

## Taxonomic study of two *Trichogramma* species from Uruguay (Hym.: Trichogrammatidae)

C. BASSO, B. PINTUREAU & G. GRILLE

Estudio taxonómico de dos especies de *Trichogramma* del Uruguay (Hym.: Trichogrammatidae).

La variación de diferentes caracteres, por ejemplo morfológicos y morfométricos, compatibilidad reproductiva y esterases separadas por electroforesis, fue analizada en varias líneas de dos especies de *Trichogramma* comunes en Uruguay. Los caracteres morfológicos y morfométricos indicaron que una de las especies es *T. pretiosum*, ya conocida en Uruguay, y la otra es probablemente *T. exiguum*, y no la especie próxima *T. fuentesi*. *Trichogramma exiguum* es señalada por primera vez en Uruguay. Las dos especies, *T. pretiosum* y *T. exiguum*, son completamente incompatibles entre sí y mostraron importantes diferencias en sus esterases lo cual puede ser utilizado como caracteres diagnósticos. Como en otras especies ya estudiadas, una alta variabilidad intraespecífica fue constatada. Esta variabilidad podría corresponder a un complejo de especies próximas en *T. exiguum*.

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**Palabras claves:** parasitoides oófagos, sistemática, lucha biológica, morfología, cruzamientos, esterases.

### INTRODUCTION

*Trichogramma* are minute parasitoids of numerous insect eggs, mainly of Lepidoptera, widely used in biological control. Because of their small size and of the complexity of their taxonomy, many *Trichogramma* species have been only recently described. Systematics of this genus is particularly based on the male antenna and genitalia, but the existence of closely related species has prompted use of other characters such as reproductive compatibility, allozymes and minor morphological traits (PINTO and STOUTHAMER, 1994).

In Uruguay, the number of recorded *Trichogramma* species is probably well below

the number of existing species. Only four species were identified in this country, *T. pretiosum* Riley, *T. galloi* Zucchi, *T. distinctum* Zucchi (BASSO and MOREY, 1991) and a new species (BASSO and PINTUREAU, 1999).

Most *Trichogramma* species are highly variable in morphological characters (PINTO, *et al.*, 1989; PINTUREAU and DAUMAL, 1995) and interpopulation reproductive compatibilities (PINTO, *et al.*, 1991; PINTUREAU, 1991). The present paper provides new data on variation of some characters, i.e. morphology and morphometric characters, reproductive compatibility, and some allozymes, in two American species. Studies were carried out on materials collected in Uruguay. Among studied strains, one shows a

thelytokous reproduction which may be induced by bacteria of the genus *Wolbachia* (Stouthamer, *et al.*, 1990a, 1990b; ROUSSET, *et al.*, 1992; LOUIS, *et al.*, 1993). This strain, infected by *Wolbachia* (JAGER, *et al.*, 1997), was subjected to thermic and antibiotic treatments to confirm that the Bacteria are responsible for the mode of reproduction and to obtain necessary males for the morphological studies.

## MATERIALS AND METHODS

**Material.** Uruguayan *Trichogramma* collected on four hosts, *Diatraea saccharalis* (Fabricius) (Lep.: Pyralidae), *Heliothis zea* (Boddie) (Lep.: Noctuidae), *Bonagota cranaodes* (Meyrick) and *Argyrotaenia sphaeropa* (Meyrick) (Lep.: Tortricidae), infesting 4 host plants, allowed to establish eleven strains (Table 1). Among these strains, three originated from North-West Uruguay (Artigas departamento), and eight originated from South Uruguay (Montevideo and Canelones departamentos). Moreover, three species from Colombia (identified by D. L. VIN-

CENT) and Cuba (identified by J. RODRÍGUEZ and B. PINTUREAU) served as reference for species identification in the crossing experiments (Table 1).

Strains were reared in the laboratory on *Ephestia kuehniella* Zeller eggs (Lep.: Pyralidae) at 25°C, 70% RH and L:D 16:8.

**Thermic and antibiotic treatments of the thelytokous strain.** The thelytokous strain 191 was treated with high temperatures (30°C) and tetracycline. The 30°C temperature was used from G1 to G18. At G14, half of the line submitted to 30°C was used to establish a new line which was placed at 25°C. Sex ratios were calculated from 100 individuals at each generation (G1 - G18 at 30°C, G15 - G19 at 25°C). Another line placed at 25°C was supplied with honey (1/3 honey, 2/3 water) containing chlorhydrate tetracycline (250 mg/ml) from G1 to G9. Then, the line was only supplied with honey (without antibiotics). Its sex ratio was calculated from 100 individuals at each generation until G14.

Bacteria are responsible for the reproductive mode since thermic and antibiotic treatments increased the male percentage. In the thermic treated line, the presence of male

Table 1. - List and origin of studied strains of *Trichogramma* from South America and Cuba

Strain number	Species	Host	Host Plant	Origin (country, city, region)	Year of collection
22	<i>T. pretiosum</i> (1)	<i>D. saccharalis</i>	Sugar cane	Uruguay, Bella Unión (2)	1992
54	<i>T. pretiosum</i> (1)	<i>D. saccharalis</i>	Rice	Uruguay, Bella Unión (2)	1992
60	<i>T. fuentesi</i>	<i>D. saccharalis</i>	Sugar cane	Cuba, Holguin	1990
115	<i>T. pretiosum</i> (1)	<i>H. zea</i>	Corn	Uruguay, Bella Unión (2)	1992
172	<i>T. exiguum</i> (1)	<i>B. cranaodes</i>	Vine	Uruguay, Melilla (3)	1993
183	<i>T. exiguum</i> (1)	<i>A. sphaeropa</i>	Vine	Uruguay, Melilla (3)	1995
191	<i>T. pretiosum</i> (1)	<i>A. sphaeropa</i>	Vine	Uruguay, Las Piedras (4)	1993
192	<i>T. exiguum</i> (1)	<i>B. cranaodes</i>	Vine	Uruguay, Las Piedras (4)	1993
231	<i>T. exiguum</i> (1)	<i>A. sphaeropa</i>	Vine	Uruguay, Joanicó (4)	1994
234	<i>T. pretiosum</i> (1)	<i>A. sphaeropa</i>	Vine	Uruguay, Joanicó (4)	1994
252	<i>T. exiguum</i> (1)	<i>A. sphaeropa</i>	Vine	Uruguay, Joanicó (4)	1995
271	<i>T. exiguum</i> (1)	<i>B. cranaodes</i>	Vine	Uruguay, Joanicó (4)	1995
320	<i>T. exiguum</i>	?	?	Colombia	1982
340	<i>T. pretiosum</i>	?	?	Colombia	1982

(1) Species identified in the present work; (2) Departamento of Artigas; (3) Departamento of Montevideo; (4) Departamento of Canelones.

individuals was observed as early as G2, but a high percentage (about 25%) of these individuals was not observed before G12. The percentage increased to reach about 40% in the next six generations. The return at 25°C from G14 did not obviously modify the percentage of males in the next five generations. In the antibiotic treated line, males appeared at G3 but remained at a low percentage (about 15%) during the next six generations. After stopping the treatment, the male percentage was not modified in the next five generations. These treatments allowed to obtain a sufficient number of males for the morphological studies.

**Morphological and morphometric analyses.** Males of the 11 Uruguayan strains were mounted on slides in Hoyers medium after cleaning in a 10% KOH solution for some minutes. Genitalia and antennae of each individual were observed and compared.

Some strains belong to a species difficult to determine because of their resemblance with two closely related species which can easily be confused. So, males of one of these strains (strain 183) were morphometrically analyzed. Measurements were generally taken on 10 individuals. Six characters were measured: length (FL) and basal width (BFW) of the antennal flagellum, length of longest seta on flagellum (SL), genital capsule length (GL), ventral ridge length (VRL), and distance between the paramere (PM) tips and the volsellar digit (VS) tips. From these characters, four ratios were calculated: BFW/FL, SL/BFW, VRL/GL, and «distance between the PM tips and the VS tips»/GL.

**Reproductive compatibility.** Reciprocal crosses were performed between two Uruguayan strains (172 and 192) and a Cuban strain (60) belonging to *T. fuentesi* Torre (RODRÍGUEZ, *et al.*, 1994), between a Uruguayan strain (115) and a Colombian strain (340) belonging to *T. pretiosum*, and between a Uruguayan strain (172) and a Colombian strain (320) belonging to *T. exiguum* PINTO &

PLATNER. In G1, the sex ratio of the offspring was calculated in non sterile crossings (1 female  $\times$  1 male). Reproductive isolation indices  $I_r$  based on these sex ratios (PINTUREAU, 1991) were calculated between the strains. Two crossings between strain 192 and strain 60 in each way (5 females  $\times$  5 males) furnished G1 individuals which were crossed in G2 (in fact, backcrosses). Sex ratio of the G2 offspring was calculated.

The reproductive compatibility was also analyzed between six Uruguayan strains. G1 intrastrain and interstrain crossings (1 female  $\times$  1 male) were performed as above-mentioned. Emerged adults were counted and sex ratio was calculated from the offspring originating from the first 8 days of oviposition. The  $I_r$  index was calculated between some strains and between species, and ANOVAs were used to compare sex ratios after Zarcosine  $\sqrt$  transformations.

**Electrophoretic analyses.** Vertical polyacrylamide gel electrophoresis was carried out according to the technique described by PINTUREAU (1987, 1993). Each bisexual Uruguayan strain was studied from 15 to 33 homogenates of about 20 individuals descending from a virgin female.

Only esterases were stained using  $\alpha$  and  $\beta$ -naphthyl-acetate. Bands were defined by their relative position on the gel (Rf) and their position relating to esterase bands known in other *Trichogramma* species (*T. brassicae* Bezdenko, *T. cacoeciae* Marchal, *T. dendrolimi* Matsumura, *T. turkestanica* Meyer, *T. cordubensis* VARGAS & CABELLO and *T. evanescens* Westwood). Intense bands were noted +++, moderately intense bands ++, and weak bands +.

The genetical interpretation of the bands was based on polymorphism cases, relative position and intensity of the bands, and data in the literature (for example, PINTUREAU, 1987, 1993). The Hardy-Weinberg equilibrium was tested at each polymorphic locus by  $\chi^2$  tests.

Although the number of studied loci is low, mean heterozygosities and Nei's genetic

distances were calculated (NEI, 1972). These values have not to be generalized to the entire genome, but can be used to compare the esterase polymorphism between strains and species.

## RESULTS

**Morphological and morphometric analyses.** All the 11 Uruguayan strains, among them a thelytokous strain, belong to species of the pretiosum group (PINTUREAU, 1994). Two morphotypes based on length of seta on male flagellum and shape of the dorsal lamina (male genitalia) were recognized. More precise observations led to conclude that the first morphotype corresponds to *T. pretiosum* (bisexual strains 22, 54, 115 and 234, thelytokous strain 191). The second morphotype (strains 172, 183, 192, 231, 252 and 271) corresponds to the «similar in structure and easily confused» species, *T. exiguum* and *T. fuentesi* (PINTO *et al.*, 1983).

These two closely related species can be differentiated by their male genitalia, male antennae and hindwings. Genitalia have a short ventral ridge (VR) in *T. fuentesi* and a long VR in *T. exiguum* (PINTO *et al.*, 1983; PINTO, 1998). The ratio VRL/GL ranges from 0.16 to 0.20 in *T. fuentesi* and from 0.44 to 0.48 in *T. exiguum*, and is equal to  $0.28 \pm 0.01$  ( $n = 4$ ) in Uruguayan strain 183 which is intermediate. Contrary to those of *T. fuentesi*, the dorsal lamina (DLA) and especially the intervolsellar process (IVP) do not reach the VS tips in *T. exiguum* (PINTO, *et al.*, 1983), and Uruguayan strain 183 is similar to the latter species for this character. The ratio «distance between the PM tips and the VS tips»/GL is also different in the two species, it ranges from 0.12 to 0.14 in *T. fuentesi* and from 0.08 to 0.10 in *T. exiguum*, and is equal to  $0.11 \pm 0.003$  ( $n = 10$ ) in Uruguayan strain 183 which is closer to the latter species.

Concerning the male antennae, the ratio SL/BFW ranges from 1.6 to 1.9 in *T. fuentesi* ( $1.80 \pm 0.03$  according to PINTO, *et al.*, 1983, and  $1.91 \pm 0.08$  according to PINTO,

1998), and from 1.9 to 2.3 in *T. exiguum* ( $2.1 \pm 0.03$  according to PINTO, *et al.*, 1978, and  $2.04 \pm 0.09$  according to PINTO, 1998), and is equal to  $1.9 \pm 0.1$  ( $n = 10$ ) in Uruguayan strain 181 which is intermediate. The ratio BFW/FL ranges from 0.19 to 0.23 in *T. fuentesi*, and is equal to  $0.17 \pm 0.002$  in *T. exiguum* (PINTO, *et al.*, 1978) and to  $0.19 \pm 0.01$  ( $n = 10$ ) in Uruguayan strain 183 which is again intermediate.

Hindwings show «setae of posterior tract increasing in length apically» in *T. fuentesi*, vs «uniformly short» in *T. exiguum* (PINTO, *et al.*, 1983). In Uruguayan strain 183, an important variability in the number and length of these setae was recorded, but the posterior tract seems closer to the *T. fuentesi* one.

The results make the Uruguayan individual identification difficult. They are intermediate between the two species *T. fuentesi* and *T. exiguum* for most characters, closer to *T. fuentesi* only for one character associated in hindwing, and closer to *T. exiguum* for two genitalic characters.

**Reproductive compatibility.** Crossings between Colombian and Uruguayan *T. pretiosum* strains furnished offspring with reduced sex ratios when compared to controls (Table 2). The Ir index between strains 115 and 340 is equal to 0.565, a value superior to Ir obtained in intraspecific crossings but inferior to most Ir obtained in interspecific crossings (PINTUREAU, 1991).

Two G1 crossings between Cuban (*T. fuentesi*) and Uruguayan (*T. exiguum* or *T. fuentesi*) strains furnished only males and were incompatible: male 172  $\times$  female 60 and male 60  $\times$  female 192. Other interstrain crossings furnished a low percentage of females, but this percentage sometimes reached those furnished by intrastrain crossings (unusually low percentages) (Table 2). The Ir indices between strains 60 and 172 or 192 were respectively equal to 0.807 or 0.771. These values are comparable or superior to those obtained in some interspecific crossings (PINTUREAU, 1991). The partial incompatibility was confirmed by the G2 crossings.

Table 2. - Reproductive compatibility observed in G1 between some *Trichogramma* strains from Uruguay and *T. pretiosum*, *T. fuentesi* and *T. exiguum*

Strains crossed	male × female	N	Offspring (1)	Sex ratio (2)
Uruguayan strain 115 × <i>T. pretiosum</i> (340)	115 × 115 115 × 340 340 × 115 340 × 340	16 15 16 16	41.2 ± 2,0 26.9 ± 2.5 24.5 ± 4.4 38.9 ± 2.8	0.753 a 0.312 b c 0.242 c 0.570 a b
Uruguayan strains 172 or 192 × <i>T. fuentesi</i> (60)	172 × 172 60 × 172 172 × 60 60 × 60 192 × 60 60 × 192 192 × 192	9 10 3 29 7 5 8	47.3 ± 4.8 (4) 29.5 ± 6.8 24.0 ± 9.5 32.6 ± 1.8 15.0 ± 3.4 9.2 ± 2.9 34.0 ± 6.5	0.070 a 0.027 a 0 a 0.396 a 0.181 a 0 a 0.129 a
Uruguayan strain 172 × <i>T. exiguum</i> (320)	172 × 172 320 × 172 172 × 320 320 × 320	15 45 (3) 45 (3) 15	37.7 ± 2.7 (4) 30.6 ± 2.6 31.2 ± 2.8 41.8 ± 2.8	0.740 a 0.331 b 0.249 b 0.743 a

(1) Number of descendants reaching the imaginal stage.

(2) Total number of females (all pairs)/total number of individuals. Ratios followed by different letters are significantly different ( $p < 0.05$ ) according to the Scheffe test (one way ANOVA after  $2\arcsin\sqrt{\phantom{x}}$  transformation of sex ratio of each pair;  $p < 0.0001$  for the 4 crossings with *T. pretiosum*,  $p = 0.0078$  for the 7 crossings with *T. fuentesi*, the important variability and unusual low sex ratio in controls explain the absence of differences according to the Scheffe tests;  $p < 0.0001$  for the 4 crossings with *T. exiguum*).

(3) Three replicates ( $N = 15+13+17$  for  $320 \times 172$ , and  $N = 16+21+8$  for  $172 \times 320$ ) carried out at different but near times, the first replicate was simultaneous to the controls  $172 \times 172$  and  $320 \times 320$ .

(4) Two replicates carried out at very different times.

Surprisingly, in the «5 males  $60 \times 5$  females 192» cross, no G1 offspring were obtained (in the previous «1 male × 1 female» G1, only males were obtained) and no G2 crossings were performed. In the reciprocal cross, G1 individuals were obtained allowing to make 20 pairs. Out of these 20 G2 crossings, 10 were sterile and the remaining 10 crossings furnished 0.6% of females. So, the strains 172 and 192 do probably not belong to the species *T. fuentesi*.

Crossing between the Colombian strain of *T. exiguum* and the Uruguayan strain 172 furnished results similar to those obtained with *T. pretiosum* strains, i.e. offspring with reduced sex ratios (Table 2). The Ir index between strains 172 and 320 is equal to 0.608, a high value for two intraspecific strains and a low value for two closely related species (PINTUREAU, 1991). Nevertheless, the Uru-

guayan strain 172 being closer to *T. exiguum* than to *T. fuentesi*, it is temporarily identified as *T. exiguum*.

Reciprocal interspecific crosses between Uruguayan *T. pretiosum* and *T. exiguum* furnished only males and Ir was always equal to 1. These species are thus incompatible (Table 3). Fecundity is not affected by the interspecific crossings, except in one case: the crossing «male 252 (*T. exiguum*) × female 115 (*T. pretiosum*)» furnished a reduced offspring (Table 3). Reciprocal intraspecific crosses (interstrain or intrastrain crossings) always furnished more than 50% females with a high variability (Table 3).

In *T. pretiosum*, Ir was calculated between strains 115 and 234 and was equal to 0, indicating a total compatibility. Only one significant difference between sex ratios (male 115 × female 234 and male 234 × female 234)

Table 3. - Reproductive compatibility observed in G1 between Uruguayan strains identified as *T. pretiosum* and *T. exiguum*

Type of crossing	male × female	N	Offspring (i)	Sex ratio (2)
Intraspecific in <i>T. pretiosum</i>	115 × 115	10	82.7 ± 3.1	0.707 ab
	234 × 234	11	57.5 ± 5.5	0.555 b
	234 × 115	9	63.8 ± 4.0	0.699 ab
	115 × 234	5	71.0 ± 4.0	0.746a
Intraspecific in <i>T. exiguum</i>	183 × 183	7	66.1 ± 3.2	0.823 a
	271 × 271	5	32.6 ± 3.7	0.675 a
	252 × 252	5	47.8 ± 7.1	0.690a
	172 × 172	15	37.7 ± 2.7	0.740 a
	252 × 271	6	30.7 ± 3.4	0.840 a
	271 × 252	3	45.7 ± 5.5	0.759 a
	172 × 271	4	50.0 ± 12.1	0.648 a
	271 × 172	5	76.6 ± 10.7	0.606 a
	271 × 183	2	67.0 ± 7.9	0.794 a
	183 × 271	3	47.2 ± 12.1	0.554 a
Interspecific between <i>T. pretiosum</i> and <i>T. exiguum</i>	115 × 183	8	60.6 ± 8.7	0
	115 × 252	12	53.2 ± 2.5	0
	183 × 115	6	58.3 ± 10.6	0
	252 × 115	6	18.8 ± 4.4	0

(1) Number of descendants reaching the imaginal stage.

(2) Total number of females (all pairs)/total number of individuals. Ratios followed by different letters are significantly different ( $p < 0.05$ ) according to the Scheffe test (one way ANOVA after arcsine<sup>1/2</sup> transformation of sex ratio of each pair,  $p = 0.007$  for the 4 crossings in *T. pretiosum*,  $p = 0.881$  for the 10 crossings in *T. exiguum*). For 115 × 115 and 172 × 172, results already appeared in Table 2; in the present table it is a new replicate of the crossing 115 × 115 and one of the previous replicates of the crossing 172 × 172.

was recorded, indicating a father influence (Table 3). A two way ANOVA (factors: mother strain and father strain) showed a significant effect on the offspring sex ratio of the father strain ( $p = 0.014$ ) and of the interaction between the two factors ( $p = 0.019$ ), but no effect of the mother strain ( $p = 0.418$ ).

In *T. exiguum*, Ir was calculated between three pairs of strains. The Ir index relating to strains 252 and 271 was equal to 0 (total compatibility), and the Ir indices relating to strains 271 and 181 and to strains 271 and 172 did not reach 0.2 (0.163 and 0.113, respectively) indicating a very partial incompatibility. Nevertheless, no significant differences between sex ratios were recorded (Table 3). Two one way ANOVAs tested the effect of the father (4 strains) associated to the same mother (strain 271), and the effect of the mother (4 strains) associated to the same father

(strain 271). No father ( $p = 0.587$ ) or mother ( $p = 0.818$ ) effects were detected.

**Allozymic variation.** Eighth esterase bands were stained in *T. pretiosum*, and only one was variable. In *T. exiguum*, 4 to 6 bands were stained and most of them were variable (Fig. 1). Genetical interpretation of the bands concluded to the presence of 4 loci in *T. pretiosum* and of 3 loci in *T. exiguum* (in the first species, alleles at one locus encode for 4 bands, and in the two species, alleles at another locus encode for 2 bands).

*Trichogramma pretiosum*: Est 1 (+++), allele 0.10; Est 1' (+-+-+), allele 0.02-0.04-0.06-0.09; Est 2 (+++), alleles 0.18bis in strains 22 and 115, and 0.19 in strains 54 and 234; Est 5' (+-+++), allele 0.40-0.43. The locus Est 2 furnished a band more intense than in other studied strains of

*T. pretiosum* or other species (PINTUREAU, 1993), and regulating genes may be responsible for the strong enzyme activity.

*Trichogramma exiguum*: Est 1 (+++), allele 0.13 in strains 172, 183, 192, 231 and 252, and alleles 0.13 and 0.18 in strain 271; Est 2 (++), allele 0.22; Est 5' (++-+++), allele 0.44-0.47 in strains 172, 192 and 252, alleles

0.44-0.47 and 0.47-0.50 in strains 183, 231 and 271.

Allelic frequencies appear in table 4 and mean heterozygosities (that cannot be generalized to the entire genome) in figure 1. The most variable esterases were recorded in strain 271 of *T. exiguum*. The Hardy-Weinberg equilibrium was tested in polymorphic

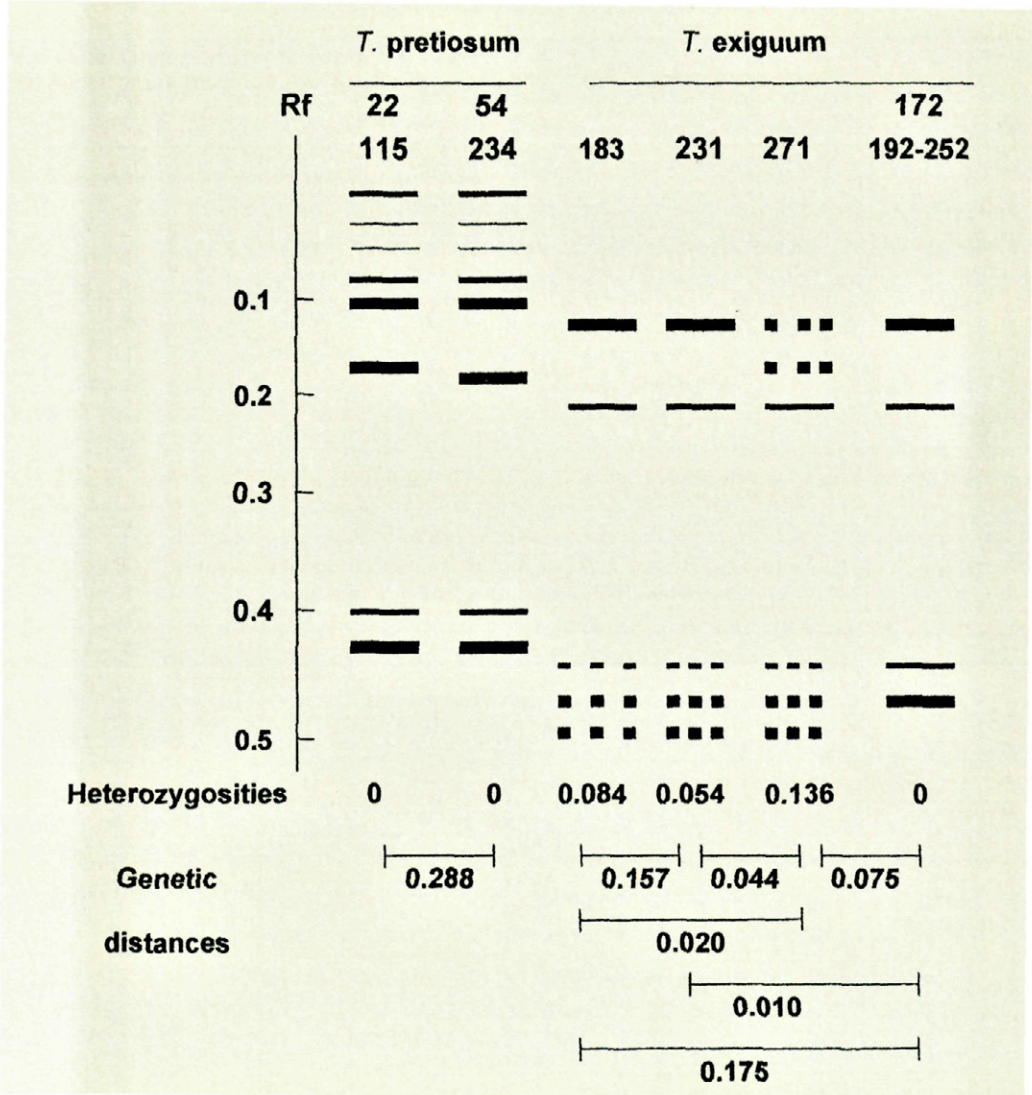


Fig. 1. - Esterase bands for Uruguayan strains (indicated by numbers) of *Trichogramma*. The band thickness takes into consideration the band intensity; the bands represented by a discontinuous line are not present in all individuals of a strain (polymorphism cases). Mean heterozygosities in each strain and Nei's genetic distances between strains calculated from four esterase loci.

loci and was always respected, except for Est 5' in strain 231 ( $p < 0.01$ ).

Nei's genetic distances, that cannot be generalized to the entire genome, are infinite between the two species, indicating no common esterase bands. In *T. pretiosum*, two sets of strains differentiated by Est 2 can be distinguished (Fig.1). In *T. exiguum*, the most distant strains, differentiated by Est 5', are 183 and the set 172, 192 and 252 (Fig. 1).

## DISCUSSION

Although a high variability exists in the studied Uruguayan strains of *Trichogramma*, it seems that they belong to the two species *T. pretiosum* and *T. exiguum*, the latter being recorded for the first time in this country. These two species are totally incompatible, what confirms results obtained by PINTO, *et al.* (1978), and easily discriminated by esterase characters. *Trichogramma exiguum* is closely related to *T. fuentesi*. Nevertheless, Uruguayan strains show morphological charac-

ters slightly closer to those of *T. exiguum*, a strong but not complete reproductive incompatibility with *T. fuentesi*, and esterases very different from those of *T. fuentesi*. In Cuban *T. exiguum* (RODRÍGUEZ, *et al.*, 1997) as in Uruguayan strains, the locus Est 5' is active, vs the locus Est 5 in *T. fuentesi* (PINTUREAU, 1993; RODRÍGUEZ, *et al.*, 1996a).

In *T. pretiosum*, the variability induces different levels of interstrain reproductive incompatibilities as previously observed (PINTO, 1998). Esterase differences between populations from Uruguay and populations from Mexico (PINTUREAU, 1993), Cuba, Peru and USA (RODRÍGUEZ, *et al.*, 1996b), show the high variability of this species. New alleles Est 2<sup>0.18bis</sup> and Est 2<sup>0.19</sup> were recorded in place of Est 2<sup>0-20bis</sup> (Mexico) or Est 2<sup>0-22</sup> (other 3 countries), and new allele Est 5<sup>0.40-0.43</sup> was recorded in place of Est 5<sup>0.38-0.41</sup> (Mexico), Est 5<sup>0.41-0.44</sup> (Cuba, Peru), Est 5<sup>0.42-0.45</sup> (USA), Est 5<sup>0.44-0.47</sup> (Peru) or Est 5<sup>0.45-0.48</sup> (USA).

In *T. exiguum* too, the variability induces reproductive incompatibilities. This variabi-

Table 4. - Allelic frequencies at four esterase loci in different Uruguayan strains (indicated by numbers) of *T. pretiosum* and *T. exiguum*

Locus	Allele	<i>T. pretiosum</i>		<i>T. exiguum</i>			
		22,115	54,234 252	172,192,	183	231	271
Est 1	0.10	1	1	-	-	-	-
	0.13	-	-	1	1	1	0.976
	0.18	-	-	-	-	-	0.024
Est 1'	0.02-0.04-						
	0.06-0.09	1	1	-	-	-	-
	0(1)	-	-	1	1	1	1
Est 2	0.18bis	1	-	-	-	-	-
	0.19	-	1	-	-	-	-
	0.22	-	-	1	1	1	1
Est 5'	0.40-0.43	1	1	-	-	-	-
	0.44-0.47	-	-	1	0.214	0.852	0.476
	0.47-0.50	-	-	-	0.786	0.147	0.524

(1) Null allele.



lity 15 obvious for the morphological characters but also involves biochemical characters: populations from Uruguay show some esterase differences with populations from Cuba and Peru (RODRÍGUEZ, *et al.*, 1997). New alleles Est 1<sup>0.13</sup> and Est 1<sup>0.18</sup> were recorded in place of Est 1<sup>0.10</sup>, and new alleles Est 5<sup>0.44-0.47</sup> and Est 5<sup>0.47-0.50</sup> were recorded in place of Est 5<sup>0.45-0.48</sup>.

Individuals named *T. fasciatum* (PERKINS) and redescribed by Nagarkatti and Nagaraja (1971) were considered as «close to, if not conspecific to» *T. exiguum* by PINTO, *et al.* (1978), and then placed in the species *T. fuentesi* by PINTO, *et al.* (1983). Individuals redescribed by NAGARKATTI and NAGARAJA (1971) originated from different South American countries and especially from Argentina which is a country geographically close to Uruguay. The represented individual shows a VR of the *T. fuentesi* type, but a ratio «distance between the PM tips and the VS tips» /GL equal to 0.11 and thus close to the ratio recorded in *T. exiguum* and Uruguayan indi-

viduals. It is possible that populations identified as *T. exiguum* or *T. fuentesi* constitute in fact a complex of more than two species, and that populations from southern part of South America correspond to a third species.

Geographic distributions of the two studied species are apparently different in South America. *Trichogramma pretiosum* is present in almost all countries, but *T. exiguum* was only recorded from Chile, Colombia, Peru (ZUCCHI and MONTEIRO, 1997), Guiana and Argentina (DE SANTIS and FIDALGO, 1994). The closely related species *T. fuentesi* was only recorded from Peru and Argentina and these records have to be confirmed.

## ACKNOWLEDGMENTS

Fulvia García Roa (Instituto Colombiano Agropecuario) provided Colombian *Trichogramma*. This study was supported by grant UB 9405 from ECOS (France-Uruguay cooperation).

## ABSTRACT

BASSO, C.; B. PINTUREAU & G. GRILLE, 1999: Taxonomic study of two *Trichogramma* species from Uruguay (Hym.: Trichogrammatidae). *Bol. San. Veg. Plagas*, 25 (3): 373-382.

Variation of different characters, i.e. morphological and morphometric characters, reproductive compatibility and esterases separated by electrophoresis, was analyzed in several strains of two common *Trichogramma* species from Uruguay. Morphological and morphometric characters showed that one of the species is *T. pretiosum*, already known in Uruguay, and that the other species is probably *T. exiguum*, and not the closely related species *T. fuentesi*. *Trichogramma exiguum* is recorded for the first time in Uruguay. The two species *T. pretiosum* and *T. exiguum* are completely incompatible and show important esterase differences which can be used as diagnostic characters. As in other species already studied a high intraspecific variability was noted. This variability could correspond to a complex of closely related species in *T. exiguum*.

**Key words:** egg parasitoids, systematics, biological control, morphology, crossina, esterases.

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(Recepción: 1 junio 1999)

(Aceptación: 13 setiembre 1999)