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Multivariate Analyses of Elytral Spot Patterns in the Phytophagous Ladybird Beetle *Epilachna vigintioctopunctata* (Coleoptera, Coccinellidae) in the Province of Sumatera Barat, Indonesia

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ABSTRACT—We analyzed geographic trends of elytral spot variations in the phytophagous ladybird beetle *Epilachna vigintioctopunctata* in the Province of Sumatera Barat, Indonesia. An UPGMA analysis revealed two distinct clusters of populations, one of which was further subdivided into two minor clusters. A principal component analysis also indicated the presence of the two major clusters. However, a closer analysis of the data suggested that the two clusters represented a complicated intraspecific variation rather than two distinct taxa. The dichotomy of the populations seemed to be caused by the pausity of samples at particular altitudes, which makes a continuous series of variation as if they are composed of two distinct subsets. The first two principal components accounted for approximately 90% of the variance. The first component mainly expressed the number of elytral spots, and the scores increased with an increase of altitude. The second component expressed the frequencies of particular types of spot confluences.

INTRODUCTION

The phytophagous ladybird beetle *Epilachna vigintiocto-punctata* (Fabricius), which is a serious pest of solanaceous crops and is widespread from southeast Asia through Australia, exhibits a wide range of infra- and interpopulational variation in their elytral spot pattern [1–4]. Katakura *et al.* [3] mentioned that individual beetles of *E. vigintioctopunctata* in the Province of Sumatera Barat, Indonesia, possessed 6 to 14 spots on each elytron, and some of these spots were often coalesced. They recognized two forms, formae A and B, in the Sumatran *E. vigintioctopunctata* that were identical in structural character but were different in body coloration, pronotal and elytral spot variations and vertical distribution. Later, Abbas *et al.* [1] divided populations of *E. vigintiocto-punctata* in the same area into four groups based on their spot pattern variations. Group I, occurring in the coastal plains and inland lowlands, and Group IV, confined to the highlands, were two extremes of the spot pattern variations, the latter having many more non-persistent spots and confluences, larger body size and more advanced melanism than the former. Group I consisted of forma A individuals while Group IV was made up of forma B individuals, but the two groups were connected with each other via the intermediate groups II and III. On the basis of these findings, Abbas *et al.* [1] considered that the two forms of Katakura *et al.* [3] represent a largely altitude-linked intraspecific variation rather than two distinct but closely related species.

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The present paper aims to confirm these previous results and conclusions through multivariate analyses of the data provided by Abbas *et al.* [1]. It also aims to elucidate major components involved in the geographic variation of elytral spot patterns in west Sumatran *E. vigintioctopunctata*.

MATERIALS AND METHODS

Area surveyed (Fig. 1)

The Province of Sumatera Barat occupies the western part of Central Sumatra, ranging from 0°54'N to 3°30'S, and 98°36'E to 101°53'E. The coastal plain is very narrow, only a few to 25 km in width, and soon gives way to the steep western slope of the Barisan Mountains, which run parallel to the coast with numerous peaks higher than 2,000 m. The eastern slope of the mountains is far less steep, forming a plateau 200 to 1,000 m high [3].

Specimens

Abbas *et al.* [1] reported that, although individuals of both sexes showed a similar pattern of geographic variation, females had a slightly but significantly larger number of spots than males from the same locality. For the sake of brevity, then, we used only male specimens in the present analyses. From the materials used by Abbas *et al.* [1], we chose 53 population samples (Fig. 1), each of which contained more than 20 males. The number of specimens examined and the elevation of the collected site of each population are listed in Appendix.

Spot patterns

The standard elytral spot patterns of *E. vigintioctopunctata* are given in Figure 2. The basic pattern consists of six black "persistent" spots (*1–6*) on each elytron. This pattern may be modified by the addition of one to eight "non-persistent" spots (*a-h*) on each

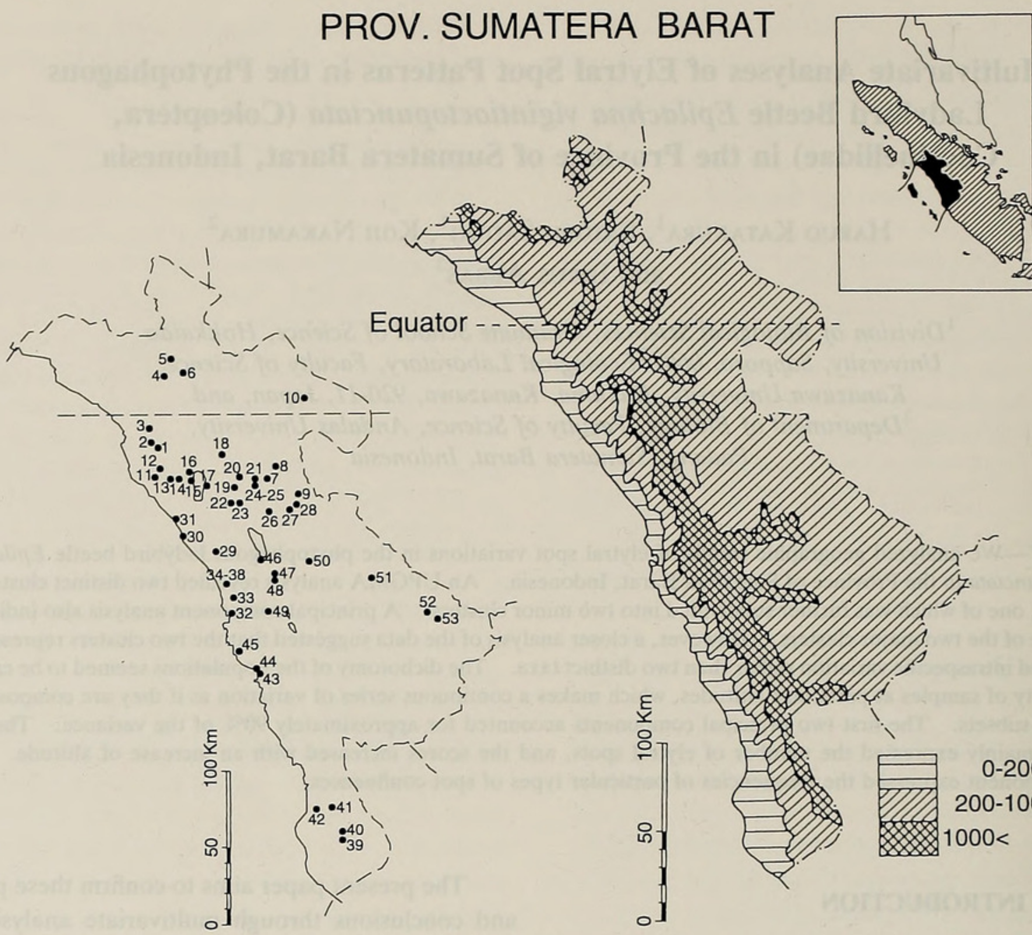

Fig. 1. Relief map of Sumatera Barat (modified from Katakura *et al.* [3]) and provenance of the studied populations. Populations were shown with the code numbers given in Appendix.

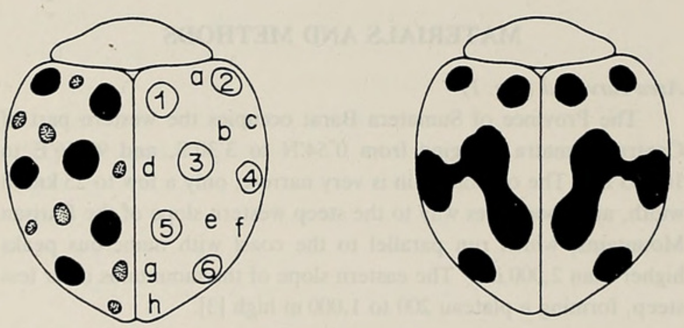

Fig. 2. Left: Standard elytral spot patterns of *E. vigintioctopunctata*, showing codes for persistent spots (*1–6*) and non-persistent spots (*a-h*). Right: The confluence of spots exemplified by *4*+*3*+*5*. (From Katakura *et al.* [3])

elytron, or by the enlargement and confluence of several spots [2]. Spot *a* is very rare and did not appear in the present materials [3]. All other non-persistent spots appeared with various frequencies according to populations. In addition, the following seven types of confluences, all between persistent spots, and combinations of some of these confluences were recognized: *1-2*, *1-3*, *2-4*, *3-4*, *3-5*, *4-6*, and *5-6*.

Data analyses

We treated each non-persistent spot (except *a*) and each type of confluence as a character in the following analyses. We recorded the presence or absence of each non-persistent spot and each confluence type for each specimen. If an individual had a joint confluence consisting of more than two spots (as shown in Figure 2 right where spots *4-3-5* fused), the individual was regarded to have two or more confluences (e.g., *3–4* and *3–5*). We then calculated percentage ratios of respective non-persistent spots and confluence types for each population. As the raw data set, we used arcsine transformed values [9] of these percentage ratios.

As a measure of the dissimilarity of elytral spot pattern variations, we calculated the Euclidean distances between populations from the raw data. Then a dendrogram using UPGMA (unweighted pair group method using arithmetic means [8]) was depicted. Next, we carried out a principal component analysis based on the covariance matrix of the fourteen characters (seven non-persistent spots plus seven confluence types) for all the 53 populations to extract the major components of interpopulational variations. Finally, to visualize the geographic pattern of variations, contour lines of the principal component scores standardized between 1 (the maximum score) and 0 (the minimun score) [8] were drawn on the map at an interval of 0.1, using Delaunay triangulation [5] and linear interpolation techniques. Delaunay triangulation and the drawing of the contour lines were performed on a SAS graph program package (Proc G3D, Proc Gcontour [6]).

RESULTS

Figure 3 is a UPGMA dendrogram based on the dissimi-

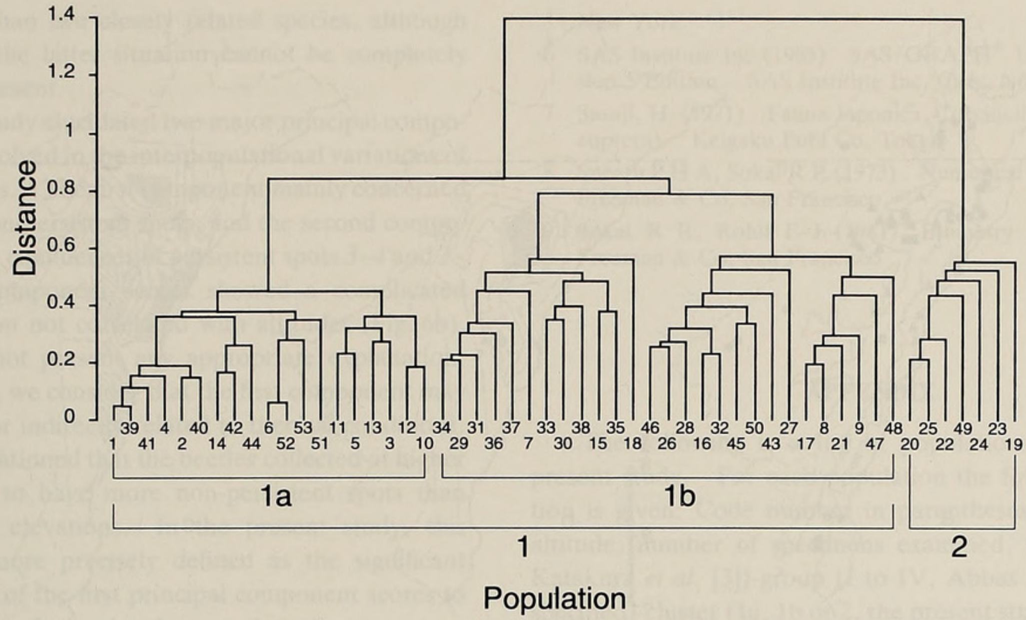

FIG. 3. A dendrogram resulting from UPGMA cluster analysis of elytral spots of 53 populations of *E. vigintioctopunctata* in Sumatera Barat. Populations were shown with the code numbers given in Appendix.

larity of the elytral pattern variation between populations. There are two major population clusters. One of the two clusters is further subdivided into two minor clusters. We hereafter call these three clusters 1a, 1b and 2 as indicated in Figure 3. Figure 5a shows the distribution of these three clusters. Cluster 2 (denoted by rectangles) occupies the mountain area whereas the population clusters 1a (circles) and 1b (triangles) are distributed in lower altitudes.

The results of the principal component analysis are summarized in Table 1. The first two components account for approximately 90% of the variance. The first component (explaining 72% of the variance) has positive and comparatively high loadings for all non-persistent spots and confluences *3–4* and *3–5*. On the other hand, the second component (17% of the variance) has high positive loadings for confluences *3–4*, *3–5*, and weak negative loadings for all non-persistent spots. In other words, the first component mainly expresses the frequencies of non-persistent spots, whereas the second component mainly expresses the frequencies of particular types of spot confluences.

TABLE 1. Loadings of the 14 characters (seven non-persistent spots and seven types of confluences) on the first two principal components (PCs)

Character	PC1	PC2
b	0.277	−0.254
C	0.313	−0.156
d	0.310	−0.173
e	0.251	−0.121
f	0.256	−0.136
g	0.375	−0.180
h	0.466	−0.212
1–2	0.131	0.051
1–3	0.017	0.003
2–4	0.012	−0.002
3–4	0.382	0.531
3–5	0.251	0.587
4–6	0.019	0.060
5–6	0.125	0.372
Explained variance (%)	71.5	16.7

Population projections on the first two axes (Fig. 4) show that the two major clusters recognized by UPGMA (1a+b, 2) are also clearly separated by the scores of the first principal component. On the other hand, the two minor clusters 1a and 1b are contiguous along the first axis. The three clusters are not separable along the second axis, and there was a very large variation in the minor cluster 1b.

Geographical variations of the first two principal components are shown in Figure 5b and 5c. The score of the first

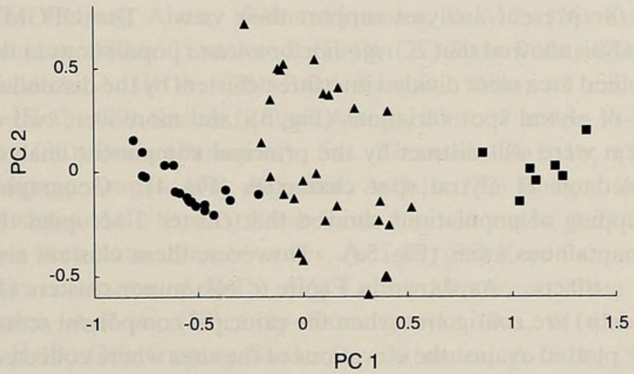

FIG. 4. Projections of principal component scores of 53 populations onto the first two axes. Symbols denote the clusters recognized by UPGMA cluster analysis: circles, cluster 1a; triangles, cluster 1b; rectangles, cluster 2.

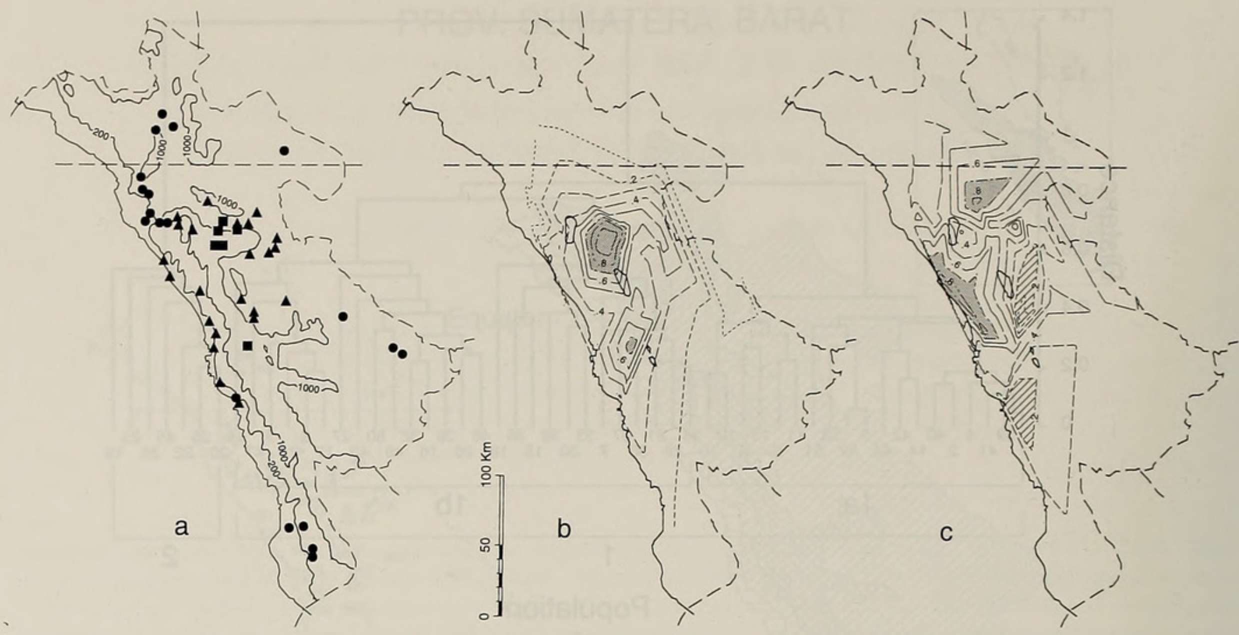

FIG. 5. a: Distribution of the three clusters recognized by UPGMA analysis; symbols are the same with those of Fig. 4. Contour lines are for 200 m and 1,000 m above sea level. b and c: Geographic variations of the first (b) and the second (c) principal component scores, expressed by contour lines; areas with scores higher than 0.8 are shaded, and in c, those lower than 0.2 hatched.

component (Fig. 5b) tends to become large at higher altitudes, with two conspicuous peaks of scores amid the Barisan mountains. On the other hand, the scores of the second component vary geographically in a complicated manner (Fig. 5c). The areas with large second component scores occupy the middle part of the west coast of Sumatera Barat, and a mountainous area just south of the equator. The lowest scores are found near a crater lake and on the western slope of the southern part of the mountain regions. Consequently, a steep east-west cline of the second component scores runs near the central part of Sumatera Barat across the mountains.

DISCUSSION

Abbas *et al.* [1] considered that the two forms of *E. vigintioctopunctata* reported by Katakura *et al.* [3] were a largely altitude-linked infraspecific variations. The results of the present analyses support their view. The UPGMA analysis showed that *E. vigintioctopunctata* populations in the studied area were divided into three clusters by the dissimilarity of elytral spot variations (Fig. 3), and moreover, two of them were still distinct by the principal component analysis based on 14 elytral spot characters (Fig. 4). Geographic mapping of populations showed that cluster 2 occupied the mountainous areas (Fig. 5a). However, these clusters may be artifacts. As shown in Figure 6, two minor clusters (1a and 1b) are contiguous when the principal component scores are plotted against the elevations of the sites where collected, and the distinction of the two major clusters (1 and 2) seems to be largely dependent on the pausity of population samples around 800 m altitude. It is likely that if an adequate number of populations are sampled around this altitude, the

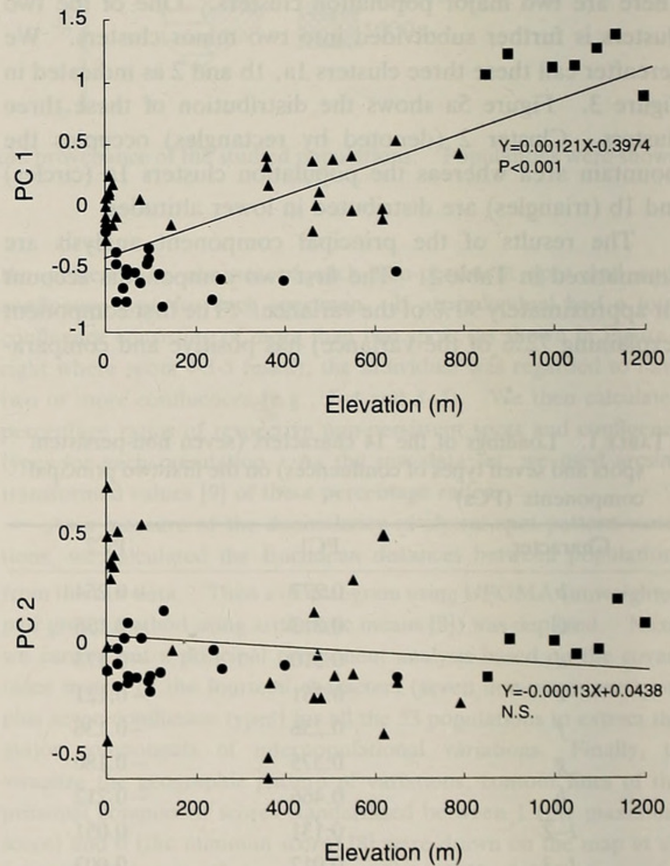

FIG. 6. Relationships between elevations of collected sites and the first two principal component scores of populations.

two major clusters will be united and form a continuous array of variation. The regression coefficient of the first component to elevations is highly significant ($P<0.001$). Thus, we regarded clusters 1 and 2, or forma A and B to be infraspecific variations rather than two closely related species, although the possibility of the latter situation cannot be completely ruled out at the present.

The present study elucidated two major principal components that were involved in the interpopulational variations of elytral spot patterns. The first component mainly concerned the incidence of non-persistent spots, and the second component concerned the confluences of persistent spots *3–4* and *3–5*. The second component scores showed a complicated geographic variation not correlated with altitudes (Fig. 6b), for which we cannot present any appropriate explanation. On the other hand, we consider that the first component may be either directly or indirectly related to thermal conditions. Abbas *et al.* [1] mentioned that the beetles collected at higher elevations tended to have more non-persistent spots than those from lower elevations. In the present study, this relationship was more precisely defined as the significant positive regression of the first principal component scores to elevation (Fig. 6a). It is also known that the temperate conspecific populations of *E. vigintioctopunctata* in Japan usually have a full number of 14 spots on each elytron [2, 7]. These facts suggest that the number of elytral spots, or degree of melanization in *E. vigintioctopunctata*, increases with the decrease of ambient temperatures. Both environmental and genetic factors seem to be responsible for the determination of elytral spot patterns in *E. vigintioctopunctata* [1]. Yet, it is still unknown whether the increase of melanization *per se* is adaptive in cooler conditions or it is a byproduct of other adaptive characters that change with the change of thermal conditions.

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APPENDIX

The following is a list of populations studied in the present study. For each population the following information is given: Code number in parentheses, locality name, altitude, number of specimens examined, forma (A or B, Katakura *et al.* [3])-group (I to IV, Abbas *et al.* [1]; ?, not specified)-cluster (1a, 1b or 2, the present study) to which the population was assigned.

(1) Padang Kotomarapak, 70m, 45 males, A-I-1a; (2) Kapundung, 100m, 22 males, A-I-1a; (3) Kinali, 25m, 42 males, A-I-1a; (4) Tinggam Sinurut, 650m, 35 males, A-I-1a; (5) Panti, 240m, 20 males, A-I-1a; (6) Ampang Gadang, 260m, 22 males, A-I-1a; (7) Piladang, 550m, 29 males; B-III/IV-1b; (8) Napar-Tarok (Payakumbuh), 510m, 60 males, AB-III/IV-1b; (9) Halaban, 790m, 24 males, A-III-1b; (10) Koto Ameh-Koto Masjid-Koto Ateh, Pangkalan, 130m, 25 males, A-?-1a; (11) Manggopoh, 40m, 82 males, A-I/II-1a; (12) Sungai Jaring, 50m, 31 males, A-I/II-1a; (13) Kampung Tangah-Desa Sikabu, 80m, 37 males, A-I/II-1a; (14) Batukarak, 100m, 49 males, A-I/II-1a; (15) Muko-muko, 465m, 46 males, A-I/II-1b; (16) Batu Anjing-Sawar Panjang, 470m, 52 males, A-I/II-1b; (17) Kakuban-Sungai Batang, 465m, 50 males, AB-III-1b; (18) Palupuh-Panorama Baru-Bukittingi, 620m, 25 males, AB-?-1b; (19) Ladang Cakiah, 900m, 30 males, B-IV-2; (20) Biaro, 850m, 122 males, B-IV-2; (21) Padang Tarab, 650m, 51 males, B-IV-1b; (22) Pandai Sikek, 1100m, 39 males, B-IV-2; (23) Gunung Merapi, 1140m, 53 males, B-IV-2; (24) Pincuran Puti, 1050m, 30 males, B-IV-2; (25) Tanjung Alam, 1000m, 65 males, B-IV-2; (26) Koto Panjang, 480m, 35 males, B-III-1b; (27) Batu Bulat, 620m, 31males, A III-1b; (28) Balai Tangah, 550m, 43 males, A-III-1b; (29) Kasiak (Lubuk Alang), 20m, 39 males, A-II-1b; (30) Pauh (Pariaman), 5m, 27 males, A-II-1b; (31) Simpang Ampar, 10m, 40 males, A-II-1b; (32) Bungus, 2m, 51 males, A-II-1b; (33) Pasar Baru, 25m, 32 males, A-II-1b; (34) Ikur Koto, 10m, 66 males, A-II-1b; (35) Koto Panjang, 20m, 27 males, A-II-1b; (36) Lubuk Minturum, 80m, 58 males, A-II-1b; (37) Lolo Gunungsarik, 10m, 94 males, A-II-1b; (38) Gunung Sarik, 5m, 30 males, A-II-1b; (39) Dusun Baru, 50m, 90 males, A-I-1a; (40) Tanjung Pondok, 50m, 20 males, A-I-1a; (41) Sungai Gemuruh, 25m, 28 males, A-I-1a; (42) Indrapula, 25m, 32 males, A-I-1a; (43) Painan, 2m, 22 males, A-I/II-1b; (44) Salido, 5m, 64 males, A-I/

II-1a; (45) Api-api, 2m, 37 males, A-I/II-1b.(46) Kasiak (Sumani)-Sikumbang-Padang Belimbing, 365m, 69 males, A-III-1b; (47) Solok, 360m, 23 males, A-III-1b; (48) Kotobaru, 360m, 28 males, A-III-1b; (49) Batang Barus, 1200m, 21 males, B-III/IV-2; (50) Padang Sibusuk, 150m, 40 males, A-I/II-1b; (51) Rasam Tapanggang, 350m, 22 males, A-I/II-1a; (52) Suigai Kambuik-Sungai Daleh, 120m, 29 males, A-I-1a; (53) Gunung Medan, 95m, 44 males, A-I-1a.