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Viability of rearing *Telenomus* sp. (Hymenoptera, Scelionidae), an egg parasitoid of *Helicoverpa armigera* (Lepidoptera, Noctuidae), under laboratory conditions

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The genus *Telenomus* Haliday is composed by hymenopteran egg parasitoids which attack insect hosts of the orders Lepidoptera and Hemiptera, mainly. In Portugal, in processing tomato fields, the parasitoid *Telenomus* sp. presence is almost coincident with the first tomato fruitworm eggs.

From May until September of 2004, six processing tomato fields in the Ribatejo region (central Portugal) were monitored weekly, 50 plants were observed per field and tomato fruitworm eggs were collected.

The established targets were: verify the possibility of rearing *Telenomus* parasitoids under laboratory conditions by investigating which host was the most adequate for mass rearing among the species used as hosts, and by evaluating the biological characteristics of the reared parasitoids.

Telenomus sp. specimens were tested regarding their host range possibilities. The species investigated as hosts were the noctuids *Autographa gamma* (Linnaeus), *Chrysodeixis chalcites* (Esper), *Helicoverpa armigera* (Hübner), *Peridroma saucia* (Hübner) and *Thysanoplusia orichalcea* (Fabricius), and the pyralid *Ephestia kuehniella* Zeller.

Rearing of *Telenomus* was possible under laboratory conditions, at 25±1°C, 65±10% RH and 12:12 (L:D). The development time, from oviposition to emergence, was of about 20 days.

During laboratory rearing, some factors indicated a possible existence of superparasitism situations. Moreover, the number of females that emerged increased throughout the generations.

C. chalcites proved to be the most appropriated host, with the highest parasitism rate, together with the lowest rate of larvae emergence and collapsed eggs. *A. gamma* showed a high rate of collapsed eggs, whereas *H. armigera* showed a low parasitism rate. Finally, *E. kuehniella* eggs were rejected because of their small size.

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Key words: *Telenomus* sp., egg parasitism, laboratory rearing, hosts.

INTRODUCTION

Quality standards imposed by international markets have tended to limit pesticide use dramatically.

Tomato crops are grown worldwide, both outdoors and under glasshouse conditions, either for fresh market consumption or for processing. Portugal is the 8th world producer and the 5th in Europe of processing tomatoes.

Some noctuid species are known to be key, occasional or potential pests in tomato processing agroecosystem. The larvae attack the host plant (fruit, leaves or roots, depending on the noctuid species). In processing tomato fields, the larvae are one of the main pests, and are responsible for the application of pesticides. It is therefore crucial to investigate alternative control measures to minimize pest attacks, namely throughout biological control.

The tomato fruitworm, *Helicoverpa armigera* (Hübner) (Lepidoptera, Noctuidae) is considered to be a key pest of processing tomato agroecosystem, because it severely attacks the fruits (ARAÚJO, 1990; HOFFMAN *et al.*, 1991). *H. armigera* is naturally limited by a group of hymenopteran parasitoids, namely by species of the genus *Telenomus* Haliday (Hymenoptera, Scelionidae) (FIGUEIREDO *et al.*, 2003, DUARTE *et al.*, 2005).

This genus has about 500 described species, all egg parasitoids which attack mostly Lepidoptera and Hemiptera, but also Diptera and Neuroptera, thus being quite important in the regulation of pest populations (POLASZEK & KIMANI, 1990; POLASZEK *et al.*, 1993).

The potential of *Telenomus* sp. as a biological control agent has encouraged attempts for their mass rearing for later releases in both agrarian fields and forests (BUSTILLO & DROOZ, 1977; ORR, 1988; CAVE, 2000). In Portuguese processing tomato fields, the parasitoid *Telenomus* sp. was found to be the most precocious and time synchronized with the first *H. armigera* eggs. This parasitoid has an accurate sense for host egg detection in the field (ARAÚJO, 1990). Field observations showed that the natural parasitism rate on noctuids of *Telenomus* and *Trichogramma* Westwood (Hymenoptera, Trichogrammatidae) are variable among the years (ARAÚJO, 1990; GONÇALVES *et al.*, 2005b; GONÇALVES *et al.*, 2006).

The identification of *Telenomus* species occurring in Portugal is not completed yet, but there are indications that exists one species, *Telenomus laeviceps* Foerster (Polaszek & Figueiredo, *personal communication*). Male genitalia slides are under preparation to be identified by specialists.

In this study, the research targets were: check the viability of rearing *Telenomus* under laboratory conditions, as well as at finding out which is the most adequate host and at evaluating the biological characteristics of the reared parasitoids.

MATERIAL AND METHODS

Both the *Telenomus* parasitoid and the noctuid hosts were collected as eggs in processing tomato fields, in the Ribatejo region, in central Portugal, from May to September 2004. Eggs of *Ephestia kuehniella* (Lepidoptera, Pyralidae) were obtained from a laboratory reared strain (GONÇALVES *et al.*, 2005a).

Telenomus specimens were investigated for host range suitability. Lepidopteran hosts used were the pyralid *E. kuehniella* and the noctuids *Autographa gamma*, *Chrysodeixis chalcites*, *Helicoverpa armigera*, *Peridroma saucia* and *Thysanoplusia orichalcea*. The noctuids were reared in the laboratory at 25±1°C, 65±10% RH, 12:12 (L:D), on an artificial diet based on corn (POITOUT & BUES, 1974).

The eggs used in the assays were less than 48 hours old. Eggs were laid on paper stripes and small paper pieces were cut off, without direct egg handling, since the eggs can be easily damaged. A minimum of twenty replicates per host species was established, which was not possible to reach for all host species and all parameters.

The assays were conducted at room temperature, from mid September to late November 2004. Upon emergence, male and female specimens of *Telenomus* were placed in glass vials, covered with cotton, along with a honey solution (80% in water), as recommended by some authors (GERLING, 1972; GERLING & SCHWARTZ, 1974; STRAND & VINSON, 1983; AGBOKA *et al.*, 2002). Host eggs were placed in rectangular paper cards following the method of CRUZ (2000). Each egg card was identified regarding the host species, date of exposure to parasitoids and number of available host eggs. Eggs were

replaced depending on egg availability resulting from lepidoptera laboratory rearing. The parameters used for testing host suitability were: total parasitism rate, parasitism rate per female, sex-ratio, female and male longevity, development time, rate of larvae emergence, rate of unviable eggs (eggs parasitized, *i. e.*, with darkened chorion, but from which no parasitoid emerged) and rate of collapsed eggs (eggs that collapsed before parasitoid or larvae emergence). The results are shown in percentages (except for longevity and development time, which are expressed in days) because different numbers of eggs were used for each replicate.

Correlation tables were built, using the non-parametric Kendall's concordance coefficient, appropriate for measuring the degree of association between more than two variables. The biological parameters of laboratory rearing were tested using Kruskall-Wallis non-parametric tests. The significance level was 5% for all analyses. Tests were performed in STATISTICA 6.0. and tables were produced in MICROSOFT EXCEL XP.

RESULTS

E. kuehniella eggs were rejected by *Telenomus*. Three species from those tested as hosts adapted well to laboratory rearing, namely *H. armigera*, *C. chalcites* and, to a somewhat lower extent, *A. gamma*. The remaining noctuids tested, *P. saucia* and *T. orichalcea*, did not adjust to laboratory rearing, probably because of the low quality of the eggs, possibly caused by non-optimal temperature and/or relative humidity rearing conditions (temperature, humidity and photoperiod). Rejection by the parasitoids was not a major cause for non suitability of these hosts. Results achieved for the three noctuids well adapted to laboratory rearing are summarized in Table 1.

The highest parasitism rate, both total and per female, were found in *C. chalcites*, together with the lowest larvae emergence rate. *C. chalcites* presented, as well, the highest sex ratio. The highest rate of collapsed eggs was observed for *A. gamma* and the highest rate of larvae emergence for *H. armigera*.

To measure the degree of association between parameters a correlation matrix was built (Table 2). As expected, the number of eggs available for parasitoids was positively correlated with the rate of larvae emergence. For a higher number of eggs, the probability of them having different ages is higher. Under laboratory rearing conditions different generations overlap, and the generations were positively correlated with development time. Female age was positively correlated with development time too.

Table 1. **Total parasitism rate (%), parasitism rate per female (%), sex ratio (%), female longevity (days), male longevity (days), development time (days), larvae emergence rate (%), unviable eggs rate (%), collapsed eggs rate (%).**

	A. gamma	n	*C. chalcites*	n	*H. armigera*	n
Total parasitism rate	30.5±37.0 a	26	67.5±34.4 b	48	41.1±37.7 a	47
Parasitism rate per female	20.9±30,7 a	26	33.3±31.6 bc	48	24.8±32.1 ac	47
Sex-ratio	59.5±41.7 a	13	62.3±29.3 bc	42	53.8±35.9 ac	30
Female longevity	43.1±19.5 a	4	31.6±21.1 a	17	35.1±22.0 a	13
Male longevity	37.1±20.2 a	7	30.0±19.4 a	15	29.1±17.9 b	14
Development time	19.6±10.3 a	14	20.6±7.6 b	43	21.2±10.7 ab	31
Larvae emergence rate	0.7±2.6 a	26	0.6±7.9 a	48	19.5±27.2 b	47
Unviable eggs rate	8.0±4.7 a	26	5.0±8.0 a	48	4.0±7.2 a	47
Collapsed eggs rate	57.2±43.7 a	26	22.7±34.5 b	48	19.8±26.9 b	47

Values followed by different letters are significantly different (Kruskall-Wallis tests; $p<0.05$).

Table 2. **Multiple correlation table with Kendall's concordance coefficient, obtained for the data of laboratory rearing of *Telenomus* with different hosts. ***

	Valid N	Kendall	Z	P-level
Assays *vs.* parasitism rate	32	0,07575	0,60929	0,54233
Assays *vs.* sex ratio	32	-0,03965	-0,31890	0,74980
Assays *vs.* development time	32	0,06007	0,48319	0,62896
Assays *vs.* larvae emergence rate	31	0,15158	1,19797	0,23093
Assays *vs.* collapsed eggs rate	31	0,01898	0,15002	0,88075
Assays *vs.* unviable eggs rate	31	0,11132	0,87977	0,37899
Assays *vs.* female longevity	17	-0,04584	-0,25678	0,79735
Assays *vs.* male longevity	21	-0,18191	-1,15356	0,24868
Assays *vs.* parasitism rate per female	32	0,05516	0,44370	0,65726
Number of eggs *vs.* parasitism rate	111	0,07186	1,11800	0,26357
Number of eggs *vs.* sex ratio	111	0,07414	1,15353	0,24869
Number of eggs *vs.* development time	111	0,05228	0,81333	0,41603
Number of eggs *vs.* larvae emergence rate	110	0,24419	3,78135	**0,00016**
Number of eggs *vs.* collapsed eggs rate	110	-0,09700	-1,50147	0,13324
Number of eggs *vs.* unviable eggs rate	110	-0,04608	-0,71364	0,47545
Number of eggs *vs.* female longevity	65	0,16431	1,93484	0,05301
Number of eggs *vs.* male longevity	68	0,11647	1,40439	0,16020
Number of eggs *vs.* parasitism rate per female	111	0,10440	1,62407	0,10436
Parental sex ratio *vs.* parasitism rate	111	-0,07840	-1,21967	0,22259
Parental sex ratio *vs.* sex ratio	111	-0,08797	-1,36863	0,17111
Parental sex ratio *vs.* development time	111	0,04282	0,66627	0,50524
Parental sex ratio *vs.* larvae emergence rate	110	-0,00554	-0,08576	0,93166
Parental sex ratio *vs.* collapsed eggs rate	110	0,08489	1,31452	0,18867
Parental sex ratio *vs.* unviable eggs rate	110	0,08874	1,37426	0,16936
Parental sex ratio *vs.* female longevity	65	-0,16311	-1,92068	0,05477
Parental sex ratio *vs.* male longevity	68	-0,12062	-1,45453	0,14580
Parental sex ratio *vs.* parasitism rate per female	111	-0,07344	-1,14262	0,25320
Generation *vs.* parasitism rate	111	0,10493	1,63253	0,10257
Generation *vs.* sex ratio	111	0,16002	2,48953	**0,01279**
Generation *vs.* development time	111	0,30498	4,74482	**0,00000**
Generation *vs.* larvae emergence rate	110	0,07769	1,20303	0,22897
Generation *vs.* collapsed eggs rate	110	-0,07873	-1,21916	0,22278
Generation *vs.* unviable eggs rate	110	0,10348	1,60246	0,10905
Generation *vs.* female longevity	65	0,04511	0,53118	0,59529
Generation *vs.* male longevity	68	0,03484	0,42007	0,67443
Generation *vs.* parasitism rate per female	111	-0,11455	-1,78213	0,07473
Female age *vs.* parasitism rate	110	0,06384	0,98866	0,32283
Female age *vs.* sex ratio	110	0,04916	0,76119	0,44654
Female age *vs.* development time	110	0,24124	3,73564	**0,00019**
Female age *vs.* collapsed eggs rate	110	0,01904	0,29487	0,76810
Female age *vs.* unviable eggs rate	110	0,10990	1,70187	0,08878
Female age *vs.* female longevity	65	-0,10667	-1,25609	0,20908
Female age *vs.* male longevity	68	-0,11270	-1,3586	0,17427
Female age *vs.* parasitism rate per female	110	0,05876	0,90999	0,36283

Table 2. **Multiple correlation table with Kendall's concordance coefficient, obtained for the data of laboratory rearing of *Telenomus* with different hosts. * (Cont.)**

	Valid N	Kendall	Z	P-level
Female number *vs.* parasitism rate	111	0,14381	2,23733	**0,02526**
Female number *vs.* sex ratio	111	0,19817	3,08307	**0,00205**
Female number *vs.* development time	111	0,23346	3,63218	**0,00028**
Female number *vs.* larvae emergence rate	110	-0,11476	-1,77718	0,07554
Female number *vs.* collapsed eggs rate	110	-0,16056	-2,48629	**0,01291**
Female number *vs.* unviable eggs rate	110	0,16737	2,59175	**0,00955**
Female number *vs.* female longevity	65	0,19526	2,29935	**0,02148**
Female number *vs.* male longevity	68	0,19720	2,37793	**0,01741**
Female number *vs.* parasitism rate per female	111	-0,23879	-3,71514	**0,00020**
Hosts *vs.* parasitism rate	110	0,03600	0,55748	0,57720
Hosts *vs.* sex ratio	110	-0,02218	-0,34354	0,73119
Hosts *vs.* development time	110	0,08971	1,38913	0,16479
Hosts *vs.* larvae emergence rate	109	0,45089	6,94940	**0,00000**
Hosts *vs.* collapsed eggs rate	109	-0,23744	-3,65959	**0,00025**
Hosts *vs.* unviable eggs rate	109	-0,04539	-0,69950	0,48424
Hosts *vs.* female longevity	65	0,04964	0,58449	0,55889
Hosts *vs.* male longevity	68	-0,02999	-0,36158	0,71766
Hosts *vs.* parasitism rate per female	110	0,02507	0,38818	0,69788

*Values of correlation in bold are significantly different for p<0,05.

Female number was positively correlated with parasitism rate, sex-ratio, development time, unviable egg rate and female and male longevity, whereas it was negatively correlated with parasitismrate per female and rate of collapsed eggs.

The host species was positively correlated with larvae emergence rate, and negatively correlated with collapsed eggs rate. More *H. armigera* larvae emerge, and *A. gamma* had a higher number of collapsed eggs. These correlations can be explained if it is assumed that the analysis attributed an increasing value to the species as follows: *A. gamma* - *C. chalcites* - *H. armigera*.

DISCUSSION

E. kuehniella eggs are too small and were rejected by *Telenomus* females. Small eggs do not provide minimal resources for the development of these parasitoids; this was already reported for *Telenomus* which were offered other small hosts (*e. g.* FEDDE, 1977).

H. armigera had the highest larvae emergence rate. This could have hindered the performance, because the larvae are cannibalistic and may have eaten the surrounding eggs (possibly parasitized) after emergence. Cannibalism also creates difficulties for laboratory rearing. It is necessary to individualize each larvae, instead of rearing several larvae in "rearing boxes", which facilitates both cleaning and feeding of the larvae. This problem could be avoided by killing embryos within the eggs with U.V. radiation or freezing; however these techniques may decrease the eggs nutritional quality for the development of parasitoids.

A. gamma was not a good host for the laboratory rearing of *Telenomus* because of the high rate of collapsed eggs. Since these noctuids were not reared under optimal temperature and humidity conditions for their development, this fact may indicate that this noctuid has a low capacity of resisting changes in the environmental conditions at this stage of its life cycle. The parasitism rates

for this noctuid were the lowest. Although the results showed the highest longevity of both female and male parasitoids for this host, they can be bias caused by the low number of replicates for this parameter. *A. gamma* could be a good potential host for *Telenomus* laboratory rearing if, under different conditions of temperature and humidity, the rate of collapsed eggs decreased.

The most appropriated noctuid for *Telenomus* rearing seems to be *C. chalcites*. For this host the highest parasitism rates were obtained, both in total (which was significantly different from the others noctuids) and per female, high sex-ratios, low larvae emergence rates and low rates of both unviable and collapsed eggs.

The development time for *Telenomus*, from oviposition until adult emergence was about twenty days for all hosts. The longevity in adults was variable, with females living a little longer than males (respectively 34.4 ± 21.7 and 31.9 ± 19.1 days).

As far as the specimens of *Telenomus* continued to be reared for more generations under laboratory conditions, the sex-ratio increases, which may be desirable for their adaption for biological control purposes, and it is in accordance with the LMC (local mate competition) theory (HAMILTON, 1967; BAYRAM *et al.*, 2004). The genetic variability throughout generations may decrease, therefore crossing different *Telenomus* strains becames important in order to maintain a good quality of the parasitoids reared. The identification of *Telenomus* present in the field trials were not fully confirmed to the species level yet, but it is assumed that only one species of this genus is present in this agroecosystem, acting as a noctuid parasitoid. It was observed that generation after generation the viability of parasitized eggs did not decrease. Another fact that supports this assumption is related to the haplodiploid mode of reproduction in *Telenomus,* where fertilized eggs originate females and unfertilized eggs originate males. If fertilization problems existed due to crossing incompatibility between different *Telenomus* species, they would lead to a significant increase in the proportion of males. Our results, however, showed that the proportion of females increased with generations.

The development time did increase throughout the generations, which can be explained by the fact that the parasitoids were kept at room temperature until mid November (2nd and 3rd generations). Temperature seems to influence directly the hatching period of *Telenomus* (*e.g.* CHABY-OLAYE *et al.*, 1997; CHABY-OLAYE *et al.*, 2001).

The increase in unviable eggs rate with the increase in the number of females may be explained by superparasitism (VAN ALPHEN & VISSER, 1990; RABINOVICH *et al.*, 2000). This phenomenon may have occurred because of the limited number of available eggs and the impossibility for females to search for other egg resources. Since only one *Telenomus* specimen develops inside a noctuid egg, multiple ovipositions would lead to competition for the resources among the developing parasitoid larvae, and usually older larvae kill small ones. RABINOVICH *et al.* (2000) found that, in *Telenomus fariai* Lima, a gregarious egg endoparasitoid of heteropterans, larval mortality occurs when the number of larvae exceeded the resource capacity of the parasitized egg. Hence, mortality may occur if we accept that only one specimen of *Telenomus* could totally develop within a noctuid egg.

Some authors believe that superparasitism can be an adaptive strategy for insect parasitoids in some situations, increasing the probability of gaining offspring from hosts (VAN ALPHEN & VISSER, 1990). For example, for solitary parasitoids, such as *Telenomus*, VAN ALPHEN & VISSER (1990) did observe that laying more than one egg in a host could be advantageous when the risk that a conspecific parasitoid searching for the same resource is high, because the progeny has a higher chance of surviving. Another situation where superparasitism is beneficial occurs when the host is able to encapsulate parasitoid eggs. Hosts hemocytes can form a multice-

llular capsule enveloping and killing the parasitoid egg. In hosts where evasion of encapsulation is impossible, multioviposition in a parasitoid host may be adaptive when encapsulation of the first egg laid in the host has exhausted the hemocyte supply of the host (VAN ALPHEN & VISSER, 1990).

One important characteristic for a good performance by a natural enemy is the synchronization between its field activity and the life cycle of the host pest. In processing tomato, the two major groups of egg parasitoids belong either to the genus *Telenomus*, which have been reported as parasitoids attacking *H. armigera* eggs earlier in the processing tomato growing season, or to the genus *Trichogramma* spp., which appear later. While *Telenomus* are solitary parasitoids, trichograms are gregarious, which results in the production of multiple progeny from one parasitized noctuid egg. Species of the genus *Trichogramma* have a higher increase rate in their population levels, but have a shorter life span when compared with *Telenomus*, both in field and in laboratory (ARAÚJO, 1990; *personal observation*). *Telenomus* have other characteristics that increase its potential as biological control agents, like other parasitoids reared for biological control purposes, such as simplicity of the diet when adults (honey solution), absence of known hyperparasitoids, reduced size which implies little space necessary for rearing and ability to use alternative hosts, like demonstrated in this study. It is known that the characteristics of the tomato plant itself may interfere with egg parasitism rates, as pointed out by KENNEDY (2003) since the glandular trichomes present in the tomato plants may work as traps for the egg parasitoids, particularly the smaller ones, while the larger species, as *Telenomus*, are less vulnerable to these plant defences.

All these characteristics indicate that the *Telenomus* parasitoids have great potential as biological control agents (ORR, 1988).

CONCLUSIONS

This study demonstrates that it is possible to rear specimens of the genus *Telenomus* under laboratory conditions, using noctuids captured in processing tomato fields as hosts. Our results demonstrated that *C. chalcites* was the most adequate host based both on studied parameters and on the easy handling of the host in the laboratory rearing.

The parasitism capacity of *Telenomus* and its previous use by some researchers in biological control programmes are indicators of its great potential for pest control. The release of mass reared *Telenomus* in processing tomato fields might be a quite useful procedure to be followed in the future. This will be possible throughout the development and improvement of mass rearing techniques, the investigation of the *Telenomus*' ability to parasitize other host eggs and a better taxonomical knowledge of the species present in a specific ecosystem.

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RESUMEN

DUARTE S., C. I. GONÇALVES, E. FIGUEIREDO, J. A. QUARTAU, A. MEXIA, F. AMARO. 2006. Viabilidad de cría de *Telenomus* sp. (Hymenoptera, Scelionidae), un parásito de huevos de *Helicoverpa armigera* (Lepidoptera, Noctuidae), bajo condiciones de laboratorio. *Bol. San. Veg. Plagas*, **32**: 513-521.

El género *Telenomus* Haliday es compuesto por himenópteros parásitos del huevo que atacan huéspedes de insectos de las órdenes Lepidoptera y Hemiptera, principalmente.

En Portugal, en campos de tomate de industria, la presencia de *Telenomus* sp. es casi coincidente con los primeros huevos del oruga del tomate.

Desde mayo hasta septiembre de 2004, seis campos de tomate de industria en la región de Ribatejo (Portugal central) fueron monitorizados semanalmente, 50 plantas fueron observadas por campo y los huevos de la oruga del tomate fueron recogidos.

Los objetivos establecidos eran: verificar la posibilidad de cría de los parásitos de *Telenomus* sp. bajo condiciones de laboratorio investigando qué huésped era más adecuado y evaluando las características biológicas de los parásitos creados.

Los especimenes de *Telenomus* sp. fueron estudiados con respecto su capacidad de parasitar la gama de huéspedes: *Autographa gamma* (Linnaeus), *Chrysodeixis chalcites* (Esper), *Helicoverpa armigera* (Hübner), *Peridroma saucia* (Hübner) and *Thysanoplusia orichalcea* (Fabricius), y el piralideo *Ephestia kuehniella* Zeller.

El cría de *Telenomus* era posible bajo condiciones de laboratorio, en 25±1°C, 65±10% HR y 12:12 (L:D). El tiempo de desarrollo, desde la oviposición a la eclosión, era de cerca de 20 días.

Durante la cría en laboratorio, algunos factores indicaron una existencia posible de situaciones de superparasitismo. Por otra parte, el número de hembras que emergieron aumentó a través de las generaciones.

C. chalcites demostró ser el huésped más apropiado, con la tasa más alta de parasitismo, junto con el índice más bajo de la eclosión de larvas y huevos colapsados. *A. gamma* demostró un alto índice de huevos colapsados, mientras que *H. armigera* demostró una tasa baja de parasitismo. Finalmente, los huevos de *E. kuehniella* fueron rechazados debido a su tamaño pequeño.

Palabras clave: *Telenomus* sp., parásitos de huevos, cría en laboratorio, huéspedes.

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