EGG PARASITOIDS OF TABANIDS IN MANITOBA: PREVALENCE, TAXONOMY, BEHAVIOUR, AND USE OF POLYMERASE CHAIN REACTION-RESTRICTION FRAGMENT LENGTH POLYMORPHISM (PCR-RFLP) TO ESTABLISH HOST-PARASITE INTERRELATIONSHIPS

A Thesis Submitted to the Faculty of Graduate Studies

The University of Manitoba

by

Mahmood Iranpour

In Partial Fulfillment of the Requirements of the Degree of Doctor of Philosophy

Department of Entomology

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES
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MAHMOOD IRANPOUR

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

Doctor of Philosopy

Dedicated to my wife,

Shahin

and our children,

Arman and Rosa

for their much tried patience.

And to my parents

for their support.

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ABSTRACT

Tabanid egg masses were collected from different locations in Manitoba (Canada) during the summers of 1996-2000. Two scelionid parasitoids (*Telenomus* species A and B) were reared from horse fly egg masses (*Hybomitra* spp.) and another scelionid parasitoid (*Telenomus* species C) along with one trichogrammatid parasitoid (*Trichogramma semblidis* (Aurivillius)) were reared from deer fly egg masses (*Chrysops* spp.). The taxonomy and host-associations of ten *Telenomus* species recorded from eggs of Tabanidae in different geographical regions of the world were reviewed and type specimens of these species were compared with specimens reared in Manitoba. Based on characters on the metasoma, mating behaviour and host partitioning, three scelionid parasitoids reared from tabanid eggs in Manitoba were undescribed. These three species were described and an identification key for New World species (including new species from Manitoba, and one Palaearctic species) was provided.

The prevalence of tabanid egg parasitoids among and within the collected egg masses was evaluated. During 1996-1999, the range of prevalence of parasitism among the egg masses was from 87.5% (1999) to 93.8% (1998) for *Hybomitra nitidifrons nuda* (McDunnough) egg masses and from 72.4% (1996) to 94.1% (1998) for *Chrysops aestuans* Van der Wulp egg masses. Prevalence of parasitoids within egg masses was evaluated using the chorion of individual eggs. In multi-layered egg masses laid by *H. n. nuda*, the range of parasitism was from 28.2% to 37.3%. Approximately 36% of eggs were also damaged which produced neither parasitoids nor hosts. In single-layered egg masses laid by *C. aestuans*, prevalence of parasitism within egg masses was 40% to 55% with approximately 21% damaged eggs. Co-existence of *Telenomus* species A and B in more than 85% of collected egg masses of *Hybomitra* spp. and *Telenomus* species C and

T. simblidis in 79% of collected egg masses of *Chrysosp* spp. was an indication of intense competition between parasitoids in their associated hosts. Host specificity, host finding strategies, host partitioning and emergence patterns of parasitoids, mating and oviposition behaviours, acceptance of stored and frozen tabanid egg masses by parasitoids, parasitoid development inside the host, and overwintering strategies of tabanid egg parasitoids were studied.

Telenomus species A, B, and C were arrested by the extracts from fresh egg masses and tips of female abdomens of some species of tabanids. Laboratory experiments and direct observations revealed that these egg parasitoids were not highly host specific. They might attack species other than their primary hosts in the same genus or even other genera in the family Tabanidae.

Materials extracted from primary hosts and from cattail, *Typha* sp. (the main plant used by tabanids as oviposition sites), did not attract parasitoids from a distance. However, parasitoids were able to detect by direct contact the kairomones extracted from their host. After contact, parasitoids were arrested by extracts for 3 to > 600 seconds depending on host species and concentration of the extract. In this experiment, parasitoids were rarely arrested by extracts from species of a genus other than their primary hosts.

In multi-layered egg masses, there was a fine level of host partitioning between *Telenomus* species A and B. The prevalence of species A and B in the top layers of an egg mass was not statistically different. However, in the centre of egg masses laid by *Hybomitra* spp. occurrence of species A with its longer metasoma was significantly higher than that of species B with its shorter metasoma.

Male scelionids emerged first and emergence of females took four days under laboratory conditions. Males competed with each other to mate with newly emerged

females. The successful male mated with a female soon after her emergence. For species A and B, preparation for mating took 15-20 minutes and mating lasted 30 seconds. Under laboratory conditions, females were ready to lay eggs 24 h after mating and tabanid egg masses that were newly laid or up to three days old were acceptable for parasitoids. In the field, I frequently observed that one to six parasitoids attacked tabanid eggs while the female tabanid was laying eggs. Females of species A and B survived for seven to eight days after emergence in the laboratory when given 5% sucrose.

Unparasitized egg masses that were incubated at 25, 20, and 15°C were accepted by *Telenomus* species A and B for up to three, four, and 10 days after incubation, respectively. However, parasitoids would not parasitize previously frozen egg masses. Under field conditions, development of *Telenomus* species A and B took 18 days from egg to adult. However, they completed their life cycle in 11 days under laboratory conditions (25°C, 50% RH, and 18h:6h light:dark intervals).

To study host-parasitoid interactions, it was necessary to identify the tabanid egg masses. Rearing and a molecular technique were examined to identify tabanid egg masses. The molecular method was a fast and accurate technique to associate adults of tabanids with their egg masses. Polymerase chain reaction (PCR) and subsequent restriction fragment polymorphism (RFLP) analysis were used to differentiate 35 species of adult horse flies and deer flies (Diptera: Tabanidae) representing five genera [*Atylotus* (1 sp.), *Chrysops* (11 spp.), *Haematopota* (1 sp.), *Hybomitra* (17 spp.), and *Tabanus* (5 spp.)] from Manitoba. Initial PCR-RFLP analysis using amplicons of two mitochondrial tRNA genes and the internal transcribed spacer (ITS) of nuclear ribosomal DNA (rDNA) could be used to distinguish only a limited number of species from others in the collection. However, PCR-RFLP analysis using the rDNA intergenic spacer (IGS)

between the 28S and 18S rRNA genes generated restriction fragment profiles that could be used to differentiate all 35 species from one another. There was a small degree of intraspecific variation among fragment patterns observed when multiple individuals of a species were examined. The rDNA IGS therefore provided a target sequence with sufficient variation to identify individual tabanids to the species level. Then using molecular markers, 56 egg masses of horse flies and deer flies collected in Manitoba were matched with their associated adults. Descriptions of egg masses of five species (*H. n. nuda*, *Hybomitra lasiophthalma* (Macquart), *Chrysops mitis* Osten Sacken, *C. aestuans*, and *Chrysops excitans* Walker) of tabanids along with colour pictures are provided.

FOREWORD

This thesis is written in paper style, with a review of pertinent literature necessary for understanding the overall body of research contained in Chapter II. Individual manuscripts (Chapters III-VII) are prepared as independent submissions to scientific journals. Chapter III has been submitted to The Canadian Entomologist. Chapter IV has been submitted to The Journal of Medical Entomology. Chapter VIII contains an general discussion of the research contained herein.

CHAPTER I

GENERAL INTRODUCTION

The family Tabanidae originated in the mid-Mesozoic (approximately 180 million years ago) and diversified with the mammals, their primary hosts (Downes 1971). Through female blood-feeding, tabanids are capable of inflicting direct injury, causing blood loss, and transmitting pathogens, with the potential for spreading more than 30 disease agents, including viruses, bacteria, protozoa, and helminths to their hosts (Krinsky 1976). Some species bite humans, but only a few are biological vectors of human pathogens, e.g. *Chrysops silaceus* Austen which transmits loiasis. As mechanical vectors of pathogens, the flies are primarily of veterinary importance, but sometimes they can transmit disease pathogens to humans, including anthrax, tularaemia, and possibly Lyme disease (Chainey 1993). In addition, they cause losses to livestock producers and dairymen by reducing weight gains and milk production. Some species readily attack humans and may be extremely annoying in recreational and work areas (Anthony 1962).

Tabanidae are present in temperate and tropical regions of the world. In certain areas of North America, Tabanidae are among the most important pests of livestock and humans. Generally, they are called horse flies, but several other names are used, including gad flies, stouts, elephant flies, buffalo flies, moose flies, bulldogs, clegs, deer flies, and greenheads for Nearctic species of salt marsh *Tabanus* (Chainey 1993; McKeever and French 1997). Approximately 4,350 species have been described, including 335 species and subspecies in North America north of Mexico (Burger 1995). There are 144 described species distributed throughout Canada and Alaska, except in the

northern tundra zone where trees are absent (Teskey 1990). In Manitoba, there are 50 species in five genera: *Hybomitra*, *Tabanus*, *Chrysops*, *Atylotus*, and *Haematopota*.

Adult tabanids in North America are reasonably well known taxonomically. However, the systematics of the immature stages has not kept pace with the taxonomy based on adults. Descriptions of larvae are available for many species, but detailed descriptions of tabanid egg masses are sketchy and anecdotal, with descriptions scattered throughout the literature (Teskey 1990). In the United States, descriptions of larvae and pupae have been published for some species of tabanids (Goodwin 1976; Pechuman 1981). Teskey (1969) provided descriptive details of the immature stages for about 20% of the described species in the Nearctic region as well as descriptions of and keys to the larvae and pupae for 81 of 144 Tabanidae species found in Canada. Teskey (1969) suggested that the keys should be used with caution because information for many species and the range of variation to be expected in some species were not available. Even if complete keys for the immature stages of all species in Canada were available, it would still be necessary to rear larvae to the third or fourth instar for accurate identification.

Despite their great diversity and importance, tabanids have received inadequate attention. In the late 1980s, worldwide active research programs on tabanids included 50 programs in systematics and distribution, 15 in economic entomology and 5 in disease transmission (Foil 1989). Specific characters in their life history and behaviour make them a relatively difficult group to study. Difficulties in rearing have also impeded the study of Tabanidae under laboratory conditions. The major obstacles to studying tabanids have been caused by their prolonged life cycle (1 to 3 years) (Pechuman 1981); by the

reluctance of adults to accept blood through membranes; by their habit of indiscriminate oviposition in cages, often under conditions not conducive to maturation of the eggs; by the refusal of the larvae to feed or by their cannibalistic behaviour; by mortality resulting from physiological disorders during ecdysis and pupation and by disease throughout development; and by the inhibitory effect of cage retention reproduction (Thompson and Krauter 1978). Also for some species, it is difficult to determine to what degree they are characteristically selective in their oviposition sites. Most available information has been derived from observation rather than experimentation. Therefore, many aspects of tabanid life history as reported in the literature are confusing and contradictory (Foster et al. 1973).

Their powers of flight, prolonged emergence, soil-dwelling habitat of larvae near water for much of their lives, and their extensive breeding sites have made it difficult to manage tabanid populations. Control methods are divided into three categories: environmental modification and physical control, which provide a relatively permanent solution; chemical control, which is the most widely used method and usually causes temporary reduction in tabanid populations, and biological control, which rarely has been attempted, although a number of natural enemies prey on tabanids. Silhouette-type traps are often used to decrease the numbers of flies feeding on cattle, horses, and humans; however, their efficacy is not established and particularly with partially autogenous species, trapping will have minimal influence on subsequent generations, because they already will have laid at least one batch of eggs. Design modification of traps and addition of bait such as carbon dioxide provide some relief from persistent attacks and could be used as a component of integrated control programmes (Anderson 1985).

Some insecticides have been used against adults and larvae with equivocal results. The impracticality of treating large semiaquatic areas and the probability of effects on non-target organisms have limited the use of larvicides. Space sprays and direct applications of insecticides to domestic animals and vegetation against adults in turn have some difficulties. Space sprays and direct applications to animals and vegetation are effective for only a short time. Because of the forest canopy, it is difficult to get adequate spray coverage of forested areas and frequent penetration of forested areas is impractical. Failure to achieve long-term control with insecticides is attributed, in part, to female dispersal and their ability to reinfest previously sprayed areas quickly. A number of chemicals repel tabanids when applied, but they are short-lived in the field. Moreover, some formulations have caused skin and other disorders such as epidermal peeling, dermatitis, exfoliation, excessive salivation, nasal discharge, and blistering of skin in cattle, horses, and humans. The impregnation of clothing with repellents may be more effective than applications to skin. Impregnation of clothing with a quick knockdown insecticide is a more promising approach (Anderson 1985).

Tabanids are preyed upon by a variety of natural enemies. From the numerous descriptive and incidental accounts, there is a rich fauna and flora of predators, parasites, and pathogens as natural enemies of tabanids. However, there is no record of pathogenic viruses or rickettsiae of tabanids and the records for fungi, bacteria, and protozoa are sparse. Larvae have a thick protective cuticle and their predatory habit reduces the likelihood of their ingesting infective stages of pathogens in the soil, two factors which keep tabanids relatively disease free. Mermithid nematodes may be excellent biological control agents. However, for mass production, artificial media are needed which has not

yet been achieved for mermithids in general. Bacteria are rarely considered primary invaders of healthy insects. Therefore, their success depends on entering the haemolymph when the insect is in a weakened condition, then overpowering its defence system. In contrast to these potential pathogens, *Bacillus thuringiensis* Berliner is capable of initiating infection in healthy insects; however, tabanid larvae do not ingest *B. thuringiensis* spores from the soil because the larvae are predaceous.

The phycomycete fungi (*Coelomomyces*, *Entomophthora*, and *Tabanomyces*) isolated from tabanids are all obligate parasites and there is a variable degree of pathogenicity. However, methods have not yet been devised for their mass production on artificial media. Therefore, their use as biological control agents remains restricted until a method to mass rear tabanid larvae on artificial media is developed (Poinar 1985).

Egg parasitoids are frequently reported from different countries as one of the most efficient natural enemies of Tabanidae; however, no thorough study has been conducted of any species. *Trichogramma* and *Telenomus* are frequently reared, but the systematics of these parasitoids and their hosts are poorly known. Taxonomic descriptions and keys are needed for accurate identification. Dispersal capabilities, overwintering strategies, microhabitat preferences, and seasonal host succession of these parasitoids are unknown. There are no descriptions or keys for tabanid egg masses and individual eggs in the literature. Therefore, there is a great need for more detailed descriptions of egg masses and identification of their associated parasitoids. Anderson (1985) mentioned that a few tabanid parasitoids are not host specific and species such as *Trichogramma minutum* Riley and *Telenomus emersoni* (Girault) parasitize a variety of host species in the field.

He concluded that since some species had been reared for one or two generations in the laboratory, their potential colonization was not unlikely.

Although there are some reports on the occurrence of tabanid egg parasitoids in Canada 20 years ago or more, there is no detailed information about prevalence, capability, and biology of tabanid egg parasitoids. Therefore, it is important to study these beneficial parasitoids and their role in population dynamics of tabanids. In our preliminary survey in Manitoba in 1996, more than 90% of tabanid egg masses were parasitized by scelionid parasitoids at high prevalence of parasitism within the egg masses. Our initial objectives were to examine the behaviour of these parasitoids, to determine their impact on tabanid populations and to explore the possibilities of manipulating these parasitoids in an integrated pest management programme in Manitoba. In our first collections, which comprised *Trichogramma* and *Telenomus* spp., we believed that all *Telenomus* reared from tabanid egg masses were *T. emersoni*. However, as we gained more detailed information about the hosts attacked, and about the specific behaviour of the wasps, we went back to reexamine a greater number of specimens. Our specimens were compared with type specimens of known species in North America and with closely related Palaearctic species, and we determined that there were three new, ecologically distinct *Telenomus* spp. To study host parasitoid interactions, it became necessary to associate tabanid egg parasitoids with their specific hosts. Therefore, the objectives of the research project were expanded after the preliminary results were obtained. The objectives of this study were: 1) to identify parasitoids and their hosts in Manitoba; 2) to determine prevalence of parasitoids at

several locations in the field; 3) to obtain information on parasitoid biology and behaviour, host-parasitoid interactions, and host-finding strategies of the parasitoids.

CHAPTER II

REVIEW OF PERTINENT LITERATURE

Classification and geographical distribution of Tabanidae - Except for some oceanic islands, tabanids are distributed worldwide from temperate to tropical regions and from sea level to about 5000 metres above sea level. Based on cladistic analysis, it is believed that they evolved first in southern Gondawana and then radiated northwards (Chainey 1993).

Tabanidae have been divided into three subfamilies (Chvala et al. 1972). The subfamily Pangoniinae, containing the more primitive Tabanidae, is divided into four tribes: Pangoniini, Scionini, Philolichini, and Scepsidini. The Pangoniini is regarded as the more primitive of these tribes. The subfamily Chrysopsinae is divided into three tribes: Bouvieromyiini, Chrysopsini, and Rhinomyzini. The subfamily Tabaninae is divided into three tribes: Diachlorini, Haematopotini, and Tabanini. Chainey (1993) raised the tribe Scepsidini to a subfamily without any tribes.

In Canada, the subfamily Pangoniinae consists of two tribes, Pangoniini, with three genera (*Goniops*, *Stonemyia*, *Apatolestes*) and Scionini with one genus. There are three genera of Chrysopsinae, two genera belonging to the tribe Chrysopsini (*Silvius* and *Chrysops*) and one genus belonging to the tribe Bouvieromyiini (*Merycomyia*). There are four genera of Tabaninae in Canada, one genus in the tribe Haematopotini (*Haematopota*) and three genera in the tribe Tabanini (*Hybomitra*, *Atylotus*, and *Tabanus*).

Importance of Tabanidae - The economic importance of tabanids is well known because of their medical and veterinary importance, both by direct injury and by transmission of disease pathogens. Adult tabanids take a considerable quantity of blood and cause annoyance and irritation resulting in interruption of feeding (Pechuman 1981).

Some people feel the bite when a female tabanid tries to pierce the skin or probes deeply for an adequate flow of blood, and usually little swelling, reddening or irritation occurs after the bite. However, some people have a moderate reaction, which lasts from a few minutes to several hours. A few people respond with allergic reactions involving extensive swelling, erythema, itching, and related effects. This tabanid allergic reaction is primarily associated with salt marsh tabanids, particularly *Tabanus nigrovittatus* Macquart (green head). The salt marsh tabanid problem extends from Nova Scotia to Florida and along the Gulf Coast to eastern Texas. The activity of *Chrysops* species is usually restricted to smaller geographical areas than that of *Tabanus* spp. Deer flies move from the salt marsh into adjacent woods and other vegetation but not far into open fields. They are seldom a problem on beaches or on boats but may be more serious than *T. nigrovittatus* on golf courses, in camp grounds, parks, and along the wooded margins of cultivated fields. Species of *Chrysops* tend to feed on the head and arms and are much more attracted to a moving host than one standing still (Hansens 1979).

Tashiro and Schwardt (1953) reported that the average blood meal from a cow was 0.111 ml, 0.082 ml, and 0.340 ml for *Hybomitra lasiophtalma* (Macquart), *Tabanus quinquevittatus* Wiedemann, and *Tabanus sulcifrons* Macquart, respectively. They also found that in New York, the daily blood loss of one cow due to these species was 352 ml, 59 ml, and 109 ml, respectively. Since the blood does not immediately coagulate at the

feeding site, there is a loss in addition to that taken by the fly. Perich et al. (1986) found that heifers exposed to attacks by an average of 90 flies per animal per day for 84 days gained 0.08 kg per animal per day less than heifers protected from horse flies. Unprotected heifers were 16.9% less efficient in feed utilization than protected heifers. They also estimated more than $10 potential total loss per head per year in weight gain in heifers exposed to tabanids for 84 days. Losses to U. S. beef cattle production due to tabanid attacks were estimated to be $54 million in stocker cattle alone (Drummond 1987).

Adult tabanids are known as vectors of several pathogenic organisms of humans and animals, including viruses, bacteria, rickettsia or rickettsia-like organisms, trypanosomes, and filarial worms (Pechuman 1981). Most diseases agents are transmitted mechanically while tabanids are feeding upon their hosts. Because of the painfulness of tabanid bites, the host makes an effort to dislodge the fly. Then the dislodged fly returns to complete its feeding but may select a nearby host. The new host may then receive disease-causing organisms if the first one was infected.

Loa loa (Guyot) (loiasis), *Elaeophora schneideri* Wehr and Dikmans (elaeophorosis), and *Trypanosoma theileri* Laveran are known to be biologically transmitted by tabanids (Pechuman 1981). Anthony (1962) believed that the relatively large size of the mouth parts and biting habits of tabanids potentially enable them to transmit pathogens to humans and animals. He mentioned that anthrax (in livestock), tularaemia (in humans and rabbits), loiasis (in humans), anaplasmosis (in cattle), trypanosomiasis (in horses, camels, dogs, donkeys, cattle, mules, sheep, and goats), equine infectious anaemia (in horses, mules, and donkeys), and vesicular stomatitis (in

horses, cattle, and swine) have been transmitted by adult tabanids under field and/or laboratory conditions. Krinsky (1976) published a review article on animal disease agents transmitted by horse flies and deer flies. He mentioned 31 different disease agents, categorized in four groups (viruses, bacteria, protozoa, and helminths) which were transmitted mechanically or biologically under laboratory or field conditions. He believed that tabanids exhibited various adaptations related to blood-feeding which increase the probability of transmission of disease agents from one animal to another. Some of these adaptations are the anautogenic habit in females, telmophagy (cutting through the skin to feed from a pool of blood), imbibing a large blood meal, long engorgement time, and interrupted feeding.

Foil (1989) mentioned that *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt & Brenner the casual agent of Lyme disease, was found in four *Chrysops* spp., three *Hybomitra* spp., two *Tabanus* spp., and two mosquitoes collected in an area where *Ixodes scapularis* Say was present. He believed that the range of Lyme disease had expanded in the USA outside the range of *I. scapularis* and epidemiological studies should be designed to examine the possibility of transmission by tabanids.

Tabanid Biology

Hovering and Mating - It is believed that swarming flight and hovering are of significance in the mating of certain members of the family Tabanidae (Bailey 1948). Males of *Tabanus* species usually gather at dawn and dusk around a landmark, where they hover in a fairly stationary group. Landmarks vary, but the common feature is relatively open and free of obstruction. Forest-meadow margins, roads, shrubs, and a

driveway are some reported landmarks (Gaugler and Schutz 1989; Bailey 1948). The mating system of *Hybomitra arpadi* (Szilady) was studied at a subarctic, treeline site near Churchill, Manitoba by Smith et al. (1994). They found that males aggregated in a linear clearing on a hilltop in spruce-larch forest, and hover at a height of 13 cm. They also reported that hovering occurred only on sunny days with the temperature more than 12° C for periods of up to 11 h.

Flight - Tabanids are strong fliers; however, some environmental factors influence their flight activities. Miller (1951) reported that maximum adult activities occurred at 20-22.8° C, a water saturation deficit of 9-12 mmHg, and light intensity of 6000-7500 lumens per 0.093 m^2. Meteorological factors such as barometric pressure, temperature, evaporation, total sky radiation, wind velocity, and relative humidity influenced flight activity of female tabanids (Burnett and Hays 1974). Raymond (1979) found that the reactions of various species to maximum temperature were different and that optimum temperature for *Hybomitra* spp. was lower than that for other genera. Amano (1985) found that radiation, relative humidity, and wind velocity were decisive factors affecting flight activity; however, the importance of each factor, varied according to species. He indicated that wind velocity had primarily a mechanical action in inhibiting flight activity; temperature was of little importance, except in limiting initiation of flight activity; solar radiation had a strong influence; relative humidity was a flight depressant for tabanids. McElligott and Galloway (1991b) reported that the morning onset of tabanid activity was related to temperature, but the cessation of activity in the evening was affected by either temperature or light. They observed low flight activity at temperatures

below 20°C and at light intensity levels below 1000 lumens/m^2 regardless of temperature. Tabanids are known to be strong fliers. Hocking (1953) calculated that with energy available from a full crop of nectar, *Hybomitra affinis* (Kirby) could fly 100 km. Sheppard and Wilson (1976) studied flight range of Tabanidae in a Louisiana bottom land forest with marked adults. They used Fire-Orange and Signal-Green in self-marking devices. They recaptured marked female tabanids uniformly from 0.8 to at least 6.8 km from release points.

Temporal and Daily Distribution - Golini and Wright (1978) reported the flight period of nearly 85% of the sampled population of Tabanidae near Guelph, Ontario, consisting mainly of *Chrysops frigidus* Osten Sacken, *H. lasiophthalma*, and *Hybomitra epistates* (Osten Sacken), occurred from the end of May until mid-July, whereas, *T. quinquevittatus*, *Tabanus lineola* Fabricius, and *H. epistates* were active till mid-September. Baribeau and Maire (1983) collected 43 species of tabanids in a subarctic locality of Quebec. *Chrysops aestuans* Van der Wulp was the dominant species, followed by *Hybomitra pechumani* Teskey and Thomas, *H. lasiophthalma*, *C. frigidus*, and *Hybomitra zonalis* (Kirby). The flight period began in mid-May with the greatest abundance from mid-June to mid-July. McElligott and Galloway (1991a) collected 20 tabanid species in three genera using Manitoba horse fly traps in Manitoba. They found that *Hybomitra lurida* (Fallén) and *Hybomitra nitidifrons nuda* (McDunnough) reached maximum abundance in late May to early June; *Hybomitra illota* (Osten Sacken) and *H. lasiophthalma* in early June; *H. affinis*, *H. arpadi*, and *H. zonalis* in mid-June; and *H. epistates* and *H. pechumani* in late June to early July. *Hybomitra trepida* (McDunnough)

peaked twice in late June and early August. They also reported that the greatest tabanid abundance and diversity occurred during June and few flies were on the wing at the study area after mid-July. McElligott and Galloway (1991b) studied daily activity patterns of horse flies in northern and southern Manitoba. *Hybomitra epistates* and *H. pechumani* were most active during late morning or mid day; *H. arpadi*, and *H. zonalis* in early afternoon; *H. affinis*, *H. illota*, and *H. lasiophthalma* in late afternoon; and *H. n. nuda* and *H. lurida* in early evening.

Feeding Behaviour - For the production of eggs, a blood meal is needed by most tabanid species as a protein source. Some species are autogenous and develop at least the first egg batch without blood and some species are non-haematophagous. Both females and males feed on nectar at flowers, and may be found at sap exuding from a wounded tree or at aphid honeydew. Adults have been observed to visit pools and drink while in flight (Chainey 1993; Bosler and Hansens 1974; Stoffolano 1983). Using the Cold Anthrone method, Kniepert (1980) analysed the diet of 7002 females and 2436 males. He found that 53% of the females and 69% of the males had fed on nectar. Hayakawa (1986) found that about 93% of all collected specimens (females of 70 species and males of 14 species) and 20-30% of flies in most species had pollen on their bodies.

Sheppard and Wilson (1977) studied the relationship of horse fly host-seeking activity to the edges of wooded areas in southern Louisiana. They found that host-seeking activity was significantly greater in open areas within 130 m of woods than inside woods within 15-18 m of the edge or 237 m or greater from the wood's edge in the open. Colour, movement, odour, carbon dioxide emission and body heat are the most

important stimuli which tabanids use to locate their hosts. Vale and Phelps (1974) believed that the odour of tabanid hosts induced anemotaxis from a distance and visual stimuli assisted final orientation. Carbon dioxide is a known attractant to female tabanids; however, each tabanid species has a definite individual response to CO_2 (Roberts 1971). McElligott and McIver (1987) found that CO_2 was operative for host seeking females of *Hybomitra* spp. over a short to moderate distance from the host; however, in general, visual cues were the most important long-range attractant for tabanids. For 17 tabanid species, Magnarelli and Anderson (1980) determined preferred feeding sites on cattle. More than 82% of 628 females of *Chrysops* spp. fed on the head region; however, the sides and backs of animals were generally attractive to female *Hybomitra* spp. and *Tabanus* spp. They captured 2559 vertebrate host-seeking tabanids in woodlands and pastures. Of these 251, (10%) contained partial blood meals in the midgut. Reactions to antisera showed that engorged tabanids had fed upon bovine (57%), horse (14%), dog (9%), rabbit (8%), bovine-deer (7%), deer (4%), and an unidentified mammal (1%).

Oviposition - Tabanids lay eggs in compact masses approximately 4-8 days after a blood meal, depending on temperature. They lay eggs on warm sunny days, usually from 0900 to 1700 h, with peak activity from 1200 to 1300 h. Egg masses are laid on the stems of plants or under surfaces of leaves overhanging water or wet soil (Teskey 1990). Egg masses are found occasionally in very dry situations. Arrangement, shape, and dimension of egg masses and numbers of eggs in the mass are considerably different in some species. The eggs are laid in moderately uniform rows, each egg glued to the adjacent

ones and to the substrate at an angle of 15 to 90 degrees. Most *Chrysops* lay only single-layered egg masses, whereas others of this genus and most species of other genera deposit egg masses with two to four tiers. Fresh egg masses are rather pallid in colour but soon darken to brown or blackish. Usually eggs are securely glued together; however, eggs of some species are held together loosely. Each egg mass contains 100 to 800 eggs, the larger numbers being from some species of *Tabanus* and *Hybomitra*. Eggs hatch within 4-6 days under normal conditions. All eggs in a mass hatch at the same time in the morning, after some warming by sun (Teskey 1990).

Many tabanids have been observed to produce their eggs autogenously. Under natural conditions, Rockel (1969) demonstrated autogeny of the American salt marsh deer fly, *Chrysops fuliginosus* Wiedemann. Anderson (1971) found that the salt marsh tabanids, *Chrysops atlanticus* Pechuman and *T. nigrovittatus*, were autogenous. McElligott and Galloway (1991b) studied parity of host-seeking tabanids in Manitoba. They found that for three species, parity increased rapidly over the flight season. In *H. lurida* and *H. n. nuda*, parity reached 100% within 14 and 17 days of their appearance respectively. However, parity of *H. trepida* increased to 100% within 28 days of its appearance and declined to 40% 14 days later, and returned to 100% after another two weeks.

Larva - Newly hatched larvae drop to the substrate which usually consists of water or wet soil and sink to the bottom or are carried by the current to the shoreline (Teskey 1990). The first instar larvae do not feed, but immediately after the first molt and following sclerotization of the mouthparts, the second instars begin feeding (Terterian

1985). Larvae of Tabaninae are carnivorous and the larval head capsule has a filtering apparatus that might strain food materials from the water. However, larvae of *Chrysops* spp. have never been observed to attack living invertebrates (Teskey 1990). Many species of Tabaninae are cannibalistic and/or predaceous on various soil invertebrates. Larvae of *Chrysops* spp. are thought to feed on vegetable material (Goodwin et al. 1985). But Burger (personal communications) believes that *Chrysops* spp. feed on small prey too.

Larvae of species of Tabaninae pass through 7-11 instars, whereas larvae of *Chrysops* spp. molt five to seven times. In middle latitudes, larvae take 9-10 months to develop. Some tropical and subtropical species may have two generations per year, and some far northern species may require two to three or more years to complete their life cycle (Teskey 1990). Also many of the very large species probably take at least two years to develop (Burger, personal communications). Pechuman (1981) reported that in New York, most tabanids have one generation a year. However, *T. lineola* and *Tabanus similis* Macquart may occasionally develop from egg to adult in one season. Some individuals of large species of *Tabanus* such as *Tabanus atratus* Fabricius may take 2-3 years to complete their development. In New York, as far as is known, all species overwinter as larvae. In the appropriate season, full grown larvae migrate to drier situations to pupate in a vertical position just below the surface of the substrate (Teskey 1990). Drees (1987) found that last instars of some Afrotropical *Tabanus* species constructed mud cylinders before the pupal stage to ensure pupal survival, where mud became dry and cracked during the dry season.

Tabanid larvae have been collected from almost all types of wetland habitats. Larvae of *Chrysops* spp. mostly were found on the margins of streams or ponds in a substrate containing a moderate to high amount of mineral soil ingredients (Teskey 1969). Whereas, those of Tabaninae were collected more in swamps and marshes away from running water. For most species of *Hybomitra* and *Atylotus*, the substrates of their habitats are of organic origin. Larvae of *Tabanus* are frequently found in habitats which contain more mineral soil constituents. There are some exceptions to the above generalization. Larvae of *C. frigidus*, *Chrysops nigripes* Zetterstedt, and *Chrysops excitans* Walker were usually found in habitats typical of *Hybomitra* spp. Larvae of *Tabanus reinwardtii* Wiedemann and *Tabanus marginalis* Fabricius were collected from the banks of streams and in lentic habitats (Teskey 1969). Some species have rather restricted habitats. Larvae of *Tabanus fairchildi* Stone and *Hybomitra criddlei* (Brooks) live among stones and gravel in the bed of fast moving streams. Other species such as *Hybomitra minuscula* (Hine), *H. trepida*, and *Hybomitra itasca* Philip, apparently have larvae adapted to acidic bog-like conditions. Those of *T. marginalis* and *Chrysops vittatus* Wiedemann were collected in almost all types of wetlands and larvae of *T. nigrovittatus*, and *C. fuliginosus* live in salt marshes. Larvae of *T. quinquevittatus* were collected in relatively drier conditions than most tabanids (Teskey 1969). Burger et al. (1981) studied the larval habitats and reared some common *Chrysops* species in New Hampshire. They stated that of 60 *Chrysops* species whose larval stages were known, for only 55 species were detailed descriptions of the larval habitat available. They found that the greatest diversity of *Chrysops* species occurred predominantly in lentic habitats such as ponds, lakes, ditches, and in bogs or swamps. Thirty five (65%) species and 10 (18%)

species were entirely or predominantly lentic and lotic respectively. Seven species (13%) were collected from both lentic and lotic habitats.

Pupa – Pupation occurs in the larval habitats and the pupal stage lasts one to three weeks, depending on the temperature and species. Adults emerge in the morning, and after a short rest, expanding their wings, and harden the cuticle, they fly (Teskey 1990).

Molecular systematics of Tabanidae

Hudson and Teskey (1976) used isozyme electrophoresis of esterase enzymes, in addition to differences in size and colour of characters, to segregate two forms of *Hybomitra typhus* (Whitney). Jacobson et al. (1981) distinguished two genetically distinct populations of *T. nigrovittatus* by starch gel electrophoresis of enzymes. The analysis of cuticular hydrocarbon profiles has been used to differentiate morphologically similar *Tabanus* species (Hoppe et al. 1990) and to compare three cryptic species in the *T. nigrovittatus* complex (Sutton and Carlson 1997). However, cuticular hydrocarbons may vary with the stage, age, and sex of insects (Pomonis and Mackley 1985; Pomonis 1989) and therefore may not be suitable for associating different stages of the same species. In comparison to hydrocarbons and proteins, DNA is more stable and does not vary in different developmental stages (Taylor et al. 1996).

Natural Enemies

Eggs, larvae, pupae, and adults of tabanids are preyed upon by a variety of natural enemies. Eggs are attacked by hymenopterous parasitoids, various insect predators, and

fungi. Larvae and pupae are preyed upon and parasitized by vertebrates and invertebrates such as Diptera, Hymenoptera, and Nematoda. They are also infected by protozoan and fungal pathogens. Adult tabanids are attacked by a variety of vertebrate and invertebrate natural enemies.

Natural Enemies of Eggs

Parasitoids - Hymenopterous parasitoids have frequently been reported from eggs by many tabanid workers. Scelionid species have been reported as egg parasitoids since 1916. Girault (1916) published the first paper describing *Phanurus emersoni* (= *T. emersoni*). He distinguished this species from its three other allied species from the United States. Parman (1928) worked on parasitism and biological aspects of *T. emersoni* in Texas. He reported 97% parasitism in collected egg masses of *Tabanus hyalinipennis* Hine (=*Tabanus dorsifer* Walker) from which 6 to 83 parasitoids emerged. Parman (1928) mentioned that the adult parasitoids mated as soon as they emerged and oviposition began from 4 to 24 hours after emergence. Fresh eggs were preferred by females and they never parasitized eggs more than five to six hours old. One to eight females were observed ovipositing on the same egg mass at the same time. Average parasitism was 60% in each single egg mass, with the highest being 93%. He also mentioned that from three masses which were attacked by a collected female, 114 adults emerged. The males died within a few hours after mating and females died soon after oviposition. The majority of adults did not live longer than two days in the breeding jars and none for more than five days. He concluded that parasitoids had destroyed 50% or more of the tabanid eggs during the most favourable seasons, and 10% or less in the most

unfavourable seasons. Webb and Wells (1924) reported an average of 60% of parasitism in eggs of *T. dorsifer* by *T. emersoni* in United States. In Saskachevan, Cameron (1926) reported up to 30% parasitism of *C. aestuans* and *Chrysops mitis* Osten Sacken by *T. emersoni*. Philip (1931) stated that up to 12% of deer fly eggs were parasitized by *T. emersoni* in Minnesota. Schwardt (1936) reported heavy parasitism of egg masses of *Tabanus vivax* Osten Sacken by *T. emersoni* in Arkansas. Hatton (1948) reported that 11 of 14 tabanid egg masses were parasitized by *T. emersoni* in British Columbia. In southern Ontario, James (1963) collected 54 egg masses of *C. aestuans*. Of those, 57.4% were parasitized by *T. emersoni* and *T. minutum*. The mean number of parasitized eggs per mass was 17.8 (6.5%). Parasitism of *Tabanus* spp. by *T. emersoni* has also been reported by different researchers from Louisiana, Alabama, New Jersey, and Nevada (MacCreary 1940; Jones and Anthony 1964; Jackson and Wilson 1966; Orminati and Hansens 1974; Middlekauff and Lane 1980).

Mayr (1877) from Germany, Olsuf'ev (1935) from Russia, and Nikol'skaya (1948) from Austria reported up to 30% parasitism of *Tabanus* egg masses by *Telenomus angustatus* Thomson (=*Telenomus tabani* Mayr). Skufin (1949) cited in Anderson (1985) reported parasitism of *Chrysops relictus* Meigen by *T. angustatus* from Russia. Rastegaeva (1965) reported 40% parasitism in tabanid eggs by *T. angustatus* from Russia. Kozlov (1967) and Auroi (1981) reported up to 15% parasitism in egg masses of *Hybomitra bimaculata* (Macquart) by *T. angustatus* in Switzerland. Patton and Cragg (1913) reported emergence of *Telenomus benefactor* Crawford from tabanid egg masses in Sudan. Nikol'skaya (1948) also reported parasitism of *Tabanus biguttatus* Wiedemann egg masses by *T. benefactor* in Africa. Crawford (1913), Schwardt (1936), Tashiro and

Schwardt (1953), and Bailey (1948) reported up to 100% parasitism of *Goniops chrysocoma* (Osten Sacken) and *H. lasiophthalma* eggs by *Telenomus goniopis* Crawford form Maryland, Arkansas, New York and Massachusetts. Marchand (1920) and Nikol'skaya (1948) reported parasitism of *Tabanus kingi* Austen egg masses by *Telenomus kingi* Crawford from Sudan. Egg parasitism of *H. bimaculata* (Auroi 1981) and *Tabanus* sp. (Nikol'skaya 1948) by *Telenomus oophagus* Mayr were reported from Switzerland and Central Asia. Goodwin (1976) reported more than 50% parasitism of *Merycomyia whitneyi* (Johnson) by *Telenomus tabanivorus* Ashmead in South Carolina, also Drees (1982) reported parasitism of *H. lasiophthalma* eggs by the same parasitoid from Ohio. Up to 100% parasitism of *Tabanus atratus* Fabricius eggs by *T. tabanivorus* was reported by Hart (1895), Ashmead (1895), Hine (1903, 1907), Muesebeck (1979), Tashiro and Schwardt (1953), Jones (1953), and Jones and Anthony (1964) from Massachusetts, south to Florida, west to Illinois. Jackson and Wilson (1966) worked on parasitism of tabanid eggs in four areas of southern Louisiana. They collected *Telenomus tabanivorous* only from eggs of large *Tabanus* species such as *T. atratus* and *Tabanus stygius* Say. However, *Telenomus emersoni* was collected from eggs of small *Tabanus* species such as *T. lineola*. They also reared an undescribed species of *Telenomus* from eggs of small species of *Tabanus* and eggs of *Chrysops* spp. They reported that *T. tabanivorus* parasitized 0 to 100% of each egg mass of *T. stygius* Say in Louisiana (Jackson and Wilson 1966), 0 to 51% of each egg mass of *Tabanus* sp. in Alabama (Dukes and Hays 1971), and 0 to 53% of each egg mass of *Chrysops* sp. in Alabama. Medvedev et al. (1983) reported parasitism of tabanid egg masses by *Telenomus inclinis* Le from Vietnam. Also 0 to 100% of tabanid egg masses were parasitized by scelionids

and trichogrammatids reported from USA (Herms 1927; Jackson and Wilson 1966; McAtee 1911; Roberts and Dicke 1964; Jones and Anthony 1964; Middlekauff and Lane 1980; Hays 1960), India (Fletcher 1922; Isaac 1924; Basu et al. 1954), Sudan (Kings 1910), and Java (Nieschulz 1927). James and Hays (1971) studied seasonal distribution and parasitism of tabanid eggs in Alabama. In their study, the seasonal average hatch and parasitism were 54.1% and 14%, respectively with almost 32% of the eggs which producing neither larvae nor parasitoids. They also mentioned that parasitoids attacked fresh tabanid egg masses, sometimes while the female tabanid was laying eggs.

Nikol'skaya (1948) described the adults of both sexes of *T. oophagus* Mayr which was reared from the eggs of *Tabanus* spp. in the Republic of Karakalpak, south of the Aral Sea in central Asia. He presented a key to this species and the four other species of *Telenomus* that parasitize the eggs of Tabanidae. These were *T. benefactor* from *T. taeniola* Palisot de Beauvois in the Anglo-Egyptian Sudan and *T. biguttatus* in Nyasaland, *T. kingi* from *T. kingi* in Sudan, *T. tabanivorus* from *T. atratus* in the United States and *T. tabani* from *Tabanus* sp. in Germany, Australia, European Russia and Karakalpak.

Trichogrammatids are the second most important group of tabanid egg parasitoids reported by many investigators. Parasitism of *Chrysops* sp. eggs by *T. minutum* was reported by Drees (1982) from Ohio, Cameron (1926) from Saskatchewan, James (1963) from Ontario, and Philip (1931) from Minnesota reported up to 36% parasitism of *C. aestuans* by *T. minutum*. Cameron (1926) found that 4 to 36% of *C. mitis* eggs were parasitized by *T. minutum*. Martin (1927) stated that *T. minutum* was known to parasitize over one hundred and fifty hosts in the orders Lepidoptera, Coleoptera, Hymenoptera,

Megaloptera, Neuroptera, Diptera, and Hemiptera. However, Lepidoptera were the favoured hosts. Martin reported *Sialis infumata* Newn., *Chrysops striatus* Osten Sacken, *C. excitans*, and *H. lasiophthalma* as new hosts for *T. minutum*. He found that only one larva was able to develop to maturity in the eggs of *Sialis* and *Chrysops*; however, *Tabanus* eggs usually had enough food for three parasitoids. He believed that the parasitoid probably overwintered in the egg, larval or pupal stage in some lepidopterous eggs. Fattig (1946) and Martin (1927) reported parasitism of *Chrysops univittatus* Macquart by *T. minutum* from Georgia and *H. lasiophthalma* from Michigan. James (1963) found that 6% of egg masses laid by *Hybomitra* and *Tabanus* were parasitized by *T. minutum* in Ontario. However, it is possible that some of *Trichogramma* specimens were mistakenly identified as *T. minutum*. In New York, Tashiro and Schwardt (1953) reported 78% parasitism of deer flies egg masses by *Trichogramma semblidis* (Aurivillius), which destroyed the majority of eggs. Nagarkatti and Jeyasingh (1974) reared *T. semblidis* from *Tabanus macer* Bigot (=*Tabanus dorsilineus* Wiedemann) in India. They used the eggs of rice moth, *Corcyra cephalonica* Stainton, as a host for laboratory studies of *T. semblidis*. The parasitoid readily accepted rice moth eggs and development was satisfactory. They believed that parasitoid ability to attack other lepidopterous hosts would probably be an advantage when tabanid eggs were scarce. They reported that *T. semblidis* parasitized 34.5% of eggs laid by *T. macer* in their study area. Olsuf'ev (1935) found that *Trichogramma evanescens* Westwood parasitized up to 30% of eggs laid by *Chrysops caecutiens* (Linnaeus) in Russia. Skuf'in (1949) reported parasitism of *C. relictus* egg masses by *T. evanescens* in Russia.

Rastegaeva (1965) studied the role of hymenopterous parasitoids in the reduction of the number of horse flies in the Omsk region of western Siberia, Russia. She believed that scelionid and trichogrammatid parasitoids caused considerable mortality of the tabanid eggs. She found that nearly 40% of eggs were parasitized by *T. tabani* and *T. evanescens*, but 60% were destroyed, as the female parasitoids punctured more eggs than they actually used for oviposition. Menon (1957) reported parasitism of *Atylotus* sp. egg masses by *Centrodora* sp. (Hymenoptera: Encyrtidae) in India. Parasitism of egg masses of *Chrysops* sp., *Tabanus rubidus* Wiedemann, *Tabanus striatus* Fabricius and *T. taeniola* by *Lathromeris* sp. (Hymenoptera: Chalcidoidea) was reported by Kryger (1904) from Denmark, Patton and Cragg (1913) from India, Patton and Evans (1929) from India and King (1910) from Africa, respectively. Pechuman (1981) found that approximately 50% of collected egg masses had some degree of parasitism. He believed that parasitism must have some effect on tabanid populations in New York. Barrion and Litsinger (1984) collected *Tabanus* egg masses from rice, maize and coconut fields in 14 Philippine provinces. They observed 85% parasitism in 94 collected egg masses of *Tabanus* spp. by *Telenomus dignus* Gahan. They mentioned that *Tabanus* spp. and the rice stem borers, *Scirpophaga incertulas* (Walker) and *Scirpophaga innotata* (Walker) (Lepidoptera: Pyralidae) oviposit on the tops of leaves where *T. dignus* attack both, although *Tabanus* eggs were larger and more slender than those of the rice stem borer species. They believed that *Tabanus* eggs could maintain *T. dignus* in fields when populations of rice stem borer species were low.

Philip (1931) found that a species of *Anaphoidea* (Hymenoptera: Mymaridae) parasitized the eggs of *C. aestuans* in Minnesota, but considered its occurrence less

common than that of *T. emersoni* and *T. semblidis*.

Predators - Predation of fresh tabanid eggs by insects belonging to the Lepidoptera, Diptera, Coleoptera, and Orthoptera was reported by Schwardt (1936), Jackson and Wilson (1965), Johnson and Hays (1973), Orminati and Hansens (1974), Middlekauff and Lane (1980), Pechuman (1981), and Magnarelli et al. (1982).

Jackson and Wilson (1965) reported some predators of horse fly and deer fly eggs in Louisiana. They observed *Orchelimum vulgare* Harris (Orthoptera: Tettigoniidae) feeding on eggs of *Tabanus* spp. and *Chrysops* spp., *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae) feeding on *Chrysops* spp. eggs, and *Collops bipunctatus* Say (Coleoptera: Melyridae) feeding on *Tabanus* spp. eggs. During this study, they found that numerically, the tettigoniid appeared to be more important as a predator in limiting the tabanid population than the two beetle species in the study area. Under lab conditions, eggs of *Tabanus lineola*, *T. nigrovittatus*, and *Tabanus fuscicostatus* Hine were exposed to two adult coccinellids, *C. maculata* and *Hippodamia convergens* Guerin-Meneville. All six egg masses offered to *C. maculata* were consumed within 24 hrs. *Hippodamia convergens* fed on all tabanid eggs offered, but not so readily as *C. maculata*. Only 1/2 to 3/4 of the four egg masses were consumed by *H. convergens* after 72 hrs. Jackson and Wilson (1965) observed that *C. bipunctatus* fed slowly on the *Tabanus* eggs for 10 min. during a 30-min. observation period.

Johnson and Hays (1973) studied some predators of immature tabanids in Alabama. They evaluated natural predation on tabanid egg masses in the field from May through July in 1969 and 1970. They found that daily predation ranged from 0 to 5.96%

of the egg masses examined. Based on the percentage eaten and numbers of egg masses preyed upon, predation per mass was 1.86% and 2.35% on average in 1969 and 1970, respectively. They examined 1646 and 2178 egg masses in 1969 and 1970, and of these, 19 (1.2%) and 32 (1.5%) egg masses were preyed upon, respectively. They believed that egg predation was not a significant factor controlling the population size of tabanids under natural conditions. Johnson and Hays (1973) observed larvae and adults of *C. maculata*, larvae of *Nola sorghiella* Riley (Lepidoptera: Nolidae), and adults of *Sepedon* sp. (Diptera: Sciomyzidae) feeding on *Chrysops* spp. egg masses.

Fungi- *Aspergillus*, *Beauveria*, and *Fusarium* were cultured from eggs of *C. relictus* (Koval and Andreeva 1971). Fungal infections of eggs of *C. relictus* (Koval and Andreeva 1971) and *Tabanus autumnalis* Linnaeus (Andreeva 1972) by *Aspergillus flavus* Link have been reported from Russia. Infection of *C. relictus* eggs by *Beauveria densa* (Link and Picard) was also reported by Koval and Andreeva (1971) and Andreeva (1972) from Russia.

Natural Enemies of Larvae and Pupae

Parasitoids – A small number of species of Bombyliidae and Tachinidae (Diptera), and Diapriidae and Pteromalidae (Hymenoptera) parasitize larvae and pupae of tabanids (Anderson 1985).

Villa lateralis (Say) (Bombyliidae) emerged from two larvae of *Tabanus annulatus* Macquart (= *H. lasiophthalma*) which were collected in Louisiana (Jones and Bradley 1923). Larvae of both *H. lasiophthalma* and *Hybomitra typhus* (Whitney) which

were collected in woodland pool habitats in Ontario were parasitized by *V. lateralis* (Teskey 1969). Parasitism of *Leucotabanus annulatus* (Say) larvae by *V. lateralis* was also reported from Mexico (Painter and Painter 1965), and by *Villa* sp. from Florida (Goodwin, 1976). In southern Ontario, 3 of 13 larvae of *Hybomitra trispila* (Wiedemann) were parasitized by *Carinosillus novaeangliae* (West) (Tachinidae) (James 1963). In the USA, Sabrosky and Arnaud (1965) reported parasitism of *H. trispila* by *C. novaeangliae*. Philip (1931), Hays (1958), Sabrosky and Arnaud (1965), Goodwin (1968), and Teskey, (1969) reported parasitism of larvae and pupae of five *Hybomitra* spp. and five *Tabanus* spp. by *Carinosillus tabanivorus* (Hall) (Tachinidae) from Ontario, Pennsylvania, Wisconsin, Michigan, and Tennessee. In Michigan, Hays (1958) found that 16% of the tabanid larvae collected in sphagnum bogs were parasitized by *C. tabanivorus*. He reported that the most frequent host was *H. trepida*; however, two other species of *Hybomitra* as well as one *Chrysops* larva were parasitized by *C. tabanivorus*. Jones and Anthony (1964) and Sabrosky and Arnaud (1965) reported parasitism of *Tabanus* sp. larvae by *Ormia punctata* Robineau-Desvoidy (Tachinidae). From the USA, Jones and Anthony (1964), Sabrosky and Arnaud (1965) and Goodwin (1968) reported emergence of *Phasiops flavus* Coquillett (Tachinidae) from pupae of *Tabanus petiolatus* Hine and *Tabanus trimaculatus* Palisot de Beauvois. Jones and Bradley (1923) reported larval parasitism of *T. trimaculatus* by *P. flavus* in Louisiana. Jones (1953) observed parasitism of *Tabanus* larvae by *P. flavus* in Florida. Goodwin (1968) reported parasitism of a *Chrysops* sp. pupa by fly larvae in Tennessee. One male of *Vibrissotheresia pechumani* Reinhard (Tachinidae) emerged from a larva of *T. reinwardtii* collected from the muddy bank of a small creek near Ithaca (Tashiro and

Schwardt 1953). Ashmead (1896), Cameron (1926), Philip (1931), Miller (1951), James (1952), James (1963), Teskey (1969), Burks (1979), and Burger et al. (1981) reported larval and pupal parasitism of six *Chrysops* spp. (up to 20.8%) and two *Hybomitra* spp. (up to 13.9%) by *Diglochis occidentalis* (Ashmead) (Pteromalidae). Cameron (1926) reared *D. occidentalis* from the pupae of *Chrysops mitis*, and *C. excitans*. Miller (1951) dissected parasitized pupae of *Tabanus* at Churchill, Manitoba. In dissected pupae he found numerous larvae of *D. occidentalis* which filled the body cavities to capacity. In one case, he observed that 90 chalcidoids emerged in the insectary from a single *Tabanus* pupa. James (1952) collected 15 larvae of *Tabanus* spp. and 38 larvae of *Chrysops* spp. at Churchill, Manitoba. He found 13.9% of *Tabanus* larvae and 20.8% of *Chrysops* larvae were parasitized by *D. occidentalis*. James (1952) believed that *D. occidentalis* was an important factor in the natural control of tabanids in his study area. In one case, he observed 110 adults of *D. occidentalis* to emerge from one pupa. The average from seven pupae of *Tabanus* spp. was 45.5; however, from 15 pupae of *Chrysops* spp., the average was 16.1. James (1963) studied larval habitats, development, and parasites of some Tabanidae in southern Ontario. He reared *D. occidentalis* from *C. aestuans* larvae which were collected at Gunter and St. Ola, Ontario. He reported 7.5% parasitism of 106 larvae and pupae of *C. aestuans* with an average of 12 adults per parasitized pupa. He found that the parasite oviposited on both the larva and pupa of *C. aestuans*, and in view of these facts, *D. occidentalis* could not be considered strictly as a pupal parasitoid. Teskey (1969) collected 25 *Chrysops* and *Hybomitra* pupae parasitized by *D. occidentalis* from 18 localities in Canada and the USA. Burger et al. (1981) stated that *D. occidentalis* was common in 1970 at the Colebrook Trout Hatchery site, New Hampshire. They

believed that it might occasionally be abundant enough at particular sites to reduce adult emergence of *Chrysops*, and therefore affect rearing success in the laboratory. Fuller (1937) reported parasitism of the immature stages of *Tabanus neobasalis* Taylor by *Spilomicrus* sp. (Diapriidae) in Australia. Parasitism of the immature stages of *Chrysops nigribimbo* Whitney, *H. trepida*, *T. marginalis*, *T. nigrovittatus*, and *T. reinwardtii* by *Trichopria* sp. (Diapriidae) has been reported by James (1963), Teskey (1969), and Magnarelli and Anderson (1980). They reported up to 4% parasitism of collected tabanid larvae. Cameron, (1926), Bailey (1947), Jones (1953), Jones and Anthony (1964) and Goodwin (1968) reported up to 50% parasitism of immature stages of *C. mitis*, *T. fairchildi*, *T. lineola*, *T. nigrovittatus*, and *T. reinwardtii* by *Trichopria* sp. from North America. Magnarelli and Anderson (1980) worked on parasitism of immature *T. nigrovittatus* by *Trichopria* sp. near *tabanivora* on Cat Island, Milford, Connecticut. They collected 334 larvae and 87 pupae in 1976. Parasitism in 1976 and 1977 was 4.5% and 4.6%, respectively. Numbers of parasites emerging from *T. nigrovittatus* pupae varied, with an average of 46.4 per parasitized pupa.

Predators - Tabanid larvae are attacked from the time of hatching until pupation by a variety of vertebrate and invertebrate predators (Anderson 1985). Some species of shore birds, fish, mites, and insects prey upon tabanid larvae.

Larger tabanid larvae are included in the diets of shore birds (Pechuman 1981). Johnson and Hays (1973) analysed crop or stomach contents of 31 birds collected around tabanid larval habitats in Alabama. They found that only one bird, a solitary sandpiper, contained a tabanid larva that belonged to the genus *Tabanus*. Predation of tabanid larvae

by fish has been reported by Segal (1936) and Hine (1906) both cited in Anderson (1985). Johnson and Hays (1973) removed 401 bluegills and red ear sunfish from a pond in Alabama. Fish were dissected and stomach contents of each fish were examined under a dissecting microscope. No tabanids were found in any of these fish, although the authors had previously found two *Chrysops* sp larvae in the digestive system of a bluegill. Many freshly hatched larvae were entangled and died in webs of tetranychid mites on leaves around the egg masses (Pechuman 1981). In Alabama, fire ants, *Solenopsis* sp., were observed to forage in the tabanid larval habitats and sting, kill, and eat larvae of one *Tabanus* sp. (Johnson and Hays 1973). Miller (1951) reported that the larvae of the tipulid, *Prionocera* sp., preyed readily on larvae of *Chrysops* spp. and were considered an important factor in biological control. Akchmetbekova et al. (1982) reported predation of tabanid larvae by predaceous beetles in Kazakhstan.

Nematodes - Nematode parasites of Tabanidae belong to two families, Mermithidae and Steinernematidae. The Mermithidae contain the majority of nematode parasites of Tabanidae, including the genera *Amphibiomermis*, *Bathymermis*, *Eurymermis*, *Gastromermis*, *Hexamermis*, *Paramermis*, *Mermis*, and *Mesomermis*. *Neoaplectana* sp., one species of Steinernematidae has been obtained from tabanid larvae in Russia. One species of nematode belonging to the family Diplogasteridae was reported from a California tabanid (Poinar 1985). Shamsuddin (1966) found 16-37% infection by *Bathymermis* in *Chrysops furcatus* Walker larvae collected in Alberta. Lane and Poinar (1985) reported 37.5% infection by *Pheromermis* sp. in *Hybomitra* sp. larvae collected in California. Poinar (1985) reviewed nematode parasites reared from larvae of Tabanidae.

He mentioned one species of Diplogasteridae in *Chrysops* reported from USA; over 20 species of Mermithidae in *Chrysops* spp., *Hybomitra* spp., and *Tabanus* spp. from Russia, Canada, USA, and Germany; and one species of Steinernematidae from a larva of *Tabanus* sp. from Russia.

Fungi - There are many reports of fungi from tabanids in the USA, Russia, and African countries. Ninety-five per cent of *T. autumnalis*. were infected by *Entomophthorales* sp. (Andreeva 1972). Andreeva (1975) reported that 24 species of fungi, including the genera *Aspergillus*, *Beauveria*, *Fusarium*, and *Metarrhizium*, infected larvae and pupae of tabanids. She also found that approximately 26% of collected immature stages of tabanids in the Ukraine died from fungal infections. Laird (1976) found that 12-20% of tabanid larvae collected in the Ukraine were infected by *Metarrhizium anisopliae* (Metchnikoff). Poinar (1985) tabulated pathogenic fungi isolated from Tabanidae. He mentioned one report of *Beauveria densa* (Link and Packard) in eggs and larvae of *C. relictus* in Russia; three reports of *M. anisopliae* from larvae of *Tabanus* spp., *Hybomitra* spp., and *Chrysops* spp. in Russia; and two reports of *Tabanomyces milkoi* (Dudka and Koval) from larvae of *C. relictus* and *T. autumnalis* in Russia.

Bacteria- Thomas and Poinar (1973) isolated *Bacillus cereus* Frankland and Frankland from pupae of *C. vittatus* and larvae of *Hybomitra* sp. They also reported *Pseudomonas aeruginosa migula* (Schroeter) from larvae of *Hybomitra* sp. and *Tabanus* sp. Both *B. cereus* and *P. aeruginosa* are ubiquitous and probably occur in all larval tabanid habitats. During events such as molting or injury, the insects are under stress, so bacteria enter the

haemocoel and can cause lethal septicaemia. Unlike *B. cereus* and *P. aeruginosa*, which are rarely considered primary invaders of healthy insects, *B. thuringiensis* is a primary pathogen and can initiate infection in healthy insects (Poinar 1985). Kadyrova et al. (1977) tested four formulations of *B. thuringiensis* against larvae of *T. autumnalis*. They observed 25% and 18-50% mortality in larvae and pupae of tabanids with dosages of 2-7 g/m^2 under laboratory conditions and in the field.

Protozoa - Protozoan pathogens isolated from tabanids mostly belong to the class Microsporidia. *Thelohania tabani* Gingrich was the first microspordia described from a tabanid (Gingrich 1965). *Nosema* sp., *Octosporea tabani* Levchenko, and *Stempellia lairdi* Levchenko and Andreeva have been isolated from *Tabanus* sp. in Russia (Levchenko 1975; Levchenko and Andreeva 1977; Andreeva and Levchenko 1977). *Thelohania tabani* and some other unidentified microspordia have been reported from larvae of *Tabanus* spp. in the USA (Gingrich 1965). Up to 63% of *T. autumnalis* larvae were infected by another microspordia (Andreeva and Levchenko 1978). *Cometoides pechumani* Anderson and Magnarelli, a nonpathogenic eugregarine microspordia infected 89% of *C. fuliginosus* larvae (Anderson and Magnarelli 1978).

Natural Enemies of Adults

Parasites - Insects, mites, nematodes, protozoa, and fungi have been reported as parasites of tabanid adults. Spratt and Wolf (1972) reported less than 1% infestation of *Dasybasis* sp. with *Bactromyiella* sp. (Diptera: Tachinidae) in Australia. Thompson et al. (1980) found 3.8% infestation in adult *H. lasiophthalma* infested with larvae of a bombyliid.

They removed one to seven larvae per infested host from the abdomen near the terminal segments. Nicholls (1920) reported infestation of adult *Tabanus* by a chalcid, *Perilampsus,* from Tasmania. The larvae were found attached to the proboscis of the host.

Mites attacked *Chrysops*, *Tabanus*, and *Dasybasis* adults (Philip 1931; Davies 1959). Boshko and Sklyar (1981) observed *Rhipicephalus turanicus* Pomerantsev and Matikashvili (Acari: Ixodidae) attached at the base of the hypopharynx of a female *Tabanus leleani* Olsufev. Leprince et al. (1988) examined 25,000 adult tabanids collected in Louisiana and eastern Canada. They found a male *Boophilus annulatus* (Say) (Acari: Ixodidae) located between the wing and the calypter of a female *Tabanus americanus* Forster.

Philip (1931) found a nematode within the abdomen of a fresh specimen of *Tabanus astuta* (Osten Sacken) collected at International Falls, Minnesota. Females of *H. arpadi* were attacked by the nematode, *Paramermis hybomitrae* Rubtsov and Bey-Bienko (Rubtsov and Bey-Bienko 1974).

Fungal infection of adult *Chrysops* sp. by *Coelomomyces* sp. (Phycomycetes) was reported by Steinhaus and Marsh (1962) from west Africa. Anderson and Magnarelli (1979) reported mycotic infection in adults of the saltmarsh horse fly, *T. nigrovittatus* by *Entomophthora* sp. Mullens et al. (1983) collected *Atylotus thoracicus* (Hine) in a sphagnum bog, Cortland County, New York. They found one female to be infected with *E. tabanivora*. Polyakov and Aivazyan (1986) collected adults of 11 species of *Hybomitra*, *Chrysops*, and *Haematopota* in northern Sakhalin. They found *A. flavus* in

all species, *Aspergillus* sp. in two species and another species of *Aspergillus* in three species.

Jones (1953) observed dragonflies while they were capturing tabanid adults. He observed the sphecid wasp, *Bembix carolina* (Fabricius) (Hymenoptera: Sphecidae), capture six tabanids from a horse in 15 minutes. He also reported tabanids entangled in spider webs. Roberts and Wilson (1967) studied predation of horse flies by two species of bembicine wasp in southern Louisiana. They determined the effect of the predators on tabanid populations on cattle, and the number of flies placed in individual burrows by adult wasps while rearing the larvae. They observed that population densities of two or more wasps per animal considerably reduced numbers of tabanids. They reported *Stictia* sp. females carried only tabanids (3.1 daily) to their burrows, whereas female *Bembix texana* Cresson carried diverse prey items, including members of the family Sarcophagidae, Stratiomyiidae, Syrphidae, and Tabanidae. Davies (1959) observed predation of *Vespula maculata* (Linnaeus) (Hymenoptera: Vespidae) on *Chrysops* in Algonquin Park, Ontario. Nazarova and Baratov (1981) reported five *Bembix* spp. (Sphecidae) which preyed upon 10 *Tabanus* and one species *Hybomitra* in Tadzhikistan. Apart from *Bembix* species, they had also reported two species of wasps belonging to the genus *Pseudovespula* (Vespidae) which preyed upon tabanids in Tadzhikistan. They believed that predatory wasps of the genera *Bembix* and *Paravespula* were promising biological agents for controlling the blood-sucking Diptera, tabanids in particular. The nyssonine wasps (Hymenoptera: Sphecidae: Nyssoninae) are the most frequently reported tabanid adult predators (Hine 1906; Evans 1957, 1966; Evans et al. 1982).

Jones (1953) observed the lizard, *Anolis carolinensis* Voigt, stalking and capturing large species of horse flies. Burns and Chapin (1969) studied arthropods in the diet of the cattle egret, *Bubulcus ibis* (Linnaeus), in southern Louisiana. They found that 9% of cattle egret diet was composed of Diptera, particularly Tabanidae. Lane et al. (1983) observed Barn Swallows, *Hirundo rustica* Linnaeus, and Song Sparrows, *Melospiza melodia* (Wilson), which were preying on *Apatolestes actites* Philip and Steffan (Diptera: Tabanidae) on a sandy beach and adjacent hillsides in California. Ellis (1993) found that 31% of the total sample volume of dietary items of Rafinesque's big-eared bat, *Plecotus rafinesquii* Lesson, was made up of male tabanids.

Control Methods

Environmental Management and Physical Control - Parman (1928) collected approximately 20 to 25 million of tabanid eggs along a stream over a distance of 14 miles. The following year, he observed a 50 per cent decrease in the number of flies in the area where the eggs had been collected compared to a similar area not so treated.

Schwardt (1936) mentioned that since egg masses were usually deposited on emergent vegetation and projecting debris in and around ponds and streams, destruction of vegetation and debris and/or egg masses would reduce the number of tabanids in the following years. On the other hand, when the eggs are concentrated on the remaining vegetation, eggs can be more easily destroyed. Sudden changes of water level in ponds were destructive to certain species of tabanid larvae and pupae (Schwardt 1936; Pechuman 1981). McMahon and Gaugler (1993) found that larval densities of *T. nigrovittatus* were higher in well drained salt marsh areas. They explained that the

development of anaerobic conditions in sod at high water levels discouraged larvae from burrowing into the sod. Webb and Wells (1924) believed that drainage in the Antelope Valley of California and Nevada would significantly reduce the numbers of *Hybomitra sonomensis* (Osten Sacken) and *Tabanus punctifer* Osten Sacken. Anderson (1985) mentioned that increasing of water level at the proper time may effectively control tabanids.

Wild animals protect themselves from tabanid bites by submerging much of their body into ponds and lakes (Pechuman 1981). Domestic animals congregate and swish their tails to keep the adults off their bodies to achieve some relief (Webb and Wells 1924). Ralley et al. (1993) observed individual and group behaviour of pastured cattle in response to attack by biting flies. They described the formation of grazing lines and bunching as group behavioural responses and head tosses, foot stomps, ear flicks and tail switches as individual responses to biting fly attack. Keeping domestic animals in closed buildings during periods of persistent attack can be helpful (Olsuf'ev and Lelep 1935; Gunarova and Toriska 1977). Burlap or canvas blankets and hoods may be used for work animals (Hine 1906; Webb and Wells 1924; Pechuman 1981). Doorway curtains and screening were effective in keeping tabanids out of barns and houses (Morgan et al. 1972). To reduce the numbers of flies feeding on humans, light coloured hats, trousers, and long sleeved shirts were suggested (Hansens 1947). Removal of trees and bushes reduced harbourage for adult tabanids (Hansens and Rabin 1981). Conversely, making barriers by the strategic planting of vegetation may inhibit salt marsh species from reaching upland areas (Morgan and Lee 1977). Using trapping devices is the most

promising control for adults. Shiny black panels covered with a sticky substance and baited with dry ice were effective (Pechuman 1981).

Silhouette-type traps such as Manning, Harris, canopy, box, adhesive-coated panels, and helium-filled weather balloons all baited with or without carbon dioxide are often used to trap host-seeking females. These devices have been used by many tabanid workers to decrease the numbers of adults (Anderson 1985).

Chemical Control - In chemical control of the larval stage, the relative persistence of the particular chemical is more important than the chemical's relative toxicity. Due to its persistence, dieldrin was used frequently in North America and Africa. Hansens (1956) applied granulated insecticide (dieldrin a 1, 2 and 5% formulated on ground tobacco stems) to control larval green head populations. He found that 5% dieldrin eliminated larvae from the treated marsh for two years; however, this strategy seriously damaged other animal life.

To disperse toxicants into the air or onto surfaces where flies tend to congregate (e. g. water, vegetation, and host animals), fixed wing aircraft, helicopters, portable compressed air sprayers, vehicle-mounted mist blowers and ultra low volume sprayers used (Anderson 1985). Skuf'in (1949) applied kerosene to entire ponds to trap the flies as they were drinking water from pools. Pyrethrins, permethrin, methoxychlor, thanite, dichlorovos, naled, and trichlorfon as fast-acting, nonpersistent insecticides have been used successfully directly on domestic animals to kill and repel flies (Anderson 1985). Applications were sometimes made more than once per day or usually two to three times per week. Space sprays are used to control flies inside barns and sheds; however, it may

provide only one day of adequate protection (Adkins 1974). Aerial application of DDT was successful in marshes in Massachusetts from 1955 to 1963 for the control of *T. nigrovittatus* (Spender 1971), although less promising results were reported by other authors using chlorinated hydrocarbons.

A number of non-lethal chemicals such as N, N- diethyl-m-toluamide, ethyl hexanediol, dimethyl phthalate; 2, 2, 4-trimethyl-1,3-pentanediol, have been used as tabanid repellents (Anderson 1985). The impregnation of clothing with repellents has been suggested by different authors. Deet-impregnated clothing (Catts 1968), clothes impregnated with N, N-diethylamide or benzoic acid (Potapov et al., 1972), and resmethrin-impregnated clothing provided varying results to keep tabanids away from humans (Anderson 1985). However, Burger (personal communications) believes that Deet is relatively ineffective as a repellent for tabanids and instead Citronella works much better but does not last as long as Deet for mosquitoes. Foil et al. (1990) studied sublethal effects and mortality of tabanids induced by fenvalerate treatment of cattle. They observed 27.4 to 38.8% reduction in feeding time for tabanids feeding on cows sprayed with fenvalerate. They also found 31% reduction in engorgement weight of *T. fuscicostatus*. Leprince et al. (1991) evaluated pyrethroid ear tags and spray treatment of cattle against horse flies under field conditions in Louisiana. They collected of 2922 females belonging to three *Tabanus* species were collected. They found 3%, 9%, 15%, 67%, and 79% mortality in controls, permethrin tag, fenvalerate tag, 0.01% fenvalerate spray, and 0.02% fenvalerate spray, respectively. Foil et al. (1991) worked on survival and dispersal of horse flies feeding on cattle sprayed with a sublethal dose of fenvalerate. Total of 15,465 tabanids belonging to 17 species and five genera marked individually and

divided between control and fenvalerate-sprayed bullocks. They recaptured 6.6% for the control and 3.4% for the fenvalerate treatment. They also found that dispersal of tabanids was not affected by treatment. Ralley et al. (1993) found that the numbers of tail switches and foot stomps were significantly less in the herd which was sprayed with cypermethrin (0.1% active ingredient) than those in the control herd.

CHAPTER III

Three new Nearctic species of *Telenomus* (Hymenoptera: Scelionidae) attacking tabanid eggs (Diptera: Tabanidae) in Canada with an identification key to the New World species

Introduction

The genus *Telenomus* is the most specios genus of the family Scelionidae (subfamily Telenominae) with over 500 described species worldwide (Johnson 1984). Species of *Telenomus* parasitize the eggs of a variety of insects in the orders Lepidoptera, Hemiptera, Diptera, and Neuroptera (Polaszek et al. 1993). There are 11 species groups of Nearctic *Telenomus* including the *Telenomus tabanivorus* (Ashmead) species group, with members attacking Tabanidae, Asilidae, and Stratiomyiidae (Johnson 1984).

There are 10 described species (with many synonyms) in the *tabanivorus* group distributed among all geographical regions of the World (Anderson 1985). According to Johnson (1984, 1992), these 10 valid, accepted species and their synonyms are as follows: *Telenomus angustatus* (Thomson) (=*tabani* Mayr; =*coccivorus* Mayr; =*oophagus* Nikol'skaya; =*praetabani* Szab'o) and *Telenomus promachivorus* (Gahan) are Palaearctic; *Telenomus benefactor* Crawford, and *Telenomus kingi* Crawford are Afrotropical; *Telenomus emersoni* (Girault), *Telenomus goniopis* Crawford and *Telenomus tabanivorus* are Nearctic; *Telenomus tabanocida* Crawford is Neotropical; *Telenomus dignus* (Gahan) (=*Phanurus matsumurai* Ishida) and *Telenomus inclinis* Le occur in the Oriental region. Anderson (1985) reviewed papers on the natural enemies of tabanids including species of *Telenomus* attacking tabanid egg masses in the Old World. These egg parasitoids are as follows: *T. angustatus* reared from *Chrysops relictus* Meigen in Russia (Sku'fin 1949), from *Hybomitra bimaculata* (Macquart) in Switzerland (Auroi 1981; Kozlov 1967), from *Tabanus* sp. in Germany, Austria, and Russia (Mayr 1877; Nikol'skaya 1948; Olsuf'ev 1935), and from eggs of an unidentified tabanid species

in Russia (Rastegaeva 1965). *Telenomus benefactor* was reported from the Sudan (Patton and Cragg 1913), and Africa (Nikol'skaya 1948). *Telenomus kingi* was reared from *Tabanus kingi* Austen in the Sudan (Marchand 1920; Nikol'skaya 1948)

In Canada, *T. emersoni* was reported from Saskatchewan (Cameron 1926), British Columbia (Hatton 1948), and Ontario (James 1963); in the USA from Minnesota (Philip 1931), Texas (Girault 1916; Parman 1928; Webb and Wells 1924), Delaware (MacCreary 1940), New Jersey (Orminati and Hansens 1974), California (Webb and Wells 1924), Nevada (Middlekauff and Lane 1980), Arkansas (Schwardt 1936), Louisiana (Jackson and Wilson 1966), and Alabama (Jones and Anthony 1964). Many species of *Chrysops, Hybomitra*, and *Tabanus* (all Diptera: Tabanidae) have been reported as hosts for *T. emersoni* in the Nearctic region.

In the USA, *T. goniopis* has been reported from Maryland (Crawford 1913), New York (Tashiro and Schwardt 1953), Massachusetts (Bailey 1948), and Arkansas (Schwardt 1936). *Goniops chrysocoma* (Osten Sacken) (Diptera: Tabanidae), *Hybomitra lasiophthalma* (Macquart), and *Tabanus atratus* Fabricius have been reported as hosts for *T. goniopis*. In the USA, *T. tabanivorus* has been reported from South Carolina (Goodwin 1976), Ohio (Drees 1982), Massachusetts south to Florida, west to Illinois (Hart 1895; Ashmead 1895; Hine 1903, 1907; Muesebeck 1979; Tashiro and Schwardt 1953; Jones 1953; Jones and Anthony 1964), Louisiana (Jackson and Wilson 1966), and Alabama (Dukes and Hays 1971). Tabanid hosts reported for *T. tabanivorus* are *Merycomyia whitneyi* (Johnson), *H. lasiophthalma*, *T. atratus*, *Tabanus stygius* Say, and *Chrysops* sp.

This chapter involves taxonomy of parasitoids, in which three new species of *Telenomus* reared from egg masses of *Hybomitra nitidifrons nuda* (McDunnough) and

Chrysops aestuans Van der Wulp (Diptera: Tabanidae) are described. An illustrated key for identification of New World species, along with one common species from the Palaearctic is provided.

Materials and methods

More than 1000 egg masses of Tabanidae were collected at 12 locations south and east of Winnipeg, Manitoba, Canada in 1997-2000 and incubated at 25±1° C and 50% RH with 18L:6D for 1-10 days. Approximately 1000 parasitoids were sorted into three groups, scelionids that emerged from *H. n. nuda* eggs, scelionids from *C. aestuans* and trichogrammatids from *C. aestuans*. Specimens were mounted on points and slides. Antennae, wings, legs, metasoma, and male genitalia were prepared on slides for proper identification. Specimens of described species, including the type series, were borrowed from the following institutions:

CNCI	Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada,
UMNH	National Museum of Natural History, Washington, DC, USA
ZISP	Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia.

The type material (in UMNH) of the following *Telenomus* species were examined: *T. benefactor* (Figs. I.60-63), *T. dignus* (Figs. I.64-67), *T. emersoni* (Fig. I.53), *T. goniopis* (Figs. I.49, I.56), *T. kingi* (Figs. I.68-70), *T. tabanocida* (Figs. I.50, I.57), and *T. tabanivorus* (Figs. I.51, I.54, I.58-59). Specimens of *T. angustatus* (det. M.A. Kozlov)

(Figs. I.52, I.55) from Russia were compared with Huggert's (1983) redescription of Thomson's type. However, the identification key was prepared only for New World species, with one exception. Because of the proximity of Nearctic and Palaearctic regions, the most widely distributed species of tabanid egg parasitoid in the Palaearctic, *T. angustatus*, is included in the key. The holotype for each species of three new species is deposited in the CNCI; paratypes are distributed to CNCI, USNM, and the J.B. Wallis Museum, Department of Entomology, University of Manitoba, Canada.

Characters and Measurements

Morphological terms used in this paper are those defined and illustrated by Masner (1980) and Masner and Denis (1996). The definitions of some morphological terms along with details on measurements are listed below.

Head

Eye height - Maximal distance between upper and lower orbit of eye; measured in frontal view.

Head height - Maximal distance between lower margin of clypeus and top of vertex; measured in frontal view.

Head length - Maximal distance between frons and postgena; measured in lateral view.

Head width - Maximal distance between outer margins of eyes; measured in dorsal view.

Interorbital space - Shortest distance on frons between inner orbits; measured in frontal view.

Malar space - Distance between lower orbit of eye and mandibular condyle; measured in frontal view.

Occipital carina - Carina bordering posterior margin of gena; posterior view.

OD - Diameter of lateral ocellus measured from dorsal view.

Temple - Region behind eye; measured in dorsal view.

Antenna

Antennal segments in relative proportions - Maximal length/maximal width; measured in lateral view.

Clava (A7-A11) /A2-A6 - Relative length; measured in lateral view.

Mesosoma

Mesosoma height - Maximal distance between top of mesoscutum and lowermost point of mesopleuron; measured in lateral view.

Mesosoma length - Maximal distance between anterior margin of pronotum and posterior margin of nucha; measured in dorsal view.

Mesosoma width - Maximal distance between posterolateral corners of pronotum in front

of tegulae; measured in dorsal view.

Metasoma

Metasoma length - Maximal distance between anterior margins of T1 and apex of metasoma; measured in dorsal view.

Metasoma width - Maximal distance, usually across posterior end of T2; measured in dorsal view.

T1 length/width ratio - Tergite one measured in dorsal view.

T2 length/width ratio - Tergite two measured in dorsal view.

Grooves - Depressions between costae.

Striae - Fine impressed scratches posterior to grooves.

Length of grooves on T1 - Maximal length of grooves on the meson of tergite one; measured in dorsal view.

Length of grooves on T2 - Maximal length of grooves on the meson of tergite two; measured in dorsal view.

Grooves and striae on T2/length of T1 - Maximal length of grooves combined with striae on T2/maximal length of T1; measured in dorsal view.

General habitus of the body, length of the metasoma, length of T1, hump on T1, and striae on T1 and T2 are valuable as diagnostic characters in species identification.

Telenomus species A, new species (chosen name *T. hybomitrae*)

(Figs. 3.1, 3.9, 3.12, 3.15, 3.22, 3.29, I.1, I.4, I.7, I.10, I.13, I.16, I.19, I.22, I.25, I.28, I.31, I.34, I.37, I.40, I.43, I.46)

Material Examined -18 females, 12 males

Holotype: female (CNCI No. 22674), Canada, Manitoba, Hwy #11, 3 km East of Elma, 49°56′N, 95°54′W, 15 June, 1999, M. Iranpour. Allotype: Canada, Manitoba, Hwy #11, 3 km East of Elma, 49°56′N, 95°54′W, 15 June, 1999, M. Iranpour. Paratypes: 4 ♀♀, 6 ♂♂, Canada: MB, Hwy #12, Piney, 49° 5′ N, 95° 57′ W, 11 June, 1998, M. Iranpour; 13 ♀♀, 5 ♂♂, Canada, Manitoba, Hwy #11, 3 km East of Elma, 49°56′N, 95°54′W, 15 June, 1999, M. Iranpour.

Diagnosis

Female - Body distinctly elongate; head in dorsal view distinctly transverse (16:28); metasoma distinctly elongate (58:20); T1 hump-like in lateral view, grooves absent medially or extremely short; grooves and striae combined on T2 not longer than length of T1; T6 slightly transverse (7:9); apex of metasoma (small part of T6 and entire T7) not covered by fore wing in resting position. **Male** - T1 in dorsal view with sub-triangular smooth area at meson, medially with basal short grooves in form of punctures, wrinkled on corners; T1 in dorsal view with delicate hump that is slightly higher than top of T2.

Description

Holotype female - Length 0.97 mm, body colour black, all coxae dark brown, femora and tibiae, except bases and apices, dark brown, legs otherwise yellow, radicle and base of A1 brown, antennae otherwise dark brown; wings clear.

Head in dorsal view distinctly transverse (16:28), slightly wider than mesosoma (28:25); vertex from occipital carina to anterior ocellus with impressed coriaceous sculpture, with some whitish setae; temple behind eye 3.3 OD, with impressed coriaceous sculpture extending from posterior orbit to occipital carina; head in lateral view higher than long (25:16), as high as mesosoma (25:25); upper postgena along outer orbit coriaceous, lower postgena smooth; eye height:length (15:12); head in frontal view slightly wider than high (27.5:25); eye height:malar space (15:10); eye height:interorbital space (15:15), inner orbits divergent toward mouthparts; antennal scrobe weakly developed, frons predominantly smooth and highly shining, lower portion of frons along inner orbit coriaceous, upper part of frons between orbits smooth; malar region and gena predominantly with fine coriaceous sculpture; occiput smooth; antennal segments (A1-A11) in relative proportions (15:3); (5:3); (4:2); (3:2); (2:2); (2:2); (2:4); (3:4); (3:5); (3:5); (5:4); clava (A7-A11) longer than A2-A6 (18:15); sensillar formula on A8-A11 1:2:2:1, sensillum located on tip of A8, upper 1/3 of A9 and A10, and middle of A11.

Mesosoma in dorsal view longer than wide (34:25); mesoscutum wider than long (19:25), with whitish decumbent hairs, and impressed coriaceous sculpture; scutellum transverse (5:10), with decumbent whitish pilosity, predominantly smooth, with few peripheral punctures; dorsellum rugulose; mesosoma in lateral view longer than high

(30:25), slightly convex; side of pronotum predominantly coriaceous; netrion smooth; mesopleuron with coriaceous sculpture, mesopleural depression strongly developed, mesopleural chain foveolate with deep and regular pits; metapleuron smooth with distinct pit near middle.

Metasoma in dorsal view distinctly elongate, longer than mesosoma and head combined (58:43), longer than wide (58:20); T1 moderately transverse (7.5:13), predominantly smooth, grooves reduced to few minute pits along anterior margin, anterior corners wrinkled, T1 with 1 bristle on each side of hump and 3 bristles on lateral margin; T2 elongate (26:20), with 14 short grooves along anterior margin, grooves slightly longer than wide, extending 1/8 of T2 length, longest grooves combined with striae on T2 less than medial length of T1 (3:7.5), length of T2:length of T1 (26:7.5); T2 to T7 with 3 bristles on each side of tergite; T3 to T7 in relative length:width (4:16), (3.5:13), (4.5:11), (7:9), (4:4); T1 in lateral view with moderate hump, top of hump exceeding top of T2.

Allotype male - Differs from female as follows: body size smaller, length 0.84 mm, width 0.27 mm; body colour lighter than in female, pedicel and A1 light brown, otherwise antennae dark brown, upper head and mesosoma dark brown, lower head, frons, and lower mesosoma lighter, legs yellow, metasoma black.

Head transverse (15:27); length of eye:temple:OD (9:5:2); postgena in lateral view with coriaceous sculpture, with more long bristles; head in frontal view with inner orbits subparallel; gena and malar region with coriaceous sculpture more extensive than

in female; antennal segments (A1-A12) in relative proportions (14:3); (3.5:3); (2.5:2); (2.5:2.5); (4:3); (3:2); (3:3); (2.5:3); (2.5:3); (2.5:3); (2.5:3); (5:2); Mesosoma longer than wide (32:23).

Metasoma longer than wide (47:19); entire metasoma covered by fore wing; tergites T1 to T7 in relative proportions (5:14); (26:19); (5:17); (4:14); (3:10); (3.5:8); (3:5); T1 in dorsal view medially with basal short grooves in form of punctures, wrinkled on corners, T1 with sub-triangular smooth area at meson; T1 in lateral view with only delicate hump but with top slightly higher than top of T2.

Remarks

Telenomus species A is closely related to *T. tabanivorus*, with its distinctly elongate habitus and convex T1 in the female (Figs. I.16, I.22, I.58). However, it differs from the latter species by having long striae on T2, 4 more grooves on base of T2, shorter body size, a smaller hump (Figs. 3.1, 3.5), and shorter length of T3-T7. In the female, it differs from *Telenomus* species B by having a hump on T2, shorter striae on T2, and an elongate metasoma (Figs. 3.1, 3.2).

Variation

In total, 238 females and 42 males were examined and the following variation was observed. Body length varied because of the telescopic nature of the metasoma (female 1.05±0.02 mm; male 0.85±0.01 mm). T1 in some individuals without any basal grooves and in some punctuate. Number of grooves on T2 13-16 in females and 11-13 in males.

Combined basal grooves and striae on T2 slightly longer than in the holotype, but never longer than length of T1. Height of hump on T1 variable, but always present. In male hump is not as distinct as in female.

Hosts

Eggs of *Hybomitra nitidifrons nuda*. Also, reared from eggs of *H. lasiophthalma*.

***Telenomus* species B, new species (chosen name *T. utilis*)**

(Figs. 3.2, 3.10, 3.13, 3.16, 3.23, 3.30, 3.36, I.2, I.5, I.8, I.11, I.14, I.17, I.20, I.23, I.26, I.29, I.32, I.38, I.41, I.44, I.47)

Material Examined 12 females, 9 males

Holotype: female (CNCI No. 22675), Canada, Manitoba, Hwy #11, 3 km East of Elma, 49°56′N, 95°54′W, 15 June,1999, M. Iranpour. ALLOTYPE: Canada, Manitoba, Hwy #11, 3 km east of Elma, 49°56′N, 95°54′W, 15 June, 1999, M. Iranpour. Paratypes: 11 females, 8 males, Canada, Manitoba, Hwy #11, 3 km East of Elma, 49°56′N, 95°54′W, 15 June, 1999, M. Iranpour.

Diagnosis

Female - Body elongate; head in dorsal view distinctly transverse; metasoma elongate; T1 flat, with long basal grooves; grooves and striae on T2 longer than length of T1;

metasoma covered by fore wings in resting position. **Male** - T1 in dorsal view medially with basal long grooves, extending more than half of T1 length, length of grooves identical across T1.

Description

Holotype female - Length 1.05 mm, body colour black, all coxae dark brown, femora and tibiae, except bases and apices, dark brown, legs otherwise yellow, radicle and base of A1 brown, antennae otherwise dark brown; wings clear.

Head in dorsal view distinctly transverse (17:29), wider than mesosoma (29:25); vertex from occipital carina to anterior ocellus with impressed coriaceous sculpture, and some whitish setae; temple behind eye more than 3.3 OD, with impressed coriaceous sculpture extending from posterior orbit to occipital carina; head in lateral view higher than long (28:18), as high as mesosoma (28:28); upper postgena along outer orbit coriaceous, lower postgena smooth; eye height:length (17:13); head in frontal view slightly wider than high (29:28); eye height:malar space (16:10); eye height:interorbital space (15:15), inner orbits divergent toward mouth parts; antennal scrobe weakly developed, frons predominantly smooth and highly shining, lower portion of frons along inner orbit to antennal scrobe coriaceous, upper part of frons between orbits smooth; malar region and gena predominantly with fine coriaceous sculpture; occiput smooth; antennal segments (A1-A11) in relative proportions (16:3); (5:3); (3.5:2); (2:3); (3:3); (2:2); (2.5:3); (3:4); (3:4); (3:4); (5:3.5); clava (A7-A11) slightly longer than A2-A6

(18:17); sensillar formula on A8-A11 1:2:2:1, sensillum located on tip of A8, upper 1/3 of A9 and A10, and middle of A11.

Mesosoma in dorsal view slightly longer than wide (35:25); mesoscutum wider than long (20:25), with whitish decumbent hairs, with impressed coriaceous sculpture; scutellum transverse (14:7), with decumbent whitish pilosity, predominantly smooth, with few peripheral punctures; dorsellum rugulose; mesosoma in lateral view longer than high (30:26), slightly convex; side of pronotum predominantly coriaceous; netrion smooth; mesopleuron with coriaceous sculpture, mesopleural depression strongly developed, mesopleural chain foveolate with deep and regular pits; metapleuron smooth with distinct pit near middle.

Metasoma in dorsal view elongate, longer than mesosoma and head combined (59:44), longer than wide (59:24); T1 distinctly transverse, wider than long (5:17), anterior half with grooves, posterior half smooth; grooves on T1 well developed, deep, extending ½ of T1 length, twice or more longer than wide, more or less same length across T1 width; T1 with 2 bristles on dorsum and each lateral margin; T2 elongate (32:24), with 12-13 long grooves along anterior margin, grooves much longer than wide (2-3 times); grooves and striae extending one third of T2 length, longest striae combined with grooves on T2 longer than medial length of T1 (10:5), length of T2:length of T1 (32:5); T2 to T7 with 3 bristles on each side of tergite; T3 to T7 in relative length:width (5:21), (4:17), (5:12), (5:7), (3:4.5); T1 in lateral view slightly higher than T2.

Allotype male - Differs from female as follows: body size smaller, length 0.92 mm, width 0.29 mm; body colour lighter, pedicel and A1 light brown, otherwise antennae dark brown, upper head and upper mesosoma dark brown, lower head, frons, and lower mesosoma lighter, legs yellow, metasoma black.

Head transverse (17:29); length of eye:temple:OD (9:6:2); post gena in lateral view with coriaceous sculpture and more long bristles; head in frontal view with inner orbits subparallel; more extensive coriaceous sculpture in genal and malar regions; antennal segments (A1-A12) in relative proportions: (16:3); (4:3); (4:2.5); (4:2.5); (4:3.5); (3:2.5); (2.5:3); (3:3.5); (3:3.5); (3:3.5); (3:4); (5.5:3.5). Mesosoma length:width (34:24).

Metasoma longer than wide (45:23); tergites T1 to T7 in relative proportions: (4:13); (28:24); (4:21); (3:17); (2:12); (2:8); (3:5); T1 in dorsal view medially with basal long grooves identical in size, extending more than half of T1 length.

Remarks

Telenomus species B is closely related to *T. angustatus* in colour, size, presence of grooves on T1 and T2, and striae on T2. However, it differs from *T. angustatus* by having shorter striae on T2, shorter grooves on T1 and T2 (Figs. 3.2, 3.8, I.52), and a longer metasoma. In the female, it differs from *Telenomus* species A by its lack of a hump on T2, the longer striae on T2, and its shorter metasoma (Figs. 3.1, 3.2, I.16, I.17).

Variation

In total, 60 females and 95 males were examined and the following variation was observed. Body length varied because of the telescopic nature of the metasoma (female 0.99±0.01 mm; male 0.87±0.03 mm). Number of grooves on T1 11-13 in females and 10-13 in males. Number of grooves on T2 13-15 in females and 11-13 in males. Length of combined grooves and striae on T2 variable but always longer than T1 length.

Hosts

Eggs of *Hybomitra nitidifrons nuda*. Also reared from eggs of *H. lasiophthalma*.

***Telenomus* species C, new species (chosen name *T. chrysopsis*)**

(Figs. 3.3, 3.11, 3.14, 3.17, 3.24, 3.31, I.3, I.6, I.9, I.12, I.15, I.18, I.21, I.24, I.27, I.30, I.33, I.36, I.39, I.42, I.45, I.48)

Material Examined 12 females, 10 males.

Holotype: female (CNCI No. 22676), Canada, Manitoba, Hwy #11, 1 km East of Elma, 49°54′N, 95°54′W, 1 July, 1998, M. Iranpour. Allotype: Canada, Manitoba, Hwy #11, 1 km East of Elma, 49°54′N, 95°54′W, 1 July, 1998, M. Iranpour. Paratypes: 11 females, 9 males, Canada, Manitoba, Hwy #11, 1 km East of Elma, 49°54′N, 95°54′W, 1 July, 1998, M. Iranpour.

Diagnosis

Female - Body elongate; head in dorsal view slightly transverse; metasoma elongate, covered by fore wings; T1 with long basal grooves and no hump in lateral view; T2 with basal grooves and short, undeveloped striae. **Male** - T1 flat, with long basal grooves, extending more than half of T1 length, length of grooves identical across T1. Base of T2 with grooves and short, undeveloped striae.

Description

Holotype female - Length 0.66 mm, body colour black, all coxae dark brown, femora and tibiae, except bases and apices, dark brown, legs otherwise yellow, radicle and A1 dark brown, antennae otherwise light brown; wings clear.

Head in dorsal view transverse (13:20), slightly wider than mesosoma (20:17); vertex rounded into occiput, vertex from occipital carina to anterior ocellus with impressed coriaceous sculpture, and some whitish setae; temple behind eye 5 OD, with impressed coriaceous sculpture extending from posterior orbit to occipital carina; head in lateral view higher than long (18:12), as long as mesosoma (18:18); upper postgena along outer orbit coriaceous, lower postgena smooth; eye height:length (11:8); slightly wider than high (20:18); eye height:malar space (9:6); eye height:interorbital space (11:13), inner orbits divergent toward mouthparts; antennal scrobe weakly developed, frons smooth and shining, lower portion of frons along inner orbit coriaceous, upper part of frons smooth; malar region and gena predominantly coriaceous, with small part smooth near malar sulcus; occiput smooth; antennal segments (A1-A11) in relative proportions

(10:2); (4:2); (1.5:1.5); (2:1.5); (1.5:1.5); (1:1.5); (1.5:2); (2:2.5); (3:3); (3:3); (4:3); clava (A7-A11) longer than A2-A6 (14:11); sensillar formula on A8-A11, 1:2:2:1, sensillum located on tip of A8, upper 1/3 of A9 and A10, and middle of A11.

Mesosoma longer than wide (24:17); mesoscutum wider than long (13: 17), with whitish decumbent hairs, and coriaceous sculpture; scutellum transverse (10:5), with decumbent whitish pilosity, predominantly smooth, with few peripheral punctures; dorsellum rugulose; mesosoma in lateral view longer than high (23:18); side of pronotum predominantly coriaceous; netrion smooth; mesopleuron with coriaceous sculpture, mesopleural depression strongly developed; mesopleural chain with shallow irregular pits; metapleuron smooth with distinct pit near middle.

Metasoma in dorsal view elongate (32:20), slightly shorter than mesosoma and head combined (32:33), longer than wide (32:17); T1 moderately transverse, wider than long (9:2), partly smooth, grooves developed, extending more than half of T1 length, T1 with 1 bristle on each side dorsally; T2 elongate (20:17), with 12 short and shallow grooves along anterior margin, slightly longer than wide, striae not developed, length of T2:length of T1 (20:2); T2 to T7 with 3-4 bristles on each side of tergite; T3 to T7 in relative length:width (2:14), (2:10), (2:8), (3:5), (2.5:4); T1 in lateral view flat, lower than T2-T7.

Allotype male - Differs from female as follows: body length, 0.65 mm width, 0.18 mm; colour lighter, antennae light brown, upper head and mesosoma dark brown, lower head, lower mesosoma lighter, legs yellow, metasoma dark brown.

Head transverse (12:20); length of eye:temple:OD (5:5:1); postgena in lateral view with coriaceous sculpture, with more long bristles; head in frontal view with inner orbits subparallel; predominantly with coriaceous sculpture; antennal segments (A1-A12) in relative proportions (11:2); (4:2); (2:1.5); (1.5:1.5); (2:2); (1.5:1.5); (1.5:2); (1.5:2); (2:2); (2:2); (2:2); (4:2). Mesosoma longer than wide (24:17).

Metasoma longer than wide (35:15); tergites T1 to T2 in relative proportions (3:7); (17:15); (4:14); (4:13); (2:11); (2:7); (2:5); each tergite with 4 bristles on each side.

Remarks

Telenomus species C is closely related to *T. tabanocida* with its small body and grooves on T1 (Figs. 3.3, 3.7, I.21, I.50). However, in the female, it differs from the latter species by having more basal grooves (12 or more compared to 10) on T2, and shorter body length (Fig. 3.24, 3.28). It differs from *Telenomus* species A by its smaller body, lack of hump on T1 and lack of striae on T2 (Figs. 3.1, 3.3). *Telenomus* species C differs from *Telenomus* species B by its smaller body and lack of striae on T2 (Figs. I.20, I.21).

Variation

In total, 121 females and 90 males were examined and the following variation was observed. Body length varied because of the telescopic nature of the metasoma (female 0.72±0.01 mm; male 0.67±0.01 mm). Number of grooves on T1 6-9 in females

and 8-11 in males. Number of grooves on T2 10-12 in females and 9-11 in males.

Hosts

Eggs of *C. aestuans.* Also reared from eggs of *Chrysops excitans* Walker and *Chrysops mitis* Osten Sacken.

Key to females of New World and the most common Palaearctic *Telenomus* attacking tabanid eggs

1 - T1 convex, with hump; basal grooves on T1 extremely short, puncture-like or absent (Figs. 3.1, 3.5).....2

- T1 flat, with no hump; basal grooves on T1 longer extending at least ¼ of tergite length (Figs. 3.2, 3.4, 3.6, 3.7, 3.8).....3

2(1) - T2 basally without striae (Fig. 3.5); relative length of T2:T3-T7 (27:43); mesosoma in lateral view convex, body length 1.3 to 1.5 mm; ratio clava:A2-A6 (20:16); height of head:height of mesosoma (27:34); scutellum transverse (6:15); hump on T1 distinctly convex (Fig. 3.5); T2 moderately elongate (27:22). From eggs of *Tabanus* spp. (Fig. 3.26).....***T. tabanivorus***

- T2 basally with grooves and striae extending as long as length of T1 or slightly less (Fig. 3.1); relative length of T2:T3-T7 (30:33); mesosoma in lateral view not convex; ratio clava:A2-A6 (17:16); height of head:height of mesosoma (23:25); scutellum not as transverse as above (7:12); hump on T2 less convex (Fig. 3.1); T2 more elongate (30:20). From eggs of *Hybomitra spp.* (Fig. 3.22). ***Telenomus* species A, new species**

3(1) - Grooves on T2 long, with well developed long striae; grooves and striae on T2 longer than length of T1 (Figs. 3.2, 3.6, 3.8).....4

-Grooves on T2 short, without well developed striae; grooves and striae on T2 shorter than length of T1 (Figs. 3.3, 3.4, 3.7).....6

4(3) - T2 basally with 10 long, deep, and wide grooves, and 2-3 long striae mesally (Fig. 3.6); body length 1.17 mm, width 0.34 mm; dorsum of mesosoma convex in lateral view; A1 longer (18.25:3.6); mesosoma width:height (30:32); metasoma length:width (60:27). From eggs of *G. chrysocoma* (Fig. 3.27).....***T. goniopis***

- T2 basally with 10-14 grooves, and many striae (Figs. 3.2, 3.8); body length 0.99 mm or less, width 0.30 mm or less; dorsum of mesosoma not convex; A1 shorter (15:3); mesosoma smaller, width:height (27:27); metasoma length:width (57 or less:22).....5

5(4) - T2 basally with 10 deep and long grooves, striae very long, extending half length of T2 or slightly less, grooves and striae on T2 twice the length of T1 or more (Fig. 3.8), metasoma shorter (47:22), length of T2:T3-T7 (28:14); height of head:height of mesosoma (25:30); body less elongate (95:30)..... ***T. angustatus***

- T2 basally with 14 grooves, not as deep as above, striae shorter, extending 1/6 to 1/7 of length of T2, grooves and striae on T2 equal in length to T1 or slightly more (Fig. 3.2); metasoma more elongate (57:22), relative proportion of T2:T3-T7 (32:20); height of head:height of mesosoma (25:27); body more elongate (104:28). From eggs of *Hybomitra spp.* (Fig. 3.23)..... ***Telenomus* species B, new species**

6(3) - Basal grooves on T1 very short, extending 1/8 of T1, basal striae almost as wide as long (Figs. 3.4, 3.25)……..…..***T. emersoni***

- Basal grooves on T1 long, extending 1/2 to ¾ length of T1, basal grooves on T1 2 to 3 times longer than wide (Figs. 3.3, 3.7)……..……..……7

7(6) - Basal grooves on T1 extending about 3/4 length of T1 (Fig. 3.3); smaller individuals, body 0.7 mm long, 0.21 mm wide; tip of fore wing extending beyond tip of metasoma (99 microns longer); head length:width:height (14:21:18); eye length:temple (7:5); A1 length:width (11:2); clava length:width (15:3); mesosoma length:width:height (27:17:18); scutellum with scattered punctures; T2 length:width (21:17); T3-T7 length:width (12:17); metasoma length:width (35:17). From eggs of *Chrysops* spp. (Fig. 3.24)…….…. ***Telenomus* species C, new species**

- Basal grooves on T1 extending1/2 or less length of T1 (Fig. 3.7); larger individuals, body length about 0.85 mm, width 0.25 mm (Fig. 3.28); tip of fore wing reaching tip of metasoma or slightly more (40 microns more); head length:width:height (15:25:22); eye length:temple (10:4); A1 length:width (14:2); clava length:width (15:4.5); mesosoma length:width:height (27:20:23); scutellum with denser punctures; T2 length:width (24:18); T3-T7 length:width (23:18); metasoma length:width (50:18). From tabanid eggs in Trinidad (Fig. 3.28)……..

……..…..…..***T. tabanocida***

Key to males of New World and the most common Palaearctic *Telenomus* attacking tabanid eggs

1 - A3-A5 elongate, rectangular, A6-A11 distinctly transverse (Fig. 3.37); wing shortened, front wing (49:10), hind wing (32:24); middle and hind legs short and stout..... ***T. emersoni***

- A3-A5 slightly elongate, spherical, A6-A11 spherical (Fig. 3.36); wings normally developed, front wings (63:16 or bigger), hind wings (52:6 or bigger); middle and hind legs are more elongate and more slender.....2

2(1) - Grooves on T1 extending maximum to ¼ of T1 length at meson or punctuate (Figs. 3.15, 3.18); T1 long, ¼ of T2 length or longer (Figs. 3.29, 3.33); T1 slightly elevated. 3

- Grooves on T1 well developed, extending at least ½ of T1 length (Figs. 3.16, 3.17, 3.19, 3.20, 3.21); T1 shorter, 1/5 of T2 length or shorter (Figs. 3.30, 3.31, 3.32, 3.34, 3.35); T1 flat.....4

3(2) - Striae on T2 present, basal grooves on T2 deep and long (2-3 times longer than wide) (Fig. 3.15), basal grooves on T1 irregular in length, wrinkled at corners (Fig. 3.15). From eggs of *Hybomitra* spp. (Fig. 1.29)..***Telenomus* species A, new species**

- Striae on T2 absent, basal grooves on T2 shallow and short, as long as wide (Fig. 3.18), basal grooves on T1 regular in length, punctuate, not wrinkled at corners (Fig. 3.18). From eggs of *Tabanus* spp. (Fig. 3.33).....***T. tabanivorus***

4(2) - Striae on T2 present.....5

- Striae on T2 absent.....7

5(4) - Only fine striae at meson of T2, extending ¼ of T2 length or shorter; T2 basally with 8 grooves, wide at meson (Fig. 3.19); body larger, length:width:height (110:33:30); eye height:malar space (19:9); metasoma distinctly elongate (53:25). From eggs of *G. chrysocoma* (Fig. 1.34).....***T. goniopis***

- Distinct striae on the middle of T2, extending 1/3 of T2 length or longer; T2 basally with 11 or more narrow grooves (Figs. 3.16, 3.21); body smaller (Fig. 3.30).....6

6(5) - Base of T2 with grooves and striae 3 times longer than length of T1 (Fig. 3.21); body larger, length:width:height (86:29:27); metasoma longer, length:width (40:24). ..... ***T. angustatus***

- Base of T2 with grooves and striae as long as T1 or slightly longer (Fig. 3.16); body smaller, length:width:height (78:26:23); metasoma shorter, length:width (35:20). From eggs of *Hybomitra* spp. (Fig. 3.30).....**T*elenomus* species B, newspecies**

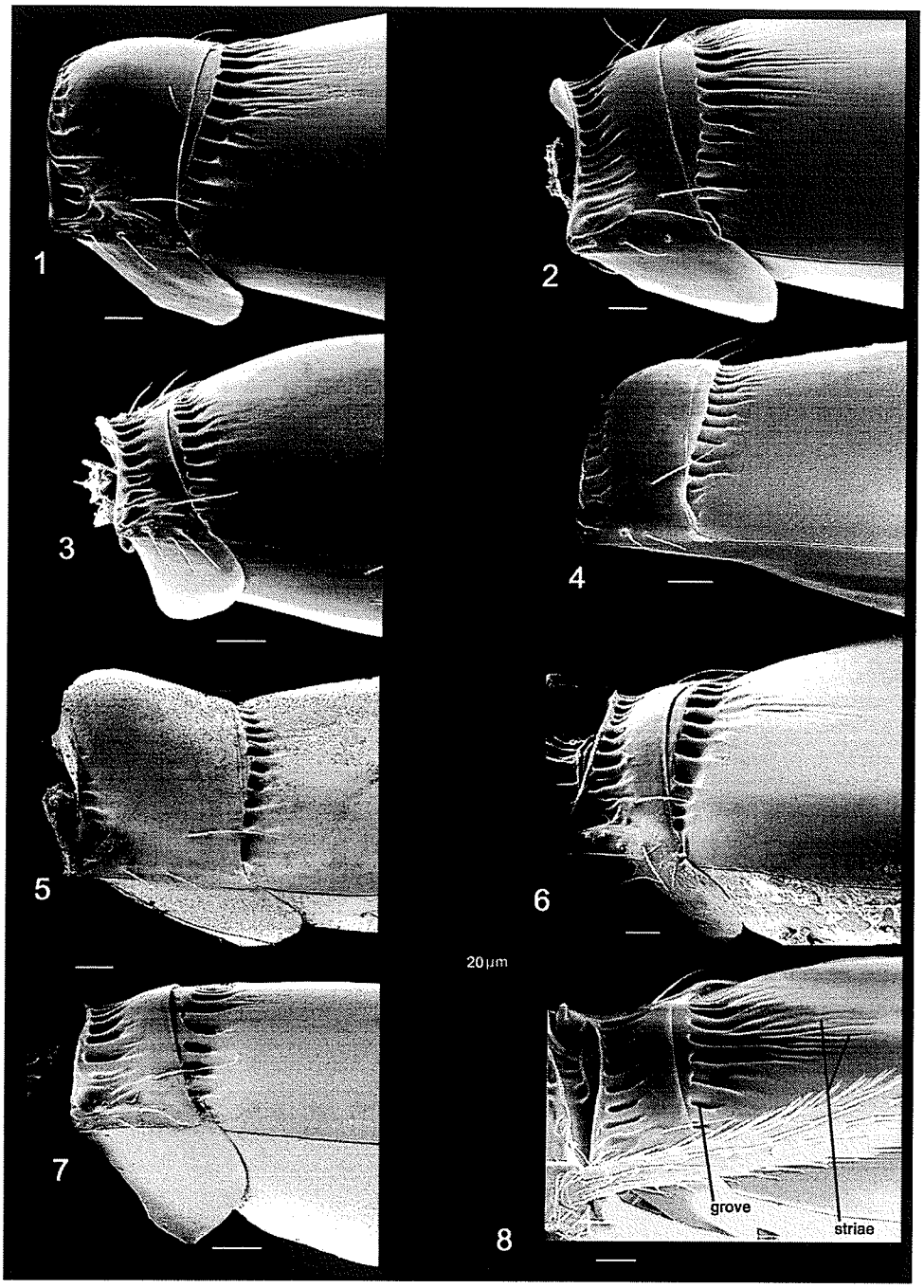
7(4) - T1 basally with grooves extending at least to ½ of T1 length (Fig. 3.17); basal grooves on T2 slightly longer than its width (Fig. 3.17); eye height:malar space (10:5); colour black. From eggs of *Chrysops* spp. (Fig. 3.31).....
..... ***Telenomus* species C, new species**

- T1 basally with grooves extending only 1/3 of T1 length (Fig. 3.20); basal grooves on T2 2-3 times longer than wide; eye height:malar space (13:5); colour light brown (Fig. 3.35).....***T. tabanocida***

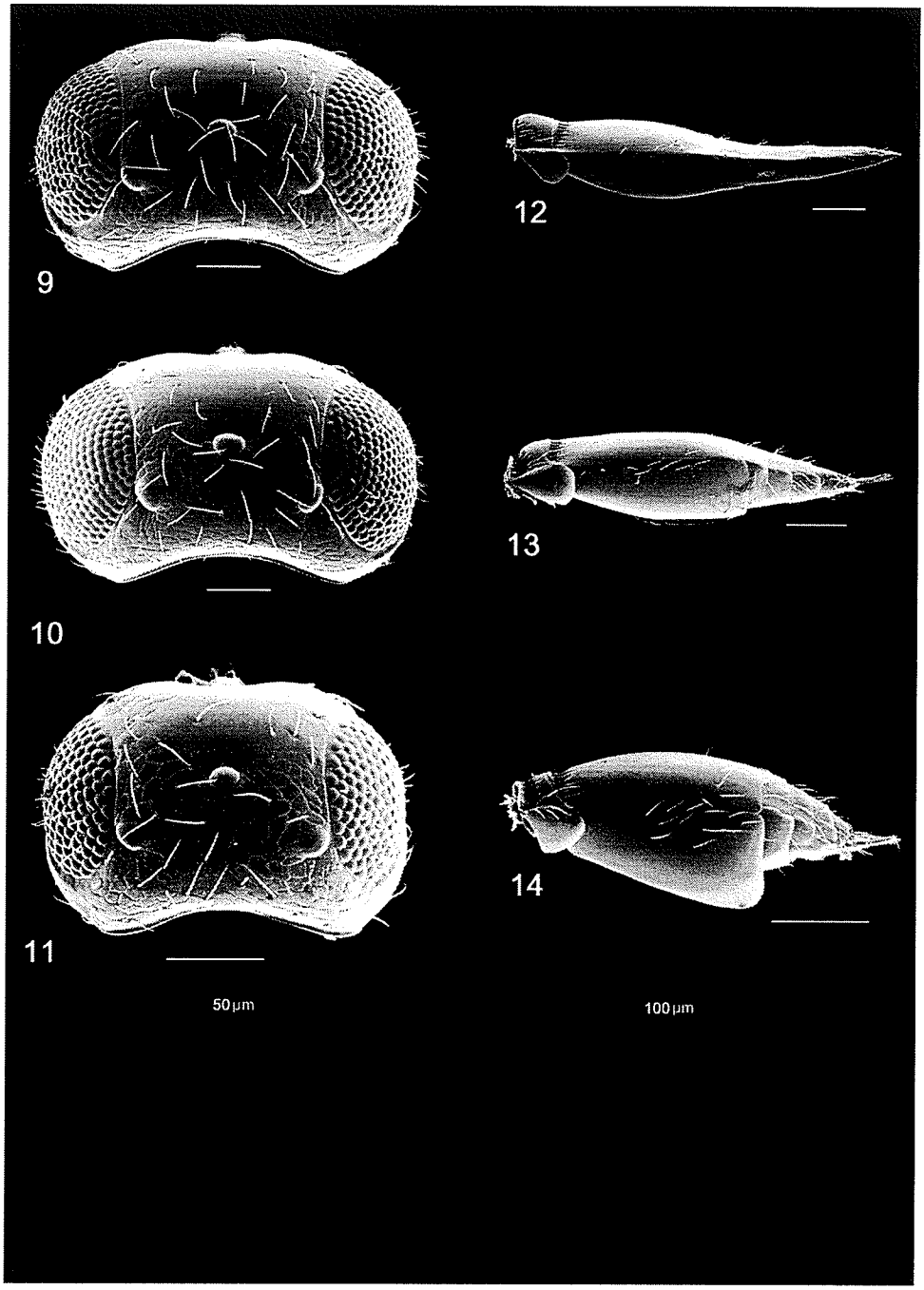
Discussion

Researchers have recognized the impact of scelionid egg parasitoids on tabanid populations in many studies (Crawford 1913; Girault 1916; Webb and Wells 1924; Cameron 1926; Parman 1928; Philip 1931; Schwardt 1936; Bailey 1948; Hatton 1948; Tashiro and Schwardt 1953; James 1963; Jones and Anthony 1964; Jackson and Wilson 1966; Dukes and Hays 1971; Goodwin 1976; Muesebeck 1979; Drees 1982). In southeastern Manitoba, more than 90% of the tabanid egg masses collected had been attacked by scelionid parasitoids, and about 70% of the individual eggs within egg masses were either parasitized or damaged by parasitoids (Iranpour and Galloway 2001).

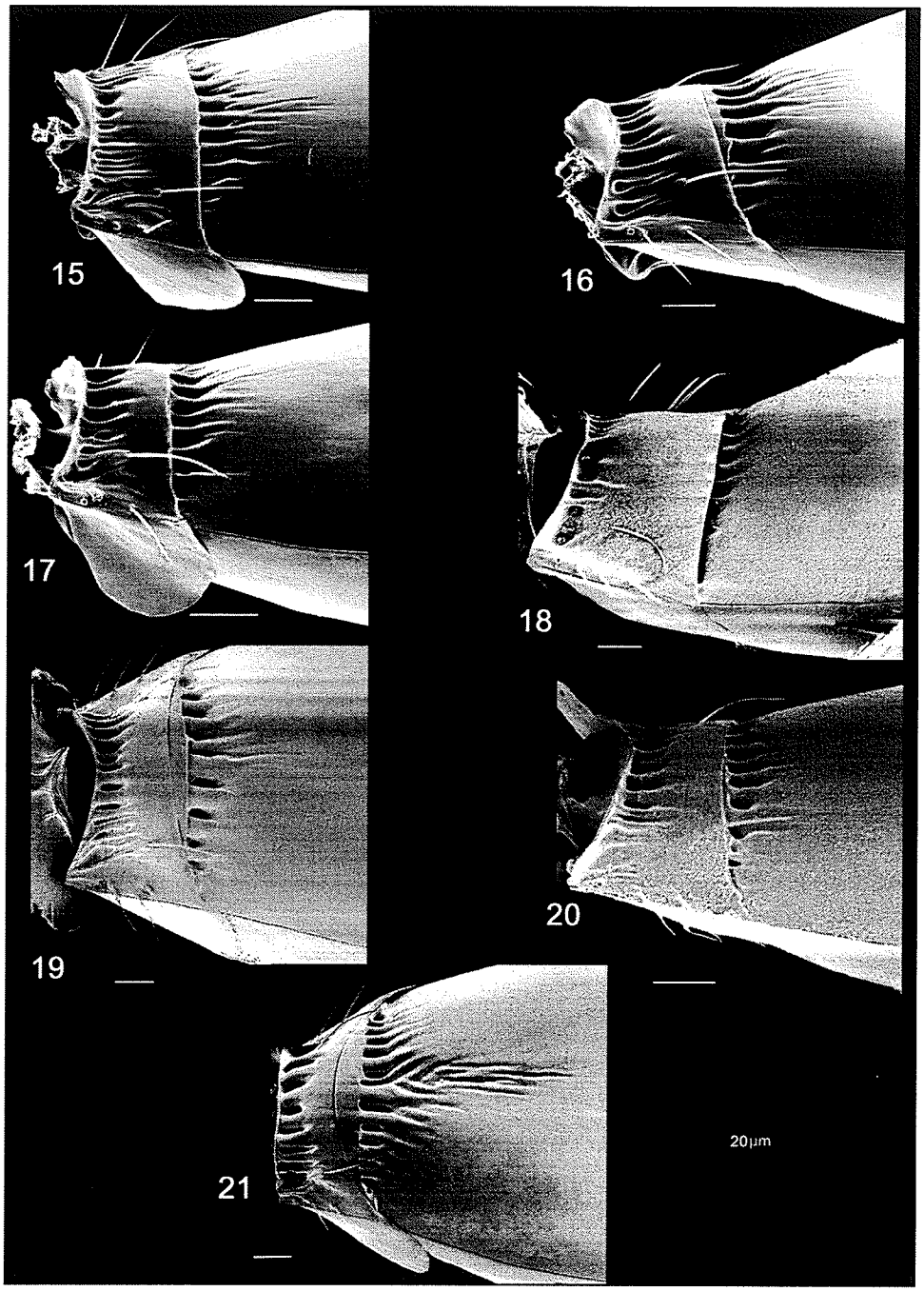
Before we can understand the nature of the relationship between these important egg parasitoids and their tabanid hosts, to consider their application in biological control programmes, we must be confident of the taxonomic status of the parasitoids under study. Upon close examination of the scelionids attacking tabanid eggs in a rather restricted area of Manitoba, we discovered three previously undescribed species. It is not unreasonable to expect that additional new species may be encountered, and careful collection, host association and rearing of parasitoids from tabanid egg masses should yield exciting results. A detailed systematic and biogeographic analysis of the *Telenomus* spp. which parasitize tabanid eggs was beyond the scope of this project. However, such an extensive study is clearly warranted.



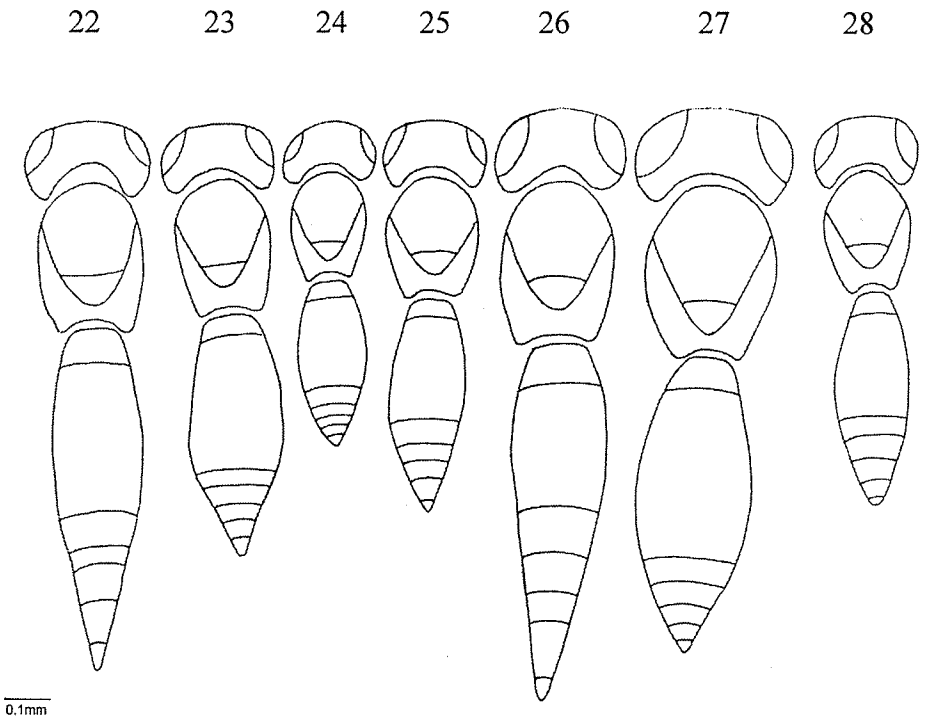
Figs. 3.1-8. T1 and T2 (females) in dorso-lateral view. 1, *Telenomus* species A; 2, *Telenomus* species B; 3, *Telenomus* species C; 4, *T. emersoni*; 5, *T. tabanivorus*; 6, *T. goniopis*; 7, *T. tabanocida*; 8, *T. angustatus* (each scale bar is equal to 20 microns).



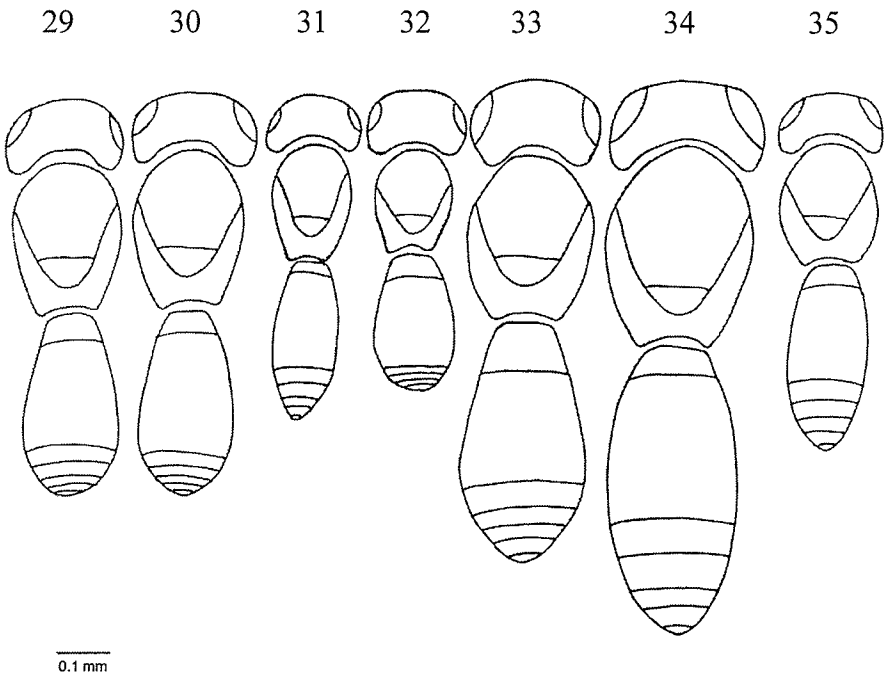
Figs. 3.9-14. 9-11, prosoma in dorsal view (females), each scale bar is equal to 50 microns; 12-14, metasoma in lateral view (females), each scale bar is equal to 100 microns; 9, 12 *Telenomus* species A; 10, 13 *Telenomus* species B; 11, 14 *Telenomus* species C.



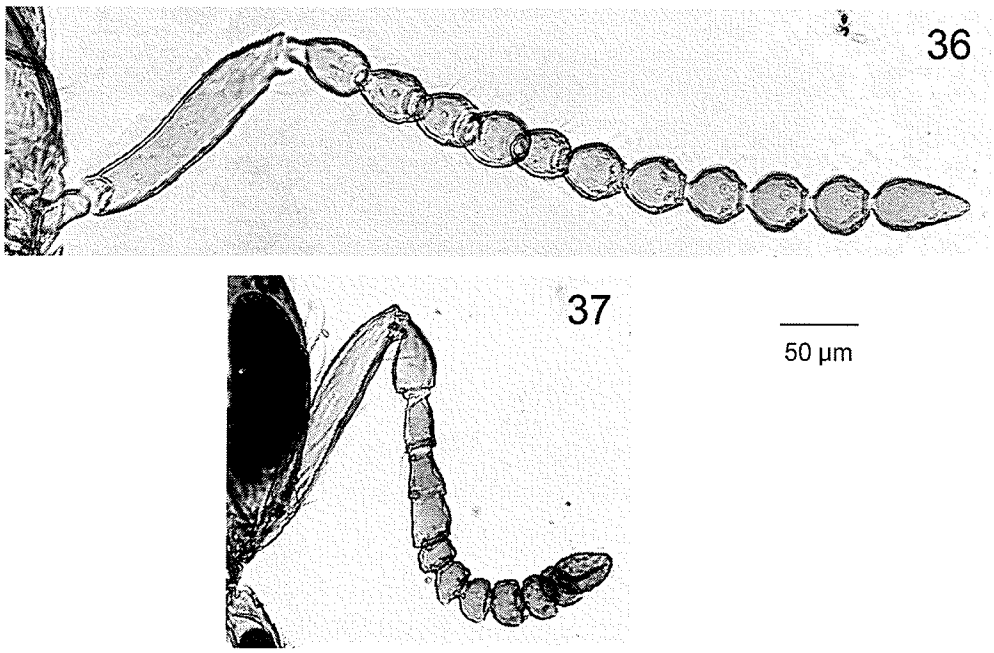
Figs. 3.15-21. T1 and T2 (males) in dorso-lateral view. 15, *Telenomus* species A; 16, *Telenomus* species B; 17, *Telenomus* species C; 18, *T. tabanivorus*; 19, *T. goniopis*; 20, *T. tabanocida*; 21, *T. angustatus* (each scale bar is equal to 20 microns).



Figs. 3.22-28. Body habitus (females) in dorsal view. 22, *Telenomus* species A; 23, *Telenomus* species B; 24, *Telenomus* species C; 25, *T. emersoni*; 26, *T. tabanivorus*; 27, *T. goniopis*; 28, *T. tabanocida*



Figs. 3.29-35. Body habitus (males) in dorsal view. 29, *Telenomus* species A; 30, *Telenomus* species B; 31, *Telenomus* species C; 32, *T. emersoni*; 33, *T. tabanivorus*; 34, *T. goniopis*; 35, *T. tabanocida*.



Figs. 3.36-37. Male antennae. 36, *Telenomus* species B; 37, *T. emersoni*.

CHAPTER IV

Molecular Characterization of Tabanidae (Diptera) in Manitoba Using the PCR-RFLP Method

Introduction

In certain areas of North America, flies of the family Tabanidae are among the most medically and economically important pests of livestock and humans. Through female blood feeding, tabanids are capable of inflicting direct injury, causing blood loss and transmission of pathogens, with the potential of spreading more than 30 disease agents, including viruses, bacteria, protozoa, and helminths to their hosts (Krinsky 1976). For example, the daily blood consumption from one cow due to *Hybomitra lasiophthalma* (Macquart), *Tabanus quinquevittatus* Wiedemann, and *Tabanus sulcifrons* Macquart was 352, 59, and 109 ml respectively (Tashiro and Schwardt 1953). In addition, hosts expend energy attempting to avoid painful bites. Pastured cattle exhibit the formation of grazing lines, bunching, head tosses, foot stomps, ear flicks, and tail switches in response to tabanid attacks (Ralley et al. 1993). Tabanids can affect the weight and fitness of their hosts as well. In a study by Perich et al. (1986), heifers exposed to attacks by an average of 90 flies per animal per day for 84 days gained 80 grams less per animal per day than those protected from horse flies.

Members of Tabanidae are present in temperate and tropical regions of the world. Approximately 4,350 species have been described, including 335 species and subspecies in North America north of Mexico (Burger 1995). There are 144 described species distributed in all parts of Canada and Alaska, except in the northern tundra zone where trees are absent (Teskey 1990). In Manitoba, there are 50 species in five genera: *Hybomitra*, *Tabanus*, *Chrysops*, *Atylotus*, and *Haematopota*. Adult tabanids are reasonably well known taxonomically in North America. However, the systematics of the

immature stages have not kept pace with that based on adult stages. Descriptions of larvae are available for some species, but detailed descriptions of tabanid egg masses are sketchy and anecdotal, with descriptions scattered throughout the literature (Teskey 1990). In the United States, descriptions of larvae and pupae have been completed for some species of tabanids (Goodwin 1976; Pechuman 1981). Teskey (1969) provided descriptive details of the immature stages for about 20% of the described species in the Nearctic region as well as descriptions of and keys to the larvae and pupae for 81 of 144 Tabanidae species found in Canada. Teskey (1969) suggested that the keys should be used with caution because information for many species and the range of variation to be expected in some species were not available. Even if a key for the immature stages of all species in Canada was available, it would still be necessary to rear larvae to the third or fourth instar for accurate identification. The major problems in studying the biology and taxonomy of larvae and pupae of species of Tabanidae include their inaccessibility in the soil, prolonged life cycle (one to three years), adult reluctance to mate and feed under laboratory conditions, aberrant oviposition behaviours in cages, refusal of larvae to feed, cannibalism in larvae, and mortality during ecdysis and pupation (Thompson and Krauter 1978).

Molecular methods have been used for tabanid systematics by some investigators. Hudson and Teskey (1976) used isozyme electrophoresis of esterase enzymes, in addition to differences in size and colour of characters, to segregate two forms of *Hybomitra typhus* (Whitney). Jacobson et al. (1981) distinguished two genetically distinct populations of *Tabanus nigrovittatus* Macquart by starch gel electrophoresis of enzymes.

The analysis of cuticular hydrocarbon profiles has been used to differentiate morphologically similar *Tabanus* species (Hoppe et al. 1990) and to compare three cryptic species in the *T. nigrovittatus* complex (Sutton and Carlson 1997). However, cuticular hydrocarbons may vary with the stage, age, and sex of insects (Pomonis and Mackley 1985; Pomonis 1989) and therefore may not be suitable for associating different stages of the same species.

Compared to hydrocarbons and proteins, DNA is more stable and does not vary in different developmental stages (Taylor et al. 1996). Thus, DNA analysis may be useful for identifying tabanids in the adult and immature stages. This became important in a study on wasps (Scelionidae and Trichogrammatidae) that parasitize tabanid eggs, where we discovered high prevalence of parasitism in egg masses collected in the field, but it was impossible to associate the parasitoids correctly with the species of tabanids which had laid the eggs. Our hypothesis was that the larvae that hatched from an egg mass could be identified by molecular analysis if we could identify molecular markers which were consistent between larvae and adults. Therefore, the purpose of this study was to use the polymerase chain reaction (PCR) and subsequent restriction fragment length polymorphism (RFLP) analysis to characterize adults of horse fly and deer fly species representing five genera (*Atylotus, Chrysops, Haematopota, Hybomitra,* and *Tabanus*) in Manitoba, Canada. Regions of mitochondrial DNA (mtDNA) and nuclear ribosomal DNA (rDNA) were amplified from adult flies for PCR-RFLP analysis to examine intraspecific and interspecific variations in the collected species. The effectiveness of the selected DNA regions for species identification and potential applications of the PCR-

RFLP technique for the classification of the immature stages is discussed. The results of our analysis of the larval stages and the application to the ecology of the egg parasitoids will be published in a subsequent paper.

Materials and Methods

Adult Collection and Identification - Adult tabanids were collected in Manitoba (Fig. 4.1) during June and July of 1999 and 2000. Manitoba Horse Fly Traps and aerial nets were used on hot, sunny days from 1000 to 1600 h to collect adult flies. Specimens were transported to the laboratory in a cooler and stored at –25°C in sealed plastic containers. Approximately 10 weeks after field collection, tabanids were removed from the freezer and identified to species using the identification key of Teskey (1990). The identified adults were then stored in 95% ethanol at
–25°C for DNA extraction.

DNA extraction - Total DNA was isolated by the procedure of Taylor et al. (1996) with modifications. For each individual adult, the thorax was ground to a fine powder in liquid nitrogen with a prechilled mortar and pestle. To this was added 400 µl lysis buffer (100 mM NaCl, 100 mM EDTA, 100 mM Tris, 0.5% SDS, pH 7.5) and 4 µg proteinase K and the lysis mixture was incubated at 55°C for 3 h with occasional mixing. Following the incubation, 2 µg RNase A was added and incubated at 37°C for 20 min. To this, 100 µl

phenol was added and the mixture was incubated at 55°C for 10 min. After the incubation, 100 µl chloroform:isoamyl alcohol (24:1) was added and the sample was centrifuged at 12,000 g for 5 min. The aqueous phase was removed and a second DNA extraction was done with an equal volume of chloroform:isoamyl alcohol (24:1) and the sample was then centrifuged at 12,000 g for 5 min. The aqueous phase was transferred to a fresh 1.5-ml microfuge tube and the DNA was precipitated with the addition of two volumes of cold 95% ethanol and 0.08 volumes 8 M LiCl and incubated at –60°C for 1 h. The sample was then centrifuged at 12,000 g for 20 min at 4°C. The DNA pellet was washed with 70 % ethanol, centrifuged at 12,000 g for 7 min at 4°C, vacuum-dried, and resuspended in 50 to 100 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.4).

PCR amplification - Eight primers (Table 4.1) were used to amplify four target sequences in the total DNA. Two regions from mtDNA were amplified: (1) primers P4 and P5 amplified a region including the 3' end of the NADH dehydrogenase I gene, the entire tRNA leucine ($tRNA^{leu}$) gene, and the 5' end of the large subunit ribosomal RNA gene (Fig. II.2b), and (2) primers P6 and P7 amplified a region including the 3' end of the cytochrome *b* gene, the complete tRNA serine ($tRNA^{ser}$) gene, and the 5' end of the NADH dehydrogenase I gene (Fig. II.2a). Two additional regions from nuclear rDNA were amplified: (1) primers CS_{249} and FL amplified the internal transcribed spacer (ITS) between the 18S and 28S rRNA genes including the 5.8S rRNA gene (Fig. II.2d), and (2) primers FL-3 and P2 amplified the intergenic spacer (IGS) between the 28S and 18S rRNA genes (Fig. II.2C).

All amplifications were carried out in a 50 µl total volume using a Techne Unit Genius Thermocycler (Techne Incorporated, Princeton, NJ). Each reaction mixture consisted of 5 µl *Taq* Extender 10x reaction buffer (Stratagene, La Jolla, CA), 200 µM each of dATP, dTTP, dCTP, and dGTP (Invitrogen, Carlsbad, CA), 1.5 mM $MgCl_2$, 20 pmol of each primer, 1.25 U *Taq* DNA polymerase (Invitrogen), 1.25 U *Taq* Extender (Stratagene), and approximately 10 ng of total DNA. The reaction mixtures for all mtDNA and nuclear rDNA targets were subjected to an initial denaturation at 93°C for 3 min. Cycling parameters for both mtDNA targets were 1 min at 93°C , 1 min at 42°C, and 1 min at 72°C for a total of 35 cycles. For the nuclear rDNA targets, reaction mixtures were subjected to 1 min at 93°C, 1 min at 50°C, and 1 min at 72°C for 30 cycles for the ITS, and 1 min at 93°C, 1 min at 50°C, and 10 min at 65°C for 20 cycles for the IGS. To assess the efficiency of the amplification, 5 µL aliquots of PCR products were separated on 1% agarose gels prepared with 1x TBE buffer (89 mM Tris-HCl, 89 mM boric acid, and 20 mM EDTA), stained with ethidium bromide, and visualized under ultraviolet light.

Restriction endonuclease digestions - Eight to 12 µl of amplified DNA were digested with 2.5 U each of *Hin*fI, *Mbo*I, *Rsa*I, *Taq*I, *Alu*I, *Hpa*II, *Msp*I, *Hae*I, *Eco*RV, and *Cfo*I according to manufacturer's specifications (Invitrogen). All restriction digest reactions were stopped after 2 h with the addition of 3.0 µl gel loading buffer (40% (w/v) sucrose, 0.25% bromophenol blue, and 20mM EDTA) to each reaction tube. Digested DNA fragments were separated on a 1.5% agarose gel in 1x TBE buffer, stained with ethidium

bromide, and photographed under UV light. The length of each restriction fragment was estimated using the 1 kb Plus DNA Ladder (Gibco BRL) (Fig. II.1). Only fragments ranging in size from 100-3000 bp were considered in determining diagnostic bands for each species.

Results

Mitochondrial tRNA genes - PCR amplification of the mitochondrial tRNA genes was done for one individual each of *Hybomitra nitidifrons nuda* (McDunnough), *H. lasiophthalma,* and *Chrysops aestuans* Wulp. P4-P5 amplification of the $tRNA^{leu}$ gene produced a single PCR product of approximately 320 bp for each species (Figs. II.3, 4). Amplification of the $tRNA^{ser}$ gene with primers P6 and P7 yielded an amplicon of 350 bp for all species (Figs. II.4, 5). Restriction digestions of PCR products were done using *Alu*I, *Hin*fI, *Taq*I, *Rsa*I, *Hpa*II, and *Eco*RV. For P4-P5 amplicons, no sites were present for *Hin*fI, *Hpa*II, *Eco*RV, and *Taq*I. *Rsa*I digestions resulted in three fragments (140, 130, and 50 bp) for all three species. There was a single *Alu*I site for *H. n. nuda*, which was absent in *H. lasiophthalma* and *C. aestuans*, which produced two fragments 260 and 60 bp in size. For P6-P7 PCR products, no sites were present for *Taq*I, *Hin*fI, and *Alu*I. A single *Rsa*I site in *C. aestuans* resulted in two fragments (280 and 70 bp) but was absent in the other two species. Restriction digestions with *Hpa*II and *Eco*RV each produced two fragments (150 and 200 bp with *Hpa*II; 230 and 120 bp with *Eco*RV) for all three species.

Internal Transcribed Spacer - Primers CS_{249} and FL were used to amplify the ITS for single individuals of 11 species [*H. n. nuda, H. lasiophthalma, Hybomitra epistates* (Osten Sacken), *Hybomitra illota* (Osten Sacken), *Hybomitra affinis* (Kirby), *Hybomitra zonalis* (Kirby), *Hybomitra trepida* (McDunnough), *Hybomitra arpadi* (Szilady), *Tabanus similis* Macquart, *Tabanus marginalis* Fabricius, and *C. aestuans*]. A single PCR product of approximately 1,500 bp was produced for all species except *C. aestuans,* for which the amplicon was about 1,800 bp. Restriction digestions were done using *Rsa*I, *Msp*I, *Taq*I, *Cfo*I, and *Hae*I (Appendix II, Figs. 6-10). *Rsa*I digestions resulted in two fragments for all species. Fragment sizes were 1,350 and 150 bp except for *H. epistates* (850 and 650 bp) and *C. aestuans* (1,450 and 350 bp). *Msp*I did not cleave the amplicons except for *H. arpadi,* producing two fragments (1,110 and 400 bp). The *Taq*I digestions produced three fragments (650, 600, and 550 bp) for *C. aestuans* and two fragments of approximately 950 and 550 bp in size for the remaining ten species. The *Cfo*I digestion resulted in two fragments of 900 and 600 bp in size for *T. similis*, but the enzyme did not cleave PCR products for the other ten species. There were no *Hae*I sites present in amplicons for any of the eleven species.

Intergenic Spacer - Amplification of the IGS using primers FL-3 and P2 resulted in a wide range of amplicon sizes for the 35 species representing five genera studied, and usually resulted in multiple amplicons per species (Table 4.2; Figs. II.11-25). For seven species [*Hybomitra brennani* (Stone), *Hybomitra liorhina* (Philip), *Hybomitra pechumani* (Teskey & Thomas), *H. trepida, Chrysops indus* Osten Sacken, *Chrysops nigripes* Osten

Sacken, and *Atylotus calcar* Teskey], a single PCR product ranging in size from approximately 4.0 to 8.0 kb was produced. For *C. indus, H. pechumani,* and *H. trepida*, for which more than one individual was tested, the size of the amplicon was the same for all individuals within a species. For the remaining species, multiple PCR products, from 2 to 11 per individual, were produced, with a wide range in sizes from approximately 1.7 to 8.5 kb. In most cases, intraspecific variation in the size and number of amplicons was observed when more than one individual of a species was examined (Table 4.2).

The complete amplification product for each individual was digested using *Hin*fI, *Mbo*I, *Rsa*I, and *Taq*I, and up to eleven fragments were produced for each species (Figs. II.26-42). Restriction fragment patterns for the most typical individual of each species are shown in Figs. 4.2 and 4.3. Restriction fragment sizes of bands which were bright in appearance and comprised the diagnostic profile for each species are presented in Table 4.3. In most cases, restriction fragment patterns were identical for all individuals of the same species, as illustrated by the data for *H. epistates* (Fig. 4.4). However, in *H. affinis, Hybomitra frontalis* (Walker), *H. illota, H. n. nuda,* and *H. zonalis*, some intraspecific variation was observed (Figs. II.33, 35, 38, 40). For example, the *Rsa*I and *Hin*fI digestions for individuals 3 and 5 of *H. frontalis* had a 150 and 160 bp fragment, respectively, which was absent in the other four individuals (Fig. 4.5). As well, individual 6 had a unique 1,400 bp *Rsa*I fragment.

Discussion

The development of molecular markers to assist in the identification and taxonomy of species of Tabanidae is valuable because of their medical and economic importance. The choice of an appropriate target sequence for analysis is essential for classifying individuals to the desired taxonomic level. Regions from mtDNA and nuclear rDNA were examined for their suitability for species identification of adult tabanids.

Restriction digestions of PCR products of mitochondrial tRNA genes for leucine and serine were not effective for differentiating the three species examined. Using six restriction endonucleases, only two enzymes produced fragment patterns that were diagnostic for a particular species. The *Alu*I digestion of the $tRNA^{leu}$ gene PCR product differentiated *H. n. nuda* from *H. lasiophthalma* and *C. aestuans* while the *Rsa*I digestion of the $tRNA^{ser}$ gene PCR product distinguished *C. aestuans* from *H. n. nuda* and *H. lasiophthalma.* No single combination of restriction enzyme and PCR product successfully differentiated all three species from one another.

Mitochondrial DNA is useful for studies of population biology and phylogenetics because of its maternal transmission, abundance, relatively rapid evolution, and large number of coding sequences (Moritz et al. 1987). While mtDNA in vertebrates tends to evolve about five to ten times faster than nuclear DNA (nDNA) (Brown et al. 1979), mtDNA and nDNA in *Drosophila* evolve at approximately equal rates (Powell et al. 1986; Solignac et al. 1986; Vawter and Brown 1986). PCR-RFLP analysis using mitochondrial tRNA genes has been used in an attempt to differentiate two species of Calliphoridae (Taylor et al. 1996) and four species of *Muscidifurax* (Taylor et al. 1997). In each study, initial screening with 27 restriction endonucleases resulted in only a

limited number of combinations of enzymes and PCR products that could be used to distinguish one species from another. Therefore, mitochondrial tRNA genes may be more useful in studies of distantly related species when data from many tRNA genes are combined (Simon et al. 1994).

Nuclear ribosomal DNA in eukaryotes is a multigene family comprised typically of several hundred repeat units arranged in head-to-tail tandem arrays. For example, the rDNA gene family in *Drosophila melanogaster* Meigen consists of approximately 250 tandem repeat units located in the nucleolar organizers of both X and Y chromosomes (Ritossa 1976). Each repeat unit contains an 18S, 5.8S, and 28S rRNA gene separated by two internal transcribed spacers (ITS-1 and ITS-2). The IGS separates repeat units from one another. Ribosomal DNA evolves by concerted evolution which is the nonindependent evolution of repetitive DNA sequences resulting in a sequence similarity that is greater within than among species (reviewed by Elder and Turner 1995). Different regions of the repeat unit evolve at different rates. The coding regions evolve slowly than the spacer regions, making the IGS and ITS especially useful for the study of phylogenies of closely related species (reviewed by Hillis and Dixon 1991).

In this study, using restriction digestions of the ITS (ITS-1 and ITS-2, including the 5.8S rRNA gene), we were able to differentiate groups of species, and in some cases individual species, from one another. Digestions with *Cfo*I and *Msp*I only cleaved PCR products for *T. similis* and *H. arpadi,* respectively, and thus we were able to distinguish these two species from the other 10 species. The *Rsa*I digestion produced two different fragment profiles for *H. epistates* and *C. aestuans,* but the enzyme failed to cleave the

amplicon for the remaining nine species. *Taq*I digestions resulted in identical fragment patterns for all species, except *C. aestuans,* which had a unique profile.

PCR-RFLP analysis of the nuclear rDNA ITS with five restriction enzymes therefore provided better resolution for species identification than did the mtDNA tRNA genes. The restriction fragment patterns with some enzymes were used to distinguish certain species from one another, but for the most part could only be used to identify groups of species which shared the same profiles. No single enzyme was capable of distinguishing all eleven species from one another. PCR-RFLP analysis of the rDNA ITS may therefore not be useful for differentiating

a large number of species from one another, but may rather be effective for identifying individual species or a small number of species from a larger subset using the appropriate restriction enzymes. While the spacer regions in rDNA evolve more rapidly than the coding regions, the ITS is somewhat more conserved compared to the IGS (reviewed by Hillis and Dixon 1991). Sequence analysis of the ITS may provide a higher degree of resolution, compared to PCR-RFLPs, for inferring phylogenetic relationships of closely related species. This has been used for other genera of Diptera including *Aedes* (Wesson et al. 1992), *Drosophila* (Schloetter et al. 1994), *Culex* (Miller et al. 1996), and *Anopheles* (Hackett et al. 2000).

Since the IGS evolves the fastest among rDNA spacer regions, it has the potential to provide a region more suitable than mtDNA or tRNA genes or the rDNA ITS for identification of closely related species using PCR-RFLP analysis. PCR amplification of the IGS for 35 species of Tabanidae resulted in a wide range of amplicon sizes (1.7 to 8.5

kb). Concerted evolution homogenizes sequences of rDNA repeat units, including the IGS, within a species. For eight species, amplification of the IGS resulted in a single PCR product. However, anywhere from 2 to 11 amplicons per individual were produced for the remaining species. Variation in the length and sequence of rDNA spacer regions is often observed within individuals, a pattern of concerted evolution which has been demonstrated in *D. melanogaster* (Coen et al. 1982). Often, size differences in the IGS within individuals of a species are due to variation in the number of smaller subrepeat sequences within the IGS. Unequal crossing-over between subrepeats within the IGS is a mechanism by which such variation in repeat size within an individual may arise (Coen and Dover 1983). Variation in the number of subrepeats has been shown to be the source of IGS size variation in insect genera such as *Calliphora* (Schafer et al. 1981), *Chironomus* (Israelewski and Schmidt 1982), *Drosophila* (Tautz et al. 1987), *Glossina* (Cross and Dover 1987), and *Aedes* (Baldridge and Fallon 1992; Wu and Fallon 1998), and could be the cause of the multiple PCR products of the IGS for some Tabanidae species presented here.

The presence of and variation in numbers of multiple PCR products in some species did not hinder the effectiveness of RFLPs in differentiating species. This is due, presumably, to the likelihood that the multiple amplicons produced from one IGS are subsets of the same master sequence. However, the sum of restriction fragment sizes often did not add up to the size of the original PCR product. Comigration of bands very similar in size may have resulted in them being recorded as single fragments which could account for fragment size sums less than that of the original amplicon size. Multiple PCR

products in certain individuals may have produced variable fragment patterns which could explain why the fragment size sum was sometimes greater than the original amplicon size. In some cases, the sum of the restriction fragments was very close to the sum of the original PCR product.

PCR-RFLP analysis of the IGS revealed very little intraspecific variation in fragment patterns, and the interspecific variation was great enough to differentiate successfully all 35 species from one another. For 20 species, multiple individuals of each species were analyzed. For sixteen of these species (*H. affinis, H. arpadi*, *H. epistates*, *H. lasiophthalma*, *Hybomitra lurida* (Fallén), *H. pechumani*, *H. trepida*, *C. aestuans*, *Chrysops dawsoni* Philip, *Chrysops excitans* Walker, *Chrysops frigidus* Osten Sacken, *C. indus*, *Chrysops mitis* Osten Sacken, *Chrysops venus* Philip, *T. marginalis*, and *T. similis*), no intraspecific variation was observed among individuals of the same species. Even among individuals of *H. affinis, H. lurida,* and *Chrysops furcatus* Walker isolated from Churchill, Manitoba (the most northerly location from which flies were obtained), restriction fragment patterns were identical to those individuals of their respective species from the southern regions of the province. While minor intraspecific differences in restriction fragment patterns were observed for the remaining four species (*H. frontalis, H. illota, H. n. nuda,* and *H. zonalis*), more than 80% of the fragments were common to all individuals of the same species and therefore diagnostic for these four species. It is possible that some of these intraspecific variations may be reflected at the population level. For example, two individuals of *H. frontalis* had variant *Rsa*I and *Hin*fI fragment profiles (Fig. 4.5). Based on the colour pattern of the adult body, McAlpine (1961) found

eight different morphs of *H. frontalis* in the Nearctic region. He suggested that the *H. frontalis* complex of northern, eastern, and western forest regions consisted of a continuous population with different morphs (McAlpine 1961). The variation in the IGS restriction patterns reported here may be a reflection of the morphological variation present among the morphs. Similar relationships between morphological and molecular variation may also be reflected in the other tabanid species which displayed intraspecific variation in the IGS. Morphological and molecular data from additional individuals, along with biological information about populations of these species, would be necessary to reach conclusions about species boundaries. For the remaining 15 species, only one individual was available for analysis, thus, levels of intraspecific variation could not be determined.

Tabanids are difficult to manipulate under laboratory conditions. This is the main reason that the taxonomy of the immature stages is not well known. The PCR-RFLPs of the IGS have the potential to be used to identify the immature stages quickly and effectively by comparing restriction fragment profiles of DNA from egg masses or larvae to the adult profiles presented here. This has been investigated by the authors with several egg masses collected in Manitoba and the results will be published in a subsequent paper. RFLP analysis of the IGS may also be used to confirm the identification of adult flies based on classical morphological keys. For DNA isolation, it is possible to use a part of the body which is not crucial for morphological identification, such as the thorax, and preserve the rest of the body for future reference.

PCR-RFLP analysis of the rDNA IGS was a very effective, inexpensive, and rapid technique for providing molecular markers for the identification of individual tabanids to the species level. The use of RFLPs of the rDNA ITS and mitochondrial tRNA genes was much less successful. RFLPs of the IGS, using only four restriction enzymes, successfully differentiated 35 species representing five genera of Tabanidae in Manitoba. Each restriction enzyme produced species-specific restriction fragment profiles, so that initial screening with a large number of enzymes was unnecessary. Despite its rapid rate of evolution and size heterogeneity within species, the IGS was not too variable for species identification using PCR-RFLP analysis. These characteristics of the IGS may complicate sequence analysis using this region, and the ITS might still be a more suitable target for sequencing and phylogenetic analysis of Tabanidae. A low degree of intraspecific variation in fragment profiles was observed, even when isolates from as far north as Churchill were compared with those from southern Manitoba. The molecular data obtained from this study support the species boundaries that have been established by morphological data, which implies that the adult taxonomy of this group of insects is well studied at the species level. Thus, molecular markers, such as IGS-RFLPs, can aid in the identification process, providing a rapid and accurate method to screen and identify individual tabanids and their immature stages.

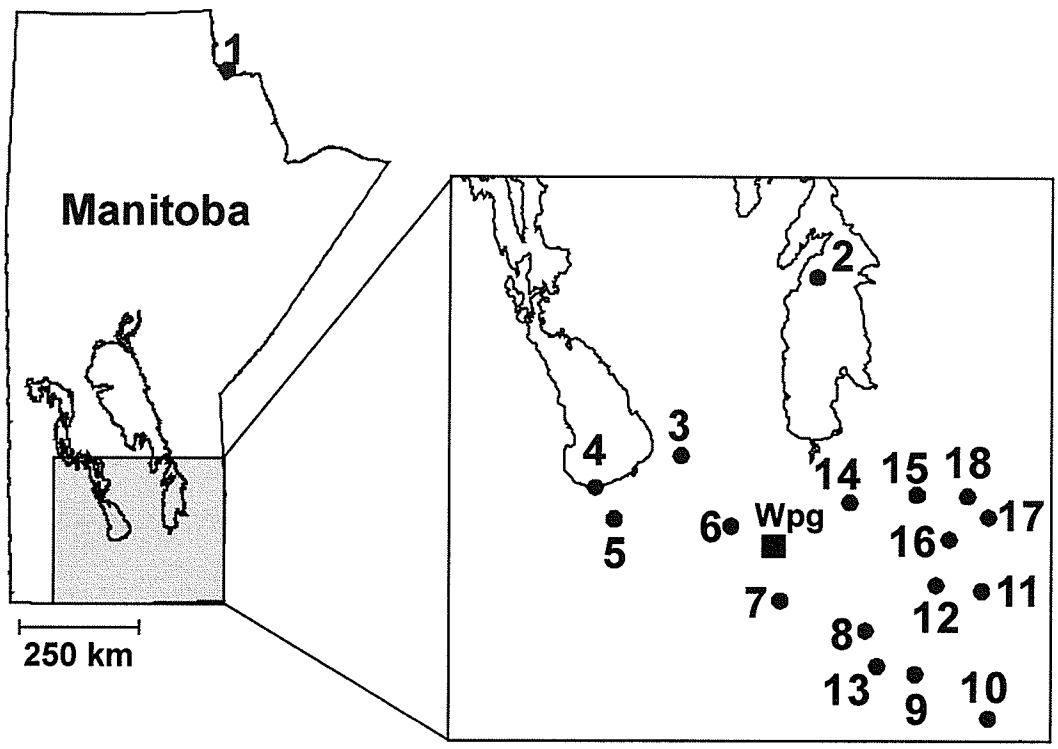


Fig. 4.1. Manitoba locations from which adult tabanids were collected in June and July of 1999 and 2000. Numbers on map correspond to the following locations: 1, Churchill; 2, Hecla Island; 3, West Shoal Lake; 4, Delta Beach; 5, Portage la Prairie region; 6, St. Charles Rifle Range, Winnipeg; 7, Glenlea Research Station; 8, Marchand; 9, Woodridge; 10, Sprague; 11, McMunn; 12, Hadashville; 13, Sandilands; 14, Beausejour; 15, Seven Sisters; 16, Elma; 17, White Lake; 18, Pinawa; Wpg, Winnipeg.

Fig. 4.2. A gel showing restriction digestions of IGS PCR products with (A) *Hin*fI, (B) *Mbo*I, (C) *Rsa*I, and (D) *Taq*I. Lanes (geographic locations in parentheses): 1, *Hybomitra affinis* (Woodridge); 2, *H. arpadi* (Woodridge); 3, *H. brennani* (Woodridge); 4, *H. criddlei* (Woodridge); 5, *H. epistates* (Woodridge); 6, *H. frontalis* (Elma); 7, *H. illota* (Woodridge); 8, *H. lasiophthalma* (Hadashville); 9, *H. liorhina* (St. Charles Rifle Range, Winnipeg); 10, *H. longiglossa* (McMunn); 11, *H. lurida* (Elma); 12, *H. nitidifrons nuda* (Woodridge); 13, *H. pechumani* (Elma); 14, *H. pediontis* (West Shoal Lake); 15, *H. tetrica* (Portage la Prairie region); 16, *H. trepida* (White Lake); 17, *H. zonalis* (Elma); 18, *Tabanus atratus* (Glenlea Research Station); 19, *T. marginalis* (Elma); 20, *T. reinwardtii* (Beausejour); 21, *T. similis* (Portage la Prairie region); 22, *T. vivax* (Woodridge); 23, *Chrysops aestuans* (Elma); 24, *C. ater* (Woodridge); 25, *C. dawsoni* (Woodridge); 26, *C. excitans* (Woodridge); 27, *C. frigidus* (Elma); 28, *C. furcatus* (McMunn); 29, *C. indus* (Elma); 30, *C. mitis* (Woodridge); 31, *C. sackeni* (White Lake); 32, *C. venus* (Marchand); 33, *Atylotus calcar* (Delta Beach); 34, *Haematopota americana* (Pinawa); L, 1 kb Plus DNA Ladder (Band sizes for ladder: 100, 200, 300, 400, 500, 650, 850, 1,000, 1,650, 2,000, and 3,000 bp).

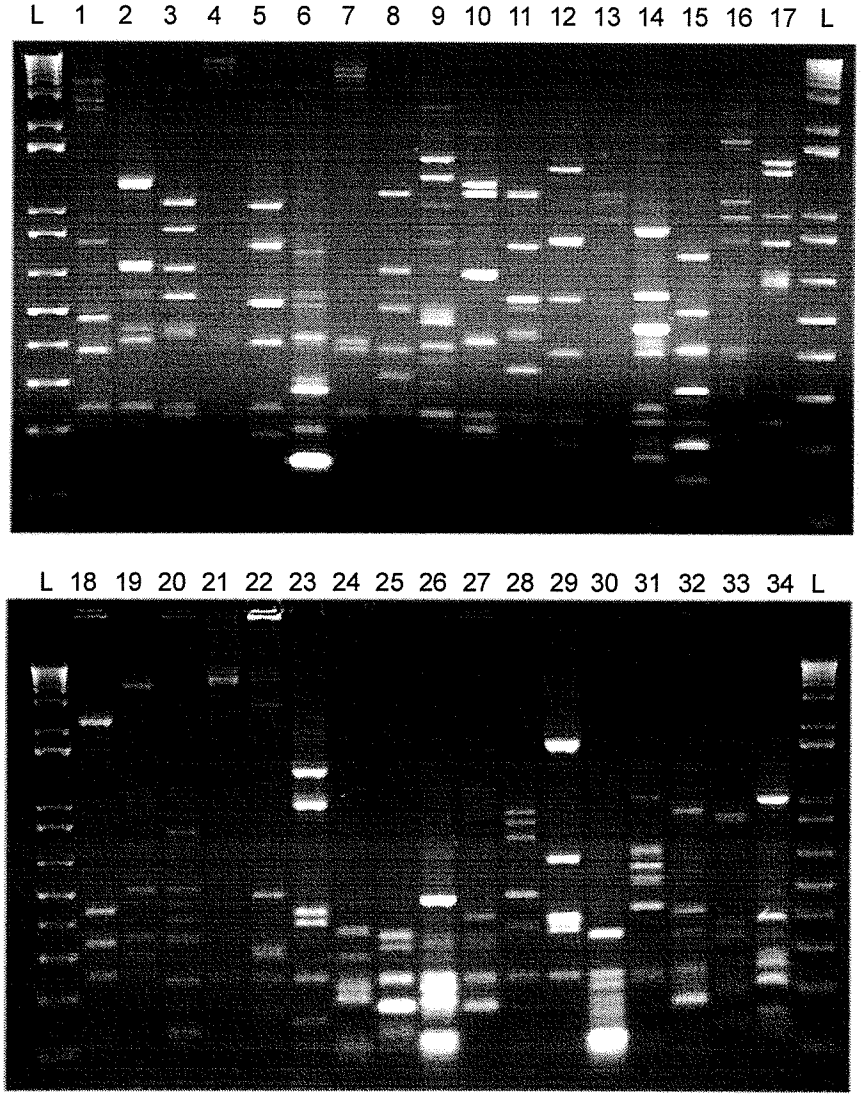

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 L
L 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 L

Fig. 4.2A

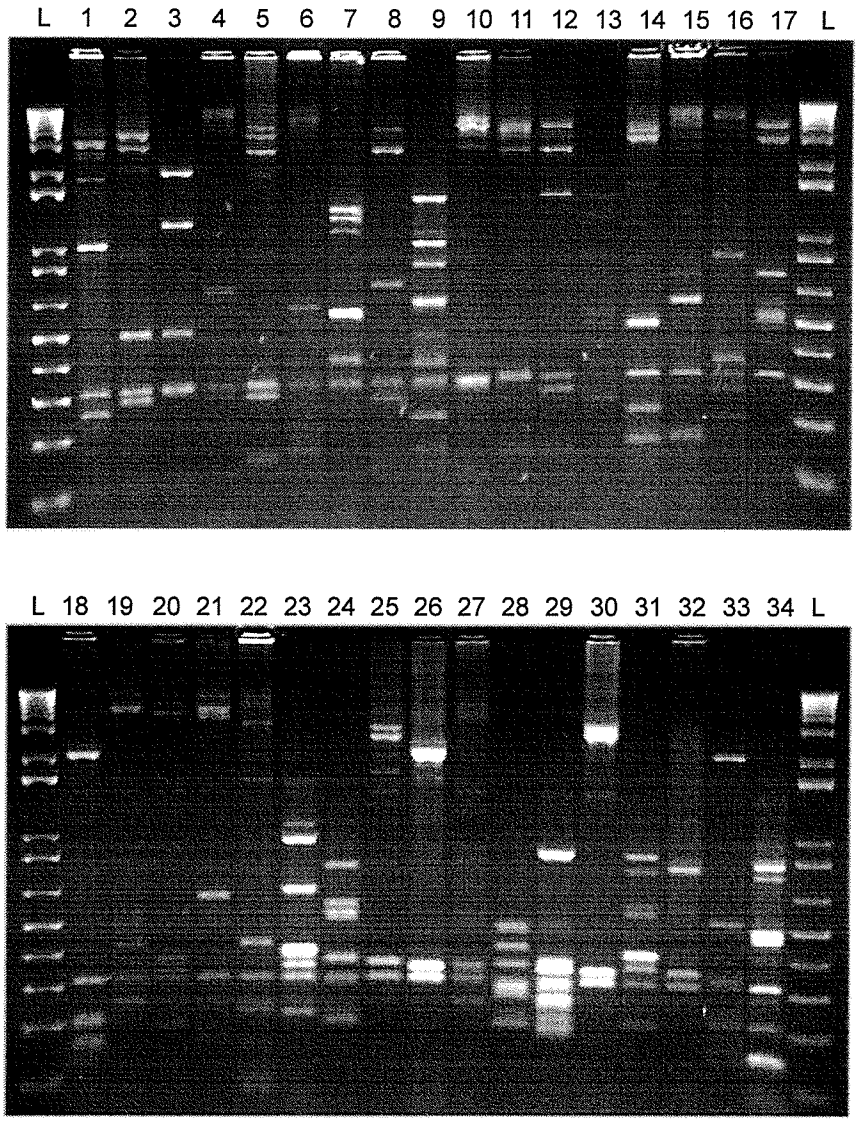

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 L
L 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 L

Fig. 4.2B

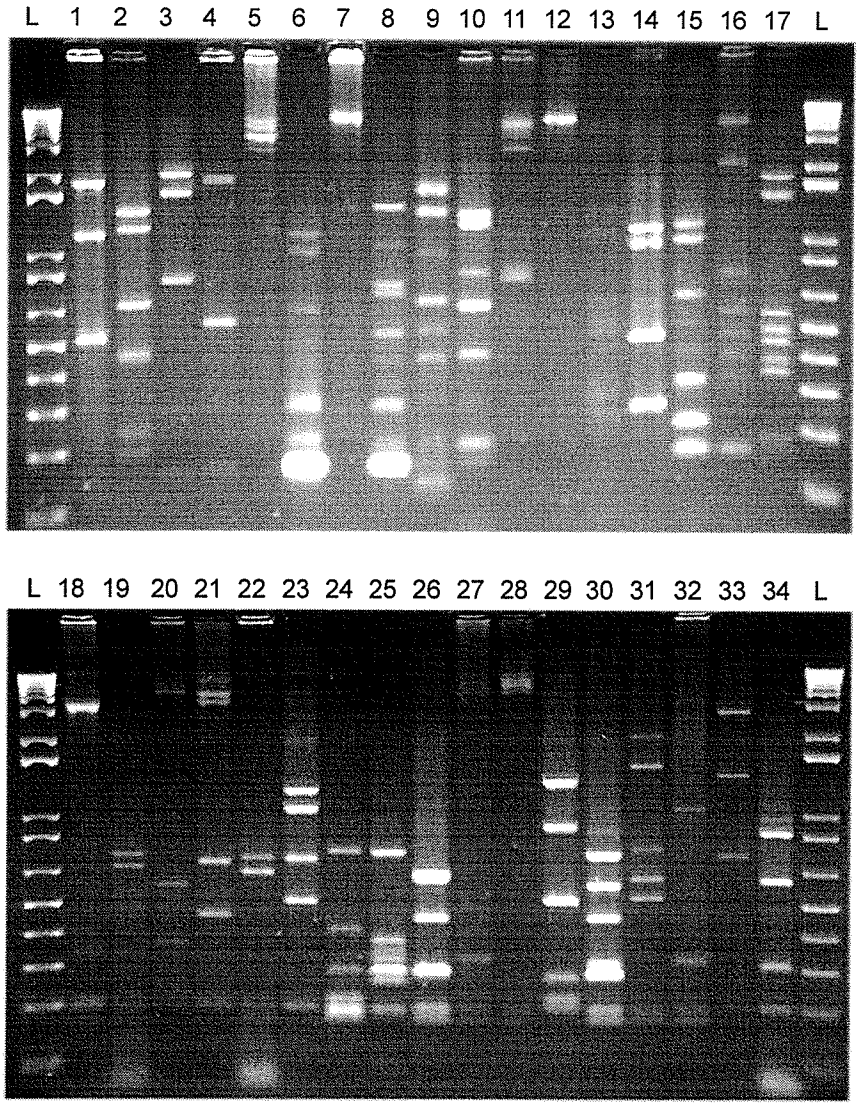

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 L
L 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 L

Fig. 4.2C

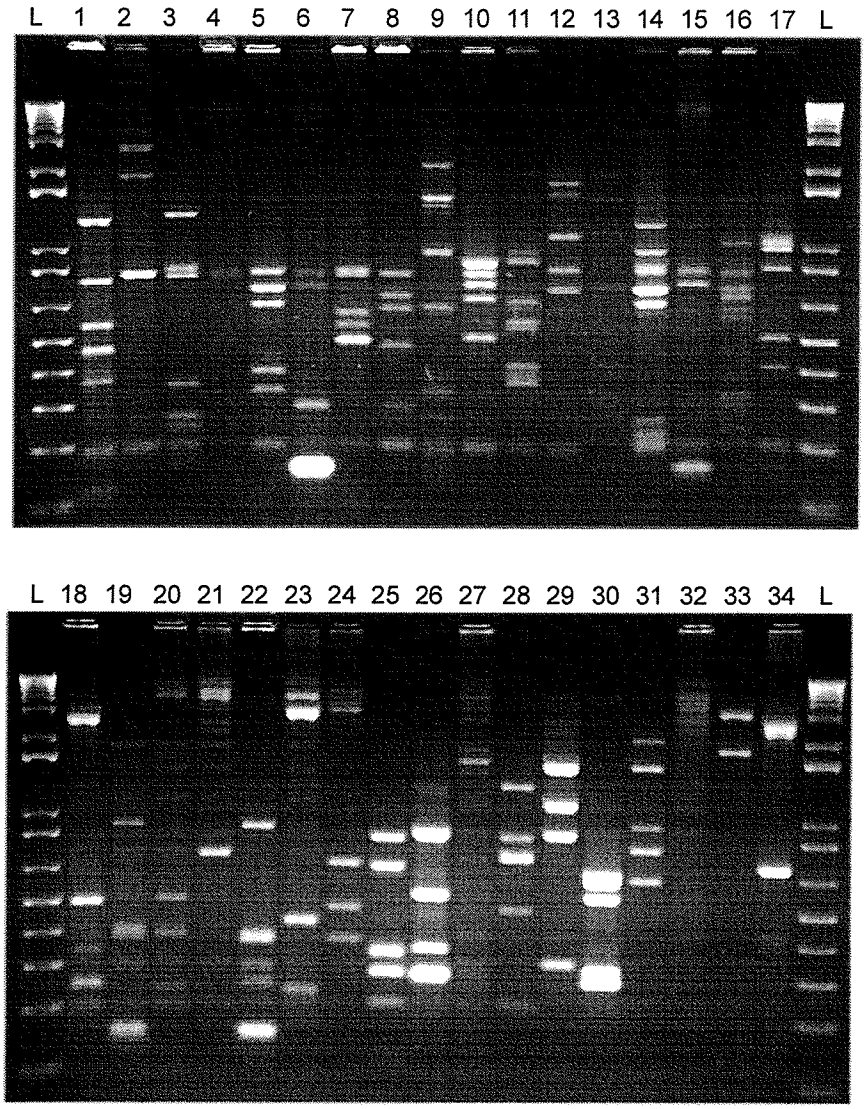

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 L
L 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 L

Fig. 4.2D

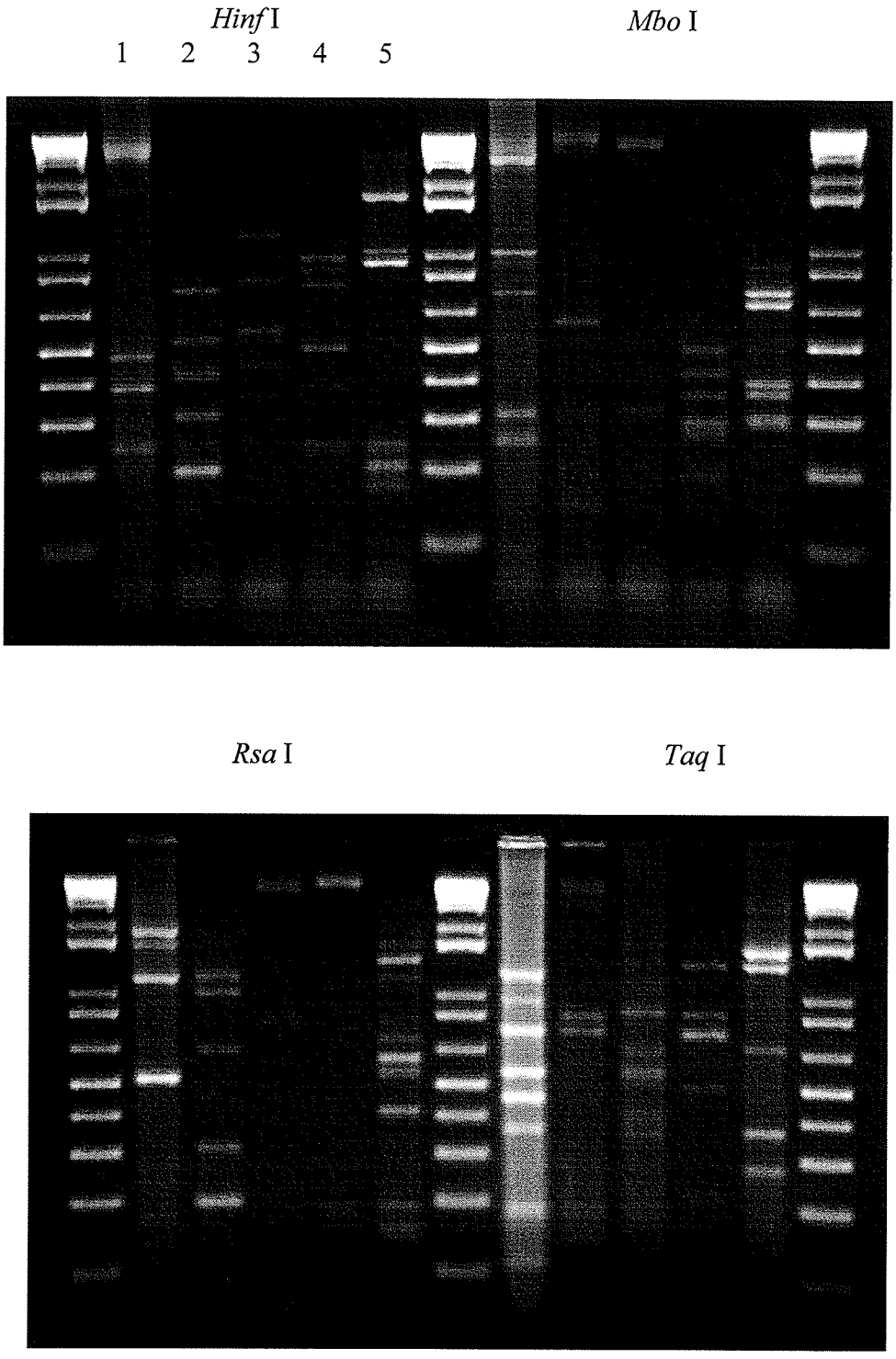


Fig. 4.3. A gel showing restriction digestions of IGS PCR products of Tabanidae collected from Churchill, Manitoba. Lanes: 1, *Hybomitra affinis*; 2, *H. frontalis*; 3, *H. lurida*; 4, *Chrysops furcatus*; 5, *C. nigripes*; L, 1 kb Plus DNA Ladder (Band sizes for ladder: 100, 200, 300, 400, 500, 650, 850, 1,000, 1,650, 2,000, and 3,000 bp).

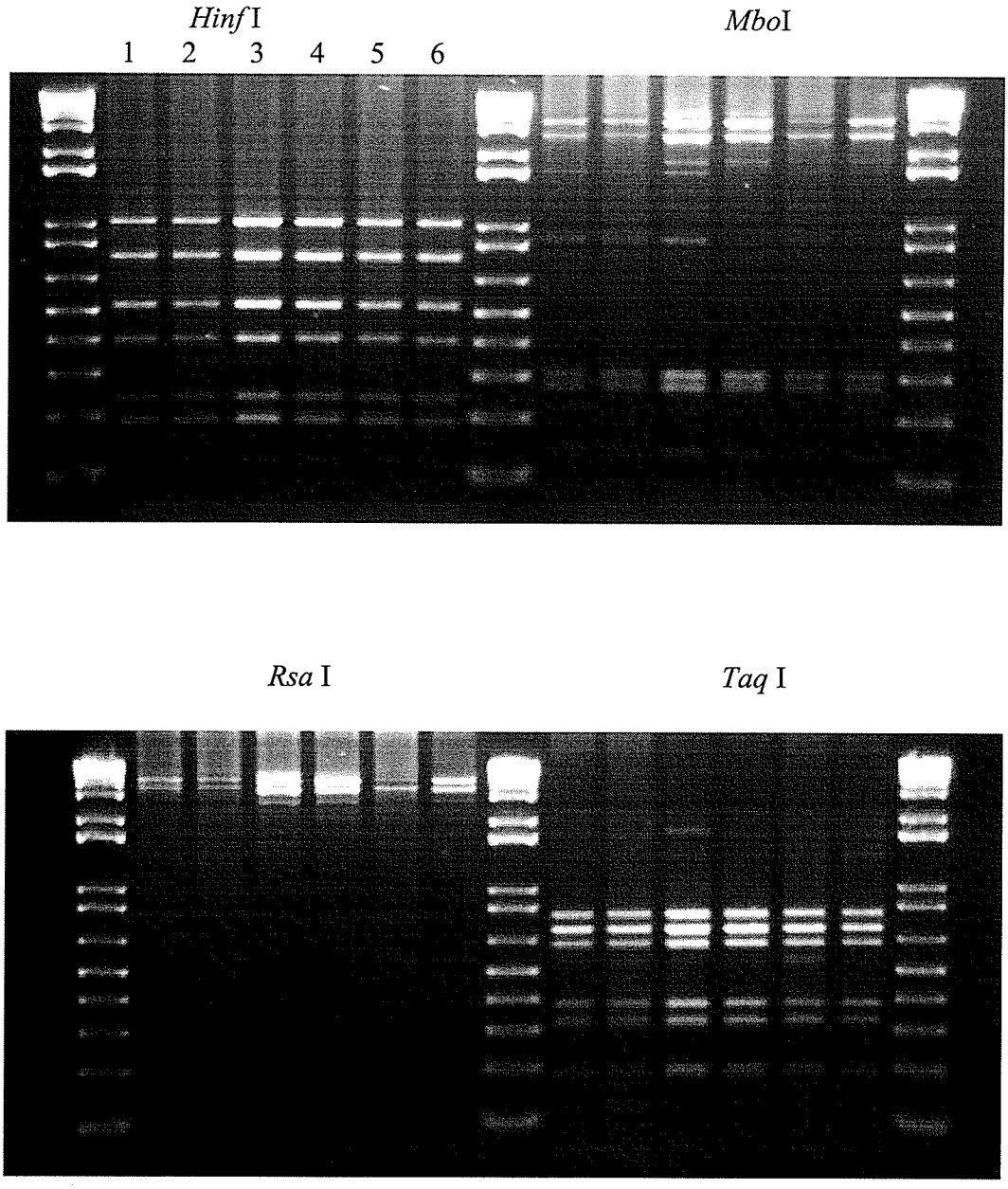


Fig. 4.4. A gel showing restriction digestions of the IGS PCR products of *Hybomitra epistates* with four restriction enzymes. Lanes 1-6 correspond to geographic locations from which individuals were collected: 1, Hecla Island; 2, McMunn; 3, Portage la Prairie region; 4, Sandilands; 5, Seven Sisters; 6, Woodridge; L, 1 kb Plus DNA Ladder (Band sizes for ladder: 100, 200, 300, 400, 500, 650, 850, 1,000, 1,650, 2,000, and 3,000 bp).

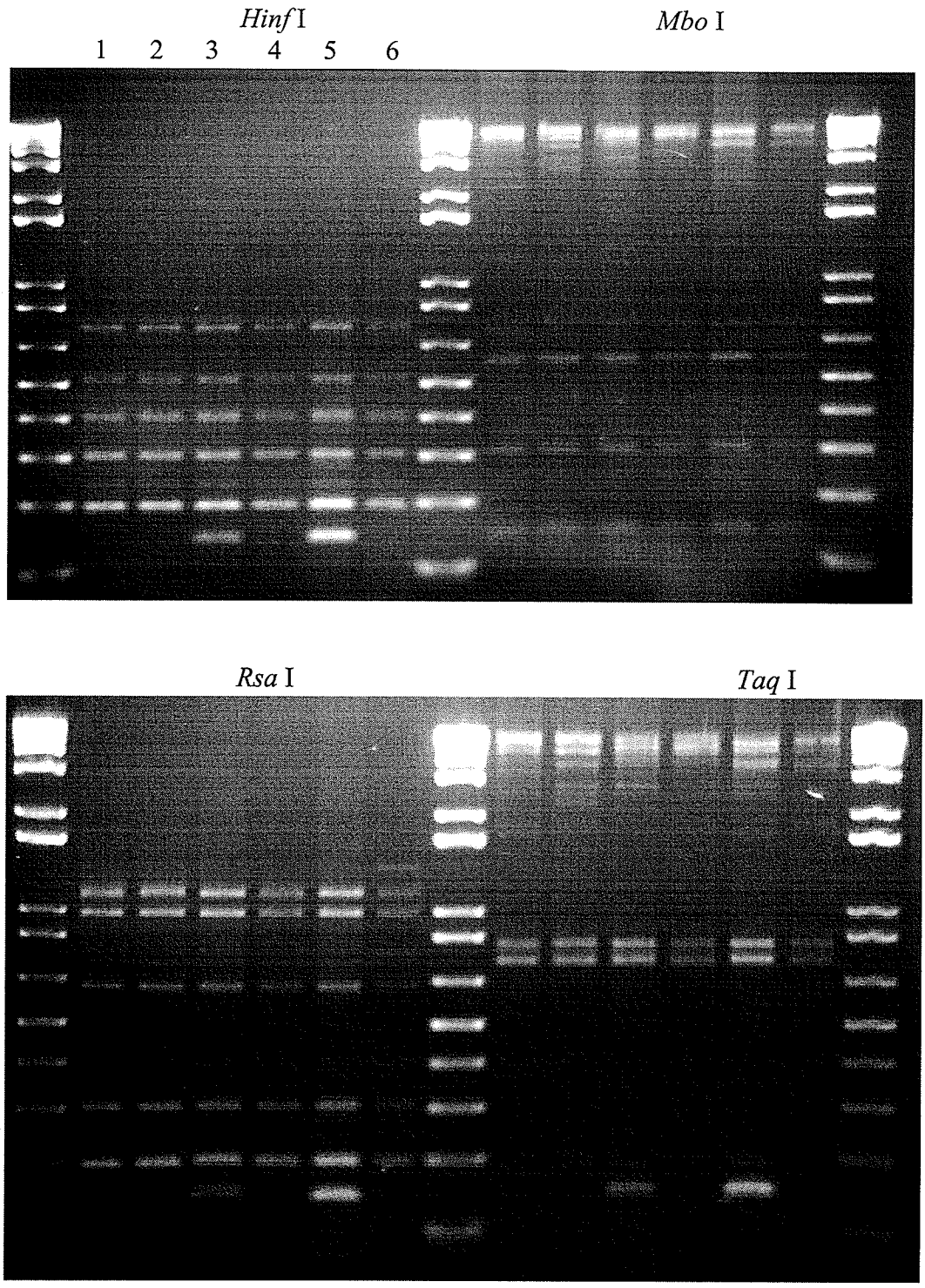


Fig. 4.5. A gel showing restriction digestions of the IGS PCR products of *Hybomitra frontalis* with four restriction enzymes. Lanes 1-6 correspond to geographic locations from which individuals were collected: 1, McMunn; 2, Seven Sisters; 3, West Shoal Lake; 4, Portage la Prairie region; 5, Sprague; 6, Woodridge; L, 1 kb Plus DNA Ladder (Band sizes for ladder: 100, 200, 300, 400, 500, 650, 850, 1,000, 1,650, 2,000, and 3,000 bp).

Table 4.1. Sequences and genomic locations of PCR primers used to amplify tRNA genes, ITS and IGS of tabanids in Manitoba.

Primer	Sequence (5' to 3')	Genomic Location (bp)
P4[a]	GGTCCCTTACGAATTTGAATATATCCT	12,559-12,585[b]
P5[a]	GAGTTCAAACCGGCGTAAGCCAGGT	12,854-12,878[b]
P6[c]	ACATGAATTGGAGCTCGACCAGT	11,523-11,545[b]
P7[c]	GGTACATTACCTCGGTTTCGTTATGAT	11,841-11,867[b]
CS249[d]	TCGTAACAAGGTTTCCG	1,953-1,969[e]
FL	GCTGCACTATCAAGCAAC	3,554-3,571[e]
FL-3	GATTATGCCTGAACGCCT	48-66[e]
P2	ATACTTAGACATGCATGGC	6,898-6,915[e]

[a] Taylor et al. (1996).

[b] *Drosophila yakuba* mtDNA map (Clary and Wolstenholme 1985).

[c] Pruess et al. (1992).

[d] Schlotterer et al. (1994).

[e] *Drosophila melanogaster* rRNA gene sequences (Tautz et al. 1988).

Table 4.2. Approximate sizes of the ribosomal IGS for 35 Tabanidae species collected in Manitoba. The number of individuals examined per species is in parentheses ([a]fragments not common in all individuals).

Species	Size of IGS (bp)
Atylotus calcar (1)	4,800
Chrysops aestuans (5)	7,000[a], 6,000, 4,900, 3,700
C. ater (1)	5,800, 4,700, 4,500, 3,900, 3,000, 2,700, 2,500, 2,400, 2,100, 2,000, 1,700
C. dawsoni (5)	3,800, 3,500
C. excitans (4)	7,000[a], 3,800, 3,500, 3,000, 2,900, 2,400
C. frigidus (6)	7,300[a], 6,500[a], 5,500[a], 4,900[a], 4,700[a], 4,500[a], 4100, 3,700
C. furcatus (1)	8,000, 7,000, 5,800, 5,200, 4,500, 4,300, 3,700, 3,200, 1,700
C. indus (6)	4,000
C. mitis (5)	3,700, 3,300, 3,000[a], 2,700[a], 2,500
C. nigripes (1)	4,500
C. sackeni (1)	4,300, 3,900, 2,900
C. venus (6)	6,000, 4,000, 3,800, 3,600, 3,400, 3,200
Haematopota americana (1)	3,500, 3000
Hybomitra affinis (6)	
H. arpadi (5)	4,700, 4,400, 4,100[a], 3,900[a], 3,200[a]
H. brennani (1)	5,000[a], 4,500, 3,700[a]
H. criddlei (1)	4,500
	8,500, 8,00, 7,500, 7,000, 6,500, 5,800, 5,300, 4,700, 4,300

Species	Size of IGS (bp)
H. epistates (7)	4,200, 3,800, 3,000[a], 2,500[a]
H. frontalis (6)	7,500[a], 7,000, 6,500, 6,000, 5,300, 4,500[a], 4,000[a], 3,800, 3,100[a], 2,800[a]
H. illota (5)	6,000[a], 5,500, 5,000, 4,500, 4,100, 3,800, 3,200
H. lasiophthalma (6)	6,000[a], 5,000[a], 4,500, 4,000
H. liorhina (1)	5,000
H. longiglossa (1)	5,800, 5,000, 4,500, 4,000
H. lurida (6)	5,500[a], 5,100[a], 4,800[a], 4,700[a], 4,500[a], 4,300[a], 4,100[a], 3,900[a], 3,500[a], 3,400[a]
H. nitidifrons nuda (5)	4,500, 3,800[a]
H. pechumani (5)	7,500
H. pediontis (1)	6,500, 6,000, 5,000, 4,500, 3,700, 3,100, 2,900
H. tetrica (1)	8,000, 7,000, 6,000, 5,100, 4,400, 3,700, 3,400, 3,200
H. trepida (6)	8,000
H. zonalis (10)	5,500[a], 4,500[a], 4,000[a], 3,500, 2,700[a], 2,000[a], 1,700
Tabanus atratus (1)	3,400, 2,700
T. marginalis (3)	7,500, 6,500[a], 5,800[a], 5,200, 5,000[a], 4,900[a], 4,700[a], 4,500[a], 4,200, 3,800
T. reinwardtii (1)	7,800, 6,400, 5,600, 3,800
T. similis (3)	7,500, 6,000[a], 5,800[a], 5,200[a], 5,000, 4,700, 4,500[a], 4,200[a], 3,800
T. vivax (1)	6,500, 5,800, 4,200, 2,700

Table 4.3. Restriction fragment sizes (fragments which are bright in appearance and comprise the diagnostic profile) for 35 species of tabanids collected in Manitoba inferred from restriction digestions of the IGS. The number of individuals examined is in parentheses.

Species	Enzyme	Size of Diagnostic Bands (bp)
Atylotus	*Hinf* I	910, 380, 340, 230, 210, 200
calcar	*Mbo* I	2200, 520, 330, 320, 240, 220, 200, 190, 180
(1)	*Rsa* I	2600, 1500, 750, 200
	Taq I	3000, 1850
Chrysops	*Hinf* I	1330, 1050, 450, 420, 230, 160
aestuans	*Mbo* I	1170, 1000, 700, 410, 380, 320, 240
(5)	*Rsa* I	1310, 1130, 700, 520, 200
	Taq I	3000, 450, 250
Chrysops ater	*Hinf* I	380, 300, 230, 210, 190, 100
(1)	*Mbo* I	800, 620, 560, 410, 320, 230
	Rsa I	750, 420, 290, 220, 200
	Taq I	3000, 720, 510, 400
Chrysops	*Hinf* I	350, 310, 230, 180, 150
dawsoni	*Mbo* I	3000, 2800, 370, 320
(5)	*Rsa* I	740, 380, 360, 340, 300, 280, 200
	Taq I	870, 680, 380, 320, 230
Chrysops	*Hinf* I	460, 230, 210, 190, 180, 120
excitans	*Mbo* I	2200, 360, 320
(4)	*Rsa* I	630, 450, 300, 200, 190
	Taq I	1000, 560, 380, 300
Chrysops	*Hinf* I	1100, 950, 420, 330, 230, 180
frigidus	*Mbo* I	360, 320, 260
(6)	*Rsa* I	330, 200
	Taq I	1800
Chrysops	*Hinf* I	920, 840, 740, 490, 360, 230
furcatus	*Mbo* I	520, 460, 380, 320, 300, 230
(1)	*Rsa* I	200
	Taq I	1400, 900, 750, 500, 230

Chrysops indus (6)	*Hinf* I	1780, 630, 400, 380, 230
	Mbo I	930, 380, 320, 250, 230, 180
	Rsa I	1350, 900, 520, 280, 230, 200
	Taq I	1650, 1200, 850, 320
Chrysops mitis (5)	*Hinf* I	350, 230, 220, 180, 160, 120
	Mbo I	3000, 360, 320
	Rsa I	750, 610, 450, 320, 280, 200, 180
	Taq I	650, 600, 300, 280
Chrysops nigripes (1)	*Hinf* I	1800, 1050, 950, 230, 200, 180
	Mbo I	750, 670, 390, 380, 320
	Rsa I	1450, 1300, 830, 700, 600, 550, 410, 190, 170
	Taq I	1600, 1500, 670, 380, 280
Chrysops sackeni (1)	*Hinf* I	1100, 700, 600, 530, 420, 230
	Mbo I	900, 830, 620, 560, 410, 380, 320, 220
	Rsa I	2100, 1600, 800, 600, 530, 200
	Taq I	2200, 1630, 950, 800, 650
Chrysops venus (6)	*Hinf* I	920, 410, 330, 230, 210, 180
	Mbo I	800, 360, 320
	Rsa I	1150, 320, 200
	Taq I	no site
Haematopota americana (1)	*Hinf* I	1000, 380, 280, 250, 220, 160
	Mbo I	830, 810, 490, 480, 320, 220, 180
	Rsa I	870, 630, 310, 210
	Taq I	2400, 720
Hybomitra affinis (6)	*Hinf* I	830, 490, 390, 240
	Mbo I	2800, 1900, 1080, 310, 270
	Rsa I	1850, 1220, 530
	Taq I	1300, 800, 570, 470, 380, 200, 130
Hybomitra arpadi (5)	*Hinf* I	1250, 670, 450, 420, 240
	Mbo I	2900, 500, 310, 290
	Rsa I	1400, 1220, 660, 480
	Taq I	2700, 1850, 800, 200
Hybomitra brennani (1)	*Hinf* I	1100, 900, 670, 560, 440, 240, 230
	Mbo I	2000, 1250, 510, 310
	Rsa I	2100, 1770, 830
	Taq I	1430, 880, 830, 370, 280, 250, 200, 180
Hybomitra criddlei (1)	*Hinf* I	420, 240
	Mbo I	700, 310, 300
	Rsa I	1890, 600
	Taq I	850, 820, 480, 200
Hybomitra epistates (7)	*Hinf* I	1120, 800, 530, 420, 240, 190
	Mbo I	2800, 310, 280, 150
	Rsa I	no site
	Taq I	830, 750, 650, 390, 340, 200

Hybomitra	*Hinf* I	750, 520, 410, 300, 200
frontalis	*Mbo* I	600, 310, 150
(6)	*Rsa* I	1250, 1000, 630, 300, 200
	Taq I	830, 750, 300, 150
Hybomitra	*Hinf* I	420, 390, 240
illota	*Mbo* I	1500, 1320, 1200, 580, 400, 310
(5)	*Rsa* I	no site
	Taq I	850, 620, 570, 500, 200
Hybomitra	*Hinf* I	1250, 670, 520, 400, 330, 270, 240
lasiophthalma	*Mbo* I	2800, 750, 310, 280
(6)	*Rsa* I	1500, 770, 730, 530, 290, 200, 150
	Taq I	820, 700, 620, 480, 290, 200
Hybomitra	*Hinf* I	1600, 1440, 510, 480, 410, 240
liorhina	*Mbo* I	1600, 1000, 850, 640, 500, 400, 310, 240
(1)	*Rsa* I	1750, 1450, 980, 680, 530, 430, 150
	Taq I	2140, 1600, 1550, 1000, 650, 340, 260, 200
Hybomitra	*Hinf* I	1300, 1250, 670, 420, 240, 220
longiglossa	*Mbo* I	310
(l)	*Rsa* I	1400, 1300, 850, 650, 430, 200, 180
	Taq I	930, 830, 750, 680, 510, 200
Hybomitra	*Hinf* I	1275, 790, 560, 440, 340, 240
lurida	*Mbo* I	320
(6)	*Rsa* I	830
	Taq I	930, 700, 570, 420, 380, 200
Hybomitra	*Hinf* I	1540, 790, 560, 400, 240, 200
nitidifrons	*Mbo* I	310, 280, 120
nuda	*Rsa* I	no site
(5)	*Taq* I	1650, 1130, 830, 750, 200
Hybomitra	*Hinf* I	900, 800, 750, 570, 450, 370, 300, 150
pechumani	*Mbo* I	550, 450, 420, 310, 250
(5)	*Rsa* I	580, 450
	Taq I	1050, 900, 850, 720, 580, 480, 450, 300, 170
Hybomitra	*Hinf* I	900, 560, 470, 440, 400, 270, 240, 200, 180
pediontis	*Mbo* I	520, 310, 230, 180
(1)	*Rsa* I	1220, 1000, 480, 270
	Taq I	1330, 950, 850, 750, 650, 250, 220, 200
Hybomitra	*Hinf* I	750, 530, 400, 310, 290, 240, 200, 150
tetrica	*Mbo* I	670, 310, 180
(1)	*Rsa* I	1250, 1050, 680, 320, 220, 170
	Taq I	850, 800, 200, 170
Hybomitra	*Hinf* I	1850, 1200, 1000, 850, 630, 410, 330, 320, 240,
trepida	*Mbo* I	220
(6)	*Rsa* I	850, 380, 310, 280, 200
	Taq I	2100, 800, 580, 480, 400, 320, 170
		1150, 830, 750, 710, 630, 600, 310, 300, 220, 200

Hybomitra zonalis (10)	*Hinf* I	1600, 1540, 980, 830, 650, 240
	Mbo I	3000, 760, 530, 310
	Rsa I	1800, 1530, 600, 480, 450, 390, 350
	Taq I	1140, 1000, 850, 510, 410, 200
Tabanus atratus (1)	*Hinf* I	2300, 420, 330, 230
	Mbo I	2200, 320, 220, 180, 150
	Rsa I	350, 210
	Taq I	2800, 500, 260, 200
Tabanus marginalis (3)	*Hinf* I	520, 330, 300, 230
	Mbo I	440, 320, 270
	Rsa I	750, 690, 210, 150
	Taq I	920, 400, 170
Tabanus reinwardtii (1)	*Hinf* I	830, 520, 400, 330, 230, 140
	Mbo I	400, 340, 320, 270, 200
	Rsa I	610, 370, 240, 210
	Taq I	520, 400, 240, 200
Tabanus similis (3)	*Hinf* I	230
	Mbo I	630, 320, 270, 180
	Rsa I	730, 460, 210
	Taq I	780
Tabanus vivax (1)	*Hinf* I	500, 310, 230, 170, 130
	Mbo I	450, 320, 250, 170
	Rsa I	750, 640, 210, 190
	Taq I	960, 400, 310, 280, 200, 170

CHAPTER V

Identification of tabanid egg masses in southern Manitoba using the PCR-RFLP method

Introduction

The medical and veterinary importance of tabanids is well known because of direct injury and transmission of pathogens to livestock, wildlife and humans through female blood feeding. In certain areas of North America, species of Tabanidae are among the most serious pests of livestock and humans. Tabanidae are capable of transmitting mechanically or biologically more than 30 disease-causing agents including viruses, bacteria, protozoa, and helminths, (Krinsky 1976). In addition to suffering blood loss, hosts expend energy in attempting to avoid painful bites. Formation of grazing lines, bunching, head tosses, foot stomps, ear flicks, and tail switches by pastured cattle are some group and individual behaviours in response to tabanid attack in Manitoba, Canada (Ralley et al. 1993). Heifers exposed to attacks by an average 90 flies per animal per day for 84 days gained 80 g per animal per day less than those protected from horse flies (Perich et al. 1986). In New York, the daily blood loss in one cow due to attack by *Hybomitra lasiophthalma* (Macquart), *Tabanus quinquevittatus* Wiedemann, and *Tabanus sulcifrons* Macquart was estimated to be 352 ml, 59 ml, 109 ml, respectively (Tashiro and Schwardt 1953).

Species of Tabanidae occur in temperate and tropical regions of the World with over 4350 described species and 335 species and subspecies in North America north of Mexico (Burger 1995). Based on adult characters, there are 144 described species distributed in all parts of the Canada and Alaska except the northern most tundra zone, where trees are absent (Teskey 1990). In Manitoba, there are 50 species in five genera: *Hybomitra*, *Tabanus*, *Chrysops*, *Atylotus*, and *Haematopota*. Adult tabanids are reasonably well known taxonomically in North America. However, the detailed

description of immatures has not been as extensive as that of adults. Tabanid egg masses have been described briefly and descriptions are scattered through the literature. Therefore, there is a great need for more detailed descriptions of tabanid egg masses (Teskey 1990). Taxonomic descriptions of some larvae and pupae are available. Teskey (1969) provided descriptions and keys to the larvae and pupae of 81 out of 144 species of Tabanidae occurring in Canada. Larvae and pupae of the remaining species are unknown. Because of these gaps and the absence of information on the range of variation to be expected in some species, Teskey (1969) suggested that his key should be used with caution. In the USA, regional studies have been conducted on taxonomy of larvae and pupae of some species of tabanids by different investigators (Goodwin 1976; Pechuman 1981; Burger 1977). However, systematics of immature stages in the Nearctic are better known than in other geographical regions of the World (J.F. Burger, personal communication). Even if complete keys for immature stages of all species in Canada were available, for accurate identification it would be necessary to rear larvae to the third or fourth instar. This is difficult due to high mortality of early instars.

The major problems in the study of biology and taxonomy of larvae and pupae of species of Tabanidae are due to the inaccessibility of these life stages because of their living in aquatic habitats, their prolonged life cycles (1-3 years), reluctance of adults to mate and feed under laboratory conditions, their unnatural oviposition behaviour in cages, refusal of the larvae to feed, cannibalistic behaviour, and mortality during ecdysis and pupation (Thompson and Krauter 1978).

Molecular methods have been used for tabanid taxonomy by some investigators. Hudson and Teskey (1976) segregated two forms of *Hybomitra typhus* (Whitney) by size

and colour of characters and also by differences in the appearance of esterase isozymes shown by electrophoresis. They found that specimens recorded as *H. typhus* comprise more than one species. Jacobson et al. (1981) distinguished two genetically distinct species of *Tabanus nigrovittatus* Macquart by starch gel electrophoresis of enzymes. Identification of tabanids by analysis of cuticular hydrocarbons was studied by Hoppe et al. (1990). In this study, *Tabanus abdominalis* Fabricius, *Tabanus limbatinevris* Macquart, and *T. sulcifrons* were differentiated by comparison of hydrocarbon profiles. Sutton and Carlson (1997) used cuticular hydrocarbons to differentiate three cryptic species in the *T. nigrovittatus* complex. Cuticular hydrocarbons vary with stage, age, and sex of insects (Pomonis and Mackley 1985; Pomonis 1989); therefore, this technique may not be suitable to associate different stages of the same species.

DNA is more stable than enzymes and does not vary in different stages (Taylor et al. 1996). Thus, DNA analysis may be useful for identifying tabanids in the adult and immature stages. PCR-RFLP analysis of the rDNA intergenic spacer (IGS) between the 28S and 18S rRNA genes was successfully used to generate restriction fragment profiles that differentiated 35 species of adult tabanids from one another (Chapter IV). There was a small degree of intraspecific variation among fragment patterns when multiple individuals of a species were examined. The rDNA IGS therefore provided a target sequence with sufficient variation to identify individual adult tabanids to the species level. Molecular identification of tabanid egg masses became important in a study on wasps (Scelionidae and Trichogrammatidae) that parasitize tabanid eggs. In southern Manitoba, there was high prevalence of parasitism in egg masses collected in the field (Iranpour and Galloway 2002); however, it was impossible to associate the parasitoids

correctly with species of tabanids which had laid the eggs. Therefore, the purpose of this study was to use polymerase chain reaction (PCR) and subsequent restriction fragment length polymorphism (RFLP) analysis to associate adults of horse fly and deer fly species with their egg masses on the basis of molecular diagnostic profiles established for adult tabanids (Chapter IV). Morphological and molecular descriptions of egg masses of two species of *Hybomitra* and three species of *Chrysops* will be provided. The potential of the selected DNA region and the application of PCR-RFLP technique for tabanid immature identification and classification of the immature stages is discussed.

Materials and Methods

Adult and egg mass collection - Adult tabanids were collected in Manitoba (Fig. 5.1) during June and July of 1999 and 2000. Manitoba Horse Fly Traps and aerial nets were used on hot, sunny days from 1000 to 1600 h to collect adult flies. Specimens were transported on ice to the laboratory in a cooler and stored at –25°C in sealed plastic containers. Approximately 10 weeks after field collection, tabanids were removed from the freezer and identified to species using the identification key of Teskey (1990). Identified adults were then stored in 95% ethanol at –25°C for DNA extraction.

Tabanid egg masses were also collected in southern Manitoba (Fig. 5.1) in June and July of 1999 and 2000. Egg masses were carried on ice to the laboratory, labeled and kept in an incubator (25°C and 50% RH) in petri dishes. When the eggs hatched, first instar larvae from each egg mass were stored at –25°C in 95% ethanol for DNA extraction and the chorions were kept in petri dishes at –20 °C as voucher material.

During field collection, egg masses of three species were collected along with the adult tabanids which laid the egg masses. The adults were identified using the key of Teskey (1990) and the eggs masses were kept until first instar larvae hatched and then the larvae were used for molecular identification as previously described.

DNA extraction - Total DNA was isolated based on the procedure of Taylor et al. (1996) with modifications. A thorax from one adult was ground to a fine powder in liquid nitrogen with a prechilled mortar and pestle. To this was added 400µl lysis buffer (100 mM NaCl, 100 mM EDTA, 100 mM Tris, 0.5% SDS, pH 7.5) and 4µg proteinase K and the lysis mixture was incubated at 55°C for 3 h with occasional mixing. Following the incubation, 2 µg RNase A were added and incubated at 37°C for 20 min. To this, 100µl phenol were added and the mixture was incubated at 55°C for 10 min. After incubation, 100 µl chloroform:isoamyl alcohol (24:1) were added and the sample was centrifuged again at 12,000 g for 5 min. The aqueous phase was removed and a second DNA extraction was done with an equal volume of chloroform:isoamyl alcohol (24:1) and the sample was then centrifuged at 12,000 g for 5 min. The aqueous phase was transferred to a fresh 1.5-ml microfuge tube and the DNA was precipitated with the addition of two volumes of cold 95% ethanol and 0.08 volumes 8 M LiCl and incubated at –60°C for one h. The sample was then centrifuged at 12,000 g for 20 min at 4°C. The DNA pellet was washed with 70 % ethanol, centrifuged at 12,000 g for 7 min at 4°C, vacuum-dried, and resuspended in 50 to 100 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.4). The same procedure was used for DNA extraction for first instar larvae.

PCR amplification - Primers FL-3 [5-GATTATGCCTGAACGCCT-3 with genomic location 48-66 *Drosophila melanogaster* Meigen rRNA gene sequences (Tautz et al. 1988)] and P2 [5-ATACTTAGACATGCATGGC-3 with genomic location 6,898-6,915 *D. melanogaster* rRNA gene sequences (Tautz et al. 1988)] were used to amplify the intergenic spacer (IGS) between the 28S and 18S rRNA genes (Fig. II.2). Amplification was carried out in a 50 µl total volume using a Techne Unit Genius Thermocycler (Techne Incorporated, Princeton, NJ). Each reaction mixture consisted of 5 µl *Taq* Extender 10x reaction buffer (Stratagene, La Jolla, CA), 200 µM each of dATP, dTTP, dCTP, and dGTP (Invitrogen, Carlsbad, CA), 1.5 mM $MgCl_2$, 20 pmol of each primer, 1.25 U *Taq* DNA polymerase (Invitrogen), 1.25 U *Taq* Extender (Stratagene), and approximately 10 ng of total DNA. The reaction mixtures were subjected to an initial denaturation at 93°C for 3 min. Reaction mixtures were subjected to 1 min at 93°C, 1 min at 50°C, and 10 min at 65°C for 20 cycles. To assess the efficiency of the amplification, 5 µl aliquots of PCR products were separated on 1% agarose gels prepared with 1x TBE buffer (89 mM Tris-HCl, 89 mM boric acid, and 20 mM EDTA), stained with ethidium bromide, and visualized under ultraviolet light.

Restriction endonuclease digestions - Eight to 12 µl of amplified DNA were digested with 2.5 U each of *Hin*fI, *Mbo*I, *Rsa*I, *Taq*I according to manufacturer's specifications (Invitrogen). All restriction digest reactions were stopped after 2 h with the addition of 3.0 µl gel loading buffer (40% (w/v) sucrose, 0.25% bromophenol blue, and 20mM EDTA) to each reaction tube. Digested DNA fragments were separated on a 1.5% agarose gel in 1x TBE buffer, stained with ethidium bromide, and photographed under

UV light. The lengths of each restriction fragment from adult tabanids were compared with that of larvae using the 1 kb Plus DNA Ladder (Gibco BRL) (Fig. II.1).

Results

Approximately 500 tabanid egg masses were collected during the summers of 1999 and 2000. After sorting them by size, shape, colour, and number of layers, 56 egg masses were selected for DNA extraction following with enzyme digestion by *Hinf*I. Restriction patterns of egg masses were compared with adult molecular markers (Fig. 4.2, Table 4.3) for egg mass identification. Fifty-six egg masses were identified for *Hybomitra nitidifrons nuda* (McDunnough) (33), *H. lasiophthalma* (2), *Chrysops excitans* Walker (17), *Chrysops aestuans* Wulp (2), and *Chrysops mitis* Osten Sacken (2) (Fig. 5.2).

IGS of one egg mass from each species along with IGS of associated adult species were amplified and digested by *Hinf*I, *Mbo*I, *Rsa*I, and *Taq*I (Fig. 5.3). There was a match between adults and immatures of the same species, except for a few fragment differences, which were observed in *H. n. nuda* (*Mbo*I, *Taq*I) and *C. aestuans* (*Rsa*I). Five egg masses of *H. n. nuda* were collected immediately after adult female finished their oviposition. DNA was extracted from the first instar larvae that emerged from egg masses and then IGS was amplified and digested by *Hinf*I, *Mbo*I, *Rsa*I, and *Taq*I (Fig. 5.4). There was no interspecific variation among these individuals and the fragment profile was the same as that for adult *H. n. nuda* (Fig. 5.2, Table 5.3, Fig. II.38).

Identified egg masses were examined under the dissecting microscope and described based on colour, number of layers, maximum length of egg mass, maximum

width of egg mass, height of egg mass, length of individual eggs, and number eggs within the egg mass (Table 5.1).

Hybomitra nitidifrons nuda (Fig. III.1): Initially white or cream-coloured. More or less uniform egg masses in length, width, height and shape. Colour milky white immediately after oviposition, turning to brown after 24 h and then dark brown to black. Egg masses consist of five layers, the fifth layer on the top with only 5-10 eggs. Egg mass has regular shape with a sharp tail pointed toward the ground. Means of maximum length, width, and height were 11.7±0.5, 6.3±0.2, and 2.7±0.1 mm, respectively (95% C.L., n=10). Mean number of individual eggs within the egg mass were 735±44 (95% C.L., n=40) with 2±0.1mm length for individual eggs (95% C.L., n=10). The chorion of the hatched eggs was very delicate and transparent.

Hybomitra lasiophthalma (Fig. III.2): Initially white or cream-coloured. More or less uniform egg masses in length, width, height and shape. Colour milky white immediately after oviposition and then turning to dark, shiny black after a couple of hours. Egg masses of three layers, the third layer on the top with fewer eggs than the other layers. Egg mass with more or less regular shape, without tail. Means of maximum length, width, and height were 9.2±0.3, 5.2±0.2, and 3.7±0.2 mm, respectively (95% C.L., n=10). Mean number of individual eggs within the egg mass were 490±27 (95% C.L., n=10). Individual eggs 2.2±0.1mm long (95% C.L., n=10), larger than that of *H. n. nuda* in length and width, and the chorion of the hatched eggs was thicker than other species, opaque, and easy to separate in alcohol.

Chrysops excitans (Fig. III.3): Initially white or cream-coloured. Egg masses were not uniform in length, width, height and shape. Some egg masses were strongly elongate, three to four times longer than wide, the width of two ends more or less similar; the second type spindle-shaped, shorter, one end much wider than the other end which was tail-shaped and pointed toward the ground, colour of the old egg mass brown in both forms. Egg masses of three layers, the third layer on the top with fewer eggs than the other layers. Egg mass with more or less regular shape some with and some without tail. Means of maximum length, width, and height were 9.8±0.4, 3.8±0.1, and 2.2±0.1 mm, respectively (95% C.L., n=15). Mean number of individual eggs within the egg mass was 462±74 (95% C.L., n=15). Mean length of individual eggs was 1.7±0.1mm (95% C.L., n=15), the chorion of the hatched eggs very delicate and transparent.

Chrysops mitis (Fig. III.4): Initially white or cream-coloured. Egg masses were not uniform in length, width, height and shape. Some individuals strongly elongate, spindle-shaped, three to four times longer than wide, the second type shorter, triangular from dorsal view, one end much wider than the other end which was pointed toward the ground, colour of the old egg mass light brown in both forms. Egg masses consisted of 3-four layers, the top layer with 15-20 eggs, with more or less regular shape. Means of maximum length, width, and height were 8.8±0.7, 3.4±0.1, and 1.6±0.1 mm, respectively (95% C.L., n=5). Mean number of individual eggs within the egg mass was 545±55 (95% C.L., n=2). Individual eggs shorter than that of the other examined species, 1.5±0.1mm long (95% C.L., n=10), the chorion of the hatched eggs very delicate and

transparent. In total, the sizes of the egg mass and individual eggs were smaller than that of *C. excitans*.

Chrysops aestuans (Fig. III.5): Initially white or cream-coloured. Egg masses were not uniform in length, width, height and shape in general. Some individuals were strongly elongate (four to six times longer than wide) and some shorter (one to two times longer than wide). Colour milky white immediately after oviposion and then turning to shiny, dark black after a couple of hours. Egg masses consisted of 1 layer, with irregular shape. Means of maximum length, width, and height were 10.4±0.8, 3.4±0.2, and 0.5±0.1 mm, respectively (95% C.L., n=15). Mean number of individual eggs within the egg mass was 217±17 (95% C.L., n=40). Individual eggs were 1.8±0.1 mm long (95% C.L., n=15), the chorion of the hatched eggs very delicate, transparent and smoky brown in colour.

Discussion

Although tabanid investigators occasionally have reared late instar larvae of tabanids under laboratory conditions to acceptable size for identification (Hine 1906; Marchand 1917; Segal 1936; Roberts and Dicke 1964; Roberts 1966; Teskey 1969; Tarmudji 1984; Mckeever and French 1992; Burger 1977), great care is required for months or years for far northern species (Teskey 1990) with poor success. In addition, optimum laboratory conditions and feeding might be different from one species to another. Therefore, it seems impossible to apply one single rearing technique for all species. This could be one of the reasons that anatomical descriptions of tabanid egg masses are not well known although there are considerable differences among some

species in arrangement, shape, and dimensions of egg masses and also number of eggs in the mass (Teskey 1990).

It is possible to use molecular markers in species identification of tabanids in all stages. In the fragment pattern of IGS after restriction enzyme by *Hinf*I, *Mbo*I, *Rsa*I, and *Taq*I, there was enough interspecific variation to distinguish adults of different species from each other. On the other hand, intraspecific variations were small and more than 80% of restriction fragments were shared by different individuals of the same species. Some of these variations can be seen in Fig. 3.3B and 3.3D for two individuals of *H. n. nuda*. In restriction digestion by *Mbo*I, the first individual (adult) had two extra bands (about 3000 bp and 1850 bp); in restriction digestion by *Taq*I, this individual had one extra band (more than 1850 bp). In restriction digestion of IGS by *Taq*I, the other individual of *H. n. nuda* (larva) provided two bands (~ 470 bp and ~ 575 bp), which were absent in adults. Based on results inferred from restriction digestion of IGS by *Hinf*I of 47 individuals of *H. n. nuda* (39 larvae and 8 adults), it can be concluded that there are at least two forms of *H. n. nuda* in Manitoba. Form I (such as individuals 2, 9, and 16 in Fig. 5.2) had 2 extra fragments (1850 bp and 1000 bp) which were absent in form II (such as individuals 1, 4, 5, and 6 in Fig. 5.2, both individuals in Fig. 5.3A, and all individuals in Fig. 5.4A). However, digestion of IGS of this species by *Hinf*I provided 6 unique restriction fragments (1540, 790, 560, 400, 240, and 200 bp) which can be used as molecular markers for this species in adult and immature stages. Restriction digestion of IGS by other enzymes can also be used to confirm identification of this species. By comparing restriction patterns of two individuals in Fig. 5.3, five individuals in Fig. 5.4, and seven individuals in Chapter 4 (Fig. 4.2 and Fig. II.38), it is evident that restriction

enzyme of IGS by *Mbo*I and *Taq*I provides 4 (>3000, 310, 280, 120 bp) and 5 (1650, 1130, 830, 750, and 200 bp) consistent fragments, respectively, can be used to reconfirm identification of this species by *Hinf*I. Molecular characterization of different stages of tabanids using PCR-RFLP seems promising for further systematic studies on tabanids, particularly immature stages.

PCR-RFLP for this purpose is fast, inexpensive and accurate; however, preliminary studies in each region must be carried out. First adult tabanids should be collected and identified, followed by DNA extraction, amplification of IGS, and enzyme digestion. We believe that the molecular data inferred from PCR-RFLP of IGS can be used by other tabanid investigators in North America; however, this should be confirmed by further studies. The second step would be collecting tabanid egg masses and rearing them to first instar larvae (to avoid contamination of tabanid DNA by parasitoid DNA, extraction of DNA from egg masses is not recommended). Some of the first instar larvae can be used for DNA extraction and the rest may be reared to subsequent instars for morphological description or can be kept in alcohol as voucher materials. After molecular characterization, tabanid egg masses can be described based on physical characters.

In this study, egg masses were collected mainly in two types of habitats in different locations (Fig. 5.1). The first type of habitat was ditches with wet soil and/or shallow, stagnant water beside highways and provincial roads. Most of these egg masses were identified as *H. n. nuda* and *H. lasiophthalma*. The second habitat was ponds and ditches with deep water where *Chrysops* spp. egg masses were collected. Since about 50 species of tabanids occur in Manitoba (Teskey 1990), there should be many more habitats where tabanids lay their eggs. Our egg collection yielded five species, which are

described in Table 5.1. It seems that combinations of some morphological characters such as number of tiers, colour, number of eggs within egg masses, length of individual eggs, and general configuration of the egg mass can be used to identify tabanid egg masses. In this study, it was found that in some species, characters such as length, width, and height of egg masses vary from one individual to another.

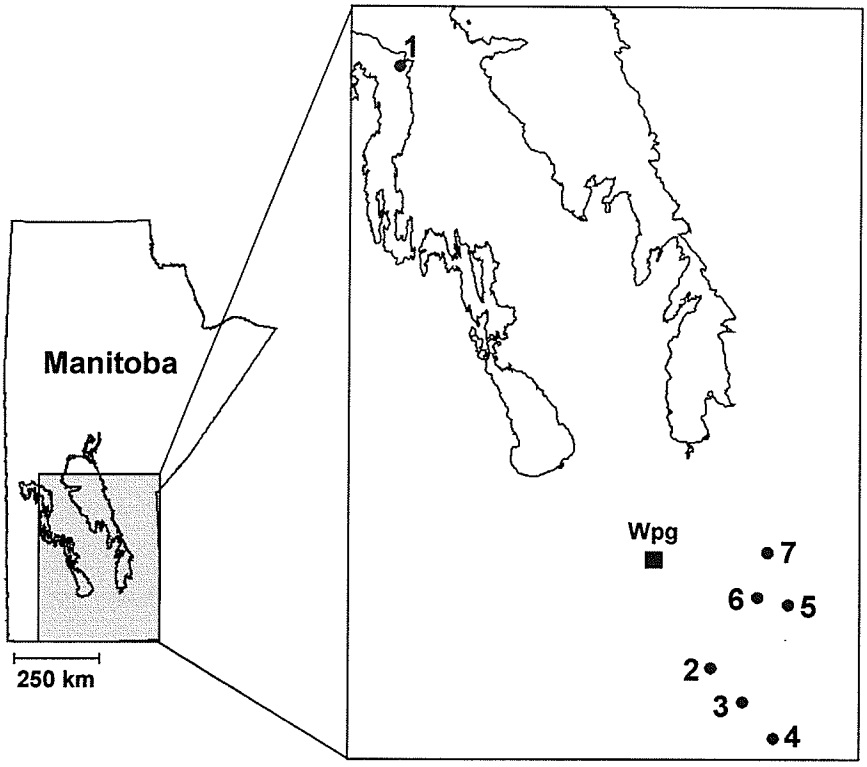
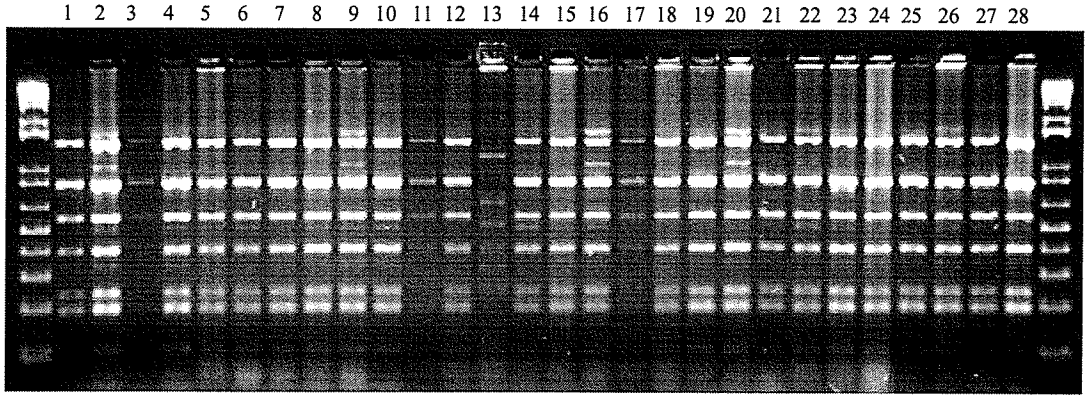


Fig. 5.1. Locations in Manitoba where tabanid adults and their egg masses were collected.

1-Easterville (Denbeigh Point)
2-Marchand
3-Woodridge
4-Piney
5-McMunn
6-Hadashville
7-Elma
WPG-Winnipeg

Fig. 5.2. A gel showing restriction digestion of IGS by *Hinf* I of first instar larvae reared from 56 egg masses of Tabanidae collected at the following locations of Manitoba (1-12, 14, 15-29, 31-34, and 36, *Hybomitra nitidifrons nuda*; 13, 30, *Hybomitra lasiophthalma*; 35, 37, 38, 42-54, 56, *Chrysops excitans*; 40, 41 *Chrysops aestuans*; 39, 55, *Chrysops mitis*):

17	Easterville (Denbeigh point), 27 June, 1999
4-12	Elma, 18 June, 1999
13, 14-16	Elma, 23 June, 1999
18-28, 35, 36	Elma, 28 June, 1999
46-48	Elma, 29 June, 2000
40	Elma, 12 July, 1999
55	Elma, 14 July, 2000
41	Elma, 18 July, 1999
45-	Junction of Hwy #1 and 11, 19 June, 2000
49,52	Junction of Hwy #1 and 11, 29 June, 2000
50, 53, 54	Junction of Hwy #1 and 11, 4 July, 2000
51	Junction of Hwy #1 and 11, 10 July, 2000
38,39	Junction of Hwy #1 and 11, 12 July, 1999
56	Junction of Hwy #1 and 11, 18 July, 2000
42-44	Junction of Hwy #1 and 11, 23 July, 1999
34	Marchand, 25 June, 1999
37	McMunn, 4 July, 1999
33	Piney, 6 July, 2000
1	Woodridge, 9 June, 1999
2-3	Woodridge, 15 June, 1999
31	Woodridge, 22 June, 2000
29-30	Woodridge, 29 June, 1999
32	Woodridge, 6 July, 2000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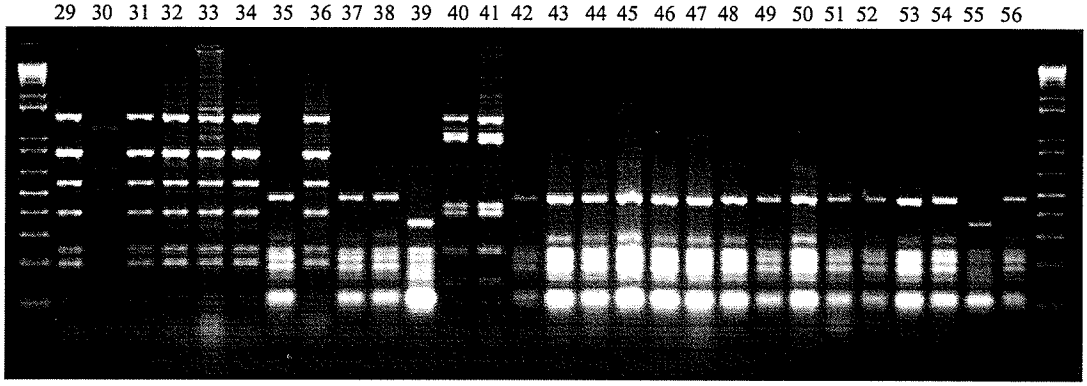
29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56

Fig. 5.3. A gel showing restriction digestion of IGS (A, *Hinf*I; B, *Mbo*I; C, *Rsa*I; D, *Taq*I) of adults and larvae of five species of Tabanidae collected at the following locations of Manitoba:
1-*Hybomitra lasiophthalma* (adult), Hadashvile, 18 July, 2000
2-*Hybomitra lasiophthalma* (larva), Elma, 23 June, 1999
3-*Hybomitra nitidifrons nuda* (adult), Woodridge, 19 June, 2000
4-*Hybomitra nitidifrons nuda* (larva), Woodridge, 29 June, 1999
5-*Chrysops aestuans* (adult), Elma, 10 July, 2000
6-*Chrysops aestuans* (larva), Elma, 12 July, 1999
7-*Chrysops excitans* (adult), Woodridge, 19 June, 2000
8-*Chrysops excitans* (larva), junction of Hwy #1 & 11, 23 July, 1999
9-*Chrysops mitis* (adult), Woodridge, 19 June, 2000
10-*Chrysops mitis* (larva), junction of Hwy #1 & 11, 12 July, 1999

1 2 3 4 5 6 7 8 9 10

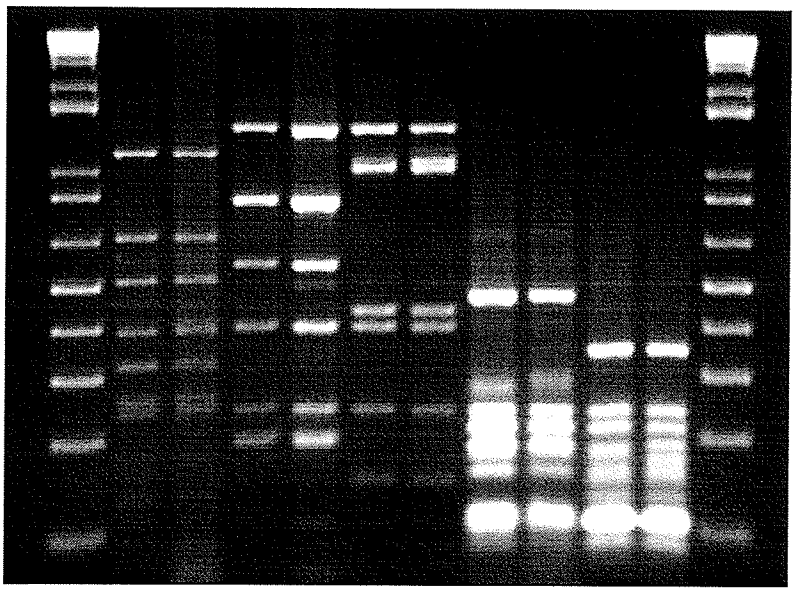

Fig. 3A

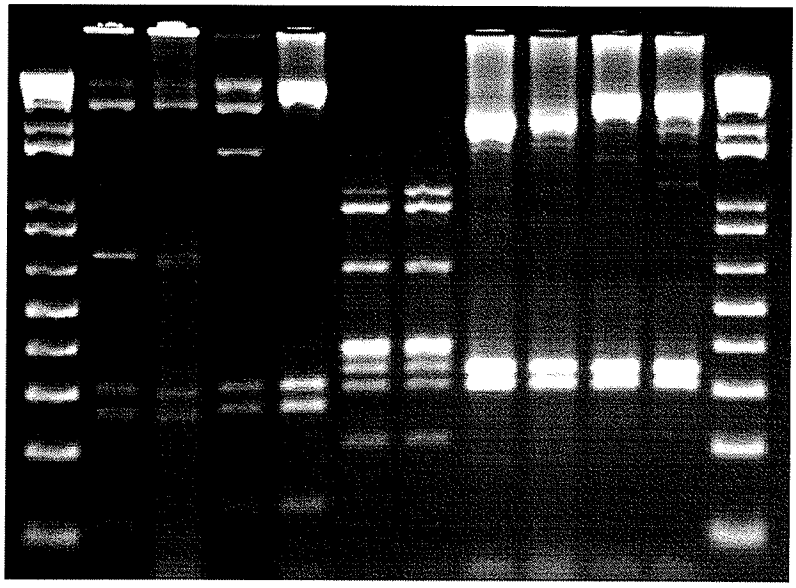

Fig. 3B

1 2 3 4 5 6 7 8 9 10

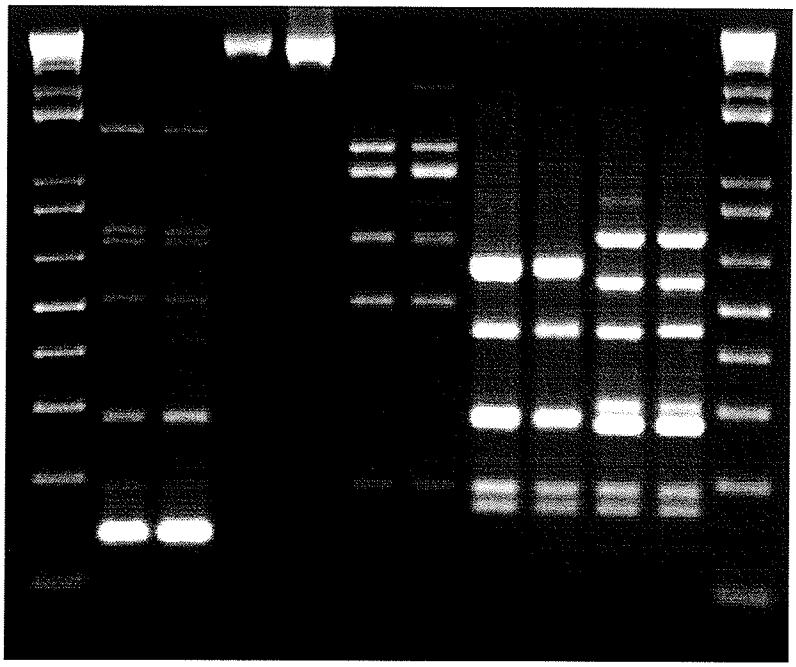

Fig. 3C

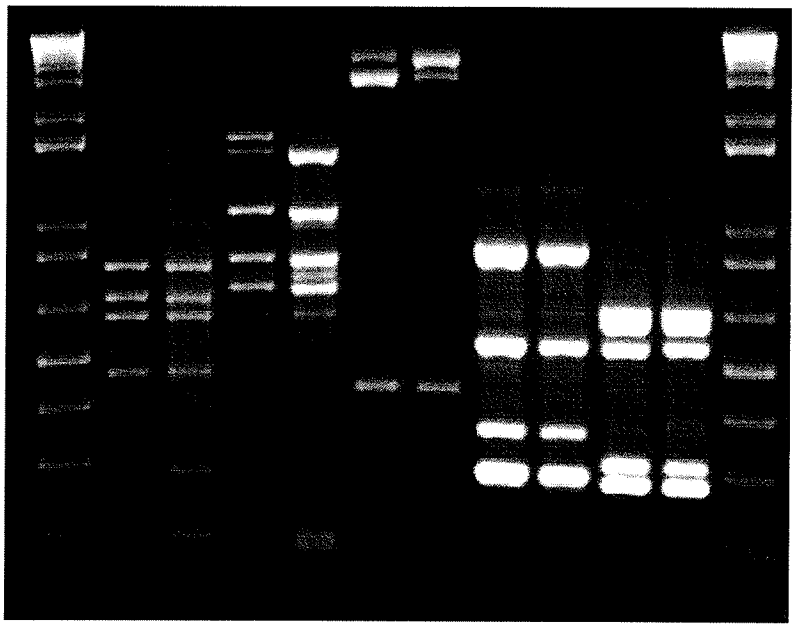

Fig. 3D

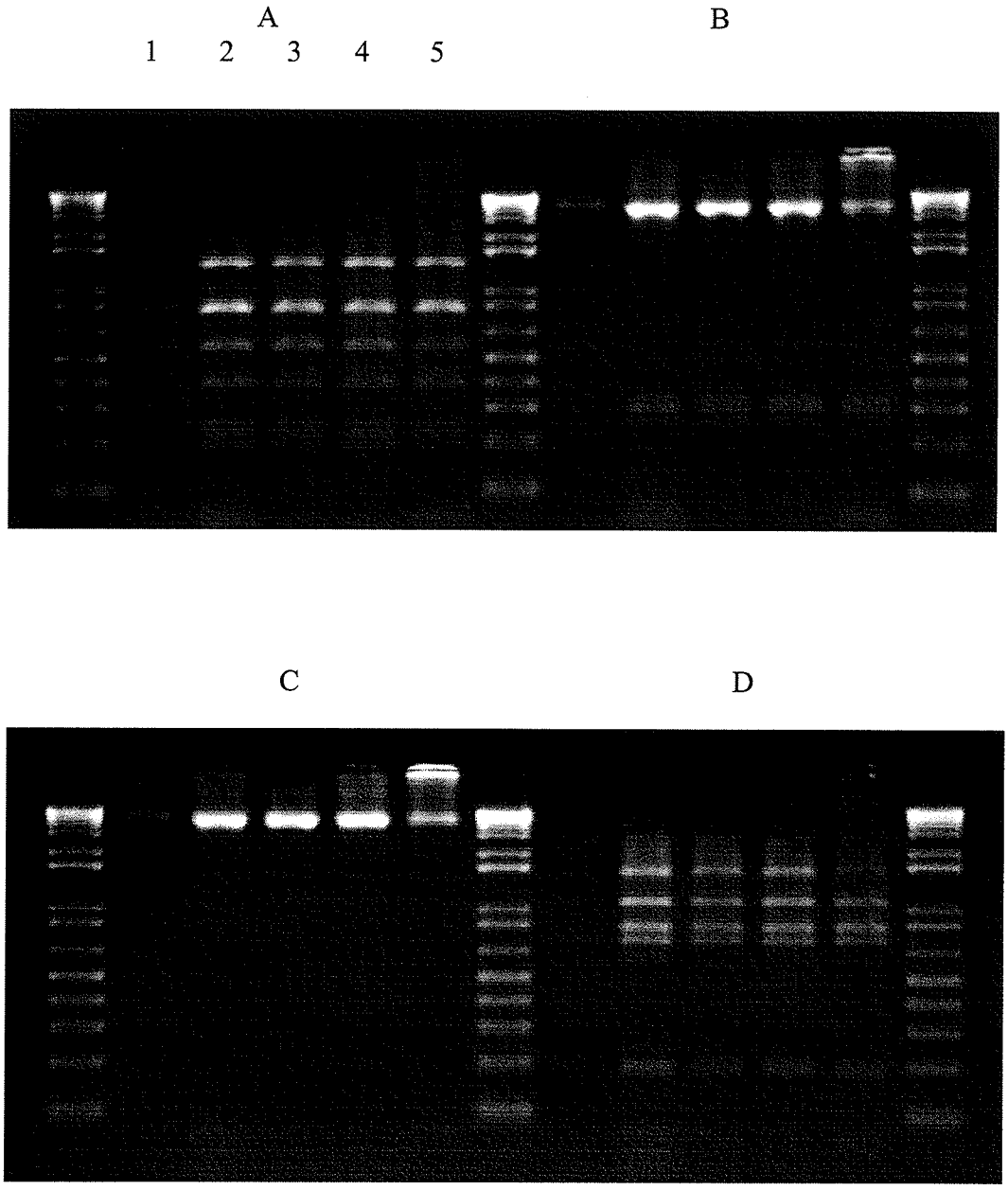

Fig. 5.4. A gel showing restriction digestion of IGS (A, *Hinf*I; B, *Mbo*I; C, *Rsa*I; D, *Taq*I) of five egg masses of *Hybomitra nitidifrons nuda* immediately after oviposition, at the following locations in Manitoba:
1-Woodridge, 6 June, 1999
2-Woodridge, 7 June, 1999
3-Elma, 12 June, 2000
4-Piney, 14 June, 2000
5-Woodridge, 10 June, 1997

Table 5.1. Physical descriptions of egg masses belonging to five species of tabanids collected in southern Manitoba and identified by PCR-RFLP. Numbers in parentheses represent the number of egg masses examined. All measurements (mean±SE) are in millimetres.

Egg mass character	*Hybomitra n. nuda*	*Hybomitra lasiophthalma*	*Chrysops aestuans*	*Chrysops excitans*	*Chrysops mitis*
colour	dark brown to black	jet black	black	brown	dark brown to black
# of layers	5	3	1	3	3-4
Max length	11.7±0.5 (n=10)	9.2±0.3 (n=10)	10.4±0.8 (n=15)	9.8±0.4 (n=15)	8.8±0.7 (n=5)
Max width	6.3±0.2 (n=10)	5.2±0.2 (n=10)	3.4±0.2 (n=15)	3.8±0.1 (n=15)	3.4±0.1 (n=5)
Max height	2.7±0.1 (n=10)	3.7±0.2 (n=10)	0.5±0.02 (n=15)	2.2±0.1 (n=15)	1.6±0.1 (n=5)
Length of egg	2.0±0.1 (n=10)	2.2±0.1 (n=10)	1.8±0.1 (n=15)	1.7±0.1 (n=15)	1.5±0.1 (n=10)
# of eggs in egg mass	734.7±43.6 (n=40)	489.6±26.7 (n=10)	217.0±17.2 (n=40)	461.6±74.4 (n=15)	544.5±54.5 (n=2)

CHAPTER VI

Prevalence of four tabanid egg parasitoids in southern Manitoba: their biology, behaviour, and interactions

Introduction

Flies of the family Tabanidae occur in temperate and tropical regions of the World with approximately 4350 described species (Burger 1995). There are 335 species and subspecies in North America north of Mexico, 144 of those in Canada (Burger 1995; Teskey 1990). In certain areas of North America, they are among the most serious pests of livestock and humans. Female tabanids are of particular medical and economic importance because of direct injury and transmission of pathogens to livestock, wildlife and humans through blood-feeding. Species of Tabanidae are potentially capable of transmitting more than 30 disease agents, viruses, bacteria, protozoa, and helminths to their hosts mechanically and biologically (Krinsky 1976). In New York, the daily blood loss of one cow due to *Hybomitra lasiophthalma* (Macquart), *Tabanus quinquevittatus* Wiedemann, and *Tabanus sulcifrons* Macquaert was 352 ml, 59 ml, and 109 ml respectively (Tashiro and Schwardt 1953). In addition to blood loss, hosts expend energy in attempting to avoid painful bites. Formation of grazing lines, bunching, head tosses, foot stomps, ear flicks, and tail switches by pastured cattle were some group and individual behavioural responses to tabanid attack in Manitoba, Canada (Ralley et al. 1993). Heifers exposed to attacks by an average 90 flies per animal per day for 84 days gained 80 g per animal per day less than those protected from horse flies (Perich et al. 1986).

Adult female tabanids generally lay their egg masses 4 to 8 days after a blood-meal on vegetation overhanging water in a wide varieties of habitats (Teskey 1990). Embryonic development takes 6 to 11 days depending on species and temperature (Teskey 1990; McKeever and French 1992). Eggs hatch simultaneously, and the larvae

drop to the water or wet soil beneath, where they overwinter in the larval stage. Larvae undergo 5 to 11 instars which take one to three years to compelete (Teskey 1990). Fully grown larvae migrate to drier areas where they pupate and remain in the pupal stage for 1 to 4 weeks, depending on species and latitude (McKeever and French 1997). Their wings expand and their exoskeleton hardens as soon as they emerge; they then mate and females seek hosts. Adults are able to fly at least 1.7 to 6.8 kilometers from larval areas depending on species (Sheppard and Wilson 1976).

Environmental management, physical control, and chemical control have been used to control tabanid populations. However, their powers of flight, prolonged emergence, soil-borne larval habitat near water for much of their lives, and their extensive breeding sites have been made it difficult to manage tabanid populations (Anderson 1985). Biological control has rarely been attempted although a number of natural enemies have been reported.

Eggs, larvae, pupae, and adults of tabanids are attacked by a variety of natural enemies. Eggs are attacked by hymenopterous parasitoids, various insect predators, and fungi; larvae and pupae are partly controlled by vertebrates, dipterous and hymenopterous parasitoids, nematodes, and microbial pathogens (Anderson 1985). However, tabanid egg parasitoids are the most frequent natural enemies that have been reported by different investigators.

Menon (1957) reared a *Centrodora* sp. (Hymenoptera: Encyrtidae) from eggs of *Atylotus* sp. in India. Up to 40% of *Chrysops caecutiens* (Linnaeus) and *Chrysops relictus* Meigen eggs were parasitized by *Trichogramma evanescens* Westwood in Russia (Olsuf'ev 1935; Skuf'in 1949; Rastegaeva 1965). Up to 36% of egg parasitism of

different species of *Chrysops* spp., *Hybomitra* spp., and *Tabanus* spp. by *Trichogramma minutum* Riley has been reported from Minnesota, (Philip 1931), Michigan, (Martin 1927), Georgia, (Fatting, 1946), Ohio, (Drees 1982), Ontario, (James 1963), and Saskatchewan, (Cameron 1926). *Trichogramma semblidis* (Aurivillius) has been reared from 78% of *Chrysops* egg masses in New York, (Tashiro and Schwardt 1953). There are 10 described species (with many synonyms) of *Telenomus* reported to attack tabanid eggs in many geographical regions (Anderson 1985). According to Johnson (1984, 1992), these 10 species and their synonyms are as follow:

Telenomus angustatus (Thomson) (=*tabani* Mayr; =*coccivorus* Mayr; =*oophagus* Nikol'skaya; =*praetabani* Szabo) and *Telenomus promachivorus* (Gahan) are Palaearctic; *Telenomus benefactor* Crawford, and *Telenomus kingi* Crawford are Ethiopian; *Telenomus emersoni* (Girault), *Telenomus goniopis* Crawford, and *Telenomus tabanivorus* are Nearctic; *Telenomus tabanocida* Crawford is Neotropical; *Telenomus dignus* (Gahan) (=*Phanurus matsumurai* Ishida) and *Telenomus inclinis* Le are Oriental.

In the Old World, *T. angustatus* has been reared from *C. relictus* in Russia (Skuf'in 1949), from *Hybomitra bimaculata* (Macquart) in Switzerland (Auroi 1981; Kozlov 1967), from *Tabanus* sp. in Germany, Austria, and Russia (Mayr 1877; Nikol'skaya 1948; Olsuf'ev 1935), and from eggs of a tabanid species in Russia (Rastegaeva 1965) with up to 30% to 40% parasitism within egg masses. *Telenomus benefactor* was reported from Sudan (Patton and Cragg 1913), and Africa (Nikol'skaya 1948). *Telenomus kingi* was reared from *Tabanus kingi* Austen in Sudan (Marchand 1920; Nikol'skaya 1948)

In Canada, *T. emersoni* has been reported from Saskatchewan (Cameron 1926), British Columbia (Hatton 1948) and, Ontario (James 1963) with up to 30% parasitism within egg masses; in the USA from Minnesota (Philip 1931), Texas (Girault 1916; Parman 1928; Webb and Wells 1924), Delaware (MacCreary 1940), New Jersey (Orminati and Hansens 1974), California (Webb and Wells 1924), Nevada (Middlekauff and Lane 1980), Arkansas (Schwardt 1936), Louisiana (Jackson and Wilson 1966), and Alabama (Jones and Anthony 1964) with 13% to 90% parasitism within egg masses. Many different species of *Chrysops*, *Hybomitra*, and *Tabanus* have been reported as hosts for *T. emersoni* in the Nearctic region.

In the USA, up to 100% parasitism by *T. goniopis* has been reported from Maryland (Crawford 1913), New York (Tashiro and Schwardt 1953), Massachusetts (Bailey 1948), Arkansas (Schwardt 1936). *Goniops chrysocoma* (Osten Sacken), *H. lasiophthalma*, and *Tabanus atratus* Fabricius have been reported as hosts for *T. goniopis*. In the USA, *T. tabanivorus* has been reported from South Carolina (Goodwin 1976), Ohio (Drees 1982), Massachusetts south to Florida, west to Illinois (Hart 1895; Ashmead 1895; Hine 1903, 1907; Muesebeck 1979; Tashiro and Schwardt 1953; Jones 1953; Jones and Anthony 1964), Louisiana (Jackson and Wilson 1966), Alabama (Dukes and Hays 1971) with 50% to 100% parasitism within egg masses. Hosts for *T. tabanivorus* were *Merycomyia whitneyi* (Johnson), *H. lasiophthalma*, *T. atratus*, *Tabanus stygius* Say, and *Chrysops* spp.

Although tabanid egg parasitoids are frequently reported, there is little detailed information on prevalence, biology and behaviour of a single species. Taxonomy of this group of parasitoids is also unclear and it is probable that many new species remain to be

described. Study of different aspects of tabanid egg parasitoids and their hosts in Manitoba began in the summer of 1996 and continued until 2000. The objectives of this part of project were to determine prevalence of tabanid parasitoids in Manitoba, to study parasitoid biology and behavior, host-parasitoid interactions, and possibility of storage of host eggs and laboratory rearing of parasitoids.

Materials and Methods

Tabanid egg masses were collected from six locations in southern Manitoba in the summers of 1996-99: Woodridge, Sprague, McMunn, Hadashville, Seven Sisters, and Elma (Fig. 4.1). Egg masses were put in petri dishes and transported to the laboratory in an insulated cooler. After labeling, each egg mass was placed individually in a Petri dish and kept in an incubator at laboratory conditions (25°C, 50% RH, and 18L:6D intervals under fluorescent light). Egg masses were examined daily under the microscope and a few individual eggs were dissected to follow parasitoid development. About 24 h before the parasitoids were expected to emerge, egg masses were removed from the petri dishes to disposable flasks (13 x 8.5 x 3.5 cm, Fig IV.6) and sealed with cotton wool wrapped in paper towel. When the parasitoids started to emerge, they were provided with a 5% sucrose solution and water in a 2cm x 0.5cm wrapped dental cotton glued in the bottom of a 2 cm x 0.5 cm plastic container. The cotton was kept moist by adding water every second day.

Approximately 1000 3-5 day old tabanid egg masses were collected from Sprague, McMunn, Hadashville, and Seven Sisters and were kept under laboratory conditions to rear adult parasitoids. Reared parasitoids were used to make laboratory

observations on mating and oviposition behaviour, determining longevity and sex ratio, and carrying out laboratory experiments.

Acceptance of stored and previously frozen tabanid egg masses by *Telenomus* A and B - Thirty newly laid, unparasitized egg masses of *Hybomitra nitidifrons nuda* (McDunnough) were collected from the study areas and stored at 15, 20, and 25°C (10 egg masses each temperature) at 50% RH and 18L:6D intervals under flourescent light. Fourteen unparasitized egg masses of *Chrysops aestuans* Van der Wulp were also kept under laboratory conditions for this experiment. Five individual eggs were dissected daily to study embryonic development of the host. Stored egg masses were exposed to scelionid parasitoids on different days after incubation to determine acceptance by parasitoids.

Five fresh egg masses of *H. n. nuda* were collected in the field and stored at –25°C for 10 days. Frozen egg masses were kept under laboratory conditions and then were exposed to large numbers of *Telenomus* species A and B. After 24 hr, egg masses removed from the exposure chamber and were kept under laboratory conditions for three weeks.

Parasitoid development inside the tabanid eggs - Forty 1-2 day-old parasitized egg masses were collected and kept in petri dishes at 12, 15, 20, and 25°C at 50% RH and 18D:6L intervals under flourescent light (10 egg masses for each treatment). Egg masses were examined daily and 10-30 individual eggs from each egg mass were dissected under the microscope to determine the stage of development at each temperature.

Prevalence of parasitoids - To estimate the prevalence of parasitoids, egg masses of horse flies and deer flies were collected each year from 1996-1999. In 1998, 96 multi-layered egg masses and 153 single-layered egg masses were collected from Woodridge, and Elma, respectively. Both locations were visited at three-day intervals in June and July and on each visit, all observed egg masses were numbered and dated, and left in the field for 10-12 days to allow enough time for both host and parasitoid to develop and emerge normally. Empty egg masses were collected and stored separately in 70% ethanol to estimate prevalence of parasitoids among and within the egg masses. On each visit, two or three parasitized egg masses were collected to rear parasitoids under laboratory conditions for species identification. Forty-six multi-layered egg masses (40 parasitized and six unparasitized egg masses) and 69 single-layered egg masses (63 parasitized and six unparasitized egg masses) were selected to determine per cent parasitism, and the proportion of damaged and healthy individual eggs within tabanid egg masses. Using fine dissecting pins, individual eggs of selected egg masses were separated under a dissecting microscope, then eggs were categorized as parasitized (having an exit hole and meconium) damaged (thick and flat with solid materials inside), or successfully hatched (transparent, empty and with a delicate chorion), and then the percentage for each category was calculated.

Host partitioning and emergence patterns of parasitoids - To study host partitioning of parasitoids attacking egg masses of *H. n. nuda* and emergence patterns of scelionid egg parasitoids of tabanids in Manitoba, 31 parasitized egg masses were marked in Sprague,

Woodridge, and Elma. About three days before the parasitoids completed their development, the egg masses were collected from the field and parasitoids reared under laboratory conditions from each egg mass. Ten individual eggs were dissected to examine the stage of development of parasitoids. Twenty egg masses with fully developed parasitoids were placed in 70% ethanol immediately before emergence of the parasitoids. The surface layers of eggs were then separated from centre layers; individual eggs in each group were separated and sorted into three groups of eggs (parasitized, damaged, and hatched) and then parasitoids were removed from the parasitized eggs for species identification. Prevalence of parasitism was calculated for each parasitoid species in the surface versus centre of the egg mass.

Before the parasitoids began to emerge, 11 additional egg masses (five horse fly egg masses and six deer fly egg masses) were placed in a glass tube (length=10 cm and inside diameter=2 cm), sealed with cotton wool and paper towels. These were incubated under laboratory conditions. At 8 h intervals, adult parasitoids that emerged were stored in 70% ethanol for determination of sex and species identification.

Overwintering strategy of parasitoids - In late August, 1997 and May, 1998, 60 soil samples, including surface litter, were taken from Elma and Seven Sisters (each location 30 samples) where both parasitoids and hosts were abundant during this study. Samples were taken randomly from both sides of ditches (15 samples each side) from the edge of the water to five meters away from the water. Each sample consisted of soil and litter from a 50 x 50 cm area to a depth of 15 cm. Fifteen samples from each location were put in Berlese funnels for five days and emerging invertebrates were collected in 70%

ethanol. A flotation technique was used for the rest of the samples. Samples were added to a large bucket and then an appropriate volume of water was poured into the bucket. After stirring, soil particles settled to the bottom and organic debris such as leaves and branches floated on the top of the water. Coarse organic debris was washed in the bucket and removed by hand and the rest of the materials were placed on a fine sieve and washed with tap water. Invertebrates on the sieve were stored in 70% ethanol, sorted under a stereo microscope and hymenopterous parasitoids were removed.

Results

Mating behaviour – Male specimens actively walked all around and over the egg mass after emerging and frequently examined exit holes with their antennae. When a male detected that another parasitoid was emerging, it kept its head very close to the exit hole where the female was expected to emerge and the male repeatedly probed the exit hole with its antennae. When the female was not close to the exit hole, the male moved back and forth on its legs and covered the exit hole with its thorax, keeping the tip of its abdomen bent. The legs were firmly positioned around the exit hole and the wings were spread to shield the area from other males. Defending males stayed in this position for hours and would fight with other approaching males. Males used their antennae and head to ward off other males, which approached from the front. They used their wings and legs to repel males coming from left and right sides and hind legs and tip of the abdomen were used to keep off other males coming from the rear. When the emerging wasp appeared in the exit hole, the male touched it with its antennae. If the wasp inside the hole was a male, the guarding male would walk away. If the wasp was a female, the male would

immediately mount the female as it emerged from the egg. The female often ran away while the male was on her back, grasping her with his legs. While they were running around, other males attacked the mating male and attempted to dislodge it from its position, but in most cases the first male was successful in mating. Preparation for mating and getting the female stationary, took 15-20 minutes. During mating, which took about 30 seconds, the front legs of the male were between the head and thorax of the female, the middle legs were between the thorax and abdomen, and the hind legs held the hind legs of the female to keep her steady. During mating, the female's antennae were held divergent but the male's antennae were close to each other and vibrating between the antennae of the female. The tip of the abdomen of the female was tipped up, and the ovipositor was extended and ventrally directed. The abdomen of the male was bent down and the genitalia were applied between the base of the ovipositor and ventral part of the abdomen. After mating, the female was refractory to mating again; other males were able to distinguish between unmated and mated females. To observe mating behaviour, 23 pairs of parasitoids were studied under the dissecting microscope. After mating, each pair was kept in 70% alcohol for species identification. There were 19 pairs of *Telenomus* species A and four pairs of *Telenomus* species B. Identification of captured males and females supported my initial matching of males and females for species A and B.

Oviposition behaviour of *Telenomus* species A & B - Based on field and laboratory observations, newly laid and up to three day-old tabanid egg masses were acceptable to parasitoids. In this study, the majority of tabanid egg masses were found on leaves of *Typha* spp. (cattail) overhanging the water. On several occasions it was observed that

female tabanids fly around the cattail plants for a few minutes and eventually landed on a leaf of the selected plant and started to lay eggs. In the field, *H. n. nuda* completed an egg mass within 50-60 minutes and frequently it was observed that 1- 6 female *Telenomus* were present and stinging eggs while the female tabanid was laying eggs. When the egg mass was completed, some individual eggs in the centre of the egg mass were not accessible to parasitoids that arrived subsequent to completion of the egg mass. However, female parasitoids used any space among eggs and cattail leaves to parasitize eggs in the bottom layers. In these cases, a parasitoid would hold its wings vertically over the body to permit movement of the abdomen into the small space available. Some parasitoids walked under the leaf and parasitized eggs from beneath, through the cattail leaf. Female parasitoids walked around the egg mass and frequently stopped and examined the eggs with their antennae. When they found an acceptable egg, they moved forward, and held the abdomen above the selected egg, and pierced the egg with their ovipositor. Oviposition time was 10-15 seconds after selecting an appropriate egg. In *C. aestuans* egg masses, neither *T. semblidis* nor *Telenomus* species C were observed while a female tabanid was laying eggs. However, several times I observed *T. semblidis* arriving on newly laid egg masses before *Telenomus* species C.

Acceptance of stored and previously frozen tabanid egg masses by parasitoids - In the laboratory, eggs of *H. n. nuda* hatched in 5.4±0.4 (C.L. 95%, n=10 egg masses) and 7.9±0.5 (C.L. 95%, n=10 egg masses) days at 25°C and 20°C, respectively, and increased to 19.4±0.8 (C.L. 95%, n=10 egg masses) days at 15°C. Eggs of *C. aestuans* hatched in 8.0±0.6 (C.L. 95%, n=14 egg masses) days at 25°C. Egg masses incubated at 25, 20, and

15°C were acceptable for parasitoids for up to three, four and 10 days after incubation, respectively. No previously frozen egg masses were parasitized by parasitoids.

Parasitoid development inside the tabanid eggs and adult life span - At 25° C, eggs, larvae, and pupae of *Telenomus* species A or B took a mean of 1.1± 0.3 (C.L. 95%, n=17), 6.8±0.32 (C.L. 95%, n=22), and 3.1±0.28 (n=28) days to develop to the next stage, respectively; at 20° C, these developmental times were 2.3±040 (n=14), 9.3±0.36 (n=18), and 5.5±1.02 (n=21) days, respectively. The pupal stage took 15.0±0.72 (n=10) and 21.1±0.92 (n=13) days at 15° C and 12° C, respectively. At 25° C, *Telenomus* species C developed from egg to adult in 12 to 13 days; however, at 15° C, *T. semblidis* developed from egg to adult in 30 days. At Woodridge, development of parasitoids inside the tabanid eggs took 18 days from egg to adult and unparasitized eggs of tabanids hatched after 10-12 days in early June. Adults of both species A and B lived for up to five days under laboratory conditions (25±1°C, 50% RH and 18L:6D intervals under flourescent light) without any water or nutrients. However, providing 5% sucrose solution increased female life span to 7-8 days at this temperature. Females lived for 12-16 days with sucrose and water at 11° C.

Prevalence of parasitoids - In the ditch at Woodridge, *H. n. nuda* (Fig. IV.2) was the most abundant species of horse fly, but occasionally egg masses of *H. lasiophthalma* were also collected at this breeding site (Fig. IV.1). *Telenomus* species A (Fig. IV.5) and *Telenomus* species B emerged from egg masses of *H. n. nuda* and *H. lasiophthalma,* under laboratory conditions. At the second breeding site (pond, Fig. IV.3), *C. aestuans*

(Fig. IV.4) was the dominant species; however, 20 egg masses of other species including *Chrysops mitis* Osten Sacken and *Chrysops excitans* Walker were also collected. *Telenomus* species C was reared from egg masses of all three species. A few egg masses of an unidentified deer fly species produced scelionid parasitoids which appear to be different from *Telenomus* species A, B and C. Too few specimens of this *Telenomus* species were reared to allow preparation of a formal description.

Prevalence of parasitism among and within the collected egg masses from 1996 to 1999 was calculated and summarized in Table 6.1. On average, 85.3% of deer fly egg masses and 91.5% of horse fly egg masses were parasitized by scelionid and/or trichogrammatid parasitoids each year. On average, parasitism within the egg masses was 39.1% and 33.5% for deer fly and horse fly egg masses, respectively, between 1996 and 1999. In 1998, prevalence of parasitism among and within egg masses was studied in detail from larger numbers of egg masses collected in the field (Table 6.2 and 6.3). In that year, 153 deer fly egg masses and 96 horse fly egg masses were examined. Prevalence of parasitism among the egg masses was 94.1% and 93.8%, respectively. In the same year, parasitism within the egg masses was 46.4% and 34.5% for deer fly and horse fly egg masses, respectively.

In the summer of year 2000, 13 egg masss of *H. n. nuda* (9777 individual eggs) were selected and prevalence of *Telenomus* species A and *Telenomus* species B was determined. *Telenomus* species A and *Telenomus* species B emerged from 15.4±2.9% (C.L. 95%) and 13.5±3.6% (C.L. 95%) of examined eggs respectively; 44.4±5.8% (C.L. 95%) of eggs failed to hatch and 26.0±6.7% (C.L. 95%) of eggs successfully produced tabanid larvae.

Host partitioning and emergence patterns of parasitoids - Host-partitioning was studied in the 20 egg masses (14875 individual eggs) of *H. n. nuda* in 2000. In the surface layer of eggs, 11.1± 2.5% (C.L. 95%) of eggs were parasitized by *Telenomus* species A, 12.5±3.1% (C.L. 95%) by *Telenomus* species B and 72.7±5.3% (C.L. 95%) produced neither parasitoids nor host larvae. The rest of the eggs (3.7± 2.1%, C.L. 95%) successfully hatched. In the centre of the egg masses, *Telenomus* species A and *Telenomus* species B emerged from 22.0±3.5% (C.L. 95%) and 10.9±4.1% (C.L. 95%) of eggs respectively and 17.04±3.8 (C.L. 95%) failed to hatch; however, the rest of eggs (50.1±8.4%, C.L. 95%) produced tabanid larvae.

Male of all three scelionid species, males started to emerge at least two hours before females and waited for their mates on the egg mass. Emergence period for *Telenomus* species A from *H. n. nuda* egg masses was four days long and approximately 27% of males and 34% of females emerged on the first day of the emergence period. On the second day, 43% of males and 42% of females, on the third day, 25% of males and 22% of females, and on the fourth day the rest of parasitoids emerged. However, 53% of males and 43% of female *Telenomus* species B emerged on the first day, 37% of males and 33% of females on the second day, 9% males and 17% of females on the third day, and the rest of the parasitoids emerged on the fourth day of the emergence period. Females of *Telenomus* species C emerged in four days and male parasitoids emerged within two days in *C. aestuans* egg masses,. On the first day, 55% of males and 10% of females emerged. On the second day, 45% of males and 39.5% of females appeared, on the third day, 40.5% of females, and on the fourth day the rest of females (10.0%)

emerged. For all three species, the maximum number of parasitoids emerged in the morning during the emergence period (Table IV.1 and 2)

Sex ratios - Sex ratios of parasitoids were determined for parasitoids which emerging from 40 egg masses of horse flies and deer flies. Sex ratios were 0.67:0.33 and 0.57:0.43 (female:male) for *Telenomus* species A and B, respectively. The sex ratio for *Telenomus* species C emerging from *C. aestuans* (deer fly) egg masses was 0.67:0.33 (female:male). Chi-square showed significant difference among the sex ratios of species A and B (df=1, χ^2=8.59, P=0.005).

Overwintering strategy - No tabanid egg parasitoids were found among the insects collected from samples using Berlese funnels and the flotation method.

Discussion

The prevalence of tabanid egg parasitoids among the collected egg masses and within egg masses has been evaluated by different investigators during the last century. The range of parasitism among the egg masses has been reported as 13% to 100% for different hosts and scelionid parasitoids. Since the age of egg masses collected from the field is rarely determined, it is difficult to evaluate prevalence below 100%. Parman (1928) mentioned that *T. emersoni* did not parasitize egg masses older than five to six hours. In Manitoba, the scelionid species accepted egg masses up to four days old, depending on temperature throughout the development period. It is likely that some of the egg masses in previous studies were collected while they might still have been acceptable

to parasitoids. In this study, egg masses were marked and left in the field until all parasitoids had emerged, and then they were transported to the laboratory. In 1998, the prevalence of parasitism among the egg masses was 93.8% for *H. n. nuda* egg masses and 94.1% for *C. aestuans* egg masses. Because the egg masses were collected from a few habitats and locations where they were accessible by vehicle, it is possible that this evaluation is biased for overall prevalence of parasitoids in all breeding sites. However, if this estimate is typical for all breeding sites, then the parasitoids have a very efficient host-finding strategy to locate their hosts over extremely extensive tabanid oviposition habitats. Simultaneous parasitism by *Telenomus* species A and B in more than 85% of collected egg masses and *Telenomus* species C and *T. semblidis* in 79% of *C. aestuans* is an indication of intense competition between parasitoids in their associated hosts.

Parasitism within the egg masses has also been evaluated by different tabanid researchers. From 30% to 100% parasitism by *Telenomus* species have been reported within the egg masses. In at least two reports (Tashiro and Schwardt 1953; Rastegaeva 1965), more than 20% of the eggs within the egg masses produced neither parasitoids nor hosts. Previous evaluations may not be precise because in the majority of studies, estimates were made based on few egg masses collected in a single visit per location per season or the number of collected egg masses was not reported. In the present study, two locations were visited regularly for the entire tabanid season and the chorion of eggs within egg masses was used to determine the prevalence of parasitoids and also the study was continued for five consecutive summers. In multi-layered egg masses laid by *H. n. nuda* in 1998 at Woodridge, 34.5% of eggs within the egg masses were parasitized by *Telenomus* species A and B and 36.3% of eggs produced neither parasitoids nor hosts.

Because the egg masses were collected from the field after all parasitoids had emerged, it was not possible to estimate the contribution of species A from B to parasitism within the egg masses. Measuring the diameter of exit hole did not help to identify the responsible parasitoid for parasitized eggs because body size in *Telenomus* species A and B is similar. Therefore, in the summer 2000, a complementary study was conducted to determine the contribution of each species to parasitism of tabanid eggs. In those egg masses which were simultaneously attacked by both species, 15.36% and 13.46% of eggs parasitized by *Telenomus* species A and B, respectively. There was no significant difference ($P \leq 0.05$; t-test) between contributions of these two parasitoids to parasitizing individual eggs of *H. n. nuda*. In another experiment on host partitioning, prevalence of *Telenomus* species A was not significantly different ($P \leq 0.05$) from that of species B on the surface layers of eggs. However, *Telenomus* species A parasitized significantly more eggs ($P \leq 0.05$) in the centre of the egg masses. Females of *Telenomus* species A were more successful in the centre of egg masses probably because they have a longer metasoma. This character provides the opportunity for this species to access eggs in the centre of the egg mass. In single-layered egg masses attacked only by *Telenomus* species C or by both *Telenomus* species C and *T. semblidis*, 40% to 55% of eggs were parasitized. Parasitism in single-layered egg masses was higher than in multi-layered egg masses (36.3%) probably because all eggs in the former were exposed to parasitoids. In multi-layered egg masses, at least some of the eggs in the centre of the egg mass are not accessible to parasitoids. In single-layered egg masses, 40% to 44% of eggs were parasitized by *Telenomus* species C and about 10% to 11% of eggs were parasitized by *T. semblidis*.

The percentage of unhatched eggs in multi-layered egg masses was considerably higher than in single-layered egg masses (36% compared to 21% or less). Since, the percentage of unhatched eggs in unparasitized egg masses was 3% to 10%, it can be concluded that *Telenomus* species are responsible for the mortality in some eggs and that they may damage eggs physically as they are parasitizing adjacent eggs. It seems that *T. semblidis* does not contribute substantially to damaging eggs because in those egg masses attacked only by *T. semblidis*, only about 9% of the eggs failed to hatch, similar to the proportion of unhatched eggs in unparasitized egg masses (9.9%). Approximately 73% of eggs in the surface layers did not hatch while in the centre only 17% of eggs failed to hatch. This again supports the conclusion that an increase in parasitism by parasitoids causes more inadvertent damage to the surface eggs.

Mass production of *Telenomus* species attacking tabanid eggs of their natural hosts is impractical at this time because rearing tabanids under laboratory conditions has not been successful. The major problems are the prolonged life cycle of tabanids (1-3 years), adult refusal to accept blood, refusal to mate under lab conditions, unnatural oviposition behaviours in cages, refusal of the larvae to feed, cannibalistic behaviour, and mortality during ecdysis and pupation (Thompson and Krauter 1978).

Using an alternate host for scelionid species for laboratory rearing is restricted because of their limited host range. Some *Telenomus* species attack more than one host species within one genus, such as *Telenomus clisiocampae* Riley, which is restricted to hosts of the genus *Malacosoma* (Lepidoptera: Lasiocampidae). However, in some other *Telenomus* species, the host range is broader, such as *Telenomus alsophilae* Viereck, which attacks 17 genera of Geometridae and Noctuidae (Fedde 1977). It is unlikely that

the host range of a single species of *Telenomus* crosses ordinal boundaries (Johnson 1984; Masner, personal communications). In very rare cases, crossing ordinal boundaries in host range was reported for a single *Telenomus* species, such as Barrion and Litsinger (1984), who reported that *T. dignus* attacked eggs of *Tabanus* sp. (Diptera: Tabanidae), *Scirpophaga incertulas* (Wlk.), and *Scirpophaga innotata* (Wlk.) (Lepidoptera: Pyralidae) in the Philippines. It is possible that parasitoids of *S. incertulas* and *S. innotata* were mistakenly identified as *T. dignus*. According to results of this study in Manitoba, each *Telenomus* sp. attacks eggs of one or more species of tabanid in the same genus. For example, *Telenomus* species A and B parasitized eggs of *H. n. nuda* and *H. lasiophthalma*; *Telenomus* species C attacked eggs of *C. aestuans*, *C. excitans*, and *C. mitis*. It would not be surprising if more tabanid species are reported as hosts for these parasitoids in future studies. However, if a single species attacks the eggs of a few species in a genus, what do tabanid egg parasitoids do in Manitoba from August of a particular year, when no tabanid eggs are available, to early June of the following year?

Studies on overwintering strategies of tabanid egg parasitoids can help to answer this question. There are two possibilities for these parasitoids to pass through the cold Canadian winter. The first possible overwintering strategy could be to remain as adults in the organic litter, where the temperature is moderated by snow cover during winter, and for them to become active in late May shortly before tabanid oviposition activity starts. The second scenario would involve a second host from another family in Diptera (such as *T. alsophilae* which has different hosts in two families of Lepidoptera) in which its eggs can be used by parasitoids to overwinter in immature stages until the following year. If the second scenario occurs, perhaps this second host can be studied as an alternative host

for tabanid egg parasitoids for lab rearing purposes. All attempts to find a second host for these egg parasitoids in Manitoba and to determine their overwintering strategy were unsuccessful.

Trichogrammatid species are the second group of tabanid egg parasitoids which have been reared, mainly from *Chrysops* spp. eggs. Using alternative hosts for *T. minutum*, *T. evanescens*, and *T. semblidis* is possible because these species are frequently reported as polyphagous egg parasitoids (Anderson 1985). However, being polyphagous, they can easily switch to non-target hosts. Moreover, their contribution to tabanid egg mortality is marginal and mainly restricted to one genus.

Mass production of tabanid egg parasitoids is impractical until a method to mass rear tabanid eggs is developed or appropriate alternative hosts are found. However, it is possible to take parasitized egg masses from one location with a high level of parasitism to another location. Parman (1928) collected 10-12 gallons of parasitized egg masses (20,000,000 to 25,000,000 eggs) and placed them in tin cans and buckets along a river in another location. He found a higher prevalence of parasitism in the treated location than in the control. We found that parasitized egg masses can be stored at 5°C to 10°C and carried to another location to release the parasitoids simultaneously. However, to make such an operation more efficient, there is a great need to study responses of different stages of parasitoids to temperature and light to avoid high parasitoid mortality during storage.

Table 6.1- Prevalence (%) of parasitism by *Telenomus* species A and B among and within the egg masses of *Hybomitra* spp. egg masses; *Telenomus* species C and *Trichogramma semblidis* among and within the egg masses of *Chrysops* spp. Egg masses were collected from 1996-1999 in southern Manitoba.

	Prevalence among egg masses		Prevalence within egg masses	
	Chrysops spp.	*Hybomitra* spp.	*Chrysops* spp.	*Hybomitra* spp.
1996	72.4 (29)*	92.8 (14)	22.6 (29)	28.2 (14)
1997	89.1 (64)	91.7 (108)	42.3 (15)	37.3 (15)
1998	94.1 (153)	93.8 (96)	46.4 (63)	34.5 (40)
1999	85.7 (70)	87.5 (64)	45.1 (10)	33.8 (10)

*- Numbers in parenthesis represent the number of egg masses examined.

Table 6.2- Prevalence of three scelionid and one trichogrammatid parasitoid among the egg masses of *Hybomitra nitidifrons nuda* and *Chrysops aestuans*, summer of 1998, Manitoba, Canada.

Host Species	Parasitoids	Egg masses attacked (%)
Hybomitra nitidifrons	*Telenomus* species A & B	85.44 (82)*
nuda	*Telenomus* species A	5.21 (5)
(multi-layered egg masses)	*Telenomus* species B	3.12 (3)
	Unparasitized	6.25 (6)
Chrysops aestuans	*Telenomus* species C & *Trichogramma semblidis*	79.09 (121)
(single-layered egg masses)	*Telenomus* species C	11.11 (17)
	T. semblidis	3.92 (6)
	Unparasitized	5.88 (9)

*- Numbers in parentheses represent the number of egg masses attacked by the associated species.

Table 6.3- Parasitism within the egg masses of *Hybomitra nitidifrons nuda* and *Chrysops aestuans* parasitized by three scelionid species (*Telenomus* species A, B, and C) and one trichogrammatid species (*Trichogramma semblidis*), summer of 1998, Manitoba, Canada.

Host species	Type of egg masses	Number of examined eggs	Parasitized eggs %	Damaged* eggs %	Hatched eggs %
H. nitidifrons nuda (multi-layered egg masses)	Parasitized egg masses (n=40)	29387	34.5±4.39	36.3±4.51	29.2±4.84
	Unparasitized egg masses (n=6)	4408	0	2.7±1.37	97.3±1.37
C. aestuans (single-layered egg masses)	Egg masses attacked by both parasitoids (n=40)	6948	44.1±6.08 *Tel.* 9.91±1.77 *Tri.*	18.6±3.32	27.4±6.53
	Egg masses attacked by only *Telenomus* species C (n=17)	2953	40.8±9.34	21.3±5.29	37.9±12.03
	Egg masses attacked by only *T. semblidis* (n=6)	1298	11.1±4.06	8.7±3.33	80.3±4.00
	Unparasitized egg masses (n=6)	1042	0	9.9±2.61	94.5±2.61

*- Damaged eggs produced neither parasitoids nor tabanid larvae.

CHAPTER VII

Arrestment of *Telenomus* A and *Telenomus* B (Hymenoptera: Scelionidae) by a kairomone extracted from their hosts, *Hybomitra* spp. (Diptera: Tabanidae)

Introduction

In the nature, a vast array of chemical and environmental cues provide information to parasitoids to exploit their hosts. Semiochemicals have a critical role in enabling parasitoids to locate and identify hosts. Chemical cues originate from hosts and/or their environment. Since, the host benefits by being as inconspicuous as possible, indirect cues to its location are often the only information available to foraging female parasitoids to direct them to the host habitat (Tumlinson et al. 1992). Then host-produced close-range kairomones, which are more directly linked to the host, help parasitoids to recognize their hosts. These semiochemicals, often found in host by-products, act as arrestants and/or stimulate the parasitoids to search more intensively in the near vicinity. For example, parasitoids have been observed to respond at close range to oral secretions (Corbet 1971; Loke and Ashley 1984), cuticle (Burks and Nettles 1978), silk (Weseloh 1987), faeces (Jones et al. 1971; Loke and Ashley 1984) and scales (Jones et al. 1973). Many of the host-produced close-range kairomones have been identified and they tend to be relatively non-volatile (Tumlinson et al. 1992). Two main strategies are known for scelionid parasitoids to locate their hosts from a distance. Three species of *Telenomus* have been reported to be phoretic on the adults of their hosts, *Telenomus beneficiens* (Zehntner) (Van Vuuren 1935), *Telenomus calvus* Johnson (Buschman and Whitcomb 1980), and *Telenomus dignus* (Gahan) (Fernando 1971). This strategy allows egg parasitoids to access newly laid eggs without spending time and energy to locate the host, and also allows them to disperse to new habitats, an adaptation which may be particularly important for parasitoids with dispersive hosts (Orr 1988). The second strategy is to respond to environmental cues which are used to help egg parasitoids to find suitable

habitats which likely contain hosts (Tumlinson et al. 1992; Vecher 1981). *Telenomus* species (Hymenoptera: Scelionidae) are egg parasitoids of a wide variety of insects in the orders Lepidoptera, Hemiptera, Diptera, and Neuroptera (Johnson 1984). The existence and characterization of kairomones involved in host-location and selection by *Telenomus* species have been documented by different investigators. *Telenomus remus* Nixon and *Telenomus heliothidis* Ashmead have been studied with respect to kairomones released by their hosts. *Telenomus remus* parasitizes eggs of the *Spodoptera* pest complex (Lepidoptera: Noctuidae), serious pests of a large range of agricultural crops. Gerling and Schwartz (1974) found that *T. remus* was not able to find its hosts by volatile chemical cues in a Y-shaped olfactometer. Nordlund et al. (1983) reported that host-seeking behaviour of female *T. remus* was increased in Y-tube, Petri dish, and greenhouse bioassays by abdominal tips of female *Spodoptera frugiperda* (J.E. Smith) as well as (Z)-9-tetradecene-1-ol acetate and (Z)-9-dodecene-1-ol acetate. Nordlund et al. (1987) reported that accessory glands of *S. frugiperda* and *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae) contained kairomones that influenced the host recognition by *T. remus*. Gazit et al. (1996) reported that *T. remus* females were arrested after contacting kairomones extracted from eggs of *S. frugiperda*. Because arrestment occurred only upon physical contact of a wasp with the kairomone, they believed there was no involvement of volatile attraction of extracted materials. Strand and Vinson (1982) reported that the material present in the accessory gland of adult female *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) acted as an egg recognition kairomone for *T. heliothidis*. They also observed that the parasitoid failed to respond to the kairomone unless it was associated with a target, which was the size and shape of normal hosts.

There are 10 described species of *Telenomus* attacking tabanid egg masses in different geographical regions of the world. Tabanid eggs are laid about 4-8 days following a blood meal, depending on temperature (Teskey 1990). Usually on warm sunny days, female tabanids deposit their eggs in masses on the stems of plants, mainly cattail (*Typha* spp.) or under surfaces of leaves overhanging water or wet soil, where larvae develop (Teskey 1990). In the literature, there are no reports on the host-finding strategy of tabanid egg parasitoids, except in the study by Vecher (1981). He made laboratory and field observations on two species of tabanid egg parasitoids, a *Telenomus* sp. and a *Trichogramma* sp. He believed that these parasitoids were able to find the general location where eggs of tabanids were most likely to be encountered, then they detected eggs when they made physical contact.

During the summers of 1996-2000, hundreds of tabanid egg masses were collected in southern Manitoba. More than 90% of collected egg masses had been attacked by scelionid parasitoids, and about 70% of the individual eggs within the egg masses were either parasitized or damaged by parasitoids (Iranpour and Galloway 2002). To take better advantage of these beneficial insects, it is essential to understand the major factors that affect their host-seeking behaviour. We need to know how they use chemical cues to locate their hosts and to determine the sources, identity and functions of the semiochemicals that are used by parasitoids to locate their hosts. The objective of this study was to extract any semiochemicals from adult tabanids, their fresh egg masses, and breeding habitats that may assist scelionid parasitoids in their search for tabanid egg masses. Laboratory and field observations were made on host-finding and recognition behaviour of scelionid parasitoids.

Materials and Methods

Parsitoid and host collection - Five to 10 day old egg masses of *Hybomitra* spp. and *Chrysops* spp. (Diptera: Tabanidae) were collected along ditches and ponds, east and southeast of Winnipeg, Manitoba (Canada) in the summers of 1998-2000. They were kept individually in disposable flasks, and the flasks were sealed with cotton wool and incubated at 25±2 °C and 50% RH under 16L:8D supplied by fluorescent light intervals. As male parasitoids started to emerge, they were provided with 5% sucrose solution and water from 2 cm x 0.5 cm cotton rope glued in the bottom of a plastic container of the same size, and distilled water was added to the rope every three days. Time and date of female emergence were recorded for each flask and one to two day old naïve female parasitoids were used for each experiment.

Manitoba horse fly traps were used to collect adult female tabanids in the same location as egg masses. Adults were collected from 10:00 to 15:00 and transported to the laboratory on ice in an insulated cooler. Adults were stored at –25 °C for 24 h in plastic containers, and then were identified to species and kept in separate vials for material extractions on the same day. Fresh tabanid egg masses were collected in the field as soon as oviposition was completed. Fresh egg masses of *Hybomitra nitidifrons nuda* (McDunnough) were placed in vials and transported to the laboratory on ice in an insulated cooler. Materials from fresh egg masses were extracted in the laboratory within two hours of collection.

Extractions - The abdomen was removed from a female tabanid and ground in a mortar in the presence of liquid nitrogen. Then 100 ml of hexane were added and stirred slowly for 10 minutes. The hexane was removed to a glass graduated cylinder and another 100 ml of hexane were added to the mortar for an additional five minutes and stirred. The two hexane samples were combined. Nitrogen gas was used to evaporate the hexane to the desired concentrations. During evaporation, different concentrations were made from the original extract and stored in small vials at –25 °C. Extracts were used to measure arrestment time of parasitoids reared from tabanid egg masses.

Bioassays - Twenty µl of each extract concentration were applied to a 1 x 1 cm piece of filter paper (Whatman, No. 1) as the treated surface. Treated filter papers were dried at room temperature for 10 minutes and then were placed on the centre of a circular filter paper and both were put in an uncovered petri dish (9.5 cm diameter) (Fig.V.1). Bioassays were carried out inside a clean Plexiglass cage (30 x 30 x 30 cm) (Fig.V.2) with fine screen on top, back, right and left sides. The front of the cage had a sliding door to regulate the desired space to access the inside of the cage. Because both *Telenomus* A and B emerged from the same egg masses, and it was not possible to separate them when they were alive, the examined parasitoids were captured after each experiment for species identification. Each wasp was captured from the rearing flask under a stereomicroscope using an aspirator. The wasp was carried inside the cage beside the treated area and, with the aid of camel hair brush, moved on to the treated paper (in preliminary experiments, wasps were not attracted to the treated filter papers from a distance, therefore they were placed directly on treated filter papers). The arrestment response was determined by using

a stopwatch to measure the retention of the parasitoid on the treated filter paper. The measurement began when the wasp touched the filter paper and was terminated when it left. If the wasp returned to the treated surface within 30 seconds, this period of time was included in the arrestment time and the timing was continued. Filter papers treated with 20 µl of hexane were used as controls. For bioassay tests, naïve female wasps (1 to 2 days old) were examined for each treatment at laboratory conditions (22±1 °C and RH 57±2 %) from 9:00 to 3:00.

Response of *Telenomus* species A to the extract from *H. n. nuda* on different days after extraction - Material was extracted from the abdomens of five *H. n. nuda* individual females. Different concentrations, 0.0002, 0.002, 0.02, 0.2, and 0.4 female equivalents, were made from the original extracts from the *H. n. nuda* abdomen and stored in small vials at –25 °C. Then 11 filter papers were treated simultaneously for each concentration and kept at room temperature and humidity to test in subsequent days. Every day, one set of the treated filter paper was used to measure arrestment time. For bioassay tests, 5-12 naïve female *Telenomus* species A were examined at each concentration. Analysis of covariance, linear regression estimation and two-way ANOVA (SAS version 8.2) were used for data analysis. Bonferroni adjustments were applied to the significance level to adjust for multiple pairs compared.

Arrestment of parasitoids species A and B by different concentrations of extract from abdomens of *H. n. nuda* - Nine to 20 females of each of *Telenomus* species A and B were exposed to different concentrations of extract from *H. n. nuda*. In this experiment,

0.0002, 0.002, .02, 0.2, 0.4, 0.8, 1.6 female equivalent concentrations were made from the original extract from the abdomens of *H. n. nuda*. Then the arrestment of females of species A and B at different concentrations was measured. Confidence limits (95%) were calculated for means at each concentration to compare arrestment of *Telenomus* species A and B.

Response of parasitoids A and B to extract from *Typha* sp. (cattail) - Leaves of *Typha* sp. (cattail), the plant normally used by tabanids for oviposition at the related field site, were collected from the oviposition sites of horse flies and deer flies. Plant materials were transported to laboratory in sealed plastic bags in an insulated cooler. They were weighed on the same day and placed in a glass chamber (100 cm long x 20 cm diameter) for 24 h. The system was connected to a filter pump for suction. Pentane (200 ml) was used to collect and dissolve any possible volatile component released from the cattails for 24 h. Then the pentane volume was diminished using nitrogen gas to the desired concentrations (0.7, 7, 14 g cattail equivalents). Ten parasitoids of each species were examined at each concentration. Then analysis of covariance was used to analyse the data.

Response of parasitoid species A and B to fresh egg masses and four-year-old egg masses - Fresh and four-year-old egg masses of *H. n. nuda*, were used to examine the response of parasitoid species A and B. Ten parasitoids of each species were examined for each treatment and 95% confidence limits were calculated for mean arrestment time.

Response of parasitoid species A, B, and C to extracts from different host species - Material was extracted from abdomens of two to five individual females of the following species: *H. n. nuda*, *Hybomitra lasiophthalma* (Macquart), *Hybomitra affinis* (Kirby), *Hybomitra epistates* (Osten Sacken), *Hybomitra arpadi* (Szilady), *Hybomitra lurida* (Fallén), *Hybomitra illota* (Osten Sacken), *Tabanus similis* Macquart, *Chrysops indus* Osten Sacken, *Chrysops aestuans* Wulp, *Chrysops mitis* Osten Sacken, *Chrysops excitans* Walker (Diptera: Tabanidae); *Stratiomyia* sp. (Diptera: Stratyomiidae); *Brochymena quadripustulata* (Fabricius) (Hemiptera: Pentatomidae); *Phlyctaenia tertialis* (Gnenée) (Lepidoptera: Pyralidae). Then 0.2 female equivalent concentrations were made for all extracts and six to 21 parasitoids were examined for bioassay tests. Two way analysis of variance and least square means multiple comparison were used to compare the arrestment time of *Telenomus* species A, B, and C in the presence of extracts from different hosts and suspected hosts.

Attraction of parasitoid species B by extracts from cattail and fresh egg masses of hosts - In this experiment, the effect of volatile extracts from cattail and fresh egg masses of *H. n. nuda* to attract parasitoid species B was examined. Fresh egg masses of *H. n. nuda* were placed in 100 ml of hexane and shaken gently for 10 minutes. After removing the first 100 ml hexane, another 100 ml hexane were added and shaken for an additional five minutes. Nitrogen gas was used to evaporate the excess hexane to the desired concentrations (0.1, 0.2, 0.4, 0.8, 1.6 egg mass equivalents). Different concentrations (0.5, 1, 2, 4, 8, 16, 32 g leaf equivalents) of extract from cattail were also made as previously described. Pieces of filter papers (1x1 cm) were treated with different

concentrations of extracts of fresh egg mass and cattail then they were used in a Y-shaped olfactometer to test the volatile effect of the extracts. For a control, filter papers treated with 20µl hexane were used through all the experiments. Control and treated filter papers were placed at the two top ends of the Y-shaped tube and the ends were sealed with a piece of cotton wool. Twenty individual parasitoids of species B were released (one parasitoid at a time) into the bottom of the tube and the numbers of parasitoids walking toward the control and treatment ends were recorded. Those parasitoids which stayed in the bottom part of the tube for more than five minutes were removed and replaced. Chi-square analysis and Armitage Trend Test were used to analyse the data.

Results

Three species of scelionid parasitoids were reared from collected egg masses: *Telenomus* A and B emerged from egg masses of *H. n. nuda* and *Telenomus* species C emerged from egg masses of *C. aestuans*.

Response of *Telenomus* species A to extract from *H. n. nuda* on different days after extraction - Arrestment of parasitoid species A at five concentrations (0.0002, 0.002, 0.02, 0.2, 0.4 female equivalents) of material extracted from the abdomens of *H. n. nuda* was measured from 24 h after treating filter paper up to 11 days (Table 7.1). For any given day, the relationship between concentration and arrestment time was non-linear. Therefore a transformation used to produce linearity. ANCOVA used to analysis arrestment time and then logged concentrations used to plot arrestment time in different days (Fig.V.3). There was a relationship between different concentrations and arrestment

times for days 1-6, where the higher concentrations caused longer arrestment times. Concentration effect (df=5, F=103.26) and day effect (df=10, F=24.96) were significant (P<0.0001) and also interaction between concentration and day was significant (df=32, F=3.94, P<0.0001). The slope of regression lines varied from one day to another. Slopes of lines from Day 1 to Day 6 were significantly different from control (P<0.0001 for Day 1 to 5, and P<0.0008 for Day 6). The slopes of Day 7 to 11 were not significantly different from the control. After Bonferroni corrections for different numbers of comparisons, it became possible to compare arrestment time of different concentrations within each day. For Day 7 to Day 11, none of arrestment times were significantly different from the control. However, for Day 1 to Day 6, arrestment times for concentrations 0.2 and 0.4 were significantly different from the control (D1, D2, D3, D4, P<0.0001 for both concentrations; D5, P<0.0002, P<0.0001; D6, P<0.0024, P<0.0006); for concentration 0.02, arrestment times for Day 1 and Day 2 were significantly higher than the control (Day 1, P<0.0001; Day 2, P<0.001). Arrestment times for 0.02 female equivalent concentrations for Day 3 to Day 6 were not significantly different. Arrestment times for 0.0002, and 0.002 for Day 1 to Day 6 were also equal to the control.

Arrestment of parasitoids species A and B by different concentrations of extract from abdomens of *H. n. nuda* - Arrestment of parasitoid species A and B at seven concentrations of material extracted from the abdomen of *H. n. nuda*, was measured 10 minutes after filter papers were treated with extract (Table 7.2). Arrestment of both species of parasitoids to 0.0002 female equivalents was not significantly different from the control (C.L. 95%). For both parasitoids, arrestment time was significantly longer

than the control at concentrations of 0.002, 0.02, and 0.2 female equivalents (C.L. 95%). In both species of parasitoids, arrestment time diminished when concentrations increased to 0.4, 0.8, and 1.6 female equivalents (C.L. 95%). For all six concentrations from 0.002 to 1.6 female equivalents, arrestment time for species B was significantly greater than for species A (C.L. 95%).

Response of parasitoids A and B to extract from *Typha* sp. (cattail) - Arrestment times for parasitoids A and B at three concentrations of cattail extracts were measured (Table 7.3). The estimated slope was –0.124 (P=0.036) for logged concentration on arrestment time of both species of A and B. The lower the concentration used, the higher the arrestment time observed.

Response of *Telenomus* species A and B to fresh and four-year-old egg masses - To examine visual cues from host egg masses, arrestment times of parasitoid species A and B by fresh and four-year-old tabanid egg masses were measured (Table 7.4). Both parasitoids were arrested by fresh egg mass for more than 600 seconds. However, arrestment times on four-year-old egg masses were not significantly greater than for controls.

Response of parasitoid species A, B, and C to extracts from different host species - Arrestment times for parasitoids A and B in response to extracts from abdomens of the following species were measured: seven species of *Hybomitra*, one species of *Tabanus*, four species of *Chrysops*, (all Tabanidae); one species of *Stratiomyia* (Stratiomyiidae);

one species of *Brochymena* (Pentatomidae); one species of *Phlyctaenia* (Pyralidae) (Table 7.5). Arrestment of parasitoid C by extracts from abdomens of *H. n. nuda* and *C. aestuans* (host of parasitoid species C) was also measured. There were parasitoid species effects, host species effects, and interactions between parasitoid species and host species. The first analysis of variance was carried out for arrestment of *Telenomus* A and B to extracts from *Hybomitra* species and the control. There was a significant parasitoid species effect ($df=1$, $F=106.36$, $P<0.001$), host species effect ($df=7$, $F=37.47$, $P<0.001$), and interaction between two variables ($df=7$, $F=7.51$, $P<0.001$). Looking at arrestment time of parasitoid species A by extracts from *Hybomitra* species, three different groups were found (Table 7.5). Arrestment time for species A in response to extracts from *H. n. nuda* was significantly higher than that of extracts from other *Hybomitra* species and the control ($P<0.001$). The second group was *H. lasiophthalma*, *H. affinis*, *H. arpadi*, *H. lurida*, *H. epistates*, and *H. illota* for which response by species A to these hosts was significantly different from *H. n. nuda* and the control ($P<0.001$). The third group was the rest of species including control, for which arrestment of species A to these species was different from *H. n. nuda* and *H. lasiophthlama* ($P<0.001$). For *Telenomus* species A, the second and third groups had overlap for some species (Table 7.5).

For species B, there were three non-overlapping groups (Table 7.5). The response for species B to *H. n. nuda* was the highest, then the second group was *H. arpadi*, *H. lasiophthalma*, *H affinis*, and *H. lurida*. The third group was the rest of examined species including control. Parasitoid species B was arrested by the extracts from *H. affinis*, *H. arpadi*, *H. lasiophthalma*, *H. lurida*, and *H. n. nuda* for significantly longer period than

parasitoid species A ($P<0.001$); however, it was not significantly different to the extracts from *H. epistates* and *H. illota*.

For the rest of the extracts examined (*Tabanus* spp., *Chrysops* spp., *Stratiomyia* sp., *Brochymena* sp., *Phlyctaenia* sp.), arrestment times for species A were not significantly different from the control ($df=8$, $F=2.73$). However, for species B, responses to extracts from *T. similis* and *C. excitans* were different from the control ($P=0.05$; $P<0.0001$).

Arrestment times of species A, B, and C in response to extracts from *H. n. nuda*, and *C. aestuans* were different. ANOVA analysis showed significant host effect ($df=2$, $F=49.10$, $P<0.0001$) parasitoid effect ($df=2$, $F=9.58$, $P=0.0002$) and interaction ($df=4$, $F=18.70$, $P<0.0001$). Response of species A and B to extracts from *H. n. nuda* were significantly different from the control ($P<0.0001$); however, response to *C. aestuans* was not significantly different from the control. Arrestment of *Telenomus* species C by material extracted from *H. n. nuda* was not significantly different from the control but response of this species to material extracted from *C. aestuans* was significantly different from that of the control ($P\leq0.0006$).

Attraction of *Telenomus* species B by extracts from cattail and fresh egg mass of host - Different concentrations of extracts from fresh egg masses and cattail were used to measure attraction of *Telenomus* species B in a Y-shaped olfactometer (Table 7.6-7). The number of *Telenomus* species B attracted to different concentrations of *H. n. nuda* egg mass extracts was not significantly different from the control ($P=0.5275$). Likewise the

number of *Telenomus* species B attracted to different concentrations of cattail extraction was not significantly different from control (P=0.5850)

Discussion

Egg parasitoids of tabanids find their hosts in two phases, locating the suitable host habitat followed by location of the host itself. So far, the only long-range kairomone that has been identified is a sex pheromone of the host which helps the parasitoid to find its host (Tumlinson et al. 1992). However, Tumlinson et al. (1992) believed that such semiochemicals likely exist and help parasitoids to locate their hosts from long distance. Such long-range kairomones play a role, particularly in situations where there is little likelihood that the parasitoid can use a pheromone or an indirect chemical cue from the habitat to locate its host. In the case of tabanid egg parasitoids, Vecher (1981) believed that parasitoids were able to locate the habitats where they can possibly find the host, then by short-range flights and random walking, recognize the suitable host eggs after direct contact. However, in several cases in the field, we observed that 1 to 6 parasitoids were at tabanid egg masses within minutes after female tabanids started to lay eggs. These parasitoids parasitized tabanid eggs while the female tabanid was ovipositing. It is unlikely that the parasitoids can locate the oviposition site by random search in a few minutes particularly in Manitoba where there are extensive tabanid oviposition habitats. Moreover, in several cases, we collected only one tabanid egg mass in an extensive habitat with parasitoids in it. It seems that tabanid egg parasitoids may be using a host finding strategy which is much more efficient than random search. Although, phoresy is used by some *Telenomus* species (Van Vuuren 1935; Buschman and Whitcomb 1980; Fernando 1971), after several years of collecting adult tabanids, identification and

manipulating of live and dead specimens, the authors have never observed a single scelionid parasitoid associated with adult tabanids, either on the body of adults or in collecting traps. On the other hand, we did not observe any attraction of parasitoids to material extracted from fresh tabanid egg masses and cattail in a Y-tube olfactometer. Although it appears that there are no volatile cues from either fresh egg masses or cattail to help parasitoids to locate their host from long distance, there may be long-range kairomones from the host or its habitat, which direct parasitoids to their hosts. It is also possible that visual cues from cattail or other materials at tabanid oviposition sites help scelionid parasitoids to locate their hosts. Also if parasitoids overwinter in the tabanid oviposition site, then in the following year they are already in the suitable habitat for finding their hosts.

From examination of materials extracted from fresh tabanid egg masses and adult females, there are one or more components in the female tabanid abdomen which arrest scelionid parasitoids to stay and explore the eggs. Under laboratory conditions, *Telenomus* A was able to detect the material extracted from the abdomen of its host (*H. n. nuda*) in different concentrations ranging from 0.002 to 1.6 female equivalents or more. At concentrations above 0.2 female equivalent, this kairomone was stable enough to arrest the parasitoids after storage under laboratory conditions for at least six days after extraction. Although some authors reported that tabanid egg parasitoids only parasitize newly laid egg masses (Parman 1928), I observed both in the laboratory and the field, that these scelionid parasitoids attacked egg masses which were up to 4-5 day old.

Telenomus B also responded to different concentrations of material extracted from abdomens of *H. n. nuda*. *Telenomus* A and B both responded to concentrations equal to

0.002 female equivalent or more. Arrestment time increased with concentration from 0.002 to 0.02 and 0.2 female equivalents. However, for both species A and B, increasing concentration to 0.4, 0.8, and 1.6 female equivalent did not increase the arrestment time. Arrestment time for species B was significantly longer than for species A for all concentrations examined. More studies are needed to find the reasons for this difference between species A and B. *Telenomus* A and B did not respond to different concentrations of material extracted from cattail leaves, and the arrestment time for these parasitoids was not significantly longer than for controls. It seems that cattail has no material soluble in pentane to arrest parasitoids. An arrestment test of *Telenomus* A and B for fresh and four-year-old egg mass indicated that the physical aspects of the egg mass did not play a role in arresting parasitoids to stay and explore the egg masses.

Parasitoids A and B were arrested by extracts from the abdomens of seven species of *Hybomitra*, including the known host species for these parasitoids, *H. n. nuda*. Arrestment of species B to extracts from *T. similis* and *C. excitans* was also significantly longer than for the control. It can be concluded that *H. n. nuda* is the primary host for these egg parasitoids, but they might parasitize other species in the same genus or even other genera of Tabanidae. Unfortunately, we were not able to find egg masses of other *Hybomitra* species in the field to rear parasitoids to support laboratory results. Arrestment time for species B was significantly longer than for species A in all seven species of *Hybomitra* examined which implies stronger interaction between this species and it hosts. Arrestment times for *Telenomus* A and B by material extracted from abdomen of *Tabanus* spp., *Chrysops* spp., (all Diptera: Tabanidae), *Stratiomyia* sp. (Diptera: Stratiomyiidae), *Brochymena* sp. (Hemiptra: Pentatomidae), *Phlyctaenia* sp.

(Lepidoptera: Pyralidae), were not longer than for the control. Response of species B to species of other genera, implies that this species might have wider range of hosts among members of the family Tabanidae.

For species C, material extracted from only two species were tested. Arrestment time of species C by materials extracted from abdomen of *C. aestuans* (known host for this parasitoid) was significantly longer than for that of *H. n. nuda* and the control.

In conclusion, it seems that tabanid egg parasitoids may not be highly host specific. They might naturally parasitize other species in the same genus or other genera of the same family. Existing material in the body of the host which arrests parasitoids is promising for future studies to find alternative hosts for these egg parasitoids. This material should be extracted and identified in future studies. After finding appropriate candidates, this material might encourage parasitoids to accept alternative hosts under laboratory conditions.

Table 7.1. Mean arrestment times (seconds) for *Telenomus* species A (5-12 individuals for each treatment) by different concentrations (female equivalents) of material extracted from abdomen of adult female of *Hybomitra nitidifrons nuda* for 1 to 11 day-old extracts.

Age of extracts (days)	0.0002	0.002	0.02	0.2	0.4	Control
1	2.3 ±0.20	6.4±1.10	34.7±5.60	71.1±19	74.1±2.80	2.6±0.31
2	2.0±0.10	8.3±0.50	30.4±5.20	59.2±7.80	73.3±11.20	2.1±0.30
3	-	5.5±0.50	23.7±3.10	50.2±6.60	70.1±8.70	1.6±0.10
4	-	2.7±0.40	20.5±3.40	38.5±3.30	55.2±7.20	1.8±0.20
5	-	2.8±0.40	12.6±1.00	36.8±4.70	47.1±4.50	2.7±1.10
6	-	2.4±0.30	4.5±0.30	29.2±6.60	32.8±5.00	1.7±0.30
7	-	-	2.7±0.20	23.8±6.10	27.0±2.50	2.4±0.60
8	-	-	-	15.7±2.40	20.2±2.50	2.3±0.50
9	-	-	-	12.9±2.40	15.8±1.40	2.1±0.60
10	-	-	-	8.8±2.00	12.4±1.40	2.1±0.30
11	-	-	-	4.5±0.50	10.4±1.00	2.4±0.80

Table 7.2. Arrestment time (seconds, C.L. 95%) of female *Telenomus* species A and B to hexane extracts from the abdomens of *Hybomitra nitidifrons nuda*. Numbers of examined parasitoids are in parentheses.

Concentrations (female equivalents)	A	B
0.0002	2.1±0.1 (12)	2.1±0.1 (10)
0.002	17.5±1.7 (15)	153.6±10.3 (10)
0.02	59.9±5.5 (19)	165.3±22.4 (13)
0.2	106.8±7.8 (16)	186.8±6.7 (9)
0.4	99.3±8.0 (13)	145.6±8.7 (9)
0.8	84.2±4.3 (18)	112.5±10.4 (9)
1.6	75.2±9.2 (15)	99.5±5.5 (10)
Control	2.3±0.2 (19)	2.3±0.3 (20)

Table 7.3. Arrestment time (seconds) of tabanid egg parasitoids (10 individuals for each species of *Telenomus* species A and B) to possible volatile pentane extracts from cattail leaves (*Typha* sp.), a common plant used by tabanids for oviposition (310 g leaves in glass chamber for 24 h, extracts accumulated in 9 ml pentane).

Cattail equivalent	Species A	Species B
0.7 g	1.8±0.5	1.5±0.2
7 g	1.5±0.1	1.5±0.1
14 g	1.1±0.1	1.2±0.2
Control	1.7±0.1	1.5±0.2

Table 7.4. Arrestment time (seconds) of tabanid egg parasitoids (*Telenomus* spp., 10 individuals for each species) to fresh and four-year-old egg masses of *Hybomitra nitidifrons nuda*.

Treatment	Species A	Species B
Fresh egg mass	>600	>600
Four-year-old egg mass	3.0±0.6	2.8±0.3
Control	2.2±0.2	2.3±0.3

Table 7.5. Arrestment time (seconds)* for tabanid egg parasitoids to material extracted from abdomens of adult females of different species of insects (0.2 female equivalents).

Treatment	Species A	Species B	Species C
Hybomitra n. nuda	106.8±9.50 (16)[a]	186.8±12.40 (10)[a]	1.9±0.10 (6)[a]
H. lasiophthalma	26.7±4.10 (20)[b]	115.2±8.60 (12)[b]	-
H. affinis	27.0±3.20 (11)[bc]	104.0±7.50 (10)[b]	-
H. epistates	9.0±1.40 (10)[bc]	29.2±5.40 (10)[c]	-
H. arpadi	21.6±5.30 (10)[bc]	127.1±12.30 (12)[b]	-
H. lurida	14.7±2.80 (21)[bc]	99.9±8.70 (10)[b]	-
H. illota	2.6±0.30 (13)[bc]	12.5±2.20 (10)[c]	-
Tabanus similis	2.0±0.10 (13)[c]	2.9±0.40 (9)[e]	-
Chrysops indus	2.3±0.20 (10)[c]	2.2±0.20 (10)[c]	-
C. aestuans	1.8±0.10 (10)[c]	2.1±0.20 (10)[c]	48.2±4.30 (9)[b]
C. mitis	2.2±0.10 (11)[c]	1.9±0.10 (9)[c]	-
C. excitans	2.1±0.20 (14)[c]	2.3±0.20 (14)[e]	-
Stratiomyia sp.	2.0±0.10 (10)[c]	1.9±0.10 (9)[c]	-
Brochymena quadripustulata	2.2±0.20 (9)[c]	2.3±0.30 (10)[c]	-
Control (20µl hexane)	2.1±0.40 (13)[c]	2.3±0.30 (11)[c]	1.9±0.5 (10)[a]

* - Means followed by the same letter within columns not significantly different (ANOVA and Least Squares Means multiple comparison).

Table 7.6. Attraction of *Telenomus* species B in a Y-tube olfactometer by material extracted from fresh egg masses laid by *Hybomitra nitidifrons nuda*.

Concentrations	Number of parasitoids attracted	
(egg mass equivalent)	Treatment	Control
0.1	12	8
0.2	9	11
0.4	8	12
0.8	10	10
1.6	9	11

Table 7.7. Attraction of *Telenomus* species B in Y-tube olfactometer by material extracted from cattail (*Typha* sp.).

Concentrations	Number of parasitoids attracted	
leaf weight equ. (g)	Treatment	Control
0.5	12	8
1	9	11
2	8	12
4	10	10
8	9	11
16	11	9
32	8	12

CHAPTER VIII

GENERAL DISCUSSION

Although insecticides are currently our primary means of controlling populations of insect pests, there are a number of factors such as government regulations, economic constraints, environmental concerns, and pest resistance which restrict their applications. Biological control is considered one of the valuable tactics to control insect pests in agriculture and public health to avoid the disadvantages of using insecticides. Among biological agents, egg parasitoids mainly belong to the Scelionidae, Trichogrammatidae, and Mymaridae and have been reported as effective natural enemies of insect pests.

Species of Mymaridae attack eggs of other insects, mostly eggs laid in concealed situations, such as in plant tissue, under scales, or in the soil. Hosts of mymarid parasitoids are primarily Hemiptera and Homoptera, though eggs of species of Orthoptera, Psocoptera, Coleoptera, Lepidoptera, and Diptera are also used.

Species of Trichogrammatidae are all egg parasitoids of the large holometabolous orders and of Hemiptera, Homoptera, Orthoptera, and Thysanoptera. Species of this family appear to be more habitat-specific than host-specific. Species of *Trichogramma* have been used extensively in biological control programmes of various pests, particularly Lepidoptera (Goulet and Huber 1993). *Trichogramma semblidis* was one of the tabanid egg parasitoids that was reared from egg masses of *C. aestuans* in this study in Manitoba. This species is also reported as an egg parasitoid of species of other insects in the orders Lepidoptera and Coleoptera, a wide host range indeed for this species.

The Scelionidae is a large family of parasitic insects, the members of which specialize in egg parasitism of insects and arachnids. Although the host range of

scelionids varies widely, most species are considered monophagous or oligophagous (Krombein et al. 1979) which is desirable in potential biological control agents. It has not been reported that the host range for a single species of *Telenomus* crosses ordinal boundaries (Johnson 1984). Therefore they are generally more host specific than trichogrammatid parasitoids. Fewer than 30 species of *Telenomus* have been used in classical biological control and several of these have produced excellent results (Orr 1988). Species of the *T. tabanivorus* group attack eggs of Tabanidae, Asilidae, and Stratiomyidae. In Manitoba three scelionid species were reared from collected tabanid egg masses.

The prevalence of tabanid egg parasitoids among the collected egg masses has been evaluated by different investigators during the last century. The range of parasitism among the egg masses has been reported as 13% to 100% for different hosts and scelionid parasitoids. During 1996 to 1999, the range of prevalence of parasitism among the egg masses collected in Manitoba was from 87.5% in 1999 to 93.8% in 1998 for *H. n. nuda* egg masses and 72.4 % in 1996 to 94.1% in 1998 for *C. aestuans* egg masses. Within egg masses, from 30% to 100% parasitism by *Telenomus* species has been reported. In at least two reports (Tashiro and Schwardt 1953; Rastegaeva 1965), more than 20% of the eggs within egg masses produced neither parasitoids nor hosts. In Manitoba, in multi-layered egg masses laid by *H. n. nuda*, the range of parasitism within the egg masses was from 28.2% (1996) to 37.3% (1997). In addition, approximately 36% of eggs produced neither parasitoids nor hosts. In single-layered egg masses, which were attacked by *Telenomus* species C and/or *T. semblidis*, 40% to 55% of eggs were parasitized. Prevalence of parasitoids in single-layered egg masses was higher than in

multi-layered egg masses, perhaps because all eggs in the former are exposed to parasitoids. In single-layered egg masses, 40% to 44% of eggs were parasitized by *Telenomus* species C, but about 10% to 11% of eggs were parasitized by *T. semblidis*. Based on the previous reports and the current study in Manitoba, it seems that egg parasitoids naturally have considerable impact on tabanid populations.

Since the majority of researchers have reported high prevalence of tabanid egg parasitoids in their study area, the necessity of mass rearing and releasing these parasitoids is questionable. Accurate and extensive studies are needed in a variety of tabanid oviposition habitats to evaluate the prevalence of egg parasitoids. If the prevalence of parasitoids is low in a particular habitat, it might be useful to mass produce and release these egg parasitoids in target regions or simply to carry parasitized egg masses from one tabanid oviposition site to another. Nevertheless, mass production of *Telenomus* species attacking tabanid eggs on their natural hosts is unlikely because rearing tabanids under laboratory conditions has not been successful. Using an alternative host for lab rearing scelionid species is also restricted because of their limited host range. However, for a better understanding about these beneficial insects, it is necessary to study their taxonomy and biology.

One major stumbling block to a fuller exploitation of this group has been the lack of a solid systematic base. Even with recent improvements in the systematics, many species remain to be discovered and described. In the subfamily Telenominae, for example, more than 600 species have been described, that this represents only 10-25% of the estimated total number of existing species (Orr 1988). *Telenomus* is the largest genus of the family Scelionidae with over 500 described species worldwide (Johnson 1984).

There are 10 described species of *Telenomus* attacking tabanid eggs in all geographical regions of the World (Anderson 1985). Since these egg parasitoids are monophagous or oligophagous and there are approximately 4350 described species of tabanids worldwide, it seems that 13 described species (including three species reared in Manitoba) of tabanid egg parasitoids would be a small proportion of the real fauna of this group of parasitoids. Discovery of three new species in southeastern Manitoba also implies that there are many more species to be found and described around the world.

At the begining of this research project, I thought that there were two species of parasitoids attacking tabanid egg masses (*T. emersoni* and *T. semblidis*); however, closer examination revealed that there were three undescribed *Telenomus* species occurring in Manitoba, species A and B attacking egg masses of some species of horse flies, and species C parasitizing eggs of some species of deer flies. *Telenomus* species C was much smaller than species A and B; it had a different body habitus and different characters on the metasoma; it was also reared only from *Chrysops* spp. eggs. *Telenomus* species C was quite different from *Telenomus* species A and B. Species A and B were more challenging to recognize because they emerge from the same egg mass and have similar body size and habitus. Eventually, fine scale host partitioning and characters on the metasoma helped us separate females of species A from B. Moreover, association of mating males and females supported our initial matching of males and females of species A and B. Therefore, for accurate identification of tabanid egg parasitoids, it is critical to know their behaviour and interactions with their hosts.

The most challenging part of this project was study host-parasitoid interaction because it was necessary to identify tabanid egg masses. The first attempt to identify

tabanid egg masses using available information was not successful because there were no suitable descriptions of egg masses. Then, for more than a year, I tried to rear larvae from eggs. However, early instar larvae were not morphologically appropriate for identification and high mortality during the process of larval development caused major problems for raising tabanid larvae to maturity. Therefore, molecular techniques were explored to find a fast and accurate method to identify immature stages of Tabanidae.

Molecular tools have been used widely in insect systematics during the last two decades. However, application of molecular techniques (particularly DNA-based methods) in other aspects of entomological research has been used during 1990s.

To my knowledge, there are only a few studies in which isozyme and cuticular hydrocarbons have been used to segregate two or three cryptic species of tabanids (Hudson and Teskey 1976; Jacobson et al. 1981; Hoppe et al. 1990; Sutton and Carlson 1997). I examined several regions of mitochondrial and nuclear DNA to find an informative region to distinguish adults of different species. After two years of laboratory work, I found that IGS was an appropriate DNA target to characterize adults of 35 species of tabanids so they could be matched with first instar larvae reared from field-collected egg masses. After successful molecular characterization of adults, the same procedure was applied to the egg masses and first instar larvae to associate egg masses with adults of each species.

PCR-RFLP for this purpose was fast, inexpensive and accurate; however, preliminary molecular studies on adult tabanids in each geographical region must be carried out. Since individuals collected from Churchill had the same restriction fragment pattern as individuals of the same species collected in southeastern Manitoba, this

molecular data inferred from PCR-RFLP of IGS in Manitoba might be useful to other tabanid investigators on the same continent; however, this should be confirmed by further collection and molecular studies. This database for Tabanidae can be supplemented by adding data for Tabanidae of other regions. Then this can be used for revision of the family and particularly improving the knowledge of systematics of immature stages. Eventually identification of immature stages of tabanids would be useful for better understanding of host-parasitoid relationships.

Egg masses were collected mainly in two types of habitats in different locations. The first type of habitat was ditches with wet soil and/or shallow, stagnant water beside the highways and provincial roads. Most of these egg masses were identified as *H. n. nuda* and *H. lasiophthalma*. The second habitat included ponds and ditches with deep water where *Chrysops* spp. egg masses were collected. Since there are about 50 species of tabanids in Manitoba (Teskey 1990), there should be many more habitats where tabanids lay their eggs in this province.

I found that egg parasitoids have considerable impact on tabanid populations in Manitoba and it is likely that they have the same impact on tabanid populations in other locations. Now with available data on taxonomy, prevalence, biology and behaviour of tabanid egg parasitoids and also the molecular tools for DNA fingerprinting of immature stages of Tabanidae, the foundation is laid for future research on tabanids and their egg parasitoids in Canada and other regions. Using these data, future studies may be designed to explore new species of egg parasitoids, possible alternative hosts for parasitoids, host finding strategy, storage of parasitized eggs in proper laboratory conditions for mass

release purposes, host-parasitoid interactions, overwintering strategy of parasitoids in arctic regions, and systematics of the immature stages of tabanid.

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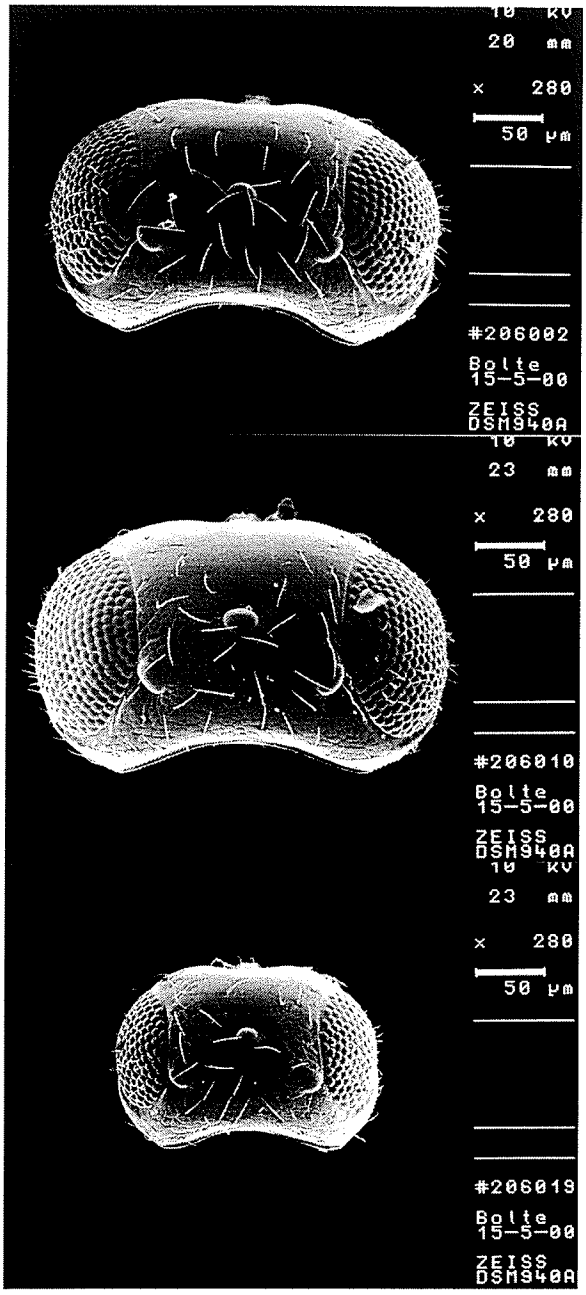
Appendix I. Electron microscopy of *Telenomus* species which parasitize eggs of Tabanidae.



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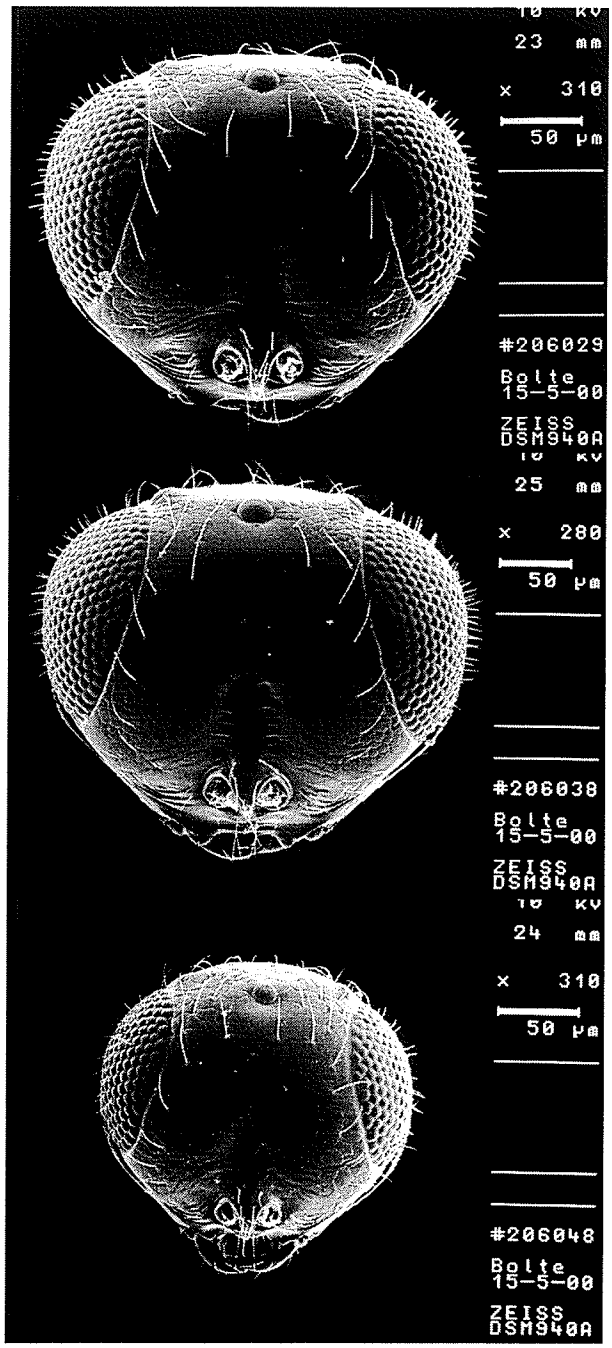
Figs. I.1-3. Prosoma in dorsal view (females). 1, *Telenomus* species A; 2, *Telenomus* species B; 3, *Telenomus* species C.



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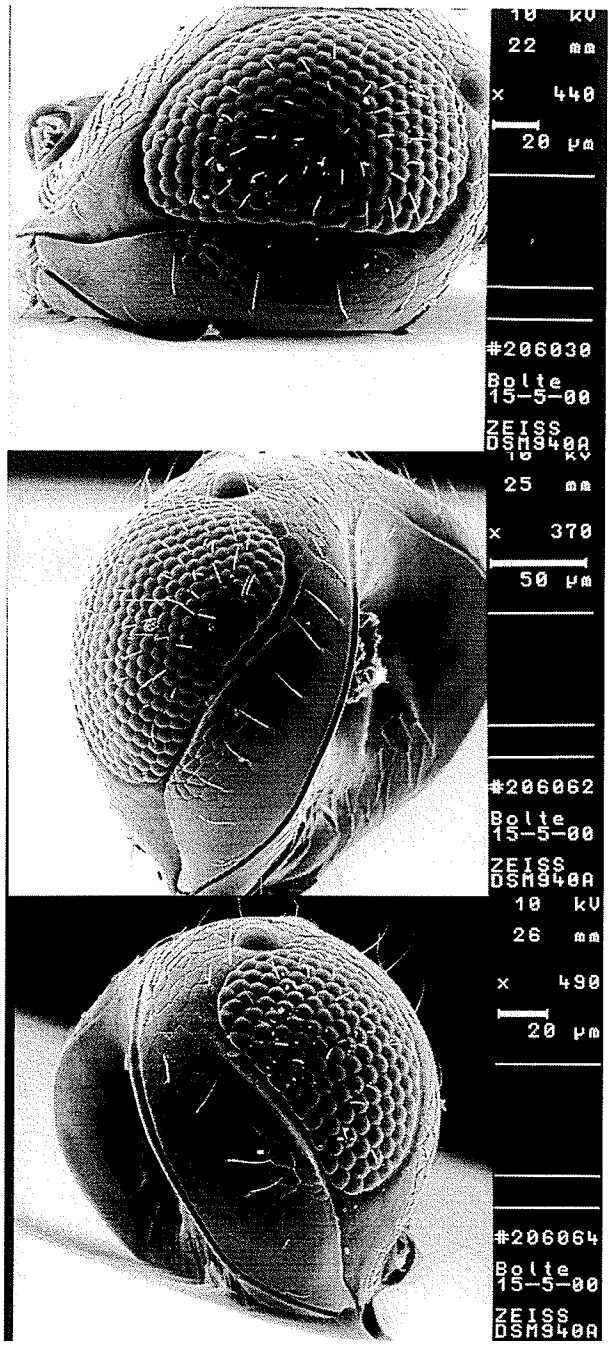
Figs. I.4-6. Prosoma in frontal view (females). 4, *Telenomus* species A; 5, *Telenomus* species B; 6, *Telenomus* species C.



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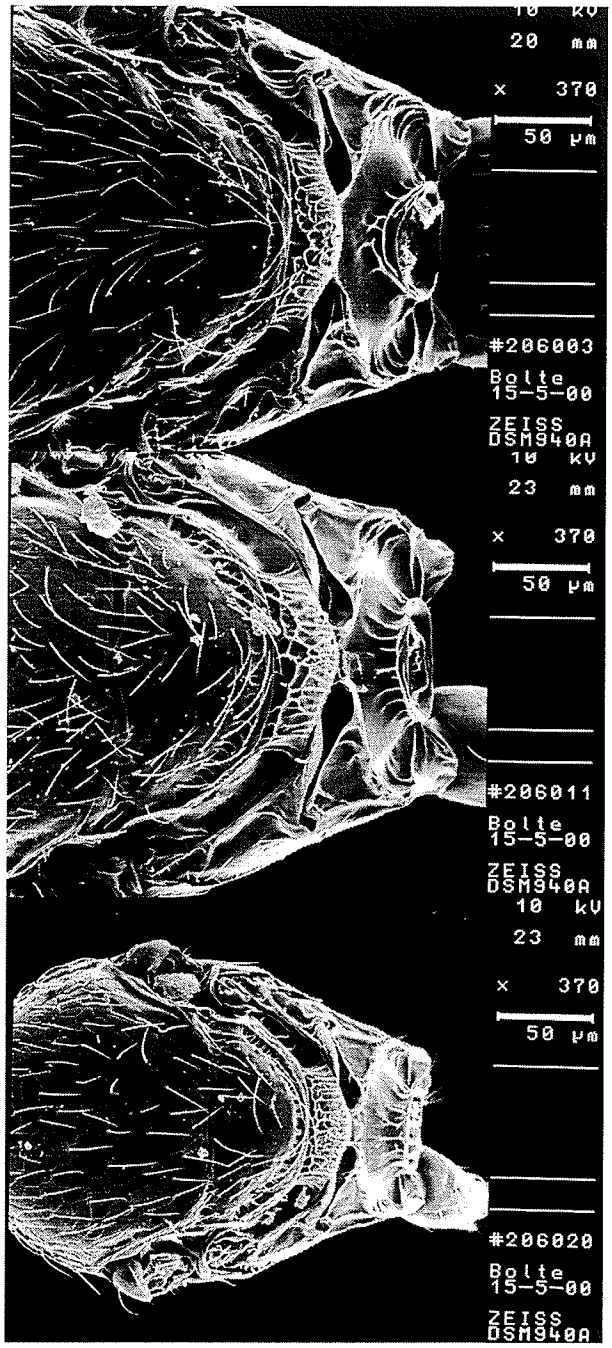
Figs. I.7-9. Prosoma in lateral view (females). 7, *Telenomus* species A; 8, *Telenomus* species B; 9, *Telenomus* species C.



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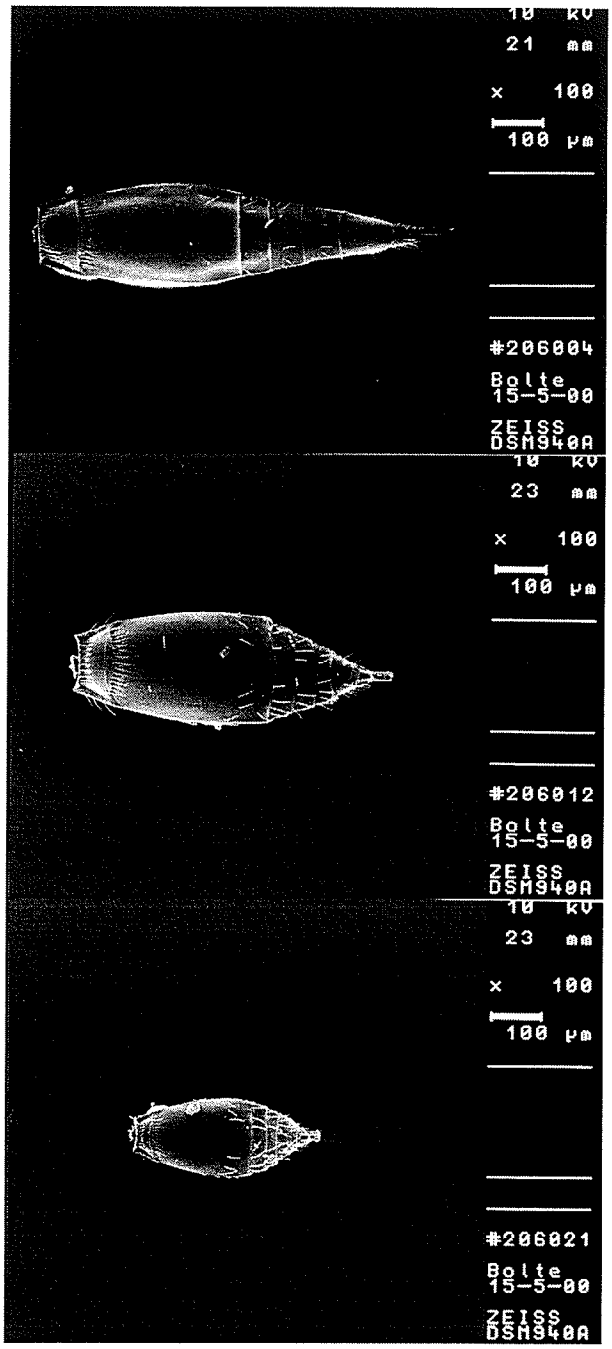
Figs. I.10-12. Mesosoma in dorsal view (females). 10, *Telenomus* species A; 11, *Telenomus* species B; 12, *Telenomus* species C.



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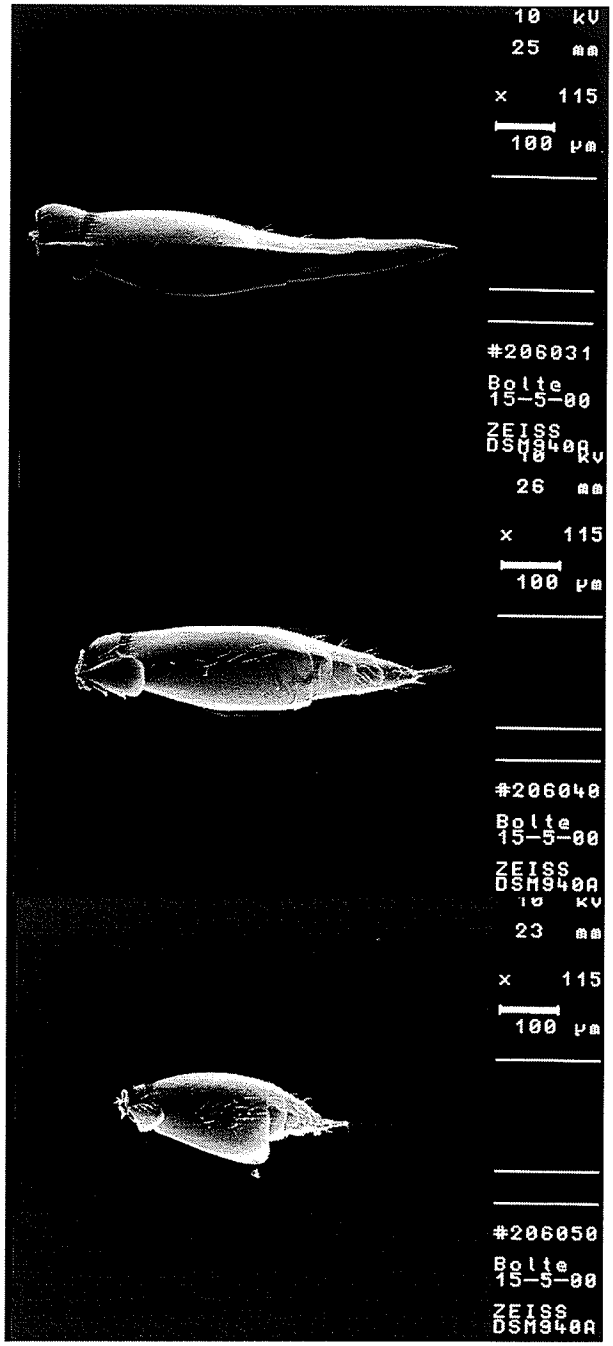
Figs. I.13-15. Metasoma in dorsal view (females). 13, *Telenomus* species A; 14, *Telenomus* species B; 15, *Telenomus* species C.



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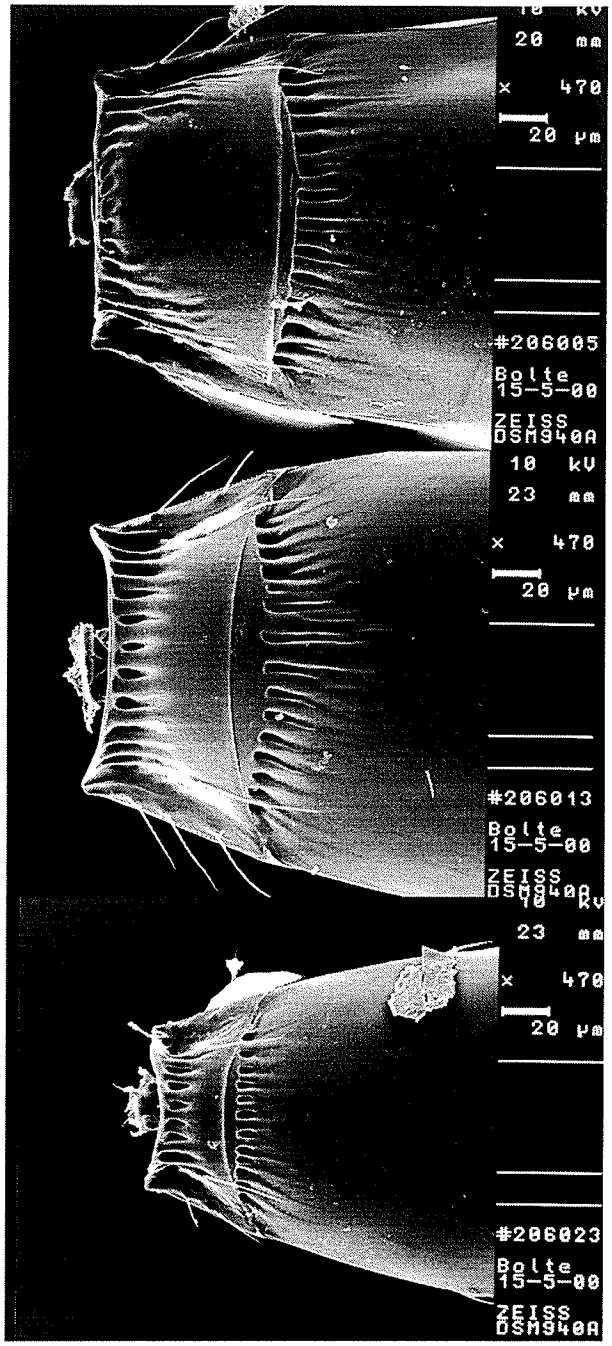
Figs. I.16-18. Metasoma in lateral view (females). 16, *Telenomus* species A; 17, *Telenomus* species B; 18, *Telenomus* species C.



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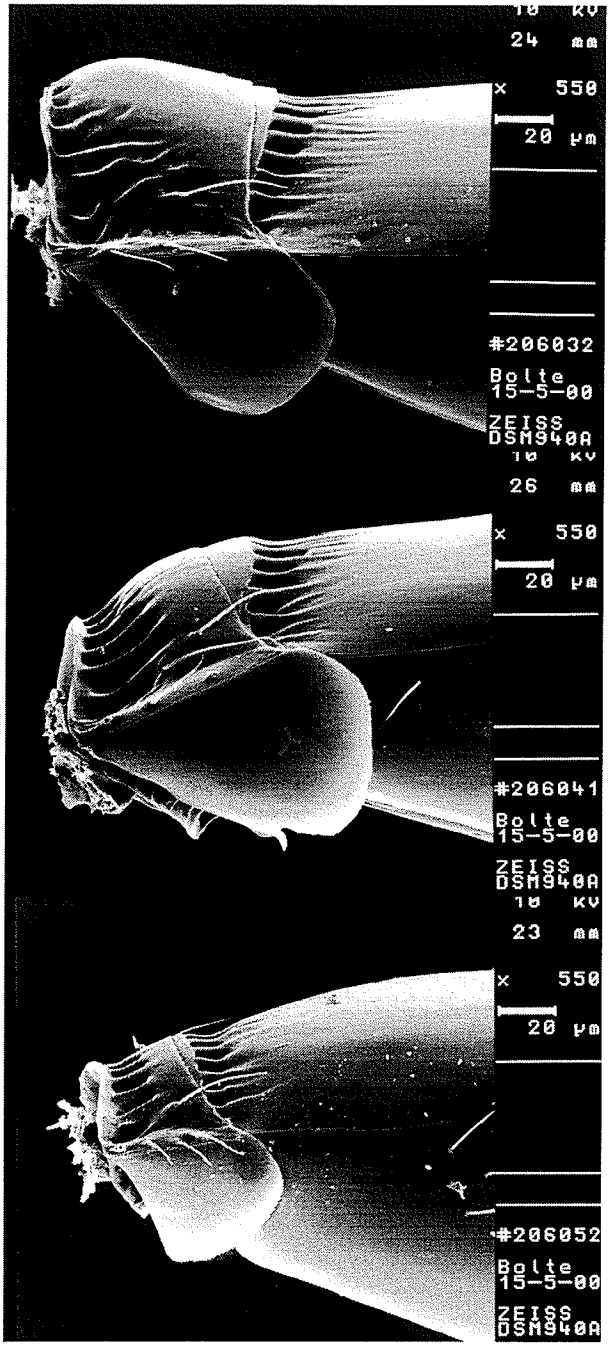
Figs. I.19-21. T1 in dorsal view (females). 19, *Telenomus* species A; 20, *Telenomus* species B; 21, *Telenomus* species C.



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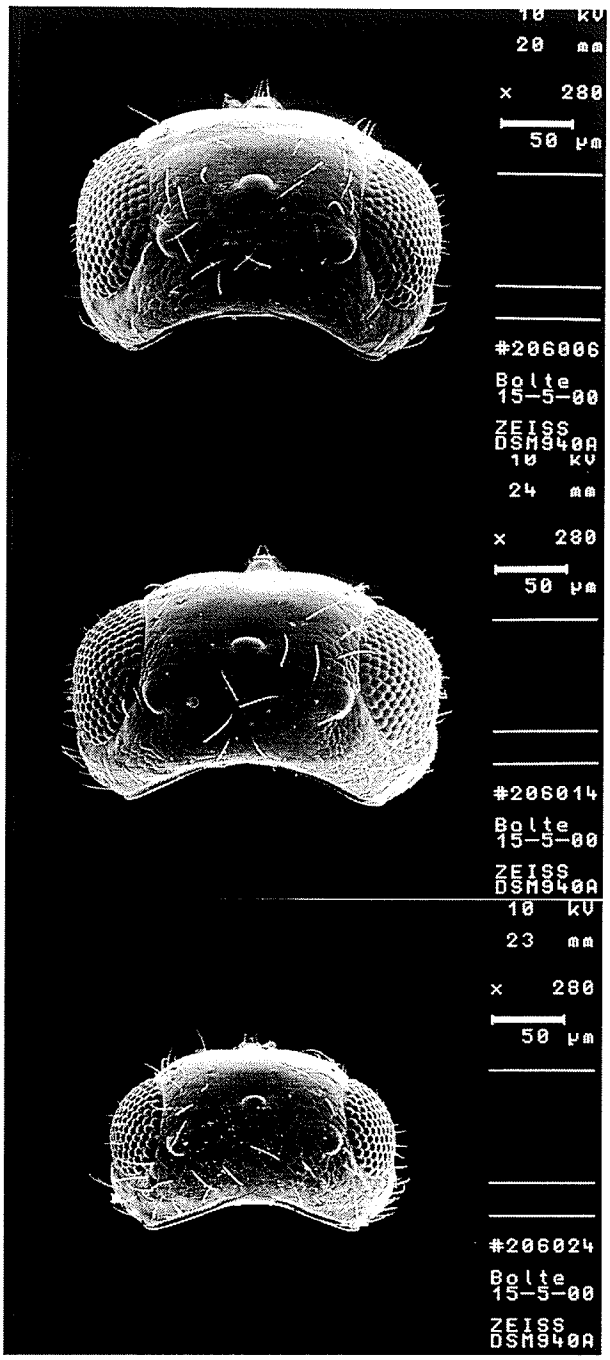
Figs. I.22-24. T1 in lateral view (females). 22, *Telenomus* species A; 23, *Telenomus* species B; 24, *Telenomus* species C.



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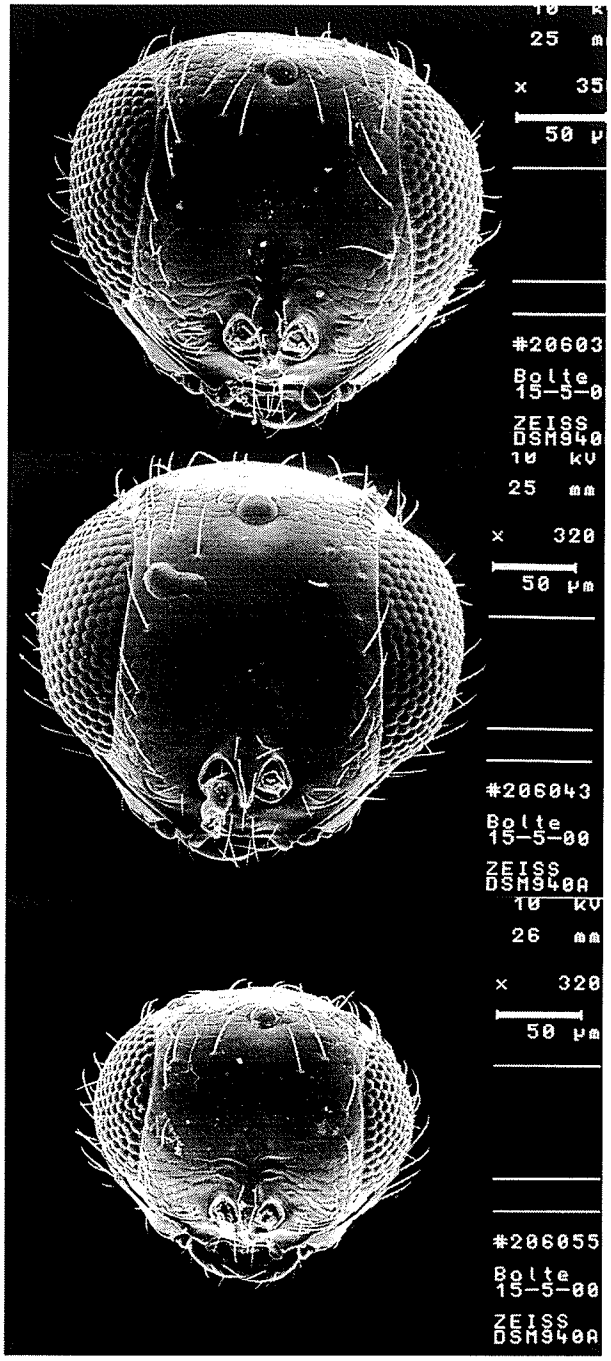
Figs. I.25-27. Prosoma in dorsal view (males). 25, *Telenomus* species A; 26, *Telenomus* species B; 27, *Telenomus* species C.



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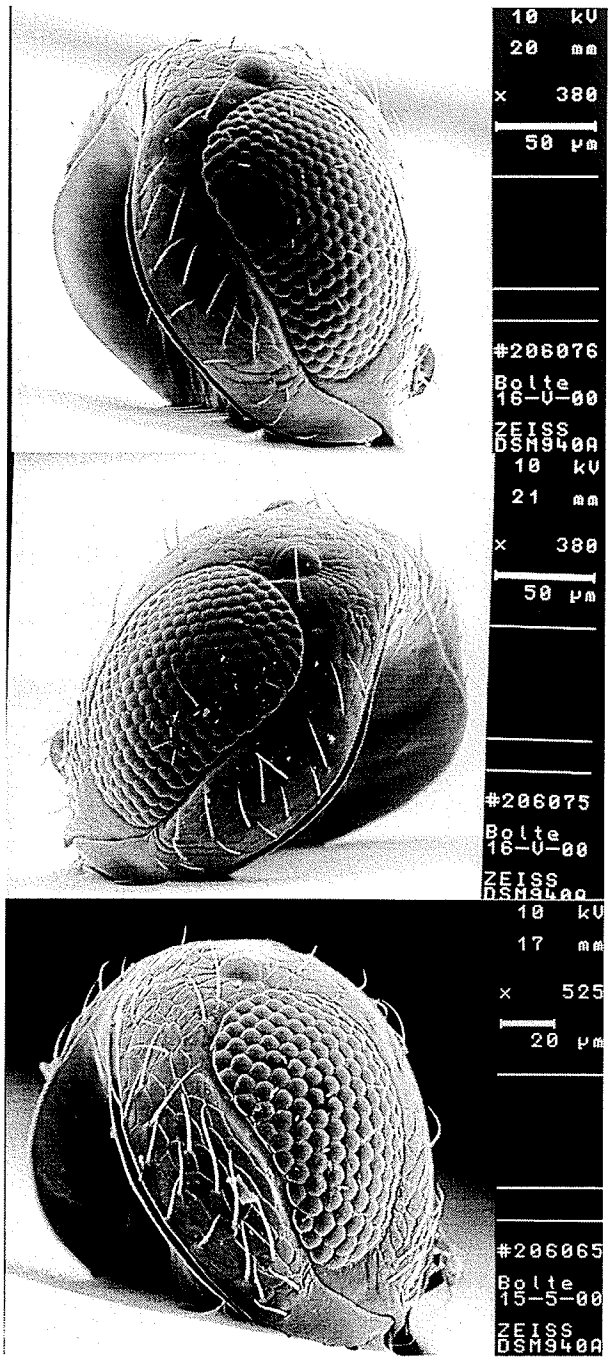
Figs. I.28-30. Prosoma in frontal view (males). 28, *Telenomus* species A; 29, *Telenomus* species B; 30, *Telenomus* species C.



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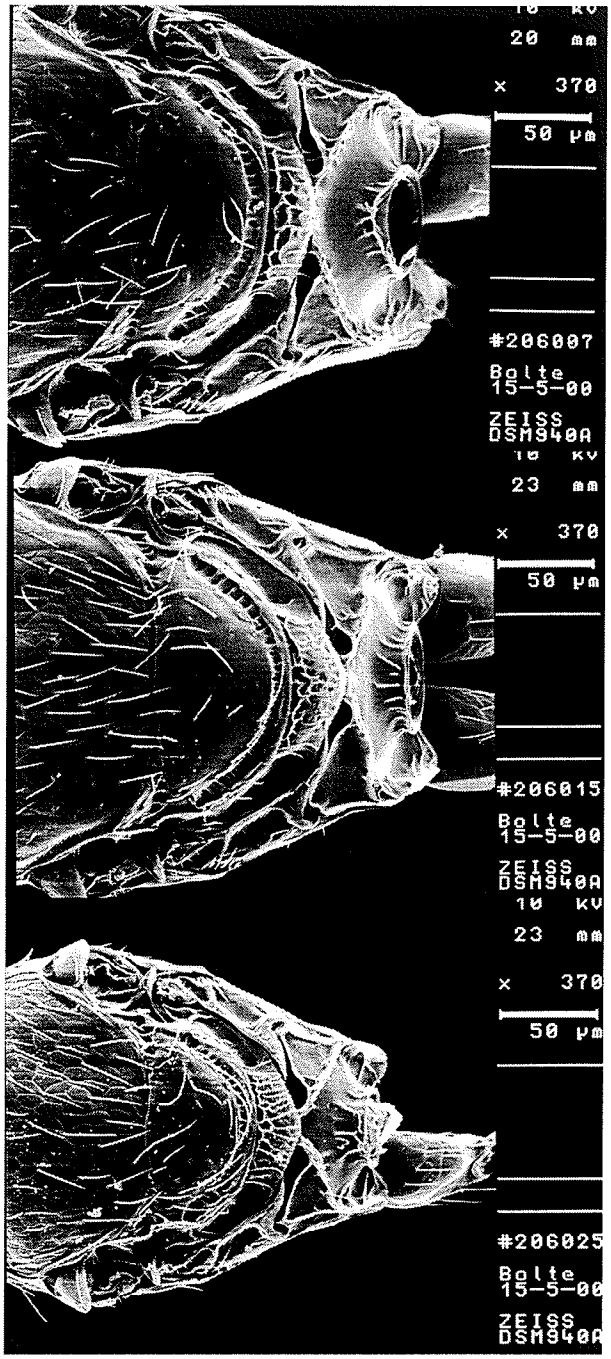
Figs. I.31-33. Prosoma in lateral view (males). 31, *Telenomus* species A; 32, *Telenomus* species B; 33, *Telenomus* species C.



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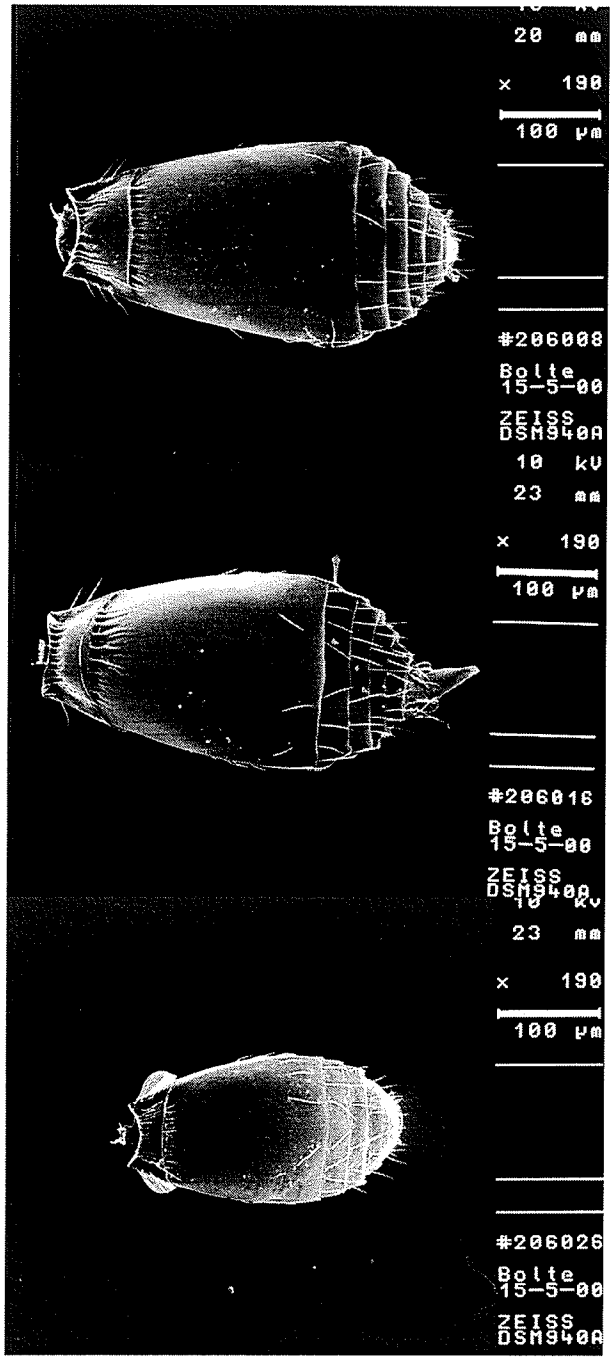
Figs. I.34-36. Mesosoma in dorsal view (males). 34, *Telenomus* species A; 35, *Telenomus* species B; 36, *Telenomus* species C.



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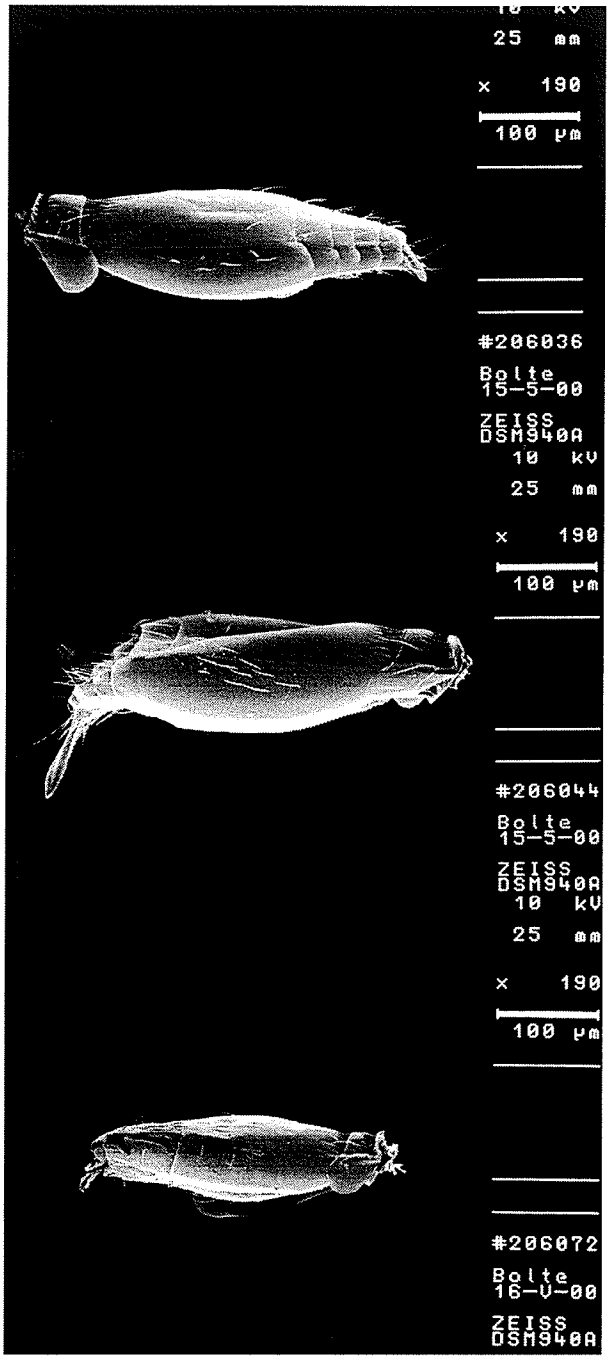
Figs. I.37-39. Metasoma in dorsal view (males). 37, *Telenomus* species A; 38, *Telenomus* species B; 39, *Telenomus* species C.



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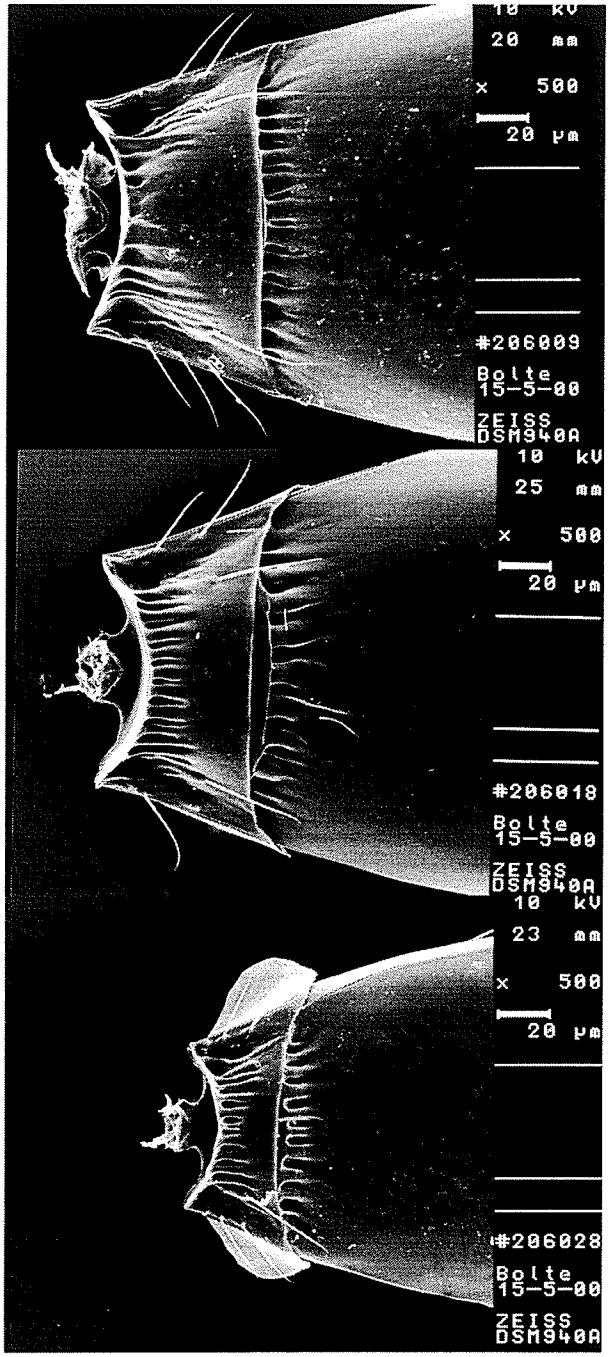
Figs. I.40-42. Metasoma in lateral view (males). 40, *Telenomus* species A; 41, *Telenomus* species B; 42, *Telenomus* species C.



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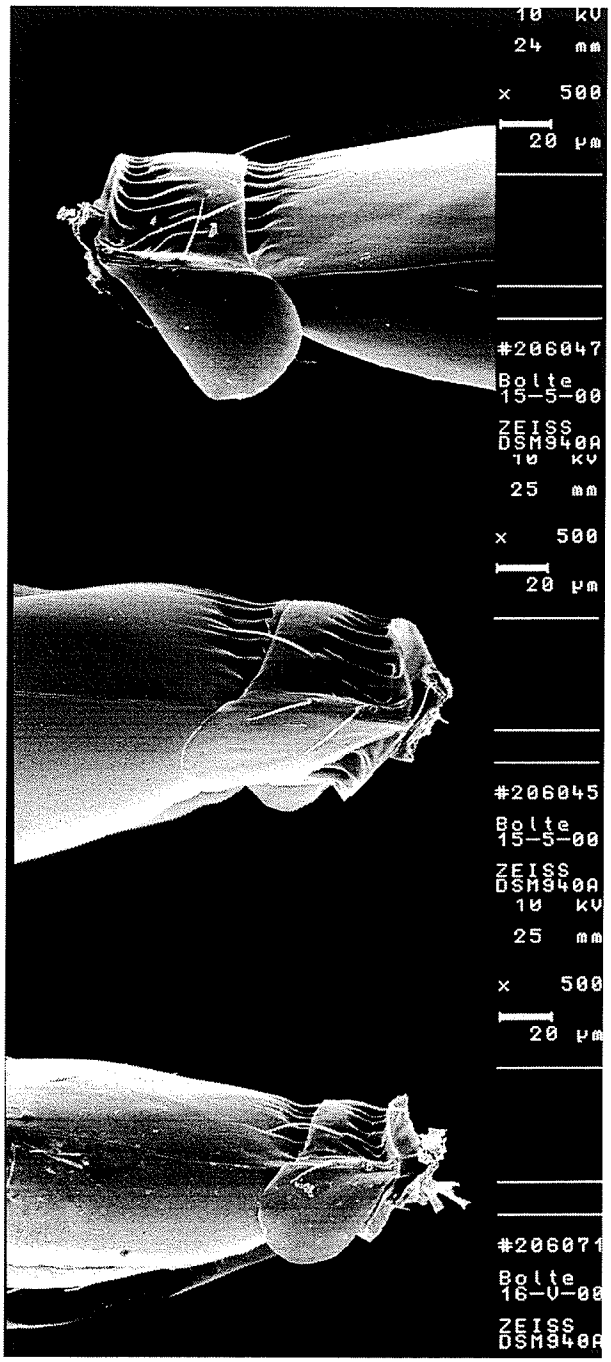
Figs. I.43-45. T1 in dorsal view (males). 43, *Telenomus* species A; 44, *Telenomus* species B; 45, *Telenomus* species C.



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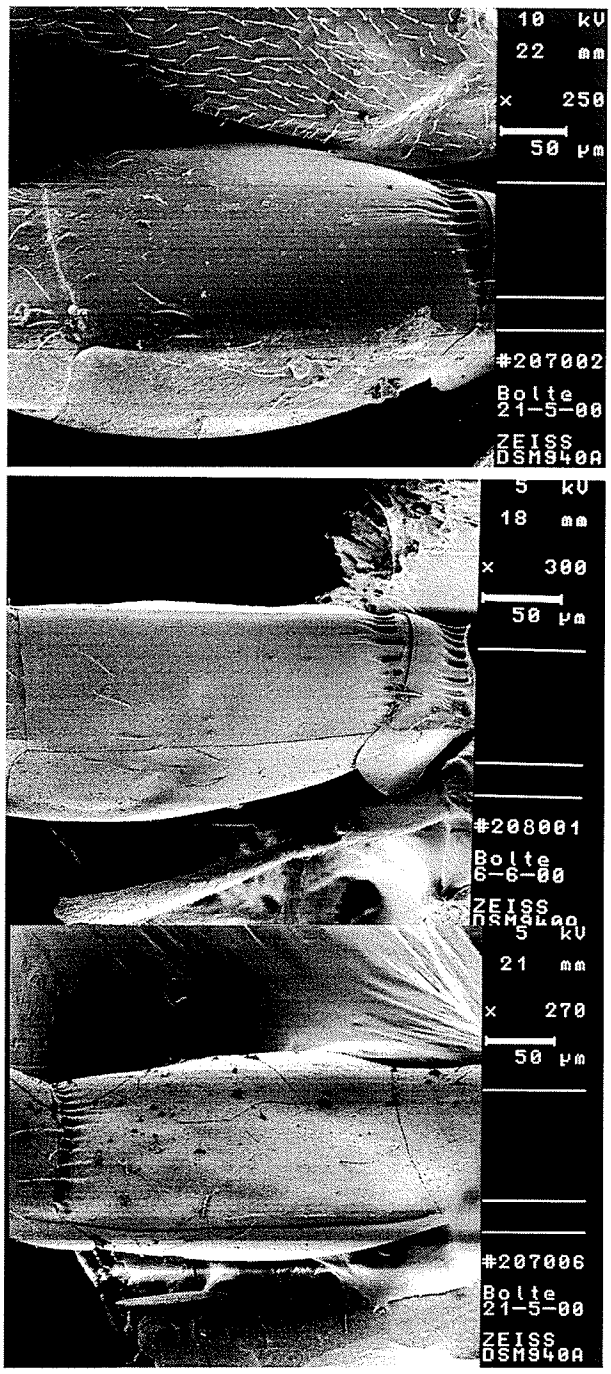
Figs. I.46-48. T1 in lateral view (males). 46, *Telenomus* species A; 47, *Telenomus* species B; 48, *Telenomus* species C.



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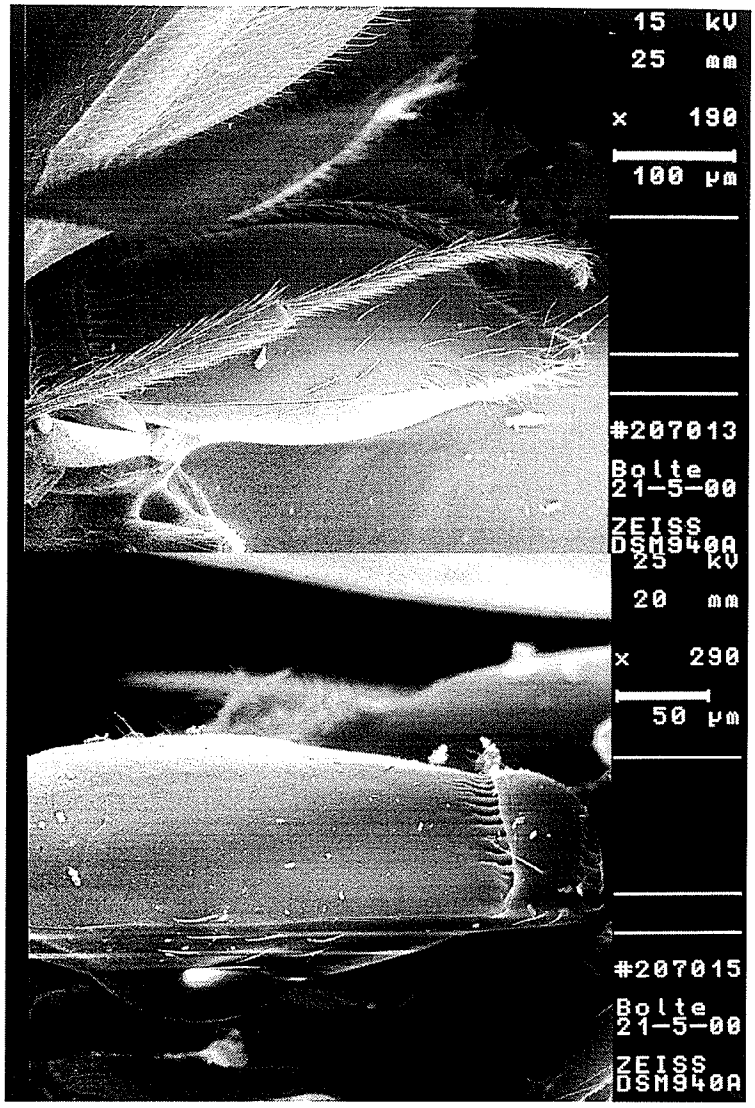
Figs. I.49-51. T2 in dorso-lateral view (females). 49, *Telenomus goniopis*; 50, *T. tabanocida*; 51, *T. tabanivorus*.



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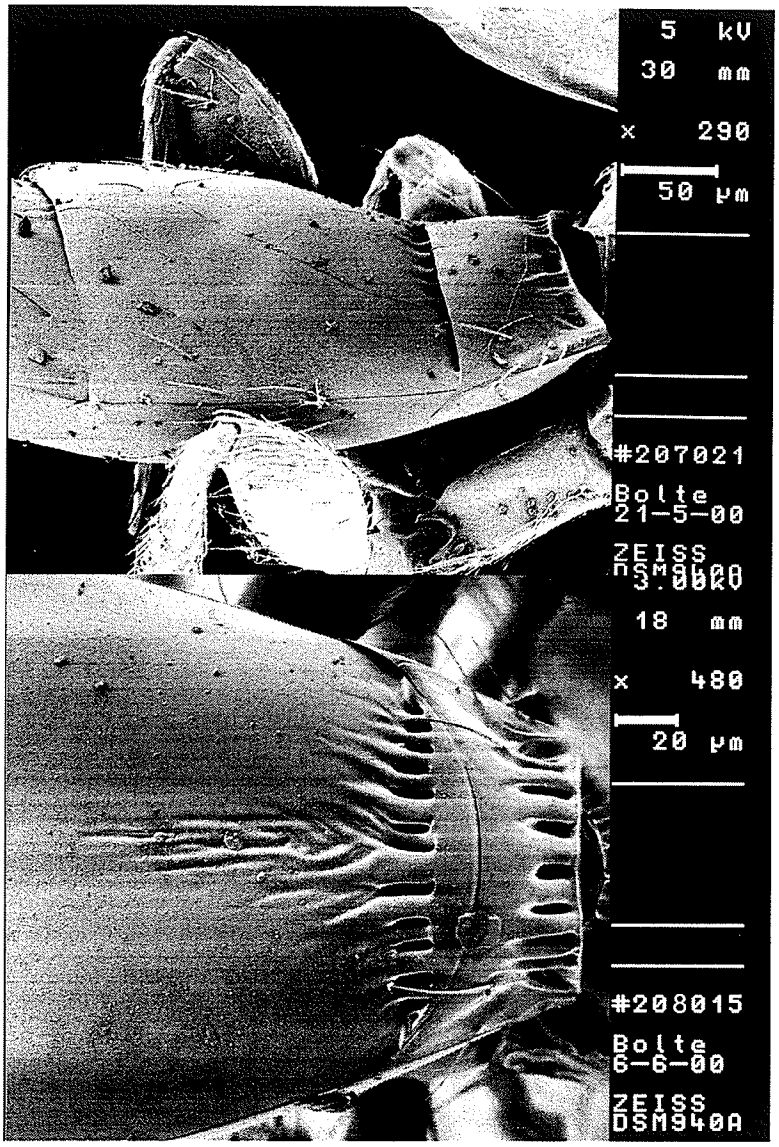
Figs. I.52-53. T1 & T2 in dorso-lateral view (females). 52, *Telenomus angustatus*; 53, *T. emersoni*.



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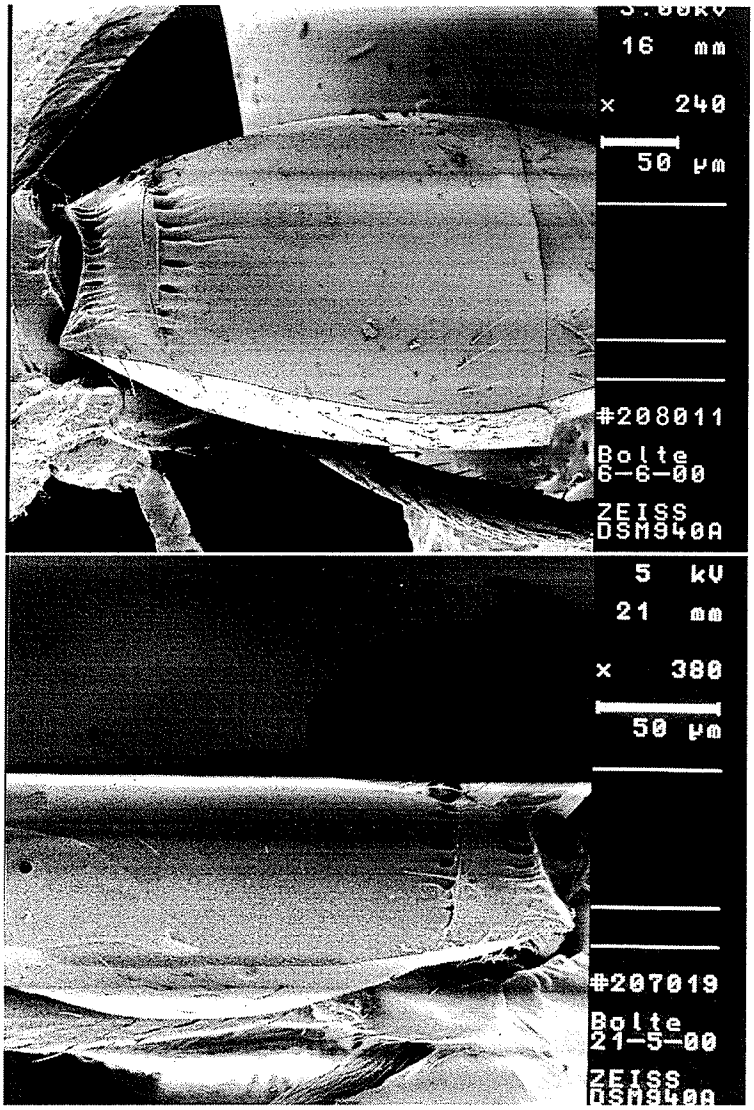
Figs. I.54-55. T1 & T2 in dorso-lateral view (males).
54, *Telenomus tabanivorus*; 55, *T. angustatus*.



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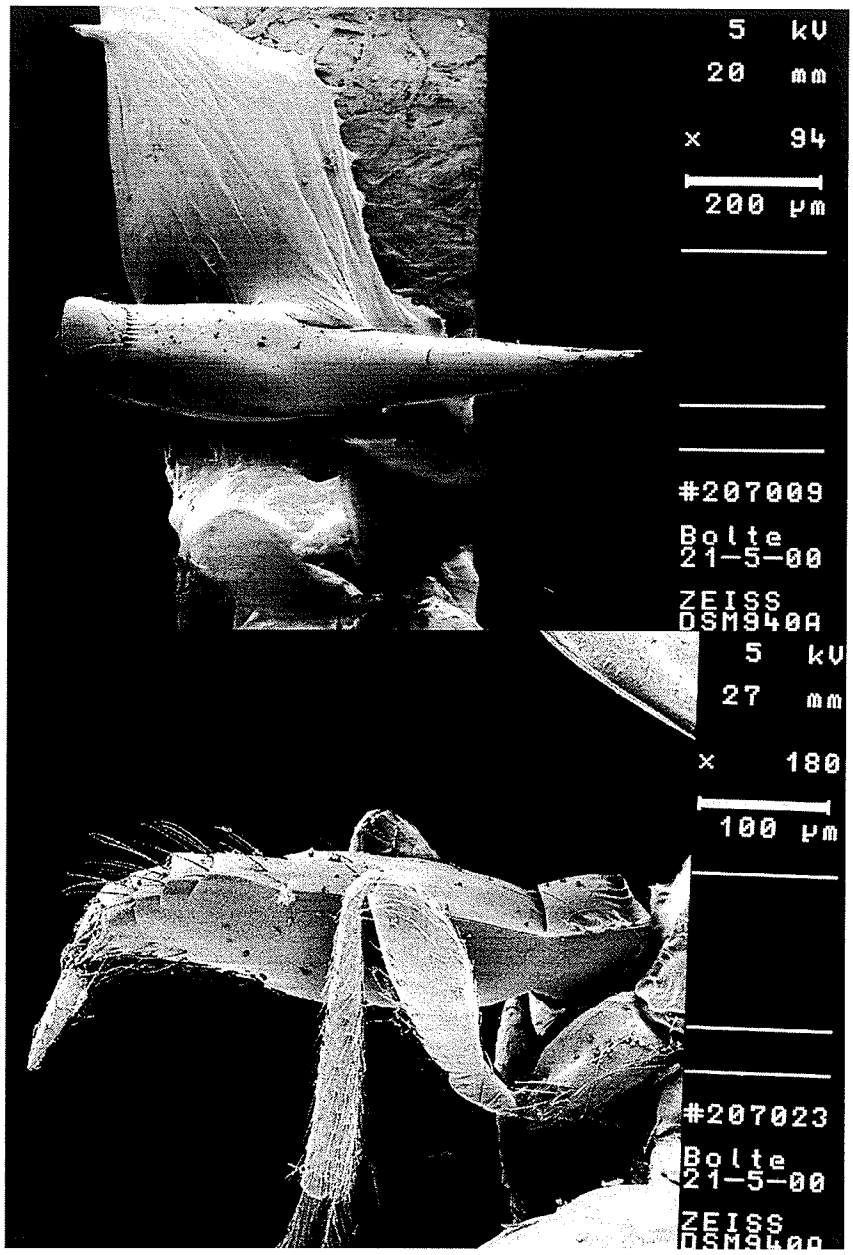
Figs. I.56-57. T1 & T2 in dorso-lateral view (males).
56, *Telenomus goniopis*; 57, *T. tabanocida*.



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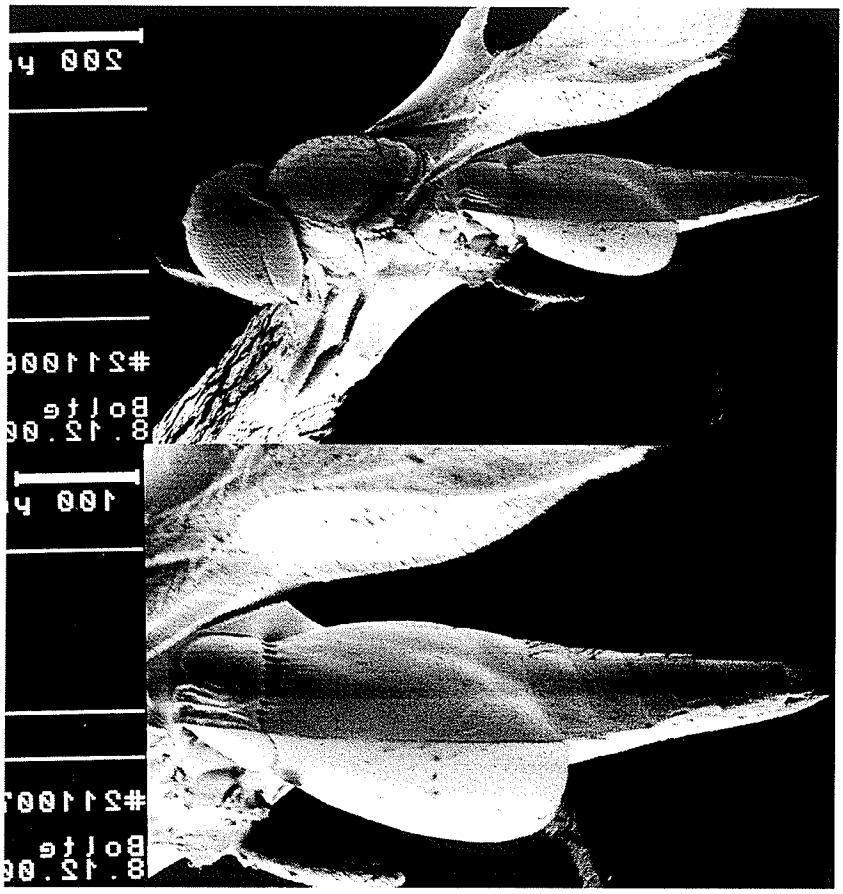
Figs. I.58-59. Metasoma of *Telenomus tabanivorus* in dorso-lateral view (58, female; 59, male).



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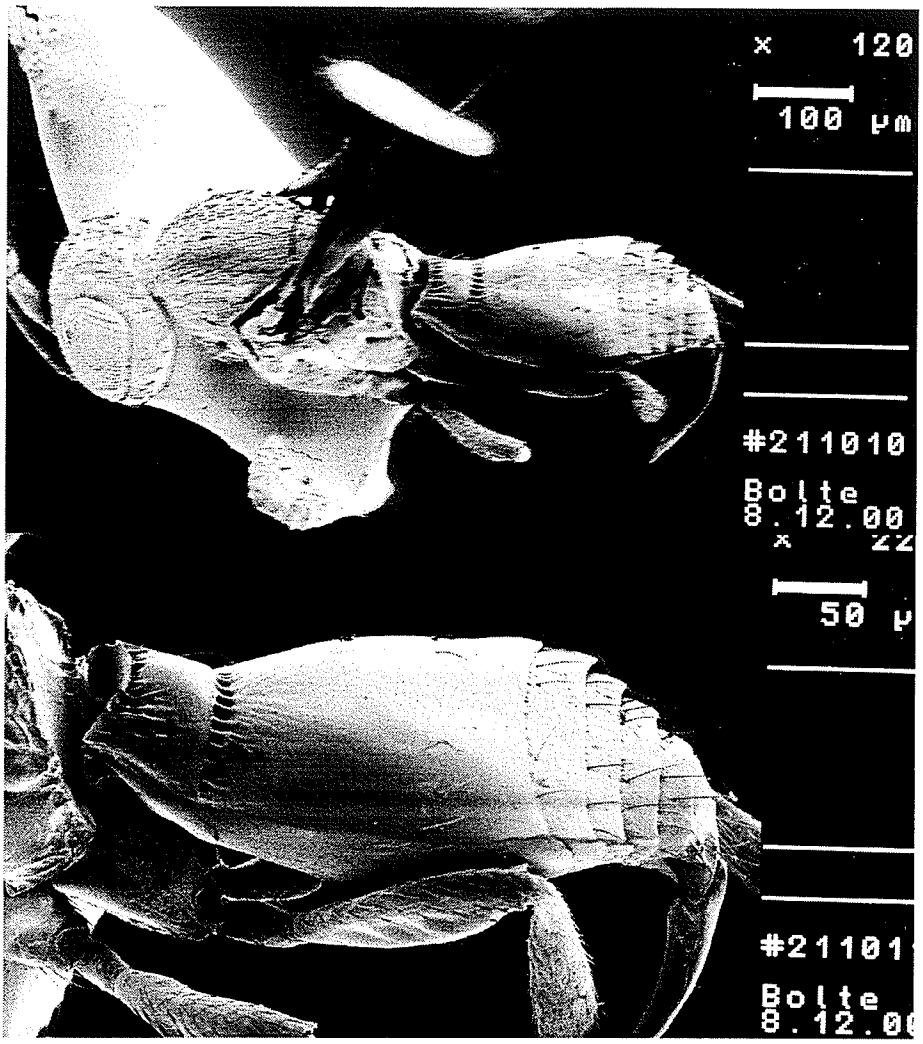
Figs. I.60-61. *Telenomus benefactor* in dorso-lateral view (female).



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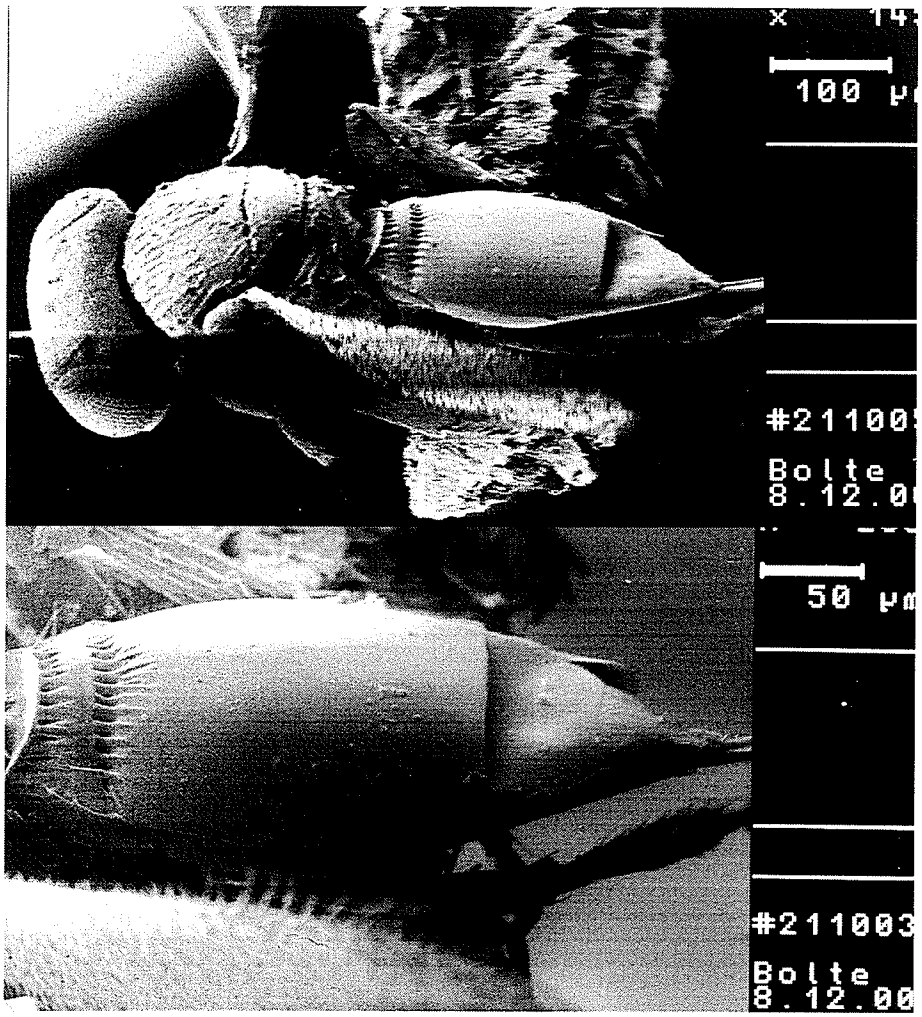
Figs. I.62-63. *Telenomus benefactor* in dorso-lateral view (male).



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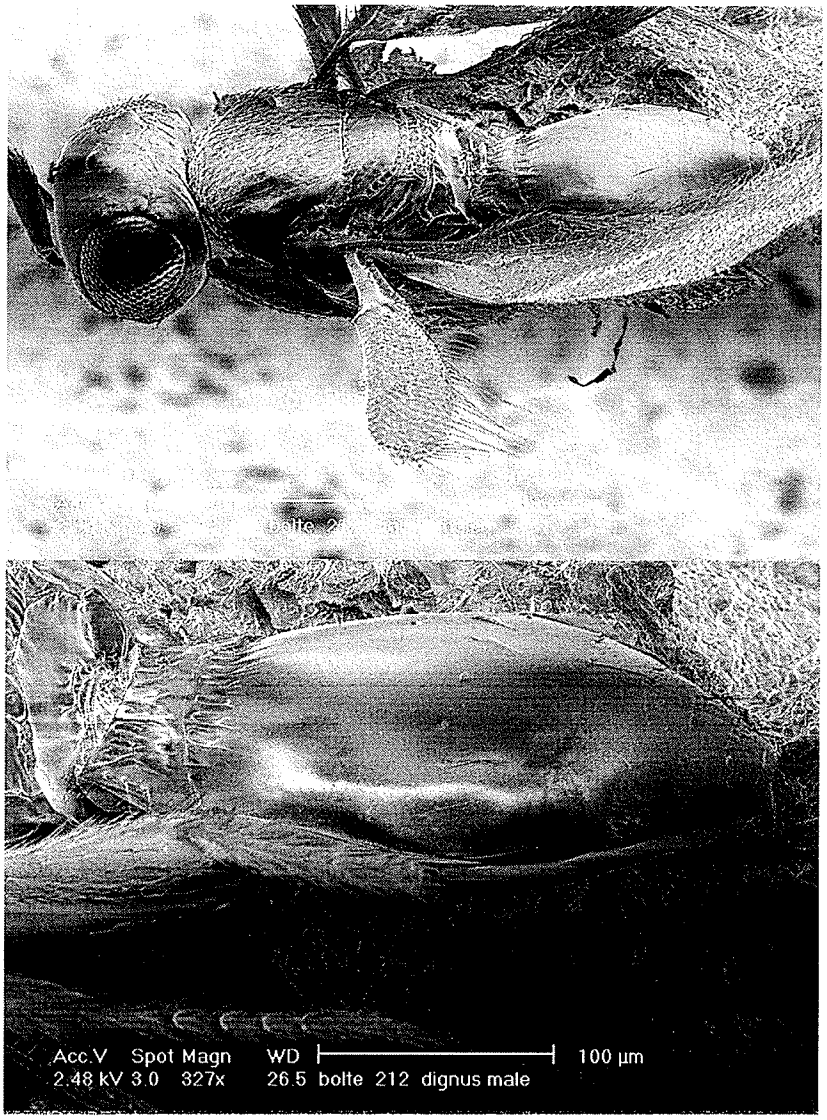
Figs. I.64-65. *Telenomus dignus* in dorso-lateral view (female).



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Figs. I.66-67. *Telenomus dignus* in dorso-lateral view (male).

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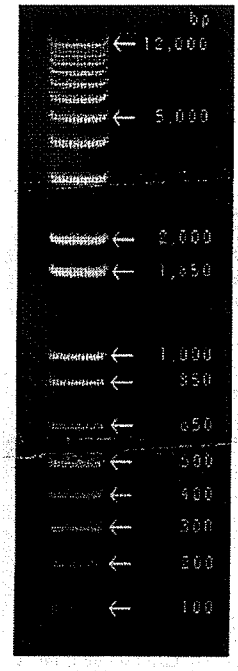
Figs. I.68-70. *Telenomus kingi* in dorso-lateral view (female).

Appendix II. Restriction digestion of two mitochondrial genes and two nuclear spacers (ITS and IGS) of Tabanidae, Manitoba, 1996-2001.



Structure of 1 Kb Fragment:

5′-GATCC_____G
G_____CCTAG-5′
5′ 3′
(996 bp)$_n$

Notes:
During 1% agarose gel electrophoresis with Tris-acetate (pH 7.5) as the running buffer, bromophenol blue migrates together with the 500 bp band.

The 1650 bp band is generated from pUC. The bands smaller than 1000 bp are derived from lambda DNA.

1 Kb PLUS DNA LADDER
0.7 µg/lane
0.9% agarose gel
stained with ethidium bromide

Fig. II.1. 1 kb Plus Ladder was used to estimate DNA fragments.

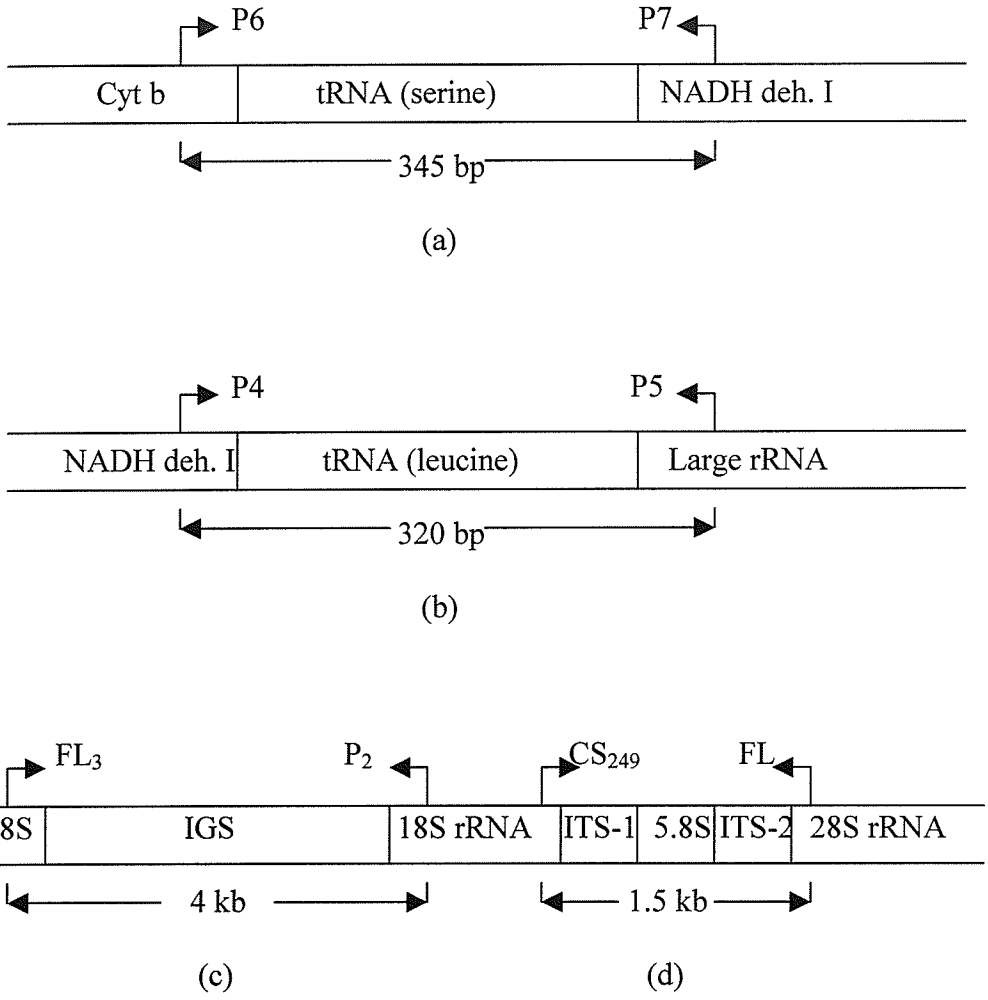


Fig. II.2. Four amplicons have been examined for molecular characterization of 35 species of Tabanidae in Manitoba, (a) and (b) mitochondrial DNA; (c) and (d) nuclear DNA.

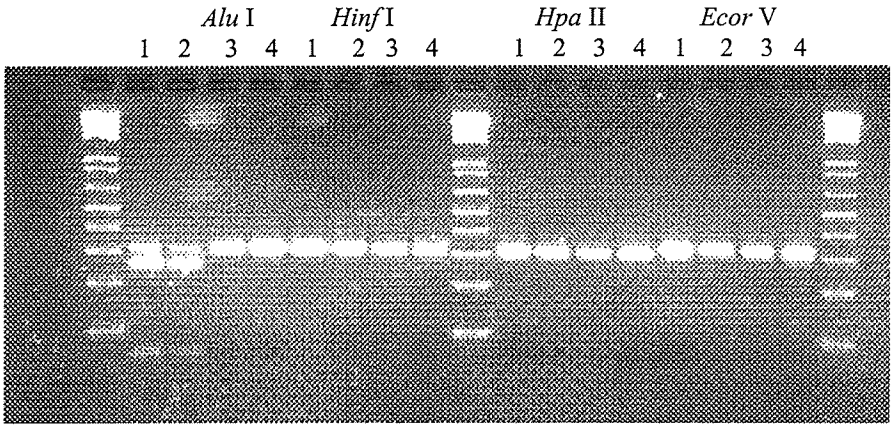


Fig. II.3. A gel showing restriction digestion of tRNA (leucine) gene (mitochondrial DNA) of three species of tabanids by four enzymes. Specimens collected at the following locations:
1-*Hybomitra nitidifrons nuda* (egg mass), Woodridge, 15 June, 1998
2-*H. nitidifrons nuda* (adult), Elma, 20 June, 1998
3-*H. lasiophthalma* (adult), Woodridge, 15 June, 1998
4-*Chrysops aestuans* (adult), Elma, 12 July, 1998

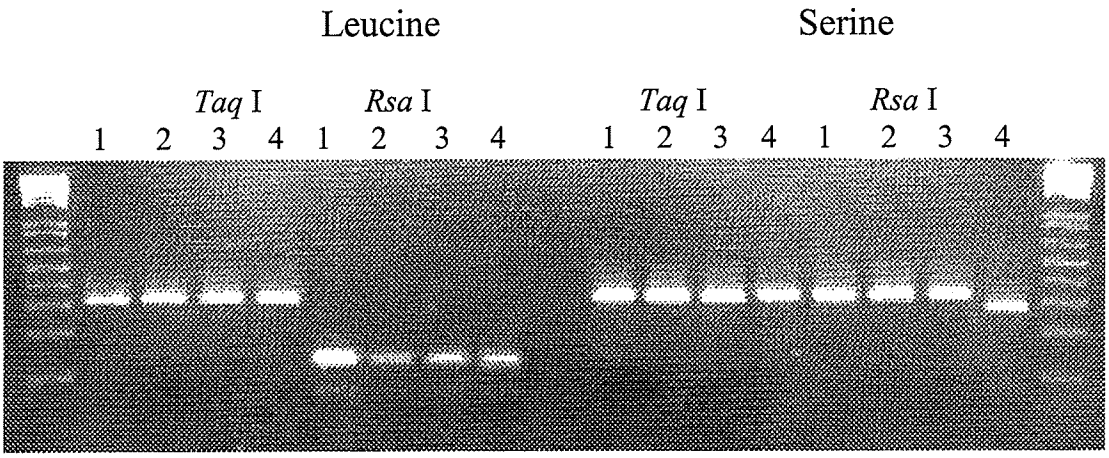


Fig. II.4. A gel showing restriction digestion of tRNA (leucine) gene and tRNA (serine) gene (mitochondrial DNA) of three species of tabanids by two enzymes. Specimens collected at the following locations:
1-*Hybomitra nitidifrons nuda* (egg mass), Woodridge, 15 June, 1998
2-*H. nitidifrons nuda* (adult), Elma, 20 June, 1998
3-*H. lasiophthalma* (adult), Woodridge, 15 June, 1998
4-*Chrysops aestuans* (adult), Elma, 12 July, 1998

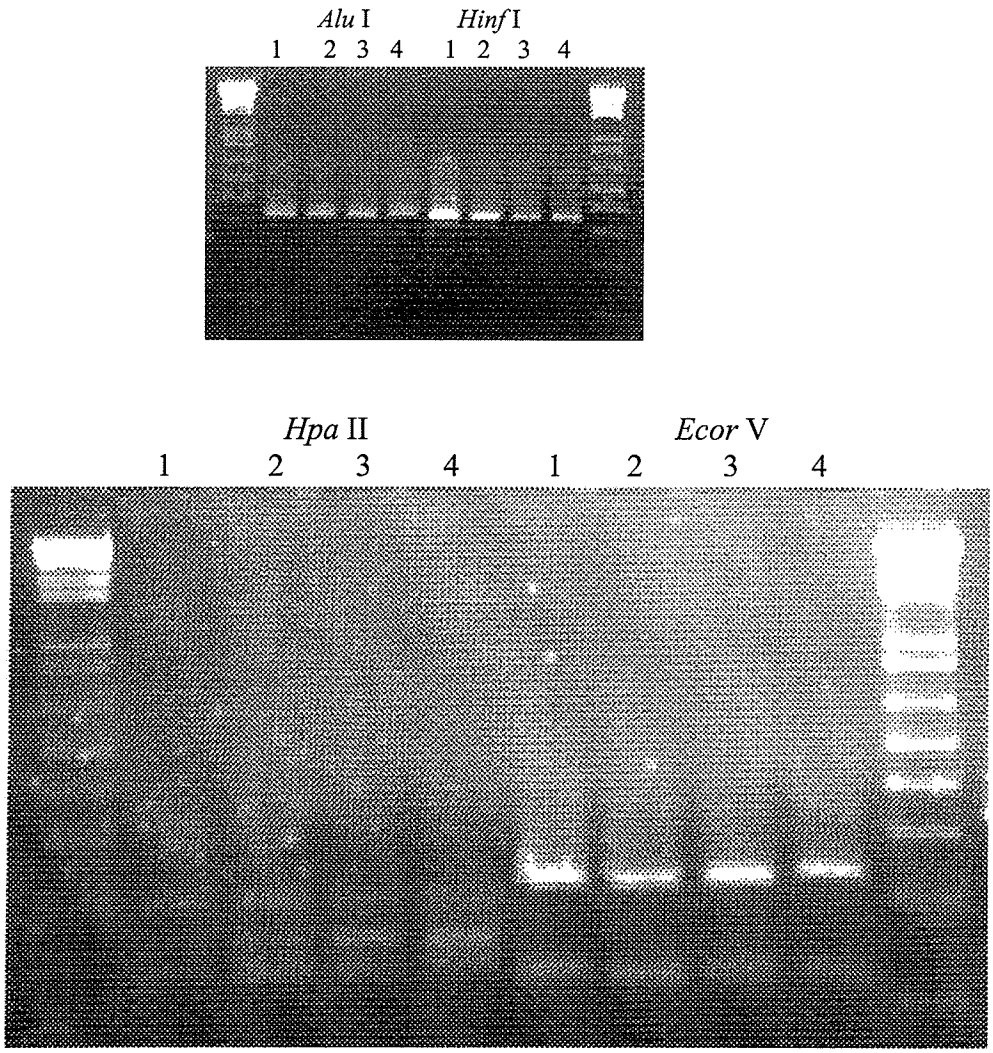


Fig. II.5. A gel showing restriction digestion of tRNA (Serine) gene (mitochondrial DNA) of three species of tabanids by four enzymes. Specimens collected at the following locations:
1-*Hybomitra nitidifrons nuda* (egg mass), Woodridge, 15 June, 1998
2-*H. nitidifrons nuda* (adult), Elma, 20 June, 1998
3-*H. lasiophthalma* (adult), Woodridge, 15 June, 1998
4-*Chrysops aestuans* (adult), Elma, 12 July, 1998

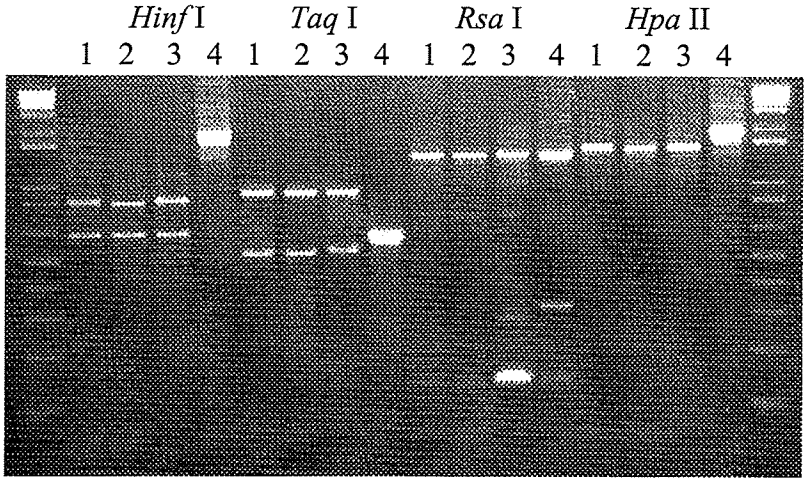


Fig. II.6. A gel showing restriction digestion of ITS of three species of tabanids by four enzymes (*Hinf* I, *Taq* I, *Rsa* I, and *Hpa* II). Specimens were collected at the following locations:
1-*Hybomitra nitidifrons nuda* (egg mass), Woodridge, 15 June, 1998
2-*Hybomitra nitidifrons nuda* (adult), Elma, 20 June, 1998
3-*Hybomitra lasiophthalma* (adult), Woodridge, 15 June, 1998
4-*Chrysops aestuans* (adult), Elma, 12 July, 1998

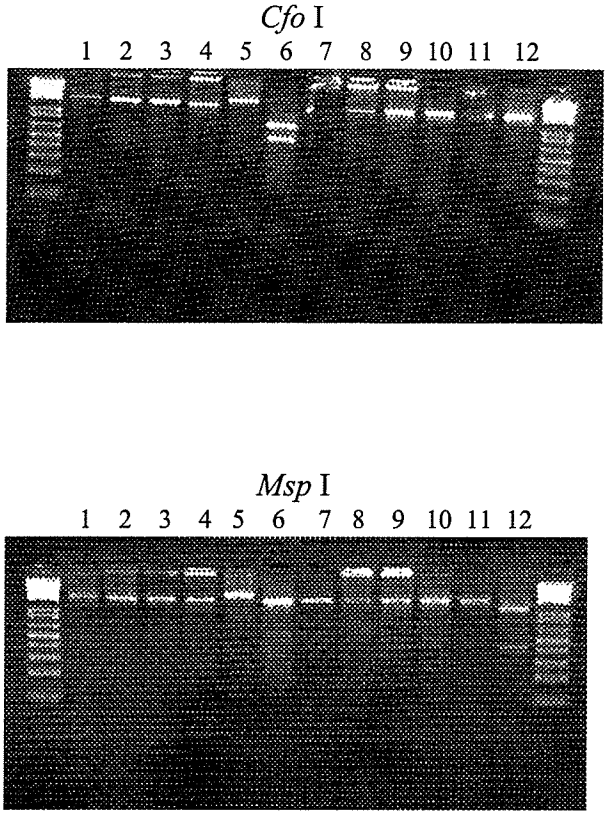


Fig. II.7. A gel showing restriction digestion of ITS of 11 species of tabanids by *Cfo* I and *Msp* I. Specimens were collected at the following locations:
1- *Hybomitra nitidifrons nuda* (egg mass), Woodridge, 12 June, 1998
2- *H. n. nuda*, Elma, 15 June, 1998
3- *H. lasiophthalma*, Woodridge, 16 June, 1998
4- *H. epistates*, Woodridge, 15 June, 1998
5- *Chrysops aestuans*, Elma, 15 July, 1998
6- *Tabanus similis*, Beausejour, 4 July, 1998
7- *T. marginalis*, Beausejour, 4 July, 1998
8- *H. illota*, Woodridge, 16 June, 1998
9- *H. affinis*, Woodridge, 18 June, 1998
10- *H. zonalis*, Elma, 4 July, 1998
11- *H. trepida*, Seven Sisters, 25 July, 1998
12- *H. arpadi*, Woodridge, 18 June, 1998

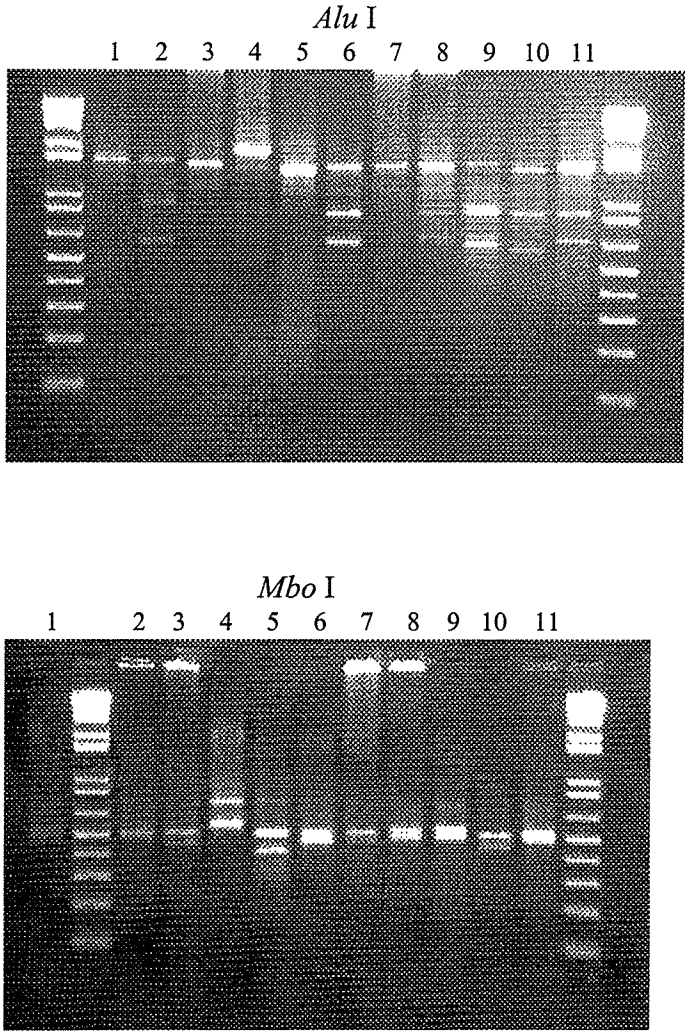


Fig. II.8. A gel showing restriction digestion of ITS of 11 species of tabanids by *Alu* I and *Mbo* I. Specimens were collected at the following locations:
1- *Hybomitra nitidifrons nuda* (egg mass), Woodridge, 12 June, 1998
2- *H. lasiophthalma*, Woodridge, 16 June, 1998
3- *H. epistates*, Woodridge, 15 June, 1998
4- *Chrysops aestuans*, Elma, 15 July, 1998
5- *Tabanus similis*, Beausejour, 4 July, 1998
6- *T. marginalis*, Beausejour, 4 July, 1998
7- *H. illota*, Woodridge, 16 June, 1998
8- *H. affinis*, Woodridge, 18 June, 1998
9- *H. zonalis*, Elma, 4 July, 1998
10- *H. trepida*, Seven Sisters, 25 July, 1998
11- *H. arpadi*, Woodridge, 18 June, 1998

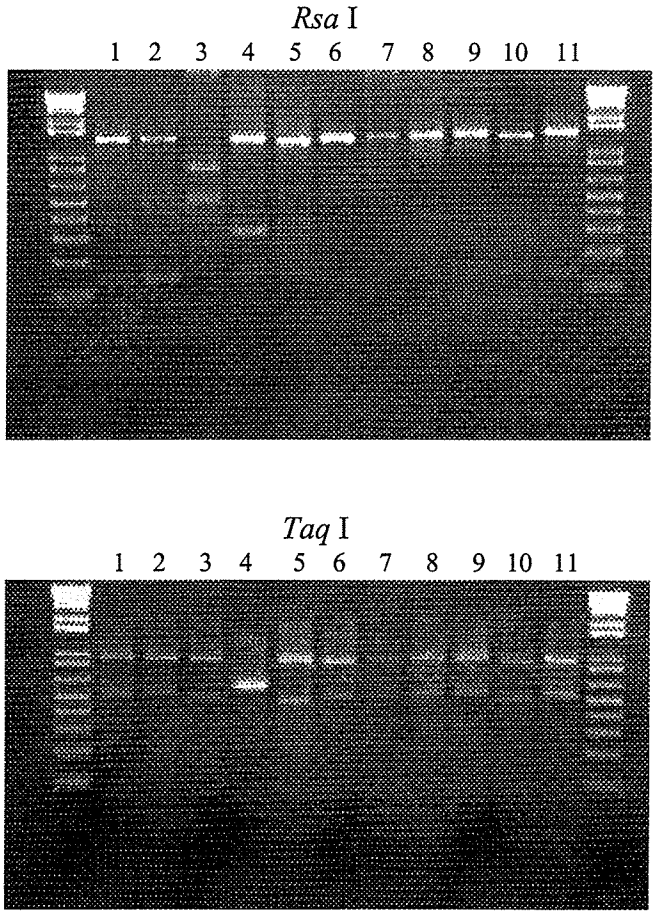


Fig. II.9. A gel showing restriction digestion of ITS of 11 species of tabanids by *Rsa* I and *Taq* I. Specimens were collected at the following locations:
1- *Hybomitra nitidifrons nuda* (egg mass), Woodridge, 12 June, 1998
2- *H. lasiophthalma*, Woodridge, 16 June, 1998
3- *H. epistates*, Woodridge, 15 June, 1998
4- *Chrysops aestuans*, Elma, 15 July, 1998
5- *Tabanus similis*, Beausejour, 4 July, 1998
6- *T. marginalis*, Beausejour, 4 July, 1998
7- *H. illota*, Woodridge, 16 June, 1998
8- *H. affinis*, Woodridge, 18 June, 1998
9- *H. zonalis*, Elma, 4 July, 1998
10- *H. trepida*, Seven Sisters, 25 July, 1998
11- *H. arpadi*, Woodridge, 18 June, 1998

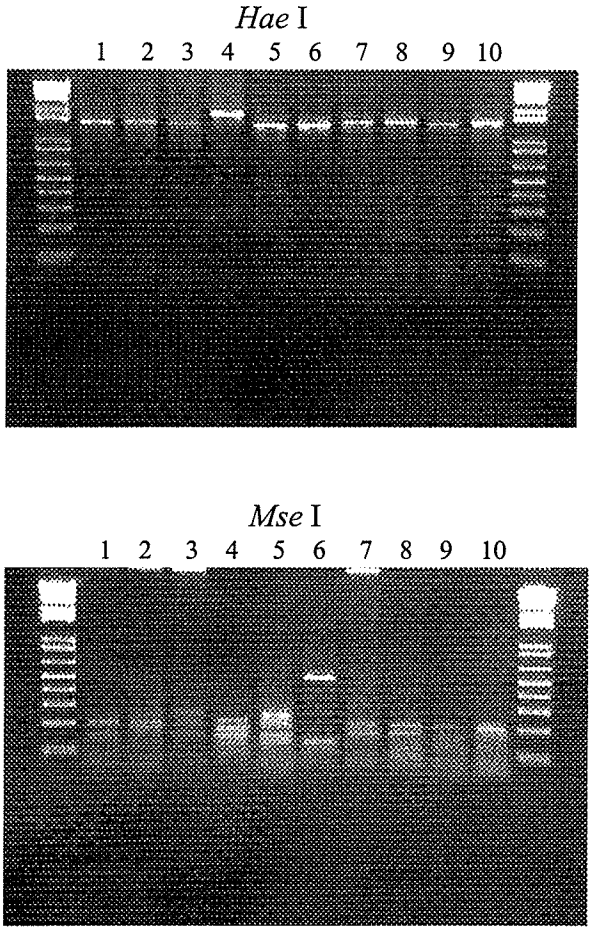


Fig. II.10. A gel showing restriction digestion of ITS of 11 species of tabanids by *Hae* I and *Mse* I. Specimens were collected at the following locations:
1- *Hybomitra nitidifrons nuda* (egg mass), Woodridge, 12 June, 1998
2- *H. lasiophthalma*, Woodridge, 16 June, 1998
3- *H. epistates*, Woodridge, 15 June, 1998
4- *Chrysops aestuans*, Elma, 15 July, 1998
5- *Tabanus similis*, Beausejour, 4 July, 1998
6- *T. marginalis*, Beausejour, 4 July, 1998
7- *H. illota*, Woodridge, 16 June, 1998
8- *H. affinis*, Woodridge, 18 June, 1998
9- *H. trepida*, Seven Sisters, 25 July, 1998
10- *H. arpadi*, Woodridge, 18 June, 1998

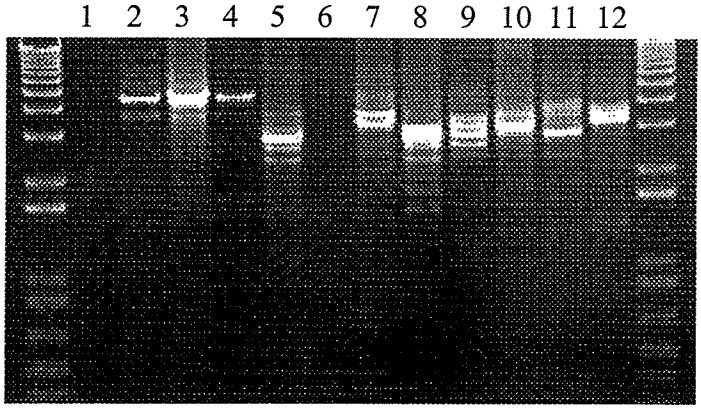


Fig. II.11. A gel showing amplified IGS of *Chrysops aestuans*, *C. excitans*, and *C. mitis* (4 individuals for each) collected at the following locations in Manitoba:
1-*C. aestuans*, Elma, 23 July, 1999
2-*C. aestuans*, Sandilands, 12 July, 1999
3-*C. aestuans*, Woodridge, 25 June, 1999
4-*C. aestuans*, Woodridge, 14 July, 1999
5-*C. excitans*, McMunn, 8 June, 1999
6-*C. excitans*, Seven Sisters, 7 June, 1999
7-*C. excitans*, Whiteshell, 18 July, 1999
8-*C. excitans*, Woodridge, 25 June, 1999
9-*C. mitis*, Elma, 23 June, 1999
10-*C. mitis*, McMunn, 10 June, 1999
11-*C. mitis*, Whiteshell, 18 July, 1999
12-*C. mitis*, Woodridge, 14 July, 1999

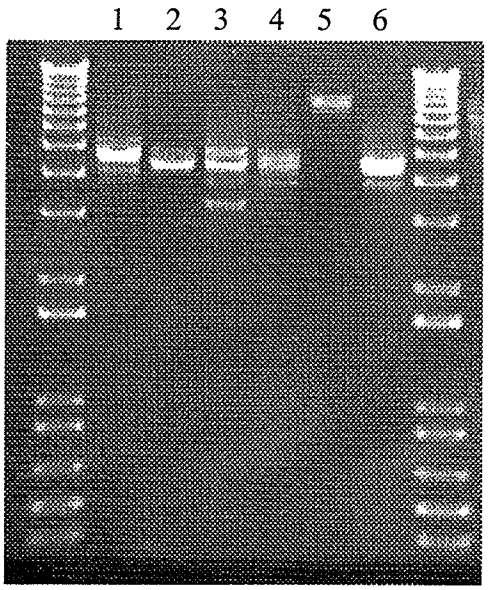


Fig. II.12. A gel showing amplified IGS of six individuals of *H. affinis* collected at the following locations in Manitoba:
1-Elma, 23 June, 1999
2-McMunn, 8 June, 1999
3-Sandilands, 12 July, 1999
4-Seven Sisters, 7 June, 1999
5-Seven Sisters, 3 August, 1999
6-Woodridge, 14 July, 1999

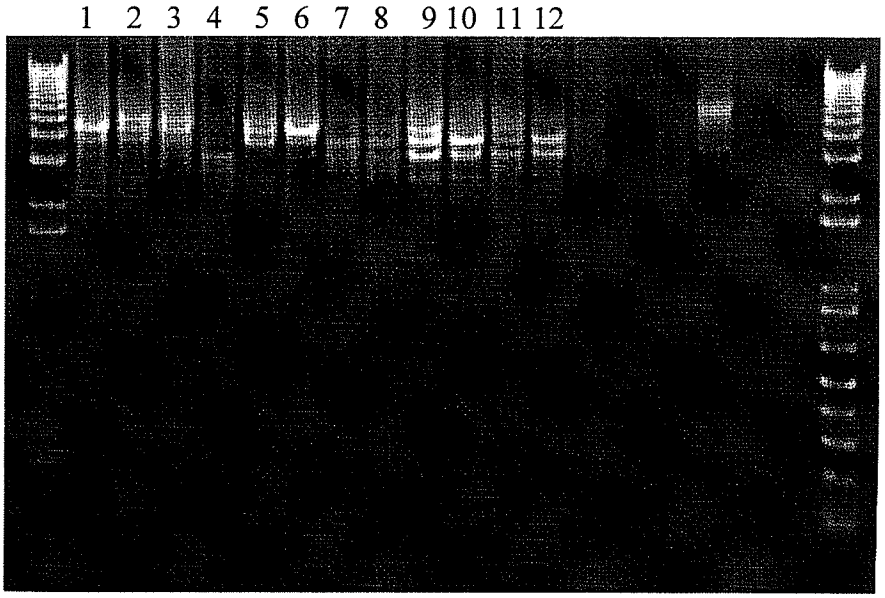


Fig. II.13. A gel showing amplified IGS of *H. arpadi* (1-6) and and *H. epistates* (7-12) collected at the following locations in Manitoba:

1-Elma, 23 June, 1999
2-McMunn, 8 June, 1999
3-Sandilands, 12 July, 1999
4-Seven Sisters, 3 Aug., 1999
5-Seven Sisters, 7 June, 1999
6-Woodridge, 25 June, 1999
7-Hecla Island, 22 July, 1999
8-McMunn, 8 June, 1999
9-Portage La Prairie, 21 July, 1999
10-Sandilands, 12 July, 1999
11-Seven Sisters, 7 June, 1999
12-Woodridge, 14 July, 1999

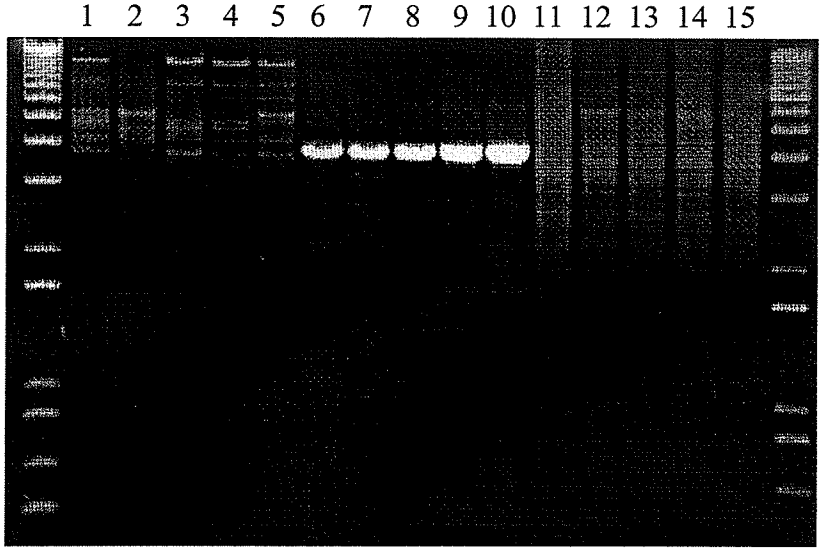


Fig. II.14. A gel showing amplified IGS of *Chrysops frigidus*, *C. indus*, and *C. venus* (five individuals for each) collected at the following locations in Manitoba:
1-*Chrysops frigidus*, Elma, 23 June, 1999
2-*C. frigidus*, Sandilands, 12 July, 1999
3-*C. frigidus*, Sprague, 20 July, 1999
4-*C. frigidus*, Whiteshell, 18 July, 1999
5-*C. frigidus*, Woodridge, 14 July, 1999
6-*C. indus*, Elma, 23 June, 1999
7-*C. indus*, McMunn, 10 June, 1999
8-*C. indus*, Sandilands, 12 July, 1999
9-*C. indus*, Seven Sisters, 7 June, 1999
10-*C. indus*, Woodridge, 14 July, 1999
11-*C. venus*, Woodridge, 14 July, 1999
12-*C. venus*, Piney, 17 July, 1999
13-*C. venus*, Whiteshell, 14 July, 1999
14-*C. venus*, Woodridge, 17 July, 1999
15-*C. venus*, Seven Sisiters, 14 July, 1999

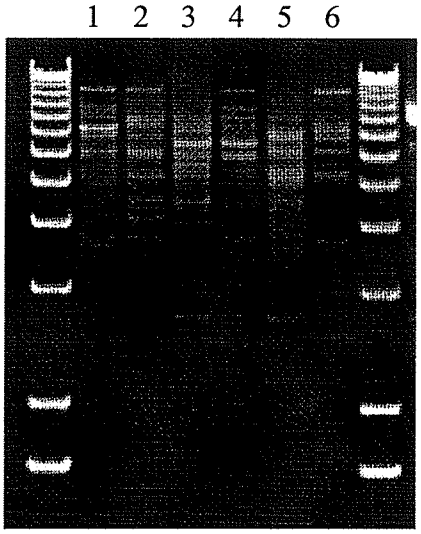


Fig. II.15. A gel showing amplified IGS of six individuals of *H. frontalis* were collected at the following locations in Manitoba:
1-McMunn, 4 July, 1999
2- Portage La Prairie, 21 July, 1999
3- Seven Sisters, 3 August, 1999
4- Sprague, 20 July, 1999
5- West Shoal Lake, 29 July, 1999
6- Woodridge, 14 July, 1999

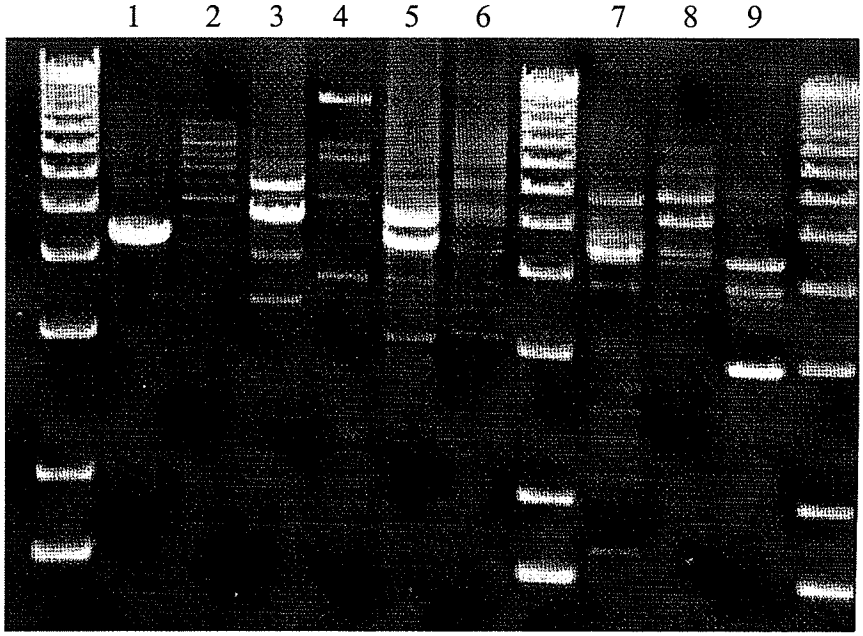


Fig. II.16. A gel showing amplified IGS of six species of *Hybomitra* spp. and three species of *Chrysops* spp. collected at the following locations in Manitoba:
1-*Hybomitra brennani*, Woodridge, 14 July, 1999
2-*H. criddlei*, Woodridge, 25 June, 1999
3-*H. longiglossa*, McMunn, 7 June, 1999
4-*H. microcephala,* Sandilands, 12 July, 1999
5-*H. pediontis*, West Shoal Lake (Interlake), 29 July, 1999
6-*H. tetrica*, Portage La Prairie, 21 July, 1999
7-*Chrysops ater*, Woodridge, 14 July, 1999
8-*C. furcatus*, McMunn, 7 June, 1999
9-*C. sackeni*, Whiteshell, 18 July, 1999

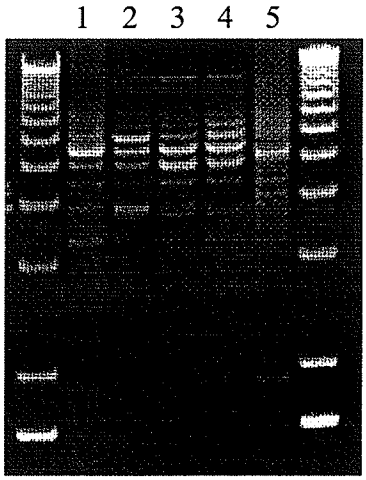


Fig. II.17. A gel showing amplified IGS of five individuals of *H. illota* collected at the following locations in Manitoba:
1-Delta Marsh, 13 July, 1999
2-Elma, 23 June, 1999
3-McMunn, 8 June, 1999
4-Seven Sisters, 3 August, 1999
5-Woodridge, 14 July, 1999

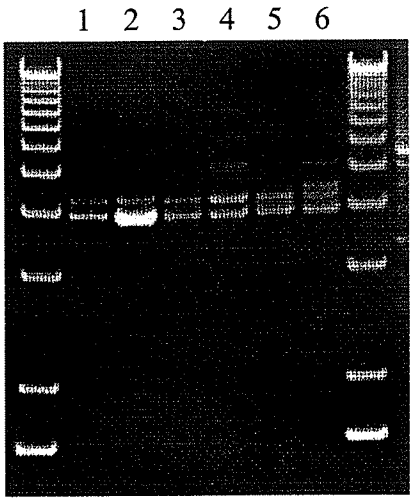


Fig. II.18. A gel showing amplified IGS of six individuals of *H. lasiophthalma* collected at the following locations in Manitoba:
1-Elma, 23 June, 1999
2-McMunn, 8 June, 1999
3-Sandilands, 12 July, 1999
4-Seven Sisters, 7 June, 1999
5-Woodridge, 25 June, 1999
6-Elma, 15 June, 1999

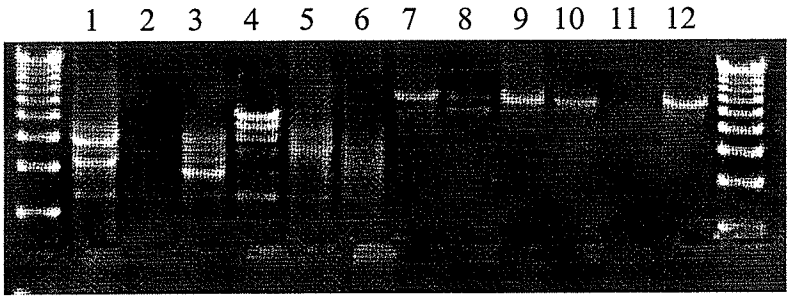


Fig. II.19. A gel showing amplified IGS of *H. lurida* (1-6) and *H. pechumani* (7-12) collected at the following locations in Manitoba:
1-Elma, 23 June, 1999
2-McMunn, 8 June, 1999
3-McMunn, 13 June, 1999
4-Sandilands, 12 July, 1999
5-Seven Sisters, 7 June, 1999
6-Woodridge, 19 June, 1999
7-Elma, 23 June, 1999
8-McMunn, 4 July, 1999
9-Sandilands, 18 July, 1999
10-Seven Sisters, 20 July, 1999
11-Sprague, 20 July, 1999
12-Woodridge, 14 July, 1999

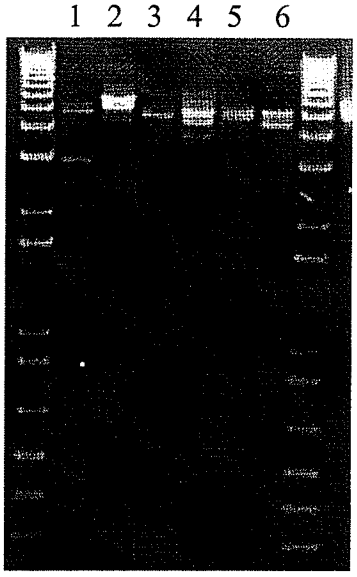


Fig. II.20. A gel showing amplified IGS of six individuals of *Tabanus marginalis* collected at the following locations in Manitoba:
1-Elma, 7 July, 1999
2-Portage La Prairie, Prov. Rd. # 240, 21 July, 1999
3-Sandilands, 12 July, 1999
4-Seven Sisters, 19 June, 1999
5-Whiteshell, 18 July, 1999
6-Woodridge, 14 July, 1999

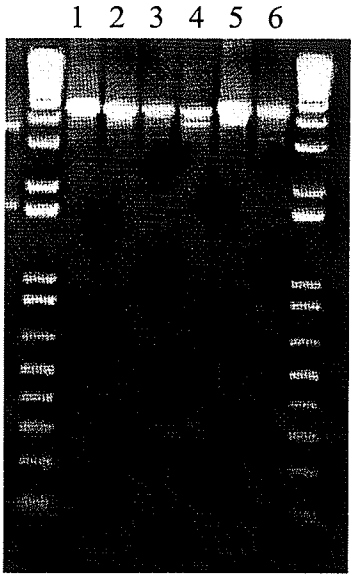


Fig. II.21. A gel showing amplified IGS of six individuals of *H. nitidifrons nuda* collected at the following locations in Manitoba:
1-Sandilands, 15 June, 1999
2-McMunn, 8 June, 1999
3-Seven Sisters, 7 June, 1999
4-Seven Sisters, 7 June, 1999
5-Woodridge, 25 June, 1999
6- Woodridge, 25 June, 1999

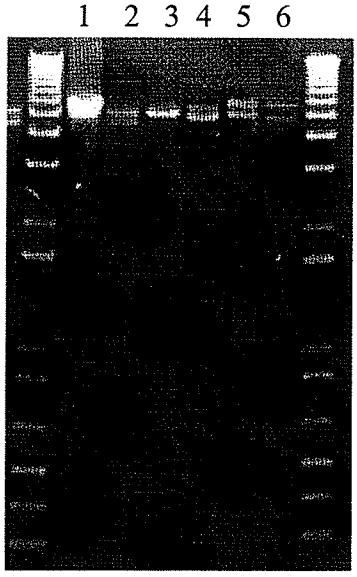


Fig. II. 22. A gel showing amplified IGS of six individuals of *Tabanus similis* collected at the following locations in Manitoba:
1-Elma, 23 June, 1999
2-Glenlea Research Station, 11 July, 1999
3-Seven Sisters, 19 June, 1999
4-Seven Sisters, 3 Aug., 1999
5-West Shoal Lake, 29 July, 1999
6-Woodridge, 25 June, 1999

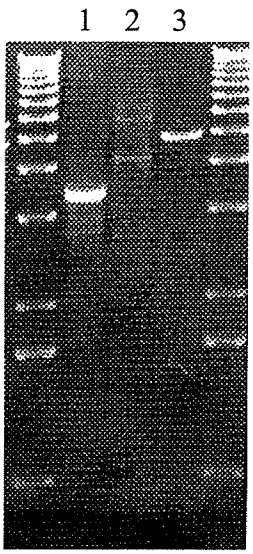


Fig. II. 23. A gel showing amplified IGS of (1) *Tabanus atratus*, (2) *T. vivax*, and (3) *Atylotus calcar* collected at the following locations in Manitoba:
1-Glenlea Research Station, 11 July, 1999
2-Woodridge, 14 July, 1999
3-Delta Marsh, 13 July, 1999

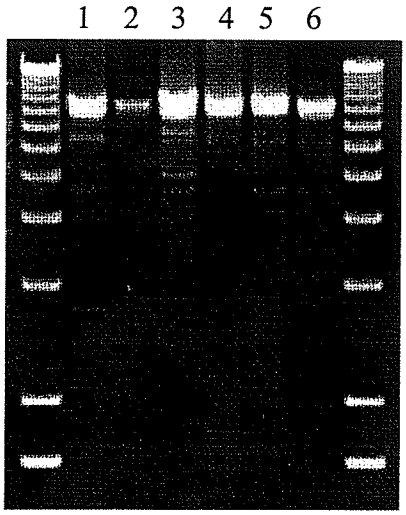


Fig. II.24. A gel showing amplified IGS of six individuals of *H. trepida* collected at the following locations in Manitoba:

1-Elma, 7 July, 1999
2-McMunn, 8 June, 1999
3-Sprague, 20 July, 1999
4-Whiteshell, 18 July, 1999
5-Woodridge, 14 July, 1999
6-McMunn, 5 July, 1999

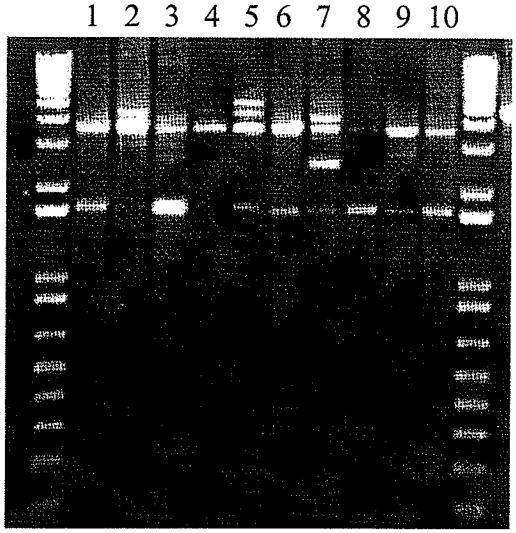


Fig. II.25. A gel showing amplified IGS of 10 individuals of *H. zonalis* collected at the following locations in Manitoba:

1-Elma, 23 June, 1999
2-McMunn, 5 July, 1999
3-Sandilands, 12 July, 1999
4-Seven Sisters, 18 July, 1999
5-Woodridge, 25 June, 1999
6-Elma, 25 June, 1999
7-McMunn, 8 June, 1999
8-Sandilands, 15 July, 1999
9-Whiteshell, 18 July, 1999
10-Woodridge, 28 June, 1999

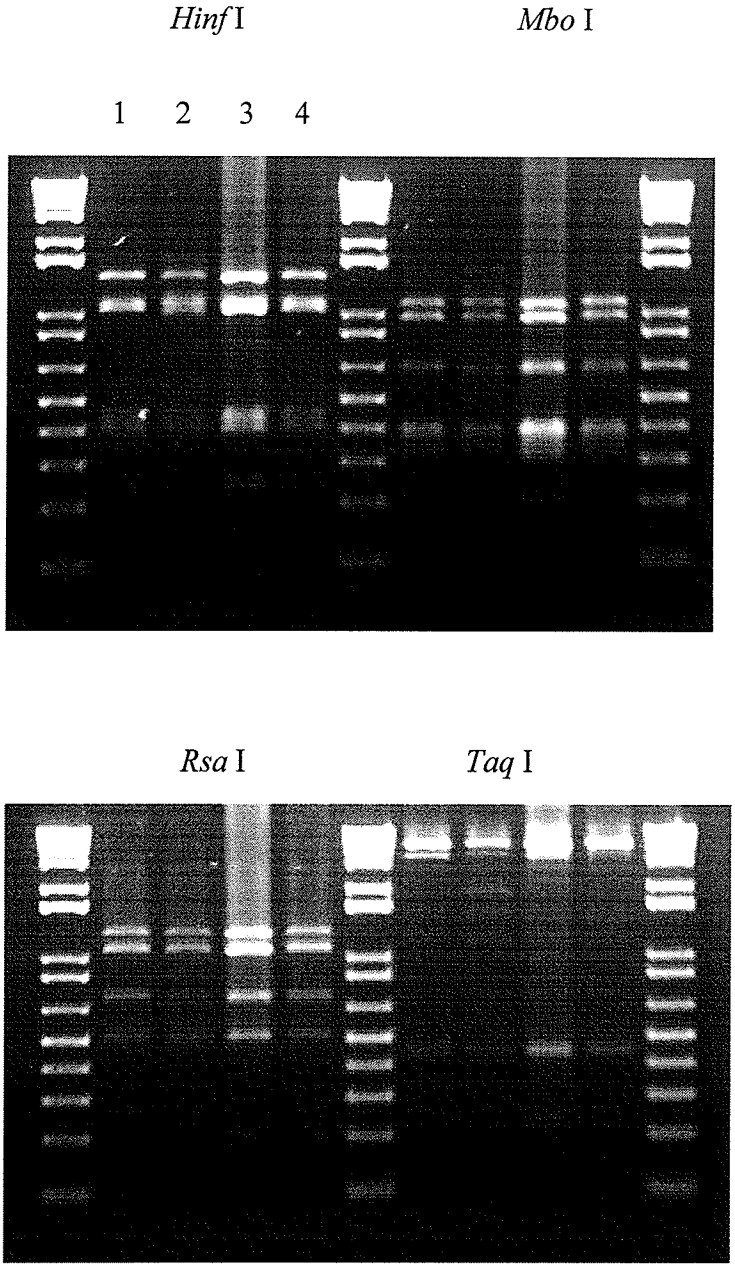


Fig. II.26. A gel showing restriction digestion of IGS of four individuals of *Chrysops aestuans* collected in Manitoba at the following locations:
1-Elma, 23 July, 1999
2-Sandilands, 12 July, 1999
3-Woodridge, 25 June, 1999
4-Woodridge, 14 July, 1999

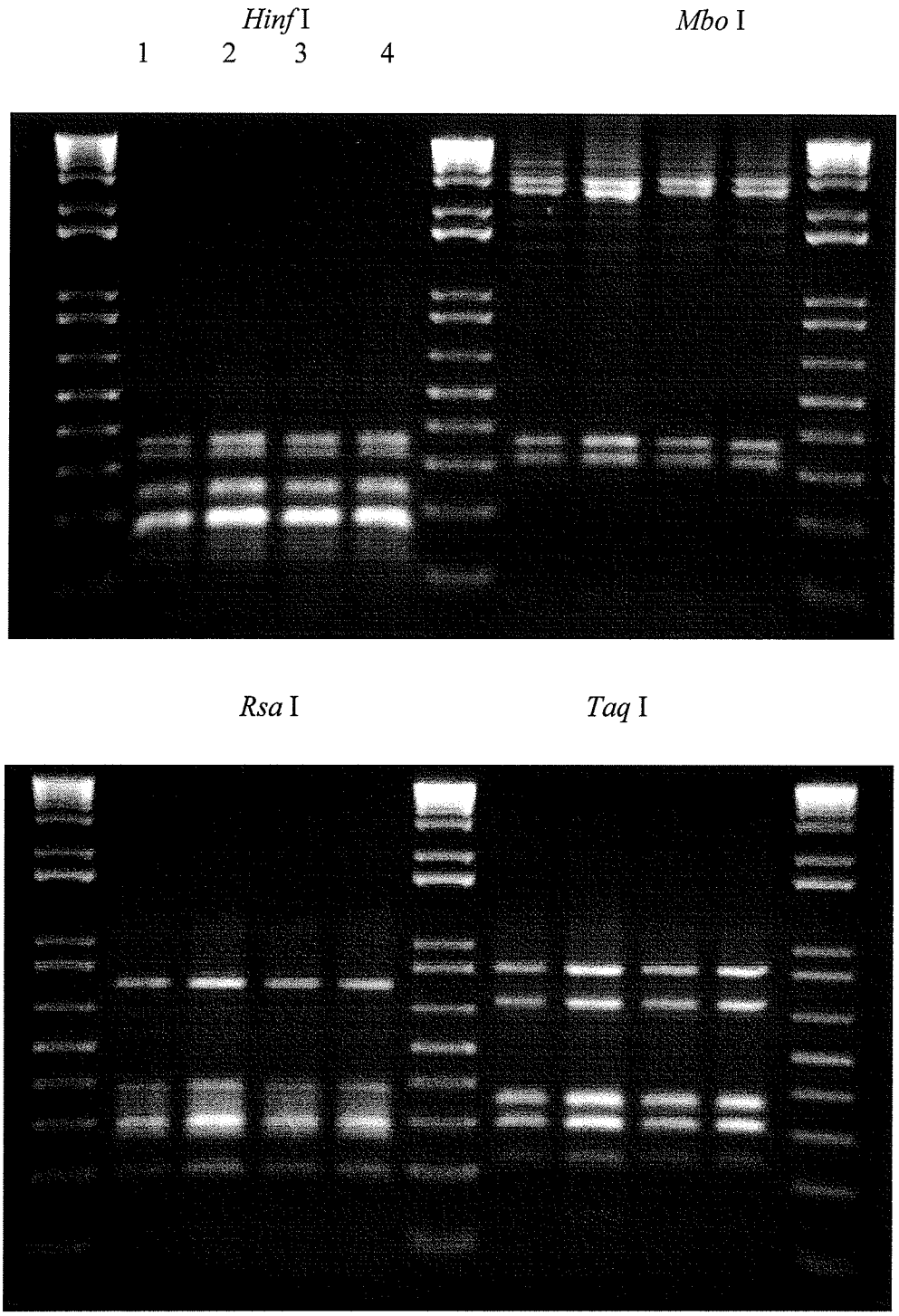


Fig. II.27. A gel showing restriction digestion of IGS of four individuals of *Chrysops dawsoni* collected in Manitoba at the following locations:
1-Woodridge, 19 June, 2000
2-Elma, 25 June, 2000
3-Sandilands, 1 July, 2000
4-Woodridge, 23 June, 2000

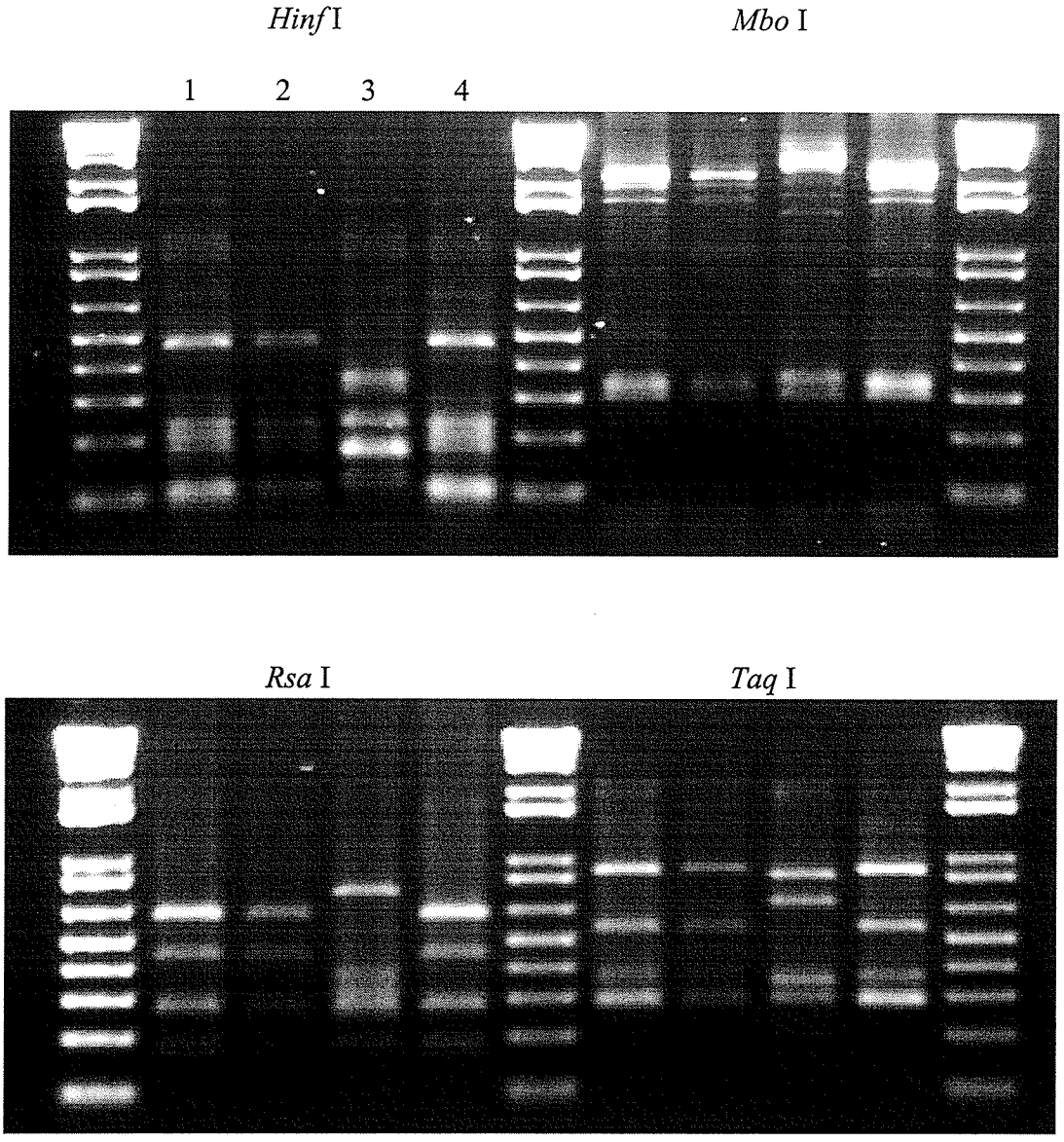


Fig. II.28. A gel showing restriction digestion of IGS of three individuals of *Chrysops excitans* (1, 2, and 4) and one individual of *C. dawsoni* (3) collected at the following locations in Manitoba:
1-McMunn, 8 June, 1999
2-Seven Sisters, 7 June, 1999
3-Whiteshell, 18 July, 1999
4-Woodridge, 25 June, 1999

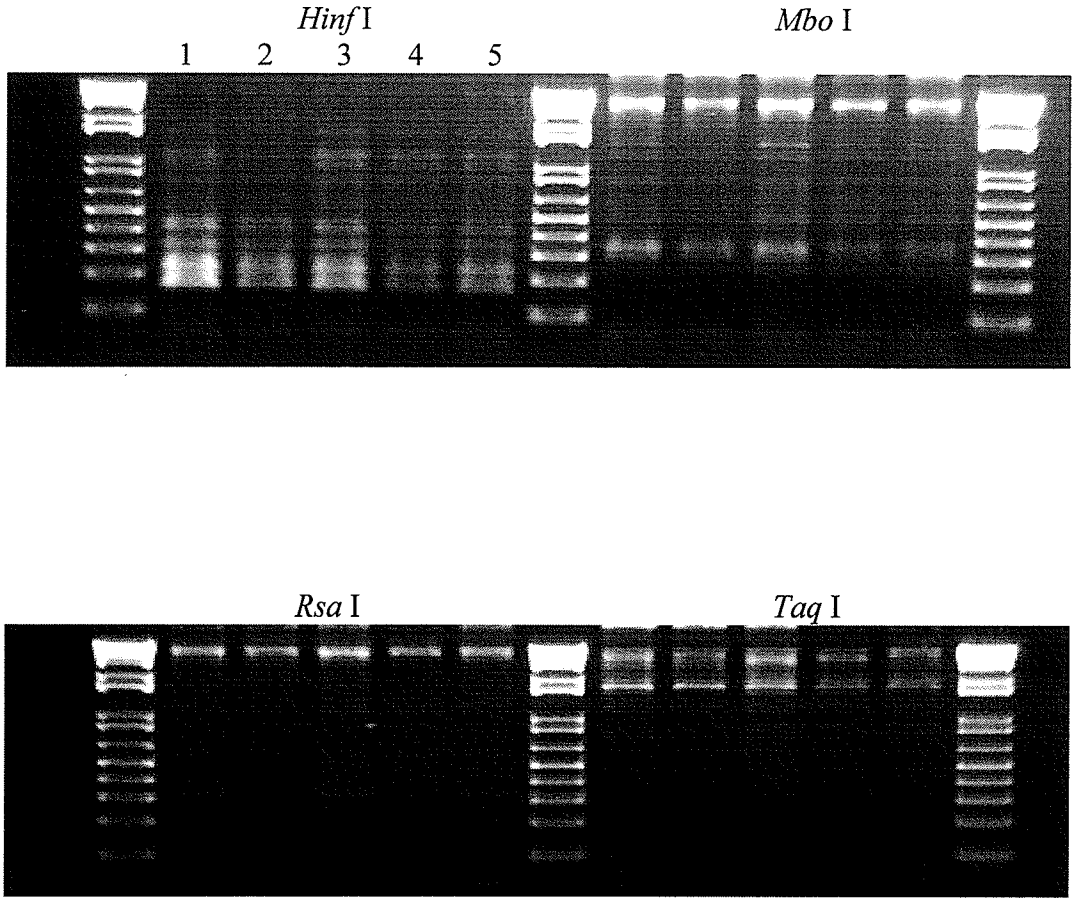


Fig. II.29. A gel showing restriction digestion of IGS of five individuals of *Chrysops frigidus* collected at the following locations in Manitoba:
1-Elma, 23 June, 1999
2-Sandilands, 12 July, 1999
3-Sprague, 20 July, 1999
4-Whiteshell, 18 July, 1999
5-Woodridge, 14 July, 1999

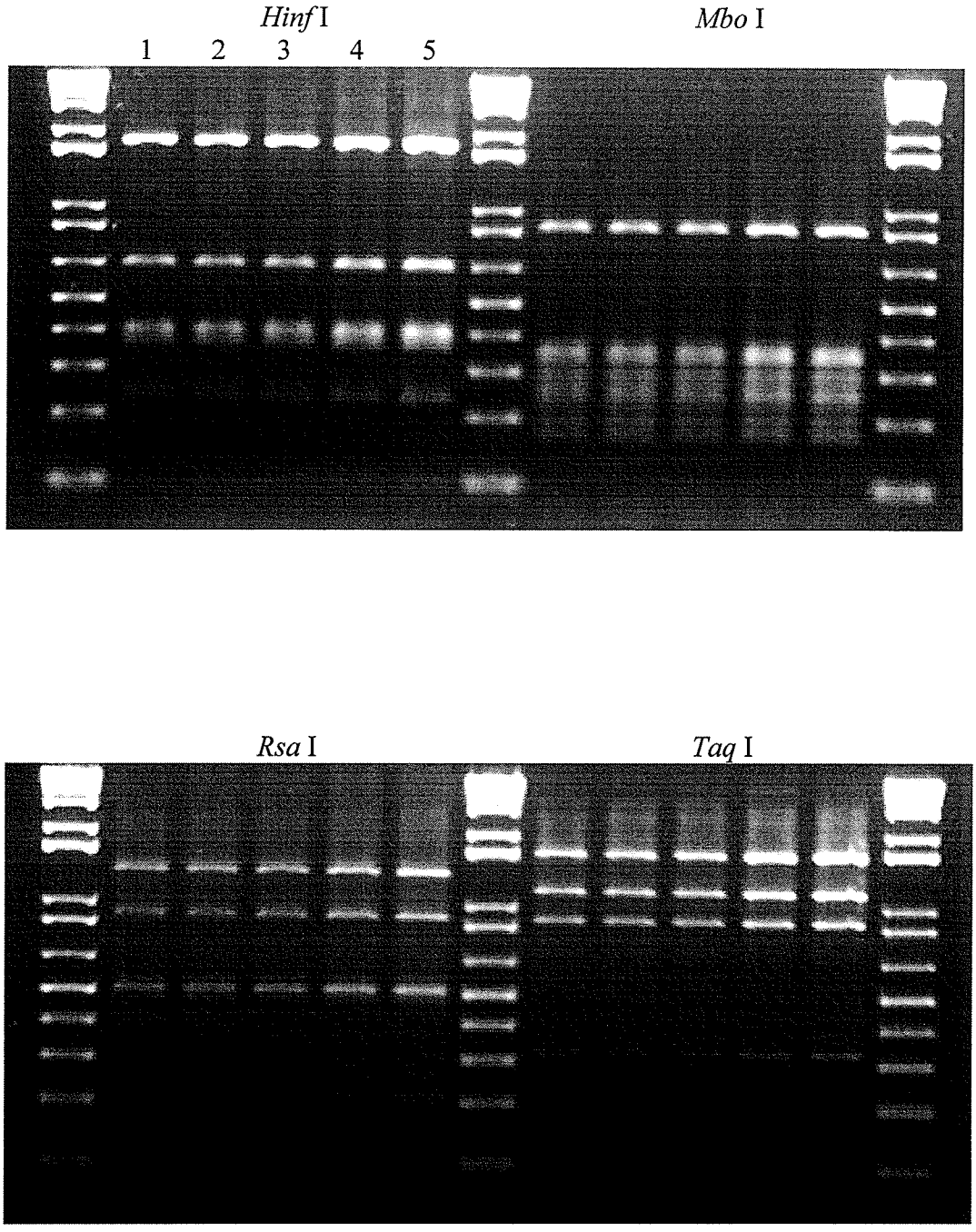


Fig. II.30. A gel showing restriction digestion of IGS of five individuals of *Chrysops indus* collected at the following locations in Manitoba:
1-Elma, 23 June, 1999
2-McMunn, 10 June, 1999
3-Sandilands, 12 July, 1999
4-Seven Sisters, 7 June, 1999
5-Woodridge, 14 July, 1999

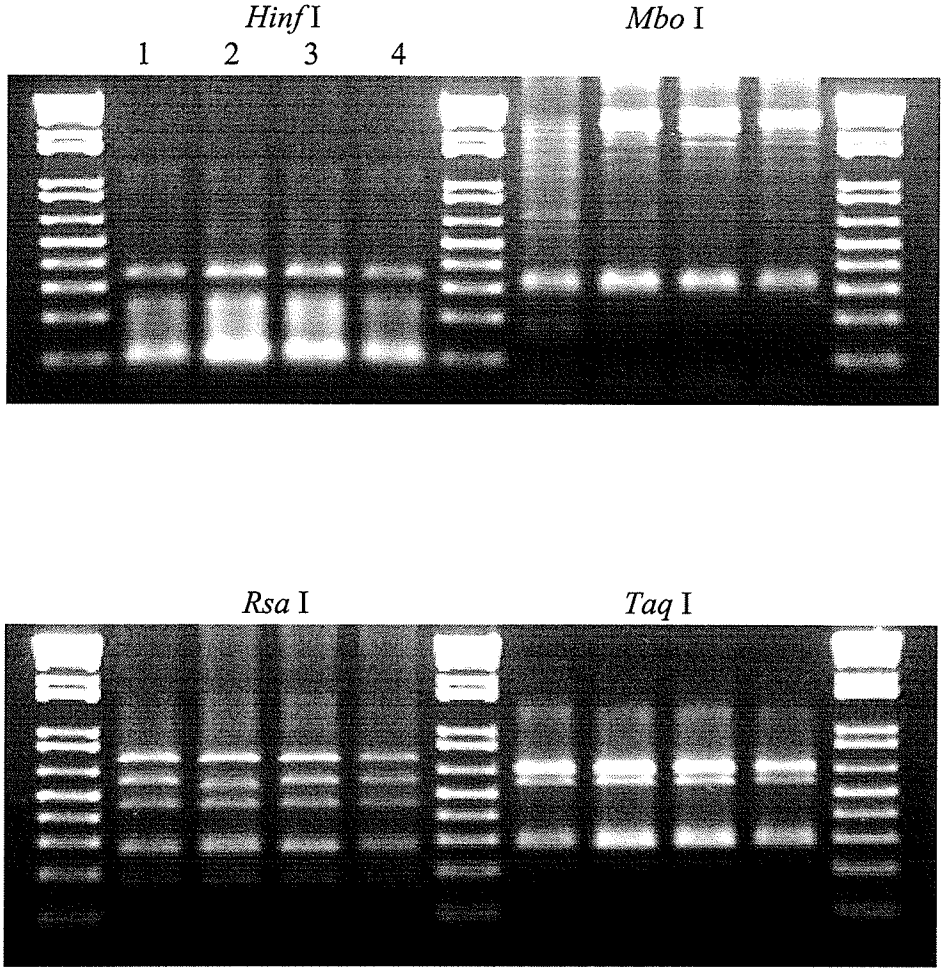


Fig. II.31. A gel showing restriction digestion of IGS of four individuals of *Chrysops mitis* collected at the following locations in Manitoba:
1-Elma, 23 June, 1999
2-McMunn, 10 June, 1999
3-Whiteshell, 18 July, 1999
4-Woodridge, 14 July, 1999

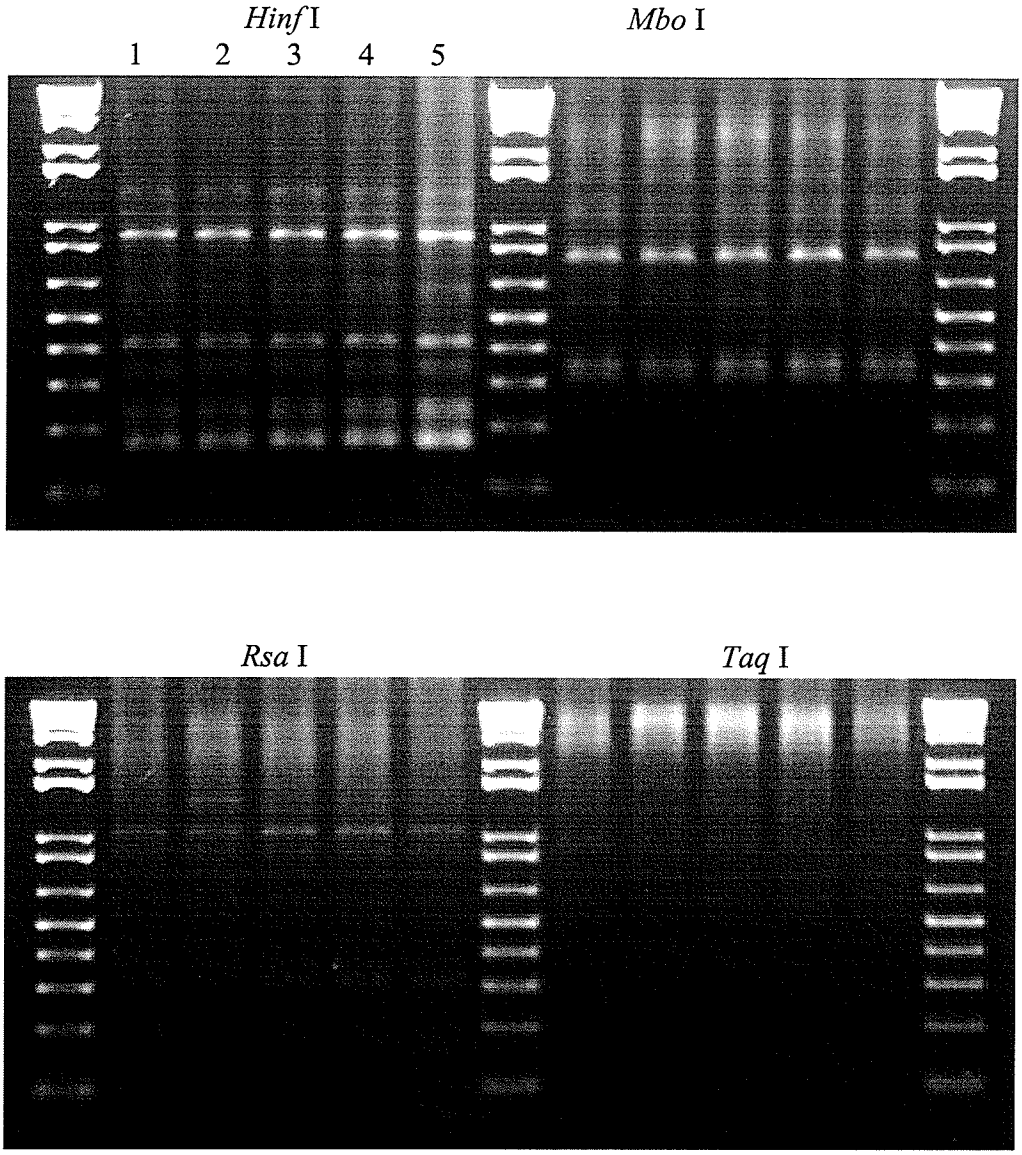
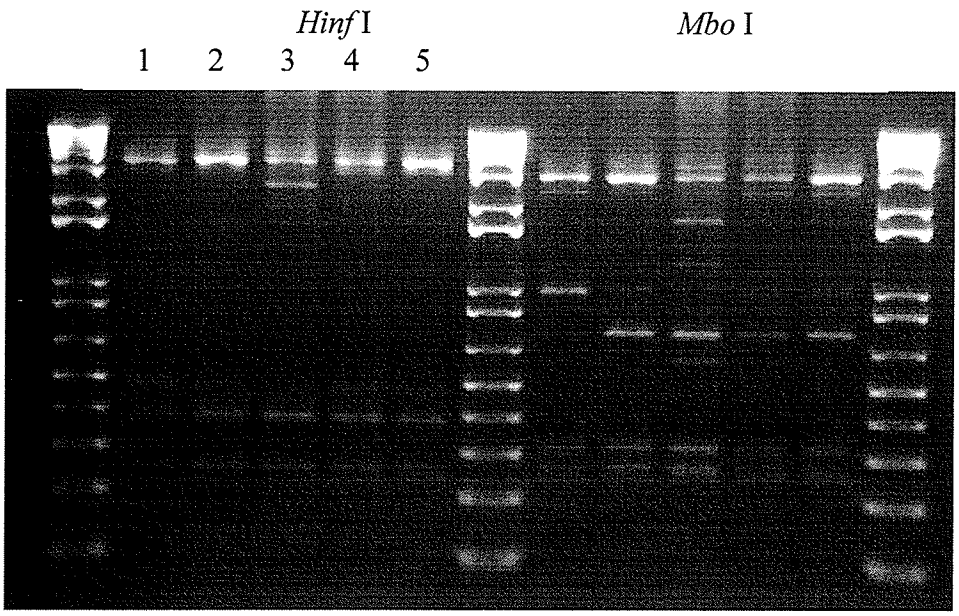


Fig. II.32. A gel showing restriction digestion of IGS of five individuals of *Chrysops venus* collected at the following locations in Manitoba:
1-Woodridge, 14 July, 1999
2-Piney, 17 July, 1999
3-Whiteshell, 14 July, 1999
4-Woodridge, 17 July, 1999
5-Seven Sisters, 14 July, 1999



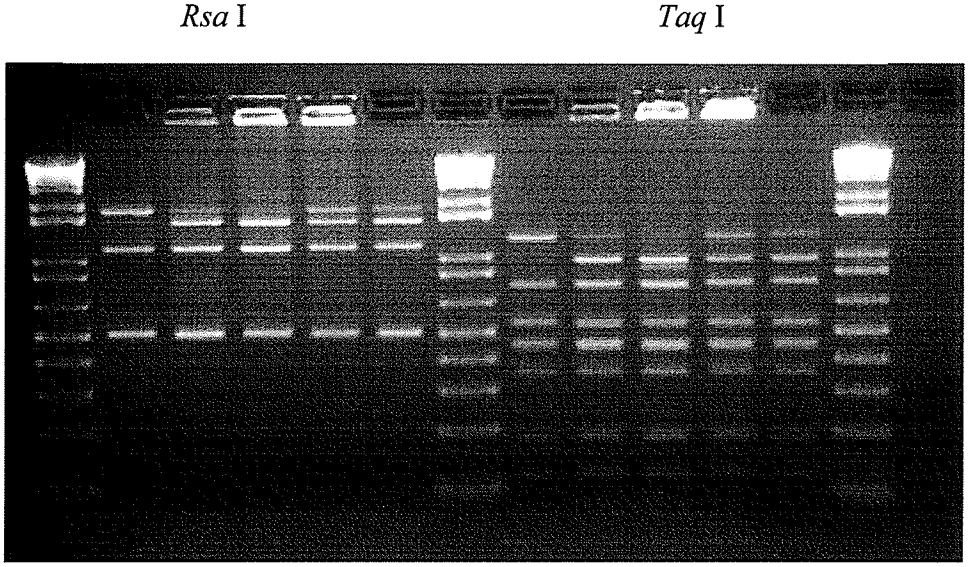


Fig. II.33. A gel showing restriction digestion of IGS of five individuals of *Hybomitra affinis* collected at the following locations in Manitoba:
1-Elma, 23 June, 1999
2-McMunn, 8 June, 1999
3-Sandilands, 12 July, 1999
4-Seven Sisters, 7 June, 1999
5-Seven Sisters, 3 August, 1999

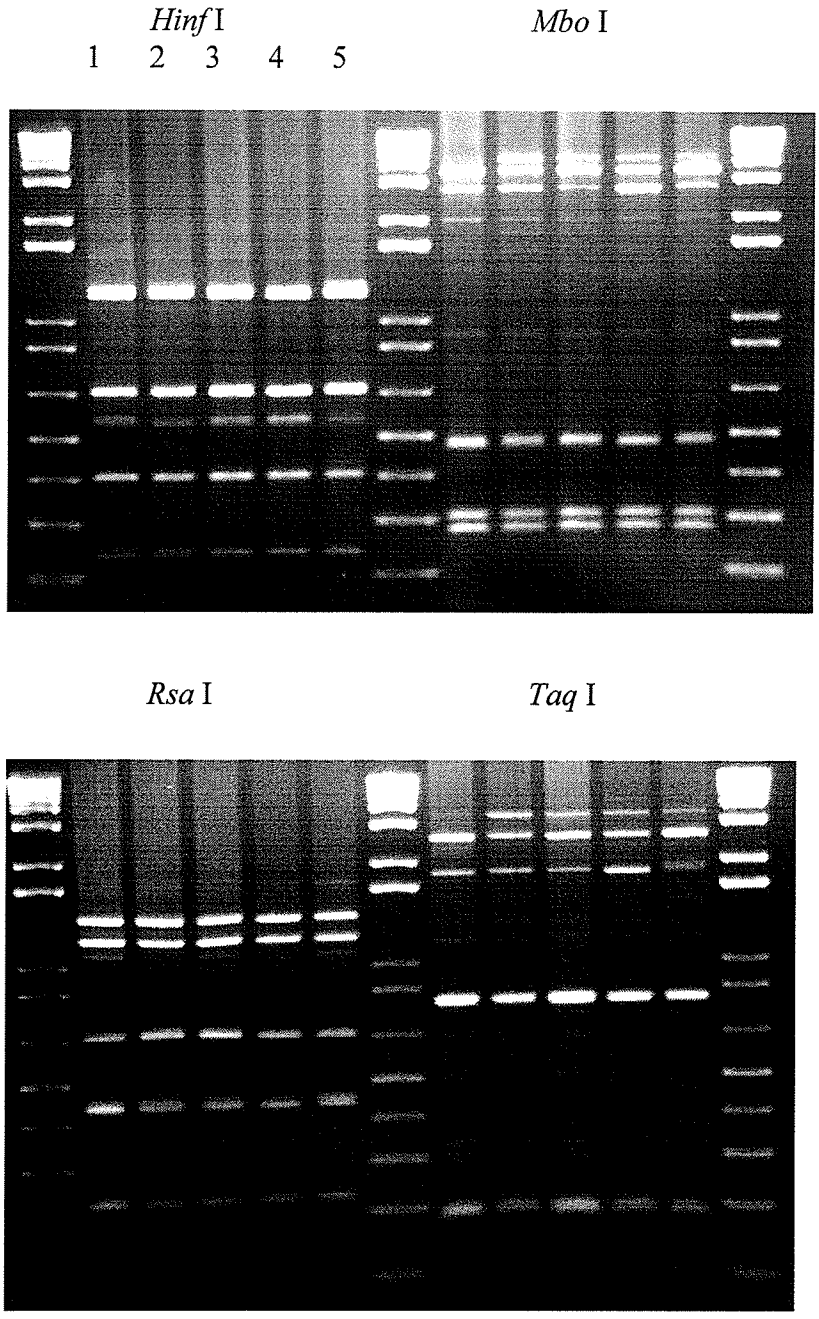


Fig. II.34. A gel showing restriction digestion of IGS of five individuals of *Hybomitra arpadi* collected at the following locations in Manitoba:

1-Elma, 23 June, 1999
2-McMunn, 8 June, 1999
3-Sandilands, 12 July, 1999
4-Seven Sisters, 3 Aug., 1999
5-Seven Sisters, 7 June, 1999
6-Woodridge, 25 June, 1999

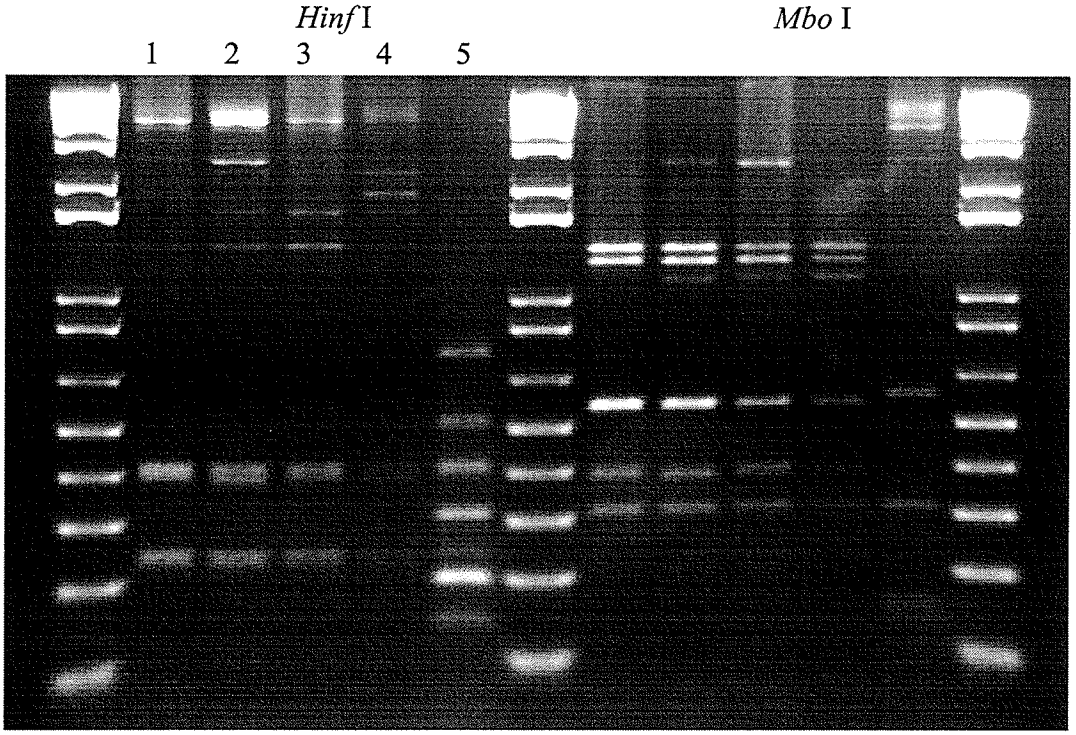


Fig. II.35. A gel showing restriction digestion of IGS of four individuals of *Hybomitra illota* (1-4) and one unknown of *Hybomitra* sp. (5) collected at the following locations in Manitoba:
1-Delta Marsh, 13 July, 1999
2-Elma, 23 June, 1999
3-McMunn, 8 June, 1999
4-Seven Sisters, 3 August, 1999
5-Woodridge, 14 July, 1999

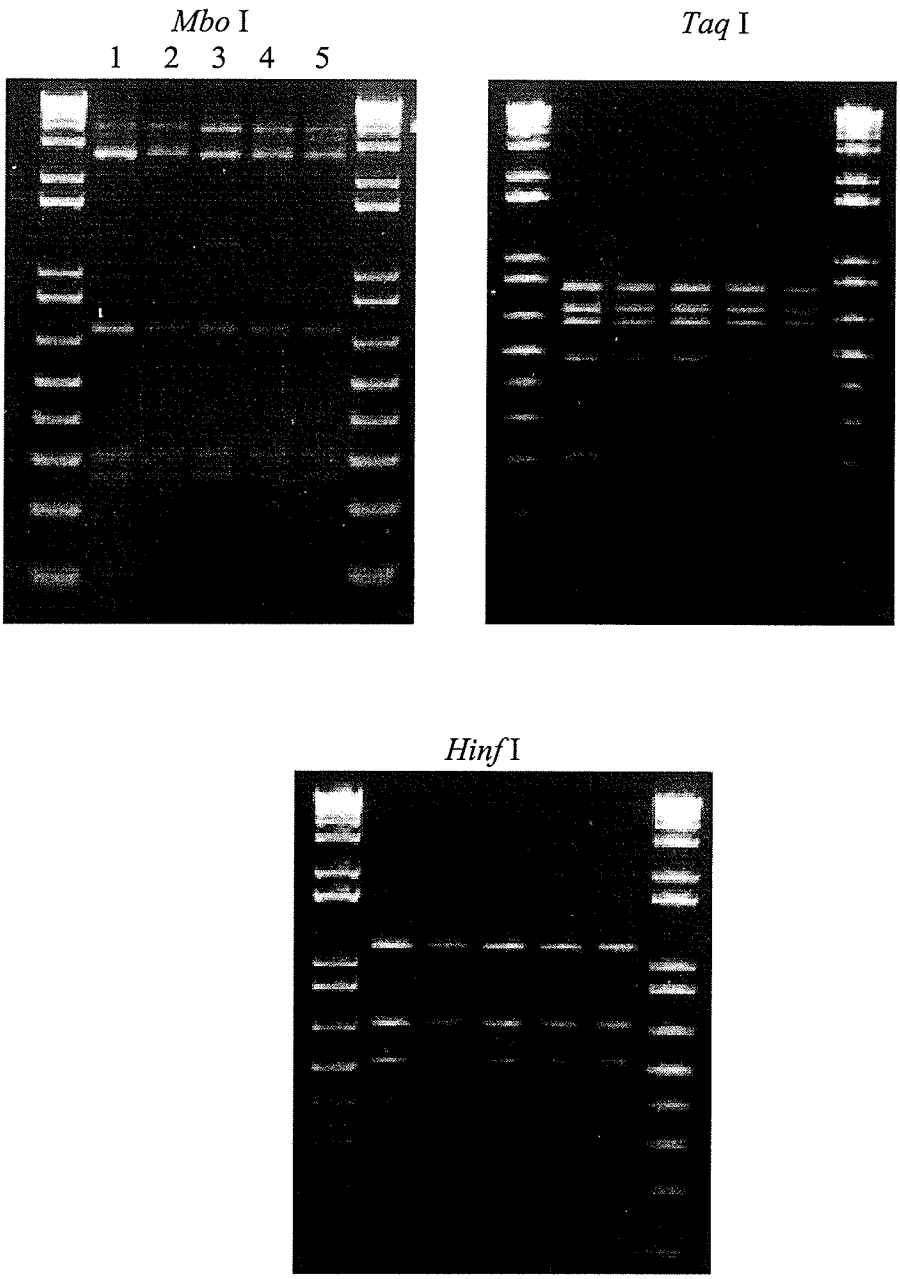


Fig. II.36. A gel showing restriction digestion of five individuals of *Hybomitra lasiophthalma* collected at the following locations in Manitoba:
1-Elma, 23 June, 1999
2-McMunn, 8 June, 1999
3-Sandilands, 12 July, 1999
4-Seven Sisters, 7 June, 1999
5-Woodridge, 25 June, 1999

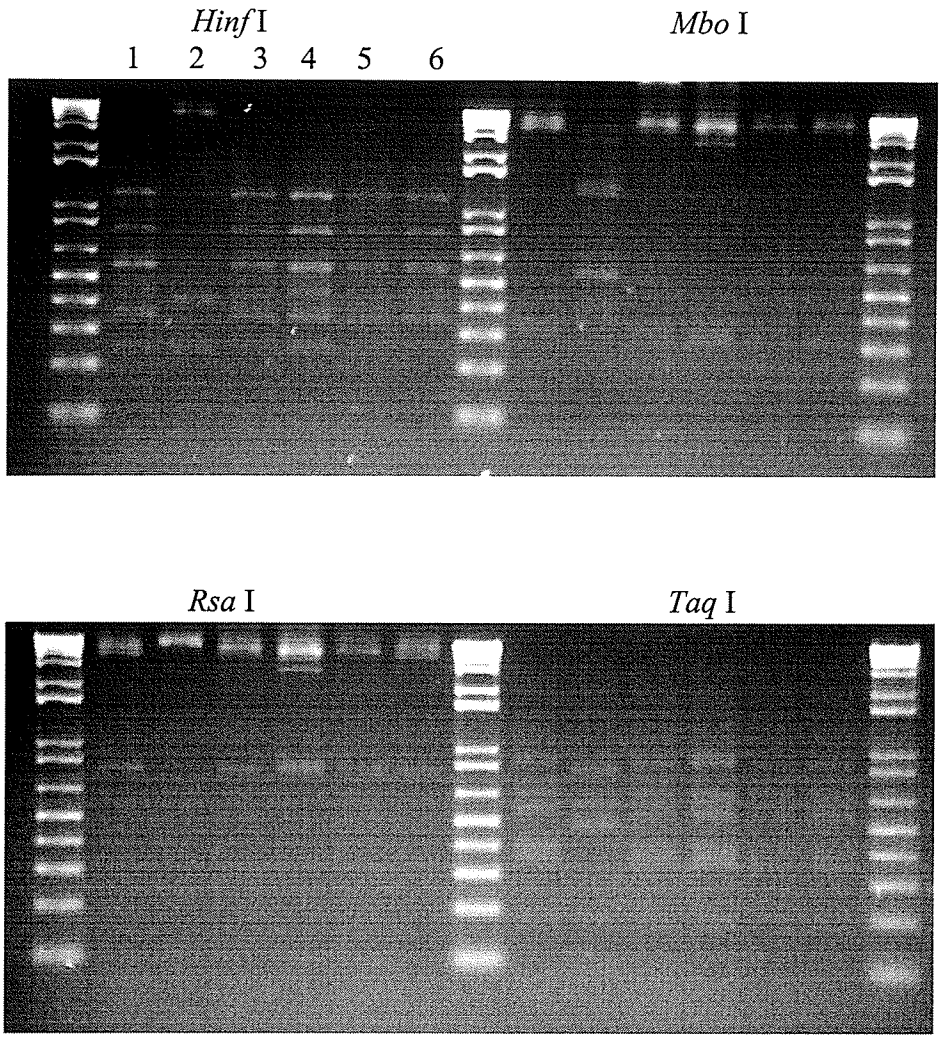


Fig. II.37. A gel showing restriction digestion of IGS of five individuals of *Hybomitra lurida* (1 & 3-6) and 1 unknown *Hybomitra sp.* (2) collected at the following locations in Manitoba:
1-Elma, 23 June, 1999
2-McMunn, 8 June, 1999
3-McMunn, 13 June, 1999
4-Sandilands, 12 July, 1999
5-Seven Sisters, 7 June, 1999
6-Woodridge, 19 June, 1999

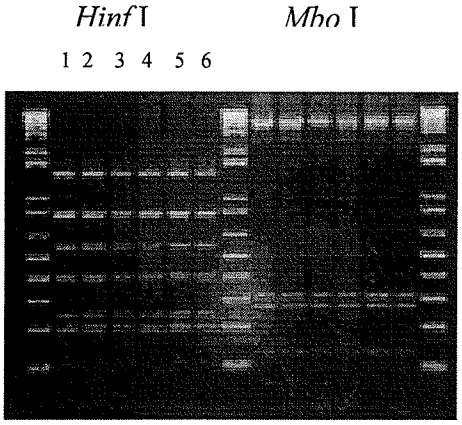


Fig. II.38. A gel showing restriction digestion of IGS of six individuals of *Hybomitra nitidifrons nuda* collected in Manitoba at the following locations:

1-Sandilands, 15 June, 1999
2-McMunn, 8 June, 1999
3-Seven Sisters, 7 June, 1999
4-Seven Sisters, 7 June, 1999
5-Woodridge, 25 June, 1999
6-Woodridge, 25 June, 1999

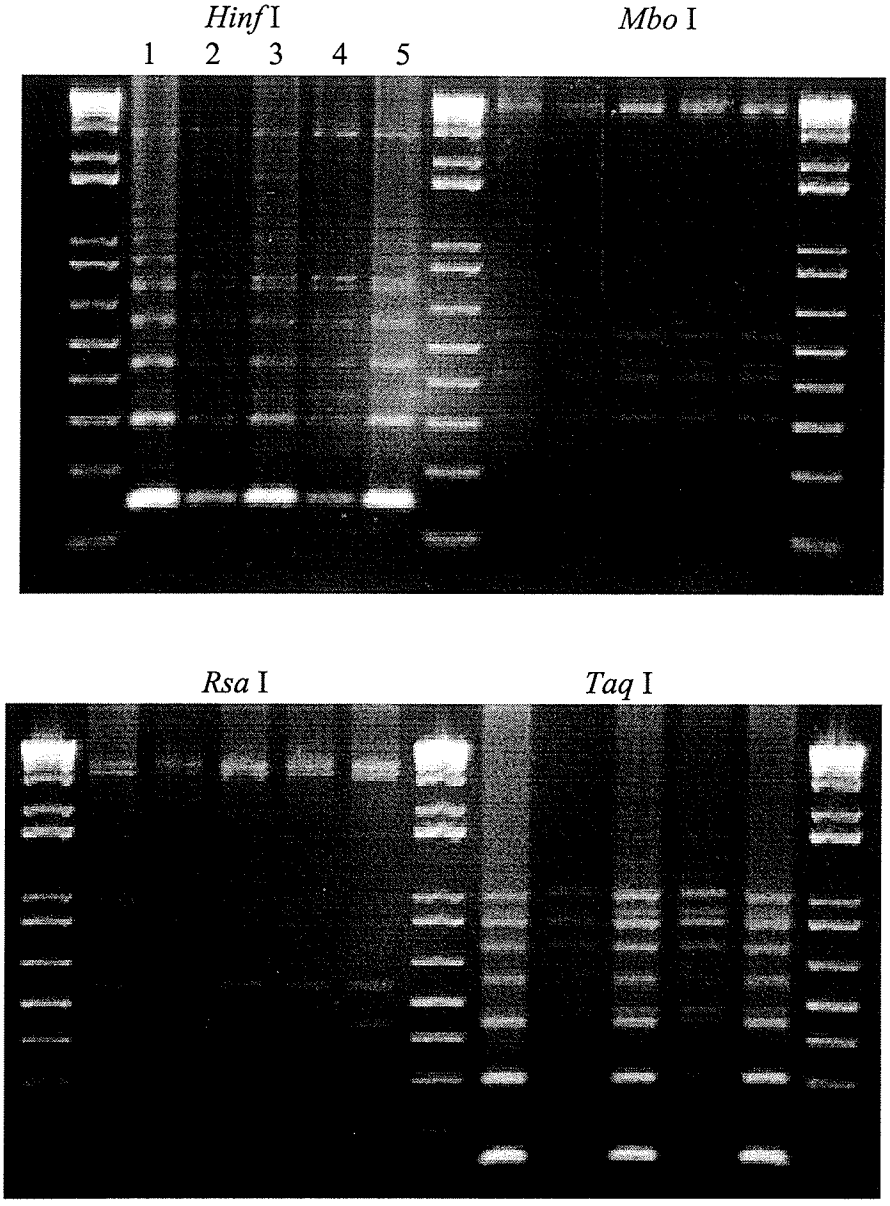


Fig. II.39. A gel showing restriction digestion of IGS of five individuals of *Hybomitra pechumani* collected at the following locations in Manitoba:
1-Elma, 23 June, 1999
2-McMunn, 4 July, 1999
3-Sandilands, 18 July, 1999
4-Seven Sisters, 20 July, 1999
5-Woodridge, 14 July, 1999

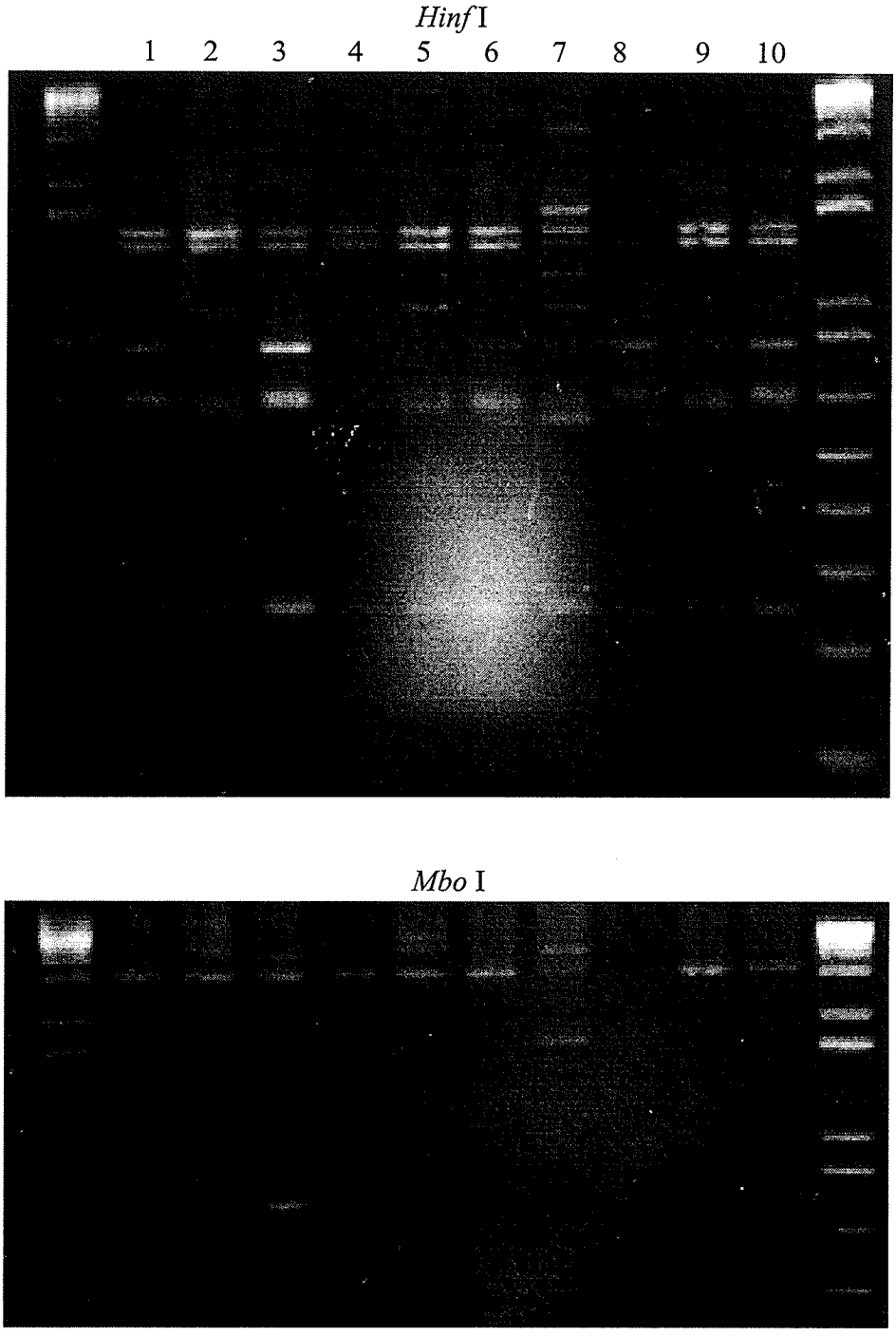
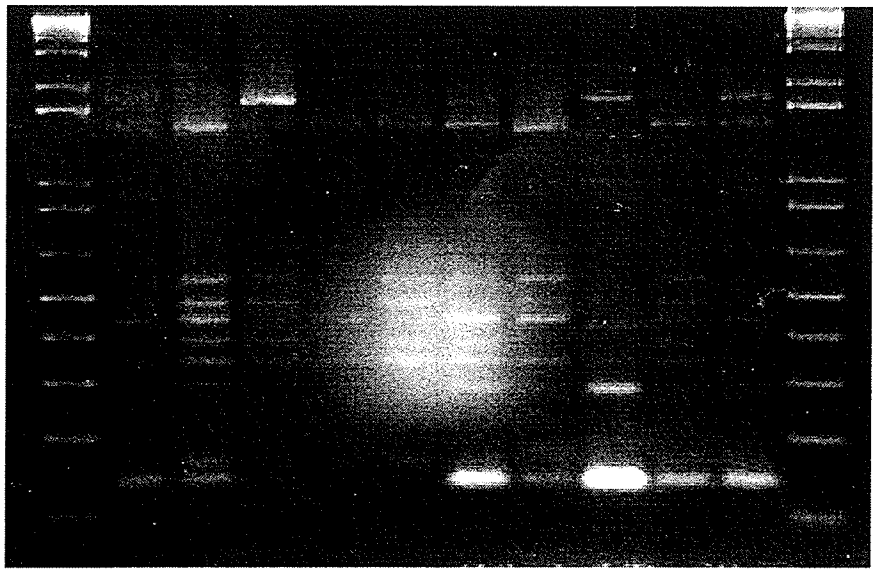


Fig. II.40. A gel showing restriction digestion of 10 individuals of *Hybomitra zonalis* by *Hinf*I and *Mbo*I. Adults collected at the following locations in Manitoba:

1-Elma, 23 June, 1999
2-McMunn, 5 July, 1999
3-Sandilands, 12 July, 1999
4-Seven Sisters, 18 July, 1999
5-Woodridge, 25 June, 1999
6-Elma, 25 June, 1999
7-McMunn, 8 June, 1999
8-Sandilands, 15 July, 1999
9-Whiteshell, 18 July, 1999
10-Woodridge, 28 June, 1999

Rsa I

1 2 3 4 5 6 7 8 9 10



Taq I

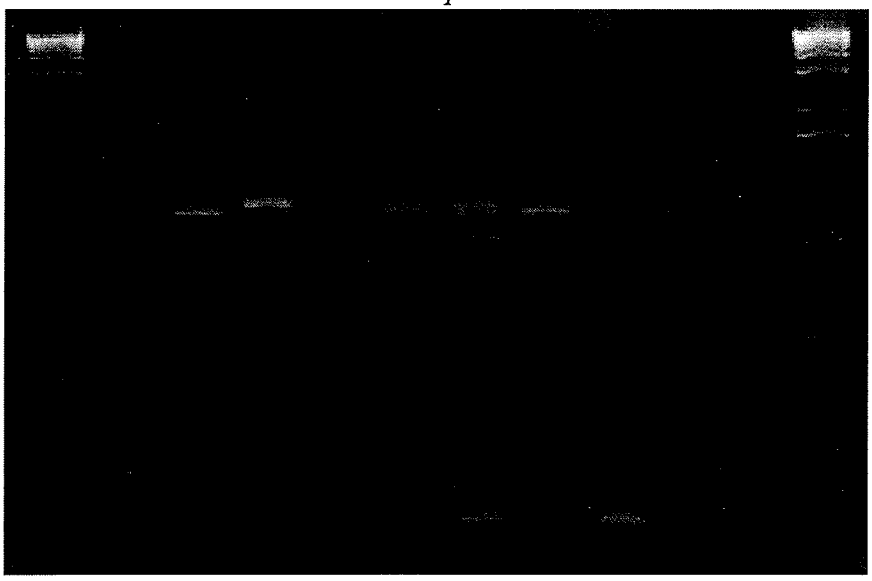


Fig. II.41. A gel showing restriction digestion of 10 individuals of *Hybomitra zonalis* by *Rsa*I and *Taq*I. Adults collected at the following locations in Manitoba:

1-Elma, 23 June, 1999
2-McMunn, 5 July, 1999
3-Sandilands, 12 July, 1999
4-Seven Sisters, 18 July, 1999
5-Woodridge, 25 June, 1999
6-Elma, 25 June, 1999
7-McMunn, 8 June, 1999
8-Sandilands, 15 July, 1999
9-Whiteshell, 18 July, 1999
10-Woodridge, 28 June, 1999

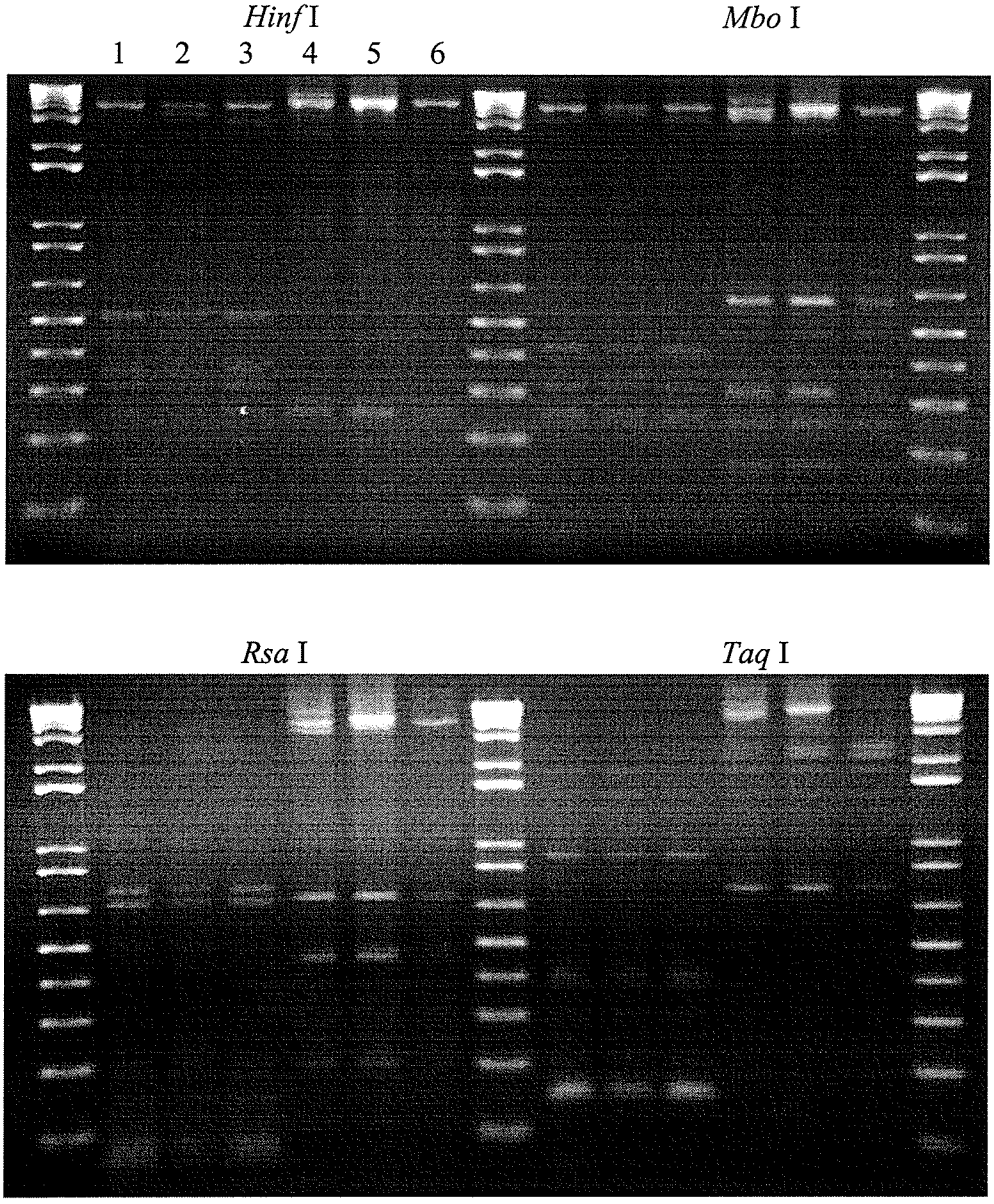


Fig. II.42. A gel showing restriction digestion of IGS of three individuals of *Tabanus marginalis* (1-3) and three individuals of *T. similis* (4-6) collected in Manitoba at the following locations:

1-Elma, 4 July, 2000
2-Woodridge, 14 July, 2000
3-Whiteshell, 20 July, 2000
4-Portage La Prairie, Rd. # 227, 23 June, 2000
5-Hadashville, 29 June, 2000
6-Beausejour, 10 July, 2000

Appendix III. Tabanid egg masses were collected in Manitoba and identified by PCR-RFLP. Fig. 1. *Hybomitra nitidifrons nuda*, Fig. 2. *H. lasiophthalma*, Fig. 3. *Chrysops excitans*, Fig. 4. *C. mitis*, Fig. 5. *C. aestuans*.

Fig. 1

Fig. 2

Fig. 3

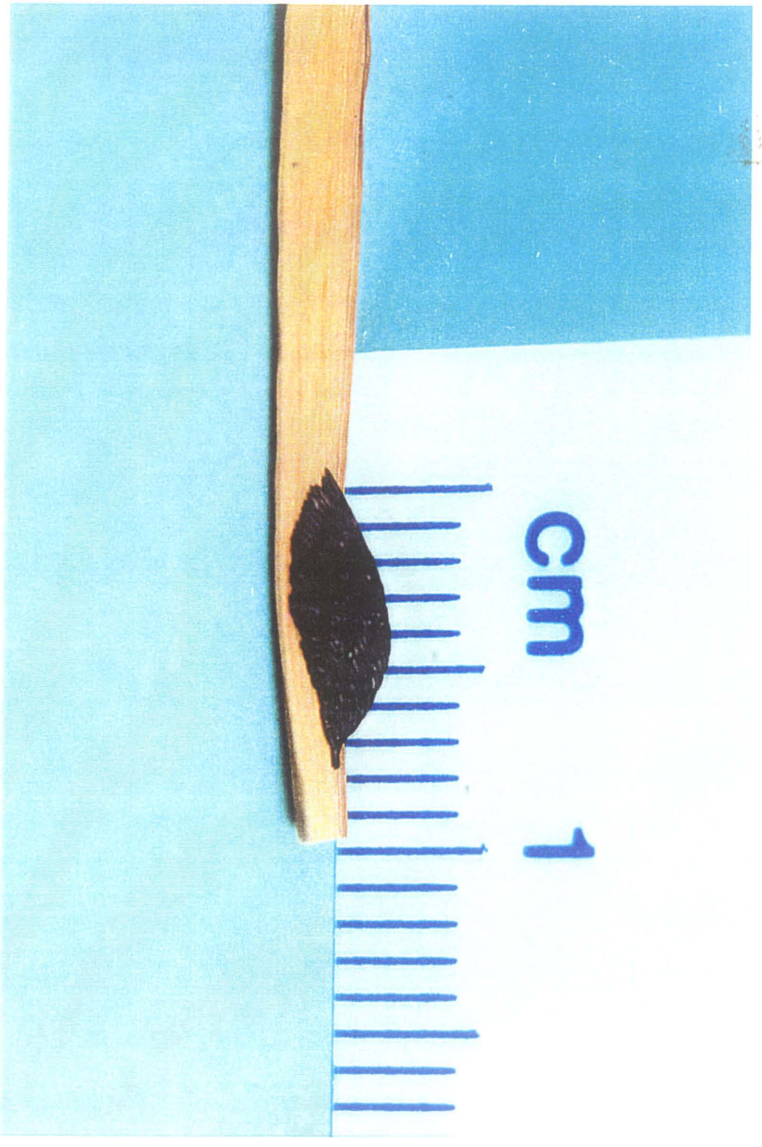


Fig. 4

Fig. 5

Appendix IV. Emergence period of parasitoid species *Telenomus* A, B and C from tabanid egg masses; breeding sites; rearing flask.

Table IV.1. Emergence patterns of *Telenomus* species A and B from five egg masses of horse flies under laboratory conditions (25°C, 50% RH, and 16L:8D light intervals) in Manitoba. Numbers are percentages of adults that emerged from five egg masses for each time interval.

Day	Time	Species A		Species B	
		Female	Male	Female	Male
1	0900	26.4	20.6	25.4	36.2
	1700	7.8	6.2	17.8	17.0
2	0100	6.4	2.8	10.0	13.0
	0900	19.8	27.6	13.8	19.2
	1700	15.8	12.4	9.6	4.8
3	0100	5.2	4.8	1.4	3.4
	0900	12.0	12.4	9.2	3.4
	1700	4.8	7.6	6.0	2.0
4	0100	1.8	5.6	1.0	-
	0900	-	-	3.6	1.0
	1700	-	-	2.2	-

Table IV.2. Emergence pattern of *Telenomus* species C from six egg masses of deer flies under laboratory conditions (25˚C, 50% RH, and 16L:8D light intervals). Numbers are percentages of adults emerged from six egg masses for each time.

Day	Time	Female	Male
1	0900	7.0	45.0
	1700	3.0	10.0
2	0100	1.5	6.0
	0900	35.0	39.0
	1700	3.0	-
3	0100	-	-
	0900	35.0	-
	17	5.5	-
4	0100	10.0	-
	0900	-	-
	1700	-	-

Fig. IV. 1. Typical breeding site for *Hybomitra nitidifrons nuda*, southern Manitoba, summer of 1997.

Fig. IV. 2. *Hybomitra nitidifrons nuda* laying eggs on cattail (*Typha* sp.), Woodridge, Manitoba, summer of 1997.

Fig. IV. 3. Typical breeding site for *Chrysops aestuans*, Elma, Manitoba, summer of 1997.

Fig. IV. 4. *Chrysops aestuans* laying eggs on cattail (*Typha* sp.), Elma, Manitoba, summer of 1997.

Fig. IV.5. Female (top) and male (bottom) of *Telenomus* species A reared from eggs of *Hybomitra nitidifrons nuda*, and *H. lasiophthalma*.

Fig. IV.6. Disposable flasks were used to rear tabanid egg parasitoids.

Appendix V. Petri dish with parasitoid on impregnated filter paper; Plexiglass cage.

Fig. V.1. Petri dish with parasitoid arrested to filter paper impregnated to extract from abdomen of *Hybomitra nitidifrons nuda*.

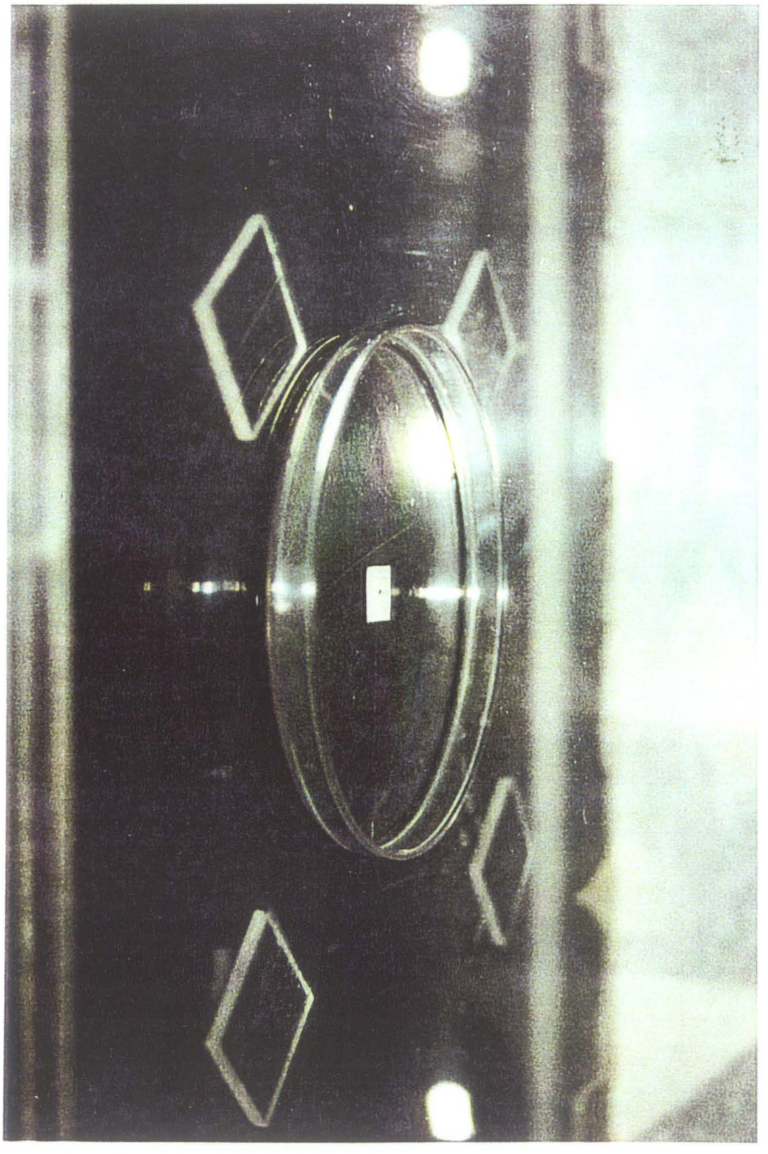


Fig. V.2. A Plexiglass® cage was used to measure arrestment time of *Telenomus* species A, B, and C by extracts from their hosts.

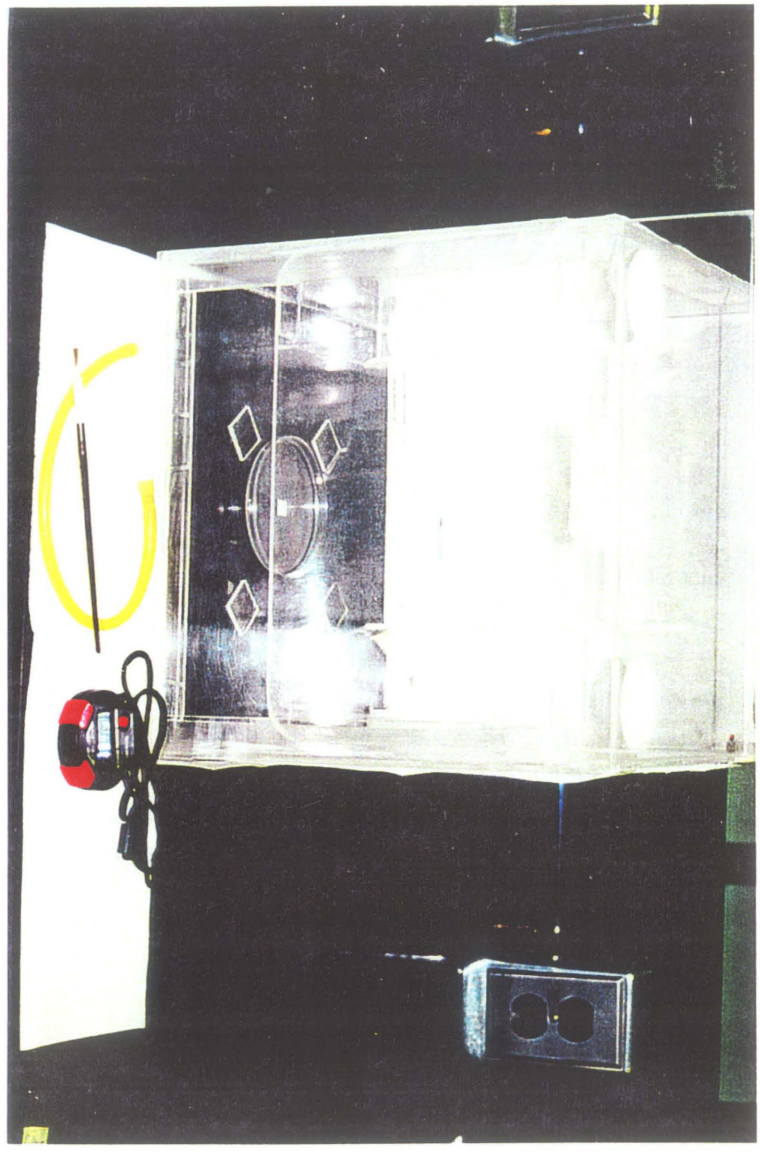
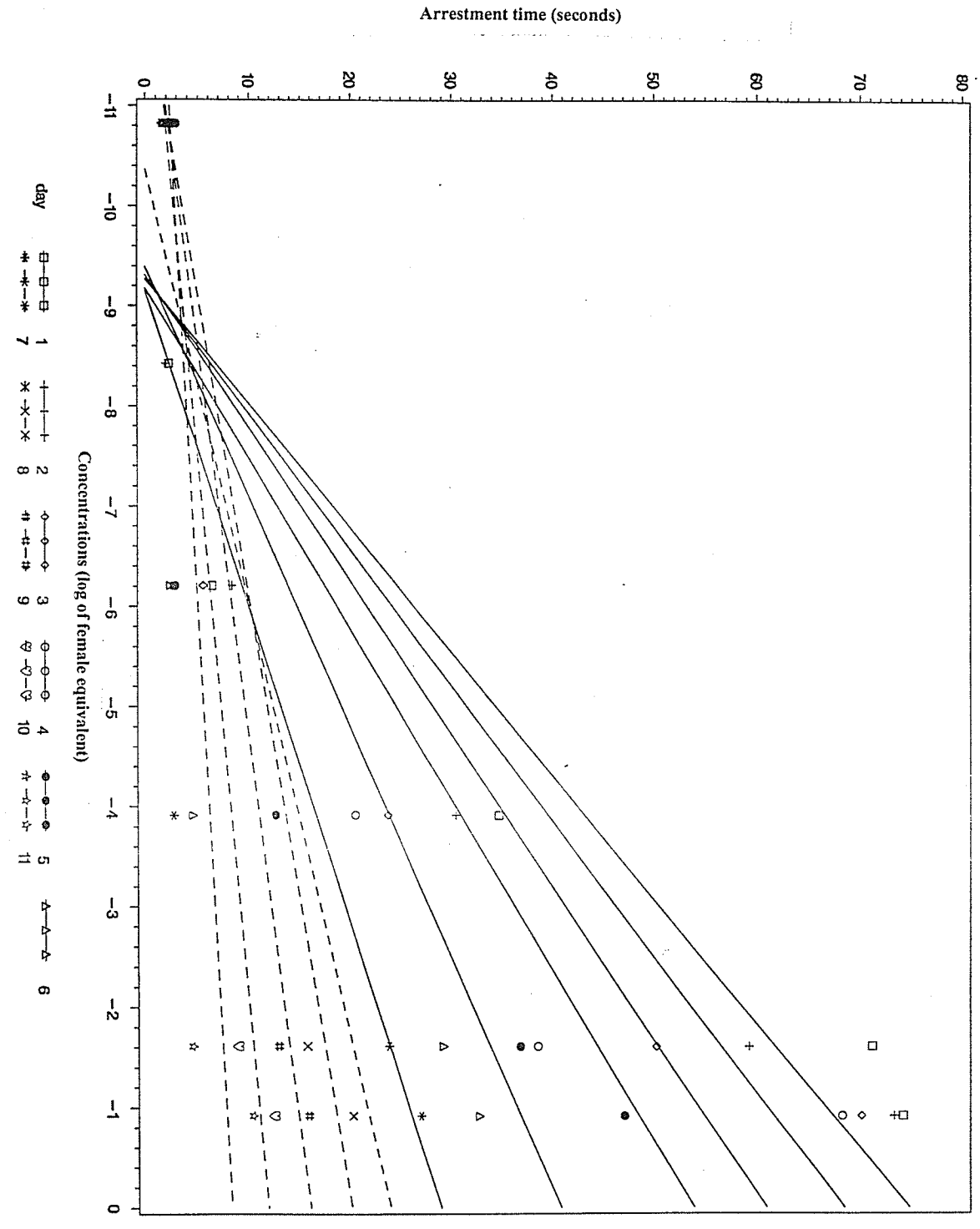


Fig.V.3. Arrestment time of parasitoid A to extract from abdomen of *Hybomitra nitidifrons nuda* on different days (1-11) after extraction (concentration of extract on logarithmic scale).


Arrestment time (seconds)
0
10
20
30
40
50
60
70
80
Concentrations (log of female equivalent)
-11
-10
-9
-8
-7
-6
-5
-4
-3
-2
-1
0
day
1
2
3
4
5
6
7
8
9
10
11