First report of pupal parasitoids of filth-breeding flies (Diptera) from bovine manure in northeastern Mexico

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Abstract—A total of 2702 individuals representing nine species of Pteromalidae (Chalcidoidea) plus at least one species of Diapriidae, *Trichopria haematobiae* (Ashmead) (Diaprioidea), were reared as pupal parasitoids of filth-breeding flies in bovine manure during a 2-year study in northern Tamaulipas, Mexico. The Pteromalidae included two undescribed species of *Spalangia* Latreille plus *Spalangia cameroni* Perkins, *S. drosophilae* Ashmead, *S. endius* Walker, *S. nigroaenea* Curtis, *Muscidifurax raptor* Girault and Sanders, *M. zaraptor* Kogan and Legner, and *Pachycrepoideus vindemiae* (Rondani). *Spalangia nigroaenea* accounted for 71% of all the pupal parasitoids and all six *Spalangia* species for 94% of the parasitoids over the 2 years. Based on its preponderance, *S. nigroaenea* is recommended as a candidate for inundative releases within integrated pest management programs to help control fly pests of cattle in northeastern Mexico.

Résumé—Au cours d'une étude de deux ans dans le nord de l'état de Tamaulipas, Mexique, nous avons élevé à partir de fumier de bovins un total de 2702 parasitoïdes des pupes de mouches des immondices; ces individus représentent neuf espèces de Pteromalidae (Chalcidoidea), ainsi qu'au moins une espèce de Diapriidae, *Trichopria haematobiae* (Ashmead) (Diaprioidea). Les Pteromalidae comprennent deux espèces inédites de *Spalangia* Latreille en plus de *Spalangia cameroni* Perkins, *S. drosophilae* Ashmead, *S. endius* Walker, *S. nigroaenea* Curtis, *Muscidifurax raptor* Girault et Sanders, *M. zaraptor* Kogan et Legner et *Pachycrepoideus vindemiae* (Rondani). *Spalangia nigroaenea* constitue 71 % et les six espèces de *Spalangia* ensemble 94 % de tous les parasitoïdes des pupes obtenus au cours des deux années. À cause de cette prépondérance, nous recommandons de retenir *S. nigroaenea* comme candidat potentiel pour des libérations massives pour aider à contrôler les mouches nuisibles du bétail dans le cadre des programmes de lutte intégrée dans le nord-est du Mexique.

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Introduction

Several species of filth-breeding flies (Diptera) are important livestock pests in Mexico. Economically the most important fly pests of cattle are the house fly, *Musca domestica* L., stable fly, *Stomoxys calcitrans* (L.), and horn fly, *Haematobia irritans* (L.) (Muscidae) (Martinez and Lumaret 2006). These flies affect livestock health by transmitting disease through biting and loss of blood and causing stress that results in weight loss and reduced milk production. They also have an impact on public health and, as a nuisance problem, on public relations,

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and they reduce the productivity of personnel working in livestock and poultry facilities (Carballo 1993).

The use of chemical insecticides is the most common and intensively applied method of controlling filth-breeding fly pests of livestock, but several reports indicate that some fly species that were initially highly susceptible to insecticides now include populations that show resistance (Cilek and Greene 1994; Almazan *et al.* 2001). For sustainable management of pest flies it is imperative to reduce dependency on pesticides by developing other, more environmentally friendly methods of control. It has been shown that inundative release of mass-reared hymenopterous pupal parasitoids is a biological-control method that sometimes successfully suppresses house and stable fly populations that have a negative impact on livestock production (Meyer *et al.* 1990). This method has the additional advantages of preventing the development of immature flies to the adult (pest) stage, ease and safety of application, and cost effectiveness. However, inundative releases of pupal parasitoids are not always successful in reducing pest fly populations, and the factors that limit their effectiveness are not obvious. Among these factors, the host preferences and host-finding abilities of different parasitoid species vary and because of this, releasing more than one parasitoid species may be more effective and convenient than releasing only one species (Petersen *et al.* 1983; Kaufman *et al.* 2001). Identifying the native parasitoids in a region is an important first step in determining which species are best manipulated within integrated pest management (IPM) programs to achieve maximum control of pest species while minimizing pesticide use and conserving beneficial insects.

The livestock area of northern Tamaulipas in northeastern Mexico is mostly dedicated to beef cattle, though a few ranches with 10–20 cows are dedicated to the production of milk and its derivatives. Fly infestations are prevalent, and protection based on insecticides has contributed to the permanence of or increase in populations of pest flies in the area. The species composition of the pupal parasitoids attacking pest flies of cattle developing in bovine manure in northern Tamaulipas has not previously been investigated. The objective of the present study was to identify and quantify the species in order to generate information on which species might be mass-reared for inundative control within future sustainable IPM programs.

Materials and methods

The study was conducted during 2005 and 2006 on five and four cattle ranches, respectively, located in northern Tamaulipas, Mexico, at 26°02′N, 98°12′W, 25°52′N, 98°30′W, 25°43′N, 98°50′W, 25°32′N, 99°21′W, and 25°12′N, 99°10′W. Three of the ranches (1, 3, and 4) were small dairy ranches and the other two (2 and 5) were beef-cattle ranches. The study region is about 25 m above sea level and the average annual rainfall is 653 mm. Conditions are subtropical, semi-arid, and warm, with median, high, and low temperatures of 22.6, 27.4, and 17.8 °C and median, high, and low relative humidity values of 80%, 100%, and 50%, respectively. On each ranch, 10 fresh cattle droppings were collected every 2 weeks and placed in plastic trays in close proximity to confined livestock. From 7 to 10 samplings per ranch were made from 8 March to 1 June 2005 and from 11 to 16 samplings per ranch from 13 March to 24 August 2006, covering the period of greatest abundance of cattle pest flies (Loera 2005, unpublished report).[2] Droppings were left exposed for 7–8 days and then taken to the laboratory of the Rio Bravo experiment station of the Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias, where they were crumbled by hand in a container containing 40 L of water. Pupae were extracted when they floated and then maintained in petri dishes (14.5 mm diameter) at room temperature (23 ± 1 °C) for 5 months. From 20 to 30 puparia were placed in each petri dish. To prevent the escape of emergent parasitoids, the petri dishes were covered with Parafilm. Emerged parasitoids were preserved in ethanol diluted to 70% with water. Puparia from which neither flies nor parasitoids emerged were dissected to recover unemerged parasitoids. All pupal parasitoids were identified by G.A.P. Gibson. Host associations of the reared parasitoids were not determined because the host puparia and fly species emerging from the puparia were not identified, but the probable fly hosts of the reared species are discussed, based on the

[2] G.J. Loera. 2005. Dinámica poblacional de dípteros asociados a las excretas de ganado bovino en el norte de Tamaulipas, Mexico. Reporte interno Campo Experimental Río Bravo, Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias.

Table 1. Hymenopterous parasitoids (females/males) emerged from fly puparia collected from bovine manure in northern Tamaulipas, northeastern Mexico, in 2005.

	Spalangia nigroaenea	*Spalangia cameroni*	*Spalangia drosophilae*	*Spalangia endius*	*Spalangia* n. sp. 1	*Spalangia* n. sp. 2	*Muscidifurax raptor*	*Trichopria haematobiae*
Ranch 1	169/135	33/20	4/0	0/1	3/2	22/13	0/0	8/3
Ranch 2	105/59	34/19	1/0	6/5	0/0	0/0	0/0	0/0
Ranch 3	120/77	18/16	0/1	1/0	0/0	0/0	0/0	0/0
Ranch 4	146/67	41/14	0/1	8/0	0/0	2/0	0/0	0/0
Ranch 5	57/47	40/21	14/6	0/0	0/0	2/0	2/10	0/0
Total females/males	597/385	166/90	19/8	15/6	3/2	26/13	2/10	8/3
Total	982	256	27	21	5	39	12	11

results of other studies. Comprehensive host lists for each of the described species of Pteromalidae reared are given in Noyes (2003). Because of the experimental method used, Eucoilidae (Hymenoptera: Cynipoidea) were also reared, but these were not identified further because they are parasitoids of larvae. Voucher specimens of all reared parasitoid species are deposited in the Canadian National Collection of Insects, Arachnids and Nematodes, Agriculture and Agri-Food Canada, Ottawa, and in the Universidad Autonoma de Tamaulipas, Ciudad Victoria, Tamaulipas, Mexico.

Results

We reared a total of 1353 (823 ♀♀, 520 ♂♂) pupal parasitoids during 2005 (Table 1) and 1349 (669 ♀♀, 633 ♂♂, 47 unsexed) during 2006 (Table 2), representing at least 10 species. Included were at least one species of the genus *Trichopria* Ashmead (*T. haematobiae* (Ashmead) (Diaprioidea: Diapriidae)), as well as *Pachycrepoideus vindemiae* (Rondani), *Muscidifurax raptor* Girault and Sanders, *M. zaraptor* Kogan and Legner (Hymenoptera: Pteromalidae), and six species of *Spalangia* Latreille (Chalcidoidea: Pteromalidae). The *Spalangia* species included two undescribed species plus *S. cameroni* Perkins, *S. drosophilae* Ashmead, *S. endius* Walker, and *S. nigroaenea* Curtis. *Muscidifurax zaraptor* and *P. vindemiae* were not recovered in 2005 (Table 1), but all 10 species were reared in 2006 (Table 2). Rearings in 2006 also included 1 female belonging to the genus *Trichopria* plus 47 individuals belonging to the genus *Spalangia* that were not identified to species or sexed (Table 2). The latter could not be identified because they were pupae or were insufficiently preserved, but most were likely *S. nigroaenea*.

In 2005 the six identified *Spalangia* species accounted for 98% of all the parasitoids, and one species, *S. nigroaenea*, accounted for 72.6%. The other species, with percent composition in parentheses, were *S. cameroni* (19.0%), *Spalangia* n. sp. 2 (2.9%), *S. drosophilae* (2.0%), *S. endius* (1.6%), *M. raptor* (0.9%), *T. haematobiae* (0.8%), and *Spalangia* n. sp. 1 (0.4%). *Spalangia nigroaenea*, *S. cameroni*, and *S. drosophilae* occurred on all five ranches; *S. endius* was recovered from four ranches, *Spalangia* n. sp. 2 from three ranches, and *Spalangia* n. sp. 1, *M. raptor*, and *T. haematobiae* from only one ranch.

In 2006 the six identified *Spalangia* species accounted for 90.5% of all the parasitoids and *S. nigroaenea* for 68.8%. The other species, with percent composition in parentheses, were *S. cameroni* (6.4%), *S. drosophilae* (4.9%), *T. haematobiae* (4.4%), *S. endius* (3.9%), *M. zaraptor* (2.4%), *Spalangia* n. sp. 2 (2.3%), *M. raptor* (1.6%), *P. vindemiae* (1.1%), and *Spalangia* n. sp. 1 (0.7%). The two unidentified *Spalangia* species represented an additional 3.5% of the parasitoids. *Spalangia nigroaenea*, *S. cameroni*, and *Spalangia* n. sp. 2 occurred on all four ranches; *S. drosophilae*, *S. endius*, *Spalangia* n. sp. 1, *M. zaraptor*, and *T. haematobiae* were recovered from three ranches and *M. raptor* and *P. vindemiae* from two ranches. A single unidentified individual belonging to the genus *Trichopria* was also reared from one ranch.

Estimates of percent parasitism, total and by females only, on the ranches during 2005 and 2006 are given in Table 3. All the parasitoids reared are solitary species, so percent parasitism is based on the premise that only one parasitoid emerged from each puparium. It is estimated that neither flies nor parasitoids emerged from 10% of the puparia. In 2005 the highest percent parasitism was recorded on ranches 1 and 4. A total of 229 female parasitoids were recovered on ranch 1, where *S. nigroaenea* was 1.2–3.0 times more numerous

than on the other ranches and *Spalangia* n. sp. 2 females were 11 times more numerous than on ranches 4 and 5. On ranch 4, where 197 female parasitoids were recovered, *S. cameroni* females were up to 2.3 times more numerous than on the other ranches and *S. nigroaenea* was the second most numerous among the ranches. In 2006 the highest percent parasitism was recorded on ranch 2, where all 10 identified parasitoid species were present and 228 female parasitoids were recovered; *S. nigroaenea* was as numerous on ranch 2 as on ranch 1 and 1.4–1.6 times more numerous than on the other ranches. On ranch 1, where females of only five species of parasitoids were recorded, *S. nigroaenea* females were 1.4–1.7 times more numerous than on the other ranches and *T. haematobiae* females were 6.5–8.7 times more numerous than on ranches 2 and 3. Females of six and seven parasitoid species were reared on ranches 3 and 4, respectively.

Discussion

About a dozen species of Pteromalidae plus *Trichopria* spp. are commonly found to attack puparia of filth-breeding flies throughout the world (Legner *et al.* 1976). Hernández-Hernández *et al.* (2004) previously reported rearing the pteromalids *Spalangia longepetiolata* Bouček, *S. cameroni*, *S. endius*, *S. nigroaenea*, *M. raptor*, and *Urolepis rufipes* (Ashmead) from cattle manure on dairy farms in Aguascalientes in central Mexico. The identity of *S. longepetiolata* requires confirmation because this species was introduced into California from Africa in the late 1960s (Legner 1978) but was not subsequently recovered until the report of Hernández-Hernández *et al.* (2004). Based on Noyes (2003), our rearings of *S. drosophilae* and *M. zaraptor* represent new distribution records for Mexico, though all the species we reared in northeastern Mexico have also been reported from Texas.

Loera (2005, unpublished report)[2] showed that populations of fly pests of cattle in northern Tamaulipas consist primarily of *M. domestica* (35%–49%) and *H. irritans* (39%–48%) plus the less-frequent species *S. calcitrans* (1%–10%) and the little house fly, *Fannia canicularis* (L.) (Diptera: Fanniidae) (2%–11%). These species are all known hosts of one or more of the seven named species of Pteromalidae that we reared (Noyes 2003). The two undescribed species we reared, *Spalangia* n. sp. 1 and *Spalangia* n. sp. 2, are morphologically similar to *S. drosophilae* and *S. haematobiae* Ashmead, respectively. Comprehensive information on their known distributions and hosts will be given when they are described, but host associations observed from museum specimens indicate that *Spalangia* n. sp. 1 is a parasitoid of *H. irritans,* whereas *Spalangia* n. sp. 2 is a parasitoid of *H. irritans* and *S. calcitrans* plus other species in the families Anthomyiidae and Sepsidae (unpublished data). *Spalangia drosophilae* has been reported as a parasitoid of *H. irritans*, *M. domestica*, and *S. calcitrans* plus other flies in the families Chloropidae, Drosophilidae, Sarcophagidae, and Sepsidae (Noyes 2003).

We also reared *S. cameroni*, *S. endius*, *S. nigroaenea*, *M. raptor*, and *P. vindemiae*, which are the five species of pupal parasitoids most commonly reported in other studies of fly pests encountered in intensive animal production (Floate *et al.* 1999; Skovgård and Jespersen 1999; Hogsette *et al.* 2001; Birkemoe *et al.* 2004; Gibson and Floate 2004; Hernández-Hernández *et al.* 2004). All these species have wide distributions (Noyes 2003) and may have been introduced into Mexico and throughout the world along with livestock. In our study, *S. nigroaenea* represented 71% of the parasitoids reared from unidentified filth-breeding flies. In Brazil, Sereno (2000) reported that *S. nigroaenea* was responsible for 91.5% of all parasitism of the horn fly in cattle manure, whereas Marchiori *et al.* (2000*a*) reported that *S. drosophilae* (40.8%) and *S. nigroaenea* (35.6%) were the predominant species parasitizing muscoid puparia in cattle manure. Research data available from the midwestern United States of America indicate that *S. nigroaenea* is the commercially available parasitoid that is most likely to attack both house fly and stable fly puparia in feedlots. Releases of *S. nigroaenea* and *M. zaraptor* on cattle feedlots in Illinois significantly reduced production of house flies and stable flies over a 3-year period (Weinzierl and Jones 1998). In a study of puparia collected on dairy farms in northern Illinois, Olbrich and King (2003) also reported that *S. nigroaenea* accounted for most of the parasitoids (71.6%) reared from the house fly, whereas *S. endius* and *S. cameroni* accounted for only 6.2% and 4.6%, respectively. *Spalangia nigroaenea* accounted for 19.7% of the parasitoids reared from stable fly puparia compared with 27.5% for *S. nigra* Latreille, 25.3% for *S. endius*, and 21.9% for *Muscidifurax* spp. Geden *et al.* (2006) further

Table 2. Hymenopterous parasitoids (females/males) emerged from fly puparia collected from bovine manure

	Spalangia nigroaenea	*Spalangia cameroni*	*Spalangia drosophilae*	*Spalangia endius*	*Spalangia* n. sp. 1
Ranch 1	139/139	13/7	0/0	0/0	0/0
Ranch 2	138/194	17/22	10/8	21/19	3/4
Ranch 3	84/75	4/3	0/1	4/4	1/1
Ranch 4	98/61	12/9	31/16	2/2	0/1
Total females/males	459/469	46/41	41/25	27/25	4/6
Total	928	87	66	52	10

*Total number of individuals only.

Table 3. Percent parasitism of unidentified fly species by hymenopterous parasitoids, total and females alone, based on the numbers of puparia and emerged parasitoids collected from bovine manure in northern Tamaulipas, Mexico, during 2005 and 2006.

	No. of puparia		No. of emerged parasitoids*		Percent parasitism*	
Site	2005	2006	2005	2006	2005	2006
Ranch 1	2544	3166	229/413	190/368	9.0/16.2	6.0/11.6
Ranch 2	2433	3257	146/229	228/507	6.0/9.4	7.0/15.6
Ranch 3	1985	1886	139/233	100/195	7.0/11.7	5.3/10.3
Ranch 4	2188	2419	197/279	150/279	9.0/12.8	6.2/11.5
Ranch 5	1642	—	115/199	—	7.0/12.1	—
Total	10 792	10 728	826/1353	668/1349	7.7/12.5	6.2/12.6

*Female parasitoids / total parasitoids.

reported that *S. nigroaenea*, *S. cameroni*, *S. endius*, and *M. raptor* all attacked and produced progeny on house, stable, and horn flies as well as the black dump fly, *Hydrotaea aenescens* (Weidemann) (Muscidae), and the flesh fly *Sarcophaga bullata* Parker (Sarcophagidae). In that laboratory study, *S. nigroaenea* had similar rates of parasitism on the house fly, stable fly, and black dump fly.

Two of the species we reared, *M. raptor* and *S. cameroni*, are among the most commonly used parasitoids in intensive systems of livestock and poultry production (Chavarrias 2006). Skovgård (2006) reported that indoor releases of *S. cameroni* and *M. raptor* on dairy farms in Denmark significantly increased parasitism of the house fly from 5.3% to about 29%, with *S. cameroni* contributing approximately 71.5%–72.3%. Although *S. cameroni* has a longer development time than *M. raptor* (about 26 *vs.* 14 days at 27 °C), and a lower attack rate (up to 10 *vs*. 20 hosts per female each day), it has a longer life-span (about 3 *vs.* 2 weeks), is less sensitive to pesticides, and is highly effective at locating pupae buried deep in the breeding substrate (Geden 1996, 1997, 1999, 2002; Geden *et al.* 2006).

Pachycrepoideus vindemiae is a primary or facultative hyperparasitoid of a wide diversity of Diptera in the families Anthomyiidae, Calliphoridae, Muscidae, Tachinidae, and Tephritidae (Noyes 2003). Although Pickens and Miller (1978) showed that *P. vindemiae* can significantly increase the mortality of house fly pupae in a poultry house, they noted that the species is a relatively poor searcher and is probably best used in programs where fly breeding is concentrated in a small area. In a field comparison of mass releases of *P. vindemiae* and *M. zaraptor* in cattle feedlots, *M. zaraptor* represented more than 95% of parasitoids recovered from naturally occurring pupae and *P. vindemiae* fewer than 1% (Petersen *et al.* 1992).

The species of Pteromalidae discussed above attack mature puparia, whereas *Trichopria* species attack young puparia. Unidentified *Trichopria* species have been commonly found as parasitoids of the horn fly in Brazil and the United States of America (see references in Marchiori *et al.* 2000*b*; Geden *et al.* 2006), but little is known of their biology. *Trichopria haematobiae* was described originally by Ashmead (1893) as a parasitoid of the horn fly.

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Spalangia n. sp. 2	*Spalangia* sp.*	*Muscidifurax raptor*	*Muscidifurax zaraptor*	*Pachycrepoideus vindemiae*	*Trichopria haematobiae*	*Trichopria* sp.
8/8	0	0/0	4/1	0/0	26/23	0/0
2/5	5	6/1	19/3	8/5	4/1	1/0
4/1	5	0/3	0/0	0/0	3/2	0/0
3/0	37	0/0	2/3	2/0	0/0	0/0
17/14		6/15	25/7	10/5	33/26	1/0
31	47	21	32	15	59	1

Conclusions

Management of manure to reduce pest fly populations is seldom practiced on most cattle ranches in Mexico. As herd size increases, pest pressures are often aggravated by large quantities of animal waste and crowded conditions, which promote pest fly populations and the spread of external parasites. However, studies of pest fly parasitoids, including their identification, behaviour, and seasonal fluctuation, are very scarce. Our recovery of two undescribed *Spalangia* species, as well as new distribution records, demonstrate that much remains to be done even to document the diversity of the parasitoid fauna of filth-breeding flies in northeastern Mexico. Management of cattle pests in the region usually relies on insecticides as the single control tactic; this has aggravated resistance problems in pest populations and inadvertently destroyed natural enemies. Our study revealed at least 10 species of indigenous pupal parasitoids that could be used in biological control as part of IPM programs to control pest flies of cattle. Mexican commercial laboratories for mass-rearing parasitoids produce only *S. endius*, but this species likely is not the correct choice for release everywhere. *Spalangia nigroaenea* is reported to be one of the most effective parasitoids of the house fly, stable fly, and horn fly in studies from other regions. In our study, *S. nigroaenea* was 10 times more prevalent than *S. endius*, indicating that it is better adapted to the conditions in northern Tamaulipas and therefore may be more suitable for inundative releases in the region. Use of the native parasitoids of a region is an essential factor in increasing the probability of success in biological control.

The information gained from our study is an initial step in establishing more effective control of pest flies using parasitoids in IPM programs in northeastern Mexico. However, the effectiveness of each of the parasitoid species in controlling the different fly species in the region remains to be determined, as do the appropriate parasitoid release rates relative to fly species composition and population levels, if effective biological control is to be achieved. It is also suggested that recovered parasitoids may be reared for release in combination as they occur in nature.

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