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Overwintering Biology and Sex Ratio of the Parasitoid *Trichopria Columbiana* Ashmead (Hymenoptera: Diapriidae) in North Texas

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PURPOSE: This technical note summarizes the results of investigations from November 2007 to July 2008 into the overwintering biology of *Trichopria columbiana* Ashmead, a parasitoid wasp native to the United States that impacts populations of the biocontrol agent *Hydrellia pakistanae* Deonier. Information regarding behavior, overwintering life stage and location, and winter sex ratio is provided and discussed.

BACKGROUND: *Trichopria columbiana* (Figure 1) is a North American wasp that parasitizes a wide range of dipteran hosts, including the native *Hydrellia discursa* Deonier and *H. bilobifera* Cresson. In addition, *T. columbiana* has been found to parasitize the introduced biocontrol agent *Hydrellia pakistanae* Deonier, a leaf-miner of hydrilla (*Hydrilla verticillata* (L.f.) Royle) (Coon 2000). Parasitism by *T. columbiana* has been speculated to play a role in limiting *H. pakistanae* field establishment and spread, as well as subsequent population growth (Deonier 1998, Grodowitz et al. 2009).

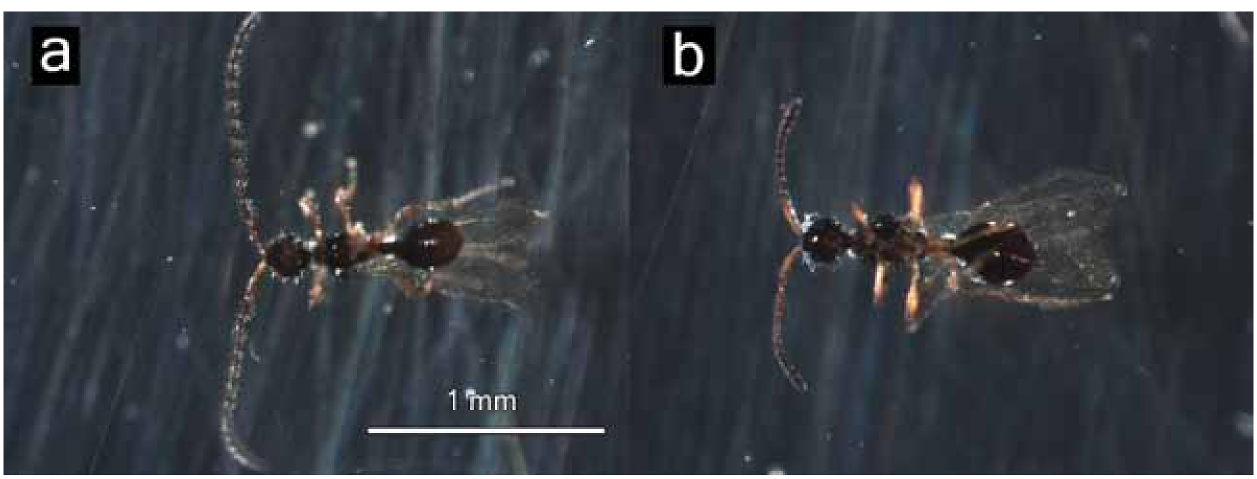

Figure 1. Male (a) and female (b) *Trichopria columbiana*. Males are readily distinguished from females by the threadlike, filiform antennae, as opposed to the shorter, clavate antennae found in females.

The *T. columbiana* - *H. pakistanae* predator-prey relationship has only recently been investigated. Researchers have examined the biology (Coon 2000) and host-selection of *T. columbiana* and its effect

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on *H. pakistanae* populations during summer months, when *H. pakistanae* actively feeds on hydrilla (Grodowitz et al. 2009). *Trichopria columbiana* has been reported to parasitize late instar-pupal hosts most frequently, and with greater survival rates than early instars (Grodowitz et al. 2009).

It is now becoming clear that *H. pakistanae* overwinters primarily as first and second-instar larvae by tunneling into the stem of the hydrilla plant (Harms and Grodowitz, in preparation). Such overwintering behavior occurs in North Texas from November to April. In summer months, however, *H. pakistanae* larvae (all instars) are found mining between the epidermal layers of hydrilla leaves, providing accessible host resources for *T. columbiana*. Because *T. columbiana* prefers late-instar and pupal hosts, it is important to understand how a shift in the presence of preferred host life stages, as well as relative absence of accessible hosts in stem vs. leaf, influences parasitoid abundance and behavior during the winter months.

In order to investigate the overwintering biology of *T. columbiana*, preliminary observations of potential overwintering sites were made during the winter months of 2006-2007. Based on these observations, a study was undertaken during the winter months of 2007-2008 to examine the winter biology of *T. columbiana*. This information may aid in more efficient rearing and release procedures by producing the highest number of *H. pakistanae* with minimum parasitism, leading to higher success rates in the management of hydrilla.

MATERIALS AND METHODS: Replicated quantitative data were collected from two *H. pakistanae* rearing ponds at the Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, Texas (33º 04’45” N, 96º 57’ 30” W) between the months of November 2007 and July 2008. Four methods were used to quantitatively examine *T. columbiana* overwintering, including sampling via floating soap-traps, pond-edge debris Berlese extraction, fresh hydrilla Berlese extraction, and hydrilla stem examination.

Adult collection. Four floating soap-traps were placed within hydrilla beds in each of two 0.1-hectare ponds once a month from November 2007 through June 2008. However, beginning in May 2008, floating soap-traps were placed in only one pond because the second pond was unexpectedly de-watered. Traps were filled with pond water and approximately 1-2 mL of liquid antibacterial hand soap was added to break surface tension. Traps were left in the ponds for approximately 4 hr (± 30 min) mid-day, then retrieved and processed. All trapped insects were preserved in 70% ethanol for later identification. Insect quantities are reported as an average of all traps in both ponds per sampling date.

In addition, pond-edge debris such as leaf-litter and cut grass was collected from the ponds’ edges monthly, November until March. Five large handfuls of debris were collected randomly, weighed, and placed in Berlese funnels (60-watt bulbs) to extract invertebrates. Debris samples were allowed to remain in Berlese funnels for at least seven days, until dried. Collections were discontinued in March because new growth along the ponds’ edges made sampling difficult and debris became minimal after this time.

Hydrilla was collected from the study ponds monthly, November until June. Five plant samples were randomly taken from each pond, weighed, placed in Berlese funnels, and allowed to dry for one week. If the samples were not dry after one week, they were allowed to remain longer, until dry. Extracted invertebrates were preserved in 70% ethanol for later identification. As indicated previously, during June 2008, only one pond was sampled due to unexpected draining of the other research pond.

Larvae and pupae collection. The possibility of *T. columbiana* overwintering as larvae/pupae within the host *H. pakistanae* was also examined. Ten apical hydrilla stem pieces, averaging 10 cm in length, were collected monthly from each pond and examined for *Hydrellia* larvae. Stem length, stem weight, number of whorls, number of damaged leaves, and number and life stage of *Hydrellia* spp. immatures present were recorded. In order to determine whether the *Hydrellia* spp. immatures had been parasitized, stems were placed, post-examination, in separate 1-L glass rearing containers fitted with mesh tops and incubated at 24-27 ºC. Containers were monitored daily for one month to record adult parasitoid emergence.

Sex ratio. *Trichopria columbiana* has been previously observed to display geographic parthenogenesis; southern populations are often nearly completely female (unpublished data). Because of this, all collected specimens were sexed to determine overwintering sex ratio. In addition to individuals collected during the course of this study, several years worth of previously collected *T. columbiana* specimens were examined and sexed. These specimens were collected both from Lake Texana, TX and from rearing ponds at the LAERF. They were not collected using a standardized method; i.e., specimens were either aspirated from the water's surface, collected by Berlese extraction of fresh field-collected hydrilla, or reared from host pupae.

Statistical analyses. Data were analyzed using STATISTICA version 9.0 (StatSoft ®, Inc., 2009, Tulsa, OK) and included analysis of variance (ANOVA) and Neuman-Keuls (NK) for mean separation. Statements of significance refer to an alpha level of 0.05, unless otherwise noted.

ANOVA was used to differentiate changes in number of *T. columbiana* throughout the study, including soap-trap collections, pond-edge debris Berlese extractions, and hydrilla Berlese extractions. A Neuman-Keuls post-hoc test was used to separate means into statistically different groups.

RESULTS AND DISCUSSION

Adults. *Trichopria columbiana* adults were collected, although in low numbers, from floating soap-traps throughout the winter. This is probably due to low levels of foraging, possibly in native aquatic vegetation, of which some exists in nearly every rearing pond at the LAERF.

Adult *T. columbiana* were found along the ponds' edge from November until April (Figure 2a), reaching densities in one sample as high as 5619 wasps per kilogram of pond-edge debris. A significant trend was found in the decline of *T. columbiana* numbers in pond-edge debris from December to March ($y = -0.99x + 300246.9$, df = 1, 2, $F = 154.64$, $R^2 = 0.99$, $p = 0.006$) (Figure 2b) and probably corresponds to mortality and the lack of new cohorts developing during the winter months.

Berlese extraction from fresh hydrilla resulted in the collection of adult wasps throughout the entire study, though there was a significant decline after December (Figure 3). This is likely a combination of adults foraging in the hydrilla plant material at the time of sampling as well as newly emerging adults caught by the extraction method. Numbers of wasps in hydrilla began to increase again in May and June, which corresponds to warmer temperatures, increased daylength, and an increased availability of host insects (Harms and Grodowitz, in preparation).

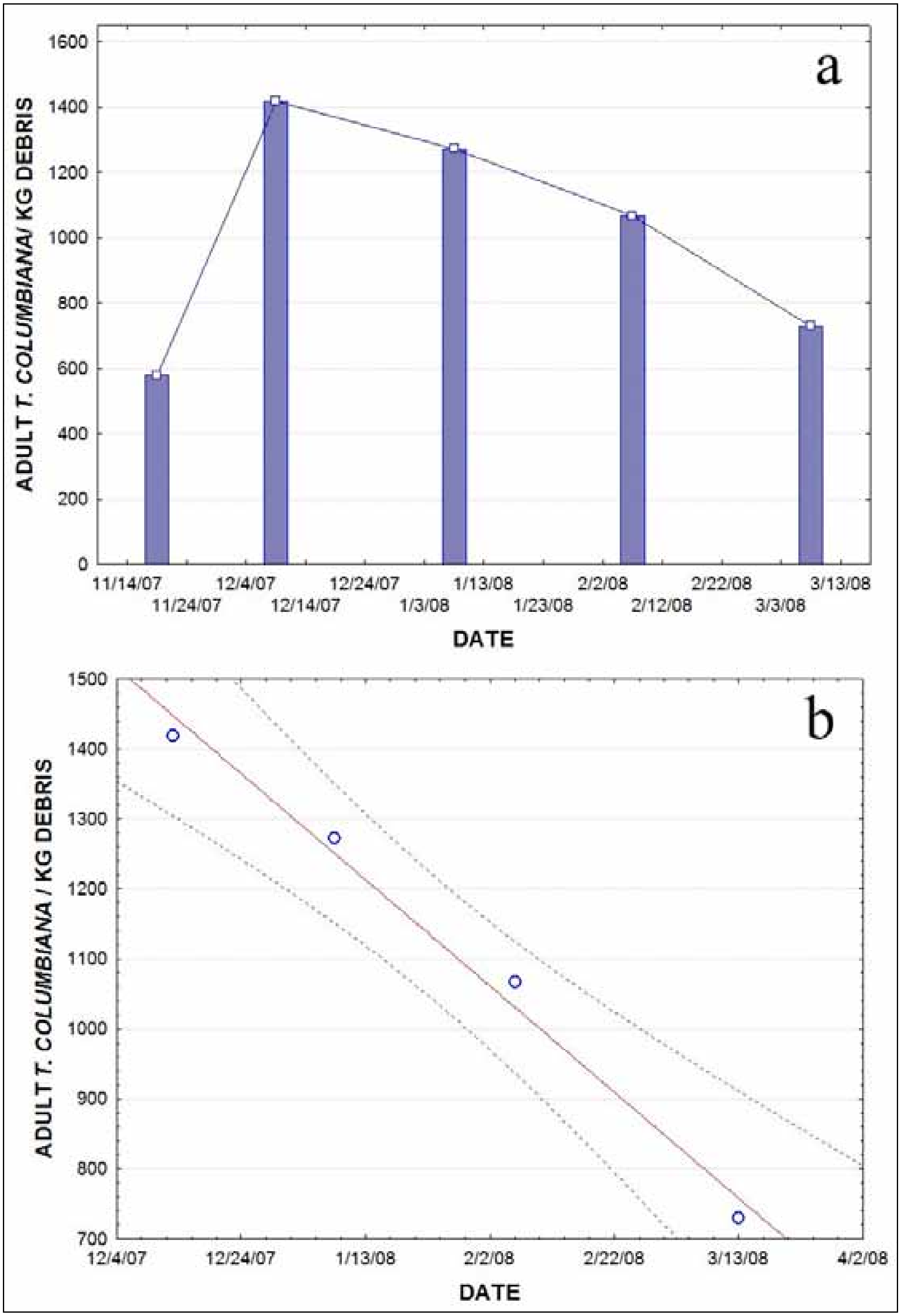

Figure 2. Mean wasps per kilogram of pond-edge debris recovered through (a) Berlese extraction, and (b) regression analysis on December through March sample means ($y = -0.99x + 300246.9$, df = 1, 2, $F = 154.64$, $R^2 = 0.99$, $p = 0.006$).

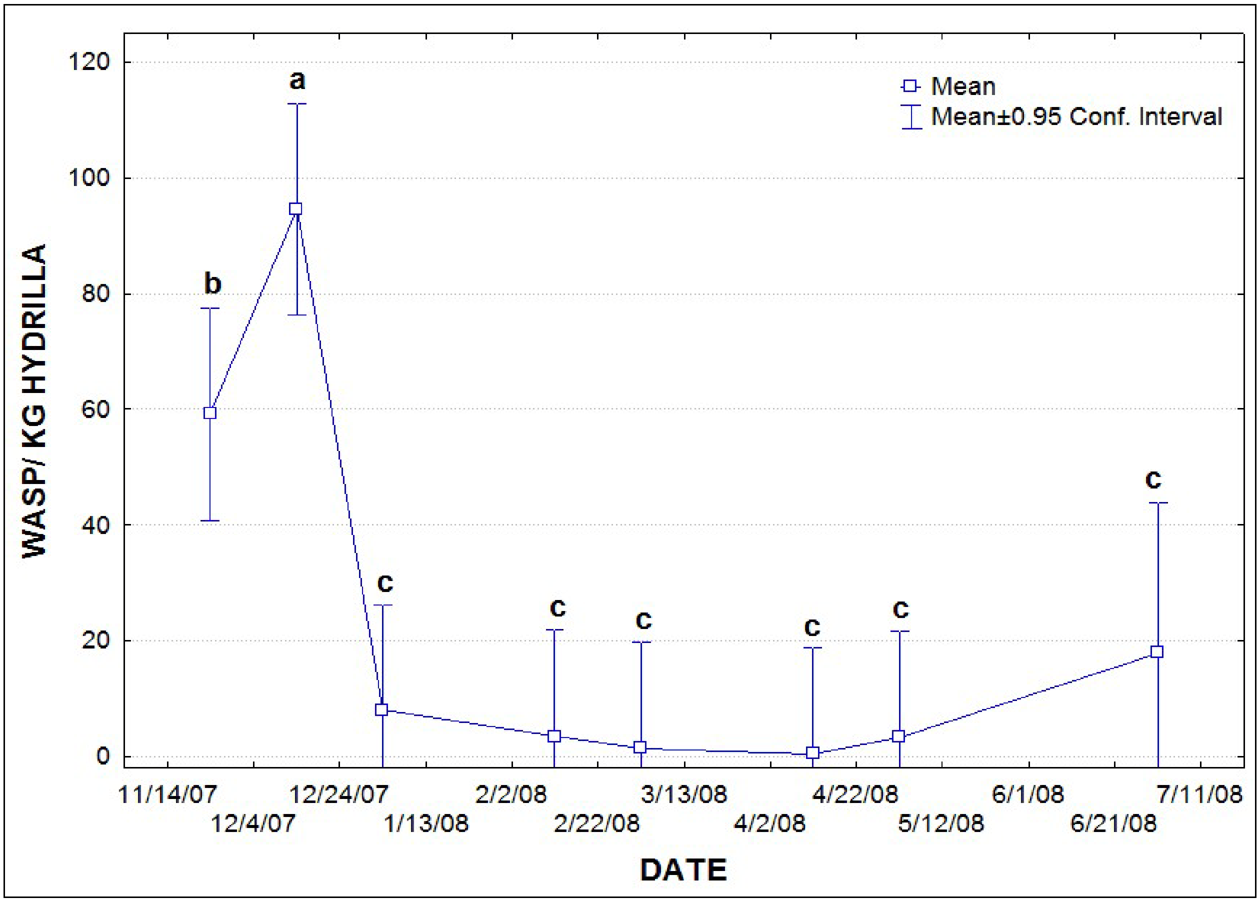

Figure 3. Mean wasps per kilogram of hydrilla plant material recovered through Berlese extraction (One-way ANOVA, df = 7, 67, F = 14.242, p < 0.0001). Vertical bars denote 0.95 confidence intervals. Letters denote significantly different means (Student Neuman Keuls post-hoc test).

Larvae and pupae. *Trichopria columbiana* adults were reared from hydrilla stems throughout the study. On two dates (November and April), the number of observed *H. pakistanae* larvae in the leaves was exceeded by the number of *T. columbiana* adults reared. It is not clear if the *H. pakistanae* larvae were hyperparasitized, though this is unlikely because early instar *T. columbiana* will reportedly eliminate any other competition from the host (Coon 2000). A more likely explanation is that some *H. pakistanae* larvae were parasitized before entering the stem for the winter, and were thus not counted during the leaf examinations. It is also not clear at this point whether *T. columbiana*'s foraging mechanisms allow for detection of larvae within the hydrilla stem. In that regard, more research is needed.

Trichopria columbiana apparently overwintered primarily in the adult life stage, in organic debris on the edge of the research ponds. The wasps appear to rest in pond-edge vegetation, and occasionally venture out onto the water surface searching for a suitable host when temperatures become warmer. Because hydrilla, and suitable host life stages, are available in limited numbers during winter months, a dramatic decline in parasitism of *H. pakistanae* was observed. It is possible, though not examined in the current study, that native hosts, such as *H. bilobifera* and *H. discursa*, may be utilized as winter hosts. It is not clear how abundant either of these native *Hydrellia* spp. are during winter months, but their presence may allow *T. columbiana* parasitism to remain active year-round.

Sex ratio. A total of 15,377 specimens of *T. columbiana* were collected and sexed, of which 14,776 were collected in the current study, and 601 were reared from host pupae or represented past collections. A total of four males were found during the current study, giving a winter sex ratio of 1:3694 (male: female). In addition, only one male was identified from past rearing/collections, giving an estimated sex ratio of 1:600. The first author has observed a similar ratio at field sites in Texas and Alabama (Harms, unpublished data). There is some evidence, though, that in the northern range of *T. columbiana* the sex ratio is nearly 1:1[1]. The reasons for this geographic parthenogenesis are unknown, and more work is needed to examine the significance to *T. columbiana* populations. Regardless, it appears that male *T. columbiana* are rare in nature, at least in North Texas.

These results have several implications. First, biocontrol agent release should coincide with high *H. pakistanae* populations and low parasitism. This may occur in early spring, or opportunistically if parasitism levels are observed to decline during the summer. By releasing large numbers of biocontrol agents into field sites at a time when parasitism is low, the likelihood of establishing agents and impacting hydrilla populations increases. Although adult parasitoid abundance along the ponds' edge is relatively great during winter months, it is not clear why parasitism historically has been low in spring and increases throughout the growing season (Grodowitz et al. 2009). One explanation may be that adult parasitoid fitness decreases during winter months (possibly indicated by a decrease in abundance) and so parasitism levels are low at a time when *Hydrellia* spp. populations begin to increase during spring months. In addition, because *T. columbiana* aggregates along the ponds' edge during winter months, the timely application of insecticides to impact parasitoid populations may allow fly populations to increase more rapidly and with less restriction throughout the growing season.

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