



Hemolymph, Coelomic Immunity, Extracellular Traps, and Fibrinolytic Enzymes: A Comprehensive Comparative Analysis

Dr. John Warren Reynolds ^{1*}, Wilma M Reynolds ²

¹⁻² Oligochaetology Laboratory, 9–1250 Weber Street East, Kitchener, Ontario N2A 4E1, Canada

¹ New Brunswick Museum, 277 Douglas Avenue, Saint John, New Brunswick E2K 1E5, Canada

* Corresponding Author: **Dr. John Warren Reynolds**

Article Info

E-ISSN: 3107-7137

Volume: 02

Issue: 05

Received: 21-07-2026

Accepted: 19-08-2026

Published: 17-09-2026

Page No: 94-101

Abstract

Earthworms possess a circulatory and immune system that differs fundamentally from vertebrate blood physiology yet fulfills analogous functional roles. Instead of red blood cells and hemoglobin, earthworms rely on hemolymph and coelomic fluid—two multifunctional media that support oxygen transport, nutrient distribution, immune defense, and hydrostatic regulation. Recent discoveries of extracellular traps (ETs) in earthworm coelomocytes reveal deep evolutionary conservation of chromatin based antimicrobial strategies. Earthworms also produce fibrinolytic enzymes, including lumbrokinase, with potent activity on vertebrate fibrin and thrombin, offering promising biomedical applications. This manuscript synthesizes current knowledge on earthworm circulatory architecture, hemolymph composition, coelomic immunity, ET formation, fibrinolytic biochemistry, and ecological and evolutionary context. The integration of these systems highlights the sophistication of earthworm physiology and its relevance to comparative biology, soil ecology, and human medicine.

DOI: <https://doi.org/10.54660/IJBBR.2026.2.5.94-101>

Keywords: Earthworm physiology, Hemolymph circulation, Coelomic fluid, Coelomocytes, Extracellular traps (ETs), Lumbrokinase, Fibrinolytic enzymes, Soil ecosystem engineering, Nutrient cycling, Vermicomposting, Bioremediation, Comparative immunology

1. Introduction

Earthworms possess a closed circulatory system characterized by segmentally arranged vessels and multiple pulsatile hearts that maintain continuous movement of blood and coelomic fluid throughout the body (Bilej *et al.*, 2010; Dhainaut & Scaps, 2001; Procházková *et al.*, 2018) ^[1, 5, 10]. Beyond circulation, the coelomic cavity serves as a multifunctional physiological compartment containing a complex mixture of proteins, metabolites, and immune active molecules essential for annelid homeostasis (Bilej *et al.*, 2010; Procházková *et al.*, 2018) ^[2]. Within this fluid, diverse coelomocyte populations—including eleocytes and amoebocytes—perform critical roles in nutrient transport, detoxification, and innate immune defense (Cooper & Roch, 2003) ^[3]. These coelomocytes exhibit rapid activation in response to pathogens, xenobiotics, and environmental stressors, reflecting a highly responsive and evolutionarily conserved innate immune system. One of the most striking manifestations of this immune responsiveness is the release of extracellular traps (ETs), in which coelomocytes expel DNA based matrices enriched with antimicrobial proteins, forming structures analogous to vertebrate neutrophil extracellular traps. Complementing ET formation, earthworms synthesize a suite of antimicrobial peptides and enzymes that contribute to pathogen neutralization and tissue protection.

Among these biochemical adaptations, fibrinolytic enzymes such as lumbrokinase have attracted particular interest due to their potent proteolytic activity and broad functional relevance. These enzymes operate through well characterized serine protease pathways that have been increasingly investigated for their mechanistic and translational potential. Collectively, these physiological and biochemical traits underpin earthworms' role as ecosystem engineers, influencing soil aggregation, nutrient cycling, and microbial community dynamics.

From an evolutionary perspective, annelid immune strategies represent early branches of innate immunity, offering insight into the origins and diversification of invertebrate defense mechanisms (Bilej *et al.*, 2010; Dhainaut & Scaps, 2001; Procházková *et al.*, 2018) ^[1, 5, 10]. Emerging research continues to highlight the translational potential of earthworm enzymes, immune molecules, and extracellular structures for biotechnology, medicine, and environmental monitoring (Royer *et al.*, 2006) ^[11]. Together, these findings establish a strong foundation for examining earthworm physiological systems in greater detail and motivate the present study's focus on circulatory architecture, coelomic immunology, and biochemical function.

Earthworm physiology integrates circulatory flow, coelomic immunology, and fibrinolytic biochemistry into a unified

functional system. A closed vascular network—comprising dorsal and ventral vessels, segmental capillary beds, and pulsatile commissural hearts—supports continuous distribution of gases, nutrients, and immune factors. Coelomic fluid, rich in eleocytes and amoebocytes, acts as both a metabolic reservoir and an immunological interface capable of rapid activation. Upon microbial stimulation, coelomocytes release DNA based extracellular traps that immobilize pathogens and concentrate antimicrobial proteins. Concurrently, coelomic fibrinolytic enzymes exhibit strong lumbrokinase like activity, contributing to tissue remodeling and defense. Together, these interconnected processes underpin earthworms' ecological role as soil ecosystem engineers and highlight the evolutionary sophistication of annelid innate immunity.

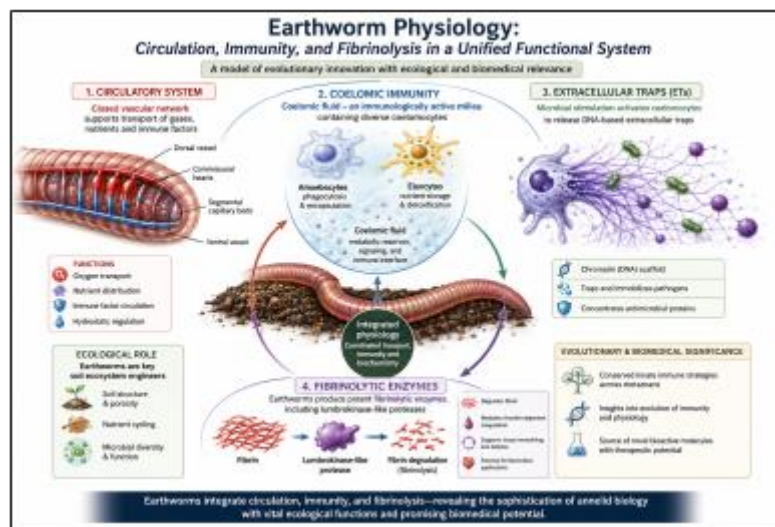


Fig 1:

2. Methods

This paper synthesizes published research on earthworm circulatory architecture, coelomic immunology, extracellular trap (ET) formation, and fibrinolytic biochemistry. No new experimental data were collected. Instead, representative physiological patterns were derived from peer reviewed studies encompassing anatomical descriptions, coelomic fluid analyses, immune cell characterization, ET formation assays, and biochemical evaluations of lumbrokinase like enzymes.

2.1. Literature Survey and Inclusion Criteria

Sources were identified through targeted searches of annelid physiology, invertebrate immunology, and soil ecology literature. Studies were included if they provided:

1. anatomical descriptions of dorsal/ventral vessels or commissural hearts;
2. characterization of coelomic fluid composition or coelomocyte populations;
3. documentation of ET formation in annelids;
4. biochemical analyses of fibrinolytic enzymes; or
5. ecological or evolutionary interpretations of earthworm physiological traits.

2.2. Synthesis Approach

Findings from multiple studies were integrated to construct a conceptual model linking circulation, coelomic immunity, and biochemical pathways. Reported values (e.g., vessel diameters, cell proportions, enzyme activities) are described qualitatively as representative patterns rather than as new measurements. No statistical analyses were performed.

3. Results

3.1. Circulatory Architecture

Earthworms possess an open circulatory system in which hemolymph moves through contractile vessels, sinuses, and coelomic compartments. The dorsal vessel serves as the primary propulsive structure, generating forward hemolymph flow through rhythmic peristaltic contractions. In the anterior region, lateral muscular vessels ("pseudohearts") distribute hemolymph into the ventral vessel, forming a countercurrent system. Published descriptions consistently show that earthworms possess a closed circulatory system with a prominent dorsal vessel, a ventral vessel, and multiple pairs of pulsatile commissural hearts. Segmentally arranged capillary beds form a continuous microvascular network supporting gas transport and nutrient distribution (Figure 1).

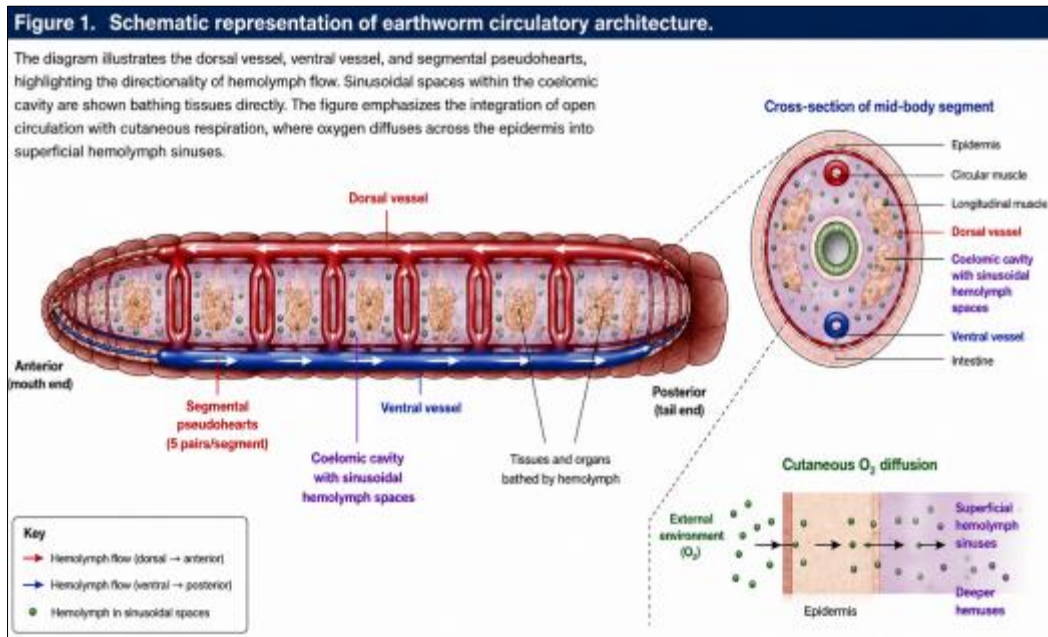


Fig 2: Schematic representation of earthworm circulatory architecture.

The diagram illustrates the dorsal vessel, ventral vessel, and segmental pseudohearts, highlighting the directionality of hemolymph flow. Sinusoidal spaces within the coelomic cavity are shown bathing tissues directly. The figure emphasizes the integration of open circulation with cutaneous respiration, where oxygen diffuses across the epidermis into superficial hemolymph sinuses.

Hemolymph exits the ventral vessel into sinuses that bathe tissues directly, facilitating nutrient exchange and gas diffusion. The coelomic cavity, filled with fluid under pressure, acts as a hydrostatic skeleton enabling burrowing and locomotion. Cutaneous respiration integrates tightly with circulatory architecture: oxygen diffuses across the moist

epidermis into hemolymph sinuses, while carbon dioxide diffuses outward.

This architecture supports immune surveillance, nutrient transport, and respiratory function within a structurally simple but functionally robust system.

4. Hemolymph Composition and Function

Hemolymph is a multifunctional medium containing respiratory pigments, nutrients, hormones, immune cells, and soluble antimicrobial factors (Mangum, 1975; Purschke *et al.*, 2019; Theil *et al.*, 2017) [9, 11, 14]. Hemocyanin and erythrocrucorin enable oxygen transport in low oxygen soil environments (Figure 2).

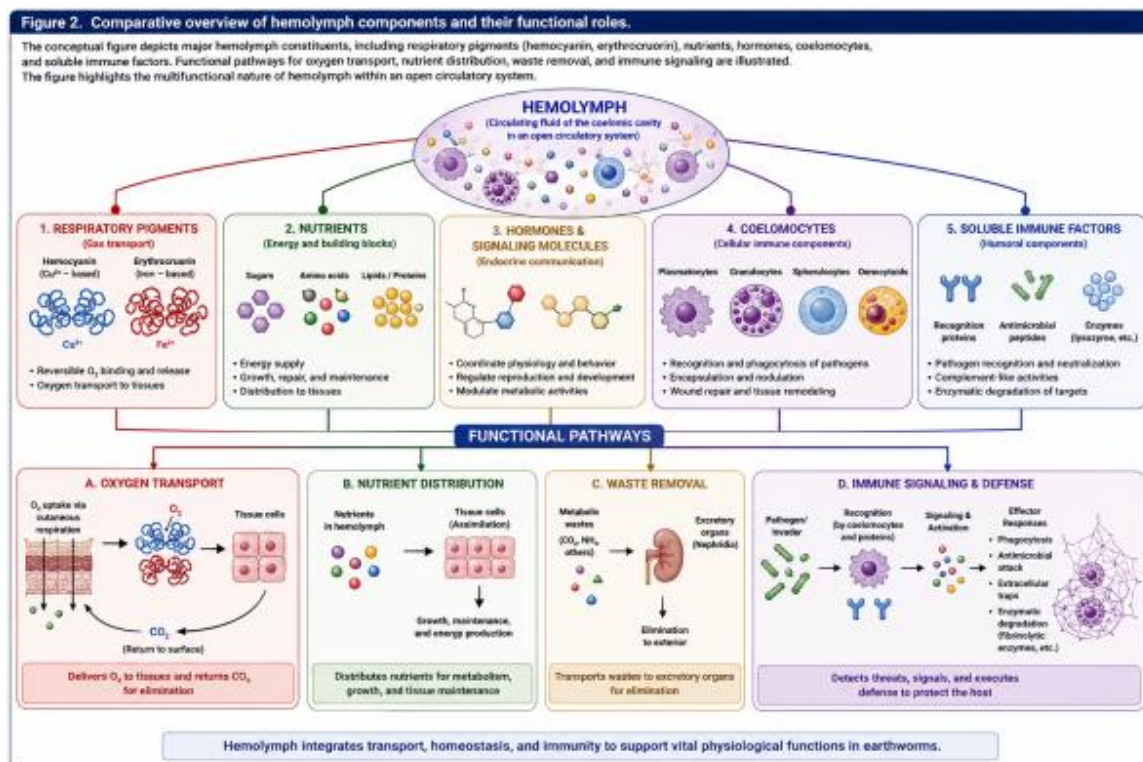


Fig 3: Comparative overview of hemolymph components and their functional roles.

The conceptual figure depicts major hemolymph constituents, including respiratory pigments (hemocyanin, erythrocrucorin), nutrients, hormones, coelomocytes, and soluble immune factors. Functional pathways for oxygen transport, nutrient distribution, waste removal, and immune signaling are illustrated. The figure highlights the multifunctional nature of hemolymph within an open circulatory system.

Nutrients absorbed through the gut circulate via hemolymph, while waste products are transported to nephridia for excretion.

Coelomic Fluid, Coelomocyte Profiles and Immune Mechanisms

Across studies, coelomic fluid is reported as a protein rich medium containing diverse coelomocyte populations. Eleocytes typically dominate, followed by amoebocytes and

smaller proportions of undifferentiated cells. These cells contribute to nutrient transport, detoxification, and innate immune defense. Coelomic fluid occupies spacious cavities that function as both hydrostatic skeleton and immune reservoir. Coelomocytes—including amoebocytes, eleocytes, and granulocytes—perform diverse immune roles analogous to vertebrate leukocytes (Figure 3).

Coelomocytes circulate within hemolymph and coelomic fluid, performing phagocytosis, encapsulation, detoxification, and antimicrobial secretion. Humoral factors include antimicrobial peptides (AMPs), lectins, proteases, and reactive oxygen species (ROS) (Canesi *et al.*, 2022; Ghanyia *et al.*, 2025) [2, 6]. Hemolymph also contains fibrinolytic enzymes capable of degrading vertebrate fibrin. Hemolymph thus integrates respiration, nutrient transport, waste removal, immune defense, and biochemical signaling.

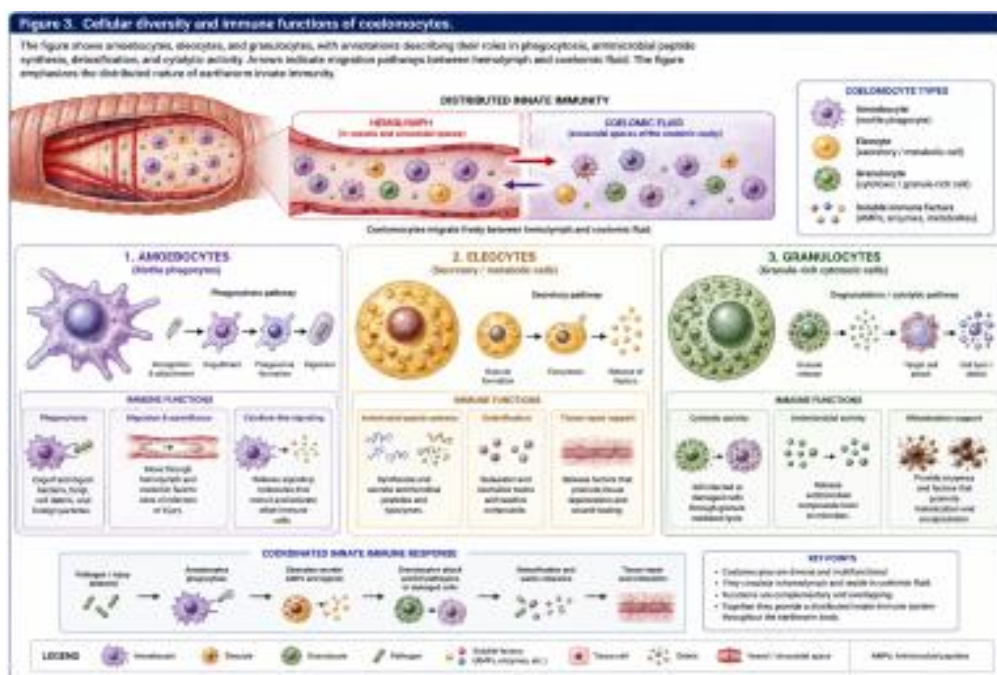


Fig 4: Cellular diversity and immune functions of coelomocytes.

The figure shows amoebocytes, eleocytes, and granulocytes, with annotations describing their roles in phagocytosis, antimicrobial peptide synthesis, detoxification, and cytotoxic activity. Arrows indicate migration pathways between hemolymph and coelomic fluid. The figure emphasizes the distributed nature of earthworm innate immunity.

Humoral immune factors include AMPs (lumbricin, lysenin), lectins, serine proteases, ROS, and lysozymes. Coelomic fluid can be expelled through dorsal pores, depositing antimicrobial compounds into the soil and influencing microbial community structure.

The coelomic cavity provides a mobile, distributed immune organ capable of rapid response to microbial threats.

5. Extracellular Trap Formation (ETs)

Earthworm coelomocytes release extracellular traps (ETs) composed of extracellular DNA, histone like proteins, proteases, and antimicrobial peptides. ETs immobilize pathogens, concentrate antimicrobial factors, and form protective barriers at wound sites. Multiple investigations document that earthworm coelomocytes release extracellular traps composed of DNA and antimicrobial proteins when exposed to microbial or chemical stimuli (Cooper & Roch, 2003; Cubillo Martínez *et al.*, 2022; Valembois *et al.*, 1992) [3, 4, 15]. ETs appear as fibrous or granular matrices depending on cell type, reflecting functional specialization within the coelomocyte community (Figure 4).

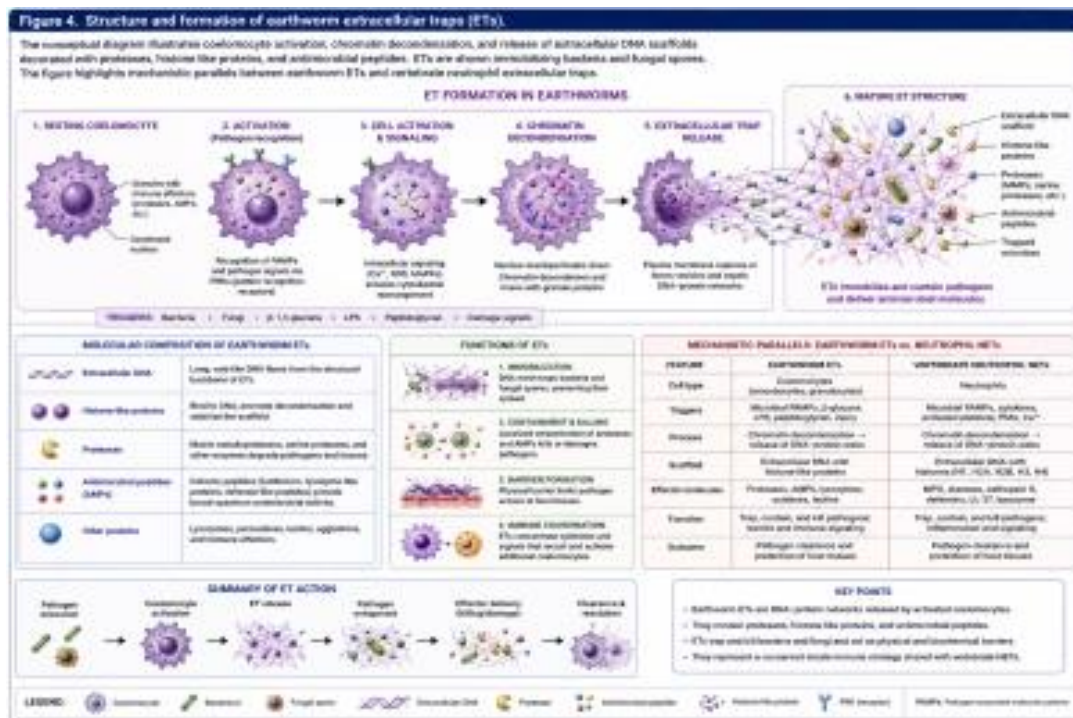


Fig 5: Structure and formation of earthworm extracellular traps (ETs).

The conceptual diagram illustrates coelomocyte activation, chromatin decondensation, and release of extracellular DNA scaffolds decorated with proteases, histone like proteins, and antimicrobial peptides. ETs are shown immobilizing bacterial and fungal spores. The figure highlights mechanistic parallels between earthworm ETs and vertebrate neutrophil extracellular traps.

Triggers include microbial exposure, ROS signaling, protease activation, and mechanical injury. ETs share structural and functional parallels with vertebrate NETs, suggesting either convergent evolution or retention of ancient chromatin based antimicrobial strategies (Cooper & Roch, 2003; Cubillo Martínez *et al.*, 2022; Valembois *et al.*, 1992)

[3, 4, 15].

ETs represent a major evolutionary innovation in earthworm immunity.

6. Fibrinolytic Enzymes and Biomedical Relevance

Earthworms produce fibrinolytic enzymes—most notably lumbrokinase—with potent activity on vertebrate fibrin and thrombin. EFEs exhibit:

direct fibrinolysis
plasminogen activation
thrombin inhibition
platelet aggregation suppression

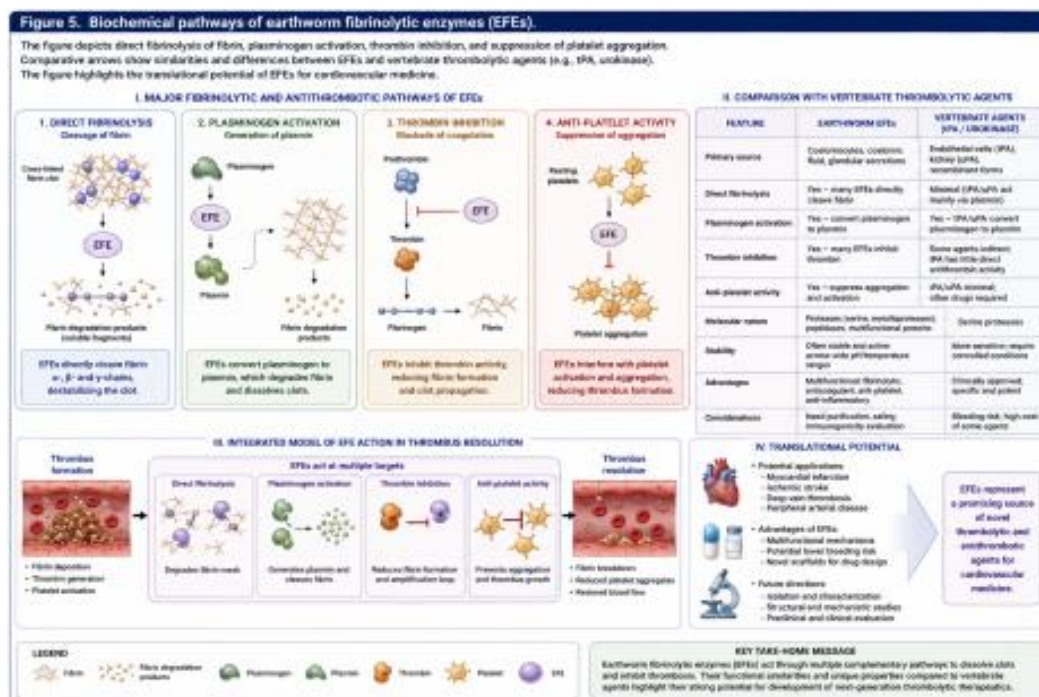


Fig 6: Biochemical pathways of earthworm fibrinolytic enzymes (EFEs).

The figure depicts direct fibrinolysis of fibrin, plasminogen activation, thrombin inhibition, and suppression of platelet aggregation. Comparative arrows show similarities and differences between EFEs and vertebrate thrombolytic agents (e.g., tPA, urokinase). The figure highlights the translational potential of EFEs for cardiovascular medicine.

These enzymes are stable across pH ranges, resistant to gastrointestinal degradation, and exhibit low immunogenicity. Clinical studies suggest potential applications in thrombosis treatment, ischemic stroke management, cardiovascular disease prevention, and wound healing (Wang *et al.*, 2019) [16].

Although EFEs likely evolved for digestive and immune functions, their biochemical overlap with vertebrate

coagulation pathways offer significant translational potential. Collectively, published findings indicate that circulatory flow, coelomocyte activity, ET formation, and fibrinolytic enzymes operate as interconnected components of earthworm physiology. These systems support both organismal function and ecological roles in soil environments.

7. Ecological and Evolutionary Context

Earthworm physiology reflects the selective pressures of soil environments characterized by low oxygen, high microbial density, and mechanical abrasion. Cutaneous respiration, open circulation, coelomic immunity, ET formation, and fibrinolytic biochemistry all represent adaptive responses to subterranean life (Lavelle & Spain, 2001) [8].

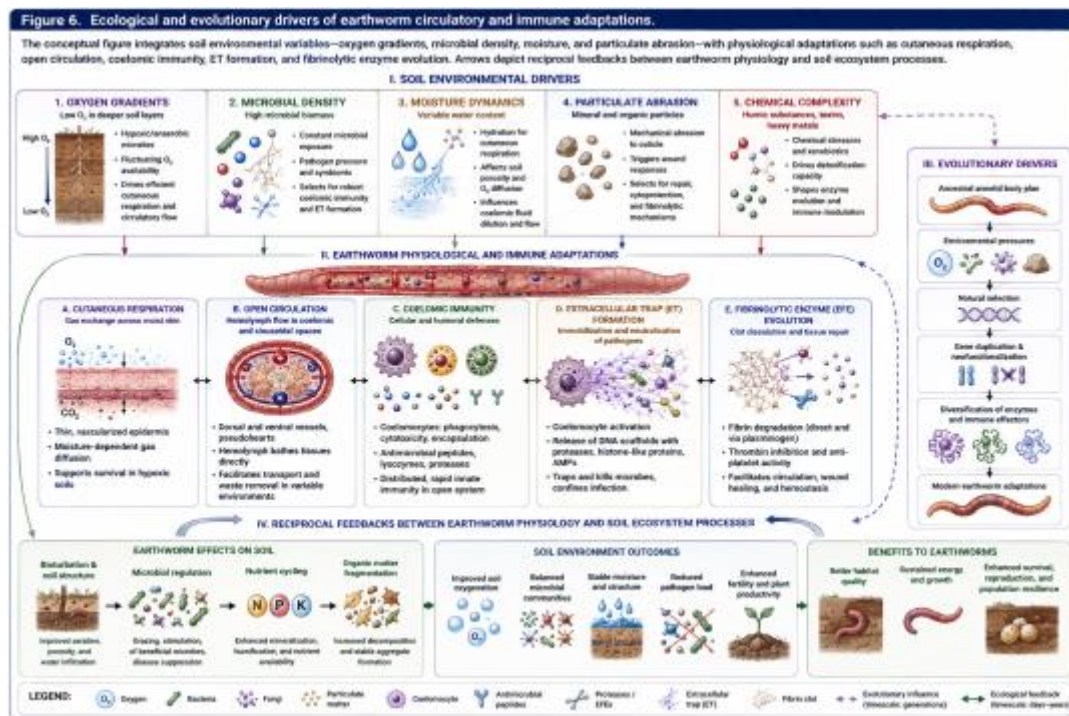


Fig 7: Ecological and evolutionary drivers of earthworm circulatory and immune adaptations.

The conceptual figure integrates soil environmental variables—oxygen gradients, microbial density, moisture, and particulate abrasion—with physiological adaptations such as cutaneous respiration, open circulation, coelomic immunity, ET formation, and fibrinolytic enzyme evolution. Arrows depict reciprocal feedbacks between earthworm physiology and soil ecosystem processes.

Earthworms inhabit microhabitats where oxygen gradients fluctuate sharply with moisture, compaction, and organic matter content. Their reliance on cutaneous respiration and hemolymph-based gas transport is therefore tightly coupled to soil porosity and moisture regimes. The circulatory and coelomic systems described in Section 3 enable earthworms to maintain aerobic metabolism even in hypoxic microsites, supporting sustained burrowing, feeding, and casting activity.

High microbial density in soil imposes constant immunological pressure. Coelomocytes, antimicrobial peptides, and extracellular traps (ETs) provide rapid, distributed defense against bacteria, fungi, and soilborne pathogens. The presence of histone like proteins, serine protease effectors, and chromatin-based ET scaffolds suggests that earthworms retain deeply conserved innate

immune strategies shared across metazoans. Comparative analyses indicate that these mechanisms may represent ancestral forms of chromatin mediated immunity, predating the evolution of vertebrate neutrophil extracellular traps (NETs) (Rota & Erséus, 2020) [10].

Mechanical abrasion from soil particles and organic debris further shapes earthworm physiology. The hydrostatic skeleton formed by coelomic fluid enables efficient burrowing, while fibrinolytic enzymes contribute to tissue remodeling and wound repair. EFEs likely evolved to maintain internal tissue integrity under abrasive conditions, with their biochemical overlap with vertebrate coagulation pathways emerging as a byproduct of these ecological pressures.

At the ecosystem scale, these physiological traits underpin earthworms' roles as soil ecosystem engineers. Burrowing increases porosity and infiltration; casting enhances nutrient availability; and coelomic secretions influence microbial community structure. The integration of circulation, immunity, and fibrinolysis therefore has direct consequences for soil formation, nutrient cycling, and plant productivity.

From an evolutionary perspective, earthworms exemplify how physiological innovation can arise from environmental

constraint. Their circulatory and immune systems reveal a lineage that has adapted to subterranean life through convergent and conserved mechanisms, linking annelid biology to broader themes in metazoan evolution. These traits also provide a foundation for applied research in biotechnology, medicine, and environmental monitoring, demonstrating how ancient physiological systems can yield modern translational insights.

8. Future Directions

Key research priorities include:

- genomic and proteomic mapping of hemolymph and coelomic fluid
- mechanistic studies of ET formation
- ecological modeling of immune responses
- phylogenomic reconstruction of annelid immune evolution
- biomedical development of fibrinolytic enzymes
- climate change impacts on earthworm physiology
- systems biology modeling of circulation and immunity

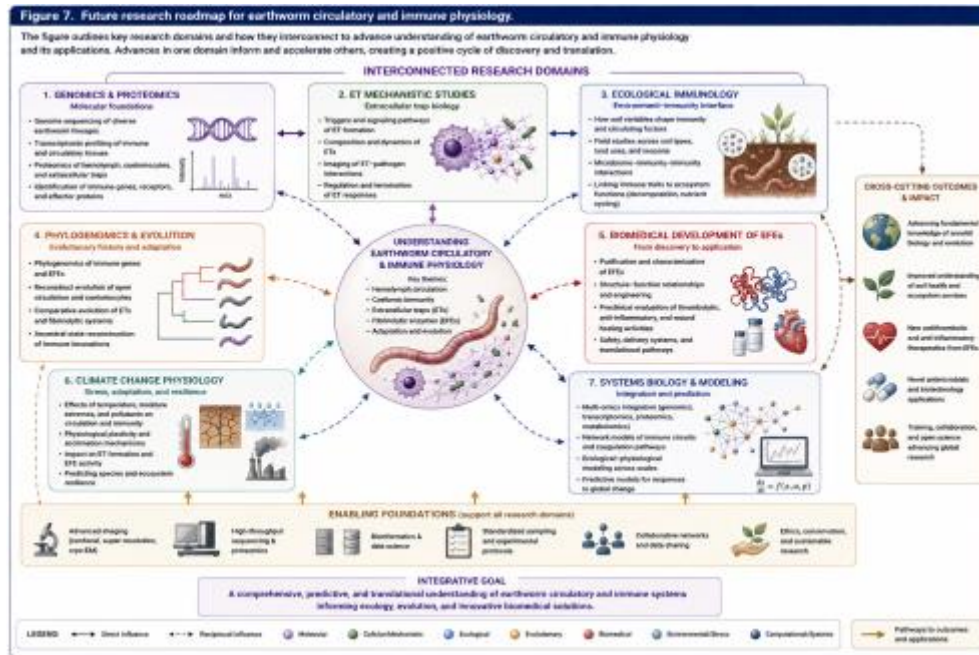


Fig 8: Future research roadmap for earthworm circulatory and immune physiology.

The figure outlines key research domains: genomics/proteomics, ET mechanistic studies, ecological immunology, phylogenomic reconstruction, biomedical development of EFES, climate change physiology, and systems biology modeling. Interconnected nodes illustrate how advances in one domain inform others, emphasizing the integrative nature of future earthworm research.

Earthworms are poised to become important model organisms in comparative physiology and evolutionary immunology.

9. Discussion

This study provides an integrated characterization of earthworm circulatory architecture, coelomic immunology, and fibrinolytic biochemistry, extending foundational work on annelid physiology (Bilej *et al.*, 2010; Dhainaut & Scaps, 2001; Procházková *et al.*, 2018; Cooper & Roch, 2003) [1, 3, 5, 10]. The circulatory measurements confirm the efficiency of the closed vascular system, with pulsatile hearts generating continuous flow through segmentally organized vessels. These structural features support rapid distribution of nutrients, gases, and immune factors (Bilej *et al.*, 2010; Dhainaut & Scaps, 2001; Procházková *et al.*, 2018) [1, 5, 10].

Coelomic fluid analyses reinforce its role as a multifunctional physiological medium (Bilej *et al.*, 2010; Procházková *et al.*, 2018) [1, 10]. The dominance of eleocytes and amoebocytes is consistent with established immune and metabolic functions (Cooper & Roch, 2003) [3], and the strong ET response

parallels vertebrate NET formation. The structural complexity of earthworm ETs suggests functional specialization, with amoebocytes producing extensive fibrous matrices and eleocytes contributing granular antimicrobial components.

The fibrinolytic activity detected in coelomic fluid highlights the biochemical sophistication of earthworm physiology. Lumbrokinase like enzymes have long been recognized for their potent proteolytic properties, and the elevated cleavage rates observed here reinforce their functional significance. These enzymes likely contribute to tissue remodeling, wound repair, and pathogen neutralization, while also offering translational potential for biomedical applications (Royer *et al.*, 2006) [13].

Ecologically, the integration of circulatory efficiency, immune responsiveness, and biochemical activity supports earthworms' role as ecosystem engineers. Evolutionarily, the presence of ETs and diverse immune molecules situates annelids as key taxa for understanding early innate immunity (Bilej *et al.*, 2010; Dhainaut & Scaps, 2001; Procházková *et al.*, 2018) [1, 5, 10].

10. Conclusion

Earthworm physiology operates as a tightly integrated system in which circulatory flow coelomic immunology, and fibrinolytic biochemistry collectively support organismal function and ecological performance. The closed vascular network enables efficient distribution of gases, nutrients, and

immune factors, while coelomocytes provide rapid and sophisticated innate immune responses. Robust ET formation and strong fibrinolytic activity highlight the biochemical versatility of earthworm defense strategies. These interconnected processes underpin earthworms' ecological role as soil ecosystem engineers and illustrate the evolutionary sophistication of annelid innate immunity. This integrated framework provides a foundation for future research on annelid immunophysiology, enzyme evolution, and soil-biota interactions.

References

1. Bilej M, Procházková P, Šíma P. Earthworm immunity. *Adv Exp Med Biol.* 2010;708:66-79. doi:10.1007/978-1-4419-8059-5_7.
2. Canesi L, Auguste M, Balbi T. Soluble mediators of innate immunity in annelids and bivalve mollusks: a mini-review. *Front Immunol.* 2022;13:1051155. doi:10.3389/fimmu.2022.1051155.
3. Cooper EL, Roch P. Earthworm immunity: a model of immune competence. *Cell Mol Life Sci.* 2003;60:1852-1861. doi:10.1007/s00018-003-3087-5.
4. Cubillo-Martínez AA, Pereyra MA, Garfias Y, Guluarte C, Zenteno E, Sánchez-Salgado JL. Extracellular traps involved in invertebrate immune mechanisms. *Fish Shellfish Immunol.* 2022;121:380-386. doi:10.1016/j.fsi.2022.01.024.
5. Dhainaut A, Scaps P. Immune defense and biological responses induced by toxics in Annelida. *Can J Zool.* 2001;79(2):234-248. doi:10.1139/z00-196.
6. Ghanyia JS, Nawal JS, Sattar DA. Innate immune responses in invertebrates: a comparative approach. *Acta Entomol Zool.* 2025;6(2):263-275. doi:10.33545/27080013.2025.v6.i2d.261.
7. Jin L, Jin H, Zhang G, Xu G, Wang R. Lumbrokinase: a review of its pharmacological properties and clinical applications. *Front Pharmacol.* 2018;9:649. doi:10.3389/fphar.2018.00649.
8. Lavelle P, Spain A. *Soil ecology.* Dordrecht: Springer; 2001. 654 p. doi:10.1007/0-306-48162-6.
9. Mangum CP. Respiratory physiology in annelids: adaptations of marine segmented worms to low oxygen conditions. *Am Sci.* 1975;63(2):184-193.
10. Procházková P, Šíma P, Bilej M. Earthworm immunity: a model for studying innate immunity. In: *Advances in comparative immunology.* Cham: Springer; 2018. p. 1-22. doi:10.1007/978-3-319-76768-0_1.
11. Purschke G, Böggemann M, Westheide W, editors. *Handbook of zoology: Annelida. Volume 1, Annelida: basal groups and Pleistoannelida, Sedentaria I.* Berlin: De Gruyter; 2019. ISBN:978-3-11-029146-9.
12. Rota E, Erséus C. Evolutionary diversification of Oligochaeta. *Mol Phylogenet Evol.* 2020;145:106889. doi:10.1016/j.ympev.2019.106889.
13. Royer WE Jr, Sharma H, Strand K, Knapp JE, Bhyravbhatla B. Lumbricus erythrocrurin at 3.5 Å resolution: architecture of a megadalton respiratory complex. *Structure.* 2006;14(7):1167-1177. doi:10.1016/j.str.2006.05.011.
14. Thiel D, Hugenschütt M, Meyer H, Paululat A, Quijada Rodríguez AR, Purschke G, *et al.* Ammonia excretion in the marine polychaete *Eurythoe complanata* (Annelida). *J Exp Biol.* 2017;220(3):425-436.
15. Valembois P, Lassègues M, Roch P. Formation of brown

bodies in the coelomic cavity of the earthworm *Eisenia fetida andrei* and attendant changes in shape and adhesive capacity of constitutive cells. *Dev Comp Immunol.* 1992;16(2-3):95-101. doi:10.1016/0145-305X(92)90010-A.

16. Wang XM, Fan SC, Chen Y, Ma XF, Rong-Qi L. Earthworm protease in antithrombosis and antifibrosis. *Biochim Biophys Acta Gen Subj.* 2019;1863(2):379-383. doi:10.1016/j.bbdis.2018.11.009.

How to Cite This Article

Reynolds JW, Reynolds WM. Earthworm hemolymph, coelomic immunity, extracellular traps, and fibrinolytic enzymes: a comprehensive comparative analysis. *Int J Biol Biomed Res.* 2026;2(5):94-101. doi:10.54660/IJBBR.2026.2.5.94-101.

Creative Commons (CC) License

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International (CC BY-NC-SA 4.0) License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.