



## C A P Í T U L O 6

# ADVANCES IN THE DESIGN AND CONTROL OF CYBORG HISSING COCKROACHES: MACHINE LEARNING, MOTION TRACKING, AND TEMPERATURE CONTROL WITH A PERSPECTIVE ON SEARCH AND RESCUE OPERATIONS

**Eduardo Gracidas Reyes**

Universidad de las Américas Puebla, Departamento de  
Computación, Electrónica y Mecatrónica

**Gerardo Ulises Díaz Arango**

Universidad Veracruzana, Facultad de Instrumentación Electrónica

**ABSTRACT:** This study investigates the use of Madagascar hissing cockroaches (*Gromphadorhina portentosa*) as cyborg insects for search and rescue operations. We review recent advances in machine learning, motion tracking, and environmental control systems for characterizing and controlling cockroach locomotion. A novel motion tracking and temperature control system was developed to examine how thermal conditions impact cockroach movement patterns. Experimental results indicate that locomotor performance peaks between 18-30°C, with decreased activity at higher temperatures, contrary to predictions based on ectotherm physiology. Maximum velocity, acceleration, and detention percentage emerged as the most temperature-sensitive parameters. Our findings remark the importance of precise environmental control in cockroach locomotion studies and cyborg development. We discuss current limitations in stimulation methods, control interfaces, and power systems that must be addressed to enable practical search and rescue applications. This work provides a foundation for further research on environmental influences and locomotor control in cyborg cockroach systems.

**KEYWORDS:** *Gromphadorhina portentosa*, environmental control, locomotion, temperature, cyborg cockroaches

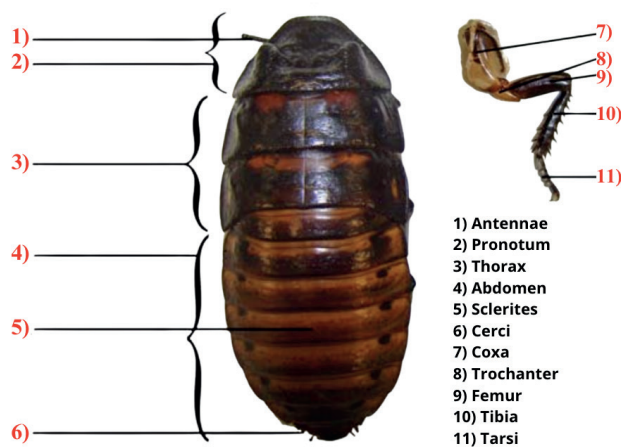
## 1. INTRODUCTION

The giant Madagascar cockroach (*Gromphadorhina portentosa*) is an insect endemic to the island of Madagascar and belongs to the order Blattodea and family Blaberidae. Its natural habitat lies in the understory of humid tropical rainforests,

where it hides among the dry leaf litter covering the forest floor. This species reaches a length of between 5 and 10 cm and is characterized by its robust, heavily sclerotized body and coloration, which ranges from light brown to black. Unlike most cockroach species, *G. portentosa* is wingless and produces a distinctive hissing sound, which it uses in social or defensive contexts (Monahan et al., 2023).

This species exhibits marked sexual dimorphism. Males can be identified by the presence of horn-like protrusions on the dorsal pronotum, as well as by their longer, thicker, and hair-covered antennae. They are also generally larger than females, which have the ability to internally incubate eggs; after a gestation period of approximately 60 days, the offspring are born as fully formed nymphs. These nymphs reach sexual maturity between five and seven months of age (Mulder and Shufran, 2016).

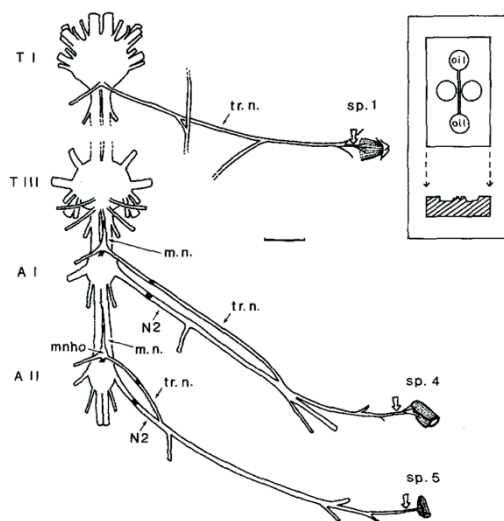
The anatomy of *G. portentosa* has been studied to establish a well-defined model for insect research (Heyborne et al., 2012). Fig. 1 illustrates the external anatomy of a male Madagascar hissing cockroach, which is representative of both sexes, as almost the same external anatomical features are present in females.



**Fig. 1.** External anatomy of the Madagascar hissing cockroach (Heyborne et al., 2012).

Hissing cockroaches also exhibit great social capabilities and can communicate through hissing sounds. The ability of *G. portentosa* to produce this characteristic sound is attributed to a structural modification in the trachea that connects spiracle 4 to the lateral longitudinal tracheal trunk, as illustrated in Fig. 2 (Nelson, 1979). Additionally, the diameter of the spiracle was found to influence sound production, with spiracle 4 exhibiting a significantly larger diameter than the others, thereby enhancing its role in acoustic signaling (Nelson, 1979).

As a result of an evolutionary anomaly, the fourth spiracle in *G. portentosa* underwent modification in its neural wiring, diverging from the primitive pattern observed in the other spiracles. This evolutionary adaptation also endowed the fourth spiracle with elongated muscles, enabling amplitude modulation and additional functions beyond its original respiratory role (Nelson, 1979).



**Fig. 2.** Dorsal dissection of the ventral nerve of *G. Portentosa*. Innervations of the first (thoracic), fourth and fifth (abdominal) spiracles (Nelson, 1979).

The evolutionary adaptation of the fourth spiracle of *G. portentosa* has led to its unique social capabilities. A study demonstrated that in aggressive interactions, the individual emitting the hiss was more likely to be the dominant and victorious male, suggesting a correlation between hissing and social dominance (Nelson and Fraser, 1980). In courtship contexts, hissing is a critical component of reproductive success. Muted males exhibited the lowest copulation rates, indicating that the absence of acoustic signaling significantly reduced their mating success. However, when courtship hissing was played back during interactions involving muted males, both their chances of copulation and female receptivity markedly increased. Furthermore, in normal males, hissing frequently occurs during copulation, reinforcing the conclusion that this sound plays an essential role in courtship communication and mating behavior (Nelson and Fraser, 1980).

In scientific research and education, Madagascar cockroaches have been used for various purposes. It is commonly employed in classroom settings because of its size and ease of handling. It has also been studied to test insecticides and investigate

their physiology, neurobiology, and social ecology. In recent years, it has become one of the primary species used in the development of “BioBots” or cyborg insects, owing to the relative ease with which electrodes can be inserted and electronic devices can be installed (Monahan et al., 2023).

Among the advantages of using hissing cockroaches over other invertebrates for research is the fact that, unlike most mammals and even other cockroach species, the Madagascar hissing cockroach is not subject to ethical, health, or environmental regulations, which makes it particularly suitable for experimental research. It has even been proposed as an alternative to traditional laboratory species commonly employed in pharmacological testing, such as rats or rabbits, for the evaluation of immune responses and virulence (Chua et al., 2017). Nevertheless, recent studies recommend the administration of anesthetics to sedate cockroaches and other invertebrates prior to experimental procedures, even in the absence of explicit regulatory requirements, as this practice helps to minimize the potential for pain (John, 2011).

Another advantage of using the Madagascar cockroach in experiments is that it is not considered a vector of contagious diseases in humans. Other cockroach species, such as the American cockroach, have been identified as vectors of concern in hospitals and clinics, capable of transmitting serious diseases to humans, including fungal infections caused by *Candida glabrata* and *Candida krusei* (Khodabandeh et al., 2020). The German cockroach is also noteworthy, as it is considered a vector of bacterial infections, such as *Pseudomonas* and *Staphylococcus* species (Menasria, 2014). Nevertheless, some fungal species have been identified in Madagascar cockroaches that could potentially cause allergies or illnesses in immunocompromised individuals (Yoder, 2008), although there is still insufficient evidence to classify this species as a public health risk.

However, Madagascar hissing cockroaches have been studied for purposes beyond laboratory experiments. Natural and human-made disasters pose persistent threats to public safety, particularly in conflict zones and high-risk areas. The infrastructural damage caused by such catastrophes often complicates search and rescue (SAR) operations, making them logistically challenging and financially demanding. Consequently, many survivors remain trapped beneath the debris, resulting in preventable loss of life. To address these challenges, recent research has explored the integration of robotic systems to improve the efficiency of SAR operations by reducing exploration and debris removal times, as well as by extending the range of accessible areas (Liu et al., 2007). Nevertheless, current robotic technologies still face significant limitations that hinder their practical implementation in real-world settings.

Thus, the main goal of developing these BioBots is to aid in locating people trapped under rubble following natural disasters such as earthquakes, tsunamis, or landslides. Owing to their small size, ability to adhere to various surfaces, relatively high speed, and low cost, cyborg cockroaches are particularly well-suited for operating in complex environments that require high mobility and access to narrow, dark spaces.

Cyborg cockroaches can be massively deployed following a catastrophe to search inaccessible areas and cover large zones. This swarm of cyborg insects can rapidly determine whether individuals are trapped under debris or in hazardous situations, significantly reducing the time required by human rescue teams during search operations.

However, developing these cyborg cockroaches faces several limitations that must be addressed before making them suitable for SAR operations. For instance, these cockroaches are extremely sensitive to environmental conditions, such as temperature, humidity, and light, which have been shown to significantly influence the locomotion of other ectothermic insect species (Abram, 2017). Specifically, “hot” thermal ecotypes of ectothermic insects, such as Madagascar hissing cockroaches, tend to perform better at higher body temperatures, exhibiting faster locomotion, lower sociability, and elevated activity levels (Michelangeli et al., 2017). Despite the fact that ectothermic insects tend to improve their locomotion at warm temperatures, extremely high temperatures can negatively affect them. For instance, *Porcellio laevis* has been shown to experience increased physiological stress when exposed to temperatures of 32°C, and prolonged exposure may lead to desiccation or, in some cases, induced coma (Bozinovic, 2016).

Locomotion is a critical factor in the development of cyborg hissing cockroaches, as it is intended to be regulated through stimulation signals or other electronic systems. Therefore, quantifying and understanding how different environmental conditions influence cockroach locomotion is essential for achieving effective control.

Thus, this study aims to synthesize the key findings on *Gromphadorhina portentosa* in the field of cybernetics, with particular emphasis on its application as a biorobotic platform for search and rescue (SAR) operations. Specifically, we examine how machine learning, signal processing, and data acquisition techniques have been employed across different studies to control the locomotion of cyborg cockroaches and how various environmental conditions influence their movement. In addition, this review discusses the current limitations of these approaches, outlines prospective research directions, and considers potential pathways for technological development, thereby providing a comprehensive overview of the state of the field. Finally, we present our contributions to the characterization of this species with respect to its cybernetic control under diverse environmental conditions.

The structure of this work is organized as follows. Section 2 presents the current state of the art regarding the use and development of *G. portentosa* as a biorobotic platform. Section 3 introduces our research on the characterization of *G. portentosa* under different environmental conditions and the development of our own motion-tracking and environmental control system, including preliminary results on cockroach stimulation in specific environments. Section 4 discusses the limitations of the current approaches, outlines future work, and proposes potential directions for further research. Finally, Section 5 summarizes the main findings and presents the conclusions of the study.

## 2. CURRENT RESEARCH

Research on *G. portentosa* as a cybernetic platform has focused on locomotion, neural stimulation, and control interface development. These avenues aim to (1) characterize the natural movement patterns of this cockroach species, (2) identify optimal stimulation parameters and interface mechanisms, and (3) design effective human-cockroach interfaces capable of directing or modulating the behavior of cyborg cockroaches. Notably, no published studies to date have employed robust and precise environmental control in their experimental procedures or thoroughly characterized cockroach locomotion under varying environmental conditions.

Another important line of research that has not been extensively explored is the energy supply for the electronic system carried by cockroaches. Electronic backpacks are often too heavy for cockroaches and require high amounts of power for operation; therefore, some researchers have focused on developing or finding efficient alternatives to commonly used power sources, such as LiPo batteries.

### 2.1 Locomotion

Most efforts to characterize the locomotion of *G. portentosa*, both in natural and stimulated conditions, rely on the use of inertial measurement units (IMUs) with multiple degrees of freedom combined with machine learning algorithms. In one study, for example, a single cockroach was equipped with an electronic backpack containing a CC2530 microcontroller, a five degree of freedom ADXL335 accelerometer, and a two degree of freedom LPY410ALTR gyroscope, enabling the automated classification of movement types through machine learning techniques (Cole et al., 2017).

No stimulation techniques were employed to control the movement of the Madagascar cockroach; instead, the study focused on analyzing its “natural” locomotion within a circular arena. Three key zones were identified in the arena: the periphery, transition zone, and central area. Cockroach movement was classified

into four categories: stationary, free movement (within the central zone), clockwise, and counterclockwise locomotion (Cole et al., 2017). Fig. 3 displays the key zones and their distance from the center. To monitor the cockroach position, a 30-fps webcam was used and synchronized with the data from the IMU sensor.

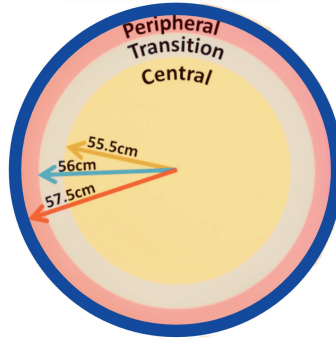


Fig. 3. Key zones of the circular arena, as proposed by Cole et al. (2017).

The results indicated that the best-performing classification model was a Support Vector Machine (SVM), using a dataset with 1.5-second segments and incorporating temporal, frequency, and wavelet-based features—a total of 117 features (Cole et al., 2017)

Another study developed an autonomous navigation system without external movement capture systems by integrating electronic backpacks that incorporated a distance Time of Flight sensor (ToF) VL53L0X and a Panasonic thermal infrared sensor AMG8833 of an 8x8 pixel matrix. In combination, both sensors were intended to detect human presence and obstacle proximity using machine learning to reduce stop times in the cyborg cockroach (Ariyanto et al., 2024).

Cockroaches were placed at the center of a green reference circle located within a maze constructed using wooden boards. This reference circle was positioned to evaluate the system's ability to prevent the cockroaches from hiding or remaining stationary in a corner of the maze. Data was transmitted via an electronic system directly connected to the computer (Ariyanto et al., 2024).

Four machine learning models were evaluated for human detection: random forest, decision tree, SVM, and K-nearest neighbor (KNN). Since initial tests showed that the random forest model yielded the best results, it was selected for implementation in the 32-bit microcontroller embedded in the electronic backpack (Ariyanto et al., 2024).

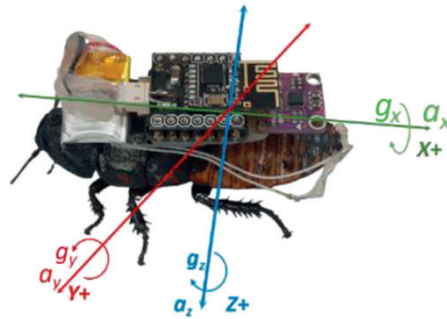


Fig. 4. Cyborg hissing cockroach carrying an electronic backpack equipped with a gyroscope and wireless transmitter, following electrode insertion into the cerci (Ariyanto et al., 2023).

The results showed that the cockroaches exited the maze in 150 s or less in 82.3% of the cases when the right antenna was stimulated and in 94.1% of the cases when the left antenna was stimulated. Human detection reached an accuracy of 92.5% at distances shorter than 25 cm and 70% at distances greater than 1 m. The improvement in locomotion becomes evident when considering that, prior to the system's implementation, the cockroaches failed to escape in 66% of the cases (with only a 34% success rate) (Ariyanto et al., 2024).

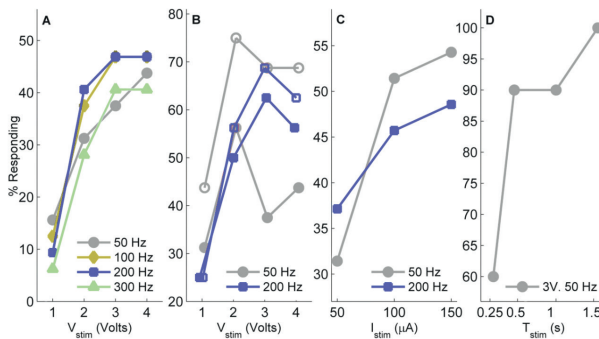
Trials have also been conducted to optimize movement by combining stimulation and machine learning. A study developed three cyborg cockroaches (BioBots), placed them in a circular arena, and used a machine learning algorithm to automatically stimulate them, aiming to reduce inactivity and undesired movements (e.g., escaping to the center). Each cockroach was equipped with a backpack containing an MPU9250 9-DOF motion sensor, BMP280 temperature and pressure sensor, 32-bit SAMD21G18 microcontroller, NRF24L01 transmitter, and 3.7V 50mAh rechargeable battery (Ariyanto et al., 2023). A 50 Hz square wave with a 50% duty cycle was used for stimulation, and a graphical interface in Python (Tkinter) was developed to control and monitor the system's performance. Fig. 4 displays one of the cyborgs hissing cockroaches with the implemented electronic backpack.

Among the models tested, the SVM yielded the best results, with an accuracy of 0.9985 for inactivity detection and 0.9864 for movement (Ariyanto et al., 2023). Performance improved significantly: the search rate increased by 68%, the distance traveled by 70%, and the inactivity time decreased by 78%.



## 2.2 Stimulation

To investigate the optimal stimulation parameters, such as signal amplitude, frequency, waveform, and other characteristics, a study inserted electrodes measuring 3 to 5 cm in length and 0.005 inches in diameter into the cerci and antennae of *G. portentosa*, with an additional electrode placed in the abdomen to serve as a ground (Erickson et al., 2015). During the experimental procedure, only one antenna was stimulated at a time to analyze the lateral response, while both cerci were stimulated simultaneously to induce escape behavior.



**Fig. 5.** Percentage of test subjects (cockroaches) that responded to different stimuli: A) monopolar voltage-varying signal, B) bipolar voltage-varying signal, C) current-controlled pulses, and D) different stimulation durations (Erickson et al., 2015).

Fig. 5 shows the cockroach responses to the different tested stimulation signals. It can be observed that, depending on the voltage, current, polarity, and stimulation duration, a greater number of test subjects (cockroaches) responded to the stimuli. For variable monopolar voltage signals, the highest response rate (41–47%) was achieved with voltages of approximately 3V and frequencies between 100 and 200 Hz. For variable bipolar voltage signals, the response rate was higher with voltages of 2V at 50 Hz (56%). With variable current signals, the best results were obtained at 150  $\mu$ A and 50 Hz. Finally, the optimal stimulation duration was found to be 1.5 s, yielding a 100% success rate (Erickson et al., 2015).

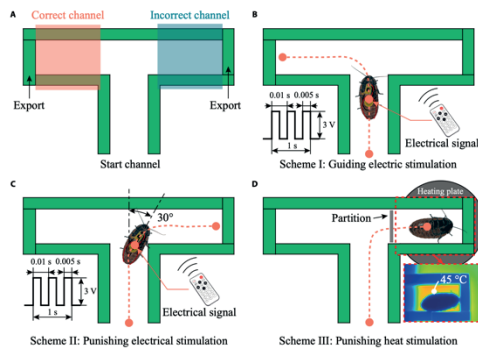
Nevertheless, statistical analysis revealed a weak correlation between the stimulation signals intended to induce locomotion and the actual movements. Both the Pearson correlation coefficients ( $r$ ) and the corresponding  $p$ -values ( $p$ ) were consistently low across all examined variables (Erickson et al., 2015). This limited statistical significance hinders the replicability of the findings and reduces their applicability in future research efforts.

Similarly, research has been conducted to examine how different electrical waveforms affect the locomotion of *G. portentosa* during its stimulation. Three types of stimulation signals were tested: two bipolar waveforms (square and nerve-like) and one monopolar square waveform. The amplitude and frequency parameters were kept constant across all experiments, with values set at 3.3V and 50 Hz, respectively, using a 50% duty cycle (Liu et al., 2024).

The experimental sessions lasted for 120 min, with four trials per signal type using different cockroaches. Each cercus received 10 one-second stimulations, spaced by five-minute rest intervals, followed by a 30-minute break between stimulation sets. In total, 12 cockroaches were used (Liu et al., 2024).

The effectiveness rates were 76.25%, 88.75%, and 96.25% for the monopolar square, bipolar square, and bipolar nerve-like signals, respectively. Although the bipolar square waveform was identified as optimal, the results suggest that the bipolar nerve-like signal provides greater effectiveness and finer control. Thus, the study's focus on the effectiveness-to-intensity ratio may have overlooked the broader value of controllability and stimulation quality (Liu et al., 2024).

Another study evaluated whether cerci stimulation in *G. portentosa* induced spatial learning and memory formation. For this purpose, a "T"-shaped maze was constructed to train the cockroaches, with each arm of the maze representing a possible choice (one designated as correct and the other as incorrect) (Yu et al., 2025). Fig. 6 displays the T-shaped maze and a visual illustration of the different experimental procedures used to test memory training.



**Fig. 6.** T-maze and visual methodology used to evaluate spatial learning in Madagascar hissing cockroaches: (A) T-shaped maze structure, (B) Method 1 – learning by guided stimulation, (C) Method 2 – punitive stimulation, (D) Method 3 – thermal punishment (Yu et al., 2025).

Three different training methodologies were proposed.

**Guiding stimulation:** Unilateral electrical stimulation of one cercus before the cockroach decided to steer it toward a specific choice.

**Punitive stimulation:** electrical stimulation was applied after selecting an incorrect option.

**Thermal punishment:** exposure to a heat source exceeding 40°C following an incorrect choice.

The experimental parameters were varied in terms of the stimulus frequency and memory retention. The stimulation frequency ranged from 10 to 100 Hz, and memory retention was assessed both in the short term (30 min) and long term (24 h). A total of 36 cockroaches were used: six for determining the optimal stimulation parameters and 30 for testing each training method (10 per method) (Yu et al., 2025).

Among the results, guiding stimulation proved to be the most effective in the short term, achieving a memory score of  $85.5 \pm 0.15\%$ , based on the average number of times the cockroaches chose the correct path in five T-maze trials after stimulation sessions. All 10 cockroaches in this group showed a preference for the correct path (Yu et al., 2025).

In the long-term evaluation, no significant differences were observed between guiding and punitive stimulation; only four out of ten cockroaches retained the correct preference in both groups. The least effective method overall was thermal punishment, which performed poorly in both short- and long-term assessments (Yu et al., 2025).

## 2.3 Interface development

There have also been attempts to introduce cyborg hissing cockroaches to the market for educational and entertainment purposes. *RoboRoach* was the first company to introduce them to the market in 2013, but it is not an open-source product (Stojnić, 2017). However, a study attempted to develop a brain-computer interface using SSVEP signals to control hissing cockroaches using the RoboRoach system.

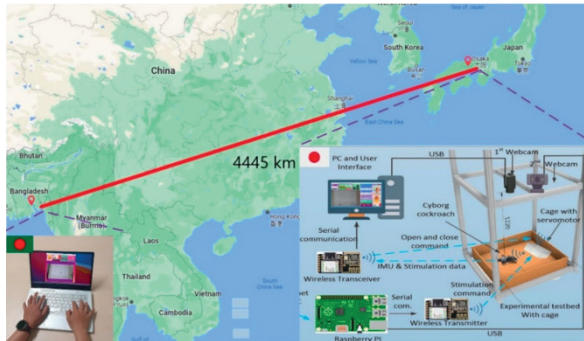
The brain-computer interface and stimulation system used to control the cyborg cockroaches demonstrated relatively high accuracy, achieving  $86 \pm 10.4\%$  and  $89.5 \pm 15\%$ , respectively (Li and Zhang, 2016). To assess the system's performance, an S-shaped maze was constructed, and two experimental methodologies were employed: (1) control evaluation, where a single participant directed the cockroach, and (2) an entertainment-based approach involving a competition between two participants, each controlling a cyborg cockroach (Li and Zhang, 2016).

The results of this study demonstrated the feasibility of using electroencephalographic (EEG) signals to control the cyborg cockroaches. However, the level of control remains quite limited, as the success rate was only 20% on the S-shaped track and approximately 40% on an obstacle course (Li and Zhang, 2016).

The implementation of cameras is also of great interest in cyborg cockroach research because they can help in SAR operations by detecting trapped humans in real time. A study implemented a high-resolution camera, an ESP32 microcontroller, and a battery of 125mAh (Rasakatla et al., 2022).

The stimulation electrodes were placed on both the antennae and cerci, with the abdomen serving as the ground connection. Experiments were conducted in a maze with obstacles to evaluate the navigation capabilities of the cockroach, demonstrating that the system was optimal and reliable. However, connectivity poses a significant limitation owing to the limited communication range, which causes interruptions in video streaming. Additionally, the weight of the electronic backpack overloaded the cockroach and reduced its mobility (Rasakatla et al., 2022).

Efforts have also been made to control cyborg cockroaches over long distances. One study developed a teleoperated user interface (UI) to monitor and control a cyborg cockroach between two different countries, Bangladesh and Japan (Ariyanto et al., 2022). Virtual Network Computing (VNC) technology was employed to facilitate teleoperation, enabling real-time video streaming, whereas radio communication was used locally to control the movements of the cyborg cockroach (Ariyanto et al., 2022).



**Fig. 7.** Cyborg cockroaches controlled over long distances (Bangladesh and Japan) using VNC technology and RFC (Ariyanto et al., 2022).

Fig. 7 illustrates the main stages of communication between the human operator in Bangladesh, the control station in Japan, and the cyborg cockroach. VNC communication was established between a computer located in Bangladesh and a Raspberry Pi 4 in Japan, with a user interface (UI) developed using the Tkinter library (Ariyanto et al., 2022). The experimental setup also included a webcam, wireless transceiver, servomotor, and computer, which were used to enable live video streaming and remotely open or close the cockroach containment gate. The

UI displays various elements, including stimulation parameters, live video feed, cockroach position and direction, and gate status (Ariyanto et al., 2022).

Five cyborg cockroaches were used in the experiments. The test involved placing an obstacle within the arena and instructing the teleoperator to guide the cockroach to avoid the obstacle and subsequently enter the containment area, at which point the gate would be opened to admit the cockroach. The stimulation signal used for navigation was a square waveform with a 50% duty cycle (Ariyanto et al., 2022). They successfully developed a teleoperation UI for controlling cyborg cockroaches over long distances with a delay of 0.275s, which is acceptable for these usages (Ariyanto et al., 2022).

## 2.4 Energy

Efforts to develop effective and reliable energy sources for cyborg cockroaches have been limited to date. However, only one study has investigated the use of solar energy as a sustainable power supply. Researchers developed an ultra-thin organic solar cell module mounted on the back of the cockroach, which generated 17.2mW of power using an area of 777 and a thickness of only 4. Under simulated sunlight conditions (100mW/), the module achieved an energy conversion efficiency of 7.96%. Following 30 min of exposure to simulated sunlight, the system could power the cyborg cockroach for over two hours using a 40mAh battery (Takei et.al, 2022).

## 3. CHARACTERIZATION UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

### 3.1 Environmental control and motion tracking system development

Given the noticeable lack of research on how different environmental conditions influence cockroach locomotion and its control as a cyborg, we have been working on characterizing the natural locomotor behavior of this species under varying conditions, with particular attention to temperature, as it appears to be the most influential factor for *G. portentosa* locomotion. To measure and study the influence of temperature on cockroach locomotion, a proper motion-tracking system and an environmental control chamber were developed.

Existing studies have employed circular arenas to monitor cockroach locomotion using different sensing technologies, including webcams, inertial measurement units (IMUs), or a combination of both (Ariyanto et al., 2023). Although effective to some extent, these systems have significant limitations. For instance, they often require large experimental spaces to allow unrestricted motion, depend on high-resolution video acquisition systems, or necessitate the attachment of relatively

heavy IMU-based backpacks onto the cockroach body. Each of these requirements introduces potential sources of bias, either by constraining the experimental design, increasing the cost, or altering the natural behavior of the insect due to the added mechanical load.

Alternative approaches, such as those proposed by Whitmire, who utilized Kinect-based tracking to monitor insect motion in circular arenas (Whitmire et al., 2013), address some of these issues by removing the dependence on high-resolution cameras and minimizing the need for invasive wearable sensors. Nevertheless, they still share a critical limitation with other circular arena systems: the large physical area makes it difficult to exert precise control over environmental variables such as light, temperature, and humidity. Moreover, in cases where IMU-based backpacks are used, the locomotor activity of the insect can be significantly influenced by the additional weight, as documented by Rasakatla et al. (2022).

A previous study proposed a motion-tracking system based on a frictionless polystyrene ball. The system employed two USB optical mice (Razer Spectre II, Carlsbad, CA) with a resolution of 1800 DPI and a sports ball cake pan equipped with five air ports. Compressed air was introduced through these ports to achieve semi-flotation of the polystyrene ball, allowing for near-frictionless movement (Erickson et al., 2015). This design was adapted from earlier systems described in previous studies (Lott et al., 2007; Hedrick et al., 2007).

The system operated by placing a cockroach on top of a polystyrene ball and securing it to a fixed support using Velcro. As the cockroach attempted to walk, it induced the rotation of the ball, which was tracked by two optical mice positioned orthogonally (i.e., at 90° relative to each other). The resulting movement data were sampled at a constant rate and recorded on a computer using LabVIEW, as described by Erickson et al. (2015), or using other software tools.

However, the system described by Erickson lacks the capability to control environmental variables and relies on proprietary, non-open-source software packages. This represents a significant limitation for subsequent studies that either aim to investigate the effects of environmental conditions on GP or lack access to the required software tools.

Moreover, the use of compressed air to generate a frictionless effect on the polystyrene ball requires costly, energy-intensive, and noisy components (such as an air compressor), which are generally undesirable and introduce an unnecessary layer of complexity to the system implementation.

To develop our own motion-tracking system, we adapted the design proposed by Erickson, addressing some of its reported limitations. For instance, instead of relying on the compressed air method to reduce friction, we implemented frictionless bearings that require no energy and generate less noise. Additionally, we replaced

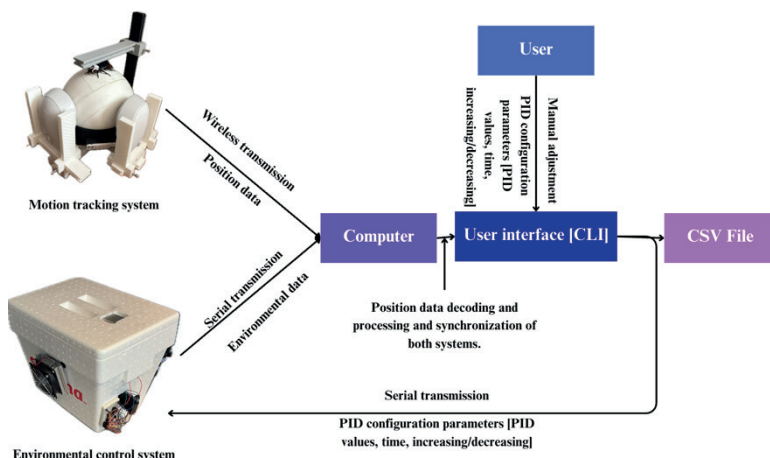
the polystyrene ball with a PLA 3D-printed ball, achieving a better fit within the structure and further reducing the friction. Finally, we 3D-printed lateral supports for both optical mice and a vertical support for attaching the cockroach to the center of the ball with Velcro. The entire structure is shown in Fig. 7.



**Fig. 8.** Motion-tracking system 3D-printed structure.

Because few environmental control systems are available for this type of experimental procedure, we designed one from the ground up. The system incorporates four environmental sensors: two GY-30 modules for measuring light intensity and two SHT45 sensors for monitoring the humidity and temperature. These high-precision sensors allowed us to accurately assess the environmental conditions within the polystyrene cage. Temperature regulation was achieved using two Peltier modules with fans, one dedicated to heating and the other to cooling, whereas an ESP32 microcontroller processed the sensor data and controlled the Peltier modules via PWM signals through a high-power H-bridge.

In combination, we were able to simultaneously track the locomotion of the cockroaches and control their surrounding environment during the experiment. Communication between the two systems is shown in Fig. 9. It can be observed that the synchronization between both systems is achieved through a command line interface (CLI) interface in Python running on a computer that receives the information of both optical mice and the environmental control system in parallel.



**Fig. 9.** Synchronization of both systems: motion tracking and environmental control through a CLI interface running on a computer.

## 3.2 Experimental design

For our experimental procedures, we randomly selected 42 hissing cockroaches from a colony kept at room temperature (between 24°C and 27°C) and fed only carrots and dog kibble. The only discrimination parameter was their length, as cockroaches below 5 cm were avoided because they were too young to place the Velcro and move the frictionless ball.

Prior to the experimental sessions, all subjects underwent an invasive procedure to prepare them for the movement monitoring system. This procedure involved gently filing the dorsal surface of the thorax and pronotum (Heyborne et al., 2012) using fine sandpaper. Once the attachment area was adequately exposed, a small amount of instant adhesive (commercial brand: Kola Loka) was applied, to which a Velcro fragment bearing a unique identification number for each individual was attached. This intervention was conducted with utmost care to minimize any physiological or behavioral alterations after manipulation. The highest ethical standards were rigorously observed to reduce induced pain as much as possible, along with post-intervention care to ensure survival and well-being.

Subsequently, the treated individuals were housed in groups of five within individual plastic containers, where they remained at rest for a minimum of 12 h. This rest period aimed to ensure physical recovery after the marking procedure and to prevent any potential effects caused by crowding or social stress, which could negatively influence locomotor behavior during testing, as documented in previous research (Clark and Moore, 1995).



Each experimental session lasted for approximately 25 min. This duration was established to encompass not only the initial acclimation phase of the subject to the new thermal conditions but also to allow sufficient time to record more stable and representative locomotor patterns of the animal. During the sessions, various relevant environmental variables (such as ambient temperature, illumination, and the individual's position within the environmental control chamber) were recorded in real-time. The sampling frequency was approximately 28 Hz, which was deemed adequate to accurately capture the locomotion of the test subjects.

To avoid cumulative adverse effects (such as physical fatigue, habituation to the experimental environment, or thermal stress), each individual was limited to a maximum of four sessions per day. Furthermore, a minimum two hour rest interval was established between consecutive sessions to allow for proper physiological recovery and passive re-acclimation to the baseline conditions of temperature and darkness in which the participants were originally maintained.

Experimental trials were carefully scheduled and randomly distributed throughout the day to minimize any potential confounding influence of circadian rhythms on cockroach locomotor performance, thereby reducing the risk of biased results. The role of circadian rhythms in cockroach locomotion behavior has already been demonstrated, with previous studies showing that cockroaches exhibit significantly enhanced locomotor activity during nocturnal phases (Simon et al., 2018; Satterly et al., 2023).

To further control for this factor, regardless of the specific time of day when the experiments were conducted, all sessions were performed under standardized low-light conditions. Illumination levels were consistently maintained below 30 lux, which ensured a stable visual environment and reduced the possibility of light-induced behavioral alterations.

This methodological choice was particularly relevant given that light intensity and photoperiod have been reported to influence locomotion patterns in cockroaches, at least in the species *Periplaneta americana* L. (Zhukovskaya et al., 2017). Consequently, the adopted design provided a robust framework for minimizing external temporal and environmental sources of variability, thus allowing a more reliable assessment of locomotor behavior under controlled experimental conditions.

From the constructed database, various parameters were derived to evaluate the locomotion of the test subjects under different temperature conditions. The primary and most fundamental parameters correspond to those employed in the system described by Erickson et al.. These parameters are velocity ( ) and gyro  $\omega$  ( ) calculated using equations 1 and 2 (Erickson et.al, 2015).

$$v(t) = \sqrt{\dot{y}_1^2 + \dot{y}_2^2} \quad (1)$$

$$\omega(t) = \frac{x_1 + x_2}{2 \cdot R} \quad (2)$$

Where  $\dot{y}_1^2$  and  $\dot{y}_2^2$  are the first derivative of each movement plane obtained by the central difference method and  $R$  is the radius of the polystyrene sphere (which is 6cm for this case). The acceleration was obtained by calculating the velocity derivative using the central difference method according to equation 3.

$$\alpha(t) = \frac{v_{i+1} - v_{i-1}}{2 \cdot \Delta t} \quad (3)$$

The maximum velocity and acceleration were computed using the `max()` Python function, considering the absolute values of the velocity and acceleration. The average acceleration and velocity were obtained using equations 4 and 5, respectively.

$$v_{prom} = \frac{1}{N} \sum_{i=1}^N v_i \quad (4)$$

$$\alpha_{prom} = \frac{1}{N} \sum_{i=1}^N \alpha_i \quad (5)$$

The total trajectory was determined using the Pythagorean theorem, considering two planes of movement, as shown in equation 6.

$$d = \sqrt{(x_{i+1} - x_i)^2 + (y_{i+1} - y_i)^2 + (x_{2,i+1} - x_{2,i})^2 + (y_{2,i+1} - y_{2,i})^2} \quad (6)$$

Another parameter of interest was the detention percentage, which was defined as the proportion of time during which the cockroach exhibited any movement. This parameter was calculated using equation 7, where  $N$  denotes the length of the distance vector obtained from equation 6.

$$\%detention = \frac{1}{N} \sum \{ \begin{matrix} d_{i+1} - d_i > 0, 0=0 \\ d_{i+1} - d_i \leq 0, 0=1 \end{matrix} \} * 100\% \quad (7)$$

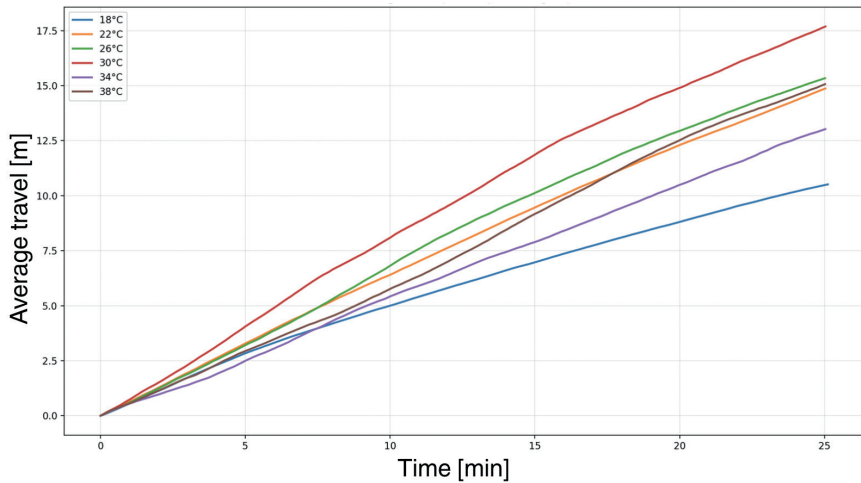
According to the previously described experimental design, a mixed-model approach was employed to evaluate the effects of temperature on the various locomotion parameters obtained. This statistical method was chosen because it enables the analysis of data in which the same subjects are assessed under different conditions (in this case, different temperature levels), thereby controlling for intra-individual variability and enhancing the statistical power and reliability of the findings.

After detecting significant overall differences, a post-hoc analysis with significance correction was performed to determine which specific pairs of thermal conditions exhibited statistically significant differences in the results. In this analysis, all variables derived from the processing of locomotion data, such as tortuosity, maximum velocity and acceleration, average velocity and acceleration, angular turn, total distance traveled, and percentage of time in detention, were considered dependent variables.

The results obtained from the statistical analysis were graphed as a heatmap with every parameter and comparison group. Box plots were also generated to graphically visualize the most relevant statistical information from each temperature group and parameter.

### 3.3 Results and discussion

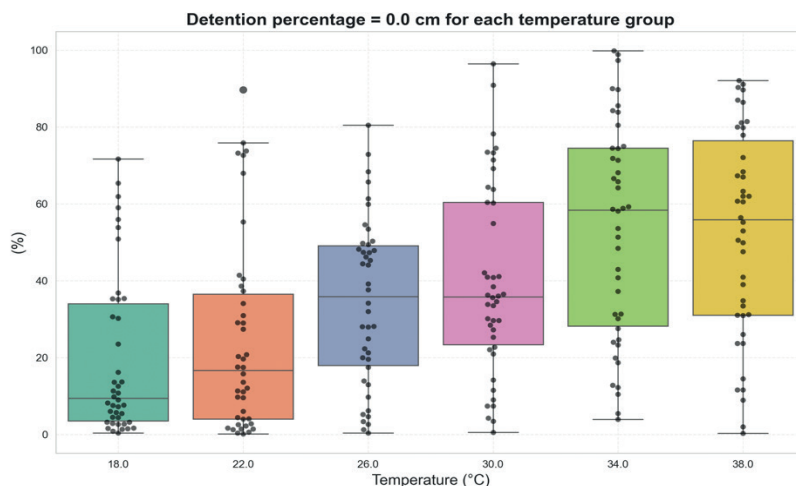
As shown in Fig. 10, the average travel distance per temperature group was the highest at 30°C compared to the other temperatures. It is also noticeable that the average travel increases significantly between 18 and 30°C; beyond this interval, the travel begins to decrease, showing a less linear pattern.



**Fig. 10.** Average group travel for each temperature.

From our results, the maximum velocity exhibited a more linear trend than the total travel, with the lowest value observed at 18°C and the highest at 38°C. There was a significant increase in the maximum velocity between 18 and 30°C, whereas the differences between 30 and 38°C were minimal. The same pattern is not observed for the average velocity, as a linear increase occurs only between 18°C and 26°C, whereas beyond 30°C, the average velocity decreases significantly.

The detention percentages shown in Fig. 11 indicate that the 18 and 22°C groups had the lowest stop times, whereas the 34 and 38°C groups exhibited the highest stop times. This suggests that lower temperatures promote more continuous and linear movement, whereas at higher temperatures, cockroaches are more likely to remain stationary.



**Fig. 11.** Detention percentages for each temperature group.

The outcomes derived from the mixed-effects models are shown in Fig. 12. Panel (A) illustrates the estimated coefficients on a logarithmic scale, whereas panel (B) presents the corresponding p-values. This representation provides a comprehensive overview by simultaneously displaying the magnitude of the estimated effects and the statistical evidence supporting them.

The outcomes derived from the mixed-effects models are shown in Figs. 12 and 13. Fig. 12 illustrates the estimated coefficients on a logarithmic scale, whereas Fig. 13 presents the corresponding p-values. This representation provides a comprehensive overview by simultaneously displaying the magnitude of the estimated effects and the statistical evidence supporting them.

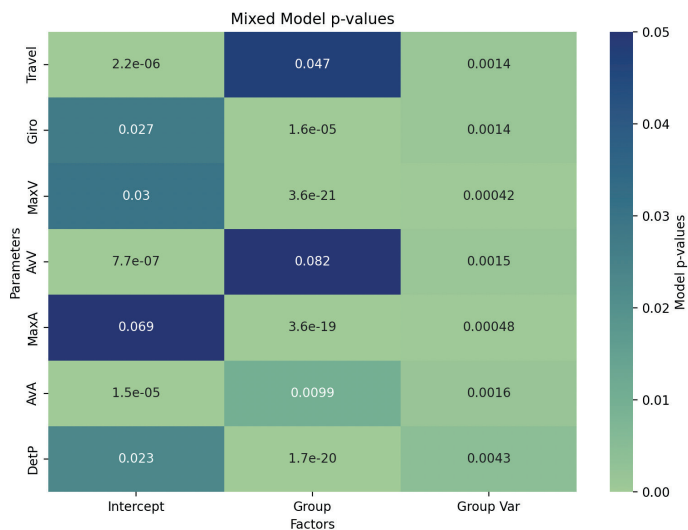
The coefficient distribution revealed that maximum acceleration, mean acceleration, maximum velocity, and explored area exhibited the most pronounced effect sizes, indicating that these locomotor parameters are particularly sensitive to thermal variation. The magnitude of these coefficients underscores the relevance of acceleration- and velocity-related measures as primary indicators of environmentally induced changes in locomotor performance.

Maximum acceleration, mean acceleration, maximum velocity, detention percentage, and giro were significantly associated with temperature groups. In contrast, travel approached the conventional significance threshold ( $p < 0.05$ ) but remained near the boundary of the null hypothesis, suggesting a minimal effect of temperature. The average velocity, explored area, and tortuosity did not achieve statistical significance, implying that these metrics remained comparatively stable across thermal conditions and were less responsive to temperature-induced effects.



Fig. 12. Logarithmic scale coefficients obtained from mixed models.

After performing the mixed models, a post-hoc analysis was performed to determine the temperature effects on each parameter among the groups. The resulting p-values are visualized as a heatmap in Fig. 14. This analysis revealed that the most statistically significant parameters were maximum velocity (MaxV), maximum acceleration (MaxA), and detention percentage (Det.P). Other parameters, such as total travel, average velocity, and acceleration, were also significant, but only within the 18–30°C range. Notably, none of the parameters reached statistical significance at higher temperatures (30–34, 34–38, and 30–38°C), indicating a clear breakpoint at 30°C. Most of the significant differences were observed between low and high temperatures.



**Fig. 13.** P-values obtained for each parameter from mixed models.

Although maximum velocity and detention percentage appear to be the most statistically significant locomotion parameters, travel distance and acceleration also play a notable role in cockroach locomotion at temperatures below the suggested breakpoint of 30°C. This observation has important implications for the ectothermic hypothesis in *G. portentosa*, as it suggests that this species does not enhance locomotion at substantially high temperatures, contrary to previous reports. In fact, locomotion seems to be positively influenced only within the temperature range of 18–30°C; beyond this breakpoint, locomotion not only fails to improve but also declines in most parameters, including velocity, acceleration, and overall movement, as indicated by an increase in the detention percentage. One possible explanation for this pattern is analogous to that proposed for *Porcellio laevis*: cockroaches may experience discomfort or pain at temperatures above 30°C, leading to reduced locomotor activity. Alternatively, the opposite hypothesis could apply at higher temperatures; the cockroach may feel more comfortable, reducing its motivation to escape or move actively within the experimental setup.

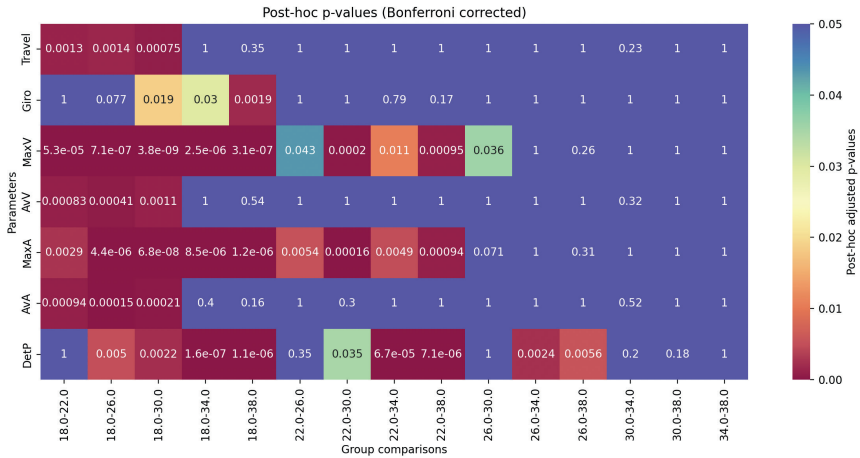


Fig. 14. P-value heatmap after mixed models and post-hoc analysis.

## 4. LIMITATIONS AND FURTHER WORK

Our results indicate that cockroach locomotion behavior does not entirely conform to the predictions of the ectothermic hypothesis. The precise mechanisms underlying the observed decrease in locomotion beyond 30°C remain unclear, and further research should aim to determine whether these effects are driven by psychological responses, physiological changes or unconsidered environmental variables. Additionally, the potential influences of population factors such as sex, body length, age, and nutritional status were not accounted for in this analysis and therefore remain unknown. Nevertheless, these population variables do not appear to exert a significant effect on cockroach locomotion; at least within the conditions tested, they seem insufficient to counteract the impact of temperature, suggesting that their influence on locomotor behavior is likely to be minimal.

Although our system is effective in controlling temperature and light conditions and tracking cockroach locomotion, it is limited in its ability to regulate other relevant parameters, such as noise, vibrations, and humidity. Therefore, future investigations should consider these factors and seek to enhance the proposed system to incorporate their control. Research on the potential influence of sound on this cockroach species is scarce, aside from studies on their characteristic hissing; however, external noise may still affect behavior by inducing alert responses. Humidity also appears to be an important factor in cockroach locomotion, although its influence does not seem as critical as that of light or temperature. However, future studies should examine whether this parameter exerts measurable effects. In contrast, vibrations are not expected to directly influence *G. portentosa* behavior but could interfere with the

motion-tracking system. Therefore, implementing stabilizers or isolation mechanisms is advisable to ensure that recorded signals accurately reflect cockroach movement rather than external mechanical disturbances.

Regarding the current literature, further developments are required in control interfaces, stimulation parameters and methods, as well as in the physiological and behavioral characterization of cockroaches, in order to achieve real-life applications of cyborg hissing cockroaches.

Among these areas, the control interfaces appear to be the most developed. However, they may also be the least significant at this stage, as their full potential will only be realized once the other fields reach a comparable level of maturity.

In contrast, stimulation methods represent the least developed area of research. Although considerable improvements have been made in the design of electronic backpacks for cockroaches, very limited progress has been made in refining the stimulation signals or methods. For example, only two studies identified effective stimulation parameters, and none incorporated DACs or other electronic components that could enhance the signal quality and precision.

Motion tracking can be considered the second most developed field, as there are already some proposals aimed at achieving standardized systems. Nevertheless, this study has some important limitations. Video-tracking approaches require very large experimental arenas, onboard IMU-based systems face challenges in energy efficiency, and optical mouse-based methods, such as those proposed by Erickson and implemented in our own system, still require improvements in terms of stability.

## 5. CONCLUSIONS

As highlighted in the current state of the art, the control of hissing cockroaches using stimulation signals and novel interfaces remains in the early developmental stage. In recent years, there have been no substantial advances in improving cockroach control, and the currently available approaches are insufficient for real-world applications. Moreover, certain research avenues, such as studies on hissing cockroach social interactions and physiological processes, have been largely neglected over time, with the most significant contributions dating back to several decades. More effort must be made to develop these fields before considering deploying cyborg cockroaches during SAR operations, as current advances only allow minimum control over cyborg cockroach locomotion, and the interfaces and power sources are still inefficient for real-time streaming, as they face many communication problems as well as short-time battery duration.



Our approach to investigating the effects of temperature on cockroach locomotion is only the first step in a long process of characterizing *G. portentosa*. This is perhaps the most important path to follow, as it is necessary to understand the most relevant parameters that affect locomotion and behavior to achieve efficient control, even under varying external conditions or with specimens with high behavioral variability.

Our results emphasize that temperature plays a decisive role in hissing cockroach locomotion. Therefore, future studies must ensure that this parameter is carefully controlled within the experimental conditions. This will help guarantee that the results obtained truly reflect the variables of interest rather than uncontrolled temperature effects.

Moreover, as environmental factors have been shown to significantly influence cockroach behavior, future research should also explore the impact of other variables, such as light, humidity, and noise. A comprehensive understanding of how these external conditions affect cockroach behavior and locomotion is essential, as controlling them may ultimately represent the most critical step in ensuring the reliability and consistency of experiments.

Additionally, the statistical analysis demonstrated that the ectothermic hypothesis regarding *G. portentosa* is not as previously assumed. Temperature has a positive effect on cockroach locomotion, but this effect appears to be restricted to the range of 18–30°C. Beyond this point, higher temperatures not only fail to enhance locomotion but also lead to a decline.

This distinctive response may explain the discrepancies observed in previous studies. Experimental procedures conducted at temperatures close to 30°C may have benefited from increased locomotor activity, whereas those performed at temperatures below approximately 26°C may have been negatively affected by reduced activity levels.

Therefore, future research on *G. portentosa* locomotion should incorporate more precise and comprehensive environmental controls, at least for studies specifically addressing locomotion, to ensure accurate and comparable results.

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