Argas (Persicargas) persicus (Oken, 1818) (Ixodida: Argasidae) in Sicily with considerations about its Italian and West-Mediterranean distribution

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Summary:

Recently, in the province of Trapani (Western Sicily), some overwintering specimens of the argasid tick *Argas (Persicargas) persicus* (Oken, 1818) were observed and collected. Morphological and genetic analysis were utilized in order to reach a definitive identification. The species was found in two semi-natural sites where, having been found repeatedly, its presence does not appear accidental. Moreover the characteristics of the Sicilian findings seem to exclude a human-induced spread. This record, the first regarding Sicily and South Italy, is discussed together with the previous doubtful citations for Italy. These findings revalue not only all the old citations for Italy but also the hypothesis that the Mediterranean distribution of this argasid is of a natural origin.

KEY WORDS: Ixodida, Argasidae, geographic distribution, Italy.

Résumé: Argas (Persicargas) persicus (Oken, 1818) (Ixodida: Argasidae) en Sicile avec quelques considérations sur sa distribution en Italie et dans la Méditerranée occidentale

Récemment, des exemplaires de tique des volailles Argas (Persicargas) persicus (Oken, 1818) en diapause ont été observés et récoltés dans la province de Trapani (Sicile occidentale). L'identification a été confirmée par les analyses morphologiques et génétiques. L'espèce, présente dans deux zones semi-naturelles, a été retrouvée plusieurs fois, ce qui fait paraître sa présence comme non accidentelle. En plus, les caractéristiques des découvertes siciliennes paraissent exclure une diffusion anthropique. Cette signalisation, la première concernant la Sicile et l'Italie du sud, est discutée avec d'autres précédentes citations suspectées mais non confirmées pour l'Italie. Ces découvertes remettent en valeur non seulement les anciennes citations pour l'Italie, mais aussi l'hypothèse que la distribution méditerranéenne de la tique des volailles est effectivement d'origine naturelle.

MOTS CLÉS: Ixodida, Argasidae, distribution géographique, Italie.

INTRODUCTION

rgas (Persicargas) persicus (Oken, 1818) (Ixodida: Argasidae) is today considered native in Turanian-Central Asia as a parasite of arboreal nesting birds, and has successfully adapted to coexistence with domestic fowl. Probably via human transport, it has spread throughout the continents, where it survives practically exclusively in association with domestic fowl (chickens, turkeys, Helmeted Guinea fowls, and others) and infrequently pigeons (Hoogstraal et al., 1979, 1981; Hoogstraal, 1985; Keirans & Durden, 2001). In some countries this tick is the most important poultry ectoparasite (Arends, 2003; Shah et al., 2004; Nicholson

et al., 2009). It is rare in modern poultry production, but it finds a habitat very compatible with its biology in breeding houses (Axtell, 1999).

Beginning more or less fifty years ago, through the use of the SEM (scanning electron microscope) and above all a virological-biosystematic approach, the interpretation of this taxon has changed dramatically with the discovery of closely related species (describing them as new or resurrecting incorrect synonymies) put together in the subgenus *Persicargas* Kaiser, Hoogstraal & Kohls, 1964 (Hoogstraal *et al.*, 1979; Hoogstraal, 1985). So numerous old published records of *A. persicus* became doubtful and the distribution of this species needed to be confirmed often (e.g. Easton, 2006). Nevertheless its presence in America (Kohls *et al.*, 1970; Keirans & Durden, 2001), Africa (Eastwood, 1972; Sylla *et al.*, 2004), India (Gosh *et al.*, 2007), Australia (Petney *et al.*, 2004) is sure nowadays.

In western parts of its palearctic areal this tick is known in the Middle East, Egypt, Libya and Maghreb on the Southern Mediterranean side and Anatolia, the Balkans (reaching Slovakia in the North and Trieste, Italy, in the West), Corsica and Spain in the North (Rageau, 1972; Doss *et al.*, 1978; Nosek *et al.*, 1980; Manilla, 1986, 1998; Travassos Santos Dias, 1994; Osacar-Jeminex *et al.*, 1998;

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Anonymous, 1999, 2000, 2001). Apparently there are not any sure records for Italy, excluding the only one from Trieste very near to the oriental boundary with the Balkans (Starkoff, 1958b; Manilla, 1998; Iori *et al.*, 2005). Recently, in two sites of the province of Trapani (Western Sicily), some overwintering specimens of an argasid tick belonging to *Argas (Persicargas)* were observed and collected. Morphological and genetic analyses were utilized in order to reach a definitive identification. This record, the first regarding Sicily and South Italy, will be discussed together with the previous doubtful citations for Italy.

MATERIALS AND METHODS

Leica MZ16 stereo microscope and a Leitz Diaplan FSA/20 microscope were used both for morphological observations and for photographs taken with a Leica DFC320 digital camera and LAS (Leica Application Suite) applied software Version 2.5.0 R1.

The DNA extraction was obtained from a single leg of two specimens (one for each site described in the "Results") using the salting-out protocol (Miller et al., 1988). 16S rDNA PCR amplifications and sequencing were performed using primers and conditions as previously described in Petney et al. (2004). The 16S gene portions were aligned in Clustal X ver. 1.64b (Thompson et al., 1997) with representative argasid tick 16S sequences, searched on GenBank (www.ncbi.nlm.nih. gov). We carried out a phylogenetic reconstruction by Maximum Parsimony (MP), (Kluge & Farris, 1969) and Neighbour-Joining (NJ) (Saitou & Nei, 1987) analyses using PAUP 4.0, b. 10 (Swofford, 2001) and by the Bayesian method using MrBayes 3.1 (Ronquist & Huelsenbeck, 2003). Neighbour-Joining trees were constructed with distances computed using the best-fit model obtained with MODELTEST 3.04 (Posada & Crandall, 1998). The GTR model was the best one selected (Tavarè, 1986), corrected for rate heterogeneity among sites with a Gamma distribution (Yang, 1993). MP was carried out using the heuristic search algorithm, with 100 random-taxon-replicates for all analyses. The analysis was performed with ACCTRAN optimization and tree bisection TBR branch swapping, considering all characters as unordered and equally weighted, and gaps treated as fifth state. A strict consensus tree was calculated when there was more than one tree. Non-parametric bootstrapping with heuristic searches of 2000 replicates for MP and NJ was used to assess confidences of branches in MP and NJ. The Bayesian analysis was performed with clade support assessed by posterior probability. Four Markov chains, one heated and three cold, were allowed to run for 2 million generations using random starting trees. Trees were sampled every 100 generations, yielding 8,000 samples of the Markov chain after a "burn-in" of 2,000 generations.

A sequence from a species (*Ornithodorus gurneyi*, Warburton, 1926, GenBank Acc. No AY436767) belonging to subfamily Ornithodorinae, the sister-group of subfamily Argasinae, and already utilized in previous studies (Petney *et al.*, 2004) was chosen as an outgroup. Besides, some argasid tick sequences, found in GenBank, are used as a comparison and included in the analyses.

RESULTS

FINDINGS

n 2 February 2008 in site A (see below), some specimens of an argasid tick were observed and photographed in nature during winter entomological samplings under the bark of *Eucalyptus camaldulensis* Dehnhardt, 1832. In the following winter, on 8 December 2008, the same observation recurred in site B. Consequently *ad hoc* researches were repeated on 21 December 2008 (site B) and 17 January 2009 (site A) collecting the specimens listed below.

Site A was in the Natural Reserve "Foce del Fiume Belice", Castelvetrano, Trapani (Sicily), 37° 34′ 44″ N - 12° 53′ 15″ E; site B in Misiliscemi Guarrato, Trapani (Sicily), 37° 55′ 01″ N - 12° 33′ 57″ E.

Both sites contain small groups of isolated River Red Gum trees which were isolated (site A) (Fig. 1C, 1E) or near a deserted house (site B) (Fig. 1B, 1D), in neither case were there poultry nearby. Old nests of medium-sized unknown birds were present. Probably they belong to the Common Magpie, *Pica pica* Linnaeus, 1758, less probably they should be looked for among arboreal Columbidae, other Corvidae or small Falconidae.

MATERIAL EXAMINED

Site A: 1 male, 1 female conserved by ISE-CNR Sassari (1 female utilized for genetic analysis), 1 female by section of Parasitology DipSAB Cagliari; site B: 2 males, 1 female, 2 nymphs by ISE-CNR Sassari (1 nymph utilized for genetic analysis), 3 males, 2 females by section of Parasitology DipSAB Cagliari. All specimens have been stored in 70 % or 95 % ethanol.

MORPHOLOGY

All specimens, without ventral paired organs, have oval idiosoma, wider behind (Fig. 1F). Postpalpal setae are present. The anterior subdorsal seta of tarsal segment IV is single. The peripheral cells of the dorsum have a single large setiferous pit occupying most of the surface area; the cells are large, mostly rectangular or sub-rectangular

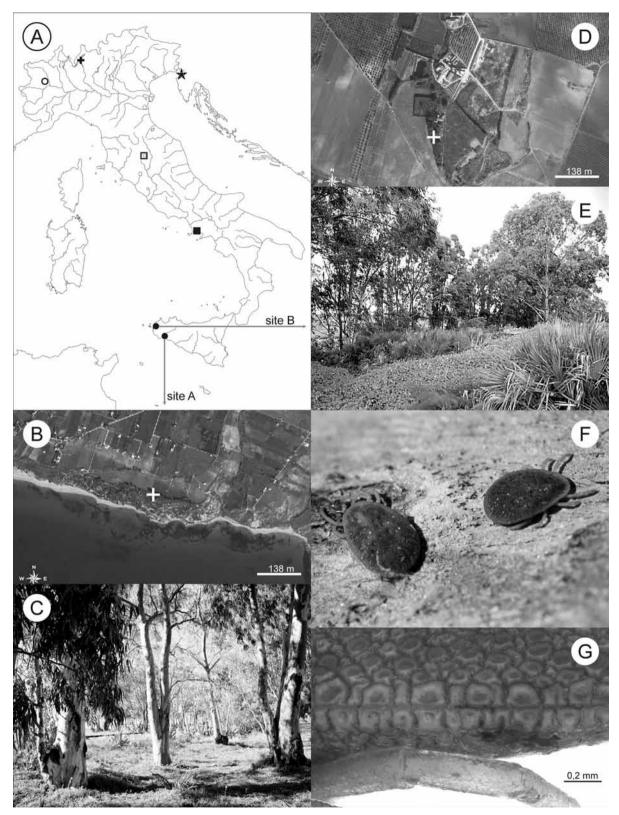


Fig. 1. – A) *Argas persicus*: map of records in Italy; star = Starkoff (1958a, b, 1959) and Manilla (1990 [1991]); cross = Walter & Massa (1987); open circle = Durio *et al.* (1982) and Manilla (1982); open square = Tarello (2001) and Tarello & Riccieri (2003); solid square = Latreille (1824); solid circles = new sites. B) Natural Reserve "Foce del Fiume Belice", Castelvetrano, Trapani (Sicily), aerial photography (by Google Earth); white cross = site A. C) The same, landscape photography of site A (by Marcello Romano). D) Locality Misiliscemi Guarrato, Trapani (Sicily), aerial photography (by Google Earth); white cross = site B. E) The same, landscape photography of site B (by Luigi Barraco). F) Two specimens of *Argas persicus* on River Red Gum, after the removal of the bark, in site A (by Marcello Romano). G) *Argas persicus*: peripheral cells of dorsum.

(Fig. 1G). These and other morphological characteristics (integument, capitulum, genital area, legs, etc.) fit very well with the descriptions of *Argas (Persicargas) persicus* (Oken, 1818) provided by Kaiser *et al.* (1964), Filippova (1966), Khols *et al.* (1970), Manilla (1998).

GENETIC ANALYSIS

The two Sicilian sequences of the 16S gene fragment (448bp) were identical and have been registered by GenBank under No GU451248. The nucleotide divergences among this sample and the other *Argas* sequences published in GenBank ranged from 0,5 % (with *A. persicus* from Australia) to 16 % (*A. vulgaris* AF401404 and *A. polonicus* AF401403). The phylogenetic reconstruction showed that the closest relative to the argasids from

Trapani (Sicily) were other *A. persicus* samples, as suggested by morphological investigations. All sequences belonging to *A. persicus* indeed constitute a cluster separated from the other *Argas* species in all phylogenetic analyses, supported by high bootstrap and posterior probability values (Fig. 2).

DISCUSSION

he identification, as *Argas* (*Persicargas*) *persicus*, of the argasid ticks from Sicily is certain. Both morphological and genetic analysis confirm it. The latter was indispensable due to the lack of keys to identify the species of the subgenus *Persicargas* (or the

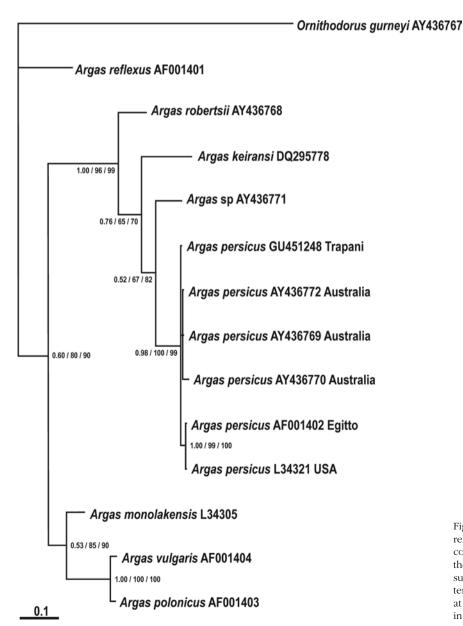


Fig. 2. – Consensus of proposed phylogenetic relationships obtained with the 168 data. The consensus diagrams summarize the results of the three reconstruction methods, with their support values for single nodes, Bayesian posterior probability, MP and NJ bootstrap values at the nodes. Argasid tick sequences as found in GenBank are included.

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corresponding group of species following Klompen & Oliver, 1993).

All Italian records of this species, with two exceptions, were ancient and not well-documented (Fig. 1A). Starkoff (1958a, 1958b, 1959) found A. (P.) persicus in the collection of the Natural History Museum of Trieste. The specimens, mixed with Argas (Argas) reflexus (Fabricius, 1794), were sampled in a loft (infested by pigeons) in Trieste, a city in the furthest East of Italy, near the Balkans. This record was paired by Manilla (1990 [1991]) who reported the finding in poultry-pen near Trieste. These are considered the only two sure records for Italy by both Manilla (1998) and Iori et al. (2005). Starkoff (1958a, 1958b, 1959), like Manilla (1982) at first, was convinced that the species was following the expansion of the Collared Dove, Streptopelia decaocto (Frivaldszky, 1838), from the Balkans to the West. So he immediately rejected an ancient citation of Latreille (1824) for Naples. A second very confused record was that of Manilla (1982) who reports a personal communication regarding a finding on an owl (Strigiformes) in Piedmont: but this record corresponds exactly to that of Durio et al. (1982), which appeared in the same issue of the same journal, about a female of A. (A.) reflexus (Fabricius, 1794). Afterwards Walter & Massa (1987) reported a sampling of 107 engorged larvae on the Great Tit, Parus major Linnaeus, 1758, near Lake Como in Lombardy. Finally, in the Checklist of the Italian Fauna, Bernini et al. (1995) cited A. (P.) persicus both in North and Peninsular Italy, but in a successive update (Castagnoli & Nannelli, 2003) the report for Central Italy was rejected. The reason for this is unknown. Lastly, the presence of A. (P.) persicus around Lake Trasimeno, via the finding of Aegyptianellainfection on wild birds, was hypothesized by Tarello (2001) and Tarello & Riccieri (2003).

In the Trapani province the species was found in two semi-natural sites where, having been found repeatedly, its presence does not appear accidental. The Sicilian habitat is very similar to the wild ones described by Filippova (1966) regarding Central Asia. The findings agree very well with the overwintering behaviour of the species when it develops on wild birds (Filippova, 1966). Poultry is absent in the vicinities of the infested trees and, even if possible, a passage from domestic to wild birds appears very improbable. So the presence of *A. (P.) persicus* in Sicily seems to be natural, excluding a human-induced spread.

Surely these findings have been favoured by the peculiar, easy to inspect, smooth bark of the River Red Gum, which is shed in long ribbons. In other situations the observation of this species could be very difficult and actually, except in Central Asia, the species was rarely collected in its natural habitat (Filippova, 1966).

These records revalue not only all the old citations for Italy but also the hypothesis that the Mediterranean

distribution of this argasid is of a natural origin (rejected by Hoogstraal *et al.*, 1979). A Central-Asian-Mediterranean distributional pattern is, as a matter of fact, recognized and common (Vigna Taglianti *et al.*, 1992). Species with this distributional pattern can be very abundant in Central Asia but scarce, rare or scattered in the Mediterranean area. The natural expansion of *A. (P.) persicus* in the Saharo-Sindian and Indo-Malayan regions would be natural via migrating birds as demonstrated in other tick species by Hoogstraal *et al.* (1961, 1963).

CONCLUSIONS

rgas (Persicargas) persicus is commonly considered a highly specialized wide-spread ectoparasite of chickens. The risk of its vector activity is high above all in traditional poultry houses, but not in modern ones. In the NW-Mediterranean countries, Italy, France, Spain and Portugal, the risk seems generally low (see for example the analysis of [Simpson], 2006) both due to the good sanitary conditions of poultry breeding and for the record scarcity of this species in wild habitats.

Nevertheless it is very difficult to find *A. (P.) persicus* in natural habitats, mainly as overwintering adults, and its populations can be easily underestimated. Its distribution in NW-Mediterranean could be not only natural but also much vaster than believed. Even if presently this argasid is really rare, the alert level should be high. The number of suitable habitats could increase as a result of global climatic change. Consequently the contact between wild and domestic birds could also increase, causing pathogen transmission and major damage to farms.

ACKNOWLEDGEMENTS

he authors wish to thank Albertina Bernardi Iori, of the Department of Public Health Sciences "G. Sanarelli" - University "La Sapienza" of Rome, for providing references. Gario Zappi for his indispensable translation of some parts of the Filippova book. Bruno Massa, of the S.En.Fi.Mi.Zo. Department - University of Palermo, for providing us with information about the probable nesting bird species. Two anonymous referees for their valuable comments on the manuscript.

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Reçu le 31 mars 2010 Accepté le 9 juin 2010