

***Myrmeleon punicanus* n. sp., a new pit-building antlion (Neuroptera Myrmeleontidae) from Sicily and Pantelleria**

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Abstract

A new species of antlion, *Myrmeleon punicanus* n. sp. (Neuroptera Myrmeleontidae), is described from Sicily and Pantelleria. The new taxon belongs to the *formicarius*-group thanks to the absence of pilula axillaris in the male hind wings, the characteristics of the male genitalia and the ecological traits. Moreover, the validity of the new species is confirmed by a DNA based phylogenetic reconstruction in which it is compared with closely related taxa. A preliminary description of the third instar larva of *M. punicanus* n. sp. is also given. Its habitat is the Mediterranean shrub land in which the larvae build pit-traps near shelters. Due to its ecological requirements a possible endangered status cannot be ruled out because of the anthropogenic habitat fragmentation.

Key words: Neuropterida, Italy, Mediterranean, *Myrmeleon formicarius*-group.

Among the Western Palearctic species of *Myrmeleon* Linnaeus 1767 there is a group characterised by the absence of pilula axillaris in the hind wing of the male. Three known species belong to this group in the Mediterranean area: the Siberian-European *Myrmeleon formicarius* Linnaeus 1767, the West-Mediterranean *Myrmeleon gerlindae* Hoelzel 1974 and the East-Mediterranean *Myrmeleon noacki* Ohm 1965. They can be defined as “sylvestral” living in wood or wood-like habitats: *M. formicarius* slightly more euryoecious; *M. gerlindae* and *M. noacki* exclusively linked to xerophilous Mediterranean woods and lush shrub lands (Ohm, 1965; Hözel, 1974; Aspöck *et al.*, 1980, 2001; Stange, 2004).

M. noacki is present, as far as we know, in the South Balkan Peninsula and Anatolia, not reaching the oriental border of Italy (Aspöck *et al.*, 2001); *M. gerlindae* in Morocco, the Iberian Peninsula, South France, reaching the western border of Liguria and the whole of Sardinia (Aspöck *et al.*, 2001; Molinu *et al.*, 2007; Badano and Letardi, 2010; Badano, 2011). In the Italian Peninsula only *M. formicarius* was known, being present everywhere in the North but in the South colonising both high mountains such as Pollino (Principi, 1952), Aspromonte (Schmid, 1972) or Etna (Letardi and Pantaleoni, 1996) and the remains of fresh deciduous woods also near the coast, such as Circeo (Letardi and Pantaleoni, 1996), Castelporziano (Letardi and Maltzoff, 2001) or Foresta Umbra on Gargano (Grandi, 1955).

It was not without surprise that, in the year 2000, one of us (RAP), during a short trip to Pantelleria Island, Straits of Sicily, found many *Myrmeleon* larvae living in the sclerophyllous wood covering the central mountain. Unfortunately transfer to the laboratory was very difficult, many larvae died and only few adults emerged. Nevertheless, it was immediately clear that these specimens belonged to a new taxon of the group *formicarius*, but the scarcity of adults stopped further investigations.

About 10 years later, it became possible to have other larvae from Pantelleria. Thanks to a scout, in exactly the

same point and in a further one 15 larvae were found that we received alive. Moreover, during another short trip in September 2010, the same author (RAP) conducted special research in order to find the taxon in suitable habitats in Sicily. Thanks to a colleague with a deep knowledge of the territory, this turned out to be successful, finally having enough specimens to conclude the study and describe a new species here.

Materials and methods

Rearing and classical morphology

All the adults were exclusively obtained from rearing larvae collected in the field. These were reared in small cylindrical containers a third full of loose sand. The prey were live Yellow Mealworm larvae, *Tenebrio molitor* L. 1758 (Coleoptera Tenebrionidae), of an adequate size. Rearing was carried out in a dedicated room with a mean temperature of 24 °C and 60% relative humidity. During the winter the larvae were moved into an unconditioned room to simulate natural conditions.

A Leica MZ9.5 stereomicroscope was used for morphological observations while a Leica MZ16 stereomicroscope equipped with a DFC320 digital camera was utilized both for morphological measurements and for photographs which were subsequently elaborated using LAS (Leica Application Suite) applied software Version 2.5.0 R1. The software Adobe Photoshop CS5 Extended Version 12.0 was utilized for post-shoot image processing.

The length of the adults was measured from the vertex of the head to the tip of the abdomen. The length of the wings was measured longitudinally from the insertion to the apex, and the width was taken as the maximum width perpendicular to the length measurement line. The body length of larvae was measured from the head (excluding jaws) to the tip of abdomen. The length of the head capsule was measured ventrally from the clypeolabrum to the proximal margin, the width was taken just below the eye tubercles, at the point of maximum width,

the length of the mandibles was measured from the tip to the base (Cesaroni *et al.*, 2010; Pantaleoni *et al.*, 2010).

The male genitalia were prepared by maceration in 10% KOH (potassium hydroxide) in cold water for several hours and subsequently stained in a saturated solution of Chlorazol Black in 95% ethanol. After examination they were washed in acetic acid and subsequently in ethanol.

When not otherwise specified, the specimens have been deposited in the authors' collections at ISE-CNR Sassari.

DNA taxonomy

Different DNA taxonomy approaches were used: the Automated Barcode Gap Detector (Puillandre *et al.*, 2012), the K/theta method (Birkay *et al.*, 2010), and the Generalised Mixed Yule Coalescent model (Pons *et al.*, 2006), in order to support the description of the new species. These methods rely on different assumptions and use different techniques so their congruent results will provide strong support.

The ABGD (Puillandre *et al.*, 2012) is based on the idea of a barcoding gap between species, but instead of using a fixed threshold (e.g. 3%, as for barcoding known faunas: Hebert *et al.*, 2003), it recursively searches for the most likely value of the threshold between intraspecific and interspecific genetic distances, given a set of prior intraspecific divergences. Only an aligned dataset of sequences is needed as input for this method.

The K/theta method, formerly called the four times rule (Birkay *et al.*, 2010), is rooted on population genetics theory and instead of using thresholds on genetic distances, it looks for clusters in a phylogeny from mitochondrial loci, with clusters that satisfy the rule of within-cluster distances lower than four times the distance to the closer cluster of sequences. A rooted phylogeny and the matrix of genetic distances calculated from the phylogeny are used as input for this method.

The Generalized Mixed Yule Coalescent (GMYC) model (Pons *et al.*, 2006) identifies units of diversity from DNA taxonomy based on the branching patterns in a phylogeny, where it searches for the maximum likelihood threshold between speciation/extinction events and within-species coalescent processes, and therefore identifies distinct evolutionary entities akin to species and also provides confidence intervals for the most likely solutions. A rooted chronogram, with only the ingroup included, is used as input for this method.

Genetic datasets

The dataset comprised 21 specimens, covering five species of the genus *Myrmeleon* (including the new one), plus *Distoleon tetragrammicus* F. 1798 belonging to the same family, and *Chrysoperla lucasina* Lacroix 1912 belonging to a different family (Chrysopidae) to be used as an outgroup. The species chosen within the genus *Myrmeleon* represent two species belonging to the *formicarius*-group (*M. gerlindae* and *M. formicarius*), one for which we have a dense population sample (*Myrmeleon inconspicuus* Rambur 1842), one lacking the pilula axillaris but not belonging to the *formicarius* group (*Myrmeleon caliginosus* Hoelzel et Ohm 1983) in order

to improve the effectiveness of the GMYC model in finding the threshold between speciation events and coalescent processes (Pons *et al.*, 2006).

DNA extraction was performed with the Quiagen QIAmp Microkit, using a leg from each individual. Voucher specimens are deposited in the authors' collection. DNA was amplified for each individual, for a 612bp fragment of the cytochrome c oxidase subunit I (COI) gene using primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer *et al.*, 1994). Cycle conditions comprised initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 90 s, and a final extension step at 72 °C for 7 min. Cycle sequencing reactions were set up using PCR primers and the ABI Big Dye Terminator v1.1 kit, and run on an ABI 3770 automated sequencer.

Chromatograms were checked visually for potential mis-readings; the alignment was trivial and was obtained with a text editor and then checked visually with MacClade 4.08 (<http://macclade.org/>). Given that COI is a protein coding gene, we looked for potential problems such as gaps, ambiguously aligned positions, frame shifts and stop codons but no such problems were detected.

The complete alignment was then reduced by omitting duplicate sequences; this alignment was used as input on the ABGD webpage (<http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html>), using the default settings searching on a set of prior minimum genetic distances ranging from 0.001 to 0.1. Then we selected the results from priors between 0.005 and 0.01 for this locus (Puillandre *et al.*, 2012).

Phylogenetic reconstructions

The reduced dataset including unique sequences only was used to reconstruct the phylogeny. ModelGenerator v0.85 (Keane *et al.*, 2006) was used to search the best evolutionary model, which resulted GTR+I. This evolutionary model was then implemented in Bayesian reconstructions with BEAST v1.6.1 (Drummond and Rambaut, 2007). The settings for BEAST included uncorrelated lognormal relaxed clock, estimated from a normal distribution; speciation tree prior from a Yule process with lognormal distribution of birth rate process; all other priors as default settings; all operators as default settings, except for integer random walk of branch rate categories set to tuning = 10; length of MCMC chain set to 100,000,000; trees saved every 1,000 generations and subsequent burn-in discarding the first 50% of the trees. Phylogenetic reconstructions were run through the Oslo Bioportal (www.bioportal.uio.no).

The tree obtained by BEAST was used as the backbone for the K/theta method, identifying all possible clusters that satisfied the 4 times rule. The matrix of genetic distances was calculated as cophenetic distances from the tree with R 2.14.0 (R Development Core Team, 2011) package ape 3.0 (Paradis *et al.*, 2004). The R package splits 1.0-11 (<https://r-forge.r-project.org/projects/splits>) were used to run the GMYC model on the phylogenetic tree obtained by BEAST, after pruning the tree from the outgroup, whose presence

could bias the estimate of the parameters. Moreover, the R package ape was also used to calculate matrices of uncorrected pairwise distances from the alignment, in order to compare the distances within and between species, as it is commonly done for DNA barcoding (e.g. Birký *et al.*, 2011).

***Myrmeleon punicanus* n. sp.**

Diagnosis

Small *Myrmeleon* with a predominantly blackish habitus; pronotum with a characteristic and discriminating pattern; wings hyaline with dark-and-pale dashed veins, in the forewing the cubital fork is slightly more basal than the fork of the Media posterior, male hind wing without pilula axillaris; abdomen dark brown with yellowish-brown posterior margins of sternites and tergites, 8th tergite with dorsal yellowish-brown marks. Larva sylvester, with dark habitus and black round spots on the underside of the posterior femora.

Holotype

♂ in alcohol: “Pantelleria: Monastero di sopra / (Sicilia, Trapani); ex larva / 28.V.2000 R.A. Pantaleoni leg.” // HOLOTYPE / *Myrmeleon punicanus* n. sp. / Pantaleoni and Badano, 2012 [coll. Museo Civico di Storia Naturale “G. Doria”, Genova].

Paratypes

8♂♂ in alcohol: “Pantelleria: Monastero di sopra / (Sicilia, Trapani); ex larva / 28.V.2000 R.A. Pantaleoni leg.”, 1♂; “Pantelleria: Monastero di sopra / (Sicilia, Trapani); ex larva 23.V.2010 A. Corso leg. // 18-sspan1”, 1♂; “Pantelleria: Bugeber / (Sicilia, Trapani); ex larva 23.V.2010 A. Corso leg.”, 1♂; “Pantelleria: Bugeber / (Sicilia, Trapani); ex larva 23.V.2010 A. Corso leg.”, 1♂ [coll. Naturhistorische Museum, Wien]; “Pantelleria: Bugeber / (Sicilia, Trapani); ex larva 23.V.2010 A. Corso leg.”, 1♂ [coll. Horst and Ulrike Aspöck, Wien]; “Pantelleria: Bugeber / (Sicilia, Trapani); ex larva 23.V.2010 A. Corso leg.”, 1♂ [coll. Rinaldo Nicoli Aldini, Bologna]; “Mazara del Vallo: Gorghi Tondi / (Sicilia, Trapani); ex larva / 10.IX.2010 R.A. Pantaleoni leg. // 77-sic1”, 1♂; “Mazara del Vallo: Gorghi Tondi / (Sicilia, Trapani); ex larva / 10.IX.2010 R.A. Pantaleoni leg.”, 1♂.

7♀♀ in alcohol: “Pantelleria: Monastero di sopra / (Sicilia, Trapani); ex larva 23.V.2010 A. Corso leg. // 19-sspan2”, 1♀; “Pantelleria: Bugeber / (Sicilia, Trapani); ex larva 23.V.2010 A. Corso leg.”, 2♀♀; “Pantelleria: Bugeber / (Sicilia, Trapani); ex larva 23.V.2010 A. Corso leg.”, 1♀ [coll. Horst and Ulrike Aspöck, Wien]; “Pantelleria: Bugeber / (Sicilia, Trapani); ex larva 23.V.2010 A. Corso leg.” [coll. Agostino Letardi, Roma], 1♀; “Mazara del Vallo: Gorghi Tondi / (Sicilia, Trapani); ex larva / 10.IX.2010 R.A. Pantaleoni leg” 2♀♀.

1♀ pinned: “Mazara del Vallo: Gorghi Tondi / (Sicilia, Trapani); ex larva 10.IX.2010 R.A. Pantaleoni leg.” [coll. Museo Civico di Storia Naturale “G. Doria”, Genova].

Further examined specimens

5 III instar larvae in alcohol: “Pantelleria: Monastero di sopra / (Sicilia, Trapani); ex larva 23.V.2010 A. Corso leg.” 3 specimens; “Pantelleria: Bugeber / (Sicilia, Trapani); ex larva 23.V.2010 A. Corso leg.” 2 specimens.

2 III instar larvae still under rearing: “Mazara del Vallo: Gorghi Tondi / (Sicilia, Trapani); IX.2011 M. Romano leg.”.

Locus typicus

Pantelleria: Monastero di sopra, (Sicilia, Trapani); 36°46'35.87"N 11°58'57.10"E.

Derivatio nominis

The name of this species is a Latin adjective that means “at/in the Carthaginian [*i.e.* Punic] manner” and refer to the similarity between the known and presumable distribution of the new *Myrmeleon* and the early dominion of Cartago.

Description of the adult

Body length 22.0 mm (min-max 20.2-23.5); forewing male length 21.9 mm (20.4-24.7), female length 24.1 mm (22.4-25.5), ratio width/length (both sexes) 0.24; hind wing male length 20.4 mm (19.0-22.7), female length 23.0 mm (21.0-24.3), ratio width/length (both sexes) 0.23. General colouring dark-brown or blackish with few yellowish-brown patches on the pronotum and abdomen (figure 1). Head (figure 2B): vertex shadow-black with a deep-black pattern; occiput black; superior half of frons black, inferior whitish, the pale area subdivided by a darkish line; clypeus predominantly whitish with two central spots joint with the median dark line of the frons; antennae dark brown, scape with the distal portion pale; labrum brownish; last two segments of the maxillary palpi brown; last segment of the labial palpi black. Thorax: pronotum (figure 2A) dark brown, whitish margined anteriorly and with a irregularly pale vertical area in the middle; mesonotum and metanotum completely dark brown except the posterior border of the scutellum whitish bordered and a central paler area of the mesonotal prescutum. Legs (figure 3A): prothoracic legs dark brown; meso- and metathoracic legs with coxae and tarsi dark brown, femora almost completely dark (3rd pair) or only in the inner face (2nd pair), tibiae dark in the inner face; tibial spurs a little shorter than the first tarsomere. Wings (figure 3B): membrane hyaline; pterostigma distinct, dark brown; venation predominantly dark with alternating pale dashes; in the forewing the cubital fork is slightly more basal than the fork of the Media posterior; hind wing with 5 presectoral cross-veins; male hind wing without pilula axillaris. Abdomen (figure 4E): shorter than the wings; dark, each segment with the distal border of sternites and tergites pale; pleural membranes dark-brown; 8th tergite with conspicuous yellowish brown coloration, visible dorsally as a pair of parallel sinuous lines. Male terminalia as in figures 4A, 4B, male genitalia as in figure 5; female terminalia as in figures 4C, 4D.

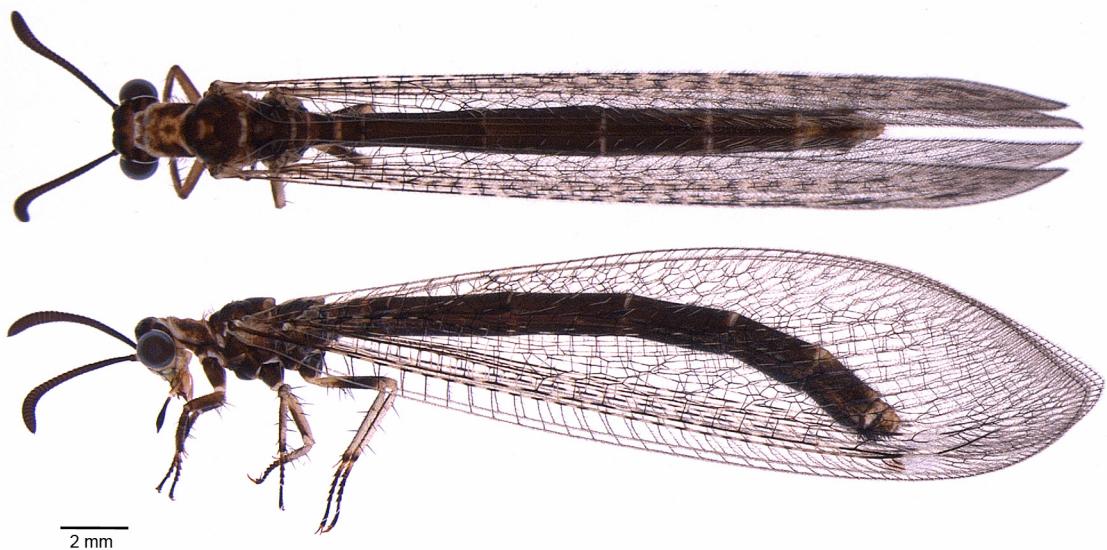


Figure 1. *Myrmeleon punicanus* n. sp.: habitus, dorsal (above) and lateral (below) view [Mazara del Vallo: Gorghi Tondi, paratype ♀].
 (In colour at www.bulletinofinsectology.org)



Figure 2. *Myrmeleon punicanus* n. sp.: A, head and pronotum, dorsal view [Mazara del Vallo: Gorghi Tondi, paratype ♂]; B, head, frontal view [Pantelleria: Bugeber, paratype ♀ with cutted head]; *Myrmeleon gerlindae* Hoelzel 1974; C, head and pronotum, dorsal view; D, head, frontal view [Alghero: Capoccacia (Sardinia), 1.V.2010, D. Badano leg., ♀].
 (In colour at www.bulletinofinsectology.org)

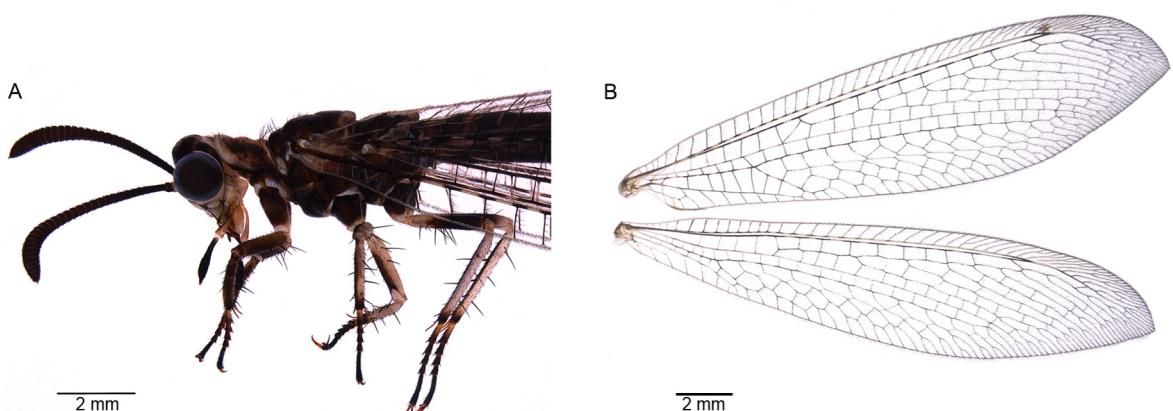


Figure 3. *Myrmeleon punicanus* n. sp.: A, head and thorax, lateral view [Mazara del Vallo: Gorghi Tondi, paratype ♀]; B, wings [Mazara del Vallo: Gorghi Tondi, paratype ♀ pinned].
(In colour at www.bulletinofinsectology.org)

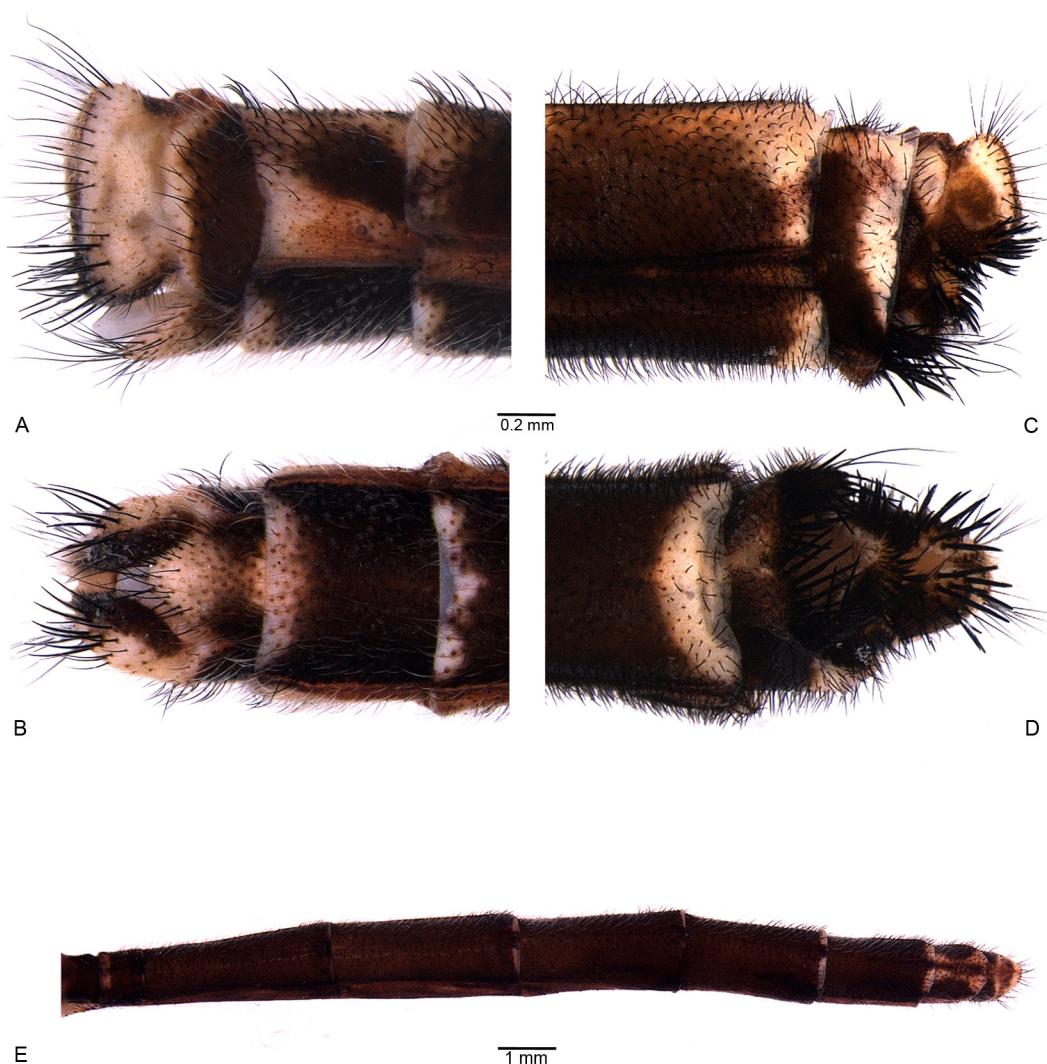


Figure 4. *Myrmeleon punicanus* n. sp.: male terminalia, A, lateral view, B, ventral view [Mazara del Vallo: Gorghi Tondi, paratype ♂]; female terminalia, C, lateral view, D, ventral view [Mazara del Vallo: Gorghi Tondi, paratype ♀]; E, male abdomen, dorsal view [Mazara del Vallo: Gorghi Tondi, paratype ♂].
(In colour at www.bulletinofinsectology.org)

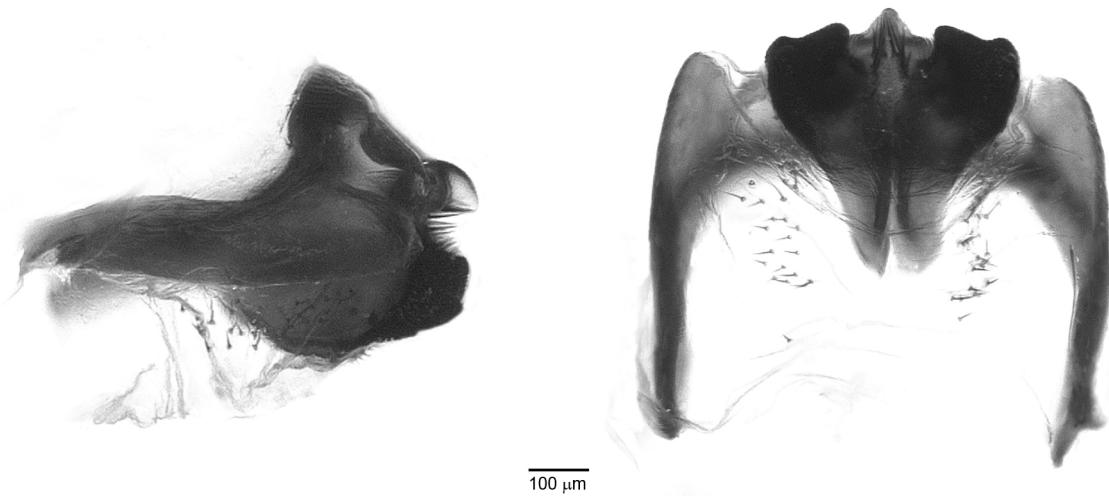


Figure 5. *Myrmeleon punicanus* n. sp.: male genitalia, gonarcus-paramere complex (= complex of gonocoxites 9 + gonocoxites 11 *sensu* Aspöck and Aspöck, 2007), lateral (left) and ventral (right) view [Pantelleria: Bugeber, paratype ♂ dissected].

Preliminary description of third instar larva (figure 6)

Average body length 8.6 mm; head capsule length 1.9 mm (min-max 1.8-2.0), head capsule width 1.6 mm (1.5-1.7), mandible length 1.9 mm (1.8-1.9), ratio head capsule length/width 0.84, ratio head capsule length/mandible length 0.97. General colouring dark brown with numerous darker marks and areas, ventral side much clearer with a large median pale ochre stripe bordered by black spots; setae mostly black. Head sub-rectangular, longer than wide; ocular tubercles not prominent; mandibles relatively long and strong equipped with three sub-parallel and equidistant pairs of teeth of approximately equal size; labial palpi composed of 3 articles in addition to the basal one (the “prelabial lobe” by authors). External edge of the mandibles covered by long setae, short and stout setae are present among the teeth. On the dorsal side of the mandibles there are sparse and short setae more numerous toward the edge; along the ventral side, in the portion of the mandibles external to maxillae there are dense and short setae, distributed from the base to the height of the second pair of teeth. Dorsal side of the head brown with two darker spots near middle length and two posterior V-shaped darker marks; ventral side of the head with two pairs of markings: the first one situated in the anterior portion of the head capsule and composed by two convergent spots, drawing a V-shaped mark; the second pair is more posterior and lateral in position and represented by oblique stripes; a couple of elongated marks is present along the sides. Inferior side of hind tibiae and femora presents round dark spots. Abdominal 8th sternite equipped with a pair of odontoid processes; 9th sternite with a poorly developed pair of palettes, each one equipped with four digging setae, of which the external pair is the longest and stoutest, more anteriorly there is a second group of four digging setae, neatly disposed along a line and parallel to the setae of the palettes.

Variability

The variability in colouring among adults is of minor importance, therefore appearing as a uniform and little variable species. Only the contrast of the yellowish-brown dorsal markings of 8th tergite is less evident in some individuals.

Ecological notes and distribution

Both the finding places, known directly by the authors, (and probably the third too: Bugeber, Pantelleria) are shrub lands (so called *macchia* locally). The larvae built their pits in small patches of incoherent soil often near a shelter rather than under a cover. Both localities are the remains of wider regional shrub cover, and the species is probably still endangered as a consequence of the severe fragmentation of its habitat. This fragmentation will make it difficult to define the pre-anthropic-disturbance range. In any way its presence on the volcanic island Pantelleria appears to be a consequence of a recent colonization more probably from the nearby African coast, specifically from Cape Bon peninsula, than from Sicily. Then the hypothesis of a distribution comprising at least Northern Tunisia and Western Sicily seems the more acceptable.

DNA Taxonomy

The 21 analysed individuals (table 1) provided 19 different haplotypes. The five putative species in the genus *Myrmeleon* were supported by Bayesian posterior probabilities of 1.0 (figure 7). All three methods supported the existence of the five species in the analysed COI dataset for *Myrmeleon*, confirming the status of *M. punicanus* as a new species. The ABGD and the K/theta method provided unambiguous support for the five species; the GMYC provided a maximum likelihood solution of five species that was significantly preferred to the null model of only one single entity (likelihood of



Figure 6. *Myrmeleon punicanus* n. sp.: 3rd instar larva, dorsal (above), ventral (middle) and lateral (below) view [Pantelleria: Bugeber].
(In colour at www.bulletinofinsectology.org)

Table 1. Accessions of COI of *Myrmeleon* and outgroups deposited in GenBank.

GenBank N.	Species	Origin	Vaucher N.
JQ864059		Italy, Sicily	1-incsic2
JQ864060		Italy, Emilia Romagna	2-incbo1
JQ864061		Greece, Epirus	5-incegre2
JQ864062		Greece, Epirus	6-incegre1
JQ864063	<i>Myrmeleon inconspicuus</i>	Italy, Lombardy	7-incpo1
JQ864064		Italy, Tuscany	9-incfo2
JQ864065		Tunisia, Tunis	40-inctu1
JQ864066		Romania, Dobruja	41-incro1
JQ864067		Italy, Sardinia	49-inclaz
JQ864068		Italy, Piedmont	22-forpie1
JQ864069	<i>Myrmeleon formicarius</i>	Italy, Piedmont	23-forpie2
JQ864070		Italy, Tuscany	24-forfol1
JQ864071		Italy, Sicily	42-forsic1
JQ864072	<i>Myrmeleon gerlindae</i>	Italy, Sardinia	35-gersar1
JQ864073		Italy, Liguria	54-gerlig1
JQ864074	<i>Myrmeleon caliginosus</i>	Tunisia, Tunis	56-caltun1
JQ864075		Italy, Sicily	77-sic1
JQ864076	<i>Myrmeleon punicanus</i> n. sp.	Italy, Sicily (Pantelleria)	18-sspan1
JQ864077		Italy, Sicily (Pantelleria)	19-sspan2
JQ864078	<i>Distoleon tetragrammicus</i> Myrmeleontidae	Italy, Sardinia	48-tetra1
JQ864079	<i>Chrysoperla lucasina</i> Chrysopidae	Italy, Sardinia	11-chry

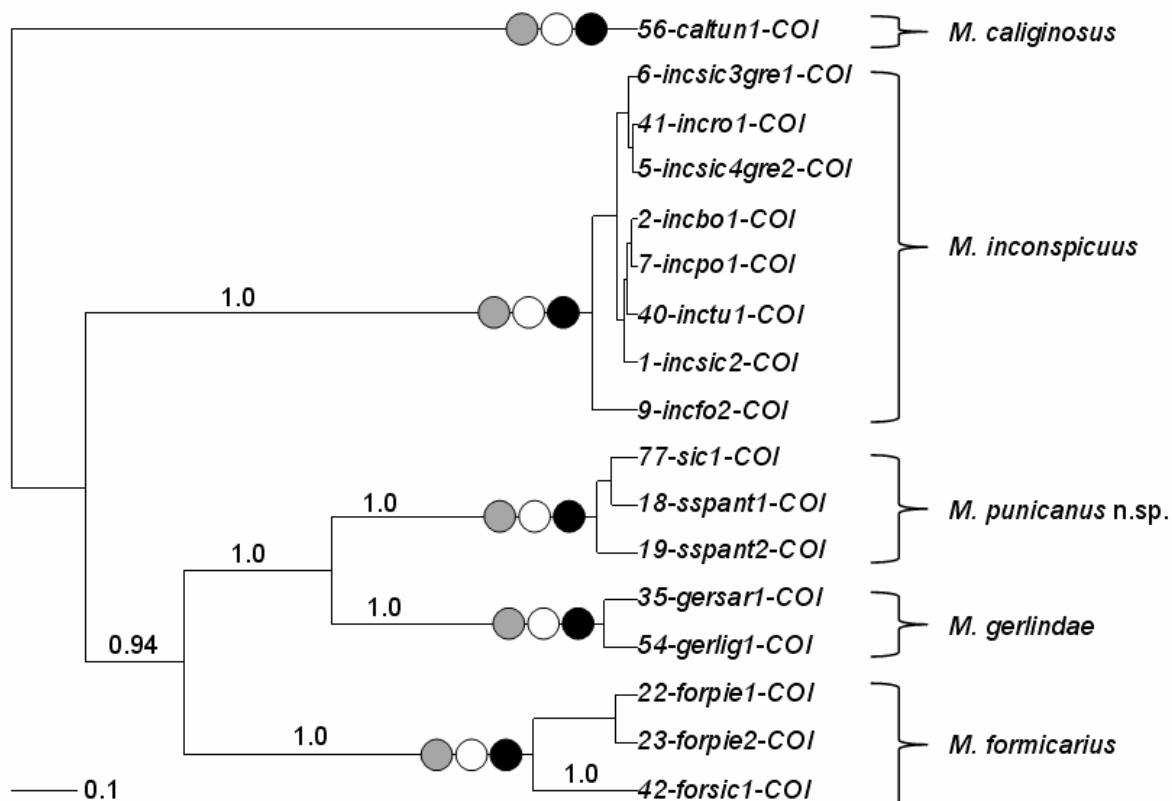


Figure 7. Phylogenetic relationships of cytochrome c oxidase subunit I COI in the genus *Myrmeleon*. The consensus of 50,000 sampled trees from a Bayesian analysis is shown in the rooted phylogeny (outgroups omitted), displaying all compatible groupings and with average branch lengths proportional to numbers of substitutions per site under the GTR+I substitution model. Posterior probabilities above 0.9 are shown above each branch; support values for within-species relationships are not shown for very short branches. Circles indicate clusters and singletons identified as potential species by ABGD (grey circles), K/theta (white circles) and GMYC model (black circles).

null model = 44.384; likelihood of GMYC model = 48.702; likelihood ratio test = 8.636; p-value = 0.034); the confidence interval of the most likely solutions spanned from five to seven, with the most conservative estimate of the GMYC being exactly five species, as for the other two methods.

Comments and comparative notes

Both the male genitalia and DNA sequences, as well as the absence of pilula axillaris in male hind wing, confirm that *Myrmeleon punicanus* n. sp. belongs to the *formicarius*-group comprising in the Western Palearctic: *M. formicarius*, *M. noacki* and *M. gerlindae*. As comparison, the illustrations of head and pronotum of *M. gerlindae* are given in figures 2C, 2D. *M. caliginosus*, a species whose distribution reaches the South Mediterranean border from Africa, also lacks pilula axillaris, but it belongs to another group, as confirmed once again by DNA analysis and the male genitalia.

The adults of *M. punicanus* can be easily recognised among all the species of the West-Palearctic area (*sensu* H. Aspöck *et al.*, 2001) on the basis of the evident external characteristics such as the pigmentation of the pronotum, pigmentation of the wing venation, the shape of the wings, size, overall colouring of the thorax and abdomen, etc. These characters also separate the species from the other species of the *formicarius*-group to which it belongs (figure 2).

Current knowledge on the *Myrmeleon* larvae does not still permit a morphological discrimination within the *formicarius*-group. Conversely the larvae of the group are differentiated from all the known antlion larvae of the Western Palearctic fauna by the round dark spots on the inferior side of femora and tibiae of the hind pair of legs (Brauer, 1853; Hagen, 1873; Redtenbacher, 1884; Friheden, 1973; Steffan, 1975; Gepp, 2010; Badano and Pantaleoni, unpublished data). Nevertheless the 3rd instar larva of *M. formicarius* is easily recognizable from the very similar larvae of *M. punicanus* and *M. gerlindae* (and very probably also *M. noacki*) simply by its noticeable larger size, above all of the jaws. For comparison a set of 16 *formicarius*-larvae from all parts of Italy was measured as follows: average body length 8.9 mm; head capsule length 2.3 mm (min-max 2.1-2.5), head capsule width 2.0 mm (1.8-2.3), mandible length 2.4 mm (2.1-2.6), ratio head capsule length/width 0.88, ratio head capsule length/mandible length 1.05 (unpublished data).

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References

- ASPÖCK U., ASPÖCK H., 2007.- Phylogenetic relevance of the genital sclerites of Neuropterida (Insecta: Holometabola).- *Systematic Entomology*, 33: 97-127.
- ASPÖCK H., ASPÖCK U., HÖLZEL H., 1980.- *Die Neuropteren Europas. Eine zusammenfassende Darstellung der Systematik, Ökologie und Chorologie der Neuropteroidea (Megaloptera, Raphidioptera, Planipennia) Europas.*- Goecke & Evers, Krefeld, Germany.
- ASPÖCK H., HÖLZEL H., ASPÖCK U., 2001.- Kommentierter Katalog der Neuropterida (Insecta: Raphidioptera, Megaloptera, Neuroptera) der Westpaläarktis.- *Denisia*, 2: 1-606.
- BADANO D., 2011.- I Neuroterti (Neuropterida) della Liguria, p. 54. In: *Atti XXIII Congresso Nazionale Italiano di Entomologia*, Genova 13-16 Giugno 2011.
- BADANO D., LETARDI A., 2010.- A review of the Neuropterida of Liguria (north-west Italy), pp. 83-87. In: *Proceedings of the 10th International Symposium on Neuropteryology* (DEVETAK D., LIPOVŠEK S., ARNETT A. E., Eds), 22-25 June 2008, Piran, Slovenia.
- BIRKY C. W. JR., ADAMS J., GEMMEL M., PERRY J., 2010.- Using population genetic theory and DNA sequences for species detection and identification in asexual organisms.- *PLoS ONE*, 5 (5): e10609. doi:10.1371/journal.pone.0010609
- BIRKY C. W. JR., RICCI C., MELONE G., FONTANETO D., 2011.- Integrating DNA and morphological taxonomy to describe diversity in poorly studied microscopic animals: new species of the genus *Abrochtha* Bryce, 1910 (Rotifera: Bdelloidea: Philodinavidae).- *Zoological Journal of the Linnean Society*, 161: 723-734.
- BRAUER F., 1853.- Vergleichende Beschreibung der Larven des *Myrmecoleon formicarius* L. und *M. formicalyx* T.- *Verhandlungen des Zoologisch-Botanischen Vereins in Wien (Sitzungsberichte)*, 3: 144-145.
- CESARONI C., NICOLI ALDINI R., PANTALEONI R. A., 2010.- Larvae of *Gymnocnemia variegata* (Schneider, 1845) and *Megistopterus flavicornis* (Rossi, 1790) (Neuroptera Myrmeontidae), pp. 135-144. In: *Proceedings of the 10th International Symposium on Neuropteryology* (DEVETAK D., LIPOVŠEK S., ARNETT A. E., Eds), 22-25 June 2008, Piran, Slovenia.
- DRUMMOND A. J., RAMBAUT A., 2007.- BEAST: Bayesian evolutionary analysis by sampling trees.- *BMC Evolutionary Biology*, 7: 214. doi:10.1186/1471-2148-7-214
- FRIHEDEN J., 1973.- Morphological characteristics of North-European myrmeontid larvae (Neuroptera).- *Entomologica Scandinavica*, 4: 30-34.
- 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.- *Molecular Marine Biology and Biotechnology*, 3 (5): 294-299.
- GEPP J., 2010.- Ameisenlöwen und Ameisenjungfern. Myrmeontidae. Eine weltweite Betrachtung unter besonderer Berücksichtigung Mitteleuropas. Die Neue Brehm-Bücherei, Bd. 589.- Westwarp Wissenschaften, Germany.
- GRANDI G., 1955.- Campagna di ricerche dell'Istituto di Entomologia dell'Università di Bologna alla "Foresta Umbra" (Gargano).- *Annali, Accademia Italiana di Scienze Forestali*, 4: 405-418.

- HAGEN H. A., 1873.- Die Larven von *Myrmeleon*.- *Stettiner Entomologische Zeitung*, 34: 249-295, 377-398.
- HEBERT P. D. N., CYWINSKA A., BALL S. L., DEWAARD J. R., 2003.- Biological identifications through DNA barcodes.- *Proceedings of the Royal Society of London B - Biological Science*, 270: 313-321.
- HÖLZEL H., 1974.- Ein neuer trichterbauender Ameisenlöwe aus Südwesteuropa (Planipennia).- *Nachrichtenblatt der Bayerischen Entomologen*, 23: 81-85.
- KEANE T. M., CREEVEY C. J., PENTONY M. M., NAUGHTON T. J., MCLNERNEY J. O., 2006.- Assessment of methods for amino acid matrix selection and their use on empirical data shows that ad hoc assumptions for choice of matrix are not justified.- *BMC Evolutionary Biology*, 6: 29. doi:10.1186/1471-2148-6-29
- LETARDI A., MALTZEFF P., 2001.- Neuroteridi e Mecotteri della Tenuta Presidenziale di Castelporziano e delle aree limitrofe (Neuroptera, Raphidioptera, Mecoptera).- *Bollettino dell'Associazione Romana di Entomologia*, 56: 49-62.
- LETARDI A., PANTALEONI R. A., 1996.- I Neuroteroidei W-Paleartici della collezione del Museo di Zoologia dell'Università di Roma (Neuropteroidea).- *Fragmenta Entomologica*, 28: 277-305.
- MOLINU A., SASSU A., PANTALEONI R. A., 2007.- Neuropterida of the Asinara Island (NW Sardinia, Italy).- *Annali del Museo civico di Storia naturale di Ferrara*, 8: 111-115.
- OHM P., 1965.- *Myrmeleon noacki* nov. sp., eine neue Myrmeleontiden-Art von der Balkan-Halbinsel (Neuroptera).- *Fragmenta Balcanica Musei Macedonici Scientiarum Naturalium*, 5: 107-114.
- PANTALEONI R. A., CESARONI C., NICOLI ALDINI R., 2010.- *Myrmeleon mariaemathildae* n. sp.: a new Mediterranean pit-building antlion (Neuroptera Myrmeleontidae).- *Bulletin of Insectology*, 63 (1): 91-98.
- PARADIS E., CLAUDE J., STRIMMER K., 2004.- APE: analyses of phylogenetics and evolution in R language.- *Bioinformatics*, 20: 289-290.
- PONS J., BARRACLOUGH T. G., GOMEZ-ZURITA J., CARDOSO A., DURAN D. P., HAZELL S., KAMOUN S., SUMLIN W. D., VOLGER A. P., 2006.- Sequence based species delimitation for the DNA taxonomy of undescribed insects.- *Systematic Biology*, 55: 595-609.
- PRINCIPI M. M., 1952.- Ricerche zoologiche sul Massiccio del Pollino (Lucania-Calabria). VI. Neuroterti.- *Annuario dell'Istituto e Museo di Zoologia dell'Università di Napoli*, 4 (10): 1-22.
- PUILLANDRE N., LAMBERT A., BROUILLET S., ACHAZ G., 2012.- ABGD, Automatic Barcode Gap Discovery for primary species delimitation.- *Molecular Ecology*, 21 (8): 1864-1877.
- REDTENBACHER J., 1884.- Überischit der Myrmeleontiden-Larven. *Denkschriften der Mathematisch-Naturwissenschaftlichen Classe der Kaiserlichen Akademie der Wissenschaften*, 48: 335-368.
- SCHMID H., 1972.- Neuropteren aus dem Aspromonte, zugleich ein Beitrag zur tiergeographischen Stellung dieses Gebirges.- *Entomologische Zeitschrift*, 82: 249-253.
- STANGE L. A., 2004.- A systematic catalog, bibliography and classification of the world antlions (Insecta: Neuroptera: Myrmeleontidae).- *Memoirs of the American Entomological Institute*, 74: 1-565.
- STEFFAN J. R., 1975.- Les larves des fourmiliens [Planipennes: Myrmeleontidae] de la faune de France.- *Annales de la Société entomologique de France*, 11 (2): 383-410.
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