCTGTTAAGCAAAGT-TTTTGTTTTACAACAATGAGTTTGTTTTAGC [ 870 ]  
Ausaris\_palleola TGACGTTGGACCTCGTCTCTTTGT---TCTCCTGCGGGTG---CAACAA [ 867 ]  
Cyclidia\_substigmaria CGCAGTTCGGGAGCCCGTCTGTC---CTCTCAACGGGGG---CGCGGT [ 873 ]  
Drepana\_arcuata CGCCGTTCCGGGAGCCCGTTCG---CTCCCTCGGGGA---CGCTGC [ 887 ]  
Falcaria\_bilineata CGCCGTTCCGGGAGCCCATTCGT-----ACCTTTCG-GGGT---CACGGT [ 870 ]  
Drepana\_curvatula CGTCTGCTAAACAAGATTTTGTGTTTGGCCACATTGAGTTTGTTTTCAGC [ 871 ]  
Drepana\_curvatula2 CGTCTGCTAAACAAGATTTTGTGTTTGGCCACATTGAGTTTGTTTTCAGC [ 871 ]  
Drepana\_falcataria CGCCGTTCCGGGAGCCCGTTCG---CTCCTCTCGGGGA---CGCTGC [ 887 ]  
Ennomos\_autumnaria TGCCTTTCGGGAGTCTCCGTTTCTAGTTCGCTAGTTTGGGA----- [ 867 ]  
Epicopeia\_hainesii CGCCGTTCCGGGAGTCCCGTCTCC---CCCTCGCGGGGG---TGGGGC [ 871 ]  
Euparyphasma\_maxima CGCCGTTCCGGGAGCCCATAGTGC---CCCTAGC-GGGA---CACTGT [ 873 ]  
Euthyatira\_pudens CGCCGTACAAGC----- [ 838 ]  
Habrosyne\_pyritoides CGCCGTTCCGGGAGCCCATAGTGC---GCCCTCG-GGGA---CACTGT [ 872 ]  
Lyssa\_zampa CGTCTGACGGGAGCC-----ACCGTTGTGGGTTTCGGCCGC [ 866 ]  
Maucrauzata\_maxima CTCGGTTCGGGAGCCCATTTGT-----CCTTAGCGAGGT---CACTGT [ 872 ]  
Microblepsis\_acuminata CGCCGTTCCGGGAGCCCATTTGT-----ACCCCTCG-GGGT---CACTGT [ 869 ]  
Nordstromia\_grisearia CGCCGTTCCGGGAGCCCGTTCG---CCCTCGCGGGG---CCGCGG [ 877 ]  
Ochropacha\_duplaris CGCCGTTCCGGGAGCCCATTTGCG---ACCCTC---GGC---CGCTGT [ 868 ]  
Oreta\_loochooana CGCCGTTCCGGGAGCCCGTTGT-----GCCCTCAC-GGGT---CGCAGC [ 869 ]  
Oreta\_pulchripes CGCCGTTCCGGGAGCCCGTTGT-----GCCCTCAC-GGGT---CGCAGC [ 869 ]  
Oreta\_rosea CGCCGTTCCGGGAGCCCGTTGT-----GCCCTCAC-GGGT---CATAGC [ 870 ]  
Oreta\_turpis CGCCGTTCCGGGAGCCCGTTGT-----GCCCTCAC-GGGT---CGCAGC [ 869 ]  
Pseudothyatira\_cym. CGCCGTTCCGGGAGCCCATCGGTG---TCCCTCAC-GGGT---CACTGT [ 872 ]  
Psychostrophia\_melanargia CGCCGTTCCGGGAGTACCATCGTTCG---CCTC---GGC---GGCGG [ 865 ]  
Sabra\_harpagula CGCCGTTCCGGGAGCCCATTCGT-----ACCTCTCG-GGGT---CACGGT [ 869 ]  
Nothus\_lunus CGCTGTTCCGGGCTACTGCGGTC---ACCCTCG-GGGC---GGTCTG [ 873 ]

Tethea_consimilis	CGCCGTTCCGGGAGCCCCACGGTG-----TCCCCCCC-GGGC---CACCGT	[ 870 ]
Tethea_taiwana	CGCCGTTCCGGGAGCCCCACGGCG-----ACTCTC---GGGT---CACCGT	[ 868 ]
Tethea_or	CGCCGTTCCGGGAGCCCCACGGTG-----TCCCTC---GGGT---CACTGT	[ 868 ]
Tetheela_fluctuosa	CGCCGTTCCGGGAGCCCCATAGTG-----CCCTCGC-GGGG---TACTGT	[ 870 ]
Thyatira_batis	CGCCGTTCCGGGAGCCCCATAGCG-----TCCTC---GGGT---CGCTGT	[ 868 ]
Thyatira_batis2	NN	[ 900 ]
Tridrepana_flava	CTTCGTCCGGGAGCCCGTGCGCA-----CCTCTCGCGGGT---CGCGTA	[ 872 ]
Tridrepana_unispina	CTTCGTCCGGGAGCCCGTGCGCA-----CCTCTCGCGGGT---CGCGTA	[ 872 ]
Watsonalla_binaria	CGCCGTTCCGGGAGCCCCATCGT-----ACCCCTCG-GGGT---CACTTT	[ 869 ]
Watsonalla_cultraria	CGCCGTTCCGGGAGCCCCATCGT-----ACCCCTCG-GGGT---CACTTT	[ 869 ]
Watsonalla_uncinula	CGCCGTTCCGGGAGCCCCATTGTGC-----ACCTTTCG-GGGT---CACTTT	[ 871 ]
Cilix_glaucata	CGCCGTTCCGGGAGCCCCAGCGGGTGTGCGCCCTCGCGGGTTCGCCGCGGT	[ 881 ]
Falcaria_lacertinaria	CGCCGTTCCGGGAGCCCCATCGT-----ACCTTTCG-GGGT---CACGGT	[ 870 ]
Habrosyne_aurorina	CGCCGTTCCGGGAGCCCCATAGTG-----TCCTTTAC-GGGT---CACTGT	[ 878 ]
Jodis_putata	TGCCCTCCGGGAGTCTTCGCTTC-----CTTGTGTGTGTGA-----	[ 862 ]
Neodaruma_tamanukii	CGCCGTTCCGGGAGCCCCATTGTA-----CCCTTAC-GGGG---CACTGT	[ 870 ]

[	910	920	930	940	950]
[	.	.	.	.	.]

Accinctapubes_albifasciata	NN	[ 950 ]
Agnidra_scabiosa	GG-----GACCGT-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 909 ]
Auzata_superba	GG-----GACCGT-----GACGTG-CGCCGATCGGTTGTCGTACGGTA	[ 906 ]
Ausaris_micacea	GG-----GACCGT-----GACGTG-CGCCGATCGGTTGTCGTACGGTA	[ 895 ]
Ausaris_palleola	GGAGTTTGGTTCC-----GGCGC--CGCCGATGCCATTGGACGGTA	[ 908 ]
Cyclidia_substigmatica	GG-----GACCGC-----GACTGT-CGCCGACCGGCGTTCGTACGGTA	[ 910 ]
Drepana_arcuata	CG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 924 ]
Falcaria_bilineata	GG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 907 ]
Drepana_curvatula	AG-----GACCGT-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 896 ]
Drepana_curvatula2	AG-----GACCGT-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 896 ]
Drepana_falcataria	CG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 924 ]
Ennomos_autumnaria	-----TACCGT-----GAAGTG-CACCGACCGGCGTTCGTACGGTA	[ 902 ]
Epicopeia_hainesii	GG-----TACCGT-----GACGGA-CGCCGACCGGCGTTCGTACGGTA	[ 908 ]
Euparyphasma_maxima	GG-----GACCGT-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 910 ]
Euthyatira_pudens	-----GGTAGT-----GACGAG-CGCCGACCGGCGTTCGTACGGTA	[ 873 ]
Habrosyne_pyritoides	GG-----GACCGT-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 909 ]
Lyssa_zampa	CGATGGTGACCGT-----CGCGAA-CGCCGACCGGCGTTCGTACGGTA	[ 908 ]
Maucrauzata_maxima	GG-----GACCGT-----GACTTG-AGCCGACCGGCGTTCGTACGGTA	[ 909 ]
Microblepsis_acuminata	GG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 906 ]
Nordstromia_grisearia	TG-----GACCGT-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 914 ]
Ochropacha_duplaris	GG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 905 ]
Oreta_loochooana	GG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 906 ]
Oreta_pulchripes	GG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 906 ]
Oreta_rosea	GG-----GACCGC-----GACGTG-CACCGACCGGCGTTCGTACGGTA	[ 907 ]
Oreta_turpis	GG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 906 ]
Pseudothyatira_cym.	GG-----GACCGT-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 909 ]
Psychostrophia_melanargia	GG-----TACCGT-----GACGGA-CGCCGACCGGCGTTCGTACGGTA	[ 902 ]
Sabra_harpagula	GG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 906 ]
Nothus_lunus	AG-----TACCG-----GACGGA-CGCCGACCGGCGTTCGTACGGTA	[ 909 ]
Tethea_consimilis	GG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 907 ]
Tethea_taiwana	GG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 905 ]
Tethea_or	GG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 905 ]
Tetheela_fluctuosa	GG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 907 ]
Thyatira_batis	GG-----GACCGT-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 905 ]
Thyatira_batis2	NN	[ 950 ]
Tridrepana_flava	CG-----GACCGTTACACAGACAAT-AGCCGACCGGCGTTCGTACGGTA	[ 916 ]
Tridrepana_unispina	CG-----GACCGATCAACAGACAAT-AGCCGACCGGCGTTCGTACGGTA	[ 916 ]
Watsonalla_binaria	GG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 906 ]
Watsonalla_cultraria	GG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 906 ]
Watsonalla_uncinula	GG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 908 ]
Cilix_glaucata	TG-----GACCGC-----GACGTG-CGTCGACCGGCGTTCGTACGGTA	[ 918 ]
Falcaria_lacertinaria	GG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 907 ]
Habrosyne_aurorina	GG-----GACCGT-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 915 ]
Jodis_putata	-----GACCGC-----GAGGTGTACCAGCCGCGTTCGTACGGTA	[ 898 ]
Neodaruma_tamanukii	GG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 907 ]

[	960	970	980	990	1000]
[	.	.	.	.	.]

Accinctapubes_albifasciata	NN	[ 1000 ]
Agnidra_scabiosa	TGAA-T-----TG-AC-----GAAACGCGCACCGGTTCC	[ 936 ]
Auzata_superba	TAAG-T-----TG-AC-----GAAACGCGTACCGGTTT	[ 933 ]
Ausaris_micacea	AG-----GACCGC-----GACGTG-CGCCGACCGGCGTTCGTACGGTA	[ 915 ]



Nothus\_lunus CG-----CGCGTCCGGCCCGACGCAAGGCGTCGTCGTATT-TCTCATT [977]  
 Tethea\_consimilis CA-----ACGCGTCCGGCCCGACGCAAGGCAACGTCGTA---TCCCACG [975]  
 Tethea\_taiwana TT-----GCGCGTCCGGCCCGACGCAAGGCAACGTCGTATCCTCCGACG [975]  
 Tethea\_or -----ACGCGTCCGGCCCGACGCAAGGCAACGTCGTA---TCTTGCG [971]  
 Tetheela\_fluctuosa TA-----GCGCGTCCGGCCCGACGCAAGGCAACGTCGTA---TCCTTCG [974]  
 Thyatira\_batis TA-----ACGCGTCCGGCCCGACGCAAGGCAACGTCGTG---TCCGGCG [971]  
 Thyatira\_batis2 NNN [1050]  
 Tridrepana\_flava AT-----ACGCGTCCGGCCCGACGCAAGGCAACGTCGTA---TCACGAG [997]  
 Tridrepana\_unispina TA-----ACGCGTCCGGCCCGACGCAAGGCAACGTCGTA---TCGAGAG [997]  
 Watsonalla\_binaria GC-----GCGCGTCCGGCCCGACGCAAGGCAACGTCGTA---TCCTATG [974]  
 Watsonalla\_cultraria GC-----GCGCGTCCGGCCCGACGCAAGGCAACGTCGTA---TCCTATG [974]  
 Watsonalla\_uncinula GC-----GCGCGTCCGGCCCGACGCAAGGCAACGTCGTA---TCCTATG [976]  
 Cilix\_glaucata TA-----GCGCGTCCGGCCCGACGCAAGGCAACGTCGTG---TCCGGTG [1003]  
 Falcaria\_lacertinaria AT-----GCGCGTCCGGCCCGACGCAAGGCAACGTCGTA---TCCTATG [975]  
 Habrosyne\_aurorina TA-----AGCGCGTCCGGCCCGACGCAAGGCAACGTCGTA---TCCTACG [984]  
 Jodis\_putata T-----CGCGTCCGGCCCGACGCAAGGCAACGTCGTA---TCCTTCC [964]  
 Neodaruma\_tamanukii TA-----GCGCGTCCGGCCCGACGCAAGGCAACGTCGTA---TCCAATG [974]

[ 1060 1070 1080 1090 1100 ]  
 [ . . . . . ]

Accinctapubes\_albifasciata NNN [1100]  
 Agnidra\_scabiosa TCCTGCCCCGAGCGCGGACGT-CGGTGCGGCGCGCCTGT-CGTGCGAGCCG [1027]  
 Auzata\_superba TCCTGCCCCGAGTGCGGACGT-CGGTGCGGCGCGTCTGT-TGTCGCCGCCG [1022]  
 Ausaris\_micacea TACTCCCTA-----GAGTGTATTGTCCCTGC---GAGTGCTG [979]  
 Ausaris\_palleola TCCTGCCCCGAGTGCGGACGT---GGTGCCTGTGCCCTGT-GGCTGCCG--- [1017]  
 Cyclidia\_substigmatica TCCTGCCACAGCGCGGACTC-GGGTGCGGCGCGCCTGT-CGTGCTGCCG [1026]  
 Drepana\_arcuata TTCTGCCCGAGTGCGAATTC-GAGTGCGGCGCGTCTGT-TGTCGCCGCCG [1055]  
 Falcaria\_bilineata TCCTGCCCCGAGTGCGGACGT-CGGTGCGGCGCGCCTGT-CGTGCTGCCG [1023]  
 Drepana\_curvatula --CTCGCA-----GAGTGCCTTATCCCT-----GTGCTG [977]  
 Drepana\_curvatula2 --CTCGCA-----GAGTGCCTTATCCCT-----GTGCTG [977]  
 Drepana\_falcataria TTCTGCCCCGAGTGCGAATTC-GAGTGCGGCGCGTCTGT-TGTCGCCGCCG [1055]  
 Ennomos\_autumnaria TCCTGCCCCGAGTGCGGACGT-AGATGCGGCGCGCCTGT-CGTTGACGCC [1016]  
 Epicopeia\_hainesii TCCTGCCCCGAGTGCGGACGT-GGGCGCGCGCGCCTGT-CGTGCGAGCCG [1023]  
 Euparyphasma\_maxima TCCTGCCCCGAGTGCGGACGT-CGGTGCGGCGCGCCTGT-CGTGCTGCCG [1032]  
 Euthyatira\_pudens FCC-GCCAAAGTGCGGACGG-TCATACGAC---TTGTATGTCGCGCTCG [981]  
 Habrosyne\_pyratoides TCCTGCCCCGAGTGCGGACGT-CGGTGCGGCGCGCCTGT-CGTGCTGCCG [1030]  
 Lyssa\_zampa TCCTGCCCCAAGTGCGGACGT-GGATGCTGCGCGCCTGTGATCGCCGCCG [1024]  
 Maucrauzata\_maxima TCCTGCCCCGAGTGCGGACGT-CGGTGCGGCGCGTCTGT-CGTGCGAGCCG [1031]  
 Microblepsis\_acuminata TCCTGCCCCGAGCGCGGACGT-AGGTGCGGCGCGCCTGT-CGTGCGAGCCG [1022]  
 Nordstromia\_grisearia TCCTGCCCCGAGTGCGGACGT-TGGTGCGGCGCGCCTGT-CGTGCGAGCCG [1035]  
 Ochropacha\_duplaris TCCTGCCCCAAGTGCGGACGT-CGGTGCGGCGCGCCTGT-CGTGCTGCCG [1020]  
 Oreta\_loochooana TCCTGCCCCGAGTGCGGACGT-TGGTGCGGCGCGCCTGT-TGTCGAGCCG [1026]  
 Oreta\_pulchripes TCCTGCCCCGAGTGCGGACGT-TGGTGCGGCGCGCCTGT-TGTCGAGCCG [1026]  
 Oreta\_rosea TCCTGCCCCAAGTGCGGACGT-TGGTGCGGCGCGCCTGT-CGTGCGAGCCG [1027]  
 Oreta\_turpis TCCTGCCCCGAGTGCGGACGT-TGGTGCGGCGCGCCTGT-TGTCGAGCCG [1026]  
 Pseudothyatira\_cym. TCCTGCCCCGAGTGCGGACGT-CGGTGCGGCGCGCCTGT-CGTGCTGCCG [1024]  
 Psychothropia\_melanargia TCCTGCCCCGAGTGCGGACGT-GGGTGCGGCGCGCCTGT-CGTGCGAGCCG [1017]  
 Sabra\_harpagula TCCTGCCCCGAGTGCGGACGT-AGGTGCGGCGCGCCTGT-TGTCGCCGCCG [1022]  
 Nothus\_lunus CTCTGCCTGTGCGCGGACGT-GGGTGCGGCGCGCCTGT-CGTGCTGCCG [1025]  
 Tethea\_consimilis TCCTGCCCCGAGTGCGGACGT-TGGTGCGGCGCGCCTGT-CGTGCGAGCCG [1023]  
 Tethea\_taiwana TCCTGCCCCGAGTGCGGACGT-CGGTGCGGCGCGCCTGT-CGTGCTGCCG [1023]  
 Tethea\_or TCCTGCCCCAAGTGCGGACGT-GGGTGCGGCGCGCCTGT-CGTGCTGCCG [1019]  
 Tetheela\_fluctuosa TCCTGCCCCGAGTGCGGACGT-CGGTGCGGCGCGCCTGT-CGTGCTGCCG [1022]  
 Thyatira\_batis TCCTGCCACAGTGCGGACGT-CGGTGCGGCGCGCCTGT-CGTGCGAGCCG [1019]  
 Thyatira\_batis2 NNN [1100]  
 Tridrepana\_flava TCCTGCCCCGAGTGCGGACGT-GAGTGCGGCGCGTCTGT-TGTCGCCGCCG [1045]  
 Tridrepana\_unispina TCCTGCCCCGAGTGCGGACGT-GCGTGCGGCGCGTCTGT-TGTCGCCGCCG [1045]  
 Watsonalla\_binaria TCCTGCCCCGAGTGCGGACGT-CGGTGCGGCGCGCCTGT-CGTGCTGCCG [1022]  
 Watsonalla\_cultraria TCCTGCCCCGAGTGCGGACGT-CGGTGCGGCGCGCCTGT-CGTGCTGCCG [1022]  
 Watsonalla\_uncinula TCCTGCCCCGAGTGCGGACGT-CGGTGCGGCGCGCCTGT-CGTGCTGCCG [1024]  
 Cilix\_glaucata TCCTGCCCCGAGCGCGGACGT-CGGTGCGGCGCGTCTGT-TGTCGCCGCCG [1051]  
 Falcaria\_lacertinaria TCCTGCCCCGAGTGCGGACGT-CGGTGCGGCGCGCCTGT-CGTGCTGCCG [1023]  
 Habrosyne\_aurorina TCCTGCCCCGAGTGCGGACGT-CGGTGCGGCGCGCCTGT-CGTGCGGCCG [1032]  
 Jodis\_putata TCCTGCCCCGAGTGCGGAGA-GGGTGCGGCGCGCCTGT-CGTGCTGCCG [1012]  
 Neodaruma\_tamanukii TCCTGCCCCGAGTGCGGACGT-AGGTGCGGCGCGCCTGT-CGTGCTGCCG [1022]

[ 1110 1120 1130 1140 1150 ]  
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Accinctapubes\_albifasciata NNN [1150]  
 Agnidra\_scabiosa TGC-AGTCTCTGACA-T---GTGCGGCTCTGTCTGCGATGATTCAGTT [1072]  
 Auzata\_superba TGC-AGTCTCTGACA-T---GTACGCGTCTGTCTGCGATGATTCAGTT [1067]







Psychostrophia_melanargia	GGTATTAATTGGTGTGGCTTTTTGACTTTATTAGAACGTAAGTTTATG	[ 1261 ]
Sabra_harpagula	NN	[ 1267 ]
Nothus_lunus	TGTATTAATTGGGGTTGCATTTTAACTTTATTAGAACGTAATATTAG	[ 1259 ]
Tethea_consimplis	NN	[ 1268 ]
Tethea_taiwana	TGTTTTAGTGGGGTAGCTTTTTTAACTTTATTAGAACGTAAGTTTATG	[ 1259 ]
Tethea_or	AGTATTAGTAGGGGTGGCTTTTTTAACTTTATTAGAACGTAAGTTTATG	[ 1255 ]
Tetheela_fluctuosa	AGTTTTAGTAGGAGTTGCTTTTTTAACTTTATTAGAACGTAAGTTTATG	[ 1264 ]
Thyatira_batis	GGTATTAGTAGGAGTTGCTTTTTTAACTTTATTAGAACGTAAGTTTATG	[ 1263 ]
Thyatira_batis2	NN	[ 1350 ]
Tridrepana_flava	GGTATTGATTGGGGTAGCTTTTTTAACTTTATTAGAACGTAAGTTTATG	[ 1279 ]
Tridrepana_unispina	AGTTTTAGTTGGTGTGCTTTTTTAACTTTATTAGAACGTAAGTTTATG	[ 1281 ]
Watsonalla_binaria	AGTTTTAATTGGAGTTGCTTTTTTAACTTTATTAGAACGTAAGTTTATG	[ 1258 ]
Watsonalla_cultraria	AGTTTTAATTGGAGTTGCTTTTTTAACTTTATTAGAACGTAAGTTTATG	[ 1266 ]
Watsonalla_uncinula	NN	[ 1269 ]
Cilix_glaucata	AGTTTTAATTGGGGTTGCTTTTTTAACTTTATTAGAACGTAAGTTTATG	[ 1296 ]
Falcaria_lacertinaria	GGTMTTAAATTGGGGTTGCTTTTTTAACTTTATTAGAACGTAAGTTTATG	[ 1267 ]
Habrosyne_aurorina	NN	[ 1277 ]
Jodis_putata	GGTTTTAATTGGGGTTGCTTTTTTAACTTTATTAGAACGTAAGTTTATG	[ 1254 ]
Neodaruma_tamanukii	AGTTTTAGTGGGTGTGCTTTTTTAACTTTATTAGAACGTAAGTTTATG	[ 1258 ]

[ 1360 1370 1380 1390 1400 ]  
[ . . . . . ]

Accinctapubes_albifasciata	GTTATATTCAAATTCGAAAAGGGCCTAATAAAGTTGGATTTATAGGTATT	[ 1389 ]
Agnidra_scabiosa	GTTATATTCAAATTCGTAAGGTCCTAATAAATTAGGTATTATAGGATTA	[ 1310 ]
Auzata_superba	GTTATACTCAAACCTCGAAATGGCCCTAATAAGTTGGGATTAATAGGATTA	[ 1305 ]
Ausaris_micacea	GTTATATTCAAACTCGTAAAGGCCAATAAATTTGGGATTTATAGGATTA	[ 1259 ]
Ausaris_palleola	GTTACATTCAACTTCGTAAGGTCCTAATAAATTAGGTTTATAGGATTA	[ 1299 ]
Cyclidia_substigmata	GTTATATTCAAATTCGAAAAGGTCCTAATAAAGTTGGATTAATAGGGGTT	[ 1323 ]
Drepana_arcuata	GTTATATTCAAATTCGTAAGGTCCTAATAAATTAGGATTTATAGGATTA	[ 1349 ]
Falcaria_bilineata	GTTATATTCAGATTCGTAAGGCCCTAATAAATTAGGTTTATAGGTTTA	[ 1317 ]
Drepana_curvatula	GTTATATTCAAATTCGTAAGGACCTAATAAATTAGGTTTATAGGGATA	[ 1268 ]
Drepana_curvatula2	GTTATATTCAAATTCGTAAGGTCCTAATAAATTAGGATTTATAGGATTA	[ 1268 ]
Drepana_falcataria	GTTATATTCAAATTCGTAAGGACCTAATAAATTAGGTTTATAGGGAATA	[ 1349 ]
Ennomos_autumnaria	GTTATATTCAGATTCGTAAGGTCCTAATAAAGTTGGTTTTATAGGAATT	[ 1301 ]
Epicopeia_hainesii	GCTATATTCAGTTACGTAAGGACCTAATAAAGTTGGTTTATAGGGATT	[ 1317 ]
Euparyphasma_maxima	GTTATATTCAAATTCGTAAGGTCCTAATAAAGTTGGATTTATAGGTATT	[ 1326 ]
Euthyatira_pudens	NN	[ 1276 ]
Habrosyne_pyritoides	NN	[ 1314 ]
Lyssa_zampa	GTTATATTCAAATTCGAAAAGGGCCAAATAAGGTTGGTTATTTAGGAATT	[ 1310 ]
Maucrauzata_maxima	GTTATATTCAAATTCGTAAGGTCCTAATAAATTAGGTTTATAGGATT	[ 1325 ]
Microblepsis_acuminata	NN	[ 1317 ]
Nordstromia_grisearia	GTTACATTCAAATTCGAAAAGGTCCTAATAAGTTGGGTTTATAGGGATA	[ 1330 ]
Ochropacha_duplaris	GTTATATTCAAATTCGTAAGGTCCTAATAAAGTTGGATTTATAGGAATT	[ 1306 ]
Oreta_loochooana	GTTATATTCAGATTCGTAAGGTCCTAATAAAGTTGGATTTATAGGTTTA	[ 1320 ]
Oreta_pulchripes	GTTATATTCAAATTCGTAAGGTCCTAATAAAGTTGGATTTATAGGTTTA	[ 1320 ]
Oreta_rosea	GTTACATTCAGATTCGTAAGGCCCTAATAAAGTTGGATTTAATGGGCTA	[ 1321 ]
Oreta_turpis	GTTATATTCAGATTCGTAAGGTCCTAATAAAGTTGGATTTATAGGTTTA	[ 1320 ]
Pseudothyatira_cym.	NN	[ 1319 ]
Psychostrophia_melanargia	GTTACATTCAAATTCGAAAAGGTCCTAATAAAGTTGGTTTATAGGAATT	[ 1311 ]
Sabra_harpagula	NN	[ 1317 ]
Nothus_lunus	GATATATTCAAATTCGTAAGGTCCTAATAAATTAGGATTTATAGGTATT	[ 1309 ]
Tethea_consimplis	NN	[ 1318 ]
Tethea_taiwana	GTTATATTCAAATTCGTAAGGACCTAATAAAGTTGGTTTATAGGAGTT	[ 1309 ]
Tethea_or	GTTATATTCAAATTCGTAAGGCCCTAATAAAGTTGGTTTATAGGAGTT	[ 1305 ]
Tetheela_fluctuosa	GCTATATTCAAATTCGTAAGGTCCTAATAAAGTTGGTTTATAGGGAATT	[ 1314 ]
Thyatira_batis	GTTATATTCAAATTCGTAAGGTCCTAATAAAGTTGGATTTATAGGAATT	[ 1313 ]
Thyatira_batis2	NN	[ 1400 ]
Tridrepana_flava	GTTATATTCAAATTCGAAAAGGCCCTAATAAATTAGGATTTCTGGGTTA	[ 1329 ]
Tridrepana_unispina	GTTATATTCAAATTCGTAAGGTCCTAATAAATTAGGTTTATAGGGTTA	[ 1331 ]
Watsonalla_binaria	GTTATATTCAGATTCGTAAGGTCCTAATAAATTAGGATTTATAGGCTTA	[ 1308 ]
Watsonalla_cultraria	GTTATATTCAGATTCGTAAGGTCCTAATAAATTAGGATTTATAGGCTTA	[ 1316 ]
Watsonalla_uncinula	NN	[ 1319 ]
Cilix_glaucata	GTTATATTCAACTCCGTAAGGCCCTAATAAATTAGGATTTTATAGGAATT	[ 1346 ]
Falcaria_lacertinaria	GTTATATTCAGATTCGTAAGGCCCTAATAAATTAGGTTTATAGGTTTA	[ 1317 ]
Habrosyne_aurorina	NN	[ 1327 ]
Jodis_putata	GATATATTCAAATTCGTAAGGTCCTAATAAAGTTAGGTTTATAGGAGTT	[ 1304 ]
Neodaruma_tamanukii	GTTATATTCAAATTCGTAAGGTCCTAATAAAGTTGGGTTTATAGGAATT	[ 1308 ]

[ 1410 1420 1430 1440 1450 ]  
[ . . . . . ]

Accinctapubes_albifasciata	TTACAGCCTTTTCTGATGCTATTAAATTATTTAGAAAAGAACAAATATA	[ 1439 ]
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Agnidra\_scabiosa [1360]  
 Auzata\_superba [1355]  
 Ausaris\_micacea [1309]  
 Ausaris\_palleola [1349]  
 Cyclidia\_substigmaria [1373]  
 Drepana\_arcuata [1399]  
 Falcaria\_bilineata [1367]  
 Drepana\_curvatula [1318]  
 Drepana\_curvatula2 [1318]  
 Drepana\_falcataria [1399]  
 Ennomos\_autumnaria [1351]  
 Epicopeia\_hainesii [1367]  
 Euparyphasma\_maxima [1376]  
 Euthyatira\_pudens [1326]  
 Habrosyne\_pyritoides [1364]  
 Lyssa\_zampa [1360]  
 Maucrauzaata\_maxima [1375]  
 Microblepsis\_acuminata [1367]  
 Nordstromia\_grisearia [1380]  
 Ochropacha\_duplaris [1356]  
 Oreta\_loochooana [1370]  
 Oreta\_pulchripes [1370]  
 Oreta\_rosea [1371]  
 Oreta\_turpis [1370]  
 Pseudothyatira\_cym. [1369]  
 Psychostrophia\_melanargia [1361]  
 Sabra\_harpagula [1367]  
 Nothus\_lunus [1359]  
 Tethea\_consimilis [1368]  
 Tethea\_taiwana [1359]  
 Tethea\_or [1355]  
 Tetheela\_fluctuosa [1364]  
 Thyatira\_batis [1363]  
 Thyatira\_batis2 [1450]  
 Tridrepana\_flava [1379]  
 Tridrepana\_unispina [1381]  
 Watsonalla\_binaria [1358]  
 Watsonalla\_cultraria [1366]  
 Watsonalla\_uncinula [1369]  
 Cilix\_glaucata [1396]  
 Falcaria\_lacertinaria [1367]  
 Habrosyne\_aurorina [1377]  
 Jodis\_putata [1354]  
 Neodaruma\_tamanukii [1358]

[ 1460 1470 1480 1490 1500 ]  
 [ . . . . . ]

Accinctapubes\_albifasciata [1489]  
 Agnidra\_scabiosa [1410]  
 Auzata\_superba [1405]  
 Ausaris\_micacea [1359]  
 Ausaris\_palleola [1399]  
 Cyclidia\_substigmaria [1423]  
 Drepana\_arcuata [1449]  
 Falcaria\_bilineata [1417]  
 Drepana\_curvatula [1368]  
 Drepana\_curvatula2 [1368]  
 Drepana\_falcataria [1449]  
 Ennomos\_autumnaria [1401]  
 Epicopeia\_hainesii [1417]  
 Euparyphasma\_maxima [1426]  
 Euthyatira\_pudens [1376]  
 Habrosyne\_pyritoides [1414]  
 Lyssa\_zampa [1410]  
 Maucrauzaata\_maxima [1425]  
 Microblepsis\_acuminata [1417]  
 Nordstromia\_grisearia [1430]  
 Ochropacha\_duplaris [1406]  
 Oreta\_loochooana [1420]  
 Oreta\_pulchripes [1420]  
 Oreta\_rosea [1421]  
 Oreta\_turpis [1420]



Accinctapubes_albifasciata	ATTAATTTTAATTTAGGAATTTTATTTTTTTTATGTTGCACAAGAANNNN	[ 1589 ]
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Auzata_superba	GTTAGATTTAATTTAGGTATTTTATTTTTTTCCCTTGTAAAGAGTGGG	[ 1505 ]
Ausaris_micacea	ATTAGATTTAATTTAGGGGTTTTATTTTTTTCCCTTGTATTAGTTAGG	[ 1459 ]
Ausaris_palleola	ATTAGTTTTAATTTAGGGATTTTATTTTTTTCCCTTAAATTAGTTGGGG	[ 1499 ]
Cyclidia_substigmaria	ATTAGTTTTAATTTAGGGGTTTTATTTTTTTTATGTTGTACAAGAGTTGG	[ 1523 ]
Drepana_arcuata	ATTAGATTTAATTTAGGTGTATTATTTTTTTT-TCTTGTATTAGAATAGG	[ 1548 ]
Falcaria_bilineata	ATTAGTTTTAATTTAGGGATATTATTTTTTTTCTTGTATTAGTGTGG	[ 1517 ]
Drepana_curvatula	ATTAGATTTAATTTAGGGGTATTATTTTTTTTCTTGTATTAGATTATG	[ 1468 ]
Drepana_curvatula2	ATTAGATTTAATTTAGGTGTATTATTTTTTTT-TCTTGTATTAGAATAGG	[ 1467 ]
Drepana_falcataria	ATTAGATTTAATTTAGGTGTATTATTTTTTTTCTTGTATTAGATTATG	[ 1549 ]
Ennomos_autumnaria	ATTAGATTTAATTTAGGTATTATTTTTTTTCTTGTACTAGTTTAGG	[ 1501 ]
Epicopeia_hainesii	TTAAGATTTAATTTGGGGGTTTTATTTTTTTTTCGCTACTAGTTGGG	[ 1517 ]
Euparyphasma_maxima	ATTAATTTTAATTTAGGAATTTTATTTTTTTCTTGTGTACAAGTTAGG	[ 1526 ]
Euthyatira_pudens	NN	[ 1476 ]
Habrosyne_pyritoides	NN	[ 1514 ]
Lyssa_zampa	ATTAGATTTAATTTAGGTATTATTTTTTTANNNNNNNNNNNNNNNNNN	[ 1510 ]
Maucrauzata_maxima	ATTAGATTTAATTTAGGGTTTTATATTTTTTTTCTTGTATTAGTGTGG	[ 1525 ]
Microblepsis_acuminata	NN	[ 1517 ]
Nordstromia_grisearia	ATTAGCTTTAATTTAGGGTTTTATTTTTTTTCTTGTATTAGAGTTGG	[ 1530 ]
Ochropacha_duplaris	ATTAGATTTAATTTAGGAATTTTATTTTTTTCTTGTGTACAAGTTAGG	[ 1506 ]
Oreta_loochooana	ATTAGTTTTAATTTAGGGATATTATTTTTTTTCTTGTATTAGAGTTTC	[ 1520 ]
Oreta_pulchripes	ATTAGTTTTAATTTAGGGATATTATTTTTTTTCTTGTATTAGAGTTTC	[ 1520 ]
Oreta_rosea	ATTAGTTTTAATTTAGGGATATTATTTTTTTTCTTGTATTAGAGTTTC	[ 1521 ]
Oreta_turpis	ATTAGTTTTAATTTAGGGATATTATTTTTTTTCTTGTATTAGAGTTTC	[ 1520 ]
Pseudothyatira_cym.	NN	[ 1519 ]
Psychostrophia_melanargia	ATTAGATTTAATTTAGGTATTTTATTTTTTTTGTCTGCACTAAATTAGG	[ 1511 ]
Sabra_harpagula	NN	[ 1517 ]
Nothus_lunus	ATTAGATTTAGATTAGGTTTATTTATTTTTTTNNNNNNNNNNNNNNNN	[ 1509 ]
Tethea_consimilis	NN	[ 1518 ]
Tethea_taiwana	ATTAGATTTAATTTAGGTATTATTTTTTTTGTGTACTAGTTTAGG	[ 1509 ]
Tethea_or	ATTAGATTTAATTTAGGGGTTTTATTTTTTTCTTGTGTACTAGATTGGG	[ 1505 ]
Tetheela_fluctuosa	ATTAGATTTAATTTAGGTATTTTATTTTTTTTCTTGTGTACTAGTTTAGG	[ 1514 ]
Thyatira_batis	ATTACATTTAATTTAGGGGTTTTATTTTTTTTATGTTGTACTAGAATAGG	[ 1513 ]
Thyatira_batis2	NN	[ 1600 ]
Tridrepana_flava	GTTAGATATAATTTAGGTATATTATTTTTTTTCTTGTATTAGATTAGG	[ 1529 ]
Tridrepana_unispina	ATTAGTTTTAATTTAGGGGTTTTATTTTTTTTCCCTTGTATTAGATTGGG	[ 1531 ]
Watsonalla_binaria	ATTAGATTTAATTTAGGGATTTTATTTTTTTTCTTGTATTAGAGTAGG	[ 1508 ]
Watsonalla_cultraria	ATTAGATTTAATTTAGGGATTTTATTTTTTTTCTTGTATTAGAGTAGG	[ 1516 ]
Watsonalla_uncinula	NN	[ 1519 ]
Cilix_glaucata	ATTATGTATAATTTAGGTATTATTTTTTTTCTTGTATTAGATTAGG	[ 1546 ]
Falcaria_lacertinaria	ATTAGTTTTAATTTAGGGATATTATTTTTTTTCTTGTATTAGTGTTC	[ 1517 ]
Habrosyne_aurorina	NN	[ 1527 ]
Jodis_putata	ATAGTNN	[ 1504 ]
Neodaruma_tamanukii	ATTAGATTTAATTTAGGGATTTGTTTTTTCTTGTGTACTAGTTTAGG	[ 1508 ]

{ 1610 }  
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Accinctapubes_albifasciata	NNNNNNNNNNNNNNNNNN	[ 1606 ]
Agnidra_scabiosa	NNNNNNNNNNNNNNNNNN	[ 1527 ]
Auzata_superba	GGTTTACCCTATTATAG	[ 1522 ]
Ausaris_micacea	AGTATACACGGTAATAA	[ 1476 ]
Ausaris_palleola	GGTTTATAGAGTTATAG	[ 1516 ]
Cyclidia_substigmaria	AGTTTATACTGTAATAG	[ 1540 ]
Drepana_arcuata	AGTTTATACTATTATAA	[ 1565 ]
Falcaria_bilineata	AGTTTATACAGTTATAA	[ 1534 ]
Drepana_curvatula	-----	[ 1468 ]
Drepana_curvatula2	AGTTTATACTATTATAA	[ 1484 ]
Drepana_falcataria	AGTNNNNNNNNNNNNNN	[ 1566 ]
Ennomos_autumnaria	GGTTTATACTGTAATNN	[ 1518 ]
Epicopeia_hainesii	GGTTTATACATTAATAA	[ 1534 ]
Euparyphasma_maxima	TGTTTATACTGTTATAG	[ 1543 ]
Euthyatira_pudens	NNNNNNNNNNNNNNNNNN	[ 1493 ]
Habrosyne_pyritoides	NNNNNNNNNNNNNNNNNN	[ 1531 ]
Lyssa_zampa	NNNNNNNNNNNNNNNNNN	[ 1527 ]
Maucrauzata_maxima	AGTTTACACAATTATAA	[ 1542 ]
Microblepsis_acuminata	NNNNNNNNNNNNNNNNNN	[ 1534 ]
Nordstromia_grisearia	GGTTTATATAGTGATAA	[ 1547 ]
Ochropacha_duplaris	GGTTTATACTGTAATAA	[ 1523 ]
Oreta_loochooana	AGTCTATACTGTTATAA	[ 1537 ]
Oreta_pulchripes	AGTCTATACTGTTATAA	[ 1537 ]
Oreta_rosea	TGTTTATACAGTTATGA	[ 1538 ]

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Oreta_turpis          AGTCTATACTGTTATAA  [1537]
Pseudothyatira_cym.  NNNNNNNNNNNNNNNNN  [1536]
Psychostrophia_melanargia  GGTATATACTGTAATAG  [1528]
Sabra_harpagula      NNNNNNNNNNNNNNNNN  [1534]
Nothus_lunus         NNNNNNNNNNNNNNNNN  [1526]
Tethea_consimilis    NNNNNNNNNNNNNNNNN  [1535]
Tethea_taiwana       GGTTTATACAGTTATAG  [1526]
Tethea_or            TGTTTATACAGTTATAG  [1522]
Tetheela_fluctuosa   GGTTTATTTGTTATAA  [1531]
Thyatira_batis       AGTTTATACAGTTATAG  [1530]
Thyatira_batis2      NNNNNNNNNNNNNNNNN  [1617]
Tridrepana_flava     GGTTTATACAATTATAG  [1546]
Tridrepana_unispina  GGTTTATACTGTTATAA  [1548]
Watsonalla_binaria   GGTTTATCCTACTATAA  [1525]
Watsonalla_cultraria AGTTTATACTACTATAA  [1533]
Watsonalla_uncinula  NNNNNNNNNNNNNNNNN  [1536]
Cilix_glaucata       GGTTTTNNNNNNNNNNNN  [1563]
Falcaria_lacertinaria AAANNNNNNNNNNNNNN  [1534]
Habrosyne_aurorina   NNNNNNNNNNNNNNNNN  [1544]
Jodis_putata         NNNNNNNNNNNNNNNNN  [1521]
Neodaruma_tamanukii GGTTTATACTGTTATAG  [1525]
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END;

BEGIN CODONS;
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    N: 639-1167,
    1: 3-636\3 1170-1617\3 1617,
    2: 1-637\3 1168-1615\3,
    3: 2-638\3 1169-1616\3;
  CODESET * UNTITLED = Universal: all ;
END;

BEGIN LABELS;
  CharGroupLabel CAD COLOR=(RGB 0.01978 0.08762 0.73315 );
  CharGroupLabel 28s COLOR=(RGB 0.05776 0.73321 0.00855 );
  CharGroupLabel ND1 COLOR=(RGB 0.73324 0.04479 0.03615 );
END;

BEGIN SETS;
  CHARSET CAD = 1-638;
  CHARSET 28s = 639-1167;
  CHARSET ND1 = 1168-1617;
  CHARPARTITION CAD = Default: 1-1617;
  CHARPARTITION ND1 = CAD: 1-638, 28s: 639-1167, ND1: 1168-1617;
  CHARPARTITION * 28s = CAD: 1-638, 28s: 639-1167, ND1: 1168-1617;
END;

BEGIN ASSUMPTIONS;
  OPTIONS DEFTYPE=unord PolyTcount=MINSTEPS ;
END;

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**APPENDIX C: MUSCLES OF THE ANAL SEGMENT IN *DREPANA ARCUATA* AND  
*TETHEA OR***

Muscles were examined in two representative species, one with reduced anal prolegs (*Tethea or*) and another without anal prolegs (*Drepana arcuata*) in order to help propose a model for the mechanistic changes that accompanied the transition from crawling to anal scraping (Chapter 5).

***Methods***

The last five segments (A6-A10) of the larval abdomen of two representative species, *T. or* ( $n = 6$ ) and *D. arcuata* ( $n = 10$ ), preserved in 80% ethanol, were dissected. This was done by cutting at either the dorsal or ventral midline and pinning the body open, or by cutting at both the dorsal and ventral midlines to create a parasagittal section. The viscera and loose fat of the body cavity were removed, leaving only the muscles intact. The muscles of A6-A10 were examined in detail with an Olympus dissection microscope (SZX12, Olympus, Japan), and were drawn and photographed using a Pixelink Megapixel firewire camera (PLA642) attached to the microscope. Muscles were examined, identified based on origins and insertion points, and named according to (Eaton, 1988).

## **Results**

### **Tethea or**

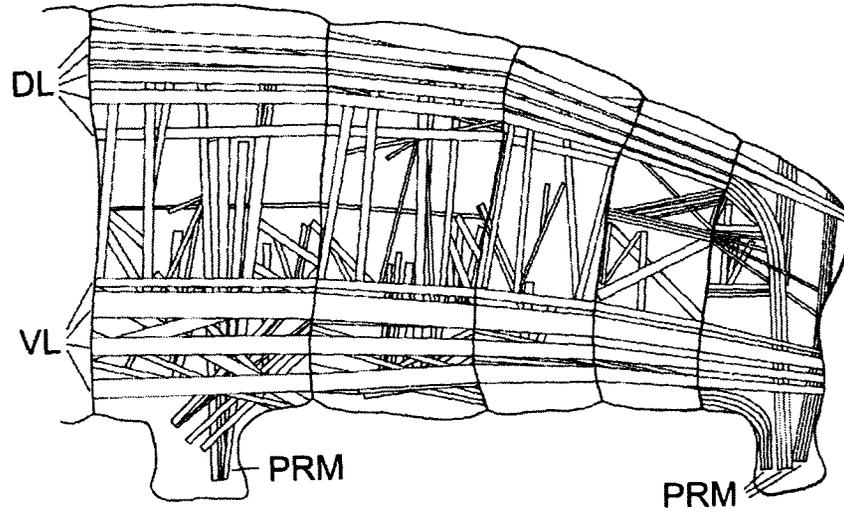
As in *M. sexta* (see Eaton, 1988), in the last five abdominal segments of *T. or*, the dorsal longitudinal muscles run parallel to each other, dorsal to the spiracles, arising on the tergal antecostae of the previous segment and inserting on the posterior edge of the tergal antecostae of the next segment (Fig. C.1a). The ventral longitudinal muscles also run parallel to each other, but arise on the sternal antecostae of the previous segment and insert on the posterior edge of the sternal antecostae of the next segment. All other body muscles are lateral to the dorsal and ventral longitudinal muscles. The planta retractor muscles arise on the ventral region of the tergum near the middle of the segment, posterior to the spiracle, and insert on the tendon of the planta. In the anal segment, the planta retractor muscles insert on the tendon of the planta, arising ventral to the ventral longitudinal muscles, at the location where the two most ventral dorsal longitudinal muscles should arise, or just ventral to the anus on the posterior edge of the sternum.

### **Drepana arcuata**

As in *T. or*, the dorsal and ventral longitudinal muscles arise on the tergal and sternal antecostae, respectively, of the previous segment and insert on the posterior edge of the tergal and sternal antecostae of the next segment (Fig. C.1b). The planta retractor muscles found in the sixth abdominal segment arise on the ventral region of the tergum near the middle of the segment, posterior to the spiracle, and insert on the tendon of the planta. In the anal segment, since *D. arcuata* lacks anal prolegs, three groups of muscles insert on the ventral edge of the sternum, arising on the pleural region near the anus.

**Fig. C.1.** Muscles of the abdominal segments 6 - 10 in a caterpillar with a reduced anal proleg (*Tethea or*) (**a**) and a caterpillar with no anal proleg (*Drepana arcuata*) (**b**). DL = dorsal longitudinal muscles; VL = ventral longitudinal muscles; PRM = planta retractor muscles.

a



b

