NEW RECORDS OF PIPUNCULIDAE ATTACKING PROCONIINE SHARPSHOOTERS (AUCHENORRHYNCHA: CICADELLIDAE: PROCONIINI)

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Five records of Pipunculidae (Diptera) attacking proconiine sharpshooters (Auchenorrhyncha: Cicadellidae) are documented here for the first time. *Eudorylas alternatus* (Cresson) is documented as a parasitoid of *Cuerna obtusa* Oman and Beamer and *Oncometopia orbona* (Fabricius) is recorded as being attacked by an apparently undescribed species of *Eudorylas* (Pipunculidae). Records of unidentified pipunculid larvae are also recorded from *Cuerna kaloostiani* Nielson, *Cuerna curvata* Oman & Beamer, and *Cuerna* sp. near *striata* (Walker) – *septentrionalis* (Walker). We describe these observations, summarize the data for them and explore the potential of Pipunculidae as biological control agents for pest proconiines such as glassy-winged sharpshooter (*Homalodisca vitripennis* (Germar)). We also reveal the utility of DNA barcoding for identifying pipunculid larvae.

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Introduction

With the exception of the big-headed fly genus *Nephrocerus* Zetterstedt which attack crane fly adults (Tipulidae), pipunculids are parasitoids of leafhoppers and planthoppers (Hemiptera: Auchenorrhyncha). They typically attack second instar larvae, although some parasitize adults (Waloff and Jervis 1987). Big-headed flies are found in almost every terrestrial habitat world-wide including agricultural ecosystems. Their larvae develop fully within their host, typically emerging from the dorsum of the abdomen of adult hosts after a rapid development. Hosts are usually rendered sterile or are killed by these parasitoids. Larvae normally pupariate in the leaf litter or soil. Development is variable with multivoltine species typically eclosing from the puparium within a few days to weeks and univoltine species overwintering in the substrate (Waloff 1980; Skevington and Marshall

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1997). The effects of pipunculid parasitization on planthoppers and leafhoppers have been documented by numerous scientists, most recently by May (1979), Chandra (1980), Waloff (1980), Lauterer (1981), Huq (1984, 1986a, 1986b), Ylonen and Raatikainen (1984), Yano (1985), and Skevington and Marshall (1997). Parasitized hosts are sometimes recognizable by their swollen abdomen and sluggish movements.

Recorded rates of parasitism vary from fractions of a percent to nearly 100 percent in local populations. For example, Hartung and Severin (1915) found *Circulifer tenellus* (Baker) (beet leafhopper, Cicadellidae) with up to 47% parasitism by two pipunculid species and Skevington and Marshall (1997) recorded parasitism rates of *Cuerna striata* by *Eudorylas* sp. near *alternatus* to be as high as 89%. Despite the importance of pipunculids as parasitoids, few rearing records exist for Pipunculidae, particularly in North America (Skevington and Marshall 1997). Data on host ranges are available for more than 52 European species of Pipunculidae (Skevington and Marshall 1997) while in the Nearctic Region only 16 species have received such documentation (Skevington and Marshall 1997; Moya-Raygoza et al. 2004; Koenig and Young 2007).

The potential value of Pipunculidae for biological control has stimulated some work on the bionomics of this family. For example, research into the control of the potato leafhopper, *Empoasca fabae* (Harris), a major pest of alfalfa in mid-western and eastern USA and Canada, involved exploration within Europe for natural enemies to be introduced to the United States (Jervis 1992). *Chalarus* specimens were reared for this effort but apparently were never released. Similarly, European species of *Chalarus* were considered for introduction into New Zealand for control of Frogatt's apple leafhopper, *Edwardsiana crataegi* (Douglas), populations of which are insecticide resistant (Jervis 1992). A release was never made because of concerns about adding yet another foreign species to the New Zealand fauna (pers. comm. M. De Meyer).

We decided to investigate the potential of these flies as parasitoids of Glassy-winged Sharpshooter (GWSS, *Homalodisca vitripennis* (Germar) (Cicadellidae, Proconiini)) in 2005. This species is native to the southeastern USA and northeastern Mexico, from Augusta, Georgia to Leesburg, Florida, west to ValVerde and Edwards counties in Texas, south to Mexico (Turner and Pollard 1959; Triapitsyn and Phillips 2000). It has become a serious pest of grapes in California where it was introduced in 1989 (Sorensen and Gill 1996; Hoddle 2004). Glassy-winged sharpshooters are effective vectors of *Xylella fastidiosa* Wells et al. (Eubacteria), the causative agent of Pierce's Disease in grapes, which has severely damaged vineyards in southern and central California (Hoddle 2004). Considerable effort has been expended to find egg parasitoids of GWSS and other pest leafhoppers, but little effort to date has been made to study their nymphal parasitoids (Goolsby and Setamou 2005; Irwin and Hoddle 2005; Pilkington et al. 2005). Finding a larval parasitoid for GWSS would be a great advance in potential biological control programs for the species. Although we have not discovered such a parasitoid, the discovery of several pipunculid parasitoids (described below) attacking related proconiine species is encouraging.

Methods and Materials

Adult pipunculids and leafhoppers were either killed with cyanide and pinned or collected into 100% alcohol. Specimens are deposited in the Canadian National Collection of Insects, Arachnids and Nematodes (CNC) and the Illinois Natural History Survey Collection (INHS). The CNC specimens are all labelled with a unique number (either in the format JSS # *n* or CNCD # *n*). Pipunculid larvae were collected into 70% alcohol (RR) or 100% alcohol (JHS). Voucher data for the material used in this study are available in Appendix 1.

Field work contributing to this study was conducted by two teams. Roman Rakitov collected the Arizona specimens while conducting general fieldwork there in 2003. John Goolsby coordinated fieldwork in Texas where his team was searching for potential biological control candidates for GWSS. When possible, leafhoppers were killed and dissected in the lab to search for parasitoids. When no lab facilities were available, leafhoppers were examined in the field for evidence of parasitism. Although leafhoppers that are parasitized by third instar pipunculids may be recognized in the field by their sluggish behaviour and swollen abdomens, we found no behavioural changes in cicadellids parasitized by first instar larvae. Dissection of a random series of leafhoppers in the field (by removing their abdomens and squeezing out the contents) thus allowed discovery of parasitized populations of leafhoppers. Even though very small, first instar pipunculids are easy to see as they crawl around.

Pipunculid larvae and adults collected in the survey were sequenced in an effort to match the identity of the immatures with the adult specimens. DNA was extracted and a 658 base pair fragment of the COI gene (now referred to as *cox*1 in the 'barcoding' literature) was amplified using the primer pair LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994). Methods used follow Hebert et al. (2003). Relevant sequences were deposited in GenBank (Appendix 1).

Parsimony and neighbour-joining analyses were performed with PAUP* (Swofford 2001). *Chalarus* sp. was defined as the outgroup for all analyses, as this is the putative basal genus of Pipunculidae (Rafael and De Meyer 1996; Skevington and Yeates 2000). The heuristic search procedure was used with stepwise-addition and 100 random replications. The heuristic search option was used with tree bisection-reconnection branch swapping, MULPARS, and random addition of taxa. Multistate characters were treated as non-additive.

Results and Discussion

Arizona

Between 13 and 18 April 2003, 33 *Eudorylas alternatus* puparia were obtained by R. Rakitov from pipunculid larvae developing within *Cuerna obtusa* in Arizona (Appendix 1). From these puparia, 19 adult pipunculids (10 females, 9 males) were reared. The leafhoppers were collected in forests of *Pinus edulis* and *P. ponderosa*. Note that the identification of these flies is tentative, despite being based on examination of the *E. alternatus* holotype. Confirmation will only be possible in the context of a complete revision of *Eudorylas*. The best current key to Nearctic eudorylines (Hardy 1943) does not work and over half of the species in the genus are undescribed (Skevington unpublished data). These flies appear to be conspecific with the flies reared from *Cuerna striata* in Ontario, Canada (Skevington and Marshall 1997). Although there is minor genitalic variation, their *cox*1 sequences differ by only 0.5%. This is typical of genetic distances among species of Pipunculidae (Skevington et al. 2007).

Rakitov (personal communication) also reports records of pipunculized specimens of *Cuerna kaloostiani* from Arizona, *Cuerna curvata* from California, and *Cuerna* sp. near *striata – septentrionalis* from Utah. The parasitized cicadellids and extracted pipunculid larvae supporting these records are in the INHS collection. These pipunculids are likely also species of Eudorylini, but further research is needed to corroborate this hypothesis.

Texas

On 20 October 2005, we dissected two first instar pipunculid larvae out of adult *Oncometopia orbona* at Yegua Creek, Texas (from ten *O. orbona* that were dissected). All efforts to rear this species of pipunculid from additional leafhoppers failed. Larval pipunculids are unidentifiable to species and in most cases, even to genus. In an effort to identify the larvae, we extracted DNA from one specimen and sequenced *cox*1. The generic identity of this larva was hypothesized based on phylogenetic placement of this sequence within a large matrix being prepared for a paper on the phylogeny of Pipunculidae (Skevington et al . unpublished data). Parsimony analysis using this dataset supported the placement of the larva as a member of the genus *Eudorylas* (the closest relative, *E. alternatus*, was 14.2% different based on pairwise analysis). This generic identification was expected, given that the other two identified pipunculids recorded as attacking proconiines were species of *Eudorylas*. Based on this discovery, we added 54 morphospecies of Eudorylini from the southern USA to the *cox*1 dataset and found a match (specimen CNCD3333) – the uncorrected pairwise distance between the two specimens is 0.6%, within the range of typical intraspecific genetic distances for pipunculids (Skevington et al. 2007). Assigning a name to this fly continues to be a problem. It cannot be identified with existing keys and will only be named in the context of a planned revision of the Eudorylini (Skevington, in prep). What we have learned though is where this species is likely to occur. Comparing CNCD3333 with other female pipunculids in the Canadian National Collection of Insects and the United States National Museum collection, turned up five specimens of this species (listed as *Eudorylas sp. TX8* in Appendix 1). As a result, we now know that this species occurs from College Station and Yegua Creek, Texas (Houston area) to Greenville, Mississippi, and appears to be at least bivoltine. Flight times are from April to May and September.

This example illustrates the power of DNA barcoding to associate immature stages with adults. It also illustrates how important it is to continue to work towards modern revisions of these flies. One of us (JHS) has been routinely DNA barcoding all of the species that he includes in revisions for five years (Skevington 2005b; Skevington 2006; Skevington and Földvári 2007; Skevington and Kehlmaier 2008), but a concerted effort is clearly needed to barcode as many species of adult pipunculids as possible. Doing so will open up research on biological control and facilitate ecological studies of these important flies.

Given the oligophagous nature of most pipunculids, we speculate that the species attacking *O. orbona* will also be found in *H. vitripennis* as both of these proconiines occur in the same habitats at the same time of year. Further research is warranted to collect, rear and evaluate this species of pipunculid as a potential biological control agent of *H. vitripennis* where it is invasive in California. Revision of Nearctic Eudorylini is also clearly a priority. It is likely that over 200 species occur in the Nearctic Region and only 38 valid species are currently described (Skevington 2005a). Most of these are not identifiable using current resources.

Acknowledgments

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Appendix 1 – Material Examined (Voucher data)

Pipunculidae: Pipunculinae: Eudorylini: *Eudorylas alternatus* (Cresson): USA, AZ, Coconino Co., 2.5 miles S Tusayan, "10X" Campground, 35°56'16.3" N, 112°07'48.7" W, R. Rakitov, 9♂, 10♀, 11 puparia, 3 third instar larvae, collected in *Pinus edulis* & *Pinus ponderosa* forest, host collection date 11.iv.2003, pupation dates 13-18.iv.2003, adult emergence dates 9-13.v.2003, host *Cuerna obtusa* Oman and Beamer, JSS# 13848-13849 (CNC), 13850 (INHS), 13851 – 3 legs removed for sequencing – GenBank # DQ349219, 13852-13854 (CNC), 13855 (INHS), 13856-13869, 13871-13881 (CNC).

***Eudorylas sp. nr. alternatus* (Cresson)** Canada, ON, Sideroad 25, 5 km SE Arkell, 1♂, host collection date 27.iv.1993, pupation dates 1.v.1993, adult emergence date 20.v.2003, host *Cuerna striata* Walker, JSS#12590 (CNC) – 3 legs removed for sequencing – GenBank # DQ349219.

***Eudorylas sp. TX8:* larvae:** USA, TX, Lee Co., Yegua Creek, 30°17'28" N, 96°15'39" W, 82 m, J. Skevington, 20.x.2005, 2 first instar larvae (one per host), host *Oncometopia orbona* (Fabricius) adults (one voucher JSS#16947 listed below), JSS#16853, one larva destroyed for sequencing – GenBank # DQ337627 (CNC); **adult females:** USA, TX, Brazos Co., College Station, Lick Creek Park, 30°38' N, 96°20' W, 17. Iv. 2006, Malaise trap, R. A. Wharton, CNCD3333 – GenBank # FJ860147 (CNC); USA, MS, Lafayete Co., F. M. Hull, v.1951, CNCD4914, iv.-v.1946, CNCD4914 (CNC); MS, Greenville, 11.ix.1922 (2 specimens), CNCD4916-7 (CNC).

Cicadellidae: Cicadellinae: Proconiini: *Oncometopia orbona* (Fabricius): USA, TX, Lee Co., Yegua Creek, 30°17'28" N, 96°15'39" W, 82 m, J. Skevington, 20.x.2005, host of first instar Eudorylini larva (larva destroyed for sequencing), 1 adult ♀, JSS#16947 (CNC).