



Corpses in aquatic environments: two human forensic cases with associated chironomid (Insecta: Diptera: Chironomidae) larvae

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ABSTRACT

Forensic entomology is a criminalistic tool that provides relevant information about circumstances of death and time elapsed until a corpse is discovered. However, it is rarely applied in aquatic environments because knowledge is scarce and fragmented. This paper summarizes the information available and contributes to the knowledge on Chironomidae in forensic cases by reporting on two human forensic cases from contrasting freshwater environments in which *Cricotopus* sp. and *Goeldichironomus holoprasinus* larvae were found. It discusses the advantages and limitations of using Chironomidae in forensic investigations to establish the postmortem submersion interval (PMSI) and other relevant information that they may provide about possible shifts in the location of the corpses. Because of the lack of basic biological data on these species, conclusions cannot be drawn about time of death. We highlight the need for further studies, mainly on basic biological information, to support Chironomidae as useful tool for aquatic forensic cases.

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Introduction

Forensic entomology is a useful criminalistic tool for determining the circumstances and date of death by analysing carrion insects and other associated arthropods. There is solid information supporting the use of forensic entomology in terrestrial environments. Sarcosaprophagous terrestrial arthropods, mainly Diptera and Coleoptera, colonize the corpse sequentially in a predictable ecological succession directly related to trophic requirements and body decomposition. This enables the approximate date of death or postmortem interval (PMI) to be inferred and provides information on the stage of decay, season of the year in which the death occurred and possible shifts in location of corpses^{1,2}.

Death by accidental drowning, suicide, natural disasters or even murder occurs frequently around the world, with about 360,000 drowned persons every year³. In contrast to terrestrial cases, aquatic deaths are often more difficult to study using forensic entomology, mainly because available knowledge is scarce and fragmented. A further drawback in

the use of this discipline in aquatic environments is that aquatic insects feed on algae, decaying plant matter or preys, or parasitize other organisms, but no truly necrophagous aquatic insect has been reported. Nevertheless, facultative necrophagy should not be ruled out, because Trichoptera larvae have been reported feeding on dead squawfish^{4,5}. In addition, difficulties to conduct experimental studies underwater and the small number of previous forensic case reports on submerged human bodies, still limit the application of aquatic entomology as a useful tool for criminalistics⁶.

Five decomposition phases have been described under experimental conditions on pig carrion under water: submerged fresh, early floating, early floating decay, advanced floating decay and sunken remains^{2,7}. Temperature, water chemistry, water flow, corpse condition and covering, as well the presence of flora and fauna, have a strong influence on the types and rates of decomposition processes⁸.

Date of death based on postmortem changes and rates of decomposition needs to be estimated with careful consideration because these processes are generally slower and more disturbed under water than in the air⁶. The effort to estimate the postmortem submersion interval (PMSI) – the interval from the time when the body enters the water to the time of its discovery – or to infer a possible movement of the body or cause of death has been increasing recently (e.g. ⁹). For example, the sum of the average daily temperatures to which a body is exposed, expressed in accumulated degree days, in combination with the measurement of total aquatic decomposition, has been used to estimate the PMSI¹⁰. The study of the benthic organisms associated to corpses also provides relevant information for estimating the PMSI. For such purpose, some studies conducted under experimental conditions by submerging pig^{11–14} or rat carcasses^{15,16}, provided information on macroinvertebrate assemblages, colonization patterns and functional feeding group composition. Additional information from forensic reports describing the aquatic biota associated to human corpses has been reported in^{6,9,17–20}.

In particular, insects with aquatic or semi-aquatic stages which can be associated with corpses could provide relevant information in investigations of death scenarios. Among insects, the most important are Ephemeroptera, Plecoptera, Trichoptera and the dipterans Chironomidae and Simuliidae²¹. However, their use should be undertaken considering that the development of the community is highly dependent on available colonizers, body size, season of immersion, water temperature, acidity, current speed and depth⁶.

Members of the family Chironomidae inhabit an enormous variety of aquatic ecosystems, from moist soils to pools in tree-holes, and from low-oxygen lake sediments to fast-flowing mountain streams, and frequently dominate aquatic insect communities in both abundance and species richness²². Most of them deposit their eggs on firm substrata such as macrophytes, and the egg masses and young larvae drift. The first larval instar is generally planktonic and disperses passively, while advanced instars are commonly benthic and disperse actively or passively²³.

The purpose of this paper is to report two human forensic cases from contrasting freshwater environments in which only chironomid larvae were found as associated fauna. It analyzes the biological information on the *Cricotopus* sp. and *Goeldichironomus holoprasinus* Goeldi specimens collected in each case, their correlation with the data recorded during the autopsy, the type of aquatic environment where the corpses were found, and the possible shift in location of the corpse, and discusses the advantages and limitations in the use of Chironomidae in forensic investigations. It is important to

emphasize the need for further studies to support Chironomidae as useful tool for aquatic forensic cases.

Material and methods

Study area

The cases described in the present study are based on corpses found in Buenos Aires Province, Argentina. This area belongs to the Biogeographic Pampean District of the Neotropical Region²⁴. It is a vast grassy plain covering the central area of Argentina, with temperate humid climate, mean annual temperature 16°C, maximum rainfall mainly occurring in spring and autumn, with annual mean precipitation 600–1200 mm. The natural vegetation in this region is grassland, with no natural perennial plants except *Celtis tala* Gill. ex Planch and *Salix humboldtiana* Willd., which grow in isolated areas with specific soil conditions²⁴. Any forested areas consist of introduced species.

In the Pampean region, sinuous streams flow across fertile soils formed by Quaternary loess deposition containing large amounts of clay in the B horizon and plentiful organic matter in the upper layers²⁵. These streams generally present brownish water due to the high levels of suspended solids, silty sediment in the benthos, lack of riparian forest vegetation, high nutrient levels, development of rich, dense macrophyte communities, and low current velocities due to the very gentle slope of the surrounding terrain. The water in Pampean streams has alkaline pH, high conductivity, elevated dissolved oxygen and nutrient concentrations, and relatively high concentration of dissolved phosphorus and nitrates²⁶.

Description of cases

Case 1.

A corpse of a man 1.70 m tall, weighing about 95 kg, was discovered in spring in an asynanthropic area in a Pampean stream. The corpse was found 3 m away from the shore, floating in supine position, dressed, with no signs of external violence, with most of its surface covered in silty sediments. In the discovery area, stream width varies from 12 to 20 m, and no floating macrophytes were present at the time of discovery. When the body was found, water temperature was 17°C, with an average of 18.7°C (14.2°C–19.7°C) over the previous week, without rains, and relative humidity was about 62.6%.

The disappearance had been reported 7 days before the body was found. The autopsy reported that death had occurred 3 to 5 days prior to discovery of the body. According to the decomposition stages described in^{1,11}, the corpse was in the initial putrefactive phase, in the early floating stage of decay, with distended abdomen projected above the water.

Case 2.

The corpse of a man 1.80 m tall, weighing about 60 kg, was discovered in winter in a hemisynanthropic area covered in dense forest near Luján City. It was found at the bottom of an uncovered inspection pit with an opening of 0.75 × 0.75 m and 1.70 m deep, with the bottom containing a little wastewater and rainwater, mud, tree leaves and garbage. The temperature during the months prior to discovery had been slightly higher than the mean value for the area, averaging 14.3°C (11.2°C–18.3°C), average precipitation was 54.3 mm, and average relative humidity 71.9%. The corpse was partially submerged,

in prone position, dressed and covered by tree leaves. According to decomposition stages described in^{1,11}, the corpse showed an advance stage of decay, colliquative with individualized organs phase, with no sign of violent death. According to the autopsy, death presumably occurred 45 days prior to discovery.

Methodology

The entomological evidence was collected from the corpses at the place of discovery with fine-tipped tweezers and fixed in 70% ethanol. Permanent microscope slides were prepared by clearing specimens with 10% KOH; neutralizing with glacial acetic acid; dehydrating in 80%, 96% and 100% ethanol and mounting in Canada Balsam, following²⁷. External features were observed under microscope (Leica DM1000) and photographed (Leica DFC290) for morphological identification. The specimens were identified based on specialized literature^{27,28} and housed in the ILPLA collection (Instituto de Limnología 'Dr Raúl A. Ringuelet', La Plata, Buenos Aires, Argentina).

Results

Entomological findings

Cricotopus sp. (Orthoclaadiinae: Orthoclaadiini) (Figure 1) (n = 41). Larvae were collected from the cadaver discovered in the Pampean stream. According to the size and presence of preimaginal structures in the larger larvae, they were identified as fourth stage larvae, and smaller, third stage larvae were also found.

Biological considerations

The genus *Cricotopus* is highly diverse and widely distributed^{29,30}. *Cricotopus* species have been recorded in environments ranging from lotic waters such as pristine streams in upland tropical rainforest, to highly degraded coastal streams and manmade urban drainages. The in-stream microhabitat used by the *Cricotopus* species varies from sand beds to submerged vegetation, or algal and cyanophyte colonies³⁰. In Argentina, this genus has been found in streams ranging from fast-flowing mountain streams^(e.g. 31) to slow-flowing Pampean streams^(e.g. 32).

Goeldichironomus holoprasinus (Chironominae: Chironomini) (Figure 2) (n = 21). Larvae were collected from the corpse found in an inspection pit. According to the size and presence of preimaginal structures in the larger larvae, they were identified as fourth stage larvae, and smaller second and third stage larvae were also found.

Biological considerations

The genus *Goeldichironomus* occurs mainly in the tropics and subtropics of Central and South America³³. The species collected in the second case, *G. holoprasinus*, is one of the most abundant and widespread species of chironomid reported in man-made and natural systems. It occurs mainly in small standing water bodies, organically enriched and depleted of oxygen, and is commonly a pioneer species which often invades temporary water bodies²⁷. It may also occur in sediments, in or on plants and in floating mats of vegetation and wood, under conditions ranging from oligotrophic to hypereutrophic^{27,34}. The species

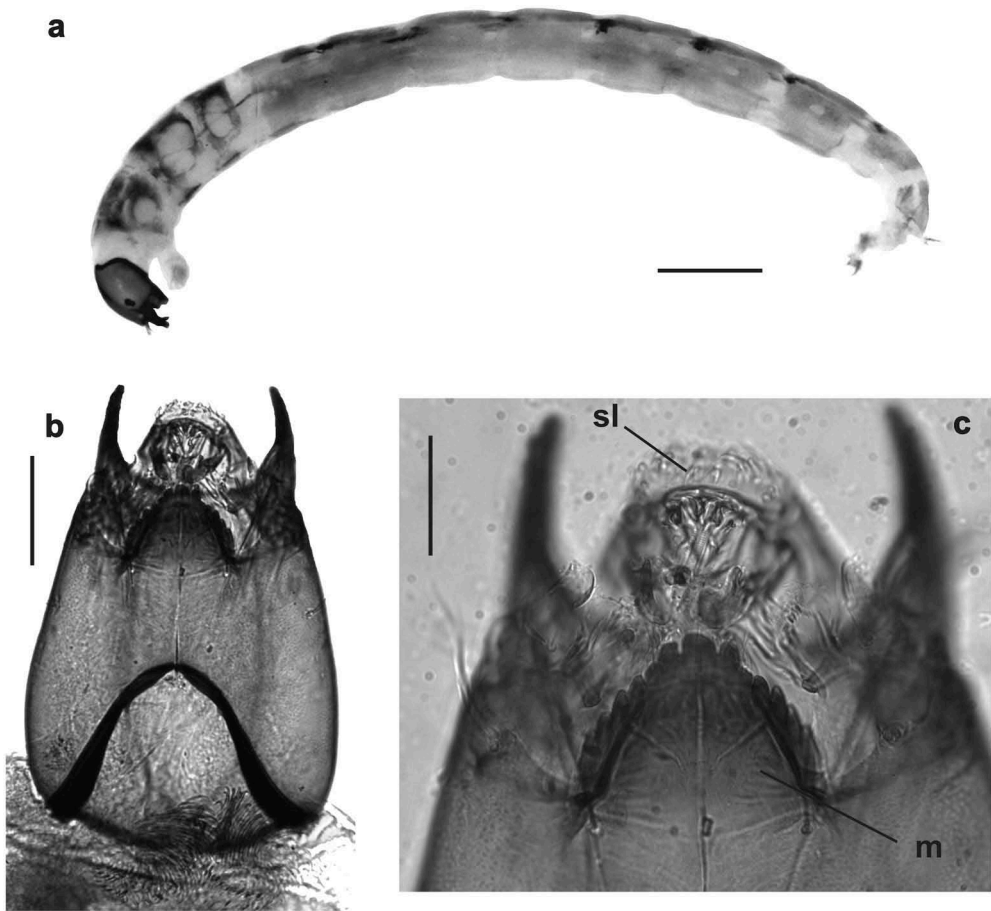


Figure 1. *Cricotopus* sp. (a), entire larva, (b) head, ventral view, (c) details of the anterior region of the head, ventral view. sl: labral seta 1; m: mentum. Scale bars: (a) = 500 µm; (b) = 100 µm; (c) = 50 µm.

has been reared under laboratory conditions at room temperature (18–33°C) in an average of 18 days (16–20) from the time of oviposition to the emergence of the adults³⁵.

Discussion and conclusion

Some of the most relevant questions faced by the forensic sciences are those related to the time of death and whether the death occurred at the site where the body was found or the corpse was moved. In the aquatic environment, the degree of decomposition and other features of the corpse, as well as its position in the water column, provide important information to attempt to answer these questions. Studies on human corpses have shown that a body can be expected to rise to the surface within a few days (2 days in summer), whereas at cold or near-freezing water temperatures, resurfacing of the body can be delayed for several weeks (2 or 3 weeks in winter) to several months²⁰.

Chironomid immatures are considered of particular interest in forensic investigations because they inhabit diverse aquatic habitats, are commonly present at high densities,

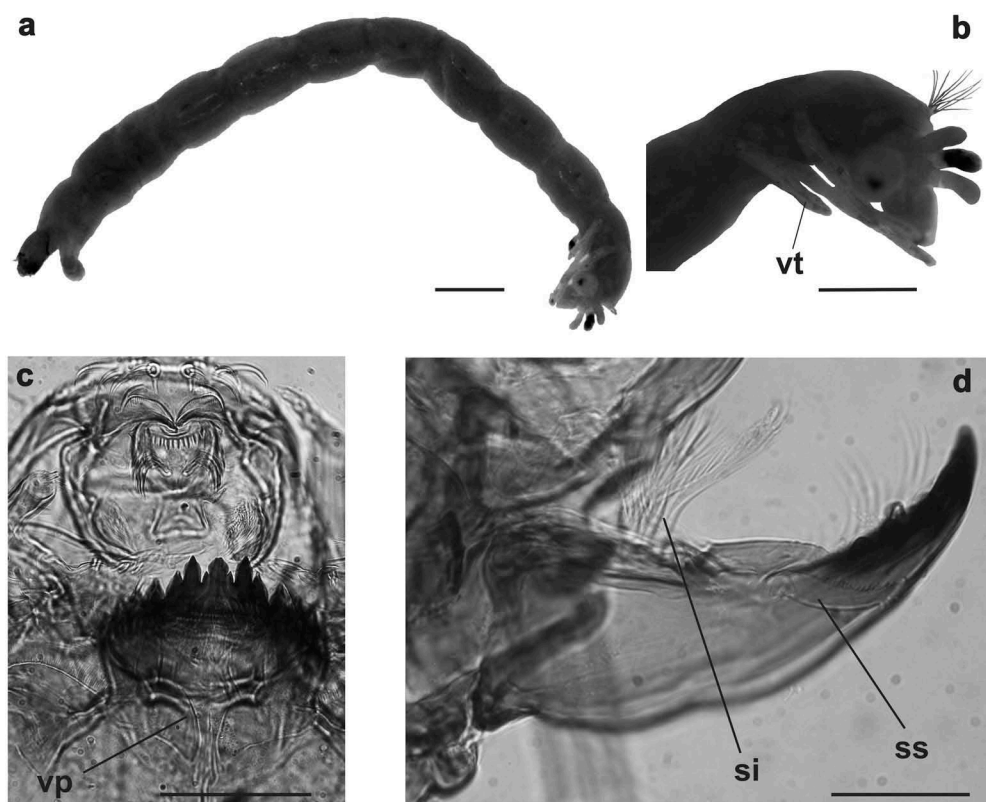


Figure 2. *Goeldichironomus holoprasinus* (a) entire larva, (b) details of the two pairs of posterior ventral tubules, the first one forked (c) details of the anterior region of the head, ventral view, (d) mandible, lateral view; si: seta interna; ss: large seta subdentalis, toothed along its inner margin; vp: ventromental plates, closed medially; vt: ventral tubules. Scale bars: (a) = 500 μm ; (b) = 200 μm ; (c) = 100 μm ; (d) = 50 μm .

and are able to colonize submerged carcasses in a short period of time, seeking food and shelter^{12,15,16}. Moreover, they are good environmental indicators because they have specific requirements of temperature, pH, oxygen concentrations, pollution, salinity, depth and current velocity³⁶. Due to these specific requirements, chironomid assemblages can differ between aquatic habitats, even in zones within the same environment³⁷.

Chironomids are therefore good indicators of the submersion of corpses^{17,38} and provide relevant information that can help elucidate where the death occurred. They could also provide valuable evidence of possible changes in the location of the body during decay (floating and sunken stages), as well as other movements produced by the water, such as whether the corpse was on the shore and later swept up in the current¹⁴. Finally, in some instances, they can provide information about intentional transfer of corpses^{6,21}.

The collector–gatherer chironomids, which feed on fine particulate matter derived from the natural decay of organic matter and from the biofilm formed on corpses, are not actively involved in the decay of a body. According to studies conducted under experimental conditions,¹⁵ as well as based on human forensic cases,^{9,20} chironomids have been

considered helpful as indicators of the PMSI, because their larvae are not affected by the activity of postmortem scavengers in the submerged corpse. Contrarily, Barrios and Wolff¹⁴ cast doubt on the usefulness of chironomids because they are not associated to any specific stage of decay and can reach the body at different instars.

Therefore, we consider that chironomids as entomological evidence to establish the PMSI should be used with great caution. Each forensic case should be analysed considering the knowledge of the specific life cycles carried out under controlled laboratory conditions similar to those present in the water body where the corpse was found. The place where the body was removed should be studied in detail to rule out the possibility of cross-contamination, i.e. larvae that may have developed on the substrate before the body contact with the water, in accordance with¹⁴.

The corpse in the first case report in the present study was found in an early floating phase in the forensic scenario. This stage of decay agrees with the autopsy information. Moreover, the absence of terrestrial fauna such as necrophagous dipterans and coleopterans, which typically colonize emerged surfaces, enabled us to corroborate the stage of decay. Estimated time of death (3 to 5 days previously, according to the autopsy report) is within the range reported in previous studies regarding the time a corpse takes to float in the same season under similar environmental conditions (e.g.²⁰).

Chironomids probably reached the body in advanced larval stage, either passively by drifting or when the corpse contacted the benthos, as well as by active movements of the larvae. Although in our study, the presence of *Cricotopus* sp. larvae was not useful for estimating the PMSI, it supports the found scene in the stream, where immatures of this genus naturally inhabit. In conclusion, the sediment and biofilm covering the corpse, as well as the chironomid larvae and the absence of aerial fauna allow us to confirm that the body had remained submerged and would not have been transferred or moved since it entered the stream.

In the second case reported, according to the advanced decomposition stage of the corpse, the PMI provided by medical autopsy was higher than 45 days. Because the uncovered inspection pit received wastewater, rainwater, dust and tree leaves, the water level varied daily, and the body was discovered when it was partially submerged in the bottom. In this case, the *G. holoprasinus* larvae collected might have been growing in the sediment of the inspection pit and contacted the body. Larvae use the body as a substrate to establish themselves and a place to find food. No relevant assumption can be made regarding the age of the larvae, since it is not known when they contacted the corpse. Based on the entomological evidence obtained by experts in the autopsy, we infer that the absence of terrestrial fauna could be due to the body having been cyclically covered and washed by wastewater. Therefore, the presence of *G. holoprasinus* in this type of environment, organically enriched and depleted in oxygen, as well as the absence of terrestrial fauna, would be taken as evidence that the body remained in that place and was cyclically covered and washed by wastewater. Any shift in the location of the corpse since it entered this environment could thus be ruled out.

This contribution is the first report of both genera associated to human corpses in forensic cases, considering that *Cricotopus* sp. larvae have previously been reported only in association with rat carcasses from streams¹⁵. We point out that both genera provide novel information regarding the structure of the insect community associated to dead bodies in submerged contexts and could therefore be used as biological evidence in court

cases involving submersed and/or floating human remains. However, further studies are required for specific identification and laboratory rearing to learn about the life cycle of the chironomids in different aquatic conditions. The results will enable inferences to be made about the activity of these taxa during the submerged instances in order to know when and in which larval instar they reach the corpses, and to estimate the postmortem submersion interval (PMSI). These reports of chironomids recorded in forensic situations in Argentina begin a new area of basic research, providing new information which would be useful in resolving criminal investigations. However, we emphasize the need for further studies on the biology of Chironomidae species in forensic cases to enable dipterans to be widely used as a tool for aquatic forensic cases.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

1. Smith KGV. A manual of forensic entomology. London: Trustees of the British Museum (Natural History); 1986. 205.
2. Byrd JH, Castner JL. Insects of forensic importance. In: Byrd JH, Castner JL, editors. Forensic entomology: the utility of arthropods in legal investigations. 2nd ed. Boca Raton (FL): CRC Press; 2009. p. 39–126.
3. WHO (World Health Organization). Violence and injury prevention and disability. 2018. [cited 18 Feb 2019]. Available from: <http://www.who.int/mediacentre/factsheets/fs347/en/>
4. Brusven MA, Scoggan AC. Sarcophagous habits of Trichoptera larvae on fish. Entomol News. 80;1969:103–105.
5. Fenoglio S, Merritt RW, Cummins KW. Why do no specialized necrophagous species exist among aquatic insects? Freshw Sci. 2014;33(3):711–715. doi:10.1086/677038
6. Merritt RW, Wallace JR. The role of aquatic insects in forensic investigations. In: Byrd JH, Castner JL, editors. Forensic entomology: the utility of arthropods in legal investigations. 2nd ed. Boca Raton (FL): CRC Press; 2009. p. 271–319.
7. Benbow M, Tomberlin J, Tarone A. Carrion ecology, evolution, and their applications. Boca Raton (FL): CRC Press; 2015.
8. Stuart BH, Ueland M. Chapter 17: decomposition in aquatic environments. In: Schotsmans EMJ, Márquez-Grant N, Forbes SL, editors. Taphonomy of human remains: forensic analysis of the dead and the depositional environment: forensic analysis of the dead and the depositional environment. New York (NY): John Wiley & Sons Ltd; 2017. p. 235–247.
9. González Medina A, Soriano Hernando Ó, Jiménez Ríos G. The use of the developmental rate of the aquatic midge *Chironomus riparius* (Diptera, Chironomidae) in the assessment of the postsubmersion Interval. J Forensic Sci. 2015;60(3):822–826. doi:10.1111/1556-4029.12707
10. Reijnen G, Gelderman HT, Oude Grotebevelsborg BFL, Reijnders UJL, Duijst WLJM. The correlation between the Aquatic Decomposition Score (ADS) and the post-mortem submersion interval measured in Accumulated Degree Days (ADD) in bodies recovered from fresh water. Forensic Sci Med Pathol. 2018;14(3):301–306. doi:10.1007/s12024-018-9987-5

11. Payne JA, King EW. Insect succession and decomposition of pig carcasses in water. *J Georgia Entomol Soc.* 7;1972:153–162.
12. Vance GM, VanDyk JK, Rowley WA. A device for sampling aquatic insects associated with carrion in water. *J Forensic Sci.* 1995;40(3):479–482.
13. Heo CC, Marwi AM, Jeffery J, Omar B. Insect succession on a decomposing piglet carcass placed in a man-made freshwater pond in Malaysia. *Trop Biomed.* 25;2008:23–29.
14. Barrios M, Wolff M. Initial study of arthropods succession and pig carrion decomposition in two freshwater ecosystems in the Colombian Andes. *Forensic Sci Int.* 2011;212:164–172. doi:10.1016/j.forsciint.2011.06.008
15. Keiper JB, Chapman EG, Foote BA. Midge larvae (Diptera: chironomidae) as indicators of postmortem submersion interval of carcasses in a woodland stream: a preliminary report. *Forensic Sci.* 1997;42(6):1074–1079.
16. Arimoro FO. Colonization and invertebrate succession on mammalian carcasses in Ethiopia, Niger delta, Nigeria. *Appl Sci Res J.* 1;2013:7–21.
17. Hawley DA, Haskell NH, McShaffrey DG, Williams RE, Pless JE. Identification of a red “fiber”: chironomid larvae. *J Forensic Sci.* 1989;34(3):617–621.
18. Hobischak NR, Anderson GS. Freshwater-related death investigations in British Columbia in 1995–1996. A review of coroners cases. *Can Soc Forensic Sci J.* 1999;32(2–3):97–106.
19. Wallace JR, Merritt RW, Kimbirauskas R, Benbow ME, McIntosh M. Caddisflies assist with homicide case: determining a postmortem submersion interval using aquatic insects. *J Forensic Sci.* 2008;53(1):219–221.
20. Myskowiak JB, Masselot G, Fanton L, Schuliar Y. Freshwater invertebrates and Wagner’s parsimony method (WPM): tools for the submersion time estimation of a cadaver found in a natural aquatic environment. Description of a sampling protocol. *La Rev Médecine Légale.* 2010;1:47–60. doi:10.1016/j.medleg.2010.08.002
21. Keiper JB, Casamatta DA. Benthic organisms as forensic indicators. *J North Am Benthol Soc.* 2001;20(2):311–324.
22. Ferrington L. Global diversity of non-biting midges (Chironomidae; Insecta-Diptera) in freshwater. *Hydrobiologia.* 2008;595:447–455. doi:10.1007/s10750-007-9130-1
23. Pinder LCV. The habitats of chironomid larvae. In: Armitage PD, Cranston PS, Pinder LCV, editors *The Chironomidae: biology and ecology of non-biting midges.* London: Chapman & Hall; 1995. p. 107–135.
24. Cabrera ÁL, Willink A. *Biogeografía de América Latina.* 2a edición corregida. Washington: Monografía 13. Serie de Biología. Secretaría General de la Organización de los Estados Americanos; 1980.
25. Papadakis J. *El suelo, con especial referencia a los suelos de América Latina.* Buenos Aires: Editorial Albatros; 1980.
26. Feijóo C, Lombardo R. Baseline water quality and macrophyte assemblages in Pampean streams: a regional approach. *Water Res.* 2007;41(7):1399–1410. doi:10.1016/j.watres.2006.08.026
27. Epler JH. Identification manual for the larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern United States, including Florida. Special Publication SJ2001-SP13. North Carolina. Department of Environment and Natural Resources, Raleigh, NC, and St. Johns River Water Management District, Palatka, FL, 2001. [Updated 1 January 2016]. [cited 1 March 2019]. Available from: <http://home.earthlink.net/~johnnepler/index.html>
28. Silva FL, Pinho LC, Wiedenbrug S, Dantas GPS, Siri A, Andersen T, Trivinho-Strixino S. Chapter 16.2 - Family Chironomidae. In: Hamada N, Thorp JH, Rogers, editors. *Thorp and Covich’s freshwater invertebrates.* United Kingdom, London: Academic Press; 2018. p. 661–700.
29. Drayson N, Cranston PS, Krosch MN. Taxonomic review of the chironomid genus *Cricotopus* v. d. Wulp (Diptera: chironomidae) from Australia: keys to males, females, pupae and larvae, description of ten new species and comments on *Paratrichocladus* Santos Abreu. *Zootaxa.* 2015;3919(1):1–40. doi:10.11646/zootaxa.3919.1.1

30. Krosch MN, Cranston PS, Baker AM, Vink S. Molecular data extend Australian *Cricotopus* midge (Chironomidae) species diversity, and provide a phylogenetic hypothesis for biogeography and freshwater monitoring. *Zool J Linn Soc.* 2015;175(3):496–509. doi:10.1111/zoj.12284
31. Mauad M, Siri A, Donato M. Does type of substratum affects Chironomid Larvae assemblage composition? A study in a river catchment in Northern Patagonia, Argentina. *Neotrop Entomol.* 2017;46(1):18–28. doi:10.1007/s13744-016-0429-3
32. Cortelezzi A, Paggi AC, Rodríguez M, Rodrigues Capítulo A. Taxonomic and nontaxonomic responses to ecological changes in an urban lowland stream through the use of Chironomidae (Diptera) larvae. *Sci Total Environ.* 2011;409(7):1344–1350. doi:10.1016/j.scitotenv.2011.01.002
33. Pinder LCV, Reiss F. The larvae of Chironominae (Diptera: chironomidae) of the Holarctic region. Keys and diagnoses. In: Wiederholm T, editor. Chironomidae of the Holarctic region. Keys and diagnose. Part I: larvae. *Ent Scan Suppl.* Sweden, Lund: Entomological Society of Lund; 1983. Vol. 19. p. 293–435.
34. Cranston PS, Epler JH. The larvae of Tanypodinae (Diptera: chironomidae) of the Holarctic Region – keys and diagnoses. In: Andersen T, Cranston PS, Epler JH, editors. Chironomidae of the Holarctic region – keys and diagnoses. Part 1. Larvae. *Ins Syst Evol Suppl.* Sweden, Lund: Entomological Society of Lund; 2013. Vol. 66. p. 39–135.
35. Zilli F, Marchese M, Paggi AC. Life cycle of *Goeldichironomus holoprasinus* Goeldi (Diptera: chironomidae) in Laboratory. *Neotrop Entomol.* 2009;38(4):472–476.
36. Olafsson J. Vertical microdistribution of benthic chironomid larvae within a section of the littoral zone of a lake. *Netherlands J Aquat Ecol.* 1992;26(2–4):397–403.
37. Sæther OA. Chironomid communities as water quality indicators. *Ecography.* 1979;2(2):65–74.
38. Wolkomir R, Wolkomir J. When scientists become sleuths. *Natl Wildl.* 30;1992:8–15.