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Case Report

First records of *Synthesiomyia nudiseta* (Diptera: Muscidae) from forensic cases in Italy

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ABSTRACT

The knowledge of the fauna associated with carrions and cadavers for a specific region plays a fundamental role in the estimation of the time since death in forensic cases. In the last years global warming and globalization have affected the insect species distribution. This phenomenon is affecting also the species of forensic interest associated with the cadaver decomposition. The species distribution shift, in the forensic context, has been mainly observed in Diptera of different family: Calliphoridae, Stratiomyidae and Phoridae. In the last decade the presence of the carrion feeding species, *Synthesiomyia nudiseta* (Diptera: Muscidae), was reported from forensic cases in Spain and in the last year from Italy where the species was collected from 5 bodies in different decomposition stages in the Genoa district. All the records concern indoor cases with the presence of other species belonging to the first colonization waves (e.g. Calliphoridae, Sarcophagidae).

Different hypothesis about the presence of the species in Italy can be suggested, but the molecular analysis and the importation records support the introduction trough commercial exchanges with Asian countries instead of a variation in the species distribution area from the Iberian Peninsula.

1. Introduction

In 2004 and 2010 Turchetto and Vanin [1–3] highlighted the effects of global warming and globalisation on the distribution and phenology of forensically important species, underlying the need of updated species lists on a regional scale.

Newly introduced species can (1) have different developmental rates and different phenology/seasonality, when compared with their original distribution area; (2) compete with the autochthonous carrion-breeding entomofauna, altering the structure of the communities colonizing a body; (3) be a cause of the misinterpretation of a body transfer; (4) be misidentified if the entomologist working on the case is not well prepared and lacks a global view of the subject.

To our knowledge, after a deep analysis of the available literature, the taxon of forensic interest mostly affected in Europe by the global warming/globalisation is the Diptera order with some species already reported to have changed their distribution such as *Hermetia illucens* (Diptera, Stratiomyidae), *Megaselia scalaris* (Diptera, Phoridae) and *Chrysomya albiceps* (Diptera, Calliphoridae) [1,4–7]. In 2013 another species of forensic interest, *Synthesiomyia nudiseta* (Diptera: Muscidae), already reported from human cadavers in South and North America and East-Asia [8–11] was described from forensic cases in Spain [12]. The authors of this article reported the presence of the species from different localities on the Mediterranean (Alicante) and Atlantic (Ourense) coast and the centre of the country (Madrid) indicating a wide spread of the species [12]. In September 2011, 4 adults of this species were collected in the city centre of Naples (Southern Italy), "in a rather dirty place with dog excrements and urban waste" [13].

In Europe *S. nudiseta* is known as well from the Canary Islands, Madeira Islands, Azores, Malta (www.faunaeur.org) and Mainland Portugal [14]. The 20 °C annual isotherm was considered the border of the distribution of this tropicopolitan species [15].

Synthesiomyia nudiseta is a saprophagous organism with larvae developing both on animal and plant decomposing organic matter. Larvae have been reported as well from animal and human excrements. Larvae, that seem to prefer carrions and cadavers as a food source, can be facultative carnivorous. Pupae are often found among the victim's clothes and the pupal cages are often covered by a silky white substance with adhered debris (Fig. 1). *Synthesiomyia nudiseta* is mainly sinanthropic in all its distribution area and it has been reported only from a few outdoor cases, being more prevalent in indoor contexts [12,16,17]. Developmental data, in the

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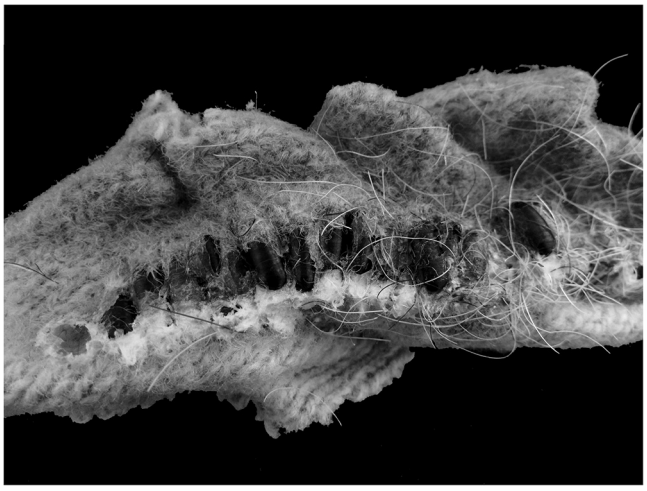

Fig. 1. Pupae of *S. nudiseta* embedded in the clothes fibers of the victim.

range 12–30 °C, are available for South American, Asian [9,18–20] and European populations [12].

Despite the availability of a lot of information and identification material (keys and descriptions) for the adults and the larval stages [12,15] little information is available for the puparia [15].

This note deals with the first findings of *S. nudiseta* from forensic cases in Italy.

The species was found in 5 cases that occurred indoors in North-West Italy between December 2015 and September 2016 (Table 1) for which an initial forensic investigation was requested by the sanitary or the judicial authorities. All the bodies were discovered in the Genoa district. The area is composed of a tight coast facing the Gulf of Genoa with mountains reaching directly the sea. The area is about 500 km from the Spanish border (Fig. S1, Supplementary materials). The average summer temperature on the coast is about 23–24 °C.

The aims of this article are to increase the knowledge about the distribution area of *S. nudiseta* and to describe the puparium of the species. A short description, especially of the puparium, and some illustrations are here reported to avoid future misidentification of this species that in some European regions seems to be not a rare species.

Furthermore, due to the geographical position, the findings here reported play an important role in understanding the phenomena of species occurrence on cadavers and species spread, potentially influenced by the climate change and globalization and it clearly illustrates the consequence of a new introduced species in a forensic context.

2. Material and methods

Entomological samples were collected, fixed, stored and analysed following the guidelines of the European Association for Forensic Entomology [21]. Microscopic observations were carried out using a Leica M60 and a Keyence VHX-S90BE digital microscope, equipped with Keyence VH-Z250R and VH-Z20R lens and VHX-2000 Ver. 2.2.3.2 software (Keyence). In addition, in order to perform morphological and molecular analysis of the samples the protocol reported by Tuccia et al. [22] was followed. Temperatures were obtained from the meteorological station of Genoa Sestri Ponente. Morphological identifications were performed using specific identification keys [15,23,24] and by comparison with the already identified specimens in the collection of one of the Authors (SV). When necessary identifications were confirmed via molecular analysis.

Total DNA extraction was performed using QIAamp DNA Mini Kit (QIAGEN, Redwood City, CA, USA). The protocol 'DNA Purification from Tissue' (QIAGEN) was used according to manufacturer's instructions. Digestion by Proteinase K (100 μg/ml) (Promega) was performed overnight on the tissues after removal of the cuticle as in Tuccia et al. [22]. Sterile deionized water was used to elute DNA. Polymerase Chain Reaction was carried out on mitochondrial COI gene choosing a 710 bp long region and commonly used as a molecular target in insects' barcoding. Universal LCO-1490 Forward primer (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO-2198 Reverse primer (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) were used as described by Folmer et al. [25]. Mitochondrial NAD5 gene was amplified using the forward 5′-CCAAAATATTCWGATCAHCCYTG-3′ and reverse primer 5′-GGATTAACTGTTTGTTATWCTTTTCG-3′ as described by Zehner et al. [26].

PROMEGA GoTaq® Flexi Polymerase protocol was followed in order to prepare a master mix reaction of 20 μl final volume: 4 μl of colourless GoTaqFlexi Buffer (5×), 2 μl of $MgCl_2$ (25 mM), 0.5 μl of each primer (10 pmol/μl), 0.5 μl of Nucleotide Mix (10 mM),

Table 1
Most relevant information about the cases.

Case	Body discovery	Scene	Body	Collected insects (developmental state)	Average temperature from the closest meteorological station (°C)	Estimated mPMI (days)	Estimation based on developmental data published by
1	13.12.2015	Indoor	AcD—M	*S. nudiseta* (LIII; P)** *C. vomitoria* (LIII; A)*	13.1 (max 15.5–min 11.3)	10–14	[12] NA
2	13.06.2016	Indoor	AcD—M	*S. nudiseta* (LII; LIII; P)** *S. argyrostoma* (LIII)* *C. vicina* (LIII; P)*	19.9 (max 22.1–min 17.2)	8–10	[12] [37] [39,40]
3	26.08.2016	Indoor	AcD—B	*S. nudiseta* (LIII)*** *L. sericata* (LIII)***	25.6 (max 29.1–min 22.7)	3–4	[12] [37]
4	04.09.2016	Indoor	AdD—S	*S. nudiseta* (LIII; P)*** *S. argyrostoma* (LIII)*	25.3 (max 29.6–min 21.9)	6–7	[12] [38]
5	21.09.2016	Indoor	AcD	*S. nudiseta* (LIII)** *S. argyrostoma* (LIII)* *L. sericata* (P)**	24.0 (max 28.5–min 20.5)	3–5	[12] [38] [37]

NA, not available; AcD, active decay; AdD, advanced decay; B, bloated; F, fresh; M, mummified; S, skeletonization; A, adult; E, eggs; LI, larvae I instars; LII, larvae II instars; LIII, larvae III instars; LIIIpf, larvae III instars postfeeding; P, pupae; Pu, empty puparia. Asterisk indicates the number of specimens collected and analysed: *1–10; **10–100; ***>100.