

การพบแอมฟิพอดในศพสามารถระบุสาเหตุเบื้องต้น แห่งพฤติการณ์การตาย เพื่อประโยชน์ในงาน นิติวิทยาศาสตร์และการสืบสวน

The detection of amphipods in a corpse
can preliminary identify the cause of
death for forensic science and
investigation benefits

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■ บทคัดย่อ

ปัจจุบันแมลงและสัตว์ข้อปล้องเข้ามามีบทบาทและเป็นประโยชน์ต่อนิติวิทยาศาสตร์ รวมถึงสัตว์น้ำขนาดเล็กที่มีเปลือกแข็งในน้ำ (แม่น้ำ ทะเล) ด้วยเช่นกัน พวกมันสามารถช่วยให้นักกฎหมายและนักนิติวิทยาศาสตร์คาดการณ์ช่วงเวลาหลังการเสียชีวิตโดยการศึกษาลักษณะวงจรชีวิต พฤติกรรม และถิ่นที่อยู่อาศัย ซึ่งมีความจำเพาะแตกต่างกันไปตามชนิดของมัน บทความนี้มีวัตถุประสงค์เพื่อศึกษาความสำคัญของ Amphipod (แอมฟิพอด) ที่มีประโยชน์ต่อนิติวิทยาศาสตร์และกระบวนการยุติธรรม แอมฟิพอดเป็นสัตว์น้ำจืดขนาดเล็กเป็นนกก้นที่พบตามพื้นดินใต้น้ำ และเป็นหนึ่งในรายงานส่วนน้อยที่พบสัตว์เปลือกแข็งประเภท Crustaceans (ครัสเตเชียน) ไม่กี่ชั่วโมงหลังความตาย จากการชันสูตรเบื้องต้นพบแอมฟิพอดภายในกระเพาะอาหารและหลอดลมของศพชายหนุ่มที่เสียชีวิตในแม่น้ำเจ้าพระยา พื้นที่ สภ.ปากเกร็ด จังหวัดนนทบุรี วิธีการ คือเก็บตัวอย่างแอมฟิพอดในกระเพาะอาหารและหลอดลมของศพนำมาสแกนด้วย micro-CT ทำให้

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ทราบข้อมูลของแอมฟิพอด ซึ่งเป็นประโยชน์อย่างยิ่งทางนิติวิทยาศาสตร์ โดยการประมาณช่วงเวลาหลังการตายให้ใกล้เคียงกับความจริงมากที่สุด และอธิบายถึงเหตุหรือพฤติการณ์เบื้องต้นของการเสียชีวิต อันจะนำมาซึ่งประโยชน์ในกระบวนการยุติธรรมเพื่อหลีกเลี่ยงในการตีความผิดและข้อผิดพลาดในการประเมินช่วงเวลาหลังการเสียชีวิตและเหตุแห่งการเสียชีวิตให้ใกล้เคียงกับช่วงเวลาจริงมากที่สุด บทความนี้ถือเป็นหนึ่งในบทความที่มีประโยชน์ต่อพนักงานสอบสวนและแพทย์นิติเวชที่ปฏิบัติหน้าที่ในสถานที่เกิดเหตุ และการชันสูตรพลิกศพ

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■ ABSTRACT

Nowadays, insects and arthropods have become beneficial to forensic science. This also includes crustaceans in the water (river, sea). They can help the entomologist and forensic scientist reconstruct and predict the postmortem interval by studying their characteristics, life cycle, behavior, and habitat. The purpose of this article is to study the importance of amphipods, which are useful for forensic science and the justice system. Amphipods are small freshwater animals known as terrestrial or aquatic predators. There is a paucity of reports on the presence of crustaceans on a dead body after death for a few

hours. Based on the autopsy, amphipods were found in the stomach and trachea of a young man's body found in the Chao Phraya River, Pak Kret Police Station service area, Nonthaburi Province. By collecting amphipod samples from the corpse and micro-CT scanning examination, the postmortem interval can be estimated to be accurate to the true value by using the information from the analysis of amphipods. Moreover, the incident and cause of death can be achieved, leading to the advantages of the justice system with the resolution of misinterpretation and postmortem evaluation errors. This article can be considered a consultative tool for investigation officers and forensic pathologists who work on crime scene investigation and autopsy.

Keywords: arthropods, crustaceans, forensic, postmortem

■ Instruction

Arthropods, including insects, arachnids, scorpions and crustaceans, are the largest and most numerous zoological taxon on earth. Arthropods are present in a wide variety of locations commonly used by humans, and they have been reported also from crime scenes.

During the last three decades, the utility of forensic entomology in death investigation

has been largely documented in Europe, America, Australia, and Asia by several case studies (Campobasso & Introna, 2001) and experimental works that represent the references for the discipline.

It is worth mentioning that such cases and experiments show the potentiality of this discipline in the forensic pathology investigation field not only in Post Mortem Interval (PMI) estimation but also in drug detection, cadaver transfer and victim identification (Marchetti; *et al.*, 2013). Therefore, the arthropods found at a crime scene and on a victim's body can assist many types of forensic investigation (Amendt; *et al.*, 2007). In particular, they can provide information about time since death, season of death, primary crime scene, movement or concealment of the remains following death, specific sites of trauma on the body (Benecke & Lessig, 2001), use of drugs and neglect of children (Benecke; *et al.*, 2004), elders (Archer; *et al.*, 2015) and victim's identification when the body is removed from the initial decomposition site.

Despite being highly informative, arthropods have the potential to strongly alter and modify the crime scene and the victim's body.

Therefore, given the complexity of crime scene investigations and the necessity to respond to the six W questions (i.e. What, Where, How, When, Who and Why) and to

understand the dynamics of violent crimes, the forensic entomological knowledge is of utmost importance, and the forensic pathologists should be aware of the damage that arthropods can cause, together with the useful information they can provide. These are very important to be benefic to the officer in the investigation and the victims to get justice. (Viero; *et al.*, 2019)

■ Objective

1. To study characteristic life cycle, behavior, habitat and types of amphipods.
2. To study amphipods found in corpses for the benefit of determining the preliminary cause of death.
3. Utilization of amphipods found in corpses for the benefit of the justice process.

■ Conceptual Framework

Ecological Role of Amphipods

Amphipods are a major order of crustaceans that stands out for their local abundance (both in terms of density and biomass), diversity, ecological importance and role as hosts for parasites. More than 10,000 species are currently described (Horton; *et al.*, 2020) and colonize all aquatic ecosystems (freshwater, brackish and marine), including extreme habitats like ground water (Jayanthi; *et al.*, 2023), some

are live in the calm water, some stay in polar and hydrothermal sites, (Poltermann, 2001) and to a lesser degree, (Shader; *et al.*, 2004) some terrestrial ones (Friend & Richardson, 1986). Amphipods not only inhabit multiple habitats, but also fill different ecological niches and support several ecosystem services (Bojko, 2019), (Best & Stachowicz, 2014). They are omnivorous, trophically versatile and opportunistic, having a great diet adaptability (Fano; *et al.*, 1982). Many species are assigned to the functional feeding group of shredders, feeding mainly on leaf material, but some species are also predators of a wide range of invertebrates including conspecifics and congenics (cannibalism) (Kelly & Dick, 2002), (Piscart; *et al.*, 2009), (Isart; *et al.*, 2009). Amphipods are often significant components of other aquatic systems where they contribute to nutrient recycling and provide high-quality food for a variety of animals, including crayfish, fish, amphibians, water birds, and semiaquatic mammals. (Thorp & Christopher, 2015) Amphipods usually have a laterally compressed, comma-shaped body, no carapace, and three pairs of uropods. Sexes are separate and species of most families are sexually dimorphic in the size and form of the gnathopods. Unlike many other crustaceans, amphipods do not have pelagic larvae or manca. (Hughes & Shane, 2016)

Amphipods comprise an order of crustacea (MacNeil *et al.*, 1997), shrimp-like in form, which contains mostly marine and freshwater forms. While some species are terrestrial, they still require moist habitats. These terrestrial species are sometimes referred to as “lawn shrimp” due to their similarity to true shrimp.

■ Main point

General Description and Life Cycle of Amphipods

Amphipods are elongate and more or less compressed laterally. They do not have a carapace (the hard covering of the thorax common in other crustacea), and seven (rarely six) of the thoracic segments are distinct and bear leg like appendages. The abdominal segments are more often or less fused, and so the thoracic segments make up most of the body (Borror; *et al.*, 1989). They have two pairs of antennae, with one pair usually very small. The eyes usually are well developed, but are sometimes reduced or lacking. Members of this order have chewing mouthparts (Smith & Whitman, 1992).

Adult amphipods range from 5 mm. to 20 mm. ($\frac{3}{16}$ to $\frac{3}{4}$ inch) in length. *Arcitalitrus sylvaticus* (Haswell) reaches a length of 8 mm. ($\frac{3}{16}$ inch), *Talitroides topitotum* are 7 mm. in length and *Talitroides allaudi*

about 3.5 mm. Aquatic species are often whitish but are seen in other colors also. The color of terrestrial species varies from pale brown to greenish to brownish black when alive, but they often turn red when they die. (Vanin & Silvano, 2011)

Amphipods are sometimes misidentified as springtails (Insecta: Collembola). Springtails (Figure 1) (Collembola, Copyright © 1997-2024) are also very commonly found

in moist areas, sometimes in vast numbers. However, springtails are not crustacea, but insect-like arthropods with only three pairs of legs, one pair of antennae and usually a furcula (a forked structure) on the 4th abdominal segment. The group of springtails that might be confused with amphipods also has a tubular structure on the last abdominal segment.



Figure 1 A photo of the springtail (*Isotoma habitus*): From Amateur Entomologists' Society Site from <https://www.amentsoc.org>

Eggs are deposited within a brood pouch on the underside (Pinto, 1990) of the adult female amphipod's body. The eggs hatch in one to three weeks. The young amphipods resemble the adults and leave the pouch during the next one to eight days when the female has her first molt during mating. The molt usually takes about one hour. And most species complete their life cycle (egg to adult) in one year or less (Smith and Whitman, 1992).

Steps to collect specimens.

Collection of entomological evidence at the case A0814/49 (Figure 2) samples of amphipod and preserved samples:

- Preserved samples include specimens placed directly into 80% ethyl alcohol (label the details).

After collected amphipods from the body. Confirm the species of amphipods. It's useful for entomologist to estimate as close as the truth minimum of post mortem interval (minPMI) and the initial cause of death.



Figure 2 Amphipods specimen

Steps to study amphipods by Micro-CT scanning

Each amphipod was preserved in 80% ethanol, was dehydrated in an ethanol, transferred into hexamethyldisilazane for several hours and air dried for 24 hours. No stain was used. Amphipod was scanned in a SkyScan 1172 microtomograph. To scan whole body the following were used: voltage (~ 80 kV) and current (~ 50 – 100 μ A) were tailored for each scan based on body size and density (Duband; *et al.*, 2011), to obtain maximum signal to noise ratio and optimal contrast. The reconstructions of raw images dataset and “cleaning” was performed using the Bruker-Skyscan free software to reconstruct and process the images, permitting not only reconstruction, but also

virtual slices used to achieve the final volume rendering reconstructions. A detailed description of the procedure was published (Alba, 2014).

■ Result

After examining the morphology to know the life cycle, habitat and behavior of amphipod (Lowry & Springthorpe, 2001), which is classified in the Kingdom Animal, Phylum Arthropoda, Class Crustacea (Lowry, 2010), Order Amphipoda, Family Hyalidae.

External morphology in general is:

(1) The body is flat, the dorsal is curved. The body is approximately 1 - 10 mm. long and is gray - white in color. (Figure 2)

¹ This scale is ocular micrometer scale 10 times magnification under micro-CT scanning.

(2) Amphipod's body is divided into 3 parts: head, thorax, and abdomen. (Figure 4)

- The head has 1 pair of eyes, 2 pair of antennae, and a mouth.

- The thorax has 7 segments, consisting of 7 pairs of walking legs, with the first 4 pairs extending forward and the last 3 pairs extending backward.

- The abdomen has 6 segments, consisting of 3 pairs of uropods (tail-limbs) that help with balance under water, similar to the rudder of a ship

(3) Amphipods live in fresh water. Their food is mostly bacteria that live on decaying plant and animal remains. Amphipods are good decompose and good indicator for environment.

(4) Amphipods are live in the surface of the ground beneath the calm river. It stays to a depth of not less than 7-8 meters.

After collected specimens from trachea (Figure 3 T1, T2) and gastric (Figure G1, G2) and studied under micro-CT scanning. The results are classified as follows:

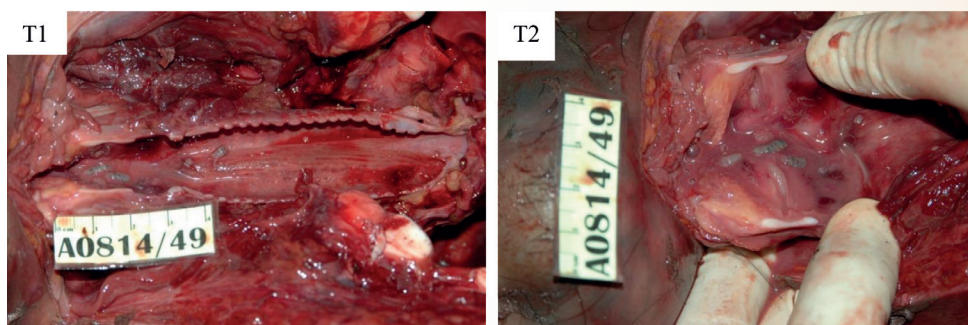


Figure 3. Amphipods in trachea

T = Trachea

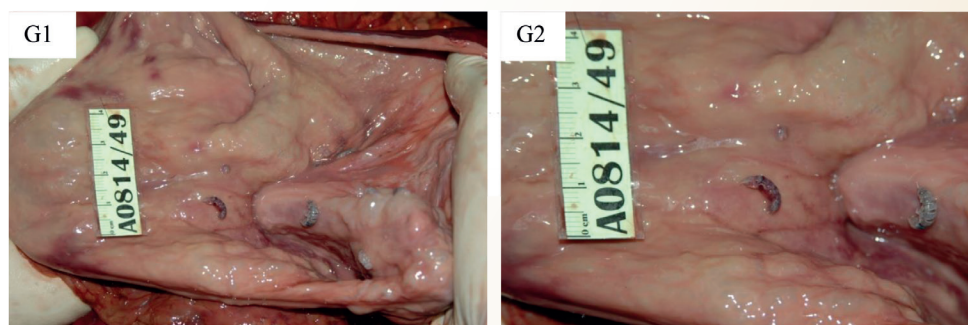


Figure 3. Amphipods in gastric

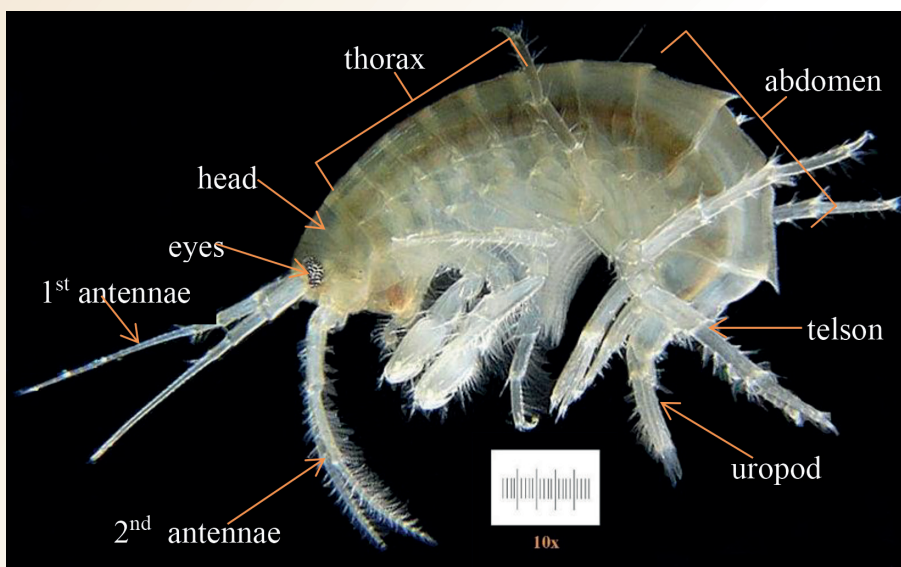


Figure 4. Amphipod structure

Family Hyalidae

Parhyale hawaiiensis (Dana, 1853)²
(McLaughlin et al., 2005)

General characteristics: The body is flat on the sides (Figure 5). Males and females have different characteristics. The first pair of antennae is $\frac{3}{4}$ of the length of the second pair (Figure 5A1, Figure 5A2). The second pair of gnathopod claws, the 5th segment is small, the 6th segment is bigger than the 5th and oval-like shaped (Figure 5G2). Uropod is a biramus with the inner ramus being smaller and longer than the outer ramus (Figure 5U3).

The telson has two lobes (cleft) (Figure 5T).

Habitat found spread wide from Southeast Asia. China Sea to the Atlantic Ocean and from this discovery, a male body was found. Drowned under the Chao Phraya River in the area of Pak Kret Police Station, Nonthaburi Province (Pak Kret Police Station currently, it is a subsidiary branch near the Nonthaburi Pier, Rama 4 Bridge area). An autopsy found amphipods in the trachea and gastric of the deceased. Samples were collected and then studied under a micro-CT scanning in order to know the external shape.

² It is a binomial naming system which is an international principle used throughout the world.

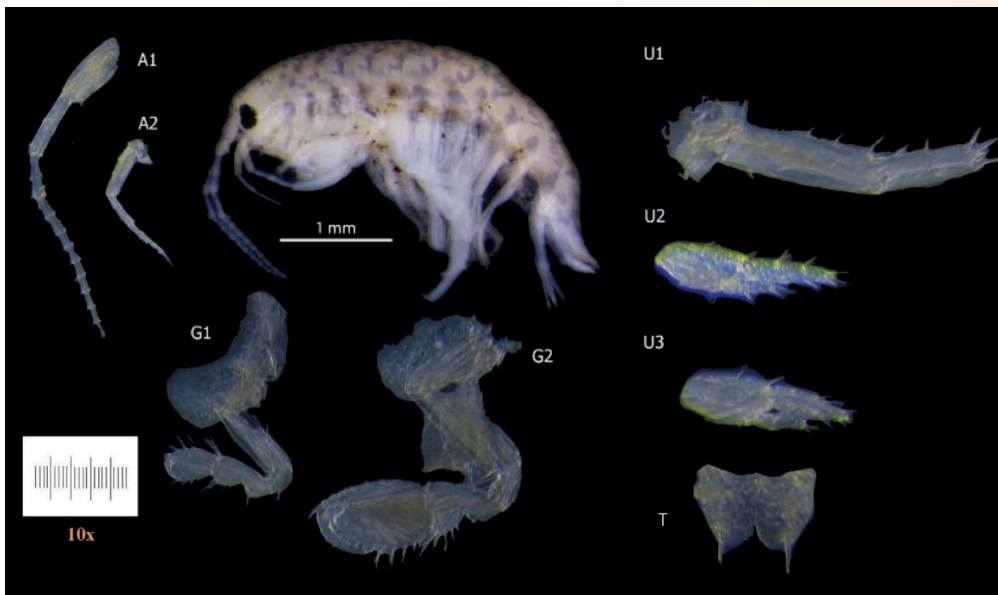


Figure 5 *Parhyale hawaiiensis* (Dana,1853) from micro-CT scanning

A = Antenna

G = Gnathopod (prehensile legs)

U = Uropod (like rudder of a ship)

T = Telson

Amphipods have sexual reproduction. While swimming, the male will hold onto the female, called an amplexus. After that, the male will release sperm to the female. The fertilized eggs are stored in the brood chamber between the female's legs. After that, they will hatch into larvae. They look very similar to their parents. In some Amphipod species, the mother and father also help each other care for the larvae for a period after they are released from the brood chamber.

Amphipods were found inside the trachea and gastric of corpses, which is considered an extremely important jointed animal in

forensic science. It shows that in addition to finding aquatic insects, amphipods can also be found, which is very possible, especially in countries in the region, hot and humid like Thailand

Investigators' initial report on this body was that found submerged under water. The corpse's palms and soles of the feet were wrinkled and pale and stained with mud. Which matches the habitat characteristics of amphipods *Parhyale hawaiiensis* (Dana, 1853) that live on the underwater surface. The man had choked on mud inside his trachea and gastric and found amphipods inside.

■ Conclusion

This report therefore would like to announce the amphipods are useful and help in forensic investigations and justice by studying the characteristics of the amphipods. As a result, the victims were treated fairly and the initial circumstances of the death could be explained as drowning. The corpse's palms and soles of the feet were wrinkled, pale and stained with mud. After autopsy amphipods *Parhyale hawaiiensis* (Dana, 1853) were found in trachea and gastric. It's shown that the body was dead at least 6-12 hours in conjunction with the process of decomposition and the discovery of amphipods inside and the corpse sank to a depth of at least 7-8 meters, which can be determined from habitat of them. It can be said that this report is only a small part of reports from Thailand only and very useful to forensic science and justice.

■ Suggestion

Amphipod is a small crustacea-like animal belonging to (Class Crustacea), of which there are many species that live in fresh water and seawater. Amphipod has many forensic and judicial benefits, such as:

- Amphipod can be used as an indicator of water quality, as some amphipod can only live in oxygen-rich water, and some

amphipod can only live in water that contains various toxins or pollutants.

- Amphipod can be used as an indicator of the death time of a corpse, as some amphipod destroys the corpse of a dead animal floating in the water, so studying the destruction of amphipod corpses will help to know the time of death of that corpse (Post Mortem Interval = PMI).

- Amphipod can be used as an indicator of an accident or murder because some amphipod destroys the bodies of dead people floating in the water. If a corpse has amphipod's cuts or teeth marks on a non-face part, it could mean that the corpse died before floating in the water, but if the corpse has amphipod's cuts or teeth marks only on its face, it could mean that the corpse died after floating in the water.

- Amphipods in this article is *Parhyale hawaiiensis* (Dana,1853) found in trachea and gastric. They live in the ground water. All studied can help the the pathologist to estimate the Post Mortem Interval (PMI) as soon as the truth ($_{min}$ PMI) and help the officer to know the body sank to a depth of at least 7-8 meters and dead at least 6-12 hours. Finally, summarize primary cause of death a case A0814/49 was drowning. It can be seen that amphipod is very useful for forensic science and helps in solving cases in the justice system.

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■ References

- Alba-Tercedor, J. (2014). From the sample preparation to the volume rendering images of small animals: A step by step example of a procedure to carry out the micro-CT study of the leafhopper insect *Homalodisca vitripennis* (Hemiptera : Cicadellidae). *Bruker Micro-CT Users Meeting 2014*, 260–288.
- Amendt, J., Campobasso, C. P., Gaudry, E., Reiter, C., LeBlanc, H. N., & Hall, M. J. (2007). Best practice in forensic entomology-standards and guidelines. *Int. J. Legal Med.* 121, 90–104. doi: 10.1007/s00414-006-0086-x.
- Archer, M. S., Bassed, R. B., Briggs, C. A., & Lynch, M. J. (2005). Social isolation and delayed discovery of bodies in houses: the value of forensic pathology, anthropology, odontology and entomology in the medico-legal investigation. *Forensic Sci. Int.* 151, 259–265. doi: 10.1016/j.forsciint.2005.02.016.
- Benecke, M. & Lessig, R. (2001). Child neglect and forensic entomology. *Forensic Sci. Int.* 120, 155–159. doi: 10.1016/S0379-0738(01)00424-8.
- Benecke, M., Josephi, E., & Zweihoff, R. (2004). Neglect of the elderly: forensic entomology cases and considerations. *Forensic Sci. Int.* 146(Suppl), S195–S199. doi: 10.1016/j.forsciint.09.061.
- Best, R. J. & Stachowicz, J. J. (2014). Phenotypic and phylogenetic evidence for the role of food and habitat in the assembly of communities of marine amphipods. *Ecology*, 95, 775-786.
- Bojko, J. (2019). Amphipod disease: Model systems, invasions and systematics-Introduction to DAO Special 8. Dis. *Aquat. Organ*, 136, 1-2.
- Borror, D. J., Triplehorn, C. A., & Johnson, N. F. (1989). *An Introduction to the Study of Insects*. Harcourt Brace Jovanovich.
- Campobasso, C. P., & Introna, F. (2001). The forensic entomologist in the context of the forensic pathologist's role. *Forensic Sci. Int.* 120, 132-139. doi: 10.1016/S0379-0738(01)00425-X.
- Collembola. (n.d.). *The Amateur Entomologists' Society*. <https://www.amentsoc.org>
- Duband, S., Forest, F., Clemenson, A., Debout, M., & Pèoc'h, M. (2011). Postmortem injuries inflicted by crawfish: morphological and histological aspects. *Forensic Science*

- International*. 206(1-3), 49-51. doi: 10.1016/j.forsciint.2010.08.006.
- Fano, E. A., Rossi, L., & Basset, A. (1982). Fungi in the diet of three benthic invertebrate species. *Bolletino Zool*, **49**, 99-105.
- Friend, J. A., & Richardson, A. M. (1986). Biology of terrestrial amphipods. *Annu. Rev. Entomol*, **31**, 25-48.
- Horton, T., Lowry, J., De Broyer, C., Bellan-Santini, D., Coleman, C. O., Daneliya, M., Dauvin, J. C., Fiser, C., Gasca, R., Grabowski, M., et al. (2020, April 10). *World Amphipoda Database*. Available online. <http://www.marinespecies.org/amphipoda>
- Hughes, E. L., & Ahyong, S. T. (2016). Collecting and Processing Amphipods. *Journal of Crustacean Biology*. **36**(4), 584-588, <https://doi.org/10.1163/1937240X-00002450>.
- Jayanth, H. S., Geetha, K. B., Vijay, V., & Parkash, M. (2023). Post-mortem freshwater animal predation - a case report. *Egyptian Journal of Forensic Science*, **13**(4).
- Piscart, C., Dick, J. T. A., McCrisken, D., & MacNeil, C. (2009). Environmental mediation of intraguild predation between the freshwater invader *Gammarus pulex* and the native *G. duebeni celticus*. *Biol. Invasions*, **11**, 2141-2145.
- Kelly, D. W., & Dick, J. T. A. (2002). Montgomery, W.I. The functional role of *Gammarus* (Crustacea, Amphipoda): Shredders, predators, or both?. *Hydrobiologia*, **485**, 199-203.
- Lowry, J. (2010). *Arcitalitrus sylvaticus* (Haswell, 1879). *World Register of Marine Species*. <http://www.marinespecies.org/aphia.php?p=taxdetails&id=555665>
- Lowry, J. K., & Springthorpe, R. T. (2001). Amphipoda: Families. *Crustacea.net*. <http://www.crustacea.net/crustace/amphipoda/index.htm>.
- MacNeil, C., Dick, J. T. A., & Elwood, R. W. (1997). The trophic ecology of freshwater *Gammarus* (Crustacea: Amphipoda); problems and perspectives concerning the functional feeding group concept. *Biol. Rev. Camb. Philos. Soc.*, **72**, 349-364.
- Marchetti, D., Arena, E., Boschi, I., & Vanin, S. (2013). Human DNA extraction from empty puparia. *Forensic Sci. Int.* **229**, 26-29. doi: 10.1016/j.forsciint.2013.03.043.
- McLaughlin, P. A., Camp, D. K., Angel, M. V., Bousfield, E. L., Brunel, P., Brusca, R. C., & Cadien, D. (2005). Common and Scientific Names of Aquatic Invertebrates from the United States and Canada: Crustaceans. *American Fisheries Society Special Publication*, **31**, pp. 545.
- Pinto, L. J. (1990). Occasional Invaders. In Story, K., & Moreland, D. (eds.). (pp. 833-867). Franzak & Foster Co. Cleveland. 1990
- Piscart, C., Genoel, R., Doledec, S., Chauvet, E., & Marmonier, P. (2009). Effects of intense agricultural practices on hetero-

- trophic processes in streams. *Environ. Pollut*, **157**, 1011-1018.
- Poltermann, M. (2001). Arctic sea ice as feeding ground for amphipods - Food sources and strategies. *Polar Biol*, **24**, 89-96.
- Sheader, M., Van Dover, C. L., & Thurston, M. H. (2004). Reproductive ecology of *Bouvierella curtirama* (Amphipoda: Eusiridae) from chemically distinct vents in the Lucky Strike vent field, Mid-Atlantic Ridge. *Mar. Biol*, **144**, 503-514.
- Smith, E. H. & Whitman, R. C. (1992). *Field Guide to Structural Pests*. National Pest Management Association. Dunn Loring, VA.
- Thorp, H. J. & D. Christopher Rogers. (2015). Amphipods are important constituents of many river and stream ecosystems. *Science Direct*. <https://www.sciencedirect.com/topics/earth-and-planetary-sciences/amphipod>.
- Vanin, S. & Silvano, Z. (2011). Post-mortal lesions in freshwater environment. *Forensic Science International*. **212**(1-3), e18-20, e1-284 ,e4. doi: 10.1016/j.forsciint. 2011.05. 028. Epub 2011 Jun 25.
- Viero, A. M., Montisci, G., Pelletti & Vanin, S. (2019). Crime scene and body alterations caused by arthropods: implications in death investigation. *International Journal of Legal Medicine*. **133**(1), 307-316. doi: 10.1007/s00414-018-1883-8.

