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Diversity and geographic distribution of the indigenous and exotic parasitoids of the olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae), in Southern France

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Abstract: The olive fruit fly, *Bactrocera oleae* (Dipt., Tephritidae), is the most important pest of olive crops in the world. Economic losses associated to the limited efficiency of pesticides and natural regulation require the development of new alternatives. A classical biological control program was thus implemented in 2007 in France with two main objectives: (1) test the efficiency of a new exotic parasitoid, *Psyttalia lounsburyi* (Hym., Braconidae) on the olive fruit fly populations and (2) understand how intraspecific hybridization could affect the demographic success of exotic biocontrol agents and, more generally, invasive species. In 2008, more than 43,000 *P. lounsburyi* were consequently introduced in 60 sites located in Southern France, covering the whole geographic distribution of olive crops in this country. The pluri-annual surveys realised between 2007 and 2010 gave the opportunity to better document the dynamics of olive fruit fly populations as well as the associated communities of parasitoids. Main results on these two topics are outlined here in a view to stimulate collaborative research and more precisely document the community ecology of *B. oleae* and its natural enemies in the Mediterranean area and elsewhere.

Key words: *Bactrocera oleae*, barcoding, classical biological control, *Psyttalia lounsburyi*, parasitoids community

Introduction

The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is a major pest of olive crops worldwide and its damage represents 5% of the total olive production (Nardi *et al*., 2005). This pest is reported in South and Central Africa, Canary Islands, the Near and Middle East, California, Central America and is widespread in the Mediterranean basin (Daane & Johnson, 2010) which represents 98% of the cultivated olive trees in the world (Montiel Bueno & Jones, 2002).

The management of *B. oleae* populations is still based on conventional control strategies and particularly insecticides which are incompatible with the development of organic productions (Daane & Johnson, 2010). Biological control attempts using parasitoids were developed to control the olive fruit fly populations and several parasitoids were found to parasitize *B. oleae* in different countries (Daane *et al*., 2011), including Africa, its supposed native area (Nardi *et al*., 2005). Even though several species were found to be potentially effective, laboratory and field investigations are still under progress (Daane *et al*., 2011).

A classical biological control program was implemented in France in 2007 with two main objectives: (i) to assess the efficiency of a new potential biological control agent,

Psyttalia lounsburyi (Silvestri, 1913) (Hymenoptera, Braconidae), in Southern France, and (ii) to use classical biological control as an experimental frame for testing hypothesis in invasion biology. With regard to this second objective, we more precisely investigated whether intra-specific hybridization could favour the establishment of invasive species (Malausa *et al*., 2010a; Cheyppe-Buchmann *et al*., 2011). This led us to release in the field a total of 43,000 *P. lounsburyi* originating either from Kenya or from South Africa, and labour intensive pre- and post-release surveys were carried out to try to recover established populations.

In addition to the initial objective, these surveys gave also a good opportunity to better document the dynamics of the olive fruit fly as well as the associated community of parasitoids. Integrating results of 2009 and 2010 and using new approaches (multivariate statistics and molecular characterization), we update here the results given by Malausa *et al*. (2010b).

Material and methods

Field releases and surveys

The introduction of the Kenyan and South African strains of *P. lounsburyi* was realised in 2008 and detailed in Malausa *et al*. (2010b). In brief, about 43,000 individuals were spread over 60 locations with a factorial design (20 locations for each strain and 20 other ones with a mix of both origins). In order to monitor the establishment and local dynamics of the strains, pluri-annual surveys were organized between 2007 and 2010. For each year and in each location, 1000 olives were collected and brought back to the laboratory. The emergence of all insects was checked and all the individuals were visually sorted and kept in alcohol for further morphological or molecular analysis. It is noteworthy that no chemical treatments were normally realized on the restricted area of the olive sampling, even on sites with conventional oleiculture.

Additional individuals of *Eupelmus* and *Pnigalio* were respectively provided by Gérard Delvare and Marco Gebiola (Dipartimento di Entomologia e Zoologia Agraria “F. Silvestri” – Facoltà di Agraria – Università degli Studi di Napoli “Federico II” – Portici, Italy). These individuals were previously unambiguously characterized using morphological and/or molecular markers.

Molecular characterization

Genomic DNA was extracted with the prepGEM insect Kit (Zygem). The cytochrome oxidase c subunit I (COI) was amplified using the primer LCO 1490 (5’-GGTCAACAAATCAT AAAGATATTGG-3’) and HCO 2198 (5’-TAAACTTCAGGGTGACCAAAAAATCA-3’) (Folmer *et al.,* 1994). PCR amplifications were usually carried out in a total volume of 25µl using the Phusion High-Fidelity DNA polymerase 530l kit (FINNZYMES, Espoo, Finland) (1X Phusion HF buffer, 200µM of dNTPs, 0.5µM of each primer and 0.125 U Phusion enzyme Taq polymerase) and 2µl of DNA. PCR thermal cycling program was (i) initial denaturation at 98°C for 30s, (ii) 35 cycles of the three steps: 98°C for 10 s, 52°C, for 15 s, 72°C for 15 s, and (iii) final extension at 72°C for 5 min. Sequences were corrected manually. Multiple alignment and phylogenetic analysis were both performed using MEGA 5 (Tamura *et al*., 2011).

Statistical analysis

Multivariate analysis performed by R version 2.11.1 (R Development Core Team 2010) was used to investigate to relationships between insect’s abundance (both olive fruit fly and

parasitoids) and different factors: distance to sea, altitude, latitude, year and pest management practices. All variables displayed a significant level of multicolinearity, thus we used Principal Component Analysis (hereafter PCA) to identify subsets of variables with a comparable effect on fruit fly and parasitoid abundance. We performed the PCA using "princomp" procedure, and we relied on the correlation matrix to generate PCA scores to standardize the scale of variables. Because of substantial missing data (~30%) for pest management practices, we did not include this variable in the PCA but we checked for its distribution along the PCA axes afterwards. The analysis included 11 variables, and we retained as significant the principal components that accounted for more variance than expected under a homogenous distribution (> 9.1% of variance per component).

Results and discussion

Olive fruit fly and parasitoids distribution and abundance

The mean abundance of *Bactrocera oleae* was respectively 28, 24, 39 and 16 flies per 100 olives in 2007, 2008, 2009 and 2010 (Fig. 1). For most of the locations and dates, infestation rates were less than 30 flies per 100 olives. Nevertheless, very high infestation rates (more than 100 flies per 100 olives) were locally observed (3 locations in 2007, 1 in 2008, 6 in 2009 and 1 in 2010). One site located in the department of Pyrénées-Orientales had more than 100 flies per 100 olives each year.

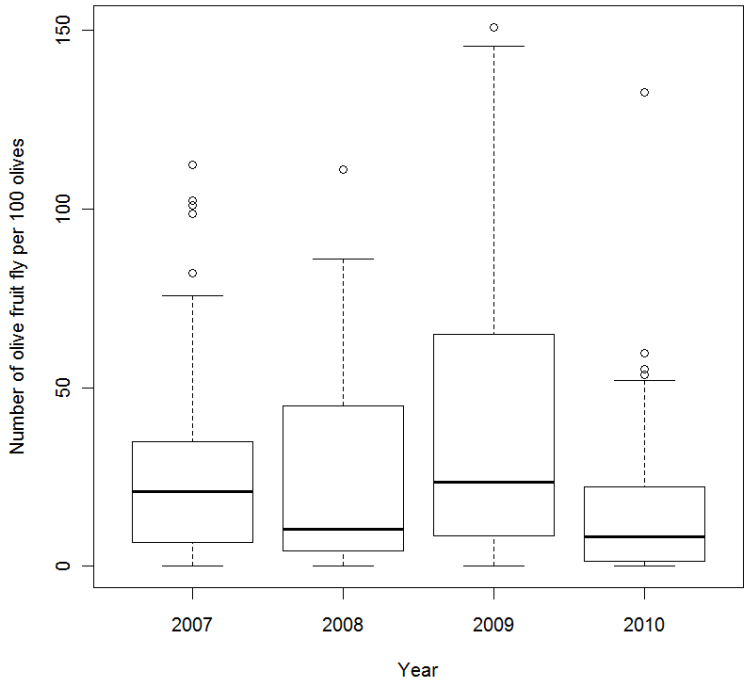

Figure 1. Abundance of *Bactrocera oleae* in Southern France between 2007 and 2010.

With regard to the olive fruit fly, the number of parasitoids appears to be low since the mean "apparent parasitism rate" (*i.e.* number of parasitoids emerged divided by the number of flies and parasitoids emerged) was 3.3%, 0.4%, 1.9% and 0.2 % in 2007, 2008, 2009 and

2010, respectively. The Corsica island is of particular interest insofar as (i) most of the parasitoids and highest "apparent parasitism rate" were found there (57.27%, 10.26%, 48.28% and 4%); (ii) this is the only place where the four genera of native parasitoids were found and where the presence of parasitoids was stable across years (at least for two of the four locations). On the continental side of France, only 3 "hotspots" of parasitoids were observed and disseminated in 3 different Departments: Aude (with a total of 26 individuals), Hérault (with a total of 90 individuals) and Pyrénées Orientales (with a total of 189 individuals).

The PCA evidences some correlations between the annual abundances of pests and parasitoids (all species included) and some geographical data. In particular, 56% of the variability was explained by the two first components of the PCA. As shown in Figure 2, a negative correlation was found on the first component between, on one side, the olive fruit flies and parasitoids abundance and, on the other side, geographical variables (altitude, latitude and distance to sea). The second component evidences negative correlation between olive fruit flies and parasitoids abundance for all years except 2007. The third component (explaining 12% of the variability) shows that there is no correlation between abundance of flies and year (data not shown). Finally, there is no clear correlation between the pest management practices and the abundance of flies and parasitoids, but results show a tendency to have more flies in organic crops (Fig. 2).

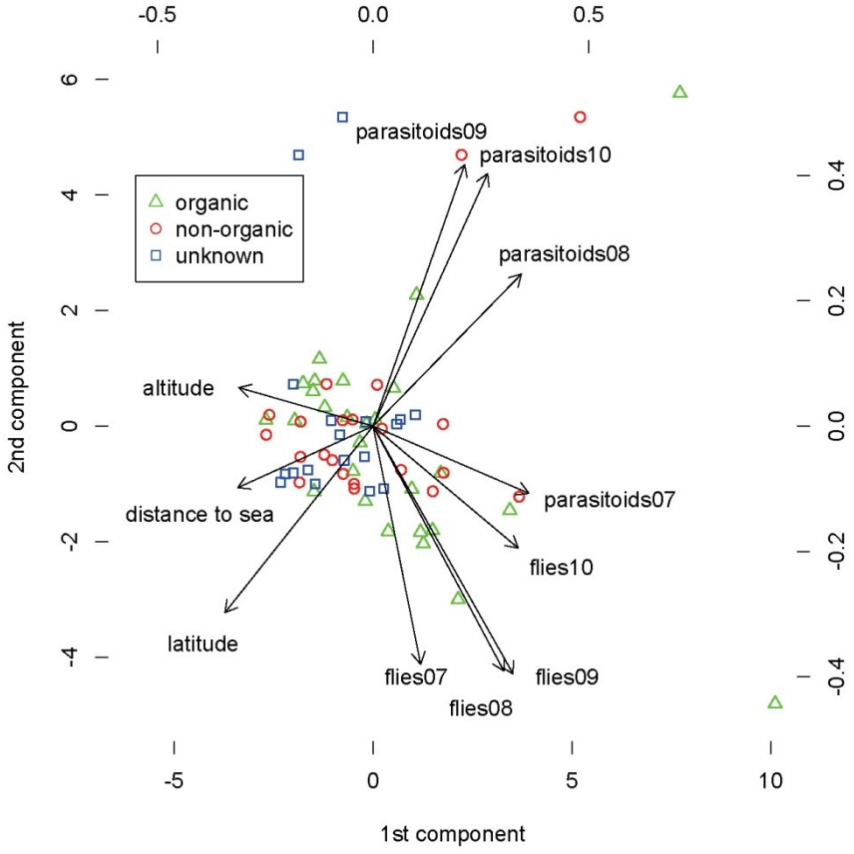

Figure 2. Influence of geographic parameters and cultural practices on the abundances of *Bactrocera oleae* and its parasitoids (all species included). The two first components of the Principal Component Analysis were represented here. They respectively represent 38% and 18% of the overall variability. The pest management practices were plotted afterwards.

Diversity of the parasitoids

A total of 120 individuals were successfully molecularly characterized and main haplotypes are shown in the Figure 3. Even if this analysis was performed on a relatively short part of a single gene, this method allowed the discrimination between related species within the main genera (*Psyttalia*, *Pnigalio* and *Eupelmus*).

Concerning the introduced *Psyttalia lounsburyi*, only 20 individuals were recovered during our 4-year field survey: 16 in 2008 (distributed on 10 sites) and 4 in 2009 (on 2 sites). There is no site where *P. lounsburyi* was recovered two successive years. No individuals were collected in 2010. Four haplotypes were found and they logically correspond to previously identified ones (Cheyppe-Buchmann *et al*., 2011). Even if the latency time of introduced exotic insects could be long (Kiritani & Yamamura, 2003), the establishment of *P. lounsburyi* in Southern France seems doubtful after 3 years of post-release survey done in a wide variety of sites and different local conditions. The inability of this African species to overwinter under the French climate could partly explain this failure (R. Exilien & M. Thaon, unpublished data).

Some specimens of another *Psyttalia* species, *P. concolor*, were collected in 2008 (6 individuals in 1 site) and in 2009 (48 individuals in 1 site). This species was found only in Corsica where, to our knowledge, no releases were done. Since this species had been nevertheless released many times in the Mediterranean Basin (Delucchi, 1957), two hypotheses can be proposed: either these individuals came from an established population (Malausa *et al*., 2008), either these individuals are transient ones, originating from Italia where this species is established. It is noteworthy that the genetic diversity in this species seems to be rather low with only 2 haplotypes.

With 452 individuals collected between 2007 and 2010 on 22 sites, *Pnigalio* is the most abundant genus found to parasitize the olive fruit fly in France. This confirms data obtained in other countries (Boccaccio & Petacchi, 2009; Daane & Johnson, 2010). Although the taxonomy of *Pnigalio* and the status of *P. mediterraneus* Ferrière & Delucchi, 1957, and *P. agraules* (Walker, 1839) were controversial (Askew, 1984; Gebiola *et al*., 2009), the molecular characterization provided evidences that all individuals can be identified as *Pnigalio mediterraneus*. A large number of haplotypes was found in agreement with the diversity found on Italian individuals provided by Marco Gebiola.

With 120 individuals collected between 2007 and 2010 on 9 sites, *Eupelmus* is the second most abundant genus on *B. oleae* in Southern France. All these individuals are distributed into two main groups corresponding to *E. urozonus* Dalman, 1820 and *E. martellii* Masi, 1941 in quite the same proportions (respectively 55% and 45%). A geographical pattern can be observed since *E. martellii* was found only in the continental side of France while most of the *E. urozonus* were found in Corsica. It is noteworthy that both *E. urozonus* and *E. martellii* were clearly differentiated from the species *E. microzonus* Förster, 1860, that might occur in the vicinity of olive trees.

Finally, few specimens (4 in 2007 and 3 in 2009) of the genus *Eurytoma* were collected during our study. Based on morphological characters, we identified this species as *E. martellii* Domenichini, 1960*,* even if two distinct genetic groups seem to occur.

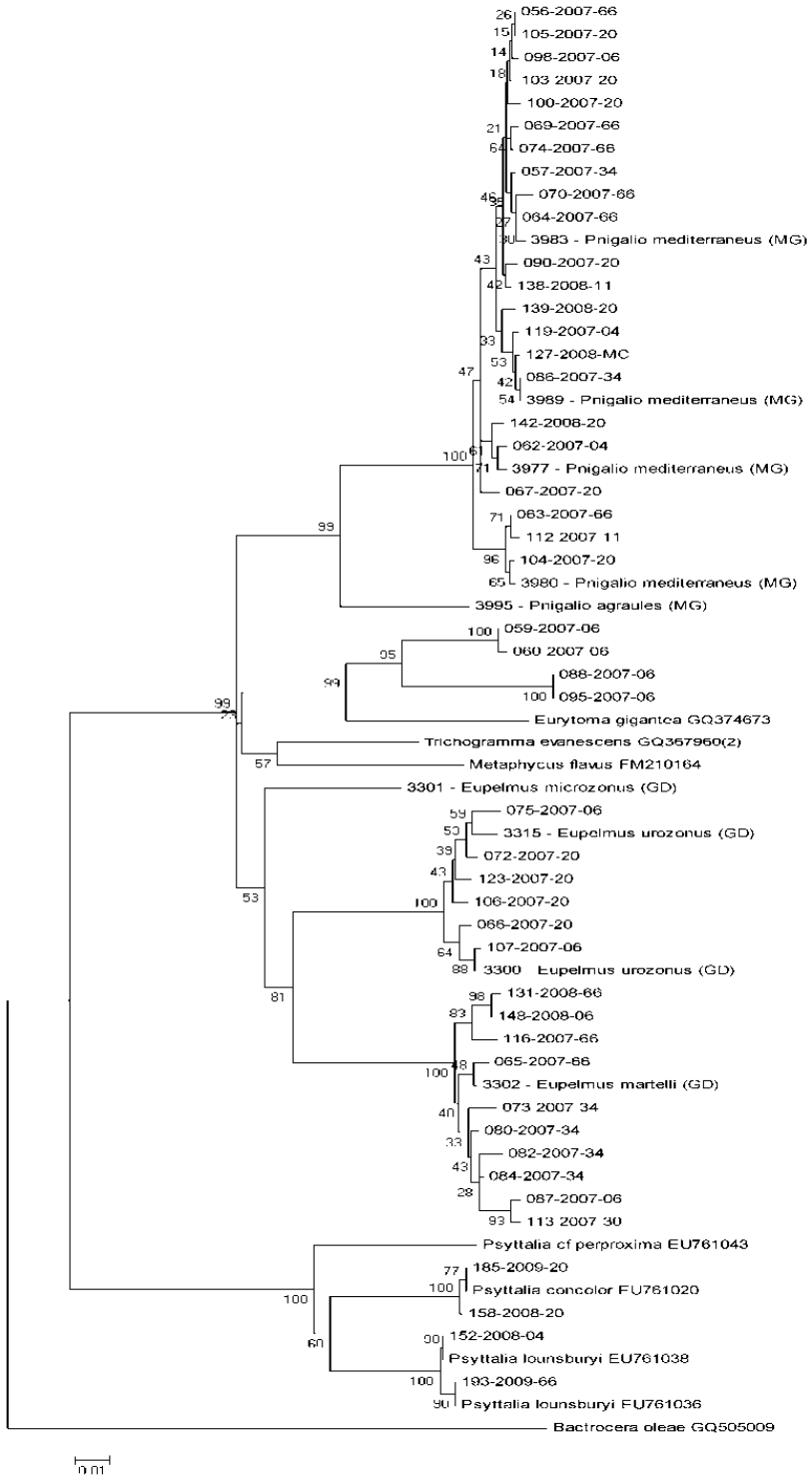

Figure 3. Genetic diversity of parasitoids associated to *B. oleae* in France. This consensus phylogenetic tree was based on 393pb of the Cytochrome oxidase c sub-unit I. (Neighbor-Joining; p-distance; bootstrap: 1000 replicates). Sequences used were obtained from the 2007-2010 field survey, from other individuals previously identified by Gérard Delvare or Marco Gebiola (repectively GD and MG in the figure) or from Genbank. For the two first cases, the left side of the code corresponds to an intrinsic identifier. For the individuals caught in the field, central and right parts of the code respectively correspond to the year of release and the geographical origin (04 = Alpes-de-Haute-Provence; 06 = Alpes-Maritimes; 11 = Aude; 20 = Corsica; 30 = Gard; 34 = Hérault; 66 = Pyrénées-Orientales; MC = Principality of Monaco).

Conclusion

With regard to the initial objective of testing the role of intraspecific hybridization on the establishment of the biocontrol agent *P. lounsburyi*, this labour-intensive field survey unfortunately brought pessimistic results in so far as no perennial populations of *P. lounsburyi* have been found. This work nevertheless provides relevant information about the abundance and diversity of both the olive fruit fly and its native natural enemies. Geo-climatic conditions seem indeed to be the main driver of the abundance of these insects with decreasing gradient from the sea-side to the hinterland. Although the number of parasitoids found was low, partial negative correlation between pest and natural enemies abundances suggest that some regulation may occur. The optimization of this ecosystem service may be useful to decrease the damage caused by *B. oleae* but care must be taken to precisely understand ecological interaction between *B. oleae*, the main native parasitoids as well as other potential interacting species. In this context, barcoding approach has proved to be useful to discriminate between closely related species such as in the genus *Eupelmus*.

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References

Askew, R. R. 1984: Species of *Pnigalio* and *Chrysocharis* (Hymenoptera: Eulophidae) parasitic on Tischeriidae (Lepidoptera), with the description of a new species. Entomol. Gazette 35: 103-109.

Boccaccio, L. & Petacchi, R. 2009: Landscape effects on the complex of *Bactrocera oleae* parasitoids and implications for conservation biological control. Biocontrol 54: 607-616.

Cheyppe-Buchmann, S., Bon, M. C., Warot, S., Jones, W., Malausa, T., Fauvergue, X. & Ris, N. 2011: Molecular characterization of *Psyttalia lounsburyi*, a candidate biocontrol agent of the olive fruit fly, and its *Wolbachia* symbionts as a pre-requesite for future intraspecific hybridization. Biocontrol (Online first).

Daane, K. M. & Johnson, M. W. 2010: Olive fruit fly: Managing an ancient pest in modern times. Ann. Rev. Entomol. 55: 151-169.

Daane, K. M., Johnson, M. W., Pickett, C. H., Sime, K. R., Wang, X. G., Nadel, H., Andrews Jr. J. W. & Hoelmer, K. A. 2011: Biological controls investigated to aid management of olive fruit fly in California. Calif. Agric. 65: 21-28.

Delucchi, V. 1957: Les parasites de la mouche des olives. Entomophaga 2: 107-118.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. 1994: DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3:294-299.

Gebiola, M., Bernardo, U., Monti, M. M., Navone, P. & Viggiani, G. 2009: *Pnigalio agraules* (Walker) and *Pnigalio mediterraneus* Ferrière and Delucchi (Hymenoptera: Eulophidae): two closely related valid species. J. Nat. Hist. 43: 2465-2480.

Kiritani, K. & Yamamura, K. 2003: Exotic insects and their pathways for invasion. In: Invasive species: vectors and management strategies, eds. Ruiz, G. & Carlton, J. T., Island Press, Washington, USA: 44-67.

Malausa, J. C., Blanchet, A., Bon, M. C., Cheyppe-Buchmann, S., Groussier-Bout, G., Jones, W., Pickett, C., Ris, N., Thaon, M. & Fauvergue, X. 2010a: Introductions of the African parasitoid *Psyttalia lounsburyi* in South of France for the classical biological control of *Bactrocera oleae*: will hybridization affect establishment and population growth? IOBC-WPRS Bull. 53: 49-55.

Malausa, J. C., Auguste-Maros, A., Cheyppe-Buchmann, S., Groussier-Bout, G., Ris, N., Thaon, M., Warot, S. & Fauvergue, X. 2010b: Introductions of the African parasitoid *Psyttalia lounsburyi* in South of France for classical biological control of *Bactrocera oleae*. IOBC-WPRS Bull. 59: 163-170.

Malausa, J. C., Rabasse, J. M. & Kreiter, P. 2008: Les insectes entomophages d'intérêt agricole acclimatés en France métropolitaine depuis le début du 20ème siècle. OEPP Bull. 38: 136-146.

Montiel Bueno, A. & Jones, O. 2002: Alternative methods for controlling the olive fly, *Bactrocera oleae*, involving semiochemicals. IOBC-WPRS Bull. 25: 1-11.

Nardi, F., Carapelli, A., Dallai, R., Roderick, G. K. & Frati, F. 2005: Population structure and colonization history of the olive fly, *Bactrocera oleae* (Diptera, Tephritidae). Mol. Ecol. 14: 2729-2738.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011: MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution (in press).