

## Discrimination between Two Geographical Biotypes of the Aphid Parasitoid, *Aphidius matricariae* Hal. (Hymenoptera: Aphidiidae).

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### ABSTRACT

Cereal aphids are the serious pests attacking cereal crops, particularly wheat and barley in Egypt. The aphid parasitoid *Aphidius matricariae* is widely distributed in almost all the Mediterranean countries and has a wide range of hosts in agro-ecosystems. The morphological, biological and electrophoretic analysis of protein parameters were used for discrimination between the two geographical biotypes; the native (Egyptian) and exotic (Syrian) biotypes of *A. matricariae*. No significant differences were found in almost all the measurements and biological data between the two biotypes. Cross mating experiments between the two biotypes succeeded in all cases to reproduce fertile offspring able to continue for successive generations, confirming that the two biotypes belong to the same parasitoid species, *A. matricariae*. Exotic biotype contains more protein contents than the native biotype. The slight differences between the two biotypes may be due to the environmental differences in their two geographical habitats.

**Key Words:** Cereal aphids, morphology, biology, biotypes, *Aphidius matricariae*.

### INTRODUCTION

Aphids are one of the insect groups whose economic importance increases with the development of agriculture (Stary, 1976). Cereal aphids are the serious pests attacking cereal crops, particularly wheat, barley and corn not only in Egypt but also in many other countries; i.e. Southern Russia, Iran, Afghanistan, and countries bordering the Mediterranean Sea (Kindler *et al.*, 1991).

Importation and colonization of natural enemies associated with cereal aphids has great potential as an effective method for their control. Aphid parasitoids are one of the groups of which utilization in biological control has given significant results in many countries. (Stary, 1976).

Through the Egyptian-American collaborative project, Agricultural Technology Utilization and Transfer (ATUT), "Wheat crop protection based on plant resistance and biological control" which was carried out during 1997-2001, certain cereal aphid exotic parasitoids were imported from different countries to provide additional mortality factors to the indigenous ones, against key cereal aphid species in Egypt as well as in the US (Final report of project, 2001). The survey for such included Syria, Morocco, and Iran, in localities near the reported areas of the origin of key cereal aphid species and from habitats of climatic patterns similar to those in Upper Egypt, was carried out to search for effective parasitoid species or biotypes (Gonzalez, *et al.*, 1979&1980). Different parasitoid species attacking pests in different geographical areas and also, genetic, behavioral, and bio-systematic differences among populations of parasitoids described under one species name were found (Gonzalez, *et al.*, 1980&1990; Unruh *et al.* 1989). *Aphidius matricariae* Haliday (Hymenoptera: Aphidiidae) was imported from Syria for evaluation showed high potential under laboratory and field conditions against cereal aphid species in Egypt. It is noteworthy to mention that this species has been recorded by several authors as an indigenous parasitoid against different aphid species in Egypt. Identification of the native and exotic biotypes of *A. matricariae* was confirmed by Dr. P. Stary, Institute of

Entomology, Academy of Science of the Czech Republic.

*A. matricariae* is widely distributed in almost all the Mediterranean countries and has a wide range of hosts in agro-ecosystems; *Aphis affinis* Del Guercio, *A. craccivora* Koch Iraq, *A. fabae* Scopoli- Italy, and Cyprus, *A. gossypii* Glover - Italy, *Myzus persicae* Sulzer - Algeria, Portugal, Corse, France, Italy, and Israel, *Rhopalosiphum maidis* Fitch - Gruzia, *R. padi* Linne - Portugal, and *Schizaphis graminum* Rondani - France (Stary, 1976), and on cereal aphid species; *R. padi*, *S. graminum*, and *R. maidis*, in Egypt (El-Heneidy *et al.*, 2001).

The objective of the present work is to study the difference between the two geographical biotypes; the native and exotic of the parasitoid *A. matricariae*. Morphological, biological and electrophoretic analysis of protein parameters were used for discrimination.

### MATERIALS AND METHODS

#### Host and Parasitoid Stock Cultures

Detailed rearing conditions and methods for the laboratory stock cultures of both the host; *Rhopalosiphum padi* and the native and exotic biotypes of its parasitoid, *A. matricariae* were described by Adly (2002). Both the aphid species *R. padi* and its native biotype of *A. matricariae* were collected from wheat fields in Egypt, while the exotic biotype was collected from Abo-Kamal region, Syria (the border between Syria and Iraq).

#### Cross Mating

To ensure that the two tested biotypes, the Egyptian (native) and the Syrian (exotic) belong to the same identified parasitoid species *A. matricariae*, an experiment was carried out under the laboratory conditions of (20±1°C), photoperiod L:D 16:8 and R.H. 50-70%. Couples from unmated females from the exotic and males from the native and vice-versa of both biotypes were allowed to mate in glass vials for 24 hours (n=15) and provided with droplets of honey as excess of food. Mated females of each parasitoid biotype, native and exotic were provided individually with 100-120 nymphs of *R. padi* (2nd and 3rd instars) on wheat plants.

cages until their death. Parasitized aphids were left on the wheat plants until mummies formation. The mummies were collected and maintained in small vials until adults' emergence. For two generations, the duration (egg to adult emergence), survival rate, emergence rate and sex ratio were recorded.

### Morphological Studies

Immature stages (egg, larval instars, pre-pupa and pupa) of the parasitoid were recognized by their morphological characteristics. The immature stages were measured and drawn by a square glass lens placed in a research microscope.

### Biological Studies

One hundred *R. padi* nymphs, (almost 2nd and 3rd nymphal instars), were placed on wheat seedlings, cultivated in small pots and kept in small cages (30 individuals/replicate and treatment  $n=30$ ). In each cage, aphids were exposed to 10 mated parasitoid females for one hour. Thereafter, parasitoid females were removed, and the cages were placed inside incubators at a constant temperature ( $20\pm1^{\circ}\text{C}$ ), photoperiod L:D 16:8 and R.H. 50-70% to determine the duration of different parasitoid stages (egg, larval instars, pre-pupa and pupa). Parasitized aphids were dissected daily by very fine needles, in a drop of Ringer's solution using a stereomicroscope.

### Mass Rearing

Exotic and native biotypes of the parasitoid *A. matricariae* cultures were mass reared separately in cloth cages by releasing mated females on wheat seedlings infested with *R. padi*. After 8-9 days, formed mummies were collected and kept in vials until emergence of parasitoid adults (Adly, 2002). Newly emerged adults from the two biotypes of the laboratory cultures were collected and kept frozen at  $0^{\circ}\text{C}$  to be used for electrophoretic analysis of protein.

### Electrophoretic Analysis of Protein

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of the native and exotic females of *A. matricariae* was used in 10% gels with the buffer system of Laemmli (1970). For the analysis, samples of protein extracts (30  $\mu\text{l}$ ) of the native and exotic females of *A. matricariae* were run in the gel with Bio-Rad (Richmond, California) molecular weight standard protein (consisting of Myosin, B-galactosidase, bovine serum albumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme with the respective molecular weight values: 205, 119, 98, 52, 36, 30 and 22 KD, respectively). The gel was stained with Coomassie brilliant blue, after removing the excess dye with acetic acid, the gel was scanned using computerized scanner (Bio-Image 1-D) to determine the number of protein bands and their  $R_f$  values.

## RESULTS AND DISCUSSION

Because of the few differences between the two biotypes of *A. matricariae*, the following description and data concluded the sharing characteristics of the both

biotypes as well as the excogitations in each biotype when found.

### *A. matricariae* Haliday 1834

#### Synonymy

*Aphidius* (*Aphidius*) *cirsii* Haliday 1834. *Aphidius* (*Aphidius*) *arundinis* Haliday 1834. *Aphidius phorodontis* Ashmead 1889. *Aphidius chrysanthemi* Marshall 1896. *Aphidius polygoni* Marshall 1896. *Aphidius lychnidis* Marshall 1896. *Aphidius valentinus* Quilis 1931. *Aphidius affinis* Quilis 1931. *Aphidius arundinis* Haliday var. *obscuriforme* Quilis 1931. *Aphidius discrytus* Quilis 1931. *Aphidius merceti* Quilis 1931. *Aphidius baudysi* Quilis 1931. *Aphidius renominatus* Hincks 1943. *Aphidius nigrileteus* Smith 1944. (Stary, 1976) and *Aphidius matricariae*.

#### Cross Mating

In case of exotic female with native male and native female with exotic male, in the first generation, the respective following bio-data were obtained: successful cross mating ratios were 73.33 and 60%, the durations, (egg to adult emergence) ranged 13-15 days of the both, total numbers of mummies/female (m/f) and percentage of adult's emergence were 27.7 (13-48) and 17.7 (4-29) mummies/ female, 58.28 and 72.7% and sex ratio (females: males) were 1:0.93 and 1: 1.09 on *R. padi*. Respective data, in case of mated females of the same biotype (native female + male and exotic female + male), the duration (egg to adult emergence) ranged 12-14 days in both; total number of mummies/ female(m/f), percentage of adult emergence and sex ratio were estimated as,  $110.87 \pm 54.62$ , 80.74% and 1: 0.97 on *R. padi* for the exotic,  $66.83 \pm 26.39$ , 74.36% and 1: 0.91 on *R. padi* for the native biotype, respectively. Statistical analysis showed significant difference between total number of mummies/female and percentage of adult emergence for the first generation and mated females of the same biotype. In the second generation, successful cross mating ratio was 100%, the duration (egg to adult emergence) ranged 12-14 days of the both, total number of mummies/female (m/f) and percentage of adult emergence were 25.2 (10-35) and 11.1 (3-15) mummies/ female, 61.4 and 83.2% and sex ratio (females: males) were 1:1 and 1: 1.3 on *R. padi* in case of exotic female with native male and native female with exotic male, respectively. Statistical analysis showed no significant difference between total number of mummies/female and percentage of adult emergence for the first and second generations.

### Morphological and Biological Characteristics of *A. matricariae*

#### Egg

Newly deposited eggs of both biotypes of *A. matricariae* are not obvious. One day old eggs are spherical in shape, shiny and transparent with very delicate chorions. The chorion appears with two helix layers at  $16\times40\times$ . The egg ( $< 24$  h.) measures 0.08 mm in diameter (Fig.1a). It's size increases gradually and the circumference of the chorion becomes irregular helix, measuring 0.17 mm long and 0.15 mm wide. The embryo

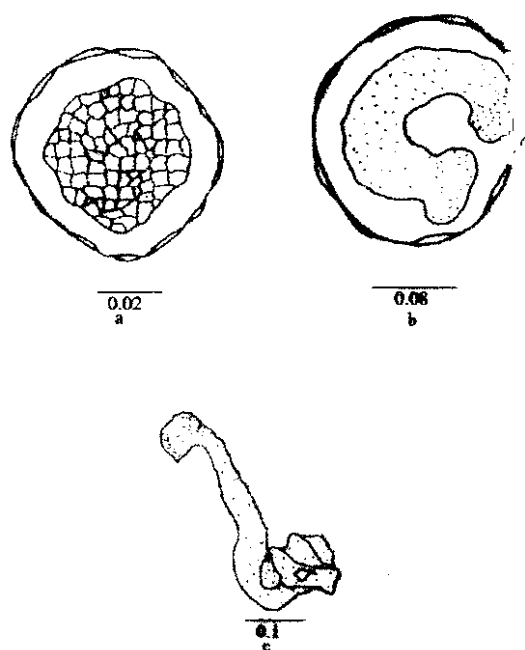


Fig. (1): *Aphidius matricariae*, egg.  
a = The egg < 24h.  
b = Egg in the late stage.  
c = Larva before hatching.

becomes clearly visible and characterizes to head and body wall, but the segmentation is not apparent inside the egg (<48 h) (Fig. 1b). Before hatching, the head is clearly differentiated to its final shape, but without any differentiation of the embryo's body. The cells of the trophamnion start to expand and continue to increase in size and number (> 48h). Hatching process has never been observed. The 1<sup>st</sup> instar larva is extruded out of its egg (Fig. 1c). Hatching is mostly accomplished by the mandibles not by the growth and activity pressure of the larva (Schlinger and Hall, 1959). The egg averages  $0.14 \pm 0.08$  mm long, and  $0.12 \pm 0.06$  mm at the greatest width (Table 1). The incubation period averaged  $2.7 \pm 0.47$  days for the native biotype, and  $2.67 \pm 0.48$  days for the exotic biotype.

### Larva

*A. matricariae* has three larval instars. Available literatures on larval development in Aphidiidae suggested different views. However, Veval (1942) and Shalaby and Rabasse (1979) mentioned four larval instars for the same parasitic species. Obtained results agreed with the findings of O'Donnell (1987a) who depend in his study on the qualitative and quantitative characters and summarized his study as follows; aphidiid larvae have three larval instars; first larval instar, second instar has two stages, early 2<sup>nd</sup> instar (1<sup>st</sup> intermediate stage) and late 2<sup>nd</sup> instar (2<sup>nd</sup> intermediate stage) and third larval instar.

### First Larval Instar

Easily recognized (Fig. 2a); caudate and mandibulate type. Body wall translucent white; consisting of head and

thirteen distinct segments. Head, relatively large and triangular, representing 1/5 of the body length and possesses a distinct mouth opening. Labium and labrum present. Mandible sickle-shaped with sclerotized tip (Fig. 2c&d). O'Donnell (1987 b) stated that mandibles are not used for feeding, but may be used for a temporarily attachment during locomotion. Chow and Sullivan (1984) mentioned that they are used for fighting supernumerary larvae. Body wall slightly curved, tapered from the middle towards apex, distinguished into three thoracic segments, nine abdominal segments and a terminal abdominal cauda (tail). The larva has ventral processes projecting from thoracic segments of the thorax during telescoping motion. These processes which disappear at the late stage of the 1<sup>st</sup> larval instar are probably excess cuticle that allow larva to grow without the necessity for too many ecdysis (O'Donnell 1987b). Metathoracic segment and all abdominal segments have irregular spines of varied lengths dorsally and generally directed posteriorly. Spines are present on the whole length of tail but concentrated dorsally (Fig. 2b). Internally, the oesophagus extends as a small tube into the body of larva. Alimentary canal is dilated blindly in the 7<sup>th</sup> segment of the abdomen (Fig. 2e). The larva averages 0.51 mm long and 0.15 mm at its greatest width; head capsule 0.11 mm long and 0.13 mm wide (Table 1); mandible of the native biotype (Fig. 2c) measures 0.036 mm long and 0.014 mm wide, at the base, opposed to 0.037 mm long and 0.017 mm wide at the base for the exotic biotype (Fig. 2d) (Table 2).

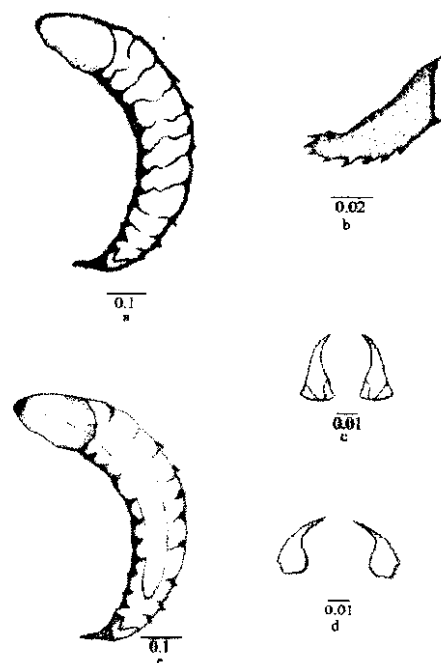


Fig. (2): *Aphidius matricariae*, 1<sup>st</sup> larval instar.  
a = The first larval instar.  
b = Tail of the 1<sup>st</sup> larval instar.  
c = Mandible of the native biotype.  
d = Mandible of the exotic biotype.  
e = Alimentary canal of the 1<sup>st</sup> larval instar.

Table (1): Averages length and width (mm) of immature stages of the parasitoid species *Aphidius matricariae*.

| Stage             | Head                        |                             | Body wall                   |                             |
|-------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                   | Long ± SD                   | Wide ± SD                   | Long ± SD                   | Wide ± SD                   |
| Egg               |                             |                             | 0.14 ± 0.08<br>(0.04-0.27)  | 0.12 ± 0.06<br>(0.04-0.22)  |
| 1st larval instar | 0.11 ± 0.008<br>(0.1-0.12)  | 0.13 ± 0.013<br>(0.1-0.15)  | 0.51 ± 0.022<br>(0.49-0.56) | 0.15 ± 0.015<br>(0.14-0.19) |
| 2nd larval instar | 0.18 ±<br>(0.14-0.23)       | 0.22 ± 0.037<br>(0.16-0.28) | 0.59 ± 0.07<br>(0.45-0.71)  | 0.23 ± 0.04<br>(0.16-0.28)  |
| 3rd larval instar | 0.64 ± 0.088<br>(0.47-0.82) | 1.06 ± 0.091<br>(0.89-1.18) | 1.15 ± 0.15<br>(0.87-1.34)  | 0.58 ± 0.91<br>(0.4-0.71)   |
| Pre-pupa          |                             |                             | 1.36 ± 0.18<br>(1.1-1.65)   | 0.57 ± 0.06<br>(0.49-0.68)  |
| Pupa              |                             |                             | 1.12 ± 0.21<br>(0.82-1.45)  | 0.45 ± 0.04<br>(0.38-0.52)  |

Table (2): Averages length and width (mm) of mandibles of larval instars of the two biotypes of the parasitoid species *Aphidius matricariae*.

| Biotype | 1st larval instar              |                                | 3rd larval instar              |                                |
|---------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
|         | Long ± SD.                     | Wide ± SD                      | Long ± SD.                     | Wide ± SD                      |
| Native  | 0.036 ± 0.002<br>(0.034-0.039) | 0.014 ± 0.002<br>(0.012-0.019) | 0.049 ± 0.003<br>(0.044-0.054) | 0.037 ± 0.005<br>(0.029-0.047) |
| Exotic  | 0.037 ± 0.003<br>(0.034-0.042) | 0.017 ± 0.002<br>(0.015-0.022) | 0.048 ± 0.004<br>(0.042-0.054) | 0.046 ± 0.008<br>(0.03-0.054)  |

Second Larval Instar

Hymenopteriform in shape. Body of early stage is translucent, slightly increasing in curvature (Fig. 3a). Head becomes nearly quadrate with an apical unvaginated mouth opening leads to long oesophagus and enlarged gut. Mandible invisible or absent and possess oral lobes. Thoracic segments with no dorsal spines, but projections are observed during telescoping movement. Abdomen has dorsal spines with small bristles. Cauda (tail) shorter than of the 1st instar, blunt and covered with spines (Fig. 3b).The late stage of this instar becomes strongly curved (Fig. 3c). Head smaller than thoracic segments, shorter and flattened interiorly, compared with the early stage (the ratio between head to body wall of the early stage is more than late stage of the same instar). Spines disappear and a well-developed nervous system is present. Cauda much reduced, with no spines. Feeding is still, by pharyngeal pumping as in the early stage. Shalaby and Rabasse (1979) studied the morphology of the immature stages of this species reported the absence of tail and the presence of mandibles during the second larval instar. Body of the 2nd larval instar measures 0.59 mm long and 0.23 mm wide (n = 25 larvae) and the head capsule 0.18 mm long and 0.22 mm wide. (Table 1).

Third Larval Instar

Hymenopteriform in shape, strongly curved (Fig. 4a) with quite short head. Mouth parts well developed and sclerotized. Mandible stout triangular, sclerotized and dense sclerotization at the sharp pointed tips. (Fig. 4b&c).

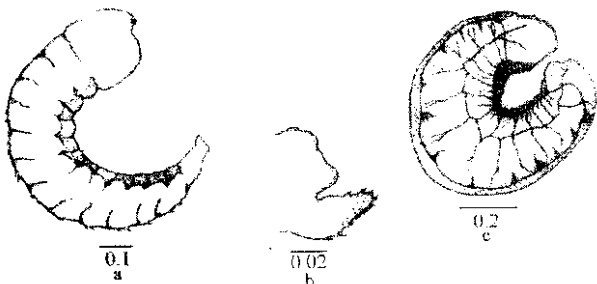


Fig. (3). *Aphidius matricariae*, 2nd larval instar.  
a = Early stage of the second larval instar.  
b = Tail of the 2nd larval instar.  
c = Late stage of the second larval instar.

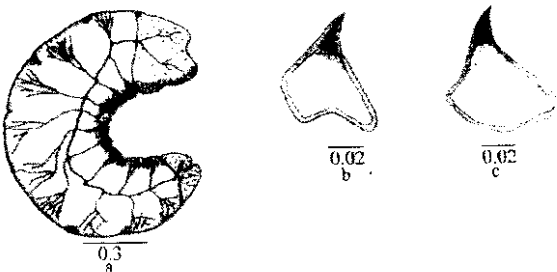


Fig. (4). *Aphidius matricariae*, 3rd larval instar.  
a = The 3rd larval instar.  
b = Mandible of the native biotype.  
c = Mandible of the exotic biotype

Thorax and abdomen are composed of nine segments, with an atrophy rounded cauda (tail) and no external appendages on abdominal segments. Nervous and tracheal systems are developed during this instar. Body of 3<sup>rd</sup> larval instar measures 1.15 mm long and 0.58 mm wide ( $n = 25$  larvae), head capsule 0.64 mm long and 1.06 mm wide (Table 1). Mandible, 0.049 mm long and 0.037 wide in the native biotype, opposed to 0.048 mm long and 0.046 mm wide in the exotic biotype (Table 2). The present results confirmed what reported by O'Donnell (1987a) that there are only three instars of *A. colemani*. Shalaby and Rabasse (1979) stated that the third larval instar of *A. matricariae* has an inconspicuous mandible, which disagreed with the present data. The average duration of the first, second and third larval instars were  $2.7 \pm 0.47$ ,  $2.53 \pm 0.51$  and  $1.4 \pm 0.5$  days for the native biotype and  $1.6 \pm 0.5$ ,  $2.77 \pm 0.43$  and  $2.77 \pm 0.43$  days for the exotic biotype, respectively.

### The Prepupa

Perpupal stage begins with the discharging of meconium. It could be differentiated from the last larval instar by the presence of the bi-lateral cephalic dark spots corresponding to the future pupal compound eyes (Fig. 5a). Average dimensions ( $n = 22$  pre-pupa) are: 1.36 mm long and 0.57 mm wide (Table 1). The average duration was  $1.17 \pm 0.38$  days for the native biotype and  $1.17 \pm 0.38$  days for the exotic biotype.

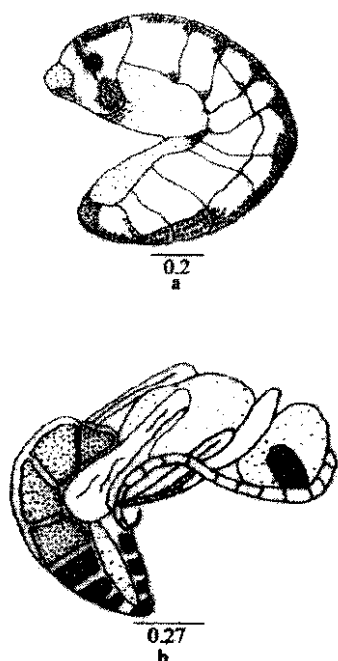


Fig. (5): *Aphidius matricariae*.

a = Pre-pupa.

b = Pupa.

### The Pupa

The pupa is a free type (exarate). Initially, it is creamy in color. Compound eyes are visible but colorless. Later, the appendages are fully formed and compound eyes are red in color and then they turn to brick red. At the end of

this stage, the pupa gradually turns darker in color and the appendages, antennae and wing pads are obviously fully formed (Fig. 5b). Average dimensions ( $n = 22$  pupae) are: 1.12 mm long and 0.45 mm wide. (Table 1). The average duration was  $5.5 \pm 0.51$  days for the native biotype and  $5.53 \pm 0.51$  days for the exotic biotype.

### Electrophoretic Analysis of Protein

Relatively few quantities and qualitative changes were seen in the electrophoretic pattern of the two biotypes (Fig. 6a&b). Twenty-three protein bands are found in the native biotype opposed to 21 bands in the exotic biotype. Bands no. 3, 11 and 12 ( $R_f$  values of 0.157, 0.406 and 0.417) were present in the native and absent in the exotic, while an extra band no. 20 (with  $R_f$  value of 0.710) was present in the exotic. The two biotypes match each other in 20 protein bands. The most important observation in the electrophoretic pattern is the increased density of most of the protein bands in the exotic biotype in comparison with the native. This increased density is much evident in bands no. 2, 10 and 18. On the other hand, three bands, i.e., no. 13, 14 and 15 showed decreased density in the exotic biotype (Table 3).

Table (3): The electrophoretic pattern of the native and exotic biotypes of *A. matricariae*.

| Bands | Native biotype |       |       | Exotic biotype |       |       |
|-------|----------------|-------|-------|----------------|-------|-------|
|       | Norm'd         | Band  | Rf    | Norm'd         | Band  | Rf    |
| 1     | 100.00         | 3.41  | 0.117 | 132.28         | 3.46  | 0.111 |
| 2     | 100.00         | 0.43  | 0.138 | 321.48         | 1.05  | 0.132 |
| 3     | 100.00         | 0.21  | 0.157 |                |       |       |
| 4     | 100.00         | 0.39  | 0.240 | 189.56         | 0.56  | 0.235 |
| 5     | 100.00         | 0.57  | 0.273 | 114.75         | 0.50  | 0.269 |
| 6     | 100.00         | 3.34  | 0.291 | 124.31         | 3.18  | 0.286 |
| 7     | 100.00         | 2.73  | 0.313 | 120.77         | 2.53  | 0.306 |
| 8     | 100.00         | 2.26  | 0.342 | 133.05         | 2.31  | 0.336 |
| 9     | 100.00         | 3.74  | 0.362 | 121.63         | 3.49  | 0.355 |
| 10    | 100.00         | 1.44  | 0.395 | 405.59         | 4.47  | 0.400 |
| 11    | 100.00         | 2.45  | 0.406 |                |       |       |
| 12    | 100.00         | 1.27  | 0.417 |                |       |       |
| 13    | 100.00         | 5.46  | 0.452 | 98.43          | 4.12  | 0.447 |
| 14    | 100.00         | 12.70 | 0.475 | 87.26          | 8.49  | 0.472 |
| 15    | 100.00         | 8.99  | 0.506 | 91.30          | 6.29  | 0.504 |
| 16    | 100.00         | 16.32 | 0.574 | 129.14         | 16.15 | 0.571 |
| 17    | 100.00         | 3.48  | 0.599 | 203.93         | 5.44  | 0.599 |
| 18    | 100.00         | 0.61  | 0.625 | 566.09         | 2.64  | 0.621 |
| 19    | 100.00         | 7.27  | 0.664 | 144.22         | 8.03  | 0.662 |
| 20    |                |       |       | 0.00           | 0.33  | 0.710 |
| 21    | 100.00         | 3.44  | 0.769 | 186.30         | 4.91  | 0.767 |
| 22    | 100.00         | 11.43 | 0.837 | 145.00         | 12.69 | 0.835 |
| 23    | 100.00         | 3.51  | 0.862 | 123.37         | 3.32  | 0.859 |
| 24    | 100.00         | 4.56  | 0.939 | 173.21         | 6.05  | 0.932 |

In conclusion, no significant differences existed between almost all the measurements and biological data of the two biotypes of *A. matricariae*. Slight differences were found in some morphological scene such as: base of mandibles, it was straight (Fig. 2c) and convex (Fig. 2d) in the first larval instar of the native and exotic biotypes, respectively. Respective bases of the third larval instar were concave (Fig. 4b) and convex (Fig. 4c). Cross mating experiments between the two biotypes succeeded

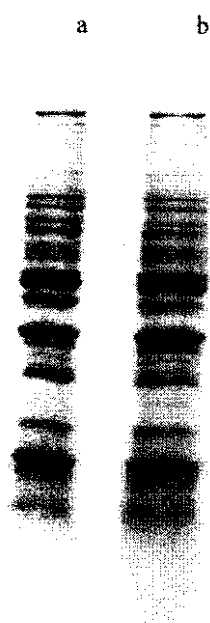


Fig. (6): The electrophoretic pattern of two biotypes of *Aphidius matricariae*.

- a: 23 Protein bands in the native biotype.  
 B: 21 protein bands in the exotic biotype.

in all cases to reproduce offspring able to continue for successive generations. Exotic biotype contains more protein than the native biotype. This protein content is expressed in the increased density of most of the protein bands (Fig. 6). Similarity in protein pattern is quantitative rather than qualitative, therefore the two different geographical biotypes may be considered as more or less (subspecies) belonging to the same species *A. matricariae*. The slight differences in the morphological, biological and the electrophoretic pattern analysis may be due to the environmental differences and geographical habitats of the two studied biotypes.

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