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Forensic evaluations on a crime case with monospecific necrophagous fly population infected by two parasitoid species

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ABSTRACT

The entomoforensic investigation carried out on a decaying corpse found indoor has shown an unusual insect cenoses, that created some problems in estimating the actual time since death. Except for very few beetles, the body had been colonized by a monospecific population of the fly, *Hydrotaea capensis* Weidemann 1818 (Diptera, Muscidae), parasitized by two species of parasitoid wasps of the superfamily Chalcidoidae: *Nasonia vitripennis* (Walker, 1836) (Hymenoptera, Pteromalidae) and *Tachinaephagus zealandicus* Ashmead 1904 (Hymenoptera, Encyrtidae). The possible implications of the parasitoids on the fly population growth and density, the life cycle length, the maggots' and pupae survival and their hindering the PMI evaluation and other entomologico-forensic inferences are discussed.

Keywords: Forensic Entomology, P.M.I., Fly monospecific population, Hydrotaea capensis, Fly parasitoids, Nasonia vitripennis, Tachinaephagus zealandicus

Introduction

The entomoforensic science is nowadays used mostly for estimating the postmortem interval (P.M.I.) [1-10], even if various new applications, such as drug and chemical assumption, toxins or xenobiotics presence, abuse and rape before the death and so on [11-13], are in progress. The time since death is calculated by taking into account the largest number of variables, both intrinsic to the involved species and extrinsic to the insects, interfering one other. Among the first group of variables, we can include the kind of species feeding on the remains, the number of specimens, the

time of development of the species, the composition of arthropod biocenoses, the successional patterns, the autoecological and synecological features of each species.

In the second group we can include all the external factors referred to the macro-ecosystem of the environment and to the micro-ecosystem of the body: the kind of biotope, the season, the climatic conditions, the composition of the local fauna and flora, the soil or litter composition, xenobiotic pollution, treatments with pesticides or other anthropic interferences. Regarding the corpses we have also to consider, the position and the state of the body (indoors, outdoors, burned, buried, hanging, under branches, underneath or inside plastic bags, etc.), the age and the general status (newborn, young, old, ill, whole, torn, with injuries or wounds, etc.) and the rate of decomposition of the human remains.

Nevertheless the most widespread, and less known, biotic factor affecting the growth and the survival of the insects is the presence of their parasites and parasitoids[1]. Arthropod parasitology is an immense world, only a very little part of which has been studied so far: the parasitology of useful bred insects (honey bees, silk worms, etc.), the parasitology of arthropods of medical and veterinary interest (considering the arthropods as vectors of parasites pathogenic for humans or animals) and, only recently, the parasitology applied to biological pest management. The last applications which include the control of fruit flies, in agriculture, and the elimination of flies inducing myasis, in the veterinary science, employ parasitoids more than true parasites.

Many species of Hymenopteran have been introduced for such purposes, often in an uncontrolled fashion in countries and environments far away and different from their native area.

The pest flies, against which this biological control is performed, are often the same species or very closely related to the species found in corpse, and parasites and parasitoids are not strictly selective. Therefore we can reasonably suppose that many of them could also infest the flies used as PMI indicators, altering their development or killing them and, consequently, hindering entomoforensic evaluations [14, 15].

Case history

Crime scene

In mid September 2000 in a house of Eraclea, a little town in the inland of Venice (N-E Italy), the decaying remains of a young woman were found. The house, empty and with tightly shut doors and windows, Venetian blinds included, had not been inhabited for long time. The environmental temperature had been warm during the summer (25-30°C daily average) whereas indoors a temperature a few degrees lower, but more constant, had been recorded. The body, partially dressed, lying face-downward, was near the front door. The tiled floor and the walls bore trace of dried blood, as if the women had dragged herself towards the way out. The uncovered skin was drying out, except for the organic matter, in ammoniacal fermentation, protruding out from a wound in the skull (the crime weapon was later identified as the hooked support of a radiator). No papers nor other objects identifying the woman were found.

The investigators called us saying that the body was full of “a lot of worms and mouse faeces”, instead, as we arrived at the scene of the crime we found a very large number of maggots, pupae and puparia. The fly larvae of all instars were feeding on the uncovered skin parts and, among the hairs, on the matter in ammoniacal fermentation, whereas many pupae and pupal cases were found on the driest parts and especially on the clothes. Very few necrophagous beetles were

present, while no other arthropod was found in the room. It must be presumed that the insects had come in through a little space under the door, attracted by the cadaver's smell. At first sight, both the maggots and the pupae seemed to belong all to just one species. Pupae and puparia were characterized by two conspicuous prothoracic horns (fig. 1); some flies had successsfully emerged as indicated by an empty puparium missing an apical hood, while others pupae had been killed as indicated by one or two parasitoid emergence holes.



Fig. 1. *Full view on puparium*

Forensic entomology remarks

The largest possible amount of beetles and flies was collected from the corpse at the crime site and immediately taken alive to the our laboratory, in the Biological Department, University of Padova, a few kilometers away from the death recovery site. Through an additional collection, made in the mortuary during the autopsy, the same type of specimens were collected.

Materials and methods

In the laboratory the few Coleoptera found (4 adults, 6 pupae and 2 larvae) were prepared and determined with specific keys [5, 16-18].

The maggots were certainly all of the same species, but they appeared to be of a species not common on carrion and not easy to identify. A portion of the collected larvae, of each instar, were killed by dropping them into hot water and then stored in alcohol 70% for further study [5, 19]. The remaining specimens were reared to obtain the adults for a sure species identification and to calculate the length of the complete life cycle.

Breeding was carried out in small cages covered with a fine-meshed net, with damp sand on the bottom and the maggots were fed on rotten minced meat [19, 20]. Temperature and humidity were maintained near to those measured inside the house at the moment of the crime discovery (25°C; 70% rh., in average). Individuals beginning to pupariate were daily carefully collected and

transferred into dryer cages, as naturally occurs, without food and wet sand, to avoid the bacterial and fungal attack during the quiescent phase.

The emerged adults (fig. 2) were collected and some of them were dry prepared, pinned and determined, while others were transferred alive into new clean cages with food and wet sand. These were mantained through two generations to study two full life cycles. With the intention of obtaining diapausing pupae, the offspring of the third generation were kept without food in a dry, airtight jar and left in a cooler thermostatic room (10°C) for six months.



Rearing the flies, we also obtained some small parasitoid wasps, responsible for the holes observed in the pupa cases (fig. 3). Some adults were sent to taxonomic specialists for species' determination.

All the observations of the insects or of the diagnostic parts of them were performed under a stereomicroscope (Leica MZFL III) and a microscope (Leica DMR); photographs were taken with a digital camera (Nikon D1X and Leica DC 200).

Results and Discussion

The collected beetles belonged to two families, usually associated with carrion: the skin beetle *Dermestes maculatus* Payne (Coleoptera: Dermestidae) and the bone beetles *Necrobia rufipes* De Geer and *Necrobia violacea* L. (Coleoptera: Cleridae). In Italy *D. maculatus* and *N. rufipes* are widespread beetles, while *N. violacea* is not a common species, reported only from the northern regions [16, 21] and never cited, as far as we know, in the forensic literature. According to Mégnin [1] and Smith [5], these insects are typical of the 5th-7th waves, arriving at the remains after the butyric fermentation, when the carrion is drying out or is almost dry. Many other findings confirm the time of their arrival in the last stages of decay. They are gnawers on dry skin, sinews and bones and probably maggot predators [5, 22]; nevertheless in our case their small amount could not affect the fly population.



Fig. 3. *Emerged adults (parasitoids)*

The identification of the collected maggots and pupae and of the adults from the rearing confirmed that the body had been colonized by a monospecific population of the genus *Hydrotaea* Robineau-Desvoidy (=*Ophyra*) (Diptera:Muscidae). The genus was determined using as diagnostic features the mouthparts and the anterior and posterior spiracles (fig. 4.1) of the larvae [23-25] and the prothoracic spiracular horns of the puparium (fig. 4.2). The species was determined as *Hydrotaea*

capensis (Widemann, 1818), determination confirmed by A. Pont (personal communication, 2001)[2]. This is a

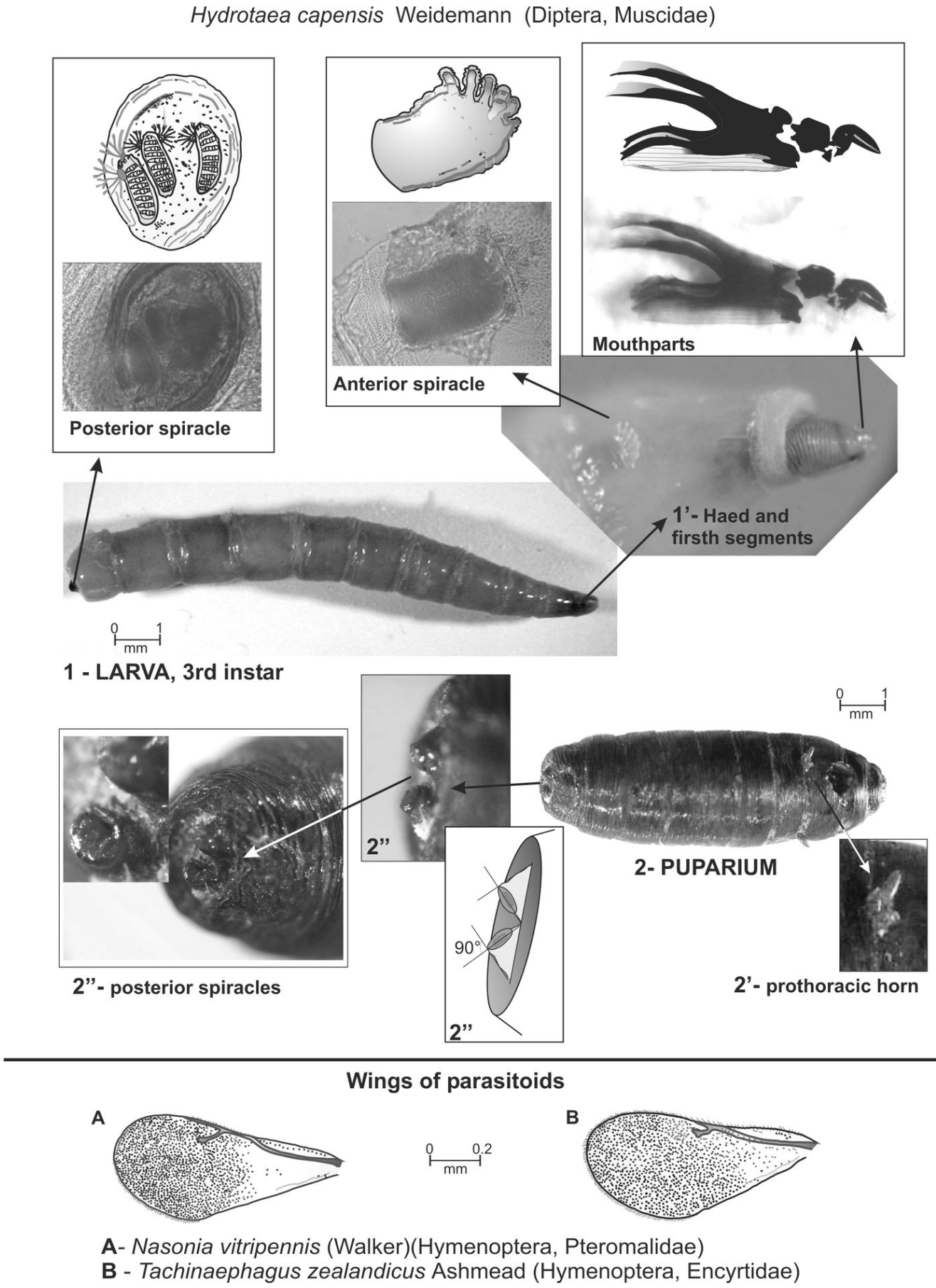

Fig. 4. *Anatomical features*

Mediterranean and Afrotropical species, known, but not widely in northern Italy [26-28].

According to many Authors [26, 29, 30] different species of the genus *Hydrotaea* feed on dry faeces, manure, dung, carcasses and lavatories in the summer, the adults being most abundant in August. In human remains the species appears during the period of ammoniacal fermentation (i.e. four months after death and over); K. G. V. Smith [5] wrote that "if the body has not been exposed to open air for that period of time, these may be the only maggots or puparia present and the time of their arrival may be affected" and this seems to be the same situation we found. Mégnin [1] found flies of this genus in corpses about one year old. From the rearings we obtained two complete cycles of *H. capensis*, from eggs to adults. In both the experiments, adults emerged after 12-17 days (the whole life cycle being of 15 days, in average, at 25°C).

Together with the adult flies, in the rearing cages some adults of parasitoid wasps were also found, belonging to two different species of Hymenoptera Chalcidoidae. Specialists identified them as the Pteromalidae *Nasonia vitripennis* (Walker, 1834) and as the Encyrtidae *Tachinaephagous zealandicus* Ashmed, 190_4 [31] (fig. 4 A, B).

Hydrotaea capensis

The number of holed puparia in the two experiments were 5% and 10% respectively. In the experiment with the maggots kept in the jar hermetically closed, after six months we found 66 pupae and puparial cases: 42 unholed and 24 pierced (36% of the total), 20 of which by a single hole, 4 by two or three holes (Tab I). The only wasps present were, in the last experiment, *N. vitripennis*, species reported as winner competitor, in the long run, over other parasitoids [41]. The dissections yielded some unholed still pupae, parasitized by 6-8 adult wasps, ready for eclosion (fig. 3), as observed by Nasser and Eraky [42].

breedings	time of eclosion	pierced puparia
1st	12-17 days	5%
2nd	24-30 days	10%
3th	6 months	36%

Tab. 1. *Time of eclosion of* Hydrotaea capensis *and percent of parasitized papae and puparia*

The former is a polyphagous species, living in various habitats, and which may also parasitize several Cyclorrhapha dipterans [32-34]; its gregarious larvae develop inside the fly pupae, attached to the host's nymphs and also into maggots paralyzed by their venom or, as idiobiont ectopararasites, on nymphs of Cyclorrhapha flies. Cosmopolitan in its distribution, this species is the only European of the genus.

The latter wasp species, *T. zealandicus*, probably native to Australia and New Zealand, is reported as a parasitoid of synanthropic flies (Calliphoridae, Muscidae, Sarcophagidae), that attacks the third instar of the maggots, the postfeeding or the prepupae [35, 36]. It has been introduced into the Southern Hemisphere and several States of U.S.A., as a biological control agent against myasis-inducing flies [37-40]. This is the first record of *T. zealandicus* for the Paleartic Region [31].

Conclusions

This murder appears as a textbook case, as if it had been performed in laboratory under controlled conditions. The indoor environment was constant in temperature and humidity, the insect fauna

was very simple, and there was no evidence that a vertebrate had disturbed the corpse. Crime scene conditions were reproduced in our laboratory in order to establish the length of the whole life cycle of *Hydrotaea capensis*, a datum not reported in literature, for an estimation of the exact time when this crime was committed. In fact in long-term deaths the medico-legal inferences about the time since death are very difficult inasmuch as the degradative process decelerates, getting to a final steady state. In most cases a criminal investigation and a medical evaluation may be aided by the entomological observations.

However in this case, owing to the lack of other insects, usually present in the early stages of decomposition, it was only possible to state that: the murder was committed into the house (the same room), the body had been inside for four months or more and nobody had come in before the police's discovery of the corpse. The presence of only a *Hydrotea capensis* population and the lack of the usual cadaveric fauna could be explained by the physical isolation of the remains and by the ability of *H. capensis* to find an indoor corpse [5].

Final remarks

Until today, lacking any kind of clue (neither documents nor personal objects, nor registration of the fingerprints, nor even adisappearance report), the victim has not been nameidentified. All that it is possible to say about her is that she was a young Caucasic woman, probably from Eastern Europe. The entomological inferences agree with those of the forensic-pathologists and legal-doctors: death had occurred at least four months prior to discovery. Surely the lack of the necrophagous biodiversity and the presence of the parasitoids did not help the entomologists to precisely establish the PMI.

The person guilty of this murder has not yet been found, as it often happens when the crime involves irregular immigrants, but it may be linked with prostitution or drug dealing.

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References

1. P. Mégnin, La fauna des cadavres, Encyclopédie scientifique des aide-mémoires, Masson, Gautier, Villard et Fils, Paris, 1894.
2. G.F. Bornemissza, An analysis of arthropod succession in carrion and the effects of its decomposition on the soil fauna.Aust. J. Zool., 5 (1957) 1-12.
3. P. Nuorteva, Sarcosaprophagous insects as forensic indicators, In C.G. Tedeschi, W.G. Eckert, L.G. Tedeschi (eds.). Forensic medicine and environmental hazards, vol. II, Sanders, Philadelphia, 1977, pp. 10721095.
4. Y.Z. Erzinçlioglu, The application of entomology in forensic medicine. Med. Sci. Law. 23 (1983) 57-63.
5. K.G.V. Smith, A Manual of Forensic Entomology, British Museum (Natural History), London, 1986. pp. 1-205.

6. E.P. Catts, M.L. Goff, Forensic entomology in criminal investigations. Annu. Rev. Entomol. 37 (1992) 253272.
7. M.L. Goff, Estimation of Postmortem Interval Using Arthropod Development and Successional Pattern. Forensic Sc. Rev. 5 (1993) 82-94.
8. M. Leclercq, C. Verstraeten, Entomologie et médicine légale. L'entomofaune des cadavres humains: sa succession par son interprétation, ses résultats, ses perspectives. J. Med. Lég. Droit Méd. 36 (1993) 205222.
9. G.S. Anderson, S.L. Van Laerhoven, Initial studies on insect succession on carrion in Southwestern British Columbia. J. Forensic Sci. 41 (1996) 617-625.
10. M.I. Marchenko, Medicolegal relevance of cadaver entomofauna for the determination of the time of death. Forensic Sci. Internat. 120 (2001) 89-109.
11. B. Bourel, G. Tournel, V. Hedouin, M. Deveaux, M.L. Goff, D. Gosset, Morphine extraction in necrophagous insect remains for determining ante-mortem opiate intoxication. Forensic Sci. Int. 120 (1-2) (2001) 127131.
12. R. Gagliano-Candela, L. Aventaggiato, The detection of toxic substances in entomological specimens. Int. J. Legal. Med.114(4-5) (2001) 197-203.
13. D. Komar, O. Beattie, Postmortem insect activity may mimic perimortem sexual assault clothing patterns. J. Forensic Sci. 43(4) (1998) 792-796.
14. J.A. Payne, W.R.M. Mason, Hymenoptera associated with pig carrion. Proceeding of the Entomological Society of Washington 73 (1971) 153-162.
15. M. Turchetto, S. Vanin, An approach to fly parasitoid interference on entomoforensic evaluations. XXII Congress of the Italian Society of Parasitology, SoIPa, Parassitologia 44, (Suppl.1) (2002) 105.
16. A. Porta, Fauna Coleopterorum Italica 2 Voll.+ 3 suppl. 1(1923) 285, 2(1926) 405, 1 suppl. (1934) 208, 2 suppl (1949), 3 suppl. (1959) 344, Stabilimento Tipografico Piacentino, Piacenza.
17. R. Perrier, Fauna de la France Coléoptères V (1927) 192 Delagrave Paris.
18. R. Perrier, Fauna de la France Coléoptères VI (1927) 230 Delagrave Paris.
19. W. D. Lord, J. F. Burger, Collection and Preservation of Forensically Important Entomological Materials, JFSCA 28 (1983) 936-944.
20. M. Turchetto, Implicazioni entomologico-forensi dell'introduzione in Italia della mosca neotropicale Hermetia illucens L. (Diptera: Stratiomyiidae), Rivista Italiana di Medicina Legale 22 (6) (2000) 1279-1290.
21. P.Audisio, G. Gobbi, G. Liberti, G. Nardi, Coleoptera Polyphaga IX (Bostrichoidea, Cleroidea, Lymexyloidea), in A. Minelli, S. Ruffo, S. La Posta (eds.) Checklist delle specie della fauna italiana, 54 (1995) Calderini, Bologna.
22. P. Kulshrestha, D.K. Satpathy, Use of beetles in forensic entomology, Forensic Sci. Int. (1-2) (2001) 127-31.
23. A. Cuthberston, Biological notes on some Diptera in Southern Rhodesia. Occasional Papers of the Rhodesian Museum 4 (1935) 11-28.
24. H. F. Chu, The immature insects, WM. C. Brown Company Publisher, Dubuque, Iowa (1949) 234 pp.
25. H.J. Teskey, Key to families - larvae, in J. F. MacAlpine, B. V. Peterson, G. E. Shewell, H.J. Teskey, J. R. Vocheroth, D. M. Wood (eds.), Manual of Neartic diptera. Monograph 27, Vol. 1. Research Branch Agriculture Canada, Quebec, Canada (1981) 125-147.
26. A.C. Pont, Muscidae, in K.G.V. Smith (ed.), Insects and other arthropods of medical importance, The Trustes of the British Museum (Natural History), London (1973) 251-270.

27. A. Pont, L. Matile, Découverte de quelques Insectes de J. P. Mégnin; identité d' Ophyra cadaverina Mégnin (1894). Bulletin de la Société Entomologique de France 85 (1980) 41-43.
28. K. Gorodkov, A.C. Pont, R. Rozkošý, Diptera Muscoidea. In A. Minelli, S. Ruffo, S. La Posta (eds.), Checklist delle specie della fauna italiana, Vol. 76 1995 Calderini, Bologna.
29. G.S. Graham-Smith, Observation on the habits and parasites of common flies. Parassitology 8 (1916) 440544.
30. B. Greenberg, Flies and Disease. 2 Vol, viii + 856pp; x + 447 pp. Princenton University Press, 1971-73 Princenton.
31. M. Turchetto, C. Villemant-Aït Lemkaden, S. Vanin Two fly parasitoids collected during an entomoforensic investigation: the widespread Nasonia vitripennis (Hymenoptera Pteromalidae) and the newly recorded Tachinaephagus zealandicus (Hymenoptera Encyrtidae). Boll. Soc. entomol. ital. 135 (2003) 109115.
32. D.C. Darling, J.H. Werren, Biosystematics of Nasonia (Hymenoptera: Pteromalidae): Two New Species Reared from Birds' Nests in North America, Ann.Entomol. Soc. Am., 83 (1990) 352-370.
33. E.F. Legner, The status of Nasonia vitripennis as a natural parasite of the house fly, Musca domestica, Canadian Entomologist 99 (3) (1967) 308-309.
34. P. Blanchot, Inventaire des Parasitoïdes de Mouche synanthropes recensés en France, EPHE Biologie et Evolution des Insectes 7/8 (1994/1995) 111-119.
35. M. A. Ferreira de Almeida, A. Pires do Prado, C.H. Geden, Influence of temperature on development time and longevity of Tachinaephagus zealandicus (Hymenoptera: Encyrtidae), and effects of nutrition and emergence order on longevity, Envirin. Entomol. 31 (2002) 375-380.
36. G.S. Olton, Biology of Tachinaephagus zealandicus (Hymenoptera: Encyrtidae), parasitoid of synanthropic Diptera, Can. Ent. 106 (1974) 785-800.
37. D. M. Bishop, A. C. G. Heath, N. A. Haack, Distribution, prevalence and host associations of Hymenoptera parasitic on Calliphoridae occurring in flystrike in New Zealand, Medical and Veterinary Entomology 10 (1996) 365-370.
38. D. M. Bishop, Parasitic Hymenoptera reared from dung- breeding Diptera in New Zealand, New Zealand Entomologist 21 (1998) 99-106.
39. B. R. Subba Rao, B. R. S. Rao, A revision of Tachinaephagus Ashmead (Hymenoptera: Encyrtidae) with descriptions of four new species, Bulletin of Entomological Research 68 (1978) 65-73.
40. J. S. Noyes, Encyrtidae. Fauna of New Zealand 13 (1988) 1-188.
41. H.G. Wylie, Oviposition restraint of Nasonia vitripennis (Hymenoptera: Pteromalidae) on hosts parasitized by other hymenopterous species. Can. Ent. 102 (1970): 886-894.
42. M.A.K Nasser, S.A. Eraky, Biology and the impact of the pupal parasite, Nasonia vitripennis (Walker) on three dipteran species. Assiut Journal of Agricoltural Sciences 25 (1994) 173-180.
43. J. Amendt, R. Krettek, C. Niess, R. Zehener, H. Bratzke, Forensic entomology in Germany, Forensic Science International 113 (2000) 309-314.
44. M. Grassberger, C. Frank, Temperature-related development of the parasitoid wasp Nasonia vitripennis as forensic indicator. Medical and Veterinay Entomology 17 (2003) 257-262.
45. D. B. Rivers, L. Denlinger, Redirection of metabolism in the flesh fly, Sarcophaga bullata, following envenomation by the ectoparasitoid Nasonia vitripennis and correlation of metabolic effects with the diapause status of the host. Journal of Insect Physiology 40 (1994) 207-213.
46. D. B. Rivers, L. Denlinger, Fecundity and development of the ectoparasitic wasp Nasonia vitripennis are dependent on host quality. Entomologia Experimentalis et Applicata 76 (1995) 15-24.

Notes

1. A parasitoid is a symbiotic species that always kills its host. ↑
2. A. Pont distinguishs *Hydrotaea capensis* Wiedemann from the very similar american species, *H. aenescens* Wiedemann, by the palpi, black in the first and yellow in the second. ↑