

## HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL INVESTIGATIONS ON POST-MORTEM INJURIES CAUSED BY ANTS AND LITERATURE REVIEW

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**Abstract:** Ants are arthropods that may inflict post-mortem skin damages on human corpses, often interfering with forensic investigations. A histochemical and immunohistochemical study was performed on skin samples of a human corpse found in an open suburban area in Southern Italy, showing extensive attack by *Tapinoma nigerrimum* Nylander, 1856 (Hymenoptera: Formicidae). The histological findings were compared to the available literature data about post-mortem skin damages caused by arthropods, also conducting an extensive survey of forensic literature on the subject.

**Keywords:** ants, histological investigation, post-mortem damages, skin, superficial lesions.

### INTRODUCTION

Several species of necrophagous arthropods may inflict post-mortem damages on skin that may be erroneously attributed by pathologists to ante-mortem lesions [1, 2] possibly leading to incorrect assessment of post-mortem interval (PMI) and/or causes of death. Ants are among insects that have been reported as agents of post-mortem damages on the skin of human and animal bodies [3].

In order to investigate in detail how ants inflict these post-mortem damages, a histochemical and immunohistochemical study was performed on skin samples collected from a human corpse on which occurred an extensive attack by *Tapinoma nigerrimum* Nylander, 1856 (Hymenoptera: Formicidae) [4]. Moreover, in order to compare our data to those available about post-mortem skin damages caused by arthropods, an extensive, interdisciplinary survey of forensic literature was conducted, including detailed taxonomical information about arthropod groups and about conditions of corpses undergoing the attacks.

### MATERIALS AND METHODS

Skin samples were collected during the autopsy of a 48-year-old man, found dead in an open suburban area of Cosenza (Region of Calabria, Southern Italy). The body showed clear signs of attack from the ant *T. nigerrimum*, which were also collected live on the corpse. The lesions on the body were red and irregular, and ants were observed while feeding on skin fragments along their edges [4]. The samples, obtained from the abdominal skin in regions with or without lesions, were prepared for histochemical and immunohistochemical investigations aimed to establish the type of post-mortem damage inflicted by ants. Samples were fixed in formaldehyde 10% (Bioptica, Milano, Italy), embedded in Paraplast (Sigma-Aldrich, St. Louis, Missouri, USA) and serially sectioned at 5 µm with a microtome (Biocut, Leica Biosystems, Wetzlar, Germany). Some sections were used for histochemical investigations and others for immunohistochemistry.

Histochemical investigations were performed by staining with haematoxylin-eosin and by a vitality stain, the Masson's trichromic, using an aniline blue kit

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according to a standard protocol (Bio-Optica, Milan, Italy).

Immunohistochemical investigations were performed using anti-human cytokeratin mouse monoclonal antibodies, clones AE1/AE3 (DAKO Denmark A/S, Glostrup, Denmark), diluted 1:50. Sections prepared and stained as previously mentioned were observed under an optical microscope (Leica DM4, Leica Microsystems, Wetzlar, Germany) connected to a digital camera (Leica Microsystems, Wetzlar, Germany).

## RESULTS

The results of histological analyses on skin samples obtained from post-mortem lesions inflicted by ants are shown in Fig. 1. Sections of damaged skin stained by haematoxylin-eosin exhibited an anomalous detachment of the epidermal layer, which appeared raised in blisters of different size and shape (Fig. 1a). The large blisters had a transparent content, while the small ones contained an amorphous material (Fig. 1a and inset). The Masson's trichromic stain showed an amorphous material located at the base of the large blisters and some epithelial cells still attached to the upper layer of the dermis (Fig. 1b). This material, stained in red, apparently deeply penetrated the epidermal layer, reaching in some places the upper layer of the dermis. In another section stained by hematoxylin-eosin, some large swellings were characterized by a complete detachment of the epidermal layer from the underlying dermis (Fig. 1c and d). The complete detachment between these two skin layers was clearly visible by Masson's trichrome stain (Fig. 1e).

The immunohistochemical investigations by anti-cytokeratin antibodies showed an intense staining in the part of the blister detached from the epithelium and also (scattered) in the region overlying the dermis. However, in other parts of the same blister no epithelial cells were seen attached to the dermis (Fig. 1f). Signs of damage were also detected in the middle and innermost layer of the dermis, as red-stained and wavy collagen fibers (Fig. 1e).

Control samples collected from intact skin showed normal layers without signs of degeneration (Fig. 1g).

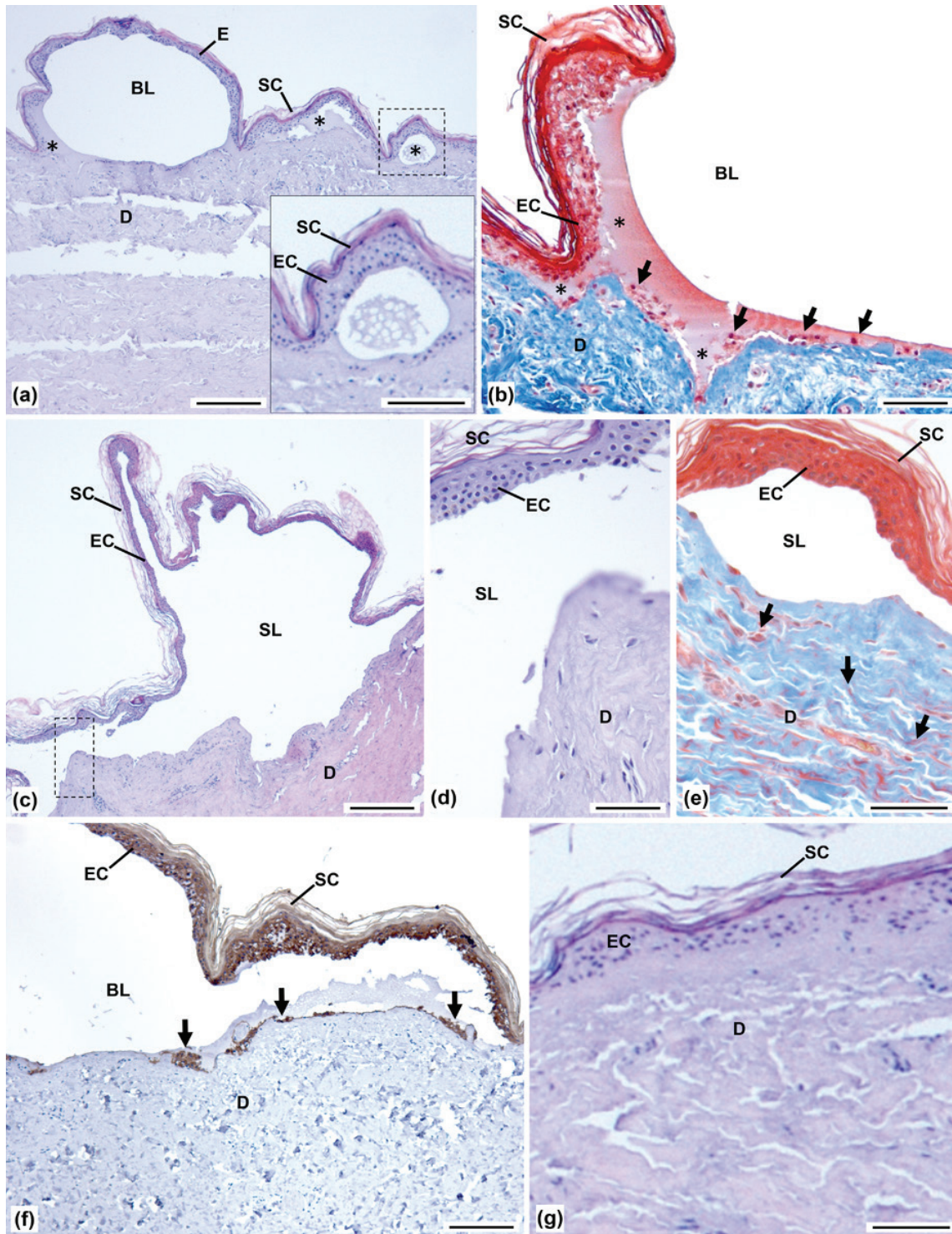
## DISCUSSION

Several reports describe the role of ants as necrophagous insects able to inflict post-mortem

skin damages to human corpses. Generally, these damages are characterized by lesions with irregular and serpiginous outlines on the skin surface [1, 3, 5] but the presence of "papulae" was also reported in one case [6]. Up to date, only two species of ants have been identified as agents of these damages to human corpses, namely *T. nigerrimum* and *Crematogaster scutellaris* (Olivier, 1792). Recently, damages by unidentified species of the genera *Atta* Fabricius, 1805 and *Cephalotes* Latreille, 1802 were also reported [6-8] (Table 1). Data on necrophagous behavior of ants have also been obtained by experimental studies on animal models. In a Brazilian study on insects of forensic relevance using rabbit carcasses as model, *Camponotus rufipes* (Fabricius, 1775) and the genus *Pheidole* Westwood, 1839 were able to inflict small lesions on nose and ears [9]. A study on successional patterns of insect fauna on pig carcasses in Italy reported the activity of *C. scutellaris* as agent of serpiginous damages on skin [10]. During an experimental study involving ant activity on pig carcasses in Brazil, a total of 39 ant species were collected. Among them, three species, *Atta laevigata* (Smith, 1858), *Camponotus sericeiventris* (Guérin-Méneville, 1838), *Pheidole radoszkowskii* Mayr, 1884 and one genus, *Solenopsis* Westwood, 1840, were ascertained to have caused skin damages [11]. In a recent experimental study on pig carcasses in the state of Mississippi (United States) a hybrid genotype, *Solenopsis invicta x richteri*, was reported as agent of skin damages [12]. Post-mortem alterations of human skin by cockroaches (Blattodea) and crustaceans (Siphonostomatoida, Amphipoda and Decapoda) have also been reported (Tables 1 and 2).

Experimental studies on post-mortem alterations caused by arthropods in animals reported damages by *Dermestes maculatus* De Geer, 1774 (Coleoptera: Dermestidae) [13], *Necrobia rufipes* De Geer, 1775 (Coleoptera: Cleridae) [14], *Agelaiia fulvofasciata* (De Geer, 1773; Hymenoptera: Vespidae) [15], *Notiothauma reedi* MacLachlan, 1877 (Mecoptera: Eomeropidae) [16] and *Procambarus clarkii* Girard, 1852 (Decapoda: Cambaridae) [17].

Studies involving post-mortem skin damages of forensic relevance caused by arthropods are based on general descriptions without detailed histological analyses, except studies concerning scavenging activities on human bodies by *T. nigerrimum* [7], Blattodea [18], *Austropotamobius pallipes* Lereboullet, 1858 (Decapoda: Astacidae) [19] and *Gammarus pulex* (Linnaeus, 1758; Amphipoda: Gammaridae) [20] (Tables 1 and 2). In the study on *A. pallipes* individuals



**Figure 1.** Histological analyses on skin samples obtained from post-mortem lesions inflicted by *Tapinoma nigerrimum*. (a) Anomalous detachment of the epidermal layer, raised in blisters of different size, some transparent and others containing an amorphous material (asterisks) (scale bar: 250µm). Inlay: detail of the outlined region, showing a small blister containing the amorphous material. Stained by hematoxylin-eosin (scale bar: 100µm). (b) Detail of a large blister showing an amorphous material at its base (asterisks) and some epithelial cells attached to the upper layer of the dermis (arrows). Stained by Masson's trichrome (scale bar: 100µm). (c) Large swelling with an irregular shape. Stained by hematoxylin-eosin (scale bar: 250µm). (d) Detail of the region outlined in Fig. 1c, showing the complete detachment of the epidermal layer from the underlying dermis. Stained with hematoxylin-eosin (scale bar: 100µm). (e) Detail of a large swelling showing the complete detachment between the epidermal layer and the dermis and loosely detached red and wavy collagen fibers within the dermis (arrows). Stained by Masson's trichrome (scale bar: 100µm). (f) Intense immunohistochemical reaction to anti-cytokeratin antibodies showing the presence of epithelial cells in the part of the blister detached from the epithelium and in the region overlying the dermis (arrows) (scale bar: 250µm). (g) Detail of intact skin. Stained with hematoxylin-eosin (scale bar: 100µm). Abbreviations: BL, blister lumen; D, dermis; E, epidermis; EC, epidermal cells; SC, stratum corneum; SL, swelling lumen.

**Table 1.** Post-mortem skin damages caused by insects on human bodies: reported taxa, case number, type of damage, discovery area, season and corresponding reference. Abbreviations: DF, ants from subfamilies Dolichoderinae and Formicidae also reported; NR, not reported

Taxon	Case no.	Reported damage	Discovery area (country)	Season	Reference	
<i>Tapinoma nigerrimum</i> (Hymenoptera: Formicidae)	1	Injuries (1-4.5mm) with serpiginous edges	Rural (Italy)	Spring	[7]	
	2	Injuries with serpiginous edges and scalloped areas, bright red to brown	Suburban (Italy)	Spring	[4]	
<i>Crematogaster scutellaris</i> (Hymenoptera: Formicidae)	3	Injuries with serpiginous edges and scalloped areas; papulae	Indoor (Indoor)	Summer	[6]	
<i>Atta</i> sp. (Hymenoptera: Formicidae)	4	Scalloped injuries surrounding dry, orange-colored skin	Rural (Brazil)	Autumn-winter	[8]	
	5	Scalloped injuries surrounding dry, orange-colored skin	Urban/rural (Brazil)	Spring-summer	[8]	
<i>Cephalotes</i> sp. (Hymenoptera: Formicidae)	6 (DF)	Superficial skin injuries	Forest (Brazil)	Winter-spring	[8]	
	7	Parchment skin	Indoor (Australia)	NR	[22]	
	8	Irregular parchment areas with serpiginous edges and skin loss	Bushland (Australia)	Summer	[23]	
	9	Irregular lesions with skin loss	Rural (Australia)	Summer	[23]	
	10	Dry irregular areas with skin loss and serpiginous edges	Indoor (Australia)	NR	[23]	
	11	Converging lesions with irregular edges	Indoor (Spain)	NR	[24]	
	12	Irregular and scalloped parchment areas with serpiginous edges and skin loss	Rural (Italy)	NR	[5]	
	13	Skin surface injuries	Rural (Italy)	NR	[5]	
	Formicidae (Hymenoptera)	14	Abrasions with serpiginous edges	Public park (Italy)	NR	[25]
		15	Abrasions with serpiginous edges	Garden (Italy)	NR	[25]
16		Abrasions with serpiginous edges	NR (Italy)	NR	[25]	
17		Abrasions with serpiginous edges	Indoor (Australia)	NR	[26]	
18		Dry irregular areas and serpiginous edges	Indoor (Australia)	NR	[26]	
19		Semi-circular lesions in the body regions in contact with the surface	Urban (Australia)	NR	[26]	
20		Serpiginous lesions, dry and yellow-brown, or red with passive oozing of blood	Urban (Australia)	NR	[27]	
21		Multiple irregular brown lesions (1-4mm)	Urban (Italy)	Summer	[28]	
22	Irregular parchment areas with serpiginous edges	NR (Turkey)	NR	[1]		
Blattodea	23	Superficial, irregular lesions, reddish-brown	Indoor (Canada)	NR	[18]	
	24	Superficial, irregular lesions, reddish-brown	Indoor (Canada)	NR	[18]	
	25	Excoriations (3-18mm)	Indoor (Canada)	NR	[18]	
	26	Skin abrasions	NR	NR	[29]	

**Table 2.** Post-mortem skin damages caused by crustaceans on human bodies: reported taxa, case number, type of damage, discovery area, season and corresponding reference. Abbreviation: NR, not reported

Taxon	Case no.	Reported damage	Discovery area (country)	Season	Reference
Caligidae (Siphonostomatoida)	1	Oval punctate lesions	Sea (Australia)	NR	[22]
<i>Gammarus fasciatus</i> (Amphipoda: Gammaridae) and other unidentified crustaceans	2	Scavenging and feeding	Lake (Canada)	NR	[30]
	3	Scavenging and feeding	River (Canada)	NR	[30]
	4	Scavenging and feeding	River (Canada)	NR	[30]
	5	Scavenging and feeding	River (Canada)	NR	[30]
Brachyura	6	Scavenging and feeding	Lake (Canada)	NR	[30]
<i>Excirolana</i> sp. (Isopoda: Cirolanidae)	7	Punctured skin lesions	Sea (NR)	NR	[29]
<i>Natatolana woodjonesi</i> (Isopoda: Cirolanidae)	8	Oval punctate lesions	Sea (NR)	NR	[29]
<i>Gammarus pulex</i> (Amphipoda: Gammaridae)	9	Erythematous lesions, not hemorrhagic and with irregular edges (3-10mm)	Canal (France)	NR	[20]
	10	Erythematous lesions, hemorrhagic and with irregular edges	Lake (France)	NR	[20]
<i>Niphargus elegans</i> (Amphipoda: Niphargidae)	11	Lesions (1-2mm deep), occasionally converging into larger reddish areas	River (Italy)	Summer	[31]
<i>Austropotamobius pallipes</i> (Decapoda: Astacidae)	12	Epidermal lesions, poorly hemorrhagic	Lake (France)	NR	[19]
<i>Procambarus clarkii</i> (Decapoda: Cambaridae)	13	Scavenging and feeding	River (Italy)	Summer-autumn	[32]

found on the body of a woman submerged in an artificial freshwater lake, signs of the attack by the crustacean (extensive hemorrhagic lesions) were detected on several body parts. The histological analyses revealed detachment and loss of the epidermal layer over an intact dermis, compatible with feeding activity by the crustacean [19]. The other crustacean species for which a post-mortem scavenging activity has been reported is the shrimp *G. pulex*. The interesting features of this activity are focal lesions, histologically characterized by loss of all epidermal layers but the stratum corneum, yielding a raised “bubble” aspect. Sometimes the “bubbles” contained serum with red blood cells and excisions at their sides, presumably the access point for shrimp feeding. Based on the intense hemorrhagic aspect of lesions, the authors advanced the hypothesis of emission of anticoagulants by *G. pulex* [20]. The skin lesions involving Blattodea were histologically similar to those caused by *A. pallipes* [18, 19].

The previous study on *T. nigerrimum* [7] apparently involved a prolonged attack by ants, causing a larger mechanical detachment of the epidermis and

extensive changes to the dermis structure. Based on these observations, the authors advanced the hypothesis of a digestion by chemicals released from glands of the ant mouth parts before active feeding: these secretions could have weakened the skin compactness, making it suitable for consumption [7].

The present histological study shows for the first time a blistering and swelling appearance of post-mortem skin lesions by *T. nigerrimum*. These data support the hypothesis that ants produce secretions suitable for pre-oral digestion of the skin, able to undermine the structure of the epidermal layer and causing extensive skin swelling in preparation for mechanical attacks by ant mouthparts. This hypothesis may be supported by the observation of papulae in a human body attacked by *C. scutellaris* [6]. Previous studies in ants have described propharyngeal and other mouthpart glands able to produce digestive enzymes used for pre-oral digestion [21]. Further studies will be necessary to verify the presence and the structure of these glands in *T. nigerrimum*, together with biochemical studies on the composition of the secretion.

### Conflict of interest

The authors declare that they have no conflict of interest.

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