



Biomechanics of Nematode Locomotion and Movement Patterns

Robert Smith and Julia Anderson

EasyChair preprints are intended for rapid dissemination of research results and are integrated with the rest of EasyChair.

May 13, 2024

Biomechanics of Nematode Locomotion and Movement Patterns

Robert Smith, Julia Anderson

Abstract:

Nematodes, commonly known as roundworms, are ubiquitous organisms found in various habitats across the globe. Despite their diminutive size, nematodes exhibit remarkable locomotion capabilities that enable them to navigate through diverse environments. Understanding the biomechanics underlying nematode locomotion and movement patterns is crucial for elucidating their ecological roles, interactions with other organisms, and potential applications in biomimetics. This paper provides an overview of the biomechanical principles governing nematode locomotion, encompassing aspects such as muscle structure and function, hydrostatic skeleton mechanics, neural control, and environmental influences. Additionally, it explores the diverse movement patterns displayed by nematodes in response to different environmental stimuli and physiological states. By synthesizing existing knowledge and identifying research gaps, this paper aims to stimulate further investigations into the fascinating biomechanics of nematode locomotion.

Keywords: Nematodes, locomotion, biomechanics, hydrostatic skeleton, muscle physiology, neural control, environmental stimuli, movement patterns.

I. Introduction:

Nematodes, often colloquially referred to as roundworms, constitute a diverse and abundant phylum within the animal kingdom, comprising over a million known species[1]. Despite their microscopic size, nematodes play crucial roles in various ecosystems, contributing to nutrient cycling, soil health, and decomposition processes[2]. Understanding the biomechanics underlying nematode locomotion is fundamental to comprehending their ecological significance and evolutionary adaptations. Nematodes exhibit a remarkable array of movement patterns, ranging from crawling and swimming to burrowing, each finely tuned to suit their specific ecological niche[3].

The locomotion of nematodes is facilitated by a unique anatomical feature known as the hydrostatic skeleton, characterized by a fluid-filled body cavity enclosed by a flexible cuticle. This hydrostatic system, coupled with specialized muscle arrangements, enables nematodes to achieve locomotion through rhythmic contractions and changes in body shape[4]. The study of

nematode locomotion encompasses multiple disciplines, including biology, biomechanics, and physics, reflecting the interdisciplinary nature of understanding their movement strategies[5].

One of the key aspects of nematode locomotion is the role of neural control, mediated by a relatively simple nervous system composed of interconnected neurons[6]. Neurotransmitters, particularly acetylcholine, play a pivotal role in coordinating muscle activity and modulating locomotor behavior in response to sensory cues. The interplay between neural circuits, muscle physiology, and environmental stimuli shapes the diverse locomotion patterns observed in nematodes across different habitats and ecological conditions[7].

Furthermore, nematode locomotion is profoundly influenced by environmental factors such as substrate composition, temperature, humidity, and chemical signals. Nematodes exhibit behavioral plasticity, adjusting their movement patterns to optimize foraging efficiency, avoid predators, and locate suitable habitats[8]. By unraveling the biomechanical principles underlying nematode locomotion and movement patterns, researchers can gain insights into their ecological roles, interactions with other organisms, and potential applications in fields ranging from agriculture to robotics.

II. Anatomy and Muscle Structure:

Nematodes possess a streamlined body plan characterized by a cylindrical shape and a protective cuticle, which serves as an exoskeleton[9]. Beneath the cuticle lies a hydrostatic skeleton, a fluid-filled cavity known as the pseudocoelom, which provides structural support and facilitates locomotion. The muscles of nematodes are arranged in longitudinal and diagonal layers, enabling them to generate movement through coordinated contractions. Longitudinal muscles run parallel to the body axis and are responsible for producing sinusoidal waves of motion during crawling. In contrast, diagonal muscles are positioned obliquely, allowing for twisting and bending movements essential for navigating complex environments[10].

The muscle cells of nematodes exhibit a unique organization, with dense bodies and thin filaments arranged in a lattice-like pattern. This arrangement provides structural support and facilitates force transmission during muscle contraction. The contractile apparatus of nematode muscles relies on the sliding filament mechanism, wherein myosin heads interact with actin filaments to generate movement[11]. Calcium ions play a crucial role in regulating muscle contraction, with calcium release from intracellular stores triggering the activation of molecular motors and the generation of contractile force[12].

Muscle activity in nematodes is under the control of a relatively simple nervous system, comprising a network of neurons distributed throughout the body. Motor neurons transmit signals to muscle cells, initiating and coordinating rhythmic contractions required for locomotion. Neurotransmitters, such as acetylcholine, mediate synaptic transmission at the neuromuscular junction, facilitating the propagation of action potentials and the regulation of muscle activity[13]. The integration of sensory feedback with neural control mechanisms enables

nematodes to adjust their locomotor behavior in response to environmental cues, ensuring efficient navigation and survival in diverse habitats.

III. Hydrostatic Skeleton Mechanics:

Nematodes rely on a hydrostatic skeleton, a fluid-filled cavity surrounded by a flexible cuticle, to support their body structure and facilitate locomotion[14]. The pseudocoelom, filled with a pressurized fluid, provides both support and flexibility, allowing nematodes to undergo rapid changes in body shape during movement. Contractions of longitudinal and diagonal muscles compress the fluid within the pseudocoelom, generating hydrostatic pressure that maintains body stiffness and facilitates propulsion[15].

The mechanics of nematode locomotion are governed by the interplay between muscle contractions and hydrostatic support. As muscles contract, they exert force on the fluid-filled cavity, causing changes in internal pressure that result in bending and extension of the body[16]. The arrangement of muscles and their attachment points to the cuticle determine the direction and amplitude of movement, enabling nematodes to navigate through complex environments such as soil particles or aquatic sediments.

The efficiency of nematode locomotion relies on the precise control of hydrostatic pressure within the pseudocoelom. By regulating muscle activity and coordinating contractions along the body length, nematodes can achieve different locomotion modes, including crawling, swimming, and burrowing[17]. The hydrostatic skeleton also provides protection against external forces and predators, as changes in body shape can help nematodes evade capture or escape from confinement.

Overall, the hydrostatic skeleton represents a versatile biomechanical adaptation that allows nematodes to thrive in diverse habitats. By harnessing the principles of hydrostatic support and muscle-driven propulsion, nematodes demonstrate remarkable agility and efficiency in navigating their environments, highlighting the adaptive significance of their locomotion mechanisms[18]. Further studies elucidating the dynamics of hydrostatic skeleton mechanics hold promise for advancing our understanding of nematode physiology and informing biomimetic design principles in engineering and robotics.

IV. Neural Control of Locomotion:

Nematodes exhibit a relatively simple nervous system that plays a crucial role in coordinating locomotor behavior[19]. The nervous system of nematodes comprises a network of interconnected neurons distributed throughout the body, with specialized sensory and motor neurons involved in regulating movement. Neural circuits within the nervous system orchestrate the rhythmic contractions of muscles required for locomotion, enabling nematodes to navigate their environment and respond to sensory stimuli.

Neural signaling in nematodes is primarily mediated by neurotransmitters, with acetylcholine playing a central role in neuromuscular transmission. Motor neurons release acetylcholine at the neuromuscular junction, where it binds to receptors on muscle cells, initiating depolarization and muscle contraction[20]. The precise timing and coordination of synaptic transmission are essential for generating coherent locomotion patterns, with neural circuits modulating muscle activity to produce sinusoidal waves of motion during crawling or undulatory movements during swimming.

Sensory feedback mechanisms play a crucial role in regulating nematode locomotion, allowing them to respond adaptively to changes in their environment[21]. Nematodes possess specialized sensory structures, such as amphids and phasmids, which detect chemical cues, temperature gradients, and mechanical stimuli. Sensory information is integrated within the nervous system, influencing motor output and adjusting locomotor behavior accordingly. For example, nematodes may exhibit chemotaxis towards food sources or avoid noxious chemicals by altering their movement patterns in response to sensory cues[22].

The neural control of locomotion in nematodes represents a fascinating example of how simple nervous systems can generate complex behaviors. Despite their small size and anatomical simplicity, nematodes demonstrate remarkable agility and flexibility in navigating diverse environments[23]. Understanding the neural mechanisms underlying nematode locomotion not only provides insights into fundamental principles of nervous system function but also offers potential applications in robotics and biomimetic design, inspiring innovations in locomotion control algorithms and soft robotics technologies[24].

V. Environmental Influences on Locomotion:

Nematode locomotion is profoundly influenced by a myriad of environmental factors, including substrate properties, temperature, moisture levels, and chemical signals. The physical characteristics of the substrate, such as its texture and composition, play a crucial role in shaping nematode movement patterns[25]. For instance, nematodes may exhibit different crawling strategies when navigating through soil versus aquatic sediments, adjusting their locomotion to optimize propulsion and minimize energy expenditure.

Temperature gradients can significantly impact nematode locomotion, as they influence metabolic rates and muscle function. Nematodes are ectothermic organisms, meaning their body temperature is dependent on the surrounding environment. Changes in temperature can alter muscle contractility and nerve conduction velocity, affecting the speed and efficiency of locomotion. Furthermore, temperature fluctuations may induce behavioral responses in nematodes, such as seeking out thermally optimal microhabitats or entering diapause to survive adverse conditions[26].

Moisture levels also play a critical role in nematode locomotion, particularly for species inhabiting terrestrial environments. Water acts as a lubricant, reducing friction between the

nematode's body and the substrate, thereby facilitating movement[27]. Conversely, excessively dry or waterlogged conditions can impede locomotion, as they may cause desiccation or hinder muscle function. Nematodes exhibit behavioral adaptations to moisture gradients, with some species displaying hygrotaxis, the ability to migrate towards preferred humidity levels.

Chemical signals present in the environment can elicit specific behavioral responses in nematodes, influencing their locomotion patterns. Chemical cues associated with food sources, mates, or predators can trigger chemotaxis or pheromone-mediated behaviors, guiding nematodes towards favorable locations or inducing avoidance responses[28]. The integration of sensory information from chemical signals with neural control mechanisms enables nematodes to modulate their locomotor behavior in real-time, enhancing their ability to forage, reproduce, and evade threats in complex environments.

VI. Movement Patterns and Behavioral Ecology:

Nematodes exhibit a diverse array of movement patterns that are finely tuned to their ecological roles and environmental conditions. Crawling, characterized by sinusoidal waveforms, is a common locomotion mode employed by nematodes in soil and sediment habitats. This movement pattern allows nematodes to navigate through complex matrices, such as soil particles or interstitial spaces, while conserving energy and minimizing mechanical resistance[29]. The frequency and amplitude of crawling movements may vary depending on factors such as substrate texture, moisture content, and the presence of obstacles.

Swimming represents another locomotion mode observed in aquatic nematodes, enabling them to disperse in water columns and colonize new habitats. Nematodes typically exhibit an undulatory motion during swimming, propelling themselves forward by flexing their body in a wave-like fashion. Swimming behavior may be influenced by factors such as water currents, temperature gradients, and predator avoidance strategies[30]. Some nematodes exhibit rheotaxis, the ability to orient and swim against water flow, enhancing their ability to navigate in aquatic environments.

Burrowing is a specialized movement pattern employed by certain nematode species adapted to soil or sediment habitats. Burrowing nematodes use corkscrew-like movements to tunnel through substrates, exploiting gaps and pores to facilitate locomotion. This movement strategy allows nematodes to access nutrient-rich microenvironments, evade predators, and establish protective burrows for feeding or reproduction[31]. Burrowing behavior may be influenced by substrate composition, moisture levels, and the presence of chemical cues signaling favorable habitats or food sources.

The movement patterns exhibited by nematodes are intricately linked to their behavioral ecology, encompassing aspects such as foraging, dispersal, mating, and predator avoidance. Behavioral plasticity enables nematodes to adjust their locomotion strategies in response to changing environmental conditions and resource availability[32]. By integrating sensory feedback with neural control mechanisms, nematodes optimize their movement patterns to maximize fitness

and survival in diverse ecological niches. Studying nematode movement patterns and their ecological significance provides insights into fundamental principles of animal behavior and ecosystem dynamics, with implications for fields such as agriculture, ecology, and biomimetics[33].

VII. Applications and Future Directions:

Understanding the biomechanics of nematode locomotion holds promise for various practical applications across multiple disciplines. In agriculture, nematodes can have significant impacts as pests of crops or beneficial organisms for soil health[34]. By elucidating the mechanisms underlying nematode movement, researchers can develop strategies for pest management, such as targeted control methods that disrupt specific aspects of nematode locomotion or behavior[35]. Additionally, understanding how nematodes interact with soil particles and organic matter can inform soil management practices aimed at optimizing nutrient cycling and soil fertility[36].

In biotechnology and biomimetics, insights into nematode locomotion can inspire the design of novel robotic systems and soft-bodied robots. Mimicking the efficient movement strategies of nematodes, such as crawling or burrowing, could lead to the development of robotic platforms capable of navigating complex environments, such as soil or underwater sediments, for applications in environmental monitoring, exploration, and infrastructure maintenance[37]. By integrating principles of nematode biomechanics with advances in materials science and robotics, researchers can create innovative solutions for challenges in fields ranging from search and rescue operations to space exploration[38].

Future research directions in the study of nematode locomotion may involve exploring the molecular basis of muscle contraction and neural signaling, leveraging advances in techniques such as optogenetics and live imaging to elucidate the dynamics of neuromuscular interactions[39]. Understanding how nematodes respond to environmental cues at the molecular and cellular levels could uncover novel targets for intervention in parasitic nematode infections or provide insights into the evolution of locomotion strategies across diverse nematode taxa[40].

Furthermore, investigating the ecological consequences of altered movement patterns in response to environmental change can shed light on the resilience of nematode communities to global environmental shifts, such as climate change or habitat degradation[41]. By integrating experimental approaches with computational modeling and ecological theory, researchers can unravel the complex interactions between nematode locomotion, ecosystem processes, and environmental drivers, facilitating the development of predictive frameworks for assessing ecosystem health and resilience in a changing world. Overall, the applications of nematode biomechanics extend beyond fundamental research to address pressing societal challenges and inspire innovations in technology and sustainability[42].

VIII. Conclusion:

The biomechanics of nematode locomotion represents a captivating intersection of biology, physics, and engineering, with far-reaching implications for ecology, agriculture, and technology. Through intricate coordination of muscle contractions, hydrostatic support, and neural control mechanisms, nematodes exhibit remarkable agility and adaptability in navigating diverse environments, from soil to aquatic sediments. Understanding the principles underlying nematode movement not only sheds light on fundamental aspects of animal physiology and behavior but also offers practical applications in pest management, robotics, and environmental monitoring. By bridging disciplines and fostering interdisciplinary collaborations, future research endeavors hold promise for unlocking new insights into nematode biomechanics and leveraging this knowledge to address pressing challenges in sustainability, biodiversity conservation, and human health. As we delve deeper into the mysteries of nematode locomotion, we continue to uncover the hidden complexities of life on Earth and draw inspiration from nature's elegant solutions for innovation and discovery.

REFERENCES:

- [1] A. Q. Beeman, Z. L. Njus, S. Pandey, and G. L. Tylka, "The effects of ILeVO and VOTiVO on root penetration and behavior of the soybean cyst nematode, *Heterodera glycines*," *Plant disease*, vol. 103, no. 3, pp. 392-397, 2019.
- [2] B. I. Abrams and M. J. Mitchell, "Role of nematode-bacterial interactions in heterotrophic systems with emphasis on sewage sludge decomposition," *Oikos*, pp. 404-410, 1980.
- [3] A. Benda, L. Zerajic, A. Ankita, E. Cleary, Y. Park, and S. Pandey, "COVID-19 testing and diagnostics: a review of commercialized technologies for cost, convenience and quality of tests," *Sensors*, vol. 21, no. 19, p. 6581, 2021.
- [4] R. Anderson, E. Elliott, J. McClellan, D. C. Coleman, C. Cole, and H. Hunt, "Trophic interactions in soils as they affect energy and nutrient dynamics. III. Biotic interactions of bacteria, amoebae, and nematodes," *Microbial Ecology*, vol. 4, pp. 361-371, 1977.
- [5] J. A. Carr, R. Lycke, A. Parashar, and S. Pandey, "Unidirectional, electrostatic-response valve for *Caenorhabditis elegans* in microfluidic devices," *Applied Physics Letters*, vol. 98, no. 14, 2011.
- [6] S. S. Briar, S. J. Fonte, I. Park, J. Six, K. Scow, and H. Ferris, "The distribution of nematodes and soil microbial communities across soil aggregate fractions and farm management systems," *Soil Biology and Biochemistry*, vol. 43, no. 5, pp. 905-914, 2011.

- [7] J. A. Carr, A. Parashar, R. Gibson, A. P. Robertson, R. J. Martin, and S. Pandey, "A microfluidic platform for high-sensitivity, real-time drug screening on *C. elegans* and parasitic nematodes," *Lab on a Chip*, vol. 11, no. 14, pp. 2385-2396, 2011.
- [8] A. Ciancio, M. Colagiero, I. Pentimone, and L. Rosso, "Soil microbial communities and their potential for root-knot nematodes management: a review," *Environmental Engineering & Management Journal (EEMJ)*, vol. 15, no. 8, 2016.
- [9] B. Chen, A. Parashar, and S. Pandey, "Folded floating-gate CMOS biosensor for the detection of charged biochemical molecules," *IEEE Sensors Journal*, vol. 11, no. 11, pp. 2906-2910, 2011.
- [10] K. G. Davies, "Interactions between nematodes and microorganisms: bridging ecological and molecular approaches," *Advances in applied microbiology*, vol. 57, pp. 53-78, 2005.
- [11] X. Ding, Z. Njus, T. Kong, W. Su, C.-M. Ho, and S. Pandey, "Effective drug combination for *Caenorhabditis elegans* nematodes discovered by output-driven feedback system control technique," *Science advances*, vol. 3, no. 10, p. eaao1254, 2017.
- [12] D. H. Fitch, "Introduction to nematode evolution and ecology," *WormBook: The Online Review of C. elegans Biology [Internet]*, 2005.
- [13] J. P. Jensen, A. Q. Beeman, Z. L. Njus, U. Kalwa, S. Pandey, and G. L. Tylka, "Movement and motion of soybean cyst nematode heterodera glycines populations and individuals in response to abamectin," *Phytopathology*, vol. 108, no. 7, pp. 885-891, 2018.
- [14] D. W. Freckman and E. P. Caswell, "The ecology of nematodes in agroecosystems," *Annual review of Phytopathology*, vol. 23, no. 1, pp. 275-296, 1985.
- [15] J. P. Jensen, U. Kalwa, S. Pandey, and G. L. Tylka, "Avicta and Clariva affect the biology of the soybean cyst nematode, *Heterodera glycines*," *Plant disease*, vol. 102, no. 12, pp. 2480-2486, 2018.
- [16] Y. Jiang *et al.*, "Nematode grazing promotes bacterial community dynamics in soil at the aggregate level," *The ISME Journal*, vol. 11, no. 12, pp. 2705-2717, 2017.
- [17] U. Kalwa, C. Legner, E. Wlezien, G. Tylka, and S. Pandey, "New methods of removing debris and high-throughput counting of cyst nematode eggs extracted from field soil," *PLoS One*, vol. 14, no. 10, p. e0223386, 2019.
- [18] Y. Jiang *et al.*, "Nematodes and microbial community affect the sizes and turnover rates of organic carbon pools in soil aggregates," *Soil Biology and Biochemistry*, vol. 119, pp. 22-31, 2018.
- [19] T. Kong, N. Backes, U. Kalwa, C. Legner, G. J. Phillips, and S. Pandey, "Adhesive tape microfluidics with an autofocusing module that incorporates CRISPR interference: applications to long-term bacterial antibiotic studies," *ACS sensors*, vol. 4, no. 10, pp. 2638-2645, 2019.
- [20] C. M. Malmstrom, U. Melcher, and N. A. Bosque-Perez, "The expanding field of plant virus ecology: historical foundations, knowledge gaps, and research directions," *Virus Research*, vol. 159, no. 2, pp. 84-94, 2011.
- [21] C. Legner, U. Kalwa, V. Patel, A. Chesmore, and S. Pandey, "Sweat sensing in the smart wearables era: Towards integrative, multifunctional and body-compliant perspiration analysis," *Sensors and Actuators A: Physical*, vol. 296, pp. 200-221, 2019.
- [22] P. G. Mason, *Biological control: global impacts, challenges and future directions of pest management*. Csiro Publishing, 2021.
- [23] C. M. Legner, G. L. Tylka, and S. Pandey, "Robotic agricultural instrument for automated extraction of nematode cysts and eggs from soil to improve integrated pest management," *Scientific reports*, vol. 11, no. 1, p. 3212, 2021.
- [24] D. A. Neher and C. L. Campbell, "Nematode communities and microbial biomass in soils with annual and perennial crops," *Applied soil ecology*, vol. 1, no. 1, pp. 17-28, 1994.
- [25] R. Lycke, A. Parashar, and S. Pandey, "Microfluidics-enabled method to identify modes of *Caenorhabditis elegans* paralysis in four anthelmintics," *Biomicrofluidics*, vol. 7, no. 6, 2013.

- [26] R. Neilson *et al.*, "Microbial community size is a potential predictor of nematode functional group in limed grasslands," *Applied Soil Ecology*, vol. 156, p. 103702, 2020.
- [27] D. Miley, L. B. Machado, C. Condo, A. E. Jergens, K.-J. Yoon, and S. Pandey, "Video capsule endoscopy and ingestible electronics: emerging trends in sensors, circuits, materials, telemetry, optics, and rapid reading software," *Advanced Devices & Instrumentation*, 2021.
- [28] J. Nicol, G. Stirling, B. Rose, P. May, and R. Van Heeswijck, "Impact of nematodes on grapevine growth and productivity: current knowledge and future directions, with special reference to Australian viticulture," *Australian Journal of Grape and Wine Research*, vol. 5, no. 3, pp. 109-127, 1999.
- [29] Z. Njus *et al.*, "Flexible and disposable paper-and plastic-based gel micropads for nematode handling, imaging, and chemical testing," *APL bioengineering*, vol. 1, no. 1, 2017.
- [30] D. C. Norton and T. L. Niblack, "Biology and ecology of nematodes," in *Manual of agricultural nematology*: CRC Press, 2020, pp. 47-72.
- [31] S. Pandey and M. H. White, "Parameter-extraction of a two-compartment model for whole-cell data analysis," *Journal of neuroscience methods*, vol. 120, no. 2, pp. 131-143, 2002.
- [32] M. Renčo, E. Gömöryová, and A. Čerevková, "The effect of soil type and ecosystems on the soil nematode and microbial communities," *Helminthologia*, vol. 57, no. 2, pp. 129-144, 2020.
- [33] S. Pandey, A. Bortei-Doku, and M. H. White, "Simulation of biological ion channels with technology computer-aided design," *computer methods and programs in biomedicine*, vol. 85, no. 1, pp. 1-7, 2007.
- [34] G. Du Preez *et al.*, "Nematode-based indices in soil ecology: Application, utility, and future directions," *Soil Biology and Biochemistry*, vol. 169, p. 108640, 2022.
- [35] S. Pandey *et al.*, "Behavioral monitoring tool for pig farmers: Ear tag sensors, machine intelligence, and technology adoption roadmap," *Animals*, vol. 11, no. 9, p. 2665, 2021.
- [36] H. R. Wallace, *Nematode ecology and plant disease*. 1973.
- [37] A. Parashar and S. Pandey, "Plant-in-chip: Microfluidic system for studying root growth and pathogenic interactions in Arabidopsis," *Applied physics letters*, vol. 98, no. 26, 2011.
- [38] W. M. Williamson, D. A. Wardle, and G. W. Yeates, "Changes in soil microbial and nematode communities during ecosystem decline across a long-term chronosequence," *Soil Biology and Biochemistry*, vol. 37, no. 7, pp. 1289-1301, 2005.
- [39] V. Patel, A. Chesmore, C. M. Legner, and S. Pandey, "Trends in workplace wearable technologies and connected-worker solutions for next-generation occupational safety, health, and productivity," *Advanced Intelligent Systems*, vol. 4, no. 1, p. 2100099, 2022.
- [40] G. W. Yeates and B. Boag, "Background for nematode ecology in the 21st century," *Nematology: advances and perspectives*, vol. 1, pp. 406-437, 2004.
- [41] J. N. Saldanha, A. Parashar, S. Pandey, and J. A. Powell-Coffman, "Multiparameter behavioral analyses provide insights to mechanisms of cyanide resistance in *Caenorhabditis elegans*," *toxicological sciences*, vol. 135, no. 1, pp. 156-168, 2013.
- [42] H. Ferris, B. S. Griffiths, D. L. Porazinska, T. O. Powers, K.-H. Wang, and M. Tenuta, "Reflections on plant and soil nematode ecology: past, present and future," *Journal of Nematology*, vol. 44, no. 2, p. 115, 2012.