Examination of fecal pellet physical characteristics of an invasive drywood termite, Cryptotermes dudleyi (Isoptera: Kalotermitidae): A potential approach for species marker and non-destructive monitoring method

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Abstract. Cryptotermes, one of the major drywood termite genera in Indonesia, is a cryptic species that isolates their presence inside a wood piece. Due to its cryptic lifecycle hidden away inside wood, monitoring its presence and also identifying the corresponding species has been a difficult process. One of the Cryptotermes species, native to Java island, Indonesia, is Cryptotermes dudleyi. In this preliminary study, we used C. dudleyi as a species model to find out whether fecal pellet physical characteristics can be used as a stable species marker to assist in non-destructive monitoring surveillance. The characteristics used were maximum diameter, diagonal width of maximum diameter, and 2D surface area and area perimeter. The study used fecal pellets from orphaned and mature colonies and three different dietaries (grass, hardwood, and softwood) to check whether the characteristic value of fecal pellets is narrow over various influences. The results showed that each characteristic tends to have unique mean and also its unique value range which depends on their collection site condition. Due to unique means and value range, the species marker become not so accurate and not robust enough as consequences in accommodating these unique means and value range. On the other hand, employing completely new clustering based on like-like axiom on individual fecal pellets may results in robust species marker as long as adequate data comparison from other species fecal pellets to validate the species marker is available.

1. Introduction
Drywood termite has been characterized for its habit to dwell and feed in a single dry wood (a one-piece nester) which put the colony in a protected environment [1,2]. Most of the time, this protected environment is a part of human living space which makes drywood termites a pest since they can attack various objects made of wood in human space such as furniture, flooring, up to the body of house itself [3,4]. This condition makes monitoring the drywood infestation to be important especially for the prevalent environment like wooden building or various materials made of cellulose [4]. Several approaches have been developed in the monitoring of drywood termite infestation. These methods ranging from finding fecal pellet next to the wood, finding termite’s wings near the wood, conducting
focused light over the wood to wood scan using x-ray tomography [4-9]. The various development in monitoring method makes the monitoring of drywood termite has been easier than before. On the other hand, the main issue of identifying the species of drywood termites remain a difficult process.

The concern for identifying the species of drywood termite has become more relevant nowadays due to the drywood termite strict in festation that favored wood low moisture level and the surrounding environment warm temperature [10-12]. While normally such conditions limit drywood infestation and its potential to spread to other region, current era of globalized trade with modern housing equipped with controlled temperature has turned our house into the most viable place for drywood termite to breed. This situation makes drywood termites that were distributed fragmentally based on regions seasonal weather and general climate to be unrestricted and can cross over to other regions easily [4,13,14]. To combat this issue, the ability to identify and monitor the species of drywood termites has become important especially in the context of goods transfer across the world and also the biosecurity of the corresponding area.

The easiest way to identify termite down to species level is through the morphological features. Regarding the drywood termite, the morphospecies method might not be the easiest to conduct, not because of the difficulty in distinguishing the morphological features between species, but due to the difficulty in procuring the specimen in the first place. The only feasible method to get the specimen is through wood dissection [4,15]. While wood dissection manages to get the specimen out, our past experience results in a high number of destroyed specimens due to dissection shear force effected to termite inside the gallery in the wood. This situation makes the viable number of specimens to be used become smaller. Adding the fact that soldier was found in low abundance in drywood termite makes the wood dissection sometimes yielded no viable specimen to be identified. To counter this issue, a method to identify drywood termite without the specimen need to be developed.

One of the promising ideas is through fecal pellet as a mean to identify the species that may be useful for non-destructive approach for drywood termite monitoring and inspection. Earlier studies had showed that fecal pellet hydrocarbon composition can track the termite species without the specimen [16]. Another past study also showed, albeit ambiguously, that the fecal pellet of Cryptotermites dudleyi and Cryptotermes cynocephalus can be distinguished based on length and width dimension [17]. While this result might indicate the viability of fecal pellet physical characteristics as species differentiator, we are interested on whether these physical characteristics can be relatively unchanging under various condition and feed type. On this basis, we try to verify and conduct in-depth study regarding the suitability of physical characteristics of fecal pellets of drywood termite to be a species marker. To do so, we used a native drywood termite of Java Island, C. dudleyi, a well-known dry-wood termite pest as the species model in our study.

2. Materials and Methods

2.1 Termite source and fecal pellets collections

The colony of drywood termite C. dudleyi that was used for short-term laboratory scale experiment with various dietaries subjection, was collected from Bogor, Indonesia. The mature colony of drywood termite that was used for long term field scale was collected from Termite Rearing Laboratory, Research Center for Biomaterials LIPI, Cibinong, Bogor, Indonesia.

2.2 Fecal pellets observation

2.2.1 Orphaned colony-laboratory scale experiment

The experiment was conducted using three different feed sources (bamboo, rubber tree, and pine tree) representing grass, hardwood, and softwood. All of the feed sources were cut into blocks of 2 x 1 x 1 cm. For each feed source, 100 workers and 5 soldiers of orphaned colonies of C. dudleyi were introduced into 10 blocks of feed source and kept in a dark room. After 6 months, accumulated pellets were collected to be measured. An orphaned colony of at least two years old that was fed with hardwood was also added to the test as a comparison data, later referred as L.A1 in the study.
2.2.2 Mature colony-field scale experiment.
The collection of C. dudleyi fecal pellets were from four different types of hardwood timbers that has been infested for years, estimated more than 10 years. The termites were collected through through chipping the wood. The fecal pellets were collected from inside the infested wood and then put into sealed zipper bags for further measurement.

2.3 Fecal pellets measurement
The collected fecal pellets from the laboratory scale and field collection was counted and labeled as indicated in Table 1.

| ID label | Experiment set-up | Termite feed | Fecal pellets (pieces) |
|----------|-------------------|--------------|-----------------------|
| F.C1     | Field scale       | Hardwood     | 434                   |
| F.C2     | Field scale       | Hardwood     | 470                   |
| F.C3     | Field scale       | Hardwood     | 392                   |
| L.A1     | Laboratory scale  | Hardwood     | 546                   |
| L.B      | Laboratory scale  | Grass        | 284                   |
| L.R      | Laboratory scale  | Hardwood     | 378                   |
| L.P      | Laboratory scale  | Softwood     | 168                   |

These fecal pellets were observed using Keyence VHX 6000 (Keyence Corp., Osaka, Japan) using 150x magnifications over 225 stitched collated pictures for each type of feed. The measurement was conducted using auto grain function from Keyence Communications Tools (Keyence Corp., Osaka, Japan) with measured characteristics of area, perimeter, maximum diameter, minimum diameter (widest width), and diagonal width of maximum diameter.

2.4 Data analysis
The measured characteristics were then turned into monotonic variables as described below,

\[
\text{Shape Ratio (SR)} = \frac{\text{diagonal width}}{\text{maximum length}}
\]

\[
\text{Enclosure Ratio (ER)} = \frac{\text{perimeter}}{2(\text{max diameter} + \text{diagonal width})}
\]

The Shape Ratio (SR) score indicates the general outline of fecal pellets. The closer the number to 1, the pellet’s shape will be like a ball or cube while closer to 0 will indicate extreme ellipse or rectangle like shape. The Enclosure Ratio (ER) score indicates further classification of the shape ratio score in which closer to one might indicates a cube or rectangle while closer to 0 will indicates a ball or ellipse. The ER score of more than 1 indicates perimeter that was too wavy and meandering that might not well represent the perimeter.

At first, these monotonic variables, area and perimeter were tested according to several factors such as the generalized effect of colony condition between orphaned-laboratory scale, and mature field scale, the generalized effect of feeding materials (grass, softwood and hardwood) and specially for the condition of the orphaned colonies, the effect of different hardwood, albeit not yet identified in the field case, and the effect of consuming hardwood in different colony condition. We were interested in the effect of these factors in shaping the physical characteristics of the produced fecal pellets. These comparison tests were conducted using Kruskal Wallis test. To complete the comparison at collection site level, we also conducted site-to-site comparison using Wilcox test.

The data calculation was conducted in R (version 3.6.2, R Core Team, https://www.R-project.org/). The Kruskal-Wallis and Wilcox test were conducted using rstatix R package (version 0.6.0, A.
The test was grouped based on factors to consider as mentioned previously and direct site to site comparison. The test also compared each collection site to the base mean of the pooled data for each characteristic. Data presentation was using ggpubr (version 0.4.0, A. Kassambara, https://CRAN.R-project.org/package=ggpubr) and ggplot2 r packages [18].

3. Results
The study managed to collect 1,296 fecal pellets from field scale collection (F.C1 = 434, F.C2 = 470, F.C3 = 392) and 1,376 fecal pellets from laboratory scale experiment (L.A1 = 546, L.R = 378, L.B = 284, L.P = 168). All of these fecal pellets were measured and then presented in boxplot together with the result of Wilcoxon test against the base mean of the corresponding characteristics are shown in Figure 1.

**Figure 1.** The boxplot of characteristics value of observed characteristic (A) Shape Ratio (SR), (B) Enclosure Ratio (ER), (C) Area, and (D) Perimeter from each collection site. The post-hoc marker was the p value adjusted significance generated using unpaired Wilcoxon test against the base mean of the corresponding characteristics, ns: not significant
Based on Figure 1, the spread of the characteristics value was not equally distributed with a lot of outlier, especially in enclosure ratio (ER) data that showed anomaly. The anomaly was indicated by some ER value from C1 and C2 that exceed 1 which indicated the perimeter has extreme waviness. This condition made generalized interpretation of fecal pellets shape in these collection site to be prone to mistake once closing to score 1 (Figure 1B).

Another observation also showed that there was a clear difference \((p = < 2 \times 10^{-16})\) in fecal pellets 2D surface area between the laboratory experiment (L.A1, L.R, L.B, and L.P) and the field experiment (F.C1, F.C2, and F.C3), see Figure 1C. A similar observation was also observed in perimeter category \((p = < 2 \times 10^{-10})\). The only parameter that was not significantly different between the two experiment was the shape ratio \((p = 0.34)\). An observation from Figure 1C also showed increasing size of fecal pellet from orphaned colony (L.R and L.A1) to mature colony (F.C1-3).

Due to the limitation of the graphical marker, only the Wilcox test against the base mean of all of the corresponding value was used as the reference in deriving the significant difference in Figure 1. The rest of the comparison between factors was tested and the results are presented in Table 2 below.

| Factors | Character          | n  | Statistic | df | p   | p.adj | p.adj. signif |
|---------|--------------------|----|-----------|----|-----|-------|---------------|
| Global effect from different colony condition (orphaned-laboratory and mature-field colony) | Shape Ratio (SR) | 2672 | 6.900685223 | 1 | 0.00862 | 0.00862 | ** |
|        | Enclosure Ratio (ER) | 2672 | 798.9699715 | 1 | 9.04E-176 | 9.04E-176 | **** |
|        | Area               | 2672 | 1207.134831 | 1 | 1.72E-264 | 1.72E-264 | **** |
|        | Perimeter          | 2672 | 1404.987676 | 1 | 1.73E-307 | 1.73E-307 | **** |
| Global effect from different feeding material regime (hardwood, softwood and grass) | Shape Ratio (SR) | 2672 | 185.7544109 | 2 | 4.61E-41 | 4.61E-41 | **** |
|        | Enclosure Ratio (ER) | 2672 | 226.3501713 | 2 | 7.06E-50 | 7.06E-50 | **** |
|        | Area               | 2672 | 849.1043295 | 2 | 4.16E-185 | 4.16E-185 | **** |
|        | Perimeter          | 2672 | 849.6756433 | 2 | 3.13E-185 | 3.13E-185 | **** |
| Feeding effect of different hardwood in mature-field colony | Shape Ratio (SR) | 1296 | 54.38302957 | 2 | 1.55E-12 | 1.55E-12 | **** |
|        | Enclosure Ratio (ER) | 1296 | 757.6002249 | 2 | 3.08E-165 | 6.16E-165 | **** |
|        | Area               | 1296 | 260.4235504 | 2 | 2.82E-57 | 2.82E-57 | **** |
|        | Perimeter          | 1296 | 267.7196189 | 2 | 7.34E-59 | 7.34E-59 | **** |
| Feeding effect between hardwood, softwood, and grass in orphaned-laboratory colony | Shape Ratio (SR) | 1376 | 186.8519923 | 3 | 2.92E-40 | 5.84E-40 | **** |
|        | Enclosure Ratio (ER) | 1376 | 37.21191773 | 3 | 4.15E-08 | 4.15E-08 | **** |
|        | Area               | 1376 | 474.9808627 | 3 | 1.26E-102 | 2.52E-102 | **** |
|        | Perimeter          | 1376 | 463.8393194 | 3 | 3.27E-100 | 6.54E-100 | **** |
| Feeding hardwood effect in different colony state (orphaned-laboratory and mature-field colony) | Shape Ratio (SR) | 2220 | 9.648243082 | 1 | 0.0019 | 0.0019 | ** |
|        | Enclosure Ratio (ER) | 2220 | 598.4695654 | 1 | 3.60E-132 | 3.60E-132 | **** |
|        | Area               | 2220 | 746.6913517 | 1 | 2.10E-164 | 2.10E-164 | **** |
|        | Perimeter          | 2220 | 953.9219429 | 1 | 1.86E-209 | 1.86E-209 | **** |

The result in Table 2, clearly demonstrated that for each factor accounted in this study, all of them showed a stark contrast in characteristic value which unwittingly expands the range for physical characteristics value that can be attributed to *C. dudleyi*. To better understand the scope of range existed for *C. dudleyi* according to our measured physical characteristics, multiple random, direct site to site comparison under Wilcox test was used to test the limit of physical characteristics that belong to *C. dudleyi*. The result was presented in Table 3.
Table 3. *Wilcoxon* test results for all of the possible combination in two measured characteristics and two monotonic characteristics

| Combination | Shape Ratio (SR) | Enclosure Ratio (ER) | Area (μm²) | Perimeter (μm) |
|-------------|------------------|----------------------|------------|----------------|
|             | gr.1 | gr.2 | P       | p_adj | p_adj.signif | P       | p_adj | p_adj.signif | P       | p_adj | p_adj.signif |
| F.C1        | 3.47E-12 | 3.82E-11 | ****   | 1.01E-125 | 1.82E-122 | ****   | 2.54E-06 | 7.62E-06 | ****   | 7.04E-51 | 5.63E-50 | ****   |
| F.C1        | 1.86E-08 | 1.86E-07 | ****   | 1.13E-125 | 2.15E-124 | ****   | 1.28E-33 | 5.12E-33 | ****   | 2.71E-07 | 8.13E-07 | ****   |
| F.C1        | 1.41E-14 | 1.97E-13 | ****   | 1.58E-154 | 3.32E-153 | ****   | 2.30E-50 | 1.84E-49 | ****   | 1.76E-111 | 3.17E-110 | ****   |
| F.C1        | 0.000228 | 0.001824 | **     | 5.62E-127 | 1.12E-125 | ****   | 1.54E-87 | 2.46E-86 | ****   | 4.15E-117 | 8.72E-116 | ****   |
| F.C1        | 0.013 | 0.078 | ns     | 3.72E-111 | 6.32E-110 | ****   | 1.11E-106 | 2.00E-105 | ****   | 3.38E-112 | 6.42E-111 | ****   |
| F.C1        | 1.11E-34 | 2.22E-33 | ****   | 4.45E-79 | 7.12E-78 | ****   | 4.26E-77 | 5.54E-76 | ****   | 7.88E-80 | 1.10E-78 | ****   |
| F.C2        | 0.412 | 0.568 | ns     | 0.006 | 0.024 | *     | 8.65E-52 | 7.79E-51 | ****   | 5.21E-33 | 2.08E-32 | ****   |
| F.C2        | 0.284 | 0.568 | ns     | 1.01E-31 | 1.21E-30 | ****   | 2.45E-34 | 1.23E-33 | ****   | 1.04E-45 | 7.28E-45 | ****   |
| F.C2        | 0.026 | 0.13 | ns     | 1.63E-35 | 2.28E-34 | ****   | 1.44E-70 | 1.73E-69 | ****   | 8.57E-71 | 9.43E-70 | ****   |
| F.C2        | 1.14E-17 | 1.82E-16 | ****   | 3.62E-45 | 5.43E-44 | ****   | 1.26E-107 | 2.39E-106 | ****   | 4.96E-108 | 8.43E-107 | ****   |
| F.C2        | 1.30E-17 | 1.95E-16 | ****   | 1.23E-18 | 1.11E-17 | ****   | 3.87E-78 | 5.81E-77 | ****   | 4.74E-76 | 5.69E-75 | ****   |
| F.C3        | 0.076 | 0.304 | ns     | 6.05E-20 | 6.05E-19 | ****   | 2.29E-95 | 3.89E-94 | ****   | 3.26E-97 | 4.89E-96 | ****   |
| F.C3        | 0.164 | 0.492 | ns     | 1.48E-24 | 1.63E-23 | ****   | 9.22E-115 | 1.94E-113 | ****   | 2.60E-112 | 5.20E-111 | ****   |
| F.C3        | 1.55E-13 | 1.86E-12 | ****   | 2.02E-34 | 2.63E-33 | ****   | 2.93E-108 | 5.86E-107 | ****   | 6.47E-108 | 1.04E-106 | ****   |
| F.C3        | 5.68E-18 | 9.66E-17 | ****   | 6.03E-13 | 4.82E-12 | ****   | 4.10E-78 | 5.81E-77 | ****   | 1.15E-77 | 1.50E-76 | ****   |
| L.A1        | 0.003 | 0.021 | *     | 0.002 | 0.01 | **     | 8.59E-05 | 0.0001718 | ****   | 0.007 | 0.007 | **     |
| L.A1        | 9.66E-20 | 1.84E-18 | ****   | 9.72E-09 | 6.80E-08 | ****   | 1.60E-60 | 1.76E-59 | ****   | 1.24E-63 | 1.24E-62 | ****   |
| L.A1        | 1.94E-14 | 2.52E-13 | ****   | 0.852 | 0.852 | ns     | 1.30E-45 | 7.80E-45 | ****   | 9.52E-39 | 4.76E-38 | ****   |
| L.R         | 5.15E-08 | 4.64E-07 | ****   | 0.022 | 0.066 | ns     | 1.74E-56 | 1.74E-55 | ****   | 7.93E-61 | 7.14E-60 | ****   |
| L.R         | 5.59E-19 | 1.01E-17 | ****   | 0.025 | 0.066 | ns     | 5.75E-47 | 4.03E-46 | ****   | 1.39E-40 | 8.34E-40 | ****   |
| L.B         | 3.91E-38 | 8.21E-37 | ****   | 6.97E-06 | 4.18E-05 | ****   | 0.751 | 0.751 | ns     | 0.002 | 0.004 | **     |

Note. gr. = group; p = p value; p_adj = p value adjusted; p_adj.signif. = p value adjusted significance
The results of Wilcoxon test in Table 3 paint a better approximation of how different the value of each characteristic from each collection site and the range of physical characteristics value. It was interesting to note in the SR characteristic from the field collection that only F.C1 that was different significantly in value compared to the rest. On the other hand, the L.R and L.A1 from the laboratory scale was not so different in SR value compared to F.C2 and F.C3 from the field collection. This result may suggest that SR value are stable across most of the collection site that feed on hardwood, the SR value itself is too wide in range that this stable characteristic value might not yield much meaningful information on the fecal pellets formation. Regarding the rest of the characteristics in Table 3, the only other interesting observation was the surface area between L.B (bamboo) and L.P (pine) was not significantly different although the SR characteristics showed a stark difference from each other for these two samples.

![Figure 2](image)

**Figure 2.** Pooled histogram from seven collection site for characteristic (A) Shape Ratio (SR), (B) Enclosure Ratio (ER), (C) Area, and (D) Perimeter. The data was split into 200 bin per characteristic to visualize the break in the data continuity. The vertical dashed line in the middle of each plot was the median marker

Summing up the observation made from Figure 1 together with Table 1 and Table 2 indicated that although various factors comparison showed stark contrast to the rest characteristics value other than in SR, once the individual collection site was directly compared to each other, the results still showed a significant difference between collection site. Therefore, due to each collection site resulted in
significantly different physical characteristic’s value that differs from one another, we can state that the range of physical characteristics value that belongs to *C. dudleyi* comes from the entire data set almost without repetition other than for SR characteristic.

To get the best approximation of the physical characteristic’s value range from each variable, we have built a histogram from each characteristic to visualize the range as presented in Figure 2. The pooled distribution data as presented in figure 2 showed that SR has normal distribution which explains its *Kruskal Wallis* test result. On the other hand, the other characteristics distribution showed unequal distribution with some cases of gradual leaning favoring one side (skewness) or a series of choppy hills across the plot. The observed skewness indicated step-change in fecal pellets features with prominent example in ER histogram plot (Figure 2B) and presence of gap in termite fecal pellet sizes as shown in Figure 2C and 2D.

The result depicted in figure 2 demonstrated that generally fecal pellets of *C. dudleyi* will have these characteristic’s value range in which certain smaller range may corresponds to details regarding the species condition whether it was the colony condition or its feed material. The generalized characteristic’s means and standard deviation shown below in Table 4.

![Table 4. Generalized physical characteristics of the observed *C. dudleyi*

| Characteristic | mean  | median | SD    | IQR   | MAD  |
|----------------|-------|--------|-------|-------|------|
| SR             | 0.6582| 0.6569 | 0.0512| 0.0672| 0.0497|
| ER             | 0.8631| 0.8523 | 0.0359| 0.0286| 0.0193|
| Area (um2)     | 263530.65| 260374.50| 61231.52| 95104.25| 69960.19|
| Perimeter (um) | 2046.28| 2029.00| 280.92| 445.25| 326.17|

Note. SD = Standard Deviation, IQR = Interquartile Range; MAD = Median Absolute Deviation

4. Discussion
The physical characteristics of fecal pellets from *Cryptotermes dudleyi* have been shown to be distributed in large spread of value for each corresponding characteristic (Figure 1 and 2). This range of value was made through data composite by combining each collection site characteristic’s value. The need to pool all of the data into one arise due to each collection site individual means and value range data which showed to be significantly different than other sites (Table 3). The key issue remains on what drive the different means for each value range as observed in each collection site.

To address this key issue, we tried group comparison to assess factors that may influence the formation of fecal pellets. While we acknowledge that our data combination was actually shaped by two clumped-factors (colony condition tied with variance in feeding materials) that cannot be separated further cleanly, this data can still give a generalized view of the two factors that was the effect of different colony condition and feeding materials at larger scale once it constituent sub-factors were also analyzed. In our case, the analysis was done by comparing a lot of interrelated factors. These interrelated factors can be seen after the *global factors* in Table 2.

Our analysis showed that there is a clear effect (*p* < 2 x 10^-16) from the condition of the colony to the fecal pellet formation, in which an orphaned, newly inducted colony will generally yield smaller size fecal pellet than a mature colony. To understand this further, the comparison results in feeding hardwood between two colony condition and direct site-to-site comparison between site in field scale compared to L.R confirmed that they are different in means although the characteristics value range can overlap each other at some range position (Figure 1). This is the first report that documented this condition. There are several biological factors that might answer this observation such as stress reaction after being removed and inducted in new place, and change in general caste composition that favored certain caste [4,19]. Further studies are required to find which exact factor that play into this observed condition.

Another observation also showed that there was a clear effect of different feeding materials. The finding in the orphaned colony found that fecal pellet made of pinewood and grass to be smaller in size and also similar to each other, as indicated by smaller value of surface area and perimeter compared to
fecal pellet produced by eating hardwood (Table 2). While this seems to indicate that different feeding material can result in different means value for the characteristics, feeding on the same diet (hardwood) also gave significantly different means with their corresponding value range.

As additional note, while we only restricted our study to physical characteristics measurement, we also observed that some fecal pellet is more fragile compare to other with special note to fecal pellet produced by *C. dudleyi* eating pinewood (L.P). The impression of fragile was indicated by finding a lot of broken fecal pellet that was cut off in the middle or having its cone section cut off which makes them unviable to be measured. The fragileness observed might be related to the type of feed since fecal pellets produced from other source was not as fragile as fecal pellets derived from pinewood (softwood).

To understand the varied sizes of fecal pellets, the mechanism behind the production of fecal pellets would certainly help. As past studies have reported, drywood termite fecal pellets will typically have six-sided/hexagon shape in the middle bounded by a dome in one side and a cone in the other side. This typical shape is most likely generated due to the molding effect of termite anus sphincter muscle that shape the feces into pellets [11]. Based on regular diet of drywood termite, water will be absorbed to the greatest level which could explain the pellet form of the fecal matter as form of water conservation [12,20]. As comparison, past study and our experience in collecting termite feces in subterranean termite found wet and sticky fecal matter since subterranean termite lives in location with water abundance [21]. The pellets also served a function as plug hole to cut off the colony from outside disturbance when needed [1].

After discussing the possibility of factors that influence the varied sizes of fecal pellets, the question is the usage of these physical characteristics as species marker. Our current study clearly indicates that the features of fecal pellets characteristics are too wide and far from being uniform (Table 2). Trying to use them directly as a definitive species marker is possible but due to the value range that is too wide, the filter created through the measured characteristics web will most likely introduced a lot of false positive from other drywood termite species that shared similar measured characteristics to our current data. This will become a hit and miss situation with more miss to be expected.

To find a solution to not-so-sensitive physical value range of *C. dudleyi*, we believe that the solution can be derived by clustering the individual fecal pellets based on its physical characteristics measurement through an axiom with the end goal to reach a data uniformity or a data fingerprint. This approach was noticed once we divided the pooled data back into the group comparison and then to site to site comparison which for each smaller group comparison yielded more detail-oriented information. While this idea is doable to be executed through the existing data, the question remains on how much group that actually exists in our data. Through machine learning, clustering the existing data using clustering algorithm such as k-means clustering is very feasible [22], but we do not have other references, e.g., other drywood termite species fecal pellets, to compare the clustering results. Therefore, we cannot validate our data fingerprint for now.

In the end, our study has demonstrated that the characteristics means value was highly depended on factors such as colony condition and type of dietary materials. We have also given the range of species marker and indicated its usage drawback of possibility to have high rate of false positive. And finally, we come up with an approach to turn existing measurement parameters into more robust species marker for the future works.

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