

Diverse mechanisms of feeding and movement in Cyclorrhaphan larvae (Diptera)

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Direct observation, filmed behaviour and morphological analysis were used to investigate mechanisms of larval feeding and movement in 20 species of Cyclorrhapha (Diptera). Feeding mechanisms refer to techniques of gathering food close to the pharynx entrance prior to sucking it in. A total of 12 mechanisms were recorded. Contrasting mechanisms were associated with saprophagy, phytophagy, mycophagy and zoophagy. Larvae had role-specific, compartmentalised bodies. The rear compartment grips substrates enabling the front ones to scan for food by sweeping from side to side or up and down. The front compartments extend or lunge to gather food or grip substrates during locomotion. Lower cyclorrhaphans had more disparate mechanisms than higher cyclorrhaphans. In the latter, the size, shape and movements of the thorax, pseudocephalon and head skeleton are diverse. Influences on this diversity are food quality and access conditions at development sites. Movement capabilities and functions were established for many components.

Keywords: feeding mode; performance; head skeleton; larval head; locomotion

Introduction

The Cyclorrhapha is one of the better supported and biodiverse of higher taxa proposed within the Diptera (Ferrari 1987; Collins and Wiegmann 2002; Wiegmann et al. 2011; Lambkin et al. 2013). One of its characteristics is a larval head comprising a fleshy pseudocephalon and an inverted head skeleton (Keilin 1915; Roberts 1971; Courtney et al. 2000; Rotheray and Gilbert 2008). In higher cyclorrhaphans (= Schizophora) the head has three sections: the oral cavity, atrium and cibarium (Hennig 1952; Roberts 1970). The oral cavity is an external hollow or pocket formed between the lobes of the pseudocephalon, at the rear of which is the entrance to the pharynx (Hennig 1952; Roberts 1970; Courtney et al. 2000; Rotheray and Gilbert 2008). The mandibles are ensheathed within these lobes and each has an opening for the mandible apex or mandibular hook. The tubular atrium extends from the oral cavity to the salivary duct and is supported by the intermediate sclerite (Roberts 1970). The cibarium runs from the salivary duct to the back of the head skeleton and is supported by the basal sclerite (Roberts 1969; Courtney et al. 2000).

In lower cyclorrhaphans (= Aschiza), an atrium and oral cavity are absent except for the derived, Eristalinae (Syrphidae) in which an atrium is present (Hartley 1963). In the Lonchopteridae, the pseudocephalon forms an open trough (Rotheray and

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Gilbert 2008), while in the Platypterozoidea and Syrphoidea, the pseudocephalon surrounds the head skeleton (Rotheray et al. 2004; Rotheray and Gilbert 2008).

Despite an extensive literature, the movement capabilities and functional roles of the various sclerites and sections of the cyclorrhaphan larval head are poorly understood (Ferrar 1987; Courtney et al. 2000; Rotheray and Gilbert 2008). A notable exception is the basal sclerite. Dowding (1967) demonstrated how this part of the head skeleton functions as a pump for drawing in food and that the cibarial ridges within it separate microbes from liquids. Based on this analysis, the presence of cibarial ridges indicates a microbial diet and a saprophagous feeding mode (Dowding 1967; Ferrar 1987). Phytophagy and zoophagy (predation) also have indicator features: mandibular teeth in phytophages and long, narrow, head skeletons in predators (Roberts 1971; Ferrar 1987). However, limited taxon sampling and unresolved phylogeny make the generality of indicator morphology difficult to assess (Ferrar 1987).

Dowding (1967) analysed movement capabilities by direct observation and determined the points of origin and insertion of associated muscles. For example, originating on the inner margins of the dorsal arms or cornua of the basal sclerite and inserting on the sides and roof of the cibarium and atrium, are bands of muscle revealing how the pump and its valves work. Furthermore, from muscle dimensions, the power output of the pump has been assessed: short, wide muscles indicate a powerful pump suiting high viscosity food; long, thin bands indicate a weak pump for low viscosity food (Roberts 1971). On the outer sides of these cornua, additional muscles insert or originate and functions such as the following have been deduced: head skeleton suspensor muscles, mandibular elevator and depressor muscles, head skeleton protractor and retractor muscles and labial lobe retractor muscles (Hartley 1963; Roberts 1971; Hanslik et al. 2010; Wipfler et al. 2013).

Combining knowledge of morphology, muscles and behaviour into robust explanations of feeding mechanisms (i.e. explaining the biomechanics of ingestion or how food is gathered into the pharynx) has not often been attempted, for an example, see Roberts (1971), and is problematic for a variety of reasons. For instance, greater emphasis is usually given to the impacts of feeding, rather than the behaviour itself and morphological descriptions rarely consider performance, i.e. the fit of morphology and behaviour to environmental variables. However, performance is the only way to assess how larvae are adapted. Neglecting performance is understandable given that preserved more than live material is studied and feeding is usually too fast to see what is going on (Roberts 1971; Tinkeu and Hance 1998). Also, it occurs frequently in difficult to observe situations. In this paper we attempt to overcome these problems using, especially, filmed behaviour. Our objective is to investigate variability in larval feeding and movement across a range of cyclorrhaphan exemplars. By movement we refer not just to locomotion, but movement capabilities of individual components. We also assess performance and search for commonalities.

Materials and methods

Third stage larvae of 20 species were collected live from natural development sites (Table 1). Where necessary, reared adults confirmed identity and names follow Chandler (1998 + updates at: www.dipteristsforum.org.uk). Larvae were kept in

Table 1. Details of the 20 species of cyclorrhaphan Diptera studied in this paper, including development sites and collection details.

Species	Family	N	Feeding mode	Access	Food quality	Development site	Collection details
Lower Cyclorrhapha (= Aschiza)							
<i>Lonchoptera lutea</i> Panzer	Lonchopteridae	1	saprophagy	restricted+	watery liquid	fallen leaves	<i>Acer pseudoplatanus</i> leaves, coll. Jan 2013, Edinburgh
<i>Polyporivora picta</i> (Meigen)	Platyezidae	10+	mycophagy	restricted+++	hard solid	fungal fruiting body	<i>Trametes versicolor</i> , Sept. 2007, Stirlingshire
<i>Microdon analis</i> (Macquart)	Syrphidae	3	zoophagy	restricted+	integument	ant nests	<i>Formica</i> sp nest under bark on <i>Pinus</i> stump, April 2013, Inverness-shire
<i>Melangyna cincta</i> (Fallén)	Syrphidae	6	zoophagy	no restriction	integument	aphid colonies	<i>Phyllaphis fagi</i> colonies on leaves of <i>Fagus sylvatica</i> , June 2013, Cumbria
Higher Cyclorrhapha							
(= Schizophora)							
<i>Setisquamadonchaea fumosa</i> (Egger)	Lonchaeidae	10+	saprophagy	restricted+ to ++	soft solid, oily to watery liquid	dead plant stems	decaying stem bract of <i>Heracleum mantezianum</i> , Sept 2012, Stirlingshire
<i>Lonchaea sylvatica</i> Beling	Lonchaeidae	3	myophagy	restricted++	powder to oily liquid	dead wood	sooty fungi and decaying cortical tissue under <i>Betula</i> bark, April 2013, Perthshire
<i>Lonchaea haekmani</i> Kovalev	Lonchaeidae	5	saprophagy	restricted++	oily to watery liquid	dead wood	under bark of <i>Populus tremula</i> , April 2013, Inverness-shire
<i>Palloptera trimacula</i> (Meigen)	Pallopteridae	10+	saprophagy	restricted+ to ++	soft solid, oily to watery liquid	dead plant stems	decaying stem of <i>Heracleum sphondylium</i> , Jan. 2013, Midlothian
<i>Tephritis vespertina</i> (Loew)	Tephritidae	2	phytophagy	restricted++	soft solid	flowerheads	<i>Hypochoeris radiata</i> , June 2013, Edinburgh
<i>Chaetostomella cylindrica</i> (Robineau-Desvoidy)	Tephritidae	10+	phytophagy	restricted++ to +++	hard to soft solid	flowerheads	<i>Centaurea nigra</i> , August 2012, Inverness-shire
<i>Acidia cognata</i> (Wiedemann)	Tephritidae	10+	phytophagy	restricted+++	soft solid	leaf miner	<i>Tussilago farfara</i> , October 2013, Inverness-shire
<i>Meiosimyza platycephala</i> (Loew)	Lauxaniidae	3	saprophagy	restricted+	watery liquid	fallen leaves	<i>Acer, Fagus</i> and <i>Quercus</i> leaves, Jan 2010, East Lothian, Stirlingshire
<i>Coelopa (Fucomyia) frigida</i> (Fabricius)	Coelopidae	5	saprophagy	restricted+	soft solid, oily to watery liquid	dead seaweed	mixed, unidentified seaweeds, July 2013, Isle of Canna, Inner Hebrides
<i>Clusia flava</i> (Meigen) (Clusiidae)	Clusiidae	5	saprophagy	restricted++	watery liquid	dead wood	in soft, decayed whitewood of fallen <i>Betula</i> , April 2013, Inverness-shire

(Continued)

Table 1. (Continued).

Species	Family	N	Feeding mode	Access	Food quality	Development site	Collection details
<i>Neophylomyza acygllossa</i> (Villeneuve)	Mitichidae	1	saprophagy	restricted++	watery liquid	dead wood	in soft, decayed whitewood of fallen <i>Betula</i> , April 2013, Perthshire
<i>Chymomyza costata</i> (Zetterstedt)	Drosophilidae	1	saprophagy	restricted+ to ++	soft solid, oily to watery liquid	dead wood	under bark on <i>Pinus</i> stump, April 2013, Inverness-shire
<i>Stegana coleoptrata</i> (Scopoli)	Drosophilidae	6	myophagy	restricted++	powder to oily liquid	dead wood	sooty fungi and decaying cortical tissue under <i>Betula</i> bark, April 2013, Perthshire
<i>Botanophila seneciella</i> (Meade)	Anthomyiidae	10+	phytophagy	restricted++	soft solid	flowerheads	<i>Senecio jacobaea</i> , July 2013, Isle of Canna, Inner Hebrides
<i>Pegomya solemiss</i> (Meigen)	Anthomyiidae	10+	phytophagy	restricted+++	soft solid	leaf miner	<i>Rumex acetosa</i> , Sept. 2013, Edinburgh
<i>Calliphora vomitoria</i> (Linnaeus)	Calliphoridae	10+	saprophagy	restricted+	soft solid, oily to watery liquid	carion	decaying <i>Salmo salar</i> , June 2013, Edinburgh

cool, dark conditions in containers with small quantities of food. The type of food consumed was confirmed by direct observation (Table 1).

Spatial access and food quality varied at the development sites and, for each species the following were recorded. Access was categorised as: 'no restriction' indicates barriers absent; 'restricted+' indicates squeezing sometimes required, i.e. manipulating the body through liquids or spaces less than larval height or width; 'restricted++' indicates squeezing usually required; and 'restricted+++' indicates excavation or tunnelling necessary. Food quality was categorised as: 'powder'; 'firm solid'; 'soft solid'; 'oily liquid'; and 'watery liquid'. For saprophages, food was further assessed as 'shallow' or 'deep' (i.e. about or more than larval height) and occurring on either 'smooth' or 'convoluted' substrates (with cracks and crevices or networks of fibres).

Morphology involved in feeding and movement was analysed by measurement and description. Larvae were prepared for description by fixation in Kahle's solution (Ferrar 1987), which causes larvae to become extended and translucent enabling recognition of inverted components. Where necessary, integumental detail was made visible by dropping a solution of methyl blue on to a preserved larva and allowing it to air dry. After examination, methyl blue was removed with alcohol. To examine the head two methods were used. Head skeletons were removed from puparia by soaking in hot potassium hydroxide (KOH) for about 10 minutes, then washed in acetic acid and stored in glycerol or 70% ethanol. In the second method, the front of a preserved larva was cut off, soaked in hot KOH for 10–15 minutes, tissue surrounding the head skeleton removed with pins and the preparation washed in acetic acid and stored in 70% ethanol. These preparations provide *in situ* views of the head skeleton and surrounding integument. To determine where tendons attach to mandibles, head skeletons were obtained as above and the tendons carefully exposed from surrounding tissue using pins in a solid watch glass containing 70% ethanol. Mandibles and their tendons were examined in methanol or dry in a watch glass containing glass beads.

Preparations and preserved larvae were examined using a Wild M5 stereo microscope and an Olympus BX51 compound microscope. In stereo microscopy, measurements were made using an eyepiece graticule attached to the microscope and are accurate to 0.1 mm. In compound microscopy, measurements were made using Olympus morphometrics software, AnalySIS docu v5. The following measurements were taken: lengths of the abdomen, thorax, basal sclerite, intermediate sclerite and mandible and, to quantify taper at the front end, height of the base of the first abdominal segment and height of the apex of the prothorax.

Feeding and locomotion were investigated by direct observation and filming. To make observations and films, larvae within containers were either carefully exposed by removing surrounding material with a pin or a larva was placed in a Petri dish containing food or water and allowed to settle for several minutes. Observations were made using a stereo microscope. Feeding was sometimes stimulated by starving larvae for up to 24 h. Larvae were filmed using a Canon PowerShot G10 camera together with Canon Conversion Lens Adapter LA-DC58K and a Zarf Enterprises LNS-G10/30 adapter fitted to a stereo microscope. A Canon RS60-E3 remote switch was used as the shutter release. Films were examined using iMovie11 initiated on an Apple MacBook Pro computer in real time, slow and fast time, reverse time and freeze-frame.

Between three and 10 larvae per species were observed, except for *Neophyllomyza acyglossa* (Villeneuve) (Milichiidae) in which only a single specimen was available and films were made of single individuals of all species except *Polyporivora picta* (Platypezidae) and *Chaematostomella cylindrica* (Tephritidae). The following terms described segment and head skeleton movement: **sweeping** – lateral displacement of the front end of a larva relative to a stationary rear end; **lunging** – downward and/or forward movement of the prothorax, pseudocephalon, head skeleton and mandibles; **contracting** – a segment or body section moving backwards such that it creases and/or collapses; **extending** – a segment or body section recovering from a contracted state; **folding** – a body segment moving backwards by doubling up into an adjacent segment usually at the border or, doubling up on itself; **unfolding** – a body segment recovering from a folded state; **protracting** – head skeleton moving forwards out of the thorax; **retracting** – head skeleton moving backwards into the thorax; **pivoting** – head skeleton see-sawing up and down or from side to side; **elevating** – mandible abducting or moving up; **depressing** – mandible adducting or moving down.

Based on freeze-frames and timed sequences from films, the following were measured appropriate to particular species: **lunge distance** – relative to the anterior spiracles, the distance the head skeleton protracts; **pivot distance, mandible** – relative to the intermediate sclerite apex, the angle between the points of maximum elevation and depression of the mandible measured at the apex of the mouthhook; **pivot distance, head skeleton** – the angle between points of maximum movement up and down of the head skeleton measured at the apex of the mandibular hook and relative to the fulcrum point at the base of the intermediate sclerite; **lunge time** – the time taken for the head skeleton to make one lunge, i.e. for the head skeleton to protract and retract or pivot. See Supplemental Material, Measurements pdf for images illustrating some of these measures.

Still images were taken using a camera attached to a stereo or compound microscope. Terminology for head skeletons follows Courtney et al. (2000), with minor modifications from Rotheray and Gilbert (2008). The material studied here, including films and images, is deposited in the collections of the National Museums Scotland.

Results

Cyclorrhapha

About 30 hours were spent observing larvae of 20 cyclorrhaphan species (Table 1). About 5 hours of film were recorded (films available as Online Supplemental Material). All species gathered food close to the opening of the pharynx prior to sucking it in and had compartmentalised bodies, i.e. independently mobile sections not corresponding to the head, thorax and abdomen. During feeding and locomotion, the uniform segments of the rear compartment were immobile while the tapered segments of front compartments swept and lunged (Film 1).

More than the rear compartment, segments of front compartments are able to crease, collapse, fold, turn and, in some cases, twist and rotate, i.e. these compartments are more flexible and manoeuvrable. For the rear compartment, the emphasis in direction of movement is towards the head and this is reflected at segment borders where the segment behind folds under the one in front (Figure 1). In the front sections, the direction of folding at segment borders is reversed (Figure 1).

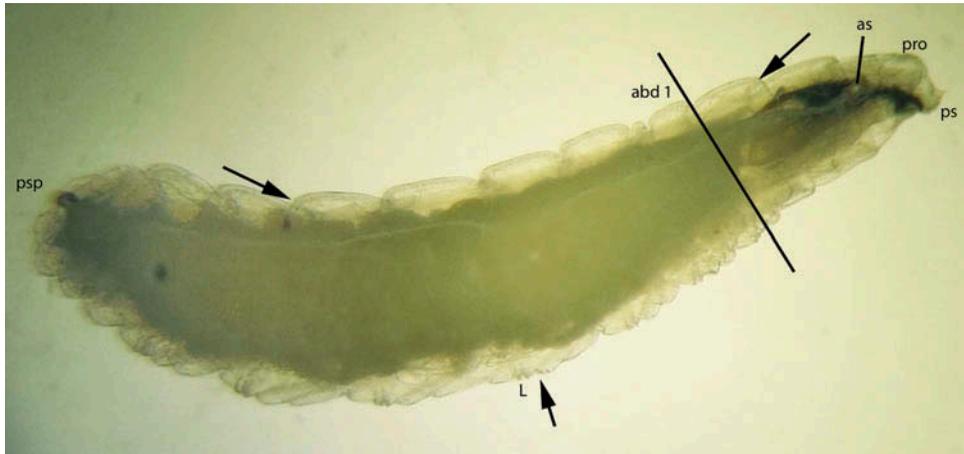


Figure 1. *Palloptera trimacula*, whole preserved larva, lateral view, length 5.5 mm, head to the right. Arrows dorsal to the rear and thoracic body compartments indicate directions segments fold into another at the borders; line through the larva is the border between the thorax and abdomen. abd1 = abdominal segment 1; as = anterior spiracle; L = locomotory spicules straddling the border between abdominal segments 3 and 4, arrow indicates position of the border; pro = prothorax; ps = pseudocephalon; psp = posterior spiracle.

Furthermore, compared with dorsal halves, ventral halves of the segments of the front compartments are, from back to front, increasingly folded into the segment behind. This imposes a downward inclination to these compartments, mirroring the direction of feeding and locomotion (Figure 1).

Lower Cyclorrhapha

Compared with higher Cyclorrhapha, the labium is diverse in form and active in gathering food. Body compartments vary in number and none of the species studied have an atrium, an oral cavity or an anal lobe (spicule-coated projection round the anus).

Saprophagy: Lonchoptera lutea (Lonchopteridae)

The larva of this species feeds on watery biofilm, i.e. a matrix enclosed, microbial accretion (Hall-Stoodley et al. 2004) coating smooth substrates, such as fallen leaves, dead wood, stones, etc. where access is restricted+ to ++ (Table 1). The segments of the rear compartment, anal segment to the metathorax, are up to 3× wider than long, dorso-ventrally flattened and protected by sclerotised, dorsal plates. The ventral surface is coated in spicules. The plates of the anal and seventh abdominal segment and the metathoracic and first abdominal segment are fused. The front compartment, mesothorax to the head, is cylindrical and narrow relative to the rear compartment (Figure 2). The head is an open trough, the sides of which each bear an antenna and a maxillary organ (Figures 3A and 3B). The apex of the trough has a bar-shaped, labial lobe on each side of which is an extrudable, tapered, setae-coated projection. Extending from the head skeleton into each side of the trough is a sclerotised rod,

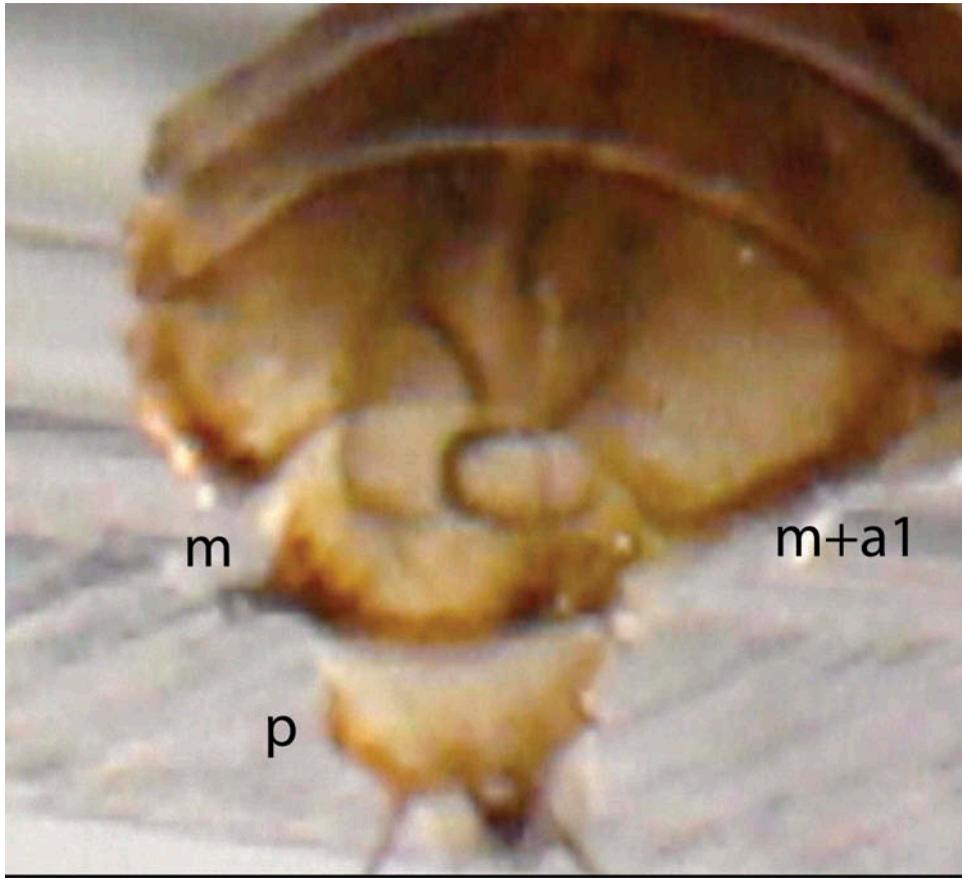


Figure 2. *Lonchoptera lutea*, larva with extended front compartment, still from a film. p = prothotax; m = mesothorax; m+a1 = fused metathorax + first abdominal segment.

the parastomal bars of higher Cyclorhapha and into the floor of the trough, paired labial rods which are joined by a lightly sclerotised strip, the ventral bridge of higher Cyclorhapha. Each mandible consists of three or four sclerites which are not in the same plane (Figure 3B). The first sclerite articulates with the labial rods, the next two change plane and extend up through the fleshy sides of the trough and end in a large, oval-shaped sclerite. From this oval sclerite is a short projection orientated in another plane, at the end of which is a hook (Figure 3B). The hooks form an arch over the opening to the pharynx, at the base of the trough (Figure 3A). Cibarial ridges are present.

In feeding, this larva sweeps and lunges repeatedly at a rate of about one lunge per 0.1 sec (Film 2, Figures 4A–D). Lunges consist of the front compartment extending down and across the substrate by which action biofilm is shovelled on to the trough (Figure 4A). During shovelling, the extrudable lobes swung round in front (Figure 4B), the trough tilts up and the oval mandibular sclerite twists backwards, the mandibular hooks elevate, the sides of the trough flick backwards and the pump in the basal sclerite sucks biofilm into the pharynx (Films 2–3, Figures 4C and 4D).

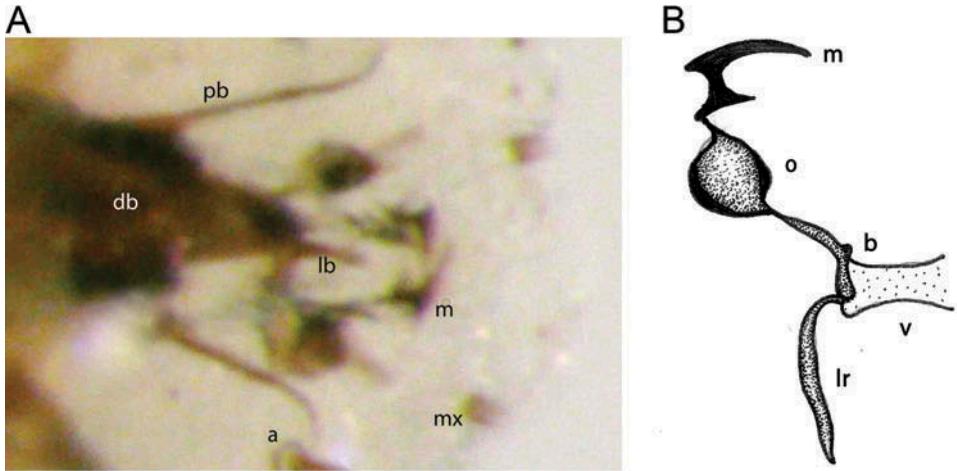
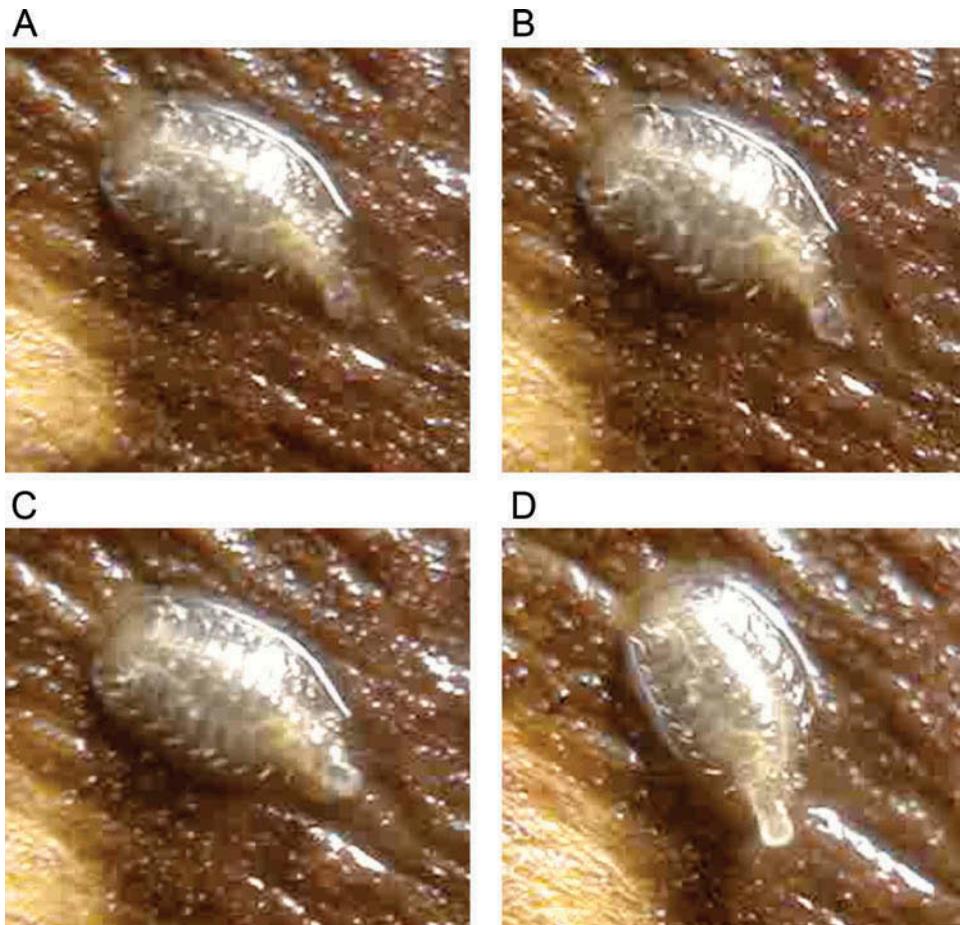


Figure 3. *Lonchoptera lutea*, head skeleton. (A) *Lonchoptera lutea*, head skeleton. pseudocephalon and apex of head skeleton, still from Film 3. a = base of antenna; db = dorsal bridge; lb = labrum; m = mandibular hooks; mx = maxillary organ; pb = parastomal bar; (B) drawing of the mandible and articulation with the labium. b = basal mandibular sclerite(s); lr = labial rod; o = oval mandibular sclerite; m = mandibular hook; v = ventral bridge.

Action of the pump is revealed by inward movement of the dorsal cornua and there is one pumping action per lunge (Film 3). The head skeleton is fixed and does not protract or retract (Film 4). The dorsal plates are inflexible and do not fold during locomotion, but raise and lower in relation to the position of the peristaltic movement wave. Direction changes are due to the front compartment pressing against the substrate at an angle relative to the previous position. Alternatively, S-shaped, sideways motion occurs. With the anal segment and the pseudocephalon gripping the substrate, opposing contraction/expansion of the lateral margins of the first few abdominal segments presses one side against the substrate while the other expands, lifts and lowers. When the expanded side touches the substrate, inducing a characteristic rocking motion, the prothorax and pseudocephalon move to a position lateral to the start position and by repetition of these movements, the body shifts sideways (Film 2). This larva is unable to make sharp changes in direction, twist round and it avoids moving on narrow substrates, such as leaf petioles. It is, however, able to squeeze through spaces less than its height, such as between wet, overlapping leaves.

Mycophagy: Polyporivora picta (Platypezidae)

This larva excavates tunnels through fruiting bodies of the fungus, *Trametes versicolor* (L.) Lloyd (Polyporaceae) where access is restricted+++ (Table 1). Rear compartment segments, anal segment to the metathorax, are subcylindrical in cross-section and coated in transverse rows of spicules (Figure 5). Segments of the middle compartment, mesothorax to the pseudocephalon, are wedge-shaped. Pseudocephalon is retractile and small, only about two thirds as high, half as long and less than half as wide as the prothorax. From the ventral margin of the pseudocephalon extends an



Figures 4. *Lonchoptera lutea*, feeding on biofilm coating dead wood, stills from Film 2. (A) Pseudocephalon lowered into biofilm; (B) extrudable cones swing round to front of pseudocephalon; (C) pseudocephalon lifted up; (D) limit of pseudocephalon lifting.

elongate, sclerotised, oral plate, nearly as long as the head skeleton (Figure 6A). The front compartment is the retractile head skeleton which lacks cibarial ridges. Apically, it has three pairs of conspicuous labial teeth (Figures 6 and 7). A gap exists between the rows of teeth (Figure 6B) and on either side of the upper two pairs of teeth are the poorly sclerotised, translucent mandibles, which articulate with the labium posteriorly and have a serrated apical margin (Figure 7). The entrance to the pharynx is between the upper pair of teeth.

To feed the larva grips the tunnel with the rear compartment and lunges against the face. Lunges consist of the middle compartment contracting and raising and the head skeleton retracting; these actions place the exposed, labial teeth high in the tunnel. Extending and lowering from these contracted states presses the labial teeth into and moves them down across the tunnel face, and the space between the rows of labial teeth fills with loosened and torn fungal hyphae and, if necessary, the



Figure 5. *Polyporivora picta*, preserved puparium, lateral view, head to the right, length 5 mm.



Figures 6. *Polyporivora picta*, head skeleton from a puparium. (A) Lateral view, labial teeth to the right, length 0.6 mm; (B) apical view of labial teeth.

mandibles depress to guide them towards the pharynx where they are sucked in by the pump. Ensuring the labial teeth are fully exposed during lunging, the ventral part of the pseudocephalon is pulled backwards and out of the way probably by contraction of muscles attached to the oral plate. Peristaltic waves keep the larva pressed up against the tunnel face. Differential contraction of the lateral margins enables the front end to alter the direction of lunging and to excavate a tunnel wide enough for the body to pass through. Tunnels extend many times body length and curve gently

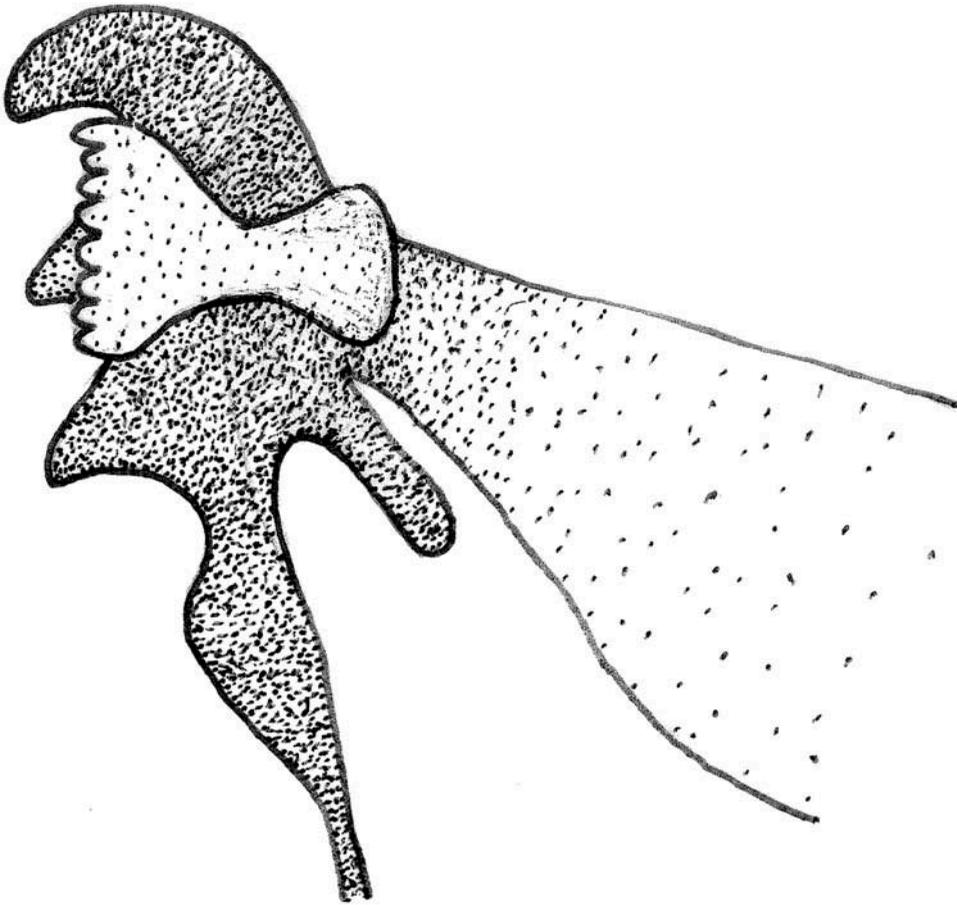


Figure 7. *Polyporivora picta*, labial teeth and mandible with serrated margin outside the upper two labial teeth.

sideways or up or down. They penetrate the fungus in all directions and open out on both the dorsal and ventral surfaces.

Zoophagy: Microdon analis and Melangyna cincta (Syrphidae)

The larva of *M. analis* lives in galleries excavated by *Formica* ants (Hymenoptera, Formicidae) under bark of fallen pine trees and stumps (*Pinus sylvestris* L., Pinaceae) where access is restricted+ and feeds on their early stages (Table 1). The larva of *M. cincta* feeds mainly on the aphid, *Phyllaphis fagi* (L.) (Hemiptera, Aphididae) which forms colonies on the undersides of leaves of beech, *Fagus sylvatica* L. (Fagaceae). Infested leaves often curl up and access is unrestricted to restricted+ (Table 1). Aphids are surrounded and covered in varying amounts of secreted, white flocculence and honeydew is present, some of which is in the form of wax-coated droplets. Uniquely, these two species have antennomaxillary organs borne on elongate, cylindrical

projections at the apex of the pseudocephalon. Between the projections is a shallow groove, along which the head skeleton protracts and retracts.

Microdon analis: rear compartment, anal segment to the metathorax, is hemispherical or domed in shape and rigid, although not sclerotised, and ventrally coated in fine setae. The metathorax is divided into an upper, basal section and a lower apical one, the latter being concealed along with the front compartments, under the basal section with the border between them being a continuation of a band of setae that circumvents the entire ventral margin of the body. The middle compartment, mesothorax to the pseudocephalon, is narrow in relation to the rear section and highly retractile. The relatively large mandibles are at the front of the head skeleton (Figure 8) and are thin and blade-like with serrated ventral margins, the eight or so posterior teeth being longer and rotated backwards and inwards (Figure 9). Posteriorly, the mandible is inflated and the rear face has an oval cavity which articulates with a peg-like, labial projection (Figure 9). A conspicuous, curved, tapering apodeme extends from the postero-ventral margin, round which attaches a tendon for the mandibular muscles (Figure 9). The apex of the labium is separate and comprises a pair of flat, elongate-oval sclerites, the labial sclerites, and between them, a triangular sclerite, the labial plate, which has a rounded apex and curves posteriorly to articulate with the basal section of the labium. The rear, inside margin of the labial plate bears upright spines.

During movement, the middle compartment extends to slightly beyond the marginal band of setae and sweeps up to 70 degrees either side of the longitudinal midline (Figures 10A and 10B). Locomotion is slow and, if a barrier or obstacle is encountered, the larva contracts the body at the point of contact and by shuffling backwards and sideways, attempts to bypass it. The larva can also rotate the entire body on a

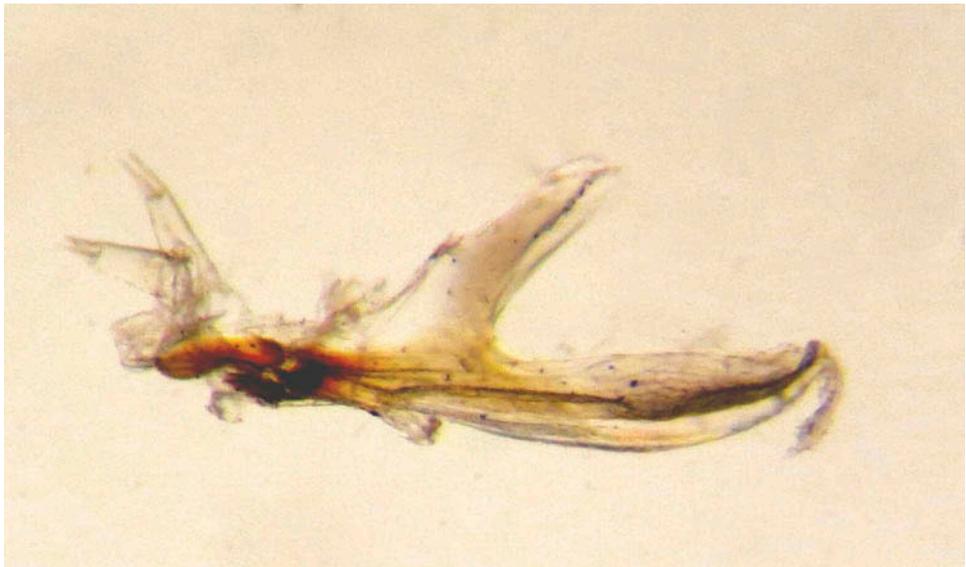


Figure 8. *Microdon analis*, head skeleton, removed from a puparium, lateral view, mandibles to the left, length 1.4 mm.

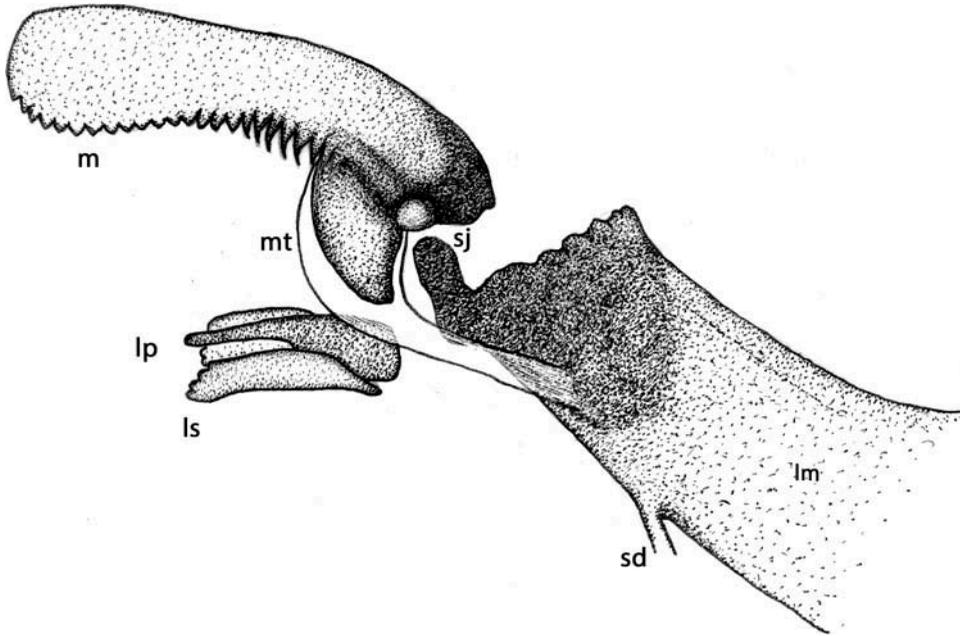
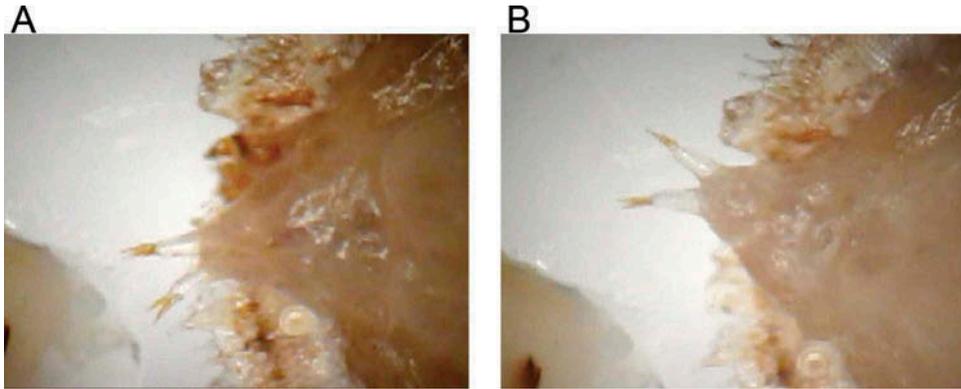


Figure 9. *Microdon analis*, drawing of the articulation point between the mandible and the labium, lateral view, length of mandible 0.3 mm. lm = labium; lp = labial plate; ls = labial sclerite; m = mandible; mt = muscle tendon; sd = salivary duct; sj = socket joint.



Figures 10. *Microdon analis*, sweeping, stills from a film sequence lasting 5 sec: (A) to the left; (B) to the right.

point, but does not grip the substrate with mandibles. Feeding consists of repeated lunges into the prey via protraction and retraction of the head skeleton. When protracting, the mandibles are depressed to a shallow 25–40 degrees angle relative to the horizontal. At the limit of protraction, the labial sclerites and plate depress to almost a right angle and the mandibles rise to the horizontal and may separate slightly, these actions expose the opening of the pharynx ready for food to be sucked

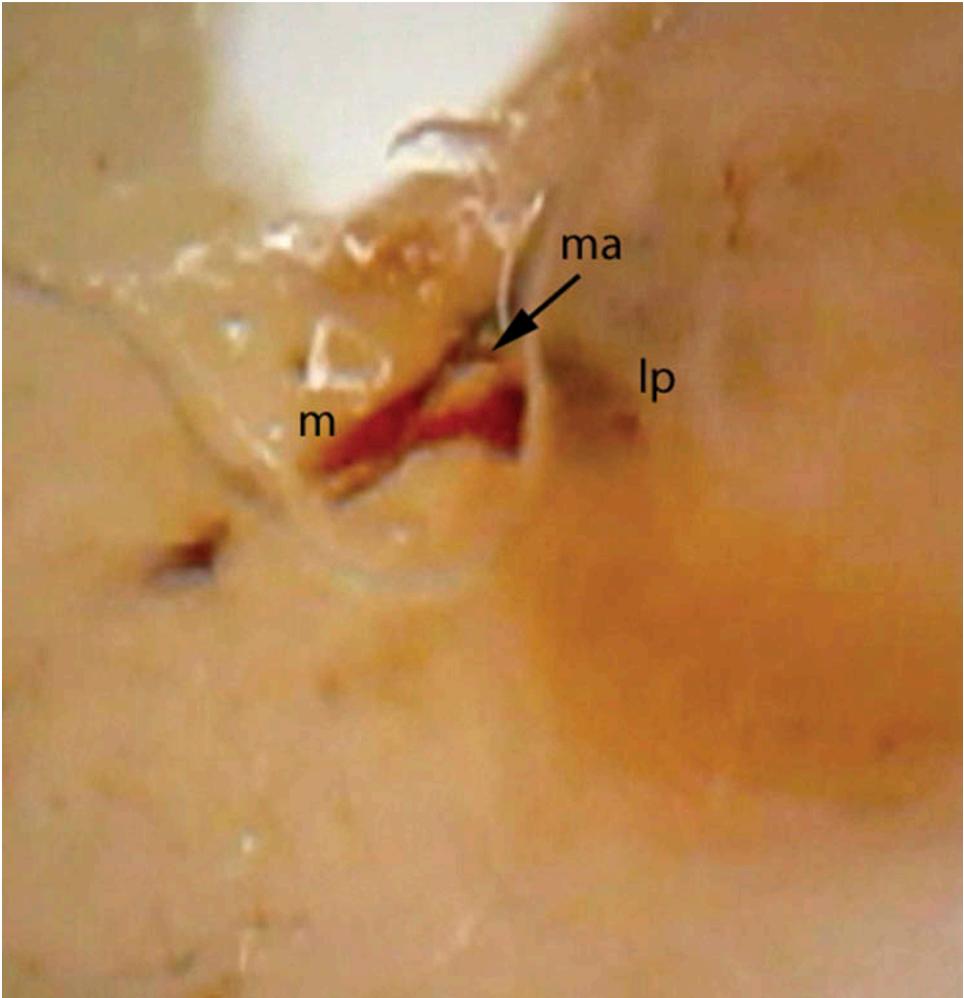


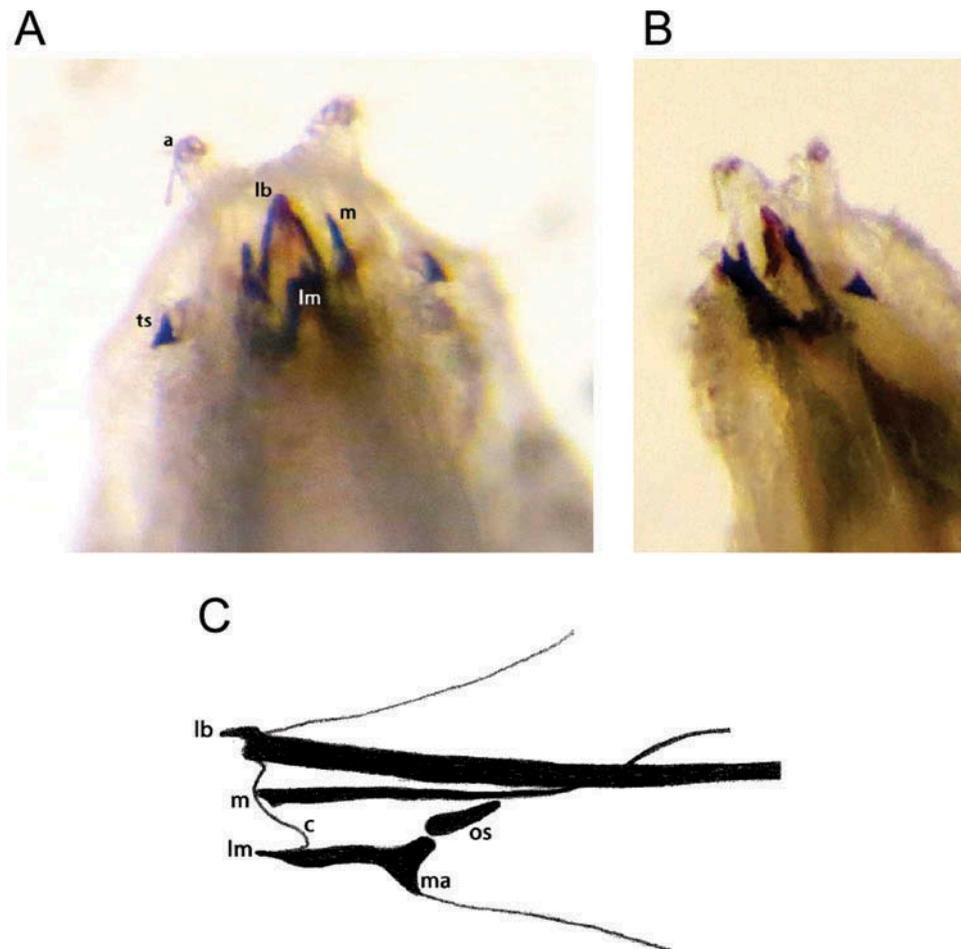
Figure 11. *Microdon analis*, position of mandibles and labial plate + sclerites during a feeding lunge inside a *Lasius* ant larva (Formicidae), still from Film 5. lp = labial plate and sclerites; m = mandible, ma = apodeme at base of mandible.

in (Film 5, Figure 11). The pump operates independently of lunging and 3–8 pumping actions occur per lunge. By altering the direction of protraction, the head skeleton can reach different areas inside the prey, but no seal is made between it and the larva and prey fluids spill from the wound.

Melangyna cincta: at rest, the larva of this species is similar to *M. analis* in that the front margin is the metathorax with the mesothorax and prothorax contracted underneath, but the metathorax is not fixed into two sections and when feeding or moving, these segments unfold. The larva is subcylindrical, highly flexible and the entire body has a complex, repeated, segmental pattern of integumental grooves and lines along which the integument collapses during movement. Rear compartment segments, anal segment to the prothorax posterior to the anterior spiracles, lack

vestiture and parts of the ventral surface are smooth. The apex of the anal segment has a smooth-surfaced, transverse, grasping bar. The prothorax overlaps the mesothorax. Antero-laterally the prothorax has a pair of black, triangular-shaped sclerites that are basally attached to the integument (Figure 12A and 12B). The labrum and labial plate are sclerotised and taper together at the front of the head skeleton (Figures 12A and 12B). The labial plate articulates with the labial sclerites which are embedded in the sides of the head skeleton with muscles inserting on a postero-ventral apodeme (Figure 12C). The mandibles are bar-shaped, taper posteriorly, lack muscles and are embedded into the lateral margins of the head skeleton (Figure 12C).

This larva is the most prehensile of those studied here. It sweeps by holding on with the rear end and moving the front of the body up to about 75 degrees to either side. Unlike other species, the border between the rear and front compartments is not



Figures 12. *Melangyna cincta*, preserved larva. (A) Prothorax and apex of head skeleton, apico-ventral view; (B) prothorax and apex of head skeleton, lateral view; (C) drawing of apical end of the head skeleton, lateral view. a = antenna; c = connecting tissue; lb = labrum; lm = labium; m = mandible; ma = postero-ventral muscle attachment apodeme; os = labial sclerite; ts = triangular sclerite.

fixed and, depending on the extent of sweeping, the body bends at any point from about the sixth abdominal segment to the mesothorax. To initiate forward locomotion, muscles contract in front of the anus and a crease forms across the body. This pulls the grasping bar on to the substrate and a peristaltic wave moves forward. The sides of the body bulge with body fluids which forces the ventral surface against the substrate and gripping power moves along the body. When the peristaltic wave reaches the prothorax, it inclines and either holds on using a prominent, mid-ventral pad or, the tip of the head skeleton is pressed against the substrate and held there, presumably by suction pressure from the pump. Simultaneously, the crease at the rear end fills out and the grasping bar lifts to release the anal segment which is pulled forward and another peristaltic wave begins. When it reaches the prothorax, it lifts and extends forward and ahead of the previous position and so, forward locomotion occurs. The maximum rate recorded was one body length in 2.4 seconds.

The larva can also raise the body up as far as the fourth and fifth abdominal segments, and move forward with just the rear three or four segments while the front end remains elevated (Film 6). If flocculence is encountered when lunging for food, it probes it with the head skeleton and if it sticks, the larva wipes it off on the substrate. If a wax-coated droplet of honeydew is encountered, it pierces it and imbibes or spills the contents. If it encounters an aphid with any part of the ventral surface of the anterior compartments, the larva shuffles backwards until it can bend the prothorax and capture it. Capture involves holding it in place with sticky saliva, contraction of the prothoracic apex to form a cup-shape into which the prey is drawn and simultaneously exposes the triangular, lateral hooks to further grip the prey. Feeding occurs by protraction of the head skeleton co-ordinated with contraction of the prothorax which presses the sharp apices of the labrum and labial plate against the prey until it is pierced and, usually, the prey is lifted from the substrate. To extract food, the head skeleton protracts and retracts repeatedly and the labium lowers so that prey fluids enter the head skeleton and are sucked in by the pump (Film 7, Figure 13). Recorded lunge rates were fairly constant at one lunge per 0.4–0.5 second. From capture to wiping off the remains at the end of feeding, handling time in a filmed sequence was 2 minutes 20 seconds, during which about 311 lunges were made.

Higher Cyclorrhapha

Compared with lower Cyclorrhapha, the labium is inactive in food gathering. The upper arm articulates with the mandibles, while the lower arm comprising labial plate and labial sclerites is incorporated into a new structure, the atrium. Also new is an oral cavity and an anal lobe, and food gathering includes a functional partnership between the pseudocephalon and mandibles. Four compartments are usually present: anal segment to abdominal segment 2; abdominal segment 1 to the anterior spiracles; anterior spiracles to the apex of the prothorax and, the head skeleton.

Saprophagy: *Silba fumosa* (Egger), *Lonchaea hackmani* Kovalev (Lonchaeidae); *Paloptera trimacula* (Meigen) (Pallopteridae); *Meiosimyza platycephala* (Loew) (Lauxaniidae); *Coelopa frigida* (Fabricius) (Coelophidae); *Clusia flava* (Meigen) (Clusiidae); *Neophyllomyza acyglossa* (Villeneuve) (Milichiidae); *Chymomyza costata* (Zetterstedt) (Drosophilidae); *Calliphora vomitoria* (Linnaeus) (Calliphoridae).

These larva feed on watery to soft-solid biofilm in narrow, concealed spaces where access is restricted + to ++ (Table 1). However, *C. frigida* and *C. vomitoria*



Figure 13. *Melangyna cincta*, depression of the labium at the end of a feeding lunge inside a *Phyllaphis fagi* aphid (Aphididae), still from Film 7.

also feed in less constrained places where volumes of decay can be many times larval heights (Table 1). Rear compartment segments relatively uniform and locomotory spicules straddle the borders between segments, always with fewer spicules on the segment in front; those spicules may incline forward and those behind incline backwards. Similar spicules coat the anal lobe and the anterior face of the prothorax, and reduced numbers are usually present at the border between abdominal segment 1 and the metathorax. The pseudocephalon comprises a pair of lobes each of which ensheathes a mandible with a gap ventro-apically through which the mandible protrudes (Figure 14). Anastomising cirri cover the lateral and ventral margins of the lobes and lead towards the pharynx between the mandible bases (Figure 14). Mandibles comprise two parts or sections: a subrectangular base and a curved, distal mandibular hook (Figures 15 and 16). The mandibular base bears two apodemes to which elevator muscles attach at the upper, inner point and depressor muscles underneath the lower, outer point (Figure 16). The inside face of the mandibular hook is often flattened or scalloped (Figure 14). The mandibular base is often indented medially (Figure 16). A hinge joint exists between the mandible and the intermediate sclerite, with a ridge on the rear face of the mandible (Figure 17) fitting into a groove on the front margin of the intermediate sclerite (Figure 18). The anterior end of the



Figure 14. *Coelophora frigida*, preserved larva, pseudocephalon, length, 1.3 mm, ventral view.

intermediate sclerite is inflated to accommodate the groove and greatly more sclerotized relative to the base and the ventral bridge at this end of the intermediate sclerite (Figure 15). Dental sclerites extend under the base of each mandible and are enveloped in depressor muscle tendon (Figure 16). The basal sclerite is variably sclerotised but usually more in the upper half and the cornua are more or less parallel (Figure 15).

Feeding and movement consist of sweeping and lunging (Film 1, Figure 19A–C). Lunging is inclination of the prothorax and except for *C. flava*, in which the head skeleton is fixed in the thorax, involves protraction and retraction of the head skeleton and depression of the mandibles (Films 8–11, Figure 20A–E).

As one lunge ends:

- abdominal segments 8–2 are extended and stationary;
- abdominal segment 1, the metathorax, mesothorax and the prothorax to the level of the anterior spiracles are contracted;

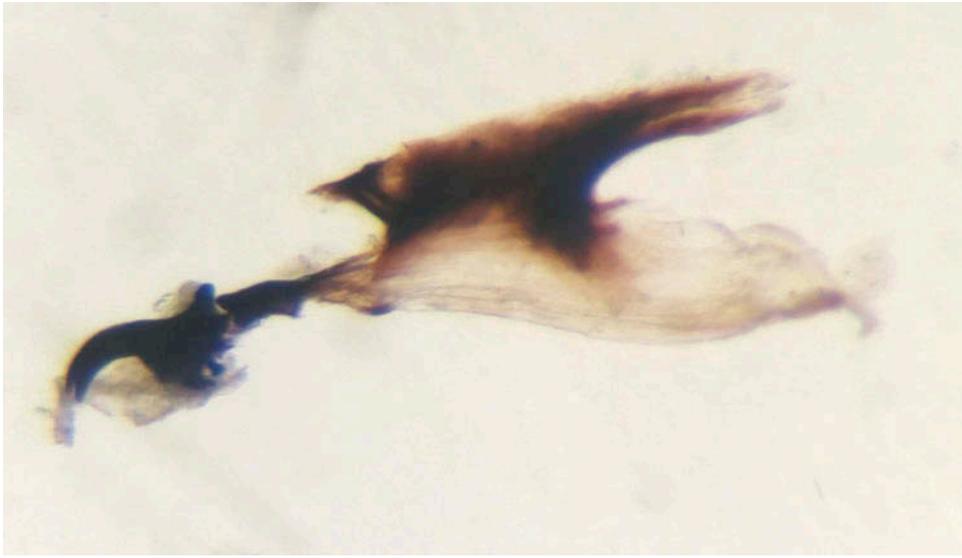


Figure 15. *Silba fumosa*, head skeleton removed from a puparium, length 1.3 mm, mandibles to the left.

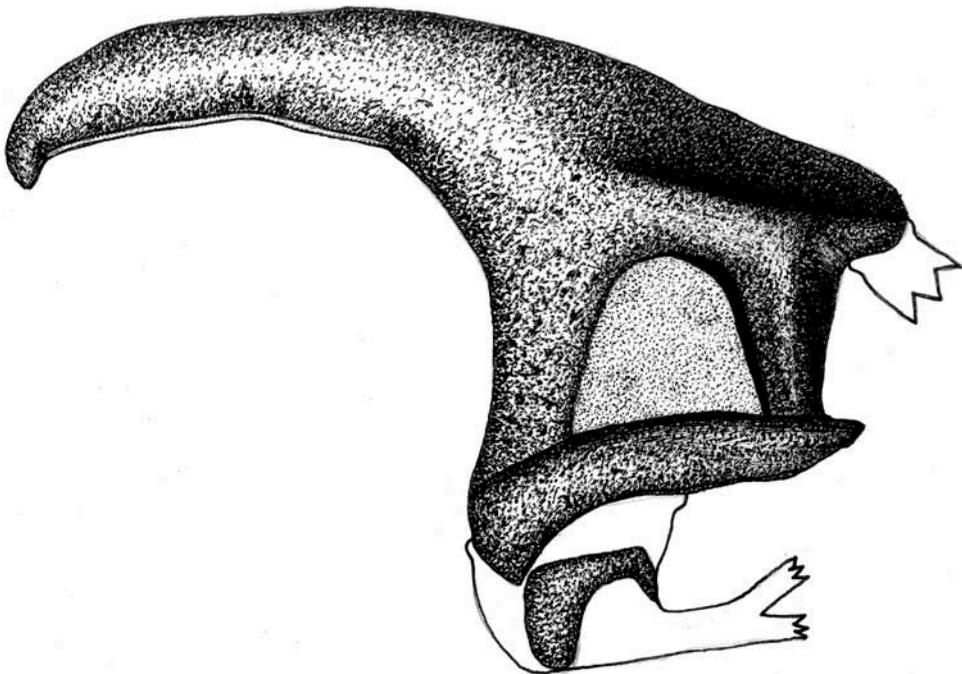


Figure 16. *Calliphora vomitoria*, drawing of the left hand mandible, dental sclerite and tendons, outside lateral view, length 0.4 mm.

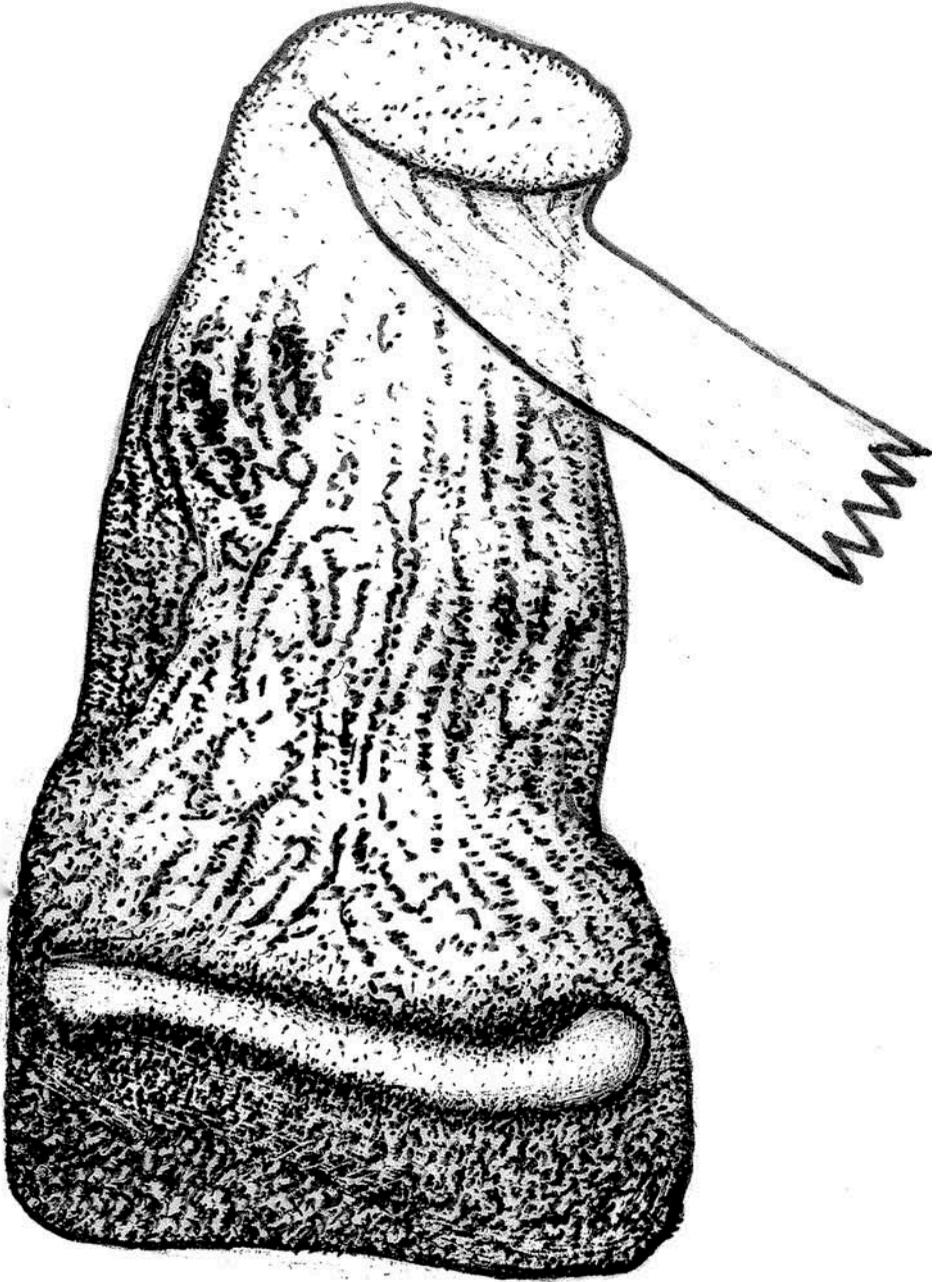


Figure 17. *Calliphora vomitoria*, drawing of the posterior face of the right hand mandible, elevator tendon on the upper margin and ridge of the hinge joint with the apex of the intermediate sclerite (Figure 18) across the lower margin, height 0.1 mm.

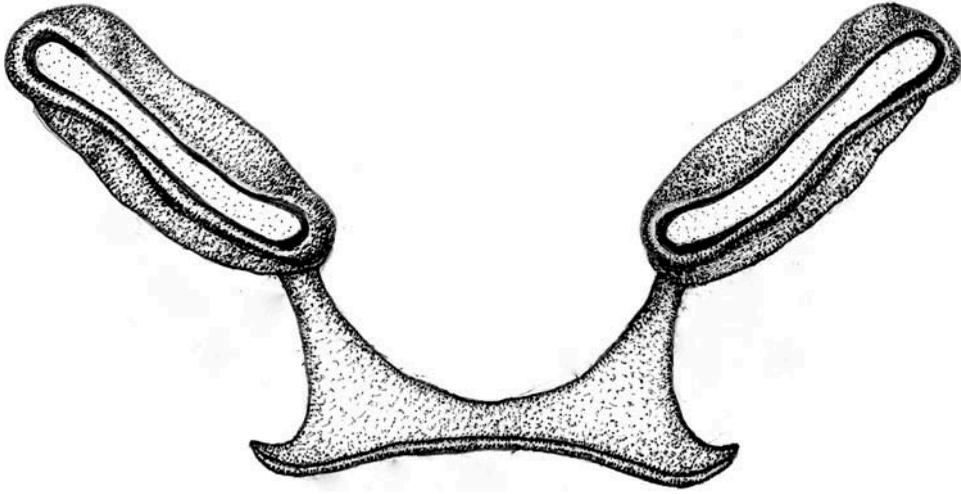
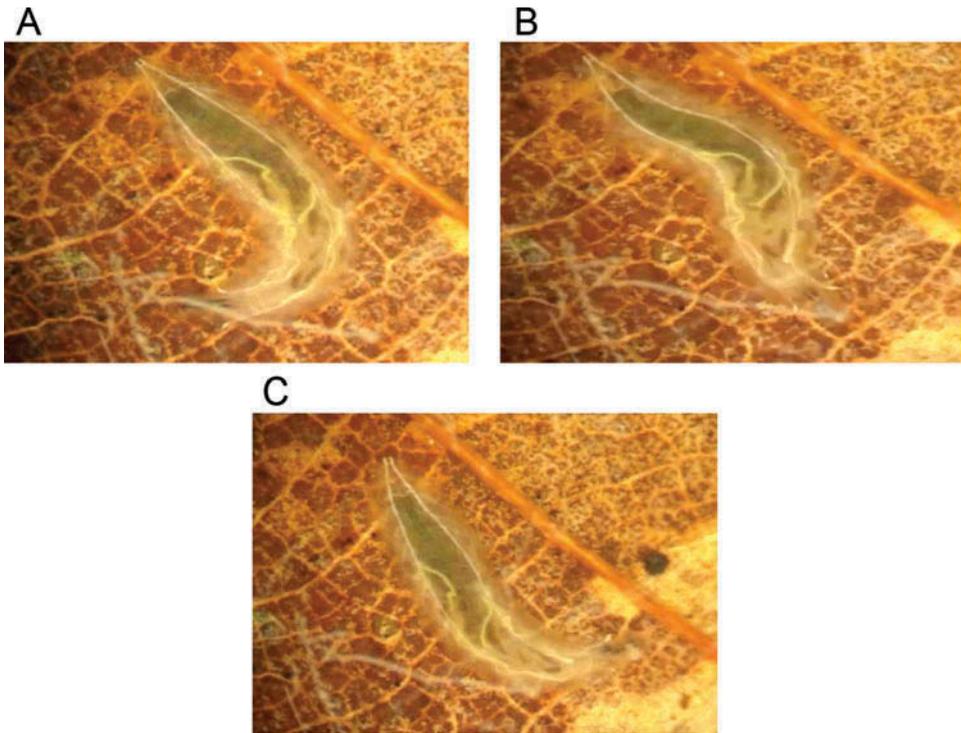
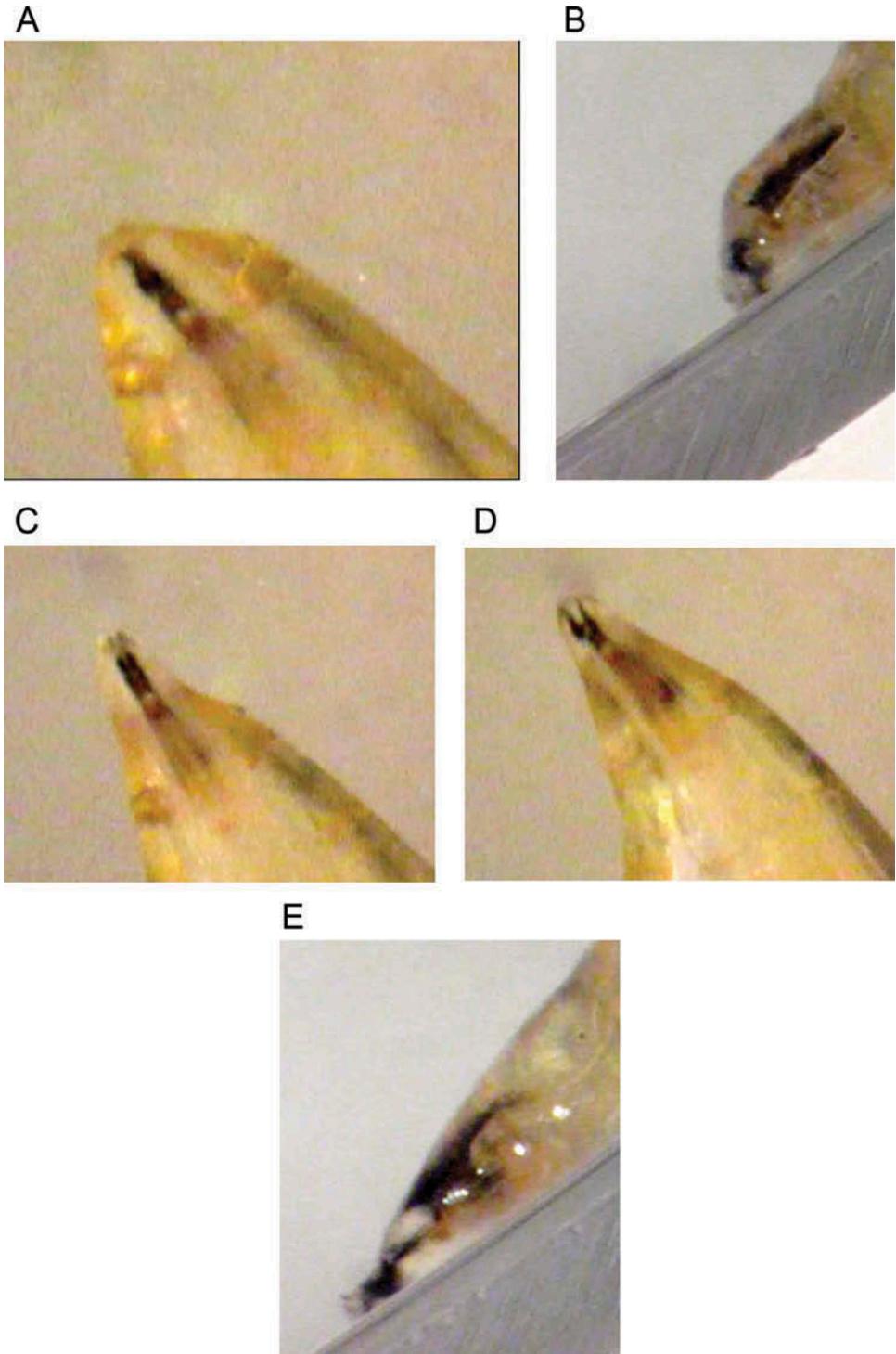


Figure 18. *Calliphora vomitoria*, drawing of the anterior face of the intermediate sclerite with inclined grooves of the hinge joint formed with a ridge on the posterior face of the mandible (Figure 17), maximum width 0.2 mm.



Figures 19. *Meiosimyza platycephala*, sweeping on the surface of a dead leaf, stills from Film 1 of a sequence lasting 12 sec in real time. (A) Limit reached of sweeping right; (B) sweeping back to the midline; (C) sweeping left.



Figures 20. *Silba fumosa*, a lunge taking about 1 sec. (A) Start of a lunge, ventral view, from Film 8; (B) start of a lunge, lateral view from Film 9; (C–E) mandible positions at the limit of extension: (C) ventral view; (D) mandibles starting to depress and separate, ventral view; (E) on the substrate, lateral view.

- the prothorax beyond the spiracles is inclined;
- the pseudocephalon and head skeleton are retracted and the mandibles depressed (Figure 20A and 20B).

A new lunge begins when:

- the head skeleton pivots and protracts, the mandibles elevate and the prothorax beyond the anterior spiracles rises and extends; these actions bring the mandibles together which closes the oral cavity, tapers the pseudocephalon and moves these compartments towards food or a substrate (Figure 20C);
- at the limit of extension, the prothorax beyond the anterior spiracles inclines down and the mandibles depress and separate and the labial lobe retracts; these actions extend the mandibular hooks from their sheaths, opens the oral cavity and exposes the pharynx (Figure 20D);
- on reaching a firm substrate, the entire ventral length of the mandibles are briefly parallel with it (Figure 20E), i.e. the mandibular hooks are in the food, the oral cavity is open and food is gathered into it by retraction of the head skeleton and depression of the mandibles (Figures 20B and 21);
- the mandibles depress to their limit which lifts the oral cavity (Figure 20B) and biofilm within the oral cavity is sucked into the head skeleton by the pump in the basal sclerite; from inward movement of the dorsal cornua, there is more than one pumping action per depression.

In deep quantities of biofilm, food may be gathered by repeated elevation and depression of the mandibles alone. Extra-oral digestion was not observed in these species, i.e. saliva secreted prior to ingestion. Lunge times were within the range of one lunge per 0.4–0.9 sec (Table 2). The distances head skeletons protract from the thorax were 29–100% of head skeleton length (Table 2). The range of angles over which mandibles elevate and depress was greater below than above the horizontal axis (Table 2). During depression, the mandibles separate to about three times their distance apart at elevation (Figure 21).

Forward locomotion starts with contraction across the ventral and lower lateral margins of the seventh abdominal segment anterior to the anus, which presses the anal lobe against the substrate, and is followed by a peristaltic wave passing along the body. When the wave reaches the prothorax, a lunge takes place and the mandibles depress and grip the substrate. This momentarily blocks the next wave and, at the segment border with the prothorax, the mesothorax folds and slides forward over the prothorax as far as the anterior spiracles (Figure 22) and the front of the prothorax slides over the pseudocephalon (Figure 23A and 23B). At the next wave, the mandibles elevate and a lunge occurs which moves the anterior end ahead of the previous position. The main difference between feeding and locomotory lunges is that, in locomotion, the



Figure 21. *Palloptera trimacula*, mandibles fully extended and separated on lowering into food, antero-ventral view, still from Film 10.

mandibles partially depress to grip the substrate, whereas in feeding, the mandibles depress to their limit.

Silba fumosa; (Lonchaeidae); *Palloptera trimacula* (Pallopteridae)
and *Chymomyza costata* (Drosophilidae)

Compared with other saprophages, these larvae were most similar to each other. Cibarial ridges are present and lunge times are similar and neither fast nor slow relative to the range of other saprophages (Table 2). Lunge distances are neither long nor short relative to the range of the others (Table 3). Thoracic segments taper the least (Table 3). Length of the thorax as a proportion of overall body length is at the high end of the range for all saprophages (Table 3). The pseudocephalon is longer than wide and, in *S. fumosa*, the oral cavity is open (i.e. the lobes of the pseudocephalon separate from the base of the mandibles); the oral cavity is closed in the other two species (i.e. by fusion of the lobes along their dorsal and apical margins), like an upside-down bowl. The inside margin of the mandibular hook is rounded and the anterior face without spicules in *S. fumosa*. In the other two species, the mandibular hook is flattened and the anterior fold has spicules.

Table 2. Lunge times, lunge distance (as proportion of head skeleton length) and mandible and head skeleton pivot angles measured from films of the larva of 14 species of higher Cyclorrhapha (= Schizophora), x = unscorable.

Species	Lunge time mean and (range) (in seconds)	Lunge distance	Mandible pivot above (°)	Mandible pivot below (°)	Head skeleton pivot (°)
Saprophagy					
<i>Setisquamalonchaea fumosa</i>	0.62 (0.6–0.7)	55	20	90	x
<i>Paloptera trimacula</i>	0.8 (0.7–0.9)	66	20	90	x
<i>Chymomyza costata</i>	0.74 (0.6–1)	57	10	90	x
<i>Coelopa (Fucomyia) frigida</i>	0.54 (0.4–0.6)	81	0	20–40	x
<i>Calliphora vomitoria</i>	0.28 (0.2–0.4)	100	10	80	x
<i>Lonchaea hackmani</i>	0.44 (0.3–0.5)	29	10	80	x
<i>Neophyllomyza acyglossa</i>	0.94 (0.6–1.5)	30	10	80	x
<i>Meiosimyza platycephala</i>	0.54 (0.4–0.8)	38	0	20–70	x
Phytophagy					
<i>Tephritis vespertina</i>	0.5 (0.4–0.7)	x	10	30	40
<i>Botanophila seneciella</i>	0.56 (0.5–0.6)	x	10	40	70
<i>Acidia cognata</i>	0.66 (0.6–0.8)	x	15	20	100
<i>Pegomya solennis</i>	0.36 (0.3–0.4)	x	10	60	90
Mycophagy					
<i>Lonchaea sylvatica</i>	0.8 (0.6–1.2)	9	10	90	20
<i>Stegana coleoprata</i>	1.62 (0.4–2)	13	20	90	20

Coelopa (Fucomyia) frigida (Coelophidae) and
Calliphora vomitoria (Calliphoridae)

Typically, these larvae are immersed in deep volumes of material and, compared with other saprophages, they are similar. Fully extended, the middle body compartments of both species form a gradual taper from back to front, but importantly, this gradual taper is disguised by the extent to which individual segments fold into one another so that this body compartment appears to taper in a stepped manner, i.e. across segment borders succeeding segments appear smaller abruptly rather than gradually; compare Figure 1 with Figure 24. Also, their oral cavities are bridged anteriorly and *C. frigida* has crenulated ridges coating the bridge and the fused ends of the cephalic lobes (Figure 14). In contrast, *C. vomitoria* has a smooth bridge with a spoon-shaped, black sclerite embedded within it, extending from which, into the oral cavity, is a pair of lightly sclerotised, oval-shaped plates (Figures 25 and 26). The inner faces of the mandibular hooks are scalloped (Figure 14) and both larvae have extensive networks of spicules for gripping substrates which extend from the locomotory organs to circumvent segment borders (Figure 24). The intermediate sclerite is block-shaped not

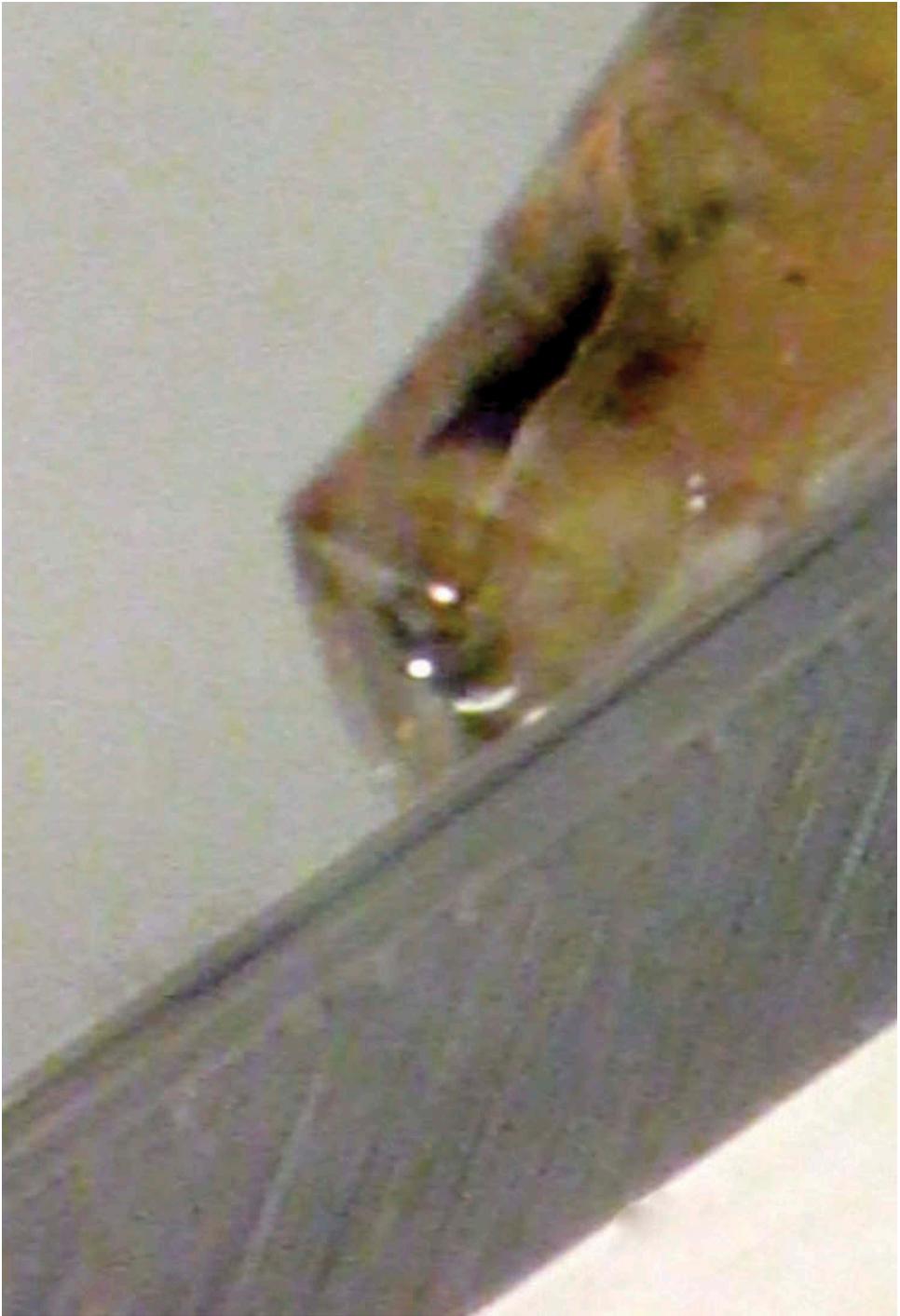
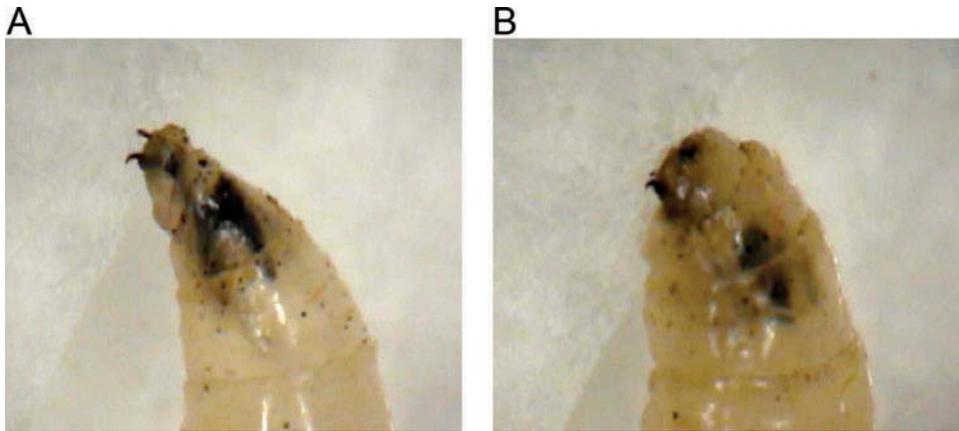


Figure 22. *Silba fumosa*, mesothorax folding over prothorax during locomotion, lateral view, still from Film 9.



Figures 23. *Coelopha frigida*, stills from a film. (A) Mandibles gripping a substrate at the end of a peristaltic wave. (B) Prothorax folded over the pseudocephalon during the next locomotory wave.

bar-shaped in *C. vomitoria* and sclerotisation of the ventral cornu ends abruptly midway along its length. The vertical plate in *C. frigida* is remarkably short. Both species have cibarial ridges and both initiate sweeping by thrusting the middle compartment in a more or less anterior direction and bending it round to one side or the other, almost reaching a position parallel with the rear section (Figure 27). Lunging takes place repeatedly during sweeping and the segments of the middle and front compartments are highly retractile, for example, only in these two species did the mesothorax fold over the anterior spiracles (Figure 23A and 23B). With the middle compartment sweeping, the front one may incline and twist so that the oral cavity covers a wide range of directions and angles. Mandibular depression in *C. vomitoria* exposes the sclerotised plates between the mandibular hooks (Film 11, Figure 26). Lunging in *C. vomitoria* was the most rapid of the saprophages examined here and this species had the longest lunge distance (Film 12, Table 2). *C. frigida* also had a relatively fast, long lunge (Table 2). Unusually, in *C. vomitoria* saliva was secreted during locomotion and both species ingested food in shallow films using mechanisms similar to *S. fumosa*, *P. trimacula* and *C. costata*.

Lonchaea hackmani (Lonchaeidae)
and *Neophyllomyza acyglossa* (Milichiidae)

These larvae feed particularly on biofilm in cracks and crevices and coating networks of fibres (Figure 28, Table 1). During locomotion they are able to move forwards by gripping the substrate with the abdomen alone, and great flexibility and range of movement exists in the front body compartment and the head skeleton. For instance, the prothorax and head skeleton can twist and pivot from side to side (Film 13) and the prothorax can fold on itself (Figures 29 and 30). In *L. hackmani*, the prothorax can rotate 360 degrees (Film 13) and, uniquely, the intermediate sclerite of *N. acyglossa* can bend (Film 14, Figure 31A–D). Lunge distances were, however, the shortest recorded (Table 2). Yet lunge times differed, being the second fastest in *L.*

Table 3. Body measurements and proportional lengths taken from larvae of 16 species of higher Cyclorhapha (= Schizophora) that were preserved after filming. x = unscorable for *C. flava* due to fusion between the basal and intermediate sclerites (summed length of both sclerites reported under column headed 'Basal sclerite length').

Species	Abdomen length (mm)	Thorax length (mm)	Body length (mm)	% thorax of body length	Height base of metathorax (mm)	Height apex of prothorax (mm)	% prothorax apex of metathorax base	Basal sclerite length (mm)	Intermediate sclerite length (mm)	Mandible length (mm)	Head skeleton length (mm)	% head skeleton length of body
Saprophagy												
<i>Setisquamalonchaea fumosa</i>	6.7	2.3	9	25	0.9	0.4	44	0.9	0.2	0.2	1.3	14
<i>Palloptera trimacula</i>	3.9	1.6	5.5	29	1.2	0.8	66	0.9	0.2	0.2	1.3	23
<i>Chymomyza costata</i>	4.9	1.3	6.2	28	0.3	0.2	66	1.3	0.3	0.3	1.9	21
<i>Coelopa (Fucomyia) frigida</i>	11.2	1	12.2	8	1.7	0.5	29	1.5	0.3	0.4	2.1	17
<i>Calliphora vomitoria</i>	12.8	3.2	16	20	1.4	0.5	35	1	0.2	0.4	1.6	10
<i>Lonchaea hackmani</i>	7.3	1.7	8	21	0.4	0.1	25	0.2	0.2	>0.1	0.5	6
<i>Neophylomyza acygllosa</i>	3.5	1	4.5	22	0.5	0.1	20	0.2	0.2	>0.1	0.5	13
<i>Meiostomyza platycephala</i>	3.4	1.1	4.5	24	0.5	0.3	60	1	0.3	0.3	1.6	35
<i>Clusia flava</i>	6.8	1.9	8.7	21	0.1	0.1	0	0.3	x	>0.1	0.4	4
Phytophagy												
<i>Tephritis vespertina</i>	3.3	0.7	4	17	1.2	0.6	50	0.2	0.1	>0.1	0.4	10
<i>Chaetostomella cylindrica</i>	4.7	1.6	6.1	26	1.7	0.7	41	0.4	0.1	0.1	0.6	9
<i>Botanophila seneciella</i>	3.8	2.8	6.6	42	1.3	0.6	46	0.5	0.1	0.1	0.7	10
<i>Acidia cognata</i>	4.1	1.6	6.7	23	1.5	0.7	46	0.3	0.2	0.1	0.6	9
<i>Pegomya solennis</i>	6.4	1.7	8.1	20	1.2	0.5	41	0.2	0.2	0.1	0.5	6
Mycophagy												
<i>Lonchaea sybatica</i>	4.2	1.3	4.5	28	0.6	0.3	50	0.7	0.2	0.2	1.1	24
<i>Stegana coleoprata</i>	3.5	1	4.5	22	0.8	0.5	62	0.4	0.1	0.2	0.7	15



Figure 24. *Coelopa frigida*, preserved larva, abdominal segment 1, thorax and pseudocephalon, lateral view, length 2.9 mm.

hackmani and the slowest in *N. acyglossa* (Table 2). Also, relative to other saprophages, mandibles and oral cavities were relatively small, but the relative thorax length was neither long nor short although the thorax taper was the highest recorded (Table 3). Cibarial ridges are lacking and, in both species, the basal sclerite is distinctive being not greatly sclerotised with a short ventral cornu (Figure 32). The intermediate sclerite of *N. acyglossa* is uniquely long (Figure 32).

Meiosimyza platycephala (Loew) (Lauxaniidae)
and *Clusia flava* (Meigen) (Clusiidae)

These larvae feed on watery biofilm coating relatively smooth substrates where access is restricted + to ++ (Table 1). The oral cavity of *M. platycephala* is closed by a fleshy bridge, its surface coated with eight transverse rows of conspicuous, upstanding, apically sclerotised seta-like projections which also cover the inflated sides of the pseudocephalon and either side of the mandibular hooks (Figures 33A and 33B). This larva gathers food into the oral cavity by brushing with these setae (Films 1 and 15). The mandible base is narrow and elongate and the atrium is wide relative to the width of the mandibles (Figure 34). The atrium is supported dorsally by the sclerotised apex of the epipharyngeal plate and cibarial ridges are present. When feeding the larva sweeps from side to side (Film 1, Figures 19A–C) and lunges repeatedly; the lunge distance is fairly short and the lunge time not particularly fast or slow (Table 2). Throughout lunging, the mandibles are inclined and parallel, i.e. they do not elevate and only depress over the range 20–70 degrees



Figure 25. *Calliphora vomitoria*, preserved larva, pseudocephalon, ventral view, length 0.6 mm.

(Table 2), and they do not separate by much (Film 15, Figure 34). When feeding, the larva avoids obstacles such as prominent leaf veins. However, it crosses over such obstacles during locomotion, is able to make sharp changes in direction, such as over the edges of overhanging leaves, and by flattening its body can squeeze between adhering leaves.

Clusia flava: prothorax ovoid with a flattened apex surrounded by sensilla with antennae and maxillary organs almost flat to the surface (Figures 35–37). The centre of this apex leads to the inverted pseudocephalon from which project the tips of the lightly sclerotised mandibular hooks. This larva feeds by spot-sucking watery biofilm coated and soaked into soft, decayed, annual layers of sapwood of dead trees and branches (Table 1). Apart from the mandibular hooks, the head skeleton lacks sclerotisation, is translucent and short (Table 3). Like the pseudocephalon, it is fixed in an inverted position and does not protract or retract. The intermediate sclerite is fused to the basal sclerite, and cibarial ridges and dental sclerite are absent. Unusually, the anterior and ventral margins of the mesothorax and metathorax are coated in spicules; spicules also coat much of the ventral and



Figure 26. *Calliphora vomitoria*, pseudocephalon with mandibles and cutting plates depressed, still from a film, apical view.

dorsal margins of the rear body compartment. Apart from an inverted head skeleton, this larva is remarkable for its slow movement and highly flexible, middle body compartment (Film 16). The middle body compartment can twist from side to side, rotate 360 degrees, and each segment can collapse and fold into the one behind. Feeding lunges consist of the middle compartment bending down and the apex of the prothorax being placed on to the substrate and remaining in this position for up to 4 minutes.

Phytophagy: Tephritis vespertina; Chaetostomella cylindrica; Acidia cognata (Tephritidae); *Botanophila seneciella* and *Pegomya solennis* (Anthomyiidae).

The larvae of all five species are found within living plant structures (Table 1). *T. vespertina*, *C. cylindrica* and *B. seneciella* develop in different species of Asteraceae flowerheads, squeezing between florets and removing tissue (Figures 38A and 38B). Access is similar in flowerheads and restricted++. Unlike the other two species, the larva of *C. cylindrica* completes development by tunnelling through the hard receptacle at the base of the flowerhead. *A. cognata* and *P. solennis* mine leaves of



Figure 27. *Calliphora vomitoria*, larva feeding on decayed *Salmo*, still from Film 12.

Tussilago farfara L. (Asteraceae) and *Rumex acetosa* L. (Polygonaceae), respectively, and feed on live mesophyll (Figures 39 and 40). Access is restricted+++; however, access in leaves of *R. acetosa* is even more restricted as they are less than half as thick as those of *T. farfara* (<0.1–0.2 mm versus 0.4–0.6 mm, respectively, $n = 3$ leaves per plant species and measurements made at two equidistant points across the middle of each leaf).

All phytophages have mandibles with short, wide mandibular hooks; accessory teeth are usually present (little developed in *B. seneciella* (Figure 41D–F), absent in *C. cylindrica*) and elevator and depressor apodemes are more or less aligned one under the other, with the result that the base is crescent-shaped in lateral view (Figure 41A–G). Cibarial ridges are absent and dental sclerites reduced or absent. The intermediate sclerite is completely sclerotised and, in the tephritids, abuts smoothly with a forward projection of the basal sclerite (Figure 41A–C). The labial sclerite is developed and, in lateral view, appears as a curved, ventral bar between the mandible and the intermediate sclerite (Figure 41C and 41E). The valve at the end of the ventral cornu is weak (i.e. not sclerotized) and windows and areas of poor sclerotisation are present in both the dorsal and ventral cornua (Figure 41A–D and 41G). The pseudocephalon is dome-shaped (Figure 42), with a slightly elongate



Figure 28. *Lonchaea hackmani*, development site, convoluted substrate with wet decay under bark of fallen *Populus tremula*, Scotland, Inverness-shire, Strathspey, April 2013.

or oval-shaped oral cavity, cirri are absent and the ventral margin has a translucent, curved oral plate, sometimes with a small, sclerotised section (entirely translucent in *P. solennis* and *C. cylindrica*) and slightly shorter than the length of the head skeleton.

Sweeping is less pronounced and consists of downward rather than sideways movement of the middle body compartment. As one lunge ends:

- abdominal segments 8–2 are stationary;
- abdominal segment 1, the metathorax, mesothorax and the prothorax to the level of the anterior spiracles are extended;
- the prothorax beyond the spiracles is inclined;
- the pseudocephalon is extended dorsally and collapsed and/or folded into the prothorax ventrally and the head skeleton is inclined and the mandibles depressed;

A new lunge begins when:

- the head skeleton pivots up by about 10–20 degrees with respect to the horizontal position (Table 2) and the mandibles rise; dorsally, the pseudocephalon folds slightly and ventrally it unfolds (Figure 43A);



Figure 29. *Lonchaea hackmani*, prothorax unfolding during a lunge, still from Film 13.

- as pivoting ends and the mandibles are against plant tissue, the head skeleton starts pivoting in the opposite direction which pulls the mandibles down across plant tissue; the mandibles pivot down by up to 60 degrees (Table 2), and dorsally, the pseudocephalon unfolds and ventrally folds (Figure 43B);
- for most of the downward pivot, the mandibles do not depress until the end of the pivot when they depress to their limit; rasped material fills the oral cavity and is sucked in by the pump in the head skeleton.

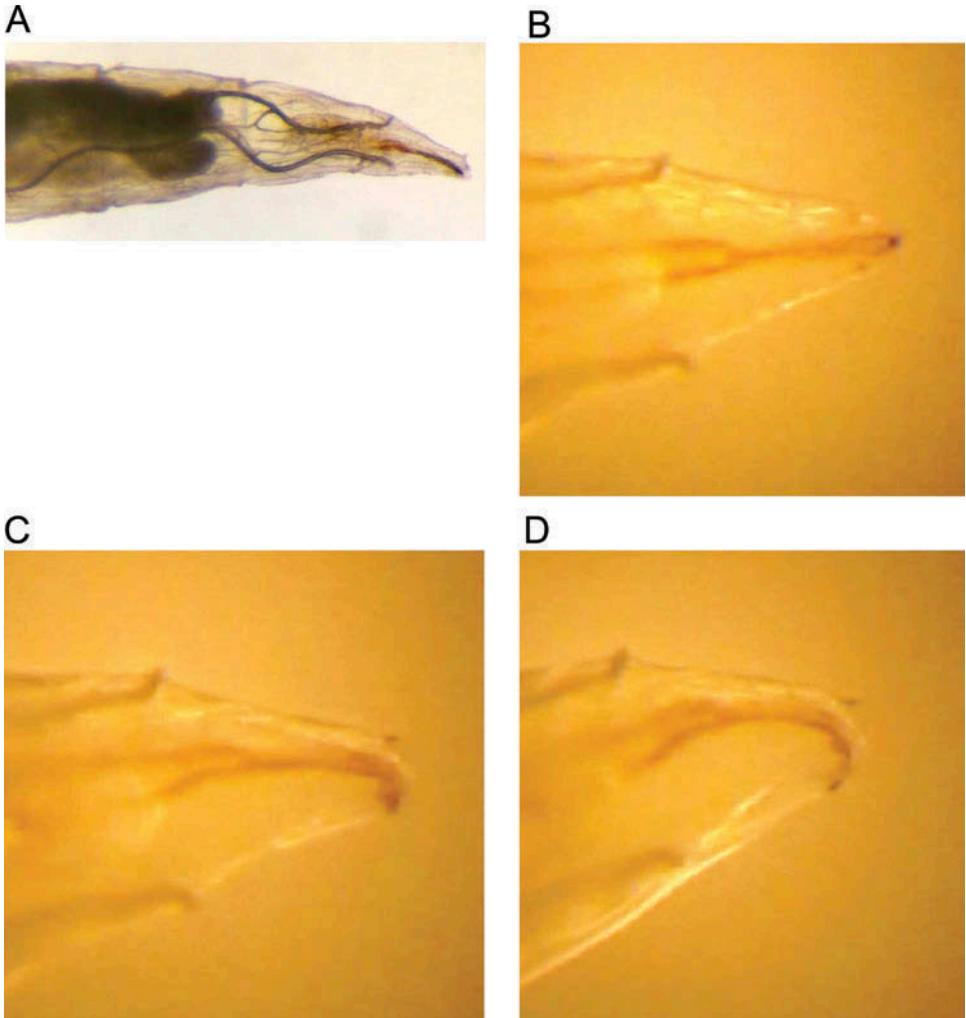
Flowerhead feeders: body short and broad (Figures 38, 44–46, Table 3). Spicules are extensive, except for being reduced on the middle abdominal segments, metathorax and mesothorax and only present on antero-dorsal and antero-ventral margins of the prothorax. *T. vespertina* with mandibular muscle apodemes straight, not curved (Figures 41A) and mandible almost as long as wide with one accessory tooth. Mandible are longer than wide in *B. seneciella* and *C. cylindrica*; *B. seneciella* has a tapered apex to the mandibular hook (Figure 41(f)). In living larvae, the upper margin of the dorsal cornu is bent inwards along the window and the front body compartment is able to rotate 360 degrees. In all three species, holes are made in floret tissues, the margins of which decay and the larva can be surrounded by moist to wet decayed material on which it may feed (Figure 38A). Larvae start development by feeding towards the base of florets, but readily reverse direction. Lunge times in



Figure 30. *Neophyllomyza acyglossa*, prothorax unfolding during a lunge, still from a film.

T. vespertina were 0.4–0.7 sec per lunge and 0.5–0.6 sec per lunge in *B. seneciella*; lunge times were not measured in *C. cylindrica* (Table 2).

Leaf miners: body elongate (Figures 38, 47 and 48, Table 3). Spicules are extensive and circumvent segment borders, especially on the anterior abdominal segments and the metathorax. Unlike *A. cognata* which is symmetrical, in *P. solennis* the pseudocephalon is asymmetrical with a longer lower side, i.e. when feeding the shorter margin faces the upper epidermis of the leaf (Figure 49). The pseudocephalon in *P. solennis* is more tapered than in *A. cognata*, such that the antennae are approximated whereas, in *A. cognata*, antennae are separated by about 1.5× their width. The lateral margins of the pseudocephalon in *P. solennis*



Figures 31. *Neophyllomyza acyglossa*. (A) Preserved larva, anterior end, lateral view, length 4.5 mm. Lunging, stills from Film 14: (B) limit of forward extension; (C) bending of the intermediate sclerite; (D) limit of bending in the intermediate sclerite.

have three square-shaped studs on the long side and six on the short side (Figure 49); studs are absent in *A. cognata*. The mandibular muscle apodemes curve backwards in both species. The mandibles are symmetrical in *A. cognata* with one accessory tooth each, but asymmetrical in *P. solennis* with the longer, lower mandible having a strongly curved, apical mandibular hook and two, short, accessory teeth and the lower mandible with a similar strong, curved apex to the mandibular hook and three, elongate accessory teeth (Figure 50). The prothorax in *P. solennis* has a conspicuous, lozenge-shaped, mid-ventral projecting bar (Figure 42). These larvae excavate leaf tissue in very different ways.

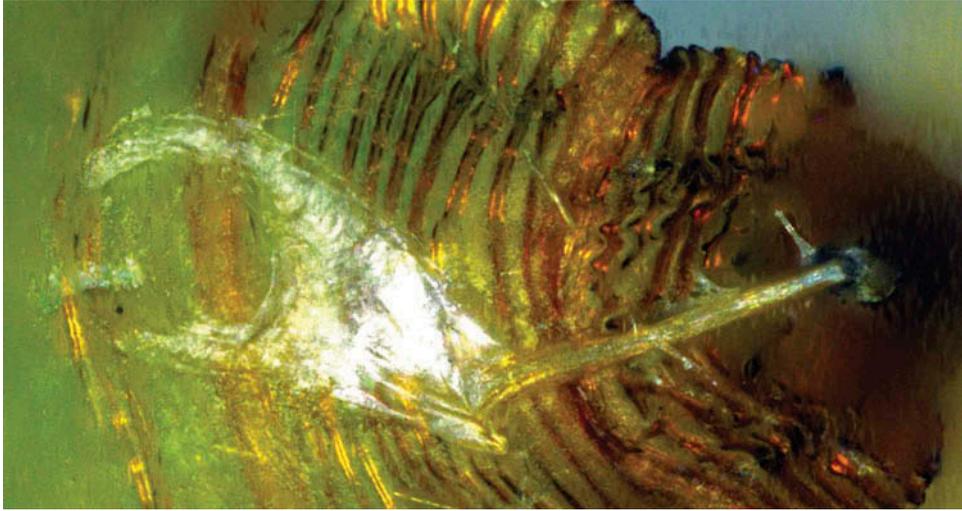
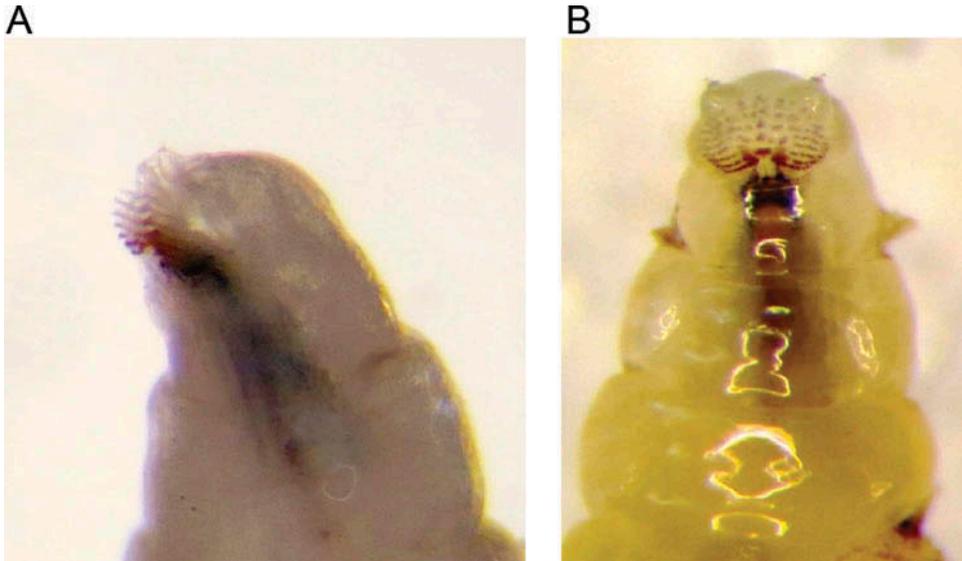


Figure 32. *Neophyllomyza acyglossa*, head skeleton, *in situ* lateral view from a puparium, length 0.4 mm.



Figures 33. *Meiosimyza platycephala*, preserved larva, pseudocephalon; (A) lateral view; (B) ventral view, length 0.2 mm.

Acidia cognata: feeding on its side this larva removes tissue in a series of long, crescent-shaped feeding tracks. Each track consists of succeeding numbers of arc-shaped excavations of tissue; both tracks and arcs are traceable by remnants of tissue left on the upper epidermis (Figure 40). These traces are not frass, which is otherwise scattered through the mine as lumps of moist, brown material (Figure 39).

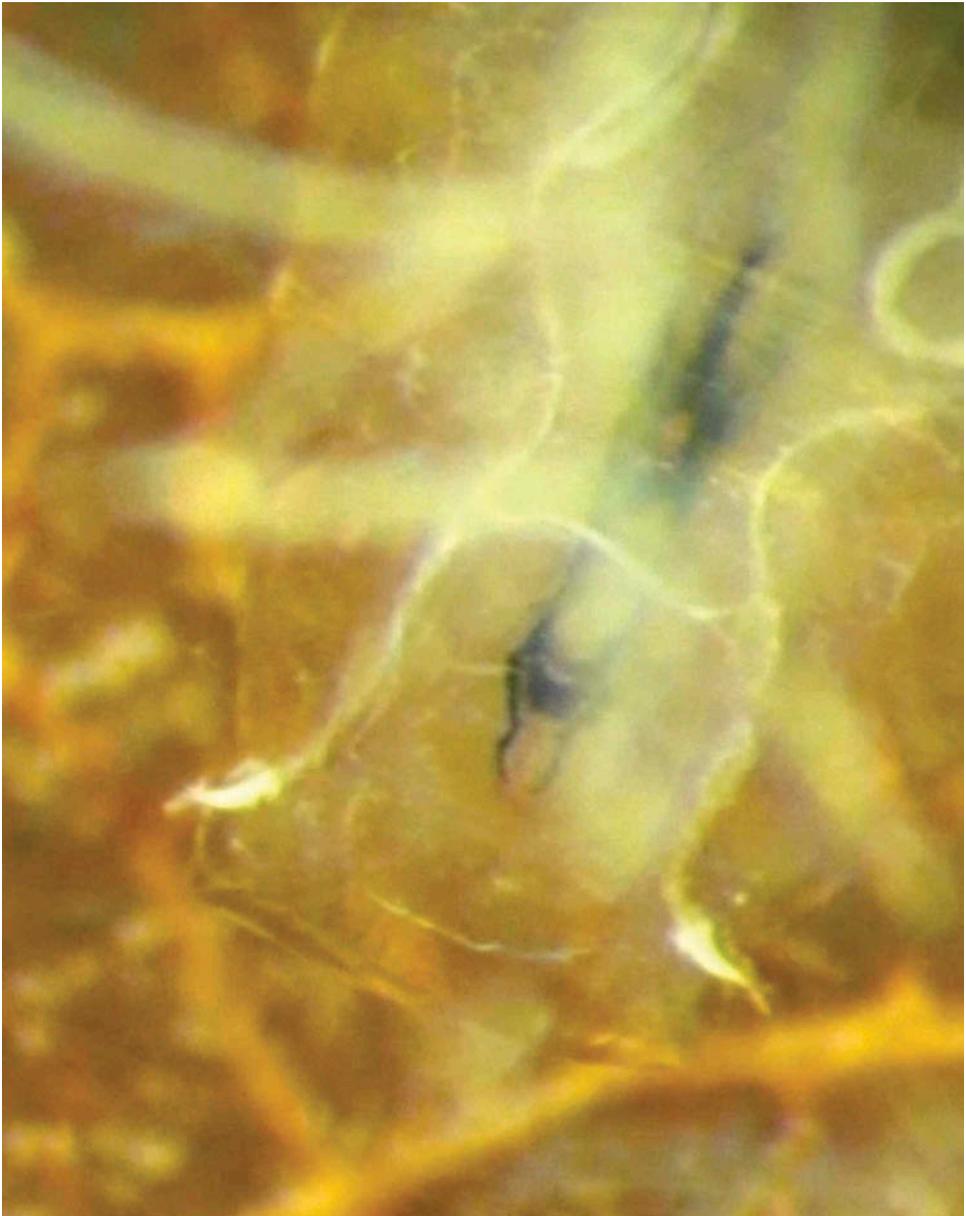


Figure 34. *Meiosimyza platycephala*, anterior end, dorsal view, still from Film 15.

Track direction is influenced by barriers, such as leaf veins, leaf edges, damaged areas and presumably, other larvae in the leaf. Arcs are made by the front compartment sweeping down across the mine face during which lunging occurs continuously with each lunge removing a line of tissue. To lunge, the rear compartment grips the upper and lower layers of the leaf, the middle compartment extends and the front compartment lifts and the mandibles elevate slightly and are placed against the

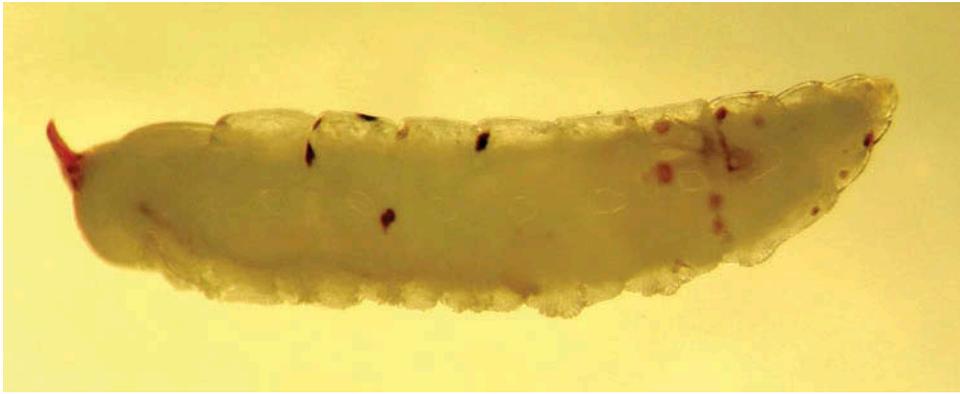


Figure 35. *Clusia flava*, preserved larva, lateral view, head to the right, length 8.7 mm.



Figure 36. *Clusia flava*, thorax, position at maximum forward extension, dorsal view, still from Film 15.

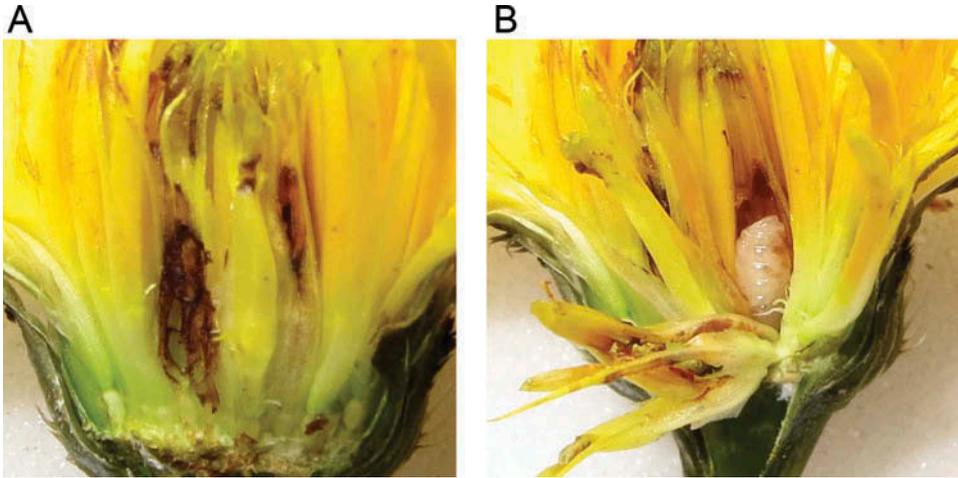
mine face. The head skeleton pivots down and the mandibles depress slightly and a line of tissue is removed (Film 17). The prothorax moves slowly down across the mine face with the head skeleton pivoting continuously; a small arc so filmed, took about 74 seconds to create and involved about 114 head skeleton pivots, i.e. lunge time was about one lunge per 0.6 sec. At the end of an arc, the larva moves into the



Figure 37. *Chusia flava*, prothorax, apical view still from Film 15.

excavated space, lifts the prothorax and begins to make another arc and so, a track gradually forms.

Pegomya solennis: the larva also feeds on its side but instead of tracks and arcs, oval-shaped cavities are formed in the leaf, but little tissue is left behind to indicate the pattern of feeding. To start lunging, the larva lifts the front compartment and the mandibles elevate slightly and are pressed against the mine face (Film 18, Figure 43A). The head skeleton pivots and a line of tissue is removed by the mandibles which depress slightly in the process. At the limit of depression, the fleshy, prothoracic bar is adjacent to the mandibles (Figure 43B). The front compartment then lifts and the process repeats. Lunge times, the time for a line of tissue to be removed, were about twice as fast as *A. cognata* and varied from about 0.1 to 0.3 second. When a space has been created in front of the larva, the prothorax



Figures 38. *Tephritis vespertina*. (A) Feeding damage caused by the third stage larva inside the flowerhead of *Hypochoeris radicata* L. (Asteraceae); (B) larva *in situ*.

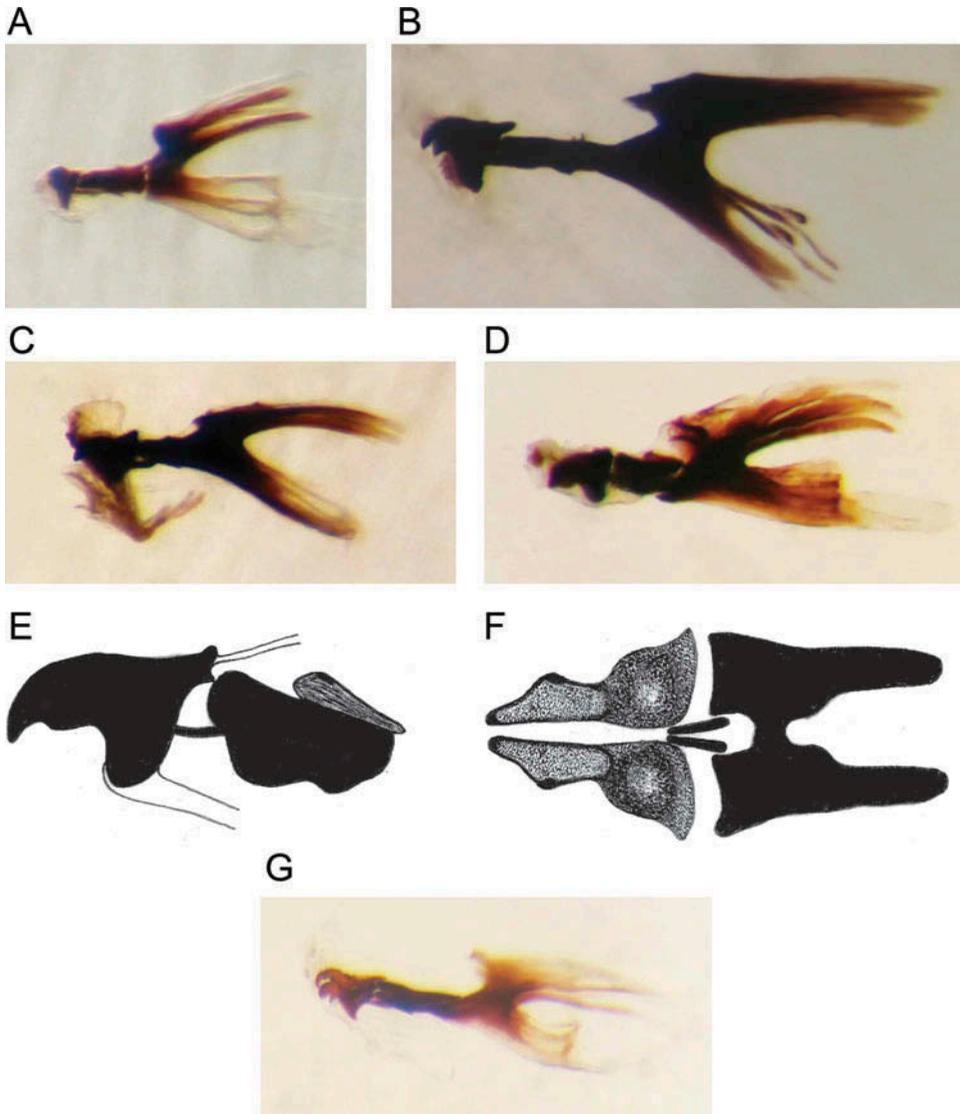


Figure 39. *Acidia cognata*, leaf mine in *Tussilago farfara* L. (Asteraceae), mine opened with larva *in situ*.



Figure 40. *Acidia cognata*, leaf mine in *Tussilago farfara* L. Asteraceae: t = between the arrows, part of a feeding track; a = arcs within a feeding track.

extends, the head skeleton pivots and protracts, and the mandibles elevate and grip the mine face. A peristaltic wave pushes the larva up against the mine face and another cycle of lunges start (Film 18). Blotch-shaped excavation occurs via the direction of lunging altering slightly. This depends where the mandibles grip the mine face prior to peristalsis; if below the longitudinal axis of the body, then lunging is in that direction, if above, then in that direction.



Figures 41. Head skeletons of phytophagous species, lateral views, mandibles to the left. (A) *Tephritis vespertina*, length 0.3 mm; (B) *Acidia cognata*, length 0.6 mm; (C) *Chaetostomella cylindrica*, length 0.6 mm; (D) *Botanophila seneciella*, length 0.7 mm. *Botanophila seneciella*, drawing of mandible and intermediate sclerite: (E) lateral view with muscle tendons; (F) ventral view; (G) *Pegomya solennis*, length 0.5 mm.

Mycophagy: Lonchaea sylvatica (Lonchaeidae)
and *Stegana coleoprata* (Drosophilidae).

The larvae of *L. sylvatica* and *S. coleoprata* feed on sooty moulds growing under bark lenticels of fallen aspen, *Populus tremula* L. and especially birch, *Betula* spp. (Figure 51). They can also feed on soft solid to firm biofilm under bark, more so

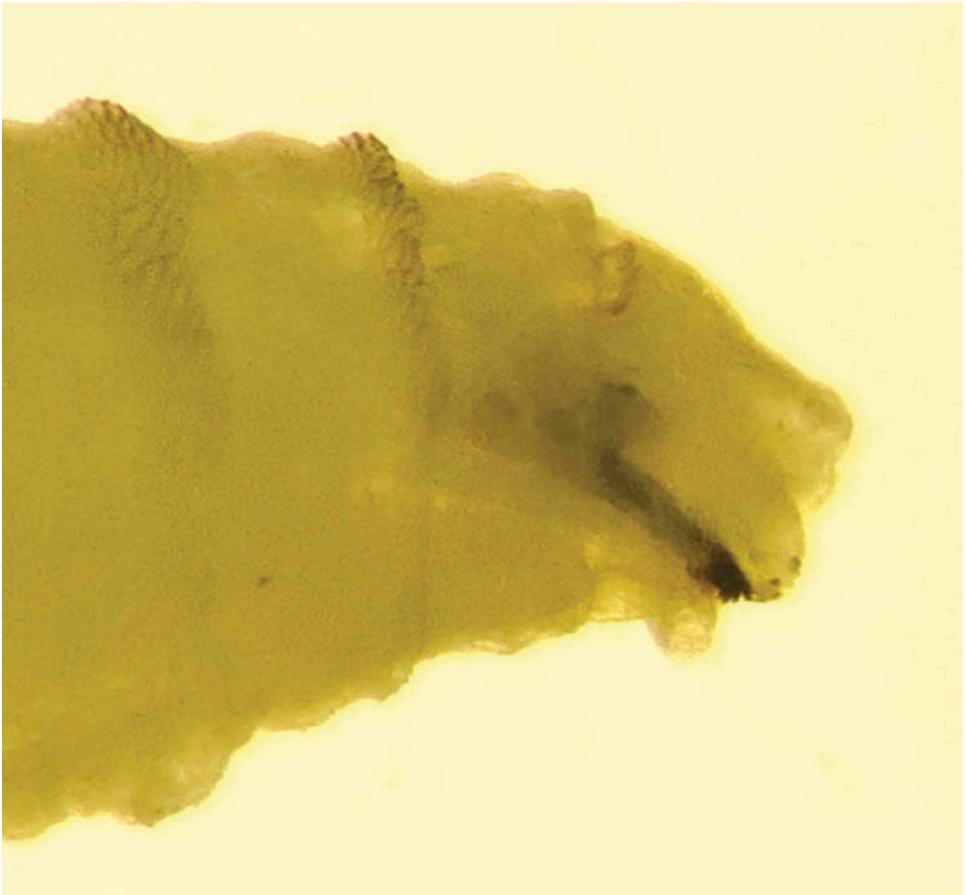
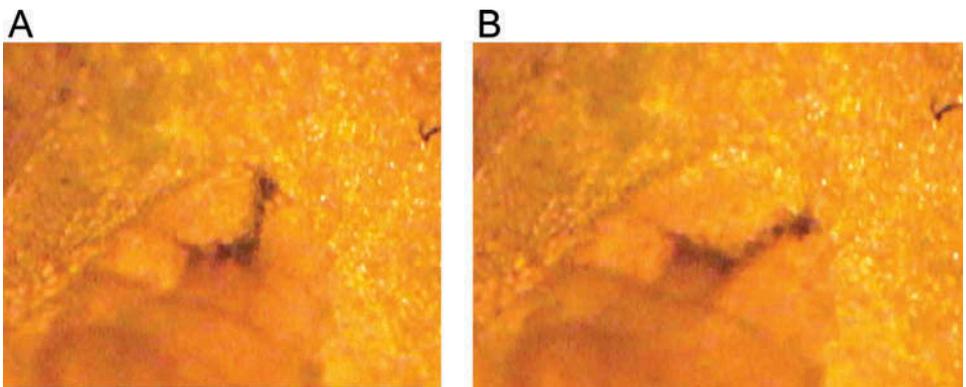


Figure 42. *Pegomya solennis*, preserved larva, thorax and pseudocephalon, head to the right, lateral view.



Figures 43. *Pegomya solennis*, larva feeding in a leaf mine on *Rumex acetosa* (Polygonaceae), stills from Film 18. (A) Position at the start of a feeding lunge; (B) position at the end of a feeding lunge.



Figure 44. *Botanophila seneciella*, preserved larva, lateral view, head to the right, length 2 mm.



Figure 45. *Tephritis vespertina*, preserved larva, lateral view, head to the right, length 4 mm.



Figure 46 *Chaetostomella cylindrica*, preserved larva, lateral view, head to the right, length 6.3 mm.



Figure 47. *Pegomya solennis*, preserved larva, lateral view, head to the right, length 8.1 mm.



Figure 48. *Acidia cognata*, preserved larva, lateral view, head to the right, length 5.7 mm.



Figure 49. *Pegomya solennis*, preserved larva, pseudocephalon, ventral view.

L. sylvatica. The heights of these spaces is often less than the heights of larvae and access is restricted++ to +++. The distinctive features of these two species are the shapes of the prothorax, the pseudocephalon and the position, size and shape of the mandibles. Like phytophages, the prothorax is truncate anteriorly, rather than tapered (Figure 52), the cephalic lobes are fused and the pseudocephalon is dome-shaped lacking cirri. The mandibles are blunt-tipped and, in resting position, they incline almost at right angles relative to the intermediate sclerite (Figure 52). Oral plates are present with a small region of dark sclerotisation (Films 19–20, Figures 53 and 54). Mandibles are similar to those of saprophages with rectangular bases and non-aligned apodemes for muscle attachment (Figure 52). Compared with *L. sylvatica*, the prothorax in *S. coleoptrata* is proportionately more narrow, segment borders are coated in spicules, the anterior spiracles are more dorsal in position and the mandibles are elongate and fused medially. The mandibles of *S. coleoptrata* are also long relative to the length of the head skeleton of the other higher cyclorrhaphan larvae studied here (Table 3). Other features of *S. coleoptrata* are the short, deep intermediate sclerite aligned smoothly with the basal sclerite (i.e. it abuts the basal sclerite evenly), the greater degree of sclerotisation indicated by black colouration and the absence of cibarial ridges and dental sclerites. The narrow development site means that when moving and feeding the body is compressed, less sweeping takes place, lunging occurs repeatedly over the same part of the substrate and feeding tracks tend to be straight or in slightly curving lines. Both species have relatively short head skeleton pivots and lunge distances and slow lunge rates (Table 2, Films 19–20). The mandibles barely move apart during elevation and depression, but unlike phytophages, they extend from the pseudocephalon (Films 19–20).



Figure 50. *Pegomya solennis*, mandibles removed from a puparium, ventral view, maximum length 0.1 mm.



Figure 51. Development site of *Lonchaea sylvatica* and *Stegana coleoptrata*, sooty fungi under lenticels of *Betula* bark, from a cut branch.

Performance consequences

Lonchoptera lutea: in this larva, the high width to length ratios of rear compartment segments and flattening enhance the ability to hold on and squeeze between adhering substrates with the sclerotised plates protecting the dorsal surface. Fusion between the plates of the metathorax and first abdominal segment probably reinforces the rear compartment during shovelling, i.e. fusion braces against the tendency of the rear compartment to move when the front one shovels forward. The fused plates of the seventh abdominal and anal segments similarly brace the rear end at the start of peristalsis. However, flattening and sclerotisation restrict manoeuvrability and lonchopterid larvae do not make sharp changes in direction, move effectively along narrow substrates nor move round sharp angles such as leaf edges. Conversely, the width of abdominal segments facilitates the sinusoidal mode of locomotion which, among the species studied here, is unique.

The relatively narrow front compartment facilitates shovelling, but as a feeding mechanism, shovelling seems less effective compared with brushing, scooping and spot-sucking, the other feeding mechanisms observed for feeding on watery biofilm (Table 4). For instance, in shovelling, the trough moves forward and the tendency will be for biofilm to move with it. There is also the tendency for biofilm to flow off the end when the trough is lifted up. Furthermore, the trough is fleshy and, compared

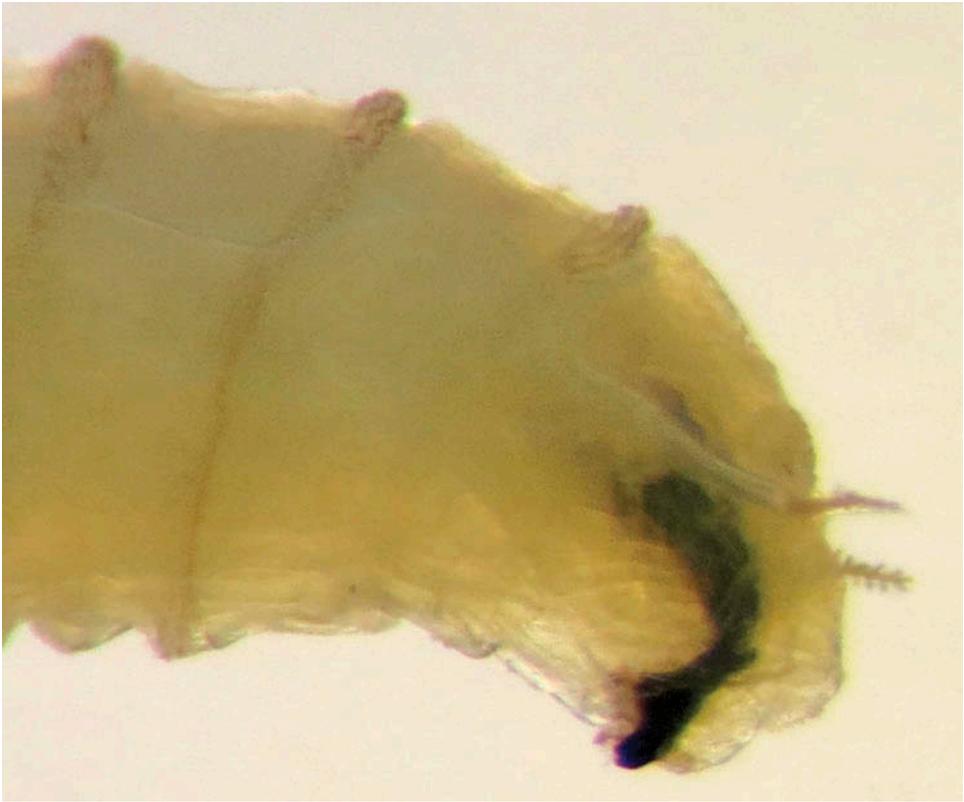


Figure 52. *Stegana coleoptrata*, preserved larva, thorax and head, head to the right, lateral view, length 1.3 mm.

with sclerotised structures, poor at penetrating into and loosening other than watery biofilm.

Mitigating against these limitations, the floor of the trough is supported mechanically by the sclerotised labial rods which originate on the basal sclerite and, relative to it, angle down by about 35 degrees. This imposes a fixed inclination to the trough which supports shovelling. Contraction of labial muscles may flatten the fleshy apex which further encourages biofilm on to the trough. To prevent it flowing off the trough, it tilts up at the end of shovelling and the labial muscles probably relax allowing the margin to expand with body fluids and so act as a barrier. The trough also has a pair of extrudable, tapering cones which, as it is lifted up, swing round in front and may also help prevent biofilm flowing off.

Apart from sclerotised labial rods supporting the floor of the trough, the sides are supported by parastomal bars and basal mandibular sclerites, and muscle action encourages the flow of biofilm towards the pharynx. In locomotion the mandibles do not grip substrates and are not important. In any case, this would be complicated as the mandibles are not at the front of the body and are orientated at right angles to its long axis.

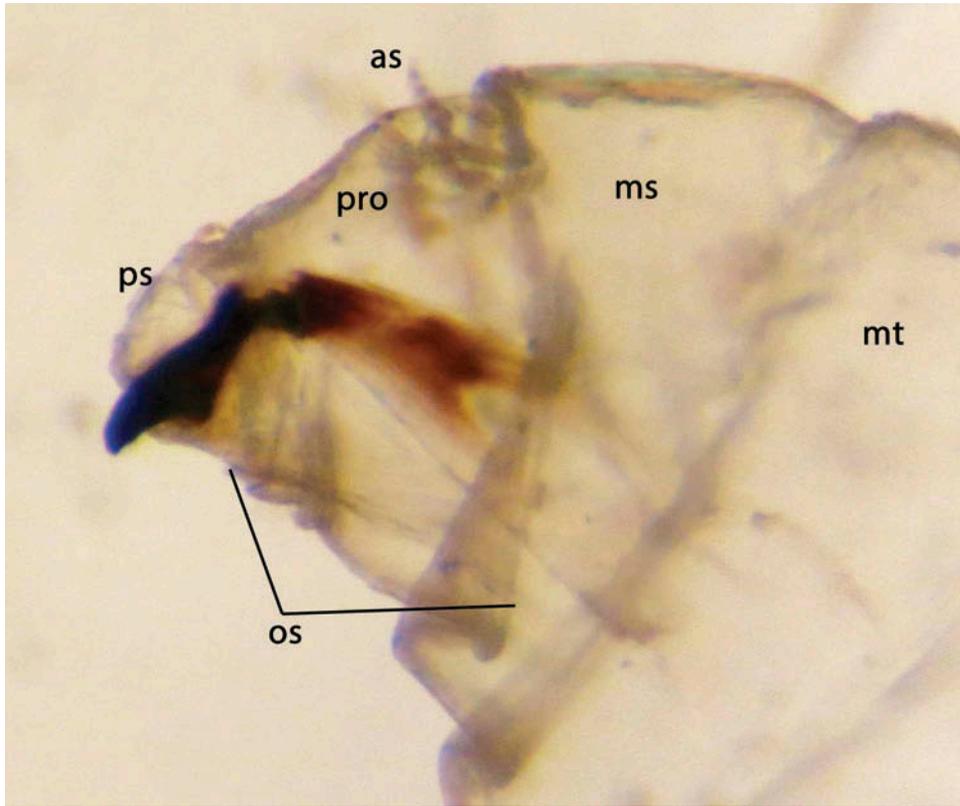


Figure 53. *Stegana coleoptrata*, thorax and head cleared in KOH, lateral view, head to the right, as = anterior spiracle; ms = mesothorax; mt = metathorax; os = oral sclerite; pro = prothorax; ps = pseudocephalon.

Polyporivora picta: the cylindrical body shape of this larva is more efficient than a flattened one for tunnelling. The ability of the segments of the rear compartment to contract, and hence shorten and broaden, is also important for gripping the sides of the tunnel. By frictional force, holding on is supported by the short, stiff setae which cover the body. The tapered shape of the middle compartment creates space for lunging and the ability of the head skeleton to pivot and protract and retract provides a fine level of adjustment for placing the labial teeth against the tunnel face. Moving the labial teeth down the tunnel face tends to force ripped and torn hyphae towards the entrance to the pharynx ready to be sucked in. This process is probably facilitated by the mandibles which depress to guide rasped hyphae between the upper labial teeth, but are otherwise of minor significance. The oral plate attached to the ventral margin of the pseudocephalon (Figure 6A) supports rasping by action of muscles attached to it which contract and so pull back the pseudocephalon to expose fully the labial teeth.

Microdon analis: as with *L. lutea*, the high width to length ratios of the abdominal segments enable this larva to move sideways and rotate on a point which is advantageous in confined ant galleries. Elongate projections supporting the antennomaxillary

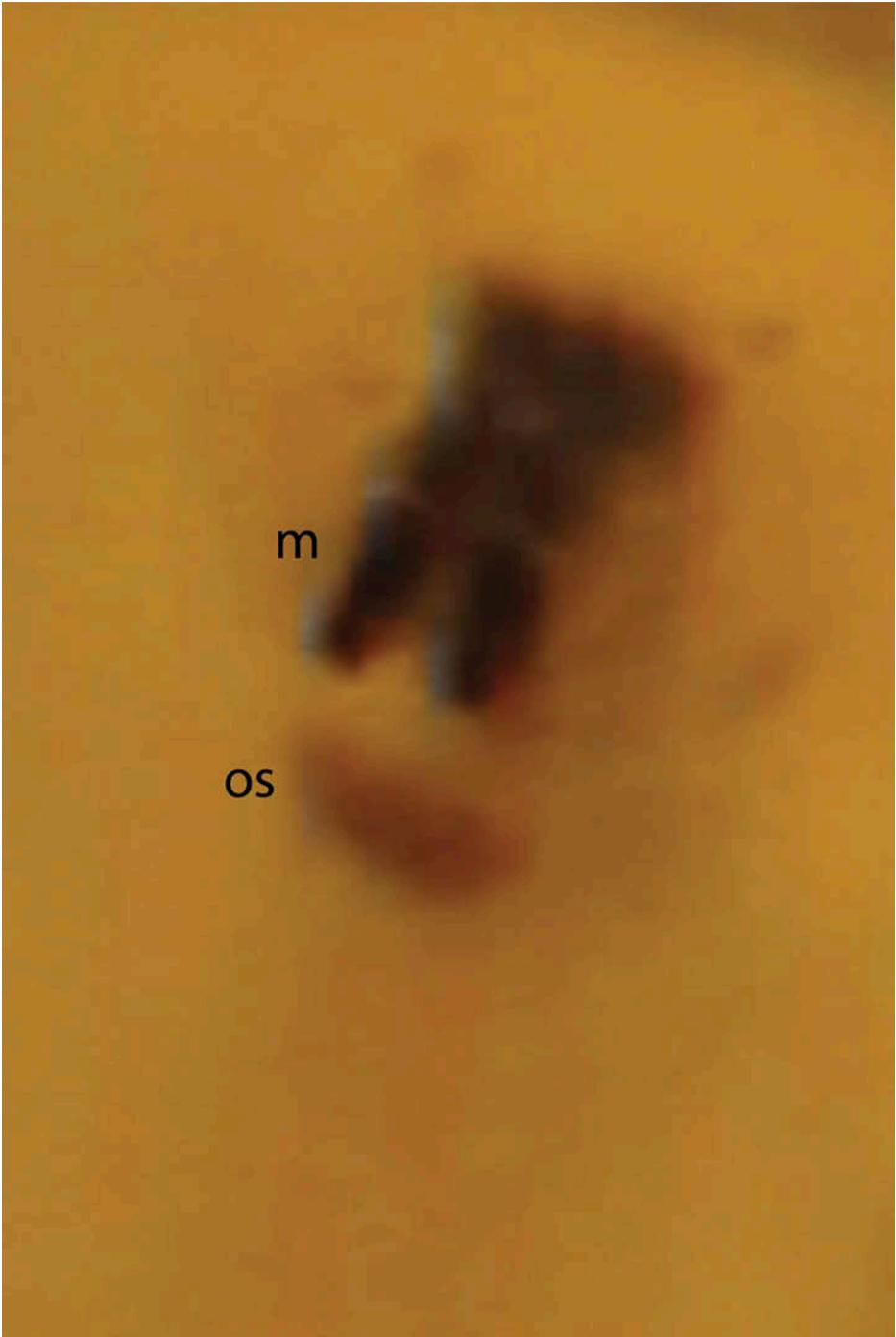


Figure 54. *Lonchaea sylvatica*, still from Film 19 showing small, sclerotised part of the oral plate (os), appearing below the inclined mandibular hooks (m), ventral view.

Table 4. Larval feeding mechanisms of 20 species of Cyclorrhapha (Diptera).

Species	Feeding mode	Feeding mechanism	Main features
<i>Lonchoptera lutea</i>	saprophagy	shovelling watery biofilm	open trough at the front of the head
<i>Polyporivora picta</i>	mycophagy	rasping firm compacted fungal hyphae	external labial teeth
<i>Microdon analis</i>	zoophagy	cutting into prey	serrated mandibles, spiked labial plate
<i>Melangyna cincta</i>	zoophagy	cutting into prey	tapered, piercing labrum and labium, sticky saliva and triangular sclerites for gripping prey
<i>Silba fumosa</i> ; <i>Palloptera trimacula</i> ; <i>Chymomyza costata</i>	saprophagy	scooping oily to soft solid biofilm	horizontal protraction of head skeleton, elongate oral cavity and pseudocephalon that extends and contracts evenly, inclined mandibular movement; rectangular base to the mandible with non-aligned elevator and depressor tendons
<i>Coelopa frigida</i> ; <i>Calliphora vomitoria</i>	saprophagy	scooping deep quantities of oily to soft solid decay	stepped, highly flexible and retractile front and middle compartments; bridged oral cavity coated in serrated ridges in <i>C. frigida</i> and cutting plates in <i>C. vomitoria</i>
<i>Meiosimyza platycephala</i>	saprophagy	brushing watery biofilm	bridged oral cavity with coating of setae
<i>Clusia flava</i>	saprophagy	spot-sucking watery biofilm	inverted oral cavity; flat tipped ovoid prothorax
<i>Lonchaea hackmani</i> ; <i>Neophyllomyza acyglossa</i>	saprophagy	scooping biofilm from convoluted substrates	highly flexible and retractile front and middle compartments, small oral cavity and mandibles
<i>Lonchaea sylvatica</i> ; <i>Stegana coleoprata</i>	mycophagy	scraping sooty fungi (and firm biofilm)	pivoting, barely protracting head skeleton, dome-shaped pseudocephalon that alternately folds dorsally and ventrally in co-ordination with head skeleton pivoting, oral plates present, relatively large mandibles with a rectangular base

(Continued)

Table 4. (Continued).

Species	Feeding mode	Feeding mechanism	Main features
<i>Tephritis vespertina</i> ; <i>Chaetostomella cylindrica</i> ; <i>Botanophila seneciella</i>	phytophagy	rasping floret tissue	pivoting, barely protracting head skeleton, dome-shaped pseudocephalon that alternately folds dorsally and ventrally in co-ordination with head skeleton pivoting, oral plates present, mandibles with a lozenge-shaped base, body short and broad
<i>Acidia cognata</i> ; <i>Pegomya solemnis</i>	phytophagy	rasping mesophyll inside leaves	pivoting, barely protracting head skeleton, dome-shaped pseudocephalon that alternately folds dorsally and ventrally in co-ordination with head skeleton pivoting, oral plates present, mandibles with a lozenge-shaped base, body long and narrow

organs extend their reach during sweeping for prey. Prey capture was not observed, but presumably when the antennomaxillary organs sense prey, the head skeleton protracts, the mandibles grab it in a pincer-like action, the head skeleton retracts and the front compartment contracts. These actions pull the prey under the upper section of the metathorax where the prey is pierced. How this occurs is unclear. By depressing the labial plate and sclerites, space would be created between them and the mandibles, and the prey is possibly pulled into this space where it is pierced by the spines at the back of the labial plate and the long, incurved teeth at the back of the mandibles. Additional force would be available to pierce and tear prey if it was rubbed against the floor of the ant gallery. Such processes are facilitated by the position of the mandibles at the front of the head skeleton. The ability of the pump to work independently of the mandibles, i.e. several pumping actions per lunge, means that food can be imbibed simply by protracting the head skeleton into the prey body. During pumping, the mandibles are relatively inactive and unimportant. However, unlike *M. cinctus*, no seal is made between the larva and its prey and some prey fluids and tissue spill out and are lost.

Melangyna cincta: at the start of peristalsis, this larva prevents slippage using a unique, grasping bar at the apex of the anal segment. High levels of collapsibility and extendability underpin the fine control and exceptional flexibility of this larva. These features suit locomotion on the varied topography of plant substrates. Such abilities are enhanced by the shifting border between the two main body compartments which, in particular, modify the distance over which lunges are made. Once a prey aggregation is located, it is an advantage not to disturb it as more than one aphid is required for development. Having a modifiable lunge distance is energy-efficient because larvae can stay close with short lunges capturing the nearest aphids. The unique

overlap between, particularly the prothorax and mesothorax, means that anterior spiracles are removed from a position where they might interfere with feeding and frees the prothorax to retract into a cup-shape to hold prey in combination with sticky saliva and the triangular-shaped sclerites on the lateral margins of the prothorax. Such a mechanism for holding prey creates a seal and prevents loss of prey fluids and tissues. Overlap may also provide space for the head skeleton to pivot and protract and retract when the prey is lifted up, i.e. protractor and retractor muscles originate further backwards than if no overlap occurred. Unlike *M. analis*, the labrum and labial plate are tapered and project together at the front of the head skeleton, which is an efficient shape and position for piercing prey. The mandibles have no active role in feeding. They lack muscles and are embedded in and support the sides of the head skeleton. This creates a nozzle at the apex of the head skeleton which increases velocity of intake by concentrating sucking power (Figure 12C). To suck in food, like *M. analis* and *P. picta*, the labium depresses to expose the nozzle entrance. By altering the direction of protraction, the head skeleton can reach different areas inside the prey. Lifting prey up has two advantages, alarm pheromones emitted by captured aphids are dispersed harmlessly above aphid aggregations and it prevents prey escaping by walking away (Roberts 1970; Rotheray and Gilbert 2011).

Higher Cyclorhapha: observation of movement in Petri dishes reveals that at the start of peristalsis, spicules on the anal lobe are pressed against the substrate and probably prevent the anal segment from slipping. As peristalsis moves forward, gripping power is maintained by spicule-bearing integument either side of segment borders spreading out forwards anterior of the borders and backwards behind it; the spicules are often orientated this way too. At these borders there are always more spicules on the posterior than the anterior segment which probably reflects the emphasis for forward more than reverse locomotion, i.e. there is a greater need to resist slipping backwards than forwards. Spicules presumably provide gripping power by frictional forces, the results of which were probably the effective locomotion observed when larvae moved on dry, filter paper as opposed to slipping, body rocking and lack of progress which occurred on a substrate they found problematic to grip, the smooth, dry surface of a plastic Petri dish. The segments of the rear compartment are relatively uniform in size and shape which provides an even spread of gripping power, especially during sweeping and lunging when this compartment is stationary and the middle and front ones are mobile.

The middle body compartment sweeps and rarely grips a substrate, which explains the relative absence of spicules. The division between the middle and the front compartments is just ahead of the anterior spiracles. Beyond the anterior spiracles, the prothorax inclines or bends to reach food or a substrate. The anterior spiracles are rigid structures that do not bend or collapse, and this may explain why the border between these compartments is beyond them. In addition, in this position, the anterior spiracles remain elevated during lunging and better protected from immersion and blockage with loose or wet material. Their position also effects locomotion. This is because, when the mandibles depress to grip a substrate, the next peristaltic wave causes the mesothorax to fold over the stationary prothorax but usually the fold stops just behind the anterior spiracles (Figure 22), perhaps because the spiracles might be damaged or would cause damage if they were taken into a fold. This limit on folding is breached in *C. frigida* and *C. vomitoria* (Figure 23B). In these species, the small size of the prothorax relative to the mesothorax appears to make

this possible. It means that, when folding into one another, there is space between them sufficient to accommodate the anterior spiracles. When the mandibles grip the substrate, the face of the prothorax, the anterior fold, is also pressed against the substrate (Figure 23A and 23B). In almost all species, the anterior fold is coated in spicules that probably protect the prothorax and help in holding on.

The border between the middle and front compartments is also seen in the puparium. The anterior spiracles are on the antero-dorsal margin of the puparium in all higher cyclorhaphan species examined here. The prothorax beyond the spiracles is inclined as it is during lunging but, in the puparium, bending goes further and the prothorax is turned to almost 180 degrees into the puparium. At its end, the pseudocephalon remains attached and within it are the mandibles followed by the rest of the head skeleton. Hence, the apex of the puparium is the point where it inclines just ahead of the anterior spiracles.

Among the species examined here, the form of both the head skeleton and the pseudocephalon is variable but in general, the head skeleton protracts and retracts, and the mandibles depress which extends the mandibular hooks from their sheathes in the pseudocephalon. The mandibular hooks gather food or, in locomotion, grip a substrate. Between the mandibles is the oral cavity into which food is gathered prior to it being sucked into the pharynx, which at this point, is in the form of the nozzle-like atrium.

Saprophagy: compared with mycophagy and phytophagy in which firm or compacted tissue needs removing and zoophages in which prey have to be captured and pierced, food gathering in saprophages appears less challenging. Liquidised food needs gathering for which the suction pump in the head skeleton appears well suited. As do ensheathed mandibles which, by virtue of the fleshy sides of the pseudocephalon, provide a greater surface area than mandibular hooks alone for gathering liquid food and larvae are usually immersed in and surrounded by food (Table 1). Although saprophages share some features, in the nine species assessed, seven distinctive feeding mechanisms were recorded (Table 4).

In most saprophages, the mandibular base is subrectangular and indented (Figure 16). Indentation probably adds mechanical resistance to forces that might distort the mandible when the mandibular hooks are gripping or pulling across hard substrates. Furthermore, the mandibular hook is often flat to scalloped which, by a greater surface area, is an effective shape for gathering liquid or soft food. The mandibles usually move in an inclined plane, i.e. they separate on depression. Inclination opens and closes the oral cavity and, in locomotion, separated mandibles make two points of contact with the substrate, helping to anchor and stabilise the front end of the body.

A hinge joint links the mandibles to the intermediate sclerite. It consists of a groove in the anterior face of the intermediate sclerite which articulates with a ridge on the rear face of the mandible (Figures 17 and 18). The joint slopes inward relative to the horizontal plane and it is this slope that enables inclined mandibular movement. The muscles controlling mandibular movement are inserted via tendons to slight apodemes or projections, elevator muscles to the inner, upper corner and depressor muscles to the outer, lower corner of the rectangular base (Figure 16). Because of the width of the mandible and their position on it, these apodemes are not in the same vertical plane but displaced laterally from one another. This arrangement means they work with the joint during movement i.e. independent of the joint itself;

elevator muscles would tend to pull the mandible up and towards the midline, whereas depressor muscles would tend to pull the mandible down and away from the midline. Due to the position of the dental sclerites below and under the mandible base and that depressor tendon envelops them (Figure 16), mandibular depression pulls the rear margin of the oral cavity back and down which, in conjunction with retraction of the labial lobe, exposes the opening to the atrium and acts as a critical backstop, helping to retain food in the oral cavity.

The pseudocephalon and hence, the oral cavity, is longer than wide in saprophages and at the border with the prothorax, the pseudocephalon folds and unfolds evenly as a unit, unlike the pseudocephalon of phytophages and mycophages, see below. This shape and pattern of folding optimises the degree the oral cavity is projected to gather food. The characteristic systems of anastomosing ridges or cirri that coat the sides of the oral cavity and lead to the opening of the atrium presumably both protect the pseudocephalon and guide fluids into the cavity, but details of the latter process are unclear. Despite spicules coating the anterior face of the prothorax, the prothorax and indeed other parts of the integument are often covered in scars, appearing as black marks on the integument (Figures 23A and 35).

Silba fumosa, *Palloptera trimaculata* and *Chymomyza costata*: the larva of these three species are the most generalised of saprophages examined here. For instance, over the range recorded for all saprophages, their middle and front body compartments are not especially manoeuvrable, lunge times are neither fast nor slow, and head skeleton protraction distances are neither long nor short. Their most distinctive features are the middle and front body compartments which taper the least but are relatively long. The lack of taper suggests they are not specialised for extracting food from particularly confined spaces. The relatively long middle and front compartments might be an adaptation for optimising reach, but given that these larvae are usually immersed in and surrounded by biofilm, there seems to be no particular advantage of a long over a short reach.

Food gathering in *S. fumosa* appears even more generalised compared with the other two species in that this species has an open oral cavity, the inside margin of the mandibular hook is rounded not flattened and the prothoracic anterior face lacks spicules. The advantage of a closed oral cavity is that biofilm will be isolated inside simply by placing it down into biofilm. With an open cavity, the two lobes have to be drawn and held together. Compared with a flattened or scalloped mandibular hook, biofilm will tend to flow either side of a rounded state and, without protection for the anterior fold, food gathering may be confined to a subset of places where the chances of injury are slight, such as smooth biofilm on smooth substrates.

Lonchaea hackmani and *Neophyllomyza acyglossa*: compared with other saprophages, the distinctive features of these two species are their relatively small mandibles and oral cavities and short, tapered middle and front body compartments. These features suggest that the muscles of these body sections are correspondingly short and approximated. Also distinctive is their unusual capability to fold the prothorax (Figures 29 and 30). All these features contribute to an ability to make fine, controlled movement. Such control facilitates food gathering from inside crevices and coating networks of fibres where these larvae usually feed.

The most obvious difference between these two species is the elongate, flexible intermediate sclerite of *N. acyglossa*. The significance of this remarkable feature is

presumably the enhancement it gives to manipulating the head skeleton in food gathering.

Meiosimyza platycephala: one of the features of this species is that the middle and front compartments do not contract or fold but remain extended during sweeping. This is probably a consequence of decaying leaves being relatively flat and where the mechanical resistance of watery biofilm is low. Facilitating the feeding mechanism of this species are the rows of setae-like projections covering the bridge across the front of the oral cavity. When the oral cavity is lowered into biofilm and retracted, these setae brush the substrate that not only gathers biofilm but also creates a wave of it which bulges up into the oral cavity. These setae project further than the ventral margin of the thorax and, during protraction, the front section must lift to prevent them brushing biofilm away on the outstroke. The setae are protected by sclerotisation. The lunge distance and range of mandible movement is low, but with the oral cavity filled by bulges of biofilm, a wide range of movement is not required. Compared with other higher cyclorrhaphan saprophages, the mandibles play a reduced role in food gathering. The wide atrium supported by a sclerotised apex to the epipharyngeal plate facilitates the flow of biofilm through to the basal sclerite where microbes are separated from excess water by the cibarial ridge filtering mechanism.

For protection and effective grasping, spicules are extensive in *M. platycephala*, covering the ventral and dorsal surfaces. Furthermore, the larva is able to flatten its body which facilitates squeezing through confined spaces.

Clusia flava: feeding lunges were the slowest of the species recorded here, taking minutes not seconds to complete. Most lunging time is spent with the apex of the prothorax pressed against sapwood and this is when ingestion takes place via repeated pumping. Repeated pumping probably draws biofilm into the head skeleton not only from the surface covered by the apex but also from biofilm soaked into and from the surrounding area, aided by an eversible channel that can form across the flattened apex. This explains the length of time the head is pressed against the substrate during lunging.

The flattened apices of the prothorax and pseudocephalon facilitate spot-sucking. The surrounding sensilla probably enable the pressure applied to the substrate to be assessed. An inverted, tubular oral cavity facilitates capillary action and some biofilm will enter without suction from the pump. Depression of the mandibles opens the entrance to the oral cavity, but the mandibles have little further role in food gathering. Cibarial ridges are absent and their loss implies a continuous passage of food, which facilitates prolonged sucking over one spot. However, a poorly sclerotised head skeleton suggests that pumping actions are relatively weak, i.e. the head skeleton is not braced or buttressed against distortion caused by contracting muscles. Absence of cibarial ridges and food entering the oral cavity by capillary action might mean that a strong pump, and hence a large, well-sclerotised head skeleton, is not so important.

The presence of spicules on the dorsum and ventrum of the thorax is unusual as is the slow rate of locomotion. Spicules probably reflect a need to protect the integument, as suggested by the sometimes, numerous black scars on the integument (Figure 35). Slow locomotion probably reflects a compromise between the difficulties of squeezing through sapwood and minimising energy expenditure. Even when removed from sapwood, this larva retains a slow rate of progress.

Coelopha frigida and *Callicera vomitoria*: the distinctive feature of these two species is the wide range of angles and directions achieved during sweeping and lunging. Such high levels of manoeuvrability are facilitated by a high level of folding between increasingly smaller and tapered segments of the middle and front body compartments. Such manoeuvrability takes advantage of food which, in a deep volume, is available in almost any direction. Furthermore, both species have a fleshy bridge across the anterior margin of the oral cavity and food can be gathered by simply extending the oral cavity forward. The crenulated ridges coating the distal end of the oral cavity in *C. frigida* presumably assist scooping food and/or protect its surface. In *C. vomitoria*, the operation of the bridge is more complex due to the presence within it of the oral sclerite and the paired oral plates. These plates incline during mandibular depression, which helps cut through and isolate portions of food. The rapid isolation of food that these plates facilitate in part explains the very rapid rate of feeding observed in this species. The other requirement of rapid feeding is a pump strong enough to withstand the forces involved. This may explain the high level of sclerotisation in the basal sclerite of *C. vomitoria*. It was not possible to confirm if saliva was secreted extra-orally during feeding in *C. vomitoria*, but at the rate of ingestion recorded, extra-oral digestion seems unlikely. Apart from scalloped mandibles, another feature aiding rapid ingestion in these two species is a wide atrium which by the provision of space, facilitates ingestion of lumpy or soft-solid food.

Mycophagy and phytophagy: compared with saprophages, food gathering in mycophages and phytophages is a mechanically demanding process. The mandibles must engage relatively hard or compacted tissue with sufficient force to fragment it. The mycophages and phytophages examined here share certain features that facilitate this process. For instance, to enhance leverage, the head skeleton pivots or see-saws up and down rather than, as in saprophages, protracting and retracting forwards and backwards.

In saprophages, the pseudocephalon extends back and forth evenly. With up and down movement, this does not occur and dorsal and ventral margins fold in opposing directions during pivots, i.e. when the head skeleton pivots up, the dorsal margin of the pseudocephalon folds or collapses while the ventral margin extends and unfolds and the reverse on moving down. This is facilitated by the domed rather than elongate shape of the pseudocephalon. This imposes, however, the problem of ensuring the oral cavity remains open and does not collapse on the down stroke. This is critical because food is gathered into it during the down stroke. In saprophages, dental sclerites keep the oral cavity open during mandibular depression, but in the mycophages and phytophages examined here, dental sclerites are absent. The oral cavity is kept open by an oral plate that extends from the rear margin of the oral cavity under the head skeleton. This structure can be almost as long as the basal sclerite and moves in co-ordination with it. A pivoting head skeleton also explains the truncate shape of the prothoracic apex in mycophages and phytophages. Compared with the narrow taper of the prothorax in saprophages, a truncate apex in mycophages and phytophages probably increases space and modifies the angles of protractor and retractor muscles, enabling them to pivot the head skeleton.

Finally, in saprophages the mandibular hooks are relatively long, narrow, sharp-tipped and separate on depression. These features are not as effective for tearing plant and fungal tissue as the mandibular hooks of mycophages and phytophages which are parallel-moving, short, wide and blunt-tipped. This type of mandibular hook

concentrates rasping in a smaller area and protects the tips by spreading their surface area. In most phytophages, surface area is additionally increased by accessory teeth.

Lonchaea sylvatica and *Stegana coleoprata*: these larvae feed most frequently on sooty fungi. But they can also feed on firm biofilm, both food types co-occurring at their development sites under the bark of fallen wood (Figure 51, Table 1). The narrow range of head skeleton and mandibular movement that characterises these larvae facilitates repeated lunging at the same place on the substrate. This appears to be required to loosen and gather hard food. Such a requirement for repeated lunging and spatial restrictions within development site probably explains the linear shape of feeding tracks. The morphological differences between *L. sylvatica* and *S. coleoprata* suggest that the latter species is more completely modified for feeding on hard food. For instance, the prothorax of *S. coleoprata* is more wedge-shaped and the anterior margins of segments are coated in spicules. These characteristics probably facilitate feeding in restricted spaces, perhaps by feeding in a twisted or sideways position. Also, as a proportion of head skeleton length the mandibles of *S. coleoprata* are twice as long as those of *L. sylvatica* (50% versus 25%) and they are fused together. Length and fusion adds mechanical strength to the mandibles.

Other distinctive head skeleton features of *S. coleoprata* are the greater degree of sclerotisation, absence of cibarial ridges and a shorter, deeper intermediate sclerite which is smoothly aligned with the basal sclerite. These features brace the head skeleton to a higher level than that of *L. sylvatica*. A deep intermediate sclerite also provides space for fragments of food to mix with saliva. The absence of cibarial ridges means that food is not concentrated in the head skeleton but passes rapidly through it.

Tephritis vespertina, *Chaetostomella cylindrica*, *Acidia cognata*, *Botanophila seneciella* and *Pegomya solennis*: in lunging, these phytophagous larvae maintain the mandible in one position, rather than it depressing continuously. This enhances rasping power, but elevator and depressor muscles must work in conjunction to hold the mandible in position. This may, in part, explain the vertical alignment between elevator and depressor muscle apodemes, and hence the characteristic shape of the mandible base in phytophages. An additional influence on the position of the depressor apodeme is avoiding interference with a short mandibular hook. The depressor apodeme would probably be in the way if located in the same relative position as in saprophages.

Lunges of this type involve the intermediate and basal sclerites because mandibular muscles originate on the basal sclerite and these sclerites must be strong enough to withstand the forces involved to hold the mandible in one position. Added strength is exemplified by the characteristic, complete sclerotisation of the intermediate sclerite and heavy sclerotisation of the vertical plate. Furthermore, in the tephritids, additional bracing occurs via smooth alignment between the intermediate and basal sclerites, similar to that of *S. coleoprata*. However, the cornua in phytophages appear weak. There is abrupt termination of sclerotisation partway along the ventral cornu, and there are windows and areas lacking sclerotisation in the dorsal cornu. In phytophagous larvae, cibarial ridges are absent and, possibly as a consequence, the terminal valve is reduced. There is probably a trade off on the ventral cornu between the need to resist distortion via sclerotisation and the reduced need for sclerotisation resulting from the loss of cibarial ridges and terminal valve. This explains the characteristic pattern of sclerotisation in the ventral cornu. A factor explaining the

pattern of sclerotisation in the dorsal cornu is probably space. The films reveal that, in live phytophagous larvae, the dorsal cornu bends along these windows. Bent over dorsal cornua reduce the height of the head skeleton. This may compensate for lack of space caused by up and down movement of the head skeleton and narrow, confined feeding places.

With greater forces required to gather food, phytophagous larvae require a very firm grip of the substrate. This is in part facilitated by the restricted spaces they occupy, such that lateral and dorsal aspects of the body are in contact with substrates. For instance, in leaf mines, larvae are on their sides and hold on with spicule-coated, lateral margins and, in flowerheads, larval bodies are, on all sides, in contact with the substrate. Furthermore, the two leaf mining larvae have unfolded bodies when feeding whereas flowerhead larvae are folded and are, in consequence, short but broad. These shapes are retained even when larvae are removed from their development sites and they suggest adaptive processes to conserve body size within confined spaces and optimise body shape for holding on.

The larva of *C. cylindrica* is unusual among the flowerhead species in feeding on the receptacle. Receptacle tissue is dense and hard relative to that of the florets and there are certain features of the larva of *C. cylindrica* absent in the two other flowerhead feeders that may facilitate gathering this harder quality of food. To better brace the head skeleton, it is almost completely sclerotised and the front margin of the basal sclerite tapers and makes a complete junction with the intermediate sclerite. Also, the basal sclerite is braced more effectively by the symmetrical angle between the two cornua (Figure 41C). In the other flowerhead species, the angle of divergence suggests that the ventral cornu is mainly involved in bracing (Figure 41A and 41D).

Comparing the two leaf mining larvae, there are considerable differences between them. The larva of *P. solennis* is characterised by an extraordinary group of characters, unique among the species studied here, a crenulated pseudocephalon, a conspicuous fleshy bar on the prothoracic apico-ventral margin, and asymmetrical pseudocephalon and mandibles. The prothoracic bar helps prevent rasped food from being moved past the oral cavity during lunging, and because fluids cannot be compressed, the crenulations on the pseudocephalon allow excess fluids to pass between them. Asymmetry creates a level of flattening that suits the narrow leaves of the food plant used by this species. In particular, the relative shape and position of the two mandibles means that teeth on both can be brought into action when lunging. These remarkable features are absent in the other similar-sized leaf miner examined here, *A. cognata*, but this species mines a leaf about twice as thick.

Discussion

Feeding mechanisms

We investigated larval mechanisms of feeding and movement in 20 cyclorrhaphan species (Table 1). Films were especially informative through being able to repeat sequences and slow or speed them up. They revealed features and movement not detectable by direct observation and 12 separate feeding mechanisms were recorded (Table 4). Most species had some distinctive features, six were altogether unique and exclusive mechanisms were associated with feeding mode (Table 4). Other contrasts were unexpected, such as seven feeding mechanisms in 10 saprophages. Lower

cyclorrhaphans were more disparate than higher cyclorrhaphans and mechanisms even differed between cases of species within the same family (Table 4).

Films helped explain performance. For instance, in saprophages and zoophages, head skeletons move in and out of the thorax, but see-saw up and down in mycophages and phytophages. These opposing styles of movement optimise leverage for gathering liquid versus compacted food respectively. Furthermore, in saprophages, the mandibles separate as they depress which opens the oral cavity to receive food; in mycophages and phytophages, the absence of such movement enhances rasping power by concentrating it to a confined area. Again, both characteristics are appropriate for food type and explained by the angle of inclination of the hinge joint between the mandible and the intermediate sclerite. Certain small features were found to have important roles. Thus dental sclerites in saprophages, whose rotation is part of mandibular depression, expose the rear margin of the oral cavity to help retain food. In phytophages and mycophages, the oral cavity is kept open not by dental sclerites but by an inconspicuous oral plate.

We also found contrasts in movement capabilities that helped characterise feeding mechanisms. For example, among saprophages, *C. vomitoria* had almost three times faster and two times longer lunges than *N. acyglossa*, the species with the slowest and shortest lunges. Mechanistically, these differences are explained by the armoured oral cavity of *C. vomitoria* which enables food to be isolated by simply placing the oral cavity into it and the intermediate sclerite of *N. acyglossa* which bends to enable food gathering from narrow crevices and between fibres, but in so doing, extends lunge times. We were able to specify functions for structures whose roles were unclear, such as eversible projections in lonchopterid larvae and windows in basal sclerites. We also found features that appear not to have been assessed or even noticed previously, such as oral plates, diversity in the form and folding pattern of the pseudocephalon and remarkable asymmetry in the leaf-mining larva of *P. solennis*.

Feeding mechanisms and diversification

Feeding and movement diversity in so small a sample of species demonstrates the adaptability of the cyclorrhaphan larva and, based on Wiegmann et al. (2011), species sharing a feeding mechanism are convergent. Such diversity suggests caution if *Drosophila* or *Calliphora* larvae are used to model or stand proxy for the cyclorrhaphan larval stage. It also suggests that much remains to be discovered and raises the probability of cyclorrhaphan evolution being driven by changes in larval features, an idea discussed by Rohdendorf (1974); switches between larval feeding modes were suggested by Wiegmann et al. (2011) as a partial explanation of episodic radiation in higher Cyclorrhapha.

Pulses of diversification following colonisation of novel development sites made possible by innovations in larval morphology may amplify episodic radiation. Candidate innovations possibly amplifying cyclorrhaphan diversification include a compartmentalised body, a protractable head skeleton, replacement of the labium by the mandible in food gathering, and development of an atrium and an oral cavity. Later stages may have been amplified by recurring modifications in the size, shape and movement ranges of the mandibles, atrium, oral cavity and pseudocephalon.

The head skeleton in Lonchopteridae is, like Brachyceran outgroups, fixed in the thorax and cannot protract and retract independently. Based on sequences for the

lower Cyclorrhapha recovered by Collins and Wiegmann (2002), Rotheray and Gilbert (2008) and Wiegmann et al. (2011), a protractable head skeleton is an innovation originating in the Platypezoidea and persists almost unmodified across the Cyclorrhapha. An exception is the Clusiidae where the head skeleton has become fixed in the thorax as part of the development of a spot-sucking feeding mechanism. The performance advantage of a protractable head skeleton was suggested when comparing feeding and movement in lonchopterid versus other cyclorrhaphans. A protractable head skeleton extends reach, enables food to be gathered from enclosed spaces and is energy-efficient because, as food is gathered, the rest of the body can be immobile.

In the Lonchopteridae and Platypezoidea the labium, which is located beneath the pharynx, plays a more significant role in food gathering than the mandibles which are to the side or slightly above it. In the basal Microdontinae of the Eumuscomorpha (Syrphoidea + higher Cyclorrhapha), the labium retains an important role but the mandibles have become significant in prey capture. Relative to the states found in the Platypezoidea, two significant changes have occurred in the Microdontinae that enhance the ability of the mandible to catch prey. It sticks out in front of the head skeleton and leverage is gained by increasing its mobility. But with increased mobility, extra articulation is required which may explain the socket-like joint between the labium and the mandible. In the higher Cyclorrhapha, the mandible retains the Microdontine position at the front of the head skeleton but replaces the labium as the main food gathering structure.

In the Platypezoidea and Syrphoidea, the labium bifurcates. The upper bifurcation articulates with the mandible and becomes the intermediate sclerite in higher Cyclorrhapha and the apex of the lower bifurcation separates from, but articulates with, its basal section (Rotheray and Gilbert 2008). Depression of this articulated apex not only exposes the entrance to the pharynx, but in the Platypezoidea, this is the main food gathering part of the head skeleton (= the labial teeth in *P. picta*). In the Syrphoidea, this apex becomes the labial plate and the paired labial sclerites. In higher Cyclorrhapha, these sclerites are incorporated into a novel feature, the atrium (Rotheray and Gilbert 2008).

Development of the atrium is simple to envisage from states in the Syrphoidea. Dorsally, the roof of the pharynx and, laterally, the labial rods simply develop forward. The floor of the pharynx also extends and embeds within it, the labial plate and sclerites while retaining the fleshy, labial lobe at the apex. This lobe protects the entrance to the pharynx and is retracted during feeding (Hartley 1963; Roberts 1971). These developments create the atrium, a nozzle-like extension to the pharynx. The nozzle in Syrphinae, formed by membrane embedding the mandibles and connecting with the labrum and the labial plate, is analogous. In the Syrphinae, the mandibles are incorporated and fixed into the nozzle, but the labial apex (labial sclerites and plate) retains the plesiomorphic state of independent mobility. In higher cyclorrhaphans, this is reversed with the mandibles independently mobile and the labial apex becoming fixed into the floor of the atrium.

The dimensions of the atrium are labile. For example, the atrium is shortest and deepest in *S. coleoprata* which suits feeding on hard food where a short but deep atrium better braces the head skeleton for rasping compacted food and, being deep, provides space for rasped particles to mix with saliva. The atrium is longest in *N. acyglossa* and length, combined with remarkable flexibility, enables the

proportionally small mandibles to reach into and gather food from crevices and networks of fibres.

In higher Cyclorrhapha, the space between the ensheathed mandibles, which exists because each mandible articulates with one of the paired dorsal bifurcations of the labium and the space between the bifurcations is wider than the width of the sheaths, is the oral cavity. The performance advantages of an oral cavity are fleshy lobes for gathering food and a space to hold it against the nozzle ready for sucking up. However, as with the atrium, its characteristics are labile. For example, relatively long and open in forward lunging saprophages that gather liquid or soft biofilm and short and closed in phytophages and mycophages that rasp food.

A shared feature of all larvae is a compartmentalised body, i.e. divided into functionally independent sections. The rear section grips a substrate while the front ones sweep and lunge for food and lead direction changes in locomotion. A functionally divided body has been observed previously. For instance, Chandler (1969) recorded the predatory larva of the syrphid, *Episyrphus balteatus* (Degeer) (Diptera, Syrphidae) casting for prey, i.e. with a stationary rear end, the front of the body lifts and scans either side; Rotheray (1987) used rates of casting to assess predatory potential in these syrphids. Roberts (1971) explained that holding on with the rear section was important to prevent slippage during movement.

Many feeding mechanism differences recorded here were due to variations in manoeuvrability. Observation of outgroup Brachyceran larvae, *Rhagio scolopaceus* (Linnaeus) (Rhagionidae) ex soft, wet *Betula* sapwood, *Systemus pallipes* (von Roser) (Dolichopodidae) ex sap runs on trees and several unidentified *Medetera* spp. (Dolichopodidae) under *Acer*, *Betula* and *Pinus* bark suggests that compartmentalisation is not unique, but is more developed in cyclorrhaphans. Outgroup larvae were similar in having a relatively stationary rear end while the front one moved from side to side. The border between the two was, like most lower cyclorrhaphans, at the metathorax/mesothorax although, just as often, the front of the body turned from a position further towards the rear end, suggesting less compartmentalisation. As with *L. lutea*, the head skeleton in outgroups was not independent, but fixed in the thorax. In outgroup larvae, peristaltic body wall contractions were slight and less pronounced than in cyclorrhaphans, and body sections and segments creased rather than folding up or contracting. Brief though these observations are, they suggest that, relative to the lower Brachycera, compartmentalisation is enhanced in cyclorrhaphan larvae, which has probably facilitated developments in manoeuvrability.

Filmed behaviour

Only a few studies report the use of film to analyse larval movement. Berrigan and Lighton (1993) videoed locomotion in third stage larvae of *Protophormia terraenovae* Robineau-Desvoidy (Calliphoridae). Compared with limbed animals, they found peristalsis a costly mode of transport and recorded, but did not explain, discontinuous movement especially of the head. We also found interrupted movement of the head in most saprophagous higher cyclorrhaphans. It is explained by the mandibles which, at the end of a peristaltic wave, are gripping the substrate. When another wave reaches them, it is momentarily held up and the resulting pressure apparently causes the mesothorax to fold over the prothorax (Figures 20B–E, 21, 22). At the point where the mandibles release their grip, the head pauses or even contracts slightly before

peristaltic pressure carries it forward, the thorax extends and the mandibles grip ahead of the previous position and so jerky locomotion occurs. Not all larvae progress in this way. Lower cyclorrhaphans and most phytophages do not grip substrates with their mouthparts and saprophagous higher cyclorrhaphans, such as *L. hackmani* and *N. acy glossa*, rarely do.

Berrigan and Lighton (1993) quantified locomotion using stride length which was the distance the larva travelled during a peristaltic wave and stride frequency which was the number of waves per unit time. Harrison and Cooper (2003) used rates of peristaltic waves, and also head skeleton movements, to compare the performance of larvae of *Megaselia scalaris* Loew (Phoridae) with *Drosophila melanogaster* Meigen (Drosophilidae). Sewell et al. (1975) measured feeding activity in drosophilds by rates of head skeleton activation. Apart from peristaltic waves, we found four measures captured the types of movement recorded here. Head skeleton movements, activations or protraction and retraction were part of a wider movement also involving the pseudocephalon and prothorax referred to here as lunging and lunge distance is a measure of how far forward the head skeleton travels during lunging and lunge time is the time of a lunge. Pivot angles of head skeletons and mandibles express the range of movement in these structures. Using these measures we found characteristic ranges according to species, feeding mode and feeding mechanism. For example, the narrow versus wide pivot distances in the mandibles of the drosophilds, *S. coleoprata* versus *C. costata*, the see-sawing of head skeletons in phytophages compared with the horizontal movement in saprophages and for brushing biofilm, the narrow range of mandibular movement in the saprophage, *M. platycephala*, compared with the wider range when scooping in the similarly, saprophagous *P. trimacula*.

In respect of brushing, Semelbauer and Kozánek (2012) provide scanning electron microscope (SEM) images of a bridge covered in setae-like projections from species of two additional lauxaniid genera. Hence brushing may be widespread in the Lauxaniidae. Also, Rotheray and Horsfield (2013) suggest that spot-sucking is general in larval Clusiidae. Suggesting that an independently mobile, labial apex is a general Syrphoidea character, Barr (1995) reported it in *Microdon* and Nishida et al. (2002) found it in the Neotropical syrphid, *Allograpta centropogonis* Nishida (Diptera, Syrphidae). However, each of the three species of Lonchaeidae studied here is very different from one another (Table 4). This does not support Hennig (1952) who commented that, in the Lonchaeidae, changes in larval feeding habit have been made without morphological adaptation.

Support from musculature

The musculature of higher cyclorrhaphan larvae has been studied many times (Hewitt 1908; Thompson 1928; Miller 1933; Hartley 1963; Crossley 1965, 1978; Roberts 1969, 1970, 1971; Sinclair 1992; Hanslik et al. 2010; Wipfler et al. 2013). Most studies involve species of Calliphoridae or Drosophilidae, but Sinclair (1992) compared mandibles and their musculature across the Brachycera. However, only a few authors attempt to integrate evidence from muscles with observations of feeding behaviour (Hartley 1963; Roberts 1969, 1970, 1971; Harrison and Cooper 2003).

Crossley (1965) showed that the musculature of the thorax and first and last abdominal segments differed from other segments. Such a pattern supports the differential functioning of a compartmentalised body not corresponding to the

head, thorax and abdomen that we report here. Some studies describe depressor muscles inserting on the dental sclerite as well as the mandible (Hewitt 1908; Thompson 1928; Roberts 1970). Such insertions are congruent with our observations. Roberts (1969) found that, in *C. vomitoria*, a thin band of muscle originates on the dorsal surface of the thorax and inserts on to the expanded basal end of the oral sclerite. Action of this muscle is congruent with our observations of exposure of plates in the oral cavity. In *Calliphora vicina* Robineau-Desvoidy (Calliphoridae), which also has an oral sclerite, this band of muscle appears to be the one referred to by Hanslik et al. (2010) as the cephalic lobe retractor.

Wipfler et al. (2013) refer to muscles originating on the dorsal surface of the pseudocephalon and inserting on the ventral surface. Our observations suggest that, as part of peristalsis, the pseudocephalon empties and fills with body fluids. The muscles reported by Wipfler et al. (2013) may function as a valve, controlling entry of body fluids into the pseudocephalon. They may contract to close the pseudocephalon which would be advantageous during protraction of the head skeleton. On relaxation and co-ordinated with mandibular depression, body fluids flow into and expand the pseudocephalon and hence, expose the oral cavity ready for it to receive food.

Some muscles reported by Hanslik et al. (2010) and Wipfler et al. (2013) are not apparent in other studies of the same or congeneric taxa, such as Miller (1933) and Roberts (1969, 1971). For example, Hanslik et al. (2010) and Wipfler et al. (2013) refer to mandibular accessor muscles in *C. vicina* and *D. melanogaster* which originate on the dorsal surface of the prothorax and apparently insert on the dorsal margin of the mandible. In the calliphorid, drosophilid and indeed in nearly all the higher cyclorrhaphan saprophages we studied, depression of the mandibles extends the mandibular hooks from their sheathes and moves them horizontally. This has the effect of flattening the anterior margin of the oral cavity which allows entry of food and exposes the mandibular hooks to help gather it. The movement appears to be set by the nature of the hinge joint, but mandibular hook accessor muscles might also be involved, although unlike elevators and depressors, we found no tendon for their insertion on the mandible and previous workers, such as Miller (1933) and Roberts (1969, 1971), do not refer to them. Furthermore, in the mandible of the phytophages, there is no equivalent place for accessor muscles to insert. If mandibular hook accessor muscles have been correctly interpreted, they may be a saprophagous feature. Finally, both Hartley (1963) and Roberts (1970) depict muscles attached to the mandibles of syrphine mouthparts. We examined larvae of *Epistrophe eligans* (Harris), the species investigated by Hartley (1963) and *Syrphus ribesii* (Linnaeus) used by Roberts (1970), and indeed the actual slide preparations of Hartley (1963). But we were unable to confirm these muscles nor indeed syrphine mandibles capable of independent movement. In both these species and *M. cincta*, mandibles were embedded in the membranous side margins of the head skeleton.

Indicators of feeding mode, access and food quality

None of the feeding mode indicators were supported in all species examined here (Roberts 1971; Ferrar 1987). Cibarial ridges were absent in three saprophages: *C. flava*, *L. hackmani* and *N. acyglossa*. Mandibular teeth were absent in two phytophages, *C. cylindrica* and *B. seneciella*, and a narrow head skeleton is not a feature of the zoophage, *M. analis*.

Cibarial ridges are absent or reduced in other saprophages, such as certain Camillidae, Chiromyidae (Rotheray 2011) and Heleomyzidae (Skidmore 1966; Rotheray 2012). Ferrar (1979) and Rotheray (2012) discuss the possibility that, even if cibarial ridges are present, food may not necessarily filter through them. Advantages of bypassing ridges are faster rates of ingestion and obtaining nutrients from the decay matrix and bypassing them is a step towards their loss altogether. Vestigial or loss of function in cibarial ridges is indicated in the head skeleton by a lack of sclerotisation and a poorly defined valve at the apex of the ventral cornu (Figure 32). These features are also present in *C. vomitoria*, indicating that although cibarial ridges are present, they are reduced or bypassed. Furthermore, some apparently saprophagous lauxaniid larvae developing on dead leaves and possess mandibular teeth (Semelbauer and Kozánek (2012). Perhaps these lauxaniids use their teeth to loosen dry and accreted biofilm or leaf tissue itself.

Of higher cyclorrhaphan saprophages examined here, all but *C. flava* are distinguished by feeding mechanism characters: long, narrow mandibular hook, often flattened or scalloped; rectangular mandible base with non-aligned elevator and depressor apodemes; inclined hinge joint resulting in mandibles separating on depression; dental sclerites present; base of the intermediate sclerite not sclerotised; head skeleton protracting more or less horizontally; oral cavity longer than wide; and pseudocephalon with cirri and extending and contracting evenly during lunges.

C. flava is not distinguished by the above characters and differs in having a unique set of features for spot-sucking: an ovoid prothorax with a flattened apex; an inverted oral cavity; and a fixed head skeleton. The latter is also a feature of *L. lutea*. Both these species feed on watery biofilm coating smooth substrates, but the apparent convergence of a fixed head skeleton is not real. By outgroup comparison, it is a plesiomorphic feature in *L. lutea* and a derived feature in *C. flava*.

Higher cyclorrhaphan phytophages are distinguished by the following characters that also relate to feeding mechanisms: short, wide mandibular hook, often with accessory teeth; mandible base lozenge or crescent-shaped with more or less, vertically aligned apodemes; hinge joint horizontal so that mandibles barely separate on depression; mandibles braced against a sclerotised intermediate sclerite and intermediate sclerite sometimes braced against the basal sclerite; head skeleton pivoting up and down during lunges and barely extending from the prothorax; oral plate present; and pseudocephalon without cirri and dome-shaped in lateral view with dorsal and ventral margins extending and contracting in opposing directions during lunging; oval-shaped oral cavity.

The two higher cyclorrhaphan mycophages examined, *L. sylvatica* and *S. coleoptrata*, possess most of the phytophagous characters noted above, but can be distinguished by the saprophagous shape of their mandibles, i.e. a rectangular base with non-aligned muscle apodemes and also by the position of the mandibles, which, when at rest, are inclined, almost at a right angle to the rest of the head skeleton.

Although only two lower cyclorrhaphan zoophages were studied, we observed and filmed movement, but not prey capture, in the cyclorrhaphan predator, *Phaonia gobertii* (Mik) (Muscidae) which develops on other larvae under bark of fallen trees (Skidmore 1985). This species has the predation indicator of a long, narrow head skeleton, but head skeletons of other muscid predators are not like this (Skidmore 1985). Of other higher cyclorrhaphan predators we examined, two have long, narrow head skeletons, *Megamerina dolium* (Fabricius) (Megamerinidae) and

Leucopsis puncticornis Meigen (Chamaemyiidae) and one does not, *Speccafrons halophila* (Duda) (Chloropidae) (see Supplemental Material, Head Skeletons pdf). A feature shared by all these predators is, however, a sickle-shaped mandible which suits piercing. A notable contrast between the two syrphid predators examined here was the seal made between prey and the larva of *M. cincta* and the lack of a seal in *M. analis*. Sealing wounds to prevent loss of food is presumably advantageous. Roberts (1971) describes how the predatory muscid, *Limnophora riparia* (Fallén), keeps open wounds with scissors-like sclerites and makes a seal round them by pressing the prothorax against it. The mandibles of *P. gobertii* and *M. dolium* also have similar sclerites (see Supplemental Material, Head Skeletons pdf). Sickle-shaped mandibles, mechanisms for keeping open the wound and making a seal are also features of ectoparasitic larvae. For instance, *Neottiophilum praeustum* (Meigen) (Diptera, Piophilidae) is a bird brood ectoparasite (Keilin 1924). It has a sickle-shaped mandibular hook (Rotheray and Hancock 2012), paired scrapers on the underside of the pseudocephalon (Keilin 1924) that probably keep the wound open and break up clots of blood, and it presumably seals the wound by pressing the prothorax against it. Hence, sickle-shaped mandibular hooks, mechanisms for keeping open wounds and sealing round them indicate a predatory or ectoparasitic larva.

Dental sclerites were absent in all phytophages and mycophages examined here, but they are present in other apparent phytophages such as *Chiastocheta rotundiventris* Hennig which develops in *Trollius* flowerheads, *Egle minuta* (Meigen) in *Salix* catkins, *Pegomyia rubivora* (Coquillett) in *Filipendula* stems (Anthomyiidae), *Suillia laevifrons* (Loew) in *Luzula* stems and *Suillia ustulata* (Meigen) in *Sambucus* stems (Heleomyzidae) (see Supplemental Material, Head Skeletons pdf). Furthermore, the mandibular muscle apodemes are of the non-aligned saprophagous type in *P. rubivora* and *S. laevifrons* but of the aligned phytophagous type in the other species (Head Skeletons pdf). If indicator features and behaviours of more than one feeding mode are present in a larva, this may indicate a species with mixed feeding modes (in the above: phytophagy and saprophagy). These examples also suggest that species are diverse with respect to characters associated with mixed feeding modes.

Feeding mode associated features may reflect the physical properties of food rather than mode per se. Saprophagous features optimise gathering food that is essentially a liquid. Phytophagous/mycophagous features are effective for food that is firm and compacted and features associated with predation/ectoparasitism work well for membrane-enclosed, liquid food.

Larval development sites vary in conditions of spatial access and food quality (Table 1) and body shape and armature indicate some of these conditions. Larvae with restricted++ access include *L. lutea* and *M. platycephala* which squeeze between wet, closely adhering leaves. These larvae are dorso-ventrally flattened which suits squeezing. Flattening is fixed in *L. lutea*, but not in *M. platycephala*. To aid holding on, the ventral surfaces of both are extensively coated in spicules. Their dorsal surfaces are protected from mechanical damage, in *L. lutea* by sclerotised plates and by spicules in *M. platycephala*. A larva with more restricted access is *C. flava* which squeezes between layers of decay-softened whitewood, but it is subcylindrical, not flattened. It was the slowest moving of all the larvae studied here and slow movement may reflect the optimal speed with which it is able to squeeze through decayed whitewood. To protect and help it move forward, it has spicules on the

ventral and dorsal surfaces of both the abdomen and the thorax. However, black scars on the integument suggest frequent wounding (Figure 35).

Access is most restricted in the mycophages and the two leaf mining species and they must continuously excavate or tunnel through compacted tissue. They are also coated in spicules for holding on and protection, particularly at segment borders, less so in *L. sylvatica*. Leaf mining larvae are long and narrow, and flowerhead developing larvae are short and broad which are solutions to preserving body size in different types of confined environments. Front body compartments of both mycophages and phytophages are laterally flattened, more ventrally than dorsally, which suits reaching into and excavating tissue in narrow spaces. In the leaf miner, *P. solennis*, lateral flattening reaches an extreme with an asymmetrical pseudocephalon. Hence spatial constraints at development sites result in adaptive changes to body shape and spicule distribution, the former matching available spaces and the latter protecting the integument and also facilitating holding on. Consequently, from the small sample of species studied here, larval body shapes and spicule patterns are indicators of access conditions at development sites.

Indicators of food quality and other circumstances exist in the head skeleton. For instance, windows in the dorsal cornu enable it to bend which appears to be a space-saving device and indicates that the larva feeds in a confined space. A set of contrasting, co-varying features indicate abilities to feed on firm versus watery food. Hence, the head skeletons of the mycophages/saprophages studied here are heavily sclerotised, the mandibles are relatively large and the head skeleton is braced against distortion. There is also a well-developed and heavily sclerotised labial sclerite. These states contrast with those of species like *L. hackmani*, *N. acyglossa*, *M. platycephala* and *C. flava* that feed on oily to watery biofilm and which have small mandibles, relatively little sclerotisation and bracing and inconspicuous labial sclerites. Similar modifications in head skeleton and other features to firm and soft or liquid food also occur in Platypezidae (Rotheray et al. 2004) and phytophagous Syrphidae (Rotheray and Gilbert 2011). These results show the importance of food quality on trophic morphology, with combinations of certain features indicative of particular conditions.

A major objective of this study was to examine an overlooked yet critical process: the means by which cyclorrhaphan larvae consume food. The range of mechanisms and behaviour we recorded shows, unequivocally, that cyclorrhaphan larvae are far from being monotonously the same in this respect and that considerable morphological and behavioural diversification has occurred. However, documenting such diversity is limited by the very real problems of few cyclorrhaphan species being known in their larval stages, lack of appropriate observations and the paucity of preserved material on which to base morphological analyses. A reason why larvae are poorly known is suggested in works such as McAlpine et al. (1987), Papp and Darvas (1998) and Brown et al. (2010). In these monographs, chapters dealing with individual families of Cyclorrhapha are all too often reduced due to lack of data to treating larvae in superficial and simplistic ways, with the unfortunate consequence of reinforcing neglect due to lack of perceived significance. We hope the data presented here contribute to breaking down the cycle of neglect and ignorance affecting a clearly significant stage in a clearly significant lineage.

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Supplemental material

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