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The complete mitogenome of the European mantis, *Mantis religiosa*, from Italy: implications for the origin of North American mantis population

Andrea LUCHETTI[1], Lino OMETTO[2], Omar ROTA-STABELLI[3]
[1]*Department of Biological, Geological and Environmental Sciences, University of Bologna, Italy*
[2]*Department of Biology and Biotechnology, University of Pavia, Italy*
[3]*Center Agriculture Food Environment C3A, University of Trento/Fondazione Edmund Mach, Italy*

Abstract

The European mantis, *Mantis religiosa* L. (Mantodea Mantidae), is distributed all over Southern Europe, Africa and Asia, and has been reported as alien species in North America. Here we present the mitogenome sequence of an Italian individual and compare it with previously sequenced Chinese and Canadian samples. The assembled mitogenome has a length of 15,530 nucleotides and includes 13 protein coding genes, two ribosomal RNA genes, 23 tRNA genes (including the additional Arginine tRNA already observed in other *M. religiosa* mitogenomes), and the control region. Based on the inferred phylogenetic relationships, the Canadian sample is more closely related to the Italian than to the Chinese one, in line with the putative European origin of the North American invasive population. Time-calibrated phylogeny dated the divergence among extant European Mantis lineages at 2.33 million years ago, consistent with the first appearance of *M. religiosa* fossils. Our results support a European origin of the North American *M. religiosa* population and suggest that selective processes acting on mitogenome may have contributed to its adaptation in the new area.

Key words: Mantidae, *Mantis religiosa*, mitogenome, time-calibrated phylogeny.

Introduction

The European mantis, *Mantis religiosa* L. (Mantodea Mantidae), is among the most common mantis species. It is widely distributed all over Southern Europe, Africa and Asia, and has been reported as having been introduced in North America from Europe (Cannings, 2007; Battiston and Fontana, 2010). In the last decades, the warmer temperatures associated to the global climate changes also caused a northward range expansion of this species in Europe (Linn and Griebeler, 2015). *M. religiosa* is considered as of Least Concerns by the IUCN, although specific threats are recognized locally: in fact, some European countries included this species in protection and conservation programs (Battiston, 2016).

Understanding the genetic structure and the evolutionary history of threatened species could be important to better analyse the processes underlying the current population distribution pattern and to manage conservation plans (Frankham, 2003). In fact, the analysis of genetic markers may help in understanding the extent of genetic diversity (including loss of variability or inbreeding), which may also inform about possible population bottlenecks, the population size variation through time, the gene flow between populations or even the possibility of adaptive variations (Hedrick, 2001). Moreover, at the same time, DNA-based methods are also useful to identify and monitor alien/invasive species (Darling and Blum, 2007). Genetic analyses helped to identify and track invasions of some exotic/invasive insects such as, for example, termites (Scicchitano *et al*., 2018; Ghesini *et al*., 2020), flies (Kremmer *et al*., 2017; Rota-Stabelli *et al*., 2019) or the infamous brown-marmorated stink bug *Halyomorpha halys* (Stal) (Gariepy *et al*., 2014; Cesari *et al*., 2018).

To this goal, however, it is necessary to develop specific genetic resources necessary to provide useful markers for population delimitation and genetic diversity inferences.

The complete mitogenome of *M. religiosa* has already been sequenced from a Chinese (Ye *et al*., 2016) and a Canadian (Jia *et al*., 2019) sample: they show the very same structure, including a specific additional Arginine tRNA (*trnR*). Samples from Europe are still missing, impairing the understanding of inter-population divergence and the correct inference of the colonization route of the introduced North American population. Here we report the sequencing of the mitogenome from an Italian sample: our dated phylogenies and molecular evolution studies clarify the *M. religiosa* population structure and suggest an episodic mitogenomic adaptation event.

Materials and methods

The sample (stored under the accession praySGP13 at MoZoo Lab, Department BiGeA, University of Bologna) was collected in San Giovanni in Persiceto (44°38'27"N 11°11'06"E), Italy. Total DNA was isolated from legs with Stratec DNA Isolation Kit (Invisorb) and subjected to Next Generation Sequencing with the Illumina HiSeq2000 platform. Two libraries with 350 bp and 550 bp insert size, respectively, were sequenced to produce 125 bp pair ends. Raw reads were trimmed with Trimmomatic using default settings (Bolger *et al*., 2014) and assembled with Platanus Genome Assembler (Kajitani *et al*., 2014). The mitogenome was deposited in NCBI Genbank under the accession number MZ153073.

We obtained full mitogenomes of Mantidae species available from NCBI Genbank (last accessed in December

2020) as well as the sequence of the cryptic mantis *Sibylla pretiosa* Stal, which was used as outgroup. We then extracted the protein coding genes (PCGs) sequences from each mitogenome and aligned them using the Clustal W algorithm implemented in Mega X (Kumar *et al*., 2018). Finally, Maximum Likelihood and Bayesian phylogenetic analyses were carried out on the concatenated PCGs nucleotide alignments using IQ-Tree (Trifinopulos *et al*., 2016), with 1000 ultrafast bootstrap replicates, and with BEAST v. 1.8 (Drummond and Rambaut, 2007), ran for 20×10^6 generations, respectively. Both analyses were performed using the GTR+G+I substitution model. The Bayesian tree was time calibrated using the first appearance of a Mantidae fossil, namely *Eobruneria tessellata* Cockerell, dated at 35.5 million years ago (Cockerell, 1913) (Mya; Paleobiology Database at http://fossilworks.org/, last accessed December 2020): the calibration was implemented with an exponential distribution and a soft maximum bound at 150 Mya. The likelihood ratio test, as implemented in Mega X, rejected ($P = 1.9 \times 10^{-44}$) the hypothesis of substitution rate constancy among branches; therefore, a lognormal relaxed clock was used to model the substitution rate heterogeneity across lineages. The Birth-Death model was used as tree prior.

We used the branch-site test implemented in PAML (Yang, 2007) to evaluate whether the mitochondrial PGCs underwent positive selection along the Canadian *M. religiosa* lineage. In this test (branch-site model A, test 2; Yang *et al*., 2005), the level of selective pressure $\omega = d_N/d_S$ can vary both among sites in the coding region and across branches on the tree (model = 2, NSsites = 2). The null model fixed $\omega_2 = 1$ (fix_omega = 1, omega = 1), whereas the positive selection model allowed $\omega_2 > 1$ (fix_omega = 0, omega = 1) in the foreground species, i.e. in the Canadian *M. religiosa* lineage. A Likelihood ratio test (LRT) was subsequently used to test for the best fitting model. Moreover, aBSREL test (Smith *et al*., 2015) was also used to check for episodic positive selection events on the Canadian *M. religiosa* branch. The aBSREL test implements a branch-site model and infers the optimal number of ω categories to which sites on particular branches are assigned.

Results and discussion

The assembled mitogenome is 15,530 base pairs (bp) long and includes 13 PCGs, two ribosomal RNA genes, 23 tRNA genes (thus, including the additional *trnR*), and the control region (figure 1). The same structure and gene order was also found in previous *M. religiosa* sequencing (Ye *et al*., 2016; Jia *et al*., 2019). Nucleotide content is highly AT-rich (76.8%), as commonly reported in insects

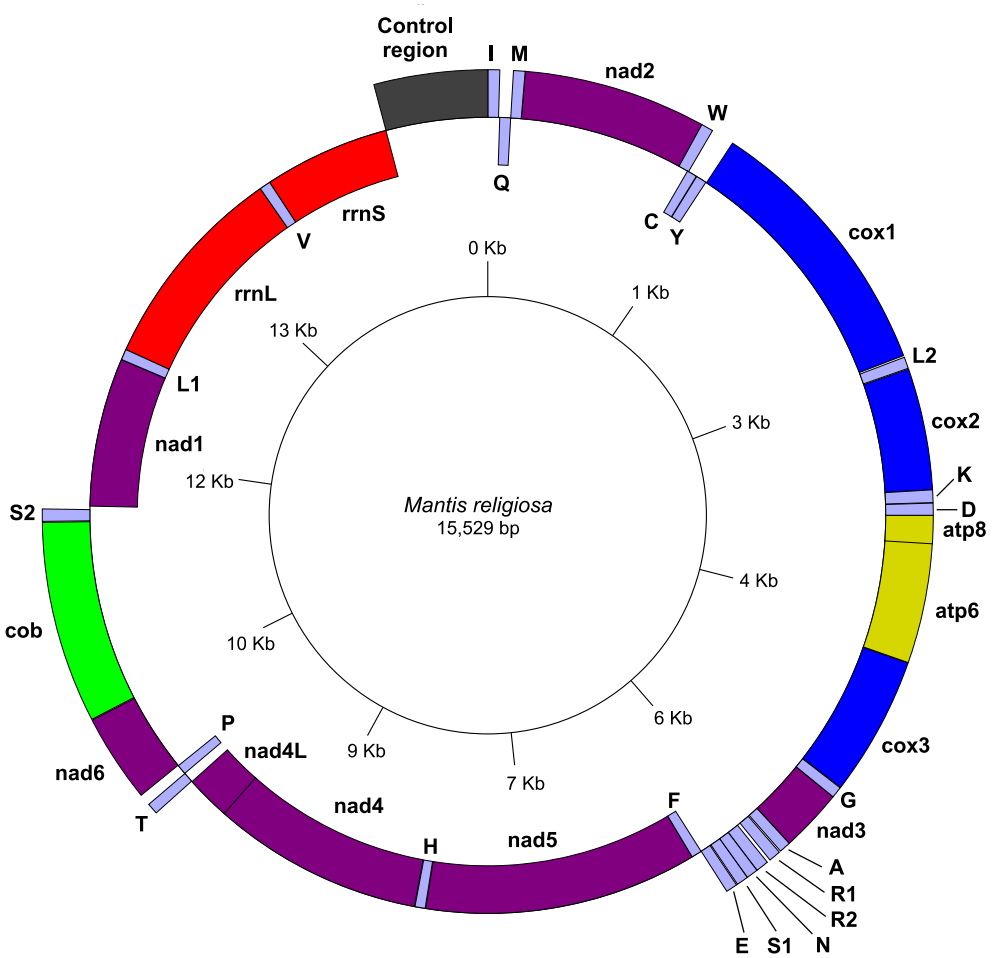

Figure 1. Map of the sequenced *Mantis religiosa* mitochondrial genome.

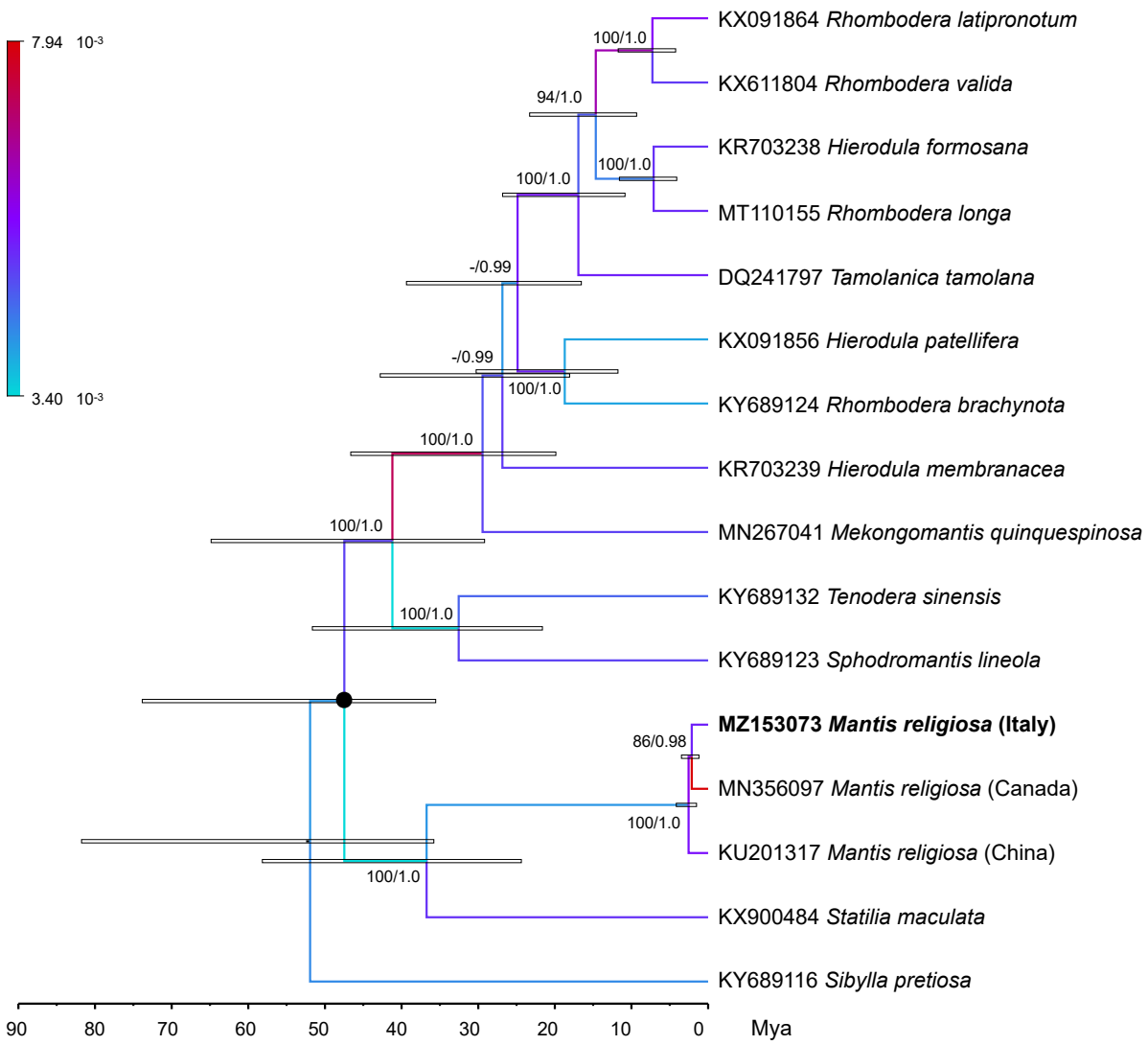

Figure 2. Time-calibrated phylogenetic tree of Mantidae based on concatenated mitochondrial genes. Number at nodes represent Maximum Likelihood bootstrap/Bayesian posterior probability (dash indicates bootstrap values <50). Timescale (Million years ago, Mya) is reported below the tree; the fossil-calibrated node is indicated by the black dot. Bars at nodes represent the age 95% high posterior density. Branches are colored based on the substitution rate, as indicated in the upper left legend which indicates the number of nucleotide substitutions/site/million years. NCBI Genbank accession numbers of mitogenomes are also reported; presently sequenced sample is highlighted in bold.

(Cameron, 2014). The start codon of most PCGs is ATN (with N being any nucleotide) but for the *cox1*, where it is TTG. Stop codon was always TAA, except for *cox2* and *nad5*, where it was incomplete (T--) and likely completed after the addition of AA upon transcription (Boore *et al*., 1999). The overall nucleotide variability, calculated as the proportion of different nucleotides between sequences, across PCGs of the three *M. religiosa* samples was 1.68%, the most variable gene being the *nad6* (3.20%) and the least variable being the *nad5* (1.20%). The Italian mantis was slightly more similar to the Canadian sample, with a divergence of 1.63%, than to the Chinese one (divergence = 1.73%).

Maximum Likelihood and Bayesian tree shared the same topology, although two nodes were not supported by the Maximum Likelihood analysis (figure 2). The phylogenetic relationships are in line with previous analyses (Ye *et al*., 2016; Zhang *et al*., 2018); the three *M. religiosa* samples are correctly clustered together, in sister relationship with the *Statilia maculata* (Thunberg) mitogenome. Timing of cladogenetic events indicated the divergence of *M. religiosa* from its sister lineage at about 36.7 Mya, in full agreement with previous analyses (Vidal-García *et al*., 2020). Moreover, the divergence among extant European mantis linages was dated back to 2.55 Mya with a 95% high posterior density of 1.95 Mya - 4.14 Mya. This is consistent with the first appearance of *M. religiosa* fossils that occurred between 3.6 Mya and 2.6 Mya (Beier, 1967).

The Italian and the Canadian *M. religiosa* samples resulted more closely related to each other, confirming a European origin of the Canadian population (Cannings, 2007; Battiston and Fontana, 2010). It is interesting to note that along the branch leading to the Canadian sample there is an increase of the nucleotide substitution rate

Table 1. Maximum likelihood estimates of ω for the site classes ω_0 (under purifying selection in both background and foreground branches), ω_1 (under neutral evolution in both background and foreground branches), ω_{2a} (under neutral evolution in the background and positive selection in the foreground branches) and ω_{2b} (under purifying selection in the background and positive selection in the foreground branches). The foreground branch is the Canadian *M. religiosa* lineage and values were estimated by the Branch-site model A.

Site class	ω_0	ω_1	ω_{2a}	ω_{2b}
Proportion	0.98509	0.01446	0.00044	0.00001
Background ω	0.01524	1.00000	0.01524	1.00000
Foreground ω	0.01524	1.00000	236.5	236.5

(figure 2). Additional results of the PAML analysis indicate that this rate acceleration is associated with an increase in non-synonymous substitution rate due to the action of positive selection ($P < 0.001$; table 1). The aBSREL test confirmed this finding, indicating episodic positive selection on the Canadian *M. religiosa* branch ($P < 0.01$; $\omega_1 = 0.036$; $\omega_2 = 0.044$; $\omega_3 = 382.0$). Positive selection on mitochondrial PCGs has been correlated with local adaptation, with special regard to temperature conditions (Florencia Camus *et al*., 2017; Lajbner *et al*., 2018; Li *et al*., 2018; Coyle *et al*., 2019), including in introduced insect populations that experience novel environmental conditions (Li *et al*., 2016). We can thus hypothesize that after its recent introduction in Canada from Europe, the *M. religiosa* population underwent a phase of adaptation to a colder environment.

Overall, our results provide evidence for a European origin of the Canadian *M. religiosa* population and suggest that selective processes may have contributed to the fast spread of the introduced population.

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Authors' addresses: Andrea LUCHETTI (corresponding author: andrea.luchetti@unibo.it), Department of Biological, Geological and Environmental Sciences, *Alma Mater Studiorum* Università di Bologna, via Selmi 3, 40126 Bologna, Italy; Lino OMETTO, Department of Biology and Biotechnology, University of Pavia, via Ferrata 9, 27100 Pavia, Italy; Omar ROTA-STABELLI, Center Agriculture Food Environment C3A, University of Trento/Fondazione Edmund Mach, via E. Mach 1, 38098 San Michele all'Adige, Italy.

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