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Morphological and Genetic Variation in the Genus *Tetramesa* and its Parasitoids (Hymenoptera: Chalcidoidea) and its Application to Iranian Biogeography

A thesis presented for the degree of Doctor of Philosophy

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2004

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THIS THESIS DEDICATED TO THE MEMORY OF

PROFESSOR MORTEZA ESMAILI

AND

MY PARENTS, WIFE AND DAUGHTER

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Full Title of Thesis **Morphological and Genetic Variation in the Genus *Tetramesa* and its Parasitoids (Hymenoptera: Chalcidoidea) and its Application to Iranian Biogeography**

Summary:

The aim of this thesis was to investigate the evolution and ecology of parasitoid chalcidoid wasps associated with two allopatric grass species, *Leucopoa pseudosclerophylla* (Krivot.) Bor. and *L. sclerophylla* (Boiss. & Hohen.) V. Krecz & Bobrov in south-east, south-west and northern Iran. The associated chalcidoid wasp genus *Tetramesa* (=*Isosoma* Walk.; =*Harmolita* Motsch.) (Hymenoptera: Chalcidoidea: Eurytomidae) is considered exclusively phytophagous during larval development with some species forming distinct galls and some live without any indication of a galling habit. Multidisciplinary approaches were used to clarify the morphological and molecular genetic variation of the chalcidoid wasps: 1) morphometrics, 2) phylogenetic and phylogeographic relationships, 3) community structure.

Morphometric analyses were carried out to study variation in morphological characters among geographic populations of *Tetramesa* spp. The northern populations feed on *L. sclerophylla*, while southern populations are restricted to *L. pseudosclerophylla*. Morphometric analyses of 19 characters showed that 4 and 5 of these characters displayed significant differences between populations of *Tetramesa* based on locality (3 sites) and region (3 sites) respectively. Discriminant function and cluster analyses showed the strongest distinction between geographic groups and exhibited geographically correlated variation in size for some morphological characters.

The structure of the parasitoid communities associated with *Tetramesa* species in the two grass species was also explored. A semi-quantitative food web was constructed based on the total parasitism rates. The food web comprised two subcommunities, which contained 15 species organized into five trophic levels. The most striking characteristic of the food web was the extreme specificity of most of the species in the community. The outcome of this high level of specialization was a compartmentalized food web. The result also showed that the parasitoids of most host species had relatively consistent dominant hierarchies.

The genetic structure of the *Tetramesa* parasitoids, *Eurytoma* spp., *Sycophila* spp. (Eurytomidae), *Pediobius* spp. (Eulophidae), and *Chlorocytus* spp. (Pteromalidae) was investigated using sequence data from the mitochondrial cytochrome oxidase subunits (CO) I and II genes. Phylogeographic patterns of *Sycophila* spp. were investigated using a 778 base pair region of the COI gene which identified 5 haplogroups comprising 25 haplotypes in 10 populations. Considerable genetic variation and divergence was observed within and between haplogroups, especially haplogroups I and II which may comprise several new taxa. The sequence divergences within haplogroups ranged between 0.1 and 1.0% and among haplogroup values varied between 2.4 and 6.4%. Based on data from sequence divergence of other taxa and estimated divergence times it is hypothesized that divergence between haplogroups probably started from the late Pliocene and continued during the Pleistocene, probably in refugial areas followed by colonization events during interglacial periods.

Abstract

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Acknowledgements

First and foremost I would like to express my appreciation and gratitude to my supervisor, Prof. Michael Bruford for his guidance, constructive criticism, enormous patience, invaluable comments, and encouragement throughout the course of this study. I thank him for taking me into his lab when everything was not going smoothly, and giving me the opportunity to carry out this thesis.

In particular I am most grateful for the help and advice I received during the initial stage of the Ph.D programme in Iran from my first Iranian supervisor, Prof. Morteza Esmaili.

I am most grateful to Dr. H. A. Dawah, my former supervisor who kindly first accepted me as a Ph. D student and for his useful guidance during the initial stage of the Ph. D programme, invaluable comments and reading the related sections of the thesis

I thank Dr. R. Khajeh, Iranian supervisor for his help and advice in Iran.

I am deeply grateful to the people and organizations listed below for their contribution for the successful conclusion of this Ph. D. thesis:

Shahid-Bahonar University of Kerman, Ministry of Science, Research and Technology of Iran, Cardiff University, and Embassy of Islamic Republic of Iran (Dr. Khodaii and Dr. Shams) in London for providing me with study leave and sponsorship.

Drs. Ciara Dodd and Carlos Fernandes for their valuable advice and help on molecular work.

Drs. M. Kharusy, S. Manzari, Cristiane Silveria and Mr. B. Rowson for their useful comments on morphometrics, and also Mr. Paul McGeoghan for his guidance on statistical analyses.

Dr. F. Termeh (Plant Pests and Disease Research Institute, Tehran, Iran), Mr. S. M. Mirtajaddini (Dept. of Biology, Shahid-Bahonar University, Kerman), Mr. B. Hamzeh (Research Institute of Forests and Rangelands, Tehran), Iran for their help in identification of grass species.

Dr. H. A. Dawah for his help in identification of the larvae and adult wasps.

Dr. M. Dastanpour (Dep. of Geology, Shahid-Bahonar University, Kerman) for the information on coordinates and geography, Dr. Vaziri for his help in photography.

Dr. Lora, R. Stevens (Dept. of Geoscineces, University of Nebraska, USA); Dr. Herb. E. Wright (University of Minnesota, USA); Prof. C. Neil Roberts (School of Geography, University of Plymouth, UK) for their comments on palaeoclimate of Iran.

Dr. Mark Jervis for his advice and help during this study.

Mike Turner of the SEM unit for his assistance with scanning electron microscopy, Vyv Williams and Guy Pitt for photography. Alborz Research Centre of Research Institute of Forests and Rangelands, Karaj, Iran for the help in sample collection from northern Iran (Alburz Mountains).

The friendly members of the G10 Lab, such as Dr. Trinidad Perez for her help in selecting the map and Dr. Ciara Dodd for proofing reading the final part of my thesis.

The many people and organizations who gave valuable help collecting the grasses in the field in Iran including Shahid-Bahonar University of Kerman, The Iran; Natural Resources Research Centre of the ministry of Djahad-Sazandegi (Kerman, Fars, Khorasan and Chalous) especially Mr. Naseri in the Kerman branch, I am indebted to him for his invaluable help and for organizing the sampling programmes in Kerman and in other parts of the country. My special thanks go to Mr. M. Mirtajaddinin and Mr. Pourmirzaii for accompanying me in sampling at several sites in the Kerman province. I also thank Mr. Takallozadeh at the School of Agriculture, Shahid-Bahonar University for their help and assistance related to photography. I thank Mrs. Dr. Azmayeshfard (Agriculture College, Tehran University) for her help in Iran and also Dr. Ebrahimi (Plant Pests and Disease Research Institute, Tehran, Iran) for his information about the morphological studies in Iran.

My colleagues, Ciara, Carlos, Shamie, Simon Shyler, Reza, Babak and Mozaffar at Cardiff for all the encouragement, help and patience throughout the course of my study.

Lastly, I am deeply indebted to my parents, wife and daughter for all their friendship, belief, support and dedication, especially my wife and daughter who have borne the many difficulties encountered in producing this thesis.

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CHAPTER 1

Introduction

1.1 Biological diversity of chalcid wasps

The superfamily Chalcidoidea, (commonly referred to as chalcids or chalcidoids), is one of the most diverse groups of parasitic Hymenoptera biologically, numerically and morphologically. It comprises about 21,250 described species in over 2000 genera (Gaston 1991). It has been estimated that there may be more than 400,000 species and, if so, this group would prove to be far more specious even than ichneumonoids (Noyes, 1978, 1990, 1998). The number of Chalcidoid families range from nine (Riek 1970) to 24 (Nikol'skaya 1952). This cosmopolitan superfamily is found in all zoogeographic regions and in all habitats, but appears generally to be most diverse in the tropics. Despite their omnipresence, they remain one of the poorest known groups of parasitic Hymenoptera, possibly because of their small size, morphological similarities, biological diversity and numerical abundance.

Parasitoid biology within Hymenoptera reaches its most elaborate development in the Chalcidoidea. They have diverse host relationships and dietary specialisation and their host range is thought to exceed all groups of insects except the order Diptera (Grissel & Schauff 1997). Most species are parasitoids (see section 1.2 below). Some species are phytophagous, either as seed-eaters or gall formers or develop in plant stems, especially grasses. Phytophagous chalcidoids are known from six families. Some species are both phytophagous and entomophagous (e.g. some *Eurytoma* species), but most species are primary, secondary and tertiary parasitoids (Hyperparasitoids) (Gauld & Bolton 1988). Biological control is dependent to a considerable extent for success on correct identification of both pests and their parasites or predators (Miller & Rossman

1995). Some hymenopteran species are considered as pests (such as some gall wasps), but most species are highly beneficial being regarded as enemies of insect pests and have been used extensively in the biological control of insect pests (De Bach 1964; Greathead, 1986; Godfray 1994). For example the parasitoid wasp *Apoanagyrus* (*Epidinocarsis*) *lopezi* De Santis (Hymenoptera: Encyrtidae) has been used in the biological control programme against the Cassava mealybug, *Phenacoccus manihoti* Mat.-Ferr. (Homoptera: Pseudococcidae) which is an important agricultural pest in Africa (Zeddies *et al.* 2001).

The superfamily Chalcidoidea is almost certainly a holophyletic group, or if including the Mymarommatidae, is likely to be monophyletic (Gibson, 1986). The current classification of chalcid wasps is based mainly on adult morphology. There are many problems among different taxa within chalcidoidea. Morphological examination alone is thought likely to be inadequate for revising the taxonomy of chalcid wasps (Claridge 1989; Dawah 1986) because of the existence of species complexes within this group. Additionally, intraspecific phenotypic variation in chalcid wasps potentially confounds species boundaries and cryptic diversity, common in chalcid wasps, implies that there are many more species than are currently identified.

Despite many efforts to resolve the taxonomy of chalcid wasps over a long period of time, their situation remains remarkably poorly understood (Zerova 1978; Bouček 1988; Graham 1970; Bouček and Rasplus 1991). Several techniques have been used to resolve the taxonomic situation of cryptic and closely related species such as morphometrics (see chapter 3 for references), mate choice (Khasimuddin & DeBach 1976; Dawah 1987, 1988a; Kennis & Mills 1998; Sundaralingman *et al.* 2001; Dawah *et al.* 2002), protein electrophoresis (Pungerl 1986; Dawah 1987; Loxdale 1994; Kennis & Mills 1998; Dawah *et al.* 2002, Al-Barrak *et al.* 2004), host preference studies (e.g.

Dawah 1988a; Sundaralingam *et al.* 2001; Dawah *et al.* 2002) and molecular genetics (see Chapter 4).

1.2 The family Eurytomidae (Chalcidoidea)

The family Eurytomidae is the largest group among the Hymenoptera, comprising approximately 1400 species in 87 genera (Noyes 1998). The most interesting genus of the family Eurytomidae is *Tetramesa* Walker, which contains some 130 species world-wide (Zerova 1976, 1978; Zerova *et al* 2003).

The family Eurytomidae display a wide range of habits. The majority of species are parasitic attacking a great variety of immature insects including Diptera, Coleoptera, Hymenoptera or Arachnida or eggs of Heteroptera and Orthoptera. Some species are phytophagous, feeding on the tissues of at least 10 plant families, as seed-feeders, borers, gall makers or as inquilines in other insects galls (Zerova 1978).

There is disagreement with regard to higher level classification especially at the generic and subgeneric levels. For example, separation of some of the genera including *Eurytoma* and *Tetramesa* is almost impossible (see Claridge 1961c; Bouček 1988). Several studies have been carried out using morphological and biological characters to separate different species in the family Eurytomidae (e.g. Claridge 1959 a, b, 1961 a, b; Abdul-Rassoul 1976; Zerova 1978, 1989; Dawah 1987, 1988 b).

1.3 Hymenoptera associated with grasses

The grasses (Gramineae) make up 20% of the vegetation in the world (Heywood 1978). They are ecologically and economically the most important components of agroecosystems because of their special significance in providing the cereal crops as well as in providing grazing for livestock (Duffey *et al* 1974; Crowder & Chneda 1982).

Many grasses and cereals are attacked by phytophagous Hymenoptera (e.g. *Tetramesa*). These herbivores are themselves attacked by 11 genera of Hymenopteran parasitoids in the U.K (Fig. 1.1) (Claridge & Dawah 1994). These parasitoids are highly specific to *Tetramesa* and other phytophagous hosts belonging to the family Eurytomidae, including species of the Eulophidae; *Pediobius*, the Pteromalidae; *Chlorocytus* and *Homoporus*, and the Eurytomidae; *Eurytoma* and *Sycophila*. There are also other species of the Eupelmidae; *Eupelmus*, and *Macroneura* and of the ichneumonidae; *Endromopoda* that attack *Tetramesa* larvae and also other parasitoid larvae. The biology, taxonomy and community structure of *Tetramesa* and of the major parasitoid genera in Europe and other parts of the world have been studied, but little has been done about Hymenoptera associated with grasses in Iran.

1.3.1 *Tetramesa* Walker

Tetramesa Walker (Eurytomidae: Chalcidoidea) is distributed mainly in the temperate zones of the northern hemisphere (about 120 species), with a few species in the southern hemisphere such as Africa (at least 8 species), New Guinea and Australia (2 or 3 species), and New Zealand (3 species, probably all introduced from Europe) (Bouček 1988). There are about 75 species in the Palaearctic region (Abdul-Rassoul 1976); 37 in the British Isles (Fitton *et al.* 1978); 63 in America north of Mexico (Peck 1963); 61 in the former Soviet Union (Zerova 1976) and 30 in Hungary (Erdos 1960)

The larvae of *Tetramesa,* so far as it is known, are exclusively phytophagous and associated with grasses (Poaceae). In many species they live in the central cavity of the internodes and feed on the inner tissues of the wall. Some species cause swelling at the places attacked resulting in the growth of characteristic galls on the stem, e.g. *T. calamagrostidis* (Von Schlechtendal 1891) or flowering part, e.g. *T. hyalipennis* (Walk),

other species live singly in or above the nodes of flowering stems e.g. *T. longicornis* (Walk) without formation of a distinct gall (Claridge & Dawah 1994). Some species decrease the productivity of the flowering head or reduce the weight of the seed (Claridge 1961c; Spears 1978; Dawah 1987) and are regarded as serious pests of cereal crops (Claridge 1961c; Bouček 1988). Other species show little external evidence of their presence and no obvious galling.

Numerous species of *Tetramesa* have been reared from Poaceae in different parts of the world. Many species have been regarded as major pests of cereals and range land in North America (Phillips 1920; Spears & Barr 1985) and the former Soviet Union (Zerova 1976). However, there is no evidence to show that they are serious agricultural pest in the U.K (Claridge 1961c). Members of this genus are extremely host specific (Phillips 1920; Claridge 1961c; Dawah 1987). *Tetramesa* larvae are attacked by some parasitoid species, including species of *Pediobius* Walker (Eulophidae), *Eurytoma* Illiger and *Sycophila* Walker (Eurytomidae), *Chlorocytus* Graham and *Homoporus* sp. (Pteromalidae) and *Enderomopoda* sp. (Ichneumoidea) (Claridge & Dawah 1994).

Tetramesa species are difficult to identify because of their morphological conservation and this problem is complicated by the fact that several species may live on a single host plant. At the species level several features have been used by various authors, such as sculpturing of the propodeum or petiole, shape of the abdomen, antenna, head, pronotum and lateral profile of the female abdomen as diagnostic characters, but most of these characters are subject to intraspecific variation and overlap between different species (Dawah 1987). Moreover, Claridge (1958, 1961b) emphasised the importance of biological characters particularly host specificity and mode of larval life in the confirmation of the taxonomic status of closely related species of *Tetramesa.* All

published identification keys have been concerned mainly with the identification of females since males have even fewer reliable external characters.

Several authors in various countries have studied the taxonomy and biology of *Tetramesa* species (e.g. Claridge 1959a, 1961a, b; Erdös 1960, Zerova 1976, 1978; Dawah 1987; Zerova *et al.* 2003a; Çam 2004). Zerova *et al.* (2003a) described new species of *Tetramesa* from Turkey (*Tetramesa anatolica* sp. nov.). Roskam (1982) stated that the taxonomic characters of the final instar larvae could be useful for identification and separation of *Tetramesa* at generic and species level. Dawah (1987) believed that because of slight structural diversity within the genus, other criteria must be used. He attempted to solve this difficulty by using additional evidence such as host preference, mate choice and protein electrophoresis. He confirmed the previous morphological separation of eight species of *Tetramesa* by using starch gel electrophoresis of ten enzyme systems.

1.3.2 *Eurytoma* Illiger, 1807

Eurytoma (Eurytomidae: Chalcidoidea) is cosmopolitan in distribution (Burks 1971). About 450 species have been described worldwide (Bugbee 1967). Fitton *et al.* (1978) lists forty described species of *Eurytoma* occurring in Britain. Attempts have been made to delimit the genus from other genera within the family, but the extent of the genus is still rather uncertain. Burks (1971) stated "Many species are placed in *Eurytoma* not because they are greatly like the type species, but because it has not as yet been possible to place them elsewhere."

Some species of *Eurytoma* are phytophagous, but the majority of species are either primary or secondary parasitoids on various orders such as Hymenoptera, some Coleoptera, Lepidoptera and Diptera (Burks 1971). Some species are of biological and

economic importance. Phytophagous species such as *E. suecica* Von Rosen are pests of wheat (Rosen 1956). Some species such as *E.amygdali* End are seed-eaters (Nikol'skaya 1952).

Because of the presence of many species within the genus, they have been clustered into 17 groups (Abdul-Rassoul 1976). The *Eurytoma appendigaster* species complex is one of these groups (Abdul-Rassoul 1976) which were first established under *E. appendigaster* (Swederus) by Claridge (1959b). He included eight species in the group, five of which were new (Dawah 1986). Several authors (Claridge 1959; Erdös 1960; Zerova 1976; Abdul-Rassoul 1976; Dawah 1988b) have studied the biology and taxonomy of the *appendigaster* group. Abdul-Rassoul (1976) carried out a comprehensive study of the *Eurytoma* species. All known species of the *E. appendigaster* group are parasites on *Tetramesa* in grasses (Dawah 1986). Some of them are parasitic only for a short period early in larval life. After consuming the host, the *Eurytoma* larvae then feeds on plant tissue in the same way as phytophagous species (Dawah 1986). Intraspecific variability of diagnostic morphological characters makes taxonomy of the genus extremely difficult. A taxonomic study of the *E. appendigaster* group was carried out by Claridge (1959b) who introduced the most important morphological characters to identify the species of this group such as the shape of face, antennae and mesepisternum. Taxonomic notes on some species of the *E. appendigaster* group were also presented by Graham (1970). According to Roskam (1982), taxonomic characters of the final instar larvae are useful for separation at the genus and species level. Claridge and Askew (1960) showed that egg structure maybe useful for separation of some species groups of *Eurytoma* such as the *Eurytoma rosae* Ferriéré group.

1.3.3 *Sycophila* Walker, 1871

The taxonomic status of the genus *Sycophila* has for a long time been a matter of much controversy (Bouček 1974; Burks 1971; Zerova 1989). These difficulties originate when taxonomists want to use the name *Sycophila* with regard to the synonymy. Little is known about the early stages and biology of this genus. Most species are ectoparasitoids of Diptera, Hymenoptera or Lepidoptera larvae living in plant galls (Claridge 1959b). Six species of *Sycophila* are known to occur in Britain (Fitton *et al* 1978). Claridge (1959b) first recorded *Sycophila mellea* attacking *Tetramesa* species in *Festuca rubra* (L.) and *Elymus repens* (L.) in Britain. He described the genus as being probably the most distinct genus of Eurytomidae within the Palaearctic region. This genus differs from *Eurytoma* in the presence of a distinct stigma or submarginal band (Claridge 1951b).

Sycophila mellea (Curtis, 1831) attacks different species of *Tetramesa* in grasses. A taxonomic study of the S. *mellea* was carried out by Anga (1991) who used morphometric analysis, enzeme electrophoresis and Scanning Electron Microscopy (S.E.M.) techniques and concluded that *Sycophila mellea* is a species complex.

Two new species of *Sycophila*, S. *emarginata* sp. n. and *S. gilva* sp. n. were described by Abdul-Rassoul (1980) during his studies on Iraqi chalcid wasps. The first species was reared from Cynipid galls (genus *Isocolus* Foerster) in the flower bracts of the safflower *Carthamus oxyacanthus* M. Bieberstein (Compositae). The second species was reared from Cynipid galls on leaves of a *Quercus* spp. in Iraq.

1.3.4 *Pediobius* Walker

Pediobius (Eulophidae: Chalcidoidea) is a genus comprising species which are usually small in size and often with brilliant metallic colouring. Some species of *Pediobius* are reported as primary or secondary parasitoids of the larvae or pupae of

Lepidoptera, Coleoptera, Diptera and Hymenoptera, and also attack the egg sacs of spiders (Araneae), Thysanoptera and egg cases of Mantidae (Mantodea). Several species of *Pediobius* are hyperparasites (Gibson *et al* 1997). It is worldwide in distribution, but it appears that Africa contains the largest number of species, while very few are known from the Neotropical region (Kerrich 1973). Fitton *et al* (1978) listed 20 species of *Pediobius* from Britain. Peck *et al* (1964), has described more than 20 species in C.S.R (Czechoslovakia). About 32 species are known from the Nearctic region (Gibson *et al* 1997). It is a large group and amongst the most striking of the Chalcidoidea, and contains many species of economic importance.

A group of closely related species of *Pediobius* known as the *P. eubius* complex attack phytophagous chalcid wasps especially *Tetramesa* species and their parasitoids (Dawah 1988a). The taxonomy of this complex is very complicated. Several attempts have been made for resolving taxonomic situation of this complex in Europe (Graham 1959, 1963; Bouček 1965; Dawah 1986). Dawah (1988a) separated the *P. eubius* complex into nine distinct species based on morphological studies, mate-choice experiments and allozyme electrophoresis experiments. He produced a key to nine species of *Pediobius* reared from different species of grasses. Based on these studies he described six new species. Recently Dawah *et al.* (2002) described a new species, *P. obscurus* attacking *T. linearis* in the grass *E. repens* in the U.K.

1.3.5 *Chlorocytus* Graham, 1956

The genus *Chlorocytus* forms part of the stenomalina group of genera within the Pteromalidae. There are 15 species of *Chlorocytus* listed for the British Isles (Fitton *et al* 1978), at least 20 species in the West-Palaearctic region (Bouček & Rasplus 1991), more than 12 species in the Nearctic region (Gibson *et al* 1997) and at least two species in

Australia (Bouček 1988). *Chlorocytus* species are entomophagous parasites of stem-feeding Hymenoptera, Diptera and Coleoptera. Most species of *Chlorocytus* overwinter as fully fed larvae within the host plant (Graham & Claridge 1965). *Chlorocytus* is a difficult genus taxonomically, because members show a remarkable lack of useful morphological characteristics for the separation of species. The taxonomy of the genus has been studied by several workers (e.g. Graham & Claridge 1965; Dawah 1989).

1.4 Geographical variation

One of the major problems facing taxonomists is the failure to distinguish the variation within and between species which leads in many cases to single species being described and given different names, and also of different species considered as a single species when relying exclusively on morphological characters. Geographic variation is a major feature of many taxa. Variation among individuals of a population or among the populations of a species and among different species is referred to respectively as intraspecific and interspecific variation. Mayr (1963) pointed out that "every population of a species differs from all others" and "the degree of differences between populations of a species ranges from almost complete identity to distinctness almost of species". The populations of some species have a large geographical distribution while the populations of other species have a restricted geographical range because some populations are able to adapt to a wider interval of environmental conditions and extend their distribution whilst other populations are not able to adapt to such diverse conditions, which results in a restricted distribution. Several factors may influence the geographic distribution of a species, ecological characteristics of a species and/or historical processes such as dispersal and vicariance (Ridley 2004). Many species are expected to have a history of range contractions and expansions which have occurred during the evolution of the

species especially during the ice age, which led to great changes in their distribution so that some species went extinct, some dispersed to new areas and some survived in refugia and later expanded again (Hewitt 2000). The study of geographical variation is of importance for determining the status of a given species. Understanding the nature of genetic variation within species and levels of genetic divergence between species and their phylogenetic relationships, studying population structure and the evolutionary forces which shape it within species are fundamental issues in evolutionary studies and also an important step toward developing effective conservation strategies (Zehr & Voytek 1999). The study of geographic variation has been very important in understanding processes of speciation, since species revealing high degree of genetic variation are often more likely to undergo speciation. Such studies have revealed that many species show structuring among populations parallel to that observed among closely related species (Avise *et al.* 1987; Avise, 1994, 2000).

Intraspecific divergence is promoted by partial or complete geographic isolation in many taxa and this may lead to reproductive isolation (Mayr 1963). However reproductive isolation can be considered as a by-product of divergence in allopatric populations (Ridley 2004). Adjacent populations of a species may differ from each other in a number of characteristics. Two different patterns of geographical distribution are identified in geographically differentiated forms of a particular species: Allopatric, when populations of a species are completely separated from one another and parapatric, when populations are in contact but with nonoverlaping geographic ranges and that are separated along a narrow border where interbreeding may occur (Futuyma 1998; Mayr & Ashlock 1991).

A 'subspecies' has been defined as "a geographically defined aggregate of local populations which differ taxonomically from other subdivisions of the species" (Mayr

1940, 1963, 1970; Mayr & Ashlock 1991). Subspecific variation is spatially circumscribed subdivision within a species, characterized by reduced gene flow with other populations of the species. Each subspecies can be identifiable on the basis of its phenotype and its geographic location (Mayr 1940; Mayr & Ashlock 1991). Different subspecies are often reproductively compatible, forming a narrow or wide hybrid zone, but interbreeding is expected to be reduced between subspecies compared with interbreeding within subspecies. However there is controversy when applying this concept to some taxa. Subspecies might have different geographic distributions (parapatric or allopatric) and these are often thought of as equivalent to 'geographical races', a term that has been used interchangeably by taxonomists who study mammals, birds, and insects (Mayr & Ashlock 1991). A race is a population or group of populations in a species which is located in a particular area and has gene frequencies that are different from other races in the species. Phenotypic characteristics may differ among races and the races of one species are capable of interbreeding with one another. As there are few specific criteria to designate a population as a subspecies, some authors have abandoned using this concept (Ridley 2004). Many characters display independent patterns of geographic variation and many loci are polymorphic (heterozygous) and vary in allele frequency from one population to another. There is no concordance among the characters that could effectively lead to distinction among subspecies or races based on one character, rather than a number of characters. Thus there is no obvious limit to the possible number of subspecies within one species. Polytypic species are often referred to as species that are divided into subspecies. Recently, the incorporation of genetic traits for identifyting subspecies has been proposed (Avise & Ball 1990). Mayr and Ashlock (1991) pointed out that members of a subspecies must share three features: first, to have a unique geographic range; second, to have phylogenetically concordant phenotypic

characters that can be described; and third, to have a unique natural history in comparison to other subspecies within that species.

When there is a gradual change in a character (or frequency of an allele) along a geographic range, the term 'cline' is given to such a series of contiguous populations in which a certain character changes gradually (Mayr & Ashlock 1991; Futuyma 1998). An example is clines in the frequency of the adh^F allele at the alcohol dehydrogenase locus of *Drosophila melanogaster* in three continents (Oakeshott *et al* 1982). However, clines in different characters are often discordant and they have different geographic patterns.

Geographic variation in morphological characters is not only due to the direct effects of environmental processes on phenotypes (phenotypic plasticity), the heritability of some morphological characters has been demonstrated in some species of birds (for instance see: Alatalo & Gustafsson 1988; Martin 1991) and Hemiptera (Margaritopoulos *et al.* 2000). However it is important to notice that the contribution of molecular phylogenetics is an important criterion in order to test the relative contribution of factors, such as selection and divergence in allopatry as causes of geographical variation in morphological characters (Puorto *et al.* 2001; Malhotra *et al.* 1996). Several factors can affect the genetic variation in populations such as mutation, gene flow, random genetic drift, natural selection, and environmental factors like climate, resources, disease and ecological processes. An example of geographic variation caused by genetic drift can be seen in the house mouse (*Mus Musculus* L.) on the island of Maderia (Britton-Davidian *et al.* 2000). This study showed rapid chromosomal evolution. Natural selection could not be the only factor to explain the geographic variation observed and the variation was better explained by genetic drift. However selection and drift can act simultaneously in populations and measuring the contribution of theses forces requires detailed study.

Gene flow is the movements of individuals among populations that have an impact in changing the spatial distribution of genes (Slatkin 1985). Genetic differences between populations can thus be decreased by migration. Reduction in gene flow among populations leads to increased geographic differentiation. Geographic variation may exist without restriction in gene flow, if selective differences among environments are high enough to prevail over the effects of gene flow (Endler 1977). Estimation of population parameters, such as gene flow can be helpful for understanding the dynamics and evolution of populations. The rate of gene flow is associated with natural selection and genetic drift and also determines whether local populations act as independent evolutionary units. Although gene flow traditionally has been considered as a homogenizing force, it can potentially give rise to divergence among subpopulations by producing unique combinations of alleles (Slatkin 1987). Several studies have proven that natural selection associated with shifts in ecology can give rise to rapid divergence and affects the rate of evolution of reproductive isolation, even in the presence of gene flow (Orr & Smith 1998).

Populations from different habitats that differ in some characteristics, make up several different morphs. These morphological types are given the name 'ecotypes' which are genetically distinct forms or locally adapted populations that are consistently found in certain habitats (Futuyma 1998, Rolán-Alvares *et al.* 2004). This word has been mostly applied by botanists to identify a phenotypic variant of a species that is located in a particular habitat.

1.5 Species concepts

The concept of 'species' has been a controversial subject in biology over a long period of time. Taxonomists have used morphological or phenetic characters to recognize

species. However, often even diagnostic morphological characters are not completely discriminatory and show some variation. Identifying species is a difficult task because as yet there is no consensus on the definition of a species (Cracraft 1997). In practice identifying the species using phenetic or morphological characters has been reasonably successful, when taxonomists argue about species concepts, they are often not arguing about how species are defined in practice (Blackman 1995).

It is clear to all that species show variation. This biological diversity has been the subject of two schools of thinking, 'population thinking' and 'typological thinking' (Mayr 1976). In order to refer to typological thinking it is appropriate to define the typological species concept. Mayden (1997) identified 24 species concepts, many of them are variations on a 'main' concept (see Mayden 1997). Before the modern neo-Darwinian synthesis, species had traditionally been defined by reference to a morphological 'type'. Based on the typological or morphological species concept a species is composed of all individuals that are sufficiently similar to the type specimen of the species (Mayr 2001).

Phenetic species concepts (also known as classical species concepts, morphospecies concepts, Linnean species concept) assert that a species is a set of organisms that are phenotypically similar and that are different in some way from other sets of organisms. Based on this concept phenotypic similarity is all that matters in recognising separate species (see Cronquist 1978).

In fact the typological species concept is the old version of the phenetic species concept. The phenetic species concept was later developed to the numerical species concept. Several statistical techniques were used by numerical taxonomists for describing the phenetic similarity of organisms. Based on this concept a species could then be identified as a set of organisms of sufficient phenetic distinctness (Ridley 2004). One advantage of the phenetic concept is that it includes both sexual and asexual organisms. It

also could apply to past (extinct) species. The Biological Species Concept (BSC) (Mayr 1942, 1963, 2001) is based on the criterion of interbreeding. Based on this concept a species is a population or a group of populations, whose members have the potential to interbreed with one another in nature, producing viable and fertile offspring, and are reproductively isolated of other such groups and thus can not produce viable, fertile offspring with members of other species. (Dobzhansky 1935; Mayr 1942). This concept applies only to organisms reproducing through sexual reproduction. How does reproductive isolation between species happen? This phenomenon usually occurs by isolating mechanisms (barriers) which are specific to each species and are defined by Mayr (2001) as "biological properties of individual organisms that prevent the interbreeding of populations of different species where they are sympatric". Isolating barriers ultimately give rise to genetic isolation between closely-related species. The BSC fits within population genetics, defines species in terms of the presence or absence of gene exchange and is completely concerned with the mechanisms by which sexually reproducing animals maintain their independent existence in sympatry. A community of interbreeding organisms can compose a gene pool. So in this concept the gene pool can be identifiable as species (Ridley 2004). Although the BSC is the most widely species concept, it has limitations, some of them irresolvable (Cracraft 1987, Donoghue 1985). It is only applicable for extant species and it does not include the past evolutionary history of the species and also only applicable to sexual organisms (Templeton 1989).

The problems associated with the biological species concept led to the creation of phylogenetic species concept (PSC). The phylogenetic species concept is based on Cracraft (1989) and a species is "an irreducible (basal) cluster of organisms, diagnosably distinct from other such clusters, and within which there is a parental pattern of ancestry and descent". PSC considers the present status of species and also their phylogenetic

history (see Nixon & Wheeler 1990; Baum 1992). In contrast to the BSC, the PSC can be applicable to both sexual and asexual organisms. The PSC is experimentally valuable in identifying recently diverged or otherwise cryptic species. It seems population genetics and systematics can complement one another using phylogenetic techniques in the study of populations and species (Goldstein & DeSalle 2003).

1.6 Molecular markers in phylogeographic studies of insects

Several molecular methods have been used by population geneticists and systematists to address and resolve questions and hypotheses at the population level in molecular systematics, population ecology and evolutionary biology (Parker *et al* 1998, Hoy 1994). So far, many investigations have been limited to electrophoretic analysis of proteins in different taxa of chalcid wasps associated with different host plant species (e.g. Eurytomidae: Dawah 1987, 1988b; Eulophidae: Dawah 1988a, Dawah *et al* 2002; Pteromalidae: Dawah 1989; Cynipidae: Stone & Sunnucks 1993, Stone *et al* 2001).

However recently DNA sequencing has become the most widely used method for creating comparative molecular data and for phylogenetic reconstruction (Hillis *et al* 1996, Hoy 1994, Caterino *et al* 2000, Miaymoto and Cracraft 1991). Insect molecular systematics has developed rapidly during recent years, especially the advent of the polymerase chain reaction (PCR) (Mullis *et al* 1986, Saiki *et al* 1985) and its development has had a major impact on systematics, making it possible to carry out direct sequencing from complex genomic DNA (Klepp *et al* 1971, Mullis *et al* 1986, Mullis and Faloona 1987, Hillis *et al.* 1996). This method is now recognized to be one of the most powerful techniques in population biology when using different genes of the mitochondrial genome (Parker *et al.* 1998). Most systematics questions can be explored using DNA sequencing including the intraspecific variability and phylogeny of

organisms, although this method is time consuming and expensive, particularly for investigations where many samples must be studied (Hoy 1994).

Some applications of DNA sequencing to molecular systematics are:

- Quantifying the scope of genetic variation and construction of molecular phylogenies in populations and species;
- Clarifying the sources of genetic variation and assessing evolutionary changes within populations and species;
- Explanation of the processes that are responsible in genetic variation; illuminating timing of speciation events which can be used for reconstruction of evolutionary changes in different species. (Hewitt 1998, Hoy 1994).

Animal mitochondrial DNA (mtDNA) has been the most commonly used genetic marker for the first generation of phylogeographic investigations. The animal mitochondrial genome is a small and closed circular molecule usually 14 kilobases (kb) to more than 30 kb in size (Moritz *et al.* 1987; Brown 1985) and in most taxa has 37 genes including 2 ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes, and 13 protein coding (messenger RNA) genes which code for subunits of enzymes functioning in electron transport or ATP synthesis. In addition to these genes, a control region or "A+ T-rich" region (in insects), about 0.8 kilobase long, appears to exercise control over mtDNA replication and RNA transcription. There are no introns, repetitive DNA, pseudogenes and intergenic sequences (Avise *et al* 1987; Avise 1994; Harrison 1989; Simon 1994).

MtDNA has several properties that make it particularly attractive as a genetic marker in population biology (Avise *et al* 1987; Moritz *et al* 1987, Harrison 1989). The mtDNA genome of animals is haploid, usually maternally inherited, and thus has an effective population size approximately one-quarter that of the nuclear genome. Consequently

demographic processes will be particularly important in determining frequencies of mtDNA haplotypes (Avise *et al.* 1987; Avise *et al* 1984) and also most lines of evidence confirm that it does not undergo recombination (Avise 1991).

The mtDNA genome has a relatively rapid rate of evolution, perhaps up to 10 times faster than nuclear DNA (Brown *et al* 1979, 1982; Vawter and Brown 1986).
MtDNA is a mosaic molecule with faster and slower evolving DNA regions. The faster evolving regions have been used in population genetic structure of many animals and especially insects. Gene flow mediated by male dispersal will not affect spatial patterns of variation for mtDNA (Avise 1991, 1994). The sequences located between these faster evolving regions, allow the design of 'conserved' primers referred to as Universal primers (Roehrdanz 1995; Simon *et al* 1994; Lunt *et al* 1998) and allow us to address phylogenetic questions at various taxonomic levels and study variation within and between closely related populations and species. Indeed a high resolution of intraspecific patterns of geographical variation in phylogeographic investigations can be assessed using mtDNA (Avise 2000).

The most popular genes in animal mtDNA which have been used extensively in phylogeographic studies of insects are cytochrome oxidase I (COI), cytochrome oxidase II (COII) and cytochrome *b* (cyt *b*). Other genes including NADH dehydrogenase subunit 2, isoleucine transferase RNA and the control region are less important. Because of the properties mentioned above, mtDNA has been used in more than 80% of phylogeographic studies (Avise 1998), relating the phylogenetic history of the taxa to past and present geographical patterns (Bermingham & Moritz 1998; Kelt & Brown 2000). However in phylogeographic studies it is important to disentangle current processes from historical events that influence population genetic structure of taxa. Nested clade analysis is one approach that has been used to partially achieve this

(Templeton 1998, 2001). Nested clade analysis was used to explore the current population structure and population history of the populations of an Iberian peninsula lizard, *Lacerta schreiberi* Bedriaga, in order to test several hypotheses about the history of colonization and persistence (Paulo *et al* 2002).

A problem associated with mtDNA which has been explored through many studies during the last decade, is the existence of mitochondrial-like sequences in the nuclear genome of many organisms (nuclear mitochondrial pseudogenes or Numts) and their contamination of mtDNA during PCR amplification (Zhang & Hewitt 1996). The presence of Numts in the Hymenoptera has been reported for gallwasps (Antonis Rokas, Graham Stone, see http://www.pseudogene.net). Many mitochondrial regions have been found to be integrated into the nuclear genome, including protein-coding, rRNA-coding, and noncoding regions- the control regions, rRNA genes, cytochrome b and genes for the subunit I and II of the cytochrome oxidase (COI-COII) (Zhang & Hewitt 1996). It seems that much larger proportion of Numts exists in plants than metazoans (Blanchard & Schmidt 1995). Failure in discovering presence of Numts can produce robust, believable, but incorrect genetic phylogenies (Bensasson *et al.* 2001). Among closely related species, it is possible to find differences in Numts abundance, for instance Numts are very abundant in *Sitobion* aphids, but occurred less often in four other aphid genera that were studied (Sunnucks & Hales 1996).

1.7 Parasitoids and life history strategies

Parasitoids are insects whose larvae feed exclusively on the bodies of their hosts, ultimately killing them (Godfray 1994). Parasitoidism has been observed in several insect orders including Hymenoptera. It is estimated that about 80% of parasitoid species belong to the Hymenoptera (Quicke 1997). The life history strategies of parasitoids have

been investigated over the years by many researchers (e.g. Askew & Shaw 1986). Researchers have attempted to divide the parasitoids by examining their life histories and have described two main types, koinobionts and idiobionts. In the koinobionts type the host is only partially paralysed by the wasp's venom. The host continues to grow and is only killed when the parasitoid reaches maturity. With idiobionts the host is completely paralysed by the wasp's venom and its development is stopped (Askew & Shaw 1986). However there are several distinctions between these two types based on biological characteristics and the most important ones are reviewed here (and see Quicke 1997): koinobionts are endoparasitoids and they develop inside the body cavity of their hosts (some ectoparasitoid koinobionts are known, but are unusual), while idiobionts are ectoparasitoids and they develop outside of the body of their hosts, sucking nutrients through the host's skin. Idiobionts have broader host ranges than koinobionts. They attack more host species and they often have highly developed means of locating and reaching concealed hosts, while koinobionts have very precise physiological adaptations to specific hosts. In koinobionts oviposition occurs on exposed hosts which means the host is still active and is able to survive, while for idiobionts oviposition occurs on concealed hosts and the host is totally paralysed and is unable to protect itself. In koinobionts the development time is long, with larval development postponed in the early stages to allow the host to develop. In idiobionts the development period is short, the host is totally paralysed and the parasitoid consumes it rapidly. Egg production in koinobionts is pro-ovigenic, that is, they produce many, small eggs that are fully developed at the moment the wasp hatches, while egg production in idiobionts is synovigenic, in other words they produce a few, large eggs that grow sequentially over the life of the adult wasp. Koinobionts have specific venom that only applies to a small number of host

species, while idiobionts have general venom that works on a variety of host species (Quicke 1997).

1.8 Gall and gall forming wasps

Plant galls may be defined as any consistent plant deformation that occurs in response to feeding or other stimuli by different organisms such as insects, mites, viruses, bacteria, fungi, and nematodes. The organisms feed within these structures and are identifiable from other insect-generated structures by having active differentiation and growth of plant tissues (Gagné 1989; Crespi *et al* 1997; Williams 1994). The major causes of galls are mites and insects. The gall making insects includes 6 orders: Coleoptera (beetles), Lepidoptera (moths and butterflies), Homoptera (aphids), Thysanoptera (thrips), Diptera (flies), and Hymenoptera (sawflies and wasps) (Figure 1.2). The three major groups of insects that cause galls are aphids and their relatives, gall midges and gall wasps (Williams 1994; Claridge & Dawah 1994).

The most common group of gall making insects is the gall wasps. Most of these belong to the family Cynipidae but a few sawflies and chalcid wasps also cause galls. The gall wasps mostly attack oaks, roses and related plants. The galls may be found on any part of the plant and in diverse forms or shapes. The gall serves both as a shelter and a food source. The gall insect is also protected from parasites and predators, especially general ones.

Among chalcid wasps, most species of the genus *Tetramesa* (Family: Eurytomidae) make distinct galls in grass stems. Some species of *Tetramesa* show little external evidence of their occurrence and no obvious galling (Claridge & Dawah 1994). Based on these differences *Tetramesa* species could be divided into galling and non-galling species. For example *Tetramesa calamagrostidis* (V. Schlecht) can cause stunting

of grasses. In some species the galls contain only a single chamber occupied by one larva, while in others the gall includes several chambers and a single *Tetramesa* larva lives in each chamber. Some species such as *T. linearis* (Walker) do not cause galls. The larvae live in separated hardened cells in the stem wall and not in the central stem cavity, so there are a number of larval cells together. As a result the stem is badly stunted reducing plant development (Philips 1920).

The study of galls (as model systems) and their occupant insects is a valuable approach in studies of population dynamics (species richness, gall diversity and the factors responsible in the evolution of gall structure) and other evolutionary issues like altruism (Stone & Schönrogge 2003). Gall induction in all insects at the molecular level remains unknown; the only theory is that of Cornell (1983) which states insect introduce foreign DNA or RNA into the plant's genome near the galling sites. Secretions produced by the insect make the plant tissue susceptible to viral attack. As a result of DNA/RNA transmission, changes in growth structure will take place that result in creating the gall. This theory has not yet been confirmed. Some gall-inducing stimuli that are located in different parts of insect's body are transferred by insect. For example in aphids they are located in saliva and transfer at the time of feeding; in *Pontania* Costa sawflies, they are located in maternal secretions and transfer during oviposition, in a species of cynipid gall wasp they are located in larval secretions (Stone & Schönrogge 2003). Other studies show that galls on the leaves of *Erythrina* which are produced by chalcid wasp, are not the result of chemicals secreted by chalcid wasp, but is the result of the plant's chemicals (Van staden & Davey 1994).

1.9 Iranian biogeography

The following brief summary of present knowledge of Iranian biogeography is based on the recent review by Zehzad *et al.* (2002).

The country of Iran covers an area of 1,623,779 km^2 and constitutes a large part of the Iranian plateau. It is bordered in the north by the Caucasus mountains, the Middle Asian natural regions and the Caspian Sea; in the east by the eastern part of the Iranian plateau (Afghanistan and adjacent west Pakistan) and the Baluch-Sindian region; in the west by the Anatolian and Mesopotamian regions; and in the south by the Persian Gulf and Oman Sea, which are connected to the Indian Ocean. The principal highlands are included of four distinct mountainous areas: Alburz in the north, the Zagros Mountains in the West, the Kopet-Dagh and north Khorassan ranges in the north-east and the Jebal Barez and Baluchestan Mountains in the central to southeast. In addition, the northwest Iranian mountain area and the central Iranian mountain area constitute more or less definite highlands in the inner part of the country.

Iran is predominantly an arid and semi-arid country, but the northern slope of the Alburz ranges and the Caspian lowlands receive 800 to 2000 mm annual rainfall and are the most humid part of the country. The Dasht-e Kavir and Dasht-e Lut deserts are the driest parts with less than 150 mm annual precipitation. From a bioclimatic point-of-view, SABETI divided the country into 14 bioclimatic types based on Emberger's method (Zehzad *et al* 2002).

From the zoogeographical point of view there is no absolute overall scientific assignment. The mountain ranges divide the country into three separate climatic and biotic regions: the Caspian Sea littoral; the central plateau and the Persian Gulf littoral with the Khuzistan Plain.

The Caspian Sea littoral comprises the northern slopes of the Alborz Mountains and the Caspian Plain, a narrow strip of land, forest covered, with a Mediterranean climate. The average temperature ranges between 10° and 35° C, and the average relative humidity between 70% and 100%. Water sources are abundant, keeping the region green throughout the year.

The central plateau, located between the Alborz and Zagros ranges of mountains, is very mountainous in the northwest where the ranges originate and is a somewhat lower desert in the east. The climate is dry, with average temperatures between 0° and 40° C, with hot, dry summers and cold, snow-bound winters.

The Persian Gulf littoral and Khuzistan plain, to the south of the foothills of the Zagros Mountains, has a tropical climate. The average temperature ranges between 12° and 50° C. The average relative humidities range between 40% and 80%, the highest values being along the coastal plain. The coastal plains become broader as the Zagros Mountains lose height towards Pakistan.

There are seven desert plains and depressions: the Dasht-e Kavir in Central Iran, the Dasht-e Lut Desert, the Sistan and Jazmurian depressions in the southeast, the Khuzestan Plain in the southwest, the Moghan Steppe in the northwest and the Turkman-Sahra steppe in the northeast. There are more than 20 lakes with the largest one, the saline Urumia Lake, covering 4,868 km^2 in the northwest. Unfortunately, there is no reliable picture of the zoogeographic relationships or of the degree of endemism of the Iranian fauna.

Based on plant geography, Iran is divided into three distinct phytogeographic regions: the Hyrcanian region in the north, the Nubo-Sindian region in the south, and the Irano-Turanian region in the rest of the country, which occupies more than two-thirds of the land-surface.

About 5% of the national territory is under protection for conservation purposes. The majority of the protected areas are located in the Irano-Turanian region (Zehzad *et al* 2002).

1.10 Morphological and molecular studies in Iran

Many morphological studies have been carried out on Iranian fauna but despite the continuous investigations that have been made by Iranian and international zoologists, Iran still is one of the least explored countries in the Middle East. The taxonomy, biology and ecology of different taxa of invertebrates and vertebrates have been investigated by Iranian and international zoologists.

Some morphological studies have been carried out on different taxa of Hymenoptera, some examples are: recording six new parasitoid wasps (Ebrahimi 1993), three new records of Symphyta (Ebrahimi 1995), and other studies (Lotfalizadeh & Ebrahimi 2001; Ebrahimi 2002, 2004; Chahartaghi, Azmayeshfard & Ebrahini 2002a, b). Although some molecular investigations have been carried out on the vertebrate fauna of Iran, there are little combined morphological and molecular investigations on insect fauna in Iran. Morphological and mitochondrial DNA variation of the sunn pest, *Eurygaster integriceps* Put. (Heteropetra: Scutelleridae) which is an important pest on wheat and barely in Iran, has recently been investigated using morphometrics and restriction fragment length polymorphism (RFLP) analyses (Sarafrazi 2001). Nucleotide sequence data from the gene regions COI, 16S, 28S, and ß-tubulin has been carried out to investigate genetic variation among the Palaearctic parasitoid, *Microctonus aethiopoides* Loan (Hymenoptera: Brachonidae) which is a parasitoid of weevils in the genera *Sitona* Germar and *Hypera* Germar (Coleoptera: Curculionidae) collected in Australia, Iran, New Zealand, the United States, and 10 European countries (Vink *et al* 2003).

The taxonomy, biology and ecology of Hymenoptera associated with grasses have been little studied in Iran and there is no record for some taxa belonging to different families of chalcid wasps. There has been no record of the presence of the genera *Tetramesa*, *Eurytoma* and *Sycophila* (Family Eurytomidae), *Pediobius* (family Eulophidae), and *Chlorocytus* (Family Pteromalidae) associated with grasses in Iran (Ebrahimi *pers. comm*).

1.11 Aims

The overall aim of this Ph D thesis is to investigate the geographic and genetic structure of the genus *Tetramesa* and its parasitoids associated with the grasses *Leucopoa pseudosclerophylla* and *L. sclerophylla*, which are assemblages of species complexes using morphometric and molecular analyses. The phylogeography of *Tetramesa* spp. and its parasitoids will be assessed across different regions of Iran and some U.K species also will be examined for comparative purposes. All chapters have their own reference lists followed by tables and figures.

The detailed aims of this research are:

1. To investigate the geographic variation in morphological characters among populations of *Tetramesa* using morphometric analysis (Chapter 3).
2. To study the genetic structure and phylogeography of *Tetramesa* and its parasitoids associated with grasses across Iran using partial DNA sequences of the mitochondrial cytochrome oxidase I (COI) and cytochrome oxidase II (COII) genes (Chapter 4).
3. To investigate the community structure of *Tetramesa* and its parasitoids associated with *Leucopoa pseudosclerophylla* and *L. sclerophylla* (Chapter 5).

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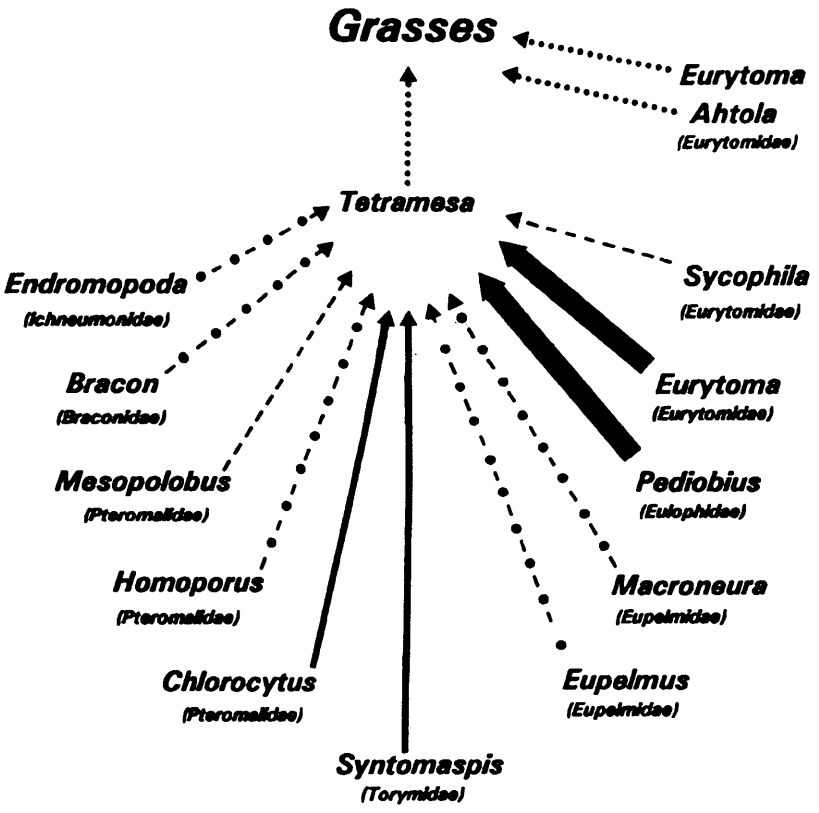
Table 1.1 Principal groups of gall-causing organisms. (After Williams 1994)

Organisms	Order	Family
Viruses		
Bacteria		
Mycoplasmas		
Actinomycetes		
Fungi (16 orders)		
Angiosperms		
Protozoa		
Rotifera		
Nematoda		
Arachnida	Acarina:	Eriophyidae
		Tarsonemidae
		Tetranychidae
Insecta:	Thysanoptera:	Thripidae
	Homoptera:	Adelgidae
		Aphalaridae
		Aphididae
		Cercopidae
		Coccidae
		Pemphigidae
		Phylloxeridae
		Psyllidae
		Triozidae
	Lepidoptera: (17 families)	
	Coleoptera:	Apionidae
		Cerambycidae
		Chrysomelidae
		Curculionidae
		Scolytidae
	Diptera:	Agromyziae
		Anthomyidae
		Cecidomyiidae
		Chloropidae
		Tephritidae
	Hymenoptera:	Blasticotomidae
		Cynipidae
		Tenthredinidae
		Eurytomidae

Figure 1.1 Generalized food web of the UK phytophagous Eurytomidae and their parasitoids associated with grasses in the U.K. Parasitism relationships are shown by different line conventions which indicate frequency of attack: thick solid lines indicate relationships observed on more than 60 separate occasions, thin solid lines between 30 and 50, dashed lines between 20 and 30, and dot-dashed lines between 5 and 10. (Source: Claridge and Dawah 1994).



CHAPTER 2

Materials and Methods

2.1 Materials and specimen preparation

2.1.1 Grass collection

As there was no information on the presence of chalcid wasps associated with the grasses (Gramineae) in Iran, a wide range of localities were initially considered in South-east, South-west and the north of Iran and different grass species were collected from these localities (Figure 2.1, see Appendix 3.1 in chapter 3). The sampling sites were chosen according to known records of these grass species in the sampling regions (Bor 1970; Ghahreman 1987, 1989). Two samples were carried out a year from each site, one early summer (June) and the other in September. The first sample was taken in order to study the host-parasitoid relationships and to discover which species attack which. The second sample was taken in order to obtain the parasitoids which already have eaten the host body.

2.1.2 Identification of the grass species

Different species of grasses were kept separately in large polythene bags. Each polythene bag was tagged with the name of the locality, the date of collection and the name of the species of grass. The bags were kept outdoors in open air to simulate natural conditions. Samples of grasses were mounted on sheets of paper (40x29 cm) and preserved for recording and accurate identification. The grasses were identified using Bor (1970) and Ghahreman (1987, 1989 in Persian). Names of Iranian grasses were taken from Flora Iranica (Bor 1970) and Flora of Iran (Ghahreman 1987, 1989 in Persian). Grass identifications were confirmed by Dr. F. Termeh (Plant Pests and Disease

Research Institute, Tehran, Iran); S.M.Mirtadjaddini (Department of Biology, Shahid-Bahonar University of Kerman, Iran) and B. Hamzeh (Research Institute of Forests and Rangelands, Tehran, Iran).

2.1.3 Insect rearing

The rearing method of *Tetramesa* and their parasitoids was as described by Varley (1947). This method has been employed by other researchers (Claridge 1961; Graham and Claridge 1965; Abdul-Rassoul 1976; Dawah 1986; Henneicke *et al.*. 1992) for rearing parasitic Hymenoptera from grasses. Each grass stem was dissected with a scalpel in the lab, during winter (from Mid October onward) when the larvae within were in final instar stage and were fully fed. The advantage of using final instar larvae is that they are in diapause and that the insect spends most of its life in this stage (Henneicke *et al.* 1992). During dissection care was taken not to damage or injure the larvae inside the stem. The larvae and pupae of the parasitoids were removed from the grass stems together with cuticle exuviae and any remains of *Tetramesa*. Larvae and pupae were labelled with a code number and placed individually in small gelatine capsules (10 mm X 7 mm) through to the adult stage (see Noyes 1982). The gelatine capsules were stored in a cool place outdoors, to facilitate pupation and emergence. Regular fortnightly checks were made to check for the growth of mould. During May to August each year, the gelatine capsules were checked daily for emerging adults. Adults were removed from the gelatine capsules after emergence and kept isolated in 75x25 mm glass vials which were covered with nylon net or muslin tops. Each vial contained a strip of filter paper and 25% honey-water solution was used for feeding emerged adults.

There was high mortality of *Tetramesa* and its parasitoids (range from 33.8%-100%) (see appendices 2.1 and 2.2). Therefore, the number of emerged specimens of *Tetramesa* and its parasitoids does not reflect the number of specimens reared. Some

adults were obtained using emergence boxes of different sizes. Some grass stems were placed in emergence boxes and were left outdoors in natural conditions. A small hole was made on the top of each box and a glass tube (75 x 25 mm) was placed inversely on the hole. The emerging insects were trapped into the glass vial, as they were attracted by the light coming through the hole. This technique was effective for obtaining some adult insects (Dawah 1986).

2.1.4 Identification of the insect larvae and adults

All the larvae of parasitic wasps obtained from the grass stems were studied using an Olympus binocular dissecting microscope. The larvae were identified to genus level based on morphological characters and descriptions (Henneicke *et al.* 1992; Dawah and Rothfritz 1996). The adult insects were also identified to genus level. All the specimens were compared with species of the same genus in the Professor M. F. Claridge and H.A. Dawah collections.

2.2 Molecular methods

2.2.1 DNA extraction

Initially it was necessary to examine several different methods of extracting DNA from chalcid wasp adults with regard to yield, amplification of DNA and quality of sequence. Two different extraction methods were tested and compared including high salt precipitation and an extraction kit [QIAamp DNA Mini Kit (Qiagen)]. Pilot studies were carried out to test the efficiency of these methods using other parasitic wasps, *Nasonia* sp. and *Spalangia camerouni* (family: Pteromalidae) due to the paucity of specimens. DNA was extracted from individual specimens (frozen, ethanol preserved and

dried). For each set of extractions a negative control was used to check for contamination.

2.2.1.1 High salt precipitation

This method is a modification by Collins *et al.* (1987) of a technique developed by Livak (1984) and hereafter called the Collins' method. Individual specimens were homogenised in a total volume of 100 µl of preheated high salt extraction (Livak) buffer containing 0.8M Nacl, 0.16 M Sucrose, 0.06M EDTA, 0.5% SDS, 0.1 M Tris Base (pH 8.6). Homogenisation was carried out in 1.5-ml microfuge tubes using a hand held pellet mixer. A separate pestle was used for each sample. The homogenates were incubated in a water bath at 65°C for 30 min followed by addition of 14 µl 8M potassium acetate to a final concentration of 1M. After incubating on ice for 30 min, samples were centrifuged at room temperature for 15 min at 13000 rpm. Supernatant was transferred to a clean Eppendorf tube to which 200µl ice-cold 100% ethanol was added and the samples were incubated at –20°C for 1.5-2 hours to precipitate the DNA. Centrifugation was carried out at 13000 rpm for 15 min to pellet the precipitated DNA. The supernatant was discarded and the DNA pellet was then washed with 70% ethanol by centrifugation at 13000 rpm for 5 min. The supernatant was discarded and the pellets were air-dried for 20 min at room temperature, or vacuum dried for 5 minutes. The pellet was re-suspended in 20-50µl TE buffer (10mM Tris-HCL, 1mM EDTA, pH 8.0) by heating at 65°C for 10 min in a water bath. Samples were stored at 4°C.

2.2.1.2 QIAamp DNA extraction kit

DNA extraction was carried out using the Tissue Protocol (QIAamp DNA Mini Kit (Qiagen). Individual specimens were homogenised in 180µl of ATL buffer.

Homogenisation was carried out in 1.5-ml plastic microcentrifuge tubes using a hand held pellet mixer followed by addition of 20 µl proteinase K. A separate pestle was used for each sample. The samples were then mixed by vortexing and incubated in a hybridisation oven containing a rocking platform at 56°C overnight. After incubation the samples were centrifuged briefly to remove drops from the inside of the lids. Two hundred µl AL buffer was added to the samples and mixed by pulse-vortexing for 15 seconds followed by incubation at 70°for 10 minutes. The solutions were briefly centrifuged to remove drops from inside the lids. Two hundred µl of 100% ethanol was added to each tube, and mixed by pulse-vortexing for 15 seconds. After mixing, the 1.5-ml microcentrifuge tubes were briefly centrifuged to remove drops from inside the lids.

The mixtures (including the precipitate), but not insect remains were carefully applied to the QIAamp spin columns (in a 2-ml collection tube) without wetting the rim. Centrifugation was carried out at 6000 g (8000 rpm) for 1 minute. The QIAamp spin Columns were placed in the clean 2-ml collection tubes and the tubes containing the filtrate were discarded. Five hundred µl AW1 buffer was added to the columns without wetting the rim and were centrifuged at 6000 g (8000 rpm) for 1 minute. The columns were placed in the clean 2-ml collection tubes and the collection tubes containing the filtrate were discarded. Five hundred µl AW2 buffer was then added to the columns without wetting the rim, which was centrifuged at full speed (13,000 rpm) for 3 minutes. The QIAamp spin columns were placed in clean 1.5-ml microcentrifuge tubes and the collection tubes containing the filtrate were discarded. The columns were spun for 1 min at 13000 rpm to remove excess buffer AW2 and placed in clean collection tubes. Two hundred µl AE buffer was added to the QIAamp spin columns and was incubated at room temperature for 5 minutes and then centrifuged at 6000 g (8000 rpm) for 1 minute. The last step was repeated again, so the DNA was extracted in two sets. All DNA extractions

were run on 1.3% agarose gel stained with Ethidium-bromide (0.5 μg/ml) at 90 V for 1 hr, viewed under a UV transilluminator and then amplified using mitochondrial primers.

2.2.2 Laboratory techniques for developing a mitochondrial marker

2.2.2.1 Selecting primers

General insect mitochondrial primers for COI, COII, and Cyt b were used to amplify wasp DNA. A list of primers, which were used in this study together with the original reference, is shown in Table 2.2.

The location of oligonucleotide primers used for amplification and sequencing of the mitochondrial COI and COII and Cyt b genes are shown in Figures 2.2 and 2.3.

2.2.2.1.1 Cytochrome Oxidase Subunit I (COI) primers

For amplifying a partial fragment of the COI gene (831 bp), "Jerry" (Forward) and "Pat" (Reverse) primers were used (Simon *et al.* 1994). "Jerry" has been used to amplify coleopterans, thysanopterans, homopterans, psocopterans, lepidopterans, and hemipterans (Simon *et al.* 1994). "Pat" works for gerrids, weevils, mosquitoes, flies, and lepidopterans (Simon *et al.* 1994).

For amplifying a partial fragment of the COI gene (473 bp), the primers "Ron" (Forward) and "Nancy" (Reverse) were used (Simon *et al.* 1994). These primers has been reported to work well with virtually all insects tested, including lepidopterans, dipterans, coleopterans, thysanopterans, hemipterans, homopterans and collembolans (Simon *et al.* 1994).

2.2.2.1.2 Cytochrome Oxidase Subunit II (COII) primers

Several pairs of primers were used for amplifying different parts of the COII gene as follows:

A region of COII mtDNA (825 bp long) was amplified using the primers "StLeu" (Forward) (Machado *et al.* 1996) and "AtAsp" (Reverse) (Willis *et al.* 1992, Machado *et al.* 1996). Machado *et al.* (1996) utilized these primers to amplify this gene in fig wasps. Willis *et al.* (1992) used "AtAsp" for amplifying the entire COII gene in honey bees (Genus *Apis*). For amplifying a specific region of the gene (748 bp) the primers "A-tLEU" (Forward) and "B-tLYS" (Reverse) (Liu and Beckenbach 1992) were used. These primers were utilized by Liu and Beckenbach (1992) for amplifying the COII gene of five orders of insects.

A fragment of approximately 830 bp was amplified using the primers "tRNA-LEU" (Forward) (Willis *et al.* 1992) and "At-Asp" (Reverse) (Machado *et al.* 1996). For amplifying a fragment, 877 bp, the primers "A-tLEU" (Forward) (Liu and Beckenbach 1992) and "B-ATPase 8" (Reverse) (Simon *et al.* 1994) were used. These primers amplify the COII gene successfully for the ants (genus *Lasius* niger). (Simon *et al.* 1994). A region of COII mtDNA, 385 bp long, was amplified using the primers "A-298" (Forward) and "B-tLYS" (Reverse) (Liu and Beckenbach 1992). The first primer is well conserved in all insects studied (Simon *et al.* 1994). For amplifying a fragment (523 bp) the primers "Pierre" (Forward) and "Barbara" (Reverse) (Simon *et al.* 1994) were utilized. The first primer is well conserved in wasps (Simon *et al.* 1994). For amplifying a region (514 bp) the primers "A-298" (Forward) (Liu and Beckenbach 1992) and "B-ATPase8" (Reverse) (Simon *et al.* 1994) were used.

2.2.2.1.3 Cytochrome Oxidase Subunit I (COI) and subunit II (COII) primers

For amplification of a target region of mtDNA (COI-COII) (625 bp), the primers "S2792" (Forward) and "Muscid" (Reverse) were used (Taylor *et al.* 1997).

The 5' ends of these primers are located at bp 2773 and 3400 of the *Drosophila yakuba* Burla mtDNA map (Clary & Wolstenholme 1985), respectively. These primers

have been used in amplifying a partial fragment of the COI-COII region of *Muscidifurax* spp. (Hymenoptera: Pteromalidae) (Taylor *et al.* 1997).

2.2.2.1.4 Cytochrome *b* (Cyt *b*)

Several pairs of primers were used for amplifying different parts of the Cyt b gene. A region of Cyt *b* mtDNA (1071 bp) was amplified using the primers "CB1L" (Forward) (Simon *et al.* 1994) and "TRs" (Reverse). As Simon *et al.* (1994) pointed out, the first primer works in insects. The second primer was used for the ant *Tetraponera rufoniger* Lowe (Simon *et al.* 1994).

A 750 bp fragment long was amplified using the primers "CB1" (Forward) and "TRs" (Reverse). These primers were successfully used for the ant *T rufoniger* (Jermiin and Crozier 1994). For amplifying a fragment (755 bp) the primers "CB1L" (Forward) and "CB2" (Reverse) were used. A fragment of (434 bp) long was amplified using the primers "CB1" (Forward) and "TRs" (Reverse). These primers work in bees, ants and *Drosophila* (Simon *et al.* 1994).

2.2.2.2 PCR amplification

All the primers mentioned above (listed in Table 2.1) were tested on DNA extracted from *Tetramesa* sp., *Eurytoma* sp., *Sycophila* sp., *Pediobius* sp., and *Chlorocytus* sp. from Iran and also some UK chalcid wasps. PCR conditions and thermocycling programmes for all primers pairs were optimised.

2.2.2.2 1 Cytochrome Oxidase Subunit I (COI) primers

2.2.2.2 1.1 "Jerry" and "Pat"

For optimisation of these primers a combination of methods were used for each population, such as changing concentrations of reaction mixture components, using

touchdown programmes and using different annealing temperatures in the PCR programme. These reaction conditions and PCR programmes for populations of *Tetramesa* spp., *Eurytoma* spp., *Sycophila* spp., *Pediobius* spp., and *Chlorocytus* spp. from Iran and also some UK populations (*Eurytoma. flavimana* Boheman, *E. appendigaster* (Swederus), *E. pollux* Claridge, *E.* sp. nr. *apicalis* Walker, *Chlrocytus phalaridis* Graham, *Pediobius claridgei* Dawah, *P. deschampia* Dawah, *P. planiventris* (Thomson), *P.* sp. nr. *claridgei* Dawah) are given in Table 2.3.

2.2.2.2 1.2 "Ron" and "Nancy"

PCR reaction conditions and thermocycling programmes which were performed for populations of *Tetramesa, Eurytoma, Sycophila, Pediobius* and *Chlorocytus* from Iran and U.K are shown in Table 2.4.

PCR reaction conditions and thermocycling programmes were performed for other primers in cytochrome oxidase subunits I and II and cytochrome *b* that represented in Tables 2. 5. to 2. 15.

2.2.2.3 Agarose gel electrophoresis

PCR's were run on 1.3% agarose gel stained with EtBr (0.5 μg/ml) at 90 V for 1 hr and viewed under a UV transilluminator. All PCRs contained a negative control. A 100 bp ladder size standard (MBI Fermentas) was run on each gel with the PCR to give an indication of the size of the target band that was being amplified.

2.2.2.4 Sequencing

2.2.2.4 1 Purifying PCR samples

PCR samples for sequencing were cleaned to remove inhibitors using the GeneClean Turbo for PCR Kit (BIO 101). The process of cleaning up carried out in three stages:

1. Twenty µl PCR reaction was placed in a microcentrifuge tube. Five volumes (100 µl) of GENECLEAN Turbo salt was added and mixed well. The microcentrifuge tubes were labelled and then vortexed briefly. After vortexing, the tubes were microcentrifuged. The solutions were transferred to a GENECLEAN Turbo Cartridge and labelled. The liquids were spun at 13,000 rpm for 5 seconds.
2. 500µl of GENECLEAN Turbo wash solution was added to the filter. The solution was spun for 5 seconds. The catch tubes were emptied and the washing was repeated again. The catch tubes were then emptied and spun for 4 minutes to dry GENECLEAN Turbo Cartridge.
3. The GENECLEAN Turbo Cartridge were transferred to a 1.5 ml GENECLEAN Turbo Catch Tube. The caps were removed from the GENECLEAN Turbo Catch Tube and were set aside. Thirty µl GENECLEAN Turbo Elution Solution was added and left for 5 minutes in room temperature. The solution was spun for 1 minute to transfer eluted DNA to the Catch Tube. The GENECLEAN Turbo Cartridges were discarded and the tubes were capped.

The cleaned PCR products were sequenced using the ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit. The sequencing kit was diluted, to produce a master mix containing 2 parts sequencing kit: 1 part 5X buffer: 1 part sterile water (see Chippindale *et al.* 1998). The sequencing PCR was carried out with 2 µl of

DNA, 2 µl of the sequencing mix and 1 µl of either forward or reverse primers at 1.6 pmol/µl concentration. The sequencing PCR programme with a total reaction volume of 5 µl per sample was as follows: 96°C for 10 seconds, 50°C for 5 seconds and 60°C for 4 minutes for 25 cycles.

2.2.2.4 2 Purification of sequencing product

Different purification methods were used for ABI 377 and ABI 3100.

2.2.2.4.2.1 Cleaning up the sequencing PCR using ethanol/sodium acetate precipitation (for the ABI 377)

Thirteen µl of 3M sodium acetate and 12.5 µl of 95% ethanol was mixed and was added to each 0.5 ml eppendorf tube. Five µl of PCR sample was then added to the tube and vortexed for about 10 seconds. The tubes were left at room temperature for 15 minutes. The samples were centrifuged at 13,000 r.p.m for 25 minutes, and the supernatant discarded. Sixty two and half µl of 70% ethanol was added to the tube, vortexed briefly so that the pellets were rinsed with the ethanol. The samples were then centrifuged at 13,000 r.p.m for 5 minutes. The supernatant was discarded and the pellets were vacuum dried for around 15 minutes. The pellets were stored at –20°C.

2.2.2.4.2 2 Purification of sequencing product using isopropanol (for the ABI 3100)

Twenty seven ml 100% Isopropanol and 15.75 ml polished water were mixed. This mixture is sufficient for about 40 samples. 90 µl of the Isopropanol mix was placed to each 0.5 ml eppendorf tube. Five µl of PCR samples were transferred from 0.2 ml PCR strips to the Isopropanol mix and vortexed for 20-30 seconds. The samples were left to precipitate at room temperature for 10 minutes. The samples were centrifuged for 30

minutes at 13,000 r.p.m. The supernatant was removed carefully and 150 μl of 70% isopropanol was added to each sample. The samples were mixed by inverting the eppendorf tubes. The samples were then spun for 10-12 minutes at 13,000 r.p.m. The supernatant was removed and the pellets vacuum dried for 10-15 minutes. The pellets were stored at –20°C.

Analytical methods and their results are presented in chapter 4.

2.4 References

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Table 2.1 Location, coordinates (longitude and latitude) and elevation for sample sites of grass species, *Leucopoa pseudosclerophylla*, *Leucopoa sclerophylla* and *Festuca arundinacea* across Iran during 3 years from 1997 to 1999.

Province	Collection Sites and codes	latitude	Longitude	Elevation (m)
Kerman	1a Sirch	30 07 15 N	57 27 10 E	3750
(South-east)	F2a Bidouieh	30 11 40 N	57 31 30 E	3520
	2b Deh-Salah	30 20 00 N	57 17 00 E	2300
	2d Deh Lo-Lo, Kouhpayeh	30 03 15 N	57 15 12 E	3620
	4b Seyahbenouieh, Rabour	29 15 10 N	56 17 07 E	4250
Fars	7 Dasht-e-Morghab	29 15 08 N	52 17 07 E	2300
(South-west)	7a Sheshpir (Shiraz-Sepidan Rd.)	30 10 10 N	52 25 02 E	2150
	7b Sepidan-Yasuj Rd.	30 15 06 N	51 10 12 E	2780
Tehran	9b Gachsar (Karaj-Chalous Rd)	36 45 11 N	50 45 30 E	3940
(North)	9c Sirachal (Karaj-Chalous Rd.)	35 43 07 N	50 40 20 E	4100

Table 2.2. List of the PCR primers utilised for amplifying the COI, COII, Cytochrome *b* genes.

Primer	Sequence	Direction	Reference
COI			
S2792	5'-ATACCTCGACGTTATTCAGA-3'	Forward	Taylor *et al.* 1997
Jerry	5'-CAACATTTATTTTGATTTTTTGG-3'	Forward	Simon *et al.* 1994
Pat	5'-TCCAATGCACTAATCTGCCATATTA-3'	Reverse	Simon *et al.* 1994
Ron	5'-GGATCACCTGATATAGCATTCCC-3'	Forward	Simon *et al.* 1994
Nancy	5'-CCCGGTAAAATTAAAATATAAACTTTC-3'	Reverse	Simon *et al.* 1994
COII			
Muscid	5'-TCAATATCATTGATGACCAAT-3'	Reverse	Taylor *et al.* 1997
StLeu	5'-ATGGCAGATTAGTGCAAT-3'	Forward	Machado *et al.* 1996
A-tAsp	5'-GGCCGTCTGACAAACTAATGTTAT-3'	Reverse	Willis *et al.* 1992
tLeu	5'-CCGGGAATTCGAATGAGTGCATTGAACTTA-OH-3'	Forward	Willis *et al.* 1992
A-tLEU	5'-ATGGCAGATTAGTGCAATGG-3'	Forward	Liu & Beckenbach 1992
B-tLYS	5'-GTTTAAGAGACCAGTACTTG-3'	Reverse	Liu & Beckenbach 1992
B-ATPase8	5'-TCATCTTATAGGTACTATTTGAGG-3'	Reverse	Simon *et al.* 1994
A-298	5'-ATTGGACATCAATGATATTGA-3'	Forward	Liu & Beckenbach 1992
Pierre	5'-AGCGCCTCTCCTTTAATAGAACA-3'	Forward	Simon *et al.* 1994
Barbara	5'-CCACAAATTTCTGAACATTGACCA-3'	Reverse	Simon *et al.* 1994
Cyt b			
CB1	5'-TATGTACTACCATGAGGACAAATATC-3'	Forward	Simon *et al.* 1994
TRs	5'-TATTTCTTTATTATGTTTTCAAAAC-3'	Reverse	Simon *et al.* 1994
CB1L	5'-CCATCCAACATCTCAGCATGATGAAA-3'	Forward	Simon *et al.* 1994
CB2	5'-ATTACACCTCCTAATTTATTAGGAAT-3'	Reverse	Simon *et al.* 1994

Table 2.3. PCR reaction parameters for the primers "Jerry" and "Pat" (COI). (T: touchdown programme; *E: Eurytoma; C; Chlorocytus; P: Pediobius*;).

Population	Primer (μM)	$MgCl_2$ (mM)	dNTPs (mM)	Taq (U/μl)	DNA (μl)	BSA mg/ml	T_{ANN} °C (Cycles)
Tetramesa spp.	0.5	1.5-3	0.4-0.8	0.03;0.04	0.75-2	0.1	52 (35) 53(5) 51(5) 49(25) T 53(5) 51(5) 49(24) T 55(5) 53(5) 51(15) T 54(5) 52(5) 50(25) T 60(5) 58(5) 55(25) T 60(5) 57(5) 55(5) 53(20) T 60(2) 58(2) 56(2) 52(2) 50(32) T
Eurytoma spp. *E. flavimana* *E. appendigaster* *E. pollux* *E.* sp. nr. *apicalis*	0.5	1.5-6	0.4-0.8	0.03-0.05	1-3.5	0.1	52 (35); 48(35) 57(5) 54(5) 51(35) T 57(5) 54(10) 51(30) T 57(5) 54(5) 51(20) T 57(5) 54(5) 51(15) T 54(5) 52(10) 50(30) T 57(5) 54(5) 51(15) 49(20) T 57(3) 54(3) 51(44) T 53(5) 51(5) 49(35) T 60(5) 58(5) 55(25) T 60(2) 58(2) 56(2) 52(2) 50(32) T
Sycophila spp.	0.5	2-4	0.4-0.8	0.03;0.04	1-3	0.1	52 (35) 53(5) 51(5) 49(24) T 53(5) 51(5) 49(25) T 60(5) 58(5) 55(25) T 60(5) 58(5) 55(30) T 60(5) 57(5) 55(5) 53 (20) T 60(2) 58(2) 56(2) 52(2) 50(32) T
Chlorocytus spp. *C. phalaridis*	0.5	2-7	0.4-0.8	0.03-0.05	1-3.5	0.1	52(35, 40, 50), 48(35, 40), 50(35) 53(5) 51(5) 49(24) T 53(5) 51(5) 49(25) T 55(5) 53(5) 51(25) T 55(5) 53(5) 51(30) T 60(5) 58(5) 55(25) T 53(5) 51(5) 49(35) T 57(5) 54(5) 51(45) T 57(5) 54(10) 51(30) T 57(5) 54(10) 51(35) T 57(3) 54(3) 51(55) T 54(10) 52(35) T 57(5) 54(5) 51(15) 49(20) T 57(5) 54(5) 51(5) 49(35) T 57(5) 54(5) 51(5) 49(40) T 55(5) 53(5) 51(15) 49(15) T 60(2) 58(2) 56(2) 52(2) 50(32) T
Pediobius spp. *P. claridgei* *P. deschampia* *P. planiventris* *P.* sp. nr. *claridgei*	0.5	2-5	0.4-0.8	0.03;0.04	1-5	0.1	52 (35), 50(35) 55(5) 53(5) 51(25) T 53(5) 51(5) 49(24) T 55(5) 53(5) 51(30) T 60(2) 58(2) 56(2) 52(2) 50(27) T 60(2) 58(2) 56(2) 52(2) 50(32) T

Table 2.4 PCR reaction parameters for the primers "Ron" and "Nancy" (COI). (T: touchdown programme; S: set up programme; *P: Pediobius; E: Eurytoma; C; Chlorocytus*).

Population	Primer (µM)	$MgCl_2$ (mM)	dNTPs (mM)	Taq (U/µl)	DNA (µl)	T_{ANN} °C (Cycles)
Tetramesa spp.	0.5	2-4	0.2-0.8	0.04	1-2	56 (30), 54 (30)
Sycophila spp.						54 (5) 52 (5) 50 (24) T
Pediobius spp.						60 (2) 58(2) 56 (2) 52 (2) 50 (32) T
P. claridgei						45 (5) 55 (35) S
P. deschampia						
P. planiventris						
P. sp. nr. *claridgei*						
Eurytoma pollux	0.5	2-4	0.2-0.8	0.04	1-2	56 (30)
E. flavimana						54 (5) 52 (5) 50 (24) T
Chlorocytus spp.						60 (2) 58 (2) 56 (2) 52 (2) 50 (32) T
C. phalaridis						

Table 2.5 PCR reaction parameters for the primers "StLeu" and "AtAsp" (COII) (T: touchdown programme; *C; Chlorocytus*).

Population	Primer (µM)	$MgCl_2$ (mM)	dNTPs (mM)	Taq (U/µl)	DNA (µl)	T_{ANN} °C (Cycles)
Tetramesa spp.	1.0	1.5, 2	0.8	0.025	2	50 (35), 47 (35), 52 (35) 47 (1) 47 (30) 47 (1) T
Chlorocytus spp.	1.0	1.5	0.8	0.025	2	47 (1) 47 (30) 47 (1) T
C. phalaridis						
Pediobius spp.	1.0	2	0.8	0.025	2	47 (1) 47 (30) 47 (1) T 52 (35)

Table 2.6 PCR reaction parameters for the primers "A-298" and "B-ATPase 8" (COII) (T: touchdown programme, S: set up programme; *P: Pediobius*).

Population	Primer (µM)	$MgCl_2$ (mM)	dNTPs (mM)	Taq (U/µl)	DNA (µl)	T_{ANN} °C (Cycles)
Tetramesa spp.	0.5, 1.0	1.5-4	0.4-0.8	0.025, 0.03	1-3	50 (30), 50 (35)
Eurytoma spp.						45 (5) 55 (35) S
Sycophila spp						60 (2) 58 (2) 56 (2) 52 (2) 50 (32) T
Chlorocytus spp.						56 (2) 54 (2) 52 (2) 50 (2) 48 (32) T
Pediobius spp.	0.5	1.5-6	0.4-0.8	0.03, 0.04	2	50 (30), 50 (35), 52 (30)
P. claridgei						58 (10) 51 (25) T
P. planiventris						54 (3) 52 (3) 50 (3) 48 (3) T
P. sp. nr. *claridgei*						56 (4) 53 (4) 50 (32) T
						60 (2) 58 (2) 56 (2) 52 (2) 50 (32) T
						56 (2) 54 (2) 52 (2) 50 (2) 48 (32) T
						45 (5) 55 (35) S

Table 2.7 PCR reaction parameters for the primers "A-298" and "B-tLys" (COII) (*P: Pediobius*).

Population	Primer (μM)	$MgCl_2$ (mM)	dNTPs (mM)	Taq (U/μl)	DNA (μl)	T_{ANN} °C (Cycles)
Tetramesa spp.	1.0	1.5, 2.5	0.8	0.025	2	48.5 (35)
Eurytoma spp.						
Sycophila spp.						
Chlorocytus spp.						
Pediobius spp.						
P. claridgei						
P. planiventris						
P. sp. nr. claridgei						

Table 2.8 PCR reaction parameters for the primers "A-tLeu" and "B-$ATP_{ase}8$" (COII) (*C; Chlorocytus*).

Population	Primer (μM)	$MgCl_2$ (mM)	dNTPs (mM)	Taq (U/μl)	DNA (μl)	T_{ANN} °C (Cycles)
Chlorocytus spp.	1.0	2	0.8	0.025	2	50 (35), 54 (35)
C. phalaridis						

Table 2.9 PCR reaction parameters for the primers "tLeu" and "AtAsp" (COII) (*C; Chlorocytus*).

Population	Primer (μM)	$MgCl_2$ (mM)	dNTPs (mM)	Taq (U/μl)	DNA (μl)	T_{ANN} °C (Cycles)
Chlorocytus spp.	1.0	1.5	0.8	0.025	2	52 (30), 47 (30)
C. phalaridis						47 (1) 47 (30) 47 (1)
						56 (1) 56 (30) 56 (1)
						59 (1) 59 (30) 59 (1)
						60 (1) 60 (30) 60 (1)
						62 (1) 62 (30) 62 (1)
						65.7 (1) 65.7 (30)
						65.7 (1)

Table 2.10 PCR reaction parameters for the primers "B-tLYS" and "A-tLEU" (COII) (*C; Chlorocytus).*

Population	Primer (μM)	$MgCl_2$ (mM)	dNTPs (mM)	Taq (U/μl)	DNA (μl)	T_{ANN} °C (Cycles)
Tetramesa spp.	1.0	2	0.8	0.025	2	50 (35)
Chlorocytus spp.	1.0	2, 3	0.8	0.025	2	45 (35), 51 (35), 57
C. phalaridis						(35), 51 (30), 52 (30),
						54 (30)

Table 2.11 PCR reaction parameters for the primers "Pierre" and "Barbara" (COII) (*P: Pediobius*).

Population	Primer (μM)	$MgCl_2$ (mM)	dNTPs (mM)	Taq (U/μl)	DNA (μl)	T_{ANN} °C (Cycles)
Tetramesa spp. *Eurytoma* spp. *Sycophila* spp. *Chlorocytus* spp. *Pediobius* spp. (IR) *Pediobius* spp. (U.K) *P. planiventris* *P.* sp. nr. *claridgei*	1.0	4	0.8	0.04	2, 5	48.5 (35), 60 (35)

Table 2.12 PCR reaction parameters for the primers "S2792" and "Muscid" (COI-COII) (*E: Eurytoma; C; Chlorocytus; P: Pediobius*).

Population	Primer (μM)	$MgCl_2$ (mM)	dNTPs (mM)	Taq (U/μl)	DNA (μl)	BSA mg/ml	T_{ANN} °C (Cycles)
Tetramesa spp	0.8;1	1.5-2.5	0.2-0.8	0.04, 0.025	2	-	48 (35), 48 (30), 48 (55), 55 (35),
Eurytoma spp. *Eurytoma pollux* *E. flavimana* *E. appendigaster* *E. sp. nr. apicalis*	0.8	2.5-3	0.2-0.6	0.04, 0.025	2	-	55 (35)
Sycophila spp	0.8	2-3	0.2-0.6	0.04, 0.025	2	-	55 (30), 55 (35)
Chlorocytus spp. *C. phalaridis*	0.8	1.5-3	0.2-0.8	0.04, 0.025	1, 2	BSA:0.004 Bataine:0.05 M	48(35)
Pediobius spp. *P. claridgei* *P. deschampia* *P. planiventris* *P. sp. nr. claridgei*	0.8	2-2.5	0.4-0.8	0.04, 0.025	2	-	48 (35), 55 (35)

Table 2.13 PCR reaction parameters for the primers "CB1" and "TR_s" (Cytochrome b) (*P. Pediobius*).

Population	Primer (μM)	$MgCl_2$ (mM)	dNTPs (mM)	Taq (U/μl)	DNA (μl)	BSA Mg/ml	T_{ANN} °C (Cycles)
Tetramesa spp.	0.5, 1.0	3-6	0.6, 0.8	0.03, 0.05	2, 3	0.1	52 (35), 45 (35), 45 (5) 55(30) S 45 (5) 50 (5) 55(30) S 58 (2) 56 (2) 54 (2) 52 (2) 50(32) T
Eurytoma spp.							
Sycophila spp.							
Chlorocytus spp.							
Pediobius spp (IR)							
Pediobius spp (UK)							
P. planiventris							
P. sp. nr. *claridgei*							

Table 2.14 PCR reaction parameters for the primers "CB1L" and "CB2" (Cytochrome b) (*P. Pediobius*).

Population	Primer (μM)	$MgCl_2$ (mM)	dNTPs (mM)	Taq (U/μl)	DNA (μl)	BSA mg/ml	T_{ANN} °C (Cycles)
Tetramesa spp.	0.5, 1.0	3-6	0.8	0.03,0.05	2, 3	0.1	45 (10) 50(25) S 45 (5) 50 (5) 55(30) S 60 (2) 58 (2) 56 (2) 52 (2) 50 (32) T
Chlorocytus spp.							
Eurytoma spp.	0.5, 1.0	3-6	0.8	0.03,0.05	2, 3	0.1	45 (35), 45 (10) 50(25) S 45 (5) 50 (5) 55(30) S 60 (2) 58 (2) 56 (2) 52 (2) 50 (32) T
Sycophila spp.							
Pediobius spp.							
P. claridgei							
P. deschampia							
P. planiventris							
P. sp. nr. claridgei							

Table 2.15 PCR reaction parameters for the primers "CB1" and "CB2" (Cytochrome b) (*P. Pediobius*).

Population	Primer (μM)	$MgCl_2$ (mM)	dNTPs (mM)	Taq (U/μl)	DNA (μl)	BSA Mg/ml	T_{ANN} °C (Cycles)
Tetramesa spp.	0.5	2.5, 3	0.8	0.03, 0.04	2	0.1	45 (35), 53 (35), 52 (5) 50 (5) 48 (5) 45 (20) T
Eurytoma spp.							
Sycophila spp.							
Chlorocytus spp.							
Pediobius spp. (IR)							
Pediobius spp. (UK)							
P. planiventris							
P. sp. nr. *Claridgei*							
P. deschanpia							

Table 2.16 The chalcid wasps which reared from two grass species, *Leucopoa pseudosclerophylla* and *Leucopoa sclerophylla* in south-east, south-west and northern Iran during 1997 to 1999.

Grass species	Parasitic wasp (Family)	Taxon
Leucopoa pseudosclerophylla	Eurytomidae	*Tetramesa* spp.
		Eurytoma spp.
		Sycophila spp.
	Eulophidae	*Pediobius* spp.
	Pteromalidae	*Homoporus* spp.
		Chlorocytus spp.
	Eupelmidae	*Macroneura vesicularis*
	Torymidae	*Syntomaspis baudysi*
Leucopoa sclerophylla	Eurytomidae	*Tetramesa* spp.
		Eurytoma spp.
		Sycophila spp.
	Eulophidae	*Pediobius* spp.
	Pteromalidae	*Chlorocytus* spp.
	Eupelmidae	*Macroneura vesicularis*
	Torymidae	*Syntomaspis baudysi*

Figure 2.1: Map of the grass sampling sites in south-east, south-west and north in Iran. Abbreviations are given in Table 2.1.

Figure 2.2 The relative location of oligonucleotide primers used for amplification and sequencing of different regions of mitochondrial COI and COII genes (Adapted from Simon *et al* 1994).

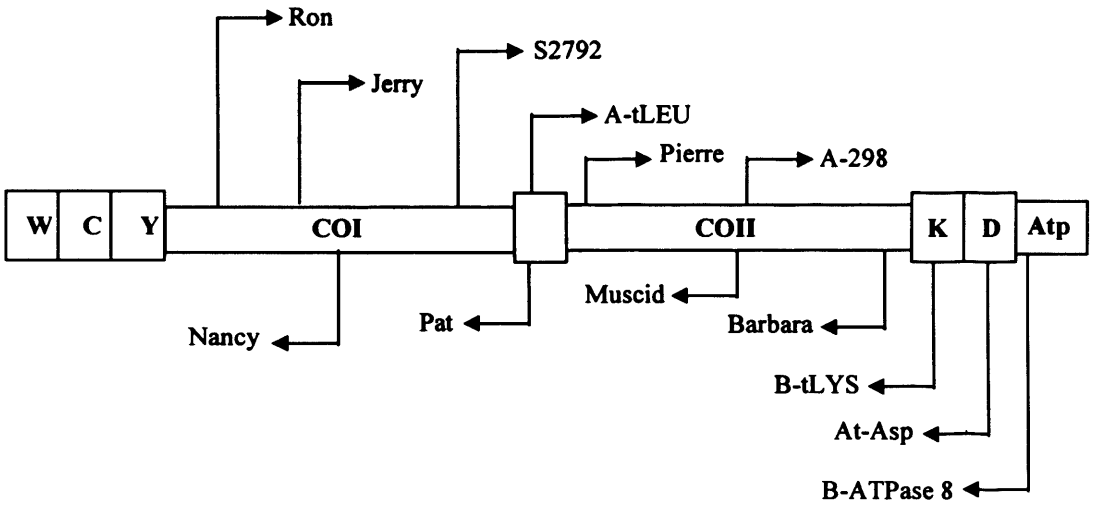


Figure 2.3. The relative location of oligonucleotide primers used for amplification and sequencing of the mitochondrial cytochrome b gene (Adapted from Simon *et al* 1994).

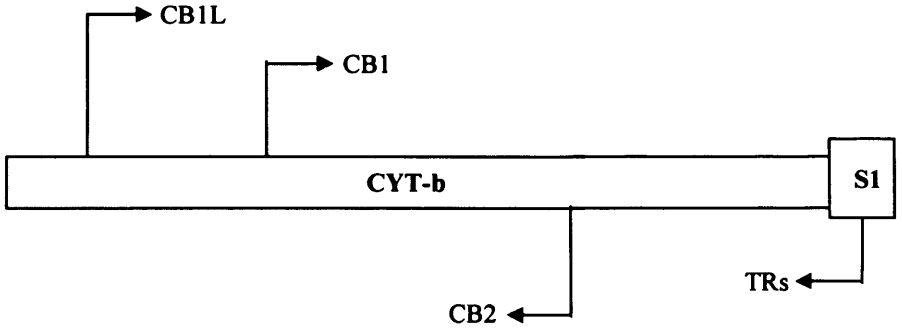
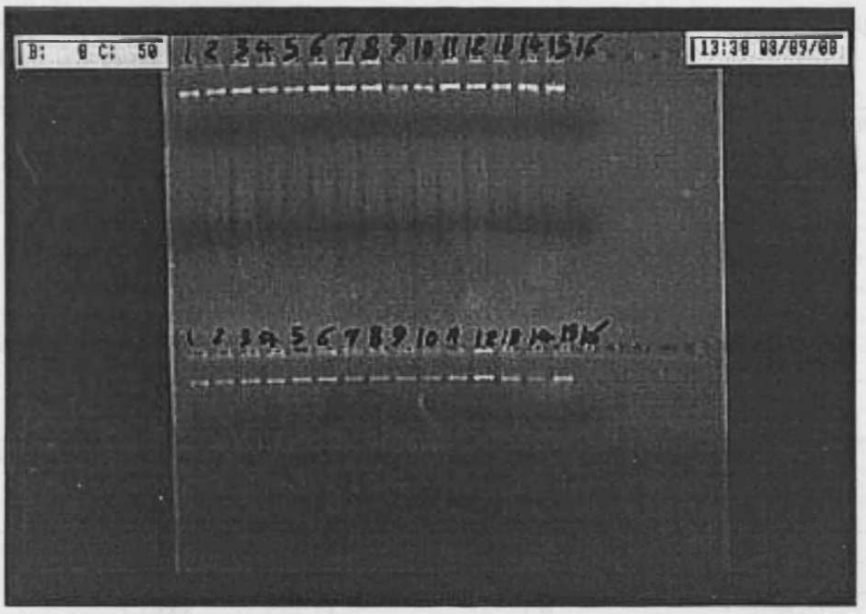


Figure 2.4 QIAamp DNA extraction checking gel of individuals of *Tetramesa* sp. from southeast, southwest and northern Iran.



Appendix 2.1

Number of the specimens of *Tetramesa* and its parasitoids reared from the grass *Leucopa pseudosclerophylla* together with the number of specimens that died and percentage of mortality for all the sampling sites (southern Iran).

Species	Year	No. of specimens reared	No. of specimens died	% of mortality
Tetramesa spp.	1997	653	315	46.0
	1998	662	250	37.7
	1999	445	180	40.4
Eurytoma spp.	1997	162	70	43.2
	1998	174	80	46.0
	1999	124	50	40.3
Sycophila spp.	1997	121	55	45.4
	1998	142	55	38.7
	1999	87	37	42.5
Homoporus spp.	1997	36	14	38.8
	1998	40	15	37.5
	1999	29	13	44.8
Syntomaspis baudysi	1997	13	6	46.2
	1998	24	11	45.8
	1999	11	6	54.5
Pediobius spp.	1997	66	29	44.0
	1998	62	24	38.7
	1999	57	24	42.1
Chlorocytus spp.	1997	212	100	47.2
	1998	238	99	41.6
	1999	166	65	39.2
Macroneura vesicularis	1997	24	9	37.5
	1998	59	20	33.8
	1999	97	45	46.4

Appendix 2.2

Number of the specimens of *Tetramesa* and its parasitoids reared from the grass *Leucopa sclerophylla* together with the number of specimens that died and percentage of mortality for all the sampling sites (northern Iran).

Species	Year	No. of specimens reared	No. of specimens died	% of mortality
Tetramesa spp.	1997	487	227	46.6
	1998	496	220	44.4
	1999	235	93	39.5
Eurytoma spp.	1997	23	10	43.5
	1998	19	8	42.1
	1999	14	5	35.7
Sycophila spp.	1997	61	25	41.0
	1998	61	20	32.8
	1999	63	22	35.0
Syntomaspis baudysi	1997	25	25	100
	1998	33	33	100
	1999	50	50	100
Pediobius spp.	1997	21	10	48.0
	1998	32	13	40.6
	1999	15	7	46.7
Chlorocytus spp.	1997	187	85	45.5
	1998	151	60	39.7
	1999	85	33	38.8
Macroneura vesicularis	1997	61	30	49.2
	1998	40	17	42.5
	1999	74	25	33.8

CHAPTER 3

Geographical variation in morphological characters among *Tetramesa* populations in Iran as derived from a morphometric analysis

3.1 Introduction

Parasitic Hymenoptera are among the most difficult taxa of insects to identify and classify because of their uniform morphology. Some described species are comprised of complexes of morphologically closely related species or sibling species (Claridge 1961; Claridge & Askew 1960; Abdul-Rassoul 1976; Dawah 1986, 1987, 1988a; Dawah *et al.* 2002; Albarrak 2001). Among natural populations of these insects, morphological similarity occurs so much that often no single character is diagnostic and sufficient to separate them. Even within a genus, species show extremely uniform morphology while genetic differentiation may have occurred (Dawah *et al.* 2002). Lack of distinguishable morphological characters among closely related species, the presence of sibling species and intraspecific variability make their separation based on morphological characters alone very difficult (Claridge *et al.* 1997). The taxonomic status of cryptic species is, however, of importance for evolutionary biologists, ecologists and applied biologists who seek the evolutionary and genetic relationships of the nominal species under study. Different explanations may be inferred, for example with regard to intraspecific differentiation in host use and other ecological patterns, when nominally conspecific populations are classified as separate host-specific sibling species (Adams & Funk 1997). The above-mentioned difficulties have made it necessary for taxonomists to look for other methods and complementary studies to delimit species (Gauld 1986, Powell & Walton 1989).

In this study, populations of *Tetramesa* were reared from two grass species, *L. pseudosclerophylla* and *L. sclerophylla* collected from several localities in Iran (see

Table 3.1). As populations of *Tetramesa* were obtained from two host grass species, the aim of this chapter was to test the hypothesis that geographical and host associated populations are differentiated from each other and can be detected as sibling species. The study was performed in order to determine whether there had been significant morphological differentiation between the populations (based on locality and region-host plant) especially northern populations, which were obtained from the grass, *L. sclerophylla* compared to the southern populations (S.E and S.W) which were obtained from the grass *L. pseudosclerophylla.* The results obtained will be compared with the results of the molecular phylogeny of populations

One of the approaches used to establish the relative taxonomic status of species and populations is morphometric analysis. Morphometrics continue to play a significant role in the rapidly expanding fields of evolutionary biology, quantitative genetics and evolutionary studies of development (Reyment *et al.* 1984; James & McCulloch 1990; Roth & Mercer 2000). The word morphometric comes from two Greek words, 'morpho' meaning form and 'metrien' meaning to measure. It involves the quantitative analysis of form using measurements of morphological structures. Morphological data can be summarised numerically and expressed graphically to show possible morphological relationships between populations. (Daly 1985). Multivariate morphometric analyses are carried out either to group organisms together (cluster) or separate them (discriminate) and provide statistical methods which allow the interrelationships between many variables to be studied (Gauch 1982, James & McCulloch 1990, Reyment *et al.* 1984, Sneath & Sokal 1973, Sokal & Rohlf 1981). Multivariate morphometric studies have been carried out on different insect taxa to analyse population variation because insect exoskeletons retain their form throughout adult life and can be easily measured (Sneath & Sokal 1973). Examples of morphometric approaches to the study of morphological

variation in different insect populations include morphological variation between populations of *Apis cerana* (Fabricius) in Thailand and the Malaysian peninsula (Sylvester *et al.* 1998), morphometric analyses on populations of *Eubazus* (Nees) (Hymenoptera: Braconidae), parasitoids of European *Pissodes* spp. weevils (Coleoptera: Curculionidae) (Kenis & Mills 1998) and morphometrics of selected allopatric populations of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) (Kimani-Njogu *et al.* 1997).

Species discrimination in host associated populations has been studied using morphometric techniques in four species of *Tetramesa* in the UK (Albarrak 2001) and in some genera of leafhoppers and planthoppers, e.g. *Alnetoidia* Dlabola (Gillham, 1989), *Oncopsis* Burmeister (Claridge & Nixon 1986), *Nephotettix* spp. (Haslam, 1984; Ramakrishnan 1983), *Mullerianella* complex species (Booji 1981) and *Alebra* spp. (Wilson 1979). Examples of a multivariate morphometric approach for studying morphological variation in different insect species include morphometric and molecular analysis of the *Encarsia inaron* species-group (Hymenoptera: Aphelinidae), parasitoids of whiteflies (Hemiptera: Aleyrodidae) (Manzari *et al.* 2002), identification of the *Diadegma* Foerster species (Hymenoptera: Ichneumonidae, Campopleginae) attacking the diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) (Azidah *et al.* 2000), discrimination of three species of phlebotomine sand flies (Diptera: Psychodidae) (Añez *et al.* 1997), revision of the Indo-Australian parasitic wasp genus *Macrobracon* Szépligeti (Hymenoptera: Braconidae) (Chishti & Quicke 1994), morphological variation of *Aphidius* Nees species (Hymenoptera: Aphididae) reared from seventeen aphid-hosts species (Pungerl 1986).

In light of these earlier morphometric studies, it was assumed that an analysis of the extent of morphological variation between populations of *Tetramesa* could therefore

be very useful using morphometric approaches with some expectation of discriminatory power. So far no morphometric studies had been carried out on populations of *Tetramesa* in Iran, so little is known about the morphological variation in this taxon. Thus, the aim of this part of study was to investigate morphological variation in *Tetramesa* populations using traditional multivariate analysis of distance measurements among morphological landmarks. It was also important to see whether morphometrics can be used as a useful method to population discrimination.

3.2 Materials and Methods

3.2.1 Specimens and sample size

Morphometric analyses were carried out on eight populations of *Tetramesa* reared from two grass species *L. pseudosclerophylla* (Southeastern and Southwestern Iran) and *L. sclerophylla* (Northern Iran) during three years from 1997 to 1999. In total, 80 females were measured in this study. Sample sizes for each population and their geographical region are shown in Table 3.1. For the map of collection sites see Figure 2.1 in chapter 2. The largest distance between sampling sites was 979 km (between sites 9b and 4b) and the smallest distance was 20 km (between sites 2d and 1a).

3.2.2 Character choice

Nineteen morphological characters were scored altogether, four from the head, three from the thorax, five from the antennae, one from the wing, two from the fore leg, two from the mid leg, and 2 from the hind leg. Some characters have been previously used in the taxonomy of Chalcidoidea which is standard practice in traditional morphometric study on this group of insects (Claridge 1961: Graham 1969, 1970; Bouček 1988; Bouček & Rasplus 1991; Zerova 1978, 1989) and others were chosen

specifically for taxonomic analysis of *Tetramesa* (H.A. Dawah *pers. comm.*). Other characters were selected on the basis of being convenient to measure and would give accurate and reliable results. This is standard practice in morphometric work on this group of insects. Description and illustrations of all measurements are given in Table 3.2 and Figure 3.1.

3.2.3 Sample preparation and measurement

The adult female insects used in this study were euthanased by putting them in the freezer at –20° for about 20 minutes. The antennae, legs and wings were removed under a binocular microscope using an entomological pin. Measurements were made from the right-hand side of the body for all insects. All body parts were cleaned using water and 70% ethanol. The head, thorax, antennae, wing, fore leg, mid leg and hind legs were mounted with water-soluble adhesive onto a slide. Five adult insects were mounted on each slide. When mounting samples, care was taken to arrange all the parts in a line and the same position.

Photographs of all specimens were taken at National Museum and Galleries of Wales (NMGW), Entomology Department using a video camera and binocular microscope with a ring light connected to a PC running the programme Auto-Montage v3.04 Syncroscopy. A standard lighting setting, iris opening and magnification were used throughout. Images were then analyzed with Aequitas IA v1.32 Dynamic Data Links calibrated with a micrometer (Figure 3.2). Before the measurements were made, the microscope magnification was calibrated and kept unchanged throughout the measurements. Before doing the measurements, land marks were selected. The morphological characters used had very clear measuring landmarks.

3.2.4 Consistency test

Consistency and accuracy of measurements is the most important point in morphometric analysis, so before doing any measurement, a test to establish the consistency and accuracy of the procedure was performed. For this reason, a few specimens were first observed in order to determine appropriate landmark points for use in measuring. Once the points were determined, the procedure of measurements was performed. A character was selected and the measurement was performed for ten insects on the first day and the same character was measured on the second day using the same samples in order to check the accuracy and consistency of measurements. In this case, the length of stigmal vein was measured. The data were analyzed using Analysis of Variances (ANOVA). Consistency was also achieved in that one person performed all of the measurements and all measurements were taken from the right-hand side of body. All measurements were recorded in millimeters. The analyses were all carried out on untransformed data. (Appendix 3.1).

3.2.5 Data analysis

Statistical analyses were conducted using the statistical package SPSS Base 10 (SPSS Ink 1999). Three common multivariate methods have primarily been used in morphometric analysis: principal components analysis (PCA), discriminant function analysis (DFA) and cluster analysis (CA).

3.2.5.1 Principal components analysis (PCA)

Principal components analysis is used as a data reduction technique to analyse relationships among variables and among individuals within a single sample, to discriminate size and shape components and because it attributes no *a priori* subdivision of the sample into discrete units, to identify groups in the sample (Sneath & Sokal 1973,

Reyment *et al.* 1984). PCA explains the maximum amount of total variation by decreasing the original variables to a limited set of independent variables or principal components, which are linear combinations of the original variables (Quicke 1993, Wiley 1981). PCA has often been used to analyse both intrapopulation (Kharusy 2000, Bilton 1993) and interpopulation morphological variation (Kimani-Njogu 1997, Haas & Tolley 1998). In PCA the first component accounts for the highest amount of the total variance in the data set. The following component accounts for the next highest amounts of the total variance and so on. Most of the variation can usually be summarised with only a few components, therefore a two-dimensional graph can be constructed from the data with many variables and the graph uses the components as axes. Here we used PCA to analyse interpopulation morphological variation in size among *Tetramesa* populations reared from *L. pseudosclerophylla* (in South-east and South-west Iran) and *L. sclerophylla* (in Northern Iran) based on locality and also region analysis.

3.2.5.2 Discriminant function analysis (DFA)

Discriminant function analysis (DFA) or Canonical discriminant analysis on distance measurements is a statistical method for comparing two or more groups (populations). The objective is to find a linear combination of characters that can maximise the morphological variation present among populations and discriminate between them (James & McCulloch 1990, Lubishew 1962, Gillham & Claridge 1994). Discriminant function analysis has proved to be a powerful tool for discriminating taxa in parasitic Hymenoptera (Krzanowski 1990, Chishti & Quicke 1994). Like principal components analysis, discriminant function analysis summarises variation in a data set and can be used to produce transformed axes, which yield maximum discrimination between groups (Reyment *et al.* 1984, Pungerl 1986). Discriminant function analysis is used to assign specimens in a sample to a group which they most resemble (Norušis

1994). This method requires *a priori* designation of groups and is used to allocate doubtful specimens to the group which they most closely resemble (Blackith & Reyment 1971) and is useful for studies of geographical variation (Claridge & Gillham 1992). In the discriminant function analysis, the eigenvalue represents a measure of a function's ability to discriminate between groups (populations) (Norušis 1994). Records show that components bigger than one are of special importance. Wilks' Lambda represents the discriminatory power of a variable, as the discriminatory power of a variable increases, the smaller Wilks' Lambda become. Its values range from 0.0 to 1.0. Small values indicate strong group differences, while values close to 1.0, show no differences.

In this study DFA was used to discriminate between *Tetramesa* populations (interpopulation morphological variation) reared from *L. pseudosclerophylla* (in south-east and south-west Iran) and *L. sclerophylla* (in Northern Iran) once based on locality and also based on region-host plant (Geographical and host-associated populations).

3.2.5.3 Cluster analysis

Cluster analysis is a technique used to classify populations or taxa based on their similarity or dissimilarity using matrices of coefficients of similarity or dissimilarity (Euclidean distance). Cluster analysis methods can be used as an "accuracy tool" to detect the similarity of closely related groups especially when other techniques are not helpful for classifying them. Relationships among the groups are usually expressed in a dendrogram. The hierarchical cluster analysis uses different distances to define the differences or similarities between groups. The squared Euclidean distance is used frequently as a distance measure for clustering groups (SPSS Base 10). The table of proximity matrix shows the matrix of proximities between groups (populations). In this section squared Euclidean distance was used which is a measure of dissimilarity. Larger values show populations which are very different and smaller values indicate populations

which are very similar. Here we used hierarchical cluster analysis to detect interrelationships among *Tetramesa* populations reared from *L. pseudosclerophylla* (in South-east and South-west Iran) and *L. sclerophylla* (in Northern Iran) once based on locality and also based on region-host plant.

3.3 Results

3.3 1 Grass collection and identification

Nine species of grasses were identified which are shown in Appendix 3.2. When the grass species *Leucopoa pseudosclerophylla* and *Lecopoa sclerophylla* were dissected initially, a reasonable number of chalcid wasps belonging to different taxa were collected from them, so this study was focused on these two grass species. These two grass species were collected regularly from south-east, south-west and north of Iran in 1997, 1998 and 1999 (see Table 2.1 in chapter 2).

3.3 2 Insect rearing and identification

The larvae of parasitic wasps were identified to genus level using the identification key and general characters as described by Dawah & Rothfritz (1996). The adults were identified at the genus level using the identification key by Peck (1964) and Bouček & Rasplus (1991). The result of identifications showed the presence of eight genera belonging to five families of chalcidoid wasps: Eurytomidae, Pteromalidae, Eulophidae Eupelmidae and Torymidae. Three Genera (*Tetramesa, Eurytoma* and *Sycophila*) belonging to family Eurytomidae; two genera (*Chlorocytus* and *Homoporus*) belonging to family Pteromalidae; one genus (*Pediobius*) belonging to family Eulophidae; one genus (*Macroneura*) belonging to family Eupelmidae and one genus (*Syntomaspis*) belonging to family Torymidae were identified. The Chalcid wasps (larvae

and adult) collected from *L. pseudosclerophylla* and *L. sclerophylla*, during three years (from 1997 to 1999) are shown in Table 2.16. Dr. H. A. Dawah confirmed all the identifications of all the Hymenoptera reared from grasses.

3.3.3 Consistency test

The result of consistency test using analysis of variances (ANOVA) showed that there were no significant differences between measurements ($P = 0.870$) (Table 3.3), indicating that all measurements were consistent and accurate.

3.3.4 Data analysis

The analysis was conducted in two ways: first based on locality (topic A) and second based on region (topic B). In A, eight populations were considered, (Table 3.4). In B, three populations were considered (Table 3.5). In B analysis three subpopulations from southeast Iran were considered as a single population (which were reared from the grass *L. pseudosclerophylla*), three subpopulations from southwest Iran were considered as population two (which were reared from *L. pseudosclerophylla*) and two subpopulations from northern Iran were considered as population three (which were reared from *L. sclerophylla*) and the statistical methods were performed separately.

A. Morphometric analysis in *Tetramesa* populations based on locality

3.3.5. Principal components analysis (PCA)

Principal components analysis was performed for all morphological characters. Five components were extracted for the analysis. A scatter diagram of female *Tetramesa* with respect to the first and second principal components obtained using all 19 characters is presented in Figure 3.3. Component matrix (factor loading) for first two principal

components is presented in Table 3.6 which is the same for both locality and region analyses. The component score coefficient matrix show the contribution of characters; i.e. the characters which have high scores, they have more contribution on different principal components. In other words, these scores help us to find the variables, which may be more important in the separation of populations. Length of antennal segment 1, distance between lower left pronotum and lower right mesoscutum, bread of thorax and tarsal segment 1 of hindleg had the highest influences on first principal component (see Table 3.6).The specimens of population eight (code 9c, northern Iran) which were reared from *L. sclerophylla* are weakly separated from other seven populations with some overlap, while the individuals of other populations show much overlap with each other (Fig. 3.3). The first two principal components together accounted for 73.3% of the total variance. Therefore we were unable to detect clear isolated clusters in *Tetramesa* populations reared from *L. psudosclerophylla* and *L. sclerophilla* from eight localities in South-eastern, South-western and Northern Iran, although population eight was partially separated from the other seven populations.

3.3.6 Discriminant function analysis (DFA)

A summary of canonical discriminant functions considering 19 variables for female *Tetramesa* across the eight localities across Iran is given in Table 3.6, including the levels of variance and the significance for seven functions. Low values of Wilks' Lambda test (the smaller the lambda, the greater the difference among groups) and statistically significance Chi-square values (Table 3.7) show that, in particular the first three functions are significant predictors. The first function accounted for 67.8% of the total variation a, the second function for 19.0%, and the third function for 6.8% which together accounted for 93.6% of the total variation in the data.

Table 3.8 shows the standardised canonical discriminant function coefficients that determine the relative importance of the characters in discriminating the populations. The characters HL2 (tarsal segment 2 of hind leg) and W1 (stigmal length) show the highest magnitudes (1.330 and –1.135 respectively) at function 1; whereas the characters H1 (distance between two top ocelli) and T3 (length of petiolar foramen) show the highest magnitudes (1.636 and –1.119 respectively) at function 2. These functions contribute most to the separation between populations. A plot of the first two canonical variates for the *Tetramesa* populations is shown in Fig. 3.4 showing the degree of inter-population difference. Population 8 (code 9c, northern Iran) is completely separated from other populations. Population 7 (code 9b, northern Iran) seems also to be somewhat separated from other populations, but the separation is not complete.

Table 3.9 shows the result of reclassification of individuals according to the original discriminant functions, derived with *a priori* specified group membership. The table indicates that in population 8 (code 9c, north Iran), 100% of individuals are reclassified correctly into their original group. The individuals of populations 2 (code 2d, S.E), 4 (code 7, S.W), 5 (code 7a, S.W), 6 (code 7b, S.W), and 7 (code 9b, North) resembled each other, since 80% of individuals of these populations are reclassified correctly into their original groups. In populations 1 (code 1a, S.E) and 3 (code 4b, S.E) 50% and 60% of individuals are reclassified correctly into their original groups respectively. In total, 76.3% of original grouped cases were correctly classified.

3.3.7 Cluster analysis

Table 3.10 shows a population proximity matrix between *Tetramesa* populations based on squared Euclidean distance. This is a dissimilarity matrix. Higher values indicate populations which are very different, while smaller values indicate populations which are very similar. The dendrogram of cluster analysis based on data of squared

Euclidean distances between populations of *Tetramesa* which were reared from two allopatric host plants, *L. pseudosclerophylla* and *L. sclerophylla* across eight locations in Northern, South-eastern and South-western Iran is shown in Fig. 3.5. Two main branches are shown, one that combines seven populations from South-eastern, South-western and Northern Iran and the other that represents one Northern population (9c). The population 9c from North that were reared from *L. sclerophylla*, showed a relatively large phenotypic distance from those collected from southeast, southwest and also northern Iran.

B. Morphometric analysis in *Tetramesa* populations based on region

Three populations were studied, southeast samples (subpopulations) as population 1, Southwest samples (subpopulations) as population 2 and Northern samples (subpopulations) as population 3. The multivariate statistical tests were performed and the results are given below.

3.3.8 Principal Components Analysis (PCA)

Five components were extracted for the analysis. A scatter diagram of female *Tetramesa* with respect to first and second principal components obtained using all 19 characters. The results were similar to the locality analysis so they are not presented again.

3.3.9 Discriminant function analysis (DFA)

Summary of canonical discriminant function is shown in Table 3.11 which considers 19 morphological characters for female individuals from the three geographic regions across Iran including the levels of variance and the significance for two functions. The table indicates that function 1 carries 94.9% of the variance and function 2

shows 5.1% variance. Both functions together therefore accounted for 100% of total variation in the data.

Table 3.12 represents the standardised canonical discriminant function coefficients. As the table shows the characters T3 (length of petiolar foramen), H1 (distance between two top ocelli), H3 (length of compound eye) and HL2 (tarsal segment 2 of hindleg) show the highest magnitudes (-1.399, 1.296, -1.268, and 1.255 respectively) at function 1 and the character T3 (length of petiolar foramen) show the highest magnitude (-1.297) at function 2 that contribute more to the separation between populations.

A graphical presentation of the first two canonical variates is shown in Figure 3.6, indicating the degree of inter-population differences. Here we can see that populations of *Tetramesa* can largely be classified into geographic groups based on region-host plant. Also as the graph shows, the northern population is separated from the southern populations.

Table 3.13 shows the results of reclassification of individuals according to the original discriminant functions, derived with *a priori* specified group membership. The correct self-classification rate is 83.3% for populations 1 and 2 (Southern populations) and 85% for population 3 (Northern populations). As a whole about 84% of original grouped cases were correctly classified.

3.3.10 Cluster analysis

Table 3.14 shows the population proximity matrix between *Tetramesa* populations based on squared Euclidean distance. The dendrogram of cluster analysis based on data of squared Euclidean distances between three populations of *Tetramesa* which were reared from two allopatric host plants, *L. pseudosclerophylla* and *L. sclerophylla* in three regions in Iran is shown in Figure 3.7. Two main branches are

recovered, one that combines populations one and two from the southeast and southwest respectively and the other that represent northern population (population three). The northern population that was reared from *L. sclerophylla* shows a very large phenotypic distance from Southern populations (south-eastern and south-western populations).

3.4 Discussion

There are no studies concerning multivariate analysis of morphological characters in *Tetramesa* populations associated with grasses in Iran. The aim of this study was to investigate morphological variation in eight *Tetramesa* populations collected from Southeast, Southwest and Northern Iran based on two analyses, locality and region-host plant. The result of this morphometric analysis reveals variation for size in some morphological characters among geographic and host associated populations of *Tetramesa* (northern and southern populations) based on region analysis.

Considering the canonical discriminant analysis based on locality, population 9c (Northern Iran) is completely separated from other populations at function 1, while the other northern population (9b) is almost completely separated from other populations (except one individual). This can again be seen on the dendrogram of cluster analysis, when the population 9c does not cluster with any other population, revealing a clear separation. Canonical discriminant function analysis produces the strongest separation between geographic (allopatric) populations based on region analysis. Since the separation of Northern and Southern populations can be confirmed by cluster analysis it is possible to discriminate clearly the two geographic clusters. For example, Georgini & Baldanza (2004) measured 20 morphological characters of two populations of *Encarsia Sophia* (Girault and Dodd) (Hymenoptera: Aphelinidae) from different geographical areas (Pakistan and Spain), which are parasitoids of greenhouse whitefly *Trialeurodes*

vaporariorum (Westwood), and the sweetpotato whitefly *Bemisia tabaci* (Gennadius) species-complexes (Hemiptera: Aleyrodidae). As with the present study, they were able to discriminate completely between Pakistani and Spanish individuals using the canonical discriminant function analysis.

Albarrak (2001) carried out morphometrics using relevant morphological characters of the head, thorax, wing and leg measurements to study differentiation between four *Tetramesa* species in the UK. Although he provided evidence to separate these taxa, the approach was not successful in discriminating between the host associated sympatric populations of *T. hyalipennis* (Walker) reared from two host grasses, *Elymus repens* (L.) and *E. farctus* (Viv.). The result of the present study demonstrates that according to locality analysis, the tarsal segment 2 of the hind leg, stigmal vein, distance between the two top ocelli, length of petiolar foramen and, for region analysis the length of the petiolar foramen and distance between two top ocelli, length of compound eye and tarsal segment 2 of hindleg were the most important characters that showed differentiation between *Tetramesa* populations in Iran. This agrees with the finding of Al-Barrak (2001), Ghajarieh (2003) and Claridge (1961) who showed that the leg, wing and head characters are among the most important in separating eurytomid wasps.

The first two canonical variates for *Tetramesa* populations accounted for 93.6% and 100% of total variation based on location and region-host plant respectively so it can be concluded following Reyment *et al.* (1984) the variation, since it is more than 80%, is biologically meaningful in this case.

Overall 24% and 16% of individuals based on locality and region-host plant respectively were misclassified into different groups. This result indicates some degree of intrapopulation variation; for example Kharusy (2000) and Bilton (1993) found similar results on their work on the grain borer, *Prostephanus truncatus* (Horn) (Coleoptera:

Bostrichidae) and the Diving beetle, *Hydroporus glabriusculus* Aubé (Coleoptera: Dytiscidae) respectively.

This study demonstrates that morphometric analysis of morphological characters can discriminate among geographic groups based on size variation and accurately classify individuals of Northern and Southern populations of *Tetramesa* in Iran (Fig. 3.7). These populations can be separated into two main morphological groups corresponding to northern and southern populations on the basis of selected morphological characters, their geographical distribution (regions) and host plants. Geographic variation is widespread in animal populations that have a large geographic distribution (Mayr 1963). As a result the populations located in different geographical areas which may subdivided by geographical barriers are locally adapted to the physical and biotic factors of their environment (Futuyma 1998). The morphological variation among northern and southern populations of *Tetramesa* may be due to environmental pressures that gave rise to selection for certain morphological characters. Margaritopoulos *et al.* (2000) found that morphological variation between clones of aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) reared from tobacco and other hosts were likely to be under genetic control, and the effect of temperature also on morphological variation in aphids has been demonstrated (Blackman & Spence 1994). As northern and southern populations of *Tetramesa* were reared from two host plant species, *L. sclerophylla* and *L. pseudosclerophylla* respectively, this implies that each geographic and host associated population is restricted to one grass species. However it remains possible that phenotypic plasticity between northern and southern populations of *Tetramesa* has been induced. Other evidence is needed to support the existence of morphological and genetic differentiation in Northern and Southern populations of *Tetramesa* in Iran. For example, host-shift experiments could be done to investigate the effect of host food plant.

Investigations at the molecular level such as phylogeographic analysis among the *Tetramesa* populations are also necessary in order to achieve an additional non phenotypic perspective of the variation among populations.

3.5 References

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Table 3.1. Collection sites of eight populations of *Tetramesa* from Iran. (*L. p: Leucopoa pseudosclerophylla; L. s: Leucopoa sclerophylla).*

Province	Collection Sites and codes	Longitude	Latitude	Sample size	Host plant
Kerman	1a Sirch	30 07 15 N	57 27 10 E	10	*L. p*
(South-east)	2d Deh Lo-Lo, Kouhpayeh	30 03 15 N	57 15 12 E	10	*L. p*
	4b Seyahbenouieh, Rabour	29 15 10 N	56 17 07 E	10	*L. p*
Fars	7 Dasht-e-Morghab	29 15 08 N	52 17 07 E	10	*L. p*
(South-west)	7a Sheshpir (Shiraz-Sepidan Rd.)	30 10 10 N	52 25 02 E	10	*L. p*
	7b Sepidan-Yasuj Rd.	30 15 06 N	51 10 12 E	10	*L. p*
Tehran	9b Gachsar (Karaj-Chalous Rd)	36 45 11 N	50 45 30 E	10	*L. s*
(North)	9c Sirachal (Karaj-Chalous Rd.)	35 43 07 N	50 40 20 E	10	*L. s*

Table 3.2 Morphological characters used for populations of *Tetramesa* from Iran.

Character	Description
H1	Distance between two top ocelli
H2	Distance between compound eye and ocellus
H3	Length of compound eye
H4	Distance between the lower corner of compound eye and ocellus
T1	Distance between lower left pronotum and lower right mesoscutum
T2	Breadth of thorax
T3	Length of petiolar foramen
W1	Stigmal vein (from start of postmarginal vein to uncus)
AN1	Length of scape
AN2	Length of antennal segment 1
AN3	Length of antennal segment 2
AN4	Length of antennal segment 3
AN5	Length of clava (club)
FL1	Tarsal segment 1 of foreleg
FL2	Tarsal segment 2 of foreleg
ML1	Tarsal segment 1 of midleg
ML2	Tarsal segment 2 of midleg
HL1	Tarsal segment 1 of hindleg
HL2	Tarsal segment 2 of hindleg

Table 3.3 Test of consistency which used to determine accuracy of the measurements.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.467	9	.163	.461	.870
Within Groups	3.533	10	.353		
Total	5.000	19			

Table 3.4 Population grouping used for locality analysis. Site and collection data for eight populations of *Tetramesa* from Iran are shown.

Collection site	Population Code	Province	Geographic location	Sample size	Host plant
Sirch	1a	Kerman	S.E Iran	10	*L. pseudosclerophylla*
Deh Lo Lo, Koohpayeh	2d	Kerman	S.E Iran	10	*L. pseudosclerophylla*
Seyahbenouieh, Rabor	4b	Kerman	S.E Iran	10	*L. pseudosclerophylla*
Ghader-Abad, Dasht-e-Morghab	7	Fars	S.W Iran	10	*L. pseudosclerophylla*
Sheshpir, Shiraz-Sepidan Road.	7a	Fars	S.W Iran	10	*L. pseudosclerophylla*
Sepidan-Yasuj Road.	7b	Fars	S.W Iran	10	*L. pseudosclerophylla*
Karaj-Chalus Road.	9b	Tehran	North Iran	10	*L. sclerophylla*
Sirachal, Karaj-Chalus Road.	9c	Tehran	North Iran	10	*L. sclerophylla*
			Total samples	80	

Table 3.5 Population grouping used for region-host plant analysis. Site and collection data for three geographic populations of *Tetramesa* from Iran are shown.

Population number	Subpopulations	Geographic region	Sample size	Host plant
1	1a,2d,4b	S.E Iran	30	*L. pseudosclerophylla*
2	7,7a,7b	S.W Iran	30	*L. pseudosclerophylla*
3	9b,9c	N. Iran	20	*L. sclerophylla*
		Total samples	80	

Table 3.6 Component matrix (factor loading) for two principal components extracted based on all morphological characters for female *Tetramesa* sp.

	Component	
	1	2
H1	.804	.258
H2	.628	-.001
H3	.802	.406
H4	.796	.349
T1	.861	.274
T2	.866	.258
T3	.721	.351
W1	.320	.553
AN1	.925	9.876E-02
AN2	.837	.145
AN3	.802	6.225E-02
AN4	.712	-.025
AN5	.760	.299
FL1	.762	-.451
FL2	.766	-.507
ML1	.847	-.428
ML2	.777	-.485
HL1	.865	-.365
HL2	.836	-.459

Table 3.7 Summary of canonical discriminant function for female *Tetramesa* based on locality analysis ($p < 0.001$).

Test of functions	Eigenvalue	% of Variance	Wilks' Lambda	Chi-square	df	Significance
1	7.127	67.8	0.013	304.670	63	0.000
2	1.997	19.0	0.108	156.955	48	0.000
3	0.713	6.8	0.323	79.572	35	0.000
4	0.357	3.4	0.554	41.619	24	0.014
5	0.246	2.3	0.752	20.072	15	0.169
6	0.048	0.5	0.937	4.594	8	0.800
7	0.018	0.2	0.982	1.261	3	0.738

Table 3.8 Standardised canonical discriminant function coefficients for the first three functions on the basis of locality analysis. Asterisks represent those values that contribute most strongly to the separation among populations.

Character	Function		
	1	2	3
H1	0.183	1.636*	-0.112
H2	0.344	0.325	0.605
H4	-0.768	-0.477	-1.342
T2	0.252	0.845	0.679
T3	-0.481	-1.119*	-1.204
W1	-1.135*	0.393	0.743
AN2	0.322	-0.929	0.763
HL1	0.072	-0.901	-0.527
HL2	1.330*	0.480	0.448

Table 3.9 Results of a reclassification of individuals of *Tetramesa* populations using the original discriminant functions. 76.3% of original grouped cases were correctly classified. Percentage of individuals correctly classified are in the diagonal.

Population	Predicted group membership (%)							
	1a	2d	4b	7	7a	7b	9b	9c
1a	**50**	10	0	10	20	10	0	0
2d	20	**80**	0	0	0	0	0	0
4b	10	20	**60**	0	0	10	0	0
7	10	10	0	**80**	0	0	0	0
7a	10	10	0	0	**80**	0	0	0
7b	10	0	0	0	10	**80**	0	0
9b	0	0	0	0	10	10	**80**	0
9c	0	0	0	0	0	0	0	**100**

Table 3.10 Population proximity matrix based on squared Euclidean distance between eight *Tetramesa* populations according to locality analysis (dissimilarity matrix).

Pop	1	2	3	4	5	6	7	8
1								
2	0.400							
3	2.407	1.777						
4	0.221	0.215	2.759					
5	0.786	1.589	4.892	0.966				
6	1.663	1.140	0.754	1.762	4.288			
7	0.730	0.662	1.735	0.728	1.859	0.909		
8	4.748	6.226	10.275	5.316	2.736	9.092	4.803	

Table 3.11 Summary of canonical discriminant function for female *Tetramesa* based on region-host plant analysis ($p < 0.001$).

Test of functions	Eigenvalue	% ofVariance	Wilks' Lambda	Chi-square	df	Significance
1	4.229	94.9	.156	138.508	12	.000
2	0.228	5.1	.815	15.274	5	.009

Table 3.12 Standardised canonical discriminant function coefficients for the first three functions on the basis of region-host plant analysis. Asterisks represent those values that contribute most strongly to the separation among populations.

	Function	
Character	1	2
H1	1.296*	.659
H3	-1.268*	.054
T2	.758	.944
T3	-1.399*	-1.297*
W1	-.521	.323
HL2	1.255*	-.624

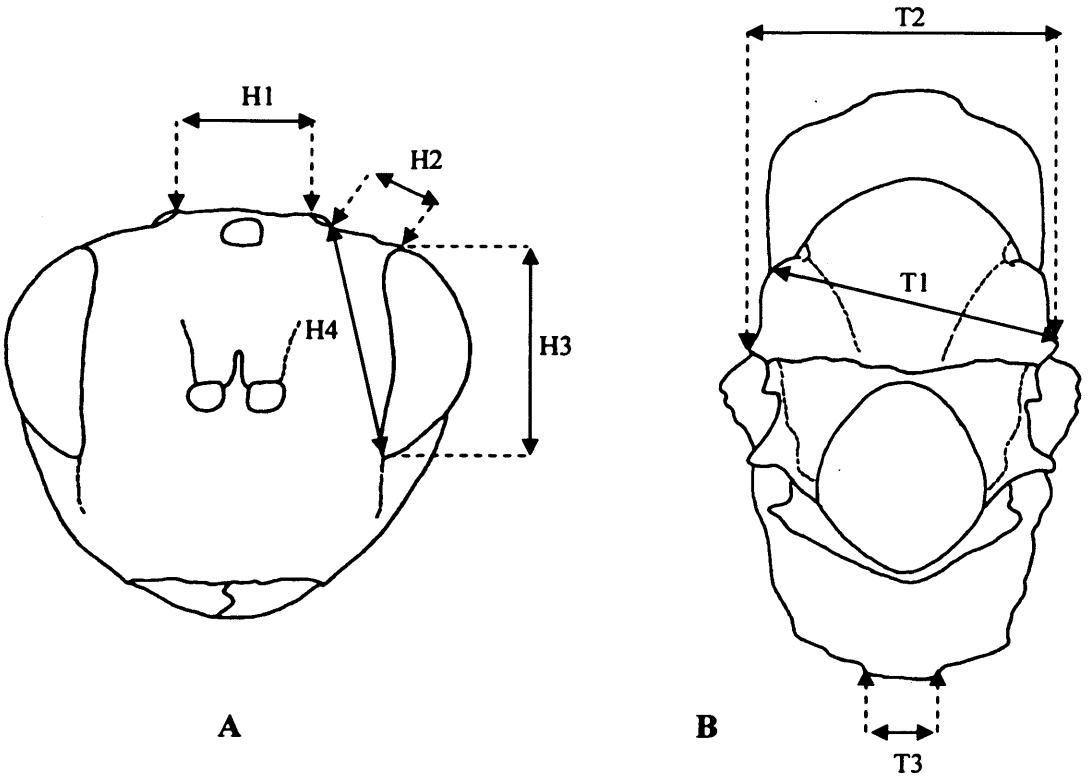
Table 3.13 Results of a reclassification of individuals of *Tetramesa* populations using the original discriminant functions. 83.8% of original grouped cases were correctly classified. Percentage of individuals correctly classified are shown in the diagonal.

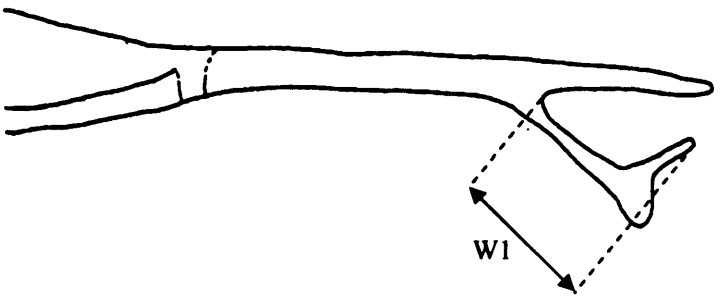
	Predicted Group Membership		
Population	1	2	3
1	**83.3**	16.7	.0
2	16.7	**83.3**	.0
3	.0	15.0	**85.0**

Table 3.14 Population proximity matrix based on squared Euclidean distance between three *Tetramesa* populations according to region-host plant analysis.

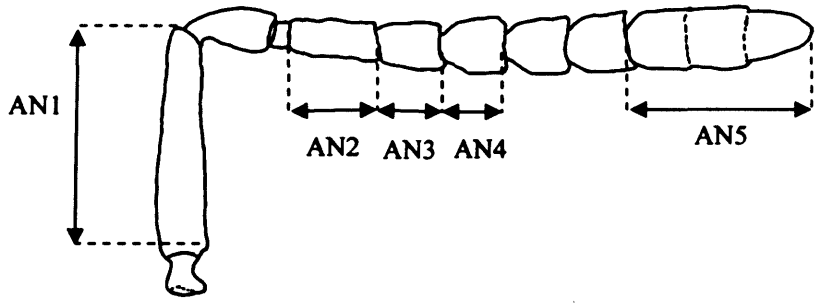
Pop	1	2	3
1			
2	2.421		
3	404.685	455.390	

Figure 3.1 Morphological characters used for the morphometric analysis of populations of *Tetramesa* (A. head, B. thorax, C. wing (stigmal vein), D. antenna, E. fore, mid and hind legs).



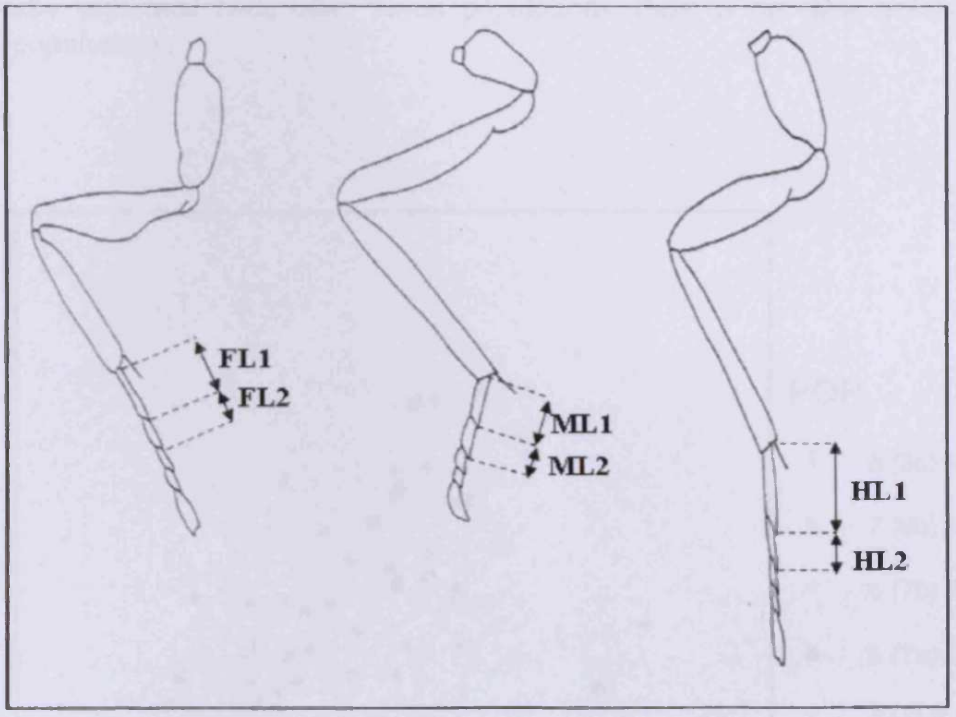


C



D

Figure 3.1 (continued) Morphological characters used for the morphometric analysis of populations of *Tetramesa* (A. head, B. thorax, C. wing, D. antenna, E. fore, mid and hind legs).



E

Figure 3.2 The video camera, binocular microscope, and computer which was used for morphometric analysis of morphological characters among *Tetramesa* populations (see text for the details).

Figure 3.3 Plot of the first two principal components for eight *Tetramesa* populations based on Locality using all nineteen morphological characters. The first two components accounted for 73% of the total variance. Although the population 8 from northern Iran (Circled) partially separated from other seven populations, there is no clear isolated cluster among populations.

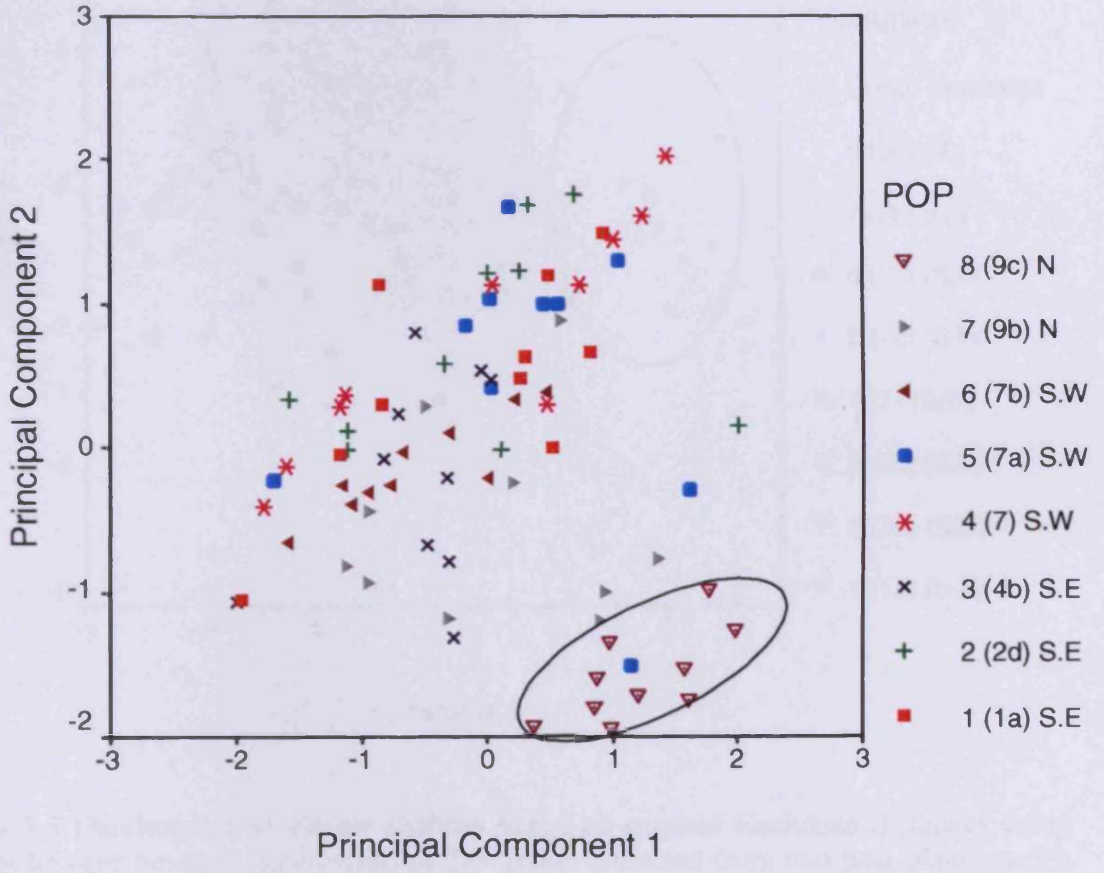


Figure 3.4 Group centroids of the first two discriminant funtions for females of eight *Tetramesa* populations which reared from two grass species, *Leucopa pseudosclerophylla* and *Leucopa sclerophylla* in Iran. Population 8 from northern Iran (circled) is completely separated from other populations, but the separation of other northern population (7) is not complete.

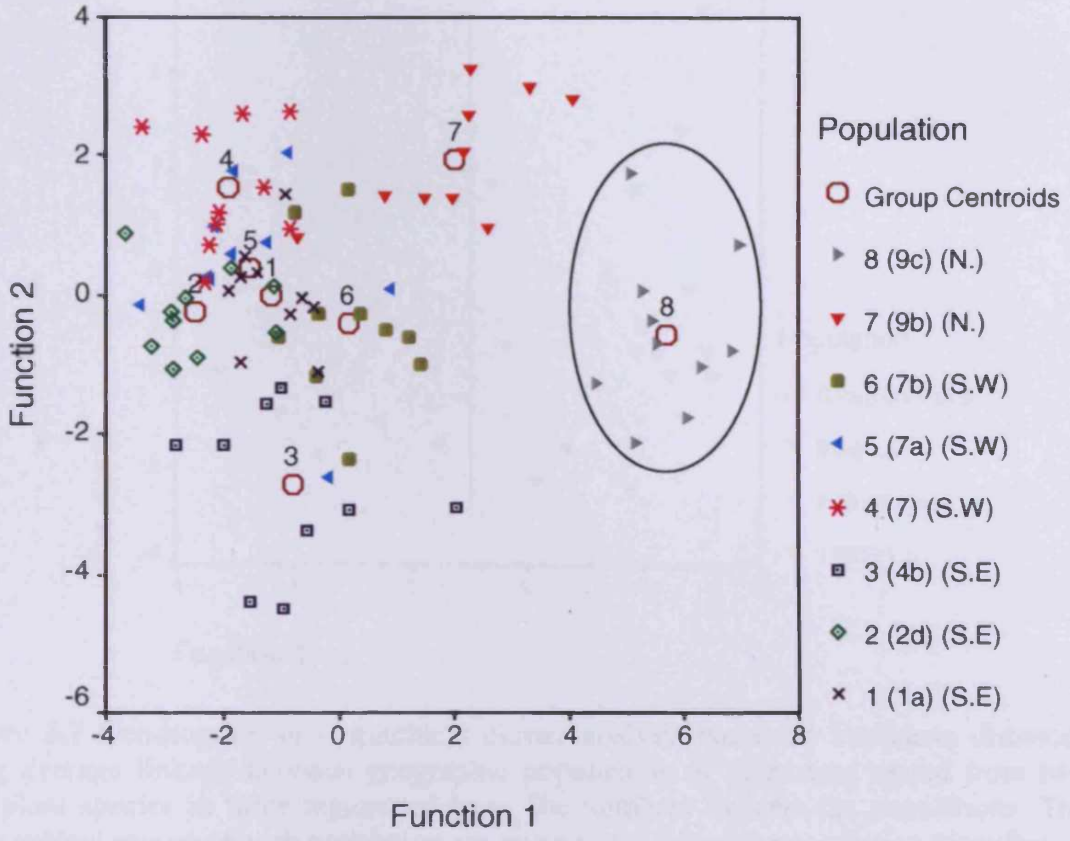


Figure 3.5 Dendrogram of cluster analysis based on squared Euclidean distances using average linkage between populations of *Tetramesa* collected from two host-plant species in eight localities of Iran. The geographical region of each population are given to the left of the population identifier.

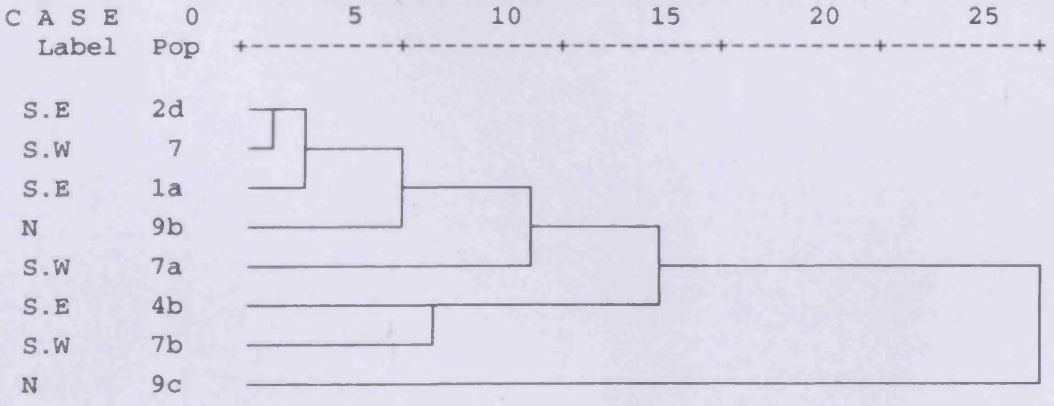


Figure 3.6 Group centroids of the first two discriminant functions for females of three *Tetramesa* populations which were reared from two grass species, *Leucopa pseudosclerophylla* and *Leucopa sclerophylla* in Iran. Northern population is separated from southern populations.

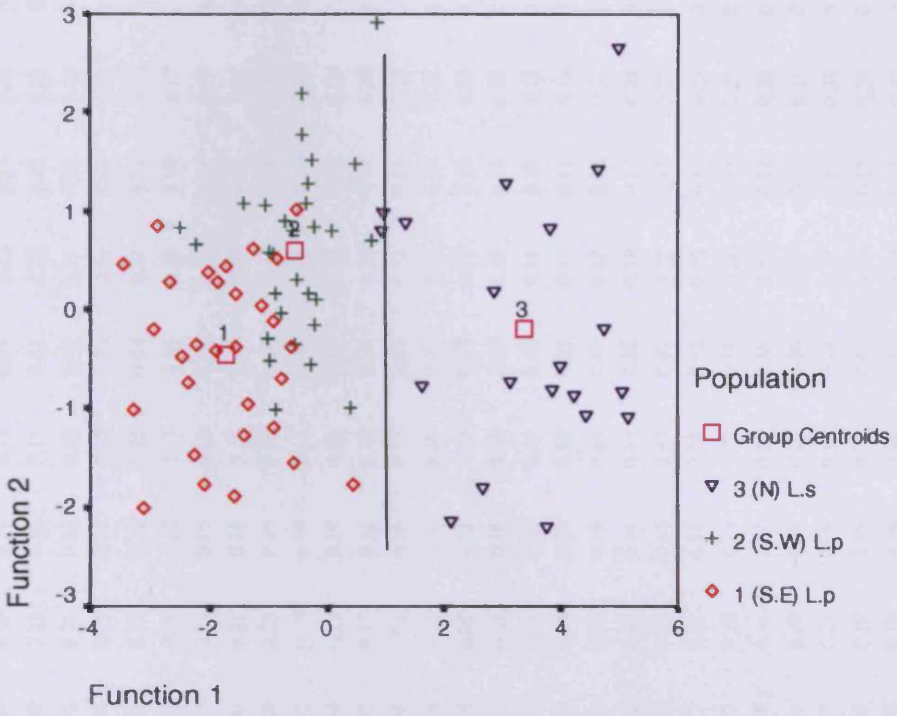
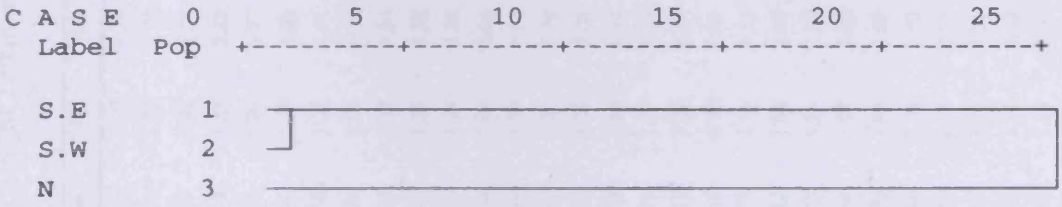


Figure 3.7 Dendrogram of hierarchical cluster analysis based on Euclidean distances using average linkage between geographic populations of *Tetramesa* reared from two host-plant species in three regions of Iran. The numbers indicate the populations. The geographical region of each population are given to the left of the population identifier.



Appendix 3.1 Morphometric data of eight *Tetramesa* populations from Iran.

Population	H1	H2	H3	H4	T1	T2	T3	W1	AN1	AN2	AN3	AN4	AN5	FL1	FL2	ML1	ML2	HL1	HL2
1.00	0.59	0.21	0.67	0.79	1.30	1.32	0.32	0.37	0.60	0.21	0.16	0.14	0.46	0.22	0.14	0.24	0.14	0.36	0.18
1.00	0.56	0.19	0.62	0.74	1.23	1.25	0.31	0.34	0.59	0.20	0.17	0.13	0.41	0.22	0.14	0.28	0.17	0.33	0.18
1.00	0.55	0.21	0.62	0.75	1.22	1.21	0.29	0.35	0.58	0.21	0.16	0.15	0.41	0.23	0.13	0.23	0.13	0.33	0.17
1.00	0.58	0.22	0.65	0.78	1.27	1.29	0.31	0.38	0.58	0.21	0.15	0.13	0.40	0.21	0.13	0.22	0.14	0.36	0.18
1.00	0.46	0.18	0.55	0.65	0.96	0.97	0.24	0.31	0.48	0.15	0.11	0.09	0.34	0.19	0.13	0.19	0.12	0.27	0.15
1.00	0.51	0.19	0.57	0.66	1.05	1.06	0.28	0.33	0.51	0.17	0.12	0.10	0.40	0.23	0.11	0.20	0.13	0.27	0.15
1.00	0.47	0.19	0.55	0.68	1.39	1.41	0.35	0.32	0.47	0.15	0.12	0.13	0.38	0.19	0.10	0.17	0.12	0.27	0.16
1.00	0.53	0.20	0.59	0.70	1.08	1.09	0.26	0.32	0.55	0.17	0.15	0.13	0.38	0.20	0.11	0.19	0.12	0.29	0.15
1.00	0.59	0.21	0.61	0.75	1.22	1.24	0.32	0.37	0.57	0.21	0.14	0.13	0.37	0.23	0.14	0.24	0.14	0.35	0.17
1.00	0.59	0.22	0.63	0.77	1.25	1.28	0.33	0.37	0.60	0.23	0.15	0.14	0.40	0.21	0.14	0.26	0.15	0.38	0.19
2.00	0.53	0.21	0.62	0.76	1.24	1.29	0.31	0.36	0.56	0.18	0.14	0.11	0.44	0.22	0.12	0.22	0.13	0.33	0.16
2.00	0.50	0.19	0.56	0.68	1.03	1.05	0.28	0.33	0.49	0.17	0.14	0.13	0.39	0.19	0.11	0.20	0.11	0.29	0.16
2.00	0.50	0.20	0.56	0.69	1.04	1.07	0.27	0.34	0.51	0.17	0.13	0.13	0.37	0.19	0.13	0.20	0.12	0.28	0.14
2.00	0.59	0.21	0.67	0.79	1.25	1.30	0.32	0.40	0.58	0.21	0.16	0.15	0.43	0.23	0.13	0.23	0.13	0.33	0.17
2.00	0.56	0.20	0.66	0.77	1.21	1.24	0.31	0.37	0.58	0.21	0.16	0.16	0.42	0.20	0.12	0.22	0.12	0.32	0.16
2.00	0.54	0.21	0.59	0.74	1.14	1.17	0.31	0.37	0.55	0.20	0.15	0.13	0.40	0.27	0.16	0.23	0.14	0.32	0.16
2.00	0.61	0.22	0.68	0.79	1.27	1.33	0.35	0.43	0.62	0.22	0.18	0.16	0.45	0.30	0.19	0.32	0.16	0.42	0.22
2.00	0.57	0.20	0.62	0.75	1.21	1.22	0.31	0.39	0.58	0.21	0.15	0.14	0.42	0.19	0.13	0.23	0.13	0.35	0.17
2.00	0.47	0.19	0.57	0.68	0.99	1.00	0.26	0.33	0.48	0.17	0.12	0.12	0.39	0.17	0.11	0.16	0.12	0.25	0.14
2.00	0.54	0.21	0.60	0.73	1.15	1.15	0.29	0.35	0.54	0.18	0.14	0.14	0.40	0.22	0.13	0.21	0.11	0.32	0.16
3.00	0.50	0.22	0.56	0.72	1.08	1.09	0.29	0.34	0.52	0.19	0.14	0.13	0.38	0.19	0.12	0.21	0.12	0.32	0.15
3.00	0.54	0.20	0.61	0.73	1.15	1.18	0.30	0.35	0.54	0.21	0.15	0.15	0.41	0.22	0.12	0.23	0.14	0.33	0.17
3.00	0.52	0.21	0.61	0.74	1.08	1.09	0.30	0.39	0.57	0.21	0.14	0.13	0.42	0.20	0.14	0.23	0.14	0.33	0.17
3.00	0.49	0.22	0.57	0.70	1.08	1.09	0.28	0.35	0.53	0.20	0.15	0.12	0.38	0.20	0.13	0.22	0.14	0.42	0.20
3.00	0.44	0.18	0.53	0.64	0.91	0.90	0.24	0.32	0.48	0.16	0.11	0.11	0.35	0.17	0.11	0.20	0.12	0.28	0.16
3.00	0.52	0.21	0.58	0.70	1.12	1.17	0.30	0.33	0.54	0.20	0.14	0.13	0.40	0.17	0.11	0.21	0.12	0.31	0.15
3.00	0.44	0.16	0.52	0.62	1.24	1.26	0.31	0.38	0.57	0.21	0.15	0.12	0.42	0.24	0.13	0.24	0.14	0.33	0.17
3.00	0.43	0.17	0.50	0.60	1.19	1.19	0.30	0.36	0.58	0.19	0.15	0.15	0.41	0.23	0.13	0.24	0.14	0.31	0.17
3.00	0.44	0.15	0.52	0.61	1.18	1.20	0.29	0.35	0.56	0.19	0.16	0.16	0.41	0.30	0.17	0.22	0.13	0.32	0.18
3.00	0.43	0.16	0.50	0.60	1.16	1.18	0.30	0.35	0.56	0.20	0.15	0.15	0.40	0.20	0.13	0.21	0.10	0.31	0.16
4.00	0.49	0.20	0.58	0.70	1.04	1.07	0.26	0.33	0.49	0.17	0.13	0.13	0.39	0.18	0.11	0.21	0.12	0.26	0.14
4.00	0.51	0.19	0.56	0.71	1.05	1.08	0.26	0.34	0.49	0.17	0.12	0.13	0.41	0.18	0.11	0.20	0.12	0.28	0.15

4.00	0.48	0.19	0.54	0.68	0.98	0.95	0.25	0.33	0.47	0.14	0.12	0.11	0.37	0.20	0.11	0.18	0.12	0.25	0.15
4.00	0.58	0.22	0.63	0.76	1.26	1.29	0.29	0.37	0.58	0.19	0.15	0.13	0.40	0.22	0.14	0.26	0.16	0.35	0.18
4.00	0.49	0.19	0.56	0.68	0.99	1.00	0.24	0.33	0.48	0.16	0.13	0.11	0.37	0.20	0.11	0.19	0.11	0.26	0.14
4.00	0.65	0.22	0.68	0.81	1.39	1.41	0.34	0.41	0.63	0.22	0.16	0.14	0.45	0.22	0.13	0.25	0.15	0.38	0.20
4.00	0.63	0.19	0.67	0.80	1.32	1.36	0.31	0.41	0.62	0.21	0.16	0.13	0.43	0.26	0.14	0.25	0.14	0.37	0.18
4.00	0.57	0.20	0.64	0.77	1.20	1.18	0.29	0.38	0.57	0.18	0.14	0.14	0.42	0.20	0.11	0.23	0.14	0.31	0.18
4.00	0.63	0.23	0.68	0.80	1.32	1.34	0.33	0.39	0.64	0.22	0.16	0.13	0.44	0.22	0.14	0.24	0.15	0.38	0.19
4.00	0.60	0.22	0.63	0.76	1.27	1.29	0.32	0.37	0.59	0.22	0.16	0.15	0.42	0.24	0.13	0.24	0.13	0.34	0.18
5.00	0.57	0.20	0.60	0.74	1.12	1.10	0.29	0.38	0.58	0.21	0.15	0.15	0.39	0.22	0.15	0.21	0.13	0.32	0.17
5.00	0.55	0.21	0.60	0.74	1.23	1.25	0.28	0.37	0.55	0.21	0.13	0.13	0.39	0.21	0.12	0.21	0.13	0.33	0.16
5.00	0.50	0.19	0.53	0.66	1.03	1.04	0.25	0.34	0.50	0.16	0.12	0.10	0.34	0.17	0.10	0.20	0.12	0.29	0.14
5.00	0.52	0.20	0.62	0.75	1.20	1.24	0.28	0.38	0.56	0.20	0.16	0.14	0.42	0.20	0.11	0.23	0.14	0.31	0.17
5.00	0.53	0.21	0.61	0.74	1.21	1.24	0.30	0.39	0.60	0.20	0.16	0.14	0.42	0.29	0.19	0.31	0.18	0.48	0.20
5.00	0.60	0.23	0.64	0.78	1.33	1.36	0.34	0.37	0.62	0.21	0.15	0.16	0.43	0.28	0.17	0.30	0.18	0.42	0.21
5.00	0.57	0.22	0.62	0.75	1.29	1.31	0.30	0.40	0.59	0.20	0.15	0.14	0.40	0.20	0.12	0.24	0.15	0.36	0.18
5.00	0.58	0.20	0.64	0.76	1.34	1.38	0.28	0.36	0.60	0.21	0.15	0.13	0.43	0.23	0.14	0.23	0.14	0.35	0.17
5.00	0.59	0.22	0.64	0.77	1.34	1.37	0.32	0.38	0.62	0.22	0.16	0.15	0.44	0.24	0.14	0.24	0.14	0.36	0.18
5.00	0.55	0.21	0.61	0.74	1.28	1.28	0.31	0.40	0.58	0.22	0.16	0.14	0.40	0.21	0.12	0.20	0.12	0.31	0.16
6.00	0.55	0.23	0.61	0.75	1.21	1.25	0.28	0.34	0.56	0.19	0.16	0.14	0.41	0.24	0.13	0.23	0.14	0.34	0.16
6.00	0.49	0.20	0.55	0.68	1.03	1.05	0.27	0.34	0.52	0.17	0.14	0.13	0.39	0.20	0.12	0.22	0.12	0.31	0.16
6.00	0.46	0.21	0.54	0.66	0.99	0.97	0.24	0.32	0.49	0.16	0.12	0.12	0.37	0.17	0.11	0.21	0.12	0.29	0.15
6.00	0.54	0.21	0.59	0.72	1.16	1.18	0.29	0.29	0.53	0.19	0.16	0.14	0.39	0.19	0.14	0.20	0.13	0.32	0.16
6.00	0.53	0.19	0.56	0.68	1.06	1.09	0.26	0.29	0.52	0.16	0.14	0.12	0.37	0.19	0.11	0.21	0.12	0.29	0.15
6.00	0.52	0.23	0.61	0.75	1.19	1.21	0.28	0.30	0.58	0.19	0.15	0.12	0.39	0.23	0.13	0.26	0.13	0.34	0.17
6.00	0.51	0.21	0.58	0.70	1.11	1.13	0.27	0.31	0.54	0.18	0.13	0.13	0.40	0.18	0.13	0.22	0.12	0.32	0.16
6.00	0.53	0.19	0.58	0.71	1.08	1.09	0.28	0.31	0.54	0.18	0.13	0.11	0.38	0.20	0.13	0.21	0.13	0.34	0.16
6.00	0.54	0.22	0.62	0.74	1.27	1.31	0.29	0.31	0.59	0.22	0.16	0.15	0.41	0.21	0.13	0.25	0.14	0.35	0.17
6.00	0.48	0.20	0.54	0.70	1.07	1.10	0.28	0.28	0.51	0.18	0.13	0.13	0.37	0.18	0.11	0.22	0.13	0.29	0.15
7.00	0.52	0.22	0.53	0.68	1.07	1.10	0.24	0.29	0.53	0.18	0.15	0.13	0.38	0.18	0.12	0.20	0.12	0.29	0.16
7.00	0.55	0.22	0.58	0.71	1.12	1.16	0.27	0.31	0.56	0.19	0.15	0.12	0.39	0.20	0.11	0.22	0.12	0.31	0.16
7.00	0.54	0.20	0.54	0.68	1.06	1.08	0.23	0.27	0.52	0.17	0.14	0.12	0.37	0.18	0.11	0.21	0.13	0.30	0.17
7.00	0.59	0.23	0.63	0.77	1.27	1.28	0.28	0.40	0.58	0.21	0.17	0.16	0.42	0.26	0.16	0.29	0.19	0.43	0.23
7.00	0.57	0.23	0.61	0.76	1.19	1.21	0.29	0.38	0.58	0.19	0.15	0.14	0.41	0.30	0.17	0.28	0.18	0.35	0.23
7.00	0.58	0.22	0.60	0.74	1.22	1.24	0.24	0.28	0.59	0.21	0.18	0.13	0.39	0.22	0.14	0.24	0.12	0.34	0.18
7.00	0.52	0.20	0.53	0.66	1.02	1.06	0.25	0.29	0.53	0.18	0.14	0.14	0.38	0.20	0.12	0.21	0.13	0.31	0.17

7.00	0.55	0.22	0.62	0.75	1.18	1.20	0.29	0.38	0.61	0.19	0.15	0.15	0.43	0.30	0.16	0.27	0.18	0.38	0.22
7.00	0.57	0.23	0.65	0.79	1.30	1.31	0.29	0.29	0.61	0.20	0.15	0.13	0.45	0.19	0.13	0.24	0.15	0.32	0.18
7.00	0.53	0.20	0.53	0.66	1.12	1.15	0.27	0.38	0.52	0.19	0.13	0.11	0.41	0.24	0.16	0.27	0.16	0.36	0.17
8.00	0.59	0.23	0.60	0.74	1.22	1.24	0.29	0.28	0.59	0.24	0.14	0.15	0.42	0.26	0.16	0.26	0.18	0.42	0.22
8.00	0.56	0.22	0.60	0.74	1.26	1.29	0.28	0.30	0.59	0.18	0.15	0.13	0.42	0.29	0.18	0.29	0.17	0.41	0.22
8.00	0.54	0.21	0.59	0.72	1.14	1.16	0.29	0.29	0.57	0.19	0.15	0.14	0.38	0.26	0.15	0.28	0.18	0.41	0.21
8.00	0.57	0.22	0.61	0.76	1.24	1.28	0.29	0.27	0.59	0.21	0.15	0.15	0.39	0.27	0.15	0.29	0.19	0.40	0.21
8.00	0.58	0.23	0.62	0.76	1.23	1.27	0.29	0.27	0.61	0.20	0.15	0.15	0.44	0.29	0.17	0.29	0.18	0.42	0.23
8.00	0.58	0.20	0.62	0.74	1.21	1.24	0.31	0.28	0.58	0.21	0.14	0.14	0.41	0.27	0.17	0.30	0.19	0.45	0.23
8.00	0.57	0.24	0.64	0.78	1.33	1.35	0.33	0.31	0.63	0.23	0.18	0.16	0.41	0.27	0.17	0.29	0.19	0.44	0.23
8.00	0.59	0.21	0.65	0.77	1.29	1.31	0.30	0.30	0.61	0.22	0.17	0.16	0.41	0.31	0.20	0.31	0.17	0.45	0.23
8.00	0.60	0.24	0.63	0.77	1.37	1.40	0.32	0.34	0.63	0.22	0.18	0.15	0.42	0.30	0.18	0.32	0.18	0.49	0.24
8.00	0.56	0.23	0.62	0.76	1.30	1.35	0.30	0.32	0.59	0.24	0.17	0.16	0.41	0.28	0.18	0.31	0.18	0.43	0.24

Appendix 3.2

Different grass species collected preliminary from localities in south-east, south-west and north Iran.

Host plant	Province	Collection sites	Collection Site code	Geographical region
L. pseudosclerophylla	Kerman	Sirch	1a	Southeastern Iran
		Deh-Salah, Kouhpayeh	2b	Southeastern Iran
		Deh Lo-Lo, Kouhpayeh	2d	Southeastern Iran
		Seyahbenouieh, Rabour	4b	Southeastern Iran
	Fars	Dasht-e-Morghab, Shiraz	7	Southwestern Iran
		Sheshpir (Shiraz-Sepidan Rd.	7a	Southwestern Iran
		Sepidan-Yasuj Rd.	7b	Southwestern Iran
	Khorasan	Dizbad, Neishbour	8	Northeastern Iran
L sclerophylla	Tehran	Karaj-Chalous Rd.	9b	Northern Iran
		Sirachal (Karaj-Chalous Rd.)	9c	Northern Iran
Stipa arabica Trin. & Rupr.	Kerman	Kerman-Sirch Rd.	1b	Southeastern Iran
		Kerman-Kouhpayeh Rd.	2c	Southeastern Iran
		Simk, Kouhpayeh	2e	Southeastern Iran
		Vamegh-Abad, Kouhpayeh	2f	Southeastern Iran
		Pabdana, Zarand	6	Southeastern Iran
Calamagrostis pseudophragmites (Haller f.) Koeler	Kerman	Sirch	1a	Southeastern Iran
		Kerman-Sirch Rd.	1b	Southeastern Iran
		Bidouieh, Kouhpayeh	2a	Southeastern Iran
		Deh Lo-Lo, Kouhpayeh	2d	Southeastern Iran
		Dahmardan (Negar-Qaleaskar Rd.)	3a	Southeastern Iran
		Hararon (Kerman-Baft Rd.)	3c	Southeastern Iran
Elymus elongatiformis (Drobow) Assadi	Kerman	Sirch	1a	Southeastern Iran
		Dahmardan (Negar-Qaleaskar Rd.)	3a	Southeastern Iran
		Hararon (Kerman-Baft Rd.)	3c	Southeastern Iran
Alopecurus arundinaceus Poir.	Kerman	Hararon (Kerman-Baft Rd.)	3c	Southeastern Iran
Alopecurus pratensis L.	Kerman	Dar-e-Nem, Sardouieh	5	Southeastern Iran
Festuca arundinacea (Schreb)	Kerman	Bidouieh, Kouhpayeh	2a	Southeastern Iran
Dactylis glomerata L.	Kerman	Dahmardan (Negar-Qaleaskar Rd.)	3a	Southeastern Iran
	Tehran	Karaj	10a	Northern Iran

CHAPTER 4

Genetic variation and mitochondrial DNA phylogeography of chalcid wasp populations in Iran

4.1 Introduction

Phylogeography is a powerful approach for exploring historical demographic events associated with biogeography, including the relative roles of gene flow, population bottlenecks, population expansions and vicariance in shaping current geographical patterns of genetic variation (Avise 1994). Patterns of genetic differentiation at any geographical scale may reveal valuable information pertaining to contemporary processes such as secondary contact, hybridization and also processes such as past fragmentation and range expansion (Zink 1994; Templeton *et al.* 1995).

Advances in molecular genetic markers particularly mitochondrial DNA (mtDNA) have in particular revolutionised insect population genetic studies and have made it possible to reveal sequential patterns of population differentiation, reflecting these historical links in geographical space in order to make spatio-temporal inferences about the evolutionary processes at the population level (Templeton 1998; Alexandrino 2002) which is the subject of intraspecific phylogeography (Avise *et al.* 1987; Roderick 1996). Although this approach has been recently investigated for a large number of insect species (e.g. Gómez-Zurita *et al.* 2000; Brunner *et al.* 2004; Mardulyn 2001; Andersen *et al.* 2000; Cooper *et al.* 1995; Ibrahim *et al.* 1996), surprisingly little is known about population structure and rates of gene flow between local populations of insect natural enemies (Roderick 1996; Hopper *et al* 1993).

Highlighting the importance of evolutionary processes (contemporary and historical) is less well established in the order Hymenoptera and especially in the genus *Tetramesa* and their parasitoids. Intraspecific phenotypic variation and the existence of

many aggregate sibling species in a number of taxa within the chalcidoidea (Dawah 1986; Dawah *et al.* 2002; Boucek 1988; Graham 1970) have excited taxonomists to find other criteria in the field of molecular systematics for resolving these taxonomic issues. However, few molecular studies have been carried out on eurytomid wasps and their parasitoids to date.

Studies so far have been limited to protein electrophoresis analysis on the British *Tetramesa* species complex (Dawah 1987) and the British *Eurytoma* species complex (Eurytomidae) (Dawah 1988b), the British *Pediobius* species complex (Eulophidae) (Dawah 1988a; Dawah *et al.* 2002), the British *Chlorocytus* species complex (Pteromalidae) (Dawah 1989), the marble gallwasp, *Andricus kollari* (Hartig) (Stone *et al.* 2001), *A. quercuscalicis* (Cynipidae) (Stone & Sunnucks 1993), and the *Trichogramma minutum* Riley complex (Trichogrammatidae) (Pinto *et al* 1992). Phylogenetic studies have been done recently on British wasps including the *Tetramesa* species complex (Al-Barrak 2001) using random amplified polymorphic DNA markers (RAPD-PCR), on the *Sycophila* species complex (Ghajarieh 2003) and the *Pediobius* species complex (Shamie 2004) using DNA sequencing.

There are no phylogenetic and phylogeographic studies on assemblages of *Tetramesa* (Fa: Eurytomidae) and their parasitoids including the genera *Eurytoma* and *Sycophila* (Fa: Eurytomidae), *Pediobius* (Fa: Eulophidae) and *Chlorocytus* (Fa: Pteromalidae) in Iran and the regional chalcid wasp fauna of Iran and surrounding states is very poorly known. The present study represents the first record of these taxa in the country. The herbivore group *Tetramesa* is associated with host grass species *L. pseudosclerophylla, L. sclerophylla* and *F arundinacea* in Iran. *Tetramesa* is attacked by several taxa of parasitoids mentioned above. The distribution of *Tetramesa* is related to the distribution of its host plants, *L. pseudosclerophylla* and *L. sclerophylla*. The first

host plant is distributed through southeastern and southwestern Iran, while *L. sclerophylla* is restricted to northern areas of Iran. These grass species are restricted to mountain slopes at different elevations between 2000m to more than 4000 m and are geographically isolated.

The climatic fluctuations of the Pleistocene and Holocene in the Middle East has had a major effect on the geographical distribution of taxa. Evidence suggests that Quaternary climatic-vegetational changes were the most likely phenomena affecting for example, avian geographic differentiation in the Palearctic region (Moreau 1954. 1955). The paleoclimate of Iran is not very well understood, because of paucity of studies in the region. Data come from two sites in the Zagros Mountains: Lakes Zeribar and Mirabad (western Iran) and few data on Alborz Mountains (northern Iran). There is no data on eastern and south-eastern Iran and eastern Iran may have had a different climate than the west.

During the last glacial period (70,000-10,000 YBP), the climate was cold and dry and a relatively arid climatic condition has been suggested for the western Iran based on information obtained from Lake Zeribar (Snyder *et al.* 2001). Vegetation composed of a "desert steppe" and grasses made up to 10-20%. The grasses were present at a higher percentage during 50-35 KYBP and the climate was slightly wetter (and possibly warmer) than the interval from 35 to 11.5 KYBP. During 15-17 KYBP (Heinrich 1) and 13 to 11.5 KY BP (Younger Dryas) the climate was drier and severe climatic conditions occurred during these two periods. In the early Holocene (~ 11 to 7 KY BP) the climate changed to a considerably warmer phase and the percentage of the grasses went up to 50% of vegetation at Lake Zeribar.

Based on the seasonality hypothesis (Stevens *et al.* 2001), the early Holocene was slightly drier than the late Holocene and contained wet winters and protracted dry

summers (similar to the modern Mediterranean climate) and as a result Pistachio trees were present. During the mid Holocene, changes happened so that there were wetter springs (and thus shorter summers) and as a result oak trees (Quercus spp.) became dominant over pistachio (similar to the present day). Wright Jr & Thorpe (2003) based on pollen studies confirmed the abundance of grasses in Lake Zeribar in Southwestern Iran both before and after the transition from the last glacial to the Holocene as the climate changed from cold and dry to warmer and not quite so dry.

Palynological and geomorphological studies suggest that during the last glacial period (Würm) the climate was cold and dry and some lakes were formed in central Iran as a result of lower evaporation rates (Haffer 1997). During the last glacial stage (Würm, maximum 20 KY BP) the temperature was at least 5 to 8°C lower than today in the northern part of Iran and was 3-4°C in the Zagros Mountains. During the early Würm glacial period there was a brackish lake and of shallow freshwater and oligohaline waters in the Kerman region in Central Iran which showed temporarily increased precipitation (Haffer 1977). A humid-cold climatic condition was dominant throughout the Middle East during the early Würm glacial period. Based on pollen studies (Wright Jr & Thorpe 2003), the grasses (Poaceae) were abundant in southwest Iran both before and after the transition from the last glaciation to the Holocene, as the climate changed from cold and dry to warmer and not quite so dry. The uplift of the Alburz Mountains extended into the Pleistocene (Haffer 1977) and the formation of the Zagros Mountains in the southern part of the Iranian Plateau dates back to the late Miocene (10 MYBP) (Macey *et al.* 1998).

In this chapter, phylogenetic and phylogeographic patterns and population differentiation of *Tetramesa* and their parasitoids, *Eurytoma*, *Sycophila*, *Pediobius* and *Chlorocytus* reared from host grass species *L. pseudosclerophylla*, and *F. arundinacea* in ten sampling localities in south-eastern and south-western Iran and *L. sclerophylla* in

northern Iran, was investigated for the first time using partial mitochondrial cytochrome oxidase subunit I (COI) and cytochrome oxidase subunit II (COII) DNA sequencing. The aim of this chapter is to investigate the genetic structure of the taxa under study and also to elucidate the contribution of population structure and/or population history as processes explaining the geographical distribution of mtDNA haplotypes in the genus *Tetramesa* and its parasitoids. Some questions addressed in this chapter include: (i) Is there any variability in mtDNA within and between wasps populations for each taxon?; (ii) Is there any genetic differentiation in mtDNA between wasps from different host plant species and region (especially southern and northern populations) with regard to the formation of Zagros and Alburs Mountains?; (iii) what are the relative contributions of contemporary and historical processes in shaping the present day geographical patterns of genetic variation?

4.2 Materials and Methods

4.2.1 Grass collections and sample preparation

The grass stems were collected from several localities in Iran (Figure 4.1) and were dissected and the larvae and pupae of chalcid wasps were removed. The larvae and pupae were labelled and placed individually in small gelatine capsules through to adult stage (see chapter 2). Some adults were stored in 95% ethanol. Some live adults from each taxon, about 4-5 days after emergence, were labelled and placed individually in small plastic tubes and deep-frozen at –70°C. Some U.K grasses (Table 4.2) were also dissected in the lab and the chalcid wasps that were reared from them were labelled and placed individually in gelatine capsules in the same way. Live adults a few days after emergence were placed in small plastic tubes and deep-frozen at –70°C. The samples

from each genus, from Iran and the U.K, which were used for DNA extraction, are listed in Tables 4.1 and 4.2 respectively.

4.2.2 DNA Extraction and amplification

DNA was extracted from individual adults (Frozen, ethanol preserved and dried specimens) using the Tissue Protocol, QIAamp DNA Mini Kit, (Qiagen) following the manufacturer's recommendations, (see chapter 2). PCR amplification was performed in a GeneAmp 9700 thermal cycler (PE Applied Biosystems). The final optimised PCR condition and thermocyclying programmes for each taxon and primers pairs are summarised in Tables 4.3 to 4.5. Although several primers, were tested (either general insect primers or Hymenoptera-specific): "Ron" and "Nancy" (Simon *et al.* 1994); "StLeu" (Forward) (Machado *et al.* 1996) and "AtAsp"(Reverse) (Willis *et al.* 1992, Machado *et al.* 1996); "A-tLEU" (Forward) and "B-tLYS" (Reverse) (Liu and Beckenbach 1992); "tRNA-LEU" (Forward) (Willis *et al.*1992) and "At-Asp" (Reverse) (Machado *et al.* 1996); "A-tLEU" (Forward) (Liu and Beckenbach 1992) and "B-ATPase 8" (Reverse) (Simon *et al.* 1994); "A-298" (Forward) and "B-tLYS" (Reverse) (Liu and Beckenbach 1992); "Pierre" (Forward) and "Barbara" (Reverse) (Simon *et al.* 1994); "A-298" (Forward) (Liu and Beckenbach 1992) and "B-ATPase8" (Reverse) (Simon *et al.* 1994) and several primers pairs for Cytochrome *b* as well, it was not possible to obtain a regular and reliable amplification of the target fragments, corresponding to complete or partial COII and Cytochrome *b* sequences, in *Tetramesa*, *Eurytoma*, *Sycophila*, *Chlorocytus* and *Pediobius* populations (see chapter 2).

For amplifying a partial fragment of the COI gene (831 bp), the "Jerry" and "Pat" primers were used (Simon *et al* 1994). For amplification of another target region of mtDNA (COI-COII) (625 bp), the primers "S2792" and "Muscid" were used (Taylor *et al* 1997). The 5' ends of these primers are located at bp 2773 and 3400 of the *Drosophila*

yakuba mtDNA map (Clary & Wolstenholme 1985), respectively. These primers have been used in amplifying a partial fragment of the COI-COII region of *Muscidifurax* spp. (Hymenoptera: Pteromalidae) (Taylor *et al.* 1997). These primers are shown in Table 4.3. For optimisation of these primers and the others mentioned above, a combination of methods were used for each population, such as changing concentrations of reaction mixture components, using touchdown PCR programmes and using different annealing temperatures in the PCR programme (see chapter 2) (Tables 4.4 and 4.5 in this chapter).

PCR products were run on a 1.3% agarose gel and stained with ethidium-bromide at 90 V for 1 hr (0.5 μg/ml) and viewed under a UV transilluminator. All PCR's contained a negative control. A 100 bp ladder size standard (MBI Fermentas) was run on each gel with the PCR to give an indication of the size of the target band that was being amplified.

4.2.3 Sequencing

PCR products were purified to remove inhibitors using the GeneClean Turbo for PCR Kit (BIO101), and sequenced in the forward and reverse direction with the ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) using a 9700 thermal cycler (Perkin-Elmer). The sequencing kit was diluted, to produce a master mix containing 2 parts sequencing kit: 1 part 5X buffer: 1 part sterile water. The sequencing PCR was carried out in 5 μl reaction volumes containing 2 μl of purified DNA , 2 μl of the sequencing mix and 1 μl of either forward or reverse primers at 1.6 pmol/μl concentration. The sequencing PCR programme with a total reaction volume of 5 μl per sample was as follows: 96°C for 10 seconds, 50°C for 5 seconds and 60°C for 4 minutes for 25 cycles. Purification of the sequencing products was carried out by Ethanol/Sodium Acetate and isopropanol precipitation as recommended by PE

Biosystems (see chapter 2). Fragments were separated on ABI 377 and ABI 3100 Automated Sequencers.

4.2.4 Sequence alignment and $tRNA_{LEU}$ secondary structure

COI and COII sequences were aligned and edited using the program Sequencher v.3.1.2 (GENECODES^{TM}). Gaps were treated as fifth character state in all analyses.The secondary structure of tRNA sequences was determined using an existing arthropod $tRNA_{LEU}$ secondary structure model (Nardi *et al.*. 2001).

4.2.5 Amino acid translation

COI and COII sequences translated into amino acid codons with the invertebrate genetic code (*Drosophila*), using the appropriate reading frame (2nd for COI and 1st for COII). The reading frame of COI and COII was obtained by aligning the existing chalcid wasp sequences, *Ceratosolen capensis* (Grandi) (Hymenoptera: Agaonidae) (AF200377), *C. bisulcatus* (AF200375), *Blastophaga malayana* (AF200373) and *B. intermedia* (AF200372) (Weiblen 2001) from GenBank to existing sequences. The above chalcid species were used as outgroups for the subsequent analysis.

4.2.6 Molecular characterization of MtDNA

Base composition, pairwise uncorrected and corrected sequence divergences were calculated using PAUP 4.0b10 (Swofford 1998). The number of transitions (T_s) and transversions (T_v) and the T_s/T_v ratios (R) between pairs of sequences were calculated using MEGA v2.01 (Kumar *et al.*. 2001).

4.2.7 Phylogenetic analysis

Phylogenetic analysis was conducted using PAUP 4.0b10 (Swofford 2002). Neighbour joining (NJ) trees were constructed using 10,000 bootstrap permutations with PAUP v. 4.0b10 (Swofford 2002), using corrected distances. The optional evolutionary model for the sequences was determined using Modeltest version 3.06 (Posada & Crandall 1998) which identified the best distance estimator for *Chlorocytus* to be K81uf and for *Sychophila* to be HYK+I (I = 0.8505). Maximum likelihood (ML) trees were constructed using 100 bootstrap permutations using the evolutionary model of best fit as above.

An overall phylogenetic analysis was conducted for taxa in which the corrected distance estimator was identified as 'General Time Reversible',GTR+G (Γ = 0.4670).

4.2.8 Phylogeographic patterns of mtDNA variation

4.2.8.1 Minimum spanning network

Sequence haplotypes were used to create a minimum spanning network (MSN) based on the number of substitutions between haplotypes designed for each taxon using the programmes TCS v 1.13 (Clement *et al.* 2001) and Minspnet (Excoffier 1993).

4.2.8.2 Nested clade analysis

The haplotype network that created for the MSN was divided into nested clades of haplotypes using TCS v 1.13 (Clement *et al.* 2001). GeoDIS 2.2 (Posada *et al.* 2000) was used in order to test for significant association between haplotypes and geographic location. GeoDIS calculates two measures of geographic distance. The clade distance (D_c) that measures how geographically widespread the individuals are from that clade, and the nested clade distance (D_n), which measures the distance of clades from the

geographic centre of their nesting category (Templeton *et al.*. 1995). In other word D_c measures the geographical spread of haplotypes within a clade, whilst D_n measures how a clade is geographically distributed relative to other clades in the same higher-level nesting category.

GeoDIS determines whether any of the distances are significantly small or large at the 5% level. The 'inference key' presented by Templeton (2004) was used where significant D_c and/or D_n values were observed. Hence nested clade analysis may discriminate between the effects of contemporary events such as gene flow, versus historical processes including fragmentation, range expansion, isolation by distance or long-distance dispersal (Templeton 1998; Alexandrino *et al.*. 2002).

Sequence haplotypes were identified manually in Sequencher. Haplotype (h) and nucleotide diversity (π) were calculated for each population of *Sycophila* and *Chlorocytus* using Arlequin v.2.000 (Schneider *et al.* 2000). Pairwise comparisons of population and analysis of molecular variance (AMOVA) were used to determine the degree of variation within and between populations according to the regions north, south-east and south-west, using Arlequin v.2.000 (Schneider *et al.* 2000). Gene flow between population pairs was calculated using the formula: $Nm= 0.5[(1/Fst) - 1]$ (Takahata & Palumbi 1985) using a minimum of 1000 permutations for significance. A Mantel test (Smouse *et al.* 1986; Smouse & Long 1992) was used to determine whether there was a relationship between geographic distance (*d*) and genetic distance (Fst) using Arlequin v.2.000 (Schneider *et al.* 2000). Great circle distances (km) between populations were calculated using the programme Earth (Byers 1999).

4.2.8.3 Mismatch distribution analysis

Mismatch distributions were calculated for *Sycophila* using Arlequin v.2.000 (Schneider *et al.* 2000). The purpose of this analysis is to calculate the distribution of

pairwise nucleotide differences among DNA sequences by estimating tau τ (expansion time), θ_0 and θ_1 (mutation parameters) based on a sudden demographic expansion model (Rogers 1995; Schneider & Excoffier 1999). The date of the expansion can be estimated from the following formula which presented by Nei and Tajima (1981, eq. 5) as: $u = 2\mu k$. where k is average number of nucleotide sites per haplotype; μ is the mutation rate per nucleotide and u is the sum of per-nucleotide mutation rates in the region of DNA under study.

Based on a sudden expansion model, (Rogers 1995), t (time in generation since expansion), N_0 (female effective population size before expansion) and N_1 (female effective population size after expansion) were calculated using the following equations:

$\Theta_0 = 2uN_0$; $\Theta_1 = 2uN_1$, and $\tau = 2ut$

A mutation rate of 1.2%-2.8%/ site per Myrs was considered based on COI mitochondrial DNA evolution (Brower 1994; Danforth 1999) and a generation time of one year was used (Claridge 1961; Dawah *Pers. Comm.*).

4.3 Results

4.3.1 DNA extraction

It was possible to extract DNA using either of the two methods tested. The second method (Tissue Protocol, QIAamp DNA Mini Kit) (Qiagen) was successful for the vast majority of the samples. As a higher yield of DNA (as observed on agarose gels) was obtained using the tissue protocol, this method was used to extract DNA from the majority of the samples. This procedure was used for extraction of DNA from samples frozen at –70°C, ethanol preserved and dried samples. DNA extraction from live samples, samples frozen at –70°C or specimens preserved in 100% ethanol (Dessauer *et al.* 1990; Post *et al.* 1993; Chippindale *et al.* 1998) or cold storage in 70% ethanol (Quicke 1999)

have been shown to be more successful in yielding DNA amenable to PCR amplification, while samples which have been air-dried rapidly at low humidity, have a high quality and high amount of DNA (Chippindale *et al.* 1998). In this study samples which were frozen at –70°C and ethanol preserved samples (Figure 2.4) showed a high amount of DNA, but the air-dried samples were not so successful in yielding DNA amenable to PCR amplification.

The methods and results of these molecular methods are presented in Chapter 4.

4.3.2 Molecular characterization of mtDNA

A total of 1089 bp of mtDNA was sequenced for each taxon. However the size of the sequenced region was larger than this as 1089 bp was used in this study to minimize ambiguous calls at the ends of sequences. Of 1089 bp region, 621 was in the COI, 68 in tRNA and 254 in the COII. A 53 bp insertion between 3' end of COI and tRNA, was not included in analysis. Sequence alignment for COI, COII and tRNA are presented in Appendices 4.1, 4.2 and 4.3. The sequences were A + T rich (77.2%) in COI and 83% in COII (Table 4.6). In the COI gene, first and second codon positions were less A-T biased (70.3% and 66.9%), while third codon positions were extremely A-T biased (94.3%) (Table 4.6). In the COII gene, the position was similar. First and second codon positions were less A-T biased (72.6% and 80%), while third codon position were highly A-T biased (95.5%) (Table 4.6). Ts/Tv ratios (R) in the COI gene (778 bp) for all taxa are presented in Table 4. 7. In the COI gene, the highest Ts/Tv ratio was found in the third codon position (3.367 ± 2.407) (in the *Pediobius*), while the least Ts/Tv ratio was observed in the second codon position (0.400 ± 0.594) (in the *Chlorocytus*). In the COII gene, the highest Ts/Tv ratio was found in the third codon position (2.889 ± 1.347 in the *Sycophila*), while the least Ts/Tv ratio was observed in the first and third codon positions

(0.000 ± 0.000 in the *Tetramesa*) and second codon position (0.000 ± 0.000) for the *Sycophila*) (Table 4.9).

4.3.3 Amino acid translation

The amino acid translation for *Sycophila, Eurytoma, Pediobius* and *Chlorocytus* COI haplotypes is presented in Appendix 4.4. The amino acid translation for *Sycophila, Tetramesa, and Eurytoma* COI and COII are presented in Appendices 4.5 and 4.6 respectively.

4.3.4 Phylogenetic analysis

Sequence divergence, corrected (HYK+I, I = 0.8505) for *Sycophila* haplotypes and uncorrected *p* distances among taxa are presented in Appendices 4.7 and 4.8 respectively. Uncorrected *p* distance values within ingroups ranged between 0.000 (0%) and 0.039 (39%) for all pairwise comparisons of nucleotide sequences. Uncorrected *p* distances within *Sycophila* haplotypes varied between 0.1-6.8%.

The Neighbour-Joining phenogram based on GTR + G (G = 0.4670) (Figure 4. 2) was constructed for all taxa (*Tetramesa*, *Eurytoma* and *Sycophila*) including outgroups. Based on the NJ tree there is a high bootstrap values support for *Tetramesa* (100%) and *Eurytoma* (100%), but a lower bootstrap values support (64%) for *Sycophila. Tetramesa* individuals from S.E, S.W. and also from one northern site (9C2) are clustered with each other, while *Tetramesa* from other northern site (T9B1) was distinct from other *Tetramesa* individuals. Within *Eurytoma* sp. the individuals from S.W and S.E (E7.1 and E1A1) were clustered, while E7A1 was not grouped with others. Although several *Sycophila* individuals from south-east and south-west were grouped with each other but two individuals from south-west and one individual from north were not clustered with

the other individuals. In addition, surprisingly, one individual of *Sycophila* from south-east (4B1) was grouped with *Tetramesa* and *Eurytoma* clusters.

The maximum likelihood tree (Figure 4.3) although indicating high bootstrap values support for *Tetramesa* (100%) and *Eurytoma* (100%), but a lower bootstrap value for *Sycophila*. NJ and ML trees showed similar topologies. The Neighbour Joining phenogram for all wasp taxa is presented in Fig. 4.2. The maximum likelihood tree for all wasp taxa is shown in Fig. 4.3.

4.3.5 The $tRNA_{LEU}$ secondary structure

Wasp tRNA sequences were aligned and inserted between the 3' end of COI and the 5' end of the tRNA to accommodate a variable length non-transcribed region between COI and tRNA genes in some species (Figure 4. 4). Alignment gaps are a common feature of RNA sequences and are necessary to preserve base pairing and structure as this can be more conserved than the nucleotide sequence (Kjer 1995).

4.3.6 Phylogeographic patterns of mtDNA variation

4.3.6.1 Haplotype distribution and diversity

A 778 base pair (bp) region of the COI gene was sequenced for samples of each taxon for phylogeographic analysis (for sample sizes see Tables 4.1 and 4.2). The result of sequence analysis indicated 25 haplotypes for *Sycophila* from Iran, (samples and location of each haplotype found in this study is presented in Table 4. 14), 16 for *Eurytoma* from Iran and the U.K, 8 for *Pediobius* from Iran and the U.K, 6 for *Chlorocytus* from Iran. There were 84 variable characters in the 778 bp data set for *Sycophila*, 160 for *Eyrytoma*, 61 for *Pediobius*, and 71 for *Chlorocytus* (Tables 4.10 to 4.13). The most common haplotype for *Sycophila* was haplotype 3, occurring in 17.24%

of samples. Seventeen haplotypes were represented by a single individual (Table 4.14). Haplotype (h) and nucleotide diversity (π) for *Sycophila* are shown in Table 4.15. In *Chlorocytus* the most common haplotype was haplotype 1 and was present in 66.7% of individuals. Three haplotypes were represented by a single individual (Table 4.16). Haplotype (h) and nucleotide diversity (π) for *Chlorocytus* are presented in Table 4.17.

An analysis of molecular variance (AMOVA) was carried out for *Sycophila* to estimate the proportion of the genetic variation of populations located in different geographical regions, and also variation among populations within each region and variation within populations. When we compare two haplogroups I and II (Northern and Southern haplotypes), as in Table 4.18, most variation occurs within populations (67.51%) rather than among groups. Gene flow between northern and southern haplotypes was calculated (Fst = 0.32487) which was equivalent to 1.04 individuals per generation implying that genetic variation between northern and southern haplotypes (haplogroups I and II) was high although some gene flow has occurred. When we compare all the populations in the Table 4.19, the greatest variation again appears within populations (93.03%) and gene flow was estimated as 6.68 individuals per generation (Fst = 0.06968). Considering the haplogroup II only (northern haplotypes), as Table 4. 20 shows, most variation is again observed within populations (94.17%) and the amount of gene flow between populations was 8.1 individuals per generation (Fst = 0.05830). When haplogroup I (Southern haplotypes) is considered, as in Table 4.21, most variation also occurs within populations (62.32%) rather than among populations and the gene flow between populations was estimated 0.83 individuals per generation, much less than that of the haplogroup II (northern haplotypes). This indicates that genetic differentiation in haplogroup I (southern haplotypes) is higher than in haplogroup II (northern haplotypes). When south-east haplotypes vs. south-west haplotypes within haplogroup 1 were

compared again the most variation was observed within populations (63.39%). Gene flow between south-east and south-west populations was 0.87 individuals per generation (Fst = 0.36615) indicating high genetic variation between these regions (Table 4. 22).

Pairwise Fst comparisons between *Sycophila* populations are presented in Table 4.23. Significant genetic differentiation between the majority of populations was observed. Fst values ranged between 0.1155 and 0.9365 (Table 4.23). Significant differentiation was found between all pairs of *Sycophila* populations except for 2b vs. 1a, 2b and 2d vs. F2a, 7a and 7b vs. F2a, 2d vs. 2b, 7, 7a, 7b, 9b and 9c vs. 2b, 7a and 7b vs. 7, 9b vs. 7a, 9b and 9c vs. 7b, 9b vs. 9c.

The Mantel Test was, however not significant (p = 0.531) indicating no association between genetic distance (Fst) and geographic distance (*d*).

4.3.6.2 Minimum spanning network

As mentioned previously 25 haplotypes were identified from 58 individuals in *Sycophila* populations in 10 populations from south-easth, south west and northern Iran. The MSN was composed of two main clusters (haplogroups I and II) that are well separated (Fig. 4.5). The two haplogroups were separated by approximately 18 substitutions. The first haplogroup contains south-east and south-west haplotypes and the second haplogroup comprises some northern haplotypes. Three other haplogroups (haplogroups III, IV and V) were also recognised. Haplogroup III was composed of two south-western haplotypes (haplotypes 19 and 17). Haplogroup IV was comprised of one south-western haplotype (haplotype 9) and Haplogroup V was composed of one south-east haplotype (haplotype 5). Haplotype 3 (from haplogroup I) includes populations 1a, 2d and F2a from south-east Iran and was the most abundant haplotype. Haplotype 5 (from haplogroup V) includes population 4b from south-east Iran, represents a divergent lineage separated by approximately 40 substitutions from its nearest neighbour. There

was an unresolved relationship between haplotypes 1, 16 and 7 from haplogroup I and between haplotypes 27, 21, 26, 28 and 29. Haplotypes 3 and 4 composed of south-east populations which contains populations from *F. arundinacea* in addition to the two other host grass species, *L. pseudosclerophylla* and *L. sclerophylla.*

4.3.6.3 Neighbour Joining and Maximum Likelihood trees for *Sycophila* haplotypes

The Neighbour Joining phenogram of *Sycophila* sp. is presented in Fig. 4.6. It contains 2 separate clusters that may comprise two new cryptic taxa, with strong bootstrap support (98%). The second group is internally divided. The first cluster comprises exclusively southern haplotypes that show relatively little variation. The second cluster contains northern populations and some southern haplotypes. The NJ tree is supported by minimum spanning network. ML tree for *Sycophila* haplotypes is presented in Figure 4.7. NJ and ML trees showed relatively the same topologies.

4.3.6.4 Nested clade analysis (NCA)

A 3-level nested cladogram was constructed for *Sycophila* haplotypes (Fig. 4.8). The nested design was interpreted using an inference key (Templeton 2004). The result for clades 2-3 was unfortunately that the sampling scheme is inadequate to discriminate between fragmentation, range expansion and isolation by distance while restricted gene flow with isolation by distance was the result inferred for clade 3-2. For whole cladogram there was insufficient evidence to discriminate between long-distance movements of the organism and the combined effects of gradual movements during a past range expansion and fragmentation. This illustrates that NCA is not able to provide detailed inference unless large sample sizes are available. The Great Circle Distances are presented in Table 4. 24.

4.3.6.5 Mismatch distribution analysis

For *Sycophila* sp. when we consider the whole network, the observed mismatch distribution did correspond to the expected model of a sudden expansion (Fig. 4.9). SSD goodness-of-fit value (0.023, p = 0.254) was not significant. The observed distribution appeared to follow a complex multimodal pattern. The observed distribution pattern shows that *Sycophila* populations comprise three distinct genetic groups or shows the evidence for three demographic expansions happening a long time apart. The estimated date of initial expansion of *Sycophila* populations was calculated as occurring approximately between 92,545-216,006 years before present.

However the mismatch distribution analysis was also carried out for haplogroups I and II in *Sycophila*. For haplogroup I which includes southern haplotypes (Fig. 4.10), the expansion date was calculated as t = 162,362-378,961. The effective population sizes before expansion was 29,472-68,790 and after expansion was 364,977-851,873. The observed mismatch distribution for haplogroup I was not corresponded to the expected model of sudden expansion (Figure 4. 10). This was supported by the small confidence intervals at p = 0.050 or the 95% level, and a significant SSD goodness-of-fit value (0.0336, p = 0.020). The distributions were bimodal, suggesting that haplogroup I has undergone a recent demographic expansion.

4.4 Discussion

4.4.1 Molecular characterization of mtDNA

There is much information available on mtDNA sequences in insects (Simon *et al.* 1994) which could be used for comparison within and between taxa. MtDNA genes have been widely used as genetic markers in insect phylogenetics and much information is available on mtDNA sequences that could be used to compare phylogenetic

relationships within and between populations or species. The COI sequences studied here were highly A + T rich (77.2%) falling at the upper end of the described ratio range of between 63% and 88% found in many insect orders such as Lepidoptera (Brown *et al.* 1994), Orthoptera (Litzenberger & Chapco 2001), and Diptera (Mitchell *et al.*. 1993; Clary & Wolstenholme 1985). The COII sequences in the present study were also highly A + T biased (83%). This high bias in COI-COII A + T content has been demonstrated in other wasp mtDNA sequences, for example 75% in pollinating fig wasps in the family Agaonidae (Weiblen 2001).

In the present study A + T bias was most dominant in the third codon position in the COI and COII genes (94.3% and 95.5% respectively). This similarity has also been reported in pollinating fig wasps (Weiblen 2001) and also other insect species (Clary & Wolstenholme 1985; Crozier & Crozier 1993). Several lines of evidence indicate that the A + T content of some Hymenoptera are greater in comparison with the Diptera, while others are not (Dowton & Austin 1995). In the present study the transition: transversion ratios in the COI sequences for *Pediobius* and *Sycophila*, were >1.0 (despite the dominant transversion bias in parasitic Hymenoptera: see below). Transition: transversion ratios in the COII sequences for *Sycophila* also indicated a transition substitution bias. These results imply that multiple substitutions have not occurred at the same time and the sequences have not reached a saturated divergence level and as a result COI can be regarded as a useful marker in phylogenetic analysis studies in *Pediobius* and COI-COII in *Sycophila* (Weiblen 2001). However transition: transversion ratios in COI for *Eurytoma* and *Chlorocytus* were very close to 1 which would not indicate the dominance of transition and/or transversion in these taxa. High transition substitution bias has been demonstrated in many closely related species; however a transversion bias has been seen to be dominant among parasitic Hymenoptera (Dowton & Austin 1995,

Ghajarieh 2003). Dowton & Austin (1995, 1997) found that the A-content is higher in parasitic wasps compared with nonparasitic wasps and concluded that this difference may be associated with a parasitic life style. However, Castro *et al.* (2002) found an increased rate of molecular evolution in parasitic Hymenoptera compared with parasitic Diptera. They suggested that the pattern of higher mitochondrial and nuclear molecular variation in parasitic Hymenoptera may be due to the fact that the speciation rate is higher in endoparasitic Hymenoptera than in endoparasitic Diptera, especially when speciation in the former occurs in relation to founder events.

The COI sequence divergences within *Sycophila* haplogroups IV and V were not calculable, but within other haplogroups, it ranged between 0.1 -1.0% (SE ± 0.001 - 0.002). High level of variation was observed within haplogroups I (0.7%) and II (1.0%), which is similar to the sequence divergence of other insects, for example 0.4% for *Choristoneura fumiferana* Clements species complex (Lepidoptera: Tortricidae) (Sperling & Hickey 1994), and 0.5% for *Heliconius* butterflies (Brower 1994). The sequence divergence between *Sycophila* haplogroups varied between 2.4-6.4% (SE ± 0.005-0.009 respectively), the upper end of which range surpasses values that have been reported for some closely related species (Landry *et al.* 1999; Spicer 1995; Spicer unpublished observation; Hartsough, C. D. B. *et al.* unpublished observation). Sequence divergence of 2.8% is reported between solitary and eusocial populations of the genus *Lasioglossum* Curtis (Hymenoptera) that highly suggests they are distinct species (Danforth 1999). The presence of high sequence divergences between some haplotypes of *Sycophila* in this study suggests the existence of cryptic species and probably several distinct taxa (0.13%-6.8%).

4.4.2 Haplotype and haplogroup diversity in *Sycophila*

Sycophila populations from southern and northern Iran showed a wide mtDNA variation. Of 60 individuals screened, 25 haplotypes were identified from a 778 bp fragment of the mtDNA COI gene. The high number of haplotypes recorded for *Sycophila* shows that the COI gene is variable and can be used as a valuable marker for phylogeographic analysis of some parasitic wasps. Five haplogroups were observed in the minimum spanning network (Fig. 4. 5). Haplogroup I comprised the main cluster of haplotypes in the network. It contained southern haplotypes which were the majority of samples sequenced. This haplogroup was connected to haplogroup II by a minimum of 18 substitutions. Haplogroups III, IV and V were connected to haplogroup II by 19, 17, and 40 substitutions respectively.

The grouping of sequences into haplogroups I to V was firmly supported by the NJ and ML trees. Haplogroup I was separated from other haplogroups, while haplogroups II, III, IV and V are clustered with each other with 98% bootstrap support values. However there was no high bootstrap value for the haplotypes within haplogroup I. Since the haplotypes in haplogroup I contained short branch lengths in the trees, this haplogroup may represent the most recent and common *Sycophila* lineages in Iran. The pattern in haplogroups II to V which are connected by high numbers of substitutions in the network may indicate the presence of cryptic species or several distinct taxa or these haplogroups may represent more ancient extant lineages within a single taxon. More phylogenetic analysis in the future will help to create improved estimates of phylogeny within the *Sycophila* haplotypes.

4.4.3 Mismatch distribution, and geographical distribution of haplogroups in *Sycophila*

Two alternative explanations (scenarios) could explain mismatch distribution analysis. First, when we consider the whole network, we can see that observed distribution follows a complex multimodal pattern and could infer that the *Sycophila* lineages have undergone three demographic expansions from the first expansion date (Figure 4.9). The expansion date was estimated at between 92,545-216,006 years BP, during the late Pleistocene and before the last glacial period. Unfortunately, fossil records for insects are rare especially in the Hymenoptera. Parasitic Hymenoptera first appear in the fossil record during the early Jurassic (130-160 Myr B. P) (Rasnitsyn 1980). The only known documented record in the family Eurytomidae comes from Yoshimoto (1975) who recorded two species of *Eurytoma* from the Neogene period (Miocene) (around 24 Myr BP) and one species of *Eudecatoma* (*Sycophila*) from Palaeogene period (Oligocene) (around 34 Myr BP). These two fossil records were found in North America. The time of origin of Eurytomids can date back to no later than the Cretaceous, since many recent chalcid genera, including Eurytomids are known from the Palaeogene period (Yoshimoto 1975). The second explanation is that, the three peaks observed in the mismatch distribution reflect the presence of three separate taxa or cryptic species. It seems likely that the second scenario is more probable because of the presence of large numbers of substitutions between the mtDNA haplogroups.

Interestingly haplogroup V, which comprises haplotype 5 from south-east Iran, constitutes a divergent lineage and possibly a new taxon. This haplogroup (haplotype) is located in the extreme south in an isolated mountain region with the highest elevation (4250 m) of the entire region in comparison to the other geographical sites (Figure 4.1). This haplogroup (haplotype) together with other divergent haplogroups (II, III, and IV)

may represent more ancient extant lineages which survived in isolated vicariant refugia during Pleistocene climatic fluctuations.

The bimodal pattern in the mismatch distribution for haplogroup I is also indicative of the presence of divergent lineages that may also represent cryptic taxa. The result of the NCA for clade 3.2 is inferred as restricted gene flow with isolation by distance. This clade comprises south-western and south-eastern haplotypes located in haplogroup I, except haplotypes 1 and 7. Most haplotypes are from south-western Iran and haplotypes 2, 3 and 4 contains individuals from different areas. The majority of haplotypes in this clade were separated by between 1 and 4 substitutions. A linear relationship between mtDNA sequence divergences and the time of splitting events has been calculated for recently diverged arthropod taxa by Brower (1994) suggesting a constant substitution rate of 1.1 to 1.2% per Myr and based on this, the average mtDNA divergence rate is estimated at 2.3% per Myr. For Hymenoptera different sequence divergences have been proposed for closely related species. For instance as mentioned in section 4.4.1 a sequence divergence between populations within a genus of Hymenoptera of 2.8% has been proposed (Danforth 1999). Extrapolation of this rate to the haplogroups recorded here, imply that divergence between haplogroups probably started from the late Pliocene and subsequent divergences continued during the Pleistocene, probably in the refugial areas followed by some colonisation events during inter-glacial periods when the climate was warmer (See section 4.4.5).

Surprisingly *Sycophila* parasitizes *Tetramesa* in *L. pseudosclerophylla* and *L. sclerophylla* groups with *Sycophila* also parasitizing *F. arundinacea* in the main south-eastern and south-western haplotypes. Therefore a question remains as to whether parasitism by *Sycophila* of *Tetramesa* from *Festuca* represents a recent evolutionary event?

4.4.4 Nested clade analysis and restricted gene flow with isolation by distance

In haplogroup I (southern haplotypes), restricted gene flow with isolation by distance was only inferred for clade 3.2. This indicates that historical demographic processes have had an important impact on genetic variation in this group. These processes seem to have had a great influence on the genetic architecture of many other insect taxa as revealed by NCA, including Coleoptera (Mardulyn 2001; Gómez-Zurita *et al.*. 2000) and Odonata (Turgeon & McPeek 2002). Restricted gene flow inferred for clade 3-2 was present over long geographic distances (maximum 500 km), between region 1a (south-east) versus region 7 (south-west), and region 2d (south-east) versus region 7 (south-west), and region 1a (south-east) versus region 7a (south-west). This suggests that *Sycophila* individuals have experienced long-distance dispersal and colonization. Some lines of evidence indicate that gene flow among populations of insects decrease with geographical distance to reinforce this idea (Britten *et al.*. 1995; Costa & Ross 1993; Loxdale *et al.*. 1985; McCauley & Eanes 1987). Isolation among populations can give rise to changes in phenotypic characters (Thomas *et al.*. 1998; Dempster 1991) and isolation by distance in organisms with a short dispersal range may have an important influence on genetic differentiation between populations. Long distance gene flow may occur between intermediate populations via 'stepping stone' habitats (Peterson 1996). Most insect species exist in heterogeneous habitats in particularly closed populations consisting of a single habitat patch that contains essential resources such as food and a reproduction site so that most individuals spend their lifetimes in this environment (New *et al.*. 1995; Southwood 1977). In this case a restricted gene flow may take place because individual insects are confined to these separated patches and as a result populations may be structured over small geographical distances. Population structuring and restricted (limited) gene flow has been indicated in

other insects (West 1996; Rosenburg 1989; Sokal *et al.*. 1987). Restricted gene flow together with other processes such as resource or habitat fragmentation in agricultural environments (especially in host-parasitoid interactions) due to climatic fluctuations or habitat alteration can dramatically affect the distribution of haplotypes that can survive in island habitats (Mader 1984; Templeton *et al.*. 1995; Cronin 2004). This may be the case for haplotypes 5 in the south-east and haplotypes 9, 17 and 19 in the south-west of Iran. However it was not possible to detect the relative importance of demographic historical versus contemporary processes in shaping the genetic variation of haplotypes at some highest level clades and the whole cladogram, which may imply that recent historical events are acting or indicate the weakness of the tests because of insufficient sample sizes (Avise 2000, Templeton *et al.* 1995).

NCA was, however not able to separate contemporary and historical events in the whole cladogram in all five haplogroups. This may mean that sampling over the geographical range has been insufficient and suggests a sampling scheme for future studies. *L. pseudosclerophylla* is distributed across southeastern and southwestern regions of Iran, west Pakistan, Afghanistan and Northern Iraq and is restricted to mountain areas. *L. sclerophylla* is distributed in northern and north-western Iran as well as in Turkey. Turkey is important as a potential source population and centre of genetic diversity during the Pleistocene (Rokas *et al.*. 2003). It was not possible to arrange an optimal sampling scheme in the present study because of the wide geographical distribution and taxonomic uncertainty of *Sycophila* (which may be considered as a species complex). As a result, *L. pseudosclerophylla* was collected from southeastern and southwestern regions and *L. sclerophylla* was collected from northern Iran. For future studies more samples need to be collected from additional and intermediate geographical areas (i.e. Mashad in north-eastern, Zagros Mountains in southwestern, Tabriz in North-

western Iran) and more extensively (i.e. from other countries) in order to be able to identify the effects of demographic processes and glacial refugia throughout its extensive geographical range with the aim of identifying the centre of genetic diversity for the species. This approach, coupled with morphological studies to clarify the taxonomic status of the *Sycophila* complex, ecological studies of host-parasitoid interactions and other theoretical and empirical studies are essential to place the phylogeographic patterns in context and firmly to relate the patterns observed in the network with the proposed scenarios.

The molecular markers used in this thesis were not able to detect any genetic differentiation between *Tetramesa* populations, which seems puzzling given that the 778 bp fragment of the mtDNA COI gene was monomorphic for *Tetramesa* populations yet polymorphic in *Sycophila* (see Dawah 1987, 1988b; for polymorphic parasitoid, *Eurytoma. danuvica* Erdös attacking the monomorphic host, *Trtramesa. eximia* Giraud using enzyme electrophoresis and see Dawah 1987, 1988b for polymorphic parasitoid, *Pediobius deschampsiae* Dawah attacking polymorphic host, *T. petiolata* (Walker) using enzyme electrophoresis and see Al-Barrak 2001 for polymorphic host, *T. petiolata*).. One possible explanation is that *Sycophila* could be evolving to adapt to the morphological differences between *Tetramesa* populations on different host plants in different regions (Al-Barrak 2001; Ghajarieh 2003). In contrast, *Tetramesa* may be showing phenotypic differences (as revealed by morphometrics in this study) but not genotypic, for a variety of reasons including 1) a recent adaptive radiation to different host plants, hence not enough time for molecular divergence to accumulate; 2) a recent population bottleneck reducing genetic variation or 3) extensive gene-flow homogenising genetic variation over a large spatial scale. In support of hypothesis 3, it appears that *Tetramesa* spp. seems to be highly vagile (H. A. Dawah Pers. Communication), so it is possible that the existence

of gene flow between populations swamps any local selection pressure. However, without population genetic analysis using polymorphic markers, the relative merits of these explanations cannot be assessed.

4.5 References

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Table 4.1. Sample size of chalcid wasps from Iran from which DNA was extracted.

Site	*Tetramesa.* spp.	*Eurytoma.* spp.	*Sycophila.* spp.	*Pediobius.* spp.	*Chlorocytus.* spp.	Grass species
1a	10	10	10	-	10	*L.pseudosclerophylla*
2a	-	1	10	-	-	*Festuca arundinacea*
2b	-	-	1	-	-	*L.pseudosclerophylla*
2d	10	5	10	-	10	*L.pseudosclerophylla*
4b	10	9	10	-	10	*L.pseudosclerophylla*
7	10	10	8	6	2	*L.pseudosclerophylla*
7a	5	10	4	3	5	*L.pseudosclerophylla*
7b	6	-	5	1	3	*L.pseudosclerophylla*
9b	5	-	5	-	5	*L.sclerophylla*
9c	5	-	5	-	5	*L.sclerophylla*

Table 4.2. Sample size of chalcid wasps from U.K which were used for DNA extraction.

Wasp population	Code	sample size	Grass species
Eurytoma pollux	E.p	1	*Calamagrostis epigejos* (L.) Roth.
Eurytoma appendigaster	E.a	1	*Deschampsia cespitosa* (L.) Beauv.
Eurytoma sp.nr apicalis	Esa	5	*Elymus repens* (L.) Gould, Madrono
Eurytoma flavimana	Ef	11	*E. repens*
Pediobius planiventris	PBs	1	*Brachypodium sylvaticum* (Huds.) Beauv.
Pediobius sp.nr claridgei	Per	1	*E repens*
Pediobius sp.	PPp	1	*Phleum pratense* L.
Pediobius deschampiae	PDc	1	*Deschampsia cespitosa* (L.) Beauv.
Chlorocytus phalaridis	CPa	1	*Phalaris arundinacea* L.

Table 4.3. List of the PCR primers selected for amplifying the COI-COII genes.

Primer	Sequence	Direction	Reference
COI			
S2792	5'-ATACCTCGACGTTATTCAGA-3'	Forward	Taylor *et al.* 1997
Jerry	5'-CAACATTTATTTTGATTTTTTGG-3'	Forward	Simon *et al.* 1994
Pat	5'-TCCAATGCACTAATCTGCCATATTA-3'	Reverse	Simon *et al.* 1994
COII			
Muscid	5'-TCAATATCATTGATGACCAAT-3'	Reverse	Taylor *et al.* 1997

Table 4 4. Final optimised PCR reaction parameters for "Jerry" and "Pat" (COI) (831 bp).

Population	Grass	Primer (μM)	$MgCl_2$ (mM)	dNTPs (mM)	Taq (U/μl)	DNA (μl)	T_{ANN} °C (Cycles)
Tetramesa spp	*L. s*	0.5	2	0.4	0.04	1	53(5) 51(5) 49 (24) T
Tetramesa spp	*L. p*	0.5	2	0.4	0.04	0.75	55(5) 53(5) 51 (15) T
Eurytoma spp *E. sp. nr apicalis*	*L. p* *E. repens*	0.5	2	0.4	0.04	1	55(5) 53(5) 51 (25) T
E. flavimana	*E. repens*	0.5	2	0.6	0.04	1	57(5) 54(5) 51(20) T
E. flavimana *E. appendigaster*	*E. repens* *D. cespitosa*	0.5	4	0.6	0.03	2	53(5) 51(5) 49 (24) T
Sycophila spp	*L. p* *L. s* *F. arundinacea*	0.5	3	0.6	0.04	1.5	53(5) 51(5) 49(24) T
Chlorocytus spp	*L. p*	0.5	2	0.4	0.04	1.5	53(5) 51(5) 49(25) T
Pediobius spp *P. planiventris* *P. sp.nr. claridgei* *p.* spp *P. deschampia*	*L. p* *B. sylvaticum* *E. repens* *P. pratens* *D. cespitosa*	0.5	3	0.8	0.03	2	52 (35)

Table 4.5. Final optimised PCR reaction parameters for "S2792" and "Muscid" (COI-COII) (608 bp).

Population	Grass species	Primer (μM)	$MgCl_2$ (mM)	dNTPs (mM)	Taq (U/μl)	DNA (μl)	T_{ANN} °C (Cycles)
Tetramesa spp	*L. p (S.E &S.W)*	0.8	2	0.8	0.04	2	48 (45)
Tetramesa spp	*L. p (S.E)* *L. s*	0.8	2.5	0.8	0.04	2	48 (35)
Eurytoma spp *Eurytoma spp* *E. pollux* *E. appendigaster* *E. sp. nr apicalis* *E. flavimana*	*L. p* *F. arundinacea* *C. epigejos* *D. cespitosa* *E. repens* *E. repens*	0.8	2.5	0.4	0.04	2	55(35)
Sycophila spp	*L. p* *L. s* *F. arundinacea*	0.8	2.5	0.2	0.025	2	55(30)
Chlorocytus spp	*L. p* *L. s* *P. arundinaceus*	0.8	2.5	0.2	0.04	2	55(35)
Pediobius spp *P. planiventris* *P. sp.nr. claridgei* *p. spp* *P. deschampia*	*L.p* *B. sylvaticum* *E. repens* *P. pratens* *D. cespitosa*	0.8	2	0.8	0.04	2	48 (35)

Table 4.6 Average frequencies base composition between Iranian wasp genera, *Sycophila*, *Tetramesa* and *Eurytoma*. All frequencies are given in percent.

Gene	Codon position	T	C	A	G
COI	1st	37.4	9.5	32.9	20.1
	2nd	47.3	18.1	19.6	15
	3rd	52.1	1.4	42.2	4.3
	Mean	45.6	9.7	31.6	13.1
COII	1st	34.4	8.6	38.2	18.7
	2nd	56.6	13.8	24	5.6
	3rd	50	0.8	45.5	3.7
	Mean	47	7.8	35.9	9.4
tRNA		41.5	6	41.8	10.7
insertion		38.4	4.3	49	8.4
All		45.4	8.7	34.1	11.8

Table 4.7 Number of Transitions (s), transversions (v) and transition/transversion ratio (R) for wasp species using COI sequences (778 bp) calculated using uncorrected-*p* distances.

Wasp		COI (1st, 2nd,3rd)	1ST	2ND	3RD
Sycophila	R	3.418 ± 1.733	2.280 ± 1.262	n/c	3.238 ± 1.340
	s	0.018 ± 0.002	0.003 ± 0.002	0.000 ± 0.000	0.017 ± 0.004
	v	0.006 ± 0.001	0.000 ± 0.000	0.000 ± 0.000	0.050 ± 0.007
Eurytoma	R	1.098 ± 0.404	0.933 ± 0.564	0.583 ± 0.430	1.046 ± 0.394
	s	0.021 ± 0.003	0.007 ± 0.003	0.002 ± 0.001	0.055 ± 0.007
	v	0.031 ± 0.004	0.008 ± 0.003	0.001 ± 0.001	0.083 ± 0.010
*Pediobius*Iran	R	4.400 ± 2.528	n/c	n/c	3.367 ± 2.407
	s	0.009 ± 0.002	0.003 ± 0.002	0.002 ± 0.002	0.021 ± 0.006
	v	0.002 ± 0.001	0.000 ± 0.000	0.000 ± 0.000	0.006 ± 0.004
Chlorocytus	R	0.917 ± 0.324	1.000 ± 0.992	0.400 ± 0.594	0.783 ± 0.281
	s	0.013 ± 0.002	0.008 ± 0.003	0.003 ± 0.002	0.028 ± 0.005
	v	0.018 ± 0.003	0.008 ± 0.003	0.004 ± 0.002	0.042 ± 0.007

Table 4.8 Number of Transitions (s), transversions (v) and transition/transversion ratio (R) for wasp species using COI long sequences (621 bp) in the phylogenetic analysis calculated using uncorrected-*p* distances.

Wasp		COI (1st, 2nd,3rd)	1ST	2ND	3RD
Sycophila	R	2.681 ± 1.026	2.286 ± 1.353	0.250 ± 0.182	2.600 ± 1.195
	s	0.022 ± 0.003	0.007 ± 0.003	0.002 ± 0.001	0.057 ± 0.009
	v	0.011 ± 0.002	0.001 ± 0.001	0.003 ± 0.003	0.028 ± 0.007
Eurytoma	R	n/c	n/c	n/c	n/c
	s	0.002 ± 0.001	0.003 ± 0.003	0.000 ± 0.000	0.003 ± 0.003
	v	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Tetramesa	R	n/c	n/c	n/c	n/c
	s	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.001
	v	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000

Table 4.9 Number of Transitions (s), transversions (v) and transition/transversion ratio (R) for wasp species using COII sequences (254 bp) in the phylogenetic analysis calculated using uncorrected-*p* distances.

Wasp		COII (1^{st},2^{nd},3^{rd})	1^{ST}	2^{ND}	3^{RD}
Sycophila	R	3.178 ± 1.367	n/c	0.000 ± 0.000	2.889 ± 1.347
	s	0.018 ± 0.005	0.012 ± 0.006	0.000 ± 0.000	0.042 ± 0.014
	v	0.006 ± 0.002	0.000 ± 0.000	0.003 ± 0.003	0.015 ± 0.006
Eurytoma	R	n/c	n/c	n/c	n/c
	s	0.005 ± 0.003	0.000 ± 0.000	0.000 ± 0.000	0.016 ± 0.011
	v	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Tetramesa	R	0.200 ± 0.196	0.000 ± 0.000	1.000 ± 1.051	0.000 ± 0.000
	s	0.002 ± 0.001	0.000 ± 0.000	0.005 ± 0.003	0.000 ± 0.000
	v	0.008 ± 0.002	0.009 ± 0.005	0.005 ± 0.003	0.010 ± 0.005

Table 4.10 Variable positions in the 778 bp segment of the COI gene defining 26 different haplotypes of *Sycophila* across Iran. ; dots (.) indicate identical nucleotides.

	Nucleotide position																																						
Haplotype	0 0 1	0 0 7	0 1 3	0 3 7	0 6 1	0 8 2	0 8 5	0 9 1	1 0 0	1 0 9	1 1 2	1 1 8	1 2 1	1 3 0	1 3 3	1 4 5	1 4 8	1 5 1	1 5 4	1 6 0	1 6 3	1 6 9	1 7 8	2 0 8	2 2 3	2 2 9	2 3 8	2 4 1	2 4 4	2 7 7	2 7 8	2 8 0	2 8 1	2 8 6	2 9 2	2 9 8	3 1 0	3 1 3	3 2 2	3 2 8	3 4 3	3 5 8	3 6 7
SHT1	A	G	G	T	T	T	G	T	G	T	C	G	G	T	A	T	G	T	T	A	T	C	G	A	G	A	G	C	A	G	T	G	T	T	A	A	T	A	T	T	A	G	T
SHT2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	T	.	.	.	.	A	.	.	.	C	.	.	.	G	.	.	.	.	.	.	.	.
SHT3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	A	G	.	.	A	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.
SHT4	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	.	C	.	.	.	.	.	A	.	.	.	.	.	.	.	G	.	.	.	.	C	.	.	.
SHT5	.	A	C	.	.	C	A	.	A	A	T	A	A	.	G	G	.	C	.	.	.	T	A	.	A	.	A	T	G	A	.	A	A	G	.	.	G	T	A	.	T	A	.
SHT6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	T	.	.	.	.	A	.	.	.	C	.	.	.	G	.	.	.	.	.	.	.	.
SHT7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A	G	A	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.
SHT8	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	C	.	C	T	.	.	.	.	A	.	.	.	C	.	.	.	G	.	.	.	.	.	.	.	.
SHT9	.	.	.	.	.	.	.	.	.	A	T	.	A	.	.	.	.	.	.	G	.	T	.	.	.	.	A	T	.	.	C	C	.	.	.	.	.	.	A	.	.	A	C
SHT10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	A	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.
SHT11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	A	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.
SHT12	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	A	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.
SHT13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	A	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.
SHT15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	A	.	.	.	.	.	.	.	G	.	.	.	.	C	.	.	.
SHT16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	A	T	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.
SHT17	G	A	T	.	.	.	A	.	.	.	T	.	.	.	.	A	A	.	.	.	.	T	.	.	.	.	A	T	.	.	.	.	.	.	G	G	.	T	.	.	G	A	.
SHT18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	A	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.
SHT19	.	A	T	.	.	.	A	.	.	.	T	.	.	.	.	A	A	.	.	.	.	T	.	.	.	.	A	T	.	.	.	.	.	.	G	G	.	T	.	.	G	A	.
SHT20	.	.	.	.	.	.	.	.	.	.	T	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	A	T	G	.	.	.	.	G	G	.	.	.	.	.	.	A	.
SHT21	.	.	.	.	.	.	.	.	.	.	T	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	A	T	G	.	C	.	.	G	G	.	.	.	.	.	.	A	.
SHT23	.	.	.	.	.	.	.	.	.	.	T	.	A	.	G	.	.	.	.	.	.	T	.	.	.	.	A	T	G	.	.	.	.	G	G	.	.	.	.	.	.	A	.
SHT26	.	.	.	C	.	.	.	.	.	.	T	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	A	T	.	.	.	.	.	G	G	.	.	.	.	.	.	A	.
SHT27	.	.	.	.	.	.	.	.	.	.	T	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	A	T	G	.	.	.	.	G	G	.	.	.	.	.	.	A	.
SHT28	.	.	.	.	.	.	A	.	.	.	T	.	.	.	G	.	.	.	.	.	C	T	.	.	.	.	A	T	G	.	.	.	.	G	G	.	.	.	.	.	.	A	.
SHT29	.	.	A	.	C	.	.	A	C	.	T	.	.	.	G	.	.	.	.	.	.	T	.	.	.	.	A	T	G	.	.	.	.	G	.	.	.	.	.	.	.	A	.

Table 4.10 Continued

Haplotype	Nucleotide position																																									
	3	3	3	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7
	7	8	9	1	3	3	4	4	5	5	6	0	1	3	4	5	5	5	5	6	6	7	8	8	8	9	9	0	1	3	4	4	7	7	8	1	1	4	5	5	5	6
	9	5	7	5	0	3	5	8	1	4	3	5	7	8	7	3	4	6	9	2	5	1	3	6	9	5	8	4	3	1	1	7	1	9	2	2	8	2	1	4	5	9
SHT1	**A**	**T**	**T**	**A**	**G**	**T**	**A**	**T**	**T**	**C**	**T**	**A**	**G**	**T**	**T**	**A**	**T**	**A**	**A**	**T**	**A**	**G**	**T**	**T**	**A**	**A**	**T**	**T**	**A**	**A**	**T**	**G**	**A**	**T**	**T**	**G**	**A**	**T**	**T**	**T**	**C**	**T**
SHT2	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHT3	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	G	.	.	.	C	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHT4	.	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHT5	.	.	A	G	T	A	.	.	C	T	G	.	T	.	C	G	C	T	.	.	.	A	.	.	G	T	.	.	G	T	.	.	G	.	.	A	.	.	.	C	.	.
SHT6	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHT7	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHT8	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHT9	.	C	.	.	A	G	.	.	.	T	G	.	.	.	.	G	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	A	.	.	.	.	.	.	.	.	.	A
SHT10	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHT11	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHT12	.	.	.	.	.	G	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHT13	.	.	.	.	.	G	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHT15	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHT16	G	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHT17	.	C	.	.	A	G	.	.	.	T	G	.	.	.	.	G	.	.	T	.	.	.	C	.	.	G	.	.	.	T	.	.	G	.	C	T	.	.	.	T	.	.
SHT18	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	w	.	.	.	.	.	.	.	.	.	.	.
SHT19	.	C	.	.	A	G	.	.	.	T	G	.	.	.	.	G	.	.	T	.	.	.	C	.	.	G	.	.	.	T	.	.	G	.	C	T	.	.	.	T	.	.
SHT20	.	C	.	.	A	A	.	.	.	T	G	.	.	.	.	G	.	.	T	.	.	.	.	.	.	.	.	C	.	T	.	.	.	.	.	T	G	C	.	.	.	.
SHT21	.	.	.	.	.	G	G	.	.	T	A	G	.	.	.	G	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	G	C	.	.	.	.
SHT23	.	C	.	.	A	G	.	.	.	T	G	.	.	.	.	G	.	.	T	.	.	.	.	.	G	.	.	C	.	T	.	.	.	.	.	T	G	C	.	.	T	.
SHT26	.	C	.	.	C	G	.	.	.	T	G	.	.	.	.	G	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	.	T	G	C	.	.	.	.
SHT27	.	C	.	.	A	G	.	.	.	T	G	.	.	.	.	G	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	G	C	C	.	.	.
SHT28	.	.	.	.	A	G	.	C	.	T	G	.	.	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	G	C	.	.	.	.
SHT29	.	.	.	.	A	G	.	.	.	T	G	.	.	C	.	G	.	.	T	.	.	.	.	.	.	.	.	C	.	T	.	.	.	.	.	T	G	C	.	.	.	.

Table 4.11 Variable positions in the 778 bp segment of the COI gene defining 16 different haplotypes of *Eurytoma* sp. in Iran and the U.K; dots (.) indicate identical nucleotides.

	Nucleotide position																																									
Haplotype	001	004	007	013	019	022	028	036	037	040	043	052	058	064	067	073	094	100	101	103	106	109	118	121	133	139	142	145	148	151	154	157	160	163	166	169	181	184	189	196	199	202
EHT1	A	T	A	A	T	T	A	G	A	A	A	G	T	A	A	A	A	A	T	A	G	A	A	A	A	T	T	T	A	T	A	T	A	A	T	T	T	T	T	T	A	T
EHT2	.	.	.	.	.	.	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
EHT4	.	.	.	.	.	.	.	.	.	.	.	.	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.
EF1	.	.	.	.	.	.	.	.	G	.	.	A	A	G	T	G	.	T	.	.	.	.	.	.	G	A	A	.	.	.	T	.	C	T	.	.	.	.	.	A	.	C
EF2	.	.	.	.	.	.	.	.	.	.	.	.	A	G	T	.	.	T	.	.	.	.	.	.	.	A	A	.	.	.	T	.	C	T	.	.	.	.	.	A	.	C
EF3	.	.	.	.	.	.	.	.	.	.	.	A	A	G	T	.	.	T	.	.	.	.	.	.	.	A	A	A	.	C	T	.	.	T	.	.	.	.	.	A	.	.
EF4	.	.	.	.	.	.	.	.	.	.	.	A	A	G	T	.	.	T	.	.	.	.	.	.	.	A	A	.	.	.	T	.	C	T	.	.	.	.	.	A	.	C
EF5	.	.	.	.	.	.	.	.	.	.	.	A	A	G	T	.	.	T	.	.	.	.	.	.	.	A	A	A	.	C	T	.	.	T	.	.	.	.	.	A	.	.
EF6	.	.	.	.	.	.	.	.	.	.	.	A	A	G	T	.	.	T	.	.	.	.	.	.	.	A	A	A	.	C	T	.	.	T	.	.	.	.	.	A	.	.
EF7	.	.	.	.	.	.	.	.	.	.	.	A	A	G	T	.	.	T	.	.	.	.	.	.	.	A	A	A	.	C	T	.	.	T	.	.	.	.	.	A	.	.
EF8	.	.	.	.	.	.	.	.	.	.	.	A	A	G	T	.	.	T	.	.	.	.	.	.	.	A	A	A	.	C	T	.	.	T	.	.	.	.	.	A	.	.
EP1	.	.	T	.	C	.	.	.	.	G	.	A	C	T	.	.	G	.	.	G	A	.	.	.	.	.	A	A	G	.	T	.	G	.	.	.	.	A	.	.	.	.
EF2A1	T	.	.	T	.	.	G	.	G	.	.	A	C	.	T	.	.	T	.	.	A	.	.	G	.	.	.	A	G	.	.	.	T	G	.	.	.	.	.	.	T	.
ESPA4	.	A	.	.	.	.	G	.	.	.	.	.	.	G	T	.	G	.	.	.	A	.	T	.	.	A	A	A	G	.	T	C	.	.	C	C	.	.	.	.	T	.
EA1	.	.	.	.	.	A	.	.	.	G	.	A	.	T	T	.	.	.	C	.	A	.	.	.	.	A	A	A	G	.	T	.	.	.	.	.	A	A	.	.	.	.
ESPA5	.	A	.	T	.	A	.	A	T	.	G	.	A	G	T	.	.	T	.	.	A	T	T	.	.	.	.	A	.	.	G	.	T	.	A	.	.	A	.	A	T	.

Table 4.11 Continued

	Nucleotide position																																								
Haplotype	2 0 5	2 0 8	2 1 4	2 1 7	2 2 0	2 2 3	2 2 6	2 2 7	2 3 2	2 3 5	2 4 4	2 5 6	2 6 2	2 6 5	2 6 8	2 7 4	2 7 7	2 7 8	2 8 0	2 8 1	2 8 3	2 9 2	2 9 5	3 0 4	3 0 7	3 1 0	3 1 3	3 1 9	3 2 2	3 2 9	3 3 1	3 3 4	3 4 0	3 4 3	3 5 2	3 5 3	3 5 5	3 5 8	3 7 9	3 8 2	3 9 7	4 0 3
EHT1	A	T	A	T	T	A	A	T	A	T	A	A	A	A	A	A	A	A	A	T	A	A	T	T	A	A	C	T	A	C	T	A	T	A	T	G	A	A	A	A	A	A
EHT2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
EHT4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
EF1	.	.	.	.	.	G	G	.	G	.	.	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	A	.	T	.	.	.	.	C	.	.	.	.	T	.
EF2	T	.	.	.	.	G	G	C	.	C	.	.	.	.	.	G	.	C	.	.	.	G	.	.	.	.	A	.	T	.	.	.	.	.	.	.	.	T	.	T	.
EF3	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	A	.	T	.	.	.	.	.	.	.	G	.	.	T	.
EF4	.	.	.	.	.	G	G	C	.	C	.	G	.	.	.	G	.	C	.	.	.	G	.	.	.	.	A	.	T	.	.	.	.	.	.	.	.	T	.	T	.
EF5	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	A	.	T	.	.	.	.	.	.	.	G	.	.	T	.
EF6	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	A	.	T	.	.	.	.	.	.	.	G	.	.	T	.
EF7	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	A	.	T	.	.	.	.	.	.	.	G	.	.	T	.
EF8	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	A	.	T	.	.	.	.	.	.	.	G	.	.	T	.
EP1	.	.	.	C	.	T	.	.	T	A	.	.	.	.	T	.	.	T	.	A	G	.	.	.	.	.	T	A	T	.	.	T	.	.	.	T	A	.	T	T	T
EF2A1	.	A	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	A	.	.	.	T	.	T	.	.	.	.	.	T	T
ESPA4	.	A	G	.	.	.	.	.	T	A	.	.	T	G	.	.	.	C	T	A	.	.	.	.	.	.	G	A	.	.	.	.	A	.	.	A	T	.	.	T	T	.
EA1	.	A	.	.	.	T	.	.	T	A	.	.	.	.	T	.	.	T	G	.	.	.	C	.	T	.	T	A	T	.	.	T	T	.	.	.	T	.	.	T	.	T
ESPA5	T	A	.	.	.	.	.	C	T	.	T	G	C	T	T	.	G	T	.	.	.	G	.	A	T	T	A	A	.	T	A	T	A	T	.	.	.	.	.	T	T	T

Table 4.11 (Continued)

Nucleotide position Haplotye	409	412	415	421	430	433	447	451	455	460	463	469	472	478	481	485	500	505	517	520	524	525	526	529	535	538	541	550	553	556	559	562	565	568	571	574	580	583	589	601	611	613
EHT1	**T**	**T**	**T**	**T**	**A**	**A**	**T**	**T**	**T**	**A**	**A**	**T**	**A**	**A**	**A**	**T**	**A**	**A**	**A**	**T**	**A**	**C**	**T**	**T**	**C**	**T**	**A**	**A**	**A**	**A**	**A**	**T**	**A**	**T**	**A**	**T**	**A**	**T**	**T**	**A**	**G**	**A**
EHT2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
EHT4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
EF1	A	A	.	A	.	.	.	A	A	.	T	.	.	.	.	.	T	.	.	A	.	.	.	.	T	.	.	.	.	G	.	.	G	.	.	A	.	.	A	.	.	G
EF2	G	A	.	A	.	.	.	A	A	.	T	.	.	.	.	.	T	.	.	A	.	.	.	.	T	.	.	.	.	G	T	.	G	.	.	A	.	.	A	.	.	G
EF3	A	A	.	A	.	.	.	A	A	.	T	.	.	.	.	.	T	.	.	A	.	.	.	.	T	.	G	.	G	G	T	.	.	.	.	A	.	.	A	.	.	.
EF4	G	A	.	A	.	.	.	A	A	.	T	.	.	.	.	.	T	.	.	A	.	T	.	.	T	.	.	.	.	G	T	.	G	.	.	A	.	.	A	.	.	G
EF5	A	A	.	A	.	.	.	A	A	G	T	.	.	.	.	.	T	.	.	A	.	.	.	.	T	.	.	.	G	G	T	.	.	.	.	A	.	.	A	.	.	.
EF6	A	A	.	A	.	.	.	A	A	.	T	.	.	.	.	.	T	.	.	A	.	.	.	.	T	.	G	.	G	G	T	.	.	.	.	A	.	.	A	.	.	.
EF7	A	A	.	A	.	.	.	A	A	.	T	.	.	.	.	.	T	.	.	A	.	.	.	.	T	.	G	.	G	G	T	.	.	.	.	A	.	.	A	.	.	.
EF8	A	A	.	A	.	.	.	A	A	.	T	.	.	.	.	.	T	.	.	A	.	.	.	.	T	.	G	.	G	G	T	.	.	.	.	A	.	.	A	.	A	.
EP1	.	A	A	A	.	.	.	.	A	.	T	C	.	.	.	.	.	G	G	.	T	T	A	.	.	.	.	.	.	.	.	A	G	.	.	.	.	.	A	.	.	T
EF2A1	A	A	A	A	T	T	.	.	A	.	T	.	G	.	.	.	.	G	G	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	T	.	.	.	A	G	.	T
ESPA4	A	A	A	A	.	G	.	A	A	.	T	A	.	.	.	.	.	.	T	A	.	.	.	.	T	.	.	.	G	.	.	.	.	.	.	.	.	.	.	G	A	.
EA1	.	G	A	A	.	.	.	.	A	.	T	.	.	G	.	C	.	.	.	A	.	.	A	.	.	.	.	G	.	.	.	A	.	.	.	.	.	C	A	.	A	T
ESPA5	A	A	A	.	G	T	A	A	.	.	.	.	.	.	T	.	.	.	.	G	T	T	A	A	T	.	.	.	.	G	.	A	.	A	.	A	T	.	.	.	.	T

Nucleotide position Haplotype	619	622	628	631	632	634	640	641	644	646	649	652	653	655	663	671	673	676	685	688	691	697	724	730	736	743	745	752	760	766	767	769	774	778
EHT1	**T**	**A**	**T**	**A**	**A**	**T**	**T**	**T**	**A**	**A**	**T**	**A**	**A**	**A**	**T**	**T**	**A**	**G**	**A**	**A**	**A**	**A**	**T**	**A**	**A**	**G**	**A**	**A**	**A**	**T**	**T**	**T**	**A**	**A**
EHT2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
EHT4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
EF1	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	A	.	.	.	T	.	.	.	A	.	.	.	.	.	A	.	.
EF2	.	.	.	.	.	.	A	C	.	.	A	.	.	.	G	.	.	A	.	.	.	T	.	.	.	A	.	.	.	.	.	A	.	.
EF3	.	.	A	.	.	.	A	.	.	.	A	.	.	.	.	.	.	A	.	.	.	T	.	.	.	A	.	.	.	C	.	A	.	.
EF4	.	.	.	.	.	.	A	C	.	.	A	.	.	.	G	.	.	A	.	.	.	T	.	.	.	A	.	.	.	.	.	A	.	.
EF5	.	.	G	.	.	.	A	.	.	.	A	.	.	.	.	.	.	A	.	.	.	T	.	.	.	A	.	.	.	C	.	A	.	.
EF6	.	.	A	.	.	.	A	.	.	.	A	.	.	.	.	.	.	A	.	.	T	T	.	.	.	A	.	.	.	C	.	A	.	.
EF7	.	.	A	.	.	.	A	.	.	.	A	.	.	.	.	.	.	A	.	.	T	T	.	.	.	A	.	.	.	C	.	A	.	.
EF8	.	.	A	.	.	.	A	.	.	.	A	.	.	.	.	.	.	A	.	.	.	T	.	.	.	A	.	.	.	.	.	A	.	.
EP1	A	G	A	.	T	A	.	.	.	T	A	.	T	.	.	.	.	A	T	.	T	.	.	.	G	A	T	T	.	.	.	A	.	.
EF2A1	.	.	A	.	.	.	.	.	.	.	A	.	.	.	.	.	.	A	.	.	T	.	.	G	.	.	T	T	.	.	.	A	.	G
ESPA4	A	T	A	T	.	A	.	.	T	.	A	T	.	.	.	.	.	A	.	G	T	G	.	T	.	.	.	T	.	.	A	.	T	.
EA1	A	.	A	G	C	A	.	.	.	T	A	.	T	T	.	.	.	A	.	.	T	.	C	G	.	A	T	G	.	.	.	A	.	.
ESPA5	.	.	A	.	G	A	A	.	.	.	A	T	T	.	.	C	T	A	.	.	T	.	.	.	.	A	.	T	T	.	.	A	.	.

Table 4.12 Variable positions in the 778 bp segment of the COI gene defining 8 different haplotypes of *Pediobius* sp. in Iran and the U.K ; dots (.) indicate identical nucleotides.

	Nucleotide position																														
Haplotype	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	2	2	2	2	2	3	3	3	3	3	3	3	2	4	4
	1	3	4	6	7	73	0	0	2	2	4	5	6	6	8	0	3	4	7	8	0	1	1	2	2	6	6	7	8	0	3
	4	4	3	4	0		6	9	1	4	5	4	3	6	4	5	8	4	1	6	7	0	3	2	3	4	7	0	2	9	0
PHT1	C	T	A	G	C	A	A	C	G	T	A	C	T	T	T	A	A	A	A	T	A	A	A	A	A	T	A	T	T	A	A
PHT2	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PHT3	T	.	G	.	T	.	.	T	A	.	.	T	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
PHT4	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
PDC1	T	C	.	.	T	.	G	T	.	.	.	T	.	.	.	T	.	G	.	A	.	.	T	G	.	.	.	.	.	.	G
PER1	T	.	.	.	T	.	.	T	A	.	.	A	.	.	A	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	G
PPP1	T	.	T	A	T	G	.	T	A	A	G	T	G	C	.	T	G	G	G	.	G	G	T	G	G	.	.	C	C	.	G
PBS1	T	.	.	A	T	.	.	.	.	.	G	T	.	.	A	T	.	G	.	.	.	.	T	.	.	C	G	.	.	G	G

	Nucleotide position																														
Haplotye	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7
	4	5	6	8	9	0	3	5	5	6	7	8	8	8	9	1	3	4	6	6	7	7	8	9	9	0	5	5	6	7
	9	3	8	1	6	5	8	3	9	8	1	3	6	9	8	3	7	0	4	6	6	9	2	1	7	6	4	7	6	8
PHT1	C	C	C	A	A	A	T	T	T	T	T	T	T	G	T	T	G	T	T	A	G	A	T	G	A	A	T	G	C	A
PHT2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PHT3	.	.	.	.	.	.	.	.	G	.	.	.	.	A	.	T	.	.	.	.	.	.	.	A	.	.	C	A	.	.
PHT4	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	C	A	.	.
PDC1	.	.	.	.	G	.	C	.	G	C	.	C	C	A	.	T	.	.	.	.	.	G	.	A	G	.	C	A	.	.
PER1	T	.	.	.	.	.	.	.	.	.	.	.	.	A	.	T	.	.	.	T	.	.	C	A	.	G	.	A	T	.
PPP1	.	T	T	.	.	.	.	.	A	.	G	.	.	A	.	C	A	.	C	.	A	.	.	T	.	.	.	T	.	G
PBS1	.	.	.	G	.	G	C	C	.	.	.	.	.	A	C	T	.	C	.	.	.	.	.	A	.	.	.	A	.	.

Table 4.13 Variable positions in the 778 bp segment of the COI gene defining 6 different haplotypes of *Chlorocytus* sp.across Iran; dots (.) indicate identical nucleotides.

Haplotype	Nucleotide position																																									
	007	013	022	044	067	082	094	101	104	121	124	145	148	154	175	196	202	205	227	229	244	268	307	310	313	316	322	329	334	343	356	358	361	382	412	448	451	463	464	468	484	517
CHT1	A	A	T	T	T	C	T	C	C	A	T	T	A	A	A	T	T	A	C	T	T	A	T	T	T	A	T	T	A	A	C	T	C	C	A	T	A	T	A	T	G	A
CHT2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CHT3	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.
CHT4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.
CHT5	G	T	.	A	A	T	A	T	T	G	A	A	G	G	T	A	C	T	T	A	A	T	C	A	A	G	A	C	T	T	T	A	T	T	T	.	T	A	T	C	A	G
CHT6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Haplotype	Nucleotide position																												
	535	559	562	568	571	589	614	622	628	637	646	652	653	657	671	682	716	721	730	733	746	747	748	750	754	757	759	760	772
CHT1	C	A	T	T	T	A	T	A	T	C	A	T	T	T	T	A	T	T	T	T	C	C	T	A	T	A	G	A	C
CHT2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.
CHT3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CHT4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CHT5	T	G	G	A	A	T	C	T	A	T	G	A	A	A	A	G	A	A	A	.	G	A	A	T	C	T	.	T	T
CHT6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.

Table 4.14 Haplotype distribution by population, available sample sizes and number of haplotypes for *Sycophila* sp.

Population/ Haplotype	1a	F2a	2b	2d	4b	7	7a	7b	9b	9c	N
SYCHT1	8										8
SYCHT2	1	1				1					3
SYCHT3		3	1	6							10
SYCHT4		4		2							6
SYCHT5					7						7
SYCHT6					1						1
SYCHT7					1						1
SYCHT8					1						1
SYCHT9						1					1
SYCHT10						1					1
SYCHT11		1				2					3
SYCHT12						1					1
SYCHT13						2					2
SYCHT15							1				1
SYCHT16							1				1
SYCHT17							1				1
SYCHT18							1				1
SYCHT19								1			1
SYCHT20									1		1
SYCHT21									1		1
SYCHT23									1		1
SYCHT26										1	1
SYCHT27										1	1
SYCHT28										1	1
SYCHT29										2	2
N	**9**	**9**	**1**	**8**	**10**	**8**	**4**	**1**	**3**	**5**	**58**
Haplotypes	**2**	**4**	**1**	**2**	**4**	**6**	**4**	**1**	**3**	**4**	

Table 4.15 Haplotype and nucleotide diversities for all populations ± standard deviations for *Sycophila* sp. Estimates are based on pairwise differences.

Region	Population	h ± SD	Π ± SD
SE	1a	0.2222 ± 0.1662	0.0023 ± 0.0017
SE	2b	1.0000 ± 0.0000	0.0000 ± 0.0000
SE	2d	0.4286 ± 0.1687	0.0039 ± 0.0026
SE	4b	0.5333 ± 0.1801	0.0299 ± 0.0163
SW	7	0.9286 ± 0.0844	0.0101 ± 0.0060
SW	7a	1.0000 ± 0.1768	0.02164 ± 0.0147
SW	7b	1.0000 ± 0.0000	0.0000 ± 0.0000
N	9b	1.0000 ± 0.2722	0.0094 ± 0.0076
N	9c	0.9000 ± 0.1610	0.0108 ± 0.0070
SE	F2a	0.7500 ± 0.1121	0.0059 ± 0.0036
	Overall	**0.9292 ± 0.0169**	**0.0259 ± 0.0129**

Table 4.16 Haplotype distribution by population, available sample sizes and number of haplotypes for *Chlrocytus* sp.

Population/ Haplotype	1a	2d	4b	7a	7b	9b	9c	Pal	N
CHLHT1	5	5		4	1	1	2		18
CHLHT4		2							2
CHLHT3			4						4
CHLHT2				1					1
CHLHT6						1			1
CHLHT5								1	1
N	**5**	**7**	**4**	**5**	**1**	**2**	**2**	**1**	**27**
Haplotypes	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**1**	

Table 4.17 Haplotype and nucleotide diversities for all populations ± standard deviations for *Chlorocytus* sp. Estimates are based on pairwise differences.

Region	Population	h ± SD	Π ± SD
SE	1a	0.0000 ± 0.0000	0.0000 ± 0.0000
SE	2d	0.4762± 0.1713	0.0006 ± 0.0007
SE	4b	0.0000 ± 0.0000	0.0000 ± 0.0000
SW	7a	0.4000 ± 0.2373	0.0005 ± 0.0007
SW	7b	1.0000 ± 0.0000	0.0000 ± 0.0000
N	9b	1.0000 ± 0.5000	0.0013 ± 0.0018

Table 4.18 AMOVA based on pairwise difference between two haplogroups for *Sycophila* populations. Locations are partitioned by haplogroups I (Southern haplotypes) and haplogroup II (northern haplotypes).

	% Variance
Among groups	- 3.01
Among populations within regions	35.50
Within populations	67.51
Fst	0.32487
Nm	1.04

Table 4.19 AMOVA based on pairwise difference between haplotypes for *Sycophila* populations when all populations are considered.

	% Variance
Among populations	6.97
Within populations	93.03
Fst	0.06968
Nm	6.75

Table 4.20 AMOVA based on pairwise difference between haplotypes for *Sycophila* populations within haplogroup II (northern haplotype) only.

	% Variance
Among poulations	5.83
Within populations	94.17
Fst	0.05830
Nm	8.1

Table 4.21 AMOVA based on pairwise difference between haplotypes for *Sycophila* populations within haplogroup I (southern haplotype) only.

	% Variance
Among poulations	37.68
Within populations	62.32
Fst	0.37677
Nm	0.83

Table 4.22 AMOVA based on pairwise difference between haplotypes for *Sycophila* populations in haplogroup I (southern haplotype). Locations are partitioned by south-east and south-wast regions.

	% Variance
Among regions	−3.15
Among populations within regions	39.77
Within populations	63.39
Fst	0.36615
Nm	0.87

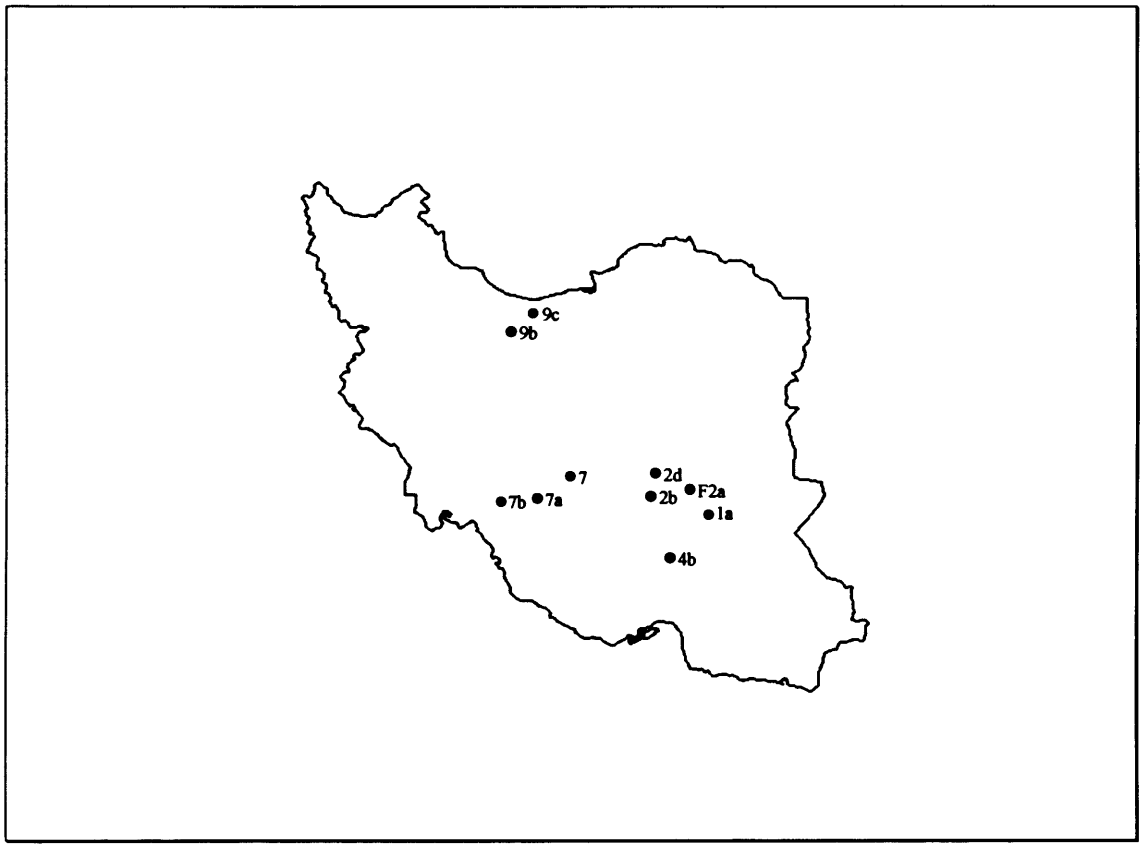
Table 4.23 Pairwise Fst comparisons (below diagonal) and Nm (above diagonal) between populations of *Sycophila* sp. Significance values: * P<0.05, ** P<0.01, *** P<0.001, ns = not significanct.

popolation	1a	F2a	2b	2d	4b	7	7a	7b	9b	9c
1a	-	0.29	0.11	0.16	0.29	0.60	0.67	0.03	0.08	0.11
F2a	0.6314 ***	-	-20.5	4.73	0.30	3.83	2.56	0.09	0.14	0.16
2b	0.8181 ns	-0.0250 ns	-	-1.20	0.82	73.02	-1.84	nc	0.22	0.25
2d	0.7527 ***	0.0956 ns	-0.7142 ns	-	0.29	1.19	1.18	0.06	0.10	0.13
4b	0.6323 **	0.6236 **	0.3795 ***	0.6302 ***	-	0.40	0.65	0.81	0.56	0.48
7	0.4552 ***	0.1155 *	0.0068 ns	0.2961 **	0.5540 **	-	-36.21	0.20	0.27	0.27
7a	0.4288 **	0.1631 ns	-0.3741 ns	0.2979 *	0.4350 *	-0.0140 ns	-	1.71	0.67	0.57
7b	0.9365 ***	0.8481 ns	1.0000 ns	0.9008 ***	0.3828 ***	0.7194 ns	0.2260 ***	-	0.26	0.31
9b	0.8622 **	0.7849 ***	0.6986 ns	0.8340 **	0.4696 *	0.6532 **	0.4285 ns	0.6562 ns	-	16.07
9c	0.8160 ***	0.7591 ***	0.6640 ns	0.7981 ***	0.5114 **	0.6472 ***	0.4681 *	0.6181 ns	0.0301 ns	-

Table 4. 24 Great circle distances (km) between two points calculated from latitude and longitude coordinates using the programme Earth (Byers, 1999).

a	1a	F2a	2b	2d	4b	7	7a	7b	9b	9c
1a	-									
F2a	10.73	-								
2b	28.68	27.68	-							
2d	20.56	30.41	31.16	-						
4b	148.69	159.28	153.88	129.46	-					
7	508.05	516.31	496.67	487.91	387.60	-				
7a	483.87	490.60	467.41	465.01	386.84	102.71	-			
7b	603.57	610.13	586.56	584.85	505.65	154.63	120.12	-		
9b	962.99	968.93	934.38	957.10	979.44	845.51	747.52	723.44	-	
9c	886.60	885.53	858.32	878.92	890.27	734.24	637.70	609.24	115.21	-

Figure 4.1 Location, coordinates (latitude and longitude) and elevation for sample sites in Iran.



Province	Collection Sites and codes	Latitude	Longitude	Elevation (m)
Kerman	1a Sirch	30 07 15 N	57 27 10 E	3750
(South-east)	F2a Bidouieh	30 11 40 N	57 31 30 E	3520
	2b Deh-Salah	30 20 00 N	57 17 00 E	2300
	2d Deh Lo-Lo, Kouhpayeh	30 03 15 N	57 15 12 E	3620
	4b Seyahbenouieh, Rabour	29 15 10 N	56 17 07 E	4250
Fars	7 Dasht-e-Morghab	29 15 08 N	52 17 07 E	2300
(South-west)	7a Sheshpir (Shiraz-Sepidan Rd.)	30 10 10 N	52 25 02 E	2150
	7b Sepidan-Yasuj Rd.	30 15 06 N	51 10 12 E	2780
Tehran	9b Gachsar (Karaj-Chalous Rd)	36 45 11 N	50 45 30 E	3940
(North)	9c Sirachal (Karaj-Chalous Rd.)	35 43 07 N	50 40 20 E	4100

Figure 4.2 The Neighbor Joining phenogram for *Sycophila*, *Tetramesa* and *Eurytoma* for COI and COII genes based on GTR + G model, (G = 0.4670). bootstrap values are displayed where clusters diverge.

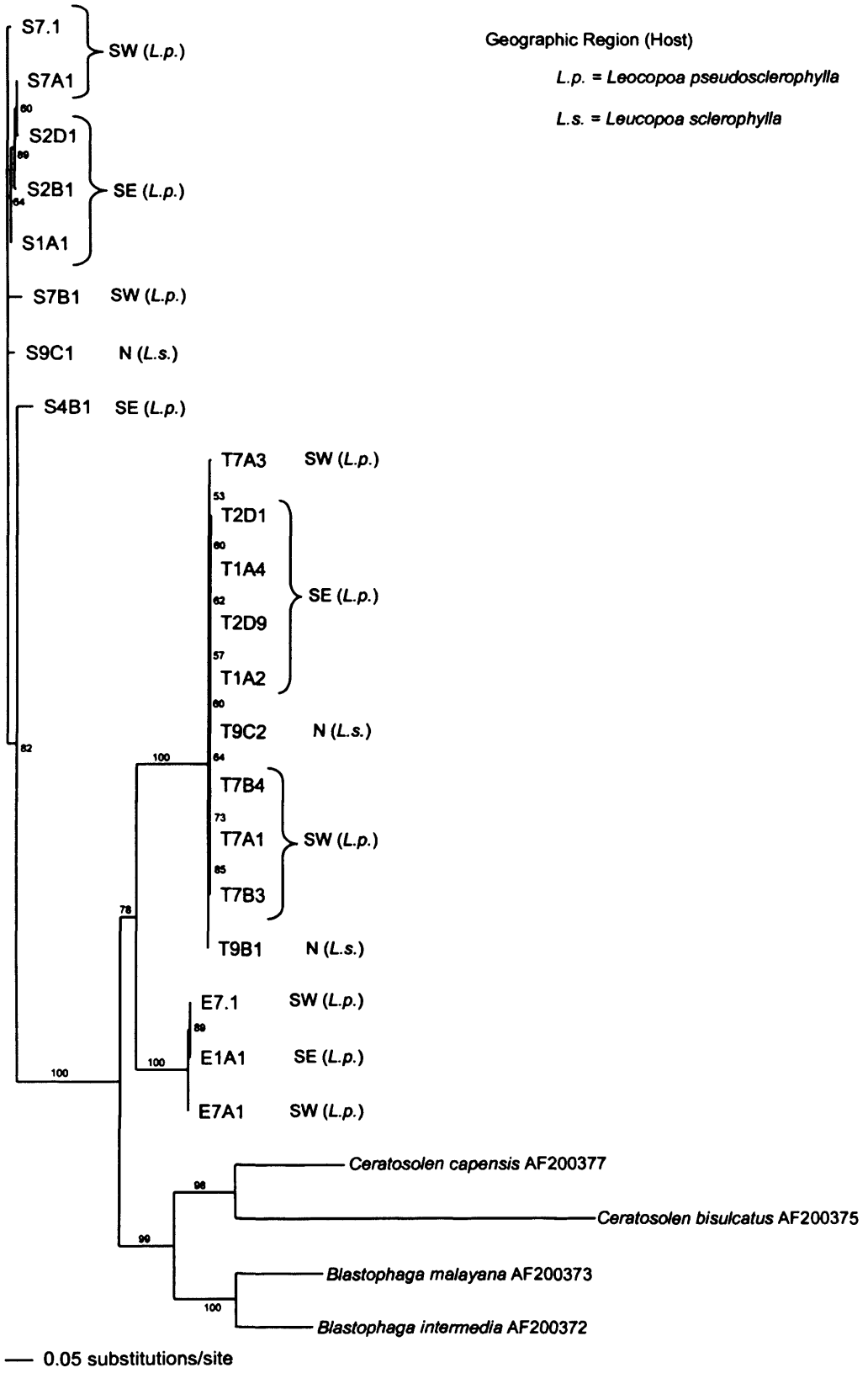


Figure 4.3 The Maximium Likelihood tree with bootstrap values for *Sycophila*, *Tetramesa* and *Eurytoma* for COI and COII gene based on the GTR + G (G = 0.4670).

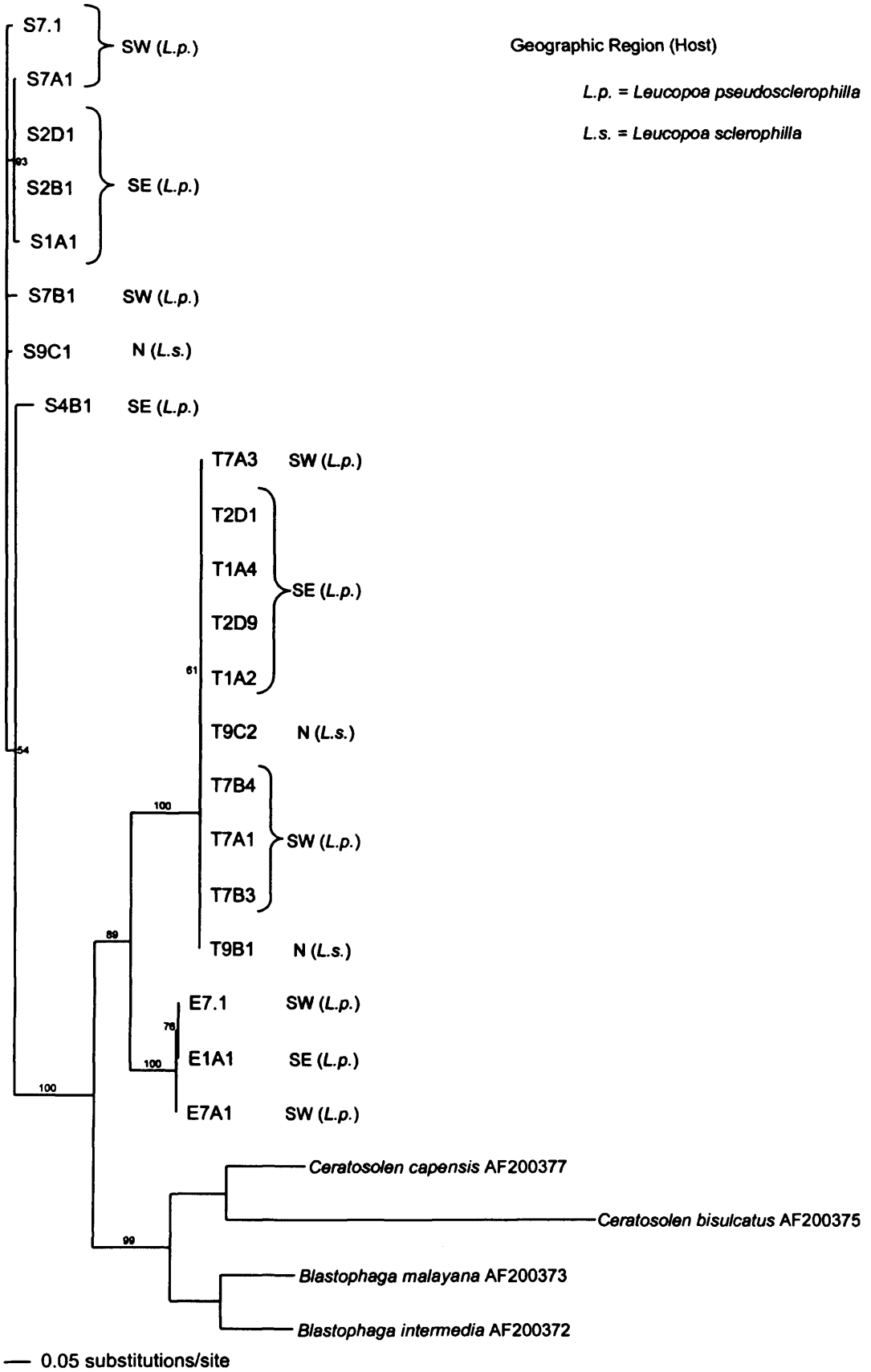


Figure 4.4 : Alignment of tRNA $_{LEU}$ to show secondary structure. Shaded areas indicate areas of complimentary base pairing in stems.

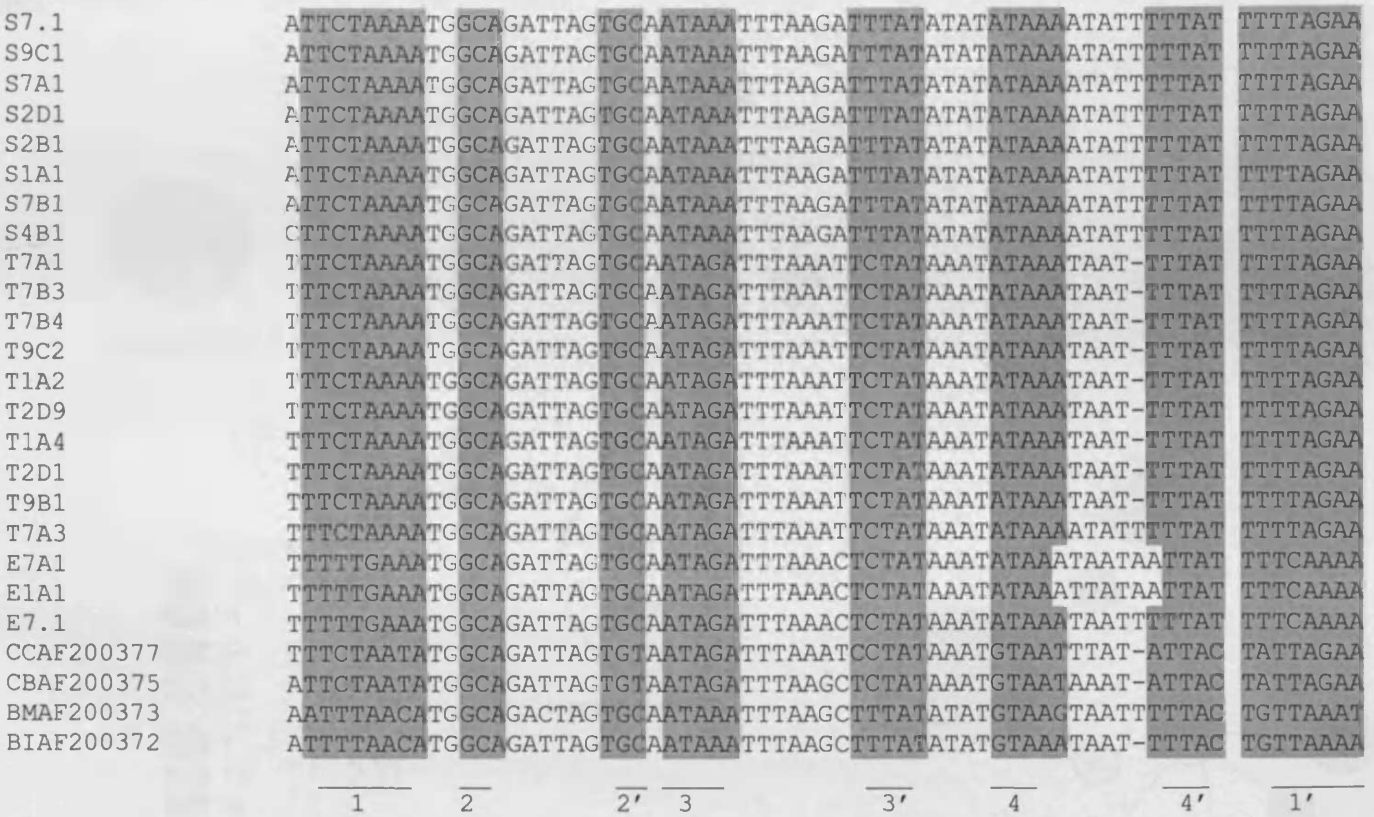


Figure 4.5 A minimum spanning network of the *Sycophila* haplotypes representing 5 haplogroups: haplogroup I (southern haplotypes), haplogroup II (northern haplotypes), haplogroup III (southwest haplotypes), haplogroup IV (southwest haplotype), haplogroup V (southeast haplotype). The size of haplotypes is relatively proportional to their frequency. The locations are represented by pie charts. The number of substitutions between haplotypes are shown on the lines. The dashed lines represent ambiguous, but the most parsimonious connections between haplotypes.

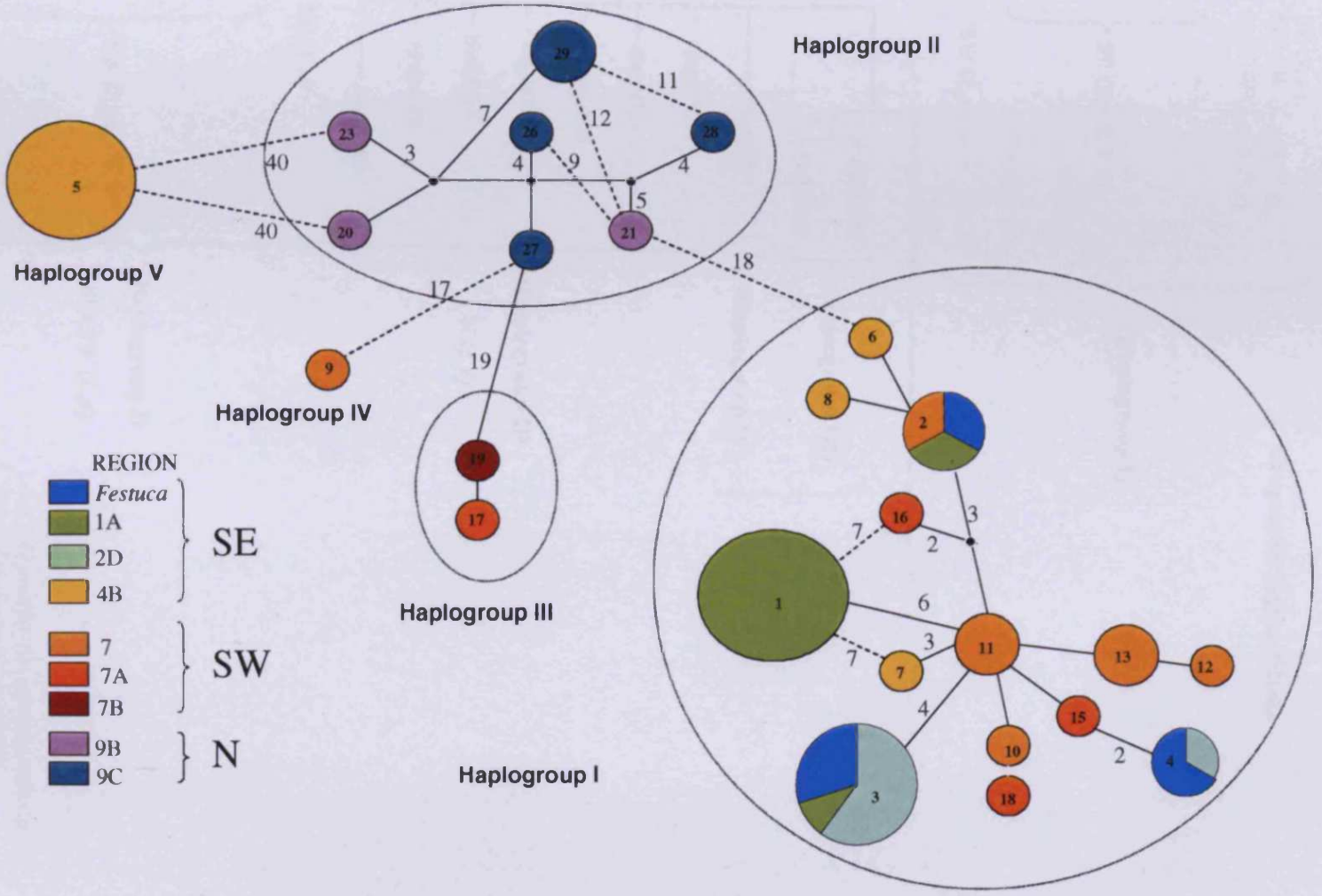


Figure 4.6 The Neighbour Joining phenogram for *Sycophila* haplotypes with 1000 bootstrap replication. Bootstrap support values are displayed where clusters diverge.

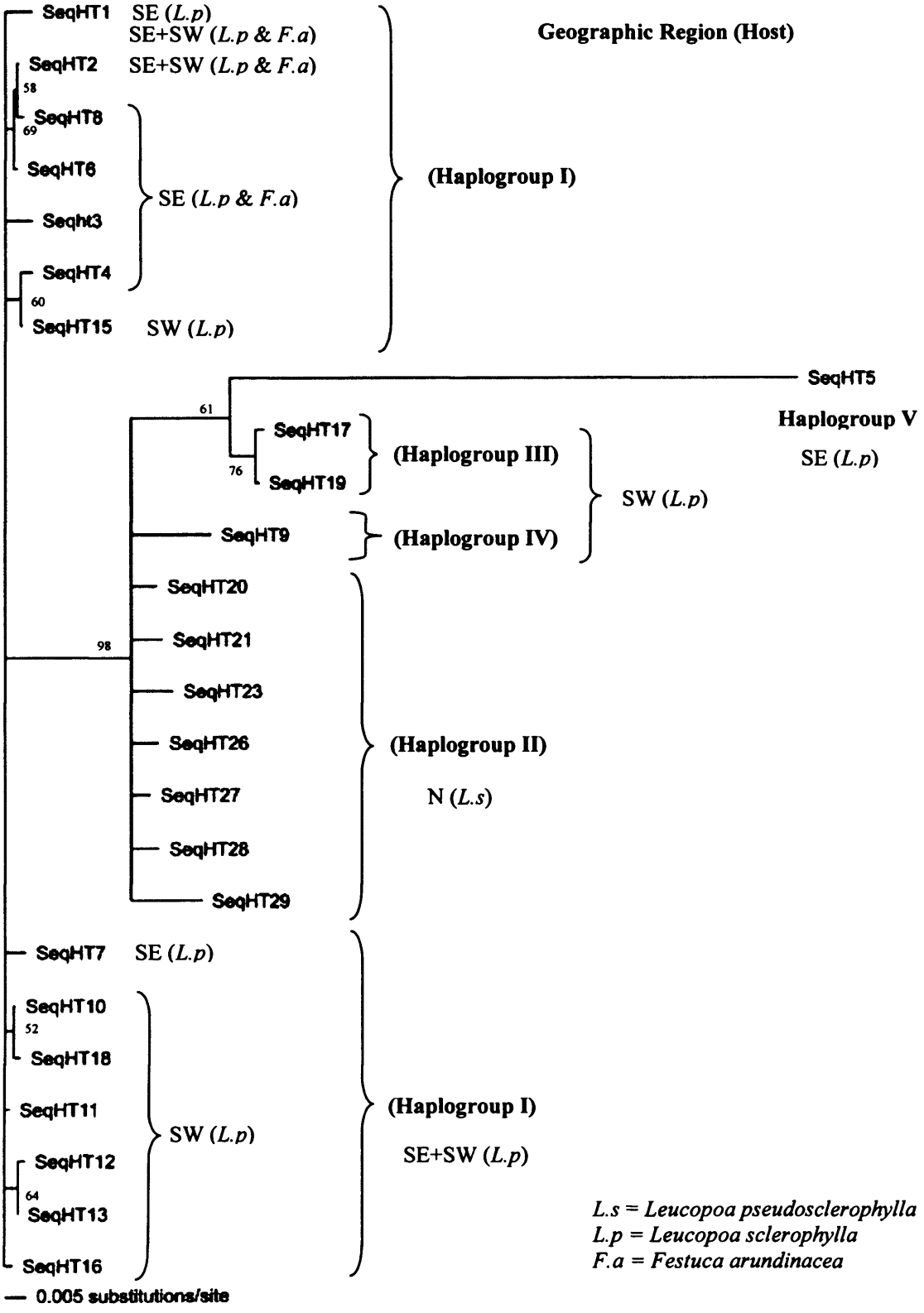


Figure 4.7 The maximum likelihood tree with bootstrap values for *Sycophila* haplotypes. Bootstrap support values are displayed where clusters diverge.

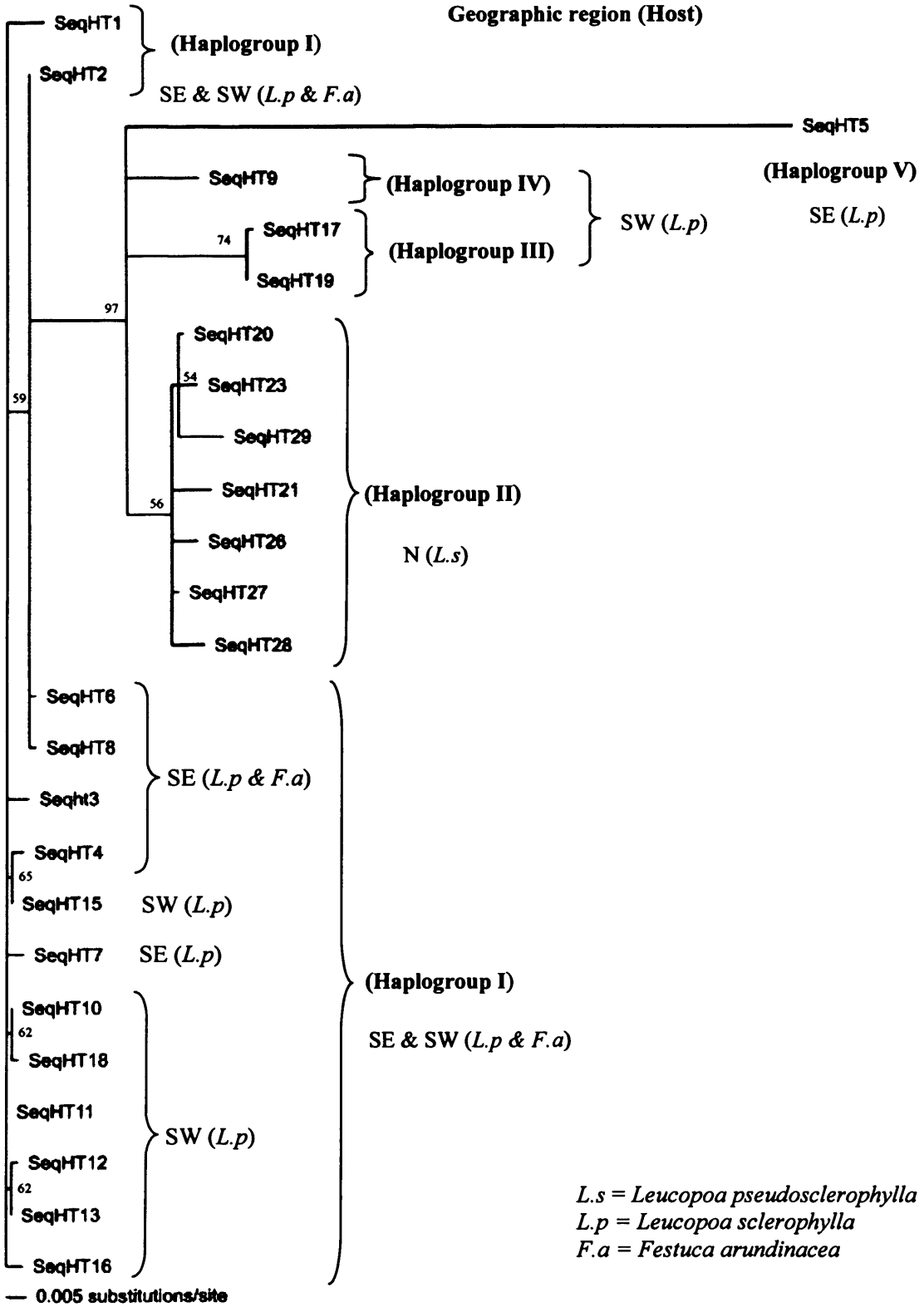


Figure 4. 8 Nested cladogram for *Sycophila* populations. Each line in the network represent a single mutational change. Roman numerals represent haplotype numbers. 0 indicates an interior node in the network that was not present in the sample and is therefore an inferred intermediate haplotype between the two nearest haplotypes in the network that differed by two or more mutations. Haplotypes are grouped into 1-step clades, 1-step clades into 2-step clades and 2-step clades nested into 3-step clades.

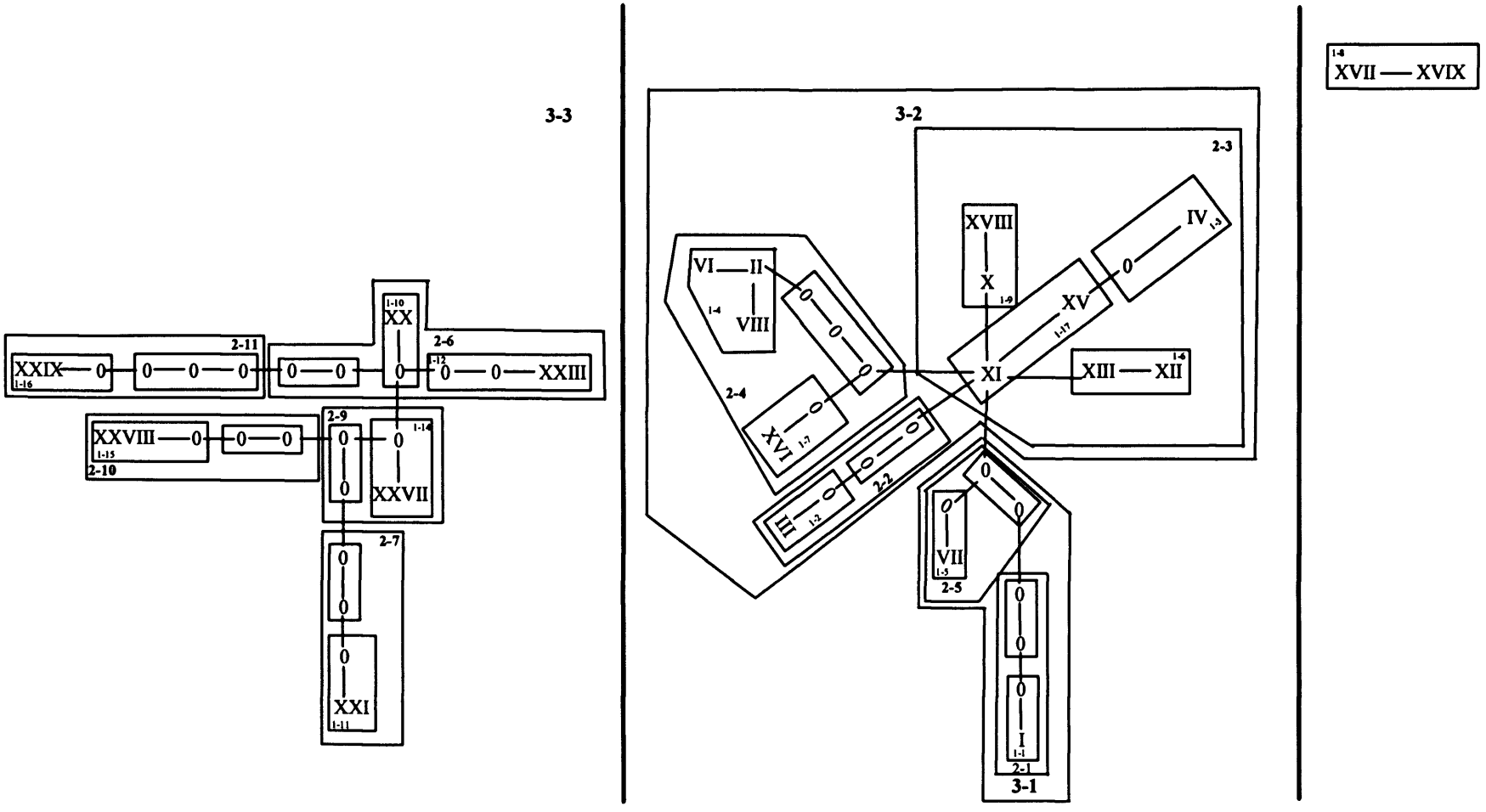


Figure 4. 9 Mismatch distribution of *Sycophila* populations including whole network (including upper and lower 95% confidence intervals (τ = 4.035; θ_0 = 19.086; θ_1 = 28.711; SSD p = 0.254).

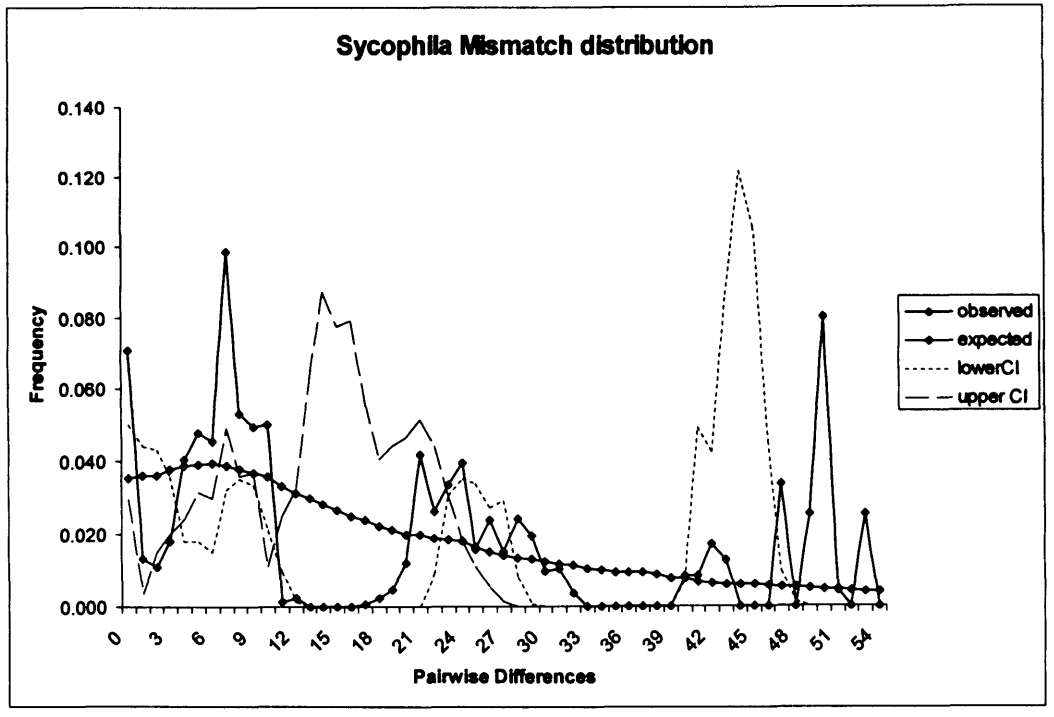
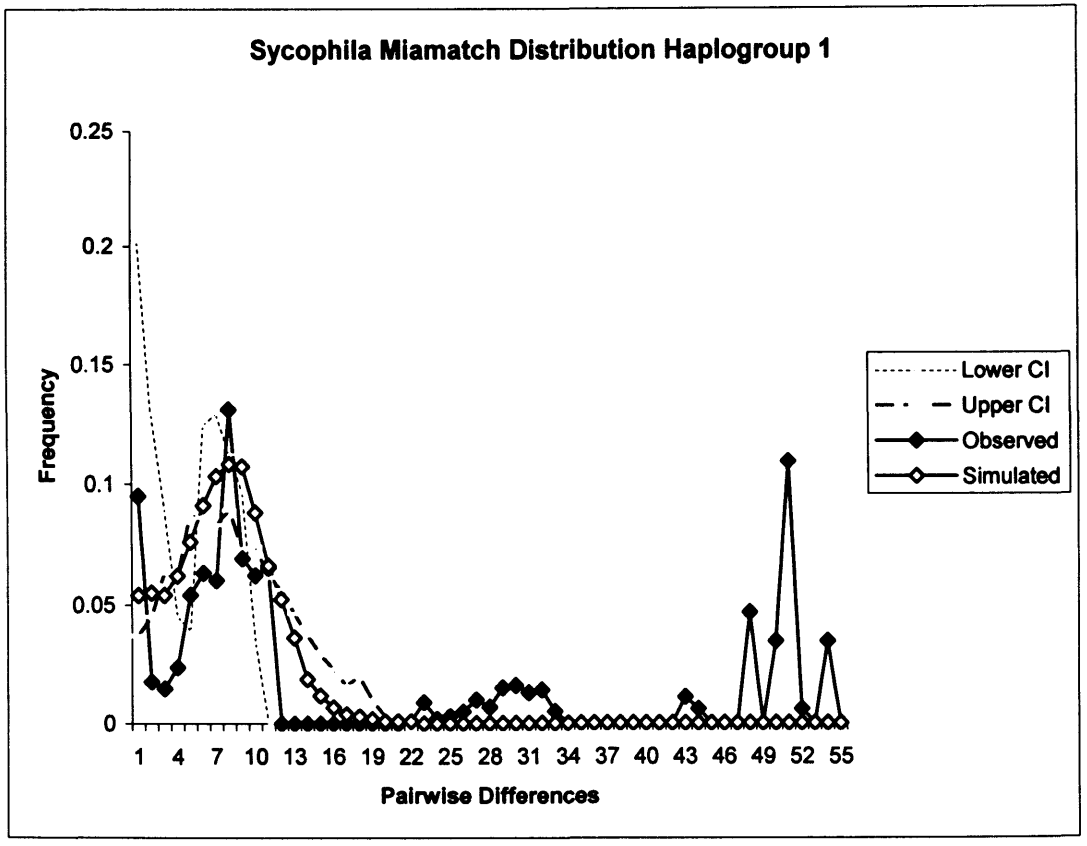


Figure 4. 10 Mismatch distribution of haplogroup I *Sycophila* populations (including upper and lower 95% confidence intervals ($\tau = 7.079$; $\theta_0 = 1.285$; $\theta_1 = 15.913$; SSD p = 0.020).



Appendix 1: Alignment of 875 bp of COI and COII sequences. Ingroup taxa include *Sycophila* (S), *Tetramesa* (T), *Eurytoma* (E). Outgroup taxa are *Ceratosolen capensis* (Grandi) (Hymenoptera: Agaonidae) (CC), *C. bisulcatus* (CB), *Blastophaga malayana* (BM) and *B. intermedia* (BI). Codon start position 2. Identical nucleotides are shown by (.).

Domain = COI: Codon Start = 2

```
#S7.1         G GCA ACT ATA ATT ATT GCG GTT CCT ACT GGA ATT AAA ATT TTT AGG TGA TTA GCT TCA ATA AAT GGA ATA AAA ATT AT
#S7A1         . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ..G ... ... ... ..
#S7B1         . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#S2D1         . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ..
#S9C1         . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ... ..
#S2B1         A ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ..
#S1A1         . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ..C ... ... ... ..
#S4B1         A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ... ... ... ... ..G ... ... ..
#T7A3         T ..T ... ... ... ... ..A A.. ... ... ... ... ... ... ... ..A ... A.. ... ... T.. ... ... ... ... C.. .A
#T2D1         T ..T ... ... ... ... ..A A.. ... ... ... ... ... ... ... ..A ... A.. ... ... T.. ... ... ... ... C.. .A
#T9B1         T ..T ... ... ... ... ..A A.. ... ... ... ... ... ... ... ..A ... A.. ... ... T.. ... ... ... ... C.. .A
#T1A4         T ..T ... ... ... ... ..A A.. ... ... ... ... ... ... ... ..A ... A.. ... ... T.. ... ... ... ... C.. .A
#T2D9         T ..T ... ... ... ... ..A A.. ... ... ... ... ... ... ... ..A ... A.. ... ... T.. ... ... ... ... C.. .A
#T1A2         T ..T ... ... ... ... ..A A.. ... ... ... ... ... ... ... ..A ... A.. ... ... T.. ... ... ... ... C.. .A
#T9C2         T ..T ... ... ... ... ..A A.. ... ... ... ... ... ... ... ..A ... A.. ... ... T.. ... ... ... ... C.. .A
#T7B4         T ..T ... ... ... ... ..A A.. ... ... ... ... ... ... ... ..A ... A.. ... ... T.. ... ... ... ... C.. .A
#T7A1         T ..T ... ... ... ... ..A A.. ... ... ... ... ... ... ... ..A ... A.. ... ... T.. ... ... ... ... C.. .A
#T7B3         T ..T ... ... ... ... ..A A.. ... ... ... ... ... ... ... ..A ... A.. ... ... T.. ... ... ... ... C.. .A
#E7.1         A ..T ... ... ... ... ..T ..A ... ..A ..T ... ... ... ... ..A ... ... ..A ..T ... ... ... ... ... ... .A
#E7A1         A ..T ... ... ... ... ..T ..A ... ..A ..T ... ... ... ... ..A ... ... ..A ..T ... ... ... ... ... ... .A
#E1A1         A ..T ... ... ... ... ..T ..A ... ..A ..T ... ... ... ... ..A ... ... ..A ..T ... ... ... ... ... ... .A
#CCAF200377   A ATT ... ... G.A ... ..T ... ... ... ... ... ... G.A ... ..A ... ... ..A ... ... .C. A.. G.T ... T.A .A
#CBAF200375   A ... ..A ... G.A ... .G. ... ..G ... ... ... ... G.. .A. ..T ... G.T TG. A.T T.. T.. ... TC. ... T.. .A
#BMAF200373   A AT. ..A ... ... ... ..A ..A ... ..A ..T ..C ... ... ... ..A ..G ... ... ... ... ... ... ... ... ... ..
#BIAF200372   A ... ..A ... ... ... ..T ... ..A ... ... ... ... ... ... ..A ... ..G ..G ... ... .GA ... ... ... T.. .A

#S7.1         A TTT AGA GTT TCA AAT TTA TGG CTC TTA GGT TTT TTA TTT TTA TTT ACT GTA GGT GGA TTA ACT GGA ATT ATT TTA TC
#S7A1         . ... ... ... ... ... ... ... T.G ... ... ... ..G ... ... ... ... ... ... ... ... ... ..T ... ... ... ..
#S7B1         . ... ... ... ... ... ... ... T.G ... ... ... ..G ... ..G ... ... ... ... ..T ... ... ..T ... ... ... ..
#S2D1         . ... ... ... ... ... ... ... T.G ... ... ... ..G ... ... ... ... ... ... ... ... ... ..T ... ..C ... ..
#S9C1         . ... ... ... ... ... ... ... T.G ... ..G ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ..
#S2B1         . ... ... ... ... ... ... ... T.G ... ... ... ..G ... ... ... ... ... ... ... ... ... ..T ... ... ... ..
#S1A1         . ... ... ... ... ... ... ... T.G ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ..
```

```
#S4B1
#T7A3
#T2D1
#T9B1
#T1A4
#T2D9
#T1A2
#T9C2
#T7B4
#T7A1
#T7B3
#E7.1
#E7A1
#E1A1
#CCAF200377
#CBAF200375
#BMAF200373
#BIAF200372

#S7.1
#S7A1
#S7B1
#S2D1
#S9C1
#S2B1
#S1A1
#S4B1
#T7A3
#T2D1
#T9B1
#T1A4
#T2D9
#T1A2
#T9C2
#T7B4
#T7A1
#T7B3
#E7.1
```

```
#E7A1        A ... ... ... ... ... ... ..A ... ... ... ..T ... ... ... ... ..A ..T ... ... ... ..A ... ... ... ..T ..
#E1A1        A ... ... ... ... ... ... ..A ... ... ... ..T ... ... ... ... ..A ..T ... ... ... ..A ... ... ... ..T ..
#CCAF200377  . ... ..A ... C.. ... ... A.A A.. ... ... ..T ... ... ..T ..T ... ..T ... ... ... ... ... ... ... ..A ..
#CBAF200375  . ... G.. G.. T.A ... G.G ... ... ... ... ..T ... ... ..T ... ... ..T ... ... ... ..A ..G ..T ... ..T ..
#BMAF200373  A ... ... ... ... ... ... A.. ... ... ... ... ... ... ..T ..G ... ..T ... ... ... ... ... ... ... ..T ..
#BIAF200372  A ... ... ... ... ... ... A.. ... ... ... ..T ... ... ..T ..T ... ..T ... ... ... ..A ... ..T ... ... ..

#S7.1        A GTA TTT GCT ATT TTT GGA AGG TTT ATT TAT TGA TTT CCT TTT ATT TTT GGG GTT ACT ATA AAT AAA AAA TTA TTA AA
#S7A1        . ... ... ... ... ... ..G ... ... ... ... ... ... ... ..C ... ... ..T ... ... ... ... ... ... ... ... ..
#S7B1        . ... ... ... ... ... ..G ... ... ... ... ... ... ... ..C ... ... ..T ... ... ... ... ... ... ... ... ..
#S2D1        . ... ... ... ... ... ..G ... ... ... ... ... ..C ... ..C ... ... ..T ... ... ... ... ... ... ... ... ..
#S9C1        . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#S2B1        . ... ... ... ... ... ..G ... ... ... ... ... ... ... ..C ... ... ..T ... ... ... ... ... ... ... ... ..
#S1A1        . ... ... ... ... ... ..G ..T ... ... ... ... ... ... ..C ... ... ..T ... ... ... ... ... ... ... ... ..
#S4B1        . ..G ... ... ... ... ..T ..A ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ..
#T7A3        T ..R ... ..A ... ... ... ..A ... ... ... ... ... ... A.A ... AGA ..T A.. GTG ... ... ... ... .G. ... ..
#T2D1        T ..G ... ..A ... ... ... ..A ... ... ... ... ... ... A.A ... AGA ..T A.. GTG ... ... ... ... .G. ... ..
#T9B1        T ..G ... ..A ... ... ... ..A ... ... ... ... ... ... A.A ... AGA ..T A.. GTG ... ... ... ... .G. ... ..
#T1A4        T ..G ... ..A ... ... ... ..A ... ... ... ... ... ... A.A ... AGA ..T A.. GTG ... ... ... ... .G. ... ..
#T2D9        T ..G ... ..A ... ... ... ..A ... ... ... ... ... ... A.A ... AGA ..T A.. GTG ... ... ... ... .G. ... ..
#T1A2        T ..G ... ..A ... ... ... ..A ... ... ... ... ... ... A.A ... AGA ..T A.. GTG ... ... ... ... .G. ... ..
#T9C2        T ..G ... ..A ... ... ... ..A ... ... ... ... ... ... A.A ... AGA ..T A.. GTG ... ... ... ... .G. ... ..
#T7B4        T ..G ... ..A ... ... ... ..A ... ... ... ... ... ... A.A ... AGA ..T A.. GTG ... ... ... ... .G. ... ..
#T7A1        T ..G ... ..A ... ... ... ..A ... ... ... ... ... ... A.A ... AGA ..T A.. GTG ... ... ... ... .G. ... ..
#T7B3        T ..G ... ..A ... ... ... ..A ... ... ... ... ... ... A.A ... AGA ..T A.. GTG ... ... ... ... .G. ... ..
#E7.1        T ..T ... ... ... ... ... ..A ... ... ... ... ... ... A.A T.. .CA ..A T.A ... ... ... ... ... .G. ... ..
#E7A1        T ..T ... ... ... ... ... ..A ... ... ... ... ... ... A.A T.. .CA ..A T.A ... ... ... ... ... .G. ... ..
#E1A1        T ..T ... ... ... ... ... ..A ... ... ... ... ... ... A.A T.. .CA ..A T.A ... ... ... ... ... .G. ... ..
#CCAF200377  T ... ... ... ... ... ... ..T ... ... ... ... ... ... ..A T.. .AC T.. A.A GTA ... ... C.. ... .G. ... ..
#CBAF200375  T ... ... ..A ... A.A ... ..T ... G.. .T. ... ... ..A ..A ..A AGA ..A A.. TA. T.. ... C.. ... ... ... ..
#BMAF200373  . ..C .A. ..A ... ... ... ..A ... ... ... ... ... ... ..A T.. ... ..A T.A .AA T.. ... C.. ... .G. ... ..
#BIAF200372  T ..T .A. ... ... ..A ... ..A ... ... ... ... ... ... ..A T.. ... ..A ... .G. ... ... C.. ... .G. ... ..

#S7.1        A ATT CAA TTT TTA ATA TTA TTT TTA GGG GTA AAT TTA ACT TTT TTT CCT CAA CAT TTT TTA GGG TTA AGT GGT ATA CC
#S7A1        . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ..A ... ..G ..
#S7B1        . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ..A ... ... ..
#S2D1        . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ..A ... ... ..
#S9C1        . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ..
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#S2B1
#S1A1
#S4B1
#T7A3
#T2D1
#T9B1
#T1A4
#T2D9
#T1A2
#T9C2
#T7B4
#T7A1
#T7B3
#E7.1
#E7A1
#E1A1
#CCAF200377
#CBAF200375
#BMAF200373
#BIAF200372

#S7.1      T CGG CGT TAT TCA GAT TAT CCA GAT TCA TTT TTA TGT TGA AAT GTA GTT TCA TCA ATT GGT TCT GTA GTT TCA TTA GT
#S7A1
#S7B1
#S2D1
#S9C1
#S2B1
#S1A1
#S4B1
#T7A3
#T2D1
#T9B1
#T1A4
#T2D9
#T1A2
#T9C2
#T7B4
#T7A1
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#T7B3          A ..T ..G ... ... ... ... G.T ... ... ... ... ... ... ... ... ... A.T T.G ... ... ... ..A ... A.. A.. ... ..T A.
#E7.1          . ..A ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... T.A ..T ... ... ... ..A A.T A.. ..T ... A.
#E7A1          . ..A ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... T.A ..T ... ... ... ..A A.T A.. ..T ... A.
#E1A1          . ..A ... ... ... ... ... ..T ... ... ... A.. ... ... ... ... ... T.A ..T ... ... ... ..A A.T A.. ..T ... A.
#CCAF200377    . ..A ..A ... AGG ... ... ... GTT .A. ... ... ... ... ... ... ... T.. ... ..T ... ..G ... T.. T.A ... ..T T.
#CBAF200375    . ..A ..A ... ..T ... ... ... G.T .A. A.. ... ... ... ... ... AA. C.. ..T ... T.A ..G ..A A.. T.A ... ..T T.
#BMAF200373    . ..T ..A ... ..T ... ... ..C ..T ... A.. ... ... ... ... ... T.. T.A ... ... ... ..A T.T A.C ... ... A.
#BIAF200372    A ..T ..A ... ..T ... ... ... ... A. ... ... ... ... ... ... T.. T.A ..T ..T ... ..A ... T.. A.. ..T ..T A.

#S7.1          T AGT ACT TTT ATA TTT TTT TAT ATT ATT TGA GAT GCT TTT ATT TTA AAA CGT TTA TTA ATT TTT GTG AAA AGA TTA AA
#S7A1           . G.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#S7B1           . G.. ... ... ... ... ... ... ... ... G.. ... ... ... ..C ... ... ... ... ... ... ... ..T ... ... ... ..
#S2D1           . G.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#S9C1           . G.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ..G ... ..
#S2B1           . G.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#S1A1           . G.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#S4B1           . G.. ... ... ... ... ... ... ... ... G.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#T7A3           A ..A ... A.A T.. ... ... .T. ... T.A ..G ..A AGA ..A ..A .CT GTT ... A.T ... ... ... T.A ... .AT ... ..
#T2D1           A ..A ... A.A T.. ... ... .T. ... T.A ..G ..A AGA ..A ..A .CT GTT ... A.T ... ... ... T.A ... .AT ... ..
#T9B1           A ..A ... A.A T.. ... ... .T. ... T.A ..G ..A AGA ..A ..A .CT GTT ... A.T ... ... ... T.A ... .AT ... ..
#T1A4           A ..A ... A.A T.. ... ... .T. ... T.A ..G ..A AGA ..A ..A .CT GTT ... A.T ... ... ... T.A ... .AT ... ..
#T2D9           A ..A ... A.A T.. ... ... .T. ... T.A ..G ..A AGA ..A ..A .CT GTT ... A.T ... ... ... T.A ... .AT ... ..
#T1A2           A ..A ... A.A T.. ... ... .T. ... T.A ..G ..A AGA ..A ..A .CT GTT ... A.T ... ... ... T.A ... .AT ... ..
#T9C2           A ..A ... A.A T.. ... ... .T. ... T.A ..G ..A AGA ..A ..A .CT GTT ... A.T ... ... ... T.A ... .AT ... ..
#T7B4           A ..A ... A.A T.. ... ... .T. ... T.A ..G ..A AGA ..A ..A .CT GTT ... A.T ... ... ... T.A ... .AT ... ..
#T7A1           A ..A ... A.A T.. ... ... .T. ... T.A ..G ..A AGA ..A ..A .CT GTT ... A.T ... ... ... T.A ... .AT ... ..
#T7B3           A ..A ... A.A T.. ... ... .T. ... T.A ..G ..A AGA ..A ..A .CT GTT ... A.T ... ... ... T.A ... .AT ... ..
#E7.1           A ... ..A A.A T.. ... ... .T. ... T.A ..G ... AGA ..A ..A .CT ... ... ..A A.T ... ... ... A.A ... .AT ... ..
#E7A1           A ... ..A A.A T.. ... ... .T. ... T.A ..G ... AGA ..A ..A .CT ... ... ..A A.T ... ... ... A.A ... .AT ... ..
#E1A1           A ... ..A A.A T.. ... ... .T. ... T.A ..G ... AGA ..A ..A .CT ... ... ..A A.T ... ... ... A.A ... .AT ... ..
#CCAF200377     A ... ... T.A ..A TAT ... ... A.A .T. ... ..A .T. ... ... TGA .C. ... ..T ... ..A G.T G.. ... ... A.A .GT .A. AC. ..
#CBAF200375     A ..G ..A GCA T.T ... ... GGG ... G.. .T. ..A .GA A.. T.A .C. ..T ... ..A AA. ... ... ... AGT GG. .A. A.. ..
#BMAF200373     A GCA ..A A.A TAT ... A.A .TA ... ..A ... ..A T.. ... .A. AAT C.. ... ..A G.T G.. ... ... T.A ... .A. .CT ..
#BIAF200372     . G.. ..A A.A T.T ... A.A .T. ... T.A G.. ..A ..A A.. T.. .GT C.. ... ..A G.. A.T TG. ... A.T ..G .A. ACT ..

#S7.1          T AAT ATA ATT GAA ATA ATT ATA AAT TTT CCT CCT AGA GAT CAT TCA TTT ATA GAA ATT CCT AAA TTA GTA ATG GG
#S7A1           . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#S7B1           . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
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#S2D1        . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ... ... ... ... ... .G. ..
#S9C1        . ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... ..
#S2B1        . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ... ... ... ... ... .G. ..
#S1A1        . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ... ... ... ... ... .A. ..
#S4B1        . ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ..T ... ... ... ... ... ... ... .C. ... ..
#T7A3        . .TA TC. ..A ..G TG. T.A T.. ... ... ... ... ..T ..A ... ... ... CAT ... ... ... ... A.T TAC ..T CT
#T2D1        . .TA TC. ..A ..G TG. T.A T.. ... ... ... ... ..T ..A ... ... ... CAT ... ... ... ... A.T TAC ..T CT
#T9B1        . .TA TC. ..A ..G TG. T.A T.. ... ... ... ... ..T ..A ... ... ... CAT ... ... ... ... A.T TAC ..T CT
#T1A4        . .TA TC. ..A ..G TG. T.A T.. ... ... ... ... ..T ..A ... ... ... CAT ... ... ... ... A.T TAC ..T CT
#T2D9        . .TA TC. ..A ..G TG. T.A T.. ... ... ... ... ..T ..A ... ... ... CAT ... ... ... ... A.T TAC ..T CT
#T1A2        . .TA TC. ..A ..G TG. T.A T.. ... ... ... ... ..T ..A ... ... ... CAT ... ... ... ... A.T TAC ..T CT
#T9C2        . .TA TC. ..A ..G TG. T.A T.. ... ... ... ... ..T ..A ... ... ... CAT ... ... ... ... A.T TAC ..T CT
#T7B4        . .TA TC. ..A ..G TG. T.A T.. ... ... ... ... ..T ..A ... ... ... CAT ... ... ... ... A.T TAC ..T CT
#T7A1        . .TA TC. ..A ..G TG. T.A T.. ... ... ... ... ..T ..A ... ... ... CAT ... ... ... ... A.T TAC ..T CT
#T7B3        . .TA TC. ..A ..G TG. T.A T.. ... ... ... ... ..T ..A ... ... ... CAT ... ... ... ... A.T TAC ..T CT
#E7.1        . .GA TC. ... ... TG. ... G.. .GA ... ... ..A ... ..A ... ..T ... TAT ... ... ... ... G.T TAT .A. AT
#E7A1        . .GA TC. ... ... TG. ... G.. .GA ... ... ..A ... ..A ... ..T ... TAT ... ... ... ... G.T TAT .A. AT
#E1A1        . .GA TC. ... ... TG. ... G.. .GA ... ... ..A ... ..A ... ..T ... TAT ... ... ... ... G.T TAT .A. AT
#CCAF200377  . .C. TC. ... ... TG. T.A ... ... .A. ... T.A GC. ... ... ..T ... TAT ... G.A ..A ..G G.T ACT .AT .A
#CBAF200375  . .T. TC. T.A ... TG. ..A ... .C. .A. ... ... .T. ..A ... ..T ... GAT ... T.A ..A ... G.. T.T TAT A.
#BMAF200373  . GTA TC. T.A ... TG. T.. ... TC. .A. ... G.. CCG ... ... ... A.. TAT ... C.C ... C.. A.T AA. GAA .A
#BIAF200372  . GT. TCT T.A ..G TGG T.. ... TC. .A. ... G.A CCT ... ... ... A.. TAT ... C.. ..A ... ... A.. GAA .A

Domain=COII: Codon Start=1
#S7.1        ATT TCT TAT TGA CAT CAA ATT ATA CTT CAA GAT GCT GGG TCT TCT GTT ATA GAA GCT ATA ACT TAT TTT TAT GAT GAA
#S7A1         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#S7B1         ... ... ... ... ... ... ... ... T.A ... ... ... ... ... ... A.. ... ..G ... ... ... ... ... ... ...
#S2D1         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#S9C1         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#S2B1         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#S1A1         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#S4B1         ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ... ... ... ..G ... ... ... ... ... ...
#T7A3         ... ... ... ... ... ... ... ... T.A ... ... ... .CA ... ... A.. ... ... AA. ... .T. .TG ... ... ... T.T
#T2D1         ... ATA ATA ... ... ... TA. TCT T.. ... ... ... .CA ... ... A.. ... ... AA. ... .T. .TG ... ... ... T.T
#T9B1         ... ATA ATA ... ... ... TA. TCT T.. ... ... ... .CA ... ... A.. ... ... AA. ... .T. .TG ... ... ... T.T
#T1A4         ... ATA ATA ... ... ... TA. TCT T.. ... ... ... .CA ... ... A.. ... ... AA. ... .T. .TG ... ... ... T.T
#T2D9         ... ATA ATA ... ... ... TA. TCT T.. ... ... ... .CA ... ... A.. ... ... AA. ... .T. .TG ... ... ... T.T
#T1A2         ... ATA ATA ... ... ... TA. TCT T.. ... ... ... .CA ... ... A.. ... ... AA. ... .T. .TG ... ... ... T.T
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#T9C2        ... ATA ATA ... ... ... TA. TCT T.. ... ... ... .CA ... ... A.. ... ... AA. ... .T. .TG ... ... ... T.T
#T7B4        ... ATA ATA ... ... ... TA. TCT T.. ... ... ... .CA ... ... A.. ... ... AA. ... .T. .TG ... ... ... T.T
#T7A1        ... ATA ATA ... ... ... TA. TCT T.. ... ... ... .CA ... ... A.. ... ... AA. ... .T. .TG ... ... ... T.T
#T7B3        ... ATA ATA ... ... ... TA. TCT T.. ... ... ... .CA ... ... A.. ... ... AA. ... .T. .TG ... ... ... T.T
#E7.1        ..A G.. .TA ... ... ... TA. .AT T.. ... ... ... .CT ... ..A ... ... ... AA. ... .T. ATA ... ... ... ATT
#E7A1        ..A G.. .TA ... ... ... TA. .AT T.. ... ... ... .CT ... ..A ... ... ... AA. ... .T. ATA ... ... ... ATT
#E1A1        ..A G.. .TA ... ... ... TA. .AT T.. ... ... ... .CT ... ..A ... ... ... AA. ... .T. ATA ... ... ... ATT
#CCAF200377 ... ..A GTA ... GGA ... ..A .AT T.A ... ... ..A A.A ..A C.A A.. ... C.. AA. ... GTG AT. ... C.. ..A TTT
#CBAF200375 ... G.. ... ATT GG. ... T.A GG. T.A ... ..A A.A AAT ... C.A ..A ... AT. TT. T.. T.. G.. .A. C.. ... AT.
#BMAF200373 ... ..A .T. ... GGA ... ... ... ..A ..G ... ..A A.A ..C C.A A.. ... C.. AA. ... .T. .T. ... C.. ... T.T
#BIAF200372 ... ... .TA ... GG. ... ..A ... T.A ... ... ... ..T ..G AT. AC. ... ... AA. ... .T. .T. ..A C.. ... T.T

#S7.1        GCT ATG ATA ATT TTA GTA ATA ATT ATT ATA TTA ATT TTA TAT ATG TTA ATT TTT ATA GTA TTT AAT AAA TTT ACT GAT
#S7A1        ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .A. ... ... ... ... ...
#S7B1        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .A. ... ... ... ... ...
#S2D1        ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .A. ... ... ... ... ...
#S9C1        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .A. ... ... ... ... ...
#S2B1        ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .A. ... ... ... ... ...
#S1A1        ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .A. ... ... ... ... ...
#S4B1        ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ..A ... G.. ... ... ..T .A. ... ... ... ... ...
#T7A3        ... ..A T.. G.. A.T A.. ... ... ... ..T ..G ... A.. ... ..A ..T ... ... ... A.T AAA ... ... ..A GT. A..
#T2D1        ... ..A T.. G.. A.T A.. ... ... ... ..T ..G ... A.. ... ..A ..T ... ... ... A.T AAA ... ... ..A GT. A..
#T9B1        ... ..A T.. G.. A.T A.. ... ... ... ..T ..G ... A.. ... ..A ..T ... ... ... A.T AAA ... ... ..A GT. A..
#T1A4        ... ..A T.. G.. A.T A.. ... ... ... ..T ..G ... A.. ... ..A ..T ... ... ... A.T AAA ... ... ..A GT. A..
#T2D9        ... ..A T.. G.. A.T A.. ... ... ... ..T ..G ... A.. ... ..A ..T ... ... ... A.T AAA ... ... ..A GT. A..
#T1A2        ... ..A T.. G.. A.T A.. ... ... ... ..T ..G ... A.. ... ..A ..T ... ... ... A.T AAA ... ... ..A GT. A..
#T9C2        ... ..A T.. G.. A.T A.. ... ... ... ..T ..G ... A.. ... ..A ..T ... ... ... A.T AAA ... ... ..A GT. A..
#T7B4        ... ..A T.. G.. A.T A.. ... ... ... ..T ..G ... A.. ... ..A ..T ... ... ... A.T AAA ... ... ..A GT. A..
#T7A1        ... ..A T.. G.. A.T A.. ... ... ... ..T ..G ... A.. ... ..A ..T ... ... ... A.T AAA ... ... ..A GT. A..
#T7B3        ... ..A T.. G.. A.T A.. ... ... ... ..T ..G ... A.. ... ..A ..T ... ... ... A.T AAA ... ... ..A GT. A..
#E7.1        .TA ..A T.. G.A A.T ... ... ... ... ..T ... ... A.. ... ..A ... ... ... ... ... AAA ... ... ..A .T. A..
#E7A1        .TA ..A T.. G.A A.T ... ... ... ... ..T ... ... A.. ... ..A ... ... ... ... ... AAA ... ... ..A .T. A..
#E1A1        .TA ..A T.. G.A A.T ... ... ... ... ..T ... ... A.. ... ..A ... ... ... ... ... AAA ... ... ..A .T. A..
#CCAF200377 ..A ..A G.. ... G.. T.. T.. ... ... GC. AA. T.A ... ... ..T A.T TGA A.A ... T.. A.A ... ... ... ... ...
#CBAF200375 ATA ..A T.T T.A A.. TAT T.T ... .GA T.T CA. G.A A.. ... TCT A.T T.. A.. G.T T.. .G. TCA ... .CA TTA ...
#BMAF200373 ... T.A ... ... A.T C.. ... ... T.A ..T ... T.G ..T ... T.A ... ..A A.A ... T.. AA. TC. .T. ... GT. A..
#BIAF200372 TTA ..A ... ... A.T T.. ... ... ..A G.. ... T.A ..T ... ..A ... .GA A.A ... .CT AG. TTA ... ... TT. ..A
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#S7.1        CGG TTT TTA TTA GAA GGC CAG TTT ATT GAA ATT TTT TGA ACT TTA GTT CCT ATA GGA TTT TTA ATA TTT TTA GCA ATT
#S7A1        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#S7B1        ... ... ... ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ...
#S2D1        ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#S9C1        ... ... ... ... ... ..T ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ...
#S2B1        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#S1A1        ..A ... ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ...
#S4B1        ..T ... ... ..G ..G ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#T7A3        TTA AAA ... ... ... ..T ..A ..A ... ... ... A.. ... ..A G.T ... ... ... TTT A.A ... ..T ... ... ... ...
#T2D1        TTA AAA ... ... ... ..T ..A ..A ... ... ... A.. ... ..A G.T ... ... ... TTT A.A ... ..T ... ... ... ...
#T9B1        TTA AAA ... ... ... ..T ..A ..A ... ... ... A.. ... ..A G.T ... ... ... TTT A.A ... ..T ... ... ... ...
#T1A4        TTA AAA ... ... ... ..T ..A ..A ... ... ... A.. ... ..A G.T ... ... ... TTT A.A ... ..T ... ... ... ...
#T2D9        TTA AAA ... ... ... ..T ..A ..A ... ... ... A.. ... ..A G.T ... ... ... TTT A.A ... ..T ... ... ... ...
#T1A2        TTA AAA ... ... ... ..T ..A ..A ... ... ... A.. ... ..A G.T ... ... ... TTT A.A ... ..T ... ... ... ...
#T9C2        TTA AAA ... ... ... ..T ..A ..A ... ... ... A.. ... ..A G.T ... ... ... TTT A.A ... ..T ... ... ... ...
#T7B4        TTA AAA ... ... ... ..T ..A ..A ... ... ... A.. ... ..A G.T ... ... ... TTT A.A ... ..T ... ... ... ...
#T7A1        TTA AAA ... ... ... ..T ..A ..A ... ... ... A.. ... ..A G.T ... ... ... TTT A.A ... ..T ... ... ... ...
#T7B3        TTA AAA ... ... ... ..T ..A ..A ... ... ... A.. ... ..A G.T ... ... ... TTT A.A ... ..T ... ... ... ...
#E7.1        ... .A. A.. ... ... ..T ..A A.A ... ... ... A.. ... ... A.T A.. ..A ... ATT ... ... ..T ... ... ... ...
#E7A1        ..A .A. A.. ... ... ..T ..A A.A ... ... ... A.. ... ... A.T A.. ..A ... ATT ... ... ..T ... ... ... ...
#E1A1        ... .A. A.. ... ... ..T ..A A.A ... ..G ... A.. ... ... A.T A.. ..A ... ATT ... ... ..T ... ... ... ...
#CCAF200377  GTA AA. ... ACT AG. ..A A.T ... G.. ..C C.. G.. ... ..A A.T A.. ..A ... TT. G.A ... T.. C.. ... ..T T..
#CBAF200375  TTA A.A AAT AGG .GC T.A AGA GGC .AA ... .GG AAG ... ... ... T.G ..A TCC TT. CAA AAG TGG AA. ... A.. G..
#BMAF200373  TTA AAA A.. A.T C.T ..A ..A A.A ... ... G.A A.. ... ..A G.. A.. ... ... ATT A.. ... T.. ..C ... ..T T.C
#BIAF200372  TTA AAA A.. ..T C.T AAT ..A GCA T.A ... ..A ..A ... ... A.T ... ..A ..T ATT G.. ... G.T ... ... ..T ...

#S7.1        CCT TCA TTA AAA GTT TTA TA
#S7A1        ... ... ... ... ... ... ..
#S7B1        ... ... ... ..G ... ... ..
#S2D1        ... ... ... ... ... C.. ..
#S9C1        ... ... ... ... ... ... ..
#S2B1        ... ... ... ... ... ... ..
#S1A1        ... ... ... ... ... ... ..
#S4B1        ... ... ... ... ... ... ..
#T7A3        ... ... ... ... A.. ... ..
#T2D1        ... ... ... ... A.. ... ..
#T9B1        ... ... ... ... A.. ... ..
#T1A4        ... ... ... ... A.. ... ..
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#T2D9        ... ... ... ... A.. ... ..
#T1A2        ... ... ... ... A.. ... ..
#T9C2        ... ... ... ... A.. ... ..
#T7B4        ... ... ... ... A.. ... ..
#T7A1        ... ... ... ... A.. ... ..
#T7B3        ... ... ... ... A.. ... ..
#E7.1        ... ..C ... ..G ..A ... ..
#E7A1        ... ..C ... ..G ..A ... ..
#E1A1        ... ..C ... ..G ..A ... ..
#CCAF200377 ... ... ... C.T T.G A.. A.
#CBAF200375 T.. ... A.C T.T A.A .AT A.
#BMAF200373 ..A ..G ... C.T T.A ..G ..
#BIAF200372 ... ..T ... C.T C.. ... ..
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Appendix 2: Alignment of 68 bp of tRNA sequences. Ingroup taxa include *Sycophila* (S), *Tetramesa* (T), *Eurytoma* (E). Outgroup taxa are *Ceratosolen capensis* (Grandi) (Hymenoptera: Agaonidae) (CC), *C. bisulcatus* (CB). Identical nucleotides are shown by (.), gaps are shown by (-).

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#S7.1        ATTCTAAAAT GGCAGATTAG TGCAATAAAT TTAAGATTTA TATATATAAA ATATTTTTAT TTTTAGAA
#S7A1        .......... .......... .......... .......... .......... .......... ........
#S7B1        .......... .......... .......... .......... .......... .......... ........
#S2D1        .......... .......... .......... .......... .......... .......... ........
#S9C1        .......... .......... .......... .......... .......... .......... ........
#S2B1        .......... .......... .......... .......... .......... .......... ........
#S1A1        .......... .......... .......... .......... .......... .......... ........
#S4B1        C......... .......... .......... .......... .......... .......... ........
#T7A3        T......... .......... .......G.. ....AT.C.. ..A..... .......... ........
#T2D1        T......... .......... .......G.. ....AT.C.. ..A..... TA.....-.. ........
#T9B1        T......... .......... .......G.. ....AT.C.. ..A..... TA.....-.. ........
#T1A4        T......... .......... .......G.. ....AT.C.. ..A..... TA.....-.. ........
#T2D9        T......... .......... .......G.. ....AT.C.. ..A..... TA.....-.. ........
#T1A2        T......... .......... .......G.. ....AT.C.. ..A..... TA.....-.. ........
#T9C2        T......... .......... .......G.. ....AT.C.. ..A..... TA.....-.. ........
#T7B4        T......... .......... .......G.. ....AT.C.. ..A..... TA.....-.. ........
#T7A1        T......... .......... .......G.. ....AT.C.. ..A..... TA.....-.. ........
#T7B3        T......... .......... .......G.. ....AT.C.. ..A..... TA.....-.. ........
#E7.1        T..T.G.... .......... .......G.. ....AC.C.. ..A..... TA........ ...C.A..
#E7A1        T..T.G.... .......... .......G.. ....AC.C.. ..A..... TA..AA.... ...C.A..
#E1A1        T..T.G.... .......... .......G.. ....AC.C.. ..A..... T...AA.... ...C.A..
#CCAF200377 T......T.. .......... ..T....G.. ....ATCC.. ..A..G...T T...A..-.C .A......
#CBAF200375 .......T.. .......... ..T....G.. .....C.C.. ..A..G...T .A..A..-.C .A......
#BMAF200373 .A.T...C.. ......C... .......... .....C.... .....G...G TA.......C .G...A.T
#BIAF200372 ...T...C.. .......... .......... .....C.... .....G.... TA.....-.C .G...A..
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Appendix 3: Alignment of 778 bp of COI sequences. *Sycophila* haplotypes (SHT), *Pediobius* haplotypes (PHT), *Eurytoma* haplotypes (EHT), *Chlorocytus* haplotypes (CHT). Codon start position 2. Identical nucleotides are shown by (.).

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Domain=COI: CodonStart=2
#SHT1  A CCT GGG TTT GGG TTA GTT TCT CAT ATA ATT TGT AAT GAA AGA ATA AAA AAA GAA ACA TTT GGT GCT ATA GGA ATA AT
#SHT2  . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT3  . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT4  . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT5  . ... ..A ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT6  . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT7  . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT8  . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT9  . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT10 . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT11 . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT12 . ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT13 . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT15 . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT16 . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT17 G ... ..A ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT18 . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT19 . ... ..A ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT20 . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT21 . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT23 . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT26 . ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT27 . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT28 . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT29 . ... ... ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ..
#PHT1  . ... ..A ... ..T C.. ... ... ... ... ... ... ... ..G ... ... ... ... ... GTT ... ..G .T. ..C ... ... ..
#PHT2  . ... ..A ... ..T C.. ... ... ... ... ... ... ... ..G ... ... ... ... ... GTT ... ..G .T. ..T ... ... ..
#PHT3  . ... ..A ... ..T ... ... ... ... ... ... ... ... ..G ..G ... ... ... ... GTT ... ..G .T. ..T ... ... ..
#PHT4  . ... ..A ... ..T ... ... ... ... ... ... ... ... ..G ... ... ... ... ... GTT ... ..G .T. ..C ... ... ..
#PDC1  . ... ..A ... ..T ... ... ... ... ... ... ..C ... ..G ... ... ... ... ... GTT ... ..G .T. ..T ... ... ..
#PER1  . ... ..A ... ..T ... ... ... ... ... ... ... ... ..G ... ... ... ... ... GTT ... ..G .T. ..T ... ... ..
#PPP1  . ... ..A ... ..T ... ... ... ... ... ... ... ... ..G ..T ... ... ... ... GTT ... ..A .T. ..T ..G ... ..
#PBS1  . ... ..A ... ..T ... ... ... ... ... ... ... ... ..G ... ... ... ... ... GTT ... ..A .T. ..T ... ... ..
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```
#EHT1   . ... ..A ... ..A ... A.. ... ... ... ... ... .GA ... ... ... ... ..G ... ..T ... ..A ..A ... ... ... ..
#EHT2   . ... ..A ... ..A ... A.. ... ... ... ... ... .GA ... ... ... ... ..G ... ..T ... ..G ..A ... ... ... ..
#EHT4   . ... ..A ... ..A ... A.. ... ... ... ... ... .GA ... ... ... ... ..G ... ..T ... ..G ..A ... ... ... ..
#EF1    . ... ..A ... ..A ... A.. ... ... ... ... ... .GG ... ... ... ... ... ... ... ... ..G ... ... ..G ... ..
#EF2    . ... ..A ... ..A ... A.. ... ... ... ... ... .GA ... ... ... ... ..G ... ... ... ..G ... ... ... ... ..
#EF3    . ... ..A ... ..A ... A.. ... ... ... ... ... .GA ... ... ... ... ... ... ... ... ..G ... ... ... ... ..
#EF4    . ... ..A ... ..A ... A.. ... ... ... ... ... .GA ... ... ... ... ... ... ... ... ..G ... ... ... ... ..
#EF5    . ... ..A ... ..A ... A.. ... ... ... ... ... .GA ... ... ... ... ... ... ... ... ..G ... ... ... ... ..
#EF6    . ... ..A ... ..A ... A.. ... ... ... ... ... .GA ... ... ... ... ... ... ... ... ..G ... ... ... ... ..
#EF7    . ... ..A ... ..A ... A.. ... ... ... ... ... .GA ... ... ... ... ... ... ... ... ..G ... ... ... ... ..
#EF8    . ... ..A ... ..A ... A.. ... ... ... ... ... .GA ... ... ... ... ... ... ... ... ..G ... ... ... ... ..
#EP1    . ... ..T ... ..A ... A.C ... ... ... ... ... .GA ..G ... ... ... ... ... ..C ... ... ..A ... ... ... ..
#EF2A1  T ... ..A ... ..T ... A.. ... ... ..G ... ... .GG ... ... ... ... ... ... ..C ... ..A ... ... ... ... ..
#ESPA4  . ..A ..A ... ..A ... A.. ... ... ..G ... ... .GA ... ... ... ... ..G ... ..T ... ..G ... ... ... ... ..
#EA1    . ... ..A ... ..A ... A.. ..A ... ... ... ... .GA ..G ... ... ... ... ... ..T ... ... ... ... ... ... ..
#ESPA5  . ..A ..A ... ..T ... A.. ..A ... ... ... ... ... ... ..G ... ... ..G ... ... ... ..G ... ... ... ... ..
#CHT1   . ... ..A ... ..A ... ..A ... ... ... ... ... ... ... ... T.. ... ... ... ... ... ..A T.. ... ... ... ..
#CHT2   . ... ..A ... ..A ... ..A ... ... ... ... ... ... ... ... T.. ... ... ... ... ... ..A T.. ... ... ... ..
#CHT3   . ... ..A ... ..A ... ..A ..A ... ... ... ... ... ... ... T.. ... ... ... ... ... ..A T.. ... ... ... ..
#CHT4   . ... ..A ... ..A ... ..A ... ... ... ... ... ... ... ... T.. ... ... ... ... ... ..A T.. ... ... ... ..
#CHT5   . ... ... ... ..T ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A T.A ... ... ... ..
#CHT6   . ... ..A ... ..A ... ..A ... ... ... ... ... ... ... ... T.. ... ... ... ... ... ..A T.. ... ... ... ..

#SHT1   T TAT GCG ATA ATT TCT ATT GGG GTT TTA GGT TTC ATT GTG TGG GCT CAT CAT ATA TTT ACA GTA GGT ATG GAT GTT GA
#SHT2   . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ..
#SHT3   . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ..
#SHT4   . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ..C ..
#SHT5   . ..C ..A ... ... ... ... ..A ... ... ..A ..T ... ..A ..A ... ... ... ..G ... ... ... ..G ... ..C ... ..
#SHT6   . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT7   . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ..
#SHT8   . ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ..
#SHT9   . ... ... ... ... ... ... ... ... ... ..A ..T ... ... ..A ... ... ... ... ... ... ... ... ... ... ... ..
#SHT10  . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ..
#SHT11  . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ..
#SHT12  . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ..
#SHT13  . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ..
#SHT15  . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ..
#SHT16  . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ..
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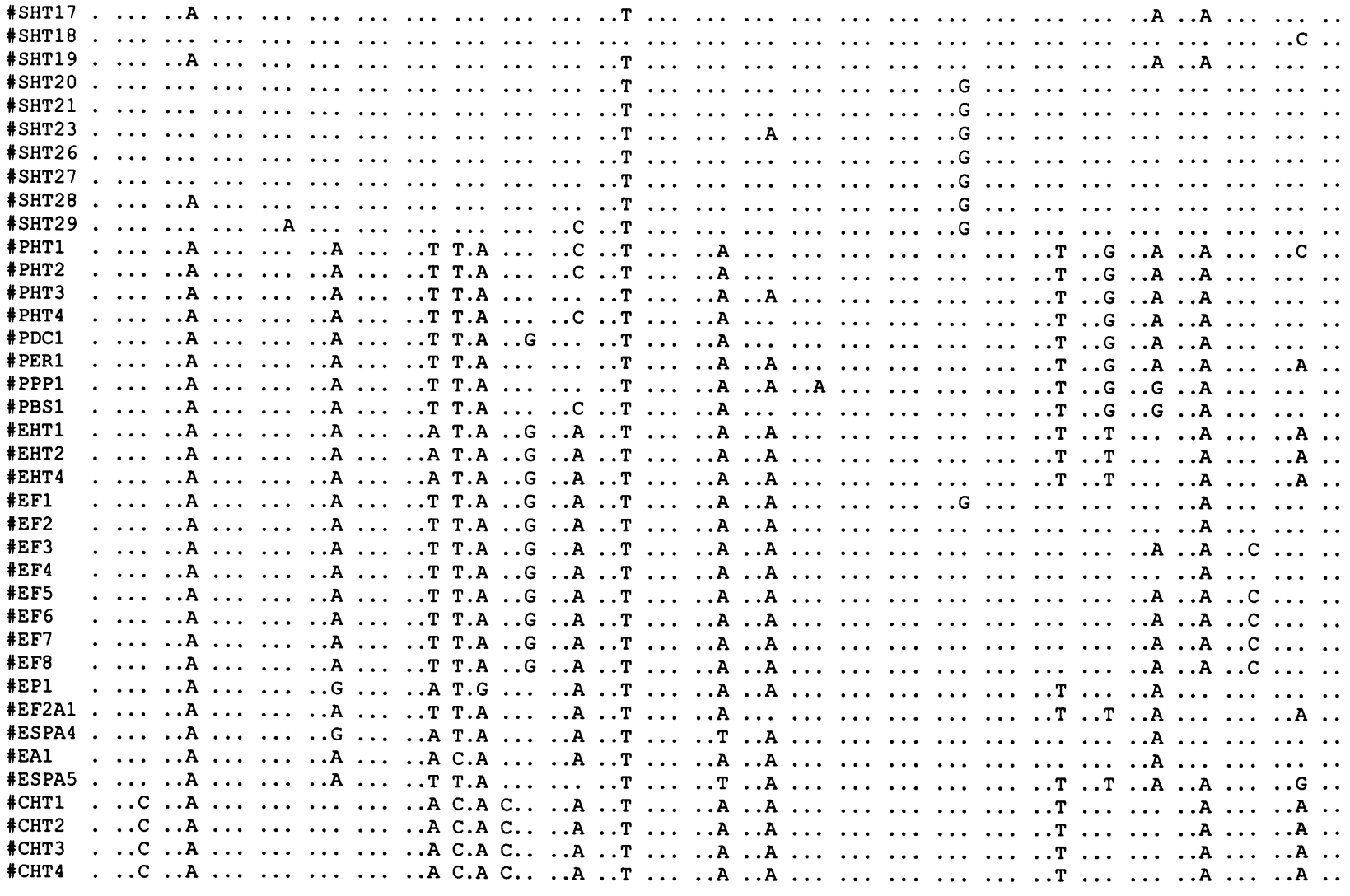
#SHT17 . ... ..A ... ...T ... ...A ..A ... .
#SHT18 . ...C ..
#SHT19 . ... ..A ... ...T ... ...A ..A ... .
#SHT20 . ...T ... ...G ... ...
#SHT21 . ...T ... ...G ... ...
#SHT23 . ...T ... .A ... ...G ... ...
#SHT26 . ...T ... ...G ... ...
#SHT27 . ...T ... ...G ... ...
#SHT28 . ... ..A ... ...T ... ...G ... ...
#SHT29 . ...A ... ...C ..T ... ...G ... ...
#PHT1 . ... ..A ... .A ... ..T T.A ... ..C ..T ... ..A ... ...T ..G ..A ..A ... ..C ..
#PHT2 . ... ..A ... .A ... ..T T.A ... ..C ..T ... ..A ... ...T ..G ..A ..A ... .
#PHT3 . ... ..A ... .A ... ..T T.A ... .T ... ..A ..A ... ...T ..G ..A ..A ... .
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#EF2 . ... ..A ... .A ... ..T T.A ..G ..A ..T ... ..A ..A ... ...A ... .
#EF3 . ... ..A ... .A ... ..T T.A ..G ..A ..T ... ..A ..A ... ...A ..A ..C ... ..
#EF4 . ... ..A ... .A ... ..T T.A ..G ..A ..T ... ..A ..A ... ...A ... .
#EF5 . ... ..A ... .A ... ..T T.A ..G ..A ..T ... ..A ..A ... ...A ..A ..C ... ..
#EF6 . ... ..A ... .A ... ..T T.A ..G ..A ..T ... ..A ..A ... ...A ..A ..C ... ..
#EF7 . ... ..A ... .A ... ..T T.A ..G ..A ..T ... ..A ..A ... ...A ..A ..C ... ..
#EF8 . ... ..A ... .A ... ..T T.A ..G ..A ..T ... ..A ..A ... ...A ..A ..C ... ..
#EP1 . ... ..A ... .G ... ..A T.G ... ..A ..T ... ..A ..A ... ...T ... ..A ... ...
#EF2A1 . ... ..A ... .A ... ..T T.A ... ..A ..T ... ..A ... ...T ..T ..A ... .A ..
#ESPA4 . ... ..A ... .G ... ..A T.A ... ..A ..T ... ..T ..A ... ...A ... ...
#EA1 . ... ..A ... .A ... ..A C.A ... ..A ..T ... ..A ..A ... ...A ... ...
#ESPA5 . ... ..A ... .A ... ..T T.A ... .T ... ..T ..A ... ...T ..T ..A ..A ... ..G ..
#CHT1 . ..C ..A ... ...A C.A C.. ..A ..T ... ..A ..A ... ...T ... .A ... ..A ..
#CHT2 . ..C ..A ... ...A C.A C.. ..A ..T ... ..A ..A ... ...T ... .A ... ..A ..
#CHT3 . ..C ..A ... ...A C.A C.. ..A ..T ... ..A ..A ... ...T ... .A ... ..A ..
#CHT4 . ..C ..A ... ...A C.A C.. ..A ..T ... ..A ..A ... ...T ... .A ... ..A ..

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#CHT5  . ... ..A ... ... ..A ... ..A T.A ... ..A ..T ... ..A ... ..A ... ... ... ... ..T ... ..A ... ... ..G ..
#CHT6  . ..C ..A ... ... ... ... ..A C.A C.. ..A ..T ... ..A ..A ... ... ... ... ... ..T ... ... ..A ... ..A ..

#SHT1  T ACA CGT GCT TAC TTT ACT AGG GCA ACT ATA ATT ATT GCG GTT CCT ACT GGA ATT AAA ATT TTT AGG TGA TTA GCT TC
#SHT2  . ... ..C ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT3  . ... ..C ... ... ... ... ..A ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ... ... ... ..
#SHT4  . ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT5  . ... ... ... ..T ... ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ... ..
#SHT6  . ... ..C ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT7  . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ..G ... ..
#SHT8  . ... ..C ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT9  . ..G ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT10 . ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT11 . ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT12 . ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT13 . ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT15 . ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT16 . ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT17 . ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT18 . ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT19 . ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT20 . ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT21 . ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT23 . ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT26 . ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT27 . ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT28 . ... ..C ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT29 . ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#PHT1  . ..T ... ... ..T ... ..A TCA ..T ... ... ... ... ..T A.. ... ..A ... ... ... ... ... ..T ... ... ... ..
#PHT2  . ..T ... ... ..T ... ..A TCA ..T ... ... ... ... ..T A.. ... ..A ... ... ... ... ... ..T ... ... ... ..
#PHT3  . ..T ... ... ..T ... ..A TCA ..T ... ... ... ... ..T A.. ... ... ... ... ... ... ... ..T ... ... ... ..
#PHT4  . ..T ... ... ..T ... ..A TCA ..T ... ... ... ... ..T A.. ... ..A ... ... ... ... ... ..T ... ... ... ..
#PDC1  . ..T ... ... ..T ... ..A TCA ..T ... ... ... ... ..T A.. ... ... ... ... ... ... ... ..T ... ... ... ..
#PER1  . ..T ... ... ..T ... ..A TCA ..T ..A ... ... ... ..T A.. ... ... ... ... ... ... ... ..T ... ... ... ..
#PPP1  . ..T ..G ..C ..T ... ..A TCA ..T ... ... ... ... ..T A.. ... ... ... ... ... ... ... ..T ... ... ... ..
#PBS1  . ..T ... ... ..T ... ..A TCA ..T ..A ... ... ... ..T A.. ... ... ... ... ... ... ... ..T ... ... ... ..
#EHT1  . ... ..A ... ..T ... ... TCA ..T ... ... ... ... ..T ..A ... ..A ..T ... ... ... ... ..A ... ... ..A ..
#EHT2  . ... ..A ... ..T ... ... TCA ..T ... ... ... ... ..T ..A ... ..A ..T ... ... ... ... ..A ... ... ..A ..
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#EHT4   . ... ..A ... ..T ... ... TCA ..T ... ... .C. ... ..T ..A ... ..A ..T ... ... ... ... ..A ... ... ..A ..
#EF1    . ..C ... ... ..T ... ... TCA ..T ... ... ... ... ..A ..A ..C ..A ..T ... ... ... ... ... ..G ... ..G ..
#EF2    . ..C ... ... ..T ... ... TCA ..T ... ... ... ... ..A ..A ..C ... ..T ... ... ... ... ... ..G C.. ..A ..
#EF3    . ... ... ... ..T ... ... TCA ..T ... ... ... ... ..A ..A ... ..A ..T ... ... ... ... ..A ..G ... ..A ..
#EF4    . ..C ... ... ..T ... ... TCA ..T ... ... ... ... ..A ..A ..C ..A ..T ... ... ... ... ... ..G C.. ..A ..
#EF5    . ... ... ... ..T ... ... TCA ..T ... ... ... ... ..A ..A ... ..A ..T ... ... ... ... ..A ..G ... ..A ..
#EF6    . ... ... ... ..T ... ... TCA ..T ... ... ... ... ..A ..A ... ..A ..T ... ... ... ... ..A ..G ... ..A ..
#EF7    . ... ... ... ..T ... ... TCA ..T ... ... ... ... ..A ..A ... ..A ..T ... ... ... ... ..A ..G ... ..A ..
#EF8    . ... ... ... ..T ... ... TCA ..T ... ... ... ... ..A ..A ... ..A ..T ... ... ... ... ..A ..G ... ..A ..
#EP1    . ..G ..A ... ..T ... ... TCA ..T ..A ... ... ... ..T ..A ... ..A ..T ... ... ..C ... ..T ... ... ... ..
#EF2A1  . ..T ..G ... ..T ... ... TCA ..T ... ... ... ... ..T ... ... ..A ... ... ... ... ..C ..A ... ... ..A ..
#ESPA4  C ... ..A ..C ... ... ... TCA ..T ... ... ... ... ..T ... ... ..A ... ... ..G ... ... ..A ... ... ... ..
#EA1    . ... ..A ... ..T ... ... TCA ... ..A ... ... ... ..T ..A ... ..A ... ... ... ... ... ..T ... ... ... ..
#ESPA5  . ..T ..A ..A ..T ... ... TCA ..T ..A ... ... ... ..A ... ... ... ... ... ... ... ... ..A ... C.. ... ..
#CHT1   . ..T ..A ... ..T ... ..A TCA ... ... ... ... ... ..T A.. ... ..A ... ... ... ... ... ..A ... C.T ... ..
#CHT2   . ..T ..A ... ..T ... ..A TCA ... ... ... ... ... ..T A.. ... ..A ... ... ... ... ... ..A ... C.T ... ..
#CHT3   . ..T ..A ... ..T ... ..A TCA ... ... ... ... ... ..T A.. ... ..A ... ... ... ... ... ..A ... C.T ... ..
#CHT4   . ..T ..A ... ..T ... ..A TCA ... ... ... ... ... ..T A.. ... ..A ... ... ... ... ... ..A ... C.T ... ..
#CHT5   . ..T ..A ... ..T ... ... TCA ... ... ... ... ... ..A A.. ..C ... ... ... ... ... ... ..A ... ... ... ..
#CHT6   . ..T ..A ... ..T ... ..A TCA ... ... ... ... ... ..T A.. ... ..A ... ... ... ... ... ..A ... C.T ... ..

#SHT1   A ATG AAC GGA ATA AAA ATT ATA TTT AGA GTT TCA AAT TTA TGG TTG TTA GGT TTT TTA TTT TTA TTT ACT GTA GGT GG
#SHT2   . ..A ... ... ... ... ... ... ... ... ... ... ... ... ... C.. ... ... ... ..G ... ... ... ... ... ... ..
#SHT3   . ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ... ..
#SHT4   . ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ... ..
#SHT5   . ..A ..T ..G ... ... ... ... ... ... ... ... ... ... ..A ..A A.. ..G ... ... ... ... ... ... ... ..G ..
#SHT6   . ..A ... ... ... ... ... ... ... ... ... ... ... ... C.. ... ... ... ..G ... ... ... ... ... ... ..
#SHT7   . ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ... ..
#SHT8   . ..A ... ... ... ... ... ... ... ... ... ... ... ... C.. ... ... ... ..G ... ... ... ... ... ... ..
#SHT9   . ..A ..T ... ... ... ... ... ... ... ... ... ... ... C.C ... ... ... ... ... ... ... ... ... ... ..
#SHT10  . ..A ... ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ... ..
#SHT11  . ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ... ..
#SHT12  . ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ... ..
#SHT13  . ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ... ..
#SHT15  . ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ... ..
#SHT16  . ..A ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ... ..
#SHT17  . ..A ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ..G ... ... ... ... ..
#SHT18  . ..A ... ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ... ..
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#SHT19 . ..A ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ..G ... ... ... ... ..
#SHT20 . ..A ..T ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ..G ... ... ... ... ... ..
#SHT21 . ..A ..T ..G ... ... ... ... ... ... ... ... ... ... ... ... C.. ... ..G ... ..G ... ... ... ... ... ..
#SHT23 . ..A ..T ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ..G ... ... ... ... ... ..
#SHT26 . ..A ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ..G ... ... ... ... ... ..
#SHT27 . ..A ..T ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ..G ... ... ... ... ... ..
#SHT28 . ..A ..T ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ..G ... ... ... ... ... ..
#SHT29 . ..A ..T ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ... ... ..
#PHT1  . ..A ..T ... ... ... ... .AG ... .AT A.. .AT .GA A.. ..A A.T ... ... ... A.T ... ... ... ... ... ..A ..
#PHT2  . ..A ..T ... ... ... ... .AG ... .AT A.. .AT .GA A.. ..A A.T ... ... ... A.T ... ... ... ... ... ..A ..
#PHT3  . ..A ..T ... ... ... ... .AG ... .AT A.. .AT .GA A.. ..A A.T ... ... ... A.T ... ... ... ... ... ..A ..
#PHT4  . ..A ..T ... ... ... ... .AG ... .AT A.. .AT .GA A.. ..A A.T ... ... ... A.T ... ... ... ... ... ..A ..
#PDC1  . ..A ..T ..G ... ... ... .AG ... .AT A.. .AT .GA A.. ..A A.T ... ..A ... A.T ... ... ... ... ... ..A ..
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#SHT18    . ... ... ... ... ... ..G ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ..
#SHT19    . ... ..G ... ..T ... ... ... ... ... ..C ... ... ... ..G ... ... ... ... ... ... ... ... ... ..
#SHT20    . ... ..G ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ..
#SHT21    . ... ..G ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT23    . ... ..G ... ..T ... ... ... ... ... ..G ... ... ... ... ... ... ... ... ..C ... ... ... ... ..
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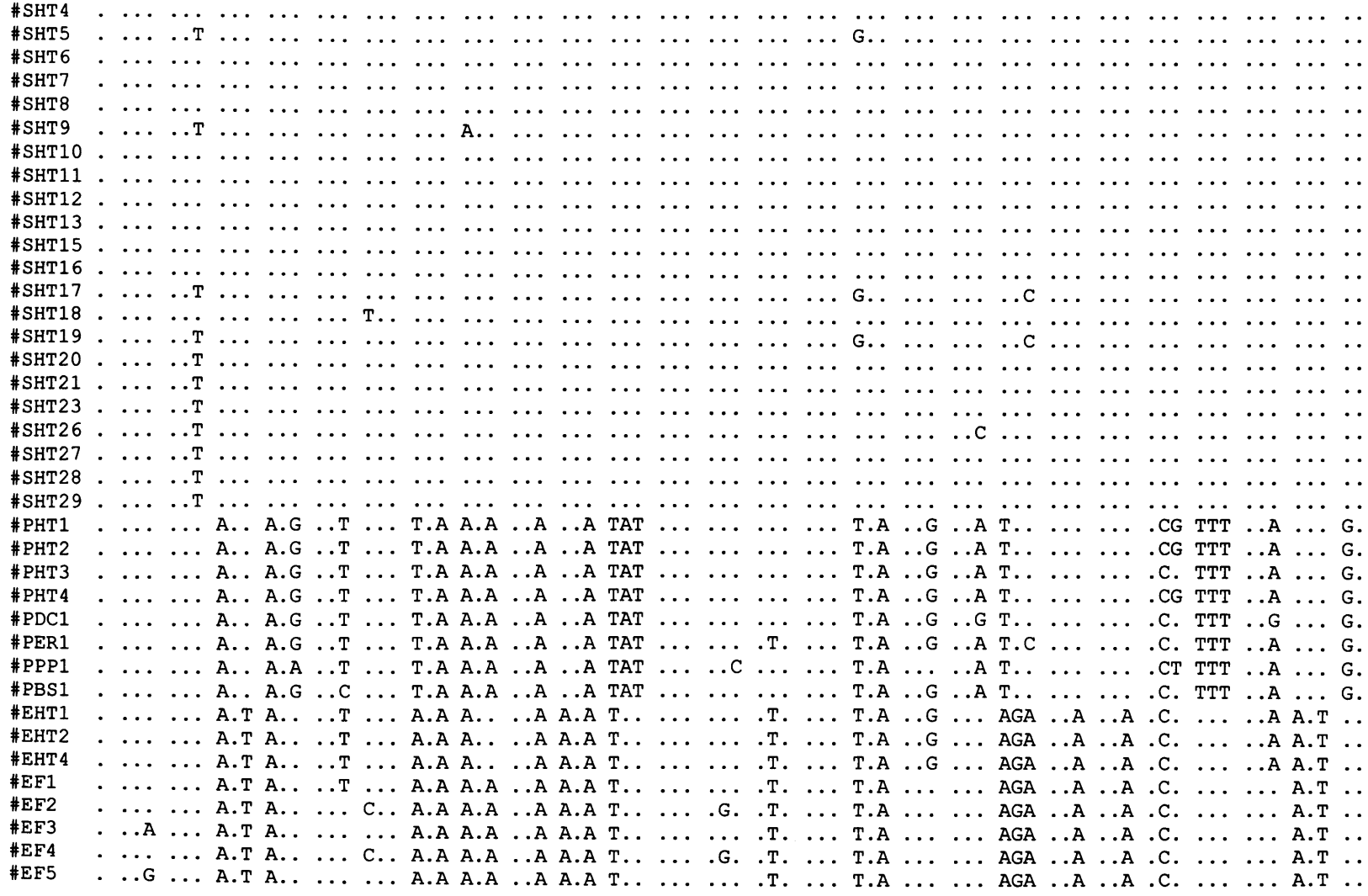
```
#SHT26 . ... ..G ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT27 . ... ..G ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT28 . ... ..T ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT29 . ... ..G ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ... ..
#PHT1  . ... ..T ... ..T ... ... ... ..T ..A ... ..T ... ... ..G ... ..T .A. ... ... ... ... A.T T.A ..T ... G.
#PHT2  . ... ..T ... ..T ... ... ... ..T ..A ... ..T ... ... ..G ... ..T .A. ... ... ... ... A.T T.A ..T ... G.
#PHT3  . ... ..T ... ..G ... ... ... ..T ..A ... ..T ... ... ... ... ..T .A. ... ... ... ... A.T T.A ..T ... G.
#PHT4  . ... ..T ... ..T ... ... ... ..T ..A ... ..T ... ..C ..G ... ..T .A. ... ... ... ... A.T T.A ..T ... G.
#PDC1  . ... ..T ... ..G ... ... ..C ..T ..A ... ..T ..C ..C ... ... ..T .A. ... ... ... ... A.T T.A ..T ... G.
#PER1  . ... ..T ... ..T ... ... ... ..T ..A ... ..T ... ... ... ... ..T .A. ... ... ... ... A.T T.A ..T ... G.
#PPP1  . ... ..T ... ... ... ... ... ... ..A ... ..T ... ... ... ... ..T .A. ... ... ... ... A.C T.A ..T ... G.
#PBS1  . ... ..C ... ..T ... ... ... ..T ..A ... ..T ... ... ... ... ..T .AC ... ... ... ... A.T T.A ..T ... G.
#EHT1  . ... ... ... ... ... ... ... ... ..A ... ... ... ... ... ..T ... ... ... A.. ... ... ... ... T.A ..T ... ..
#EHT2  . ... ... ... ... ... ... ... ... ..A ... ... ... ... ... ..T ... ... ... A.. ... ... ... ... T.A ..T ... ..
#EHT4  . ... ... ... ... ... ... ... ... ..A ... ... ... ... ... ..T ... ... ... A.. ... ... ... ... T.A ..T ... ..
#EF1   . ... ... ..G ... ... ..G ... ..A ..A ... ... ... ... ... ... ... ... ... A.. ... ... ... ... T.A ..T ... ..
#EF2   . ... ... ..G ..T ... ..G ... ..A ..A ... ... ... ... ... ... ... ... ... A.. ... ... ... ..G T.A ..T ... ..
#EF3   . ... ..G ..G ..T ... ... ... ..A ..A ... ... ... ... ... ... ... ... ... A.. ... ... ... ..G T.A ..T ... ..
#EF4   . ... ... ..G ..T ... ..G ... ..A ..A ... ... ... ... ... ... ... ... ... A.. ... ... ... ... T.A ..T ... ..
#EF5   . ... ..G ..G ..T ... ... ... ..A ..A ... ... ... ... ... ... ... ... ... A.. ... ... ... ..G T.A ..T ... ..
#EF6   . ... ..G ..G ..T ... ... ... ..A ..A ... ... ... ... ... ... ... ... ... A.. ... ... ... ... T.A ..T ... ..
#EF7   . ... ..G ..G ..T ... ... ... ..A ..A ... ... ... ... ... ... ... ... ... A.. ... ... ... ... T.A ..T ... ..
#EF8   . ... ..G ..G ..T ... ... ... ..A ..A ... ... ... ... ... ... ... ... ... A.. ... ... ... ... T.A ..T ... ..
#EP1   . ... ... ... ... ..A ..G ... ..A ... ... ... ... ... ... ... ... ... A.. ... ... ... A.T T.A ... ..G ..
#EF2A1 . ... ... ... ... ..A ... ... ..T ... ... ... ... ... ... ... ... ... A.G ... ... ... ..T T.A ..T ... ..
#ESPA4 . ... ..G ... ... ... ... ... ..A ... ... ... ... ... ... ..T ... ... ... A.G ... ... ... ... T.A ... ..T ..
#EA1   . ..G ... ... ... ..A ... ... ..A ... ... ... ..C ... ... ... ... ... ... A.. ... ... ... A.T T.A ... ... ..
#ESPA5 . ... ... ..G ... ..A ... ..A ..A ..A ... ..T ... ... ..T ... ... ... A.. ... ... ... A.T T.A ..T ... ..
#CHT1  . ... ... A.. ... ... ... ... ..T ..A ... ... ... ... ... ... ... ... .A. ACT ... ... ... A.T T.A ... ... ..
#CHT2  . ... ... A.. ... ... ... ... ..T ..A ... ... ... ... ... ... ... ... .A. ACT ... ... ... A.T T.A ... ... ..
#CHT3  . ... ... A.. ... ... ... ... ..T ..A ... ... ... ... ... ... ... ... .A. ACT ... ... ... A.T T.A ... ... ..
#CHT4  . ... ... A.. ... ... ... ... ..T ..A ... ... ... ... ... ... ... ... .A. ACT ... ... ... A.T T.A ... ... ..
#CHT5  . ... ... A.. ..G ..G ... ..A ..A ..A ... ... ... ... ..T ... ... .A. ACT ... ... ... A.T C.A ... ..T ..
#CHT6  . ... ... A.. ... ... ... ... ..T ..A ... ... ... ... ... ... ... ... .A. ACT ... ... ... A.T T.A ... ... ..

#SHT1  T GGT TCA GTA GTT TCA TTA GTT GGT ACT TTT ATA TTT TTT TAT ATT ATT TGA GAT GCT TTT ATT TTA AAA CGT TTA TT
#SHT2  . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
#SHT3  . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
```


#SHT4 . ...
#SHT5 . ... ..T ... ... G.. ... ...
#SHT6 . ...
#SHT7 . ...
#SHT8 . ...
#SHT9 . ... ..T ... ... A.. ... ...
#SHT10 . ...
#SHT11 . ...
#SHT12 . ...
#SHT13 . ...
#SHT15 . ...
#SHT16 . ...
#SHT17 . ... ..T ... ... G.. ... .C ... ...
#SHT18 . ... T.. ... ...
#SHT19 . ... ..T ... ... G.. ... .C ... ...
#SHT20 . ... ..T ... ...
#SHT21 . ... ..T ... ...
#SHT23 . ... ..T ... ...
#SHT26 . ... ..T ... ...C ... ...
#SHT27 . ... ..T ... ...
#SHT28 . ... ..T ... ...
#SHT29 . ... ..T ... ...
#PHT1 . ... A.. A.G ..T ... T.A A.A ..A ..A TAT ... ... T.A ..G ..A T.. ... ... .CG TTT ..A ... G.
#PHT2 . ... A.. A.G ..T ... T.A A.A ..A ..A TAT ... ... T.A ..G ..A T.. ... ... .CG TTT ..A ... G.
#PHT3 . ... A.. A.G ..T ... T.A A.A ..A ..A TAT ... ... T.A ..G ..A T.. ... ... .C. TTT ..A ... G.
#PHT4 . ... A.. A.G ..T ... T.A A.A ..A ..A TAT ... ... T.A ..G ..A T.. ... ... .CG TTT ..A ... G.
#PDC1 . ... A.. A.G ..T ... T.A A.A ..A ..A TAT ... ... T.A ..G ..G T.. ... ... .C. TTT ..G ... G.
#PER1 . ... A.. A.G ..T ... T.A A.A ..A ..A TAT ... ... .T. ... T.A ..G ..A T.C ... ... .C. TTT ..A ... G.
#PPP1 . ... A.. A.A ..T ... T.A A.A ..A ..A TAT ... ..C ... ... T.A ... ..A T.. ... ... .CT TTT ..A ... G.
#PBS1 . ... A.. A.G ..C ... T.A A.A ..A ..A TAT ... ... T.A ..G ..A T.. ... ... .C. TTT ..A ... G.
#EHT1 . ... A.T A.. ..T ... A.A A.. ..A A.A T.. ... ... .T. ... T.A ..G ... AGA ..A ..A .C. ... ..A A.T ..
#EHT2 . ... A.T A.. ..T ... A.A A.. ..A A.A T.. ... ... .T. ... T.A ..G ... AGA ..A ..A .C. ... ..A A.T ..
#EHT4 . ... A.T A.. ..T ... A.A A.. ..A A.A T.. ... ... .T. ... T.A ..G ... AGA ..A ..A .C. ... ..A A.T ..
#EF1 . ... A.T A.. ..T ... A.A A.A ..A A.A T.. ... ... .T. ... T.A ... ... AGA ..A ..A .C. ... ... A.T ..
#EF2 . ... A.T A.. ... C.. A.A A.A ..A A.A T.. ... .G. .T. ... T.A ... ... AGA ..A ..A .C. ... ... A.T ..
#EF3 . ..A ... A.T A.. ... ... A.A A.A ..A A.A T.. ... ... .T. ... T.A ... ... AGA ..A ..A .C. ... ... A.T ..
#EF4 . ... A.T A.. ... C.. A.A A.A ..A A.A T.. ... .G. .T. ... T.A ... ... AGA ..A ..A .C. ... ... A.T ..
#EF5 . ..G ... A.T A.. ... ... A.A A.A ..A A.A T.. ... ... .T. ... T.A ... ... AGA ..A ..A .C. ... ... A.T ..

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#EF6     . ..A ... A.T A.. ... ... A.A A.A ..A A.A T.. ... ... .T. ... T.A ... ... AGA ..A ..A .C. ... ... ... A.T . .
#EF7     . ..A ... A.T A.. ... ... A.A A.A ..A A.A T.. ... ... .T. ... T.A ... ... AGA ..A ..A .CT ... ... ... A.T . .
#EF8     . ..A ... A.T A.. ... ... A.A A.A ..A A.A T.. ... ... .T. ... T.A ... ... AGA ..A ..A .C. ... ... ... A.T . .
#EP1     . ..A ... T.. A.. ..T ... A.. A.A ..A ..A T.. ... ... .T. ... T.A ... ... AGA ... ..A .CT ... ... ..A A.T . .
#EF2A1   . ..A ... A.T A.. ..T ... A.A A.A ..A A.A T.. ... ... .T. ... T.A ... ... AGA ..A ..A .CT ... ... ..A A.T . .
#ESPA4   . ..A ..T A.. A.. ..T ... T.A A.A ... A.A T.. ... ... .T. ... T.A ... ... AGA ..A ..G .CT ... ... ..G A.T . .
#EA1     . ..A ..G C.. A.. ..T ... A.. A.A ..A ... T.. ... ... .T. ... T.A ... ... AGA ..A ..A .CT ... ... ..A A.T . .
#ESPA5   . ..A ... ... A.. ... ... A.A A.A ... ..A T.. ... ... .T. ... C.. ... ... AGA ..A ..A .CT ... ... ..A A.T . .
#CHT1    A ... AG. A.. A.C ... ... A.. A.A A.A ... ..A T.T ... ... .T. ... T.A ... ... AGA A.. ... .C. ... ... ... A.T A.
#CHT2    A ... AG. A.. A.C ... ... A.. A.A A.A ... ..A T.T ... ... .T. ... T.A ... ... AGA A.. ... .C. ... ... ... A.T A.
#CHT3    A ... AG. A.. A.C ... ... A.. A.A A.A ... ..A T.T ... ... .T. ... T.A ... ... AGA A.. ... .C. ... ... ... A.T A.
#CHT4    A ... AG. A.. A.C ... ... A.. A.A A.A ... ..A T.T ... ... .T. ... T.A ... ... AGA A.. ... .C. ... ... ... A.T A.
#CHT5    A ..A AG. A.. A.. ... ... A.. A.G A.A ..A A.A TAT ... ... .T. ... ..A ... ... AGG A.. ... .C. ... ... ... A.T A.
#CHT6    A ... AG. A.. A.C ... ... A.. A.A A.A ... ..A T.T ... ... .T. ... T.A ... ... AGA A.. ... .C. ... ... ... A.T A.

#SHT1    A ATT TTT GTG AAA AGA TTA AAT AAT ATA ATT GAA ATA ATT ATA AAT TTT CCT CCT AGA GAT CAT TCT TTT ATA GAA
#SHT2    . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#SHT3    . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#SHT4    . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#SHT5    . ... ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ...
#SHT6    . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#SHT7    . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#SHT8    . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#SHT9    . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ...
#SHT10   . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#SHT11   . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#SHT12   . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#SHT13   . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#SHT15   . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#SHT16   . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#SHT17   . ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... T.. ... ... ... ... ... ...
#SHT18   . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#SHT19   . ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... T.. ... ... ... ... ... ... ...
#SHT20   . ... ... ..T ... ..G ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#SHT21   . ... ... ..T ... ..G ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#SHT23   . ... ... ..T ... ..G ... ... ... ... ... ..C ... ... ... ... ... ... T.. ... ... ... ... ... ... ...
#SHT26   . ... ... ..T ... ..G ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#SHT27   . ... ... ..T ... ..G ... ... ... ... ... ..C ... ... ... ... ..C ... ... ... ... ... ... ... ... ...
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#SHT28 . ... ... ..T ... ..G ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ...
#SHT29 . ... ... ..T ... ..G ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ...
#PHT1  . ..A .A. A.A ... .AT ... ... ... TC. ..A ... TG. T.A ... TCA .A. ... ..G ... T.. ..C ..A .A. TAT ...
#PHT2  . ..A .A. A.A ... .AT ... ... ... TC. ..A ... TG. T.A ... TCA .A. ... ..G ... T.. ..C ..A .A. TAT ...
#PHT3  . ..A .A. A.A ... .AT ... ... ... TC. ..A ... TG. T.A ... TCA .A. ..C ..A ... T.. ..C ..A .A. TAT ...
#PHT4  . ..A .A. A.A ... .AT ... ... ... TC. ..A ... TG. T.A ... TCA .A. ..C ..A ... T.. ..C ..A .A. TAT ...
#PDC1  . ..A .A. A.A ... .AT ... ... ... TC. ..A ... TG. T.A ... TCA .A. ..C ..A ... T.. ..C ..A .A. TAT ...
#PER1  . ..G .A. A.A ... .AT ... ... ... TC. ..A ... TG. T.A ... TCA .A. ... ..A ... T.. ... ..A .A. TAT ...
#PPP1  . ..A .A. A.A ... .AT ... ... ... TC. ..A ... TG. T.A ... TCA .A. ... ... ... T.. ..C ..A .A. TAT ..G
#PBS1  . ..A .A. A.A ... .AT ... ... ... TC. ..A ... TG. T.A ... TCA .A. ... ..A ... T.. ..C ..A .A. TAT ...
#EHT1  . ... ... A.A ... .AT ... ... .GA TC. ... ... TG. ... G.. .GA ... ... ..A ... ..A ... ... ... TAT ...
#EHT2  . ... ... A.A ... .AT ... ... .GA TC. ... ... TG. ... G.. .GA ... ... ..A ... ..A ... ... ... TAT ...
#EHT4  . ... ... A.A ... .AT ... ... .GA TC. ... ... TG. ... G.. .GA ... ... ..A ... ..A ... ... ... TAT ...
#EF1   . ... ... A.A ... .AT ... ... .GA TC. ... ... TG. ... ... .GA ... ... ..A ... ..A ... ..A ... TAT ...
#EF2   . ... ... A.A ... .AT ... ... .GA TC. ... ... TG. ... ... .GA ... ... ..A ... ..A ... ..A ... TAT ...
#EF3   . ... ... A.A ... .AT ... ... .GA TC. ... ... TG. ... ... .GA ... ... ..A ... ..A ..C ..A ... TAT ...
#EF4   . ... ... A.A ... .AT ... ... .GA TC. ... ... TG. ... ... .GA ... ... ..A ... ..A ... ..A ... TAT ...
#EF5   . ... ... A.A ... .AT ... ... .GA TC. ... ... TG. ... ... .GA ... ... ..A ... ..A ..C ..A ... TAT ...
#EF6   . ... ... A.A ... .AT ... ... .GA TC. ... ... TG. ... ... .GA ... ... ..A ... ..A ..C ..A ... TAT ...
#EF7   . ... ... A.A ... .AT ... ... .GA TC. ... ... TG. ... ... .GA ... ... ..A ... ..A ..C ..A ... TAT ...
#EF8   . ... ... A.A ... .AT ... ... .GA TC. ... ... TG. ... ... .GA ... ... ..A ... ..A ... ..A ... TAT ...
#EP1   . ... ... A.A ... .AT ... ... .GA TC. ... ..G TG. ... ..T .GG ... ... ... ... ..A ... ..A ... TAT ...
#EF2A1 . ... ... A.A ... .AT ... ... .GA TCG ... ... TG. ... G.T .GA ... ... ... ... ..A ... ..A ... TAT ..G
#ESPA4 . ... ... A.A ... .AT ... ... .GA TCT ... ... TG. ... G.. .GA ... ... ... ... ..A ... A.. ... T.T ...
#EA1   . ... ... A.A ... .AT ... ..C .GA TCG ... ... TG. ... ..T .GA ... ... ..G ... ..A ... ..A ... TAT ...
#ESPA5 . ... ... A.A ... .AT ... ... .GA TC. ... ... TG. ... ... .GA ... ... ... ..T ..A ... ..A ... TAT ...
#CHT1  T ... ... A.A ... TAT A.T ... ... TCT ... ... TG. ... ... CC. .A. ... ..A ... T.. ... ... ..C .AT ...
#CHT2  T ... ... A.A ... TAT A.T ... ... TCT ... ... TG. ... ... CC. .A. ... ..A .A. T.. ... ... ..C .AT ...
#CHT3  T ... ... A.A ... TAT A.T ... ... TCT ... ... TG. ... ... CC. .A. ... ..A ... T.. ... ... ..C .AT ...
#CHT4  T ... ... A.A ... TAT A.T ... ... TCT ... ... TG. ... ... CC. .A. ... ..A ... T.. ... ... ..C .AT ...
#CHT5  T ... ... A.A ... .AT A.. ... ... TC. ... ... TG. ... ... G.A ... ..C ... ..T T.. ... ... ... .AT ...
#CHT6  T ... ... A.A ... TAT A.T ... ... TCT ..C ... TG. ... ... CC. .A. ... ..A ... T.. ... ... ..C .AT ...
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Appendix 4: Amino acid translation for wasp COI haplotype sequences using the second reading frame. S=*Sychophila*, P=*Pediobius*, E=*Eurytoma*, C=*Chlorocytus*.

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#SHT1  PGFGLVSHMI CNESMKKETF GAMGMIYAMI SIGVLGFIVW AHHMFTVGMD VDTRAYFTSA TMIIAVPTGI KIFSWLASMN
#SHT2  .......... .......... .......... .......... .......... .......... .......... ..........
#SHT3  .......... .......... .......... .......... .......... .......... .......... ..........
#SHT4  .......... .......... .......... .......... .......... .......... .......... ..........
#SHT5  .......... .......... .......... .......... .......... .......... .......... ..........
#SHT6  .......... .......... .......... .......... .......... .......... .......... ..........
#SHT7  .......... .......... .......... .......... .......... .......... .......... ..........
#SHT8  .......... .......... .......... .......... .......... .......... .......... ..........
#SHT9  .......... .......... .......... .......... .......... .......... .......... ..........
#SHT10 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT11 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT12 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT13 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT15 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT16 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT17 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT18 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT19 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT20 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT21 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT23 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT26 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT27 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT28 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT29 .......... .......... .........M .......... .......... .......... .......... ..........
#PHT1  .......... ........V. .VI..... ...L...... .......... .......... .....I.... ..........
#PHT2  .......... ........V. .VI..... ...L...... .......... .......... .....I.... ..........
#PHT3  .......... ........V. .VI..... ...L...... .......... .......... .....I.... ..........
#PHT4  .......... ........V. .VI..... ...L...... .......... .......... .....I.... ..........
#PDC1  .......... ........V. .VI..... ...L...... .......... .......... .....I.... ..........
#PER1  .......... ........V. .VI..... ...L...... .......... .......... .....I.... ..........
#PPP1  .......... ........V. .VI..... ...L...... .......... .......... .....I.... ..........
#PBS1  .......... ........V. .VI..... ...L...... .......... .......... .....I.... ..........
#EHT1  .....I.... .S........ .......... ...L...... .......... .......... .......... ..........
#EHT2  .....I.... .S........ .......... ...L...... .......... .......... .......... ..........
#EHT4  .....I.... .S........ .......... ...L...... .......... .......... ..T....... ..........
```

```
#EF1     .....I.....  .S.........  ..........  ...L......  ..........  ..........  ..........
#EF2     .....I.....  .S.........  ..........  ...L......  ..........  ..........  ..........
#EF3     .....I.....  .S.........  ..........  ...L......  ..........  ..........  ..........
#EF4     .....I.....  .S.........  ..........  ...L......  ..........  ..........  ..........
#EF5     .....I.....  .S.........  ..........  ...L......  ..........  ..........  ..........
#EF6     .....I.....  .S.........  ..........  ...L......  ..........  ..........  ..........
#EF7     .....I.....  .S.........  ..........  ...L......  ..........  ..........  ..........
#EF8     .....I.....  .S.........  ..........  ...L......  ..........  ..........  ..........
#EP1     .....I.....  .S.........  ..........  ...L......  ..........  ..........  ..........
#EF2A1   .....I.....  .S.........  ..........  ...L......  ..........  ..........  ..........
#ESPA4   .....I.....  .S.........  ..........  ...L......  ..........  ..........  ..........
#EA1     .....I.....  .S.........  ..........  ...L......  ..........  ..........  ..........
#ESPA5   .....I.....  ..........  ..........  ...L......  ..........  ..........  ..........
#CHT1    ..........  .....L.....  .S........  ...L......  ..........  .....I....  ..........
#CHT2    ..........  .....L.....  .S........  ...L......  ..........  .....I....  ..........
#CHT3    ..........  .....L.....  .S........  ...L......  ..........  .....I....  ..........
#CHT4    ..........  .....L.....  .S........  ...L......  ..........  .....I....  ..........
#CHT5    ..........  ..........  .S........  ...L......  ..........  .....I....  ..........
#CHT6    ..........  .....L.....  .S........  ...L......  ..........  .....I....  ..........

#SHT1    GMKIMFSVSN  LWLLGFLFLF  TVGGLTGIIL  SNSSIDIVLH  DTYYVVAHFH  YVLSMGAVFA  IFGSFTYWFP  FIFGVTMNKK
#SHT2    ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
#SHT3    ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
#SHT4    ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
#SHT5    ..........  ...M......  ..........  ..........  ..........  ..........  ..........  ..........
#SHT6    ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
#SHT7    ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
#SHT8    ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
#SHT9    ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
#SHT10   ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
#SHT11   ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
#SHT12   ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
#SHT13   ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
#SHT15   ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
#SHT16   ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
#SHT17   ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
#SHT18   ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
#SHT19   ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
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#SHT20 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT21 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT23 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT26 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT27 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT28 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT29 .......... .......... .......... .......... .......... .......... .......... ..........
#PHT1  ....K.NIYS M.I...I... .......... .......... .......... .......... .......... SM..LSL.Q.
#PHT2  ....K.NIYS M.I...I... .......... .......... .......... .......... .......... SM..LSL.Q.
#PHT3  ....K.NIYS M.I...I... .......... .......... .......... .......... .......... SM..LSL.Q.
#PHT4  ....K.NIYS M.I...I... .......... .......... .......... .......... .......... .M..LSL.Q.
#PDC1  ....K.NIYS M.I...I... .......... .......... .......... .......... .......... SM..LSL.Q.
#PER1  ....K.NIYS M.I...I... .......... .......... .......... .......... .........S SM..LSL.Q.
#PPP1  ....K.NIYS M.I...I... .......V.. .......... .......... .......... .......... .M..LFL.Q.
#PBS1  ....K.NIYS M.I...I... .......... .......... .......... .......... .......... SM..LSL.Q.
#EHT1  ....K...T. ..M..... .......... .......... .......... .......... .......... MFS.L.....
#EHT2  ....K...T. ..M..... .......... .......... .......... .......... .......... MFS.L.....
#EHT4  ....K...T. ..M..... .......... .......... .......... .......... .......... MFS.L.....
#EF1   ....K...T. .......... .......... .......... .......... .......... .......... M.S.L.....
#EF2   ....K...T. .......... .......... .......... .......... .......... .......... M.S.L.....
#EF3   ....K...T. .......... .......... .......... .......... .......... .......... M.S.L.....
#EF4   ....K...T. .......... .......... .......... .......... .......... .......... M.S.L.....
#EF5   ....K...T. .......... .......... .......... .......... .......... .......... M.S.L.....
#EF6   ....K...T. .......... .......... .......... .......... .......... .......... M.S.L.....
#EF7   ....K...T. .......... .......... .......... .......... .......... .......... M.S.L.....
#EF8   ....K...T. .......... .......... .......... .......... .......... .......... M.S.L.....
#EP1   ....K...T. ...M..... .......... .......... .......... .......... .......... M.S.L.....
#EF2A1 ....K...T. .......... .......... .......... .......... .......... .......... M.S.L.....
#ESPA4 ....K...T. ...M..... .......... .......I.. .......... .......... .......... M.S.L.....
#EA1   ....K...T. .......... .......... .......... .......... .......... .......... M.S.L.....
#ESPA5 ....K...T. .......... .......... .......... .......... .......... ........Y. MFS.L....N
#CHT1  ....K.N.T. ......I... .......... .......I.. .......... .......... ........Y. MM..ML..Q.
#CHT2  ....K.N.T. ......I... .......... .......I.. .......... .......... ........Y. MM..ML..Q.
#CHT3  ....K.N.T. ......I... .......... .......I.. .......... .......... ........Y. MM..ML..Q.
#CHT4  ....K.N.T. ......I... .......... .......I.. .......... .......... ........Y. MM..ML..Q.
#CHT5  ....K.N.T. ......I... .......... .......I.. .......... .......... ........Y. MM..LS..Q.
#CHT6  ....K.N.T. ......I... .......... .......I.. .......... .......... ........Y. MM..ML..Q.
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#SHT1  LLKIQFLMLF LGVNLTFFPQ HFLGLSGMPR RYSDYPDSFL CWNVVSSIGS VVSLVGTFMF FYIIWDAFIL KRLLIFVKSL
#SHT2  .......... .......... .......... .......... .......... .......... .......... ..........
#SHT3  .......... .......... .......... .......... .......... .......... .......... ..........
#SHT4  .......... .......... .......... .......... .......... .......... .......... ..........
#SHT5  .......... .......... .......... .......... .......... .......... ...V...... ..........
#SHT6  .......... .......... .......... .......... .......... .......... .......... ..........
#SHT7  .......... .......... .......... .......... .......... .......... .......... ..........
#SHT8  .......... .......... .......... .......... .......... .......... .......... ..........
#SHT9  .......... .......... .......... .......... .......... .....S.... .......... ..........
#SHT10 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT11 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT12 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT13 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT15 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT16 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT17 .......... .......... .......... .......... .......... .......... ...V...... ..........
#SHT18 .......... .......... .......... .......... .......... ...L...... .......... ..........
#SHT19 .......... .......... .......... .......... .......... .......... ...V...... ..........
#SHT20 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT21 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT23 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT26 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT27 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT28 .......... .......... .......... .......... .......... .......... .......... ..........
#SHT29 .......... .......... .......... .......... .......... .......... .......... ..........
#PHT1  M.....F.M. F......... .......... ........Y. ...IL..V.. MM..LS.LY. ...L.ES..S F..VMYM.N.
#PHT2  M.....F.M. F......... .......... ........Y. ...IL..V.. MM..LS.LY. ...L.ES..S F..VMYM.N.
#PHT3  M.....F.M. F......... .......... ........Y. ...IL..V.. MM..LS.LY. ...L.ES..S F..VMYM.N.
#PHT4  M.....F.M. F......... .......... ........Y. ...IL..V.. MM..LS.LY. ...L.ES..S F..VMYM.N.
#PDC1  M.....F.M. F......... .......... ........Y. ...IL..V.. MM..LS.LY. ...L.ES..S F..VMYM.N.
#PER1  M.....F.M. F......... .......... ........Y. ...IL..V.. MM..LS.LY. .F.L.ES..S F..VMYM.N.
#PPP1  M.....F.M. F......... .......... ........Y. ...IL..V.. MM..LS.LY. ...L.ES..S F..VMYM.N.
#PBS1  M.....F.M. F......... .......... ........Y. ...IL..V.. MM..LS.LY. ...L.ES..S F..VMYM.N.
#EHT1  W.....M.M. I...T..... .......... .........M ....L..... II..MS.ML. .F.L..SLMS ..I...M.N.
#EHT2  W.....M.M. I...T..... .......... .........M ....L..... II..MS.ML. .F.L..SLMS ..I...M.N.
#EHT4  W.....M.M. I...T..... .......... .........M ....L..... II..MS.ML. .F.L..SLMS ..I...M.N.
#EF1   W.......M. I...T..... .......... .........M ....L..... II..MS.ML. .F.L..SLMS ..I...M.N.
#EF2   W.......M. I...T..... .......... .........M ....L..... II..MS.ML. CF.L..SLMS ..I...M.N.
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#EF3   W.......M. I...T..... .......... .........M ....L..... II..MS.ML. .F.L..SLMS ..I...M.N.
#EF4   W.......M. I...I..... .......... .........M ....L..... II..MS.ML. CF.L..SLMS ..I...M.N.
#EF5   W.......M. I...T..... .......... .........M ....L..... II..MS.ML. .F.L..SLMS ..I...M.N.
#EF6   W.......M. I...T..... .......... .........M ....L..... II..MS.ML. .F.L..SLMS ..I...M.N.
#EF7   W.......M. I...T..... .......... .........M ....L..... II..MS.ML. .F.L..SLMS ..I...M.N.
#EF8   W.......M. I...T..... .......... .........M ....L..... II..MS.ML. .F.L..SLMS ..I...M.N.
#EP1   W.....M.M. I......... .......... .........M ...IL..... LI..IS.LL. .F.L..S.MS ..I...M.N.
#EF2A1 W.....M.M. I...T..... .......... .........M ....L..... II..MS.ML. .F.L..SLMS ..I...M.N.
#ESPA4 W.....M.M. I...T..... .......... .........M ....L..... MI..LS.ML. .F.L..SLMS ..I...M.N.
#EA1   W.....M.M. I...T..... .......... .........M ...IL..... LI..IS..L. .F.L..SLMS ..I...M.N.
#ESPA5 W.....M.M. I......... .......... .........M ...IL..... .I..MS.LL. .F.L..SLMS ..I...M.N.
#CHT1  W.....F.M. I......... ....M..... ........YT ...IL..M.. MI.MMS.LF. .F.L..SI.S ..II..M.YI
#CHT2  W.....F.M. I......... ....M..... ........YT ...IL..M.. MI.MMS.LF. .F.L..SI.S ..II..M.YI
#CHT3  W.....F.M. I......... ....M..... ........YT ...IL..M.. MI.MMS.LF. .F.L..SI.S ..II..M.YI
#CHT4  W.....F.M. I......... ....M..... ........YT ...IL..M.. MI.MMS.LF. .F.L..SI.S ..II..M.YI
#CHT5  W.....F.M. I......... ....M..... ........YT ...IL..M.. MI.MMS.MY. .F.M..SI.S ..II..M.NM
#CHT6  W.....F.M. I......... ....M..... ........YT ...IL..M.. MI.MMS.LF. .F.L..SI.S ..II..M.YI

#SHT1  NNMIEMIMNF PPSDHSFME
#SHT2  .......... .........
#SHT3  .......... .........
#SHT4  .......... .........
#SHT5  .......... .........
#SHT6  .......... .........
#SHT7  .......... .........
#SHT8  .......... .........
#SHT9  .......... .........
#SHT10 .......... .........
#SHT11 .......... .........
#SHT12 .......... .........
#SHT13 .......... .........
#SHT15 .......... .........
#SHT16 .......... .........
#SHT17 .......... .S.......
#SHT18 .......... .........
#SHT19 .......... .S.......
#SHT20 .......... .........
#SHT21 .......... .........
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#SHT23 .......... .S.......
#SHT26 .......... .........
#SHT27 .......... .........
#SHT28 .......... .........
#SHT29 .......... .........
#PHT1  ..SM.WL.SY ...Y..YY.
#PHT2  ..SM.WL.SY ...Y..YY.
#PHT3  ..SM.WL.SY ...Y..YY.
#PHT4  ..SM.WL.SY ...Y..YY.
#PDC1  ..SM.WL.SY ...Y..YY.
#PER1  ..SM.WL.SY ...Y..YY.
#PPP1  ..SM.WL.SY ...Y..YY.
#PBS1  ..SM.WL.SY ...Y..YY.
#EHT1  .SS..W.VS. ...E...Y.
#EHT2  .SS..W.VS. ...E...Y.
#EHT4  .SS..W.VS. ...E...Y.
#EF1   .SS..W..S. ...E...Y.
#EF2   .SS..W..S. ...E...Y.
#EF3   .SS..W..S. ...E...Y.
#EF4   .SS..W..S. ...E...Y.
#EF5   .SS..W..S. ...E...Y.
#EF6   .SS..W..S. ...E...Y.
#EF7   .SS..W..S. ...E...Y.
#EF8   .SS..W..S. ...E...Y.
#EP1   .SS..W.IS. ...E...Y.
#EF2A1 .SS..W.VS. ...E...Y.
#ESPA4 .SS..W.VS. ...E.T.F.
#EA1   .SS..W.IS. ...E...Y.
#ESPA5 .SS..W..S. ...E...Y.
#CHT1  ..S..W..PY ...Y...N.
#CHT2  ..S..W..PY ..KY...N.
#CHT3  ..S..W..PY ...Y...N.
#CHT4  ..S..W..PY ...Y...N.
#CHT5  ..S..W..E. ...Y...N.
#CHT6  ..S..W..PY ...Y...N.
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Appendix 5: Amino acid translation for wasp COI sequences using the second reading frame. S=*Sychophila*, T=*Tetramesa*, E=*Eurytoma*. Outgroups are: C. C = *Ceratosolen capensis* (Grandi) (Hymenoptera: Agaonidae) (AF200377), C. B = *C. bisulcatus* (AF200375), B. M = *Blastophaga malayana* (AF200373) and B. I = *B. intermedia* (AF200372).

```
#S7.1        ATMIIAVPTG IKIFSWLASM NGMKIMFSVS NLWLLGFLFL FTVGGLTGII LSNSSIDIVL HDTYYVVAHF HYVLSMGAVF
#S7A1        .......... .......... .......... .......... .......... .......... .......... ..........
#S7B1        .......... .......... .......... .......... .......... .......... .......... ..........
#S2D1        .......... .......... .......... .......... .......... .......... .......... ..........
#S9C1        .......... .......... .......... .......... .......... .......... .......... ..........
#S2B1        .......... .......... .......... .......... .......... .......... .......... ..........
#S1A1        .......... .......... .......... .......... .......... .......... .......... ..........
#S4B1        .......... .......... .......... ....M..... .......... .......... .......... ..........
#T7A3        ......I... ......M..L ....LK.... ...M...... ..I....... ........I. .......... ........?.
#T2D1        ......I... ......M..L ....LK.... ...M...... ..I....... ........I. .......... ..........
#T9B1        ......I... ......M..L ....LK.... ...M...... ..I....... ........I. .......... ..........
#T1A4        ......I... ......M..L ....LK.... ...M...... ..I....... ........I. .......... ..........
#T2D9        ......I... ......M..L ....LK.... ...M...... ..I....... ........I. .......... ..........
#T1A2        ......I... ......M..L ....LK.... ...M...... ..I....... ........I. .......... ..........
#T9C2        ......I... ......M..L ....LK.... ...M...... ..I....... ........I. .......... ..........
#T7B4        ......I... ......M..L ....LK.... ...M...... ..I....... ........I. .......... ..........
#T7A1        ......I... ......M..L ....LK.... ...M...... ..I....... ........I. .......... ..........
#T7B3        ......I... ......M..L ....LK.... ...M...... ..I....... ........I. .......... ..........
#E7.1        .......... .......... .....K...T ...M...... .......... .......... .......... ..........
#E7A1        .......... .......... .....K...T ...M...... .......... .......... .......... ..........
#E1A1        .......... .......... .....K...T ...M...... .......... .......... .......... ..........
#CCAF200377 I..V...... ..V....... TSV.LK.T.T ...V...I.. ........VV .....L..MM .......... ..........
#CBAF200375 ...V.G.... ..VY..VCTL Y.S.FKI..P L..VF..I.. .....TS.VM .A.AAL.V.. .......... ..........
#BMAF200373 M......... .......... .....I.N.A ...I...I.. ..I.....V. ........I. .......... .........Y
#BIAF200372 .......... .......... S...FKLN.T I..I...I.. ..I......V ........I. .......... .........Y

#S7.1        AIFGSFIYWF PFIFGVTMNK KLLKIQFLML FLGVNLTFFP QHFLGLSGMP RRYSDYPDSF LCWNVVSSIG SVVSLVSTFM
#S7A1        .......... .......... .......... .......... .......... .......... .......... ......G...
#S7B1        .......... .......... .......... .......... .......... .......... .......... ......G...
#S2D1        .......... .......... .......... .......... .......... .......... .......... ......G...
#S9C1        .......... .......... .......... .......... .......... .......... .......... ......G...
#S2B1        .......... .......... .......... .......... .......... .......... .......... ......G...
#S1A1        .......... .......... .......... .......... .......... .......... .......... ......G...
#S4B1        .......... .......... .......... .......... .......... .......... .......... ......G...
#T7A3        .......... .M.S.IV... .W.....FLM .......... .......... ......A... ....IL.... .MI.FM..ML
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#T2D1        .......... .M.S.IV... .W.....FLM .......... .......... ......A... ....IL.... .MI.FM..ML
#T9B1        .......... .M.S.IV... .W.....FLM .......... .......... ......A... ....IL.... .MI.FM..ML
#T1A4        .......... .M.S.IV... .W.....FLM .......... .......... ......A... ....IL.... .MI.FM..ML
#T2D9        .......... .M.S.IV... .W.....FLM .......... .......... ......A... ....IL.... .MI.FM..ML
#T1A2        .......... .M.S.IV... .W.....FLM .......... .......... ......A... ....IL.... .MI.FM..ML
#T9C2        .......... .M.S.IV... .W.....FLM .......... .......... ......A... ....IL.... .MI.FM..ML
#T7B4        .......... .M.S.IV... .W.....FLM .......... .......... ......A... ....IL.... .MI.FM..ML
#T7A1        .......... .M.S.IV... .W.....FLM .......... .......... ......A... ....IL.... .MI.FM..ML
#T7B3        .......... .M.S.IV... .W.....FLM .......... .......... ......A... ....IL.... .MI.FM..ML
#E7.1        .......... .MFS.L.... .W.....M.M .I...T...S .......... .......... M....L.... .II..M..ML
#E7A1        .......... .MFS.L.... .W.....M.M .I...T.... .......... .......... M....L.... .II..M..ML
#E1A1        .......... .MFS.L.... .W.....M.M .I...T.... .......... .......... M....L.... .II..M..ML
#CCAF200377  .......... .LFYWMV..Q .W...H.F.. .I........ .......... ........VY ....L...F. .LL.FL.SLY
#CBAF200375  ..M...VF.. .LMS.IYL.Q ....M..WS. .....F.... .......... ........AY M...KL..L. .ML.FL..AF
#BMAF200373  .......... .LF..LKL.Q .W...H.W.. .I........ .......... .......... M...LL.... .FI..MA.MY
#BIAF200372  ..L....... .LF...S..Q .W...H.WV. .I........ ....S..... .........Y ....LL.... .LI.FIG.MF

#S7.1        FFYIIWDAFI LKRLLIFVKS LNNMIEMIMN FPPSDHSFME IPKLVM
#S7A1        .......... .......... .......... .......... .....S
#S7B1        ....V..... .......... .......... ..S....... ......
#S2D1        .......... .......... .......... .......... .....S
#S9C1        .......... .......... .......... .......... ......
#S2B1        .......... .......... .......... .......... .....S
#S1A1        .......... .......... .......... .......... .....K
#S4B1        ....V..... .......... .......... .......... ....A.
#T7A3        ..F.L.ESLM SV.I...L.N ..MSM.WLL. ....E...H. ...IYI
#T2D1        ..F.L.ESLM SV.I...L.N ..MSM.WLL. ....E...H. ...IYI
#T9B1        ..F.L.ESLM SV.I...L.N ..MSM.WLL. ....E...H. ...IYI
#T1A4        ..F.L.ESLM SV.I...L.N ..MSM.WLL. ....E...H. ...IYI
#T2D9        ..F.L.ESLM SV.I...L.N ..MSM.WLL. ....E...H. ...IYI
#T1A2        ..F.L.ESLM SV.I...L.N ..MSM.WLL. ....E...H. ...IYI
#T9C2        ..F.L.ESLM SV.I...L.N ..MSM.WLL. ....E...H. ...IYI
#T7B4        ..F.L.ESLM SV.I...L.N ..MSM.WLL. ....E...H. ...IYI
#T7A1        ..F.L.ESLM SV.I...L.N ..MSM.WLL. ....E...H. ...IYI
#T7B3        ..F.L.ESLM SV.I...L.N ..MSM.WLL. ....E...H. ...IYI
#E7.1        ..F.L..SLM S..I...M.N ..SS..W.VS ....E...Y. ...VYK
#E7A1        ..F.L..SLM S..I...M.N ..SS..W.VS ....E...Y. ...VYK
#E1A1        ..F.L..SLM S..I...M.N ..SS..W.VS ....E...Y. ...VYK
```

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#CCAF200377 .MF.MLES.W SN.VV..MSK T.TS..WL.. Y.SA....Y. V..VTN
#CBAF200375 ..G.VLEGIL SN.K...SGK M.ISL.WM.T Y..ME...D. L..VFY
#BMAF200373 .ML.M.ES.N NQ.VV..L.K S.VSL.WF.S Y.AP...IY. L.QIKE
#BIAF200372 .MF.LGE.IF CQ.VIC.I.K T.VSL.WF.S Y.AP...IY. L...ME
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Appendix 6: Amino acid translation for wasp COII sequences using the first reading frame. S=*Sychophila*, T=*Tetramesa*, E=*Eurytoma*. Outgroups are: C. C = *Ceratosolen capensis* (Grandi) (Hymenoptera: Agaonidae) (AF200377), C. B = *C. bisulcatus* (AF200375), B. M = *Blastophaga malayana* (AF200373) and B. I = *B. intermedia* (AF200372).

```
#S7.1        ISYWHQIMLQ DAGSSVMEAM TYFYDEAMMI LVMIIMLILY MLIFMVFNKF TDRFLLEGQF IEIFWTLVPM GFLMFLAIPS
#S7A1        .......... .......... .......... .......... ......Y... .......... .......... ..........
#S7B1        .......... .....I.... .......... .......... ......Y... .......... .......... ..........
#S2D1        .......... .......... .......... .......... ......Y... .......... .......... ..........
#S9C1        .......... .......... .......... .......... ......Y... .......... .......... ..........
#S2B1        .......... .......... .......... .......... ......Y... .......... .......... ..........
#S1A1        .......... .......... .......... .......... ......Y... .......... .......... ..........
#S4B1        .......... .......... .......... .......... ..V...Y... .......... .......... ..........
#T7A3        .......... ..A..I..N. IL...Y..LV IM...I..M. .F...IK..L VNLK.....L ...I..V... FM.I......
#T2D1        .MM...YSF. ..A..I..N. IL...Y..LV IM...I..M. .F...IK..L VNLK.....L ...I..V... FM.I......
#T9B1        .MM...YSF. ..A..I..N. IL...Y..LV IM...I..M. .F...IK..L VNLK.....L ...I..V... FM.I......
#T1A4        .MM...YSF. ..A..I..N. IL...Y..LV IM...I..M. .F...IK..L VNLK.....L ...I..V... FM.I......
#T2D9        .MM...YSF. ..A..I..N. IL...Y..LV IM...I..M. .F...IK..L VNLK.....L ...I..V... FM.I......
#T1A2        .MM...YSF. ..A..I..N. IL...Y..LV IM...I..M. .F...IK..L VNLK.....L ...I..V... FM.I......
#T9C2        .MM...YSF. ..A..I..N. IL...Y..LV IM...I..M. .F...IK..L VNLK.....L ...I..V... FM.I......
#T7B4        .MM...YSF. ..A..I..N. IL...Y..LV IM...I..M. .F...IK..L VNLK.....L ...I..V... FM.I......
#T7A1        .MM...YSF. ..A..I..N. IL...Y..LV IM...I..M. .F...IK..L VNLK.....L ...I..V... FM.I......
#T7B3        .MM...YSF. ..A..I..N. IL...Y..LV IM...I..M. .F...IK..L VNLK.....L ...I..V... FM.I......
#E7.1        MAL...YNF. ..A.....N. IM...IV.LV I....I..M. ......K..L IN.YM....M ...I..II.. I..I......
#E7A1        MAL...YNF. ..A.....N. IM...IV.LV I....I..M. ......K..L IN.YM....M ...I..II.. I..I......
#E1A1        MAL...YNF. ..A.....N. IM...IV.LV I....I..M. ......K..L IN.YM....M ...I..II.. I..I......
#CCAF200377 ..V.G.MN.. ..S.PI.QN. VI.HEF..V. VLL..AKL.. IIWM.LM... ..VN.TS.N. VDLV..II.. LV.LL..F..
#CBAF200375 .A.IG.LG.. ETN.P..MFL SDYH.MM.FL MYF.SFQVM. SIFIVLCS.S L.LMNSGWSG K.SK...L.S LQKWN.TVS.
#BMAF200373 ..F.G..... ..S.PI.QN. IF.H.Y.L.. IL..LI.LF. L.MM.LNSM. VNLKMIH..M ..VI..VI.. II.L...F..
#BIAF200372 ..L.G.M... ....IT..N. IFLH.YL... IL..MV.LF. ..SM.ASL.. FELKMFHN.A L.ML..I..I IV.V......

#S7.1        LKVL
#S7A1        ....
#S7B1        ....
#S2D1        ....
#S9C1        ....
#S2B1        ....
#S1A1        ....
#S4B1        ....
```

```
#T7A3         ..I.
#T2D1         ..I.
#T9B1         ..I.
#T1A4         ..I.
#T2D9         ..I.
#T1A2         ..I.
#T9C2         ..I.
#T7B4         ..I.
#T7A1         ..I.
#T7B3         ..I.
#E7.1         ....
#E7A1         ....
#E1A1         ....
#CCAF200377 .HLM
#CBAF200375 IYMY
#BMAF200373 .HL.
#BIAF200372 .HL.
```

Appendix 7: Pairwise uncorrected distances (upper diagonal) and corrected distances (lower diagonal) for COI sequences of *Sycophila* haplotypes. Uncorrected distance estimator is uncorrected p, and corrected distance estimator is HYK85 + I.

	SH1	SH2	SH3	SH4	SH5	SH6	SH7	SH8	SH9	SH10	SH11	SH12	SH13	SH15	SH16	SH17	SH18	SH19	SH20	SH21	SH23	SH26	SH27	SH28	SH29
SH1	-	0.0103	0.0129	0.0116	0.0604	0.0090	0.0090	0.0116	0.0283	0.0090	0.0077	0.0103	0.0090	0.0090	0.0090	0.0373	0.0103	0.0360	0.0270	0.0270	0.0309	0.0270	0.0270	0.0283	0.0309
SH2	0.0110	-	0.0103	0.0090	0.0643	0.0013	0.0090	0.0013	0.0283	0.0064	0.0051	0.0077	0.0064	0.0064	0.0064	0.0373	0.0077	0.0360	0.0283	0.0244	0.0309	0.0270	0.0270	0.0257	0.0334
SH3	0.0141	0.0108	-	0.0090	0.0643	0.0116	0.0090	0.0116	0.0334	0.0064	0.0051	0.0077	0.0064	0.0064	0.0090	0.0399	0.0077	0.0386	0.0309	0.0296	0.0334	0.0296	0.0296	0.0296	0.0360
SH4	0.0124	0.0093	0.0094	-	0.0681	0.0103	0.0077	0.0103	0.0347	0.0051	0.0039	0.0064	0.0051	0.0026	0.0077	0.0411	0.0064	0.0399	0.0321	0.0309	0.0347	0.0309	0.0309	0.0270	0.0373
SH5	0.1902	0.1777	0.1770	0.1788	-	0.0630	0.0630	0.0630	0.0540	0.0630	0.0643	0.0630	0.0643	0.0656	0.0630	0.0553	0.0643	0.0540	0.0514	0.0553	0.0514	0.0553	0.0527	0.0527	0.0540
SH6	0.0096	0.0013	0.0123	0.0107	0.1773	-	0.0103	0.0026	0.0270	0.0077	0.0064	0.0090	0.0077	0.0077	0.0077	0.0360	0.0090	0.0347	0.0270	0.0231	0.0296	0.0257	0.0257	0.0244	0.0321
SH7	0.0096	0.0094	0.0094	0.0080	0.1773	0.0108	-	0.0103	0.0321	0.0051	0.0039	0.0064	0.0051	0.0051	0.0077	0.0386	0.0064	0.0373	0.0296	0.0283	0.0321	0.0283	0.0283	0.0296	0.0347
SH8	0.0125	0.0013	0.0123	0.0108	0.1766	0.0026	0.0109	-	0.0296	0.0077	0.0064	0.0090	0.0077	0.0077	0.0077	0.0360	0.0090	0.0347	0.0296	0.0257	0.0321	0.0283	0.0283	0.0244	0.0347
SH9	0.0405	0.0393	0.0486	0.0507	0.1543	0.0371	0.0466	0.0417	-	0.0321	0.0309	0.0334	0.0321	0.0321	0.0296	0.0321	0.0334	0.0309	0.0231	0.0257	0.0231	0.0231	0.0219	0.0270	0.0270
SH10	0.0096	0.0066	0.0066	0.0052	0.1765	0.0080	0.0053	0.0080	0.0463	-	0.0013	0.0039	0.0026	0.0026	0.0051	0.0386	0.0013	0.0373	0.0270	0.0257	0.0296	0.0283	0.0257	0.0244	0.0321
SH11	0.0081	0.0052	0.0052	0.0039	0.1777	0.0066	0.0039	0.0066	0.0438	0.0013	-	0.0026	0.0013	0.0013	0.0039	0.0373	0.0026	0.0360	0.0283	0.0270	0.0309	0.0270	0.0270	0.0257	0.0334
SH12	0.0111	0.0080	0.0080	0.0066	0.1766	0.0094	0.0066	0.0094	0.0490	0.0039	0.0026	-	0.0013	0.0039	0.0064	0.0373	0.0051	0.0360	0.0309	0.0296	0.0334	0.0296	0.0296	0.0283	0.0360
SH13	0.0096	0.0066	0.0066	0.0052	0.1777	0.0080	0.0053	0.0080	0.0464	0.0026	0.0013	0.0013	-	0.0026	0.0051	0.0386	0.0039	0.0373	0.0296	0.0283	0.0321	0.0283	0.0283	0.0270	0.0347
SH15	0.0095	0.0066	0.0066	0.0026	0.1781	0.0080	0.0052	0.0080	0.0461	0.0026	0.0013	0.0039	0.0026	-	0.0051	0.0386	0.0039	0.0373	0.0296	0.0283	0.0321	0.0283	0.0283	0.0270	0.0347
SH16	0.0096	0.0066	0.0094	0.0080	0.1780	0.0080	0.0080	0.0080	0.0418	0.0052	0.0039	0.0066	0.0053	0.0052	-	0.0360	0.0064	0.0347	0.0270	0.0257	0.0296	0.0257	0.0257	0.0244	0.0321
SH17	0.0599	0.0569	0.0622	0.0643	0.1331	0.0545	0.0601	0.0542	0.0490	0.0597	0.0569	0.0570	0.0598	0.0594	0.0548	-	0.0399	0.0013	0.0270	0.0321	0.0270	0.0270	0.0257	0.0283	0.0334
SH18	0.0110	0.0080	0.0080	0.0066	0.1777	0.0094	0.0066	0.0094	0.0489	0.0013	0.0026	0.0053	0.0039	0.0039	0.0066	0.0625	-	0.0386	0.0283	0.0270	0.0309	0.0296	0.0270	0.0257	0.0334
SH19	0.0569	0.0542	0.0594	0.0615	0.1299	0.0518	0.0573	0.0515	0.0463	0.0569	0.0542	0.0543	0.0570	0.0566	0.0521	0.0013	0.0597	-	0.0257	0.0309	0.0257	0.0257	0.0244	0.0270	0.0321
SH20	0.0365	0.0374	0.0417	0.0436	0.1479	0.0354	0.0398	0.0396	0.0303	0.0352	0.0374	0.0419	0.0396	0.0394	0.0355	0.0354	0.0374	0.0333	-	0.0103	0.0051	0.0077	0.0039	0.0090	0.0103
SH21	0.0366	0.0313	0.0395	0.0414	0.1577	0.0294	0.0377	0.0334	0.0347	0.0332	0.0353	0.0398	0.0375	0.0373	0.0335	0.0442	0.0353	0.0420	0.0110	-	0.0129	0.0116	0.0090	0.0116	0.0154
SH23	0.0434	0.0418	0.0462	0.0481	0.1467	0.0397	0.0443	0.0441	0.0302	0.0395	0.0418	0.0465	0.0441	0.0439	0.0399	0.0356	0.0417	0.0334	0.0053	0.0140	-	0.0103	0.0064	0.0116	0.0129
SH26	0.0371	0.0358	0.0400	0.0420	0.1427	0.0337	0.0382	0.0380	0.0307	0.0380	0.0358	0.0403	0.0380	0.0378	0.0339	0.0359	0.0402	0.0338	0.0081	0.0126	0.0111	-	0.0064	0.0116	0.0154
SH27	0.0365	0.0352	0.0394	0.0413	0.1512	0.0332	0.0376	0.0374	0.0282	0.0331	0.0352	0.0396	0.0374	0.0372	0.0334	0.0333	0.0352	0.0312	0.0039	0.0095	0.0067	0.0067	-	0.0077	0.0116
SH28	0.0396	0.0340	0.0406	0.0359	0.1670	0.0320	0.0407	0.0318	0.0375	0.0318	0.0340	0.0385	0.0362	0.0360	0.0321	0.0381	0.0339	0.0359	0.0096	0.0126	0.0126	0.0127	0.0081	-	0.0141
SH29	0.0441	0.0472	0.0519	0.0539	0.1679	0.0450	0.0500	0.0497	0.0372	0.0447	0.0472	0.0523	0.0497	0.0494	0.0452	0.0470	0.0471	0.0446	0.0110	0.0173	0.0141	0.0174	0.0125	0.0158	-

Appendix 8: Pairwise uncorrected p distances (upper diagonal) and GTR + G (G = 0.4670) corrected distances for wasps taxa. Ingroup taxa include *Sycophila* (S), *Tetramesa* (T), *Eurytoma* (E). Outgroup taxa are *Ceratosolen capensis* (Grandi) (Hymenoptera: Agaonidae) (CC), *C. bisulcatus* (CB), *Blastophaga malayana* (B.m) and *B. intermedia* (B.i).

	S7.1	S7A1	S7B1	S2D1	S9C1	S2B1	S1A1	S4B1	T7A3	T2D1	T9B1	T1A4	T2D9	T1A2	T9C2	T7B4	T7A1	T7B3	E7.1	E7A1	E1A1	CC	CB	BM	BI
S7.1	-	0.0234	0.0265	0.0255	0.0181	0.0234	0.0244	0.0499	0.1966	0.1976	0.1976	0.1976	0.1976	0.1976	0.1976	0.1976	0.1976	0.1976	0.1900	0.1900	0.1900	0.2646	0.3316	0.2452	0.2401
S7A1	0.0245	-	0.0329	0.0064	0.0223	0.0064	0.0106	0.0520	0.2019	0.2029	0.2029	0.2029	0.2029	0.2029	0.2029	0.2029	0.2029	0.2029	0.1911	0.1911	0.1911	0.2699	0.3326	0.2463	0.2454
S7B1	0.0279	0.0352	-	0.0350	0.0276	0.0329	0.0361	0.0488	0.2062	0.2072	0.2072	0.2072	0.2072	0.2072	0.2072	0.2072	0.2072	0.2072	0.1996	0.1985	0.1996	0.2688	0.3326	0.2516	0.2401
S2D1	0.0270	0.0064	0.0377	-	0.0265	0.0085	0.0106	0.0552	0.2019	0.2029	0.2029	0.2029	0.2029	0.2029	0.2029	0.2029	0.2029	0.2029	0.1932	0.1911	0.1932	0.2678	0.3316	0.2484	0.2454
S9C1	0.0188	0.0236	0.0290	0.0284	-	0.0244	0.0234	0.0467	0.1977	0.1987	0.1987	0.1987	0.1987	0.1987	0.1987	0.1987	0.1987	0.1987	0.1921	0.1921	0.1921	0.2678	0.3348	0.2516	0.2433
S2B1	0.0246	0.0064	0.0352	0.0086	0.0259	-	0.0127	0.0520	0.2019	0.2029	0.2029	0.2029	0.2029	0.2029	0.2029	0.2029	0.2029	0.2029	0.1900	0.1900	0.1900	0.2678	0.3316	0.2452	0.2444
S1A1	0.0258	0.0108	0.0389	0.0108	0.0248	0.0130	-	0.0510	0.1977	0.1987	0.1987	0.1987	0.1987	0.1987	0.1987	0.1987	0.1987	0.1987	0.1879	0.1858	0.1879	0.2635	0.3284	0.2431	0.2391
S4B1	0.0576	0.0599	0.0556	0.0639	0.0526	0.0600	0.0583	-	0.2000	0.1998	0.1998	0.1998	0.1998	0.1998	0.1998	0.1998	0.1998	0.1998	0.1932	0.1911	0.1932	0.2688	0.3316	0.2505	0.2444
T7A3	0.3611	0.3757	0.3870	0.3778	0.3657	0.3732	0.3651	0.3711	-	0.0000	0.0011	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1466	0.1445	0.1466	0.2498	0.3253	0.2390	0.2380
T2D1	0.3634	0.3781	0.3894	0.3801	0.3680	0.3755	0.3674	0.3704	0.0000	-	0.0011	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1466	0.1445	0.1466	0.2508	0.3263	0.2390	0.2380
T9B1	0.3644	0.3759	0.3905	0.3779	0.3690	0.3766	0.3652	0.3714	0.0011	0.0011	-	0.0011	0.0011	0.0011	0.0011	0.0011	0.0011	0.0011	0.1466	0.1445	0.1466	0.2508	0.3263	0.2390	0.2370
T1A4	0.3634	0.3781	0.3894	0.3801	0.3680	0.3755	0.3674	0.3704	0.0000	0.0000	0.0011	-	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1466	0.1445	0.1466	0.2508	0.3263	0.2390	0.2380
T2D9	0.3634	0.3781	0.3894	0.3801	0.3680	0.3755	0.3674	0.3704	0.0000	0.0000	0.0011	0.0000	-	0.0000	0.0000	0.0000	0.0000	0.0000	0.1466	0.1445	0.1466	0.2508	0.3263	0.2390	0.2380
T1A2	0.3634	0.3781	0.3894	0.3801	0.3680	0.3755	0.3674	0.3704	0.0000	0.0000	0.0011	0.0000	0.0000	-	0.0000	0.0000	0.0000	0.0000	0.1466	0.1445	0.1466	0.2508	0.3263	0.2390	0.2380
T9C2	0.3634	0.3781	0.3894	0.3801	0.3680	0.3755	0.3674	0.3704	0.0000	0.0000	0.0011	0.0000	0.0000	0.0000	-	0.0000	0.0000	0.0000	0.1466	0.1445	0.1466	0.2508	0.3263	0.2390	0.2380
T7B4	0.3634	0.3781	0.3894	0.3801	0.3680	0.3755	0.3674	0.3704	0.0000	0.0000	0.0011	0.0000	0.0000	0.0000	0.0000	-	0.0000	0.0000	0.1466	0.1445	0.1466	0.2508	0.3263	0.2390	0.2380
T7A1	0.3634	0.3781	0.3894	0.3801	0.3680	0.3755	0.3674	0.3704	0.0000	0.0000	0.0011	0.0000	0.0000	0.0000	0.0000	0.0000	-	0.0000	0.1466	0.1445	0.1466	0.2508	0.3263	0.2390	0.2380
T7B3	0.3634	0.3781	0.3894	0.3801	0.3680	0.3755	0.3674	0.3704	0.0000	0.0000	0.0011	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	-	0.1466	0.1445	0.1466	0.2508	0.3263	0.2390	0.2380
E7.1	0.3355	0.3332	0.3543	0.3401	0.3362	0.3322	0.3268	0.3438	0.2282	0.2287	0.2270	0.2287	0.2287	0.2287	0.2287	0.2287	0.2287	0.2287	-	0.0032	0.0021	0.2486	0.3156	0.2367	0.2401
E7A1	0.3330	0.3307	0.3508	0.3325	0.3337	0.3297	0.3193	0.3368	0.2222	0.2227	0.2210	0.2227	0.2227	0.2227	0.2227	0.2227	0.2227	0.2227	0.0032	-	0.0032	0.2465	0.3124	0.2336	0.2369
E1A1	0.3347	0.3324	0.3534	0.3392	0.3354	0.3314	0.3259	0.3429	0.2277	0.2281	0.2265	0.2281	0.2281	0.2281	0.2281	0.2281	0.2281	0.2281	0.0021	0.0032	-	0.2476	0.3156	0.2367	0.2401
C. a	0.6398	0.6693	0.6641	0.6694	0.6596	0.6618	0.6373	0.6552	0.5470	0.5501	0.5514	0.5501	0.5501	0.5501	0.5501	0.5501	0.5501	0.5501	0.5545	0.5434	0.5464	-	0.2986	0.2316	0.2391
C. b	1.0753	1.0737	1.0723	1.0698	1.0797	1.0725	1.0406	1.0629	1.0083	1.0133	1.0088	1.0133	1.0133	1.0133	1.0133	1.0133	1.0133	1.0133	0.9404	0.9199	0.9370	0.8448	-	0.3432	0.3263
B. m	0.5513	0.5570	0.5805	0.5675	0.5741	0.5539	0.5432	0.5823	0.5306	0.5288	0.5260	0.5288	0.5288	0.5288	0.5288	0.5288	0.5288	0.5288	0.5102	0.4985	0.5087	0.4896	1.1573	-	0.1764
B. i	0.5299	0.5504	0.5306	0.5534	0.5350	0.5491	0.5280	0.5486	0.5171	0.5179	0.5137	0.5179	0.5179	0.5179	0.5179	0.5179	0.5179	0.5179	0.5157	0.5038	0.5142	0.5221	1.0107	0.2959	-

CHAPTER 5

The structure of the parasitoid communities associated with *Tetramesa* species feeding on *Leucopa pseudosclerophylla* and *Leucopa sclerophylla* in Iran

5.1 Introduction

A community is defined as "a group of species having a high degree of spatial and temporal concordance, and in which the members mutually interact to some extent" (Askew & Shaw 1986) or "an assemblage of species populations which occur together in space and time" (Begon *et al.* 1990). Understanding the structure and dynamics of insect communities and, for example identifying parasitoids that attack the host species, is of principal interest to understand both the ecological and evolutionary affinities between host and parasitoids. Recognizing the types of interaction in insect communities has in particular been possible by analyzing the host-parasitoid assemblages associated with a particular plant (Strong *et al.* 1984).

The phytophagous larvae of many small insect species live inside plant tissue and complete their development in this space, usually supporting a number of parasitoid insect species (1-20 sometimes more) that together compose a structured community (Askew 1980). One advantage of studying parasitoid communities is that it is relatively easy to recognize the major trophic interactions and to quantify them in comparison with other communities such as predators and prey (Martinez *et al.* 1999). However, one problem of studying parasitoid communities is the identification of parasitoid species, mostly because of the presence of many sibling and closely related species. It should be emphasized therefore that parasitoids especially the majority of hymenopterous parasitoids, can still claim to be one of the most taxonomically challenging groups of insects (Jones *et al.* 1997).

Community ecologists have constructed food webs to understand the structure of assemblages of species. Food webs describe the trophic relationships between sets of interacting species and hence are an essential tool in community ecology (Polis & Winemiller 1996). A parasitoid web is a subset of a traditional food web that comprises hosts (and possibly food plants), parasitoids and hyperparasitoids (Memmott & Godfray 1992, 1994).

There are three different kinds of parasitoid food webs: connectance, semi-quantitative, and quantitative (Memmot & Godfray 1993; Memmott *et al.* 1994). Connectance webs indicate whether species of parasitoids have been recorded from particular hosts; semi-quantitative food webs can include information about the relative abundance of different species of parasitoids attacking each species of host; quantitative food webs in addition show the density of different host and parasitoid species (Memmott & Godfray 1994). A few semi-quantitative food webs have been constructed for superfamilies of Hymenoptera among which are British cynipid gall wasps (Askew 1961) and British eurytomid chalcid wasps associated with Poaceae (Dawah *et al.* 1995).

The communities of grass-infesting chalcid wasps have been poorly studied, especially those attacking non-economic grasses. Claridge & Dawah (1994) primarily studied the general community structure of some of the more common British species of grass-feeding *Tetramesa* and their parasitoids. In their study the general food webs of phytophagous Eurytomidae and their parasitoids associated with five grass species were constructed. In a wider study, Dawah *et al.* (1995) investigated the parasitoid assemblages associated with 18 species of chalcid wasps feeding on 10 grass species which were sampled quantitatively between 1980 and 1992 at 24 sites in Wales and England to examine food web structure, the size and composition of the parasitoid complexes, and structures of the local communities. Tscharntke *et al.* (2001) compared

the parasitoid communities associated with grasses in Germany and Britain to examine geographic consistency in community composition. They also examined characteristics of host plant and ecological factors of the host insects.

Parasitoid communities of grass-feeding chalcid wasps, especially eurytomid hosts, have several special characteristics. Eurytomid hosts have very diverse lifestyles. They are rare or abundant, many are true herbivores, many are parasitoids, gallers and non-gallers, gregarious or solitary, and they live within the tissues of their host plants throughout their larval stages (Tscharntke 1994; Claridge & Dawah 1994; Dawah *et al.* 1995; Price *et al.* 1997).

The aim of this study was to investigate the parasitoid communities associated with *Tetramesa* sp. feeding on *L. pseudosclerophylla* and *L. sclerophylla* in Iran. The community structure of *Tetramesa* sp. and their parasitoids associated with these two grass species has not previously been investigated.

5.2 Materials and methods

Sampling of *L. pseudosclerophylla* and *L. sclerophylla* from several localities in Iran was carried out from 1997 to 1999 (see Table 5.1 for sampling sites and Figure 2.1 in chapter 2 for the map). Sampling sites are shown in Figs 5.1, 5.2, and 5.3.

5.2.1 Insect rearing

For the rearing method of *Tetramesa* and their parasitoids see chapter 2.

5.2.2 Host-parasitoids relationships and semi-quantitative food web

Plant stems were dissected in August and throughout the winter in order to determine which species attack which and to calculate percentage of parasitism by each parasitoid species and to determine the dominance hierarchies of the hosts and their

parasitoids in space and time. Host remains were identified to find out the trophic interaction between Hymenopteran species. The trophic interrelationships between grasses, herbivores and parasitoids were summarised diagrammatically (Figures 5.4 and 5.5). A semi-quantitative food web was constructed using total percentage of parasitism of *Tetramesa* sp. feeding on *L. pseudosclerophylla* and *L. sclerophylla* (Figure 5.6). The type and thickness of the lines represent an approximate indication of the frequency of the trophic relationships. In other words, they reflect feeding rates averaged across all sites and years. The semi-quantitative food web was based on 5937 successful rearings of the sum of herbivores (2978) and parasitoids (2959) in *L. pseudosclerophylla* and *L. sclerophylla*. In order to distinguish the wasp species in each grass species, *Tetramesa* sp. and the parasitoids in *L. pseudosclerophylla* were marked with sp.1 while *Tetramesa* sp. and parasitoids in *L. sclerophylla* were marked with sp.2.

5.2.3 Parasitism rate

The percentage of parasitism (P) by one particular species of parasitoids (primary parasitoids) were calculated by dividing the number of parasitoids reared (P_1) on the sum of the total number of *Tetramesa* reared (T) plus the number of all parasitoids reared (P_0) (each *Tetramesa* individual was consumed by one parasitoid) as presented in the following equation:

$$P = P_1/T + P_0$$

The percentage of parasitism by hyperparasitoids (P) was calculated by dividing the number of the hyperparasitoid species reared (Pz) by the sum of the total number of *Tetramesa* reared (T) plus the number of all the parasitoids reared (P_0) minus the number of the primary parasitoids (P_b) which were attacked by the hyperparasitoids (this is because *Tetramesa* larva when being parasitized either by the primary parasitoid or

indirectly by the hyperparasitoids which attack the primary parasitoids) as presented in the following equation:

$$P = Pz/(T + P_0) - P_b$$

Since the number of reared specimens of *Tetramesa* and its parasitoids were used to calculate the percentage of parasitism, the differential mortality between species could not have affected the results of the community structure.

5.3 Results

5.3.1 Host-parasitoids relationships and food web structure

As mentioned above the host remains were identified to find out the trophic interaction between Hymenopteran species. The trophic interrelationships between grasses, herbivores and parasitoids were determined and are presented diagrammatically for each grass species (Figures 5.4 and 5.5). A semi-quantitative food web of *Tetramesa* species and their parasitoids associated with *L. pseudosclerophylla* and *L. sclerophylla* from several localities (Southeast, Southwest and Northern Iran) was determined (Figure 5.6). The grass-herbivore-parasitoid food web comprised two subcommunities, which contained 15 species. The first subcommunity associated with *L. pseudosclerophylla* comprised eight species (Figure 5.4), while the second subcommunity associated with *L. sclerophylla* comprised seven species (Fig 5.5) [Total number of species (herbivore and parasitoids) was eight for *L. pseudosclerophylla* and seven for *L. sclerophylla* and as *Macroneura vesicularis* linked the two subcommunities, it was counted for both. The total number of parasitoid species for the first subcommunity was seven and for the second one was six]. These species were organised into five trophic levels (Figure 5.6). There was extreme specificity of host parasitoids especially the lower two consumer trophic levels, herbivores and primary parasitoids, whereas the top two trophic levels,

hyperparasitoids and tertiary parasitoids, comprised more generalised omnivores. The outcome of this high level of specialisation was a compartmentalised food web and that only *Macroneura vesicularis* linked the two systems.

5.3.2 Herbivorous and parasitoid insects associated with *Leucopa pseudosclerophylla*

One herbivorous species (*Tetramesa* sp.1) was reared from *L. pseudosclerophylla. Tetramesa* sp.1 was attacked by a number of parasitoids (seven species). These were: *Eurytoma* sp.1, *Sycophila* sp.1 (Eurytomidae), *Pediobius* sp.1 (Eulophidae), *Homoporus* sp.1, *Chlorocytus* sp.1 (Pteromalidae), *Macroneura vesicularis* (Retzius) (Eupelmidae) and *Syntomaspis baudysi* Bouček (Torymidae) (Figures 5.4 and 5.6).

1. *Tetramesa* sp.1

Tetramesa sp.1 was the only herbivore insect reared from *L. pseudosclerophylla.* One to 12 distinct larval cells together was observed above the stem nodes, (figure 5.11, see below) but sometimes maximum 21 larval cells were observed. The pupa of *Tetramesa* sp.1 is shown in Figures 5.7 and 5.8. Adults (Figure 5.10) emerge during March and April. Figure 5.9 shows a *Tetramesa* pupa in the stem. Both sexes were reared from larvae removed from stems of *L. pseudosclerophylla*, but males were less abundant than females. The sex ratio in some species of *Tetramesa* is 1:1 (i.e. *Tetramesa calamagrostidis*) and in some species the sex ratio found to be female biased (i.e. *T. linearis*). *Tetramesa* sp.1 does not form characteristic galls in stems of *L. pseudosclerophylla.* The galls are similar to those in *Ammophila arenaria* (Claridge & Dawah 1994) being not completely obvious, but they are arranged like those of *Tetramesa linearis* in the stems of *Elymus repens* consisting of a collection of cells in the

stem wall (Claridge 1961) (Figure 5.11). The number of *Tetramesa* sp.1 and its parasitoids reared in the grass *L. pseudosclerophylla* is shown in Table 5.2.

Tetramesa sp.1 occurred in all localities where *L. pseudosclerophylla* was collected, but its abundance differed in different localities (Fig 5.12). The highest number of *Tetramesa* sp. associated with *L. pseudosclerophylla* was found in South-east populations (Deh Lo-Lo, Kouhpayeh, code 2d) in 1997 and 1998 and Seyahbenouieh, Rabour (code 4b) in 1998, while the lowest number was also found in south-east populations, Sirch (code 1a) in 1999 and Seyahbenouieh, Rabour (code 4b) in 1999.

2. *Eurytoma* sp.1

Eurytoma sp.1 is a primary parasitoid of *Tetramesa* sp.1 in the stems of *L. pseudosclerophylla*. As many as one to nine larvae were found each inhabiting a single *Tetramesa* cell in the stem. A large number of larvae were reared from stems of *L. pseudosclerophylla*. Larvae of *Eurytoma* sp.1 are whitish with a barrel-like body shape distinctly broader in the mid-region and tapering gradually anteriorly and posteriorly. All body segments are covered with conspicuous setae, of different sizes. The head is usually circular in anterior view. The final instar larvae when disturbed are very active compared with those of *Tetramesa*. Adults (Figure 5.13) emerge during late March, April and early May.

3. *Sycophila* sp.1

Sycophila sp.1 attack *Tetramesa* sp.1 in the stems of *L. pseudosclerophylla*. *Sycophila* sp.1 was reared from larvae removed from the stems of *L. pseudosclerophylla*. From one to four larvae were found in the stems of *L. pseudosclerophylla*. Larvae of *Sycophila* sp.1 are dirty white in colour and the body is barrel-shaped, tapering anteriorly and posteriorly. The integument is translucent with the contents of the digestive system

visible beneath which is purple in colour. The antennae are reduced to short knobs. The setae are greatly reduced and present only on some body segments (Fig 5.14). Adults (Fig5.15) emerge during April and May and even early June.

4. *Pediobius* sp.1

Pediobius sp.1 is a secondary parasitoid of *Tetramesa* sp.1 in *L. pseudosclerophylla*. It also parasitises *Eurytoma* sp.1 in this grass. *Pediobius* sp.1 was found only in south-western localities. This species was reared from stems of *L. pseudosclerophylla*. From one to five larvae were found in the stems of *L. pseudosclerophylla* in these localities. The larvae are white in colour and the head has a telescopic extension going into the body. The antennae are inconspicuous (Figure 5.16). Adults (Figure 5.17) emerge during April and May.

5. *Homoporus* sp.1

Homoporus sp.1 parasitises *Tetramesa* sp.1 in. *L. pseudosclerophylla*. This species was reared from the stems of *L. pseudosclerophylla* in south-eastern and south-western localities. As many as one to three larvae were found in the stems of *L. pseudosclerophylla* in South-eastern and south-western localities. In *Homoporus* sp.1 larvae, the head is triangular in anterior view and significantly narrower than the thoracic segments. The papilliform antennae are visible on the head. Adults emerge during late April to May.

6. *Chlorocytus* sp.1

Chlorocytus sp.1 parasitises *Pediobius* sp.1, *Eurytoma* sp.1, and *Tetramesa* sp.1 in the stems of *L. pseudosclerophylla*. A large series of both sexes were reared from stems of *L. pseudosclerophylla* in south-eastern and south-western localities. As many as

five larvae were found in the stems of this grass species. *Chlorocytus* sp.1 larvae are big, active and whitish. A special characteristic of the larvae is the presence of a pair of protuberances above cleapeal area, which makes it easy to identify this larva from other similar larvae (Figure 5.18). Adults (Figure 5.19) emerge during March and April.

7. *Macroneura vesicularis* (Retzius)

It was found that *M. vesicularis* parasitizes *Tetramesa* sp.1, *Eurytoma* sp.1, *Homoporus* sp.1, and *Syntomaspis baudysi* in stems of *L. pseudosclerophylla.* In this study *Macroneura vesicularis* was reared from larvae removed from stems of *L. pseudosclerophylla.* Body of the larvae is covered with several rows of bristle-like setae. The fully-grown larva is greyish white in colour and a dark area could be seen along the full length of the larva, which is because of the contents of the digestive tube. Adults emerge during May and June.

8. *Syntomaspis baudysi* Bouček

Syntomaspis baudysi parasitises *Tetramesa* sp.1 in *L. pseudosclerophylla. Syntomaspis baudysi* was only found in population 2 (Deh Lo-Lo, Kouhpayeh, Kerman province, code 2d, Southeast Iran). This species was reared from stems of *L. pseudosclerophylla.* The larvae are very active, strongly flexed venturally and have a hairy appearance (Fig 5.20). Adults emerge during May.

5.3.3 Herbivorous and parasitoid insects associated with *Leucopoa sclerophylla*

One herbivorous species (*Tetramesa* sp.2) was reared from *L. sclerophylla* and six parasitoid species were reared from *Tetramesa* sp.2. These were: *Eurytoma* sp.2, *Sycophila* sp.2 (Eurytomidae), *Pediobius* sp.2 (Eulophidae), *Chlorocytus* sp.2

(Pteromalidae), *Macroneura vesicularis* (Retzius) (Eupelmidae) and *Syntomaspis baudysi* Bouček (Torymidae) (Figures. 5.5 and 5.6).

1. *Tetramesa* sp.2

Tetramesa sp.2 was the only herbivore insect reared from *L. sclerophylla*. This species is very similar morphologically and biologically to *Tetramesa* sp.1 reared from *L. pseudosclerophylla*. From one to fifteen distinct larval cells were observed above the stem nodes, but sometimes a maximum of 24 larval cells were found. Adults emerge during February, March and April. Both sexes were reared from stems of *L. sclerophylla*, but males occurred very less often than females. *Tetramesa* sp.2 does not form the characteristic galls in the stems of *L. sclerophylla* such as *Tetramesa* sp.1 does in *L. pseudosclerophylla* (Figure 5.21). *Tetramesa* sp.2 occurs in both localities in northern Iran where the host grass was collected (Figure 5.12). The highest number of *Tetramesa* sp.2 was found in population 7 (Karaj-Chalous Road. code 9b, northern Iran) in 1997, while the lowest number was also found in this population in 1999. The number of *Tetramesa* sp.2 and its parasitoids reared in the grass *L. sclerophylla* is shown in Table 5.3.

The specimens of *Tetramesa* reared from *L. pseudosclerophylla* and *L. sclerophylla* were compared with all the species of *Tetramesa* (especially European species) and found to be a new species (Madjdzadeh *et al.* 2005).

2. *Eurytoma* sp.2

Eurytoma sp.2 parasitises *Tetramesa* sp.2 in the stems of *L. sclerophylla*. One to three larvae were found each inhabiting a single *Tetramesa* cell in the stem. The *Eurytoma* sp.2 is very similar in the larval and adult stage to *Eurytoma* sp.1 reared from

Tetramesa sp.1 in *L. pseudosclerophylla*. This species was not found in Sirachal (Code 9c).

3. *Sycophila* sp.2

Sycophila sp.2 attacks *Tetramesa* sp. 2 in the stems of *L. sclerophylla*. *Sycophila* sp.2 was reared from larvae removed from stems of *L. sclerophylla*. Figure 5.22 shows a pupa of *Sycophila* sp.2. One to five larvae were found in the stems of *L. sclerophylla* in northern localities. Adults emerge during April and May.

4. *Pediobius* sp.2

Pediobius sp.2 attacks *Tetramesa* sp.2 and *Eurytoma* sp.2 in the stems of *L. sclerophylla*. This species was in found the form of mature larvae in the stems of *L. sclerophylla*. *Pediobius* sp.2 is a secondary parasitoid of *Tetramesa* sp.2 in this grass. It also parasitises *Eurytoma* sp.2 in the stems of this grass. In most stems only one larva was found in the stems of *L. sclerophylla* in northern localities. It was not possible to obtain adult specimens of this species as all the larvae died because of infestation.

5. *Chlorocytus* sp.2

Chlorocytus sp.2 parasitises *Pediobius* sp.2, *Eurytoma* sp.2, and *Tetramesa* sp.2 in stems of *L. sclerophylla*. A large series of both sexes was reared from larvae removed from stems of *L. sclerophylla* in northern localities. One to eight larvae were found in the stems of this grass species. Adults emerge during April.

6. *Macroneura vesicularis* (Retzius)

Macroneura vesicularis parasitises *Tetramesa* sp.2, *Eurytoma* sp.2, and *Syntomaspis* sp.2 in the stems of *L. sclerophylla*. *M. vesicularis* was reared from stems of

L. sclerophylla. In most stems only one larva was found in this grass species. Adults emerge during May and June.

7. *Syntomaspis baudysi* Bouček

Syntomaspis baudysi parasitises *Tetramesa* sp.2 in *L. sclerophylla*. This species was reared from larvae removed from stems of *L. sclerophylla*. From one to three larvae were found in the stems of *L. sclerophylla*. Unfortunately all the larvae which were reared in northern localities, died because of infestation.

5.3.4 Parasitism rate

Percentage parasitism of *Tetramesa* sp.1 and *Tetramesa* sp.2 for each site and year are presented in Tables 5.4 and 5.5 respectively. The whole infestation/parasitism is given in the Table 5.6. Figure 5.23 shows the variation in the number of the parasitoids of *Tetramesa* sp.1 in *L. pseudosclerophylla* between sites and years in southeast and southwest Iran. Figure 5.24 represents the variation in the number of parasitoids of *Teramesa* sp.2 in *L. sclerophylla*. It was clear that the dominant parasitoid at a site in one year may not be dominant in subsequent years in the same site.

5.3.5 Species richness

The relationship between sample sites and number of parasitoids reared for *Tetramesa* species associated with *L. pseudosclerophylla* and *L. sclerophylla* during three years from 1997 to 1999 are shown in Figures 5.25, 5.26 and 5.27. The result shows that the number of parasitoid species among six populations reared from *L. pseudosclerophylla* in southern Iran was approximately similar for the years 1997, 1998 and 1999. The number of parasitoids reared from *L. sclerophylla* among two populations

(7 and 8) in northern Iran was also similar for the years 1997, 1998 and 1999 (see Figure 5.25).

5.4 Discussion

The aim of this study was to investigate the structure of the parasitoid assemblages associated with *Tetramesa* sp. feeding on *L. pseudosclerophylla* and *L. sclerophylla* collected from eight localities in southeast (three sites), southwest (three sites) and northern Iran (two sites).

5.4.1 Food web structure

The grass-herbivore-parasitoid food web comprised 15 species (Figure. 5.6). In this study it was found that the number of parasitoids attacking *Tetramesa* sp.1 feeding on *L. pseudosclerophylla* and *Tetramesa* sp.2 feeding on *L. sclerophylla* were seven and six respectively. The most striking characteristic of the food web was the extreme specificity of both herbivores and primary parasitoids and because of the specificity of most of the species in the community, the food web was compartmentalised. The generalist hyperparasitoid, *M. vesicularis* tied together the other portions of the web. *M. vesicularis* has also been reared from other endophagous hosts of quite different groups living in non-grass host plants (Morris 1938; Erdös 1960). However, although it is easy to say how many hosts a particular parasitoid can attack, it is difficult to determine all the hosts of a particular parasitoid.

This study revealed that most parasitoid species studied here were idiobionts. This characteristic is found in communities on endophytic hosts (Hawkins 1994, Dawah *et al* 1995, Askew & Shaw 1986). In addition hyperparasitoid species were facultative, rather than obligate and most species showed a narrow host range. This characteristic is found in communities of herbaceous plants (Zwölfer 1979, 1980; Askew & Shaw 1986).

There are two anomalies in this system: first, the depth of the food web was uncharacteristic, because *Chlorocytus* sp.1 and *Chlorocytus* sp.2 were recorded as tertiary parasitoids in this study. This is due to the large number of feeding relationships that have been observed in *L. pseudosclerophylla* and *L. sclerophylla*. It seems likely that tertiary parasitoids are more widespread than the existing data indicate. The parasitoids that attack gall-making insects produce communities that containing tertiary parasitoids have not been reported widely. The second anomaly was the extreme host specificity of the idiobiont primary parasitoids. Most idiobionts are characterised however, by a broad host range (Askew & Shaw 1986; Hawkins 1994), but some idiobionts specialise when their hosts are consistently abundant as is the case in this study, although they are physiologically capable of utilising a broader range of host species than they actually use (Askew & Shaw 1986; Tscharntke 1992).

5.4.2 Species richness

As Lawton (1999) argued the relationship between regional species richness and local species richness can be shown using two models, Type I and Type II. The first model says that local richness is directly proportional to, but less than, regional richness, while in Type II local richness is not directly proportional to regional richness. He mentioned that the majority of ecological systems are Type I. In this present study, the number of parasitoids reared from *Tetramesa* sp.1 associated with *L. pseudosclerophylla* through six localities across southeast and southwest was similar. The number of parasitoid species reared from *Tetramesa* sp.2 associated with *L. sclerophylla* was the same in the two sites (9b and 9c) in northern Iran during 1997, 1998 and 1999 was also similar (Figures 5.25, 5.26 and 5.27).

In this study it was found that *Pediobius* sp. attacking *Tetramesa* feeding on *L. pseudosclerophylla* were found in the southwest and northern Iran but not from the

southeast of Iran. *L. pseudosclerophylla* is very common and widespread in southwest as well as in southeast Iran; *Tetramesa* feeding on *L. pseudosclerophylla* were found everywhere their host exists, and therefore parasitoids may not be limited by dispersal between sites. Since not every species can live everywhere, and chance or isolation excludes others, with a few notable exceptions (e.g. Dawah *et al* 1995), it should not be expected that every species found in a regional pool will be found in every local community (Zobel 1992).

5.4.3 Species abundance

It is likely that parasitoid abundance is constrained simply by differences in host finding abilities, or perhaps because some of the primary parasitoids are preferred by hyperparasitoids. This study revealed that *S. baudysi* and *Homoporus* sp.1 attacking *Tetramesa* sp.1 in *L. pseudosclerophylls* and *Eurytoma* sp.2 and *Pediobius* sp.2 in *L. sclerophylla* are rare, probably because they are fairly generalised and are not efficient at locating hosts of any particular host species (Rasplus 1994; Dawah *et al.* 1995). Also as it could be observed that *Chlorocytus* sp.1, *Eurytoma* sp.1, and *Sycophila* sp.1 in *L. pseudosclerophylla* and *Chlorocytus* sp.2 and *Sycophila* sp.2 in *L. sclerophylla* are among the abundant species. The abundance of parasitoids may be related to their ability in finding hosts (see Godfray 1994), fecundity, larval competitive ability and adult longevity (Huffaker *et al.* 1977; Force 1974). It could be a combination of all these factors; one or more factors are responsible for parasitoid relative abundance. Dawah *et al* (1995) found that variation in parasitism rates both between and within host species could not be explained by the variables investigated (gall/non-gall, host geographic range, mean *Tetramesa* log-density, variability in host density, number of hosts per stem, number of co-occurring hosts, grass type, host voltinism), so the forces affecting host utilisation rates remain unknown.

This present study revealed that the dominant parasitoid at a site in one year might not be dominant in a subsequent year in the same site. For example, *Eurytoma* sp.1 (Figure 5.23) was the dominant parasitoid at site 1a in 1997, while *Sycophila* sp.1 was dominant at the same site in 1998, and again *Eurytoma* sp.1 was dominant in 1999 at the same site. It must be mentioned that *Eurytoma* sp.1 was more abundant in some populations, while *Chlorocytus* sp.1 was more abundant in other populations. It was also noticed that some parasitoids had similar abundance in different populations. For example, *Eurytoma* sp.1 had similar abundance in population 2d in 1997 and 1999 and in population 7b in 1999.

The variation in the number of the parasitoids of *Tetramesa* sp.2 in *L. sclerophylla* between sites and years is shown in Figure 5.24. Some parasitoids were dominant through time at a site. For example, *Chlorocytus* sp.2 was dominant at site 9b in 1997, 1998 and 1999. This species was more abundant in most populations. Some parasitoids also had similar abundance in different populations. For example, *S. baudysi* had similar abundance in population 9b in 1999 and in population 9c in 1998. Many factors may give rise to fluctuations in abundance in insects, such as seasonal and other changes in the weather, changes in food supply, the attacks of natural enemies including micro-organisms causing disease (Solomon 1985) and habitat destruction (Cronin 2004).

It was found that most of the local parasitoid communities showed consistent dominance hierarchies (Figures 5.23 and 5.24). This agrees with the finding of Dawah *et al* (1995) who found most of the local parasitoid communities they studied showed relatively consistent dominance hierarchies. Their study attempted to identify the ecological factors giving rise to this consistency, but they were unable to determine these factors (Dawah *et al* 1995).

In conclusion, despite the fact that the Dawah *et al.* (1995) study focused on 10 grass species sampled quantitatively between 1980-1992 at 24 sited in UK, they concluded that the patterns of parasitoid species richness in the grass based communities were readily predictable, but the dynamics of the communities were not. The result of the present study is in agreement with Dawah *et al.* (1995)

5.5 References

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Table 5.1. Localities across Iran where the host grasses collected during 3 years from 1997 to 1999. For sampling code see chapter 2.

Region	Province	Sampling site	Year	Grass species	No. Stems
Southeast	Kerman	1a	1997	*L. pseudosclerophylla*	688
Southeast	Kerman	1a	1998	*L. pseudosclerophylla*	1166
Southeast	Kerman	1a	1999	*L. pseudosclerophylla*	608
Southeast	Kerman	2d	1997	*L. pseudosclerophylla*	318
Southeast	Kerman	2d	1998	*L. pseudosclerophylla*	112
Southeast	Kerman	2d	1999	*L. pseudosclerophylla*	208
Southeast	Kerman	4b	1997	*L. pseudosclerophylla*	181
Southeast	Kerman	4b	1998	*L. pseudosclerophylla*	192
Southeast	Kerman	4b	1999	*L. pseudosclerophylla*	241
Southwest	Fars	7	1997	*L. pseudosclerophylla*	1021
Southwest	Fars	7	1998	*L. pseudosclerophylla*	1407
Southwest	Fars	7	1999	*L. pseudosclerophylla*	451
Southwest	Fars	7a	1997	*L. pseudosclerophylla*	125
Southwest	Fars	7a	1998	*L. pseudosclerophylla*	81
Southwest	Fars	7a	1999	*L. pseudosclerophylla*	100
Southwest	Fars	7b	1997	*L. pseudosclerophylla*	281
Southwest	Fars	7b	1998	*L. pseudosclerophylla*	321
Southwest	Fars	7b	1999	*L. pseudosclerophylla*	298
North	Tehran	9b	1997	*L. sclerophylla*	405
North	Tehran	9b	1998	*L. sclerophylla*	340
North	Tehran	9b	1999	*L. sclerophylla*	285
North	Tehran	9c	1997	*L. sclerophylla*	297
North	Tehran	9c	1998	*L. sclerophylla*	562
North	Tehran	9c	1999	*L. sclerophylla*	340

Table 5.2 Number of *Tetramesa* sp.1 and its parasitoids reared together with number of stems (collected and infested) in each site and year in the grass *Leucopoa pseudosclerophylla.*

Region	Sampling site	Year	Stem collected	Stem infested	*Tetramesa* sp.	*Eurytoma* sp.	*Sycophila* sp.	*Homoporus* sp.	*Syntomaspis* sp.	*Pediobius* sp.	*Chlorocytus* sp.	*Macroneura* sp.	Total parasitoids
South-east	1a	1997	688	59	90	18	6	6	0	0	18	0	48
		1998	1166	102	99	31	41	15	0	0	40	27	154
		1999	608	60	44	21	12	10	0	0	16	25	84
	2d	1997	318	108	184	23	61	7	13	0	15	24	143
		1998	112	44	150	17	45	5	24	0	12	32	135
		1999	208	52	102	12	27	0	11	0	20	0	70
	4b	1997	181	48	120	30	19	12	0	0	38	0	99
		1998	192	57	142	38	24	10	0	0	49	0	121
		1999	241	73	61	30	31	10	0	0	23	19	113
South-west	7	1997	1021	62	88	30	15	5	0	20	27	0	97
		1998	1407	83	110	37	18	6	0	23	32	0	116
		1999	451	6	74	12	0	5	0	16	2	53	88
	7a	1997	125	58	77	51	15	6	0	30	68	0	170
		1998	81	37	70	31	10	4	0	16	40	0	101
		1999	100	45	82	37	12	4	0	21	53	0	127
	7b	1997	281	57	94	10	5	0	0	16	46	0	77
		1998	321	81	91	20	4	0	0	23	65	0	112
		1999	298	63	82	12	5	0	0	20	52	0	89
		Total	7799	1095	1760	460	350	105	48	185	616	180	1944

Table 5.3 Number of *Tetramesa* sp.2 and its parasitoids reared together with number of stems (collected and infested) in each site and year in the grass *Leucopoa sclerophylla.*

Region	Sampling site	Year	Stem collected	Stem infested	*Tetramesa* sp.	*Eurytoma* sp.	*Sycophila* sp.	*Syntomaspis* sp.	*Pediobius* sp.	*Chlorocytus* sp.	*Macroneura* sp.	Total parasitoids
North	9b	1997	405	64	363	23	38	0	17	164	21	263
		1998	340	86	242	19	27	0	11	106	12	175
		1999	285	32	102	14	33	22	6	51	47	173
	9c	1997	297	25	124	0	23	25	4	23	40	115
		1998	562	51	254	0	34	33	21	45	28	161
		1999	340	44	133	0	30	28	9	34	27	128
		Total	2229	302	1218	56	185	108	68	423	175	1015

Table 5.4 Percentage parasitism (%) of *Tetramesa* sp.1 parasitoids in each site in the grass *Leucopoa pseudosclerophylla.*

Region	Year	Sampling site	*Eurytoma* sp.1	*Sycophila* sp.1	*Homoporus* sp.1	*S. baudysi.*	*Pediobius* sp.1	*Chlorocytus* sp.1	*M. vesicularis*
South-east	1997	1a	13.0	4.3	4.3	0	0	7.2	0
South-east	1998	1a	12.3	16.2	5.9	0	0	9.1	5.9
South-east	1999	1a	16.4	9.4	7.8	0	0	7.0	10.9
South-east	1997	2d	7.0	18.7	2.1	4.0	0	2.8	4.3
South-east	1998	2d	6.0	15.8	1.8	8.4	0	2.5	3.9
South-east	1999	2d	7.0	15.7	0	6.4	0	6.4	0
South-east	1997	4b	13.7	8.7	5.5	0	0	9.6	0
South-east	1998	4b	14.4	9.1	3.8	0	0	10.6	0
South-east	1999	4b	17.2	17.8	5.7	0	0	7.5	10.9
South-west	1997	7	16.2	8.1	2.7	0	8.1	8.6	0
South-west	1998	7	16.4	8.0	2.7	0	7.5	8.4	0
South-west	1999	7	7.4	0	3.1	0	6.2	1.2	7.4
South-west	1997	7a	20.6	6.1	2.4	0	9.7	16.2	0
South-west	1998	7a	18.1	5.8	2.3	0	7.0	14.0	0
South-west	1999	7a	17.7	5.7	1.9	0	7.7	14.8	0
South-west	1997	7b	5.8	2.9	0	0	7.0	15.8	0
South-west	1998	7b	9.9	2.0	0	0	8.9	18.7	0
South-west	1999	7b	7.0	2.9	0	0	9.4	17.5	0

Table 5.5 Percentage parasitism (%) of *Tetramesa* sp.2 parasitoids in each site in the grass *Leucopoa sclerophylla.*

Region	Year	Sampling site	*Eurytoma* sp.2	*Sycophila* sp.2	*S. baudysi*	*Pediobius* sp.2	*Chlorocytus* sp.2	*M. vesicularis*
North	1997	9b	3.7	6.1	0	1.4	21.1	2.1
North	1998	9b	4.6	6.5	0	1.4	20.4	2.9
North	1999	9b	5.1	12.0	8.0	2.1	15.3	12.0
North	1997	9c	0	9.6	10.5	1.7	7.5	12.1
North	1998	9c	0	8.2	8.0	2.7	8.7	3.4
North	1999	9c	0	11.5	10.7	3.4	10.3	7.7

Table 5.6 Total percentage of infestation/parasitism in two grass species, *Leucopoa pseudosclerophylla* (*L. p*) and *Leucopoa sclerophylla* (*L. s*).

	Herbivore	Primary parasitoids				Hyperparasitoids								
						Pediobius sp.		*Chlorocytus* sp.			*M. vesicularis*			
Grass	*Tetramesa* sp	*Eurytoma* sp.	*Sycophila* sp.	*Homoporus* sp.	*S. baudysi*	*Tetramesa* sp.	*Eurytoma* sp.	Tetramesa sp.	*Eurytoma* sp.	*Pediobius* sp.	*Tetramesa* sp.	*Eurytoma* sp.	*Homoporus* sp.	*S. baudysi*
L. p	14	12.4	9.4	2.8	1.3	3.8	1.2	9.7	4.9	2.4	2.3	1.2	0.6	0.8
L. s	13.5	2.5	8.3	-	4.8	2.0	1.0	15.2	1.5	2.3	5.4	1.8	-	0.7

Figure 5.1 A view of sample collection site of *L. pseudosclerophylla* (1a, Sirch, Kerman) in Southeast Iran.

Figure 5.2 A view of sample collection site of *L. pseudosclerophylla* (7b, Sepidan-Yasuj Rd.) in Southwest Iran.

Figure 5.3 A view of sample collection site of *L. sclerophylla* (9c, Sirachal) in Northern Iran.

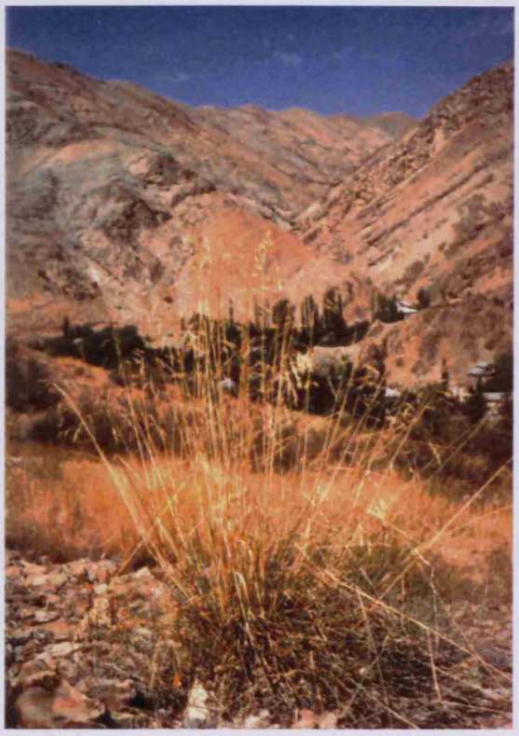

Figure 5.4 Community interrelationships in *L. pseudosclerophylla*.

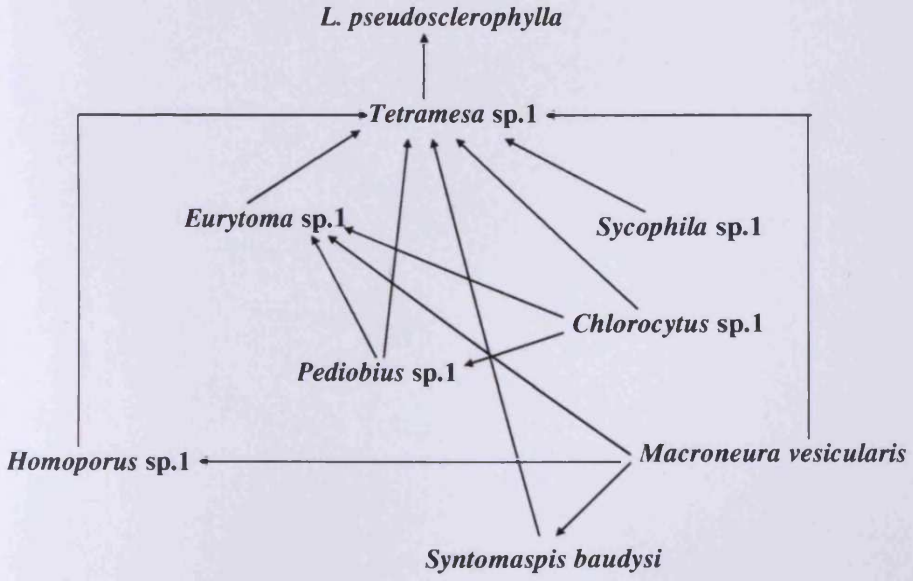

Figure 5.5 Community interrelationships in *Leucopoa sclerophylla*.

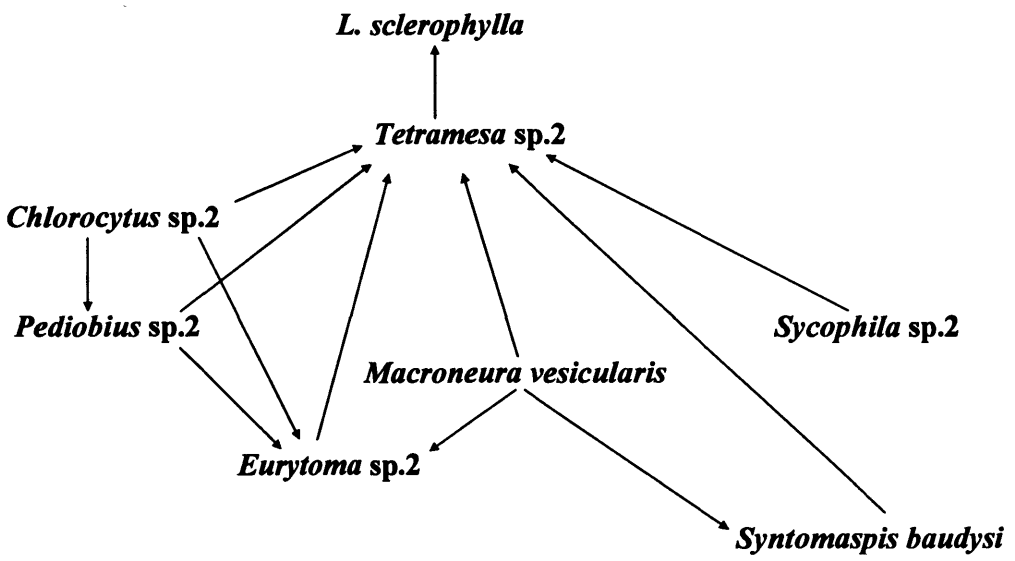

Fig 5.6 A semi-quantitative food web for the grass-herbivore-parasitoid community in two grass species, *Leucopoa pseudosclerophylla* and *Leucopoa sclerophylla* collected from eight localities in Southeast, Southwest and Northern Iran.

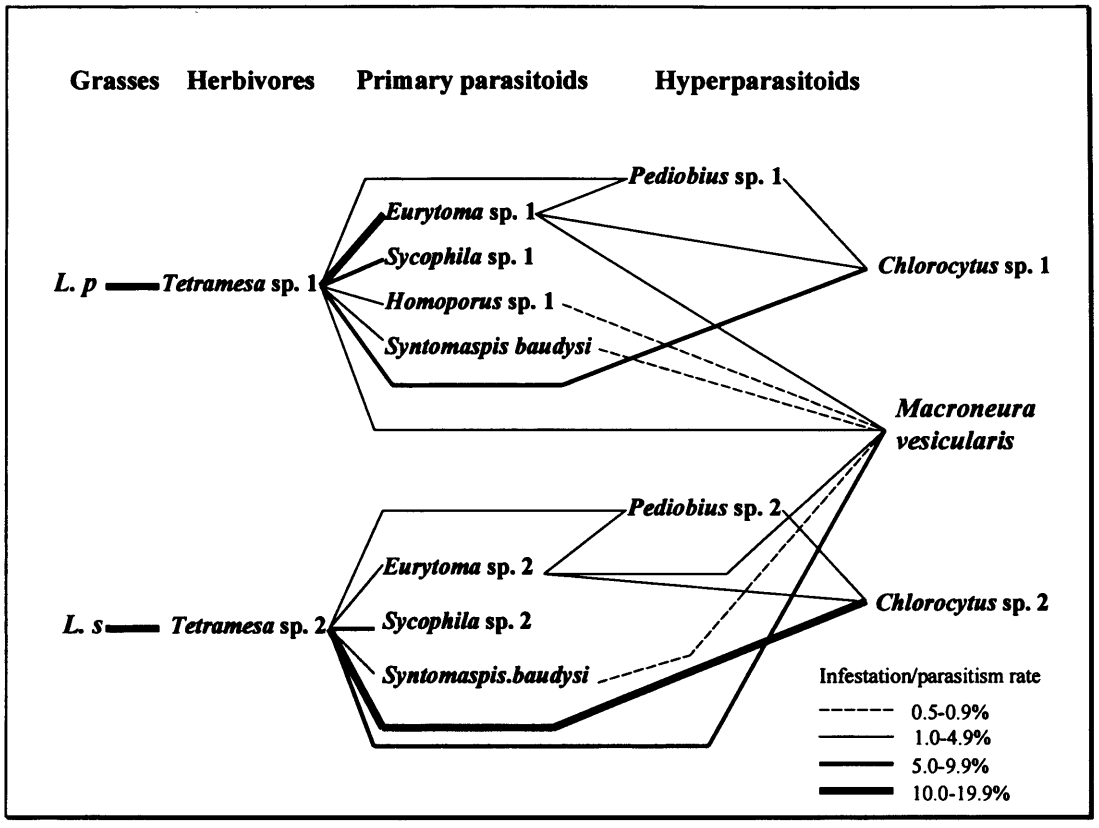

Figure 5.7 Pupa of *Tetramesa* sp.1 reared from stems of *Leucopoa pseudosclerophylla* (dorsal view).

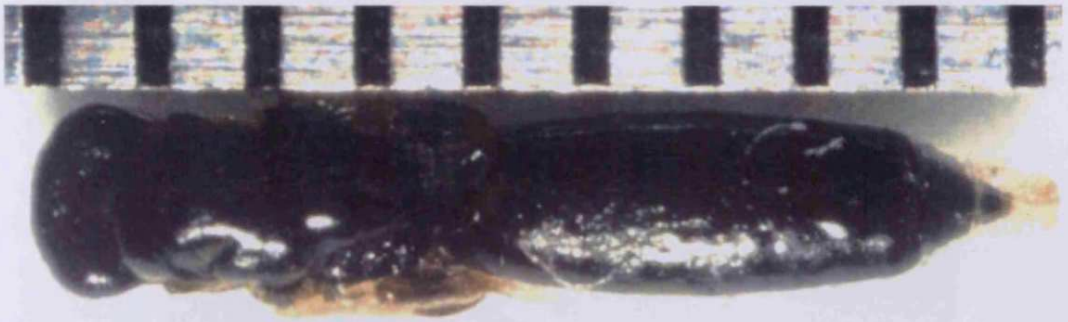

Figure 5.8 Pupa of *Tetramesa* sp.1 reared from stems of *Leucopoa pseudosclerophylla* (lateral view).

Figure 5.9 A pupa of *Tetramesa* sp.1 inside the stem of *Leucopoa pseudosclerophylla*

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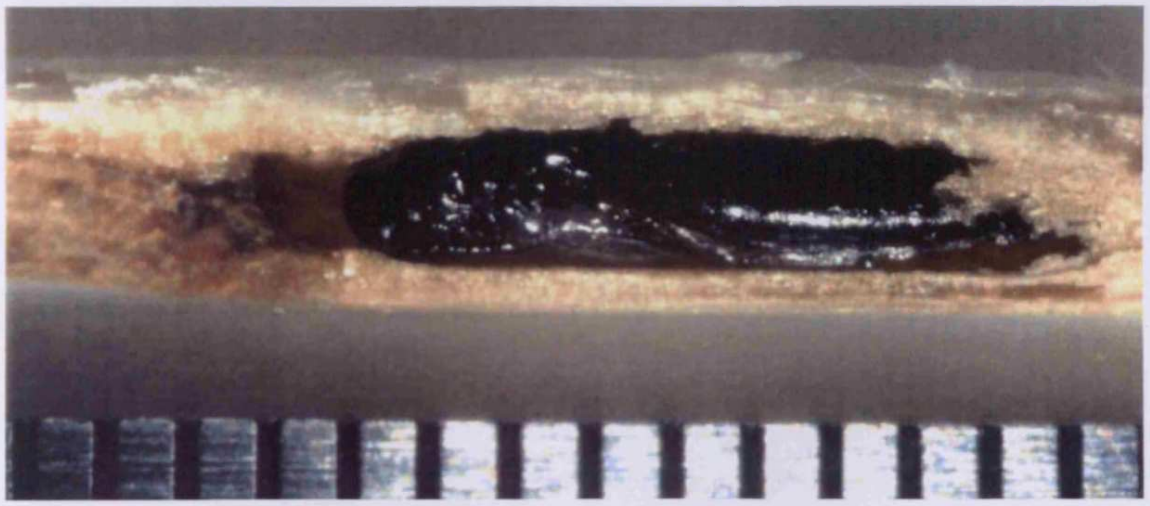


Figure 5.10 Adult of *Tetramesa* sp.1 (female) reared from stems of *Leucopoa pseudosclerophylla*.

Figure 5.11 Stem-galls of *Tetramesa* sp.1 on *Leucopoa pseudosclerophylla*.

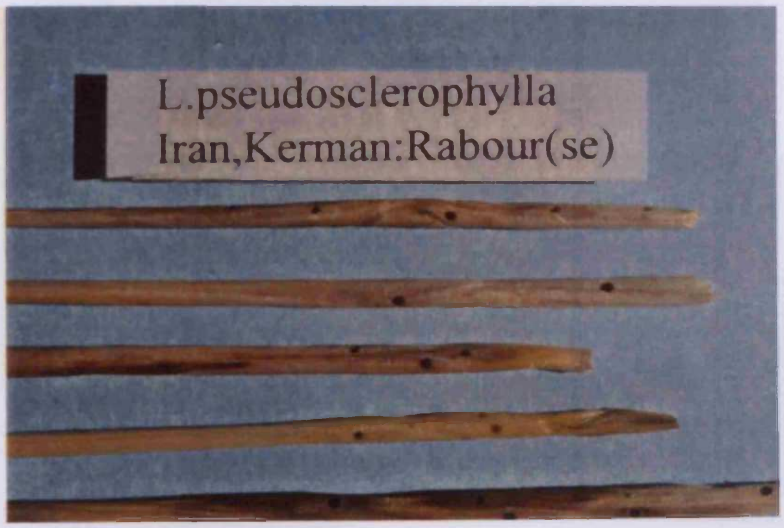


Figure 5.12 Number of *Tetramesa* sp. over 1997, 1998 and 1999 for eight populations. Populations 1 to 6 belong to *Leucopoa pseudosclerophylla* and populations 7 and 8 belong to *L. sclerophylla*. Populations' codes are: 1=1a, 2=2d, 3=4b, 4=7, 5=7a, 6=7b, 7=9b, and 8=9c. For population code see Table 5.1).

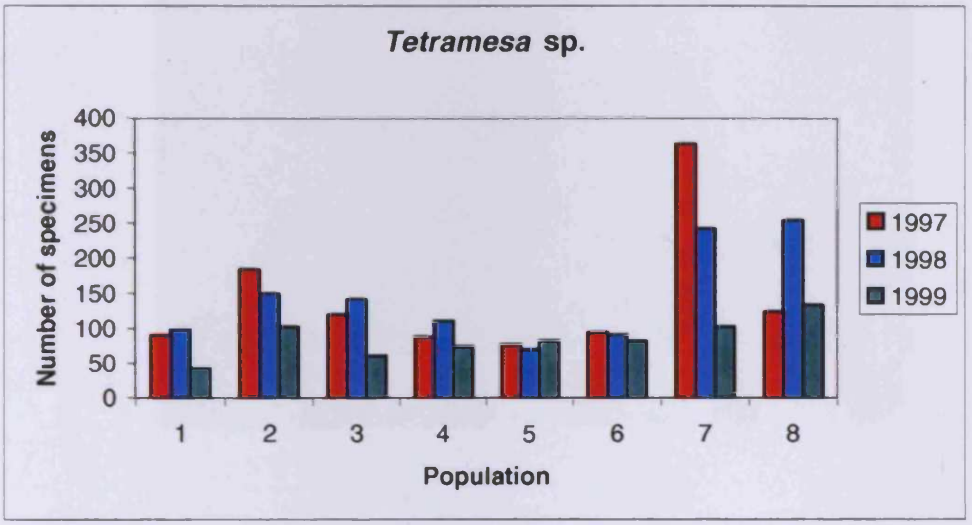


Figure 5.13 Adult of *Eurytoma* sp.1 (male) reared from *Leucopoa pseudosclerophylla*.

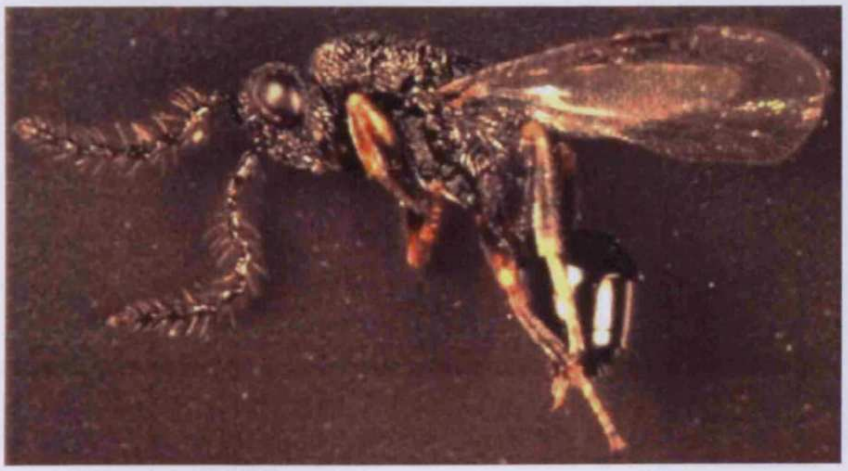


Figure 5.14 Larva of *Sycophila* sp.1 in *Leucopoa pseudosclerophylla* (ventral view). Distance between lines is 1 mm.

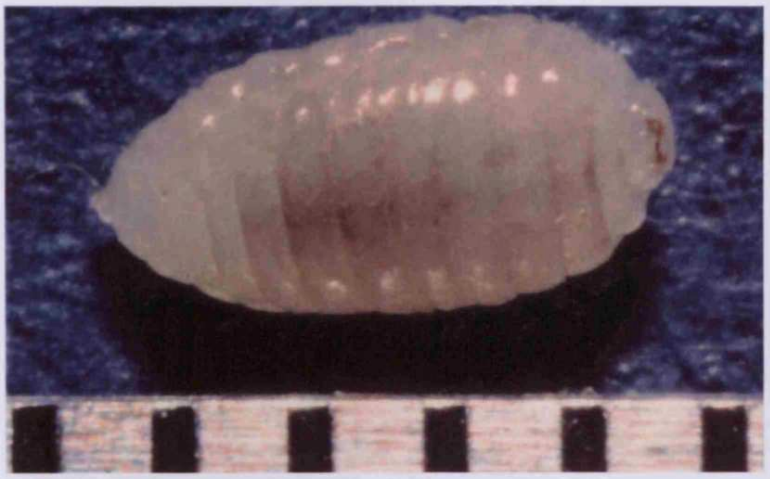


Figure 5.15 Adult of *Sycophila* sp.1 (female) reared from *Leucopoa pseudosclerophylla*.

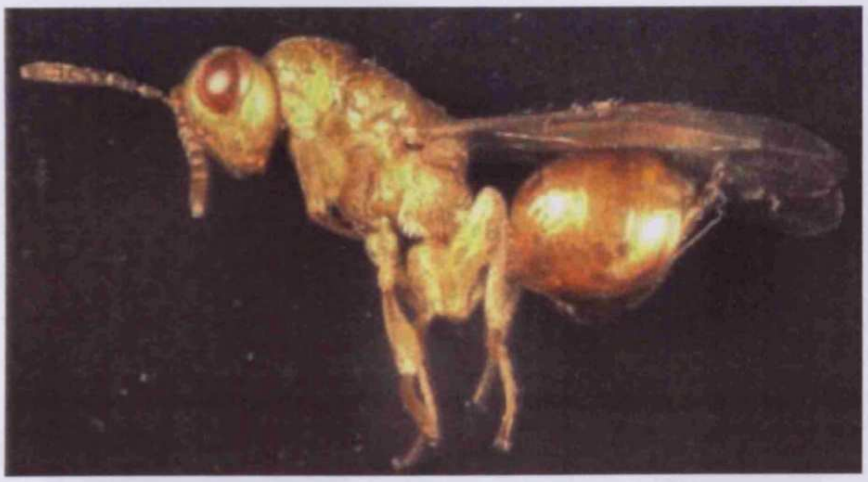


Figure 5.16 Larva of *Pediobius* sp.1 reared from *Leucopoa pseudosclerophylla*.

Figure 5.17 Adult of *Pediobius* sp.1 reared from *Leucopoa pseudosclerophylla*.

Figure 5.18. Larva of *Chlorocytus* sp.1 reared from *Leucopoa pseudosclerophylla*. The protuberances above the clypeal area are indicated by the arrows.

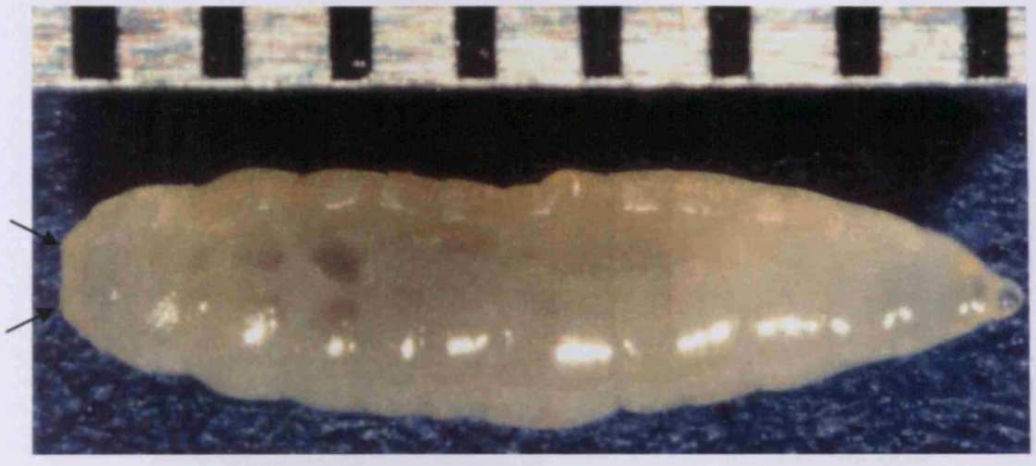


Figure 5.19. Adult of *Chlorocytus* sp.1 reared from *Leucopoa pseudosclerophylla*.

Figure 5.20. Larva of *S. baudysi* reared from *Leucopoa pseudosclerophylla*.

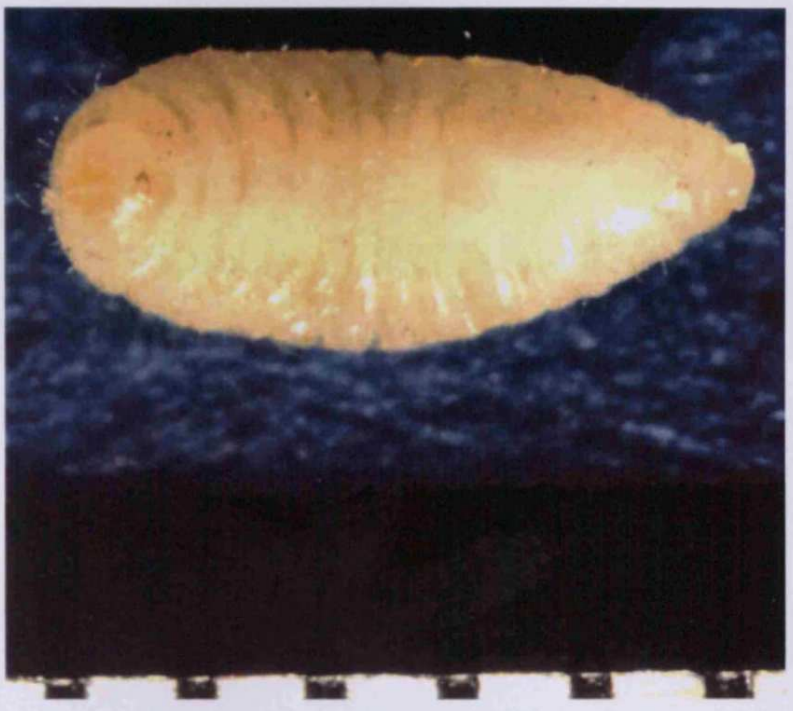


Figure 5.21 Stem-galls of *Tetramesa* sp.2 on *Leucopoa sclerophylla*.

Figure 5.22 pupa of *Sycophila* sp.2 reared from *Leucopoa sclerophylla* in Northern Iran (lateral view). Distance between lines is 1 mm.

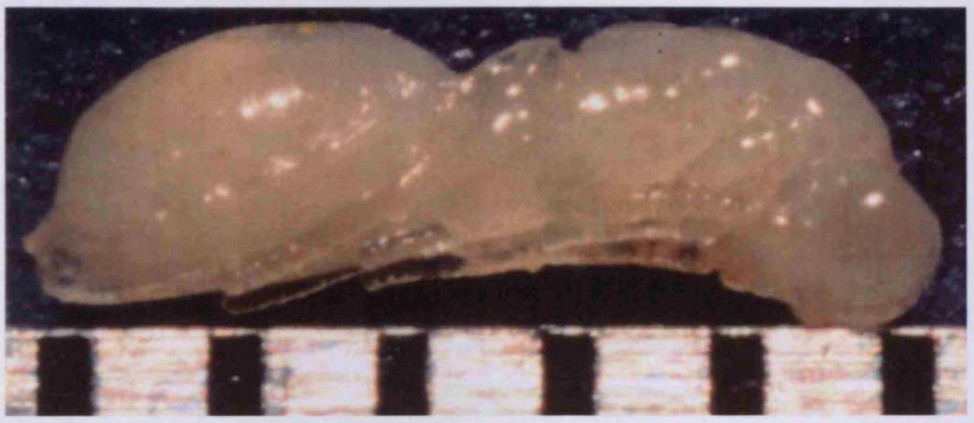


Figure 5.23 Percentage parasitism of *Tetramesa* sp. by seven different parasitoid species over 1997, 1998 and 1999 For six populations in the grass *L. pseudosclerophylla* collected from Southeast and Southwest Iran.

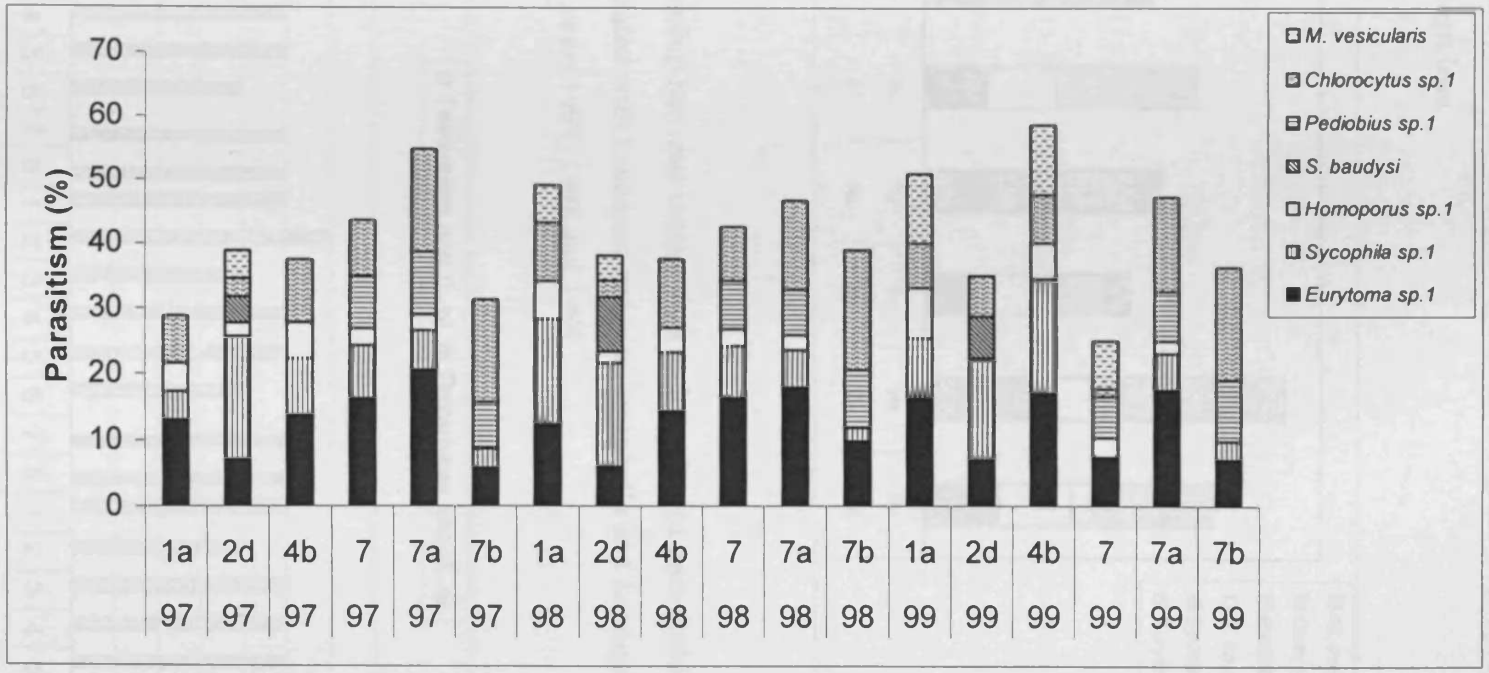


Figure 5.24 Percentage parasitism of *Tetramesa* sp. by six different parasitoid species over 1997, 1998, and 1999 for two populations in the grass *Leucopoa sclerophylla* collected from northern Iran.

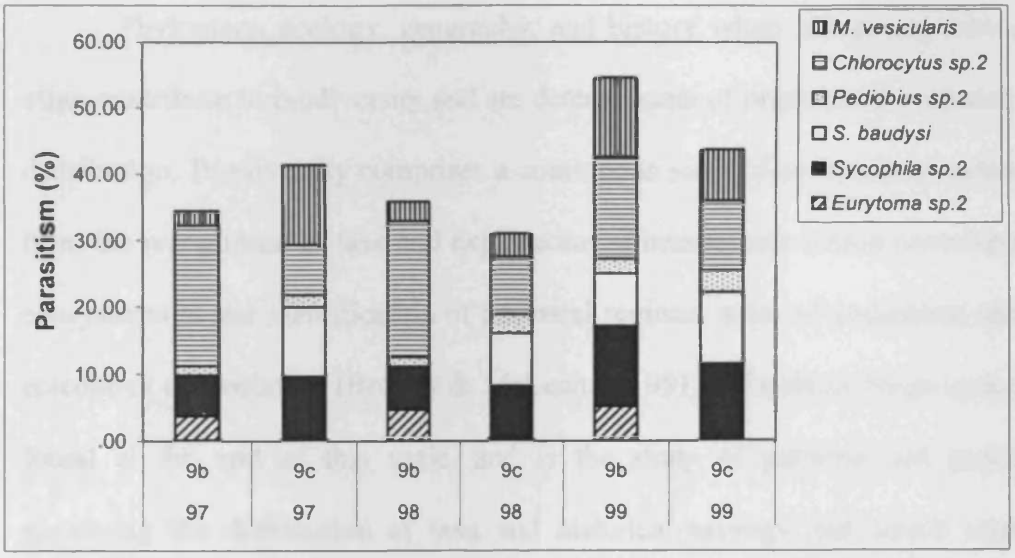
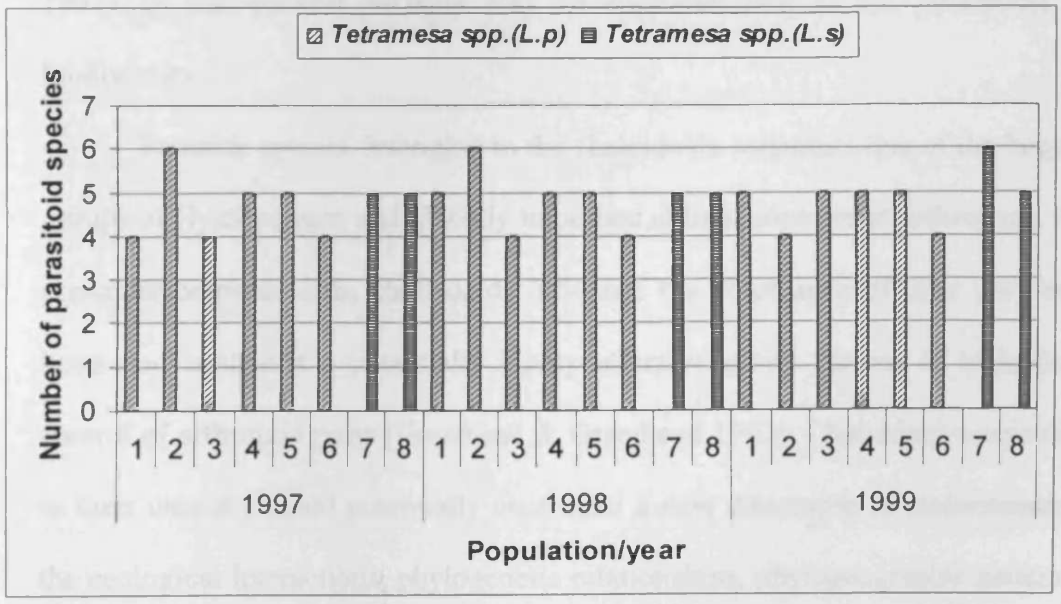


Figure 5.25 Relationship between sample sites and number of parasitoids reared for *Tetramesa* sp. associated with *Leucopoa pseudosclerophylla* and *Leucopoa sclerophylla* for the years 1997, 1998 and 1999.



CHAPTER 6

General Discussion

6.1 Background

Phylogeny, ecology, geography, and history when interacting with each other contribute to biodiversity and are determinants of organismal evolution and distribution. Biodiversity comprises a continuous scale of associations extending from the recognition of taxa and explanation of interactions within contemporary ecosystems to the identification of ancestral regions, areas of endemism and the epicentres of evolution (Brooks & McLennan 1991). Historical biogeography is found at the end of this scale, and is the study of patterns and processes governing the distribution of taxa and historical ecology and which contains macroevolutionary processes in community development. So with phylogenetic analysis, evidence from geological history and ecological interactions may suggest hypotheses about the production of biological diversity and evolution of community structure in a strictly evolutionary framework (Brooks & McLennan 1991). In this context parasites play an important role in the generation of biodiversity.

Parasitic species belonging to the chalcidoids constitute one of the largest groups of Hymenoptera and globally important natural enemies of arthropods. As predators or parasitoids, chalcidoids influence the abundance of their prey and hosts and constitute a potentially highly effective group for use in biological control of arthropod pests (Greathead & Greathead 1992). Chalcidoid wasps due to their ubiquity could potentially contribute a new dimension to understanding the ecological interactions, phylogenetic relationships, phylogeographic patterns, and the complex history of many species in many geographic regions.

From a conservation point of view, knowledge of the phylogenetic history and patterns of host-parasite assemblages is critical to our understanding of the age and duration of faunal associations, recognition of areas of endemism, speciation and the historical biogeographic structure of ecosystems and communities. Complex decisions in fields such as conservation biology can be assisted using data from phylogeography together with information on palaeogeography and palaeoclimatology (Brooks *et al.* 1992; Feldman & Spicer 2002; Zheng *et al.* 2003).

In order to provide a better understanding of the molecular and morphological characteristics and host-parasitoid interaction of the taxa under study, a multidisciplinary investigation was performed using morphometrics, molecular genetics and community ecology to examine the determinants of biological diversity of chalcidoid wasps associated with *L. pseudosclerophylla* and *L. sclerophylla* in Iran. Before this study, no information was available about the presence and distribution of chalcidoid wasps associated with grasses in Iran.

This thesis examined morphological variation in geographical populations of *Tetramesa*, community structure, and genetic diversity of chalcid wasp species, specifically the phylogeography of *Sycophila* sp. which is possibly a cryptic species complex in Iran. I attempted to evaluate the historical and contemporary factors that could have affected the present day genetic structure of *Sycophila*. Since *Tetramesa* sp. was the only herbivore species that could be reared from the two grass species, *L. pseudosclerophylla* and *L. sclerophylla* in this investigation, I used it to study morphological variation in geographically distinct and different host associated populations using morphometrics. Unfortunately due to paucity of reared specimens, morphological markers were

not applied to the associated parasitoid species to investigate morphological differentiation in these taxa. However, the parasitoids associated with *Tetramesa* showed genetic variation using molecular markers. Despite many efforts to resolve the taxonomy and systematics of chalcid wasp species over a long period of time, their status remains remarkably poorly understood, especially because traditional taxonomy has failed to resolve difficulties among suspected cryptic species (Bouček 1988; Bouček & Rasplus 1991; Graham 1969, 1970;; Zerova 1978).

6.2 Discussion

Tetramesa sp. comprises many populations inhabiting different geographical regions in Iran and they seem to be found everywhere their host food plant is found. One important question is whether these populations, especially northern and southern populations are morphologically closely related populations or cryptic species. Geographic variation is a prominent feature in many widespread species (Mayr 1963) since populations of a species adaptively diverge to survive in the diverse physical and biotic conditions of their environments (Futuyma 1998). *Tetramesa* species, which have a wide geographical range, must experience different selection pressures in different sections of their geographical range. Some phytophagous species are relatively restricted (oligophagous) or very restricted (monophagous; Fox & Morrow 1981) at any one locality with regard to host plant and their hosts may provide sufficient raw material for allopatric speciation where these populations become isolated by geographical barriers, although the presence of local food plant races shows that partial barriers to gene flow already exist (see Feder *et al.* 2003 for

details concerning speciation in the apple maggot fly, *Rhagoletis pomonella* complex).

So far no single species of *Tetramesa* has been recorded to attack different allopatric grass species, although *Tetramesa* parasitizing sympatric closely species of grasses have already been recorded (Dawah 1987). Different models of speciation make different assumptions about the evolution of isolating mechanisms (e.g. Bush 1975) and in the absence of geographic isolation, postzygotic barriers to gene-flow (Turelli *et al.* 2001) have been posited as the main factors that contribute to genetic differentiation during adaptive divergence. For example, recently assortative mating (Kondrashov & Shpak 1998) has been considered as a key mechanism ensuring reproductive isolation in sympatry over time. This has been directly quantified in a recent study of sympatric host races of the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) feeding on different host plants using genetic and biogeochemical markers (see Malausa *et al.* 2005).

The result of morphometric analysis carried out in this study successfully discriminated two host adapted populations of *Tetramesa* sp. (northern and southern populations) reared from *L. sclerophylla* and *L. pseudosclerophylla* respectively using discriminant function and cluster analysis. In a similar way, three Eurasian allopatric populations of fruit fly, *Drosophila lummei*, were successfully separated based on differences in wing morphology using both principal components and discriminant function analysis (Haas & Tolley 1998). The status of the *Tetramesa* populations studied here remains unclear and may be consistent with the existence of recently diverged allopatric species (Bush 1975; White 1978; Mopper & Strauss 1998). However, morphometric data alone cannot

provide strong enough evidence to consider these two populations as sibling species because first, no molecular differentiation could be detected between these populations using DNA sequence data for a fragment of the mitochondrial COI and COII genes. Second, no data is available on gene flow between the populations – important since it seems that this species is highly mobile (H. A. Dawah Pers. Communication).

Nowadays, new taxa defined on the basis of morphology are routinely studied using neutral genetic markers (Caterino *et al.* 2000; Kruse & Sperling 2001; Manzari *et al.* 2002). Here, the existence of little or no genetic differentiation among geographical locations for *Tetramesa* may indicate that allopatric divergence has occurred recently. Alternatively, there may in fact be significant gene flow among the populations studied here, but selection has been strong enough to maintain morphological differentiation despite gene flow (Orr & Smith 1998, Rice & Hostert 1993). This explanation can only be tested using polymorphic markers (such as microsatellites, Bruford and Wayne 1993) which can accurately measure gene-flow in recently diverged populations.

Some studies have shown that morphological variation in size is likely to be environmentally influenced (Atchley 1983; Daly 1985). However other lines of evidence suggest that much morphological variation is under genetic control (Margaritopoulos *et al.* 2000; Bilton 1993; Kharuzy 2000). Although discriminant function analysis on distance measurements can maximize the morphological variation among populations, it fails to address the question of partitioning size and shape within populations (Gillham & Claridge 1994). In order to fully understand the geographical variation it is therefore important to obtain as much information as possible on the molecular phylogeography of taxa

under study (Gubitz *et al* 2000), which makes it possible to posit hypotheses in order to test the importance of selection and divergence in allopatric populations as reasons for geographic variation in morphology (Puorto *et al.* 2001; Malhotra *et al.* 1996). *Sycophila* populations showed great genetic diversity in this study from which one may infer that the host (*Tetramesa*) populations are also genetically different. The results obtained in this investigation indicate that application of morphometric and phylogeographic studies may give rise to a clearer picture and provide the background in terms of revaluating the taxonomic position of cryptic and closely related species of Hymenoptera. This is especially vital for the herbivores and parasitoids used in biological control policies in Integrated Pest Management (IPM) programmes to control insect pests. One explanation for the observed geographical divergence in morphological characters among two populations of *Tetramesa* occurring in the northern and southern regions of Iran is that they are not necessarily homogeneous morphological and genetic entities and represent two geographical host-adapted forms. It is possible that host-adapted characteristics that evolve in allopatry are more effective in isolating populations than characteristics that evolve in sympatry and there are many who feel that geographic isolation is necessary for divergence (Mayr 1963). It is therefore possible that these morphologically similar, host-adapted geographical forms of *Tetramesa* may be considered as an example of superficially cryptic allopatric speciation in insects.

There are several potential reasons why *Tetramesa* populations did not show genetic variation in this study and these remain unresolved. For example, a recent adaptive radiation to different host plant species may have occurred more rapidly than could be tracked using a molecular marker such as the Mt CO I

gene, less likely, a recent population bottleneck could have eliminated neutral genetic diversity in this species, alternatively extensive gene-flow between populations could have resulted in the complete homogenization of alleles. As *Tetramesa* is highly vagile (H. A. Dawah Pers. Communication), the existence of gene flow between populations could potentially swamp any localised selective forces. These explanations should be considered with caution until future studies of polymorphic markers have been carried out.

Tetramesa species and their parasitoids may be able to play an important role in ecological studies. Understanding the community structure of herbivorous insects and their parasitoids is important because insect herbivores comprise a major component of the global animal biodiversity, representing approximately 57% of 1.75 million described species (See Price 2002); herbivores also have significant impacts on productivity, decomposition, nutrient cycling and other important ecosystem-level processes (see Kremen *et al.* 1993); and many insect herbivores are highly mobile, and may therefore be expected to migrate rapidly in response to shifting climate zones. Understanding the mechanisms underlying host-parasitoid interactions depends on the resolution of taxonomic problems, because of the presence of cryptic species within herbivores and parasitoid wasps. However knowledge of the evolutionary relationships among coexisting species is also of major importance. Studies have emphasized in using phylogenies in community ecology (Losos 1996; Thompson *et al.* 2001) and increasing interest in the role of history on contemporary ecological community structure (Brooks & McLennan 1991; Greene 2005; Vitt & Pianka 2005). Accordingly the interaction of phylogeny with community ecology has been noted (Brooks & McLennan 1991, 2002; Grancolas 1998; Nel *et al.* 1998).

The dissection of *L. pseudosclerophylla* stems from southeast and southwest populations and *L. sclerophylla* from northern populations showed that *Tetramesa* spp. is the only hymenopteran herbivore of these two grass species. The parasitoids attacking *Tetramesa* were both primary parasitoids and hyperparasitoids, all were idiobionts and all hyperparasitoids reared were facultative. Polyphagous parasitoids attack different host species and are common on most hosts (Askew 1980: Jervis 1980; Hawkins & Goeden 1984; Dawah *et al.* 1995) and the polyphagous species associated with *L. pseudosclerophylla* and *L. sclerophylla* were *Chlorocytus* sp. and *M. vesicularis*.

The result of this study showed that local species richness is proportional to regional richness (type I; See page ...), consistent with finding of Dawah *et al.* (1995) and Cornell (1985). They found that the species that occur on the host regionally will also be found everywhere host populations were present. Different factors may affect species richness and the number of insect species associated with host plants is expected to correlate with whether the plants are common and widespread or local and rare (Southwood 1984). In this study it was found that *Pediobius* sp. attacking *Tetramesa* feeding on *L. pseudosclerophylla* and *L. sclerophylla* were found in the southwest and northern Iran but not from the southeast Iran. So why do similar habitats in different regions have different number of species? One possibility is that the different histories of the areas have led to occupancy by different clades or species groups (Latham & Ricklefs 1993; Schluter & Ricklefs 1993; Qian & Ricklefs 1999; Ricklefs 2002) and that different clades have different potential for diversification (Farrell *et al.* 1991; Sanderson & Donoghue 1996; Dodd *et al.* 1999; Gardezi & da Silva 1999) and hence the different numbers of extant species. Another possibility is that the areas

differ in the length of time they have been occupied (Brown *et al.* 2000; Ricklefs 2002). Although *Tetramesa* are found everywhere their host exists, since not every species can live everywhere, and chance or isolation can exclude others, it should not be expected that every species found in a regional pool will be found in every local community (Colywan 2003; Lawton 1999;; Zobel 1992).

My study showed that most local parasitoid communities have consistent dominance hierarchies (Figures 5.23 and 5.24, chapter 5), agreeing with the findings of Dawah *et al.* (1995). In community ecology the composition of local-scale communities depends upon the regional species pool (Morin 1999) and the composition of the regional species pool is, in turn, determined by large-scale biogeographical patterns (Ricklefs & Schluter 1993; McPeek & Brown 2000). Investigating how present-day terrestrial insect assemblages are structured along an environmental gradient and on different host species, will help to use this information to develop hypothesis as to how community structure and composition may change in the future.

It is predicted that climate change will have significant impacts on the distribution, phenology and abundance of many species over the next decades (Root *et al.* 2003). Climate change can give rise to changes in community structure, and may also result in extinctions for some species (Bale *et al.* 2002; Thomas *et al.* 2004). For instance, it is not clear whether the present-day community interactions, based on the semi-quantitative food web constructed in this study, is similar to that of the interactions in the past. Recent climatic changes such as global and/or regional warming have already affected the distribution and abundance of many taxa (e.g. Parmesan 1999; Root *et al.* 2003) due to differential response rates which may give rise to a decoupling of present

day ecological interactions, the establishment of new relationships and eventual drastic changes in the structure and composition of present-day communities (Harrington *et al.* 2001; Voigt *et al.* 2003; Hughes 2000).

Molecular data provide a very useful independent source of information with which to test evolutionary hypotheses since characters are strictly heritable, unambiguous, homologous, and more amenable to quantitative treatments (Graur & Li 2000) and are now often considered important criteria to resolve taxonomic difficulties associated with species complexes (for example see Starkey *et al.* 2003; Kai *et al.* 2002; Zink *et al.* 2002). Molecular markers have increased our understanding of the genetic structure of populations and studies apply different analytical techniques to gain more information from genetic data (Sunnucks 2000), especially important since natural populations often have complex geographies and histories (Hey & Machado 2003).

Systematics and population genetics have been used common molecular methods to test hypothesis in molecular systematics and evolution. However, hymenoptera show very little variation in allozymes, maybe as a result of haplodiploidy (Crespi 1991; Blanchetot & Packer 1992). Although some studies had been carried out on the taxonomy and biology of *Sycophila* sp. associated with grasses in other parts of the world, traditional methods were not able to resolve the taxonomic situation of this species complex (Bouček 1974; Burk 1971; Zerova 1989; Anga 1991) and very few molecular studies have been carried out.

DNA sequencing has become the most popular source of data for phylogenetic reconstruction (Hoy 1994) and can provide significant information about phylogenetic relationships and phylogeographic patterns in closely related

insect species that cannot be inferred from morphology (Parker *et al.* 1998; Simon *et al.* 1994; Zhang & Hewitt 1996; Emerson *et al.* 2001). Nowadays phylogeographic studies are becoming increasingly used in Hymenoptera (e.g. Rokas *et al.* 2003) and this approach yielded significant genetic differentiation among the geographical populations of *Sycophila* studied. In phylogeographic studies it is important to explore and separate contemporary processes such as restricted gene flow, secondary contact and hybridization from the historical events such as past fragmentation and range expansion (Zink 1994; Templeton *et al.* 1995).

The molecular data from the mtDNA showed the existence of large genetic differentiation among *Sycophila* populations (the parasitoid of *Tetramesa* associated with the three host grass species, *L. pseudosclerophylla, L. sclerophylla and F. arundinacea*). Five genetically distinct haplogroups comprising 25 haplotypes were found. Although the occurrence of host associated sibling species in many phytophagous (e.g. two host-adapted forms of *T. hyalipennis*; see Al-Barrak *et al.* 2004) and parasitoid (e.g. *Sycophila*, Ghajarieh 2003) chalcidoid wasps has been used as evidence in support of sympatric speciation, an appreciation of the phylogenetic relationships and phylogeographic patterns observed between haplotypes of *Sycophila* spp. provides the means by which patterns of colonisation and demographic history can be inferred (DeSalle & Grimaldi 1991).

Wolbachia, which are a group of intracellular bacteria found in the reproductive tissues of many invertebrates and which are inherited almost solely through maternal cytoplasm exhibit several mechanisms that cause female biased sex ratios, including cytoplasmic incompatibility which is an important

mechanism in sympatric and allopatric speciation (Werren 1998), e.g. in *Drosophila melanogaster* (see Reed & Werren 1995; Rousset & Solignac 1995) and *Nasonia* wasps (Breeuwer *et al.* 1992). The chalcidoid wasps studied here tested positive for the presence of *Wolbachia* and populations infected with different strains or combinations of *Wolbachia* could therefore become reproductively isolated. Jeyaprakash and Hoy (2000) indicated that as many as 76% of insects and spider species could be infected (Knight 2001). If *Wolbachia* does indeed drive speciation and so many species are infected, this is a potential explanation as to why insects and spiders are amongst the most specious group of organisms. Possibly some associations of symbiotes with insects may promote speciation through effects on reproductive isolation of the host populations. A detailed study of *Wolbachia* infecting Iran's chaclidoid wasps would therefore be merited in the future.

Studies of historical biogeography are now routinely using molecular phylogenies to investigate the history of genealogical connections among regions (phylogeography; e.g. Brown & Lomolino 1998; Nelson & Plantnick 1981). In many regions of the world historical biogeographical studies are lacking and contemporary ecology may not shed light on the processes that explain the large-scale distribution of evolutionary clades in the present day, for example with the *Sycophila* sp. studied here (Wiley 1988; van Veller *et al.* 2002;Wiens & Donoghue 2004).

Testing hypotheses regarding the past status of *Sycophila* populations in Iran is problematic because first, almost all research on the climatic history of Iran and the Near East concerns the transition from the last glaciation to the

Holocene (Wright Jr *Pers. Comm.*); second, almost all the data that is available from this region has been retrieved from only two sites, Lakes Zeribar and Mirabad in the Zagros Mountains (south-west Iran). Third, for the Poaceae as a whole, their presence or absence in a region inferred from pollen studies may not reflect the presence or absence of these grasses in reality because there is no way currently to distinguish cultivated from wild grasses by pollen analysis (Wright Jr *Pers. Comm.*).

Taking into account the difficulties mentioned above, it seems likely that the biogeographic pattern in *Sycophila* haplotypes is consistent with a continuous postglacial colonization (via a stepping-stone mechanism) and range expansion by a few founder populations with serial bottlenecking processes. Although this can only be confirmed with more studies in the future, which have a wider geographical sampling. For example, although NCA was not able to detect range expansions, these may still have played an important role in the biogeographic pattern of *Sycophila*. NCA in some cases fails to detect range expansions especially in the case of recent events (Paulo *et al.* 2002), especially where sampling is insufficient.

Sycophila populations are likely to have been distributed throughout Iran and adjacent areas before the Pleistocene (during the Neogene period), but there is little information on the presence of Poaceae at that time. According to a Pleistocene allopatric speciation model, separation of populations into different glacial refugia will have generated intraspecific divergence, with isolated gene pools being protected from mixing by hybrid zones during interglacial periods (Hewitt 1996). An alternative view is that Pleistocene environmental changes inhibited allopatric speciation by repeatedly altering species distributions and

thus prevented accumulation of evolutionary changes (Zink & Slowinski 1995). These alternative hypotheses can be evaluated by reconstructing phylogenies and estimating species divergence time (Goropashnaya 2003). If we consider the sequence divergence among Hymenoptera as determined for the genus *Lasioglossum* as 2.8% per Myr (Danforth 1999), it seems that haplogroup V diverged from haplogroup I (especially from haplotypes in the south-east Iran) around 2,285,714 YBP, during the late Pliocene, (close to a species divergence time consistent with a Pleistocene speciation model). Divergence between haplotypes V and II took place around 1,892,857 YBP which coincides with the start of the Pleistocene. It is possible that this divergence happened just after a range expansion during an interglacial period, with haplogroup II being confined to northern refugial areas. In the next stage in a south-ward expansion, haplogroup I diverged from haplogroup II (especially from haplotypes in the south-west Iran) around 1,035,714 YBP. Divergence of haplogroup III from haplogroup II took placed around 1,000,000 YBP followed by divergence of haplogroup IV from haplogroup II around 857,142 YBP with both haplogroups III and IV being confined to south-west Iran (in the Zagros Mountains). The detectable demographic expansion of *Sycophila* is estimated at between 92,545-216,006 YBP. Before these dates, there was a glacial period when the climate was colder and although grasses were present (Wright Jr & Thorpe 2003; Wright Jr *Pers. Comm.*) most *Sycophila* populations might have been confined to refugia in the restricted moist areas mentioned above. This explanation is based on studies (Haffer 1977; Nadler *et al* 1973) which show that populations of some bird and mammal species were restricted to moist refuge areas of the Turkey-Lebanon region in the west and along the base of the central Asian Mountains to

the east, during glacial periods (Haffer 1977). Haffer (1977) argues that many animal populations in this region experienced morphological and genetic differentiation at this time due to geographic isolation. During population range expansion, which covers the interglacial period (when the climate was warmer), grasses were ubiquitous (Wright Jr & Thorpe 2003; Wright Jr *Pers. Comm.*) and *Sycophila* populations are likely to have extended their range and occupied new areas, especially in the southeast areas where they must also have parasitized *Tetramesa* in *F. arundinacea*. After this stage and in the subsequent glacial period, populations may have become disconnected and confined to moist areas in the southwest, north and a few in the southeast. This population isolation possibly continued until the early last glacial period (70,000 YBP) when grasses were also present (Stevens *et al* 2001; Wright Jr & Thorpe 2003). The most recent population expansion is likely to have occurred from 50,000 YBP and the populations re-established and this situation continued during the transition period from the late glacial period to the Holocene. In the early Holocene, *Sycophila* populations are likely to have occupied a greater area in different environments when grasses provided around 50% of the pollen recorded from 11,000 to 7,000 YBP (Stevens *et al* 2001) a situation which continues to the present date. The present geographic distribution of *Sycophila*, NJ and ML trees, and the MSN network indicate that the southern haplotypes which comprised haplogroup I may represent a recently diverged lineage and alludes to the presence of cryptic taxa. The four other haplogroups from the north and south-west seem likely to be more anciently diverged lineages that survived in isolated refugial areas during the glacial period. Unfortunately, although Iran is an important center of diversity for several taxa, there is little information about

intra-specific genetic diversity in this region to provide comparisons (Rokas *et al.* 2003).

The presence of high sequence divergences between five haplogroups implies that *Sycophila* may comprise a complex of taxa and several evolutionary lineages whose taxonomic status is unclear. However it is difficult to provide a definitive resolution to this issue because of lack of information on morphological characters and their separation. 'Species' have been regarded as the fundamental units of analysis in areas of ecology and biogeography (Riddle & Hafner 1999). Species identification is essential for distribution and habitat studies (Pilgrim *et al.* 2002), is important in determining biological diversity, and also constitutes a framework for conservation strategies (Zehr & Voytek 1999). However defining a "species" or even "subspecies" is problematic and there are controversies to identify them as either taxonomic or evolutionary entities (Templeton 1989). A complete taxonomic revision of many insect taxa at different levels of hierarchy (family, genus, and/or species level) (Brunner *et al.* 2004; Gentile *et al.* 2002) is required in insect phylogenetic studies. According to the phylogenetic species concept (Cracraft 1989; Packer & Taylor 1997) all five haplogroups could, in principle, be considered as phylogenetic species.

At present several factors decrease global species biodiversity, such as anthropogenic climate change which have resulted in perceptible changes in plant and animal characteristics (Root *et al.* 2005); destruction of natural ecosystems such as grasslands and forests (Hopkins 2005); sensitivity to temperature (Thuiller *et al.* 2005) and over the last century species extinctions have increased about 1,000 times their normal level (Hopkin 2005). Because of these difficulties, scientists are attempting to measure biodiversity at the taxon level (Purvis &

Hector 2000) to understand species vulnerability and to allow projections of the future distribution of species under different climatic conditions (Thuiller *et al.* 2005). Although parasitic Hymenoptera are tiny in size and are not often considered worthy of conservation, insects as a whole are a major components of the ecosystem worldwide (Erwin 1986). Not only endangered and threatened species (and habitats), but also native natural enemies are considered in conservation decisions which are important for pest control or population regulation. Urbanization and agriculture by humans are the major factors contribute threaten to native insects (Howarth 1991). Insects are ideal subjects with which to investigate theories in relation to conservation biology.

One critical issue in conservation biology is to define population units for management strategies. Since management is dependent on identification of taxonomic units in their current state, a distinction between evolutionary significant units (ESUs) and management units (MUs) was suggested by Moritz (1994a): ESUs are defined as populations of individuals that are monophyletic in mtDNA yet may differ from another in allele frequencies at nuclear loci and are now being widely applied in conservation policies (Riddle & Hafner 1999). With regard to conventional taxonomy they often correspond to species or subspecies boundaries, but in some situations can extend to isolated populations (Karl & Bowen 1999). MUs differ significantly in allele frequencies at both mtDNA and nuclear loci, regardless of the phylogenetic distinctiveness of those alleles. The phylogenetic species concept has been successfully applied in identifying conservation units of various species, for example in the endangered tiger beetle *Cicindela dorsalis* Horn (Vogler *et al.* 1993) where mtDNA and allozyme variation placed populations into evolutionary distinct units and showed that

differences between subspecies were equivalent to those between related *Cicindela* species. The genetically divergent haplogroups of *Sycophila* studied here could also potentially be recognized as evolutionary significant units regardless of their taxonomic status. However, identifying the haplogroups as species, subspecies or ESUs could be problematic without having knowledge and evidence from other disciplines (Kelt & Brown 2000; Goldstein et al. 2000; Moritz 1994b).

As the genus *Sycophila* is reported here from Iran for the first time, morphological analyses have not been carried out within this genus, and there are no known diagnostic characters to support the proposed evolutionary taxa. Complementary data from nuclear molecular markers, alternative mtDNA genes, morphology, ecological studies of host-parasitoid interactions and biogeography are therefore necessary to corroborate the taxon limits that have been suggested by mtDNA here (Leache & Reeder 2002; Hewitt 1998; Hare 2001; Roderick 1996).

6.3 Future studies

The taxonomic situation of the chalcid wasps studied here especially *Sycophila* and its five mitochondrial haplogroups remains unclear, and a taxonomic revision of these groups is necessary. It is interesting that new taxa have been hinted at in this molecular study, but molecular phylogenetics needs to be accompanied with data from morphology, morphometrics, host-associated interactions and data from other mitochondrial and nuclear markers in order to verify the presence of new taxa. In addition a cytotaxonomic study (Karyology) would be a potentially useful tool since this approach has successfully discriminated sibling species in the *Encarsia strenua* species-group (Giorgini &

Baldanza 2004). This method may be especially useful when there is little sequence divergence in mitochondrial DNA among populations and this possibly could be a valuable tool for studying *Tetramesa* sp. which did not show any detectable genetic variation in the present study.

The herbivore *Tetramesa,* which is regarded as a host for *Sycophila* showed morphological variation across geographical areas using morphometrics, but did not show any genetic variation for the fragment of the mitochondrial COI gene studied here. It may be worthwhile investigating the phylogenetic relationships and phylogeographic patterns in this species and its parasitoids in the future using other mitochondrial genes and/or nuclear markers, because these studies are likely to be particularly useful if they include both the herbivores and parasitoids that are in the same level of ecological interaction (Brown *et al* 1994). It also would be interesting to discover if the same process of parallel cladogenesis has occurred with Chalcidoids and their associated *Wolbachia.* More detailed information on *Wolbachia* phylogeny and taxonomy may contribute to a greater understanding of the phylogeny of the host populations.

In addition, there is unfortunately no information on the genetic diversity and phylogeographic patterns in the host plant species *L. pseudosclerophylla* and *L. sclerophylla.* This would be an excellent avenue for future studies using molecular markers such as plant chloroplast DNA or the ITS region, especially since there is no information about the divergence time of the grass species studied here and this may help to explore the date of divergence between *Sycophila* lineages. In addition, information on the paleoclimate, paleoecology and paleobiogeography of these host plant species would be useful in order to detect the demographic forces that have influenced their (and their host and

parasitoids) present day genetic diversity. It is important to mention that further ecological and biological studies on other herbivores and their parasitoids from different geographical regions needs to be done, because these will prepare sufficient information about community structure which give rise to a clear understanding of host-parasitoid interactions.

Extensive samples especially from intermediate geographical areas should be collected to complete the verification of phylogeographical patterns of mitochondrial DNA observed in *Sycophila* haplogroups and for identifying other refugial areas, to detect the effect of historical processes and to explore the centers of genetic diversity. A combination of all the data mentioned above will provide a clear perspective of the diversity observed in *Sycophila* and it may be possible to relate the phylogeographical patterns observed in the network with the proposed scenarios.

6.6 References

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Vestnik zoologii, **39**(1): 65–66, 2005

UDC 595.792(55)

A NEW SPECIES OF *TETRAMESA* (HYMENOPTERA, EURYTOMIDAE) FROM IRAN

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Accepted 1 October 2004

A New Species of *Tetramesa* (Hymenoptera, Eurytomidae) from Iran. Madjdzadeh M., Zerova M., Dawah H. — *Tetramesa leucospae* Zerova et Madjdzadeh, sp. n. is described from Iran (type locality: Karadi-Chalous). New species belongs to *cereipes* group.

Key words: Hymenoptera, Eurytomidae, new species, Iran.

Новый вид рода *Tetramesa* (Hymenoptera, Eurytomidae) из Ирана. Мадждзадех М., Зерова М., Давах Н. — Описан новый вид *Tetramesa leucospae* Zerova et Madjdzadeh, sp. n. из Ирана (типовая местность: Каради-Халуз). Новый вид относится к группе видов *cereipes*.

Ключевые слова: Hymenoptera, Eurytomidae, новый вид, Иран.

Tetramesa leucospae Zerova et Madjdzadeh, sp. n.

Material. Holotype ♀, Iran, Karadi-Chaluos, RD, 14.03.1999 ex *Leucospa sclerophylla* (Poaceae), leg. Madjdzadeh; paratype ♀ with the same label but 9.03.1999. Holotype and paratype in collection of Schmalhausen Institute of Zoology National Academy of Sciences of Ukraine (Kyiv).

Female (fig. 1). Body length 3.8–4 mm (holotype 3.8 mm). Body slender, black; gaster long. Antero-lateral edges of pronotum with large yellow spots. Mandibles and palpi dark; tegulae black, venation dark-yellow; disc of fore wing hyaline; all coxae black, hind femora predominantly black, fore and mid femora yellow at base and at tip, dark-rufous in the middle; tibiae and tarsi yellow except fifth segments of all tarsi which are brown; scape, pedicel and funicle black. Vertex and dorsum of thorax finely reticulate. Head and thorax almost bare.

Head from above stout, only slightly wider than pronotum; POL : OOL as 14 : 9; in frontal view head about 1.25 times as wide as high. Eyes black, bare. Malar space almost as long as height of eye; genae slightly curved. Lower margin of clypeus very slightly produced, weakly curved; tentorial pits distinct; supraclypeal area vertically striate. Face provided with a smooth oblong elevation in the middle. Antennae inserted above the mid-point of face; antennal scape long, reaching lower margin of median ocellus, 2.3 times longer than pedicel; the latter nearly twice as long as wide. Antenna with one distinctly transverse anellus; first flagellar segment elongated, 2.6 times as long as wide, 2nd and 3rd segments slightly longer than wide (length to width as 10 : 7); 4th and 5th segments somewhat shorter than 3rd or 2nd; 6th segment appears separated from adjacent segments, 7th segment closely applied to 8th. Rhinaria well developed; 1st flagellar segment with short hairs, segments 2nd to 6th with rather long hairs.

Mesosoma somewhat flattened dorsally; pronotum slightly more than twice as wide as long, reticulate all over. In lateral view, mesepisternum without angle anterior to mid coax. Scutellum as long as mesoscutum. Propodeum heavily rugulose, with distinct

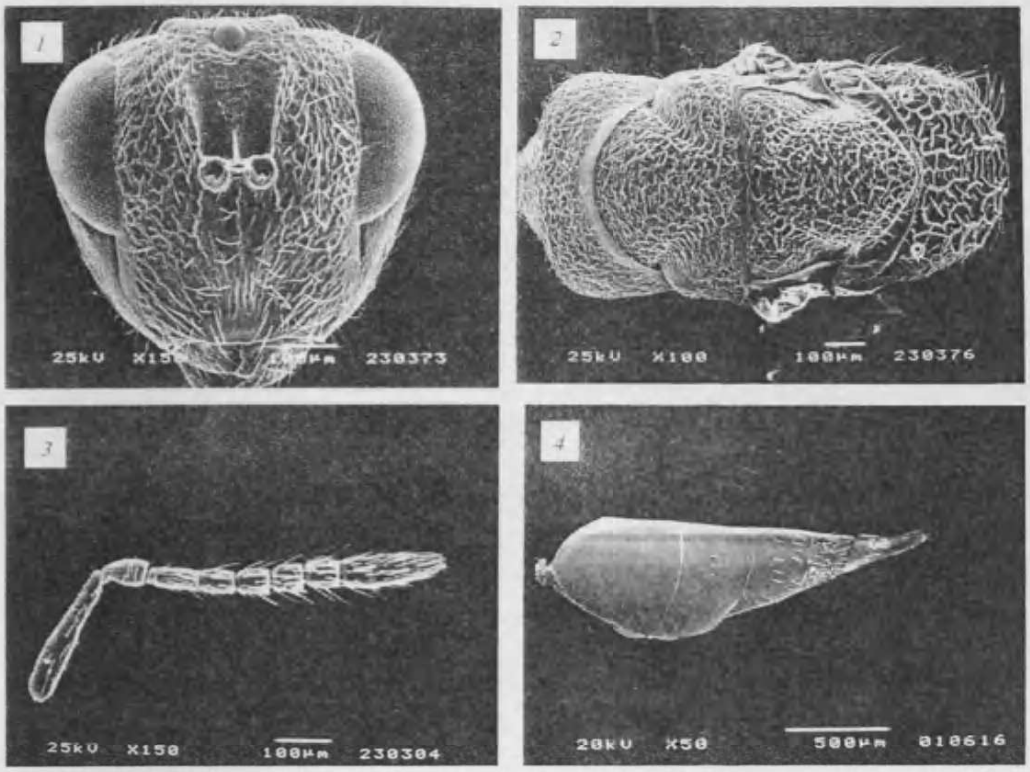

Fig. 1. *Tetramesa leucospae*: *1* — head, frontal view; *2* — mesosoma; *3* — female antenna; *4* — metasoma.
Рис. 1. *Tetramesa leucospae*: *1* — голова, вид спереди; *2* — мезосома; *3* — усик самки; *4* — метасома.

impressed medial furrow. Fore wings clothed with very short sparse hairs; marginal vein as long as postmarginal vein which is a little longer than stigmal vein. Hind coxae small, very finely reticulate.

Metasoma elongated, almost twice as long as mesosoma; petiolus in lateral view very short, lamelliform. Fourth gastral segment is the longest. Fifth to 7th segments with alutaceous sculpture. Ovipositor sheaths slightly projecting.

Male unknown.

Discussion. The new species most closely resembles *T. cereipes* Erdös, 1955, differing in shorter flagellar segments in female, more distinct sculpture of face, longer abdomen and sculpture of propodeum with median carina.

Remarks. In addition to specimens reared from stems of *Leucospa sclerophylla*, we examined also some specimens reared from another species of *Leucospa* (♀, Iran, Rabour, Kerman, 30.03.1999, ex *Leucospa pseudosclerophylla*, leg. Madjdzadeh; ♀ with the same label but 1.04.1999; ♂, Iran, Sirch, Kerman, 22.03.1998, ex *L. pseudosclerophylla*, leg. Madjdzadeh; ♂, with the same label but 23.03.1998).

These specimens differ from the type specimen by some shorter 3–5 flagellar segments in female. This material is believed to represent another form of the same species — *T. leucospae* but only molecular analysis could support or disprove this. Currently, we refer only the Northern population reared from *Leucospa sclerophylla* to the new species.

Erdös J. Studia Chalcidologica Hungarica // Ann. Hist.-Natur. Mus. Nation. Hungaricas (SN). — 1955. — 6. — P. 285–300.