



# Ant-observer: A new approach for automatic acquisition and autonomous analyses of individual species abundance and interactions

Jiaxin Hu<sup>\*</sup>, Taylor A. Bogar, Yi-Fei Gu, Benoit Guénard

School of Biological Sciences, The University of Hong Kong, Hong Kong, SAR, China

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## ABSTRACT

Traditional methods, such as direct observations or manually photographing bait stations, are limited in both data collection efficiency and accuracy. Consequently, they fail to capture the dynamic changes and complex relationships between ant species. We developed a novel system as a potential solution that combines processed consumer-grade camera equipment and software that acquires data needed for in situ experiments. This software features a user-friendly graphics user interface (GUI) for data collection and post-hoc analysis from videos captured by the camera equipment. Researchers can use this system to (1) count species abundance per frame; (2) calculate species speed; (3) detect ant interactions; and (4) count the abundance of different subcastes within species on baits. To demonstrate the complete workflow, we applied this system on ant videos collected in Hong Kong. Practical guides (supplement materials) detailing suggestions for camera equipment and software operations in both field and laboratory studies are provided. We present the results of ten in situ experiments of ants (each lasting 1–1.5 h) conducted in Hong Kong through processed consumer-grade camera equipment, with videos analyzed using the newly developed software. The analysis captured a range of variables related to ant foraging behavior, including resource discovery time, nestmate recruitment rate, and interspecific interactions. Our results confirm that the developed software provides an integrated and efficient approach for data collection, extraction, and analysis in in situ experiments, enhancing the capabilities of insect ecology research.

## 1. Introduction

Interspecific competition is considered one of the main driving forces shaping community structure and ecological dynamics, with important implications for determining species diversity and composition (Denno et al., 1995; Kaplan and Denno, 2007; Schoener, 1983; Tokeshi, 2009). At the community level, ecologists have been devoted to explore the mechanisms underlying species coexistence through the lens of the competitive exclusion principle (Achury et al., 2020; Connor and Simberloff, 1979; Roughgarden, 1983) and yet, in many communities more species appear to coexist than can be accounted for by the number of limiting resources (Laird and Schamp, 2008). This has led to considerable debate on the role that interspecific competition plays in shaping communities (Lyu and Alexander, 2022; Nottebrock et al., 2017). In this context, dominance hierarchies have become one of the dominant paradigms in ant ecology, but their consistency is limited (Stuble et al., 2017). Therefore, exploring more effective ways to document ant behavior may help reduce these limitations and provide a more comprehensive understanding.

Ants are an ideal model organism for studying interspecific competition. Their high abundance and ubiquity within most terrestrial ecosystems (Schultheiss et al., 2022; Wilson, 1987) and impressive species diversity with over 15,900 species and subspecies globally (Bolton, 2024) make them particularly suitable to address such ecological questions. In addition, ants occupy and exploit various ecological niches and habitats, sculpting the biodiversity of many other organisms through their interactions and diverse ecological roles they play (De Castro Solar et al., 2016; Parker and Kronauer, 2021). Meanwhile, ant ecological research is anchored in understanding ant communities through the emergence of interspecific competition for territories, food resources or nest sites among other factors (Neumann and Pinter-Wollman, 2022; Warren et al., 2020). Through the study of ant ecology, we can systematically identify and comprehend ant coexistence mechanisms, and thus understand the impact of interspecific competition on community structure, dynamics or disruption (Hart et al., 2017; Kaplan and Denno, 2007; Loreau and De Mazancourt, 2013; Wong et al., 2021; Wong et al., 2022).

Baiting has been commonly adopted for collecting data on insects,

<sup>\*</sup> Corresponding author.

E-mail address: [hujiaxin1206@126.com](mailto:hujiaxin1206@126.com) (J. Hu).

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Table 1

Research questions using baiting in multiple studies with summary of methods used for extracting data, with a focus on studies published in the past 5 years (2018–2023), but also including historical studies.

Research focuses	Observation Methods
Foraging ant collection (Gutiérrez et al., 2020; Wang et al., 2001; Warren et al., 2023)	Manual recording and collection performed at regular intervals
Ant food exploitation (Lach et al., 2019; Youngsteadt et al., 2023)	Manual recording and collection performed at regular intervals
Species prevention and control (Oi et al., 2022; Sunamura et al., 2022)	Manual recording and collection performed at regular intervals
Ant competition (Achury et al., 2020; Dáttilo and MacGregor-Fors, 2021; Feener Jr et al., 2008; Fellers, 1987)	Manual recording and collection performed at regular intervals
Behavioral dominance (Antoniazzi et al., 2021; Lebrun and Feener Jr, 2007; Lee et al., 2021; Warren et al., 2023)	Manual recording and collection performed at regular intervals
Ant competition (Video camera continuous recording)	Laboratory (60 h) (Cordonnier et al., 2020); Laboratory (Trigos-Peral et al., 2021); Field (84 h) (Warren et al., 2020);
Ant food exploitation (Video camera continuous recording)	Laboratory (Armbrrecht and Gallego, 2007); Laboratory and field (Cabrera et al., 2021)
Ant behavior (Video camera continuous recording)	Field (6 h) (Gray et al., 2018); Laboratory (Larabee and Suarez, 2015); Field (7.5 h) (Pearce-Duvet et al., 2011)
Species prevention and control (Video camera continuous recording)	Field (Castracani et al., 2023); Laboratory (Currie and Stuart, 2001)

especially for studying ecological interactions, food resource acquisition, and ecosystem functions (e.g., myrmecochory) within ant communities (Wang et al., 2001). This method utilizes a standardized observation area (e.g. white dish or white background) for quantifying the number of ant individuals (i.e. ant abundance) and characterizing competitive interactions among ants on shared resources (Hölldobler and Wilson, 1990). Despite its common use, many studies relying on baiting often collect interaction data through images or human observations across multiple bait stations checked at different time periods (Oi et al., 2022; Wang et al., 2001; Warren et al., 2023; Youngsteadt et al., 2023). The practical limitations of time constraints and human involvement, however, often result in data being collected for a few seconds every 5 to 10 min per bait station, leading to numerous interactions being overlooked, low resolution in abundance variation and potentially inadequate estimation of species abundance (Table 1). Consequently, such baiting practices may yield scarce photographic datasets that fail to capture the full spectrum of occurred interactions, and thus hamper the in-depth ecological information needed for ant studies.

Some researchers have implemented video cameras to address the data scarcity issue as videos can provide a comprehensive view of ant interactions. These studies, however, are limited to either controlled laboratory conditions (Cordonnier et al., 2020; Larabee and Suarez, 2015; Trigos-Peral et al., 2021) or dealing with small sample sizes in field conditions, due to heavy and fragile filming equipment (Castracani et al., 2023; Pearce-Duvet et al., 2011). Lightweight and low-cost camera equipment is often unable to achieve the resolution and clarity needed to photograph insects, especially for small organisms like ants. Moreover, handling large video datasets can be challenging since most data are processed through manual extraction from the videos. Such processing is a lengthy task that may necessitate the participation of many individuals (e.g., 17 trained undergraduate students in Modlmeier et al., 2019), potentially leading to inconsistencies and biases in the dataset.

In recent years, computer-assisted video analysis has been applied in video processing to reduce the burden of manual data analysis (Caci et al., 2013; Fonio et al., 2016; Gao et al., 2024; Gelblum et al., 2015). Notable advances such as obtaining the movement trajectory of ants and detecting ant individuals have been achieved (Gal et al., 2020; Haalck

Table 2

Function comparison between other software and the proposed Ant-Observer.

Software/reference	Research Focuses	Differences from Ant-Observer
DeepLabCut (Mathis et al., 2018)	Deep neural network-based transfer learning. This allows the application for knowledge gained by the model on a previous task to improve the generalization ability of another task. Excellent results can be obtained with very little training data.	Not applicable for counts of ants aggregated and layered on baits. Only applicable to a small amount of field data or laboratory data
AntTracker (Sabattini et al., 2023)	AntTracker can segment individual ants, track their movements, and classify whether they are carrying leaves/loads using a convolutional neural network	Not applicable for counts of ants aggregated and layered on baits. Unable to discriminate intraspecific size differences.
AntVis (Hu et al., 2020)	AntVis is a tool for exploring ant movement data collected from the video recording of ants moving on tree branches	Not applicable for counts of ants aggregated and layered on baits.
Imirzian et al. (2019)	Develops deep learning-based computer vision algorithms to track foraging ants frame-by-frame through video footage captured under natural conditions on the tropical forest floor at night.	Not applicable for counts of ants aggregated and layered on baits. Unable to discriminate intraspecific size differences.
CATER (Haalck et al., 2023)	Combines an unsupervised probabilistic detection mechanism with a globally optimized environment reconstruction pipeline that enables precise quantification of behavior in natural environments.	Not applicable for counts of ants aggregated and layered on baits. Focuses on tracking individual species rather than producing detailed abundance counts of large numbers of ants in the field.
Silva et al. (2023)	Method to understand the abundance and distribution of ants in colonies based on convolutional neural networks.	Not applicable for counts of ants aggregated and layered on baits. Unable to discriminate intraspecific size differences.
Cao et al. (2020)	Uses an online multi-object tracking (MOT) framework to track individual ants efficiently and with high accuracy.	Not applicable for counts of ants aggregated and layered on baits. Only applicable to a small amount of field data or laboratory data
AntCounter (Bustamante and Amarillo-Suárez, 2016)	This program estimates ant abundance by monitoring their movement in and out of the nest entrance, including individuals entering and leaving the nest.	Not applicable for counts of ants aggregated and layered on baits. Unable to discriminate intraspecific size differences.
anTraX (Gal et al., 2020)	Algorithm and software package for high-throughput video tracking of color-tagged insects.	Not applicable for counts of ants aggregated and layered on baits. Focuses on tracking the paths of limited swarms of insects, rather than the number of insects that gather while foraging in the field.
Tracktor (Sridhar et al., 2019)	Image-based tracking freeware designed to perform single-object tracking in noisy environments or multi-object tracking in unified environments.	Not applicable for counts of ants aggregated and layered on baits. Only applicable to a small amount of field data or laboratory data
ToxTrac (Rodriguez et al., 2018)	Trox is an open-source executable software for image-based tracking of multiple organisms in the	Not applicable for counts of ants aggregated and layered on baits. Only applicable to a small

(continued on next page)

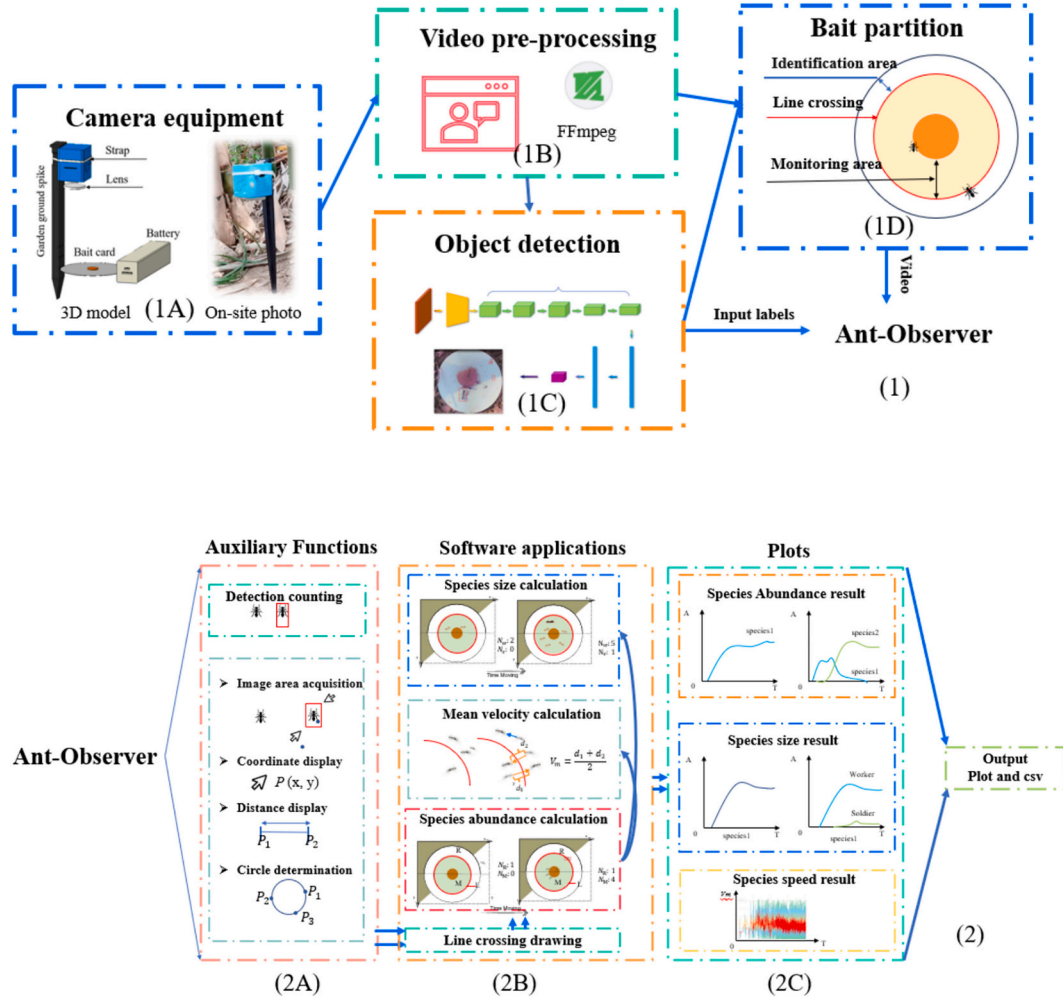
**Table 2** (continued)

Software/reference	Research Focuses	Differences from Ant-Observer
	laboratory. The software is easy to use, flexible and powerful, capable of handling multiple species and quickly analyzing data	amount of field data or laboratory data

et al., 2023), yet fall short of fully meeting the needs for in situ ant studies (see Table 2). When using food bait to attract social insects like ants, it often leads to individuals aggregated around the baits, sometimes with two to three layers of workers. Such scenarios pose challenges for detection algorithms to identify clustered individuals, potentially resulting in erroneous estimation of ant abundance. To circumvent such problems, some studies have estimated numbers using a ranked scale (e.g. 0 = 0 ants; 1 = 1 ant; 2 = 2–5 ants; 3 = 6–10 ants; 4 = 11–20 ants; 5 = 21–50 ants; 6 ≥ 50 ants) to represent the relative abundance of species rather than actual counts (Griffiths et al., 2018; Parr et al., 2016). Although existing ant counting software, such as AntCounter and other programs (Bustamante and Amarillo-Suárez, 2016; Silva et al., 2023), has been developed to streamline the

traditional manual processing, their primary focus remains on laboratory data rather than field applications. Expanding computer-assisted video analyses to field data can provide additional benefits in processing comprehensive ecological data. For instance, it enables simultaneous extraction of multiple ecologically relevant data per frame, including species discernment, recruitment duration, the number of recruits, and ant moving speed, facilitating continuous observation over a specified period. Therefore, an operating system that concurrently collects and analyzes in situ data with minimal interventions and at high efficiency and accuracy is imperative for ecologists.

In order to solve the above problems, we developed a software with an intuitive interactive interface that allows to post-analyze the data obtained from existing object detection in order to efficiently extract and present relevant information from videos. The camera equipment requires a specially processed wireless camera that is focused on the bait station to record data (Appendix 3). The software is used to analyze video taken by the hardware, with minimal manual operations for data collection required for in situ experiments in field and laboratory environments. Here, we hypothesize that our system will have the following advantages over traditional methods: 1) Utilizing our approach will mitigate cost of experimental equipment 2) This method will increase sampling convenience and increase quantity of acquired data, and 3)



**Fig. 1.** (1) Workflow of video pre-processing. (1A) Demonstration of camera equipment: the left side shows a 3D model of the equipment, and the right side shows actual photos of the equipment in the field. (1B) Video pre-processing: Batch preprocessing of video sets using FFmpeg (Appendix 2). (1C) Object detection: Performs object detection on the video to obtain 'labels file' (Appendix 2). (1D) Bait partition: set the parameters of software 'linecrossing' according to the bait settings. (2) The software workflow used to extract and calculate information about ant behaviors and characteristics from field video. (2 A) Auxiliary functions: four different auxiliary functions to help users configure 'linecrossing' more easily. (2B) Software applications: functions that can be realized by the software. (2C) Plots: software to plot the results.

**Table 3**

Specifications of the analyzed videos. The label is the tag assigned to the video. The sampling location consists of the site name and associated coordinates. Species name provides the identity of the species observed foraging and interacting at the bait station. The center of the bait card on the video was determined by using the Ant-Observer auxiliary function 'Circle determination'. The best 'line crossing' radius was selected using the least squared method for the best estimation of ant abundance.

Label (Video)	Sampling Location	Coordinates (lat./long.)	Species names	Circle center	Statistics	Best line crossing radius
TC2by22s05 (Video 1)	Tung Mui Ancient Trail	22.29, 113.96	<i>Pheidole taipoana</i> / <i>Diacamma</i> sp.	0.50,0.53	Mean	0.38
TC2b22s10 (Video 2)	Tung Mui Ancient Trail	22.29, 113.95	<i>Aphaenogaster</i> sp.	0.50,0.56	Median	0.32
TC2b22s01 (Video 3)	Tung Mui Ancient Trail	22.29, 113.95	<i>Meranoplus bicolor</i>	0.53,0.54	Median	0.34
TC2b22s14 (Video 4)	Tung Mui Ancient Trail	22.29, 113.95	<i>Pheidole pيلي.</i> / <i>Odontoponera denticulata</i> .	0.50,0.51	Median	0.36
TC2fy22s13 (Video 5)	Tung Mui Ancient Trail	22.29, 113.97	<i>Pheidole elongicephala</i> cf. / <i>Tapinoma</i> sp.	0.51,0.60	Median	0.38
TC1b22s14 (Video 6)	Tung Mui Ancient Trail	22.29, 113.95	<i>Meranoplus bicolor</i>	0.51,0.5	Median	0.4
TC1b22s04 (Video 7)	Tung Mui Ancient Trail	22.29, 113.95	<i>Pheidole pيلي.</i>	0.5,0.37	Mean	0.345
TC2b22s12 (Video 8)	Tung Mui Ancient Trail	22.29, 113.95	<i>Pheidole pيلي.</i>	0.52,0.5	Median	0.41
TC1b22s07 (Video 9)	Tung Mui Ancient Trail	22.29, 113.95	<i>Pheidole pيلي.</i>	0.48,0.56	Mean	0.42
TC2b22s16 (Video 10)	Tung Mui Ancient Trail	22.29, 113.95	<i>Pheidole pيلي.</i>	0.50,0.51	Median	0.4

Will reduce the computation and time needed to collect data. So, predictions will be 1) it is cheaper than other previously existing methods, 2) it can collect more data in less time than traditional methods.

## 2. Materials and methods

### 2.1. Camera equipment

The camera equipment (Appendix 3) was selected to be rugged and lightweight (83 g), ensuring reliable operation throughout the field monitoring period (Fig. 1A). Each camera setup cost approximately \$31 USD (Appendix 3) and consists of three main modules: the imaging module, the power supply module, and the fixing module. These components, detailed further in Appendix 3, minimizes potential technical failures and facilitate deployment and operation under challenging field conditions.

2.2 Video pre-processing.  
We selected the SQ23 camera (Appendix 3) as the video capture device based on cost-effectiveness. The camera is capable of generating a video file in AVI format with a resolution of 1260\*1080 pixels and a frame rate of 25 FPS at 5-min intervals during continuous shooting. To achieve high-accuracy analysis of the underlying data information of the video, we performed the following pre-processing steps before inputting the video into the software.

#### 2.1.1. Video converting and resizing

We used the open-source software FFmpeg (<https://ffmpeg.org/>) to convert and resize the video file, in order to avoid the impact of camera shooting direction (i.e., horizontal or vertical) and to ensure that the bait card occupies as much of the video frame as possible. We first batch merged and converted the video files from AVI to MP4, and then cropped the largest square from the rectangular video frame while aligning the center of the video before and after processing. The resolution of output videos was uniformly converted to 1080 × 1080 pixels (or any other 1:1 resolution).

#### 2.1.2. Ant individual detection and classification

Our software focuses on the post-hoc analysis of the data that the user obtains from the object detection model. Before using our software for in-depth analyses of video data, users are required to preprocess the format-converted and size-adjusted video using an object detection algorithm. The YOLO v5 (You Only Look Once – Real Time Object Detection System: <https://github.com/ultralytics/yolov5>) algorithm is recommended for this software because it is able to accurately locate the pixel positions of the target ants and identify their classes (label file). After the user applies the object detection algorithm to analyze the video, the software will further process the detected data identified by the algorithm (for more information on input files [label files and video formats], see Appendix 2). In particular, it collects data about ants on the bait station by post analyzing the results of object detection, and then calculates ant abundance and ant speed in and out of the 'line

crossing'.

### 2.2. Ant video sampling

Bait stations were installed in the Hong Kong area in April 2022 at forest edge or in peri-urban areas. Each bait consisted of a slice of honey chicken sausage (~ 20 mm in diameter and 2 mm in height) and a white plastic dish (6 cm in diameter) flushed with the ground. The white plastic dish provided an ideal backdrop for observing and recording the ants' behavior, and this setup method helped to reducing interferences and improve the accuracy of data collection. Camera equipment was installed next to each bait station to continuously record videos for one hour. We selected 10 videos (~20 GB, see Table 3) to test and demonstrate the functionality of the Ant-Observer software.

### 2.3. Software workflow

Although the camera equipment captures real-time information from bait stations as videos, this large-scale data requires a lot of time and effort for processing and analysis. To streamline these tasks, we have developed a user-friendly software with a standalone graphics user interface (GUI) to automate data processing. Four functions were designed to reduce human interventions and workload (Fig. 1).

#### 2.3.1. Auxiliary functions

Four auxiliary functions have been developed to optimize data processing efficiency and user experience, including coordinate display, distance display, circle determination, and image area acquisition. The detailed auxiliary function descriptions are available in Appendix 2.

#### 2.3.2. Calculation of insect abundance

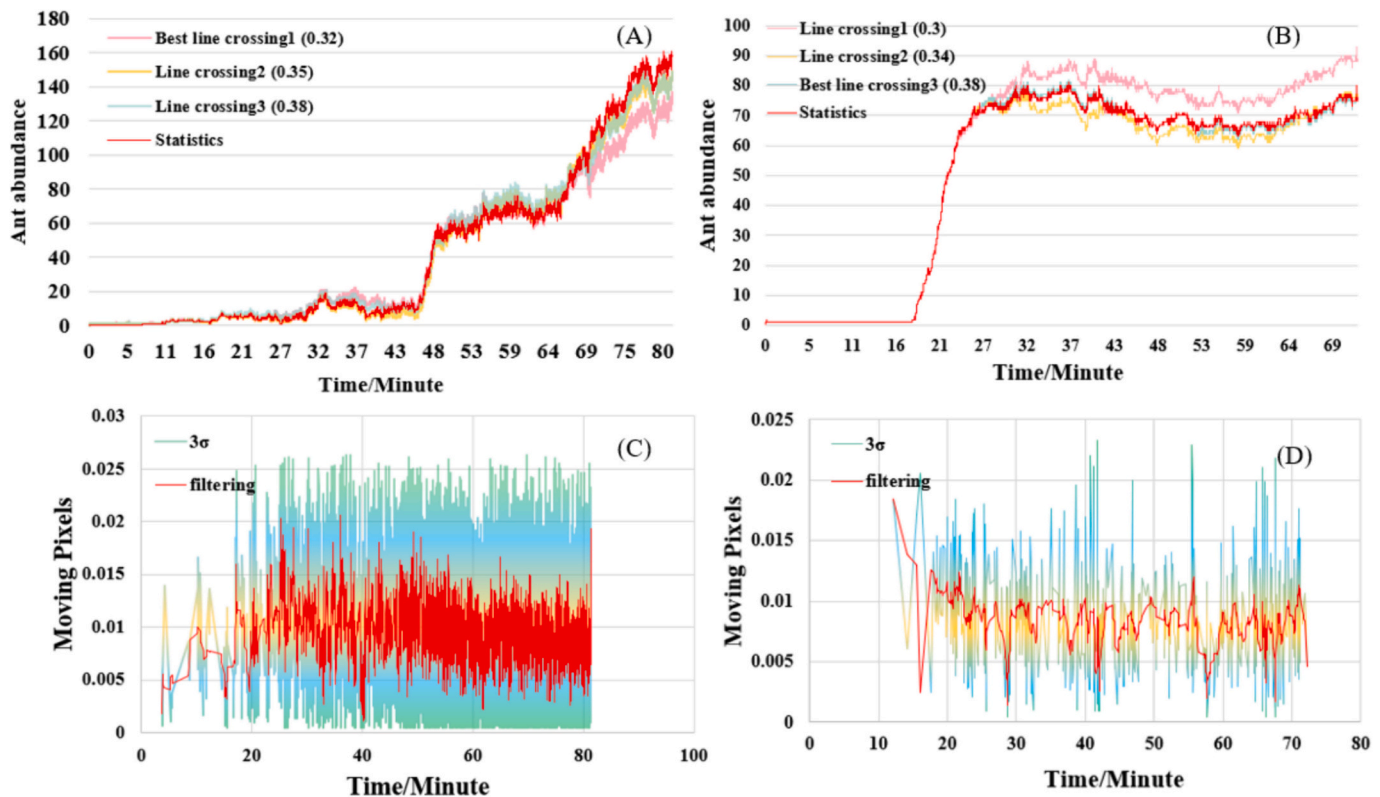
Three algorithms were proposed in this software to count ants on the bait:

##### (1) Line crossing count

This function helps users define a 'line crossing' state to evaluate the dynamics of ants entering and leaving the bait station instead of counting the number of individuals gathered around the bait, which is nearly impossible to count accurately. Specifically, this function will divide the bait station area into three parts (see Appendix 2) defined below:

**Identification area:** Designated area for the classification of species. The software applies the identification area based on object detection to identify individual ants. The specific identification area is situated within the edge of the bait station. It is recommended to place it at 2/3 of distance from the centre to the edge of the bait station. This ensures that the identification area is far enough from the resource and has a suitable distance between ants (without stacking), which effectively reduces the recognition error (Appendix 2).





**Fig. 2.** Result of the two videos (TC2b22s10 (Video 2) and TC2b22s01 (Video 3)) showing ant abundance and movement calculated by the software. The x-axis of all graphs represents the time of the video in minutes. Ant abundance of TC2b22s10 (Video 2) (A) showing *Aphaenogaster* sp. and of TC2b22s01 (Video 3) (B) showing *Meranoplus* sp. In both (A) and (B), the y-axis is the abundance, while the different line colors in the figure represent the results of different 'line crossing' calculations, with the red line representing the statistics result, which is the aggregated result of all 'line crossings'. (C) and (D) show the moving pixels of *Aphaenogaster* sp. and *Meranoplus* sp., respectively. In both (C) and (D), the y-axis is the moving pixels of the ants. The  $3\sigma$  line is the result after detected outliers, and the filtering is the result after the noise was reduced by moving mean filtering. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Line crossing:** The designation of the 'line crossing' is a core element of the entire method. The user should establish a 'line crossing' within Ant-Observer, which will be used to record the movement of ants entering and exiting the designated 'Monitoring area'. In this way, the software is able to accurately count the abundance of ants on the bait. The 'line crossing' is any circle centered on the bait station with a radius between  $2/3$  and  $1/3$  of the bait station's radius. In addition, the size of the 'line crossing' was set to optimize individual ant recognitions in the videos with lower quality. For example, if the edge of the bait station is blocked by debris such as fallen leaves or dirt, the user could avoid the influence of such debris through a reduction of the size of the 'line crossing' appropriately. The 'line crossing' on the bait station separates identification and monitoring areas. The 'line crossing' monitors the entries and exits of ants into the bait station area, to accurately obtain the total number of ants near the bait. Since the 'line crossing' is positioned further from the bait, the algorithm can monitor changes in the number of ants in real time by counting individual ants crossing the 'line crossing', mitigating the problem that individual ants near the bait could be difficult to distinguish from each other due to their high density and overlapping positions.

**Monitoring area:** The software utilizes the 'label files' generated by object detection to evaluate the number of ants entering and exiting the 'line crossing'. In order to solve the problem of ants congregating on resources, which leads to failure of object detection, the software adopts the concept of monitoring area (Fig. 1). Within the monitoring area, the number of ant individuals is not determined by the number of targets detected by object detection, but by post-hoc analysis evaluating the number of ant individuals entering and exiting the 'line crossing'. As such, estimation on the number and distribution of ants in the

monitoring area can be more accurate.

## (2) Species size count

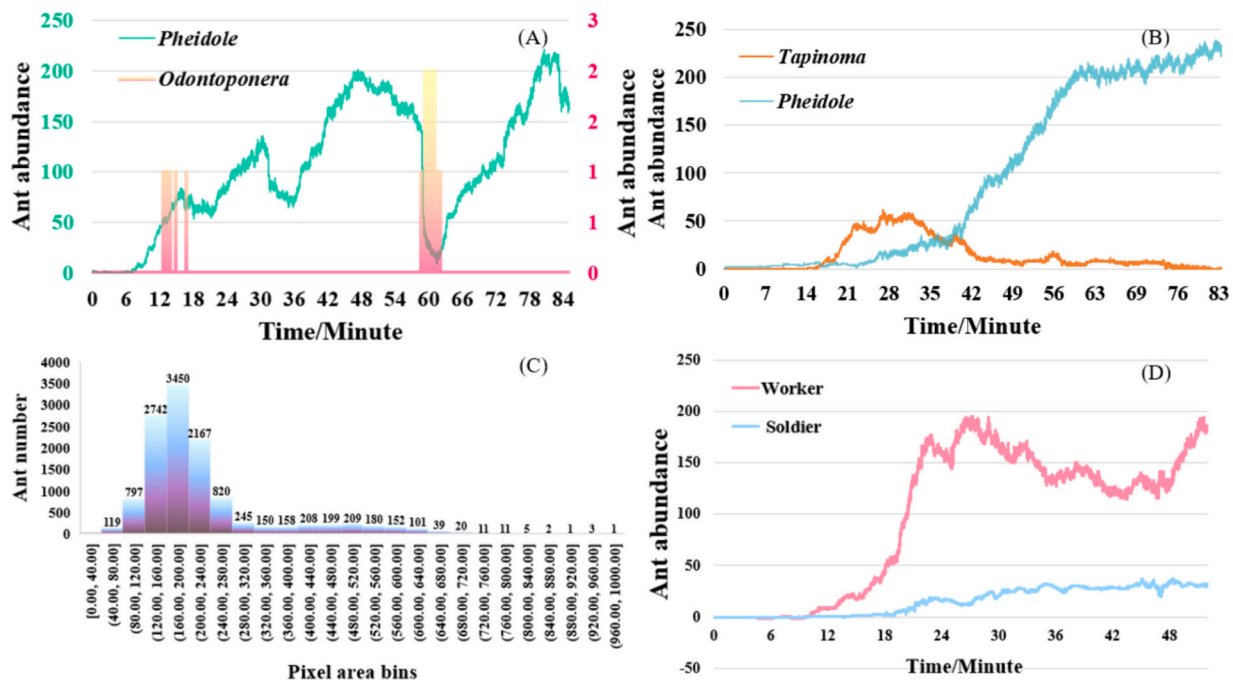
This software is able to calculate the abundance of different-sized ants of the same species on bait stations. When an individual crosses the 'line crossing', the software will capture its contour and calculate the area of the contour. Users need to divide the range of ants in the area of different sizes by the contour areas (Appendix 2), with each range representing one ant size. Ants of different sizes are judged based on the user-defined threshold matrix of contour area (consisting of upper and lower limits selected for each size category). Employing this method, the software can calculate the changes in abundance of ants of different sizes on the bait station on a frame-by-frame basis, starting from the initial frame.

## (3) Detection counting method

For large size species (e.g., species total length  $\geq 8$  mm), we recommend using object detection directly (i.e., choose the detection counting method) if minimal overlapping or stacking of individuals near the baits is observed. This method can recognize individuals and capture their positions and respective abundance from each frame of the video based on the detection results previously trained by the users themselves. (see Appendix 2).

### 2.3.3. Mean displacement

The mean velocity of individual ants is a key factor affecting their resource discovery and exploration abilities, which relates to the



**Fig. 3.** The results of the three videos (TC2b22s14 (Video 4), TC2fy22s13 (Video 5), TC2by22s05 (Video 1)) showing ant interaction and ant body size identification using our software. In videos TC2b22s14 (Video 4) (A), the x-axis is the video time (minute) and the y-axis is the abundance of *Pheidole* sp. (primary; left) and *Odontoponera denticulata* (secondary; right). The different colors of the lines in the figure represent the abundance of different species. (B) Similarly, the x-axis in TC2fy22s13 (Video 5) is the video time (minute) and the y-axis is the abundance of ants. The different colors of lines in the figure represent the abundance of different species. (C) The histogram shows (TC2by22s05, Video 1) with the distribution of pixel areas of ants crossed by 'line crossing', where the x-axis represents bins of pixel area (each bin width is 40), and the y-axis represents the number of ants in each bin (see Appendix 2 Section 4.3). (D) The abundance of different sizes of dimorphic ants (TC2by22s05, Video 1), where the x-axis is the video time (minute), and the y-axis is the abundance of the ants. The different colors of the lines in the figure represent the abundance of different size bins.

probability of randomly finding and then dominating resources (Feener Jr et al., 1988; Fellers, 1987). In the context of resource competition, the dominance of resource occupation may be determined by the ability of a species to discover resources. This software can estimate the mean velocity of each species by tracking the displacement of each individual in adjacent frames (displacement here refers to moving pixels, see Appendix 2).

### 2.3.4. Results visualization

The software uses a GUI to visualize the results from the operations including line crossing plotting, line crossing counting, detection counting, and average displacement (the details are shown in Appendix 2).

### 2.3.5. Feasibility analysis

To evaluate the reliability of the software developed, we performed ant abundance count for nine videos in Table 3 using both software and a 'manual (traditional)' methods. The manual (traditional) method involves taking photos of the bait station every 5 min, and then using the ImageJ (Schindelin et al., 2015) to manually mark and count ant abundance on the photos. We measured the time required to complete the counting task for the software and the traditional method separately and performed data analysis. In order to compare the two sets of data results (ant abundance counted by software and traditional methods) for significant differences, we first used the Kolmogorov-Smirnov test to assess the normality of the data. Next, non-parametric tests (Mann-Whitney *U* test) were used to test for differences between the two data sets. Finally, the correlation between the two sets of data was tested by Spearman's rank correlation. The tests were performed in stats package in R.

## 3. Results

### 3.1. Design of apparatus

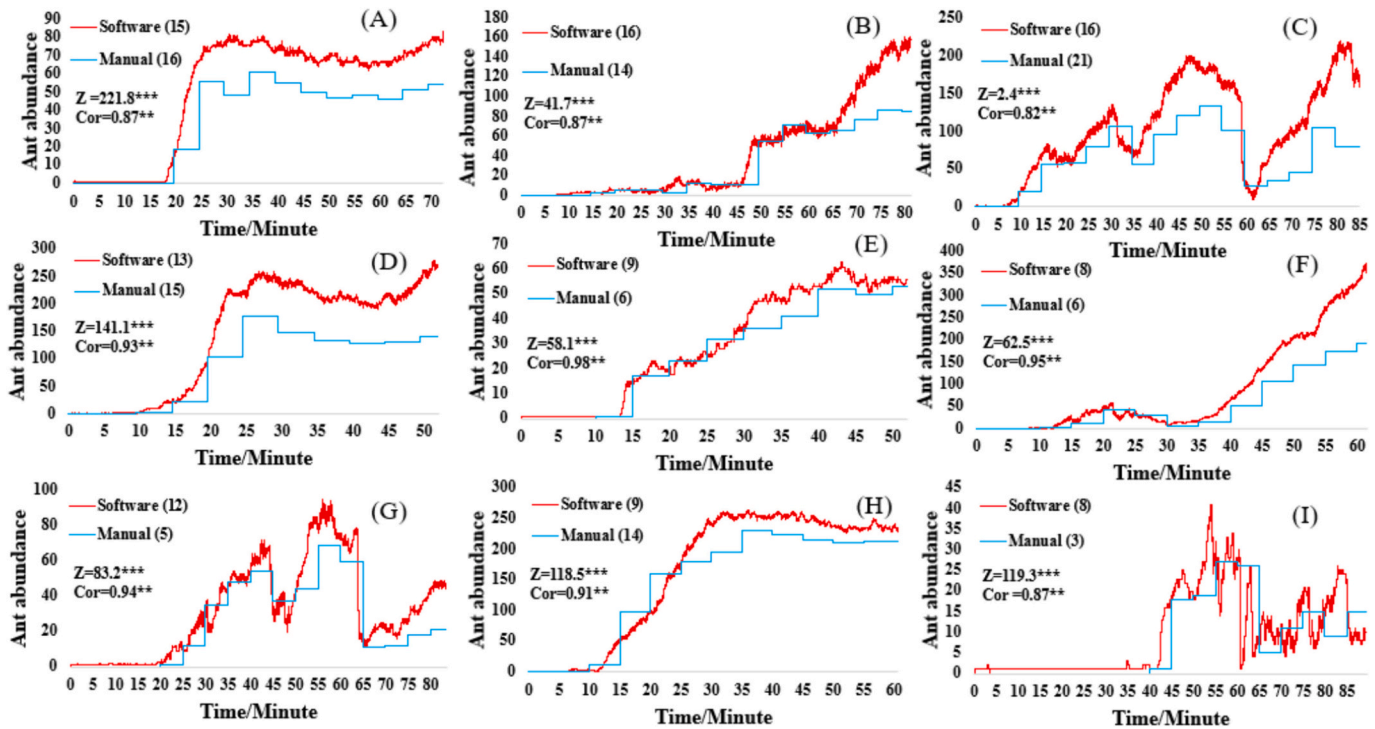
Based on the formula given in Appendix 3, we can calculate that the optimal lens magnification at a distance of 1.2 bait card size ( $\theta$ ) is 2.41. Considering the deviation of the camera lens and thickness, the actual optimal lens should be larger than the calculated one, and the standard lens has a price advantage. Therefore, we set the lens magnification to 3, and the optimal distance to about 8.5 cm (from camera to bait card). For a 3× lens, a distance of 8.5–10 cm is needed for taking clear pictures of ants.

Compared to other cameras, our camera equipment has the advantages of portability and affordability (Appendix 1). Although the characteristics are not exactly comparable between cameras, our equipment can achieve the required quality for the in situ experiments while balancing the benefits of being light weight and affordable. In addition, this camera equipment has a simple setup process. For more experienced researchers, it takes only 20–40 s to set up an additional camera equipment on the bait station.

### 3.2. Software applications

#### 3.2.1. Abundance of species

We used 3 'line crossings' to calculate the abundance of species (TC2b22s10 (Video 2) and TC2b22s01 (Video 3)) and determined that the best radii were 0.35 and 0.38 respectively (based on the least squares method, as shown in Fig. 2, A&B). Although the calculation time required by multiple 'line crossing' is longer than that of traditional methods (Appendix 1), the 'line crossing' method allows one to obtain more data, requiring only a small amount of manual operation. The 'line crossing' method provides data with higher accuracy and richer details,



**Fig. 4.** Results of using manual (traditional) and the new software to count ant abundance in nine videos. The x-axis shows the time of the video (minute) and the y-axis shows the species abundance. The different line colors in the figure represent the abundance of species according to both methods used to estimate abundance. The parentheses in the data labels represent the time in minutes used by the different methods. In the bottom left corner of each subfigure (Fig. 4 A-I), the Z represents the z-value of nonparametric test and the Cor represents the correlation between abundance in function of the counting approach. The ‘\*\*\*’ indicates a statistical significance level of  $p < 0.01$  and ‘\*\*\*\*’ represents  $p < 0.001$ . (A) Detail of ant abundance estimate in video TC2b22s01 (Video 3). (B) Detail of ant abundance estimate in video TC2b22s10 (Video 2). (C) Detail of ant abundance estimate in video TC2b22s14 (Video 4). (D) Detail of ant abundance estimate in video TC2by22s05 (Video 1). (E) Detail of ant abundance estimate in video TC1b22s14 (Video 6). (F) Detail of ant abundance estimate in video TC1b22s04 (Video 7). (G) Detail of ant abundance estimate in video TC2b22s12 (Video 8). (H) Detail of ant abundance estimate in video TC1b22s07 (Video 9). (I) Detail of ant abundance estimate in video TC2b22s16 (Video 10).

which effectively solves the problems of data bias caused by ant stacking and data discontinuity due to insufficient manpower in traditional methods.

### 3.2.2. Displacement analysis

For the ant displacement data calculated in TC2b22s01 (Video 3) and TC2b22s10 (Video 2) (Fig. 2, C&D), we use the statistical “3 $\sigma$ ” criterion to detect outliers, that is, according to the mean  $\mu$  and standard deviation  $\sigma$  of the whole sample, data outside  $\mu - 3\sigma$  and  $\mu + 3\sigma$  are considered as outliers and are excluded. We use moving average filter to reduce the noise of the displacement data after removing outliers, and then set the filter window width to 11, which can effectively reduce the noise of the data and preserve the original features of the data at the same time. Therefore, the use of software greatly improves the amount of data collected, allowing the collection of phenomena that difficult to observe by traditional methods such as the movement speed of ants and the characterization of ant behavior.

### 3.2.3. Ant interactions

Interactions were observed in the video TC2b22s14 (Video 4), where the resource was discovered by *Pheidole pili* after 7 min, at which point recruitment occurred. After 60 min, *Odontoponera denticulata* occurred on the same resource, prompting an interaction with the *P. pili* workers, which led to a dramatic decrease in *P. pili* abundance before rebounding. Video TC2fy22s13 (Video 5) had a similar situation where *Pheidole elongicephala* discovered the resource in the second minute following the bait station placement, and then proceeded to recruit. However, in the 6th minute, *Tapinoma* sp. demonstrated a faster recruitment and dominated the resource for a period of time. After 30th

minute, *P. elongicephala* successfully displaced *Tapinoma* sp. and dominated the resource again (Fig. 3, A&B).

### 3.2.4. Size identification

For TC2by22s05 (Video 1), we set 0.385 as the ‘line crossing’ radius for the size calculation. Based on the entry and exit data and the image area obtained, we defined the pixel area of 100–350 pixel area as worker and 500–700 pixel area as soldier (Fig. 3C). The results showed that the worker abundance started to increase sharply at the 20th minute and then declined slightly at the 30th minute, until around the 46th minute, when the worker number began to rise slowly again. These results are consistent with the video (Fig. 3D).

### 3.2.5. Feasibility analysis

We performed manual (traditional) counting and software counting of different videos and compared the ant abundance obtained by the two methods (Fig. 4). The results of non-parametric tests surface a significant difference in the two methods ( $p < 0.01$ ). The results showed a high correlation between the two methods (all of video’s correlation are  $> 0.8$  ( $p < 0.01$ )). The Fig. 4 illustrates the results of the nonparametric tests and correlation analyses. However, we also found that manual (traditional) counting significantly underestimated the ant abundance in the second half of the videos, when abundance tends to be at its peak, which was different from software counting. This is likely due to the overlap of ants on the resource, making it difficult for the manual counts to accurately identify each individual ant.

We further compared the time efficiency of the two methods. We found that for a 1–1.5 h video ( $N = 9$ ), manual counting took 8–20 min ( $N = 9$ ), while software counting was dependent on the number of line



crossings set by the user and computer performance. Taking the computer we used (i9–10,900 CPU@2.80GHz) as an example, software counting also takes 14–16 min if one line crossing is set. However, software counting has a significant advantage over manual counting in that it can provide more dimensional and higher precision data. The software supports multi-process computing, allowing users to process multiple video data sets in parallel (see Appendix 2), further improving computing efficiency and data processing capabilities.

#### 4. Discussion

Interspecific competition has important implications for determining species diversity and composition (Hood et al., 2021; Mahaut et al., 2020). Since traditional methods are unable to record the total ant abundance that have visited the bait, the lack and inadequacy of the data obtained from this method may lead to a decrease in the credibility and validity of the results. To address this problem, we designed a lightweight and user-friendly camera equipment and built a system to automatically collect and process data from videos.

We demonstrated that our low-cost camera equipment can be deployed in field conditions during baiting experiments to provide various information such as ant species discrimination, abundance, size, and movement speed. With this information, we demonstrated the complex interactions observed in ant communities that could be missed by traditional methods. For instance, the pattern seen in TC2b22s14 (Video 4) resembles a retention event used in tradition methods to score competitive interactions (Lebrun and Feener Jr, 2007). However, instead of a simple score, our software allows for in-depth analysis of the change in abundances between the two species during interactions. This information can help researchers to better understand the structure of the ant community, its dynamics and the behavior of ant species in different habitats and under various environmental conditions.

The camera equipment is conceived for a wider range of stand-alone surveillance applications and uses efficient and flexible hardware components. In relation to other research equipment (such as GoPro+ or Canon cameras) used in previous studies, we selected camera equipment that is economical, field-practical (<100 g), and with comparable video quality in relation to previous but more expensive equipment. In addition, this equipment has a wide range of application and is not limited to a specific type of camera; virtually any similar camera can be applied to the camera equipment, simply by selecting the appropriate lens according to the research requirements through the provide the full name formula (see Appendix 2). The camera equipment can be applied to record ants, other invertebrates or plants, and only requires the user to be flexible in determining the type and parameters of the lens used according to specific research requirements. Our camera equipment can thus be adapted to different research needs by adjusting the components so that it can work properly in different environments.

The camera equipment can be easily deployed in different environments, while reducing human interference and errors, to monitor and record ant activity in real time. The camera equipment can be deployed by researchers during traditional food baiting with only an extra five minutes to set up 16 cameras in an entire area. In contrast, researchers using the traditional method were only able to examine a few food baits and had to check them at each fixed time period, causing unnecessary distractions (Wang et al., 2001) and wasted time.

In addition, for data extraction from the collected videos (pictures), the software we designed has advantages over traditional methods:

- (1) The video data collected is continuous and can reflect the dynamic changes of ant colonies during foraging. Compared with traditional methods, we are able to capture more details from the video, such as ant interactions, ant recruitment, speed, body size range, etc.
- (2) Extracting data through software greatly reduces labor costs and errors. The researcher needs to spend a lot of time and energy to

observe and record by traditional methods which is prone to subjective judgment and inaccuracy. For the software method, researchers are able to perform simultaneous calculations on multiple videos and obtain more accurate and objective data by simply operating the software. Although for the time being, the time taken to calculate ant abundance using software is comparable to the time required by traditional methods, this problem can be solved by using computers with higher performance, which would become increasingly accessible with time.

- (3) Extracting data through software enables to obtain more detailed and rich data than traditional methods. For the traditional method, the researcher can only manually calculate ant abundance on the data obtained by taking photos (videos), so the amount of data obtained is limited. However, using software to extract the data, it is possible to obtain data on ant abundance in each second of the video, which can make a greater contribution to the understanding ant ecology.
- (4) Using software to extract the data may be more accurate than manually. For the traditional method, it is difficult to accurately count the exact number of ants stacked on the food bait using the naked eye, while the software uses the line crossing method to avoid this problem, greatly reducing the possibility of double counting or omission of counting.
- (5) Through this software, data that are difficult to obtain using traditional methods, can here be acquired. For instance, traditional methods usually collect data from bait stations about ant species and abundance, while data extraction with our software can also provide additional data on speed, behavior, the number of polymorphic species and their respective individuals, etc. Overall, these data can help researchers gain a deeper understanding of ant behavior patterns and coexistence mechanisms of ant communities and make a greater contribution to the understanding of ant ecology.

Some limitations exist, however, when using this system that need to be considered in future research.

- (1) The measurement of ant body size is influenced by the lighting condition. Sunlight may cause the ant's shadow to deform, resulting in a deviation in the program's estimation of ant volume. In order to optimize the reliability of the measurements, it is recommended to avoid placing the bait stations in direct sunlight to reduce errors caused by uneven lighting and to reduce the risk of camera damage due to high temperatures.
- (2) Video quality can be challenging under nocturnal conditions in the field. While infrared night-vision cameras are beneficial for capturing footage in low-light conditions, our testing revealed that the video quality obtained solely through infrared capabilities is inadequate for identifying ant species. Alternatively, the use of light-emitting diode (around 70 lm) as an auxiliary light source can significantly improve video clarity (Appendix 3). Hence, it is recommended to employ light-emitting diode for nighttime recording or to select a superior infrared camera designed for high-quality imaging. A similar approach can also be considered for darker habitats in which light is relatively limited.
- (3) Our software may encounter difficulties in estimating the number of fast-moving ants on the bait station, which is due to the insufficient frame rate of the camera to capture the rapid changes of ants (our camera using 25 frames per second). To improve the accuracy of calculation, it is suggested that users use a higher frame rate camera to obtain more detailed data and reduce potential errors. Based on the model selected, this may also increase costs and weight. It should be noted, however, that these represent special cases and only a relatively small percentage of ants overall, which traditionally were not included.



- (4) Some behaviors of ants can pose challenges to the software calculation. For instance, a few ant species may bury food resources, especially liquid food (Módra et al., 2020; Wen et al., 2021), thus making it impossible for object detection to find ants' position accurately. However, this problem is not specific to our new system, as other traditional methods will be confronted to similar limitations.

Although this system focuses on ant baiting experiments, the software's application could also be applied to central-place foraging scenarios. For example, it can be used to monitor the flux of ants in and out at the nest entrances to assess ant activities. In addition, it can also be applied in studies on other arthropods. Ants are among the most challenging organisms to quantify in food bait experiments, due to ant behaviors, such as stacking on resources, and mass recruitment which can result in hundreds or thousands of individuals recruited over a small area, making it extremely difficult to calculate species abundance on bait stations. Therefore, since this system is able to count ant abundance, it can be assumed that the system is equally (or possibly more) effective for other insects and other invertebrates with swarming behavior (e.g., crickets, bedbugs, cockroaches, beetles, snails) as long as they move through a 2D plan (new issue may arise with flying or swimming organisms due to the need for camera focus which would require a different approach). In addition, this system should be highly adaptable and could be used for studies that require assessment of insect abundance such as for community ecology, biological control (e.g. responses to pheromone traps or measuring predator/prey interactions), or pest control (efficiency of particular pesticides to attract targeted species).

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## CRediT authorship contribution statement

**Jiaxin Hu:** Conceptualization, Methodology, Software, Data curation, Writing – original draft, Writing – review & editing. **Taylor A. Bogar:** Software, Validation, Data curation, Writing – review & editing. **Yi-Fei Gu:** Software, Validation, Writing – review & editing. **Benoit Guénard:** Supervision, Conceptualization, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known financial or personal conflict of interest that could have influenced the work reported in this paper.

## Data availability

The guidelines for software, video preprocessing and verification video are available at.

[https://connecthkuhk-my.sharepoint.com/:f/g/personal/u3008597\\_connect\\_hku\\_hk/EjTN94DNSZxFrT-L-fwrjJ8By3lcoLtfqHwyu5rNxOIog?e=NhnMMH](https://connecthkuhk-my.sharepoint.com/:f/g/personal/u3008597_connect_hku_hk/EjTN94DNSZxFrT-L-fwrjJ8By3lcoLtfqHwyu5rNxOIog?e=NhnMMH).

Software is available at <https://github.com/Jx1206/Ant-Observer>.

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