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A morphological study of phlebotomine sandflies and their role in the transmission of human leishmaniasis, with special reference to Namibia

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TABLE 2 Comparison of data of three members of the Southern African SYNPHLEBOTOMUS group

♀♀ n =	ROSSI A[east] 17 mm	ROSSI B[west] 23 mm	GROVEI 21 mm
Wing length	2.3-2.7	1.87-2.27	1.85-2.3
breadth	0.67-0.83	0.47-0.64	0.56-0.73
Antenna 3	0.25-0.295	0.208-0.276	0.184-0.245
Ascoid 3	0.075-0.087	0.05-0.065	0.058-0.075
c/b 3	0.93-1.13	1.15-1.57	0.80-1.10
Antenna 4	0.115-0.14	0.098-0.128	0.09-0.115
Ascoid 4	0.075-0.095	0.052-0.067	0.065-0.086
c/b 4	1.0-1.15	1.28-1.57	0.95-1.15
Ascoid formula	2/III-XV	2/III-XV	2/III-XV
Labrum	0.285-0.34	0.248-0.295	0.245-0.305
Antenna 3/Labrum	0.82-1.16	0.77-1.06	0.70-0.94
Palp 2	0.15-0.18	0.12-0.17	0.12-0.18
Palp 3	0.18-0.22	0.14-0.20	0.15-0.20
Palp 4	0.16-0.21	0.14-0.20	0.14-0.20
Palp 5	0.40-0.56	0.42-0.56	0.30-0.48
Spermatheca	6-8 segments	6-8 segments	6-8 segments

♂♂ n =	ROSSI A 28 mm	ROSSI B 28 mm	GROVEI 14 mm
Wing length	1.75-2.7	1.87-2.27	1.72-1.89
breadth	0.55-0.69	0.47-0.63	0.44-0.53
Antenna 3	0.22-0.28	0.21-0.27	0.164-0.203
Ascoid 3	0.055-0.075	0.03-0.05	0.045-0.062
c/b 3	1.12-1.47	1.7-2.3	0.96-1.15
Antenna 4	0.10-0.13	0.10-0.14	0.08-0.097
Ascoid 4	0.055-0.075	0.035-0.05	0.047-0.066
c/b 4	1.09-1.45	1.7-2.4	1.05-1.26
Ascoid formulae	a] 2/III-IX; 1/X-XIII	a] 2/III-XIII; 1/XIII b] 2/III-XI; 1/XII-XIII c] 2/III-XV d] 2/III-XIII	a] 2/III-IX; 1/X-XIII b] 2/III-X; 1/XI-XIII
Labrum	0.20-0.26	0.18-0.23	0.17-0.23
Antenna 3/Labrum	0.97-1.2	0.97-1.10	0.86-1.01
Palp 2	0.12-0.13	0.08-0.14	0.11-0.12
Palp 3	0.15-0.20	0.14-0.17	0.12-0.15
Palp 4	0.15-0.21	0.14-0.17	0.12-0.16
Palp 5	0.36-0.46	0.34-0.47	0.32-0.42
Style	0.18-0.20	0.16-0.20	0.13-0.15
Coxite	0.32-0.35	0.32-0.36	0.25-0.28
Coxal lobe	0.10-0.12	0.08-0.11	0.08-0.10
Paramere	0.19-0.23	0.20-0.24	0.18-0.20
Aedeagus	0.09-0.11	0.08-0.11	0.08-0.11
Genital Pump	0.14-0.17	0.15-0.18	0.12-0.15

3.3.2 Differences in ascoid lengths.

Table 2 also shows the distinct difference, between **P. rossi** east and west of both sexes, in the length of the longest ascoids on antennal segments 3 & 4.

3.4. COMPUTER ANALYSIS.

Certain morphological features and the observation of certain habitat preferences suggest that **P. rossi s.l.** may comprise 2 species (hereafter termed **P. rossi** species A [east] and **P. rossi** species B [west]). Furthermore, the females of **P. rossi s. l.** are difficult to differentiate from **P. grovei** in areas of sympatry. Discriminant function analysis was used to develop linear equations that permit the correct identification of **P. rossi** species A and **P. rossi** species B and these from **P. grovei**.

3.4.1 Discriminant Function Analysis (DFA).

Data were obtained for these analyses by measuring nineteen morphological features (see Appendix 1) on each specimen. The data were then analysed using the programme BMDP 7M (Dixon & Brown, 1981). The samples were made up as follows:

P. rossi species A - 23 females, 28 males;

P. rossi species B - 17 females, 28 males.

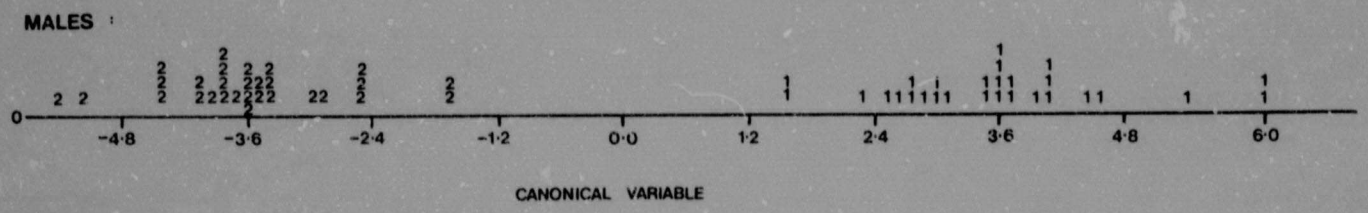
P. grovei - 21 females.

P. rossi sp. A and B were categorised as such on distribution. In the first analysis the females of *P. rossi* sp. A and *P. rossi* sp. B were analysed separately from the females of *P. grovei*. It was also necessary to analyse the males of *P. rossi* s.l., these being grouped as such on distribution, as there is no obvious difference in the morphology of the male terminalia of sp. A and sp. B. It was not necessary to analyse the males of *P. rossi* s.l. and *P. grovei* as the morphology of their terminalia is diagnostic. In the second analysis the data from the females of *P. rossi* sp. A, *P. rossi* sp. B and *P. grovei* were analysed. *P. grovei* was grouped as having a shorter antennal segment 3 in conjunction with a narrower wing and shorter ascoid 3 than *P. rossi* sp. A. It was separated from sp. B on distribution as well as differences in the c/b ratios (see Table 2).

3.4.2 Results of DFA of *P. rossi* s.l.

On the basis of three variables, namely ascoid 3, ascoid 4 and c4 [see Appendix 1, Fig. 143], the programme classified the female specimens as being 100% correctly grouped [Fig. 9]. The F-test (analysis

Fig. 9 Histogram showing the discrimination [in both sexes] between P. rossi sp. A and P. rossi sp. B.



1 : P. rossi sp. A
2 : P. rossi sp. B

Fig. 9

of variance) value at 3 and 36 d.f. at $p<0.001$ was 214.67, suggesting the difference in length of the chosen variables of sp. A and sp. B to be highly significant. In the analysis of the males, three variables namely ascoid 3, ascoid 4 and the combined length of antennal segments 4 and 5, classified the specimens as being 100% correctly grouped [Fig. 9]. The F-test value at 3 and 52 d.f. at $p<0.001$ was 227.92 again suggesting a highly significant difference between sp. A and sp. B.

3.4.3 Results of DFA of *P. rossi* s.l. and *P. grovei*.

In this analysis of female specimens one designated as ***P. grovei*** was grouped as a ***P. rossi*** sp. A. The programme chose 3 variables, namely antennal segment 3 length, ascoid 4 length and the length of c4. The F-test value at $p<0.001$ at 3 and 56 d.f. for ***P. rossi*** sp. A and ***P. rossi*** sp. B was 134.12; for ***P. rossi*** sp. B and ***P. grovei*** 82.50; and for ***P. rossi*** sp. A and ***P. grovei*** 45.11. Additional material acquired since this analysis has been added to the graphical plots [Fig. 10] produced by the programme, calculated according to the following linear equations.

Fig. 10 Computer plot showing the graphical separation of P. rossi sp. A, P. rossi sp. B and P. grovei.

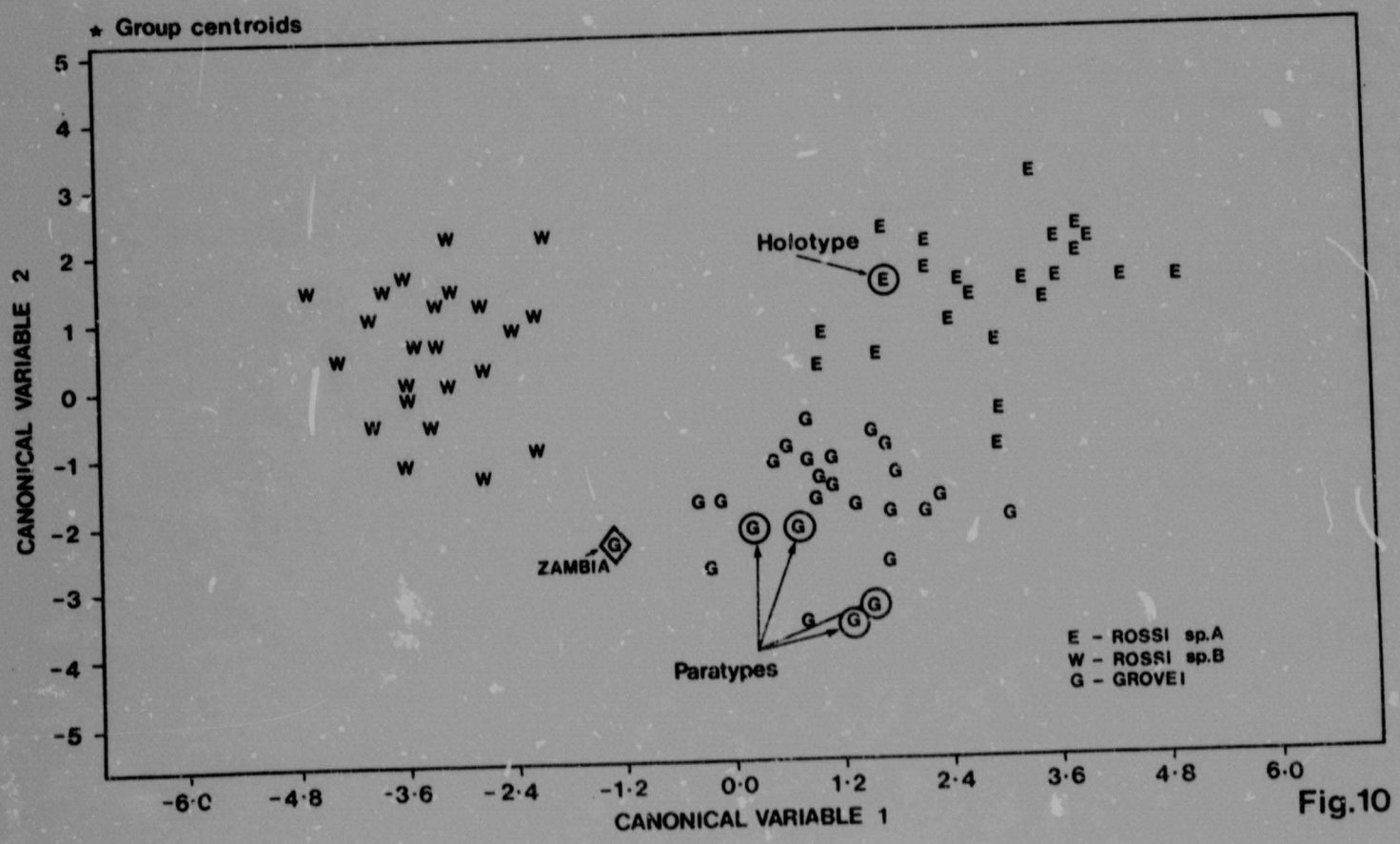

★ Group centroids
Holotype
Paratypes
ZAMBIA
E - ROSSI sp.A
W - ROSSI sp.B
G - GROVEI
CANONICAL VARIABLE 2
CANONICAL VARIABLE 1
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6·0
-4·8
-3·6
-2·4
-1·2
0·0
1·2
2·4
3·6
4·8
6·0

Fig.10

FEMALES -

CV_1 = -7.50934 + 0.01666 (antennal segment 3) + 0.26519 (ascoid 4) - 0.18493 (r4).

CV_2 = -10.35202 + 0.07828 (antennal segment 3) - 0.05609 (ascoid 4) - 0.05868 (r4).

MALES -

CV_1 = -2.93134 - 0.05468 (antennal segment 3) + 0.26301 (ascoid 4).

CV_2 = -16.22804 + 0.05468 (antennal segment 3) + 0.05951 (ascoid 4).

3.5 DISCUSSION.

The known distribution of P. rossi sp. B is from Windhoek in central Namibia, southwards to Okiep in the northern Cape of the Republic of South Africa. This distribution coincides with that of diagnosed leishmaniasis cases, with the exception of three cases from Otavi and Grootfontein in northern Namibia (Grové, 1970) [Fig. 2]. These three cases fall within the distribution of another potential vector species Phlebotomus (Synphlebotomus) grovei, Downes (1971), but the habitat does not fit the general habitat preference expected for this species. However, collecting in this area has been insufficient to draw any conclusions as to the role of P. grovei as a vector. No cases of leishmaniasis have been diagnosed from within the distribution of P. rossi sp. A.

The histogram plot [Fig. 9], of both sexes, of P.

rossi sp. A and *P. rossi* sp. B shows them to be morphologically distinct. It may be suggested that *P. rossi* sp. A and B are simply ends of a geographic cline. This explanation is undermined by the following facts. Phlebotomine collections have been made in the central area separating the two populations, from various potential microhabitats including rock hyrax warrens. No *P. rossi* specimens have been collected [Fig. 2]. Rock hyrax occur throughout southern Africa wherever suitable habitat prevails. There is no indication of intermediate morphological characters in any of the measurements taken from specimens of *P. rossi s.l.* collected either from N to S or from E to W (pers.obs.).

The computer plot [Fig. 10] suggests that features used to group the females of *P. rossi s.l.* and *P. grovei* are good enough to separate most specimens. Table 2 compares the measurements of the three species and the linear equations [pg. 44] enable one to key out the difficult specimens.

SECTION TWO - East Africa.

3.6 INTRODUCTION.

The specific identification of a vector species is important when determining its distribution and undertaking control measures. The role of the three **Synphlebotomus** species in the dissemination of leishmaniasis in Kenya is not fully understood. **Phlebotomus martini** Parrot (1936) was described from 2♂ and 9♀ from Ethiopia, Diré Daoua, iv/v/vi.1935. **Phlebotomus celiae** Minter (1962) was described from 10♂ and 10♀ from Kenya, Kauriro in the Kitui district and collected from termite hills during 1960 and 1961 and **P. vansomerenae** Heisch, Guggisberg & Teesdale (1956) was described from 3♂ and 15♀ specimens from Kenya, Nuu, Ngomeni and Tseikuru. All three species have at some stage been suggested as vectors of CL and VL (Lysenko, 1971; Minter, 1963; Minter & Wijers, 1963; Wijers & Minter, 1962; Wijers, 1963; Wijers & Ngoka, 1974). Wijers and Minter (1962) suggest that the only species likely to be vectors of VL (kala-azar) are **P. martini** and **P. vansomerenae**. Minter & Wijers (1963) concluded that **P. martini** is

the principal natural vector of kala-azar in Kenya, with P. vansomerenae and P. celiae possibly involved as secondary vectors in certain areas. Today, it is well known that the females of these three species are indistinguishable (Killick-Kendrick & Ward, 1981; Kaddu, 1986), so although it can possibly be surmised that P. martini is the vector in foci other than those where more than one species of the Synphlebotomus complex are known to occur, it cannot be taken for granted that it is the vector in areas of sympatry as in the Kitui focus and possibly in the adjacent Machakos district (including the Masinga focus) [Fig. 3]. The males of the 3 species are easily recognisable by the morphology of the terminalia and the ascoid formulae [Figs 11-13; Table 3]. The specific identification of the females may have been further complicated by the apparently incorrect association of paratype material of P. celiae and P. vansomerenae.

3.7 METHODS.

The available type material of P. celiae and P. vansomerenae was borrowed from the British Museum (Natural History). It was not possible to acquire any of the type series of P. martini as they are lodged in Algeria which does not communicate with South



Fig.11



Fig.12



Fig.13

Figs 11 - 13. Male terminalia of *P. martini*, *P.celiae*, and *P. vansomerenae*.

TABLE 3 Comparison of data of three members of the East African SYNPHLEBOTOMUS group

♀♀	CELIAE	VANSOMERENAE	MARTINI
n =	40	27	54
	mm	mm	mm
Wing length	1.6-2.1	1.7-2.25	1.8-2.3
breadth	0.46-0.64	0.48-0.64	0.53-0.70
Antenna 3	0.148-0.20	0.146-0.20	0.157-0.222
Ascoid 3	0.045-0.067	0.059-0.079	0.06-0.082
c/b 3	0.86-1.24	0.81-1.0	0.78-1.07
Antenna 4	0.075-0.095	0.082-0.104	0.082-0.104
Ascoid 4	0.05-0.07	0.059-0.084	0.059-0.084
c/b 4	0.95-1.20	0.9-1.10	0.83-1.07
Ascoid formula	2/III-XV	2/III-XV	2/III-XV
Labrum	0.19-0.23	0.22-0.26	0.27-0.34
Antenna 3/Labrum	0.74-0.89	0.57-0.85	0.55-0.70
Palp 2	0.10-0.13	0.11-0.14	0.127-0.19
Palp 3	0.125-0.16	0.14-0.175	0.155-0.205
Palp 4	0.123-0.165	0.12-0.185	0.137-0.19
Palp 5	0.215-0.425	0.22-0.45	0.195-0.50
Spermatheca	7-9 segments	7-9 segments	7-8 segments

♂♂	CELIAE	VANSOMERENAE	MARTINI
n =	10	08	30
	mm	mm	mm
Wing length	1.5-1.7	1.65-1.95	1.56-2.05
breadth	0.41-0.51	0.52-0.57	0.45-0.60
Antenna 3	0.15-0.20	0.18-0.205	0.169-0.235
Ascoid 3	0.034-0.044	0.05-0.056	0.056-0.066
c/b 3	1.15-1.69	1.0-1.16	0.97-1.21
Antenna 4	0.078-0.09	0.08-0.10	0.083-0.11
Ascoid 4	0.038-0.048	0.053-0.061	0.055-0.07
c/b 4	1.31-1.65	1.04-1.19	0.95-1.24
Ascoid formulae	2/III-XIII	2/III-IX; 1/X-XIII	2/III-X; 1/XI-XIII
Labrum	0.165-0.195	0.19-0.205	0.22-0.265
Antenna 3/Labrum	0.89-1.05	0.89-1.03	0.77-0.97
Palp 2	0.09-0.115	0.105-0.12	0.115-0.16
Palp 3	0.12-0.15	0.125-0.16	0.135-0.18
Palp 4	0.115-0.15	0.145-0.16	0.125-0.165
Palp 5	0.24-0.33	0.235-0.32	0.275-0.46
Style	0.125-0.17	0.14-0.155	0.13-0.175
Coxite	0.285-0.32	0.275-0.305	0.26-0.33
Coxal lobe	0.115-0.135	0.105-0.115	0.05-0.075
Paramere	0.18-0.22	0.16-0.19	0.17-0.215
Aedeagus	0.095-0.11	0.075-0.09	0.075-0.105
Genital Pump	0.125-0.165	0.125-0.16	0.135-0.17

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