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Quantitative measurement of stag beetle behaviors in low light and complex background conditions using DAMM (detect any mouse model)

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Aggressive behavior in insects plays a crucial role in various ecological and evolutionary processes, influencing resource acquisition, mate competition, and social organization. Stag beetles (Lucanidae) represent a compelling model system for studying aggression, particularly male-male combat. However, studying their interactions in a contest test with low light—a common condition for nocturnal species—and a complex background to mimic their natural environment remains challenging due to poor visibility and tracking limitations. Here, we adapted Detect Any Mouse Model (DAMM), a machine learning-driven tracking framework, to detect and track the position of each male stag beetle of two species (*C. mniszechi* and *C. speciosus*), followed by the calculation of several essential behavior endpoints to quantitatively analyze their behaviors with a conspecific during the test. The calculated object detection metrics indicated that the DAMM performed well in detecting and classifying objects. Meanwhile, based on the behavior endpoints, *C. mniszechi* displayed more robust behaviors during the test compared to *C. speciosus*, which was indicated by the tendency of this species to secure the food tube while fighting another contestant to defend their position. In addition, the calculated fighting-related-behavior endpoints also showed consistent results with the manual observation, highlighting the validity of the calculated endpoints. To sum up, the adaptation of DAMM to study insect behavior enables researchers to quantify their behaviors and automation that would undoubtedly lead to unprecedentedly detailed and objective insights into the complex behaviors of insects, especially stag beetles, contributing to a deeper understanding of their ecology, evolution, and the intricate workings of the natural world.

Keywords Behaviors, DAMM, Machine learning, Movement tracking, Stag beetle

Fighting is one of the animal behaviors that occurs during a competition for limited resources for their survival and reproduction[1]. In some animals, including stag beetles, this behavior might even occur when battling for females[2]. Outcomes of this behavior are influenced by various factors, including their sexually selected traits, such as ornaments and weapons that are found in a very wide range of taxa and often evolve into larger-sized weapons[1]. One group of insects with an amazing diversity of weapons is stag beetles (Family: Lucanidae, Subfamily: Lucaninae)[3]. The male gender of this insect utilizes their exaggerated mandibles to fight for access to sap sites, territory, and females[4,5]. However, although mandible and body size are reliable indicators in predicting the winning chances of a beetle, other factors, including their fighting ability, also influence their contest

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interactions[6]. Moreover, due to the high diversity in the mandible size and shapes within and across species of stag beetles, the displayed fighting behaviors are highly variable and thus increase the complexity of analyzing these behaviors[1,3]. Unfortunately, despite the importance of this behavior to be studied for understanding the evolution and diversification of their weapon forms, this behavior has received little scientific attention[1].

Previously, several researchers had tried to deeply study the fighting behaviors of stag beetles. For example, Goyens et al. described the male stag beetle (*Cyclommatus metallifer*) fighting behaviors by observing various behavior parameters, including battle duration, actions during the battle, mandible regions used to bite, and behavior after settling the battle in the lab with conditions that mimicked a natural environment, and related them to the measured morphological parameters, such as body and mandible size[2]. Another prior study by Chen et al.. also investigated the assessment strategy of another species of stag beetle (*Cyclommatus mniszechi*) by pairing them with another male beetle and evaluating the output together with other data, including their mandible length. In their study, the behavior observations were conducted in an acrylic contest arena with sawdust at the bottom, a piece of half-cut wood at the center as a feeding station, and dry oak leaves on the surface as the resting zones[3]. However, while the behavior observations in these studies were carried out in an environment with a complex background due to all of the applied components to mimic the natural environment, they still relied on manual scoring of specific actions, although one of the studies utilized a program for a computer-based review of previously recorded videos or live observations, which can be subjective and time-consuming[7]. On the other hand, in our previous study, we had implemented DeeplabCut (DLC), an open-source Python package for animal pose estimation, to automatically label each essential body part of the stag beetle and evaluate their complex social behaviors based on their body part location. However, since we wanted to increase the accuracy of each body part tracking without the necessity of conducting numerous attempts of model training, the visibility of each body part became the priority of the study. Thus, the test was conducted in a small acrylic tank with an artificial light source underneath the tank, considering the difficulty that occurred when recognizing each body part location in a complex background by using this method. This condition was not reflecting the condition in their natural environment, which might change the animals' natural behavior[8]. Taken together, although some methods, such as DLC, might still have the potential to automatically analyze stag beetles' behaviors in a complex background, the development of novel methodologies is always necessary to provide a more rigorous and objective analysis as an alternative method to evaluate their complex interactions in such a background.

C. mniszechi is one of the stag beetles that is commonly used to study the fighting behaviors of Lucanidae due to their eagerness to engage in male-male battles in competition for resources[9]. Their habitat is lowland forests below approximately 750 m in southeast China, Vietnam, and northern Taiwan[10]. Adult *C. mniszechi* is known to feed on plant secretions and fruits[3]. While males generally possess larger body and mandible sizes compared to females, their sizes are highly variable[11]. Typically, during a fight with another stag beetle, *C. mniszechi*, with its long, tusked, and curved mandibles would grasp the head or body of the opponent from above and then lift it[3,12]. Previous studies have shown that, before physical contact occurs, males primarily rely on self-assessment strategies. This is likely because *C. mniszechi* has limited visual ability under low-light conditions and therefore cannot effectively use visual threat signals, such as assessing the size of an opponent's enlarged mandibles. As contests escalate and the mandibles become interlocked, males shift to mutual assessment strategies that involve direct physical interaction. [9]. Given the high aggression level of male *C. mniszechi* and their peak activity at dusk and during nighttime, an automated computer-based behavioral analysis would be particularly useful to enable objective and quantitative evaluation of contest behaviors under low-light experimental conditions that closely resemble natural feeding-site competition[9,12].

Considering these factors, it was necessary to develop a novel method that is able to accurately detect and track the positions of insects, such as stag beetles, in an environment that is similar in nature to a low-contrast environment. Unfortunately, traditional object tracking often fails when applied in a low-contrast environment for small objects like stag beetles. Moreover, observing and quantifying the behaviors of stag beetles also possesses several inherent challenges due to their small size, rapid movements, and often cryptic lifestyles, which can make detailed observation difficult. However, recent advancements in the computer vision field have made significant achievements under controlled conditions, but these tools struggle with occlusion, variable lighting, and diverse backgrounds. Detect Any Mouse Model (DAMM) model is one is a state-of-the-art object detection model built upon deep learning principles, specifically leveraging the Mask R-CNN architecture that was originally developed for tracking mice in complex laboratory conditions. Previously, Kaul et al. used a pre-trained Mask R-CNN object detector model to perform instance segmentation, validated DAMM performance using zero-shot and few-shot inference, and integrated the SORT algorithm for robust tracking of mice[13].

Finally, based on the previous studies mentioned above, the current study aimed to utilize DAMM to quantitatively analyze the fighting behaviors of male *C. mniszechi* under low-light conditions in a test tank with various components to mimic their natural habitat. In addition, the same training dataset was also used to detect *Cyclommatus speciosus*, another stag beetle species with natural habitat on Vella Lavella island, inhabiting tropical, humid, and mountainous rainforest environments and it isparticularly notable for its aggressive-looking mandibles. By using the same dataset generated during the analysis process of *C. mniszechi*, we aimed to evaluate the capability of this method in analyzing different species of stag beetle, considering the relatively similar morphology of both species. The usage of this generated dataset should be able to shorten the whole process of analysis, leaving only a relatively short fine-tuning process to produce more accurate and reliable data. This study hypothesized that, although this method could not specifically label each body part of the animals, DAMM could accurately label the center mass of each animal in every frame, thus assisting in various behavior endpoint calculations to determine the outcome of each animal's encounter. We believe that the present study could highlight the utility of DAMM in offering a significant opportunity to transition from descriptive accounts of insect behavior to more precise quantitative analyses and, therefore, provide valuable insights into the evolutionary pressures shaping weaponry and aggressive strategies in insects.

Materials and methods

Study organism

Males *Cyclommatus mniszechi*, a native species in Taiwan, and *Cyclommatus speciosus*, with mean and SD of the body (head to elytra) and mandible lengths of 2.752 ± 0.260 cm and 1.187 ± 0.182 cm, and 3.700 ± 0.273 cm and 1.898 ± 0.406 cm, respectively, were used in the current study. The larvae were bred independently with fermented sawdust in a 250 ml plastic cup, while the adults were reared independently with a slightly damp dead *Sphagnum* moss in a 250 ml plastic cup and fed with insect jelly. All of the animals were maintained in the lab facility with a controlled temperature ranging from 21 to 24 °C with a 12:12 h light and dark cycle and artificial light as the light source. Prior to the test, each animal was placed and isolated in the resting zone to undergo an acclimation process and was not fed during this period.

Contest experiments

The behavior test was conducted in an acrylic enclosure (32 × 18 × 30 cm) with two resting zones (8 × 18 cm) and one central zone (16 × 18 cm) that were separated with acrylic dividers (Fig. 1). A matte black foam with some dead *Sphagnum* moss was used to provide an environment that resembles their natural habitat. These components were also necessary to minimize reflections and to assist the beetles in walking naturally. In the central zone, a piece of half-cut wood with a feeding station was placed at the center of the arena to facilitate competition between individuals. The feeding station contained a food tube that was made from a 1.5 ml vial tube that was filled with insect jelly (Fig. 1). All of the lateral sides of the test tank were covered by black plastic boards, except for one side where the camera was placed, to reduce external visual disturbances around the enclosure. The contests were started by removing the acrylic dividers and were conducted after 18:00 in a totally dark environment, considering they are nocturnal animals[3,9]. In recording their behaviors, two synchronized infrared (IR)-sensitive cameras (Yaba IR-KA2812A, MINS Co., Ltd., Taoyuan, Taiwan) with 850 nm IR filters linked with a hybrid digital video recorder (Yaba HDVR-DHA405H5N, MINS Co., Ltd., Taoyuan, Taiwan) were used to record the stag beetle's behaviors from the top view. The recorded videos were in a Full HD resolution (1920 × 1080 pixels) at 30 frames per second (fps). However, due to technical difficulties, some videos of *C. speciosus* were recorded at 15 fps. During the recording process, brief interventions were necessary when the wood log was displaced or when a stag beetle became hidden under the wood, entangled in the Sphagnum moss, or remained upside down for an extended period. A total of six videos of *C. mniszechi* with a duration of two hours were recorded, while for *C. speciosus*, a total of six videos with a duration of 1 h were recorded, with a total sample size of 12 stag beetles for each species.

DAMM adaptation pipeline

In the present study, the original DAMM implementation was modified for fine-tuning the model on our local GPU systems rather than using Google Colab (https://github.com/backprop64/DAMM (accessed on 25th June 2025)). This adjustment was necessary considering the large size of the dataset, which could make cloud computations very expensive. Overall, the present locally adapted DAMM pipeline consists of six steps, as shown in Fig. 2.

Video input

All of the selected videos were loaded using a Python script for further processing. Prior to the analysis process, the integrity of each video was checked by using Python scripts to confirm that the files were not corrupted and whether there were broken frames or unreadable segments to ensure the quality of the data that would be entered into the analysis pipeline.

Frame extraction

A total of 2596 frames were extracted from the entire stag beetle video dataset by utilizing OpenCV, which supports various video formats, such as .mp4, .avi, .mkv, .mov, and .flv. These extracted frames were uniformly sampled across all videos, regardless of the length of the video, to ensure balanced representation and model

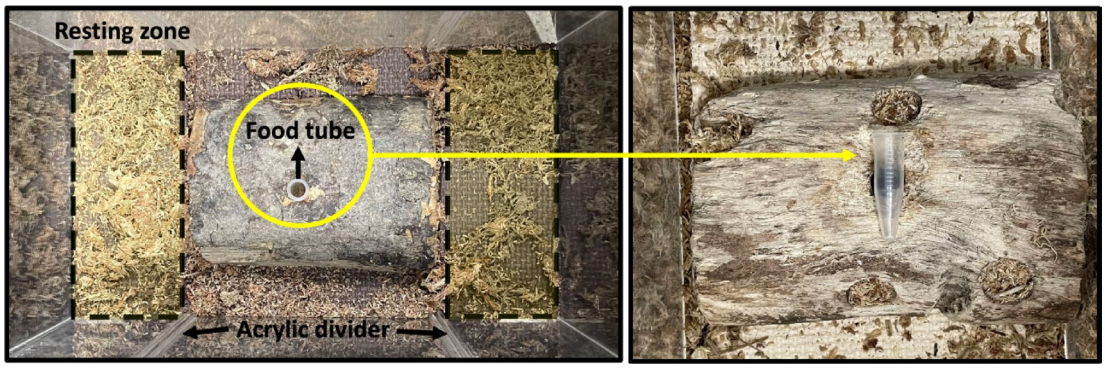

Fig. 1. The overhead view of a contest arena. The test enclosure consisted of two resting zones on the left and right sides, with the food tube in the center of the enclosure (left), inside a wooden log (right).

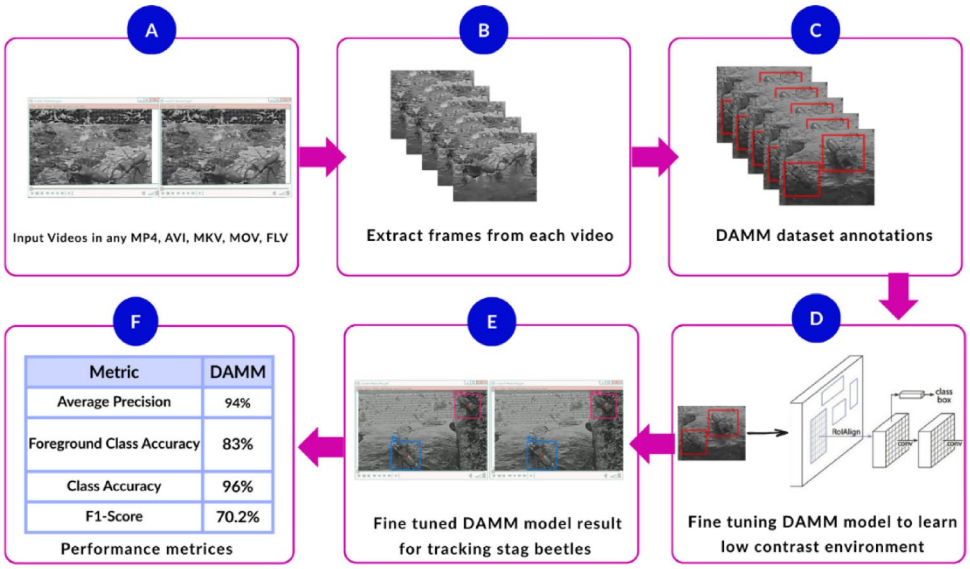

Fig. 2. The workflow of DAMM adaptation in the current study. (**A**) Video input. (**B**) Frame extraction. (**C**) Dataset annotations. (**D**) Fine-tuning the model for low-light conditions. (**E**) The animal tracking process. (**F**) Performance metric evaluation.

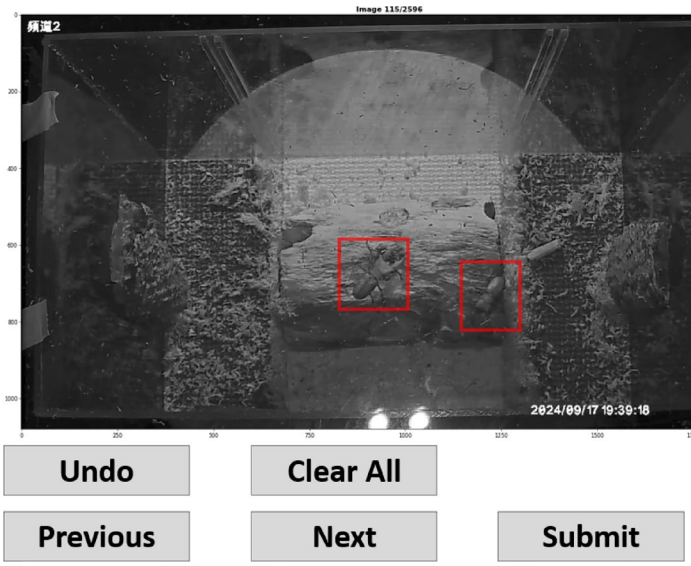

Fig. 3. The GUI (Graphical User Interface) of the customized developed tool for DAMM dataset annotation.

generalization, and also ensured the balanced representation of frames across the dataset to prevent the model from over-fitting or bias towards specific light conditions.

Dataset annotations

For the annotations process, a custom interactive bounding box annotation tool with support from matplotlib, OpenCV, and PIL was developed in the current study. By using this tool, users could easily draw bounding boxes on sampled frames that are extracted from videos. Furthermore, this tool also allows users to fully control image navigation, undo or clear annotations, and save the annotations' progress. After the bounding boxes were drawn, annotations were stored in a COCO-style format in the metadata.json file. Overall, this tool was developed to ensure consistency, scalability, and user-friendly manual annotations across a large dataset. The graphical user interface (GUI) of this tool is shown in Fig. 3.

Fine-tuning DAMM

DAMM utilizes the Mask R-CNN model from the library of Detectron2, a powerful detection framework based on PyTorch. However, a fine-tuning process was still necessary to ensure that the model could recognize stag

beetles in different environments. The first step for fine-tuning was the modification of the model architecture for a custom dataset containing stag beetles. The key parameters for this fine-tuning include the learning rate, which controls model learning weights during the training process. The value of ROI_HEADS.NUM_CLASSES was set to 1 since the model is only concerned with detecting stag beetles. In addition, the maximum number of iterations for the model to learn was also set to 100 to avoid over-fitting on the dataset.

DAMM inference on videos

For inference on videos to track stag beetles in a low contrast environment, the process was initiated by setting up necessary configurations, including paths for fine-tuned model weights, configuration files, video directory path, and output directory. For each video in the directory, the DAMM tracker performed object detection and tracking using the SORT algorithm, which assigned unique IDs to the detected stag beetles and tracked their position in every frame (Fig. 4). The key parameter involved in the tracking process was a confidence score, which was set at 0.5 as the minimum confidence required for the detection to be considered valid. During the tracking process, the model outputs the bounding boxes of the detected objects in a single frame. Afterward, the SORT algorithm was applied to these detections, which would assign a unique ID for each stag beetle to track their movement in the next frames. Next, the final results were visualized and saved in the selected directory. In addition, these inference scripts also output .csv files that consist of all necessary information regarding the bounding boxes, animals' IDs, and centroid coordinates of the detected animals for further analysis.

Performance matrices

In evaluating the performance of the object tracking system, the following important metrics, which were Average Precision, Class Accuracy, Foreground Classification Accuracy, and F1-score, were calculated since each metric offered insights into different aspects of the model's performance.

Average precision (AP) First, the precision score was also used in object detection for the evaluation of precision and recall at various thresholds of the intersection over union (IOU) metric. Average precision is the area under the precision-recall curve. A higher AP means that the model would perform better for object detection[14]. It is defined as:

$$\mathrm{AP} = \sum_{\mathrm{n}} (\mathrm{R_{n+1}}\text{-}\mathrm{R_1}) * \mathrm{P_{interp}}(\mathrm{R_{n+1}})$$

Where:

- $\mathrm{R_n}$: Is recall at step n.
- $\mathrm{P}(\mathrm{R_n})$: Is precision at recall R_n.
- $\mathrm{P_{interp}}(\mathrm{R_{n+1}})$: Interpolated precision at the recall level R_{n+1}.

Class accuracy Class accuracy is a metric that evaluates the overall performance of the classifier in assigning correct labels[15]. It measures the proportion of all correctly classified bounding boxes relative to the total number of predicted boxes. This metric is defined as:

$$\text{Class Accuracy} = \frac{\text{Correctly Classified Boxes}}{\text{Total Predicted Boxes}}$$

Fig. 4. One of the representatives analyzed frames that show the detection of two *C. mniszechi* with bounding boxes and a unique ID assigned for each individual stag beetle (yellow color: ID_0 and blue color: ID_1).

Foreground classification accuracy Foreground classification accuracy is a metric that evaluates how the classifier performs in classifying foreground objects[16]. This metric measures the proportion of correctly classified foreground boxes out of the total number of foreground boxes. The main focus of this metric is to differentiate between foreground actual objects and the background. This metric is defined as:

$$\text{FG Accuracy} = \frac{\text{Correctly Classified Foreground Boxes}}{\text{Total Foreground Boxes}}$$

F1-score The F1-score, which combines both precision and recall into a single metric, was also calculated to provide a balanced measure that penalizes both false positives and false negatives. This score is very useful when dealing with imbalanced datasets or when both false positives and false negatives carry significant consequences[17]. The F1-score is defined as:

$$\text{F1 - Score} = 2 \times \frac{\text{Precision} \times \text{Recall}}{\text{Precision} + \text{Recall}}$$

Behavior endpoint calculations

The centroid coordinates of each animal generated by DAMM, which were saved as a .csv file, were used to calculate several essential behavior endpoints, including average speed that represents their overall locomotor activity, average angular velocity, which reflects the animal's movement orientation, and fractal dimension and entropy that were used to evaluate their movement complexity. Meanwhile, to assess their position preference during the contest, two endpoints, which were the average distance to the food tube and the total time spent in the rest area, were also calculated. Average speed was obtained by calculating the total distance the stag beetle had moved within a defined arena, divided by the total time of the observation, while average angular velocity was calculated by dividing the total angular displacement in degrees by the total time interval of the movement. Meanwhile, the fractal dimension and entropy were calculated based on the previous publication with minor modifications[18]. In this study, fractal dimension was used as an index of movement complexity. It describes how tortuous or irregular an animal's movement trajectory is within the arena. Lower fractal dimension values indicate relatively straight, directed movements, whereas higher values reflect more complex, exploratory, or erratic movement patterns. Thus, fractal dimension provides insight into how structured or variable a stag beetle's locomotion is during behavioral interactions. Entropy was used to quantify the variability and unpredictability of movement over time. Higher entropy values indicate more irregular and less predictable movement patterns, while lower entropy values reflect more stereotyped or repetitive behavior. In the context of stag beetle behavior, entropy helps capture changes in movement organization that may be associated with different behavioral states, such as exploration, contest escalation, or rest. Furthermore, some socially related behavior endpoints were also calculated as the average distance between beetles and the total interaction time. The total interaction time between beetles was calculated as the total time when the distance between beetles was less than 6 cm, with the speed ≥ 0.01 cm/s. The first parameter was set considering the length of the central body to the tip of the mandible of the tested stag beetles, which were 2–3 cm; thus, the closer distance between each centroid (< 6 cm) indicated a high probability of interaction between beetles. In addition, another condition regarding their speed was also applied to avoid the false positive results, since, based on the manual observation, it was found that some of the beetles were close to each other with a near-complete lack of body movement (freezing) and did not interact with another beetle. In addition, to verify this behavior endpoint, manual observation was also conducted to count how many times the fighting sequence occurred during the test. Here, the interactions between beetles were counted as fighting when both beetles utilized their mandibles in direct physical contact with each other, which might lead to biting or lifting, or one contestant aggressively approached another contestant, which might result in a face-to-face physical contact or cause another contestant to run away. A fighting sequence was considered finished when there was a distinct winner and loser, which was indicated when the loser either decided to run away, fell off the tree log, or stayed apart from the winning beetle[2]. The contestants were declared winners in a contest test when they won in most of the fighting sequences according to the manual observation. For the sample video without any observed fighting sequence, the winner was decided based on which beetle secured the area around the food tube longer than the other contestant.

Statistical analysis

In the present study, the comparison of behavior endpoints between two species and between winner and loser beetles was analyzed by using an unpaired t-test. This test was chosen considering the data distribution normality of each group that was tested by using the Shapiro-Wilk and Kolmogorov-Smirnov tests, which later confirmed that each dataset passed the normality tests, prior to the t-test. A significant difference between groups is marked as * if $P<0.05$, ** if $P<0.01$, *** if $P<0.001$, and **** if $P<0.0001$. Meanwhile, in evaluating the correlation between the social interaction endpoints that were calculated from trajectories that were automatically generated by DAMM with the manual observation results in the form of fighting behavior count, the Pearson correlation test was conducted, as the majority of the data passed the distribution normality test. All of these statistical analyses and the graphs were carried out and plotted, respectively, by using GraphPad Prism (GraphPad Software version 8.0.2, La Jolla, CA, USA).

Ethical note

We conducted a statistical power analysis in all of the calculated behavior endpoints in every tested group to obtain information regarding the appropriateness of the used sample size in the present study to ensure that

sufficient statistical power was achieved while minimizing the number of animals used[19]. The required sample size was estimated using the following standard equation:

$$n = \left(\frac{Z\sigma}{E}\right)^2$$

where Z is the value from the table of probabilities of the standard normal distribution for the desired confidence level, E is the margin of error, and σ is the standard deviation. Based on the analysis results, the applied animal number in the present study (6 pairs for each species) was sufficient to provide data with a 95% confidence interval (CI) and a margin of error of 6 units. Meanwhile, to minimize stress on the stag beetles, we reduced unnecessary handling and ensured that only one observer was present in the room throughout the experiment. We followed ethical guidelines throughout the study, with all procedures approved by the Institutional Animal Care and Use Committee.

Results

Model performance

The current DAMM model performance was evaluated using object detection metrics, such as average precision, class accuracy, foreground class accuracy, and the F1 score. Since the current model was trained based on the *C. mniszechi* dataset, performance metrics were only calculated for this model. The model achieved a high average precision of 94%, which indicates that the majority of predicted bounding boxes were correct and relevant, as true positives were minimal (Fig. 5). Similarly, it also attained a class accuracy of 96% and a foreground class accuracy of 83%, which reflects strong performance in both overall and target-specific classifications. The overall F1 score is 70.2%, demonstrating that the DAMM performs well in detection and classifying objects, maintaining strong precision.

Observed behavior during fighting contest of *C. mniszechi*

Next, the x and y coordinates of each animal in every sample video output by DAMM from the current method were used to calculate several behavior endpoints that are important in observing the outcome of the recorded fighting contest. Here, the average speed, the distance between beetles, and the distance to the food tube of each stag beetle every minute were calculated. Based on the results from the sample videos, the winner could be easily indicated by the relatively closer and more stable value of the distance to the food tube value, which was also confirmed by manual observation (Fig. 6). Furthermore, higher locomotor activity in the loser than the winner was also observed since the losing beetle usually ran away after they lost in the contest (Fig. 6)[2]. In addition, in sample video 1, two troughs in the distance to the food tube of beetle 0 after beetle 1 had already steadily claimed the food tube were observed, together with two peaks of average speed in beetle 1. These phenomena indicated the battle occurrences during the contest that happened two times, which were later verified by manual observation (Fig. 6A; Table 1). However, this pattern might not be clearly shown in all of the sample videos, as some of the beetles were found to frequently approach the food tube and directly move away when they saw another contestant, as shown in sample videos 3 and 6. Next, while the outcome of the fighting contest in every sample video was relatively easy to determine, such as beetle 0 in sample videos 2 and 5 and beetle 1 in sample videos 3 and 4, by considering their closer distance to the food tube after the fighting contest was initiated for several minutes (Fig. 6B-E), a change in the winner position was observed in the sample video 5 as highlighted in Fig. 6F that was later confirmed by manual observation. In addition, the number of frames in which the position of the animals could not be detected by the system was also calculated, with an average of 2.9%, which highlighted the relatively high accuracy of the current method in assigning a specific ID for each individual.

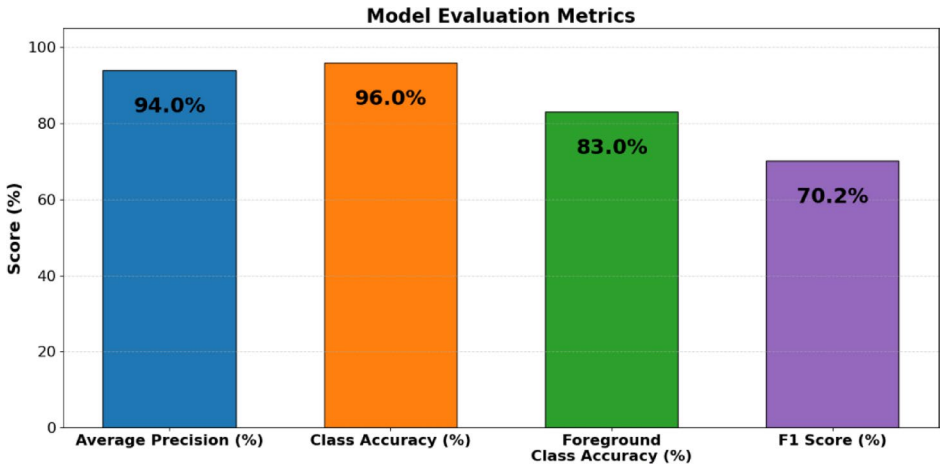

Fig. 5. The performance metrics of the current DAMM model from the *C. mniszechi* dataset that include average precision (94.0%), class accuracy (96.0%), foreground class accuracy (83.0%), and F1 score (70.2%).

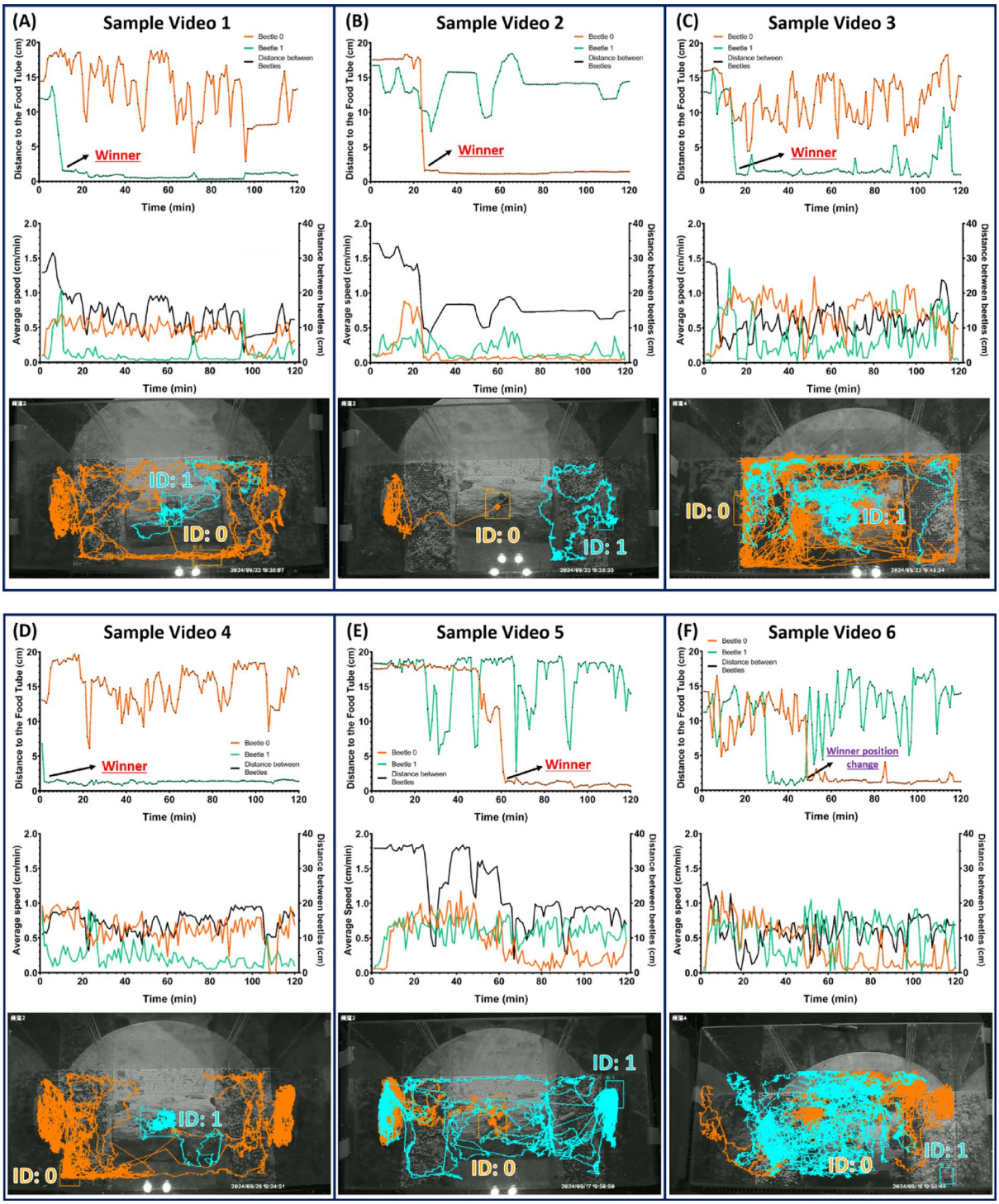

Fig. 6. Calculated distance to the food tube, average speed, and distance between beetles of the tested *C. mniszechi* throughout 120 min of contest test and representative figure of the condition when the winning beetle had been decided, including their movement trajectories, during the contest test from sample video (**A**) 1, (**B**) 2, (**C**) 3, (**D**) 4, (**E**) 5, and (**F**) 6.

Collectively, the calculated behavior endpoints that were based on the generated trajectories by DAMM in the current method could greatly help in distinguishing the winner contest.

Behavior endpoint differences between winner and loser *C. mniszechi*

To provide more insight regarding the behavior differences between the winner and loser *C. mniszechi*, several additional behavior endpoints were also calculated, and their values were statistically compared between these groups. The winning group consisted of an individual beetle in each sample video that had successfully defeated

Sample video	Beetle ID	Average speed (cm/s)	Average Angular Velocity (°/s)	Average distance to the food tube (cm)	Total of time spent in the rest area (%)	Fractal dimension	Entropy	Untracked frames (%)	Average distance between beetles (cm)	Total interaction time (%)	Fighting behavior count (times)
1	0	0.447	24.878	13.273	70.656	0.989	1.024	0.041	14.112	1.220	2
	1	0.119	5.713	1.587	6.002	0.367	0.576	1.002			
2	0	0.128	6.089	4.478	19.585	0.422	0.493	0.000	17.632	0	0
	1	0.189	10.502	13.896	94.583	0.472	0.782	1.955			
3	0	0.704	27.004	11.872	62.776	0.606	0.989	8.167	12.363	9.032	17
	1	0.341	16.176	3.243	12.567	0.571	0.858	1.152			
4	0	0.634	27.602	15.262	93.428	1.550	0.979	6.666	14.746	0.724	3
	1	0.224	10.363	1.364	0.146	0.429	0.795	0.000			
5	0	0.432	18.801	8.847	49.509	0.515	0.879	0.2111	23.886	1.381	2
	1	0.598	26.747	16.122	87.961	0.804	0.979	3.228			
6	0	0.378	17.599	5.260	27.356	0.874	0.807	1.110	12.199	9.835	13
	1	0.554	23.867	10.764	68.967	0.808	1.012	11.310			

Tsble 1. Summary of the calculated behavior endpoints of each *C. mniszechi* throughout 120 min of every sample video.

another beetle in the fights and stayed near the food tube for most of the time. From the calculations, the overall results of two behavior endpoints, which were average distance to the food tube and average speed, were consistent with the chronology data from Fig. 6, which were statistically higher in the winner beetles compared to the losers (Fig. 7A&C), that also caused the loser beetle group to have a statistically higher preference to stay longer in the rest area (Fig. 7B). Consistently, in terms of their movement orientation, the loser beetles displayed a statistically higher turning angle than the winners, which might be due to the loser beetles that were often moving around the tank since they could not secure the food tube for themselves (Fig. 7D). Furthermore, regarding their movement complexity, although no statistical differences were observed in the fractal dimension, a statistically higher entropy value was shown in the loser group compared to the winner group, which might hint at their unpredictable movement pattern (Fig. 7E&F). Finally, the body and mandible lengths between the winner and loser were also measured to evaluate the correlation between the outcome of the fighting contest and these physical factors. While winners exhibited significantly longer body lengths, no statistically significant difference in mandible length was detected. The observed trend toward slightly longer mandibles in winners warrants further investigation with larger sample sizes. Nevertheless, these results indicated that the body lengths might potentially play a significant role in the outcome of a fighting contest in this species (Fig. 7G&H).

Observed behavior during fighting contest of *C. speciosus*

Interestingly, based on the results, DAMM with the current dataset of *C. mniszechi* and a short fine-tuning process displayed a relatively feasible performance in the detection and tracking processes of *C. speciosus*, as shown in the average untracking frames percentage of 1.94%, which was even lower than the ones found in *C. mniszechi* data above during the one-hour video (Table 2). In terms of the fighting contest, by observing the calculated behavior endpoints, the patterns of the distance to the food tube and the average speed of some of the winning beetles were relatively distinct from the losers, which was similar to *C. mniszechi*, as shown with the lower distance to the food tube and average speed of beetle 0 for sample video 1 and beetle 1 for sample videos 2 and 3 (Fig. 8A-C). However, the determination of the winning beetle of this species in some cases was found to be more complicated if solely based on the calculated behavior endpoints. For example, in sample videos 4 and 5, while beetle 1 and beetle 0, respectively, showed a similar pattern to the winning beetle in sample videos 1 to 3 in their behavior endpoints, the manual observation found that these beetles were not the winner (Fig. 8D&E). Moreover, there was even a case where no beetles claimed the position around the food tube, although they were observed to fight each other (Fig. 8F). Next, the behavior endpoints between the winners and losers that were determined by manual observation were also compared. From the results, no statistical differences were found in every behavior endpoint comparison (Supplementary Fig. 1), although, some notable differences that showed a similar pattern with *C. mniszechi* data, such as average distance to the food tube, average speed, average angular velocity, and body length, are still worth to be mentioned (Supplementary Fig. 1A, C, D, & G). Taken together, *C. speciosus* possessed relatively different behaviors during the fighting contest compared to *C. mniszechi*.

Behavior endpoint differences between *C. mniszechi* and *C. speciosus* in the fighting contest

To provide more comprehensive and accurate comparisons regarding the behavior differences between these two stag beetle species that were hinted at in the previous section, each calculated behavior endpoint was statistically compared. Interestingly, no statistical differences were observed in every behavior endpoint comparison between these stag beetles. However, after closer investigation, some differences in each behavior aspect are still worth mentioning, including the tendency of relatively lower movement complexity of *C. speciosus* than *C. mniszechi* (Fig. 9A-D). Meanwhile, in terms of their social interaction, *C. speciosus* seemed to have more intense interaction with their conspecific, and the winners did not prefer to stay closer to the food tube and stayed longer in the rest area than *C. mniszechi* winners (Fig. 9E-I).

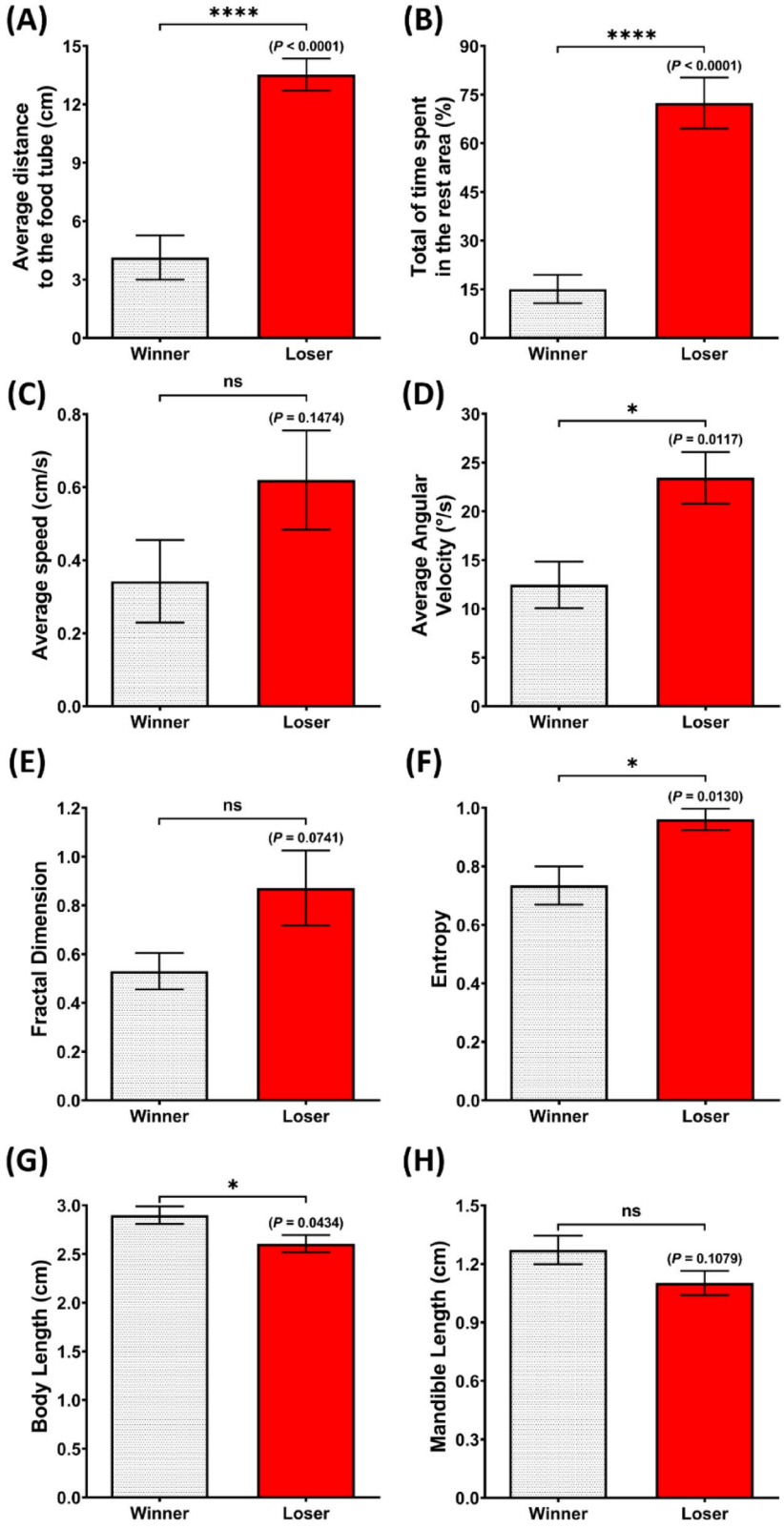

Fig. 7. Calculated (**A**) average distance to the food tube, (**B**) total percentage of time in the rest area, (**C**) average speed, (**D**) average angular velocity, (**E**) fractal dimension, as an index of movement complexity, (**F**) entropy, to quantify the variability and unpredictability of movement over time, (**G**) body length, and (**H**) mandible length of the winner and loser *C. mniszechi* throughout the 120-minutes of contest test from all of the sample videos. The data are expressed as mean with SEM and were analyzed by unpaired t-test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; $n = 6$).

Sample video	Beetle ID	Average speed (cm/s)	Average Angular Velocity (°/s)	Average distance to the food tube (cm)	Total time spent in the rest area (%)	Fractal dimension	Entropy	Untracked frames (%)	Average distance between beetles (cm)	Total interaction time (%)	Fighting behavior count (times)
1	0	0.234	13.808	4.850	14.861	0.251	0.868	0.000	12.397	8.843	19
	1	0.800	26.100	10.819	48.467	0.787	0.905	2.565			
2	0	0.552	9.856	9.654	36.271	0.950	0.681	0.439	9.165	12.167	4
	1	0.301	6.108	3.776	9.823	0.280	0.512	0.000			
3	0	0.361	12.272	3.861	6.322	1.224	0.740	0.985	11.515	8.974	7
	1	0.431	19.554	10.189	50.489	0.612	0.964	14.032			
4	0	0.534	13.350	11.640	64.390	0.099	0.825	3.428	12.565	4.611	4
	1	0.200	4.591	2.699	2.354	0.118	0.508	0.236			
5	0	0.350	14.576	6.899	32.387	0.239	0.661	0.333	13.417	7.078	8
	1	0.721	24.032	11.176	57.037	1.473	0.820	1.257			
6	0	0.135	5.961	13.010	78.693	0.096	0.538	0.000	19.564	1.248	1
	1	0.118	5.033	12.765	100.000	0.193	0.568	0.000			

Table 2. Summary of the calculated behavior endpoints of each *C. speciosus* throughout 60 min of every sample video.

Comparison of the social interaction endpoints from DAMM and manual observation

Since a similar pattern between the comparison of the average distance between beetles and fighting behavior counts for both stag beetle species was observed in the previous section (Fig. 9G&I), it would be intriguing to evaluate the accuracy of the social interaction endpoints that were calculated from trajectories that were automatically generated by DAMM in a comparison with the manual observation results in a form of fighting behavior count. Therefore, the Pearson correlation test in each dataset that consists of all sample videos for every stag beetle species was carried out to address this matter. From the results, the Pearson r value in both datasets was more than 0, indicating that there was a positive correlation, meaning that as one variable increases, the other tends to increase as well[20]. However, this r value was found to be in a different magnitude for each dataset. A higher Pearson r value (0.966) was observed in *C. mniszechi* data, together with the statistically significant *P* value (0.002) (Fig. 10A). This r value indicated a very strong positive linear relationship between the two calculated behavior endpoints, which was also supported low *P* value that indicates that the correlation is statistically significant. Meanwhile, a lower Pearson r value was found in *C. speciosus* data (0.401), which indicates a moderate correlation between those behavior endpoints (Fig. 10B)[21]. Moreover, no statistical difference was observed based on the calculated *P* value (0.430). Overall, the behavior endpoint that was calculated based on the automatically generated trajectories by DAMM has a positive correlation to the behavior endpoint that was calculated based on manual observation in both species, highlighting the potency of this method as an alternative method to evaluate social behaviors in stag beetles without the necessity of laborious manual work.

Discussion

Here, we successfully utilized DAMM to develop a novel tracking method that is able to accurately detect the position of stag beetles over time in a low-contrast environment with a relatively complex background. By using this method, we were able to obtain the x and y coordinates of each beetle automatically, enabling us to quantitatively analyze the behaviors of male *C. mniszechi* and *C. speciosus* during a contest test. In addition, besides utilizing DAMM, the performance of other tracking systems that are generally used for animal movement tracking was also tested, which were idTracker.ai and UMATracker, to better evaluate the tracking performance of the proposed method[22,23]. idTracker.ai is a computer vision-based software tool that employs deep learning techniques for tracking and analyzing the behavior of individuals in a group. Meanwhile, UMATracker is an open-source image-based tracking software for quantitative analysis in animal experiments. Unfortunately, neither tracker was able to accurately label the beetles, especially when their position was in the rest area. Thus, as shown in Supplementary Fig. 2, both trackers could only track the movement of one of the beetles once it climbed to the log where the food tube was located. These results were plausible since idTracker.ai and UMAtracker were designed for animals that are clearly distinguishable from the background, and they require even lighting to avoid reflections that can confuse the tracking algorithms. In addition, they also perform best in a static camera and non-changing background, which, in this case, these requirements were difficult to fulfill to provide an optimal condition for observing the fighting behaviors of stag beetles. Therefore, the test results show that the proposed method with DAMM outperformed idTracker.ai and UMATracker in analyzing animal movements in the current video conditions.

The DAMM was originally developed for mouse tracking in a very complex environment. Here, fine-tuned DAMM achieved an impressive average precision, suggesting that the model effectively localized and identified relevant instances with minimal false positives. Meanwhile, although the F1 score was relatively lower, it still reflects a reasonable balance between precision and recall. The fine-tuned model also achieved high class accuracy, which supports model robustness to identify the correct class in a very complex environment. Therefore, the DAMM results demonstrate that the model adapts effectively to a new biological domain, as its original design was for mice. DAMM was originally developed for tracking mice in a highly complex environment that is based

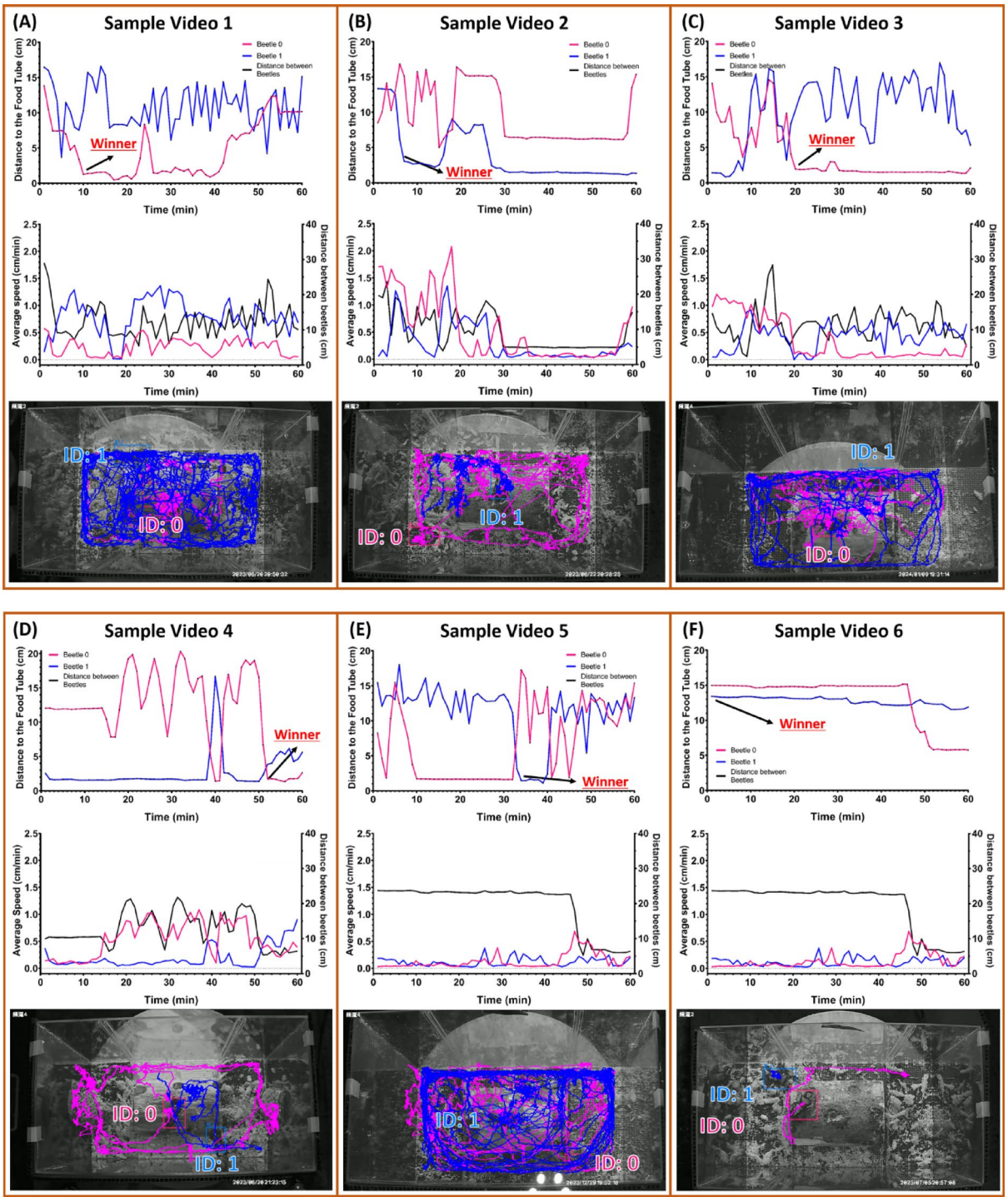

Fig. 8. Calculated distance to the food tube, average speed, and distance between beetles of the tested *C. speciosus* throughout 60 min of contest test and representative figure of the condition when the winning beetle had been decided, including their movement trajectories, during the contest test from sample video (**A**) 1, (**B**) 2, (**C**) 3, (**D**) 4, (**E**) 5, and (**F**) 6.

on Mask R-CNN architecture. In this study, the DAMM was fine-tuned and achieved an impressive average precision of 94%. A previous study by Fang et al. utilized Mask R-CNN architecture for multi-target detection and segmentation for autonomous driving in complex scenes and achieved 62.62% of mean average precision (mAP) for target detection[24]. Similarly, Shen et al. also used Mask R-CNN for instance segmentation in the grape cluster field by fusing an attention mechanism and achieved 60.1% average precision for the object detection task[25]. Thus, compared to other previous studies, this value of average precision is considered high, suggesting that the model effectively localized and identified relevant instances with minimal false positives. In simpler terms, the

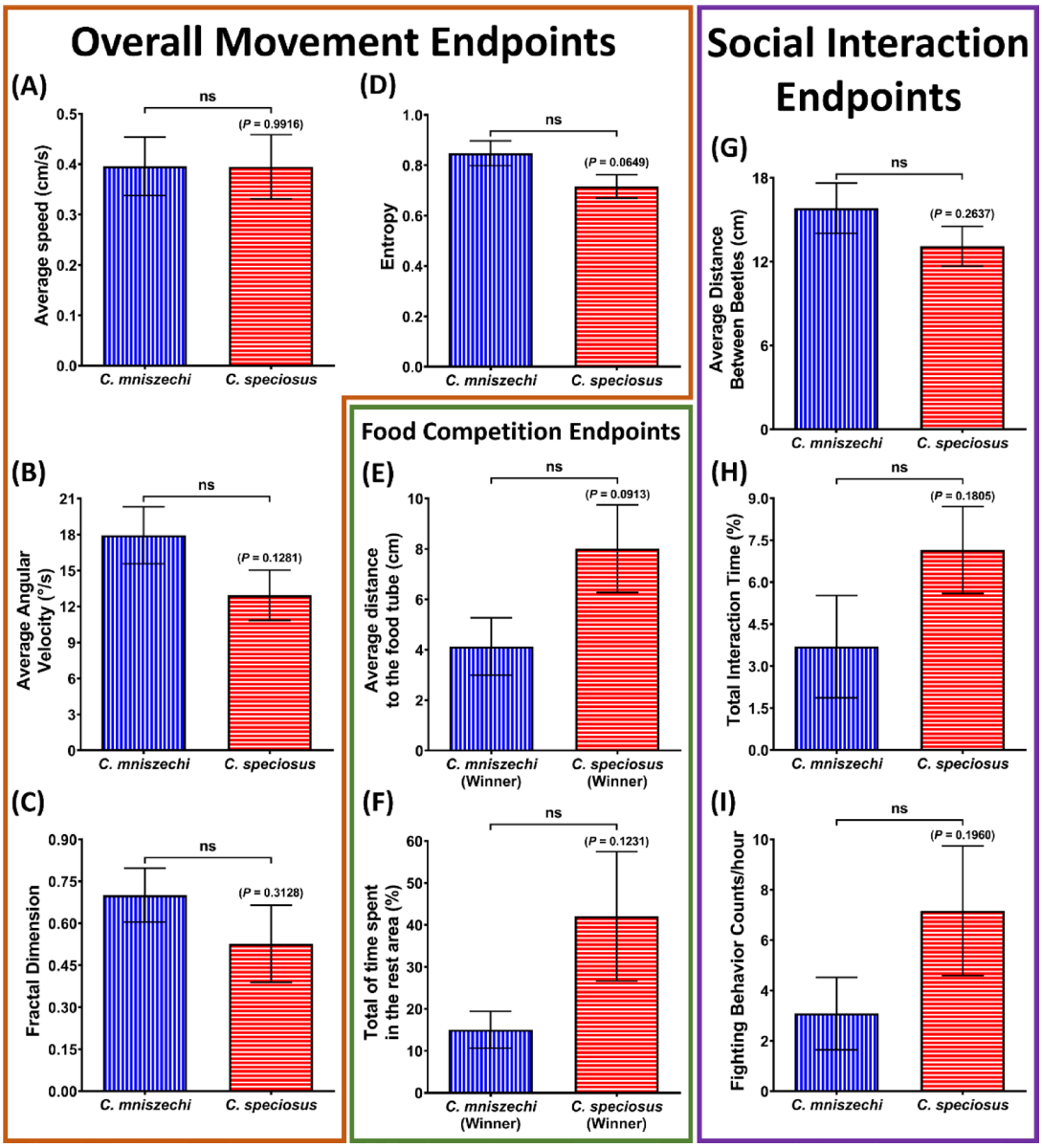

Fig. 9. Comparisons of overall movement endpoints ((**A**) average speed, (**B**) average angular velocity, (**C**) fractal dimension, as an index of movement complexity, and (**D**) entropy, to quantify the variability and unpredictability of movement over time), food competition endpoints of the respective winner beetles ((**E**) average distance to the food tube and (**F**) total percentage of time in the rest area), and social interaction endpoints ((**G**) average distance between beetles, (**H**) total interaction time, and (**I**) fighting behavior counts per hour) between *C. mniszechi* and *C. speciosus* from all of the sample videos. The data are expressed as mean with SEM and were analyzed by unpaired t-test ($n = 12$, except for Fig. 9E-I ($n = 6$)).

model correctly found the stag beetles in most of the frames and rarely confused background elements as stag beetles. In addition, our fine-tuned model also reached a relatively high score in class accuracy (96%), suggesting a correct identification class in a very complex environment. This result is important in complex scenes where multiple background objects' noise could confuse the model. Furthermore, the current DAMM also achieved foreground class accuracy of 83%, which indicates how accurate the model is in correctly recognizing the stag beetles. Foreground refers to the region of interest of the stag beetles instead of the background environment. Additionally, we also calculated the F1 score, which was 70.2%. Based on the previous study by Hongjie et al. in utilizing Mask R-CNN for automated identification and extraction of oil well sites, with an achieved average precision and F1 score of 60.93% and 61.59%, respectively, this value reflects a reasonable balance between precision and recall given task challenging nature of the task, showing that the model handled the challenging detection task reasonably well[26]. Finally, based on the manual observation in each analyzed video, the current DAMM model also performed sufficiently in accurately tracking the identity of the beetles, which usually becomes a major issue in multi-agent tracking, especially in a complex environment with occlusions. From

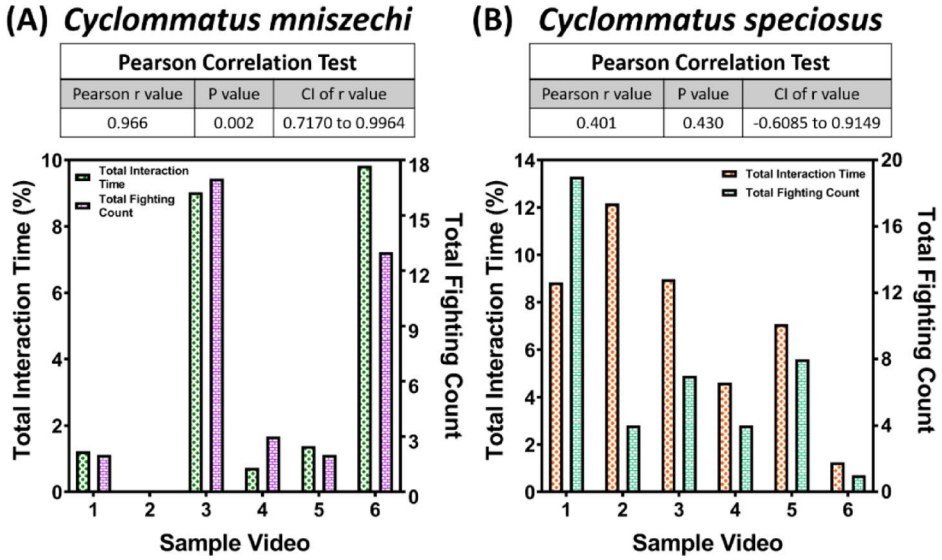

Fig. 10. Comparisons of calculated total interaction time percentage and total fighting count between (**A**) *C. mniszechi* and (**B**) *C. speciosus* in each sample video. In sample video 2 from Fig. 10A, no fighting behavior was observed by the manual observation method throughout the video, and the calculated total interaction time by the current method was also zero when the video was analyzed. Each dataset was analyzed by a Pearson correlation test to evaluate the correlation between these two calculated behavior endpoints. The calculated Pearson r value and P value for (**A**) were 0.966 and 0.002, respectively, while for (**B**) were 0.401 and 0.430, respectively.

the analyzed videos, although in some frames the current DAMM model faced some difficulties in the identity tracking during occlusion that occurred during their fighting sequence, we found that, eventually, the identity of each animal was still preserved after the encounters, maintaining the accuracy in the fighting outcome (winner position). In summary, DAMM was successfully implemented in a biological domain of stag beetle interaction analysis, which demonstrated the potential of Mask R-CNN-based models to transfer effectively across diverse and complex visual contexts and highlighted the adaptability and generalization capability beyond its original use case.

In this study, the calculated behavior endpoints that were based on the automatically generated trajectories by DAMM could greatly help in deciding the winner of each contest test for *C. mniszechi*. Based on these behavior endpoints, especially the distance to the food tube, most of the winning beetles were decided in the early time of the fighting contest (< 20 min), while the others were shown to be winners more than 30 min after the fighting contest was initiated. This phenomenon is plausible since the battles seem to depend on the beetle's motivation and opportunities that occur during the scuffle, with some battles starting hesitatingly and gradually intensifying, or even pugnacious from the beginning[2]. However, for the beetles that won the fight shortly after the test was initiated, there might be a relatively large size difference between the two beetles, as indicated in the statistically longer body length of the winning beetles observed in the current study, since generally, the largest beetle would win easily, settling the battle quickly[2]. Therefore, although literature was still vague on the practical advantage of longer mandibles in stag beetle battles, the current finding emphasized that the majority of the winner beetles possess longer mandibles compared to the losers, as also shown in other stag beetle species[4,27] and rhinoceros beetles[28,29]. Nevertheless, this finding showed the potency of the calculated behavior endpoints to assist researchers during a quantification process of a contest test as here, these DAMM-based calculated behavior endpoints indicated consistent outcomes regarding the winner beetles with the results from the manual observation, including the relatively more complicated results from sample video 6 that showed a winner change around ~ 50 min of the video (Fig. 6F). Taken together, these results indicated that this species prefers to secure the provided food tube, as in four out of six sample videos, the winning beetles arrived at the food station and fed on the jelly before the contests started. This phenomenon might also explain why the beetles that arrived at the food station were most likely to win fights since they possessed higher energy reserves while the losers' demand for energy supply drove the animals' motivation to find food, explaining the statistically higher total of time spent in the rest area, together with higher average angular velocity and entropy, that were observed during the test[9]. On the other hand, *C. speciosus* displayed relatively distinct behaviors from *C. mniszechi* in the contest test. To the best of our knowledge, this is the first study that evaluated the behaviors of *C. speciosus* in a contest test. From the results, no statistical differences were observed in all of the calculated endpoints between the winners and losers of this species. These findings highlighted that despite winning the fighting sequences, the majority of the winners did not directly dominate the feeding station throughout the test, as shown by *C. mniszechi*. Due to these differences, the overall behavioral performances of these stag beetles were also compared. Interestingly, no statistical differences were observed, although some substantial differences in the values of several endpoints might be worth taking into account since the absence of statistical differences might be due to the low sample

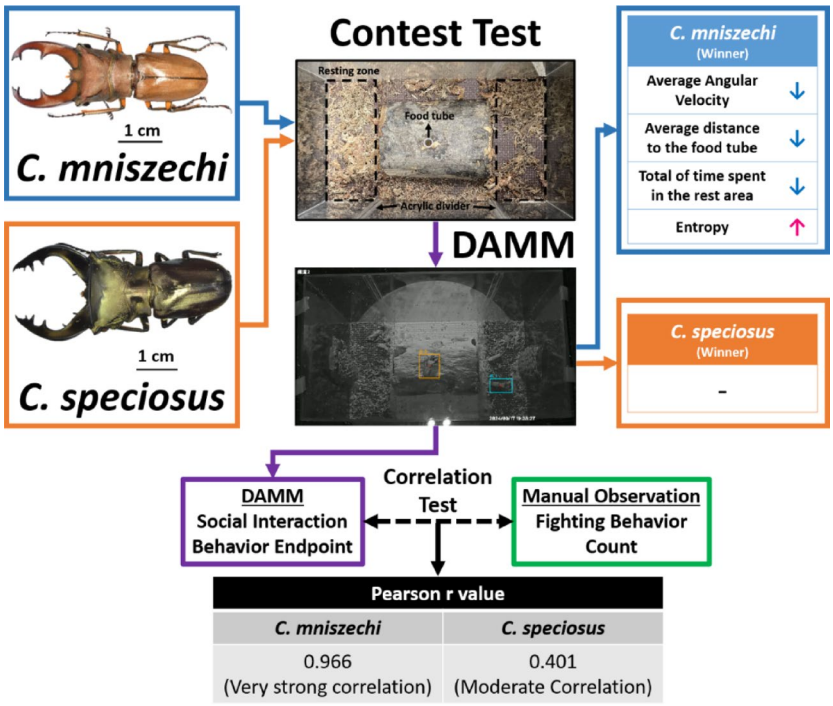

Fig. 11. Summary of the present study in applying DAAM to detect two species of stag beetles (*C. mniszechi* and *C. speciosus*) in a low-light condition with a complex background. The differences in behavior endpoints were observed in the winner *C. mniszechi* compared to the losers, while these differences were not found in *C. speciosus* (↓: lower, ↑: higher, -: no statistical differences). The correlation test between two calculated fighting behavior endpoints showed a positive correlation in both species.

size that was used in the current study, that would be intriguing to be addressed in future studies[30]. To sum up, despite their similar ratio in body and mandible lengths, winners and losers of *C. mniszechi* and *C. speciosus* displayed relatively different behaviors to each other in a contest test.

Next, considering the important role of stag beetles, especially *C. mniszechi*, as one of the animal models in studying animals' fighting behavior, it is important to establish a method that could quantify the fighting occurrence of these animals during test[3]. Thus, based on the generated trajectories by DAMM, the social interaction between animals was quantified as a total interaction time. In addition, to evaluate the accuracy and validity of this behavior endpoint, a correlation test was conducted to evaluate the correlation between this behavior endpoint and the total fighting count by manual observation of each sample video. Based on the correlation test, a very strong correlation was found in the *C. mniszechi* data, which indicated that this behavior endpoint could represent the social interactions that occurred during the test. Meanwhile, for *C. speciosus*, only a moderate correlation was observed. After further investigations, we found that this result was plausible since in some of the sample videos, there was a contestant who stayed close to another contestant without any social interaction, or the fighting sequences occurred in a short duration but in a more frequent manner, resulting in differences in the output of the total interaction time and fighting count by manual observation. Nevertheless, based on the positive correlation that was found in the correlation test between the two endpoints, we concluded that the current method could also be used as an alternative approach to analyzing the social behaviors of stag beetles, especially *C. mniszechi* and *C. speciosus*, without the necessity of laborious manual work.

Conclusion

To sum up, this study successfully applied DAMM to evaluate stag beetle behaviors during a contest test to output a rich dataset of quantitative information based on the generated tracking data, including the centroid positions of each detected beetle across every frame of the video, along with a unique identification label for each individual. Based on the results, *C. mniszechi* was found to be a more suitable stag beetle compared to *C. speciosus* in the contest test due to their robust behavior in capturing a food tube while fighting with another contestant. In addition, the calculated behavior endpoints based on the generated trajectories also showed relatively consistent results with manual observation, signifying the validity of the output data (Fig. 11). However, several limitations were still faced by this method. First, the current method is not able to perform a pose estimation, which identifies and tracks the positions of specific body parts of an animal to extract detailed information about an animal's posture, movement kinematics, and interactions with its conspecifics. Second, while the current study showed its capability to detect and track the movement of *C. speciosus* by using the training dataset of *C. mniszechi* with only a short fine-tuning process, ones have to keep in mind that these stage beetles shared a relatively similar morphology, thus, an annotation process might be required to provide a new training dataset

for other species that possess a different morphology. In addition, this might also be the reason for the lower r value of *C. speciosus* data in Fig. 10. Therefore, we believe that another annotation process that is specific to this species could generate better results. Nevertheless, this highlights the performance of DAMM in evaluating independent data (*C. speciosus* videos) by using only the training dataset that was derived from *C. mniszechi* videos, although ones have to keep in mind that even during dataset training, only several frames in *C. mniszechi* videos were used, leaving other unannotated frames as unseen data. Finally, we also observed that there were untracked frames in some of the sample videos that reached ~ 14% in the worst case, as shown in Tables 1 and 2. Based on our observation, this occurred when the animals were hiding or strangled in the wood log or *Spaghnum* moss, respectively, flying around the tank, or staying in some corners of the tank with a very low brightness that was even difficult for human eyes to identify their position. However, we believe that this limitation can be overcome with more precise fine-tuning, by conducting analysis using a dataset that was specifically trained for this species as an example, and better lighting and video recording conditions. Nonetheless, the current method could potentially open up exciting new avenues for quantitative insect behavior research in the future, such as comparing fighting behaviors across different stag beetle species or between males exhibiting different mandible morphologies to further enhance our understanding of the evolution and function of their fighting behaviors and impressive weapons, and investigate the influence of various environmental factors, such as temperature and humidity, on stag beetle fighting behavior under low light conditions that could provide valuable ecological insights. Finally, the current DAMM-based method offers a promising starting point for automated detection and tracking, and with potential fine-tuning and adaptation for pose estimation, it could provide a powerful tool for analyzing the intricate dynamics of stag beetle combat, opening up exciting new avenues for quantitative insect behavior research.

Data availability

All of the recorded videos that were used in the present study can be found at (https://zenodo.org/records/15377746) for *C. mniszechi* and (https:/zenodo.org/records/15627979) for *C. speciosus* . All of the tracked videos and the full set of annotations used in the present study can be found at (https://zenodo.org/records/15632270 and https:/zenodo.org/records/18467837).

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Author contributions
G.A. performed statistical analyses, summarized results, and manuscript writing, C.H.H and M.Y. prepared samples and carried out experiments, I.M.T. drafted the manuscript, C.D.H. coordinated the project and directed the study, and C.P.L. obtained financial support, and was responsible for study design. All of the authors reviewed, approved, and contributed to the final version of the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

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