

FIRST RECORD OF *THEMIRA NIGRICORNIS* (MEIGEN, 1826) (SEPSIDAE: DIPTERA) IN ROMANIA

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Abstract. This report on the occurrence of *Themira nigricornis* (Meigen, 1826) (Sepsidae: Diptera) on Romanian territory represents the first record of this species within south-eastern Europe. Adults of *T. nigricornis* were collected in spring, in the second half of April, from an experimental setting placed in an urban location (Bucharest) in southern Romania. The bait consisted of domestic pig carcass in advanced decomposition stage, placed on soil ground and surrounded by vegetation. The visiting specimens were located around the posterior segment of the pig carcass. The registered abundance of adult individuals was relatively low, with 10 specimens distinguished during two weeks interval. The species was taxonomically characterized and identified. This study, documenting the presence of *T. nigricornis* in the southern part of Romania, expanded the Fauna Europaea distribution range record of this species, assigning the southernmost location of *T. nigricornis* in Europe.

Résumé. Ce rapport sur la présence de *Themira nigricornis* (Meigen, 1826) (Sepsidae: Diptera) sur le territoire roumain représente la première mention de cette espèce dans l'Europe du Sud-Est. Les adultes de *T. nigricornis* ont été recueillies pendant le printemps, dans la seconde moitié d'Avril, à partir d'un cadre expérimental placé dans un emplacement urbain (Bucarest) dans le sud de la Roumanie. L'appât consistait en une carcasse de porc en phase de décomposition avancée, placée sur le sol et entourée de végétation. Les spécimens en visite ont été trouvés dans le segment postérieur de la carcasse de porc. L'abondance enregistrée des individus adultes a été relativement faible, avec 10 spécimens démarqués au cours de deux semaines. Cette étude, en documentant la présence de *T. nigricornis* dans la partie du sud de la Roumanie, a élargi l'aire de répartition de cette espèce dans la Faune Europaea, par l'attribution de *T. nigricornis* au point le plus au sud d'Europe.

Key words: *Themira nigricornis*, first mention, pig carcass, Romania, distribution.

INTRODUCTION

The Sepsidae family, known as the black scavengers flies because of their coprophagous preference, comprises 37 genera and 318 species worldwide (Ozerov, 2005). The adults were found on dung (Snowball, 1944), animal and plant decaying material (Ozerov, 1989, 1999 a). These insects were predominantly located on human and animal excrements (Iwasa, 1980; Ozerov, 1991), playing an important role in faeces decomposition processes (Papp, 1971).

Species belonging to this family (Pont & Meier, 2002) are of relatively small size, reaching 6–12 mm in length. Morphologically, they resemble ants with narrow waist, the majority of individuals presenting pronounced sexual dimorphisms, of the legs and the male's 4th sternites. The forelegs of the male are used during courtship and mounting for holding onto the base of the female wing.

Themira species are characterized by the absence of supraalar seta (Pont & Meier, op. cit.). Adult males are easily recognized by the ornate fore-legs, and by the prominent form and colour that varies from black to yellow of the mid tarsomeres. The females are recognised by the absence of postpronotal setae, pruinose katepisternum, and partially yellow legs.

T. nigricornis, fully described and illustrated by Ozerov (1999 b), was first identified almost two centuries ago (1826) by Meigen, but no certain location and

holotype/syntype of female were indicated for this incidence (Ozerov, 2005). An earlier report of this species was recorded on American territory by Steyskal (1946). Since then, the presence of *T. nigricornis* was reported in western, central, eastern and northern European countries including Austria, Great Britain (Incl. Shetlands, Orkneys, Hebrides and Man Is.), Czech Republic, Danish mainland (Incl. Borholm I), Finland, French mainland, Germany, Hungary, Latvia, Lithuania, Norwegian mainland, Poland, Central, East, South and Northwest Russia, Slovakia, Sweden (Incl. Gotland I), Switzerland, The Netherlands, Ukraine (Fauna Europaea, Version 2.6.2, de Jong, 2013) and Italy (Ozerov, 2005). So far, the south-eastern limit for the spread range of this species did not include Romania.

The current study reports the first occurrence of *T. nigricornis* on Romanian territory, in the southern part of the country, containing a brief description of the associated experimental settings, taxonomic identification of adult specimens collected from a pig decomposed carcass.

MATERIAL AND METHODS

Experimental setting

T. nigricornis specimens were identified during a forensic experimental model investigation in Bucharest, Romania. This urban site is located 100 m above sea level (44°27'10"N, 26°05'04"E) and belongs to a temperate climate region. The experimental setting consisted of a pig carcass weighing approximately 15 kg, placed on the ground, in a natural park area surrounded by spring vegetation (grass and trees), and protected by 50 cm x 100 cm metal cage against vertebrate scavengers (Fig. 1). The bait was exposed for approximately 5 months during cold period (December-February) and beginning of warm period (March-April). The spatial-temporal, environmental and experimental model features describing the occurrence of this species in Romania were summarized in table 1.

Sampling and taxonomical characterization

Insect sampling was performed using an entomological net, and the specimens were preserved in 75% ethanol at room temperature, until further investigations.

T. nigricornis taxonomic identification was performed according to the taxonomic keys for this species (Pont & Meier, 2002). The identification of main



Fig. 1 - Experimental setting for *T. nigricornis* appearance site. Pig carcass after 5 month exposure.

Table 1

Characteristics of experimental settings for *T. nigricornis* occurrence.

Characteristic	Experimental data
Location	Bucharest, Romania, 44°27'10"N L26°05'04"E
Period	15 – 30.IV.2013
Environmental temperature (°C)	20.05 ± 2.1°C
Relative humidity (%)	57.06 ± 8.0%
Precipitation (mm)	0.53 ± 0.09 mm
Experimental model	Pig carcass in advanced decomposition stage after 5 months of outdoor exposure in a park type setting
Environmental conditions	Arboreal vegetation (<i>Aesculus hippocastanum</i> , <i>Tilia</i> sp., <i>Ulmus</i> sp.), rich cover of small plants and shrubs
Abundance	10 specimens (7 males and 3 females)

diagnosis characters was performed using a stereomicroscope Stemi2000 (Zeiss) at 3 x (1.9 to 225x) magnification scale.

DNA barcoding

T. nigricornis genomic DNA was extracted from two different adult individuals using DNeasy Blood & Tissue Kit (Qiagen), following an adapted protocol that included an additional initial cellular disruption step. The insect habitus suspended in 200 µl TE was disrupted at 20°C using a Cell Homogenizer SpeedMill PLUS (Analytik Jena), by applying continuous 50 Hz-power for 12 minutes at 20°C, in the presence of 5 Zirconia Beads II per tub (Invitex). After centrifugation of the cell extract at 10.000 x g for 1 min, the soluble fraction was further purified following the manufacturer procedure. The concentration and purity of the resulted DNA were measured with a NanoDrop1000 (Thermo Scientific).

DNA barcoding was performed based on PCR amplification of cytochrome oxidase subunit I (COI) mitochondrial gene fragment (Folmer et al., 1994). PCR amplification was carried out using a Mastercycler ProS System (Eppendorf). The reaction mixture contained 200 ng insect genomic DNA, 100 pmols of forward (LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3') and reverse (HC02198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') invertebrates' COI gene specific primers (Folmer et al., 1994), 1 unit of Taq DNA polymerase (Thermo Scientific), 1x Taq buffer, 2.5 mM MgCl₂, 0.1 mM dNTP (Thermo Scientific), in a total volume of 50 µl. The amplification reaction consisted of an initial incubation step of 1 min at 94°C, followed by 5 cycles of 30 sec at 94°C (denaturation), 1.5 min at 45°C (annealing) and 1 min at 72°C (extension), followed by 35 cycles of 30 sec at 94°C, 1.5 min at 51°C, 1 min at 72°C and a final extension step of 5 min at 72°C. The reaction performed in the absence of DNA was used as negative control.

The resulted DNA fragments (710 bp) were analysed by electrophoresis on 1% agarose gels. The amplified COI gene fragments were purified using QIAquick PCR Purification Kit (Qiagen), and sequenced (Macrogen) on both strands, using the cloning primers. Sequence similarity was determined by BLAST-NCBI screening tool (<http://www.ncbi.nlm.nih.gov>).

RESULTS

Spatial and temporal characteristics of T. nigricornis occurrence in Bucharest – Romania

The insects were observed in the second half of April, 2013, when the average temperature raised to $20.05 \pm 2.1^\circ\text{C}$. The precipitation and relative humidity during this interval corresponded to 0.53 ± 0.09 mm and $57.06 \pm 8.0\%$, respectively. The insects were located on the surface of the pig carcass, in the vicinity of the posterior section. A total of ten specimens, counting seven males and three females, were collected during midday period over the two week interval. Noteworthy, the presence of *T. nigricornis* on the carcass was simultaneous with other necrophagous species belonging to Diptera and Coleoptera Orders, including members of Calliphoridae, Muscidae, Cleridae and Silphidae families.

Taxonomic identification of T. nigricornis

Taxonomic identification of this species was carried out based on the taxonomic keys provided by Pont & Meier (2002). The analysis of male adult specimen (Fig. 2) indicates a series of characteristics of *T. nigricornis* species, as follow: black thorax and abdomen, and habitus presenting orange-yellow genal area (Fig. 2A); the front femure (Fig. 2B) exhibiting several posterodorsal setae

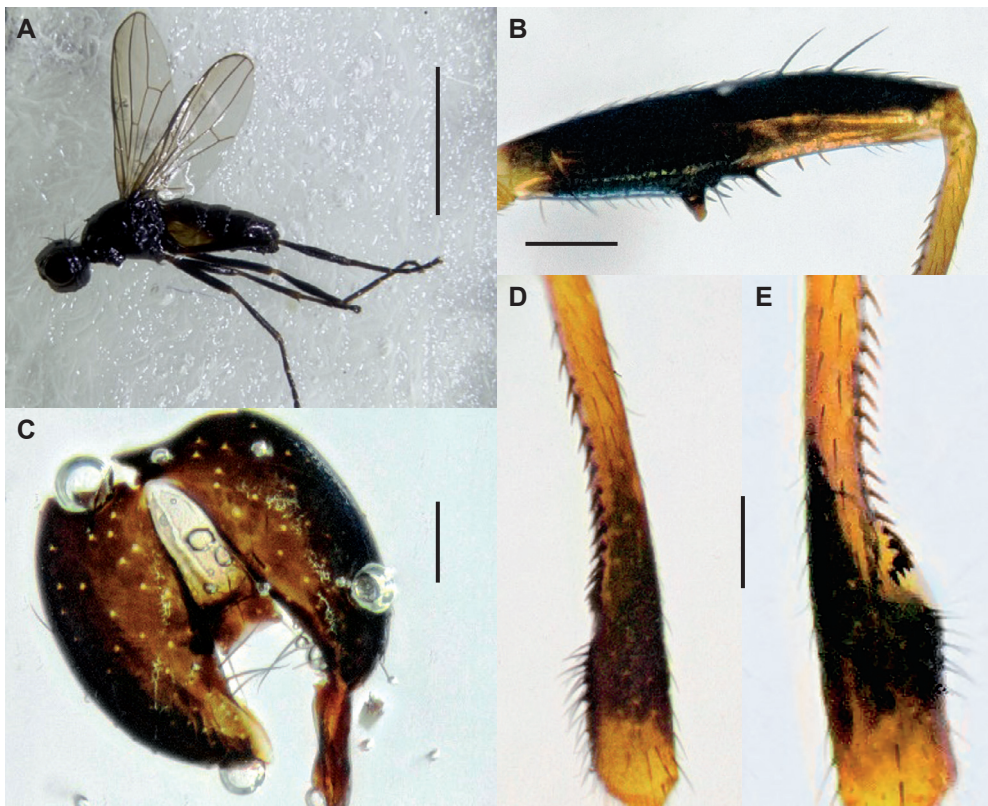


Fig. 2 - Stereomicroscopic images of *T. nigricornis*. A, habitus; B, fore femure anterior view; C, hypopygium; D, fore tibia, anterior view; E, fore tibia, view posterior. Scales (in mm): A – 2; B-E – 0.5.

in the apical half, and hypopygium as part of the copulatory apparatus (Fig. 2 C); yellow coloured fore tibia except of the apical half region, as shown by the anterior (Fig. 2 D) and posterior (Fig. 2 E) views.

These corroborated elements correspond to key morphological characteristics of *T. nigricornis*, indicating that the isolated organism corresponds to this species.

DNA barcoding identification of T. nigricornis

PCR amplification of mitochondrial cytochrome C oxidase I gene (COI) using specific primers for invertebrates was obtained for 2 distinct adult individuals. DNA sequencing of both TN1 and TN2 amplicons indicate 100% sequence identity over a 622-bp gene fragment.

The TN1 sequence was assigned GenBank accession number KJ007735.

Sequence similarity search using BLAST screening indicated the closest match of TN1 sequence (99% identity) with the *T. nigricornis* COI gene fragment (Laamanen et al., 2005; accession number AJ832117).

DISCUSSION

This report represents the first mentioning of *T. nigricornis* presence in Romania.

The location of the insect on a pig carcass in advanced decomposition stage confirmed the preference of this species for such environments.

The adult abundance in the forensic experimental setting was relatively low, only 10 individuals (males and females) being observed on the site over 2 weeks period. Such low density is in accordance with previous statements (Pont & Meier, 2002) mentioning that *T. nigricornis* was only occasionally abundant. Moreover, the appearance season of *T. nigricornis* on Romanian territory in early spring, when temperatures raised to about 20°C, is in accordance with prior reports on the main occurrence period of this species within end of March - August interval, corresponding to the warm season (Pont & Meier, op. cit.). The identity of this species was confirmed both taxonomically and by DNA barcoding based on mitochondrial cytochrome C oxidase I gene (COI) amplification and sequencing.

To date, the worldwide spread range of this species covered the nearctic USA region (Alaska, Connecticut, Illinois, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania), and Palaearctic regions of Asia including Kazakhstan, Korea, Kyrgyzstan, Pakistan, Turkmenistan, South Korea and Japan (Pont & Meier, op. cit.; Ozerov, 2005).

In Europe, the distribution of *T. nigricornis* species covered 19 countries (Pont & Meier, op. cit.), with a species widespread incidence in the central and northern European zones and eastwards to Russia (Far East province), but with only specific locations in certain European countries such as Denmark (East Jutland and North East Zealand), Norway (northwards to Nord Traendelag), Sweden (Skane to Torne Lappmark), and Finland (northwards to Kuusamo).

In this respect, the current report on the presence of *T. negricornis* on the southern Romanian territory contributed to update the distribution map of this species on the European range (Fig. 3), indicating the southernmost location of this Sepsidae species within Eastern Europe.

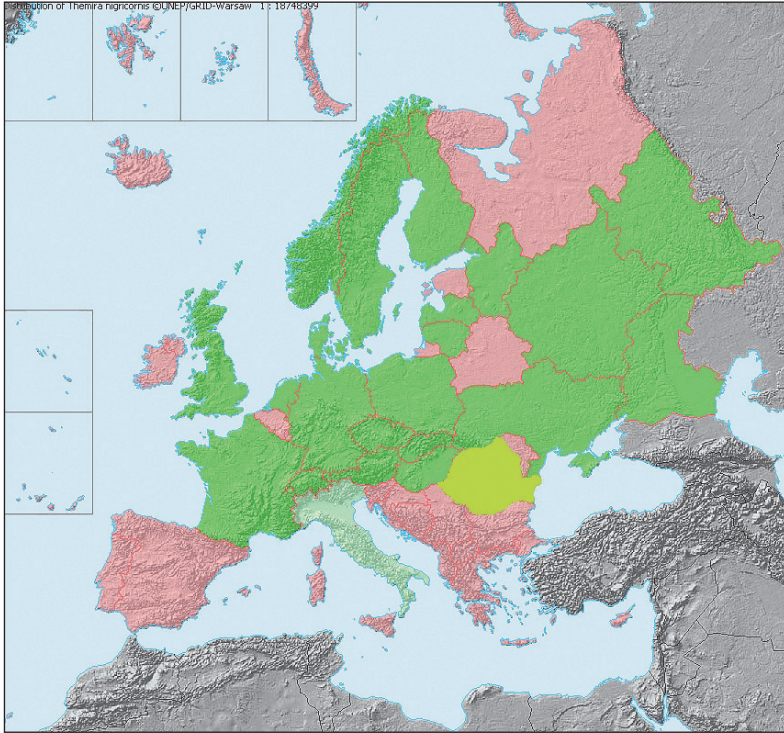


Fig. 3 - Updated map of *T. nigricornis* distribution in Europe (modified from <http://www.faunaeur.org>). Legend: Presence - dark green, doubtful presence - light green, absence - pink, new record - bright green.

ACKNOWLEDGEMENTS

We thank the administration of “Grigore Antipa” National Museum of Natural History, Bucharest, Romania for technical assistance with the experimental settings. This work was supported for COI gene fragment sequencing, by the Romanian Academy RO1567-IBB05/2013 project of the Institute of Biology Bucharest.

PRIMA SEMNALARE A SPECIEI *THEMIRA NIGRICORNIS* (MEIGEN, 1826) (SEPSIDAE: DIPTERA) ÎN ROMÂNIA

REZUMAT

Acest raport privind semnalarea speciei *Themira nigricornis* (Meigen, 1826) (Sepsidae: Diptera) pe teritoriul românesc reprezintă prima înregistrare a acestei specii în sud-estul Europei. Adulții speciei *T. nigricornis* au fost colectați în anotimpul primăvara, în a doua jumătate a lunii aprilie, în cadrul unui experiment amplasat în spațiu urban (București), în partea de sud a României. Au fost utilizate carcase de porc domestic (*Sus scrofa domesticus*) aflate într-un stadiu de descompunere avansată, amplasate direct pe sol și înconjurată de vegetație. Exemplarele de *T. nigricornis* au fost localizate în segmentul posterior al carcaselor. Abundența a fost relativ scăzută, constând în 10 exemplare colectate într-un interval de două săptămâni. Specia a fost caracterizată și identificată taxonomic. Acest studiu, ce documentează prezența speciei *T. nigricornis* în sudul României, extinde arealul de distribuție semnalat de Fauna Europaea, desemnând localizarea în partea de sud a Europei.

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Received: June 18, 2014

Accepted: August 22, 2014

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