ECLOSION OF *PHYSOCEPHALA TIBIALIS* (SAY) (DIPTERA: CONOPIDAE) FROM A *BOMBUS* (APIDAE: HYMENOPTERA) HOST: A VIDEO RECORD

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Some members of Conopidae and other families of flies require development within hymenopteran hosts. Rearing of parasitized Apoidea provides valuable life history and ecological data but is rarely documented. Greater emphasis on gathering and analyzing rearing data is required. Analysis of a new video record of *Physocephala tibialis* (Say) reared from *Bombus impatiens* Cresson provides detailed evidence of the use of the ptilinum, mouthparts, and legs for eclosion within Conopidae. The previous literature on Conopidae/Apoidea rearing is reviewed.

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Introduction

Some species of Conopidae (Diptera) develop exclusively within hymenopteran hosts. Eggs are deposited inside adult bees or wasps. Following emergence from the egg, the larva grows, develops, and pupates entirely within the body of the host. Following death of the host and often after an overwintering period, the adult conopid ecloses from the host's corpse (Freeman 1966). While this behaviour is well noted within the conopid literature, careful rearing of parasitized hymenopteran hosts is only rarely documented.

Meijere (1904), Freeman (1966), and Smith (1959, 1966) summarized known host records and life histories for Conopidae, including many records of development within bee hosts. However, they did not distinguish between studies that relied on confirmed rearing records and studies that did not. Several studies tried to establish host records for Conopidae based exclusively on associations with host species (Rasmussen and Cameron 2004; Rocha-Filho et al. 2008) or on the discovery of eggs on pinned specimens (Stuckenberg 1963; Couri and Pont 2006; Couri and Barros 2010; Couri et al. 2013). These records must be considered

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tentative, as they do not confirm the successful development of Conopidae within a given host. While most confirmed rearing records are based on dead or obviously parasitized bees being held until parasitoids emerge, a few (Paxton et al. 1996; Polidori et al. 2005) were based on careful observation of host nests until parasitoids were seen to emerge.

Past rearing efforts have also produced other important observations on conopid life history. Schmid-Hempel et al. (1990) and Otterstatter et al. (2002) compared the relative parasitism rates and overlapping phenologies of competing parasitoid species. Knerer and Atwood (1967) reared one species of Conopidae, *Thecophora occidensis* (Walker), from six different species of halictine bees. They proposed a complex life history for *T. occidensis*, including phenological host switching. Müller (1994) observed self-burying behaviour in parasitized specimens of *Bombus terrestris.* Unfortunately, the conopid species reared out was never identified. Malfi et al. (2014) reared 39 specimens of *Physocephala tibialis* from three species of *Bombus*. They provided evidence of differential successful parasitism rates among the host species. Self-burying behaviour was also observed in parasitized bees, with a differential frequency of this behaviour depending on host species.

Several authors used rearing experiments to observe directly the process of eclosion from the host by Conopidae. Cumber (1949) reared *Physocephala rufipes* from *Bombus agrorum*, and noted that "the [conopid] adult emerges by pushing aside the anterior segments with its ptilinum." Polidori et al. (2005) observed an adult *Zodion cinereum* emerging from a ground nest of *Andrena agilissima* (Andrenidae) with the ptilinum "still being inflated rhythmically, indicating that they were freshly emerged adults." Koeniger et al. (2010) reared *Physocephala paralleliventris* from two different species of honey bees (*Apis cerana*, *A. koschevnikovi*) in Borneo. They observed an active period of walking for about fifteen minutes prior to inflation of the wings. They theorized that this active stage was necessary for conopids to emerge from the leaf litter in which the host bee was buried.

For other families of Diptera, video recordings of parasitoid emergence have been informative. Downing (1995) observed ptilinal expansion in *Amobia* (Sarcophagidae), which cleptoparasitizes mud-tube nesting wasps of *Trypoxylon* (Sphecidae). Strohm (2011) recorded cleptoparasitic members of Drosophilidae using ptilinal expansion to break out of the closed brood cells of their hymenopteran hosts.

The video recording presented here produced both life history data and behavioural observations for one species of Conopidae.

Materials and Methods

As part of ongoing research examining the impact of parasitism on bumble bees (Hymenoptera: Apidae: *Bombus*) in northern Virginia (Malfi and Roulston, 2014; Malfi et al. 2014), 445 foraging bumble bees were collected at Blandy Experimental Farm (Boyce, VA, USA, 36.09°N, 78.06°W) in June and July of 2012. These bees were maintained until death in the lab by housing bees in aquaria partially filled with soil and leaf litter and providing them sugar water *ad libitum* (for details, see Malfi et al. 2014). After death, bees were examined for the presence of a conopid parasite. Often this could be determined with minimal disturbance of the bee corpse as the conopid pupa frequently occupies the entire abdominal cavity of its host and a large, last-instar larva can be seen externally to move

within the bee. A total of 120 bumble bee workers belonging to three different species (*B. bimaculatus*, *B. griseocollis*, *B. impatiens*) were parasitized with conopid larvae. In September 2012, 94 conopid pupae harvested from these parasitized bees were placed in individual vials in a household refrigerator (~4˚C) and left there until April, 2013,when they were placed on a lab bench in a room at 21.5° C and monitored for emergence. The emergence of one parasitoid was observed in progress. After the corpse of its host, a *Bombus impatiens* worker, began to move, one author (ADS) recorded the emergence process with a cell phone camera held up to the eyepiece of a dissecting microscope. Recording of emergence began at 8:50 AM on June 2nd, 2013, and continued for eight minutes, at which point the fly had completed its exit from the host.

Results

Thirty-nine of the conopid pupae emerged as adults and were identified as *Physocephala tibialis*. A 30-second edited version as well as the full eight-minute video may be viewed at (VIDEO). The edited video begins with the head of the conopid already visible, emerging from the ventral anterior of the bee abdomen. The ptilinum is seen fully inflated, with the antennae deflected to the ventral surface of the head (Figs. 1A, 1D). The mouthparts are displaced posteriorly, between the forelegs. At full inflation, the ptilinal sac appears to be equal in volume to the rest of the head. Following full inflation, the ptilinum deflates, but is still extruded (Figs. 1B, 1E). As the conopid pulls the rest of its body from the host, the ptilinum continues rhythmically inflating and deflating, though not to a volume equal to that visible as the head is first emerging. The conopid uses its legs and mouthparts as levers to pry itself from the host's body. When the conopid body is fully emerged from the host, it begins to walk around with the deflated ptilinal sac still visible. Throughout the eclosion process, it appears that the antennae, mouthparts, and legs are already sclerotized and dark. The sclerites of the head, thorax, and abdomen appear pale and unsclerotized. The wings are not inflated to any extent throughout the video. The adult *P. tibialis* is included with other voucher specimens that have been deposited in the University of Guelph Insect Collection, Guelph, ON.

Discussion

Schizophora (Diptera) have a membranous invagination in the head that is visible in adults only as a ptilinal fissure (Réaumur 1738; Becker 1882; Cumming and Wood 2009). The first description of the ptilinum in Calliphoridae (Réaumur 1738) included the suggestion that it was used to burst the puparium wall through expansion. Early research on the structure and function of the ptilinum (reviewed in Laing 1935; Atkins 1949) was limited to Calliphoridae and Drosophilidae. Strickland (1953) examined 150 species from over 40 schizophoran families and found that, in all cases, the ptilinum is lined with microscopic scales that are used to improve the puparium-bursting capabilities of the ptilinum. Žďárek et al. (1986) and Žďárek and Denlinger (1992) revealed pressure changes within the ptilinum and associated structures during eclosion and bodily inflation of the adult. Reid et al. (1987)

defined a series of phases, including ptilinal expansion in the 'extrication behaviour' of Sarcophagidae. Our observations suggest that Conopidae demonstrate the same patterns and behaviours of eclosion as those observed in Calliphoridae, Drosophilidae, and Sarcophagidae.

Strickland (1953) noted that the ptilinum of Conopidae is larger, thicker, and covered with more varied types of scales than that of any other fly family examined. His detailed drawings of *Physocephala furcillata* indicate that the base and length of the mouthparts as well as the ptilinum are covered with sclerotized scales. He contended that these scales assist with eclosion of the fly and subsequent digging from a subterranean location. The species we observed, *P. tibialis*, is morphologically similar to *P. furcillata* (Camras 1957). Malfi et al. (2014) demonstrated an induced digging behaviour in bees parasitized by *P. tibialis*. Our video demonstrates the use of the mouthparts and ptilinum as part of both eclosion and subsequent digging.

For most species of Conopidae, there are no host records confirmed through rearing. The known records for Conopidae reared from Apoidea are summarized here (Table 1). The seven genera listed represent only 11.9% of the approximately 59 extant genera and subgenera (Gibson and Skevington 2013; Gibson et al. 2013).

Using DNA barcoding approaches to associate larvae, pupae, or adults of parasitoids with each other and their host species (e.g., Smith et al. 2006, 2007) may add ecological and evolutionary data to rearing experiments. A search of the cytochrome oxidase *c* subunit I (COI) sequences available on GenBank (June 10, 2014) revealed 45 species of Conopidae,

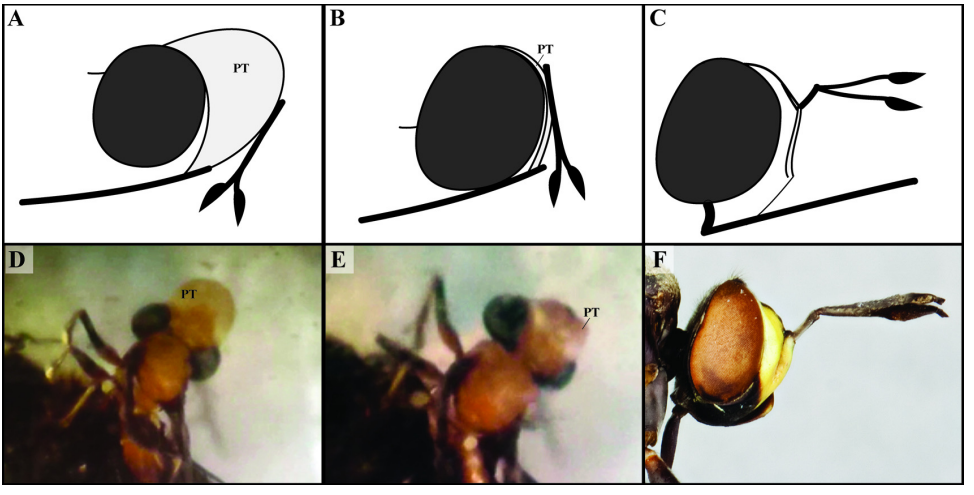

FIGURE 1. (A) *Physocephala tibialis.* Lateral view with ptilinum (PT) inflated. (B) Lateral view with ptilinum deflated. (C) Lateral view after sclerotization. (D) Dorsal view with ptilinum (PT) inflated. (E) Dorsal view with ptilinum deflated. (F) Lateral view after sclerotization (different specimen of same species). Photo supplied by T. Burt, Canadian National Collection of Insects. (D) and (E) are still images extracted from the VIDEO. Sclerotized adult head width for (D) and (E) = 3.69 mm. Line diagrams were created with Adobe Illustrator, based upon the video.

TABLE 1. Tabanidae species and number of specimens collected in 2011 and 2012 using Malaise traps and sweep netting, with abundance records.

Parasitoid	Host	Region	Reference
Dalmannia signata Chen	*Lasioglossum scitulum* (Smith)	Japan	Maeta and MacFarlane 1993
Myopa buccata (Linnaeus)	*Andrena japonica* (Smith)	Japan	Maeta and MacFarlane 1993
	A. scotica Perkins	Sweden	Paxton et al. 1996
M. rubida (Bigot)	*A. vierecki* Cockerell	California	MacSwain and Bohart 1947
M. testacea (Linnaeus)	*A. scotica* Perkins	Sweden	Paxton et al. 1996
Physocephala aurifrons Walker	*Centris analis* (Fabricius)	Brazil	Santos et al. 2008
P. bennetti Camras	*C. analis*	Brazil	Santos et al. 2008
	Xylocopa frontalis (Olivier), *X. submordax* Cockerell	Trinidad	Camras 1996
P. bimarginipennis Karsch	*X. carinata* Smith, *X. flavorufa* Degeer	Kenya, Uganda	Smith and Cunningham-Van Someren 1970
P. bipunctata (Macquart)	*Euglossa anodorhynchi* Nemésio	Brazil	Melo et al. 2008
	Centris analis (Fabricius)	Brazil	Santos et al. 2008
P. cayennensis Macquart	*C. analis*	Brazil	Santos et al. 2008
P. inhabilis (Walker)	*Megachile maculata* Smith	Brazil	Stuke and Cardoso 2013
	Centris analis	Brazil	Santos et al. 2008
P. furcillata (Williston)	*Bombus vagans* Smith	Ontario	MacFarlane and Pengelly 1975
P. marginata (Say)	*Apis mellifera* Linnaeus	Washington	Van Duzee 1934
	Bombus fervidus (Fabricius)	Ontario	MacFarlane and Pengelly 1975
	Megachile mendica Cresson	North Carolina	Krombein 1967
P. obscura Matsumura	*Megachile willughbiella* (Kirby)	Japan	Maeta 1997
	Bombus ardens Smith, *B. diversus* Smith	Japan	Maeta and MacFarlane 1993
P. paralleliventris Kröber	*Apis cerana* Fabricius, *A. koschevnikovi* Enderlein	Borneo	Koeniger et al. 2010
P. pusilla (Meigen)	*Megachile rotundata* Fabricius	Mongolia, France	Seidelmann 2005, Tasei 1975
P. rufipes (Fabricius)	*Bombus agrorum* Fabricius	England	Cumber 1949
	B. terrestris Linnaeus	???	Meijere 1904
	B. lapidarius (Linnaeus), *B. lucorum* (Linnaeus), *B. pascuorum* (Scopoli), *B. terrestris*	Switzerland	Schmid-Hempel and Schmid-Hempel 1988
P. rufithorax Kröber	*Centris analis*	Brazil	Santos et al. 2008
P. sagittaria (Say)	*Apis mellifera*	Washington	Van Duzee 1934
	Bremus auricomus Robertson	Illinois	Frison 1926

TABLE 1 continued...

P. spheniformis Camras	*Centris analis*	Brazil	Santos et al. 2008
P. soror Kröber	*Centris analis*	Brazil	Santos et al. 2008
P. texana (Williston)	*Apis mellifera*	Washington, Wyoming	Van Duzee 1934, Riedel and Shimanuki 1966
	Nomia melanderi Cockerell	Idaho	Foote and Gittins 1961
	Bombus bifarius Cresson, *B. californicus* Smith, *B. flavifrons* Cresson, *B. occidentalis* Greene	Alberta	Otterstatter et al. 2002
P. tibialis (Say)	*B. bimaculatus* Cresson, *B. griseocollis* DeGeer, *B. impatiens* Cresson	Virginia	Malfi et al. 2014
P. vittata (Fabricius)	*Megachile maritima* (Kirby)	Netherlands	Meijere 1904
P. wulpi Camras	*Xylocopa artifex* Smith, *X. augusti* Lepeletier, *X. splendidula* Lepeletier	Argentina	Stuke et al. 2011
Physocephala sp.	*Bombus* (9 spp.)	Massa-chussetts	Gillespie 2010
Physoconops fronto (Williston)	*Megachile perihirta* Cockerell	California	Bohart and MacSwain 1940
Sicus ferrugineus (Linnaeus)	*Bombus lucorum*, *B. pascuorum*, *B. terrestris*	Switzerland	Schmid-Hempel and Schmid-Hempel 1988
Thecophora occidensis (Walker)	*Lasioglossum forbesii* (Roberts), *L. laevissimus* (Smith), *L. lineatulum* (Crawford), *Halictus ligatus* Say, *H. rubicundus* (Christ), *Evylaeus cinctipes* (Provancher)	Ontario	Knerer and Atwood 1967
	Halictus confusus Smith	Indiana	Dolphin 1979
	H. confusus, *H. rubicundus*, *Lasioglossum cinctipes* (Provancher), *L. imitatus* (Smith), *L. lineatulum*, *L. forbesii*	Ontario	Smith 1966
Zodion cinereum (Fabricius)	*Andrena agilissima* (Scopoli)	Italy	Polidori et al. 2005
	Andrena prostomias Pérez	Japan	Maeta and MacFarlane 1993
Z. fulvifrons Say	*Apis mellifera*	South Dakota	Severin 1937
Z. obliquefasciatum (Macquart)	*Nomia melanderi*	???	Howell 1967
Z. vsevolodi Zimina	*Ceratina flavipes* Smith, *C. japonica* Cockerell, *C. megastigmata* Yasumatsu and Hirashima, *Chalicodoma spissula* (Cockerell), *Hylaeus thoracicus* Fabricius	Japan	Maeta and MacFarlane 1993

including three species of *Physocephala*. The use of DNA methods of identification, when target taxa have already been sequenced, greatly increases the value of studies that record immature parasites in hosts but, for methodological reasons, are unable to rear out adults (Gillespie 2010; Malfi and Roulston 2014).

Our study is an example of the added information about parasitoids that can be gained through careful rearing. Previous theories about the function of the ptilinum and the process of eclosion from the host have been strengthened with video evidence.

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