Rev. Chilena Ent.
2001, *28*: 39 - 46

LARVAL MORPHOLOGY OF THE PURE STRAIN Arg294 OF *ANASTREPHA FRATERCULA* (WIEDEMAN) (DIPTERA: TEPHRITIDAE) FROM ARGENTINA AND A COMPARISON WITH *CERATITIS CAPITATA*.

Delprat M. A., Manso F. C., Cladera J.L.[1]

ABSTRACT

The South American fruit fly, *Anastrepha fratercula* (Wiedeman) is an economically important pest distributed in the range from south of Texas to temperate areas in South America. In this paper, a number of characters usually employed in species determination, are described on the external morphology of larval stages of individual belonging to the genetically homogeneous lab strain Arg294. A comparison between larvae of this species and *Ceratitis capitata* (Wiedeman) is also provided here because this information may be useful for species identification in phytosanitary posts of Chile and Argentina, where they share host and economic impact.
Key words: Tephritidae, larval morphology, *Anastrepha fraterculus*, *Ceratitis capitata*, anal lobes, spiracle, sensilla, cephalopharyngeal skeleton.

RESUMEN

La mosca sudamericana de la fruta, *Anastrepha fratercula* (Wiedeman), es una plaga de importancia económica, que se distribuye desde la región subtropical del sur de Texas, hasta las áreas templadas de Sudamérica. En este trabajo, se describen distintos caracteres morfológicos de los estadios inmaduros, empleados para la identificación de especies en la familia Tephritidae, en la línea genéticamente homogénea de laboratorio Arg294. Se presenta además una comparación entre las larvas del tercer estadio de *A. fratercula* y *Ceratitis capitata*, que puede resultar de utilidad para identificar ambas especies en las barreras fitosanitarias de Chile y Argentina, donde estas moscas comparten hospederos e importancia económica.
Palabras clave: Tephritidae, morfología larvaria, *Anastrepha fraterculus*, *Ceratitis capitata*, lóbulos anales, espiráculos, sensilla, esqueleto cefalofaríngeo

INTRODUCTION

The South American fruit fly, *Anastrepha fratercula* (see Artigas, 1994), is a neo-tropical tephritidae found from southern Texas, in the USA, to Uruguay, and Argentina, in South America (Stone, 1942). In South America, where it is a pest of economic importance, can be found from subtropical northern to temperate central areas.

Early population studies on the chromosomes (Mendes, 1958; Bush 1962; Solferini & Morgante, 1987) and isoenzymes (Morgante et al., 1980; Morgante & Malavasi, 1985; Steck, 1991) of this species have been reported. In samples from northern and central populations in Argentina variations in both, chromosome morphology (Lifschitz et al., 1999) and isoenzymes (Vilardi et al., 1994) have been found more recently; variations in larval morphology have been also observed in some samples (F.Manso, unpublished).

It is not yet clear whether natural variation occurring within the species *A. fratercula* would supports the idea that a "complex" of sibling (Bush, 1962) or cryptic (Solferini & Morgante, 1987) species, may be found under such a taxon. Definitive clarification of this problem, will come only after flies from different geographical regions, are crossed to well characterised standard strains (Man-

[1]IGEAF, CICVyA, CNIA, INTA, Castelar, Argentina. E-mail: jcladera@cnia.inta.gov.ar
(Recibido: 9 de abril del 2001. Aceptado: 26 de septiembre del 2001)

so & Basso, 1999). However, experiments involving crossing of mutant traits, and chromosomal or isozymic variants, have not been reported.

A protocol for rearing individual strains of *A. fratercula* on artificial diet has been developed (Manso, 1999) making it possible to perform cross between both genetic and geographic variants. In order to initiate such laboratory studies, strains have to be reared to play the role of the genetic "wild type" strain. In this paper one reared or "standard" strains from Argentina is introduced for that purpose (Arg294).

The immature stages have been used for species identification in the family Tephritidae (Green, 1929; Philips, 1946; Berg, 1979; White & Elson-Haris, 1992) and, in the genus *Anastrepha* (Steck & Warton, 1988; Steck & Malavasi, 1988; Steck et al., 1990). It would also be helpful to have a diagnosis by which the larval stages may be separated from other *Anastrepha* (see Greene, 1929, and more details in White and Elson–Harris, 1992). So a careful description of the larva of this strain seemed an appropriate starting point.

Another tephritidae fruit pest, namely the Mediterranean fruit fly, *Ceratitis capitata* (Wiedeman) is also present in some fruit producing areas of Argentina and other South American countries, where *A. fratercula* is present. A quick way to tell apart the two larvae at phytosanitary posts has often been requested. Although the general features of *C. capitata* larva have been described (Back and Pemberton, 1918), distinctive traits have not been reported. The third larval stage of *A. fratercula* was also compared to *C. capitata* in this work, in order to find characters allowing easy separation of larvae from both species.

MATERIALS AND METHODS

Origin of strain Arg294:

Infested guava fruits (*Psidium guajava*) were collected at Ituzaingo, 25 km west of Buenos Aires, on 21 March 1994. The adults were determined to be *A. fratercula*, using a pictorial key (Steyskal, 1977). Note that, other than *A. fratercula,* no *Anastrepha* species has ever been described in this area. Positive identification was confirmed by the local expert Eng. Norma Vaccaro, INTA Concordia, Argentina (voucher specimens are available on request). The progeny of a number of pairs were obtained for different purposes, like studying if they were compatible, performing crosses between chromosome types, etc. The results of these experiment are reported elsewhere (Lifschitz et al., 1999; Manso & Basso, 1999).

After two years of rearing, one of these pair, bred as the pure strain Arg294, was used for the present study. Flies were reared in single pairs according to the previously described protocol (Manso, 1999): the adults were fed on 2:1 brown sugar: corn hydrolysed protein. Eggs were collected on artificial fruits made of 2% agar 0.2% red dye covered in ParafilmR. Larvae were fed on a carrot diet (Manso, 1999).

Observations of the morphology:

Most procedures and descriptions were done following Steck & Warton (1988). Larvae from the laboratory culture, were taken and immersed in water and heated to boiling for ~2 min. After cooling, larvae were placed in 50% ethanol (~5min), and stored in 70% ethanol for study of external structures. Then, the specimens were placed in chloride-lacto-phenol for 3 days, and mounted in Faure's medium to observe the cephalic-pharyngeal skeleton according to Steck & Warton (1988).

A sample (n=28) of 3rd stage was studied. Smaller samples (n=5 each) of 2nd stage and 1st stage were studied for comparisons with them. Measurements were taken on cephalopharyngeal skeleton (1st, 2nd, and 3rd stages), and on anterior spiracle, posterior spiracle and caudal segment (only in 3rd stage).

RESULTS

External Morphology of Late 3rd Stage

The body is elongate, 4.5 to 4.7 fold longer than wide, pointed anteriorly, with thin, smooth, integument (actual size: length=8.5 to 10mm, width=2.1 to 2.5mm). Separate and conical spinules appear distributed on all abdominal segments, in short, staggered rows. On the ventral side they occur on each abdominal segment, distributed in 8 to 9 rows along discrete fusiform areas; and in 1 row on all 3 thoracic segments. On the dorsal side they are absent.

Anterior End. On the ventral view of the head, antennal and maxillary sensory organs are located on well-developed cephalic lobes above the mouth

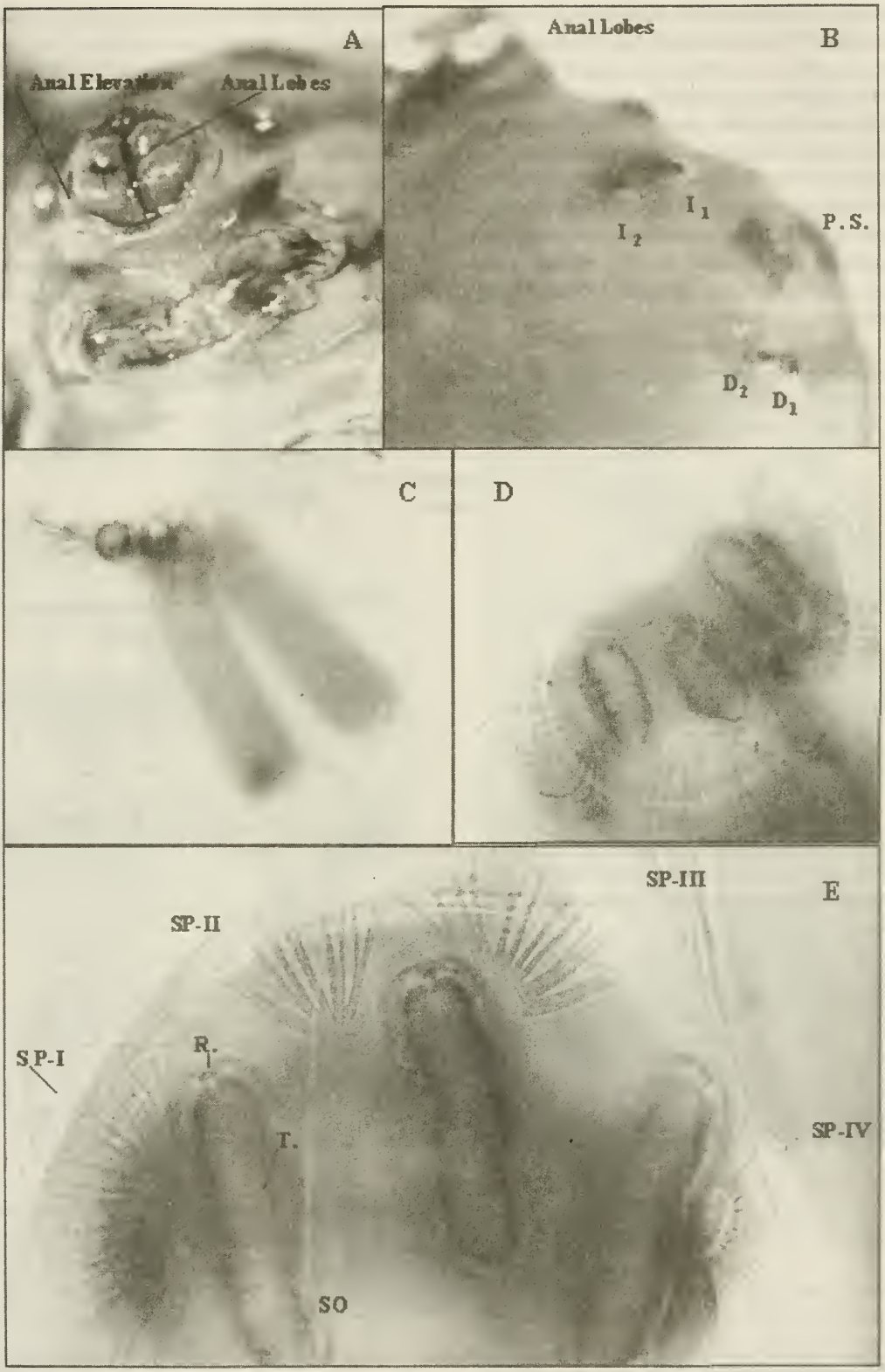

Figure 1. Cephalic lobe. A: Ventral view of head. B: Schematic. C: Oral ridges in second stage. C': Schematic. D: Oral ridges in third stage. D': Schematic. E: Anterior spiracles of second stage. F: Anterior spiracles of third stage.

hooks (Fig. 1A-B). Antennal sensory organs appear two-segmented with a sclerotized, cylindrical basal collar and apical knob-like sense organs. Maxillary sensory organs are cylindrical, apically truncate, bearing several peg-like sensillia. Stomal organs are minute clusters of sensilla (Fig. 1A-B). Oral ridges (Fig. 1D), 8-10 per side, are well developed with irregular shape. Small accessory plates are present. Anterior spiracles (Fig. 1F) with a cylindrical trunk are apically bilobed and sharply flared; 11-13 tubules per side; width of anterior spiracles: 0.21-0.23mm.

Posterior End. Anal lobes (Fig. 2A) are elevated, clearly bilobed, and encircled by 2-3 rows of spinules. Sensillia-bearing tubercles of the caudal segment are shown on Fig. 2B. We were able to distinguish 2 dorsal (D1, D2), 1 lateral (L), and 3 intermediate (I1, I2, I3) tubercles with sensilla. Intermediate sensilla I1 and I2 appear on moderately developed tubercles; remaining sensilla, on weak tubercles. Posterior spiracles (Fig. 2E) are located above the horizontal midline and have three slits, well-developed rimae and trabeculae. Slits length/ width ratio=3-4 (actual size: length:0.079-

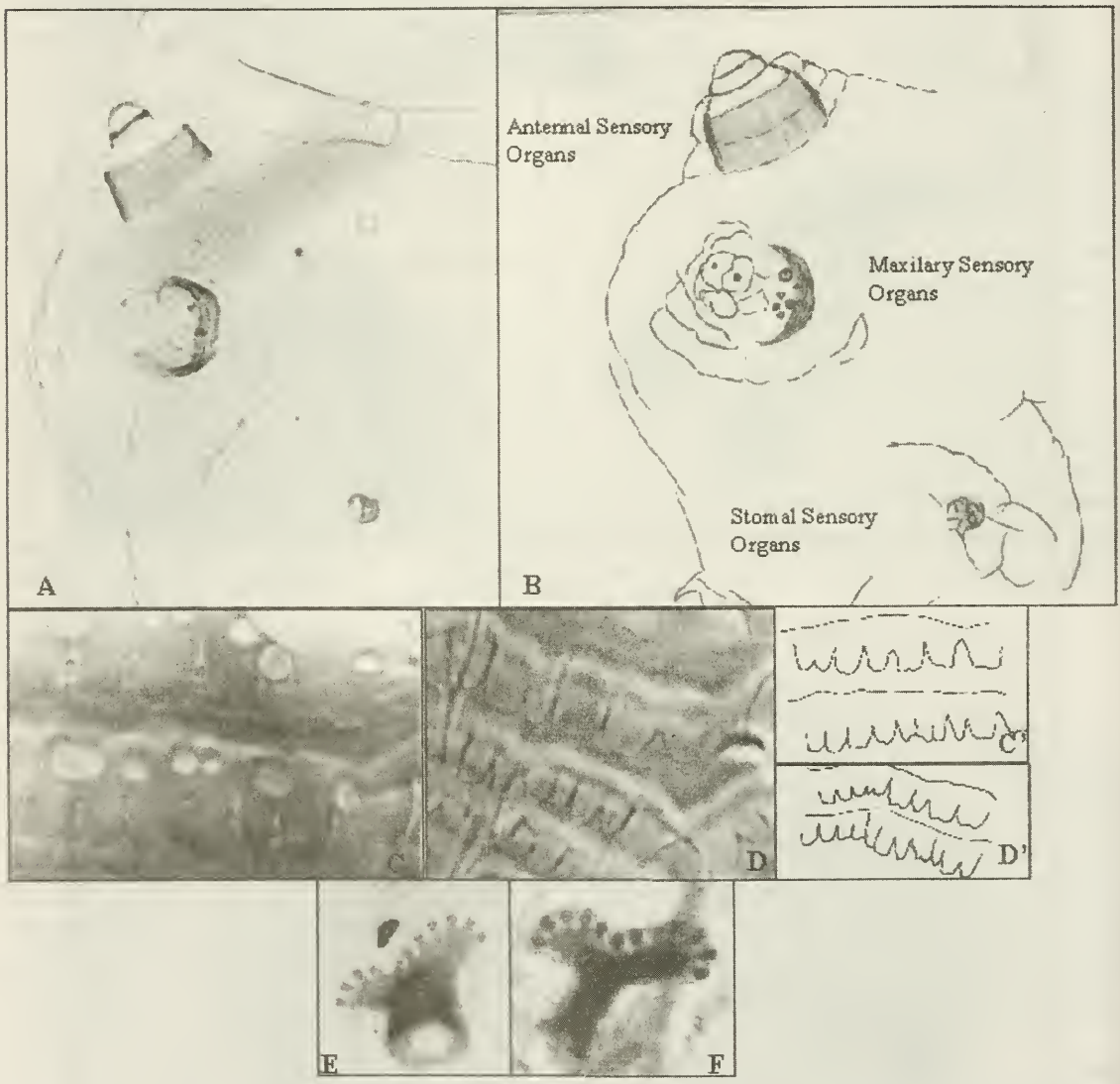

Figure 2: Anal Lobe. A: Ventral view of 8th. abdominal segment. B: Lateral view of 8th. abdominal segment (I: Intermediate tubercles, D: Dorsal tubercles, P.S.: Posterior Spiracles). C: Posterior spiracles of 1st stage larva. D: Posterior spiracles of 2nd stage. E: Posterior spiracles of 3rd stage (S.P.: Spiracular Processes; R: Rima; T: trabeculae; SO: Spiracular Opening).

0.0958mm; width: 0.021-0.029mm; n=18). Spiracular processes (Fig. 2E) are four (SP-I to SP-IV) bundles of hairs (called "trunks") with varying number of branches (called "tips") in medial and external third. SP-I and SP-IV with 11-16 trunks and 1, 2 or more than 3 tips per trunks; SP-III and SP-II with 8-10 trunks and 1 or 2 tips per trunk.

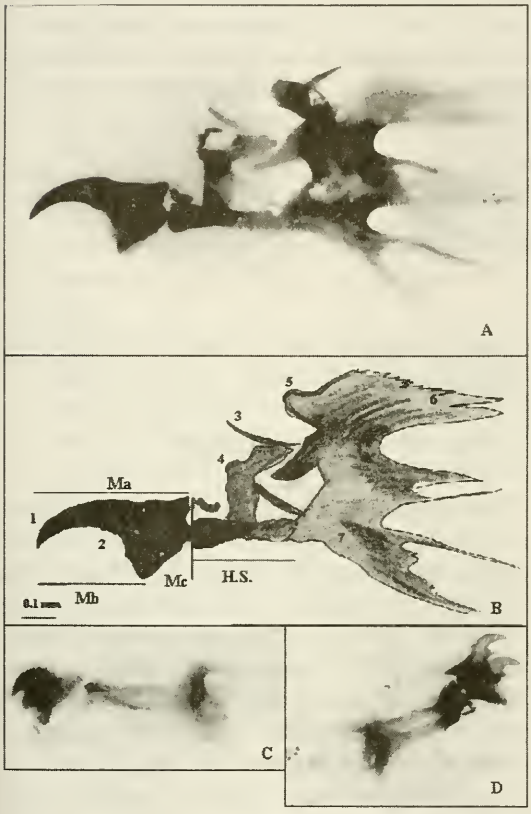

Figure 3: Cephalopharyngeal skeleton. A: Third stage. B: Schematic. C: First stage. D: Second Stage. 1: Mouthhook, 2: Preapical tooth, 3: Parastomal bar, 4: Anterior sclerite, 5: Dorsal arch, 6: Dorsal cornu, 7: Ventral cornu, 8: Epipharingeal sclerite, 9: Labial sclerite. Ma, Mb, and Mc: Mandible Measurements. H.S.: Hypopharyngeal Sclerite.

Cephalopharyngeal Skeleton of 3rd larval stage

Total length, from tip of mandible to end of ventral cornu is 1 to 1.3mm. Two separate sclerites were studied in detail, the mandible falciform and the hypopharyngeal sclerite.

The mandible falciform, (Fig. 3A-B), is heavily sclerotized, occasionally (4 individuals in 28) single-toothed, with a vestigial preapical tooth; total length (lateral view) 0.83-0.91mm. The mean values for the remainder measures were Ma=0.24mm, Mb=0.15mm, Mc=0.16mm.

Hypopharyngeal sclerite is H-shaped in dorsal view; width at bridge 0.166-0.175mm; in lateral view, length (=0.17mm) about twice height.

Remainder sclerites are: parastomal bar, anterior forks, dorsal cornu, ventral cornu, anterior sclerite, and dental sclerite. The parastomal bar is long and thin, usually bent medially, length: 0.208-0.233; the anterior forks are heavily sclerotized; the dorsal cornu is narrowly connected at dorsal bridge; the ventral cornu is trough-shaped, with 7 pharyngeal ridges; the anterior sclerite is irregularly shaped and developed; and the dental sclerite is small and inconspicuous, or absent. One reviewer of the paper pointed that the parastomal bar and the labial sclerite in Fig 3 are not in their expected location; the parastomal bar must usually be observed immediately above the hypopharyngeal sclerite, and the labial sclerite, in the ventral region of the cephalopharyngeal skeleton, between the mandibles and the hypopharyngeal sclerite.

Comparison among Larval Stages

External morphology. The oral ridges (Fig. 1C), 7-8 per side, look in 2nd stage very similar to 3rd stage (Fig. 1D), but in 1st stage they are absent. Also, in 2nd stage (Fig. 1E) the anterior spiracle is bilobed, with 10-11 tubules, but in 1st stage it is only a minute pore (not shown in figure). There are 3 spiracular openings in 2nd stage (Fig. 2D) with thinner rimae and fewer trabeculae than 3rd; length/width ratio = 2.1-2.2 (actual legth=0.13-0.14mm); spiracular processes are similar to 3rd, with 1-2 tips per trunk. In the 1st stage (Fig. 2C) there are only 2 spiracular openings with two bundles of hair.

Cephalopharyngeal skeleton. Its total length, in the 2nd stage, from tip of mandible to end of ventral cornu is = 0.51mm, in 1st stage = 0.18. In 2nd stage (Fig. 3D) Ma=0.12mm, Mb=0.09mm, Mc=0.11mm, and in 1st stage (Fig. 3C) Ma=0.06, Mb=0.04, Mc=0.06. The hypopharyngeal sclerite, in 2nd stage is =0.085mm, but in 1st stage the hypopharyngeal sclerite is fused to the pharyngeal sclerite.

The comparison among larval stages, using the various features illustrated on Figs. 1, 2, and 3, is summarised in Table 1. A rule of thumb: 1st stage, anterior spiracles are absent and posterior spiracles show only two openings, whereas in 2nd stage an-

TABLE 1: COMPARISON AMONG THREE LARVAL STAGES OF *ANASTREPHA FRATERCULA* (WIED.).

	Third Stage	Second Stage	First Stage
Anterior Spiracles	Bilobed	Bilobed	A minute pore
Number of tubules	10-13	10-11	-
Oral Ridges	8 to 10	7 to 8	Absent
Mouthhook			
Sclerotised	Heavily	Weakly	Weakly
Preapical tooth	Occasional and vestigial	Small	Big
Hypopharyngeal Sclerite	Heavily sclerotised	Sclerotised	Weakly sclerotised
Anal Lobes	Bilobed	Bilobed	Entire
Posterior Spiracles	3 openings	3 openings	2 openings
Spiracular Hairs	4 bundles of branched hairs in variable number	4 bundles of fewer number of branched hairs than 3rd	2 bundles with 2 or 3 hairs per bundle

terior spiracles are present and posterior spiracles show three openings. To distinguish 2nd from 3rd stage is more difficult. Although the mouth hook is amber in 2nd and heavily sclerotized in 3rd stage, for a safe distinction, a dissection of the cephalophryngeal skeleton may be necessary to visualise the mandible shape, showing the well-developed subapical tooth in 2nd stage which is absent or only vestigial in 3rd stage (see Fig. 3A-D).

Differences with the Larva of *Ceratitis capitata*

The general aspect does not help very much. The size of a well nourished *A. fratercula* larva is about 25% larger than *C. capitata*. Although larval color is very often dependent on diet, in our hands, rearing both species on the same carrot-based diet, towards the end of the 3rd stage, *A. fratercula* is yellowish-amber colored and *C. capitata* is white.

However, there is one key feature of the external morphology: On the posterior end of *C. capitata* there are two large rounded tubercles, with a row of small spinulles on top, visible to the naked eye. This structure is not present in *A. fratercula*. (According to Maitland, 1992, this is the place where the larva of the *C. capitata* hooks up for the jump)

After dissection of the cephalopharyngeal skeleton, the epipharyngeal and labial sclerites, obviously, present in *A. fratercula* (see Fig 3), are absent or weakly sclerotized in *C. capitata* (a good diagram of the latter was published by Back and Pemberton, 1918).

DISCUSSION

There are no previous descriptions of the larval morphology of samples of *A. fratercula* from Argentina for comparison with the present work. The reported geographic origin of the specimens previously utilised was: "undetermined" (Green, 1929; Berg, 1979); Ecuador (White & Elson-Harris, 1992); Mexico, Costa Rica, Venezuela and Brazil (Steck et al., 1990). Even though the presence of subtle morphological differences among samples of different origins may argue in favour of some differentiation along the range of this species, the existent evidence in favor of this is scant.

The study of a genetically homogeneous strain provided here the opportunity to test the taxonomic value of some traits. For instance, the occasional occurrence in Arg294 of a vestigial preapical tooth makes this trait less useful to separate the larva of this species from that of *A. serpentina*, that doesn't show a preapical tooth (Berg, 1979; White & Elson-Harris, 1992). Something similar happens with the anal lobes, supposedly "lobed" in *A.fratercula* and "bilobed" in *A.serpentina* (Berg, 1979; White & Elson-Harris, 1992). Another consideration is that specimens are not symmetrical; for instance, the number of oral ridges and of anterior spiracles tubules are not always the same on left and right sides, not even in a pure strain as Arg294.

Basically, the features of Arg294 agree with previous descriptions of *A. fratercula* 3rd stage (Berg, 1979; Steck et al., 1990; White & Elson-Harris, 1992). However, there are some noticeable exceptions in the number of tubules in anterior spiracles, the spiracular processes, the arrangement of sensilla on caudal segment, and the anal lobes.

1. The number of tubules in anterior spiracles have been ranged between 14 and 18 (White & Elson-Harris, 1992) whereas in Arg294 we found anterior spiracles with only 11-13 tubules (see Fig. 1F), about the same range (9-13) as Steck et al. (1990).
2. Spiracular processes of the posterior spiracles have been described as "bifurcados" (Berg, 1979). In Arg294 these processes (see Fig. 2E) showed 2 ramifications, but also none, 3, or more.
3. No variation in the arrangement of sensilla on caudal segment depicted in Fig. 2B was observed among 28 individuals of Arg294. It must be stressed however that some variation has been observed in our lab on larvae from northern origin (not shown). This deserves further investigation.
4. The anal lobes have been described in *A. fratercula* as entire by Green (1929) and Berg (1979); as bifid or entire by Steck et al. (1990) and as grooved, not grooved or bilobed by White & Elson-Harris (1992). All individuals studied in Arg 294 showed bilobed or bifid anal lobes (see Fig. 2A); this may obviously vary looking at a larger sample or a different strain.

It is expected than some points of the present work will be of practical use: 1) the clear distinctions pointed out for the three stages of *A. fratercula*, 2) the detailed description of the cephalopharyngeal skeleton in this species (Fig 3), and 3) some larval characters for field identification of this species from *C. capitata* larva by SENASA (Servicio Nacional de Sanidad Agropecuaria), Argentina or SAG (Servicio Agrícola Ganadero), Chile officers.

Unfortunately the information presented here does not shed any light on the controversial "complex" of siblings that may exist in the species; it was not the intention of the present research to do so. The deficiencies of this work — a species cannot be described from a single pure strain — must be overcome in the future. There is an urgent need for specimens of larvae from different sources within South America to be compared using similar methods to those presented here.

ACKNOWLEDGEMENTS

To Eng. N. Vaccaro (Instituto Nacional de Tecnología Agropecuaria, Concordia, Argentina) for helping with species identifications, to Dr. G. Steck (Florida, United States of America) for his comments on an early version of the manuscript, to Dr. Hennessy for his thorough review and to Dr. M. Viscarret for her suggestions.

Proyecto 81048 del Instituto Nacional de Tecnología Agropecuaria. Paper Gen No.: 968.

M.A.Delprat is fellow of CONICET (Consejo Nacional de Investigación Científica y Técnica), Argentina; present address: University of Patras, Greece.

REFERENCES

Artigas, J.N. 1994. "Entomología Económica". Vol I; pp 329-332.

Back, E. A. & C. E. Pemberton, 1918. U. S. Dept. Agriculture Bull. *536*:1-118. Cited in: "La mosca Mediterránea, Guía de Laboratorio/I". L. A. Quesada Allué, editor. Publication of Instituto de Investigaciones Bioquímicas, Fundación Campomar. Buenos Aires, Argentina.

Berg, G.H., 1979. Pictorial key to fruit fly larvae of the family Tephritidae. Organismo Internacional Regional de Sanidad Agropecuaria. San Salvador.

Bush, G.L., 1962. The cytotaxonomy of the larvae of some Mexican fruit flies in the genus *Anastrepha* (Tephritidae Diptera). Psyche *69* (3) 87-101.

Greene, C.T., 1929. Characters of the larvae and pupae of certain fruit flies. J. Agric. Res. *38*: 489-498.

Lifschitz, E.; F. Manso & A. Basso, 1999. Karyotype study of the South American Fruit-fly *Anastrepha fraterculus* Wied. in Argentina. pp. 21-24. IN: Ortiz G editor; The south American fruit fly, *Anastrepha fraterculus* (Wied.); advances in artificial rearing, taxonomic status and biological studies. Publication of International Atomic Energy Agency.200pp.

Manso, F., 1999. Breeding technique of *Anastrepha fraterculus* for genetic studies pp. 25-30. IN: Ortiz G editor; The south American fruit fly, *Anastrepha fraterculus* (Wied.); advances in artificial rearing, taxonomic status and biological studies. Publication of International Atomic Energy Agency.200pp.

Manso, F. & A. Basso, 1999. Notes on the present situation of *Anastrepha fraterculus* in Argentina. pp. 147-162. IN: Ortiz G editor; The south American fruit fly, *Anastrepha fraterculus* (Wied.); advances in artificial rearing, taxonomic status and biological studies. Publication of International Atomic Energy Agency.200pp.

Mendes, L.O.T., 1958. Observacoes citológicas em "Moscas das frutas". Bragantia N°7, Vol *17*: XXIX-XL.

Morgante, J. S.; A. Malavasi & G. L. Bus, 1980. Biochemical

systematics and evolutionary relationships of neo-tropical *Anastrepha*. Ann. Entomol. Soc. America. *73*: 622-630.

Morgante, J.S. & A. Malavasi, 1985. Genetic variability in populations of the South American fruit fly *Anastrepha fraterculus* (Tephritidae). Rev. Brasileira. Genet *8*: 241-247.

Philips, V.T., 1946. The biology and identification of trypetid larvae (Diptera: Trypetidae) Memoirs of the American Entomological Society *12*:1-161.

Solferini, V.N. & J.S. Morgante, 1987 Karyotype study of eight species of *Anastrepha* (Diptera Tephritidae) Caryologia *40*(3) 229-241.

Steck, G.J., 1991. Biochemical systematics and population genetic structure of *Anastrepha fraterculus* and related species (Diptera Tephritidae) Ann. Entomol. Soc. America. *84*: 10-28.

Steck, G.J. & A. Malavasi, 1988. Description of immature stages of *Anastrepha bistrigata* (Diptera : Tephritidae) Ann. Entomol. Soc. America. *81*(6): 1004-1009.

Steck, G.J. & R.A. Warton, 1988. Description of immature stages of *Anastrepha interrupta, A. limae*, and *A. grandis* (Diptera : Tephritidae) Ann. Entomol. Soc. America. *81*(6): 994-1003.

Steck, G. J.; L. E. Carroll; H. Caledonio-Hurtado & J. Guillen-Aguilar, 1990. Methods for identification of *Anastrepha* larvae (Diptera: Tephritidae), and key to 13 species. Proc. Entomol. Soc. Washington. *92*(2): 333-346.

Steyskal, G.C., 1977. Pictorial Key to the species of the Genus Anastrepha (Diptera: Tephritidae), Entomological Society of Washington, Washington.

Stone, A., 1942 The fruit flies of the genus *Anastrepha*. Miscellaneous Publication of the United States Department of Agriculture, *439*, 1-112.

Vilardi, J.; B. Saidman; A. Alberti; G. Calcagno; A. Basso; F. Manso & J. Cladera, 1994. Isoenzymatic and chromosomal analyses in three Argentine populations of *Anastrepha fraterculus*. p. VIII-XIV. Fourth Internl. Symp. Fruit Flies Econ. Import. Sand Key, Florida, USA. June 5-10, 1994.

White, I. M. & M. Elson-Harris, 1992. Fruit flies of economic significance: Their identification and bionomics. CAB International and ACIAR (eds.), 600pp.