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ABSTRACT

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8 Conservation of indigenous breeds of ruminants is crucial for offering alternatives to
9 commercial breeds. Moreover, it is part of long-term strategies in the agri-food sector to sustain
10 supplies by ensuring genetic resource diversity to overcome climate change and the food crisis.
11 Malin is the only native sheep breed in Malaysia. Due to traits such as heat tolerance and
12 disease resistance, Malin sheep are considered an invaluable biological heritage. However,
13 breeders and industrial producers are not interested in Malin because of their low commercial
14 value and slow growth rate. Hence, this breed is neglected, its population is fragmented, and
15 its numbers are dwindling, without data updates. Therefore, current information regarding
16 Malin sheep is needed, including the latest geographical distribution and phenotypic
17 characterization. First, we determined the population distribution using information from the
18 State Department of Veterinary Services. Data were then collected from 15 studied locations
19 in Pahang, Perak, and Kelantan via purposive sampling. Six qualitative traits and seven
20 morphometric traits were recorded for 152 Malin sheep. These traits were quantitatively
21 analyzed using multi-variate statistical tools to define the best measure to represent body
22 conformation when comparing Malin sheep across studied locations. Findings showed that the
23 Malin *Ne* population is very small. Morphologically, most Malin sheep exhibit light-brown
24 wool with a course wool type; convex head shape, curved and horned in males but polled in
25 females; and white hoof color. Imputation for missing body weight values in one population
26 was successfully performed based on imputation regression modelling prior to downstream
27 analyses. Kruskal-Wallis tests indicated that the median value of all morphometric traits except
28 female body weight differed significantly between all studied locations. The highest correlation
29 was observed between chest girth and body length in males ($\rho=0.76$) and chest girth and body
30 weight ($\rho=0.76$) in females. Non-metric dimensional scaling showed that sheep maintained by
31 smallholders in Pahang and Kelantan are similar phenotypically, but with smaller size

32 compared with Perak. These findings suggest that phenotypic traits can help evaluate and
33 compare sheep body conformation and thus provide an opportunity to distinguish and clarify a
34 herd's position, thereby highlighting populations requiring management attention.

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36 **Keywords:** Malin, Morphometrics traits, Phenotypic characterization, Morphological traits,
37 Geographical distribution, sheep

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INTRODUCTION

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The Anthropocene era is witnessing biodiversity loss, which dramatically affects species adaptation. For example, intraspecific genetic diversity provides a critical basis for evolutionary changes, such as adaptation to new environmental conditions [1,2]. In today's extreme environments, adaptation traits for livestock include tolerance to climate changes and ability to adapt to poor-quality diets [3]. To ensure sustainability of the livestock industry, researchers are actively bioprospecting for breeds with higher adaptation potential, as this is necessary to respond adequately to challenges associated with climate change, food security and livelihood needs, and conservation of natural resources [3].

The growing demand for animal food products is met through industrial systems. Nonetheless, traditional livestock systems still contribute to the livelihoods of 70% of the world's rural poor. Smallholders and mixed crop-livestock systems are predicted to continue serving as the backbone of pro-poor agricultural growth in developing countries through 2030 [3,4]. The diversity of these production systems and cultures with regard to farm animal species such as sheep (*Ovis aries*), which is based on a long history of domestication, has created diverse, locally adapted breeds across global geographic regions. Unfortunately, comprehensive data on many locally adapted or indigenous breeds are scarce, which could adversely affect long-term food security and agriculture sustainability [5].

Malin sheep, which derives from Malaysia (MAL) Indigenous (IN), is the only indigenous sheep breed in Malaysia [6,7]. Compared with exotic sheep breeds from temperate regions, Malin sheep are hardy, exhibiting heat tolerance, highly resistance to diseases such as internal parasites, and tolerance of a poor-quality diet [5,8,7]. The adaptive capacity of Malin sheep provides a glimpse of the range of genetic diversity among the world's livestock. Nevertheless, imported, exotic sheep breeds such as Dorper and Boer are in high demand in local markets due to their meat quality [9,10] whereas Malin sheep are regarded as having a

65 lower meat composition [11] wool of little benefit to the livestock industry [12], small body
66 size, and slow production rate [6]. These factors have caused local herders to choose exotic
67 breeds over Malin sheep (DVS, personal communication). However, the lack of interest in
68 Malin sheep has caused their population to decline nationwide. In addition, no regular updates
69 regarding the Malin sheep population have been conducted, and no proper risk status has been
70 calculated.

71 The most recent study of the Malin breed was conducted in 2014 by Mastura [7] and
72 involved the Malin nucleus herd maintained by the NIBV (National Institute of Biodiversity
73 Veterinary) in the Jerantut district, located in Pahang, East Coast region of Peninsular Malaysia.
74 In their study, Mastura [7] examined various phenotypic characteristics of Malin sheep,
75 including morphologic and morphometric traits. The FAO [5] stated that recording the
76 geographical distribution of animals is an integral component of phenotypic characterization.
77 However, as the findings pertained only to Malin sheep in the NIBV population, which is well-
78 managed and adequately supervised by DVS personnel, comprehensive studies of other Malin
79 sheep smallholders are warranted. Therefore, this study was conducted as a primary survey of
80 Malin herd locations and phenotypic characteristics across Peninsular Malaysia, including
81 other local farmers nationwide with variable livestock management methods. The field
82 characterization of these small Malin herds provides information regarding the different
83 phenotypic characteristics of these sheep. The present study recorded the geographic
84 distribution and differences in morphological and morphometric traits among Malin sheep. We
85 then quantified the traits using multi-variate statistical analyses to define the best measures
86 representing body conformation when comparing Malin sheep across the studied locations.
87 These findings will help clarify herd positions and identify populations that require
88 management attention.

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MATERIALS AND METHODS

91 **Malin Farm Inventories and Documentation**

92 To clarify Malin herd positions nationwide, we collaborated with the federal DVS to
93 determine the locations of small Malin shepherders. Inventories of Malin sheep populations
94 were obtained manually via email correspondence and telephone by the state DVS after local
95 herders assented to participation in a sheep census. Our research team also conducted phone
96 calls and population searches of the Malin breed based on data from internet searches and
97 personal communications. Raw data on herd locations and population sizes were compiled into
98 a master spreadsheet and later shared with the federal DVS.

99 **Farm Visits and Data Collection**

100 This study was conducted between October 2019 and February 2020. All procedures
101 involving animals performed in this study were approved by the Animal Ethics and Care
102 Committee at UKM (FST/2019/SHAIRAH/20-MAR/999/-MAR-2019-DEC-2021) and by the
103 DVS Approval Committee (REF: JPV.BPI.600-1/7/1).

104 **Purposive Sampling.** Locations for the study of Malin sheep populations were chosen
105 based on a purposive sampling technique. This sampling technique depends entirely on
106 personal knowledge of an individual that can be contributed to the research [13] but cannot be
107 obtained from another individual [14,15]. We obtained additional information regarding Malin
108 sheep populations from a few active herders via telephone. However, due to logistical issues,
109 details were used only for data recording purposes and not determination of sampling locations.
110 Hence, the phenotypic data were purposely collected from 15 locations in 3 states (Pahang,
111 Perak, and Kelantan) based on recommendations from the respective state DVS personnel and
112 pandemic constraints.

113 All collected data were obtained from 152 adult female (n=102) and male (n=50) sheeps
114 from different herds in Pahang (n=59), Perak (n=30), and Kelantan (n=73). As part of the
115 genetic diversity study, the inclusion criteria were set to select for physically matured sheep

116 aged between 9 months and 3 years, as confirmed by their keepers. In addition, the FAO [16]
117 recommended that the sample size specifically for phenotypic and genotypic characterization
118 research should not exceed 10% of the total animal population and that at least 5 animals be
119 sampled from each selected population. Table 1 summarizes the population and sampling sizes
120 for the studied locations.

121 **Calculation of Effective Population Size (N_e).** Using the census population size from
122 the sampling locations, we calculated the effective population size (N_e) using the demographic
123 method based on the number of male and female animals according to Hill's formula (Equation
124 1). Only mature male sheep (sires) and female sheep selected for breeding purposes were used
125 to calculate the N_e value.

$$N_e = 4 \frac{MF}{(M+F)}$$

.... **Equation 1,**

127 where

128 M = Mature male sheep or sires;

129 F = Mature female sheep; and

130 N_e = Effective population size.

131 **Observation of Morphological Traits.** All qualitative traits for morphological
132 characterization were recorded following the FAO guidance list for sheep morphological
133 characteristics [5]. Observed traits included wool color (WC), wool type (WT), presence of
134 horn (PH), head profile (HP), hoof color (HC), and horn shape (HS). For WC, the classification
135 was based entirely on personal observation without the aid of a color chart, assisted by
136 veterinary officers. Qualitative data were tabulated in a spreadsheet and further analyzed using
137 descriptive statistics such as percentage (%), frequency, and number of individual sheep (n).
138 Chi-square (χ^2) tests for heterogeneity were carried out using MINITAB software, testing the

139 null hypothesis that different qualitative characteristics for each trait observed among the Malin
140 herds occur at random ($p < 0.05$) [17].

141 **Morphometrics data collection.** The body weight (BW) and linear body
142 measurements were recorded using an animal weighing scale and flexible measuring tape.
143 Seven body measurements were recorded, including BW, body length (BL), height at wither
144 (HW), chest circumference (CC), tail length (TL), ear length (EL), and scrotum circumference
145 (SC). Before any measurements were collected, sheep were made to stand straight in body
146 position and raise their head to avoid inaccurate measurements [18]. In addition, care was made
147 to take all morphometric measurements before sheep had their first feeding and watering for
148 that particular day in order to avoid post-feeding effects. Figure 1 illustrates the measurement
149 of sheep characteristics other than BW, and Table 2 provides descriptive details.

150
151 Morphometric data were analyzed using R software (version 3.6.3) and PAST software
152 (4.09). However, due to some missing BW data, weight was estimated using an imputation
153 method prior to downstream statistical analyses.

154 **Imputation method.** Missing data are commonly obtained using an imputation method
155 [19] to avoid reducing the sample size. This study applied a stepwise linear regression
156 imputation model to estimate missing data on sheep BW [20]. With regression imputation, the
157 information of other variables (i.e other body measurements BL, HW, etc.) is used to predict
158 the missing values in a variable (i.e body weight BW) by using a regression model. Three
159 models, Model1, Model2, and Model3, were built to generate a regression formula to examine
160 the high contribution of linear body measurements to BW readings, along with other predictors
161 (states, districts, sex). This analysis involved several addition and reduction processes of
162 independent variables until statistical significance was achieved ($p < 0.10$). Each model
163 calculated and estimated BW based on linear regression by including and excluding a number
164 of predictors, such as district, state, and sex. To choose the best model for estimating logical

165 BW readings, the model showing the highest R^2 value and lowest number of Akaike
166 Information Criteria (AIC) was considered [21]. All imputations and BW predictions were
167 performed in R using the *MASS* package.

168 ***Hypothesis testing for comparisons across states and testing for morphometric***
169 ***variable correlations.*** Prior to hypothesis testing, the Shapiro-Wilk normality test was
170 conducted to determine the data distribution. Non-parametric Kruskal-Wallis tests were
171 subsequently employed to identify statistical differences in morphometric traits between
172 studied locations among male and female sheep separately. This test compares the median
173 value of each morphometric attribute [22]. A boxplot diagram was constructed using the *ggplot*
174 package in R to display the distributions of all the morphometric data for each studied location.
175 To infer any associations between morphometric variables and determine their strength [23],
176 the correlations between BW and all linear body measurements were determined using
177 Spearman rank correlation analysis. The correlation coefficient range was between +1 and -1
178 [24]. Each coefficient value explained a different correlation relationship level, classified as
179 either very weak (value = 0.00-0.19), weak (0.20-0.39), intermediate (0.40-0.59), strong (0.60-
180 0.79), or very strong (0.80-1.00) [25].

181 ***Ordination method and visualization of sheep grouping.*** Based on the multi-
182 morphometric variables analyzed in this study, we further visualized the phenotypic
183 resemblance of Malin sheep individuals across their geographic space in comparison with
184 sheep from Jerantut using non-metric multidimensional scaling (NMDS) plots as an ordination
185 method. In NMDS analysis, coordinates of an object (each individual sheep) are plotted into
186 similarity and dissimilarity sets based on rank in 2-D scatter plots without any emphasis on the
187 magnitude of similarities or dissimilarities [26]. In an NMDS analysis, individual sheep will
188 be plotted at a closer distance when they share a greater amount of morphometric similarity;
189 conversely, they will be plotted further away when they share less similarity [27, 28, 29]. The

190 similarity measure of the Gower method was used due to the capability of measuring many
191 physical units [30, 31], such as in kilograms (kg) and centimeters (cm). Sheep from Jerantut
192 were separated as one particular population to serve as a benchmark herd, as sheep from this
193 location are likely maintained under optimal animal husbandry conditions. Different groups
194 were marked with different colors, as depicted in Table 3.

195 Following visualization using an ordination plot, multi-variate hypothesis testing was
196 performed using analysis of similarity (ANOSIM) and similarity of percentage (SIMPER).
197 ANOSIM was used to determine if there were any significant differences between Malin herd
198 populations [32], whereas SIMPER was used to determine the highest percentage of
199 morphometric variables contributing to any such differences [28].

200

RESULTS

Geographical Distribution of Malin Sheep Populations

In the present study, we characterized the geographical distribution and occurrence of Malin sheep across Peninsular Malaysia. Malin sheep populations were recorded in six states: Kedah, Perak, Kelantan, Pahang, Melaka, and Johor (Figure 2). The data were obtained from (1) correspondence with state DVS personnel and/or (2) from personal communication through telephone calls. In addition, the latest update from state DVS staff confirmed that no Malin sheep populations exist in Pulau Pinang (Penang) or across East Malaysia (Sabah and Sarawak). No information was obtained regarding the existence of Malin populations in other states, such as Terengganu, Negeri Sembilan, Kuala Lumpur, Selangor, or Perlis, however, we do believe that small and scattered Malin populations might be present.

Apart from census population size (Table 1), we also documented the number of male and female Malin sheep in each study location based on the inclusion criteria. Using this information, we then calculated the effective population size (N_e), as shown in Table 4. The overall N_e value for Peninsular Malaysia determined based on data from all sampling locations was 177. Within each farm, two locations (C-03 and D-03) had a N_e value of zero because no Malin male sheep were kept on the farm. The other 12 locations had a low N_e value (<50), and only location C-02 had a N_e value >50 .

Based on census population size, seven sampling locations had more than 30 sheep, with three locations in Pahang (C-01, C-02, C-03), two in Perak (A-01, A-02), and two in Kelantan (D-04, D-08). The remaining eight locations (C-03, D-01, D-02, D-03, D-05, D-06, D-07, and D-09) had <30 sheep. Overall, Kelantan had a higher number of Malin sheep than Perak and Pahang.

225 **Sheep Morphology Traits**

226 The phenotypic characteristics are presented in Table 5. First, in terms of WC, Malin
227 sheep had four variations (Figure 3). Overall, more than half of the studied Malin population
228 showed light brown (Lb) wool. This was followed by brown (Br) wool at 19%, and a
229 combination of 16% black and brown (Bl+Br) and 8% white and black (W+Bl). With regard
230 to WT, Malin sheep had either smooth (41%) or coarse (59%) wool. The HP was 72% straight
231 and 24% convex. Most Malin sheep had no PH, with only one-quarter of Malin sheep having
232 horns. Of sheep with horns, almost all had curvy horns, and only 3% had scurs HS (see Figure
233 4). With regard to HC, 51% of Malin sheep had white hooves, and 47% had black hooves. The
234 Chi-square χ^2 heterogeneity test (Table 5) detected significant differences in the proportion of
235 phenotypes only for WC ($p < 0.05$); therefore, we rejected that different color variations in Malin
236 sheep were random. The Lb color were found to be statistically dominant for Malin wool. None
237 of the other traits exhibited statistically significant differences.

238

239 **Sheep Morphometric Measurements**

240 *Imputation method for predicting missing BW values.* Table 6 lists all imputation
241 models. Model 1 is the full model that involves four predictors (linear body measurements,
242 states, districts, and sex); Model 2 consists of three predictors (without districts), whilst Model
243 3 is the simplest model with only two predictors (linear body measurement and sex). In Table
244 6, Model1 showed the highest R^2 value ($R^2 = 0.7123$) and the lowest AIC number (AIC = 964);
245 however, Model1 underestimated a few BW readings for adult sheep (weight <12 kg) shown
246 in Table 7. Model2 had an intermediate R^2 value ($R^2 = 0.6808$) and AIC number (AIC = 975).
247 Both Model1 and Model2 indicated that the linear body measurements making the highest
248 contribution to BW estimates were BL, CC, and HW. In contrast, Model3 had the lowest R^2
249 value and highest AIC number, and only CC contributed to the BW reading with this model.

250 Estimations for all 13 missing data are shown in Table 7, with a comparison made
251 across three models. Model 2 provides the acceptable range of body weight imputed for all
252 Malin sheep individuals, AIC number and R-values, and the significant contribution of all three
253 predictors (body measurements, states, sex). Thus, values estimated using this model are used
254 for downstream analyses.

255 ***Comparison of morphometric traits across 3 states and analysis of the correlations***
256 ***between linear body measurements.*** Shapiro-Wilk normality tests indicated that the data for
257 the majority of linear body measurements were not normally distributed. Therefore, the
258 downstream statistical analyses in the current research adopted a non-parametric statistical
259 approach for analyzing all morphometric traits. These morphometric traits were compared
260 across three states (Pahang, Perak, Kelantan), as shown in the boxplots of Figure 5. Kruskal-
261 Wallis tests were performed for all traits, separately between females and males. Each
262 morphometric trait showed significant differences ($p < 0.05$) across all studied populations,
263 except with regard to female BW ($p > 0.05$). Overall, Malin sheep populations in Pahang
264 exhibited the highest median value for five of the seven morphometric parameters, as compared
265 with Perak and Kelantan: BW($Mdn(J)=46.75$, (B)=24.5), BL($Mdn(J)=70.5$, (B)=61),
266 CC($Mdn(J)=89$, (B)=72), HW($Mdn(J)=55.5$, (B)=45.5), and SC($Mdn(J)=25.5$).

267 The strength of the correlations between linear body measurements was determined
268 using the Spearman coefficient correlation method (Figure 6) to identify the best set of
269 morphometric traits for general morphological characterization. No negative correlations were
270 detected. In male sheep, CC and BL exhibited the strongest correlation ($\rho = 0.76$), which was
271 statistically significant (Figure 6a). For females, the strongest and most significant correlation
272 was detected between CC and BW (Figure 6b), with the same correlation value as for males (ρ
273 = 0.76). All pairwise correlation analyses between morphometric traits of male sheep were
274 statistically significant with varied correlation strength, except for correlations between BW-

275 EL, HW-SC, and EL-SC (Figure 6a). In contrast, female sheep exhibited only one insignificant
276 correlation, between BW-EL (Figure 6b).

277 ***Ordination plot (NMDS) and multi-variate hypothesis testing.*** The phenotypic
278 resemblance of individual sheep across populations can be visualized using NMDS ordination
279 plots (Figures 7 and 8). We used the Malin herd from Jerantut (C-02) within the state of Pahang
280 (refer to Table 3) as a reference population for comparison with other populations because it is
281 a nucleus herd maintained in a proper care and husbandry environment by the NIBV. Figure
282 7a shows that in general, there were some overlapping individual animals from all three states.
283 Nonetheless, although two male sheep, each in Pekan and Bera (within Pahang) clustered
284 closely with males in Perak and Kelantan, many individual sheep from Jerantut scattered far to
285 the left of the NMDS-1 axis, indicating that they were phenotypically distinct from the others.
286 One of the morphometric characteristics, BW, which was mapped onto these plots, suggests
287 that those sheep were heavier than the others (Figure 7b, c, d).

288 The clustering pattern of Jerantut female sheep shown in Figure 8 was quite similar to
289 that of Jerantut male sheep, although almost all female sheep grouped closely among each other,
290 regardless of their state of origin (Figure 8 a). Across all other plots, a few females in Jerantut
291 consistently clustered together to the left of NMDS-1 axis (Figure 8b, c, d). This grouping was
292 again influenced by BW, suggesting these animals were heavier. In Figure 8c, Perak female
293 sheep were scattered close to the Jerantut females at the far right of the NMDS-1 axis, whereas
294 only a few Kelantan sheep were close to Jerantut (Figure 8d). In contrast to Pahang, there was
295 no single overlapping, and all sheep showed diverged clustering from Jerantut female sheep
296 (Figure 8b). ANOSIM revealed that comparisons for both males and females were statistically
297 significant, with all R-values <0.5.

298 Cumulative SIMPER analysis showed three variables of male sheep (BW, CC, and BL)
299 and four variables for female sheep, BW, CC, BL, and HW, were the greatest contributors to

300 the percentage of differences between the population in Jerantut and other studied locations, as
301 indicated in Table 8.

302

303

DISCUSSION

304 **Geographical distribution, low N_e , and risk status of Malin sheep.** Phenotypic
305 characterization of farm animal genetic resources for food and agriculture (FAnGR) involves
306 the systematic documentation of observed characteristics of breeds, their geographical
307 distribution, production environments, and use of resources [5]. Comprehensive data on the
308 distribution of Malin sheep populations, the adaptive capacity of breeds, and their management
309 could provide an indication of the range of options available in terms of the genetic diversity
310 of Malaysian small ruminants, yet the paucity of available data hinders these overall aims.

311 According to Mastura [7], Malin sheep populations can be found in Kelantan,
312 Terengganu, Pahang, and Negeri Sembilan. However, our recent findings indicate that Malin
313 sheep populations are distributed in different states: Kelantan, Perak, Pahang, Johor, Melaka,
314 and Kedah. In addition, recent information from the DVS showed no Malin sheep populations
315 in Penang, Sarawak, and Sabah. These differences highlight the importance of collecting and
316 regularly updating livestock information, which is a prerequisite for sustainable management
317 of animal genetic resources [33]. We also reported that overall, Kelantan has the highest
318 number of Malin sheep. This finding is in agreement with the report of [34], which described
319 Kelantan as an agrarian state in which almost all herders have small livestock farms that
320 contribute to economic growth within the east coast region of Peninsular Malaysia.
321 Furthermore, a Malin sheep population has been maintained in Kelantan since 1980 according
322 to an FAO report [35]. Very small Malin sheep populations (less than 50 animals each) are also
323 found in Melaka and Kedah, though this might be due to the limited information and survey.

324 Our sampling effort found that the Malin farm (C-02) in Jerantut, Pahang, has the
325 highest number of sheep, whereas the average size of other herd populations maintained by
326 smallholders is fewer than 30 animals. The C-02 farm is maintained by the NIBV and
327 monitored directly by the federal DVS. Farm C-02 is the nucleus group center for the Malin
328 sheep breed and plays an important role in conserving and enhancing genetic quality [7]. While
329 the population size of this nucleus herd is adequately documented, records for Malin herds
330 maintained by smallholders in other locations are also necessary in order to plan further actions.
331 Each Malin herds from smallholders across the country has the potential to be used as Farm
332 Animal Genetic Resources (FAnGR) to ensure food security and maintain genetic diversity.
333 However, effective management of FAnGR requires comprehensive knowledge that includes
334 demographic data linked to the size and structure of the population [36]. Compared to census
335 population size, N_e is the preferred measure for the assessment of the risk status of livestock
336 breeds since it is approximated based on the number of both breeding female and male
337 individuals; therefore, it allows the rate of inbreeding or loss of genetic diversity within the
338 population to be inferred [37, 36]. We must emphasize that the calculated N_e values across the
339 populations nationwide in our findings provide indications of a concerning trend that could
340 jeopardize biological conservation of the Malin breed.

341 Husemann [38] reported that N_e is a vital genetic population and biological
342 conservation indicator, as it directly translates overall population size to ideal population size
343 by showing the loss of genetic variation at a similar rate within the same population. Most N_e
344 values are lower than the actual population size. Several factors contribute to low N_e values,
345 such as an imbalance between male and female sheep, non-random breeding, and differences
346 in population size by generation [39, 40]. The smaller the N_e value, the lower the variation in
347 genetic traits inherited by subsequent generations. N_e can be considered the primary indicator
348 for monitoring the extinction risk of an animal group based on the range of N_e values [41] as

349 practiced in certain countries including UK [42], Germany [43], and Poland [44]. The overall
350 *Ne* value for Peninsular Malaysia determined based on data from all sampling locations was
351 177, which fell between 50 and 200, indicating that the breed is threatened and that the Malin
352 populations need additional conservation action [44]. Proper action, such as systematic
353 management practices and regular monitoring, should be continued.

354 Because most livestock animals do not have pedigree information or are not intensively
355 studied, estimating the *Ne* value can be carried out without the need to calculate the inbreeding
356 value within an animal population [41]. Given its risk status, the animal husbandry and
357 production systems for Malin herds kept by smallholders need upgrading to ensure proper care
358 and the long-term sustainability of this native breed. Therefore, thorough surveys that evaluate
359 the actual population sizes and then deposit these data into the Domestic Animal Diversity–
360 Information System (DAD-IS) are warranted.

361 **Phenotypic features of Malin sheep suggest their diversity and adaptability.**
362 Numerous studies have reported the phenotypic diversity of sheep breeds other than Malin (e.g.,
363 [17, 45, 46]. Recording morphological traits such as WC aids in the identification process and
364 breed characterization [47]. Variations in morphological traits are likely affected by breed
365 characteristics, geographical distribution, and the local environment [48], as well as the
366 livestock management system. Factors such as body size, growth rate, and breed type are
367 commonly considered in animal selection [49]. In addition, BW and SC are essential indicators
368 in selecting sires, as these traits are associated with genetic and reproductive fitness [50, 49].

369 The current study characterized additional phenotypic and morphological features of
370 Malin sheep both qualitatively and quantitatively. There was no specific method for
371 quantification of WC intensity in this study. However, Lb and cream color were the dominant
372 and most frequent colors of Malin sheep wool, in agreement with a report by Mastura [7] on
373 NIBV Malin. The dominance of Lb color suggests that a recessive gene controls the darker

374 color. According to Kalds [51], the Agouti gene (*ASIP*) controls the expression of yellowish
375 color through the white or tan (A^{Wt}) dominant allele (due to phaeomelanin), whereas the
376 recessive allele (A^a) controls expression of the brown and black WC through eumelanin. This
377 coat color explains the connection to the animal's ability to adapt to environmental stressors,
378 including heat stress, flies, and lice perch [52,48]. The coarse WT observed with Malin sheep
379 in the present study is similar to that reported for other breeds, such as Ethiopian Menz sheep
380 [53] and Garole sheep in India [54]. Although the wool of Menz and Garole sheep is utilized
381 for crafts, the wool of Malin sheep is considered less profitable [12], which has contributed to
382 the lack of interest in this breed among commercial livestock farmers.

383 Other features, such as HP and the HS provide information as to whether the observed
384 trait represents a primitive or adaptive response to the local environment. For instance, a
385 straight HP is a primitive characteristic trait [45]. In previous studies, Malin sheep populations
386 exhibited only a straight HP [35]; however, the present study observed the presence of a smaller
387 proportion of Malin sheep with a convex HP, likely due to environmental adaptation. Our
388 findings suggest that most Malin sheep still express these primitive features, whereas the
389 majority of other sheep breeds exhibit a convex HP [55,56,46], perhaps as a result of years of
390 domestication. Another feature impacted by domestication is HS, which has undergone so-
391 called miniaturization, as the horns of modern sheep are shorter than those of their wild sheep
392 ancestors [57], making them easier for humans to handle [58]. Horns are typically used by the
393 animals as a shield in combating predators or enemies.

394 Phenotypic characterization based on livestock observable attributes help to identify
395 and document diversity within and between breeds [18]. Multivariate analysis on body
396 conformation and morphological traits can be performed to evaluate these variables
397 simultaneously and determine any differences of groups and/or breeds, thus further provides
398 support for conservation program [27,18,29].

399 By examining morphometric traits, we found marked differences between the groupings of the
400 NIBV nucleus herd and other Malin sheep populations in graphical plots. These differences are
401 likely related to two primary factors: (i) inbreeding and (ii) low nutrient consumption. Even
402 though the specific mechanisms by which inbreeding affects a breed's genetic morphometric
403 characteristics remain unknown, several studies have reported the impact of inbreeding on
404 livestock linear body measurements. Research based on data collected over a 38-year period
405 showed that HW readings in Sardinia Anglo-Arab horses declined from 163 cm to 161 cm [59].
406 Another study on Hostein-Friesian cows that were inbred for 25 years reported decreases in
407 BW, HW, BL, and CC values. An experimental study by Cerna [60] reported that inbreeding
408 negatively affected BW after 100 days in the Czech Republic sheep breed.

409 Due to poor livestock management methods, we suspect that inbreeding is an ongoing
410 issue among Malin sheep maintained by smallholders. In support of this hypothesis, the flow
411 chart shown in Figure 11 explains the causes that eventually led to inbreeding in the Malin
412 sheep populations. In particular, many herders repeatedly use the same rams and breed them
413 with genetically related ewes, without keeping any pedigree records. Changing the ram every
414 4 to 5 years is crucial for preventing inbreeding in sheep populations [61]. The situation is
415 further exacerbated by the poor nutrient content of the sheep diet, as many animals freely graze
416 in the surrounding area. Nevertheless, we were surprised to learn that most Malin sheep
417 examined in our study were healthy, and the ease of caring for these animals is one of the main
418 reasons smallholders keep Malin sheep.

419 Although our recent findings offer updated, comprehensive data for the Malin breed
420 compared with previous research, there are still limitations that should be addressed in future
421 studies. Initially, our sampling plan was to include the Johor state in order to represent the
422 southern region and other districts by considering the Titiwangsa Mountains range, which
423 forms the backbone of Peninsular Malaysia. However, the COVID-19 pandemic affected the

424 initial plan and introduced logistical challenges. It is also imperative to revisit the Malin
425 population in Pahang, which had missing data that had to be estimated using the imputation
426 method to avoid under- or overestimation; such data could impact further management actions.
427 Collection of other data related to production traits and growth rates would also be beneficial
428 in achieving better, systematic livestock management to prevent loss of genetic diversity and
429 preserve this invaluable biological heritage.

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CONCLUSION

432 Regular inventories and field surveys of Malin sheep, including phenotypic
433 characterization and analysis of geographical distribution throughout Malaysia, can add new
434 information, indicate risk status, and ensure this breed's welfare. Differences in surrounding
435 environments and management practices that contribute to variations in phenotypic traits and
436 extreme discrepancies in morphometric features shown by Malin sheep need to be recorded
437 and addressed accordingly. Inbreeding and inefficient sheep management practices can hinder
438 conservation and rehabilitation of Malin sheep; however, with proper and resourceful animal
439 husbandry practices, Malin sheep, as shown by the NIBV, could exhibit morphometric
440 characteristics that make them commercially desirable. By applying these strategies, sheep
441 populations maintained by smallholders can provide a range of options to help ensure the
442 sustainability of Farm Animal Genetics Resources for both global and local livestock industries.

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TABLES

Table 1. Malin sheep populations and sampling sizes by studied locations.

State	Districts	Code	Population size	Sampling size
Pahang	Pekan	C-01	40	12
	Jerantut	C-02	130	28
	Bera	C-03	23	2
		C-04	60	9
Perak	Kamper	A-01	36	15
	Gopeng	A-02	39	15
Kelantan	Machang	D-01	12	5
		D-02	21	10
	Pasir Puteh	D-03	19	4
		D-04	30	8
		Jeli	D-05	8
	Pasir Mas	D-06	16	7
		D-07	15	7
		D-08	35	13
		D-09	19	10
TOTAL			152	

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Table 2. Description of morphometric measurements of Malin sheep.

Body Measurements (cm)	Descriptions
BL	The horizontal distance from the point of the shoulder to the pin bone
HW	The vertical distance from the bottom of the front foot to the highest point over wither.
CC	Placing the measuring tape around the sheep at the point of small circumference just behind the forelegs
TL	Distance from the base of the proximal tail end of the first coccygeal bone to the distal end of the last coccygeal bone
EL	Distance from the root to the end point of the ear
SC	Placing the measuring tape around the scrotum end to end.

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Table 3. Symbols and colors used to differentiate the studied locations for NMDS analysis.

Pahang		Perak		Kelantan	
Studied location	Symbol	Studied location	Symbol	Studied location	Symbol
C-01	+	A-01	▽	D-01	o
C-02	■	A-02	▼	D-02	●
C-03	X			D-03	+
C-04	*			D-04	*
				D-05	X
				D-06	△
				D-07	▲
				D-08	□
				D-09	■

C= Pahang (blue), A=Perak (green) and D=Kelantan (black).

Table 4. Effective population size of Malin sheep by study location.

States	Studied locations	Male	Female	Overall	<i>N_e</i> (sheep)
Pahang	C-01	1	12	13	3.7 ≈ 4
	C-02	24	62	86	69.2 ≈ 69
	C-03	0	2	2	0
	C-04	1	8	9	3.6 ≈ 4
Perak	A-01	8	7	15	14.9 ≈ 15
	A-02	8	7	15	14.9 ≈ 15
Kelantan	D-01	2	3	5	4.8 ≈ 5
	D-02	2	8	10	6.4 ≈ 6
	D-03	0	4	4	0
	D-04	2	6	8	6
	D-05	1	6	7	3.5 ≈ 4
	D-06	1	6	7	3.5 ≈ 4
	D-07	2	5	7	5.7 ≈ 6
	D-08	8	5	13	12.3 ≈ 12
	D-09	3	7	10	8.4 ≈ 8
Peninsular Malaysia		63	148	211	176.8 ≈ 177

Table 5. Morphological traits of Malin sheep observed in sampling locations across states.

Morphology traits	Types	States						Total N (%) n=152
		Pahang		Perak		Kelantan		
		Male N (%) n=13	Female N (%) n=38	Male N (%) n=16	Female N (%) n=14	Male N (%) n=21	Female N (%) n=50	
Wool color	Brown	0	0	8 (50)	6 (42.86)	4 (19.05)	11 (22)	29 (19.08)
	Light brown	11 (84.62)	33 (86.84)	6 (37.5)	8 (57.14)	4 (19.05)	17 (34)	79 (51.97)
	White + Black	0	1 (2.63)	2 (12.5)	0	3 (14.29)	7 (14)	13 (8.55)
	Black + Brown	0	0	0	0	10 (47.62)	15 (30.0)	25 (16.45)
	χ^2 value							74.882** * Na=6
Wool type	Smooth	5 (38.46)	10 (26.32)	9 (56.25)	4 (28.57)	14 (66.67)	20 (40.0)	62 (40.79)
	Coarse	7 (53.85)	28 (73.68)	7 (43.75)	10 (71.43)	7 (33.33)	30 (60.0)	89 (58.55)
	χ^2 value							3.959^{NS} Na=1
Head profiles	Straight	7 (53.85)	27 (71.05)	15 (93.75)	11 (78.57)	12 (57.14)	37 (74.0)	109 (71.71)
	Convex	5 (38.46)	7 (18.42)	1 (6.25)	3 (21.43)	9 (42.86)	11 (22.0)	36 (23.68)
	χ^2 value							0.652^{NS} Na=7
Horn presence	Present	10 (76.92)	0	8 (50.0)	0	20 (95.24)	1 (2.0)	39 (25.66)
	Absent	2 (15.38)	38 (100)	8 (50.0)	14 (100.0)	1 (4.76)	49 (98.0)	112 (73.68)
	χ^2 value							1.419^{NS} Na=1
Horn shape	Non-curve	0	-	0	-	0	-	0
	Curve	10 (100)	-	8 (100)	-	18 (90)	-	36 (92.31)
	Scurs	0	0	0	0	0	1 (100)	1 (2.56)
	χ^2 value							0.974^{NS} Na=2
Hoof color	Black	2 (15.38)	22 (57.89)	8 (50.0)	6 (42.86)	7 (33.33)	27 (54.0)	72 (47.37)
	White	10 (76.92)	16 (42.11)	8 (50.0)	8 (57.14)	14 (66.67)	21 (42.0)	77 (50.66)
	value							0.060^{NS} Na=3

Na = missing data and ^{NS} = not significant.

Table 6. Estimation equations for Model1, Model2, and Model3 based on different predictors, including R² and AIC values.

Model	Regression equation	R ²	AIC
Model1	BW = -49.500 +0.4001BL + 0.5629CC + 0.3994HW - 13.3700Pahang -13.9865Perak – 6.8815Gopeng + 11.2434Jerantut + 9.3150Keledang – 7.7590 Machang1 – 8.8995 Pasir Puteh 2 – 21.2670 Pasir Puteh 3 – 5.8759 Female sheep	0.7123	964.835
Model2	BW = -43.0903 + 0.1863BL + 0.6219CC + 0.3931HW – 13.5498Perak – 5.6422Female sheep	0.6808	975.545
Model3	BW = -33.5594 +0.8435CC -3.5635Female sheep	0.5765	1011.636

BW = Body weight; BL = Body length; CC = Chest circumference; HW = Height at wither.

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Table 7. Estimation of BW values for 13 missing data for sheep in Pekan and Bera based on Model1, Model2, and Model3. Bold values indicate illogical BW readings. BW from Model2 were chosen for downstream analyses

States	Districts	Sex	ID No.	Body Weight (Kg)		
				Model1	Model2	Model3
Pahang	(C-01)	Male	Mal-C01-M-01	14.63	26.35	23.78
		Female	Mal-C01-F-01	23.06	27.96	21.27
		Female	Mal-C01-F-02	16.70	21.57	26.14
		Female	Mal-C01-F-03	10.62	16.36	23.61
		Female	Mal-C01-F-04	4.82	20.48	14.33
		Female	Mal-C01-F-05	9.16	23.30	20.23
		Female	Mal-C01-F-06	14.77	24.81	20.23
		Female	Mal-C01-F-07	14.12	23.28	21.08
		Female	Mal-C01-F-08	12.29	15.89	21.92
		Female	Mal-C01-F-09	5.46	21.64	13.49
		Female	Mal-C01-F-10	10.76	16.26	20.23
	Female	Mal-C01-F-11	4.51	23.92	16.86	
	Bera (C-04)	Male	Mal-C04-M-01	12.50	25.68	22.76

M= Male; F= Female; C= Pahang; Mal= Malin sheep.

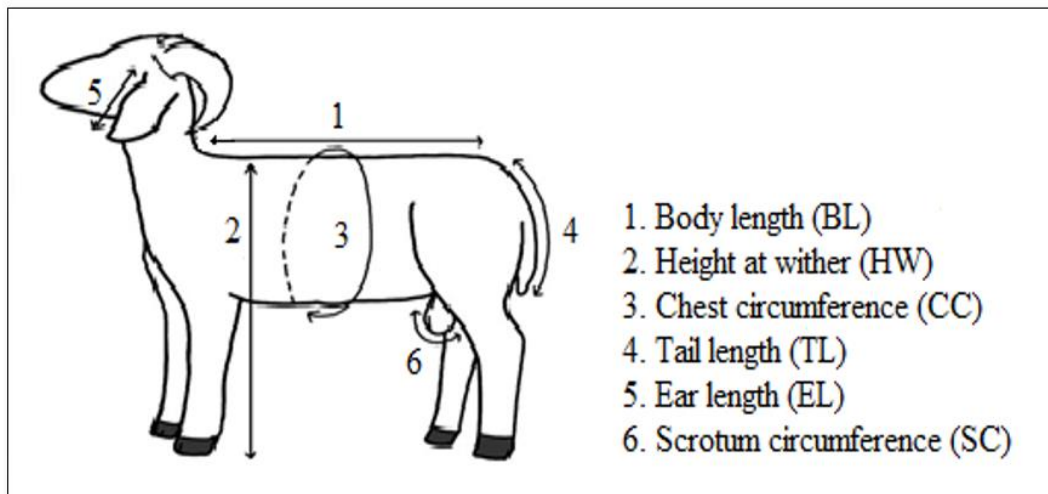
Table 8. SIMPER analysis of male and female sheep between Jerantut and other studied locations.

Sex	The highest contribution of variables to differences	Cumulative percentage of variables differences (%)
M	1. Body weight (BW) 2. Chest circumference (CC) 3. Body length (BL)	90
F	1. Chest circumference (CC) 2. Body weight (BW) 3. Body length (BL) 4. Height at wither (HW)	90

M = Male and F = Female.

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FIGURES

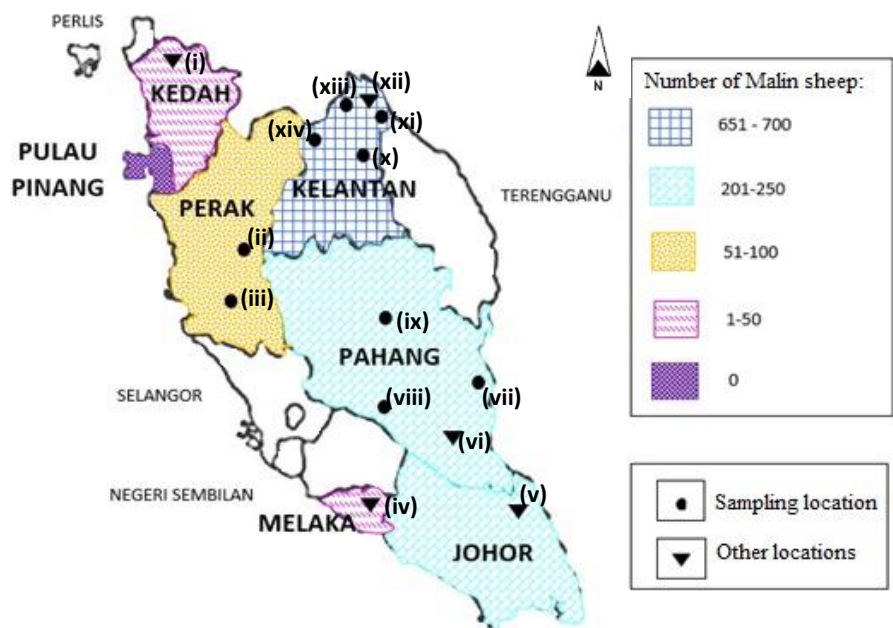


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Figure 1. Illustration of sheep linear body measurements.

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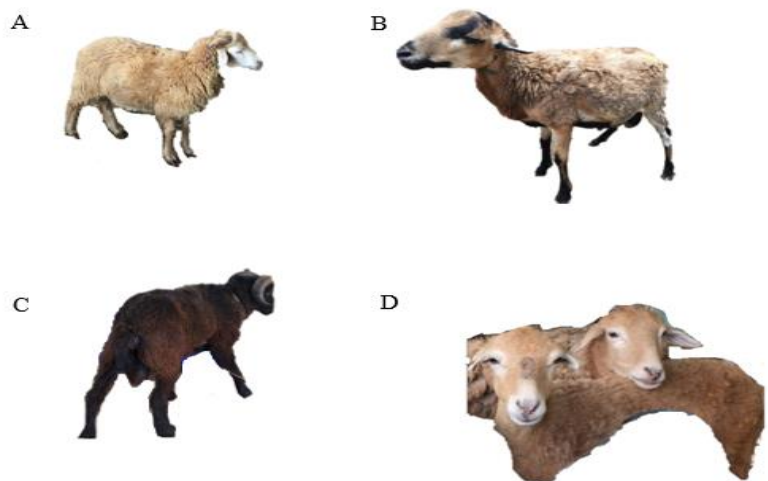
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Figure 2. Geographical distribution of Malin sheep and overall number of individual Malin sheep identified across Peninsular Malaysia. The districts involved are denoted by Roman numerals: (i) Jitra, (ii) Gopeng, (iii) Kampar, (iv) Jasin, (v) Mersing, (vi) Rompin, (vii) Pekan, (viii) Bera, (ix) Jerantut, (x) Machang, (xi) Pasir Puteh, (xii) Kota Bharu, (xiii) Pasir Mas, and (xiv) Jeli. Circles and inverted triangles denote the sampling locations involved in this study and populations that were not involved in sampling.

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Figure 3. Classification of wool color recorded in the Malin sheep populations. A = Light brown (Lb), B = White + Black (W + Bl), C = Brown + Black (Br+Bl), and D = Brown (Br).

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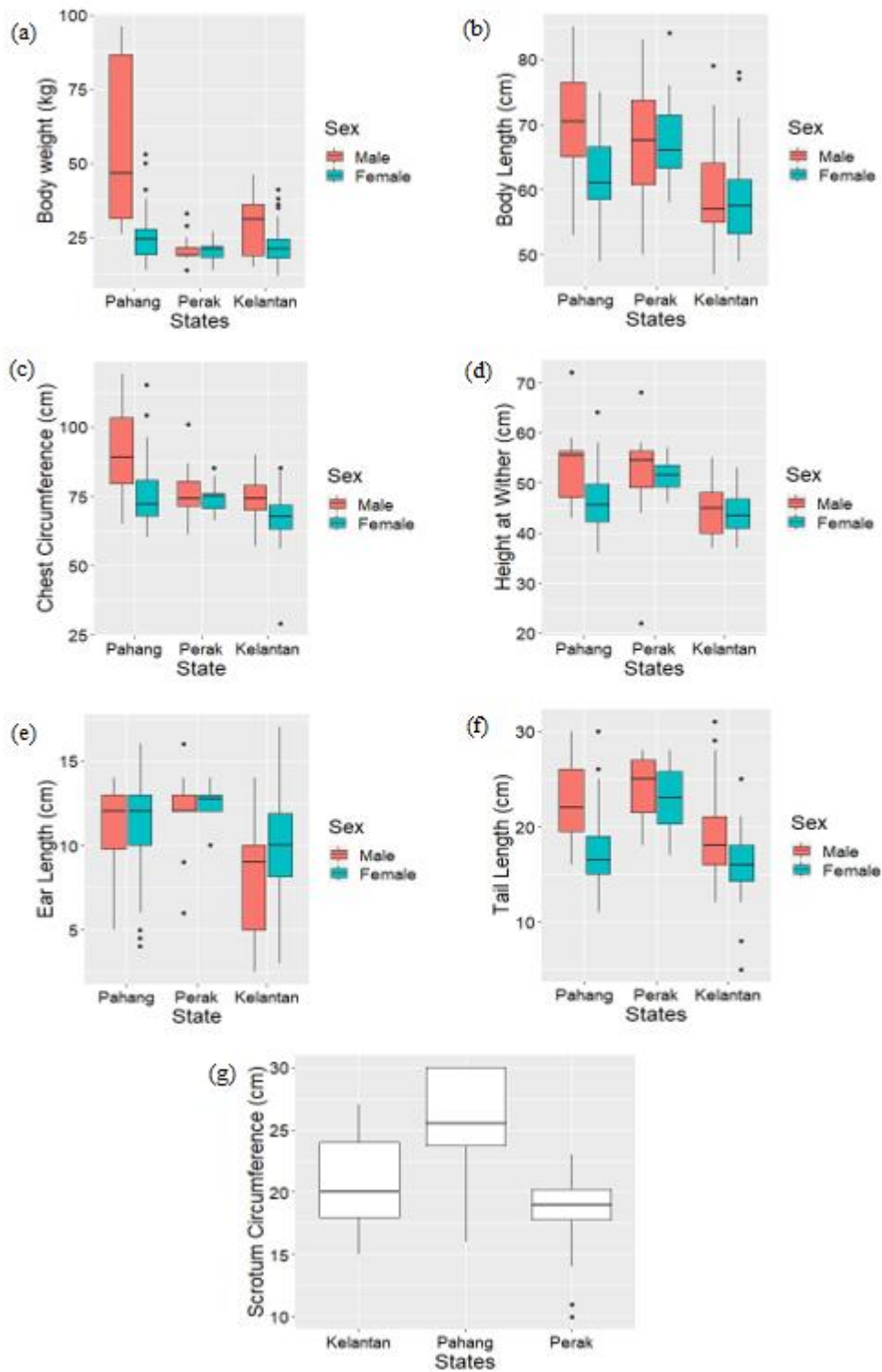
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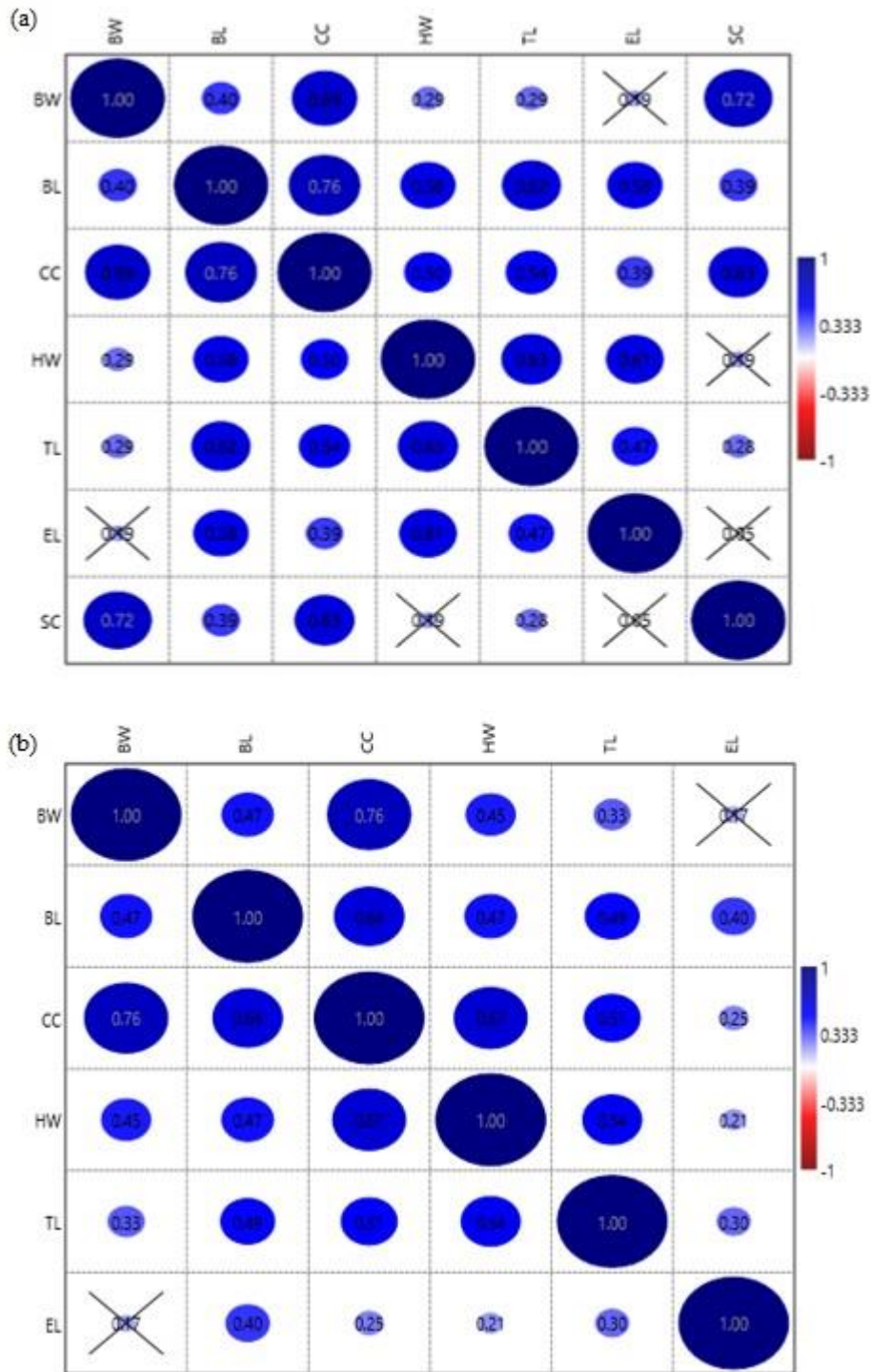
Figure 4. Horn shape of Malin sheep. A = scurs, B = polled, and C = horned.



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Figure 5. Comparison of medians of each morphometric trait via boxplot diagrams.

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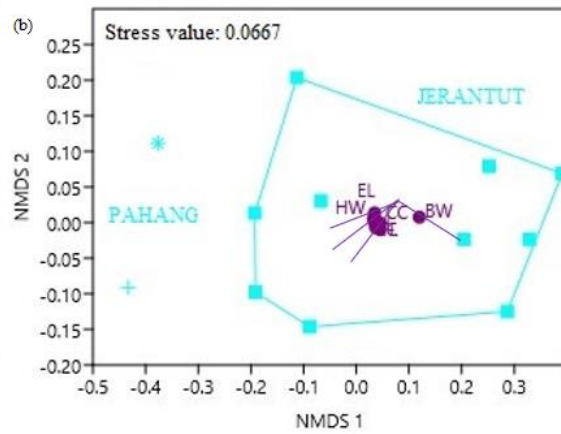
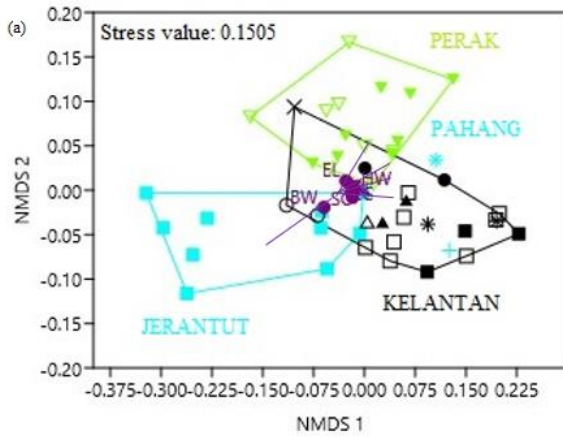
Figure 6. Spearman rank correlation tests for Malin sheep. (a) Male and (b) female. The (X) marks represent correlations that were not statistically significant ($p > 0.05$). BW = Body weight, BL = Body length, CC = Chest circumference, HW = Height at wither, TL = Tail length, EL = Ear length, and SC = Scrotum circumference.

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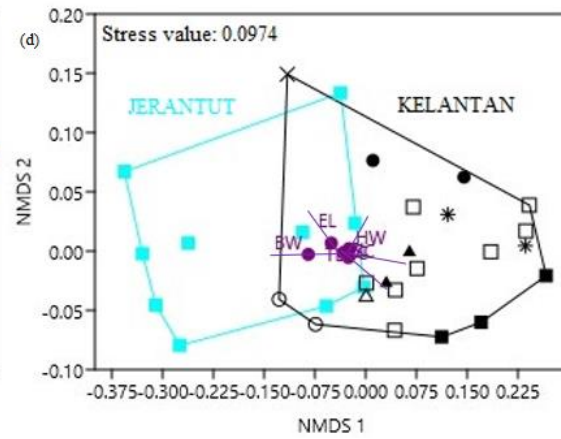
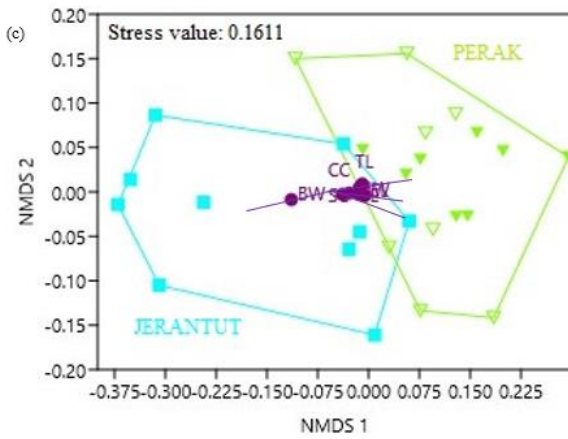
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ANOSIM
R-value = 0.478 *



ANOSIM
R-value = 0.474 ***

ANOSIM
R-value = 0.476 ***

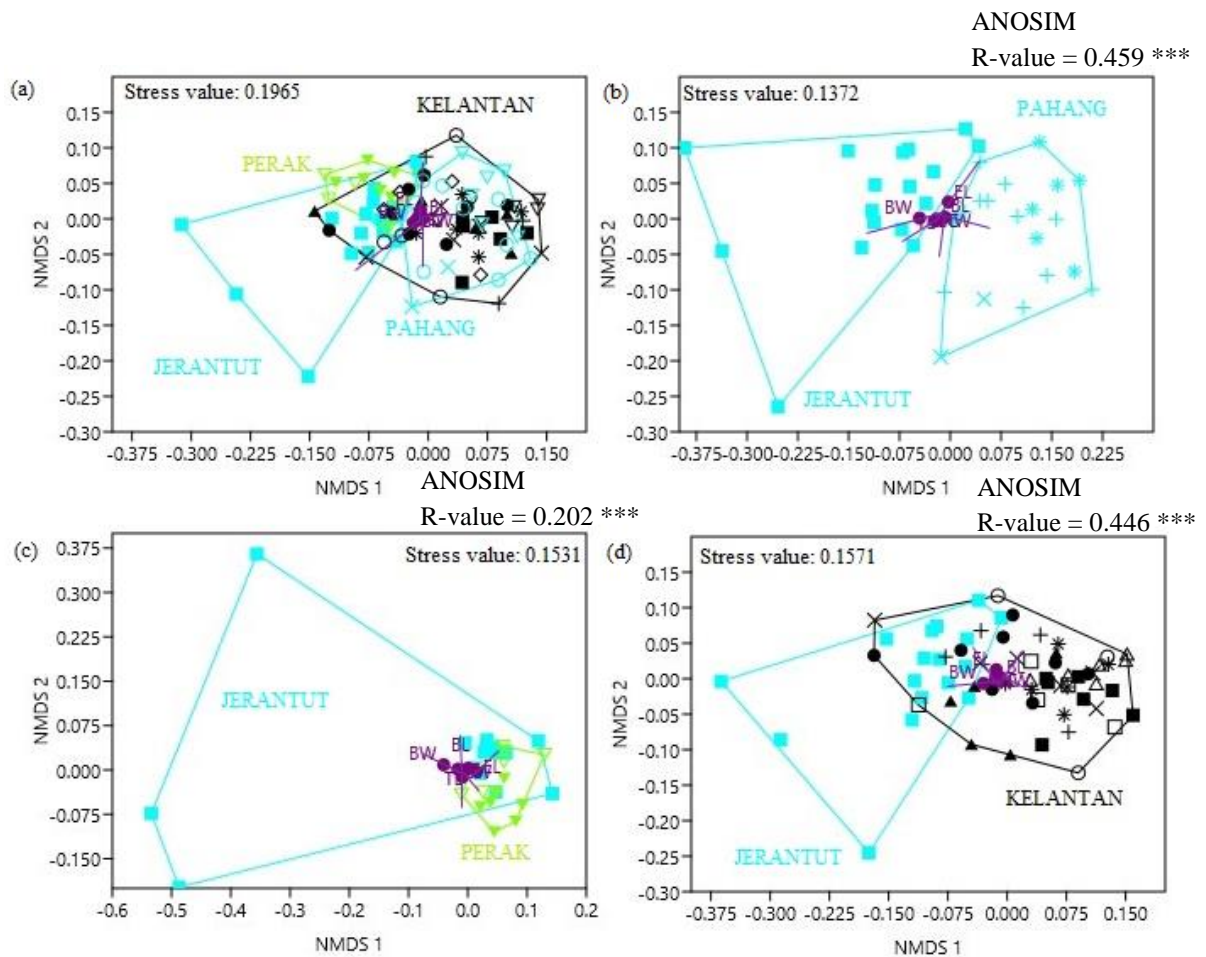


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Figure 7. NMDS plots based on Gower matrix comparing each state and the Jerantut male population. (a) Overall studied population comparisons: (b) Jerantut and Pahang (c) Jerantut and Perak, and (d) Jerantut and Kelantan. (2D stress value <math>< 0.2</math>). R-value calculated using ANOSIM, and significance is shown by asterisks (* = $p < 0.05</math>; ** = $p < 0.01</math>; *** $p < 0.01</math>).$$$

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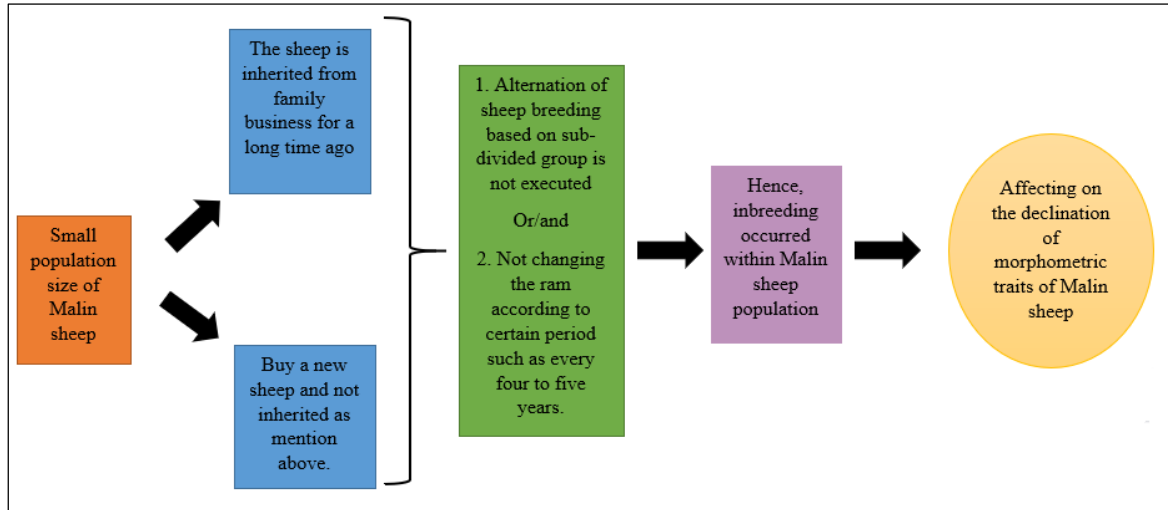
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Figure 8. NMDS plots based on Gower matrix comparing each state and the Jerantut female population. (a) Overall studied population comparisons: (b) Jerantut and Pahang, (c) Jerantut and Perak, and (d) Jerantut and Kelantan. (2D stress value <0.2). R-value calculated using ANOSIM, and significance is shown by asterisks (* = $p < 0.05$; ** = $p < 0.01$; *** $p < 0.001$).

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Figure 9. Probable causes of inbreeding in the Malin sheep population.

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